Microgram

Journal

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC 20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 1
Numbers 1-2
January - June 2003

Posted On-Line At:
Contents

2003 Information and Instructions for Microgram Journal 3

Disclaimers 7

Osmolality - A Novel and Sensitive Tool for Detection of Tampering of Beverages Adulterated with Ethanol, γ-Butyrolactone, and 1,4-Butanediol, and for Detection of Dilution-Tampered Demerol Syringes 8
James F. Wesley

Psychotria Viridis –A Botanical Source of Dimethyltryptamine (DMT) 18
Robert D. Blackledge and Charlotte M. Taylor

Evaluation of Ninhydrin Analogues and Other Electron-Deficient Compounds as Spray Reagents for Drugs on Thin Layer Chromatograms 23
Myriam Azoury, Avraham Zelkowicz, Zafir Goren, and Joseph Almog

Instrumental Separation of 3,4-Methylenedioxyamphetamine (MDA) from 1-(3,4-Methylenedioxyphenyl)-2-propanol, a Co-Eluting Compound 32
Barbara A. Vohlken and Stephen M. Layton

Potency of Cannabis Seized in Central Florida During June 2002 37
Christina J. Newell

A Study of Acids Used for the Acidified Cobalt Thiocyanate Test for Cocaine Base 40
Anna L. Deakin

1,4-Butanediol (BD) - Forensic Profile 44
Agnes D. Garcia and Allen J. Catterton

Detection and Analysis of Drugs of Forensic Interest, 1992 - 2001; A Literature Review 55
Robert F.X. Klein and Patrick A. Hays

Cover Art: “Ball and Stick” Model of Heroin (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA)
2003 Information and Instructions for Microgram Journal

[Editor’sPreface: The following information and instructions are derived from the Microgram website <http://www.dea.gov/programs/forensicsci/microgram/index.html>, and are provided here for the convenience of those subscribers who do not have access to the Internet. Updates of this material will henceforth be published only in the respective January issues for each year.]

General Information
Microgram Journal is a quarterly periodical published by the U.S. Drug Enforcement Administration's Office of Forensic Sciences, and is intended to assist and serve scientists concerned with the detection and analyses of controlled substances and other abused substances for forensic/law enforcement purposes.

Subscriptions to Microgram Journal
Microgram Journal is unclassified, and is published on the DEA public access website (see the above URL). Private citizens should use the website to access Microgram Journal. Professional scientific and law enforcement personnel may either use the website or request a subscription. Subscriptions are available electronically and in hard copy. Electronic subscriptions require Internet access. The publications themselves will not be sent electronically to any subscriber; rather, an email notification will be sent to the subscriber when the respective issue is posted on the website. Requests for hard copies are strongly discouraged, and should be limited to those offices that do not have access to the Internet, require hard copies for their libraries, or have some other valid reason (Note: “For my personal collection” is not considered to be a valid reason). Requests for hard copies are limited to one per office, and should also include formal justification. Note that due to publication delays beyond the control of the Office of Forensic Sciences, hard copies will arrive from 30 to 90 days after electronic posting on the website.

Requests to be added to the subscription list should be submitted via email to the Microgram Editor at: microgram_editor@mailsnare.net If email submission is not possible, requests should be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. All requests to be added to the Microgram mailing list should include the following Subscriber Contact Information:

* The Full Name and Mailing Address of Submitting Laboratory or Office;

* The Full Name, Title (Laboratory Director, Assistant Special Agent in Charge, Librarian, etc.), Phone Number, FAX Number, and Preferred email Address of the Submitting Individual (Note that subscriptions are mailed to titles, not names, in order to avoid subscription problems arising from future personnel changes);

* If available, the generic email address for the Submitting Laboratory or Office;

* If a generic email address is not available, one official or private email address for an individual who is likely to be a long-term employee, who has a stable email address, and who will be responsible for forwarding Microgram information to all of the other employees in the requestor’s Office (Note that only one email address per Office will be honored); and

* If requesting a hard copy mailing, justification.

Requests to be removed from the Microgram subscription list, or to change an existing subscription, should also be sent to the Microgram Editor. Such requests should included all of the pertinent Subscriber Contact Information detailed above, and also should provide the email and/or hard mail address currently being utilized for the requestor’s subscription.
Note that, due to mailing delays and/or publication timeframes, subscription requests/changes may take as long as 90 days to implement.

**Subscription Costs**
Subscriptions to *Microgram Journal* are free.

**Submissions to Microgram Journal**
*Microgram Journal* presents peer reviewed, full length Scientific Research Articles and Technical Notes on the detection and analyses of controlled substances and other abused substances for forensic/law enforcement purposes.

Manuscripts are accepted both from within and outside of DEA, and reviewers for the *Journal* are both internal (from within DEA) and external.

All submissions must be in English. Because *Microgram Journal* is unclassified, **case sensitive information should not be submitted!** All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: microgram_editor@mailsnare.net  Current versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3 ½ inch IBM® PC-compatible diskette, 100 MB Iomega® zip diskette, or an IBM® PC-compatible compact disk. **Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: "Warning - Contains Electronic Media - Do Not Irradiate."**

Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 1/2" x 11" or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. The pages should be numbered, but not stapled together.

Note that mailed submissions may be subject to lengthy handling delays beyond the control of the Office of Forensic Sciences, and electronic media sent through the mail may be destroyed en route by sanitizing procedures, despite protective measures and written warnings.

All submissions should include the following **Author Contact Information:** The Full Name and Address of Submitting Laboratory or Office, and the Full Name, Phone Number, FAX Number, and Preferred email Address of the Submitting Author.

**Scientific Research Articles** are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.) **Technical Notes** are shorter communications concentrating on a specific drug (or drug class), unusual case, novel procedure or method, or minor original research. Each article/note should be a "stand-alone" work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to full peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is received by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes should follow an abbreviated version as appropriate):
Cover Letter - Provide the Author Contact Information and pertinent correspondence (if any) for the Editor.

Title - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

Author(s)/Affiliation(s) - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author's degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page. [Note that the provided email address will be listed under the primary author's address information.]

Abstract - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under "Titles". Note that the abstract will be provided to Chemical Abstracts.

Keyword List - A minimum of five (maximum ten) abstracting keywords should be included.

Introduction - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid "Personal Communications").

Experimental (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, USE CAUTION IN DETAILING SYNTHESES OF CONTROLLED OR ABUSED SUBSTANCES, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: "Experimental details on this synthesis are not provided, in accordance with Journal policy." ] Similar cautions should be followed when discussing commercial sources of abused substances.

Results and Discussion - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

Conclusions - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or a summary paragraph in the Results and Discussion section.

Acknowledgments - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

References - Articles and notes should have all textual citations collected in an endnotes list. Within the text, references should be consecutively numbered either with superscripted Arabic numerals or in-line with Arabic numerals within parentheses (author’s choice), in accordance with their first appearance. Multiple references
should be comma delineated. Within the endnotes list, references should be consecutively numbered with Arabic numerals, as follows: Number, Period, Indent, Citation. Reference format should adhere to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Note: This is the same reference format utilized in the Selected Reference Citations in Microgram Bulletin, and also by the Journal of Forensic Sciences). Journal titles may be either spelled out in full or abbreviated using standard CASSI abbreviations. Due to their inherently transitory nature, use of website URL's as references are discouraged (but permitted if absolutely necessary). As previously noted, Personal Communications should not be utilized; however, if unavoidable, utilize the following format: Full Name, Title, Affiliation (Laboratory or Office), Location (City and State, plus Nation if not the United States), Personal Communication, Year.

Table and Figures - All Tables and Figures should be appended onto the end of the article (not imbedded in the text). Tables and Figures should be consecutively numbered with Arabic numerals, in accordance with their first citation in the text. Each Table and Figure should be "stand-alone"; that is, include sufficient descriptive information such that the reader will not have to refer back to the text to understand the Table or Figure. The Header should include the Table or Figure number and a concise title. Explanatory material, definitions of acronyms and/or abbreviations, and/or references within the Table or Figure should be designated by superscripted, lower case letters in alphabetical order, and included in dedicated footnotes at the bottom of the respective Table or Figure. Unless color is needed to enhance differentiation of the depicted material, all Tables and Figures should be in black and white (that is, avoid frivolous use of color for "artistic" purposes). Figures of spectra, chromatograms, charts, graphs, etc., should have clear and legibly labeled axes, but should not include instrument generated printoffs of experimental parameter lists.

Manuscripts submitted to Microgram Journal are required to be finished, professional quality efforts. Authors should ensure clarity, brevity, and pertinence of all information. Attention to detail in formatting, grammar, and spelling is as important as the accuracy of the presented facts. Authors are specially cautioned to conduct careful literature reviews prior to submission. At the Editor's discretion, "rough drafts" or otherwise clearly substandard and/or inappropriate manuscripts will be returned to the author(s) without review.

Manuscripts will not be retyped, but "final" versions are subject to minor to moderate Editorial rewrite to improve presentation clarity or to reformat to current Microgram Journal style.

Dual Publication - Re-publication of articles or notes of particular interest to the Microgram Journal readership will be considered if the article was originally published in a journal that is not easily accessed, and the primary author has obtained explicit, written copyright exclusion from the original publisher and consent from all co-authors. Examples include exact English translations of articles or notes originally published in a non-English language journal, unclassified and non-sensitive articles or notes originally published in a restricted journal or on a password protected website, or unclassified and non-sensitive articles or notes originally published in limited distribution newsletters or Proceedings. In general, any article or note that was published in English in a mainstream journal is not a candidate for re-publication in Microgram Journal. Authors interested in re-publishing previously published articles or notes in Microgram Journal should discuss the issue with the Microgram Editor before submitting.

Note that re-published articles should not be included as "new" articles in the respective author(s)' Curriculum Vitae.

Publication Costs - There are no costs (to the contributor) associated with publication in Microgram Journal.

Reprints - Microgram Journal does not provide reprints to authors. However, articles in Microgram Journal are not copyrighted and may be photocopied as needed.
DISCLAIMERS

1) All material published in Microgram Journal is reviewed prior to publication. However, the reliability and accuracy of all published information are the responsibility of the respective contributors, and publication in Microgram Journal implies no endorsement by the United States Department of Justice or the Drug Enforcement Administration.

2) Due to the ease of scanning, copying, electronic manipulation, and/or reprinting, only the posted copies of Microgram Journal (on www.dea.gov) are absolutely valid. All other copies, whether electronic or hard, are necessarily suspect unless verified against the posted versions.

3) WARNING!: Due to the often lengthy time delays between the actual dates of seizures and their subsequent reporting in Microgram Journal, and also because of the often wide variety of seizure types with superficially similar physical attributes, published material cannot be utilized to visually identify controlled substances currently circulating in clandestine markets. The United States Department of Justice and the Drug Enforcement Administration assume no liability for the use or misuse of the information published in Microgram Journal.

* * * * *
Osmolality - A Novel and Sensitive Tool for Detection of Tampering of Beverages Adulterated with Ethanol, γ-Butyrolactone, and 1,4-Butanediol, and for Detection of Dilution-Tampered Demerol Syringes

James F. Wesley
Monroe County Public Safety Lab
524 Public Safety Building
Rochester, NY 14614
[Email: jwesley@hushmail.com]

Abstract: Freezing point osmometry, an analytical tool used by clinical hospital laboratories and the consumer product and food industries, is investigated for its utility as a forensic screening method for detection of adulteration of commercial beverages with ethanol, γ-butyrolactone, or 1,4-butanediol, and for detection of dilution of Demerol® syringes. A comprehensive list of baseline osmolality values for various commercially available beverages, eye drops, and mouthwashes is provided. Additional potential forensic applications are discussed.

Keywords: Osmolality, Forensic Chemistry, Product Tampering, γ-Butyrolactone, GBL, 1,4-Butanediol, BD, Demerol

Introduction

Forensic drug testing laboratories have validated procedures in place for dealing with solid dosage samples and are well versed in the analysis of these types of cases. However, liquid samples containing relatively small percentages of low molecular weight substances can present analytical challenges - particularly if the supporting liquid matrix is itself a complex mixture (e.g., soda or beer). In the past, the only liquid samples submitted to this laboratory were small dropper bottles usually found to contain dilute solutions of LSD - a relatively trivial forensic challenge. More recently, however, the explosion of the "Rave/Club Drug" culture has resulted in the introduction of several different drugs and/or industrial chemicals which are also delivered in liquid form, including γ-hydroxybutyric acid (GHB) or butyrate (GHB), γ-butyrolactone (GBL), and 1,4-butanediol (BD). These may be submitted either as dilute solutions in commercial beverages or as concentrated or pure solutions in "dosing" bottles. In addition, laboratories may receive soda-type beverages, fruit drinks, or even mouthwashes seized from students and suspected of having ethanol added to them. Finally, recent terrorist events have increased public anxiety and suspicion, resulting in increased submissions of beverages suspected of having been adulterated with unknown poisons.

Many laboratories have already developed specific and robust methods for detection and identification of a few of the more commonly encountered compounds, e.g., GHB. However, there are no general methods in widespread use in forensic laboratories that are capable of rapidly and reliably detecting the presence of any soluble, low molecular weight compound (including novel compounds) in aqueous solutions. For example, the GHB substitutes 4-hydroxyvalerate (4-methyl-GHB), γ-hydroxybutyraldehyde, tetrahydrofuran (THF), and γ-aminobutyric acid (GABA) are already in use in illicit circles, but are not being tested for by most forensic laboratories. Future drug seizure cases and so-called Drug Facilitated Sexual Assault (DFSA) cases will undoubtedly involve these and still other compounds, and it is therefore important that forensic and toxicology laboratories be able to quickly detect their presence. A rapid screening method which could quickly identify "like" solutions would make it easier to separate exhibits into groups for statistical sampling and (where implicated) more advanced analytical testing. Osmolality offers the basis for such a technique.
**Principles of Freezing Point Osmometry**

When a solute is dissolved in a pure solvent (e.g., water), the physical/chemical properties of the solvent are changed. The freezing point is depressed, the boiling point is elevated, the vapor pressure is lowered, and the osmotic pressure is increased [these are the so-called colligative properties.] In actual practice, therefore, one mole [gram-molecular weight] of a non-dissociating solute dissolved in 1 kg of water decreases the freezing point by 1.86°C while exerting an osmotic pressure of about 17,000 mm Hg. There is no practical method for measuring osmotic pressure, however, freezing point depression is easily measured and has thus been a clinical and analytical tool for over 50 years. A solution with a measured freezing point depression of 1.86°C would be said to have an osmolality of 1 Osmol/kg or 1000 milliosmols/kg, expressed as 1000 mOsm/kg.

An osmometer is a device for extremely accurate and precise determinations of the concentration of homogeneous solutions by means of freezing-point measurement. This is typically done by supercooling the target solution to several degrees below its presumed freezing point and then mechanically inducing the sample to freeze. The heat of fusion liberated during the freezing process causes the sample temperature to rise to a temporary plateau where a liquid/solid equilibrium is briefly maintained. This equilibrium temperature is, by definition, the freezing point of the solution. Osmometers include a highly accurate and precise electronic thermometer to continuously determine sample temperature and measure the freezing point of the sample.

The most common current use of osmometry is in hospital toxicology laboratories, for testing serum and urine to determine electrolyte balance, diabetic acidosis, lactic acidosis, shock, stroke, and intoxication from ethanol, methanol, isopropanol, and ethylene glycol. Osmometry is also useful for monitoring rehydration therapy for treatment of severe diarrhea or to assist in recovery after collapse from over-strenuous, dehydrating exercise (such as marathons).

An Advanced 3D3 Osmometer was utilized in the present study (see additional information under Experimental). In a typical analysis, 0.25 mL of a homogeneous liquid sample is pipetted into a disposable sample cup, which is then placed into the freezing chamber maintained at -7°C. At the start of the experiment, a probe containing a thermistor and stir wire descends into the sample. Over the next minute, the sample is supercooled below its freezing point. The stir wire then vibrates, causing rapid freezing. The equilibrium temperature (i.e., the freezing point) is measured, and a microprocessor converts the freezing point to osmolality and displays the result in mOsm/kg.

Since the increase in osmolality is proportional to the molality of the solution, small molecular weight substances (i.e., with molecular weights less than 100), even when present in relatively low concentrations (1 - 5 percent) will detectably alter the osmolality. This makes osmometry an ideal general screening technique for substances such as GHB, GHB-, GBL, and BD. However, “classical” drugs of abuse (cocaine, heroin, LSD, etc.) have molecular weights that are too large to noticeably effect the osmolality of typical solutions.

**Experimental**

An Advanced 3D3 Osmometer was utilized for all osmolality experiments. Osmolality calibration standard solutions of 100 mOsm/kg and 1500 mOsm/kg were utilized this study. An American Optical T/S [Total Solids] Meter was used to measure the specific gravity of the solutions in the Demerol theft case. This (hand-held) instrument measures the refractive index of a liquid and provides a visual scale for conversion to specific gravity. It has a working measurement range of 1.000 to 1.035, which is adequate to measure dilute aqueous solutions. Commercial beverages, alcoholic beverages, mouthwashes, eye drops, and breath drops were purchased locally and used without any modification. Controlled substances and other abused substances were from laboratory stocks or seized exhibits.
**Advanced 3D3 Osmometer Evaluation**

Because most forensic chemists are unfamiliar with osmometry, the following details on the Advanced 3D3 Osmometer utilized in this study are provided as background. This instrument occupies approximately one square foot of counter space and weighs 25 lbs. It is solid state, consumes 150 watts an hour during operation, and has a small volume cooling bath design that allows for calibration and analysis within 15 minutes after powering up. The calibration is stored in RAM if power is disconnected.

The usable measurement range is 0 - 4000 mOsm/kg (more concentrated solutions can be measured after dilution). A full range of calibration standard solutions of known osmolality are supplied and validated by the manufacturer.

The instrument uses disposable 0.25 mL cuvettes (reusable cuvettes are also available). There is no auto-carousel on this model, but higher level models and other manufacturers provide this feature (some can handle up to 30 samples per hour). A typical experiment takes 2-3 minutes start to finish, and uses 0.25 mL sample. The sample is not destroyed by the osmolality analysis, and can be thawed and reanalyzed.

**Results and Discussion**

**Linearity**

A linearity study was completed using the calibration standards; results are reported in Table 1 and Figure 1.

<table>
<thead>
<tr>
<th>Expected</th>
<th>Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>1991</td>
</tr>
<tr>
<td>1000</td>
<td>988</td>
</tr>
<tr>
<td>500</td>
<td>494</td>
</tr>
<tr>
<td>250</td>
<td>261</td>
</tr>
<tr>
<td>125</td>
<td>128</td>
</tr>
<tr>
<td>62.5</td>
<td>67</td>
</tr>
<tr>
<td>31.25</td>
<td>36</td>
</tr>
<tr>
<td>15.62</td>
<td>20</td>
</tr>
<tr>
<td>7.81</td>
<td>11</td>
</tr>
<tr>
<td>3.90</td>
<td>7</td>
</tr>
<tr>
<td>1.95</td>
<td>5</td>
</tr>
<tr>
<td>0.98</td>
<td>4</td>
</tr>
</tbody>
</table>

R = 0.9999  
Slope: 0.9916  
Intercept: 3.89

**In-Run Precision**

Ten same lot samples of Mountain Dew and Diet Mountain Dew were run, alternating between the two types to check precision as well as carry-over. Results are given in mOsm/kg (see Table 2, next page). The low Coefficient of Variation (C.V.) values at both ends of the measurement range demonstrate excellent reproducibility.
Table 2 - Within-Run Precision

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Diet Mt. Dew</th>
<th>Mt. Dew</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>804</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>801</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>808</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>807</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>806</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>806</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>805</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>805</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>808</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>809</td>
</tr>
<tr>
<td>CV</td>
<td>1.7%</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

Beverage Baseline Database ²,³,⁴

A comprehensive osmolality beverage database was needed as the first step in investigating beverage tampering with low molecular weight psychoactive substances. 146 beverages were tested. Whenever possible, 16 - 20 oz plastic, screw cap beverages were selected, as these are the most likely to be adulterated for illicit purposes. 8 oz "energy drinks" in non-resealing metal cans were also tested. [Note: The full database of results is available as an Excel Spreadsheet for download (contact the author if interested).]

Sports beverage results were interesting. Although producers of sports beverages claim their products are "isotonic" (approximately equal to serum values of 275 - 295 mOsm/kg), none of the tested beverages were actually in this “physiological range”. One sports beverage had a value of 190 mOsm/kg. The remaining eleven ranged from 361 - 428 mOsm/kg. Summarized results are reported in Table 3.

Table 3 - Beverage Osmolality Database [mOsm/kg]

<table>
<thead>
<tr>
<th>Beverage Type</th>
<th>Range</th>
<th>Average</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water; Purified, Mineral, Tap</td>
<td>0-28</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Diet; Sodas, Teas</td>
<td>13-44</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>Fruit Waters</td>
<td>24-39</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td>Sports Beverages</td>
<td>190-428</td>
<td>390</td>
<td>12</td>
</tr>
<tr>
<td>Sugar Containing Sodas, Fruit Drinks</td>
<td>537-1112</td>
<td>760</td>
<td>95</td>
</tr>
<tr>
<td>Energy Drinks; Red Bull, etc</td>
<td>673-1030</td>
<td>878</td>
<td>5</td>
</tr>
</tbody>
</table>

Most commercial beverages are produced at multiple locations across the country - and in some cases, across the world. To determine the validity of using baseline data across the U.S., several different lots of each beverage from different bottling locations were checked. Data for Pepsi and Diet Pepsi are reported in Table 4 (next page). The results show some variability, but good overall consistency. However, when possible, using a control beverage in order of preference: Same lot number / same bottling location / same country is (slightly) preferred when analyzing a specific beverage tampering cases. [Note: International variability was not checked in this study, and may be significant due to different formulations in use outside the U.S.]
Table 4 - Beverage Osmolality Database [mOsm/kg]

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Osmol</th>
<th>Date</th>
<th>City, State</th>
<th>Beverage</th>
<th>Osmol</th>
<th>Date</th>
<th>City, State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsi $^2$</td>
<td>711</td>
<td>10/01</td>
<td>Augusta, ME</td>
<td>Diet Pepsi $^2$</td>
<td>13</td>
<td>10/01</td>
<td>Buffalo, NY</td>
</tr>
<tr>
<td>Pepsi $^2$</td>
<td>713</td>
<td>11/01</td>
<td>Rochester, NY</td>
<td>Diet Pepsi $^2$</td>
<td>14</td>
<td>11/01</td>
<td>Rochester, NY</td>
</tr>
<tr>
<td>Pepsi $^4$</td>
<td>726</td>
<td>6/01</td>
<td>Rochester, NY</td>
<td>Diet Pepsi $^2$</td>
<td>15</td>
<td>11/01</td>
<td>Rochester, NY</td>
</tr>
<tr>
<td>Pepsi $^2$</td>
<td>726</td>
<td>11/01</td>
<td>Buffalo, NY</td>
<td>Diet Pepsi $^2$</td>
<td>20</td>
<td>10/01</td>
<td>Augusta, ME</td>
</tr>
<tr>
<td>Pepsi $^2$</td>
<td>737</td>
<td>10/01</td>
<td>Portland, ME</td>
<td>Diet Pepsi $^4$</td>
<td>27</td>
<td>6/01</td>
<td>Rochester, NY</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet Pepsi $^2$</td>
<td>32</td>
<td>10/01</td>
<td>Portland, ME</td>
</tr>
</tbody>
</table>

*Consumer Products Database* $^2$

A sampling of mouthwashes, breath drops, and eye drops were tested to determine if osmolality might be useful for forensic cases. LSD is often dosed from small dropper bottles that originally contained eye drops or breath drops. Results are reported in Table 5. Because LSD (a very high molecular weight substance) would have minimal osmolality, the finding of a very low osmolality value for a submitted exhibit of these products would indicate probable possible substitution of a water-based fluid containing LSD for the original product. Note that to prevent swelling or shrinking of the eye, eye drops are formulated to match the osmolality of natural tears; this explains their relatively low average osmolality value versus mouthwashes and breath drops. However, even this low value is much higher than a dilute aqueous solution of LSD.

Table 5 - Consumer Products Osmolality Database [mOsm/kg]

<table>
<thead>
<tr>
<th>Type</th>
<th>Range</th>
<th>Average</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouthwash</td>
<td>2660-4900</td>
<td>3683</td>
<td>6</td>
</tr>
<tr>
<td>Breath Drops</td>
<td>13950-14130</td>
<td>14040</td>
<td>2</td>
</tr>
<tr>
<td>Eye Drops</td>
<td>270-293</td>
<td>285</td>
<td>3</td>
</tr>
</tbody>
</table>

*Estimated Osmolality Increases from Substances of Forensic Interest*

The osmolality of an adulterated beverage will be increased above its baseline in proportion to the concentration of the agent used and that agent’s molecular weight. Estimated osmolality values are reported in Table 6 (next page). Note that (where applicable) the presented results apply only to the free acid form of the material. Because of the dissociation of salt forms in solution, their actual osmolality values would be expected to be higher, in proportion to the molecular weight and concentration of each of the components. For example, a 10 percent solution of sodium γ-hydroxybutyrate, MW=126.1, completely dissociated in an aqueous solution, produces 18.2 grams of sodium cation and 81.8 grams of γ-hydroxybutyrate anion per liter. The resulting expected osmolality would therefore be 1577 mOsm/kg.

*Beverage Tampering with GBL, BD* $^2$

To determine the effect of GBL and BD on the osmolality of beverages, 20 percent V/V solutions of GBL and BD in distilled water were prepared. Using Mountain Dew and Diet Mountain Dew as test beverages, each was spiked with concentrations of GBL and BD to give final solutions ranging from 0.5 - 10 percent. The osmolalities were measured and compared to the average beverage baseline measurements. The results are reported in Tables 7 and 8 (both on next page).
Illicit use of these chemicals for recreation or for facilitation of sexual assault typically involves ingestion of 1 - 3 grams. “Dosing bottles” are usually diluted to about 30 percent of the psychoactive material; thus, a 6 mL "capful" from a "dosing bottle" contains one dosage unit. At this concentration, the “dosing bottle” solution would need to be diluted 1:5 with distilled water for testing purposes, as a 30 percent solution would exceed the osmometer’s upper measurement limit. At the lower concentrations, however, the results verify that adulterating a beverage with GBL or BD even at a level of only 0.5 percent will cause a measurable increase in the osmolality. This verifies that addition of one “dose” (1 - 3 grams) from a “dosing bottle” to a 16 - 20 oz. beverage will be detectable. This is important, because dilution into beverages is a typical route of administration for purposes of sexual assault, as the beverage flavor tends to disguise the “plastic” taste of the chemical (which has been described as akin to the taste of water from a garden hose left out on a hot day).

---

**Table 6 - Estimated Osmolality Values [mOsm/kg]**

<table>
<thead>
<tr>
<th>Substance</th>
<th>MW</th>
<th>1% Solution</th>
<th>10% Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>32.04</td>
<td>312</td>
<td>3121</td>
</tr>
<tr>
<td>Ethanol</td>
<td>46.07</td>
<td>217</td>
<td>2170</td>
</tr>
<tr>
<td>Acetone</td>
<td>58.08</td>
<td>172</td>
<td>1722</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>60.09</td>
<td>164</td>
<td>1664</td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>62.07</td>
<td>161</td>
<td>1611</td>
</tr>
<tr>
<td>GBL [γ-butyrolactone]</td>
<td>86.09</td>
<td>116</td>
<td>1161</td>
</tr>
<tr>
<td>GHB-Aldehyde [γ-hydroxybutyaldehyde]</td>
<td>88.11</td>
<td>113</td>
<td>1135</td>
</tr>
<tr>
<td>1,4-BD [1,4-Butanediol]</td>
<td>90.12</td>
<td>110</td>
<td>1110</td>
</tr>
<tr>
<td>GABA [γ-Aminobutyric Acid]</td>
<td>103.12</td>
<td>97</td>
<td>970</td>
</tr>
<tr>
<td>GHB [γ-hydroxybutyrate]</td>
<td>104.11</td>
<td>96</td>
<td>961</td>
</tr>
<tr>
<td>Methyl-GHB [4-hydroxyvalerate]</td>
<td>118.13</td>
<td>85</td>
<td>846</td>
</tr>
</tbody>
</table>

**Table 7 - Mountain Dew**

<table>
<thead>
<tr>
<th>GBL Spike</th>
<th>mOsm/kg</th>
<th>1,4-BD Spike</th>
<th>mOsm/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1856</td>
<td>10% Over-range</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>1378</td>
<td>5% 1376</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>1042</td>
<td>2% 1036</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>930</td>
<td>1% 925</td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>868</td>
<td>0.5% 867</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>805</td>
<td>Baseline 805</td>
<td></td>
</tr>
</tbody>
</table>

**Table 8 - Diet Mountain Dew**

<table>
<thead>
<tr>
<th>GBL Spike</th>
<th>mOsm/kg</th>
<th>1,4-BD Spike</th>
<th>mOsm/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>1332</td>
<td>10% 1358</td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>699</td>
<td>5% 671</td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>299</td>
<td>2% 279</td>
<td></td>
</tr>
<tr>
<td>1%</td>
<td>168</td>
<td>1% 152</td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td>100</td>
<td>0.5% 91</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>33</td>
<td>Baseline 33</td>
<td></td>
</tr>
</tbody>
</table>
Ethanol in Soda-Type Beverages

Numerous reports have indicated that some high school students occasionally "spike" their lunch beverages with alcoholic beverages. We therefore investigated the effect of ethanol on beverage osmolality. One oz [30 mL] of 80 proof vodka was added to 20 oz [590 mL] bottles of Mountain Dew and Diet Mountain Dew. Vodka was selected because it has almost no odor, and it is therefore the alcoholic beverage of choice for surreptitious adulteration by underage drinkers. One oz was selected as the minimum amount of alcohol that would probably be used, as being equivalent to one mixed bar drink. Actual adulteration amounts would likely be higher. The results are as follows:

Mountain Dew: Baseline - 807 mOsm/kg, With Vodka Spike - 1174 mOsm/kg
Diet Mountain Dew: Baseline - 26 mOsm/kg, With Vodka Spike - 332 mOsm/kg

The Case of the Missing Demerol

Theft of Demerol and other controlled substances by health care professionals is a recurring problem across the U.S. In June 1989, the author (working at the toxicology lab of St. Mary's Hospital in Rochester, New York) received a call from the Drug Enforcement Administration (DEA) regarding a Demerol theft investigation. A number of patients at a local hospital were complaining that they still had pain even after receiving their Demerol injections. Toxicology studies suggested that they had not in fact received any Demerol, implying diversion/theft by a nurse or other health-care professional. Hundreds of nurses were working at any one time, and they often worked on different nursing stations. To identify a suspect, the case agent systematically switched all nurses’ floor schedules over several days. This process demonstrated that the patient complaints only occurred when a certain nurse was on duty. The case involved 75 mg Demerol syringes. The agent reasoned that the Demerol was being removed and used by the nurse, and a unknown liquid placed back in the syringe for patient injection. Because no patient became ill, it was felt that the nurse was using one of four sterile solutions as the replacement. The agent wanted to know exactly which of the four solutions was being used so that he could confront the suspect from a basis of fact and thereby elicit a confession. The available solutions included two normal salines and two sterile waters. Osmolality and specific gravity testing were performed on a control (untampered) Demerol syringe solution, on a suspect (tampered) Demerol syringe solution, and on all four sterile solutions. An independent quantitative analysis on the suspect Demerol solution confirmed that it only had 3.9 mg of Demerol remaining - consistent with a single plunger removal of Demerol and refill with one of the sterile solutions. The osmolality and specific gravity results are reported in Table 8.

Table 8 - Osmolality and Specific Gravity Measurements in the Missing Demerol Case

<table>
<thead>
<tr>
<th>Sample</th>
<th>Osmolality [mOsm/kg]</th>
<th>Spec. Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 mg Demerol Control Syringe</td>
<td>429</td>
<td>1.037</td>
</tr>
<tr>
<td>75 mg Demerol Suspect Syringe</td>
<td>381</td>
<td>1.011</td>
</tr>
<tr>
<td>Abbott Bacteriostatic Saline</td>
<td>374</td>
<td>1.010</td>
</tr>
<tr>
<td>Lyphomed Saline</td>
<td>291</td>
<td>1.004</td>
</tr>
<tr>
<td>Quad Bacteriostatic Water</td>
<td>93</td>
<td>1.005</td>
</tr>
<tr>
<td>Abbott Sterile Water</td>
<td>1</td>
<td>1.000</td>
</tr>
</tbody>
</table>

As the results show, the specific gravity testing had limited usefulness because it could not unambiguously differentiate between all solutions. However, the osmolality testing demonstrated that Abbott Bacteriostatic Saline was most likely used to refill the syringe. The observed 381 mOsm/kg result in the suspect syringe (slightly higher than the Abbott solution), was probably due to the slight effect of the 3.9 mg of Demerol still remaining in the solution. Upon confrontation with the evidence, the nurse admitted her guilt. With the
exception of osmolality, no other laboratory method available at that time could have been employed to
differentiate between different brands of saline and water. Osmolality would clearly be a useful technique for
similar, current cases of controlled substance thefts from hospitals, pharmacies, doctors’ offices, and similar
stocks.

**Additional Potential Forensic Applications**

**Identification of Sugar-Based Beverages Substituted for Diet Beverages**

The accidental or purposeful substitution of a sugar-based beverage for a diet (sugarless) beverage can be harmful
to a diabetic individual. Several different lots of Pepsi and Diet Pepsi were tested to determine if it would be
possible to differentiate the sugar based beverage from the diet beverage. The results are as follows:

- Pepsi: 711-737 mOsm/kg (n=5)
- Diet Pepsi: 13-32 mOsm/kg (n=6)

Although only 11 different lots were tested, there is clearly enough difference between the two types of beverages
to allow a reasonable determination of diet versus sugar-based.

**Poisoning of Domestic Pets’ Water with Ethylene Glycol**

Dogs and cats are very sensitive to the poisonous effects of antifreeze (which contains ethylene glycol). Fatal
amounts are 1.4 mL/kg for cats and 6.6 mL/kg for dogs. The sweet odor and taste of ethylene glycol makes it
very attractive to animals, and it is therefore a particularly insidious poison. Osmolality is a very useful initial
screen for suspect solutions in that it will detect the presence of ethylene glycol (and also other alcohols) at very
low levels in water. Based on ethylene glycol's molecular weight of 62.02, a 1 percent solution in water would
read 161 mOsm/kg, versus a typical tap water value of approximately 3 mOsm/kg.

**Identification of Water**

Water is submitted on occasion to crime laboratories. Although osmolality cannot detect the presence of large
molecular weight compounds in water at low concentrations [i.e., most “classic” street drugs], it is an excellent
tool to identify that a submitted solution is water. Most waters tested ranged from 0 - 8 mOsm/kg. Only high-
mineral content spring waters had higher values, up to 28 mOsm/kg. Non-water solvents will not freeze and no
result will be obtained. Any polar solvent mixed into water will greatly increase its osmolality. Acids and bases
that have been added to the water will increase the osmolality and also give a pH change. For example, a solution
of 1 mL of Chlorox [5 percent hypochlorite] in 100 mL of distilled water, has a pH of 10.5 and an osmolality of
43 mOsm/kg. A solution of 1 mL of 12N HCl in 100 mL of distilled water has a pH of 1.0 and an osmolality of
243 mOsm/kg. A 1 percent solution of ethanol in distilled water has a osmolality of 158 mOsm/kg.

**Field Testing**

With results available within 15 minutes after plug-in, on only 0.25 mL of sample, the Advanced 3D3 Osmometer
instrument used in this study (or any equivalent osmometer) can be easily adapted for field testing at large concert
events from police D.U.I. vans. This would allow rapid beverage screening before submission of case samples to
the crime lab.
Limitations

“Date-Rape” Benzodiazepines in Solution

As previously mentioned, the high molecular weight of common “classic” street drugs, and their low concentration in submitted solutions, makes osmolality an ineffective screening tool for their identification. For example, a single methylphenidate (Ritalin) tablet containing 5 mg of active drug and weighing 91 mg, produced a measured osmolality of only 11 mOsm/kg when dissolved in 30 mL distilled water. Therefore, osmolality is not viable for detection of drink tampering with, e.g., flunitrazepam (Rohypnol) or other sedative benzodiazepines that are employed for drug facilitated sexual assault.

Urine in Beverages

Beverages are occasionally maliciously adulterated with urine. The osmolality of an individual’s urine varies widely [50 - 1400 mOsm/kg] and greatly depends on the person’s degree of hydration. Urea, the compound of highest concentration in the urine, varies from 0.7 - 3.3 g/100 mL, and is a better indicator of tampering than osmolality. Although a typical random urine volume of 4 - 8 oz [118 - 237 mL] may be produced, let us assume 1 oz [30 mL] was introduced into a 50 oz pot of coffee [1480 mL]. The resulting urea levels would be 14 - 67 mg/100 mL. This is easily measured with a typical urea analysis method, which usually have a dynamic range of 2 - 212 mg/100 mL.

Saliva in Beverages

Similarly, beverages are occasionally maliciously adulterated with saliva. Amylase, which is present in very high levels in saliva [20,000 units/100 mL], is a better indicator of beverage adulteration with saliva versus osmolality. A typical 0.5 mL “spit” volume in an 8 oz [237 mL] cup of coffee would result in a measured amylase of 422 units/100 mL. This is easily measured with an amylase method having a dynamic range of 1-200 units/100 mL.

Conclusions

With ever increasing case loads and limited personnel resources, crime laboratories need efficient new tools to process the disturbing increases in liquid sample submissions. Osmolality, an effective analytical tool of the hospital laboratory and food and consumer products industries, is a low cost, rapid, facile, and non-destructive screening tool for forensic chemists and toxicologists.

Acknowledgements

Special thanks to Don Wiggin from Advanced Instruments for the loan of the 3D3 osmometer, and to the Rochester Institute of Technology and Drug ID Systems for providing the samples for testing.

References


* * * * *


Brought to you by AltGov2 [www.altgov2.org]
**Psychotria Viridis** - A Botanical Source of Dimethyltryptamine (DMT)

Robert D. Blackledge, M.S.*
Naval Criminal Investigative Service Regional Forensic Laboratory
3405 Welles Street, Suite 3
San Diego, CA 92136-5018
[e-mail: rblackle@ncis.navy.mil]

Charlotte M. Taylor, Ph.D.
Missouri Botanical Garden
P.O. Box 299
St. Louis, MO 63166-0299
[e-mail: charlotte.taylor@mobot.org]

**ABSTRACT:** Dimethyltryptamine was identified by GC/MS in a sample of dried leafy material that was subsequently identified as *Psychotria viridis* (Rubiaceae), a tropical shrub native to Central and South America that has ethnobotanical use as a hallucinogen by many indigenous peoples of tropical South America. The botanical characteristics of *Psychotria viridis* are illustrated and described.

**KEYWORDS:** *Psychotria viridis*, Dimethyltryptamine, DMT, *Banisteriopsis caapi*, Ayahuasca

**Introduction**

The Naval Criminal Investigative Service Regional Forensic Laboratory (NCISRFL) in San Diego, California recently received several items that investigators had obtained from a U.S. Marine stationed in Yuma, Arizona. Item A (see Figure 1) consisted of a self-sealing plastic bag containing dried whole leaves mostly still attached...
to stem pieces. Analysis by macro and microscopic examination indicated that the material clearly was not marijuana, nor were there any visible signs that anything had been added to the leaves.

**Experimental**

Approximately 1 gram of dried leaf material was placed in a glass beaker and covered with about 3 mLs of methanol. The beaker was then heated on a hot plate in a fume hood. When the methanol volume had been reduced to about 0.5 mL, the beaker was removed from the hot plate and 1µL of the remaining extract was injected into a Hewlett-Packard 5890 Gas Chromatograph (Palo Alto, CA) equipped with a 5971 Mass Selective Detector and fitted with an HP-1 capillary column (crosslinked methyl silicone, 20 m x 0.25 mm i.d. x 2.65 µm film thickness). The column oven temperature was programmed from an initial temperature of 70°C (held for 2 min) to 200°C at 10°C/min, then held at 200°C for the final 2 minutes.

**Results**

The total ion chromatogram revealed just one strong peak above the background, as shown in Figure 2. The mass spectrum of this peak is shown in Figure 3. A library search gave N,N-dimethyltryptamine (DMT) as the
closest hit. The identification of DMT was confirmed when subsequent injection of a DMT standard produced a matching spectrum at the same retention time. DMT, an hallucinogen, is a Schedule I Controlled Substance. The dried leaves and stems were in good condition for botanical evaluation, and were matched to reference specimens of *Psychotria viridis* from Peru. DMT is known to be present in *Psychotria viridis* (1,2).

**Ethnobotanical Use of *Psychotria viridis***

A narcotic drink often called *ayahuasca* or *caapi* is made from an infusion of the bark of the so-called “Spirit Vine”, *Banisteriopsis caapi* [(Spruce ex Griseb.) C.V. Morton, Malpighiaceae] and related species of tropical rainforest lianas, by many indigenous peoples of the Amazon River basin and northwestern South America (2,3). *Ayahuasca* contains several hallucinogenic alkaloids, including harmine and harmaline, and is widely used in traditional medical rites and mystical and religious ceremonies as a purgative, a magic hallucinogen, and for prophecy, diagnosis, and telepathy. Other plants are frequently added to the infusion to alter and/or enhance the effects of the *Banisteriopsis* hallucinogens. A commonly used admixture is another plant containing DMT, which reportedly increases the intensity and duration of the *ayahuasca* intoxication. DMT is found in several plant species that grow in the same region as *Banisteriopsis*, including *Psychotria viridis*. Schultes and Hoffmann have detailed the botany, ethnobotany, and chemistry of *ayahuasca* and its common admixtures (3), and Casale and Koles have detailed the forensic analysis of a typical sample (4).

**Botanical Identification**

*Psychotria* is a large genus of shrubs and small trees found in tropical regions around the world (including about 1400 species, with perhaps 700 in the New World), and its taxonomy is somewhat complicated. Not surprisingly, several other New World tropical species are morphologically similar to *Psychotria viridis*, and at least some of these may also be used as admixtures in *ayahuasca* (3).

*Psychotria viridis* [Ruiz & Pav., Rubiaceae] can be recognized by a combination of features found on the vegetative portions of the plant, listed below and shown in Figure 1, although reproductive structures provide conclusive identification [see Figure 4 (next page) for illustrations of the reproductive characters]. *Psychotria viridis* grows naturally in wet lowland tropical forests in Cuba and northern Central America through western and central South America; it appears to be most common in Amazonian Peru and Bolivia. Because the genus *Psychotria* includes a large number of morphologically similar species, and there are other genera of the same plant family that are similar, the presence of all the characteristics listed below is needed to conclusively identify *Psychotria viridis*. Botanical identification of shredded or powdered material, or even leaves without stems, would be challenging.

- **Stems.** In the middle and lower parts of the stem, situated between the insertion points of the two opposite leaves there is a horizontal scar 0.3-1 mm wide that extends between the leaves (or leaf scars) and sometimes also connects over the tops of these scars, and along the top side of this scar there is a dense, usually furry line of fine trichomes (i.e., plant hairs) usually 0.5-1 mm long that are reddish brown when dried (Figure 4A). This combination of features is diagnostic for many species in the genus *Psychotria*, though not for any individual species [i.e., these features distinguish *Psychotria* L. Subg. *Psychotria*; other subgenera of *Psychotria* lack the well developed reddish brown trichomes inserted above the stipule scars]. On the upper stems of *Psychotria viridis* these features are obscured by a stipule (see below), which covers the trichomes; the scar actually marks the point where this structure has fallen off.

- **Stipules.** These are leafy structures that cover and protect the young developing leaves, then fall off leaving scars on the stem. The stipules are produced in pairs, and their form is distinctive for *Psychotria viridis*: They are 5-25 x 4-12 mm, elliptic in outline, sharply angled at the apex, papery to [continued on page 22]
Figure 4 - Vegetative characters of *Psychotria viridis*. A, Portion of upper stem showing, from top, a pair of well developed stipules, the bases of a pair of leaves, a stipule scar with a fringe of trichomes above it, the base of another leaf, and the scar of this last leaf's pair that has fallen off. B, Leaf, underside view with a pair of foveolae circled. C, Enlarged view of foveolae from leaf shown in B. D, Enlarged view of foveolae from the forensic sample discussed in this article. E, Enlarged view of foveolae from a different botanical specimen of *Psychotria viridis*. F, Enlarged view of a different botanical specimen of *Psychotria viridis*. C, D, E, F to 5-mm scale. A, B, C based on N. Ritter and Wood 3702 (MO), from Bolivia; E, Gentry and Jaramillo 57585 (MO), Peru; and F, Solomon and Urcullo 14103 (MO), Bolivia.
membranaceous in texture, ciliate (i.e., fringed) along the upper margins, and longitudinally flanged or winged along the middle (Figure 4A). However, stipule shape and size is quite variable among different plants, and also depends on the stipule's developmental stage and other factors such as whether the stem that produced it is reproductive or vegetative.

- **Leaves.** These (Figure 4B) are opposite in arrangement (i.e., produced in pairs along the stems), generally 5-15 x 2-6 cm, in outline generally elliptic or often widest above the middle, usually sharply angled at base and apex, papery in texture, overall smooth or infrequently with microscopic plant hairs on the lower surface, have 5-10 pairs of secondary veins, and on the lower surface usually have foveolae (see next item). The leaves are borne on petioles (i.e., leaf stalks) generally 1-10 mm long. When dry, the leaves of *Psychotria viridis* usually are gray or reddish brown. The leaves of *Psychotria viridis* are similar to a few other New World species of *Psychotria*.

- **Foveolae.** These are small pockets found on the lower leaf surface near the junction of the secondary (i.e., side) veins with the central vein. They function as shelter for tiny invertebrates such as mites that live on the plant leaf. These mites apparently often are symbiotic with the plant, taking shelter in these structures and eating fungi and herbivorous invertebrates that can damage the leaf. The foveolae (also called domatia) are distinctive for *Psychotria viridis* and a few related species: They are generally 1.5-5 mm long and 0.5-1 mm wide at the top, conical and tapered to a closed base, open and truncate or variously ornamented at the top, and situated along the sides of the central vein with the opening usually near a secondary vein (Figure 4C). These foveolae vary in shape among different plants (Figure 4C, 4D, 4E, 4F), and in number on individual leaves, and may not even be present on some leaves. Most often each leaf bears at least one pair of foveolae, which may be close to the apex; the foveolae are often more numerous on leaves from vegetative stems than on those from reproductive stems.

**Conclusions**

How does a U.S. Marine obtain plant material that grows in the Amazon basin? The suspect refused to cooperate, but an Internet sales contact was the most likely source. *Psychotria viridis* leaves in various forms (whole, broken, finely powdered, shredded) reportedly exported from Peru are offered for sale on the Internet.

**References**


Evaluation of Ninhydrin Analogues and Other Electron-Deficient Compounds as Spray Reagents for Drugs on Thin Layer Chromatograms

Myriam Azoury*, Avraham Zelkowicz, and Zafrir Goren
Division of Identification and Forensic Sciences
Analytical Chemistry Laboratory
Israel Police National Headquarters
Jerusalem 91906, Israel
[email: miriama@vms.huji.ac.il]

Joseph Almog
Casali Institute of Applied Chemistry
The Hebrew University of Jerusalem, Israel

ABSTRACT: Twenty-four electron-deficient compounds were evaluated as potential spray color-reagents for basic drugs on TLC plates. Two of them, 4-chloro-7-nitro-2,1,3-benzoxadiazole and 5,6-dimethoxyninhydrin, were superior to ninhydrin with respect to sensitivity and selectivity, and offer considerable potential.

KEYWORDS: Thin Layer Chromatography, TLC, Spray Reagents, Ninhydrin, Illicit Drugs

Introduction

Since the discovery by Dutt and Teo\textsuperscript{1} that spraying thin layer chromatographic (TLC) plates bearing drug spots with ninhydrin produces a variety of colors that can distinguish between many drugs, this reagent has been intensively used in this laboratory. The colors that are produced with ninhydrin, when correlated with the specific migration values ($R_f$) for each spot on specific TLC plates and using select solvent systems, greatly enhance the specificity of TLC for various drugs.

In forensic laboratories, the main use of ninhydrin as a spray reagent has been for detection of fingerprints, especially on porous surfaces such as paper and cardboard.\textsuperscript{2-4} However, despite its great utility, research has continued to develop even more sensitive or selective reagents. Over the last two decades a significant number of ninhydrin analogous and similar, electron deficient compounds have been synthesized and evaluated as fingerprint reagents. Some of these new reagents have displayed superior properties versus ninhydrin in their sensitivity to amino acids and latent fingerprints, particularly in the fluorescence mode.\textsuperscript{2-7}

The aim of the present study was to evaluate some of these new fingerprint detection reagents for drug detection on TLC plates. The development of new, more intense, or fluorescent colors for various drugs would increase the overall specificity and sensitivity of drug-screening TLC. Such reagents could also discriminate between drugs that produce the same color with ninhydrin.

Experimental

Drugs

The controlled substances examined in this study included the following pharmaceutical and illicit drugs: Cocaine HCl and morphine HCl (Merck, Germany), diazepam, flunitrazepam, codeine phosphate, and methadone.
HCl (Teva, Israel), lysergic acid diethylamide (LSD) (Sigma, Israel), amphetamine (Assia Chem Laboratory, Israel), heroin base, opium, and 3,4-methylenedioxymethamphetamine (MDMA) HCl (from DIFS case files), and methamphetamine, 3,4-methylenedioxymphetamine (MDA) HCl, and 3,4-methylenedioxyethylamphetamine (MDEA) HCl (synthesized at DIFS). Similar aliquots (same concentration) of each drug were deposited on TLC plates for comparison.

**Imaging Reagents**

Twenty-four potential imaging reagents were tested (see Table 1, on pages 25 - 27, for names, sources, and structural formulas). Like ninhydrin, all the compounds that were studied are molecules with electron-deficient cores. Also like ninhydrin, most of them possess the indane-dione skeleton; the remainder have quininoid or cyclobutenedione type structures. All reagents were dissolved in 95% ethanol to reach testing concentrations from 0.5 - 10%.

**TLC, Elution Solvents, and Spray Reagents**

TLC was carried out on standard silica gel plates (10 x 20 cm) containing a fluorescent indicator (254 nm) on aluminum support (Macherey-Nagel, Germany). A dioxane:xylene:ethanol:ammonia (40:30:5:5) solvent mixture was used as the mobile phase in the developing tank. After the solvent elution, the plates were dried in an oven at 120°C for 3 - 4 minutes, then cooled to room temperature. The plates were then sprayed with the reagent solution, then heated again for 10 minutes. The colors of the spots as well as background interferences were immediately recorded and photographed.

**Methods**

**1st Stage**

At the first evaluation stage, all 24 reagents were tested on TLC plates against five basic drugs: Heroin, cocaine, MDMA, diazepam, and flunitrazepam. At this stage the plates were not processed in the solvent system; rather, the drugs were spotted on the plates and the spots were treated with the reagents (5 - 10% w/v) via direct application using a pipette or cotton swab. When a color reaction was noted using these initial reagent concentrations, a lower concentration solution (0.5%) of the target reagent was attempted.

**2nd Stage**

At the second evaluation stage, only those reagents that had produced colored spots with at least one drug were investigated. At this stage, the selected color reagents were evaluated for all 14 of the above listed target drugs. In addition, in the second stage, each TLC plate bearing the drug spots was eluted using above specified the TLC solvent system, then sprayed with the reagent solution, then heated to 120°C. The results were compared versus those obtained by the ninhydrin solution routinely used in the laboratory.

**3rd Stage**

In the third stage, experimental parameters were optimized for the successful color reagents identified at the second stage. The principal optimization parameters were reagent concentration and color development temperature. Ethanolic solutions of six concentrations (0.5, 1, 2, 3, 4 and 5% v/w) were prepared for each one of the successful reagents. Each successful reagent at each given concentration was tested against each drug that it had displayed a colored spot with in Stage 2, and after elution evaluated at different development temperatures (80, 100, 120, 130, 140, 160 and 200°C). It was noted that while high reagent concentrations produced more intense colors, they also usually resulted in development of significant background colorations. High temperatures had a similar effect. Colors developed and background interferences were recorded for each set of experiments.
Table 1. Names, Sources, and Structural Formulas for Imaging Reagents  
(continued on pages 21 - 22).

<table>
<thead>
<tr>
<th>(A) [3-(dicyanomethylene)-2,3-dihydro-1H-inden-1-ylidene] malononitrile</th>
<th>(B) (1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;8&lt;/sup&gt;</td>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="A" alt="Image" /></td>
<td><img src="B" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(C) (3,4-dimethyl-2,5-dioxocyclopent-3-en-1-ylidene) malononitrile</th>
<th>(D) (5,6-dimethoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;8&lt;/sup&gt;</td>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="C" alt="Image" /></td>
<td><img src="D" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(E) (5-methoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile + (2Z)-2-(5-methoxy-1,3-dioxo-1H-inden-2(3H)-ylidene) propanenitrile (mixture of 2 compounds)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><img src="E" alt="Image" /></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(F) (3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile</th>
<th>(G) A mixture of 4- and 6-nitro-1,2-indanediones</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;8&lt;/sup&gt;</td>
<td><em>source</em>: DIFS - synthesis&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="F" alt="Image" /></td>
<td><img src="G" alt="Image" /></td>
</tr>
</tbody>
</table>
Table 1 (continued).

<table>
<thead>
<tr>
<th>(H)</th>
<th>4-chloro-7-nitro-2,1,3-benoxadiazole</th>
<th>(I)</th>
<th>alloxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NBD-chloride) source: Aldrich, Germany</td>
<td>source: Sigma, Israel</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(J)</th>
<th>2,3-dimethylandthraquinone</th>
<th>(K)</th>
<th>5,6-dimethoxyninhydrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>source: Sigma, Israel</td>
<td>source: DIFS - synthesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(L)</th>
<th>4,5,6,7-tetrachloroninhydrin</th>
<th>(M)</th>
<th>spiro[2,5-dioxacyclohexane-1,2'-indene]-1',3'-dione (ninhydrin-2-trimethyleneketal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>source: Dr. A.A. Cantu, US Secret Service</td>
<td>source: DIFS - synthesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(N)</th>
<th>pyridine analogue of ninhydrin</th>
<th>(O)</th>
<th>5-methoxyninhydrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>source: DIFS – synthesis</td>
<td>source: DIFS - synthesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(P)</th>
<th>4,5,6,7-tetrabromoninhydrin</th>
<th>(Q)</th>
<th>benzo[f]ninhydrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>source: Dr. A.A. Cantu, US Secret Service</td>
<td>source: DIFS - synthesis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(R) naphtho[fl]ninhydrin</th>
<th>(S) 5-dimethylaminoninhydrin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>source</strong>: Prof. E.R. Menzel, Texas Tech. University, Lubbock, TX, USA</td>
<td></td>
</tr>
<tr>
<td><strong>source</strong>: DIFS - synthesis(^\text{12})</td>
<td></td>
</tr>
<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td><img src="image2" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(T) ethyl (2E)-hydroxy[3-hydroxy-2,5-dioxo-4-phenylcyclopent-3-en-1-ylidene]acetate</th>
<th>(U) 2-methyl-3,4-dioxocyclobut-1-en-1-ol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>source</strong>: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA</td>
<td></td>
</tr>
<tr>
<td><strong>source</strong>: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA</td>
<td></td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure" /></td>
<td><img src="image4" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(V) 3,4-dioxo-2-phenylcyclobut-1-en-1-ol</th>
<th>(W) potassium rhodizionate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>source</strong>: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA</td>
<td></td>
</tr>
<tr>
<td><strong>source</strong>: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA</td>
<td></td>
</tr>
<tr>
<td><img src="image5" alt="Chemical Structure" /></td>
<td><img src="image6" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(X) 3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrol</th>
<th>(Y) ninhydrin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>source</strong>: Prof. R.C. West, Dept. of Chemistry, University of Wisconsin, Madison, USA</td>
<td></td>
</tr>
<tr>
<td><strong>source</strong>: Spectrum, USA.</td>
<td></td>
</tr>
<tr>
<td><img src="image7" alt="Chemical Structure" /></td>
<td><img src="image8" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>
**Results and Discussion**

Five of the twenty-four reagents examined at the first evaluation stage yielded a color reaction with at least one drug (see Table 2, next page). These were reagents E (mixture of 5-methoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) malononitrile and (2Z)-2-(5-methoxy-1,3-dioxo-1H-inden-2(3H)-ylidene) propanenitrile, F (3-oxo-2,3-dihydro-1H-inden-1-ylidene) malononitrile, H (4-chloro-7-nitro-2,1,3-benzoxadiazole), K (5,6-dimethoxy-ninhydrin), and O (5-methoxyninhydrin). Seven of the twenty-four compounds gave no visible reaction, and the remainder were rejected because of the development of intense background coloration.

At the second stage, the five preliminarily successful reagents listed above were evaluated for all 14 drugs. The results are summarized in Table 3 (see page 30), and are detailed below:

**Reagent E** (a mixture of 5-methoxy-1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene)malononitrile and (2Z)-2-(5-methoxy-1,3-dioxo-1H-inden-2(3H)-ylidene)propanenitrile), at working concentrations of 1 - 5% w/v: A yellow background is observed and the sensitivity is low; therefore, the colored spots are weak in comparison with the background.

**Reagent F** ((3-oxo-2,3-dihydro-1H-inden-1-ylidene)malononitrile), at a working concentration of 2% w/v: Intense brown-red spots are observed, mostly with amphetamines. In contrast, opiates (heroin, morphine) and cocaine produce only low intensity red colored spots. No reaction is observed with LSD. At low drug concentrations, the red colored background interferes with the colored spots.

**Reagent H** (4-chloro-7-nitro-2,1,3-benzoxadiazole), at working concentrations of 1.5 - 2% w/v: Intense brown-purple spots are formed with amphetamines, yellow spots with narcotine and papaverine in opium, blue spots with heroin, and brown spots with cocaine. An intense color reaction is also observed with LSD. In general, the colors obtained are very similar to the colors developed with ninhydrin, but the sensitivity of H is higher; therefore, a lower reagent concentration is required.

**Reagent K** (5,6-dimethoxyninhydrin), at a working concentration of 0.5% w/v: Very intense spots are formed with amphetamines, LSD and methadone. Opiates (heroin, morphine) produce weak purple spots. Strong purple spots are formed by MDMA and MDEA. Amphetamine and MDA yield milky-yellow spots.

**Reagent O** (5-methoxyninhydrin), at a working concentration of 2.5% w/v: Intense purple spots are formed with amphetamines, while opiates and LSD produce only very weak purple spots. In addition, a pink background discoloration is observed.

Of the five above reagents, H and K showed better performance versus the other three, and were therefore selected for further investigation. Optimization trials were carried out with both H and K at various concentrations and color development temperatures. The optimized parameters for H are: A 3% solution (w/v) with color development at 120°C. Under these conditions, opiates (heroin, morphine) can also be detected. The optimized conditions for K are: A 0.5-1% solution (w/v) with color development at 120°C. Under these conditions, only amphetamines show strong color reactions. It is noted for comparison that ninhydrin is typically utilized as a 10% solution.

**Conclusions**

4-Chloro-7-nitro-2,1,3-benzoxadiazole and 4,6-dimethoxyninhydrin both show good potential as spray reagents for drugs on chromatographic plates. Both reagents show some advantage over ninhydrin in their reactivity, developing more intense colors at lower reagent concentrations. Furthermore, 5,6-dimethoxyninhydrin also produces two different colors with different amphetamines: Purple spots are formed by (continued on page 30)
Table 2. Results Correlated Against Structures.

<table>
<thead>
<tr>
<th>Colored Spots Without Background Interference</th>
<th>Colored Spots with Background Discoloration</th>
<th>Negative Results, No Color Development, and No Background Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>D</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>J</td>
</tr>
<tr>
<td>K</td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>T</td>
</tr>
<tr>
<td>M</td>
<td></td>
<td>U</td>
</tr>
</tbody>
</table>

methamphetamine, MDMA, and MDEA, and milky yellow spots are formed by amphetamine and MDA. A mechanistic study of these color formation reaction may lead to a rational design of even better reagents of this family.

**Acknowledgments**: The authors are indebted to Dr. Antonio A. Cantu, Chief Chemist, US Secret Service, to Professor Robert C. West of the Chemistry Department, University of Wisconsin, Madison, and to Professor E. Roland Menzel, Director of the Center for Forensic Studies, Texas Tech University, Lubbock, for kindly providing them with some of the compounds for testing. The authors also gratefully acknowledge Ms. Lital Cohen for her technical assistance.
References

1. Dutt MC, Teo TP. Use of ninhydrin as a spray reagent for the detection of some basic drugs on thin-layer chromatograms. J Chromatography 1980;195:133.


10. Schonberg A, Singer E, Eschenhof B, Hoyer GA. Reaction of ninhydrin and of 1,2,3-indanetrione with compounds with two functional groups. A contribution to the formation of spiro compounds from ninhydrin. Chem Ber 1978;111:3058.


* * * * *
Technical Note

Instrumental Separation of 3,4-Methylenedioxyamphetamine (MDA) from 1-(3,4-Methylenedioxyphenyl)-2-propanol, a Co-Eluting Compound

Barbara A. Vohlken* and Stephen M. Layton
Florida Department of Law Enforcement
Tampa Regional Crime Laboratory
4211-A North Lois Avenue
Tampa, FL 33614
[email: barbaravohlken@fdle.state.fl.us]

ABSTRACT: Analysis of a set of mixed-component Ecstasy tablets by GC/MS indicated an apparent mixture of 3,4-methylenedioxy-methamphetamime (MDMA) and 3,4-methylenedioxyamphetamine (MDA); however, the mass spectrum for the MDA did not exactly match an MDA standard. Additional work confirmed that the presumed MDA was actually a co-eluting mixture of MDA and 1-(3,4-methylenedioxyphenyl)-2-propanol. The latter alcohol has a mass spectrum that is highly similar to MDA, but displays a molecular weight peak of 180 (versus 179 for MDA). Varying the temperature programming of the normal GC/MS run separated the alcohol.

KEYWORDS: 3,4-Methylenedioxy-methamphetamime, MDMA, 3,4-Methylenedioxyamphetamine, MDA, 1-(3,4-Methylenedioxyphenyl)-2-propanol, Ecstasy, GC/MS, Co-Elution

Introduction

Over the past few years, so-called “Ecstasy” tablets have undergone a dramatic transition in their composition. Five years ago, most Ecstasy tablets contained either 3,4-methylenedioxy-methamphetamime (MDMA), 3,4-methylenedioxyamphetamine (MDA), or (less commonly), a mixture of MDMA and MDA. More recently, however, Ecstasy tablets have often contained complex mixtures of controlled substances, control substance analogues, alternate abused substances, adulterants, diluents, and manufacturing impurities and byproducts. These mixed component tablets can offer unusual analytical challenges.

In late 2001, this laboratory received an exhibit consisting of 11 white tablets with a three-point crown imprint on one side and unmarked on the other side (photo not available), total net mass 3.3 grams. The exhibit was seized just north of Tampa, Florida, but had no other associated source information. Analysis was conducted by color testing (Marquis, cobalt thiocyanate, and Dille-Koppanyi), thin layer chromatography (TLC) (Clarke’s TB developer, visualized with acidified iodoplatinate), and gas chromatography/mass spectrometry (GC/MS). The color test results were consistent with typical (MDMA) type preparations. The Marquis test showed the usual purple, blue, and green colors, and the cobalt thiocyanate gave a slight blue reaction. After elution, spraying, and development, the TLC showed two spots consistent in both color and Rf to MDMA and MDA; however, a third spot was also noted. The first GC/MS run revealed four peaks, one with a retention time and mass spectrum corresponding to MDMA, a second with a retention time and mass spectrum very similar to MDA but with an apparent molecular ion at 180 instead of the expected 179 (for MDA), and two unknowns that did not correspond to any known controlled substances and were therefore not further analyzed. Closer examination of the “MDA” mass spectrum indicated that the fragment ion ratios at 135 relative to 136, and at 106 relative to 105, both appeared to be slightly higher than normally expected for MDA. The sample was then injected on a second GC/MS to determine if the anomalous results were an instrumental variation or a glitch of some type in the run. The second GC/MS run again revealed the same four peaks (see Figure 1, next page); the first compound (designated “A1” on Figure 1) had a retention time and mass spectrum very similar to MDA but still with the
apparent molecular ion at 180 instead of the expected 179. The second peak (designated “A2” on Figure 1) had a retention time and mass spectrum corresponding to MDMA. The two additional peaks (“A3” and “A4” on Figure 1) were also still present, but were not identified. The mass spectra of A1 through A4 are shown in Figures 2 - 5.

Figure 1.
Total Ion Chromatogram

Figure 2.

Figure 3.
Mass Spectrum of Compound A2 (MDMA)
A standard of MDA was then run on the second GC/MS, and the resulting spectra was found to be normal (i.e., displaying a typical MDA spectrum with a “proper” 179 molecular ion (See Figure 6). Since the mass spectrum of standard MDA run on the same instruments in the same time frame did not match the unknown, it was clear...
that this could not be a simple instrumental variation of the MDA spectrum. A literature search of mass spectra found no matches. The question then arose: Was the second component actually an unknown substance, or was the anomalous spectrum the result of a compound co-eluting with MDA? The presence of a third compound by TLC analysis suggested the possibility of a co-eluter.

**Experimental**

Two GC/MS instruments were utilized in the study. The first was an Agilent 5973 Mass Spectrometer interfaced with an Agilent 6890 Gas Chromatograph equipped with a 12 meter capillary column of 0.20 mm i.d. and having a 0.33 μm film thickness of methyl silicone. The temperature program was 100°C held for one minute, then ramped at 75°C per minute to 200°C, then ramped at 50°C per minute to 325°C, held for one minute. The second was a Hewlett Packard 5971A Mass Spectrometer interfaced with a Hewlett Packard 5890 Gas Chromatograph also equipped with a 12 meter capillary column of 0.20 mm i.d. and having a 0.33 μm film thickness of methyl silicone. The temperature program was 100°C with no hold, ramped at 5°C per minute to 200°C, then ramped at 25°C per minute to 325°C, held for two minutes.

**Results and Discussion**

The color testing, TLC, and GC/MS results excluded common manufacturing byproducts or “mistakes” such as N-hydroxy-3,4-methylenedioxymethamphetamine or 1-(3,4-methylenedioxyphenyl)-2-propanone-oxime. However, an unusual impurity 1-(3,4-methylenedioxyphenyl)-2-propanol had been identified in another recent case seen in this laboratory. This compound can result from reduction of excess 1-(3,4-methylenedioxyphenyl)-2-propanone in botched clandestine syntheses. 1-(3,4-Methylenedioxyphenyl)-2-propanol has a molecular weight of 180, and a base peak of 135. The mass units for the remaining peaks are nearly identical to MDA, though their abundances vary. To determine if the MDA mass spectrum anomaly was in fact the result of a co-elution with 1-(3,4-methylenedioxyphenyl)-2-propanol, the sample was injected onto the Hewlett Packard 5971A mass spectrometer with a much slower temperature programming (i.e., 5°C per minute from 100 to 200°C, then ramped at 25°C per minute to 325°C, held for two minutes). There still appeared to be a single peak for the MDA area on the ion chromatogram (see Figure 7), but the mass spectrum of the suspected MDA (taken at the peak) was now normal. However, by expanding the peak on the computer screen, a shoulder became visible (see Figure 8). The mass spectrum of this shoulder (Figure 9) was that of 1-(3,4-methylenedioxyphenyl)-2-propanol, confirmed by comparison to a spectrum copy obtained from Drug Enforcement Administration’s Southeast Laboratory (Miami, Florida). Though the peaks did not fully resolve even using the slower temperature programming, they were separated enough to obtain identifiable mass spectra for both MDA and 1-(3,4-methylenedioxyphenyl)-2-
propanol, allowing for a positive identification of the controlled substance. Since the alcohol is not controlled, further analyses (e.g., acid/base shakeouts or derivatization studies) were not required; however, such procedures could be useful for other laboratories who encounter similar mixtures or who wish to more formally isolate and identify 1-(3,4-methylenedioxyphenyl)-2-propanol.

Based on the peak heights as measured by the Agilent 5973 (total ion chromatogram), the extracted components in the mixture were approximately 22% MDMA, 35% MDA, and 3% 1-(3,4-methylenedioxyphenyl)-2-propanol, and 40% other, unidentified components (there may also have been other components which did not extract). To date no other samples of this particular mixture have been encountered at this laboratory.

* * * * *
Technical Note

Potency of Cannabis Seized in Central Florida During June 2002

Christina J. Newell
Florida Department of Law Enforcement
Orlando Regional Crime Laboratory
500 West Robinson Street
Orlando, FL 32801
[email: ch319427@pegasus.cc.ucf.edu]

ABSTRACT: The potency of cannabis seized in central Florida during the month of June, 2002, is reported. ∆9-Tetrahydrocannabinol (∆9–THC) was extracted from cannabis seizures with a mixed methanol chloroform solution, and then analyzed with gas chromatography using an external standard. The average ∆9–THC concentration was found to be 6.20%.

KEYWORDS: ∆9-Tetrahydrocannabinol, ∆9–THC, Marijuana, Cannabis, Gas Chromatography

Introduction

Cannabis remains one of the most frequently submitted substances for analysis to the Florida Department of Law Enforcement’s Orlando Regional Crime Laboratory. ∆9-Tetrahydrocannabinol (∆9–THC) is the substance responsible for most of the psychopharmacological effects that cannabis has on humans. According to the University of Mississippi’s Potency Monitoring Project, the non-normalized average potency of cannabis seizures has steadily increased since measurement began in the 1970's. The average ∆9–THC potencies were 0.90% in 1977, 2.93% in 1987, 4.53% in 1997, and 6.19% in 2002 (1). In this study, samples were collected from seizures made in central Florida and submitted for laboratory analysis during June 2002, and their respective ∆9–THC contents determined by gas chromatography (GC) using an external standard.

Experimental

Instruments and Materials

A Hewlett Packard 5890 Gas Chromatograph (GC) with a flame ionization detector was used for all analyses. The GC was equipped with an Alltech (AT-1) fused silica 10-meter capillary column with an internal diameter of 0.25 mm and having a film thickness of 0.20 µm of methyl silicone. A Mettler AE260 DeltaRange electronic analytical balance was used for weighing the samples. The external ∆9–THC standard employed was from Alltech (Lot Number 281). Methanol and chloroform (both Fisher Scientific) were used as received. A total of 36 cannabis samples obtained from 36 separate cases submitted to the laboratory in June 2002 were examined in this study. All samples were dry.

Analytical Protocol

After removing seeds and large stem pieces, the samples (roughly 200 mg) were weighed on an analytical balance (see Table 1, page 39, for exact dry weights), then covered and soaked overnight in 5 mL of methanol/chloroform 9:1 to exhaustively extract the ∆9–THC from the plant material (2). Because of the small size of the autosampler vials used on the GC, a 1.5 mL aliquot of the extract of each sample was evaporated to dryness in an autosampler.
vial, and another 1.5 mL aliquot of extract was added and the vials were sealed; this doubled the concentration of the extract. The Δ9–THC external standard was prepared to a final concentration of 1.0 mg/mL.

The GC was operated at a split ratio of 50:1. The helium flow rate was 1 mL/minute. The temperature program started at 100°C and was increased at a rate of 50°C/minute to 325°C, with a final hold for 2.25 minutes. The samples were bracketed between two standards in groups of ten. Each sample was injected in triplicate with a volume of 1 μL per injection, and the average of the three peak areas for each sample was used for quantitation. Five already extracted samples were chosen randomly and the extraction and analysis procedures were repeated on them to ensure that all of the samples had been exhaustively extracted (which they were).

Results and Discussion

The amount of Δ9–THC found in the samples ranged from 1.41% to 12.62% by dry weight (see Table 1, next page). The average Δ9–THC content was 6.20%, which is almost identical to the 2002 value reported by the University of Mississippi’s Potency Monitoring Project. Since there have been no other known studies of this type for cannabis seizures in central Florida, these values cannot be compared with local data to show a trend in cannabis potency. However, the results clearly suggest that local cannabis potencies are closely tracking national averages.

Acknowledgments

Thanks to the members of the Chemistry Section of the Florida Department of Law Enforcement Orlando Regional Crime Laboratory for their assistance with sample collection for this project. Special thanks to Dr. Frank Davis, Orlando Regional Crime Laboratory, for assistance with formulation of experimental methods and interpretation of results.

References

1. ElSohly MA, Ross SA. Potency Monitoring Project, Quarterly Report #80. National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677.

Table 1. Amount of $\Delta^9$-THC found in Central Florida Cannabis Samples

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Sample Weight (grams)</th>
<th>Percent THC by Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2095</td>
<td>6.59</td>
</tr>
<tr>
<td>2</td>
<td>0.2254</td>
<td>9.83</td>
</tr>
<tr>
<td>3</td>
<td>0.2154</td>
<td>3.79</td>
</tr>
<tr>
<td>4</td>
<td>0.2188</td>
<td>11.46</td>
</tr>
<tr>
<td>5</td>
<td>0.1609</td>
<td>6.64</td>
</tr>
<tr>
<td>6</td>
<td>0.1770</td>
<td>5.24</td>
</tr>
<tr>
<td>7</td>
<td>0.1447</td>
<td>6.02</td>
</tr>
<tr>
<td>8</td>
<td>0.1928</td>
<td>1.41</td>
</tr>
<tr>
<td>9</td>
<td>0.2079</td>
<td>2.20</td>
</tr>
<tr>
<td>10</td>
<td>0.1413</td>
<td>4.61</td>
</tr>
<tr>
<td>11</td>
<td>0.1549</td>
<td>4.46</td>
</tr>
<tr>
<td>12</td>
<td>0.2231</td>
<td>6.87</td>
</tr>
<tr>
<td>13</td>
<td>0.2185</td>
<td>8.59</td>
</tr>
<tr>
<td>14</td>
<td>0.2056</td>
<td>5.32</td>
</tr>
<tr>
<td>15</td>
<td>0.1585</td>
<td>4.74</td>
</tr>
<tr>
<td>16</td>
<td>0.2259</td>
<td>9.12</td>
</tr>
<tr>
<td>17</td>
<td>0.1230</td>
<td>3.57</td>
</tr>
<tr>
<td>18</td>
<td>0.1560</td>
<td>6.88</td>
</tr>
<tr>
<td>19</td>
<td>0.2315</td>
<td>3.94</td>
</tr>
<tr>
<td>20</td>
<td>0.1975</td>
<td>4.42</td>
</tr>
<tr>
<td>21</td>
<td>0.2168</td>
<td>7.81</td>
</tr>
<tr>
<td>22</td>
<td>0.1568</td>
<td>10.92</td>
</tr>
<tr>
<td>23</td>
<td>0.1685</td>
<td>9.82</td>
</tr>
<tr>
<td>24</td>
<td>0.1874</td>
<td>6.16</td>
</tr>
<tr>
<td>25</td>
<td>0.2202</td>
<td>6.77</td>
</tr>
<tr>
<td>26</td>
<td>0.1438</td>
<td>2.59</td>
</tr>
<tr>
<td>27</td>
<td>0.2159</td>
<td>8.69</td>
</tr>
<tr>
<td>28</td>
<td>0.2175</td>
<td>3.23</td>
</tr>
<tr>
<td>29</td>
<td>0.2219</td>
<td>12.62</td>
</tr>
<tr>
<td>30</td>
<td>0.1828</td>
<td>4.05</td>
</tr>
<tr>
<td>31</td>
<td>0.1990</td>
<td>8.56</td>
</tr>
<tr>
<td>32</td>
<td>0.1805</td>
<td>6.08</td>
</tr>
<tr>
<td>33</td>
<td>0.2217</td>
<td>5.86</td>
</tr>
<tr>
<td>34</td>
<td>0.2161</td>
<td>5.67</td>
</tr>
<tr>
<td>35</td>
<td>0.2226</td>
<td>2.23</td>
</tr>
<tr>
<td>36</td>
<td>0.1686</td>
<td>6.58</td>
</tr>
</tbody>
</table>

Mean THC Content (by Dry Weight) : 6.20%
A Study of Acids Used for the Acidified Cobalt Thiocyanate Test for Cocaine Base

Anna L. Deakin
Florida Department of Law Enforcement
Tampa Regional Crime Laboratory
4211 North Lois Avenue
Tampa, FL 33611
[Email: annadeakin@fdle.state.fl.us]

ABSTRACT: Four acids (hydrochloric, sulfuric, nitric, and acetic) were used as acidifying reagents in the “one well” cobalt thiocyanate test for cocaine base. Concentrated sulfuric, nitric, and acetic acids were found to be equally fast as concentrated hydrochloric acid (the standard acid used in the test). In addition, dilute (down to 0.1 N) hydrochloric acid was found to be as effective as concentrated hydrochloric acid. Only concentrated hydrochloric acid gave a transient blue color upon addition to the cobalt thiocyanate reagent. A number of other controlled substances, adulterants, and diluents were also tested and confirmed to not give false positives with sulfuric, nitric, acetic, or dilute hydrochloric acids.

KEYWORDS: Cocaine, Cobalt Thiocyanate, Acidified Cobalt Thiocyanate, Spot Tests, Color Tests

Introduction

The cobalt thiocyanate color test is widely used in forensic laboratories to determine the presence of cocaine salt, i.e., cocaine hydrochloride (1,2). However, the test requires a water soluble form of cocaine, and is ineffective for testing cocaine base. Therefore, a modified version of the test, the acidified cobalt thiocyanate test, is used to determine for the presence of cocaine base. The addition of an acid to the reagent allows the cocaine base to dissolve, and the color reaction can proceed. A sustained blue colored precipitate is a positive test.

There are two general procedures for running these tests. The first is to have two separate solutions prepared (one “normal” and the second acidified) and use them in two separate spot wells of a standard porcelain spot plate. The other is to run the normal (non-acidified) test first, observe for any color change, and if none then add a small amount of acid to the spot well, and again observe for any color change. This latter technique is referred to as the “one-well” method.

A literature search found that the only documented acid used for this “one well” test is concentrated hydrochloric acid (HCl). However, there is a complication when using this acid in that when it is first introduced to the cobalt thiocyanate solution, the color of the solution temporarily turns from pink to blue even if cocaine base is not present- and blue is also the characteristic color change observed for cocaine. Although this change is only temporary (as well as distinguishable to the trained eye), and there is no blue colored precipitate, it can be confusing to novices, and can potentially give ambiguous results with samples containing only trace amounts of cocaine. The latter problem can be an issue with commercial field test-kits.

In this study, a series of acids commonly utilized in most forensic/analytical laboratories were used to perform the “one well” test for cocaine base. A variety of other controlled and non-controlled substances were also studied using the same acids. In addition, the concentration of HCl used for the “one well” test was also studied to determine if the test would still be effective if a diluted version was used.
Experimental

Chemicals

Chemicals were purchased from the following vendors.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzocaine</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Matheson Coleman and Bell</td>
</tr>
<tr>
<td>Cobalt Thiocyanate</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Cocaine HCl and Base</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Diphenhydramine HCl</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>Fisher</td>
</tr>
<tr>
<td>Glucose</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>Hydrochloric Acid</td>
<td>Fisher</td>
</tr>
<tr>
<td>Inositol</td>
<td>Eastman</td>
</tr>
<tr>
<td>Lactose</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>K&amp;K Laboratories</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Mallinckrodt</td>
</tr>
<tr>
<td>Methamphetamine (case sample)</td>
<td></td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>JT Baker Chemical Co.</td>
</tr>
<tr>
<td>Nitric Acid</td>
<td>Fisher</td>
</tr>
<tr>
<td>Phencyclidine (PCP)</td>
<td>US Pharmacopeia</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Quinine HCl</td>
<td>Matheson Coleman and Bell</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>Fisher</td>
</tr>
<tr>
<td>Sulfuric Acid</td>
<td>Fisher</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>K&amp;K Laboratories</td>
</tr>
</tbody>
</table>

Prepared Reagents

Cobalt thiocyanate reagent: 2 grams of cobalt thiocyanate were dissolved in 100 mL distilled water.

Acidified cobalt thiocyanate reagent: 2 mL of concentrated HCl were added to 98 mL of above cobalt thiocyanate reagent.

Procedure

Several controlled and non-controlled substances were studied, as well as numerous case samples of cocaine base. For each sample, the following procedure was followed:

1. Add a few drops of the cobalt thiocyanate reagent to five (A-E) wells on a spot plate.
2. Add the acidified cobalt thiocyanate reagent to one well (F).
3. Add a few micrograms of solid chemical to each spot well.
4. Observe color changes (if any).
5. Add one drop of each concentrated acid to each designated well (hydrochloric to (B), sulfuric to (C), nitric to (D), and acetic to (E)).
6. Observe any new color changes in wells (B) through (E).

The effect of the concentration of HCl added to the cobalt thiocyanate solution was separately studied. Two to three drops of the cobalt thiocyanate reagent were added to several wells of a spot plate. One drop of HCl (of varying concentrations) was added to each well.

Results and Discussion

It was found that all four acids (hydrochloric, sulfuric, nitric, and acetic) produced the same test results for cocaine base (see Table 1, next page). All four concentrated acids were equally fast. In addition, no false
positives were observed with any of the other controlled substances, adulterants, and diluents tested when sulfuric, nitric, or acetic acids were substituted for concentrated HCl. Notably, only concentrated HCl gave the transient blue-colored solution when added to the "normal" (non-acidified) cobalt thiocyanate reagent that did not contain cocaine.

1. Results of Cobalt Thiocyanate + Acid

<table>
<thead>
<tr>
<th>Standard Samples</th>
<th>Cobalt Thiocyanate</th>
<th>Add HCl</th>
<th>Add H2SO4</th>
<th>Add HNO3</th>
<th>Add HAc</th>
<th>Acidified Cobalt Thiocyanate (w/ HCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine HCl</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Cocaine Free Base</td>
<td>NR</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Lactose</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Glucose</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Mannitol</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Inositol</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Tetracaine</td>
<td>Blue</td>
<td>Some disappears</td>
<td>Most disappears</td>
<td>Most disappears/yellow</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>NR</td>
<td>Slight Blue</td>
<td>Slight Blue</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Procaine</td>
<td>Blue</td>
<td>Disappears</td>
<td>Disappears</td>
<td>Disappears</td>
<td>Some disappears</td>
<td>Slight Blue</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>NR</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Caffeine</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Diphenhydramine HCl</td>
<td>Deep Blue</td>
<td>Deep Blue</td>
<td>Deep Blue/Yellow</td>
<td>Disappears</td>
<td>Disappears</td>
<td>Deep Blue</td>
</tr>
<tr>
<td>Heroin</td>
<td>Blue/Green</td>
<td>Blue/Green</td>
<td>Blue/Green</td>
<td>Blue/Green</td>
<td>Blue/Green</td>
<td>Blue/Green</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>Dirty Blue</td>
<td>Fades</td>
<td>Fades</td>
<td>Fades</td>
<td>Fades</td>
<td>Fades</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>NR</td>
<td>Fizz</td>
<td>Fizz</td>
<td>Fizz</td>
<td>Fizz</td>
<td>NR</td>
</tr>
<tr>
<td>Phencyclidine (PCP)</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Ephedrine HCl</td>
<td>Slight Blue (disappears)</td>
<td>Slight Blue</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>Slight Blue (disappears)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Quinine Sulfate</td>
<td>NR</td>
<td>Blue</td>
<td>Blue</td>
<td>Blue (Disappears)</td>
<td>Blue</td>
<td>Blue at edges (insol.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case Samples</th>
<th>Cobain Base Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Sample 1</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 2</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 3</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 4</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 5</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 6</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 7</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 8</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 9</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 10</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 11</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 12</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 13</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 14</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 15</td>
<td>NR</td>
</tr>
<tr>
<td>Test Sample 16</td>
<td>NR</td>
</tr>
<tr>
<td>Cocaine Sulfate</td>
<td>Test Sample 17</td>
</tr>
<tr>
<td>Test Sample 18</td>
<td>Blue</td>
</tr>
<tr>
<td>Test Sample 19</td>
<td>Blue</td>
</tr>
</tbody>
</table>

(NR = No Reaction)
Dilute HCl (from 1:1 down to 0.1 N) produced the same results as concentrated HCl, but also did not give the
transient blue-colored solution when added to the "normal" (non-acidified) cobalt thiocyanate reagent that did not
contain cocaine (see Table 2). When cocaine base was present, it was noted that the weaker the HCl solution, the
slower the color reaction, but it never took more than a few seconds for the blue precipitate to form, and the
overlaying solution did not turn blue even when cocaine was present. Thus, dilute HCl is as effective as
concentrated HCl for the test. The collective results suggest that substituting an alternative acid or a diluted form
of HCl for concentrated HCl for the acidified cobalt thiocyanate test would be advantageous.

Table 2. Effects of Hydrochloric Acid Dilution

<table>
<thead>
<tr>
<th>Concentration of HCl (v/v)</th>
<th>Turns solution blue?</th>
<th>Proper reaction with Coc Base?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrated</td>
<td>(12 N)</td>
<td>Yes</td>
</tr>
<tr>
<td>50%</td>
<td>(6 N)</td>
<td>No</td>
</tr>
<tr>
<td>40%</td>
<td>(4.8 N)</td>
<td>No</td>
</tr>
<tr>
<td>30%</td>
<td>(3.6 N)</td>
<td>No</td>
</tr>
<tr>
<td>20%</td>
<td>(2.4 N)</td>
<td>No</td>
</tr>
<tr>
<td>10%</td>
<td>(1.2 N)</td>
<td>No</td>
</tr>
<tr>
<td>0.80%</td>
<td>(0.1 N)</td>
<td>No</td>
</tr>
</tbody>
</table>

Acknowledgements

Thanks to Sandy Kassner and the members of the Chemistry Section at the Florida Department of Law
Enforcement, Tampa Regional Crime Laboratory, for their help and contributions to this project.

References (Not Cited in Text)

Drug Enforcement Administration, Basic Training Manual for Forensic Chemists, p. 4-8.

Velapoldi RA, Wicks MS. The use of chemical spot test kits for the presumptive identification of narcotics and

* * * * *
1,4-Butanediol (BD) - Forensic Profile

Agnes D. Garcia, B.S.*
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email: cation1072@aol.com]

Allen J. Catterton, B.S.
Drug Enforcement Administration
Southeast Laboratory
5205 NW 84th Avenue
Miami, FL 33166

ABSTRACT: 1,4-butanediol (BD), an analog and “pro-drug” of gamma-hydroxybutyric acid (GHB), is increasingly being added to so-called dietary, health, sleep aid, or sports (bodybuilding) supplements, and is also being sold on the Internet and on underground markets for purposes of illicit abuse. When so intended for human consumption, BD meets the definition of a controlled substance analog under the Controlled Substances Act, Title 21, and can be prosecuted as a Schedule I substance. A comprehensive analytical profile for BD is presented, including GC/MS, FTIR, NMR, GC/IRD, and GC/FID. Analytical parameters for the quantitative analysis of BD are also presented, along with linearity and reproducibility data.

KEYWORDS: 1,4-Butanediol, gamma-Hydroxybutyric Acid, gamma-Butyrolactone, BD, 1,4-BD, GHB, GBL, Analogs, Pro-Drug

Introduction

The widespread, illicit abuse of gamma-hydroxybutyric acid (GHB) is due to its euphoric, sedative, hallucinogenic, and alleged steroidal effects (1). Recently, GHB abusers have been switching to related compounds in an attempt to circumvent the Federal controls on GHB (2,3,4,5,6). 1,4-Butanediol (BD) and gamma-butyrolactone (GBL) (Figure 1) are the two most commonly encountered such compounds, and are considered to be both analogs and “pro-drugs” of GHB, since their chemical structures are substantially similar to GHB and they are metabolized into GHB upon ingestion and therefore produce the same psychopharmacological effects as GHB (2,3,4,5,6).

Figure 1: Diagram of Structures.
BD is an important industrial solvent and precursor with numerous applications; for this reason, it is widely available. On the underground market, BD is most commonly seen in illicit dietary, health, sleep aid, or sports (bodybuilding) “supplements”, and also as the primary ingredient or a major component in various “solvents” of nebulous makeup and dubious claimed applications. Some examples include “Dream On”, “Soma” (Photo 1), and “Rejoov” (Photo 2). Soma, for example, is labeled as a dietary supplement and sold in 32 oz bottles, and is marketed as a “sleep aid”. The label on the bottle itself states that 2.0 grams of BD have been added per 1 fluid oz. Although various warning and/or disclaimer labels are usually present on such products, there is no mention that BD is a Schedule I controlled substance if intended/sold for human consumption. All of these various supplements and solvents are commonly obtained through Internet (usually from foreign sources) and on the underground drug market, especially at “Raves” and concerts, but also at gymnasiums and similar sports/body-building venues. Not surprisingly, BD (like GHB and GBL) has also been implicated in drug facilitated sexual assaults.

Abusers of BD indicate that its ingestion results in some unpleasant side effects, including a hangover (7). Therefore, some clandestine laboratories convert BD to GBL, which is the lactone of GHB and therefore a more direct pro-drug of GHB. Methods for conversion of BD to GBL have been published in various venues [Details and methodologies not provided, per Journal policy]. However, the most commonly seen clandestine laboratories working with BD are simple “re-packaging” operations. In these laboratories, clandestine chemists dilute industrial-grade BD with water and/or other components such as flavoring agents, coloring dyes, and/or sugars, then repackage the resulting mixtures in small bottles with homemade labels on them. Such laboratories usually consist of drums of BD, flavoring agents, coloring dyes, sugars, volume dispensing pumps, and various other chemicals (see Photos 3a - 3d, next page).

The forensic analysis of BD has been previously reported (2,4,8); however, these previous studies were published in law enforcement restricted venues. Herein is reported detailed procedures and techniques that can be utilized for the comprehensive analysis of BD.
Experimental

Reagents

1,4-Butanediol standard and octane (C₈H₁₈ used as an internal standard) were obtained from Aldrich. Other solvents (such as high purity methanol and chloroform) were obtained from Baxter.

GC/MS

Gas chromatography/mass spectrometry analyses were performed on a Hewlett Packard (HP) 6890 GC interfaced with a Hewlett Packard 5973 Mass Selective Detector (MSD), using a scan acquisition from 35 to 500 amu. A crosslinked 5% phenyl methyl siloxane column (HP-5), with 0.25 mm internal diameter x 30 m and 0.25 μm film thickness, was utilized. The injection port temperature was 260°C and the detector and transfer line temperatures were 280°C. The GC oven temperature was held at 50°C initially for 2 minutes, then ramped at 35°C/min to 290°C, with a final hold of 4 minutes.

FTIR/ATR

A Nicolet Nexus 470 with a potassium bromide (KBr) beamsplitter and a deuterated triglycerine sulfate (DTGS) KBr detector, equipped with a Durascope Dicomp ATR accessory with a 3-bounce diamond ATR element, was
utilized for attenuated total reflectance IR analyses. The resolution was set at 4.000 cm⁻¹, with 32 scans between 4000 cm⁻¹ and 550 cm⁻¹. The mirror velocity was 0.6329 cm per second. BD (neat) was prepared on a KBr pellet and analyzed using the same parameters, except that the wavenumbers were set between 4000 cm⁻¹ and 400 cm⁻¹.

Aqueous samples were easily analyzed by allowing a portion of the sample to evaporate at low heat on the heating plate of the ATR instrument. However, many BD-containing “supplements” also contain color dyes, flavoring agents, and sugars. These added components form a residue with BD during evaporation, thereby making it difficult to obtain clean IR spectra. A chloroform extraction is recommended for such samples.

**GC/FTIRD**

Vapor phase infrared spectra were obtained with a HP 6890 GC/BioRad IRD II Infrared Detector using a HP 5% phenyl methyl siloxane, 25 m x 0.32 mm x 0.52 µm (HP-5) column. The temperature program was set at 50°C for 1.5 minutes, then ramped up at 35°C/min to 290°C, with a final hold of 3 minutes. Column flow was 1.5 mL per minute with an average velocity of 28 cm/sec. The inlet was set at a splitless mode with an initial temperature of 260°C. The purge gas was nitrogen at 50.0 mL per minute.

**NMR**

FT-NMR spectra were obtained using a Varian Gemini 300 nuclear magnetic resonance spectrometer, operating at 300 MHz for proton. A standard ¹H-NMR was performed, with 64 transients. Deuterated water, deuterated methanol, or deuterated chloroform can be used as solvents for BD; however, only spectra in deuterated water and deuterated chloroform are presented in this study.

**Quantitation by GC/FID**

Serial dilutions of standard were prepared in methanol, ranging in concentration from 0.0869 mg/mL to 20.67 mg/mL. The internal standard solution was prepared by dissolving octane in methanol, for a final concentration of 2.00 mg/mL. For analysis, an aliquot of the standard solution was mixed with an equal amount of the internal standard solution.

GC analyses were performed on a Hewlett Packard 6890 Gas Chromatograph with a flame ionization detector, using a 0.25 mm internal diameter x 30 m HP-5 column with a 0.25 µm film thickness. An isothermal method (90°C for 2.5 minutes) was used. One µL of the standard and each sample solution were injected using an autosampler. The injection port and detector temperatures were maintained at 260°C and 270°C, respectively.

**Results and Discussion**

**GC/MS**

The mass spectrum of BD is shown in Figure 2 (next page). Figures 3a-3g (next two pages) show a suggested fragmentation scheme. The mass spectrum has a base peak at m/z 42 and the [M-1] (molecular weight minus a hydrogen) ion at m/z 89 (Figures 3a, 3b). The peak at m/z 57 results from a loss of 32 from the M-1 fragment via a 1,3 hydride shift to form (+OH=CH=CH₂) at m/z 57 (Figure 3c). The second most abundant fragment (at m/z 44) results from the cleaving of the BD molecule to form (+OH-CH=CH₂) (Figure 3a). The loss of a water molecule from BD, followed by H-rearrangement leads to the formation of a ring (Figure 3d). When the ring cleaves, radical and charge stabilization become important (9). Thus, a second H-rearrangement occurs to yield a stable product (Figure 3e). Furthermore, the intense peak at m/z 71 is a result of several possible fragments (Figure 3f). Other peaks at m/z 42 and m/z 43 are shown below (Figures 3a, 3g).
Figure 2: The Mass Spectrum of BD Standard.

Figure 3a:

\[
\begin{align*}
\text{C}_4\text{H}_10\text{O}_2 & \quad \text{m/z 90} \\
\text{HO}^+ & \quad \text{-[CH}_3\text{CH}_2\text{OH]} \quad \text{1,3 hydrogen rearrangement} \quad \text{m/z 44} \\
\text{OH} & \quad \text{-H}_2 \quad \text{1,2 elimination} \quad \text{m/z 42} \\
\text{ketene} & \\
\end{align*}
\]

Figure 3b:

\[
\begin{align*}
\text{C}_4\text{H}_10\text{O}_2 & \quad \text{m/z 90} \\
\text{HO}^+ & \quad \text{-H}^* \quad \text{m/z 89} \\
\text{OH} & \quad \text{m/z 89} \\
\end{align*}
\]

Figure 3c:

\[
\begin{align*}
\text{m/z 89} & \quad \text{-[H} \text{HO]} \quad \text{1,3 hydrogen rearrangement} \\
\text{m/z 57} & \\
\end{align*}
\]
The FTIR/ATR spectrum of BD (Figure 4a, next page) has significant bands at 2936 and 2867 cm\(^{-1}\). The 2936 cm\(^{-1}\) peak is due to the asymmetric stretching of the methylene groups, while the symmetric stretching of the methylene groups causes the weaker 2867 cm\(^{-1}\) peak. Wagging of the methylene groups causes a series of bands from 1380 to 1150 cm\(^{-1}\). The broad band at 3300 cm\(^{-1}\) is due to inter- and intramolecular hydrogen bonding. The most prominent peak is at 1048 cm\(^{-1}\) and is due to the two primary alcohol groups (10).

*FTIR/ATR*

The FTIR/ATR spectrum of BD (Figure 4a, next page) has significant bands at 2936 and 2867 cm\(^{-1}\). The 2936 cm\(^{-1}\) peak is due to the asymmetric stretching of the methylene groups, while the symmetric stretching of the methylene groups causes the weaker 2867 cm\(^{-1}\) peak. Wagging of the methylene groups causes a series of bands from 1380 to 1150 cm\(^{-1}\). The broad band at 3300 cm\(^{-1}\) is due to inter- and intramolecular hydrogen bonding. The most prominent peak is at 1048 cm\(^{-1}\) and is due to the two primary alcohol groups (10).

*FTIR/ATR*

The FTIR/ATR spectrum of BD (Figure 4a, next page) has significant bands at 2936 and 2867 cm\(^{-1}\). The 2936 cm\(^{-1}\) peak is due to the asymmetric stretching of the methylene groups, while the symmetric stretching of the methylene groups causes the weaker 2867 cm\(^{-1}\) peak. Wagging of the methylene groups causes a series of bands from 1380 to 1150 cm\(^{-1}\). The broad band at 3300 cm\(^{-1}\) is due to inter- and intramolecular hydrogen bonding. The most prominent peak is at 1048 cm\(^{-1}\) and is due to the two primary alcohol groups (10).
When utilizing an ATR, depth of penetration in the sample can affect peak intensities. The depth of penetration of the infrared beam in the sample is a function of wavelength, i.e., the longer wavelengths will show more absorbance. This is a characteristic in ATR analyses versus analyses using the traditional KBr matrices. A sample of BD was analyzed as a neat liquid on a KBr pellet to show this difference (Figure 4b).

Figure 4a: The FTIR/ATR Spectrum of BD Standard.

Figure 4b: The FTIR Spectra of BD as a Neat Liquid on KBr Plate
The vapor phase infrared spectrum of BD is considerably simplified (Figure 5). The primary bands at 2938, 2888, and 1043 cm$^{-1}$ represent the same bands seen at approximately the same wavelengths in the FTIR/ATR spectrum. However, the -O-H stretch at 3300 cm$^{-1}$ in the FTIR/ATR spectrum shifts to 3668 cm$^{-1}$ in the vapor phase, suggesting little or no hydrogen bonding (10).

**Figure 5: The GC/FTIRD Spectra of BD Standard.**

**NMR**

The proton spectra in CDCl$_3$ showed singlet peaks at 1.4 (C2/C3 methylenes), 3.4 (C1/C4 methylenes), and 4.7 (hydroxyl protons) ppm (Figure 6). When the sample was run in D$_2$O, chemical shift increases of 0.2 ppm were observed for all peaks, i.e., singlets were found at 1.6, 3.6, and 4.8 ppm, respectively (Figure 7, next page).

**Figure 6: The Proton NMR spectra of BD standard in CDCl$_3$.**
Using the specified GC/FID parameters, BD had a retention time of 1.862 minutes, while octane (I.S.) had a retention time 1.457 minutes (see Figure 8). Area ratios of the standard/internal standard were plotted against the corresponding BD concentrations. Linear responses for BD were found to be from 0.87 mg/mL to 10.64 mg/mL (Figure 9). The correlation coefficient was 0.9997, indicating a highly linear relationship. Reproducibility for both area counts and retention times were below 2.3% RSD (Table 1).

Samples of BD could contain GBL or GHB. GHB, however, converts to GBL in heated injection ports under standard GC operating conditions, so only a GBL peak would be observed for exhibits containing GBL and/or GHB. For this reason, GBL was added to a sample of BD (to ensure that they do not co-elute), and was found to have a retention time of 1.678 minutes.
Figure 9: Linearity of BD.

\[ y = 1.0502x - 0.1916 \]
\[ R^2 = 0.9997 \]

Table 1: Reproducibility Data of BD and Octane.

<table>
<thead>
<tr>
<th>Octane</th>
<th>Area</th>
<th>1,4-butanediol</th>
<th>Area</th>
<th>Area STD/ Area ISTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time (minutes)</td>
<td>Retention Time (minutes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.460</td>
<td>204.453</td>
<td>1.853</td>
<td>139.819</td>
<td>0.684</td>
</tr>
<tr>
<td>1.460</td>
<td>208.700</td>
<td>1.854</td>
<td>139.204</td>
<td>0.667</td>
</tr>
<tr>
<td>1.461</td>
<td>216.972</td>
<td>1.856</td>
<td>144.346</td>
<td>0.665</td>
</tr>
<tr>
<td>1.460</td>
<td>212.846</td>
<td>1.854</td>
<td>144.476</td>
<td>0.679</td>
</tr>
<tr>
<td>1.461</td>
<td>212.209</td>
<td>1.855</td>
<td>142.213</td>
<td>0.670</td>
</tr>
</tbody>
</table>

average: 1.460  211.036  1.854  142.012  0.673  
%RSD: 0.04  2.23  0.06  1.73  1.19

Conclusions

A variety of techniques can be used for the analysis of BD, including GC/MS, FTIR, NMR, GC/IRD, and GC/FID. The more difficult BD samples to analyze are illicit dietary supplements and commercial solvents, due to the presence of additional components. In these instances, a chloroform extract is recommended.

Acknowledgements

The authors would like to thank Christine Sannerud (Deputy Chief, Drug and Chemical Evaluation Section, Drug Enforcement Administration (DEA)), for sharing her expertise in GHB and related samples. The authors would
also like to acknowledge Senior Forensic Chemist Donald Cooper (DEA Special Testing and Research Laboratory, Dulles, VA) and Forensic Chemist Walter Rodriguez (DEA Southeast Laboratory, Miami, FL), for their time and expertise in helping interpret the mass spectrum of BD. Special thanks also goes to Task Force Officer Brian Ballard (Atlanta, GA) for supplying Rejoov and BD clandestine laboratory pictures and valuable information on Internet purchases. Gratitude also goes to Forensic Chemist Amy Bederka (County Bureau of Identification, Raleigh City, NC) for useful conversations and pictures regarding Rejoov samples.

References


2. Chew S. 1,4-Butanediol in Liquid Exhibit. Microgram 1997;30(7):154-159.


* * * * *
Detection and Analysis of Drugs of Forensic Interest, 1992 - 2001; 
A Literature Review

Robert F.X. Klein, Ph.D.,*  
U.S. Drug Enforcement Administration  
Office of Forensic Sciences  
Laboratory Support Section  
2401 Jefferson Davis Highway  
Alexandria, VA  22301  
[email: microgram_editor@mailsnare.net]

Patrick A. Hays, B.S.  
U.S. Drug Enforcement Administration  
Special Testing and Research Laboratory  
22624 Dulles Summit Court  
Dulles, VA  20166

ABSTRACT:  The scientific literature of the detection and analysis of drugs of forensic interest, as published from 1992 through 2001, is reviewed.  1,377 references are included.

KEYWORDS:  Forensic Chemistry, Analytical Chemistry, Illicit Drugs, Controlled Substances, Review

Introduction

This review presents a 10 year survey of the detection and analysis of drugs of forensic interest, as published in the mainstream scientific literature from 1992 through 2001.  Analyses of drugs in post-ingestion biological matrices are not included, except for select studies which provide structural, spectral, and/or analytical data above and beyond routine toxicological “screening” techniques.  In addition, due to their inherently transitory nature, Internet references are not included.  Finally, forensic association newsletters and “underground” publications are not included.

Articles are first organized by overall focus, and subcategorized (where applicable) by specific drug or drug class, or instrumental technique.  The focus categories are as follows:

* Previous Reviews and Overviews  
* Analyses of Specific Drugs and Drug Groups  
  - Illicit Drugs  
  - Select Adulterants and Diluents  
  - Occluded Solvents  
* Simultaneous Analyses of Drugs in the Presence of Select Adulterants and Diluents  
* Instrumentation Focus  
* Analytical Artifacts  
* Qualitative Tests  
* Sampling Plans  
* Source Determination/Impurity Profiling  
* Source Determination/Stable Isotope Analyses  
* Comparative Analyses  
* Reference Standards
**Abbreviations**

The authors have utilized common abbreviations throughout the review; however, all terms are defined in order to avoid ambiguity with some of the more crowded or obscure acronyms, as follows:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Atomic Absorption</td>
</tr>
<tr>
<td>AP</td>
<td>Atmospheric Pressure</td>
</tr>
<tr>
<td>C-13</td>
<td>Carbon-13</td>
</tr>
<tr>
<td>CE</td>
<td>Capillary Electrophoresis</td>
</tr>
<tr>
<td>CEC</td>
<td>Capillary Electrochromatography</td>
</tr>
<tr>
<td>CGC</td>
<td>Capillary Gas Chromatography</td>
</tr>
<tr>
<td>CI</td>
<td>Chemical Ionization</td>
</tr>
<tr>
<td>CZE</td>
<td>Capillary Zone Electrophoresis</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode Array Detection</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>ECD</td>
<td>Electron Capture Detection</td>
</tr>
<tr>
<td>EKC</td>
<td>Electrokinetic Chromatography</td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact</td>
</tr>
<tr>
<td>FID</td>
<td>Flame Ionization Detection</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier Transform</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas Liquid Chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HPTLC</td>
<td>High Performance Thin Layer Chromatography</td>
</tr>
<tr>
<td>IMS</td>
<td>Ion Mobility Spectrometry</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared Spectroscopy</td>
</tr>
<tr>
<td>IRD</td>
<td>Infrared Detector</td>
</tr>
<tr>
<td>IRMS</td>
<td>Isotopic Ratio Mass Spectrometry</td>
</tr>
<tr>
<td>ITD</td>
<td>Ion Trap Detector</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid Chromatography</td>
</tr>
<tr>
<td>MECC</td>
<td>Micellar Electrokinetic Capillary Chromatography</td>
</tr>
<tr>
<td>MEKC</td>
<td>Micellar Electrokinetic Chromatography</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>MSD</td>
<td>Mass Selective Detector</td>
</tr>
<tr>
<td>MS-MS</td>
<td>Tandem Mass Spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance (Spectroscopy)</td>
</tr>
<tr>
<td>NPD</td>
<td>Nitrogen Phosphorus Detection</td>
</tr>
<tr>
<td>PDA</td>
<td>Photodiode Array</td>
</tr>
<tr>
<td>RP</td>
<td>Reverse Phase</td>
</tr>
<tr>
<td>SFC</td>
<td>Supercritical Fluid Chromatography</td>
</tr>
<tr>
<td>SHS</td>
<td>Static Headspace</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid Phase Microextraction</td>
</tr>
<tr>
<td>TD</td>
<td>Thermal Desorption</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet (Spectroscopy)</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible (Spectroscopy)</td>
</tr>
</tbody>
</table>
Previous Reviews and Overviews

The forensic analysis of illicit drugs has been the subject of a number of minor review articles and monographs over the past 10 years (1,2,3,4,5,6,7,8,9). In addition, several articles have given more general overviews of the field (10,11). Systematic approaches to substance identification have also been presented (12,13,14), and a number of scientific working groups are currently establishing national/international standards for forensic analysis of illicit drugs (15,16,17(see also:18)). Finally, forensic chemistry has been the subject of several textbooks and chapters in textbooks (19,20,21,22,23,24,25,26).

Analyses of Specific Drugs and Drug Groups

An immense amount of analytical data has been published over the past 10 years for drugs of abuse. “Comprehensive” data compilations (defined in this context as three or more analytical profiles in a specific study) have been previously provided for virtually all “traditional” drugs of abuse, but a number of updated compilations have been provided which reflect improvements in existing instrumentation and/or the advent of new instrumental techniques. In addition, “comprehensive” data compilations have been provided for a number of new drugs of abuse; these include previously unknown “designer,” “analog,” or “homolog” drugs, and also various pharmaceuticals or industrial chemicals which either had not been previously subject to abuse, or had been only rarely encountered in illicit settings. Furthermore, hundreds of studies which analyzed either specific drugs of abuse or groups of structurally related drugs of abuse, but only by one or two select analytical techniques, have also been provided. [Note that multiple citations are organized as follows: reviews, then overviews, then comprehensive studies, then by specific analytical techniques (alphabetized), and finally in reverse date order (most recent citation first).]

Controlled Substances: Over the past 10 years, the following substances were the subjects of moderate to comprehensive analytical profiling: alkyl nitrites (inhalants) (comprehensive) (27), by GC-IR (28), and by headspace GC/MS (29); Amanita Muscaria by ion-interaction HPLC (30); amphetamine by CE (31), by CE and LC (32), by GC-MS (role of self-protonation) (33), by HPLC (after acetylation) (34), by HPLC using chiral crown ether coated reversed-phase packing (35), and by HPLC using a two-dimensional column-switching chromatographic system with on-line derivatization (36); amphetamine and methamphetamine - (general review of the syntheses and analyses of phenylacetone, amphetamine, and methamphetamine) (37), in abuser’s clothing by HPLC with UV and fluorescence detection (38), by CE with cyclodextrins to determine isomers (39), by GC and GC-MS versus internal standards (40), by GC-Cl/MS (following derivatization with perfluorinated acid chlorides) (41), by headspace sampling and GC-MS (analysis of betel) (42), by HPLC (43,44), by HPLC for quantitation of clandestinely produced mixtures (45,46), by MS (47), by micro-Raman scattering (48), by surface enhanced Raman scattering detection after modification with 2-mercaptopnicotinic acid (49), and by UV/Vis spectrophotometry after derivatization with 1,2-naphthoquinone-4-sulfonic acid (50); amphetamines (review of biological/forensic issues (includes amphetamine and methamphetamine analogues)) (51), by CE (52,53), by CE (chiral) (54), by CE with added anionic chiral selectors (55), by CE and LC (56), by color tests, TLC, GC/MS, GC/IR, plus GC/IR/MS of N-acetyl derivatives (differentiation of side chain isomers of ring-substituted amphetamines (4-Me, 4-OMe, 3,4-MD- amphetamine and methamphetamine)) (57), by CZE (58), by CZE with added cyclodextrins (59), by EI-MS (N-substituted amphetamines) (60), by GC (61), by GC, HPLC, and MS (62), by GC-FTR (63), by GC/MS (64,65), by GC-MS (differentiation of acylated derivatives of methamphetamine and regioisomeric phenethylamines) (66,67), by HPLC with added cyclodextrins and using a chiral stationary phase (68), by HPLC with fluorimetric detection (69), by HPLC after derivatization with chloroformates (70), by LC with fluorometric detection after precolumn derivatization with (+)-1-(9-fluorenyl)ethylchloroformate (71), by MECC (72), by negative-ion CI-MS (73), by carbon dioxide negative-ion CI-MS (74), by SFC (75), by SFC, HPLC, GC, and CZE (76), by TLC with diazonium salts as visualization reagents (77), and by GC/FID using n-alkanes and other indirect reference standards when no internal standard is available (78); ayahuasca by GC and GC/MSD (79); barbiturates by HPLC with PDA-UV detection (80), by HPLC using 3-(18-naphthalimido)-propyl-modified silyl silica gel as a stationary phase (81), by micro-HPLC with post-column photochemical derivatization (82), by ion-trap and quadrupole MS (83), by IR and MS (84), by mercurimetric potentiometric
determination using a solid-state iodide ion-selective electrode (85), by micellar LC (86,87), by SPME and CE (88), by SPME and ion-trap GC-MS (89), by a thermally tuned tandem column (separation of barbiturates and phenylthiodyantoin amino acids) (90), and by two-dimensional overpressured layer chromatography (91); benzodiazepines by CE (92), by electrospray probe/MS (93), by free zone electrophoresis (94), by FT-Raman and FT-IR (95), by HPLC (nitrazepam, diazepam, and medazepam) (96), by HPLC (clorazepate, diazepam, and diltiazem in pharmaceuticals) (97), by HPLC-DAD (98,99), by HPLC using diode-array, electrochemical, and thermospray MS detection (100), by HPLC/electrospray MS-MS (101), by HPTLC (diazepam, chloridiazepoxide, and midazolam) (102), by MECC (103,104), by SERS after adsorption on the Ag colloidal surface (diazepam and nitrzezam) (105), by LC (alprazolam) (106), by microcolumn LC using a cholesteryl-10-undecenoate bonded phase (107), by RP-LC (flurazepam) (108), and by automated SPME-LC/EI-MS (109); 1-benzyl-1-\textit{n}-butyl-barbituric acid (comprehensive) (110); bromazepam by flow injection stopped-flow kinetic determination (111); 4-bromo-2,5-dimethoxyphenethylamine (2C-B or NEXUS) and related compounds (overview) (112), (comprehensive) (113,114), by GC and HPLC (115), by GC-MS and NMR (including analysis of derivatized samples) (116), and by LC and GC/MS (117,118,119); butotenine (comprehensive) (120,121), and by GC/MS after BSTFA derivatization (122); butotenine, psilocybin and related indole alkaloids by CE (123), by GC/MS (124), by GC/IRD (125,126), by GC/MS (includes general overview) (127), and by MS (128); [“Love Stone” (contains butotenine) (overview) (131); cathanine (\textit{alpha}-aminropriophenone) (review with 79 refs) (132), (comprehensive) (133), by NMR (enantiomeretric determination of N-acetylcathinone) (134), and by spectrophotometric detection (135); 2-chloro-4,5-methylenedioxymethamphetamine (comprehensive) (136); clenbuterol by CE (overview analysis with added CD’s) (137), by EKC (epinephrine, terbutaline, clenbuterol, and salbutamol) (138), by flow-injection fluorimetry (139), by GC after derivatization and electrospray MS (140), and by RP-HPLC (141); cocaine by CE (comprehensive analysis of coca alkaloids and sugars in illicit cocaine) (142), by free-zone CE (143), by DSC (and GC/FID) (144), by FT-IR and HPTLC (145), by flow injection analysis with amperometric detection (146), by FTIR, GC/MS, and quantitative GC (cocaine HCl in wax) (147), by GC (cocaine base) (148), by GC/MS after field testing (149), by HPLC (150,151), by HPLC and GLC (152), by an ISFET device, GC, and UV (153), by LC/AP-CI-MS (154), by MS (evaluation of fragmentation patterns) (155), by collision induced dissociation MS (156), by MECC (157), by Raman microspectroscopy (158,159), by SPME/GC/MS (methylbenzoate from cocaine) (160), by TLC, GC, UV, and GC/MS (identification of cocaine in samples in the presence of other local anesthetics) (161), and by transmission and internal reflection IR (cocaine base versus HCl) (162,163); cocaine analogs by NMR (164), cocaine base (comprehensive) (165); cocaine-N-oxide by LC and MS (166); coca tea by solid-phase extraction followed by GC-MS (167); codeline by chemiluminescence (168), by LC and LC-MS/MS (169), and by NMR (170); codeine pharmaceuticals by CE (171), by free solution CE (172), and by HPLC (173); creatine (comprehensive) (174,175), and by TLC/densitometry (176); cyclofenil (comprehensive) (177); cyclohexyl nitrite (comprehensive) (178); dexamfluramine by HPLC on a chiral column (179); diazepam by ion-trap and quadrupole mass spectroscopy (180), and by polarography (181); dihydroetorphine and etorphine (comprehensive) (182); 2,5-dimethoxy-4-ethylthiophenethylamine (2CT-2) by IR, GC/IRD, and GC/MS (183); 2,5-dimethoxy-4-(N)-propylthiophenethylamine (2CT-7) (comprehensive) (184); dimethamide (comprehensive) (185,186,187); dimethylaminorex (comprehensive) (188); dimethylamphetamine by GC, IR, and UV/VIS (stability study) (189), by GC/MS, HS-GC/MS, and LC-ESI/MS (analysis of dimethylamphetamine pyrolysis products) (190); dragon’s blood incense (overview) (191), and by GC/MS (192); ergot alkaloids (review) (193), determination and isolation by LC (194), by LC with fluorescence detection (195), and determination of ergonovine maleate by flow injection analysis with chemiluminescence detection (196); etonitazene (comprehensive) (197); fenethylline by IR, UV, and TLC (198), and by TLC, UV/VIS, and toolmarks (199); fentanyl (review) (200), and by cyclic voltametry (201); fentanyl and fentanyl analogs (review) (202), by RP-HPLC (203), and by GC, GC/MS, and IR (204); Flos Daturae by CE (205); flunitrazepam (Rohynol) (overview) (206), (comprehensive) (207,208), by color testing (screening) (209), by derivatization followed by TLC with fluorescence detection (urine screening focus) (210), by FTIR, FT-Raman, and NMR (degradation study) (211), and by screening techniques (overview) (212); 4-fluorophenylacetone, 4-fluoroamphetamine and 4-fluoromethamphetamine (comprehensive) (213); para-fluorofentanyl (comprehensive) (214), heroin by bioluminescent assay (215), by CZE (216), by rapid GC (217), by GC/MS, FTIR, and TLC (for determination of heroin base in heroin citrate) (218), by HPLC (degradation study) (219), by IR and TLC (220), by continuous flow IRMS (221), by MECC
(222,223), by NMR (224), and by TLC (225); heroin and related morphine alkaloids by HPCE (226); heroin and amphetamine by CE (227); human chorionic gonadotropin (beta subunit) by MS (228); gamma-hydroxybutyric acid (GHB) or gamma-hydroxybutyrate (review) (229), (overview) (230), (comprehensive) (231,232,233), by free zone CE with direct UV detection of GHB (234), by color testing (235), by FTIR and color testing (236), by GC/MS after extraction on a SPME fiber and derivatization with BSTFA (237), by ICP-atomic emission and MS (includes ephedrine) (238), by IR using a 3-bounce diamond ATR element (239), by microcrystal testing with cupric nitrate/silver nitrate solution (240), by NMR (241), and by SPME - GC/quadrupole ion trap spectrometry (242); gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) (interconversion study) (243,244), by CE and HPLC (245), by GC/MS with BSTFA derivatization (246), by HPLC (247), by HPLC/UV-VIS and HPLC/thermospray MS (248), gamma-butyrolactone in wine by GC/MS (249); gamma-hydroxybutyric acid (GHB), gamma-butyrolactone (GBL), 1,4-butanediol (BD), tetrahydrofuran (THF), and/or GHB/GBL analogs (overview, comprehensive) (250), (overview of analysis of GHB, GBL, and BD) (251), (overview of GHB, GABA, and various analogs) (252), by CE (253), (comprehensive) (BD in a liquid exhibit) (254), and by GC/MS and FT-IR (BD) (255); N-(2-hydroxyethyl)-amphetamine (comprehensive) (256,257); N-hydroxy-3,4-methylenedioxyamphetamine by GC-FTIR after derivatization (258); N-hydroxy-3,4-methylenedioxyamphetamine (comprehensive) (259), and by GC and GC/MS (260); imazalil (comprehensive) (261); imipramine by flow-injection extraction - spectrophotometric determination with methyl orange (262); jimson weed (overview and analysis by GC/MS: 263); ketamine (comprehensive) (264), and by GC and GC/MS (265); khat (Catha Edulis) by GC and GC/MS after derivatization with (R)-(+)alpha-methoxy-alpha-(trifluoromethyl)phenylacetic acid (266), by GC/MS (267,268,269), by GC/MS/FTIR (270), by NMR (272); lorazepam by UV (273); lysergic acid diethylamide (LSD) (review) (274), (overview, comprehensive) (275), (comprehensive) (276), by enzyme immunoassay and immunoaffinity extraction and HPLC-MS (277), by GC (278), by GC/MS using electronic pressure controls and pulsed split injection (279), by GC/MS/FTIR (280), by GLC (281), by HPLC (282), by HPLC and GLC (283), by MECC (284), by NMR (285), and by automated TLC (286); LSD and psilocin - by GC/MS and GC/MS (287), by GC and GC/MS (288), by HPLC (289), by TLC (290), by NMR and MS (291), by CE and HPLC (292), by HPLC and TLC (293), and by HPLC (294), by GC and GC/MS (includes ephedrine and pseudoephedrine) (295,296), by GC, HPLC, and TLC (297), by GC/MS (versus cannabidiol as a reference standard) (298), (versus cannabiol as a reference standard) (299), by GC/MS (300,301), by HPLC (302,303), by TLC (seeds) (304), by HPLC of neutral cannabinoids of marijuana and hashish after supercritical fluid extraction (305), by LC/MS and LC/MS-MS (306), by plant propagation (determination of viability of stem cuttings) (307), by SFC/AP-CI-MS (308), by TLC and botanical characterization of morphological features (309), by monoclonal antibody (against tetrahydrocannabinolic acid) (310), and by GC/MS (butyl cannabinoids in marijuana) (311); mescaline in hallucinogenic Cactaccae by ion-interaction HPLC (312), and in peyote by GC/MS (comparison of 6 different extraction procedures) (313); methamphetamine (related compounds) comprehensive (314), by CE (chiral analysis) (315), by diasteriomeric salt formation (316), by FT-Raman (317), by GC, HPLC, and/or CE following derivatization (for enantiomer determination) (318), by full scan GC-ion trap (319), by GC/MS (improving ion mass ratio performance at low concentrations through internal standard selection) (320), by HPLC with circular dichroism detection for determination of enantiomers (321), by IR (chiral analysis) (322,323), by NMR (chiral analysis) (324), by TD-IMS and SIMPLISMA (325), and by CE with UV and LIF detection (326); methaqualone (review) (327), and by color testing (includes mecloqualone) (328); methcathinone (ephedrone) (review) (329), (comprehensive) (330), by GC and GC/MS (methcathinone and some designer analogues) (331), by GC and GC/MS after chiral derivatization with S-(+)(-)(trifluoroacetyl)-prolyl chloride (332), by GC/MS (333), and by animal testing (potency comparison of enantiomers; includes syntheses) (334); methcathinone and cathinone (comprehensive) (335); 4-methoxyamphetamine (PMA) and 4-methoxymethamphetamine (PMMA) (comprehensive) (336), and 2-, 3-, 4-PMA, PMMA, and P2P (combined studies) (337); 3-methoxy-4,5-methylenedioxyamphetamine (comprehensive) (338,339); 2,3-methylenedioxymethamphetamines (combined studies) by GC/MS-MS (340), by LC and MS (341,342); 3,4-methylenedioxymethamphetamines (MDA’s) (general review of the syntheses and analyses of MDA’s and precursors and related compounds) (345), by MS (346), by C-13 solid-state NMR (347), by CE (348,349), by field testing (350), by FT-IR (for tablets) (351), by FTIR microscope (352), by FTIR and GC/MS (353), by GC (tablets) (354), by GC and GC/MS
by GC/MS (435), by LC (for acetylsalicylic acid, caffeine, and codeine phosphate in pharmaceuticals) (436), by RP-LC (quantitative determination of opium alkaloids in opium) (417), by GC/MS (narcotine and 1-fluoro-2,4-dinitrobenzene (405), and by displacement TLC (406); midazolam by UV/Vis (401); morphine by aqueous and nonaqueous CE (for quantitative determination of morphine in pharmaceuticals) (402), by flow-injection analysis with spectrophotometric determination (403), by HPLC with chemiluminescence detection (404), with a fluoride-selective electrode following derivatization with 1-fluoro-2,4-dinitrobenzene (405), and by displacement TLC (406); nandrolone-pare-hexloxyphenylpropionate (comprehensive) (407); nootropics/“smart drugs” (overview) (408); opiate alkaloids by CEC (409), by high-resolution electrospray ionization – IMS/MS (evaluation of opiate separation) (410), by HPLC (hydrocodone in Tussionex) (411), and by HPTLC (oxycodone in pharmaceutical solutions) (412); opium (overview of characterization methodologies: 413), by CE (414), by non-aqueous CE (415), by CE-TOF-MS (416), by GC (quantitative determination of opium alkaloids in opium) (417), by GC/MS (narcotine and papaverine in seeds) (418), by HPLC (419), by RP-HPLC on a base-deactivated stationary phase (420), by HPLC of Sep-Pak C-18 cartridge extracts (421), by MECC (422), by pyrolysis-GC (423), by synchronous excitation spectrofluorimetry (424), by TLC-UV densitometric and GC-MSD methods (425), by UV/VIS (for morphine in opium) (426), and by GC/FID, GC/MS, and GC/FTIR (headspace constituents of opium) (427); "opioid alkaloids" by CE with acidic potassium permanganate chemiluminescence detection (428), by CZE (429), by flow-injection analysis using soluble manganese(IV) for chemiluminescence detection (430), by GC/MS after oxime-TMS derivatization (431), by GC/MS (6-acetylmorphine) (432), by HPLC using porous and non-porous stationary phases (433), by HPLC-DAD (stability of morphine containing solutions) (434), by RP-HPLC (codeine and ethyl morphine HCl in pharmaceutical tablets) (435), by LC (for acetylsalicylic acid, caffeine, and codeine phosphate in pharmaceuticals) (436), (for acetylsalicylic acid, caffeine, codeine, paracetamol, pyridoxine, and thiamine in pharmaceuticals) (437), (for paracetamol, caffeine, and codeine phosphate in pharmaceuticals) (438), by RP-LC (papaverine in tablets) (439), by spectrofluorimetric detection (acetylsalicylic acid and codeine in pharmaceuticals) (440), and by spectrophotometric detection (of noscapine with bromocresol green in chloroform) (441); opium, morphine, and heroin (combined study) (comprehensive) (442); oxazepam by automatic kinetic determination (443); pentobarbital by GC/MS (444), and by HPLC (445); phencyclidine (and 1-piperidinocyclohexane-carbonitrile) (review) (446), by IMS (447), and by IR after liquid-liquid extraction from case samples (448); alpha-phenethylamine (comprehensive) (449,450), beta-phenethylamine (overview) (451,452), and by GC/MS, FTIR, and GC/IR (453); phenobarbital by CE (tablets) (454), by LC (for determination of scopolamine, hyoscyamine and phenobarbital in tablets) (455), by multivariate spectrophotometric calibration (for simultaneous determination of phenobarbital and phenytoin in tablets) (456), and by spectrophotometric determination (457); phenylpropylmethylamine (comprehensive) (458); piperazines (comprehensive) (459); psilocybe mushrooms by DNA (460,461), by IMS and GC/MS (462), by GC/MS and HPLC/UV (463), by morphological, microscopic, microchemical, and HPLC with 266 nm UV detection (464), by TLC, GC/MS, and LC/MS (465), and by TLC and GC/MS (psilocin and psilocybin in developmental mushrooms) (466); Salvia Divinorum by GC/MS (467); seconobarbital using C-13 labelled seconobarbital as an internal standard (468); sibutramine (comprehensive) (469); steroids (overview) (470), (overview, comprehensive) (471), (comprehensive) (472), by EI, CI, and CI/tandem MS (473), by GC (474), by GC/MS (475), by GC/MS after SFC isolation from aqueous matrices (476), by GC/MS and NMR after derivatization with N-methyl-N-alkylsilyl trifluoroacetamide-I-2 (477), by GLC (478), by HPLC with cyclodextrin coated columns (479), by...
HPLC with UV/Vis-particle beam MS (480), by HPLC-FTIR (481), by HPTLC and HPLC (482), by MS (of tert-butyl-dimethylsilyl ether derivatives) (483), by MS/MS (484), by quadrupole ion trap tandem MS (485), by 13C - NMR (486), by MECC (487), by MECC, gradient HPLC, and capillary GC (488), by capillary SFC with FID and ECD (489), and by TLC (490); telazol (comprehensive) (491,492); terbinafine (comprehensive) (493), and by UV and nonacqueous voltametry (494); triazolam by TD-GC (495); tricyclic antidepressants - by electrogenerated chemiluminescence (496); and tryptamines by chemiluminescence (497).

Adulterants, Diluents and Precursors (general overviews of essential chemicals and precursors) (498,499), by GC, HPLC, and CE (500), by LC (for determination of non-UV detectable organic impurities) (501), by various analytical techniques (for determination of “secondary” drugs present in cocaine, heroin, marijuana and phencyclidine) (502); acetaminophen by Raman microprobe spectroscopy (503); acetic acid (in acetic anhydride) by NMR (504), dextromethorphan by MEKC (dextromethorphan, pseudophedrine and guaifenesin) (505); diethylminoethylaniline (comprehensive) (506), cis- and trans-2,5-dimethoxy-4-beta-dimethyl-beta-nitrostyrenes by FTIR/Raman (507), dimethylsulfone (overview) (508), (removal by sublimation) (509,510), by GC/MS, IR and GC/IR (in amphetamine and methamphetamine samples) (511); dimethyl terephthalate (and dimethyl phthalate) (comprehensive) (512), differentiation of dimethylterephthalate from dimethylisophthalate by GC/FTIR (513), identification of dimethyl terephthalate in cocaine samples (comprehensive) (514); dipyrone in pharmaceuticals with a flow cell containing gold electrodes (515); ephedrine/pseudoephedrine comprehensive (516), by CE, UV, NMR, and MS (for chiral recognition of the enantiomers of ephedrine derivatives) (517), by CE on a chip with amperometric detection (for chiral analysis) (518), by acetonitrile modified CZE (for ephedrine in ephedra callus) (519), by derivative spectrophotometry and ratio spectra derivative spectrophotometry (for simultaneous determination of pseudophedrine, dextrompheniramine, and loratadine) (520), by differential-derivative spectroscopy (assy of ephedrine/theophylline containing pharmaceuticals) (521), by an ephedrine based electrode (522), by a double membrane ephedrine selective electrode (523), by flow injection - pulse amperometric detection (524), by GC (for determination of pseudophedrine and diphenhydramine) (525), by GC/MS (for determination of ephedrine alkaloids and tetramethylpyrazine in ephedra sinica Stapf) (526), by HPLC (for determination of ephedrine, pseudophedrine, norephedrine, and methylephedrine in Chinese folk medications) (527), by HPLC using chiral stationary phases (for separation of the enantiomers of ephedrine, norephedrine, and pseudoephedrine) (528), by HPLC and CE (discrimination of ephedrine and pseudoephedrine) (529), by HPTLC (for simultaneous determination of pseudophedrine and cetirizine in pharmaceuticals) (530), by LC (for N-methylphedrine, after derivatization with 9-flouroenylmethyl chloroformate) (531), by LC (for determination of pseudophedrine and carbinoxamine pharmaceuticals) (532), by proton NMR (for determination of ephedrine, pseudoephedrine, and norephedrine in bulk and dosage mixtures) (533), by RP-HPLC-UV (with data analysis to handle quantitation of overlapping peaks) (534), by SPE-LC/UV (for determination of 7 ephedrine alkaloids in herbal products) (535), by TLC and FTIR (for determination of pseudophedrine in a pseudophedrine/chlorpheniramine pharmaceutical) (536), and by impregnated TLC (for direct resolution of (+/-)-ephedrine and atropine) (537); ethoxy-1-(2-nitro-1-propenyl)benzenes by FTIR and Raman (538); guaifenesin by HPLC (539); 3-hydroxy-N-phenyl-2-naphthalene carboxamide (comprehensive) (540); lactitol in cocaine by NMR and IR (541); 4-(N-methylacetamido)-antipyrine (comprehensive) (542); paracetamol by FTIR (543), by ion chromatography (544), by reflectance NIR spectroscopy (545), by NIR transmittance spectroscopy (546), and by simultaneous stopped-flow determination and FTIR (for paracetamol, acetylsalicylic acid and caffeine in pharmaceutical formulations) (547); pheniramines by CZE (for pheniramine, chlorpheniramine, and brompheniramine) (548), and by an imprinted sensor (for chlorpheniramine) (549); phenylpropanolamine by HPLC in pharmaceutical preparations (using 4-dimethylaminobenzaldehyde) (550); procaine by spectrophotometry (using p-dimethylaminobenzaldehyde) (551); quinine by flow-injection chemiluminescence (552), and by HPLC with polarimetric detection (553); safrole by SFC extraction and GC/MS (for determination of safrole and related allylbenzenes in sassafras) (554); sugars by GC after derivatization with trimethylsilylimidazole (for quantitation of sugars in drug samples) (555), theophylline by adsorptive cathodic stripping voltammetry (556), by a flow fluororimmunosensor (557), by micellar LC and spectrophotometric detection (558), and by UV and HPLC (559); thiamine by cathodic stripping voltametry (560), by cyclic voltametry and HPLC with amperometric detection (561), by flow injection turbidimetric determination using silicotungstic acid (562), and by spectrofluorometry (563); and tripolidine by a kinetic method based on oxidation w/ KMnO4, with spectrophotometric determination (564).
Occluded Solvents in cocaine by GC/MS (565), in cocaine and heroin by headspace - GC/FID and GC/MS (566), and by SHS-GC/MS (567); in methamphetamine by SPME/GC-MS (characterization of volatile components) (568); and in pharmaceuticals (overview, emphasizing headspace - CGC and impurity profiling) (569), by CGC (570), by wide-bore CGC (571), by CGC-ITD (572), and by automated SHS - CGC-MS (573).

**Simultaneous Analyses of Drugs and Adulterants/Diluents**

A number of studies have been reported which allow simultaneous identification and quantitation of mixtures of controlled substances and adulterants without separating them into individual components. In general, such techniques may be only employed in select geographical areas in which the submitted exhibits are reasonably consistent (that is, routinely the same adulterants and diluents, at or below a threshold percentage). In most cases, they allow much more rapid sample analysis and throughput without unduly compromising the identification of the controlled substance. Such techniques have been utilized for: cocaine by IR (574,575), by Raman (576,577), by spectrometric methods (578), and by sequential second-derivative spectroscopy (579); and pharmaceuticals by NIR and Raman (580), and by spectrophotometry in conjunction with PLS-1 and PLS-2 data processing methods (581); and for determination of interferences by common diluents in street-level drugs by micro-FTIR (582).

**Instrumentation Focus**

In addition to the above studies which concentrated on specific drugs or drug groups, there have been a large number of studies which focused on specific instrumental techniques, analyzing two or more unrelated drugs or drug types in order to illustrate the utility of the described methodology, including: capillary electrophoresis: (general reviews) (583,584,585,586,587,588,589,590,591, 592,593,594,595,596,597), (general overview and reviews) (598,599), (general overview, comparing various CE techniques) (600), (review for court admissibility) (601), for separation and permanganate chemiluminescence on-line detection of some alkaloids with beta-cyclodextrin as an additive (602), for on-chip separation of amphetamine and related compounds labeled with 4-fluoro-7-nitrobenzofurazane (603), for enantioselective separations of various amphetamines and methylenedioxyamphetamine using cyclodextrins (604,605), for analysis and confirmation of synthetic anorexics in adulterated traditional Chinese medicines (606), for chiral analysis of drugs (607), for chiral identification of drug isomers (608), for chiral analysis of basic drugs using oligosaccharides (609), for chiral separation of basic drug racemates using linear, neutral polysaccharides (610), for chiral resolution of cationic drugs of forensic interest with mixtures of neutral and anionic cyclodextrins (611), for chiral separation of enantiomers of drugs using beta-cyclodextrin (612), for chiral separation of drug stereoisomers with cyclodextrins (613), for chiral separation of basic drugs using ionic and neutral polysaccharides (614), for ultra-fast chiral separation of basic drugs (615), for illicit drug seizures (616), for CE-TOF/MS of drugs of abuse (617,618), for separation of enantiomers of basic drugs (by affinity CE using a partial filling technique and &alpha;-acid glycoprotein as chiral selector) (619), for separation and identification of amphetamines, methadone, venlafaxine, and tropane alkaloids by CE-electrospray MS (620), for separation and identification of designer drugs with CE-ionspray MS (621), for determination of drug-related impurities (622), for analysis of heroin and amphetamine (623), for simultaneous chiral analysis of methamphetamine and related compounds (624), for routine analysis of methamphetamine, amphetamine, MDA, MDMA, MDEA and cocaine with dynamically coated capillaries (625), for analysis of basic pharmaceuticals by CE in coated capillaries with on-line MS detection (626), for quantitation of common illicit drugs (627,628), for chiral separation of selegiline, methamphetamine, and ephedrine using a neutral beta-cyclodextrin epichlorhydrin polymer (629), for tropane alkaloids in a plant extract (630), for CE-DAD-electrospray MS of tropane alkaloids, hyoscyamine, scopolamine, and plant extracts (631), CEC: (introductory overview) (632), for simultaneous separation of acidic, basic, and neutral organic compounds, including strong and moderate acids and bases (633), for analysis of drugs of forensic interest (634); CZE: characterization of drugs of forensic interest by CZE/electrospray ionization MS (635), for chiral separation of amphetamine and phenylephrine, using cyclodextrins (636), for chiral separation of basic drugs using cyclodextrins (637), and for chiral separation of some basic drugs (influence of the buffer organic cation) (638); EKC: for chiral separation of
drugs by electrokinetic chromatography (639), for separation of enantiomers and geometric isomers using a charged cyclodextrin (640), and for chiral separation of neutral and basic enantiomers using anionic cyclodextrins (641); MECC: (general review) (642), for chiral differentiation of pharmacologically active substances by cyclodextrin-modified MECC using a bile salt (643), for analysis of controlled substances, using different micelles (644), and for analysis of phenethylamines (645); MEKC: for separation of symaptomimetic amines of abuse and related compounds (646); non-aqueous CE: for analysis of drugs (647,648,649,650), for analysis of drugs by nonaqueous CE with electrochemical detection (651), and for analysis of tropane alkaloids and amphetamine derivatives (652); polymeth: characterisation of retention in micellar HPLC, in MEKC and in MECC with reduced flow (653), complementary use of CZE and MECC for mutual confirmation of results in forensic drug analysis (654), and the study of the CZE behavior of selected drugs and its comparison with other analytical techniques for their formulation assay (655); general: CE using polyacrylamide-coated columns (656,657), effect of methanol in sample solution on an electropherogram (658), evaluation of the use of cyclodextrins in chiral separation of basic drug substances by CE (659), improved chiral separation of basic compounds using beta-cyclodextrin and tetraalkylammonium reagents (660), quantitative aspects of the application of CE to the analysis of pharmaceuticals and drug related impurities (661), separation selectivity in chiral and achiral CE with mixed cyclodextrins (662), and use of large-volume sample stacking for selected drugs of forensic significance (663); fluorescence spectroscopy (review) (664); gas chromatography and gas chromatography/mass spectrometry: (general reviews (books)) (665,666), to distinguish amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine from other sympathomimetic amines following derivatization with propyl chloroformate (GC/Cl-MS) (667), to distinguish and quantify the enantiomers of amphetamines, phenol alkylamines, and hydroxamines following stereospecific derivatization (CGC/MS) (668), for enhanced detection of trace-level controlled substances using GC/MS with pulsed splitless injections (669), for the quantitation of cocaine, heroin, diazepam, methaqualone, codeine, and oxycodone (GC) (670), forensic analysis by GC with dual MS and NPD detection (671), by GC with surface ionization detection (672), using isotopic analogues as internal standards (673,674,675), by MS and electrospray ionization MS (676), with a programmable temperature vaporizing injector and cold on-column injector (677) by rapid GC (678), by secondary electrospray IMS/MS (679), by TLC and GC/MS (680), by wide-bore column GC-NPD (681), a dual internal standard method for screening by GLC at the one percent level (682), internal quality control of a general GC drug screen in forensic toxicology (683), use of MSD’s for identification of unknowns (684), normalization of residual ions after removal of the base peak in EI-MS (polydrug study) (685), practical determination of GC–MS limits of detection (686), sample concentrator for sensitivity enhancement in chromatographic analyses (687), SPME-GC (review) (688), and trace analysis by splitless GC/MS (689); high-performance liquid chromatography (and tandem HPLC techniques): general overview (690), (recent progress in HPLC analyses for drugs of abuse) (691), for analyses of barbiturates, LSD, MDA, and psilocybin (HPLC using continuous on-line post-elution photodiode emission with diode-array UV or thermospray-MS detection) (692), to separate and identify cocaine, morphine, heroin, codeine, papaverine, benzocaine, procaine, and lidocaine (HPLC-DAD) (693), for the rapid analysis of illicit heroin and cocaine samples (HPLC-DAD and CGC/NPD) (694), for assaying morphine and hydromorphone in pharmaceuticals (695), for direct chiral resolution of phenylalkylamines (using a crown ether chiral stationary phase (includes amphetamine and cathinone)) (696), for the simultaneous determination of triprolidine, pseudoephedrine, paracetamol, and dextromethorphan (697), for drug screening (698), for analysis of drugs of forensic interest (RP-HPLC) (699), for analysis of alkaloid drugs of forensic interest (RP-HPLC-PDA) (700), for analysis of some alkaloids on unmodified silica gel with aqueous-organic solvent mixtures (701), for determination of alkaloids in foods (multi-detector HPLC) (702), for detection in the forensic sciences (LC-PDA) (703), to determine the enantiomeric composition of abused drugs (704), for determination of illicit drugs and related substances (HPLC with an electrochemical coulometric-array detector) (705), for forensic analyses (on-line HPLC/FAB-MS) (706), for purity testing for tropane alkaloids (707), for resolution of racemic drugs (using a new chiral column based on silica-immobilized cellobiohydrolase) (708), to study the effects of chromatographic conditions on the retention indices of forensically relevant substances (RP-HPLC) (709), and for analysis of pharmaceuticals and drugs (HPLC using unmodified silica and polar solvents) (710); HPLC retention indices: (711,712,713); infrared and Raman spectroscopy: (minor review of IR and Raman for detection of narcotics) (714), (general review of Raman of narcotics and explosives) (715), FTIR and microcrystal tests for rapid identification of drugs (716), FTIR microspectrophotometry of illicit drugs (sample preparation) (717), FT-NIR for validation of controlled substance identifications (718), NIR for identification of
drugs and various adulterants/diluents (719), NIR, FT-Raman, and DR-FTIR for non-destructive identification of Chinese traditional drugs (720), GC-FTIR for screening of hallucinogenic and stimulant amphetamines (721), vapor-phase FTIR for identification of novel illicit amphetamines (722), HPTLC-FTIR for identification of LSD, MBD and atropine (723), use of a diamond anvil cell with a beam condenser and an FTIR microscope for analyses of some particulate drug mixtures (including cocaine, heroin, and methamphetamine) (724), internal reflectance spectra library (725), FT-Raman for nondestructive determination of raw plant medicinal drugs (726), filtered fiber optic Raman probes for analysis of illicit drugs (727), micro-Raman for identification of narcotics (including opioid alkaloids) (728), SERRS for drug analysis (729), and evaluation of silver substrates for SERRS of cocaine and other stimulant drugs (730); ion chromatography: for determination of ionic compounds, excipients, and contaminants in drug evidence (731); microscopy: (general overview) (732), and videomicroscopy (733); nuclear magnetic resonance spectroscopy: (general review) (734), for assessing drug enantiomeric composition (including amphetamine) (735), for chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine, and methcathinone (includes GC analyses) (736), to identify impurities in drug substances (by LC-NMR) (737), and for routine analyses (738,739); phosphorimetry: of barbital, codeine, morphine, and practolol after labelling with dansyl chloride (740); robotics and/or specialized computer programs: (overview and review) (741), for automated CGC heroin analysis (742), combined Rf and UV library search software for TLC and RPTLC (743), a computerized IR search system (744), for optimized analysis of heroin by RP-HPLC (745), to evaluate an HPLC column’s performance (746), and to optimize gradient and isocratic HPLC analyses (747); supercritical fluid chromatography: (general reviews) (748,749), and for extraction of tropane alkaloids (including cocaine) from E. coca extracts (750); thin layer chromatography: (general review) (751), TLC/DAD for forensic analyses (752), overpressured TLC (for determination of morphine, codeine, heroin, opium alkaloids, nicotine, amphetamine, cocaine, and LSD) (753), TLC for separation of cocaine, pramocaine, fentanyl, and diphenhydramine (754), and TLC with a special visualization reagent for tertiary amines (including dimethylamphetamine, flunitrazepam, methamphetamine, methaqualone, nicotine, theophylline, triazolam, and others) (755); and miscellaneous: Comparison of IR and MS for drug analyses (756).

**Analytical Artifacts**

GC and GC/MS are the current methods of choice for routine screening, identification and quantitation of controlled substances. However, the use of high-temperature injectors can produce artifacts via unimolecular rearrangements of and/or intermolecular reactions between the various components (including even the injection solvent). Artifacts are also possible in other techniques. Over the past 10 years, artifacts have been reported for: cannabinoids: nitrites in cannabinoid analyses (urine testing focus) (757,758,759); cocaine: determination of ecdyonidine methyl ester vapor pressure (760), identification of methyl esters of ecgonine as injection port produced artifacts from cocaine base (crack) exhibits (761); heroin: identification of a heroin/chloroform-impurity reaction product (762); morphine and codeine: hydromorphine and hydrocodone interference in GC/MS assays for morphine and codeine (763); phenethylamines: artifacts in the GC analysis of amphetamine and MDA (764), GC/MS identification of amine-solvent condensation products formed during analysis of drugs of abuse (from ethanol with amphetamine, MDA, and beta-phenethylamine) (765,766), conversion of ephedrine to methamphetamine and methamphetamine-like compounds during and prior to GC/MS analyses of heptafluorobutyrate and carbethoxhexafluorobutylate derivatives (urine testing focus) (767) identification of a GC/MS artifact peak as methamphetamine (768), matrix effects in the IR of methamphetamine salts (769,770), and a procedure for eliminating interferences from ephedrine and related compounds in the GC/MS analysis of amphetamine and methamphetamine (771); piperonal: an artifact in the GC analysis of piperonal (772); and miscellaneous: influence of large amounts of drugs on the peak areas of their coinjected deuterated analogues measured with APCI-LC-MS (773), and a simple software procedure to determine if a GC/MS blank injection is contaminated (774).
**Qualitative Tests**

Spot tests are a mainstay of forensic analysis of controlled substances, and offer a reliable means for very rapid screening of submitted exhibits. Over the past 10 years, the following qualitative testing studies were reported: (general overview) (775), (textbook) (776), (review of color comparisons in forensic science, including drug color tests) (777), for anhydrous ammonia (778), for cocaine (mechanistic study of the Scott Ruybal test) (779), for drugs of abuse (12 spot tests) (780), for lithium (781), for pemoline, fenozolone, and thozalinone (color tests) (782), and for red phosphorus (783,784,785).

**Sampling Plans**

Large drug seizures are almost invariably comprised of multiple units of a standard container size (for example, several thousand 1 kilogram packages of cocaine). Comprehensive analysis of such seizures is a daunting and prodigiously labor intensive task; therefore, statistically based sampling plans are utilized that enable valid assessment of an entire shipment based on analyses of a select number of representative, randomly selected exhibits. The classic study in this field (by Frank, Hinkley, and Hoffman) was reported in 1991, but is included here as critical background (786). Over the past 10 years, the following additional studies were reported: (overviews and general discussions) (787,788,789,790,791), a case studies of heroin (792,793,794).

**Source Determination/Impurity Profiling**

Determination of synthetic route origin (including processing variants) and/or geographical origin is important for developing tactical and strategic intelligence. Historically, source determination has been conducted by in-depth impurity profiling; that is, determining discriminatory marker compounds and/or ratios of marker compounds which are characteristic of origin. More recently, trace element analyses and (especially) stable isotope analyses (vide infra) have increased the confidence of geographical sourcing; this aspect of source determination is rapidly expanding. Finally, increasingly sophisticated pattern recognition techniques (often neural network based) have been employed to handle the enormous databases generated by source determination programs. A large number of source determination studies have been reported over the past 10 years: (general discussions) (795,796), (pattern recognition techniques screening for drugs of abuse (illicit amphetamines) with GC-FTIR) (797); **amphetamines**: systematic approach to profiling amphetamines (798), automated GC method for amphetamine profiling (799), amphetamine profiling in the UK (800), from arylpropenes with acetonitrile and sulfuric acid (Ritter reaction) (801), of Leuckardt amphetamine (802,803,804,805), from 1-phenyl-2-nitropropene (806,807), improved data processing for amphetamine profiling (808), from phenylacetone synthesized from phenylacetic acid (Leukardt reaction) (809), and a pan-European method for profiling amphetamines (810); **amphetamines and marijuana**: of impurities (811); **cocaine**: reviews (812,813,814), comprehensive profiling (815,816,817,818), of 2-carbomethoxy-3-alkyloxy- and heteroaryloxy substituted tropaines in cocaine (819), of 2-carbomethoxy-3-oxo analogs in cocaine (820,821), of chlorinated cocaines from cocaine treated with bleach (822, see also:823), of cuscohygrine in cocaine (824), of heteroaryl analogs in cocaine (825), of 1-hydroxy- tropacocaine in cocaine (826), of hygrine in cocaine (827), of hygrine and cuscohygrine in cocaine (828), of norcocaine in cocaine (829), of occluded solvents in cocaine (effects of microwave radiation on solvent profiles) (830), of pharmaceutical cocaine (831), of pseudococaine in cocaine (832), of trace metals in cocaine (833), of trimethoxy analogs of cocaine, cinnamoylcocaine, and tropococaine in cocaine (834), of truxillines in cocaine (835), of truxillines and similar high molecular weight impurities in cocaine (836), of illicit cocaine by X-ray Diffraclometry (also GC and GC/MSD) (837); **cocaine and heroin**: (combined studies) of trace metals in cocaine and heroin (838,839,840,841,842,843,844,845,846), and by palynology (pollen analysis) (847); of occluded solvents in cocaine and heroin (848); **ephedrine**: by microscopic examination (849); **fentanyl**: prepared from 1-phenethyl-4-piperidone (850); **heroin**: overview (851), (review) (852), of acid and neutral impurities in heroin (853), of anions and cations in heroin (854,855), of basic byproducts and adulterants in heroin (856), of impurities in heroin (857,858,859,860,861), of metal contamination in heroin (862), of O6-monoacetylmorphine in “homebake” heroin (863), of trace elements in heroin by ICP-MS (864,865), of trace organic impurities (866);
marijuana: of cannabidiol and delta-9-THC in stored marijuana (867), of impurities in hashish (868,869), of impurities in marijuana (870,871,872), of natural constituents in marijuana (reviews) (873,874), and of marijuana DNA (875,876,877,878,879,880,881,882,883); methamphetamine: review (UNDCP) (884), overview (885), generic articles on impurity profiling (886,887), of N-acetylmethamphetamine in illicit methamphetamine (888), of chloroephedrine and aziridines in methamphetamine (889), of impurities in methamphetamine (890,891), of inorganic impurities in methamphetamine (892), of methamphetamine synthesized from allylbenzene (893,894,895), of impurities in methamphetamine synthesized via HI/red P (896), of methamphetamine synthesized via HI/red P (focusing on reaction byproducts of common cold tablet ingredients) (897,898), of methamphetamine containing a hydrocarbon wax (899), of methamphetamine synthesized from pseudoephedrine tablets (900), of trace elements in methamphetamine (901), of methamphetamine seized in Australia (overview and development of a national database) (902), of methamphetamine seized in Japan (903,904, and overview: 905), and of methamphetamine seized in Korea (906); 4-methoxyamphetamine: of impurities (907); methylenedioxyamphetamine: overview of approach in Australia (908), of impurities in methylenedioxyamphetamine (909,910), of precursors, intermediates, and reaction byproducts for methylenedioxyamphetamine (911), of impurities in methylenedioxyamphetamine and amphetamine (912), of impurities in methylenedioxyamphetamine and methylenedioxyamphetamine (913,914), determination of synthetic route markers for methylenedioxyamphetamine and methylenedioxyamphetamine (915), of methylenedioxyamphetamine tablets by logo and headspace comparisons (916), of commercially available methylenedioxyphenylacetone (917), from the Ritter reaction (using safrole) (918), of methylenedioxyphenylacetone and methylenedioxyamphetamine synthesized from isosafrole (919,920), and of methylenedioxyamphetamine synthesized from nitoethane and piperonal (921); nicotine: (overview of tobacco smoke) (922); opium: of opium alkaloids (for origin determination) (923), of proteins in opium latex (924); pharmaceuticals: overview (925), of impurities (926,927,928); phenyl-2-propanone: of illicit phenylacetone synthesized from phenylacetic acid with acetic anhydride versus lead (II) acetate (929); precursors: of essential oils used as precursors in the synthesis of phenethylamine-type designer drugs (930), and testosterone undecanoate: of impurities (931).

Source Determination/Stable Isotope Analyses

Historically, processing origin could be reasonably correlated with geographical origin. However, the expansion of drug producing regions and the concomitant convergence of processing techniques, along with the international exchange or sale of precursors (for example, 3,4-methylenedioxyphenylacetone) or crudely refined controlled substances (for example, morphine or heroin base) across the world, have mandated more sophisticated analyses. Because the natural abundances of the stable isotopes of hydrogen, carbon, nitrogen, and oxygen vary across the world, and their incorporation into natural products is unaffected by subsequent illicit processing, stable isotope analyses offer a powerful tool for determining “true” geographic origin (that is, not indirectly inferred based on processing methodology). Recent advances in instrumentation (notably isotopic ratio mass spectrometry and high field nuclear magnetic resonance spectroscopy) have enabled the determination of the isotopic makeup of controlled substances with reasonable precision and accuracy. To date, only cocaine and heroin have been subjected to comprehensive studies; however, this field is expected to expand to other controlled substances over the next decade. Recent reports include: (overviews) (932,933,934); cocaine: by carbon-13 isotope analysis: (935,936), by IRMS and trace alkaloid analysis (937); cocaine and heroin: by IRMS (938), by site specific deuterium-NMR (939); and heroin: by GC/IRMS (940), and by GC/MS and GC/IRMS (941).

Comparative Analyses

Establishing commonality of origin between 2 or more exhibits requires systematic application of detailed impurity profiling. Comparative analysis does not require formal determination of synthetic, processing, or geographical origin, but rather determination of “degree of match” between profiles (usually trace-level chromatographic analyses; however, establishment of synthetic, processing, or geographical origin is a common spinoff of comparative analysis protocols). Studies reported over the past 10 years include: (general overviews)
amphetamine: computerized comparisons of Leuckart amphetamine (944); cocaine: (overview of methodologies) (945), database for comparison (946), by CGC/ECD (947), by CGC/NPD (948), comparison of crack cocaine by matching fracture lines between pieces (949), cocaine comparison court case (950), by rapid GC (951), by HPLC-DAD (952), by a neural network (953); hashish: by HPLC, GC, and AA (954); heroin: (general overview) (955), by CGC (956), computerized comparison (957), predictive model (958), harmonization study for retrospective comparisons (959,960), of SWA heroin by GC (961); marijuana: comparison by RAPD and HPLC (962); methaqualone: tablets by NIR reflectance spectra (963); methylenedioxymethamphetamine: by natural isotope abundances (964); opium: by RAPD, HPLC, and ELISA (965), pharmaceuticals: evaluation of neural networks (966); and tablets and capsules: indices of physical characteristics (967,968).

Reference Standards

Accurate analyses of controlled substances and related analogs require high purity standards, including isotopically labelled analogs. Structurally related compounds are also needed as internal standards for chromatographic analyses. Reports over the past 10 years include: (general reviews) (969,970); bufotenine (and related tryptamines): (971), butalbital: (972); cannabinoids: (973,974); cocaine: (975,976), aza analogs of cocaine (977), deuterium-labelled cocaine, cocaethylene and metabolites (978), cocaine by one step esterification of benzoylcegonine (979), 6- and 7-hydroxylated cocaines (980), C-3 alkyl analogs of cocaine (981); lysergic acid diethylamide (982,983); d- and l-methamphetamine: via optical resolution (984), “Ice” methamphetamine (985); methcathinone: (986); methohexital: (987); morphine: (988,989); polydrug: (N-ethylmethyleneaminoamphetamine, N-hydroxymethylenedioxymethamphetamine, mecloqualone, 4-methylaminorex, phendimetrazine, and phenmetrazine) (990), (O6-monoacetylmorphine, methamphetamine, methylenedioxymethamphetamine, methylenedioxyethylamphetamine, and N-methyl-3,4-methylenedioxyphenyl-2-butamine, from seized drugs) (991), and a reference garden of hallucinogenic and narcotic plants in Australia (992); (1S,2S)-pseudoephedrine: (993); and psilocybin and O-acetyl psilocybin (994).

Clandestine Laboratories

The illicit production of drugs is a dynamic and constantly changing field. Reports over the past 10 years included: (general review) (995), amphetamine (996,997,998), failed synthesis of amphetamine (999), amphetamine in methamphetamine (1000), analyses of inorganic components found in clandestine drug laboratory evidence (1001), arsenic oxide (potential reagent in methamphetamine synthesis) (1002), Birch reduction (general review) (1003) (overview of developments in the midwestern US (1004), cocaine (1005,1006), concealment and trafficking (1007), 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) (1008), ephedra (1009,1010), ephedrine and/or pseudoephedrine (1011,1012), etonitazene (1013), fentanyl (1014), freons in methamphetamine production (1015,1016,1017), hash oil (1018,1019), heroin (acetylated opium) (1020), heroin (review) (1021), hydriodic acid for methamphetamine production (1022,1023,1024), hypophosphorus acid for methamphetamine production (1025), inorganic acids (1026,1027), iodine (GC/MS identification) (1028,1029), lysergic acid amide from morning glory seeds (1030), lysergic acid diethylamide (1031), marijuana (1032,1033), methadone (1034), methamphetamine (1035,1036,1037,1038,1039,1040,1041), methamphetamine (by dissolving metal reduction) (1042,1043,1044), methamphetamine in Taiwan (1045), methaqualone and analogs (1046), methcathinone (1047), methylenedioxymethamphetamine (1048), methylenedioxyphenethylamines (1049), morphine (by dealkylation of codeine) (1050), overview of illicit drug production in the Czech Republic from the 70's through the 90's (1051), phencyclidines (1052,1053,1054), phenylacetone and methylenedioxyphenylacetone (1055,1056), piperonal (1057), polydrug (methamphetamine, phenylacetone, methylenedioxyamphetamine, and methaqualone) (1058), steroids (1059), substitution of white phosphorus for red phosphorus in hydriodic acid reduction laboratories in Idaho (1060), delta-9-THC precursors (1061), delta-9-THC acetate (1062), unusual defense to charge of MDMA manufacture (1063), and an unusual designer drug laboratory (polydrug) (1064).
Clandestine Laboratory Appraisals and Safety

The rapid expansion of clandestine laboratories in the US over the past 15 years has resulted in a large number of studies concerning proper assessment and safe dismantling, including reports on: assessment and remediation of contaminated sites (1065), clandestine laboratory production capabilities (1066), confined space laboratories (1067,1068,1069,1070), decontamination of biohazardous evidence (1071), determination of occupational exposure to cocaine by crime lab personnel (1072), determination of volumes in clandestine laboratory reaction vessels (1073), environmental impact and adverse health effects of the clandestine manufacture of methamphetamine (1074), field methods to render safe pressurized tanks of ammonia at clandestine labs (1075,1076), hydrogen sulfide fatality (1077), OSHA and NIOSH regulations (1078,1079), phosphine gas exposure from a methamphetamine laboratory investigation (1080), phosphine gas fatalities (1081,1082), phosphine gas detection and monitoring instrumentation (1083), safety training for clandestine laboratory investigators (1084,1085), supplier of 22-liter flasks put on notice (1086), training (1087,1088), triacetetonitrileperoxide causes explosion during analysis (1089), and useful websites for personnel involved in forensic laboratories and/or clandestine laboratories (1090).

Portable Instrumentation/Trace Detection

New world trade agreements and the easing of formerly restrictive national and international borders have resulted in dramatic increases in cargo transshipping and personal travel, thereby complicating drug inspection and interdiction efforts at POE’s. The need for rapid and accurate screening, and high sample throughput, requires on-site equipment capable of assessing humans, animals, and a vast array of shipping containers. In addition, on-site equipment is needed for proper assessment of clandestine laboratories. However, the typical size and operational requirements of most laboratory instrumentation preclude their use in field settings. This has resulted in a growing industry dedicated to development of man-portable, field rugged equipment for detection and identification of controlled substances. Many of the pertinent studies are proprietary, but a large number have nonetheless been reported over the past 10 years: general: (reviews) (1091,1092,1093,1094,1095), (general assessments) (1096,1097,1098,1099), appraisal of drug detection scenarios - operational analysis for drug detection (1100), and determination of high-risk cargo (1101); amperometric assay: for opiates (1102); biosensor technologies for the detection of illegal drugs (1103,1104,1105,1106,1107), antibody-based field kits for cocaine and heroin (1108), a fiber-optic cocaine biosensor (1109), an ISFET device for cocaine analysis (1110), the use of heroin esterase in the development of a biosensor (1111), and use of recombinant DNA in the design of a heroin sensor (1112); calibration standards: for narcotics detection devices (1113); correlated column micro-GC: for the detection of contraband drugs in cargo containers (1114); field ion spectrometry: (1115); gamma ray detectors: (1116,1117,1118); gas sensor arrays for drug “aroma” detection (1119); immunoassay based detection systems: (1120,1121) and similar technologies (1122); DRUGWIPE: (1123); ion mobility spectrometers: (1124,1125,1126,1127,1128), detection of cocaine and heroin by a custom built IMS (1129), detection of drugs of abuse in Customs scenarios using IMS (1130), use of fluorescence spotting to identify areas for IMS (1131), detection of methamphetamine and ephedrine in abandoned clandestine laboratories with IMS (1132), differentiation of methamphetamine versus nicotine using IMS (1133), DSP techniques for narcotic detection using IMS (1134), field applications of IMS (1135,1136), and use of SPME with IMS (1137); ion trap mobility spectrometers: (1138,1139,1140); laser-based near- and mid-IR: (1141); neutron-based technologies: (1142,1143,1144,1145,1146,1147,1148,1149,1150,1151), combined neutron and gamma ray detection (1152), evaluation of neutron techniques for illicit substance detection (1153,1154,1155), and pulsed fast neutron analysis (1156,1157); N-14 nuclear quadrupole resonance: (1158,1159,1160,1161); particle detection: cocaine phenomenology study (1162), confidence in the detection of cocaine particulates by IONSCAN and SENTOR systems (1163), particle generators for testing of particle detection equipment (1164), particle size distribution of cocaine HCI (1165), particle size analysis of six illicit heroin preparations seized in the UK (1166), use of methylene blue as a simulant for cocaine HCI and heroin HCI (1167), test material for narcotics detection equipment (1168), and voltametric determination of cocaine microparticles (1169); piezoelectric ringing: (1170); portable GC/MS: for clandestine laboratory investigations (1171,1172); Raman spectroscopy: (1173,1174,1175), minor review of applications (1176), near-IR Raman to identify illegal drugs in
solid mixtures (1177), and Raman microscopy for direct 2-D imaging of explosives and drugs (1178); human screening: of internal body packers by magnetic resonance (1179), of internal body packers by X-ray scanning (1180), of packages on persons by X-ray imaging (1181,1182), of prisoners in the Canadian Correctional Service by IMS and ion trap mobility spectroscopy (1183), and a survey of current portal technology for screening people for illicit substances (1184); SENTOR: recent developments (1185); solid-state gas sensors: (1186); surface ionization detection: (1187); surface acoustic wave (SAW) detectors: detection of taggants and volatiles by SAW/GC (1188), and portable detection system for illicit materials based on SAW resonators (1189); surface sampling: detection of drugs on vehicle surfaces (general study) (1190), and study of surface sampling procedures for improved sampling/detection protocols (1191); tandem mass spectrometry (CONDOR): (1192,1193); testbeds: chemical vapor test-beds (1194), and a nonintrusive inspection technology testbed (1195); TOF-MALDI mass spectrometry: (1196); vapor detection: analysis of volatiles from cocaine (1197,1198,1199), analysis of vapors from cocaine and heroin with the aid of SPME (1200), cocaine and heroin vapor pressures (1201), detection of cocaine in cargo containers by high volume vapor sampling (field test) (1202), and formation of methyl benzoate from cocaine HCl under different temperatures and relative humidities (1203); and X-ray technologies (1204,1205).

Surveys

Critical to total threat assessments and the monitoring the effectiveness of counter-narcotics efforts are surveys of drug use and related topics. Over the past 10 years, surveys have been reported for: amphetamine: of global amphetamine abuse (1206,1207), and of amphetamine type drugs used in Bulgaria (1208); cocaine: of adulterants in cocaine in Rome in 1996 and 1997 by GC and GC/MS (1209), of cocaine seized in Spain 1985-1993 (1210), of intralaboratory precision of cocaine analysis by CGC (1211), and of occluded solvents in cocaine 1986 - 1991 (1212); designer drugs: (review) (1213), of amphetamine-type designer drugs in Europe (1990-1996) (1214), of designer drugs in Canada (1215), of designer drugs in the European Union (1216,1217), and of designer drugs in Italy (1218); drug use (general): global trends - 2000 (1219), global trends - 1999 (1220), of drug abuse in Hungary (1221), of Irish drug seizures (1222), of drug usage in San Diego County 1990-1997 (1223), of drug contents of powders and other illicit preparations in the UK (1224), of drugs imported into the UK (1225), and of drug abuse in Western Denmark during the eighties (1226); flunitrazepam: of Rohypnol Tablets (1227); heroin: of heroin in Australia (in Sydney in 1997) (1228), of heroin in Denmark, 1981-1992 (1229), of heroin seized in France (1230), of heroin in Israel during 1992 (1231), of noscapine in heroin in Slovenia, 1997 - 1999 (1232), of heroin seized in Spain (1233), of heroin in Andaluza, Spain (1234), of heroin in the UK, 1984 to 1989 (1235), of retail level heroin purchases in the US during 1992 (1236), of the cutting of heroin in the US in the 1990's (1237), and of cutting of heroin in New York City (1238); LSD: of LSD blotter papers logos (1239); marijuana: of the THC content of cannabis cultivated in Austria (1240), of recent developments in Europe concerning licit cultivation of cannabis (1241), of the cannabinoid content of marijuana seized in Greece (1242), of delta-(9)-THC content in cannabis of Greek origin (1243), the potency of cannabis in New Zealand, 1976 to 1996 (1244), of cannabis resin and cannabis seized in the Republic of Ireland (1245), of trends in illicit cannabis cultivation in the UK and Northern Ireland (1246), of potency trends of Δ9-THC and other cannabinoids in confiscated marijuana from 1980-1997 in the US (1247), of the global situation of cannabis consumption, trafficking, and production (1248), and of recent developments in cultivation and quality of illicit cannabis (worldwide) (1249); methylenedioxymphetamines: of MDMA, MDA, MDEA, NEXUS, and MBDB tablets seen in southwestern Spain (1250,1251), and of MDMA, MDEA, and MBDB tablets seen in the United States (1252,1253,1254,1255); polydrug: of heroin, cocaine, and cannabis from British Columbia (1256), and of heroin and cocaine seized in a Swiss town (1257); UNDCP Reports (by year): World Drug Report - 2000 (1258), List of Narcotic Drugs under International Control (INCB “Yellow List”) - 1999 (1259), (INCB “Green List”) - 1999 (1260), (INCB “Red List”) - 1999 (1261), Report of the International Narcotics Control Board - 1999 (1262), Manufacture of Narcotic Drugs, Psychotropic Substances, and their Precursors - 1999 (1263), Narcotic Drugs Estimated World Requirements - 1999 (1264), Precursors and Chemicals Frequently Used in the Illicit Manufacture of Narcotic Drugs and Psychotropic Substances - 1999 (1265), Psychotropic Substances - Statistics - 1999 (1266), Terminology and Information on Drugs - 1999 (1267), the World Drug Report - 1997 (1268), and Supply of and Trafficking in Narcotic Drugs and Psychotropic Substances - 1996 (1269);
miscellaneous: of adolescents’ use of embalming fluid with marijuana and tobacco (1270), and of crime laboratory proficiency testing results 1978-1991 (1271).

Miscellaneous Topics

The following topics of peripheral interest to the analysis and detection of drugs of forensic interest were also reported from 1992 - 2001: angel trumpet: overview (1272); amphetamine: a review of U.S. statutes on methamphetamine and how they led to an increase in illicit amphetamine production (1273); ayahuasca: notes on an Ayahuasca court case in Holland (1274,1275); barbiturates: charge transfer complexes with phenytoin (1276); canines: use of activated charcoal to circumvent canine detection of concealed narcotics (1277), analysis of volatile drug components and their relevance to canine alerts (1278), characterization of the Auburn Olfactometer (1279), of cocaine on currency (1280), drug money and detection by canines (1281,1282), scientific protocol to evaluate and certify odor detection by canines (1283), and sensitivity of canines to cocaine HCl and methylbenzoate (1284); (traditional) Chinese medications: overview (1285), manufacturing flaws and misuse of Chinese herbal medicines (1286), determination of some active components in Chinese medicinal preparations by CE (1287), screening of Chinese proprietary medications for undeclared therapeutic substances by HPLC and GC/MS (1288), identification of Western medicines as adulterants in Chinese herbal medicines by HPLC and GC/MS (includes diazepam) (1289), screening for chemical drugs used to adulterate in rheumatic and analgesic traditional Chinese medicine by HPLC-DAD (includes diazepam and phenylbutazone) (1290), determination of adulterated chemical drugs in rheumatic and analgesic traditional Chinese medicine by MEKC (includes diazepam and phenylbutazone) (1291), determination of clobenzorex HCl and diazepam adulterated in anorexiant traditional Chinese medicines by MECC (1292), and determination of fluoxymesterone, methyltestosterone and testosterone in adulterated Chinese herbal preparations by HPLC (1293); cocaine: alkaloid content in Erythroxylum Coca tissue during reproductive development (1294), the base-catalyzed C-2 exchange and epimerization of 3-beta substituted 8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylates (1295), biomass accumulation and alkaloid content in leaves of Erythroxylum Coca and Erythroxylum Novogranatense Var Novogranatense grown in soil with varying pH (1296), effects of cyanoacrylate processing (for fingerprinting) on cocaine HCl trace analysis (1297), gas phase detection of cocaine by means of immunoanalysis (1298), protest against cocaine base sentencing (1299), SFC extraction of cocaine from coca leaf (1300), solubility of cocaine in gasoline (1301), and stability of cocaine in Agua Rica/Agua Madre (1302); (analysis for) controlled substances on currency: comprehensive review (covers cocaine, heroin, THC, and phentanylamines) (1303), cocaine on currency (1304,1305), cocaine on currency by GC-MS (1306), cocaine on currency by IONSCAN IMS and LC/MS with electrospray ionization or by GC-ITD/MS (1307), analysis of drugs on currency by GC/MS (1308), by MS/MS, TD-MS, and APCI-MS (1309), by tandem MS (CONDOR) (1310,1311), and screening of currency by TD/APCI-MS (1312); designer drugs (unusual): dihydrobenzofuran analogues of hallucinogens (1313), lactam analogs of fentanyl (1314), methylenedioxyisoquinolines (1315), synthesis and pharmacological evaluation of ring-methylated 3,4-methylenedioxyamphetamine (1316), reference directory of designer drugs (1317), 1,2,3,4-tetrahydroisoquinoline analogs of phenylalkylamine stimulants and hallucinogens (1318,1319), and the texts by Ann and Alexander Shulgin (1320,1321); dextromethorphan: (overview) (1322); dimethylamphethamine: mechanistic study of preparation from methylephedrine (1323); ephedrine: extraction of ephedrine from ephedra by SFC (1324); heroin: homogenization of illicit heroin samples prior to analysis (1325); inhalants: general discussions of inhalants and solvent abuse (1326,1327,1328); Internet: discussion of internet resources for forensic science (1329,1330); lysergic acid diethylamide: detection of LSD on blotter papers after processing for fingerprints (1331), and stability of LSD under various storage conditions (1332); marijuana: botanical considerations for forensic investigation of marijuana (1333), embalming fluid-soaked marijuana (compare to Elwood/Fry article) (1334), filtering effects of various household fabrics on the pollen content of hash oil (1335), identification and quantitation of 11-nor-delta(9)-tetrahydrocannabivarin-9-carboxylic acid (1336), manufacture of Cannabis Sativa for legitimate applications (1337), mineral nutrition of Cannabis Sativa L. (1338), and comments on the naming of the Duquenois and related tests for cannabis (1339); mass spectrometry: archive of mass spectral data files on CD-ROM and a computerized database (1340), ion ratio instability of a GC/MS system (1341), and poor reproducibility of in-source collisional AP MS of drugs (1342); methadone: claim that DEA chemists erred in calculating quantity of methadone that could be synthesized from precursor chemicals (1343; and response: 1344); nightshade alkaloids: historical review (1345); opium: historical review (1346), biodiversity of Papaver...
**Somniferum** L. (1347), and determination of loss on drying of opium samples using microwave ovens (1348); oxycodone: overview (1349); phencyclidine: ionic associates of phencyclidine with sulfophthaleins and azo dyes (1350); polydrug: hypnotics and sedatives not belonging to the classes of barbiturates and benzodiazepines (1351); poppy tea: case study/overview (1352); thebaine: synthesis from codeine methyl ether (1353); other topics: analysis of clandestine drug records (1354), analysis of drugs in unconventional samples (1355), analysis of false positives in drug proficiency testing (1356), analysis of a fruit juice extract that was suspected to be a narcotic beverage by GC/MS (1357), analysis of plastic packaging to trace the source of illicit drugs (1358), computerized management of a forensic analytical laboratory (1359,1360), considerations for planning and site preparation for modern laboratory instrumentation (1361,1362), development of a forensic evidence protection kit (1363), drug smuggling techniques and problems associated with analysis (1364), drug smuggling by internal body carries (1365), environmental impact of illicit narcotics cultivation and processing (1366,1367), expert evidence and forensic misconceptions of the nature of exact science (1368), GC/MS guide to ignitable liquids (1369), modification of an extraction procedure for acidic and neutral drugs (1370), neural networks in forensic science (overview/general discussion) (1371), protecting group chemistry (1372), separation and identification of drugs of abuse in drug cottons by HPLC coupled with electrochemical array detectors (1373), solid phase extraction for systematic toxicological analysis (1374), the UNDCP Dictionary of Narcotics (1375, and addendum: 1376); and an overview of the United Nations International Narcotics Control Board (1377).

**References**

15. Anonymous. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG). Microgram 2001;34(6):136. (Note: Most current iteration within review timeframe; updates many previous reports not listed here.)


32. Sadeghipour F, Varesio E, Gilroud C, Rivier L, Veuthey JL. Analysis of amphetamine by capillary electrophoresis and liquid chromatography: Application to drug seizures and


50. Falco PC, Legua CM, Cabeza AS, Serrano RP. Derivatization of amphetamine and methamphetamine with 1,2-naphthoquinone-4-sulfonic acid into solid-phase extraction cartridges. Determination of amphetamine in pharmaceutical and urine samples. Analyst 1997;122(7):673.


55. Lurie IS, Odeneal II NG, McKibben TD, Casale JF. Effects of various anionic chiral selectors on the capillary electrophoresis separation of chiral phenethylamines and achiral neutral impurities present in illicit methamphetamine. Electrophoresis 1998;19:2918.


65. Hensley D, Cody JT. Simultaneous determination of amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) enantiomers by GC-MS. J Anal Toxicol 1999;23(6):518.


430. Barnett NW, Hindson BJ, Lewis SW, Jones P, Worsfold PJ. Soluble manganese(IV); A new chemiluminescence reagent. Analyst 2001;126(10):1636. [Note: Includes morphine and codeine.]


455. Ting S. Liquid chromatographic determination of scopolamine, hyoscyamine and phenobarbital in tablets. JAOAC Int 1997;80(2):331.


477. Maume D, LeBizec B, Marchand P, Montrade MP, Andre F. N-methyl-N-alkylsilyl-


504. Hays PA, Cooper DA. Determination of the weight percent of acetic acid in acetic anhydride by 1H-nuclear magnetic resonance (NMR) spectroscopy. Microgram 2000;33(8):160.


533. Hanna GM. Determination of ephedrine, pseudoephedrine, and norephedrine in mixtures (bulk and dosage forms) by proton nuclear magnetic resonance spectroscopy. JAOAC Int 1995;78(4):946.


697. Deorsi D, Gagliardi L, Bolasco A, Tonelli D. Simultaneous determination of triprolidine, pseudoephedrine, paracetamol, and dextromethorphan by HPLC. Chromatographia 1996;43(9-10):496.


732. McCrone WC. Chemical problem solving without FTIR, EDX, NMR, XRD, etc., or Why I still use the polarized light microscope, PLM. Microscope 2000;48(3):155.


Bogusz MJ. Large amounts of drugs may considerably influence the peak areas of their coinjected deuterated analogues measured with APCI-LC-MS. J Anal Toxicol 1997;21 246.


Thornton JI. Visual color comparisons in forensic science. Forensic Sci Rev 1997;9(1):37. (Note: This is an extensive review, including drug color tests.)


852. Esseiva P, Guéniat O. The concept of drug intelligence in heroin investigation: The problem of the evaluation of “profiling evidence.” The proposal of a sequence of analysis and a scheme of interpretation for court purposes. Presentation - 1st European Meeting of Forensic Science; Lausanne, Switzerland; 1997.


867. Ross SA, El Sohly MA. CBN and delta-9-THC concentration ratio as an indicator for the age of stored marijuana samples. Bull Narc 1997/1998;(49(1,2)/50(1,2)):139.


914. Bohn M, Bohn G. Weakly basic impurities in illegally manufactured 3,4-methylendioxyamphetamine and 3,4-methylendioxyxymethylamphetamine. Proceedings of the International Association of Forensic Sciences 13th Triennial Meeting 1993;5:211.


916. Vu D-TV. Logo and headspace comparison for source determination of ecstasy seizures. Microgram 2001;34(9):244.


933. Ihle E, Schmidt HL. Multi element and on line stable isotope analysis in illicit drug characterisation. 1st European Meeting of Forensic Science, Lausanne, Switzerland, 1997.


1085. Boyd V. Dealing with heat stress; basic precautions can prevent workers in hot environments from becoming victims of serious heat-related illnesses. J Clan Lab Invest Chem Assoc


1175. Lacey RJ. Some advances in the use of Raman spectroscopy in security screening applications. IEE Conf Publ 1997;437:10.


1190. Wilson R, Brittain AH. Study to investigate the trace levels of contamination on surfaces when narcotic contraband is concealed in a vehicle. Proc SPIE - Int Soc Opt Eng 1997;2932:27.


1257. Guéniat O, Esseiva P, Ribaux O. The systematical profiling of heroin and cocaine seizures in a Swiss town: The elicitation, interpretation of links and the development of a computerized system to improve the investigation. Presentation - 1st European Meeting of Forensic Science; Lausanne, Switzerland; 1997.


1339. Mausolf N. The name of the test. Microgram 2001;34(9):235.


1343. Zedeck M. Drug Enforcement Administration (DEA) chemists erred in calculating quantity of methadone that could be synthesized from precursor chemicals. J Forensic Sci 1997;42(2):349.


1357. Ripani L, Lovera P, Muzi F, Schiavone S. GC/MS Analysis of a fruit juice extract that was suspected to be a narcotic beverage. Microgram 1994;27:149.


Microgram

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC 20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 1
Numbers 3-4
July - December 2003

Posted On-Line At:
Contents

Letter to the Editor regarding “Instrumental Separation of 3,4-Methylenedioxyamphetamine (MDA) from 1-(3,4-Methylenedioxyphenyl)-2-propanol, a Co-Eluting Compound”. 157

The Identification of 5-Methoxy-alpha-methyltryptamine (5-MeO-AMT) 158
Michelle M. Zimmerman

The Identification of N,N-Dimethylamphetamine (DMA) in an Exhibit in Malaysia 163
Kee Bian Chan, Yong Kiong Chong, and Mohamed Nazarudin

Profiling of Ecstasy Tablets Seized in Japan 169
Yukiko Makino, Shingo Kurobane, Kazuyoshi Miyasaka, and Kenichi Nagano

A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions 177
Mohammad Sarwar and John L. McDonald

A Rapid and Simple GC/MS Screening Method for 4-Methoxyphenol in Illicitly Prepared 4-Methoxyamphetamine (PMA) 184
Dieter Waumans, Noël Bruneel, Bas Hermans, and Jan Tytgat

Letrozole (Femara®) 190
Lois C. Geer and Patrick A. Hays

An Overview of DNA Methods for the Identification and Individualization of Marijuana 196
Heather Miller Coyle, Timothy Palmbach, Nicholas Juliano, Carll Ladd, and Henry C. Lee

Report of the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Conference (Montreal) 208
Joseph P. Bono

Note: In order to prevent automated theft of email addresses off the Internet postings of Microgram Journal, unless otherwise requested by the corresponding author, all email addresses reported in the Journal have had the “@” character replaced by “-at-”; this will need to be converted back (by hand) before the address can be used.

Cover Art: “Ball and Stick” Model of Cocaine (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA)
Sir:

Some clarifications on the article by authors Vohlken and Layton. The peak annotated as an unknown “A4” is almost certainly di-[1-(3,4 methylenedioxyphenyl -2- propyl)]amine, the dimer of MDA. There is an article in the April, 1985 issue of the Journal of Forensic Sciences about a similar dimer being produced during the synthesis of amphetamine. The behavior of this compound is unusual in that the GC/MS suggests that you have a low molecular weight compound at a long retention time, when in reality you are looking at only half of the molecule. The actual molecular weight is 340 or 341. This compound was synthesized at this laboratory about 15 years ago. Also of note, similar tablets (white, Rolex logo) are shown on the website dancesafe.org and described as containing MDA/MDMA and another, unidentified substance. We had a submission of similar tablets (400) in September, 2001 from the Detroit, Michigan area.

Peter Ausili
DEA North Central Laboratory, Chicago, Illinois
Technical Note

The Identification of 5-Methoxy-\textit{alpha}-methyltryptamine (5-MeO-AMT)

Michelle M. Zimmerman
Wisconsin State Crime Laboratory – Wausau
7100 Stewart Avenue
Wausau, WI 54401
[email: zimmermanmm -at- doj.state.wi.us]

ABSTRACT: The analysis of 5-methoxy-\textit{alpha}-methyltryptamine (5-MeO-AMT) via color testing and gas chromatography/mass spectrometry is presented and discussed.

KEYWORDS: 5-Methoxy-\textit{alpha}-methyltryptamine, Tryptamines, Designer Drug, Color Testing, Gas Chromatography/Mass Spectrometry, Forensic Chemistry.

Summary

In November 2002, an agency in northwestern Wisconsin submitted to the Wisconsin State Crime Laboratory in Wausau an exhibit of ten sugar cubes packaged together in foil, suspected to contain lysergic acid diethylamide (LSD). There was slight discoloration visible on approximately half of each of the ten cubes. A sample of the cubes was analyzed by color testing and gas chromatography/mass spectrometry. The results indicated not LSD but rather 5-methoxy-\textit{alpha}-methyltryptamine (aka 5-MeO-AMT or “Alpha-O”; see Figure 1).

![Figure 1: Structure of 5-Methoxy-\textit{alpha}-methyltryptamine (C_{12}H_{16}N_{2}O; mw = 204.27)](image)

Experimental

Color Tests

A purple color was observed when the sample was subjected to the \textit{para}-dimethylaminobenzaldehyde (PDMAB) reagent test. [This result, along with the fact that sugar cubes were used as the supporting media, explain why the submitting agency believed the exhibits contained LSD.]

However, a cherry red color was observed when the sample was subjected to the sodium nitroprusside reagent test, suggesting a tryptamine. This result was then compared to three tryptamine standards (Table 1). The results suggested the presence of a primary amine with an \textit{alpha}-methyltryptamine moiety (Figure 2) - and not a secondary amine with an \textit{N}-methyltryptamine moiety (Figure 3).
Gas Chromatography / Mass Spectrometry

A small portion of each sugar cube was combined and dissolved in one percent citric acid (the laboratory’s standard solution for dissolving/extracting samples suspected to contain LSD). The extract was then made basic with sodium carbonate and extracted with butyl chloride (butyl chloride is preferred because it is less dense than water (and therefore forms the upper layer) and does not require drying prior to injection on a gas chromatograph). GC/MS analysis of the extract was performed on an Agilent 6890 Gas Chromatograph equipped with an Agilent 5973N Mass Selective Detector using a 12 m x 0.20 mm HP-1 column with a film thickness of 0.33 μm (see Figure 4 for the mass spectrum). The GC oven was temperature ramped at 20°C per minute from 120°C to 260°C, then held for 4 minutes at 260°C. The mass spectrometer was scanned from m/z 35 to 305. The sample peak had a retention time of 4.66 minutes. Standards of α-methyltryptamine, N-methyltryptamine, 5-methoxy-α-methyltryptamine, and 5-methoxy-N,N-dimethyltryptamine were also run under the above conditions (retention times are presented in Table 2).
### Table 2: Retention Times for Tryptamine Standards

<table>
<thead>
<tr>
<th>Tryptamine</th>
<th>Retention Time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Methyltryptamine (AMT)</td>
<td>3.54</td>
</tr>
<tr>
<td>N-Methyltryptamine (NMT)</td>
<td>3.65</td>
</tr>
<tr>
<td>5-Methoxy-α-methyltryptamine (5-MeO-AMT)</td>
<td>4.67</td>
</tr>
<tr>
<td>5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT)</td>
<td>4.84</td>
</tr>
</tbody>
</table>

**Results and Discussion**

Upon initial review, the mass spectrum of the sample (Figure 4) was similar to those of both N-methyltryptamine (NMT) [1] (Figure 5a) and *α*-methyltryptamine (AMT) (Figure 6a), but had a molecular ion thirty mass units greater than either, which suggested the presence of a methoxy substituent. However, closer inspection of the expanded fragmentation patterns showed a loss of 15 mass units (i.e., a methyl group) in the sample spectrum (see the peak at m/z 189, Figure 4), similar to the fragmentation pattern of AMT (m/z = 159, Figure 6b) but not NMT (Figure 5b). In addition, the spectrum was clearly different versus that of 5-methoxy-N,N-dimethyl-tryptamine (Figure 7), and had a molecular ion 14 mass units lower. The collective results suggested a methoxylated *α*-methyltryptamine.

There have been several recent reports of appearances of 5-methoxy-α-methyltryptamine (5-MeO-AMT) in *Microgram Bulletin* [2]. The synthesis of 5-MeO-AMT is described in Shulgin’s *TIHKAL* [3], along with anecdotal remarks on its pharmacological effects. Several illicit drug-related Internet sites, including a message board, also have information on 5-MeO-AMT, including usage testimonials [4]. A standard of 5-MeO-AMT was obtained from a commercial source (details withheld per *Journal* policy). The mass spectra of the sample and the standard were internally consistent, and both matched the mass spectrum of 5-MeO-AMT provided by the DEA Special Testing and Research Laboratory (Dulles, Virginia) [2a]. 5-MeO-AMT is not currently listed in the U.S. Controlled Substances Act; however, it is considered to be a controlled substance analogue, and can be prosecuted as such in Federal Courts.
**Figure 5a:** Mass Spectrum of N-Methyltryptamine

**Figure 5b.** Expanded Mass Spectrum of N–Methyltryptamine

**Figure 6a:** Mass Spectrum of alpha-Methyltryptamine
Figure 6b. Expanded Mass Spectrum of alpha-Methyltryptamine

Figure 7. Mass Spectrum of 5-Methoxy-N,N-dimethyltryptamine

Acknowledgements

The author would like to thank Forensic Scientist John Nied (this laboratory) for his assistance in the identification of 5-methoxy-alpha-methyltryptamine.

References

4. The Vaults of Erowid; The Lycaeum; and The Hive.

********       ********       ********       ********
Technical Note

The Identification of \textit{d-N,N-Dimethylamphetamine (DMA)} in an Exhibit in Malaysia

Kee Bian Chan*, Yong Kiong Chong, and Mohamed Nazarudin
Narcotics Section, Forensic Division
Department of Chemistry - Malaysia
Jalan Sultan, 46661 Petaling Jaya
Malaysia
[email:  kbchan -at- kimia.gov.my]

ABSTRACT: A crystalline substance which was suspected to be methamphetamine hydrochloride was instead determined to be \textit{d-N,N-Dimethylamphetamine hydrochloride} containing traces of methamphetamine hydrochloride. Analytical data (Color Testing, GC/MS, FTIR, HPLC, Melting Point, Optical Rotation) is reported for \textit{d-N,N-Dimethylamphetamine hydrochloride}.

KEYWORDS: \textit{d-N,N-Dimethylamphetamine Hydrochloride}, Color Test, GC-MS, FTIR, HPLC, Melting Point, Optical Rotation, Forensic Chemistry.

Introduction

This laboratory recently received an exhibit consisting of approximately 200 grams of a white crystalline substance that was suspected to be methamphetamine hydrochloride. Crystalline methamphetamine hydrochloride (known locally by the street name of “syabu”) is very frequently encountered by the Central Laboratory and its nine branch laboratories. However, in this case the substance was instead determined to be \textit{d-N,N-Dimethylamphetamine hydrochloride} with traces of methamphetamine. There have been occasional literature reports of dimethylamphetamine in the United States, some of which included analytical data (\textit{vide infra}); however, those reports were in a law enforcement restricted periodical (\textit{Microgram}), and so are not generally available. More recently, crystalline dimethylamphetamine was reported to be a low prevalence drug of abuse in Japan, making its first appearance there in 1998 \textsuperscript{1}. To our knowledge, this is the first report of dimethylamphetamine in Malaysia. Dimethylamphetamine is not currently designated as a controlled substance or “dangerous drug” in Malaysia (that is, like amphetamine or methamphetamine). This paper presents a brief of our analytical findings.

Experimental

Color Test and Reagents

Marquis Reagent and Simon’s Regent: These were prepared from analytical grade reagents according to the standard formulations given in the literature \textsuperscript{2}.
GC/MS

GC/MS analysis was performed on a Shimadzu QP5050A. Column conditions: 30m x 0.25 mm i.d., film thickness 0.25 µm BPX-5 (5% phenylpolysilphenylene-siloxane), with a temperature program starting at 180 °C (2 min), then ramping 25 °C/min to 250 °C. The injection port temperature was 260 °C, and the detector and transfer-line temperatures were 280 °C.

HPLC

The chromatographic system consisted of a Hewlett Packard Series 1050 HPLC, with a variable UV-detector set at 257 nm and a HP-3396 Series II integrator. The column was a Econosphere (Alltech) 150 mm x 4.6 mm i.d. stainless steel column packed with 5 µm silica. The flow rate was set at 0.8 mL/min. Injections were made via a Rheodyne injection valve with a 20 µL loop. The mobile phase consisted of methanol/water/1N ammonia solution/1N ammonium nitrate (27:3:2:1).

FTIR

Fourier Transfer Infrared Spectroscopy was performed using a Nicolet Magna-IR Spectrometer 550. The resolution was set at 4.000 cm⁻¹, with 32 scans between 4000 cm⁻¹ and 550 cm⁻¹. The sample was determined as a KBr disc.

Melting Point

The melting point was determined using a Buchi B-545 melting point apparatus.

Polarimetry

The optical rotation of two solutions containing 0.048 grams/mL and 0.024 grams/mL of sample in distilled water were measured with a Bellingham & Stanley (London) polarimeter. The accuracy of the instrument was checked by determining the specific optical rotation of a sucrose standard solution (9.78 grams/100mL) and comparing with the literature value. An analytical grade sucrose from Mayer & Baker was used.

Results and Discussion

Color Tests

Treating the sample with the Marquis reagent gave a color change from orange to brown. A faint blue color developed slowly with the Simon’s reagent. As a tertiary amine, DMA should not produce a color change with Simon’s reagent; therefore, this result suggested the low-level presence of a secondary amine (such as methamphetamine) or some other contaminant.

GC/MS

The GC/MS chromatogram showed two peaks – a small peak preceding a much larger one. The mass spectrum of the large peak (Figure 1) was typical of phenethylamines in that it had a dominant parent ion (at m/z = 72) but otherwise only small fragment ions. The spectrum of the primary component was found to be similar to the literature DMA spectra, while the small peak was identified as methamphetamine from its mass spectrum and retention time. The low level presence of methamphetamine was consistent with the findings from the color tests.
HPLC

The HPLC chromatogram also showed two peaks, with a small peak preceding a large peak (Figure 2). The small peak was presumptively identified as methamphetamine from its retention time. The amounts of methamphetamine and DMA were estimated to be 0.5 and 98 percent, respectively, based on the relative peak areas - again, consistent with the GC and color test results.

FTIR

The FTIR spectrum of the sample is shown in Figure 3. This was compared and found to be consistent with the reference IR spectrum of \( N,N \)-dimethylamphetamine hydrochloride provided by the Forensic Science Laboratory of the Osaka Prefectural Police Headquarters (see Figure 4), and with the spectra given in the literature \(^6,7,8,9\).

Melting Point

The melting point of the sample was found to be 182 -184 °C. The melting point of \( d \)- and \( l \)- \( N,N \)-dimethylamphetamine hydrochloride is 182-183 °C, while racemic \( N,N \)-dimethylamphetamine has a melting point of 157-159 °C (Dr. Munehiro Katagi, Forensic Science Laboratory of the Osaka Prefectural Police Headquarters, personal communication, 2002). This indicated that the sample was either the \( d \)- or \( l \)- isomer.

Polarimetry

The sample was purified by recrystallization before being subjected to polarimetry measurements. After three recrystallizations the methamphetamine content was reduced to ca. 0.2 percent (the methamphetamine could not be completely removed). The specific rotation of the purified sample in aqueous medium was determined at two dilutions (0.048 grams/mL and 0.024 grams/mL), and was found to be +14.5 ° at 25 °C. The sample was thereby identified as \( d-N,N \)-dimethylamphetamine hydrochloride.

Figure 1. Mass Spectrum of Sample (\( N,N \)-Dimethylamphetamine)
Figure 2. HPLC of Sample (Methamphetamine = 7.12 minutes; N,N-Dimethylamphetamine = 7.68 Minutes)

Figure 3: FTIR Spectrum of Sample (98% N,N-Dimethylamphetamine Hydrochloride)
Conclusions

Based on the analytical findings above, the sample was determined to be d-N,N-dimethylamphetamine hydrochloride contaminated with trace methamphetamine. It is not known whether this sample was synthesized in Malaysia or imported from a neighboring country. There is a remote possibility that the synthesis or importation was done intentionally, since dimethylamphetamine is not a controlled substance in Malaysia. However, if this drug continues to appear in the local illicit drug scene, either in methamphetamine-like crystalline form or mixed with other amphetamine-type stimulants in the form of powders or tablets, then it would likely be eventually included in the list of controlled substances in Malaysia - notwithstanding reports that it exerts much lower CNS stimulant properties versus amphetamine or methamphetamine.¹⁰

Acknowledgements

The authors would like to thank Dr. Hitoshi Tsuchihashi and Dr. Munehiro Katagi (Forensic Science Laboratory of the Osaka Prefectural Police HQ) for kindly providing the specimen IR spectrum and melting points of DMA; Thomas J. Janovsky (Deputy Assistant Administrator, U.S. Drug Enforcement Administration/Office of Forensic Sciences) for providing copies of the pertinent Microgram references*; and Mohd Fauze Mohamad Ayob (Department of Chemistry Malaysia) for the FTIR analysis.

References


* Note: All issues of Microgram (November 1967 - March 2002) and the first nine issues of its successor Microgram Bulletin (April - December, 2002) were Law Enforcement Restricted publications, and are therefore unavailable to the general public.

* * * * * * * * * * * * * * * * * * * * * * * *
Technical Note

Profiling of Ecstasy Tablets Seized in Japan

Yukiko Makino*, Shingo Kurobane, Kazuyoshi Miyasaka, and Kenichi Nagano
Narcotics Control Department
Kanto-Shin'etsu Regional Bureau of Health and Welfare
Ministry of Health, Labour and Welfare
2-4-14 Nakameguro, Meguro-ku, Tokyo 153-0061 Japan
[email: ymakino-at-mbe.nifty.com]

ABSTRACT: 54 Ecstasy tablets seized in Japan during the first half of CY-2002 were analyzed to determine their physical and chemical characteristics and to derive a “snapshot” comparison with seizures made in CY’s 2000 and 2001. For physical characterization, logo, cleavage, coat, vertical view, horizontal view, diameter, thickness, weight, smell, outside color, inside color, color, toughness, capping, and logo were measured, and a photograph was taken. For chemical characterization, the tablet components were identified by GC/MS and HPLC, with quantification by HPLC. The maximum content of 3,4-methylenedioxymethamphetamine was 160 milligrams/tablet. Other tablet components detected were 3,4-methylenedioxyamphetamine, ephedrine, caffeine, ketamine, and methamphetamine. Several trends in the chemical characteristics are presented.

KEYWORDS: 3,4-Methylenedioxymethamphetamine, MDMA, Ecstasy, Characterization, Profiling, Ketamine, Forensic Chemistry.

Introduction

Abuse of 3,4-methylenedioxymethamphetamine (“Ecstasy”) has spread worldwide. Although still a very small percentage relative to worldwide consumption, the number of Ecstasy tablets seized in Japan has been rapidly increasing, with 174,000 tablets seized in 2002 [1]. Ecstasy abuse in Japan is considered to be a very serious problem, similar to methamphetamine abuse. We recently profiled Ecstasy tablets seized in Japan in CY’s 2000 and 2001, and reported the results in the Journal of Health Science [2]. We have continued to profile Ecstasy tablets using the same methodologies presented in the Journal of Health Science’s article [2]. Herein, we present the results of the profiling of Ecstasy tablets seized in Japan during the first half of CY-2002.

Experimental

Chemicals

Methamphetamine hydrochloride, ephedrine hydrochloride, and caffeine were obtained from commercial sources in Japan. 3,4-Methylenedioxyamphetamine hydrochloride (MDA), 3,4-methylenedioxymethamphetamine hydrochloride (MDMA), and 3,4-methylenedioxymethylamphetamine hydrochloride (MDEA) were obtained from the reference collection of the Narcotics Control Department Laboratory. All solvents were of HPLC grade. The 54 tablets that were analyzed in the present study were randomly selected from among seizures forfeited to the Government during the first half of CY-2002.

Physical Characteristics

Fifteen parameters (logo, cleavage, coat, vertical view, horizontal view, diameter, thickness, weight, smell, outside color, inside color, color, toughness, capping, and appearance of logo) were measured to establish the
physical characteristics of each tablet. The tablets were also photographed with a digital camera. An identification number was applied to each tablet, consisting of a logo number, color number, and serial number.

Extraction Procedure

Each tablet was placed in an agate mortar and crushed to a fine powder. A 10 mg portion of the resulting powder was dissolved in 8 mL of phosphate buffer (pH 7.0) by shaking for 5 minutes. The solution was centrifuged for 10 minutes at 3,500 rpm, and 100 μL of the supernatant liquid was transferred to a small autosampler vial for HPLC analysis. Half of the remaining solution (4 mL) was used for the identification of the basic compounds (e.g., ephedrine, MDA, MDMA, etc.), while the other half was used for the identification of the neutral compounds (i.e., caffeine). One mL of 10% Na₂CO₃ solution was used to adjust the first 4 mL aliquot of solution to pH 10.5, and the mixture was then extracted with 3 mL of chloroform by shaking for 5 minutes. The biphasic solution was then centrifuged at 3,500 rpm for 10 minutes, after which the organic layer was transferred to a vial for GC/MS analysis. The second 4 mL aliquot of solution (still at pH 7.0) was similarly extracted with 3 mL of chloroform, for identification of caffeine by GC/MS.

GC/MS Analysis

A GC/MS equipped with a Hewlett-Packard (HP) 6890 Series Gas Chromatograph, a double-focusing mass spectrometer Mstation (JEOL, Tokyo, Japan), and a data processing XMS system (JEOL, Tokyo, Japan), were used for identification of the components in the tablets. An Ultra-2 fused-silica capillary column (30 m x 0.2 mm with 0.33μm HP) was inserted directly into the ion source of the mass spectrometer, and analysis was performed in the splitless mode with Helium as the carrier gas. The GC temperature programming was run from 50 °C (1 minute) to 300 °C (4 minutes) at 10 °C /minute, with the injection port at 250 °C. Electron-impact ionization mass conditions were set as follows: Ionizing energy, 70 eV; ionization current, 300 μA; and ion-source temperature, 300 °C. Mass spectra were obtained using the scanning mode.

HPLC Analysis

A Shiseido Nanospace HPLC, equipped with a UV detector linked to a data system (S-MC, Shiseido, Tokyo, Japan), was used for qualitative and quantitative analysis of the components in the tablets. Chromatographic separation was achieved using an ODS-type semi-microcolumn (CAPCELL PAK C18 UG 120 S5, 250 mm x 1.5 mm i.d.). The mobile phase used for ephedrine, MDA, MDMA, MDEA, methamphetamine, and ketamine was 5 mmol/L SDS in 20 mmol/L KH₂PO₄-CH₃CN (65:35). The mobile phase used for caffeine was H₂O-methanol (7:3). The flow rate was maintained at 0.1 mL/minute. Separation was carried out at 50 °C for ephedrine, MDA, MDMA, MDEA, methamphetamine, and ketamine, and 35 °C for caffeine. The monitoring wavelength was 210 nm for ephedrine, MDA, MDMA, MDEA, methamphetamine, and ketamine, and 254 nm for caffeine. Good linearity for this quantitative analysis was confirmed over the concentration range of 0.1 – 0.8 mg/mL (r² = 0.9993 – 0.9997 for six compounds).

Results and Discussion

Physical Characteristics

To date, there are few reports concerning the physical or chemical characteristics of Ecstasy tablets in Japan [3,4]. To aid in quick comparison, full-color photographs of all tablets are shown in order of the amount of MDMA or MDA as an active ingredient, followed by a group containing mixed drugs (Figure 1). The characteristic physical properties are listed in Table 1. The diameters ranged from 7.1 – 10.1 mm, the widths ranged from 2.6 – 7.0 mm, and the weights ranged from 105 – 348 mg.
Chemical Characteristics

The active ingredients in each tablet were identified by GC/MS and HPLC. The detected components were MDMA, MDA, ephedrine, caffeine, ketamine, and methamphetamine. Thirty-five tablets contained MDMA as the sole active ingredient. The content range (calculated as MDMA hydrochloride) was 37 - 160 mg/tablet. One tablet contained MDA alone. The content was calculated as MDA hydrochloride at 75 mg/tablet. Eighteen tablets contained two or three active ingredients. To summarize the findings, we have noted the following trends as compared with last report [2]:

1. An increase in tablets containing ketamine.
2. An increase in tablets containing methamphetamine.
3. A decrease in tablets containing ephedrine.

We are continuing to profile Ecstasy tablets seized in Japan [4].

Figure 1. Photographs of 54 Ecstasy Tablets Seized in Japan During the First Half of CY-2002, with Logo Name and Amount of Active Ingredient.

<table>
<thead>
<tr>
<th>Tulip</th>
<th>B29</th>
<th>B29</th>
<th>XL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA 160 mg/tab</td>
<td>MDMA 157 mg/tab</td>
<td>MDMA 151 mg/tab</td>
<td>MDMA 146 mg/tab</td>
</tr>
<tr>
<td>Mitsubishi</td>
<td>cK</td>
<td>XL</td>
<td>Bird (Fry)</td>
</tr>
<tr>
<td>MDMA 103 mg/tab</td>
<td>MDMA 98 mg/tab</td>
<td>MDMA 98 mg/tab</td>
<td>MDMA 89 mg/tab</td>
</tr>
<tr>
<td>Mickey Mouse</td>
<td>Crocodile</td>
<td>CU</td>
<td>FF</td>
</tr>
<tr>
<td>MDMA 88 mg/tab</td>
<td>MDMA 87 mg/tab</td>
<td>MDMA 87 mg/tab</td>
<td>MDMA 80 mg/tab</td>
</tr>
<tr>
<td>Star</td>
<td>007</td>
<td>Smiley</td>
<td>(No Logo)</td>
</tr>
<tr>
<td>MDMA 77 mg/tab</td>
<td>MDMA 70 mg/tab</td>
<td>MDMA 69 mg/tab</td>
<td>MDMA 66 mg/tab</td>
</tr>
<tr>
<td>Image</td>
<td>Description</td>
<td>MDMA (mg/tab)</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>Mitsubishi</td>
<td>!</td>
<td>Mitsubishi Butterfly</td>
<td>MDMA 64 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitsubishi</td>
<td>MDMA 64 mg/tab</td>
</tr>
<tr>
<td>Lozenge</td>
<td>Superman</td>
<td>Lozenge Propeller</td>
<td>MDMA 61 mg/tab</td>
</tr>
<tr>
<td>Motorola</td>
<td>007</td>
<td>Motorola 007 Smiley Fish</td>
<td>MDMA 57 mg/tab</td>
</tr>
<tr>
<td>Hole</td>
<td>Smiley</td>
<td>Hole Smiley Mercedes Tower</td>
<td>MDMA 50 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hole</td>
<td>MDMA 51 mg/tab</td>
</tr>
<tr>
<td>Studio</td>
<td></td>
<td>Studio</td>
<td>MDMA 50 mg/tab</td>
</tr>
<tr>
<td>Mercedes</td>
<td></td>
<td>Mercedes</td>
<td>MDMA 46 mg/tab</td>
</tr>
<tr>
<td>Tower</td>
<td></td>
<td>Tower</td>
<td>MDMA 42 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MDMA 40 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MDMA 37 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MDMA 106 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caffeine 35 mg/tab</td>
</tr>
<tr>
<td>Tower</td>
<td>Popeye</td>
<td>Bearded Face</td>
<td>Butterfly</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
<td>--------------</td>
<td>----------------</td>
</tr>
<tr>
<td>MDMA 98 mg/tab</td>
<td>MDMA 98 mg/tab</td>
<td>MDMA 88 mg/tab</td>
<td>MDMA 80 mg/tab</td>
</tr>
<tr>
<td>Caffeine 29 mg/tab</td>
<td>Caffeine 40 mg/tab</td>
<td>Caffeine 28 mg/tab</td>
<td>Caffeine 27 mg/tab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RN</th>
<th>Yin-Yang</th>
<th>(No Logo)</th>
<th>Crown (Cross)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA 53 mg/tab</td>
<td>MDMA 38 mg/tab</td>
<td>MDMA 100 mg/tab</td>
<td>MDMA 86 mg/tab</td>
</tr>
<tr>
<td>Caffeine 6 mg/tab</td>
<td>Caffeine 62 mg/tab</td>
<td>MA 4 mg/tab</td>
<td>MA 2 mg/tab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MA 4 mg/tab</td>
<td>Ketamine 5 mg/tab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SX</th>
<th>SX</th>
<th>Spider</th>
<th>Crown (Ruff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA 84 mg/tab</td>
<td>MDMA 72 mg/tab</td>
<td>MDMA 64 mg/tab</td>
<td>MDMA 64 mg/tab</td>
</tr>
<tr>
<td>MA 3 mg/tab</td>
<td>MA 3 mg/tab</td>
<td>MA 21 mg/tab</td>
<td>MA 21 mg/tab</td>
</tr>
<tr>
<td>Ketamine 4 mg/tab</td>
<td>Ketamine 4 mg/tab</td>
<td>Ketamine 43 mg/tab</td>
<td>Ketamine 43 mg/tab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>V2K</th>
<th>Y2K</th>
<th>Y2K</th>
<th>Mitsubishi</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA 43 mg/tab</td>
<td>MDMA 38 mg/tab</td>
<td>MDMA 37 mg/tab</td>
<td>MDMA 5 mg/tab</td>
</tr>
<tr>
<td>MA 10 mg/tab</td>
<td>MA 16 mg/tab</td>
<td>MA 16 mg/tab</td>
<td>MA 2 mg/tab</td>
</tr>
<tr>
<td>Ketamine 64 mg/tab</td>
<td>Ketamine 48 mg/tab</td>
<td>Ketamine 52 mg/tab</td>
<td>Ketamine - trace</td>
</tr>
</tbody>
</table>
Figure Abbreviations: MA - Methamphetamine; MDA - 3,4-Methylenedioxyamphetamine; MDMA - 3,4-Methylenedioxymethamphetamine.

### Table 1. Select Physical and Chemical Characteristics of Ecstasy Tablets Seized in Japan During the First Half of CY-2002.

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Logo</th>
<th>Diameter (mm)</th>
<th>Thickness (mm)</th>
<th>Weight (mg)</th>
<th>Outside color</th>
<th>Active Ingredients (Percent per Tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>&quot;B29&quot;</td>
<td>8.3</td>
<td>4.7</td>
<td>287</td>
<td>blue</td>
<td>MDMA (55 %)</td>
</tr>
<tr>
<td>102</td>
<td>&quot;B29&quot;</td>
<td>8.3</td>
<td>4.5</td>
<td>285</td>
<td>brown</td>
<td>MDMA (53 %)</td>
</tr>
<tr>
<td>103</td>
<td>&quot;!&quot;</td>
<td>8.3</td>
<td>3.3</td>
<td>205</td>
<td>yellow</td>
<td>MDMA (31 %)</td>
</tr>
<tr>
<td>104</td>
<td>Bird(Fry)</td>
<td>7.1</td>
<td>3.0</td>
<td>140</td>
<td>blue</td>
<td>MDMA (64 %)</td>
</tr>
<tr>
<td>105</td>
<td>Mickey Mouse</td>
<td>7.1</td>
<td>4.3</td>
<td>225</td>
<td>brown</td>
<td>MDMA (39 %)</td>
</tr>
<tr>
<td>106</td>
<td>Motorola</td>
<td>8.5</td>
<td>3.6</td>
<td>140</td>
<td>brown</td>
<td>MDMA (36 %)</td>
</tr>
<tr>
<td>107</td>
<td>Mitsubishi</td>
<td>9.3</td>
<td>4.4</td>
<td>297</td>
<td>grey</td>
<td>MDMA (22 %)</td>
</tr>
<tr>
<td>108</td>
<td>Tower</td>
<td>8.2</td>
<td>5.0</td>
<td>325</td>
<td>yellow</td>
<td>MDMA (30 %) caffeine (9 %)</td>
</tr>
<tr>
<td>109</td>
<td>Smiley</td>
<td>7.1</td>
<td>3.5</td>
<td>168</td>
<td>orange</td>
<td>MDMA (24 %)</td>
</tr>
<tr>
<td>110</td>
<td>&quot;SX&quot;</td>
<td>8.1</td>
<td>4.3</td>
<td>218</td>
<td>orange</td>
<td>MDMA (33 %) methamphetamine (1 %) ketamine (2 %)</td>
</tr>
<tr>
<td>111</td>
<td>&quot;007&quot;</td>
<td>7.2</td>
<td>4.7</td>
<td>224</td>
<td>brown</td>
<td>MDMA (22 %)</td>
</tr>
<tr>
<td>112</td>
<td>Propeller</td>
<td>10.1</td>
<td>4.4</td>
<td>305</td>
<td>white</td>
<td>MDMA (19 %)</td>
</tr>
<tr>
<td>113</td>
<td>Crocodile</td>
<td>8.1</td>
<td>4.6</td>
<td>227</td>
<td>grey</td>
<td>MDMA (38 %)</td>
</tr>
<tr>
<td>114</td>
<td>Smiley</td>
<td>7.2</td>
<td>4.2</td>
<td>202</td>
<td>green</td>
<td>MDMA (34 %)</td>
</tr>
<tr>
<td>115</td>
<td>Fish</td>
<td>7.2</td>
<td>3.0</td>
<td>139</td>
<td>blue</td>
<td>MDMA (30 %)</td>
</tr>
<tr>
<td>116</td>
<td>&quot;FF&quot;</td>
<td>7.1</td>
<td>5.2</td>
<td>256</td>
<td>yellow</td>
<td>MDMA (31 %)</td>
</tr>
<tr>
<td>117</td>
<td>&quot;RN&quot;</td>
<td>8.1</td>
<td>3.9</td>
<td>216</td>
<td>green</td>
<td>MDMA (25 %) caffeine (3 %)</td>
</tr>
<tr>
<td>118</td>
<td>Bearded Face</td>
<td>8.2</td>
<td>5.2</td>
<td>309</td>
<td>yellow</td>
<td>MDMA (28 %) caffeine (9 %)</td>
</tr>
<tr>
<td>119</td>
<td>Yin-Yang</td>
<td>8.2</td>
<td>4.3</td>
<td>259</td>
<td>white</td>
<td>MDMA (15 %) caffeine (24 %)</td>
</tr>
<tr>
<td>120</td>
<td>&quot;Y2K&quot;</td>
<td>9.1</td>
<td>3.2</td>
<td>207</td>
<td>green</td>
<td>MDMA (18 %) methamphetamine (8 %) ketamine (23 %)</td>
</tr>
<tr>
<td>121</td>
<td>&quot;Y2K&quot;</td>
<td>9.2</td>
<td>3.4</td>
<td>209</td>
<td>green</td>
<td>MDMA (18 %) methamphetamine (8 %) ketamine (25 %)</td>
</tr>
<tr>
<td>122</td>
<td>Popeye</td>
<td>8.6</td>
<td>4.2</td>
<td>261</td>
<td>white</td>
<td>MDMA (38 %) caffeine (15 %)</td>
</tr>
<tr>
<td>No.</td>
<td>Brand</td>
<td>MDA</td>
<td>MDMA</td>
<td>Methamphetamine</td>
<td>Caffeine</td>
<td>Ketamine</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>-----</td>
<td>------</td>
<td>-----------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>123</td>
<td>Two Hearts</td>
<td>8.1</td>
<td>5.1</td>
<td>226 purple</td>
<td>MDA (24%)</td>
<td>ephedrine (18%)</td>
</tr>
<tr>
<td>124</td>
<td>Mitsubishi</td>
<td>8.2</td>
<td>5.4</td>
<td>311 white</td>
<td>MDMA (20%)</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>Armani</td>
<td>8.9</td>
<td>4.7</td>
<td>315 white</td>
<td>MDMA (19%)</td>
<td></td>
</tr>
<tr>
<td>126</td>
<td>&quot;cK&quot;</td>
<td>8.2</td>
<td>4.9</td>
<td>304 grey</td>
<td>MDMA (32%)</td>
<td></td>
</tr>
<tr>
<td>127</td>
<td>Hole</td>
<td>8.5</td>
<td>4.5</td>
<td>282 green</td>
<td>MDMA (15%)</td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>Crown(Ruff)</td>
<td>8.8</td>
<td>5.8</td>
<td>204 white</td>
<td>MDMA (25%)</td>
<td>methamphetamine (5%)</td>
</tr>
<tr>
<td>129</td>
<td>&quot;XL&quot;</td>
<td>8.2</td>
<td>4.9</td>
<td>294 orange</td>
<td>MDMA (33%)</td>
<td></td>
</tr>
<tr>
<td>130</td>
<td>Crown(Cross)</td>
<td>9.1</td>
<td>4.1</td>
<td>221 brown</td>
<td>MDMA (39%)</td>
<td>methamphetamine (1%)</td>
</tr>
<tr>
<td>131</td>
<td>&quot;CU&quot;</td>
<td>8.1</td>
<td>4.6</td>
<td>262 green</td>
<td>MDMA (33%)</td>
<td></td>
</tr>
<tr>
<td>132</td>
<td>Cellular Phone</td>
<td>9.2</td>
<td>4.3</td>
<td>274 white</td>
<td>MDMA (20%)</td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>Spider</td>
<td>9.2</td>
<td>7.0</td>
<td>260 blue</td>
<td>MDMA (25%)</td>
<td>methamphetamine (8%)</td>
</tr>
<tr>
<td>134</td>
<td>&quot;SX&quot;</td>
<td>8.1</td>
<td>4.2</td>
<td>234 brown</td>
<td>MDMA (36%)</td>
<td>methamphetamine (1%)</td>
</tr>
<tr>
<td>135</td>
<td>Tulip</td>
<td>8.4</td>
<td>6.5</td>
<td>348 brown</td>
<td>MDMA (46%)</td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>&quot;XL&quot;</td>
<td>8.2</td>
<td>4.8</td>
<td>265 orange</td>
<td>MDMA (55%)</td>
<td></td>
</tr>
<tr>
<td>137</td>
<td>Star</td>
<td>8.6</td>
<td>6.2</td>
<td>328 green</td>
<td>MDMA (23%)</td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>Mercedes</td>
<td>7.1</td>
<td>2.6</td>
<td>105 white</td>
<td>MDMA (35%)</td>
<td></td>
</tr>
<tr>
<td>139</td>
<td>Lozenge</td>
<td>7.2</td>
<td>3.3</td>
<td>161 white</td>
<td>MDMA (35%)</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>Superman</td>
<td>9.2</td>
<td>3.6</td>
<td>281 white</td>
<td>MDMA (21%)</td>
<td></td>
</tr>
<tr>
<td>141</td>
<td>Smurf</td>
<td>9.1</td>
<td>3.8</td>
<td>280 white</td>
<td>MDMA (19%)</td>
<td></td>
</tr>
<tr>
<td>142</td>
<td>&quot;007&quot;</td>
<td>8.1</td>
<td>5.0</td>
<td>311 red</td>
<td>MDMA (23%)</td>
<td></td>
</tr>
<tr>
<td>143</td>
<td>no logo</td>
<td>7.3</td>
<td>4.5</td>
<td>179 orange</td>
<td>MDMA (37%)</td>
<td></td>
</tr>
<tr>
<td>144</td>
<td>Butterfly</td>
<td>8.0</td>
<td>5.0</td>
<td>307 yellow</td>
<td>MDMA (21%)</td>
<td></td>
</tr>
<tr>
<td>145</td>
<td>Lozenge</td>
<td>7.1</td>
<td>3.3</td>
<td>165 white</td>
<td>MDMA (37%)</td>
<td></td>
</tr>
<tr>
<td>146</td>
<td>&quot;V2K&quot;</td>
<td>9.2</td>
<td>3.0</td>
<td>234 orange</td>
<td>MDMA (18%)</td>
<td>methamphetamine (4%)</td>
</tr>
<tr>
<td>147</td>
<td>Mitsubishi</td>
<td>8.0</td>
<td>5.0</td>
<td>304 yellow</td>
<td>MDMA (34%)</td>
<td></td>
</tr>
<tr>
<td>148</td>
<td>no logo</td>
<td>8.1</td>
<td>4.3</td>
<td>254 green</td>
<td>MDMA (39%)</td>
<td>methamphetamine (2%)</td>
</tr>
<tr>
<td>149</td>
<td>&quot;888&quot;</td>
<td>8.2</td>
<td>4.3</td>
<td>217 rose</td>
<td>MDMA (35%)</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>Mitsubishi</td>
<td>9.0</td>
<td>5.0</td>
<td>319 white</td>
<td>MDMA (20%)</td>
<td></td>
</tr>
<tr>
<td>151</td>
<td>Butterfly</td>
<td>9.2</td>
<td>4.4</td>
<td>326 green</td>
<td>MDMA (25%)</td>
<td></td>
</tr>
<tr>
<td>152</td>
<td>Mitsubishi</td>
<td>9.4</td>
<td>5.0</td>
<td>306 green</td>
<td>MDMA (2%)</td>
<td>methamphetamine (1%)</td>
</tr>
<tr>
<td>153</td>
<td>Smiley</td>
<td>7.1</td>
<td>3.2</td>
<td>180 orange</td>
<td>MDMA (26%)</td>
<td></td>
</tr>
<tr>
<td>154</td>
<td>Tower</td>
<td>8.1</td>
<td>5.0</td>
<td>333 yellow</td>
<td>MDMA (32%)</td>
<td>caffeine (11%)</td>
</tr>
</tbody>
</table>
Acknowledgments

The present work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan.

References


* * * * * * * * * * * * * * * * * * * * * * * * *
Technical Note

A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions

Mohammad Sarwar*, Ph.D., and John L. McDonald, B.S.
Illinois State Police
Division of Forensic Services
Forensic Science Center at Chicago
1941 W. Roosevelt Road
Chicago, IL 60608
[email: msarwar36-at-yahoo.com]

ABSTRACT: A simple, convenient, and rapid method for the identification of psilocyn in hallucinogenic mushroom/chocolate concoctions is presented. A 10% solution of acetic acid is used to extract psilocyn from the mushrooms. The acidic solution is then basified with solid sodium bicarbonate, then extracted with chloroform. The resulting extract is then back-washed to remove theobromine and caffeine from the chocolate, then concentrated and analyzed by TLC and GC/MS. The method takes about 30 minutes for mushroom/chocolate concoctions. A more simplified version of the method can be used for mushrooms, and takes about 15 minutes.

KEYWORDS: Forensic Science, Psilocyn, Extraction, Psilocybe Mushrooms, Mushroom/Chocolate Concoctions.

Introduction

Psilocyn and psilocybin, and the mushrooms containing these substances, are Schedule I substances under both Federal and Illinois state law. Psilocyn and psilocybin are hallucinogens, which act on the central nervous system to produce changes in perception, mood, and thinking ability. The effects produced by psilocyn and psilocybin are similar to those produced by LSD and mescaline (1-2). Since the mushrooms that produce these hallucinogens are easily cultivated, and spores, growing kits, and information are readily available through the Internet, increasing numbers of mushrooms and mushroom containing preparations (especially mushroom/chocolate concoctions, vide infra) are being encountered in forensic laboratories.

The life cycle of mushrooms has four stages, namely spores, the mycelium, pinhead or the primordial, and the mature fruit. The spores are actually the seeds of the fungi. Mushrooms cannot be classified as plants because they lack a root system, and do not have leaves, flowers, or the main constituent of plants, chlorophyll. Plants get their food through roots and leaves through photosynthesis, while mushrooms get their food or nutrients from the surrounding environment. The four species of mushrooms that contain psilocyn and psilocybin are strophariaceae, bolbitiaceae, coprinaceae, and cortinariaceae (3-5).

Psilocyn, psilocybin, and various other alkaloids are found naturally in all four above listed species of mushrooms. The mature fungi are sold in the underground market in both whole and powdered forms. More recently, various mushroom-containing concoctions have become popular, especially grated or powdered mushrooms in chocolate (6). A number of such cases have been received at this laboratory over the past year.

The most common analytical techniques reported in the literature for analysis of hallucinogenic mushrooms are all based on methanol extraction. In the most common procedure, the mushrooms are simply soaked in methanol overnight, and the resulting extracts condensed to near dryness and then analyzed using TLC and GC/MS (7-8). A more rapid technique involves placing the mushrooms in a closed vial with methanol, heating for a half an hour, then heating to dryness; the resulting residue is taken into 0.1 N sodium hydroxide, then extracted with...
butyl chloride. The butyl chloride extract is then back-extracted with 0.1 N sulfuric acid, and the UV spectrum recorded in acidic and basic media. The basic solution is further extracted with butyl chloride, and the extract evaporated to dryness; the resulting powder is then analyzed by IR (9). In another, longer method, the mushrooms are dried at 40 °C in an oven for 16 hours, ground, and then soaked in methanol for 24 hours. The volume is reduced and then analyzed by HPLC (10). In a more rapid method using a buffer extraction, ground mushrooms are triturated in a rotary mixer with 10% ammonium nitrate in methanol for 30 minutes, then two methanol extractions are performed, and the combined methanol extracts analyzed by HPLC (11). Quantitative determination of psilocybin and psilocybin is accomplished by stirring freeze dried mushrooms in methanol for 12 hours, followed by analysis by HPLC and TLC (12). In a more refined method, the mushrooms are extracted with methanol, and the co-extracted sugars then precipitated with acetone; the resulting solution is concentrated prior to analysis by GC/MS (13). The aqueous extraction of psilocybin was achieved by using dilute acetic acid, adjusting the pH with glacial acetic acid, and heating the contents for one hour. The pH of the solution was then raised by the addition of ammonium hydroxide, and psilocybin extracted with diethyl ether. The latter method was also applicable to pure mushrooms but was more time consuming (14).

As noted above, mushroom/chocolate concoctions have become popular. The isolation and identification of psilocybin and psilocybin from mushrooms is somewhat problematic when the mushrooms have been grated or powdered and mixed with chocolate, because chocolate is a complex matrix containing a wide variety of components, many of which are soluble in methanol. Thus, the standard methanolic extraction techniques detailed above are almost inapplicable to mushroom/chocolate concoctions. In one recently described method, the concoction is soaked in dilute sulfuric acid and then washed with chloroform or methylene chloride. The aqueous layer is then basified with sodium hydroxide to pH 10, then extracted with chloroform (15-16). However, a clean peak of psilocybin was not obtainable even after multiple washings. Moreover, psilocybin is unstable at higher pH values (17). A short review on the methods of extraction for psilocybin can be read elsewhere (18).

In general, methanolic extraction procedures are very time consuming. Most procedures either involve an “overnight” extraction or heating. In addition, methanolic extractions of psilocybe mushrooms usually co-extract other indolic compounds (and other methanol soluble components), some of which can mask the psilocybin and psilocybin peaks in GC or GC/MS analyses. And as noted above, methanolic extraction is poorly suited for mushroom/chocolate concoctions. Herein, we present a new method for the extraction of psilocybin from such concoctions. The extraction takes about fifteen minutes for pure mushrooms, and about half an hour for mushroom/chocolate concoctions. In addition, large number of samples can be analyzed in a relatively short period of time.

**Materials and Methods**

**Reagents:** (1) A 10% acetic acid by volume (Analytical Reagent); (2) Chloroform (A.R.); (3) Sodium bicarbonate (A.R.); (4) Deionized water; and (5) Ehrlichs reagent.

**Equipment:** GC/MS (HP 6890/5973), centrifuge, pestle and mortar.

**Procedure for Pure Mushrooms:**

1. About 0.2 to 0.5 gram of mushrooms are transferred into a mortar.
2. The mushrooms are covered with 10% acetic acid, and ground with the help of a pestle.
3. Another 5 mL of deionized water are added and the mixture is ground into a fine slurry.
4. The slurry is then transferred into a test tube and centrifuged for about 3 minutes.
5. The supernatant is transferred into a small beaker.
6. The supernatant is neutralized by adding small amounts of sodium bicarbonate (neutralization is judged to be complete when the foamy effervescence stops). A little excess bicarbonate is then added.
7. The resulting solution is transferred into a test tube and extracted with an equal amount of chloroform.
8. The biphasic solution is centrifuged, and the chloroform layer collected in a shell vial.
9. The chloroform extract is concentrated under air, transferred to a micro vial, and analyzed on the GC/MS.

Total extraction takes around 15 minutes. The results are shown in Figure 1.

**Procedure for Mushroom/Chocolate Concoctions:**

1. 1.0 to 2.0 gram(s) of sample is transferred into a mortar and ground with a pestle.
2. The resulting powder is covered with 10% acetic acid, and the sample is further ground with a pestle.
3. An additional 5 to 7 mL deionized water is added, and the mixture is ground for about 2 minutes, creating a thin slurry.
4. This slurry is divided into two equal portions, and each is transferred into a test tube.
5. An equal amount of chloroform is added to each tube, and the tubes are centrifuged for 3 minutes.
6. The aqueous layer is pipetted into a beaker from both of the test tubes.
7. 2 or 3 drops of this solution are placed in a test tube, and treated with the Ehrlich’s reagent; a deep purple color is indicative of presence of indolic compounds.
8. The aqueous solution in the beaker is neutralized by slowly adding sodium bicarbonate until the effervescence stops.
9. A little excess bicarbonate is added, and the pH is checked with pH paper to make sure it lies between 8 - 8.5.
10. The resulting solution is then transferred into two test tubes, and each extracted with an equal amount of chloroform.
11. The tubes are centrifuged for about 5 minutes.
12. The chloroform layers are collected into two new test tubes.
13. An excess of 2% sodium bicarbonate solution is added to each test tube.
14. After vigorous shaking, the test tubes are centrifuged for 5 minutes.
15. The chloroform layers are combined in a small beaker.
16. The chloroform extract is concentrated under air, transferred to a micro vial, and analyzed on the GC/MS.

The results are shown in Figure 2.

**Results and Discussion**

The presented acetic acid facilitated extraction of psilocyn from mushrooms is more rapid and convenient versus traditional methanolic extraction procedures, which require long time frames or potentially destructive heating. In addition, the use of sodium bicarbonate as a neutralization agent keeps the pH below 8.5, thereby avoiding base-facilitated destruction of psilocyn. The Total Ion Chromatogram (TIC) of the mushroom only sample shows a clean psilocyn peak (Figure 1). Analysis by TLC also shows only psilocyn. No psilocybin was detected - this is perhaps due to the activity of the phosphatase enzymes present in the mushrooms, which can dephosphorylate psilocybin to psilocyn in aqueous medium (19).

Analysis of mushroom/chocolate concoctions requires additional cleanup steps. Analysis of the chloroform extract at Step 12 (that is, before the extract was washed with sodium bicarbonate) showed three peaks in the TIC (Figure 2). The small peak at 4.223 minutes is due to caffeine, the broad peak at 4.50 minutes is due to theobromine, and the sharp peak at 4.837 minutes is due to psilocyn. Caffeine and theobromine (both purine alkaloids) result from the chocolate; theobromine is the main alkaloid in chocolate (2.8 - 3.5 % in cocoa), and caffeine is another major alkaloid (0.1 - 0.4 %) (20). After washing the chloroform extract (at Step 12) with 2% sodium bicarbonate solution, the amounts of theobromine and caffeine are very low (see Figure 3), resulting in a nearly clean TIC showing only psilocyn. However, the mass spectrum of psilocyn depicted in Figure 3 showed an extraneous ion 109, probably resulting from trace theobromine. When the chloroform extract was washed with
water only, the peak due to theobromine nearly disappears, and there is no extraneous 109 fragment, but the peak due to caffeine is still present (see Figure 4).

Quantitation was not performed in this study; however, the procedure allows facile identification of psilocyn in mushroom/chocolate concoctions.

The mass spectra acquired in this study for caffeine, theobromine, and psilocyn are presented in Figures 5 -7.
Figure 4. Total Ion Chromatogram of Mushroom/Chocolate Concoctions when the Chloroform Extract was Washed with Water.

Figure 5. Mass Spectrum of Caffeine.
Figure 6. Mass Spectrum of Theobromine

Figure 7. Mass Spectrum of Psilocyn.

Acknowledgements

The authors thank Forensic Scientists Joseph Gillono and Paula Bosco Szum, this laboratory, for their technical assistance.
References


Technical Note

A Rapid and Simple GC/MS Screening Method for 4-Methoxyphenol in Illicitly Prepared 4-Methoxyamphetamine (PMA)

Dieter Waumans, Noël Bruneel, Bas Hermans, Jan Tytgat*
Laboratory of Toxicology
Eduard van Evenstraat 4,
3000 Leuven Belgium
[e-mail: jan.tytgat -at- pharm.kuleuven.ac.be]

ABSTRACT: 4-Methoxyamphetamine (PMA), one of the less popular designer phenethylamines, has experienced a minor resurgence in recent years. A common method for illicit synthesis of PMA is via Leuckart reductive amination of 4-methoxyphenyl-2-propanone, which in turn is produced via peracid oxidation of anethole (1-methoxy-4-(1-propenyl)benzene, or para-propenylanisole), a major component of star anise, anise, and fennel oils. The peracid oxidation of anethole also produces 4-methoxyphenol, which can be isolated from illicitly prepared PMA via a simple and rapid procedure, and subsequently identified via GC/MS. Thus, 4-methoxyphenol is a marker compound for identification of the anethole-based production of PMA. The presented analytical methodologies represents an alternative to headspace solid-phase microextraction/mass spectral identification techniques (GC-HSPME/MS).

KEYWORDS: Anethole, Anise oil, 4-Methoxyphenol, para-Methoxyamphetamine, Impurity Profiling, Forensic Chemistry.

Introduction

The recreational use of non-medicinally accepted drugs is a phenomenon that mankind has been plagued with for many years. At present, the phenethylamines amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxymethamphetamine (MDMA) are particularly popular amongst members of certain social environments. Many other more unusual phenethylamines, such as 4-bromo-2,5-dimethoxyphenethylamine, are also encountered, but much less frequently.

The production and use of unusual phenethylamines is sometimes accidental, but is often an intentional response to laws controlling illicit drugs of abuse. Most of the common phenethylamine drugs - and virtually all of the more popular ones - are illegal, which leads clandestine chemists to engage in their illicit manufacture. However, because the laws that control illicit drugs are very specific, new and/or non-regulated drugs also occasionally appear on the underground markets. As these substances are often specifically synthesized due to their non-regulated status, they are commonly known as “designer drugs”. In Europe, some recent examples of such endeavors include 4-methylthioamphetamine (4-MTA) and 4-iodo-2,5-dimethoxyphenethylamine (2C-I).

4-Methoxyamphetamine (para-methoxyamphetamine, PMA) is a much older designer drug, which after many years of very low occurrence, has been encountered in increased frequency during the past few years - and has also been linked with a number of user deaths. However, in contrast to 4-MTA or 2C-I, PMA was already a legally restricted substance, because of its previous appearances. Due to its known high toxicity, its resurgence is somewhat surprising. PMA is usually illicitly produced via a multi-step synthesis from either anisaldehyde (4-methoxybenzaldehyde) or anethole (1-methoxy-4-(1-propenyl)benzene, or para-propenylanisole). Anethole is a major component of star anise, anise, and fennel essential oils [1]; these oils are used in vast quantities in the food and pharmaceutical industry, and so are widely available. This latter fact makes them particularly attractive precursors for clandestine chemists.
Forensic chemists are sometimes requested to determine the precursor or the synthetic route used to prepare a seized drug preparation; this information can reveal valuable information for law enforcement agencies. Analytical information for detection of Leuckart-specific impurities in PMA has been previously reported [2,3]. However, those reports focused only on impurities that confirmed the clandestine chemist’s use of the Leuckart reaction, and not on the determination of the original precursor (that is, anisaldehyde versus anethole).

We have previously reported that 4-methoxyphenol specifically derives from side reactions occurring during the peracid oxidation of anethole to para-methoxyphenyl-2-propanone (PMP2P) [4]. The synthesis of PMP2P from anethole is shown in Figure 1A: Anethole is reacted with performic or peracetic acid to yield the corresponding glycol. The glycol intermediate is subsequently converted to PMP2P by refluxing in a sulfuric acid/methanol mixture. The concomitant formation of 4-methoxyphenol during this reaction sequence is illustrated in Figure 1B: Oxidative cleavage of the propenyl double bond of anethole yields anisaldehyde (4-methoxybenzaldehyde), which in turn is oxidized by the peracid via a Baeyer-Villiger reaction to give O-formyl-4-methoxyphenol, which is further hydrolyzed to 4-methoxyphenol.

![Figure 1](image)

**Figure 1:**

A: Scheme of the peracid oxidation reaction of anethole. Anethole is reacted with a peracid ([a], typically performic or peracetic acid), after which the obtained glycol is converted to 4-methoxyphenyl-2-propanone by refluxing in a sulfuric acid/methanol mixture.

B: Overview of the 4-methoxyphenol impurity formation. The propenyl double bond of anethole is oxidatively cleaved ([a]) to yield 4-methoxybenzaldehyde. This substance is further reacted to O-formyl-4-methoxyphenol due to the presence of peracids in the reaction mixture ([b]). This compound will then be hydrolyzed to yield 4-methoxyphenol ([c]).
We have previously reported the use of headspace solid-phase microextraction - GC/MS (GC-HSPME/MS) technique for detection of 4-methoxyphenol in illicitly prepared PMA [4]; however, this methodology is not yet generally available in forensic laboratories. Herein we report a more simple and rapid technique for extraction and identification of 4-methoxyphenol.

**Experimental**

**Chemicals**

All solvents were analytical grade and were purchased from Acros Organics (Geel, Belgium). Anise oil was obtained from Taiga International NV (Breendonk-Puurs, Belgium). Unless otherwise stated, all other chemical substances were procured from Merck (Darmstadt, Germany).

**Instrumentation**

Gas Chromatography-Mass Spectrometry (GC/MS) analysis were run using an Agilent 6890 Plus Gas Chromatograph (GC) equipped with an Agilent 5973N Mass Selective Detector (MSD), with electronic pressure programming. For the GC, Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min; the column was a 30 m x 0.25 mm x 0.25 μm VF-5MS Factor-Four capillary (Varian). Oven programming was as follows: 50°C (held for 1 min), 35°C/min to 100°C, 10°C/min to 270°C (held for 5 min). A standard split/splitless liner was applied for liquid injections. The injector temperature was maintained at 280°C. For liquid injections (1 μL), the apparatus was run in splitless or split mode (1:50), depending on the nature of the sample. The mass spectrometer (MS) was operated from 36 to 400 amu in electron impact (EI) mode, with an ionization energy of 70 eV. A solvent delay of 4.00 minutes was applied.

**Performic Acid Oxidation of Anethole**

A 250 mL round-bottomed flask was equipped with a magnetic stirbar and a thermometer, and charged with a solution of 6.0 grams of anise oil in 30 mL acetone. Performic acid solution (prepared by combining 7.0 grams of 30 % hydrogen peroxide with 25.0 grams of formic acid) was added at such a rate that the reaction mixture temperature did not exceed 38°C. After addition of the performic acid solution, the reaction was allowed to sit for about 12 hours. The resulting mixture was poured into an equal volume of cold distilled water, then extracted with 2 x 50 mL dichloromethane. The yellow organic phase was isolated and washed with 75 mL of distilled water, after which the organic phase was dried over anhydrous sodium sulfate. An aliquot of 1 μL was subsequently injected on the GC/MS.

**Sample Preparation**

An aliquot of a drug preparation (100 mg for powders, 75 mg for pulverized tablet) was dissolved in 5 mL 0.1 N hydrochloric acid, after which 5 mL dichloromethane was added. The mixture was vigorously shaken for about one minute, after which the organic layer was isolated and dried over anhydrous sodium sulfate. An aliquot of 1 μL was subsequently injected on the GC/MS.

**Results and Discussion**

**4-Methoxyphenol as Specific Peracid Oxidation Marker**

We have previously analyzed star anise oil, anise oil, and fennel oil (all natural sources of anethole), and determined that 4-methoxyphenol is not naturally present in any of these essential oils. In addition, there are no literature reports of 4-methoxyphenol being present in these oils. Our analysis of commercial anisaldehyde
Merck, Acros Organics also confirmed that 4-methoxyphenol was not present. Furthermore, production of PMA from anisaldehyde (starting with the Henry condensation route (anisaldehyde and nitroethane) used by clandestine chemists) did not produce 4-methoxyphenol at any stage. These results confirm that the presence of 4-methoxyphenol is not due to natural contamination, or produced as a synthetic by-product in the illicit synthesis of PMA from anisaldehyde.

We have previously shown that 4-methoxyphenol is a specific marker for the synthesis of PMP2P via peracid oxidation of anethole [4]. In the present study, we repeated the performic acid oxidation of anethole, and analyzed the results by GC/MS (see Figure 2). Chromatogram 2a displays the total ion chromatogram (TIC) for 4.00 to 15.00 minutes (split injection 1:50). The inset (Chromatogram 2b) shows methyl chavicol (A), 4-methoxyphenol (B), O-formyl-4-methoxyphenol (C), and anisaldehyde (D). When extracting ion m/z 148, a trace of anethole is also found, as demonstrated in the extracted ion Chromatogram 2c. Methyl chavicol and anethole (both cis and trans isomers) have similar mass spectra, and identification is only possible by comparing both mass spectra and retention times.

The peaks noticed between retention time 10.00 and 13.00 are glycol derivatives (the glycol, two mono-formyl, one di-formyl, and the acetonide derivative).

**Figure 2:**
- [2a]: GC/MS analysis of the performic acid reaction mixture
- [2b]: Zooming in on [2a], we notice the presence of methyl chavicol (A), 4-methoxyphenol (B), O-formyl-4-methoxyphenol (C), and anisaldehyde (D). Their mass spectra are shown as well.
- [2c]: Extracted ion chromatogram for m/z 148 in order to find traces of anethole (E). Its mass spectrum is similar to that of (A).
The Occurrence of 4-Methoxyphenol in Illicitly Prepared PMA

Four seized samples of illicitly prepared PMA were screened for the presence of 4-methoxyphenol, using the above described procedures. The results are shown in Figure 3. Chromatogram 3a is the extract of a brownish powder, while Chromatogram 3b is the extract of a tablet (the latter tablets circulated in Belgium in 2001, and were reportedly involved in at least two deaths [5]). Chromatogram 3c is an extract of another brownish powder seized independently from 3a, while Chromatogram 3d is an extract from a powder which was contained in a capsule. In all four chromatograms, peak A is 4-methoxyphenol, while peak B is 4-methyl-5-(4-methoxyphenyl)pyrimidine, a Leuckart reaction based impurity [4]. The results indicate that all four preparations were made using anethole (most probably as anise oil) as the original precursor.

The mass spectrum and retention time of 4-methoxyphenol gave a perfect match with a commercially obtained sample, which served as the reference standard.

Conclusions

4-Methoxyphenol is a synthetic by-product formed during the peracid oxidation of anethole, and thus serves as a marker compound for PMA prepared using anethole as the original precursor. The presented extraction and identification techniques are rapid and simple. Based on our work, 4-methoxyphenol would probably not be
present if the clandestine chemist performed a distillation to purify the intermediate 4-methoxyphenyl-2-propanone; however, few clandestine chemists perform such steps. In this study, the four illicitly prepared samples of PMA contained impurities from both the peracid oxidation and the Leuckart reductive amination reaction steps.

References


*****   *****   *****   *****   *****   *****
Technical Note

Letrozole (Femara®)

Lois C. Geer* and Patrick A. Hays
U.S. Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[Email: lois.c.geer -at- usdoj.gov]

ABSTRACT: Analytical data (GC/MS, FTIR, H¹-NMR, C¹³-NMR) are reported for Letrozole (Femara), an anti-cancer drug which was submitted for a case involving androgenic steroids.

KEYWORDS: Letrozole, Femara, Anti-Cancer Drug, Analysis, Forensic Chemistry.

Introduction

This laboratory occasionally receives unusual unknowns that were seized as suspected controlled substances. Recently, we received 4.46 grams of a flocculent white powder (see Photo 1), that had been included in a group of steroids submitted from a U.S. Customs seizure in Anchorage, Alaska. The steroids included various exhibits of boldenone, methandrostenolone, oxymetholone, stanozolol, testosterone, and trenbolone; tamoxifen (an anti-cancer drug) was also seized. The shipment is believed to have originated in Nanjing, China. The original analysis was performed by the U.S. Customs Laboratory, San Francisco, California, and determined that no controlled substance was present; however, an unknown substance was determined to be present.

Photo 1

Preliminary analyses by Gas Chromatography/Mass Spectrometry (GC/MS, Figure 1) and Fourier Transform Infrared Spectroscopy (FTIR, Figure 2) gave no matches when searched in reference databases. The infrared spectrum showed an unusually strong peak in the region 2230 cm⁻¹, suggesting strong carbon-nitrogen triple bond (nitrile) stretching. Based on the GC/MS, the molecular weight was 285 atomic mass units (amu); this was confirmed by Liquid Chromatography/Mass Spectrometry (LC/MS) with chemical ionization. Further analysis by proton and carbon-13 Nuclear Magnetic Resonance (NMR) Spectroscopy (Figures 3 and 4) with quantitative
analysis suggested a molecule with the chemical formula $\text{C}_{17}\text{H}_{11}\text{N}_5$. An internet search for compounds with that formula returned letrozole as a possibility. Letrozole is an anti-cancer drug (Femara®) produced by Novartis Pharmaceuticals Corporation (East Hanover, NJ). Further analysis and comparison to spectral data and reference standard material (provided by Novartis) confirmed that the sample was letrozole (see structural formula below).

![Letrozole structure](image)

**Letrozole**

- Chemical Name: 4,4’-(1H-1,2,4-Triazol-1-ylmethylene)dibenzonitrile
- Empirical Formula: $\text{C}_{17}\text{H}_{11}\text{N}_5$
- Molecular Weight: 285.31 amu
- Melting Range: 184 - 185 °C
- Therapeutic Category: Anti-cancer
- Solubility: Freely soluble in chloroform, slightly soluble in methanol, practically insoluble in water.

Letrozole is commercially available as Femara® tablets containing 2.5 mg of letrozole. These are dark yellow, coated, slightly biconvex with beveled edges, and imprinted with “FV” on one side and “CG” on the other (see Photo 2).
Experimental

GC/MS

GC/MS spectral data were collected on an Agilent 6890 GC/5973 MSD with a J&W 30 m length x 250 μm diameter column with a 0.25 μm film thickness of DB-1. The carrier gas was Helium with a constant flow of 1.0 mL/minute. The inlet was at 280 °C, with a split ratio of 25:1. The temperature program was 250 °C for 2 minutes, 15 °C per minute ramp to 300 °C with a 10 minute hold. The transfer line was at 280 °C, the quadrupole at 150 °C, and the source at 230 °C. The mass range was 29-550 amu.

FTIR

Infrared spectra were collected on a Nicolet Nexus 570 Infrared Spectrophotometer with KBr beam splitter and DTGS KBr detector equipped with a SensIR Technologies Durascope Attenuated Total Reflectance (ATR) accessory with a single bounce ATR element with KRS-5 focusing element. The 32 scans were collected between 4000 cm⁻¹ and 400 cm⁻¹ with a resolution of 4 cm⁻¹.

NMR

One dimensional NMR analyses were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe. The sample was prepared at 17 mg/mL in deuterated chloroform (CDCl₃) containing TMS (tetramethylsilane) as the reference standard (Aldrich Chemical Co., Milwaukee, WI). The proton spectrum of the standard was obtained with 8 scans using a 45 second delay, 90° pulse, 2 second acquisition time, and oversampling by a factor of 6. The carbon spectrum of the standard was obtained with 256 scans using a 1 second delay, 45° pulse, 1.2 second acquisition time, and oversampling by a factor of 3. The sample was maintained at 25 °C. Gradient versions of the 2-Dimensional NMR experiments HSQC (correlation of hydrogens directly bonded to carbons) and HMBC (correlation of hydrogens 2, 3, or 4 bonds from carbons) were performed to make assignments (listed in Table 1). Prior to the arrival of the reference standard, structural elucidation was performed utilizing Applied Chemistry Developments (ACD, Toronto, Canada) software (HNMR Predictor, CNMR predictor, and Structure Elucidator).

Results and Discussion

This seizure represents the first time that letrozole has been submitted to a DEA laboratory as a drug exhibit. Letrozole is a non-steroidal inhibitor of the aromatase enzyme system, and acts to inhibit the conversion of androgens to estrogens (1). It is used in the first line treatment of breast cancer in post-menopausal women. It has potential for use in association with the abuse of androgenic steroids, both to prevent their conversion to estrogens, and to prevent or diminish the side effects of androgenic steroid abuse such as gynecomastia (breast enlargement). Tamoxifen, another estrogen blocker, has long been associated with androgenic steroid abuse as it is also believed to prevent gynecomastia associated with that abuse; this explains why it (Tamoxifen) was also found in this seizure.

References

1. http://www.us.femara.com

[Figures 1 - 4 and Table 1 Follow.]
Figure 1. Mass Spectrum of Letrozole.

Figure 2. FTIR of Letrozole.
Figure 3. $^1$H-NMR Spectrum of Letrozole (400.2 MHz).

Figure 4. $^{13}$C-NMR Spectrum of Letrozole (100.6 MHz).
<table>
<thead>
<tr>
<th>Carbon Chemical Shift (ppm)</th>
<th>Proton Chemical Shift (ppm)</th>
<th>Atom Number and Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>66.36</td>
<td>6.81 (singlet)</td>
<td>1 (alkyl methine)</td>
</tr>
<tr>
<td>113.29</td>
<td></td>
<td>1,1’ (benzene rings)</td>
</tr>
<tr>
<td>117.82</td>
<td></td>
<td>19, 21 (benzene rings)</td>
</tr>
<tr>
<td>128.9</td>
<td>7.70, 7.72 (doublet)</td>
<td>3, 5, 3’, 5’ (benzene rings)</td>
</tr>
<tr>
<td>132.91</td>
<td>7.28, 7.30 (doublet)</td>
<td>2, 6, 2’, 6’ (benzene rings)</td>
</tr>
<tr>
<td>141.76</td>
<td></td>
<td>4, 4’ (benzene rings)</td>
</tr>
<tr>
<td>143.68</td>
<td>8.09 (singlet)</td>
<td>5 (triazole ring)</td>
</tr>
<tr>
<td>153.05</td>
<td>8.07 (singlet)</td>
<td>3 (triazole ring)</td>
</tr>
</tbody>
</table>
ABSTRACT: The purpose of this review is to summarize the status of DNA-based methods for the identification and individualization of marijuana. In forensics, both identification of a substance as marijuana and the subsequent individualization of a sample may be desired for casework. Marijuana identification methods in the United States primarily include biochemical tests and, less frequently, DNA-based tests. Under special circumstances, DNA-based tests can be useful. For example, if the quantity of seized marijuana is extremely small and/or biochemical tests do not detect any Δ9-tetrahydrocannabinol (THC), DNA identification of plant material as Cannabis is still possible. This circumstance can arise when seeds, trace residue, tiny leaf fragments, or fine roots need to be analyzed. Methods for the individualization of marijuana include Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), and Short Tandem Repeat (STR) techniques that link an evidentiary sample to a source. Marijuana growers propagate their plants either by seed or by cloning. Seed-generated marijuana plants are expected to have unique DNA profiles analogous to a human population. Cloned marijuana plants, however, exhibit identical DNA profiles that allow for tracking of plant material derived from a common genetic lineage. The authors have validated the AFLP method for marijuana samples and are constructing a comparative database of marijuana seizure samples to estimate the expected frequency of a DNA profile match between unrelated plants. Continued development of DNA-based methods for plants can be useful for marijuana and other types of plant evidence in forensics.

KEYWORDS: Cannabis; Polymorphism (Genetics); Polymorphism; Restriction Fragment Length; Random Amplified Polymorphic DNA Technique; Tandem Repeat Sequences.
There are two main steps in most forensic classification schemes that can be applied to marijuana seizure samples. The first step involves identification of a sample. For marijuana, both biochemical (9,10) and DNA tests (11-12) are available to identify a substance as *Cannabis*. The second step is individualization (source attribution). For marijuana, several DNA-based methods are under development and will be described in later sections.

Biochemical methods to establish geographic origin of a plant have met with variable success (13-17). However, contaminants (18) and packaging (17) have shown a correlation with marijuana source. Biochemical profiling has also successfully differentiated between resinous and textile *Cannabis* (19), drug subgroups (marijuana, sinsemilla, Thai sticks, ditchweed) (7) and plant gender (20).

*Cannabis* can be seed-propagated or perpetuated through cloning (1,21,22). Seed-propagated plants are expected to have their own unique genotypes analogous to humans selected from a random population. However, plants that have been propagated through cloning should have identical genotypes like identical twins (21,22). Tracking cloned marijuana based on DNA should be relatively simple; seizure samples with identical profiles should have a common genetic source. The ability to link marijuana growers and users to a common distributor by DNA would be a useful investigative tool for narcotics enforcement. In addition, some forensic cases may be able to link a suspect and victim by matching marijuana samples. The Connecticut State Forensic Science Laboratory, along with several other research groups, is in the process of developing DNA-based methods for the individualization of plant (especially marijuana) samples that are seized from crime scenes. Different DNA-based techniques have different applications, benefits and limitations but all can be utilized to supplement existing forensic methods.

**Marijuana Drug Facts**

United States teenagers use marijuana more than any other drug according to the U. S. Government Substance Abuse and Mental Health Services Administration (23,24). For example, 20% of teenagers aged 12 to 17 years have used marijuana at least once (23,24). In comparison, only 3% of teenagers have used Ecstasy and approximately 2% report using cocaine (23,24). Marijuana prices vary depending on the quantity and quality of what is sold and where the consumer is geographically located, however; it is estimated that marijuana is a multi-billion dollar industry in the United States. One primary source of marijuana is from Mexico where the Border Patrol and U. S. Customs Service seize tons of marijuana worth millions of dollars every year (24). In addition to imported marijuana, the U.S. has a very profitable domestic marijuana growing industry (1,5,24,25).

According to the 2001 National Forensic Laboratory Information System (NFLIS) report, 36% of the analyzed drug items at the national level were identified as *Cannabis* compared to 33% as cocaine, 11% as methamphetamine and 8% as heroin, respectively (26). Considerable variation exists in drug types reported across different regions of the United States; it should be noted that these differences could result from different law enforcement strategies or laboratory analysis policies. In general, *Cannabis* is identified in 25% or more of the drug seizures for the United States regardless of geographical region. In 2001, *Cannabis* estimates for the Midwest, the Northeast, the South and the West were 47%, 36%, 36% and 23%, respectively (26).

In 1977, regional narcotics enforcement squads were replaced by a Statewide Narcotics Task Force in Connecticut (27). The Task Force is authorized to enforce the state laws concerning the manufacture, distribution, sale and possession of narcotics and controlled substances. In addition to enforcement, the Connecticut Statewide Narcotics Task Force collects and provides information regarding drug seizures for Connecticut on an annual basis (25,27). Both indoor and outdoor marijuana grow operations have been identified in Connecticut (25,27). The outdoor grow season in Connecticut begins in April and continues until harvest time in mid-September. The indoor grow season is year-round. The Statewide Narcotics Task Force, in conjunction with the Drug Enforcement Administration (DEA), sponsors and coordinates the Domestic Cannabis Eradication/Suppression Program in Connecticut (25,27). For the first nine months of 2002, statistics for the Statewide Narcotics Task Force domestic *Cannabis* eradication program indicated while greater numbers of outdoor plots were identified compared to indoor grow operations, the number of plants seized were comparable between indoor and outdoor cultivation plots (Table 1) (27). Indoor grow operations may be increasing in number and scale or are more easily...
detected based on a comparison of data from the years 1999-2002 (Table 1) (27). According to Connecticut statistics for 2002, marijuana distribution and consumption has steadily increased and the demand for high quality hydroponically grown marijuana has also increased despite the greater cost to the consumer (25,27). Marijuana cultivated in Connecticut represents a small fraction of the amount consumed by its state residents. The majority of consumed marijuana is imported from California, Texas and Mexico (25,27).

Table 1. Statewide Narcotics Task Force Cannabis Eradication Program Statistics.

<table>
<thead>
<tr>
<th>Year</th>
<th>Cultivation</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outdoor</td>
<td># Plots</td>
<td>62</td>
<td>34</td>
<td>32</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td># Plants</td>
<td>4,606</td>
<td>1,208</td>
<td>1,191</td>
<td>1,772</td>
</tr>
<tr>
<td>Indoor</td>
<td># Plots</td>
<td>5</td>
<td>11</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td># Plants</td>
<td>36</td>
<td>333</td>
<td>129</td>
<td>1,117</td>
</tr>
</tbody>
</table>

Although Connecticut is a relatively small marijuana producing state, marijuana usage still continues to be a substantial drug problem. Based on statistics from the Connecticut Department of Public Safety Controlled Substances and Toxicology Laboratory, the percentage of reported marijuana for the past three years (2000-2002) has remained stable (approximately 27%) (28). Reported marijuana drug items are only exceeded by cocaine which averages 35% of the total drug items reported (28). The majority of Cannabis items reported by the Laboratory for 2000-2002 are from four of nine Connecticut counties (Waterbury, New Haven, Hartford and Fairfield) (28). However, marijuana drug items have been identified and seized from all areas of Connecticut (28). The majority of analyzed drug items reported by the Laboratory are comprised of a single identifiable drug substance; less than 1.5% of drug items were reported as drug mixtures (28).

Marijuana Identification

Identifying a plant sample as Cannabis sativa is the first step in determining if an illegal substance has been seized. Methods for the identification of marijuana include: Botanical identification through inspection of the intact plant morphology and growth habit (1,2), microscopical examination of leaves for the presence of cystolith hairs (29-31), chemical screening tests such as the Duquenois-Levine test (32-34), THC identification through biochemical methods (10,19,33,35,36), and the use of molecular sequencing to identify DNA sequence homology to reference marijuana samples (11,12).

Biochemical Tests

Biochemical testing is the most common method for identifying plant material as marijuana. Chemical tests include those developed by Duquenois and other modifications of the original Duquenois test (32-34). Other chemical tests are the Rutgers Identification for Marijuana (RIM) technique and use of gas liquid chromatography (GLC) and high-pressure liquid chromatography (HPLC) to identify cannabinoid compounds (10,15-17,19,35,36). Occasionally, some marijuana samples can’t be identified through chemical means because little or no THC is present. Such situations include seizures of seeds not associated with marijuana plant leaves and

Brought to you by AltGov2 [www.altgov2.org]
cases where the plants have been harvested but the roots have been left at the crime scene. In these situations, DNA testing can provide a means for marijuana identification that would otherwise not be possible.

**DNA Tests**

Although three forms of DNA are present in plant cells (mitochondrial, chloroplast and nuclear), nuclear DNA sequences are most commonly used for plant species identification. DNA-based tests for the identification of marijuana include the molecular analysis of the ITS1, ITS2 and \(trnL\) intron \((11,12,63,64)\). A comparison of the ITS1 and ITS2 Polymerase Chain Reaction (PCR) product sizes in five samples of marijuana and in one sample of a close relative (\(Humulus lupulus\)) revealed a size difference between marijuana and \(Humulus\) for the ITS2 region \((11,12)\). Other tests using PCR amplification and subsequent restriction enzyme digestion of the \(trnL\) region of the chloroplast has shown that marijuana DNA profiles can be generated and compared between samples and may be useful for forensic purposes \((11,12)\).

**Marijuana Individualization**

After a forensic sample has been identified and classified, it becomes important to individualize the sample. Individualization of a sample in a forensic context means to establish a linkage between the evidentiary sample and the source (Figure 1). There are several ways that plant samples can be tested by using DNA-based methodologies in forensics: Randomly Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), and Short Tandem Repeats (STRs) (Table 2).

![Figure 1](image_url)

**Figure 1.** Potential forensic linkages based on individualized marijuana sample information. Growers sharing cloned plants can be associated based on identical, Amplified Fragment Length Polymorphisms (AFLP) profiles. Growers may be linked to major marijuana distributors and marijuana user seizure material may be traced back to a common distributor of clonal marijuana.
Table 2. A comparison of three DNA methods for the individualization of plant samples.*

<table>
<thead>
<tr>
<th>Method</th>
<th>Discrimination Power</th>
<th>Relative Cost</th>
<th>Input DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPDs</td>
<td>moderate¹</td>
<td>low</td>
<td>1-10 nanograms</td>
</tr>
<tr>
<td>AFLPs</td>
<td>high</td>
<td>moderate-high</td>
<td>1-10 nanograms</td>
</tr>
<tr>
<td>STRs</td>
<td>high</td>
<td>moderate-high</td>
<td>1-10 nanograms</td>
</tr>
</tbody>
</table>

* RAPD = Randomly Amplified Polymorphic DNAs; AFLP = Amplified Fragment Length Polymorphisms; STR = Short Tandem Repeats.
¹ Ability to distinguish between unrelated individuals.

Randomly Amplified Polymorphic DNAs (RAPDs)

Randomly Amplified Polymorphic DNA markers are generated in a single standard PCR reaction where the PCR primers consist of random sequences (typically oligomers of 10-15 bases in length). Wherever a PCR primer has sequence homology with the DNA template, it will bind and a PCR product will be formed. The PCR products are of variable size and are separated on a 1% agarose gel and stained with ethidium bromide for detection of the band pattern. No a priori knowledge of an organism’s sequence is required to perform Randomly Amplified Polymorphic DNA analysis; however, the PCR amplification conditions must be held constant to generate consistent band patterns. Randomly Amplified Polymorphic DNA marker analysis requires a single source sample for simple interpretation of band patterns. The method has been used and accepted in court for both criminal and civil cases (37,38). One well-documented plant DNA case involved the use of DNA profiles from *Palo verde* seed pods to link a suspect’s vehicle back to a homicide crime scene (37). In the *Palo verde* case, the DNA results were allowed in court but the statistical significance was not used because the representative population database consisted of too few samples (40 plants). While Randomly Amplified Polymorphic DNA marker analysis is inexpensive and simple to perform, the method has suffered from reproducibility problems between laboratories (39). The reproducibility problems may be attributed to differences in thermal cycler ramp speeds that can affect PCR primer binding to target DNA sequences. In addition, faint bands on agarose gels can be scored differently due to differences in visual assessment between analysts during the detection step (39).

Amplified Fragment Length Polymorphisms (AFLPs)

Amplified Fragment Length Polymorphism markers have been used to distinguish between individuals of many species including plants (40-49), insects (50,51), birds (52), fish (53) and bacteria (54-56). These markers are particularly useful for separating closely related individuals from inbred genetic lines (57) and on any single source sample. Amplified Fragment Length Polymorphism analysis requires the PCR amplification of restriction fragments to which adaptor oligomer sequences have been attached. The PCR primers recognize the adaptor oligomers and bind to amplify different sized DNA fragments to generate a band pattern. The DNA fragments are detected with a DNA sequencer. The sequencer has a laser that will excite the fluorescent dye that was incorporated into the DNA fragments during the PCR amplification step. Labeled DNA fragments are captured by a CCD camera as they pass by the laser and the band patterns are recorded by a computer. Computer analysis software is used to aid in interpretation and scoring of the complex band patterns generated by Amplified Fragment Length Polymorphisms. Since the extent of genetic diversity is unknown in current marijuana seizure populations, the development and validation of a marker system (such as Amplified Fragment Length Polymorphisms) that has a high power of discrimination for closely related individuals is necessary. While the procedure is more complicated than Randomly Amplified Polymorphic DNA or STR analyses, the process utilizes the same equipment and computer analysis software as current STR human identification methods. This means the cost to implement AFLP is minimal for most forensic laboratories with the exception of the Amplified
Fragment Length Polymorphisms database generation for comparative purposes. Validation of the Amplified Fragment Length Polymorphisms method for marijuana samples is complete (21,22) and our analyses of cloned marijuana (courtesy of Dr. Gary Shutler, Royal Canadian Mounted Police) has shown that clonal Amplified Fragment Length Polymorphisms profiles are highly reproducible (Figure 2). In contrast, Amplified Fragment Length Polymorphisms profiles from unrelated marijuana plants are easily distinguishable from each other using this method (Figure 3).

**Figure 2.** Amplified Fragment Length Polymorphism (AFLP) analysis of known clonal generations exhibit identical DNA profiles. Known clonal marijuana generations were propagated by the Royal Canadian Mounted Police (RCMP, Winnipeg) and were generously provided through collaboration with Dr. Gary Shutler.

**Figure 3.** Marijuana samples from unrelated cases have distinct Amplified Fragment Length Polymorphism (AFLP) profile differences. Samples #1-3 were generously provided from adjudicated cases by Dr. Eric Buel (Vermont Crime Laboratory).
Short Tandem Repeats (STRs)

Short Tandem Repeat (STR) sequences refer to repetitive elements found within nuclear DNA that are variable between individuals. The variability in the number of repeated sequences makes these elements useful for distinguishing between individuals of a population. Typically, STR analysis requires a PCR reaction using PCR primers of specific sequence that will bind and recognize a previously characterized site within the nuclear DNA. Short tandem repeat markers are the most common DNA-based method for human identity testing and these sequences are found in many organisms including plants (58,59). STRs can be used with mixtures, i.e., DNA samples from more than one source.

A few polymorphic loci have been recently identified in *Cannabis sativa* (58-61). One study identifies eleven loci that were screened through a blind test of 40 samples to confirm the reproducibility and accuracy of scoring of these candidate loci (61). This same study showed 100% concordance with our Amplified Fragment Length Polymorphisms test results. Another study describes the isolation of a single hexanucleotide repeat sequence in marijuana that was highly polymorphic when screened in a population of 108 marijuana evidentiary samples (59). A third study describes the isolation of ten STRs that were screened against a world-wide population of 255 individuals representing 33 countries (60). Five additional STR markers have been described for *Cannabis* and used to screen 93 marijuana individuals that represent drug and fiber accessions (58).

Although STR markers can be identified in plants, there is significant development and validation time required in establishing this form of testing. For example, genetic mapping to illustrate non-linkage between STR loci is needed for statistical reasons. These candidate marijuana STR markers have only recently been identified, which is the reason why the following experiments for STR loci have not yet been performed:

a) Physical mapping to chromosomes
b) Tests for locus independence
c) Typing a core set of population samples for a direct comparison of candidate loci ability to discriminate between individuals
d) Estimation of the extent of inbreeding in various populations
e) Multiplexing of loci for increased power of discrimination, sample through-put, conservation of evidence and user convenience in a single PCR amplification reaction.

It is anticipated that these types of developmental and additional validation experiments will be performed prior to adoption for forensic casework. STR testing is recognized and accepted as a valid form of DNA testing in United States courts and is extremely useful for mixed samples. The STR loci identified in marijuana should be very useful in the future for establishing forensic linkages between source and evidentiary samples.

Comparative Databases

In order to give significance to the meaning of a random match, comparative databases need to be constructed. When constructing such databases, it is important to consider the sampling strategy and the final purpose of the database. If estimating the level of genetic diversity for evolutionary purposes, a wide distribution of genetically distinct individuals can be screened. If determining a random match probability for marijuana seizure samples, it is important to have a database of seizure sample profiles for comparison. To date, one of the great difficulties in developing tests to individualize marijuana has been acquiring access to adequate numbers of marijuana samples. Nationwide (U.S.) and Connecticut State marijuana databases are under construction (62) and may be used for both establishing the extent of genetic diversity within and between seizure samples and for estimating the expected frequency of a random DNA match.
Conclusion

In the near future, marijuana DNA analysis may be performed in conjunction with chemical identification methods to extend the current capabilities for casework identification on root and seed samples of marijuana. The ability to individualize marijuana samples will further extend the role of DNA in establishing forensic linkages by using plant evidence to link homicides and other types of cases where marijuana samples may be present. The individualizing techniques being developed for marijuana may allow for the identification of a geographic source to aid in the investigation of major marijuana growers and distributors. In particular, cloned marijuana networks may be easily tracked and distributors identified through the common DNA profiles of the seizure samples (21,22,65). In addition, since marijuana samples and drug-generated funds are associated with a wide variety of criminal activities, the applications for marijuana DNA-based tests extend far beyond the obvious use for narcotics enforcement. The success of marijuana DNA typing methods could also become the foundation for using other forms of botanical evidence (grass or tree species) in criminal and civil casework (63-65).

Acknowledgements

The authors are grateful to Dr. Gary Shutler, Janet Hanniman, Sharon Abrams, Chad Johnston, Cst. Giovanni Perisichetti, Cst. Mike Crozier and Steve Towse (Royal Canadian Mounted Police-Winnipeg) for their efforts in producing cloned marijuana and providing extracted DNA. We thank members of the CT Statewide Narcotics Task Force and Captain Peter Warren for their considerable time and effort in collecting samples and providing advice on marijuana propagation. Thank you to Dr. Eric Buel for donating marijuana samples for our study. Thank you to Mr. James Jacewicz, Coordinator of Public Education for the CT Statewide Narcotics Task Force for sharing pre-publication data with us. Thank you to Joselle Germano-Presby, Elizabeth McClure Baker, Eric Carita and Leanne Kushner for their participation in the Amplified Fragment Length Polymorphisms project. Thank you to Nicholas CS Yang for assistance and critical review of the manuscript. A portion of the marijuana project is generously funded by the National Institute of Justice (NIJ grant #2001-IJ-CX-K011).

References


28. Juliano, N. Cannabis and "other drugs" reported results related to evidence submissions and items analyzed - statistical data extracted from the Laboratory Information Management System (LIMS) charted and interpreted. 2003.


43. Law JR, Donini P, Koebner RMD, Reeves JC, Cooke RJ. DNA profiling and plant variety registration.


Abbreviations: Deoxyribonucleic acid (DNA), amplified fragment length polymorphism (AFLP), short tandem repeat (STR), Δ-9-tetrahydrocannabinol (THC), National Forensic Laboratory Information System (NFLIS), Statewide Narcotics Task Force (SNTF), Drug Enforcement Administration (DEA), internal transcribed spacer (ITS), Rutgers Identification of Marijuana (RIM), gas liquid chromatography (GLC), high pressure liquid chromatography (HPLC), random amplified fragment length polymorphism (RAPD), polymerase chain reaction (PCR), amplified fragment length polymorphism (AFLP).

Terms of Use: Copyright © 2003 by the Croatian Medical Journal. All rights reserved. Apart from any relaxation permitted under national copyright laws, no part of this article may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required for a duplicate publication of the article on condition that a full reference to the source is given. Multiple copying of the contents of the article is always illegal.
The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) is an International Working Group dedicated to the development and implementation of minimum standards for the identification of drug exhibits in forensic science laboratories. The primary objectives for the Group are:

* Promote professional development in forensic drug analysis.
* Provide a means for information exchange within the forensic science community.
* Provide guidelines for drug examinations and reporting.
* Specify requirements for analysts’ knowledge, skills, and abilities.
* Establish quality assurance guidelines.
* Promote and gain international acceptance of SWGDRUG standards.

The Eighth SWGDRUG Conference was held October 7-9, 2003 in Montreal, Canada. Core committee members in attendance included:

Susan Ballou (National Institute of Standards and Technology, Gaithersburg, Maryland)
Joseph P. Bono (Drug Enforcement Administration, Office of Forensic Sciences, Arlington, Virginia)
Dr. Bob Bramley (Forensic Science Service, Trident Court, Birmingham, England)
Gary Chasteen (California Association of Criminalists, Los Angeles Country Sheriff’s Laboratory, Downey, California)
Dr. Maria Eugenia Forero (National Institute of Legal Medicine and Forensic Science, Bogotá, Colombia)
Richard Gervasoni (American Society of Crime Laboratory Directors, Montgomery County Police Department Laboratory, Rockville, Maryland)
Linda Jackson (Mid-Atlantic Association of Forensic Scientists, Virginia Division of Forensic Sciences, Richmond, Virginia)
Thomas J. Janovsky (Drug Enforcement Administration, Office of Forensic Sciences, Arlington, Virginia)
Dr. Tohru Kishi (National Research Institute of Police Science, Chiba, Japan)
Richard Laing (Health Canada, Burnaby, British Columbia, Canada)
Two core committee members were unable to attend the Fall 2003 conference:

- Dr. Erkki Sippola (European Network of Forensic Science Institutes (ENFSI), National Bureau of Investigation, Crime Laboratory, Vantaa, Finland)
- Dr. Howard Stead (Division for Policy Analysis and Public Affairs, United Nations Office on Drugs and Crime, Vienna, Austria)

The 2003 SWDRUG conference included the addition of two new Core Committee members. Dr. Maria Eugenia Forero and Mr. Etienne van Zyl were welcomed as members of the Core Committee. Dr. Forero will represent South America, and Mr. van Zyl will represent Africa. This is another step in the process of increasing representation of the Core Committee to include a member from every continent.

Accomplishments

The accomplishments of the Montreal Conference included:

1. The three published recommendations on Education and Training, Methods of Analysis, and Quality Assurance were updated. The Education and Training and Methods of Analysis Recommendations were discussed and accepted by the core committee with minor modifications. The Quality Assurance Recommendations are still under review. In order to address the concerns of forensic drug examiners internationally, one significant change was made to the Methods of Analysis for Drug Identification. This change appears in paragraph 3.5.1. The paragraph now reads:

   “For exhibits of cannabis that lack sufficient observable macroscopic and microscopic botanical detail (e.g., extracts or residues), the Δ9-tetrahydrocannabinol (THC) or other cannabinoids must be identified using the principles set forth in sections 3.1 and 3.2.”

2. After review by forensic drug examiners, the core committee approved a recommendation entitled: Code of Professional Practice for Drug Analysts.
3. After review by forensic drug examiners the core committee approved a recommendation entitled: **Validation of Analytical Techniques**.

4. Review of document entitled: **Sampling Seized Drugs for Qualitative Analysis**. This document has been returned to the subcommittee for reformatting and will be re-submitted to the forensic community for comment in the near future.

All recommendations described above are included in this report. Also included are the current SWGDRUG Glossary of Terms and contact information for all SWGDRUG Core Committee Members.

**SWGDRUG Publication (Part 2)**

The Core Committee agreed to publish a second edition of SWGDRUG recommendations which will include the following parts:

**Part I**  
**Code of Professional Practice**

**Part II**  
**Education and Training**

**Part III**  
**Methods of Analysis**
- A. Sampling for qualitative analysis (Currently under revision for additional comments)
- B. Drug Identification

**Part IV**  
**Quality Assurance**
- A. General Practices (Currently being updated)
- B. Validation of Analytical Techniques

**Issues to Be Addressed at the Next SWGDRUG Conference:**

1. Accept the “Sampling” recommendation.
2. Complete the review of the “revised” Quality Assurance document.
3. Review a glossary for the inclusion in the publication.
4. Discuss specifics of an index
5. Consider an appendix to the validation document.
6. Consider issues to be addressed at SWGDRUG Part III
   a) Uncertainty in quantitative and qualitative analyses
   b) Quantitation of controlled substances
   c) Reporting protocols (What should a report say?)
   d) Clandestine laboratories samples
   e) Inorganic Chemicals
   f) Non-controlled substances
   g) Drug Profiling
   h) Competence

* * * * * * * * * * * * * * * * * * * * * * * * *

Microgram Journal, Volume 1, Numbers 3-4 (July - December 2003)
PART I - A CODE OF PROFESSIONAL PRACTICE FOR DRUG ANALYSTS

PREFACE - This Code of Professional Practice has been written specifically for analysts. However, it is important that their managers and the technicians and others who assist them in their work are equally aware of its provisions, and they support the analyst in adhering to these. Where appropriate, the provisions are also equally applicable to the technicians in the approach to their own work.

SECTION 1: INTRODUCTION

1.1 A Code of Professional Practice is intended to provide the framework of ethical values and scientific and legal obligations within which the analyst should operate. Details are also usually provided on how alleged breaches of the Code will be investigated, what sanctions are available and how appeals should be pursued.

1.2 A Code of Professional Practice is essential to analysts and their managers in helping them carry out their duties in a proper manner and in making appropriate decisions when questions of ethics arise.

1.3 A Code of Professional Practice that is enforced and publicly available is also a powerful means of demonstrating the professional expectations of analysts and the reliability of their findings to others in the criminal justice system and the public at large.

1.4 SWGDRUG recommends that all employers of analysts develop a Code of Professional Practice and the means of dealing with breaches of the Code.

1.5 SWGDRUG further recommends that all Codes of Professional Practice for analysts should include, as a minimum, provisions relating to their professional conduct, their casework and the reporting of their results, as provided in Section 2.

SECTION 2: CODE OF PROFESSIONAL PRACTICE

2.1 Professional Conduct

Analysts should:

2.1.1 Act with honesty, integrity and objectivity;

2.1.2 Work only within the bounds of their professional competence;

2.1.3 Take reasonable steps to maintain their competence;

2.1.4 Recognize that their overriding duty is to criminal justice;

2.1.5 Declare to their employer any prior contact or personal involvement, which may give rise to conflict of interest, real or perceived;

2.1.6 Declare to their employer or other appropriate authority any pressure intended to influence the result of an examination.

2.2 Casework

Analysts should:

Microgram Journal, Volume 1, Numbers 3-4 (July - December 2003) 211
2.2.1 Ensure and be able to demonstrate that the integrity and security of evidential materials and the information derived from their analysis have been maintained while in their possession;

2.2.2 Ensure that they have a clear understanding of what the customer needs and all the necessary information, relevant evidential materials and facilities available to reach a meaningful conclusion in an appropriate timeframe;

2.2.3 Employ an appropriate analytical approach, using the facilities available;

2.2.4 Make and retain full, contemporaneous, clear and accurate records of all examinations and tests conducted, and conclusions drawn, in sufficient detail to allow meaningful review and assessment of the conclusions by an independent person competent in the field;

2.2.5 Accept responsibility for all casework done by themselves and under their direction;

2.2.6 Conduct all professional activities in a way that protects the health and safety of themselves, co-workers, the public and the environment.

2.3 Reporting

Analysts should:

2.3.1 Present advice and testimony, whether written or oral, in a clear and objective manner;

2.3.2 Be prepared to reconsider and, if necessary, change their conclusions, advice or testimony in light of new information or developments, and take the initiative in informing their employer and customers promptly of any such changes that need to be made;

2.3.3 Take appropriate action if there is potential for, or there has been, a miscarriage of justice due to new circumstances that have come to light, incompetent practice or malpractice;

2.3.4 Preserve customer confidentiality unless officially authorized to do otherwise.

APPENDIX

This appendix gives EXAMPLES to demonstrate the scope of the various provisions of the Code.

Casework

2.2.1 To ensure and be able to demonstrate that the integrity and security of evidential materials and the information derived from their analysis have been maintained while in their possession:

- Keeping a record of the chain of custody;
- Making special note of the security of sealing and packaging of the evidential materials as received;
- Preserving the evidential materials from contamination, adulteration, deterioration, loss or theft by use of appropriate working practices and utilization of suitable storage facilities with controlled access;
- Using a unique identifier for the evidential materials, any sub-sample taken from them and any accompanying documentation, that will minimize the risk of misidentification;
- Keeping the evidential materials in their original condition for future reference, insofar as this is possible;
- Securely repackaging and resealing the evidential materials after their examination;
Preserving and returning all original packaging, with original seals intact, where this is possible; Ensuring that access to the evidential materials and all documentation relating to these, before and after their examination, is restricted to authorized personnel.

2.2.2 To ensure that they have a clear understanding of what the customer needs and all the necessary information, relevant evidential materials and facilities available to reach a meaningful conclusion in an appropriate timeframe:

Conferring with the customer, if there is any uncertainty over their requirement; Establishing what work needs to be performed to provide a fit for purpose response to the customer’s requirement; Ensuring that all the requisite information and evidential materials have been submitted; Checking that all the necessary accommodation, equipment, materials and skills will be available when required; Declining to do the testing if the customer’s requirement cannot be met.

2.2.3 To employ an appropriate analytical approach, using the facilities available:

Adhering to the SWGDRUG recommendations;
Performing only those analyses that are needed to meet the specified customer requirement;
Making best use of the available resources in meeting the customer requirement;
Ensuring that the identification and quantification of any drug reflects what was present in the material submitted.

2.2.4 To make and retain full, contemporaneous, clear and accurate records of all examinations and tests conducted, and conclusions drawn, in sufficient detail to allow meaningful review and assessment of the conclusions by an independent person competent in the field:

Writing legibly;
Avoiding use of personal shorthand;
Recording all pertinent information at the time it is generated, or as soon as practicable thereafter;
Ensuring that there can be no uncertainty about what work has been carried out, how, when, where and by whom;
Complying with local jurisdictional requirements;
Consistently maintaining well-ordered casefiles and ensuring that these are available for review.

2.2.5 To accept responsibility for all casework done by themselves and under their direction:

Providing suggestions for improvement;
Ensuring that all work carried out personally and by others under their direction is in compliance with the laboratory’s procedures and protocols;
Providing clear, documented instructions to persons who do work on their behalf that might subsequently be used to support any advice or evidence they give;
Defending and justifying all work that is carried out by themselves and by others on their behalf.

2.2.6 To conduct all professional activities in a way that protects the health and safety of themselves, co-workers, the public and the environment:

Being aware of and complying at all times with current health and safety legislation;
Ensuring that all relevant risk assessments have been carried out and safe systems of work are in place and being followed;
Ensuring that others in the vicinity of their work are aware of their activities, particularly where these involve the investigation of clandestine laboratories, potential exposure to controlled drugs,
especially from bulk seizures, the use of other hazardous materials or the destruction/disposal of drugs and other hazardous materials.

Reporting

2.3.1 To present their advice and testimony, whether written or oral, in a clear and objective manner:

Adhering to the SWGDRUG recommendations;
Using lay terms wherever possible;
Explaining technical terms so that they can be properly understood;
Including only facts and objective interpretations in their advice or evidence that can be justified by the work done and the information available;
Considering and providing alternative explanations or interpretations for their findings, where appropriate;
Making clear the strengths and any limitations in their advice or evidence;
Declaring anything that might undermine the integrity of their evidence or its use (e.g., unsecured packaging; possible contamination).

2.3.2 To be prepared to reconsider and, if necessary, change their conclusions, advice or testimony in light of new information or developments, and take the initiative in informing their employer and customers promptly of any such changes that need to be made;
Accepting an on-going responsibility for any advice or evidence provided;
Immediately bringing to the attention of their employer anything that they have become aware of that might cause them to question the validity of any advice given or evidence provided;
Informing the appropriate external authorities (e.g., police, prosecutor) of their concerns;
Recording in the casefile all such new information, an assessment of its implications and the actions taken.

2.3.3 To take appropriate action if there is potential for, or there has been, a miscarriage of justice due to new circumstances that have come to light, incompetent practice or malpractice:

Informing their employer about the new circumstances;
Informing their employer about relevant concerns they have about the quality of their own work or that of others working under their direction;
Advising their employer of any relevant concerns they may have about the work, advice or evidence provided by others;
Reporting to their employer any relevant concerns that others may have made (e.g., customer complaints; criticisms in court);
Ensuring that the information is brought to the attention of the appropriate external authority.

2.3.4 To preserve customer confidentiality unless officially authorized to do otherwise:

Not disclosing information about a case unless explicitly authorized to do so by the customer, a court, or other body with the relevant statutory powers; required by the law to disclose specified information to a designated person; or an overriding duty to the court and justice system for such disclosure is recognized.
PART II - EDUCATION AND TRAINING

SECTION 1: INTRODUCTION

Part II recommends minimum education, training and experience for analysts practicing in laboratories that conduct seized drug analyses. It describes the types of activities necessary to continue professional development and reference literature required in laboratories where they practice.

1.1 Recommendations listed in Part II are intended to apply to any analyst who

Independently has access to unsealed evidential material in order to remove samples for examination;
Examines and analyzes seized drugs or related materials, or directs such examinations to be done; and
As a consequence of such examinations, signs reports for court or investigative purposes.

SECTION 2: EDUCATION AND EXPERIENCE FOR ANALYSTS

2.1 The aim of this recommendation is that all analysts recruited in the future should have at least a bachelor’s degree, while allowing existing analysts without degrees to be retained as analysts. The minimum educational requirements for analysts are either:

2.1.1 A bachelor’s degree (or equivalent, generally a three to four year post-secondary or tertiary degree) in a natural science or in other sciences relevant to the analysis of seized drugs. The degree program shall include lecture and associated laboratory classes in general, organic and analytical chemistry

or

2.1.2 By January 1, 2005, a minimum of five (5) years practical experience in the area of seized drug analysis, and demonstrated competency following the completion of a formal, documented training program and post training competency assessment.

SECTION 3: CONTINUING PROFESSIONAL DEVELOPMENT

3.1 All forensic scientists have an ongoing responsibility to remain current in their field. In addition, laboratories should provide support and opportunities for continuing professional development. Minimum continuing professional development requirements for a laboratory analyst are:

3.1.1 Twenty contact hours of training every year. Contact is defined as face-to-face interaction with an instructor or trainer in a classroom or laboratory setting. It does not include self-paced learning or distance education where the instructor has no active interaction with the student.

3.1.1.1 Training must be relevant to the laboratory's mission.

This statement is purposely broad to embrace the laboratory's broader needs such as ancillary duty assignments and supervision/management.

3.1.1.2 Training completed must be documented.

3.1.1.3 Training can be provided from a variety of sources, including, but not limited to the following:
3.1.1.3.1 Chemistry or instrumental courses taught at the post-secondary educational level

3.1.1.3.2 Instrument operation or maintenance courses taught by vendors

3.1.1.3.3 In-service classes conducted by the employer

3.1.1.3.4 In-service training taught by external providers

3.1.1.3.5 Participation in relevant scientific meetings or conferences (e.g., presenting a paper, attending a workshop, providing reports on conferences).

SECTION 4: INITIAL TRAINING REQUIREMENTS

4.1 These minimum requirements allow individual laboratories to structure their training program to meet their needs as it relates to type of casework encountered, analytical techniques, available instrumentation and level of preparedness of trainees.

4.2 There must be a documented training program, approved by laboratory management, that focuses on the development of theoretical and practical knowledge, skills and abilities necessary to examine seized drug samples and related materials. The training program must include the following:

4.2.1 Documented standards of performance and a plan for assessing theoretical and practical competency against these standards (e.g., written and oral examinations, critical reviews, analysis of unknown samples and mock casework per topic area)

4.2.2 A training syllabus providing descriptions of the required knowledge and skills in specific topic areas in which the analyst is to be trained, milestones of achievement, and methods of testing or evaluating competency

4.2.3 A period of supervised casework representative of the type the analyst will be required to perform

4.2.4 A verification document demonstrating that the analyst has achieved the required competence

4.3 Topic areas in the training program will include, as a minimum, the following:

4.3.1 Relevant background information on drugs of abuse (e.g., status of control and chemical and physical characteristics)

4.3.2 Techniques, methodologies and instrumentation utilized in the examination of seized drug samples and related materials

4.3.3 Quality assurance

4.3.4 Expert/Court testimony and legal requirements

4.3.5 Laboratory policy and procedures (such as sampling, evidence handling, safety and security) as they relate to the examination of seized drug samples and related materials.

4.4 An individual qualified to provide instruction must have demonstrated competence in the subject area and in the delivery of training.
SECTION 5: REFERENCES AND DOCUMENTS

5.1 The following references and documents must be available and accessible to analysts:

5.1.1 College/university level textbooks for reference to theory and practice in key subject areas, e.g., general chemistry, organic chemistry and analytical chemistry

5.1.2 Reference literature containing physical, chemical and analytical data. Such references include the Merck Index, Clarke’s Analysis of Drugs and Poisons, laboratory manuals of the United Nations Drug Control Program, in-house produced spectra and published standard spectra, (e.g., Mills And Roberson’s Instrumental Data For Drug Analysis, or compendiums from Pfleger or Wiley)

5.1.3 Operation and maintenance manuals for each analytical instrument


5.1.5 Laboratory quality manual, standard operating procedures, and method validation and verification documents

5.1.6 Relevant jurisdictional legislation (e.g., statutes and case law relating to controlled substances, and health and safety legislation).

* * * * * * * * * * * * * * * * * * * * * * * * *

Brought to you by AltGov2 [www.altgov2.org]
PART III B - METHODS OF ANALYSIS/DRUG IDENTIFICATION

SECTION 1: INTRODUCTION

The purpose of PART III B is to recommend minimum standards for the forensic identification of commonly seized drugs. It is recognized that the correct identification of a drug or chemical depends on the use of an analytical scheme based on validated methods and the competence of the analyst. SWGDRUG requires the use of multiple uncorrelated techniques. It does not discourage the use of any particular method within an analytical scheme and it is accepted that unique requirements in different jurisdictions may dictate the actual practices followed by a particular laboratory.

SECTION 2: CATEGORIZING ANALYTICAL TECHNIQUES

2.1 Techniques for the analysis of drug samples may be classified into three categories based on their discriminating power. Table 1 provides examples of these techniques listed in order of decreasing discriminating power from A to C.

<table>
<thead>
<tr>
<th>Table 1: Categories of Analytical Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category A</strong></td>
</tr>
<tr>
<td>Infrared Spectroscopy</td>
</tr>
<tr>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>Raman Spectroscopy</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cannabis Only:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

SECTION 3: IDENTIFICATION CRITERIA

SWGDRUG recommends that laboratories adhere to the following minimum standards:

3.1 When a validated Category A technique is incorporated into an analytical scheme, then at least one other technique (from either Category A, B or C) must be used.

3.1.1 This combination must identify the specific drug present and must preclude a false positive identification.
3.1.2 When sample size allows, the second technique should be applied on a separate sampling for quality assurance reasons. When sample size is limited, additional measures should be taken to assure that the results correspond to the correct sample.

3.1.3 All Category A techniques must have data that are reviewable.

3.2 When a Category A technique is not used, then at least three different validated methods must be employed.

3.2.1 These in combination must demonstrate the identity of the specific drug present and must preclude a false positive identification.

3.2.2 Two of the three methods must be based on uncorrelated techniques from Category B.

3.2.3 A minimum of two separate samplings should be used in these three tests. When sample size is limited, additional measures should be taken to assure that the results correspond to the correct sample.

3.3.4 All Category B techniques must have reviewable data.

3.3 For the use of any method to be considered of value, test results must be considered “positive.” While “negative” test results provide useful information for ruling out the presence of a particular drug or drug class, these results have no value toward establishing the forensic identification of a drug.

3.4 In cases where hyphenated techniques are used (e.g., gas chromatography-mass spectrometry, liquid chromatography-diode array ultraviolet spectroscopy), they will be considered as separate techniques provided that the results from each are used.

3.5 Cannabis exhibits tend to have characteristics that are visually recognizable. Macroscopic and microscopic examinations of cannabis will be considered, exceptionally, as uncorrelated techniques from Category B when observations include documented details of botanical features. Additional testing must follow the scheme outlined in sections 3.1 or 3.2.

3.5.1 For exhibits of cannabis that lack sufficient observable macroscopic and microscopic botanical detail (e.g., extracts or residues), Δ⁹-tetrahydrocannabinol (THC) or other cannabinoids must be identified utilizing the principles set forth in sections 3.1 and 3.2.

3.6 Examples of reviewable data are:

3.6.1 Printed spectra, chromatograms and photographs or photocopies of TLC plates

3.6.2 Contemporaneous documented peer review for microcrystalline tests

3.6.3 Recording of detailed descriptions of morphological characteristics for cannabis (only)

3.6.4 Reference to published data for pharmaceutical identifiers.

SECTION 4: COMMENT

These recommendations are minimum standards for the forensic identification of commonly seized drugs. However, it should be recognized that they may not be sufficient for the identification of all drugs in all circumstances. Within these recommendations, it is up to the individual laboratory’s management to determine which combination of analytical techniques best satisfies the requirements of its jurisdiction.
PART IV A - QUALITY ASSURANCE/GENERAL PRACTICES

[Note: The Recommendations in this Part Are Currently Being Re-Evaluated and Updated]

SECTION 1: INTRODUCTION

Recommendations IN PART IV involving the analysis of seized drugs are limited to qualitative analysis only. Issues involving quantitative analysis will be taken up in a later version.

It is the goal of a laboratory's drug analysis program to provide the customers of the laboratory's services access to quality drug analysis. It is the goal of these guidelines PART IV to provide a quality framework for managing the processing of drug casework, including handling of evidentiary material, management practices, analysis and reporting. These are minimum recommendations for practice.

The term “evidence” has many meanings throughout the international community. In this document it is used to describe drug exhibits which enter a laboratory system.

1.1 QUALITY MANAGEMENT SYSTEM

A documented quality management system must be established and maintained. Personnel responsible for this must be clearly designated and shall have direct access to the highest level of management concerning laboratory policy.

1.1.1 The quality management system must cover all procedures and reports associated with drug analysis.

SECTION 2: PERSONNEL

2.1 JOB DESCRIPTION

The job descriptions for all personnel should include responsibilities, duties and required skills.

2.2 DESIGNATED PERSONNEL AND RESPONSIBILITIES

An individual (however titled) may be responsible for one or more of the following duties:

2.2.1 Quality Assurance Manager: A designated person who is responsible for maintaining the quality management system (including an annual review of the program) and who monitors compliance with the program.

2.2.2 Health & Safety Manager: A designated person who is responsible for maintaining the Laboratory Health and Safety program (including an annual review of the program) and who monitors compliance with the program.

2.2.3 Technical Support Personnel: Individuals who perform basic laboratory duties, but do not analyze evidence.

2.2.4 Technician/Assistant Analyst: A person who analyzes evidence, but does not issue reports for court purposes.

2.2.5 Analyst: A designated person who
2.2.5.1 Examines and analyzes seized drugs or related materials, or directs such examinations to be done

2.2.5.2 Independently has access to unsealed evidence in order to remove samples from the evidentiary material for examination AND

2.2.5.3 As a consequence of such examinations, signs reports for court or other purposes.

2.2.6 Supervisor: A designated person who has the overall responsibility and authority for the technical operations of the drug analysis section. Technical operations include, but are not limited to protocols, analytical methodology, and technical review of reports.

2.3 QUALIFICATIONS/EDUCATION

2.3.1 Technical Support Personnel will

2.3.1.1 Have education, skills and abilities commensurate with their responsibilities AND

2.3.1.2 Have on-the-job training specific to their position.

2.3.2 Technicians/Assistant Analysts will

2.3.2.1 Have education, skills and abilities commensurate with their responsibilities AND

2.3.2.2 Have on-the-job training specific to their position.

2.3.3 Analysts will have

2.3.3.1 A bachelor’s degree (or equivalent, generally a three to four year post-secondary or tertiary degree) in a natural science or in other sciences relevant to the analysis of seized drugs. The degree program shall include lecture and associated laboratory classes in general, organic and analytical chemistry

or

2.3.3.2 By January 1, 2005, a minimum of five (5) years practical experience in the area of seized drug analysis, and have demonstrated competency following the completion of a formal, documented training program and post training competency assessment.

2.3.4 Supervisors will

2.3.4.1 Meet all the requirements of an analyst (2.3.3),

2.3.4.2 Have a minimum of two (2) years of experience as an analyst in the forensic analysis of drugs and

2.3.4.3 Demonstrate knowledge necessary to evaluate analytical results and conclusions.

2.4 INITIAL TRAINING REQUIREMENTS

2.4.1 These minimum requirements allow individual laboratories to structure their training program to meet their needs as it relates to type of casework encountered, analytical techniques, available instrumentation and level of preparedness of trainees.
2.4.2 There must be a documented training program, approved by laboratory management, which focuses on the development of theoretical and practical knowledge, skills and abilities necessary to examine seized drug samples and related materials. The training program must include the following:

2.4.2.1 Documented standards of performance and a plan for assessing theoretical and practical competency against these standards (e.g., written and oral examinations, critical reviews, analysis of unknown samples and mock casework per topic area)

2.4.2.2 A training syllabus providing descriptions of the required knowledge and skills in specific topic areas in which the analyst is to be trained, milestones of achievement, and methods of testing or evaluating competency

2.4.2.3 A period of supervised casework representative of the type the analyst will be required to perform

2.4.2.4 A verification document demonstrating that the analyst has achieved the required competence.

2.5 MAINTAINING COMPETENCE

2.5.1 Minimum annual training required for continuing professional development of analysts is twenty (20) contact hours.

2.5.1.1 Training must be relevant to the laboratory's mission.

2.5.1.2 Training completed must be documented.

SECTION 3: PHYSICAL PLANT

3.1 PHYSICAL PLANT REQUIREMENTS

3.1.1 Laboratories shall provide a healthy, safe and secure environment for its personnel and operations.

3.1.2 Laboratories must contain adequate space to perform required analytical functions and prevent contamination.

3.1.3 Chemical fume hoods must be provided. They must be properly maintained and monitored according to an established schedule.

3.1.4 A laboratory cleaning schedule must be established and implemented.

3.1.5 Adequate facilities must be provided to ensure the proper safekeeping of evidence, standards and records.

3.1.6 Appropriately secured storage must be provided to prevent contamination of chemicals and reagents.

SECTION 4: EVIDENCE CONTROL

Laboratories shall have and follow a documented evidence control system to ensure the integrity of physical
4.1 RECEIVING AND IDENTIFYING EVIDENCE

Laboratories must maintain records of requests for analysis and of the respective items of evidence. A unique identifier must be assigned to each case file or record. For chain-of-custody purposes, the evidence shall be compared to the submission documentation, any significant observations of irregularity should be documented in the case file or record, and the submitter informed promptly. This file or record must include, at least, the following:

4.1.1 Submission documents or copies
4.1.2 Identity of party requesting analysis and the date of request
4.1.3 Description of items of evidence submitted for analysis
4.1.4 Identity of the person who delivers the evidence, along with date of submission
   4.1.4.1 For evidence not delivered in person, descriptive information regarding mode of delivery and tracking information
4.1.5 Chain of custody record
4.1.6 Unique case identifier

4.2 INTEGRITY OF EVIDENCE

Evidence must be properly secured. Appropriate storage conditions shall ensure that, insofar as possible, the composition of the seized material is not altered. All items must be safeguarded against loss or contamination. Any alteration of the evidence (e.g., repackaging) must be documented in writing. Procedures should be implemented to assure that samples are AND REMAIN properly labeled throughout the analytical process.

4.3 STORAGE OF EVIDENCE

Access to the evidence storage area must be granted only to persons with authorization and access shall be controlled. A system shall be established to document the chain of custody FOR EVIDENCE IN LABORATORY CUSTODY.

4.4 DISPOSITION OF EVIDENCE

Records must be kept regarding the disposition of all items of evidence.

4.5 DOCUMENTATION PROCEDURES

All laboratory records such as results of analyses, measurements, notes, calibrations, chromatograms, spectra and reports shall be retained in a secure fashion.

SECTION 5: ANALYTICAL PROCEDURES

5.1 ANALYTICAL PROCEDURES FOR DRUG ANALYSIS

5.1.1 The laboratory shall have and follow written analytical procedures.
5.1.2 The laboratory shall have in place protocols for the sampling of evidence.

5.1.3 Work practices shall be established to prevent contamination of evidence during analysis.

5.1.4 The laboratory shall monitor the analytical processes using appropriate controls and traceable standards.

5.1.5 The laboratory shall have and follow written guidelines for the acceptance and interpretation of data.

5.1.6 Analytical procedures must be validated in compliance with Section 10.

5.1.7 The analyst shall determine the identity of a drug in a sample, and be assured that the result relates to the right submission. This is best established by the use of at least two appropriate techniques based on different principles and two independent samplings.

5.2 MINIMUM REQUIREMENTS FOR THE VERIFICATION OF DRUG REFERENCE MATERIALS FOR FORENSIC DRUG ANALYSIS.

5.2.1 The identity of certified reference materials should be verified prior to their first use.

5.2.2 The identity of uncertified reference materials must be authenticated prior to use by methods such as mixed melting point determination, Mass Spectrometry, Infrared Spectroscopy, or Nuclear Magnetic Resonance Spectrometry.

5.2.3 Verification must be performed on each new drug lot.

5.2.4 All verification testing must be documented to include the name of the individual who performed the identification, date of verification, verification test data, and reference identification.

SECTION 6: INSTRUMENT/EQUIPMENT PERFORMANCE

6.1 INSTRUMENT PERFORMANCE

Instruments must be routinely monitored to ensure that proper performance is maintained.

6.1.1 Monitoring should include the use of reference standards, test mixtures, calibration standards, blanks, etc.

6.1.2 Instrumentation performance monitoring must be documented.

6.2 EQUIPMENT

Only suitable and properly operating equipment shall be employed. Monitoring of equipment parameters shall be conducted and documented.

6.2.1 The manufacturer's operation manual and other relevant documentation for each piece of equipment should be readily available.

SECTION 7: CHEMICALS AND REAGENTS

7.1 CHEMICALS AND REAGENTS
7.1.1 Chemicals and reagents used in drug testing must be of the appropriate grade for the tests performed.

7.1.2 There must be written formulations for all chemical reagents produced within the laboratory.

7.1.3 Documentation for reagents prepared within the laboratory must include identity, concentration (when appropriate), date of preparation, identity of the individual preparing the reagents and the expiration date (if appropriate).

7.1.4 The efficacy of all test reagents must be checked prior to their use in casework. The results of these tests should be documented.

7.1.5 Chemical and reagent containers should be dated and initialed when received and also when first opened.

SECTION 8: CASEWORK DOCUMENTATION, REPORT WRITING AND REVIEW

8.1 CASEWORK

8.1.1 Documentation must contain sufficient information to allow a peer to evaluate case notes and interpret the data.

8.1.2 Evidence handling documentation should include chain of custody, the initial weight/count of evidence to be examined (upon receipt by the analyst), information regarding the packaging of the evidence upon receipt, a description of the evidence and communications regarding the case.

8.1.3 Analytical documentation should include procedures, standards, blanks, observations, results of the tests, and supporting documentation including charts, graphs, and spectra generated during an examination.

8.1.4 Casework documentation must be preserved according to written laboratory policy.

8.2 REPORT WRITING

8.2.1 Reports issued by the laboratory must meet the requirements of the jurisdiction served. These may include:

8.2.1.1 Identity of the examining laboratory

8.2.1.2 Case identifier

8.2.1.3 Identity of the contributor

8.2.1.4 Date of receipt

8.2.1.5 Date of report

8.2.1.6 Descriptive list of submitted evidence

8.2.1.7 Identity of analyst

8.2.1.8 Results/Conclusions
8.2.1.9 Analytical techniques employed

8.3 CASE REVIEW

8.3.1 The laboratory must have a written policy establishing the protocols for technical and administrative case review.

8.3.2 The laboratory must have a written policy to determine the course of action should an analyst and reviewer fail to agree.

SECTION 9: PROFICIENCY AND COMPETENCY TESTING

Each laboratory should participate in at least an annual inter-laboratory proficiency testing program and should have written protocols for testing the competency of its laboratory analysts.

9.1 PROFICIENCY TESTING

9.1.1 Laboratories shall perform proficiency testing in order to verify the laboratory’s performance in comparison to other laboratories. The frequency of the proficiency testing should be at least annually.

9.1.2 The proficiency testing samples should be representative of the laboratory's normal casework.

9.1.3 The analytical scheme should be in concert with the normal laboratory analysis procedures.

9.2 COMPETENCY TESTING

9.2.1 Laboratories will monitor the competency of their analysts. They should do so at least once a year. One of the ways of doing this is by participating in competency tests.

9.2.2 Competency testing samples should be representative of the laboratory's normal casework.

9.2.3 The analytical scheme should be in concert with the normal laboratory analysis procedures.

SECTION 10: VALIDATION AND VERIFICATION

10.1 Method validation is required to demonstrate that the method is suitable for its intended purpose.

10.1.1 For qualitative analysis the parameters that need to be checked are specificity, limit of detection, and reproducibility.

10.1.2 Minimum acceptability criteria should be described along with means for demonstrating compliance.

10.1.3 Validation documentation is required.

10.2 Laboratories adopting methods validated elsewhere should determine their own limit of detection and reproducibility.
SECTION 11: LABORATORY AUDITS

11.1 Audits of laboratory operations should be conducted at least once a year.

11.2 Records of each audit must be maintained and should include the scope, date of the audit, name of the person conducting the audit, findings, and corrective actions taken, if necessary.

SECTION 12: DEFICIENCY OF ANALYSIS

In the course of examining seized drug samples and related materials, laboratories may expect to encounter some operations or results that are deficient in some manner. Each laboratory must have a written policy to deal with such deficiencies.

12.1 This policy must include the following:

12.1.1 A definition of a deficiency as any erroneous analytical result or interpretation, or any unapproved deviation* from an established policy or procedure in an analysis.

* Deviations from established policy must have documented management approval.

12.1.2 A requirement for immediate cessation of the activity or work of the individual involved, if warranted by the seriousness of the deficiency, as defined in the written policy.

12.1.3 A requirement for administrative review of the activity or work of the individual involved.

12.1.4 A requirement for evaluation of the impact this deficiency may have had on other activities of the individual(S) or other analysts.

12.1.5 A requirement for documentation of the follow-up action taken as a result of the review.

12.1.6 A requirement for communication to appropriate employees of any confirmed deficiency which may have implications for their work.

Comment: It should be recognized that to be effective, the definition for "deficiency of analysis" must be relatively broad. As such, deficiencies may have markedly different degrees of seriousness. For example, a misidentification of a controlled substance would be very serious and perhaps require that either the methodology or the analyst be suspended pending appropriate remedial action, as determined by management. However, other deficiencies might be more clerical in nature, requiring a simple correction at the first line supervisory level, without any suspension of methodology or personnel. Thus, it may well be advantageous to identify the differing levels of seriousness for deficiencies and make the action required be commensurate with the seriousness.

SECTION 13: HEALTH AND SAFETY

The laboratory must have a documented health and safety program in place to meet the needs of the laboratory.

13.1 HEALTH AND SAFETY REQUIREMENTS

13.1.1 All personnel should receive appropriate health and safety training.

13.1.2 The drug analysis laboratory shall operate in accordance with laboratory policy and comply with any relevant statutory regulations.
13.1.3 Laboratory health, and safety manual(s) shall be readily available to all laboratory personnel.

13.1.4 Material Safety Data Sheets (MSDS) shall be readily available to all laboratory personnel.

13.1.5 All chemicals, biohazards and supplies must be stored and disposed of according to applicable government regulations and laboratory policy.

13.1.6 Safety hazards such as syringes, items with sharp edges or noxious substances should be so labeled.

SECTION 14: DOCUMENTATION

In addition to casework documentation, the forensic laboratory must maintain documentation on the following topics:

14.1 Test methods/procedures for drug analysis.

14.2 Reference standards (including source and verification).

14.3 Preparation and testing of reagents.

14.4 Evidence handling protocols.

14.5 Equipment calibration and maintenance.

14.6 Equipment inventory (e.g., manufacturer, model, serial number, acquisition date).

14.7 Proficiency testing.

14.8 Personnel training and qualification.

14.9 Quality assurance protocols and audits.

14.10 Health, safety and security protocols.

14.11 Validation data and results.

* * * * * * * * * * * * * * * * * * * * * * * * *
PART IV B - QUALITY ASSURANCE/VALIDATION OF ANALYTICAL METHODS

SECTION 1. INTRODUCTION

1.1 Definition and purpose of Validation

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. There are numerous documents that address the topic of validation but there are few validation protocols for methods specific to seized drug analysis.

1.2 Analytical scheme

An analytical scheme must be comprised of validated methods that are appropriate for the analyte.

A) The combinations of methods chosen for a particular analytical scheme must identify the specific drug of interest, preclude a false positive and minimize false negatives.

B) For quantification the method should reliably determine the amount of analyte present.

C) If validated methods are used from published literature or another laboratory’s protocols, then the methods must be verified within each laboratory.

D) Verification should, at a minimum, demonstrate that a representative set of reference materials has been carried through the process and yielded the expected results.

1.3 Individual laboratory responsibility

Each laboratory should determine whether their current standard operating procedures have been validated, verified or require further validation/verification.

1.4 Operational environment

All methods must be validated or verified to demonstrate that they will perform in the normal operational environment when used by individuals expected to utilize the methods on casework.

1.5 Documentation

The entire validation/verification process must be documented and the documentation must be retained. Documentation must include, but is not limited to the following:

- Personnel involved
- Dates
- Observations from the process
- A statement of conclusions and/or recommendations
- Authorization approval signature

1.6 Recommendation

To meet the above requirements, SWGDRUG recommends that laboratories follow the applicable provisions of Section 2 [General Validation Plan] when validating seized drug analytical methods.
SECTION 2. GENERAL VALIDATION PLAN

2.1 Purpose/Scope

This is an introductory statement that will specify what is being tested, the purpose of the testing and the result(s) required for acceptance.

2.1.1 Performance Specification

A list of specific objectives (e.g., trueness and precision) should be determined prior to the validation process.

2.1.2 Process Review

After completion of the validation process the objectives should be revisited to ensure that they have been satisfactorily met.

2.2 Analytical Method

State exactly the method to be validated. It is essential that each step in the method is demonstrated to perform satisfactorily. Steps that constitute a method for the identification and/or quantification of seized drugs may include:

Visual characterization (e.g., macroscopic examination)
Determination of quantity of sample, which may include:
  - Weight
  - Volume
  - Item count
Sampling (representative or random, dry, homogenized, etc.)
Sample preparation
  - Extraction method
  - Dissolution
  - Derivatization
  - Crystallization
Techniques for introducing the sample into the instrumentation
Instrumental parameters and specifications
  - A list of the instruments and equipment (e.g., balance and glassware) utilized
Instrument conditions
Software applications
Calculations
  - Equation(s) to be used
  - Unit specification
  - Number of measurements required
  - Reference values
  - Significant figure conventions
  - Conditions for data rejection
  - Uncertainty determination

2.3 Reference materials in validation

Appropriate reference material(s) must be used for qualitative and quantitative procedures.

2.4 Performance Characteristics
2.4.1 Selectivity

Assess the capability of the method to identify/quantify the analyte(s) of interest, whether pure or in a mixture.

2.4.2 Matrix effects

Assess the impact of any interfering components and demonstrate that the method works in the presence of substances that are commonly encountered in seized drug samples (e.g., cutting agents, impurities, by-products and precursors).

2.4.3 Recovery

May be determined for quantitative analysis.

2.4.4 Accuracy

2.4.4.1 Precision (repeatability/reproducibility)

Determine the repeatability and reproducibility of all routine methods. Conditions under which these determinations are made must be specified. [Note: Reproducibility determination may be limited to studies within the same laboratory.]

A) Within the scope of the validation, determine the acceptable limits for repeatability and reproducibility.
B) For qualitative analysis, run the qualitative method a minimum of ten times.
C) For quantitative analysis, run the quantitative method a minimum of ten times.
D) Validation criteria for non-routine methods may differ from those stated above.

2.4.4.2 Trueness

Trueness must be determined for quantitative methods to assess systematic error. Trueness can be assessed through various methods such as:

A) Comparison of a method-generated value for the reference material with its known value using replicate measurements at different concentrations.
B) Performance of a standard addition method.
C) Comparison to proficiency test results.
D) Comparison with a different validated analytical method.

2.4.5 Range

Determine the concentration or sample amount limits for which the method is applicable.

2.4.5.1 Limit of detection (LOD)

Limit of Detection (LOD) must be determined for all qualitative methods.

A) Determine the lowest amount of analyte that will be detected and can be identified.
B) The results obtained at the LOD are not necessarily quantitatively accurate.

2.4.5.2 Limit of quantitation (LOQ)

Limit of Quantitation (LOQ) must be determined for all quantitative methods. Determine
the lowest concentration that has an acceptable level of uncertainty.

2.4.5.3 Linearity

Linearity must be determined for all quantitative methods.

A) Determine the mathematical relationship (calibration curve) that exists between concentration and response over a selected range of concentrations.
B) The LOQ effectively forms the lower end of the working range.
C) Determine the level of acceptable variation from the calibration curve at various concentrations.
D) Determine the upper limits of the working range.

2.4.6 Robustness

Robustness must be determined for both qualitative and quantitative methods. Alter method parameters individually and determine any changes to accuracy.

2.4.7 Ruggedness

Ruggedness may be determined for qualitative or quantitative methods.

Alter the analysts, instrumentation and environment and assess the changes in accuracy.

2.5 Uncertainty

The contribution of random and systematic errors to method result uncertainty must be assessed and the expanded uncertainty derived for quantitative methods.

3. Quality Control

Acceptance criteria for quality control parameters should be adopted prior to implementation of the method.

4. References


###SWGDRUG GLOSSARY

These definitions were developed and adopted by the SWGDRUG Core Committee from a variety of sources including The United Nations Glossary of Terms for Quality Assurance and Good Laboratory Practices.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accreditation</strong></td>
<td>Procedure by which an accreditation body formally recognizes that a laboratory or person is competent to carry out specific tasks.</td>
</tr>
<tr>
<td><strong>Accreditation Body</strong></td>
<td>Independent science-based organization that has the authority to grant accreditation.</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>Trueness and precision compose accuracy.</td>
</tr>
<tr>
<td><strong>Analysis</strong></td>
<td>Technical operation to determine one or more characteristics of, or to evaluate the performance of, a given product, material, equipment, physical phenomenon, process, or service according to a specified procedure.</td>
</tr>
<tr>
<td><strong>Analyst</strong></td>
<td>A designated person who:</td>
</tr>
<tr>
<td></td>
<td>- Examines and analyzes seized drugs or related materials, or directs such examinations to be done.</td>
</tr>
<tr>
<td></td>
<td>- Independently has access to &quot;open&quot; (unsealed) evidence in order to remove samples from the evidence for examination.</td>
</tr>
<tr>
<td></td>
<td>- As a consequence of such examinations, signs reports for court or other purposes.</td>
</tr>
<tr>
<td><strong>Audit</strong></td>
<td>A review conducted to compare the various aspects of the laboratory’s performance with a standard for that performance.</td>
</tr>
<tr>
<td><strong>Blank</strong></td>
<td>Specimen or sample not containing the analyte.</td>
</tr>
<tr>
<td><strong>Calibration</strong></td>
<td>Set of operations that establishes, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding known values of a measurand.</td>
</tr>
<tr>
<td><strong>Certified Reference Material (CRM)</strong></td>
<td>A reference material, one or more of whose property values have been certified by a technical procedure, accompanied by or traceable to a certificate or other documentation that has been issued by a certifying body.</td>
</tr>
<tr>
<td><strong>Certifying Body</strong></td>
<td>Independent science-based organization that has the competence to grant certification.</td>
</tr>
<tr>
<td><strong>Chain of Custody</strong></td>
<td>Procedures and documents that account for the integrity of a sample by tracking its handling and storage from its point of collection to its final disposition.</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>Samples used to determine the validity of the calibration, that is, the linearity and stability of a quantitative test or determination over time. Controls are either prepared from the reference material (separately from the calibrators, that is, weighed or measured separately), purchased, or obtained from a pool of previously analyzed samples. Where possible, controls should be matrix-matched to samples and calibrators.</td>
</tr>
<tr>
<td><strong>Control Sample</strong></td>
<td>A standard of comparison for verifying or checking the finding of an experiment.</td>
</tr>
<tr>
<td><strong>Correlated Techniques</strong></td>
<td>Correlated techniques are those that have the same fundamental mechanism of characterization. For example, this would prevent the choice of two gas chromatographic tests both based on a partition mechanism (e.g., methylsiloxane</td>
</tr>
</tbody>
</table>
and phenylmethylsiloxane) or two thin layer chromatographic systems both based on an adsorption mechanism.

**Deficiency of Analysis** Any erroneous analytical result or interpretation, or any unapproved deviation from an established policy or procedure in an analysis.

**False Positive** Test result that states that a drug is present when, in fact, such a drug is not present in an amount less than a threshold or designated cut-off concentration.

**Health & Safety Manager** A designated person who is responsible for maintaining the laboratory health and safety program (including an annual review of the program) and who monitors compliance with the program.

**Independent Test Result** Result obtained in a manner not influenced by any previous results on the same or similar material.

**Laboratory** Facilities where analyses are performed by qualified personnel using adequate equipment.

**Limit of Detection:** Limit of detection (LOD) is the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty.

**Limit of Quantitation:** The limit of quantitation (LoQ) is the lowest concentration of analyte that can be determined with an acceptable level of precision and trueness.

**Linearity:** Defines the ability of the method to obtain test results proportional to the concentration of analyte.

**Method** Detailed, defined procedure for performing an analysis. See Procedure.

**Procedure** Specified, documented way to perform an activity.

**Proficiency Testing** Ongoing process in which a series of proficiency samples, the characteristics of which are not known to the participants, are sent to laboratories on a regular basis. Each laboratory is tested for its accuracy in identifying the presence (or concentration) of the drug using its usual procedures.

**Qualitative Analysis** Test that determines the presence or absence of specific drugs in the sample.

**Qualitative Test** See Qualitative Analysis

**Quality Assurance (QA)** System of activities whose purpose is to provide, to the producer or user of a product or a service, the assurance that it meets defined standards of quality with a stated level of confidence.

**Quality Assurance Manager** A designated person who is responsible for maintaining the quality management system and who monitors compliance with the program.

**Quality Management** That aspect of the overall management function that determines and implements the quality policy.

**Quality Manual** Document stating the general quality policies, procedures and practices of an organization.
Quantitative Analysis Procedure to determine the quantity of drug present in a sample.

Quantitative Test See Quantitive Analysis

Range Set of concentrations of analyte in which the error of a method is intended to lie within specified limits.

Reference Material Material or substance one or more properties of which are sufficiently well established to be used for calibrating an apparatus, assessing a measurement method, or assigning values to materials.

Repeatability Closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement.

Report Document containing a formal statement of results of tests carried out by a laboratory.

Representative Sample Statistically, a sample that is similar to the population from which it was drawn. When a sample is representative, it can be used to make inferences about the population. The most effective way to get a representative sample is to use random methods to draw it. Analytically, it is a sample that is a portion of the original material selected in such a way that is possible to relate the analytical results obtained from it to the properties of the original material.

Reproducibility Closeness of agreement between the results of successive measurements of the same analyte in identical material made by the same method under different conditions, e.g., different operators and different laboratories and considerably separated in time.

Robustness The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Sample A portion of the whole material to be tested. Statistically, it is a set of data obtained from a population.

Sampling Analytically, the whole set of operations needed to obtain a sample, including planning, collecting, recording, labeling, sealing, shipping, etc. Statistically, it is the process of determining properties of the whole population by collecting and analyzing data from a representative segment of it.

Selectivity Extent to which a method can determine particular analyte(s) in a mixture without interference from the other components in the mixture. A method that is perfectly selective for an analyte or group of analytes is said to be specific.

Specificity See Selectivity.

Standard Operating Procedures (SOPs) A written document which details the method of an operation, analysis, or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

Supervisory Chemist A designated person who has the overall responsibility and authority for the technical operations of the drug analysis section.
Technical/Assistant Analyst  A person who analyses evidence, but does not issue reports for court purposes.

Technical Support Personnel  A person who performs basic laboratory duties, but does not analyze evidence.

Test  See Analysis

Traceable  Ability to trace the history, application, or location of an entity by means of recorded identification. See also Chain of Custody.

Traceability  The property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.

Trueness:  The closeness of agreement between the average value obtained from a large set of test results and an accepted reference value.

Uncertainty:  Parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand.

Validation  Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled.

Verification  Confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. (Method works in your lab as well as where it was validated.)
Microgram

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC 20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 2
Numbers 1-4
January - December 2004

Posted On-Line At:

Brought to you by AltGov2 [www.altgov2.org]
Contents

Letter to the Editor regarding “A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions”. 3

Anise Oil as a Precursor for 2-Alkoxy-5-methoxybenzaldehydes
Dieter Waumans, Noël Bruneel, and Jan Tytgat 4

Diltiazem HCl: An Analytical Profile
DeMia E. Peters 11

Hydroxyzine: An Analytical Profile
Norman G. Odeneal II, John F. Casale, and Heidi L. Wojno 17

Mass Spectra of Select Benzyl- and Phenyl- Piperazine Designer Drugs
Hans H. Maurer 22

The Quantitation of Nimetazepam in Erimin-5 Tablets and Powders by Reverse-Phase HPLC
Yong Kiong Chong, Muzaiyanah Mohd Kaprawi, and Kee Bian Chan 27

Improvised Explosive Device Disguised as a Smoking Pipe
Richard T. Ramsey and Pam Woods 34

Identification and Determination of Carisoprodol in Tablets by Liquid Chromatography/Mass Spectrometry
Angela S. Mohrhaus and Samuel R. Gratz 36

Instructions for Authors 42

Note: In order to prevent automated theft of email addresses off the Internet postings of Microgram Journal, all email addresses reported in the Journal have had the “@” character replaced by “-at-” (unless requested otherwise by the corresponding author); this will need to be converted back (by hand) before the address can be used.

Cover Art: “Ball and Stick” Model of Methamphetamine (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA)
Sir:

While perusing the second issue of the *Journal* I noted an error in terminology in the article entitled: "A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions" by Mohammad Sarwar and McDonald. The authors twice refer to the taxonomic categories of *strophariaceae, bolbitiaceae, coprinaceae, and cortinariaceae* as the four "species" of fungi which contain psilocyn and psilocybin. These categories are not species; they are families, as indicated by the suffix -"aceae". Each of these four families contains several genera, and each genus contains multiple species. The family *cortinariaceae*, for example, contains at least eight genera and well over a thousand different species. This is a small criticism in an otherwise excellent paper, and I congratulate the authors for their work.

Robert Parsons
Indian River Crime Laboratory, Fort Pierce, Florida

* * * * * * * * * * * * * * * * * * * *
ABSTRACT: Anethole, the principal component of anise oil, is occasionally utilized as a precursor to anisaldehyde, which in turn is used as a precursor in the illicit synthesis of 4-methoxyamphetamine and 4-methoxyamphetamine. Anethole can also be utilized as a precursor for 2,5-dimethoxybenzaldehyde and 2-ethoxy-5-methoxybenzaldehyde. 2,5-Dimethoxybenzaldehyde is a precursor for designer dimethoxyphenylethylamines that are subject to abuse, such as 2C-B, DOB and DOI, while 2-ethoxy-5-methoxybenzaldehyde can be similarly used to synthesize some of the so-called "Tweetios" (methylene insertion analogs of the corresponding dimethoxy compounds). In these synthetic routes, anethole is first oxidized to anisaldehyde, which in turn is converted to 4-methoxyphenol via a Baeyer-Villiger reaction. The phenol is formylated via a Reimer-Tiemann reaction, and the resulting benzaldehyde can be methylated to give 2,5-dimethoxybenzaldehyde, or ethylated to give 2-ethoxy-5-methoxybenzaldehyde. The described procedures are of forensic and judicial interest.

KEYWORDS: Anise Oil, Anethole, Anisaldehyde, 4-Methoxyphenol, 2,5-Dimethoxybenzaldehyde, 2-Ethoxy-5-methoxybenzaldehyde, Forensic Chemistry

Introduction

Anise oil is the common trade name for the essential oils of two different plant species, *Pimpinella anisum* and *Illicium verum*. Most commercially available anise oil is derived from *Illicium verum* (also known as star anise), and is grown primarily in the Far East. Anise oil from *Pimpinella anisum* has a sweeter taste and a more agreeable odor, and is usually grown in Central Asia and the Mediterranean region.

The main component of anise oil is anethole, 4-methoxyphenyl-1-propene [1]. Both varieties of anise oil contain 80 - 90 % anethole (1a,b). The essential oil derived from fennel (*Foeniculum vulgare*) also has a high anethole content, usually 50 - 60 %. Anethole is industrially utilized as a precursor for 4-methoxyphenyl-2-propanone, a valuable chemical stock. We recently demonstrated that anethole had been used as the precursor for clandestinely prepared 4-methoxyamphetamine (PMA) or 4-methoxymethamphetamine (PMMA) through 4-methoxyphenyl-2-propanone (2). This synthetic route is analogous to the syntheses of the methylenedioxyamphetamine (MDA, MDMA, or MDEA) from 3,4-methylenedioxyphenyl-2-propanone, prepared from isosafrole.

During our study of the preparation of 4-methoxyamphetamine starting from anethole, we noted that 4-methoxyphenol [3] was formed during the performic acid oxidation of anethole in the synthesis of 4-methoxyphenyl-2-propanone (2). It was determined that 4-methoxyphenol was formed by the Baeyer-Villiger oxidation of anisaldehyde (4-methoxybenzaldehyde [2]), which was present in the reaction mixture as an impurity originating from the peracid oxidation of anethole. 4-Methoxyphenol is recovered in an industrial process using a similar per-oxidation procedure (3). Therefore, we decided to explore whether 4-methoxyphenol could be formed from anethole as the primary product (that is, not as a side-product). If so, this would represent a possible route for the preparation of several 2,5-dimethoxyphenylethylamines and 2-ethoxy-5-methoxy-phenethylamines (see Figure 1).
Figure 1: Anethole [1] is oxidized to anisaldehyde [2], which is subjected to a Baeyer-Villiger oxidation to give 4-methoxyphenol [3], which is subjected to a Reimer-Tiemann formylation to give 2-hydroxy-5-methoxybenzaldehyde [4]. Methylation gives 2,5-dimethoxybenzaldehyde [5], while ethylation gives 2-ethoxy-5-methoxybenzaldehyde [6]. Compounds 5 and 6 can be utilized as precursors for various 2,5-dimethoxylated phenethylamines or 2-ethoxylated-5-methoxylated phenethylamines. For details, see the Experimental Section.
**Experimental**

**Chemicals and Reagents**
All solvents used in this work were analytical grade and purchased from Acros Organics (Geel, Belgium). Anise oil was obtained from Taiga International NV (Breendonk-Puurs, Belgium), and originated from China (harvest year 2000) from *Illicium verum* (star anise). All other reagents were acquired from Merck (Darmstadt, Germany) or were synthesized from anethole (*vide infra*).

**Instrumentation**
Mass spectral analysis was performed on an Agilent 6890 Plus GC coupled to an Agilent 5973N MSD, and are presented in Figure 2. An HP-5-MS capillary column (30.0 m x 0.25 mm x 0.25 µm) was employed. Helium was the carrier gas, with a constant flow of 0.6 mL/min. The transfer line and ion source were operated at 280°C and 230°C, respectively. Mass spectra were recorded from 35 to 550 amu. The mass spectrometer was run in the Electron Impact (EI) mode with an ionization energy of 70 eV. A solvent delay of 4 min was applied. Oven temperature programming was as follows: 1 min at 50°C, to 100°C at 35°C/min, to 270°C at 10°C/min. This temperature was maintained until the end of the programmed run (39.48 min). Injections were done split or splitless, depending on the nature of the sample.

**Syntheses**

**Anisaldehyde (4-Methoxybenzaldehyde [2])**
A freshly prepared and stirred solution of 30 mL concentrated sulfuric acid in 150 mL water was allowed to cool down to 30°C, and anise oil (9.8 g) was added. A total of 25 g sodium bichromate was then added, at such a rate that the reaction temperature remained between 35 - 40°C. The reaction mixture was extracted four times with toluene (75 mL each), and the combined organic phases were washed twice with 5% NaOH (100 mL each), and once with water (100 mL). The organic phase was evaporated to about 20 mL, and anisaldehyde was then isolated as its bisulfite adduct. The yellow precipitate was washed with an EtOH/ether (1:1) mixture until the precipitate's color turned white (that is, similar to the bisulfite adduct generated from commercially available anisaldehyde). Setting the anisaldehyde free resulted in 4.9 g of a yellow oil with a pleasant odor. The mass spectrum was in agreement with an authentic sample. Anisaldehyde was the main product (95% by GC/MS), but several minor impurities (not further identified in this report) were noted.

**4-Methoxyphenol [3]**
Performic acid was generated by mixing 23 g 30% hydrogen peroxide with 19 mL 98 - 100% formic acid and allowing it to react for 30 minutes. The resulting mixture was added to a stirred solution of 12 mL anisaldehyde in 200 mL dichloromethane, and refluxed for 24 h. The solvent was removed via rotavap, and the resulting residue was dissolved in a mixture of 200 mL NaOH (20%) and 75 mL MeOH. This mixture was stirred for an additional hour, after which the MeOH was removed via vacuum distillation. The mixture was acidified with concentrated HCl to pH 1, and then extracted with dichloromethane (2 x 150 mL). The combined extracts were dried over anhydrous Na₂SO₄, then evaporated via rotavap to give 10.0 g of a brownish oil which solidified upon standing. Further purification gave 4-methoxyphenol as a white crystalline product. The mass spectrum was in agreement with an authentic sample.

**2-Hydroxy-5-methoxybenzaldehyde [4]**
A 500 mL three-necked round bottom flask, equipped with reflux condenser, thermometer, and magnetic stirrer, was charged with 80 g NaOH and 100 mL water and stirred until dissolved. 30 g 4-methoxyphenol was then added to the still hot and stirring solution. Once the temperature dropped to 70°C, 40 mL chloroform was added drop-wise over the course of 3.5 h, while the reaction temperature was maintained at 65 - 70°C. During the reaction, yellow-green crystals formed on top of the mixture. When all of the chloroform was added, the reaction was continued for an additional hour, after which the mixture was acidified with chilled, 10 N H₂SO₄ to pH 2 - 3. A brown oil separated on top, and was isolated, and the residual aqueous phase was extracted with dichloromethane (2 x 100 mL). The combined organic phases were dried over anhydrous Na₂SO₄, and the solvent was removed via rotavap. The resulting oil was added to the previously isolated oily layer and
steam-distilled. The distillate (2.5 L) was extracted with dichloromethane, and the organic layer isolated and washed with chilled water. The organic layer was dried over anhydrous Na₂SO₄, and the solvent was removed via rotavap. The residual yellow oil (2-hydroxy-5-methoxybenzaldehyde) weighed 23.8 g and was used in subsequent reactions without further purification.

2,5-Dimethoxybenzaldehyde [5]
A 250 mL round-bottomed flask was equipped with a reflux condenser, thermometer, and magnetic stirrer, and was charged with 14 g anhydrous potassium carbonate, 10 g 2-hydroxy-5-methoxybenzaldehyde, and 100 mL acetone, and the mixture was brought to reflux. Once the mixture was boiling, 11 g of dimethyl sulfate was added, and the reaction was refluxed. After 3.5 h, the mixture was cooled, filtered, and the solvent was removed. The residue was taken up in 100 mL of cold water, and the precipitated crystals were collected and recrystallized from water/EtOH (1:1), giving (after drying in vacuo) 8.3 g 2,5-dimethoxybenzaldehyde as faintly yellow tinted needle-shaped crystals (GC purity: 98 %+). The mass spectrum was in agreement with an authentic sample. 1H-NMR * 3.799 (s, 5-OMe), 3.893 (s, 2-OMe), 6.942 (d, J = 9.1 Hz, 1H), 7.135 (dd, J = 3.3 & 9.1 Hz, 1H), 7.326 (d, J = 3.3 Hz, 1H), 10.44 (s, 1H). 13C-NMR * 55.69, 56.06, 110.45, 113.33, 123.41, 124.98, 153.63, 156.76, 189.60 (CHO).

2-Ethoxy-5-methoxybenzaldehyde [6]
A setup similar to the one described for 2,5-dimethoxybenzaldehyde was charged with 7 g anhydrous potassium carbonate, 7 g 2-hydroxy-5-methoxybenzaldehyde, and 100 mL acetone, and the mixture was brought to reflux. Once the mixture was boiling, 5 mL diethyl sulfate was added, and the reaction was refluxed. After 3 h, the mixture was cooled, filtered, and the solvent was removed. The residue was taken up into 75 mL of cold water, and the precipitated crystals were collected and recrystallized from water/EtOH (1:1), yielding spectacularly long, needle-shaped crystals. Recrystallization from EtOH gave 5.9 g 2-ethoxy-5-methoxybenzaldehyde as faintly yellow tinted, polymorphic crystals (GC purity: 98 %+). 1H-NMR * 1.447 (t, J = 7.1 Hz, 3H), 3.794 (s, 3H), 4.106 (q, J = 7.0 Hz, 2H), 6.925 (d, J = 9.1 Hz, 1H), 7.111 (dd, J = 3.3 & 9.1 Hz, 1H), 7.317 (d, J = 3.3 Hz, 1H), 10.473 (s, 1H). 13C-NMR * 14.57, 55.63, 64.74, 110.08, 114.48, 123.47, 125.14, 153.54, 155.21, 189.72 (CHO).

Results and Discussion
The synthesis of anisaldehyde from anethole can be accomplished in several ways, for instance by reaction with ozone (4), VO₅ (5), or HNO₃ (6,7). We opted for the well-known sodium bichromate mediated oxidation. The applied procedure is a minor adaptation of a method used in the fragrance industry (6). The aldehyde was purified via its bisulfite adduct instead of distillation. Isolation as the bisulfite adduct is - in this case - a facile and low-priced alternative for purification via distillation. In fact, in the early 20th century, the bisulfite adduct of anisaldehyde was commonly traded as *aubépine cristallisée* for use in the perfume industry (*aubépine* translates from French as “hawthorn” (8)).

The synthesis of 4-methoxyphenol from anisaldehyde can be performed via the Baeyer-Villiger oxidation reaction with hydrogen peroxide or a peracid (9). We utilized performic acid in this study, but other peracids such as peracetic acid (10) or *meta*-chloroperbenzoic acid (11) work equally well. Other possibilities include sodium perborate in glacial acetic acid (12-14) or hydrogen peroxide with boric acid (15). Yields usually range between 70 % and quantitative, depending on which method was used.

The Reimer-Tiemann formylation reaction (16) is not widely utilized. Generally, low yields, several side-reactions, and easy formation of intractable tars are problematic. However, submission of 4-methoxyphenol to a Reimer-Tiemann formylation gives acceptable yields and reasonable workups. The scientific literature contains many references concerning adaptations for the Reimer-Tiemann formylation of 4-methoxyphenol, with yields usually varying between 40 - 70 %. In our study, we opted for a previously reported procedure by Wynberg and Meijer (17). Generally, this method has several advantages over the Vilsmeier-Haack formylation...
(another widely used formylation technique, but which gives poor yields in this case). Even an improved version of the Vilsmeier-Haack reaction still gave only 40 % 2,5-dimethoxybenzaldehyde after 48 h of refluxing (18).

The methylation of phenols to methoxybenzenes using dimethylsulfate is well-known. The use of dimethylsulfate requires care due to its toxicity, but it may be substituted for by less toxic and easier accessible chemicals, such as dimethyl carbonate (19).

The synthesized benzaldehydes can be used for the preparation of several “designer” phenylethylamines; 2,5-dimethoxybenzaldehyde can be applied in the synthesis of, e.g.: 2C-B (20a), 2C-I (20b), DOB (20c), DOC (20d), DOI (20e), and 2,5-DMA (20f). Other phenylethylamines can be synthesized using 1,4-dimethoxybenzene, e.g.: 2C-P (20g). 2-Ethoxy-5-methoxybenzaldehyde is a precursor for the so-called "Tweetios" (20h). Tweetios are methylene insertion analogues of the 2,5-dimethoxyphenylethylamines, where one or both methoxy groups are replaced by ethoxy groups. These compounds generally display less potency and shorter duration time than the 2,5-dimethoxy analogues, and so do not have high potential for clandestine synthesis. In this case, only the 2-ethoxy-5-methoxyphenylethylamines can be obtained.

Anethole is currently used in large quantities in the alcoholic beverage industry (e.g., for Ouzo or Ricard), and in oral hygiene products (21). It is also a valuable component in aromatherapy products. Due to this economic significance, it is unlikely that anise oil or anethole will become monitored or scheduled substances, despite their use in the illicit production of PMA and PMMA, their link with several PMA- and PMMA-related fatalities over the past few years (2,22), and/or their potential use towards synthesis of various designer phenethylamines. We are currently unaware of any examples of anise oil or anethole being used to produce designer phenethylamines, but still feel it is necessary to point this possibility, since it might become a preferred precursor in the future as chemical substance controls are gradually increased. It is also important to understand that the presence of anise oil or anethole in a clandestine laboratory does not automatically imply that the operator intended to synthesize PMA and/or PMMA; it is also possible that synthesis of a designer phenethylamine was intended. This can only be ascertained by a total review of all chemicals and notes present at the laboratory, and/or by operator interviews.

Acknowledgements

We are grateful to Prof. Dr. Roger Busson (REGA Institute, K. U. Leuven) for recording of the NMR spectra reported in this study.

References


[Note: Patents were retrieved via the Espacenet website http://gb.espacenet.com]

[Figure 2 Follows.]
Figure 2: Mass Spectra of 1 - 6.
Technical Note

Diltiazem HCl: An Analytical Profile

DeMia E. Peters
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email: demia-at-lycos.com]

ABSTRACT: Diltiazem, a potent vasodilator that is used in a wide variety of heart medications, was identified as an adulterant in several large shipments of illicit cocaine. Analytical data (gas chromatography, infrared spectroscopy, mass spectrometry, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: Diltiazem, Benzothiazepine, Calcium Channel Blocker, Vasodilator, Cocaine, Forensic Chemistry

Figure 1: Structure of Diltiazem Hydrochloride

Introduction

This laboratory recently received samples from several multi-kilogram seizures of cocaine hydrochloride (ranging from 71 - 85 % cocaine HCl) containing varying amounts of diltiazem hydrochloride (8 - 20 %) (1,2). The full chemical name for diltiazem is (2S-cis)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one (3) [Figure 1]. It is prescribed as a calcium channel blocker, and has potent vasodilating activity (4,5). This vasodilation is accomplished without additional oxygen consumption by the heart (6). These therapeutic properties have made diltiazem hydrochloride an important constituent in a myriad of heart medications which are widely prescribed for effects in combating angina, hypertension, and/or irregular heartbeats (7). Herein, we provide analytical data for diltiazem hydrochloride (8).
Experimental

Diltiazem: \( \text{C}_{22}\text{H}_{26}\text{N}_{2}\text{O}_{4}\text{S} \) 414.53 amu

Source of Diltiazem
Sigma-Aldrich, Inc. (St. Louis, Missouri); Lot #123K0968, 99%

Gas Chromatography
Instrument Agilent 6890N with a flame ionization detector
Column DB-1, 30 m x 0.25 mm x 0.25 \( \mu \)m film thickness
Injector Temperature 280° C
Oven Temperature 140° C for 1.5 min, 10° C/min to 280° C
Carrier Gas Hydrogen at 1.1 mL/min, split ratio = 25:1

Utilizing the above experimental parameters, the retention time for diltiazem HCl is 17.64 minutes. The retention time relative to cocaine is 1.52. A screening run utilizing the above parameters will detect the presence of diltiazem and allow for correct quantitation parameters to be selected.

Infrared Spectroscopy
Instrument Thermo-Nicolet Nexus 670
Number of Scans 32
Resolution 4,000
Wavenumber Range 4000 cm\(^{-1}\) to 400 cm\(^{-1}\)

Data was obtained by the use of an attenuated total reflectance (ATR) attachment on FTIR. The data was not ATR corrected [Figure 2]. In addition, spectral data was obtained with a KBr dispersion technique on FTIR [Figure 3]. The principal peaks are at 1680 cm\(^{-1}\) and 1250 cm\(^{-1}\).

Mass Spectrometry
Instrument Agilent 5973
Column DB-1, 30 m x 0.25 mm x 0.25 \( \mu \)m film thickness
Injector Temperature 280° C
Oven Temperature 90° C for 2 min, 14° C/min to 300° C
Carrier Gas Helium with split ratio = 25:1
Scan Range 34 - 550 amu

Electron impact mass spectrometry data shows a molecular ion at 414 amu and a base ion of 58 amu [Figure 4A]. When the ion abundance of this spectrum is enhanced 10x, the ions are more easily viewed [Figure 4B].

Nuclear Magnetic Resonance Spectroscopy
Analyses were performed on a Varian Mercury 400 MHz NMR. The sample was prepared at 22.4 mg/mL in deuterium oxide (D\(_2\)O) containing TSP (3-(trimethylsilyl)propionic-2,2,3,3-d4 acid, sodium salt) as the reference at 0 ppm and maleic acid as the internal standard. The maleic acid forms a singlet at 6.4 ppm. The proton spectrum of the standard was obtained with 8 scans using a 45 second delay, 90° pulse, 5 second acquisition time, and oversampling of 4 [Figure 5].
Results and Discussion

The referenced exhibits appear to be the first identified to contain diltiazem. Based on cocaine signature analysis, it appears that the diltiazem was added to the cocaine during one of the final processing stages; either: A) the base was added to cocaine base and the two were co-precipitated as hydrochloride salts; or B) the hydrochloride was added to cocaine hydrochloride and physically mixed prior to pressing into kilogram bricks.

The purpose for adulterating illicit cocaine with such an unusual (and relatively expensive) compound is unclear. A (brief) review of several websites dedicated to drug abuse does not suggest any synergistic/desirable or pseudo-therapeutic effects to co-administration of diltiazem with cocaine. Therefore, it is most likely that it was used merely as a “cut of convenience”.

Acknowledgements

The author wishes to thank Senior Research Chemist John F. Casale and Senior Forensic Chemist Pamela R. Smith (this laboratory) for their assistance. The author would also like to acknowledge Senior Research Chemist Patrick A. Hays (this laboratory) for his time and expertise in interpreting the NMR spectrum of diltiazem hydrochloride.

References


[Figures 2 through 5 follow on the next three pages.]

* * * * *
Figure 2: Uncorrected FTIR-ATR Spectrum of Diltiazem Hydrochloride

Figure 3: KBr Dispersion FTIR Spectrum of Diltiazem Hydrochloride
Figure 4a: Electron Impact Mass Spectrum of Diltiazem

Figure 4b: Electron Impact Mass Spectrum of Diltiazem (Enhanced 10x)
Figure 5: 400 MHz Proton NMR Spectrum of Diltiazem Hydrochloride in D$_2$O
Technical Note

Hydroxyzine: An Analytical Profile

Norman G. Odeneal II,* John F. Casale, and Heidi L. Wojno
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email: norm1fc -at- yahoo.com]

ABSTRACT: Hydroxyzine, an anxiolytic and antihistamine, was identified as an adulterant in several large shipments of illicit cocaine. Analytical data (gas chromatography, infrared spectroscopy, mass spectrometry, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: Hydroxyzine, Anxiolytic, Antihistamine, Cocaine, Forensic Chemistry

Figure 1: Structure of Hydroxyzine

Introduction

This laboratory recently received samples from several multi-kilogram seizures of cocaine hydrochloride containing varying amounts of hydroxyzine (2 - 20 %) (1). The full chemical name for hydroxyzine is 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]ethanol. Hydroxyzine is marketed as an anxiolytic (used in treatment of anxiety), antihistamine (allergy), and as a mild tranquilizer (2,3). It is sold in tablet form. Federal law restricts this drug to prescription use only. Herein, we provide analytical data for hydroxyzine (4).

Experimental

Hydroxyzine: C$_{21}$H$_{27}$ClN$_2$O$_2$  374.91 amu
Gas Chromatography
Instrument: Agilent 6890N with a flame ionization detector
Column: HP-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature: 280° C
Oven Temperature: 250° C Isothermal
Carrier Gas: Hydrogen at 1.1 mL/min, split ratio = 25:1

Utilizing the above experimental parameters, the retention time for hydroxyzine is 10.63 minutes. The retention time relative to cocaine is 2.85.

Infrared Spectroscopy
Infrared spectra were obtained on a Nexus 670 FT-IR equipped with a single bounce attenuated total reflectance (ATR) accessory (Figure 2).

Mass Spectrometry
Instrument: Agilent 6890 interfaced with an Agilent 5973 MSD
Column: DB-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature: 280° C
Oven Temperature: 90° C for 2 min, 14° C/min to 300° C
Carrier Gas: Helium with split ratio = 25:1
Scan Range: 34 - 550 amu
Electron Ionization: 70 eV

The Total Ion Chromatogram (TIC) for hydroxyzine is shown in Figure 3. The fragmentation pattern shows a molecular ion at m/z 374 and a base peak of m/z 201 (Figure 4). Hydroxyzine may also be further characterized by GC/MS after derivatization. Hydroxyzine (5 mg) was reacted with a mixture of 250 μL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) in 250 μL of chloroform at 80° C for 30 minutes. The mass spectrum of the resulting trimethylsilyl (TMS) derivative gives a molecule ion at m/z 446 (Figure 5).

Nuclear Magnetic Resonance Spectroscopy
One dimensional proton NMR analyses were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe. The sample was prepared at 10-30 mg/mL in deuterium oxide (D₂O) containing TSP (3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid, sodium salt) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, Wisconsin). Maleic acid was used as the internal (quantitation) standard. The proton spectrum of the standard was obtained with 8 scans using a 45 second delay, 90° pulse, 5 second acquisition time, and oversampling of 4 (Figure 6).

Results and Discussion

The referenced exhibits appear to be the first identified to contain hydroxyzine. Based on cocaine signature analysis, it appears that the hydroxyzine was added to the cocaine hydrochloride and physically mixed prior to pressing into kilogram bricks.

The purpose for adulterating illicit cocaine with such an unusual (and relatively expensive) compound is unclear. A (brief) review of several websites dedicated to drug abuse does not suggest any synergistic/desirable or
pseudo-therapeutic effects to co-administration of hydroxyzine with cocaine. Therefore, it is most likely that it was used merely as a “cut of convenience”.

Acknowledgements

The author wishes to thank Senior Research Chemist Patrick A. Hays (this laboratory) for his time and expertise in interpreting the NMR spectrum of hydroxyzine.

References


---

Figure 2: FTIR-ATR Spectrum of Hydroxyzine Hydrochloride
Figure 3: Total Ion Chromatogram of Hydroxyzine

Figure 4: Electron Ionization Mass Spectrum of Hydroxyzine
Figure 5: Electron Ionization Mass Spectrum of the Trimethylsilyl Derivative of Hydroxyzine

Figure 6: 400 MHz Proton NMR Spectrum of Hydroxyzine in D₂O
Technical Note

Mass Spectra of Select Benzyl- and Phenyl-Piperazine Designer Drugs

Hans H. Maurer
Department of Experimental and Clinical Toxicology
University of Saarland
D-66421 Homburg (Saar)
Germany
[email: hans.maurer -at- uniklinik-saarland.de]

ABSTRACT: The mass spectra of five piperazine designer drugs (N-benzylpiperazine, 1-(3,4-methylenedioxy-benzyl)piperazine, 1-(3-trifluoromethylphenyl)piperazine, 1-(3-chlorophenyl)piperazine, and 1-(4-methoxy-phenyl)piperazine) and their trimethylsilyl derivatives are presented.

KEYWORDS: Benzylpiperazines, Phenylpiperazines, Designer Drugs, Mass Spectrometry, Trimethylsilylation, Forensic Chemistry

Designer drugs of the benzyl- or phenyl- piperazine type, i.e., benzylpiperazine (BZP) itself, its methylenedioxy analogue 1-(3,4-methylenedioxybenzyl)piperazine (MDBP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP), and 1-(4-methoxyphenyl)piperazine (MeOPP), recently have gained popularity and notoriety. Seizures have been made throughout the world (1-9), and a few fatalities have been reported (10-11). The increasing abuse of piperazines in the United States resulted in the temporary placement of BZP and TFMPP into Schedule I of the Controlled Substances Act (12). BZP was permanently scheduled in March, 2004 (13); however, TFMPP is currently not controlled in the United States.

Recently, many GC/MS studies on the metabolites of piperazines (i.e., from biological fluids) and/or their acetyl or heptafluorobutyril derivatives have been published (14-24). However, most forensic drug laboratories perform GC/MS on the underivatized or trimethylsilylated derivatives of amine drugs. In Figures 1 and 2, the structures, electron-ionization mass spectra, and gas chromatographic retention indices (recorded on an Agilent GC-MSD 5972, HP-1 column, 12 m x 0.2 mm I.D., 100-310° C, 30° C/minute (25)) of the target piperazines and their trimethylsilyl derivatives are displayed. Additional data for these and several related piperazines will be published elsewhere (26-27).

References


[Editor’s Notes: * All issues of Microgram prior to January 2003 are law enforcement restricted. Selected references on the analysis of various piperazines were presented in Microgram Bulletin 2004;37(4):76.]

Figure 1: Structures, electron-ionization mass spectra, and gas chromatographic retention indices of underivatized piperazine-derived designer drugs.

Figure 2: Structures, electron-ionization mass spectra, and gas chromatographic retention indices of trimethylsilylated piperazine-derived designer drugs.
Benzylpiperazine (BZP)

mCPP  m-Chlorophenylpiperazine  Nefazodone-M (N-desalkyl-)  Trazodone-M (N-desalkyl-)

MDBP  Fipexide-M/artefact (MDBP)  Methylenedioxybenzylpiperazine  Piperonylpiperazine

MeOPP  4-Methoxypiperazine

TFMPP  Trifluoromethylphenylpiperazine
The Quantitation of Nimetazepam in Erimin-5 Tablets and Powders by Reverse-Phase HPLC

Yong Kiong Chong, Muzaiyanah Mohd Kaprawi, and Kee Bian Chan*
Narcotics Section, Forensic Division
Department of Chemistry Malaysia
Jalan Sultan, 46661 Petaling Jaya
Malaysia
[email: kbchan -at- kimia.gov.my]

ABSTRACT: The sedative-hypnotic nimetazepam in “Erimin 5” tablets and powders was quantitated by reverse phase HPLC. The selectivity, precision, and accuracy of the procedure are presented.

KEYWORDS: Nimetazepam, Erimin-5, Benzodiazepines, HPLC, Forensic Chemistry

![Structure of Nimetazepam](image)

Figure 1: Structure of Nimetazepam

Introduction

Since its appearance in illicit drug markets in Malaysia in the mid-1980’s, the benzodiazepine nimetazepam (Figure 1) has become the most commonly abused sedative in the country (midazolam and triazolam are the (distant) second and third most abused sedatives). The popularity of nimetazepam is due in part to its wide availability and relatively low price on the local black markets, and in part due to its long activity. Most of the abusers are believed to be heroin addicts, who use it as a substitute for heroin when its availability is low. More recently, however, nimetazepam has also been used as a sedative by methamphetamine abusers to help them sleep after binging (in fact, the rise in nimetazepam abuse roughly parallels the rise in methamphetamine abuse in Malaysia). The illicit use of nimetazepam is continuing to increase, as shown by the number and size of seizures made over the past few years. For example, a seizure of 310,000 tablets was made in June 2002 at a residence near the capital city (Kuala Lumpur). Tablet submissions to the Central Laboratory have been in the hundreds of thousands for each of the three years 2002 - 2004. Similar abuse of nimetazepam has been reported in neighboring countries.

The two primary forms of nimetazepam encountered in Malaysia are a commercial product (Erimin-5 tablets in blister packs (see Photos 1 - 2)) or Erimin-5 counterfeits, and an orange colored powder that appears to be either finely crushed tablets or the tablet mixture prior to tableting. Commercially prepared tablets nominally weigh...
about 170 mg and contain about 5 mg of nimetazepam each. However, as noted above, many of the Erimin-5 tablets submitted to the Narcotics Section appear to actually be counterfeit products that contain nimetazepam and/or various other benzodiazepines, notably diazepam and nitrazepam, in varying quantities.

Nimetazepam was added to the Malaysian Dangerous Drugs Act 1952 in May, 2001 and is currently the only benzodiazepine controlled in Malaysia. The analysis of nimetazepam by a variety of techniques has been previously reported (1-4), including by CE and CEC (5-7), Color Testing (8), FTIR (9), GC (10-12), HPLC and HPLC/MS (13-18), TLC (17,19), and UV/Vis (20). Herein, we report the quantitation of nimetazepam in seized tablets and powders with reverse-phase HPLC, using an external standard method.
Experimental

Chemicals
HPLC grade methanol and chloroform were purchased from Merck, while AR grade orthophosphoric acid (84%) was purchased from Ajax (Australia). Nimetazepam (free-base) standard of 100% purity was kindly provided free of charge by Sumitomo Chemical Company (Tokyo, Japan). The following benzodiazepines (as free bases) were obtained from the United Nations Drug Control Programme (UNDCP) in Vienna (Austria): Nitrazepam, bromazepam, tetrazepam, flunitrazepam, oxazepam, lorazepam, clorazepate dipotassium (salt), diazepam, flurazepam, and medazepam. Unfortunately, midazolam and triazolam standards were unavailable, and so were not run.

Instrumentation
A Hewlett Packard Series 1050 HPLC was used with the following parameters:

- Column: C-18, 5 μm particle size, 15 cm x 4.6 mm i.d. (from Alltech).
- Detector: UV at 265 nm.
- Mobile phase: Methanol:Water (50:65). The pH was adjusted to 4.0 with orthophosphoric acid (to a mixture of 500 mL of methanol and 650 mL of water was added one drop of orthophosphoric acid) (21).
- Column temperature: 25º C (ambient temperature).
- Flow rate: 1.5 mL/minute.
- Average Pressure: 155 bar.
- Injection: 20 μL by Rheodyne loop injector.
- Attenuation: 4 (Integrator).

Standard Solutions for Linearity Study and Calibration
Standard solutions containing 0.020, 0.040, 0.080, 0.120, 0.160, 0.200 and 0.240 mg/mL of nimetazepam were prepared in a mixture of methanol/chloroform (5:1) (note that the chloroform was added to better solubulize the tablet materials, and had no adverse effects on the chromatography).

Quantitative Analysis of Samples
About 70 – 100 mg of homogenized tablet material was accurately weighed into a 25 mL volumetric flask and made up to volume with a mixture of methanol/chloroform (5:1). The sample solution was ultrasonicated for 5 minutes and filtered through a 0.45 μm filter before injected onto the column. Quantitation was by external standard and with reference to the peak area of the 0.120 mg/mL nimetazepam standard.

Procedure for Standard Addition Method
(i) 350.80 mg of tablet material was weighed into a 100 mL volumetric flask, made up to volume with methanol/chloroform (5:1), and ultrasonicated for 5 minutes. (ii) 10 mL of the solution in (i) (i.e., equivalent to 35.08 mg of tablet material) was pipetted into each of five 25 mL volumetric flasks. (iii) The following aliquots of nimetazepam standard stock solution (1.00 mg/mL) were pipetted into the solutions in (ii): 0, 1, 2, 3, and 4 mL. (iv) The solutions were made up to volume (i.e., 25 mL) with methanol/chloroform (5:1). (v) The solutions were filtered through a 0.45 μm filter and injected into the HPLC. (vi) A graph of area versus concentration of nimetazepam (mg/mL) was plotted using Excel and the native nimetazepam content calculated.

Results and Discussion

Selectivity
Identification of benzodiazepines is accomplished in this laboratory by GC/MS. However, GC and GC/MS are problematic for quantitation of nimetazepam and some related benzodiazepines due to thermal degradation at injector port temperatures, and so HPLC was selected for quantitation. Because of the wide diversity of chemical structures and solubility characteristics among the benzodiazepines, no single HPLC method will separate all of
them. The specificity of the method presented herein was defined in terms of the benzodiazepines typically found in Malaysia. The identities and retention times of these benzodiazepines using the presented methodology are presented in Table 1.

Table 1: Retention Times of Benzodiazepines (HPLC)

<table>
<thead>
<tr>
<th>Benzodiazepine</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrazepam</td>
<td>6.91</td>
</tr>
<tr>
<td>Bromazepam</td>
<td>7.62</td>
</tr>
<tr>
<td>Tetrazepam</td>
<td>7.80</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>8.54</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>8.55</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>8.93</td>
</tr>
<tr>
<td><strong>Nimetazepam</strong></td>
<td><strong>9.94</strong></td>
</tr>
<tr>
<td>Clorazepate dipotassium</td>
<td>17.07</td>
</tr>
<tr>
<td>Diazepam</td>
<td>26.63</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>NE</td>
</tr>
<tr>
<td>Medazepam</td>
<td>NE</td>
</tr>
</tbody>
</table>

NE: Did not elute within 30 minutes.

Of the selected benzodiazepines, flunitrazepam, oxazepam, and lorazepam elute closest to nimetazepam, and give partially overlapping peaks. Thus, the presented HPLC method is not appropriate for samples containing these compounds. Fortunately, however, experience has shown that these three benzodiazepines are very rarely present in tablets or powders containing nimetazepam. A few samples of “Erimin-5” tablets have been found to contain diazepam instead of nimetazepam; however, diazepam elutes much later than nimetazepam. A typical HPLC chromatogram of a mixture of nitrazepam and nimetazepam is displayed in Figure 2.

Figure 2: HPLC of Nitrazepam and Nimetazepam
Note: Slight variations in Retention Times between Table 1 and Figure 2 are due to natural variations over time; the order of elution was found to be consistent from run to run.

Calibration Curve and Linearity
The calibration graph (Figure 3) for the analysis was found to be linear from 0.020 mg/mL to 0.240 mg/mL. From linear regression analysis, the correlation coefficient was better than 0.99, and the percent difference between the known concentration and the predicted concentration from the regression equation was less than 5%. In routine analyses a single point calibration was used.

Precision
The precision of the method was assessed by 10 replicate analyses of a homogenized sample of “Erimin 5” tablets. Injections were all made in triplicate and quantitation was
against the 0.120 mg/mL standard. The mean content of nimetazepam was found to be 3.1 % with a relative standard deviation of 4.4 %.

![Graph: Calibration Curve of Nimetazepam](image)

**Figure 3: Calibration Curve of Nimetazepam**

**Accuracy**

The accuracy of the method was assessed by analyzing two laboratory prepared mixtures, and re-analysis of the sample which was used for the precision study by the method of standard addition.

**Analysis of Laboratory Prepared Mixtures**

Owing to the limited supply of pure nimetazepam reference standards, only two synthetic mixtures were prepared, simulating 5 mg/tablet and 3 mg/tablet. Both the samples were prepared in lactose and contained 3.5 % and 1.7 % of nimetazepam, respectively. Replicate analyses (n = 7) of these two mixtures were made and the results assessed using the Student t-statistic:

\[
t = \left| \frac{\bar{x} - \mu}{s} \right| \sqrt{n}
\]

where

- $\bar{x}$ = sample mean (experimental value)
- $\mu$ = true value (theoretical value)
- $n$ = no. replicate (weighings)
- $s$ = standard deviation

For both samples it was found that the t value did not exceed the critical value derived by statistical analysis, showing that there was no proven evidence of difference between the experimental value and the theoretical value at 95 % confidence level.
Standard Addition Method
The same nimetazepam tablet material which was used in the precision study was re-analyzed using the standard addition method. From the standard addition calibration graph (Figure 4) the amount of nimetazepam was found to be 3.1%. This agreement with the precision study mean value shows that there is no interference from the tablet excipient materials, and thus to some extent shows that the method is accurate.

![Figure 4: Standard Addition Calibration Curve](image)

Acknowledgments
The authors would like to thank Dr. Yoji Sakito, Manager, Corporate Planning & Coordination Office, Sumitomo Chemical Company Ltd, Tokyo, Japan for the nimetazepam reference standard used in this study.

References
5. Jinno K, Sawada H, Catabay AP, Watanabe H, Haji-Sabli NB, Pesek JJ, Matyska MT. Comparison of


Technical Note

Improvised Explosive Device Disguised as a Smoking Pipe

Richard T. Ramsey* and Pamela M. Woods
Allegheny County Coroner’s Office
Division of Forensic Laboratories
Drug Chemistry Section
542 Forbes Avenue
10 County Office Building
Pittsburg, PA 15219
[email: rramsey -at- allegheny.county.pa.us]

ABSTRACT: A homemade pipe that was initially suspected to be intended for smoking controlled substances was instead found to actually be an improvised explosive device fabricated from match heads and air gun pellets.

KEYWORDS: Improvised Explosive Device, IED, Drug Paraphernalia, Pipe, Forensic Chemistry

Introduction

With the exception of needles, most drug paraphernalia items do not present a threat to the investigating officers or the forensic scientists examining the evidence. However, in a multi-exhibit case submitted to this laboratory in September, 2004 an improvised explosive device was discovered during examination and analysis. The case included six exhibits: One charred metal pipe (a typical “crack pipe”), one charred glass pipe (another typical “crack pipe”), three handmade aluminum foil pipes with charred residue (typical marijuana pipes), and one apparent pipe-like device wrapped with silver colored duct tape (see Photo 1). The seizures were made in a suburb of Pittsburg, Pennsylvania.

Photo 1

Brought to you by AltGov2 [www.altgov2.org]
Experimental

The two presumed “crack pipes” (the metal and glass pipes) were each rinsed with chloroform, and the resulting solutions screened by thin layer chromatography (TLC), using “Analtech” Silica Gel plates 250 μm thickness. A reference standard of cocaine base was also spotted on the plate. The remainder of each chloroform rinse solution was evaporated to dryness, and the resulting residue was analyzed using Fourier Transform Infrared Spectrometry (FTIR), using a Perkin Elmer Spectrum One with an ATR attachment.

The three presumed marijuana pipes (the handmade aluminum foil pipes) were unrolled and found to contain charred residues. This material was subjected to a morphological examination under a stereoscope with a 10x magnification. The Duquenois-Levine test was also performed. Finally, the pipes were each rinsed with chloroform, and the resulting solutions screened by TLC against a known reference standard of marijuana.

The last pipe was first examined visually and stereoscopically (see Photo 1). It measured 8 cm in length and 1.5 cm in diameter, and was wrapped with silver colored duct tape. As the layers of duct tape were unwrapped, an inner layer of black electrical tape was observed, and then a layer of aluminum foil. Inside of the foil, ten metal pellets were observed surrounding a plastic pen cap. The total net weight of the ten pellets was 76.9 grains. The pen cap was plastic, with a blue checked pattern on a yellow background. A standard metal shirt clip was observed on the exterior side of the pen cap. Twenty apparent match heads were found inside the pen cap. It appeared that the base of each match had been torn off, so that only the ignitable tip remained. The suspected match heads were examined using FTIR and diffuse reflectance.

Results and Discussion

The metal and glass pipes both tested positive for cocaine base. However, all three of the aluminum foil pipes tested negative for controlled substances. The final pipe was determined to actually be an IED. Upon close examination of the metal pellets, they were consistent in appearance with lead air gun pellets. The metal pellets had an hourglass shape with one end open characterized by rib marks around the outside; this is referred to as a “gereffelt” type skirt. The other end was filled and flat; this is referred to as a “wad cutter” type head design. The white material on the suspected match heads was consistent with potassium chlorate, one of the reactive ingredients used in the heads of commercially prepared matches. Although the exact function of this device is not known, detonation upon handling, disassembly, or analysis by law enforcement personnel seems unlikely. For this device to activate, an external force or an open flame would have to be introduced to ignite the match heads. The release of energy caused by the burning match heads would presumably cause an explosive effect, fragmenting the pen cap and ejecting the pellets contained within the tape. Speculating, it appears that the intended target would be an unsuspecting user.

[Editor’s Notes: Based on a brief review, references dedicated to IED’s do not include any devices of this exact description. However, there are a variety of similar, match head type IED’s, most of which would be considered to be “booby-trap” devices designed to detonate upon handling. Specific references are withheld in accordance with Journal policy.]

************************************
Identification and Determination of Carisoprodol in Tablets by Liquid Chromatography/Mass Spectrometry

Angela S. Mohrhaus and Samuel R. Gratz
U.S. Food and Drug Administration
Forensic Chemistry Center
6751 Steger Drive
Cincinnati, OH 45237
[email: amohr -at- ora.fda.gov]

[Reprinted with Permission from the FDA’s Laboratory Information Bulletin (April 2004 Edition)]

ABSTRACT: A method for the identification and determination of carisoprodol in tablet dosage form is described. Tablets were ground and carisoprodol was extracted using acetonitrile with sonication. Extracts were filtered and further dilutions were made with water. The identification of carisoprodol was accomplished using a single quadrapole mass spectrometer coupled to a liquid chromatograph with an electrospray source and positive ion detection. For determining the carisoprodol content, selected ion monitoring of the molecular ion was used. A 5 μm 2.1 x 150 mm Zorbax SB-C18 column and a mobile phase of 35 % acetonitrile (0.1 % formic acid) and 65 % water (0.1 % formic acid) at a flow rate of 0.4 mL/minute provided adequate retention. The calibration curve generated during this analysis was linear between 0.5 and 40 μg/mL for carisoprodol with a correlation coefficient, r ≥ 0.9996. Spikes of tablets gave an average recovery of 93 % for carisoprodol.

KEYWORDS: Carisoprodol, Meprobamate, Liquid Chromatography/Mass Spectrometry, ESI-LC/MS, Forensic Chemistry

Introduction

Carisoprodol is a centrally acting muscle relaxant, with analgesic properties\(^1\), making it a popular drug of abuse. Carisoprodol is widely available for purchase on the internet with or without a prescription. It is also available in combination with other analgesics, such as aspirin and codeine. Within the body, carisoprodol is metabolized to meprobamate (Figure 1), an anti-anxiety agent prescribed primarily to treat anxiety, tension, and associated muscle spasms. Meprobamate’s onset and duration of action are similar to the intermediate-acting barbiturates; however, therapeutic doses produce less sedation and toxicity than barbiturates. This conversion may account for some of the properties associated with carisoprodol. These barbiturate-mimicking properties likely contribute to its abuse\(^2\).

\[\begin{align*}
  &\text{H}_2\text{N} &\text{C} &\text{O}\text{CH}_2 &\text{C} &\text{CH}_2\text{O} &\text{C} &\text{NH}_2 \\
  &\text{CH}_2\text{CH}_2\text{CH}_3
\end{align*}\]

Figure 1: Meprobamate
Figure 2: Carisoprodol

The structure of carisoprodol (Figure 2) is such that it does not have a UV chromophore with significant absorbance. Therefore, the USP Assay method for Carisoprodol Tablets employs a liquid chromatograph equipped with a refractive index detector. Our laboratory does not currently have an operational refractive index detector, and numerous literature searches resulted in few references to carisoprodol analysis. We performed analysis of the carisoprodol tablets using a liquid chromatographic (LC) separation similar to that in the USP, but with mass selective (MS) detection. This paper describes the mass spectral identification of carisoprodol and the determination of the carisoprodol content of five individual tablets, each containing an unknown amount of carisoprodol.

Experimental

Apparatus

1. LC-MS System: Agilent (Agilent Technologies, Atlanta, GA) 1100 series LC-MSD with an electrospray source. Chemstation G1701AA version A.09.01 was used for data acquisition and processing.
2. Analytical column: Zorbax SB-C18, 2.1 x 150 mm, 5 mm (Agilent Technologies, Part # 883700-922).
3. Syringe filters: 25 mm diameter 0.45 μm Nylon syringe filters (National Scientific, Catalog #F2500-1), or equivalent.

Materials

1. Mobile phase: 35 % acetonitrile (0.1 % formic acid) and 65 % water (0.1 % formic acid). A liter of each was prepared by adding 1 mL of formic acid (88 % A.C.S. reagent, Aldrich Chemical Company, Milwaukee WI, Catalog #39, 938-8) to each respective solvent.
2. HPLC grade acetonitrile and DI water.
3. USP reference standard, Carisoprodol (Lot F). A stock standard was prepared at approximately 2 mg/mL in acetonitrile. Working standards were prepared by serial dilution with DI water at 40 μg/mL, 20 μg/mL, and 10 μg/mL.

Sample Preparation

Preparation of the carisoprodol tablets consisted of grinding five (5) individual tablets with a mortar and pestle into a fine powder. Each of the individual ground tablets was transferred into an individual 20-mL glass scintillation vial. To each vial, 10 mL of acetonitrile was added and the solutions were sonicated for 15 minutes. A portion of each solution was passed through a 25 mm 0.45 μm nylon syringe filter. Based on internet searches, the tablets were suspected of containing 350 mg carisoprodol each; therefore an additional dilution was necessary to decrease the filtrate concentration into a range suitable for MS detection. The filtrate was further diluted by taking a 100 μL aliquot, adding 10 mL DI H₂O, mixing, and then taking 100 μL of this solution to 1 mL with DI H₂O.
Method Validation – LC/MS Assay

Each of two individual tablets was spiked at a different level with a portion of the USP Carisoprodol reference standard. After grinding a single tablet, a known quantity of solid carisoprodol standard equivalent to less than that expected to be present, was added. A second tablet was ground and spiked with a portion of standard in excess of that expected to be present in the tablet. The acetonitrile was added, and the solutions were treated the same as the sample solutions, with the exception of an additional dilution. To bring the spike preparation into the calibration curve range, the final dilution was 30 µL of the spike solution diluted to a total volume of 1 mL with DI water.

Linearity of carisoprodol was established from five separate standards ranging from 0.5 µg/mL to 40 µg/mL. This concentration range was chosen to bracket the diluted sample concentrations. The plot of peak area versus concentration was linear, and the correlation coefficient, r, for carisoprodol was calculated to be 0.9996.

The limit of detection (based on signal:noise of 10:1) was determined for carisoprodol by analysis of a low level standard. The noise level was calculated from the average of ten blank injections, using the area response within the retention time window corresponding to carisoprodol. The detection limit for carisoprodol, on column, was 6.7 picograms.

**LC/MS System**

The electrospray interface was operated in positive ion scan mode with a mass range of 90-350 amu. The internal capillary voltage was set at 3000 volts. The nitrogen drying gas flow rate used was 10 L/min at 300°C, and the nebulizer pressure was set at 20 psig. For the initial screening, the MS was also operated in the full scan mode with a mass range of 90-350 amu. For the determination of carisoprodol content, the instrument was switched to selected ion monitoring (SIM) mode for more sensitivity and better peak shape. Conditions were set to monitor the protonated molecular ion at m/z 261 [M + H]+.

The mobile phase consisted of 35 % acetonitrile and 65 % DI water (both with 0.1 % formic acid), pumped through a C18 column at 0.4mL/min. The column thermostat was set at 25°C, the run time was 8 minutes, and 1.0 µL injections were made for all samples and standards.

**Data Treatment**

Total ion chromatograms were generated for all samples, spikes, standards, and blanks. Each chromatogram was integrated at the retention time corresponding to the retention time of the peak observed in the carisoprodol standard. Peaks that were observed in the blank chromatograms in the retention time range of the carisoprodol peak were small enough that their contribution to the sample peak area was considered negligible. Quantitation of carisoprodol was performed using the data obtained from the SIM ion chromatograms.

**Results and Discussion**

Initial method development for this work included use of methanol (0.1 % formic acid) in place of acetonitrile in the mobile phase, as well as use of UV detection. However, acetonitrile was chosen for the organic component of the mobile phase because it resulted in increased retention and improved peak shape for carisoprodol. Ideally, carisoprodol would generate an adequate UV signal for determining the carisoprodol content. Based on its structure, it is not expected to absorb at 280 nm or at 214 nm. UV experiments verified no absorbance from this compound, even at 20 times the injection volume used for MS detection. Therefore, quantitation was performed based on SIM data generated by the MS.

Spray chamber parameters were optimized for carisoprodol using the flow injection analysis mode of the instrument. In-source collision induced dissociation (CID) generated fragment ions and was accomplished by adjusting the instrument’s fragmentor voltage. Optimum CID conditions were obtained by injecting a 20 µg/mL
carisoprodol standard at several fragmentor voltages followed by review of the resulting mass spectra. In general, higher fragmentor voltage helps the transmission of ions through the relatively high-pressure region between the exit of the capillary and the entrance of the skimmer. At voltages of 30 V or less, very little fragmentation was observed. At voltages of 50 V or greater, excessive fragmentation occurred and there was very little signal observed at \([M + H]^+ \text{ (m/z} = 261)\). At voltages greater than 100 V, neither the protonated molecular ion nor the potassium adduct, \([M + K]^+ \text{ (m/z} = 299)\), was observed in the mass spectrum. A fragmentor voltage of 40 volts was chosen for this analysis because it allowed the detection of structurally useful fragment ions while maintaining sufficient response for the molecular ion.

For screening the samples, full scan MS data was used. Figure 3 depicts a total ion chromatogram for one of the injections of the mid-range standard, and figure 4 shows the corresponding mass spectrum for the carisoprodol peak.

Several of the ions observed in the mass spectrum are considered structurally significant. The ion observed at m/z 261 represents the protonated molecular ion \([M + H]^+\), and the ion observed at m/z 299 represents a potassium adduct \([M + K]^+\). The fragment at m/z 200 is indicative of a loss of a carbamate ion. The likely source of the m/z 158 fragment is through a McLafferty rearrangement of the m/z 200 fragment, and subsequent loss of an isopropyl group. The fragment at m/z 176 is representative of a loss of isopropylformamide from the molecular ion. Figure 5 illustrates the proposed fragmentation pathways.
For the determination of the carisoprodol content in the tablets, the SIM data was used. The protonated molecular ion (m/z = 261) was monitored in the SIM experiment. Figure 6 provides an example of a carisoprodol SIM chromatogram. The concentration of carisoprodol present in the tablets was not declared, but based on internet research, the tablets were purported to contain 350 mg carisoprodol each. Five individual tablets were assayed, with results ranging from 322 mg to 329 mg carisoprodol per tablet, and a mean concentration of 325 mg carisoprodol per tablet. This range of values represents an RSD of 1.0%. Assuming the “declared” value for the tablets is 350 mg, the average value of 325 mg/tablet translates to 93% of label claim.

Two individual tablets were each spiked with a portion of the USP Carisoprodol reference standard. Tablet 1 was spiked with carisoprodol at a level of 554 mg/g, and tablet 2 at a level of 913 mg/g. Recoveries of the carisoprodol were 90% and 95%, respectively.

Figure 5: Proposed Fragmentation Patterns for Carisoprodol by ESI-LC-MS
Conclusions

The analysis of carisoprodol tablets was performed using liquid chromatography with an electrospray interface and mass selective detection. The results obtained for five individual tablets ranged from 322 mg/tablet to 329 mg/tablet with an RSD of 1%. The accuracy of the method was demonstrated by spike recoveries of 90 – 95%. The on column detection limit was determined to be 6.7 picograms for carisoprodol. By using the technique discussed, finished dosage forms can be screened for the presence of carisoprodol and the carisoprodol content can accurately be determined.

Acknowledgements

The authors would like to thank Bryan M. Gamble for his assistance with the proposed fragmentation patterns for carisoprodol.

References


FDA Disclaimer: The Laboratory Information Bulletin is a tool for the rapid dissemination of laboratory methods (or information) which appear to work. It may not report completed scientific work. The users must assure themselves by appropriate validation procedures that LIB methods and techniques are reliable and accurate for their intended use. Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the U.S. Food and Drug Administration.

* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
INSTRUCTIONS FOR AUTHORS

General Information
Microgram Journal is a scientific periodical that is published by the U.S. Drug Enforcement Administration’s Office of Forensic Sciences, and presents peer reviewed, full length Scientific Research Articles and Technical Notes on the detection and analyses of suspected controlled substances for forensic/law enforcement purposes.

Subscriptions to Microgram Journal
Microgram Journal is unclassified, and is published on the DEA public access website (www.dea.gov). Private citizens should use the website to access Microgram Journal. Professional scientific and law enforcement personnel may either use the website or request a subscription. Subscriptions are available electronically and in hard copy. Electronic subscriptions require Internet access. The publications themselves will not be sent electronically to any subscriber; rather, an email notification of the pertinent URL will be sent to the subscriber when the respective issue is posted on the website (see additional information on email notifications, below). Requests for hard copies are strongly discouraged, and should be limited to those offices that do not have access to the Internet, require hard copies for their libraries, or have some other valid reason (Note: “For my personal collection” is not considered to be a valid reason). Requests for hard copies should indicate the number of copies required (maximum of two allowed per office), and should also include formal justification. Note that due to publication delays beyond the control of the Office of Forensic Sciences, hard copies will arrive from 30 to 180 days after electronic posting.

Requests to be added to the subscription list should be submitted via email to the Microgram Editor at: microgram_editor@mailsnare.net If email submission is not possible, requests should be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. All requests to be added to the Microgram mailing list should include the following Standard Contact Information:

* The Full Name and Mailing Address of Submitting Laboratory or Office;

* The Full Name, Title (Laboratory Director, Assistant Special Agent in Charge, Librarian, etc.), Phone Number, FAX Number, and Preferred email Address of the Submitting Individual (Note that subscriptions are mailed to titles, not names, in order to avoid subscription problems arising from future personnel changes);

* If available, the generic email address for the Submitting Laboratory or Office;

* If a generic email address is not available, one private email address for an individual who is likely to be a long-term employee, who has a stable email address, and who will be responsible for forwarding Microgram information to all of the other employees in the requestor’s Office (Note that only one email address per Office will be honored);

* If requesting hard copy mailings, the number of copies requested (two max), and justification.

Requests to be removed from the Microgram subscription list, or to change an existing subscription, should also be sent to the Microgram Editor. Such requests should included all of the pertinent standard contact information detailed above, and also should provide the email and/or hard mail address currently being utilized for the requestor’s subscription.

Note that, due to mailing delays and/or publication timeframes, subscription requests/changes may take as long as 90 days to implement.
Email Notifications
As noted above, electronic subscriptions are email based. The email provides a notification of the Microgram URL when a new issue is posted, and additional information as appropriate. Note that Microgram notices will NEVER include any attachments, or any hyperlink other than the Microgram URL. This is important, because the microgram_editor@mailsnare.net address is routinely hijacked and used to send spam, very commonly including malicious attachments. For this reason, all subscribers are urged to have current Anti-Viral, Anti-Spyware, and Firewall programs in operation.

Costs
Subscriptions to Microgram are free.

Submissions to Microgram Journal
Manuscripts are accepted both from within and outside of DEA, and reviewers for the Journal are both internal (from within DEA) and external.

All submissions must be in English. All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: microgram_editor@mailsnare.net Current versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3 ½ inch IBM® PC-compatible diskette, 100 or 250 MB Iomega® zip diskette, or an IBM® PC-compatible CD. Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: “Warning - Contains Electronic Media - Do Not Irradiate”. Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 ½" x 11" or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. The pages should be numbered, but not stapled together.

Note that mailed submissions may be subject to lengthy handling delays beyond the control of the Office of Forensic Sciences, and electronic media sent through the mail may be destroyed en route by sanitizing procedures, despite protective measures and written warnings. All submissions should include the following Contact Information: The Full Name and Address of Submitting Laboratory or Office, and the Full Name, Phone Number, FAX Number, and Preferred email Address of the Submitting Individual.

Scientific Research Articles are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.) Technical Notes are shorter communications concentrating on a specific drug (or drug class), unusual case, novel or unusual procedure or method, or minor original research. Each article/note should be a “stand-alone” work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to full peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is received by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes may follow an abbreviated version as appropriate):
Cover Letter - Provide the standard contact information and pertinent correspondence (if any) for the Editor.

Title - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

Author(s)/Affiliation(s) - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author’s degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page.

Abstract - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under “Titles”. Note that the abstract will be provided to Chemical Abstracts.

Keyword List - A minimum of five (maximum ten) abstracting keywords should be included.

Introduction - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid “Personal Communications”).

Experimental (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, USE CAUTION IN DETAILING SYNTHESSES OF CONTROLLED OR ABUSED SUBSTANCES, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: “Experimental details on this synthesis are not provided, in accordance with Journal policy.”]

Results and Discussion - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

Conclusions - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or a summary paragraph in the Results and Discussion section.

Acknowledgments - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

References - Articles and notes should have all textual citations collected in an endnotes list. Within the text, references should be consecutively numbered with superscripted Arabic numerals, or with Arabic numerals in parentheses, in accordance with their first appearance. Within the endnotes list, references should be consecutively numbered with Arabic numerals, as follows: Number, Period, Indent, Citation.
Reference format should adhere to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Note: This is the same reference format utilized in the Selected Reference Citations in Microgram Bulletin, and also (among many others) by the Journal of Forensic Sciences). Due to their inherently transitory nature, use of website URL’s as references are discouraged but permitted. As previously noted, Personal Communications should not be utilized; however, if unavoidable, utilize the following format: Full Name, Title, Affiliation (Laboratory or Office), Location (City and State, plus Nation if not the United States), Personal Communication, Year.

**Table and Figures** - All Tables and Figures should be appended onto the end of the article (not embedded in the text). Tables and Figures should be consecutively numbered with Arabic numerals, in accordance with their first citation in the text. Each Table and Figure should be “stand-alone”; that is, include sufficient descriptive information such that the reader will not have to refer back to the text to understand the Table or Figure. The Header should include the Table or Figure number and a concise title. Explanatory material, definitions of acronyms and/or abbreviations, and/or references within the Table or Figure should be designated by superscripted, lower case letters in alphabetical order, and included in dedicated footnotes at the bottom of the respective Table or Figure. Unless color is needed to enhance differentiation of the depicted material, all Tables and Figures should be in black and white (that is, avoid frivolous use of color for “artistic” purposes). Figures of spectra, chromatograms, charts, graphs, etc., should have clear and legibly labeled axes, but should not include instrument generated printoffs of experimental parameter lists.

Manuscripts submitted to Microgram Journal are required to be finished, professional quality efforts. Authors should ensure clarity, brevity, and pertinence of all information. Attention to detail in formatting, syntax, grammar, and spelling are as important as the accuracy of the facts presented. Authors are specially cautioned to conduct careful literature reviews prior to submission. At the Editor’s discretion, clearly substandard and/or inappropriate manuscripts will be returned to the authors without review.

**Manuscripts will not be retyped, but “final” versions are subject to minor to moderate Editorial rewrite to improve presentation clarity or to reformat to current Microgram Journal style.**

**Dual publication** - Re-publication of articles or notes of particular interest to the Microgram Journal readership will be considered if the article was originally published in a journal that is not easily accessed and the primary author has obtained explicit, written copyright exclusion from the original publisher and consent from all co-authors. Examples include exact English translations of articles or notes originally published in a non-English language journal, non-sensitive articles or notes originally published in a restricted journal or on a password protected website, or articles or notes originally published in limited distribution newsletters or proceedings. In general, any article or note that was published in English in a mainstream journal is not a candidate for re-publication in Microgram Journal. Authors interested in re-publishing previously published articles or notes in Microgram Journal should discuss the issue with the Microgram Editor before submitting.

Note that (in accordance with standard ethical guidelines) re-published articles should not be included as “new” articles in the respective author(s)’ Curriculum Vitae.

**Costs** - There are no costs (to the contributor) associated with publication in Microgram Journal.

**Reprints** - Microgram Journal does not provide reprints to authors. Microgram Journal may be photocopied as needed.

Questions may be directed to the Microgram Editor.
Microgram

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC 20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 3
Numbers 1-2
January - June 2005

Posted On-Line At:
<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characterization of the “Indanylamphetamines”</td>
<td>3</td>
</tr>
<tr>
<td>John F. Casale, Timothy D. McKibben, Joseph S. Bozenko, and Patrick A. Hays</td>
<td></td>
</tr>
<tr>
<td>Laboratory Analysis of the Conversion of Pseudoephedrine to Methamphetamine from Over-the-Counter Products</td>
<td>11</td>
</tr>
<tr>
<td>Robert P. Bianchi, Manoj N. Shah, David H. Rogers, and Thomas J. Mrazik</td>
<td></td>
</tr>
<tr>
<td>Spectral Characterization of 2,4-Dimethoxy-3-methylphenethylamine and Comparison to 2,5-Dimethoxy-4-methylphenethylamine (“2C-D”)</td>
<td>16</td>
</tr>
<tr>
<td>Russell A. Allred</td>
<td></td>
</tr>
<tr>
<td>Analytical Profile of Modafinil</td>
<td>27</td>
</tr>
<tr>
<td>Jeremiah A. Morris</td>
<td></td>
</tr>
<tr>
<td>Quantitation and Enantiomeric Determination of Propoxyphene Using Capillary Zone Electrophoresis</td>
<td>31</td>
</tr>
<tr>
<td>Clay P. Phelan</td>
<td></td>
</tr>
<tr>
<td>Identification and Quantitation of Hydromorphone Hydrochloride in Palladone® (Extended Time-Release) Capsules</td>
<td>39</td>
</tr>
<tr>
<td>Pamela R. Smith, Amanda K. Frohwein, Patrick A. Hays, and Ira S. Lurie</td>
<td></td>
</tr>
<tr>
<td>gamma-Hydroxybutyrate, Silver Salt (AgGHB): Identification of gamma-Hydroxybutyrate (GHB) via Conversion to the Silver Salt</td>
<td>46</td>
</tr>
<tr>
<td>James V. DeFrancesco</td>
<td></td>
</tr>
<tr>
<td>Analytical Profiles for Five “Designer” Tryptamines</td>
<td>54</td>
</tr>
<tr>
<td>Trinette K. Spratley, Patrick A. Hays, Lois C. Geer, Sam D. Cooper, and Timothy D. McKibben</td>
<td></td>
</tr>
<tr>
<td>Desloratadine: The Reaction Byproduct of the Reduction of Cold Tablets Containing Loratadine with Hydriodic Acid/Red Phosphorus</td>
<td>69</td>
</tr>
<tr>
<td>Shannon C. DiPari, Jason A. Bordelon, and Harry F. Skinner</td>
<td></td>
</tr>
<tr>
<td>Identification of Phenethylamines and Methylene dioxyamphetamines Using Liquid Chromatography Atmospheric Pressure Electrospray Ionization Mass Spectrometry</td>
<td>78</td>
</tr>
<tr>
<td>Adrian S. Krawczeniuk</td>
<td></td>
</tr>
<tr>
<td>Instructions for Authors</td>
<td>101</td>
</tr>
</tbody>
</table>

**Note:** In order to prevent automated theft of email addresses off the Internet postings of *Microgram Journal*, all email addresses reported in the *Journal* have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of Ketamine (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).
Characterization of the “Indanylamphetamines”

John F. Casale,* Timothy D. McKibben, Joseph S. Bozenko, and Patrick A. Hays
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email address withheld at author’s request]


ABSTRACT: Spectroscopic and chromatographic data are provided for 5-(2-aminopropyl)-2,3-dihydro-1H-indene 1 (the indane analog of 3,4-methylenedioxyamphetamine 2), 4-(2-aminopropyl)-2,3-dihydro-1H-indene 3 (the aromatic ring positional isomer of 1), and their respective synthetic intermediates. The data allow the identification and differentiation of 1 and 2 in illicit drug exhibits.

KEYWORDS: Indanylamphetamine, Amphetamine Analogs, Designer Drugs, Chemical Analysis, Forensic Chemistry.

Figure 1. Structural Formulas

Introduction

Clandestine laboratory operators have synthesized so-called “designer” or “analog” drugs for many years in efforts to avoid prosecution under existing statutes, and/or to produce more powerful drugs or drugs with alternate central nervous system (CNS) and/or psychoactive properties. The production (and use) of such compounds are the focus of a wide variety of texts, literature articles, and websites. The best known texts in this field, including extensive syntheses of designer/analog drugs along with detailed reports of their CNS and/or psychoactive activity levels based on self-experimentation, are PIHKAL (Phenethylamines I Have Known And Loved) and TIHKAL (Tryptamines I Have Known And Loved) by Shulgin and Shulgin [1,2].

Currently, the methylenedioxyamphetamine (3,4-methylenedioxyamphetamine (MDA, 2), 3,4-methylenedioxy-methamphetamine (MDMA), etc.) are the most popular and widely used CNS-active, psychoactive drugs on the illicit markets. Virtually all of the common MDA’s are controlled under U.S. and international statutes, encouraging the production and use of designer/analog drugs.
Additional encouragement occurred in late 2000, when the seizure of the world’s largest-ever lysergic acid diethylamide (LSD) synthesis laboratory, and the disruption of its associated distribution network [3], resulted in a major decline in LSD supplies worldwide, and an elevated demand for alternate hallucinogens. These have included traditional and well known substances such as psilocybin mushrooms, but also some unusual substances such as *Salvia divinorum* and many of the psychoactive phenethylamines and tryptamines featured in PIHKAL and TIHKAL.

Since about 2003, the indanyl analog of MDA, that is, 5-(2-aminopropyl)-2,3-dihydro-1H-indene 1 (also known as 1-(5-indanyl)-2-aminopropane, commonly abbreviated as 5-IAP or IAP (Figure 1)) has been submitted to forensic laboratories in the U.S., usually as suspected ecstasy (MDMA). 5-IAP is also commonly - but incorrectly - referred to as “indanylamphetamine” (probably a misinterpretation of the meaning of “IAP”). 5-IAP was first reported by the Nichols group in 1993 [4], and again in 1998 [5], in two studies focusing on its pharmacological activity.

Although the Nichols group does not so state, the synthesis of 5-IAP invariably produces a lesser quantity of its aromatic ring positional isomer, 4-(2-aminopropyl)-2,3-dihydro-1H-indene (4-IAP) 3. Although 4-IAP is not known (or expected) to have significant CNS stimulant activity (and therefore has minimal abuse potential), its close structural similarity to 5-IAP, and its likely presence in exhibits containing illicitly prepared 5-IAP, merits detailed spectroscopic and chromatographic delineation of the two compounds.

**Experimental**

**Chemicals and Reagents**
All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were reagent-grade and products of Aldrich Chemical (Milwaukee, WI).

**Instrumentation**
Gas Chromatography/Mass Spectrometry (GC/MS) - Mass spectra were obtained on an Agilent Model 5973 quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV and a scan range of 34-700 amu at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J & W Scientific, Rancho Cordova, CA, USA). The oven temperature was programmed as follows: initial temperature, 100 °C; initial hold, 0.0 min; program rate, 6 °C/min; final temperature, 300 °C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and a temperature of 280 °C. The auxiliary transfer line to the MSD was operated at 280 °C.

Infrared Spectroscopy (FTIR-ATR) - Infrared spectra were obtained on a Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory.

Nuclear Magnetic Resonance Spectroscopy (NMR) - Proton (1H), carbon (13C), and 2-dimensional NMR spectra were obtained on a Varian Inova 600 MHz NMR using a 5 mm Varian Nalorac Z-Spec broadband variable temperature, pulse field gradient probe (Varian, Palo Alto, CA). All compounds were dissolved in deuterochloroform (CDCl₃) containing 0.03 percent v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 25 °C. Standard Varian pulse sequences were used to acquire proton, proton-decoupled carbon, and gradient versions of COSY, HSQC, and HMBC. Data processing was performed using software from Varian and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Prediction of proton and carbon spectra was accomplished using ACD/Labs HNMR and CNMR Predictors.

**Syntheses**
The procedure of Nichols *et al.* [4] was followed for the preparation of 5-IAP 1 and its intermediates. A modification of the same procedure was utilized to prepare 4-IAP 3 and its intermediates. Due to the sensitive nature of this subject, exact experimental details and yields are not reported.
Results and Discussion

The synthetic procedure described by Nichols et al. is the most convenient route to 5-IAP, but as previously noted it produces both 4-IAP and 5-IAP (see Figure 2). To summarize, indane 4 is formylated with SnCl₄ and dichloromethyl methyl ether to give a mixture of the aldehydes 5 and 6 in about a 15:85 ratio. If desired, the aldehydes can be separated via alumina column chromatography. Condensation of the aldehydes with nitroethane gave the nitropropenes 7 and 8. Nitropropene 8 can be isolated from 7 by recrystallization from n-hexane at -76 °C. 4-IAP 3 and 5-IAP 1 are obtained from their respective nitropropenes 7 and 8 via LiAlH₄ reduction. If the intermediate products are not purified, the resulting final product will contain both 4-IAP 3 and 5-IAP 1 in about a 15:85 ratio.

Figure 2.
GC retention time data for the respective compounds are presented in Table 1. The amines were injected as their free bases since the hydrochloride ion-pairs of some phenethylamines undergo thermally induced degradation and chromatograph poorly [6]. 4-IAP and 5-IAP (15:85) are baseline resolved under the chromatographic conditions utilized (Figure 3).

**Table 1:** Gas Chromatographic Retention Times (min) for the “Indanylamphetamines” and their Synthetic Precursors. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.60</td>
</tr>
<tr>
<td>2</td>
<td>9.00</td>
</tr>
<tr>
<td>3</td>
<td>9.40</td>
</tr>
<tr>
<td>4</td>
<td>2.97</td>
</tr>
<tr>
<td>5</td>
<td>6.67</td>
</tr>
<tr>
<td>6</td>
<td>7.16</td>
</tr>
<tr>
<td>7</td>
<td>14.59</td>
</tr>
<tr>
<td>8</td>
<td>15.80</td>
</tr>
</tbody>
</table>

* Conditions given in Experimental Section.

**Figure 3.** Partial Reconstructed Total Ion Chromatogram of a Mixture of 4-IAP and 5-IAP. Peaks: 1 = 4-IAP; and 2 = 5-IAP

The IR spectra for 4-IAP and 5-IAP are illustrated in Figure 4. Comparison of the hydrochloride ion pairs reveals similar absorption patterns with the most prominent, yet subtle, differences in the C-H out-of-plane bending frequencies between 700 - 900 cm⁻¹. However, since the spectra are quite similar, additional or supplementary spectroscopic methods should be utilized for definitive identification.
Mass spectra for 4-IAP and 5-IAP, nitropropenes 7 and 8, and aldehydes 5 and 6, are presented in Figures 5 - 7, respectively (top and bottom traces). 4-IAP and 5-IAP each gave a base peak at \( m/z \) 44, but were easily distinguished by the relative abundances of ions at \( m/z \) 115 and 117 and also at \( m/z \) 128 and 131 (Figure 5). Both gave weak fragment ions as well as a weak molecule ion at \( m/z \) 175. The nitropropene intermediates 7 and 8 each gave base peak at \( m/z \) 115, but were easily distinguished by the relative abundances of ions at \( m/z \) 115 and 117 and also at \( m/z \) 141 and 145 (Figure 6). The aldehyde intermediates 5 and 6 each gave base peak at \( m/z \) 146, and were easily distinguished by the relative abundances of ions at \( m/z \) 145 and 146 (Figure 7).

The proton and carbon chemical shifts and splitting patterns for 4-IAP, 5-IAP, and their respective intermediates are presented in Tables 2 and 3, respectively. Assignments were based on proton and carbon chemical shift values, proton splitting patterns and coupling constants, and correlations between proton and carbon using the HSQC (directly bonded carbon-to-proton) and HMBC (2, 3, or 4 bond correlations between carbon and proton) experiments. The proton and carbon spectra for each structure were predicted using ACD/Labs HNMR and CNMR Predictors as an additional check. The substituent position on the indane ring was very easily determined using the aromatic proton splitting patterns. Substitution at carbon 4 resulted in 3 adjacent protons, giving a doublet, triplet, and doublet splitting pattern for the 5, 6, and 7 hydrogens, respectively. Substitution at carbon 5 resulted in a broad singlet (H-4) and two broad doublets (H-6 and H-7). The broadness of the singlet and the H-6 doublet is caused by a coupling constant less than one Hertz, typical of meta protons.

Conclusions

Analytical data is presented to assist delineating 4-IAP from 5-IAP, as well as their respective synthetic intermediates. Characterization is best achieved by GC/MS or NMR. Due to their similarities, the FTIR spectra should be supplemented with another spectroscopic method for definitive identification.
References


* * * * *

Figure 5. Electron Ionization Mass Spectra of (a) 4-IAP HCl and (b) 5-IAP HCl.
Figure 6. Electron Ionization Mass Spectra of (a) 4-[1-(nitropropenyl)]-2,3-dihydro-1H-indene 7 and (b) 5-[1-(nitropropenyl)]-2,3-dihydro-1H-indene 8.

Figure 7. Electron Ionization Mass Spectra of (a) 2,3-dihydro-1H-indene-4-carboxaldehyde 5 and (b) 2,3-dihydro-1H-indene-5-carboxaldehyde 6.
Table 2: NMR Proton Chemical Shifts (in ppm) and Splitting Patterns of 4-IAP HCl, 5-IAP HCl, and Related Compounds. Samples Run in CDCl₃ with TMS as the Reference Compound for 0 ppm.

<table>
<thead>
<tr>
<th>Proton(s)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.87 t</td>
<td>2.89-2.97 m</td>
<td>2.06 p</td>
<td>2.97 t</td>
<td>2.93 t</td>
<td>2.95 t</td>
</tr>
<tr>
<td>2</td>
<td>2.05 p</td>
<td>2.06 p</td>
<td>2.91 t</td>
<td>2.13 p</td>
<td>2.13 p</td>
<td>2.12 p</td>
</tr>
<tr>
<td>3</td>
<td>2.86 t</td>
<td>2.89-2.97 m</td>
<td>3.29 t</td>
<td>2.97 t</td>
<td>2.98 t</td>
<td>2.95 t</td>
</tr>
<tr>
<td>4</td>
<td>7.07 bs</td>
<td>--</td>
<td>7.37 bs</td>
<td>--</td>
<td>7.30 s</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>6.99 d</td>
<td>7.63 d</td>
<td>--</td>
<td>7.17 d</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.96 bd</td>
<td>7.09 t</td>
<td>7.32 t</td>
<td>7.66 bd</td>
<td>7.23 t</td>
<td>7.21 dd</td>
</tr>
<tr>
<td>7</td>
<td>7.14 d</td>
<td>7.13 d</td>
<td>7.47 d</td>
<td>7.36 d</td>
<td>7.30 d</td>
<td>7.29 d</td>
</tr>
</tbody>
</table>

CH₃ 1.38 d | 1.40 d | --    | --    | 2.40 s | 2.46 s |
CH₂ 2.82 dd| 2.86 dd| --    | --    | --    | --    |
CH 3.54 m  | 3.58 m | --    | --    | --    | --    |

bd = broad doublet, bs = broad singlet, d = doublet, dd = doublet of doublets, m = multiplet, p = pentet, s = singlet, t = triplet.

Table 3: NMR Carbon Chemical Shifts (in ppm) of 4-IAP HCl, 5-IAP HCl, and Related Compounds. Samples Run in CDCl₃ with TMS as the Reference Compound for 0 ppm.

<table>
<thead>
<tr>
<th>Carbon</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.78</td>
<td>33.08</td>
<td>33.08</td>
<td>33.41</td>
<td>31.80</td>
<td>32.92</td>
</tr>
<tr>
<td>2</td>
<td>25.44</td>
<td>25.06</td>
<td>25.45</td>
<td>25.59</td>
<td>24.82</td>
<td>25.37</td>
</tr>
<tr>
<td>3</td>
<td>32.50</td>
<td>31.55</td>
<td>31.97</td>
<td>32.61</td>
<td>32.91</td>
<td>32.74</td>
</tr>
<tr>
<td>3a</td>
<td>145.03</td>
<td>143.2</td>
<td>146.60</td>
<td>145.51</td>
<td>145.1</td>
<td>145.17</td>
</tr>
<tr>
<td>4</td>
<td>125.35</td>
<td>131.64</td>
<td>132.8 **</td>
<td>125.40</td>
<td>128.68</td>
<td>125.95</td>
</tr>
<tr>
<td>5</td>
<td>133.48</td>
<td>127.22</td>
<td>129.42</td>
<td>135.49</td>
<td>125.91</td>
<td>130.33</td>
</tr>
<tr>
<td>6</td>
<td>127.16</td>
<td>126.71</td>
<td>126.9</td>
<td>129.13</td>
<td>126.57</td>
<td>128.47</td>
</tr>
<tr>
<td>7</td>
<td>124.62</td>
<td>123.42</td>
<td>130.14</td>
<td>125.03</td>
<td>126.12</td>
<td>124.84</td>
</tr>
<tr>
<td>7a</td>
<td>143.21</td>
<td>145.0</td>
<td>152.80</td>
<td>152.28</td>
<td>145.1</td>
<td>147.04</td>
</tr>
<tr>
<td>aldehyde</td>
<td>--</td>
<td>--</td>
<td>192.98</td>
<td>192.54</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>alkene CH</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>131.91</td>
<td>134.33</td>
</tr>
<tr>
<td>alkene quaternary</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>147.86</td>
<td>146.77</td>
</tr>
<tr>
<td>CH₃</td>
<td>18.13</td>
<td>18.29</td>
<td>--</td>
<td>--</td>
<td>14.08</td>
<td>14.18</td>
</tr>
<tr>
<td>CH₂</td>
<td>40.99</td>
<td>38.94</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>CH</td>
<td>50.03</td>
<td>48.89</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

** = chemical shift determined using HMBC experiment. Peak not visible in direct carbon experiment.
Laboratory Analysis of the Conversion of Pseudoephedrine to Methamphetamine From Over-the-Counter Products

Robert P. Bianchi, BS*
Prescription Drug Research Center
Mason Enterprise Center
4031 University Drive, Suite 200
Fairfax, VA  22030
[email:  RBconsulting700 -at- aol.com]

Manoj N. Shah, PhD, David H. Rogers, PhD, and Thomas J. Mrazik, PharmD
Medical Information & Communications
McNeil Consumer & Specialty Pharmaceuticals
7050 Camp Hill Road
Fort Washington, Pennsylvania  19034


ABSTRACT: Two approaches to convert pseudoephedrine (PSE) to methamphetamine from over-the-counter (OTC) PSE products were examined. The first approach was two-step, and involved PSE extraction followed by conversion using the Birch method. Multiple-active products containing PSE and 2 - 4 actives were tested, including caplet, tablet, liquid, and liquid-filled softgel forms. The extent of conversion to methamphetamine varied among the extracts, and was up to 30.7 percent of the PSE present in the starting product. PSE extract conversion to methamphetamine was realized regardless of dosage form (i.e., whether solids, liquids, or liquid-filled softgels were used). The second approach involved direct conversion of PSE to methamphetamine using the Birch method. Materials tested included pure PSE powder, and also a combination of PSE plus an analgesic as either a powder mixture or as an OTC caplet. The extent of conversion to methamphetamine ranged from 54.1 to 67.7 percent of the PSE present in the starting material. These results provide scientific proof that PSE from solid and liquid OTC products can be converted to methamphetamine using either extraction or direct approaches (both employed by small clandestine laboratory operators). The ease and extent of PSE conversion from extracts appears to be independent of the PSE starting quantity, dosage forms, and presence of other actives.

KEYWORDS: Acetaminophen, Birch Method, Dextromethorphan, Extraction, Guaifenesin, Methamphetamine, Nazi Method, OTC PSE Products, Pseudoephedrine, Forensic Chemistry

Introduction

Methamphetamine abuse has reached widespread proportions in the United States, causing serious social, economic, and environmental problems for communities, and draining scarce law enforcement resources (1-4). Currently, thousands of small toxic laboratories (STLs) are seized annually throughout the country, especially in the Midwest and western states. With very few exceptions, these laboratories are not operated by professional chemists, but rather by “cooks” who have learned from other “cooks”, the Internet (5), or from underground publications (6). Production scales are typically one ounce or less, and are intended for personal use and/or limited distribution. It is estimated that approximately 35 percent of the methamphetamine used in the United States comes from small-scale laboratories (7).

At present, the most popular precursor used for the clandestine manufacture of methamphetamine is pseudoephedrine (PSE) contained in over-the-counter (OTC) sinus and cold preparations (8). The preferred
starting material has been single-ingredient tablets (i.e., containing no other active ingredients). The PSE in these products are extracted with alcohol, filtered, and converted to methamphetamine via the Birch reduction method (Nazi method) or one of the red phosphorus methods (9,10). The first step in this study was therefore to determine the efficiency of extracting PSE from a variety of dosage forms, with subsequent conversion to methamphetamine using the Birch reduction method, which is currently the most popular among STL “cooks.” The second approach was to directly convert PSE or PSE-containing OTC products to methamphetamine, without the preliminary extraction step, again using the Birch reduction method.

Experimental

The described experiments were conducted by National Medical Services, Willow Grove, Pennsylvania, an independent forensic laboratory accredited by the American Society of Crime Laboratory Directors – Laboratory Accreditation Board (ASCLD-LAB).

Pseudoephedrine Extraction Followed by Birch Method Conversion
A simple extraction process was performed on three multiple-active ingredient OTC products, each of which contained PSE, the pain reliever acetaminophen, and up to two other active ingredients (Table 1). The process involved grinding the tablets or caplets (modified for liquid-filled softgels), dissolving the resulting powder or liquid in denatured ethanol, filtering to isolate the solution, evaporating it to a small volume, adding acetone to precipitate the PSE, and collecting the precipitate by filtration. The procedure was conducted on a large scale (equivalent to 7.5 grams of PSE (i.e., 250 tablets/caplets or 100 liquid-filled softgels)) to simulate a typical small-scale illicit methamphetamine synthesis. The recovered PSE was directly submitted to the Birch reduction method. The method involves dissolution of the PSE in anhydrous ammonia, and then adding lithium metal (9). Quantitative analysis of the recovered PSE (and other active ingredients) from the first step, and of the methamphetamine and unreacted PSE from the second step, were performed using liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Because illicit laboratories are known to employ additional extraction techniques (in addition to the method described above), five multiple-active OTC products, each of which contained PSE, acetaminophen, and up to 2 additional active ingredients (Table 1), were submitted to a more complex extraction process. This latter procedure involved dissolution of the sample in dilute hydrochloric acid, washing with a naphtha-based organic solvent to remove polymers, waxes, and other inert ingredients, alkalinization to form PSE base, two extractions with toluene to isolate the PSE base, and then conversion of the PSE base back to the HCl salt. The complex extraction was also conducted on a 7.5 gram PSE scale (250 caplets, 100 softgels, or 600 mL of liquid). Quantitative analysis of the extracts and synthesized methamphetamine was again conducted by LC/MS/MS.

Direct Birch Method Conversion
Pure PSE powder, a mixture of PSE and acetaminophen powder, and ground caplets containing PSE and acetaminophen were directly subjected to the Birch reduction method, and the resulting products were subjected to quantitative analysis by LC/MS/MS. The quantity of PSE in each of the starting materials was 0.2 grams, 0.2 grams, and 0.18 grams, respectively.

Results and Discussion

Pseudoephedrine Extraction Followed by Birch Method Reduction
The results demonstrated PSE conversion to methamphetamine from all of the PSE precipitates subjected to the Birch method reduction (Table 2). The percentage of PSE in the starting OTC product that was converted to methamphetamine ranged from 0.3 to 30.7 percent. The conversion efficiency was comparable for PSE solid forms (ranging from 3.0 to 100 percent of the PSE in the extract being converted to methamphetamine) and PSE liquid forms (ranging from 37.5 to 100 percent of PSE in the extract being converted to methamphetamine). The starting quantity of PSE and the presence of other ingredients did not appear to affect the extent of conversion.
Direct Birch Method Conversion

Direct conversion of pure PSE powder was performed as a baseline reference. A second, powdered mixture of PSE (0.2 g) and acetaminophen (1.0 g) was used to assess the potential for interference from acetaminophen in the conversion process (since many of the OTC medications contain a high proportion of acetaminophen). A third experiment using an OTC tablet product containing PSE and acetaminophen was used to further verify the potential of direct conversion (6 tablets containing 0.18 g PSE and 3.0 g acetaminophen were ground using a mortar and pestle). The results confirmed PSE conversion to methamphetamine from all PSE forms, with 54.1 to 67.7 percent conversion of PSE to methamphetamine (Table 2). The results for PSE plus acetaminophen (i.e., powder and caplet) were comparable to those of PSE powder alone. Based on the results from the PSE extraction, it would be expected that direct conversion would also succeed for liquid and soft-gel forms.

Conclusions

The results of this study demonstrated that OTC sinus and cold preparations containing PSE can be converted to methamphetamine either directly or following a PSE extraction procedure, from both single- and multiple-active ingredient formulations. This study provides scientific proof that virtually any OTC product containing PSE can be used to manufacture methamphetamine (11,12). That is, the potential for methamphetamine production from PSE-containing OTC products is independent of dosage form (solid, liquid, or liquid-filled softgel), presence of actives, and formulation variations.

Acknowledgements

The authors thank Dr. Kevin Ballard, MD, PhD, and David Easterling, MS, National Medical Services, Willow Grove, Pennsylvania, for performing the extractions, methamphetamine synthesis, and chemical analyses for these experiments.

[Funding support: This study was funded by McNeil Consumer & Specialty Pharmaceuticals.]

References


7. Interim report from the Interagency Working Group on Synthetic Drugs to the Director of National Drug Control Policy, Attorney General, Secretary for Health and Human Services. May 23, 2005. At


11. Bremer N, Woolery RJ. The yield of methamphetamine, unreacted precursor and Birch by-product with the lithium-ammonia reduction method as employed in clandestine laboratories. Newsletter of Midwestern Association of Forensic Scientists 1999; Fall:8-16.


* * * * *

Table 1. Active Ingredients Per Dosage Unit Included in Tested OTC PSE Products.

<table>
<thead>
<tr>
<th>Product Identifier</th>
<th>Dosage Form</th>
<th>Decongestant (mg)</th>
<th>Analgesic (mg)</th>
<th>Antihistamine (mg)</th>
<th>Antitussive (mg)</th>
<th>Expectorant (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PSE Simple Extraction Followed by Birch Method Conversion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Caplet</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(500)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Tablet</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>-</td>
<td>GUA</td>
<td>(200)</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(325)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Liquid Filled</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>DXM</td>
<td>GUA</td>
<td>(100)</td>
</tr>
<tr>
<td>Softgel</td>
<td>(30)</td>
<td>(250)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PSE Complex Extraction Followed by Birch Method Conversion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Caplet</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(500)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Caplet</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>CLR</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(500)</td>
<td></td>
<td>(2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Caplet</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>DXM</td>
<td>GUA</td>
<td>(200)</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(325)</td>
<td></td>
<td>(15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Liquid Filled</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>DXM</td>
<td>GUA</td>
<td>(100)</td>
</tr>
<tr>
<td>Softgel</td>
<td>(30)</td>
<td>(250)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Liquid</td>
<td>PSE</td>
<td>APAP</td>
<td>-</td>
<td>DOX</td>
<td>DXM</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(500)</td>
<td></td>
<td>(6.25)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

APAP - Acetaminophen; CLR - Chlorpheniramine; DOX - Doxylamine; DXM - Dextromethorphan; GUA - Guaifenesin; OTC - Over-the-Counter; PSE - Pseudoephedrine.
Table 2. Results of Methamphetamine Conversion of OTC PSE Products.

<table>
<thead>
<tr>
<th>Product Identifier</th>
<th>Dosage Form</th>
<th>Quantity of PSE in Starting Extract (grams)</th>
<th>Percent PSE in Extract from Product</th>
<th>Percent PSE in Extract Converted to Methamphetamine</th>
<th>Percent PSE in Starting Products/ Material Converted to Methamphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Caplet</td>
<td>7.5</td>
<td>11.0</td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>Tablet</td>
<td>7.5</td>
<td>6.5</td>
<td>7.7</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Liquid Filled Softgel</td>
<td>3.0</td>
<td>14.8</td>
<td>37.5</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSE Simple Extraction Followed by Birch Method Conversion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSE Complex Extraction Followed by Birch Method Conversion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSE Conversion by Direct Birch Method (No PSE Extraction Step)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSE HCl Powder</td>
<td>0.2</td>
<td>67.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSE HCl + APAP Powder</td>
<td>0.2</td>
<td>65.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSE HCl + APAP Caplet</td>
<td>0.18</td>
<td>54.1</td>
<td></td>
</tr>
</tbody>
</table>

APAP - Acetaminophen; OTC - Over-the-Counter; PSE - Pseudoephedrine.

* * * * *
Spectral Characterization of 2,4-Dimethoxy-3-methylphenethylamine, and Comparison to 2,5-Dimethoxy-4-methylphenethylamine (“2C-D”)

Russell A. Allred, Ph.D.
U.S. Department of Justice
Southeast Laboratory
5205 N.W. 84th Ave.
Miami, FL  33166
[email: russell.a.allred -at- usdoj.gov]

ABSTRACT: Synthesis and analytical data for 2,4-dimethoxy-3-methylphenethylamine (2) and its hydrochloride salt (3) are described. 2 was synthesized from 2,4-dimethoxy-3-methylbenzaldehyde via trans-2,4-dimethoxy-3-methyl-β-nitrostyrene (1). The compounds were characterized by $^1$H NMR, $^{13}$C NMR, GC/MS, and FTIR. The data was compared to 2,5-dimethoxy-4-methylphenethylamine (2C-D).

KEYWORDS: Designer Drugs, Dimethoxyphenethylamines, Synthesis, Isomescaline, 2C-D, Desoxy, TIM, Forensic Chemistry

Introduction

A large number of phenethylamines derivatives are known, many of which have been reported to have CNS-stimulant and/or psychoactive properties.1 As a result, many phenethylamines compounds are listed as controlled substances. Notably, for each of these controlled substances are various possible isomers differing only in the positioning of the phenyl substituents. These positional isomers and analogues are (with few exceptions) not formally controlled; however, they may be prosecuted under the Analogue Statute of the Controlled Substances Act.

Examples of positional isomers that have circulated in the chemical underground are 2,5-dimethoxy-4-methylphenethylamine HCl (also known as “2C-D”) and 3,5-dimethoxy-4-methylphenethylamine HCl (also known as “DESOXY”).1 Recently, an exhibit containing 2C-D was received at this laboratory. Interestingly, the $^1$H NMR spectrum of 2C-D displays two singlets in the aromatic region that could potentially be confused for a doublet, albeit with a suspiciously large vicinal coupling constant (10 Hz). Trisubstituted phenethylamines may only form vicinally-derived doublets in the aromatic region if the phenyl substituents are arranged such that the two aromatic protons are $\alpha$ to each other.

An example of an isomer of 2C-D having adjacent phenyl protons is 2,4-dimethoxy-3-methylphenethylamine HCl (3).2 While NMR spectral differences between 3 and 2C-D can be predicted, it was preferable to demonstrate these differences from actual data.

The synthesis of 2,4-dimethoxy-3-methyl-β-nitrostyrene (1), 2,4-dimethoxy-3-methylphenethylamine (2), and 3 was originally reported by Merchant, et al.2 and is provided herein along with new spectroscopic data (Scheme 1). In addition, the analytical results are compared to those of the recently received 2C-D exhibit.

Experimental

Reagents: All reagents and solvents were obtained from commercial sources and unless otherwise noted were used as received. Tetrahydrofuran was dried with Na/benzophenone and distilled under nitrogen prior to use.
Scheme 1.
2,4-Dimethoxy-3-methyl-β-nitrostyrene (1-(2,4-dimethoxy-3-methylphenyl)-2-nitroethene) (1): To a nitromethane solution (30 mL) of anhydrous ammonium acetate (1.0 g, 13 mmol) was added 2,4-dimethoxy-3-methylbenzaldehyde (8.0 g, 44 mmol). The resulting mixture was stirred and heated for 20 minutes at light reflux. The solvent was then removed under reduced pressure (via rotary evaporator) while warming. The resulting orange solid was recrystallized from isopropanol, collected by vacuum filtration, and dried under vacuum (8.3 g, 85% yield). ¹H NMR (CDCl₃, 400 MHz): δ 8.12 (d, J=13.7 Hz, 1H), 7.72 (d, J=13.7 Hz, 1H), 7.34 (d, J=8.8 Hz, 1H), 6.69 (d, J=8.6 Hz, 1H), 3.87 (s, 3H), 3.74 (s, 3H), 2.15 (s, 3H); ¹³C{¹H} NMR (CDCl₃, 100 MHz): δ 162.4, 159.8, 136.0, 135.5, 129.1, 121.0, 116.3, 106.8, 61.3, 55.9, 9.0 (11 signals expected and observed) ppm. FTIR (neat/NaCl, cm⁻¹): 1621 (C=O str), 1579 (νC=O-C sym str), 1336 (νNO₂ sym str), 1107 (νC-O-C sym str). GC/MS: Rel. Rt: 2.00 (relative to methamphetamine), m/z (assignment): 223 (M⁺), 176 (base peak).

2,4-Dimethoxy-3-methylphenethylamine (2): To a 500 mL round bottom flask was added 2.1 g LiAlH₄ (56 mmol) and 70 mL dry THF. Under a nitrogen atmosphere was slowly added (via an addition funnel) 2.5 g 2,4-Dimethoxy-3-methylphenethylamine HCl (3). The reaction mixture was heated at reflux with stirring under a nitrogen atmosphere for 7 hours. After cooling the reaction mixture to ambient temperature, an equal volume of water (130 mL) was added, with the initial addition being done drop wise to minimize the vigorous reaction. The reaction mixture was extracted with EtOAc (4 x 90 mL); each extract was dried with Na₂SO₄, filtered, and combined. Removal of the solvent under reduced pressure resulted in a pale yellow oil as the crude product. This oil was redissolved in 10 mL CH₂Cl₂ and extracted with several fractions (3 - 4 mL each) of aqueous HCl (pH 2-3) until the pH of the final aqueous fraction did not increase (the latter was discarded). The combined aqueous fractions were base extracted with 2 M NaOH and CH₂Cl₂. The organic layer was collected and removal of the solvent under reduced pressure yielded 1.3 g of a clear oil (58% yield). ¹H NMR (CDCl₃, 400 MHz): δ 6.95 (d, J=8.2 Hz, 2H), 6.57 (d, J=8.4 Hz, 2H), 3.77 (s, 3H), 3.68 (s, 3H), 2.80 (t, J=7.0 Hz, 2H), 2.69 (t, J=7.0 Hz, 2H), 2.13 (s, 3H), 1.8 (br-s, N-H); ¹³C{¹H} NMR (CDCl₃, 100 MHz): δ 157.5, 157.2, 127.2, 124.5, 119.6, 106.0, 60.6, 55.5, 43.1, 34.0, 9.1 (11 signals expected and observed) ppm. FTIR (neat/NaCl, cm⁻¹): 3366 (νN-H str), 2929 (νC-H str), 1602 (νN-H bend), ~1590 (sh, νC=C-C str), 1268 (νC-O-N str) ppm. GC/MS: Rel. Rt: 1.57 (relative to methamphetamine), m/z (assignment): 195 (M⁺), 166 (base peak).

2,4-Dimethoxy-3-methylphenethylamine HCl (3). To a test tube of 0.34 g (6 mmol) dissolved in ~6 mL isopropanol was added 5-6 drops of concentrated HCl and mixed well. Crystallization was induced by addition of 0.5 mL Et₂O and cooling to ~2 °C for 2 hours. The resulting mixture was decanted, and the resulting crystalline solid was rinsed with diethyl ether and dried under vacuum, yielding 0.25 g of a white crystalline solid (63% yield). ¹H NMR (CDCl₃, 400 MHz): δ 8.32 (br-s, N-H, 3H), 7.00 (d, J=8.4 Hz, 1H), 6.57 (d, J=8.2 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.22 (br-m, 2H), 3.01 (t, J=7.1 Hz, 2H), 2.11 (s, 3H); ¹⁴N NMR (CDCl₃, 400 MHz): δ 7.05 (d, J=8.4 Hz, 1H), 6.71 (d, J=8.4 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.11 (t, J=7.6 Hz, 2H), 2.91 (t, J=7.6 Hz, 2H), 2.13 (s, 3H); ¹³C{¹H} NMR (CDCl₃, 100 MHz): δ 159.7, 158.8, 128.9, 122.6, 120.9, 107.6, 61.3, 56.1, 41.6, 29.4, 9.4 (11 signals expected and observed) ppm. FTIR (KBr, not assigned). FTIR (ATR, not assigned).
Results and Discussion

Synthesis of 1 involved a condensation/dehydration of the precursor 2,4-dimethoxy-3-methylbenzaldehyde with nitromethane in the presence of ammonium acetate (Scheme 1). Recrystallization from isopropanol provided yellow crystals of 1 in good yield. The mass spectrum of 1 (Figure 1) is consistent with its structure.

The 1H NMR spectrum of 1 (Figure 2) is consistent with formation of the expected, more stable trans isomer as evidenced by downfield chemical shifts and relatively large vicinal coupling constants compared to those typically found in the cis counterparts. The coupling constants for the alkene protons are slightly depressed with respect to comparable trans compounds due to the added electron-withdrawing effect of the nitro group.

The IR (Figures 3 and 4) spectral assignments also support the trans isomer of 1 based upon the work of By et al. (wherein related β-methyl-β-nitrostyrenes were compared and characterized by IR/Raman spectroscopy). Notably, the lower frequency for the ethylenic C=C stretching mode of 1, compared to the β-methyl-β-nitrostyrenes, can be accounted for by increased conjugation with the aromatic ring in the absence of the sterically hindering β-methyl group, allowing for a more planar conformation. On the other hand, the higher frequency observed for the symmetric NO2 stretching band of 1 can be explained by the absence of the electron donating β-methyl group.

The free base form of 2 was obtained from reduction of 1 with LiAlH4 in dry THF under an inert atmosphere (Scheme 1). The crude oily product obtained after work up of the reaction mixture was shown to contain minor amounts of impurities. Surmising that the desired product might have differing pKa value(s) from those of the impurities, 2 was successfully isolated by acid extraction with careful control of pH, followed by basic extraction. The MS, FTIR, 1H NMR spectra (Figures 5, 6, and 7, respectively) were consistent with the formation of 2.

Conversion of 2 to its hydrochloride salt was done from an isopropanolic solution mixed with a small amount of concentrated hydrochloric acid and diethyl ether, yielding a white, crystalline solid (Scheme 1) of 3. The IR spectra of 3 (Figures 8 and 9) are complicated by the broad and numerous bands displayed, particularly in the region between 3500 - 2000 cm⁻¹, as is expected for hydrated primary amine salts. The 1H NMR spectrum (Figure 10) exhibits a broad peak for the protonated amine at 8.32 ppm. Despite extensive drying of the crystalline material under vacuum, a water peak is still observed at ~1.7 ppm, likely due to the inclusion of a hydrogen bonded water molecule in the crystalline lattice of 3, suggesting the formation of a hydrate complex upon crystallization. Addition of 1 - 2 drops CD3OD to a CDCl3 solution of 3 results in a shift of the H2O peak downfield ~1.5 ppm as CD3OH is formed. In CD3OD, the 1H NMR spectrum (Figure 11) of 3 lacks peaks for the exchangeable amino and water protons. Due to a reduced solubility relative to 2 in chloroform solution, the 13C NMR spectrum of 3 was obtained in deuterated methanol.

Not surprisingly, the FTIR and mass spectra of 3 and 2C-D are fairly similar. However, differences in the substitution patterns on the phenyl ring make these compounds readily distinguishable by 1H NMR, as displayed in the spectrum (Figure 12) of the 2C-D exhibit received into this lab. The most distinguishing features are the two singlets of the phenyl protons in the spectrum of 2C-D, at 6.69 and 6.66 ppm, whereas 3 displays two doublets at 7.00 and 6.57 ppm, respectively.

It should be noted that closely related analogues such as 2,3,4-trimethoxyphenethylamine (also known as “isomescaline”) and 2,4-dimethoxy-3-thiomethylphenethylamine (also known as “TIM”) have been reported to be “non-active” (that is, having no noticeable pharmacological effects on the user.) The isostructural nature of 3 with these pharmacologically inactive compounds suggests that it is likewise inactive. However, because other dimethoxy/methyl-substituted phenethylamine isomers of 3 (e.g., 2C-D and DESOXY) are psychoactive, the situation is unclear. Regardless, these and other possible isomers can be readily distinguishable by NMR.
Acknowledgments

Notably, the concept for this project originated from former DEA Southeast Laboratory Director William D. Beazley. The author would like to thank Senior Forensic Chemist Patrick A. Hays, DEA Special Testing and Research Lab (Dulles, Virginia) for helpful information regarding the ^1^H NMR spectrum of 2C-D. Also, appreciation goes to DEA Librarian Rosemary Russo (Arlington, Virginia) for searching for and providing literature references.

References


* * * * *

Figure 1. Mass Spectrum of 1.
Figure 2. $^1$H NMR Spectrum of 1 in CDCl$_3$.

Figure 3. FTIR (KBr) Spectrum of 1.
Figure 4. FTIR (ATR) Spectrum of 1.

Figure 5. Mass Spectrum of 2.
Figure 6. FTIR (Neat, NaCl) Spectrum of 2.

Figure 7. $^1$H NMR Spectrum of 2 in CDCl$_3$. 
**Figure 8.** FTIR (KBr) Spectrum of 3.

**Figure 9.** FTIR (ATR) Spectrum of 3.
Figure 10. $^1$H NMR Spectrum of 3 in CDCl$_3$.

Figure 11. $^1$H NMR Spectrum of 3 in CD$_3$OD.
Figure 12. $^1$H NMR Spectrum of a 2C-D Exhibit in CDCl$_3$.  

* * * * *
Technical Note

Analytical Profile of Modafinil

Jeremiah A. Morris
Johnson County Sheriff’s Office
Criminalistics Laboratory
6000 Lamar
Mission, KS 66202
[email: Jeremiah.Morris @jocogov.org]

ABSTRACT: Analytical data (color tests, GC/MS, and FTIR) are reported for modafinil.

KEYWORDS: Modafinil, Provigil, Color Testing, GC/MS, FTIR, Forensic Chemistry.

Figure 1

Modafinil: 2-[(Diphenylmethyl)sulfinyl]acetamide;
C_{15}H_{15}NO_{2}S; mw = 273.36

Introduction

Modafinil (Figure 1), the active constituent of Provigil® tablets, became a Schedule IV controlled substance in January 1999. According to the manufacturer, modafinil is a CNS stimulant which possesses, “wake-promoting actions like sympathomimetic agents including amphetamine and methylphenidate, although the pharmacologic profile is not identical to that of sympathomimetic amines” [1].

Presumptive testing and instrumental data were collected to assist in the identification of submissions of modafinil tablets.

Experimental

Standard and Reagents
A reference standard of modafinil (Lot# 084K4633) was obtained from Sigma. Potassium bromide (IR grade, lot# 035261) and methylene chloride (ACS grade, lot# 040933) were obtained from Fisher. The derivatizing agent BSTFA+TMCS (99:1, lot# LA90822) was obtained from Supelco.

Methods and Instrumentation

Presumptive Color Tests: Portions of modafinil were placed in reagent wells followed by the addition of various presumptive color test reagents.

Tablet Extraction: A Provigil tablet extraction procedure was obtained from Cephalon [2]. A single tablet was ground and placed in a separatory funnel followed by the addition of 50-mL de-ionized water and 50-mL methylene chloride. The mixture was shook for approximately one minute with venting. A portion of the lower layer was drained, filtered, and evaporated to dryness, leaving a white powder residue.
**Derivatization:** A small portion of modafinil reference standard was placed in an autosampler vial followed by ~1 mL de-ionized water and ~0.5 mL BSTFA-TMCS derivatizing agent. The vial was capped tightly, mixed well, and incubated at ~70 °C for 30 minutes.

**GC/MS Analysis:** Analysis was performed with a HP 6890 GC equipped with a DB-35MS column (15 m x 0.25 mm ID, and film thickness 0.25 μm) and coupled to a HP 5973 Mass Selective Detector. The temperature during the analysis run increased from 90 °C to 300 °C at 20 °C/minute, held for 5 minutes, increased up to 310 °C at 30 °C/minute, and held for 0.5 minute. The temperatures of the injection port and transfer line were 250 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/minute. The MSD was operated in the Electron Ionization mode. Mass spectra were recorded at 70 eV, with a scanning range of m/z 40 - 400.

**FTIR Analysis:** FTIR analysis was performed using a Perkin Elmer Spectrum 1000 spectrometer. Samples were analyzed in KBr and scanned 16 times from 4000 - 400 cm⁻¹ at a resolution of 4 cm⁻¹.

**Results and Discussion**

The results of five presumptive color tests are summarized in Table 1. Based on the results, only the Marquis and Liebermann’s reagents give a positive test (however, neither is very specific or definitive).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Resulting Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marquis</td>
<td>Yellow/Orange ⇒ Brown</td>
</tr>
<tr>
<td>Liebermann’s</td>
<td>Darkening Orange</td>
</tr>
<tr>
<td>Sodium Nitroprusside</td>
<td>No Color</td>
</tr>
<tr>
<td>Cobalt Thiocyanate</td>
<td>No Color</td>
</tr>
<tr>
<td>Ehrlich’s</td>
<td>No Color</td>
</tr>
</tbody>
</table>

Underivatized modafinil (reference standard) severely degraded during GC/MS analysis, displaying five primary peaks under the specified conditions. The latest eluting compound (Rt = 11.13 minutes) had a base ion at m/z = 167 (likely a rearranged ion derived from the diphenylmethinyl fragment). Derivatizing with BSTFA resulted in a substantially more abundant peak at 11.15 minutes. The mass spectrum of this peak is shown in Figure 2. Figure 2 also suggests that the ion at m/z = 167 is the expected ion for either derivatized or underivatized modafinil. While the TMS derivative shows some degradation, the derivative is much more stable than modafinil under GC conditions, and is therefore more suitable for GC/MS analysis.

The FTIR spectrum of modafinil is presented in Figure 3. Discussions with technical staff at Cephalon indicate that the spectrum changes when extracted into methylene chloride and evaporated down, suggesting a hydrated form and an anhydrous form, or polymorphism. Figure 4 depicts the latter spectrum; comparison between the two spectra reveal minor differences in the range of 4000-3000 cm⁻¹, indicating that polymorphism is more likely.

**References**

1. Provigil® Patient Information Leaflet.
Figure 2 – Mass Spectrum of Modafinil-TMS
Figure 3 – Infrared Spectrum of Neat Modafinil

Figure 4 – Infrared Spectrum of Extracted Modafinil
Quantitation and Enantiomeric Determination of Propoxyphene Using Capillary Zone Electrophoresis

Clay P. Phelan
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
[Email Address: Clay.P.Phelan -at- usdoj.gov]

ABSTRACT: Validated Methods for the quantitation of d-propoxyphene HCl and d-propoxyphene napsylate were developed using capillary zone electrophoresis, using an uncoated capillary, a lithium phosphate buffer, and using thiamine HCl as the internal standard. The addition of a small amount of acetonitrile to the injection solvent facilitated the solubilization of d-propoxyphene napsylate. The analytes’ responses were reproducible, provided accurate recovery values, and were linear within the experimental concentration range. A chiral analysis was also conducted, using the same capillary but with 2-hydroxypropyl-β-cyclodextrin added to the run buffer. The methods were specifically developed for the analysis of pharmaceutical tablets containing d-propoxyphene HCl or d-propoxyphene napsylate, which typically are adulterated only with caffeine, aspirin, and/or acetaminophen; however, the method is applicable to analysis of a wide variety of other drugs.

KEYWORDS: Capillary Zone Electrophoresis, CZE, d-Propoxyphene HCl, d-Propoxyphene Napsylate, Chiral Analysis, Forensic Chemistry

Introduction

d-Propoxyphene is a mild narcotic analgesic found in various pharmaceutical preparations, usually as the hydrochloride or napsylate salt. These preparations typically also contain large amounts of acetaminophen, aspirin, or caffeine. This drug is prescribed for pain relief; however, it is also abused for its euphoric side effects [1], and it is therefore commonly diverted into the illicit drug trade. Currently, d-propoxyphene is a Schedule IV controlled substance in the United States; however l-propoxyphene is not controlled. This requires enantiomeric determination for all samples containing propoxyphene.

Propoxyphene is thermally labile, and will break down on a gas chromatograph. Therefore, most of the literature procedures for its analysis are based on liquid chromatographic techniques, more recently including capillary electrophoretic methods [1,2]. The analysis of controlled substances with CE, especially using specialized capillary coatings and/or run buffers, have been shown to produce highly accurate and reproducible results [3-5]. However, some of these techniques are relatively costly and complicated. The described methodologies are simple, inexpensive, and can also be utilized for a wide variety of other drugs, including the phenethylamines [6].

The first CE method for quantitation and enantiomeric determination of propoxyphene was reported in 1994 [7]. However, the methodology required the use of fairly long capillaries, resulting in long analysis times. In addition, because d-propoxyphene napsylate has a solubility limit of approximately 1.0 mg/mL in 0.01 N HCl, the quantitation samples were prepared at concentrations less than 0.6 mg/mL, and had to be sonicated for several hours prior to analysis.

Solubility problems in CE can be addressed by the use of an organic modifier in the injection solvent and/or run buffer. For d-propoxyphene napsylate, acetonitrile was determined to be an appropriate modifier. Thiamine HCl
was selected as the method internal standard, as it is commercially available, inexpensive, and not found in typical pharmaceutical preparations or in most illicit drug samples.

**Experimental**

**Preparation of Internal Standard Stock Solution**
Thiamine HCl (Sigma, St. Louis, MO) was dissolved in 0.01 N HCl, for a concentration of 1 mg/mL. The deionized water used to produce the 0.01 N HCl was obtained from a Milli Q® Gradient 10A purification system (Millipore, Bedford, MA).

**Preparation of the Achiral Buffer**
A 100 mM solution of phosphoric acid was prepared using deionized water and ≥85% reagent grade phosphoric acid (J.T. Baker, Phillipsburg, NJ). The solution was then titrated to a pH of 2.30 ±0.02 with solid lithium hydroxide (Sigma, St. Louis, MO). (Precise pH control is very important in CE, as it affects both migration times and selectivity.) The buffer was filtered prior to use through a 0.45 µm filter, using an Agilent (Wilmington, DE) Solvent Filter/Degasser. Because this buffer contains no preservatives, it was stored at 7 ºC, and was replaced every 6 to 8 weeks.

**Preparation of the Chiral Buffer**
2-Hydroxypropyl-β-cyclodextrin (Sigma, St. Louis, MO) was added to the achiral buffer such that its concentration was 20 mM. The buffer was filtered prior to use through a 0.45 µm filter.

**Preparation of Capillaries**
The capillary was prepared in-house, using a 50 µm ± 3 µm ID with a 363 µm ± 10 µm OD flexible polyamide-coated fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ). The capillary was manually cut to a nominal length of 34 cm ± 0.5 cm using a CE column cutter equipped with a diamond blade. Both ends of the capillary were inspected under a microscope to ensure that the glass edge was straight and perpendicular to the length of the capillary tubing, and also was free of debris and defects. The detector window was produced by removing the polyamide coating using a standard window maker equipped with a 7 mm heating module (MICROSOLV®, Long Branch, NJ). The coating on each end of the capillary was removed using a 2 mm heating module. The new capillary was initially conditioned at 40 ºC by flushing it with 1.0 N NaOH (5 minutes), 0.1 N NaOH (10 minutes), deionized water (5 minutes), and 100 mM lithium phosphate buffer (10 minutes). Subsequently, capillaries were conditioned once every 24 hours at 15 ºC by flushing them with 1.0 N NaOH (1 minutes), 0.1 N NaOH (2 minutes), deionized water (1 minutes), and 100 mM lithium phosphate buffer (2 minutes).

**Sample and Standard Preparation for d-Propoxyphene Hydrochloride**
d-Propoxyphene HCl standard (Sigma, St. Louis, MO) or a d-propoxyphene HCl-containing sample was accurately weighed and placed in a volumetric flask with an appropriate aliquot of thiamine HCl stock solution (1:5), and the solution was diluted to final volume with 0.01 N HCl. The concentration of the standard or sample in each solution varied between 0.2 - 0.5 mg/mL. Each solution was filtered prior to injection through a syringe equipped with a 0.45 µm filter (Acrodisc®). For the linearity studies, eight solutions of the d-propoxyphene HCl were prepared at concentrations ranging from 0.05 to 1.3 mg/mL, with the internal standard concentration constant at 0.2 mg/mL. The standard and sample solutions were diluted with 0.01 N HCl to approximately 0.05 to 0.10 mg/mL for the chiral analyses. Enantiomers were determined using the chiral buffer.

**Sample and Standard Preparation for d-Propoxyphene Napsylate**
d-Propoxyphene napsylate standard or a d-propoxyphene napsylate-containing sample was accurately weighed and placed in a 100 mL volumetric flask with 4 mL acetonitrile and sonicated of approximately 5.0 minutes. An appropriate aliquot of thiamine HCl stock solution (1:5) was added, and the solution was diluted to final volume with 0.01 N HCl. The concentration of the standard or sample in each solution varied between 0.2 - 0.5 mg/mL (the amount of acetonitrile in the final solutions was approximately 4%). Each solution was filtered prior to
injection through a syringe equipped with a 0.45 μm filter (Acrodisc®). For the linearity studies, eight solutions of the d-propanoxyphe napsylate were prepared at concentrations from 0.05 to 1.2 mg/mL, containing varying amounts of acetonitrile (0.2% to 4.8%). Additional solutions containing d-propanoxyphe napsylate were also prepared from 0.569 to 0.737 mg/mL, containing acetonitrile ranging from 6% to 14%. The appropriate aliquot of thiamine HCl stock solution (1:5) was added, and the solution were diluted to final volume with 0.01 N HCl. The standard and the sample solutions were diluted to approximately 0.05 to 0.10 mg/mL with 0.01 N HCl for the chiral analyses. Enantiomers were determined using the chiral (20 mM 2-hydroxypropyl-β-cyclodextrin) buffer.

**Capillary Electrophoresis**

Experiments were performed using a Hewlett Packard 3DCE capillary electrophoresis system (Agilent Technologies, Wilmington, DE), equipped with a diode array detector set at a wavelength of 207 nm with a bandwidth of 7 nm. For quantitations, the capillary was flushed with buffer for 2.5 minutes between injections for the HCl, and for 1.0 minutes with 0.1 N NaOH and 2.0 minutes with the achiral buffer for the napsylate. The buffer was replenished after six injections to prevent depletion of electrolytes and charge. The capillary temperature was maintained at 15 °C. The hydrodynamic injection time was 2.5 seconds at 50 mBar. The applied voltage was 14.5 kV, which was determined empirically to maintain current below 60 µA, thereby limiting Joule heating while also optimizing analysis time.

For the enantiomer determination, the capillary was flushed with buffer for 2.5 minutes between injections for both the HCl and the napsylate salts. The standard and the sample solutions were diluted to approximately 0.2 mg/mL with 0.01 N HCl. The enantiomers were determined using the chiral buffer, using the same applied voltage (14.5 kV). The d,l-propanoxyphe n standard and sample injection was set at 50 mBar of pressure for 1.5 seconds, followed by a second co-injection of 0.01 N HCl at 20 mBar for 1.0 second. The samples and standards of d-propanoxyphe napsylate and l-propanoxyphe n were injected at 50 mBar of pressure for 1.5 seconds, followed by a second co-injection of the d,l-propanoxyphe n standard at 20 mBar for 1.0 second. The buffer was not replenished after six injections, but rather was utilized until depleted.

**Results and Discussion**

The objective of the study was to accurately and rapidly quantitate d-propanoxyphe n HCl and d-propanoxyphe n napsylate by CZE without interferences from adulterants or diluents. CZE permits direct analysis without requiring extractions. The use of a simple aqueous buffer for the HCl salt reduces analysis cost and allows for simple disposal; the use of acetonitrile as an organic modifier for the napsylate salt is nearly as convenient. Because neutral compounds migrate at the rate comparable to the electroosmotic flow (EOF), while negatively charged (acidic) compounds migrate at a rate slower than the EOF, these species are not detected using the presented method. Therefore, pharmaceutical tablets that contain adulterants such as caffeine, aspirin, or acetaminophen do not interfere. For example, the analysis of a 50 mg d-propanoxyphe n napsylate tablet containing 325 mg of acetaminophen displays no peak(s) for the acetaminophen (Figure 1).

These methods were specifically developed for pharmaceutical tablets containing d-propanoxyphe n HCl or d-propanoxyphe n napsylate, which typically are adulterated only with caffeine, aspirin, and/or acetaminophen. As noted above, these adulterants do not interfere with achiral quantitation; therefore, a selectivity study was not conducted (or required for this application). In the unlikely event that counterfeit pharmaceuticals containing additional adulterants were encountered, the alternative adulterants would have to be identified by spectroscopic means, after which the sample would have to be evaluated to ensure that the selectivity requirements were met, before proceeding with CZE analysis.

The linearity study demonstrated that the calculated errors were less than five percent, and the correlation coefficients were greater than 0.998, within the specified linear range (see Table I). The linearity studies were conducted both with the method using a 1.0 minute flush with the 0.1 N NaOH followed by a 2.0 minute flush of the achiral buffer, and with the method using a 2.5 minute flush with the achiral buffer. It was determined that the use of a 0.1 N NaOH flush resulted in a higher correlation coefficient.
The precision was determined by injecting two concentrations of analyte at the lower and upper ends of the established linear range. The %RSDs for the two concentrations did not exceed 3% for the HCl or the napsylate. Furthermore, an additional five solutions ranging from 0.569 to 0.737 mg/mL of d-propoxyphene napsylate containing varying amounts of acetonitrile (6, 8, 10, 12, or 14%) gave equivalent %RSD values. The precision was determined both with the 1.0 minute flush with the 0.1 N NaOH followed by a 2.0 minute flush of the achiral buffer, and with the 2.5 minute flush with the achiral buffer. Again, it was determined that the use of a 0.1 N NaOH flush resulted in a lower, more consistent %RSDs (Tables II and III).

The accuracy (recovery) was determined by preparing three different concentrations of the analyte with each of the following adulterants: Acetaminophen, aspirin, and caffeine. The concentrations of the analytes represented the lower, middle, and upper linear ranges (i.e., 10%, 50%, and 80%), and contained the appropriate amount of the internal standard. The samples were prepared by sonicating for 5 minutes, and were also compared to non-sonicated samples. The CZE results were compared to the actual values, and did not exceed a 5.0% difference (Table IV). However, samples that were sonicated gave lower errors.

Conclusions

CZE is an effective technique for the quantitation of pharmaceutical preparations containing d-propoxyphene HCl or d-propoxyphene napsylate. Quantitative results were shown to be accurate, reproducible, and precise, and allowed analyses to be accomplished in less than 6 minutes. The use of thiamine HCl as the internal standard was convenient, did not interfere with any known controlled adulterant, and is commercially available at low cost. Chiral separations are conveniently accomplished on the same system with the use of 2-hydroxypropyl-β-cyclodextrin in the run buffer. The described system offers an approach for routine analysis that is simple, robust, practical, and inexpensive. The methodology has been applied to a broader range of illicit drugs, including synthetic opiates and phenethylamines, under the same/similar operating conditions with equal success, and has been used to analyze a large number seized exhibits over the past two years.

Acknowledgments

The author expresses appreciation to the following staff employed at the DEA Southwest Laboratory: Senior Forensic Chemist Harry F. Skinner, and Forensic Chemists Alan Randa, Dean A. Kirby, Nathan Salazar, and Nicole C. Payne-King, for their technical contributions. The author would also like to thank Librarian Rose Mary Russo of the DEA Library (Arlington, Virginia) for her assistance in acquiring references.

References


* * * * *

Table I: Linearity Study, Data for d-Propoxyphene HCl and Napsylate.

<table>
<thead>
<tr>
<th>d-Propoxyphene</th>
<th>Linear Range (mg/mL)</th>
<th>Range of % Error</th>
<th>Correlation Coefficient</th>
<th>Y Intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloride</td>
<td>0.0513 to 1.23</td>
<td>0.581 to 3.98</td>
<td>0.99991</td>
<td>0.042</td>
<td>19.30</td>
</tr>
<tr>
<td>Napsylate 1</td>
<td>0.0509 to 1.22</td>
<td>0.11 to 2.72</td>
<td>0.99995</td>
<td>0.032</td>
<td>12.7813</td>
</tr>
<tr>
<td>(with 0.1N NaOH flush)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Napsylate 1,2</td>
<td>0.101-1.22</td>
<td>0.239 to 2.05</td>
<td>0.99981</td>
<td>-0.0023</td>
<td>12.5327</td>
</tr>
<tr>
<td>(without 0.1N NaOH flush)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Standards sonicated for 5 minutes in acetonitrile before the addition of 0.01 N HCl and Thiamine HCl internal standard. Percent acetonitrile varied as follows: 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, 4.0, and 4.8, respectively.

2 The percent error was 7.6 at a concentration of 0.0509 mg/mL.

Table II: Data for Repeatability Study for d-Propoxyphene Napsylate.

<table>
<thead>
<tr>
<th>Propoxyphene Napsylate (Sonicated)</th>
<th>Repeatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Acetonitrile</td>
<td>Concentration of napsylate (mg/mL)</td>
</tr>
<tr>
<td></td>
<td>0.203</td>
</tr>
<tr>
<td>% RSD (with 0.1N NaOH flush)</td>
<td>1.46</td>
</tr>
<tr>
<td>% RSD (without 0.1N NaOH flush)</td>
<td>1.46</td>
</tr>
</tbody>
</table>
**Table III:** Data for Repeatability Study for d-Propoxyphene HCl.

<table>
<thead>
<tr>
<th>d-Propoxyphene HCl</th>
<th>Concentration (mg/mL)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.205</td>
<td>0.372</td>
</tr>
<tr>
<td></td>
<td>0.822</td>
<td>0.987</td>
</tr>
</tbody>
</table>

**Table IV:** Data for Recovery Study for d-Propoxyphene Napsylate.

<table>
<thead>
<tr>
<th>d-Propoxyphene Napsylate Recovery at 4% Acetonitrile (Sonicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adulterant</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Caffeine</td>
</tr>
</tbody>
</table>

**Table V:** Data for Recovery Study for d-Propoxyphene Napsylate.

<table>
<thead>
<tr>
<th>d-Propoxyphene Napsylate Recovery at 4% Acetonitrile (Non-Sonicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adulterant</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Caffeine</td>
</tr>
</tbody>
</table>

* N/D = not determined
**Table VI:** Data for Recovery Study for d-Propoxyphene HCl.

<table>
<thead>
<tr>
<th>Adulterant</th>
<th>Actual Percentage d-Propoxyphene HCl (%PXP) and Calculated Percent Error (% Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>%PXP= 11.27 % error= 0.621 %PXP= 45.81 % error= 0.185 %PXP= 79.37 % error= 0.460</td>
</tr>
<tr>
<td>Aspirin</td>
<td>%PXP= 10.85 % error= 0.645 %PXP= 50.71 % error= 0.424 %PXP= 77.15 % error= 0.972</td>
</tr>
<tr>
<td>Caffeine</td>
<td>%PXP= 9.23  % error= 1.89  %PXP= 51.18  % error= 0.840  %PXP= 83.11  % error= 0.0120</td>
</tr>
</tbody>
</table>

 ***

**Figure 1:** Electropherogram of Thiamine and Propoxyphene.
Figure 2: The Electropherograms for the Chiral Analysis of Propoxyphene Napsylate:
I. The racemic separation of the d,l-propoxyphene napsylate standard.
II. The chiral analysis of a sample.
III. The sample co-injected with the d,l-propoxyphene napsylate standard.
IV. The standard l-propoxyphene nasylate with the d,l-propoxyphene napsylate standard.
V. The standard d-propoxyphene nasylate with the d,l-propoxyphene napsylate standard.
Identification and Quantitation of Hydromorphone Hydrochloride in Palladone® (Extended Time-Release) Capsules

Pamela R. Smith,* Amanda K. Frohwein, Patrick A. Hays, and Ira S. Lurie
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166

ABSTRACT: Palladone® is an extended time-release formulation of hydromorphone hydrochloride. The time-release matrix presents some unusual analytical challenges (especially for quantitation). FTIR (GC/IRD), 400 MHz 1H-NMR, GC/MS, and CE (DAD) data are presented, enabling qualitative and quantitative analyses of Palladone® formulations.

KEYWORDS: Palladone®, Hydromorphone, Time-Release, Synthetic Opiate, Forensic Chemistry

Introduction

Hydromorphone is a synthetic opiate derived from morphine. It is a controlled substance (Schedule II) under the U.S. Controlled Substances Act. Palladone® is an extended time-release formulation of hydromorphone hydrochloride produced by Purdue Pharma (1). Controlled release formulations are usually solid dosage forms (capsules or tablets) that contain individual pellets that, when administered orally, slowly release the drug over a longer time frame (that is, different types of pellets in the formulation dissolve at different rates, thereby giving a lower but much longer lasting, steady-state concentration of the drug). Currently, Palladone® capsules are available in the following concentrations: 8, 12, 16, and 32 milligrams/capsule.

Synthetic opiates are popular among drug abusers, and are therefore occasionally submitted for analysis to forensic laboratories. Controlled release formulations present challenges for both qualitative and (especially) quantitative analysis. In order to accurately quantitate the drug, it must be possible to separate the drug from the matrix in a quantitative manner. This study presents both qualitative and quantitative methodologies for the analyses of Palladone® capsules.
Experimental

Chemicals and Reagents
Buffers and solutions were products of MicroSolv Technology (Eatontown, NJ). Chloroform, methanol, and acetone were products of Burdick and Jackson Laboratories (Muskegon, MI). CDCl₃, D₂O, and TMS were products of Sigma-Aldrich (Milwaukee, WI).

Qualitative Analyses

Vapor Phase Infrared Spectroscopy
The FTIR spectrum was obtained on a Nicolet 6700 FTIR GC-IRD (Figure 1).

![Figure 1. Vapor Phase FTIR of Hydromorphone.](image)

Nuclear Magnetic Resonance Spectroscopy
One dimensional NMR analyses of hydromorphone and Palladone® were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe.

Hydromorphone hydrochloride has four isomeric forms (two keto [Ia, Ib] and two enol [IIa, IIb] forms (Figure 2)), each exhibiting two possible N-methyl orientations) under acidic conditions in either D₂O or CD₃OD. The relative proportions of these forms in solution depends on the solvent and the solution pH. Use of NMR for identification and quantitation of hydromorphone hydrochloride is therefore not recommended, because great care would be required to identify and properly integrate the signals generated by the four keto-enol forms. However, by utilizing a basic extraction with sodium bicarbonate into CDCl₃, only one form of hydromorphone base is observed (Figure 3).
Figure 2. Keto-Enol Structures of Hydromorphone Hydrochloride.

Figure 3. Hydromorphone Base in CDCl$_3$.
Workup of Palladone® for NMR Analysis: Weigh 20 milligrams of Palladone® into a 15 mL centrifuge tube. Add 0.5 mL saturated sodium bicarbonate in D₂O and 1 mL CDCl₃ containing 0.03% TMS, and sonicate for 15 minutes. This produces a white emulsion, which is then centrifuged. The CDCl₃ (lower) layer is then isolated and dried over anhydrous sodium sulfate, filtered, and transferred to an NMR tube. The resulting spectrum (Figure 4) displays both the hydromorphone peaks and the peaks from the capsule matrix material(s) (compare with Figure 3).

Figure 4. Palladone® (basified) in CDCl₃ (Note: Brackets indicate location of hydromorphone base peaks for identification).

Gas Chromatography/Mass Spectrometry
Instrument: Agilent 6890N with an Agilent 5973 MSD
Column: DB-1, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature: 280 °C
Oven Temperature: 90 °C for 2 minutes, 14 °C/minute to 280 °C
Carrier Gas: Helium with split ratio = 25:1
Scan Range: 34 - 550 amu
Electron Ionization: 70 eV

Hydromorphone has a chemical formula of C₁₇H₁₉NO₃, and a molecular weight of 285.34. The mass spectrum shows the molecular ion (which is also the base peak) at m/z 285 (Figure 5).

Brought to you by AltGov2 [www.altgov2.org]
Quantitative (Capillary Electrophoresis) Procedures

As noted above, Palladone® presents some challenges for quantitative analysis. When utilizing GC, HPLC, or many modes of CE, the peak shapes are broad and non-symmetrical, causing difficulties for quantitation. In this study capillary electrophoresis (CE) was employed utilizing a dynamic coating with a chiral run buffer. Previous studies have shown that this method has improved peak shape over dynamic coatings without chiral additives (2). As shown, this approach resulted in excellent peak shape (Figure 6).

Figure 5. Mass Spectrum of Hydromorphone.

Figure 6. CE of a Palladone® Tablet; Peak Identities: (a) Hydromorphone; and (b) Procaine (IS).
An additional study was conducted to determine a method for quantitative recovery of hydromorphone hydrochloride from Palladone® capsules. The recovery study showed that using a mixture of 20% methanol : 80% injection solvent to prepare the sample gives a recovery of greater than 96 percent from any of the four Palladone® concentrations (Figure 7).

![Figure 7](image)

**Figure 7.** Recovery of Hydromorphone Hydrochloride from Palladone®.

**Capillary Electrophoresis Procedures**

Run Buffer: CElixir accelerator solution B (pH 2.5) + 50 mM 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) (Sigma). Weigh 1576 mg of HP-β-CD into a 50 mL Erlenmeyer flask. Pipette 20.0 mL of CElixir accelerator solution B (pH 2.5) and shake vigorously. Filter into 22 mL Teflon PVA vials (Cole Parmer) using a 0.45 µm, 25 mm regenerated cellulose filter.

Injection Solvent: Weigh 1,034 milligrams of sodium phosphate monobasic into a 100 mL volumetric flask. Dilute to volume with HPLC grade water. Adjust to approximately pH 2.6 using phosphoric acid added dropwise. Transfer contents into a 2000 mL volumetric flask with the aid of HPLC grade water. Dilute to volume with HPLC grade water. This final solution contains 3.75 mM phosphate (pH 3.2).

Internal Standard Stock Solution (ISSS): Weigh an appropriate amount of procaine hydrochloride into a volumetric flask to obtain a final concentration of approximately 1.0 mg/mL. Dilute to volume with 20% methanol : 80% injection solvent.

Standard Solution: Weigh an appropriate amount of standard hydromorphone hydrochloride into a volumetric flask to obtain a final concentration of approximately 0.10 mg/mL. Pipette an appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/mL. Dilute to volume with 20% methanol : 80% injection solvent. Filter approximately 1.0 mL of solution with a 0.45 µm, 25 mm regenerated cellulose filter into a 2.0 mL glass vial (Agilent part # 5182-0567). Care should be taken to ensure that there are no air bubbles on the bottom of the glass vial. Cap the vial with a polypropylene cap (Agilent part # 5182-9697).

Sample Preparation: Weigh an appropriate amount of sample into a volumetric flask so that the final hydromorphone hydrochloride concentration is approximately equal to that of the standard solution. Pipette an appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/mL. Dilute to volume with 20% methanol : 80% injection solvent. If the sample is a time-release preparation, it should be sonicated for
at least one hour. Filter approximately 1.0 mL of sample solution with a 0.45 μm, 25 mm regenerated cellulose filter into a 2.0 mL glass vial (Agilent part # 5182-0567). Once again, care should be taken to ensure that there are no air bubbles on the bottom of the glass vial. Cap the vial with a polypropylene cap (Agilent part # 5182-9697).

Instrumental Conditions:
- Capillary Electrophoresis: HP 3D instrument operated in CE mode
- Capillary: 50 μm i.d. x 32.2 cm (23.7 cm length to detector)
- Capillary Temperature: 15 °C
- Conditioning: 0.1 N NaOH; 1 minute H₂O CELixir Reagent A (MicroSolv CE); 2 minutes CELixir Reagent B, pH 2.5 (MicroSolv CE)
- Run Buffer: CELixir Reagent B, pH 2.5 (MicroSolv CE) + 7.88% (w/v) HP-β-CD (hydroxypropyl-β-cyclodextrin)
- Voltage: 20 kV
- Injection: Sample – 50mbar x 2 seconds followed by water at 35 mbar x 1 second
- Total run time/sample: 6 minutes

Validation Criteria:
- Linearity Range: 0.0217 mg/mL – 0.9774 mg/mL
- Repeatability: RSD < 0.81 %
- Accuracy: E % < 3.9 %
- Correlation Coefficient (R²): 0.99995

References

* * * * *
**gamma-Hydroxybutyrate, Silver Salt (AgGHB): Identification of gamma-Hydroxybutyrate (GHB) via Conversion to the Silver Salt**

**James V. DeFrancesco**  
U.S. Department of Justice  
Drug Enforcement Administration  
North Central Laboratory  
536 S. Clark St., Suite 800  
Chicago, IL 60605  
[email: james.v.defrancesco -at- usdoj.gov]

**ABSTRACT:** A practical method for the identification of *gamma*-hydroxybutyrate (GHB) via infrared analysis of the corresponding silver salt is presented. The method is facile and robust, and complements the GC/MS analysis of GHB derivatives.

**KEYWORDS:** *gamma*-Hydroxybutyrate, *gamma*-Hydroxybutyric Acid, GHB, Sodium Oxybate, Infrared Spectrophotometry, IR, Silver Nitrate, Precipitation, Derivatization, GC/MS, Forensic Chemistry

**Introduction**

The continuing abuse of *gamma*-hydroxybutyric acid (GHB) and *gamma*-hydroxybutyrate (also commonly abbreviated as “GHB”) has prompted the forensic community to develop an array of analytical methodologies to identify it in its various forms (1). As new salt forms of GHB have been encountered, forensic chemists have relied primarily on infrared spectrophotometry (IR) for identification (2-5). In conjunction with IR, derivatization of GHB (usually via silylation) with subsequent analysis by Gas Chromatography/Mass Spectrometry (GC/MS) has served as a complementary means of identification of the organic ligand (2).

The silver nitrate test has been a staple in the analytical laboratory for many years; however, its use is typically limited to the presumptive identification of simple halides and commonly encountered polyatomic ions by precipitation, often followed by a solubility test for the precipitate. This study was initiated to investigate the use of the silver nitrate test as a fast and easy method to presumptively identify GHB in the field. However, the resulting silver salt precipitate (i.e., AgGHB) has proven to be quite valuable for more rigorous laboratory identification.

There are several advantages to converting GHB from a group I or II metal salt (e.g., LiGHB, NaGHB, KGHB, or Ca(GHB)₂) into AgGHB. The most immediate and practical advantage is increased stability with respect to water absorption. The silver salt is far less hygroscopic than any of its group I or II metal counterparts. This property gives the analyst a much longer time frame in which to conduct further analyses. Thus, the preparation and direct characterization of the AgGHB salt directly by IR, or by subsequent derivatization followed by GC/MS analysis, increases the specificity and accuracy of the analysis.

**Experimental**

**Intrumentation**

The IR spectra were collected by two instruments: A Nicolet 6700 FT-IR equipped with a single-bounce diamond ATR accessory, and (for KBr windows) with an ATI Mattson Genesis Series FT-IR. The GC/MS data were obtained using a Agilent 6890 GC equipped with a 5873 MSD (EI, 70 eV) and HP-5MS column (30 m long x 0.32 mm ID x 0.25 μm thickness) heated from 90 °C to 280 °C at 10 °C/minute.
Precipitation of AgGHB from Samples

The following methodology effectively yields AgGHB in acceptable purity. The procedure first precipitates all ions by addition of silver nitrate, then isolates AgGHB from the other silver salts based on solubility.

1. Place five drops of an aqueous sample or a small amount (100 – 200 mg) of a solid sample in a test tube. Add 5 drops of deionized (DI) water and 5 drops of 1 N AgNO₃ (aq) and mix well.

2. If no precipitate is formed, then there are probably no interfering anions (proceed to step 3). If a precipitate is formed, add 1 mL of DI water to test the solubility of the precipitate. If the precipitate dissolves with this additional water, then proceed to step 3. (Note that at high concentrations, AgGHB forms a precipitate that readily dissolves in excess water.) If the precipitate does not dissolve with excess water, separate the precipitate by decantation, centrifugation, or filtration. This (non-dissolving) precipitate is most likely the silver salt of a halide or a polyatomic anion. Collect the remaining (clear) liquid. Add one more drop of AgNO₃ solution to ensure that all remaining interfering ions are precipitated. If a second crop precipitate forms, repeat the above process until no further precipitation occurs. Proceed to step 3.

3. Add an equal volume of alcohol (methanol, ethanol, or isopropanol) to the recovered solution to precipitate any AgGHB. If a precipitate forms, collect it by centrifugation, and dry it under an air or nitrogen purge at ~70 °C. If no precipitate forms, add additional alcohol (you may need to add up to 2 - 3 equivalent volumes of alcohol). In solution, the AgGHB precipitate is finely divided and moves like a lyotropic liquid crystal (opalicious). A convenient mechanism to remove residual alcohol, water, and remaining organic impurities is to add ether, mix, and then decant and discard the ether. The precipitate forms initially as a white powder, but may darken over time due to heat and exposure to air. This degradation (to perhaps a silver oxide, carbonate, etc.) does not appear to affect the IR spectrum - a testament to the robustness of the technique.

4. Obtain an IR spectrum. If the spectrum appears to contain excess water (broadened bands) during a KBr pellet analysis, allow the chamber to purge with nitrogen for about 15 minutes. If the same effect is observed during an ATR analysis, allow the material to simply air dry. Both purging with nitrogen and air drying will remove the water and considerably sharpen the spectral bands.

5. An optional test to perform is direct silylation of AgGHB followed by GC/MS analysis. Derivatization can be accomplished with bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing a small amount of trimethylchlorosilane (TMCS), followed by heating. To guard against the introduction of silver metal ions onto the GC column, the derivatization solution should be passed over solid NaCl to capture residual silver metal ion in the form of AgCl. The resulting solution is then analyzed by GC/MS.

Results and Discussion

Analysis of AgGHB by IR

Group I and II metal salts of GHB are notoriously hygroscopic, as evidenced by the IR spectral bands which become severely blurred upon absorption of atmospheric moisture. One of the major advantages of converting these salts into AgGHB is that the silver salt is considerably less hygroscopic than any of the common Group I and II metal salts of GHB. This property is demonstrated in Figure 1, which shows the IR spectrum of a wet AgGHB sample isolated from an actual exhibit containing both a GHB salt and GBL. Several of the bands which initially appeared blurry became much sharper as the IR chamber was purged with nitrogen (which effectively drove off the residual water). In Figure 2, the transmission IR spectrum of AgGHB (i.e., acquired as a KBr window) is compared to that of the ATR spectrum. The five step procedure described above was successfully used to convert the Na, Li, K, and Ca salts of GHB into AgGHB (see Figure 3 for the IR spectra of these four salts).
Interfering Components
There are numerous components that can potentially affect the purity of AgGHB. However, few interferences were observed. Halides and other common polyatomic anions are removed by precipitation with excess AgNO₃, prior to recovery of AgGHB (i.e., while the solution is still aqueous). Sugar, a common component in liquid GHB exhibits, had no effect on the purity of the AgGHB in controlled experiments. This absence of carry-over was most likely due to sugar’s solubility in the lower alcohols. The same is true for AgNO₃, which remains in solution upon addition of the alcohol.

As noted earlier, excessive heating and prolonged exposure to air can cause minor degradation of the AgGHB. However, this darkening does not appear to affect the IR spectrum. This is demonstrated in Figure 4, which shows that the IR spectrum of a nearly four year old sample of AgGHB is indistinguishable from that of a freshly prepared sample.

Further Use of AgGHB
Further attempts to characterize AgGHB versus the Na, Li, K, and Ca GHB salts proved to be fruitless. Techniques such as HPLC and proton NMR cannot differentiate AgGHB from other GHB salts.

Conclusions
The precipitation method described above is an effective and selective method for removal of GHB salts from solution. Interferences by halide and common polyatomic anions and components such as AgNO₃ and sugar are minimized via filtration of unwanted precipitates and washings of the target precipitate with selected solvents at key points in the isolation scheme. The major advantage of forming the silver salt of GHB is its increased stability, which in turn affords the chemist greater opportunity for further testing. Perhaps the primary benefit of this work is the increased specificity. The conversion to and characterization of GHB as the silver salt, followed by secondary derivatization and characterization with a silylating reagent, significantly increases the specificity of the analysis, and thus yields a more in-depth identification.

Acknowledgements
The author would like to thank Forensic Chemist Scott J. Tschaekofske of the Minnesota Department of Public Safety, Bureau of Criminal Apprehension, for extensive discussions concerning this topic.

References
Figure 1: Effect of Moisture on IR Spectrum of AgGHB (Transmission).
Figure 2: Transmission and Reflectance IR Spectra of AgGHB.
**Figure 3a:** IR/ATR Spectra of AgGHB (top), NaGHB, LiGHB, KGHB, and Ca(GHB)$_2$.

**Table 1:** Frequencies for IR/ATR Spectral Bands of AgGHB, NaGHB, LiGHB, KGHB, and Ca(GHB)$_2$ (in cm$^{-1}$).

<table>
<thead>
<tr>
<th></th>
<th>AgGHB</th>
<th>NaGHB</th>
<th>LiGHB</th>
<th>KGHB</th>
<th>Ca(GHB)$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3241</td>
<td>3318</td>
<td>3318</td>
<td>3113</td>
<td>3091</td>
</tr>
<tr>
<td>2945</td>
<td>2959</td>
<td>3227</td>
<td>2945</td>
<td>2941</td>
<td></td>
</tr>
<tr>
<td>2877</td>
<td>2942</td>
<td>2954</td>
<td>2886</td>
<td>1587</td>
<td></td>
</tr>
<tr>
<td>1552</td>
<td>2870</td>
<td>2932</td>
<td>2845</td>
<td>1544</td>
<td></td>
</tr>
<tr>
<td>1512</td>
<td>1555</td>
<td>2877</td>
<td>2777</td>
<td>1451</td>
<td></td>
</tr>
<tr>
<td>1418</td>
<td>1451</td>
<td>1574</td>
<td>2714</td>
<td>1417</td>
<td></td>
</tr>
<tr>
<td>1398</td>
<td>1405</td>
<td>1555</td>
<td>1564</td>
<td>1407</td>
<td></td>
</tr>
</tbody>
</table>

(Continued Next Page)
(Table 1, Continued)

<table>
<thead>
<tr>
<th>AgGHB</th>
<th>NaGHB</th>
<th>LiGHB</th>
<th>KGHB</th>
<th>Ca(GHB)$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1349</td>
<td>1329</td>
<td>1438</td>
<td>1480</td>
<td>1312</td>
</tr>
<tr>
<td>1292</td>
<td>1272</td>
<td>1409</td>
<td>1452</td>
<td>1238</td>
</tr>
<tr>
<td>1232</td>
<td>1229</td>
<td>1357</td>
<td>1393</td>
<td>1082</td>
</tr>
<tr>
<td>1162</td>
<td>1156</td>
<td>1282</td>
<td>1361</td>
<td>1032</td>
</tr>
<tr>
<td>1049</td>
<td>1066</td>
<td>1222</td>
<td>1318</td>
<td>936</td>
</tr>
<tr>
<td>1026</td>
<td>1015</td>
<td>1167</td>
<td>1220</td>
<td>910</td>
</tr>
<tr>
<td>952</td>
<td>946</td>
<td>1092</td>
<td>1056</td>
<td>868</td>
</tr>
<tr>
<td>904</td>
<td>920</td>
<td>1055</td>
<td>1019</td>
<td>810</td>
</tr>
<tr>
<td>870</td>
<td>881</td>
<td>954</td>
<td>914</td>
<td>752</td>
</tr>
<tr>
<td>800</td>
<td>867</td>
<td>914</td>
<td>874</td>
<td>664</td>
</tr>
<tr>
<td>763</td>
<td>774</td>
<td>881</td>
<td>859</td>
<td>613</td>
</tr>
<tr>
<td>698</td>
<td>753</td>
<td>778</td>
<td>752</td>
<td>600</td>
</tr>
<tr>
<td>592</td>
<td>666</td>
<td>711</td>
<td>687</td>
<td>537</td>
</tr>
<tr>
<td>567</td>
<td>635</td>
<td>672</td>
<td>548</td>
<td></td>
</tr>
<tr>
<td>576</td>
<td>581</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>550</td>
<td>540</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3b:** IR/ATR Spectra of AgGHB (top), NaGHB, LiGHB, KGHB, and Ca(GHB)$_2$
(Expanded View of 3500 - 2400 cm$^{-1}$).
Figure 3c: IR/ATR Spectra of AgGHB (top), NaGHB, LiGHB, KGHB, and Ca(GHB)$_2$ (Expanded View of 1700 - 530 cm$^{-1}$).

Figure 4: IR/ATR Spectra of Freshly Prepared and Aged AgGHB.
Analytical Profiles for Five “Designer” Tryptamines

Trinette K. Spratley,* Patrick A. Hays, Lois C. Geer, Sam D. Cooper, and Timothy D. McKibben  
U.S. Department of Justice  
Drug Enforcement Administration  
Special Testing and Research Laboratory  
22624 Dulles Summit Court  
Dulles, VA 20166  
[email: Trinette.K.Spratley -at- usdoj.gov]

ABSTRACT: Analytical data (Color Tests, GC/FID, GC/MS, FTIR/ATR, ¹H-NMR, and HPLC) for five hallucinogenic “designer” (synthetic) tryptamines is reported. The compounds (5-methoxy-N,N-diisopropyltryptamine hydrochloride (5-MeO-DIPT); 5-methoxy-N-methyl-N-isopropyltryptamine base (5-MeO-MIPT), 5-methoxy-α-methyltryptamine hydrochloride (5-MeO-AMT), N,N-dipropyltryptamine hydrochloride (DPT), and 5-methoxy-N,N-dimethyltryptamine base (5-MeO-DMT)) are increasingly encountered in forensic, crime, and toxicology laboratories.

KEYWORDS: Tryptamines, Analogues, Hallucinogens, Color Testing, GC/MS, ¹H-NMR, FTIR/ATR, HPLC, Forensic Chemistry

Introduction

Over the past six months, this laboratory has received over 40 referral drug samples suspected of containing hallucinogenic “designer” tryptamines. Some hallucinogenic tryptamines (e.g., N,N-dimethyltryptamine (DMT), psilocybin, bufotenine, etc.) are naturally produced in fungi, plants, and animals, but these “designer” tryptamines are non-naturally occurring compounds that are produced in laboratories [1]. 5-Methoxy-N,N-diisopropyltryptamine hydrochloride (5-MeO-DIPT), 5-methoxy-N-methyl-N-isopropyltryptamine base (5-MeO-MIPT), 5-methoxy-α-methyltryptamine hydrochloride (5-MeO-AMT), N,N-dipropyltryptamine hydrochloride (DPT), and 5-methoxy-N,N-dimethyltryptamine base (5-MeO-DMT) (Figure 1) are all synthetically produced analogues of known hallucinogenic tryptamines, and have been submitted with increasing frequency to federal, state, and local forensic, crime, and toxicology laboratories throughout the United States. On September 29, 2004, 5-MeO-DIPT (also known by its street names of “Foxy” and “Foxy-Methoxy”) became...
federally regulated as a Schedule I Controlled Substance [2]. As of the submission date of this article (April, 2005), 5-MeO-AMT, 5-MeO-MIPT, DPT, and 5-MeO-DMT are not yet specifically listed in the Controlled Substance Act (CSA); however, individuals trafficking in these substances can be prosecuted under the Analogue Statute of the Controlled Substances Act [3]. Herein, we report analytical data (Color Tests, GC/FID, GC/MS, FTIR, NMR, and HPLC) for these five tryptamines.

**Experimental**

**Color Test Reagents**

*Ehrlich’s Reagent:* 0.5 g of *para*-dimethylaminobenzaldehyde (p-DMAB) in a mixture containing 50 mL of ethyl alcohol and 50 mL concentrated hydrochloric acid [5]. *Marquis Reagent:* 100 mL formaldehyde in 1000 mL concentrated sulfuric acid [5].

**Fourier Transfer Infrared Spectroscopy/Attenuated Total Reflectance (FTIR/ATR)**

FTIR spectra were collected on a Thermo Nicolet Nexus 670 FTIR with a potassium bromide (KBr) beam splitter and a deuterated triglycine sulfate (DTGS) KBr detector, equipped with a single bounce Durascope Attenuated Total Reflectance (ATR) accessory. Thirty-two (32) scans were collected between 4000 cm⁻¹ and 400 cm⁻¹, with a resolution of 4.0 cm⁻¹.

**Gas Chromatography/Flame Ionization Detector (GC/FID)**

GC analyses were performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector, using a J & W Scientific DB-1 column with a 30 m x 0.25 mm ID and 0.25 µm film thickness. Instrumental parameters include an injector temperature of 280 °C, hydrogen carrier gas with a flow rate of 1.1 mL/minute, a split ratio of 25:1, and nitrogen make-up gas. The detector temperature was 280 °C. The oven temperature was initially held at 100 °C for 1 minute, then ramped at 12 °C/minute to 280 °C and held for 9 minutes. The concentration for each of the tryptamine analogues was 4 mg/mL in chloroform with a 1 µL injection.

**Gas Chromatography/Mass Spectrometry (GC/MS)**

GC/MS spectra were collected on an Agilent 6890N GC interfaced with an Agilent 5973N Mass Selective Detector (MSD) using a scan acquisition from 34 to 550 amu. A J & W Scientific DB-1 column with a 30 m x 0.25 mm ID and 0.25 µm film thickness was utilized. The injection port temperature was set at 280 °C. The
carrier gas was Helium with a split ratio of 25:1 and constant flow of 1 mL/minute. The oven temperature was initially held at 100 °C for 1 minute, then ramped at 12 °C/ minute to 300 °C and held for 7 minutes. A volume of 1 μL containing a concentration of 4 mg/mL of each tryptamine analogue in chloroform was injected.

**High Performance Liquid Chromatography (HPLC)**

HPLC analyses were performed on a Hewlett Packard (HP) Series 1100 HPLC equipped with an ultraviolet lamp and diode array detector (DAD). A volume of 20 μL containing a concentration of 0.4 mg/mL of each tryptamine analogue diluted in phosphate buffer was injected onto a Whatman Partisil 5 ODS 3, 3.2 x 125 mm column, and scanned from 220 nm – 340 nm with a threshold of 1.0 mAU. An HPLC gradient program was utilized with an initial 20 minute ramp from 95:5 phosphate buffer/methanol to 70:30 phosphate buffer/methanol. This was held for 6 minutes. This was followed by a 10 minute ramp from 70:30 phosphate buffer/methanol to 20:80 phosphate buffer/methanol and held for 4 minutes. The pump flow was 0.76 mL/minute with a total run time of 45 minutes.

**Nuclear Magnetic Resonance (‘H-NMR)**

Proton NMR analyses were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe, or on a Varian Unity 500 MHz NMR with a 5 mm Varian Indirect Detection probe. The samples were prepared at 10 - 30 mg/mL in deuterium oxide (D₂O) containing 3-(trimethylsilyl) propionic-2,2,3,3-d4 acid, sodium salt (TSP) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, WI). The proton spectra were obtained with 8 scans using a 45 second delay and 90° pulse.

**Results and Discussion**

**Color Testing**

Testing each of the tryptamine analogues with the Ehrlich’s reagent produced the same change in color from purple to blue, except for DPT HCl, which produced a violet color change and 5-MeO-MIPT which changed from purple to a faint blue. In the presence of the Marquis reagent, each tryptamine analogue produced the same color change from yellow to black, except for DPT HCl which gave a yellow color only, as shown in Table 1.

**Table 1:** Results of Color Testing.

<table>
<thead>
<tr>
<th>“Designer” Tryptamine</th>
<th>Ehrlich’s Reagent</th>
<th>Marquis Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>N,N-DPT HCl</td>
<td>violet</td>
<td>yellow</td>
</tr>
<tr>
<td>5-MeO-DMT</td>
<td>purple to blue</td>
<td>yellow to black</td>
</tr>
<tr>
<td>5-MeO-MIPT</td>
<td>purple to faint blue</td>
<td>yellow to black</td>
</tr>
<tr>
<td>5-MeO-DIPT HCl</td>
<td>purple to blue</td>
<td>yellow to black</td>
</tr>
<tr>
<td>5-MeO-AMT HCl</td>
<td>purple to blue</td>
<td>yellow to black</td>
</tr>
</tbody>
</table>

**GC/FID**

The tryptamine analogues were first injected separately to establish an absolute retention time, followed by an injection of a mixture containing the tryptamine analogue and tryptamine itself (as an internal standard) to establish a relative retention time. Based upon the relative retention times, each tryptamine was fully resolved, as shown in Table 2.

**HPLC**

The HPLC chromatograms show that each tryptamine has the same ultraviolet spectra (UV) and molar absorptivity due to the UV detection of identical chromophores. The retention time is utilized to distinguish each tryptamine, noting that in aqueous acid, each one has a λmax at 276 nm. DPT HCl has a λmax at 280 nm, as shown in Table 3.
Table 2: Results of GC/FID Analyses.

<table>
<thead>
<tr>
<th>“Designer” Tryptamine</th>
<th>Absolute Retention Time</th>
<th>Relative Retention Time vs. Tryptamine (10.547 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeO-AMT</td>
<td>12.732 minutes</td>
<td>1.207</td>
</tr>
<tr>
<td>5-MeO-DMT</td>
<td>12.946 minutes</td>
<td>1.227</td>
</tr>
<tr>
<td>DPT</td>
<td>13.625 minutes</td>
<td>1.292</td>
</tr>
<tr>
<td>5-MeO-MIPT</td>
<td>14.272 minutes</td>
<td>1.353</td>
</tr>
<tr>
<td>5-MeO-DIPT</td>
<td>15.195 minutes</td>
<td>1.441</td>
</tr>
</tbody>
</table>

Table 3: Results of HPLC Analyses.

<table>
<thead>
<tr>
<th>“Designer” Tryptamines</th>
<th>Retention Time</th>
<th>λ_{max} in Aqueous Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MeO-AMT</td>
<td>9.325 minutes</td>
<td>276 nm</td>
</tr>
<tr>
<td>5-MeO-DMT</td>
<td>10.280 minutes</td>
<td>276 nm</td>
</tr>
<tr>
<td>5-MeO-MIPT</td>
<td>11.961 minutes</td>
<td>276 nm</td>
</tr>
<tr>
<td>5-MeO-DIPT</td>
<td>17.051 minutes</td>
<td>276 nm</td>
</tr>
<tr>
<td>DPT</td>
<td>17.016 minutes</td>
<td>280 nm</td>
</tr>
</tbody>
</table>

FTIR/ATR
The infrared spectra of 5-MeO-AMT HCl (Figure 2) is a primary amine salt which shows N-H stretching in the region of 3256 cm⁻¹. 5-MeO-DIPT HCl (Figure 3) and DPT HCl (Figure 5) are tertiary amine hydrochlorides which exhibit a N-H stretch in the region of 3156 cm⁻¹ to 3186 cm⁻¹. In the region of 3000 cm⁻¹ to 3035 cm⁻¹, 5-MeO-MIPT (Figure 4) and 5-MeO-DMT (Figure 6) exhibit an aromatic C-H stretch.

GC/MS
The mass spectra are displayed in Figures 7 - 11. Each of the base peaks are attributed to alpha cleavage of the amine side chain, with the exception of 5-MeO-AMT (Figure 7). 5-MeO-AMT produces a base peak at m/z 161 due to alpha cleavage and proton transfer to the indole moiety. 5-MeO-AMT has a molecular ion at m/z 204 and a prominent peak at m/z 44. 5-MeO-MIPT (Figure 8) gives a molecular ion at m/z 246 with a base peak at m/z 86. The ion at m/z 160 suggests the loss of the methyl-isopropylamine side chain (C₆H₁₂N). 5-MeO-DIPT (Figure 9) gives a molecular ion at m/z 274 with a base peak at m/z 114 (C₇H₁₆N⁺). The fragmentation at m/z 160 suggests the loss of 114 from the molecular ion. DPT (Figure 10) gives a molecular ion at m/z 244 and a base peak at m/z 114, with fragments at m/z 130 due to a loss of C₆H₁₀N and m/z 144 due to the loss of C₇H₁₆N from the molecular ion. 5-MeO-DIPT (Figure 11) gives a molecular ion at m/z 218, with a fragmentation at m/z 160 due to the loss of the base peak at m/z 58 (C₃H₈N⁺). Each mass spectrum has a different molecular ion and a different base peak, except for 5-MeO-DIPT (Figure 9) and DPT (Figure 10). These compounds have a base peak at m/z 114. Alpha cleavage is a dominant reaction of amines which produces the base peak in N-alkylamines and α-substituted primary amines, leading to the loss of the largest alkyl group [4].

NMR
The NMR spectra are displayed in Figures 12 - 16. All five compounds were easily distinguishable by proton NMR. The 5-methoxy substituted tryptamines have the same peak patterns and very similar chemical shifts in the aromatic region and methoxy region: 4 aromatic protons (2 doublets, a singlet, and a doublet of doublets) and 3 protons at 3.9 ppm (singlet for the methoxy group). DPT is not substituted at position 5, giving a different aromatic peak pattern for 5 protons (2 doublets, 2 triplets, and one singlet), and these signals have different chemical shifts from the 5-methoxy compounds, as shown in Figure 1.
All five compounds have unique and easily interpretable peak patterns. A singlet at 2.8 - 2.9 ppm indicates an N-CH₃; integration will determine if the peak represents a mono- or di-methyl group. Doublets at 1.2 - 1.3 ppm indicate methyls bonded to a methine that are beta to the amine nitrogen (Figures 12-14). Integration of these doublets and their associated methines will determine if the group is a disopropylamine, monoisopropylamine, or a simple N-CHR-CH₃. The spectrum of DPT (Figure 15) contains a triplet at 0.9 ppm integrating to 6 protons, indicating 2 methyls bonded to 2 methylenes (multiplets at 1.6-1.7 ppm) bonded to 2 more methylenes (multiplet at 3.1 ppm); i.e., an N,N-dipropyl group. Ethyl amine protons are found as triplets or multiplets above 3 ppm.

References


Figure 2: FTIR Spectrum of 5-Methoxy-α-methyltryptamine HCl.
Figure 3: FTIR Spectrum of 5-Methoxy-N,N-diisopropyltryptamine HCl.

Figure 4: FTIR Spectrum of 5-Methoxy-N-methyl-N-isopropyltryptamine Base.
Figure 5: FTIR Spectrum of N,N-Dipropyltryptamine HCl.

Figure 6: FTIR Spectrum of 5-Methoxy-N,N-dimethyltryptamine Base.
Figure 7: Mass Spectrum of 5-Methoxy-α-methyltryptamine.

Figure 8: Mass Spectrum of 5-Methoxy-N-methyl-N-isopropyltryptamine.
Figure 9: Mass Spectrum of 5-Methoxy-N,N-diisopropyltryptamine.

Figure 10: Mass Spectrum of N,N-Dipropyltryptamine.
Figure 11: Mass Spectrum of 5-Methoxy-N,N-dimethyltryptamine.
Figure 12: Proton NMR (400 MHz) of 5-Methoxy-α-methyltryptamine (with Insets).

^1H NMR (400 MHz, D$_2$O) $\delta$ ppm 7.45 (d, J=8.90 Hz, 1 H) 7.30 (s, 1 H) 7.18 (d, J=2.45 Hz, 1 H) 6.94 (dd, J=8.90, 2.45 Hz, 1 H) 3.91 (s, 3 H) 3.65 - 3.74 (m, J=7.50, 6.65 Hz, 1 H) 6.50 Hz, 1 H) 3.10 (dd, J=14.80, 6.50 Hz, 1 H) 3.03 (dd, J=14.80, 7.50 Hz, 1 H) 1.38 (d, J=6.65 Hz, 3 H).
Figure 13: Proton NMR (500 MHz) of 5-Methoxy-N,N-diisopropyltryptamine (with Insets).

$^1$H NMR (500 MHz, D$_2$O) δ ppm 7.43 (d, J=8.80 Hz, 1 H) 7.25 (s, 1 H) 7.07 (d, J=2.42 Hz, 1 H) 6.92 (dd, J=8.88, 2.42 Hz, 1 H) 3.88 (s, 3 H) 3.53 - 3.77 (m, J=6.40 (x6) Hz, 1 H) 3.20 (dd, J=10.50, 6.00 Hz, 2 H) 1.35 (d, J=6.64 Hz, 6 H) 1.32 (d, J=6.55 Hz, 6 H).
Figure 14: Proton NMR (400 MHz) of 5-Methoxy-N-methyl-N-isopropyltryptamine (with Insets).

$^1$H NMR (400 MHz, D$_2$O) $\delta$ ppm 7.45 (d, J=8.90 Hz, 1 H) 7.30 (s, 1 H) 7.17 (d, J=2.45 Hz, 1 H) 6.96 (dd, J=8.80, 2.45 Hz, 1 H) 3.90 (s, 3 H) 3.55 - 3.69 (m, J=6.70 (x6) Hz, 1 H) 3.37 - 3.55 (m, 1 H) 3.05 - 3.33 (m, 3 H) 2.81 (s, 3 H) 1.31 (d, J=6.46 Hz, 3 H) 1.23 (d, J=6.26 Hz, 3 H).
Figure 15: Proton NMR (400 MHz) of N,N-Dipropyltryptamine (with Insets).

$^1$H NMR (400 MHz, D$_2$O) δ ppm 7.65 (d, J=7.83 Hz, 1 H) 7.54 (d, J=8.12 Hz, 1 H) 7.29 (s, 1 H) 7.29 (ddd, J=8.14, 7.07, 1.03 Hz, 1 H) 7.20 (ddd, J=7.73, 7.29, 0.93 Hz, 1 H) 3.40 (dd, J=8.31, 7.14 Hz, 2 H) 3.17 (dd, J=8.22, 7.14 Hz, 2 H) 3.07 - 3.14 (m, 4 H) 1.60 - 1.74 (m, 4 H) 0.93 (t, J=7.38 Hz, 6 H).
Figure 16: Proton NMR (400 MHz) of 5-Methoxy-N,N-dimethyltryptamine (with Insets).

$^1$H NMR (400 MHz, D$_2$O) δ ppm 7.47 (d, J=8.90 Hz, 1 H) 7.32 (s, 1 H) 7.20 (d, J=2.45 Hz, 1 H) 6.97 (dd, J=8.90, 2.45 Hz, 1 H) 3.91 (s, 3 H) 3.46 (t, J=7.43 Hz, 2 H) 3.20 (t, J=7.43 Hz, 2 H) 2.92 (s, 6 H).
Desloratadine: The Reaction Byproduct of the Reduction of Cold Tablets Containing Loratadine with Hydriodic Acid/Red Phosphorus

Shannon C. DiPari,* Jason A. Bordelon, and Harry F. Skinner
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA  92081
[Reprinted with Permission from the Journal of the Clandestine Laboratory Investigating Chemists Association 2005;15(1):4-11. Note that the original article did not contain an Abstract or Keyword list, and those provided below are by the Editor. Note also that this version has been reformatted to Microgram Journal standards, and additionally has had minor errors corrected.]

ABSTRACT: Production of methamphetamine via hydriodic acid/red phosphorus reduction of over-the-counter pseudoephedrine products that contain other active co-ingredients will generate products that are contaminated with those co-ingredients and/or their reduction byproducts. In the case of pseudoephedrine products containing loratadine, the final product will contain desloratadine. Identification of desloratadine in methamphetamine therefore provides an indication of the commercial product used as the precursor in the synthesis.

KEYWORDS: Desloratadine, Loratadine, Pseudoephedrine, Methamphetamine, Hydriodic Acid, Red Phosphorus, Reduction, Trace Analysis, Impurity Profiling, Forensic Chemistry

Introduction

Clandestine methamphetamine laboratories are prevalent in the United States. One of the primary synthetic methods encountered is the reduction of ephedrine or pseudoephedrine with hydriodic acid/red phosphorus.¹ When first encountered and for many years thereafter, commercial hydriodic acid and red phosphorus were used in the reduction. In recent years, however, hydriodic acid and red phosphorus purchases have been restricted by law, forcing the clandestine laboratory operators to search for alternative sources. Red phosphorus is commonly obtained by the use of matchbooks and flares whereas hydriodic acid needs to be synthesized by the clandestine laboratory operator. The synthetic methods to generate hydriodic acid use iodine and either red phosphorus or other reactive phosphorous compounds such as hypophosphorous acid or phosphorous acid.²

Due to the increased restrictions on obtaining pure precursor ephedrine or pseudoephedrine, most clandestine laboratory operators are utilizing common cold tablet preparations.³⁴ These cold tablet preparations contain either ephedrine or pseudoephedrine, and often other ingredients such as cough suppressants, analgesics, expectorants, or antihistamines. Common co-ingredients include acetaminophen, brompheniramine, chlorpheniramine, dextromethorphan, diphenhydramine, doxylamine, guaifenesin, and triprolidine. When these compounds are present in the ephedrine/pseudoephedrine reduction mixtures, they will be carried through the reaction sequence unchanged, or will produce characteristic byproducts that are identifiable by GC/MS.³⁻⁶

The identification of these compounds or their byproducts in clandestinely produced methamphetamine can assist the analyst in determining which cold tablet preparation was used as the precursor source. The ratios of these byproducts relative to methamphetamine are usually very low in the final product. However, they can be easily extracted and identified.⁷ As new cold products become available on the market, clandestine laboratory operators will use them to obtain pseudoephedrine and manufacture methamphetamine. Any new co-products contained in...
these cold tablets have the potential to produce impurities not previously encountered by the forensic analyst. A recent example is tablets containing loratadine, which are already being used in methamphetamine production. Loratadine is the active ingredient in Claritin®, which recently changed from a prescription to an over-the-counter medication.

**Experimental**

Reactions
Loratadine, red phosphorus and 30 mL of 57 % hydriodic acid were refluxed (boiling point = 120 °C) in a round bottom flask fitted with a condenser. The reactions were monitored by removal of aliquots with subsequent analysis. The aliquots were sampled initially, once the mixture began to reflux, and then at every hour. The progress of each reaction was monitored as a decrease of the precursor and the formation of desloratadine. The progress was also monitored by detection of intermediates and byproducts.

**Gas Chromatography**
These analyses were performed using an Agilent Technologies 6890N Gas Chromatograph equipped with electronic pneumatic control and a flame ionization detector. A 10.0 m x 0.32 mm i.d. fused-silica capillary column coated with 0.52 μm DB-5 (Agilent Technologies) was employed. Hydrogen was the carrier gas, with an average linear velocity of 40 cm/sec (constant flow). The injection port and detector were maintained at 280 °C. The samples were extracted into ether, and one μL of each sample was injected in split mode (25:1). The oven temperature was programmed as follows: Initial temperature 130 °C, hold for 1.0 minute, then increase temperature 25 °C per minute to 280 °C, hold for 1.0 minute (total run time = 10.0 minutes).

**Gas Chromatography/Mass Spectrometry**
The electron impact (EI) mass spectra were obtained using an Agilent Technologies Mass Spectrometer. The spectrometer was equipped with a 5973 mass selective detector and 6890N gas chromatograph. A 30.0 m x 0.25 mm i.d. fused-silica capillary column coated with 0.25 μm HP5-MS (Agilent Technologies) was employed. Helium was the carrier gas with an average linear velocity of 40 cm/sec (constant flow). The injection port and ion sources were set at 240 °C and 180 °C, respectively. One μL from each of the samples was injected in split mode (30:1). The oven temperature was programmed as follows: Initial temperature 100 °C for 1.0 minute, then increase temperature 20 °C per minute to 280 °C, hold for 10.0 minutes (total run time = 20 minutes). The mass spectrometer was scanned over an m/z range of 40 - 500. The transfer lines were maintained at 280 °C.

**Infrared Spectrophotometry**
The infrared spectra were obtained in potassium bromide on a Nicolet Magna 560 Fourier Transform Infrared (FTIR) Spectrophotometer. The infrared spectra were also obtained by attenuated total reflectance (ATR) on an Avatar 370 FTIR Spectrophotometer.

**Results and Discussion**
The intermediates and byproducts in the synthesis of methamphetamine utilizing ephedrine/pseudoephedrine via HI/red P are well documented. For example, if chlorpheniramine or 1-(4-methylphenyl)-1-(2-pyridyl)-3-pyrolidinopropane (commonly referred to as “reduced triprolidine”) are present in a methamphetamine sample, then the precursor source of the pseudoephedrine also certainly contained chlorpheniramine or triprolidine, respectively. Whenever a new compound shows up in the finished product, this indicates that a new, previously unused cold tablet preparation has probably been used as the precursor source.

Recently, a methamphetamine sample was analyzed in this laboratory and was found to contain a new compound not seen in previous exhibits. The compound was in very low concentration in comparison to the methamphetamine, indicating it was probably not added as a cutting agent. The gas chromatogram is shown in Figure 1. The compound eluted on the gas chromatograph in the same general area of other “byproduct amines”
produced from the previously discussed pseudoephedrine pharmaceutical preparations. The mass spectrum was easily obtained by employing an extraction technique to enhance these compounds. That is, the methamphetamine sample (50 to 100 mg) was dissolved in 2-3 mL of water and made pH 8/9 basic with sodium bicarbonate. The basic solution was then extracted with 2 mL of hexane. The majority of the methamphetamine remained in the aqueous solution and did not extract into the hexane. Neutrals and “byproduct amines” are enhanced over the methamphetamine and also over the most common cutting agent dimethylsulfone. The extracted solution was further enhanced by evaporating it to dryness on a hot plate at 90º C, using a stream of air for about two minutes. Phenyl-2-propanone, methamphetamine, and residual dimethylsulfone are more volatile and evaporate, leaving only the suspected “byproduct amine.” In the present case, after the compound was isolated, the mass spectrum was easily obtained. The mass spectrum of the compound gave a base peak of 280 amu and parent ion of 310 amu, as shown in Figure 2.

Several new products containing pseudoephedrine and previously unreported antihistamines including fexofenadine, cetirizine, and loratadine are now commercially available. The molecular weights are as follows: Fexofenadine (mw: 501.7), cetirizine (mw: 388.9) and loratadine (mw: 382.9). A “D” at the end of the proprietary name of the antihistamine product denotes that the product also contains the decongestant pseudoephedrine. The ratio of fexofenadine HCl to pseudoephedrine HCl is 60:120 mg/tablet. The ratio of cetirizine HCl to pseudoephedrine HCl is 5:120 mg/tablet. The ratio of loratadine to pseudoephedrine sulfate is 5:120 or 10:240 mg/tablet. The loratadine containing product has recently been converted from a prescription to
an over-the-counter product, making it much easier to obtain than the other two products. Based on the ratio of
the “byproduct amine” to methamphetamine in the methamphetamine exhibits, and the mass spectrum of the new
compound, the loratadine product with pseudoephedrine was suspected to be the source of the new byproduct.
The structures of fexofenadine, cetirizine, and loratadine are shown above.

The chemical name of loratadine is ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-
ylidene-1-piperidine carboxylate. Loratadine is also known as 8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta-
[1,2-b]pyridine-11-ylidene-1-piperidine carboxylic acid ethyl ester. Based on the structure of loratadine, the
reaction with HI was suspected to cleave the amide group and reduce the double bond. Both of these structural
changes are consistent with the cleavage of the amide/ester groups and the reduction of the double bond, as
previously observed with triprolidine. The expected products would have molecular weights of 310.8 and 312.8
amu, respectively.

Loratadine was obtained by extracting commercial tablets containing only loratadine as the active ingredient. The
ground tablets were extracted with chloroform, recrystallized with acetonitrile/ether, and air-dried on a hot plate.
The mass spectrum and infrared spectrum of the solid matched a standard of loratadine (USP Cat. #137020). The
mass spectrum and infrared spectra (KBr and ATR) are shown in Figures 3 - 5.

The isolated loratadine was refluxed with the same ratio of hydriodic acid and red phosphorus often used in
methamphetamine manufacture, and the reaction monitored by gas chromatography. The retention times for the
reaction product and loratadine were 7.12 and 9.24 minutes, respectively. The reaction product formed as soon
the reaction mixture was heated. The gas chromatograph retention time and the mass spectrum of this reaction
product are the same as the unknown compound encountered in the methamphetamine exhibits. Loratadine was
also refluxed with only hydrochloric acid, and this mixture produced the same reaction product.

The unknown compound had to be an acid cleaved amide product with no reduction of the double bond, since
hydrochloric acid is not capable of reducing the double bond. The identity of the unknown compound is
4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene-1-piperidine, or more simply
desloratadine. The identity was confirmed by comparison with extracted desloratadine from pharmaceutical
tablets containing desloratadine. The methamphetamine precursor therefore had to be tablets containing
loratadine and pseudoephedrine, since commercial tablets containing desloratadine do not contain
pseudoephedrine. The mass spectrum for desloratadine is shown in Figure 6. The structure of desloratadine is
shown below.

![Desloratadine structure](image)

The main byproduct formed when manufacturing methamphetamine using loratadine-containing pseudoephedrine
tablets is desloratadine. However, in some of the methamphetamine samples containing desloratadine another
“byproduct amine” was also detected. The compound elutes just after desloratadine. The mass spectrometer
total ion chromatogram is shown in Figure 7. This compound has a base peak of 82 amu with an ion at 267 amu and a suspected parent peak of 310 amu. The mass spectrum is shown in Figure 8. This compound has not yet been identified, and will be addressed in future studies.

**Conclusions**

Use of cold tablet preparations as the source of pseudoephedrine has presented challenges in the identification of the trace amounts of the “byproduct amines” in methamphetamine samples. These challenges will continue as new pharmaceutical combinations with pseudoephedrine are made available. The identification of these byproducts in clandestinely produced methamphetamine can help analysts in determining which cold tablet preparations were used as the precursor source, and to link specific exhibits and cases, or both.

**References**


[Figures 1 - 8 Follow.]
Figure 1. Gas Chromatogram of a Methamphetamine Sample Containing Dimethylsulfone and the Unknown “Byproduct Amine.” Retention times: Dimethylsulfone: 0.579 Minutes; Methamphetamine: 1.289 Minutes; and Unknown “Byproduct Amine”: 7.122 Minutes.

* * * * *

Figure 2. Mass Spectrum of the Previously Unseen “Byproduct Amine.”
Figure 3. Mass Spectrum of Loratadine.

Figure 4. Infrared Spectrum of Loratadine in KBr.
Figure 5. Infrared Spectrum of Loratadine by ATR.

* * * * *

Figure 6. Mass Spectrum of Desloratadine.
Figure 7. Total Ion Chromatogram of Desloratadine from Reaction of Loratadine with Hydriodic Acid/Red Phosphorus.

Figure 8. Mass Spectrum of Secondary Unknown Product.
Identification of Phenethylamines and Methylenedioxyamphetamine Analogues Using Liquid Chromatography Atmospheric Pressure Electrospray Ionization Mass Spectrometry

Adrian S. Krawczeniuk
U.S. Department of Justice
Drug Enforcement Administration
Northeast Laboratory
99 - 10th Avenue, Suite 721
New York, NY 10011
[email: Adrian.S.Krawczeniuk -at- usdoj.gov]

ABSTRACT: A liquid chromatography - mass spectrometric (LC/MS) procedure utilizing atmospheric pressure electrospray ionization (API-ES) was developed for the identification of phenethylamines, methylenedioxyamphetamine analogs, and other related compounds of forensic interest. An evaluation of three Phenomenex Synergi C-18 columns (Hydro-RP, Polar-RP, Fusion-RP) was performed using 22 compounds of interest to determine optimum selectivity. The method utilizes an isocratic buffered system of 10 mM ammonium formate pH 3.7 - acetonitrile along with diode array detection at 280 nm and 210 nm. Ionization is effected via electrospray in positive mode, resulting in a protonated pseudomolecular ion for the compounds of interest. Electrospray parameters were optimized via flow injection analysis and collision induced dissociation experiments were performed to optimize fragmentation of the compounds of interest. Sample preparation was minimal, and there was no need to derivatize.

KEYWORDS: Phenethylamines, Methylenedioxyamphetamine Analogues, LC/MS, Electrospray, Collision Induced Dissociation, Forensic Chemistry

Introduction

Gas chromatography/mass spectrometry (GC/MS) is considered the standard technique for the identification of sympathomimetic amines such as phenethylamines and structurally related substituted compounds [1-5]. However, many of these compounds exhibit mass spectra with a very predominant base peak and very low molecular and fragment ions, which can make the identification challenging. Software normalization techniques have been employed in discriminating mass spectra of amines by removing the dominant base peak and normalizing the spectrum to a lower residual ion [5]. Derivatization techniques utilizing perfluorinated anhydrides such as heptafluorobutyryl (HFB), pentafluoropropionyl (PFP), or trifluoroacetyl (TFA), or silylating derivatives such as BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) and MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide), have also been widely employed, and both improve specificity and produce more readily identifiable mass spectra [6-7].

A liquid chromatograph coupled with atmospheric pressure ionization (LC-API), in either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) mode, provides an alternative technique to GC/MS for the identification of illicit drugs. LC/MS has been used successfully to analyze a broad range of compounds, with applications in forensic and clinical toxicology [8-12], anti-doping testing [13], and therapeutic drug monitoring [14]. LC/MS is ideal for thermolabile, low molecular weight compounds, non-volatile compounds, and/or highly polar drugs, eliminating the need for derivatization.

Atmospheric pressure ionization (API) is a relatively soft ionization technique generating either protonated [M+H]⁺ or deprotonated pseudomolecular ions [M-H]⁻, or multiply charged ions; these are formed through ion
evaporation in ESI, and through gas-phase chemical ionization in APCI. API sources yield low fragmentation, which would normally preclude the use of single quadrupole instruments. However, in-source collision dissociation (CID) can take place in the ion source, allowing for fragmentation of analyte ions via collisions with neutral molecules from residual solvent and gas molecules. This results in bond cleavages and rearrangements that are representative of the molecular structure of the molecule.

This study presents an LC/MS method using electrospray ionization (ESI) for the separation and confirmation of phenethylamines (PEAs), methylenedioxyamphetamines (MDAs), and other compounds routinely encountered in illicit drug seizures. HPLC separations were optimized using three different C-18 stationary phases, and CID experiments were performed in order to obtain mass spectra with fragmentations characteristic for each compound examined.

**Experimental**

**LC/MS Methodology**

Analyses were performed using an Agilent Technologies 1100 series high pressure liquid chromatograph (HPLC), including a quaternary pump, vacuum degasser, autosampler, thermostatted column compartment, diode array detector, and coupled to an Agilent Technologies 1100 series Model SL single quadrupole mass spectrometer equipped with an electrospray ionization interface. Nitrogen drying gas was generated using a nitrogen generator (Agilent Technologies 5183-2003) coupled to a Jun Air air compressor.

Chromatographic separations were evaluated using three different Phenomenex Synergi columns (15 cm x 3.0 mm, 4 μ 80 A) - Hydro-RP, Polar-RP and Fusion-RP. A mobile phase of 10 mM ammonium formate pH 3.7: Acetonitrile (88:12) delivered at a flow rate of 0.5 mL/minute was used to elute the compounds of interest. The column temperature was thermostatically controlled at 40 °C. An injection volume of 2 μL was used.

Mass analyses were performed in scan mode from a mass range m/z 50 - 350 measuring positive pseudomolecular ions. The fragmentor was set at 150 V for all compounds except phenethylamine which was run at 70 V in order to observe the pseudomolecular ion. Spray chamber parameters were as follows: 12.0 L/minute drying gas, 350 °C drying gas temperature, 40 psig nebulizer, 4000 V capillary voltage.

All illicit samples examined were dissolved in the mobile phase and filtered thru a 0.45 μ nylon membrane filter prior to analysis. Flow injection analysis was performed on all compounds examined by varying the fragmentor voltage from 100 - 240 V in increments of 20 V. Nebulizer pressure, capillary voltage, and drying gas flow were operated as per manufacturer’s specifications. Complete system control and data evaluation was carried out using the Agilent Chemstation for LC/MS.

**Reagents**

All drug standards were obtained from the reference collection of the DEA Northeast Laboratory. Standards were prepared at a concentration of 0.05 mg/mL diluted in 10 mM ammonium formate pH 3.7. Ammonium formate (99.995+ %), formic acid (95 - 97 %), and acetonitrile (LC/MS Chromasolv grade) were obtained from Sigma Aldrich, St. Louis, MO. Ultrapure water from a Millipore Gradient 10-Elix 3 system (Billerica, MA) was used to prepare all buffers in the study.

**Results and Discussion**

Optimization of the HPLC conditions was performed using an ammonium formate buffer at pH 3.7 and acetonitrile as the organic modifier. Acetonitrile was chosen as opposed to methanol because it gave more symmetrically shaped peaks and efficiently resolved the compounds of interest. PEAs and MDAs are ideal candidates for positive ion ESI because low pH buffers completely ionize basic compounds (i.e., resulting in protonated species). A total of 22 compounds including structurally similar sympathomimetic amines, MDAs,
and adulterants routinely encountered in illicit seizures were evaluated on three Phenomenex Synergi C-18 columns (Hydro-RP, Polar-RP and Fusion-RP).

Selectivity data for each of the three columns evaluated are listed in Tables 1 - 3. All three columns gave similar selectivities for the 12 most commonly encountered substrates (phenylpropanolamine, phenethylamine, ephedrine, pseudoephedrine, methylephedrine, amphetamine, dimethylamphetamine, methamphetamine, phenetermine, 3,4-MDA, 3,4-MDMA, 3,4-MDEA, and MBDB) (see Tables 1 - 3 and Figures 1 - 3). The Polar-RP column (an ether linked phenyl phase with hydrophilic endcappping) exhibited slightly more retentiveness for the N-substituted MDA analogs MDEA, MBDB, and N,N-dimethyl-MDA, along with dimethylamphetamine and ketamine. The adulterant caffeine, commonly encountered in MDMA seizures submitted to our laboratory, was retained on the Polar-RP column, but co-eluted with MDMA on the Hydro-RP and Fusion-RP columns (Tables 1 - 3). Case submissions containing both illicit tablets and powders were analyzed using the established LC/MS procedure (see Figures 13 - 14). Results were verified using a GC/MS method. Both the Hydro-RP and Polar-RP columns have been used interchangeably for routine case submissions, with good success.

Figures 4 - 10 show the mass spectra of 17 PEAs and MDAs examined under the ESI conditions specified. All compounds examined (except phenethylamine) exhibited a protonated pseudomolecular ion using a fragmentor of 150 V. Decreasing the voltage to 70 V revealed the protonated pseudomolecular ion for phenethylamine.

Methamphetamine and phenetermine exhibit similar GC/MS fragmentation patterns, but are readily differentiated under ESI conditions. Both compounds are easily resolved on the Hydro-RP and Fusion-RP columns (see Figure 3). These isomers are more closely resolved on the Polar-RP column with a resolution of 1.55 (HPLC) and 1.22 (MS) using the half-width method calculation. Methamphetamine and phenetermine both exhibit a pseudomolecular ion of m/z 150, but are easily differentiated by their characteristic fragment ions, with methamphetamine exhibiting a m/z 119 product ion and phenetermine exhibiting a m/z 133 product ion (see Figure 4). The ability to resolve these isomeric pairs chromatographically and differentiate them by their mass spectral fragmentation patterns allows for facile identification of these two compounds. Phentermine and 4-methoxymethamphetamine are unresolved on the three columns tested - but this combination has never been encountered at our laboratory (see Figure 3).

The MDAs were resolved on all three columns (see Figure 1). N-hydroxy-3,4-MDA was strongly retained on the Hydro-RP, and eluted in 44 minutes. However, the Polar-RP and Fusion-RP columns offered a more efficient elution, with retention times less than eleven minutes. N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) is readily resolved from its regioisomeric MDA derivatives (i.e., 3,4-MDEA, and N,N-dimethyl-3,4-MDA) on all three columns. All three compounds exhibit a protonated pseudomolecular ion at m/z 208. 3,4-MDEA and N,N-dimethyl-3,4-MDA exhibit indistinguishable ESI mass spectra (Figures 7). 3,4-MDEA and MBDB are readily differentiated by their product ions, with MBDB exhibiting product ions at m/z 177 and 135 while MDEA exhibits product ions at m/z 163 and 133 (see Figure 7).

3,4-MDA exhibits a pseudomolecular ion at m/z 180, with product ions at m/z 163, 133, and 105, while 3,4-MDMA exhibits a pseudomolecular ion and base peak at m/z 194, with similar product ions (see Figure 5). All the substituted MDA analogs examined (except 3,4-MDEA and N,N-dimethyl-3,4-MDA) are readily distinguishable by their pseudomolecular ions. In addition, all of the MDAs are resolved on all three columns, thereby allowing for differentiation even of 3,4-MDEA and N,N-dimethyl-3,4-MDA via retention time matching.

In the present study, a fragmentor voltage of 150 V was chosen in order to observe a protonated pseudomolecular ion and sufficient fragmentation product ions that would allow for conclusive identification of each compound examined. Table 4 provides a summary of the relative abundances of the five major ions for each compound examined. The protonated molecular ion was the base peak for 12 of the 22 compounds examined.

Flow injection analysis allows for direct sample injection into the mass spectrometer and was used in order to optimize API-MS parameters. This allowed for rapid method development. CID experiments via flow injection analysis were performed on all compounds by varying the fragmentor voltages from 100 - 240 V.
Varying the fragmentor voltages had the greatest impact on the rate of fragmentation as observed during the CID of 3,4-MDMA (Figure 12). The protonated pseudomolecular ion $m/z$ 194 is most abundant at 100 V and gradually decreases as the fragmentor voltage is ramped to 240 V. At 100 V, the pseudomolecular ion is the base peak, and there are minimal fragment product ions. As the fragmentor voltage is increased, characteristic product ions are observed, and increase in intensity (Figure 11).

Fragmentor voltages were optimized for each respective compound (see Table 5). The sensitivity of higher mass ions was higher at lower fragmentor voltages, while the sensitivity of lower mass ions increased at higher fragmentor voltages. Area response sensitivity gradually decreased for compounds examined as a function of increasing fragmentor voltage (see Figure 15).

In-source CID has been shown to produce similar fragmentations as conventional CID in the collision cell of a tandem mass spectrometer (MS-MS) - but not necessarily of the same intensities. A requirement for in-source CID is a complete separation of the compounds being studied, as opposed to conventional CID using a tandem MS, where a precursor ion is specifically selected, followed by fragmentation [15,17,18]. A disadvantage of in-source CID is that since all ions are fragmented there is no mechanism to elucidate which product ions originated from which precursor ion [18]. In addition, no commercial in-source CID mass spectral libraries exist at present, requiring the user to create an in-house CID mass spectral library for the compounds of interest [16].

In conclusion, in-source fragmentation using a single quadrupole mass spectrometer allows for the positive identification of PEAs and MDAs. The instrumentation is user friendly, provides the ability to perform rapid method development using in-source CID, and offers extracted ion monitoring to deconvolute complex chromatograms. The LC/MS method has been implemented at our laboratory, and has been instrumental in confirming the presence of PEAs, MDAs, and common adulterants found in complex illicit mixtures. This provides a complementary and/or alternative means of identification of PEAs and MDAs.

Acknowledgments

Special thanks to Forensic Chemist Ramona S. Montreuil and Supervisory Chemist Ed J. Manning (both at this laboratory) for their editorial suggestions in reviewing this manuscript.

References


* * * * *

[Table 1 - 5 and Figures 1 - 15 Follow.]
Table 1. Selectivity of Compounds Examined on Phenomenex Synergi Hydro-RP.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS Rt (minutes)</th>
<th>RRt (Methamphetamine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacinamide</td>
<td>1.93</td>
<td>0.32</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>2.87</td>
<td>0.47</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>2.90</td>
<td>0.47</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>2.99</td>
<td>0.49</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>3.72</td>
<td>0.61</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>3.73</td>
<td>0.61</td>
</tr>
<tr>
<td>Benzylpiperazine</td>
<td>4.08</td>
<td>0.67</td>
</tr>
<tr>
<td>Methyleneephedrine</td>
<td>4.22</td>
<td>0.69</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>4.97</td>
<td>0.81</td>
</tr>
<tr>
<td>Caffeine</td>
<td>5.06</td>
<td>0.83</td>
</tr>
<tr>
<td>3,4-MDA</td>
<td>5.57</td>
<td>0.91</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>6.12</td>
<td>1.00</td>
</tr>
<tr>
<td>3,4-MDMA</td>
<td>6.66</td>
<td>1.09</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>7.06</td>
<td>1.15</td>
</tr>
<tr>
<td>Phentermine</td>
<td>7.36</td>
<td>1.20</td>
</tr>
<tr>
<td>N,N-Dimethyl-3,4-MDA</td>
<td>7.62</td>
<td>1.25</td>
</tr>
<tr>
<td>4-Methoxymethamphetamine</td>
<td>7.64</td>
<td>1.25</td>
</tr>
<tr>
<td>Ethylamphetamine</td>
<td>8.50</td>
<td>1.39</td>
</tr>
<tr>
<td>3,4-MDEA</td>
<td>9.30</td>
<td>1.52</td>
</tr>
<tr>
<td>Ketamine</td>
<td>11.01</td>
<td>1.80</td>
</tr>
<tr>
<td>MBDB</td>
<td>12.30</td>
<td>2.01</td>
</tr>
<tr>
<td>N-Hydroxy-3,4-MDA</td>
<td>44.24</td>
<td>7.23</td>
</tr>
</tbody>
</table>

* * * * *
Table 2. Selectivity of Compounds Examined on Phenomenex Synergi Polar-RP.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS Rt (minutes)</th>
<th>RRt (Methamphetamine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacinamide</td>
<td>2.39</td>
<td>0.37</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>3.15</td>
<td>0.49</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>3.22</td>
<td>0.50</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>3.65</td>
<td>0.57</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>4.07</td>
<td>0.63</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>4.19</td>
<td>0.65</td>
</tr>
<tr>
<td>Methylephedrine</td>
<td>4.83</td>
<td>0.75</td>
</tr>
<tr>
<td>Benzylpiperazine</td>
<td>4.86</td>
<td>0.75</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>5.05</td>
<td>0.78</td>
</tr>
<tr>
<td>3,4-MDA</td>
<td>6.30</td>
<td>0.98</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>6.44</td>
<td>1.00</td>
</tr>
<tr>
<td>Phentermine</td>
<td>6.84</td>
<td>1.06</td>
</tr>
<tr>
<td>Caffeine</td>
<td>7.88</td>
<td>1.22</td>
</tr>
<tr>
<td>3,4-MDMA</td>
<td>7.99</td>
<td>1.24</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>8.20</td>
<td>1.27</td>
</tr>
<tr>
<td>4-Methoxymethamphetamine</td>
<td>8.34</td>
<td>1.30</td>
</tr>
<tr>
<td>Ethylamphetamine</td>
<td>8.79</td>
<td>1.36</td>
</tr>
<tr>
<td>N,N-Dimethyl-3,4-MDA</td>
<td>10.33</td>
<td>1.60</td>
</tr>
<tr>
<td>N-Hydroxy-3,4-MDA</td>
<td>10.59</td>
<td>1.64</td>
</tr>
<tr>
<td>3,4-MDEA</td>
<td>11.12</td>
<td>1.73</td>
</tr>
<tr>
<td>Ketamine</td>
<td>12.31</td>
<td>1.91</td>
</tr>
<tr>
<td>MBDB</td>
<td>13.10</td>
<td>2.03</td>
</tr>
</tbody>
</table>

* * * * *
Table 3. Selectivity of Compounds Examined on Phenomenex Synergi Fusion-RP.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MS Rt (minutes)</th>
<th>RRt (Methamphetamine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacinamide</td>
<td>2.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>3.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>3.58</td>
<td>0.53</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>3.80</td>
<td>0.63</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>3.84</td>
<td>0.64</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>3.94</td>
<td>0.66</td>
</tr>
<tr>
<td>Benzylpiperazine</td>
<td>4.28</td>
<td>0.71</td>
</tr>
<tr>
<td>Methylephedrine</td>
<td>4.94</td>
<td>0.73</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>4.98</td>
<td>0.83</td>
</tr>
<tr>
<td>3,4-MDA</td>
<td>5.76</td>
<td>0.96</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>6.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Caffeine</td>
<td>6.04</td>
<td>1.01</td>
</tr>
<tr>
<td>3,4-MDMA</td>
<td>6.77</td>
<td>1.13</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>6.78</td>
<td>1.13</td>
</tr>
<tr>
<td>Phentermine</td>
<td>7.19</td>
<td>1.20</td>
</tr>
<tr>
<td>4-Methoxymethamphetamine</td>
<td>7.44</td>
<td>1.24</td>
</tr>
<tr>
<td>N,N-Dimethyl-3,4-MDA</td>
<td>7.64</td>
<td>1.27</td>
</tr>
<tr>
<td>Ethylamphetamine</td>
<td>8.13</td>
<td>1.36</td>
</tr>
<tr>
<td>3,4-MDEA</td>
<td>9.32</td>
<td>1.55</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10.70</td>
<td>1.78</td>
</tr>
<tr>
<td>N-Hydroxy-3,4-MDA</td>
<td>10.93</td>
<td>1.82</td>
</tr>
<tr>
<td>MBDB</td>
<td>11.96</td>
<td>1.99</td>
</tr>
</tbody>
</table>

* * * * *
Table 4. Mass Ion Abundances and % Relative Intensity of Mass Ion Abundances at 150 V for compounds examined.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion#1(m/z)</th>
<th>Ion#2(m/z)</th>
<th>Ion#3(m/z)</th>
<th>Ion#4(m/z)</th>
<th>Ion#5(m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacinamide</td>
<td>123(100%)</td>
<td>124(7.4%)</td>
<td>80 (3.3%)</td>
<td>50(2.4%)</td>
<td>78(1.3%)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>152(100%)</td>
<td>110(18.2%)</td>
<td>102(14.7%)</td>
<td>153(9.3)</td>
<td>174(3.3%)</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>105(100%)</td>
<td>79(5.7%)</td>
<td>103(4.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylephedrine</td>
<td>180(100%)</td>
<td>162(21.0%)</td>
<td>181(12.6%)</td>
<td>135(5.9%)</td>
<td>163(2.5%)</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>134(100%)</td>
<td>135(13.6%)</td>
<td>117(7.1%)</td>
<td></td>
<td>152(6.7%)</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>148(100%)</td>
<td>166(33%)</td>
<td>149(10.9%)</td>
<td>135(4.3%)</td>
<td>167(4.0%)</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>148(100%)</td>
<td>166(13.9%)</td>
<td>149(11.9%)</td>
<td>133(3.5%)</td>
<td>167(1.8%)</td>
</tr>
<tr>
<td>Benzylpiperazine</td>
<td>177(100%)</td>
<td>178(12.2%)</td>
<td>91(4.9%)</td>
<td>85(1.2%)</td>
<td></td>
</tr>
<tr>
<td>Amphetamine</td>
<td>91(100%)</td>
<td>119(59.7%)</td>
<td>136(9.0%)</td>
<td>120(6.3%)</td>
<td>65(0.9%)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>195(100%)</td>
<td>196(10.3%)</td>
<td>138(4.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4-MDA</td>
<td>163(100%)</td>
<td>164(11.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>150(100%)</td>
<td>91(82.8%)</td>
<td>119(70.5%)</td>
<td>151(11.7%)</td>
<td>92(6.8%)</td>
</tr>
<tr>
<td>3,4-MDMA</td>
<td>163(100%)</td>
<td>194(73.3)</td>
<td>164(11.0%)</td>
<td>195(9.5%)</td>
<td>135(6.7%)</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>164(100%)</td>
<td>119(15.9%)</td>
<td>91(15.4%)</td>
<td>165(13.2%)</td>
<td>92(1.2%)</td>
</tr>
<tr>
<td>Phentermine</td>
<td>133(100%)</td>
<td>91(63.4%)</td>
<td>150(11.9%)</td>
<td>134(10.9%)</td>
<td>105(7.9%)</td>
</tr>
<tr>
<td>N,N-Dimethyl-3,4-MDA</td>
<td>208(100%)</td>
<td>163(35.3%)</td>
<td>209(13.6%)</td>
<td>164(4.0%)</td>
<td>135(2.6%)</td>
</tr>
<tr>
<td>4-Methoxymethylamphetamine</td>
<td>149(100%)</td>
<td>180(37.6%)</td>
<td>121(14.1%)</td>
<td>150(11.6%)</td>
<td>181(4.6%)</td>
</tr>
<tr>
<td>Ethylamphetamine</td>
<td>164(100%)</td>
<td>91(32.8%)</td>
<td>119(32.7)</td>
<td>165(13.0%)</td>
<td>120(3.2%)</td>
</tr>
<tr>
<td>3,4-MDEA</td>
<td>208(100%)</td>
<td>163(70.4%)</td>
<td>209(14.1%)</td>
<td>135(5.1%)</td>
<td>133(4.7%)</td>
</tr>
<tr>
<td>Ketamine</td>
<td>238(100%)</td>
<td>240(32.3%)</td>
<td>239(14.5%)</td>
<td>207(12.7%)</td>
<td>179(6.9%)</td>
</tr>
<tr>
<td>MBDB</td>
<td>208(100%)</td>
<td>135(50.1%)</td>
<td>177(40.3%)</td>
<td>209(14.3%)</td>
<td>147(6.0%)</td>
</tr>
<tr>
<td>N-Hydroxy-3,4-MDA</td>
<td>163(100%)</td>
<td>196(17.8%)</td>
<td>164(10.9%)</td>
<td>105(7.1%)</td>
<td>135(6.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* * * * *
Table 5. Fragmentor Voltage Optimization of Mass Ions for Compounds Examined.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion#1 (Voltage)</th>
<th>Ion#2</th>
<th>Ion#3</th>
<th>Ion#4</th>
<th>Ion#5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacinamide</td>
<td>123(120V)</td>
<td>124</td>
<td>80</td>
<td>50</td>
<td>78</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>152(100)</td>
<td>110</td>
<td>93</td>
<td>153(120)</td>
<td>174(3.3%)</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>122(70)</td>
<td>105</td>
<td>79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylephedrine</td>
<td>180(100)</td>
<td>162</td>
<td>181</td>
<td>135</td>
<td>163</td>
</tr>
<tr>
<td>Phenylpropanolamine</td>
<td>134(140)</td>
<td>135</td>
<td>117</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Ephedrine</td>
<td>148(160)</td>
<td>166</td>
<td>149</td>
<td>135</td>
<td>167</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>148(160)</td>
<td>166</td>
<td>149</td>
<td>133</td>
<td>167</td>
</tr>
<tr>
<td>Benzylpiperazine</td>
<td>177(100)</td>
<td>178</td>
<td>91</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Amphetamine</td>
<td>91(180)</td>
<td>119</td>
<td>136</td>
<td>120</td>
<td>65</td>
</tr>
<tr>
<td>Caffeine</td>
<td>195(140)</td>
<td>196</td>
<td>138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4-MDA</td>
<td>163(140)</td>
<td>164</td>
<td>180</td>
<td>205</td>
<td>133</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>150(100)</td>
<td>91</td>
<td>119</td>
<td>151</td>
<td>92</td>
</tr>
<tr>
<td>3,4-MDMA</td>
<td>163(160)</td>
<td>194</td>
<td>164</td>
<td>195</td>
<td>135</td>
</tr>
<tr>
<td>Dimethylamphetamine</td>
<td>164(100)</td>
<td>119</td>
<td>91</td>
<td>165</td>
<td>92</td>
</tr>
<tr>
<td>Phentermine</td>
<td>133(140)</td>
<td>91</td>
<td>150</td>
<td>34</td>
<td>105</td>
</tr>
<tr>
<td>N,N-Dimethyl-3,4-MDA</td>
<td>208(120)</td>
<td>163</td>
<td>209</td>
<td>164</td>
<td>135</td>
</tr>
<tr>
<td>4-Methoxymethamphetamine</td>
<td>149(160)</td>
<td>180</td>
<td>121</td>
<td>150</td>
<td>181</td>
</tr>
<tr>
<td>Ethylamphetamine</td>
<td>164(100)</td>
<td>91</td>
<td>119</td>
<td>165</td>
<td>120</td>
</tr>
<tr>
<td>3,4-MDEA</td>
<td>208(100)</td>
<td>163</td>
<td>209</td>
<td>135</td>
<td>133</td>
</tr>
<tr>
<td>Ketamine</td>
<td>238(120)</td>
<td>240</td>
<td>239</td>
<td>207</td>
<td>179</td>
</tr>
<tr>
<td>MBDB</td>
<td>208(100)</td>
<td>135</td>
<td>177</td>
<td>209</td>
<td>147</td>
</tr>
<tr>
<td>N-Hydroxy-3,4-MDA</td>
<td>163(160)</td>
<td>196</td>
<td>164</td>
<td>105</td>
<td>135</td>
</tr>
</tbody>
</table>

* * * * *
Figure 1. Total Ion Chromatogram of 5 Components Mixture of 3,4-MDA Analogs. (a) 3,4-MDA, (b) 3,4-MDMA, (c) N,N-Dimethyl-3,4-MDA, (d) 3,4-MDEA, (e) MBDB.
Figure 2. Total Ion Chromatogram of 12 Component Drug Mixture. (a) Niacinamide, (b) Acetaminophen, (c) Ephedrine, (d) Benzylpiperazine, (e) Amphetamine, (f) 3,4-MDA, (g) Methamphetamine, (h) 3,4-MDMA, (i) Phentermine, (j) 3,4-MDEA, (k) Ketamine, (l) MBDB.
Figure 3. Total Ion Chromatogram of Mixture of 7 amines of interest. (a) Ephedrine, (b) Amphetamine, (c) Methamphetamine, (d) Dimethylamphetamine, (e) Phentermine, (F) 4-Methoxymethamphetamine, (g) Ethylamphetamine.
Figure 4. ESI Mass Spectra of Methamphetamine (top) and Phentermine (bottom).
Figure 5. ESI Mass Spectra of 3,4-MDA (top) and 3,4-MDMA (bottom).
Figure 6. ESI Mass Spectra of Ethylamphetamine (top), Amphetamine (middle), and Dimethylamphetamine (bottom).
Figure 7. ESI Mass Spectra of 3,4-MDEA (top), MBDB (middle), and N,N-Dimethyl-3,4-MDA (bottom).
Figure 8. ESI Mass Spectra of N-Hydroxy-3,4-MDA (top), 4-Methoxymethamphetamine (middle), and Ketamine (bottom).
Figure 9. ESI Mass Spectra of Pseudoephedrine (top), Ephedrine (middle), and Methylephedrine (bottom).
Figure 10. ESI Mass Spectra of Phenylpropanolamine (top) and Phenethyamine (bottom).
Figure 11. Collision Induced Dissociation of 3,4-MDMA.

Figure 12. Flow Injection Analysis of 3,4-MDMA Monitored at $m/z$ 194. Fragmentor Ramped from 100 V - 240 V at 20 V Increments.
Figure 13.  Total Ion Chromatogram of Illicit Tablets Containing (A) Ephedrine, (B) Caffeine, (C) Methamphetamine, and (d) 3,4-MDMA, on a Hydro-RP Column.

* * * * *

Figure 14.  Total Ion Chromatogram of Illicit Powder Containing (A) Amphetamine, (B) Methamphetamine, and (C) Caffeine, on a Polar-RP Column.
Figure 15. Fragmentor Voltage Optimization for Compounds of Interest.

* * * * * * * * * * * * * * * * * * * * * * * * * *
INSTRUCTIONS FOR AUTHORS

General Information

*Microgram Journal* is a scientific periodical that is published by the U.S. Drug Enforcement Administration’s Office of Forensic Sciences, and presents peer reviewed, full length Scientific Research Articles and Technical Notes on the detection and analyses of suspected controlled substances for forensic/law enforcement purposes.

Subscriptions to *Microgram Journal*

*Microgram Journal* is unclassified, and is published on the DEA public access website ([www.dea.gov](http://www.dea.gov)). Private citizens should use the website to access *Microgram Journal*. Professional scientific and law enforcement personnel may either use the website or request a subscription. Subscriptions are available electronically and in hard copy. Electronic subscriptions require Internet access. The publications themselves will not be sent electronically to any subscriber; rather, an email notification of the pertinent URL will be sent to the subscriber when the respective issue is posted on the website (see additional information on email notifications, below). Requests for hard copies are strongly discouraged, and should be limited to those offices that do not have access to the Internet, require hard copies for their libraries, or have some other valid reason (Note: “For my personal collection” is not considered to be a valid reason). Requests for hard copies should indicate the number of copies required (maximum of two allowed per office), and should also include formal justification. Note that due to publication delays beyond the control of the Office of Forensic Sciences, hard copies will arrive from 30 to 180 days after electronic posting.

Requests to be added to the subscription list should be submitted via email to the *Microgram* Editor at: microgram_editor@mailsnare.net If email submission is not possible, requests should be mailed to: *Microgram* Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. All requests to be added to the *Microgram* mailing list should include the following Standard Contact Information:

* The Full Name and Mailing Address of Submitting Laboratory or Office;

* The Full Name, Title (Laboratory Director, Assistant Special Agent in Charge, Librarian, etc.), Phone Number, FAX Number, and Preferred email Address of the Submitting Individual (Note that subscriptions are mailed to titles, not names, in order to avoid subscription problems arising from future personnel changes);

* If available, the generic email address for the Submitting Laboratory or Office;

* If a generic email address is not available, one private email address for an individual who is likely to be a long-term employee, who has a stable email address, and who will be responsible for forwarding *Microgram* information to all of the other employees in the requestor’s Office (Note that only one email address per Office will be honored);

* If requesting hard copy mailings, the number of copies requested (two max), and justification.

Requests to be removed from the *Microgram* subscription list, or to change an existing subscription, should also be sent to the *Microgram* Editor. Such requests should included all of the pertinent standard contact information detailed above, and also should provide the email and/or hard mail address currently being utilized for the requestor’s subscription.

Note that, due to mailing delays and/or publication timeframes, subscription requests/changes may take as long as 90 days to implement.
Email Notifications (Additional Comments)
As noted above, electronic subscriptions are email based. The email provides a notification of the Microgram URL when a new issue is posted, and additional information as appropriate. Note that Microgram notices will NEVER include any attachments, or any hyperlink other than the Microgram URL. This is important, because the microgram_editor@mailsnare.net address is routinely hijacked and used to send spam, very commonly including malicious attachments. For this reason, all subscribers are urged to have current Anti-Viral, Anti-Spyware, and Firewall programs in operation.

Costs
Subscriptions to Microgram are free.

Submissions to Microgram Journal
Manuscripts are accepted both from within and outside of DEA, and reviewers for the Journal are both internal (from within DEA) and external.

All submissions must be in English. All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: microgram_editor@mailsnare.net Current versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3 ½ inch IBM® PC-compatible diskette, 100 or 250 MB Iomega® zip diskette, or an IBM® PC-compatible CD. Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: “Warning -Contains Electronic Media - Do Not Irradiate”. Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 1/2" x 11" or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. The pages should be numbered, but not stapled together.

Note that mailed submissions may be subject to lengthy handling delays beyond the control of the Office of Forensic Sciences, and electronic media sent through the mail may be destroyed en route by sanitizing procedures, despite protective measures and written warnings. All submissions should include the following Contact Information: The Full Name and Address of Submitting Laboratory or Office, and the Full Name, Phone Number, FAX Number, and Preferred email Address of the Submitting Individual.

Scientific Research Articles are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.) Technical Notes are shorter communications concentrating on a specific drug (or drug class), unusual case, novel or unusual procedure or method, or minor original research. Each article/note should be a “stand-alone” work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to full peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is received by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes may follow an abbreviated version as appropriate):
**Cover Letter** - Provide the standard contact information and pertinent correspondence (if any) for the Editor.

**Title** - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

**Author(s)/Affiliation(s)** - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author’s degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page.

**Abstract** - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under “Titles”. Note that the abstract will be provided to *Chemical Abstracts*.

**Keyword List** - A minimum of five (maximum ten) abstracting keywords should be included.

**Introduction** - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid “Personal Communications”).

**Experimental** (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, **USE CAUTION IN DETAILING SYNTHESSES OF CONTROLLED OR ABUSED SUBSTANCES**, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: “Experimental details on this synthesis are not provided, in accordance with *Journal* policy.”]

**Results and Discussion** - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

**Conclusions** - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or a summary paragraph in the Results and Discussion section.

**Acknowledgments** - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

**References** - Articles and notes should have all textual citations collected in an endnotes list. Within the text, references should be consecutively numbered with superscripted Arabic numerals, or with Arabic numerals in parentheses, in accordance with their first appearance. Within the endnotes list, references
should be consecutively numbered with Arabic numerals, as follows: Number, Period, Indent, Citation. Reference format should adhere to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Note: This is the same reference format utilized in the Selected Reference Citations in Microgram Bulletin, and also (among many others) by the Journal of Forensic Sciences). Due to their inherently transitory nature, use of website URL’s as references are discouraged but permitted. As previously noted, Personal Communications should not be utilized; however, if unavoidable, utilize the following format: Full Name, Title, Affiliation (Laboratory or Office), Location (City and State, plus Nation if not the United States), Personal Communication, Year.

**Table and Figures** - All Tables and Figures should be appended onto the end of the article (not embedded in the text). Tables and Figures should be consecutively numbered with Arabic numerals, in accordance with their first citation in the text. Each Table and Figure should be “stand-alone”; that is, include sufficient descriptive information such that the reader will not have to refer back to the text to understand the Table or Figure. The Header should include the Table or Figure number and a concise title. Explanatory material, definitions of acronyms and/or abbreviations, and/or references within the Table or Figure should be designated by superscripted, lower case letters in alphabetical order, and included in dedicated footnotes at the bottom of the respective Table or Figure. Unless color is needed to enhance differentiation of the depicted material, all Tables and Figures should be in black and white (that is, avoid frivolous use of color for “artistic” purposes). Figures of spectra, chromatograms, charts, graphs, etc., should have clear and legibly labeled axes, but should not include instrument generated printoffs of experimental parameter lists.

Manuscripts submitted to Microgram Journal are required to be finished, professional quality efforts. Authors should ensure clarity, brevity, and pertinence of all information. Attention to detail in formatting, syntax, grammar, and spelling are as important as the accuracy of the facts presented. Authors are specially cautioned to conduct careful literature reviews prior to submission. At the Editor’s discretion, clearly substandard and/or inappropriate manuscripts will be returned to the authors without review.

**Manuscripts will not be retyped, but “final” versions are subject to minor to moderate Editorial rewrite to improve presentation clarity or to reformat to current Microgram Journal style.**

**Dual publication** - Re-publication of articles or notes of particular interest to the Microgram Journal readership will be considered if the article was originally published in a journal that is not easily accessed and the primary author has obtained explicit, written copyright exclusion from the original publisher and consent from all co-authors. Examples include exact English translations of articles or notes originally published in a non-English language journal, non-sensitive articles or notes originally published in a restricted journal or on a password protected website, or articles or notes originally published in limited distribution newsletters or proceedings. In general, any article or note that was published in English in a mainstream journal is not a candidate for re-publication in Microgram Journal. Authors interested in re-publishing previously published articles or notes in Microgram Journal should discuss the issue with the Microgram Editor before submitting.

Note that (in accordance with standard ethical guidelines) re-published articles should not be included as “new” articles in the respective author(s)’ Curriculum Vitae.

**Costs** - There are no costs (to the contributor) associated with publication in Microgram Journal.

**Reprints** - Microgram Journal does not provide reprints to authors. Microgram Journal may be photocopied as needed.

Questions may be directed to the Microgram Editor.
Microgram

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC  20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 3
Numbers 3-4
July - December 2005

Posted On-Line At:
Contents

Analysis and Characterization of Designer Tryptamines using Electrospray Ionization Mass Spectrometry (ESI-MS)
Sandra E. Rodriguez-Cruz

Assessment of the Volatility (Smokeability) of Cocaine Base Containing 50 Percent Mannitol: Is it a Smokeable Form of “Crack” Cocaine?
John F. Casale

Levamisole: An Analytical Profile
Ann Marie M. Valentino and Ken Fuentecilla

Rapid Chiral Separation of Dextro- and Levo- Methorphan using Capillary Electrophoresis with Dynamically Coated Capillaries
Ira S. Lurie and Kimberly A. Cox

Reduction of Phenylephrine with Hydriodic Acid/Red Phosphorus or Iodine/Red Phosphorus: 3-Hydroxy-N-methylphenethylamine
Lisa M. Kitlinski, Amy L. Harman, Michael M. Brousseau, and Harry F. Skinner

Synthesis of trans-4-Methylaminorex from Norephedrine and Potassium Cyanate
Walter R. Rodriguez and Russell A. Allred

Identification of a New Amphetamine Type Stimulant: 3,4-Methylenedioxy-(2-hydroxyethyl)amphetamine (MDHOET)
Carola Koper*, Elisa Ali-Tolppa, Joseph S. Bozenko Jr., Valérie Dufey, Michael Puetz, Céline Weyermann, Frantisek Zrcek

Analysis and Characterization of Psilocybin and Psilocin Using Liquid Chromatography - Electrospray Ionization Mass Spectrometry (LC-ESI-MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID)
Sandra E. Rodriguez-Cruz

Specificity of the Duquenois-Levine and Cobalt Thiocyanate Tests Substituting Methylene Chloride or Butyl Chloride for Chloroform
Amanda J. Hanson

The Identification of 1-Dehydromethandrostenolone
Robert D. Blackledge

Note: In order to prevent automated theft of email addresses off the Internet postings of Microgram Journal, all email addresses reported in the Journal have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of 3,4-Methylenedioxymethamphetamine (MDMA) (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).
Analysis and Characterization of Designer Tryptamines using Electrospray Ionization Mass Spectrometry (ESI-MS)

Sandra E. Rodriguez-Cruz, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
[email: sandra.e.rodriguez-cruz -at- usdoj.gov]

[Presented in Part at the ASMS 17th Sanibel Conference on Mass Spectrometry - Mass Spectrometry in Forensic Science and Counterterrorism, Clearwater Beach, FL (January 28 - February 1, 2005).]

ABSTRACT: The analysis and characterization of 12 “designer” tryptamines by electrospray ionization mass spectrometry (ESI-MS) are presented. Molecular weights were confirmed based on the experimental observation of protonated and deprotonated pseudo-molecular ions in the positive and negative ion modes, respectively. Standard tandem mass spectrometry (MS²) experiments were also performed, and the results provided for the characterization of various fragmentation signatures, useful for the future analysis of currently unknown, similar compounds. The fragmentation spectra obtained from collision-induced dissociation (CID) experiments (35 eV) were also compiled as part of an in-house mass spectral library. Results from selected MS³ experiments are presented and their use in structural elucidation is discussed. For comparison, the gas chromatography/mass spectrometry (GC/MS) data for the tryptamines are also included and discussed.

KEYWORDS: Tryptamines, Analogues, Designer Drugs, Electrospray Ionization-Mass Spectrometry, ESI-MS, Pseudo-molecular Ion, Fragmentation, Tandem Mass Spectrometry, Collision-Induced Dissociation, GC/MS, Forensic Chemistry.

Introduction

“Designer drugs” are compounds with structures that are very similar to controlled substances, but that are not specifically controlled. Also known as “analogues,” most of these compounds have never been previously encountered or characterized, and so are not present in commercial spectral libraries; therefore, they can represent unusual challenges for forensic laboratories. Definitive identification of such drugs usually requires in-depth analysis using multiple and complementary techniques, including infrared spectroscopy (IR), gas chromatography/mass spectrometry (GC/MS), and nuclear magnetic resonance (NMR).

For decades, the use of electron ionization (EI) mass spectrometry as a detector for gas chromatography (GC) instruments has been a main step in the structure elucidation process used by analytical and synthetic chemists [1]. Electron ionization mass spectra of many thousands of compounds, normally collected under 70 eV of energy, are readily available from reference databases [2] and instrument manufacturers [3]. The capabilities of mass spectrometry, however, have greatly expanded with the more recent development of specialized ionization techniques like electrospray ionization (ESI) [4] and atmospheric pressure chemical ionization (APCI) [5], making possible the analysis of many polar and thermally labile compounds that were not amenable to GC/MS analyses. Through the ESI process, ions in solution are transported into the gas phase by a series of solvent evaporation and Coulomb explosion steps, preserving the original intact ions and introducing them into the
vacuum-housed mass analyzer without significant fragmentation. As a result, this process produces singly and/or multiply charged ions which, upon mass spectrometric analysis, provide direct molecular weight information - a critical step in identification.

In addition, the interface of an ESI source to an ion-trap mass spectrometer provides for not only molecular weight determinations, but also for tandem and MS² fragmentation analysis of intact gas-phase ions via collision-induced dissociation (CID) experiments using a target gas [5]. By performing these experiments under controlled conditions, further structural elucidation can be accomplished, and the additional spectra generated can be collected and stored as part of a laboratory-generated library.

Certain synthetic tryptamines produce hallucinogenic effects in humans [6,7]. These properties can be expected based on the structural similarities between these tryptamines and some naturally occurring hallucinogenic tryptamines such as psilocybin, psilocin, dimethyltryptamine, bufotenine, and ibogaine. By slightly modifying the structures of these latter substances, synthetic chemists have developed novel “designer” tryptamines with very similar, new, modified, unusual, and/or otherwise desirable psychedelic properties. Synthetic details for the preparation of some designer tryptamines have been available to the public for many years [8]. However, the scientific literature provides only very limited information regarding their analysis and characterization [9-13]. Most of the available literature focuses on the extraction of the naturally available tryptamines from their source [14], or the analysis and detection of their metabolites in animal biological fluids [15-18].

This paper presents the analysis of various designer tryptamines using ESI-MS. Figure 1 illustrates the core structure of a tryptamine-type molecule, while Table 1 lists the 12 compounds investigated in this work, along with details of their structure. Some of these compounds were recently acquired during a law enforcement investigation targeting their open sale on the internet. Initially, these compounds were identified at this laboratory using IR and NMR spectroscopy, GC, and GC/MS techniques. Analysis by ESI-MS provides complementary information that is valuable for the full characterization of these compounds. For most of the compounds, the generation of pseudo-molecular ions via ESI provides molecular weight information that is not available via GC/MS analysis. MS² fragmentation experiments further complement the structural information obtained from GC/MS, and allow the additional characterization of thermally labile compounds.

**Experimental**

Solutions for each of the analogues were prepared by dissolving the appropriate amount in methanol to obtain a final concentration of approximately 10 μg/mL. Solutions were introduced into the mass spectrometer using a ThermoFinnigan Surveyor autosampler. Sample injections (10 μL) were loaded into a methanol constant flow (200 μL/min) provided by a ThermoFinnigan Surveyor solvent pump.

Mass spectra were collected using a ThermoFinnigan LCQ Advantage MAX ion-trap mass spectrometer equipped with an ESI source and operated using the Xcalibur software (Version 1.4) provided by the manufacturer. The ESI voltage needle was kept at 5.0 kV, generating a spray current of approximately 0.3 μA. The sheath and auxiliary sweep gas flows (nitrogen) were operated at 40 and 10 units, respectively. Conditions inside the source were as follows: Capillary temperature 300 °C; capillary voltage ±30 V; and tube lens voltage ±15 V. Negative and positive mass spectra were collected in the centroid mode for the m/z range of 50 to 550. Each scan collected was composed of three microscans using a maximum ion injection time of 50 milliseconds. During ion storage, the trap was operated with the automatic gain control (AGC) set point at 5 x 10⁶ ions and Helium (99.999% purity) was used as the trapping gas. After sequential ejection from the trap, ions were detected using a conversion dynode (±14.7 kV) and electron multiplier (-750 V) assembly. The pressure within the mass analyzer region was kept at 6.7 x 10⁻⁶ Torr by using a turbomolecular pump. MS² experiments were performed by isolating the desired precursor ions using an isolation window of 3.0 m/z units. The isolated ions were then subjected to normalized collision energies between 25 and 35 eV (%) in order to generate characteristic fragmentation.
Helium (99.999% purity) was used as the collision gas, and ions were activated during 30 milliseconds using an activation q value of 0.25.

Experimental data were analyzed using the qualitative analysis program provided within the Xcalibur software suite. In addition, all MS² fragmentation data were incorporated into an in-house spectral library.

For the GC/MS data (displayed in the Appendices), solutions of the 12 analogues were prepared in methanol at a concentration of 1 mg/mL. Samples were introduced into the gas chromatograph using 1.0 µL injections. The GC oven program used was: initial temperature: 90 °C (1 minute hold), ramp to 300 °C (20 °C/minute); final temperature: 300 °C (5 minute hold). Helium was used as the carrier gas at a constant flow of 1 mL/minute. Mass spectra were obtained using a ThermoFinnigan PolarisQ ion-trap mass spectrometer controlled by the Xcalibur software.

Results and Discussion

ESI-MS Experiments

Figures 2a and 2b present the positive and negative ion mode full-scan electrospray mass spectra obtained for 4-acetoxy-N,N-diisopropyltryptamine (9). The singly protonated and singly deprotonated pseudo-molecular ions are clearly represented at m/z 303 and 301, respectively, indicating a molecular weight of 302 for this compound. Similar spectra were obtained for the other 11 analogues. The molecular weights determined from all of these experiments are included in Table 1. Under the experimental conditions utilized, the signal intensity for the negative ion spectra was observed to be somewhat dependent on the structure of the compounds, specifically the presence of methoxy, acetoxy, or hydroxy groups. Molecules containing such functionalities were observed to produce more intense deprotonated pseudo-molecular ions.

Full-scan electrospray analysis was especially useful for the identification and differentiation of 4-hydroxy-N,N-diisopropyltryptamine (7) and 4-acetoxy-N,N-diisopropyltryptamine (9). The latter compound is thermally unstable, and standard GC/MS analyses gave highly similar spectra (see Appendices). However, ESI-MS provided accurate molecular weight information, allowing an easy identification.

ESI-MS² Experiments

In addition to collecting full-scan ESI spectra for these molecules, ESI-MS/MS (ESI-MS²) fragmentation experiments were also performed in order to obtain structural information that would complement the information previously obtained via GC/MS experiments. ESI-MS² fragmentation experiments were performed in the positive ion mode at various normalized collision energies. The spectra generated at 35 eV were considered to be the most useful, and so were exported and compiled into an in-house library for future use in the identification of unknowns.

The Appendices contain the ESI-MS² spectra obtained for each of the tryptamines investigated using a normalized collision energy of 35 eV (top panels). After isolation of the singly protonated pseudo-molecular or parent species, the ions were subjected to collisions with the target gas Helium, producing fragments that partially characterize the original molecule. For all the tryptamines investigated, major fragments observed correspond to two types of dissociations, both due to cleavages on the aliphatic side chain of the molecule. The first cleavage is between the nitrogen and the alpha carbon, with the second cleavage occurring between the alpha and beta carbons (see Figure 1). The former process results in the production of ammonia or a neutral primary or secondary amine (depending on the amine substituents), with the charge being transferred to the indole-containing fragment. The latter fragmentation process produces a charged amine, along with a neutral indole-containing fragment. Both of these fragmentation processes are illustrated in Figure 3 for the case of 5-methoxy-N,N-methyisopropyltryptamine (11).
For compounds with the same molecular weights, the fragmentation patterns obtained from the ESI-MS² experiments were useful for elucidating structures. For example, 4-acetoxy-N,N-methylisopropyltryptamine (8) and 5-methoxy-N,N-diisopropyltryptamine (10) have the same molecular weight of 274. In both cases, the protonated and deprotonated pseudo-molecular ions are observed at m/z 275 and 273, respectively, not allowing for their distinction. However, ESI-MS² analysis produced different fragmentation patterns for these compounds (see Appendices 8 and 10). For 4-acetoxy-N,N-methylisopropyltryptamine (8), the major fragment is observed at m/z 202, with additional fragments at m/z 160 and 86. Whereas for 5-methoxy-N,N-diisopropyl-tryptamine (10), the major fragment is observed at m/z 114, with additional fragments at m/z 102 and 174.

The usefulness of ESI-MS² analyses can also be illustrated by again comparing 4-hydroxy-N,N-diisopropyltryptamine (7) and 4-acetoxy-N,N-diisopropyltryptamine (9). As mentioned before, these compounds are virtually undistinguishable by GC/MS analysis, due to the thermal instability of the 4-acetoxy group. In addition to providing direct molecular weight information, ESI-MS² analysis produces distinctive fragments that allow their specific characterization. For 4-acetoxy-N,N-diisopropyltryptamine (9), fragments are produced at m/z 202, 160, 114, and 102. Whereas for 4-hydroxy-N,N-diisopropyltryptamine (7), fragments are produced at m/z 160, 114, and 102. While the fragment at m/z 202 is the predominant fragment produced from the former compound, it is absent from the latter, providing a marker ion for differentiation.

**GC/MS Experiments**

The Appendices also include the standard EI spectra obtained using a GC/MS system (bottom panels). Not surprisingly, most of the spectra are characterized by the presence of one major peak (base peak) corresponding to fragmentation of the sigma bond between the alpha and beta carbons. For 10 of the compounds (tryptamines 2 - 11), alpha cleavage [19] results in retention of the charge by the amine group. For alpha-methyltryptamine (1) and 5-methoxy-alpha-methyltryptamine (12), the most favorable fragmentation, inductive cleavage [19], results in charge migration to the indole-containing fragment, producing peaks at m/z 130 and 160, respectively. An additional hydrogen rearrangement process is also involved, resulting in major peaks at m/z 131 and 161, respectively. GC/MS experiments also produce characteristic signatures for the different amine substituents. For example, m/z 86 is characteristic of the N,N-methyleisopropyl and N,N-diethyl groups, while m/z 114 is characteristic of the N,N-dipropyl or N,N-diisopropyl functionalities. For these two latter isomeric species, the additional presence of ions at m/z 72 and 86, respectively, provides a useful distinguishing factor.

Additional bond cleavages in the tryptamine molecule result in the generation of specific fragmentation signatures. These can be of great use when interpreting data generated by either GC/MS or ESI-MS² techniques. For example, the presence of an ion at m/z 144 is indicative of a non-substituted indole moiety, after cleavage of the sigma bond between the amine nitrogen and the alpha carbon. The same type of cleavage leads to the generation of peaks at m/z 174 and 202 for compounds where the indole contains a methoxy or acetoxy functionality, respectively. As previously mentioned, cleavage between the alpha and beta carbons produces a high-intensity peak at m/z 86, due to the generation of the C₄H₉⁺N=CH₂ fragment, which can be produced from the diethylamine or methylisopropylamine group. Through the same process, the generation of m/z 114 is indicative of the C₅H₉⁺N=CH₂ fragment, consistent with either dipropylamine or diisopropylamine. For dimethyltryptamine compounds, the analogous peak will appear at m/z 58 due to C₃H₇⁺N=CH₂. As observed for alpha-methyltryptamine (1) and 5-methoxy-alpha-methyltryptamine (12), cleavage between the alpha and beta carbons also produces signature ions at m/z 130 and 160, characteristic of an unsubstituted and a methoxy-substituted indole, respectively.

**ESI-MS² Experiments**

The use of ESI combined with a quadrupole ion trap as the mass analyzer provides the enhanced capabilities of MS² experiments. To illustrate the usefulness of this type of analysis, ESI-MS² experiments were performed on N,N-dipropyltryptamine (5) and N,N-diisopropyltryptamine (6). As illustrated in the Appendices, the fragments...
generated during MS\(^2\) analysis do not allow for the unambiguous differentiation of these two compounds, although the presence of \(m/z\) 86 (5% intensity) for \(N,N\)-dipropyltryptamine (5) does suggest that they are in fact different. By isolation and fragmentation of the singly protonated pseudo-molecular ions at \(m/z\) 245 using a collision energy of 25 eV (%), major ions at \(m/z\) 144 and 114 are generated for both compounds. However, further isolation and fragmentation of the \(m/z\) 114 ion, using a collision energy of 30 eV, leads to significantly different MS\(^3\) spectra (see Figures 4 and 5). Fragmentation of the \(m/z\) 114 ion produces a fragment at \(m/z\) 86 for \(N,N\)-dipropyltryptamine and a fragment at \(m/z\) 72 for \(N,N\)-diisopropyltryptamine. These ions correspond to the loss of the neutral fragments \(\text{CH}_2=\text{CH}_3\) and \(\text{CH}_2=\text{CHCH}_3\), respectively. These losses are characteristics of the dipropyl and diisopropyl groups, respectively, providing a tool for differentiation. In fact, further fragmentation (MS\(^4\)) of the \(m/z\) 86 ion, produced from the fragmentation of \(N,N\)-dipropyltryptamine, generates a peak at \(m/z\) 58, consistent with the loss of a second \(\text{CH}_2=\text{CH}_2\) molecule from the remaining dipropyl chain (data not shown). Similar MS\(^4\) analysis of the \(m/z\) 72 ion from \(N,N\)-diisopropyltryptamine would produce additional information regarding the second diisopropyl group; however, the additional loss of 42 would produce an ion at \(m/z\) 30, which is below the observable low-mass limit of 50 for this instrument. These ESI-MS\(^3\) results are in agreement with the GC/MS data obtained for \(N,N\)-dipropyltryptamine and \(N,N\)-diisopropyltryptamine, where the presence of \(m/z\) 86 and 72 is a basis for differentiation.

The complementary value of the GC/MS and ESI-MS data can be better illustrated using \(\alpha\)-methyltryptamine (1). The GC/MS spectrum does not provide molecular weight information, but it does indicate the presence of a non-substituted indole (\(m/z\) 130), with the presence of a phenyl group further confirmed by \(m/z\) 77. Although the ion at \(m/z\) 44 is indicative of \(\text{C}_6\text{H}_5\text{N}\), no confirmation of the \(-\text{NH}_2\) group is obtained. By direct observation of the pseudo-molecular ion, the ESI-MS data provide a direct determination of molecular weight at 174. The ESI-MS\(^2\) spectrum, while simple and only containing one major ion at \(m/z\) 158, provides a signature indicating the loss of ammonia from the protonated intact molecule, confirming the presence of a \(-\text{NH}_2\) substituent.

Conclusions

The analysis of 12 tryptamine analogues using ESI-MS has been presented. Full scan analysis in the positive and negative ionization modes allowed the observation of singly protonated and singly deprotonated ions, providing molecular weight information. MS\(^2\) experiments allowed the fragmentation of pseudo-molecular ions to be investigated under controlled experimental conditions, providing structural information for each one of the compounds, and making possible the observation of fragmentation signatures. Particularly useful was the generation of ESI full-scan and MS\(^2\) spectra for the thermally-labile compound 4-acetoxy-\(N,N\)-diisopropyltryptamine, which provided a distinction from 4-hydroxy-\(N,N\)-diisopropyltryptamine. MS\(^3\) experiments were also performed and results provided an additional technique for the differentiation of compounds with the same molecular weight and similar MS\(^2\) spectra. The results presented are in agreement with those obtained using standard GC/MS techniques, and show the utility of ESI-MS as a complementary analytical technique that can be used in conjunction with GC/MS, NMR, and IR spectroscopy in the structural characterization of tryptamines.

Acknowledgements

The author is grateful to DEA Southwest Laboratory Administrative Assistant D. A. Perez, and also to DEA Southwest Laboratory Forensic Chemists J. M. Katz, N. C. Payne, and H. F. Skinner, for useful discussions and review of the manuscript.

References


****Law Enforcement Restricted.

---


---

**Figure 1. Core Structure of the Tryptamines**

**Table 1. Designer Tryptamines Investigated (Refer to Figure 1 for Substituent Positions).**

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>alpha-Methyltryptamine</td>
<td>H</td>
<td>H</td>
<td>-Me</td>
<td>H</td>
<td>H</td>
<td>174</td>
</tr>
<tr>
<td>2</td>
<td>N,N-Dimethyltryptamine</td>
<td>-Me</td>
<td>-Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>188</td>
</tr>
<tr>
<td>3</td>
<td>N,N-Diethyltryptamine</td>
<td>-Et</td>
<td>-Et</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>216</td>
</tr>
<tr>
<td>4</td>
<td>N,N-Methylisopropyltryptamine</td>
<td>-Me</td>
<td>-iPr</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>216</td>
</tr>
<tr>
<td>5</td>
<td>N,N-Dipropyltryptamine</td>
<td>-Pr</td>
<td>-Pr</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>244</td>
</tr>
<tr>
<td>6</td>
<td>N,N-Diisopropyltryptamine</td>
<td>-iPr</td>
<td>-iPr</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>244</td>
</tr>
<tr>
<td>7</td>
<td>4-Hydroxy-N,N-diisopropyltryptamine</td>
<td>-iPr</td>
<td>-iPr</td>
<td>H</td>
<td>-OH</td>
<td>H</td>
<td>260</td>
</tr>
<tr>
<td>8</td>
<td>4-Acetoxy-N,N-methylisopropyltryptamine</td>
<td>-Me</td>
<td>-iPr</td>
<td>H</td>
<td>-OAc</td>
<td>H</td>
<td>274</td>
</tr>
<tr>
<td>9</td>
<td>4-Acetoxy-N,N-diisopropyltryptamine</td>
<td>-iPr</td>
<td>-iPr</td>
<td>H</td>
<td>-OAc</td>
<td>H</td>
<td>302</td>
</tr>
<tr>
<td>10</td>
<td>5-Methoxy-N,N-diisopropyltryptamine</td>
<td>-iPr</td>
<td>-iPr</td>
<td>H</td>
<td>H</td>
<td>-OMe</td>
<td>274</td>
</tr>
<tr>
<td>11</td>
<td>5-Methoxy-N,N-methylisopropyltryptamine</td>
<td>-Me</td>
<td>-iPr</td>
<td>H</td>
<td>H</td>
<td>-OMe</td>
<td>246</td>
</tr>
<tr>
<td>12</td>
<td>5-Methoxy-alpha-methyltryptamine</td>
<td>H</td>
<td>H</td>
<td>-Me</td>
<td>H</td>
<td>-OMe</td>
<td>204</td>
</tr>
</tbody>
</table>
Figure 2. (a) Positive and (B) Negative Ion Mode Electrospray Ionization Mass Spectra for 4-Acetoxy-\(N,N\)-diisopropyltryptamine (6).
Figure 3. Chemical Diagram Illustrating the Two Main Fragmentation Processes Observed During the MS² Analysis of Tryptamines.
Figure 4. Full Mass, MS$^2$ and MS$^3$ Spectra Obtained for N,N-Dipropyltryptamine (5).
Figure 5. Full Mass, MS² and MS³ Spectra Obtained for N,N-Diisopropyltryptamine (6).
Appendix 1. \textit{alpha}-Methyltryptamine: ESI-MS\textsuperscript{2} (top) and GC/MS (bottom).
Appendix 2. *N,N*-Dimethyltryptamine: ESI-MS (top) and GC/MS (bottom).
Appendix 3. *N,N*-Diethyltryptamine: ESI-MS\(^2\) (top) and GC/MS (bottom).
Appendix 4. *N,N*-Methylisopropyltryptamine: ESI-MS² (top) and GC/MS (bottom).
Appendix 5. *N,N*-Dipropyltryptamine: ESI-MS$^2$ (top) and GC/MS (bottom).
Appendix 6. \(N,N\)-Diisopropyltryptamine: ESI-MS\(^2\) (top) and GC/MS (bottom).
Appendix 7. 4-Hydroxy-N,N-diisopropyltryptamine: ESI-MS\(^2\) (top) and GC/MS (bottom).
Appendix 8. 4-Acetoxy-N,N-methylisopropyltryptamine: ESI-MS (top) and GC/MS (bottom)
Appendix 9. 4-Acetoxy-\(N,N\)-diisopropyltryptamine: ESI-MS\(^2\) (top) and GC/MS (bottom).
Appendix 10. 5-Methoxy-N,N-diisopropyltryptamine: ESI-MS (top) and GC/MS (bottom).
Appendix 11. 5-Methoxy-N,N-methylisopropyltryptamine: ESI-MS (top) and GC/MS (bottom).
Appendix 12. 5-Methoxy-\(\alpha\)-methyltryptamine: ESI-MS\(^2\) (top) and GC/MS (bottom).
Technical Note

Assessment of the Volatility (Smokeability) of Cocaine Base Containing 50 Percent Mannitol: Is it a Smokeable Form of “Crack” Cocaine?

John F. Casale
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email address withheld at author’s request]

ABSTRACT: A defendant convicted of possession and use of “crack” cocaine claimed on appeal that cocaine base containing 44 percent mannitol was not a “smokeable form,” and therefore that Federal Sentencing Guidelines for “crack” cocaine were not applicable in his sentencing. To investigate this claim, a sample of “crack” cocaine was made by mixing molten illicit cocaine base with an equal weight of mannitol, then cooled to form a solid “rock” that was visually consistent with typical exhibits of “crack” cocaine. A sample of this formulation was heated in a device similar to a crack pipe, and the resulting vapors were collected by dissolution into chloroform. Analysis of the resulting solution by GC/MS identified cocaine, thereby confirming that “crack” formulated from equal parts cocaine base and mannitol is smokeable.

KEYWORDS: Cocaine, Mannitol, Crack, Gas Chromatography/Mass Spectrometry, Forensic Chemistry.

Introduction

“Crack” cocaine has been a major drug of abuse since the early 1980’s. The term “crack” is the most common street name used for cocaine base that is smoked. It can be in a rock-like, powder, or oil-like form, and can also be adulterated or diluted (“cut”) with a virtually unlimited number of substances; some of the most common include benzocaine, procaine, mannitol, acetaminophen, aspirin, and phenacetin. “Crack” is not a scientific term; however, for sentencing purposes, “crack” has been defined as cocaine base.

Because of the violence associated with trafficking and sale of “crack” cocaine, and its unusually high potential for addiction, the U.S. Congress mandated more punitive sentences for cocaine base versus cocaine hydrochloride. Some recent judicial rulings have decreed that cocaine base exhibits must be in a “smokeable form” in order to impose Federal Sentencing Guidelines for “crack” cocaine [1]. Recently, a defendant who had been convicted for possession and use of “crack” cocaine claimed on judicial appeal that cocaine base containing 44 percent mannitol (that is, the sample he had) was not a “smokeable form” of cocaine base. Herein, we report the results of experiments designed to determine if a “crack” cocaine sample containing 50 percent mannitol is a “smokeable form” of cocaine base.

Experimental

Materials
Illicitly prepared cocaine base (m.p. 84 - 88 °C) was obtained from the reference collection of this laboratory. Pharmaceutical cocaine base and mannitol were obtained from Merck Chemical (Rahway, NJ) and
Sigma-Aldrich Chemical (Milwaukee, WI), respectively. Chloroform was a distilled-in-glass product of Burdick and Jackson Labs (Muskegon, MI).

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The MSD was operated in the EI mode with an ionization potential of 70 eV, a scan range of 34-700 amu, and 1.34 scans/sec. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μm) (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100 °C; initial hold, 0.0 min; program rate, 6 °C/min; final temperature, 300 °C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and a temperature of 280 °C. The auxiliary transfer line to the MSD was also operated at 280 °C.

Formation of “Crack” Cocaine Containing 50 Percent Mannitol
Illicit cocaine base (1.0 gram) was placed into a 15 mL beaker and heated on a laboratory hotplate until it melted into an oil. Mannitol (1.0 gram) was added and stirred for about 1 minute, resulting in a uniform oil. Upon cooling to room temperature, the oil solidified, after which it was broken into small, off-white to yellowish “rocks” that were visually consistent with typical exhibits of “crack” cocaine (m.p. 86 - 93 °C).

Vaporization of Crack Cocaine Containing 50 Percent Mannitol
The melting points of pharmaceutical grade cocaine base and cocaine hydrochloride are 98 °C and 195 °C, respectively [2]. Illicitly prepared cocaine base and cocaine hydrochloride have lower melting points since they contain cocaine-related impurities resulting from the crude illicit processing methodologies [3], and illicit cocaine base containing 50 percent mannitol would be expected to have an even lower melting point (in this case, however, it was actually several degrees higher than the illicit cocaine base used to prepare it).

A device similar to a “crack” pipe was fashioned from a 9-inch Pasteur pipette by inserting a tight plug of glass wool into the larger opening. The pipette was loaded with a small “rock” of the formulated “crack” (approximately 20 mg), and then fitted into a 125 mL suction filtration flask containing 5 mL of chloroform. A slight vacuum was applied to the flask and the section of the pipette containing the “rock” was gently heated with a propane flame. The “rock” vaporized within seconds (with some charring), and the produced vapors were drawn by the vacuum into the chloroform. The resulting solution was analyzed via GC/MS to determine what substances were trapped from the vapors. The chloroform was subsequently evaporated to dryness under a stream of nitrogen to provide 9 milligrams of solid material, which was also analyzed by GC/MS.

Results and Discussion
“Crack” cocaine containing 50 percent mannitol was easily prepared. Upon gentle heating with a propane flame, the mixture melted and boiled within a few seconds, giving white-colored vapors. Analysis of the chloroform solution of these vapors by GC/MS confirmed primarily cocaine and a small amount of methylecgonidine (Figures 1 – 3); mannitol was not detected. Methylecgonidine is a well-known byproduct of cocaine degradation due to heat, and has been previously documented [4]. The mass spectrum of the recovered cocaine was identical to the pharmaceutical standard. Quantitative analysis of the solid resulting from evaporation of chloroform solution confirmed that approximately 90 percent of the cocaine base present in the original “rock” was delivered into the chloroform.

Conclusions
“Crack” cocaine containing 50 percent mannitol is a “smokeable form” of cocaine base. Although beyond the scope of this study, similar results may be reasonably expected from “crack” cocaine made from any other common adulterant or diluent, regardless of their relative proportions.
References


---

Figure 1. Total ion chromatogram of chloroform soluble vapors from heating crack cocaine containing 50 percent mannitol. Peak identification: Methylecgonidine (7.72 min) and Cocaine (21.32 min).
Figure 2. Electron Ionization Mass Spectrum of the Recovered Methylecgonidine.

Figure 3. Electron Ionization Mass Spectrum of the Recovered Cocaine.
Levamisole: An Analytical Profile

Ann Marie M. Valentino* and Ken Fuentecilla
U.S. Department of Justice
Drug Enforcement Administration
Northeast Laboratory
99 10th Avenue, Suite 721
New York, NY 10011
[email: ann.marie.m.valentino -at- usdoj.gov]

ABSTRACT: Levamisole, an antineoplastic cancer medication used in the treatment of colon cancer, has been identified in numerous submissions of illicit cocaine hydrochloride. Analytical methodologies and data (gas chromatography, capillary electrophoresis, infrared spectroscopy, mass spectroscopy, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: Levamisole, Cocaine, (l)-Tetramisole, Ergamisol, Ketrax, Solaskil, Forensic Chemistry

Figure 1: Structure of Levamisole

Introduction

Over approximately the past two years, this laboratory has received numerous cocaine submissions containing various amounts of levamisole, (S)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole [1,2]. Levamisole is the levor enantiomer of tetramisole, and is a synthetic imidazothiazole derivative that has been widely used in the treatment of worm infestations in both humans and animals. In 1990 the U.S. Food and Drug Administration approved the use of levamisole in combination drug therapy with another cancer drug, fluorouracil, for patients to treat some advanced cases of colon cancer [3]. Analytical data for levamisole is provided.

Experimental

Levamisole: C_{11}H_{12}N_{2}S; mw = 204.3 amu [2]

Source: Sigma-Aldrich, Inc. (St. Louis, MO); Lot #073K3602
Gas Chromatography

Instrument: Agilent 6890N with a flame ionization detector
Column: HP-5, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature: 270 °C
Oven Temperature: 215 °C for 5.5 min, 45 °C to 250 °C for 1.4 min
Carrier Gas: Helium ramped flow 2.7 mL/min for 5.5 min to 5 mL/min
Split Ratio: 75:1

The retention time for levamisole is 2.99 minutes under the above experimental parameters. The retention time relative to cocaine is 0.58.

Capillary Electrophoresis

Instrument: Agilent HP3D CE Capillary Electrophoresis System with a diode array detector
Column: Bare fused silica capillary, 50 μm ID, 40 cm LEF
Run Buffer: Microsolve DEA Custom Chiral, Phenethylamine and Propoxyphene Buffer containing 78.8 mg/mL 2-hydroxypropyl-β-cyclodextrin
Detector: 200 nm, reference 480 nm
Voltage: 20 kV
Cassette Temperature: 15 °C
Precondition: Flush 1.0 min 0.1 NaOH
Flush Sequence: 1.0 min Water; 1.0 min Microsolve CElírix A; 2.0 min Microsolve DEA Custom Chiral, Phenethylamine and Propoxyphene Buffer containing 78.8 mg/mL 2-hydroxypropyl-β-cyclodextrin
Injection Parameters: Pressure 35.0 mbar, 2.0 sec sample vial
Injection Solvent: Pressure 35.0 mbar 1.0 sec water

Note: The above instrumental parameters enables resolution of dextro- and levo- enantiomers of tetramisole [4].

Infrared Spectroscopy

Instrument: Thermo-Nicolet Nexus 670
Number of Scans: 16
Resolution: 4.000 cm⁻¹
Wavenumber Range: 4000 cm⁻¹ to 650 cm⁻¹

Data was obtained by the use of an attenuated total reflectance (ATR) attachment on FTIR [Figure 2]. The data was not corrected.

Mass Spectrometry

Instrument: Agilent 5973
Column: HP-5 MS, 30 m x 0.25 mm x 0.25 μm film thickness
Injector Temperature: 255 °C
Oven Temperature: 90 °C for 1.35 min, 35 °C/min to 290 °C
Carrier Gas: Helium with a 35:1 split ratio
Scan Range: 40 - 550 amu

The electron impact mass spectrum is presented in Figure 3.

Nuclear Magnetic Resonance Spectroscopy

Data was obtained using 1D proton Nuclear Magnetic Resonance on a Varian Mercury 400 MHz NMR. The sample was prepared at 25.2 mg/mL in deuterated methanol (CD3OD) containing TMS (tetramethylsilane) as the reference at 0 ppm. The proton spectrum of the standard was obtained with 8 scans using a 1.0 second delay, 45
degree pulse, and a 2.99 second acquisition time. Data from sweep width of 6410 Hz was stored in 32K data points [Figure 4].

Results and Discussion

The presence of pharmacologically active adulterants and inactive diluents found in illicit cocaine seizures is common. Many of these adulterants cause pulmonary and systemic reactions, and therefore may contribute to the toxicity of the cocaine. However, after a brief internet inquiry concerning adulterating illicit cocaine with levamisole, it is unclear as to why this relatively expensive compound is being used.

References


Figure 2: Uncorrected FTIR-ATR Spectrum of Levamisole Hydrochloride.
**Figure 3:** Electron Impact Mass Spectrum of Levamisole.

**Figure 4:** 400 MHz Proton NMR Spectrum of Levamisole Hydrochloride in CD$_3$OD.
Rapid Chiral Separation of Dextro- and Levo- Methorphan using Capillary Electrophoresis with Dynamically Coated Capillaries

Ira S. Lurie* and Kimberly A. Cox
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email: ira.s.lurie -at- usdoj.gov]

ABSTRACT: The chiral differentiation of the Dextro- and Levo- Methorphan is obtained in under 4 minutes with excellent peak shapes using capillary electrophoresis with dynamically coated capillaries. Dynamic coating of the capillary surface is accomplished by rapid flushes of 0.1 N sodium hydroxide, water, a buffer containing a polycation coating reagent, and a reagent containing methanol and a polyanionic coating reagent containing hydroxypropyl-beta-cyclodextrin.

KEYWORDS: Dextromethorphan, Levomethorphan, Chiral Analysis, Capillary Electrophoresis, Dynamically Coated Capillaries, Forensic Chemistry

Introduction

Dextromethorphan is an antitussive agent commonly found in Over-the-Counter (OTC) cough and cold pharmaceuticals (and more recently in Ecstasy (MDMA) mimic or combination tablets). Levomethorphan is a narcotic analgesic that is not commercially available, and therefore is not commonly submitted to forensic laboratories. Nonetheless, the differentiation and identification of these enantiomers is important in the United States, since Dextromethorphan is not controlled while Levomethorphan is a Schedule II controlled substance.

However, the differentiation of Dextro- and Levo- Methorphan is challenging. Methorphan is a tertiary amine which is not amenable to derivatization; therefore, the use of chiral derivatizing reagents (to form diastereomers for analysis on an achiral gas chromatography (GC) or high performance liquid chromatography (HPLC) column) is not a viable approach. Instead, relatively expensive chiral columns are required to resolve the two isomers using either GC or HPLC [1].

Capillary electrophoresis (CE) allows for the separation of enantiomers on conventional capillaries by utilizing run buffers containing chiral additives. Micellar electrokinetic chromatography (MEKC) [2], and electrokinetic chromatography (ECC) [3], both with single wavelength UV detection, and free zone capillary electrophoresis (CZE) with secondary equilibria and PDA-UV detection [4], have all been previously used to resolve the enantiomers of methorphan. However, run times in excess of 15 minutes were required.

For the separation of basic drugs at low pH, faster, more precise migration times and higher plate counts are obtained using dynamically coated versus uncoated capillaries. The use of a chiral additive (such as a cyclodextrin) imparts the additional selectivity needed for the analysis of enantiomers [5]. Using the procedure developed by Chevigne and Janssens [6], the capillary, after base hydrolysis, is sequentially coated with a polycation and then with a polyanion. The run buffer (with or without an added cyclodextrin) is the final coating
reagent. This process produces coated capillaries with a higher and more robust electroosmotic flow (EOF) at lower pH values versus uncoated capillaries. In addition, the coated capillary surface has more favorable kinetics. In the present study, the rapid chiral analysis of methorphan using a dynamically coated capillary approach is reported.

**Experimental**

**Chemicals**
Standards of Dextro- and Levo- Methorphan were obtained from the reference collection of this laboratory. Sodium hydroxide 0.1 N, CElixir A (pH 2.5), CElixir B (pH 2.5), and CElixir B (pH 2.5) with 0.95 % (w/v) hydroxypropyl-β-cyclodextrin, were all acquired from MicroSolv Technology (Long Branch, NJ). Hydroxypropyl-β-cyclodextrin (HP-β-CD) was obtained from Sigma (St. Louis, MO). HPLC-grade methanol was obtained from Burdick and Jackson (Muskegon, MI). Deionized and high purity water (that is, HPLC-grade water) was obtained from a Millipore Synergy 185 water system (Bedford, MA).

**Instrumentation and Procedures**
An Agilent Model HP1D CE Capillary Electrophoresis System fitted with a diode array detector (Waldbronn, Germany) was used for CE separations. New, bare silica capillaries were conditioned following the same procedure used for regular analyses. That is, the capillaries were first flushed with 0.1N sodium hydroxide for 1 minute, followed by water for 1 minute, then CElixir Reagent A for 1 minute, and finally the run buffer for 2 minutes. Either 2.0 mL CE glass vials or 1.0 mL polypropylene vials are used as reservoirs. For glass vials, waste vials were filled with 500 µL of water. Flush, run buffer, standard and sample vials were filled with 1000 µL of liquid (for the sodium hydroxide 0.1 N vial add 500 µL to a polypropylene vial). When polypropylene vials were used, waste vials were filled with 250 µL of water, while all others were filled with 500 µL of liquid.

**Standard and Sample Preparation**
The injection solvent consisted of 75 mM phosphate monobasic, adjusted to pH 2.6 with phosphoric acid, and diluted 1:20 with HPLC-grade water. Alternatively, injection solvent concentrate (MicroSolv) was diluted 1:20 with HPLC-grade water.

For standard solutions, an appropriate amount of standard Dextro- and Levo- Methorphan was weighed into an appropriate volumetric flask and diluted to volume with injection solvent, in order to obtain a final concentration of approximately 0.05 mg/mL of each component. These were sonicated for 15 minutes, then filtered. For sample solutions, an appropriate amount of powder was weighed into a volumetric flask and diluted to volume with injection solvent, in order to obtain a final concentration approximately equal to that of standard. These were also sonicated for 15 minutes, then filtered. All standard and sample solutions were filtered with 0.45 µm Nylon syringe filters (MicroSolv).

**Capillary Electrophoresis Conditions**
For the chiral separation either a 50 µm ID 32 cm (23.5 cm to the detector) fused silica capillary obtained from Polymicro Technologies (Phoenix, AZ) or a 50 µm ID 33 cm (24.5 cm to the detector) pre-made capillary (Agilent) were used, at 15 °C. The run buffer consisted of 15 % methanol and 85 % (CElixir Reagent B (pH 2.5) + 0.95 % HP-β-CD). For all CE runs a 50 mbar pressure injection of 2 second duration was used, followed by a 35 mbar pressure injection of water for 1 second. For electrophoresis, an initial 0.5 minute linear voltage ramp from 0 V to the final voltage of 20 kV was used.

**Results and Discussion**
Dahlen and Lenz used CZE with an uncoated capillary and with added HP-β-CD to resolve Dextro- and Levo- Methorphan in under 16 minutes [4]. In the present study, using the same run buffer but on a dynamically coated
capillary, identical results were obtained in under 4 minutes (see Figure 1). Highly precise separations were obtained, as demonstrated by excellent run-to-run migration time precision (% RSD = 0.1, n = 7). Because the peaks are so narrow, identification can be difficult based on migration time alone; however, co-injection of sample and either standard eliminates any ambiguities. Relative migration time data (relative to Dextromethorphan) of solutes commonly found with Dextromethorphan is given in Table 1. The non-controlled substances are included because they and Dextromethorphan are commonly combined in various OTC pharmaceuticals. The controlled substances are included since they and Dextromethorphan are occasionally identified in Ecstasy (MDMA) mimic and combination tablets.

If needed, n-butylamphetamine can be used as an internal standard in this method for the quantitation of Methorphan.

The present procedure is compatible with previously reported methodology for the CE analysis of a wide variety of seized drugs using the same capillary with dynamic coatings [5]. Classes of compounds that can be analyzed using this methodology (using higher CD concentrations than used in this study) include the phenethylamines and the methylenedioxyphenethylamines. Specific compounds that can be analyzed using this methodology include propoxyphene, cocaine, oxycodone, heroin, lysergic acid diethylamide (LSD), opium, psilocybe mushrooms, gamma-hydroxybutyrate (GHB), and gamma-butyrolactone (GBL).

References


5. Lurie IS, Hays PA, Parker K. Capillary electrophoresis analysis of a wide variety of seized drugs using the same capillary with dynamic coatings. Electrophoresis 2004;25:1580-1591. [Note: Substrates analyzed in the study include the enantiomers of norpseudoephedrine, pseudoephedrine, ephedrine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxymethamphetamine (MDEA), and propoxyphene].


[Table 1 and Figure 1 Follow.]
### Table 1. Relative Migration Times of Solutes Commonly Found with Dextromethorphan.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Relative Migration Time *</th>
</tr>
</thead>
<tbody>
<tr>
<td>d,l-Methamphetamine HCl</td>
<td>0.798</td>
</tr>
<tr>
<td>Phenylpropanolamine HCl</td>
<td>0.836</td>
</tr>
<tr>
<td>d,l-Pseudoephedrine HCl</td>
<td>0.841</td>
</tr>
<tr>
<td>MDMA HCl</td>
<td>0.850</td>
</tr>
<tr>
<td>d,l-Ephedrine HCl</td>
<td>0.851</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>0.874</td>
</tr>
<tr>
<td>n-Butylamphetamine HCl (internal standard)</td>
<td>0.919</td>
</tr>
<tr>
<td>Diphenhydramine HCl</td>
<td>0.973</td>
</tr>
<tr>
<td><strong>Dextromethorphan</strong></td>
<td><strong>1.000</strong></td>
</tr>
<tr>
<td>Levomethorphan</td>
<td>1.010</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>2.371</td>
</tr>
<tr>
<td>Guaifenesin</td>
<td>2.388</td>
</tr>
</tbody>
</table>

* Relative to Dextromethorphan. Note that the concentration of the cyclodextrin used in this study was insufficient to resolve the enantiomeric pairs of the listed phenethylamines and methylenedioxyphenethylamines; therefore, only one (the average) RMT is reported.

---

**Figure 1.** Electropherogram of a standard mixture of a) Dextromethorphan and b) Levomethorphan. A 33 cm (24.5 cm to the detector window) x 50 μm ID fused-silica dynamically coated capillary was used. Solute concentration of each enantiomer was approximately 0.05 mg/mL (CE conditions are described in the Experimental section).
Reduction of Phenylephrine with Hydriodic Acid/Red Phosphorus or Iodine/Red Phosphorus: 3-Hydroxy-N-methylphenethylamine

Lisa M. Kitlinski, Amy L. Harman, Michael M. Brousseau, and Harry F. Skinner*
U. S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
[email: harry.f.skinner -at- usdoj.gov]

ABSTRACT: In an effort to decrease illicit methamphetamine production within the United States, many pharmaceutical companies are now substituting phenylephrine for pseudoephedrine in many of their Over-the-Counter consumer products intended for treatment of the symptoms of the common cold, allergies, and related maladies. Because these products are the favored source of pseudoephedrine for illicit production of methamphetamine, and also because many clandestine laboratory operators are chemically naïve, it is expected that phenylephrine-containing products will occasionally be utilized in erroneous efforts to produce methamphetamine. Submission of phenylephrine to reduction conditions typically utilized in clandestine methamphetamine laboratories (hydriodic acid/red phosphorus or iodine/red phosphorus) produced 3-hydroxy-N-methylphenethylamine, commonly referred to as “Reduced Phenylephrine” or “Reduced PE.” Standard analytical data for “Reduced PE” are presented.

KEYWORDS: 3-Hydroxy-N-methylphenethylamine, Phenylephrine, Reduction, Methamphetamine, Pseudoephedrine, Reduced Phenylephrine, Reduced PE, Hydriodic Acid, Red Phosphorus, Iodine, Clandestine Laboratories, Forensic Chemistry

Introduction

Clandestine methamphetamine laboratories are epidemic in many areas of the United States. One of the primary synthetic methods that has been used for production of methamphetamine over the past 25 years is the reduction of ephedrine or pseudoephedrine with hydriodic acid/red phosphorus (HI/red P) [1]. In past efforts to combat illicit methamphetamine production, federal and state authorities restricted the sale of bulk ephedrine and pseudoephedrine, and closely monitored the sale and use of hydriodic acid and red phosphorus [2]. In response, clandestine laboratory operators began utilizing Over-the-Counter (OTC) consumer products containing ephedrine or pseudoephedrine, began generating hydriodic acid in situ with iodine and red phosphorus (I₂/red P) [3], and also turned to alternate syntheses, notably the lithium/ammonia reduction [4]. A number of states countered these initiatives with a variety of additional restrictions on the sale of ephedrine- and pseudoephedrine-containing OTC products, including purchase limits, access restrictions, and identification/signature requirements, and also further restricted sales of I₂ and red P. In turn, clandestine laboratory operators in those states resorted to so-called “road trips” to purchase ephedrine- and pseudoephedrine-containing OTC products, either traveling to states that had no restrictions, or purchasing the maximum allowable amounts at dozens or even hundreds of stores within states that had restrictions in place. In addition, as acquisition of I₂ and red P became increasingly problematic, clandestine laboratory operators turned to a variety of I₂- and red P-containing consumer products, and also began using other phosphorus compounds as substitutes for red P.

Most recently, the Combat Methamphetamine Epidemic Act (enacted in March 2006) placed federal restrictions on the sale of ephedrine-, pseudoephedrine-, and phenylpropanolamine-containing OTC products (again,
purchase limits, access restrictions, and identification/signature requirements) [5]. In total, federal and state imposed restrictions have dramatically reduced the number of methamphetamine laboratories in some states.

Initially, the pharmaceutical companies that produced ephedrine and pseudoephedrine-containing OTC products were strongly opposed to the imposition of restrictions on their sale. However, as they began to recognize the extent and increase of methamphetamine abuse, and the salient role that ephedrine- and pseudoephedrine-containing OTC products played in illicit methamphetamine manufacture, they began to create alternate formulations that contained phenylephrine (3-(1-hydroxy-2-methylaminoethyl)phenol, sometimes abbreviated as “PE”), as a substitute for ephedrine/pseudoephedrine. The structures of phenylephrine and ephedrine/pseudoephedrine are shown below.

![Phenylephrine and Ephedrine/Pseudoephedrine Structures](image)

Phenylephrine (mw = 167.1)  Ephedrine/Pseudoephedrine

Phenylephrine-containing products have been available for OTC sale in Europe for many years. The first of these reformulated products became available to consumers in the United States in January 2005, and has gained rapid acceptance among American consumers.

However, because many clandestine laboratory operators are chemically naïve, and do not understand that phenylephrine and ephedrine/pseudoephedrine are different compounds, it is expected that these new phenylephrine-containing OTC products will eventually be utilized in erroneous efforts to produce methamphetamine. The purpose of this study is to identify the products, byproducts, and intermediates formed during the reduction of phenylephrine with HI/red P or I2/red P.

**Experimental**

**Reactions**

Reagents were obtained from Aldrich Chemical Company. Ten grams of phenylephrine HCl, 3 grams of red phosphorus, and 30 mL of 57% hydriodic acid were refluxed at about 120 °C in a round-bottom flask fitted with a reflux condenser. Alternately, 10 grams of phenylephrine HCl, 20 grams of iodine, 3 grams of red phosphorus, and 18 mL of water were similarly refluxed. The reactions were monitored by removal of aliquots at timed intervals, with subsequent analysis via gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The aliquots were taken before heating, once the mixture began to reflux, and every 5 minutes thereafter until the reaction was complete. The progress of the reactions was monitored as a decrease of phenylephrine and the formation of the end product. Various intermediates and byproducts were also monitored.

**Gas Chromatography**

Analyses were performed using an Agilent Technologies 6890N Gas Chromatograph equipped with electronic pneumatic control and a flame ionization detector. A 10.0 m x 0.32 mm i.d. fused-silica capillary column coated with 0.52 μm DB5 (Agilent Technologies) was employed. Hydrogen was used as the carrier gas, with an
average linear velocity of 40 cm/sec (constant flow). The injection port and detector were both maintained at 280 °C. For each analysis, 1 μL of the sample was injected in split mode (25:1). The oven temperature was programmed as follows: Initial temperature 130 °C, hold for 1.0 minute, then increase 25 °C per minute to 280 °C, and hold for 1.0 minute (total run time = 10.0 minutes). Relative retention times (relative to methamphetamine) are shown in Table 1.

**Gas Chromatography/Mass Spectrometry**

Electron impact mass spectra (70 eV) were obtained using a 5973 Agilent Technologies Mass Selective Detector equipped with a 6890N Gas Chromatograph. A 30.0 m x 0.25 mm i.d. fused-silica capillary column coated with 0.25 μm HP5-MS (Agilent Technologies) was used. Helium was used as the carrier gas with an average linear velocity of 40 cm/sec (constant flow). The injection port and ion sources were set at 240 °C and 180 °C, respectively. For each analysis, 1 μL of the sample was injected in split mode (30:1). The oven temperature was programmed as follows: Initial temperature 130 °C, hold for 1.0 minute, then increase 30 °C per minute to 280 °C, and hold for 5.0 minutes (total run time = 11.6 minutes). Mass spectra were scanned over an m/z range of 40 - 500.

**Infrared Spectrophotometry**

Infrared spectra were obtained using a Nicolet Avatar 370 FTIR Spectrophotometer operated in the attenuated total reflectance (ATR) mode. Sixteen scans were collected at a resolution of 4.0 cm⁻¹.

**Results and Discussion**

One popular preparation of the reformulated OTC “PE” allergy/cold medications contains 10 milligrams of phenylephrine hydrochloride per tablet, which is easily extracted with methanol. The base was precipitated from a saturated solution of the hydrochloride by basifying to pH 11 with ammonium hydroxide (pH control is important, see below). The infrared spectra of phenylephrine hydrochloride and base are shown in Figures 1 and 2, respectively. The full-scale and expanded mass spectra of phenylephrine are shown in Figures 3 and 4, respectively.

Based on the analogous reduction of ephedrine/pseudoephedrine [1], the HI/red P reduction of phenylephrine was expected to result in loss of the benzylic hydroxyl, thereby producing 3-hydroxy-N-methylphenethylamine, also known as “Reduced Phenylephrine” or less commonly, “Reduced PE”. The structures of 3-hydroxy-N-methylphenethylamine and methamphetamine are shown below.

3-Hydroxy-N-methylphenethylamine  “Reduced Phenylephrine” (mw = 151.1)

Methamphetamine

The reduction mechanism was similarly expected to parallel that of the HI/red P reduction of ephedrine/pseudoephedrine; that is, through an iodophenylephrine intermediate that can be inferred by detection of the
corresponding aziridine. The phenolic hydroxyl was not expected to be reduced by HI/red P [6]. Similarly, phenylacetone-like and naphthalene-like compounds (that are detected as byproducts during the HI/red P reduction of ephedrine/pseudoephedrine [7]) were not expected to form during the HI/red P reduction of phenylephrine, due to the presence of the phenolic hydroxyl group.

The analytical results were consistent with these postulates. An iodophenylephrine intermediate was apparently formed, as verified by detection of the corresponding aziridine compound, 1-methyl-3-(meta-hydroxyphenyl)-aziridine (mw = 148.1), confirmed by mass spectrometry (see Figure 5). This iodo intermediate was in turn reduced to 3-hydroxy-N-methylphenethylamine (hereafter “Reduced PE”). The full-scale and expanded mass spectra of Reduced PE are shown in Figures 6 and 7, respectively. As expected, no phenylacetone-like or naphthalene-like byproducts were observed.

Interestingly, the reduction of phenylephrine was much more facile than the corresponding reduction of ephedrine/pseudoephedrine. In fact, the aziridine was already present in the aliquot that was removed prior to heating (see Figure 8), and Reduced PE was already the major product after just 5 minutes of reflux (see Figure 9). Some trace level intermediate peaks were also noted, but were not identified. Complete conversion to Reduced PE, with essentially no byproduct formation, occurred within 30 minutes of reflux (see Figure 10). In additional experiments, phenylephrine that was added directly to 57 % HI sitting at room temperature, without applied heat or added red P, formed some of the aziridine and Reduced PE within one day. This facile conversion is most likely due to the influence of the phenolic hydroxyl group. Mass spectrometry and NMR data (not shown) also confirm that only the benzylic hydroxyl was reduced, in agreement with previous work on compounds containing both an alkyl amine and phenolic hydroxyl [6].
The extraction process for Reduced PE was more challenging than the corresponding extraction of methamphetamine. Because Reduced PE can form a phenolate salt, the extraction can only be accomplished in a narrow pH range (expected to be between the pKa’s of methamphetamine hydrochloride and phenol; that is, corresponding to about pH 11). To determine the optimal extraction pH, a series of test solutions were made, each using a set amount of reaction mixture adjusted to different pH values via dropwise addition of ammonium hydroxide. The resulting solutions were then extracted with a chloroform/isopropanol (2:1) mixture, and the extracts analyzed by GC. As expected, as the pH in the respective test tubes approached pH 11 (as measured by multi-range pH paper), the amount of Reduced PE in the extracts increased, but as the pH increased past pH 11, the amount of Reduced PE in the extracts decreased.

The solvent system used for the above extractions was unusual. Reduced PE does not readily extract from a pH 11 solution into ether, hexane, or camping type fuel (extraction solvents that are typically used at clandestine methamphetamine laboratories). It is thought that a zwitterion is formed between the phenolic hydroxyl and the free amine. Since a zwitterion has salt-like characteristics, its solubility in water and polar solvents is enhanced. The chloroform/isopropanol (2:1) mixture improves the partition coefficient of Reduced PE, allowing its extraction from the aqueous solution. This solvent mixture has previously proven effective in extracting similar zwitterionic substances form aqueous solutions, including morphine, psilocybin, and lysergic acid.

The infrared spectrum of Reduced PE base (as obtained by evaporation of the above chloroform/isopropanol (2:1) extracts) is shown in Figure 11.

Conclusions

As the availability of ephedrine/pseudoephedrine-containing OTC products decrease, it is expected that some chemically naïve clandestine laboratory operators will attempt to produce methamphetamine from substitute phenylephrine-containing OTC products, thereby producing Reduced PE. It is in fact quite likely that such substitutions have already been attempted; however, it is also quite likely that these syntheses failed due to the loss of Reduced PE during the extraction procedures typically utilized in illicit methamphetamine production. Similarly, some clandestine laboratory operators will attempt to produce methamphetamine from mixtures of pseudoephedrine- and phenylephrine-containing OTC products, but these efforts will only result in lower apparent yields of methamphetamine, again due to the loss of Reduced PE during extractions.

Furthermore, the reduction of phenylephrine-containing OTC products using the lithium/ammonia process (Birch reduction) will likely be attempted, and will probably yield Reduced PE - though some other unknown product is also possible (this will be addressed in future research). Regardless of the reduction technique, however, the use of phenylephrine as a substitute for ephedrine/pseudoephedrine in illicit methamphetamine production will probably be short-lived, since Reduced PE is believed to have no significant CNS stimulant effects [8], and clandestine laboratory operators will quickly become educated about phenylephrine through drug abuse websites, bulletin boards, and chat-rooms on the Internet, consumer complaints, and discussions with fellow laboratory operators. Nonetheless, it is necessary for forensic analysts to be able to identify these products, and understand their sources.

References


* Law Enforcement Restricted.

[Figures 1 - 11 and Table 1 Follow.]
Figure 1. Infrared Spectrum (ATR) of Phenylephrine HCl.

Figure 2. Infrared Spectrum (ATR) of Phenylephrine Base.
Figure 3. Mass Spectrum of Phenylephrine.

Figure 4. Expanded Mass Spectrum of Phenylephrine.
Figure 5. Mass Spectrum of 1-Methyl-3-(meta-hydroxyphenyl)aziridine

Figure 6. Mass Spectrum of 3-Hydroxy-N-methylphenethylamine (Reduced Phenylephrine).
Figure 7. Expanded Mass Spectrum of 3-Hydroxy-N-methylphenethylamine (Reduced Phenylephrine).

Figure 8. Gas Chromatogram of the Initial Mixture of Phenylephrine and Hydriodic Acid.
Figure 9. Gas Chromatogram after 5 Minutes of Reflux of Phenylephrine and Hydriodic Acid.

Figure 10. Gas Chromatogram after 30 Minutes of Reflux of Phenylephrine and Hydriodic Acid.
Figure 11. Infrared Spectrum of 3-Hydroxy-N-methylphenethylamine Base (Reduced Phenylephrine Base).

Table 1. Methamphetamine Relative Retention Times (RRT’s) for Gas Chromatography

<table>
<thead>
<tr>
<th>RRT</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.48</td>
<td>Dimethylsulfone</td>
</tr>
<tr>
<td>0.79</td>
<td>P2P / Amphetamine</td>
</tr>
<tr>
<td>0.83</td>
<td>cis-1,2-Dimethyl-3-phenylaziridine</td>
</tr>
<tr>
<td><strong>1.00</strong> (1.26 min.)</td>
<td><strong>Methamphetamine</strong></td>
</tr>
<tr>
<td>1.08</td>
<td>trans-1,2-Dimethyl-3-phenylaziridine</td>
</tr>
<tr>
<td>1.67</td>
<td>Chloroephedrine (Chloromethamphetamine)</td>
</tr>
<tr>
<td>1.69</td>
<td>Ephedrine / Pseudoephedrine</td>
</tr>
<tr>
<td>1.80</td>
<td>Aziridine Phenylephrine</td>
</tr>
<tr>
<td>1.94</td>
<td>Oxazolidine Pseudoephedrine</td>
</tr>
<tr>
<td>1.96</td>
<td>Dimethylphthalate</td>
</tr>
<tr>
<td>1.96</td>
<td>Reduced Phenylephrine</td>
</tr>
<tr>
<td>1.98</td>
<td>Oxazolidine Ephedrine</td>
</tr>
<tr>
<td>2.67</td>
<td>Phenylephrine</td>
</tr>
<tr>
<td>3.99</td>
<td>232-1</td>
</tr>
<tr>
<td>5.14</td>
<td>232-2</td>
</tr>
</tbody>
</table>
Synthesis of trans-4-Methylaminorex from Norephedrine and Potassium Cyanate

Walter R. Rodriguez,* M.S. and Russell A. Allred, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Southeast Laboratory
5205 NW 84th Avenue
Miami, FL 33166
[Email: walter.r.rodriguez -at- usdoj.gov]

ABSTRACT: An unusual and previously undocumented synthesis for trans-4-methylaminorex was determined to be in use at a clandestine laboratory. Conventional references unanimously describe the use of norephedrine (phenylpropanolamine, 2-amino-1-phenylpropan-1-ol) and cyanogen bromide to synthesize cis-4-methylaminorex. In this case, use of norephedrine and potassium cyanate gave predominantly trans-4-methylaminorex. This new synthesis is explored, and its intermediates and byproducts are characterized.

KEYWORDS: 4-Methylaminorex, Oxazoline, Norephedrine, Phenylpropanolamine, Potassium Cyanate, Diastereomers, Clandestine Laboratory, Controlled Substance Analogue, Isomer, Forensic Chemistry

Introduction

In December 2004, a clandestine laboratory raid was conducted at a private residence in Ft. Lauderdale, Florida. The operator was an educated chemist (degree in Chemical Engineering). In his post-Miranda statements, he indicated that he had been synthesizing “Euphoria” (4-Methylaminorex, also known as: U4Euh, Ice*, 4-MAR, Intellex) in his home since June 2004, and stated that he was capable of producing batches as large as 1 kilogram. He also admitted to manufacturing lesser quantities of amphetamine, methamphetamine, and 3,4-methylenedioxyamphetamine. The chemicals and materials seized at the site supported his claims.

Of particular interest was 20 kilograms of a white powder alleged to be potassium cyanate (later confirmed to be a cyanate salt (cation not identified)). This compound had never been previously reported as a primary reagent at a clandestine laboratory. By the cook’s account, he followed an internet recipe for synthesis of trans-4-methylaminorex from norephedrine and potassium cyanate.

This statement was surprising in that exhaustive literature searches indicated that the only reported syntheses starting with norephedrine used cyanogen bromide (not potassium cyanate) and produced cis (not trans) 4-methylaminorex [1]. The internet recipe (which had been posted on a website dedicated to drug abuse) was derived from the work of Fodor and Koczka [2], who investigated the stereochemistry of the conversion of 2-ureidoalcohols to oxazolidines. Included were the conversions of ephedrine and pseudoephedrine to the corresponding 2-ureidoalcohols, followed by their cyclization to their corresponding oxazolidines. Extrapolating from these results, the internet author theorized that the same reaction sequence could be applied to norephedrine, resulting in trans-4-methylaminorex [3] (Figure 1).

Klein et al. reported that the cyanogen bromide method is stereoselective and proceeds with retention of configuration at the benzylic carbon (C-1) of norephedrine [4]. Thus, norephedrine produces cis-4-methyl-
aminorex and norpseudoephedrine yields trans-4-methylaminorex (Figure 1). Therefore, if the classic synthesis (with cyanogen bromide) had been used, the trans isomer could only have been synthesized from norephedrine if stereochemical inversion was first performed on norephedrine to produce norpseudoephedrine [4]. This “stereoinversion” requires a tedious series of steps that is unlikely to ever be attempted in a clandestine setting. In contrast, in the potassium cyanate synthesis, trans-4-methylaminorex is allegedly generated directly from norephedrine without preliminary “stereoinversion” at C-1.

**Figure 1.** The Diastereomers of 4-Methylaminorex (the Corresponding Enantiomers Have Been Omitted for Clarity).

Analysis of the seized exhibits confirmed that trans-4-methylaminorex was in fact the major product. Herein, the synthesis of trans-4-methylaminorex from norephedrine and potassium cyanate is characterized.

**Legal Issues**

When 4-methylaminorex was first temporarily controlled [5] in the summer of 1987, very little was known regarding the individual optical isomers of both the cis and trans forms [6]. Since the clandestine procedure employed at the time resulted in the production of the racemic cis isomer, there was no evidence that the trans isomer had any abuse potential, much less if it even existed in the clandestine market. As a result, only the cis isomer was explicitly controlled [7]. This marked the first and to date the only time a specific diastereomer has been listed as a controlled substance.

The production of trans-4-methylaminorex therefore raised an interesting legal issue. Since cis-4-methylaminorex is a Schedule I controlled substance, it can be inferred that “…its salts, isomers, and salts of isomers…” would also be Schedule I controlled substances. However, the term isomer, as defined in 21 CFR 1300.01(b)(21) means “…the optical isomer except as used in… [Schedules I(d) and II(b)].” cis-4-Methylaminorex is specifically listed as a stimulant in Schedule I(f). Since trans-4-methylaminorex is not an optical isomer of cis-4-methylaminorex, the “isomer” provision does not apply and it is not formally controlled.

Therefore, the legal issue is whether trans-4-methylaminorex is a controlled substance analogue. Under the Controlled Substance Analogue provision of the Controlled Substances Act, it must first be demonstrated that trans-4-methylaminorex has a chemical structure that is substantially similar to the chemical structure of controlled substance in Schedule I or II. The controlled substance in this case is cis-4-methylaminorex. The diastereomeric relationship between these two compounds clearly satisfies the requirements of the first “prong” of the provision.
Secondly, \textit{trans}-4-methylaminorex must exhibit a stimulant effect that is substantially similar to or greater than the stimulant effect of \textit{cis}-4-methylaminorex -OR- must be represented or intended to have a stimulant effect that is substantially similar to or greater than the stimulant effect of \textit{cis}-4-methylaminorex. The rank order of potencies of the four enantiomers of 4-methylaminorex has been shown to be:

\[\text{trans}-4\text{S,5S} > \text{cis}-4\text{S,5R} \sim \text{cis}-4\text{R,5S} > \text{trans}-4\text{R,5R}\]

in several pharmacological studies \cite{6,8-11}. One group of researchers suggested that the \textit{trans}-4S,5S- isomer may have sufficient abuse potential to warrant its classification as a Schedule I controlled substance \cite{10}. Thus, the second “prong” of the Controlled Substance Analogue provision is also met.

Finally, the \textit{trans}-4-methylaminorex seized in this case was specifically stated by the clandestine chemist to be “Euphoria,” which is the generic street nomenclature for 4-methylaminorex without any stereochemical (\textit{cis} or \textit{trans}) designation \cite{12,13}. Therefore, all three “prongs” of the Controlled Substance Analogue provision are satisfied, and it is virtually certain that Federal prosecution of \textit{trans}-4-methylaminorex as a “controlled substance analogue” would be successful. In this case, the clandestine chemist was convicted of manufacture of a controlled substance.

\textit{Experimental}

Reagents were obtained from Sigma-Aldrich, and were used without further purification.

Gas chromatograph/mass spectrometry (GC/MS) data was obtained from an Agilent 6890 Gas Chromatograph (GC) coupled to an Agilent 5973 Mass Selective Detector (MSD) operating in electron impact (EI) mode. The mass spectral scan range was m/z 34 to 520. The ion source and quadrupole temperature zones were set to 230 °C and 150 °C, respectively. The interface was heated to 280 °C. The GC was equipped with a 30 meter ZB-1 column with an internal diameter of 0.25 mm and a 0.25 µm film thickness (Phenomenex). The inlet was set to 250 °C and the carrier gas was Helium with a constant flow rate of 1.3 mL/min. The oven was ramped from 100 °C - 295 °C at 35 °C/min, with a 6.43 minute hold at 295 °C, for a total run time of 12 minutes.

Fourier Transform $^1$H Nuclear Magnetic Resonance (FT-NMR) analyses were performed on a Varian Mercury-plus spectrometer operating at 400 MHz. Eight scans were collected for each sample. Internal reference standards were not used.

Fourier Transform Infrared (FTIR) spectra were collected on a Thermo-Nicolet Nexus 470 FTIR equipped with a SensIR Technologies Durascope 3-bounce ATR attachment. The scan range was 4000 cm$^{-1}$ to 550 cm$^{-1}$, with a 4 cm$^{-1}$ resolution. 32 scans were collected for each sample.

Molecular drawings, 3-D optimizations, and IUPAC names were generated with ACD Labs ChemSketch software, version 7.0.

\textit{Preparation of \textit{trans}-4-Methylaminorex}

Because this synthesis is no longer readily accessible on the internet, experimental details have been omitted, in accordance with \textit{Microgram Journal} policy. Law enforcement personnel with a legitimate need to know should contact the authors for further information.

\textit{Results and Discussion}

It is uncommon for trained, professional chemists to produce illicit drugs. It is even more unusual that a genuinely new clandestine manufacturing process is encountered. It was helpful in this case that the cook not
only documented his reaction, batch, and scale-up information, but also was willing to discuss his “work” in a
detected a hearing. As detailed above, the synthesis of trans-4-methylaminorex was performed using a procedure
partially described on the internet and further expanded by the clandestine chemist [14]. A scaled-down version
of this method was used in this study.

Analysis of the reaction mixture from the illicit method after the first 2.5 hours revealed the presence of
N-(2-hydroxy-1-methyl-2-phenethyl)urea, 4-methyl-5-phenyl-1,3-oxazolidin-2-one and unreacted norephedrine
(see Figure 2). No trans-4-methylaminorex was detected up to this point.

Figure 2. The Components of the Reaction Mixture from the Illicit Method after the First
2.5 Hour Reflux Period. Only One of Each Enantiomeric Pair Is Shown.

The final reaction mixture was similar in composition to the actual evidence seized from the clandestine
laboratory. In addition to trans-4-methylaminorex, small amounts of cis-4-methylaminorex were also found. The
crude mixture also contained 4-methyl-5-phenyl-1,3-oxazolidin-2-one, N-(2-hydroxy-1-methyl-2-phenethyl)urea,
and unreacted norephedrine. For this study, this mixture was cleaned up prior to isolation of the final product.

It is likely that the synthesis occurs through the intermediate 2-ureidoalcohol alpha-methyl-beta-hydroxy-
phenethylurea [15] which cyclizes to the oxazoline with inversion of configuration at C-1 [2]. Therefore, the
synthesis was also conducted by the initial production and isolation of N-(2-hydroxy-1-methyl-2-phenethyl)urea
intermediate. The general stoichiometric reaction is shown below:

Norephedrine + Potassium Cyanate + H₂O → Norephedrine-Urea + KOH  (Equation 1)

The pH of the solution immediately upon complete dissolution of the reactants was about 5-6. After the reaction
had gone to completion, the pH of the remaining liquid was about 10-11, supporting Equation 1.
It is generally accepted that the reaction of cyanates in water does not proceed via the cyanate but via isocyanic acid [16-18]. This scenario (Scheme 1) involves nucleophilic attack by the amino group of norephedrine on the somewhat positively polarized carbon of isocyanic acid, with a subsequent proton shift from the amino group of norephedrine.

Scheme 1

This mechanism shows that the chiral centers remain unchanged during the formation of the urea intermediate. Since norephedrine produces trans-4-methylaminorex, inversion of configuration must occur via an intramolecular SN₂-type attack at the benzylic carbon by the carbonyl group of the urea portion of the intermediate. This can occur because N-(2-hydroxy-1-methyl-2-phenethyl)urea can achieve a favorable conformation to allow the reaction to occur [2]. The pseudo-5-membered ring conformation places the carbonyl oxygen in proximity to the benzylic carbon, enabling the SN₂ type attack. This conformation is depicted in the 3-D image shown below (Figure 3).

Figure 3. Three Dimensional Image of N-[(1S,2R)-(2-Hydroxy-1-methyl-2-phenethyl)]urea in a Conformation Enabling SN₂ Attack at the Benzylic Carbon (C-2) by the Ureido Carbonyl.

Note the changes in the numbering system of the various compounds. The benzylic carbon in norephedrine is designated as C-1. The same carbon is labeled as C-2 in the urea intermediate, and as C-5 in 4-methylaminorex. Thus, the (1R,2S) isomer of norephedrine becomes the (1S,2R) isomer of the urea intermediate, which is subsequently converted to the trans-(4S,5S)- isomer of 4-methylaminorex.
The small amount of the oxazolidinone detected in the reaction mixture at the half-way point is probably formed by the attack of the benzylic hydroxyl on the carbonyl of the urea (Figure 5), liberating a molecule of ammonia into the solution (presumably as NH₂OH), which may also contribute to the already moderately high pH observed at the end of the reaction (Equation 1).

Acknowledgements

The authors would like to thank DEA Librarians RoseMary Russo and Lavonne Wienke for their assistance in searching for and providing the literature references cited herein.

References


3. 4-Methylaminorex synth w/o CNBr. www.thehive.org 2001 (Note: Lapsed Website).


5. Lawn JC. Schedules of controlled substances; temporary placement of 2-amino-4-methyl-5-phenyl-2-oxazoline (4-methylaminorex) into Schedule I. Federal Register 1987;52:30174.


---

**Figure 6.** EI Mass Spectra. Top: 4-Methyl-5-phenyl-1,3-oxazolidin-2-one; Middle: N-(2-Hydroxy-1-methyl-2-phenethyl)urea; Bottom: *trans*-4-Methyaminorex.
Figure 7. $^1$H NMR, 8 scans, *trans*-4-Methylaminorex.
Figure 8. $^1$H NMR, 8 scans, N-(2-Hydroxy-1-methyl-2-phenethyl)urea.
Figure 9. FTIR. 32 scans, trans-4-Methylaminorex.
Figure 10. FTIR. 32 scans, N-(2-Hydroxy-1-methyl-2-phenethyl)urea.
Identification of a New Amphetamine Type Stimulant: 3,4-Methylenedioxy-N-(2-hydroxyethyl)amphetamine (MDHOET)

Carola Koper*
Netherlands Forensic Institute
P. O. Box 24044
2490 AA The Hague, The Netherlands
[email: c.koper -at- nfi.minjus.nl]

Elisa Ali-Tolppa
National Bureau of Investigation
P.O. Box 285
FIN- 01301 Vantaa, Finland

Joseph S. Bozenko Jr.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, Virginia  20166, USA

Valérie Dufey
Laboratoire Police Scientifique de Lyon
31 Avenue Franklin Roosevelt
69134 Ecully, France

Michael Puetz
Bundeskriminalamt
Thaerstrasse 11
65193 Wiesbaden, Germany

Céline Weyermann
Institute de Police Scientifique
University of Lausanne, Batiment de Chimie
CH 1015 Lausanne-Dorigny, Switzerland

Frantisek Zrcek
Police of Czech Republic
Institute of Criminalistics Prague
P.O. Box 62/KUP/Strojnicka 27
17089 Prague, Czech Republic

ABSTRACT: 3,4-Methylenedioxy-N-(2-hydroxyethyl)amphetamine (MDHOET), an MDA derivative, was identified in Ecstasy-type tablets seized in France, and subsequently in exhibits seized in Austria, The Netherlands, Switzerland, and the United Kingdom. This unusual amphetamine type stimulant (ATS) was submitted as an unknown to seven European laboratories participating in a sponsored ATS profiling program. Six
of the seven laboratories successfully identified MDHOET upon initial analysis. Analytical data from gas chromatography, infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance spectroscopy are presented.

**KEYWORDS:** 3,4-Methylenedioxy-\(N\)-(2-hydroxyethyl)amphetamine, MDHOET, MDMA, Ecstasy, ATS, CHAMP, Forensic Chemistry

**Introduction**

In December 2004, one thousand white Ecstasy-type tablets with the “Euro” logo were seized in Saint Etienne, France (see Photo 1). These tablets were subsequently determined to actually contain a combination of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-\(N\)-(2-hydroxyethyl)amphetamine (MDHOET; see Figure 1) [1,2]. Subsequent to this initial submission, six additional seizures containing MDHOET were made in Europe (see Table 1), including powders in the Netherlands (three separate submissions), white tablets with a “LOVE” logo (no photo) in Austria and Switzerland, and fragments of tablets in the United Kingdom. MDMA was present as a co-ingredient in five of the seven cases, but when present was always at a lower percentage versus MDHOET.

**Figure 1.** 3,4-Methylenedioxy-\(N\)-(2-hydroxyethyl)amphetamine (MDHOET)

MDHOET can be synthesized similarly to MDMA; for example, by reductive amination of 3,4-methylenedioxyphenyl-2-propanone (piperonylmethylketone or PMK) with ethanolamine, using a reducing agent (e.g., sodium cyanoborohydride) or via catalytic hydrogenation (e.g., H\(_2\) over Platinum) [1,2]. Not much is known about the physiological effects of this drug; it is reported to have very limited activity, presumably due to its relatively high polarity [2]. It is unknown whether the drug was intentionally synthesized as a non-controlled “designer drug,” or instead was an erroneous synthesis of 3,4-methylenedioxymethylamphetamine (MDEA); that is, by mistakenly using ethanolamine instead of ethylamine.

As MDHOET had not, to our knowledge, been previously encountered in Ecstasy-type tablets or powders, it represented an ideal test compound for submission to the seven laboratories currently participating in a project entitled: “Collaborative Harmonisation of Methods for Profiling of Amphetamine Type Stimulants” (CHAMP). Exhibits of the “Euro” tablets seized in France were used as the test samples.
Experimental

Gas Chromatography - Mass Spectrometry (GC/MS)
An Agilent 6890 GC coupled to a 5973 Mass Selective Detector system (MSD) was used. The column that was used was a HP Ultra-1 (length: 12 m, inner diameter: 0.22 mm, film thickness: 0.3 μm). Helium was used as the carrier gas (1 mL/minute, split ratio 50:1). The GC oven was programmed from 110 °C (1 minute hold) to 275 °C at a rate of 40 °C/minute. The carrier gas velocity was set at 40 cm/second (1.0 mL/minute, constant flow rate) and the inlet temperature was set at 275 °C. The injected volume was 1 μL. The scan range was m/z 35 to 450. A solvent delay of 0.8 minute was applied. The temperature of the MS transfer line was 300 °C.

Liquid Chromatography Mass Spectrometry (LC-MS/MS)
An Agilent 1100 Series HPLC system with autosampler and an Agilent 1100 series LC/MSD Trap ion trap MS was used, with Agilent LC/MSD Trap software version 5.2 (Bremen, Germany). The column was a Phenomenex Luna C18 (3 mm x 150 mm, 3 mm). The eluent A was 0.01 M ammonium acetate with 0.1 % formic acid and eluent B was acetonitrile with 5 % 0.01 M ammonium acetate and 0.1 % formic acid, in gradient run. The gradient used was 20 - 100 % eluant B over 0 - 10 minutes and 100 % eluant B from 10 - 25 minutes. The flow rate was 0.3 mL/minute, and the column temperature was 30 °C. The injection volume was 10 μL.

The electrospray ionisation ESI technique was used, in the positive ion mode. Operating parameters of the ESI ion source were as follows: Drying gas temperature was 350 °C, drying gas flow 9.0 L/minute, nebuliser gas pressure 40 psi, end plate voltage -3500 V, and end plate offset -500 V. Ion trap parameters were as follows: Accumulation time was 43 ms and averages 5, rolling averaging off, and ion charge control on. The fragmentation amplitude was increased from 30 % to 200 % from the set value of 1.0 V. AutoMS(n) mode was used. The scan range was m/z 50 - 500.

Fourier Transform Infrared Spectroscopy (FT-IR)
A Thermo-Nicolet Nexus 670 was used. Scans were recorded from 4000 cm⁻¹ to 400 cm⁻¹; an average of 32 scans was taken. Spectra were obtained using an attenuated total reflectance (ATR) attachment (data not corrected).

Nuclear Magnetic Resonance Spectroscopy
Analyses (see Figures 6 and 7) were performed on a Varian Mercury 400 MHz NMR using a Varian Nalorac 5 mm indirect detection, pulse field gradient (PFG), variable temperature probe, with PulseTuneTM. The sample was prepared at 26.8 mg/mL in deuterium oxide (D₂O) containing TSP (3-(trimethylsilyl)propionic-2,2,3,3-d₄ acid, sodium salt) as the 0 ppm reference, and maleic acid (5 mg/mL) as the internal standard for quantitation (maleic acid exhibits a singlet at 6.4 ppm). The proton spectrum of the standard used to determine the purity of the synthesized reference compound was obtained with 8 scans using a 45 second delay, 90° pulse, 5 second acquisition time, and oversampling of 4. Confirmation of the structure of the synthesized reference compound was performed using proton, carbon, COSY, HSQC, and HMBC NMR spectra, and Advanced Chemistry Developments Structure Elucidator software program (ACD/Labs, Toronto, Canada).

Synthesis of Reference Material
MDHOET was synthesized by reductive amination of 3,4-methylenedioxyphenyl-2-propanone (PMK, 4.52 grams) with ethanolamine hydrochloride (25 grams) in isopropanol (IPA) and sodium cyanoborohydride (NaCNBH₃, 1.1 grams) as the reducing agent [1]. PMK was added to ethanolamine hydrochloride in IPA and vigorously stirred. NaCNBH₃ was added and the mixture was stirred, adjusting the pH (as needed) with HCl in IPA at room temperature. After 3 days, 300 mL of water was added, and the mixture was made strongly acidic with 37 % HCl. The resulting solution was extracted 3 times with 100 mL CH₂Cl₂, which was discarded. The remaining aqueous phase was then made basic with 25 % NaOH, then extracted 3 times with 100 mL CH₂Cl₂. The resulting organic extracts were combined and dried over anhydrous Na₂SO₄. The solvent was removed under vacuum, yielding a clear, slightly viscous oil (2.82 grams). This oil was dissolved in IPA containing HCl and diluted with diethyl ether, precipitating 3.85 grams (68 %) MDHOET HCl as an off-white powder.
Results and Discussion

Laboratory Results
Subexhibits of the “Euro” tablets seized in Saint Etienne, France were distributed to the participating laboratories as “blind” samples (that is, with no indication that they were a test, and no instructions on recommended methods for analysis). This resulted in the use of a variety of techniques, of which GC/FID, GC/MS, FT-IR were the most frequently employed (see Table 2). The composition of the tablets (approximately 2% MDMA (as base), caffeine, and approximately 7% MDHOET (as base)) was an unusual complicating factor. Six of the seven partners properly identified MDHOET; the lone exception missed its presence during initial screening due to its co-elution with caffeine during the GC/FID analysis used in their laboratory. To confirm the identification, one laboratory synthesized a reference standard, a second laboratory used NMR spectroscopy (1H, 13C-, COSY and HETCOR), and a third laboratory did both. In addition to MDMA and MDHOET, caffeine, sorbitol and cellulose were identified by some of the laboratories (using GC, 1H-NMR, and/or FT-IR).

Mass Spectrometry
All laboratories taking part in the round robin used mass spectrometry for the elucidation of the structure of the unknown analyte. Apart from GC/MS, both liquid chromatography - mass spectrometry (LC/MS) and capillary electrophoresis - mass spectrometry (CE/MS) were used. When LC/MS, CE/MS or ion-trap GC/MS was used, the M+H ion was present at m/z 224 (Figure 2). However, if quadrupole GC/MS was used, derivatization (for example, silylation or acetylation) was necessary to determine the molecular ion.

Upon GC/MS analysis of a non-derivatized sample, both MDMA (Retention index (RI) 1515) and MDHOET (RI 1865) were detected [3]. The mass spectrum of MDHOET is displayed in Figure 3. No molecular ion could be identified. Besides the base peak at m/z 88 (CH₃CH=NHCH₂CH₂OH), other characteristic fragment ions are visible at m/z 44, m/z 70 (-18 (H₂O) from m/z 88), m/z 135 (-88 (CH₃CH=NHCH₂CH₂OH)) and m/z 163 (-H₂O). The two fragments at m/z 70 and m/z 88 both suggest the presence of an -OH functionality (that is, loss of H₂O).

Acetylation of MDHOET gave two derivatives, in agreement with the presence of two active hydrogens (NH and OH). The major compound was the di-acetylated derivative (Figure 4, molecular ion at m/z 307), while the minor compound was the mono-acetylated compound (molecular ion at m/z 281; spectrum not shown). Comparison of the mass spectrum of MDHOET with that of its amphetamine analogue, N-(2-hydroxyethyl)amphetamine as described by Cry et al. and Carpenter et al. [4,5], further corroborates this interpretation.

Fourier Transform Infrared Spectrometry
The FTIR-ATR spectrum of the synthesized MDHOET HCl is shown in Figure 5. The principal wavebands are at 1031 and 1249 cm⁻¹, with additional characteristic bands at 3369, 2949 and 1578 cm⁻¹.

NMR Spectroscopy
The 1H- and 13C-NMR spectra of the synthesized MDHOET in D₂O are shown in Figures 6 and 7. The two active hydrogens are not visible due to deuterium exchange with the solvent (when dissolved in CDCl₃, both resonances are visible as broad singlets between 9.2 - 9.3 ppm). The 1H-NMR spectrum of the original sample dissolved in D₂O is shown in Figure 8. In addition to MDHOET, MDMA, sorbitol, and caffeine are observed. The assignment of both the 1H- and 13C- resonances of MDHOET are given in Table 3.

Conclusions
Although MDHOET is quite unlikely to ever become a significant drug of abuse, future encounters are probable, both in Europe and elsewhere. The analytical data presented in this article should enable facile analyses of this unusual “designer drug,” in accordance with SWGDRUG protocols [6].
Acknowledgements

The Sixth Framework Program of the European Commission is gratefully acknowledged for funding this project. The authors would like to thank the following persons for their contributions: Laura Aalberg (Vantaa, Finland), Fabrice Besacier (Ecull, France), Jiri Bolehovsky (Prague, Czech Republic), Rainer Dahlenburg (Wiesbaden, Germany), Susanne Dieckmann (Wiesbaden, Germany), Laurence Dujourdy (Ecull, France), Pierre Esseiva (Lausanne-Dorigny, Switzerland), Céline Delaporte (Lausanne-Dorigny, Switzerland), Henk Huizer (The Hague, The Netherlands), Libuse Kawulokova (Prague, Czech Republic), Eric Lock (The Hague, The Netherlands), Anneke Poortman (The Hague, The Netherlands), Milan Prazak (Prague, Czech Republic), Erkki Sippola (Vantaa, Finland), and Pavel Tomicek (Prague, Czech Republic) [See address header for respective addresses.]

References


Table 1. Seizures of MDHOET in Europe During the Time Frame December 2004 - March 2006.

<table>
<thead>
<tr>
<th>Country</th>
<th>Date</th>
<th>Logo</th>
<th>Wt., Diam., Width</th>
<th>Contents</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>12/04</td>
<td>Euro</td>
<td>282 mg, 9.1 mm, 3.6 mm</td>
<td>MDHOET, MDMA, caffeine</td>
<td>1000 tablets</td>
</tr>
<tr>
<td>Netherlands</td>
<td>02/05</td>
<td>-</td>
<td>-</td>
<td>MDHOET, MDMA</td>
<td>10 g powder</td>
</tr>
<tr>
<td></td>
<td>06/05</td>
<td>-</td>
<td>-</td>
<td>MDHOET, MDMA</td>
<td>20 g powder</td>
</tr>
<tr>
<td></td>
<td>03/06</td>
<td>-</td>
<td>-</td>
<td>MDHOET, MDMA</td>
<td>1.8 g powder</td>
</tr>
<tr>
<td>Austria</td>
<td>05/05</td>
<td>Love</td>
<td>190 mg, 6.7 mm, 4.1 mm</td>
<td>MDHOET</td>
<td>50 tablets</td>
</tr>
<tr>
<td>Switzerland</td>
<td>05/05</td>
<td>Love</td>
<td>278 mg, 7.1 mm, 4.5 mm</td>
<td>MDHOET, MDMA</td>
<td>Not Reported</td>
</tr>
<tr>
<td>U.K.</td>
<td>04/05</td>
<td>Prob.</td>
<td>-</td>
<td>MDHOET</td>
<td>Tablet Fragments</td>
</tr>
</tbody>
</table>
Table 2. Overview of the Techniques Used to Identify MDHOET.

<table>
<thead>
<tr>
<th>LAB</th>
<th>GC/FID</th>
<th>HPLC</th>
<th>GC/MS</th>
<th>GC/MS</th>
<th>LC-MS/MS</th>
<th>DLC</th>
<th>CE-MS/MS</th>
<th>FTIR</th>
<th>NMR</th>
<th>Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deriv.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>X</td>
<td>Quad</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>X</td>
<td>Quad</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Quad</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>X</td>
<td>X</td>
<td>Quad</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td>Quad/Ion Trap</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ion Trap</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td>X</td>
<td>Quad</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Assignment of $^1$H- and $^{13}$C-Resonances of MDHOET

<table>
<thead>
<tr>
<th>Position</th>
<th>Proton (ppm, Peak Multiplicity, Coupling Constant)</th>
<th>Carbon (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$</td>
<td>1.28 (d, $J = 6.6$ Hz)</td>
<td>18.0</td>
</tr>
<tr>
<td>CH$_3$-CH</td>
<td>3.6 (ddq, $J = 5.8, 8.7, 6.6$ (x3))</td>
<td>58.4</td>
</tr>
<tr>
<td>CH$_3$-CH-CH$_2$</td>
<td>3.07 (dd, $J = 13.8, 5.8$ Hz), 2.80 (dd, $J = 13.8, 8.7$ Hz)</td>
<td>41.2</td>
</tr>
<tr>
<td>N-CH$_2$-CH$_2$-OH</td>
<td>3.28 (td, $J = 13.3, 5.3$ Hz), 3.21 (td, $J = 13.1, 5.2$ Hz)</td>
<td>49.1</td>
</tr>
<tr>
<td>N-CH$_2$-CH$_2$-OH</td>
<td>3.85 (t, $J = 5.2$ Hz)</td>
<td>59.7</td>
</tr>
<tr>
<td>O-CH$_2$-O</td>
<td>5.96 (s)</td>
<td>104.1</td>
</tr>
<tr>
<td>phenyl #1</td>
<td>n/a</td>
<td>132.5</td>
</tr>
<tr>
<td>phenyl #2</td>
<td>6.85 (d, $J = 1.47$ Hz)</td>
<td>112.6</td>
</tr>
<tr>
<td>phenyl #3</td>
<td>n/a</td>
<td>150.4</td>
</tr>
<tr>
<td>phenyl #4</td>
<td>n/a</td>
<td>149.2</td>
</tr>
<tr>
<td>phenyl #5</td>
<td>6.88 (d, $J = 7.92$ Hz)</td>
<td>111.6</td>
</tr>
<tr>
<td>phenyl #6</td>
<td>6.80 (dd, $J = 7.92, 1.47$ Hz)</td>
<td>125.7</td>
</tr>
<tr>
<td>NH, OH</td>
<td>Exchanged with Solvent</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 2. LC-MS/MS Mass Spectrum of MDHOET HCl.
Figure 3. Mass Spectrum of MDHOET HCl.

Figure 4. Mass Spectrum of the Double-Acetylated Derivative of MDHOET HCl.
Figure 5. Uncorrected FTIR-ATR Spectrum of Synthesized Reference MDHOET HCl.

Figure 6. Proton NMR Spectrum of Synthesized Reference MDHOET HCl in D₂O.
Figure 7. Carbon NMR Spectrum of Synthesized Reference MDHOET HCl in D$_2$O.

Figure 8. Proton NMR Spectrum of one of the French “Euro” Tablet in D$_2$O (Containing MDHOET HCl, MDMA HCl, Caffeine, and Sorbitol).
Technical Note

Analysis and Characterization of Psilocybin and Psilocin Using Liquid Chromatography - Electrospray Ionization Mass Spectrometry (LC-ESI-MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID)

Sandra E. Rodriguez-Cruz, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
[email: sandra.e.rodriguez-cruz -at- usdoj.gov]

ABSTRACT: The rapid analysis of psilocybin and psilocin using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) is presented. Full-scan MS experiments provide molecular weight information, but little fragmentation. Similarly, collision-induced-dissociation (CID) experiments generate only a limited number of fragments. However, source-induced-dissociation (SID) experiments result in more extensive fragmentation. The combined results from these complementary techniques allows for the more complete characterization of psilocin and the thermally-labile psilocybin.

KEYWORDS: Psilocybin, Psilocin, Thermally-Labile, Liquid Chromatography-Mass Spectrometry (LC/MS), Tandem Mass Spectrometry, Collision-Induced-Dissociation, Source-Induced-Dissociation.

Introduction

Direct analysis of thermally-labile compounds using gas chromatography - mass spectrometry (GC/MS) is limited or impossible due to degradation caused by the high injector and column temperatures. Although derivatization is useful in many cases, direct analysis of the compounds of interest is always preferable. The development of the electrospray ionization (ESI) technique has enabled the transfer of thermally-labile compounds from solution into the gas phase without significant degradation [1]. The use of ESI in combination with liquid chromatography - mass spectrometry (LC/MS) techniques therefore provides a powerful analytical tool for the analysis of heat sensitive compounds.

A “classic” example of such a thermally labile compound is psilocybin, a powerful hallucinogen found in over 100 species of mushrooms, including *Psilocybe azurescens*, *Stropharia cubensis*, and *Psilocybe mexicana* [2-4]. Psilocybin is the phosphorylated ester of psilocin (Figure 1). The phosphate ester in psilocybin is delicate, and analysis of psilocybin-containing substrates by standard analytical techniques is therefore problematic. Because both psilocybin and psilocin are classified under Schedule I of the United States Controlled Substances Act, their analyses are important for forensic/law enforcement purposes.

Previous reports on the analysis of hallucinogenic mushrooms include descriptions of various extractions of the material, followed by instrumental analysis using liquid chromatography, gas chromatography, and mass spectrometry techniques [5-11]. In actuality, most of these analyses allow the detection of psilocin only, since the psilocybin did not survive the extraction and/or analysis. In addition, both psilocin and psilocybin have been indirectly analyzed following derivatization [12], and more recently, directly analyzed with the use of LC/MS.
and tandem mass spectrometry (MS/MS) techniques [13]. Unfortunately, even these advanced techniques give limited information beyond molecular weights.

However, when combined with multiple fragmentation techniques, LC-ESI-MS enables more complete characterization of thermally labile compounds. Collision-induced-dissociation (CID; MS/MS) can generate some fragment ions. For small compounds like tryptamines, a greater amount of dissociation and information can usually be obtained by performing source-induced-dissociation (SID) experiments, where ions are fragmented within the electrospray interface before they reach the mass analyzer.

Herein, a method is presented for the separation and characterization of psilocybin and psilocin using LC-ESI-MS in combination with CID (MS/MS) and SID experiments.

**Experimental**

Experiments were performed using a ThermoFinnigan LCQ Advantage MAX quadrupole ion-trap mass spectrometer equipped with an electrospray ionization source and interfaced to a Surveyor HPLC system (solvent pump, autosampler/column, and photodiode array detector).

Liquid chromatography conditions were investigated in order to provide for the best separation possible during the shortest analysis time. Separations were performed using a Phenomenex Prodigy column (150 x 4.6 mm; 5 µm), and an isocratic flow of 89 % Solvent A and 11 % Solvent B. Solvent A is H₂O with 0.1 % (v/v) formic acid, while Solvent B is acetonitrile with 0.1 % (v/v) formic acid. The eluent flow rate was 400 µL/minute. Standard solutions of psilocin (Sigma Chemical) and psilocybin (Alltech) were prepared at concentrations of 20 µg/mL in Solvent A.

Sample injections of 10 µL were loaded into the isocratic flow and introduced into the mass spectrometer using the ESI interface. The transfer capillary was maintained at a temperature of 250 °C, while the capillary and tube lens were kept at 20 and 15 V, respectively. Nitrogen (99 %; 100 ± 20 psi) was used as both the sheath and auxiliary gas, and operated at 50 and 20 units, respectively.

Mass spectrometry data were collected in the positive ion mode using the full-scan and tandem (MS/MS) modes in order to provide both molecular weight and structural information. MS/MS experiments were performed using

**Figure 1.** Chemical Structures of Psilocybin (Left) and Psilocin (Right).
a standard collision energy of 35 eV. Source-induced-dissociation (SID) experiments were performed using variable energies between 25 and 40 eV. Helium (99.999%; 40 ± 10 psi) was used as both the trapping and collision gas.

Instrument control, data collection and analysis were performed using the Xcalibur software (version 1.4) provided by the instrument manufacturer.

**Results and Discussion**

Figure 2 shows the total ion chromatogram (TIC), UV-based chromatogram, and full-scan ESI mass spectral data obtained during a 10 minute isocratic separation (11% Solvent B). Psilocybin elutes at 5.5 minutes, while psilocin elutes at 7.4 minutes. Clear separation is obtained and the full-scan spectra show the pseudo-molecular (M+H\(^+\)) ions for psilocybin and psilocin at m/z 285 and 205, respectively. The full-scan ESI spectrum for psilocybin also shows a peak at m/z 307, corresponding to the (M+Na\(^+\)) ion. The ESI data for psilocin also shows a small fragment ion at m/z 160. This experiment allows for the separation, detection, and determination of the molecular weights for these two compounds.

Figure 3 shows the tandem (MS/MS) fragmentation data obtained during the chromatographic separation of psilocybin and psilocin. During standard collision-induced dissociation (CID) experiments at 35 eV, psilocybin dissociates into two main fragments. The fragment observed at m/z 205 corresponds to loss of a neutral phosphate (HPO\(_3\)) moiety (80 Da), while the fragment at m/z 240 results from the loss of neutral dimethylamine (HN(CH\(_3\)_2); 45 Da). Dissociation of psilocin is dominated by the loss of dimethylamine, producing a fragment at m/z 160. The dissociation patterns observed for psilocybin and psilocin are typical of tryptamine-type fragmentations previously reported [14,15], and are also in agreement with recent tandem MS experiments using a triple-quadrupole mass analyzer [13].

Source-induced-dissociation (SID) experiments provide an alternative fragmentation technique for compounds that show a limited number of fragments under MS/MS conditions. During SID, electrospray-generated ions are subjected to high-energy collisions with the background gas within the relatively high-pressure capillary-skimmer region of the ionization interface. As a result, characteristic fragments are generated and mass analysis provides additional structural information. Figures 4 and 5 show SID data obtained for psilocybin and psilocin, respectively, using dissociation energies of 25, 30, 35, and 40 eV.

For psilocybin, SID experiments result in the formation of multiple fragments. In addition to the fragments at m/z 240 and 205 observed with CID, other characteristic fragments are observed at m/z 222, 160, 142, and 115. The former three fragments correspond to loss of H\(_2\)O, HPO\(_3\), and H\(_3\)PO\(_4\) from the m/z 240 species, while the peak at m/z 115 is characteristic of the indole moiety. The fragment at m/z 160 can also be generated from the loss of HN(CH\(_3\)_2) from m/z 205. As observed in the SID spectra, the sodiated psilocybin ion at m/z 307 does not undergo significant dissociation under these conditions. This is probably reflective of the greater stability of this species, due to the higher affinity of the phosphate group for sodium.

Increased fragmentation is also observed from SID experiments on psilocin. After production of m/z 160, subsequent dissociation reactions result in the appearance of fragments at m/z 142 and 132, due to loss of H\(_2\)O and CH\(_3\)_2CH\(_2\), respectively. The fragments observed at m/z 115 and 117 are again characteristics of the indole group, with and without the loss of H\(_2\).

**Conclusions**

The presented LC-ESI-MS techniques allow for the direct, facile separation and identification of psilocybin and psilocin. The LC conditions used separated the two compounds in less than 8 minutes. Mass spectrometry
experiments in the full-scan and MS/MS mode provided molecular weight and partial structural information. Source-induced-dissociation experiments provided complementary fragment information, allowing for a much more complete structural characterization of the two species. The presented techniques illustrate the utility of LC-ESI-MS, CID, and SID experiments for the analysis and characterization of thermally-labile compounds.

References


Figure 2. Total Ion Chromatogram, UV Chromatogram, and Full-Scan ESI Spectra Showing the Separation and Detection of Psilocybin (MW = 284) and Psilocin (MW = 204).
**Figure 3.** Total Ion Chromatogram and MS/MS Spectra Showing the Fragmentation of Psilocybin and Psilocin under Standard Collision-Induced-Dissociation Conditions at 35 eV.
Figure 4. SID Spectra for Psilocybin Obtained Using Fragmentation Energies of 25, 30, 35, and 40 eV.
Figure 5. SID Spectra for Psilocin Obtained Using Fragmentation Energies of 25, 30, 35, and 40 eV.
Technical Note

Specificity of the Duquenois-Levine and Cobalt Thiocyanate Tests Substituting Methylene Chloride or Butyl Chloride for Chloroform

Amanda J. Hanson
Wisconsin State Crime Laboratory - Madison
4626 University Avenue
Madison, WI 53705-2156
[e-mail: hansonaj-at-doj.state.wi.us]

ABSTRACT: The use of alternative solvents in the Duquenois-Levine and Cobalt Thiocyanate tests were explored due to substandard results with recently purchased lots of chloroform. Methylene chloride provided satisfactory results when substituted for chloroform in both tests. Butyl chloride provided satisfactory results in the Duquenois-Levine test.

KEYWORDS: Duquenois-Levine, Cobalt Thiocyanate, Marijuana, Cocaine, Chloroform, Methylene Chloride, n-Butyl Chloride

Introduction

The Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test (Scott test) are proven screening tests for the presence of marijuana and cocaine, respectively. The organic solvent traditionally used in these tests is chloroform. However, chloroform recently purchased by this laboratory produced little or no color change when performing the Duquenois-Levine and Cobalt Thiocyanate tests. Shortly after opening, this chloroform became yellow to green in color, at which point it was unsuitable to perform these tests. According to the manufacturer, this unusual decomposition of the chloroform was due to insufficient amounts of preservatives. This experience led to the investigation of using alternative organic solvents, specifically methylene chloride and n-butyl chloride, in the Duquenois-Levine and Cobalt Thiocyanate tests.

Experimental

Reagents and Solvents
Hydrochloric acid, methylene chloride, and n-butyl chloride were obtained from Fisher Scientific. Acceptable quality chloroform was obtained from OmniSolv. The Duquenois reagent was prepared by adding 10 grams of vanillin and 5 milliliters of acetaldehyde to 500 milliliters of ethanol. The vanillin, acetaldehyde, and ethanol were obtained from Kodak, EM Science, and Fisher Scientific, respectively. The cobalt thiocyanate reagent was prepared by dissolving ten grams of cobalt (II) thiocyanate in a mixture of 490 milliliters of distilled water and 500 milliliters of glycerin. The cobalt (II) thiocyanate and glycerin were obtained from Aldrich Chemical and Fisher Scientific, respectively.

Procedures
The Duquenois-Levine test was performed on 17 different substances using chloroform, methylene chloride, and butyl chloride as the organic solvent. The test was performed by placing approximately 10 to 20 milligrams of a target substance in a glass test tube, then 10 drops of the Duquenois reagent. After shaking, 10 drops of concentrated hydrochloric acid were added, and the tube was again shaken. Any color that resulted after the
hydrochloric acid step was recorded. Twenty drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate into two layers. Any color that transferred into the organic layer was recorded (Table 1). This procedure was repeated for each target substance by substituting methylene chloride or butyl chloride for chloroform.

The cobalt thiocyanate test was performed on 14 different substances using chloroform, methylene chloride, and butyl chloride. The test was performed by placing approximately 2 to 4 milligrams of a target substance in a glass test tube, then 5 drops of cobalt thiocyanate reagent. After shaking, 1 or 2 drops of concentrated hydrochloric acid were added, and the tube was again shaken. Ten drops of chloroform were then added, and the tube was vortexed, then allowed to settle and separate into two layers. The final color of the chloroform (organic) layer was recorded (Table 2). This procedure was repeated for each target substance by substituting methylene chloride or butyl chloride for chloroform.

**Results and Discussion**

The results for the Duquenois-Levine test using either methylene chloride and butyl chloride were consistent with results obtained using chloroform. The marijuana became purple with the addition of the Duquenois reagent and hydrochloric acid. Upon addition of the organic solvent, the purple color transferred to the organic layer, indicating a positive test for cannabinoids. The color was consistent in all tests involving marijuana, regardless of the solvent used. None of the remaining 16 substances tested gave the characteristic purple color in the organic solvent layer.

Similarly, the results of the Cobalt Thiocyanate test were equivalent whether chloroform or methylene chloride was used. However, the results for the butyl chloride were mixed. Addition of the cobalt thiocyanate reagent to cocaine hydrochloride resulted in the surface of the particles turning a bright blue (faint blue for cocaine base). The solution changed back to pink upon adding one or two drops of hydrochloric acid and mixing. Addition of 10 drops of chloroform, vortexing, and allowing the solution to settle resulted in a blue organic layer for both cocaine hydrochloride and cocaine base. The test had similar results when methylene chloride was substituted for chloroform. In the case of butyl chloride, however, the organic layer stayed clear, giving an inconclusive test. Diphenhydramine and lidocaine also gave blue organic layers with either chloroform and methylene chloride. These compounds are known false positives for cocaine. However, in the case of butyl chloride, the organic layers were clear for diphenhydramine and white for lidocaine. The other ten materials had consistent negative test results for all three organic solvents.

**Conclusions**

Methylene chloride may be substituted for chloroform in both the Rapid Modified Duquenois-Levine test and Cobalt Thiocyanate test. Similarly, butyl chloride may be substituted for chloroform in the Duquenois-Levine test. However, butyl chloride was not a reliable substitute solvent for use in the Cobalt Thiocyanate test. Methylene chloride also works well as an extraction solvent in place of chloroform.

[Tables 1 and 2 Follow.]
### Table 1. Duquenois-Levine Test Results

<table>
<thead>
<tr>
<th>Material</th>
<th>Chloroform</th>
<th>Methylene Chloride</th>
<th>Butyl Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aqueous/organic</td>
<td>aqueous/organic</td>
<td>aqueous/organic</td>
</tr>
<tr>
<td>Allspice</td>
<td>brown/clear</td>
<td>brown/clear</td>
<td>brown/clear</td>
</tr>
<tr>
<td>Celery Flakes</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
</tr>
<tr>
<td>Chamomile</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
</tr>
<tr>
<td>Chamomile Tea</td>
<td>yellow/clear</td>
<td>green/clear</td>
<td>green/clear</td>
</tr>
<tr>
<td>Coffee</td>
<td>brown/clear</td>
<td>brown/clear</td>
<td>brown/clear</td>
</tr>
<tr>
<td>Dill Seed</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
</tr>
<tr>
<td>Hops</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
</tr>
<tr>
<td>Ginger</td>
<td>orange/orange</td>
<td>orange/orange</td>
<td>orange/clear</td>
</tr>
<tr>
<td>Ginseng</td>
<td>brown/brown</td>
<td>green/clear</td>
<td>green/clear</td>
</tr>
<tr>
<td>Marijuana</td>
<td>purple/purple</td>
<td>purple/purple</td>
<td>purple/purple</td>
</tr>
<tr>
<td>Marjoram</td>
<td>yellow/clear</td>
<td>green/clear</td>
<td>green/clear</td>
</tr>
<tr>
<td>Mint</td>
<td>green/clear</td>
<td>green/clear</td>
<td>green/clear</td>
</tr>
<tr>
<td>Sage</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
<td>yellow/clear</td>
</tr>
<tr>
<td>Salvia Divinorum</td>
<td>green/clear</td>
<td>brown/clear</td>
<td>green/clear</td>
</tr>
<tr>
<td>Thyme</td>
<td>yellow/clear</td>
<td>green/clear</td>
<td>green/clear</td>
</tr>
<tr>
<td>Tobacco</td>
<td>brown/clear</td>
<td>brown/clear</td>
<td>brown/clear</td>
</tr>
<tr>
<td>White Pepper</td>
<td>orange/yellow</td>
<td>orange/yellow</td>
<td>orange/yellow</td>
</tr>
</tbody>
</table>

### Table 2. Cobalt Thiocyanate Test Results

<table>
<thead>
<tr>
<th>Material</th>
<th>Chloroform</th>
<th>Methylene Chloride</th>
<th>Butyl Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>organic layer</td>
<td>organic layer</td>
<td>organic layer</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Cocaine</td>
<td>blue</td>
<td>blue</td>
<td>clear</td>
</tr>
<tr>
<td>Cocaine Base</td>
<td>blue</td>
<td>blue</td>
<td>clear</td>
</tr>
<tr>
<td>Dextrose</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>blue</td>
<td>blue</td>
<td>clear</td>
</tr>
<tr>
<td>Heroin</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Inositol</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>blue</td>
<td>blue</td>
<td>white</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>MDMA</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Morphine</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Procaine</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Soap</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>clear</td>
<td>clear</td>
<td>clear</td>
</tr>
</tbody>
</table>

* * * * *
The Identification of 1-Dehydromethandrostenolone

Robert D. Blackledge
Naval Criminal Investigative Service
Regional Forensic Laboratory
3405 Welles St. Ste. 3
San Diego, CA 92136
[email: bigpurple-at-cox.net]

ABSTRACT: A recent steroid seizure was identified as 1-dehydromethandrostenolone, a positional isomer of methyltestosterone. GC/MS results are reported.

KEYWORDS: 1-Dehydromethandrostenolone, 1,(5α)-Androsten-17α-methyl-17β-ol-3-one, Anabolic Steroid, GC/MS, Forensic Chemistry

Introduction

The Naval Criminal Investigative Service (NCIS) Regional Forensic Laboratory (San Diego, California) recently received 22 white capsules containing a dark granular material, a suspected steroid (see Photo 1). The exhibits were seized in San Diego by NCIS personnel from a member of the U.S. military who was believed to a member of a steroid trafficking group (details not available). Analysis of a methanolic extract by GC/MS indicated a mixture of niacinamide and an unknown steroid with the same molecular weight as methyltestosterone (302), but with a different retention time and fragmentation pattern. Library searches on the unknown spectrum were inconclusive.

Experimental

1-Dehydromethandrostenolone standard was acquired from Steraloids (Code A4450-000; www.steraloids.com/pages/page028.html). GC/MS data were acquired using an HP 6890 GC interfaced with a HP 5972A MSD. The GC was equipped with a 30 m x 250 μm x 25.0 μm J&W DB-5MS capillary column, with an initial temperature of 70 ºC (2 minutes), then a ramp of 20 ºC/minute up to 300 ºC, then held for 15 minutes.

Results and Discussion

Figure 1a shows the Total Ion Chromatogram resulting from analysis of the methanolic extract. The first peak was identified as niacinamide (Figure 1b). The second peak (Figure 2a) was apparently isomeric with methyltestosterone (Figure 2b). After consultation with DEA laboratory personnel, the second peak was tentatively identified as 1-dehydromethandrostenolone (1,(5α)-Androsten-17α-methyl-17β-ol-3-one). Analysis of a commercial standard confirmed 1-dehydromethandrostenolone (Figure 2c; Note that the variations in relative mass fragment abundances between Figures 2a and 2c are due to acquisition on different mass spectrometers).
1-Dehydrotestosterone is controlled under Schedule III of the U.S. Controlled Substances Act. It is a positional isomer of methyltestosterone, with the only difference being the location of the double bond within the steroid “A” ring (see Figure 3). In 1-dehydrotestosterone the double bond is between the 1 and 2 carbons, whereas in methyltestosterone it is between the 4 and 5 carbons. Both 1-dehydrotestosterone and methyltestosterone are classified as anabolic steroids.

**Figure 1.** (a) Total Ion Chromatogram; (b) Mass Spectrum of Niacinamide (7.3 Minutes).
Figure 2a. Mass Spectrum of Unknown Steroid (13.67 Minutes in Figure 1a).

Figure 2b. Mass Spectrum of Methyltestosterone Standard. Contrast the Peaks at $m/z$ 107 and 122 in Figure 2a Versus the Peaks at $m/z$ 105 and 124 in this Spectrum.
Figure 2c. Mass Spectrum of 1-Dehydromethandrostenolone Standard.

Figure 3. Structural Formulae

1 - The NCIS San Diego Laboratory ceased operations in early 2006.
Microgram

Journal

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC 20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 4
Numbers 1-4
January - December 2006

Posted On-Line At:
Contents

Identification of Bufotenine in Yopo Seeds via GC/IRD  
Robert D. Blackledge and Clay P. Phelan  
3

Analytical Profiles for 3,4,5-, 2,4,5-, and 2,4,6-Trimethoxyamphetamine  
Kenji Tsujikawa, Tatsuyuki Kanamori, Kenji Kuwayama, Hajime Miyaguchi,  
Yuko Iwata, and Hiroyuki Inoue  
12

A New, Highly Specific Color Test for Ketamine  
Mohammad Sarwar  
24

Eszopiclone (Lunesta™): An Analytical Profile  
Roxanne E. Franckowski and Robert A. Thompson  
29

Isolation of cis-Cinnamoylcocaine from Crude Illicit Cocaine via Alumina  
Column Chromatography  
John F. Casale, Enrique L. Piñero, and Elizabeth M. Corbeil  
37

The Characterization of 4-Methoxy-N-ethylamphetamine Hydrochloride  
John F. Casale, Patrick A. Hays, Trinette K. Spratley, and Pamela R. Smith  
42

Quantitation of Cocaine by Gas Chromatography-Flame Ionization Detection Utilizing  
Isopropylcocaine as a Structurally Related Internal Standard  
Enrique L. Piñero and John F. Casale  
47

Dehydrochlormethyltestosterone: An Analytical Profile  
Eric S. Wisniewski and Patrick A. Hays  
54

Qualitative and Quantitative Analysis of Ionamin 30 Capsules (Containing  
a Time-Release Formulation of Phentermine)  
Nicole R. Edwards  
66

Information and Instructions for Authors  
70

---

Note: In order to prevent automated theft of email addresses off the Internet postings of Microgram Journal, all email addresses reported in the Journal have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of Δ⁹-Tetrahydrocannabinol (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).
Identification of Bufotenine in Yopo Seeds via GC/IRD

Robert D. Blackledge*
U.S. Naval Criminal Investigative Service
Regional Forensic Laboratory - San Diego
3405 Welles St., Suite 3
San Diego, CA  92136 1
[.email:  bigpurple -at- cox.net]

Clay P. Phelan
U.S. Department of Justice
Drug Enforcement Administration
South Central Laboratory
10150 E. Technology Blvd.
Dallas, TX  75220
[.email:  clay.p.phelan -at- usdoj.gov]

ABSTRACT: The analysis of seeds from yopo (*Anadenanthera peregrina*) by GC/IRD and GC/MS is presented. The GC/IRD technique is easily able to discriminate between bufotenine (present in yopo seeds) and its positional isomer psilocin.

KEYWORDS: Yopo, *Anadenanthera Peregrina*, Bufotenine, Psilocin, Tryptamines, GC/IRD, GC/MS, Forensic Chemistry

Introduction

Yopo (*Anadenanthera peregrina*) is a tree that is native to the open plains of South America (1,2). Its leaves, bark, and seeds (sometime called “beans”) reportedly contain bufotenine (5-hydroxydimethyltryptamine), dimethyltryptamine (DMT), and 5-methoxydimethyltryptamine (5-MeO-DMT) (1-6). The seeds are ground with a mortar and pestle into a snuff-like powder that is used by indigenous peoples in various religious rituals. Because the various tryptamines that are present in yopo are hallucinogenic, the seeds are also subject to abuse, and so are irregularly encountered in forensic laboratories (3).

Recently, this laboratory (NCIS - RFL - San Diego) received a zip-lock plastic bag that contained approximately 20 suspected yopo seeds (see Photo 1, next page). The exhibit had been confiscated from a U.S. Navy member in Japan (no further details).

Analysis of any substrate containing bufotenine by GC/MS is complicated by the similarity of its mass spectrum with that of its positional isomer psilocin (4-hydroxydimethyltryptamine). Bufotenine and psilocin are both controlled under Schedule I of the U.S. Controlled Substances Act, but bufotenine-containing substrates are

1 The NCIS San Diego Laboratory ceased operations in early 2006.
submitted far less commonly to forensic laboratories than psilocin-containing substrates. Analysis and discrimination of the isomers is usually accomplished using a combination of GC and GC/MS, with confirmation (if needed) by additional GC/MS analysis of their respective N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives (7,8). However, GC/IRD is both simpler and gives distinct and easily distinguished spectra (8). Herein, we report the analysis of yopo seeds using a combination of GC/IRD and GC/MS, and compare and contrast the respective spectra for bufotenine and psilocin.

**Experimental**

*Standard Preparation:* Bufotenine monooxalate and psilocin standards (Sigma, St. Louis, MO) were provided by the DEA Southwest Laboratory. For GC/MS and GC/IRD analyses, a small amount (not weighed) of these standards were placed in glass vials and dissolved in a few drops of methanol.

*Sample Preparation:* Using a scalpel blade, the thin hard dark brown outer coating was removed from one of the seeds. The inside, uniform, light brownish-yellow material was placed in a mortar, covered with saturated sodium bicarbonate, and macerated with a pestle. After sitting for several minutes, the resulting solution was transferred to a separatory funnel and extracted with a small amount of chloroform. The extract was filtered through a cotton plug in a disposable Pasteur pipette. After concentrating via evaporation, the extract was analyzed by GC/MS at the NCIS - RFL - San Diego, and also by both GC and GC/IRD at the DEA Southwest Laboratory.

*Gas Chromatography:* An Agilent Technology 6890N GC equipped with a flame ionization detector was used. The GC was fitted with a 10 m x 0.10 mm i.d. capillary column coated with 0.34 μm 5 % phenylmethyl siloxane (J&W DB-5). The GC was operated in a split mode of approximately 50:1. The injector port and detector temperatures were maintained at 280 °C. The oven temperature program was as follows: Initial temperature, 100 °C for 1 minute, ramped up to 280 °C at 25 °C per minute, with a final hold of 1.5 minutes. Hydrogen was used at an average velocity of 99 cm/second.

*Gas Chromatography/Mass Spectrometry:* An Agilent Technology 6890 GC interfaced to an Agilent Technology 5972A Mass Selective Detector was used. The GC was fitted with a 30 m x 0.25 mm i.d. capillary column with 0.25 μm 5 % polyphenylmethyl siloxane (J & W DB-5MS). The GC was operated in a split mode of 50:1. Helium was used as a carrier gas at a column flow rate of 28 cm/second. The injection port temperature was maintained at 250 °C. The oven temperature program was as follows: Initial temperature, 70 °C for 2
minutes, ramped up to 300 °C at 20 °C per minute, with a final hold of 15 minutes. The MSD transfer line was maintained at 280 °C. The MSD was operated at 70 eV.

Gas Chromatography/Infrared Spectroscopy: A Varian/Digilab GC/IRD was used. The GC was fitted with a 25 m x 0.32 mm i.d. capillary column with 0.52 μm 5 % phenylmethyl siloxane (HP-5). The GC was operated in a splitless mode, with a purge delay time of 0.50 minute. Helium was used as the carrier gas at a column flow rate of 36 cm/second. The injector port temperature was maintained at 275 °C. The oven temperature program was as follows: Initial temperature, 70 °C for 1 minute; ramped up to 300 °C at 25 °C per minute, with a final hold of 3 minutes. The flow cell and transfer line temperatures were maintained at 250 °C.

Results and Discussion

The workup procedure gave a chloroform extract that was surprisingly clean, and that contained a significant amount of bufotenine based on the intensities of the GC, GC/MS, and GC/IRD signals. Clearly, even less than one seed would have provided sufficient sample for analysis and identification. The psilocin eluted prior to the bufotenine on all three instrument systems, and the elution time for the yopo seed extract matched the bufotenine standard (the seed extract’s greater concentration caused some peak broadening). Figures 1 through 6 show, respectively, the GC, GC/MS, and GC/IRD instrumental results for the seed extract, the psilocin standard, and the bufotenine standard.

Although others have reported that the seeds (or beans) contain DMT and 5-MeO-DMT in addition to bufotenine (1-6), in fact only bufotenine was found in the seeds in this case. A portion of these seeds were sent to James S. Miller, Ph.D., Curator and Director at the William L. Brown Center for Plant Genetic Resources, Missouri Botanical Garden [P.O. Box 299, St. Louis, MO  63166-0299]; Dr. Miller confirmed that the seeds were from Anadenanthera peregrina, “Yopo.”

References

1. Anonymous. Cebil and yopo (Anadenanthera spp.). http://www.a1b2c3.com/drugs/var003.htm
Figure 1. Chromatographs of Psilocin and Bufotenine.
Figure 2. TIC and Mass Spectrum of Yopo Seed Extract.
Figure 3. TIC and Mass Spectrum of Psilocin Standard.
Figure 4. TIC and Mass Spectrum of Bufotenine Standard.
Figure 5. GC/IRD Spectra of Bufotenine Standard and Yopo Seed Extract.
Figure 6. GC/IRD Spectra of Psilocin Standard.
Analytical Profiles for 3,4,5-, 2,4,5-, and 2,4,6-Trimethoxyamphetamine

Kenji Tsujikawa,* Tatsuyuki Kanamori, Kenji Kuwayama, Hajime Miyaguchi, Yuko Iwata, and Hiroyuki Inoue
National Research Institute of Police Science
6-3-1, Kashiwanoha, Kashiwa
Chiba 277-0882, Japan
[Email: tujikawa-at-nrips.go.jp]

ABSTRACT: Analytical profiles (Marquis color testing, infrared spectroscopy, nuclear magnetic resonance, thin layer chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry) are presented for 3,4,5-trimethoxyamphetamine, 2,4,5-trimethoxyamphetamine, and 2,4,6-trimethoxyamphetamine. The data allows identification and differentiation of these positional isomers.

KEYWORDS: 3,4,5-Trimethoxyamphetamine, 2,4,5-Trimethoxyamphetamine, 2,4,6-Trimethoxyamphetamine, TMA, Positional Isomers, Marquis, IR, NMR, TLC, HPLC, GC/MS, Forensic Chemistry

Introduction

Most of the trimethoxyamphetamines (TMAs) are hallucinogens (1). There are six different positional isomers, that differ only in the respective positions of the three methoxy groups on the benzene ring (see Figure 1, next page). Of the six isomers, 3,4,5-trimethoxyamphetamine (TMA-1), 2,4,5-trimethoxyamphetamine (TMA-2), and 2,4,6-trimethoxyamphetamine (TMA-6) are more important than other three isomers, both from the perspective of their legal status and their circulation in Japanese drug markets. Unlike in the United States, positional isomers of hallucinogenic phenethylamines are not automatically controlled under Japanese statutes. Thus, TMA-1 is controlled by the Narcotics and Psychotropics Control Law in Japan, while TMA-2 is currently uncontrolled (but is anticipated to be scheduled in the near future), and TMA-6 is currently uncontrolled. In Japan, TMA-1 is usually sold as a solid, while TMA-2 and TMA-6 are more commonly sold in liquid forms, usually mixed with pigments, flavors, and sometimes other psychoactive compounds. Currently, abuse of 2,3,4-trimethoxyamphetamine (TMA-3), 2,3,5-trimethoxyamphetamine (TMA-4), and 2,3,6-trimethoxyamphetamine (TMA-5) have not been reported in Japan.

Because the legal status of the TMAs vary by structure in Japan, it is important to be able to identify and differentiate between (at least) TMA-1, TMA-2, and TMA-6. To our knowledge, no methods have been reported for such differentiation. Herein, we present analytical data (color testing, infrared spectroscopy (IR), nuclear magnetic resonance (NMR), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography/mass spectrometry (GC/MS)) for TMA-1, TMA-2, and TMA-6.

Experimental

Syntheses: Authentic standards of hydrochloride salts of TMA-1, TMA-2, and TMA-6 were synthesized in our laboratory using previously reported procedures (1). All other chemicals used were of analytical grade.

Color Testing: Marquis reagent was prepared by adding one drop of formaldehyde to 1 mL of concentrated sulfuric acid (2). The sample was placed in a depression of spot plates, and 3 drops of the reagent were added. [The TLC spray reagents are reported below.]
FTIR: A Shimadzu FTIR-8900 Fourier Transform Infrared Spectrophotometer was used. The substrates were analyzed using the standard potassium bromide method. Thirty-two scans were collected between 4000 and 450 cm$^{-1}$, with a resolution of 4.0 cm$^{-1}$.

NMR: Proton NMR analyses were performed on a JEOL JNM-ECP600 NMR spectrometer. The samples were prepared at approximately 10 mg/mL in methanol-$d_4$ (CD$_3$OD), using added tetramethylsilane (TMS) as the 0.0 ppm reference.

TLC: TLC analyses were performed using the method of Takahashi et al. (3), with a minor modification. The analyses were carried out on silica gel plates (10 x 10 cm) containing a fluorescent indicator (254 nm) on glass support (Merck, Darmstadt, Germany). The respective hydrochlorides of each TMA were dissolved in methanol at concentrations of 10, 1, and 0.1 mg/mL. These were applied manually on the plates with a microsyringe. A solvent mixture of chloroform/methanol/25 % aqueous ammonia (75:25:3 v/v/v) was used as the mobile phase. After development and evaporation of the mobile phase, the compounds were detected by UV (254 nm) and by spraying with Dragendorff or fluorescamine reagents (prepared as follows):

Dragendorff reagent: Bismuth hydroxide (0.9 g) was dissolved in concentrated hydrochloric acid (2 mL), and potassium iodine (3 g) dissolved in water (3 mL) and 70 % aqueous acetic acid (45 mL) were then added (4).

Figure 1. The TMA Positional Isomers.
Fluorescamine reagent: Fluorescamine (0.5 mg) was dissolved in acetone (1 mL) (2). The spots were observed under UV (365 nm).

**HPLC**: HPLC analyses were performed using the method of Kikura-Hanajiri et al. (5), with a minor modification. A Shimadzu LC-10ADvp series equipped with an SPD-M10Avp diode array detector set at 230 nm was used. The column was a Symmetry C18 column (Waters, 150 mm x 2.1 mm i.d., 3.5 μm) protected by an OptiGuard C18 guard column (Optimize technology), and was operated at 40 °C. The mobile phase, delivered at a flow rate of 0.2 mL/min, was a gradient of a mixture of acetonitrile-methanol (7:3 v/v) (B) in 10 mM ammonium formate (pH 3.5) (A): 0-1 min, 10 % B; 1-24 min, from 10 % to 33 % linear gradient of B in A.

**Sample Prep**: A volume of 20 μL containing 10 μg/mL of each trimethoxyamphetamine hydrochloride dissolved in distilled water was injected.

**GC/MS**: GC/MS analyses were performed using a GCMS-QP5050A (Shimadzu) equipped with a DB-5MS capillary column (Agilent technologies, 30 m x 0.25 mm i.d., 0.25 μm film thickness). The temperature of the injector and the interface was set at 250 °C. The oven temperature was held at 50 °C for 1 min, then raised to 300 °C at 15 °C/min, then held for 3 min. Helium was used as the carrier gas (head pressure 52.8 kPa, column flow 1.0 mL/min at 50 °C, constant pressure). The mass spectrometer was operated under electron ionization (EI) mode. One microliter samples were injected in the splitless mode. Sample Prep: For the free bases, the respective hydrochloride salts (200 μg) were dissolved in 1 mL of distilled water, basified to pH 12 with 1 M sodium hydroxide, and extracted with 1 mL of ethyl acetate. The extract was transferred to a GC vial. For the trifluoroacetylated derivatives, 100 μL of trifluoroacetic anhydride and 100 μL of ethyl acetate was added to 50 μg of the respective hydrochloride salt, and the mixture heated at 55 °C for 20 min. After evaporation of excess reagents, the residue was redissolved in 1 mL of ethyl acetate, and transferred to a GC vial.

**Results and Discussion**

**Color Testing**: The Marquis reagent reacted the three TMAs to give the following colors: TMA-1: Red; TMA-2: Pale yellow; and TMA-6: Orange. Different ring substitution patterns are known to give different colors with the Marquis reagent (6); however, the color differences between TMA-1, TMA-2, and TMA-6 were distinct and (somewhat) unexpected.

**IR**: The IR spectra of the three TMA hydrochloride salts are shown in Figure 2. The spectral patterns in the fingerprint region (< 1500 cm⁻¹) were completely different, and could therefore be used to unambiguously identify and differentiate the compounds.

**NMR**: The Proton NMR spectra are shown in Figure 3. The splitting patterns in the aromatic region were different for TMA-2 (two singlet peaks) versus TMA-1 and TMA-6 (one singlet peak). TMA-2 has two chemically nonequivalent protons, while TMA-1 and TMA-6 have two chemically equivalent protons. TMA-1 and TMA-6 could be distinguished by chemical shifts of their aromatic protons. The respective values for TMA-1 and TMA-6 were 6.56 ppm and 6.25 ppm. These values did not agree with those predicted from the empirical rule (7) (6.13 ppm for TMA-1 and 6.00 ppm for TMA-6), but the relative difference was consistent.

**TLC**: The Rf values of TMA-1, TMA-2, and TMA-6 using the described system were 0.69, 0.65, and 0.59, respectively. Although the spots were very close, they could be differentiated from one another. Table 1 (next page) shows the detection limits by the UV (254 nm) and various detection reagents. The sensitivities of the reagents were in decreasing order: Fluorescamine reagent, Marquis reagent, and Dragendorff reagent. However, the fluorescamine and Dragendorff reagents gave minimal color differences between the three isomers (green fluorescence under UV (365 nm) for the fluorescamine reagent, and orange for the Dragendorff reagent). On the other hand, spraying with Marquis reagent gave different colors, as follows: TMA-1: Orange but immediately fading; TMA-2: yellow; and TMA-6: Orange then changing to purple-red.
Table 1. Detection Limits (milligrams) of the TMAs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>UV (254 nm)</th>
<th>Dragendorff</th>
<th>Fluorescamine</th>
<th>Marquis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA-1</td>
<td>2</td>
<td>10</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>TMA-2</td>
<td>1</td>
<td>5</td>
<td>0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>TMA-6</td>
<td>2</td>
<td>10</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

HPLC: Figure 4 shows the HPLC chromatogram of a mixture of the three TMAs.

GC/MS: Figure 5 shows the total ion chromatograms (TICs) of the nonderivatized and trifluoroacetylated (TFA-derivatized) TMAs. The nonderivatized TMAs all displayed tailing, and TMA-1 and TMA-2 were not baseline resolved. However, the TFA-derivatives displayed improved peak shapes and enhanced separation between TMA-1 and TMA-2.

Figure 6 shows the EI mass spectra. The spectra of nonderivatized TMAs were similar, and it was especially difficult to discriminate between TMA-1 and TMA-2. However, the TFA-derivatives (though also similar) were sufficiently different for differentiation.

References


[Figures 2 - 6 Follow (Note: Figure 4 is Between Figures 2c and 3a in Order to Improve Layout).]
Figures 2a-b. IR Spectra of the Hydrochloride Salts of TMA-1 and TMA-2.
Figure 2c. IR Spectra of the Hydrochloride Salt of TMA-6.

Figure 4. HPLC Chromatogram of a Mixture of the Three TMAs (Detection: UV 230 nm). Retention times (Minutes): TMA-1 - 10.7, TMA-2 - 11.9, and TMA-6 - 18.7.
Figure 3a. Proton NMR (600 MHz) of TMA-1: $^1$H-NMR (CD$_3$OD) δ: 6.56 (2H, s), 3.84 (6H, s), 3.74 (3H, s), 3.58-3.51 (1H, m), 2.87 (1H, dd, $J = 13.7$, 7.1 Hz), 2.80 (1H, dd, $J = 13.5$, 7.4 Hz), 1.29 (3H, d, $J = 6.6$ Hz).
Figure 3b. Proton NMR (600 MHz) of TMA-2: $^1$H-NMR (CD$_3$OD) $\delta$: 6.81 (1H, s), 6.70 (1H, s), 3.86 (3H, s), 3.84 (3H, s), 3.78 (3H, s), 3.54-3.48 (1H, m), 2.87 (1H, dd, $J = 13.6$, 6.9 Hz), 2.79 (1H, dd, $J = 13.7$, 6.9 Hz), 1.25 (3H, d, $J = 6.7$ Hz).
**Figure 3c.** Proton NMR (600 MHz) of TMA-6: $^1$H-NMR (CD$_3$OD) $\delta$: 6.25 (2H, s), 3.82 (6H, s), 3.81 (3H, s), 3.44-3.38 (1H, m), 2.87 (1H, dd, $J = 13.0$, 6.0 Hz), 2.84 (1H, dd, $J = 13.3$, 6.8 Hz), 1.23 (3H, d, $J = 6.7$ Hz).
Figure 5. Total Ion Chromatograms of the Three Non-Derivatized and TFA-Derivatized TMAs.

[Retention Indices: Non-Derivatized: TMA-1 - 1724; TMA-2 - 1739; TMA-6 - 1771; TFA-Derivatized: TMA-1 - 1814; TMA-2 - 1830; TMA-6 - 1849.]
Figure 6a. EI Mass Spectra of the Three Non-Derivatized TMAs.
Figure 6b. EI Mass Spectra of the Three TFA-Derivatized TMAs.
Technical Note

A New, Highly Specific Color Test for Ketamine

Mohammad Sarwar, Ph.D
Forensic Research Laboratory
Center for Excellence in Molecular Biology
University of the Punjab
Lahore, Pakistan
[email: m sarwar36 -at- yahoo.com]

ABSTRACT: A new color test for the screening/presumptive identification of ketamine is reported. Treatment of ketamine with alkaline gold bromide produces a deep purple color within approximately one minute that changes to dark, blackish-purple within approximately two minutes. The color, color change, and time frames constitutes a highly specific screening test for ketamine. Of particular note, the test is negative for amphetamine, methamphetamine, MDA, MDMA, and phencyclidine (PCP), all of which are occasionally encountered in combination with ketamine.

KEYWORDS: Ketamine, Gold Bromide, Color Test, Screening Test, Forensic Chemistry

Introduction

Ketamine (Figure 1) is a medical and veterinary anesthetic and a controlled substance (Schedule III in the United States). Due to its anesthetic and hallucinogenic properties, ketamine is increasingly abused (1-3). Because its synthesis is challenging, its presence in illicit drug markets is almost universally due to diversion of pharmaceutical stocks. It is available in powder, liquid, and solid dosage forms, and for abuse purposes is smoked, snorted, injected, or taken orally. More recently, ketamine is being increasingly encountered as an added component in Ecstasy-type tablets. Other controlled substances that are occasionally encountered mixed with ketamine in Ecstasy-type tablets include (but are not limited to): Amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and phencyclidine (PCP).

Figure 1. Ketamine ( (+/-)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone); C₁₃H₁₆ClNO; m.w. (Base) = 237.7, (HCl) = 274.2.
There are numerous analytical methods for the identification of ketamine in forensic and toxicological laboratories (4-13); however, until recently only one color test (the Janovsky reagent (11,14)) was available for screening purposes. Unfortunately, although moderately specific the Janovsky reagent (alkaline meta-dinitrobenzene) is rather insensitive (detection limit about 1.25 milligrams) and is therefore infrequently used for screening of mixed samples or solutions. In early 2007, Morris reported a modified cobalt thiocyanate color test for ketamine that is highly specific (15); however, it is also rather insensitive (detection limit also about 1.25 milligrams). Herein, a new presumptive color test for the preliminary screening of ketamine is reported. The test is simple, easy to perform, nearly twice as sensitive as the Janovsky and Morris tests, and highly specific.

Experimental

Materials: All standards used were from Sigma, Alltech, and Matheson. Gold bromide and sodium hydroxide were both analytical grade.

Reagents: A 0.5 % solution of gold bromide was prepared in deionized water, resulting in a brownish yellow colored solution. A 0.2 M solution of NaOH was also prepared in deionized water.

Method: One drop of 0.5 % gold bromide solution and one drop of the 0.2 M NaOH solution were combined in a spot plate well. A small amount of the substrate was added to the spot well and mixed, and the color monitored over approximately the next 2 minutes.

Results and Discussion

A literature search indicates that gold bromide has not been previously reported for color testing; however, acidified gold bromide has been used in a rather obscure microcrystal test for caffeine (16-18).

As noted in the Experimental section, the alkaline gold bromide test reagent is brownish-yellow in color. Upon treatment with the test reagent, ketamine gives a deep purple color within approximately one minute, that turns to a dark, blackish-purple color within approximately two minutes. A blank does not produce any color changes. Forty-seven other compounds (illicit drugs, adulterants, and diluents) which are frequently encountered in forensic laboratories were also tested (see Table 1, next page). A few of these compounds produced the same purple color as ketamine, but in all such cases there was a major time difference for the development of the color. A few compounds having hydroxyl or phenolic groups (acetaminophen, ascorbic acid, lactose, mannitol, morphine, and sucrose) gives the purple color almost instantaneously. Similarly, heroin (both standard and street-level) also gives the purple color almost instantaneously (the observation that heroin standard gives a positive test confirms that the positive test for street-level heroin was not just due to the presence of morphine or sugars). However, none of the tested compounds gave the color and color change like ketamine over the two minute time frame. In addition, none of the other amine drugs tested gave a positive test, including those most commonly encountered in combination with ketamine in illicit samples. Ketamine is not commonly encountered in combination with heroin, morphine, or the other tested diluents that do give an instantaneous color development - nonetheless, if a nearly instantaneous color change is observed, the test cannot be used for presumptive identification of ketamine.

As noted in the Introduction, the Janovsky and Morris tests have limits of detection of approximately 1.25 milligrams, in both cases requiring additional efforts to maximize sensitivity. The limit of detection for the presented test, with no special efforts to maximize sensitivity, was 0.8 milligrams - nearly twice as sensitive.

The initial purple color may be due to the formation of a complex between the gold and the ketamine. The cause for the change of color from purple to dark blackish-purple is unknown; however, it may be due to a redox reaction that produces a small amount of colloidal gold.
Table 1. Color Testing Results.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Observation of Color</th>
<th>Compound</th>
<th>Observation of Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>+</td>
<td>Acetaminophen</td>
<td>+</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>-</td>
<td>Alprazolam</td>
<td>-</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>-</td>
<td>Ascorbic acid</td>
<td>(+)</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>Benzocaine</td>
<td>-</td>
</tr>
<tr>
<td>Diazepam</td>
<td>-</td>
<td>Bromazepam</td>
<td>-</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>-</td>
<td>Butalbital</td>
<td>-</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>-</td>
<td>Caffeine</td>
<td>-</td>
</tr>
<tr>
<td>Heroin</td>
<td>+</td>
<td>Clonazepam</td>
<td>-</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>-</td>
<td>α-Lactose</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>Lidocaine</td>
<td>-</td>
</tr>
<tr>
<td>Codeine Base</td>
<td>-</td>
<td>Lorazepam</td>
<td>-</td>
</tr>
<tr>
<td>MDMA</td>
<td>-</td>
<td>Manitol</td>
<td>+</td>
</tr>
<tr>
<td>Cocaine base</td>
<td>-</td>
<td>Sodium Chloride</td>
<td>-</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>-</td>
<td>Nicotinamide</td>
<td>-</td>
</tr>
<tr>
<td>Morphine</td>
<td>+</td>
<td>Nitrazepam</td>
<td>-</td>
</tr>
<tr>
<td>MDA</td>
<td>-</td>
<td>Oxazepam</td>
<td>-</td>
</tr>
<tr>
<td>PCP</td>
<td>-</td>
<td>Pentazocine</td>
<td>-</td>
</tr>
<tr>
<td>Butalbital</td>
<td>-</td>
<td>Quinine</td>
<td>-</td>
</tr>
<tr>
<td>Cocaine HCl</td>
<td>-</td>
<td>Stearic acid</td>
<td>-</td>
</tr>
<tr>
<td>Fentanyl Citrate</td>
<td>-</td>
<td>Temazepam</td>
<td>-</td>
</tr>
<tr>
<td>Phentermine</td>
<td>-</td>
<td>Triazolam</td>
<td>-</td>
</tr>
<tr>
<td>Quinine HCl</td>
<td>-</td>
<td>Calcium Carbonate</td>
<td>-</td>
</tr>
<tr>
<td>Codeine HCl</td>
<td>-</td>
<td>Butalbarbital</td>
<td>-</td>
</tr>
<tr>
<td>Morphine Sulfate</td>
<td>+</td>
<td>Sodium Carbonate</td>
<td>-</td>
</tr>
</tbody>
</table>

**Conclusions**

The presented color test can be used either as a standalone screen or in combination with either or both the Janovsky reagent or the new, modified cobalt thiocyanate test by Morris. The three tests are highly complementary in that only a few (and different) compounds interfere with each test; therefore, use of any two and certainly all three would constitute a uniquely specific screen and presumptive identification of ketamine, either alone or in combination with other controlled substances and/or adulterants, so long as at least 1.25 milligrams of ketamine is present in each test sample.
Acknowledgements

I am greatly indebted to the Forensic Science Center at Chicago, Illinois (USA) for providing access to the facilities and necessary materials to conduct this research. The assistance of Art Weathers (Forensic Scientist III, Forensic Science Center at Chicago) in conducting this work is highly appreciated. The assistance of Imran Majeed (Assistant Research Officer, Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan) in preparing this manuscript is also highly appreciated.

References


* * * * *
Eszopiclone (Lunesta™): An Analytical Profile

Roxanne E. Franckowski, M.S.* and Robert A. Thompson, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA  20166
[ email:  roxanne.e.franckowski -at- usdoj.gov]

ABSTRACT: Eszopiclone (Lunesta™) is a nonbenzodiazepine hypnotic/sedative prescribed for treatment of insomnia. Analytical data (gas chromatography, mass spectrometry, infrared spectroscopy, ultra performance liquid chromatography, and proton and carbon-13 nuclear magnetic resonance spectroscopy) for eszopiclone are presented.

KEYWORDS: Lunesta™, Eszopiclone, Hypnotic, Sedative, Forensic Chemistry

Introduction

The DEA Special Testing and Research Laboratory recently received a sample of eszopiclone (Figure 1) to add to its reference standards collection. Eszopiclone (the active “S” enantiomer of zopiclone) is a nonbenzodiazepine hypnotic/sedative prescribed for treatment of chronic (long-term) insomnia. It is currently marketed in tablet form as Lunesta™, in concentrations of 1, 2, or 3 milligrams per tablet (see Photo 1) (1). Although it has a reduced potential for abuse versus classic benzodiazepine hypnotic/sedatives, it is a Schedule IV controlled substance, and federal law restricts it to prescription use. Based upon its potential for abuse, and the limited literature available concerning its analysis, herein are provided GC, GC/MS, FTIR-ATR, UPLC, and ¹H- and ¹³C-NMR data for eszopiclone.

Figure 1. Eszopiclone.

Photo 1. 3 Milligram Tablet
(Note: Diameter is 6.4 Millimeters).
**Experimental and Discussion**

**Eszopiclone**

Source: Sepracor Canada, LTD. (Windsor, Nova Scotia, Canada)
Lot Number / Purity: 029-0014 RS / 99.9 %
Chemical Formula / CAS Number: C17H17ClN6O3 / [138729-47-2]
Molecular Weight: 388.81 amu
Melting Point: 202 - 203 °C (2)
Solubility: [Chloroform: Soluble; Methanol: Somewhat Soluble; Deionized H2O: Somewhat Soluble]

**Gas Chromatography (GC)**

Instrument: Agilent 6890 equipped with a Flame Ionization Detector (FID)
Column: DB-1, 30 m x 0.25 mm I.D, 0.25 µm film thickness
Injector Temperature: 280 °C
Oven Temperature: 100 °C for 1 minute, 12 °C/minute to 280 °C, 7 minute hold
Carrier Gas: Hydrogen at 1.1 mL/minute, split ratio = 25:1

Approximately 8.95 milligrams and 8.63 milligrams of eszopiclone were added to 2 mL of methanol and 2 mL of chloroform, respectively, and vortexed until dissolved; eszopiclone took longer to dissolve in methanol than in chloroform. Utilizing the described experimental parameters, both solutions displayed a major chromatographic peak at 21.14 minutes. In addition, both solutions displayed the same chromatographic pattern with minor early eluting peaks - possibly due to eszopiclone breakdown. The chromatogram of the chloroform solution is illustrated in Figure 2.

**Gas Chromatography/Mass Spectrometry (GC/MS)**

Instrument: Agilent 6890 Gas Chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD)
Column: DB-1, 30 m x 0.25 mm I.D., 0.25 µm film thickness
Injector Temperature: 280 °C
Oven Temperature: 100 °C for 2 minutes, 14 °C/minute to 300 °C, 10 minute hold
Carrier Gas: Helium at 1.0 mL/minute, split ratio = 25:1
Scan Range: 34-550 amu
Electron Ionization: 70 eV

In methanol, one major peak at approximately 18 minutes was observed in the Total Ion Chromatogram (TIC) (Figure 3), with minor early eluting peaks as noted above. The fragmentation pattern shows a base peak at m/z 143 with other mass fragments at m/z 245, 99, 112, 217, 139, and 56 (Figure 4). The molecular ion was not detected.

**Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR)**

Instrument: Thermo-Nicolet Nexus 670 FTIR Spectrometer equipped with SensIR Dura-Scope Attenuated Total Reflectance (ATR) Accessory (1-Bounce Diamond/KRS-5 Focusing)

The eszopiclone standard was analyzed directly without preparation. Figure 5 (full spectrum) and Figure 6 (fingerprint region) illustrate the baseline-corrected spectra. The following is a list of assignments and corresponding wavenumbers (cm⁻¹): Aromatic C-H stretch (3077), aliphatic C-H stretch (2941, 2837, 2789), ester carbonyl stretch (1730), amide carbonyl stretch (1713), CH₂ bend (1462), CH₃ bend (1417), tertiary aromatic amine (1370), aliphatic C-N (1290, 1238, 1140), C-O stretch (1086), and C-Cl stretch (848) (3).
**Ultra Performance Liquid Chromatography (UPLC)**

**Instrument:** Waters ACQUITY Ultra Performance Liquid Chromatograph (UPLC) equipped with Waters 2996 Photo Diode Array (PDA) Detector

**Column:** 2.1 mm x 100 mm Waters ACQUITY UPLC BEH C18, 1.7 μm

**Mobile Phase:** A: 100 mM Phosphate buffer, pH 1.8; B: Acetonitrile

**Flow Rate:** 0.43 mL/minute

**Linear Gradient:** 98 % to 35 % A over 10 minutes, 35 % A for 2 minutes

A 100 mM phosphate buffer, pH 1.8, was added to 2.32 milligrams of eszopiclone until a 25.0 mL final volume was obtained. The solution was then sonicated for 15 minutes. Utilizing the above parameters, one peak at a retention time of 3.73 minutes was observed (Figure 7). Figure 8 illustrates the UV spectrum between the wavelengths 220 - 340 nm. The maximum UV absorbance is 301 nm.

**Nuclear Magnetic Resonance (NMR) Spectroscopy**

1H- and 13C-NMR spectra (see Figures 9 and 10, respectively) were acquired on a Varian Mercury Plus 400 MHz instrument using a Nalorac 5 mm indirect detect pulse field gradient (PFG) probe at 25 °C. (1H parameters: Number of scans (nt) = 8, pulse width (pw) = 45 °, relaxation delay (d1) = 5 s, acquisition time (at) = 2.5 s; 13C parameters: nt = 4098, pw = 45 °, d1 = 1 s, at = 1.3 s, complete proton decoupling). Spectra were processed using ACD's SpecManager software (Applied Chemistry Development Inc.©, Toronto, Canada). Eszopiclone was prepared in CDCl3 containing 0.05 % v/v tetramethylsilane (TMS; Aldrich Chemical Co., Milwaukee, WI) at 16.84 mg/mL. Chemical shifts (δ) are reported in parts per million (ppm) using TMS (0.0 ppm) as the reference standard. 1H data are reported as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant, number of protons present. 1H-NMR (400 MHz, CDCl3) δ 8.90 (d, J = 2.5 Hz, 1H), 8.85 (d, J = 2.5 Hz, 1H), 8.52 (d, J = 8.9 Hz, 1H), 8.40 (d, J = 2.4 Hz, 1H), 8.02 (s, 1H), 7.80 (d, J = 8.9, 2.5 Hz, 1H), 3.65 (br m, 1H), 3.54 (br m, 1H), 3.25 (br s, 2H), 2.42 (br m, 2H), 2.26 (s, 3H), 2.22 (br m, 1H), 2.05 (br m, 1H). 13C-NMR (100 MHz, CDCl3) δ 165.4, 162.9, 155.6, 153.4, 148.4, 147.8, 146.8, 143.9, 138.1, 133.4, 128.3, 116.1, 79.1, 54.5, 52.3, 46.1, 44.1.

**Acknowledgements**

The authors wish to thank Senior Forensic Chemist Patrick A. Hays (this laboratory), for his time and NMR expertise, and Senior Forensic Chemist Dr. Edward S. Franzosa (this laboratory), for his time and help with imaging the tablets.

**References**

Figure 2. Gas Chromatogram of Eszopiclone in Chloroform.

Figure 3. GC/MSD Total Ion Chromatogram of Eszopiclone.
Figure 4. Electron Ionization Mass Spectrum of Eszopiclone.

Figure 5. FTIR-ATR Spectrum of Eszopiclone.
Figure 6. FTIR-ATR Spectrum of Eszopiclone, Fingerprint Region.

Figure 7. Ultra Performance Liquid Chromatogram of Eszopiclone.
Figure 8. 220 - 340 nm UV Spectrum of Eszopiclone from UPLC. UV max = 301 nm.

Figure 9a. 400 MHz $^1$H-NMR Spectrum of Eszopiclone in CDCl$_3$ (See Next Page for Assignments).
Figure 9b. Assignments of Protons for Eszopiclone (See Figure 9a for the Spectrum).

Figure 10. 100 MHz $^{13}$C-NMR Spectrum of Eszopiclone in CDCl$_3$. 

* * * * *
Technical Note

Isolation of cis-Cinnamoylcocaine from Crude Illicit Cocaine via Alumina Column Chromatography

John F. Casale*, Enrique L. Piñero, and Elizabeth M. Corbeil
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email address withheld at author’s request]

ABSTRACT: The isolation procedure of gram quantities of cis-cinnamoylcocaine from crude cocaine base is provided. Isolation was achieved through classical alumina column chromatography and recrystallization. The procedure will enable forensic scientists to obtain a standard of cis-cinnamoylcocaine for cocaine signature analyses and related research.

KEYWORDS: cis-Cinnamoylcocaine, Column Chromatography, Isolation, Cocaine Signature Analyses, Forensic Chemistry

Introduction

Cocaine signature analyses have become routine in many forensic laboratories. These analyses are intended for both sample-to-sample comparison work (tactical intelligence) and geographic origin classification (strategic intelligence). Several chromatographic methods have been published over the past 15 years which utilize cis-cinnamoylcocaine (Figure 1), a naturally occurring product in coca, as one of the target compounds (1-6). Since cis-cinnamoylcocaine is not commercially available, standard material must be either synthesized or isolated from illicit cocaine. However, the synthesis procedure with followup purification by preparative high performance liquid chromatographic (HPLC), as reported by By, Lodge, and Sy (7), is problematic for forensic laboratories not staffed for synthetic work or equipped with a preparative HPLC. Similarly, the isolation from

![Figure 1. cis-Cinnamoylcocaine.](image-url)
illicit cocaine utilizing ion-pair chromatography, as reported by Moore (8), yields only milligram quantities and cannot be scaled up. Herein, we provide a simple chromatographic procedure for the isolation of gram quantities of cis-cinnamoylcocaine from crude cocaine base.

**Experimental**

**Materials:** A crude cocaine base exhibit containing approximately 13 percent cis-cinnamoylcocaine was acquired from the research collection of this laboratory. All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were of reagent-grade quality and were products of Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4 percent (w/w).

**Gas Chromatography/Mass Spectrometry (GC/MS):** Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100 °C; no hold, program rate, 6 °C/min; final temperature, 300 °C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280 °C. The auxiliary transfer line to the MSD was operated at 280 °C.

**Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR):** Spectra were obtained on a Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Spectra were collected using 32 scans between 4000 cm⁻¹ and 400 cm⁻¹ at a resolution of 4 cm⁻¹.

**Proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR):** Spectra were obtained on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac indirect detection, variable temperature, pulse field gradient probe with PulseTune® (Varian, Palo Alto, CA). The compound was dissolved in deuterochloroform (CDCl₃) containing 0.03 percent v/v tetramethylsilane (TMS) as the 0 ppm reference. The temperature of the sample was maintained at 25 °C. Standard Varian pulse sequences were used to acquire the proton spectra. Processing of data was performed using software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

**Isolation of cis-Cinnamoylcocaine:** Crude cocaine base (170 grams containing approximately 13 percent cis-cinnamoylcocaine) was dissolved into one liter of warm diethyl ether/hexane (1:1) and eluted on a glass chromatographic column (100 cm x 5.5 cm ID) containing 1.0 kilogram of basic alumina (150 mesh). The column was then eluted with 1.0 liter of diethyl ether, followed by 1.0 liter of diethyl ether/chloroform (1:1). The bulk of the cis-cinnamoylcocaine was contained in the diethyl ether fractions. The combined diethyl ether fractions were evaporated in vacuo to an oil (34 grams of 55 percent cis-cinnamoylcocaine), which was chromatographed again on 1.0 kilogram of basic alumina (same size column) using the following series of solvents: 500 mL diethyl ether/hexane (1:2), 500 mL diethyl ether/hexane (1:1), 500 mL diethyl ether/hexane (2:1), 500 mL diethyl ether/hexane (5:1), and 1500 mL diethyl ether. The diethyl ether/hexane (5:1) and 1500 mL diethyl ether fractions were then combined and evaporated in vacuo to a light yellow oil (9.9 grams of 88 percent cis-cinnamoylcocaine). The resulting oil was chromatographed again on 1.0 kilogram of basic alumina (same size column) using 500 mL diethyl ether/hexane (1:2), 500 mL diethyl ether/hexane (1:1), 500 mL diethyl ether/hexane (2:1), 500 mL diethyl ether/hexane (5:1), and 2000 mL diethyl ether. The first 750 mL of the diethyl ether fractions were combined and evaporated in vacuo to a clear oil (7.0 grams of 96 percent cis-cinnamoylcocaine) which crystallized slowly upon standing. The product was recrystallized from diethyl ether/petroleum ether (20 - 40 °C) to give 6.17 grams of 99 percent pure cis-cinnamoylcocaine as a white solid (28 percent recovery).
Results and Discussion

Crude cocaine base contains (mostly) cocaine, lesser amounts of both cis- and trans- cinnamoylcocaine, numerous other tropane alkaloids, and various processing impurities and byproducts. Under the described chromatographic procedures, cocaine and trans-cinnamoylcocaine predominate in the hexane/diethyl ether fractions and elute prior to cis-cinnamoylcocaine. Although cocaine and trans-cinnamoylcocaine have some carryover, cis-cinnamoylcocaine is enriched significantly in the diethyl ether fractions. More polar cocaine impurities such as norcocaine, egnonine, and benzoylecgonine are retained by the alumina column. Two additional alumina column passes of the enriched cis-cinnamoylcocaine, followed by recrystallization, were sufficient to give an analytically pure (99 percent or better) sample. The FTIR-ATR, 1H-NMR, and GC/MS spectra are illustrated in Figures 2 - 4, respectively. The reported procedure can be utilized to isolate cis-cinnamoylcocaine even from refined illicit cocaine exhibits containing as little as 3 percent cis-cinnamoylcocaine. Samples of cis-cinnamoylcocaine should be stored in amber glass bottles or in a dark location, as observations suggest that isomerization to trans-cinnamoylcocaine occurs over extended periods of time.

Acknowledgments

The authors are indebted to Senior Forensic Chemist Patrick A. Hays (this laboratory) for his assistance in acquiring the NMR data.

References


[Figures 2 - 4 Follow.]
Figure 2. Infrared Spectrum (FTIR-ATR) of cis-Cinnamoylcocaine.

Figure 3. Proton NMR Spectrum of cis-Cinnamoylcocaine.
Figure 4. Gas Chromatography/Mass Spectra (70 eV EI) of cis-Cinnamoylcocaine.
The Characterization of 4-Methoxy-N-ethylamphetamine Hydrochloride

John F. Casale*, Patrick A. Hays, Trinette K. Spratley, and Pamela R. Smith
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA  20166
[email address withheld at author’s request]

ABSTRACT: The synthesis, analysis, and characterization of 4-methoxy-N-ethylamphetamine hydrochloride is presented. Analytical data (gas chromatography/mass spectrometry, Fourier transform infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: 4-Methoxy-N-ethylamphetamine, Phenethylamine, Synthesis, Analysis, Forensic Chemistry

Introduction

In late 2004, this laboratory received a white crystalline substance submitted as an unknown suspected phenethylamine (seizure exhibit) for identification and characterization. It was thought that this compound might be one of the many esoteric phenethylamine “designer drugs” described in PIHKAL (1). Preliminary screening indicated that the sample contained a single component and was relatively pure. Utilizing proton nuclear magnetic resonance (1H-NMR) spectroscopy and a computerized structural elucidation program, the compound was tentatively identified as 4-methoxy-N-ethylamphetamine hydrochloride (Figure 1). Surprisingly, this compound is not detailed in PIHKAL, and furthermore has few literature citations, including on websites dedicated to drug abuse. It therefore constitutes a new phenethylamine-type “designer drug.” For this reason, and also to confirm the tentative identification via direct spectral comparisons, it was synthesized and fully characterized via gas chromatography/mass spectrometry (GC/MS), Fourier transform infrared spectroscopy (FTIR), and 1H-NMR spectroscopy.

Figure 1. 4-Methoxy-N-ethylamphetamine.
**Experimental**

**Chemicals, Reagents, and Materials:** All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI). 4-Methoxyamphetamine HCl (the starting material for the synthesis) was acquired from the reference collection of this laboratory.

**Gas Chromatography/Mass Spectrometry (GC/MS):** Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100 °C; no hold, program rate, 6 °C/min; final temperature, 300 °C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280 °C. The auxiliary transfer line to the MSD was operated at 280 °C.

**Infrared Spectroscopy (FTIR-ATR):** Spectra were obtained on a Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Spectra were collected using 32 scans between 4000 cm\(^{-1}\) and 400 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\).

**Proton Nuclear Magnetic Resonance Spectroscopy (\(^1\)H-NMR):** Spectra were obtained on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac indirect detection, variable temperature, pulse field gradient probe with PulseTune® (Varian, Palo Alto, CA). The compound was dissolved in deuterium oxide (D\(_2\)O) containing 0.05 percent (by weight) 3-(trimethylsilyl)propionic-2,2,3,3-d\(_4\) acid, sodium salt (TSP) as a 0 ppm reference and 5 mg/mL maleic acid as the quantitative internal standard. The temperature of the sample was maintained at 25 °C. Standard Varian pulse sequences were used to acquire proton, proton-decoupled carbon, and gradient versions of COSY, HSQC, and HMBC. Processing of data was performed using software from Varian and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Structural elucidation was performed manually and by using ACD/Labs Structure Elucidator® software.

**Syntheses:**

4-Methoxy-N-acetylamphetamine: 4-Methoxyamphetamine HCl (5.00 grams, 24.8 mmol) was dissolved into 25 mL of water in a 500-mL Erlenmeyer flask, followed by addition of 250 mL of saturated aqueous sodium bicarbonate, with stirring for several minutes. Acetic anhydride (21.6 grams, 211 mmol) was then added slowly and stirred for 2 hours at room temperature. The reaction was extracted with methylene chloride (3 x 100 mL). The extracts were combined, dried over anhydrous sodium sulfate, and evaporated in vacuo to give 4-methoxy-N-acetylamphetamine as a light yellow oil (5.0 grams, 99 percent purity, 97.5 percent yield).

4-Methoxy-N-ethylamphetamine HCl: A flame-dried 1-liter round bottom flask fitted with an addition funnel and water-cooled condenser was charged with 100 mL diethyl ether containing 1.0 M LiAlH\(_4\) (100 mmol). Approximately 75 mL of anhydrous diethyl ether containing 4-methoxy-N-acetylamphetamine (5.0 grams, 24.2 mmol) was added dropwise over 30 minutes, followed by an additional 125 mL of anhydrous diethyl ether, and the mixture was refluxed overnight. The reaction was quenched slowly, in sequence, with 4.0 mL of water, 4.0 mL of 15 percent aqueous NaOH, and 12 mL of water, and was then stirred for approximately 30 minutes. The lithium and aluminum salts were removed via suction filtration through a Celite pad, which was washed with an additional 100 mL diethyl ether. The filtrate was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give a clear oil. The oil was reconstituted in 35 mL isopropanol, treated with isopropanolic HCl until pH 5, and then diluted to approximately 800 mL with diethyl ether. The resulting precipitate was collected via suction filtration, washed with additional diethyl ether to remove traces of excess HCl, and desiccated under vacuum to remove residual solvent to give 4-methoxy-N-ethylamphetamine HCl as a white crystalline powder (3.22 grams, 58 percent yield).
Results and Discussion

Independent synthesis, spectral characterization, and comparison of authentic 4-methoxy-N-ethylamphetamine HCl to the submitted unknown confirmed its identity. The infrared spectrum (Figure 2) displays an absorbance pattern that is consistent with a secondary amine halogen (HCl) ion-pair and a para disubstituted aromatic ring. The mass spectrum (Figure 3) gives fragments at m/z 72 (base peak), 121, and 192, all consistent with a methoxy-substituted-N-ethylamphetamine. The 1H-NMR spectrum (Figure 4) exhibited two doublets at 7.0 and 7.3 ppm, integrating to 2 protons each, typical of a para substituted benzene. The singlet at 3.8 ppm integrates to 3 protons and corresponds to the methoxy group (supported by 13C-NMR (not shown)). The multiplet at 3.5 ppm integrates to 1 proton and corresponds to the methine (which is bonded to the methyl group (a doublet at 1.2 ppm integrating to 3 protons), the methylene group (2 doublet of doublets at 2.8 and 3.1 ppm integrating to 1 proton each), and the nitrogen). The methylene proton chemical shifts (2.8 and 3.1 ppm) confirm that they are bonded to the benzene ring. The remaining proton peaks (the multiplet at 3.2 ppm integrating to 2 protons and the triplet at 1.3 ppm integrating to 3 protons) are of the N-ethyl group. The spectrum peaks below 3.6 ppm are, as expected, nearly identical to those of MDEA (Figure 5).

The starting material used in this synthesis (4-methoxyamphetamine, also known as “PMA”) is itself a controlled substance that is abused worldwide (especially in North America and Europe); therefore, it is quite unlikely that the synthetic procedure described herein was utilized by the original clandestine chemist - nor is it likely to be utilized in the future by any clandestine chemists. The choice of this synthetic route was based on convenience, since 4-methoxyamphetamine was available from this laboratory’s reference collection. Although not investigated, the clandestine chemist in this case probably started his synthesis from 4-methoxyphenyl-2-propanone. It is doubtful whether he intended to make 4-methoxy-N-ethylamphetamine, as comments made in PIHKAL concerning the homologous compound 4-methoxy-N-methylamphetamine (also known as 4-methoxymethamphetamine, PMMA) would suggest that 4-methoxy-N-ethylamphetamine is only a moderate stimulant with minimal (if any) hallucinogenic properties. In addition, both PMA and PMMA are toxic compounds that have been implicated in numerous deaths over the past four decades, and it is likely that 4-methoxy-N-ethylamphetamine would display similar toxicity.

Conclusions

The gas chromatography and infrared and mass spectra of 4-methoxy-N-ethylamphetamine are expected to be similar to its 2- and 3-methoxy substituted analogs. 1H-NMR provides unequivocal identification. Although quite unlikely, if this compound becomes more common in illicit markets, the acronym “PMEA” is an obvious choice.

References


[Figures 2 - 5 Follow.]
Figure 2. Infrared Spectrum (FTIR-ATR) of 4-Methoxy-N-ethylamphetamine HCl.

Figure 3. Gas Chromatography/Mass Spectra of 4-Methoxy-N-ethylamphetamine; Normalized (Upper Spectrum) and Enhanced 10x (Lower Spectrum).
Figure 4. $^1$H-NMR Spectrum of 4-Methoxy-N-ethylamphetamine HCl.

Figure 5. $^1$H-NMR Spectrum of 4-Methoxy-N-ethylamphetamine HCl and 3,4-Methylenedioxy-N-ethylamphetamine (MDEA).
Quantitation of Cocaine by Gas Chromatography-Flame Ionization Detection Utilizing Isopropylcocaine as a Structurally Related Internal Standard

Enrique L. Piñero* and John F. Casale
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[Email address withheld at author’s request]

ABSTRACT: The quantitation of cocaine by gas chromatography-flame ionization detection using isopropylcocaine as a structurally related internal standard is presented. The selectivity, precision, and accuracy of the method are detailed. The facile, multi-gram synthesis of isopropylcocaine standard from cocaine (via two different routes) is described.

KEYWORDS: Isopropylcocaine, Synthesis, Gas Chromatography, Flame Ionization Detection, Cocaine Quantitation, Internal Standard, Forensic Chemistry

Introduction

The analysis of cocaine exhibits has been a major task in forensic, crime, and toxicological laboratories over the past 20 - 25 years. Federal Sentencing Guidelines (1), as well as some state criminal statutes, require quantitative analysis of cocaine exhibits. In addition, the accurate assay of cocaine is also a critical element for laboratories that are conducting cocaine signature analyses (2-3). In the DEA's Cocaine Signature Program (CSP), target alkaloids are quantitated relative to the amount of cocaine present, not to the total sample weight. Therefore, even samples that are cut, either with an adulterant or a diluent, can still be analyzed for signature purposes as though they were uncut. However, this technique requires highly accurate quantitations of all of the target alkaloids.

Several gas chromatographic methods have been developed and validated for cocaine quantitation (4-8). These methods utilize an internal standard (ISTD) to improve the precision of the quantitative analysis; however, the ISTDs utilized in these studies (tetraphenylethylene, morphine HCl, cyclobenzapine HCl, methylpalmitate, or eicosane) are not structurally related to cocaine, and in fact in most instances have dissimilar chemical and physical properties. Thus, the presence of impurities, possible acid hydrolysis of cocaine (9), dirty injection ports, and the formation of artifacts (10), can decrease the accuracy of the assay (11). The use of a structurally related internal standard (SR-ISTD) minimize the factors that affect the resulting analyte signal (in this case cocaine), since the SR-ISTD will have virtually the same response to the detector (3). The gas chromatographic method presented herein employs isopropyl cocaine as the SR-ISTD. Isopropylcocaine is not commercially available; however, also as described herein it can be synthesized from cocaine and commercially available reagents (see Figure 1, next page).

Experimental

Materials: Pharmaceutical cocaine base and hydrochloride were obtained from Merck Chemical (Rahway, NJ). Chloroform was a distilled-in-glass product of Burdick and Jackson Laboratories (Muskegon, MI). All other...
Figure 1. Synthetic Routes to Isopropylcocaine.
reagents and chemicals were reagent-grade quality products of the Sigma-Aldrich Chemical Company (Milwaukee, WI). Illicit refined cocaine HCl was acquired from the reference collection of this laboratory.

Syntheses (Acid Hydrolysis Route):

Ecgonine HCl: Refined illicit cocaine HCl (250 grams, 0.736 mol) was combined with water (500 mL) and concentrated hydrochloric acid (25.0 mL) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was gently refluxed, with stirring, for 6 days. Benzoic acid precipitated from the solution upon cooling. The reaction mixture was extracted with chloroform (5 x 500 mL) to remove benzoic acid and methyl benzoate. The aqueous phase was then added slowly, with stirring, to acetone (7.2 liters) to precipitate ecgonine HCl. The precipitate was captured via suction filtration, washed with additional acetone (1.5 liters), then dried to provide ecgonine HCl as a white powder (107 grams, 65 percent yield).

Ecgonine Isopropyl Ester HCl: Ecgonine HCl (40.0 grams, 0.18 mol) was combined with isopropanolic HCl (2.0 liters, 0.14 grams/mL) in a 5-liter round-bottom flask fitted with a water-cooled condenser. The solution was gently refluxed with stirring, for 3 days. The isopropanol was evaporated in vacuo to an oil. The oil was dissolved in water (500 mL), adjusted to pH 10 with concentrated NaOH, and extracted with methylene chloride (3 x 200 mL). The combined extracts were washed with water (3 x 400 mL) and brine (200 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to a clear oil (42.0 grams). The oil was dissolved in anhydrous diethyl ether (500 mL), and isopropyl ecgonine HCl was precipitated by adding ethereal HCl (0.05 grams/mL) until a pH of 4 was achieved. The ether was decanted from the crystalline product, and acetone (400 mL) added with stirring. The product was captured via suction filtration, washed with additional acetone (400 mL) and diethyl ether (400 mL), then dried to provide ecgonine isopropyl ester HCl as a white powder (32.0 grams, 67 percent yield).

Isopropylecocaine: Ecgonine isopropyl ester HCl (31.1 grams, 0.118 mol) was combined with pyridine (200 mL) and benzoyl chloride (19.8 grams, 0.142 mol) in a 1-liter round-bottom flask fitted with a drying tube. After stirring for 1 hour, acetone (400 mL) was added to precipitate isopropylecocaine HCl. The product was captured via suction filtration, washed with additional acetone (2 x 200 mL) and diethyl ether (200 mL), then dried to provide isopropylecocaine HCl as a white powder containing a small amount of pyridine HCl. The product was dissolved in water (100 mL), adjusted to pH 9 with solid sodium carbonate, then extracted with methylene chloride (0.05 grams/mL) until a pH of 4 was achieved. The ether was decanted, and the aqueous phase was washed with methylene chloride (3 x 200 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to provide isopropylecocaine HCl as a white crystalline powder (31.6 grams, 81 percent yield, 99+ percent purity).

Syntheses (Base Hydrolysis Route):

Benzoylecgonine: Pharmaceutical cocaine base (70.6 grams, 0.233 mol) was combined with water (250 mL) and dioxane (350 mL) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was heated at 55 °C for 9 days. The reaction mixture was evaporated in vacuo to provide crude benzoylecgonine tetrahydrate as a white powder. The powder was washed with diethyl ether (2 x 400 mL) to remove any remaining cocaine base, then dried to give 67.5 grams of benzoylecgonine tetrahydrate. The product was dissolved in boiling acetone (750 mL), cooled to room temperature, diluted with diethyl ether (2.25 liters), and allowed to stand overnight at 5 °C. The resulting crystalline product was captured via suction filtration, washed with additional diethyl ether (600 mL), then dried to provide anhydrous benzoylecgonine as a white powder (53.5 grams, 79 percent yield).

Isopropylecocaine: Anhydrous benzoylecgonine (30.7 grams, 0.106 mol) was combined with methylene chloride (500 mL) and 1',1'-carbonyldimidizole (17.2 grams, 0.106 mol) in a 1-liter round-bottom flask fitted with a drying tube, and stirred overnight. Isopropanol (26.8 grams, 0.447 mol) was added, and the solution was stirred for 6 days. The reaction was extracted with 3 N HCl (2 x 200 mL). The combined aqueous extracts were washed with methylene chloride (200 mL), adjusted to pH 9 with concentrated ammonium hydroxide, and extracted with methylene chloride (3 x 200 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give a clear oil (30.0 grams). The oil was dissolved in petroleum
ether (20 - 40°C boiling range, 300 mL) and allowed to stand overnight, resulting in precipitation of the imidizole by-product. The solution was suction filtered to remove this byproduct, and the filtrate evaporated in vacuo to give a clear oil which crystallized upon standing. This was recrystallized from petroleum ether, then dried to provide isopropylcocaine base as a white powder (23.3 grams, 66 percent yield, 99+ percent purity).

Gas Chromatography - Flame Ionization Detection (GC-FID): Analyses were performed with an Agilent (Palo Alto, CA) Model 6890N gas chromatograph. One mL of the prepared solutions was placed into an autosampler vial for analysis under the following conditions: A 30 m x 0.25 mm ID fused-silica column coated with 0.25 μm HP-1 (Agilent) was used. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/minute. The injection port and flame ionization detector were maintained at 280 °C. Samples (2 μL) were injected in the split mode (25:1) by an Agilent 7683 Series Auto Injector. The oven temperature was programmed isothermally at 250 °C for 7.00 minutes. Nitrogen was used as the auxiliary make-up gas for the detector.

Structurally Related Internal Standard Stock Solution: Isopropylcocaine base was dissolved into chloroform at a concentration of 0.9 mg/mL (equivalent to 1.0 mg/mL isopropylcocaine hydrochloride). The solution was stored at 4 °C when not in use. Solutions can be stored for one year at 4 °C without detectable degradation. The solution should be warmed to room temperature before use.

Standard Solutions for Linearity Study and Calibration: Individual solutions containing 0.038, 0.087, 0.23, 0.44, 0.63, 0.83, 1.00, 1.53, and 2.03 mg/mL of cocaine base in chloroform were prepared. Each also contained 0.18 mg/mL of the SR-ISTD.

Standard and Sample Preparation: About 18 to 20 mg of cocaine hydrochloride (or 16 to 18 mg for cocaine base) was accurately weighed (to the nearest 0.01 mg) into a 50 mL Erlenmeyer flask, and 5.0 mL of the SR-ISTD stock solution and 20 mL of chloroform containing 50 μL of diethylamine were added. The solutions were allowed to sit for 5 minutes. Aliquots of standard and sample solutions were transferred to autosampler vials for analyses.

Results and Discussion

The synthesis of isopropylcocaine is relatively simple, and can be performed on a large scale with common glassware and reagents. The mass spectrum of isopropylcocaine is illustrated in Figure 2. Isopropylcocaine was selected as the SR-ISTD for several reasons. Its close structural similarity to cocaine means it will have a similar FID response. Second, it has excellent chromatographic properties, and does not interfere with any other coca alkaloids or commonly encountered diluents and adulterants (see Figure 3 for chromatographic profiles of illicit cocaine base and illicit cocaine HCl). Third, only a small amount (about 5 mg) is needed for each analysis. Fourth and finally, it was found to be very stable. A stock solution stored for up to one year at 4 °C in chloroform yielded no detectable hydrolysis or degradation products, and produced the same number of integrated area counts over that entire time frame.

The linearity of the method was confirmed over the concentration range listed in the Experimental Section, and linear regression analysis determined the correlation coefficient (R²) as 0.9999 (Figure 4). The average error difference between the known concentrations and the predicted concentrations was +/- 0.75 percent between 0.44 mg/mL and 1.53 mg/mL. For routine analyses, a single point calibration of approximately 0.75 mg/mL was used. Method selectivity was excellent; the identities and retention times of some common adulterants and diluents using the presented methodology are shown in Table 1 (reported retention times are relative to cocaine). Other coca alkaloids and common cutting agents do not interfere with cocaine or isopropylcocaine. The precision of the method was determined using the nine linearity concentrations, with five replicate injections per concentration. The resulting calculated Relative Standard Deviation (RSD) for each concentration was less than 0.21 percent, and in some instances was as low as 0.02 percent. The accuracy of the method was tested over a 14 month period by having eleven different chemists quantitate a secondary cocaine standard (having a known cocaine

Brought to you by AltGov2 [www.altgov2.org]
concentration of 84.6 percent) against the pharmaceutical cocaine standard during routine casework. Over that time period, 188 quantitative observations for the secondary standard were recorded. The average value obtained was 84.7 percent, with a range of 83.4 - 86.0 percent. The RSD for all 188 observations was found to be 0.58 percent. The overall absolute error of the assay was determined to be less than 1 percent.

Acknowledgments

The authors wish to thank Supervisory Forensic Chemist Valerie Colley (this laboratory) for providing assistance with the analytical data.

References


[Table 1 and Figures 2 - 4 Follow.]
Table 1. Relative Retention Times (RRT) of Some Common Adulterants/Diluents and Coca Alkaloids.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RRT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecgonine methyl ester</td>
<td>0.48</td>
</tr>
<tr>
<td>Benzocaine</td>
<td>0.49</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.52</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.61</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>0.65</td>
</tr>
<tr>
<td>Procaine</td>
<td>0.76</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Isopropylcocaine</strong></td>
<td><strong>1.14</strong></td>
</tr>
<tr>
<td>cis-Cinnamoylcocaine</td>
<td>1.36</td>
</tr>
<tr>
<td>trans-Cinnamoylcocaine</td>
<td>1.78</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Figure 2. Electron Ionization Mass Spectrum of Isopropylcocaine.
Figure 3. Capillary Gas Chromatographic Profiles of (Upper) 86.1 Percent Illicit Cocaine Base Exhibit and (Lower) 86.2 Percent Illicit Cocaine HCl Exhibit. Peak Identification: 1 = Tropacocaine, 2 = Norcocaine, 3 = Cocaine, 4 = Isopropylcocaine (SR-ISTD), 5 = cis-Cinnamoylcocaine, and 6 = trans-Cinamoylcocaine.

Figure 4. Calibration Curve for the Isopropylcocaine Internal Standard Methodology.
Dehydrochlormethyltestosterone: An Analytical Profile

Eric S. Wisniewski*
U.S. Department of Justice
Drug Enforcement Administration
Mid-Atlantic Laboratory
1440 McCormick Dr.
Largo, MD 20774
[email address withheld at author’s request]

Patrick A. Hays
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166

[Previously Reported in Part as an Intelligence Alert; see: Microgram Bulletin 2006;39(7):87.]

ABSTRACT: Analytical data (GC, GC/MS, FTIR, HPLC, ¹H- and ¹³C- NMR) for the analysis and identification of dehydrochlormethyltestosterone ((17β)-4-chloro-17-hydroxy-17-methylandrosta-1,4-dien-3-one) is presented. Historical background is also included.

KEYWORDS: Dehydrochlormethyltestosterone, Chlorodehydromethyltestosterone, Turanabol, Turinabol, Anabolic Steroid, Controlled Substance, Analysis, Forensic Chemistry

Introduction

The Drug Enforcement Administration Mid-Atlantic Laboratory recently received a submission of steroids and steroid-related exhibits that were seized during a consent search of a residence in Winchester, Virginia. The exhibits included 15 bottles, each labeled “Turanabol,” “Chlorodehydromethyltestosterone,” and “Golden Triangle Pharmaceuticals” (see Photo 1). Despite identical appearances (same bottle type, labeling, lot number, and number of tablets (100)), six of the bottles contained nondescript orange capsules while nine bottles contained nondescript yellow capsules (see Photo 2, next page). Subsequent analyses confirmed that the orange capsules contained dehydrochlormethyltestosterone as the only active ingredient, while the yellow capsules contained primarily dehydrochlormethyltestosterone with minor amounts of stanozolol and methandrostenolone (see structures, next page). This is believed to be the first submission of dehydrochlormethyltestosterone to the DEA laboratory system (1).

Dehydrochlormethyltestosterone is a Schedule III controlled substance in the United States and is also listed in the 2006 Prohibited List/World Anti-Doping Code. It gained notoriety as a result of the East German Olympic doping scandals that were fully exposed after the fall of the Berlin wall (2). Data from
East German medical personnel involved in the doping indicated that dehydrochlormethyltestosterone produced dramatic increases in speed and strength, but with detrimental side effects such as deepening of the voice, increased acne, and body hair growth. Other, long term side-effects ranged from liver damage to severe gynecological disorders (2). This steroid is no longer legitimately produced, and appears to be available only as an illicitly-prepared product on the black market.
As with nearly all anabolic steroids, dehydrochlormethyltestosterone has multiple name variations, including (but not limited to): Dehydrochloromethyltestosterone, chloredheydromethyltestosterone, chlorodehydromethyltestosterone, 4-chlorodehydromethyltestosterone, 4-chloromethandienone, 4-chlor-1-dehydro-17α-methyltestosterone, 1,4-androstadien-4-chloro-17α-methyl-17β-ol-3-one, and 4-chloro-17β-hydroxy-17α-methylandrost-1,4-dien-3-one. The most common trade name for dehydrochlormethyltestosterone, Oral-Turinabol®, is often abbreviated as “OT” in both the scientific literature and on internet websites dedicated to anabolic steroid use/abuse (2,3).

Not surprisingly, most of the scientific literature dedicated to the analysis of dehydrochlormethyltestosterone has a toxicological focus (that is, analysis of biological fluids for dehydrochlormethyltestosterone metabolites for detection of doping (4,5). Although there are a number of reports of submissions of dehydrochlormethyltestosterone to forensic and crime laboratories (6), complete forensic analysis of dehydrochlormethyltestosterone has not been previously reported, and even standard reference texts in the field (e.g., 7,8,9) do not contain data for this compound. Herein, we report analytical data (GC, GC/MS, FTIR-ATR, HPLC, and 1H- and 13C- NMR) for the analysis and identification of the title steroid. In addition, because this is the first comprehensive report for this steroid, an in-depth analysis of the NMR data is presented.

**Experimental**

**Standard:** A reference standard of dehydrochloromethyltestosterone was obtained from Steraloids (Newport, RI).

**Gas Chromatography (GC):** GC screening was conducted using an Agilent 6890N (Waldbrom, Germany) equipped with flame ionization detector (FID). The sample was dissolved in methanol and injected into the instrument using the parameters below.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Agilent 6890N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>HP-5 (5 % phenyl/95 % methyl silicone); 12 m x 0.2 mm i.d. x 0.33 μm thickness</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>Helium at 1.0 mL/min</td>
</tr>
<tr>
<td>Temperatures</td>
<td>Injector: 270 °C</td>
</tr>
<tr>
<td></td>
<td>Detector: 280 °C</td>
</tr>
<tr>
<td></td>
<td>Oven Program: 175 °C for 1 min</td>
</tr>
<tr>
<td></td>
<td>15 °C/min to 280 °C</td>
</tr>
<tr>
<td></td>
<td>Hold at 280 °C for 4 min</td>
</tr>
<tr>
<td>Injection Parameters</td>
<td>Split ratio = 60:1, 1 mL injected</td>
</tr>
</tbody>
</table>

**Gas Chromatography/Mass Spectrometry (GC/MS):** An Agilent 6890N gas chromatograph equipped with an Agilent 5973 Mass Selection Detector (MSD) (Waldbrom, Germany) was used in the electron ionization (EI) mode to obtain mass spectra of samples and standards. Instrumental parameters are listed below. Agilent’s MS Interpreter (Version 0.9) was used to derive the relative abundances of the molecular ion cluster.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Agilent 6890N with Agilent 5973 Mass Selection Detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>HP-5MS (5 % phenyl/95 % methyl silicone); 15 m x 0.25 mm x 0.25 μm thickness</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>Helium at 1.0 mL/min</td>
</tr>
<tr>
<td>Temperatures</td>
<td>Injector: 280 °C</td>
</tr>
<tr>
<td></td>
<td>Oven Program: 150 °C for 0.5 min</td>
</tr>
<tr>
<td></td>
<td>30 °C/min to 300 °C</td>
</tr>
<tr>
<td></td>
<td>Hold at 300 °C for 1.5 min</td>
</tr>
<tr>
<td>Injection Parameters</td>
<td>Split ratio = 75:1, 1 mL injected</td>
</tr>
<tr>
<td>Detector</td>
<td>Quadrupole Mass Detector</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------</td>
</tr>
</tbody>
</table>
| Temperatures | Transfer Line: 280 °C  
                MS Quad: 150 °C  
                MS Source: 230 °C |
| Acquisition Mode | Scan |
| Solvent Delay Time | 0.5 minutes |
| Scan Parameters | Mass Range: 40 - 450 amu  
                  Sample #: 3 (2n = 8 samples taken at each mass)  
                  Resulting Scan Rate = 1.84 scans/sec |

**Fourier Transform Infrared Spectrometry - Attenuated Total Reflectance (FTIR-ATR):** Infrared spectroscopy was performed using a Thermo Nicolet Nexus 670 Fourier Transform Infrared Spectrometer (FTIR) (Madison, WI) equipped with a Golden Gate Attenuated Total Reflectance (ATR) detector. The sample was prepared by extraction of the capsule matrix with methanol followed by evaporation. The IR spectrum was collected by averaging 24 scans with a resolution of 4.0 wavenumbers (cm⁻¹).

**High Performance Liquid Chromatography (HPLC):** HPLC was conducted using an Agilent 1100 Series instrument (Waldbronn, Germany) using ultraviolet (UV) detection. The sample was dissolved in methanol and injected into the instrument using the parameters below (10).

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Agilent 1100 Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Waters Xterra RP18 (4.6 x 150 mm, 3.5 mm)</td>
</tr>
</tbody>
</table>
| Mobile Phase | 80 % Water (W): 20 % Acetonitrile (A) hold for 3 min  
               Ramp to 55 % W: 45 % A for 2 min and hold for 8 min  
               Ramp to 35 % W: 65 % A for 3 min and hold for 10 min  
               Ramp to 10 % W: 90 % A for 5 min and hold for 9 min |
| Temperature | 45 °C |
| Detection Wavelength | 225 nm |
| Injection Volume | 5 mL |
| Injection Solvent | Methanol |

**Nuclear Magnetic Resonance (NMR) Spectroscopy:** One and two dimensional (1D and 2D) NMR experiments were performed on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac pulse field gradient (PFG) indirection detection probe (Varian Inc., Palo Alto, CA). Standard Varian pulse sequences were employed. The sample and standard were prepared in deuterated methanol (CD₃OD) with tetramethylsilane (TMS) added (approximately 0.05 % v/v) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, WI). The ¹H-NMR spectrum of the standard was obtained with 8 scans using a 45 second delay, 90 ° pulse, 2 second acquisition time, and oversampling of 6. The ¹³C-NMR spectrum of the standard was obtained with proton decoupling; 2,000 scans were acquired, using a 1 second delay, 45 ° pulse, 1.2 second acquisition time, and oversampling of 3. Samples were maintained at 25 °C. Standard Varian gradient versions of 2D NMR experiments were performed to help make assignments, including homonuclear COSY (2 - 4 bond proton-proton through bond correlations), NOESY (proton - proton spatial nearness correlations for protons < 4 angstroms apart), heteronuclear HSQC (proton to directly bonded carbon correlations), and HMBC (2, 3, or 4 bond proton to carbon correlations). Structural elucidation was performed utilizing Applied Chemistry Developments (ACD/Labs, Toronto, Canada) software (HNMR Predictor, CNMR predictor, and Structure Elucidator).
Results and Discussion

Gas Chromatography (GC): The chromatogram is not shown. Methandrostenolone, dehydrochlormethyltestosterone, and stanozolol eluted at 7.96, 9.32, and 10.29 minutes, respectively. The peak shape for stanozolol was broad in comparison to the other steroids. The mixture in the yellow tablets was not formally quantitated, but was estimated as roughly 100 : 5 : 2.5 dehydrochlormethyltestosterone : methandrostenolone : stanozolol. Table 1 lists the relative retention times for cocaine, heroin, and six other steroids with similar chromatography.

<table>
<thead>
<tr>
<th>Drug (GC)</th>
<th>RRt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>0.580</td>
</tr>
<tr>
<td>Mesterolone</td>
<td>0.813</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.822</td>
</tr>
<tr>
<td>Heroin</td>
<td>0.829</td>
</tr>
<tr>
<td>Methyltestosterone</td>
<td>0.836</td>
</tr>
<tr>
<td>Methandrostenolone</td>
<td>0.854</td>
</tr>
<tr>
<td>Testosterone Acetate</td>
<td>0.882</td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>0.990</td>
</tr>
<tr>
<td><strong>Dehydrochlormethyltestosterone</strong></td>
<td><strong>1.000</strong></td>
</tr>
<tr>
<td>Stanozolol</td>
<td>1.103</td>
</tr>
<tr>
<td>Testosterone Isocaproate</td>
<td>1.188</td>
</tr>
</tbody>
</table>

Gas Chromatography/Mass Spectrometry (GC/MS): The mass spectra of dehydrochlormethyltestosterone, methandrostenolone, and stanozolol are shown in Figures 1 - 3, respectively. Dehydrochlormethyltestosterone displayed a molecular ion at m/z 334. Analysis of the molecular ion cluster (i.e., for C_{20}H_{27}O_{2}Cl) revealed close agreement with the theoretical values obtained from the MS Interpreter program, confirming the molecular formula and the presence of a chlorine atom (Table 2). Of note, the spectra did not give a satisfactory match with any compound in the instrument’s database, indicating both that the compound is not a different steroid and that dehydrochlormethyltestosterone is not entered.

<table>
<thead>
<tr>
<th>Mass (amu)</th>
<th>Theoretical (Relative Abundance)</th>
<th>Experimental (Relative Abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>334</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>335</td>
<td>22.73</td>
<td>22.87</td>
</tr>
<tr>
<td>336</td>
<td>34.84</td>
<td>34.92</td>
</tr>
<tr>
<td>337</td>
<td>7.53</td>
<td>7.41</td>
</tr>
<tr>
<td>338</td>
<td>0.93</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Fourier Transform Infrared Spectrometer - Attenuated Total Reflectance (FTIR-ATR): The IR spectrum of the reference standard is shown in Figure 4. The spectrum displayed major absorbances for O-H (3485 cm⁻¹), C-H (2947 cm⁻¹) and C=O (1655 cm⁻¹). Comparison of the reference standard with the sample is shown in Figure 5. The direct comparison did not show a high quality match; it is suspected that either polymorphism or the presence of other soluble capsule materials in the extract caused the differences in the spectra. Again, neither spectrum gave a satisfactory match with any compound in the instrument’s database, indicating both that the compound is not a different steroid and that dehydrochlormethyltestosterone is not entered.
High Performance Liquid Chromatography (HPLC): The chromatograms for dehydrochlormethyltestosterone standard and a mixture of dehydrochlormethyltestosterone and stanozolol standards (roughly 5 : 95) are shown in Figure 6. The two peaks resulting from the dehydrochlormethyltestosterone - stanozolol mixture did not resolve using this method (inset in Figure 6). However, they are resolved by GC or GC/MS, enabling each to be identified. Table 3 lists the relative retention times of a series of similarly sized steroids. Figure 7 shows the UV spectrum of dehydrochlormethyltestosterone.

Table 3. HPLC Relative Retention Times (Asterisks denote steroids analyzed at an earlier date; the retention times were adjusted relative to the dehydrochlormethyltestosterone).

<table>
<thead>
<tr>
<th>Drug (LC)</th>
<th>RRt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxymesterone</td>
<td>0.64</td>
</tr>
<tr>
<td>Boldenone*</td>
<td>0.70</td>
</tr>
<tr>
<td>Nandrolone*</td>
<td>0.73</td>
</tr>
<tr>
<td>Methandrostenolone</td>
<td>0.75</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.79</td>
</tr>
<tr>
<td>Methyltestosterone</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Dehydrochlormethyltestosterone</strong></td>
<td><strong>1.00</strong></td>
</tr>
<tr>
<td>Stanozolol</td>
<td>1.02</td>
</tr>
<tr>
<td>Testosterone Acetate</td>
<td>1.37</td>
</tr>
<tr>
<td>Methenolone Acetate*</td>
<td>1.46</td>
</tr>
<tr>
<td>Nandrolone Propionate*</td>
<td>1.52</td>
</tr>
<tr>
<td>Testosterone Propionate*</td>
<td>1.77</td>
</tr>
<tr>
<td>Nandralone Phenylpropionate*</td>
<td>1.86</td>
</tr>
<tr>
<td>Testosterone Phenylpropionate*</td>
<td>1.98</td>
</tr>
<tr>
<td>Testosterone Isocaproate*</td>
<td>2.14</td>
</tr>
<tr>
<td>Testosterone Cypionate*</td>
<td>2.34</td>
</tr>
<tr>
<td>Methenolone Enanthate*</td>
<td>2.40</td>
</tr>
<tr>
<td>Nandralone Decanoate*</td>
<td>2.58</td>
</tr>
<tr>
<td>Testosterone Decanoate*</td>
<td>2.62</td>
</tr>
<tr>
<td>Testosterone Undecylanate*</td>
<td>2.68</td>
</tr>
</tbody>
</table>

Nuclear Magnetic Resonance (NMR) Spectroscopy: The $^1$H-NMR spectrum of the reference standard are shown in Figures 8a - b. Spectral assignments are summarized in Table 4 (next page). The proton, carbon, and HSQC experiments showed that the unknown molecule contained 20 carbons and 26 non-exchangeable hydrogens. There were 6 quaternary, 5 methine, 6 methylene, and 3 methyl carbons. Adding the carbons (20), non-labile protons (26), oxygens (2), and chlorine (1 based on the MS data), gives a molecular weight of 333 Daltons. The remaining mass (1 Dalton) is due to an exchangeable proton. Using the HMBC NMR data, it was determined that there is one carbonyl carbon (180 ppm), 1 - 3 bonds from 4 alkene carbons at 126.5, 128.6, 158.9, and 166.1 ppm, two of which are protonated, with the hydrogens (6.32 and 7.33 ppm) coupled to each other ($J = 10.1$ Hz). This corresponds well to a doubly conjugated ketone on ring “A” with the carbonyl at position 3, protonated alkene carbons at positions 1 and 2, and quaternary alkene carbons at positions 4 and 5 (meaning position 4 has a substituent, presumably the chlorine). Far removed is a quaternary carbon at 82.0 ppm, indicating that it is bonded to oxygen (likely bonded to the exchangeable proton). Assuming that this is a common steroid ring structure, placement of the carbon (82 ppm) bonded to oxygen would be at the 17 position. This accounts for all but the methyl group, and since the 82 ppm carbon is a quaternary carbon, the methyl group is attached at the 17 position. Comparison of the experimental data with that predicted with the ACD software showed very good agreement. In addition, comparison of the $^1$H-NMR spectrum of the unknown to methandrostenolone showed they were nearly identical below 2.0 ppm, indicating that the B, C, and D rings are the same.
Table 4. NMR Data and Assignments.

<table>
<thead>
<tr>
<th>Position</th>
<th>Carbon ppm</th>
<th>Proton ppm</th>
<th>#H</th>
<th>Type</th>
<th>Coupling Constants (J) (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>158.9</td>
<td>7.33</td>
<td>1</td>
<td>bd</td>
<td>10.1</td>
</tr>
<tr>
<td>2</td>
<td>126.5</td>
<td>6.32</td>
<td>1</td>
<td>d</td>
<td>10.1</td>
</tr>
<tr>
<td>3</td>
<td>180.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>128.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>166.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6a</td>
<td>30.1</td>
<td>2.4</td>
<td>1</td>
<td>td</td>
<td>13.6(x2), 5.2</td>
</tr>
<tr>
<td>6b</td>
<td>30.1</td>
<td>3.27</td>
<td>1</td>
<td>dt</td>
<td>13.6, 3.2(x2)</td>
</tr>
<tr>
<td>7a</td>
<td>33.5</td>
<td>0.99</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>7b</td>
<td>33.5</td>
<td>2.02</td>
<td>1</td>
<td>abdq</td>
<td>13.6, ~3.9, ~3.9, 3.2</td>
</tr>
<tr>
<td>8</td>
<td>37.5</td>
<td>1.84</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>54.9</td>
<td>1.06</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>48.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11a (or 15)</td>
<td>24.1</td>
<td>1.33</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>11b (or 15)</td>
<td>24.1</td>
<td>1.81</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>12a</td>
<td>32.7</td>
<td>1.31</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>12b</td>
<td>32.7</td>
<td>1.61</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>47.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>51.0</td>
<td>1.23</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>15a (or 11)</td>
<td>24.4</td>
<td>1.39</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>15b (or 11)</td>
<td>24.4</td>
<td>1.63</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>16a</td>
<td>39.1</td>
<td>1.68</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>16b</td>
<td>39.1</td>
<td>1.87</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>82.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>14.7</td>
<td>0.94</td>
<td>3</td>
<td>s</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>19.6</td>
<td>1.35</td>
<td>3</td>
<td>s</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>26.1</td>
<td>1.16</td>
<td>3</td>
<td>s</td>
<td>-</td>
</tr>
</tbody>
</table>

b = Broad, d = Doublet, m = Multiplet, abdq = Broad Doublet of Quartets, s = Singlet, and t = Triplet. Many coupling constants could not be determined due to the complexity of the $^1$H-NMR spectrum.

Final confirmation of the compound’s identity was achieved via comparison of mass spectral fragmentation patterns, GC retention times, and proton and carbon NMR spectra, with the reference standard.

Acknowledgements

The authors gratefully acknowledge Forensic Chemist Esther Chege and Senior Forensic Chemist Charles Matkovich, both of the Mid-Atlantic Laboratory, for their assistance running NMR experiments.

References

1. According to internal DEA intelligence.

3. Internet searches for dehydrochlorimethyltestosterone, chlordihydrotestosterone, Turanabol, and/or Oral-Turanabol return a multitude of websites selling or providing advice for use/abuse of this steroid.


[References Continued on Page 65.]

---

Figure 1. Mass Spectrum of Dehydrochlorimethyltestosterone.

---

Figure 2. Mass Spectrum of Methandrostenolone.
Figure 3. Mass Spectrum of Stanozolol.

Figure 4. The Infrared Spectrum (FTIR-ATR) of Dehydrochlormethyltestosterone Reference Standard.
Figure 5. Infrared Spectrum (FTIR-ATR) of Sample of Orange Colored Capsule’s Methanol Soluble Materials (Upper Trace) Compared to the Reference Standard (Lower Trace).

Figure 6. The HPLC UV Chromatogram (225 nm Detection) for Dehydrochlormethyltestosterone Standard (13.377 Minutes). The Inset Shows the Chromatogram for the 5 : 95 Mixture of Dehydrochlormethyltestosterone (13.334 minutes) and Stanozolol (13.543 minutes) Standards.
Figure 7. HPLC UV Spectrum of Dehydrochlormethyltestosterone in Methanol.

Figure 8a. $^1$H-NMR of Dehydrochlormethyltestosterone Reference Standard in CD$_3$OD. Dimethylsulfone (Listed as ISTD) was used to Quantitate the Standard.
Figure 8b. Alkyl Region of Proton Spectrum of Figure 8a, Expanded to Show Peak Splitting Patterns.

References (Continued from Page 61)

6. Smith PR (Special Testing and Research Laboratory), personal communication, 2007 (based on reports made to the Clandestine Laboratory Investigating Chemists Association Bulletin Board).


10. Method developed by DEA Senior Forensic Chemist David Rees of the Mid-Atlantic Laboratory.
Technical Note

Qualitative and Quantitative Analysis of Ionamin 30 Capsules
(Containing a Time-Release Formulation of Phentermine)

Nicole R. Edwards
U.S. Department of Justice
Drug Enforcement Administration
Mid-Atlantic Laboratory
1440 McCormick Dr.
Largo, MD 20774
[email: nicole.r.edwards-at-usdoj.gov]

ABSTRACT: Analysis of a time-release formulation of phentermine required sonication in water for 60 minutes, in order to release the active compound from the matrix.

KEYWORDS: Phentermine, Ionamin 30, Time-Release Formulation, Analysis, HPLC, 1H-NMR, Sonication, Forensic Chemistry

Introduction

The Mid-Atlantic Laboratory recently received a large submission of multiple exhibits allegedly containing various forms of phentermine. The exhibits were seized in Laurel, Maryland (no further details). One exhibit included 1,494 yellow capsules (14 x 5 millimeters), each labelled as “Ionamin 30” and containing brown resin beads and white powder (see Photos 1 and 2, next page). Ionamin 30 is a time-release formulation of phentermine containing 30 milligrams of phentermine in a cationic exchange resin complex (1). However, preliminary analyses of methanol and chloroform extracts of the capsule contents using GC, GC/MS, and NMR indicated no controlled substances. Further research on drug-resin complexes revealed that the time-release mechanism in these capsules involves a water-permeable/acid insoluble barrier that allows the substance to be slowly released into the body. Herein, a method for the analysis of this type of formulation is presented. The method may be useful for other time-release formulations.

Experimental

Chemicals and Reagents: Phentermine standard was acquired from this laboratory’s reference collection. All other chemicals were of reagent-grade quality or better.
**HPLC**: Analyses were performed using a Agilent 1100 Series High Performance Liquid Chromatograph. Acquisition Parameters are summarized below:

- **Column**: RP18 Waters Symmetry Shield; 3.5 µm particle size, 150 mm x 4.6 mm
- **Detector**: Diode Array (Detection at 210 nm)
- **Temperature**: 30 °C
- **Flow Rate**: 1.0 mL/minute
- **Injection Volume**: 3 µL
- **Buffer**: 4000 mL HPLC grade water, 9.6 grams sodium phosphate monobasic, adjusted to pH 2.3 with phosphoric acid, 8.0 mL hexylamine, and 50 milligrams sodium azide
- **Mobile Phase**: 2.3 pH buffer:acetonitrile (85:15)

**Standard Solution**: A standard solution of phentermine was prepared at approximately 0.5 mg/mL with 0.3 mg/mL resorcinol in 95:5 buffer:acetonitrile.

**Sample Solution**: A portion of sample was accurately weighed into a volumetric flask, a small amount of room temperature water added, and the mixture was sonicated for at least 60 minutes. The resulting solution was diluted with additional water to give an estimated phentermine concentration of approximately 0.5 mg/mL. The diluted solution was filtered through a 0.2 micron filter before injection onto the HPLC.

**Quantitative Procedure**: Inject 3 µL of the solution onto the HPLC. The preferred wavelength for phentermine is 210 nm with a bandwidth of 10 nm.

**1H-NMR**: Analyses were performed using a Varian Mercury-Plus 400 MHz NMR using a 5 mm Varian Nalorac indirect detection, variable temperature, pulse field gradient probe with PulseTune® (Varian, Palo Alto, CA). The compound was dissolved in deuterated water (D₂O) containing 1 percent (w/w) 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt as the reference compound. The temperature of the sample was maintained at about 21 °C. Standard Varian (vNMR Version 6.1) pulse sequences were used to acquire the proton spectra. Eight scans were acquired for each spectrum. Processing of data was performed using software from Applied Chemistry Development Laboratory, Version 8 (Toronto, Canada).

**Results and Discussion**

Phentermine is an appetite suppressant (anorectic) used in the management of obesity (1,2). Because it is also a stimulant that is subject to abuse, phentermine is a Schedule IV controlled substance under the U.S. Controlled Substances Act.
Analyses of standard preparations of phentermine is straightforward (3,4). However, time-release formulations of pharmaceuticals require preliminary workup to release the active ingredient from the matrix. In the present study, attempted dissolution of the contents of an Ionamin 30 time-release formulation of phentermine in either methanol or chloroform was ineffective. The resin used in the Ionamin formulation is water-permeable, and it was found that sonication in water was sufficient to release the trapped phentermine. The release was time-dependent; detectable amounts of phentermine (sufficient for a qualitative determination) were released after 10 minutes of sonication, but complete release (required for accurate quantitation) required 60 minutes of sonication (see Figures 1 and 2). The sonicated solution can also be dried down and reconstituted in chloroform/methanol for GC and/or GC/MS analysis, or in deuterated water for NMR analysis (Figures 3a and b).

The white powder in the capsules was not identified, but according to the literature it is a mixture of lactose, magnesium stearate, and titanium dioxide (1).

References


Figure 1. Ten Minute HPLC Qualitative Interval Study of 30 mg Phentermine Capsule.
Figure 2. Ten Minute HPLC Quantitation Interval Study of 30 mg Phentermine Capsule.

Figure 3a. $^1$H-NMR Spectrum of Ionamin 30 Capsule in Deuterated Water.

Figure 3b. $^1$H-NMR Spectrum of Phentermine Hydrochloride Standard in Deuterated Water.
Information and Instructions for Authors for Microgram Journal

General Information
Microgram Journal is a scientific periodical published by the U.S. Drug Enforcement Administration’s Office of Forensic Sciences, that presents peer reviewed, full length Scientific Research Articles and Technical Notes on the detection and analyses of suspected controlled substances for forensic/law enforcement purposes.

Access to Microgram Journal
Microgram Journal is unclassified, and is published on the DEA public access website (at: www.dea.gov/programs/forensicsci/microgram/index.html). At this time, Microgram Journal is available only electronically, and requires Internet access. Professional scientific and law enforcement personnel may request email notifications when new issues are posted (such notifications are not available to private citizens). The publications themselves are never sent electronically (that is, as attachments).

Requests to be added to the email notification list should preferably be submitted via email to the Microgram Editor at: microgram-2007 -at- mailsnare.net Requests can also be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. All requests to be added to the Microgram email notification list should include the following Standard Contact Information:

* The Full Name and Mailing Address of Submitting Laboratory or Office;
* The Full Name, Title (Laboratory Director, Assistant Special Agent in Charge, Librarian, etc.), Phone Number, FAX Number, and Preferred email Address of the Submitting Individual (Note that email notifications are mailed to titles, not names, in order to avoid problems arising from future personnel changes);
* If available, the generic email address for the Submitting Laboratory or Office;
* If a generic email address is not available, one private email address for an individual who is likely to be a long-term employee, who has a stable email address, and who will be responsible for forwarding Microgram information to all of the other employees in the requestor’s Office (Note that only one email address per Office will be honored).

Requests to be removed from the Microgram email notification list, or to change an existing email address, should also be sent to the Microgram Editor. Such requests should include all of the pertinent Standard Contact Information detailed above, and also should provide both the previous and the new email addresses.

Email notification requests/changes are usually implemented within six weeks.

Email Notifications (Additional Comments)
As noted above, the email notification indicates which issue has been posted, provides the Microgram URL, and additional information as appropriate. Note that Microgram e-notices will NEVER include any attachments, or any hyperlink other than the Microgram URL. This is important, because the Microgram email address is routinely hijacked and used to send spam, very commonly including malicious attachments. For this reason, all subscribers are urged to have current anti-viral, anti-spyware, and firewall programs in operation. However, in order to ensure that the email notifications are not filtered as spam, the microgram-2007 -at- mailsnare email address must be “whitelisted” by the Office’s ISP.
**Costs**

Access to *Microgram Journal* is free.

**Submissions to Microgram Journal**

Manuscripts are accepted both from within and outside of DEA, and reviewers are both internal (from within DEA) and external.

All submissions must be in English. All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: microgram-2007@mailsnare.net  *Current* versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 2401 Jefferson Davis Highway, Alexandria, VA 22301. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3 ½ inch IBM® PC-compatible diskette, 100 or 250 MB Iomega® zip diskette, or an IBM® PC-compatible CD. *Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: “Warning - Contains Electronic Media - Do Not Irradiate”*. Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 1/2” x 11” or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. All photos and figures should also be submitted as stand-alone attachments, not only embedded in the manuscript. The pages should be numbered, but not stapled together.

Note that mailed submissions may be subject to lengthy handling delays beyond the control of the Office of Forensic Sciences, and electronic media sent through the mail may be destroyed *en route* by sanitizing procedures, despite protective measures and written warnings. All submissions should include the following  

**Contact Information:**  The Full Name and Address of Submitting Laboratory or Office, and the Full Name, Phone Number, FAX Number, and Preferred email Address of the Submitting Individual.

**Scientific Research Articles** are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.)  

**Technical Notes** are shorter communications concentrating on a specific drug (or drug class), unusual case, novel or unusual procedure or method, or minor original research. Each article/note should be a “stand-alone” work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is *received* by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes may follow an abbreviated version as appropriate):

**Cover Letter** - Provide the standard contact information and pertinent correspondence (if any) for the Editor.

**Title** - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the
Manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

Author(s)/Affiliation(s) - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author’s degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page.

Abstract - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under “Titles”. Note that the abstract will be provided to Chemical Abstracts.

Keyword List - A minimum of five (maximum ten) abstracting keywords should be included. Unless inappropriate, the last keyword pair should always be “Forensic Chemistry.”

Introduction - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid “Personal Communications”).

Experimental (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, USE CAUTION IN DETAILING SYNTHESSES OF CONTROLLED OR ABUSED SUBSTANCES, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: “Experimental details on this synthesis are not provided, in accordance with Journal policy.”]

Results and Discussion - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

Conclusions - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or the summary paragraph in the Results and Discussion section.

Acknowledgments - Optional - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

References - Articles and notes should have all textual citations collected in an endnotes list. Within the text, references should be consecutively numbered with superscripted Arabic numerals, or with Arabic numerals in parentheses, in accordance with their first appearance. Within the endnotes list, references should be consecutively numbered with Arabic numerals, as follows: Number, Period, Indent, Citation. Reference format should adhere to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Note: This is the same reference format utilized in the Selected Reference Citations in Microgram Bulletin, and also (among many others) by the Journal of Forensic Sciences). Due to their inherently transitory nature, use of website URL’s as references are discouraged but are permitted. As
previously noted, Personal Communications should not be utilized; however, if unavoidable, utilize the
following format: Full Name, Title, Affiliation (Laboratory or Office), Location (City and State, plus
Nation if not the United States), Personal Communication, Year.

Table and Figures - All Tables and Figures should be appended onto the end of the article (not
embedded in the text). Tables and Figures should be consecutively numbered with Arabic numerals, in
accordance with their first citation in the text. Each Table and Figure should be “stand-alone”; that is,
include sufficient descriptive information such that the reader will not have to refer back to the text to
understand the Table or Figure. The Header should include the Table or Figure number and a concise
title. Explanatory material, definitions of acronyms and/or abbreviations, and/or references within the
Table or Figure should be designated by superscripted, lower case letters in alphabetical order, and
included in dedicated footnotes at the bottom of the respective Table or Figure. Unless color is needed to
enhance differentiation of the depicted material, all Tables and Figures should be in black and white (that
is, avoid frivolous use of color for “artistic” purposes). Figures of spectra, chromatograms, charts,
graphs, etc., should have clear and legibly labeled axes, but should not include instrument generated
printoffs of experimental parameter lists.

Manuscripts submitted to Microgram Journal are required to be finished, professional quality efforts. Authors
should ensure clarity, brevity, and pertinence of all information. Attention to detail in formatting, syntax,
graham, and spelling are as important as the accuracy of the facts presented. Authors are specially cautioned to
conduct careful literature reviews prior to submission. At the Editor’s discretion, clearly substandard and/or
inappropriate manuscripts will be returned to the authors without review.

Manuscripts will not be retyped, but “final” versions are subject to minor to moderate Editorial rewrite to
improve presentation clarity or to reformat to current Microgram Journal style.

Dual publication - Re-publication of articles or notes of particular interest to the Microgram Journal
readership will be considered if the article was originally published in a journal that is not easily accessed and the primary
author has obtained explicit, written copyright exclusion from the original publisher and consent from all co-
authors. Examples include exact English translations of articles or notes originally published in a non-English
language journal, non-sensitive articles or notes originally published in a restricted journal or on a password
protected website, or articles or notes originally published in limited distribution newsletters or proceedings. In
general, any article or note that was published in English in a mainstream journal is not a candidate for re-
publication in Microgram Journal. Authors interested in re-publishing previously published articles or notes in
Microgram Journal should discuss the issue with the Microgram Editor before submitting.

Note that (in accordance with standard ethical guidelines) re-published articles should not be included as “new”
articles in the respective author(s)’ Curriculum Vitae.

Costs - There are no costs (to the contributor) associated with publication in Microgram Journal.

Reprints - Microgram Journal does not provide reprints to authors. Microgram Journal may be photocopied (or
printed off the website) as needed.

Questions may be directed to the Microgram Editor.

* * * * *
DISCLAIMERS

1) All material published in Microgram Journal is reviewed prior to publication. However, the reliability and accuracy of all published information are the responsibility of the respective contributors, and publication in Microgram Journal implies no endorsement by the United States Department of Justice or the Drug Enforcement Administration.

2) Due to the ease of scanning, copying, electronic manipulation, and/or reprinting, only the posted copies of Microgram Journal (on www.dea.gov) are absolutely valid. All other copies, whether electronic or hard, are necessarily suspect unless verified against the posted versions.

3) WARNING!: Due to the often lengthy time delays between the actual dates of seizures and their subsequent reporting in Microgram Journal, and also because of the often wide variety of seizure types with superficially similar physical attributes, published material cannot be utilized to visually identify controlled substances currently circulating in clandestine markets. The United States Department of Justice and the Drug Enforcement Administration assume no liability for the use or misuse of the information published in Microgram Journal.

* * * * *          * * * * *          * * * * *          * * * * *          * * * * *
Microgram

Journal

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC  20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 5
Numbers 1-4
January - December 2007

Posted On-Line At:
## Contents

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis and Identification of N,N-Dimethylcathinone Hydrochloride</td>
<td>3</td>
</tr>
<tr>
<td><em>Terry A. Dal Cason</em></td>
<td></td>
</tr>
<tr>
<td>Quantitation of the Major Alkaloids in Opium from <em>Papaver Setigerum DC</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Sini Panicker, Heidi L. Wojno, and Lewis H. Ziska</em></td>
<td></td>
</tr>
<tr>
<td>Analysis of Fatty Acids in Marijuana (<em>Cannabis Sativa Leaf</em>)</td>
<td>20</td>
</tr>
<tr>
<td><em>Nadia Fucci</em></td>
<td></td>
</tr>
<tr>
<td>The Characterization of Three FLY Compounds (2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY)</td>
<td>26</td>
</tr>
<tr>
<td><em>Erin C. Reed and Gregory S. Kiddon</em></td>
<td></td>
</tr>
<tr>
<td>Comparison of the Novel Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (AccuTOF-DART™) and Signature Analysis for the Identification of Constituents of Refined Illicit Cocaine</td>
<td>34</td>
</tr>
<tr>
<td><em>Jeri D. Ropero-Miller, Peter R. Stout, Nichole D. Bynum, and John F. Casale</em></td>
<td></td>
</tr>
<tr>
<td>The Isolation, Identification, and Quantitation of Dimethyltryptamine (DMT) in <em>Mimosa Hostilis</em></td>
<td>41</td>
</tr>
<tr>
<td><em>Jack A. Fasanello and Andrea D. Placke</em></td>
<td></td>
</tr>
<tr>
<td>Five Year Indices</td>
<td>53</td>
</tr>
<tr>
<td>Information and Instructions for Authors</td>
<td>61</td>
</tr>
</tbody>
</table>

---

**Note:** In order to prevent automated theft of email addresses off the Internet postings of *Microgram Journal*, all email addresses reported in the *Journal* have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of Testosterone (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).
Synthesis and Identification of N,N-Dimethylcathinone Hydrochloride

Terry A. Dal Cason  
U.S. Department of Justice  
Drug Enforcement Administration  
North Central Laboratory  
536 S. Clark Street  
Chicago, IL 60605  
[email: terry.a.dalcason -at- usdoj.gov]

ABSTRACT: The syntheses and analyses of N,N-dimethylcathinone and N-ethylecathinone are presented and discussed.

KEYWORDS: N,N-Dimethylcathinone, N-Ethylecathinone, \( \alpha \)-Aminopropiophenones, Synthesis, Analysis, Forensic Chemistry

**Introduction**

Although substantial information has been published concerning the analysis and identification of cathinone (2-amino-1-phenyl-1-propanone, \( \alpha \)-aminopropiophenone)\(^a\) and methcathinone (2-methylamino-1-phenyl-1-propanone, \( \alpha \)-methylaminopropiophenone) [1-6], very little analytical data has been published to assist in the identification of the structural analog N,N-dimethylcathinone hydrochloride (hereafter “dimethylcathinone”).\(^b\) A recent seizure of a very large quantity of this drug [7] has spurred interest in its analysis.

While various synthetic aminopropiophenones are popular drugs of abuse in Europe [8,9], with the minor exceptions of methcathinone and methylone (N-methyl-3,4-methylenedioxypropiophenone) [10], this interest has not been matched in the United States (U.S.). The abuse of khat (Catha edulis), which contains small amounts of

\(^a\) “Natural” cathinone (from khat (Catha edulis)) is the S enantiomer; however, in order to avoid confusion, “cathinone” is understood to be a generic (common) term for 2-amino-1-phenyl-1-propanone, and the stereochemistry (R, S, or R/S) is specified as appropriate.

\(^b\) *Editor’s Note:* Dimethylcathinone is occasionally referred to as “dimethcathinone” (a now superceded common name). Dimethylcathinone is the proper nomenclature.
(2S)-(−)-cathinone [4,11-16], has been endemic in the east African communities within the U.S., but cathinone is virtually never encountered as a clandestinely synthesized or extracted drug, possibly because of its instability in free base form. Under U.S. law, cathinone, khat, and methcathinone are all Schedule I Controlled Substances. Currently (early 2008), dimethylcathinone is not scheduled; however, prosecution of this compound (as a Schedule I drug) would be conducted under the tenets of the Controlled Substances Analogue Enforcement Act.

Quite surprisingly, racemic dimethylcathinone is approximately equipotent with both racemic cathinone and racemic amphetamine, while the (2S)-(−)-enantiomer is nearly equipotent with both (2S)-(−)-cathinone and (2S)-(−)-amphetamine [17,18]. This is in direct contrast with (2S)-(−)-dimethylamphetamine, which has been found in drug discrimination studies to be only approximately one tenth as potent an analeptic agent (CNS stimulant) as (2S)-(−)-amphetamine [17]. This unexpected potency likely explains the recent appearances of dimethylcathinone in clandestine markets.

(2S)-(−)-Dimethylcathinone can be synthesized from (1R,2S)-(−)-N-methylephedrine by oxidation with potassium permanganate [5,19,20] or any of a variety of chromium compounds, most often sodium or potassium dichromate [21-27]. Alternatively, racemic dimethylcathinone can be prepared from 2-bromopropiophenone by reacting with dimethylamine [17,28-32]. Herein, the synthesis and analysis of dimethylcathinone are presented and discussed. For comparative purposes, analytical data for N-ethylcathinone (hereafter “ethylcathinone”), an isomeric structural analog of dimethylcathinone, are also presented.

**Experimental**

**Instrumentation:** Solid state Fourier Transform infrared (FTIR) spectra were acquired as a potassium bromide matrix, with a Thermo Nicolet Model 6700 Fourier Transform Infrared Spectrophotometer. Gas phase infrared (IRD) spectra were obtained using a Bio-Rad (now ASAP) IRD II infrared detector interfaced to an Agilent 6890 Gas Chromatograph (GC) with an HP-5 30 m x 0.32 mm x 0.25 m column in splitless mode from 80°C (2.0 min) at 15°C/min to 270°C (2.0 min). Mass spectra were acquired using an Agilent 5973 Mass Selective Detector (MSD) attached to an Agilent 6890 GC. This GC had the same type column as above but used a program from 80°C (2.0 min) to 20°C/min to 240°C (0.5 min) in split mode (100:1). Nuclear magnetic resonance (NMR) spectra were acquired at 400 MHz using a Varian Mercury 400 NMR. The compounds were analyzed as the hydrochloride salts in deuterium oxide (D_2O). Melting points were determined with a Thomas-Hoover “Unimelt” apparatus. Polariometry on the (2S)-(−)-dimethylcathinone HCl was performed using a Perkin-Elmer Model 241 Polarimeter with a 10 cm (1 decimeter) sample cell. All data were acquired at the DEA North Central Laboratory with the exception of the NMR spectra, which were provided by the DEA Special Testing and Research Laboratory (Dulles, VA).

**Syntheses and Melting Points:** Racemic dimethylcathinone and racemic ethylcathinone were prepared by reacting 2-bromopropiophenone (Aldrich Chemical Co., Milwaukee, WI) with dimethylamine or ethylamine, respectively, as aqueous free bases at -8°C (ice-salt bath). (2S)-(−)-Dimethylcathinone HCl was prepared by oxidizing (1R,2S)-(−)-N-methylephedrine HCl (Aldrich) with a sodium dichromate/sulfuric acid solution at -5°C (ice-salt bath). The hydrochloride salts of all compounds were prepared by the addition of a 5% isopropanol/HCl solution to a chloroform solution of the respective free base.

Racemic dimethylcathinone HCl, mp = 206-206.5°C
Racemic ethylcathinone HCl, mp = 186-188°C
(2S)-(−)-Dimethylcathinone HCl, mp = 197.5-200°C, [α] = −52.5° (H_2O, 1%), T = 21°C

**Gas Chromatography - Mass Spectrometry (GC/MS):** The mass spectra of dimethylcathinone and ethylcathinone were acquired as the free bases in chloroform, prepared by dissolving the HCl salts in water, adding saturated sodium carbonate, and extracting into chloroform. The resulting extracts were then passed through a disposable pipette containing a pledget of glass wool and into a glass vial, then introduced into the GC/MS. The mass
spectra of N-acetylethylcathinone was acquired by adding acetic anhydride to a solution of ethylcathinone in chloroform, and immediately injecting the mixture into the GC/MS (the acetylation occurs in the injection port).

**Infrared Spectroscopy (FTIR):** The two compounds were analyzed as the hydrochloride salts in a compressed potassium bromide matrix.

**Infrared Spectroscopy (IRD):** The two compounds were introduced to the IRD as free bases in chloroform through an Agilent 6890 GC.

**NMR Spectrometry:** The 400 MHz proton NMR spectra were acquired by the DEA Special Testing and Research Laboratory (Dulles, VA). Maleic acid was used as an internal standard (peak at 6.40 ppm). Hydrogen exchange with the D₂O solvent is responsible for the HOD resonance at 4.80 ppm. The 0.00 ppm reference peak is from 3-(trimethylsilyl)propionic-2,2,3,3- d₄ acid, sodium salt, present in the D₂O solvent (Aldrich).

**Color Tests:** Although ethylcathinone HCl is a secondary amine, its response to the secondary amine test is almost imperceptible, with only a slight bluish ring forming in a porcelain spot plate after a short period of time. Both of the cathinone analogs give a dull orange with Chen’s reagent. Ethylcathinone starts to respond to the reagent in about 90 seconds, while dimethylcathinone starts to respond in about 180 seconds. Full dissipation of the initial “Robin’s Egg Blue” color of the reagent mixture requires 10 minutes or more, giving an orange-brown color. Preparation of the reagents is given in Reference 1.

**Results and Discussion**

The GC retention times of dimethylcathinone and ethylcathinone are very close using the column and parameters specified in the Instrumentation section (dimethylcathinone 7.07 min; ethylcathinone 7.19 min). The resulting mass spectra are typical of simple phenethylamines (Figures 1a-b). The molecular ions are nearly imperceptible, and a large base peak is observed at m/z = 72, indicative of the respective immonium ions. However, ethylcathinone also has a significant ion at m/z = 44, from loss of ethylene from the m/z = 72 ion [33]. This allows easy differentiation of the two compounds, a distinction that is further enhanced by conversion of ethylcathinone to N-acetylethylcathinone with acetic anhydride. N-Acetylethylcathinone gives a mass spectrum having a very large ion at m/z = 114 (72 + 42) and a small molecular ion at m/z = 219 (177 +42) (Figure 1c). Dimethylcathinone (a tertiary amine) does not react with acetic anhydride. A detailed elucidation of the fragmentation patterns for methamphetamine and related compounds has recently been published [34].

Although GC/MS is the method of choice for identification of many compounds, infrared spectrophotometry may be preferable for dimethylcathinone. The solid state FTIR of a purified sample gives a distinctive spectrum that also allows identification of the salt form (Figures 2a-b). When available, IRD offers the convenience of GC/MS without the sample preparation often required for FTIR. IRD spectra, although lacking the fine structure seen in solid state FTIR spectra, are nonetheless distinct and avoid potential difficulties which may occur with some compounds due to polymorphism (Figures 3a-b).

Proton NMR also gives distinct spectra for dimethylcathinone and ethylcathinone. The spectra are easily distinguished by the resonances for the dimethylamino versus ethylamino groups, between 1.30 and 3.30 ppm (Figures 4a-b).

Color tests (presumptive tests, field tests) are often useful in determining the initial direction of an analysis. In the case of cathinone-based compounds, however, the beta-keto group appears to have an adverse effect on several commonly used reagents (e.g., Marquis and secondary amine), by slowing or preventing the color responses typically observed for simple secondary phenethylamines. One test that is somewhat useful is the Chen’s Test. When a blank is prepared from the three components comprising the reagent, a “Robin’s Egg Blue” precipitate results. This is the initial response of this reagent to the cathinone compounds tested to date. However, when
allowed to sit undisturbed for periods of up to 10 minutes, the blue precipitate dissipates, leaving a clear orange to
orange-brown solution. In contrast, the corresponding aminoalcohols typically give an immediate purple response
[10].

Melting points are useful in determining if a pure enantiomer or the racemate of dimethylcathinone is present.
Enantiomers can be more rigorously identified with polarimetry. However, caution is needed when performing
polarimetry on any of the α-aminopropiophenones. Cathinone free base is known to racemize quickly in
hydrolytic solvents (methanol, ethanol, etc.), but less rapidly in chloroform or methylene chloride. The
propensity for enantiomeric dimethylcathinone to racemize is unknown. The oxalate and hydrochloride salt forms
of dimethylcathinone are stable as dry powders.

Acknowledgements

The authors wish to thank Senior Forensic Chemists Patrick A. Hays and Trinette K. Spratley (both of the DEA
Special Testing and Research Laboratory, Dulles, VA) for acquiring and providing the NMR spectra.

References

2. Noggle FT, DeRuiter J, Valaer A, Clark CR. GC-MS analysis of methcathinone and its major
3. Noggle FT, DeRuiter J, Hayes L, Clark CR. Stereochemical analysis of methcathinone prepared by
5. Savenko VG, Semkin EP, Sorokin VI, Kazankov SP. Expert Examination of Narcotic Substances
Obtained from Ephedrine; Recommended Methods; L.V. Razina, Ed.; U.S.S.R. Ministry of the Interior.
All Union Scientific Research Institute of Polygraphy (Publisher), Moscow, 1989, pp. 1-20.
6. Lurie IS, Klein RFX, Dal Cason TA, LeBelle MJ, Brenneisen R, Weinberger RE. Chiral resolution of
cationic drugs of forensic interest by capillary electrophoresis with mixtures of neutral and anionic
2007;40(8):81. (b) Even more recently, 4-methylmethcathinone (another positional isomer of
dimethcathinone) was identified in Australia (Karen Blakey, Queensland Health Scientific Services,
personal communication, February 19, 2008).
8. Peter Roesner (Altenholz, Germany), personal communication, November 9, 2007. [Dr. Roesner
estimates that perhaps 20 illicit cathinone analogs have been encountered. His mass spectra library,
Designer Drugs 2008, contains a large number of cathinone analogs, including many legitimately
prepared as research chemicals.]
of two new designer drugs with an α-aminophenone structure: 4'-Methyl-α-pyrrolidinohexanophenone


* Law Enforcement Restricted Issue.

---

** Figure 1a. Mass Spectrum of Dimethylcathinone. **

---
Figure 1b. Mass Spectrum of Ethylcathinone.

Figure 1c. Mass Spectrum of N-Acetyleneptylcathinone.
Figure 2a. FTIR of Dimethylcathinone HCl.

Figure 2b. FTIR of Ethylecathinone HCl.
Figure 3a. IRD of Dimethylcathinone.

Figure 3b. IRD of Ethylcathinone.
Figure 4a. 400 MHz Proton NMR of Dimethylethylone HCl in D$_2$O.

Figure 4b. 400 MHz Proton NMR of Ethylcathinone HCl in D$_2$O.
Quantitation of the Major Alkaloids in Opium from *Papaver Setigerum* DC

**Sini Panicker** and **Heidi L. Wojno***

U.S. Department of Justice  
Drug Enforcement Administration  
Special Testing and Research Laboratory  
22624 Dulles Summit Court  
Dulles, VA 20166  
[Email: sini.x.panicker -at- usdoj.gov]

**Lewis H. Ziska**  
Crop Systems and Global Change Laboratory  
U.S. Department of Agriculture  
Agricultural Research Service  
10300 Baltimore Avenue  
Beltsville, MD 20705  
[Email: lewis.ziska -at- ars.usda.gov]

**ABSTRACT:** Quantitation of morphine and other major alkaloids in opium gum from specially cultivated *Papaver setigerum* DC (“Wild Poppy”) is presented. *Papaver setigerum* plants (n = 14) were grown in an atmosphere containing a slightly elevated level of carbon dioxide (390 ppm). Opium gum collected from the capsules of the mature plants was analyzed for morphine, codeine, thebaine, noscapine, and papaverine, using capillary electrophoresis (CE). Morphine was confirmed at an average of 2 percent by weight. Codeine, noscapine, and papaverine were also detected; however, thebaine was below the limits of quantitation by the employed CE method, and could only be detected by gas chromatography/mass spectrometry.

**KEYWORDS:** *Papaver setigerum*, *Papaver somniferum*, Opium Poppy, Wild Poppy, Opium, Opium Alkaloids, Quantitation, Forensic Chemistry

**Introduction**

There are more than one hundred species under the genus *Papaver* that produce alkaloids in the specialized cells called laticifers. However, only two naturally occurring species, *Papaver somniferum* L (Photo 1, next page) and *Papaver setigerum* DC (Photo 2, next page) produce morphine in significant quantities [1]. *Papaver somniferum* is commonly known as opium poppy, and is cultivated around the world for both licit and illicit purposes. *Papaver setigerum*, also known as “wild poppy,” is native to the Mediterranean region and Canary Islands. Although several previous reports indicate that *Papaver setigerum* opium contains morphine (*vide infra*), to date it has never been reported to have been used for licit or illicit morphine production.

Scientific publications on *Papaver setigerum* reveal varying opinions amongst scientists in labeling it as a separate species or as the subspecies of *Papaver somniferum*. One of the earliest publications on opium poppies from Fulton [2] suggested a close relationship between *Papaver somniferum* and *Papaver setigerum*. Farmilo et al. [3] was the first to report the presence of morphine in the pods, buds, and leaves of *Papaver setigerum*.

---

*a Current Address: Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601.
Photo 1. Typical *Papaver Somniferum* Plant. Note the Horizontal Score Marks on the Capsules.

Photo 2. *Papaver Setigerum* Plant Grown for this Study. Note the Smaller, Elongated Capsules (Compare with Photo 1).
Farmilo et al. also indicated that the *Papaver setigerum* chromosome count is a tetraploid (n = 22), whereas *Papaver somniferum* is a diploid (n = 11), which suggests that *Papaver somniferum* could not have evolved from *Papaver setigerum* as many scientists had previously suggested. Farmilo’s article also compared and contrasted sketches of *Papaver somniferum* and *Papaver setigerum* plants, including drawings of their pods (which differ significantly in appearance). The information supported earlier work by Sugiura [4], who disagreed with the concept of *Papaver setigerum* as a direct ancestor to *Papaver somniferum*, and instead regarded them as separate species, with the possibility of a common ancestor.

One of the earliest (1956) studies on the quantitation of morphine in *Papaver setigerum* opium came from Asahina et al., who reported a morphine content of 5.1% [5]. A detailed publication from the same group in 1957 [6] described two sets of experiments with *Papaver setigerum* plants, reporting morphine values of 5.1% and 7.3% (surprisingly high). The other alkaloids were reported at: Codeine (0.9 and 0.8%); thebaine (2.1 and 1.6%); papaverine (1.9 and 2.6%); and narcotine (also known as noscapine, 0.1 and 0.1%). The paper also reported the relative differences in the alkaloids present in opium from *Papaver somniferum* cultivations in Iran, India, and Turkey versus those present in *Papaver setigerum* opium. A later study from La Valva et al. [7] found no morphine, codeine, and thebaine in the *Papaver setigerum* populations from Mediterranean France and southern Italy; however, this latter finding is atypical. Subsequently, Garnock-Jones et al. [8] confirmed alkaloids in the air-dried capsules of New Zealand cultivations of *Papaver setigerum* (morphine 0.4%; codeine 0.5%; papaverine 1.6%; narcotine 1.3%; thebaine not reported), but also noted that the alkaloid concentrations were “much lower” in wild plants. Garnock-Jones et al. also declared *Papaver setigerum* to be a subspecies of *Papaver somniferum*. Aside from the negative alkaloid values reported by La Valva et al., these reports collectively established *Papaver setigerum* as a separate species (or possibly a rather distantly related subspecies), under the genus *Papaver*, with the potential of producing minor to significant amounts of morphine.

In the era of numerous internet vendors for seeds of both *Papaver somniferum* and *Papaver setigerum*, the forensic science community has a salient interest in the actual alkaloid composition of *Papaver setigerum*, and in the potential use of its opium for illicit purposes. As detailed above, the literature is inconsistent and in some cases is contradictory. The U.S. Controlled Substances Act does not differentiate between species (or subspecies) of *Papaver*. Thus, *Papaver setigerum* is not formally controlled (by name) in the United States; however, opium and related products (such as poppy straw) from *Papaver setigerum* are controlled (Schedule II) if they contain morphine, codeine, and/or thebaine. Of note, opium cultivation laws in many other countries specifically prohibit cultivation of *Papaver setigerum*.

The opium analyzed in this study came from an environmental research project conducted by the U.S. Department of Agriculture, where the primary goal was to study the effects of a slight increase in atmospheric carbon dioxide (CO₂) on *Papaver setigerum* plant growth and alkaloid production [9]. The Mauna Loa Observatory (Hawaii) reports the average ambient atmospheric CO₂ level to be 385 ppm [10]. However, the actual ambient atmospheric CO₂ level is thought to be slightly higher, as was reported by Ziska et al. [11]. Therefore, the study was conducted at a 390 ppm CO₂ level, which is believed to provide more valid data on actual alkaloid production in the wild. Fourteen *Papaver setigerum* plants were cultivated in controlled environment grow chambers in an atmosphere containing 390 ppm CO₂. Opium gum was obtained by lancing the mature pods from the plants, in the same manner that opium from *Papaver somniferum* plants is obtained, and was analyzed using a capillary electrophoresis methodology [12].

**Experimental**

**Seeds:** Seeds of *Papaver setigerum* DC were obtained from the Institut fur Pflanzengenetik und Kulturpflanzenforschung in Gatersleben, Germany.

**Cultivation and Harvesting:** The study was conducted using controlled environment chambers (EGC Corporation, Chagrin Falls, OH), with the chamber set at 390 ppm CO₂ for 24 h day⁻¹. The actual average 24 h CO₂ values were 389 +/- 12.1 ppm. The seeds were sown by hand in 2.6 L pots filled with a 4:1:1 mixture of...
sphagnum, perlite, and vermiculite. Floral initiation occurred at about 70 days after sowing. There were approximately 8-10 capsules per plant. The scoring of the mature capsules began about two weeks after the loss of the floral petals. Scoring was done using a razor blade, making 2 to 3 one-millimeter deep incisions on the capsule surface. For each capsule, opium gum was collected over a 24 h period on aluminum foil, allowed to air dry for 72 hours, and then weighed. The timing and harvesting techniques match those typically used for *Papaver somniferum*.

*Capillary Electrophoresis (CE):* Opium alkaloid standards were obtained from the reference collection of the DEA Special Testing and Research Laboratory (Dulles, VA). All CE-grade reagents and run buffer solutions were obtained from Microsolv™ Technology (Eatontown, NJ). High Performance Liquid Chromatography grade methanol was obtained from Burdick and Jackson (Muskegon, MI). High purity, deionized water was obtained from a Millipore Milli-Q-Gradient A10 water system (Bedford, MA). An internal standard stock solution of tetracaine hydrochloride was prepared by weighing 25 mg into a 100 mL volumetric flask and diluted to volume with a 1:11 mixture of methanol and 3.75 mM phosphate buffer (pH 3.2). To obtain the tetracaine internal standard working solution, 6 mL of the tetracaine HCl internal standard stock solution was diluted to volume with 3.75 mM phosphate buffer (pH 3.2) in a 50 mL volumetric flask. Appropriate amounts of morphine, codeine, thebaine, noscapine, and papaverine base standards were weighed into a 100 mL volumetric flask in order to obtain an approximate final concentration of 0.025 mg mL⁻¹ for each compound. Ten mL of the internal standard stock solution was pipetted into the above-mentioned volumetric flask and diluted to volume with a 1:11 mixture of methanol and 3.75 mM phosphate buffer. Approximately 500 µL of the solution was filtered using 0.45 µm regenerated cellulose Titan filter and transferred to a 1.0 mL polypropylene CE injection vial.

Appropriate amounts of the opium gum samples were weighed into a volumetric flask in order to obtain a concentration of morphine similar to that of the standard. The flask was filled to half volume with methanol and sonicated for 30 minutes at 55°C to completely extract the alkaloids. The flask was then cooled, and diluted to volume with 3.75 mM phosphate buffer (pH 3.2). 400 µL of the above solution was added to 2.0 mL of the internal standard working solution. Approximately 500 µL of the above solution was filtered using a 0.45 µm regenerated cellulose Titan filter and transferred to a 1.0 mL polypropylene CE injection vial. An Agilent Model HP3DCE capillary electrophoresis system equipped with a diode array detector (Waldbronn, Germany) was used for alkaloid analysis and quantitation, as described by Lurie *et al.* [12]. All experiments were carried out with fused silica 32 cm (23.5 cm to detector window) x 50 µm I.D. pre-made capillaries obtained from Agilent Technologies (Part No: G1600-63211).

*Gas Chromatography/Mass Spectrometry (GC/MS):* Analyses were conducted using an Agilent (Palo Alto, CA) Model 5973 Quadrupole Mass Selective Detector (MSD) interfaced with an Agilent Model 6890 Gas Chromatograph (GC). The GC contained a J&W Scientific (Rancho Cordova, CA) 30 m x 0.25 mm I.D. fused silica capillary column coated with a film thickness of 0.25 µm DB-1. The injection port was maintained at 280°C. The oven was programmed with an initial temperature of 90°C, holding for 2 minutes, then 14°C per minute increase until 300°C, holding for another 10 minutes. One mL portions of the methanol extracts of the opium samples were placed into autosampler vials for analysis.

**Results and Discussion**

Morphine, codeine, noscapine, and papaverine were all detected and quantitated using the CE method described in the Experimental section. Thebaine was detected in the opium using the GC/MS method described in the Experimental section; however, CE quantitation of thebaine was not possible because of its low levels (the TIC indicated thebaine between 0.01 and 0.3%). The low thebaine content was unexpected in view of the value previously reported by Asahina *et al.* [6]. The quantitation results for morphine, codeine, noscapine, and papaverine for the opium samples collected from all 14 plants are presented in Table 1. The amounts of opium obtained from each plant are also presented in Table 1. The average levels were 2% morphine, 3% codeine, 10% noscapine, and 5% papaverine, all by weight of opium. The noscapine quant was stunningly high (5x) relative to
the morphine content, and interestingly, is very similar (on a weight percent basis) to the noscapine content of opium from *Papaver somniferum* cultivations in South and Central America [12].

Studies at the Special Testing and Research Laboratory have shown that the morphine content in *Papaver somniferum* opium varies among growing regions. However, on average, *Papaver somniferum* opium contains about 10 - 13% morphine [13]. These latter results do not derive from controlled cultivations; however, as noted above, it is assumed that these plants were grown at an average ground level CO₂ level of 390 ppm. The electropherograms of typical *Papaver setigerum* and *Papaver somniferum* opiums are shown in Figure 1.

Based on the results of this study, although *Papaver setigerum* opium contains some morphine, it is not a viable source of opium for illicit poppy cultivators. Cultivation and the manual harvesting of opium poppy capsules by lancing and subsequent hand-collection are very time-consuming and labor-intensive processes. *Papaver setigerum* plants are much smaller in size compared to *Papaver somniferum* plants; furthermore, the capsules in *Papaver setigerum* are also very small and elongated in shape (between 10 - 16 millimeters in length and between 4 - 7 millimeters in diameter). In contrast, *Papaver somniferum* capsules are more or less globular in shape and much larger in size (typically between 20 and 40 millimeters in diameter). For these reasons, the total amount of opium that can be obtained from a field of *Papaver setigerum* poppies is much lower than from an equal sized field of *Papaver somniferum* poppies. The much lower morphine content, much lower total opium yield, and the increased time and labor needed to harvest the opium from the small, elongated pods, all explain why *Papaver setigerum* has never received much attention in the areas of either licit or illicit cultivation and morphine production. Based on this study, it would appear that this disinterest is justified.

**Acknowledgements**

The authors would like to thank Thomas M. Duncan and Elizabeth R. Pascual, Supervisory Chemists, DEA Special Testing and Research Laboratory (Dulles, VA), for their support and encouragement.

**References**


13. From the compiled opium data obtained from the analyses of thousands of opium samples at the DEA Special Testing and Research Laboratory (Dulles, VA).

* * * * *

Table 1. Alkaloid Composition of Papaver setigerum Opium
(Note: Thebaine was Detected, not Quantified).

<table>
<thead>
<tr>
<th>Plant Number</th>
<th>Weight of opium obtained from each plant (mg)</th>
<th>Morphine%</th>
<th>Codeine%</th>
<th>Papaverine%</th>
<th>Noscapine%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.1</td>
<td>2.5</td>
<td>3.0</td>
<td>5.2</td>
<td>11.4</td>
</tr>
<tr>
<td>2</td>
<td>24.1</td>
<td>2.4</td>
<td>2.9</td>
<td>5.0</td>
<td>10.9</td>
</tr>
<tr>
<td>3</td>
<td>38.8</td>
<td>2.3</td>
<td>2.7</td>
<td>4.7</td>
<td>10.3</td>
</tr>
<tr>
<td>4</td>
<td>27.6</td>
<td>2.5</td>
<td>2.2</td>
<td>4.8</td>
<td>9.8</td>
</tr>
<tr>
<td>5</td>
<td>35.2</td>
<td>3.1</td>
<td>1.8</td>
<td>5.1</td>
<td>10.4</td>
</tr>
<tr>
<td>6</td>
<td>34.2</td>
<td>2.1</td>
<td>2.9</td>
<td>5.4</td>
<td>10.8</td>
</tr>
<tr>
<td>7</td>
<td>82.6</td>
<td>2.3</td>
<td>2.0</td>
<td>5.5</td>
<td>11.2</td>
</tr>
<tr>
<td>8</td>
<td>52.3</td>
<td>2.4</td>
<td>3.0</td>
<td>4.4</td>
<td>9.8</td>
</tr>
<tr>
<td>9</td>
<td>67.9</td>
<td>2.3</td>
<td>2.1</td>
<td>5.5</td>
<td>10.5</td>
</tr>
<tr>
<td>10</td>
<td>23.1</td>
<td>1.4</td>
<td>3.5</td>
<td>4.6</td>
<td>10.9</td>
</tr>
<tr>
<td>11</td>
<td>27.6</td>
<td>2.5</td>
<td>2.6</td>
<td>4.2</td>
<td>9.6</td>
</tr>
<tr>
<td>12</td>
<td>21.4</td>
<td>2.0</td>
<td>3.2</td>
<td>4.9</td>
<td>10.1</td>
</tr>
<tr>
<td>13</td>
<td>16.9</td>
<td>2.8</td>
<td>2.7</td>
<td>3.3</td>
<td>8.5</td>
</tr>
<tr>
<td>14</td>
<td>19.8</td>
<td>1.7</td>
<td>2.4</td>
<td>3.8</td>
<td>9.0</td>
</tr>
<tr>
<td>Average</td>
<td>36.4</td>
<td>2.3</td>
<td>2.6</td>
<td>4.7</td>
<td>10.2</td>
</tr>
<tr>
<td>Std Dev</td>
<td>19.132</td>
<td>0.427</td>
<td>0.495</td>
<td>0.660</td>
<td>0.832</td>
</tr>
<tr>
<td>Min</td>
<td>1.4</td>
<td>1.8</td>
<td>3.3</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Max</td>
<td>3.1</td>
<td>3.5</td>
<td>5.5</td>
<td>11.4</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Electropherograms of Typical *Papaver setigerum* (Top Trace) and *Papaver somniferum* (Bottom Trace) Opiums. Time (x-axis) Differs for the Two Runs Because They Were Completed on Distantly Removed Dates (the older the capillary, the longer the retention times; however, the retention order and relative retention times are consistent). The *Papaver somniferum* Opium Electropherogram Presents Data from Opium Poppies Recently Grown in Afghanistan.
Technical Note

Analysis of Fatty Acids in Marijuana (Cannabis Sativa Leaf)

Nadia Fucci, Ph.D.
Catholic University of the Sacred Heart
Institute of Legal Medicine
Largo Francesco Vito
1-00168 Rome, Italy
[email: nadiafucci-at-unicatt.it]

ABSTRACT: Various fatty acids (palmitic, myristic, oleic, and stearic acids) were identified in 20 marijuana (cannabis leaf) samples recently seized on the illicit market in Rome, Italy. Samples were analyzed by gas chromatography/mass spectrometry to determine delta-9-tetrahydrocannabinol, other minor cannabinoid congeners, and fatty acids. Although cannabis seeds and the oil derived from those seeds are known to be rich in fatty acids, this is believed to be the first study demonstrating the presence of fatty acids in marijuana. The potential value of the results in source determination and comparative analyses is discussed.

KEYWORDS: Marijuana, Fatty Acids, Myristic Acid, Palmitic Acid, Oleic Acid, Stearic Acid, Analysis, GC/MS, Forensic Chemistry

Introduction

Cannabis preparations, especially marijuana (cannabis leaf), are the most widely abused illicit drugs in the world. Because of the significant economic and social impact associated with abuse of marijuana and related products, extensive effort is expended to monitor their production, trafficking, and use. These efforts include source determination (i.e., geographic origin) [e.g., 1-4] and comparative analysis (i.e., sample - sample comparisons) [e.g., 5-8]. Approaches have included classic impurity profiling (including cannabinoid quants and ratios), various DNA-based analyses, and isotope ratio analyses (primarily based on δ^{13}C and δ^{15}N) [e.g., 9].

Because hemp fiber, seeds, and seed oils all have potential economic value, analysis of Cannabis sativa has not been limited to leaf or leaf-derived products. One sub-topic of interest is the presence of fatty acids in the seeds and fruits of cannabis [10-13]. The seeds and seed oils of cannabis contain a wide variety of fatty acids in economically viable amounts. Samir et al. [12] reported the concentration of fatty acids and the relative percentage of unsaturated and saturated fatty acids in a number of different samples of cannabis seeds, and noted that climate and growing conditions seemed to influence the composition of these compounds in the different samples. Furthermore, Bagci et al. [13] showed that high amounts of individual fatty acids, along with various minor components (tocopherol and tocotrienols) was useful in assessing chemotaxonomic relationships among different varieties of cannabis. Collectively, these results suggest that fatty acids may be of value in source determination and/or comparative analysis of cannabis seeds or seed oils.

However, there do not appear to be any studies reporting the presence of fatty acids in marijuana itself (i.e., cannabis leaf). In this study, a simple gas chromatography/mass spectrometry (GC/MS) method was developed for the determination of fatty acids in marijuana, and was successfully demonstrated on 20 samples seized in Rome, Italy. The technique is sensitive and accurate. The potential value of the results in source determination and comparative analyses is discussed.
Experimental

Materials and Methods: All chemical and reagents employed were of analytical grade. Fatty acid standards were purchased from Sigma-Aldrich. A mixture of the standards, each at a concentration of 0.1 mg/mL, was used for method development. Twenty marijuana samples from the illicit Roman market were analyzed. The samples were stored in the dark in a dry-box prior to analysis. Individual samples (100 milligrams) were extracted with 0.5 mL of chloroform solution at room temperature, and an aliquot injected into the GC/MS.

Instrumentation: GC/MS analyses were performed on a Model Focus-HP Gas Chromatograph fitted with a split-splitless injector (270°C) equipped with a HP-1 capillary column (12 m x 0.2 mm I.D.) coated with 0.3 μm thickness of methylsilicone. The temperature program ramped from 70°C to 280°C at 10°C/min, with a 5 min final hold. Helium was employed as the carrier gas, at a column head pressure of 10 psi. The GC was connected to an HP 5971A Mass Analyzer operating at 70 eV EI over 40-500 a.m.u. in selected ion monitoring (SIM) mode.

Results and Discussion

Cannabis seeds and seed oils have been shown to contain up to 20 fatty acids [10-13]. Analysis of the 20 marijuana samples selected for this study confirmed the (varying) presence of myristic, palmitic, oleic, and/or stearic acids (see Tables 1-3 and Figures 1-4). Table 1 presents the IUPAC names and formulas for the respective acids. Table 2 presents the respective retention times and the ions selected for SIM analysis. Table 3 presents the results by sample (ratio’d against the combined cannabidiol (CBD) and cannabinol (CBN) content). Figures 1-4 display typical SIM chromatograms of the respective acids.

The acids varied dramatically by sample. All four acids were present in only 10 of the 20 samples (1,2,4,5,10,11,13,15,16, and 17). Three acids (myristic, palmitic, and stearic) were present in samples 3 and 18; two acids (myristic and palmitic) were present in samples 6,7,12, and 19; only one acid (oleic) was present in samples 9,14 and 20; and finally, no fatty acids were detected in sample 8. The geographic origin for samples 6 and 7 was alleged to be the Netherlands, and in fact those samples had similar delta-9-tetrahydrocannabinol contents (about 1%) and similar impurity profiles, and were also similar in their fatty acid profile (but with only two acids present and with actual origin unknown, the results are interesting but of only curiosity value). Nonetheless, these results suggest that the fatty acid profile of marijuana may be useful for comparative analyses (sample - sample comparisons). The analysis is easy, quick, and sufficiently sensitive for small sample amounts. Although unlikely [12], the method may also be useful for source determination; however, such an advance would require a much larger database of authentics (samples of known origin).

Additional research is planned for more sensitive determination of fatty acids in marijuana by derivatizing the acids prior to analysis. The results will be the subject of a future report.

References


---

Table 1. IUPAC Names and Formulas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chemical Name</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic Acid</td>
<td>Tetradecanoic Acid</td>
<td>C\textsubscript{14}H\textsubscript{30}O\textsubscript{2}</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>Hexadecanoic Acid</td>
<td>C\textsubscript{16}H\textsubscript{34}O\textsubscript{2}</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>9-Octadecenoic Acid\textsuperscript{a}</td>
<td>C\textsubscript{18}H\textsubscript{36}O\textsubscript{2}</td>
</tr>
<tr>
<td>Stearic Acid</td>
<td>Octadecanoic Acid</td>
<td>C\textsubscript{18}H\textsubscript{36}O\textsubscript{2}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Geometric Isomer (\textit{cis} or \textit{trans}) not determined.
Table 2. Retention Times and Ions Chosen for Selected Ion Monitoring (SIM) Analysis.

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>R.T.</th>
<th>TARGET ION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>8:06</td>
<td>228-185</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>10:06</td>
<td>256-213</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>11:19</td>
<td>264-282</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>11:34</td>
<td>284-241</td>
</tr>
</tbody>
</table>

Table 3. Results Obtained for the Marijuana Samples Analyzed in GC/MS.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>M/(CBD+CBN)</th>
<th>P/(CBD+CBN)</th>
<th>O/(CBD+CBN)</th>
<th>S/(CBD+CBN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>0.30</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>0.50</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0.20</td>
<td>N</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>0.30</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.03</td>
<td>0.20</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>0.02</td>
<td>0.78</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>0.03</td>
<td>0.8</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>N</td>
<td>N</td>
<td>0.01</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>0.06</td>
<td>0.20</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>11</td>
<td>0.01</td>
<td>0.48</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>12</td>
<td>0.03</td>
<td>0.70</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>0.05</td>
<td>0.18</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>14</td>
<td>N</td>
<td>N</td>
<td>0.01</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>0.02</td>
<td>0.28</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>16</td>
<td>0.05</td>
<td>0.20</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>17</td>
<td>0.03</td>
<td>0.18</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>18</td>
<td>0.05</td>
<td>0.15</td>
<td>N</td>
<td>0.01</td>
</tr>
<tr>
<td>19</td>
<td>0.02</td>
<td>0.6</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>N</td>
<td>N</td>
<td>0.02</td>
<td>N</td>
</tr>
</tbody>
</table>

M = Myristic Acid  
P = Palmitic Acid  
O = Oleic Acid  
S = Stearic Acid  
CBD = Cannabidiol  
CBN = Cannabinol  
N = None Detected
Figure 1. Myristic Acid (GC/MS Analysis in SIM Mode).

Figure 2. Palmitic Acid (GC/MS Analysis in SIM Mode).
Figure 3. Oleic Acid (GC/MS Analysis in SIM Mode).

Figure 4. Stearic Acid (GC/MS Analysis in SIM Mode).
Technical Note

The Characterization of Three FLY Compounds (2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY)

Erin C. Reed*
Office of the Ohio Attorney General
30 E. Broad Street, 14th Floor
Columbus, OH 43215
[email: ereed -at- ag.state.oh.us]

Gregory S. Kiddon
Office of the Ohio Attorney General
Ohio Bureau of Criminal Identification and Investigation
1560 State Route 56 SW
London, OH 43210
[email: gkiddon -at- ag.state.oh.us]

ABSTRACT: The analysis and characterization of 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']-difuran-4-yl)-2-aminoethane hydrochloride (2C-B-FLY), 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']-difuran-4-yl)-2-aminopropane hydrochloride (3C-B-FLY), and 1-(8-bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane hydrochloride (Bromo-DragonFLY) are presented. Gas chromatography/mass spectra (GC/MS), gas chromatography/infrared spectra (GC/IRD), and solid phase Fourier transform infrared (FTIR) spectra are presented.

KEYWORDS: 2C-B-FLY, 3C-B-FLY, Bromo-DragonFLY, Phenethylamines, GC/MS, GC/IRD, FTIR, Forensic Chemistry

Introduction

A large number of phenethylamine derivatives are abused for their hallucinogenic properties [1,2]. Recent submissions to various crime laboratories [e.g., 3,4], as well as commentary on various Internet websites and similar venues that are dedicated to drug abuse, indicate an increasing interest in a specific group of phenethylamine analogs referred to as the FLY compounds. Three of the better known FLY compounds are: 1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']-difuran-4-yl)-2-aminoethane hydrochloride (2C-B-FLY), 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b;4,5-b']-difuran-4-yl)-2-aminopropane hydrochloride (3C-B-FLY), and 1-(8-bromobenzo[1,2-b;4,5-b']difuran-4-yl)-2-aminopropane hydrochloride (Bromo-DragonFLY) (see Figure 1, next page). Several other FLY compounds are known but are more obscure. The “FLY” designation allegedly derives from the two “wing-like” furan or dihydrofuran rings that are fused on the opposite sides of the central benzene ring, giving an insect-like appearance with the bromo substituent as the head and the ethylamine or isopropylamine substituent as the tail.

At present (early 2008), none of the above FLY compounds are formally controlled in the United States. However, their core structures are highly similar to the Schedule I hallucinogens 4-bromo-2,5-dimethoxy-phenethylamine (2C-B) and 4-bromo-2,5-dimethoxyamphetamine (DOB). For this reason, they could potentially be prosecuted under the tenets of the Controlled Substances Analogue Enforcement Act.
Due to the scarcity of known standards and consequent lack of instrumental data, the identification of these compounds has been hindered. In order to address this issue, mass spectra, gas phase FTIR spectra, and solid phase FTIR spectra are presented for 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY.

![Structures of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY](image)

**Figure 1.** Structures of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY

**Experimental**

*Standards:* 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY standards were synthesized and provided by the Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences at Purdue University [5,6].

*Gas Chromatography/Mass Spectrometry (GC/MS):* Spectra were acquired using an Agilent Model 6890N GC equipped with an Agilent Model 5973 quadrupole mass-selective detector (MSD). The MSD was operated using 70 eV E.I. ionization. The GC was fitted with a 30 m x 0.25 mm I.D. fused silica capillary column coated with 0.52 μm 5% phenylmethyl siloxane (HP-5MS), and was operated using a 50:1 split ratio. The injector port was maintained at 250°C. The oven temperature program was as follows: Initial temperature 200°C (2 minutes), ramped to 280°C at 20°C per minute (final hold 14 minutes). Helium was used as a purge gas at a rate of 39 cm/second.
Gas Chromatography/Infrared Spectroscopy (GC/IRD): Spectra were acquired using an Agilent Model 6890N GC interfaced with a BioRad Infrared Detector II. The GC was fitted with a 30 m x 0.32 mm I.D. fused silica capillary column coated with 0.52 μm 5% phenylmethyl siloxane (HP-5), and was operated in splitless mode. The injector port temperature was maintained at 250ºC. The oven temperature program was as follows: Initial temperature 55ºC (1 minute), ramped to 275ºC at 25ºC per minute (final hold 6 minutes). The flow cell and transfer line were maintained at 300ºC. Helium was used as a carrier gas at a flow rate of 2 mL/minute.

Fourier Transform Infrared Spectroscopy (FTIR-ATR): Spectra were acquired using a Perkin Elmer Spectrum One Spectrophotometer with a universal attenuated total reflectance (UATR) accessory. Spectra were collected using 4 scans between 4000 cm⁻¹ and 500 cm⁻¹.

Results and Discussion

The FLY compounds are alleged to be potent hallucinogens, and they and various other hallucinogenic phenethylamines and tryptamines are often represented to be LSD. The FLY compounds have been submitted to forensic laboratories both in liquid form and on blotter paper. The mass spectra of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY are presented in Figures 2-4. Unlike many simpler phenethylamines, the mass spectrum of each compound displayed a molecular ion peak. Also present are the fragmentation patterns which are characteristic of naturally occurring bromine isotopes. For each compound, alpha cleavage is responsible for the base peak. For this reason it is important to ensure that data is collected at a mass range with a minimum below m/z = 30. The gas and solid phase FTIR spectra of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY are presented in Figures 5-7 and 7-10, respectively. [Note: References are listed on page 33.]

Figure 2. Mass Spectrum of 2C-B-FLY.
Figure 3a. Mass Spectrum of 3C-B-Fly (Bromo-Fly).

Figure 3b. Mass Spectrum of 3C-B-FLY (Bromo-FLY), Normalized to the 254 ion.
Figure 4a. Mass Spectrum of Bromo-DragonFLY.

Figure 4b. Mass Spectrum of Bromo-DragonFLY, Normalized to the 142 Ion.
Figure 5. GC/IRD of 2C-B-FLY.

Figure 6. GC/IRD of 3C-B-FLY (Bromo-FLY).

Figure 7. GC/IRD of Bromo-DragonFLY.
Figure 8. FTIR/ATR Spectrum of 2C-B-FLY.

Figure 9. FTIR/ATR Spectrum of 3C-B-FLY (Bromo-FLY).
**References**


TECHNICAL NOTE

Comparison of the Novel Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (AccuTOF-DART™) and Signature Analysis for the Identification of Constituents of Refined Illicit Cocaine

Jeri D. Ropero-Miller, Ph.D.*, Peter R. Stout, Ph.D., and Nichole D. Bynum, M.S.
RTI International
Center for Forensic Sciences
3040 Cornwallis Road
Research Triangle Park, NC  27709
[email:  jerimiller -at- rti.org]

John F. Casale, B.S.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA  20166

ABSTRACT: The characterization of 25 illicit cocaine samples by a novel application of direct analysis in real time (DART) sample introduction coupled with time-of-flight mass spectrometry (TOF-MS) and cocaine signature analyses is provided. The AccuTOF-DART™ analysis of the cocaine samples resulted in the detection of most analytes, although some compounds were not detected. This new technique is easy, rapid, requires very little sample, and can be used to screen even complex mixtures. Potential applications, including use for signature analyses of controlled substances, are discussed.

KEYWORDS: Cocaine Signature Analyses, DART, TOF-MS, Screening Test, Forensic Chemistry

Introduction

Time-of-flight mass spectrometry (TOF-MS) using exact mass determination has the potential to greatly improve drug screening in forensic laboratories [1-4]. A TOF-DART instrument, which couples a TOF mass spectrometer with a direct analysis in real time (DART) ion source, has been recently introduced. The instrument easily and rapidly screens samples for a wide range of compounds, and requires only minute amounts of sample and little sample preparation. Both sample preparation and sample screening for multiple drug analytes can be completed in minutes with the TOF-DART, whereas conventional cocaine signature analyses or controlled substances screening may take 8 hours or longer. Figure 1 compares the analysis of controlled substances by traditional GC/MS to the novel screening by TOF-DART. The instrument provides sufficient selectivity and accurate elemental composition assignment through exact mass determination, resulting in analytical identification for a wide variety of small molecules, such as drugs and unknown substances (e.g., adulterants, manufacturing solvents, and byproducts), with minimal sample preparation. TOF-DART detects a variety of controlled substances in solid samples or solution preparations [5-6].

In addition to routine sample analysis, AccuTOF-DART™ may have potential as an adjunct technique for signature analyses. While such analyses have become routine in many forensic laboratories, these programs could still benefit from a rapid screening method to identify controlled substances [7]. A procedure with minimal to no...
sample preparation would complement existing methods. Determination of complex mixtures of drugs, adulterants, and diluents can help law enforcement track high-level dealers of illicit substances and identify new local or national illicit manufacturing trends. Herein, we provide a direct comparison of cocaine signature and AccuTOF-DART™ analyses of 25 refined illicit cocaine samples.

**Experimental**

**Materials:** Twenty-five DEA confiscated cocaine hydrochloride samples were obtained from the National Institute of Drug Abuse's drug supply repository for research (Bethesda, MD). Polyethylene glycol (used as the calibrating reagent) was of reagent-grade quality, and was obtained from Sigma Aldrich Chemical (St. Louis, MO). Cocaine analyte standards were purchased from Cerilliant (Austin, TX) as hydrochloride salt solutions in methanol (cocaine, anhydroecgonine methyl ester, cocaethylene, norcocaine) or acetonitrile (benzoylecgonine), all at 1 mg/mL.

**AccuTOF-DART™ Analyses:** Analyses were performed at the RTI International’s Center for Forensic Sciences using a JEOL USA, Inc. (Peabody, MA) AccuTOF-DART™. The analyses were conducted using positive modes of the DART ion source. The source was operated with a ring lens voltage of 5 V, an orifice 1 voltage of 20 V, and an orifice 2 voltage of 5 V. Electrodes 1 and 2 of the DART source were set to 150 V and 350 V, respectively, while the DART temperature was set to 300°C. The detector was optimized at 2,200 V. The AccuTOF-DART™ was calibrated with polyethylene glycol prior to each sample run. The samples were introduced into the ion source by dipping a glass probe into the sample and passing this through the stream. When available, the mono-isotopic M+H values of the cocaine analytes were verified using certified drug standard solutions.

**Cocaine Signature Analyses by Gas Chromatography/Mass Spectrometry (GC/MS):** Cocaine signature analyses were conducted by gas chromatography/mass spectrometry, as reported by Casale et al. and briefly described herein [7-11]. Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm I.D. fused-silica capillary column coated with 0.25 μm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100°C; no hold, program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280°C. The auxiliary transfer line to the MSD was operated at 280°C.

**Results and Discussion**

Table 1 contains the theoretical M+H values of the target analytes that were detected in the cocaine exhibits by cocaine signature analyses. All values are reported to 0.001 mmu with the exception of petroleum ether (which has a very low mass and thus a larger expected mass error).

The results of the AccuTOF-DART™ analysis of the 25 cocaine samples in comparison to the multi-technique signature analyses are in Table 2. Anhydroecgonine methyl ester (AEME) and cinnamoylcocaine were easily detected in 23 out of the 25 samples, as shown in the AccuTOF-DART™ spectra depicted in Figures 1A - B. In all samples, there was an ion present at m/z = 290.139, which is the M+H value of C_{16}H_{19}NO_{4}. This is the molecular formula of the isomeric pair benzoylecgonine (BE) and norcocaine, which have identical (and therefore indistinguishable) masses. Figure 2 shows the presence of the ion at 290.169 in an analyzed sample. The theoretical value of BE and norcocaine is 290.139. Although the difference of 0.030 mmu is not optimal, it may be due to an interferent present at a similar mass, resulting in a skewed m/z value. This is a problem that is frequently encountered during TOF-DART analysis. For example, known analytes may be analyzed sequentially and subjected to the same calibration, but while one peak will generate an M+H value 1 or 2 mmu from its
theoretical value, the other will have a difference of more than 10 mmu. Cocaine has a theoretical M+H value of 304.154 and the actual value, as seen in the analysis of a sample in Figure 2, is only 0.002 mmu higher than expected, while this is not the case with the BE/norcocaine isomer. In a recent study, the isomeric pair was analyzed by increasing the orifice 1 voltage to 90, which generated distinguishable ion fragmentation patterns [5]. However, this was done with methanolic standards at a high concentration, and was unsuccessful when analyzing the illicit cocaine samples used in this study.

Tropacocaine and truxillines were present in 5 and 7, respectively, of the cocaine samples (Figures 3A - B), while 3',4',5'-trimethoxy cocaine and cocaethylene were undetected. Of the solvents and adulterants/diluents detected by cocaine signature analyses, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) (Figures 4A and B), and dimethylterephthalate (Figure 1A) were all identified by AccuTOF-DART™.

The AccuTOF-DART™ allowed for the rapid introduction and analysis of 25 illicit cocaine samples without the need for sample preparation. However, although this direct analysis resulted in rapid production of data, it also gave inconsistent results. In addition, because the introductions of the powdered samples were done manually, the outcome was analyst dependent (not ideal for signature analyses, where consistency of analysis is critically important). Many samples required multiple analyses to verify the presence or absence of the target analytes. Although analytes such as AEME and cinnamoylcocaine were easily detected in most of the samples, AEME is likely present as an artifact generated from truxillines during analysis. Other analytes such as tropacocaine and 3',4',5'-trimethoxy cocaine were minimally detected, if at all.

Conclusions

The AccuTOF-DART™ is a novel approach to forensic analysis; however, its use in the analysis of refined illicit cocaine in this study proved ineffective for detecting the presence of the many compounds that are used to trace a cocaine sample to its geographic origin. In an effort to increase laboratory production, forensic laboratories may wish to utilize AccuTOF-DART™ as a rapid screening test for preliminary sample-to-sample comparison work, which could then be confirmed by more thorough analyses.

Acknowledgments

This research investigation was funded in part by the National Institute of Justice (NIJ Award 2006-DN-BX-K019).

References


* * * * *

Table 1. Theoretical Mono-Isotopic Mass+H of Analytes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Theoretical Mono-Isotopic Mass+H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anydroecgonine methyl ester</td>
<td>182.117</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>290.139</td>
</tr>
<tr>
<td>Caffeine</td>
<td>58.958</td>
</tr>
<tr>
<td>Cinnamoylcocaine</td>
<td>330.169</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>318.169</td>
</tr>
<tr>
<td>Dimethylterephthalate</td>
<td>195.064</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>89.052</td>
</tr>
<tr>
<td>(Iso-/n-)Propyl acetate</td>
<td>103.068</td>
</tr>
<tr>
<td>Lactose</td>
<td>343.116</td>
</tr>
<tr>
<td>Mannitol</td>
<td>303.079</td>
</tr>
<tr>
<td>Methyl ethyl ketone</td>
<td>73.064</td>
</tr>
<tr>
<td>Methyl isobutyl ketone</td>
<td>101.096</td>
</tr>
<tr>
<td>Norcocaine</td>
<td>290.138</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>87.90</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>58.985</td>
</tr>
<tr>
<td>3’,4’,5’-Trimethoxycocaine</td>
<td>393.178</td>
</tr>
<tr>
<td>Tropacocaine</td>
<td>246.141</td>
</tr>
<tr>
<td>Truxillines</td>
<td>658.325</td>
</tr>
</tbody>
</table>
Table 2. The Number of Samples, out of the Total 25 Analyzed, That Tested Positive for the Various Analytes, Using the AccuTOF-DART™ System and Cocaine Signature Analysis.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Cocaine Signature Analyses</th>
<th>AccuTOF-DART™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhydroecgonine methyl ester</td>
<td>ND</td>
<td>23</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>Cinnamoylcocaine</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Norcocaine</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>3',4',5'-Trimethoxyecgonine</td>
<td>25</td>
<td>ND</td>
</tr>
<tr>
<td>Tropococaine</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Truxillines</td>
<td>25</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 1. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Cocaine, Anhydroecgonine Methyl Ester (AEME), and Dimethylphthalate; and (B) Cinnamoylcocaines.
Figure 2. AccuTOF-Dart™ Spectra of an Illicit Cocaine Sample Showing the Presence of Possible Norcocaine and Benzoylcegonine (both at \( m/z = 290.17 \); see Expansion Window).

Figure 3. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Truxillines; and (B) Tropacocaine (in Expansion Window).
Figure 4. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Methyl Ethyl Ketone (MEK); and (B) Methyl Isobutyl Ketone (MIBK).
The Isolation, Identification, and Quantitation of Dimethyltryptamine (DMT) in *Mimosa hostilis*

Jack A. Fasanello* and Andrea D. Placke  
U.S. Department of Justice  
Drug Enforcement Administration  
Northeast Laboratory  
99 Tenth Avenue, Suite 721  
New York, NY 10011  
[email: jack.a.fasanello -at- usdoj.gov]  


**ABSTRACT:** Dimethyltryptamine (DMT) was extracted from the root bark of *Mimosa hostilis* via three methods, using methanol (direct or via Soxhlet) and acetic acid (direct only), respectively. The product from the direct methanol extraction was used in both qualitative and quantitative analysis, while the product from the acetic acid extraction (isolated in crystal form after workup) was used for qualitative analysis. FTIR/ATR, GC/MS, GC/IRD, ¹H-NMR, and HPLC data are presented. Quantitative analysis by ¹H-NMR and HPLC indicated 0.9 percent and 0.8 percent DMT, respectively, in the analyzed samples.

**KEYWORDS:** *Mimosa hostilis*, Dimethyltryptamine, DMT, Extraction, Analysis, Forensic Chemistry

**Introduction**

Tryptamines are substituted indole compounds which are both naturally occurring and synthetically manufactured. Many tryptamines, including dimethyltryptamine (DMT, Figure 1), have hallucinogen properties, and are therefore listed as Schedule I drugs under the U.S. Controlled Substances Act (21 CFR 1308.11). DMT is present in many plants and their seeds, including in *Mimosa hostilis* and *Psychotria viridis* [1-3], and can be abused by smoking, injection, or ingestion of either these natural materials or their crude or purified extracts, either alone or in combination with other extracts (e.g., Ayahuasca [4]). *Mimosa hostilis* and similar natural plant materials are not formally controlled (by name) in the United States; however, they are controlled (Schedule I) if they are shown to contain DMT or other controlled hallucinogens. Despite their controlled status, a number of DMT-containing natural products, including *Mimosa hostilis*, are openly marketed on the Internet.

![Figure 1. Structure of Dimethyltryptamine (DMT; C₁₂H₁₆N₂, m.w. = 188.27).](image-url)
Clandestine DMT extraction laboratories are occasionally seized by law enforcement agencies [e.g., 5]. The basis of this report was the seizure of an unknown plant material (Photo 1) at a clandestine MDMA (Ecstasy) laboratory in rural Pennsylvania. GC/MS analysis of a methanolic extraction of the material identified DMT. Upon debriefing, the defendant in the case indicated that material was root bark from *Mimosa hostilis*. Similar seizures of this material have been made at other clandestine laboratory sites in the United States, and subsequent analyses of those exhibits confirmed that they also contained DMT.

![Photo 1. *Mimosa hostilis* Root Bark Seized at Clandestine Lab in Pennsylvania.](image)

**Experimental**

**Methanol Extraction:** The root bark was cut into small pieces then ground in a blender to produce a very fine powder. For direct extraction, methanol was added to the powder, heated to 60°C with stirring for 1 hour, and then filtered. This step was repeated three more times, except the re-extractions were carried out for only 5 - 10 minutes each. The combined extracts were evaporated to a residue over steam, then reconstituted as needed for analysis. For Soxhlet extraction, the powdered material was placed in an extraction thimble, placed in a Soxhlet, and extracted with 50 mL of methanol for approximately 50 volumes. The solvent was evaporated to a residue over steam, then reconstituted as needed for analysis.

**Acetic Acid Extraction:** The root bark was cut into small pieces then ground in a blender to produce a very fine powder. A 3% acetic acid solution was added to the powder, and the resulting suspension was stirred for approximately two hours. The solution was filtered and transferred to a separatory funnel, made basic with sodium hydroxide, and then extracted with methylene chloride. The methylene chloride solution was isolated, and the aqueous later was re-extracted with a second volume of methylene chloride. The combined extracts were dried over magnesium sulfate, filtered, and evaporated to give a crystalline material.

**Fourier Transform Infrared with Attenuated Total Reflectance (FTIR/ATR)**

**Instrument:** Perkin-Elmer Spectrum One FTIR.

**Data collection:** Four scans were collected between 650 cm⁻¹ and 4000 cm⁻¹.

**Resolution:** 4 cm⁻¹.

**Sample:** Crystals from the acetic acid extraction.
Gas Chromatograph/Mass Spectrometer (GC/MS)
Column: HP-5, 30 m x 0.25 mm x 0.25 µm column.
Temperature program: 90°C - 120°C @ 35°C/min; initial time 1.35 min, then 120°C - 290°C @45°C/min; initial time 0.55 min, final hold time 8.5 min.
Injection port temperature: 300°C.
Transfer line temperature: 280°C.
Ionization source: Electron ionization (EI).
Mass analyzer: Quadrupole.
Scan range: 40 - 525.
Quadrupole temperature: 150°C.
MS source temperature: 230°C.
Sample preparation: Residue from the methanol extraction, reconstituted in methanol.

Gas Chromatograph/Infrared Detector (GC/IRD)
Instrument: Agilent 6890 GC/Varian IRD Detector.
Column: HP-5, 25 m x 320 µm x 0.52 µm column.
Split mode: 5:1.
Temperature program: 100°C for 1.50 min, ramp @ 35°C/min to 120°C, hold for 0.55 min, then ramp @ 40°C/min to 290°C, final hold for 8.13 min.
Inlet temperature: 270°C.
Injection volume: 2 µL.
Constant column flow: 2.0 mL/min.
Transfer line temperature: 280°C.
Flow cell temperature: 280°C.
KBr windows.
Optical resolution: 8.
1.5 scans/sec.
Sample: Residue from the methanol extraction, reconstituted in chloroform.

Proton Nuclear Magnetic Resonance (1H-NMR)
Instrument: Mercury 400 MHz.
Number of transients: 8.
Relaxation delay: 45 seconds.
Pulse: 90°.
Sweep width: 6393.9 Hz.
Temperature: 25°C.
Sample preparation for qualitative analysis: Crystals from the acetic acid extraction, reconstituted in 1 mL CD3OD.
Sample preparation for quantitative analysis: 5.0 g Mimosa hostilis extracted via the methanol extraction procedure, yielding 1.52 g residue. Added 28.0 mg to 1 mL CD3OD, with 5.544 mg maleic acid added as the internal standard.

High Performance Liquid Chromatography (HPLC)
Instrument: Agilent 1100 Series HPLC.
Column: Phenomenex Partisil 5µm ODS-3 (C-18).
Mobile phase: Phosphate buffer pH 2.5:methanol (90:10).
Injection: 5 µL.
Flow rate: 1.0 mL/min.
Detection: 280 nm.
Run time: 8 minutes.
Sample preparation: 9.9 g Mimosa hostilis extracted via methanol extraction procedure, with the residue reconstituted in 100 mL methanol.
Results and Discussion

The extraction of DMT from *Mimosa hostilis* was completed using two different solvents, methanol (direct or via Soxhlet) and acetic acid (direct only). The methanol extraction gave the maximum recovery of DMT for qualitative and quantitative analysis; however, the extract included other soluble plant impurities. The extraction efficiency using methanol was identical whether done directly or via Soxhlet. The acetic acid extraction gave a very clean, pure product, but in lower yield versus the methanol extraction.

**FTIR/ATR:** The crystals from the acetic acid extraction procedure produced a clean spectrum (Figure 2).

**GC/MS:** DMT eluted at 6.06 minutes using the described method. The spectra showed a base peak at $m/z = 58$ and the molecular ion at $m/z = 188$, along with smaller peaks at $m/z = 44, 77, \text{ and } 130$ (Figures 3 and 4).

**GC/IRD:** DMT eluted at 6.88 minutes using the described method (Figure 5).

$^1$H-NMR (Qualitative): The singlet at 2.35 ppm is due to the two N-methyl groups, the two triplets at 2.70 ppm and 2.95 ppm correspond to the alpha and beta methylene groups. The multiplet at 7.00 ppm corresponds to protons 2, 5, and 6 on the indole. Finally, the two doublets at 7.25 ppm and 7.50 ppm correspond to protons 4 and 7 on the indole. A slight shift was observed in the extract versus a DMT standard; this was due to pH differences (the spectrum was obtained from DMT acquired using the acetic acid extraction procedure, which involved an acid base workup). (Figure 6). (Quantitative): Using the direct methanol extract, DMT was determined to be 0.9% weight/weight in *Mimosa hostilis* (Figure 7 and Table 1). Using the direct methanol extract, DMT was determined to be 0.9% weight/weight in *Mimosa hostilis* (Figure 8 and Table 2).

**HPLC:** DMT eluted in under 3 minutes. Using the methanol extract, DMT was determined to be 0.8% weight/weight in *Mimosa hostilis* (Figure 9 and Table 3).

Acknowledgments

The authors would like to thank the Laboratory Director Thomas Blackwell, Supervisory Chemists Christopher Guglielmo and Ann Marie O’Neill, Senior Forensic Chemist Michelle Camilleri, and Forensic Chemists Christopher Benintendo and Ken Fuentecilla (all of this laboratory), and Senior Forensic Chemist Patrick Hays (DEA Special Testing and Research Laboratory, Dulles, VA).

References


* Law Enforcement Restricted Publication.
Figure 2. FTIR/ATR of a DMT Standard (Top Trace) and DMT from the Acetic Acid Extraction Procedure (Bottom Trace). [Note: The DMT Standard was Recrystallized from Chloroform.]
Figure 3. GC/MS Total Ion Chromatogram of DMT (Methanol Extract).

Figure 4. GC/MS Data of DMT (Methanol Extract). [Note: Molecular Ion at m/z = 188.]
Figure 5. GC/IRD Data of DMT (Methanol Extract). [Note: DMT eluted at 6.88 Minutes.]
Figure 6a. Full-Scale NMR Data of DMT (Acetic Acid Extract).

Figure 6b. Expanded Spectrum from 2 to 4 ppm. See Results and Discussion for Peak Assignments.
**Figure 6c.** Expanded Spectrum from 6.5 to 7.6 ppm. See Results and Discussion for Peak Assignments.

**Figure 7a.** NMR Quantitation of DMT (Direct Methanol Extract); See Table 1.
Figure 7b. NMR Quantitation of DMT (Direct Methanol Extract); Expansion; See Table 1.

<table>
<thead>
<tr>
<th>Table 1. NMR Quantitation of DMT (Direct Methanol Extract); See Figure 7.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Amount of Plant Material (g)</td>
</tr>
<tr>
<td>Amount of extraction product (g)</td>
</tr>
<tr>
<td>Sample Amount (mg)</td>
</tr>
<tr>
<td>Molecular Weight of Sample</td>
</tr>
<tr>
<td>Solvent</td>
</tr>
<tr>
<td>Internal Standard (I.S.)</td>
</tr>
<tr>
<td>Molecular Weight of I.S.</td>
</tr>
<tr>
<td>I.S. Amount (mg)</td>
</tr>
<tr>
<td>Peaks Chemical Shift (ppm)</td>
</tr>
<tr>
<td>Integral Value</td>
</tr>
<tr>
<td>Number of protons represented</td>
</tr>
<tr>
<td>Quantitation Value</td>
</tr>
<tr>
<td>Average purity of extracted material</td>
</tr>
<tr>
<td>Amount of DMT in <strong>Mimosa hostilis</strong></td>
</tr>
</tbody>
</table>
Figure 8a. NMR Quantitation of DMT (Methanol - Soxhlet Extract); See Table 2.

Figure 8b. NMR Quantitation of DMT (Methanol - Soxhlet Extract); Expansion; See Table 2.
Table 2. NMR Quantitation Results of DMT (Soxhlet Extract); See Figure 8

<table>
<thead>
<tr>
<th>Sample Amount (mg)</th>
<th>Molecular Weight of Sample</th>
<th>Solvent</th>
<th>Internal Standard (I.S.)</th>
<th>Molecular Weight of I.S.</th>
<th>I.S. Amount (mg)</th>
<th>Peaks Chemical Shift (ppm)</th>
<th>Integral Value</th>
<th>Number of protons represented</th>
<th>Quantitation Value</th>
<th>Amount DMT in Mimosa hostilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1045.0</td>
<td>188.3</td>
<td>CD3OD</td>
<td>Maleic Acid</td>
<td>116.07</td>
<td>6.118</td>
<td>[6.25..6.33] [7.01..7.17] [7.33..7.39] [7.55..7.61]</td>
<td>32.58 2.575 1.032 0.999</td>
<td>2 1 1 1</td>
<td>1.501% 0.601% 0.582%</td>
<td>0.894%</td>
</tr>
</tbody>
</table>

Figure 9. HPLC Quantitation of DMT (Methanol Extract); See Table 2.

Table 3. HPLC Quantitation of DMT (Methanol Extract); See Figure 9.

<table>
<thead>
<tr>
<th></th>
<th>Concentration (mg/ml)</th>
<th>RT 1 (minutes)</th>
<th>RT 2 (minutes)</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Average Area</th>
<th>Quant Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMT Standard</td>
<td>0.2924</td>
<td>2.685</td>
<td>2.702</td>
<td>2304.82</td>
<td>2366.98</td>
<td>2335.904</td>
<td>100.00</td>
</tr>
<tr>
<td>DMT Extract</td>
<td>99.00</td>
<td>2.686</td>
<td>2.688</td>
<td>6302.68</td>
<td>6339.69</td>
<td>6321.187</td>
<td>0.799</td>
</tr>
</tbody>
</table>
Five Year Index (by Author)


Garcia AD, Catterton AJ. 1,4-Butanediol (BD) - Forensic profile. Microgram Journal 2003;1(1-2):44-54.


Rodriguez-Cruz SE. Analysis and characterization of psilocybin and psilocin using liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) with collision-induced-dissociation (CID) and source-induced-dissociation (SID). Microgram Journal 2005;3(3-4):175-82.


Sarwar M, McDonald JL. A rapid extraction and GC/MS methodology for the identification of psilocybin in mushroom/chocolate concoctions. Microgram Journal 2003;1(3-4):177-83.


* * * * *

Five Year Index (by Compound or Topic)


1,4-Butanediol: Garcia AD, Catterton AJ. 1,4-Butanediol (BD) - Forensic profile. Microgram Journal 2003;1(1-2):44-54.


Psilocybin and psilocin (analysis by LC-ESI-MS): Rodriguez-Cruz SE. Analysis and characterization of psilocybin and psilocin using liquid chromatography - electrospray ionization mass spectrometry (LC-ESI-MS) with collision-induced-dissociation (CID) and source-induced-dissociation (SID). Microgram Journal 2005;3(3-4):175-82.


DISCLAIMERS

1) All material published in Microgram Journal is reviewed prior to publication. However, the reliability and accuracy of all published information are the responsibility of the respective contributors, and publication in Microgram Journal implies no endorsement by the United States Department of Justice or the Drug Enforcement Administration.

2) Due to the ease of scanning, copying, electronic manipulation, and/or reprinting, only the posted copies of Microgram Journal (on www.dea.gov) are absolutely valid. All other copies, whether electronic or hard, are necessarily suspect unless verified against the posted versions.

3) WARNING!: Due to the often lengthy time delays between the actual dates of seizures and their subsequent reporting in Microgram Journal, and also because of the often wide variety of seizure types with superficially similar physical attributes, published material cannot be utilized to visually identify controlled substances currently circulating in clandestine markets. The United States Department of Justice and the Drug Enforcement Administration assume no liability for the use or misuse of the information published in Microgram Journal.
Information and Instructions for Authors for Microgram Journal

General Information
Microgram Journal is a scientific periodical published by the U.S. Drug Enforcement Administration’s Office of Forensic Sciences, that presents peer reviewed, full length Scientific Research Articles and Technical Notes on the detection and analyses of suspected controlled substances for forensic/law enforcement purposes.

Access to Microgram Journal
Microgram Journal is unclassified, and is published on the DEA public access website (at: www.dea.gov/programs/forensicsci/microgram/index.html). At this time, Microgram Journal is available only electronically, and requires Internet access. Professional scientific and law enforcement personnel may request email notifications when new issues are posted (such notifications are not available to private citizens). The publications themselves are never sent electronically (that is, as attachments).

Requests to be added to the email notification list should preferably be submitted via email to the Microgram Editor at: DEA-Microgram-2008 -at- mailsnare.net Requests can also be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 8701 Morrissette Drive, Springfield, VA 22152. All requests to be added to the Microgram email notification list should include the following Standard Contact Information:

* The Full Name and Mailing Address of Submitting Laboratory or Office;

* The Full Name, Title (Laboratory Director, Assistant Special Agent in Charge, Librarian, etc.), Phone Number, FAX Number, and Preferred email Address of the Submitting Individual (Note that email notifications are mailed to titles, not names, in order to avoid problems arising from future personnel changes);

* If available, the generic email address for the Submitting Laboratory or Office;

* If a generic email address is not available, one private email address for an individual who is likely to be a long-term employee, who has a stable email address, and who will be responsible for forwarding Microgram information to all of the other employees in the requestor’s Office (Note that only one email address per Office will be honored).

Requests to be removed from the Microgram email notification list, or to change an existing email address, should also be sent to the Microgram Editor. Such requests should include all of the pertinent Standard Contact Information detailed above, and also should provide both the previous and the new email addresses.

Email notification requests/changes are usually implemented within six weeks.

Email Notifications (Additional Comments)
As noted above, the email notification indicates which issue has been posted, provides the Microgram URL, and additional information as appropriate. Note that Microgram e-notices will NEVER include any attachments, or any hyperlink other than the Microgram URL. This is important, because the Microgram email address is routinely hijacked and used to send spam, very commonly including malicious attachments. For this reason, all subscribers are urged to have current anti-viral, anti-spyware, and firewall programs in operation. However, in order to ensure that the email notifications are not filtered as spam, the DEA-Microgram-2008 -at- mailsnare email address must be “whitelisted” by the Office’s ISP.
Access to Microgram Journal is free.

Submissions to Microgram Journal
Manuscripts are accepted both from within and outside of DEA, and reviewers are both internal (from within DEA) and external.

All submissions must be in English. All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: DEA-Microgram-2007@mailsnare.net. Current versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 8701 Morrissette Drive, Springfield, VA 22152. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3½ inch IBM® PC-compatible diskette, 100 or 250 MB Iomega® zip diskette, or an IBM® PC-compatible CD. Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: “Warning - Contains Electronic Media - Do Not Irradiate”.

Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 1/2” x 11” or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. All photos and figures should also be submitted as stand-alone attachments, not only embedded in the manuscript. The pages should be numbered, but not stapled together.

Scientific Research Articles are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.) Technical Notes are shorter communications concentrating on a specific drug (or drug class), unusual case, novel or unusual procedure or method, or minor original research, again excluding in post-ingestion human/animal biological matrices. Each article/note should be a “stand-alone” work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is received by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes may follow an abbreviated version as appropriate):

Cover Letter - Provide the standard contact information and pertinent correspondence (if any) for the Editor.

Title - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the
manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

Author(s)/Affiliation(s) - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author’s degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page.

Abstract - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under “Titles”. Note that the abstract will be provided to Chemical Abstracts.

Keyword List - A minimum of five (maximum ten) abstracting keywords should be included. Unless inappropriate, the last keyword pair should always be “Forensic Chemistry.”

Introduction - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid “Personal Communications”).

Experimental (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, USE CAUTION IN DETAILING SYNTHESES OF CONTROLLED OR ABUSED SUBSTANCES, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: “Experimental details on this synthesis are not provided, in accordance with Journal policy.”]

Results and Discussion - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

Conclusions - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or the summary paragraph in the Results and Discussion section.

Acknowledgments - Optional - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

References - Articles and notes should have all textual citations collected in an endnotes list. Within the text, references should be consecutively numbered with superscripted Arabic numerals, or with Arabic numerals in brackets, in accordance with their first appearance. Within the endnotes list, references should be consecutively numbered with Arabic numerals, as follows: Number, Period, Indent, Citation. Reference format should adhere to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Note: This is the same reference format utilized in the Selected Reference Citations in Microgram Bulletin, and also (among many others) by the Journal of Forensic Sciences). Due to their inherently transitory nature, use of website URL’s as references are discouraged but are permitted. As
previously noted, Personal Communications should not be utilized; however, if unavoidable, utilize the following format: Full Name, Title, Affiliation (Laboratory or Office), Location (City and State, plus Nation if not the United States), Personal Communication, Year.

**Table and Figures** - All Tables and Figures should be appended onto the end of the article (not embedded in the text). Tables and Figures should be consecutively numbered with Arabic numerals, in accordance with their first citation in the text. Each Table and Figure should be “stand-alone”; that is, include sufficient descriptive information such that the reader will not have to refer back to the text to understand the Table or Figure. The Header should include the Table or Figure number and a concise title. Explanatory material, definitions of acronyms and/or abbreviations, and/or references within the Table or Figure should be designated by superscripted, lower case letters in alphabetical order, and included in dedicated footnotes at the bottom of the respective Table or Figure. Unless color is needed to enhance differentiation of the depicted material, all Tables and Figures should be in black and white (that is, avoid frivolous use of color for “artistic” purposes). Figures of spectra, chromatograms, charts, graphs, etc., should have clear and legibly labeled axes, but should not include instrument generated printoffs of experimental parameter lists.

Manuscripts submitted to *Microgram Journal* are required to be finished, professional quality efforts. Authors should ensure clarity, brevity, and pertinence of all information. Attention to detail in formatting, syntax, grammar, and spelling are as important as the accuracy of the facts presented. Authors are specially cautioned to conduct careful literature reviews prior to submission. At the Editor’s discretion, clearly substandard and/or inappropriate manuscripts will be returned to the authors without review.

**Manuscripts will not be retyped, but “final” versions are subject to minor to moderate Editorial rewrite to improve presentation clarity or to reformat to current *Microgram Journal* style.**

**Dual publication** - Re-publication of articles or notes of particular interest to the *Microgram Journal* readership will be considered if the article was originally published in a journal that is not easily accessed and the primary author has obtained explicit, written copyright exclusion from the original publisher and consent from all co-authors. Examples include exact English translations of articles or notes originally published in a non-English language journal, non-sensitive articles or notes originally published in a restricted journal or on a password protected website, or articles or notes originally published in limited distribution newsletters or proceedings. In general, any article or note that was published in English in a mainstream journal is not a candidate for re-publication in *Microgram Journal*. Authors interested in re-publishing previously published articles or notes in *Microgram Journal* should discuss the issue with the *Microgram* Editor before submitting.

Note that (in accordance with standard ethical guidelines) re-published articles should not be included as “new” articles in the respective author(s)’ *Curriculum Vitae*.

**Costs** - There are no costs (to the contributor) associated with publication in *Microgram Journal*.

**Reprints** - *Microgram Journal* does not provide reprints to authors. *Microgram Journal* may be photocopied (or printed off the website) as needed.

Questions may be directed to the *Microgram* Editor.

* * * * *
Contents

The Use of Dipropionylmorphine as a Structurally-Related Internal Standard for Gas Chromatographic Quantitation of Heroin  
Susan C. Kerr and John F. Casale  
3

Rapid Screening of Seized Drug Exhibits Using Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)  
Sandra E. Rodriguez-Cruz  
10

Discovery of an Interesting Temperature Effect on the Sensitivity of the Cobalt Thiocyanate Test for Cocaine  
Jim W. McGill, Crystal A. Dixon, David Ritter, and Joanna D. Sides  
26

Identification of N-Methylbenzylamine Hydrochloride, N-Ethylbenzylamine Hydrochloride, and N-Isopropylbenzylamine Hydrochloride  
Ramona M. Sanderson  
36

Isolation of Methamphetamine from 1-(1',4'-Cyclohexadienyl)-2-methylaminopropane (CMP) Using Potassium Permanganate  
Fracia S. Martinez, Daniel M. Roesch, and James L. Jacobs  
46

Information and Instructions for Authors  
55

Note: In order to prevent automated theft of email addresses off the Internet postings of Microgram Journal, all email addresses reported in the Journal have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of 3-Chlorophenylpiperazine (mCPP; Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).
The Use of Dipropionylmorphine as a Structurally-Related Internal Standard for Gas Chromatographic Quantitation of Heroin

Susan C. Kerr, B.S.* and John F. Casale, B.S.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[ email address withheld at author’s request ]

ABSTRACT: Dipropionylmorphine is utilized as a structurally similar internal standard for quantitation of illicit heroin via gas chromatography with flame ionization detection. The described method has excellent selectivity, precision, and accuracy, with a relative standard deviation of less than 0.2 percent and a correlation coefficient of 0.99999. The quantitative results were in excellent agreement with other quantitative methods. The synthesis of high-purity dipropionylmorphine from morphine is detailed.


Introduction

The quantitative determination of illicit heroin (Figure 1) and related opium alkaloids is important for forensic, toxicological, and judicial purposes. Many methods have been published for this purpose, including using, for example, high pressure liquid chromatography (HPLC) [1], capillary electrophoresis (CE) [2], nuclear magnetic resonance (NMR) [3], and gas chromatography (GC) [4-6].

Internal standards are routinely incorporated in quantitative methods. Structurally similar internal standards are preferred because they improve method accuracy and precision. However, most analytical methods that employ internal standards use non-structurally related hydrocarbons such as \( n \)-tetracosane [4], \( n \)-octacosane [4], and \( n \)-triacontane [5] because of their wide availability, high purity, high stability, and relative ease in handling. In contrast, methods using structurally related internal standards are uncommon.

Specifically looking at gas chromatography analysis with flame ionization detection (GC/FID), an ideal internal standard should have similar chemical and physical properties as well as comparable FID responses to the analyte of interest. As such, the structurally related internal standard would maximize precision and accuracy [6] and minimize issues related to reactivity, absorption, solubility, and inlet/on-column degradation [4,7]. Diacetylnalorphine has been previously utilized as a structurally similar internal standard for heroin quantitation [8], and satisfies the above criteria. However, it is not an ideal compound for routine use because of the relatively high cost of its precursor (nalorphine), and its moderate instability in solution (three months at 4°C).

To date, only diacetylnalorphine has been reported for use as a structurally similar internal standard for heroin quantitation. However, dipropionylmorphine (Figure 1) was previously utilized as a target compound for derivatization reagent. A priori, dipropionylmorphine would be an ideal internal standard for heroin quantitation by GC/FID, providing it is non-coincident with any other opium alkaloids or typical adulterants and diluents. Herein we report the facile synthesis and successful use of dipropionylmorphine as a structurally similar internal standard for GC/FID analysis of heroin.
Experimental

Reagents: All solvents were obtained from Burdick and Jackson Laboratories (Muskegon, MI). Diethylamine, propionylchloride, ammonium hydroxide, alumina, and activated carbon were obtained from Sigma Aldrich Inc. (St. Louis, MO). Heroin hydrochloride and morphine hydrochloride standards were obtained from this laboratory’s reference collection.

Synthesis of Dipropionylmorphine: Morphine hydrochloride monohydrate (40.0 g, 0.124 mol) was combined with 800 mL of acetonitrile and propionyl chloride (51.1 g, 0.552 mol) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was refluxed gently, with stirring, for 22.5 hours. Upon cooling, the reaction mixture was split into four 200 mL aliquots. Each portion was added slowly with stirring to a mixture of isooctane (1.0 liter) and diethyl ether (800 mL), causing crude dipropionylmorphine hydrochloride to precipitate from solution. The crystals were captured via suction filtration and washed with anhydrous diethyl ether (200 mL). All four crops of crude material were then dissolved into 400 mL of water and filtered. The filtrate was washed with isooctane (500 mL), then with anhydrous diethyl ether (400 mL). The solution was adjusted to pH 9 with concentrated ammonium hydroxide and extracted with methylene chloride (2 x 300 mL). The extracts were combined, dried over anhydrous sodium sulfate, filtered, and treated with activated carbon (2 g). The carbon was removed by filtering through a celite pad. The filtrate was evaporated in vacuo to provide approximately 40 grams of 96 percent dipropionylmorphine base. The material was chromatographed on a basic alumina column (600 g containing 4 percent H2O) using methylene chloride. The first 800 mL of eluate was collected and evaporated in vacuo to a clear oil. The oil was dissolved into a minimal volume of anhydrous diethyl ether in a flask, and the flask was scratched to crystallize dipropionylmorphine base as a chromatographically pure (99+ percent) white powder (34.2 g, 70 percent yield).

Gas Chromatography - Flame Ionization Detection (GC/FID): An Agilent 6890N GC with a DB-5 column (30 m x 0.25 mm I.D., 0.25 µm film thickness) was utilized. The oven temperature program began at 205°C (1 minute hold), ramped to 240°C at 12°C/minute (5 minute hold), ramped at 4°C/minute to 275°C (1 minute hold), and then ramped at 15°C/minute to 285°C (2.33 minute hold). The carrier gas was hydrogen (99.999 percent UHP) at a flow rate of 0.9 mL/minute, with a split ratio of 25:1. The injector and detector temperatures were maintained at 280°C.

Gas Chromatography - Mass Spectrometry (GC/MS): An Agilent 6890N GC/MSD with a DB-1 column (30 m x 0.25 mm I.D., 0.25 µm film thickness) was utilized. The oven temperature program began at 90°C (2 minute hold), ramped to 300°C at 14.0°C/minute (10.0 minute hold). The carrier gas was ultra high purity Helium at a flow of 1.0 mL/minute, with a split ratio of 25:1. The injector and detector temperatures were maintained at 280°C. The mass spectrum of dipropionylmorphine is presented in Figure 2.

Internal Standard Stock Solution: A stock solution of dipropionylmorphine was prepared at 1 mg/mL in chloroform. The solution was used at room temperature and can be stored at 4°C for up to two years without detectable degradation.

Standard and Sample Preparation: Approximately 18 - 20 mg of heroin hydrochloride standard was accurately weighed into a 50 mL Erlenmeyer flask. 5.00 mL of the internal standard stock solution and 20 mL of chloroform (containing 50 µL of diethylamine) were added to the Erlenmeyer flask and the solution was allowed to sit for 5 minutes. Samples were prepared in the same manner with slight modifications in sample weight to maintain sample concentration within the linear range of the method (see below). Aliquots of both standard and sample were transferred to separate autosampler vials for analysis.

Linearity and Precision: Nine individual concentrations of heroin hydrochloride were prepared (at 0.106, 0.207, 0.418, 0.611, 0.757, 0.807, 1.011, 1.632, and 2.039 mg/mL) with the internal standard, as described above. All nine concentrations were utilized to calculate the method linearity and precision.
Results and Discussion

High purity dipropionylmorphine is easily prepared from morphine hydrochloride. As expected, heroin and dipropionylmorphine give highly similar FID responses. The selectivity of dipropionylmorphine on a DB-5 column was excellent, with no interferences with any of the opium alkaloids, adulterants, and diluents typically present in illicit heroin (see Figure 3 and Table 1). The stock solution was stable for over 2 years at 4°C (no detectable degradation or hydrolysis, and consistent FID peak area and height counts (see Figure 4)).

The method linearity was determined over the concentration range stated in the Experimental section. The calculated correlation coefficient (R²) was 0.99999 (see Figure 5). The method precision was determined using all nine of the solutions listed in the Experimental section, with seven replicate injections per solution. The Relative Standard Deviations (RSDs) ranged from 0.04 to 0.17 percent. The method accuracy was determined by quantitating 11 illicit samples that had previously been analyzed in this laboratory via proton nuclear magnetic resonance (1H-NMR) and capillary electrophoresis (CE). The average difference for the three methods was determined to be 2.6 percent absolute (see Table 2).

Finally, the GC/FIDs of four different types of heroin are presented in Figure 6. Highly refined samples, such as Southeast Asian (SEA/4) and South American (SA) heroin, as well as crudely refined samples, such as Southwest Asian (SWA/A) and Mexican black tar (MEX) heroin, can all be routinely quantitated utilizing this method.

References:


* Law Enforcement Restricted Publication.
Table 1. Relative Retention Times (RRT) of Some Common Adulterants and Alkaloids.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RRT (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>0.16</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>0.17</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.22</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>0.23</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.27</td>
</tr>
<tr>
<td>Procaine</td>
<td>0.30</td>
</tr>
<tr>
<td>Cocaine</td>
<td>0.39</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.51</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.54</td>
</tr>
<tr>
<td>Acetylcodeine</td>
<td>0.61</td>
</tr>
<tr>
<td>O6-Monoacetylmorphine</td>
<td>0.62</td>
</tr>
<tr>
<td>Heroin</td>
<td>0.75</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Dipropionylmorphine</strong></td>
<td><strong>1.00</strong></td>
</tr>
<tr>
<td>Papaverine</td>
<td>1.03</td>
</tr>
<tr>
<td>Noscapine</td>
<td>1.53</td>
</tr>
</tbody>
</table>

---

Table 2. Comparison of 11 Samples Quantitated by Different Methods.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% by CE</th>
<th>% by NMR</th>
<th>% by GC/FID using Dipropionylmorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.7</td>
<td>N/A</td>
<td>10.9</td>
</tr>
<tr>
<td>2</td>
<td>83.6</td>
<td>N/A</td>
<td>81.2</td>
</tr>
<tr>
<td>3</td>
<td>74.2</td>
<td>72.2</td>
<td>71.8</td>
</tr>
<tr>
<td>4</td>
<td>86.3</td>
<td>N/A</td>
<td>86.3</td>
</tr>
<tr>
<td>5</td>
<td>93.3</td>
<td>93.2</td>
<td>89.4</td>
</tr>
<tr>
<td>6</td>
<td>86.0</td>
<td>N/A</td>
<td>86.1</td>
</tr>
<tr>
<td>7</td>
<td>80.9</td>
<td>78.0</td>
<td>77.8</td>
</tr>
<tr>
<td>8</td>
<td>47.6</td>
<td>N/A</td>
<td>45.8</td>
</tr>
<tr>
<td>9</td>
<td>56.5</td>
<td>58.0</td>
<td>55.2</td>
</tr>
<tr>
<td>10</td>
<td>11.2</td>
<td>10.9</td>
<td>10.7</td>
</tr>
<tr>
<td>11</td>
<td>10.2</td>
<td>11.9</td>
<td>9.7</td>
</tr>
</tbody>
</table>
Figure 1. Structures of Heroin (Left) and Dipropionylmorphine (Right).

---

Figure 2. Mass Spectrum of Dipropionylmorphine.

Microgram Journal, Volume 6, Numbers 1-2 (January - June, 2008)
**Figure 3.** Capillary GC/FID Profile of a Typical Street Heroin Sample Containing Several Adulterants. Peak Identification: 1 = Caffeine, 2 = Lidocaine, 3 = Cocaine, 4 = Acetylcodeine, 5 = O6-Monoacetylmorphine, 6 = Heroin, 7 = Internal Standard, 8 = Papaverine, 9 = Noscapine.

**Figure 4.** GC/FID Comparison of Internal Standard Prepared on May 14, 2004 (Upper) and Internal Standard Prepared on November 6, 2007 (Lower); No Loss in Peak Area Detected. Peak Identification: 1 = O6-Monoacetylmorphine, 2 = Heroin Standard (contains a small amount of O6-Monoacetylmorphine), 3 = Dipropionylmorphine Internal Standard.
**Figure 5.** Linearity for Heroin with Dipropionylmorphine Internal Standard.

**Figure 6.** GC/FID Comparison of Four Types of Heroin. Southeast Asian (SEA/4) 86.3 % Heroin Hydrochloride, South American (SA) 86.1 % Heroin Hydrochloride, Mexican (MEX) 9.8 % Heroin as Hydrochloride, and Southwest Asian (SWA/A) 57.6 % Heroin Base. Peak Identification: 1 = Acetylcodine, 2 = O6-Monoacetylmorphine, 3 = Heroin, 4 = Dipropionylmorphine Internal Standard, 5 = Papaverine, 6 = Noscapine.
Rapid Screening of Seized Drug Exhibits Using Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

Sandra E. Rodriguez-Cruz, Ph.D.
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
[email: Sandra.E.Rodriguez-Cruz-at-usdoj.gov]

[Presented in Part at the 2007 American Society for Mass Spectrometry (ASMS) Fall Workshop - The Art of Open Air Ionization on Surfaces, Philadelphia, PA (November 9, 2007).]

ABSTRACT: Desorption electrospray ionization mass spectrometry (DESI-MS), an extension of the electrospray ionization technique, is utilized for the rapid screening and/or pre-analysis of multi-unit exhibits. Multiple real-case analyses are presented, including hydrocodone tablets, Ecstasy tablets containing MDMA/methamphetamine mixtures, prednisone tablets, alprazolam tablets, marijuana, counterfeit sildenafil citrate tablets containing sildenafil base, counterfeit oxycodone tablets containing fentanyl, breath mints containing Δ9-tetrahydrocannabinol, a chocolate-coated opium bar, and an unknown liquid containing a mixture of gamma-butyrolactone and gamma-hydroxybutyric acid. Analyses were conducted directly on the samples, in most cases with no sample preparation, and the results were obtained in less than 30 seconds per sample. Additionally, the use of DESI-MS/MS enabled identification of the controlled substances and adulterants in the samples.

KEYWORDS: Desorption Electrospray Ionization Mass Spectrometry, DESI-MS, DESI-MS/MS, Controlled Substances, Screening, Analysis, Forensic Chemistry.

Introduction

Forensic laboratories with large caseloads need high-throughput sampling and identification techniques in order to reduce turn-around times while fulfilling the requirements of the judicial system. For exhibits composed of multiple units, even if the units are visually indistinguishable, the standard analytical protocol requires a pre-analysis (screening) on each individual or selected unit to determine composition, before either combination of the units into a homogeneous composite, or separation of the units into several mutually exclusive subgroups, based on the results. For decades, screening was done with a combination of visual inspection, color tests, microcrystal tests, thin-layer chromatography, microscopy, and similar techniques. However, most such techniques are only presumptive, and evolving laboratory policy and procedures mandate the identification of all controlled substances in each individual unit prior to composition.

The capabilities of analytical instruments (including screening instruments) have been greatly extended with the addition of powerful detectors, autosampling devices, data handling computers, and library searching abilities. However, increasing sophistication usually equates to longer analyses and the generation of very large amounts of data, increasing turnaround times. Furthermore, sample preparation, extractions, and chemical derivatization steps are still often necessary in order to conclusively identify active ingredient(s). Currently, gas chromatography - mass spectrometry (GC/MS) is the most commonly used technique for the screening and preliminary identification of forensic exhibits. However, depending on instrument parameters, even short GC
analyses can take five minutes between injections. For a case with 25 samples, this already translates into more than two hours of analysis, not including sample preparation time and data processing. Standard GC and GC/MS analyses usually take considerably longer. Pre-analyses/screening of individual units can also be done with infrared (FTIR) or Raman spectroscopy; however, these techniques are best suited to pure or uncut samples, and are usually not very fast. For these reasons, there is a continuing need for instrumental techniques that can very rapidly analyze large numbers of samples, including samples with multiple components.

A rapid, confirmational screening technique for the analysis of benzodiazepines in drinks using direct electrospray probe / mass spectrometry has been reported [1]. However, although able to identify unknown samples during rapid screening, an extraction procedure was required prior to analysis. Fast analysis of multiple drugs of abuse in urine and hair using liquid chromatography - mass spectrometry techniques have been reported [2,3]. More recently, Cooks et al. [4] reported the development of a new, ambient sampling technique, desorption electrospray ionization (DESI). This technique allows for the rapid analyses of samples under ambient conditions and without any type of sample preparation, using mass spectrometry as the detector. Also recently, Cody et al. developed another technique for rapid atmospheric sampling using mass spectrometry [5]. Direct analysis in real time (DART) interfaced with time-of-flight mass spectrometry also allows the analysis of samples without the need for sample preparation or extraction procedures. The introduction of these techniques represents a significant step forward in the development of high-throughput analyses. Their application to the analysis of forensic samples could have a great impact on the quantity and quality of data generated by forensic chemists, especially for the rapid confirmatory screening of controlled substances.

During DESI, charged solvent droplets are directed towards the surface of a sample, resulting in the formation of singly and/or multiply charged species similar to those observed under normal electrospray conditions [6,7]. The samples can be analyzed either directly or after deposition onto a non-conducting surface. The interface of the DESI technique with a mass spectrometer (DESI-MS) or a tandem mass spectrometer (DESI-MS/MS) allows for the formal identification of the charged species. It has been proposed that the ion formation process during DESI occurs via at least three mechanisms [4]. The first involves a molecule-pick-up process resulting from the impact of charged solvent droplets onto the sample surface. This mechanism is believed to be responsible for the ESI-like spectra generated during DESI. The second involves a charge and momentum transfer leading to desorption of the sample molecules as ions. Indirect evidence for this mechanism is obtained by the generation of DESI spectra for carotenoid compounds, which otherwise are not ionized using ESI. The third involves the volatilization or desorption of neutral species followed by gas-phase ionization via ion/molecule reactions.

Herein, multiple applications of DESI-MS and DESI-MS/MS for the screening and analysis of various controlled substances are presented. Our previous studies are also summarized here, as they further demonstrate the application of this recently developed technique for forensic analyses [8]. Samples of licit as well as illicit origin were investigated, and the rapid identification of the controlled active ingredient(s) was/were achieved. Analyses were conducted directly on the samples, in most cases with no sample preparation, and were completed in less than 30 seconds per sample.

**Experimental**

Experiments were performed using a Thermo Fisher Scientific LCQ Advantage MAX quadrupole ion-trap mass spectrometer equipped with an IonMAX atmospheric pressure ionization source and an ESI probe (San Jose, CA). This system was interfaced to a Thermo Fisher Scientific Surveyor HPLC system. For the DESI experiments, the desorbing solvent was delivered by the built-in syringe pump on the mass spectrometer or by the solvent delivery pump of the HPLC system. These two options allowed for delivery of solvent at flow rates between 2 and 100 μL per minute. Depending on experimental needs, the desorbing solvent was a mixture of methanol, deionized water (0.1 percent formic acid), and/or acetonitrile (0.1 percent formic acid). The solvent was directed to the ESI interface without further modification.
The atmospheric pressure ionization chamber was kept opened to the laboratory atmosphere and the automatic high voltage shut-off of the IonMAX chamber was disabled in order to allow sampling. Once constant ESI solvent, voltage, and current were achieved, direct sampling was performed. As during routine electrospray operation, the droplet desolvation process was aided using Nitrogen (99 percent; 100 ± 20 psi) as both the sheath and auxiliary gas, operated at 50 and 20 units, respectively. The ESI source transfer capillary was maintained at a temperature of 250°C, while the capillary and tube lens were kept at 30 and 15 V, respectively.

Instrument control, data collection and analysis were performed using the Xcalibur software (version 1.4) provided by the instrument manufacturer. Mass spectrometry data were collected in the positive ion mode using either the real-time view provided by the Tune Plus program module or through the Sequence module provided by the software. By setting up the mass analyzer to collect both full-scan and MS² data, molecular weight and structural information was obtained. Collision-induced dissociation experiments were performed after optimization of the collision energy (typically 25-35 percent eV) for the analyte. Helium (99.999 percent; 40 ± 10 psi) was used as both the trapping and collision gas. Under these conditions, the fragmentation data obtained from DESI-generated ions can be directly compared to the laboratory-generated standard spectral library previously developed using ESI-generated ions.

Samples were obtained from seized exhibits submitted to the laboratory for forensic analysis, and were analyzed as received (i.e., with no preparation or derivatization steps prior to the DESI-MS and/or DESI-MS/MS analyses). Sampling was performed by positioning a flat portion of the material in question, using non-conducting (Teflon®) tweezers, slightly below the entrance to the mass spectrometer, within the electrospray plume region (see Photo 1). The samples were positioned to obtain a 45 degree angle (approximately) between the flat surface to be analyzed and the electrospray emitter (optimal sample positioning has been addressed by Cooks et al. [4]). Samples were analyzed for 1 to 5 seconds, and sufficient solvent-only collection time (approximately 1 minute) was allowed between samples in order for the signal background to return to low levels.

**Results and Discussion, I - Previous Studies**

Previously, this laboratory used DESI-MS and DESI-MS/MS for the rapid analyses of various tablets and marijuana [8]. The objectives and results of those studies (summarized below) demonstrated the use of these techniques for pre-analysis/screening purposes.

**Objective 1:** To demonstrate that the technique can detect the presence of one or more controlled substances in the presence of other major matrix components. Analyses were conducted on authentic Vicodin® tablets and on illicit Ecstasy-type tablets suspected to contain 3,4-methylenedioxymethamphetamine (MDMA). Each Vicodin® tablet contained 5 mg of hydrocodone bitartrate, 500 mg of acetaminophen, and various tablet binding components [9]. Figure 1 shows the ambient sampling of five of these tablets. The total-ion-current (TIC) (upper) trace shows the real-time sampling of the tablets (completed in less than 6 minutes), with each peak representing a different tablet. The bottom five panels display the full-scan mass spectra for each tablet. The molecular ions at m/z 152 and 325 correspond to protonated acetaminophen (MW = 151 Da) and the sodium-bound dimer of acetaminophen (2M+Na⁺), respectively. The peak at m/z 300 is due to protonated hydrocodone (MW = 299 Da). It is noteworthy that the presence of acetaminophen does not interfere with the detection of hydrocodone, even though the concentration of the latter is 100 times lower. The observed relative intensities of the two components vary from sample to sample due to the variable (non-reproducible) positioning of the tablets within the ESI plume.

Figure 2 shows the ambient sampling of five suspected Ecstasy tablets. The full-scan mass spectral data shows a major component at m/z 194, consistent with MDMA (MW = 193 Da), and additional peaks at m/z 163 and 150. The peak at m/z 163 corresponds to a fragment commonly observed during analysis of the methylenedioxymethamphetamine. The peak at m/z 150 suggests the presence of methamphetamine (MW = 149
Further chemical analysis confirmed the presence of MDMA hydrochloride and methamphetamine hydrochloride at concentrations of 57.0 and 9.5 milligrams/tablet, respectively.

**Objective 2:** To demonstrate that the methodology is free of interferences and/or cross-contamination (carry-over) from previously tested samples. Analyses were conducted on authentic prednisone and Vicodin® tablets. The first and third tablets were prednisone (MW = 358 Da), while the second and fourth tablets were Vicodin® (same as above). Figure 3 shows the results from the alternating sampling of the four tablets. The prednisone tablets (left panels) display a molecular ion at $m/z$ 359. The Vicodin® tablets (right panels) again display the molecular ions for acetaminophen and hydrocodone. No interferences or cross-contamination (carry-over) are observed in any of the spectra.

**Objective 3:** To demonstrate that the methodology can identify controlled substances. DESI-MS/MS analyses were conducted on 23 authentic Xanax® tablets; each tablet contained 2 milligrams of alprazolam (MW = 308 Da). Figure 4 shows the ambient sampling for four of these tablets. The fragmentation data contains the major fragment ions generated upon dissociation of the $m/z$ 309 ion; comparison with a previously generated library standard confirmed alprazolam. For a total tablet weight of 260 milligrams, this represents detection of the active ingredient at a concentration of 0.7 percent. Of note, the MS/MS data obtained using DESI can be directly compared with the laboratory-generated ESI library using standard MS/MS conditions. Thus, the development of a DESI-MS/MS specific library is not necessary.

**Objective 4:** To demonstrate that the methodology can analyze plant material. Cooks and co-workers previously performed DESI-MS analyses on various natural products, including tomatoes and hibiscus flowers [4]. Their experiments confirmed the ability of DESI-MS to detect some of the main components in these substances, regardless of interferences generated by the complicated plant matrices. Figure 5 displays the DESI-MS data from the ambient sampling of three dried marijuana leaves. The spectrum included one major molecular ion at $m/z$ 315 and minor peaks at $m/z$ 311, 327, 341, and 359. The peak at 311 corresponds to protonated cannabinol (MW = 310 Da), while the peak at $m/z$ 315 is due to delta-9-tetrahydrocannabinol (THC; MW = 314 Da). The peaks at $m/z$ 341 and 359 are probably due to other minor cannabinoids, likely including the carboxylic acid of THC (MW = 358 Da). The identification of THC was confirmed by performing MS/MS experiments, which matched the previously generated library standard.

**Results and Discussion, II - Recent Applications**

Additional forensic applications of DESI-MS and DESI-MS/MS continue to be developed at this laboratory. The most recent applications (summarized below) include analyses of counterfeit pharmaceuticals, “medical” marijuana exhibits, gamma-butyrolactone (GBL) and gamma-hydroxybutyrate (GHB), and disguised opium formulations.

**Counterfeit Pharmaceuticals** - Counterfeit tablets and liquids are commonly submitted to this laboratory, usually pursuant to diversion investigations targeting the commercial black market. For example, the laboratory has recently received multiple exhibits of white tablets suspected to be counterfeit Viagra® (see Figure 6). These tablets all bore the characteristic logos; however, legitimate tablets are blue and contain sildenafil citrate. Analysis by DESI-MS and DESI-MS/MS confirmed sildenafil (MW = 474 Da); however, citrate (MW = 190) was not present (see Figure 6, lower panel), confirming that the tablets were counterfeits.

In another recent case, the laboratory received 9,463 round, concave, green tablets bearing “OC” and “80” inscriptions, suspected to be counterfeit or mimic Oxycontin® (see Figure 7). Again, the tablets all bore the correct logos; however, the tablets were slightly smaller than the legitimate product, and also were green throughout (the legitimate product is a compressed white powder with a colored coating). Analysis of nearly ten thousand tablets would be a daunting task for any forensic laboratory; however, the DEA evidence sampling plan allows for preliminary analysis of 29 randomly selected tablets, and if the results are consistent in all 29 tablets, formulation of a final composite for full characterization and purity determination [10]. Even screening of 29
tablets would be a tasking usually requiring many hours; however, the DESI-MS/MS analyses were completed in less than 20 minutes. The tablets actually contained very low levels of fentanyl (MW = 336 Da), not oxycodone (MW = 315 Da), confirming that they were mimics.

“Medical” Marijuana - The recent seizures of “medical” marijuana concoctions at dispensaries in California have resulted in numerous submissions of previously unseen materials suspected to contain THC. Analysis of such exhibits can be challenging, due to their wide variety and often highly complex matrices (typically foods and candies). One such case included 14 multiple colored flat squares described as “THC Breath Mints” (see Figure 8). DESI-MS/MS confirmed THC in all 14 samples, in less than 10 minutes.

Opium - Forensic laboratories occasionally receive controlled substances concealed inside food items, typically candy bars. A recent such case included 15 chocolate-covered opium bars (see Figure 9). In this case, DESI-MS analysis of the surface of the bars (i.e., the chocolate) would not indicate any controlled substance. However, analysis of a shaved piece of the suspected opium confirmed the five primary opium alkaloids: Morphine (MW = 285), codeine (MW = 299), thebaine (MW = 311), papaverine (MW = 339), and noscapine (MW = 413). The lower panel in Figure 9 shows the mass spectrum for bar #13, displaying the expected five protonated ions. The analysis of all 15 samples was completed in less than 6 minutes.

GHB/GBL Mixtures - DESI/MS analyses can also be conducted on liquids. A recent such submission consisted of a clear liquid suspected to contain the “date-rape” drug gamma-hydroxybutyrate (GHB; MW = 104 Da). Analysis was accomplished by placing a drop of the sample onto a glass slide and positioning it within the ESI plume, giving four major ions at m/z 87, 105, 173, and 191. The smaller ions are indicative of protonated GBL (MW = 86 Da) and GHB, respectively, while the two larger ions at m/z173 and 191 correspond to the GBL homo-dimer [(2GBL+H+) ] and the hetero-dimer [(GBL+GHB+H+) ], respectively (see Figure 10).

Conclusions

DESI-MS and DESI-MS/MS experiments are ideal for the rapid screening of multiple-unit exhibits. The technique provides reproducible data with a high degree of sensitivity. DESI-MS experiments will provide molecular weight information and therefore a presumptive or preliminary identification (this will need to be confirmed with a second technique like GC/MS, FTIR, or NMR). DESI-MS/MS further provides structural information via fragmentation data that can be directly compared with standard reference spectra. Analyses are accomplished in less than 30 seconds per sample, without sample preparation or extraction procedures.

Acknowledgments

The author thanks Forensic Chemists Jason A. Bordelon, Michael M. Brousseau, and Alan M. Randa (all at this laboratory) for contributions with DESI-MS data collection, and Supervisory Forensic Chemist Esther W. Chege (this laboratory) for review of the manuscript.

References


---

Photo 1. Sampling of a Tablet for DESI Analysis.
Figure 1. Rapid Sampling of Vicodin® Tablets using DESI-MS.
Figure 2. Rapid Sampling of MDMA/Methamphetamine Tablets using DESI-MS.
Figure 3. Rapid Alternating Sampling of Prednisone and Vicodin® Tablets using DESI-MS.
**Figure 4.** Fragmentation Spectra (m/z 309) Obtained During the DESI-MS/MS Sampling of 4 Xanax® Tablets. Also shown is the MS/MS Spectrum of the Alprazolam Standard.
**Figure 5.** DESI-MS and DESI-MS/MS Analysis of Cannabis Leaves. Also Shown is the MS/MS Spectrum for the THC Standard.
Figure 6. Upper Panel: Counterfeit Viagra® Tablets. Middle Panel: DESI-MS/MS Data (Fragmentation of $m/z$ 475) in the Positive Ion Mode for Legitimate (Left) and Counterfeit (Right) Tablets. Lower Panel: DESI-MS Data (Full Scan) in the Negative Ion Mode for Legitimate (Left) and Counterfeit (Right) Tablets.
Figure 7. Upper Panel: Counterfeit Oxycontin® Tablets. Middle Panel: DESI-MS Sampling of 29 Unknown Tablets. Lower Panel: DESI-MS/MS Data (Fragmentation of m/z 337) for Unknown Tablet #3 (Left) and Fentanyl Standard (Right).
Figure 8. Upper Panel: “Medical” Marijuana Breath Mints. Middle Panel: DESI-MS/MS Sampling of 14 Breath Mints. Lower Panel: DESI-MS/MS Data (Fragmentation of m/z 315) for Breath Mint #7 (Left) and MS/MS Spectrum for the THC Standard (right).
Figure 9. Upper Panel: Chocolate-Covered Opium Bars. Middle Panel: DESI-MS Sampling of 15 Opium Bars. Lower Panel: DESI-MS Spectrum of Bar #13 Containing the Expected Molecular Ions for the Five Main Opium Alkaloids (Morphine, Codeine, Thebaine, Papaverine, and Noscapine).
Figure 10. DESI-MS Spectrum Obtained from Analysis of Clear Liquid Found to Contain $\gamma$-Butyrolactone (GBL; MW = 86) and $\gamma$-Hydroxybutyric Acid (GHB; MW = 104).

* * * * *
Discovery of an Interesting Temperature Effect on the Sensitivity of the Cobalt Thiocyanate Test for Cocaine

Jim W. McGill, Ph.D.,* Crystal A. Dixon, B.A., B.S.,1 and David Ritter, Ph.D.
Department of Chemistry
MS6400
Southeast Missouri State University
One University Plaza
Cape Girardeau, MO 63701
[email: jmcgill -at- semo.edu]

Joanna D. Sides, B.S.
Missouri State Highway Patrol
Troop E Laboratory
122 South Ellis Street
Cape Girardeau, MO 63703

ABSTRACT: During investigation of the mechanism and specificity of the Scott’s (cobalt(II) thiocyanate) test for cocaine, it was discovered that the ambient temperature affected the equilibrium between the pink (negative) and the blue (positive) test results. At 4°C (~39°F) the sensitivity of the test was doubled versus room temperature (~72°F), while temperatures in excess of 40°C (~104°F) decreased the sensitivity of the test more than twofold versus room temperature. These findings can impact the storage, use, and interpretation of commercially available cocaine test kits in typical field settings that are experiencing very cold or (especially) very hot ambient temperatures. A number of recommendations are offered to minimize the effects of hot temperatures on the test kits.

KEYWORDS: Cocaine, Scott’s Test, Cobalt Thiocyanate, Presumptive Test, Color Test, Sensitivity, Temperature, Forensic Chemistry.

Introduction

The use of presumptive color tests in forensic and analytical laboratories to screen drug submissions is common [1]. Because of their ease of use and interpretation, a number of presumptive color tests for commonly submitted drugs have been incorporated into portable test kits for use by law enforcement personnel in field settings.2 These test kits are mass-produced by a number of commercial manufacturers, and typically consist of one or more small ampoules of reagents in self-contained pouches that are reasonably priced, convenient, and safe to use. The results are easily interpreted, and the used kits are easily disposed of in accordance with hazardous waste statutes. Typically, a suspected controlled substance is placed into a tube or a pouch prior to breaking a glass ampoule containing a solution of a test reagent, agitating the mixture, and observing the results (usually an obvious color change). In more complex kits, a series of ampoules is broken in sequence, and the intermediate results at each

--------

1 Current Address: Custom Sensors and Technology, 531 Axminister Drive, Fenton, MO 63026.

2 Most such kits are based on chemical tests; more recently, a number of kits based on immunoassay testing have been produced (the latter are not further addressed in this study).
step dictate whether to continue to completion. Virtually all such kits come with instructions and color charts that show the expected color(s) for positive test results, and law enforcement personnel are well trained in their use.

A positive test result is considered to be a presumptive identification for the controlled substance that was being tested for, and it would be submitted to the laboratory as such. In addition to being a preliminary identification for laboratory analysis, a positive field test is also valuable as probable cause for an arrest, a further search incident to an arrest, and/or a search warrant. Furthermore, positive field tests ease the pressure on the judicial system, as defendants very commonly plead out during preliminary hearings when faced with presumptive identifications.

However, because of the wide variety of illicit drugs with similar appearances, virtually any suspect material would almost certainly be submitted (as an unknown/suspected controlled substance) even if the field test gave a negative or inconclusive result - especially if the appearance or packaging of the material, or the circumstances of the seizure, suggested that it was a controlled substance. However, a negative or inconclusive field test would likely result in a rush analysis request to the laboratory (especially likely if suspects were being detained pending the results), which is disruptive to laboratory operations. In addition, negative/inconclusive tests can encourage guilty defendants (“guilty” in this context meaning they are fully aware of the actual identity of the suspect material) to vigorously contest judicial proceedings until the results of analysis are returned from the laboratory. Thus, reliable and accurate tests are critical.

Cobalt Thiocyanate Test for Cocaine

The cobalt thiocyanate test for cocaine was first introduced by Young in 1931 [2]. The original test employed a two percent aqueous solution of cobalt(II) thiocyanate and tin(II) chloride in an aqueous hydrochloric acid solution. A positive test displays a blue color, while a negative test remains pink (i.e., the color of the test reagent). Even from its introduction, Young and others recognized that this test, while useful for rapid presumptive testing of cocaine, was not specific [3]; i.e., various other compounds gave “positive” results when subjected to this test. A number of variations of the test have subsequently been reported, virtually all focused on improving its specificity and/or sensitivity. Accounts of the evolution of the various versions of this test, with their relative advantages and disadvantages, are well-documented in the literature [4].

The most commonly employed current incarnation of this test, known as the Scott’s test [4e], employs a three-stage sequence: 1) A 1:1 water/glycerine solution of cobalt thiocyanate is added to the suspect substance (resulting in a blue precipitate and a blue solution); 2) Concentrated hydrochloric acid is added (the blue precipitate dissolves and the liquid turns pink); and 3) Chloroform is added (the upper (aqueous) layer remains pink, while the lower (chloroform) layer turns blue). A positive result at each stage is required in order to qualify as a positive test for cocaine. This sequence is not only more specific for cocaine, but can also detect both cocaine base and cocaine hydrochloride. However, despite its significantly improved specificity versus the original (Young) test, the Scott’s test is still subject to false positive and false negative results, which have inspired continuing modifications and alternative tests. Nonetheless, its convenience and utility as a presumptive test in the hands of trained personnel have made it a mainstay in the arsenal of qualitative forensic reagents, and it is the basis for most (if not all) chemistry-based field test kits for cocaine.

Not surprisingly, the Scott’s test is one of the most frequently performed field tests. As noted above, cocaine test kits that are based on the Scott’s test are commercially produced by several different manufacturers; however, all are similar in their design and use. Typically, a qualitatively prescribed amount of the suspected cocaine is placed inside a thick transparent plastic pouch containing three secured ampoules, and the pouch is sealed. The first ampoule, containing the cobalt thiocyanate solution, is broken, the mixture is agitated, and the color and precipitate (if any) are noted. The second ampoule, containing the hydrochloric acid solution, is broken, the mixture is further agitated, and the color is again noted. Finally, the third ampoule, containing chloroform, is broken, again with agitation and observation of the colors in the two layers [5]. A positive result at each of the three stages is considered to be a presumptive identification of cocaine. Anything less than a positive result at any stage is considered to be inconclusive or negative.
However, a positive test does not confirm cocaine, and a negative test does not mean that cocaine is not present. As noted above, a number of other compounds give a “positive” test result (thus giving what is typically referred to as a “false positive”). Still other compounds can interfere with a positive result (giving a “false negative”). In addition, the test is sensitive to the quantity of test material used - both insufficient and excessive quantities of cocaine are documented to produce false negatives [4e]. The potential for chemical interference(s), and the sensitivity of the test to the quantity of cocaine present, are especially important considering the fact that illicit cocaine is almost always cut with other substances at the retail and wholesale levels, and (increasingly) even at the production level (currently (2008), it is common for cocaine kilogram bricks produced in South America to be adulterated with small to moderate percentages of diltiazem, hydroxyzine, or levamisole [6]). Adulterants and diluents can not only interfere with the test, but also decrease the actual amount of cocaine placed in the test kit.

**Mechanistic Studies of the Cobalt Thiocyanate Test**

Efforts to better understand the cobalt thiocyanate test and its limitations have been aimed at two primary areas of study: 1) Elucidation of the mechanism of the test [7]; and 2) Empirical documentation of compounds giving false positive or interfering tests. While an abundance of useful information exists in the forensic literature for each of these two areas of study, a rigorous explanation of the mechanism of the test remains elusive.

As noted above, a positive test displays a blue color in Step 1, while a negative test retains the pink color of the test reagent. Although various hypotheses have been published, the exact structures of the blue and pink complexes are unknown. It is known that octahedral cobalt(II) complexes, such as [Co(H₂O)₆]²⁺, are typically pink, whereas tetrahedral cobalt(II) complexes like [CoCl₄]²⁻ are typically an intense blue [8]. Ligand field theory explains these phenomena quite well. Critically, these changes in geometry are reversible and are often accompanied by enthalpy changes. For example, the equilibrium of octahedral and tetrahedral cobalt complex ions in the presence of chloride anion may be expressed as follows [9]:

$$\text{(Eq. 1)} \quad [\text{CoCl(H₂O)₅}]^{+}(\text{aq}) + \text{Cl}^{-}(\text{aq}) \rightarrow \text{CoCl}_2(\text{H₂O})_2(\text{aq}) + 3 \text{H₂O(l)} \quad \Delta H \approx 48 \text{ kJ/mole}$$

Since the forward reaction is endothermic (i.e., it requires heat as a reactant), raising the temperature favors the formation of the blue tetrahedral complex, while lowering the temperature favors the formation of the pink octahedral complex. This phenomenon is an excellent demonstration of Le Chatelier’s principle [10].

A reasonable inference would be that a similar change in cobalt geometry occurs in the Scott’s test. The basic equation for this analogous equilibrium (i.e., substituting thiocyanate for chloride) can be expressed as follows:

$$\text{(Eq. 2)} \quad [\text{Co(SCN)(H₂O)₃}]^{+}(\text{aq}) + 3 \text{SCN}^{-}(\text{aq}) \rightarrow [\text{Co(SCN)}₄]^{2-}(\text{aq}) + 5 \text{H₂O(l)} \quad \Delta H \sim ? \text{ kJ/mole}$$

However, in this case the exact structure of the pink octahedral and the blue tetrahedral species are unknown, and it is likely that gradual replacement of aqua with thiocyanato ligands gives a range of pink and blue colored intermediate species.

In the absence of cocaine, the equilibrium would be expected to lie to the left in an aqueous solution (where the excess water competes for the coordination sites on the cobalt). However, if cocaine is present, the substitution of two bulky, relatively hydrophobic protonated cocaine cations in the coordination sphere lends stability to the complex, rendering it more soluble in organic solvents (such as chloroform), favoring the right side of the equilibrium. That is, the cocaine serves to partly or fully exclude water from the coordination sphere, causing the equilibrium to shift to the right. Thus, the blue coordination compound responsible for a positive Scott’s test might be (R₃NH)₂Co(SCN)₄, where R₃NH⁺ represents the protonated cocaine molecule [7], as follows:

$$\text{(Eq. 3)} \quad [\text{Co(SCN)(H₂O)₃}]^{+}(\text{aq}) + 3 \text{SCN}^{-}(\text{aq}) + 2 \text{R₃NH}^+ \rightarrow (\text{R₃NH})_2\text{Co(SCN)}_4 + 5 \text{H₂O(l)}$$
Surprisingly, no studies examining the effect(s) of temperature on this equilibrium have been reported in the forensic literature. Le Chatelier’s principle predicts that applying thermal stresses to the equilibrium system will affect its position, favoring either the reactants or the products, and thereby altering the sensitivity of the test. Thus far, published studies of the sensitivity and specificity of the Scott’s test have focused on the presence of interfering analytes giving false positive or false negative results, on substituting different acids and organic solvents in the test, or on the quantities of cocaine used in the test kits [4], but none have considered temperature.

Furthermore, the various manufacturers of the test kits do not specifically address this issue in their product literature. One of the test kit manufacturers does state (in a newsletter) that “a cold test will simply show the color reactions slower than a room temperature test” [11]. However, the same newsletter also states that “[f]ield tests can be stored without concern in any container (desks, briefcases, cabinets, glove compartments, or vehicle trunks).” In view of the thermodynamics of the cobalt complexes, these assertions appear dubious.

Test kits are typically stored in vehicles prior to use, where (depending on locale) temperatures can fall below freezing during winter months and can exceed 140°F (60°C) during summer months. Two critical questions are: 1) Do the temperatures commonly achieved inside the cabin or trunk of a vehicle during winter or summer months significantly affect the sensitivity of the Scott’s test?; and 2) Can temperature-controlled storage be used to enhance the sensitivity of the Scott’s test?

**Experimental**

[Editor’s Notes: Because publication in Microgram Journal could be interpreted as an endorsement or a counter-endorsement by the U.S. Drug Enforcement Administration, the names of the test-kit manufacturers and the names of their test kits have been redacted from this article. The results apply equally to virtually any Scott’s test-based test kit.]

Cobalt(II) thiocyanate was purchased from Sigma-Aldrich. Concentrated hydrochloric acid and chloroform were purchased from Fisher Scientific. Reference standard cocaine was purchased from Sigma-Aldrich and was stored and used in the drug chemistry section of the Missouri State Highway Patrol Troop E Satellite Laboratory. Cocaine test kits used for this study were purchased from a well known manufacturer of narcotics field test kits, and were stated to be applicable for testing both cocaine salts and cocaine base. The provided instructions for use were followed, including a printed qualitative sample size indicator. Reduced temperature studies were conducted using a standard portable cooler with ice as the cooling agent. Elevated temperature studies were conducted using a standard laboratory oven.

**Stock Solution Studies**

A preliminary test was done to confirm that the equilibrium reaction operating in the Scott’s test for cocaine could in fact be manipulated by temperature. For economy, a stock solution of cobalt thiocyanate was prepared to closely match the concentration of the solution contained in the test kit ampoules. A series of calibration solutions was prepared such that the concentration of the test kit solution was within the concentration range of the calibration set, as judged by visual inspection. The actual absorbances of the solutions - measuring 0.05, 0.10, and 0.15 M in concentration - were formally measured at 517 nanometers using a Beckman DB-G UV-Vis spectrophotometer.

Using the resulting calibration curve and measuring the absorbance of the test kit solution from the ampoule, it was determined that the test kit solution was approximately 0.11 M cobalt(II) thiocyanate. A stock solution at this concentration was prepared and used in preliminary tests with various quantities of cocaine and at room, reduced, and elevated temperatures.
Reduced-Temperature Studies (with Test Kits)

A number of test kits were cooled to approximately 4°C in a small insulated cooler. While these pouches were being cooled, a benchmark test was performed using a kit at room temperature, following the manufacturer’s instructions printed on the product box. The quantity of cocaine prescribed by the circle printed on the box was measured visually and then weighed using a laboratory balance (mass = 0.5 mg). This sample was then placed into the control test kit, and the series of ampoules was broken in sequence with agitation and observation in accord with the instructions. This test result was noted and was the basis of comparison for all subsequent tests.

The quantity of cocaine (0.5 mg) and the procedures used for the benchmark test were then duplicated for a test pouch cooled to 4°C (39°F). Following this test, a second reduced-temperature test was performed using a quantity of cocaine approximately one-half the size of the recommended amount used in the initial test (0.2 mg). The quantity of cocaine was cut in half again, to approximately one-fourth the size recommended by the manufacturer (0.1 mg), and the test procedure was repeated. Duplicate tests were run to confirm each result. Trials were also performed to compare the effect of pre-cooling the test kits prior to introduction of the cocaine samples versus attempting to cause a positive or negative result to be reversed by cooling the test kits after a result was obtained at room temperature for a given sample.

Elevated-Temperature Studies (with Test Kits)

A number of test kits were warmed to either 45°C (113°F) or 60°C (140°F) in a standard laboratory oven. Another benchmark test was performed at room temperature and was used as a reference in the elevated-temperature study. The quantity of cocaine (0.5 mg) and the procedures for the benchmark test were then duplicated for test pouches warmed to 45°C and 60°C. Following each of these tests, a second set of elevated-temperature tests was performed using a quantity of cocaine approximately double the size of the recommended amount used in the initial test (1 mg).

Results and Discussion

Stock Solution Studies

Initial investigations revealed that 1 milligram of cocaine, combined in a test tube with one drop of concentrated hydrochloric acid and two drops of 0.11 M cobalt thiocyanate stock solution, resulted in the formation of blue flakes and a blue solution - a positive test. Based on literature data for the aqua complexes of cobalt(II) chloride, it was expected that decreasing the temperature of this positive test solution would shift the equilibrium toward the pink octahedral complex responsible for a negative test, while increasing the temperature would have the opposite effect. In actuality, however, cooling the blue solution to 4°C (39°F) had no significant impact on the color, while raising the temperature resulted in a change from blue to pink. At only 30°C (86°F), the solution began changing from blue to pink with blue specks. Continued heating to 60°C (140°F) resulted in a pink solution with no blue specks.

Next, the mass of cocaine was decreased to less than 0.5 milligram. This quantity was insufficient to produce a blue color in the test tube at room temperature. Raising the temperature of this solution gradually to 60°C (140°F) did not produce any significant changes in its appearance. However, cooling the solution to 4°C (39°F) resulted in a change in color from pink to blue - a positive test. Collectively, these results suggest that the reaction responsible for the change from pink to blue color in the Scott’s test for cocaine is exothermic with a ΔH on the same order of magnitude (but of opposite sign) as that for the endothermic reaction involving cobalt(II) chloride (shown in Eq. 1). The results also indicate that the temperature of a field test kit for cocaine will significantly impact the sensitivity and accuracy of its response.
Reduced-Temperature Studies (with Test Kits)

Results are summarized in Table 1. Interestingly, in one trial, the benchmark test, using 0.5 milligram of cocaine at room temperature, yielded a negative result (in that Step 3 failed to produce the requisite blue lower chloroform layer). This indicates that there is little tolerance for error in the manufacturer’s instructions for this particular test kit. In actual practice, this result would likely prompt re-testing with a larger quantity. However, cooling the negative test pouch to 4°C (39°F) resulted in a blue coloration in the chloroform layer, indicating a positive test for cocaine. Furthermore, cooling a new test kit pouch to 4°C prior to re-testing at the 0.5 milligram level resulted in a noticeably stronger positive test result at all three stages, further confirming enhanced sensitivity at lower temperatures.

To further study this finding, the quantity of cocaine was cut to approximately 0.2 mg. Testing this amount with a pouch that had been pre-cooled to 4°C again resulted in a positive result at all three stages. However, when the sample size was reduced to 0.1 mg, Steps 1 and 2 gave positive tests, but the chloroform layer did not develop a blue color in Step 3.

These results stand in contrast to the statement made in the manufacturer newsletter [11], and they confirm that storing the test kit at low temperatures - either incidentally due to weather conditions or intentionally in a portable cooler - increases its sensitivity by more than a factor of two. The results also show that cooling a test pouch that was positive at Steps 1 and 2 but negative at Step 3 is not as effective as pre-cooling it to 4°C prior to testing.

Elevated-Temperature Studies (with Test Kits)

Results are summarized in Table 2. Since the benchmark test was already at the limit for a repeatable positive test, it was expected that a relatively small temperature increase would result in a negative test - and in fact pre-warming the test kits to 45°C (113°F) gave negative results at all three stages. Not surprisingly, increasing the temperature to 60°C (140°F) also gave negative results. These temperatures are routinely attained inside a parked vehicle during warm weather, especially if the vehicle is exposed to the open sun, and even if the windows are slightly opened for ventilation.

To attempt to compensate for the reduced sensitivity observed at higher temperatures, the sample size was increased from 0.5 mg to 1 mg. In the 45°C (113°F) trials, Step 1 yielded a pink solution with no blue flakes. Continuing the test procedure through Step 3 (despite the test instructions dictating that the test be terminated after a negative result for Step 1), a faint blue chloroform layer was observed. Anyone following the test instructions, however, would never have reached this stage. Again not surprisingly, increasing the temperature to 60°C (140°F) still gave negative results with 1 mg. This confirms that elevated temperatures decrease the sensitivity of the Scott’s field test at least twofold.

Transient False Positives at Elevated Temperatures

Elevated temperatures, in addition to producing false negatives, had a further complicating factor. At both 45°C and at 60°C, a transient blue solution was observed at Step 2, which persisted for a few seconds after mixing and agitating. This transient coloration is also often observed at room temperature, just after breaking the second ampoule, even in the absence of cocaine. Presumably it is due to a temporary and localized high concentration of chloride ions prior to complete mixing of the pouch contents, but at room temperature it dissipates very rapidly with agitation. At 45 and 60°C, however, due to the thermodynamic equilibrium of the cobalt(II) chloride reaction (Eq. 1), this color is more intense and persists for a much longer time. In fact, at 60°C, even up to one minute after the initial intense blue color dissipates, the color of the solution can best be described as pink to lavender, rather than pink. This is presumably due to a sufficient concentration of the blue tetrahedral chloro complex, or of one or more of the intermediate (i.e., partially chlorinated) complexes, imparting a blue tint to the otherwise pink solution. This result is obviously directly attributable to the temperature of the pouch when used, and again could potentially lead to false positive or inconclusive tests.
Safety Issues at Elevated Temperatures

In addition to the loss of sensitivity and accuracy at elevated temperatures, two safety considerations were noted: First, the plastic clip that was used to seal the pouches (intended to prevent leakage of the reagents during agitation) can be deformed by elevated temperatures (45 - 60°C), potentially allowing the pouch to open accidentally or leak during agitation, spilling hazardous chemicals. A second concern is that chloroform boils at 61°C. Therefore, breaking the third ampoule while the kit is over 60°C will (at a minimum) result in pressurization of the pouch, and possibly cause a hazardous chemical aerosol spray to be emitted from an improperly sealed pouch. The authors are unaware of any literature reports of such problems, but the potential clearly exists.

Conclusions (and Recommendations)

The findings of this study suggest that better guidelines can and should be implemented for the storage and use of cocaine field test kits. The National Institute of Justice sets a generic standard for color test reagents and kits that recommends an ambient test temperature between 10°C and 40°C (50°F and 104°F) [12]. Based on the results of the presented study, the high temperature limit of 40°C is clearly too high for cocaine field test kits, and can result in false negatives. Furthermore, although not as critical an issue, the low temperature limit of 10°C is also too high, and needlessly sacrifices sensitivity.

Although the test kits can be stored and used at room temperature, storage at 4°C (39°F) is recommended to both enhance the sensitivity of the test and reduce the likelihood of false negatives due to low sample purity or user error. From a practical viewpoint, a single test kit can be cooled to close to this recommended temperature in approximately 10 minutes by clipping it to the front of a vehicle’s dashboard vent while running the air conditioner at maximum cooling capacity (assuming the vehicle’s A/C unit is properly operating). Maintaining a test kit clipped in this position at all times while on call would ensure the ready availability of a cooled test kit when needed, and is recommended in scenarios where law enforcement personnel have a reasonable but minor expectation of need. In cases where a larger volume of testing is anticipated, such as a planned search of a home, building, vehicle, boat, ship, or aircraft, etc., or where operational circumstances otherwise preclude using a vehicle’s air conditioner, test kits may be maintained at 4°C by storing them in a small, portable cooler with ice or cold-packs, or in a 12-volt powered portable thermoelectric cooler maintained at 39°F (4°C). In testing, it was determined that a pair of test kits can be cooled from 100°F (38°C) to 4°C in 20 minutes in a typical thermoelectric cooler (i.e., intended for use in a vehicle), and can be maintained at that temperature indefinitely if the power supply is maintained. If necessary, large quantities of test kits could be stored in a designated chemical refrigerator at an appropriate facility, to ensure a constant supply of pre-cooled test kits that can be transferred as needed to portable thermoelectric cooler in vehicles.

Finally, besides yielding recommendations for the storage and transportation of cocaine field test kits, these findings also suggest the prudence of scrutinizing other field tests (i.e., for other illicit substances) to determine whether they have similar temperature sensitivity issues.

Future studies at the authors’ laboratories include examination and quantitation of the effects of temperature on the response of known interferences in the Scott’s test for cocaine; i.e., does use at 4°C both increase the sensitivity of the test to cocaine but also to other substances that are already known to give false positives? Or (as may well be) does it further improve the specificity of the test for cocaine?

Acknowledgments

The authors wish to acknowledge the contributions of Pamela Johnson (Criminalist Supervisor) and Amie Nix (Criminalist / Drug Chemist) (both of the Missouri State Highway Patrol, Troop E Laboratory), and Dr. Bruce
Hathaway (Professor of Chemistry, Southeast Missouri State University), all of whom provided helpful discussion and suggestions during both the experimental and manuscript preparation stages of this work.

References


5. Printed instructions on the test kit box [test kit not specified, per the Editor’s comments in the Experimental section].


* * * * *

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Mass</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C (72°F)</td>
<td>0.5 mg</td>
<td>pink solution</td>
<td>(+)</td>
<td>pink upper layer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blue flakes</td>
<td></td>
<td>colorless lower</td>
</tr>
<tr>
<td></td>
<td>0 mg</td>
<td>no blue flakes</td>
<td>(-)</td>
<td>pink upper layer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink solution</td>
<td>(+)</td>
<td>colorless lower</td>
</tr>
<tr>
<td>4°C (39°F)</td>
<td>0.5 mg</td>
<td>pink solution</td>
<td>(+)</td>
<td>pink upper layer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blue flakes</td>
<td></td>
<td>blue lower</td>
</tr>
<tr>
<td></td>
<td>0.2 mg</td>
<td>pink solution</td>
<td>(+)</td>
<td>pink upper layer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blue flakes</td>
<td></td>
<td>blue lower</td>
</tr>
<tr>
<td></td>
<td>0.1 mg</td>
<td>pink solution</td>
<td>(+)</td>
<td>pink upper layer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blue flakes</td>
<td></td>
<td>colorless lower</td>
</tr>
<tr>
<td></td>
<td>0 mg</td>
<td>no blue flakes</td>
<td>(-)</td>
<td>pink upper layer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pink solution</td>
<td>(+)</td>
<td>colorless lower</td>
</tr>
</tbody>
</table>

Table 1. Results of Reduced-Temperature Study. Results are noted along with a (+) or (-) symbol to indicate a positive or negative inference. Test instructions dictate that the test ends after the first negative result. Procedure: Step 1: The first ampoule, containing the cobalt thiocyanate solution, is broken, the mixture is agitated, and the color is noted. A blue color in the solution or oily blue flakes indicates the possible presence of cocaine and permits advancement to the next step; Step 2: The second ampoule, containing the hydrochloric acid solution, is broken, the mixture is further agitated, and the color is again noted. A pink colored solution is observed but is not particularly diagnostic for cocaine; Step 3: The third ampoule, containing chloroform, is broken, again with agitation and observation of the resultant colored layers. A pink upper layer and a blue lower layer indicate the possible presence of cocaine.
<table>
<thead>
<tr>
<th>Temperature</th>
<th>Mass</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C (72°F)</td>
<td>0.5 mg</td>
<td>pink solution blue flakes (+)</td>
<td>pink solution (+)</td>
<td>pink upper layer faint blue lower (+)</td>
</tr>
<tr>
<td>45°C (113°F)</td>
<td>0 mg</td>
<td>pink solution no blue flakes (-)</td>
<td>pink solution (+)</td>
<td>pink upper layer colorless lower (-)</td>
</tr>
<tr>
<td>60°C (140°F)</td>
<td>0 mg</td>
<td>pink solution no blue flakes (-)</td>
<td>pink ~ lavender solution (?)</td>
<td>pink upper layer colorless lower (-)</td>
</tr>
<tr>
<td>45°C (113°F)</td>
<td>0.5 mg</td>
<td>pink solution no blue flakes (-)</td>
<td>pink solution (+)</td>
<td>pink upper layer colorless lower (-)</td>
</tr>
<tr>
<td>60°C (140°F)</td>
<td>0.5 mg</td>
<td>pink solution no blue flakes (-)</td>
<td>pink ~ lavender solution (?)</td>
<td>pink upper layer colorless lower (-)</td>
</tr>
<tr>
<td>45°C (113°F)</td>
<td>1 mg</td>
<td>pink solution no blue flakes (-)</td>
<td>pink solution (+)</td>
<td>pink upper layer faint blue lower (+)</td>
</tr>
<tr>
<td>60°C (140°F)</td>
<td>1 mg</td>
<td>pink solution no blue flakes (-)</td>
<td>pink ~ lavender solution (?)</td>
<td>pink upper layer colorless lower (-)</td>
</tr>
</tbody>
</table>

Table 2. Results of Elevated-Temperature Study. Results are noted along with a (+) or (-) symbol to indicate a positive or negative inference. A (?) symbol indicates an unpredicted observation that may be confusing or inconclusive to the field analyst. Test instructions dictate that the test ends after the first negative result.

Procedure: See Table 1.

* * * * *
Identification of N-Methylbenzylamine Hydrochloride, N-Ethylbenzylamine Hydrochloride, and N-Isopropylbenzylamine Hydrochloride

Ramona M. Sanderson
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott St.
Vista, CA  92081
[email: ramona.m.sanderson -at- usdoj.gov]

ABSTRACT: N-Methylbenzylamine hydrochloride, N-ethylbenzylamine hydrochloride, and N-isopropylbenzylamine hydrochloride have recently been utilized to adulterate or mimic illicit methamphetamine hydrochloride (especially “Ice” methamphetamine). The characterizations of these three alkylbenzylamines by color testing, melting point determination, GC/MS, FTIR/ATR, and ¹H-NMR are presented.

KEYWORDS: N-Methylbenzylamine, N-Ethylbenzylamine, N-Isopropylbenzylamine, “Ice” Methamphetamine, GC/MS, FTIR/ATR, ¹H-NMR, Forensic Chemistry

Introduction

Over the past 18 months, DEA and other forensic laboratories have received increasing numbers of suspected or purported high purity bulk methamphetamine hydrochloride exhibits that subsequent analyses showed to actually be a high purity alkylbenzylamine or less commonly, methamphetamine adulterated with an alkylbenzylamine [1-3]. Most of these exhibits were seized along or near the southwest border, or along the usual trafficking routes in the American southwest. In some cases, the alkylbenzylamine or methamphetamine/alkylbenzylamine mixtures

![Figure 1. Structures of Methamphetamine and the N-Alkylbenzylamines.](image-url)
were further diluted with dimethyl sulfone (a common methamphetamine “cut”). The first of these compounds encountered at this laboratory, N-methylbenzylamine hydrochloride, was submitted in early 2007. N-Ethylbenzylamine hydrochloride began to appear during the summer of 2007, and N-isopropylbenzylamine hydrochloride began to appear in late 2007 [Figure 1]. In 2008, to date (and for reasons unknown), N-isopropylbenzylamine hydrochloride appears to have become the dominant alkylbenzylamine among these submissions.

In most cases, the alkylbenzylamines were crystalline shards or crystalline powders that visually resembled “Ice” or “crystal” methamphetamine (e.g., see Photos 1 - 3). In addition, bulk exhibits (i.e., more than 2 kilograms) were packaged similarly to what is typically encountered for bulk methamphetamine (e.g., in plastic food-storage containers wrapped in cellophane and tape or in large ziplock plastic bags, etc. (e.g., see Photo 4)). And further, the bulk exhibits were smuggled similarly to other illicit drugs - and in some cases were co-smuggled with packages of other (actual) illicit drugs. For these reasons, it is widely accepted that they are being used as methamphetamine mimics (that is, as “rip-off”/sham narcotics), as opposed to “decoys” intended to divert law enforcement attention. In fact, all three alkylbenzylamines have been identified in retail (street-level) samples.

Not surprisingly, the analytical characteristics of the alkylbenzylamines are both similar and dissimilar to the simple phenethylamine drugs. One significant issue is that the various spectra may or may not be included in the libraries installed in the instruments present at most forensic laboratories. The characterization of N-methylbenzylamine, N-ethylbenzylamine, and N-isopropylbenzylamine by melting point, color testing, GC/MS, FTIR/ATR, and 1H-NMR are presented herein.
**Experimental**

Reagents

*Alkylbenzylamines*: N-Methylbenzylamine base, N-ethylbenzylamine base, and N-isopropylbenzylamine base were obtained from Sigma-Aldrich (St. Louis, MO). The respective hydrochloride salts were prepared by dissolving the free bases in acetone and adding concentrated hydrochloric acid. The resulting crystals were filtered, washed multiple times with acetone/ether (50/50), and air dried.

*Other Reagents*: Methamphetamine hydrochloride, amphetamine sulfate, phenethylamine sulfate, and dimethylsulfone were all obtained from this laboratory’s reference collection.

*Test Solutions*: Two test solutions were prepared for GC/MS Analyses: (A) Test Solution A contained approximately 0.5 mg/mL each of dimethylsulfone and the respective bases of the three alkylbenzylamines, methamphetamine, phenethylamine, and amphetamine, in diethyl ether (prepared from their respective salts by basification with 1 M NaOH followed by extraction with diethyl ether). (B) Test Solution B contained approximately 0.5 mg/mL each of dimethylsulfone and the respective salts of N-methylbenzylamine, N-ethylbenzylamine, methamphetamine, phenethylamine, and amphetamine, in methanol.

In instrumentation

*Melting Points*: Melting points for the respective hydrochloric salts were determined using an Stanford Research Systems Opti - Melt Model MPA-100 melting apparatus (Sunnyvale, CA), and are reported in Table 1.

*GC/MS*: Mass spectra (70 eV EI) were obtained using a 5975B Agilent Technologies Inert Mass Selective Detector equipped with a 6890N Gas Chromatograph. Two different columns were used: (a) An Agilent Technologies DB5-MS, 15 m, 0.25 mm i.d., fused-silica capillary column with 0.25 μm film thickness; or (b) An Agilent Technologies HP5-MS, 30 m, 0.25 mm i.d., fused-silica capillary column with 0.25 μm film thickness. Helium was used as the carrier gas with an average linear velocity of 45 cm/sec (constant flow). The injection port and ion sources were set at 280°C and 230°C, respectively. For analysis, 1 μL of the respective Test Solution was injected in split mode (50:1). The oven temperature was programmed as follows: 90°C for 1 minute, ramped at 30°C per minute to 150°C, then held there for 2.0 minutes (total run time = 5.00 minutes). The spectra were obtained by scanning over an m/z range of 40 - 500.

*FTIR/ATR*: Spectra were obtained using a Nicolet Avatar 370 FTIR Spectrophotometer operated in the ATR mode. Sixteen scans were collected at a resolution of 4.0 cm⁻¹.

*1H-NMR*: Spectra were obtained using a Varian Mercury 400 MHz NMR. The compounds were analyzed as the hydrochloride salts in D₂O (approximately 30 mg/mL) containing TSP as the 0 ppm reference (Note that the spectra are incorrectly labelled with “TMS” - TSP was actually used). Eight scans were collected, using a 90° pulse and a 2 second relaxation delay. Spectra were processed using 1.0 Hz line broadening.

Results and Discussion

The high purity and clean appearance of the various alkylbenzylamine submissions suggest that they were industrially (not clandestinely) produced. However, the large crystal forms of some submissions (especially N-isopropylbenzylamine hydrochloride) indicates that clandestine operators are recrystallizing them in order to better mimic large “Ice” methamphetamine crystals. All three alkylbenzylamines have melting points slightly higher than methamphetamine (see Table 1). Interestingly, the DEA Western Laboratory (San Francisco, CA) reported that the N-isopropylbenzylamine hydrochloride crystals in one bulk submission crushed noticeably more easily than typical “Ice” methamphetamine crystals [2]. A similar propensity was noted during this study;
however, it is unknown if that finding is universal for all three alkylbenzylamines, or instead is an anomaly for recrystallized N-isopropylbenzylamine hydrochloride from one clandestine source. Color testing by sodium nitroprusside gave positive (blue) results for all three alkylbenzylamines; however, testing with the Marquis reagent gave negative results (see below).

<table>
<thead>
<tr>
<th>N-MBA</th>
<th>N-EBA</th>
<th>N-IBA</th>
<th>Meth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sodium Nitroprusside Test

Marquis Reagent Test

1 Caution: Depending on the concentration and the age of Marquis reagent, the alkylbenzylamines can give a presumably “false” or weak positive after approximately 30 seconds (a positive Marquis test usually takes less than 10 seconds).

The total ion chromatogram (TIC) for the analysis of Test Solution A on the 15 m DB-5 column is shown in Figure 2a. All compounds were baseline separated with the exception of amphetamine and N-ethylbenzylamine. The TIC for the analysis of Test Solution B on the 30 m HP-5 column is shown in Figure 2b; the 30 m HP-5 column was able to partially resolve amphetamine and N-ethylbenzylamine. Figures 3 through 5 show the mass (GC/MS), infrared (FTIR/ATR), and nuclear magnetic resonance (¹H-NMR) spectra, respectively, for N-methylbenzylamine hydrochloride, N-ethylbenzylamine hydrochloride, N-isopropylbenzylamine hydrochloride.

The simple alkylbenzylamines are traditionally used as intermediates in organic syntheses. None of the three alkylbenzylamines are controlled, and none are believed to have appreciable CNS stimulant effects at typical methamphetamine dosage levels. The pharmacological effects of high dosages on humans are unknown [4].

Acknowledgments

The author thanks the following personnel (all of this laboratory): Jason A. Bordelon (Senior Forensic Chemist) for mentoring and valuable contributions; James L. Jacobs (Forensic Chemist, who was the first to identify N-methylbenzylamine hydrochloride at the laboratory) for standard preparations; and Michael M. Brousseau (Forensic Chemist) for acquiring the ¹H-NMR spectra.

[Note: References are posted on Page 43.]
Table 1. Melting Points.

N-Methylbenzylamine hydrochloride, mp = 180.1 - 181.4°C
N-Ethylbenzylamine hydrochloride, mp = 183.7 - 184.5°C
N-Isopropylbenzylamine hydrochloride, mp = 192.0 - 193.3°C

[Note: \textit{d}-Methamphetamine hydrochloride, mp = 172 - 174°C]

Figure 2a. GC/MS Total Ion Chromatogram (TIC) of the Sample Mixture A.

Figure 2b. GC/MS Total Ion Chromatogram (TIC) of the Sample Mixture B.
Figure 3a. Mass Spectrum of N-Methylbenzylamine.

Figure 3b. Mass Spectrum of N-Ethylbenzylamine.

Figure 3c. Mass Spectrum of N-Isopropylbenzylamine.
Figure 4a. FTIR/ATR of N-Methylbenzylamine Hydrochloride.

Figure 4b. FTIR/ATR of N-Ethylbenzylamine Hydrochloride.

Figure 4c. FTIR/ATR of N-Isopropylbenzylamine Hydrochloride.
Figure 5a. 400 MHz $^1$H-NMR Spectrum of N-Methylbenzylamine Hydrochloride in D$_2$O.

[Figures 5b and 5c Follow (Next 2 Pages).]

References


4. Per request of the DEA Office of Diversion, the National Institute of Drug Abuse (NIDA) is currently conducting studies of the pharmacology of N-methyl-, N-ethyl-, and N-isopropyl-benzylamine.
Figure 5b. 400 MHz $^1$H-NMR Spectrum of N-Ethylbenzylamine Hydrochloride in D$_2$O.
Figure 5c. 400 MHz ¹H-NMR Spectrum of N-Isopropylbenzylamine Hydrochloride in D₂O.
Isolation of Methamphetamine from 1-(1',4'-Cyclohexadienyl)-2-methylaminopropane (CMP) Using Potassium Permanganate

Fracia S. Martinez,* Daniel M. Roesch, and James L. Jacobs
U.S. Department of Justice
Drug Enforcement Administration
Southwest Laboratory
2815 Scott St.
Vista, CA  92081
[email:  fracia.s.martinez -at- usdoj.gov]


ABSTRACT: Methamphetamine illicitly prepared via active metal/ammonia (Birch) reduction of ephedrine or pseudoephedrine is commonly contaminated with 1-(1',4'-cyclohexadienyl)-2-methylaminopropane (CMP), often in significant amounts. Large percentages of CMP in methamphetamine samples result in poor quality (mixed) FTIR spectra. Preliminary treatment/cleanup of CMP-contaminated samples with potassium permanganate gives “clean” methamphetamine for FTIR analysis.

KEYWORDS: Methamphetamine, 1-(1',4'-Cyclohexadienyl)-2-methylaminopropane, Ephedrine, Pseudoephedrine, Birch Reduction, Potassium Permanganate, Forensic Chemistry

Introduction

One of the primary methods of clandestine methamphetamine synthesis is the reduction of ephedrine or pseudoephedrine utilizing an alkali metal such as lithium or sodium, and liquefied ammonia. During a typical reduction of (pseudo)ephedrine, only the hydroxyl group is reduced, producing methamphetamine. With excess alkali metal, and in the presence of an additional proton source [1-8], the aromatic ring is additionally reduced to form a cyclohexadiene (Figure 1). This product is readily generated and is consistent with Birch (Na, EtOH, NH₃) type reactions. The product produced in this reaction is known as 1-(1',4'-cyclohexadienyl)-2-methylaminopropane (CMP), or more simply, the Birch reduction product. On occasion, the CMP to methamphetamine ratio is very high in the final product of this synthesis, which can yield an undesirable, mixed infra-red spectrum (Figure 2 - second pane). Separation and identification of methamphetamine and CMP is easily accomplished by gas chromatography/mass spectroscopy, but some scientists prefer infrared spectroscopy, as it provides easy differentiation of the various phenethylamines. This paper will describe a quick, qualitative method for the elimination of CMP commonly found with methamphetamine manufactured from (pseudo)ephedrine using the lithium - ammonia reduction method [9-11].

Experimental

Reagents and Solutions:
* A 2% solution of potassium permanganate was prepared by dissolving 0.5 grams KMnO₄ in 25 mL water (use caution, potassium permanganate is a moderately strong oxidizing corrosive).
* Aqueous base (sodium hydroxide, sodium bicarbonate, etc.).
* Organic solvent (hexane, diethyl ether, or similar).
* Hydrogen chloride (HCl).
* Mixture of Methamphetamine - CMP (60:40).

**Instrumentation:**
* Nicolet Avatar 370 DTGS-Thermo Electron Corporation. Smart Golden Gate Diamond ATR with KRS-5 Lenses. Number of scans 16, resolution 4 cm⁻¹, and range 400 - 4000 cm⁻¹.
* Agilent 5973 GC-MSD quadrupole electron impact mass spectrometer system with a 30 m HP-5 MS, 0.25 mm, 0.25 μm column. Carrier gas is ultra pure Helium. Instrument parameters: Temperature 90°C to 300°C at 30°C/minute, initial time 1 minute, final hold time 6 minutes, injection port temperature 260°C, transfer line 280°C.
* LCQ Advantage Max ThermoFinnigan quadrupole ion-trap mass spectrometer equipped with an electrospray ionization source (ESI) and interfaced to a Surveyor HPLC system. Phenomenex Luna column C18 - 2.0 x 30 mm x 3 μm. Gradient flow of 95:5 to 5:95 Solvent A/Solvent B over a 10 minutes run. Solvent A is H₂O with 0.1% (v/v) formic acid, while Solvent B is acetonitrile with 0.1% (v/v) formic acid. The flow rate was 200 μL/minute. Samples were prepared using Solvent A. Mass spectrometry data were collected in the positive ion mode using the full-scan mode in order to provide molecular weight information.

**Procedure:**
1. Place 25 mg of the sample (methamphetamine/CMP) in a test tube.
2. Dissolve the sample in 3 mL of water and add 0.5 mL of 2% KMnO₄ solution, then agitate with vortex.
3. Add aqueous base (e.g., sodium hydride, sodium bicarbonate, or similar) to the test tube to make a basic solution (pH > 12).
4. Add organic solvent (3 mL hexane) to the test tube, shake, and isolate the organic layer in a new, clean vessel.
5. Bubble HCl gas through the organic extract.
6. Isolate the precipitate (filtration, evaporation, or similar), dry, and obtain an IR spectrum.

**Results and Discussion**

The potassium permanganate reaction was performed on a mixed (60:40) sample of methamphetamine and CMP. Prior to performing the potassium permanganate reaction, this sample was analyzed by mass spectrometry for confirmation of sample components (Figures 3, 4A, and 4B). Potassium permanganate was then reacted with the mixture. When CMP is reacted with potassium permanganate, the double bonds on CMP are hydroxylated. By applying this technique with an aqueous base/organic solvent extraction, the CMP sodium salt formed remained in the aqueous phase while methamphetamine passed into the organic phase, where it was isolated by precipitation as the hydrochloride salt form. The final product was then sufficiently pure to be identified by infrared spectroscopy (Figure 2). Again a mass spectrometer was used to determine the effectiveness of the reaction, and the analysis confirmed that methamphetamine had been fully isolated from CMP (Figure 5 and 6).

To verify the hydroxylation of CMP and to show that no methamphetamine is produced by this reaction, a pure sample of CMP was reacted with potassium permanganate using the described technique and then analyzed by LC/MS (Figure 7). The presence of the 186 and 220 fragments in the mass spectrum obtained indicate that a mixture of dihydroxy- and tetrahydroxy-derivatives of CMP are produced by reaction with aqueous potassium permanganate (pH > 8) [12]. There is no indication in the mass spectrum that CMP is converted to methamphetamine (no significant molecular ion at m/z 150). Methamphetamine is left unaffected when reacted with potassium permanganate (Figure 8).
Conclusions

In mixtures where the ratio of CMP to methamphetamine is high, the isolation of methamphetamine can be achieved by reacting CMP with potassium permanganate and an aqueous base. The procedure facilitates the isolation of methamphetamine from its primary by-product associated with the lithium-ammonia method of methamphetamine synthesis. It is rapid and straightforward, with few steps, and allows for convenient identification of methamphetamine using infrared spectroscopy.

Acknowledgments

The authors acknowledge the contributions and assistance of Supervisory Chemist David W. Love; Senior Forensic Chemist Sandra E. Rodriguez-Cruz, Ph.D.; and Laboratory Worker Donald G. Smith (all at this laboratory).

References


12. Internet Website (Author Not Listed). Hydroxylation. Dihydroxylated products (glycols) are obtained by reaction with aqueous potassium permanganate (pH > 8) or osmium tetroxide in pyridine. [www.cem.msu.edu/~reusch/VirtTxtJml/addene2.htm](http://www.cem.msu.edu/~reusch/VirtTxtJml/addene2.htm) (Last Accessed February, 2008).


* Law Enforcement Restricted Publication.

---

**Figure 1.** Classic Birch Route of Production with Excess Alkali Metal and Additional Proton Source.

![Chemical Diagram](attachment:image.png)
Figure 2. IR Spectra - Pre and Post Potassium Permanganate Reaction Versus Reference Standards.
**Figure 3.** Mass Spectrometer TIC Post Adding Methamphetamine Standard to CMP for a 60:40 Mixture.

**Figure 4A.** Mass Spectrum of CMP.
**Figure 4B.** Mass Spectrum of Methamphetamine.

**Figure 5.** TIC Post Potassium Permanganate Reaction.
Figure 6. Mass Spectrum of Methamphetamine Post Potassium Permanganate Reaction.

Figure 7. ESI-MS Spectrum Indicating the Presence of both Dihydroxylated and Tetrahydroxylated Derivatives of CMP.
Figure 8. Proposed Potassium Permanganate Reaction.
Information and Instructions for Authors for Microgram Journal

General Information
Microgram Journal is a scientific periodical published by the U.S. Drug Enforcement Administration’s Office of Forensic Sciences, that presents peer reviewed, full length Scientific Research Articles and Technical Notes on the detection and analyses of suspected controlled substances for forensic/law enforcement purposes.

Access to Microgram Journal
Microgram Journal is unclassified, and is published on the DEA public access website (at: www.dea.gov/programs/forensicsci/microgram/index.html). At this time, Microgram Journal is available only electronically, and requires Internet access. Professional scientific and law enforcement personnel may request email notifications when new issues are posted (such notifications are not available to private citizens). The publications themselves are never sent electronically (that is, as attachments).

Requests to be added to the email notification list should preferably be submitted via email to the Microgram Editor at: DEA-Microgram-2008 -at- mailsnare.net Requests can also be mailed to: Microgram Editor, Drug Enforcement Administration, Office of Forensic Sciences, 8701 Morrissette Drive, Springfield, VA 22152. All requests to be added to the Microgram email notification list should include the following Standard Contact Information:

* The Full Name and Mailing Address of Submitting Laboratory or Office;
* The Full Name, Title (Laboratory Director, Assistant Special Agent in Charge, Librarian, etc.), Phone Number, FAX Number, and Preferred email Address of the Submitting Individual (Note that email notifications are mailed to titles, not names, in order to avoid problems arising from future personnel changes);
* If available, the generic email address for the Submitting Laboratory or Office;
* If a generic email address is not available, one private email address for an individual who is likely to be a long-term employee, who has a stable email address, and who will be responsible for forwarding Microgram information to all of the other employees in the requestor’s Office (Note that only one email address per Office will be honored).

Requests to be removed from the Microgram email notification list, or to change an existing email address, should also be sent to the Microgram Editor. Such requests should include all of the pertinent Standard Contact Information detailed above, and also should provide both the previous and the new email addresses.

Email notification requests/changes are usually implemented within six weeks.

Email Notifications (Additional Comments)
As noted above, the email notification indicates which issue has been posted, provides the Microgram URL, and additional information as appropriate. Note that Microgram e-notices will NEVER include any attachments, or any hyperlink other than the Microgram URL. This is important, because the Microgram email address is routinely hijacked and used to send spam, very commonly including malicious attachments. For this reason, all subscribers are urged to have current anti-viral, anti-spyware, and firewall programs in operation. However, in order to ensure that the email notifications are not filtered as spam, the DEA-Microgram-2008 -at- mailsnare email address must be “whitelisted” by the Office’s ISP.
**Costs**
Access to *Microgram Journal* is free.

**Submissions to *Microgram Journal***
Manuscripts are accepted both from within and outside of DEA, and reviewers are both internal (from within DEA) and external.

All submissions must be in English. All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: DEA-Microgram-2007 -at- mailsnare.net *Current* versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: *Microgram* Editor, Drug Enforcement Administration, Office of Forensic Sciences, 8701 Morrissette Drive, Springfield, VA 22152. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3 ½ inch IBM® PC-compatible diskette, 100 or 250 MB Iomega® zip diskette, or an IBM® PC-compatible CD. *Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: “Warning - Contains Electronic Media - Do Not Irradiate”.*

Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 1/2” x 11” or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. All photos and figures should also be submitted as stand-alone attachments, not only embedded in the manuscript. The pages should be numbered, but not stapled together.

Note that mailed submissions may be subject to lengthy handling delays beyond the control of the Office of Forensic Sciences, and electronic media sent through the mail may be destroyed *en route* by sanitizing procedures, despite protective measures and written warnings. All submissions should include the following Contact Information: The Full Name and Address of Submitting Laboratory or Office, and the Full Name, Phone Number, FAX Number, and Preferred email Address of the Submitting Individual.

**Scientific Research Articles** are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.) Technical Notes are shorter communications concentrating on a specific drug (or drug class), unusual case, novel or unusual procedure or method, or minor original research, again excluding in post-ingestion human/animal biological matrices. Each article/note should be a “stand-alone” work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is *received* by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes may follow an abbreviated version as appropriate):

- **Cover Letter** - Provide the standard contact information and pertinent correspondence (if any) for the Editor.

- **Title** - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the
manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

**Author(s)/Affiliation(s)** - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author’s degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page.

**Abstract** - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under “Titles”. Note that the abstract will be provided to *Chemical Abstracts*.

**Keyword List** - A minimum of five (maximum ten) abstracting keywords should be included. Unless inappropriate, the last keyword pair should always be “Forensic Chemistry.”

**Introduction** - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid “Personal Communications”).

**Experimental** (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, **USE CAUTION IN DETAILING SYNTHESSES OF CONTROLLED OR ABUSED SUBSTANCES**, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: “Experimental details on this synthesis are not provided, in accordance with Journal policy.”]

**Results and Discussion** - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

**Conclusions** - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or the summary paragraph in the Results and Discussion section.

**Acknowledgments** - Optional - Should be brief, and include the full name, affiliation, and specific contribution made by each cited individual.

**References** - Articles and notes should have all textual citations collected in an endnotes list. Within the text, references should be consecutively numbered with superscripted Arabic numerals, or with Arabic numerals in brackets, in accordance with their first appearance. Within the endnotes list, references should be consecutively numbered with Arabic numerals, as follows: Number, Period, Indent, Citation. Reference format should adhere to the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals* (Note: This is the same reference format utilized in the Selected Reference Citations in *Microgram Bulletin*, and also (among many others) by the *Journal of Forensic Sciences*). Due to their inherently transitory nature, use of website URL’s as references are discouraged but are permitted. As
previously noted, Personal Communications should not be utilized; however, if unavoidable, utilize the following format: Full Name, Title, Affiliation (Laboratory or Office), Location (City and State, plus Nation if not the United States), Personal Communication, Year.

**Table and Figures** - All Tables and Figures should be appended onto the end of the article (not embedded in the text). Tables and Figures should be consecutively numbered with Arabic numerals, in accordance with their first citation in the text. Each Table and Figure should be “stand-alone”; that is, include sufficient descriptive information such that the reader will not have to refer back to the text to understand the Table or Figure. The Header should include the Table or Figure number and a concise title. Explanatory material, definitions of acronyms and/or abbreviations, and/or references within the Table or Figure should be designated by superscripted, lower case letters in alphabetical order, and included in dedicated footnotes at the bottom of the respective Table or Figure. Unless color is needed to enhance differentiation of the depicted material, all Tables and Figures should be in black and white (that is, avoid frivolous use of color for “artistic” purposes). Figures of spectra, chromatograms, charts, graphs, etc., should have clear and legibly labeled axes, but should not include instrument generated printoffs of experimental parameter lists.

Manuscripts submitted to *Microgram Journal* are required to be finished, professional quality efforts. Authors should ensure clarity, brevity, and pertinence of all information. Attention to detail in formatting, syntax, grammar, and spelling are as important as the accuracy of the facts presented. Authors are specially cautioned to conduct careful literature reviews prior to submission. At the Editor’s discretion, clearly substandard and/or inappropriate manuscripts will be returned to the authors without review.

**Manuscripts will not be retyped, but “final” versions are subject to minor to moderate Editorial rewrite to improve presentation clarity or to reformat to current *Microgram Journal* style.**

**Dual publication** - Re-publication of articles or notes of particular interest to the *Microgram Journal* readership will be considered if the article was originally published in a journal that is not easily accessed and the primary author has obtained explicit, written copyright exclusion from the original publisher and consent from all co-authors. Examples include exact English translations of articles or notes originally published in a non-English language journal, non-sensitive articles or notes originally published in a restricted journal or on a password protected website, or articles or notes originally published in limited distribution newsletters or proceedings. In general, any article or note that was published in English in a mainstream journal is **not** a candidate for re-publication in *Microgram Journal*. Authors interested in re-publishing previously published articles or notes in *Microgram Journal* should discuss the issue with the *Microgram* Editor **before** submitting.

Note that (in accordance with standard ethical guidelines) re-published articles should **not** be included as “new” articles in the respective author(s)’ *Curriculum Vitae*.

**Costs** - There are no costs (to the contributor) associated with publication in *Microgram Journal*.

**Reprints** - *Microgram Journal* does **not** provide reprints to authors. *Microgram Journal* may be photocopied (or printed off the website) as needed.

Questions may be directed to the *Microgram* Editor.
DISCLAIMERS

1) All material published in Microgram Journal is reviewed prior to publication. However, the reliability and accuracy of all published information are the responsibility of the respective contributors, and publication in Microgram Journal implies no endorsement by the United States Department of Justice or the Drug Enforcement Administration.

2) Due to the ease of scanning, copying, electronic manipulation, and/or reprinting, only the posted copies of Microgram Journal (on www.dea.gov) are absolutely valid. All other copies, whether electronic or hard, are necessarily suspect unless verified against the posted versions.

3) WARNING!: Due to the often lengthy time delays between the actual dates of seizures and their subsequent reporting in Microgram Journal, and also because of the often wide variety of seizure types with superficially similar physical attributes, published material cannot be utilized to visually identify controlled substances currently circulating in clandestine markets. The United States Department of Justice and the Drug Enforcement Administration assume no liability for the use or misuse of the information published in Microgram Journal.
Microgram

Journal

To Assist and Serve Scientists Concerned with the Detection and Analysis of Controlled Substances and Other Abused Substances for Forensic / Law Enforcement Purposes.

Published by:
The Drug Enforcement Administration
Office of Forensic Sciences
Washington, DC  20537

The U.S. Attorney General has determined that the publication of this periodical is necessary in the transaction of the public business required by the Department of Justice. Information, instructions, and disclaimers are published in the first issue of each year.

Volume 6
Numbers 3-4
July - December 2008

Posted On-Line At:
Contents

A Specific Screening Color Test for Diazepam 63
Mohammad Sarwar, Shaena Taylor, and Imran Majeed

An In-Depth Study of the Peruvian Base Llavada (“Washed Base”) Technique for Purification of Crude Cocaine Base 72
Danielle K. Boudreau and John F. Casale

Identification of Levamisole Impurities Found in Illicit Cocaine Exhibits 82
John F. Casale, Elizabeth M. Corbeil, and Patrick A. Hays

Identification of Diltiazem Impurities / Artifacts during the Analyses of Illicit Cocaine Exhibits Containing Diltiazem 90
John F. Casale, Pauline M. Orlando, Valerie L. Colley, and Patrick A. Hays

Etodolac: An Analytical Profile 104
Mandy C. McGehee

Determination of Cocaine in Various South American Commercial Coca Products 109
Elizabeth M. Corbeil and John F. Casale

“Crack” Cocaine: A Study of Stability over Time and Temperature 114
Laura M. Jones, Danielle K. Boudreau, and John F. Casale

The Discoloration of Illicit Drug Samples 128
James M. Moore and John F. Casale

Note: In order to prevent automated theft of email addresses off the Internet postings of Microgram Journal, all email addresses reported in the Journal have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of 2,5-Dimethoxy-4-iodophenethylamine (2C-I; Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).
A Specific Screening Color Test for Diazepam

Mohammad Sarwar, Ph.D.,* and Shaena Taylor, B.S.A.S.
Cuyahoga County Coroner’s Office
11001 Cedar Avenue
Cleveland, OH 44106
[Email: msarwar -at- cuyahogacounty.us]

Imran Majeed, M.S., M.Phil.
Forensic Research Laboratory
National Centre of Excellence in Molecular Biology
University of the Punjab
Lahore, Pakistan

ABSTRACT: A new, highly specific color test for the screening/presumptive identification of diazepam is reported. The test is a variant of the McKibben test for flunitrazepam. Treatment of diazepam with alkaline dimethylsulfoxide produces a reddish color which gradually changes to yellow with passage of time. The color instantly vanishes upon addition of water or attempted extraction with organic solvents, suggesting that the color is due to a transient charge-transfer complex. Somewhat unexpectedly, the test does not produce color with powder scraped from diazepam-containing tablets - in such cases, a chloroform extraction is required. The test is negative for other controlled substances, including other benzodiazepines, and also for various diluents and binders typically present in tablets (62 compounds were tested). The LODs were 20 μg for diazepam extracted from tablets, and 2 μg for diazepam standard. The test is particularly useful for the rapid screening of illicit Lemmon 714 (Quaalude) mimic tablets, which contain diazepam as a substitute for methaqualone.

KEYWORDS: Diazepam, Dimethylsulfoxide, Screening, Color Test, Forensic Chemistry

Introduction

Diazepam (Figure 1), most commonly known by its trade name Valium, is a benzodiazepine and a controlled substance (Schedule IV in the United States [1]). It is a potent sedative - hypnotic (CNS depressant), and is one

![Figure 1. Diazepam (7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one); C_{16}H_{13}ClN_{2}O; m.w. (Base) = 284.7; mp = 131.5-134.5°C](image-url)
of the most prescribed drugs in the world. It is also one of the top five most abused benzodiazepines, and misuse can lead to both psychological dependence and/or physical addiction [2].

In addition to Valium and numerous other licit (prescription) formulations, diazepam is found as an adulterant in heroin and as a substitute in various mimic drugs (most notably as a substitute for methaqualone in Lemmon 714 (Quaalude) mimic tablets [3]). Because its synthesis is challenging, its presence in illicit drug markets is almost universally due to diversion of pharmaceutical stocks.

There are numerous analytical methods for the identification of diazepam in forensic and toxicological laboratories [4,5], including a number of color tests; in general, however, the latter are not sensitive or specific (vide infra). Herein, a new presumptive color test for the preliminary screening of diazepam is reported. The test, which is a variant of the McKibben test for flunitrazepam [6], is simple, easy to perform, highly sensitive, and highly specific.

**Experimental**

**Materials:** Dimethylsulfoxide (hereafter DMSO) and sodium hydroxide were both acquired from Merck (Whitehouse Station, NJ). The diazepam standard was acquired from Roche Pakistan Ltd. (Lahore). Tablets containing other benzodiazepines and other tested compounds, and standard (pure) materials, were purchased from various pharmaceutical companies (see Table 1). All other chemicals used were of analytical grade or better.

**Methods:** Test Reagent A was prepared by adding 50 parts of DMSO and one part of 3M sodium hydroxide. [Note: Use of sodium hydroxide solutions below 2M or over 3M was not as effective.] Test Reagent B was prepared by adding solid sodium hydroxide to DMSO and vortexing it for approximately 2 minutes. In the latter case, the supernatant was transferred to another tube and used as the test reagent. In this study, both test reagents were freshly prepared just before use; however, subsequent studies indicated that the reagents were stable for at least 4 days. Both test reagents are colorless (see Photo 1).

**Detection of Diazepam:** A Valium tablet containing 2 mg of diazepam was ground to a fine powder. A small portion of powder (approximately 0.5 mg) was added to 1 mL of chloroform, and the resulting mixture was vortexed for approximately one minute and then centrifuged at 13,200 rpm for 3 minutes. The supernatant chloroform was isolated and evaporated to dryness, and 3 - 4 drops of the test reagent was added to the remaining residue. An immediate reddish color developed (see Photo 2). If Test Reagent A was used, the color faded to yellow after approximately 1 minute; if Test Reagent B was used, the color persisted for at least 20 minutes before gradually fading to yellow. Repeating the sequence with the diazepam standard gave the same results. Addition of 2 - 3 drops of water to either the reddish or yellow test solutions caused the color to instantly vanish. None of the other compounds tested (Table 2) gave similar results.

**Limit of Detection (LOD) from Tablets:** The above analysis was repeated on a second Valium tablet, except that five sequential extractions were performed on the same powder, and each extract isolated in separate test tubes. Upon testing, only the first three extracts displayed color; it was therefore assumed that the 2.0 mg of diazepam in the original tablet was quantitatively extracted by three serial extractions. Another tablet was extracted in the
same manner, and the first three extracts were combined to form the “stock solution.” Aliquots containing 70, 60, 50, 40, 30, 20, 10, and 5 µg of diazepam were each evaporated to dryness, and tested. The results indicate a detection limit of 20 µg.

**LOD from Diazepam Standard:** Stock solutions containing 50, 30, 20, 10, 5, 3, 2, and 1 µg of reference grade diazepam were prepared in acetone. The solutions were evaporated to dryness, and the remaining residue was tested as detailed above. The reddish color was noted down to 2 µg - one tenth the LOD for diazepam extracted from tablets. The order of magnitude difference is thought to be due to the sensitivity of the test to co-extracted impurities from the tablets (i.e., that are not present in the standard).

**Analysis of Other Substrates:** All other compounds were either analyzed as standards (pure), or were extracted from tablets using water or an organic solvent (see Table 1).

### Results and Discussion

#### Previous Studies

As noted in the Introduction, a number of color tests have been reported for the presumptive identification of diazepam; however, most are neither specific or sensitive, and others were not tested against other benzodiazepines or other controlled substances. Formaldehyde + H₂SO₄ [7], Zimmerman’s reagent (meta-dinitrobenzene + benzyltrimethylammonium hydroxide [8]), and the Janovsky reagent (meta-dinitrobenzene + KOH [9]) have been used as general screens for benzodiazepines. Of the three reagents, Zimmerman’s reagent is the most specific, producing violet/purple colors with keto-benzodiazepine derivatives such as diazepam, fludiazepam, and flurazepam. The Wagner’s test (acidic KI₃) gives a brown solution with a brown-black precipitate; however, similar results are obtained for many alkaloids, including cocaine hydrochloride [10,11]. In a commercial test kit (ingredients proprietary), a presumptive test was designed to identify the presence of diazepam, flunitrazepam, or ketamine [12]. After breaking and agitation of the two ampoules in the kit, a pale lavender color will develop for either diazepam and flunitrazepam, and a darker color for ketamine; it is unknown how this kit performs with other benzodiazepines or other controlled substances. Diazepam gives an intense red color product with the addition of picric acid (2,4,6-trinitrophenol), 3,5-dinitrobenzoic acid, or 3,4-dinitrobenzoic acid [13]; however, no other benzodiazepines or drugs were studied. Bromocresol green has been used to produce an orange colored ion-association complex with diazepam [14]; again, no other benzodiazepines or drugs were studied. Treatment of diazepam, bromazepam, and clonazepam with methanolic potassium hydroxide produces a yellow color, which could be analyzed by spectrophotometry [15]; however, a similar coloration is obtained for almost any benzodiazepine.

#### Alkaline Dimethylsulfoxide

McKibben was the first to report the use of alkaline DMSO for color testing, for screening of flunitrazepam [6]; interestingly, alkaline dimethylformamide (DMF) also worked. There were three variants of the test; in the first, the sample and the reagent were placed in a flint glass/soda lime test tube and heated at 100°C, giving a deep purple color within four minutes. In the second, the sample, reagent, and either barium oxide, barium hydroxide, or finely ground flint glass/soda lime glass were placed in a regular test tube and heated at 100°C, again giving a deep purple color within four minutes. In the third, the sample was dissolved in either DMSO or DMF and a small amount of solid sodium hydroxide added, resulting in immediate formation of a red-purple color (different than that observed in the first two variants). In all three cases, (cautious) addition of concentrated hydrochloric acid resulted in an immediate canary-yellow color.

McKibben tested over 100 different drugs, including diazepam, and determined that the tests were highly specific for flunitrazepam. Diazepam gave no color in either of the heated variants, but gave a dark orange color in the
third (unheated) variant. In all, 30 of the compounds tested by McKibben using the third (unheated) variant gave colors, including greens, yellows, and oranges.

In the current study, markedly different results were obtained, with only diazepam (reddish, see Photo 2), flunitrazepam (purple, see Photo 3), flurazepam (yellow), nitrazepam (yellow), and temazepam (green) giving colors (Table 2); however, fewer compounds (62) were tested, and the tested substrates included many non-drug compounds. Nonetheless, none of the other compounds that were tested displayed a reddish color, and the purple color displayed by flunitrazepam was similar but distinct from the reddish color produced by diazepam. Somewhat surprisingly, and in direct contrast to the McKibben reagents, the test failed to produce any color when DMF was substituted for DMSO. [Note: The colors observed with flurazepam, nitrazepam, and temazepam were not further investigated in this study; however, based on the compounds tested, the green color observed for temazepam also appears to be unique, and may constitute another definitive test. McKibben also observed a light green color for temazepam (Test Variant 3) - but noted similar colors for alprazolam, estazolam, lorazepam, and scopolamine.]

In order to further differentiate diazepam and flunitrazepam, their respective positive test solutions were each further treated (cautiously!) with 2 drops of concentrated hydrochloric acid. The reddish solution from diazepam instantly produced a faint yellow solution (see Photo 4), while the purple solution from flunitrazepam instantly produced a distinct orange solution (see Photo 5). Thus, although we feel that the initial colors allow for differentiation of diazepam from flunitrazepam, if desired this second step would rigorously confirm the identification.

The only difference between the McKibben Test/Variant 3 and the Test Reagent B used in this study is the point at which the solid sodium hydroxide is added. In the McKibben test, the sample is first dissolved in the DMSO, and then the base is added. In this study, the base is first dissolved in the DMSO, and then the sample is added. It is unclear how such a simple variation can have such a profound impact; however, the (fortuitous) difference allows for a specific test for diazepam.

**Attempted Identification of the Colored Specie:** In order to attempt identification of the colored specie, the reddish test solutions resulting from diazepam were extracted with a variety of organic solvents (acetic anhydride, acetone, acetone and chloroform, chloroform, ethyl acetate, formaldehyde, and petroleum ether). The color not only failed to extract into any of the organic solvents, in every case it vanished altogether. In addition, and as was noted in the Experimental section, addition of water to the reddish colored solutions also caused the color to instantly vanish (and the rapid fading of the reddish color when Test Reagent A was used is very likely due to the small percentage of water in that reagent). The results suggest that the reddish color results from formation of a transient charge-transfer complex that is sensitive to virtually any change in polarity or concentration. This sensitivity may explain why the test is so specific to diazepam, why the test fails with powder from tablets, why the LOD is so much higher for diazepam extracted from tablets, and finally why it fails if DMF is substituted for DMSO.

**Screening of Lemmon 714 (Quaalude) Tablets:** Actual Lemmon 714 tablets contain only methaqualone, whereas nearly all Lemmon 714 mimic tablets seen over the past 25 years have contained diazepam, sometimes adulterated with diphenhydramine [3]. Diazepam is also (uncommonly) substituted for methaqualone in Mandrax mimic tablets (widely abused in South Africa [16]). In a 1982 patent, Fischer and Morris reported a screening test to differentiate tablets containing methaqualone or mecloqualone from mimic tablets containing diazepam or diphenhydramine. Addition of about 7 drops of 85% formic acid to a sample containing methaqualone or mecloqualone from mimic tablets containing diazepam or diphenhydramine. Addition of about 7 drops of 85% formic acid to a sample containing methaqualone or mecloqualone, followed by 5 drops of 5% sodium nitrite, then 10 drops of chloroform, results in a yellow color that extracts into the chloroform layer; if diazepam is present, however, it will give a yellow color that is not extracted into the chloroform layer [17]. In the present test, methaqualone did not display any color. Thus, the two tests perfectly complement each other for rapid, facile screening of Lemmon 714 (Quaalude) or Mandrax tablets.
Photo 2 - Reddish Color from Diazepam

Photo 3 - Purple Color from Flunitrazepam

Photo 4 - Faint Yellow Color from Addition of Conc. HCl to the Reddish Solution (i.e., from Diazepam)

Photo 5 - Distinct Orange Color from Addition of Conc. HCl to the Purple Solution (i.e., from Flunitrazepam)
Acknowledgments

We are highly grateful to the Higher Education Commission of Pakistan for providing finances for the survey of illegal drugs in Pakistan. We also appreciate the help provided by the Abdul Rehman and Nasir Siddique, M.Phil. Scholars, Centre for Excellence in Molecular Biology Lahore, Pakistan.

References

2. Diazepam (See: http://en.wikipedia.org/wiki/diazepam (most recently accessed 1/15/09)).
7. See Reference 4, p. 286.
12. ODV Narco Pouch #925 (See: http://www.odvinc.com/Web%20Tubes_Pouches_Art/test_prod_desc.html (most recently accessed 1/15/09)).


---

### Table 1. Tested Compounds, Sources, Extraction Solvents, and Manufacturers.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Extraction Solvent</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Cold and Sinus Tablet</td>
<td>Acetone</td>
<td>Equal USA</td>
</tr>
<tr>
<td>alpha-Lipoic Acid</td>
<td>A/G Standard</td>
<td>Acetone</td>
<td>Sigma</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>Xanax</td>
<td>Ethanol</td>
<td>Pharmacia Pakistan Ltd.</td>
</tr>
<tr>
<td>Aspirin Sodium Salt</td>
<td>Disprin</td>
<td>Water</td>
<td>Reckitt Benckiser Pakistan</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>-</td>
<td>A/G</td>
<td>BDH</td>
</tr>
<tr>
<td>Bromazepam</td>
<td>Lexotanil Chloroform</td>
<td>Roche Pakistan Ltd.</td>
<td></td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>-</td>
<td>A/G</td>
<td>Sigma</td>
</tr>
<tr>
<td>Chlorazepate Dipotassium</td>
<td>Tranxene Water</td>
<td>Searle Pakistan (pvt.) Ltd.</td>
<td></td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>Librium Ethanol</td>
<td>Ethical Pharmaceutical Pakistan</td>
<td></td>
</tr>
<tr>
<td>Chlorpheniramine Maleate</td>
<td>Pritone Water</td>
<td>Glaxo Smith Kline Ltd. Pakistan</td>
<td></td>
</tr>
<tr>
<td>Clonazepam</td>
<td>Rivotril Acetone</td>
<td>Roche Pakistan Ltd.</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>-</td>
<td>Chloroform Sigma</td>
<td>Sigma</td>
</tr>
<tr>
<td>Codeine</td>
<td>Cocodamol Water</td>
<td>Alpharma Barnstable UK</td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>Splenda Water</td>
<td>McNeil Nutritionals LLC F. Washington</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>Valium Chloroform</td>
<td>Roche Pakistan Ltd.</td>
<td></td>
</tr>
<tr>
<td>Diclofenac Na</td>
<td>Dicloran Acetone</td>
<td>Sami Pharmaceutical Pakistan</td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine HCl</td>
<td>Cold and Sinus Tablet</td>
<td>Water</td>
<td>Equal USA</td>
</tr>
<tr>
<td>Flunitrazepam</td>
<td>Rohypnol Chloroform</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>Flurazepam</td>
<td>Dalmane Chloroform</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>Folic Acid</td>
<td>-</td>
<td>A/G Sigma</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>A/G Merck</td>
<td></td>
</tr>
<tr>
<td>Guanidine</td>
<td>-</td>
<td>A/G Sigma</td>
<td></td>
</tr>
<tr>
<td>Heroin</td>
<td>-</td>
<td>Chloroform Sigma</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Bruphen Chloroform</td>
<td>Equal USA</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Indomethacin cap. Chloroform</td>
<td>Chongging Medicine Pakistan</td>
<td></td>
</tr>
<tr>
<td>Kanamycin Monosulfate</td>
<td>-</td>
<td>A/G MP Biomedicals, Inc. Ohio, France.</td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>Ketalar Chloroform</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>A/G Merck</td>
<td></td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>-</td>
<td>A/G Acros</td>
<td></td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>-</td>
<td>A/G Sigma</td>
<td></td>
</tr>
<tr>
<td>Lorazepam</td>
<td>Aitivan Acetone</td>
<td>Spencer Pharma (pvt) Ltd. Pakistan</td>
<td></td>
</tr>
<tr>
<td>Mefenamic Acid</td>
<td>Ponstan Chloroform</td>
<td>Reckitt Benckiser Pakistan</td>
<td></td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>-</td>
<td>A/G Merck</td>
<td></td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>Splenda Water</td>
<td>McNeil Nutritionals LLC F. Washington</td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>A/G Difco Laboratory</td>
<td></td>
</tr>
<tr>
<td>Methaqualone</td>
<td>-</td>
<td>Chloroform Sigma</td>
<td></td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>-</td>
<td>A/G Sigma</td>
<td></td>
</tr>
<tr>
<td>Nalbuphine HCl</td>
<td>Loricin injection Water</td>
<td>Medicina Pharma Pakistan</td>
<td></td>
</tr>
<tr>
<td>Naproxen</td>
<td>Synflex Chloroform</td>
<td>ICI Karachi Pakistan</td>
<td></td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>-</td>
<td>A/G Sigma</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Source</td>
<td>Solvent</td>
</tr>
<tr>
<td>-----</td>
<td>------------------------</td>
<td>-------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>41</td>
<td>Nitrazepam</td>
<td>Mogadon</td>
<td>Chloroform</td>
</tr>
<tr>
<td>42</td>
<td>Oxazepam</td>
<td>Murelax</td>
<td>Chloroform</td>
</tr>
<tr>
<td>43</td>
<td>Pentazocine</td>
<td>-</td>
<td>A/G Standard</td>
</tr>
<tr>
<td>44</td>
<td>Phenolphthalein</td>
<td>-</td>
<td>A/G BDH</td>
</tr>
<tr>
<td>45</td>
<td>Phenylephrine HCl</td>
<td>Cold and Sinus Tablet</td>
<td>Water</td>
</tr>
<tr>
<td>46</td>
<td>Prednisolone</td>
<td>Prednisolone cap.</td>
<td>Ethanol</td>
</tr>
<tr>
<td>47</td>
<td>Propoxyphene</td>
<td>Algaphan</td>
<td>Water</td>
</tr>
<tr>
<td>48</td>
<td>Pyridoxine</td>
<td>-</td>
<td>A/G Sigma</td>
</tr>
<tr>
<td>49</td>
<td>Salicylic Acid</td>
<td>-</td>
<td>A/G Sigma</td>
</tr>
<tr>
<td>50</td>
<td>Sorbitol</td>
<td>-</td>
<td>A/G Sigma</td>
</tr>
<tr>
<td>51</td>
<td>Sodium Bicarbonate</td>
<td>-</td>
<td>A/G Merck</td>
</tr>
<tr>
<td>52</td>
<td>Sodium Gluconate</td>
<td>-</td>
<td>A/G GPR*</td>
</tr>
<tr>
<td>53</td>
<td>Sodium Glutamate</td>
<td>-</td>
<td>A/G Merck</td>
</tr>
<tr>
<td>54</td>
<td>Sorbic Acid</td>
<td>-</td>
<td>A/G Merck</td>
</tr>
<tr>
<td>55</td>
<td>Starch</td>
<td>-</td>
<td>A/G Merck</td>
</tr>
<tr>
<td>56</td>
<td>Succinic Acid</td>
<td>-</td>
<td>A/G Kodak</td>
</tr>
<tr>
<td>57</td>
<td>Sucralose</td>
<td>Splenda</td>
<td>Water</td>
</tr>
<tr>
<td>58</td>
<td>Sucrose</td>
<td>-</td>
<td>A/G Merck</td>
</tr>
<tr>
<td>59</td>
<td>Temazepam</td>
<td>Restoril</td>
<td>Acetone</td>
</tr>
<tr>
<td>60</td>
<td>Triazolam</td>
<td>Halcion</td>
<td>Chloroform</td>
</tr>
<tr>
<td>61</td>
<td>Tyrothricin</td>
<td>-</td>
<td>A/G Sigma</td>
</tr>
<tr>
<td>62</td>
<td>Vitamin D</td>
<td>Calcium D Tablet</td>
<td>Chloroform</td>
</tr>
</tbody>
</table>

* GPR = General Purpose Reagents; A/G = Analytical Grade (Not Extracted).

[Table 2 Follows.]
### Table 2. Test Results.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Color</th>
<th>Compound</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Acetaminophen</td>
<td>N/C</td>
<td>32 Mefenamic Acid</td>
<td>N/C</td>
</tr>
<tr>
<td>2  alpha-Lipoic Acid</td>
<td>N/C</td>
<td>33 Magnesium Sulfate</td>
<td>N/C</td>
</tr>
<tr>
<td>3  Alprazolam</td>
<td>N/C</td>
<td>34 Maltodextrin</td>
<td>N/C</td>
</tr>
<tr>
<td>4  Aspirin</td>
<td>N/C</td>
<td>35 Mannitol</td>
<td>N/C</td>
</tr>
<tr>
<td>5  Benzophenone</td>
<td>N/C</td>
<td>36 Methaqualone</td>
<td>N/C</td>
</tr>
<tr>
<td>6  Bromazepam</td>
<td>N/C</td>
<td>37 Myo-Inositol</td>
<td>N/C</td>
</tr>
<tr>
<td>7  Calcium Carbonate</td>
<td>N/C</td>
<td>38 Nalbuphine HCl</td>
<td>N/C</td>
</tr>
<tr>
<td>8  Chlorazepate Dipotassium</td>
<td>N/C</td>
<td>39 Naproxen</td>
<td>N/C</td>
</tr>
<tr>
<td>9  Chlor Diazepoxide</td>
<td>N/C</td>
<td>40 Nicotinic Acid</td>
<td>N/C</td>
</tr>
<tr>
<td>10 Chlorpheniramine Maleate</td>
<td>N/C</td>
<td>41 Nitrazepam</td>
<td>Yellow</td>
</tr>
<tr>
<td>11 Clonazepam</td>
<td>N/C</td>
<td>42 Oxazepam</td>
<td>N/C</td>
</tr>
<tr>
<td>12 Cocaine</td>
<td>N/C</td>
<td>43 Pentazocine</td>
<td>N/C</td>
</tr>
<tr>
<td>13 Codeine</td>
<td>N/C</td>
<td>44 Phenolphthalein</td>
<td>N/C</td>
</tr>
<tr>
<td>14 Dextrose</td>
<td>N/C</td>
<td>45 Phenylephrine HCl</td>
<td>N/C</td>
</tr>
<tr>
<td>15 Diazepam</td>
<td>Red</td>
<td>46 Prednisolone</td>
<td>N/C</td>
</tr>
<tr>
<td>16 Diclofenac Na</td>
<td>N/C</td>
<td>47 Propoxyphene</td>
<td>N/C</td>
</tr>
<tr>
<td>17 Diphenhydramine HCl</td>
<td>N/C</td>
<td>48 Pyridoxine</td>
<td>N/C</td>
</tr>
<tr>
<td>18 Flunitrazepam</td>
<td>Purple</td>
<td>49 Salicylic acid</td>
<td>N/C</td>
</tr>
<tr>
<td>19 Flurazepam</td>
<td>Yellow</td>
<td>50 Sorbitol</td>
<td>N/C</td>
</tr>
<tr>
<td>20 Folic acid</td>
<td>N/C</td>
<td>51 Sodium Bicarbonate</td>
<td>N/C</td>
</tr>
<tr>
<td>21 Glucose</td>
<td>N/C</td>
<td>52 Sodium Gluconate</td>
<td>N/C</td>
</tr>
<tr>
<td>22 Guanidine</td>
<td>N/C</td>
<td>53 Sodium Glutamate</td>
<td>N/C</td>
</tr>
<tr>
<td>23 Heroin</td>
<td>N/C</td>
<td>54 Sorbic Acid</td>
<td>N/C</td>
</tr>
<tr>
<td>24 Ibuprofen</td>
<td>N/C</td>
<td>55 Starch</td>
<td>N/C</td>
</tr>
<tr>
<td>25 Indomethacin</td>
<td>N/C</td>
<td>56 Succinic Acid</td>
<td>N/C</td>
</tr>
<tr>
<td>26 Kanamycin Monosulfate</td>
<td>N/C</td>
<td>57 Sucralose</td>
<td>N/C</td>
</tr>
<tr>
<td>27 Ketamine</td>
<td>N/C</td>
<td>58 Sucrose</td>
<td>N/C</td>
</tr>
<tr>
<td>28 Lactose</td>
<td>N/C</td>
<td>59 Temazepam</td>
<td>Green</td>
</tr>
<tr>
<td>29 L-Cysteine</td>
<td>N/C</td>
<td>60 Triazolam</td>
<td>N/C</td>
</tr>
<tr>
<td>30 L-Glutamine</td>
<td>N/C</td>
<td>61 Tyrothricin</td>
<td>N/C</td>
</tr>
<tr>
<td>31 Lorazepam</td>
<td>N/C</td>
<td>62 Vitamin D</td>
<td>N/C</td>
</tr>
</tbody>
</table>

N/C = No Color.
An In-Depth Study of the Peruvian Base Llavada (“Washed Base”) Technique for Purification of Crude Cocaine Base

Danielle K. Boudreau,* B.S., and John F. Casale, B.S.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email address withheld at author’s request]

ABSTRACT: An in-depth study of the Peruvian base llavada (“washed base”) technique for cleanup of crude cocaine base with ethanol is presented. Used as a substitute method for the traditional potassium permanganate purification process, the technique selectively decreases or removes many alkaloid and colored impurities. Authentic crude Peruvian cocaine base was subjected to the method, and the resulting washed base samples and their respective ethanolic washings were examined immediately thereafter and again 14 months later. The results confirm that the technique gives a whiter appearing but only slightly more pure base versus standard (unwashed) base. The fate of several alkaloid impurities is tracked. The presence of cocaethylene in illicit cocaine (resulting from transesterification of cocaine with ethanol) may be indicative of use of the base llavada technique; however, ethanol is known to be utilized in several other variants of illicit cocaine processing, so the presence of cocaethylene alone does not confirm the use of the base llavada technique.

KEYWORDS: Cocaine, Cocaine Impurities, Cocaethylene, Ethanol, Chromatographic Signature Analysis, Forensic Chemistry

Introduction

In traditional illicit cocaine production, coca leaf is processed to give crude cocaine base, which is purified with potassium permanganate to give a whiter, more refined base, which is then converted to cocaine hydrochloride [1]. Although the potassium permanganate purification is reasonably effective, the method is somewhat time intensive and technique sensitive, and potassium permanganate is both costly and difficult to acquire in cocaine processing regions. Recent interviews of South American cocaine processors indicated that a new technique is being used by some Peruvian chemists to purify their crude cocaine base. In this new variant, referred to locally as the base llavada or “washed base” technique, the crude base is first mixed with ethanol until a dough-like consistency is achieved, then wrapped in cloth and hydraulically compressed in a hydraulic press to force out as much of the ethanol solution (containing dissolved impurities) as possible. The process results in a whiter and slightly more pure product, similar in appearance to that produced by the potassium permanganate purification technique.

Prior work at this laboratory demonstrated how the use of the base llavada technique affects the signature profile of the resulting cocaine [2]. In the current study, the technique was performed on numerous samples of authentic Peruvian crude cocaine base in order to better understand what is occurring to give the whiter appearing product. The selected samples were analyzed at the time of the process (both before and after the washing), and again 14 months later. The residual ethanol/impurities solutions pressed from the samples were also analyzed both at the time of the process, and again 14 months later. This study also investigated the slow production of cocaethylene in washed samples during storage, resulting from transesterification of cocaine by the residual ethanol still remaining after the wash and pressing.
Experimental

Materials and Solvents: Ethanol and chloroform were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other reagents and chemicals were reagent-grade quality products of Sigma-Aldrich Chemical Company (Milwaukee, WI). The authentic crude Peruvian cocaine base used in this study was from the reference collection of the DEA Special Testing and Research Laboratory.

Gas Chromatography / Mass Spectrometry (GC/MS): An Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph was used to conduct all GC/MS analyses. The MSD was operated in the electron ionization mode with an ionization potential of 70 eV, scan range of 34 - 700 mass units at 1.34 scans/s. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific). Helium (99.999% UHP) was used as a carrier gas at an average linear velocity of 40 cm/s. The oven temperature was programmed as follows: Initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. Samples (1 μL) were injected in the split mode (21.5 : 1) by an Agilent 7683 Series Auto Injector. The injection temperature and the auxiliary transfer line to the MSD were both maintained at 280°C.

Gas Chromatography / Flame Ionization Detection (GC/FID) and Gas Chromatography / Electron Capture Detection (GC/ECD): GC/FID and GC/ECD analyses were performed using an Agilent 6890N gas chromatograph. Prepared solutions were placed into an autosampler vial and analyzed using previously published methods and conditions [3,4]. MSTFA was utilized as the derivatization reagent for GC/FID analyses.

Headspace - Gas Chromatography / Mass Spectrometry (HS-GC/MS): Determination of occluded ethanol was accomplished using the method described by Morello et al. [5].

Preparation of Base Llavada Samples: Seven samples from an authentic Peruvian crude cocaine base exhibit were subjected to the base llavada technique, using 90% ethanol. The amount of sample and amount of ethanol used were varied slightly from sample to sample (Table 1). The residual ethanol/impurities solution from each pressing was collected for analysis. The ethanol was evaporated in vacuo to a dark brown oil prior to analysis. All samples were then subjected to chromatographic impurity analysis to quantitatively determine the alkaloids present. All samples were stored at room temperature for 14 months and then re-examined to determine any changes that had occurred.

Table 1. Preparation of Base Llavada Samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of Cocaine Base Used* (g)</th>
<th>Amount of Ethanol Used (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>456</td>
<td>65</td>
</tr>
</tbody>
</table>

* Authentic Crude Peruvian Cocaine Base.
Figure 1. Structures of Major Alkaloids Found in Illicit Cocaine.
Results and Discussion

The base llavada technique experiments resulted in a minor (4.5% average) increase in cocaine base purity, primarily due to the partial removal of the cinnamoylccaines, truxillines, tropacocaine, and trimethoxycocaine (Figure 1). In addition (and in accordance with the information provided by the Peruvian cocaine processors), the resulting product was also noticeably whiter in appearance after the wash, due to the partial removal of the cinnamoylccaines and other colored impurities (as noted above, the residual ethanol/impurities solution is dark brown in color). Partial reconstructed chromatographic profiles of the crude cocaine base, washed cocaine base, and residual ethanol/impurities solution are illustrated in Figure 2. The retention times of the target compounds are given in Table 2. As seen in Figures 2a and 2b, the cinnamoylccaine content was reduced in the washed sample. Table 3 illustrates the effects of the wash on the relative concentrations of tropacocaine, cis-cinnamoylccaine, trans-cinnamoylccaine, trimethoxycocaine, and total truxillines. These naturally occurring alkaloids are more soluble than cocaine in ethanol, and as a result, are selectively extracted from the illicit cocaine (with minimal loss due to co-extraction of cocaine). Quantitative determinations confirmed decreases ranging from 21 - 78% relative to cocaine. The truxilline concentration was the least affected, while the trimethoxycocaine concentration was the most affected. Re-analysis of the samples 14 months later showed further decreases in alkaloid concentrations, ranging from 32 - 100%, versus the original crude cocaine base values, indicating further hydrolysis of those alkaloids over time (Table 3).

The residual ethanol/impurities solutions were also examined immediately after completion of the base llavada technique and not surprisingly were found to contain significant amounts of tropacocaine, cis-cinnamoylccaine, trans-cinnamoylccaine, trimethoxycocaine, and total truxillines relative to cocaine (Figure 2c). While the residual oil also contained some cocaine, the bulk of the material was primarily comprised of the alkaloidal impurities, approaching an order of magnitude concentration increase over their respective starting values relative to cocaine in the original crude base.

The hydrolysis of the cocaine was also examined. Hydrolysis of cocaine occurs at both ester bonds, giving ecgonine, ecgonine methyl ester, and benzoylecgonine (Figure 3). Hydrolysis of cis-cinnamoylccaine and trans-cinnamoylccaine will also occur, giving cis-cinnamoylccegnine, trans-cinnamoylccegnine, and egenone (Figure 3). The respective concentrations of the hydrolysis products was determined immediately after completion of the base llavada technique and again 14 months later. As shown in Table 4, the cleanup immediately increased these hydrolysis products from 18% to nearly 400% relative to cocaine in the resulting washed base. The residual oil also contained an increased concentration of the hydrolysis products. In addition, the ethanol remaining in the washed base samples continued to hydrolyze the cocaine, cis-cinnamoylccaine, and trans-cinnamoylccaine during the 14 month storage period, resulting in even more significant increases in the concentrations of cis-cinnamoylccegnine, trans-cinnamoylccegnine, and benzoylecgonine. When compared to the original samples, after 14 months, the hydrolysis products had increased from 57% to nearly 1,400% relative to cocaine.

Finally, although not quantitatively determined, the transesterification of cocaine to cocaethylene due to the presence of residual ethanol was also observed during storage [6]. Both the original sample and the washed base (immediately following the wash) showed no traces of cocaethylene. However, storage of the sample for 14 months allowed transesterification to occur, resulting in a measurable quantity of cocaethylene; the ethyl esters of cis- and trans-cinnamoylccaine were also detected (Figure 5). Thus, the presence of cocaethylene in illicit cocaine may be indicative of use of the base llavada technique - however, ethanol is known to be utilized in several other variants of illicit cocaine processing, so the presence of cocaethylene alone does not confirm the use of the base llavada technique.

Although increasingly utilized in Peru, the base llavada method is currently not known to be in use in either Bolivia or Colombia.
Conclusions

The *base llavada* technique is a partially effective purification technique. Ethanol selectively removes significant amounts of major alkaloid impurities from crude cocaine base, due to their higher solubility versus cocaine. In contrast to the more time intensive and costly potassium permanganate purification method, the *base llavada* technique is relatively easy and inexpensive to perform. Although this new methodology results in only a moderate increase in cocaine base purity, it does give a noticeably whiter product.

References


---

**Table 2.** Relative Retention Times (RRT) of Coca Alkaloids.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak #</th>
<th>GC/FID RT</th>
<th>GC/MS RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecgonine-di-TMS</td>
<td>1</td>
<td>5.24</td>
<td>----</td>
</tr>
<tr>
<td>Tropacocaine</td>
<td>2</td>
<td>10.96</td>
<td>17.51</td>
</tr>
<tr>
<td><em>para</em>-Fluorococaine</td>
<td>3</td>
<td>14.92</td>
<td>----</td>
</tr>
<tr>
<td>Cocaine</td>
<td>4</td>
<td>15.81</td>
<td>21.38</td>
</tr>
<tr>
<td>Benzoylecgonine-TMS</td>
<td>5</td>
<td>16.18</td>
<td>----</td>
</tr>
<tr>
<td><em>cis</em>-Cinnamoylcoaine</td>
<td>6</td>
<td>18.56</td>
<td>23.80</td>
</tr>
<tr>
<td><em>cis</em>-Cinnamoylecggonine-TMS</td>
<td>7</td>
<td>18.90</td>
<td>----</td>
</tr>
<tr>
<td><em>trans</em>-Cinnamoylcoaine</td>
<td>8</td>
<td>20.32</td>
<td>25.61</td>
</tr>
<tr>
<td><em>trans</em>-Cinnamoylecggonine-TMS</td>
<td>9</td>
<td>20.52</td>
<td>----</td>
</tr>
<tr>
<td>Trimethoxycocaine</td>
<td>10</td>
<td>24.59</td>
<td>----</td>
</tr>
<tr>
<td>Cocaethylene</td>
<td>11</td>
<td>16.41</td>
<td>22.09</td>
</tr>
<tr>
<td><em>cis</em>-Cinnamoylecggonine ethyl ester</td>
<td>12</td>
<td>----</td>
<td>24.55</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>13</td>
<td>----</td>
<td>25.95</td>
</tr>
<tr>
<td><em>trans</em>-Cinnamoylecggonine ethyl ester</td>
<td>14</td>
<td>----</td>
<td>26.31</td>
</tr>
</tbody>
</table>
### Table 3. Average Change in Alkaloid Values and (Percent Change) Relative to Percent Cocaine.

<table>
<thead>
<tr>
<th>% Cocaine</th>
<th>Original Values</th>
<th>Day 1</th>
<th>14 Months Later</th>
<th>Enriched Ethanolic Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Tropacocaine</td>
<td>0.12</td>
<td>0.07 (-41.7)</td>
<td>0.06 (-50.0)</td>
<td>1.02 (750.0)</td>
</tr>
<tr>
<td>% cis-Cinnamoyl-cocaine</td>
<td>6.36</td>
<td>4.31 (-32.2)</td>
<td>3.08 (-51.6)</td>
<td>47.13 (641.0)</td>
</tr>
<tr>
<td>% trans-Cinnamoyl-cocaine</td>
<td>4.08</td>
<td>2.68 (-34.3)</td>
<td>1.88 (-53.9)</td>
<td>32.94 (707.4)</td>
</tr>
<tr>
<td>% Trimethoxycocaine</td>
<td>0.18</td>
<td>0.04 (-77.8)</td>
<td>0.00 (-100)</td>
<td>1.02 (466.7)</td>
</tr>
<tr>
<td>% Truxillines</td>
<td>4.41</td>
<td>3.49 (-20.9)</td>
<td>3.01 (-31.7)</td>
<td>40.04 (87.9)</td>
</tr>
<tr>
<td>% EtOH relative to Cocaine</td>
<td>n/a</td>
<td>&lt;0.1%</td>
<td>&lt;0.1%</td>
<td>n/a</td>
</tr>
</tbody>
</table>

### Table 4. Average Change in Hydrolysis Product Values and (Percent Change) Relative to Percent Cocaine.

<table>
<thead>
<tr>
<th>% cis-Cinnamoyl-ecgonine</th>
<th>Original Values</th>
<th>Day 1</th>
<th>14 Months Later</th>
<th>Enriched Ethanolic Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>% trans-Cinnamoyl-ecgonine</td>
<td>0.00</td>
<td>0.18 (18.0)</td>
<td>0.57 (57.0)</td>
<td>1.45 (145)</td>
</tr>
<tr>
<td>% Ecgonine</td>
<td>0.81</td>
<td>1.20 (48.1)</td>
<td>1.51 (86.4)</td>
<td>19.02 (2248.1)</td>
</tr>
<tr>
<td>% Methylecgonine</td>
<td>0.12</td>
<td>0.48 (300.0)</td>
<td>1.57 (1208.3)</td>
<td>5.23 (4258.3)</td>
</tr>
</tbody>
</table>

[Figures 2 - 5 Follow.]
Figure 2. Partial Reconstructed GC/FID Chromatograms of: (A) Crude Cocaine Base; (B) Washed Cocaine Base; and (C) Enriched Oil. See Table 2 for Peak Identification.
Figure 3. Structures of Hydrolysis Products.
Figure 4. Partial Reconstructed GC/FID Chromatograms of: (A) Washed Cocaine Base after 1 Day; and (B) Washed Cocaine Base after 14 Months. See Table 2 for Peak Identification.
Figure 5. Partial Reconstructed Total Ion Chromatograms Showing Cocaethylene’s Development Over Time: (A) Day One; and (B) 14 Months Later. See Table 2 for Peak Identification.
Identification of Levamisole Impurities Found in Illicit Cocaine Exhibits

John F. Casale,* Elizabeth M. Corbeil, and Patrick A. Hays
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA  20166
[email address withheld at corresponding author’s request]

ABSTRACT: 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole and 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one, known levamisole degradation products, were identified in a “crack” cocaine exhibit. Spectroscopic and chromatographic data are provided for both compounds, and their presence in the sample is discussed.

KEYWORDS: Levamisole, Degradation, Cocaine Base, “Crack” Cocaine, Impurities, 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole, 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one, Forensic Chemistry

Introduction

This laboratory recently received a 1 gram portion of a “crack” cocaine (cocaine base) exhibit from another laboratory for the purpose of identifying an unknown component. The exhibit contained 79% cocaine base, 6% levamisole, and 3% of an unknown compound. The unknown had an apparent molecular weight of 202 Daltons based on the mass spectrum generated by the original laboratory, and was suspected to be a levamisole-related impurity. Upon screening, the exhibit was found to also contain trace amounts of a second unknown compound, suspected to be another levamisole-related impurity. Levamisole (Figure 1), an antineoplastic (cancer chemotherapy drug), has been a cocaine adulterant for nearly 5 years [1], but this is the first report of suspected levamisole impurities in illicit cocaine. The prevalence of levamisole in cocaine hydrochloride bricks has increased dramatically over the past year, and is currently found in 30% of all seizures (Figure 2). Herein, we report the preparative isolation, gas and liquid chromatographic-mass spectrometry, and nuclear magnetic resonance spectroscopy of both impurities. The major unknown compound (6-phenyl-2,3-dihydroimidazo[2,1b]thiazole) was confirmed via comparison to an authentic standard, while the trace unknown compound (3-(2-mercaptoethyl)-5-phenyl-imidazolidine-2-one) was synthesized to verify its identity.
Experimental

Solvents, Chemicals, and Materials: All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of reagent-grade quality and were products of Sigma-Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4% (w/w). Levamisole was part of the authentic reference collection of the DEA Special Testing and Research Laboratory. A reference standard of 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole was obtained from LGC Standards (Luckenwalde, Germany).

Gas Chromatography/Mass Spectrometry (GC/MS): GC/MS analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100°C (no initial hold); program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280°C. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and a scan rate of 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

Liquid Chromatography/Mass Spectrometry (LC/MS): Molecular weight information derived from [M+H]+ was obtained using a Waters (Milford, MA) 2525 HPLC pump fitted with a XTerra MS 150 mm x 4.8 mm, 5 μm, C-18 column. The sample was diluted to a concentration of approximately 0.2 mg/mL, and the injection volume

![Figure 2. Prevalence of Levamisole in Cocaine Hydrochloride Bricks over the past 4 Years.](image-url)
was 0.5 mL per run. The flow was optimized at 1.0 mL/min, using the following reversed-phase gradient solvents: (A) Water containing 0.1% trifluoroacetic acid, and (B) Acetonitrile. The linear gradient started at 95% A and 5% B; changed to 75% A and 25% B over 20 min, held 15 min; then changed to 5% A and 95% B over 25 min; and finally returned to 95% A and 5% B for 1 min. The HPLC eluent was introduced into a Waters Micromass ZQ single quadrupole mass spectrometer using Electrospray Ionization (ESI) with positive ion detection. The detector operated in the scan range of 40 - 500 mass units, a scan time of 0.5 sec, and an inter-scan delay of 0.1 sec.

Preparative Isolation of 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole via Alumina Column Chromatography: Approximately 900 mg of the illicit cocaine sample containing about 3% (~27 mg) of target compound was dissolved in a minimal amount of CHCl₃, and eluted on a glass chromatographic column (25 cm x 1.0 cm i.d.) containing 15 g of basic alumina (150 mesh). The column was eluted with 20 mL each of the following solvent combinations: 1) CHCl₃, 2) CHCl₃/acetone (85 : 15), 3) CHCl₃/acetone (1 : 1), and acetone. Ten mL fractions were collected and examined via GC/MS. Fractions 2 and 3 contained 17 - 22% pure target compound and were combined and evaporated to dryness. The residue was dissolved into a minimal amount of CHCl₃/hexane (1 : 1) and was chromatographed again on 15 g of basic alumina (150 mesh). The column was eluted with 20 mL each of the following solvent combinations: 1) CHCl₃/hexane (1 : 1), 2) CHCl₃, 3) CHCl₃/acetone (40 : 1), 4) CHCl₃/acetone (20 : 1), 5) CHCl₃/acetone (15 : 1), 6) CHCl₃/acetone (10 : 1), and 7) CHCl₃/acetone (6 : 1). Ten mL fractions were collected and examined via GC/MS. Fraction 5 contained the majority of the target compound (but also contained some levamisole and cocaine), and was evaporated to dryness. The residue was washed with 2 - 3 mL of petroleum ether (20 - 40OC boiling range) to remove the cocaine, and was then dried to provide a white powder (20 mg, 67% 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole and 33% levamisole).

Nuclear Magnetic Resonance Spectroscopy (NMR): Proton (¹H) NMR spectra were obtained on a Varian (Palo Alto, CA) Inova 600 MHz NMR using a 5 mm Varian Nalorac Z-Spec broadband, variable temperature, pulse field gradient (PFG) probe. The compounds were dissolved in deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference. The temperature of the samples was maintained at 25°C. Standard Varian pulse sequences were used to acquire the spectra. Data processing was performed using Applied Chemistry Development software (ACD/Labs, Toronto, Canada).

Synthesis of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one: Levamisole hydrochloride (25 mg, 0.104 mmol) was dissolved in water (5 mL), adjusted to pH 8 with aqueous NaHCO₃ (1 mL), and microwaved at 1200 watts until all the water had boiled off (about 2 - 3 minutes). The residue was dissolved in CHCl₃ (10 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated in vacuo to give the title compound as a white powder (22 mg, 95%). The reaction was repeated at pH 10 using aqueous Na₂CO₃, and gave identical results.

Results and Discussion

GC/MSD analysis of the exhibit was first conducted as a cursory assessment. Examination of the reconstructed total ion chromatogram (Figure 3a, Table 1) indicated a compound (Peak #4) closely related to levamisole was present. Peak #4 represented approximately 3% of the total ion current. Its mass spectrum (Figure 4a) produced an apparent molecular ion at m/z 202. The spectrum was markedly similar to levamisole (Figure 4b), with fragment ion shifts of minus one to two mass units for several ions, thus suggesting a levamisole-like compound with incorporation of another double bond. It did not form a TMS derivative, indicating no labile protons within the molecule. The molecular weight for this compound was confirmed via LC/MS, yielding a [M+H]+ at m/z 203, consistent with the molecular weight assignment of 202. This compound was semi-isolated as described in the Experimental section and examined via ¹H-NMR. The chemical shifts obtained were consistent with the loss of two protons within the imidazole ring and suggested that the compound was 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole. A reference standard of this compound was obtained, and its retention time and mass spectrum were identical to the unknown (Figure 1).
A trace component (Figure 3a, Peak #3) was also noted in the GC/MSD analysis, having an apparent molecular ion at \(m/z\) 222 (Figure 5a). Upon derivatization with MSTFA, this compound formed both a mono-TMS and di-TMS derivative (Figure 3b, Peaks #2 & #6), with molecular ions at \(m/z\) 294 (Figure 5b) and \(m/z\) 366 (Figure 5c), respectively. These results indicated that two labile protons were present. A mass difference of +18 Daltons from levamisole, coupled with two labile protons, suggested that the compound was an oxidation by-product of levamisole. Since 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one had been previously reported as an oxidative by-product of levamisole in aqueous solutions [2-4], it was synthesized as described in the Experimental section. The retention times and mass spectra of the synthesized standard (both underivatized and derivatized) were identical to the unknown (Figure 1).

Some Colombian-run cocaine hydrochloride laboratories have been adding levamisole to cocaine hydrochloride for nearly 5 years. Over that time period, the levamisole has always appeared to be of pharmaceutical-grade quality. Significant amounts of process impurities are rarely encountered in pharmaceutical drug products. This exhibit contained 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole at a concentration of about 50% relative to levamisole, which is quite remarkable. This impurity (and the trace amount of 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one) may have arisen from two possible sources: 1) A poorly processed or waste batch of pharmaceutical levamisole; and/or 2) Degradation of levamisole during the conversion of cocaine hydrochloride into “crack” cocaine.

The formation of significant amounts of 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole from levamisole during the conversion of cocaine hydrochloride into “crack” cocaine as currently practiced seems unlikely. The stability of levamisole in aqueous solutions has been studied at length [2-4]. In those works, the formation of up to four degradation products were tracked as a function of pH and temperature. 6-Phenyl-2,3-dihydroimidazo[2,1b]-thiazole was not detected even when levamisole was boiled to dryness in basic aqueous solutions (i.e., at pH 7 to 10). However, 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole is also a synthetic by-product from the pharmaceutical production process, due to an undesired cyclization of the first intermediate product [5]. Due to the very high relative concentration of this compound to levamisole in this sample, it appears that an impure or waste batch of levamisole made its way into the illicit drug trade.

However, as was demonstrated in the Experimental section, 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one is nearly quantitatively created from levamisole when boiled to dryness in basic aqueous solutions (i.e., at either pH 8 or 10). Therefore, the trace amount of 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one in the sample may be attributed to its formation as a by-product from levamisole during the conversion of cocaine hydrochloride into “crack” cocaine. In this case, the water was not boiled off during the “crack process,” or all of the levamisole would have been converted to this compound. The presence of large amounts of 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one in “crack” cocaine exhibits would be evidence of boiling the “crack” cocaine conversion solution to dryness.

The physiological effects and consequences of smoking “crack” cocaine adulterated with levamisole and contaminated with 6-phenyl-2,3-dihydroimidazo[2,1b]thiazole and 3-(2-mercaptoethyl)-5-phenylimidazolidine-2-one are unknown.

References


---

**Table 1.** Retention Times (RT) and Relative Retention Times (RRT) of Levamisole and Related Impurities.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT (min)</th>
<th>RRT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levamisole</td>
<td>17.25</td>
<td>0.81</td>
</tr>
<tr>
<td>222-mono-TMS*</td>
<td>19.09</td>
<td>0.90</td>
</tr>
<tr>
<td>222*</td>
<td>19.77</td>
<td>0.93</td>
</tr>
<tr>
<td>202**</td>
<td>20.99</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Cocaine</strong></td>
<td><strong>21.30</strong></td>
<td><strong>1.00</strong></td>
</tr>
<tr>
<td>222-di-TMS*</td>
<td>22.39</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* Conditions Detailed in the Experimental Section.
* 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one
** 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole

---

[Figures 3 - 5 Follow.]
Figure 3. Partial Reconstructed Total Ion Chromatograms of a Cocaine Base Exhibit Containing Levamisole Impurities. Upper (A) Is Underivatized and Lower (B) Is Derivatized. Peak Identification: 1 = Levamisole, 2 = TMS Derivative of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one, 3 = 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one, 4 = 6-Phenyl-2,3-dihydroimidazo[2,1b]thiazole, 5 = Cocaine, 6 = di-TMS Derivative of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one.
Figure 4. Electron Ionization Mass Spectrum of (A) 6-Phenyl-2,3-dihydro-imidazo[2,1b]thiazole; and (B) Levamisole.
Figure 5. Electron Ionization Mass Spectrum of 3-(2-Mercaptoethyl)-5-phenylimidazolidine-2-one: (A) Underivatized, (B) mono-TMS Derivative, and (C) di-TMS Derivative.
Identification of Diltiazem Impurities / Artifacts during the Analyses of Illicit Cocaine Exhibits Containing Diltiazem

John F. Casale,1,* Pauline M. Orlando,2 Valerie L. Colley,1 and Patrick A. Hays1

1 U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166

2 U.S. Department of Justice
Drug Enforcement Administration
South Central Laboratory
10150 Technology Boulevard
Dallas, TX 75220

[Email address withheld at corresponding author’s request]

ABSTRACT: Desacetyldiltiazem and an uncharacterized artifactual compound with an apparent mass of 354 Daltons have been observed in gas chromatographic profiles of cocaine exhibits containing diltiazem. The use of methanol as an injection solvent for cocaine samples containing sodium bicarbonate causes the formation of these compounds in the injection port; however, the use of chloroform as an injection solvent does not result in their formation. Spectroscopic and chromatographic data are provided for diltiazem, desacetyldiltiazem, and 2,3-dehydrodesacetyldiltiazem. Desacetyldiltiazem is a bona fide impurity in some cocaine exhibits, but it can also be produced as an analytical artifact. The artifact with an apparent mass of 354 Daltons is not (as postulated) 2,3-dehydrodesacetyldiltiazem, and remains unidentified.

KEYWORDS: Diltiazem, Desacetyldiltiazem, 2,3-Dehydrodesacetyldiltiazem, Injection Port Artifacts, Cocaine, Chemical Analysis, Gas Chromatography, Forensic Chemistry

Introduction

Over the past 5 years, DEA laboratories have received increasing numbers of both cocaine hydrochloride and cocaine base (“crack”) exhibits adulterated with diltiazem (Figure 1) [1]. Diltiazem is a potent vasodilator used in the treatment of angina pectoris, arrhythmia, hypertension, and related heart ailments [1]. Identification of diltiazem has typically involved GC/MS, with comparison of spectra and retention time to a standard. There are four stereoisomers of diltiazem; the isomer being utilized to adulterate cocaine is the pharmaceutical product, (+)-cis-diltiazem.

In the course of identifying suspected diltiazem in cocaine samples, slight differences between the mass spectra and retention times of the presumed diltiazem in the sample and the diltiazem standard were sometimes observed. Additionally, some samples exhibited two unknown compounds eluting just after diltiazem (Figure 2). These observations suggest that the diltiazem is degrading to other compounds during the analysis. Known diltiazem degradation products include desacetyldiltiazem, N-demethyldiltiazem, N-demethyldesacetyldiltiazem, and O-demethyldesacetyldiltiazem [2-5].
Interestingly, it was noted that significant amounts of the two unknown compounds were observed when analyzing many - but not all - cocaine base (“crack”) samples, and furthermore were rarely observed when analyzing cocaine hydrochloride samples. The two unknowns had apparent molecular ion at $m/z$ 372 and 354, respectively (Note: The molecular weight of diltiazem is 414). Since significant amounts of the first unknown compound (Figure 2, peak #2) were observed in many “crack” exhibits that contained excess sodium bicarbonate, hydrolysis to desacetyldiltiazem ($414 - 42 = 372$) was suspected. The instability of diltiazem in solution, and its hydrolysis to desacetyldiltiazem (Figure 1), are well documented [4-7]. Significant amounts of the second unknown compound (Figure 2, peak #3) were also observed in these same “crack” exhibits. Its apparent molecular ion ($372 - 18 = 354$) suggested that the compound is derived via elimination of water from desacetyldiltiazem to form 2,3-dehydrodesacetyldiltiazem (Figure 1). Of note, in most instances the two unknown components were only detected when methanol was incorporated as the injection solvent for GC/MS analyses.

Figure 1. Synthesis and Structural Formulas of Diltiazem and Related Compounds.
Herein, we report the synthesis and characterization (GC/FID, GC/MS, DESI MS, and NMR) of desacetyldiltiazem and 2,3-dehydrodesacetyldiltiazem. To identify and characterize the two unknowns, and to determine if they existed as true impurities or were only analytical artifacts, a series of chromatographic experiments were conducted on illicit cocaine samples that contained diltiazem.

**Experimental**

**Solvents, Chemicals, and Materials:** All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were reagent-grade quality and were products of Sigma-Aldrich Chemical (Milwaukee, WI). The standard of (+)-cis-diltiazem was also obtained from Sigma-Aldrich Chemical. The illicit cocaine base and cocaine hydrochloride samples were obtained from seized exhibits. Standards of desacetyldiltiazem and 2,3-dehydrodesacetyldiltiazem were synthesized at the DEA Special Testing and Research Laboratory (*vide infra*).

**Standard Solutions for Quantitative Determination of Diltiazem and Desacetyldiltiazem:** Individual CHCl₃/MSTFA solutions containing 38, 95, 189, 473, 946, and 1892 µg/mL of diltiazem hydrochloride and 14, 34, 69, 172, 344, and 688 µg/mL of desacetyldiltiazem hydrochloride, respectively, were prepared. Each solution also contained 100 µg/mL of *para*-fluorococaine as the internal standard (ISTD). Linearity was confirmed over the concentration ranges for each component and linear regression analysis determined that the correlation coefficient ($R^2$) exceeded 0.9998 for each.
Instrumentation

Gas Chromatography / Flame Ionization Detection (GC/FID): All purity determinations of cocaine, diltiazem, and desacetyldiltiazem were performed on an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. Sample preparation and chromatographic parameters for diltiazem and desacetyldiltiazem were identical to those reported by Casale and Waggoner [8], except that MSTFA was utilized as the derivatizing reagent (instead of BSA). Chromatographic parameters for all cocaine purity determinations were identical to those reported by Piñero and Casale [9].

Gas Chromatography / Mass Spectrometry (GC/MS): GC/MS analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5 : 1), at 280°C. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and a scan rate of 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

Desorption Electrospray Ionization Mass Spectrometry (DESI MS): Molecular weight information derived from [M+H]+ and MS/MS data were obtained using a Thermo (Madison, WI) Accela Liquid Chromatograph coupled with an LCQ (Madison, WI) Advantage MAX Ion Trap Mass Spectrometer. The non-ground sample was positioned near the entrance to the mass spectrometer in the 100% methanol LC eluent spray with a 100 μL/min flow rate. Atmospheric Pressure Ionization (API) parameters included a 5.0 kV spray voltage, 40 psi sheath gas, 300°C capillary temperature, and positive polarity. Full MS data was collected with a scan range of 90 - 500 m/z. MS/MS data for 415 m/z and 373 m/z were collected at 35% cid with scan ranges of 110 - 500 m/z and 100 - 500 m/z, respectively.

Nuclear Magnetic Resonance Spectroscopy (NMR): One and two dimensional NMR analyses were performed on a Varian (Palo Alto, CA) VNMRS 600 MHz NMR using a 3 mm triple resonance Varian indirect detection probe. The samples were prepared in deuterated chloroform containing tetramethylsilane (CDCl3 with TMS, Aldrich Chemical Co., Milwaukee, WI). Gradient versions of the two dimensional NMR experiments HSQC (one bond correlation of hydrogens directly bonded to carbon) and HMBC (correlation of hydrogens 2, 3, or 4 bonds from a carbon) were performed to make the assignments listed in Table 1.

Syntheses

Desacetyldiltiazem Hydrochloride: Diltiazem hydrochloride (1.00 g, 2.22 mmol) and NaOCH3 (0.21 g, 3.89 mmol) were dissolved into MeOH (15 mL) and heated at 75°C overnight in a sealed tube. The MeOH was evaporated to a minimal volume (about 0.5 mL) and then diluted with 5 mL of water and 0.25 mL of saturated aqueous Na2CO3. The solution was extracted with CHCl3 (2 x 5 mL). The combined extracts were washed with water (2 x 10 mL) and dried over anhydrous Na2SO4, filtered, and evaporated in vacuo to a semi-crystalline mass. The product was dissolved in 300 mL diethyl ether, precipitated as the hydrochloride ion-pair with the addition of sufficient ethereal hydrochloric acid, filtered, and dried to provide a white powder (666 mg, 70% yield, 97+% purity).

2,3-Dehydrodesacetyldiltiazem Base: Desacetyldiltiazem hydrochloride (150 mg, 0.37 mmol) and POCl3 (1.0 mL, 10.9 mmol) were heated at 75°C for 9 hours in a sealed tube. The reaction was cooled to 0°C and carefully quenched with cold water (2 mL), and then cold concentrated NaOH until a pH of 9 was achieved. The solution was extracted with CHCl3 (2 x 5 mL). The combined extracts were dried over anhydrous Na2SO4, filtered, and evaporated in vacuo to provide an off-white powder (150 mg, 71% yield, 98+% purity).
Results and Discussion

Two cocaine base exhibits (Base #1 and Base #2) and one cocaine hydrochloride exhibit were examined for cocaine, diltiazem, and desacetyldiltiazem by GC/FID, as detailed in the Experimental section. The base exhibits both contained sodium bicarbonate, and were specifically selected because they gave differing responses for diltiazem by GC/FID vs. GC/MS. The cocaine hydrochloride exhibit was specifically selected because it contained a relatively high level of diltiazem and trace amounts of suspected desacetyldiltiazem. Partial reconstructed GC/FID chromatograms for the diltiazem/desacetyldiltiazem determinations are illustrated in Figure 3. The quantitative data and relative retention times are given in Tables 2 and 3, respectively.

Base #1 contained 7.1% diltiazem and 0.67% desacetyldiltiazem by GC/FID utilizing CHCl₃/MSTFA. However, when analyzed by GC/MS with methanol as the injection solvent (Figure 4a), no diltiazem was detected. Instead, two unknown compounds were observed. The first was identified as desacetyldiltiazem via comparison of its mass spectrum (Figure 5b) and retention time with the synthesized standard. The second had an apparent molecular ion at m/z 354 (hereafter referred to as the “354” compound; Figure 6a). We had postulated that the “354” compound was 2,3-dehydrodesacetyldiltiazem (Figure 1), resulting from elimination of water from desacetyldiltiazem. This is analogous to the formation of methyl eecgonidine (anhydroecgonine methyl ester) from cocaine [10]. However, when the “354” compound’s mass spectrum and retention time were compared to the synthetic standard, the spectra (Figure 6a and 6b) were dissimilar, and the retention time differed by 1.5 minutes (Table 3). Thus, the “354” compound remains unidentified at this time.

When Base #1 was examined by GC/MS using CHCl₃/MSTFA as the injection solvent (Figure 4b), diltiazem was identified by its mass spectrum (Figure 5a), as well as a lower level of desacetyldiltiazem as its TMS derivative (Figure 5c). However, the “354” compound was not detected. When Base #1 was examined by DESI MS, only a small [M+H]⁺ at m/z 373 (consistent with desacetyldiltiazem (mw = 372)) was detected relative to a [M+H]⁺ at m/z 415 (diltiazem). The collective results indicate that use of methanol as the injection solvent for this exhibit results in quantitative degradation of diltiazem to desacetyldiltiazem and the “354” compound.

Base #2 was determined to contain trace diltiazem and 1.3% desacetyldiltiazem via GC/FID utilizing CHCl₃/MSTFA. When examined by GC/MS using methanol as the injection solvent (Figure 7a), trace desacetyldiltiazem was identified but no diltiazem or “354” compound were detected. When this exhibit was examined by GC/MS using CHCl₃/MSTFA as the injection solvent (Figure 7b), desacetyldiltiazem was easily identified as its TMS derivative, but again, no diltiazem or “354” compound were detected.

GC artifacts are well known when analyzing cocaine base (“crack”) exhibits that contain sodium bicarbonate. In this study, sodium methoxide and methanol were used to synthesize desacetyldiltiazem (from diltiazem) in high yield. Since the use of methanol as an injection solvent, coupled with the presence of sodium bicarbonate, will produce sodium methoxide in the injection port [10], the observed degradation of diltiazem to desacetyldiltiazem is not surprising. Since the “354” compound was not identified, the mechanism for its formation is unknown.

The cocaine hydrochloride exhibit was determined to contain 12.0% diltiazem and 0.27% desacetyldiltiazem via GC/FID. When examined by GC/MS using methanol as the injection solvent (Figure 8a), only diltiazem was identified. In this case, use of methanol did not cause degradation of diltiazem because the exhibit contained no sodium bicarbonate. Finally, when the exhibit was examined by GC/MS using CHCl₃/MSTFA as the injection solvent (Figure 8b), diltiazem and trace desacetyldiltiazem as its TMS derivative were identified, consistent with the GC/FID analysis (Figure 3c); the “354” compound was not detected in either analysis.

The cocaine hydrochloride exhibit was then converted into “crack” cocaine using the traditional process (i.e., with water and sodium bicarbonate). The quantitative GC/FID data for this exhibit is given in Table 2. The converted sample contained essentially the same percentage of cocaine, diltiazem, and desacetyldiltiazem as was found in the original hydrochloride sample. This indicates that the “crack” conversion process did not cause hydrolysis.
of diltiazem to desacetyldiltiazem. However, it is likely that prolonged storage of “crack” cocaine containing
diltiazem and sodium bicarbonate (or a stronger base (e.g., Na₂CO₃ or NaOH)) would cause slow hydrolysis to
desacetyldiltiazem. Finally, as expected, when this “crack” exhibit was examined by GC/MS using methanol as
the injection solvent (Figure 9), only desacetyldiltiazem and the “354” compound were detected.

The physiological effects and consequences of smoking “crack” cocaine adulterated with diltiazem and sodium
bicarbonate are unknown.

Conclusions

Desacetyldiltiazem and an uncharacterized artifact (the “354” compound) can be formed as analytical artifacts in
gas chromatographic profiles of cocaine exhibits containing diltiazem and sodium bicarbonate. The use of
methanol as the injection solvent for these samples causes the formation of these compounds in GC injection
ports. However, the use of CHCl₃ or CHCl₃/MSTFA as injection solvents does not promote the formation of
these artifacts. Although desacetyldiltiazem can be present at detectable levels as a bona fide impurity in some
cocaine exhibits, analysts should be aware that degradation of diltiazem to desacetyldiltiazem and the “354”
compound will occur in GC injection ports when analyzing cocaine samples containing diltiazem and sodium
bicarbonate, when using methanol as the injection solvent.

References

2. Quaglia MG, Donati E, Fanali S, Bossu E, Montinaro A, Buiarelli F. Analysis of diltiazem and its related
4. Muszalska I, Jamszol L, Grzeskowiak D. Kinetics of hydrolysis of diltiazem hydrochloride in aqueous
5. Dube LM, Mousseau N, McGilvery IJ. High-performance liquid chromatographic determination of
   diltiazem and four of its metabolites in plasma: Evaluation of their stability. Journal of Chromatography
   1988;430:103-111.
6. Andrisano V, Hrelia P, Gotti R, Leoni A, Cavrini V. Photostability and phototoxicity studies on
8. Casale JF, Waggoner RW. A chromatographic impurity signature profile analysis for cocaine using
9. Piñero EL and Casale JF. Quantitation of cocaine by gas chromatography - flame ionization detection
   utilizing isopropylecocaine as a structurally related internal standard. Microgram Journal

---

Table 1. NMR Chemical Shift (in ppm) Data for Proton and Carbon.

<table>
<thead>
<tr>
<th>position</th>
<th>cis-diltiazem HCl</th>
<th>desacetyl/diltiazem</th>
<th>2,3-dehydrodesacetyl/diltiazem</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzothiazepin proton</td>
<td>carbon</td>
<td>proton</td>
<td>carbon</td>
</tr>
<tr>
<td>2</td>
<td>5.02 d</td>
<td>54.1</td>
<td>4.83 d</td>
</tr>
<tr>
<td>3</td>
<td>5.12 d</td>
<td>71.2</td>
<td>4.29 d</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>168.2</td>
<td>-</td>
</tr>
<tr>
<td>5a</td>
<td>-</td>
<td>144.2</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>7.52 dd</td>
<td>124.6</td>
<td>7.45 dd</td>
</tr>
<tr>
<td>7</td>
<td>7.58 dt</td>
<td>132.1</td>
<td>7.47 dt</td>
</tr>
<tr>
<td>8</td>
<td>7.32 dt</td>
<td>128.5</td>
<td>7.25 dt</td>
</tr>
<tr>
<td>9</td>
<td>7.72 dd</td>
<td>135.7</td>
<td>7.64 dd</td>
</tr>
<tr>
<td>9a</td>
<td>-</td>
<td>127.6</td>
<td>-</td>
</tr>
<tr>
<td>3-acetyl C=O</td>
<td>-</td>
<td>169.8</td>
<td>-</td>
</tr>
<tr>
<td>3-acetyl CH3</td>
<td>1.90 s</td>
<td>20.4</td>
<td>-</td>
</tr>
<tr>
<td>phenyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>126.0</td>
<td>-</td>
</tr>
<tr>
<td>2,6</td>
<td>7.37 d</td>
<td>130.6</td>
<td>7.24 d</td>
</tr>
<tr>
<td>3,5</td>
<td>6.90 d</td>
<td>113.9</td>
<td>6.82 d</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>159.9</td>
<td>-</td>
</tr>
<tr>
<td>methoxy</td>
<td>3.83 s</td>
<td>55.3</td>
<td>3.74 s</td>
</tr>
<tr>
<td>(CH3)2-N-CH2-CH2</td>
<td>2.84 d</td>
<td>43.5</td>
<td>2.75 d</td>
</tr>
<tr>
<td>(CH3)2-N-CH2-CH2</td>
<td>2.92 d</td>
<td>43.0</td>
<td>2.83 d</td>
</tr>
<tr>
<td>(CH3)2-N-CH2-CH2</td>
<td>3.25 ddd</td>
<td>54.4</td>
<td>3.18 ddd</td>
</tr>
<tr>
<td>(CH3)2-N-CH2-CH2</td>
<td>3.50 ddd</td>
<td>54.4</td>
<td>3.46 ddd</td>
</tr>
<tr>
<td>(CH3)2-N-CH2-CH2</td>
<td>4.42 ddd</td>
<td>44.9</td>
<td>4.26 ddd</td>
</tr>
<tr>
<td>(CH3)2-N-CH2-CH2</td>
<td>4.58 ddd</td>
<td>44.9</td>
<td>4.54 ddd</td>
</tr>
</tbody>
</table>

* = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, s = singlet, ** indicates uncertainty with assignment of quaternary carbons

Table 2. Quantitative Data for Cocaine Exhibits Containing Diltiazem.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cocaine%</th>
<th>Diltiazem%</th>
<th>Desacetyl/diltiazem%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base #1</td>
<td>44.2</td>
<td>7.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Base #2</td>
<td>54.5</td>
<td>trace</td>
<td>1.3</td>
</tr>
<tr>
<td>Hydrochloride</td>
<td>79.9</td>
<td>12.0</td>
<td>0.27</td>
</tr>
<tr>
<td>Base #3 a</td>
<td>77.3</td>
<td>12.7</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*a Produced from the Cocaine Hydrochloride Sample.
Figure 3. Partial Reconstructed GC/FID Chromatograms of: (A) Cocaine Base Exhibit #1 Containing 44.2% Cocaine, 0.67% Desacetyldiltiazem, and 7.1% Diltiazem; (B) Cocaine Base Exhibit #2 Containing 54.5% Cocaine, 1.3% Desacetyldiltiazem, and Trace Diltiazem; (C) Cocaine Hydrochloride Exhibit Containing 79.9% Cocaine, 0.27% Desacetyldiltiazem, and 12.0% Diltiazem. Peak Identification: 1 = \textit{para}-Fluorococaine; 2 = Cocaine; 3 = Desacetyldiltiazem-TMS Derivative; and 4 = Diltiazem. CHCl₃/MSTFA was Utilized as the Injection Solvent.
Figure 4. Partial Reconstructed Total Ion Chromatograms of Cocaine Base Exhibit #1 Using: (A) Methanol as Injection Solvent; and (B) CHCl₃/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine, 2 = Desacetyldiltiazem, 3 = Diltiazem Artifact, 4 = Desacetyldiltiazem-TMS, and 5 = Diltiazem.
Figure 5. Electron Ionization Mass Spectrum of: (A) Diltiazem; (B) Desacetyldiltiazem; and (C) Desacetyldiltiazem-TMS Derivative.
Figure 6. Electron Ionization Mass Spectrum of (A) Diltiazem Artifact; and (B) 2,3-Dehydrodesacetyldiltiazem.
Figure 7. Partial Reconstructed Total Ion Chromatograms of Cocaine Base Exhibit #2 Using: (A) Methanol as Injection Solvent; and (B) CHCl₃/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine; 2 = Desacetyldiltiazem; and 3 = Desacetyldiltiazem-TMS Derivative.
Figure 8. Partial Reconstructed Total Ion Chromatograms of Cocaine Hydrochloride Exhibit Using (A) Methanol as Injection Solvent; and (B) CHCl₃/MSTFA as Injection Solvent. Peak Identification: 1 = Cocaine, 2 = Desacetyl-Diltiazem-TMS, and 3 = Diltiazem.
**Figure 9.** Partial Reconstructed Total Ion Chromatogram of Cocaine Base ("Crack") Produced from the Cocaine Hydrochloride Exhibit. Peak Identification: 1 = Cocaine; 2 = Desacetyldiltiazem; and 3 = Diltiazem Artifact.

**Table 3.** Retention Times (RT) Diltiazem and Related Impurities \(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>GC/FID RT</th>
<th>GC/MS RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>para-Fluorococaine (^b)</td>
<td>14.91</td>
<td>N/A</td>
</tr>
<tr>
<td>Cocaine</td>
<td>15.74</td>
<td>21.43</td>
</tr>
<tr>
<td>Desacetyldiltiazem-TMS</td>
<td>24.27</td>
<td>29.40</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>29.10</td>
<td>30.68</td>
</tr>
<tr>
<td>Desacetyldiltiazem</td>
<td>N/A</td>
<td>30.77</td>
</tr>
<tr>
<td>“354” Compound</td>
<td>N/A</td>
<td>31.28</td>
</tr>
<tr>
<td>2,3-Dehydrodesacetyldiltiazem</td>
<td>N/A</td>
<td>32.76</td>
</tr>
</tbody>
</table>

\(^a\) Conditions Given in Experimental Section. RT Values Given in Minutes.

\(^b\) Internal Standard.
Technical Note

Etodolac: An Analytical Profile

Mandy C. McGehee
U.S. Department of Justice
Drug Enforcement Administration
Northeast Laboratory
99 10th Avenue, Suite 721
New York, NY  10011
[email: mandy.c.mcgehee  -at-  usdoj.gov]

ABSTRACT: Etodolac (Lodine) has been identified in various submissions of illicit heroin seizures in the northeast region of the United States. Etodolac is a nonsteroidal anti-inflammatory drug used in the treatment of mild to moderate pain, and helps relieve symptoms of arthritis, such as inflammation, swelling, stiffness, and joint pain. Analytical data, including gas chromatography, infrared spectroscopy, Raman spectroscopy, mass spectroscopy and proton nuclear magnetic resonance spectroscopy are presented.

KEYWORDS: Etodolac, Heroin, Adulteration, NSAID, Analysis, Forensic Chemistry

Introduction

The presence of pharmacologically active adulterants and inactive diluents as cutting agents in illicit heroin exhibits is common, and dynamic. Over the past 9 years, this laboratory has received increasing numbers of heroin submissions containing varying amounts of etodolac (trade name Lodine, Figure 1), 1,8-diethyl-1,3,4,9-tetrahydropyranol[3,4-b]indol-1-acetic acid, a prescription nonsteroidal anti-inflammatory drug (NSAID) [1,2] (see Figure 1). Approved by the U.S. Food and Drug Administration in 1997 for acute and long term use in the management of osteoarthritis and rheumatoid arthritis, etodolac is produced by multiple pharmaceutical companies in both capsule and tablet forms [3]. Herein, standard analytical data (GC/FID, FTIR/ATR, Raman, GC/MS, and 1H-NMR) is presented for etodolac.
Experimental

Etodolac Standard: Sigma-Aldrich, Inc. (St. Louis, MO); Lot #121K4049. Because etodolac is de facto an indole propionic acid (see Figure 1), it is presumed to be a zwitterionic compound.

Gas Chromatography / Flame Ionization Detector (GC/FID):

- Instrument: Agilent 6890N with a flame ionization detector
- Column: HP-5, 30 m x 0.25 mm x 0.25 μm film thickness
- Injector Temperature: 270°C
- Oven Temperature: 175°C for 1.0 min, ramped 15°C/min to 280°C for 3.0 min
- Carrier Gas: Hydrogen ramped flow 2.5 mL/min for 5 min to 3.5 mL/min; split ratio 50 : 1

Utilizing the above experimental parameters, etodolac breaks down into four peaks, three minor peaks followed by one major peak. The retention time for the three minor peaks are 3.940, 4.974, and 5.068 minutes followed by the major peak at 6.056 minutes. The retention times relative to heroin are 0.525, 0.659, 0.676, and 0.807, respectively.

Fourier Transform Infrared Spectroscopy (FTIR/ATR):

- Instrument: Perkin Elmer Spectrum One
- Number of Scans: 16
- Resolution: 4.000 cm⁻¹
- Wavenumber Range: 4000 cm⁻¹ to 650 cm⁻¹

Data was obtained by direct analysis using an attenuated total reflectance (ATR) attachment on FTIR. The data was not ATR corrected [Figure 2].

Fourier Transform Raman Spectroscopy (FT Raman):

- Instrument: Thermo Nicolet Nexus 670 FTIR
- Number of Scans: 8
- Resolution: 8.000 cm⁻¹
- Wavenumber Range: 3701 cm⁻¹ to 100 cm⁻¹

Data was obtained by direct analysis using a Smart Golden Gate ZnSe Accessory on FTIR. The data was corrected with the automatic smooth function [Figure 3].

Gas Chromatography / Mass Spectrometry (GC/MS):

- Instrument: Agilent 5973
- Column: HP-5 MS, 30 m x 0.25 mm x 0.25 μm film thickness
- Injector Temperature: 255°C
- Oven Temperature: 90°C for 1.35 min, 35°C/min to 290°C
- Carrier Gas: Helium with split ratio = 35 : 1
- MS Quad: 150°C
- MS Source: 230°C
- Scan Range: 40 - 550 amu

Electron impact mass spectrometry data shows a molecular ion at m/z 287 and a base ion at m/z 228 [Figure 4].
Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR):

Data was obtained using a Varian Mercury 400 MHz NMR. The sample was prepared at a final concentration of 25.2 mg/mL in deuterated methanol (CD3OD) containing TMS (tetramethylsilane, Si(CH3)4) as the 0 ppm reference. The spectrum was obtained with 8 scans using a 1.0 second delay, 45° pulse, and a 2.99 second acquisition time. The scan width was 6410 Hz [Figure 5]. Note that etodolac cannot be analyzed in D2O, because it is insoluble in water.

Results and Discussion

Although the levels of adulteration have widely varied, etodolac is typically present in heroin at approximately 1% or below. With the exception of the GC/FID chromatography, the presented data is unremarkable. When etodolac is analyzed by GC/FID, four peaks are present due to the thermal breakdown of the compound (the breakdown products were not identified). The presented data will assist in the identification of etodolac.

References


Figure 2. FTIR/ATR Spectrum of Etodolac.
Figure 3. FT Raman Spectrum of Etodolac.

Figure 4. Electron Impact Mass Spectrum of Etodolac.
Figure 5. 400 MHz $^1$H-NMR Spectrum of Etodolac in CD$_3$OD.
Technical Note

Determination of Cocaine in Various South American Commercial Coca Products

Elizabeth M. Corbeil,* Ph.D., and John F. Casale, B.S.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA  20166
[email withheld at author’s request]

ABSTRACT: Cocaine content is provided for several coca products including coca tea, medicinal tonics and rubs, and alcohol. Although these products are legal in most of South America, they are considered controlled substances in the United States and in most other countries. The cocaine was separated from complex matrices utilizing trap column chromatography. Gas chromatography / mass spectrometry / selective ion monitoring was used for cocaine identification and quantitation. The amount of cocaine in for these products ranges from 0.00 - 0.65 µg/mg.

KEYWORDS: Cocaine, Coca Products, Quantitation, Mass Spectrometry, Selective Ion Monitoring, Forensic Chemistry

Introduction

Although cocaine is controlled virtually worldwide, coca is legitimately cultivated in the South American countries of Peru and Bolivia. While it is illegal to extract the cocaine from the coca leaf in all of these counties, the coca leaf and various coca leaf extracts have long been legally used to relieve fatigue, hunger, and provide nutritional value. Along these lines, Duke et al. determined that ingesting coca leaves met the recommended dietary allowance for calcium, iron, phosphorous, vitamin A, vitamin B, and vitamin E [1]. Traditional South American medicine also uses coca leaf to alleviate headaches, rheumatism, abrasions, malaria, ulcers, asthma, and parasites, and studies have shown that several of these traditional treatments are valid [2].

However, in the United States and in many other countries, it is illegal to obtain, possess, or use coca products. The U.S. Code of Federal Regulations (CFR) lists coca leaves and any derivative or preparation of coca leaves as Schedule II substances [3]. The CFR excludes substances that contain de-cocainized coca leaves and leaf extracts that do not contain cocaine and ecgonine [3]. Numerous studies have quantitated the amount of cocaine and alkaloids found in coca leaf [4-7]. Studies have also determined the percent cocaine in coca tea and how it is metabolized in the body [8-9].

Although the analyses of coca leaf, coca extracts, and illicit cocaine exhibits are routine, analyses of food, medicinal, and beauty products that contain small amounts of coca leaf or coca extracts can be challenging due to the variety and complexity of the matrices. This investigation determined the amounts of cocaine in various matrices, including coca tea, medicinal tonics, rubs, alcohol, beauty products, and food products. Cocaine was isolated from the matrices via trap column chromatography, and identified and quantitated via gas chromatography / mass spectrometry / selective ion monitoring (GC/MS/SIM).
Experimental

Materials: Chloroform was a product of Burdick and Jackson Laboratories (Muskegon, MI). Diethylamine (DEA) and acid-washed Celite 545 were products of Sigma-Aldrich Chemical (Milwaukee, WI). Isopropylcocaaine (used as an internal standard, ISTD) was synthesized in-house [10]. All standard solutions were prepared in 50 mL acid-washed glass volumetric vials. Trap column chromatography was performed using Lab Glass columns (260 mm x 22 mm). All the commercial coca products that were analyzed in this study were obtained from open markets in La Paz, Bolivia.

Gas Chromatography / Mass Spectrometry / Selective Ion Monitoring (GC/MS/SIM): Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC was fitted with a 30 m x 0.25 mm ID fused silica capillary column coated with 0.25 μm DB-1 (J&W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature 100°C; initial hold 0.0 min; program rate 6.0°C/min; final temperature 300°C; final hold 5.67 min. The injector was operated in the split mode (21.5 : 1) at 280°C. The MSD was operated in selective ion monitoring (SIM) mode. The fragment ions 82.1, 182.1, and 303.2 Daltons were monitored with a 500 millisecond dwell time for cocaine. The fragment ions 82.1, 210.2, and 331.2 Daltons were monitored with a 500 millisecond dwell time for isopropylcocaaine. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

Standard Solutions for Quantitative Determination of Cocaine: Individual CHCl₃ solutions each containing 22.50 μg/mL of isopropylcocaaine and 9.90, 20.62, 25.57, 30.51, 35.47, and 41.24 μg/mL of cocaine base, respectively, were prepared. Linearity was confirmed over the concentration ranges; linear regression analysis determined the correlation coefficient (R²) as exceeding 0.996.

Sample Preparation and Extractions: Cocaine was isolated from wax-like, aqueous, and candy-like coca products utilizing a slight modification of the trap column chromatography utilized by Moore et al. [5].

Wax-Like Samples: Between 500 - 1000 mg of wax-like samples were dissolved in 1 mL of CHCl₃ containing 22.50 μg/mL of isopropylcocaaine and 1 mL of water-saturated CHCl₃ (hereafter WSC). This solution was vortexed and then placed onto a glass chromatographic column containing 4 g of Celite 545 mixed with 2 mL of 0.36 N sulfuric acid. The column was eluted with 50 mL of WSC (discarded) followed by 50 mL WSC containing 500 μL DEA (collected and evaporated in vacuo to a residue). The residue was reconstituted in approximately 1 mL of CHCl₃, dried over anhydrous sodium sulfate, filtered, and examined via GC/MS/SIM.

Aqueous Samples: Between 2 - 10 mL of liquid samples were evaporated in vacuo to a residue. The residue was reconstituted in a mixture of 1 mL of CHCl₃, 1 mL of the CHCl₃ containing 22.50 μg isopropylcocaaine, and 3 drops of water. This solution was placed onto a glass chromatographic column containing 4 g of Celite 545 mixed with 2 mL of 0.36 N sulfuric acid. The cocaine from these samples was isolated and analyzed in the same manner as detailed above under “Wax-Like Samples.”

Candy Samples: Two pieces (approximately 8 g) of candy were ground and dissolved in water (approximately 2 mL). The solution was basified with saturated NaOH until pH 8 - 9, and the cocaine was extracted with 1 mL of CHCl₃ containing 22.50 μg isopropylcocaaine and placed onto a glass chromatographic column containing 4 g of Celite 545 mixed with 2 mL of 0.36 N sulfuric acid. The cocaine from these samples was isolated and analyzed in the same manner as detailed above under “Wax-Like Samples.”

Leaf, Vitamin, and Alcohol Samples: Cocaine was isolated from coca tea and the coca vitamin by weighing approximately 2 mg directly into a GC vial containing 1 mL of CHCl₃ containing 22.50 μg of isopropylcocaaine and 250 μL of DEA. The samples were examined via GC/MS/SIM. A 250 μL aliquot of the alcohol sample was added to 1 mL of CHCl₃ containing 22.50 μg of isopropylcocaaine spiked with 50 μL of DEA.
**Results and Discussion**

Two typical GC/MS/SIM chromatograms are shown in Figure 1. Figure 1a (Vitamins) illustrates a relatively low concentration of cocaine, while Figure 1b (Medicinal Tonic) illustrates a significant quantity of cocaine. Collectively, the analyses indicated that the products contained from 0.00 - 0.65 μg/mg cocaine (Table 1). The cocaine (if any) in the shampoo could not be determined due to the difficulty of isolating cocaine from this matrix. In addition, one of the alcohol products did not contain cocaine (see below). The appearance and manufacturer of all of the aqueous medicinal tonics was the same; their cocaine content ranged from 0.01 - 0.38 μg/mg, indicating that there were significant differences in the amount of coca leaf added to each product. All of the medicinal rubs had the same waxy appearance and a strong odor of coca leaf; their cocaine content ranged from 0.01 - 0.10 μg/mg (relatively low). Visual inspection of these latter products confirmed that only small amounts of particles were distributed (unevenly) throughout the waxy matrix. The cocaine content in the teas and the ground leaf samples ranged from 0.46 - 0.65 μg/mg. The cocaine content of the ground leaf and tea samples were consistent with coca leaf [4-7]. Both candy samples were brown, sticky substances that smelled similar to coca leaf; one had the consistency of chewing gum, while the other was more like a hard candy. They were determined to contain 0.003 - 0.01 μg/mg cocaine. The Ajayu de Coca Pachamama (liquor) was a clear liquid with a distinct alcohol smell. Despite its suggestive name, this product did not contain cocaine. The Ron Fernando Ron De Coca (liquor) was a green liquid that smelled similar to coca leaf and alcohol; it contained 0.22 μg/mg of cocaine.

**Conclusions**

Trap column chromatography can be utilized to isolate cocaine from complex bulk matrices. The utilization of GC/MS/SIM, coupled with a structurally related internal standard, gave excellent sensitivity and linearity, and could determine cocaine content down to the microgram per gram level. Utilizing the described methodology, cocaine was readily detected and determined in all the commercial products except for a shampoo and one alcoholic liquor. In the case of coca leaf, the described method was able to determine cocaine content from as little as 2 milligrams of sample.

**References**


---

**Table 1.** Coca Content of Selected Coca Products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Matrix</th>
<th>μg/mg Cocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ron Fernando Ron De Coca</td>
<td>liquor</td>
<td>Alcohol</td>
<td>0.22</td>
</tr>
<tr>
<td>Ajayu de Coca Pachamama</td>
<td>liquor</td>
<td>Alcohol</td>
<td>Not detected</td>
</tr>
<tr>
<td>Adelgazante</td>
<td>Diet</td>
<td>Aqueous</td>
<td>0.39</td>
</tr>
<tr>
<td>Anti Diabetico</td>
<td>Diabetes</td>
<td>Aqueous</td>
<td>0.15</td>
</tr>
<tr>
<td>Tos Asma</td>
<td>Asthma</td>
<td>Aqueous</td>
<td>0.29</td>
</tr>
<tr>
<td>Prostata</td>
<td>Prostate Problems</td>
<td>Aqueous</td>
<td>0.38</td>
</tr>
<tr>
<td>Parasitos</td>
<td>Parasites</td>
<td>Aqueous</td>
<td>0.33</td>
</tr>
<tr>
<td>Ulceras</td>
<td>Ulcers</td>
<td>Aqueous</td>
<td>0.01</td>
</tr>
<tr>
<td>Tonic</td>
<td>Tonic</td>
<td>Aqueous</td>
<td>0.26</td>
</tr>
<tr>
<td>Pomada Natural de Coca Contra Dolores: Reumaticos, Musculares, Varices Y Huesos</td>
<td>rheumatic, muscular, veins, and bones</td>
<td>Wax</td>
<td>0.07</td>
</tr>
<tr>
<td>Pomada Natural de Coca Para: Artritis Y Gota</td>
<td>Cream for arthritis and foot pain</td>
<td>Wax</td>
<td>0.10</td>
</tr>
<tr>
<td>Pomada Natural de Coca Para: Hemorroides</td>
<td>Cream for hemorrhoids</td>
<td>Wax</td>
<td>0.04</td>
</tr>
<tr>
<td>Pomada de Molle</td>
<td>Cream for moles</td>
<td>Wax</td>
<td>0.01</td>
</tr>
<tr>
<td>Chicle Copenhagen</td>
<td>Chewing gum</td>
<td>Candy</td>
<td>0.01</td>
</tr>
<tr>
<td>Caramelos Y ElixirEnergizante de Altura</td>
<td>Caramel candy for energy booster</td>
<td>Candy</td>
<td>0.003</td>
</tr>
<tr>
<td>Mate Windsor</td>
<td>Tea</td>
<td>Tea</td>
<td>0.59</td>
</tr>
<tr>
<td>Kokasana</td>
<td>Tea</td>
<td>Tea</td>
<td>0.46</td>
</tr>
<tr>
<td>Harina De Coca</td>
<td>Nutritional Supplement</td>
<td>Ground Leaf</td>
<td>0.65</td>
</tr>
<tr>
<td>Coca Premium</td>
<td>Nutritional Supplement</td>
<td>Ground Leaf</td>
<td>0.59</td>
</tr>
<tr>
<td>Shampoo</td>
<td>Beauty Product</td>
<td>Liquid</td>
<td>N/A</td>
</tr>
</tbody>
</table>

---
Figure 1. Partial Reconstructed Selected Ion Chromatograms of: (A) 2.73 mg of Coca Premium Vitamins Containing 0.593 µg/mg Cocaine; and (B) 5.00 g of Adelgazante Medicinal Tonic Containing 0.385 µg/mg Cocaine. Peak Identification: 1 = Cocaine; and 2 = Isopropylcocaine (ISTD).
“Crack” Cocaine: A Study of Stability over Time and Temperature

Laura M. Jones, B.S.,* Danielle K. Boudreau, B.S., and John F. Casale, B.S.
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166
[email address withheld at author’s request]

ABSTRACT: Changes in the appearance, weights, purity levels, and alkaloidal profiles of 146 laboratory-prepared “crack” cocaine exhibits stored under different temperatures and packaging types, were studied over a one year period. An accelerated aging study (elevated temperature, one month) was also performed with 2 “crack” cocaine exhibits, to simulate very long-term or higher temperature storage. The results indicate that higher purity “crack” that was prepared by the classic method is reasonably stable over 12 months if stored at or below 20°C, irrespective of incidental moisture and/or packaging type. However, extended storage times and/or elevated temperatures can result in weight loss and/or degradation, especially for samples sealed in plastic bags or heat-sealed evidence envelopes.

KEYWORDS: Cocaine, “Crack,” Stability, Storage, Degradation, Weight Loss, Forensic Chemistry

Introduction

Cocaine base, commonly referred to as “crack,” is a major drug of abuse. Forensic drug analysts routinely analyze “crack” exhibits and present their findings in court. Over the past 20 years, many instances of weight loss and degradation of stored “crack” exhibits have been noted, especially for exhibits stored for long time frames or under non-ideal conditions. Such changes can be an issue for forensic chemists when testifying at trial, particularly if the original results are not in agreement with reanalyses that were conducted within the same laboratory, at a different forensic laboratory, or independently by chemists employed by the defense.

Weight changes in cocaine hydrochloride exhibits have been previously studied, with research concluding that weight gain is often due to water absorption associated with packaging [1]. Cocaine hydrochloride degradation processes have also been previously studied, and the resulting products have been characterized [2-5]. The stability of cocaine hydrochloride in aqueous solutions has also been extensively researched [6-8]. Minor alkaloids present in illicit cocaine exhibits, such as the truxillines, have also been examined for stability over time, with the conclusion that a direct relationship exists between the sample age and the increased levels of the truxilline degradation products, i.e., the truxillic and truxinic acids [9].

However, a search of the literature found no studies on the stability of “crack” cocaine, or the products resulting from the degradation of “crack.” In this study, the stability of “crack” was monitored in two independent experiments. In the first, 146 prepared samples were stored for one year at three different temperatures (room temperature (20°C), refrigerator (5°C), and freezer (-5°C)) and two different types of packaging (standard zip-lock plastic bags and Heat Sealed Evidence Envelopes (HSEEs)). In this case, the exhibits were examined for weight loss, changes in purity, and degradation on a monthly basis. In the second experiment, two prepared samples were stored for one month at 65°C, one unsealed and one sealed in a zip-lock plastic bag. The elevated temperature was used to simulate very long term storage. In the latter case, the exhibits were analyzed at the start and finish only.
For the one year study, the “crack” was prepared via the “classical” technique. In this method, cocaine hydrochloride is dissolved in water, and an alkaline substance such as sodium bicarbonate or sodium carbonate is added to precipitate cocaine base. The solution is brought to a boil, and the cocaine base melts and forms an oil that pools on the bottom of the container. The solution is cooled, and the oil solidifies, allowing the water layer to be poured off. For the one month (high temperature) study, the “crack” was prepared via the production technique known by the street term “whipping.” In this method, water is “whipped” into the molten cocaine base prior to its solidification, to increase its bulk. The samples used for the one year study were processed using the “classical” method because it gives a reasonably uniform product (homogeneity is necessary for valid sample comparisons). The samples used for the one month study were processed using the “whipping” method because it maximizes the amount of water in the sample, and water can be considered to be the key factor in sample degradation during extended or higher temperature storage.

Other types of “crack” production techniques, including the “microwave” method, were not employed in this study because they give inhomogeneous products that contain extensive sodium bicarbonate and other processing impurities.

Figure 1 illustrates the products resulting from the degradation of cocaine and the cinnamoylcocaines in “crack.” The amounts of benzoylecgonine and the cinnamoylecgonines were tracked to determine the extent of cocaine and cinnamoylcocaine degradation, respectively (ecgonine methyl ester and ecgonine were also quantitated, but are not reported here because they result from the degradation of both cocaine and the cinnamoylcocaines). The amounts of tropacocaine and trimethoxycocaine present in “crack” were also tracked, because these alkaloids are key “marker” compounds that are used in many cocaine profiling (signature) programs [2,4,5,10].

**Experimental**

**Materials:** “Crack” cocaine was produced in-house as described below, starting with uncut, illicitly prepared cocaine hydrochloride from the laboratory inventory. Pharmaceutical cocaine base (used as the quantitation standard for all GC analyses) was obtained from Merck Chemical (Rahway, NJ). Chloroform was a distilled-in-glass product of Burdick and Jackson Laboratories (Muskegon, MI). Reagent grade diethylamine was obtained from Sigma-Aldrich Chemical Company (Milwaukee, WI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was a product of Pierce Chemical (Rockford, IL). para-Fluorococaine and isopropylcocaine (both used as internal standards (ISTDs)) were synthesized in-house.

**Gas Chromatograph / Flame Ionization Detection (GC/FID):** Quantitative analyses of cocaine were performed using isopropylcocaine as a structurally related ISTD [11]. An Agilent (Palo Alto, CA) Model 6890N gas chromatograph fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J&W Scientific, Rancho Cordova, CA) was used for cocaine quantitation. An isothermal oven temperature of 250°C was used for 7.00 min. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/min. The injection port and detector were maintained at 280°C. Samples (2 μL) were injected in the split mode (25 : 1) by an Agilent 7683 Series Auto Injector. Nitrogen was used as the auxiliary make-up gas for the detector. For all quantitations, a minimum of triplicate analyses (N = 3) were performed and results are reported as the average.

Quantitative analyses of cocaine alkaloids and their degradation products were performed using a previously detailed chromatographic impurity signature profile analysis method [2]. Analyses were conducted using an Agilent Model 6890N gas chromatograph fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1701 (J&W Scientific). The oven temperature was programmed as follows: Initial temperature 170°C; 1 min hold; program rate 4°C/min to 200°C; program rate 6°C/min to a final temperature of 275°C; 9 min hold. Samples (1 μL) were injected in the split mode (21.3 : 1) by an Agilent 7683 Series Auto Injector. The injection port and flame ionization detector were maintained at 230°C and 300°C, respectively. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/min. Nitrogen was used as the auxiliary
make-up gas for the detector. For all quantitations, a minimum of triplicate analyses (N = 3) were performed and results are reported as the average.

**Sample Preparation for Cocaine Quantitation:** About 16 - 20 mg of cocaine base were weighed (to the nearest 0.01 mg) into a 50 mL Erlenmeyer flask. Samples and standards were diluted with 20 mL of chloroform containing 50 μL of DEA and 5.0 mL of the isopropylcocaine ISTD solution (0.9 mg/mL) [11].

**Sample Preparation for Signature Analysis:** Approximately 4 - 5 mg of cocaine base was weighed (to the nearest 0.01 mg) into an autosampler vial. The samples were diluted using 250 μL of the para-fluorococaine ISTD solution (0.20 mg/mL), 250 μL of MSTFA was added, and the resulting solution was heated for 30 min at 75°C. Samples were allowed to cool to room temperature (approximately 30 min) prior to injection into the GC [2].

**Production of “Crack” Cocaine for the One Year Stability Study:** Approximately 2 kg of uncut cocaine hydrochloride were dissolved in 10 L of water, and saturated sodium bicarbonate added until pH 8 was obtained. The solution was brought to a boil, and the cocaine base melted and settled to the bottom. The solution was then allowed to cool, the water was poured off, and the solidified “crack” cocaine was removed and separated into two batches. The first batch was immediately placed into plastic bags while still wet (hereafter referred to as “fresh”), while the second was allowed to “dry out” for two hours before being placed into bags (hereafter referred to as “dry”). Although packaged wet, the fresh batch did not in fact contain a significant amount of water. Each batch was divided equally into zip-lock plastic bags and HSEEs, with approximately 4 grams of cocaine base in each bag. The samples were weighed (to the nearest 0.1 g) for initial net weights. Samples were stored at: 20°C, 5°C, and -5°C. Approximately every 30 days, one sample from each temperature condition/package type was analyzed for cocaine purity, weight change, and cocaine degradation.

**Results and Discussion**

**12 Month Study of “Classically”-Prepared Fresh and Dry “Crack” Samples**

Because the “crack” samples that were prepared for the 12 month experiments were uniform, of reasonably high purity, and contained little/no sodium bicarbonate and only incidental moisture, this study gives a “best case” scenario for stability and weight loss. The large number of samples (N = 146) allow for valid comparisons. Use of other preparative methods, or the presence of excess water, excess sodium bicarbonate, or other impurities, would have resulted in non-uniform samples and invalid comparisons (Note: Illicitly prepared “crack” samples typically vary widely in their composition, and therefore are unsuited for a study of this type).

Moisture content did not have a significant effect on purity, weight change, and/or degradation of the samples (i.e., the fresh and dry samples did not differ significantly versus each other after 12 months). Similarly, packaging type also did not play a significant role on sample stability - samples stored in either zip-lock plastic bags or HSEEs displayed similar trends when comparing purity, weight change, and degradation.
Cocaine purity results were very similar across all temperatures and packaging types, with an average purity range of 86.0 - 86.9% for HSEE stored samples, and 85.7 - 86.8% for zip-lock plastic bag stored samples. Tables 1 and 2 show the cocaine purity results for HSEE and zip-lock plastic bag stored samples, respectively.

Weight losses were highest for samples stored at room temperature, with a 2 - 5% loss after 12 months. There was a 2 - 3.5% loss at refrigerator temperature, and from a 0.5% loss to a 1.5% gain at freezer temperatures. Not surprisingly, fresh samples displayed slightly greater losses than dry samples. Tables 3 and 4 show the percent weight change over 12 months for samples stored in HSEE and zip-lock plastic bags, respectively.

Various other cocaine alkaloids were also monitored. Many of these compounds are co-extracted with cocaine from coca leaf, and are observed in most illicit cocaine exhibits [3]; others may result from the degradation of cocaine [2]. Understanding and monitoring changes in these trace alkaloids are critical for laboratories that utilize signature methodologies for comparative analyses of exhibits [4] or for intelligence-derived purposes, because even minor changes can impact cocaine classifications. Figure 2 contains the chromatographic profiles of “crack” cocaine stored at room temperature in HSEEs from start (Figure 2a) to finish (Figure 2b). Increases in ecgonine (peak #3), benzoylecgonine (peak #7), and cis- and trans-cinnamoylecgonine (peaks #9 and #11), are evident, as is a small decrease in trimethoxycocaine (peak #12).

Benzylecgonine results from the hydrolysis of cocaine [2]. From start to finish, benzylecgonine concentration changes for frozen samples were 0.05 - 0.10% (by weight), and for refrigerated samples 0.05 - 0.66% (by weight). Samples stored at room temperature, however, had more significant changes, 0.05 - 1.62% (by weight). Fresh samples showed a slightly higher rate of benzylecgonine formation versus dry samples under the same conditions; however, this rate difference was minimal compared to the effect of storage temperature. Figure 3 illustrates the benzylecgonine content in HSEEs over the 12 month study.

Cis- and trans-cinnamoylecgonine methyl esters, commonly referred to as the cinnamoylcocaines, are naturally occurring alkaloids that are co-extracted with cocaine from coca leaf. Degradation (hydrolysis) of the cinnamoylcocaines results in formation of cis- and trans-cinnamic acid and cis- and trans-cinnamoylecgonine. The results from this study indicate that there was a small increase in the cinnamoylcocaines. Again, the samples stored at freezer temperatures showed the smallest increases, while those stored at room temperature showed the highest increases (however, the relative change was small regardless of storage temperature). Figures 4 and 5 illustrate the total cinnamoylcocaine and total cinnamoylecgonine contents of “crack” stored over time in HSEEs, respectively.

Tropacocaine and trimethoxycocaine are also naturally occurring alkaloids that are co-extracted with cocaine from coca leaf [3]. These compounds are key components in classifying the origin of cocaine exhibits [10]; therefore, understanding their long-term stability is of great importance. Somewhat surprisingly, tropacocaine showed virtually no changes during the study, regardless of storage conditions and temperatures. Table 5 illustrates tropacocaine results (% by weight) for “crack” stored in HSEEs. However, trimethoxycocaine did degrade similarly to cocaine and the cinnamoylcocaines; i.e., samples stored at freezer temperatures showed very little degradation versus those stored at room temperatures. Figure 6 illustrates the trimethoxycocaine results (% by weight) for samples stored in HSEEs.

Accelerated Aging Study of “Whipped Crack” Samples

The accelerated aging (elevated temperature) study was performed to simulate both extended room temperature storage [12] and short term storage at elevated temperatures (for example, in a law enforcement officer’s vehicle in summertime heat). As detailed in the Experimental section, two “whipped crack” exhibits were prepared and stored in an oven at 65OC, one unsealed (Sample 1) and the other sealed in a zip-lock plastic bag (Sample 2). After one month, the samples were reanalyzed for purity, weight change, and degradation. Surprisingly, Sample 1 did not undergo significant degradation, with changes in alkaloid concentration very similar to the “Classical
“Crack” samples monitored in the 12-month study. Cocaine purity actually increased almost 14% from the initial quantitation, but this increase was due to the evaporative loss of the water that was “whipped” into the sample prior to the oven storage (the overall net weight decreased by 62.95 g, or 37.3% of the initial weight). The resulting cocaine base remained crystalline and showed no noticeable change in color. Figures 7a and 7b show the chromatographic profiles for the initial and final analyses, respectively. The sample did not undergo significant cocaine degradation to benzoylecgonine (peak #7); however, increased amounts of benzoic acid (peak #1) and ecomine (peak #3) were observed.

In contrast to Sample 1, however, Sample 2 degraded significantly, giving a thick, dark brown, molasses-like liquid. A similar result was reported by LeBelle et al. [4], who stored a sample of cocaine hydrochloride at 60°C and at high humidity for 13 days (liquefaction was noted after the first day). Figures 8a and 8b show the chromatographic profiles for the initial and final analyses, respectively. The cocaine decreased dramatically (57.3% to less than 1%, peak #6), while the benzoylecgonine increased equally dramatically (0.06% to 44.4%, peak #7). The chromatograms also show increased concentrations of benzoic acid (peak #1), ecomine methyl ester (peak #2), ecomine (peak #3), and cis- and trans-cinnamoylecgonine (peaks #9 and #11). The cinnamoylecgonines also degraded similarly to cocaine, resulting in elevated cinnamoylecgonines. The amount of trimethoxycocaine was also much lower, decreasing from 0.26% to 0.06%. Tropacocaine was the only alkaloid that did not undergo significant degradation, with results similar to the initial analysis. Table 6 compares the results between Samples 1 and 2. Interestingly, this cocaine exhibit experienced a net weight loss similar to the unsealed exhibit, decreasing by 52.6 g or 32.5% of the initial weight (Note: Zip-lock plastic bags are not impermeable to water vapor).

Conclusions

To prevent degradation and weight loss, “crack” is best stored at -5°C or below. However, for virtually all forensic laboratories or law enforcement agencies, such storage is not practical. Fortunately, this study indicates that relatively dry “crack” cocaine samples stored at room temperature may not undergo significant degradation within one year. However, this study used laboratory-prepared, reasonably pure samples with minimal moisture and/or sodium bicarbonate content. Retail (street) level samples that are highly adulterated or that contain excess water or sodium bicarbonate would be expected to degrade at a faster rate. Long-term storage of “crack” in sealed packaging (i.e., zip-lock plastic bags or HSEEs) may result in extensive cocaine degradation and significant weight loss. Similarly, even short-term storage of “crack” in sealed packaging at very elevated temperatures, such as within the trunk of an officer’s vehicle during summer months, can result in rapid degradation and weight loss. Finally, “crack” that contains large amounts of occluded water (e.g., “whipped crack” or similar) may undergo significant weight loss if stored unsealed, due to the evaporative loss of water.

References


* Law Enforcement Restricted Issue.

---

Table 1. Cocaine Base Purity of Heat Sealed Evidence Envelope (HSEE) Stored Samples.

<table>
<thead>
<tr>
<th>Month</th>
<th>20°C Dry</th>
<th>20°C Fresh</th>
<th>5°C Dry</th>
<th>5°C Fresh</th>
<th>(-5°C) Dry</th>
<th>(-5°C) Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>88.8</td>
<td>86.3</td>
<td>88.8</td>
<td>86.3</td>
<td>88.8</td>
<td>86.3</td>
</tr>
<tr>
<td>1</td>
<td>85.6</td>
<td>87.5</td>
<td>87.1</td>
<td>86.6</td>
<td>87.1</td>
<td>86.9</td>
</tr>
<tr>
<td>2</td>
<td>87.1</td>
<td>88.0</td>
<td>87.6</td>
<td>88.2</td>
<td>87.2</td>
<td>86.9</td>
</tr>
<tr>
<td>3</td>
<td>85.5</td>
<td>85.3</td>
<td>86.4</td>
<td>86.6</td>
<td>86.4</td>
<td>85.9</td>
</tr>
<tr>
<td>4</td>
<td>86.1</td>
<td>86.2</td>
<td>86.9</td>
<td>87.7</td>
<td>86.8</td>
<td>88.1</td>
</tr>
<tr>
<td>5</td>
<td>85.7</td>
<td>85.7</td>
<td>85.7</td>
<td>85.8</td>
<td>87.0</td>
<td>86.0</td>
</tr>
<tr>
<td>6</td>
<td>84.9</td>
<td>85.5</td>
<td>86.1</td>
<td>86.0</td>
<td>85.4</td>
<td>86.7</td>
</tr>
<tr>
<td>7</td>
<td>86.3</td>
<td>87.0</td>
<td>85.7</td>
<td>86.3</td>
<td>87.0</td>
<td>87.1</td>
</tr>
<tr>
<td>8</td>
<td>87.6</td>
<td>86.7</td>
<td>86.8</td>
<td>87.5</td>
<td>87.8</td>
<td>87.6</td>
</tr>
<tr>
<td>9</td>
<td>82.2</td>
<td>83.3</td>
<td>83.3</td>
<td>83.0</td>
<td>83.4</td>
<td>83.8</td>
</tr>
<tr>
<td>10</td>
<td>86.6</td>
<td>85.3</td>
<td>87.0</td>
<td>86.8</td>
<td>87.8</td>
<td>87.6</td>
</tr>
<tr>
<td>11</td>
<td>87.2</td>
<td>85.6</td>
<td>87.0</td>
<td>87.4</td>
<td>87.2</td>
<td>87.2</td>
</tr>
<tr>
<td>12</td>
<td>85.6</td>
<td>85.9</td>
<td>86.0</td>
<td>86.3</td>
<td>87.5</td>
<td>88.3</td>
</tr>
</tbody>
</table>
Table 2. Cocaine Base Purity of Plastic Bag Stored Samples.

<table>
<thead>
<tr>
<th>Month</th>
<th>20°C Dry</th>
<th>20°C Fresh</th>
<th>5°C Dry</th>
<th>5°C Fresh</th>
<th>(-5°C) Dry</th>
<th>(-5°C) Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>88.8</td>
<td>86.3</td>
<td>88.8</td>
<td>86.3</td>
<td>88.8</td>
<td>86.3</td>
</tr>
<tr>
<td>1</td>
<td>86.1</td>
<td>86.2</td>
<td>86.7</td>
<td>86.9</td>
<td>86.3</td>
<td>87.7</td>
</tr>
<tr>
<td>2</td>
<td>86.2</td>
<td>86.4</td>
<td>87.2</td>
<td>87.0</td>
<td>86.8</td>
<td>87.0</td>
</tr>
<tr>
<td>3</td>
<td>86.0</td>
<td>84.9</td>
<td>86.4</td>
<td>86.6</td>
<td>86.6</td>
<td>86.6</td>
</tr>
<tr>
<td>4</td>
<td>86.2</td>
<td>86.1</td>
<td>86.8</td>
<td>87.1</td>
<td>87.1</td>
<td>87.9</td>
</tr>
<tr>
<td>5</td>
<td>84.5</td>
<td>83.4</td>
<td>86.0</td>
<td>85.4</td>
<td>85.7</td>
<td>85.6</td>
</tr>
<tr>
<td>6</td>
<td>86.4</td>
<td>86.2</td>
<td>86.6</td>
<td>87.0</td>
<td>87.6</td>
<td>87.5</td>
</tr>
<tr>
<td>7</td>
<td>85.4</td>
<td>86.4</td>
<td>86.3</td>
<td>86.4</td>
<td>86.3</td>
<td>86.0</td>
</tr>
<tr>
<td>8</td>
<td>86.7</td>
<td>86.0</td>
<td>86.0</td>
<td>86.1</td>
<td>85.9</td>
<td>85.5</td>
</tr>
<tr>
<td>9</td>
<td>83.8</td>
<td>84.3</td>
<td>84.1</td>
<td>85.1</td>
<td>84.7</td>
<td>84.9</td>
</tr>
<tr>
<td>10</td>
<td>86.5</td>
<td>85.2</td>
<td>85.7</td>
<td>86.7</td>
<td>87.1</td>
<td>87.5</td>
</tr>
<tr>
<td>11</td>
<td>88.0</td>
<td>87.7</td>
<td>88.2</td>
<td>88.7</td>
<td>89.6</td>
<td>89.5</td>
</tr>
<tr>
<td>12</td>
<td>87.4</td>
<td>85.0</td>
<td>85.4</td>
<td>86.0</td>
<td>85.6</td>
<td>86.2</td>
</tr>
</tbody>
</table>

---

Table 3. Percent Weight Change of HSEE Stored Samples.

<table>
<thead>
<tr>
<th>Month</th>
<th>20°C Dry</th>
<th>20°C Fresh</th>
<th>5°C Dry</th>
<th>5°C Fresh</th>
<th>(-5°C) Dry</th>
<th>(-5°C) Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.2</td>
<td>-3.0</td>
<td>1.0</td>
<td>-2.0</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>-1.4</td>
<td>-1.1</td>
<td>0.0</td>
<td>-3.1</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>-2.3</td>
<td>-2.6</td>
<td>0.5</td>
<td>-2.6</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>-2.4</td>
<td>-4.7</td>
<td>-1.0</td>
<td>-2.8</td>
<td>0.2</td>
<td>-0.5</td>
</tr>
<tr>
<td>5</td>
<td>-1.0</td>
<td>-3.8</td>
<td>-0.5</td>
<td>-2.5</td>
<td>-0.4</td>
<td>-0.5</td>
</tr>
<tr>
<td>6</td>
<td>-1.1</td>
<td>-4.7</td>
<td>-0.5</td>
<td>-3.3</td>
<td>-0.2</td>
<td>-1.1</td>
</tr>
<tr>
<td>7</td>
<td>-2.5</td>
<td>-4.2</td>
<td>-0.6</td>
<td>-2.1</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>-3.2</td>
<td>-4.6</td>
<td>0.2</td>
<td>-2.3</td>
<td>0.7</td>
<td>-0.8</td>
</tr>
<tr>
<td>9</td>
<td>-2.0</td>
<td>-4.3</td>
<td>-2.4</td>
<td>-4.7</td>
<td>-2.7</td>
<td>-3.5</td>
</tr>
<tr>
<td>10</td>
<td>-2.2</td>
<td>-3.3</td>
<td>0.0</td>
<td>-2.5</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>11</td>
<td>-2.7</td>
<td>-3.9</td>
<td>-0.8</td>
<td>-2.6</td>
<td>0.5</td>
<td>-0.3</td>
</tr>
<tr>
<td>12</td>
<td>-2.0</td>
<td>-3.3</td>
<td>-1.6</td>
<td>-2.1</td>
<td>1.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

---

Microgram Journal, Volume 6, Numbers 3-4 (July - December, 2008)
Table 4. Percent Weight Change of Plastic Bag Stored Samples.

<table>
<thead>
<tr>
<th>Month</th>
<th>20°C Dry</th>
<th>20°C Fresh</th>
<th>5°C Dry</th>
<th>5°C Fresh</th>
<th>(-5°C) Dry</th>
<th>(-5°C) Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.8</td>
<td>-2.2</td>
<td>-0.5</td>
<td>-1.3</td>
<td>0.2</td>
<td>-0.3</td>
</tr>
<tr>
<td>2</td>
<td>-2.6</td>
<td>-3.3</td>
<td>-1.0</td>
<td>-2.8</td>
<td>0.0</td>
<td>-0.2</td>
</tr>
<tr>
<td>3</td>
<td>-2.5</td>
<td>-2.6</td>
<td>-1.0</td>
<td>-2.6</td>
<td>0.3</td>
<td>-0.5</td>
</tr>
<tr>
<td>4</td>
<td>-3.0</td>
<td>-3.8</td>
<td>-1.2</td>
<td>-2.1</td>
<td>-0.8</td>
<td>-1.0</td>
</tr>
<tr>
<td>5</td>
<td>-2.8</td>
<td>-4.0</td>
<td>-1.2</td>
<td>-2.9</td>
<td>0.0</td>
<td>-0.3</td>
</tr>
<tr>
<td>6</td>
<td>-3.2</td>
<td>-4.6</td>
<td>-1.2</td>
<td>-2.5</td>
<td>-0.2</td>
<td>-1.1</td>
</tr>
<tr>
<td>7</td>
<td>-2.7</td>
<td>-3.7</td>
<td>-1.4</td>
<td>-2.9</td>
<td>0.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>8</td>
<td>-2.8</td>
<td>-4.2</td>
<td>-1.7</td>
<td>-2.7</td>
<td>0.0</td>
<td>-0.7</td>
</tr>
<tr>
<td>9</td>
<td>-3.0</td>
<td>-4.4</td>
<td>-1.9</td>
<td>-1.7</td>
<td>0.2</td>
<td>-2.3</td>
</tr>
<tr>
<td>10</td>
<td>-2.8</td>
<td>-4.2</td>
<td>-1.6</td>
<td>-2.9</td>
<td>-0.5</td>
<td>-1.3</td>
</tr>
<tr>
<td>11</td>
<td>-2.9</td>
<td>-4.4</td>
<td>-1.8</td>
<td>-1.9</td>
<td>-0.2</td>
<td>-0.3</td>
</tr>
<tr>
<td>12</td>
<td>-2.7</td>
<td>-4.7</td>
<td>-2.1</td>
<td>-3.5</td>
<td>-0.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 5. Tropacocaine Percent in HSEE Stored Samples.

<table>
<thead>
<tr>
<th>Month</th>
<th>20°C Dry</th>
<th>20°C Fresh</th>
<th>5°C Dry</th>
<th>5°C Fresh</th>
<th>(-5°C) Dry</th>
<th>(-5°C) Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.10</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>1</td>
<td>0.11</td>
<td>0.10</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.10</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>8</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>9</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>11</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>12</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Table 6. Comparison of Sealed and Unsealed “Crack” Cocaine Stored at 65°C (Summary of Results from Accelerated Study).

<table>
<thead>
<tr>
<th>Accelerated Results (%) by weight</th>
<th>Unsealed Time = 0</th>
<th>Unsealed Time = 1 Month</th>
<th>Sealed Time = 0</th>
<th>Sealed Time = 1 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Loss</td>
<td>N/A</td>
<td>37.3% loss</td>
<td>N/A</td>
<td>32.5% loss</td>
</tr>
<tr>
<td>Cocaine Purity</td>
<td>64.3%</td>
<td>78.1%</td>
<td>57.3%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>0.12%</td>
<td>0.10%</td>
<td>0.06%</td>
<td>44.4%</td>
</tr>
<tr>
<td>Cinnamoylcocaines</td>
<td>4.41%</td>
<td>5.47%</td>
<td>3.67%</td>
<td>0.11%</td>
</tr>
<tr>
<td>Cinnamoylecgonines</td>
<td>0.05%</td>
<td>0.01%</td>
<td>0.01%</td>
<td>1.98%</td>
</tr>
<tr>
<td>Tropacocaine</td>
<td>0.07%</td>
<td>0.09%</td>
<td>0.06%</td>
<td>0.08%</td>
</tr>
<tr>
<td>Trimethoxycocaine</td>
<td>0.31%</td>
<td>0.39%</td>
<td>0.26%</td>
<td>0.06%</td>
</tr>
</tbody>
</table>

Figure 1. Cocaine and Cinnamoylcocaine Degradation Products.
Figure 2. Chromatographic Profiles of “Crack” Cocaine Stored at Room Temperature in HSEE: (A) Time = 0; and (B) Time = 12 Months. Peak Identification: 1 = Benzoic Acid-TMS; 2 = Ecgonine Methyl Ester-TMS; 3 = Ecgonine-di-TMS; 4 = Tropacocaine; 5 = para-Fluorococaine (ISTD); 6 = Cocaine; 7 = Benzoylecgonine-TMS; 8 = cis-Cinnamoylcocaine; 9 = cis-Cinnamoylecgonine-TMS; 10 = trans-Cinnamoylcocaine; 11 = trans-Cinnamoylecgonine-TMS; and 12 = Trimethoxycocaine.
Figure 3. Benzoylcegonine Percent in HSEE Stored Samples.

Figure 4. Total Cinnamoylcocaines Percent in HSEE Stored Samples.
Figure 5. Total Cinnamoylecgonines Percent in HSEE Stored Samples.

Figure 6. Trimethoxycocaine in HSEE Stored Samples.
Figure 7. Chromatographic Profiles of “Crack” Cocaine Stored Unsealed in the Accelerated Study: (A) Time = 0; and (B) Time = 1 Month. Peak Identification: See Figure 2.
Figure 8. Chromatographic Profiles of “Crack” Cocaine Stored Sealed in Accelerated Study: (A) Time = 0; and (B) Time = 1 Month. Peak Identification: See Figure 2.
"The Lost Manuscript" - A Posthumous Publication of:

The Discoloration of Illicit Drug Samples

James M. Moore† and John F. Casale‡
U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20164

[Email address withheld at corresponding author’s request]

ABSTRACT: Discoloration (browning) of illicit cocaine exhibits during long-term storage is a common but not universal phenomenon. In order to gain a better understanding of the discoloration process(es), already discolored seized samples, and a wide variety of authentic drug mixtures that similarly discolored under long-term ambient and accelerated temperature-humidity studies, were subjected to acid base workup, column chromatography, and in-depth analyses of the pertinent fractions by EI and CI GC/MS, UV/Vis, and IR. The discolored samples were all found to contain a primary aromatic amine (either procaine or benzocaine), a sugar (either lactose or dextrose), and an acid (such as cocaine hydrochloride, boric acid, benzoic acid, etc.) The rate of discoloration of the drug mixtures was both pH and temperature dependant, i.e., the rate of sample browning increased with lower pH and/or higher temperature. All discolored samples that contained procaine or benzocaine also contained N-formylprocaine or N-formylbenzocaine, respectively, and these are therefore bona fide “marker” compounds for the browning of illicit cocaine. These derivatives are believed to be formed following the degradation of lactose or dextrose to 5-hydroxymethylfurfural, which in turn degraded to formic and levulinic acids; subsequent formylation of procaine or benzocaine gave the respective “marker” compounds. A number of highly colored compounds (yellow, blue, purple, and pink) were observed in column and thin-layer chromatography of the discolored samples, and are responsible for the sample discoloration. These compounds were not identified, but are believed to derive from condensation reactions between 5-hydroxymethylfurfural with the various amines in the samples.

KEYWORDS: Cocaine Hydrochloride, Discoloration, 5-Hydroxymethylfurfural, Procaine, Benzocaine, N-Formylprocaine, N-Formylbenzocaine, Lactose, Dextrose, Forensic Chemistry

[Foreword by the Corresponding Author and the Microgram Editor: This manuscript was authored in 1974 by then Senior Forensic Chemist Jim Moore; it was intended and formatted for publication in Microgram, but apparently was never submitted. It was re-discovered on July 14, 2008 by the co-author, Senior Research Chemist John Casale. It is published here verbatim (including 1970’s formatting and scientific abbreviations), except that some experiments were repeated by the corresponding author to re-create legible figures, the above Abstract and Keyword set were provided by the Editor, and the layout was slightly reformatted by the Editor for improved readability.]

† Original author (FDA 1963-68; BNDD 1968-73; and DEA 1973-98); deceased February 24th, 1999 (see: Microgram 1999;32(4):133 (Note: Law Enforcement Restricted issue)).

‡ To whom inquiries should be addressed.
**Introduction**

This paper reports the results of a two-year investigation studying the discoloration of illicit drug samples. The study was initiated as a result of several forensic laboratories reporting problems associated with the discoloration, or “browning,” of illicit cocaine samples over a prolonged period of time. This “browning” phenomenon was of forensic interest in that there were discrepancies in the chemist’s description of the sample prior to analysis and later, upon identifying the sample during court testimony. In most cases, the samples were white when first examined but subsequently acquired a brown coloration by the time the samples were reopened for court proceedings. This change in coloration caused significant problems for the chemists in that it could be assumed that a sample mix-up had occurred, and the disposition of the case would be in doubt based upon this reasoning. Dugar, *et al.* [1] have investigated this discoloration phenomenon in contraband cocaine.

The study in this paper satisfactorily characterizes the “browning” phenomenon occurring in certain illicit samples. This characterization is based upon: (A) a thorough literature review that describes the discoloration of legitimate pharmaceutical preparations, (B) in-depth analyses of illicit samples known to have undergone the discoloration process, (C) ambient and accelerated temperature-humidity studies conducted on authentic drug mixtures, and (D) the isolation and characterization of signature compounds associated with the discoloration process in illicit and authentic samples.

**A. Literature Review**

There has been considerable study of the discoloration associated with legitimate pharmaceutical products. Blaug and Huang [2,3] have described the discoloration of amphetamine sulfate - spray dried lactose and amphetamine sulfate - dextrose mixtures when subjected to elevated temperatures. In these studies, a product of sugar decomposition, namely 5-hydroxymethylfurfural (5-HMF), was suggested to be present. Dugar *et al.* [1] also reported the presence of 5-HMF in sugar-containing illicit samples that underwent discoloration. Castello and Mattocks [4] and Duvall *et al.* [5,6] reported interaction of various primary amines with lactose and dextrose and the subsequent discoloration of such samples. In these studies, the rate of “browning” was found to be dependant upon temperature and pH. Several investigators had studied the role of 5-HMF and related substances in the discoloration of amine - sugar mixtures [7-11]. Brownley and Lachman [12] studied the formation of 5-HMF in spray-dried lactose as well as conventionally-processed lactose.

The above-referenced studies clearly demonstrated that when mixtures of primary amines (amphetamine was most often studied) and certain sugars (lactose and dextrose) were subjected to elevated temperature and humidity conditions, significant discoloration occurred. Several authors reported a relationship between the rate of discoloration and pH of the sample. Finally, most of the studies reported a relationship between drug mixtures that had discolored and the presence of 5-HMF.

**B. In-Depth Analyses of Illicit Samples**

A number of illicit drug samples known to have undergone the discoloration process were obtained from various forensic laboratories. All samples were subjected to in-depth analyses for drug constituents as well as diluents. These analyses revealed that the samples had the following elements in common: (1) all samples contained cocaine hydrochloride, (2) all samples had undergone a white-to-brown color transition after prolonged storage under unspecified conditions, (3) all samples contained either lactose or dextrose, (4) all samples contained a primary aromatic amine, such as benzocaine, and (5) all samples were acidic in nature; this acidity was due, in part, to the presence of substances such as cocaine HCl, boric acid, etc. The results of the in-depth analyses indicated a positive correlation between sample composition and the discoloration process described by other investigators.
C. Ambient and Accelerated Stability Studies of Authentic Samples

A large number of authentic samples were prepared for stability studies (see Table I). The composition of these samples was based upon elements believed responsible, in part, for the discoloration process. The authentic samples were studied under ambient conditions for about two years and under accelerated conditions for about two months.

The primary amines used in this study were benzocaine and procaine HCl. These are two widely-used adulterants associated with illicit cocaine and heroin samples, respectively. The sugars used were lactose and dextrose. Preliminary studies indicated that mannitol and sucrose did not contribute significantly to the “browning” process. Since the literature review suggested a difference in the “browning” rates of spray-dried and conventionally-processed lactose, both were used in this study. The pH of the samples was controlled over an acid range by the introduction of substances of varying acidity. In order of increasing pKa, these substances were: oxalic acid, citric acid, benzoic acid, cocaine HCl, boric acid, heroin HCl, and procaine HCl. The concentration of all compounds was varied over a wide range.

In the ambient study, the authentic samples listed in Table I were placed in glass vials with screw-on plastic caps and stored in the dark. At the end of a two-year period, the samples were examined for discoloration. Table II lists those samples that exhibited significant discoloration. The samples listed in Table I, and not included in Table II, did not exhibit significant discoloration.

A review of Table II reveals that all samples that discolored contained either lactose or dextrose, benzocaine or procaine HCl, and were distinctly acidic. It should be noted that the concentration of the acidic component did not influence the discoloration significantly. On the other hand, the majority of the samples that did discolor contained the more acidic components, (i.e., oxalic, citric, and benzoic acids). Though the cocaine HCl - benzocaine - dextrose mixtures were of higher pH values, these samples also discolored. Though the explanation for this is not clear, it may be due to decomposition of cocaine with subsequent formation of benzoic acid resulting in a decrease of pH. It is also apparent from Table II that for a sample of given acidity, benzocaine - dextrose mixtures discolored more rapidly than benzocaine - lactose mixtures. Such a distinction is not apparent for procaine-containing samples.

The samples listed in Table I were also subjected to accelerated conditions for 61 days. The humidity varied from 50 - 70%, while the temperature was gradually increased from ambient to 48°C. Fig. 1 illustrates the results of the accelerated study. As the samples discolored significantly (brown to dark brown), they were removed from the temperature-humidity chamber and noted in Fig. 1. Due to the large number of samples that discolored, only a representative cross-section are noted in Fig. 1.

The results of the accelerated stability studies can be summarized as were the ambient study described previously. Additionally, there does exist a positive correlation between the rate of discoloration of the authentic samples and increases in temperature. There is no apparent difference in the discoloration rates of samples containing the various grades of lactose, including spray-dried and conventionally-processed.

Some of the samples subjected to the accelerated study showed no significant discoloration. These included most of the two-component samples as well as those three-component mixtures that did not contain either a sugar, a primary amine, or a strong acid. Those samples that apparently contained the necessary components for “browning,” but showed no significant discoloration, were generally of a higher pH, and usually contained lactose as the sugar diluent. These included samples containing lactose mixed with heroin HCl - procaine HCl, heroin HCl - benzocaine, boric acid - procaine HCl, boric acid - benzocaine, cocaine HCl - procaine HCl, and cocaine HCl - benzocaine. In general, samples containing dextrose were found to discolor at a significantly faster rate than those containing lactose.
Table I - Composition of Authentic Samples Subjected to Ambient and Accelerated Studies

<table>
<thead>
<tr>
<th></th>
<th>2% Ox - 49% Bnzn - 49% Lac</th>
<th></th>
<th>2% Coc - 49% Proc - 49% Lac</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25% Ox - 37% Bnzn - 37% Lac</td>
<td>41</td>
<td>70% Coc - 15% Bnzn - 15% Lac</td>
</tr>
<tr>
<td>2</td>
<td>70% Ox - 15% Bnzn - 15% Lac</td>
<td>42</td>
<td>2% Coc - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>3</td>
<td>2% Ox - 49% Proc - 49% Dex</td>
<td>43</td>
<td>25% Coc - 37% Proc - 37% Lac</td>
</tr>
<tr>
<td>4</td>
<td>25% Ox - 37% Proc - 37% Dex</td>
<td>44</td>
<td>70% Coc - 15% Proc - 15% Lac</td>
</tr>
<tr>
<td>5</td>
<td>70% Ox - 15% Proc - 15% Lac</td>
<td>45</td>
<td>2% Coc - 49% Bnzn - 49% Lac</td>
</tr>
<tr>
<td>6</td>
<td>2% Ox - 49% Bnzn - 49% Dex</td>
<td>46</td>
<td>25% Coc - 37% Bnzn - 37% Lac</td>
</tr>
<tr>
<td>7</td>
<td>25% Ox - 37% Bnzn - 37% Lac</td>
<td>47</td>
<td>2% Coc - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>8</td>
<td>70% Ox - 15% Bnzn - 15% Lac</td>
<td>48</td>
<td>70% Coc - 15% Bnzn - 15% Lac</td>
</tr>
<tr>
<td>9</td>
<td>2% Ox - 49% Proc - 49% Lac</td>
<td>49</td>
<td>2% Bor - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>10</td>
<td>70% Ox - 15% Proc - 15% Lac</td>
<td>50</td>
<td>25% Bor - 37% Proc - 37% Lac</td>
</tr>
<tr>
<td>11</td>
<td>2% Cit - 49% Proc - 49% Lac</td>
<td>51</td>
<td>70% Bor - 15% Proc - 15% Lac</td>
</tr>
<tr>
<td>12</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>52</td>
<td>2% Bor - 49% Bnzn - 49% Lac</td>
</tr>
<tr>
<td>13</td>
<td>2% Cit - 49% Proc - 49% Lac</td>
<td>53</td>
<td>25% Bor - 37% Proc - 37% Lac</td>
</tr>
<tr>
<td>14</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>54</td>
<td>70% Bor - 15% Bnzn - 15% Lac</td>
</tr>
<tr>
<td>15</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>55</td>
<td>2% Bor - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>16</td>
<td>2% Cit - 49% Bnzn - 49% Lac</td>
<td>56</td>
<td>25% Bor - 37% Proc - 37% Lac</td>
</tr>
<tr>
<td>17</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>57</td>
<td>70% Bor - 15% Proc - 15% Lac</td>
</tr>
<tr>
<td>18</td>
<td>2% Cit - 49% Proc - 49% Lac</td>
<td>58</td>
<td>2% Bor - 49% Bnzn - 49% Lac</td>
</tr>
<tr>
<td>19</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>59</td>
<td>25% Bor - 37% Bnzn - 37% Lac</td>
</tr>
<tr>
<td>20</td>
<td>2% Cit - 49% Proc - 49% Lac</td>
<td>60</td>
<td>70% Bor - 15% Bnzn - 15% Lac</td>
</tr>
<tr>
<td>21</td>
<td>2% Cit - 49% Proc - 49% Lac</td>
<td>61</td>
<td>2% Her - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>22</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>62</td>
<td>70% Her - 15% Proc - 15% Lac</td>
</tr>
<tr>
<td>23</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>63</td>
<td>70% Her - 15% Proc - 15% Lac</td>
</tr>
<tr>
<td>24</td>
<td>70% Cit - 15% Proc - 15% Lac</td>
<td>64</td>
<td>2% Her - 49% Bnzn - 49% Lac</td>
</tr>
<tr>
<td>25</td>
<td>2% Bzoc - 49% Proc - 49% Lac</td>
<td>65</td>
<td>25% Her - 37% Bnzn - 37% Lac</td>
</tr>
<tr>
<td>26</td>
<td>70% Bzoc - 15% Proc - 15% Lac</td>
<td>66</td>
<td>25% Her - 37% Proc - 37% Lac</td>
</tr>
<tr>
<td>27</td>
<td>70% Bzoc - 15% Proc - 15% Lac</td>
<td>67</td>
<td>70% Her - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>28</td>
<td>2% Bzoc - 49% Proc - 49% Lac</td>
<td>68</td>
<td>25% Her - 37% Proc - 37% Lac</td>
</tr>
<tr>
<td>29</td>
<td>25% Bzoc - 37% Bnzn - 37% Lac</td>
<td>69</td>
<td>70% Her - 15% Proc - 15% Lac</td>
</tr>
<tr>
<td>30</td>
<td>70% Bzoc - 15% Bnzn - 15% Lac</td>
<td>70</td>
<td>2% Her - 49% Bnzn - 49% Lac</td>
</tr>
<tr>
<td>31</td>
<td>2% Bzoc - 49% Proc - 49% Lac</td>
<td>71</td>
<td>2% Her - 49% Proc - 49% Lac</td>
</tr>
<tr>
<td>32</td>
<td>70% Bzoc - 15% Proc - 15% Lac</td>
<td>72</td>
<td>70% Her - 15% Bnzn - 15% Lac</td>
</tr>
<tr>
<td>33</td>
<td>70% Bzoc - 15% Proc - 15% Lac</td>
<td>73</td>
<td>25% Coc - 25% Proc - 50% Ox</td>
</tr>
<tr>
<td>34</td>
<td>2% Bzoc - 49% Bnzn - 49% Lac</td>
<td>74</td>
<td>25% Coc - 25% Proc - 50% Ox</td>
</tr>
<tr>
<td>35</td>
<td>25% Bzoc - 37% Bnzn - 37% Lac</td>
<td>75</td>
<td>25% Her - 25% Proc - 50% Ox</td>
</tr>
<tr>
<td>36</td>
<td>70% Bzoc - 15% Bnzn - 15% Lac</td>
<td>76</td>
<td>50% Proc - 50% Ox</td>
</tr>
<tr>
<td>37</td>
<td>2% Coc - 49% Proc - 49% Lac</td>
<td>77</td>
<td>50% Bnzn - 50% Ox</td>
</tr>
<tr>
<td>38</td>
<td>25% Coc - 37% Proc - 37% Lac</td>
<td>78</td>
<td>50% Dex - 50% Ox</td>
</tr>
<tr>
<td>39</td>
<td>70% Coc - 15% Proc - 15% Lac</td>
<td>79</td>
<td>50% Lac - 50% Ox</td>
</tr>
<tr>
<td>40</td>
<td>2% Coc - 49% Bnzn - 49% Lac</td>
<td>80</td>
<td>50% Coc - 50% Ox</td>
</tr>
<tr>
<td>41</td>
<td>25% Coc - 37% Bnzn - 37% Lac</td>
<td>81</td>
<td>50% Her - 50% Ox</td>
</tr>
</tbody>
</table>

---

Key to Abbreviations: Ox = oxalic acid, Cit = citric acid, Bzoc = benzoic acid, Coc = cocaine HCl, Bor = boric acid, Her = heroin HCl, Proc = procaine HCl, Bnzn = benzocaine, Dex = dextrose, Lac = lactose. Various grades of lactose, including spray-dried and conventionally processed were used.
Table II - Authentic Samples that Exhibited Discoloration after a Two-Year Study under Ambient Conditions

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Sample Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19</td>
<td>Uniform dark brown to black</td>
</tr>
<tr>
<td>20, 21, 27, 28, 29, 30, 41, 42</td>
<td></td>
</tr>
<tr>
<td>1, 2, 3, 7, 8, 9, 23, 24, 25, 26, 32, 33, 40</td>
<td>Uniform light to medium brown</td>
</tr>
<tr>
<td>49, 50, 52, 53</td>
<td></td>
</tr>
<tr>
<td>16, 22, 23</td>
<td>Dark specks in white powder</td>
</tr>
</tbody>
</table>

---

**a** Ambient conditions: avg. room temperature = 24 - 26°C; humidity range = 60 - 80% RH.

**b** Refer to Table I for sample composition.

---

* * * * *

Discoloration of Authentic Samples during Accelerated Study

**Figure 1:** Graph illustrating discoloration of authentic samples subjected to accelerated conditions. As samples discolored, they were removed from temperature-humidity chamber and noted on graph. Refer to Table I for sample composition.
In summary, the results of the ambient and accelerated studies have established a clear relationship between the
discoloration of illicit samples and their composition and storage conditions. Despite this positive correlation, full
characterization of this “browning” phenomena would not be complete unless signature compounds that resulted
as by-products of the discoloration process were isolated and identified. This work is described below.

D. Isolation and Characterization of Signature Compounds

1. Chemicals and Solvents
   The formic acid used in this study was 88% analytical reagent grade and obtained from Mallinkrodt
   Chemical Works (St. Louis, Missouri). The deuterated formic acid (DCOOH) was 99% atom % D and
   was supplied by Merck and Co., Inc. (St. Louis, Missouri). All other chemicals and solvents used were of
   high quality and obtained from the usual commercial sources.

2. Drug Standards
   All drug materials were provided by the Special Testing and Research Laboratory, Drug Enforcement
   Administration.

3. Chromatographic Materials
   (a) The chromatographic partitioning columns were 250 mm in length x 22 mm i.d., and obtained from
       Kontes Glass Co. (Vineland, New Jersey).

   (b) The diatomaceous earth used in the partitioning work was Celite 545 acid-washed (AW), and
       obtained from Johns-Manville Co. (Inglewood Cliff, New Jersey).

   (c) All thin layer chromatography was done on glass plates coated with silica gel GF (250 or 2000μ
       thickness). These plates were obtained from Analtech, Inc. (Newark, Delaware).

   (d) All gas chromatographic columns were obtained from Applied Science Laboratories (State College,
       Pennsylvania) (see body of paper for dimensions). The various column packings were also obtained from
       Applied Science Laboratories. These included 3% OV-1 and 3% OV-25, all on Chromosorb WHP
       (100-120M). All internal standards were also obtained from Applied Science Laboratories.

4. Instrumentation
   (a) Gas Chromatography - The gas chromatograph work was done on a Packard 7400 gas chromatograph
       equipped with a flame ionization detector (FID) (see body of paper for other GLC parameters).

   (b) Ultraviolet Spectroscopy (UV) - All UV spectra were recorded on a Cary 14 spectrophotometer.

   (c) Infrared Spectroscopy (IR) - All IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer.

   (d) Gas Chromatography - Mass Spectrometry (GC-MS) - A Finnigan 4000 mass spectrometer was used
       in this study. It was interfaced with a Finnigan 9610 GC and Finnigan 6110 data system. The gas
       chromatograph was equipped with a 6 ft. x 2 mm i.d. glass column packed with 3% OV-1 and Gas Chrom
       Q (80-100M).

       All electron impact (EI) spectra were acquired under the following conditions: emission current - 0.35
       mA, amplifier sensitivity - 10 - 8 A/V, electron energy - 70 eV, and electron multiplier - 1600 V. The
       carrier gas was Helium and maintained at a flow rate of about 20 cc/min. The column temperature was
       programmed between 150 and 250°C, while the ionizer, separator, and transfer line temperatures were
       maintained at 250, 260, and 260°C, respectively.
All chemical ionization (CI) spectra were obtained under the following conditions: Helium was the carrier gas and methane was used as the reactant gas; the ionizer was maintained at a pressure of about 0.40 torr and a temperature of 200ºC; all other parameters are the same as for the EI study.

5. Isolation of Signature Compounds

During the in-depth analyses of the discolored, illicit samples described previously, trace amounts of unidentified impurities were detected. The methodology described below was developed in order to isolate these impurities in sufficiently pure form for spectroscopic characterization.

(a) Isolation of Signature Compounds in Illicit and Authentic, Brown Samples Containing Benzocaine

About 0.5 cc of 0.1N H₂SO₄ is mixed with 1 gm Celite 545 AW and packed moderately in a chromatographic partitioning column. An appropriate quantity of sample is dissolved in 2 cc of 0.1N H₂SO₄ and 3 gm of Celite 545 AW are added; after mixing, the sample is packed moderately above the bottom layer of the column. The column is eluted with about 50 - 75 cc of water-saturated ethyl ether. This eluate contains benzocaine and the signature compound. Cocaine and other basic drugs are retained by the column. The ether eluate is evaporated gently to dryness. Depending upon sample composition, this fraction may be sufficiently pure for spectroscopic characterization.

If additional “cleanup” is necessary, the following chromatographic procedure may be used. The residue obtained above from the ether eluate is triturated with 2 cc of 0.1N NaHCO₃; 3 gm of Celite 545 are added and mixed until fluffy; this mixture is packed moderately in a chromatographic partitioning column containing a layer of 0.5 cc 0.1N NaHCO₃ mixed with 1 gm of Celite 545 AW. The column is then eluted with 75 - 100 cc of water-saturated petroleum ether. This fraction consists primarily of benzocaine. The column is then eluted with 50 - 75 cc water-saturated ethyl ether. This fraction contains the signature compound and trace amounts of benzocaine. The ether eluate is evaporated carefully to dryness. The residue may be characterized spectroscopically or subjected to further purification using the TLC technique described below.

The residue obtained above is dissolved in a small volume of methylene chloride and spotted or streaked on a silica gel GF plate (250 or 2000µ thickness). The plate is developed with a solvent mixture of ethyl ether : petroleum ether (65:35). After development, the plate is dried and viewed under short wave UV. Both benzocaine and the signature compound appear as dark spots or bands at Rₜ values of about 0.45 and 0.17, respectively. The spot or band representing the signature compound is removed from the plate and placed in a small vial. Methanol is added to the vial and warmed gently on a steam bath. The vial is centrifuged and the methanol is decanted and evaporated carefully to dryness. The residue may be subjected to spectroscopic characterization.

Isolation of the signature compound may also be accomplished using GLC fraction collection techniques. Table III gives the appropriate GLC parameters and retention data for benzocaine, the signature compound, and internal standards.

The signature compound isolated by one or more of the chromatographic procedures given above is subjected to MS, UV, and IR characterization described later in this paper.

(b) Isolation of Signature Compounds in Illicit and Authentic Brown Samples Containing Procaine

An appropriate quantity of sample is dissolved in 2 cc of water; the solution is made basic with NaHCO₃ and 3 gm of Celite 545 AW are added; after uniform mixing, the sample is packed in a column containing a layer of 0.5 cc of 0.1N NaHCO₃ mixed with 1 gm of Celite 545 AW. The signature compound is
isolated following petroleum and ethyl ether elutions, as described above for samples containing benzocaine (ethyl ether eluate contains the signature compound as well as small quantities of procaine).

If necessary, additional TLC purification may be required as described above for benzocaine-containing samples. The adsorbent used is silica gel GF and the solvent system is ammonia-saturated chloroform : methanol (18:1). The $R_f$ values for the procaine and the signature compound are about 0.8 and 0.6, respectively. (Note: using this solvent system, acetylprocaine and the signature compound have similar $R_f$ values.)

Isolation of the signature compound may also be accomplished using GLC fraction collection techniques. Table IV gives the GLC parameters and retention data for procaine, the signature compound, and internal standard.

* * * * *

**Table III - GLC Data for Benzocaine, Internal Standards, and Signature Compound**

<table>
<thead>
<tr>
<th>Compound</th>
<th>3% OV-1 $^a$</th>
<th>3% OV-25 $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzocaine</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Signature Compound</td>
<td>5.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Eicosane</td>
<td>9.9</td>
<td>-</td>
</tr>
<tr>
<td>Hexacosane</td>
<td>-</td>
<td>9.2</td>
</tr>
</tbody>
</table>

$^a$ 6 ft. x ¼ in. i.d. column packed with 3% OV-1 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 190°C, manifold temp. = 250°C, detector temp. = 250°C; N$_2$ carrier flow = 60 cc/min.

$^b$ 6 ft. x ¼ in. i.d. column packed with 3% OV-25 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 205°C, manifold temp. = 250°C, detector temp. = 250°C; N$_2$ carrier flow = 60 cc/min.

* * * * *

**Table IV - GLC Data for Procaine, Internal Standard, and Signature Compound**

<table>
<thead>
<tr>
<th>Compound</th>
<th>3% OV-1 $^a$</th>
<th>3% OV-25 $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine</td>
<td>3.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Octacosane</td>
<td>-</td>
<td>4.3</td>
</tr>
<tr>
<td>Signature Compound</td>
<td>8.0</td>
<td>6.1</td>
</tr>
</tbody>
</table>

$^a$ 6 ft. x ¼ in. i.d. column packed with 3% OV-1 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 220°C, manifold temp. = 250°C, detector temp. = 250°C; N$_2$ carrier flow = 60 cc/min.

$^b$ 6 ft. x ¼ in. i.d. column packed with 3% OV-25 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 240°C, manifold temp. = 265°C, detector temp. = 265°C; N$_2$ carrier flow = 60 cc/min.
The signature compound isolated from procaine-containing samples by one or more of the chromatographic procedures given above are subjected to MS, UV, and IR identification outlined below.

E. Identification

1. Signature Compound in Benzocaine-Containing Samples

(a) Mass Spectral Analysis

The purified residue obtained above from benzocaine-containing samples was introduced into the GC-MS under conditions described earlier.

The EI spectrum of the signature compound was rather simple and quite similar to the EI spectrum of benzocaine (Fig. 2a and 2b). Benzocaine and the signature compound yielded molecular ions at m/e 165 and m/e 193, respectively. Both compounds exhibited prominent ions at (M-28)+, (M-45)+, and (M-73)+. In both compounds, an ion of moderate intensity was noted at m/e 65.

The CI spectra of the signature compound confirmed the molecular weight of 193 obtained from the EI spectrum. This confirmation was supported by the presence in CI of an intense quasi-molecular ion at m/e 194 as well as the expected adduct ions at (M+29)+ and (M+41)+.

The EI and CI data obtained above suggested the signature compound to be closely related to benzocaine, but substituted with a functional group of 29 mass units, such as an ethyl or formyl substituent.

(b) UV Analysis

In methanol, benzocaine yielded a UV maximum at 292 nm, while the signature compound gave a maximum at 269 nm. This hypsochromic shift of 23 nm would suggest that an electron withdrawing group had been introduced in the phenyl ring of benzocaine, probably para- to the carboxyethyl group. Inspection of the benzocaine molecule revealed that substitution on the highly reactive amino function would be a likely occurrence. The substitution of an ethyl group on the amino function would have an inductive effect which would probably increase the wavelength of UV maximum. However, the introduction of a formyl group would decrease the interaction of the lone pair of electrons of nitrogen with the phenyl ring resulting in a decrease in wavelength of UV maximum.

Since MS and UV analyses suggested the signature compound to be N-formyl substituted benzocaine, an infrared analysis was done to support this postulation.

(c) Infrared Analysis [12,13]

The infrared spectra (KBr medium) of benzocaine and the signature compound were studied and found to be similar, but not identical. The IR spectrum of the signature compound supported a formyl substituent on the amino function of benzocaine. While benzocaine exhibited three bands between 3500 and 3200 cm⁻¹ due to asymmetric, symmetric, and bonded NH₂ stretching vibrations, the signature compound exhibited only one intense band at 3310 cm⁻¹. This was strong evidence that substitution on the nitrogen function had occurred. This was further supported by the absence of the intense NH bending vibrational band found at 1630 cm⁻¹ in primary aromatic amines such as benzocaine. The substituent on the nitrogen function was probably carbonyl as supported by an additional carbonyl band at 1700 cm⁻¹ and a band at 1530 cm⁻¹ due probably to the NH bending vibration found in amides (Amide II band).
The other bands in the spectrum of the signature compound were similar to benzocaine. These intense bands were at 1685 cm⁻¹ and 1170 cm⁻¹, due to C-O stretching modes. Intense bands at 1595 cm⁻¹ and 1505 cm⁻¹, were due to the phenyl moiety; and absorption bands between 850 cm⁻¹ and 750 cm⁻¹, were due to C-H out-of-plane bending in the phenyl ring.

The combined UV, IR, and MS data suggested the signature compound to be N-formylbenzocaine (ethyl-p-formamidobenzoate). This structure is confirmed later in this paper by synthesis of the compound and its deuterated analogue and comparing its spectral data with that of the isolated signature compound. Additionally, a mechanistic interpretation of the mass spectral fragmentation of N-formylbenzocaine is presented.

2. Signature Compound in Procaine-Containing Samples

(a) Mass Spectral Analysis

The purified residue obtained above from samples containing procaine was introduced into the GC-MS under conditions described earlier.

The EI spectra of the signature compound and procaine were similar (Fig. 3a and 3b). Both spectra yielded ions at m/e 58, 65, 71, 86, 92, 99, 120, and 137. Additionally, procaine yielded an ion at m/e 164, while the signature compound produced ions at m/e 148, 192, and 249. Since procaine did not yield a molecular ion, it was not surprising that the molecular ion was not detected for the signature compound.

The CI spectrum of procaine yielded an intense quasimolecular ion at m/e 237 as well as the expected adduct ions at (M+29)+ and (M+41)+. The CI spectrum of the signature compound produced an intense quasimolecular ion at m/e 265 as well as the expected adduct ions at (M+29)+ and (M+41)+. These data suggested the molecular weight of the signature compound to be 264 amu.

The EI and CI data suggested the signature compound to be related to procaine, but with an additional substituent of 29 mass units. Since N-formylbenzocaine had been identified above, the substituent in procaine was hypothesized to be a formyl group.

b. Ultraviolet Analysis

The UV maxima of procaine and the signature compound in methanol were at 295 and 271 nm, respectively. This supported an assignment of a formyl group as a substituent on the aromatic nitrogen function in procaine (for discussion, refer above to UV analysis of benzocaine-related signature compound).

c. Infrared Analysis

The infrared spectra of procaine base (KBr medium) and the signature compound base (salt plates) were studied (signature compound base is an oil). As in benzocaine, procaine base exhibits three intense bands between 3500 and 3200 cm⁻¹ due to NH₂ stretching vibrational modes. In the signature compound, only one significant band appeared at 3300 cm⁻¹. However, unlike N-formylbenzocaine, this band was rather broad and of low to moderate intensity. This was probably due to the fact that hydrogen bonding occurred because the spectrum was obtained as an oil. Nonetheless, it was apparent that substitution on the aromatic amine function in procaine had occurred. This assignment was supported by the absence of

(Continued on Page 140)
Figure 2: Spectra regenerated on GC-MSD. Conditions utilized were identical to those published in Microgram Journal 2006;4(1-4):47-53. (a) EI mass spectrum of benzocaine. (b) EI mass spectrum of N-formylbenzocaine.
Figure 3: Spectra regenerated on GC-MSD. Conditions utilized were identical to those published in Microgram Journal 2006;4(1-4):47-53. (a) EI mass spectrum of procaine. (b) EI mass spectrum of N-formylprocaine.
the NH bending mode at 1620 cm\(^{-1}\) due to the NH\(_2\) group in procaine. The N substituent is probably a carbonyl function, as supported by a very broad and intense band at 1700 cm\(^{-1}\). This band represented a combination of two carbonyl bands, one due to the amido function and the other due to the ester moiety (note: acetylprocaine also exhibits one intense and broad band at about 1700 cm\(^{-1}\)). Further support for a carbonyl substituent on the aromatic nitrogen function arises from an intense band at 1530 cm\(^{-1}\) due to the NH bending mode found in amides (Amide II band). The other bands in the spectrum of the signature compound were similar to procaine base. These included bands at 1700 cm\(^{-1}\), in part, to the ester carbonyl stretching mode at 1270 and 1170 cm\(^{-1}\) due to the C-O stretching mode, an intense band at 1595 cm\(^{-1}\) due to the phenyl moiety, and absorption bands between 850 and 750 cm\(^{-1}\) due to C-H out-of-plane bending in the phenyl ring.

The combined UV, IR, and MS data suggested the signature compound, isolated from discolored authentic samples containing procaine, to be N-formylprocaine (2-diethylamino-p-formamidobenzoate). This structure was confirmed by the synthesis of this compound and its deuterated analogue and comparing its spectral data with that of the isolated signature compound. Additionally, a mechanistic interpretation of the mass spectral fragmentation of N-formylprocaine is given.

3. **Synthesis of N-Formylbenzocaine, N-Formylprocaine, and their Deuterated Analogues**

One gram quantities of benzocaine and procaine were dissolved in separate 10 cc portions of formic acid. One gram quantities of both compounds were also dissolved in separate 10 cc portions of deuterated formic acid (DCOOH). After about 20 hours at room temperature, the solutions were diluted with a large volume of ice water and made basic with NaHCO\(_3\). The signature compounds and small amounts of unreacted benzocaine and procaine were extracted from the NaHCO\(_3\) solution into ethyl ether. The ether extracts were passed through anhydrous sodium sulfate and evaporated to dryness. N-formylbenzocaine is a white solid, and N-formylprocaine is an oil. Both signature compounds were synthesized in greater than 90% yield.

The UV, IR, and MS spectral data for the synthesized standards were virtually identical with the signature compounds isolated from samples, thus confirming their identity as N-formylbenzocaine and N-formylprocaine.

4. **Mechanistic Interpretation of Mass Spectral Fragmentation of N-Formylbenzocaine and N-Formylprocaine** [14,15]

The N-formyl and deutero-formyl derivatives of benzocaine and procaine were synthesized as described above.

(a) **N-Formylbenzocaine** (Fig. 2b and 4)

The EI mass spectrum of N-formylbenzocaine yields a moderately intense molecular ion at \(m/e\) 193 (I). As expected, the deuterated analogue produced a molecular ion at \(m/e\) 194. An ion of moderate intensity at \(m/e\) 165 is due to expulsion of ethylene from the molecular ion with hydrogen transfer to the fragment ion (II). Though this ion could be rationalized as elimination of the formyl group with hydrogen transfer, the appearance of \(m/e\) 137 in benzocaine supports the former postulation. This assignment is confirmed by the appearance of an ion at \(m/e\) 166 in the deuterated species. The base peak at \(m/e\) 148 is due to elimination of C\(_2\)H\(_2\)O from the molecular ion with the fragment ion charge on the oxygen (III). A weak ion formed at \(m/e\) 137 is due to loss of CO and C\(_2\)H\(_2\) with a double hydrogen transfer (IV). As expected, a shift of 1 amu occurred upon deuteration. A moderately intense ion at \(m/e\) 65 is probably due to the
cyclopentadienyl ion, an ion seen frequently in the spectra of aromatic amines (V). An intense ion found at \( m/e \) 120 (VI) is due to losses of \( \text{C}_2\text{H}_5\text{O} \) and the formyl group with the formyl hydrogen transferred to the charged species. Confirmation is obtained by the appearance of an intense ion at \( m/e \) 121 in the deuterated species. A moderately intense ion at \( m/e \) 92 is probably due to the amino phenyl moiety and involves hydrogen transfer from the expelled formyl group (VII). An ion at \( m/e \) 93 in the deuterated species confirms this postulation.

**Figure 4:** Prominent ions in EI mass spectrum of N-formylbenzocaine.
N-Formylprocaine does not yield a detectable molecular ion under EI conditions. However, under CI conditions, an intense quasimolecular ion at \( m/e = 265 \) is present (VIII). Under EI, a weak ion is observed at \( m/e = 249 \) which shifts 1 amu upon deuteration (IX). This ion is due to the elimination of a methyl group from the diethylamino function in the parent molecule. An ion of low intensity occurs at \( m/e = 192 \) and is due to elimination of the diethylamino function from the molecular ion (X). Upon deuteriation, an expected shift of 1 amu is observed. An ion of moderate intensity is found at \( m/e = 99 \) and does not shift upon deuteriation. It is due to fission of C(1)-O bond with loss of hydrogen from C1 (XI). The most intense ion in the spectrum occurs at \( m/e = 86 \) and is due to fission of the C(1)-C(2) bond with charge retention on the diethylamino moiety (XII). As expected, no shift was observed upon deuteriation. Ions at \( m/e = 120 \) and 148 can be rationalized as for N-formylbenzocaine (Fig. 4).

**Figure 5:** Prominent ions in the EI and CI mass spectrum of N-formylprocaine.
5. Evaluation of N-Formylbenzocaine and N-Formylprocaine as Signature Compounds

In order to establish that the N-formyl derivatives of benzocaine and procaine were useful as signature compounds, it was necessary to demonstrate their presence in discolored authentic samples and their absence in samples that exhibited no discoloration. A number of authentic samples were analyzed for the presence of N-formylbenzocaine and N-formylprocaine. These authentic samples had been subjected to ambient and accelerated studies and exhibited either no discoloration or marked browning. Table V illustrates these results. It is evident from Table V that the N-formyl derivatives are present in those samples that discolored significantly, yet could not be detected in those samples that did not discolor. These findings supported their value as signature compounds.

* * * * *

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Study</th>
<th>Significant Discoloration</th>
<th>Presence of N-Formylbenzocaine</th>
<th>Presence of N-Formylprocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>49</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>54</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>28</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>54</td>
<td>Ambient</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>59</td>
<td>Ambient</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>47</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>64</td>
<td>Accelerated</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>65</td>
<td>Ambient</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>52</td>
<td>Accelerated</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>50</td>
<td>Ambient</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>22</td>
<td>Ambient</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* * * * *

In order to further correlate the presence of N-formyl derivatives with the browning process, the following was done. Authentic samples composed of 10% 5-HMF, 10% acid catalyst (boric acid), and 80% of either procaine HCl or benzocaine were prepared and then placed in the dark at room temperature for one day. After this time period, the samples were analyzed for the appropriate signature compounds. The N-formyl derivatives of both benzocaine and procaine were detected in these samples. Additionally, the samples exhibited marked discoloration. These results indicate that 5-HMF plays a role in the acid-catalyzed formation of the N-formyl derivatives. This is not surprising, in that 5-HMF has been associated with sugar decomposition. Furthermore, the subsequent decomposition of 5-HMF to formic acid has been reported [7,9]. Formic acid then reacts readily with either benzocaine or procaine to form the N-formyl derivatives.

Some preliminary work has been done in the isolation of additional signature compounds in an illicit sample known to have undergone discoloration. The sample consisted, in part, of cocaine HCl, boric acid, benzocaine, and dextrose. The N-formylbenzocaine signature was detected in this sample. The additional signature compounds were colored substances and isolated as described below. The discolored sample was placed on a dilute HCl - Celite 545 AW column and eluted with water-saturated ethyl ether.
as described earlier for benzocaine-containing samples. After discarding the ether eluate, the column was eluted with water-saturated chloroform. The chloroform was evaporated to less than 1 cc and transferred to the top of a neutral alumina column. The column was eluted initially with diethyl ether followed by ether-chloroform mixtures and then finally chloroform. A series of purple, blue, and pink-colored compounds eluted through the column (note: Rodd’s Chemistry of Carbon Compounds states that furfural reacts with primary amines, resulting in a ring opening and condensation with the amine to form a red-colored compound). Analysis of the combined column eluates on silica gel TLC plates revealed the presence of a number of colored substances (blue, purple, pink, and yellow). Though these colored compounds appear promising as signatures, further work must be done for their full characterization.

Summary

The study described in this paper can be summarized as follows:

A. The illicit and authentic samples that underwent discoloration, or browning, contained a primary aromatic amine, namely procaine or benzocaine, and a sugar, either lactose or dextrose; the samples also contained an acid catalyst, such as cocaine HCl, boric acid, benzoic acid, etc.

B. The rate of discoloration was pH dependant, i.e., the rate of sample browning increased as the pH of the sample decreased.

C. The rate of sample discoloration increased as the temperature of the sample increased.

D. N-Formyl derivatives of benzocaine and procaine were established as bona fide signature compounds in discolored samples. These derivatives are believed to be formed following the decomposition of 5-HMF to formic acid and the subsequent formylation of the aromatic amine function in benzocaine and procaine.

E. The presence of colored compounds (yellow, blue, purple, and pink) in the discolored samples appear promising as additional signature compounds.

Acknowledgments

I would like to thank Mrs. Susan Carr for preparing the authentic drug mixtures. I would also like to extend my appreciation to Mrs. Jean Nolan for typing the final manuscript.

References


* * * * *

Microgram Journal, Volume 6, Numbers 3-4 (July - December, 2008) 145