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A Rapid Technique for the Confirmation of Iodine and Red Phosphorus Using Direct Analysis in Real Time and Accurate Mass Spectrometry

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ABSTRACT: Iodine and red phosphorus are chemicals commonly seen in clandestine methamphetamine laboratories. Current analytical methods used for the confirmation of these chemicals include FTIR and GC/MS, usually after a derivatization or reaction with other compounds. X-ray diffraction and scanning electron microscope-energy dispersive x-ray analysis are also used to confirm these chemicals, but all of these techniques tend to be time-consuming or produce poisonous products. A novel technique, using the JEOL-IonSense AccuTOF-DART™ system, has been developed which yields accurate mass spectra usually in less than ten minutes of analysis time, with no sample preparation.

KEYWORDS: iodine, red phosphorus, Direct Analysis in Real Time, mass spectrometry, clandestine laboratory, methamphetamine, forensic chemistry

The prevalence of clandestine methamphetamine laboratories poses analytical challenges for forensic laboratories to identify the chemicals used in the clandestine process. Two of these chemicals, iodine and red phosphorus, are encountered in the syntheses involving the reduction of ephedrine or pseudoephedrine to methamphetamine via hydriodic acid [1]. If these chemicals are found at a clandestine laboratory site, their chemical identification becomes crucial in demonstrating their role in the manufacture of methamphetamine for prosecution in court.

Several presumptive screening tests and qualitative methods are currently employed to identify iodine and red phosphorus [2]. These methods typically involve conversion of the iodine or red phosphorus to other compounds, such as hydroiodic acid or white phosphorus, the handling of which may be hazardous due to the nature of these new derivatives. The derivatives are then identified via FTIR or GC/MS. X-ray diffraction and scanning electron microscope-energy dispersive spectroscopic analysis may also be used to identify iodine and red phosphorus [3-5].

The Direct Analysis in Real Time (DART™) (Ion Sense, Saugus, MA) source is beginning to see widespread use in forensic work [6-10]. A detailed description of the theory and operation of the DART source is given by Cody, et al. [11,12]. Other applications of DART span the entire field of chemistry [13-52]. In this work, the DART ion source was coupled with an accurate mass time of flight mass spectrometer (AccuTOF™, JEOL, Inc., Peabody, MA) to determine the elemental composition of solid samples held in a heated gas stream. No sample preparation was needed for the analysis of iodine and red phosphorus, and the analysis of each took less than ten minutes to complete.

Experimental

Iodine and red phosphorus were obtained from Mallinckrodt, (St. Louis, MO).

Experiments were carried out using the DART ion source coupled to a JEOL AccuTOF mass spectrometer (JMS-100LC) operated in positive-ion mode. This system was controlled by “Mass Center” software (JEOL, Inc.). The AccuTOF was tuned by infusion of reserpine (Sigma-Aldrich, Inc.) through an electrospray ion source to meet the manufacturer’s recommendations for resolution (~6000). These tune settings were then utilized for all AccuTOF-DART analyses. Daily calibration was checked by sampling a methanol (Fisher Scientific, Fair Lawn, NJ) solution of methyl stearate (Eastman Kodak, Rochester, NY) (2 mg/mL). In order to pass daily calibration, the measured mass of the [M+H]+ of methyl stearate was required to be within ± 3.0 mDa of the calculated mass for this ion (299.2950 Da).

For the iodine analysis, the measurements were taken with the ion guide peak voltage at 600 V, reflectron voltage at 910 V, orifice 1 voltage at 20 V, orifice 2 voltage at 5 V, ring lens voltage at 6 V, and an orifice 1 temperature of 80°C. The mass range was 66-600 Da. The DART ion source was used with the helium gas flow rate at 2.5 L/min, gas heater temperature at 275°C, discharge electrode needle at 4000 V, and electrode 2 at 250 V. Internal mass calibration was achieved using a dilute solution of polyethylene glycol (PEG) 600 (Chem. Service, West Chester, PA) in methanol sampled within each data file. After sampling the PEG solution, a crystal of solid iodine was held in the DART gas stream using tweezers until it sublimed. No other sample preparation was performed.

For the red phosphorus analysis, the measurements were taken with the ion guide peak voltage at 100 V, reflectron voltage at 910 V, orifice 1 voltage at 200 V, orifice 2 voltage at 10 V, ring lens voltage at 15 V, and an orifice 1 temperature of 80°C. The mass range was 38-100 Da. The DART ion source was used with the helium gas flow rate at 2.5 L/min, gas heater temperature of 350°C, discharge electrode needle at 4000 V, and electrode 2 at 250 V. Internal mass calibration was achieved using the protonated molecule of acetone (EM Science, Gibbstown, NJ) at 59.0497 Da as a drift compensation lock mass. A clean glass melting point tube (Kimble Glass, Vineland, NJ) was dipped into the liquid.
acetone and held in the DART gas stream to produce an accurate mass spectrum. Another melting point tube was then dipped into the red phosphorus and a very small amount of red phosphorus clung to the glass tube. This sample was then held in the DART gas stream. No other sample preparation was performed.

Calculated masses were determined using empirical formulas and IsoCalc version 4.1, part of Mass Spec Tools, published by ChemSW, Inc. (Fairfield, CA).

**Results and Discussion**

Figure 1 shows the AccuTOF-DART mass spectrum of solid iodine. The major ion at 253.8088 Da is from the diatomic molecule of iodine \([I_2]^+\), which has a calculated mass of 253.8090 Da. Other ions seen in the spectrum include \([I_3]^+\) at 380.7134 Da (calculated mass 380.7134 Da), \([I_4]^+\) at 507.6179 Da (calculated mass 507.6179 Da), \([I+\text{NH}_3]^+\) at 143.9290 Da (calculated mass 143.9290 Da), \([I + 2\text{H}_2\text{O}]^+\) at 162.9256 Da (calculated mass 162.9256 Da) and \([I_2 + \text{H}_2\text{O}]^+\) at 271.8265 Da (calculated mass 271.8265 Da). These are all highly characteristic ions resulting from the sublimation of iodine in the heated gas stream. Addition product ions with water and ammonia were from the atmosphere around the instrument.

Figure 2 shows the AccuTOF-DART mass spectrum of red phosphorus. The measured ions at 46.9673 Da and 62.9642 Da represent \([P+\text{O}]^+\) and \([P+\text{O}_2]^+\), respectively. The calculated masses for these ions are 46.9673 Da and 62.9636 Da. It is important to note that there are no other combinations of atoms that will give peaks at these masses.

The AccuTOF-DART analysis of iodine and red phosphorus is an extremely rapid technique that can easily be included in the forensic identification scheme for these common chemicals, with no sample preparation required.

**Acknowledgement**

The author would like to thank Dr. Robert “Chip” Cody of JEOL for his help and guidance with the analysis of these, and many other, compounds on the AccuTOF-DART.

**References**


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N-Acetylbenzocaine: Formation via Transacetylation of Benzocaine and Acetylsalicylic Acid in a Cocaine Exhibit

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ABSTRACT: N-Acetylbenzocaine was recently identified in an illicit cocaine HCl exhibit which also contained salicylic acid and traces of acetylsalicylic acid, and benzocaine. This paper discusses the analysis and characterization of N-acetylbenzocaine, as well as its transacetylation synthesis pathway. Supporting analytical data from gas chromatography/mass spectrometry, gas chromatography flame ionization detection, Fourier-transform infrared spectroscopy, and Fourier-transform nuclear magnetic resonance spectroscopy are presented.

KEYWORDS: N-acetylbenzocaine, transacetylation, synthesis, characterization, forensic chemistry

The DEA Western Laboratory recently received a white crystalline substance as a suspected cocaine exhibit. The exhibit was determined to contain 22.5% cocaine HCl, salicylic acid, and traces of acetylsalicylic acid (aspirin) and benzocaine. The exhibit also contained a significant amount of an unidentified compound. The unknown compound produced a mass spectrum (Figure 1a) having similar ions to benzocaine (Figure 1b), with an apparent molecular ion at m/z 207. The presence of an ion at m/z 43 suggested that the compound may be N-acetylbenzocaine. A literature search revealed that N-acetylbenzocaine has not been reported previously in an illicit drug exhibit. However, N-acetylbenzocaine has been identified previously as a metabolite of benzocaine in fish and guinea pigs [1-5]. The unknown compound was determined to be N-acetylbenzocaine (Figure 2) after it was synthesized and fully characterized via gas chromatography/mass spectrometry (GC/MS), infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.

Experimental
Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). N-Methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of reagent grade quality and products of Aldrich Chemical (Milwaukee, WI).

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 (GC). The GC/MSD was operated under conditions similar to those reported previously [6].

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

Nuclear Magnetic Resonance (NMR) Spectroscopy
Experiments were performed at 25°C with a Varian 600 MHz VNMRS nuclear magnetic resonance (NMR) spectrometer using a 5 mm broadband Nalorac Z-Spec probe (Varian Inc., Palo Alto, CA). Standard vendor supplied experiments were used to obtain proton and carbon (proton decoupled) spectra, and gradient versions of the heteronuclear multiple bond correlation (gHMBC) and heteronuclear single quantum coherence (gHSQC) spectra.

Gas Chromatography/Flame Ionization Detection (GC/FID)
N-Acetylbenzocaine determination: GC/FID analyses were performed with an Agilent (Palo Alto, CA) Model 6890N gas chromatograph. The sample preparation and GC/FID were operated under the same conditions as those reported previously [7]. Isopropylcoca was utilized as the internal standard and the unknown exhibit was bracketed with concentrations of 0.15 and 0.58 mg/mL of N-acetylbenzocaine (correlation coefficient, $R^2 = 0.9996$).
Synthesis

**N-acetylbenzocaine**: Benzocaine (243 mg, 1.47 mmol) was heated with 2.0 mL of acetic anhydride for 30 min. at 75°C in a sealed tube. The reaction was cooled and quenched with 10 mL of water, solid Na₂CO₃ was added until pH = 8, and the reaction was extracted with chloroform (2 x 10 mL). The extracts were combined, dried over anhydrous sodium sulfate, and evaporated in vacuo to a white powder (251 mg, 99% purity, 82% yield).

**Transacetylation of benzocaine and acetylsalicylic acid**: Benzocaine (13 mg, 0.079 mmol) and acetylsalicylic acid (26 mg, 0.14 mmol) were heated (neat) overnight at 70°C. The resulting product was dissolved in chloroform, examined via GC/MS, and found to contain a significant amount of N-acetylbenzocaine (yield not calculated).

Results and Discussion

Examination of the reconstructed total ion chromatogram for the illicit exhibit (Figure 3a) determined that cocaine (Peak #7), and salicylic acid (Peak #1) were present, in addition to lesser amounts of acetylsalicylic acid (Peak #3) and benzocaine (Peak #4). The unknown compound (Peak #6) represented over 50% of the total ion current. Its mass spectrum (Figure 1a) produced an apparent molecular ion at m/z 207. The spectrum was markedly similar to benzocaine (Figure 1b), exhibiting several ions in common, thus suggesting a benzocaine derivative. The presence of an ion at m/z 43 and a mass difference of +42 Daltons (Da) from benzocaine suggested that the compound was N-acetylbenzocaine. The unknown compound did form a TMS derivative (Figure 1c), indicating one labile proton within the molecule and consistent with a molecular weight of 207 for the underivatized moiety. The direct infrared spectrum of the exhibit (Figure 4a) indicated a significant amount of an amide present due to an apparent amide carbonyl stretch at ca. 1680 cm⁻¹ and an N-H stretch at ca. 3330 cm⁻¹.

A reference standard of N-acetylbenzocaine was synthesized and its mass spectrum and retention time were compared to the unknown compound, both derivatized and underivatized. The unknown was identical in all respects and was identified as N-acetylbenzocaine. The NMR spectrum (Figure 5), chemical shifts (Table 1), and IR (Figure 4b) of the synthesized standard are also presented. Finally, GC/FID analysis determined N-acetylbenzocaine to be 35.2% of the illicit exhibit.

N-acetylbenzocaine is not a commercially available product and the possibility that it was intentionally added as an adulterant is highly unlikely. Since salicylic acid and traces of benzocaine and acetylsalicylic acid were also present, we postulated that N-acetylbenzocaine may have arisen from transacetylation of benzocaine and acetylsalicylic. Acetylsalicylic acid (aspirin) is known to transacetylate human proteins in vivo [8] as well as transacetylate acetaminophen to its acetyl derivative [9]. The
Figure 3 - Partial reconstructed total ion chromatogram of a 22.5% cocaine exhibit containing 35.2% N-acetylbenezocaine (a) underivatized and (b) derivatized with MSTFA. Peak identification: 1 = salicylic acid, 2 = salicylic acid-di-TMS derivative + trace acetylsalicylic acid, 3 = acetylsalicylic acid, 4 = benzocaine, 5 = N-acetylbenzocaine-TMS derivative, 6 = N-acetylbenzocaine, and 7 = cocaine.

Figure 4 - Infrared spectrum (FTIR-ATR) of (a) illicit exhibit direct and (b) N-acetylbenezocaine standard.

Figure 5 - $^1$H-NMR spectrum of N-acetylbenezocaine.

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Table 1 - $^1$H-NMR and $^{13}$C-NMR chemical shifts (in ppm) and splitting patterns of N-acetylbenzocaine. Samples run in CDCl$_3$ with TMS as the reference compound for 0 ppm.

<table>
<thead>
<tr>
<th>Number</th>
<th>Benzene Ring</th>
<th>Proton</th>
<th>Carbon</th>
</tr>
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<tr>
<td>1</td>
<td>C</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CH</td>
<td>8.00 d (8.5 Hz)</td>
<td>130.8</td>
</tr>
<tr>
<td>3</td>
<td>CH</td>
<td>7.60 d (8.5 Hz)</td>
<td>118.7</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>---</td>
<td>142.0</td>
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<tr>
<td>5</td>
<td>CH</td>
<td>7.60 d (8.5 Hz)</td>
<td>118.7</td>
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<tr>
<td>6</td>
<td>CH</td>
<td>8.00 d (8.5 Hz)</td>
<td>130.8</td>
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<td>off C-1</td>
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<td></td>
<td></td>
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<tr>
<td>1</td>
<td>O-CH$_2$CH$_3$</td>
<td>4.36 q (7.2 Hz)</td>
<td>60.9</td>
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<td>1.38 t (7.2 Hz)</td>
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<td></td>
</tr>
<tr>
<td>1</td>
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<td>---</td>
<td>168.5</td>
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<td>2</td>
<td>NH-C(C=O)-CH$_3$</td>
<td>2.21 s</td>
<td>24.8</td>
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</table>

Figure 6 - Partial reconstructed total ion chromatogram of a (a) mixture of benzocaine and acetylsalicylic acid which had been heated overnight neat, and (b) mixture of cocaine HCl, benzocaine, and acetylsalicylic acid in chloroform injected immediately. Peak identification: 1 = salicylic acid, 2 = benzocaine, 3 = N-acetylbenzocaine, and 4 = cocaine.

Conclusions
Analytical data is presented to assist other forensic laboratories that encounter N-acetylbenzocaine in case exhibits. N-Acetylbenzocaine can be readily formed from benzocaine and acetylsalicylic acid with heat. Care must be taken in identifying N-acetylbenzocaine, since it can also be formed as an injection port artifact.

Acknowledgements
The authors wish to thank Senior Research Chemist Patrick A. Hays, DEA Special Testing and Research Laboratory, for his assistance in acquiring the NMR data.

References


5. Stehly GR, Meinertz JR, Gingerich WH. Effects of temperature on the elimination of benzocaine and acetylated benzocaine residues from the edible fillet of rainbow trout (Oncorhynchus mykiss). Food Addit and Contam 2000;17(5):387-92.


The Characterization of 3,4-Methylenedioxypyrovalerone (MDPV)

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ABSTRACT: The analysis and characterization of 3,4-Methylenedioxy-phenyl)-2-pyrrolidin-1-yl-pentan-1-one GC/MS Sample Not for Human Consumption.” There was no lot number or manufacturer name on the bag. The seizure was in response to a call for a vehicle off the road and stuck in the mud. The responding officer found the driver to be incoherent and confused; the driver subsequently failed a field sobriety test. The driver was requested to take a breathalyzer, which resulted in 0.00 Blood Alcohol Content. The driver declined a request for a blood test. It is not known whether the driver’s condition was a direct result of MDPV intoxication. A search of the driver provided the above mentioned bag along with pharmaceutical tablets believed to be from India. The driver stated that he was a self-employed chemist and that was the reason that he was allowed to have the bag of white powder. The tablets included 98 promethazine HCl, 1 triazolam, 2 risperidine, 4 methocarbamol, 10 baclofen, 4 bromazepam, and 4 quetiapine fumarate tablets. Also recovered were a pill crusher and a prescription bottle containing residue.

MDPV and MDPK are both abbreviations for 3,4-Methylenedioxy-phenyl)-2-pyrrolidin-1-yl-pentan-1-one (Figure 1). MDPV was first synthesized as part of a class of stimulants in 1969. MDPV is the methylenedioxy analogue of pyrovalerone, a Schedule V stimulant first synthesized in 1964. Pyrovalerone, available under the trade names Centroton and Thymergix, is used as an appetite suppressant or for the treatment of chronic fatigue.

MDPV is currently unscheduled in the United States. MDPV is found as a white or light tan powder. Users report the development of an odor when left exposed to the air. There are currently no known studies on the effects of MDPV on humans or on proper dosing. MDPV is commonly described as boosting a user’s libido, however it is also associated with extreme anxiety at higher dosages. There are no known deaths due to the use of MDPV.

Experimental

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectrum (Figure 2) was acquired using a Thermo-Nicolet Magna 560 spectrophotometer with a SensIR Durascope attenuated total reflectance (ATR) accessory. The spectrum was collected using 32 scans between 4000 cm⁻¹ and 400 cm⁻¹.

Gas Chromatography/Mass Spectrometry (GC/MS)

The mass spectrum (Figures 3a-3b) was 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. The GC was fitted with a 30 m x 0.25 mm I.D. fused silica capillary column coated with 0.50 μm 35% phenyl, 65% dimethyl arylene siloxane (DB-35MS), and was operated in splitless mode. The injection port was maintained at 250°C. The oven temperature program was as follows: Initial temperature 90°C (1 min), ramped to 300°C at 8°C/min (final hold 10 min). Helium was used as a purge gas at a rate of 60 mL/sec. Methanol was used as the solvent.

Nuclear Magnetic Resonance (NMR) Spectroscopy

1H- and 13C-NMR spectra (see Table 1, Figures 4 and 5, respectively) were acquired at 25°C on a Varian Mercury Plus 400 MHz instrument using a Nalorac 5 mm indirect detect pulse field gradient (PFG) probe. (1H parameters: Number of scans (nt) = 8, pulse width (pw) = 45°, relaxation delay
(d1) = 5 s, acquisition time (at) = 2.5 s; $^{13}$C parameters: nt = 4098, pw = $45^\circ$, d1 = 1 s, at = 1.3 s, complete proton decoupled). Spectra were processed using ACD/Labs SpecManager software (Advanced Chemistry Development Inc., Toronto, Canada). MDPV was prepared with $D_2O$ containing 5 mg/mL maleic acid (as internal standard) containing 0.05 wt % 3-trimethylsilyl-propionic-2,2,3,3-$d_4$ acid, sodium salt (TSP; Aldrich Chemical Co., Milwaukee, WI) at 16.87 mg/mL.

Chemical shifts ($\delta$) are reported in parts per million (ppm) using TSP (0.0 ppm) as the reference standard (400 MHz, $D_2O$).

**Ultraviolet (UV) Spectrophotometry**

The UV spectrum (Figure 7) was acquired using a Hewlett-Packard 845x spectrophotometer with a 1 cm cell path length. The range scanned was 220-330 nm. The sample was dissolved in methanol.

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Figure 2 - FTIR-ATR spectrum of MDPV hydrochloride.

Figure 3a - E.I. mass spectrum of MDPV.

Figure 3b - Expanded E.I. mass spectrum of MDPV.
Results and Discussion

The MDPV mass spectrum demonstrates similarity to other amines in that it gives a low-detail mass spectral fragmentation pattern. The base ion, m/z 126, however, is somewhat uncommon in drug analysis, which may prove to be of value in identifying MDPV. The resultant FTIR spectrum is very detailed with a number of sharp bands in the fingerprint region that should enable relatively facile identification. Specifically, MDPV has proven to be an analyte that is easily distinguishable from other structurally related compounds.

References
1. 1-{(3,4-Methylenedioxy)phenyl]-2-pyrrolidino-1-alkanones as stimulants. (Boehringer Ingelheim Study) 1969.
### Table 1 - Assignments, Multiplicities, and Coupling Constants.

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<th>Position</th>
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<th>$^1^H$ (ppm)</th>
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<td>doublet</td>
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<td>25.63</td>
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*apparent quartet

Figure 6 - Position of protons for MDPV (See Table 1).


Figure 7 - Ultraviolet-visible spectrum of MDPV in methanol.
The Mass Spectrum of Cocaine:
Deuterium Labeling and MS/MS Studies

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ABSTRACT: Seven derivatives of cocaine (l-2-exo-carbomethoxy-3-exo-benzoyloxytropane; Ia) were synthesized in which the hydrogen (H) atoms at various positions were replaced by deuterium (tH = D) – specifically, at the N-methyl, O-methyl, phenyl, 2-, 3-, and 4- positions, as well as at the combined 1-, 5-, 6-, and 7- positions (Ib - Ih, respectively). The mass spectra of these compounds were recorded. Elemental compositions for selected ions in the spectrum of Ia were determined using high-resolution mass spectrometry, and precursor and product ion spectra for many of these ions were studied using MS/MS. Mechanisms for many previously proposed fragmentation pathways were either confirmed or clarified, and new insights were gained into the fragmentation of Ia. Reasons for variations in relative intensities of the m/z 94 and 152 peaks between the spectra of the cocaine diastereomers are proposed.

KEYWORDS: forensic science, cocaine, mass spectrometry, fragmentation mechanisms, deuterium-labeled derivatives, high-resolution MS, MS/MS, product and precursor ions, pseudococaine.

The electron ionization (EI) mass spectra of tropane derivatives were first studied by Budzikiewicz, Djerassi, and coworkers, using the spectra of deuterated derivatives and the examination of metastable ion spectra [1,2]. Cocaine (l-2-exo-carbomethoxy-3-exo-benzoyloxytropane; Ia), a molecule of considerable forensic interest, was not included in the original studies. Subsequent workers elucidated similar basic fragmentation pathways for Ia (Figures 1-3) [3,4,5]. Many questions remain, however, regarding the nature of some fragmentations and the formation of less abundant ions. In particular, previous proposals concerning the relative intensities of the m/z 152 peak and the m/z 94/96 pair of peaks in the spectra of the cocaine diastereomers seem unsatisfactory. The present work attempts to expand upon this knowledge.

Seven labeled derivatives of Ia were prepared in which the hydrogen (H) atoms at specific positions were replaced by deuterium (tH = D; structures Ib-Ih). Mass spectra for these compounds were recorded and examined to determine the presence or absence of the labels for many of the ions represented in the cocaine spectrum. In addition, MS/MS data were collected in both the precursor (“parent”) and product (“daughter”) ion modes in order to ascertain relationships between the various ions.

Experimental Procedures
Solvents, Chemicals, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were reagent grade quality products of Sigma-Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4% (w/w). Cocaine (Ia), pseudococaine, ecgonine methyl ester, N-trideuteriomethylcocaine (Ic), and α-cocaine were from the authentic reference collection of the DEA Special Testing and Research Laboratory.
Synthesis

All syntheses were performed in flame-dried glassware and protected from moisture. Ie, If, Ig, Ih, and 2-d1-, 3-d1-, 4,4-d2-, 1,5,6,6,7,7-d6-pseudococaine were synthesized as previously described [6]. Unlabelled 2-carbomethoxy-3-tropinone was also synthesized as previously described [7]. Yields for the following syntheses were not optimized.

Cocaine (OCD3) (Ib): Anhydrous benzyloecgonine (110 mg, 0.380 mmol) was combined with CH2Cl2 (4 mL) and 1',1'-carbonyldiimidazole (63.5 mg, 0.391 mmol) in a 15 mL centrifuge tube. The reaction was allowed to stand overnight. Trideuteriomethanol (CD3OH; 100 μL) was added and the mixture allowed to stand for one day. The reaction mixture was evaporated to dryness under a stream of nitrogen at 75°C and treated with 7 mL of hot hexane. The hexane was decanted to a new tube and allowed to cool, precipitating the imidazole by-product. The hexane was filtered, evaporated in vacuo, recrystallized from hexane, and dried to provide a white powder (42 mg, 36% yield).

Cocaine (phenyl-d5) (Id): Ecgonine methyl ester hydrochloride (500 mg, 2.12 mmol) was suspended in 7 mL of dry pyridine in a 100 mL round bottom flask, to which pentadeteriobenzoyl chloride (437 mg, 3.00 mmol) was added. After stirring for 5 days, the reaction was diluted with 50 mL of dry acetone to precipitate the product. The crude product was captured by suction filtration, dissolved into water (4 mL), rendered alkaline (pH = 9) with aqueous Na2CO3, extracted with CHCl3 (1 x 8 mL), dried over anhydrous Na2SO4, filtered, and evaporated in vacuo to a crystalline mass. Approximately 1.5 g of the crude product was dissolved in a minimal amount of Et2O and loaded onto a glass chromatographic column (1 x 22 cm) containing 15 g of basic alumina (150 mesh). The column was eluted sequentially with 20 mL each of the following solvents: 1) Et2O, 2) Et2O/CHCl3 (1:1), and 3) CHCl3. Ten mL fractions were collected and examined by GC/MS, both underivatized and following derivatization with MSTFA. The first three fractions were combined and evaporated to dryness to give a clear oil (655 mg, 15% yield).

2,3-Dehydrococaine: 2-Carbomethoxy-3-tropinone (153 mg, 0.776 mmol) was combined with dry pyridine (2 mL) and benzoyl chloride (300 mg, 2.13 mmol) in a 15 mL centrifuge tube and left to stand for 1 hour. Et2O (13 mL) was added to precipitate the crude product, which was washed with additional Et2O (10 mL). The semi-crystalline material was dissolved in 0.36N H2SO4 (1 mL), washed with Et2O (2 x 14 mL), adjusted to pH 9 with Na2CO3, extracted with CHCl3 (1 x 10 mL), dried over anhydrous Na2SO4, filtered, and evaporated in vacuo to give an off-white powder (118 mg, 50% yield).

Gas Chromatography/Low Resolution-Mass Spectrometry (GC/LR-MS)

GC/LR-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 DB-1 (0.25 μm) (J & W Scientific, Rancho Cordova, CA). The oven...
Figure 2 - Previously proposed fragmentations of Ia following initial alpha-cleavage next to the N atom.
Figure 3 - Previously proposed fragmentations of Ia following initial ionization at the ester O atoms.
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 a temperature of 280°C. The MSD was operated in the electron ionization (EI) mode with an ionization energy (I.E.) of 70 eV and a scan range of 34-700 m/z units at 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

**High Resolution-Mass Spectrometry (HR-MS)**

HR-MS data were obtained on two instruments. The first was a Finnigan MAT Model 8230 system (Bremen, Germany) operating at an I.E. of 70 eV. The source temperature was 175°C. The second was an AEI Scientific Apparatus Model MS-902 system (Manchester, UK) operating at an I.E. of 70 eV. The source temperature was programmed as follows: Initial temperature, 120-150°C. Sample introduction was accomplished with a solids probe on both systems, and data was acquired at a resolution of ca. 10,000 (5% valley).

**Precursor/Product Ion Mass Spectrometry (MS/MS)**

Precursor and product ion data were obtained on a Thermo Scientific TSQ-7000 triple quadrupole MS system (Bremen, Germany). Ionization energies were varied from 5-70 eV. Sample introduction was accomplished with solids probe. Unfortunately, these data were obtained several years ago without details of the conditions used being recorded. These instruments are no longer in the authors’ laboratories.

**Results and Discussion**

Electron ionization mass spectra of Ia and of the seven D-labeled derivatives (Ib-Ih) are shown in Figures 4-6. Careful examination and comparison of these spectra reveal intensity patterns that are reasonably consistent from one spectrum to the next. The positions of individual peaks within the spectra, however, vary depending upon the presence or absence of the D label(s) in the ion(s) represented. When some or all of the D label is retained in a particular ion, the position of the peak corresponding to this ion moves to a higher m/z value in the spectrum of the D-labeled compound (the "shift technique") [8].

A compilation of m/z values for selected peaks in the spectrum of Ia and for the corresponding peaks in the spectrum of each of the derivatives is shown in Table I. In many cases, each peak in the spectrum of Ia can be correlated to a single peak in the spectrum of each derivative. When this occurs, a single fragmentation mechanism may account for formation of that ion.

In some cases, especially for lower m/z peaks, a single peak in the spectrum of Ia is represented by two or more peaks in a derivative spectrum. This indicates that two or more mechanisms may be operative in forming that ion, or that a single peak in the low-resolution spectrum may represent two or more ions. Given the multiplicity of potential precursors for many low m/z ions, this is not surprising. In a few instances, the peak correspondences between spectra are either too complex to be determined with confidence or suffer from interferences from nearby peaks. These instances are noted in Table I.

Table II summarizes the elemental compositions of ions formed during the fragmentation of Ia as determined by HR-MS. The elemental compositions confirm those determined by Shapiro, et al. [9], although the list presented here is more extensive. Table II also lists precursor and product ions for these ions as identified by MS/MS. These data represent a substantial condensation of the raw data. In order to best identify precursor-product ion relationships, emphasis was placed on scans at the lowest collision energies (5-10 eV) because product ions formed under these conditions are the least likely to undergo additional fragmentation. A schematic representation of these relationships is shown in Figure 7. Although it is tempting to use the information in Figure 7 to define EIMS fragmentation pathways for Ia, it must be remembered that both precursor and product ions formed by collisional activation in the MS/MS experiments may have different structures than those formed during EIMS [10].

The molecular ion (M+) of Ia (C17H23NO4+, m/z 303) fragments at collision energies of 5-20 eV to produce at least nine stable product ions that apparently form without the detectable intermediacy of other ions. These include the listed ions having m/z 272, 222, 198, 182, 181, 122, 97, 83, and 82 (Table III). Although product ion scans for m/z 303 did not produce peaks at m/z 288, 275, 274, 259, 244, and 155 under these conditions, other data indicate that in the EI spectrum these ions may also
Figure 5 - Electron ionization mass spectra of a) 2-deuteriococaine (Ie); b) 3-deuteriococaine (If); and 4,4-dideuteriococaine (Ig).

Figure 6 - Electron ionization mass spectra of a) 1,5,6,6,7,7-hexadeuteriococaine (Ih); and b) pentadeuriobenzoylmethylecgonine (Id).

form directly from m/z 303 (see below).

The most abundant ions formed from the M⁺ at 5-10 eV in the MS/MS experiments are those with m/z 198, 181, 83, and 82. At the collision energies listed in Table III, the m/z 182 ion accounts for only a peak of 2-4% relative intensity, which seems at odds with the fact that the m/z 182 peak is usually one of the two most intense peaks in the 70 eV EI spectrum. However, it must be remembered that peaks observed in EIMS result from the interplay of a number of factors – most importantly, the percentage of precursor ion(s) that produce the represented ion, the internal energies of both precursor and product ions, and the stability of the product ion toward further fragmentation [11]. Thus, even though relatively few m/z 303 ions appear to form m/z 182 ions directly at low collision energies, this ion is listed as a precursor ion for only one other relatively abundant ion represented in the EI spectrum (m/z 82). The m/z 182 ions formed even under the more energetic EIMS conditions seem relatively resistant to additional fragmentation, leading to a larger percentage of m/z 182 ions surviving the journey from the ion source through the analyzer to the detector.

The combined labeling and HR-MS data allow postulation of meaningful pathways for the fragmentations of cocaine ions. In making these proposals, a few guidelines were followed: a) formation of the M⁺ is assumed to take place by removing a non-bonding electron from the nitrogen (N) or oxygen (O) atoms, rather than those within the carbon framework (see Figures 1-3, for example); b) structures containing highly strained bonds were deemed less probable as structures for ions if lower-energy alternative structures could be proposed; and c) ions known to have low ionization energies (I.E.) of formation, such as carbonyl and iminium ions [12], were preferred whenever possible. Because of the low I.E. for the n-electrons on the N atom and the stability of the resulting ions, the most significant fragmentations following initial ionization are the α-cleavages shown in Figure 1. Of these, m/z 303a should be the most stable because the radical site is stabilized by conjugation with the double bond in the carbonyl group; whereas, no conjugation is possible in the other structures. Even though their lifetimes are undoubtedly very short, the ions formed in these first fragmentations serve as precursors to many of the product ions discussed below.

m/z 288. The weak m/z 288 peak reflects a loss of 15 u - a methyl radical - from the M⁺. The only D label lost in this fragmentation is that on the N-CH₃ group (Table I). The mechanism proposed in Figure 8 for its formation generates conjugated double bonds and places the final charge on the N atom. At low collision energies, this ion is one of several precursors to the m/z 166 ion (Table II). The additional loss of the phenyl ring and a H atom at C4 (shown in Figure 8) is consistent with the D labeling pattern for one of the m/z 166 ions (see below).

m/z 275. No fragment ion peak is usually observed in the EI spectrum at m/z 275. However, an ion having this m/z value is implicated as a potential intermediate during the formation of the m/z 154 ion (see below). Its intermediacy in the formation of some m/z 274 ions cannot be ruled out (see subsequent discussion).

m/z 274. It is easy to assume that the small peak at m/z 274 is
Table I - Peak Correspondence Table.

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<th>Cocaine peak $m/z$</th>
<th>O-CD$_3$ $m/z$</th>
<th>N-CD$_3$ $m/z$</th>
<th>Phenyl-$d_5$ $m/z$</th>
<th>2-$d_1$ $m/z$</th>
<th>3-$d_1$ $m/z$</th>
<th>4,4-$d_2$ $m/z$</th>
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Key: **Boldface** indicates partial or total loss of deuterium label at that position. 
-Numbers in parentheses indicate a peak of much smaller intensity. 
-Question mark (?) indicates that data cannot be interpreted with confidence.
Figure 7 - Relationships between various cocaine fragment ions as determined by MS/MS.

an isotope peak in the m/z 272 ion cluster. Alerted by the MS/MS data that an ion having m/z 274 is a precursor to the m/z 152 ion (Table II), careful analysis reveals that this peak is consistently about 0.3-0.6% too large to be due to isotope contributions alone. The spectra of the D-labeled derivatives shows partial loss of one D at C4 for this peak, plus the loss of four D atoms from positions 1, 5, 6, and 7. Loss of the D label at C2 could not be determined with certainty. Because an ion having m/z 274 reflects the loss of 29 u from the M⁺, and five of these mass units are due to H atoms, the remainder must consist of two C atoms. This loss is most easily envisioned as a H atom from either C2 or C4 plus C6 and C7 with their four attached H atoms (Figures 9 and 10). This may occur via H rearrangement followed by ethyl radical loss [1,13], or by loss of ethylene to produce m/z 275 and subsequent H radical loss from either C2 or C4. In 2-carbomethoxy-3-tropanone (II), 2,3-anhydrococaine (III), and other tropanes having some unsaturation at C2 and/or C3, peaks are observed at both M-28 and M-

m/z 259. No HR-MS or MS/MS data were recorded for this ion during this study. The loss from the M⁺ is 44 u, the mass of CO₂. All D labels are retained, indicating that no H atoms are lost during this fragmentation. It is impossible to tell from the available data which of the two carboxyl groups is lost. The mechanism in Figure 12 shows loss of the alkyl carboxyl group. Although a stepwise mechanism is shown, a nearly concerted elimination of CO₂ with methyl group migration is possible.

m/z 244. The elemental composition determined for this ion by HR-MS shows loss of C₂H₂O₂ from the M⁺. The only D label lost is that on the OCH₃ group, consistent with loss of a carbomethoxy radical.

Formation of m/z 244 is most easily depicted as resulting from m/z 303c or 303d (Figures 9 and 10). Its formation from m/z 303a cannot occur without H rearrangement, and formation from m/z 303b would likely result in either a cyclopropane ring or a diradical. “Backside” attack at C2 by the radical site at C7 in m/z 303c causes elimination of a carbomethoxy radical and generates a bicyclo[2.2.2] ion (Figure 9) [5]. Alternatively, a similar attack by a radical site at C6 produces a bicyclo[4.2.0] ion (Figure 10).

Both proposed m/z 244 ions have fragmentation options that can lead to product ions listed in Table II. Loss of ethylene by a cycloelimination reaction forms m/z 216 (identified as a product ion in the MS/MS data, even though the corresponding peak is not observed in the spectrum). The m/z 244 ion is a possible precursor to the m/z 105 ion, as well as to one or more of the m/z 122 ions [5]. Cyclic loss of benzoic acid or its corresponding radical ion produces m/z 122a₁ and 122a₂ or 122b, respectively; and migration of the charge to the benzoyl O atom generates the benzoyl ion (m/z 105).

m/z 222. This ion, which is usually represented by a peak of very low intensity in the EI spectrum, is formed directly from
Table II - Elemental Composition and MS/MS Table.

<table>
<thead>
<tr>
<th>Cocaine Ion m/z</th>
<th>Elemental Composition</th>
<th>Fragment Lost</th>
<th>Precursor Ion(s) At 5-15 eV (m/z)(^{a,b})</th>
<th>Important Product Ion(s) At 5-15 eV (m/z)(^{a,c})</th>
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<td>None</td>
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<tr>
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<td>Not Determined</td>
<td>(CH(_3))</td>
<td>(303)</td>
<td>166</td>
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<tr>
<td>274</td>
<td>Not Determined</td>
<td>(C(_2)H(_3))</td>
<td>(303)</td>
<td>(152)</td>
</tr>
<tr>
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<td>OCH(_3)</td>
<td>(303)</td>
<td>150, 122, 105, 82</td>
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<td>(100)</td>
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<td>C(_6)H(_5)CO</td>
<td>303</td>
<td>166, 82</td>
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<tr>
<td>182</td>
<td>C(<em>{10})H(</em>{16})NO(_2)</td>
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<td>303</td>
<td>150, 108, 82</td>
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<tr>
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<td>(303)</td>
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<td>C(_6)H(_5)O</td>
<td>(303)</td>
<td>140, 96, 82</td>
</tr>
<tr>
<td>154</td>
<td>C(<em>{8})H(</em>{12})NO(_2)</td>
<td>C(_6)H(_5)O</td>
<td>(275), (155)</td>
<td>(152), 122, 94</td>
</tr>
<tr>
<td>152</td>
<td>C(<em>{8})H(</em>{10})NO(_2)</td>
<td>C(_9)H(_1)O(_2)</td>
<td>274,181,154</td>
<td>122, 108, 93, 92, 59</td>
</tr>
<tr>
<td>150</td>
<td>C(<em>{9})H(</em>{12})NO</td>
<td>C(_9)H(_1)O(_3)</td>
<td>(272), (182)</td>
<td>122, 119, 93, 91, 82</td>
</tr>
<tr>
<td>140</td>
<td>C(<em>{8})H(</em>{10})NO(_2)</td>
<td>C(_9)H(_1)O(_2)</td>
<td>155</td>
<td>Not Determined</td>
</tr>
<tr>
<td>138</td>
<td>C(_7)H(_8)NO(_2)</td>
<td>C(_9)H(_1)O(_2)</td>
<td>(166)</td>
<td>Not Determined</td>
</tr>
<tr>
<td>122a</td>
<td>C(_9)H(_2)N</td>
<td>C(_9)H(_1)O(_3)</td>
<td>181, 150</td>
<td>107, 94, 91, 81</td>
</tr>
<tr>
<td>122b</td>
<td>C(_9)H(_2)O</td>
<td>C(_9)H(_1)O(_2)</td>
<td>303,272,244,222</td>
<td>105, 77</td>
</tr>
<tr>
<td>108</td>
<td>C(_9)H(_10)N</td>
<td>C(_9)H(_1)O(_4)</td>
<td>(182),(181),(152)</td>
<td>93, 42</td>
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<tr>
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<td>C(_9)H(_1)O(_3)</td>
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<td>77, 51</td>
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<td>C(_9)H(_1)O(_3)</td>
<td>303, 222</td>
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</tr>
<tr>
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<td>C(_9)H(_1)O(_4)</td>
<td>303,156</td>
<td>96, 82, 55</td>
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<tr>
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<td>C(_9)H(_1)N</td>
<td>C(_9)H(_1)O(_4)</td>
<td>303,155,97</td>
<td>94, 81, 79, 68, 42</td>
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<tr>
<td>94</td>
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<td>C(_9)H(_2)O(_4)</td>
<td>303,181,154,122,96</td>
<td>93, 78</td>
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<tr>
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<td>C(_9)H(_2)O(_4)</td>
<td>(303)</td>
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<td>Not Det.</td>
<td>(96),(94),(82)</td>
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<tr>
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<td>C(_9)H(_1)NO(_2)</td>
<td>(152)</td>
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<tr>
<td>55</td>
<td>C(_9)H(_2)N</td>
<td>C(_9)H(_1)O(_4)</td>
<td>(166),(97),(82)</td>
<td>Not Determined</td>
</tr>
<tr>
<td>51</td>
<td>(C(_4)H(_3))</td>
<td>-</td>
<td>(105), (77)</td>
<td>Not Determined</td>
</tr>
<tr>
<td>42</td>
<td>C(_9)H(_2)N</td>
<td>C(_9)H(_1)O(_4)</td>
<td>(122),(108),(96)</td>
<td>Not Determined</td>
</tr>
</tbody>
</table>

\(^{a}\text{Boldface}\) indicates the most prominent precursor or product ion(s) of the group.

\(^{b}\)Values in parentheses are proposals based on other information, most often data from product ion scans.

\(^{c}\)Values in parentheses are proposals based on other information, often data from precursor ion scans.
the M⁺ at low internal energies (Table III). The loss (81 u) is an unusual one, considering the functional groups present in the molecule. The spectra of the D-labeled derivatives show losses of the N-CD₃ label as well as four of the six D atoms on C₁, C₅, C₆, and C₇. These losses strongly suggest that the entire 5-membered ring, consisting of the N atom and C₁, C₅, C₆, and C₇, is lost as a unit, with two H atoms being transferred back to the remaining portion of the molecule. Because peaks representing ions having similar m/z values are very prominent in the spectrum (m/z 82 and 83), loss of this portion of the M⁺ as a neutral species is not unreasonable. Loss of the N atom, in addition to the even molecular mass, dictates that m/z 222 is an odd-electron ion; therefore the lost neutral species is either a molecule or a diradical.

Two fragment ions associated with m/z 222 by MS/MS are those at m/z 122 and 100. The m/z 122 ion must be C₄H₄CO₂H because it cannot contain the N atom. The mechanisms shown in Figure 13 account for these observations.

Table III. Tabulated Product Ion Spectrum of the Molecular Ion of Ia at Various Collision Energies.

<table>
<thead>
<tr>
<th>Product Ion (m/z)</th>
<th>Rel. Int. (-20 eV)</th>
<th>Rel. Int. (-13 eV)</th>
<th>Rel. Int. (-5 eV)</th>
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<tr>
<td>272</td>
<td>-</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
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<td>-</td>
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<td>4.0</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
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<td>4.0</td>
<td>10.0</td>
<td>50.0</td>
</tr>
<tr>
<td>180</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>166</td>
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<td>1.0</td>
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</tr>
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<td>5.0</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>107</td>
<td>0.5</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>1.0</td>
<td>2.0</td>
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<tr>
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<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>96</td>
<td>1.0</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>95</td>
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<td>2.0</td>
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</tr>
<tr>
<td>94</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
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<td>72.0</td>
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<td>60.0</td>
</tr>
<tr>
<td>81</td>
<td>9.0</td>
<td>13.0</td>
<td>30.0</td>
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Figure 8 - Proposed formation of m/z 288 and m/z 166 from m/z 303b.
Figure 9 - Proposed formation of $m/z$ 275a and $m/z$ 244a after initial cleavage of the C1-C7 bond ($m/z$ 303c). Subsequent fragmentations of $m/z$ 275a and $m/z$ 244a include formation of $m/z$ 274a, $m/z$ 216, $m/z$ 152, $m/z$ 122a, and $m/z$ 105.

$m/z$ 198. The combined HR-MS and MS/MS data show loss of C$_7$H$_5$O directly from the M$^+$ (Tables II and III). The only D labels lost are those on the aromatic ring, thus the neutral fragment must be a benzoyl radical (C$_6$H$_5$CO). In the spectra of $\alpha$- and $\beta$-cocaine (IVA and IVb, respectively; Figure 14a – the spectra of these two compounds are virtually identical), the peak associated with this ion is quite small, indicating that formation of this ion may require the presence of a H atom at C3, which is not present in either $\alpha$- or $\beta$-cocaine. The $m/z$ 198 ion is an important precursor to one of the $m/z$ 166 ions, which can occur by loss of a molecule of methanol (Figure 2) [4].

$m/z$ 182. The M$^+$ loses C$_7$H$_5$O$_2$ – benzoate radical – to form this ion (Tables II and III). None of the D labels except those on the phenyl ring are lost. A straightforward mechanism for its formation is shown in Figure 2 [15,16].

$m/z$ 181. Loss of benzoic acid (C$_6$H$_5$CO$_2$H) directly from the M$^+$ produces this ion (Tables II and III). The D labels on the aromatic ring are clearly lost in this fragmentation, and loss of a H atom from either C2 or C4 is supported. The $m/z$ 181 ion is implicated as an intermediate in forming ions having $m/z$ 166, 152, 122a (it cannot be C$_6$H$_5$CO$_2$H$^+$) and 108. Generation of $m/z$ 181 involves a McLafferty-type rearrangement after initial
Figure 10 - Proposed formation of \( m/z \) 275b and \( m/z \) 244b after initial cleavage of the C5-C6 bond (\( m/z \) 303d). Subsequent fragmentations of \( m/z \) 275b and \( m/z \) 244b include formation of \( m/z \) 274b, \( m/z \) 216, \( m/z \) 152, \( m/z \) 122a, and \( m/z \) 105.
Figure 11 - Losses of \( \text{C}_2\text{H}_3 \) from 2-carbomethoxy-3-tropanone (II) and 2,3-anhydrocaine (III) produce ions stabilized by aromatic conjugation.
Figure 12 - Proposed formation of the \( m/z \) 259 ion.

Figure 13 - Proposed formation of \( m/z \) 222 and its subsequent fragmentation to give the \( m/z \) 122 and \( m/z \) 100 ions.
The D labeling data is understandably complex. Although precursor ion spectra for this ion were not recorded, its most likely source is \( M^+ \) because it can be formed in a single step from \( m/z \) 303b (Figure 18) [1,2,16]. Both D labels are lost from C4, as are those from the aromatic ring and from C3, indicating that C3, C4, and the functional groups attached to C3 are lost as a single unit. This is consistent with the size of the lost neutral fragment (C\(_6\)H\(_4\)O\(_2\) = C\(_6\)H\(_4\)CO\(_2\) + CH\(_3\)=CH). The \( m/z \) 155 peak is absent from the spectra of both \( \alpha \)- and \( \beta \)-cocaine because in these compounds both the benzoate and carbomethoxy groups are located on C3, necessitating the loss of both functional groups in this fragmentation. Important product ions of \( m/z \) 155 in the MS/MS experiments include \( m/z \) 140, 96, and 82. Fragmentations that might produce \( m/z \) 140 and 82 are shown in Figure 18.

**\( m/z \) 154.** The \( m/z \) 154 peak can easily be overlooked in the group of peaks between \( m/z \) 150 and 155. The elemental composition of this ion indicates loss of C\(_6\)H\(_4\)O\(_2\) from the \( M^+ \). The spectra of the D-labeled derivatives show loss of the labels on the phenyl ring, and at least four D atoms are lost from C1, C5, C6, and C7. Loss of the benzoate group (C\(_6\)H\(_4\)O\(_2\)) along with the C6-C7 bridge (C\(_2\)H\(_4\)) is consistent with these data. The only precursor identified for \( m/z \) 154 by MS/MS was the \( m/z \) 155 ion; however, the structure for this ion must be different from the one indicated by the D labeling data because, as just discussed, \( m/z \) 155 no longer contains C3 and C4, rather than C6 and C7 (Figure 18). A more reasonable precursor for the ion represented in the EI spectrum is one having \( m/z \) 275, which is not represented in the spectrum but is a realistic intermediate to the \( m/z \) 274 ion (Figures 9 and 10).

From the MS/MS data, it appears that the \( m/z \) 154 ion may fragment to give \( m/z \) 152, 122, and 94. The structure of the \( m/z \) 122 ion that is formed under these conditions is not clear, but it seems certain that it is neither \( m/z \) 122a nor 122b because the formal loss of CH\(_3\)OH is indicated (the resultant \( m/z \) 122 ion must contain an O atom) and the benzoate group was already lost in forming \( m/z \) 154. Although a third \( m/z \) 122 ion containing both N and O is represented in the spectra of the other cocaine diastereomers at low abundance, it is not observed in the cocaine spectrum.

**\( m/z \) 152.** The elemental composition for this ion indicates the loss of C\(_6\)H\(_4\)O\(_2\) from the \( M^+ \). Both the N-CD\(_3\) and O-CD\(_3\) labels are retained, but the phenyl label is lost. Losses of D from the tropane skeleton are complex, but losses of D at C2, one D atom at C4 and four D atoms from the C1, C5, C6, and C7 (consistent with loss of the C6-C7 bridge) are supported. Other D losses occur, however, indicating that formation of this ion must follow more than one pathway. These data, along with the methyl group in the ester can be lost as either a methyl radical or a molecule of methanol.

At the remaining atoms, the H atom at C3, one H atom at C4, and one H atom from C1, C5, C6, and C7, are all lost to some degree, but only the loss at C3 is notably significant. The \( m/z \) 166 ion may fragment to produce the ions having \( m/z \) 138 and 82. The individual mechanisms shown in Figures 2 (losses at C3 and O-CH\(_3\)), 8 (losses at C2, C4, and N-CH\(_3\)), and 17 (losses at C4 and O-CH\(_3\) or N-CH\(_3\)) explain various aspects of the data [18]. Still other mechanisms are possible.

![Figure 14](image-url)  
**Figure 14** - Electron ionization mass spectra of a) \( \alpha \)-cocaine (IVa); and b) pseudococaine.

ionization at the carbonyl O atom of the benzyloxy group [5]. In this case, the charge ends up on the olefin product. Two isomeric structures for \( m/z \) 181 are possible; they appear to suffer different fates (Figures 15 and 16).

**\( m/z \) 166.** Determining the origin of the \( m/z \) 166 peak illustrates the danger of assuming there is a one-to-one correlation between ions and peaks in a low-resolution EI mass spectrum. The MS/MS data indicates that the \( m/z \) 166 ions might have at least three precursor ions - particularly the ones having \( m/z \) 288, 198, and 181. Formation of \( m/z \) 166 from \( m/z \) 288 and 181 involves loss of benzoic acid and a methyl radical, whereas production of this ion from \( m/z \) 198 involves loss of benzyol radical and a molecule of methanol. In each case, the total loss is C\(_6\)H\(_4\)O\(_2\), consistent with the elemental composition (Table II). As can be seen from the previously proposed structures for the precursor ions, it seems likely that there are at least three isomeric \( m/z \) 166 ions.

The D labeling data is understandably complex. Although the labels on the aromatic ring are lost, none of the other derivatives show complete loss or retention of individual labels. Both the OCD\(_3\) and NCD\(_3\) derivatives show partial loss of their respective labels, with somewhat more OCD\(_3\) label lost than NCD\(_3\). Fragmentation of D-labeled 2,3-anhydroecgonine methyl ester (methylconineidone; V), whose \( M^+ \) is isoelectronic with \( m/z \) 181b, shows similar behavior [17]. This means that the methyl radical is lost to some extent from the N atom, and
Figure 15 - Proposed formation of m/z 181a (by loss of the H atom at C4) and its subsequent fragmentations.
Figure 16 - Proposed formation of m/z 181b (by loss of the H atom at C2; isoelectronic with M⁺ of 2,3-anhydroecgonine methylester) and its subsequent fragmentations.
with the elemental composition, primarily indicate formal loss of a molecule of benzoic acid plus an ethyl radical. The most likely structure is the aromatic 3-carbomethoxy-N-methylpyridinium ion \([5,13,15,19]\).

Although some \(m/z\) 152 ions result from fragmentation of \(m/z\) 154 at very low collision energies (Table II), two more important precursors appear to be the \(m/z\) 274 and 181 ions. At collision energies of 6-20 eV, both are significant contributors. Both these ions are reasonable intermediates for the EI fragmentations leading to \(m/z\) 152. Formation of \(m/z\) 152 via \(m/z\) 274 involves \(\alpha\)-cleavage from the \(M^+\) with subsequent loss of the C6-C7 bridge along with a H atom from either C4 or C2, followed by cyclic loss of a molecule of benzoic acid involving a H atom at whichever C atom (C2 or C4) is still saturated (Figures 9 and 10). When \(m/z\) 152 is formed via \(m/z\) 181, this process is formally reversed (Figures 15 and 16). Although one

Figure 17 - Possible mechanisms for formation of \(m/z\) 166a and \(m/z\) 166c from \(m/z\) 181a.
might assume that the double bond in either process would form initially between C2 and C3 because it would be conjugated with the double bond in the carbonyl group at C2, there is no clear evidence that this is the case.

The intensity of the $m/z$ 152 peak for cocaine (0.4% of total peak intensities; Table IV) is significantly smaller than it is in the spectra of pseudococaine (Figure 14b), allococaine, and $\alpha$- and $\beta$-cocaine (approximately 2%). The intensity of $m/z$ 152 in the spectrum of pseudoallococaine has an intermediate value. The relative size of this peak is often used to distinguish the spectrum of cocaine from those of the other diastereomers [3,5,7,9,20]. Trying to explain why this is so has been a goal of forensic chemists for many years.

Both of the fragmentation pathways to $m/z$ 152 proposed here involve steps that are potentially sensitive to the relative stereochemistry at C2 and C3. First, loss of the C6-C7 bridge as an ethyl radical involves a 5-center H rearrangement from either C2 or C4 to the radical site at C6 or C7 (Figures 9, 10, 15, and 16). Although this rearrangement might be influenced by the relative position of the migrating H atom, it is clear that when the H atom is initially endo to the ring system the rearrangement occurs with consummate facility; in the spectrum of 2,3-anhydroecgonine methyl ester (V), this fragmentation accounts for the base peak in the spectrum (Figure 16). Even when the migrating H atom is initially exo to the ring system and becomes trans to the C6-C7 bridge, molecular models

Figure 18 - Proposed formation of $m/z$ 155 and its subsequent fragmentations.

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indicate that it can be approached by the radical site nearly as well as when the H atom is cis to the bridge.

On the other hand, cyclic loss of benzoic acid – whether from the M⁺ via the McLafferty (γ-hydrogen) rearrangement or from the m/z 274 ion by a superficially similar type of rearrangement – should proceed readily only when the carbonyl O atom and the migrating H atom can approach one another easily. This can occur by migration of a H atom from either C2 or C4, depending upon which H atoms are available (Figures 9, 10, 15, and 16). Access to an available H atom at C4 should be equally facile for all of the cocaine diastereomers in both fragmentation schemes, since one of the two available H atoms at C4 will necessarily be “cis” to the benzoate group (Figures 10 and 16). At C2, the benzoate group and single available H atom are “cis” to one another only in pseudo- and allococaine (Figure 19; also Figure 16, indicated by *; and Table IV).

When m/z 152 is formed via m/z 274, the six-membered ring in the m/z 274 ion has two π-bonds that force rigidity (Figure 20, indicated by *). Again the 2-H and 3-benzyloxy groups are cis to one another in pseudo- and allococaine, but trans to one another in cocaine and pseudocodeine. With α- and β-

cocodeine, in which both functional groups are located on C3, a choice of migrating H atoms is always offered at both C2 and C4, so that stereochemical relationships are not an issue, and a more intense m/z 152 peak is observed.

This rationalization does not explain the subtle differences that are observed – especially that the m/z 152 peak is larger in the pseudocodeine spectrum than in that of cocaine. However, it should be apparent that because more than one pathway exists for formation of m/z 152, and because of the probable difference in conformation of the ring between the 3-exo and 3-endo isomers (Figure 19), a more nuanced analysis is not possible on the basis of the data presented here.

Figure 19 - Schematic representations of stereochemical relationships present in the loss of benzoic acid from the M⁺ in the four cocaine diastereomers.

Figure 20 - Schematic representations of stereochemical relationships present in the loss of benzoic acid from m/z 274 in the four cocaine diastereomers.

m/z 150. Two ions give the m/z 150 ion as a significant product ion in the MS/MS experiments – those having m/z 272 and 182. The first instance involves overall loss of a methoxy radical plus a molecule of benzoic acid; the latter, loss of a benzoate radical and a molecule of methanol. Loss of both the OCD and phenyl labels in this ion is consistent with this interpretation. The D labels in the tropane skeleton are difficult to interpret because of interference by other peaks in this region of the spectrum. Nonetheless, it appears that there may be loss of D from C4 or from the C1, C5, C6, and C7 portion of the molecule. At low collision energies, m/z 150 loses CO to produce m/z 122a.

Mechanisms that are consistent with these data are shown in Figures 3 and 21, although additional ones are possible. Both mechanisms show the H atom involved in the cyclic losses of benzoic acid and methanol coming from C4. The two-dimensional diagrams in Figure 21 do not adequately show that the methoxy O atom and the H atom on C4 can approach each other as close as about 1.5 Å.

m/z 140. This ion appears to be formed by loss of a methyl radical from the m/z 155 ion (Table II). The pattern of D loss from the tropane skeleton is the same as that for m/z 155, but the additional loss of the O-methyl group is seen. Figure 18 depicts a mechanism that is consistent with this observation.

m/z 138. The only precursor identified for m/z 138 in the MS/MS experiments is an ion having m/z 166, but it is not clear which one. From the elemental composition, it is apparent that the lost fragment is CH₂=CH₂, not CO. Both the methyl ester and phenyl groups are lost, but the N-methyl group is retained. Losses from the tropane skeleton are harder to discern. Although the D atoms at C2 and C3 appear to be retained, three or four D atoms are lost from the d₀ derivative. This could indicate loss of the C6-C7 bridge. A simple mechanism involving a reverse cycloaddition fragmentation from m/z 166c is consistent with these data (Figure 17).
**m/z 122.** There are two ions having different elemental compositions recorded at m/z 122. High-resolution MS shows that one of these ions, designated as m/z 122a, has the formula C₈H₁₂N. The second (m/z 122b) is the benzoic acid radical ion. It is important to remember that the MS/MS studies cannot distinguish between these ions.

(a) In precursor MS/MS spectra, both m/z 181 and 150 produce m/z 122a. Mass-analyzed ion kinetic energy studies done by Shapiro, et al. also identified the m/z 181 ion as precursor [9]. These fragmentations involve loss of 59 u (the carbomethoxy group) from m/z 181 and CO from m/z 150.

The D labeling data must be analyzed with caution because of the presence of the two m/z 122 ions. Both our own (0.8% vs. 5.8%) and Shapiro’s HR-MS work [9] show that m/z 122a is significantly more abundant than m/z 122b. This is also reflected in the spectra of the D-labeled derivatives Ic and Id, in that the m/z 125 peak (reflecting the presence of the N-CD$_3$ label) is much more intense than m/z 122 in Figure 4b, but m/z 127 is only a small peak in Figure 4b (reflecting retention of the phenyl D labels). Therefore, most of the observable shifts in the spectra of the deuterated derivatives are due to m/z 122a, not m/z 122b.

Although the methyl ester and aromatic ring are absent in m/z 122a and the N-methyl group is retained, the remaining D labeling data are complex. The other derivatives show a pattern in which the D atoms at C2 are lost about 40% of the time; 25% of those at C3 are lost; and two D atoms are lost from C4 about 30% of the time, but only one D is lost about another 30% of the time. The d$_4$-derivative exhibits retention of all six D atoms (35%), loss of one D (35%), loss of five D (15%), and loss of all 6 D (15%). In this case, the losses of 5-6 D atoms are more logically associated with the m/z 122b ion or perhaps even with the m/z 119 ion, indicating that the C6-C7 bridge is probably retained in m/z 122a.

One cannot reasonably postulate a single, simple mechanism that accounts for this pattern of losses. Figures 9, 10, 15, and 21 show several possible mechanisms that together are consistent with the predominant retention of H atoms at all skeletal positions except C2 and C4 [16]. Note that m/z 122a cannot be formed easily from m/z 181b (Figure 16).

(b) Because of the elemental composition and presence of the aromatic ring, the m/z 122b ion is assigned the benzoic acid radical ion structure. An important precursor appears to be the M$^+$, although other ions undoubtedly also play a role (Table II). Initial ionization at the benzoate group, followed by McLafferty rearrangement involving removal of a H atom either from C2 or C4, leads to m/z 122b (Figure 3). Fragmentation of the m/z 122b ion leads to the benzoyl ion (m/z 105) and its known fragment ions (m/z 77 and 51) [21]. The patterns of D losses observed for m/z 105, 77, and 51 are all consistent with this assignment.

**m/z 108.** Taken as a whole, the data for the m/z 108 ion contain a contradiction that cannot be resolved without invoking two separate mechanisms. The N-CH$_3$ group is clearly retained because of the labeling and elemental composition data, but both ester groups are lost. On the tropane skeleton most of the D label at C2 is lost, most of the label at C3 is retained, and about 50% of one label at C4 is lost, as are one or two D atoms from C1, C5, C6, and C7. This strongly indicates that the C6-C7 bridge remains intact in this ion and implies that the seven C atoms in the ion consist of the N-methyl group plus all the atoms in the tropane skeleton except C2. However, the MS/MS data show that the ions having m/z 182, 181, and 152 can all act as precursors to m/z 108. In the last case, the C6-C7 bridge is no longer present. This indicates that the m/z 108 ion is formed from different precursors under EIMS and MS/MS conditions.

Formation of m/z 108 from m/z 152 involves the loss of a molecule of CO$_2$, leading to a stable aromatic ion (Table II and Figure 22a). However, production of m/z 108 from either m/z 181 or 182 seems to involve loss of the carbomethoxy group, C2, and a H atom from C5 (Figure 22b). Other fragmentation schemes, as well as other structures for m/z 108, are possible.

**m/z 105.** See discussion under m/z 122b above and Figure 3.

**m/z 100.** The m/z 222 ion appears to fragment to m/z 122b and m/z 100 at lower collision energies. Analysis of the spectra of the D-labeled derivatives for the m/z 100 ion shows retention of the O-CD$_3$ group, as well as the D atoms at C2, C3, and one D atom at C4. The N-CD$_3$ and the phenyl groups are lost. Although the pattern of losses in the d$_4$-derivative could not be determined with certainty, loss of the five-membered ring consisting of C1, the N atom, C5, C6, and C7 is likely simply because the retained mass indicated by the apparent presence of the carbomethoxy group, C2, C3, and C4 is sufficient. A mechanism for formation of this ion that both accounts for these data and is consistent with the elemental composition in Table II is shown in Figure 13.
Figure 21 - Proposed formation of $m/z$ 150, $m/z$ 122, $m/z$ 94, and $m/z$ 82 from $m/z$ 182.
Figure 22 - Possible mechanisms for formation of $m/z$ 108 from a) $m/z$ 152; and b) $m/z$ 181.
Figure 23 - Proposed formation of $m/z$ 96 and its fragments from $m/z$ 155.

Figure 24 - Proposed fragmentations of $m/z$ 82 to give $m/z$ 67 and $m/z$ 55.
$m/z$ 97. The principal precursor to this ion is the $M^+$, especially at low collision energies (Table III). Deuterium loss patterns are difficult to interpret because of the proximity of other more intense peaks in this area of the spectrum. Nonetheless, it appears that the D atoms at C2 and C3, as well as one D atom from C4, may be lost. No more than 1 D atom is lost from the remainder of the tropane skeleton, indicating that the C6-C7 bridge is retained. The N-methyl group is retained, but both ester groups are lost. A simple mechanism that explains most of these data is shown in Figure 2 [1,2,13].

$m/z$ 96. Major precursors to the $m/z$ 96 ion at low collision energies are the ones having $m/z$ 155 and 97. This strongly suggests that this ion, like those of its precursors, contains the five-membered ring portion of the tropane skeleton, rather than the six-membered ring. Losses from the D-labeled derivatives bear this out. Although there is loss of D at C2 and significant loss at C3, no more than one D atom is lost from C1, C5, C6, and C7, showing that the C6-C7 bridge is retained. The loss of the label from C4 could not be determined with certainty. The N-methyl group is retained, but both ester groups are lost. Two mechanisms are shown: the one in Figure 2 starts with $m/z$ 97 [1,2,13]; the other (Figure 23) begins with $m/z$ 155. Additional mechanisms are possible.

$m/z$ 94. In contrast to the $m/z$ 96 ion, many of the $m/z$ 94 ions appear to have an N-methylpyridinium structure [1,5,13,16]. The most important precursors for this ion at low collision energies are $m/z$ 181 and 122a. The N-methyl group is retained, as are the H atoms at C2 and C3. Both ester groups are absent, as are one H atom from C4 and four H atoms from the C1, C5, C6, and C7 portion of the molecule. This is similar to the pattern of losses exhibited by $m/z$ 152 and is consistent with loss of the C6-C7 bridge. Formation of this ion from $m/z$ 122a can occur readily via a reverse cycloaddition loss of ethylene (Figures 3, 9, 10, 15, and 21). All reasonable pathways to $m/z$ 94 from $m/z$ 181 appear to utilize $m/z$ 122a as an intermediate.

An undetermined percentage of $m/z$ 94 ions have $m/z$ 96 as their precursor, indicating that they probably have a structure in which the five-membered ring is intact (Figure 2).

Like the intensity of the $m/z$ 152 peak, the relative intensities of the $m/z$ 94 and 96 peaks also distinguish the spectrum of cocaine from those of the other diastereomers. In the spectra of cocaine and pseudocalcocaine, the peak at $m/z$ 94 is more intense than the one at $m/z$ 96; in the spectra of the other two isomers it is smaller than $m/z$ 96 (compare Figures 4a and 14b). Spectra of D-labeled derivatives of cocaine and pseudocalcocaine strongly indicate that this phenomenon is not just a simple reversal of intensities. The $m/z$ 94 peak in the cocaine spectrum is significantly larger than that produced by pseudocalcocaine, while the $m/z$ 96 peaks in both spectra have similar intensities relative to those of other nearby peaks. In addition, the $m/z$ 94 peak in the cocaine spectrum represents a greater proportion of the overall ion current (4.8%) than it does for the other isomers (2.9-3.3%) [5]. This implies that $m/z$ 94 forms more easily with cocaine than it does with the other diastereomers.

None of the individual steps involved in generation of $m/z$ 94b via either $m/z$ 155 or 97 should be sensitive to stereochemical differences in the precursor ions because C2 and C3 are lost in forming this ion (Figures 2 and 23). However, a careful examination of the other fragmentation pathways leading to $m/z$ 94a show that formation of this ion is in competition with formation of $m/z$ 152 via common intermediates: Pathways a + b vs. pathway c in Figures 9 and 10; and pathways a + c vs. pathway b in Figure 15. It is therefore tempting to conclude that the same factors that discourage formation of $m/z$ 152 should encourage formation of $m/z$ 94, and vice versa [5]. Indeed, the percent of total ion current for the $m/z$ 94 peak in the spectra of the four diastereomers is virtually opposite of what is seen for the $m/z$ 152 peak—that is, cocaine and pseudocalcocaine produce the smallest $m/z$ 152 peaks and the largest $m/z$ 94 peaks, while for pseudocalcocaine and allococaine the situation is reversed. One may conclude, then, that the relative rates of cyclic loss of benzoic acid for these compounds directly affect the ease of forming $m/z$ 152 and thereby indirectly affect formation of $m/z$ 94.

$m/z$ 83. The $m/z$ 83 ion was identified as the most abundant fragment ion produced by the $M^+$ at low collision energies, indicating that it is formed directly in one step (Table II). The N-methyl group is retained, as are most of the H atoms in the five-membered ring portion of the molecule. Carbon atoms 2, 3, and 4 and their substituents are lost in forming this ion. This ion fragments almost exclusively to give $m/z$ 82 (Figure 2) [1,2,13].

$m/z$ 82. Several important precursors to $m/z$ 82 were identified by MS/MS, so that a number of pathways for its formation are likely. At low collision energies, the $M^+$, $m/z$ 182, $m/z$ 97, and $m/z$ 83 all can produce this ion, although $m/z$ 303 and $m/z$ 182 appear to be the most important. The pattern of D losses is similar to that seen with $m/z$ 83, indicating that the structure of this ion consists of the five-membered ring of the tropane skeleton (Figure 2) [1,2,13]. A possible mechanism for formation directly from the $M^+$ is shown in Figure 24. Formation from $m/z$ 182 is shown in Figure 21.

$m/z$ 77. This ion is a known fragment of $m/z$ 105 (Figure 3). See discussion under $m/z$ 122b above.

$m/z$ 68. The ion having $m/z$ 96 is the only one that produces $m/z$ 68 as a major fragment in the MS/MS experiments. The structure of $m/z$ 68 appears to lack C2 and C3 and their substituents, which is consistent with the structure proposed for $m/z$ 96. The only label that is clearly retained is the one on the N-methyl group, although it appears that several of the D atoms on C1, C5, C6, and C7 are retained as well (the observed cluster of peaks between $m/z$ 68 and 73 in the spectrum of the d6-derivative shows no single important peak). Cyclic loss of ethylene from $m/z$ 96 could account for formation of this ion, which most likely has a methylene-azacyclopropenium structure (Figure 23).

$m/z$ 67. The peaks at $m/z$ 67 and $m/z$ 68 often move together in the spectra of the D-labeled derivatives, which gives the impression that they are related to each other in a simple manner. Although the elemental composition for this ion was not determined, it appears as an important product ion in the MS/MS spectra of $m/z$ 94 and $m/z$ 82, indicating that it may have a different structure from $m/z$ 68. It also appears that the
N-methyl label is lost, although in this area the spectrum of the N-CD_3 derivative shows more complexity than that simple analysis implies. On the other hand, formation of m/z 67 from m/z 82 can occur by loss of a methyl radical – presumably that on the N atom (Figure 24). The m/z 67 ion could form from m/z 94 by loss of HCN by a complex mechanism involving transfer of H atoms from the N-methyl group back onto the remaining C atoms (structure and mechanism not shown). This ion has a different elemental composition than that of the one formed from m/z 82. However, the presence of two separate mechanisms is not inconsistent with the D loss pattern.

m/z 59. Only two of the studied ions give m/z 59 as a product ion – m/z 182 at higher, and m/z 152 at lower, collision energies. The only D label retained is on the O-CD_3 group. That, in combination with the elemental composition, indicates that this ion is the carboxy methoxy ion. It is difficult to write a mechanism for its formation from either of these ions without invoking high-energy intermediates or significant rearrangement. Other precursors to this ion are possible.

m/z 55. Of the listed precursors to the m/z 55 ion, only m/z 82 is important at low collision energies. This involves the loss of 27 u as C_3H_2. The spectra of D-labeled derivatives indicate loss of C2, C3, and C4. The pattern of loss shown by the d_5-derivative is complex and suffers from interference by the surrounding peaks, but at least some (and perhaps most) of the labels are retained. This is consistent with the fact that m/z 82 is the precursor. One possible mechanism is shown in Figure 24.

m/z 51. This ion is a known fragment of m/z 105 and m/z 77 (Figure 3). See discussion under m/z 122b above.

m/z 42. The only D labels retained by this ion are in the N-CD_3 group and one of the D atoms on C1, C5, C6, or C7. The m/z 96 ion is the only one studied that gives m/z 42 as a significant product ion at low collision energies. A possible mechanism is shown in Figure 23.

Conclusions
Although previously proposed mechanisms for formation of many of the more abundant ions in the cocaine spectrum were confirmed in this study, details about other fragmentations were revealed. Of particular interest are explanations clarifying the subtle differences between the spectra of the cocaine diastereomers – formation of m/z 152 from m/z 274 and 181 via pathways that are sensitive to the relative stereochemistry at C2 and C3, and the apparent inverse relationship between ease of formation of m/z 152 and m/z 94. Also of interest are a) the complexities that underlie the m/z 166 peak; b) the importance of the N-containing ion having m/z 122; and c) details regarding the formation of lower abundance ions, especially those above m/z 200.

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The authors are deeply indebted to Donald A. Cooper, Senior Forensic Chemist, DEA Special Testing and Research Laboratory, ret., for obtaining the MS/MS and HR-MS data and for his ideas and comments on the manuscript.

References
Characterization of Three Methcathinone Analogs: 4-Methylmethcathinone, Methylone, and bk-MBDB

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ABSTRACT: Complete spectroscopic characterization (FTIR, FT-Raman, 1H NMR, 13C NMR, GC/MS, and EI-HRMS) is presented for the hydrochloride salts of 3,4-methylenedioxymethcathinone (methylone), 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one (bk-MBDB), and 4-methylmethcathinone (mephedrone). These three methcathinone analogs were synthesized in our laboratory as reference materials for comparison with submitted exhibits. Additionally, the identification of bk-MBDB is reported in tablets which are available over the internet.

KEYWORDS: Methcathinone, methylone, 4-methylmethcathinone, bk-MBDB, forensic chemistry

Most new designer drugs are prepared to circumvent existing legislation, to create new drugs with desirable pharmacological properties, and/or to avoid detection through normal testing protocols. Since 2006, analogs of methcathinone and structurally similar β-ketopropylamine derivatives have been intercepted in cross-border shipments by the Canada Border Services Agency (CBSA) with increasing frequency. Since many of these designer drugs are regulated in Canada under the Controlled Drugs and Substances Act, it is important to confirm the identity of these chemicals for regulatory and intelligence purposes.

The identification of new designer drugs presents certain challenges. Certified reference materials and published peer-reviewed analytical data are often unavailable when new substances are encountered. Reference standards for some analogs of methcathinone are not presently available, hindering the detection and identification of these materials.

Methcathinone (also known as ephedrine) is the β-keto analog of methamphetamine and the N-methyl derivative of cathinone, a central nervous stimulant found in leaves of the “khat” bush (Catha edulis) [1]. Methamphetamine and methcathinone syntheses are well documented in the literature and these substances can be readily prepared by reduction [2,3] and oxidation [4] of ephedrine (and pseudoephedrine), respectively (Figure 1). The names methamphetamine and methcathinone are used for these compounds regardless of their enantiopurity, which may vary depending on the methods used in their synthesis [5].

Analogs of methcathinone that possess the methylenedioxy ring substituent on the phenyl ring resemble 3,4-methylenedioxyamphetamine (MDMA, “Ecstasy”). Methylone (2a, Figure 2) is the benzylic ketone analog of MDMA. This analog was patented in 1996 as an antidepressant [6] and some analytical data was published shortly thereafter [7]. It is the main ingredient of the designer drug “Explosion,” found and reported in 2005 in the Netherlands [8]. bk-MBDB, or 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one, (2b, Figure 2) is the β-keto analog of N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), a psychoactive agent with similar pharmacology to MDMA [9]. bk-MBDB is commonly referred to as “butylene” on the internet. Recently, our laboratory analyzed two different sets of tablets that were confirmed to contain 2b by FTIR and GC/MS. The tablets in the first set were biconvex, slightly off-white in color, with an image of a dove imprinted on one side (Figure 3A). Tablets in another set, which were also found to contain fenfluramine hydrochloride (an anorectic), were round, flat, and yellow with an image of a sun imprinted on one side (Figure 3B).

Figure 1 - Ephedrine (or pseudoephedrine) undergoes reduction to give methamphetamine and oxidation to afford methcathinone.

Figure 2 - Structures of methcathinone analogs prepared in this study.

†Dr. Michael Pollard recently passed away unexpectedly at the age of 36. He will be remembered as an inspiring teacher, colleague, and friend.
In a separate case in 2008, our laboratory identified a white powder as the hydrochloride salt of 4-methylmethcathinone (2c, Figure 2). Some websites have referred to this designer drug as “mephedrone.” At that time, this analog had not been reported in the scientific literature and therefore required structural elucidation and synthesis of a reference compound to unequivocally determine its identity. Our data supports and supplements the work that was recently published on this analog [10].

To provide spectroscopic data for the hydrochloride salts of 2a and 2b, and to confirm the structure of 2c, we synthesized the hydrochloride salts of these racemic methcathinone analogs and provide complete spectroscopic (FTIR, FT-Raman, \(^1\text{H} \text{NMR}, \^{13}\text{C} \text{NMR}\)) and spectrometric characterization (GC/MS, EI-HRMS).

**Experimental Procedures**

**Chemicals, Reagents and Methods**

All solvents and reagents were purchased from Sigma-Aldrich, used without purification and were analytical grade. Derivatization grade \(N\)-methyl-\(N\)-(trimethylsilyl) trifluoroacetamide (MSTFA) was used to prepare the trimethylsilyl derivatives for GC/MS analysis. Trimethylsilyl derivatives were prepared by adding 5 mg of sample to 1 mL of 50% (v/v) MSTFA in chloroform and then heating to 70˚C for 1 h.

Sample solutions for analysis by nuclear magnetic resonance (NMR) spectroscopy were prepared with 99.9% D anhydrous DMSO-\(d_6\) in 1 mL ampoules.

**Instrumentation**

ATR-FTIR spectra were recorded on a Nicolet Avatar 370 FTIR, with single reflection diamond ATR accessory. Range: 4000 cm\(^{-1}\) - 650 cm\(^{-1}\), 16 scans and 4 cm\(^{-1}\) resolution. Raman spectroscopy was performed using a Nicolet 6700 FTIR with NXR FT-Raman module on samples in an NMR tube with laser wattage at 1.0 W and an InGaAs detector. Range: 4000 cm\(^{-1}\) - 100 cm\(^{-1}\) Raman shift, 128 scans, 1064 nm Nd-YAG excitation laser.

GC/MS data was collected using an Agilent 6890N GC with a 7683B series autosampler, 1 \(\mu\)L injection, split 150:1 - 4 mm single gooseneck liner (deactivated, no glass wool), DB5MS column (30 m × 0.25 mm × 0.25 \(\mu\)m) with constant flow (1 mL/min of helium) coupled to an Agilent 5973 Mass Selective Detector. EI operating parameters were: inlet temperature 280˚C, interface temperature 280˚C, MS source 230˚C, MS Quad 150˚C, 70 eV ionization energy. The GC oven temperature program started at an initial temperature of 100˚C with a ramp of 10˚C/min to 300˚C. The final temperature was held for 25 min (total run time 45 min).

High-resolution mass spectra (HRMS) were recorded using electron-impact ionization at the University of Ottawa Mass Spectrometry Centre on a Kratos Concept double focusing mass spectrometer with 70 eV ionization energy. All measurements are within 3 millimass units (mmu).

\(^1\text{H} \text{and} \^{13}\text{C} \text{NMR} \text{spectra were recorded in} 5 \text{mm NMR tubes on a Bruker AVANCE III 400 MHz spectrometer on solutions in DMSO-}d_6. \text{Chemical shifts are given in parts per million (ppm) (± 0.01 ppm) relative to the residual undeuterated solvent absorptions (2.50 ppm for DMSO-}d_6 \text{in} \text{H NMR spectroscopy; and 39.5 ppm for DMSO-}d_6 \text{in} \^{13}\text{C NMR spectroscopy). Coupling constants (}J\text{) are expressed in Hertz (Hz). The following abbreviations are used to designate NMR absorption patterns: }s, \text{ singlet; }d, \text{ doublet; }t, \text{ triplet; }q, \text{ quartet; }m, \text{ multiplet; }dd, \text{ doublet of doublets; AA’MM’, multiplet characteristic of the} \text{para-substituted benzene ring.}

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**Scheme 1** - Synthetic pathway used for the preparation of methcathinone analogs 2a-c.
**Synthetic Procedures**

The following synthetic pathway was used to prepare analogs 2a-c (Scheme 1). Experimental details on these syntheses are not provided, in accordance with the Journal policy.

**Results and Discussion:**

**Infrared spectroscopy and Raman spectroscopy**

The IR (Figure 4) and Raman spectra (Figure 5) collected from samples of 2a-c synthesized in our laboratory were consistent with the ATR-FTIR and FT-Raman spectra previously recorded for exhibits submitted from intercepted shipments. While IR spectroscopy has traditionally served as an important method for screening and identifying unknowns, we also present Raman spectroscopic data because it has emerged as a powerful technique in the forensic laboratory. Raman spectroscopy is a non-destructive technique which, like IR, provides a “fingerprint” spectrum of chemical compounds. In many cases, bands which are weak or completely inactive in IR tend to be strong in Raman and vice versa. Therefore, these techniques are complementary and, together, provide a more complete characterization of the molecule.

**Gas chromatography - mass spectrometry and high resolution mass spectrometry**

As shown in Figure 6, the molecular ions for analogs 2a-c were either weak or absent. As a result, each analog 2a-c was treated with MSTFA to give the thermally stable trimethylsilyl derivative for GC/MS analysis. The GC/MS data for the trimethylsilyl derivatives of 2a, 2b and 2c showed a similar fragmentation pattern and gave molecular ions of m/z 279, 293, and 249 respectively (Figure 7). The base peak for silylated 2a and 2c, each at m/z 130, corresponds to the formation of an iminium ion (C<sub>6</sub>H<sub>16</sub>NSi<sup>+</sup>) and is also characteristic of methcathinone. Silylated 2b gave a base peak of m/z 144 (C<sub>7</sub>H<sub>18</sub>NSi<sup>+</sup>) which is consistent with the extension in the alkyl chain length. The α-cleavage (M-15) fragments were found at m/z 264, 278, and 234 at low intensities for 2a, 2b and 2c derivatives respectively. The ions at m/z 149 and m/z 121 for
and \(2b\) are consistent with the methylenedioxybenzoyl cation and methylenedioxyphenyl cation (formed by subsequent loss of CO) reported for the designer drug 3,4-methylenedioxy-pyrovalerone (MDPV) [11]. The mass spectra of all TMS-functionalized derivatives of \(2a-c\) all show an ion at \(m/z\) 73 that corresponds to the \((\text{CH}_3)_3\text{Si}^+\) fragment.

Table 1 presents data from electron-impact ionization high resolution mass spectrometry (EI-HRMS) for compounds \(2a-c\).

The HRMS data for each analog agrees with molecular formula and is accurate within a 3 millimass unit error.

**Nuclear Magnetic Resonance (NMR)**

The \(^1\)H and \(^13\)C NMR spectra in DMSO-\(d_6\) are presented for the hydrochloride salts of \(2a-c\) in Figures 8-10. Data from correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments was used to assign aromatic protons for each analog and to distinguish methyl groups for \(2c\).

By preparing the amine hydrochloride salt, the N-H protons in each analog are diastereotopic and hence chemically inequivalent. As a result, these protons are observed as two broad singlets.

**Conclusion**

We have synthesized and characterized the hydrochloride salts of 3,4-methylenedioxymethcathinone (methylone), 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one (bk-MBDB), and 4-methylmethcathinone (mephedrone). The spectroscopic data collected on samples of these racemic analogs synthesized in our laboratory is consistent with the corresponding data from samples intercepted previously by the Canada Border Services Agency.
Table 1

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Figure 8 - 1H and 13C NMR data for 2a hydrochloride in DMSO-d6.
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Figure 9 - \(^{1}\text{H} \text{ and }^{13}\text{C} \text{ NMR data for } 2\text{b hydrochloride in DMSO-d}_6\).
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<th>^13C (ppm)</th>
<th>^1H (ppm)</th>
<th>Multiplicity</th>
<th>J (Hz)</th>
<th>Structure Assignment</th>
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<td>-</td>
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<tr>
<td>2</td>
<td>58.0</td>
<td>5.13</td>
<td>q</td>
<td>7.2</td>
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<tr>
<td>3</td>
<td>15.5</td>
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<td>d</td>
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<tr>
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<td>7.94</td>
<td>AA'MM'</td>
<td>8.1*</td>
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</tr>
<tr>
<td>3'</td>
<td>129.7</td>
<td>7.41</td>
<td>AA'MM'</td>
<td>8.1*</td>
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<tr>
<td>4'</td>
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<td>AA'MM'</td>
<td>8.1*</td>
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</tr>
<tr>
<td>6'</td>
<td>128.9</td>
<td>7.94</td>
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<td>8.1*</td>
<td></td>
</tr>
<tr>
<td>7'</td>
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<td>s</td>
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<td>30.6</td>
<td>2.58</td>
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</table>

* J_{apparent}

Figure 10 - ^1H and ^13C NMR data for 2c hydrochloride in DMSO-d$_6$. 
Acknowledgements
The authors would like to thank the National Anti-Drug Strategy for funding of this project, Pat Latour for performing GC/MS experiments, Mario Larouche for running NMR spectroscopy experiments, and Dr. Clem Kazakoff for HRMS analysis.

References
Characterization of 2β-(1,2,4-Oxadiazol-5-methyl)-3β-phenyltropane ("RTI-126")

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ABSTRACT: Spectroscopic and chromatographic data are provided for 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (commonly referred to as RTI-126), its 2α-epimer, and their respective synthetic intermediates. Direct comparisons of the analytical data are made to assist forensic chemists in correctly differentiating these epimeric isomers in suspected drug exhibits.

KEYWORDS: 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane, RTI-126, designer drugs, chemical analysis, forensic science

Businesses advertising and executing the sale of “legal highs” have recently flourished on the Internet. In conjunction, several Internet “chemical companies” have been publicizing the recent synthesis of many CNS-active compounds for sale that are “not for human use.” In 2010, for the first time, a cocaine-like derivative was being openly touted as a non-controlled stimulant, with an activity five-times greater than that of cocaine. The compound (Figure 1) is generically referred to as “RTI-126,” or specifically, 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane. 3β-Phenyltropane-2-carboxylic acid esters and analogs were first synthesized and studied in 1973 for their biological activity [1]. RTI-126 was first synthesized in the early 1990’s to study the binding affinities of several cocaine analogs in the dopamine, serotonin, and norepinephrine transport systems [2].

This paper presents the analytical profile of RTI-126, its 2α-epimer, and their respective synthetic intermediates (Figure 2) to assist forensic chemists who may encounter these substances in casework.

Experimental

Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All other chemicals were of reagent-grade quality and products of Sigma-Aldrich Chemical (Milwaukee, WI). Additionally, a 5 mg sample of “RTI-126” was obtained from an undisclosed source.

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Figure 1 - Structural formula of 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (RTI-126).

Fourier Transform Infrared Spectroscopy (FTIR)
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm⁻¹, gain = 8, optical velocity = 0.4747, aperture = 150, and scans/sample = 32.

Nuclear Magnetic Resonance Spectroscopy (NMR)
Proton (¹H), carbon (¹³C), and 2-dimensional NMR spectra were obtained on a Agilent (formerly Varian) 400MR 400 MHz NMR using a 5 mm Protune indirect detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). All compounds were dissolved in deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to acquire ¹H, proton-decoupled ¹³C, and gradient versions of COSY, NOESY, HSQC, and HMBC spectra. Data processing was performed using software from Agilent and Applied Chemistry Development

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Figure 2 - Synthetic route to 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 7 (RTI-126).
Figure 3 - Infrared spectra of 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 7 (RT1-126) HCl (upper) and 2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 8 HCl (lower).
Figure 4 - Electron ionization mass spectra of (a) 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 7 (RTI-126) and (b) 2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 8.
Table 1 - Gas chromatographic retention times (Rt) and Relative Retention Times (RRt) for the 3β-Phenyltropane Related Compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rt (min)</th>
<th>RRt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhydroecgonine methyl ester (2)</td>
<td>7.70</td>
<td>0.36</td>
</tr>
<tr>
<td>2α-Carboxymethoxy-3β-phenyltropane (4)</td>
<td>16.40</td>
<td>0.77</td>
</tr>
<tr>
<td>2β-Carboxymethoxy-3β-phenyltropane (3)</td>
<td>17.02</td>
<td>0.80</td>
</tr>
<tr>
<td>3β-Phenyltropane-2β-carboxylic acid-TMS (5)</td>
<td>18.30</td>
<td>0.86</td>
</tr>
<tr>
<td>2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (8)</td>
<td>19.49</td>
<td>0.92</td>
</tr>
<tr>
<td>2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropaneb (7)</td>
<td>19.89</td>
<td>0.94</td>
</tr>
<tr>
<td>2β-Carboxymethoxy-3β-benzoyloxytropane (1)</td>
<td>21.25</td>
<td>1.00</td>
</tr>
<tr>
<td>3β-Phenyltropane-2β-carboxylic acid (5)</td>
<td>22.57</td>
<td>1.06</td>
</tr>
</tbody>
</table>

aConditions given in Experimental Section  
bRTI-126

(ACD/Labs, Toronto, Canada). Structure elucidation and the prediction of $^1$H and $^{13}$C spectra was accomplished using ACD/Labs software.

**Synthesis**

The procedures of Carroll *et al.* [2, 3] were followed for the preparation of RTI-126 and its intermediates. Synthetic details and yield values are not reported.

**Results and Discussion**

The synthetic procedure (Figure 2) to give RTI-126 begins with cocaine 1, but the first intermediate compound (anhydroecgonine methyl ester) 2 can also be produced from other cocaine derivatives such as ecgonine, ecgonine methyl ester, benzoylecgonine, or anhydroecgonine (all controlled substances). It is unlikely that a Mannich-type condensation reaction would be utilized to eventually give 2, because of extremely low yields and it results in an enantiomeric (racemic) mixture. Reactions in some steps require temperatures from -45 to -78°C, thus limiting this synthesis to sophisticated laboratories equipped to operate at this range. The synthesis of 2β-carboxymethoxy-3β-phenyltropane 3 gives significant amounts of the 2α-epimer 4 and other by-products, which must be separated through chromatographic means. Saponification of 3 gives the carboxylic acid 5, which is then converted to the acid chloride 6. Formation of the oxadiazol group in 7 is very low yielding. Epimerization of 7 to 8 is accomplished via treatment with sodium methoxide.

GC retention time data for the respective compounds (Figure 2) are presented in Table 1; a mixture of 7 and 8 was baseline resolved.

The FTIR spectra for 7 and 8 as their HCl ion-pairs are illustrated in Figure 3. Comparison of the hydrochloride ion pairs reveals somewhat similar absorption patterns, however prominent differences in the C-H out-of-plane bending frequencies between 500-900 cm$^{-1}$ easily distinguish the two compounds.

Mass spectra for 7 and 8 are presented in Figure 4. Both compounds have a base peak at m/z 82, indicative of the N-methyl-pyrrolidinium ion [4]. In addition, both have multiple fragment ions with ion abundances in the same pattern, including a molecular ion at m/z 283. The two spectra are virtually identical and indistinguishable. Since the spectra are identical, additional or supplementary spectroscopic methods must be utilized for identification. Spectra of known intermediate products are included (Figures 5 and 6), in case they reside as impurities in a sample of suspected 7. The intermediates 3 and 4 (Figure 5) also gave virtually identical mass spectra, including a base peak at m/z 82, multiple fragment ions with ion abundances in the same pattern, and a molecular ion at m/z 259. However, GC retention times differentiate these compounds. Finally, the spectrum of carboxylic acid 5 is shown both underivatized and as its TMS derivative (Figure 6).

The $^1$H and $^{13}$C chemical shifts and splitting patterns for 7 and 8 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 protons using COSY (coupled protons) and NOESY (protons spatially near each other) experiments, as well as proton and carbon using the HSQC (directly bonded carbon to proton) and HMBC (2, 3, or 4 bond correlation between carbon and proton) experiments. Structure elucidation was performed on NMR data manually and by using the ACD/Labs Structure Elucidator program.

The key diagnostic feature of the proton spectrum, for differentiating compounds of this type, is the splitting pattern and coupling constants of H-3 (found at 3.3 ppm). This axial proton is coupled to three protons: H-2 (either equatorial as in structure 7 or axial as in structure 8) and the two H-4 protons. An axial-axial coupling constant is typically twice the size (about 12 Hz) of an axial-equatorial coupling (about 6 Hz). In the case of structure 7, H-2 is equatorial (H3ax-H2eq=5.7 Hz) giving H-3 a doublet of triplets peak pattern (13.1, 5.7, 5.7 Hz in a 1:2:2:1 pattern), while in the case of structure 8, H-2 is axial (H3ax-H2ax=12.1 Hz) giving H-3 a triplet of doublets pattern (12.1, 12.1, 5.7 Hz in a 1:1:2:2:1:1 pattern).

A 5 mg sample of RTI-126 was acquired from an undisclosed
Figure 5 - Electron ionization mass spectra of (a) 2β-carbomethoxy-3β-phenyltropane 3 and (b) 2α-carbomethoxy-3β-phenyl-tropane 4.
Figure 6 - Electron ionization mass spectra of (a) 3β-phenyltropane-2β-carboxylic acid 5 and (b) 3β-phenyltropane-2β-carboxylic acid TMS derivative.
Table 2 - NMR proton/carbon chemical shifts (in ppm) and splitting patterns of 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 7 (RTI-126). Samples run in CDCl₃ with TMS as the reference compound for 0 ppm.

<table>
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<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>2 eq</td>
<td>3.51</td>
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<td>dd</td>
<td>5.7, 2.8</td>
</tr>
<tr>
<td>3 ax</td>
<td>3.30</td>
<td>1</td>
<td>ddd</td>
<td>13.1, 5.7, 5.5</td>
</tr>
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<td>4 ax</td>
<td>2.67</td>
<td>1</td>
<td>td</td>
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</tr>
<tr>
<td>4 eq</td>
<td>1.78</td>
<td>1</td>
<td>ddd</td>
<td>13.1, 5.5</td>
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<tr>
<td>5</td>
<td>3.45</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
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<td>6 endo</td>
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<td>1</td>
<td>dddd</td>
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<td>3</td>
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<tr>
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<td>2.24</td>
<td>3</td>
<td>s</td>
<td>-</td>
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<tr>
<td>C-3 of oxadiazol</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-5 of oxadiazol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>phenyl (ortho, meta)</td>
<td>7.19</td>
<td>4</td>
<td>m</td>
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<tr>
<td>phenyl (para)</td>
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<tr>
<td>phenyl (quaternary)</td>
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Proton and carbon chemical shifts are in ppm referenced to TMS (0 ppm)
Type abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet

Table 3 - NMR proton/carbon chemical shifts (in ppm) of 2α-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane 8. Samples run in CDCl₃ with TMS as the reference compound for 0 ppm.

<table>
<thead>
<tr>
<th>No.</th>
<th>Proton H's</th>
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<th>J (Hz)</th>
<th>Carbon</th>
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<td>dd</td>
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<td>2 ax</td>
<td>3.75</td>
<td>1</td>
<td>dd</td>
<td>12.1, 2.2</td>
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<td>3 ax</td>
<td>3.32</td>
<td>1</td>
<td>td</td>
<td>12.1, 12.1, 5.7</td>
</tr>
<tr>
<td>4 ax</td>
<td>3.32</td>
<td>1</td>
<td>ddd</td>
<td>13.5, 12.1, ~2</td>
</tr>
<tr>
<td>4 eq</td>
<td>1.73</td>
<td>1</td>
<td>ddd</td>
<td>13.5, 5.7, 2.9</td>
</tr>
<tr>
<td>5</td>
<td>3.32</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>6 endo</td>
<td>1.79</td>
<td>1</td>
<td>dddd</td>
<td>12.2, 9.7, ~6.5, 4.7</td>
</tr>
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<td>6 exo</td>
<td>2.16</td>
<td>1</td>
<td>m</td>
<td>-</td>
</tr>
<tr>
<td>7 endo</td>
<td>2.07</td>
<td>1</td>
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<td>13.8, 9.7, 4.7</td>
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<td>1</td>
<td>dddd</td>
<td>13.8, 12.1, 6.5, 4.7</td>
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<td>N-methyl</td>
<td>2.44</td>
<td>3</td>
<td>s</td>
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<td>CH₃ on oxadiazol</td>
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<td>C-5 of oxadiazol</td>
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<tr>
<td>phenyl (ortho, meta)</td>
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<td>4</td>
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<tr>
<td>phenyl (quaternary)</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

Proton and carbon chemical shifts are in ppm referenced to TMS (0 ppm)
Type abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet
source and analyzed. GC/MS analysis of the sample revealed that 7 contributed to 96+% of the total ion chromatogram. Three impurities were detected, and were identified as 3 (~0.5%), 4 (~0.2%), and 8 (~2.7%).

Conclusions
Analytical data is presented to assist in characterizing suspected drug exhibits containing 2β-(1,2,4-oxadiazol-5-methyl)-3β-phenyltropane (RTI-126), as well as some synthetic intermediates. Characterization of RTI-126 is best achieved by combined GC/MS and FTIR or NMR spectroscopy.

References
The Characterization of Anastrozole

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Drug Enforcement Administration
Special Testing and Research Laboratory
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Dulles, VA 20166

[Email address withheld at corresponding author’s request]


ABSTRACT: The analysis and characterization of 2-[5-(1-cyano-isopropyl)-3-(1,2,4-triazolylmethyl)phenyl]-2-methylpropanenitrile (Anastrozole) is presented. Gas chromatography/mass spectrometry (GC/MS), Fourier-Transform nuclear magnetic resonance spectroscopy (FTNMR), and solid phase Fourier-Transform infrared (FTIR) spectroscopy data are presented.

KEYWORDS: anastrozole, GC/MS, NMR, FTIR, forensic chemistry

Anastrozole (Figure 1) is described as an aromatase inhibitor used in the treatment of breast cancer [1]. Anecdotal internet reports indicate that anastrozole is misused by males in the midst of an illicit steroid cycle to overcome production of excess estrogens [2]. Astra-Zeneca markets this particular drug in “Arimidex” preparations.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). Anastrozole was obtained from Accustandard (New Haven, CT). All other chemicals were reagent grade quality and products of Aldrich Chemical (Milwaukee, WI).

Figure 1 - Anastrozole.

Chemical Formula/CAS Number: C_{17}H_{19}N_{5} / [120511-73-1]
Molecular Weight: 293.35 amu
Melting Point: 81-82°C [1]
Solubility: Deionized Water: sparingly soluble; Methanol, Acetone, Ethanol, and Tetrahydrofuran: freely soluble; Acetonitrile: very soluble [3]

Nuclear Magnetic Resonance (NMR) Spectroscopy:

\(^1\)H- and \(^13\)C-NMR spectra (Figures 2 and 3, respectively) were acquired on a Varian 400MR 400 MHz instrument using an AutoX 5 mm indirect detect pulse field gradient (PFG) probe at 26°C. \(^1\)H parameters: Number of scans (nt) = 8, pulse width (pw) = 45°, relaxation delay (d1) = 1 s, acquisition time (at) = 2.6 s; \(^13\)C parameters: nt = 256, pw = 45°, d1 = 1 s, at = 1.3 s, proton decoupled). Spectra were processed using ACD/Labs SpecManager software (Advanced Chemistry Development Inc.©, Toronto, Canada). Anastrozole was prepared with CDCl$_3$ containing 0.05 wt % tetramethylsilane (TMS). Chemical shifts (δ) are reported in parts per million (ppm) using TMS (0.0 ppm) as the reference standard.

Fourier Transform Infrared Spectroscopy (FTIR):

The spectrum (Figures 4a and 4b) was acquired using a Thermo-Scientific iZ10 Spectrophotometer with a Golden Gate attenuated total reflectance (ATR) accessory. The spectrum was collected using 32 scans between 4000 cm$^{-1}$ and 400 cm$^{-1}$.

Gas Chromatography/Mass Spectrometry (GC/MS):

The spectrum (Figure 5) was 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 electron ionization. The GC was fitted with a 30 m × 0.25 mm I.D. fused silica capillary column coated with 0.50 μm 35% phenyl, 65% dimethyl arylene siloxane (DB-35MS), and was operated in splitless mode. The injection port was maintained at 250°C. The oven temperature program was as follows: Initial temperature 90°C (1 minute), ramped to 300°C at 8°C per minute (final hold 10 minutes). Helium was used as a purge gas at a rate of 60 mL/second. Methanol was used as the solvent.

Discussion

The mass spectrum of anastrozole is fairly rich in detail yielding a m/z of 209 as the base peak with the second most abundant mass of 70 roughly half the intensity of the base. The mass spectrum readily indicates anastrozole’s molecular ion at 293 mass units. The fragmentation of nitrile groups are indicated by the loss of 27 from the molecular ion as well [4]. The FTIR spectrum is very detailed with a number of sharp bands in the fingerprint region that should enable relative ease
Figure 2 - H\textsuperscript{1} NMR spectrum of anastrozole.

Figure 3 - C\textsubscript{13} NMR spectrum of anastrozole.
Figure 4 - FTIR of anastrozole: (a) full spectrum, (b) fingerprint region expanded.
for identification and discrimination. The NMR spectra of anastrozole are abundant in both proton and carbon observations enabling confirmation of the structure as well.

References:

Figure 5 - Mass spectrum of anastrozole.
Identification of Levamisole and Lidocaine Acetylation Reaction Impurities Found in Heroin Exhibits

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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: Five (5) compounds, S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate; 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one; N-acetyl-lidocaine; N-(2,6-dimethylphenyl)acetamide; and N-acetyl-N-(2,6-dimethylphenyl)acetamide were identified as impurities present in a heroin exhibit containing levamisole and lidocaine. Spectroscopic and chromatographic data are provided for characterization of these products. The presence of these impurities suggests that levamisole and lidocaine were added to morphine base prior to acetylation of morphine with acetic anhydride that produced heroin and the aforementioned compounds.

KEYWORDS: heroin, levamisole, lidocaine, acetylated products, mass spectrometry, forensic science

The Drug Enforcement Administration’s Special Testing and Research Laboratory conducted an analysis of an exhibit as a part of its Heroin Signature Program (HSP), and determined that the exhibit contained 78.4% heroin, 2.3% levamisole, 1.4% lidocaine, and other typical heroin-related alkaloids. In addition to the heroin, levamisole, and lidocaine identified in the exhibit, four other unidentified compounds of significant concentration and one compound at a trace level were observed. Examination of the total ion chromatographic profile indicated the presence of fragment ions for two of those compounds that appear to be related to levamisole impurities found in some cocaine exhibits [1]. Furthermore, fragment ions for three other compounds present in the profile indicated the possible presence of acetylated lidocaine and two lidocaine-related compounds. Although levamisole, an anitneoplactic, has been a known cocaine adulterant for more than five years [2], recently, it has become more prevalent in heroin exhibits. Some researchers have suggested that levamisole may enhance the effects of cocaine [3,4]; however, it is rarely found in heroin exhibits. Lidocaine, an anesthetic, has been a known adulterant of heroin for many years. Lidocaine and levamisole-related impurities have not been reported previously in heroin exhibits. A second heroin exhibit containing suspected levamisole-related impurities was also identified.

The results presented herein reveal the presence of levamisole and lidocaine acetylation by-products formed during illicit heroin processing. Lidocaine and levamisole were subjected to acetylation reactions, preparative isolation, gas chromatographic-mass spectrometric, and nuclear magnetic resonance analyses. These acetylation by-products were characterized as S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate; 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one; N-acetyl-lidocaine; N-(2,6-dimethylphenyl)acetamide; and N-acetyl-N-(2,6-dimethylphenyl)acetamide. Reaction mechanisms are proposed for the formation of these by-products.

Experimental
Solvents, Chemicals, 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 were products of Sigma-Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4% (w/w). Levamisole HCl, lidocaine HCl, heroin HCl, and morphine base were part of the authentic reference collection of the DEA Special Testing and Research Laboratory. A reference standard of 2,6-dimethylacetanilide (N-(2,6-dimethylphenyl)acetamide) was obtained from Sigma-Aldrich Chemical.

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 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 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.

Nuclear Magnetic Resonance Spectroscopy (NMR)
Proton (1H) spectra were obtained on a Varian (Palo Alto, CA) Model 5973 quadrupole mass-selective detector 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 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 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.
The temperature of the samples 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).

**Synthesis**

S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate: Levamisole HCl (440 mg, 1.83 mmol) was heated at 75°C with acetic anhydride (2.0 mL, 27.3 mmol) in a 15 mL capped centrifuge tube for 35 hrs. The reaction was cooled and quenched with 30 mL of water, solid Na₂CO₃ was added until pH = 9 was measured, and the reaction was extracted with CHCl₃ (2 x 75 mL). The CHCl₃ was washed with 0.36 N H₂SO₄ (to remove unreacted levamisole), dried over anhydrous sodium sulfate, and evaporated in vacuo to an orange-amber oil (120 mg crude material). The crude material was eluted with CHCl₃ on a glass chromatographic column (25 cm x 1.0 cm i.d.) containing 10 g of basic alumina (150 mesh). The first 20 mL of eluate was collected and evaporated to dryness to give the target compound as 32 mg of clear oil (yield not calculated).

3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one: Levamisole HCl (3.0 g, 12.5 mmol) and acetic anhydride (15.0 mL, 204 mmol) were refluxed in a 100 mL round-bottom flask equipped with a reflux condenser for 35 hrs. The reaction was transferred to a 250 mL flask containing 150 mL of 0.36 N H₂SO₄ and stirred for 24 hrs at 75°C. The reaction was extracted with CHCl₃ (3 x 75 mL), extracts combined, dried over anhydrous sodium sulfate, and evaporated in vacuo to a crude mixture. The crude material was eluted on a glass chromatographic column (90 cm x 4.5 cm i.d.) containing 300 g of basic alumina (150 mesh). The column was eluted with CHCl₃ (500 mL), followed by a mixture of CHCl₃/acetone (1:1). The first 200 mL of the CHCl₃/acetone (1:1) eluate was collected and evaporated to dryness to give 1.14 grams of material containing the desired compound at ca. 20% purity. Approximately 280 mg of this material was chromatographed again 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 the following solvent series: 30 mL Et₂O/CHCl₃ (1:1), 10 mL Et₂O/CHCl₃ (4:6), 10 mL Et₂O/CHCl₃ (3:7), 10 mL Et₂O/CHCl₃ (2:8), 10 mL Et₂O/CHCl₃ (1:9), 50 mL CHCl₃, 20 mL CHCl₃/acetone (19:1), 20 mL CHCl₃/acetone (18:2), 20 mL CHCl₃/acetone (17:3), 20 mL CHCl₃/acetone (16:4), 20 mL CHCl₃/acetone (15:5), 20 mL CHCl₃/acetone (14:6), 20 mL CHCl₃/acetone (13:7), 20 mL CHCl₃/acetone (12:8), 20 mL CHCl₃/acetone (11:9), 20 mL CHCl₃/acetone (10:10), 20 mL CHCl₃/acetone (9:11), 20 mL CHCl₃/acetone (8:12), 20 mL CHCl₃/acetone (7:13), 20 mL CHCl₃/acetone (6:14), 20 mL CHCl₃/acetone (5:15), 20 mL CHCl₃/acetone (4:16), 20 mL CHCl₃/acetone (3:17), 20 mL CHCl₃/acetone (2:18), 20 mL CHCl₃/acetone (1:19), 20 mL CHCl₃/acetone (0:20), and finally, 20 mL CHCl₃/acetone (0:21). The eluate was collected and evaporated to dryness to give 280 mg of material containing the desired compound at 85% purity.
Fractions of 10-mL were collected and monitored via gas chromatography. Fractions 8-11 were combined and evaporated to dryness to give the target compound as 8 mg of clear oil (yield not calculated).

N-acetyl-N-(2,6-dimethylphenyl)acetamide:
2,6-dimethylacetanilide (100 mg, 0.61 mmol) was heated at 120°C with acetic anhydride (2.0 mL, 27.3 mmol) in a 15 mL capped centrifuge tube for 22 hrs. The reaction was cooled and quenched with 10 mL of water, solid Na₂CO₃ was added until pH = 9 was measured, and the reaction was extracted with CHCl₃ (1 x 10 mL). The CHCl₃ was dried over anhydrous sodium sulfate, and evaporated in vacuo to yield 110 mg of a clear oil (yield not calculated).

N-acetyl-lidocaine: Repeated attempts to acetylate lidocaine yielded mixtures of acetyl-lidocaine, 2,6-dimethylacetanilide, and N-acetyl-N-(2,6-dimethylphenyl)acetamide which could not be separated. A typical reaction was as follows: lidocaine (130 mg, 0.48 mmol) was heated at 75°C with acetic anhydride (1.0 mL, 13.6 mmol) in a 15 mL capped centrifuge tube for 8 days. The reaction was cooled and quenched with 8 mL of 0.36 N H₂SO₄ and extracted with CHCl₃ (1 x 10 mL). The CHCl₃ was dried over anhydrous sodium sulfate, and evaporated in vacuo to yield a mixture of acetyl-lidocaine, 2,6-dimethylacetanilide, and N-acetyl-N-(2,6-dimethylphenyl)acetamide. Although N-acetyl-lidocaine (33% purity via GC-FID) could not be isolated from the by-products, its mass spectrum was obtained (yield not calculated).

**GC-MSD Analytical Artifact Experiments**
A solution of levamisole HCl (0.13 mg/mL), lidocaine HCl (0.13 mg/mL), and heroin HCl (1.07 mg/mL) was made in CHCl₃. An identical solution was prepared in MeOH. Each solution was injected under the conditions detailed previously. The resulting chromatographic profiles were examined for the formation of analytical artifacts related to acetylated levamisole and lidocaine.

**Acetylation of a Morphine, Lidocaine, and Levamisole Mixture**
A mixture of levamisole HCl (102 mg, 0.42 mmol), lidocaine HCl (102 mg, 0.35 mmol), and illicit morphine base (729 mg, 2.56 mmol) was heated at 120°C with acetic anhydride (3.0 mL, 40.9 mmol) in a 15-mL capped centrifuge tube for 2 hrs. The reaction was cooled and quenched with 40 mL of water, solid Na₂CO₃ was added until pH = 9 was measured, and the precipitated product was captured via suction filtration. The precipitate was washed further with 40 mL of water and then allowed to dry overnight at room temperature.

The precipitated product and filtrate (after CHCl₃ extraction) were each examined via GC-MSD for acetylated products of levamisole and lidocaine.

**Results and Discussion**
Initial GC-MSD analysis of two heroin signature exhibits demonstrated the presence of peaks in their reconstructed total ion chromatograms (Figure 1a and 1b, Table 1) that could not be identified without further analysis. Two unknown

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**Table 1 - Retention times (R_t) and Relative Retention Times (R_R) of levamisole, lidocaine, and related impurities resulting from acetylation.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R_t (min)</th>
<th>R_R</th>
<th>GC/MS Peak #</th>
</tr>
</thead>
<tbody>
<tr>
<td>163 compound</td>
<td>8.66</td>
<td>0.50</td>
<td>1</td>
</tr>
<tr>
<td>205 compound</td>
<td>8.99</td>
<td>0.52</td>
<td>2</td>
</tr>
<tr>
<td>caffeine</td>
<td>14.95</td>
<td>0.87</td>
<td>3</td>
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<tr>
<td>N-acetyl-lidocaine</td>
<td>15.61</td>
<td>0.90</td>
<td>4</td>
</tr>
<tr>
<td>lidocaine</td>
<td>16.30</td>
<td>0.94</td>
<td>5</td>
</tr>
<tr>
<td>levamisole</td>
<td>17.25</td>
<td>1.00</td>
<td>6</td>
</tr>
<tr>
<td>264 compound</td>
<td>20.56</td>
<td>1.91</td>
<td>7</td>
</tr>
<tr>
<td>306 compound</td>
<td>23.64</td>
<td>1.37</td>
<td>8</td>
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<tr>
<td>acetylcodeine</td>
<td>25.47</td>
<td>1.48</td>
<td>9</td>
</tr>
<tr>
<td>O₆-monocaetylmorphine</td>
<td>26.16</td>
<td>1.52</td>
<td>10</td>
</tr>
<tr>
<td>heroin</td>
<td>27.20</td>
<td>1.58</td>
<td>11</td>
</tr>
<tr>
<td>papaverine</td>
<td>29.35</td>
<td>1.70</td>
<td>12</td>
</tr>
<tr>
<td>diltiazem</td>
<td>30.69</td>
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<td>13</td>
</tr>
<tr>
<td>Triacetylmorphine</td>
<td>31.20</td>
<td>1.81</td>
<td>14</td>
</tr>
</tbody>
</table>

---

*Conditions given in experimental section.

**N-(2,6-dimethylphenyl)acetamide**

**N-acetyl-N-(2,6-dimethylphenyl)acetamide**

**3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one**

**S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate**
Figure 2 - Electron ionization mass spectrum of (a) 3-acetyl-4-phenyl-1-(2-sulfanyylethyl)imidazolidin-2-one (264 compound), (b) S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate (306 compound), (c) levamisole.
compounds (Peaks #7 and #8) were observed having apparent molecular ions at \(m/z\) 264 and \(m/z\) 306, respectively, as illustrated in Figure 2a and 2b. These compounds appeared to be related to levamisole based on the presence of ions found at \(m/z\) 132 and \(m/z\) 175. Although these two fragment ions do not directly indicate levamisole, other reported levamisole degradation products contain these ions [1]. Further examination of the spectra for peaks #7 and #8 (Figure 2a and 2b), showed the presence of an ion at \(m/z\) 43 that is indicative of an acetyl loss. The mass spectral data for these two compounds suggested that each compound was an acetylated levamisole by-product. Levamisole was acetylated as outlined in the experimental section and produced two compounds, each with identical mass spectra to peaks #7 and #8. Both synthetic compounds were isolated and their structures were elucidated via NMR (Table 2) and MS analysis. The levamisole acetylation by-products, S-\(\{2\)-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethylethanoate and 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one (Figure 3) were verified for peaks #7 and #8.

Additionally, two other compounds (Figure 1b, Peaks #1 and #2) with apparent molecular ions at \(m/z\) 163 and \(m/z\) 205, respectively, were found. Peak #1 was identified by its mass spectrum (Figure 4a) as N-(2,6-dimethylphenyl)acetamide after comparison to a known standard. Both peaks #1 and #2 appeared to be lidocaine related, based on the 2,6-dimethyl aromatic substitution (also found in lidocaine, Figure 5). Peak #2 produced a mass spectrum (Figure 4b) of 42 Daltons greater

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### Table 2 - NMR Data for levamisole and its acetylated products

<table>
<thead>
<tr>
<th></th>
<th>(6R)-6-phenyl-2,3,5,6- tetrahydroimidazo[2,1-b][1,3]thiazole</th>
<th>(4R)-3-acetyl-4-phenyl-1-(2-sulfanylethyl)-imidazolidin-2-one</th>
<th>S-{2-(4R)-3-acetyl-2-oxo-4-phenylimidazolidin-1-yl}ethyl\ethanethioate</th>
</tr>
</thead>
<tbody>
<tr>
<td>position</td>
<td>proton carbon</td>
<td>proton carbon</td>
<td>proton carbon</td>
</tr>
<tr>
<td>Phenyl ring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quaternary</td>
<td>- 142.8</td>
<td>- 141.1</td>
<td>- 141.1</td>
</tr>
<tr>
<td>ortho</td>
<td>7.34 m 126.5</td>
<td>7.29 d 125.5</td>
<td>7.26 d 125.5</td>
</tr>
<tr>
<td>meta</td>
<td>7.34 m 128.5</td>
<td>7.35 t 129</td>
<td>7.35 t 128.9</td>
</tr>
<tr>
<td>para</td>
<td>7.26 m 127.7</td>
<td>7.3 t 128.2</td>
<td>7.29 t 128.1</td>
</tr>
<tr>
<td>Imidazolidine ring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>Bonded to C2, C5, and C6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>N1-C(=)N3</td>
<td>N1-C(=)N3</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>Bonded to C2 and C4</td>
<td>N1-C(=)N3</td>
<td></td>
</tr>
<tr>
<td>C4-phenyl</td>
<td>CH 5.46 t 76.9</td>
<td>CH 5.33 dd 54.3</td>
<td>CH 5.31 dd 54.3</td>
</tr>
<tr>
<td>C5</td>
<td>CH2 3.68 t, 2.99 t</td>
<td>CH2 3.34 dd, 3.90 t</td>
<td>CH2 3.33 dd, 3.89 t</td>
</tr>
<tr>
<td>N1-CH2-CH2-S</td>
<td>N1-CH2-CH2-S</td>
<td>N1-CH2-CH2-S</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>N1-CH2-CH2-S</td>
<td>N1-CH2-CH2-S</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>N1-CH2-CH2-S</td>
<td>N1-CH2-CH2-S</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>SH bonded to C7 only</td>
<td>S-C(=)N3</td>
<td></td>
</tr>
</tbody>
</table>

Proton abbreviations: d = doublet, m = multiplet, s = singlet, t = triplet

*IUPAC Names using Advanced Chemistry Development, Inc., ACD/Name, version 12.00, Toronto, Canada.*

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Figure 3 - Structural formulae of levamisole and related acetylation by-products.
Figure 4 - Electron ionization mass spectrum of (a) N-(2,6-dimethylphenyl)acetamide (163 compound), (b) N-acetyl-N-(2,6-dimethylphenyl)acetamide (205 compound), (c) N-acetyl-lidocaine (276 compound), (d) lidocaine.
than N-(2,6-dimethylphenyl)acetamide (Figure 4a), suggesting it was the acetyl derivative of peak #1. N-(2,6-dimethylphenyl)acetamide was acetylated as outlined in the experimental section to produce N-acetyl-N-(2,6-dimethylphenyl)acetamide (Figure 5). The resulting mass spectrum was identical to peak #2; its NMR spectrum was consistent with the expected resonances (Table 3). Finally, lidocaine was acetylated with acetic anhydride to form N-acetyl-lidocaine. The resulting mass spectrum (Figure 4c) was identical to peak #4 (Figure 1b). Additionally, acetylation of lidocaine produced N-(2,6-dimethylphenyl)acetamide and N-acetyl-N-(2,6-dimethylphenyl)acetamide.

In order to demonstrate that the target compounds are not formed as analytical artifacts, solutions containing heroin, lidocaine, and levamisole were examined via GC/MS using chloroform and methanol as separate injection solvents. Examination of the resulting chromatographic profiles did not indicate the presence or formation of any acetylated lidocaine or levamisole by-products.

Finally, a mixture of illicit morphine, levamisole, and lidocaine were reacted with acetic anhydride to produce heroin. The resulting heroin sample was examined via GC/MS (Figure 1c). One acetylated levamisole by-product, S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate (306 compound), as well as all three acetylated lidocaine products were produced and detected in the resulting heroin. Additionally, these four compounds were also detected in higher concentrations in the precipitation filtrate. The presence of 3-acetyl-4-phenyl-1-(2-sulfanylethyl)imidazolidin-2-one (264 compound) was not detected since it is a degradation product of S-[2-(3-acetyl-2-oxo-4-phenylimidazolidin-1-yl)ethyl] ethanethioate (306 compound).

**Conclusions**

Characterization of the five impurities present in the heroin exhibits, in concert with the performed acetylation experiments, demonstrate that levamisole and lidocaine were added to the illicit morphine prior to the acetylation reaction that produced the heroin. It is unlikely that lidocaine and levamisole were purposely added to the morphine to enhance the effect of the final heroin product since cutting agents or adulterants are typically added to the final heroin product prior to trafficking or distribution.

**Acknowledgement**

The authors are indebted to Patrick A Hays of this laboratory for his assistance in acquiring the NMR data.
References
Chiral Separation of Methamphetamine and Related Compounds using Capillary Electrophoresis with Dynamically Coated Capillaries

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ABSTRACT: The chiral differentiation of the dextro- and levo- isomers of methamphetamine and certain precursor and/or byproducts in methamphetamine exhibits is obtained at levels down to 0.2% relative to total methamphetamine. Dynamic coating of the capillary surface is accomplished by rapid flushes of 0.1N sodium hydroxide, water, a buffer containing a polycation coating reagent, and a reagent containing a polyanionic coating reagent plus hydroxypropyl-β-cyclodextrin. The methodology has been successfully applied to samples which contain skewed ratios of d- and l-methamphetamine even at trace levels.

KEYWORDS: methamphetamine, chiral analysis, capillary electrophoresis, dynamically coated capillaries, forensic chemistry

The determination of the enantiomers of methamphetamine, its precursors, and/or by-products is important for legal and intelligence purposes [1]. Under federal sentencing guidelines, sentencing enhancement depends on whether the sample contains dextro-methamphetamine hydrochloride over 80% (ice) [2]. Isomer determination can help identify synthetic methodologies. For example, the presence of dextro-pseudoephedrine and dextro-methamphetamine could indicate the methamphetamine was produced from the reduction of pseudoephedrine.

Gas chromatography (GC) [3-5], High Performance Liquid Chromatography (HPLC) [6,7], and Capillary Electrophoresis (CE) [8-10] have all been used to determine enantiomers of phenethylamines in methamphetamine exhibits. There are some limitations for GC and HPLC for the simultaneous analysis of the above solutes. Derivatizations with chiral reagents are often required. Enantiomerically impure reagents often mask detection of low level isomers in a skewed-ratio sample. Over the past few years, the occurrence of non-racemic mixed enantiomer methamphetamine samples has been identified by the DEA laboratory system. Although chiral GC and HPLC columns are available, derivatization has been required for capillary GC. In addition, HPLC columns (chiral and achiral) typically have relatively low plate counts, which can result in poor resolution and/or long analysis times. CE can be performed without prior derivatization by employing chiral additives in the run buffer. Neutral [8] and charged cyclodextrins [9] and mixtures of these reagents [9] have been used. Dynamically coating the capillary, which gives rise to a relatively high and robust electroosmotic flow at lower pH values compared to uncoated capillaries [10-13], is well suited for chiral analysis of basic solutes. Although a dual dynamic coating procedure allowed baseline resolution of the dextro- and levo- isomers of amphetamine, methamphetamine, and pseudoephedrine, the enantiomeric separation of ephedrine and the resolution of the individual enantiomers from each other was lacking [10].

An improved dual dynamic coating procedure in terms of overall separation as well as sensitivity is presented for the analysis of methamphetamine exhibits.

Experimental

Chemicals, Material, and Reagents
Standards were obtained from the reference collection of this laboratory. Sodium hydroxide 0.1N, CElixir A (pH 2.5), CElixir B (pH 2.5), CElixir B (pH 2.5) with hydroxypropyl-β-cyclodextrin (HPBCD) 1 (Custom Chiral2 Buffer), and injection solvent concentrate (75 mM sodium phosphate, pH 2.5) were all acquired from MicroSolv Technology (Long Branch, NJ). Deionized and high purity water (HPLC-grade water) were obtained from a Millipore Synergy 185 water system (Bedford, MA).

Instrumentation and Procedures
An Agilent Model HP3D CE Capillary Electrophoresis System fitted with a diode array detector (Waldbrronn, Germany) was used for CE separations. New, bare silica capillaries were conditioned following the same procedure used for regular analysis. Capillaries were first flushed with 0.1 N 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. 1.0 mL polypropylene vials were used as reservoirs for 0.1N sodium hydroxide solution and for the run buffer; while 2.0 mL glass vials were used as reservoirs for the remaining flush solutions, waste vials, and samples. 0.1N sodium hydroxide and run buffer vials were filled with 500 μL of liquid, while samples and flush vials containing CElixir Reagent A and water, respectively, were filled with 1000 μL of liquid. Waste vials were filled with 500 μL of water.

Standard and Sample Preparation
An injection solvent concentrate was diluted with 1:20 HPLC-grade water. For standard solutions, an appropriate amount of standard dextro and levo isomers of methamphetamine HCl, amphetamine sulfate, ephedrine HCl, and pseudoephedrine HCl was added to a test tube containing sodium hydroxide and run buffer vials were filled with 500 μL of liquid, while samples and flush vials containing CElixir Reagent A and water, respectively, were filled with 1000 μL of liquid. Waste vials were filled with 500 μL of water.

1Since different lots of a cyclodextrin can vary in both the degree of substitution and the position of substituents, each time a new batch of HPBCD is received, a test mixture is analyzed; and, if necessary, a small change is made in the concentration of the HPBCD (original concentration 78 mM).
and pseudoephedrine HCl were weighed into an appropriate volumetric flask and diluted with injection solvent (after the addition by pipetting of internal standard) in order to obtain a final concentration of approximately 0.10 mg/mL of each isomer of methamphetamine, 0.008 mg/mL of the other target isomers, and 0.10 mg/mL of a racemic mixture of the internal standard (n-butylamphetamine). For sample solutions, an appropriate amount of weighed material was added into an appropriate volumetric flask and diluted with injection solvent (after the addition by pipetting of the internal standard) in order to obtain a final achiral methamphetamine concentration of approximately 0.20 mg/mL and an internal standard concentration of 0.10 mg/mL.

**Capillary Electrophoresis Conditions**

Either a 50mm ID 64.5 cm (56.0 cm to the detector) fused silica capillary obtained from Polymicro Technologies (Phoenix, AZ) or a pre-made capillary (Agilent) with the same dimensions was used at 15°C. The run buffer consisted of CElixer B (pH 2.5) with or without HPBCD. For all CE runs, a 50 mbar pressure injection of 16 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 either 20 kV (run buffer B reagent) or 30 kV (run buffer B reagent + HPBCD) was used.

**Results and Discussion**

An improved separation over previously reported methodology [10] for the dextro- and levo- isomers of methamphetamine, amphetamine, ephedrine, and pseudoephedrine was obtained by a combination of approximately doubling the length of capillary and increasing the concentration of HPBCD. As shown in Figure 1, the individual enantiomers of these solutes, as well as the enantiomers of a structurally related internal standard (n-butylamphetamine), are well resolved in less than 17 minutes.

Highly precise run-to-run separations were obtainable as demonstrated by migration time, relative migration time (relative to the 2nd internal standard peak), corrected area (area/migration time), and relative corrected area precision (relative to the 2nd internal standard peak) (%RSD ≤ 0.13, ≤ 0.05, ≤ 2.0, and ≤ 0.92, respectively, n = 5). Because of the narrow peaks and the possibility of larger shifts in migration time, identification can be difficult based on migration time alone. Therefore, the use of relative migration times or co-injection is suggested for compound identification. Day-to-day and capillary-to-capillary reproducibility is also greater using relative migration times versus absolute migration times. Relative migration time data (relative to the 2nd internal standard peak) of solutes found in methamphetamine exhibits is given in Table 1. In addition, the combination of a relatively large sample concentration and injection preceded by the stacking effect of the water plug on the large methamphetamine peak(s), allows for the determination of individual enantiomers at levels down to 0.2% relative to total methamphetamine. In comparison to previously reported dynamically coated methodology [10], this represents an approximately 4 fold improvement in detection limits.

Since implemented for routine use for intelligence analysis, thousands of samples have been successfully analyzed. An electropherogram of a sample containing d-methamphetamine and d-pseudoephedrine is shown in Figure 2.

With the current trend of enantiomeric enrichment of methamphetamine isomers [14], chiral capillary electrophoresis enables the chemist to identify even the most subtle enrichment. Trace and non-trace determination of minor isomers, both dextro and levo, is essential in determining the route of synthesis and/or post-processing techniques employed by clandestine laboratory chemists. Electropherograms of three methamphetamine exhibits seized in the same case are shown in Figure 3. The exhibits, based on their skewed ratios of l- and d-methamphetamine (see Figures 1 and 3B-D), appear to be processed using a trace enrichment procedure. However, the non-racemic ratios do not eliminate the possible use of mixed precursor material or the post-production mixing of different batches.

A question can arise whether the minor peaks in Figure 3 are d- or l-methamphetamine. The identity of the peaks is supported by the achiral profile of one of the exhibits, (similar profile for other two exhibits) which indicates that the sample only contains one peak other than the internal standard (see Figure 3A).

![Figure 1](image-url)  
**Figure 1** - Dynamically coated CE separation of standard mixture of (a) l-pseudoephedrine, (b) d-ephedrine, (c) l-amphetamine, (d) l-ephedrine, (e) d-amphetamine, (f) l-methamphetamine, (g) d-methamphetamine, (h) d-pseudoephedrine, (i) n-butylamphetamine (1), and (j) n-butylamphetamine (2). CE conditions are described in the experimental section.
Figure 2 - Dynamically coated CE separation of a methamphetamine exhibit containing (g) d-methamphetamine, (h) d-pseudoephedrine, (i) n-butyl-amphetamine (1), and (j) n-butylamphetamine (2). CE conditions are described in the experimental section.

Figure 3 - Dynamically coated CE separations of methamphetamine exhibits containing (f) l-methamphetamine, (g) d-methamphetamine, (i) n-butylamphetamine (1), and (j) n-butylamphetamine (2).
A number of re-analyses have shown that the TPC method failed to identify near-trace level isomers when compared to CE analysis. Capillary electrophoresis has no such "masking" problem and easily separates the enantiomers. A chromatogram depicting the analysis of standard \textit{d}-methamphetamine using the TPC method (see Figure 4) indicates the presence of \textit{l}-methamphetamine as an artifact at approximately the 8% level. In contrast using CE no \textit{l}-methamphetamine is detected as an artifact (see Figure 2). An analysis not possible using the GC TPC method, i.e, the detection of the \textit{l} isomer at the approximately 1% level relative to the \textit{d} isomer of methamphetamine is shown in Figure 5.

Laboratory (both domestic and foreign) have contained skewed ratios of \textit{d}- and \textit{l}-methamphetamine contrary to the normal single enantiomer and racemate historically detected. A substantive number of these exhibits contain near-trace or trace amounts of an isomer. This instance poses analytical difficulty using traditional chemical derivatization agents such as (S)-(T)-(Trifluoroacetyl)prolylchloride (TPC or TFAP) which contain impurities, mainly the other enantiomer such as the (R)-prolyl, that effectively mask detection of the minor isomer in a skewed-ratio sample. In addition to the impurity's presence, the enantiomeric excess has been observed to degrade over time, thus diminishing the already hindered discrimination power. A number of re-analyses have shown that the TPC method failed to identify near-trace level isomers when compared to CE analysis. Capillary electrophoresis has no such "masking" problem and easily separates the enantiomers. A chromatogram depicting the analysis of standard \textit{d}-methamphetamine using the TPC method (see Figure 4) indicates the presence of \textit{l}-methamphetamine as an artifact at approximately the 8% level. In contrast using CE no \textit{l}-methamphetamine is detected as an artifact (see Figure 2). An analysis not possible using the GC TPC method, i.e, the detection of the \textit{l} isomer at the approximately 1% level relative to the \textit{d} isomer of methamphetamine is shown in Figure 5.
Table 1 - Relative migration times (relative to the 2nd internal standard peak (n-butylamphetamine)) of solutes related to methamphetamine. CE conditions are described in the experimental section.

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<tr>
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<td>loratadine</td>
<td>1.180</td>
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References
Detection of Phenethylamine, Amphetamine, and Tryptamine Imine By-Products from an Acetone Extraction

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ABSTRACT: The formation of imine by-products from phenethylamines, amphetamines, and tryptamines upon an acetone extraction is presented. These imine by-products were characterized using GC/MSD and exhibited preferential cleavage at the α-carbon of the alkyl chain. Further characterization of the imine by-products of phenethylamine and tryptamine was done using IR and NMR.

KEYWORDS: phenethylamine, tryptamine, imine, acetone, schiff base, drug chemistry, forensic chemistry

In most forensic laboratories, the solvents used to extract drugs are chosen based upon their solubility properties and their ability to not interact with the drug. In fact, there are very few publications where a solvent used to extract a drug reacts with the drug and forms by-products [1-3].

This laboratory recently discovered that an additional component was formed when acetone was used to extract a sample containing a known tryptamine. Analysis by gas chromatography/mass spectroscopy (GC/MS) of the acetone extract yielded an extra peak in the total ion chromatogram that was approximately half the abundance of the known tryptamine peak. The known tryptamine peak was identified from the fragmentation pattern and retention time. The unknown peak’s fragmentation pattern exhibited a molecular ion that was 40 mass units higher than that of the known tryptamine molecular ion and was subsequently identified as the imine formed from the reaction with acetone. Primary aliphatic amines are known to react with aldehydes and ketones, typically in the presence of an acid catalyst, to produce an imine, or a carbon-nitrogen double bond, Figure 1 [4, 5].

This study reports that the following phenethylamines, amphetamines, and tryptamines form imines with acetone under mild conditions: phenethylamine; 2,5-dimethoxy-4-iodophenethylamine (2C-I); 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2); 2,5-dimethoxy-4-ethylphenethylamine (2C-E); 2,5-dimethoxy-4-n-propylthio-β-phenethylamine (2C-T-7); 2,5-dimethoxy-4-chlorophenethylamine (2C-C); 2,5-dimethoxy-4-bromo-phenethylamine (2C-B); 2,5-dimethoxyamphetamine; 4-methoxyamphetamine, 3,4-methylenedioxyamphetamine (MDA); amphetamine; tryptamine; α-methyl-tryptamine; and 5-methoxy-α-ethyl-tryptamine. This study also reports that the GC/MSD of all imine compounds showed preferential cleavage at the α-carbon on the alkyl chain. In addition to GC/MS, the imines formed from phenethylamine base and tryptamine base were characterized by Fourier transform-infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy.

Experimental
Solvents, Chemicals, and Materials
Acetone was ACS/HPLC grade from Burdick and Jackson Laboratories (Muskegon, MI). Phenethylamine base and tryptamine base were obtained from Sigma-Aldrich Chemicals (Milwaukee, WI). All other compounds were obtained from the authentic reference collection of the DEA Special Testing and Research Laboratory.

Gas Chromatography/Mass Spectrometry (GC/MS)
GC/MS analyses were performed using an Agilent Model 5975C inert XL mass-selective detector (MSD) interfaced with an Agilent Model 7890A gas chromatograph. The GC system was fitted with a 30 m x 0.250 mm ID fused-silica capillary column coated with HP-5 (0.25 µm) supplied by J & W Scientific. The injection port temperature was maintained at 280°C and was operated in the split mode (25:1). The oven temperature was programmed as follows: initial temperature, 90°C; initial hold, 2 minutes; program rate 14°C/minute; final temperature, 300°C; final hold, 10 minutes. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-550 mass units, and at 2.83 scans/second. The MS source and MSD were maintained at 230°C and 150°C, respectively.

Fourier Transform-Infrared Spectroscopy (FTIR)
FTIR analyses were performed using a Thermo Scientific Model Smart Golden Gate attenuated total reflectance (ATR)
accessory with KRS-5 focusing elements, single bounce, attached to a Thermo Scientific Model Nicolet IZ10 spectrophotometer. The FTIR parameters were as follows: number of background scans, 32; number of scans, 32; resolution, 4.000; and sample gain, 8.0.

Nuclear Magnetic Resonance Spectroscopy (NMR)

One and two dimensional NMR analyses were performed on a Varian VNMRS 600 MHz NMR using a 3 mm triple resonance Varian indirect detection probe. The samples were prepared in deuterated chloroform containing tetramethylsilane (CDCl$_3$ with TMS, Sigma-Aldrich Chemicals (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 Tables 1-2.

Synthesis

The syntheses of all the imines was performed by dissolving approximately 20 mg of the HCl salt or free base form of the phenethylamine, amphetamine, or tryptamine into 5 mL of acetone in a 20 mL centrifuge tube. Each sample was capped and vortexed for 5 to 10 sec. GC/MS analysis was performed on 1 mL aliquots.

Approximately 4 drops of phenethylamine base and 50 mg of tryptamine base were added to 2 mL GC vials and diluted with acetone to the 1.5 mL mark on the vial. The reactions were allowed to come to room temperature for 8 hours prior to performing GC/MS analysis. The acetone was evaporated with air at room temperature to obtain an oil subsequently used for the FTIR and NMR analyses.

Results and Discussion

Primary amines are known to react readily with aldehydes and ketones to form imines. In this work, a series of primary amine containing drugs, in either the free base or HCl salt forms, were dissolved in acetone and allowed to react prior to GC/MSD analysis. In all cases, an additional peak was observed in the total ion chromatograph after a short period of time, which was identified as the imine product by analysis of its mass spectrum. To further confirm the structure of the reaction products of phenethylamine base and tryptamine base with acetone, analyses by FTIR and NMR spectroscopy were performed.

The mass spectra of the imine products from the phenethylamine–type compounds are shown in Figures 2-5. All the phenethylamine-based imine products show a base peak of m/z 70, indicating predominant α-cleavage on the alkyl chain, Figure 6. The imine products with methoxy groups in the two and five positions of the aromatic ring also exhibited a smaller peak that was 31 mass units less than the molecular ion. This is due to the loss of one of the methoxy groups.

The mass spectra of the imine products formed from the

Figure 2 - Mass spectra of (a) N-isopropylidenephenethylamine and (b) 2,5-dimethoxy-4-ethyl-N-isopropylidene-phenethylamine.

Figure 3 - Mass spectra of (a) 2,5-dimethoxy-4-iodo-N-isopropylidenephenethylamine and (b) 2,5-dimethoxy-4-chloro-N-isopropylidenephenethylamine.
reaction of amphetamine and amphetamine-type compounds with acetone are shown in Figures 7-8. The mass spectra of the imine products of amphetamine and amphetamine-type compounds all exhibited a base peak of $m/z$ 84, indicating predominant $\alpha$-cleavage on the alkyl chain, Figure 9. The imine product of 2,5-dimethoxyamphetamine exhibited a smaller peak that was 31 mass units less than the molecular ion, similar to the behavior seen with the dimethoxy-substituted phenethylamines, and is due to the loss of a methoxy group. However, 4-methoxyamphetamine did not exhibit loss of its methoxy group.

The mass spectra of the imine products formed from the reaction of tryptamine, $\alpha$-methyltryptamine, and 5-methoxy-$\alpha$-ethyl-tryptamine with acetone are show in Figures 10-11. All three compounds exhibited base peaks indicating predominant $\alpha$-cleavage on the alkyl chain, Figure 12. In addition, a less intense peak is seen at $m/z$ 130 due to initial ionization of the indole ring followed by $\alpha$-cleavage or a less intense peak at $m/z$ 160 due to initial ionization of the methoxy-substituted indole ring followed by $\alpha$-cleavage, Figure 13.

In all of the cases, additional imine products were observed with longer reaction time. These peaks were either 40 mass units higher or 80 mass units higher than the initial imine product, and were subsequently identified as the imine products.

**Figure 4** - Mass spectra of (a) 2,5-dimethoxy-4-ethylthio-N-isopropylidenephentylamine and (b) 2,5-dimethoxy-4-propylthio-N-isopropylidenephentylamine.

**Figure 5** - Mass spectrum of 2,5-dimethoxy-4-bromo-N-isopropylidenephentylamine.

**Figure 7** - Mass spectra of (a) N-isopropylideneamphetamine and (b) 4-methoxy-N-isopropylideneamphetamine.

**Figure 6** - Fragmentation Mechanism of N-isopropylidene-phenethylamines.
The imine products of phenethylamine and tryptamine exhibited imine absorption bands at 1662 cm\(^{-1}\) and 1665 cm\(^{-1}\), respectively, Figures 16-17. For comparison, the IR spectra of phenethylamine base and tryptamine base standards were also run. Comparison of all the spectra shows that the imine products of phenethylamine and tryptamine were formed. Also, there was no indication that any acetone was left in the samples of the starting amine with either mesityl oxide, Figure 14, or isophorone, Figure 15. Both mesityl oxide and isophorone are condensation products formed by acetone in the presence of a base, such as amines. In the case of phenethylamine and tryptamine, the identity of the mesityl oxide and isophorone imine products were confirmed by reacting these primary amines with the appropriate ketone at 70°C for one hour. Analysis by GC/MS of these products matched the retention times and mass spectrums of the products formed from the reaction with acetone.

**FTIR-ATR**

FTIR-ATR was performed on the imine products of phenethylamine and tryptamine. An imine in the IR region exhibits an absorption band in the region 1690-1640 cm\(^{-1}\). The imine products of phenethylamine and tryptamine exhibited imine absorption bands at 1662 cm\(^{-1}\) and 1665 cm\(^{-1}\), respectively, Figures 16-17. For comparison, the IR spectra of phenethylamine base and tryptamine base standards were also run. Comparison of all the spectra shows that the imine products of phenethylamine and tryptamine were formed. Also, there was no indication that any acetone was left in the samples.
Figure 12 - Fragmentation mechanism of N-isopropylidene-tryptamines.

Figure 13 - Secondary fragmentation mechanism of N-isopropylidene-tryptamines.

Figure 14 - Structure of mesityl oxide.

Figure 15 - Structure of isophorone.

Figure 16 - FTIR spectrum of (a) N-isopropylidene-phenethylamine and (b) phenethylamine base.

Figure 17 - FTIR spectrum of (a) N-isopropylidene-tryptamine and (b) tryptamine base.
Table 1 - NMR assignments and multiplicities of N-isopropylidenetryptamine.

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<th>$^{13}$C (ppm)</th>
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Table 2 - NMR assignments and multiplicities of N-isopropylidenephenethylamine.

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Figure 18 - Position of the carbons and protons for N-isopropylidenetryptamine

Figure 19 - Position of the carbons and protons for N-isopropylidenephenethylamine
as there was no absorption band at 1710 cm\(^{-1}\). The imine absorption band for tryptamine, 1665 cm\(^{-1}\), compares well with that reported in the literature [5].

**NMR**

The NMR chemical spectra are consistent with the imine structure for the condensation products of acetone with phenethylamine and tryptamine, Tables 1-2. The proton chemical shifts for the two methyl groups of the tryptamine imine product are in good agreement with those reported earlier [5].

**References**


The Characterization of N-methylphthalimide (NMP)

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ABSTRACT: The analysis and characterization of N-methylphthalimide (NMP) is presented. Analytical data includes the results from specific color tests, gas chromatography/mass spectrometry (GC/MS), Fourier transform infrared (FTIR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy.

KEYWORDS: N-methylphthalimide, GC/MS, NMR, FTIR, forensic chemistry

N-methylphthalimide (NMP) is an indole-based heterocyclic compound (Figure 1) traditionally used as an intermediate for organic syntheses or as a building block for plastics and dyes. There are no known medical uses for NMP. Beginning in the latter part of 2009, low to moderate amounts of NMP have been detected in a variety of “ecstasy” tablets from many regions of the United States, such as those shown in Figure 2. In most cases, 3,4-methylenedioxymethamphetamine (MDMA) was the primary constituent of the illicit tablets; however, in at least one seizure from Texas, no MDMA was present. In this case, only 1-(3-trifluoromethylphenyl) piperazine (TFMPP) and caffeine were identified along with NMP.

Experimental and Results

Chemicals, Reagents, and Materials
N-methylphthalimide was obtained from Sigma-Aldrich (Milwaukee, WI).

Presumptive color tests
Sodium nitroprusside: No color change.
Marquis: No color change.

Gas chromatography/mass spectrometry (GC/MS)
The mass spectrum of NMP (Figure 3) was acquired on an Agilent Model 5975C quadrupole mass-selective detector (MDS) interfaced with an Agilent 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 40-500 amu, and 5.4 scans/s. The GC was fitted with a 15 m x 0.25 mm I.D. fused silica capillary column coated with 0.25 μm 5% phenyl 95% dimethylpolysiloxane (HP-5). A total ion chromatogram (Figure 4) is shown for a representative ecstasy tablet exhibit that contained NMP as well as other more commonly encountered adulterants.

Nuclear Magnetic Resonance (NMR) Spectroscopy
The ¹H-NMR spectrum (Figure 5) was obtained on a Varian Mercury 400 MHz spectrometer. The sample was analyzed in deuterchloroform (CDCl₃) at ambient temperature using standard Varian pulse sequences.

Fourier Transform Infrared Spectroscopy (FTIR)
The spectrum (Figure 6) was collected using a Thermo Nicolet Avatar 370 DTGS spectrometer equipped with an attenuated total reflectance (ATR) attachment. The spectrum was collected using 16 scans between 4000 cm⁻¹ and 400 cm⁻¹ at a resolution of 4.0 cm⁻¹.

References
1. [http://www.chemicalbook.com/ProductChemicalPropertiesCB8473039_EN.htm](http://www.chemicalbook.com/ProductChemicalPropertiesCB8473039_EN.htm)

Figure 1 - N-methylphthalimide
Chemical Formula/CAS Number: C₉H₇NO₂ / [550-44-7]
Molecular Weight: 161.16 amu
Melting Point: 129-132°C
Boiling Point: 286°C at 760 mm Hg
IUPAC Name: 2-methylisoindole-1,3-dione
Synonyms: 2-methyl-1H-isoindol-1,3(2H)-dion
2-methyl-1H-isoindole-3(2H)-dione
2-methyl-isoindole-1,3-dione
N-methyl-phthalimid
N-methylphthalimide
phthalimidine-N-methyl
2-methyl-1H-isoindole-1,3(2H)-dione
1H-isoindole-1,3(2H)-dione, 2-methyl-

Figure 2 - Ecstasy tablets containing N-methylphthalimide.

Brought to you by AltGov2 [www.altgov2.org]
Figure 3 - Mass spectrum of N-methylphthalimide.

Figure 4 - Total ion chromatogram for adulterated ecstasy tablet (A: dimethylsulfone, B: phthalic anhydride, C: N-methylphthalimide, D: MDMA, E: caffeine, F: palmitic acid, and G: stearic acid).
Figure 5 - NMR spectrum for N-methylphthalimide.

Figure 6 - FTIR spectrum for N-methylphthalimide.
4-Methoxyphencyclidine: An Analytical Profile

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Drug Enforcement Administration
Special Testing and Research Laboratory
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Dulles, VA 20166

[Email address withheld at corresponding author’s request]

ABSTRACT: The synthesis and characterization of 4-methoxyphencyclidine (commonly referred to as methoxydine) is discussed. Analytical data (mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: 4-methoxyphencyclidine, 1-[1-(4-methoxyphenyl)cyclohexyl]piperidine, methoxydine, designer drug, synthesis, characterization, forensic chemistry

The resulting hydrochloride crystals were captured via suction filtration and dried. The purity of the final product exceeded 99.0%. The synthetic procedure is outlined in Figure 2; the yield is not reported.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI, USA). All other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI). The intermediate, 1-(piperidin-1-yl)cyclohexane carbonitrile was part of an authentic reference collection maintained by the DEA Special Testing and Research Laboratory.

Synthesis

A solution of 4-bromoanisole in dry diethyl ether was reacted with magnesium turnings to form a Grignard reagent. A solution of 1-(piperidin-1-yl)cyclohexane carbonitrile in methylene chloride/diethyl ether (1:1) was slowly added to the Grignard with stirring. The reaction was quenched with ice and NH₄Cl, rendered basic with aqueous NaOH, and extracted with diethyl ether. The ether was acidified with methanolic HCl.

Figure 1 - Structural formula of 4-methoxyphencyclidine.

Figure 2 - Synthetic route for 4-methoxyphencyclidine.
Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters: resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Nuclear Magnetic Resonance Spectroscopy (NMR)
A proton (¹H) NMR spectrum was obtained on an Agilent VNMRS 600 MHz NMR using a 5 mm Protune broad band detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The product was dissolved in deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. A standard Agilent proton pulse sequence was used. Data processing was performed using software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Results and Discussion
The FTIR spectrum of 4-methoxyphencyclidine HCl (Figure 3) displays absorbances (2000-2700 cm⁻¹) which are consistent with an amine halogen (HCl) ion-pair and significant aliphatic CH absorbance in the region of 2800-3000 cm⁻¹. The 500-1600 cm⁻¹ region is peak (band) enriched for discriminative purposes. The ¹H-NMR spectrum (Figure 4) is distinguished from PCP, by the presence of the methoxy resonance at 3.85 ppm and the aromatic doublets at 7.0 and 7.4 ppm (indicating para-substitution of benzene). The mass spectrum of 4-methoxyphencyclidine (Figure 5) is fragment ion rich, with mass fragments of m/z 188 as the base peak and m/z 273 as the molecular ion. Analogous to PCP, an M-1 ion at m/z 272 is consistent with the loss of an ortho hydrogen from the aromatic ring and subsequent C-N bond formation, which retains the charge on the nitrogen [3].

Conclusions
Analytical data is presented to assist forensic laboratories that encounter 4-methoxyphencyclidine in case exhibits. The three presented spectral techniques each provide unequivocal characterization.

Figure 3 - Infrared spectrum (FTIR) of 4-methoxyphencyclidine HCl.
Figure 4 - Proton NMR spectrum of 4-methoxyphencyclidine HCl. S = trace CHCl₃ from CDCl₃, i = trace impurity.

Figure 5 - Electron ionization mass spectrum of 4-methoxyphencyclidine.
References
Characterization of the “Methylenedioxy-2-aminoindans”

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ABSTRACT: Spectroscopic and chromatographic data are provided for 5,6-(methylenedioxy)-2-aminoindan (commonly referred to as MDAI), 4,5-(methylenedioxy)-2-aminoindan (a positional isomer of MDAI), and their respective synthetic intermediates. Direct comparisons of the analytical data are made to assist forensic chemists in correctly differentiating between these isomers in illicit drug exhibits.

KEYWORDS: 5,6-(methylenedioxy)-2-aminoindan, 4,5-(methylenedioxy)-2-aminoindan, MDA analogs, designer drugs, chemical analysis, forensic chemistry.

5,6-(Methylenedioxy)-2-aminoindan, commonly referred to as “MDAI” (henceforth 5,6-MDAI) is a popular “research chemical” for sale over the internet at quantities of 1 gram to 10 kg. Currently, it is not specifically scheduled as a controlled substance in the United States, nor listed by the U.S. Drug Enforcement Administration as an analog of 3,4-methylenedioxyamphetamine (MDA) [1]. 5,6-MDAI 1, and its positional isomer 4,5-(methylenedioxy)-2-aminoindan (4,5-MDAI) 2, were synthesized by the Nichols group [2] in 1990 to compare their pharmacological/neurotoxological properties with 3,4-methylenedioxymethamphetamine (MDMA) 3 (Figure 1). That study found that 5,6-MDAI did substitute for MDMA in MDMA-trained rats. Although 4,5-MDAI did not show significant CNS activity, its close structural association with 5,6-MDAI merits analytical delineation of the two positional isomers. Direct comparisons of the spectroscopic and chromatographic data are presented to assist forensic chemists in correctly differentiating these positional isomers in suspected drug seizures.

Experimental
Chemical, Materials, and Reagents
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI, USA). All other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Nuclear Magnetic Resonance Spectroscopy (NMR)
Proton (¹H), carbon (¹³C), and 2-dimensional NMR spectra were obtained on an Agilent VNMR 600 MHz NMR using a 5 mm Protune broad band detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). All compounds were dissolved in deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to acquire ¹H, proton-

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Figure 1 - Structural formulas of (1) 5,6-MDAI, (2) 4,5-MDAI, and (3) MDMA.
decoupled $^{13}$C, and gradient versions of HSQC, HMBC, and 1D-NOESY spectra. Data processing was performed using software from Agilent and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Structure elucidation and the prediction of $^1$H and $^{13}$C spectra was accomplished using ACD/Labs software.

**Synthesis**

The procedures of Nichols et al. [2] were followed (Figures 2 and 3) for the preparation of 5,6-MDAI and 4,5-MDAI. Synthetic details and yield values are not reported.

**Results and Discussion**

GC retention time data for the respective compounds (Figures 2 and 3) are presented in Table 1. The amines were injected as the free bases since the hydrochloride ion-pairs of some phenethylamines undergo thermally induced degradation and chromatograph poorly [3]. 5,6-MDAI and 4,5-MDAI gave identical retention times and could not be resolved under the conditions utilized. The intermediate oximes 6 and 11 would not chromatograph as underivatized; however, each displayed excellent chromatographic properties as their trimethylsilyl (TMS) derivatives.

The FTIR spectra for 5,6-MDAI and 4,5-MDAI hydrochlorides are illustrated in Figure 4. Comparison of the hydrochloride ion pairs reveals similar absorption patterns with the most prominent differences being in the “fingerprint region” of 750-1750 cm$^{-1}$, and presence/absence differences in the C-H out-of-plane bending frequencies between 500-800 cm$^{-1}$. Since the spectra are somewhat similar, we recommend that additional or supplementary spectroscopic methods should be utilized for identification.

Mass spectra for the respective 5,6- and 4,5- substituted isomers and their intermediates are presented in Figures 5-8. Spectra produced from 5,6-MDAI and 4,5-MDAI gave an intense molecular ion at $m/z$ 177 (Figure 5), however 4,5-MDAI’s molecular ion is also the base peak for that compound ($m/z$ 160 is the base peak for 5,6-MDAI). The relative abundances for the remaining ions are quite similar. Therefore, care must be taken in differentiating 5,6-MDAI from

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_t$ (min)</th>
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<tbody>
<tr>
<td>1</td>
<td>11.53</td>
</tr>
<tr>
<td>1-TMS Derivative</td>
<td>15.36</td>
</tr>
<tr>
<td>2</td>
<td>11.53</td>
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<td>2-TMS Derivative</td>
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</tr>
<tr>
<td>5</td>
<td>13.01</td>
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<tr>
<td>6-TMS Derivative</td>
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<tr>
<td>7</td>
<td>6.86</td>
</tr>
<tr>
<td>8</td>
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<td>11-TMS Derivative</td>
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</table>

*Conditions given in the experimental section.*
Figure 3 - Synthetic route to 4,5-MDAI.
Figure 4 - Infrared spectra of (a) 5,6-MDAI HCl and (b) 4,5-MDAI HCl.
Figure 5 - Electron ionization mass spectra of (a) 5,6-MDAI and (b) 4,5-MDAI.

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Figure 6 - Electron ionization mass spectra of (a) 5,6-MDAI-TMS and (b) 4,5-MDAI-TMS.
Figure 7 - Electron ionization mass spectra of (a) 3-[3,4-(methylenedioxy)phenyl]propanoic acid and (b) 3-[2,3-(methylenedioxy)phenyl]propanoic acid.
Figure 8 - Electron ionization mass spectra of (a) 5,6-(methylenedioxy)-1-indanone and (b) 4,5-(methylenedioxy)-1-indanone.
Figure 9 - Electron ionization mass spectra of (a) 2-(hydroxyimino)-5,6-(methyleneoxy)-1-indanone-TMS and (b) 2-(hydroxyimino)-4,5-(methyleneoxy)-1-indanone-TMS.
its positional isomer (underivatized) via GC/MS, especially since both compounds elute at the same retention time. However, the mass spectra of 5,6-MDAI and 4,5-MDAI were easily differentiated as their TMS derivatives (Figure 6). Each produced a molecular ion at $m/z$ 249, but the relative intensities of ions at $m/z$ 73, $m/z$ 100, and $m/z$ 150 provided clear delineation of the two compounds. The propanoic acids 4 and 9 (Figure 7) each gave base peak at $m/z$ 135, and were easily distinguished by the relative abundances of ions at $m/z$'s 147/148 relative to $m/z$'s 176/175. Finally, the intermediate oximes 6 and 11 (Figure 9) produced similar spectra, but could be distinguished by the relative abundances of ions at $m/z$ 102 and $m/z$ 104, and at $m/z$ 159 and $m/z$ 160.

The proton and carbon chemical shifts and proton splitting patterns for 5,6-MDAI and 4,5-MDAI, are presented in Table 2. 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 correlation between carbon and proton) experiments. Both 5,6-MDAI and 4,5-MDAI had the following characteristics: 6 aromatic carbons (of which 2 were protonated), plus 3 methylene carbons, and 1 methine. The methylene with carbon at 100.7 ppm with HMBC correlated aromatic carbons indicated a methylenedioxyphenyl moiety was present. The remaining alkyl carbons formed a CH$_2$-CH-CH$_2$ group, with the methine likely bonded to nitrogen (based on its carbon and proton chemical shifts and the presence of a nitrogen based on the mass spectrum data), and the two methylenes bonded to the phenyl (based on HMBC correlations).

5,6-MDAI NMR proton and carbon spectra indicated a symmetric molecule with chemical equivalence for carbons and protons down the axis of symmetry; i.e., 1 and 3, 3a and 7a, 4 and 7, as well as 5 and 6 are chemically equivalent. The singlet at 6.66 ppm integrating to 2 hydrogens indicated that these protons were para to each other.

4,5-MDAI NMR proton and carbon spectra showed no chemical equivalence; all of the carbons and protons having unique chemical shifts. The 7.7 Hz doublets at 6.65 and 6.67 ppm indicated that these aromatic protons were adjacent to each other. While only one structure for this compound is possible based on the NMR data, assignments for positions 1, 3, 6, and 7 required confirmation using a NOESY-1D experiment (showing spatially near protons). Excitation of the 3.10 ppm proton resulted in an NOE peak at 6.67 ppm indicating these were near each other (positions 1 and 7, respectively), while excitation of the 3.13 ppm proton did not result in an NOE effect on any other aromatic proton.

**Conclusions**

Analytical data is presented to assist delineating 5,6-MDAI and 4,5-MDAI, as well as their respective synthetic intermediates. Characterization is achieved by either IR, NMR, or mass spectrometry.

**References**


![Table 2 - NMR carbon and proton chemical shifts (in ppm) and splitting patterns of the methylenedioxy-2-aminooindans and related compounds. Samples run in CDCl$_3$ with TMS as the reference compound for 0 ppm.](image-url)
Methiopropamine: An Analytical Profile

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ABSTRACT: Spectroscopic and chromatographic data are provided for methiopropamine, an internet-available compound, possessing CNS stimulant properties. Analytical data (infrared spectroscopy, mass spectrometry, and proton/carbon nuclear magnetic resonance spectroscopy) are presented for methiopropamine and its synthetic intermediates.

KEYWORDS: methiopropamine, 1-(thiophen-2-yl)-2-methylaminopropane, methamphetamine analog, designer drugs, chemical analysis, forensic chemistry.

Methiopropamine (Figure 1) is currently one of many internet-available compounds sold as “legal highs.” The IUPAC name for methiopropamine is 1-(thiophen-2-yl)-2-methylaminopropane. Although it is claimed to be legal, it is a 2-thienyl analog of the Schedule II controlled substance, methamphetamine, and may be controlled within the United States [1]. Methiopropamine was first synthesized in 1942 to compare its pharmacological properties with phenyl-related derivatives [2]. An analytical profile of methiopropamine is presented to assist forensic chemists who may encounter this substance in casework. Mass spectra of two synthetic intermediates are also given.

Experimental

Chemical, Materials, and Reagents

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI, USA). All other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Synthesis

In accordance with Journal policy, exact synthesis details are not provided. The procedure of Blicke and Burckhalter [2] was utilized (Figure 2).

Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton (¹H), carbon (¹³C), and 2-dimensional NMR spectra were obtained on an Agilent VNMRS 600 MHz NMR using a 5 mm Protune broad band detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). Samples were dissolved in deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to acquire ¹H, proton-decoupled ¹³C, and gradient versions of HSQC and HMBC spectra. Data processing was performed using software from Agilent and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Structure elucidation and the prediction of ¹H and ¹³C spectra was accomplished using ACD/Labs software.

Discussion

The FTIR spectrum of methiopropamine HCl (Figure 3) displays absorbances (2400-2800 cm⁻¹) which are consistent with a secondary amine HCl ion-pair and significant aliphatic CH absorbance in the region of 2800-3000 cm⁻¹. The 300-1500 cm⁻¹ region is peak (band) enriched for discriminative purposes.

Figure 1 - Structural formula of methiopropamine.
Figure 2 - Synthetic route for methiopropamine.

1-(Thiophen-2-yl)-2-hydroxypropane

1-(Thiophen-2-yl)-2-bromopropane

1-(Thiophen-2-yl)-2-methylaminopropane

Figure 3 - Infrared spectrum of methiopropamine HCl.
Figure 4 - $^1$H and $^{13}$C NMR spectra and associated data for methiopropamine HCl in CDCl$_3$. Proton abbreviations: bs = broad singlet, d = doublet, q = quartet, t = triplet.
The $^1$H and $^{13}$C spectra and the assignment table are found in Figure 4. A substituted propyl chain ($\text{CH}_3$-$\text{CH}$-$\text{CH}_2$-) is clearly indicated by the methyl proton doublet at 1.43 ppm, methine multiplet at 3.34 ppm, and methylene non-equivalent protons at 3.18 and 3.62 ppm (both doublet of doublets). The methine proton chemical shift of 3.34 ppm and carbon of 57.2 ppm indicates it is bonded to nitrogen and not the aromatic ring. The aromatic proton splitting patterns and coupling constants represent a continuous $\text{CH}=\text{CH}=\text{CH}$ chain, requiring substitution at C-2. The N-methyl protons are a triplet due to coupling with the two amine hydrogens (this is the HCl salt).

The mass spectrum of methiopropamine (Figure 5) is fragment ion rich, however most ions are at low abundance. Two prominent mass fragments are produced at $m/z$ 58 (base peak) and $m/z$ 97, respectively. The ion at $m/z$ 97 is from rearrangement of the 2-alkylthiophen moiety to give the thiopyrilmium ion $\text{C}_5\text{H}_5\text{S}^+$ [3]. Although a molecular ion is not observed, an M-1 ion at $m/z$ 154 is produced (analogous to the M-1 ion for methamphetamine). The mass spectra for the intermediates 1-(thiophen-2-yl)-2-hydroxypropane and 1-(thiophen-2-yl)-2-bromopropane are illustrated in Figure 6.

Prior to recrystallization of the final methiopropamine HCl product, a minor compound with slightly different chemical shifts was detected by proton NMR. One of the aromatic protons was a broad singlet, indicating that substitution was at C-3. The mass spectrum of this compound was virtually identical to that of methiopropamine, with a slightly later retention time (Table 1). It was tentatively characterized as the positional isomer 1-(thiophen-3-yl)-2-aminopropane; i.e., with the 2-methylaminopropane group at the 3 position of the thiophene ring. It should be noted that the 3-yl positional isomer is not available for sale.

**Conclusions**

Analytical data is presented to assist forensic laboratories that encounter methiopropamine in case exhibits. The three presented spectral techniques each provide unequivocal characterization.

**References**

Figure 6 - Electron ionization mass spectrum of (a) 1-(thiophen-2-yl)-2-hydroxypropane and (b) 1-(thiophen-2-yl)-2-bromopropane.

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The sale of compounds touted as “legal highs” has recently flourished on the internet. Ethylphenidate (Figure 1) is currently one of many such compounds. However, although it is claimed to be legal, it may be considered to be an analog of the Schedule II controlled substance, methylphenidate [1]; ethylphenidate being an ethyl ester vs. methylphenidate being a methyl ester. Ethylphenidate is best known as a transesterification metabolite, after methylphenidate and ethanol are consumed together [2-5]. The synthesis and pharmacology of ethylphenidate enantiomers has also been recently studied [6]. Herein, an analytical profile is presented to assist forensic chemists who may encounter this substance in casework.

**Experimental**

**Chemical, Materials, and Reagents**

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI, USA). Methylphenidate HCl was obtained from the authentic reference collection of the DEA Special Testing and Research Laboratory. All other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

**Synthesis**

In accordance with Journal policy, exact experimental details are not provided. Methylphenidate HCl was hydrolyzed to ritalinic acid, which was subsequently esterified to ethylphenidate with ethanolic HCl (Figure 2).

**Infrared Spectroscopy (FTIR)**

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

**Gas Chromatography/Mass Spectrometry (GC/MS)**

Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

**Nuclear Magnetic Resonance Spectroscopy (NMR)**

Proton (¹H), carbon (¹³C), and 2-dimensional NMR spectra were obtained on an Agilent VNMR 600 MHz NMR using a 5 mm Protune broad band detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). Samples were

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**ABSTRACT:** Spectroscopic and chromatographic data are provided for ethylphenidate, a relatively new internet-available compound, possessing CNS stimulant properties. Analytical data (infrared spectroscopy, mass spectrometry, and proton/carbon nuclear magnetic resonance spectroscopy) are presented.

**KEYWORDS:** ethylphenidate, methylphenidate analog, designer drugs, chemical analysis, forensic chemistry.

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**Figure 1** - Structural formula of ethylphenidate.

**Figure 2** - Synthetic route for ethylphenidate.
Figure 3 - Infrared spectra of (a) ethylphenidate HCl and (b) methylphenidate HCl.

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Figure 4 - $^1$H and $^{13}$C NMR spectra and associated data for ethylphenidate HCl in CDCl$_3$. Proton abbreviations: ad = apparent doublet, aq = apparent quartet, at = apparent triplet, bd = broad doublet, bs = broad singlet, d = doublet, m = multiplet, t = triplet.
References


Discussion

The FTIR spectrum of ethylphenidate HCl is remarkably similar to that of methylphenidate HCl (Figure 3). Only minor differences in some absorbance patterns can be discerned. Therefore, the use of a complementary spectroscopic/spectrometric method is recommended. The 1H and 13C NMR of ethylphenidate (Figure 4) clearly distinguish it from methylphenidate due to the presence of the ethyl ester proton pattern (4.29 ppm 1H multiplet CH2, and 1.20 ppm triplet CH3) instead of the methoxy singlet. The mass spectrum of ethylphenidate (Figure 5) gives a very weak M-1 ion at m/z 246 with major ions at m/z 84 (piperidinium ion; base peak) and m/z 91 (tropylium ion). An ion at m/z 164 is the complementary ion (ethyl phenylacetate moiety) from loss of the piperidine fragment (analogous to m/z 150 for methylphenidate; methyl phenylacetate moiety).

Figure 5 - Electron ionization mass spectrum of ethylphenidate.

dissolved in deuterchloroform (CDCl3) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to acquire 1H, proton-decoupled 13C, and gradient versions of HSQC and HMBC spectra. Data processing was performed using software from Agilent and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Structure elucidation and the prediction of 1H and 13C spectra was accomplished using ACD/Labs software.

Figure 5 - Electron ionization mass spectrum of ethylphenidate.
The Characterization of 5- and 6-(2-Aminopropyl)-2,3-dihydrobenzofuran

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ABSTRACT: The synthesis, analysis, and characterization of 5- and 6-(2-aminopropyl)-2,3-dihydrobenzofuran are discussed. Analytical data (mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy) are presented with direct comparisons of the analytical data to correctly differentiate these isomers in suspected drug exhibits.

KEYWORDS: 5-(2-aminopropyl)-2,3-dihydrobenzofuran, 6-(2-aminopropyl)-2,3-dihydrobenzofuran, benzofury, designer drug, synthesis, characterization, forensic chemistry.

6-(2-Aminopropyl)-2,3-dihydrobenzofuran, commonly referred to as “Benzofury” or “6-APB,” has become a popular “research chemical” for sale over the internet. A positional isomer, 5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APB) became available only a few weeks after 6-APB was first sold. Although not currently scheduled under the U.S. Controlled Substances Act, both may be considered to be analogs of 3,4-methylenedioxyamphetamine (MDA) [1], since an oxygen atom within the methylenedioxy moiety of MDA has been replaced with a methylene (CH₂) group (Figure 1). Analytical data is presented to assist forensic chemists who may encounter these substances in casework.

Experimental
Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All NMR solvents and other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI). 3,4-Methylenedioxyamphetamine (MDA) was obtained from the authentic reference collection maintained by the Drug Enforcement Administration’s Special Testing and Research Laboratory.

In accordance with Journal policy, exact experimental details are not provided. The procedures of Monte et al. [2] were followed (Figures 2 and 3) for the preparation of 5-APB, 6-APB, and their intermediates.

Infrared Spectroscopy (FTIR)
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and at a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, DB-1 (J & W Scientific,

Figure 1 - Structural formulas of (1) 5-APB, (2) 6-APB, and (3) MDA.

Figure 2 - Synthetic route for 5-(2-aminopropyl)-2,3-dihydrobenzofuran 1.
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 source was operated at 230°C.

**Nuclear Magnetic Resonance Spectroscopy (NMR)**

NMR spectra were obtained on a Agilent VNMRS 600 MHz NMR using a 5 mm Protune broad band detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The HCl salt of the compounds were dissolved in deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound (methanol-d₄ added dropwise to solubilize, if necessary). The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon (proton decoupled), NOESY1D, and gradient versions of 2 dimensional experiments COSY, HSQC, and HMBC. Data processing and structure elucidation was performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

**Figure 3 - Synthetic route for 6-(2-aminopropyl)-2,3-dihydrobenzofuran 2.**

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 source was operated at 230°C.

**Table 1 - Gas chromatographic retention times (Rₜ) for the (2-aminopropyl)-2,3-dihydrobenzofurans and related compounds.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rₜ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.13</td>
</tr>
<tr>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td>3</td>
<td>9.73</td>
</tr>
<tr>
<td>4</td>
<td>8.75</td>
</tr>
<tr>
<td>5</td>
<td>17.82</td>
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<tr>
<td>6</td>
<td>7.86</td>
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<tr>
<td>7</td>
<td>10.48</td>
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<tr>
<td>8</td>
<td>18.35</td>
</tr>
<tr>
<td>9</td>
<td>18.62</td>
</tr>
<tr>
<td>10</td>
<td>16.19</td>
</tr>
<tr>
<td>11</td>
<td>13.63</td>
</tr>
</tbody>
</table>

*Conditions given in the experimental section.*
The FTIR spectra for 5-APB HCl and 6-APB HCl are illustrated in Figure 4, along with MDA HCl. Comparison of the hydrochloride ion pairs reveals similar absorption patterns with the most prominent differences being in the region of 500-1750 cm\(^{-1}\). When compared to MDA HCl (Figure 4), significant differences in this region can differentiate the compounds. However, since the spectra are somewhat similar,

**Results and Discussion**

GC retention time data for the respective compounds (Figures 1-3) are presented in Table 1. All amines were injected as the free base. 5-APB and 6-APB gave virtually identical retention times and could not be resolved under the conditions utilized. Both compounds eluted approximately 1.4 minutes later than MDA in the described system.

Figure 4 - Infrared spectrum (FTIR) of (a) 5-(2-aminopropyl)-2,3-dihydrobenzofuran HCl 1, (b) 6-(2-aminopropyl)-2,3-dihydrobenzofuran HCl 2, and (c) 3,4-methylenedioxyamphetamine HCl 3.
and because 3,4-MDA is known to have differing polymorphic crystalline forms (each of which has a slightly different IR spectrum), and 5-APB and 6-APB may exhibit similar behavior, additional or supplementary spectroscopic methods should be utilized for identification.

Mass spectra for 5-APB, 6-APB, and their respective intermediates are presented in Figures 5-9. Spectra produced from 5-APB and 6-APB gave a base peak at \( m/z \) 44 and a moderate molecular ion at \( m/z \) 177 (Figure 5), with logical 2-Dalton differences from MDA (Figure 5). However, 5-APB produces a much more intense ion at \( m/z \) 134, relative to 6-APB (\( m/z \) 134 is approx. 3.5 times greater for 5-APB). Although the relative abundances for the remaining ions are quite similar, the two compounds are easily distinguished on this basis.
Figure 6 - Electron ionization mass spectra of (a) 2,3-dihydrobenzofuran-5-carboxaldehyde 4 and (b) 5-[1-(2-nitro-1-propenyl)]-2,3-dihydrobenzofuran 5.
Figure 7 - Electron ionization mass spectra of (a) 3-methoxyamphetamine 6 and (b) N-(trifluoroacetyl)-1-(3-methoxyphenyl)-2-aminopropane 7.
Figure 8 - Electron ionization mass spectra of (a) N-(trifluoroacetyl)-1-[3-methoxy-4-(chboroacetyl)phenyl]-2-aminopropane 8 and (b) N-(trifluoroacetyl)-1-[3-hydroxy-4-(chboroacetyl)phenyl]-2-aminopropane 9.
Figure 9 - Electron ionization mass spectra of (a) 6-[2-\{N-(trifluoroacetyl)amino\}propyl]-2,3-dihydrobenzofuran-3-one 10 and (b) 6-[2-\{N-(trifluoroacetyl)amino\}propyl]-2,3-dihydrobenzofuran 11.

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Figure 10 - $^1$H and $^{13}$C NMR data for 5-(2-aminopropyl)-2,3-dihydrobenzofuran HCl I.
Figure 11 - $^1$H and $^{13}$C NMR data for 5-(2-aminopropyl)-2,3-dihydrobenzofuran base I.

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<table>
<thead>
<tr>
<th>Carbon</th>
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<tr>
<td>2</td>
<td>71.1 4.55 (t, 8.7 Hz)</td>
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<tr>
<td>3</td>
<td>29.8 3.19 (t, 8.7 Hz)</td>
</tr>
<tr>
<td>3a</td>
<td>127.1 -</td>
</tr>
<tr>
<td>4</td>
<td>125.7 7.02 (bs)</td>
</tr>
<tr>
<td>5</td>
<td>131.6 -</td>
</tr>
<tr>
<td>6</td>
<td>128.6 6.91 (bd, 8.1 Hz)</td>
</tr>
<tr>
<td>7</td>
<td>109.0 6.72 (d, 8.1 Hz)</td>
</tr>
<tr>
<td>7a</td>
<td>158.6 -</td>
</tr>
<tr>
<td>1'</td>
<td>46.0 2.43 (dd, 13.4, 8.1 Hz), 2.64 (dd, 13.5, 5.3 Hz)</td>
</tr>
<tr>
<td>2'</td>
<td>48.6 3.10 (ddq, 8.1, 6.3, 5.3 Hz)</td>
</tr>
<tr>
<td>3'</td>
<td>23.5 1.11 (d, 6.3 Hz)</td>
</tr>
</tbody>
</table>

b = broad, d = doublet, q = quartet, s = singlet, t = triplet

Saturated NaHCO$_3$ D$_2$O - CDCl$_3$ Base Extraction
Figure 12: $^1$H and $^{13}$C NMR data for 6-(2-aminopropyl)-2,3-dihydrobenzofuran HCl 2.

<table>
<thead>
<tr>
<th>Carbon</th>
<th>Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>71.7 4.58 t(8.6 Hz)</td>
</tr>
<tr>
<td>3</td>
<td>29.6 3.20 t(8.6 Hz)</td>
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<tr>
<td>3a</td>
<td>126.4 -</td>
</tr>
<tr>
<td>4</td>
<td>125.4 7.16 d(7.8 Hz)</td>
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<tr>
<td>5</td>
<td>121.6 6.71 d(7.8 Hz)</td>
</tr>
<tr>
<td>6</td>
<td>136.1 -</td>
</tr>
<tr>
<td>7</td>
<td>110.2 6.63 s</td>
</tr>
<tr>
<td>7a</td>
<td>160.8 -</td>
</tr>
<tr>
<td>1'</td>
<td>40.9 2.72 dd(13.6, 8.5 Hz), 3.01 dd(13.6, 6.0 Hz)</td>
</tr>
<tr>
<td>2'</td>
<td>49.4 3.44 dqd(8.5, 6.4, 6.0 Hz)</td>
</tr>
<tr>
<td>3'</td>
<td>17.9 1.29 d(6.4 Hz)</td>
</tr>
</tbody>
</table>

d = doublet, q = quartet, s = singlet, t = triplet

1 mL CDCl$_3$ w/ 20 drops CD$_3$OD
Figure 13 - $^1$H and $^{13}$C NMR data for 6-(2-aminopropyl)-2,3-dihydrobenzofuran base 1.

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However, since both compounds elute at the same retention time, care must be taken in differentiating the spectra. Mass spectra for intermediates 4-11 are illustrated in Figures 6-9.

The proton and carbon assignments for 5-APB and 6-APB (base and HCl) are presented in Figures 10-13. Assignments were based on proton chemical shifts and peak patterns, carbon chemical shifts, HSQC (1 bond carbon to proton correlations), HMBC (2-4 bond carbon to proton correlations correlations), COSY (2-3 bond proton-proton correlations), and NOESY1D (spatial proximity between protons) spectra. Assignments were further confirmed by the ACD software.

Proton spectra for both compounds indicated the typical 1,3,4-trisubstituted benzene peak pattern (broad singlet, broad doublet, sharp doublet above 6.5 ppm), a CH₂-CH₂-O group (two triplets, 3.2 ppm and 4.5 ppm), and a CH₂-CH(NH₂)-CH₃ group (non-equivalent methylene protons that were doublet of doublets, a methine multiplet, and methyl doublet; all between 2.4 and 3.2 ppm). The molecular formula, C₁₁H₁₅NO, indicates 5 degrees of unsaturation and/or rings which are accounted for in the benzofuran ring system. This ring system is supported by HMBC correlations from the OCH₂CH₂ protons to benzene carbons, benzene protons to OCH₂CH₂ carbons, and the benzene carbon chemical shift near 160 ppm indicating it is bonded to oxygen. The NOESY1D of 6-APB base showed that H-3 (3.17 ppm) was spatially near H-4 (7.10 ppm), a narrow doublet which must be adjacent to a proton (H-5), requiring substitution of the propyl group on C-6. In addition, the predicted carbon spectra of both 5-APB and 6-APB have C-4 at approximately 125 ppm and C-7 at approximately 110 ppm, due to the strong effect of the oxygen on C-7. In the case of 5-APB base, the carbon at 109.0 ppm (C-7) has a proton doublet (8.1 Hz coupling) at 6.72 ppm; C-6 must be protonated. In the case of 6-APB, the carbon at 110.1 ppm has a proton singlet at 6.63 ppm; C-6 must be a quaternary carbon.

Conclusions
Analytical data is presented to assist forensic laboratories that encounter 5-APB or 6-APB in casework. Any combination of two of the three presented spectral techniques can provide unequivocal characterization.

References
The Characterization of Etaqualone and Differentiation from its 3- and 4-Ethyl Analogues

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ABSTRACT: The synthesis, analysis, and characterization of 3-(2-ethylphenyl)-2-methyl-quinazolin-4-one (commonly referred to as etaqualone) are briefly discussed. Analytical data (mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared spectroscopy) are presented to differentiate it from its 3- and 4-ethylphenyl analogues.

KEYWORDS: etaqualone, 3-(2-ethylphenyl)-2-methyl-quinazolin-4-one, 3-(3-ethylphenyl)-2-methyl-quinazolin-4-one, 3-(4-ethylphenyl)-2-methyl-quinazolin-4-one, designer drug, synthesis, characterization, forensic chemistry.

Although etaqualone (Figure 1, structure 2) was first synthesized and patented in 1963 [1], it has recently become a popular “research chemical” for sale over the Internet. Etaqualone is the ethyl analogue of methaqualone (Figure 1, structure 1). Illicit etaqualone has been reported recently in Europe [2]. There are several literature citations for methaqualone analogues [3-7], however, analytical data for the forensic identification of etaqualone and its 3-ethylphenyl and 4-ethylphenyl analogues (Figure 1, structures 3 and 4, respectively) are needed. Herein, we report the synthesis and analytical profiles of etaqualone, 3 and 4 (nuclear magnetic resonance, mass spectrometry, and infrared spectroscopy), to assist forensic chemists who may encounter these substances in casework.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All other chemicals and NMR solvents were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Synthesis of Etaqualone and its Positional Isomers

In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2. Briefly, N-acetylanthranilic acid was refluxed with 2-ethylaniline in the presence of PCl₃ to give etaqualone (1). 3-(3-Ethylphenyl)-2-methyl-quinazolin-4-one (3) and 3-(4-ethylphenyl)-2-methyl-quinazolin-4-one (4) were produced by utilizing 3-ethylaniline and 4-ethylaniline, respectively. All compounds were converted their respective HCl ion pair with diethyl ether containing HCl.

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were obtained on an Agilent VNMR 600 MHz NMR using a 5 mm Protune broad band detection, variable

Figure 1 - Structural formulas. 1 = methaqualone, 2 = etaqualone, 3 = 3-ethyl analogue of etaqualone, and 4 = 4-ethyl analogue of etaqualone.

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temperature, pulse field gradient probe (Agilent, Palo Alto, CA) for 4 and an Agilent 400MR using a 5 mm Proton indirect detection, variable temperature, pulse field gradient probe for 2 and 3. The samples were base extracted with sodium bicarbonate in deuterium oxide (D\textsubscript{2}O) into deuterochloroform (CDCl\textsubscript{3}) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon (proton decoupled), and gradient versions of 2 dimensional experiments COSY, NOESY, HSQC, and HMBC. Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

**Results and Discussion**

Table 1 contains the proton and carbon data for all three compounds as the free base. Assignments were carried out using proton chemical shifts and peak patterns, carbon chemical shifts, and COSY, NOESY, HSQC, and HMBC correlations.
Figure 3 - FTIR of (a) = etalalone HCl, (b) = 3-ethyl analogue of etalalone HCl, and (c) = 4-ethyl analogue of etalalone HCl.

*Microgram Journal, Volume 9, Number 2*
Figure 4 - Mass spectrum of (a) = etaqualone, (b) = 3-ethyl analogue of etaqualone, and (c) = 4-ethyl analogue of etaqualone.
The ethyl group position (i.e., ortho-, meta-, or para-) on one of the benzene rings was obvious from the proton peak patterns and COSY correlations. Each compound was easily differentiated by its NMR spectrum.

The infrared spectra of 2, 3, and 4 are illustrated in Figure 3. The FTIR (Figures 3a-c) for each compound exhibited a strong carbonyl stretch between 1712-1721 cm⁻¹, but have dissimilar absorbances between 400-1700 cm⁻¹. Most notably, the amine HCl bands for etalqualone (Figure 3a) are further downfield at ca. 2000-2350 cm⁻¹ compared to the 3- and 4-ethyl isomers having absorbances at ca. 2200-2600 cm⁻¹ (Figures 3b and 3c).

The mass spectra of 2, 3, and 4 are illustrated in Figure 4. Etaqualone produces a moderate molecular ion at m/z 264 and a base peak at m/z 249 (Figure 4a). The 3- and 4-ethyl isomers (Figures 4b and 4c) are easily differentiated from etalqualone since each produces a molecular ion as the base peak at m/z 264 and a pronounced ion at m/z 143 (etaqualone has only a minor ion at m/z 143). The 3- and 4-ethyl isomers are differentiated from each other by the relative intensity of the ion at m/z 143.

Conclusions
Analytical data are presented to assist forensic laboratories that encounter etalqualone or its 3- and 4-ethyl analogues in casework.

References
JWH-018 and JWH-022 as Combustion Products of AM2201

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ABSTRACT: The analysis of charred samples of synthetic cannabinoids led to the discovery of JWH-018 and JWH-022 as combustion products of AM-2201. The steps needed to separate these compounds for identification using GC/MS and AccuTOF-DART™ are discussed.

KEYWORDS: synthetic cannabinoids, AM2201, JWH-018, JWH-022, combustion, GC/MS, AccuTOF-DART™, forensic chemistry.

The emergence of synthetic cannabinoids continues to prove challenging to the forensic scientist. As the initially popular compounds, such as JWH-018 (1-pentyl-3-(1-naphthoyl)indole, have become controlled, several other synthetic cannabinoids have appeared to replace them for recreational use [1]. This laboratory has seen a surge in cases containing AM2201 (1-(5-fluoropentyl)-3-(1-naphthoyl)indole). As can be seen in Figure 1, AM2201 differs from JWH-018 by the replacement of a hydrogen atom on the end of the alkyl chain with a fluorine atom. In analyzing case specimens which contain AM2201, small amounts of JWH-018 and JWH-022 (1-(4-pentenyl)-3-(1-naphthoyl)indole) have been detected, interestingly, only in samples recovered from smoking devices or smoked cigar/cigarette butts. Due to the similarities in their structures (JWH-022 differs from JWH-018 by a double bond at the end of the alkyl chain), it was theorized that the JWH-018 and JWH-022 in the burnt residues may have occurred from the combustion of the plant material upon which the AM2201 was deposited.

Methods and Instrumentation

In order to test the theory of JWH-018 and JWH-022 arising as combustion products of AM2201, a “pipe” was fashioned from a section of a glass (5 mL) pipette, with copper wool inserted in one end. Plant material that contained AM2201 was packed into the end, above the wool. A vacuum line was used to draw the combustion products through the pipe as a match was used to ignite the plant material. After cooling, the pipe was rinsed thoroughly with methanol. An additional sample of the un-burnt plant material was extracted with methanol for comparison.

An Ion Sense (Saugus, MA) Direct Analysis in Real Time (DART™) ionization source coupled with a JEOL, Inc. (Peabody, MA) Accurate Time of Flight Mass Spectrometer (AccuTOF™) was used to collect data in the 65-600 Da range. The AccuTOF™ orifice 1 voltage was set to 30V. The DART™ gas, Helium (He), was heated to 275°C with a flow rate of 2.5 L/min. The methanol extracts of both the burnt and un-burnt samples were introduced into the ionization source with a glass melting point tube. More detailed information about DART™ can be found in articles by Cody, et al [2,3] and Steiner [4].

An Agilent (Little Falls, DE) dual column 7890 GC using an Agilent HP-5MS column (0.25 mm ID x 15 m x 0.25 µm film) and coupled with a 5975 mass spectrometer was used to collect

![Figure 1 - Structures of (A) 1-(5-fluoropentyl)-3-(1-naphthoyl)indole (AM2201), Formula: C_{24}H_{22}FNO, Molecular weight: 359.4 Da; (B) 1-pentyl-3-(1-naphthoyl)indole (JWH-018), Formula: C_{24}H_{23}NO, Molecular weight: 341.5 Da; and (C) 1-(4-pentenyl)-3-(1-naphthoyl)indole (JWH-022), Formula: C_{24}H_{21}NO, Molecular weight: 339.4 Da.](image)
spectra in the 14-600 m/z range. An Agilent HP-1 column (0.25 mm ID x 15 m x 0.25 µm film) was attached to a flame ionization detector (FID). The columns are joined in one split/splitless injection port using a two-hole ferrule. This allows data to be simultaneously collected from the mass spectrometer and the FID signal. Using a split ratio of 60:1, the temperature program for the GC ran from 200-300°C at a ramp rate of 30°C/min, He flow was 1.8 mL/min, the injection port was set at 290°C, the mass spectrometer transfer line was set at 300°C and the FID temperature was set at 290°C. For further separation and identification, a 30 meter HP-1MS column (0.25 mm ID x 0.25 µm film) was used under the same conditions in a different 7890/5975 GC/MS system. Using an Agilent HP-35 (0.25 mm ID x 15 m x 0.25 µm) column installed in an Agilent 6890 GC, a temperature program was run from 225-300°C at a ramp rate of 30°C/min, He flow was 1.8 mL/min, the injection port was set at 270°C and the detector was set at 280°C. An Agilent HP-1 column (0.25 mm ID x 15 m x 0.25 µm film) was also used with the Agilent 6890 GC under the same conditions.

Results and Discussion

The AccuTOF-DART™ data of the un-burnt plant material extract (Figure 2) shows a large peak for AM2201 ([M+H]+ 360.1794 Da) and nothing at the expected masses for JWH-018 ([M+H]+ calc. 342.1858 Da) and JWH-022 ([M+H]+ calc. 340.1701 Da) within the 5 mDa acceptance criteria. Figure 3 displays the resultant spectrum obtained from the methanol extract of the smoking device. JWH-018 is seen well within the acceptance range (342.1878 Da). The JWH-022 peak is slightly out of range but, as these are relatively small peaks, in a mixture spectrum, there is likely noise or other interference in the mass assignment of this peak.

Figure 4 shows the GC/MS data for the burnt plant material, with the peak at 4.61 min being AM2201 and the peak at 4.12 min showing the co-elution of JWH-018 and JWH-022 (Figure 5). Some distinction can be seen on the FID HP-1 column between these two compounds in the peak at 4.32 min (Figure 6).

It is of interest to note that very small amounts of JWH-018 and JWH-022 also appear to form as combustion products of AM2201 in the injection port of the GC. Recalling that the AccuTOF-DART™ analysis of the unburned plant material...
(Figure 2) did not show evidence of either of these products, the GC/MS data of the un-burnt material (Figures 7 and 8) shows their presence, with JWH-022 forming at a greater rate than JWH-018. The temperature to which the AM2201 is exposed in the AccuTOF-DART™ (275°C) is not as high as the temperature in the injection port of the GC (290°C), which in turn is less than the burning temperature in an actual flame. Given the relative ratio of AM2201 to the combustion products formed and, as the conversion in the injection port is not very aggressive, it is likely that the peak at 4.12 min, in unburned samples of AM2201, would not be seen under normal dilution. However, since the amount of JWH-018 in the burnt sample is roughly ten times greater, it is very probable that the JWH-018 would be in sufficient quantity for identification.

Figure 5 - (A) Co-eluted mass spectrum of JWH-018 and JWH-022; (B) reconstructed ion chromatogram showing coelution on the HP-5MS column.

Figure 6 - Enlarged portion of chromatogram from HP-1 (FID) column showing slight separation of peaks of interest (GC/MS conditions).

Figure 7 - Total ion chromatogram and (inset) reconstructed ion chromatogram of methanol extract of un-burnt plant material showing formation of JWH-018 and JWH-022 from heat of injection port. Note relative abundance of peak at 4.12 min to AM2201 peak at 4.73 min.

Figure 8 - Spectrum of peak at 4.12 min in Figure 7.
As co-elution between the two compounds does occur under the normal operating conditions of this laboratory; the identification of JWH-018 in burned samples requires additional measures. Thin Layer Chromatography does not provide significant chromatographic separation for synthetic cannabinoids [5], however, gas chromatography using an Agilent HP-35 column provided nearly baseline separation between JWH-018 and JWH-022 (Figures 9-10). While the separation is not as prominent on the GC using the HP-1 column, it did give enough separation to provide comparison to the JWH-018 standard (Figures 11 and 12). It should be noted that Figures 10 and 12 represent actual casework data of a cigarette butt containing charred AM2201. The additional peaks present are not identified in the scope of this paper.

In order to obtain a 'clean' mass spectrum of JWH-018 for structural confirmation, a 30 meter HP-1MS column afforded enough separation, with ion reconstruction, to provide a good spectrum (Figure 13).
Figure 13 - Reconstructed ion chromatogram and subtracted spectra of JWH-022 and JWH-018 run on GC/MS with 30m HP-1MS column.

References
Methamphetamine Contaminated Currency in the Birmingham, Alabama Metropolitan Area

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ABSTRACT: GC-MS Analyses of extracts of one dollar bills collected from in and nearby the Birmingham, Alabama metropolitan area in 2012 indicated that 42% of them were contaminated with methamphetamine. This is the first time that methamphetamine was identified on one dollar bills since the laboratory began testing them in 2008.

KEYWORDS: methamphetamine, cocaine, currency, gas chromatography-mass spectroscopy, trace analysis, forensic chemistry.

Analysis of one dollar bills (USD1s) acquired in and near the Birmingham, Alabama metropolitan area for cocaine contamination has been an ongoing study at the University of Alabama at Birmingham Forensic Chemistry Laboratory since 2008. Most of the bills that have been analyzed were acquired in sets of 20 USD1s from local stores and banks. Since the program inception, between 40 and 85 percent of the bills in each set have tested positive for cocaine. Other reports of cocaine contamination of U.S. currency have given values ranging from 67 to 97 percent; however, most of these reports were for analyses of higher denomination bills [1-5].

In February 2012, a set of 20 USD1s was collected from a home improvement store in north Jefferson County, Alabama, about eight miles north of Birmingham (designated as NJC 1 in Table 1 and Figure 1). The results of the analysis of the 20 USD1s were unexpected. Only eight of the collected bills tested positive for cocaine, but 17 tested positive for methamphetamine. This was the first time that methamphetamine had been identified on a set of USD1s analyzed by this laboratory. In order to determine if these findings were a one-time occurrence or rather was indicative of a fundamental change in the drug contamination of bills in the Birmingham area, additional sets of 20 USD1s were obtained and analyzed, from: A) The same store in north Jefferson County (NJC 2); B) Downtown Birmingham (B’Ham); C) Bessemer (BES, about 15 miles southwest of Birmingham); and D) Grant (about 85 miles northwest of Birmingham). In addition, the chromatograms from the previously collected and analyzed bills (i.e., 2008 – 2011) were re-examined to determine if they had also been contaminated with methamphetamine, but not recognized as such at the time.

Experimental
The USD1 sets from north Jefferson County, Bessemer, and Grant were all collected from home improvement stores, whereas the downtown Birmingham set was collected from a fast food restaurant. At each location, all 20 bills were collected from a single cash register (our previous work with cocaine contaminated USD1s had shown that there were no differences in bills collected from a single versus separate registers; that is, there was no evidence of cross contamination from cashier handling or passive contact within an individual register). Each set of bills was placed in a zip-lock plastic bag by the respective cashier, and laboratory gloves were worn by the analysts who subsequently handled the bills. The serial numbers of the bills were recorded in the order they were analyzed (subsequently, the respective Federal Reserve Bank locations were recorded for additional data evaluation; however, because the date of issue does not necessarily correspond to the year the bill was printed, the dates of issue were not recorded).

Figure 1 - Gas chromatograms of extracts showing cocaine and/or methamphetamine from USD1s.
The extraction method developed by Negruz et al. was utilized [6]. Each bill was crumpled and placed into a 20 mL vial. Ten mL of 0.1 M HCl were added, and the vial was capped and agitated on an orbital shaker (150 cycles/min) for between 30 min and overnight. The resulting solution was transferred to a 20 mL vial, basified to pH 12 with 2 M NaOH, and extracted with 1 mL of CHCl₃.

The isolated extracts were analyzed using an Agilent 6890 gas chromatograph equipped with a DB-5 MS column (30 m × 0.25 mm, 0.25 μm film thickness), interfaced with an Agilent 5973 MSD. Helium was used as the carrier gas at a constant flow of 1.0 mL/min. The injector temperature was 260°C and the split ratio was 15:1. The GC temperature program started at 70°C (no hold), ramped at 20°C/min to 250°C (6 min hold).

Cocaine and methamphetamine standards were obtained from Sigma Aldrich. All other chemicals were chromatographic or HPLC grade. The retention time of the methamphetamine standard was 4.578 ± 0.05 min, and the fragment ions of m/z 58, 91, and 134 were used for confirmation. The retention time of the cocaine standard was 11.135 ± 0.06 min, and the fragment ions of m/z of 82, 182, and 303 were used for confirmation. Representative chromatograms are shown in Figure 1; in addition to methamphetamine and cocaine, several common contaminants are labeled, including a possible nicotine metabolite (5.73 min), acetaminophen (6.04 min), diethyltoluamide (familiarly known as DEET, 7.16 min), diethyl phthalate (7.22 min), and 1,2-diphenoxycetone (8.43 min). DEET is the most commonly used insect repellent, while diethyl phthalate and 1,2-diphenoxycetone are used in production of various polymers. All of these contaminants were identified by comparison with the NIST mass spectra library [7] and were not confirmed. The chromatograms were not quantitated.

Results and Discussion
USD1s were originally chosen (in 2008) for this study both to reduce expense and because they are reportedly less likely to be contaminated with cocaine from trafficking versus any other denomination except for $100 bills [3]. Thus, in our opinion the results presented herein are more reflective of handling by cocaine and/or methamphetamine consumers, enabling local trends to be more easily identified and monitored.

The 2012 results are summarized in Table 1. In NJC 1, nine of the 20 bills were positive for methamphetamine alone, none were positive for cocaine alone, and eight were co-contaminated with both cocaine and methamphetamine. In NJC 2, none were positive for methamphetamine alone, 13 were positive for cocaine alone, and five were co-contaminated. In B’Ham, none were positive for methamphetamine alone, 14 were positive for cocaine alone, and two were co-contaminated. In BES, three were positive for methamphetamine alone, six were positive for cocaine alone, and eight were co-contaminated. In Grant, three were positive for methamphetamine alone, seven were positive for cocaine alone, and eight were co-contaminated. In total, 42% of the bills collected to date in 2012 tested positive for methamphetamine.

The chromatograms from previously conducted analyses (i.e., 2008 – 2011) were then re-examined to see if methamphetamine had actually been present on those bills but not recognized at the time. None of the chromatograms from any of the earlier analyses that were conducted using the conditions detailed in the Experimental section were positive for methamphetamine. However, one set of 10 bills collected in Birmingham in October, 2011 that had been analyzed using an alternate splitless GC method (for maximum sensitivity) did have three bills display ultra-trace-level peaks for methamphetamine. Based on these results, the contamination of USD1s with methamphetamine is a recent development in the Birmingham area.

Cocaine contamination of currency is international in scope, and has been thoroughly documented. There have been several mechanisms proposed for this contamination, including adsorption to the paper fibers, and absorption/dissolution in the various dyes that are imprinted on the paper and/or in the human sweat components and skin oils that become laced into the paper from normal handling of the bills. At the present time, the only identifiable trend is that most currency will test positive for cocaine in countries where cocaine abuse is widespread, and test negative in countries where such abuse is uncommon. According to Ebejer et al., it is currently not possible to correlate cocaine contamination with rural versus urban populations, percentage of convicted drug offenders in the area, proximity to a port of entry, geographical region, or socio-economic standing [8].

Contamination of currency with other drugs of abuse has been previously reported, although with less frequency and lower abundance versus cocaine. Heroin, morphine, O⁶-monoacetylmorphine, methamphetamine, phenylalanine, tetrahydrocannabinol, and 3,4-methylenedioxymethamphetamine have all been reported on currency [2,5,9-13].

Table 1 - Number and % of Drug Contaminated Currency.

<table>
<thead>
<tr>
<th>Set</th>
<th>Methamphetamine Only</th>
<th>Cocaine Only</th>
<th>Methamphetamine &amp; Cocaine</th>
<th>% Contaminated With Methamphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NJC 1</td>
<td>9</td>
<td>0</td>
<td>8</td>
<td>85</td>
</tr>
<tr>
<td>NCJ 2</td>
<td>0</td>
<td>13</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>B’Ham</td>
<td>0</td>
<td>14</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>BES</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>55</td>
</tr>
<tr>
<td>Grant</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>35</td>
</tr>
</tbody>
</table>

The extraction method developed by Negruz et al. was utilized [6]. Each bill was crumpled and placed into a 20 mL vial. Ten mL of 0.1 M HCl were added, and the vial was capped and agitated on an orbital shaker (150 cycles/min) for between 30 min and overnight. The resulting solution was transferred to a 20 mL vial, basified to pH 12 with 2 M NaOH, and extracted with 1 mL of CHCl₃.

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In 2001, Jenkins reported that 3 of 50 USD1s analyzed for drugs were positive for methamphetamine [2]; the bills in this study were collected from five different U.S. cities. Also in 2002, Nath et al. examined 80 bills from convenience stores in San Francisco and determined that methamphetamine could be detected on bank notes and that screening currency was indicated [11]. In 2010, Veitenheimer analyzed bundles of 1, 5, 10, 20, 50, and 100 dollar bills (10 bills per bundle) collected in 35 different cities for cocaine, codeine, heroin, MDMA, methamphetamine, and morphine. Nine of the bundles (4.3%) tested positive for methamphetamine [12]. And in 2011, Wimmer et al. identified methamphetamine on 53 of 64 Euros of mixed denominations; however, the average contamination per bill was only 7 ng, lower than cocaine (106 ng), benzoylecgonine (43 ng), heroin (41 ng), O6-monoacetyl-morphine (15.5 ng), morphine (16.5 ng), and MDMA (9 ng). Interestingly, all 64 of the Euros analyzed in this study were contaminated with cocaine [13].

As an additional measure of the randomness of the sample, the percentage of methamphetamine contaminated bills versus Federal Reserve Bank source was calculated for the Bessemer and Grant sets (Table 2). Although Birmingham is in the Atlanta Federal Reserve District, 11 of the 12 Federal Reserves were represented in the two selected sets; only the Minneapolis Federal Reserve was not encountered. Of the bills collected in BES and Grant, 27.5% were from the Atlanta Reserve Bank and 27.85% of the bills that tested positive for methamphetamine were from the Atlanta Reserve. Although the contribution from each individual Federal Reserve Bank is too small to assign any significance to them, collectively these values indicate that the location of issue is not a factor in the percentage of bills that are contaminated with methamphetamine.

Table 2 - Percent methamphetamine contamination by federal reserve banks for the Bessemer and Grant bills.

<table>
<thead>
<tr>
<th>Federal Reserve Bank</th>
<th>Number of Bills</th>
<th>% of Bills</th>
<th>Methamphetamine Positives</th>
<th>Cocaine Positives</th>
<th>% Total Methamphetamine Contaminated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlanta</td>
<td>11</td>
<td>27.5</td>
<td>5</td>
<td>8</td>
<td>27.8</td>
</tr>
<tr>
<td>Boston</td>
<td>3</td>
<td>7.5</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Chicago</td>
<td>4</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>5.6</td>
</tr>
<tr>
<td>Cleveland</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Dallas</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Kansas City</td>
<td>1</td>
<td>2.5</td>
<td>1</td>
<td>0</td>
<td>5.6</td>
</tr>
<tr>
<td>Minneapolis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>New York</td>
<td>5</td>
<td>12.5</td>
<td>4</td>
<td>3</td>
<td>22.2</td>
</tr>
<tr>
<td>Philadelphia</td>
<td>1</td>
<td>2.5</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Richmond</td>
<td>5</td>
<td>12.5</td>
<td>3</td>
<td>3</td>
<td>16.7</td>
</tr>
<tr>
<td>San Francisco</td>
<td>4</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td>Saint Louis</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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Conclusions

Methamphetamine was detected on currency for the first time in the Birmingham metropolitan area. Forty-two percent of the bills collected to date in 2012 were contaminated with methamphetamine, more than has been previously reported for any drug other than cocaine in the United States. The high percentage of contamination detected in this study, and its sudden appearance, indicates a significant change in the pattern of drug contamination of currency around Birmingham, probably reflecting higher methamphetamine abuse in the local populace. This conclusion is in agreement with and complements the findings reported in the National Substance Abuse Index, which states that methamphetamine abuse currently exceeds that of cocaine throughout the state of Alabama [14].

By the time that contamination of currency with cocaine was detected, it was already widespread. The results of this study suggest that it is possible to track significant changes in methamphetamine abuse in a specific region over time. Future studies may lead to insights into the geographical and economic factors that influence methamphetamine abuse (if any). Determining the actual mechanisms for the absorption and/or adsorption of methamphetamine to currency is potentially an important area of future research.

References:


The Characterization of 6-(2-Aminopropyl)benzofuran and Differentiation from its 4-, 5-, and 7-Positional Analogues

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ABSTRACT: The isolation, analysis, synthesis, and characterization of 6-(2-aminopropyl)benzofuran (currently and commonly referred to as 6-APB) are briefly discussed. Analytical data (infrared spectroscopy, mass spectrometry, and nuclear magnetic resonance spectroscopy) are presented to differentiate it from the 4-, 5, and 7- positional analogues.

KEYWORDS: 6-(2-aminopropyl)benzofuran, 4-(2-aminopropyl)benzofuran, 5-(2-aminopropyl)benzofuran, 7-(2-aminopropyl)benzofuran, 4-APB, 5-APB, 6-APB, 7-APB, designer drug, synthesis, characterization, forensic chemistry.

This laboratory recently received a request to confirm the identity of a suspected sample of 6-(2-aminopropyl)benzofuran and synthesize a primary standard for its identification in a number of drug exhibits. 6-(2-Aminopropyl)benzofuran (Figure 1, structure 3) is widely available through Internet vendors, and is currently marketed as “6-APB” or “Benzo fury.” Herein, we report the isolation, characterization (nuclear magnetic resonance spectroscopy, mass spectrometry, and infrared spectroscopy), and synthesis of 6-(2-aminopropyl)benzofuran 3. Additionally, data is presented for 4-(2-aminopropyl)benzofuran 1, 5-(2-aminopropyl)benzofuran 2, and 7-(2-aminopropyl)benzofuran 4 to assist forensic chemists who may encounter these substances in casework.

Experimental

Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All other chemicals and NMR solvents were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Synthesis of 6-(2-Aminopropyl)benzofuran 3 and 4-(2-Aminopropyl)benzofuran 1
In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2. The procedure of Briner et al. [1] was utilized. Briefly, bromophenol 5 was refluxed with bromoacetaldehyde 6 and NaH to give the diethyl acetyl 7, which was heated with polyphosphoric acid to give a mixture of bromobenzofurans 8 and 9. Compounds 8 and 9 were separated via silica gel column chromatography, catalytically converted to their respective 2-propanones 10 and 11, and then reductively aminated to 3 (6-APB) and 1 (4-APB). Both 1 and 3 were converted to their HCl ion-pairs.

Synthesis of 5-(2-Aminopropyl)benzofuran 2 and 7-(2-Aminopropyl)benzofuran 4
The benzofuran carbaldehydes 12 and 13 were converted to their respective benzonitrostyrenes 14 and 15, followed by LAH reduction to the amines 2 (5-APB) and 4 (7-APB). Both 2 and 4 were converted to their HCl ion-pairs.

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Figure 1 - Structural formulas. 1 = 4-(2-aminopropyl)benzofuran, 2 = 5-(2-aminopropyl)benzofuran, 3 = 6-(2-aminopropyl)benzofuran, and 4 = 7-(2-aminopropyl)benzofuran.
Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were obtained on an Agilent 400MR NMR with a 400 MHz magnet, a 5 mm Protune indirect detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The HCl ion-pair of the compound was first dissolved in CDCl₃ containing TMS as the 0 ppm reference, and later base extracted using saturated sodium bicarbonate in D₂O. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon (proton decoupled), and gradient versions of the 2 dimensional experiments HSQC, HMBC, and NOESY. Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Results and Discussion

Isolation and Characterization of 6-(2-Aminopropyl)benzofuran

Approximately 5 grams of illicit material was submitted for characterization/purification. The material was practically insoluble in CHCl₃ and had minimal solubility in cold H₂O.

A direct FTIR spectrum was non-descriptive. GC/MS analysis of the material as the TMS derivative produced one minor and two major peaks (Figure 4). Peak #1 was identified as the di-TMS derivative of succinic acid and contributed to approximately 65% of the total ion current. Peaks #2 and #3 (representing ca. 2% and 32% of the total ion current, respectively) produced nearly identical spectra having a base peak at m/z 116, a trimethylsilyl-loss ion at m/z 73, and a cluster of minor ions from m/z 244 to m/z 248 (the molecular ions could not be determined; spectra not shown). NMR analysis revealed two succinic acid molecules per amine molecule (2:1). A portion of the sample was then dissolved in boiling water, basified with saturated aqueous NaHCO₃, and extracted with CHCl₃ for GC/MS analysis. Two peaks representing 6% (peak #1) and 94% (peak #2) of the total ion current (chromatogram and spectra not shown) produced virtually identical spectra with a base peak at m/z 44 and molecular ion at m/z 175, consistent with expected ions for 1-4.

For characterization, the major component was isolated from the minor component by dissolving 1.36 grams of illicit material in 16 mL of hot water (80°C), adding 8 mL of saturated aqueous NaHCO₃, extracting with Et₂O (2 x 30 mL), drying the organic layer over anhydrous Na₂SO₄, and finally...
converting to the HCl ion-pair with Et₂O-HCl. The resulting crystalline material was washed with a minimal volume of hot acetone (minor component was soluble in hot acetone) and dried to provide 300 mg of off-white powder that was free of the minor component and 99.5+% chromatographically pure (by GC/MS). This material was examined by NMR. The carbon spectrum showed 11 peaks (8 aromatic and 3 aliphatic) while the proton spectrum showed 14 hydrogens (very broad singlet at 8.5 ppm) has 3 hydrogens (probably +NH₃), 5 aromatic hydrogens, and 6 aliphatic hydrogens. The HSQC spectrum aliphatic region revealed one methyl, one methylene, and one methine. The proton splitting patterns and chemical shifts for these aliphatic hydrogens is highly similar to methamphetamine’s aliphatic region, indicating Aryl-CH₂-CH(N)-CH₃. The aromatic proton region splitting patterns suggest a 3,4-substituted phenyl, and the HMBC, HSQC, and carbon spectra indicate that the 3,4-substitution group is CH=CH-O. The NOESY spectrum confirms that the orientation of the aliphatic group is at C-6 of the benzofuran ring. ACD/Labs Structure Elucidator software was used to process the NMR data. The compound was identified as 6-(2-aminopropyl)-benzofuran, identical to the synthesized standard.

FTIR, GC/MS, and NMR Characterization/Differentiation of 4-, 5-, 6-, and 7-(2-Aminopropyl)benzofuran

GC retention time data for the respective synthesized compounds (Figure 1) are presented in Table 1. All amines were injected as the free base. The 5- and 6- isomers (compounds 2 and 3) gave virtually identical retention times and could not be resolved under the conditions utilized. Both 2 and 3 also eluted at approximately the same retention time as MDA in the described system.

The FTIR spectra for compounds 1-4 are illustrated in Figures 5-8. All compounds appeared to exhibit polymorphism, depending on how the HCl ion-pair was crystallized. Rapid crystallization gave material with slightly different spectra versus material from slow crystallization; a previously observed phenomenon with MDA HCl as well. Comparison of the four HCl ion-pairs (both rapid and slow crystallization) reveals dissimilar patterns, with the most prominent differences being in the region of 400-1700 cm⁻¹. However, since there appears to be differing polymorphic crystalline forms of each, care must be taken in their identification via FTIR, and additional or supplementary spectroscopic methods should be utilized for identification.

The mass spectra of all four 2-aminopropylbenzofurans were nearly identical and are illustrated in Figures 9 and 10. Each produced a base peak at m/z 44 and a moderate molecular ion at m/z 175. However, 6-(2-aminopropyl)benzofuran (3) produces a much more intense fragment ion at m/z 132, relative to m/z 131 (m/z 132 for 3 has a relative abundance of 16% compared to 6% for 1, 7% for 2, and 7% for 4. Although the relative abundances for the remaining ions are quite similar, 3 can be easily distinguished on the basis of the m/z 131/132 ratio (1 = 2.9:1, 2 = 2.5:1, 3 = 1.3:1, and 4 = 2.4:1). All four
Figure 5 - FTIR of 4-(2-aminopropyl)benzofuran 1. (a) slow crystallization, (b) rapid crystallization.
Figure 6 - FTIR of 5-(2-aminopropyl)benzofuran 2. (a) slow crystallization, (b) rapid crystallization.
Figure 7 - FTIR of 6-(2-aminopropyl)benzofuran 3. (a) slow crystallization, (b) rapid crystallization.
Figure 8 - FTIR of 7-(2-aminopropyl)benzofuran 4. (a) slow crystallization, (b) rapid crystallization.
Figure 9 - Mass spectrum of (a) 4-(2-aminopropyl)benzofuran 1 and (b) 5-(2-aminopropyl)benzofuran 2.
Figure 10 - Mass spectrum of (a) 6-(2-aminopropyl)benzofuran 3 and (b) 7-(2-aminopropyl)benzofuran 4.

*Microgram Journal, Volume 9, Number 2*
Table 1: 

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$d = $ doublet, $q = $ quartet

Figure 11 - $^1H$ and $^{13}C$ NMR data for 4-(2-aminopropyl)benzofuran 1 dissolved in CDCl₃.
**5-APB base**

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</table>

$d =$ doublet, $q =$ quartet

Figure 12 - $^1H$ and $^{13}C$ NMR data for 5-(2-aminopropyl)benzofuran 2 dissolved in CDCl₃.

*Microgram Journal, Volume 9, Number 2*
Figure 13 - $^1\text{H}$ and $^{13}\text{C}$ NMR data for 6-(2-aminopropyl)benzofuran 3 dissolved in CDCl$_3$. 

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bs = broad singlet, d = doublet, q = quartet
Figure 14 - $^1$H and $^{13}$C NMR data for 7-(2-aminopropyl)benzofuran 4 dissolved in CDCl$_3$.

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Compounds can be distinguished based on a combination of retention times and the m/z 131/132 ratio; however, since 2 and 3 elute at essentially the same retention time, care must be taken in differentiating those compounds.

The proton and carbon assignments for 1-4 as the free base are presented in Figures 11-14. Assignments were based on proton chemical shifts and peak patterns, carbon chemical shifts, HSQC (1 bond carbon to proton), HMBC (2-4 bond carbon to proton), and NOESY (spatially near protons) spectra. Assignments were further confirmed using ACD Structure Elucidator software. Proton spectra from all four compounds contain small coupling doublets (~2 Hz) at about 6.7 and 7.6 ppm, which are H-3 and H-2, respectively. The other 3 aromatic proton signals fall into one of two patterns; 1) two large coupling doublets and one triplet (or apparent triplet), which results from having a series of 3 bonded methines (compounds 1 and 4); or 2) one large coupling doublet, one doublet of doublets, and one small coupling doublet due to CH=C-CH=CH series (compounds 2 and 3). HMBC spectra further distinguish positional isomers 1 from 4 by correlating C-7a (~155 ppm) to the aliphatic protons (only found with 4) or correlating C-3a (~127 ppm) to the aliphatic protons (only found with 1). Distinguishing 2 from 3 is done by HMBC correlations from C-3 to H-4 and then examining the proton peak pattern of H-4; small coupling doublet indicates 2 while a large coupling doublet indicates 3.

Conclusions

The illicit sample was identified as 6-(2-aminopropyl) benzofuran succinate (major component) containing 4-(2-aminopropyl)benzofuran succinate (minor component). The exhibit was also found to be diluted with excess succinic acid.

References


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*Conditions given in the experimental section.*
Differentiation of 3,4-Dimethylmethcathinone (3,4-DMMC) from its Dimethyl Aryl-Positional Isomers

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Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166-9509

ABSTRACT: The synthesis and characterization of six dimethylmethcathinones via mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy are discussed. Analytical data are presented to differentiate these positional analogues.

KEYWORDS: 3,4-dimethylmethcathinone, 2,3-dimethylmethcathinone, 2,4-dimethylmethcathinone, 2,5-dimethylmethcathinone, 3,5-dimethylmethcathinone, 2,6-dimethylmethcathinone, DMMC, designer drug, synthesis, characterization, forensic chemistry.

A number of methcathinone analogues have been recently reported in the literature [1-9]. The characterization of 3,4-dimethylmethcathinone (3,4-DMMC) has been reported [1], however, there are several structural analogues of 3,4-DMMC. Structural analogues are usually easily differentiated by proton nuclear magnetic resonance (¹H-NMR) spectroscopy; however, the majority of forensic laboratories are not equipped with such instrumentation, and therefore rely heavily on gas chromatography/mass spectrometry (GC/MS) and Fourier Transform infrared spectroscopy (FTIR) for the identification of drug exhibits. In many cases, GC/MS and/or FTIR do not always produce clear differentiation of structurally related analogues; therefore, standards are needed for direct comparison of spectra and retention data. Hence, we report the synthesis, characterization, and differentiation of five of the six dimethylmethcathinones (Figure 1, structures 5-9) via mass spectrometry and infrared spectroscopy, and the trace-level synthesis and GC/MS analysis of the sixth dimethylmethcathinone (structure 10). Only a trace amount of suspected 10 could be synthesized due to the effects of steric hindrance; therefore, only mass spectral data is presented for that isomer.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). 1-(2,3-Dimethylphenyl)propan-1-one, 1-(2,4-dimethylphenyl)propan-1-one, 1-(2,5-dimethylphenyl)propan-1-one, and 1-(3,5-dimethylphenyl)propan-1-one were products of Novel Chemical Solutions (Crete, NE). All other chemicals and NMR solvents were of reagent-grade quality and products of Sigma-Aldrich Chemical (Milwaukee, WI).

Synthesis of 3,4-Dimethylmethcathinone 5

In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2. Briefly, dimethylbromobenzene 1 was reacted with magnesium metal to give the Grignard 2, which was added to propionitrile to give the ketone 3, which was converted to the α-bromoketone 4, which was reacted with methylamine to give 3,4-dimethylmethcathinone 5, which was finally converted to its HCl ion-pair.

Synthesis of the 2,3-, 2,4-, 2,5-, and 3,5-Dimethylmethcathinones 6, 7, 8, and 9.

Each compound was synthesized using the appropriate dimethyl-substituted phenyl-1-propanone (analogous to 3) using the route illustrated in Figure 2.

Synthesis of 2,6-Dimethylmethcathinone 10

Several attempts to produce 10 through three different routes were unsuccessful due to the effects of steric hindrance from the 2,6-dimethyl substitution. One synthetic route produced a trace amount of suspected desired material (determined via GC/MS), but there was insufficient material for NMR
confirmation or an FTIR spectrum, and its identity is therefore unconfirmed.

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, 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).

Infrared Spectroscopy (FTIR)
Infrared spectra were obtained using a Perkin Elmer Spectrum 400 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Nuclear Magnetic Resonance Spectroscopy (NMR)
Proton (¹H), carbon (¹³C), HSQC, and HMBC NMR spectra were obtained using an Agilent 400MR NMR with 5 mm Protone indirect detection, pulse field gradient probe (Palo Alto, CA) using standard Agilent pulse sequences. The compounds were extracted into deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) using sodium bicarbonate saturated deuterium oxide (D₂O) (Sigma-Aldrich, St. Louis, MO). The sample temperature was maintained at 26°C. Data processing and structure elucidation were performed using Agilent NMR software and ACD Structure Elucidator software (Applied Chemistry Development, Toronto, Canada).

Results and Discussion
GC-MS and FTIR Differentiation of 3,4-DMMC from the 2,3-, 2,4-, 2,5-, 3,5- and (presumed) 2,6-positional analogues
GC retention time data for the respective compounds (Figure 1) are presented in Table 1. All amines were injected as their free bases. All six compounds were resolved in the described system.

Mass spectra and infrared spectra for compounds 5-10 are given in Figures 3-8 (except the FTIR for 10).

The mass spectra of all six dimethylmethcathinones gave relatively similar fragmentation patterns, but significant differences were observed. Each produced a base peak at m/z 58 and a weak molecular ion at m/z 191. Compound 5 produced ion abundances of m/z 119 > m/z 115, while compounds 6-10 produced ion abundances of m/z 115 > m/z 119. Additionally, compound 5 was easily distinguished from 6, 8, 9, and 10 by the relative abundances of ions at m/z 105 and m/z 133, where m/z 133 > m/z 105. Other significant differences between the spectra of 5-10 were also observed at low abundance ions between m/z 146 and m/z 176; each compound can be differentiated by its total spectrum.

The FTIR spectra for 5-9 gave somewhat similar secondary amine HCl ion-pair absorbances between 2400-3000 cm⁻¹ as well as a ketone stretch at 1680-1700 cm⁻¹. However, characteristic differences were observed between 400-1600 cm⁻¹, where each compound could be easily differentiated.

NMR Characterization/Differentiation
Proton chemical shifts, peak patterns, coupling constants, and assignments are presented in Table 2. Carbon chemical shifts and assignments of the base forms are presented in Table 3. Assignments were based on proton chemical shifts and peak patterns, carbon chemical shifts, HSQC (1 bond carbon to proton), and HMBC (2-4 bond carbon to proton) spectra, and were further confirmed using ACD Structure Elucidator software.

For each compound, a CH-CH₂ group is shown by a methine quartet (3.93-4.19 ppm) coupled to a methyl doublet (1.19-
Figure 3 - Mass spectrum (upper) and of FTIR (lower) of 3,4-dimethylmethcathinone 5.

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Figure 4 - Mass spectrum (upper) and of FTIR (lower) of 2,3-dimethylmethcathinone 6.
Figure 5 - Mass spectrum (upper) and FTIR (lower) of 2,4-dimethylmethcathinone 7.

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Figure 6 - Mass spectrum (upper) and FTIR (lower) of 2,5-dimethylmethcathinone 8.
Figure 7 - Mass spectrum (upper) and of FTIR (lower) of 3,5-dimethylmethcathinone 9.

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5 has benzene methyl proton signals that are so close that they produce a broad singlet and their carbon signals, although different, are within 0.3 ppm.

**Conclusions**

3,4-Dimethylmethcathinone 5 is easily distinguished from its positional isomers (6-10) via GC-MS. Each positional isomer produces a unique (although similar) mass spectrum. FTIR and NMR also delineates five of the possible six isomers (the 2,6-isomer 10 could not be synthesized in sufficient quantity for FTIR and NMR analysis).

**References**


A number of highly potent hallucinogenic phenethylamine derivatives have been encountered by law enforcement within the past year. These designer drugs are commonly referred to as “NBOMe” compounds; their structures are depicted in Figure 1 (compounds 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, and 31). They are N-(2-methoxy)benzyl derivatives of the “2C compounds” (2,5-dimethoxyphenethylamines with various substituents at C-4), first made popular as a result of their publication in PIHKAL [1]. The NBOMe series was first investigated by Heim and co-workers [2] as agonists for the 5-HT2A serotonin receptors that are associated with hallucinogenic activity, and later by Braden et al. as “superpotent” agonists for those receptors [3]. Although there have been no scientific studies on the potency of these derivatives, several illicit drug-related Internet websites recommend sub-milligram (microgram) doses, on par with LSD. Violent physical/mental episodes and deaths have been attributed to the abuse of these compounds [4, 5]. The NBOMe compounds are illicitly distributed as either uncut powders or diluted to sub-milligram doses laced into perforated blotter paper. Due to their extreme potency, forensic chemists must take great care to prevent accidental self-dosing during routine chemical analysis.

Except for three NBOMe derivatives [6], there currently is little or no spectroscopic or spectrometric data in the literature on these compounds. Herein, we report the synthesis, characterization, and differentiation of 11 commonly encountered 2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (NBOMe) derivatives from their 3- and 4-methoxybenzyl analogues (Figure 1) via mass spectrometry and infrared spectroscopy. Nuclear magnetic resonance spectroscopy of these compounds will be the subject of a later report.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). 2,5-Dimethoxy-4-bromo-phenethylamine, 2,5-dimethoxy-4-chlorophenethylamine, 2,5-dimethoxy-4-methylphenethylamine, 2,5-dimethoxy-4-
ethylphenethylamine, 2,5-dimethoxy-4-iodophenethylamine, 2,5-dimethoxy-4-nitrophenethylamine, 2,5-dimethoxy-4-ethylthiophenethylamine, 2,5-dimethoxy-4-isopropylthiophenethylamine, and 2,5-dimethoxy-4-propylthiophenethylamine were obtained from the reference materials collection maintained at this laboratory. 2,5-Dimethoxyphenethylamine and all other chemicals were of reagent-grade quality and products of Sigma-Aldrich Chemical (Milwaukee, WI).

Synthesis of NBOMe compounds (1-33)

In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2 for 2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine 1. Briefly, the appropriate 4-substituted 2,5-dimethoxyphenethylamine was condensed with the appropriate methoxy-substituted benzaldehyde, and then reduced with NaBH₄ to provide the desired product. All compounds were converted to their HCl ion-pairs with ethereal HCl.

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Results and Discussion

For the purposes of this article and clarity, abbreviations of the ensuing compounds will include the 4-substitution as commonly given for the 2C moiety + the N-benzyl addition (i.e., 2C-B = 25B-NB), and also include the position of the methoxy-substitution (i.e., 2-methoxy = 2OMe) on the benzyl moiety. Therefore 2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine 1 = 25H-NB2OMe, 2,5-dimethoxy-N-(3-methoxybenzyl)phenethylamine 2 = 25H-NB3OMe, and 2,5-dimethoxy-N-(4-methoxybenzyl)phenethylamine 3 = 25H-NB4OMe. The three 4-bromo derivatives are abbreviated 25B-NB2OMe 4, 25B-NB3OMe 5, and 25B-NB4OMe 6. This nomenclature is also utilized for compounds 7-33, as illustrated in Figure 1.

GC/MS Differentiation of the NBOMe methoxybenzyl positional isomers (2-OMe vs. 3-OMe vs. 4-OMe)

GC retention times for all 33 NBOMe compounds are presented in Table 1. All amines were injected as their free bases and were found to be relatively high boiling, late eluting compounds. The three methoxybenzyl positional isomers for each of the NBOMe compounds were resolved in the described
system. The elution order (retention time) was as followed: 4-Methoxybenzyl > 3-methoxybenzyl > 2-methoxybenzyl for each NBOMe series.

Mass spectra for compounds 1-33 are given in Figures 3-13. In general, all compounds produced a base peak ion at \( m/z \) 121 due to cleavage of the benzyl moiety and an ion at \( m/z \) 150 due to alpha-cleavage of the phenethylamine moiety. All 2-methoxybenzyl substituted compounds (1, 4, 7, 10, 13, 16, 19, 22, 25, 28, and 31) produced a tropylium ion at \( m/z \) 91 at significantly greater relative abundance than their corresponding 3- and 4-methoxybenzyl substituted analogues. Although Zuba and Sekula [6] did not report molecular ions for underivatized 10 or 13, we obtained molecular ion data for all 33 compounds. The relative abundances of the molecular ions were extremely low, however, and ranged from 0.05 to 1.0%.

25H-NB2OMe 1, 25H-NB3OMe 2, and 25H-NB4OMe 3 (Figure 3)

Compounds 1 is differentiated from 2 and 3 by the relative abundances of \( m/z \) 91 (1 = 30%, 2 = 12%, and 3 = 5%) and the relative abundances of \( m/z \) 150 and \( m/z \) 152 (\( m/z \) 150/152: 1 = 7.1, 2 = 2.1, and 3 = 1.2). Further delineation of 1 was observed by comparing ions at \( m/z \) 268 and \( m/z \) 270 (\( m/z \) 268/270: 1 = 0.8, 2 = 2.2, and 3 = 2.1). Compound 1 also produced a significant M-2 ion at \( m/z \) 299, relative to the molecular ion (\( m/z \) 299 > \( m/z \) 301), while 2 and 3 had more intense molecular ions (\( m/z \) 301 > \( m/z \) 299).

25B-NB2OMe 4, 25B-NB3OMe 5, and 25B-NB4OMe 6 (Figure 4)

Each compound produced molecular ions at \( m/z \) 379 and \( m/z \) 381, and at fragment ions \( m/z \) 199 and \( m/z \) 201, consistent with the relative abundance ratios expected for bromine substitution. All three compounds were differentiated by the relative abundances of fragment ions found at \( m/z \) 91 and \( m/z \) 150 (\( m/z \) 150/91: 4 = 2.2, 5 = 7.1, and 6 = 5.8). Although the \( m/z \) 150/91 ratio for 5 and 6 were somewhat similar, the relative abundance for \( m/z \) 150 produced by 5 was 73%, compared to 38% for 6.

25C-NB2OMe 7, 25C-NB3OMe 8, and 25C-NB4OMe 9 (Figure 5)

All three compounds were differentiated by the relative abundances of fragment ions found at \( m/z \) 91 (tropylium) and \( m/z \) 150 (\( m/z \) 150/91: 7 = 1.6, 8 = 6.1, and 9 = 5.3). Although the \( m/z \) 150/91 ratio for 8 and 9 were similar, the relative abundance for \( m/z \) 150 produced by 8 was 46%, compared to 24% for 9. Compound 7 also produced a significant M-2 ion at \( m/z \) 333, relative to the first chlorine isotope molecular ion (\( m/z \) 333 > \( m/z \) 335), while 8 and 9 had a more intense chlorine isotope at \( m/z \) 335 (\( m/z \) 335 > \( m/z \) 333).

25D-NB2OMe 10, 25D-NB3OMe 11, and 25D-NB4OMe 12 (Figure 6)

The tropylium ion (\( m/z \) 91) abundances were 10 = 28%, 11 = 13%, and 12 = 7%. Further delineation was also observed by the relative abundances of the fragment ions produced at \( m/z \) 150 and \( m/z \) 166 (\( m/z \) 150/166: 10 = 1.8, 11 = 0.9, and 12 = 0.3).

25E-NB2OMe 13, 25E-NB3OMe 14, and 25E-NB4OMe 15 (Figure 7)

The tropylium ion (\( m/z \) 91) abundances were 13 = 28%, 14 = 13%, and 15 = 6%. Further delineation was also observed by the relative abundances of the fragment ions produced at \( m/z \) 150 and \( m/z \) 180 (\( m/z \) 150/180: 13 = 1.8, 14 = 1.0, and 15 = 0.3).

25I-NB2OMe 16, 25I-NB3OMe 17, and 25I-NB4OMe 18 (Figure 8)

The tropylium ion (\( m/z \) 91) abundances were 16 = 29%, 17 = 11%, and 18 = 4%. Further delineation was also observed by the relative abundances of the fragment ions produced at \( m/z \) 150 (16 = 62%, 17 = 75%, and 18 = 39%) and \( m/z \) 278 (16 = 2%, 17 = 12%, and 18 = 5%).

25N-NB2OMe 19, 25N-NB3OMe 20, and 25N-NB4OMe 21 (Figure 9)

The tropylium ion (\( m/z \) 91) abundances were 19 = 34%, 20 = 13%, and 21 = 5%. The ratio for the relative abundances of \( m/z \) 91 and \( m/z \) 150 also differentiated the three compounds (\( m/z \) 150/91: 19 = 1.2, 20 = 4.2, and 21 = 3.3).

25P-NB2OMe 22, 25P-NB3OMe 23, and 25P-NB4OMe 24 (Figure 10)

The tropylium ion (\( m/z \) 91) abundances were 22 = 26%, 23 = 12%, and 24 = 8%. The ratio for the relative abundances of \( m/z \) 150 and \( m/z \) 194 also differentiated the three compounds (\( m/z \) 150/194: 22 = 2.1, 23 = 1.1, and 24 = 0.4). All three compounds produced a significant M-2 ion at \( m/z \) 341.

25T2-NB2OMe 25, 25T2-NB3OMe 26, and 25T2-NB4OMe 27 (Figure 11)

The tropylium ion (\( m/z \) 91) abundances were 25 = 25%, 26 = 10%, and 27 = 4%. The ratio for the relative abundances of \( m/z \) 150 and \( m/z \) 212 also differentiated the three compounds (\( m/z \) 150/212: 25 = 1.5, 26 = 0.9, and 27 = 0.3). All three compounds produced a significant M-2 ion at \( m/z \) 359.

25T4-NB2OMe 28, 25T4-NB3OMe 29, and 25T4-NB4OMe 30 (Figure 12)

The tropylium ion (\( m/z \) 91) abundances were 28 = 24%, 29 = 9%, and 30 = 4%. The ratio for the relative abundances of \( m/z \) 150 and \( m/z \) 226 also differentiated the three compounds (\( m/z \) 150/226: 28 = 1.9, 29 = 1.1, and 30 = 0.3). All three compounds produced a significant M-2 ion at \( m/z \) 373.

25T7-NB2OMe 31, 25T7-NB3OMe 32, and 25T7-NB4OMe 33 (Figure 13)

All three propyl derivatives could be differentiated by the tropylium ion relative abundances, which were nearly identical to 28-30 (see above). The ratio for the relative abundances of \( m/z \) 150 and \( m/z \) 226 also differentiated the three compounds (\( m/z \) 150/226: 31 = 1.6, 32 = 1.0, and 33 = 0.2). All three compounds produced a significant M-2 ion at \( m/z \) 373. The propyl derivatives 31-33 can be easily differentiated from their corresponding isopropyl analogues 28-30 by the ratios of the relative abundances of \( m/z \) 183 and \( m/z \) 226, where \( m/z \) 226/183 ~ 11 for 31-33 and ~3 for 28-30.
Figure 3 - Mass spectra of (a) 2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25H-NB2OMe) 1, (b) 2,5-dimethoxy-N-(3-methoxybenzyl)phenethylamine (25H-NB3OMe) 2, and (c) 2,5-dimethoxy-N-(4-methoxybenzyl)phenethylamine (25H-NB4OMe) 3.
Figure 4 - Mass spectra of (a) 2,5-dimethoxy-4-bromo-N-(2-methoxybenzyl)phenethylamine (25B-NB2OMe) 4, (b) 2,5-dimethoxy-4-bromo-N-(3-methoxybenzyl)phenethylamine (25B-NB3OMe) 5, and (c) 2,5-dimethoxy-4-bromo-N-(4-methoxybenzyl)phenethylamine (25B-NB4OMe) 6.
Figure 5 - Mass spectra of (a) 2,5-dimethoxy-4-chloro-N-(2-methoxybenzyl)phenethylamine (25C-NB2OMe) 7, (b) 2,5-dimethoxy-4-chloro-N-(3-methoxybenzyl)phenethylamine (25C-NB3OMe) 8, and (c) 2,5-dimethoxy-4-chloro-N-(4-methoxybenzyl)phenethylamine (25C-NB4OMe) 9.
Figure 6 - Mass spectra of (a) 2,5-dimethoxy-4-methyl-N-(2-methoxybenzyl)phenethylamine (25D-NB2OMe) 10, (b) 2,5-dimethoxy-4-methyl-N-(3-methoxybenzyl)phenethylamine (25D-NB3OMe) 11, and (c) 2,5-dimethoxy-4-methyl-N-(4-methoxybenzyl)phenethylamine (25D-NB4OMe) 12.
Figure 7 - Mass spectra of (a) 2,5-dimethoxy-4-ethyl-N-(2-methoxybenzyl)phenethylamine (25E-NB2OMe) 13, (b) 2,5-dimethoxy-4-ethyl-N-(3-methoxybenzyl)phenethylamine (25E-NB3OMe) 14, and (c) 2,5-dimethoxy-4-ethyl-N-(4-methoxybenzyl)phenethylamine (25E-NB4OMe) 15.
Figure 8 - Mass spectra of (a) 2,5-dimethoxy-4-iodo-N-(2-methoxybenzyl)phenethylamine (25I-NB2OMe) 16, (b) 2,5-dimethoxy-4-iodo-N-(3-methoxybenzyl)phenethylamine (25I-NB3OMe) 17, and (c) 2,5-dimethoxy-4-iodo-N-(4-methoxybenzyl)phenethylamine (25I-NB4OMe) 18.
Figure 9 - Mass spectra of (a) 2,5-dimethoxy-4-nitro-N-(2-methoxybenzyl)phenethylamine (25N-NB2OMe) \textbf{19}, (b) 2,5-dimethoxy-4-nitro-N-(3-methoxybenzyl)phenethylamine (25N-NB3OMe) \textbf{20}, and (c) 2,5-dimethoxy-4-nitro-N-(4-methoxybenzyl)phenethylamine (25N-NB4OMe) \textbf{21}.
Figure 10 - Mass spectra of (a) 2,5-dimethoxy-4-propyl-N-(2-methoxybenzyl)phenethylamine (25P-NB2OMe) \( \textbf{22} \), (b) 2,5-dimethoxy-4-propyl-N-(3-methoxybenzyl)phenethylamine (25P-NB3OMe) \( \textbf{23} \), and (c) 2,5-dimethoxy-4-propyl-N-(4-methoxybenzyl)phenethylamine (25P-NB4OMe) \( \textbf{24} \).
Figure 11 - Mass spectra of (a) 2,5-dimethoxy-4-ethylthio-N-(2-methoxybenzyl)phenethylamine (25T2-NB2OMe) 25, (b) 2,5-dimethoxy-4-ethylthio-N-(3-methoxybenzyl)phenethylamine (25T2-NB3OMe) 26, and (c) 2,5-dimethoxy-4-ethylthio-N-(4-methoxybenzyl)phenethylamine (25P-NB4OMe) 27.
Figure 12 - Mass spectra of (a) 2,5-dimethoxy-4-isopropylthio-\(N\)-(2-methoxybenzyl)phenethylamine (25T4-NB2OMe) 28, (b) 2,5-dimethoxy-4-isopropylthio-\(N\)-(3-methoxybenzyl)phenethylamine (25T4-NB3OMe) 29, and (c) 2,5-dimethoxy-4-isopropylthio-\(N\)-(4-methoxybenzyl)phenethylamine (25T4-NB4OMe) 30.
Figure 13 - Mass spectra (a) 2,5-dimethoxy-4-propylthio-\(N\)-(2-methoxybenzyl)phenethylamine (25T7-NB2OMe) 31, (b) 2,5-dimethoxy-4-propylthio-\(N\)-(3-methoxybenzyl)phenethylamine (25T7-NB3OMe) 32, and (c) 2,5-dimethoxy-4-propylthio-\(N\)-(4-methoxybenzyl)phenethylamine (25T7-NB4OMe) 33.
Figure 14 - FTIR spectra of (a) 2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine HCl (25H-NB2OMe) \( \text{1} \), (b) 2,5-dimethoxy-N-(3-methoxybenzyl)phenethylamine HCl (25H-NB3OMe) \( \text{2} \), and (c) 2,5-dimethoxy-N-(4-methoxybenzyl)phenethylamine HCl (25H-NB4OMe) \( \text{3} \).
Figure 15 - FTIR spectra of (a) 2,5-dimethoxy-4-bromo-N-(2-methoxybenzyl)phenethylamine HCl (25B-NB2OMe) 4, (b) 2,5-dimethoxy-4-bromo-N-(3-methoxybenzyl)phenethylamine HCl (25B-NB3OMe) 5, and (c) 2,5-dimethoxy-4-bromo-N-(4-methoxybenzyl)phenethylamine HCl (25B-NB4OMe) 6.
Figure 16 - FTIR spectra of (a) 2,5-dimethoxy-4-chloro-N-(2-methoxybenzyl)phenethylamine HCl (25C-NB2OMe) 7, (b) 2,5-dimethoxy-4-chloro-N-(3-methoxybenzyl)phenethylamine HCl (25C-NB3OMe) 8, and (c) 2,5-dimethoxy-4-chloro-N-(4-methoxybenzyl)phenethylamine HCl (25C-NB4OMe) 9.
Figure 17 - FTIR spectra of (a) 2,5-dimethoxy-4-methyl-N-(2-methoxybenzyl)phenethylamine HCl (25D-NB2OMe) 10, (b) 2,5-dimethoxy-4-methyl-N-(3-methoxybenzyl)phenethylamine HCl (25D-NB3OMe) 11, and (c) 2,5-dimethoxy-4-methyl-N-(4-methoxybenzyl)phenethylamine HCl (25D-NB4OMe) 12.
Figure 18 - FTIR spectra of (a) 2,5-dimethoxy-4-ethyl-N-(2-methoxybenzyl)phenethylamine HCl (25E-NB2OMe) 13, (b) 2,5-dimethoxy-4-ethyl-N-(3-methoxybenzyl)phenethylamine HCl (25E-NB3OMe) 14, and (c) 2,5-dimethoxy-4-ethyl-N-(4-methoxybenzyl)phenethylamine HCl (25E-NB4OMe) 15.
Figure 19 - FTIR spectra of (a) 2,5-dimethoxy-4-iodo-N-(2-methoxybenzyl)phenethylamine HCl (25I-NB2OMe) 16, (b) 2,5-dimethoxy-4-iodo-N-(3-methoxybenzyl)phenethylamine HCl (25I-NB3OMe) 17, and (c) 2,5-dimethoxy-4-iodo-N-(4-methoxybenzyl)phenethylamine HCl (25I-NB4OMe) 18.
Figure 20 - FTIR spectra of (a) 2,5-dimethoxy-4-nitro-N-(2-methoxybenzyl)phenethylamine HCl (25N-NB2OMe) 19, (b) 2,5-dimethoxy-4-nitro-N-(3-methoxybenzyl)phenethylamine HCl (25N-NB3OMe) 20, and (c) 2,5-dimethoxy-4-nitro-N-(4-methoxybenzyl)phenethylamine HCl (25N-NB4OMe) 21.
Figure 21 - FTIR spectra of (a) 2,5-dimethoxy-4-propyl-$N$-(2-methoxybenzyl)phenethylamine HCl (25P-NB2OMe) 22, (b) 2,5-dimethoxy-4-propyl-$N$-(3-methoxybenzyl)phenethylamine HCl (25P-NB3OMe) 23, and (c) 2,5-dimethoxy-4-propyl-$N$-(4-methoxybenzyl)phenethylamine HCl (25P-NB4OMe) 24.
Figure 22 - FTIR spectra of (a) 2,5-dimethoxy-4-ethylthio-N-(2-methoxybenzyl)phenethylamine HCl (25T2-NB2OMe) 25, (b) 2,5-dimethoxy-4-ethylthio-N-(3-methoxybenzyl)phenethylamine HCl (25T2-NB3OMe) 26, and (c) 2,5-dimethoxy-4-ethylthio-N-(4-methoxybenzyl)phenethylamine HCl (25T2-NB4OMe) 27.
Figure 23 - FTIR spectra of (a) 2,5-dimethoxy-4-isopropylthio-\(N\)-(2-methoxybenzyl)phenethylamine HCl (25T4-NB2OMe) 28, (b) 2,5-dimethoxy-4-isopropylthio-\(N\)-(3-methoxybenzyl)phenethylamine HCl (25T4-NB3OMe) 29, and (c) 2,5-dimethoxy-4-isopropylthio-\(N\)-(4-methoxybenzyl)phenethylamine HCl (25T4-NB4OMe) 30.
Figure 24 - FTIR spectra of (a) 2,5-dimethoxy-4-propylthio-N-(2-methoxybenzyl)phenethylamine HCl (25T7-NB2OMe) 31, (b) 2,5-dimethoxy-4-propylthio-N-(3-methoxybenzyl)phenethylamine HCl (25T7-NB3OMe) 32, and (c) 2,5-dimethoxy-4-propylthio-N-(4-methoxybenzyl)phenethylamine HCl (25T7-NB4OMe) 33.
FTIR Differentiation of the NBOMe methoxybenzyl positional isomers (2-OMe vs. 3-OMe vs. 4-OMe)

FTIR spectra for compounds 1-33 as the HCl ion-pairs are given in Figures 14-24. Each compound exhibited characteristic secondary amine HCl ion-pair absorbances between 2500-3000 cm⁻¹. Although each compound produced somewhat similar spectra, characteristic differences were observed between 400-1600 cm⁻¹, where each compound could be easily differentiated. Six of the 3-methoxybenzyl analogues (5, 8, 17, 20, 26, and 29) exhibited an apparent H₂O stretching band at approximately 3150-3350 cm⁻¹. The band could not be diminished even upon vacuum drying of the samples.

Conclusions
Each of the differing 4-substituted 2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamines were distinguished from their 3- and 4-methoxybenzyl analogues via mass spectrometry and infrared spectroscopy.

References
The Characterization of 2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexanone (Methoxetamine)

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ABSTRACT: The analysis, characterization, and synthesis of 2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone (commonly referred to as methoxetamine, “MXE,” or “3-Me-O-2-Oxo-PCE”) are discussed. Analytical data (nuclear magnetic resonance spectroscopy, mass spectrometry, and infrared spectroscopy) are presented and compared to the structurally similar drug ketamine.

KEYWORDS: 2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone, methoxetamine, MXE, 3-Me-O-2-Oxo-PCE, designer drug, synthesis, characterization, forensic chemistry.

The DEA Special Testing and Research Laboratory received a request to characterize an unknown compound in a suspected drug exhibit from another forensic drug laboratory. The exhibit consisted of approximately 200 milligrams of a white powder seized in the northeastern United States. The infrared spectrum of the exhibit was markedly similar to ketamine HCl. However, its mass spectrum differed from ketamine by +10 Daltons (apparent molecular weight of 247 vs. 237 for ketamine), including a base peak of +10 Daltons greater than that of ketamine. Additionally, the chlorine isotope pattern found in ketamine was not present. A mass spectral library search using the 2011 Wiley Designer Drug Library resulted in no matches. We suspected that the compound might be methoxetamine (based on the mass spectral data) and obtained 100 milligrams of sample for structural elucidation at our laboratory.

Methoxetamine or 2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexanone (Figure 1), commonly referred to as “MXE” or “3-MeO-2-Oxo-PCE,” is a new compound for sale over the Internet. Methoxetamine was originally publicized through an interview with an “underground chemist” who envisioned its dissociative properties and proposed that it would be “a stress-free version of ketamine” [1]. Although not currently scheduled under the U.S. Controlled Substances Act,

Figure 1 - Structure of methoxetamine.

methoxetamine may be considered to be an analog of ketamine (Figure 2) [2]; replacing the ortho chlorine in ketamine with a meta methoxy, and replacing the N-methyl with an N-ethyl. Herein, we report the structural elucidation of methoxetamine through nuclear magnetic resonance spectroscopy, mass spectrometry, infrared spectroscopy, and subsequent independent synthesis. The analytical data are also compared to the structurally similar drug ketamine. Additionally, analytical profiles of methoxetamine’s synthetic intermediates and its major synthetic impurity are presented to assist forensic chemists who may encounter these substances in casework.

Experimental
Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All other chemicals and NMR solvents were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI). Ketamine HCl was obtained from the reference materials collection maintained at this laboratory.

Nuclear Magnetic Resonance Spectroscopy (NMR)
NMR spectra were obtained on an Agilent VNMR-600 MHz NMR using a 5 mm Protune broad band detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The HCl salts of the samples were initially dissolved in
deuterochloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound, and later base extracted using saturated sodium bicarbonate in D₂O. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: proton, carbon (proton decoupled), and gradient versions of the 2-dimensional experiments COSY, HSQC, and HMBC. Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Synthesis of Methoxetamine
In accordance with Journal policy, exact experimental details are not provided. A procedure analogous to that of ketamine was utilized (Figure 3) for the preparation of methoxetamine and its intermediates [3].

Results and Discussion
NMR Elucidation
Proton and carbon NMR spectra as well as the assignments for methoxetamine HCl and ketamine HCl are presented in Figures 4-7. Assignments were based on proton chemical shifts and peak patterns, carbon chemical shifts, HSQC (1 bond carbon to proton correlations), HMBC (2-4 bond carbon to proton correlations), and COSY (2-3 bond proton-proton correlations) spectra. Assignments were further confirmed using ACD Structure Elucidator software.

The methoxetamine spectra (carbon and HSQC) contain 15 carbons: 1 ketone, 6 benzene (4 protonated), 1 aliphatic quaternary, 5 methylenes, and 2 methyls. The aromatic proton peak pattern for methoxetamine base clearly shows a 1,3-disubstituted benzene pattern: a triplet (7.29 ppm), a doublet (6.82 ppm), a doublet of doublets (6.82 ppm), and 1 small coupling doublet (6.75 ppm). In addition, the proton, carbon, and COSY spectra indicate the presence of an N-CH₂-NCH₂ whose methane protons are not equivalent, the presence of a methoxy singlet at 3.8-3.9 ppm, and 4 methylenes bonded to each other in an n-butyl chain (as indicated by the multiple couplings to each proton and the COSY correlations). HMBC correlations show that the butyl chain is bonded to or very nearby the ketone carbon and the quaternary aliphatic carbon. The HMBC also indicates that the N-ethyl group, the n-butyl group and the benzene ring are bonded to or very nearby the quaternary carbon. Based on the molecular weight of 247 and the NMR data, the molecular formula is C₁₅H₂₁NO₂. This formula indicates that there are 6 unsaturations and/or rings in the molecule: the benzene ring accounts for 4 and the ketone for 1, thus leaving 1 additional ring (no other unsaturations noted in spectra). The main NMR fragments are a benzene ring (with a methoxy at C3), a ketone, an N-ethyl, a quaternary carbon, and an n-butyl chain. The quaternary carbon chemical shift (69.7 ppm base) indicates it is bonded to one or more strong electron withdrawing groups. The structure of methoxetamine satisfies all this and also gives the lowest derivations of carbon chemical shifts (i.e., experimental versus calculated).

In contrast to methoxetamine, the ketamine base proton spectrum (Figure 7) displays two “doublet of doublets” (7.38 and 7.55 ppm) and two “triplet of doublets” (7.25 and 7.32 ppm) in the aromatic region, and a singlet at 2.10 ppm for the N-methyl group. The proton and carbon spectra of ketamine and methoxetamine are very different and are easily distinguished.

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Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

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Mass Spectral Elucidation
The mass spectra of methoxetamine and ketamine are shown in Figure 8. The appearance of the mass spectrum of methoxetamine is similar to that of ketamine, at least at the higher mass range. The major dissimilarities between the two spectra are a difference of +10 Daltons for the peaks of methoxetamine versus the corresponding peaks of ketamine (base peak of m/z 190 versus m/z 180; peak at m/z 204 versus m/z 194; and peak at m/z 219 versus m/z 209).

The proposed fragmentation of methoxetamine is shown in Figure 9. Due to the similarity of the structures, the major fragmentation mechanisms of methoxetamine are expected to be similar to that proposed for ketamine [4]. Initial ionization occurs at the amine nitrogen which is followed by alpha cleavage to give structure A. Structure A can undergo neutral loss of CO to yield ion B, m/z 219. The newly formed radical site in structure B can undergo secondary alpha cleavages. Loss of a hydrogen radical from structure B (pathway a) results in structure C, m/z 218. Loss of neutral ethylene from structure B (pathway b) gives structure D, m/z 191 which likewise can lose a hydrogen radical to give structure E, m/z 190. Structure B can also undergo ring closure (pathway c) to yield a radical cation (structure F) similar in stability to the parent ion. This ion can undergo further alpha cleavages to yield ions G, m/z 204 (loss of a methyl radical) and H, m/z 112 (loss of a methoxyphenyl radical).

FTIR
The FTIR spectra for methoxetamine HCl and ketamine HCl are illustrated in Figure 10. Comparison reveals somewhat similar absorption patterns, with the most prominent differences being in the region of 500-1600 cm⁻¹. An absorbance found at 1725 cm⁻¹ (due to a carbonyl stretching vibration) strongly indicates a carbonyl in the suspected methoxetamine (carbonyl
Figure 3 - Synthetic route for methoxetamine.

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<tr>
<td>4</td>
<td>21.7</td>
<td>1.62 qt(13.3,3.3 Hz), 1.85 m</td>
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<td>5</td>
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<td>1.42 t(7.3 Hz)</td>
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<td>132.9</td>
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<td>6’</td>
<td>121.2</td>
<td>6.97 dd(8.0, 2.6 Hz)</td>
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<td>OCH$_3$</td>
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<tr>
<td>NH$_2$</td>
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<td>9.55 bs, 10.18 bs</td>
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b = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet

Figure 4 - $^1$H and $^{13}$C NMR data for methoxetamine HCl.
Figure 5 - $^1$H and $^{13}$C NMR data for methoxetamine base.

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Figure 6 - $^{1}$H and $^{13}$C NMR data for ketamine HCl.
Figure 7 - $^1$H and $^{13}$C NMR data for ketamine base.

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Figure 8 - Mass spectra of (a) methoxetamine HCl and (b) ketamine HCl.
Figure 9 - Proposed fragmentation pathways for methoxetamine.

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Figure 10 - FTIR spectra of (a) methoxetamine HCl and (b) ketamine HCl.
Figure 11 - Mass spectra of (a) 3-methoxyphenyl cyclopentyl ketone, (b) \textit{alpha}-bromo-(3-methoxyphenyl)-cyclopentyl ketone, and (c) 1-[(ethylimino)(3-methoxyphenyl)methyl]cyclopentanol.
Figure 12 - Infrared spectrum (a) and mass spectrum (b) of [1-(ethylamino)cyclopentyl](3-methoxyphenyl)methanone; methoxetamine synthesis impurity.
Figure 13 - $^1$H and $^{13}$C NMR data for [1-(ethylamino)cyclopentyl](3-methoxyphenyl)methanone HCl.

*Microgram Journal, Volume 9, Number 1*
Table 1: 

<table>
<thead>
<tr>
<th>Position</th>
<th>Carbon (ppm)</th>
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<th>Structure</th>
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<td>24.7</td>
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<td>5</td>
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<td>15.9</td>
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<td>137.9</td>
<td>-</td>
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<td>114.0</td>
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<tr>
<td>3'</td>
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<td>-</td>
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<td>4'</td>
<td>118.1</td>
<td>7.05 dd(8.0, 2.2 Hz)</td>
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<td>5'</td>
<td>129.0</td>
<td>7.32 t(8.0 Hz)</td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>121.4</td>
<td>7.76 dd(8.0, 1.9 Hz)</td>
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<tr>
<td>OCH₃</td>
<td>55.4</td>
<td>3.85 s</td>
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</tr>
</tbody>
</table>

*d* = doublet, *m* = multiplet, *q* = quartet, *s* = singlet, *t* = triplet

Figure 14 - $^1$H and $^{13}$C NMR data for [1-(ethylamino)cyclopentyl](3-methoxyphenyl)methanone base.
stretch for ketamine is found at 1719 cm\(^{-1}\)). When methoxetamine HCl is compared to ketamine HCl, significant differences can differentiate the compounds, especially the absorbances at 1550-1600 cm\(^{-1}\) due to C-C stretching [5].

Synthesis

Methoxetamine was synthesized utilizing an analogous procedure for that of ketamine (Figure 3). A cyclopentyl Grignard was reacted with 3-methoxybenzonitrile to form 3-methoxyphenyl cyclopentyl ketone, which was then brominated \(\alpha\)-to the ketone. The \(\alpha\)-bromo ketone was converted to the Schiff’s base with ethyl amine, which was then heated to form methoxetamine. The NMR, FTIR, and mass spectrum of the synthesized methoxetamine were in all respects identical to the unknown compound’s spectra. Mass spectra for the three intermediates are illustrated in Figure 11. GC retention time data for the respective compounds are presented in Table 1.

A significant amount of a by-product (impurity) was produced during the synthesis of methoxetamine. The FTIR (Figure 12a) of the synthesis impurity indicated that a carbonyl was present and its mass spectrum (Figure 12b) indicated a molecular weight of 247. The impurity was easily isolated from methoxetamine HCl by its solubility in acetone. The NMR spectrum (Figures 13 and 14) illustrated that this compound, like methoxetamine, contained a 1,3-disubstituted benzene (with a methoxy group at C3), an N-ethyl group, a ketone, a quaternary carbon, and an \(n\)-butyl chain. However, the proton and carbon chemical shifts and the HMBC correlations show that the ketone is the bridge between the benzene ring and a cyclopentyl ring and this cyclopentyl ring contains the quaternary carbon which is bonded to the N-ethyl group. The isolated impurity was characterized as \[1-(\text{ethylamino})\text{cyclopentyl}(3\text{-methoxyphenyl})\text{methanone}\] (Figure 15).

References

1. Interview with a ketamine chemist or to be more precise, an arylcyclohexylamine chemist. http://www.vice.com/read/interview-with-ketamine-chemist-704-v18n2. Last accessed December 1, 2011.

Table 1 - Gas chromatographic retention times (R\(_t\)) for the methoxetamine and related compounds*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R(_t) (min)</th>
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</thead>
<tbody>
<tr>
<td>3-methoxyphenyl cyclopentyl ketone</td>
<td>14.50</td>
</tr>
<tr>
<td>1-hydroxy-cyclopentyl-(3-methoxyphenyl)-ketone-N-ethylimine</td>
<td>14.31</td>
</tr>
<tr>
<td>ketamine</td>
<td>16.51</td>
</tr>
<tr>
<td>methoxetamine</td>
<td>17.21</td>
</tr>
<tr>
<td><a href="3-methoxyphenyl">1-(ethylamino)cyclopentyl</a>methanone</td>
<td>17.35</td>
</tr>
<tr>
<td>(\alpha)-bromo-(3-methoxyphenyl)-cyclopentyl ketone</td>
<td>17.54</td>
</tr>
</tbody>
</table>

*aConditions given in the experimental section.
The Characterization of 4- and 5-Iodo-2-aminoindan

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U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166-9509
[email address withheld at authors’ request]

ABSTRACT: The synthesis, analysis, and characterization of 4- and 5-iodo-2-aminoindan (commonly referred to as “4-IAI” and “5-IAI”) are discussed. Analytical data (mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared spectroscopy) are presented and compared.

KEYWORDS: 5-iodo-2-aminoindan, 4-iodo-2-aminoindan, 2-aminoindan, 5-IAI, 4-IAI, 2-AI, designer drug, synthesis, characterization, forensic chemistry.

The Special Testing and Research Laboratory recently received a request to characterize (and eventually synthesize) an unknown compound in a suspected drug exhibit. The exhibit consisted of approximately 300 grams of a tan powder containing ascorbic acid as a diluent. The compound of interest was suspected as being 5-iodo-2-aminoindan, based partially on a mass spectrum exhibiting an apparent molecular ion of m/z 259. This molecular weight was consistent with a new compound, 5-iodo-2-aminoindan, currently advertised for sale over the Internet.

We synthesized both 4- and 5-iodo-2-aminoindan for structural elucidation and eventual confirmatory analyses at our laboratory.

5-Iodo-2-aminoindan (Figure 1, structure 1), commonly referred to as “5-IAI,” is a relatively new compound for sale over the Internet. The IUPAC name for 5-IAI is 5-iodo-2,3-dihydro-1H-inden-2-amine. Although not currently scheduled under the U.S. Controlled Substances Act, it may be considered to be an analog of amphetamine [1] (Figure 1, Structure 3); with linkage of amphetamine’s terminal methyl with the ortho position of the aromatic ring, to form an indan ring system.

5-IAI was first synthesized and reported in 1991 to study its pharmacological effects and evaluated for neurotoxicity [2,3] and a recent review on aminoindanes addressed the need for analytical profiles as well as the significant challenges in identifying these new “legal highs” [4].

Herein, we report the synthesis and structural elucidation of 5-IAI 1 and its positional isomer, 4-iodo-2-aminoindan (4-IAI) 2. 4-IAI is not yet available for purchase on the Internet. Analytical profiles (nuclear magnetic resonance, mass spectrometry, and infrared spectroscopy) of these compounds and their synthetic intermediates and impurities are presented and compared to assist forensic chemists who may encounter these substances in casework.

Experimental

Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). 2-Aminoindan and all other chemicals and NMR solvents were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Nuclear Magnetic Resonance Spectroscopy (NMR)
NMR spectra were obtained on an Agilent 400MR NMR with a 400 MHz magnet, a 5 mm Protune indirect detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The HCl salt of the compound was initially dissolved in deuterium oxide (D₂O) containing 0.05% v/v TSP as the 0 ppm reference compound, and later base extracted using saturated sodium bicarbonate into CDCl₃ containing TMS. The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon (proton decoupled), and
gradient versions of the 2-dimensional experiments HSQC, and HMBC. Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

**Infrared Spectroscopy (FTIR)**

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm\(^{-1}\); gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

**Synthesis of 5- and 4-IAI**

In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2. Briefly, 2-aminoindan (2-Al) 4 was converted to the N-trifluoroacetyl derivative 5, which was then iodinated to give a mixture of the 5-iodo-2-aminoindan-TFA derivative 6 and the 4-iodo-2-aminoindan-TFA derivative 7. Compounds 6 and 7 were separated via column chromatography (silica gel) and then deprotected to provide 1 and 2, respectively.

**Results and Discussion**

**Structural Elucidation/Confirmation of 5- and 4-IAI**

Proton and carbon NMR spectra as well as the assignments for 5-IAI are presented in Figure 3 (HCl salt in D\(_2\)O) and Figure 4 (base in CDCl\(_3\)). The NMR spectra and assignments for 4-IAI base are found in Figure 5. Assignments were based on proton chemical shifts and peak patterns, carbon chemical shifts, HSQC (1 bond carbon to proton correlations), and HMBC (2-4 bond carbon to proton correlations correlations). Assignments were further confirmed using ACD Structure Elucidator software.

NMR spectra confirmed both the 4- and the 5-IAI structures. There are 9 carbons: 3 aromatic quaternary carbons (one with the exceptionally low chemical shift, C-5 at 91-95 ppm, bonded to iodine), 3 aromatic protonated carbons (the proton peak patterns indicating a CH=CH-C=CH- sequence for the 5-iodo and a CH=CH-CH sequence for the 4-iodo compound), 2 aliphatic methylenes (almost identical in carbon and proton chemical shifts for the 5-iodo compound and very different chemical shifts for the 4-iodo compound), and 1 aliphatic methine. The methine and 2 methylene proton peak patterns

---

Figure 2 - Synthetic route for 5-IAI 1 and 4-IAI 2.
indicate CH₂-CH-CH₂ bonding with the methine likely bonded to nitrogen (51-54 ppm carbon). The carbons of the two methylenes in the 5-iodo compound are nearly equivalent, making assignment of protons to carbons initially difficult; however, due to the lack of 2<sup>nd</sup> order effects, and due to the coupling constants present, the two protons at 3.0 ppm (base spectrum) are not bonded to the same carbon, and the two protons at 3.4 ppm (base spectrum) are not bonded to the same carbon. The 5-iodo compound methylene carbons have both a 3.0 ppm proton and a 3.4 ppm proton (base spectrum). The cause of these nearly equivalent signals is the nearly symmetric appearance of the molecule. In the 4-iodo compound, there is no axis of symmetry and the methylene protons and carbons are quite far apart.

The mass spectra for 5-IAI, 4-IAI, and 2-AI are illustrated in Figure 6. Both 5-IAI and 4-IAI gave an intense molecular ion at m/z 259 and are easily distinguished by the relative intensities of ions found at m/z 115, 117, 130, and 132. Additionally, the

### Table 1: NMR Data for 5-IAI HCl

<table>
<thead>
<tr>
<th>Position</th>
<th>Carbon (ppm)</th>
<th>Proton (ppm, J)</th>
<th>Structure</th>
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<tbody>
<tr>
<td>1</td>
<td>39.8</td>
<td>3.02 dd(17.3, 3.5 Hz), 3.39 dd(17.3, 7.3 Hz)</td>
<td>![Structure Image]</td>
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<td>2</td>
<td>54.3</td>
<td>4.18 tt(7.3, 3.5 Hz)</td>
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</tr>
<tr>
<td>3</td>
<td>39.8</td>
<td>3.05 dd(17.3, 3.5 Hz), 3.43 dd(17.3, 7.3 Hz)</td>
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<tr>
<td>3a</td>
<td>144.8</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>136.7</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>139.1</td>
<td>7.66 bd(7.8 Hz)</td>
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<td>7</td>
<td>129.7</td>
<td>7.15 d(7.8 Hz)</td>
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</tr>
<tr>
<td>7a</td>
<td>142.0</td>
<td>-</td>
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</tr>
</tbody>
</table>

b = broad, d = doublet, t = triplet, s = singlet

---

Figure 3 - <sup>1</sup>H and <sup>13</sup>C NMR data for 5-IAI HCl 1.
additional or supplementary spectroscopic methods should be utilized for identification.

**Illicit Sample**

A partial reconstructed total ion chromatogram of the basic extract of the sample is illustrated in Figure 8. GC retention time data for the respective compounds are presented in Table 1, along with the synthetic intermediates. Peak #1 was identified as 2-AI and was present at a trace level. Peak #2

<table>
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<th>Position</th>
<th>Carbon (ppm)</th>
<th>Proton (ppm, J)</th>
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</tr>
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<tbody>
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<td>1</td>
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<td>H2N</td>
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<td>-53.0 *</td>
<td>3.16 tt(6.7, 4.8 Hz)</td>
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<td>3</td>
<td>42.9</td>
<td>2.66 dd(14.9, 4.8 Hz), 3.16 dd(14.9, 6.7 Hz)</td>
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<tr>
<td>3a</td>
<td>144.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>133.9</td>
<td>7.55 bs</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-91.4 *</td>
<td>-</td>
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<tr>
<td>6</td>
<td>135.4</td>
<td>7.47 dd(8.2 Hz)</td>
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<td>7</td>
<td>126.8</td>
<td>6.97 dd(8.2 Hz)</td>
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<tr>
<td>7a</td>
<td>-141.6 *</td>
<td>-</td>
<td></td>
</tr>
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b = broad, d = doublet, t = triplet, s = singlet
* = carbon value derived from HMBC

Figure 4 - $^1$H and $^{13}$C NMR data for 5-IAI base 1.

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represented approximately one percent of the ion current and produced an identical mass spectrum to synthesized 4-IAI. Peak #3 constituted approximately 92 percent of the ion current and produced an identical mass spectrum to synthesized 5-IAI. Peak #4 produced a base peak at $m/z$ 115 and a molecular ion and $m/z$ 285 (mass spectrum not illustrated), respectively, and could not be identified. Peak #5 produced a mass spectrum (Figure 9) with a molecular ion at $m/z$ 293 and a M+2 isotope abundance ratio consistent with mono-chloro substitution.

A mass of 293 Daltons with an apparent chlorine substitution suggests the compound is a chloro-iodo-2-aminoindan; however, the exact position of substitutions is not known (Figure 10). A chloro-iodo-2-aminoindan would be an expected by-product from the use of iodine monochloride (ICl) as an iodination reagent. Peaks #6-9 each produced a mass spectrum (Figure 11) with a molecular ion at $m/z$ 385; these are

<table>
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<th>Position</th>
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<th>Structure</th>
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<td>2</td>
<td>51.3</td>
<td>3.84 tt(6.9, 4.9 Hz)</td>
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<tr>
<td>3</td>
<td>48.5</td>
<td>2.68 dd(16.6, 4.9 Hz), 3.18 dd(16.6, 6.9 Hz)</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>146.5</td>
<td>-</td>
<td></td>
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<tr>
<td>4</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>135.8</td>
<td>7.54 d(7.9 Hz)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>128.4</td>
<td>6.86 dd(7.9, 7.4 Hz)</td>
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</tr>
<tr>
<td>7</td>
<td>124.5</td>
<td>7.15 d(7.4 Hz)</td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>142.6</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

b = broad, d = doublet, t = triplet, s = singlet  
* = carbon value derived from HMBC  

Solvent: CDCl$_3$ with TMS

Figure 5 - $^1$H and $^{13}$C NMR data for 4-IAI base 2.
Figure 6 - Electron ionization mass spectra of (a) 5-IAI 1, (b) 4-IAI 2, and (c) 2-AI 4.

Figure 7 - Infrared spectrum of (a) 5-IAI 1, (b) 4-IAI 2, and (c) 2-AI 4.
Figure 8 - Reconstructed total ion chromatogram of a basic extract of the illicit 5-IAI sample. Peak identification: 1 = 2-AI, 2 = 4-IAI, 3 = 5-IAI, 4 = unknown compound, 5 = suspected iodo-chloro-2-aminoindan, and 6-9 = suspected di-iodo-2-aminoindans.

Figure 9 - Electron ionization mass spectrum of suspected chloro-iodo-2-aminoindan.
consistent with di-iodo-substituted 2-aminoindans; their proposed structures are given in Figure 12. Di-iodo-substituted 2-aminoindans would be expected by-products from the use of excess iodination reagent.

Conclusions
Analytical data are presented to assist forensic laboratories that encounter 4- and/or 5-IAI in casework. Care must be employed when utilizing FTIR for characterization. Both mass spectral and NMR techniques can provide unequivocal characterization of 4- versus 5-IAI.

Table 1 - Gas chromatographic retention times ($R_t$) for the iodo-2-aminoindans and related compounds.

<table>
<thead>
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<th>Compound</th>
<th>$R_t$ (min)</th>
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<tr>
<td>2-aminoinidan</td>
<td>5.46</td>
</tr>
<tr>
<td>2-aminoinidan-TFA derivative</td>
<td>8.55</td>
</tr>
<tr>
<td>4-iodo-2-aminoinidan</td>
<td>12.22</td>
</tr>
<tr>
<td>5-iodo-2-aminoinidan</td>
<td>12.74</td>
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<tr>
<td>4-iodo-2-aminoinidan-TFA derivative</td>
<td>15.17</td>
</tr>
<tr>
<td>5-iodo-2-aminoinidan-TFA derivative</td>
<td>15.87</td>
</tr>
<tr>
<td>proposed chloro-iodo-2-aminoinidan</td>
<td>16.60</td>
</tr>
<tr>
<td>proposed di-iodo-2-aminoinidan</td>
<td>19.03</td>
</tr>
<tr>
<td>proposed di-iodo-2-aminoinidan</td>
<td>19.34</td>
</tr>
<tr>
<td>proposed di-iodo-2-aminoinidan</td>
<td>20.00</td>
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<tr>
<td>proposed di-iodo-2-aminoinidan</td>
<td>20.33</td>
</tr>
</tbody>
</table>

*Conditions given in the experimental section.*
Figure 12 - Proposed structures of illicit synthesis by-products (di-iodo-2-aminoindans).

References
Color Tests for the Preliminary Identification of Methcathinone and Analogues of Methcathinone

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ABSTRACT: The preliminary identification of methcathinone and analogues of methcathinone presents a new and growing challenge to law enforcement. Color tests remain an important tool for the preliminary testing of suspected illicit drug samples. In this study, the applicability of a range of color tests to the preliminary identification of methcathinone and analogues of methcathinone was explored. It was found that Marquis reagent is suitable for the preliminary identification of methylenedioxy-substituted analogues of methcathinone. Liebermann’s reagent was identified as an appropriate test for the preliminary identification of cathinone, methcathinone and 4-methylmethcathinone. Both of these color tests give rise to intensely yellow colored products. Liebermann’s reagent also produced yellow and orange products upon reaction with N,N-dimethylcathinone and 4-methoxy-methcathinone respectively, although these products were less intensely colored. A testing sequence incorporating these tests was utilized on seized illicit drug samples and found to be suitable for use in routine casework.

KEYWORDS: color test, methcathinone, mephedrone, designer drugs, preliminary testing, drug testing, forensic chemistry.

The abuse of methcathinone (MCAT) and analogues of methcathinone has increased markedly in jurisdictions worldwide in recent years. For example, in Australia the rate of recent use of 4-methylmethcathinone (4-MMC) by regular methylenedioxymethamphetamine (MDMA) users rose from less than 1% in 2009 to 16% in 2010 [1,2]. These β-keto analogues of amphetamines [3], are stimulants with empathogenic effects [4]. The structures of analogues of methcathinone included in this study are summarised in Table 1.

Color tests still remain an important tool for the preliminary identification of illicit drugs in spite of developments in instrumental technology and the increased portability of this technology which enables its use in the field. As recently as 2000, 86% of laboratories surveyed still frequently used color tests when testing for illicit drugs [5]. The popularity of color tests arises from the fact that they are generally simple, quick, inexpensive, and quite sensitive [6]. They are readily available and require minimal materials. These factors enable color tests to be used in the field and can be employed by those without extensive chemical backgrounds.

Results of various color tests for methcathinone and its analogues have been described in the literature [6-13]; however, no comprehensive survey has been published. Furthermore, conflicting results from different sources were identified [6,8-13]. This study assesses the suitability of a range of color test methods for the preliminary identification of methcathinone and its analogues and recommends color tests for utilisation by law enforcement.

Experimental

Illicit Drugs

Certified reference materials of the hydrochloride salts of CAT, MCAT, 4-MMC, N,N-DMC, 3-FMC, 4-MOMC, 3,4-MDMC, 3,4-MDPV, BUT, MDA, and MDMA were supplied by the National Measurement Institute’s Australian Forensic Drug Laboratory (NMI-AFDL).

Ten casework samples of illicit drugs were screened using attenuated total reflectance Fourier transform infrared spectrometry (ATR-FTIR). These were preliminary identified as containing 4-MMC (8 samples), 3,4-MDMC (1 sample) and 3,4-MDPV (1 sample). The physical form of these samples included coarse and finely divided white powders and colorless crystals in a range of shapes.

Chemicals

Color test reagent formulations are summarised in Table 2. Simon’s reagent, Scott’s test Solution 1, Chen-Kao test, and ferric chloride test were supplied by NMI-AFDL. Dille-Kopanyi Solutions 1 and 2 were from a testing kit produced by nik Public Safety. All other reagents were prepared from their constituent chemicals.

Hydrochloric acid was from BDH. Chloroform, methanol, ethanol, and pyridine were from Sigma-Aldrich. Sulfuric acid

Figure 1 - Yellow-colored products of the reaction of Marquis reagent with 3,4-MDMC (a) and Liebermann’s reagent with CAT (b).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Common Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathinone</td>
<td>CAT</td>
<td>-</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>Methcathinone</td>
<td>MCAT</td>
<td>-</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>4-Methylmethcathinone</td>
<td>4-MMC</td>
<td>Mephedrone</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>N,N-Dimethylcathinone</td>
<td>N,N-DMC</td>
<td>-</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>3-Fluoromethcathinone</td>
<td>3-FMC</td>
<td>Flephedrone</td>
<td><img src="image5.png" alt="Structure" /></td>
</tr>
<tr>
<td>4-Methoxymethcathinone</td>
<td>4-MOMC</td>
<td>Methedrone</td>
<td><img src="image6.png" alt="Structure" /></td>
</tr>
<tr>
<td>3,4-Methylenedioxymethcathinone</td>
<td>3,4-MDMC</td>
<td>Methylone</td>
<td><img src="image7.png" alt="Structure" /></td>
</tr>
<tr>
<td>3,4-Methylenedioxypyrovalerone</td>
<td>3,4-MDPV</td>
<td>-</td>
<td><img src="image8.png" alt="Structure" /></td>
</tr>
<tr>
<td>Butylone</td>
<td>BUT</td>
<td>-</td>
<td><img src="image9.png" alt="Structure" /></td>
</tr>
</tbody>
</table>
was sourced from both BDH and Sigma-Aldrich. Formaldehyde was from Unilevar. Gallic acid n-propyl ester was from TCI. Sodium nitrite and copper (II) sulfate pentahydrate were from Univar. Sodium molybdite was from Mallinckrodt. Selenious acid was from Unilab. p-Methylamino-benzaldehyde was from Fisher. Sodium nitroprusside was from M&B.

**Methodology**

All color tests were conducted in clean white porcelain spot trays, with the exception of Scott’s test which utilized clean glass culture tubes. The reagent solution(s), in quantities described in Table 2, were applied to a pin-head sized sample of drug and a paired blank well. The tray was gently agitated and any color change immediately observed and recorded. Where

<table>
<thead>
<tr>
<th>Colour Test</th>
<th>Ref</th>
<th>Reagent Formulation</th>
<th>Solution Quantities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marquis Reagent</td>
<td>18</td>
<td>9:1 sulfuric acid and 37% formaldehyde</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Liebermann’s Reagent</td>
<td>11</td>
<td>10% w/v sodium nitrite in sulfuric acid, added with cooling in water bath and swirling to absorb brown fumes</td>
<td>2 drops</td>
</tr>
<tr>
<td>Simon’s Reagent</td>
<td>18</td>
<td>Solution 1: 10% v/v acetaldehyde</td>
<td>1 drop</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 2: 10% w/v sodium nitroprusside</td>
<td>2 drops</td>
</tr>
<tr>
<td>Scott’s Test (modified)</td>
<td>18</td>
<td>Solution 1: 2% thiocyanate solution, glycerol, glacial acetic acid</td>
<td>5 drops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 2: hydrochloric acid</td>
<td>Until precipitate disappears</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 3: chloroform</td>
<td>10 drops</td>
</tr>
<tr>
<td>Chen-Kao Test</td>
<td>18</td>
<td>Solution 1: 1% v/v acetic acid</td>
<td>2 drops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 2: 1% w/v copper (II) sulfate</td>
<td>2 drops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 3: 8% w/v sodium hydroxide</td>
<td>2 drops</td>
</tr>
<tr>
<td>Ferric Chloride Test</td>
<td>18</td>
<td>5% w/v ferric chloride</td>
<td>5 drops</td>
</tr>
<tr>
<td>Cobalt Thiocyanate</td>
<td>19</td>
<td>10% w/v cobalt thiocyanate in methanol</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Frohde’s Reagent</td>
<td>19</td>
<td>5% w/v sodium molybdite in hot sulfuric acid</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Mecke Reagent</td>
<td>19</td>
<td>1% w/v selenious acid in sulfuric acid</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Ehrlich’s Reagent</td>
<td>5, 6</td>
<td>2% w/v p-methylaminobenzaldehyde in 1:1 95% ethanol and sulfuric acid</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Mandelin’s Test</td>
<td>11</td>
<td>0.5% w/v ammonium vanadates in sulfuric acid</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Gallic Acid Test</td>
<td>6</td>
<td>0.5% w/v gallic acid n-propyl ester in sulfuric acid</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Zwiker Reagent</td>
<td>5, 6</td>
<td>Solution 1: 0.5% w/v copper (II) sulfate</td>
<td>1 drop</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 2: 1:19 pyridine and chloroform</td>
<td>1 drop</td>
</tr>
<tr>
<td>Sodium Nitroprusside</td>
<td>5, 6</td>
<td>Solution 1: 1% w/v sodium nitroprusside</td>
<td>2 drops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 2: 8% w/v sodium hydroxide</td>
<td>1 drop</td>
</tr>
<tr>
<td>Dille-Kopanyi Reagent</td>
<td>5, 20</td>
<td>Solution 1: 0.1% w/v cobalt acetate in ethanol</td>
<td>2 drops</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solution 2: 1:19 isopropylamine and ethanol</td>
<td>1 drop</td>
</tr>
</tbody>
</table>

Table 2 - Color test reagent formulations.

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Results and Discussion
The results of the color tests are summarised in Table 3. Yellow colored products were yielded by the reaction of 3,4-methylenedioxy-substituted analogues of methcathinone (3,4-MDMC, 3,4-MDPV, and BUT) with Marquis reagent (Figure 1). These products likely arise from the reaction of the drug molecules with sulfuric acid in a mechanism analogous to that of the reaction of MDMA with Marquis reagent [14]. Here, the extension of the conjugation by the ketone group shifts the color of the products from blue-black observed for MDA and MDMA to yellow. Marquis reagent gave rise to no color change with any other methcathinone analogue tested. This reflects the deactivation of the aromatic ring by the electron withdrawing effects of the ketone group [10], which prevents formation of the orange color characteristic of the reaction of Marquis reagent with amines. The discriminating power of this test was calculated in order to objectively quantify the selectivity of this test. The discriminating power is the probability that two samples from two different sources (that is, samples of two different drugs) will not randomly match if a given test is performed on them. If a population contains one in N randomly matching pairs, the discriminating power is calculated using Equation 1. In this study, the pairing of each of the three methylenedioxy-substituted analogues of methcathinone with 20 “designer drug” standards yielded 60 pairs, nine of which could not be confidently discriminated using Marquis reagent. Thus, the discriminating power of Marquis reagent for methylenedioxy-substituted analogues of methcathinone was 0.85 for the sample of “designer drugs” studied, indicating that this is a highly discriminating preliminary test.

\[
DP = 1 - \frac{1}{N}
\]

Equation 1 - Calculation of discriminating power [15].

Yellow colored products also arose from the reactions of CAT, MCAT, N,N-DMC, and 4-MMC with Liebermann’s reagent (Figure 1). The mechanism of this reaction remains unclear; Liebermann’s reagent has, to date, been given little attention in the published literature. Although several possible mechanisms were considered, all entailed the persistence of unstable nitrous acid in solution and were thus deemed unlikely. An orange product was produced by the reaction of

<table>
<thead>
<tr>
<th>Colour Test</th>
<th>Marquis Reagent</th>
<th>Liebermann’s Reagent</th>
<th>Simon’s Reagent</th>
<th>Cobalt Thiocyanate</th>
<th>Froehde’s Reagent</th>
<th>Mecke Reagent</th>
<th>Mandelin’s Test</th>
<th>Gallic Acid Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>-</td>
<td>Y (B)</td>
<td>-</td>
<td>Pr/B</td>
<td>-</td>
<td>-</td>
<td>O/R</td>
<td>-</td>
</tr>
<tr>
<td>MCAT</td>
<td>-</td>
<td>Y (B)</td>
<td>-</td>
<td>Pr/B</td>
<td>-</td>
<td>-</td>
<td>O/R</td>
<td>-</td>
</tr>
<tr>
<td>4-MMC</td>
<td>-</td>
<td>Y (B)</td>
<td>-</td>
<td>Pr/B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N,N-DMC</td>
<td>-</td>
<td>Y (F)</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>3-FMC</td>
<td>-</td>
<td>-</td>
<td>B (F)</td>
<td>Pr/B</td>
<td>-</td>
<td>-</td>
<td>O/R</td>
<td>-</td>
</tr>
<tr>
<td>4-MOMC</td>
<td>-</td>
<td>O (F)</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>3,4-MDMC</td>
<td>Y (B)</td>
<td>O/B</td>
<td>Bl (F)</td>
<td>B/Pr</td>
<td>Y (B)</td>
<td>Y (B)</td>
<td>G/Br</td>
<td>Y</td>
</tr>
<tr>
<td>3,4-MDPV</td>
<td>Y (B)</td>
<td>Y/G</td>
<td>-</td>
<td>B/Pr</td>
<td>Y (B)</td>
<td>Y (B)</td>
<td>G/Br</td>
<td>Y</td>
</tr>
<tr>
<td>BUT</td>
<td>Y (B)</td>
<td>G/Br</td>
<td>Bl (F)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MDA</td>
<td>Bl (D)</td>
<td>*</td>
<td>Pi</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MDMA</td>
<td>Bl (D)</td>
<td>Br</td>
<td>Bl (D)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Key:  - = no reaction; * = not tested ; Bl = blue; Br = brown; G = green; O = orange; Pi = pink; Pr = purple; R = red; Y = yellow; (B) = bright; (D) = dark; (F) = faint. Note:  All test not shown yielded no reaction or were not tested.
4-MOMC with Liebermann’s reagent. However, this results should be confirmed by alternative preliminary identification techniques such as ATR-FTIR, with several “designer drugs” included in this study (results not shown) yielding similarly colored products. No colored product was observed when Liebermann’s reagent was applied to 3-FMC.

In some cases, ambiguous color changes resulted from the reaction of methcathinone analogues with color test reagents. For example, some blue streaking of the solution occurred upon the reaction of Simon’s reagent with 3-FMC, 3,4-MDMC and BUT; however, this was not comparable to the intense blue produced by this reagent with other secondary amines such as methamphetamine or MDMA. This likely arises from the electron withdrawing effects of the ketone group [10], which weakens the nucleophilic nature of the amine group sufficiently to prevent the nucleophilic addition of the amine to the aldehyde, the first step in enamine production [12,16,17]. Similarly, Mandelin’s reagent turned from yellow to orange upon application to analogues of methcathinone. For both of these reagents, the color change was insufficiently definitive for utilisation in routine casework.

Methcathinone and its analogues were also shown in this study not to react with a wide range of color tests commonly utilized by law enforcement, including Simon’s, Scott’s, Chen-Kao, ferric chloride, cobalt thiocyanate, Ehrlich’s, Mandelin’s, gallic acid, Zwikker, sodium nitroprusside, and Dille-Kopanyi. Froehde’s, and Mecke reagent reacted only with 3,4-methylenedioxy-substituted methcathinone analogues, producing yellow and orange-brown products respectively which, as with Marquis reagent, arise from the reaction of the drug molecule with sulfuric acid.

Given these results, Marquis reagent and Liebermann’s reagent were identified as the most appropriate color tests for the preliminary identification of methcathinone and analogues of methcathinone. Both of these color tests are relatively simple to prepare and apply to case work, as they each include only one solution. Commercial test kits for both reagents are also available. Furthermore, these reagents can easily be incorporated into testing sequences of the type typically employed by law enforcement for the preliminary identification of illicit drugs.

Based on the results of this study, a testing sequence (shown in Figure 2) incorporating Marquis and Liebermann’s reagent was been proposed and applied to casework samples. In all cases, results of the color tests were consistent with those of ATR-FTIR. These results suggested that these color tests were suitable for samples with a variety of physical characteristics, and were also sufficiently sensitive to detect the compounds of interest when sample sizes and concentrations are typical of those encountered by operational law enforcement.

Conclusions

This research has shown that color tests are a suitable method for the preliminary identification of methcathinone and analogues of methcathinone. Bright yellow colors are produced by both Marquis reagent and Liebermann’s reagent, with Marquis reagent yielding a yellow product with methylenedioxy-substituted analogues of methcathinone and Liebermann’s reagent with CAT, MCAT, N,N-DMC, and 4-MMC. The reaction of 4-MOMC with Liebermann’s reagent produces an orange product. The mechanisms of reaction of these color tests, and the reasons for the failure of other color tests to yield colored products with these drugs, have been considered. The applicability of a color testing sequence incorporating these reagents to the preliminary identification of methcathinone and analogues of methcathinone has been successfully demonstrated on casework samples.

References

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The Characterization of α-Pyrrolidinopentiophenone

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ABSTRACT: The synthesis, analysis, and characterization of α-pyrrolidinopentiophenone (commonly referred to as “alpha-PVP,” “α-PVP,” or “O-2387”) are briefly discussed. Analytical data (mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared spectroscopy) are presented.

KEYWORDS: α-pyrrolidinopentiophenone, alpha-PVP, 1-phenyl-2-(1-pyrrolidinyl)-1-pentanone, designer drug, synthesis, characterization, forensic chemistry.

This laboratory recently received a request to synthesize α-pyrrolidinopentiophenone; 1-phenyl-2-(1-pyrrolidinyl)-1-pentanone (Figure 1) as a primary standard for identification of this compound in a number of drug exhibits. Although there are two literature citations for this compound [1,2], insufficient analytical data is available for forensic identification. α-Pyrrolidinopentiophenone is not currently scheduled under the U.S. Controlled Substances Act; however, it may be considered a controlled substance analogue of 3,4-methylene-dioxypyrovalerone (MDPV, placed in Schedule I on October 21, 2011) [3]. Herein, we report its synthesis and analytical profile (nuclear magnetic resonance, mass spectrometry, and infrared spectroscopy), to assist forensic chemists who may encounter this substance in casework.

Experimental

Chemicals, Reagents, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). All other chemicals and NMR solvents were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Gas Chromatography/Mass Spectrometry (GC/MS)

Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were obtained on an Agilent 400MR NMR with a 400 MHz magnet, a 5 mm Protune indirect detection, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The sample temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon (proton decoupled), and gradient versions of the 2-dimensional experiments HSQC, and HMBC. Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Synthesis of α-Pyrrolidinopentiophenone

In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2. Briefly, 1-phenyl-1-pentanone was formed from the reaction of valeronitrile with phenylmagnesium bromide, with subsequent acidic workup. The pentanone was then brominated to form the α-bromo ketone, which was then reacted with pyrrolidine to give the title compound, which was finally converted to the HCl ion pair.

Figure 1 - Structural formula of α-pyrrolidinopentiophenone.
Results and Discussion

Structural Elucidation/Confirmation of α-Pyrrolidinopentiophenone HCl

NMR experiments (proton, carbon, COSY, NOESY, HSQC, and HMBC) were performed on the HCl ion pair dissolved in CDCl$_3$ (containing TMS as the 0 ppm reference), giving the proton spectrum and assignments found in Figure 3. The solution was base extracted with sodium bicarbonate saturated D$_2$O, and the CDCl$_3$ layer was isolated and dried with anhydrous sodium sulfate. The proton spectrum and assignments for the free base are found in Figure 4. The HCl ion pair proton spectrum shows a broad 1H singlet at 12.48 ppm indicating NH, a typical phenyl pattern at 7.56 ppm (meta, appears as a 2H triplet), 7.70 (para, appears as a 1H triplet), and 7.99 ppm (ortho, appears as a 2H doublet), and 16 aliphatic protons from 0.9-5.3 ppm. The carbon spectrum has 13 peaks translating to 15 carbons (1 ketone at 196.7 ppm, 6 aromatic in a typical 4 peak phenyl pattern, and 8 aliphatic). The HMBC, COSY, proton chemical shifts and peak patterns, and the carbon chemical shifts show the presence of a phenyl group, a pyrrolidine ring (the 4 carbons are not magnetically equivalent), and a 1,2-disubstituted pentane chain with C-1 being the ketone (there are HMBC correlations to the phenyl protons) and C-2 as a methine (whose proton and carbon chemical shifts indicate bonding to nitrogen, 5.26 ppm $^1$H, 62.7 ppm $^{13}$C) confirming the structure as α-pyrrolidinopentiophenone.

The NMR data of the base shows 21 protons and 11 carbons peaks translating to 15 carbons (1 ketone, 4 aromatic peaks that are 6 carbons, 6 aliphatic peaks that are 8 carbons). As the base, the pyrrolidine carbons produce only 2 signals (2 pair of magnetically equivalent methylenes). Comparing the HCl and base proton spectra shows what a large influence the acid has on the proton chemical shifts that are near the nitrogen. Most notably, the proton chemical shift of the methine of the 1,2-disubstituted pentane chain moves from 5.26 (HCl) to 3.91 ppm (base), while the pyrrolidine protons move from 2.0-3.8 ppm (HCl) to 1.7-2.7 ppm (base). Processing the NMR data with ACD Structure Elucidator software confirmed the structures.

The infrared and mass spectra of α-pyrrolidinopentiophenone are illustrated in Figures 5 and 6, respectively. The FTIR (Figure 5) exhibits a strong carbonyl stretch at 1681 cm$^{-1}$, aliphatic CH stretching at 2866-2958 cm$^{-1}$, and amine HCl bands at 2400-2800 cm$^{-1}$. The mass spectrum displays a weak M-2 ion at m/z 229 and base peak at m/z 126. Other ions in the spectrum are generally less than 10% of the base peak’s intensity.
<table>
<thead>
<tr>
<th>position</th>
<th>carbon (ppm)</th>
<th>proton (ppm)</th>
<th>J_{HH} (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl 1</td>
<td>135.7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2,6</td>
<td>128.6</td>
<td>7.99</td>
<td>m</td>
</tr>
<tr>
<td>3,5</td>
<td>129.4</td>
<td>7.56</td>
<td>m</td>
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<tr>
<td>4</td>
<td>135.1</td>
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<td>m</td>
</tr>
<tr>
<td>1-Pentanone</td>
<td>196.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (C=O)</td>
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<td>5.26</td>
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b = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet

Figure 3 - $^1$H and $^{13}$C NMR data for α-pyrrolidinopentiophenone HCl

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<table>
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d = doublet, m = multiplet, t = triplet

Figure 4 - $^1$H and $^{13}$C NMR data for $\alpha$-pyrrolidinopentiophenone base.
Figure 5 - FTIR of α-pyrrolidinopentiophenone HCl.

Figure 6 - Mass spectrum of α-pyrrolidinopentiophenone.
Conclusions
Analytical data are presented to assist forensic laboratories that encounter \( \alpha \)-pyrrolidinopentiophenone in casework.

References
The Characterization of 2-(5-Methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (5-MeO-BFE) and Differentiation from its N-Ethyl Analog

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Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166-9509
[email address withheld at authors’ request]

ABSTRACT: The synthesis, analysis, and characterization of 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (commonly referred to as “Head F--k”, “5-MeO-BFE”, and “dimemebfe”) and its N-ethyl analog are discussed. Analytical data (mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared spectroscopy) are presented and compared.

KEYWORDS: 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine, dimemebfe, 5-MeO-BFE, head f--k, 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine, designer drug, synthesis, characterization, forensic chemistry.

2-(5-Methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (Figure 1, structure 1), is currently sold over the Internet as “Head F--k”, and has become a popular “research chemical” for recreational drug use. Although not currently scheduled under the U.S. Controlled Substances Act, it may be considered to be an analog of the Schedule I drug 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) (Figure 1, structure 3), with the indole nitrogen replaced with an oxygen to make a benzofuran [1]. This compound was first reported in 1992 to evaluate its affinity for serotonin receptors in rats and for possible use in the design of serotonin receptor ligands [2].

Herein, we report the synthesis and structural elucidation of 1 and its N-ethyl analog, 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine (Figure 1, structure 2). Analytical profiles (nuclear magnetic resonance spectroscopy, mass spectrometry, and infrared spectroscopy) of these compounds are presented and compared to assist forensic chemists who may encounter these substances in casework.

Experimental
Chemicals, Reagents, and Materials
All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). 5-Methoxybenzofuran-yl-acetic acid was a product of Princeton Biomolecular Research (Monmouth Junction, NJ) and all other chemicals and NMR solvents were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI).

Nuclear Magnetic Resonance Spectroscopy (NMR)
NMR spectra were obtained on an Agilent VNMRS 600 MHz NMR using a Protune 5 mm broadband, variable temperature, pulse field gradient probe (Agilent, Palo Alto, CA). The samples were dissolved in deuteriochloroform (CDCl₃) containing tetramethylsilane (TMS) and the temperature was maintained at 26°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon (proton decoupled), and gradient versions of the 2-dimensional experiments HSQC, HMBC, and NOESY. Data processing and structure elucidation were performed using ACD Structure Elucidator software (ACD/Labs, Toronto, Canada).

Gas Chromatography/Mass Spectrometry (GC/MS)
Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph. The MSD

The letters “uc” have been removed to avoid problems with Internet firewalls.
Figure 2 - Synthetic route for 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine and 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine.

(5-methoxy-1-benzofuran-3-yl)acetic acid (4) → oxalyl chloride → (5-methoxy-1-benzofuran-3-yl)acetyl chloride (5) → dimethylamine / ethylamine → 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylectamide (6) / 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylectamide (7) → LAH → 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (1) / 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine (2)
was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm 100% dimethylpolysiloxane, 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 source was operated at 230°C.

Infrared Spectroscopy (FTIR)
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Synthesis of 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine HCl 1 and 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine HCl 2.

In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figure 2. Briefly, 5-methoxybenzofuran-3-yl-acetic acid 4 was converted to the acid chloride 5, which was then reacted with dimethylethylamine to give the amides 6 and 7, respectively. Amides 6 and 7 were then reduced with LAH to provide compounds 1 and 2.

Results and Discussion
GC retention time data for compounds 1, 2, 3, 4-TMS, 6, and 7 are presented in Table 1. All amines were injected as the free base. Compounds 1 and 2 were easily resolved under the conditions utilized.

The FTIR spectra for 1 HCl and 2 HCl are illustrated in Figure 3. Comparison of the hydrochloride ion pairs reveals dissimilar absorption patterns with the most prominent differences being in the region of 2400-3000 cm⁻¹, which are attributed to the tertiary (compound 1) vs. secondary (compound 2) amine HCl ion-pairs. Significant variances are also found in the region of 600-1700 cm⁻¹.

Mass spectra for 1 and 2 are presented in Figure 4. The spectra produced from 1 (Figure 4a) and 2 (Figure 4b) gave a base peak at m/z 58 and a moderate molecular ion at m/z 219. However, 2 produces much more intense ions at m/z 161, m/z 162, and m/z 219, relative to 1 (m/z 161 is ~ 1.7X, m/z 162

Figure 3 - Infrared spectrum of (a) 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine HCl 1 and (b) 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine HCl 2.
Table 1 - Gas chromatographic retention times (Rt) for the benzofuran derivatives and related compounds.

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<td>15.44</td>
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<tr>
<td>3</td>
<td>18.90</td>
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<td>6</td>
<td>19.28</td>
</tr>
<tr>
<td>7</td>
<td>18.78</td>
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</table>

*Conditions given in the experimental section.

is ~10X, and m/z 219 is ~ 4X greater for 2). Although the relative abundances for the remaining ions are quite similar, the two compounds are easily distinguished on the basis of the m/z 161/162 ratio (m/z 161/162 = 7.3:1 for compound 1 and m/z 161/162 = 1.1:1 for compound 2). The ion produced at m/z 162 for 2 is analogous to hydrogen rearrangement (hydrogen migration from the nitrogen to the benzofuran moiety), followed by \( \alpha \)-cleavage, as found for MDA and other related secondary amines [3].

The NMR assignments for the HCl ion-pairs dissolved in CDCl\(_3\) of 1 and 2 are found in Figures 5 and 6. The aromatic proton and carbon spectra are very similar, with only slight chemical shift movement. The amine proton in 1 is a broad singlet at 12.84 ppm which integrates to 1, while the amine protons in 2 are at 9.94 ppm and integrate to 2. Both

Figure 4 - Mass spectra of (a) 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine 1 and (b) 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine 2.
Figure 5 - $^1$H and $^{13}$C NMR data for 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine HCl 1.

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Table:

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b = broad, d = doublet, m = multiplet, s = singlet, t = triplet

Figure 6 - $^1$H and $^{13}$C NMR data for 2-(5-methoxy-1-benzofuran-3-yl)-N-ethylethanamine HCl 2.
compounds have a proton methoxy singlet at 3.8-3.9 ppm. The major spectral difference lies in the aliphatic region. The two methylenes appear as two peaks at 3.31 ppm $^1$H (4 protons) in I due to severe 2nd order effects. Compound 2 methylenes appear as two proton multiplets at 3.24 and 3.37 ppm. Compound 1 has two methyl proton singlets at 2.90 and 2.91 ppm, while compound 2 has a two proton multiplet at 3.11 ppm and a methyl triplet at 1.52 ppm.

Conclusions
Analytical data are presented to assist forensic laboratories that encounter 2-(5-methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine in casework. Each of the three presented spectral techniques can provide unequivocal characterization.

References
Letter to the Editor regarding: Abbreviations for 5- and 6-(2-Aminopropyl)-2,3-dihydrobenzofuran vs. 5- and 6-(2-Aminopropyl)benzofuran: A Clarification of “APB” and “APDB”

When we first published the characterization of 5- and 6-(2-aminopropyl)-2,3-dihydrobenzofuran [1] (Figure 1, upper two structures), the common Internet abbreviated slang for those compounds (and how they were sold) was “5-APB” and “6-APB”. Shortly after that publication, 5- and 6-(2-aminopropyl)benzofuran (Figure 1, lower two structures) were offered by Internet companies; those new compounds were then referred to as “5-APB” and “6-APB”, and 5- and 6-(2-Aminopropyl)-2,3-dihydrobenzofuran were subsequently referred to as “5-APDB” and “6-APDB”. This has caused confusion both in the forensic community as well as within Internet drug chat rooms. Further complicating matters, what is sometimes sold/represented over the internet as 6-APB, is in fact another active substance [2]. In order to eliminate confusion on the slang abbreviations of these four compounds, 5- and 6-(2-aminopropyl)benzofuran should be referred to as “5-APB” and “6-APB” and 6-(2-Aminopropyl)-2,3-dihydrobenzofuran should be referred to as “5-APDB” and “6-APDB.” We hope to publish the characterization of 5- and 6-(2-aminopropyl)benzofuran in the near future.

References:

John Casale
Senior Research Chemist
DEA Special Testing and Research Laboratory

Figure 1 - Structures of 5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APDB), 6-(2-aminopropyl)-2,3-dihydrobenzofuran (6-APDB), 5-(2-aminopropyl)benzofuran (5-APB), and 6-(2-aminopropyl)benzofuran (6-APB).
Identification of the synthetic cannabinoid (1-(cyclohexylmethyl)-1H-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone on plant material

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Illicit Drugs Section, Forensic Science Laboratory, ChemCentre
PO Box 1250, Bently WA 6893
[redmunds@chemcentre.wa.gov.au]

ABSTRACT: In this article we report the isolation and characterisation of a new synthetic cannabinoid. The mass spectrum highlighted structural similarities to JWH-081, however the spectrum showed no match in available libraries. The use of nuclear magnetic resonance spectroscopy along with high resolution LC/MS allowed for structural elucidation and identification of the unknown as (1-(cyclohexylmethyl)-1H-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone.

KEYWORDS: synthetic cannabinoids, naphthoylindole, spice, kronic, forensic chemistry

Since early 2011, synthetic cannabinoids have gained prominence in Australia as a form of legal highs. Governments in Australia have reacted and changed legislation banning certain synthetic cannabinoids [1-3]. These changes have resulted in a wide range of new, and currently unscheduled cannabinoids being seen across the world. The ability for these drugs to affect the same cannabinoid receptors as THC, CB1 and CB2, has been well documented over the past decade with studies carried out by many people including those by John William Huffman and co-workers [4-13]. Identification of synthetic cannabinoids is a challenging task facing forensic laboratories, with new cannabinoids continuously being detected [14-25]. Gas chromatography alone is not sufficient to identify novel synthetic cannabinoids with nuclear magnetic resonance spectroscopy and high resolution mass spectrometry required for the structural elucidation process [21-25].

Synthetic cannabinoids were first detected in plant material submitted for analysis to this laboratory in January 2011. Since this time there has been a rapid increase in the number of samples submitted with products bearing branded trademarks like “Kronic” and “Spice,” as well as unlabelled samples. In the initial stages most samples contained only a few synthetic cannabinoids, predominately JWH-018, JWH-073 and CP47,497 [2]. Western Australian legislation was initially changed banning specific cannabinoids such as JWH-018, but with the dramatic variety in synthetic cannabinoids being detected listing each one would not be practical. Legislation was further changed, this time into eight different classifications based on structure. The classes were benzyloindoles, cyclohexylphenols, dibenzopyrans, naphthoylindoles, naphthylmethylnaphthylindoles, and phenylacetylindoles [1-3]. Any synthetic cannabinoid having a structure belonging to one of these categories is considered a scheduled substance in Western Australia [1-3].

Here we report the isolation and identification of the previously unknown synthetic cannabinoid (1-(cyclohexylmethyl)-1H-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone (compound 1) found in a herbal mixture branded “Northern Lights Skunk” labelled as “Passionfruit Skunk” variety. The presence of this synthetic cannabinoid had been seen previously within the laboratory, but only in the presence of other synthetic cannabinoids and only at trace levels. This sample was the first exhibit submitted to our laboratory where this compound was the only synthetic cannabinoid present. This facilitated the isolation and characterisation.

Experimental

Chemical and reagents

All chemicals and solvents were analytical or high performance liquid chromatography (HPLC) grade. Solvents were obtained from Thermo Fisher Scientific. Chemicals were obtained from Rowe Scientific.

Extract and Isolation of Compound 1

The plant material was enclosed in a packet labelled “Northern Lights Skunk” and was red in colour. The plant material (6 g) was suspended in dichloromethane (10 mL) and the mixture was vortexed for 3 minutes. The suspension was then filtered through filter paper by vacuum filtration. The filtrate was then purified using column chromatography (60-120 mesh Silica Gel, dichloromethane eluant) to afford a pale yellow solid.

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS was run using an Agilent model 6890N GC equipped with an Agilent model 5975 mass-selective detector (MSD). The GC was fitted with a 12 m x 0.2 mm I.D. silica column coated with 0.33 µm 100% dimethylpolysiloxane (HP-1 Ultra). The injection port was maintained at 240°C and injections were performed in splitless mode. The oven temperature program was as follows: Initial temperature 60°C (0.5 min), ramped to 310°C at 25°C/min (final hold 3.5 min). Helium was used as the carrier gas at 2.5mL/min. The MSD was tuned to operate at 69.9 eV for the electron impact energy and 20.4 V for the repeller plate.

High Resolution Mass Spectrometry (HRMS)

High resolution mass spectrometry was run using an Agilent model 6540 UHD accurate mass QTOF and an Agilent 1290 Infinity LC system. The column used was a Phenomenex Kinetex 2.6 µm C18, 100 x 3.00 mm. The mobile phase was a 10 mmol ammonium formate buffer adjusted to pH 9 using 28% ammonia solution and the organic solvent was acetonitrile. The mobile phase was initially 10% organic, then after seven minutes...
50%, and after 12 minutes 100% organic. The ionization source was an Agilent Jet Stream Electron Spray source with an ionization gas of nitrogen. The column heater was set to 50°C and the flow rate was 0.5 mL/min.

**Fourier Transform Infrared Spectroscopy (FTIR)**

The infrared spectrum was acquired using a Thermo Scientific Nicolet 6700 FTIR with a Smart iTR diamond ATR accessory. Data was collected between 4000 cm⁻¹ and 550 cm⁻¹ with a resolution of 4 cm⁻¹ for 16 scans.

**Nuclear Magnetic Resonance Spectroscopy (NMR)**

Sample Preparation: The sample was dissolved in deuterated chloroform (CDCl₃). The CDCl₃ was passed through a plug of sodium sulphate before being subjected to NMR.

NMR spectra were recorded using a Bruker AVN400 (400.13 MHz for \(^1\)H, 100.62 MHz for \(^{13}\)C) spectrometer at ambient conditions. \(^1\)H and \(^{13}\)C chemical shifts were referenced to residual solvent resonances. Assignment of NMR spectra were made with the aid of DEPT, \(^1\)H-\(^1\)H COSY, HSQC (one bond \(^1\)H-\(^{13}\)C correlations) and HMBC (two- and three- bond \(^1\)H-\(^{13}\)C correlations) experiments.

\(^1\)H NMR (CDCl₃, 400.13 MHz): \(\delta\) 0.93 (m, 2H, H3'/H7' 2 x CHH), 1.15 (m, 2H, H4'/H6' 2 x CHH), 1.55 – 1.73 (m, 6H, H3'/H7' 2 x CHH, H4'/H6' 2 x CHH and H5'), 1.83 (m, 1H, H2'), 2.10 – 2.30 (m, 2H, H2''/H6'' 3 x CHH), 2.43 (m, 2H, H2''/H6'' 3 x CHH and H7''), 2.84 (m, 1H, H5''), 3.91 (d, \(J = 7.2\) Hz, 2H, H1'), 4.09 (s, 3H, OCH₃), 6.84 (d, \(J = 8\) Hz, 1H, H3''), 7.33 – 7.38 (m, 3H, H4, H5 and H6), 7.37 (s, 1H, H2), 7.49 – 7.52 (m, 2H, H6'' and H7''), 7.67 (d, \(J = 8\) Hz, 1H, H2''), 8.31 (m, 1H, H8'), 8.35 (m, 1H, H5'') and 8.46 (m, 1H, H7).

\(^{13}\)C NMR (CDCl₃, 100.61 MHz): \(\delta\) 25.7 (CH₂, C4'/6'), 26.2 (CH₂, C5'), 31.0 (CH₂, C3'/7'), 38.4 (CH, C2'), 53.7 (CH₂, C1'), 102.3 (CH, C3''), 110.3, 122.7, 123.5 (3 x CH, C4, C5 and C6), 117.6, 127.3, 137.4 (3 x C, C3, C8 and C9), 122.2 (CH, C5''), 123.0 (CH, C7), 125.78, 127.4 (2 x CH, C6'' and C7''), 125.84 (C, C1''), 126.0 (CH, C8''), 128.0 (CH, C2''), 131.6 (C, C10''), 132.3 (C9''), 138.2 (CH, C2), 157.2 (C, C4') and 191.9 (C=O).

**Ultraviolet/Visible Spectroscopy (UV-Vis)**

UV spectra were acquired on a Perkin Elmer Lambda 25 UV-Vis spectrometer, using a 1 cm path length. The sample was diluted in methanol and scanned over a range of 210-400 nm at a rate of 450 nm/min.

**Results and Discussion**

Isolation of compound 1 was relatively straight forward with an eluant of dichloromethane proving adequate for separating compound 1 from the plant derived impurities. Figure 1 shows the total ion chromatogram for the GC/MS before and after column chromatography. Purification resulted in a single peak.
for compound 1 at 13.5 minutes. This peak contained no
matches in any library databases available at the time. The mass
spectrum of the compound had a base peak of m/z (relative
intensity %) 397 (100) and three other key ions 314 (75), 185
(38) and 240 (18). Based on the mass spectrum the unknown
was suspected to contain a naphthoylindole structure due to
similarities in the mass spectrum with JWH-081. Cleavage of
the cyclohexane ring explains the m/z of 314, whilst the m/z 185
is from cleavage of the bond between the indole and ketone
carbons. The third key ion m/z 240 is explained by the cleavage
of methoxynaphthylene ring as shown in figure 2.

HRMS was performed to determine a molecular ion and a
molecular formula. The HRMS mass spectrum produced a
molecular ion (MH⁺) of m/z = 398.2135, which suggested a
calculated molecular formula of C₂₇H₂₇NO₂ (mass accuracy 4.7
ppm) with a calculated accurate mass for the molecular ion of
398.2115 Da. Using the accurate mass and retention time a
targeted MS/MS analysis was carried out. The two major
fragments present were m/z 185.0613 and 240.1397 with
157.0654 and 144.0449 as minor ions as shown in figure 3. The
calculated molecular formula for the ions observed in the
MS/MS spectrum was C₁₂H₉O for m/z 185.0613 (mass
difference 5.4 ppm) and C₁₆H₁₈NO for m/z 240.1397 (mass
difference 3.74 ppm). This data is consisted with the structure
suggested for compound 1.

NMR Experiments
The final structural elucidation of 1 was completed using ¹H
and ¹³C NMR experiments. Key signals in the ¹H NMR
spectrum (Figure 4) include a downfield doublet at 3.91 ppm,
due to the N-bound methylene protons. The doublet indicated
that the adjacent group was a CH and thus a site of additional
branching. The symmetry of the cyclohexyl group is consistent
with the numbers of signals in the alkyl regions of the ¹H and ¹³C
NMR spectra. Standout features in the aromatic region of the ¹H
NMR spectrum include two doublets at 6.84 and 7.67 ppm,
which show no additional ¹H-¹H coupling. These doublets are
due to the ortho H₂'' and H₃'' protons on the naphthyl ring.

UV-Vis spectroscopy
The UV-Vis spectrum was recorded for compound 1, with
two major peaks at λ_max = 212.7 and 320.7 nm, which is very
similar to the spectrum of JWH-081 to which compound 1 is
structurally related.

FTIR Spectroscopy
The key features in the FTIR spectrum are the CH stretches at
2921 and 2847 cm⁻¹. Due to the high level of conjugation within
compound 1 the key carbonyl stretch has likely been shifted to a
lower wave number and cannot be clearly identified due to the
large number of absorbances below 1607 wavenumbers.
Figure 4 - $^1$H NMR spectrum for compound 1.

References


The disguising of illicit materials to facilitate their trafficking is not something new; narcotics are no exception. Drug smugglers, for example, have been very active at finding new ways of modifying and/or masking various drugs to avoid their detection. Such an example is black cocaine, which is a mixture of cocaine base and/or cocaine hydrochloride with other substances carefully chosen to specifically hide the physical characteristics of the cocaine and interfere with its detection [1-5]. Once the black cocaine reaches its destination, it must then be treated chemically in order to extract the cocaine from the mixture. Several seizures of black cocaine have been made around the world, which include: religious icons made of black cocaine [6], black cocaine as a dark solid hidden in items such as a hammock [7], black cocaine as a powder in toner cartridges [8,9], as an industrial dye [10], and as an ingredient in plastic materials [11-13].

The Canada Border Services Agency (CBSA) recently submitted several exhibits of black cocaine to the CBSA’s Science and Engineering Directorate (S&E Lab). During the analysis of the black cocaine exhibits, the S&E Lab used the various commercially available narcotics field test kits and immunoassay drug tests in our possession at the time of the seizure, as well as, ion mobility spectrometry (IMS) as screening tools. It was the goal of the S&E Lab to determine which presumptive tests could be used to identify cocaine in black cocaine samples. Please note that this study is limited to the S&E Lab’s inventory of equipment and tests at the time of the seizure and is not intended to serve as a promotion tool for the equipment and tests used.

**Experimental, Results, and Discussion**

**Details of the seizure**

The S&E Lab recently received exhibits containing black granular material that was similar to crushed coal (Figure 1). The exhibits were taken from bags each weighing 20 kilograms, declared as asphalt product. The bags of asphalt were seized at a Canadian international airport and originated in Colombia.

The samples were first analysed using IMS which gave a strong positive result for cocaine in 25% of the exhibits. All of the samples were also analysed using immunoassay drug tests; while only the samples containing cocaine were tested using narcotics test kits. A more thorough analysis of the samples followed using GC/MS, LC/MS, and FTIR. The analyses indicated that the samples were composed of a hydrocarbon containing material and that 25% of the samples also contained cocaine. In addition, the analyses indicated the presence of phenyltetrahydroimidazo[1,2-a]thiazole in most of the cocaine containing exhibits. The cocaine containing exhibits were found to contain approximately 4% (w/w) cocaine calculated as the hydrochloride. The total quantity of cocaine in this shipment was determined to be 10 kg, which has an estimated resale value of $1,250,000 CAD.

**Presumptive drug testing**

The presumptive drug tests that were performed consisted of IMS, narcotic test kits (the Narcopouch from ODV Inc. and the Narcotic Identification Kit polytesting systems, also called the NIK tests), and lateral flow immunoassay-based narcotics identification tests. For this study, the lateral flow immunoassay-based narcotics identification tests used were the Rapid Solids tests from Cozart® Bioscience and the DrugID® tests from Securetec (two various types were evaluated: single test for cocaine and multiple tests for cocaine, opium, amphetamine and methamphetamine - Coc/Opi/Amph/Meth).
Following the manufacturer’s general procedure for powders, a swab was used to pick up a small amount of the powder which was then placed in the buffer solution provided with the test. The lid was then screwed on the bottle of buffer, and the bottle was shaken gently for 5 sec. Four drops of the buffer solution were then squeezed in the sample well of the cartridge. After approximately 2 min, or once the liquid had reached the end of the cartridge and the control line appeared, the result could then be interpreted. Results were positive for the samples that were known to contain cocaine and negative for the other samples (Figure 2).

Using the manufacturer’s general procedure for powders, a spatula was used to pick up a small amount of sample. The spatula was then placed in the provided buffer solution and stirred for 5 sec. The Drug ID test stick was then dipped into the buffer solution for 15 sec and placed on a horizontal surface for 3 min. Both the DrugID single drug and multi drug test sticks gave positive results for the samples containing cocaine and a negative result for samples that did not contain cocaine (Figures 3 and 4). All samples gave a negative result for opium, amphetamine, and methamphetamine on the multitest DrugID test stick.

The NIK tests were only performed on the samples containing cocaine. The sample was first treated as an unknown and the manufacturer’s general poly-testing procedures were followed. First, test A - Marquis Reagent - was performed and resulted in no obvious color change when looking at the supernatant exclusively (see Figure 5a). Using the Marquis Reagent test, cocaine can give a buff, light peach color, or no color. The next step was then to proceed to test G - Scott Reagent - for cocaine. There was no color change observed, the supernatant remained pink and the powder remained black (Figure 5b). A blue color would develop in the presence of cocaine salts; in the case of cocaine base, only the material will turn blue. The test G was repeated with more material and again, no color change could be seen, indicating a negative result. Based on the described results, the NIK Polytesting system is not able to detect cocaine in samples of black cocaine.

The tests were only performed on the samples containing cocaine. The general procedure was followed; as such, test #901 - Mayer’s reagent - was performed and resulted in no color change (negative result). This was not the expected result for cocaine, which should give a creamy white result. Despite the previous result, test #904 - for Cocaine HCl, free-base, or “crack” was performed. The reaction between the black powder and the content of the left ampoule of the pouch did not lead to a color change; the solution remained pink, which is a negative result for cocaine. Given the results described above, the Narcopouch® tests are not able to identify cocaine in samples of black cocaine.

The samples were analysed on various models of ion mobility spectrometers in our possession at the time of the seizure. Samples were run on four instruments from two different manufacturers - three bench top models (Itemiser® from Morpho Detection, Ionscan® 400B, and Ionscan® 500DT from Smiths Detection) and one handheld unit (the multi-mode
threat detector, MMTD, also from Smiths Detection). All four instruments were set to standard parameters and conditions for narcotics detection. The sampling was performed using a teasing needle which was placed in the sample bag and rubbed against the black powder, the excess material on the needle was removed by shaking the needle, and the residual powder on the needle was then transferred to the appropriate sampling material for the equipment used. All four instruments successfully identified cocaine in the samples that contained cocaine and none gave a false positive for the samples that did not contain cocaine.

Examples of plasmagrams (from the Ionscan 400B instrument) obtained for a sample containing cocaine and for a sample not containing cocaine are presented in Figures 6 and 7, respectively. The plasmagram in Figure 6 shows three peaks, one corresponding to the calibrant peak at a drift time of 9.588 msec ($K_0 1.8566$), one corresponding to the cocaine peak at a drift time of 15.345 msec ($K_0 1.1600$) and one unidentified compound coming from the sample at 12.214 msec. The plasmagram in Figure 7 only shows the calibrant peak.

Conclusions

Although efficient on solid samples, colorimetric tests such as the NIK tests and Narcopouch® have been known to present challenges when colored solids samples are tested, as the sample color may obscure the resulting color of these tests. The negative results obtained when using these colorimetric tests on the black solid samples in this study were not surprising. It is unclear if the negative results are due to the difficulty in seeing the change of color due to the black coloration of the solution or if the complex matrix is interfering and preventing the reactions of the tests with the cocaine in the sample to occur.

The other presumptive tests were efficient in the identification of cocaine, despite the complexity of the sample matrix. The lateral flow immunoassay-based narcotics identification tests required a simple sample preparation and provided the results in less than five minutes. As for the ion mobility spectrometry analysis, results were obtained in a few seconds and no sample preparation was required.

Acknowledgements

Special thanks to our colleagues at the S&E Lab of the CBSA: Pat Latour for providing the GC/MS analysis, Céline Chartrand for providing the LC/MS analysis, and Kim Lao for the ATR-FTIR analysis and for his interpretation of the GC/MS, LC/MS, and ATR-FTIR results.

References

The Separation of Cocaine and Phenyltetrahydroimidazothiazole Mixtures

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ABSTRACT: Phenyltetrahydroimidazothiazole (i.e., levamisole, dexamisole, or tetramisole) has been increasingly utilized as a cutting agent by South American illicit cocaine laboratories for the past eight years, and is now the most predominant adulterant in cocaine produced in Colombia. The salt form of illicit cocaine must be determined when possible for sentencing purposes; this is typically done via infrared spectroscopy. Several separation techniques for cocaine/phenyltetrahydroimidazothiazole mixtures are presented to assist the determination of cocaine salt form during forensic analyses. Additionally, a purification technique is presented for isolation of cocaine for isotope ratio mass spectrometric analysis, a critical component for source determination. Mixtures of cocaine (hydrochloride and base) and phenyltetrahydroimidazothiazole (85:15, 70:30, and 50:50) were prepared and separated with liquid/liquid extractions, ion-pair chromatography, and high pressure liquid chromatography. Recovered cocaine was subsequently analyzed via infrared spectroscopy, gas chromatography-flame ionization detection, and isotope ratio mass spectrometry.

KEYWORDS: Forensic chemistry, cocaine, phenyltetrahydroimidazothiazole, liquid/liquid separations, IRMS

The purity of illicit cocaine seized in the United States has steadily decreased over the past eight years. This trend is primarily due to the addition of adulterants such as phenyltetrahydroimidazothiazole (PTHIT), diltiazem, and hydroxyzine, which began around 2005 [1]. Currently, PTHIT is routinely identified in illicit cocaine samples as levamisole, tetramisole, or a non-racemic mixture of levamisole and dexamisole (Figure 1) in approximately 70% of all domestic cocaine seizures. Traditionally, PTHIT was widely used as an anti-worm medication (anthelmintic) for both humans and animals, but it is no longer approved for use in the United States or Canada due to its toxicity. The proposed explanation for the presence of PTHIT in cocaine samples is to prolong the drug’s effects [2].

In the United States, for the purposes of federal prosecution and sentencing, it is necessary to determine the salt form (base or hydrochloride) when analyzing cocaine. Chiral chromatographic methods have been developed for the separation and isomer identification of PTHIT [3-4]; however, few methods have been presented to provide a non-destructive purification of cocaine for the purpose of identifying salt forms. In addition to salt form identification, pure cocaine is needed in elemental analysis-isotope ratio mass spectrometric analyses (EA-IRMS) for the purposes of origin determination [5].

Experimental

Materials

Celite 545 and all chemicals and solvents used were reagent grade or better and were obtained from Sigma-Aldrich (St. Louis, MO). Cocaine hydrochloride (HCl) and tetramisole HCl were obtained from this laboratory’s reference materials collection.

The glass chromatographic columns used for ion-pair separations were products of Lyreux (Vineland, NJ), and were 260 mm × 22 mm i.d. with a stem length of 50 mm. Columns were prepared identically to conditions previously reported [6].

Figure 1 - Structures of levamisole and dexamisole.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR (Pittsburgh, PA) equipped with a single bounce attenuated total reflectance (ATR) accessory. Instrument parameters were: resolution = 4 cm⁻¹; gain = 8; optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Gas Chromatography/Flame Ionization Detection (GC/FID)

GC/FID analyses were performed with an Agilent (Palo Alto, CA) Model 6890N Gas Chromatograph. The sample preparation and GC/FID conditions used have been previously reported [7]. Isopropylcocaine was utilized as the internal standard.

High Performance Liquid Chromatography (HPLC)

HPLC analyses were performed with an Agilent 1200 Series Preparative Liquid Chromatograph using an autosampler equipped with a 1000 µL loop, preparative pump, thermostatted column compartment, and fraction collector. The injection volume was 430 µL. The stationary phase consisted of a silica column, Agilent Zorbax RX-SIL 4.6 mm i.d. × 100 mm, with a 1.8 µm particle size. The mobile phase consisted of the following elution gradient: An isocratic hold using 96% ammoniated hexane/4% isopropanol for 10 min, followed by a linear gradient for five min to 65% ammoniated hexane/35% isopropanol, hold for 2 min, then a linear gradient for 3 min from 65% ammoniated hexane/35% isopropanol back to the starting mobile phase, for a total run-time of 20 min. The column temperature...
was set at 20°C and the flow rate was set to 2 mL/min. Three fractions were collected from 0.5 min to 4.0 min, 4.0 min to 5.0 min, and 5.0 min to 6.0 min. The elution of cocaine and PTHIT was monitored using diode-array detection (DAD) at 254 nm, and the appropriate fractions were combined to collect the entire cocaine peak. The collected fractions were dried at 75°C under a stream of air and transferred to a 4 mL glass vial using a minimal amount of diethyl ether, which was then evaporated to obtain cocaine base powder.

**Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS)**

Cocaine samples were analyzed to determine carbon isotopes using a Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific; Pittsburgh, PA) in continuous flow mode with a ECS 4010 elemental analyzer (Costech Analytical; Valencia, CA). The elemental analyzer reactor tubes were comprised of two quartz glass tubes filled with chromium(III) oxide/silvered cobaltous oxide and reduced copper. The tubes were held at 1040°C and 640°C for combustion and reduction, respectively. Water was removed from the generated combustion gases by a trap filled with magnesium perchlorate. Nitrogen and carbon dioxide were separated with a post-reaction GC/FID reactor GC.

**Liquid/liquid Separations**

Two grams of cocaine HCl and tetramisole HCl (85:15) were prepared by weighing each compound separately and mixing thoroughly. Nineteen solvent combinations of chloroform/hexane and chloroform/ether were prepared (10:0, 9:1, 8:2, and 10% increments to 1:9). Approximately 50 mg portions of the cocaine HCl/tetramisole HCl mixture were combined with 4 mL of a selected solvent combination in 15 mL glass round-bottom centrifuge tubes. The tubes were vigorously shaken and centrifuged. The solvent was removed and filtered through a micro-filter. Insoluble material was allowed to dry overnight prior to analysis. This procedure was repeated with heating the samples at 75°C for 10 min upon addition of solvent. The samples were allowed to cool for 1 hour before filtering. The filtered solvent was then evaporated to dryness. Soluble and insoluble materials were analyzed via FTIR and GC/FID.

In addition to the chloroform/hexane and chloroform/ether combinations, aqueous/organic solvent combinations were utilized in order to separate mixtures of cocaine HCl and tetramisole HCl (85:15, 70:30, 50:50) as listed in Table 1. Fifty mg of cocaine HCl/tetramisole HCl mixture were dissolved in an aqueous solution (Solution 1) in a 15 mL glass round-bottom centrifuge tube. Five mL of chloroform was then added, and the tube was shaken vigorously, then centrifuged for approximately 2 min. The chloroform layer was removed, evaporated to dryness, and examined via FTIR/ATR and GC/FID.

Each of the cocaine HCl/tetramisole HCl mixtures (85:15, 70:30, 50:50) were converted to the base form by dissolving the mixture in boiling water and adding dilute ammonium hydroxide until the solution was basic and precipitation occurred. The mixture was allowed to cool, and the water was removed. The remaining base mixture was allowed to dry overnight. Ten 50 mg portions were combined with 5 mL of hexane or ether in 10 separate 15 mL glass round-bottom centrifuge tubes (five

### Table 1 - Liquid/liquid separations of cocaine HCl and tetramisole HCl with 5 mL chloroform.

<table>
<thead>
<tr>
<th>Solution 1</th>
<th>% Cocaine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>85:15</td>
<td>70:30</td>
</tr>
<tr>
<td>0.250 mL H₂O</td>
<td>96.5</td>
</tr>
<tr>
<td>1.0 mL H₂O</td>
<td>98.4</td>
</tr>
<tr>
<td>0.250 mL 1 N HCl/2 M NaCl</td>
<td>92.4</td>
</tr>
</tbody>
</table>

### Table 2 - Liquid/liquid separations of cocaine base and tetramisole base with hexane/H₂O (5 mL/5 mL).

<table>
<thead>
<tr>
<th>Water Washes</th>
<th>% Cocaine HCl</th>
<th>% Cocaine Base</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(85:15)</td>
<td>(70:30)</td>
</tr>
<tr>
<td>1</td>
<td>94.4</td>
<td>84.3</td>
</tr>
<tr>
<td>2</td>
<td>97.9</td>
<td>93.5</td>
</tr>
<tr>
<td>3</td>
<td>99.2</td>
<td>97.3</td>
</tr>
<tr>
<td>4</td>
<td>99.7</td>
<td>99.1</td>
</tr>
<tr>
<td>5</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 3 - Ion pair column separations of cocaine HCl and tetramisole HCl.

<table>
<thead>
<tr>
<th>Column</th>
<th>% Cocaine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>85:15</td>
</tr>
<tr>
<td>1a</td>
<td>100.0d</td>
</tr>
<tr>
<td>2b</td>
<td>100.0d</td>
</tr>
<tr>
<td>3c</td>
<td>100.0d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Cocaine</th>
<th>Corrected δ¹⁵N</th>
<th>Corrected δ¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>-9.3 ± 0.06</td>
<td>-29.3 ± 0.00</td>
</tr>
<tr>
<td>85%</td>
<td>-9.3 ± 0.05</td>
<td>-29.2 ± 0.05</td>
</tr>
<tr>
<td>70%</td>
<td>-9.2 ± 0.00</td>
<td>-29.2 ± 0.00</td>
</tr>
<tr>
<td>50%</td>
<td>-9.4 ± 0.10</td>
<td>-29.2 ± 0.05</td>
</tr>
</tbody>
</table>

a 4 g Celite 545 and 2 mL 1 N HCl/2 M NaCl
b 2 g Celite 545 and 1 mL 1 N HCl/2 M NaCl
c 4 g Celite 545 and 2 mL H₂O
d No PTHIT detected in eluent via GC/FID

### Table 4 - IRMS results from cocaine HCl and tetramisole HCl. 2a mixtures after LC- Prep cleanup procedure. b

<table>
<thead>
<tr>
<th>% Cocaine</th>
<th>Corrected δ¹⁵N</th>
<th>Corrected δ¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>-9.3 ± 0.06</td>
<td>-29.3 ± 0.00</td>
</tr>
<tr>
<td>85%</td>
<td>-9.3 ± 0.05</td>
<td>-29.2 ± 0.05</td>
</tr>
<tr>
<td>70%</td>
<td>-9.2 ± 0.00</td>
<td>-29.2 ± 0.00</td>
</tr>
<tr>
<td>50%</td>
<td>-9.4 ± 0.10</td>
<td>-29.2 ± 0.05</td>
</tr>
</tbody>
</table>

a Known values for tetramisole HCl: δ¹⁵N = -3.4; δ¹³C = -26.9
b Triplicate analysis
test tubes per solvent. All samples were heated at 75°C for approximately 5 min. Once the solutions were cooled, 5 mL of water were added to each test tube. The samples were shaken vigorously and centrifuged for 2 min. The solvent layer was removed and washed again with water. The washing process was repeated up to four times (Table 2). The washed solvent was evaporated to dryness and examined via FTIR and GC/FID.
Ion-Pair Chromatography

Three ion-pair columns were prepared to separate mixtures of cocaine HCl and tetramisole HCl (85:15, 70:30, and 50:50). Fifty mg of cocaine HCl/tetramisole HCl were dissolved in 250 µL of water, combined with 0.5 g of Celite 545, and mixed well. The resulting mixture was transferred to a column packed with a portion of Celite 545 and 1-2 mL of ion pair solution as specified in Table 3. Cocaine was eluted with 35 mL of water-saturated chloroform. Five 5 mL fractions were collected and analyzed via GC/FID for the amount of cocaine present. Appropriate fractions were combined, evaporated to dryness, and examined via FTIR.

HPLC Sample Preparation

Cocaine HCl samples adulterated with tetramisole HCl were purified by preparative liquid chromatography. Using a 25 mg equivalent of cocaine, samples were first dissolved in 1 mL of water in a 15 mL glass round-bottom centrifuge tube. A basic extraction was performed using two drops of concentrated ammonium hydroxide and 5 mL of diethyl ether. The tube was capped, shaken, and centrifuged for approximately five minutes. The ether layer was transferred to a new test tube and dried under a stream of air at 75ºC. The dried sample was then analytically transferred using small aliquots of ether to a 1.5 mL glass autosampler vial. The sample was then evaporated at 75ºC to remove all ether. When dry, the sample was diluted with 200 µL of isopropanol and 600 µL of hexane, capped and heated for approximately five minutes at 75ºC to ensure the sample was completely dissolved in injection solvent.

Results and Discussion

Liquid/liquid Separations

After washing the cocaine HCl/tetramisole HCl mixture (85:15) with several combinations of chloroform/hexane and chloroform/ether, insoluble material and soluble material were both analyzed via FTIR and GC/FID. The cocaine purity of the insoluble material and soluble material never exceeded 85% cocaine. The separation of cocaine HCl and tetramisole HCl did not improve after heating the samples upon addition of solvent. Therefore, this separation scheme was determined to be ineffective and was not further pursued.

Aqueous/organic solvent combinations were then utilized for the separation of the three cocaine HCl/tetramisole HCl mixtures. The starting mixture was initially dissolved in aque-
ous solution, and the cocaine HCl was then extracted with chloroform. The purity of cocaine isolated from these extractions was ≥ 90%, on average, between all three HCl mixtures (Table 1). The best separation was achieved using 1.0 mL of water and 5 mL of chloroform.

The three mixtures of cocaine base/tetramisole base (85:15, 70:30, 50:50) were dissolved in hexane and washed with water repeatedly. As illustrated in Table 2, tetramisole base in the 85:15 and 70:30 mixtures was completely removed from the hexane after washing with water five times due to its preferential solubility in water versus cocaine. After five washes of the 50:50 mixture, 99+% pure cocaine was obtained. FTIR spectra of the 50:50 mixture before and after the water purification are shown in Figure 2. The major areas of interference are found between 1800-1200 cm\(^{-1}\) (Figure 2, upper). After five water washes, the mentioned interferences were removed from the IR spectrum (lower Figure 2). This procedure was also performed with ether (instead of hexane); however, it was unsuccessful.

**Ion-Pair Separations**

Ion-pair chromatography has previously been utilized to separate various mixtures of cocaine and other adulterants such as lidocaine and procaine [8-11]. Based on previous success with ion-pair chromatographic separations, the same methodology was attempted with cocaine HCl and tetramisole HCl. Three separate columns were prepared with different stationary phase preparations. As shown in Table 3, Column 1 provided the best separation with pure cocaine fractions (PTHIT free) for all three mixtures tested (85, 70 and 50% cocaine HCl). FTIR spectra of the 50:50 mixture before and after purification are shown in Figure 3. Areas of interference are included to illustrate differences in the spectra before and after an ion-pair chromatographic separation. The most significant spectral interferences are shown between 960-480 cm\(^{-1}\). Pure cocaine fractions were also collected with Columns 2 and 3 for the 85:15 cocaine HCl/tetramisole HCl mixture, however, very small amounts of PTHIT were detected with the 70:30 and 50:50 mixtures.

For separations implementing an ion-pair solution, it is important to determine the salt form of cocaine in the starting material. As illustrated in Figures 2 and 3, the salt form can be determined when the purity is only 50%. Knowledge of the salt form prior to the use of a separation technique (liquid/liquid or ion-pair chromatography) will prevent an analyst from inadvertently altering the salt form of cocaine during the removal of phenyltetrahydroimidazothiazole.

![Figure 3 - FTIR spectra of cocaine HCl/tetramisole HCl (50:50) mixture (above) and cocaine HCl after separation via ion pair chromatography (below). Inserts of interfering regions are included for comparison](image-url)
**Prep-HPLC Separations**

The three mixtures of cocaine HCl/tetramisole HCl were separated via preparative-HPLC. Separations were performed in triplicate, and appropriate fractions were combined and prepared as previously described. Once the purified cocaine base was obtained and dried, samples were examined via EA-IRMS. The carbon and nitrogen isotope data were consistent among all samples analyzed, indicating the cocaine was no longer adulterated with tetramisole (Table 4). Due to the minimal variation in the isotope ratios observed in comparison to the known values of the cocaine HCl standard, it was noted the HPLC separation caused little fractionation during the purification.

**Conclusions**

Several techniques were utilized for the separation of cocaine and tetramisole mixtures. GC/FID and FTIR data were obtained to effectively determine the purity and salt form of the recovered cocaine. For mixtures of cocaine base/tetramisole base, the best method to purify the cocaine was a liquid/liquid separation employing hexane and water; five water washes successfully removed tetramisole base from the cocaine. The most successful technique for separation of cocaine HCl/tetramisole HCl utilized an ion-pair chromatographic column packed with 4 g of Celite 545 and 2 mL 1 N HCl/2 M NaCl. However, chloroform/H$_2$O extractions did remove a significant amount of the tetramisole for FTIR. The described HPLC methodology isolated the cocaine with minimal fractionation occurring for IRMS analyses of cocaine samples.

**References**

Purification and Characterization of 3-Methyl-6-[3-(trifluoromethyl)-phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872)

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ABSTRACT: The purification, identification, and characterization of 3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872) is presented. Analytical data (mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy) is included.

KEYWORDS: 3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-b]pyridazine, CL 218872, synthetic cannabinoid, GC/MS, FTIR, NMR, forensic chemistry

In June 2012, three exhibits of adulterated plant material were analyzed at the DEA North Central Laboratory and were found to contain 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872) in a mixture of synthetic cannabinoids. Two of the exhibits also contained 1-(5-fluoropentyl)-3-(2,2,3,3-tetramethylcyclopenty1)indole (XLR-11) and 1-pentyl-N-(tricyclo[3.3.1.1\(^3,7\)]dec-1-yl)-1H-indazole-3-carboxamide (AKB48). The other exhibit also contained XLR-11 and suspected 1-(5-fluoropentyl)-N-(tricyclo[3.3.1.1\(^3,7\)]dec-1-yl)-1H-indole-3-carboxamide (STS-135). To the best of our knowledge CL 218872 had not been previously identified in synthetic cannabinoid exhibits, although its presence was suspected in numerous exhibits analyzed by other laboratories in the United States [1]. First synthesized by Albright et al. in the 1970s, CL 218872 is a benzodiazepine agonist that does not appear to have been developed into a commercial pharmaceutical product [2] and is currently not scheduled in the United States. The analytical profile for CL 218872 is presented herein.

![Structure of 3-Methyl-6-[3-(Trifluoromethyl) Phenyl]-1,2,4-Triazolo[4,3-b]Pyridazine (CL 218872)](image)

In June 2012, three exhibits of adulterated plant material were analyzed at the DEA North Central Laboratory and were found to contain 3-methyl-6-[3-(trifluoromethyl) phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872) in a mixture of synthetic cannabinoids. Two of the exhibits also contained 1-(5-fluoropentyl)-3-(2,2,3,3-tetramethylcyclopenty1)indole (XLR-11) and 1-pentyl-N-(tricyclo[3.3.1.1\(^3,7\)]dec-1-yl)-1H-indazole-3-carboxamide (AKB48). The other exhibit also contained XLR-11 and suspected 1-(5-fluoropentyl)-N-(tricyclo[3.3.1.1\(^3,7\)]dec-1-yl)-1H-indole-3-carboxamide (STS-135). To the best of our knowledge CL 218872 had not been previously identified in synthetic cannabinoid exhibits, although its presence was suspected in numerous exhibits analyzed by other laboratories in the United States [1]. First synthesized by Albright et al. in the 1970s, CL 218872 is a benzodiazepine agonist that does not appear to have been developed into a commercial pharmaceutical product [2] and is currently not scheduled in the United States. The analytical profile for CL 218872 is presented herein.

Experimental
Chemicals, Reagents, and Materials

Methanol was obtained from EMD (Billerica, MA) and Fisher Scientific (Pittsburg, PA). Deuterochloform was obtained from Aldrich Chemical (Milwaukee, WI). 18MΩ water was obtained from a Millipore filtration system. A CL 218872 reference standard was obtained from Tocris Biosciences (Bristol, United Kingdom).

Gas Chromatography/Mass Spectrometry (GC/MS)

The mass spectrum of CL 218872 was acquired using an Agilent Model 5975C quadrupole mass-selective detector (MSD) interfaced with an Agilent 7890A gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV and in scan mode with a mass range of 35-500 amu. Samples were extracted with methanol and 1.0 µL was injected with a split ratio of 50:1 onto a 30 m x 250 µm I.D. x 0.25 µm 5% phenyl 95% dimethylpolysiloxane (HP-5MS) column. The oven temperature was held at an initial temperature of 80°C for 1.5 minutes, ramped at 30°C/minute to 320°C, and held for 10 minutes.

Purification by High Performance Liquid Chromatography (HPLC)

The sample was purified in order to obtain FTIR and NMR data. Purification was carried out on an Agilent 1200 series HPLC equipped with a quaternary pump, autosampler, and diode array detector. A solvent system consisting of 85:15 MeOH:H₂O, with a flow rate of 1.5 mL/min was used with a Supelco-ODS column (15 cm x 4.6 mm, 3 µm particle size), and an injection volume of 100 µL. Multiple fractions were manually collected, combined, and evaporated on a 75°C hot plate under an air stream. This method allowed for purification of the suspected CL 218872, which had the shortest retention time of the three major sample components, but gave poor chromatography due to the high sample loading.

Fourier Transform Infrared Spectroscopy (FTIR)

The spectra were collected using a diamond attenuated total reflectance (ATR) accessory on a Nicolet 6700 FTIR (Thermo
Figure 2 - EI mass spectrum of CL 218872 reference standard.

Figure 3 - FTIR spectrum of CL 218872 reference standard.

Table of Peaks (cm\(^{-1}\))

| 3115.62 | 1477.33 | 1273.68 | 1046.96 | 769.72 |
| 3061.36 | 1417.34 | 1165.44 | 992.81 | 751.95 |
| 1550.57 | 1375.30 | 1119.52 | 823.34 | 696.98 |
| 1524.30 | 1336.59 | 1099.09 | 835.42 | 670.63 |
| 1494.04 | 1314.42 | 1080.69 | 804.16 | 653.05 |
The spectrum was collected with a resolution of 4 cm$^{-1}$, spectral range of 525-4000 cm$^{-1}$, optical velocity of 0.3165 cm/s, an aperture of 80, and 8 scans/sample.

**Nuclear Magnetic Resonance (NMR)**

The purified sample and the standard were dissolved in CDCl$_3$ and spectra were acquired with standard Varian pulse sequences [3] on a Varian Model 400-MR with Varian AutoX Indirect Detection Pulse Field Gradient probe. Spectra for proton, carbon, and 2-D NMR (HSQC, HMBC, 15N CIGAR) were collected.

**Results and Discussion**

**Gas Chromatography/Mass Spectrometry (GC/MS)**

The mass spectrum of CL 218872 (Figure 2) is dissimilar to known synthetic cannabinoids [4] and to other compounds present in several mass spectral libraries routinely used for comparison purposes at the North Central Laboratory. CL 218872 has a base peak of 140 m/z and molecular ion of 278 m/z. In the mass spectrum, multiple losses of 19 m/z and a loss of 70 m/z indicated the possibility of one or more fluorine atoms which were possibly from a CF$_3$ group [5]. The DEA Northeast Laboratory (New York, New York) analyzed an exhibit presumably containing the same compound in which electrospray ionization mass spectroscopy (ESI-MS) data and accurate mass data was collected. The mass [M+H]$^+$ was determined to be 279.0852 amu, which correlated well with a proposed molecular formula of C$_{13}$H$_9$N$_4$F$_3$ [6].

**Fourier Transform Infrared Spectroscopy (FTIR)**

The spectrum of the purified component of the sample (Figure 3) contained neither ketone band nor any readily discernible bands for common functional groups, but did indicate that the compound was aromatic [7]. The best match from a search of several spectral libraries of interest was a poor quality match for fenfluramine.

**Nuclear Magnetic Resonance (NMR)**

Integration of the proton NMR (Figure 4) for the purified unknown showed a total of nine protons- one methyl singlet (3H) and six protons in the aromatic region- one triplet (1H), four doublets (4H), and one singlet (1H). The carbon NMR

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Figure 5 - 100 MHz carbon spectrum of CL 218872 reference standard.

Figure 6 - $^{15}$N-CIGAR NMR of the CL 218872 reference standard showing N-H couplings of 2-3 bond lengths and proposed couplings. (Note: No chemical shift reference used in the nitrogen dimension (F1))
(Figure 5) for the purified unknown was weak making an accurate counting of the carbon atoms difficult and was exacerbated by the fluorine splitting of the carbon signals. The peak at 131.84 ppm was split into a quartet \((J_{CF} = 33.0 \text{ Hz})\) and the peaks at 124.12 ppm and 127.47 ppm appeared to be split into doublets or quartets (depending on the line broadening used) \((J_{CF} = 3.90 \text{ Hz})\), suggesting a \(\text{CF}_3\) group. The data from the reference material showed the wide coupling characteristic of a \(\text{CF}_3\) group \((J_{CF} = 272.50 \text{ Hz})\) making it initially difficult to discern if there was a carbon multiplet or multiple carbons signals. The assignments of all protons and carbons are listed in tables in Figures 4 and 5, respectively. The 15N CIGAR experiment (2-3 bond length N-H hetero-nuclear interactions) provided data supporting the assignments for the \(H-7\) and \(H-8\) protons. Proposed nitrogen assignments are in figure 7, but further understanding of the 15N CIGAR experiment is needed to make a definitive assignment of the nitrogen signals.

References
6. Personal communication with Yuriy Uvaydov.
Technical Note

Characterization of Eleven 2,5-Dimethoxy-N-(2-methoxybenzyl)-phenethylamine (NBOMe) Derivatives and Differentiation from their 3- and 4-Methoxybenzyl Analogues - Part II

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Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166-9509

ABSTRACT: The characterization of the 33 2,5-dimethoxy-N-(2-methoxybenzyl)-, (3-methoxybenzyl)-, and (4-methoxybenzyl) phenethylamine (NBOMe) derivatives via NMR spectroscopy is presented. The data enables differentiation of all 33 compounds.


Introduction

Part I of this study presented the Fourier transform infrared and mass spectral data for the series of designer drugs commonly referred to as "NBOMe" compounds (1). Herein we present the NMR spectra (proton and carbon-13) for both the HCl salt and the free base for each of the three methoxybenzyl positional isomers for all 11 specified NBOMe compounds (Figure 1).

Experimental

Chemicals
Deuterated chloroform (CDCl₃) containing 0.05% v/v tetramethylsilane (TMS, 0 ppm reference) and deuterium oxide (D₂O) were both purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA). Sodium bicarbonate and anhydrous sodium sulfate were purchased from Sigma-Aldrich (St. Louis, MO). All 33 NBOMe HCl samples were synthesized at this laboratory.

Nuclear Magnetic Resonance Spectroscopy
All NMR spectra were obtained using the following instruments: Mercury 400 MHz NMR with a 5 mm Nalorac Pulsetune indirect detection probe; a 400MR with a 5 mm Protune Indirect Detection probe; a 400MR-DD2 with a 5 mm OneNMR Probe; or a VNMRS 600 MHz NMR with a 5 mm broadband probe (all from Agilent, Palo Alto, CA). All probes used pulse field gradients. The sample temperature was maintained at 25°C. Standard Agilent pulse sequences were used to collect the following spectra: Proton, carbon-13 (proton decoupled), and gradient versions of the 2-Dimensional experiments HSQC and HMBC. The HCl salt of each compound was dissolved in CDCl₃ and the spectra acquired. The corresponding free base of each HCl salt was then formed by basifying the respective solution with saturated sodium bicarbonate in D₂O; the isolated CDCl₃ solution was then dried with anhydrous sodium sulfate, and the spectra acquired. Data processing and structure elucidation were performed using Structure Elucidator software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).
Results and Discussion

The HCl salt and the free base of every NBOMe derivative were soluble in CDCl₃. The proton spectrum contains three basic regions of interest (see Figure 2): a) The NH region (9-10 ppm) which integrates to 2 for the HCl salt (but no signal after D₂O-sodium bicarbonate extraction); b) the aromatic region (6.5-7.5 ppm) containing the signals for the six NBOMe benzene protons (seven for 25H-NBOMe); and c) the aliphatic region (2.9-4.5 ppm) which contains the three methoxy singlets and signals for the bridging CH₂-N-CH₂-CH₂ protons. The carbon-13 spectrum (see Figure 3) is divided into five basic regions of interest: a) The three aromatic carbons bonded to oxygen (145-160 ppm); b) the other nine aromatic carbons (100-135 ppm, with the exception of 25I-NBOMe having one carbon at 80-85 ppm for the carbon bonded to iodine); c) the three methoxy carbons (about 55 ppm); d) the CH₂-CH₂ group bonded to nitrogen (about 45 ppm); and e) the last CH₂ bonded to the phenyl group (about 25 ppm). The proton spectrum definitively determines the position of the benzyl methoxy group as ortho, meta, or para. When correlated with the molecular weight and other NMR data, the specific NBOMe compound can be unambiguously identified.

Tables I-III present the proton NMR assignments for all 33 compounds as the HCl salts, while Tables IV-VI are the assignments for the same compounds as their corresponding free bases. Tables VII-IX present the carbon-13 NMR assignments for all 33 compounds as the HCl salts, while Tables X-XII are the assignments for the same compounds as their corresponding free bases. Each Table contains the 11 NBOMe compounds with the benzyl methoxy substituent in the same position (i.e., the NBOMe HCl compounds with the methoxy benzyl in the ortho position are in Table I for proton and Table VII for carbon-13, while the meta substituted compounds are in Tables II and VIII, and so on).

Proton Spectra

Common to all NBOMe compound proton spectra are the peaks in the aromatic region (6.5-7.5 ppm) with its six protons (four benzyl and two para-phenyl singlets, with the exception of 25H-NBOMe, which has seven); the methoxy region (3.6-4.2 ppm) with its three methoxy singlets and the benzyl CH₂ triplet (HCl spectra) or singlet (base spectra); and the ethylene region 2.7-3.2 ppm with its multiplets. Additional peaks, if present, can aid in the identification of a substituent at the 4-phenyl position, if that substituent also contains one or more protons.

The aromatic region may be used to determine whether the methoxy position on the benzyl ring is ortho, meta, or para, since the coupling constants for an aromatic proton change based on whether another proton it is coupled to is alpha (7-8 Hz coupling) or beta (1-3 Hz) on the ring. Using only couplings >5 Hz to describe the proton peak pattern, NB2OMe spectra contain two doublets and two triplets; NB3OMe spectra contain one broadened singlet (actually a doublet of doublets with small couplings), two doublets, and one triplet; and NB4OMe spectra have two doublets, each representing two hydrogens. In looking more closely at the coupling constants of some triplets, it is evident that they are actually doublets of doublets whose coupling constants are similar (e.g., 7 and 8 Hz). To illustrate the differences, Figure 4 shows the aromatic region of the proton spectra of the three positional isomers of 25I-NBOMe HCl dissolved in CDCl₃. The smaller coupling constants are due to meta coupling (4 bonds) and range from ~0.9 Hz to ~2.5 Hz. In 25I-NB3OMe HCl (Figure 4B), the 7.20 ppm doublet of doublets (which looks like a small triplet) represents H-2 with couplings to H-4 (~2.5 Hz) and H-6 (~1 Hz). In addition to the aromatic benzyl protons, all NBOMe proton spectra, except 25H-NBOMe, will have two singlets in the aromatic region, which are caused by the phenyl protons on C-3 and C-6 (they are para to each other and have no discernable coupling). For 25H-NBOMe, the substituent at C-4 is hydrogen, and the aromatic region will therefore show seven (not six) hydrogens, and the phenyl proton pattern will be a doublet (~8 Hz), doublet of doublets (~8, ~2 Hz), and doublet (~2 Hz), characteristic of a 2,5-disubstituted phenyl ring.
The aliphatic region is less helpful in differentiating positional isomers of NBOMe, but is still useful in identification (Figure 5). There are three tall methoxy singlets between 3.6-3.85 ppm; the benzyl CH2 triplet (HCl) between 3.9-4.2 ppm or singlet (base) between 3.7-3.8 ppm; and the CH2-CH2 multiplets between 2.9-3.2 ppm. Of note in this region is the fact that the benzyl CH2 triplet chemical shift in HCl spectra is highest in the NB2OMe spectrum, followed by the NB3OMe spectrum, and then by the NB4OMe spectrum.

If the substituent is unknown then the following procedure should help in identifying it:

1. Determine the molecular weight by high resolution mass spectrometry to derive the molecular formula. If high resolution mass spectrometry is not available, then take the molecular ion and subtract it from 300 (i.e., the nominal mass for C18H22NO3). Some example remainders are: 1 = hydrogen, 15 = methyl, 35 = chlorine, 79 = bromine, and 127 = iodine. Standard mass spectra can help identify many substituents, especially the halogens.

2. Determine how many hydrogens are present in the molecule from the proton NMR spectrum (not counting the broad singlet near 9 ppm) and subtract this number from 21. Note the location of any extra hydrogens in the proton spectrum (i.e., chemical shift, number, and peak pattern); these are on the substituent.
   a. If there are no extra hydrogens, then the extra molecular weight will be from a substituent that has no hydrogens (e.g., -NO2).
   b. If there are extra hydrogens, use their peak pattern and chemical shift, to help determine the substituent's identity.

3. Determine the number of carbons from the carbon-13 NMR spectrum and subtract from 18 for NB2OMe and NB3OMe, or from 16 for NB4OMe (the latter compounds have para substitution on the benzyl group, so the ortho and meta carbons are equivalent, and produce only two peaks for four carbons).

**Carbon Spectra**

The NB2OMe and NB3OMe carbon-13 spectra contain 12 aromatic peaks, while the NB4OMe carbon-13 spectra contain 10 aromatic peaks due to symmetry of the para-methoxyphenyl. The aliphatic portion of the NBOMe spectra contains three methoxy and three methylene carbon-13 peaks. For the HCl salts, the CH2-CH2-N carbons have chemical shifts of about 28 and 45 ppm, while the benzyl CH2 is between 45-50 ppm. The NB2OMe HCl salts have their benzyl CH2 at 46-47 ppm, while the NB3OMe HCl and NB4OMe HCl benzyl CH2 are at ~50 ppm. Similarly, the NBOMe base compounds' CH2-CH2 chemical shifts are at ~31 and ~49 ppm, while the benzyl CH2 is found at ~49 ppm (NB2OMe) or ~53 ppm (NB3OMe and NB4OMe). 25I-NBOMe compounds (HCl and base) have a distinctive aromatic carbon, bonded to iodine, at 82-84 ppm.

**Conclusions**

All of the 2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamines analyzed in this study are distinguishable from their 3- and 4-methoxybenzyl analogues via proton and carbon-13 NMR spectroscopy. A quick determination of whether an NBOMe compound is the 2-, 3-, or 4-methoxybenzyl substituted form is easily accomplished by inspection of the aromatic region of its proton NMR. In addition, a simple stepwise approach can help determine the identity of a substituent at the 4-phenyl position, using the NMR spectra and the molecular weight derived from mass spectrometry.

**Reference**

Figure 1. Structural formulas.

![Structural formula](image)

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<td>(CH₃)₂CHS</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>25T4-NB40Me (30)</td>
<td>(CH₃)₂CHS</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>25T7-NB20Me (31)</td>
<td>CH₃(CH₂)₂S</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>25T7-NB30Me (32)</td>
<td>CH₃(CH₂)₂S</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>25T7-NB40Me (33)</td>
<td>CH₃(CH₂)₂S</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
</tr>
</tbody>
</table>
**Figure 2.** Proton spectrum of 25C-NB2OMe HCl in CDCl₃ with assignments. The two singlets at 6.8 and 6.9 ppm (a) are protons on the phenyl group which are *para* to each other; the proton at 4.1 ppm (b) is a triplet due to coupling with the NH₂⁺; the tall singlets at 3.5-3.8 ppm (c) are the methoxy groups; and the multiplet at 3.1 ppm (d) is the bridging ethyl group.
Figure 3. Carbon-13 spectrum of 25C-NB2OMe HCl in CDCl₃. Aromatic carbons bonded to oxygen are at 149-158 ppm; nine remaining benzene carbons are at 110-132 ppm; methoxy carbons are at 55-57 ppm; and bridging methylenes are at 28, 45, and 47 ppm.
**Figure 4.** Proton NMR spectra of aromatic region of 25I-NBOMe HCl dissolved in CDCl₃; with the benzyl methoxy at the *ortho* (A), *meta* (B), and *para* (C) positions. *Ortho* substitution (A) is easily detected by its two apparent doublets and two apparent triplets. *Meta* substitution (B) yields one triplet, one doublet, one doublet of doublets, and one small coupling doublet of doublets (7.20 ppm). *Para* substitution (C) is demonstrated by its two doublets which contain 2 hydrogens each. The two singlets at 6.7 and 7.1 ppm are the phenyl protons at C-6 and C-3, respectively.
**Figure 5.** Proton spectra of aliphatic region of 25I-NBOMe HCl dissolved in CDCl$_3$; with the benzyl methoxy at the *ortho* (A), *meta* (B), and *para* (C) positions. The three methoxy groups are the tall singlets between 3.6-3.85 ppm; while the benzyl CH2 is the triplet between 3.9-4.2 ppm, and the CH2-CH2 bridging group are the multiplets between 2.9-3.2 ppm.
<table>
<thead>
<tr>
<th>Location of Methoxy</th>
<th>25H-NB2O1Me HCl (600 MHz)</th>
<th>25D-NB2O1Me HCl</th>
<th>25E-NB2O1Me HCl</th>
<th>25P-NB2O1Me HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCH3</td>
<td>3.74 s</td>
<td>3.67 s</td>
<td>3.70 s</td>
<td>3.70 s</td>
</tr>
<tr>
<td>CH2 NH2 CH2 CH2-Ph</td>
<td>4.17 t(5.2)</td>
<td>4.15 t(5.2)</td>
<td>4.16 t(5.1)</td>
<td>4.15 t(5.1)</td>
</tr>
<tr>
<td>CH2 NH2 CH2 CH2-Ph</td>
<td>3.11 m</td>
<td>3.09 m</td>
<td>3.10 m</td>
<td>3.10 m</td>
</tr>
<tr>
<td>5-MeOCH3</td>
<td>3.63 s</td>
<td>3.60 s</td>
<td>3.62 s</td>
<td>3.62 s</td>
</tr>
<tr>
<td>5-MeOCH3</td>
<td>3.73 s</td>
<td>3.76 s</td>
<td>3.75 s</td>
<td>3.74 s</td>
</tr>
<tr>
<td>NH2+</td>
<td>9.43 vbs</td>
<td>9.33 vbs</td>
<td>9.40 vbs</td>
<td>9.37 vbs</td>
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<table>
<thead>
<tr>
<th>4-substituent</th>
<th>2.18 s</th>
<th>2.57 q(7.6)</th>
<th>2.52 d(8.6, 6.8)</th>
<th>1.15 t(7.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Br</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
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Table I. Proton NMR Assignments ortho-Methoxyphenyl NBOMe HCl Compounds.

Abbreviations: a – apparent, b – broad, d – doublet, m – multiplet, q – quartet, s – singlet, t – triplet, vbs – very broad singlet
Table II. Proton NMR Assignments meta-Methoxyphenyl NBOMe HCl Compounds.

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<th>2SH-NB3OMe HCl</th>
<th>2SD-NB3OMe HCl</th>
<th>2SE-NB3OMe HCl</th>
<th>2SP-NB3OMe HCl</th>
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<tr>
<td>benzyl 2</td>
<td>7.26 ppm, m</td>
<td>7.23 ppm, m</td>
<td>7.23 ppm, m</td>
<td>7.23 ppm, m</td>
</tr>
<tr>
<td>4</td>
<td>6.84 dd(8.4, 7.5)</td>
<td>6.84 dd(8.3, 2.6, &lt;1)</td>
<td>6.83 dd(8.1, 2.2)</td>
<td>6.83 dd(8.4, 2.5)</td>
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<tr>
<td>5</td>
<td>7.26 ppm, m</td>
<td>7.25 ppm, m</td>
<td>7.24 ppm, m</td>
<td>7.23 ppm, m</td>
</tr>
<tr>
<td>6</td>
<td>7.08 bd(7.6)</td>
<td>7.07 bd(7.7)</td>
<td>7.07 bd(7.4)</td>
<td>7.07 bd(7.6)</td>
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<tr>
<td>location of methoxy</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>OCH3</td>
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<td>3.80 s</td>
<td>3.80 s</td>
<td>3.80 s</td>
</tr>
<tr>
<td>CH2NHCH2CH2-Ph</td>
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<td>4.03 t(5.2)</td>
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<td>3.05 m</td>
<td>3.05 m</td>
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<td>3.13 m</td>
<td>3.13 m</td>
<td>3.13 m</td>
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<td>Phenyl 3</td>
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<td>6.61 s</td>
<td>6.62 s</td>
<td>6.60 s</td>
</tr>
<tr>
<td>4</td>
<td>6.72 m</td>
<td>6.70 s</td>
<td>6.71 s</td>
<td>6.71 s</td>
</tr>
<tr>
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<td>6.71 s</td>
<td>6.71 s</td>
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<td>2-OCH3</td>
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<td>3.69 s</td>
<td>3.68 s</td>
</tr>
<tr>
<td>5-OCH3</td>
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<td>3.75 s</td>
<td>3.74 s</td>
<td>3.73 s</td>
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<tr>
<td>NH2+</td>
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<td>9.97 vbs</td>
<td>10.00 vbs</td>
<td>10.00 vbs</td>
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<td>CH2-CH3</td>
<td>propyl</td>
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<tr>
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<td>2.17 s</td>
<td>2.57 q(7.5)</td>
<td>2.52 dd(“8,7”)</td>
<td>1.15 t(7.5)</td>
</tr>
<tr>
<td></td>
<td>1.56 a-sextet(7.3)</td>
<td>0.93 t(7.3)</td>
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<table>
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<th>2SC-NB3OMe HCl</th>
<th>2SB-NB3OMe HCl</th>
<th>2SP-NB3OMe HCl</th>
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<tbody>
<tr>
<td>benzyl 2</td>
<td>7.20 dd(2.5, 1.2)</td>
<td>7.21 dd(2.5, 1.3)</td>
<td>7.21 dd(2.5, 1.3)</td>
<td>7.20 dd(2.5, 0.9)</td>
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<tr>
<td>4</td>
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<td>6.83 dd(8.0, 2.5, 1.3)</td>
<td>6.83 dd(8.4, 2.5, &lt;1)</td>
<td>6.83 dd(8.4, 2.5, &lt;1)</td>
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<tr>
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<td>7.24 dd(8.4, 7.4)</td>
<td>7.25 dd(8.0, 7.5)</td>
<td>7.25 dd(8.0, 7.6)</td>
<td>7.25 dd(8.4, 7.6)</td>
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<td>location of methoxy</td>
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<td>3</td>
<td>3</td>
<td>3</td>
</tr>
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<td>3.80 s</td>
</tr>
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<td>4.00 t(5.2)</td>
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<td>3.04 m</td>
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<tr>
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<td>6.70 s</td>
<td>6.70 s</td>
<td>6.70 s</td>
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<tr>
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<td>3.69 s</td>
<td>3.69 s</td>
</tr>
<tr>
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<td>3.82 s</td>
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<td>10.00 vbs</td>
<td>10.00 vbs</td>
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<td>Cl</td>
<td>Br</td>
<td>I</td>
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<table>
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<tbody>
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<td>7.23 dd(2.3)</td>
<td>7.22 m</td>
</tr>
<tr>
<td>4</td>
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<td>6.84 bd(8.3, 2.3)</td>
</tr>
<tr>
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<td>7.25 dd(8.3, 7.7)</td>
</tr>
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<td>7.07 bd(7.6)</td>
<td>7.07 bd(7.6)</td>
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<td>3</td>
<td>3</td>
</tr>
<tr>
<td>OCH3</td>
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<td>3.80 s</td>
<td>3.80 s</td>
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<tr>
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<td>4.02 t(5.1)</td>
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<td>3.14 m</td>
<td>3.13 m</td>
</tr>
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<td>6.84 s</td>
<td>6.76 s</td>
</tr>
<tr>
<td>4</td>
<td>6.76 s</td>
<td>6.77 s</td>
<td>6.75 s</td>
</tr>
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<td>3.70 s</td>
</tr>
<tr>
<td>5-OCH3</td>
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<td>3.81 s</td>
<td>3.82 s</td>
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<td>9.99 vbs</td>
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<td>1.64 sextet(7.3)</td>
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</table>

Abbreviations: a – apparent, b – broad, d – doublet, m – multiplet, q – quartet, s – singlet, t – triplet, vbs – very broad singlet.
Table III. Proton NMR Assignments para-Methoxyphenyl NBOMe HCl Compounds.

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<td>ppm</td>
<td>peak multiplicity</td>
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<td>d(8.7)</td>
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<tr>
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<td>6.86</td>
<td>d(8.7)</td>
<td>6.85</td>
<td>d(8.7)</td>
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<td>7.50</td>
<td>d(8.7)</td>
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<td>d(8.7)</td>
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<tr>
<td>location of methoxy</td>
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<td>4</td>
<td>4</td>
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<td>s</td>
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<td>m</td>
<td>3.11</td>
<td>m</td>
</tr>
<tr>
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<td>d(1.6)[2nd order effects]</td>
<td>6.62</td>
<td>s</td>
</tr>
<tr>
<td>4</td>
<td>6.72</td>
<td>d(1.6)[2nd order effects]</td>
<td>6.62</td>
<td>s</td>
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<td>2-OCH3</td>
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<td>s</td>
<td>3.69</td>
<td>s</td>
</tr>
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<td>vbs</td>
<td>9.84</td>
<td>vbs</td>
</tr>
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<td>CH2CH3</td>
<td>propyl</td>
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<td>q(7.5)</td>
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<td>location of methoxy</td>
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<td>OCH3</td>
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<td>(under OCH3 singlet)</td>
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<td>7.35</td>
<td>s</td>
<td>6.83</td>
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<td>3.72</td>
<td>s</td>
<td>3.71</td>
<td>s</td>
</tr>
<tr>
<td>5-OCH3</td>
<td>3.83</td>
<td>s</td>
<td>3.82</td>
<td>s</td>
</tr>
<tr>
<td>NH2+</td>
<td>9.89</td>
<td>vbs</td>
<td>9.91</td>
<td>vbs</td>
</tr>
<tr>
<td>4-substituent</td>
<td>S-C2H5</td>
<td>S-C2H3(3,2)</td>
<td>S-C2H2CH3</td>
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</tr>
<tr>
<td></td>
<td>2.89</td>
<td>q(7.4)</td>
<td>3.46</td>
<td>septet(6.8)</td>
</tr>
</tbody>
</table>

Abbreviations: a – apparent, b – broad, d – doublet, m – multiplet, q – quartet, s – singlet, t – triplet, vbs – very broad singlet
S.O.E. – 2nd order effects present
Table IV. Proton NMR Assignments ortho-Methoxyphenyl NBOMe Base Compounds.

<table>
<thead>
<tr>
<th></th>
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<tr>
<td></td>
<td>ppm</td>
<td>peak multiplicity</td>
<td>ppm</td>
<td>peak multiplicity</td>
</tr>
<tr>
<td>benzyl 3</td>
<td>6.83</td>
<td>dd(8.6)</td>
<td>6.82</td>
<td>dd(8.6)</td>
</tr>
<tr>
<td>4</td>
<td>7.21</td>
<td>m</td>
<td>7.21</td>
<td>m</td>
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<tr>
<td>5</td>
<td>6.89</td>
<td>td(7.3, 1.0)</td>
<td>6.89</td>
<td>bt(7.3)</td>
</tr>
<tr>
<td>6</td>
<td>7.21</td>
<td>m</td>
<td>7.21</td>
<td>m</td>
</tr>
</tbody>
</table>

**location of methoxy**

|            | 2 | 2 | 2 | 2 |
| OCH3       | 3.77 s | 3.75 s | 3.74 s | 3.74 s |
| CH2NH2CH2CH2-Ph | 3.80 s | 3.80 s | 3.80 s | 3.79 s |
| CH2NH2CH2CH2-Ph | 2.83 m | 2.82 m | 2.83 m | 2.82 m |
| CH2NH2CH2CH2-Ph | 2.81 m | 2.80 m | 2.81 m | 2.80 m |
| Phenyl 3   | 6.76 d(8.6) | 6.66 s | 6.67 s | 6.66 s |
| 4          | 6.69 dd(8.6, 3.1) | 6.66 s | 6.66 s | 6.65 s |
| 6          | 6.74 d(3.1) | 6.65 s | 6.66 s | 6.65 s |
| 2-OCH3     | 3.74 s | 3.73 s | 3.74 s | 3.73 s |
| 5-OCH3     | 3.74 s | 3.75 s | 3.75 s | 3.74 s |

**4-substituent**

<table>
<thead>
<tr>
<th>H</th>
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<th>CH2-CH3</th>
<th>propyl</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2.20 s</td>
<td>2.60 q(7.5)</td>
<td>2.70 dd(9.4, 6.0)</td>
</tr>
<tr>
<td></td>
<td>1.18 t(7.5)</td>
<td>1.59 dd(9.4, 7.4, 6.0)</td>
<td>0.96 t(7.4)</td>
</tr>
</tbody>
</table>

| benzyl 3 | 6.84 d(8.3) | 6.82 dd(8.2) | 6.82 dd(8.1) | 6.83 dd(8.4) |
| 4        | 7.23 t(8) | 7.22 m | 7.22 ddd(8.1, 7.4, 1.5) | 7.21 m |
| 5        | 7.20 d(8) | 7.20 m | 7.19 ddd(7.5, 1.5) | 7.19 m |

**location of methoxy**

|            | 2 | 2 | 2 | 2 |
| OCH3       | 3.77 s | 3.75 s | 3.75 s | 3.75 s |
| CH2NH2CH2CH2-Ph | 3.79 s | 3.79 s | 3.78 s | 3.79 s |
| CH2NH2CH2CH2-Ph | 2.84 m | 2.81 m | 2.81 m | 2.81 m |
| CH2NH2CH2CH2-Ph | 2.88 m | 2.79 m | 2.78 m | 2.78 m |
| Phenyl 3   | 7.39 s | 6.85 s | 7.00 s | 7.19 s |
| 4          | 6.92 s | 6.76 s | 6.74 s | 6.66 s |
| 6          | 3.79 s | 3.73 s | 3.73 s | 3.72 s |
| 2-OCH3     | 3.89 s | 3.82 s | 3.81 s | 3.79 s |

**4-substituent**

<table>
<thead>
<tr>
<th>NO2</th>
<th>Cl</th>
<th>Br</th>
<th>I</th>
</tr>
</thead>
</table>

| benzyl 3 | 6.82 d(8.3) | 6.82 d(7.9) | 6.82 bd(7.6) |
| 4        | 7.21 m | 7.21 m | 7.21 m |
| 5        | 6.89 td(7.5) | 6.88 td(7.3) | 6.89 td(7.4, 1.0) |
| 6        | 7.20 td(7.8) | 7.20 m | 7.20 m |

**location of methoxy**

|            | 2 | 2 | 2 | 2 |
| OCH3       | 3.75 s | 3.75 s | 3.75 s | 3.75 s |
| CH2NH2CH2CH2-Ph | 3.80 s | 3.80 s | 3.80 s | 3.80 s |
| CH2NH2CH2CH2-Ph | 2.82 m | 2.83 m | 2.82 m | 2.82 m |
| CH2NH2CH2CH2-Ph | 2.82 m | 2.82 m | 2.82 m | 2.82 m |
| Phenyl 3   | 6.83 s | 6.89 s | 6.82 s | 6.82 s |
| 4          | 6.69 s | 6.70 s | 6.68 s | 6.68 s |
| 6          | 3.75 s | 3.74 s | 3.74 s | 3.74 s |
| 2-OCH3     | 3.81 s | 3.80 s | 3.81 s | 3.81 s |
| 5-OCH3     | 3.90 | q(7.5) | 3.45 septet(6.7) | 2.85 dd(7.0, 7.7) |
| 4-substituent | 1.29 t(7.5) | 1.26 d(6.7) | 1.65 sextet(7.3) | 1.02 t(7.3) |

**Abbreviations:** a – apparent, b – broad, d – doublet, m – multiplet, q – quartet, s – singlet, t – triplet
### Table V. Proton NMR Assignments meta-Methoxyphenyl NBOMe Base Compounds.

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<th></th>
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<td>benzyl 2</td>
<td>6.75 ppm, m</td>
<td>6.93 ppm, bs</td>
<td>6.86 ppm, bs</td>
<td>6.86 ppm, bs</td>
</tr>
<tr>
<td>4</td>
<td>6.70 ppm, dd(8.8, 3.1)</td>
<td>6.79 ppm, dd(8.1, 2.6)</td>
<td>6.78 ppm, dd(7.8, 1.6)</td>
<td>6.77 ppm, dd(8.0)</td>
</tr>
<tr>
<td>5</td>
<td>7.21 ppm, t(7.8)</td>
<td>7.22 ppm, dd(8.1, 7.8)</td>
<td>7.21 ppm, t(7.8)</td>
<td>7.21 ppm, t(8.0)</td>
</tr>
<tr>
<td>6</td>
<td>6.88 ppm, m</td>
<td>6.91 ppm, d(7.8)</td>
<td>6.87 ppm, bd(7.8)</td>
<td>6.87 ppm, bd(8.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location of methoxy</th>
<th>OCH3</th>
<th>CH2NHCH2CH2-Ph</th>
<th>CH2NHCH2CH2-Ph</th>
<th>Phenyl 3</th>
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<tbody>
<tr>
<td>3</td>
<td>6.86 ppm, m</td>
<td>6.67 ppm, s</td>
<td>6.67 ppm, s</td>
<td>6.55 ppm, s</td>
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<tr>
<td>2-OCH3</td>
<td>3.74 ppm, s</td>
<td>3.73 ppm, s</td>
<td>3.76 ppm, s</td>
<td>3.75 ppm, s</td>
</tr>
<tr>
<td>5-OCH3</td>
<td>3.75 ppm, s</td>
<td>3.76 ppm, s</td>
<td>3.76 ppm, s</td>
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<table>
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<tr>
<th>4-substituent</th>
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<th>CH3</th>
<th>CH2-CH3</th>
<th>propyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.19 ppm, s</td>
<td>2.60 ppm, q(7.5)</td>
<td>2.54 ppm, ddd(8.2, 7.3)</td>
<td>1.18 ppm, t(7.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Position</th>
<th>25N-NB3OMe base</th>
<th>25C-NB3OMe base</th>
<th>25B-NB3OMe base</th>
<th>25i-NB3OMe base</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzyl 2</td>
<td>6.85 ppm, d(2.3)</td>
<td>6.85 ppm, m</td>
<td>6.89 ppm, m</td>
<td>6.84 ppm, bs</td>
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<tr>
<td>4</td>
<td>6.79 ppm, dd(8.3, 2.3)</td>
<td>6.78 ppm, dd(8.1, 2.5)</td>
<td>6.79 ppm, ddd(8.3, 2.6, 0.9)</td>
<td>6.78 ppm, dd(8.3, 2.6)</td>
</tr>
<tr>
<td>5</td>
<td>7.23 ppm, dd(8.3, 7.5)</td>
<td>7.22 ppm, dd(8.1, 7.8)</td>
<td>7.22 ppm, t(8.0)</td>
<td>7.22 ppm, d(8)</td>
</tr>
<tr>
<td>6</td>
<td>6.86 ppm, bd(7.5)</td>
<td>6.86 ppm, m</td>
<td>6.88 ppm, m</td>
<td>6.86 ppm, d(8)</td>
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<table>
<thead>
<tr>
<th>Location of methoxy</th>
<th>OCH3</th>
<th>CH2NHCH2CH2-Ph</th>
<th>CH2NHCH2CH2-Ph</th>
<th>Phenyl 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6.93 ppm, s</td>
<td>6.77 ppm, s</td>
<td>6.76 ppm, s</td>
<td>6.68 ppm, s</td>
</tr>
<tr>
<td>2-OCH3</td>
<td>3.82 ppm, s</td>
<td>3.75 ppm, s</td>
<td>3.74 ppm, s</td>
<td>3.75 ppm, s</td>
</tr>
<tr>
<td>5-OCH3</td>
<td>3.90 ppm, s</td>
<td>3.83 ppm, s</td>
<td>3.82 ppm, s</td>
<td>3.80 ppm, s</td>
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<table>
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<th>Br</th>
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<table>
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<tr>
<th>Position</th>
<th>25T2-NB3OMe base</th>
<th>25T4-NB3OMe base</th>
<th>25T7-NB3OMe base</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzyl 2</td>
<td>6.88 ppm, m</td>
<td>6.86 ppm, m</td>
<td>6.88 ppm, m</td>
</tr>
<tr>
<td>4</td>
<td>6.78 ppm, ddd(8.0, 2.6)</td>
<td>6.78 ppm, dd(8.1, 2.5)</td>
<td>6.78 ppm, dd(8.1, 2.3)</td>
</tr>
<tr>
<td>5</td>
<td>7.22 ppm, t(8.0)</td>
<td>7.21 ppm, t(8.1)</td>
<td>7.22 ppm, t(8.1)</td>
</tr>
<tr>
<td>6</td>
<td>6.88 ppm, m</td>
<td>6.86 ppm, m</td>
<td>6.88 ppm, m</td>
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<table>
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<th>Location of methoxy</th>
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<th>CH2NHCH2CH2-Ph</th>
<th>CH2NHCH2CH2-Ph</th>
<th>Phenyl 3</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>6.70 ppm, s</td>
<td>6.71 ppm, s</td>
<td>6.69 ppm, s</td>
<td>6.83 ppm, s</td>
</tr>
<tr>
<td>2-OCH3</td>
<td>3.76 ppm, s</td>
<td>3.76 ppm, s</td>
<td>3.76 ppm, s</td>
<td>3.76 ppm, s</td>
</tr>
<tr>
<td>5-OCH3</td>
<td>3.82 ppm, s</td>
<td>3.81 ppm, s</td>
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<table>
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<th>4-substituent</th>
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<th>S-CH(CH3)2</th>
<th>S-CH2CH2CH3</th>
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</thead>
<tbody>
<tr>
<td>2.90 ppm, q(7.5)</td>
<td>3.45 ppm, septet(6.7)</td>
<td>2.85 ppm, m</td>
<td></td>
</tr>
<tr>
<td>1.29 ppm, t(7.5)</td>
<td>1.26 ppm, d(6.7)</td>
<td>1.65 ppm, sextet(7.4)</td>
<td></td>
</tr>
<tr>
<td>1.02 ppm, t(7.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** a – apparent, b – broad, d – doublet, m – multiplet, q – quartet, s – singlet, t – triplet
Table VI. Proton NMR Assignments para-Methoxyphenyl NBOMe Base Compounds.

<table>
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<th></th>
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<tbody>
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<td>4</td>
<td>7.21</td>
<td>d(8.7)</td>
<td>7.21</td>
<td>d(8.7)</td>
<td>7.21</td>
<td>d(8.5)</td>
<td>7.21</td>
<td>d(8.8)</td>
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<td>6.84</td>
<td>d(8.7)</td>
<td>6.84</td>
<td>d(8.7)</td>
<td>6.84</td>
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<td>6.84</td>
<td>d(8.8)</td>
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<tr>
<td>5</td>
<td>6.84</td>
<td>d(8.7)</td>
<td>6.84</td>
<td>d(8.7)</td>
<td>6.84</td>
<td>d(8.5)</td>
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<td>d(8.8)</td>
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<td>7.21</td>
<td>d(8.7)</td>
<td>7.21</td>
<td>d(8.7)</td>
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<td>d(8.5)</td>
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<tbody>
<tr>
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<td>s</td>
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<td>2.18</td>
<td>t(7.3)</td>
<td>1.60</td>
<td>m</td>
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<td>t(7.3)</td>
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<table>
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<th>25C-NB40Me base ppm</th>
<th>peak multiplicity</th>
<th>25B-NB40Me base ppm</th>
<th>peak multiplicity</th>
<th>25I-NB40Me base (600 MHz) ppm</th>
<th>peak multiplicity</th>
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<tbody>
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<td>d(8.4)</td>
<td>7.20</td>
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<td>7.20</td>
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<tr>
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<td>6.85</td>
<td>d(8.4)</td>
<td>6.85</td>
<td>d(8.5)</td>
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<td>d(8.5)</td>
<td>6.84</td>
<td>d(8.3)</td>
</tr>
<tr>
<td>5</td>
<td>6.85</td>
<td>d(8.4)</td>
<td>6.85</td>
<td>d(8.5)</td>
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<td>d(8.5)</td>
<td>6.84</td>
<td>d(8.3)</td>
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<tr>
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<td>7.20</td>
<td>d(8.4)</td>
<td>7.20</td>
<td>d(8.5)</td>
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<td>7.20</td>
<td>d(8.3)</td>
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<table>
<thead>
<tr>
<th>4-substituent</th>
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<th>Cl</th>
<th>Br</th>
<th>I</th>
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<td>7.20</td>
<td>d(8.6)</td>
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<td>d(8.7)</td>
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<thead>
<tr>
<th>Location of Methoxy</th>
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<th>peak multiplicity</th>
<th>25C-NB40Me base ppm</th>
<th>peak multiplicity</th>
<th>25B-NB40Me base ppm</th>
<th>peak multiplicity</th>
<th>25I-NB40Me base (600 MHz) ppm</th>
<th>peak multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7.20</td>
<td>d(8.6)</td>
<td>7.20</td>
<td>d(8.6)</td>
<td>7.20</td>
<td>d(8.6)</td>
<td>7.20</td>
<td>d(8.6)</td>
</tr>
<tr>
<td>3</td>
<td>6.84</td>
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<td>6.85</td>
<td>d(8.7)</td>
<td>6.85</td>
<td>d(8.7)</td>
</tr>
<tr>
<td>5</td>
<td>6.84</td>
<td>d(8.6)</td>
<td>6.84</td>
<td>d(8.6)</td>
<td>6.85</td>
<td>d(8.7)</td>
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<td>d(8.7)</td>
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<td>Cl</td>
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<td>d(8.7)</td>
<td>7.23</td>
</tr>
<tr>
<td>Br</td>
<td>7.23</td>
<td>d(8.7)</td>
<td>7.23</td>
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</table>

Abbreviations: a – apparent, b – broad, d – doublet, m – multiplet, q – quartet, s – singlet, t – triplet
### Table VII: Carbon-13 NMR Assignments *ortho*-Methoxyphenyl NBOMe HCl Compounds.

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Microgram Journal 2014, Volume 11; Numbers 1-4
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<td>NO2</td>
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<td>Br</td>
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</tbody>
</table>

Notes: 600 MHz 600 MHz
Technical Note

Identification of a Dipyrone Acetylation Reaction Product Found in Some Black-Tar Heroin Exhibits

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U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
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[email withheld at author's request]

ABSTRACT: N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methyl-acetamide was identified as an impurity in a small number of Mexican black tar heroin exhibits. The presence of this compound suggests that dipyrone was added to the morphine base prior to its acetylation with acetic anhydride. Spectroscopic and chromatographic data are provided.


Introduction

Analysis of four black tar heroin exhibits submitted to this laboratory were determined to contain 38.5 - 48.1% heroin, typical heroin-related alkaloids (acetyl codeine, O6-monoacetylmorphine, etc.), and an unknown compound. The unknown eluted before heroin and contained fragment ions similar to those found for a known dipyrone injection port artifact, but had a mass of 42 Daltons higher, suggesting that it was the acetylated by-product of the dipyrone artifact, or a related impurity. Levamisole and lidocaine acetylation by-products in heroin have been recently reported from direct acetylation of morphine containing these compounds [1]. In order to determine whether a similar reaction was occurring, dipyrone was subjected to an acetylation reaction (Figure 1) and the isolated by-product was analyzed by GC/MS and NMR.

Experimental

Solvents, Chemicals, 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 were products of Sigma-Aldrich Chemical (Milwaukee, WI). Dipyrone was acquired from from the reference collection of this laboratory.

Gas Chromatography/Mass Spectrometry
GC/MS analyses were performed using an Agilent (Santa Clara, 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) and at a temperature of 280°C. The MSD was operated in the electron ionization mode at 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.
Nuclear Magnetic Resonance Spectroscopy
Proton (\(^1\)H), carbon (\(^13\)C), and 2-Dimensional NMR spectra were obtained on an Agilent VNMRS 600 MHz NMR using a 5 mm broad band detection, variable temperature, pulse field gradient probe (Agilent, Santa Clara, CA). Samples were dissolved in deuterochloroform (CDCl\(_3\)) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference compound (Cambridge Isotope Laboratories, Tewksbury, MA). The sample temperature was maintained at 25°C. Standard Agilent pulse sequences were used to acquire \(^1\)H, proton-decoupled \(^13\)C, and gradient versions of HSQC and HMBC spectra. Data processing and structure elucidation were performed using software from Agilent and Applied Chemistry Development (ACD/Labs, Toronto, Canada).

**Synthesis**

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methylacetamide: Dipyrone sodium salt (367 mg, 1.1 mmol) was heated at 110°C with acetic anhydride (3.0 mL, 41 mmol) in a 15 mL capped centrifuge tube for 2 hours. The reaction was cooled and quenched with 50 mL of water, washed with Et\(_2\)O (2 x 60 mL, discarded), extracted with CHCl\(_3\) (2 x 8 mL), and the latter extracts were combined, dried over anhydrous Na\(_2\)SO\(_4\), and evaporated in vacuo to give 242 mg of a light brown powder (85% yield). The material was sufficiently pure for chromatographic and spectroscopic analyses, and was not further purified.

**GC/MS Analytical Artifact Experiment**

Approximately 25 mg of an exhibit containing the suspect compound was dissolved into 1 mL of water and extracted with CHCl\(_3\). The extract was washed with 4 mL of 0.36N H\(_2\)SO\(_4\), dried over Na\(_2\)SO\(_4\), and analyzed via GC/MS.

**Results and Discussion**

GC/MS analysis of four heroin exhibits revealed a previously unknown peak in their total ion chromatograms (Figure 2a, Table 1). This compound (Peak #1) had an apparent molecular ion at m/z 259 (Figure 3a), and appeared to be related to a dipyrone injection port artifact (i.e., from cleavage of the methanesulfonic acid moiety) based on the presence of ions found at m/z 56, 83, 123, and 217 (Figure 3b). Further examination showed an ion at m/z 43 that is indicative of an acetyl loss. The mass spectral data suggested that the compound was an acetylated dipyrone product. Dipyrone was acetylated as outlined in the experimental section and produced a single compound, with an identical mass spectrum to peak #1. Analysis via NMR (Table 2) and GC/MS identified the compound as N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methylacetamide.

In order to demonstrate that the title compound was not formed as an injection port artifact via trans-acetylation with heroin, a heroin exhibit was extracted (see Experimental) to remove all heroin, and the remaining material re-analyzed via GC/MS. The resulting chromatographic profile confirmed that the acetylated dipyrone product was still present, thereby eliminating the possibility of trans-acetylation (Figure 2b).

**Conclusion**

Characterization of the dipyrone acetylation product present in the heroin exhibits, in concert with the performed acetylation experiments, verify that dipyrone was added to the morphine prior to its acetylation.

**Acknowledgment**

The authors are indebted to Patrick A. Hays of this laboratory for his assistance in acquiring the NMR data.

**References**

Figure 1. Structural Formulae of Dipyrone and its Acetylation Product.

Dipyrone

\[ \text{Ac}_2\text{O} \]

N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl-)N-methylacetamide
**Figure 2.** Partial reconstructed total ion chromatograms of heroin exhibits. Upper (a) heroin exhibit containing dypyrone acetylation by-product, and lower (b) heroin exhibit containing dypyrone acetylation by-product after removing heroin via extraction. For peak identification, see Table 1.
**Table 1.** Retention Times (RT) and Relative Retention Times (RRT) of the dipyrone acetylation product and heroin-related compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT (min)</th>
<th>RRT (min)</th>
<th>GC/MS Peak #</th>
</tr>
</thead>
<tbody>
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<td>23.58</td>
<td>0.85</td>
<td>1</td>
</tr>
<tr>
<td>acetylcocodeine</td>
<td>26.24</td>
<td>0.94</td>
<td>2</td>
</tr>
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<td>O6-monoacetylmorphine</td>
<td>26.40</td>
<td>0.95</td>
<td>3</td>
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<tr>
<td>heroin</td>
<td>27.85</td>
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</tr>
<tr>
<td>papaverine</td>
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</tr>
<tr>
<td>noscapine</td>
<td>33.86</td>
<td>1.21</td>
<td>6</td>
</tr>
</tbody>
</table>

*a* Conditions given in the Experimental section.  
*b* N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methylacetamide.

---

**Table 2.** NMR assignments of the dipyrone acetylation product dissolved in CDCl$_3$ at 600 MHz $^1$H, 150 MHz $^{13}$C.

<table>
<thead>
<tr>
<th>Carbon (ppm)</th>
<th>Proton (ppm)</th>
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<tbody>
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<tr>
<td>phenyl ortho</td>
<td>124.3</td>
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<tr>
<td>phenyl meta</td>
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</tr>
<tr>
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<td>$\text{CH}_3$-C(=O)-N-CH$_3$</td>
<td>21.5</td>
</tr>
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</table>

Proton Multiplicity Notes: d = doublet, s = singlet, t = triplet
Figure 3. Electron ionization mass spectrum of (a) N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-N-methylacetamide; and (b) dipyrone injection port artifact.
An Analytical Profile of Aceclofenac

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ABSTRACT: The analysis and characterization of aceclofenac (2-[(2,6-dichlorophenyl)amino]phenyl-acetoxyacetic acid) by GC/MS, ESI-Ion Trap-MS, UHPLC-QTOF-MS, LC-SQD-MS, FTIR/ATR, and NMR is presented and discussed.

KEYWORDS: Aceclofenac, Diclofenac, 1-(2,6-Dichlorophenyl)-2-indolinone, Characterization, Forensic Chemistry.

Introduction

This laboratory recently received an exhibit of oblong, pink colored tablets (Figure 1), half-scored on one face and blank on the opposite face, suspected to be Percocet™ (i.e., an oxycodoneacetaminophen preparation). The tablets had been purchased from an India-based internet pharmacy. Preliminary analysis, however, indicated that the tablets actually

Figure 1

Figure 2 - Aceclofenac
(C_{16}H_{15}Cl_{2}NO_{3}, mw = 354.185)

Figure 3 - Diclofenac
(C_{14}H_{12}ClNO_{2}, mw = 296.148)
contained a mixture of aceclofenac (2-[(2,6-dichlorophenyl)amino]phenylacetoxycetic acid, Figure 2) and acetaminophen. Aceclofenac is the glycolic (hydroxyacetic) acid ester of diclofenac (Figure 3) and is a non-steroidal anti-inflammatory drug (NSAID) with analgesic properties, typically utilized for osteoarthritis and similar afflictions (1-3). It is not available over-the-counter in the U.S., but can be easily purchased through international internet pharmacies. It is not controlled under any U.S. or state statutes at the present time, but is a prescription medication in the U.K., Italy, Spain, and elsewhere. It is widely utilized in India and other south Asian nations as a substitute for diclofenac, due to the latter drug’s unacceptable environmental impacts, most notably its lethal effects on vultures (4). Its abuse potential is considered to be low.

Due to the facile loss of glycolic acid in heated injection ports during standard GC/MS analysis, aceclofenac does not display a molecular ion, but rather a pseudomolecular ion at m/z 277, representing a thermal decomposition product. Diclofenac similarly loses water during standard GC/MS analysis, elutes at a nearly identical retention time, also displays a pseudomolecular ion at m/z 277, and displays a highly similar fragmentation pattern, indicating formation of the same breakdown product in heated injection ports (Figures 4 and 5). Therefore, GC/MS cannot be utilized to unambiguously identify or differentiate either compound.

Figure 4 - GC/MS Spectrum of Aceclofenac (Molecular Ion Not Detectable)
Previous reports have presented the characterization of aceclofenac: By mp, $^1$H- and $^{13}$C-NMR, IR, and elemental analysis (5); by mp, UV $\lambda_{\text{max}}$, FTIR, NCI-MS, and $^1$H-NMR (6); by $^1$H and $^{13}$C NMR (7); by UPLC-QTOF-MS and UPLC-QTOF-MS/MS (8); by mp (by DSC), UV/Vis, FTIR (in KBr), UPLC-QTOF-MS, and UPLC-QTOF-MS/MS (9); by FTIR (in KBr), FT-Raman, and UV/Vis (10); by mp (by DSC), UV/Vis (in 0.1N HCl), and FTIR (11); by mp (by DSC), FTIR (in KBr), and XRD (12); by FTIR and XRD (13); by mp and FTIR (in KBr) (14,15); and by UV/Vis (16-21); however, much of the published data is of lower quality, lacks experimental details, or is published in rather obscure venues that can be challenging for forensic analysts to access. Herein the characterization of aceclofenac by GC/MS, ESI-Ion Trap-MS, UHPLC-QTOF-MS, LC-SQD-MS, FTIR/ATR, and $^1$H, $^{13}$C, and $^{15}$N NMR, is presented and discussed.

**Experimental**

**Chemicals, Reagents, and Materials**

Aceclofenac and diclofenac were obtained from Sigma Aldrich. LC-grade water was obtained from a Millipore filter. LC-grade methanol, acetonitrile (ACN), and pre-mixed solutions containing 0.1% formic acid, were obtained from J.T. Baker.

**Gas Chromatography/Mass Spectrometry (GC/MS)**

Analyses were conducted on an Agilent (Santa Clara, CA) Model 7890A gas chromatograph coupled to an Agilent Model 5975C single quadrupole mass-selective detector (MSD). The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm HP-5 (Agilent). The oven temperature was programmed as follows: Initial temperature 90°C; initial hold 1.35 minutes; program rate...
35°C/minute to 120°C, hold 0.55 minutes; ramp 45°C/minute to a final temperature of 290°C, final hold 8.5 minutes. The injector was operated in the split mode (50:1) at 280°C. The carrier gas was Helium. The MSD was operated in the EI mode at 70 eV, with a scan range of 40-550 amu and a scan rate of 2 scans/second. The MSD source was operated at 230°C.

Electrospray Ionization - Ion Trap - Mass Spectrometry (ESI-Ion Trap-MS)
Tandem mass spectral analyses were conducted on a Thermo Scientific (Waltham, MA) Accela HPLC coupled to a Thermo Scientific LXQ ion trap MS (this instrument has MSn capabilities). The sample was injected directly into the spectrometer (i.e., with no chromatographic separation) using ACN (w/ 0.1% formic acid) / Millipore water (w/ 0.1% formic acid) 1:1, delivered at 0.200 mL/minute (i.e., the HPLC was only utilized to automate the injection). The spectrometer was operated in the positive ESI mode, with a scan range of 50 - 500 amu and a collision-induced-dissociation (CID) of 25 normalized energy units. The nitrogen sheath gas was at 40.0 Arb; the aux/sweep gas at 5.0 Arb; the capillary voltage at 4 kV; and the capillary temperature at 350°C.

Ultra-High Performance Liquid Chromatography - Quadrupole-Time of Flight Mass Spectrometry (UHPLC-QTOF-MS)
High-resolution mass spectral analyses were conducted on an Agilent 1290 Infinity UHPLC interfaced with an Agilent 6520 QTOF-MS. The UHPLC was fitted with a Zorbax Extend-C18 column, 2.1 mm x 50 mm, with a 1.8 μm particle size, operating at 40°C. A gradient mobile phase was utilized: Initial 30% ACN (w/ 0.1% formic acid) / 70% Millipore water to final 70% ACN (w/ 0.1% formic acid) / 30% Millipore water over 8 minutes, delivered at 0.5 mL/minute. The spectrometer was operated in the positive ESI mode with the capillary voltage at 4 kV. The nitrogen drying gas temperature was 350°C with a flow rate of 13 L/minute. The nebulizer pressure was set to 50 psi. The fragmentor voltage was set at 150 V; the skimmer voltage at 60 V; and the octopole 1 Rf Vpp at 750 V. Internal mass calibration was achieved using two reference mass ions at m/z 121.0509 and 922.0098. The mass accuracy was calculated to be 0.31 ppm.

Liquid Chromatography - Single Quadrupole - Mass Spectrometry (LC-SQD-MS)
Low-resolution mass spectral analyses were conducted on an Agilent 1200 series binary pump LC interfaced with an Agilent 6130 single quadrupole MSD (SQD-MS). The LC was fitted with an Agilent Zorbax Eclipse XDB C18 column, 4.6 mm x 50 mm, with a 1.8 μm particle size, operating at 40°C. The isocratic mobile phase was 65% ACN (with 0.1% formic acid) / 35% Millipore water, delivered at 0.75 mL/min. Nitrogen was used as both the nebulizer and drying gas. The MSD was operated in the positive ESI mode with the capillary voltage at 4 kV, drying gas flow rate of 13 L/min, nebulizer pressure of 50 psi, and a drying gas temperature of 350°C. The fragmentor voltages were set at 100 V for the first analysis and 150 V for the second analysis.

Fourier Transform Infrared / Attenuated Total Reflectance Spectroscopy (FTIR/ATR)
A Perkin Elmer (now Thermo Scientific, Waltham, MA) Frontier FTIR equipped with an ATR accessory was utilized. The spectrum was acquired using 8 scans at 4 cm⁻¹ resolution, from 4000 to 650 cm⁻¹.

Nuclear Magnetic Resonance Spectroscopy (NMR)
Aceclofenac was prepared at 15 mg/mL in dimethyl sulfoxide-d6 containing 0.05% v/v TMS.
1H, 13C, HSQC, HMBC, COSY and 1D TOCSY spectra were acquired on an Agilent VNMRS 600 MHz NMR using a broadband probe (1H parameters: 8 scans, 45° pulse width, 45 s delay between pulses, and 5 s acquisition time). 15N-HSQC and 15N-HMBC spectra were acquired on an Agilent VNMRS-DD2 400 MHz NMR using an OneNMR probe. All spectra were collected with a sample temperature of 25°C. Spectra were processed using ACD/Structure Elucidator, version 14.01, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada.

Results and Discussion

Somewhat unexpectedly, there do not appear to be any previous literature reports concerning the GC/FID or GC/MS analyses of aceclofenac - doubtless due to its facile degradation in heated injection ports. As noted in the Introduction, the standard GC/MS spectra of aceclofenac and diclofenac are markedly similar, with nearly identical retention times (approximately 6.07 minutes on the described system), identical pseudomolecular ions, and highly similar fragmentation patterns (differing only moderately in relative abundances), confirming formation of a common breakdown product.

From a mechanistic viewpoint, the loss of glycolic acid from aceclofenac and water from diclofenac would generate either a ketene (i.e., 2-(2,6-dichlorophenyl)amino-phenyl ketene, Figure 6) from abstraction of the alpha (benzylic) proton during de-esterification / dehydration, or an indolinone (i.e., 1-(2,6-dichlorophenyl)-2-indolinone, Figure 7) from intramolecular displacement of the ester or hydroxyl groups by the amine, resulting in lactamization). In fact, the mass spectrum of 1-(2,6-dichlorophenyl)-2-indolinone (22-24) is quite similar to the mass spectra of aceclofenac and diclofenac, confirming the latter mechanism. Interestingly, the mass spectra of the methyl and ethyl esters of diclofenac display their respective molecular ions at significant relative abundances (indicating greater thermal stability), but are otherwise also quite similar to aceclofenac and diclofenac (24,25).

![Figure 6](image-url) - 2-(2,6-Dichlorophenyl)amino-phenyl ketene [Note: Probably not a stable compound]

![Figure 7](image-url) - 1-(2,6-Dichlorophenyl)-2-indolinone

With GC/MS unable to unambiguously differentiate aceclofenac, diclofenac, 1-(2,6-dichlorophenyl)-2-indolinone, and potentially other diclofenac esters, additional mass spectral analyses of aceclofenac were conducted. ESI-Ion Trap-MS confirmed the molecular ion in single-stage/full-MS mode at m/z 354.00 (Figure 8a). The second-stage (MS²)
fragmentation indicated a breakdown from the molecular ion into two fragments at m/z 277.93 and m/z 250.00, both resulting from the cleavages of the acetoxyacetic acid substituent (Figure 8b). The third-stage (MS3) fragmentation indicated the loss of one chloro substituent from the fragment at m/z 250.00, giving a fragment at m/z 215.04 (Figure 8c).

Figure 8 - ESI-Ion Trap-MS of Aceclofenac: (a) Full Scan Mass Spectrum; (b) Fragmentation of the Pseudomolecular Ion at m/z 354 (MS²); (c) Fragmentation of the Product Ion at m/z 277.9 (MS³).

High-Resolution QTOF-MS confirmed the molecular ion at m/z 354.0293, corresponding to C₁₆H₁₄Cl₂NO₄ (i.e., the protonated pseudomolecular [M+H]⁺ ion; Figure 8). The sodiated adduct (C₁₆H₁₄Cl₂NO₄Na⁺) was observed at m/z 376.0110 (Figure 9).

Finally, low-resolution SQD-MS at 100 V (a moderately low collision-induced energy) again confirmed the molecular ion at m/z 354.1 (Figure 10a). At 150 V, three product ions were observed at m/z 278, 250, and 215, complementing the results obtained in the tandem MS analyses (Figure 10b).

Each of these three positive ESI mass spectral analyses easily differentiate aceclofenac versus diclofenac or any common diclofenac ester.
Figure 9 - UHPLC-QTOF-MS of Aceclofenac: (a) TIC (UHPLC Retention Data Reported in Table II); (b) High Resolution Mass Spectrum Including the Calculated Empirical Formula for the Pseudomolecular Ion and its Sodiated Adduct.

Figure 10a - SQD Mass Spectrum of Aceclofenac at 100 V (LC Retention Data Reported in Table 1).
Figure 10b - SQD Mass Spectrum of Aceclofenac at 150 V (LC Retention Data Reported in Table 1).

The FTIR/ATR spectrum (Figure 11) is unremarkable, with a broad secondary amine and carboxylic acid (hydroxyl) band from 3200 and 3400 cm\(^{-1}\), two ketone bands at 1716 and 1771 cm\(^{-1}\), and multiple, moderate to prominent phenyl ring bands from 650 to 1600 cm\(^{-1}\). The spectrum is more than adequate for identification of high purity samples.

The ¹H-NMR spectrum is presented in Figure 12. Chemical shift, peak shape, and correlation information, in conjunction with ACD/Structure Elucidator, were used for structure elucidation. The ¹H, ¹³C, and ¹⁵N assignments are reported in Table 2 (the ¹³C, HSQC, HMBC, COSY, and 1D TOCSY spectra are not shown). Proton and carbon chemical shifts are referenced to TMS.
while the nitrogen value is unreferenced. The most notable difference between the \(^1\)H-NMR spectra of aceclofenac and diclofenac is aceclofenac’s additional singlet at 4.64 ppm, from the glycolic ester methylene group. The results easily differentiate aceclofenac versus diclofenac or any common diclofenac ester.

**Acknowledgments**

I would like to thank everyone who has taken the time to review this analytical profile, in particular Senior Forensic Chemist Yurii Uvaydov and Supervisory Chemist Ann Marie O’Neill (both of this laboratory) for their constructive comments and guidance. I would also like to thank Senior Forensic Chemists Charlotte Corbett and Roxanne Franckowski of the DEA Special Testing and Research Laboratory (Dulles, VA) for their assistance with the NMR analyses and structural elucidation, and additionally to Charlotte Corbett for providing the structures for Figures 2, 3, 6, and 7, and within Table 1.

![Figure 12](attachment:image.png)

**Figure 12** - (a) 600 MHz \(^1\)H-NMR Spectrum of Aceclofenac;  
(b) Expansion between 6.1 - 7.7 ppm.
<table>
<thead>
<tr>
<th>Compound</th>
<th>UHPLC</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceclofenac</td>
<td>3.94 minutes</td>
<td>1.52 minutes</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>3.73 minutes</td>
<td>1.56 minutes</td>
</tr>
</tbody>
</table>

**Table 1** - Retention Data for Aceclofenac and Diclofenac  
(Details Provided in the Experimental Section).

**Table 2** - Structural Assignments obtained via NMR (Side Figure Shows the Numbering Protocol).

- **Position**
- **$^{13}$C or $^{15}$N (ppm)**
- **$^1$H (ppm)**
- **$^1$H multiplicity and $J_{HH}$ (Hz)**

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C or $^{15}$N (ppm)</th>
<th>$^1$H (ppm)</th>
<th>$^1$H multiplicity and $J_{HH}$ (Hz)</th>
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<tbody>
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<td>2</td>
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<td>-</td>
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<td>3</td>
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<td>d 7.8</td>
</tr>
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<td>127.7</td>
<td>7.07</td>
<td>td 7.6, 1.4</td>
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<tr>
<td>2'</td>
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<td>-</td>
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<tr>
<td>3'</td>
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<td>36.6</td>
<td>3.90</td>
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<td>-CH$_2$COOCH$_2$COOH</td>
<td>170.9</td>
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<td>-CH$_2$COOCH$_2$COOH</td>
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<td>4.64</td>
<td>s</td>
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<td>-CH$_2$COOCH$_2$COOH</td>
<td>168.9</td>
<td>13.11</td>
<td>brs</td>
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</tbody>
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[a= apparent, d = doublet, s = singlet, t = triplet, br = broad]
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-END-
Analysis of Marijuana by Liquid Chromatographic Techniques
A Literature Survey, 1990 - 2015

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ABSTRACT: A survey of the liquid chromatographic analysis of marijuana, hemp, and related preparations (e.g., hashish, hash oil, “marijuana concentrates,” “cannabis smoke condensates,” etc.) for the major phytocannabinoids, as reported during the time frame 1990 through 2015, is presented. 133 references are provided.

KEYWORDS: Marijuana, Cannabis, Hemp, Tetrahydrocannabinol, Phytocannabinoids, Liquid Chromatography, Forensic Chemistry.

Introduction

The qualitative and quantitative analysis of marijuana (Cannabis sativa L.) continues to be a significant task at most forensic laboratories. The most common techniques utilized for these analyses are GC/FID and GC/MS; however, while rapid and facile, these methodologies cannot handle thermally labile or non-volatile phytocannabinoids (such as Δ9-tetrahydrocannabinolic acid - A, THCA) without prior derivatization procedures - which tend to be time consuming, more expensive, and technique sensitive. An alternate approach for comprehensive analysis is to complement the GC-based methodology with a liquid chromatographic (LC-based) technique.

LC-based methodologies that have been utilized for this purpose range from basic (e.g., TLC with UV or spray reagent detection) to highly sophisticated (e.g., UHPLC-MS/MS or UPC2). To the author’s knowledge, although this topic is lightly covered in several general reviews of the analysis of marijuana (e.g., 1,2,3), the last comprehensive survey of these methodologies appeared 30 years ago (4).

Search Details

Searches were conducted by the Chemical Abstracts Service’s Scientific & Technical Information Network (STN)*, Google®, PubMed, by reading select forensic journals, and/or by reviewing the reference citation lists of pertinent articles. The search terms for the analytical techniques included both their fully spelled-out names and their commonly utilized acronyms, as follows:

1 Through November, 2015. Due to (normal) publication and/or abstracting delays, a small number of pre-2015 and 2015 dated references will not appear in this survey.
Automated Multiple Development (AMD);
Capillary Electrophoresis (CE);
Capillary Electrochromatography (CEC);
Electrokinetic Chromatography (EKC);
Hydrophilic Interaction Liquid Chromatography (HILIC);
High Performance Liquid Chromatography (HPLC);
High Performance Thin Layer Chromatography (HPTLC);
Incremental Multiple Development (IMD);
Liquid Chromatography (LC);
Micellar Electrokinetic Capillary Chromatography (MECC);
Optimal Performance Laminar Chromatography (OPLC, also known as overpressed layer chromatography or forced-flow TLC);
Planar Chromatography (No Acronym);
Reversed Phase High Performance Liquid Chromatography (RP-HPLC);
Reversed Phase Liquid Chromatography (RP-LC);
Supercritical Fluid Chromatography (SFC);
Thin Layer Chromatography (TLC);
Ultra High Performance Liquid Chromatography (UHPLC / UPLC);
Ultra-Performance Convergence Chromatography (UPC2 / UPCC); and
Two Dimensional Liquid Chromatography (2D-LC).

The search terms for marijuana included: Cannabinoids, cannabis, hash oil, hashish, hemp, hempseed, marihuana, marijuana, phytocannabinoids, tetrahydrocannabinol, and THC; street terms were not utilized. Analyses of rare / trace-level phytocannabinoids, or of phytocannabinoids and their metabolites in post-ingestion biological matrices, are not included in this survey. With the exception of a few reports that were re-published in *LCGC* or *American Laboratory*, “application notes,” printed “infomercials,” and similar publications also are not included. While there are no reasons to doubt the validity of the presented analyses in these latter studies, virtually all such studies are either from scientific instrumentation companies touting the capabilities of one of their instruments or are from commercial (i.e., non-government) laboratories offering for-fee testing services for “medical” or “recreational” marijuana, marijuana concentrates, or “marijuana edibles,” and are not appropriate for this survey. Finally, references concerning the heterogeneity (inhomogeneity) of cannabis and the sampling protocols to address it - pertinent especially for the accurate quantitation of “herbal” cannabis and similar preparations - are covered as a separate category.

**Results**

References are organized first by general category (e.g., Planar Techniques), then by year (in reverse chronological order), and within year by author (in alphabetical order). Except for methodologies that are deemed (by this author) to be “advanced,” techniques that are interfaced with specialized detection methods are reported under the parent technique (e.g., HPLC-MS is reported under HPLC, but HPLC-MS/MS is reported under Advanced). References that report the use of more than one LC technique are reported under both/all categories. It should be noted that in many cases the LC technique is not the focus of the referenced article, but rather was used to complement or confirm the results acquired via a different methodology. Similarly, in many other cases the analysis of cannabis is only a minor aspect of a broader technique study.

**Planar Techniques**

2015 - by TLC/MS, for determination of cannabinoids and pesticides in cannabis (5); 2014 - by TLC, as a complement to a detailed study of the Duquenois-Levine color test (6); 2013 - by TLC (7); 2010 - by TLC, to analyze the components of ayurvedic (a folk remedy from the
Indian subcontinent that includes cannabis) (8); 2009 - by HPTLC, for the quantitation of THC and the qualitative analysis of other main neutral cannabinoids in cannabis (9); by TLC, as a comparison with and complement to a detailed study of Salvia divinorum and other salvia species (10); by TLC (from the UNODC Monograph on cannabis) (11); by TLC, for determination of cannabinoids in cannabis and hemp (12); 2007 - by TLC, for determination of cannabinoids in cannabis (13); by TLC (an effort to determine optimal mobile phases for select drugs, including cannabis) (14); 2006 - by TLC, as part of a more comprehensive analysis of cannabis (15); by TLC, for determination of cannabinoids in cannabis and hemp (16); 2005 - by TLC, as part of a more comprehensive analysis of cannabis (17); by TLC (from the SWGDRUG Monograph on cannabis) (18); by HPTLC, for determination of cannabinoids in both commercially available and cannabis oils stored long term (19); 2004 - by AMD, OPLC, and TLC (a comparative study of the analysis of cannabis by different planar chromatography techniques) (20); by TLC, for determination of cannabinoids in cannabis (21); 2003 - by OPLC (a technique study of a specialized OPLC instrument capable of simultaneously running 4 to 8 samples, including cannabis) (22); 2002 - by AMD, OPLC and TLC (a preliminary version of the work detailed in Reference #20, as conducted on different plants, including cannabis) (23); by TLC, for determination of cannabinoids in “monoecious” hemp (24); by OPLC, for determination of neutral cannabinoids in hemp (25); 1998 - by TLC, for analyses of cannabis resin and cannabis in unsmoked, handrolled cigarettes (26); by TLC, for the qualitative and quantitative analyses of cannabinoids in cannabis seeds (27); 1997 - by IMD (a technique study, including cannabis) (28); 1995 - by IMD (a feasibility study, including cannabis) (29); 1994 - by TLC, as part of a comprehensive chromatographic analysis of cannabis (30); 1993 - by HPTLC (a technique study, including cannabis) (31); 1992 - by TLC, to determine the cannabinoid content of UK-grown plants (up to the 6th generation) from Moroccan, Sri Lankan, and Zambian seedstock (32); by HPTLC (a feasibility study, including cannabis) (33); by HPTLC, as part of the determination of cannabinoids in cannabis oil (34); 1990 - by TLC, for the analysis of derivatized cannabinoids (35); and by TLC, for the qualitative and quantitative analyses of cannabinoids in cannabis seeds (36).

Normal Phase LC / HPLC Techniques
2015 - by LC/MS, (37); by HPLC/DAD, for the analysis of cannabinoids and terpenes in cannabis (38); by HPLC, for the determination of 11 cannabinoids in biomass and in extracts of different varieties of cannabis (39); by HPLC and LC/MS (an overview of the marijuana testing rules in Colorado, the methods used for testing, and test results to date for “recreational marijuana”) (40); by HPLC (an overview of the marijuana testing rules in Colorado and the methods used for testing) (41); by HPLC/DAD (presenting two new, validated HPLC/DAD methods for identification and extract profiling based on the main patterns of cannabinoids and other phenolics in cannabis) (42); by HPLC, for the determination of the relative percentage of THCA and THC in cannabis, and the impact of different storage temperatures on stability (43); by LC/MS, as a complement to a DNA genotyping study (44); by HPLC, for determination of cannabinoids in “marijuana edibles” (45); by HPLC, for determination of cannabinoids in “marijuana edibles” (a feasibility study with spiked samples) (46); 2014 - by HPLC, for identification and quantitation of cannabinoids in cannabis (47); by HPLC/DAD, for quantitation of THC, THCA, CBN, and CBD in seized cannabis (48); by HPLC, to determine THC and THCA (49); by HPLC/DAD, to determine THC in hempseed oil (50); 2013 - by HPLC, for...
determination of THC following cloud point extraction of cannabis resin (51); by LC/MS, for analysis of cannabinoids in laser-microdissected trichomes of “medicinal” cannabis (52); by HPLC, for determination of potency and cannabinoid profiles (53); 2012 - by HPLC, as a complement to the voltammetric determination of THC (54); by HPLC/DAD, for the determination of THC and other major cannabinoids in cannabis cuttings and seedlings during plant growth (55); by HPLC with chemiluminescence detection, for determination of CBD in industrial-grade hemp (stated to be applicable for determination of THC in cannabis) (56); by Nano-LC, for determination of synthetic cannabinoids and THC in herbal blends (focus is on synthetic cannabinoids) (57); by LC/MS (a review of the use of LC/MS for the detection and quantitation of cannabinoids) (58); by HPLC, for the determination of cannabinoids in cannabis oil during long-term storage (59); by HPLC, for the determination of cannabinoids in cannabis resin during long-term storage (60); by HPLC, for the identification and characterization of “special types of herbal cannabis” (61); by HPLC (a study to determine the optimal solvent and conditions for extraction of THC, CBD, and CBN from cannabis resin) (62); by HPLC, for determination of THC in cannabis (63); 2011 - by HPLC, for the determination of cannabinoids in cannabis during different storage conditions (64); by HPLC/DAD, for determination of THC and (separately) THCA from cannabis after isolation via two different flash chromatography systems (65); 2010 - by HPLC/DAD, for monitoring the long term stability of cannabis resin and cannabis extracts (66); 2009 - by HPLC/DAD, for the qualitative and quantitative determination of the major cannabinoids in cannabis (67); by HPLC, as a complement to an HPTLC study (68); by LC/MS, as a complement to a DART-TOF-MS-based screening for THC in cannabis (69); by High-Temperature LC (a technique study, including analysis of THC in cannabis) (70); by HPLC, to determine cannabinoids in vaporized cannabis (71); by HPLC (from the UNODC Monograph on cannabis) (72); by HPLC, to determine the effects of tobacco on the levels of cannabinoids in vaporized cannabis (73); 2008 - by HPLC, to determine the effects of different preparation methods on the levels of cannabinoids in vaporized cannabis (74); 2007 - by HPLC, for the determination of the major cannabinoids in “medicinal grade” cannabis (75); by HPLC, for the determination of the major cannabinoids in cannabis tea (76); by HPLC, for the determination of cannabinoids in hemp (77); 2006 - by HPLC, for the determination of the major cannabinoids in “medicinal grade” cannabis (78); by HPLC, as a complement to a DNA study of drug-type versus fiber-type cannabis (79); 2005 - by HPLC/DAD and HPLC with fluorescence detection (as part of a detailed chromatographic and spectroscopic analysis of the cannabinoids in cannabis) (80); by HPLC at elevated pressure (a technique study, including analysis of THC in cannabis) (81); by HPLC/DAD, for analysis of the cannabinoids in cannabis (82); 2004 - by HPLC, for analysis of the cannabinoids in cannabis (83); by HPLC/ESI-MS (a technique study, including analysis of the cannabinoids in cannabis) (84); 2003 - by HPLC, for determination of CBD in hempseed oil (85); 2002 - by HPLC, for the determination of cannabinoids in cannabis (86); by HPLC, as a complement to a DNA (ISSR) study (87); by HPLC (an optimization study of the HPLC separation conditions for cannabinoids in cannabis, including Δ9- versus Δ8-THC) (88); 2001 - by HPLC, as a complement to a study of the supercritical fluid extraction of cannabis (89); 2000 - by Capillary LC with electrochemical detection, for determination of the cannabinoids in cannabis (90); by HPLC, for determination of the cannabinoids in hashish (91); by HPLC, for determination of the cannabinoids in cannabis (92); by HPLC with UV or fluorescence detection, for determination of THC and THCA in hemp-
containing foods (93); **1998** - by HPLC, for analysis of cannabinoids in hemp (94); **1997** - by HPLC, for the qualitative and quantitative analyses of cannabinoids in cannabis (95); **1996** - by LC/MS, for determination of the cannabinoids in hashish (96); **1995** - by HPLC, as a complement to a DNA (RAPD) study of different samples of cannabis (97); by HPLC/DAD, for the qualitative and quantitative determination of neutral and acidic cannabinoids in cannabis (for profiling purposes) (98); **1994** - by HPLC, as part of a comprehensive chromatographic analysis of cannabis (99); by HPLC, as a complement to a study of the supercritical fluid extraction of cannabis and hashish (100); **1992** - by HPLC, to determine the cannabinoid content of UK-grown plants (up to the 6th generation) from Moroccan, Sri Lankan, and Zambian seedstock (101); **1991** - by HPLC, to determine the uniformity of hashish samples (102); by HPLC (as a complement to an MECC technique study, including analysis of cannabis) (103); and **1990** - by HPLC with UV or fluorescence detection, following derivatization (a technique study, including analysis of cannabis) (104).

**Reversed Phase LC and HPLC Techniques**

**2015** - by RP-HPLC (on three different columns), for determination of the cannabinoids in cannabis and a marijuana concentrate (105); **1996** - by RP-HPLC, for analysis of “drugs of abuse,” including cannabis (106); **1994** - by RP-HPLC (a technique study, including analysis of THC in cannabis) (107); **1993** - by RP-HPLC, for determination of THC in cannabis (108); and **1990** - by RP-HPLC, to characterize the lipophilicity of natural and synthetic analogs of THC (109).

**Electrokinetic Techniques**

**2004** - by CE/ESI-MS (a technique study, also covering HPLC/ESI-MS, including analysis of the cannabinoids in cannabis) (110); **1998** - by CEC (a technique study, including analysis of THC in cannabis) (111); by CEC/DAD, for the determination of the cannabinoids in cannabis (112); and **1991** - by MECC, for the determination of the cannabinoids in cannabis (113).

**Advanced Techniques**

**2015** - by HILIC, as part of an RP-HPLC study for the determination of the cannabinoids in cannabis and a marijuana concentrate (114); by 2D-LC with chemiluminescence detection, for screening of cannabinoids in industrial-grade hemp (115); by EI-LC/MS with supersonic molecular beams (an introductory technique study, including cannabis) (116); by UPC2, for the determination of the cannabinoids in cannabis (117); **2014** - by HPLC/MS and HPLC-MS/MS, for identification and quantitation of cannabinoids in cannabis (118); **2013** - by UHPLC/MS (a technique review, including analysis of a mixture of drug standards including THC, CBD, and CBN, plus analyses of two different baked goods that contained THC) (119); by LC-MS/MS, for screening of “botanicals” (including cannabis) in food supplements (120); by UHPLC-MS/MS, for determination of THC in hemp food (121); by UHPLC-Q-ToF-MS/MS, for determination of cannabinoids in hemp seed pills (a traditional Chinese medication) (122); **2012** - by LC/ESI-MS/MS, to investigate the isomerization of CBD and THC during positive ESI analyses (123); **2011** - by HILIC (a technique study, including analysis of THC in cannabis) (124); by LC-MS/MS, for determination of cannabinoids in industrial hemp (125); by UPLC-MS/MS, for determination of THC in edible vegetable oil (126); **2010** - by UHPLC/MS (a technique study, including analysis of THC in cannabis) (127); by UPLC-MS/MS, for determination of cannabinoids in edible oil (128); **2009** - by LC/MS and LC-MS/MS, to evaluate microwave-assisted derivatization of THC (129); by UHPLC/MS, for...
determination of cannabinoids in baked goods (130); 2004 - by LC-Ion Trap-MS/MS, for analysis of the cannabinoids in cannabis (131); 2000 - by SFC, for the determination of the cannabinoids in cannabis (132); by SFC (a technique study, including analysis of THC in cannabis) (133); 1998 - by LC with thermospray-MS detection and by LC-MS/MS, for determination of the cannabinoids in cannabis (134); 1997 - by SFC with APCI-MS detection, for analysis of THC in cannabis (135); and 1993 - by HPLC with post-elution photoradiation followed by DAD or thermospray MS detection (a technique study, including analysis of THC in cannabis) (136).

Heterogeneity (Inhomogeneity) of Cannabis and Sampling Protocols to Reduce its Impact

Although the “within sample” heterogeneity of “herbal” cannabis and the resulting, inherent variability on their quantitative analyses are widely recognized (e.g., 137,138), to date there have been few studies that specifically addressed these issues (139,140). Reflecting the importance of this topic, however, a comprehensive, three-part study was recently published (141,142,143). The heterogeneity of all plant materials utilized for medicinal, nutritional, or similar purposes has also been addressed in more general terms by the U.S. Food and Drug Administration (144) and the World Health Organization (145,146).

Acknowledgment

The assistance of DEA Librarian Kristin Carr in acquiring numerous references is gratefully acknowledged.

References

[Note: In order to avoid the occasional overly wide spacings created by the use of fully justified columns, the references are provided in full page, left-justified format.]


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101. See Pitts, Neal, et al., Reference # 32.


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113. See Weinberger and Lurie, Reference #103.


117. See Thomas and ElSohly, Reference #1, Figure 4.2, page 69. This figure, which compares the separations of phytocannabinoids on UPLC versus UPC2, is not attributed in the figure caption.
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* * * * *
Enantiomeric Determination of Methamphetamine, Amphetamine, Ephedrine, and Pseudoephedrine using Chiral Supercritical Fluid Chromatography with Mass Spectrometric Detection

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ABSTRACT: Supercritical fluid chromatography-single quadrupole mass spectrometry (SFC-SQD) was utilized for the enantiomeric determination of methamphetamine, amphetamine, ephedrine, and pseudoephedrine. The effects of varying stationary phases, alcohol modifiers, and amine additives were assessed. The optimal separation for methamphetamine was achieved by a Trefoil AMY1 (150 x 2.1 mm, 2.5 μm) column using a supercritical CO₂ mobile phase containing EtOH as the co-solvent and 1% cyclohexylamine as the amine additive. The method was successfully applied for determination of the chiral composition of illicit methamphetamine, even for samples with skewed ratios of enantiomers as low as 0.1% d- or 3% l-.

KEYWORDS: Methamphetamine, Amphetamine, Ephedrine, Pseudoephedrine, Chiral Separation, Supercritical Fluid Chromatography, Mass Spectrometry, Forensic Chemistry.

Introduction

The d- and l- enantiomers of methamphetamine have dramatically different pharmacological activity levels (1,2). Despite these differences, both enantiomers are Schedule II in the U.S., due to their widespread abuse¹. In addition, samples containing 80% or more d-methamphetamine HCl are subject to higher sentences [3]. Therefore, the enantiomeric composition of methamphetamine is important from legal and medical perspectives. In addition, the chirality of methamphetamine can help indicate the possible synthetic route and precursor used in its synthesis. d-Methampheta-

¹ Pharmaceuticals containing low concentrations of l-methamphetamine, which are used as bronchodilators, are not controlled.
dramatically, from the d- enantiomer and the d,l-
racemate to samples with skewed ratios of the d-
and l- isomers. This shift confirms the current use
of P2P and resolution efforts by illicit processors.

Chromatographic enantioseparations remain the
most popular techniques for chiral analyses, and
can be accomplished using HPLC, GC, CE, and
supercritical fluid chromatography (SFC). Direct
HPLC using a chiral stationary phase (CSP) is the
most common technique for the separation of
enantiomers [6-17]. However, this method
consumes large quantities of HPLC-grade organic
solvents (which are expensive), and can display
significant peak broadening as well as longer
equilibration and analysis times. In addition,
multiple pilot columns with different CSPs are
normally needed to determine the optimal column
and conditions, another time consuming expense.

Indirect HPLC with chiral mobile phase additives
has also been utilized for chiral analyses of
phenethylamines; however, the results displayed
low efficiency in enantioselectivity [18,19].
Similarly, CE with chiral mobile phase additives
is effective, efficient, and rapid [20-24]; however,
this method suffers from low reproducibility in
migration times, and relative migration times
versus internal standards are usually required.

Another common approach involves derivatization
with a chiral reagent (to form diasteromers) with
subsequent analyses with either HPLC or (more
commonly) GC [25-34]. Though such analyses
have a better enantioselectivity, efficiency, and
reproducibility, the derivatization process is time
consuming, more expensive, and technique
sensitive, and the optical impurity in the chiral
reagents often obscures or masks the detection of
the low level isomer in a heavily skewed-ratio
sample (chiral derivatizing reagents are typically
about 98% enantiomerically pure).

SFC employs mobile phases comprised of super-
critical CO$_2$ mixed with organic modifiers (e.g.,
alcohols) and additives (e.g., acids or bases); this
results in higher diffusivity and lower viscosity
versus an HPLC mobile phase. As a result, SFC
offers high throughput capacity, much lower
solvent consumption, and a "greener," less
expensive technology. Recently, with improved
instrumentation and wider availability of chiral
columns with small particle sizes, SFC has
become a viable alternative chromatographic
technique for enantiomeric analyses [35-44].

The use of SFC on chiral amylose (AMY) or
cellulose (CEL) columns for enantomeric
determination of methamphetamine, amphet-
amine, ephedrine, and pseudoephedrine is
presented herein. Chiral analyses of seized
methamphetamine samples with skewed
enantiomer ratios are also presented. The effects
of varying the stationary phases, co-solvents, and
additives are discussed.

**Experimental**

**Chemicals**

All phenethylamine standards were obtained from
this laboratory’s reference materials collection.
Ammonium hydroxide solution, ammonium
acetate, trifluoroacetic acid (TFA), and
cyclohexylamine (CHA) were analytical grade
and obtained from Sigma (St. Louis, MO). HPLC
grade solvents, including isopropyl alcohol (IPA),
ethanol (EtOH), methanol (MeOH), and
acetonitrile were purchased from Sigma-Aldrich
(St. Louis, MO). Carbon dioxide (CO$_2$, beverage
grade) was from Air Gas (Chantilly, VA).

**Columns**

Trefoil AMY1 (tris-(3,5-dimethylphenyl-
carbamate) 2.1 x 50 mm, 2.5 μm), Trefoil CEL1
(tris-(3,5-dimethylphenylcarbamate)-cellulose, 2.1
x 50 mm, 2.5 μm), Trefoil CEL2 (tris-(3-chloro-4-
methylphenylcarbamate)-cellulose, 2.1 x 50 mm, 2.5 μm), and Trefoil AMY1 (2.1 x 150 mm, 2.5 μm) columns were provided by the Waters Corporation (Milford, MA).

**Instrumentation**

All experiments were performed on a Waters Acquity Ultra Performance Convergence Chromatography (UPC²) System. The system was equipped with a binary solvent manager, an autosampler with a partial loop volume injection system, a 2-position column oven compatible with 150 mm length columns, and was interfaced with a PDA detector and an SQD with an ESI source. A 515 pump system (an isocratic solvent manager) was used as a make-up pump, and was positioned before the mass detector. The main flow stream was split by a flow-splitter assembly before the SQD. Empower 3® software was used for system control and data acquisition.

Screening experiments were performed at 40°C with a flow rate of 1.2 mL/min. The system back pressure was set at ABPR = 2000 psi, except as noted. The gradient elution was varied to compensate for different modifiers, as specified for each experiment. The injection volume was 2 μL.

The compounds were detected in positive ESI mode with the following parameters: The ion source temperature was 150°C; nitrogen was used as the desolvation gas at a flow rate of 600 L/hr and at 400°C; the capillary voltage was 3 kV. The makeup flow was 0.6 mL/min with MeOH. In this study, Single Ion Recording (SIR) was utilized for better sensitivity and selectivity (mass spectral parameters are listed in Table 1).

**Results and Discussion**

**Screening of Chiral Columns with Different Co-Solvents and Additives**

Initial screening of stationary phases was attempted using methamphetamine on 50 mm Trefoil "pilot" columns (CEL1, CEL2, and AMY1). Based on Waters' strategy for Trefoil chiral method development, the following four step process was implemented: 1. AMY1-EtOH/IPA/ACH (33/33/33)-20 mM AmAc (B1); 2. CEL1-MeOH/IPA (50/50)-0.2% TFA (B2); 3. CEL2-EtOH/ACN (50/50)-0.2% TFA (B3); 4. AMY1-EtOH/IPA (50/50)-0.2% TFA (B4). The instrument was equilibrated at 3% B for 0.5 min, then linear gradient to 60% B over 1.5 min, then hold at 60% B for 5 min.

There was no noticeable enantiomeric separation of any of the phenethylamines with any of the screening columns and conditions. Distorted peaks were observed, possibly due to the acidic character of the large percentage of CO₂ in the mobile phase. According to a study by Ye et al., addition of an amine raises the pH of the mobile

**Table 1. Mass Spectral Parameters.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>[M+H]+</th>
<th>Cone Voltage (kV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>135.8</td>
<td>20</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>150.2</td>
<td>20</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>166.2</td>
<td>15</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>166.2</td>
<td>15</td>
</tr>
</tbody>
</table>

IPA. For samples, a 1 mg/mL stock solution in MeOH was prepared. A working sample solution at 200 μg/mL was prepared by diluting its stock solution with IPA.
phase, thereby deprotonating the analytes and improving their peak shapes and resolution [36]. Thus, three organic co-solvents (MeOH, EtOH, or IPA) doped with NH\textsubscript{2}OH or CHA were investigated. Only the AMY1 column using an alcohol and CHA demonstrated any chiral separations (and those were slight). Multiple experiments were conducted (not presented herein); based on the results, a longer AMY1 column (150 x 3.5 mm, 2.1 µm) with various alcohols as a co-solvent and CHA as an additive was selected for method optimization.

Screening of Co-Solvents with an AMY1 Chiral Column and using CHA as an Additive
As amphetamine, ephedrine, and pseudoephedrine can also be present in seized methamphetamine samples, it is useful to resolve all four compounds as well as their enantiomers in a single run, if possible. A standard mixture containing 100 µg/mL of all four phenethylamines was prepared in IPA. The effects of MeOH, EtOH, and IPA were separately assessed, each using CHA as an additive. The results are illustrated in Figures 1, 2, and 3. All four phenethylamines were separated using any of the alcohols; however, none of the alcohols resolved all four pairs of enantiomers in a single run. IPA and EtOH are both adequate co-solvents for enantiomeric separation of amphetamine; indeed, d- and l-amphetamine were well resolved using IPA, with resolution greater than 2. The four ephedrine and pseudoephedrine diastereomers were partially resolved by each of the alcohols, with MeOH giving the highest discrimination. Based on these results, MeOH/EtOH (50/50) and 0.3% CHA was utilized, and baseline resolved all four ephedrine/pseudoephedrine diastereomers (Figure 4). The amphetamine enantiomers were also baseline resolved; however, the methamphetamine enantiomers were not quite fully resolved, with the best separation being achieved using EtOH as a co-solvent, with a resolution of 0.9.

Effects of CHA Concentration and Optimization of the Chiral Separation for Methamphetamine
In order to fully resolve the separation of d- and l-methamphetamine, the CHA concentration was raised from 0.3% to 0.5% to 1%, using EtOH as the co-solvent. The flow rate was also increased to 2.5 mL/min for higher efficiency. As illustrated in Figure 5, EtOH with 1.0% CHA separated d- and l-methamphetamine with a resolution of 1.2. Under these conditions, the amphetamine enantiomers were also baseline resolved; however, the separations of ephedrine and pseudoephedrine deteriorated at 1% CHA. Higher percentages of CHA were not investigated due to ion suppression effects.

Detection Limits and Linearity of Methamphetamine Analyses
Using the optimized conditions (SIR mode), the detection limit was 0.2 µg/mL and the linearity range was 0.5 - 200 µg/mL. The detection limits for the minor isomer in a methamphetamine sample with a skewed ratio of enantiomers was also studied. Trace d-methamphetamine could be detected at 0.1%; however, trace l-methamphetamine could only be detected at 3%, due to peak tailing and limited resolution (Figure 6). In contrast to these results, the detection levels for both d- and l-methamphetamine are 0.1% using this laboratory's current CE method [37].

Chiral Determination of Methamphetamine in Seized Samples
Six illicit methamphetamine samples were analyzed using EtOH doped with 1% CHA. The chromatograms are illustrated in Figure 7. The results are compared against the analyses performed using our current CE method in Table 2. To summarize, SFC offers faster and more consistent analyses, but is not well suited for detecting trace levels of l-methamphetamine due to its lower efficiency and resolution.
Table 2. Comparison of the chiral analysis of 6 illicit methamphetamine samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>By SFC</th>
<th>By CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>d-</td>
<td>0.5% l-</td>
</tr>
<tr>
<td>2</td>
<td>d-</td>
<td>0.7% l-</td>
</tr>
<tr>
<td>3</td>
<td>d-</td>
<td>0.4% l-</td>
</tr>
<tr>
<td>4</td>
<td>l-</td>
<td>l-</td>
</tr>
<tr>
<td>5</td>
<td>d-</td>
<td>d-</td>
</tr>
<tr>
<td>6</td>
<td>35.4% l-</td>
<td>35.6% l-</td>
</tr>
</tbody>
</table>

Conclusions

Using MeOH/EtOH (50/50) and 0.3% CHA, all four phenethylamines were resolved, and all but the methamphetamine enantiomers were baseline resolved (methamphetamine 0.9). Using EtOH and 1% CHA, the methamphetamine enantiomers were fully resolved (1.2). Both experiments were carried out in less than 7 minutes.

Using SQD in positive ESI and SIR mode, the detection limits were as low as 0.2 μg/mL for methamphetamine. The method also gave a good linearity range for methamphetamine (0.5 to 200 μg/mL). However, the method can only detect l-methamphetamine at 3% or greater. Despite this limitation, the method is an excellent alternative to other GC, HPLC, and CE methods for rapid chiral analyses, with good sensitivity, selectivity, and reliability.

Acknowledgments

The author thanks Mr. Kenneth Blakeslee from Waters Corporation for his assistance and support, and the Waters Corporation for the use of the Acquity UPC² system and columns.

Figure 1. SFC chromatograms of co-solvent screening. Co-solvent: IPA with 0.6% CHA; and gradient: 8% to 15% in 4 min; 15% to 40% at 6 min. Flow rate: 1.5 mL/min. a, d-methamphetamine; b, l-methamphetamine; c, d-amphetamine; d, l-amphetamine; e, d-pseudoephedrine; f, l-pseudoephedrine; g, d-ephedrine; h, l-ephedrine. [Note: Peaks were identified via analyses of individual enantiomers.]
Figure 2. SFC chromatograms of co-solvent screening. Co-solvent: MeOH with 0.3% CHA; and gradient: 8% to 15% in 6 min. Flow rate: 1.2 mL/min.

Figure 3. SFC chromatograms of co-solvent screening. Co-solvent: EtOH with 0.5% CHA; and gradient: 8% for 4 min; 8% to 30% at 9 min. Flow rate: 2.0 mL/min.
Figure 4. SFC chromatograms of co-solvent screening. Co-solvent: MeOH/EtOH (50/50) with 1.0% CHA; and gradient: 5-7.5% in 6 min. Flow rate: 2.5 mL/min.

Figure 5. Study of CHA concentration on methamphetamine chiral separation. Co-solvent: EtOH and CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min. 1: 1.0 % CHA; 2: 0.5% CHA.
Figure 6. Detection limit of l-methamphetamine (peak at ~2.03 minutes) with a skewed ratio of enantiomers. Co-solvent: EtOH and 1.0% CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min.

Figure 7. Chiral analysis of methamphetamine samples. Co-solvent: EtOH with 1.0% CHA; and gradient: 8-30% in 6 min. Flow rate: 2.5 mL/min.
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5. Title VII of the PATRIOT Improvement and Reauthorization Act of 2005 (Public Law 109-177).


23. Lurie IS, Bozenko Jr. JS, Li L, Miller EE, and Greenfield SJ. Chiral separation of methamphetamine and related compounds


33. LeBelle MJ, Savard C, Dawson BA, Black DB, Batyal LK, Zrcek F, By AW. Chiral identification and determination of ephedrine, pseudoephedrine, methamphetamine and methcathinone by gas chromatography and nuclear magnetic
34. Husk Jr. T. Phenethylamine isomer determination via α-methoxy-α-trifluoro-methyl-phenylacetic acid (MTPA) by gas chromatography. DEA Laboratory Note, May 16, 2014.


44. Subbarao L, McCauley J, Sidhu H, Chen R. Enantiometric resolution, identification, and quantitation of chiral illicit drugs using SFC APCI MS. www.waters.com/poster [Date of Last Access July 1, 2015.]

* * * * *

“Research on Drug Evidence”

Prefacing Remarks (and a Request for Information)

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KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Review, Forensic Chemistry.

Introduction

The INTERPOL Forensic Science Symposium is a periodic meeting of crime laboratory directors and associated personnel under the auspices of the General Secretariat (1). The initial Symposium, titled as: “The First I.C.P.O.-INTERPOL Seminar on the Scientific Aspects of Police Work” and held in November 1963, marked a significant break from typical INTERPOL conferences in that the attendees were not police officials but rather forensic scientists. The Symposia are commonly referred to as “Triennial”; however, the first six were actually held in 1963, 1968, 1972, 1975, 1978, and 1980, respectively. All 18 Symposia have been held in France, at St. Cloud (a suburb of Paris) from 1963 to 1986 and since 1989 at Lyon.

The general purpose of the early Symposia was to introduce, review, and/or discuss new or improved methods for conducting forensic analyses, and to a lesser degree to coordinate select research efforts at the attendees’ laboratories (2). “Coordinating laboratories” were designated to address specific research topics, in some cases in cooperation with “assisting laboratories” from other nations. Many different forensic topics were covered, including, e.g., arson, ballistics, field tests, etc. The coordinating laboratories were tasked with presenting reports or updates at the next Symposium. Since the 11th Symposium (1995), however, the primary responsibility of the coordinating laboratories has been to provide a comprehensive review of the scientific literature in their assigned topic (i.e., published since the last Symposium), along with a presentation summarizing recent developments. This shift reflected the increasing number of researchers involved in forensic science (many of whom had minimal association with INTERPOL), the similarly increasing number of sub-disciplines (consider, e.g., the advent and rapid expansion of digital evidence and DNA analyses), and the rapidly increasing number of pertinent articles in the literature. One of the longest standing topics has been “Drugs” (more recently titled as: “Research on Drug Evidence”), with short reports
dating to the earliest Symposia and more comprehensive reviews provided since 1995.

The “Proceedings” from the first 10 Symposia were rather uneven efforts, usually consisting of little more than a syllabus provided by the organizers and a loose collection of articles, reports, and handouts provided by the coordinating laboratories. Retention of these collections was at the discretion of the attendees. Starting with the 11th Symposium, however, the Proceedings became more formalized, with bound copies of the various review articles provided in hard copy to the attendees (and subsequently, combined in book form and marketed). Starting with the 16th (2010) Symposium, the complete Proceedings have been posted on line (3a-c).

The value of the review articles from the 11th through 18th Proceedings, however, is limited by their relative obscurity. It would not be an exaggeration to state that many forensic scientists have barely or never heard of the INTERPOL Forensic Science Symposia. This is not surprising, since historically nearly all of the invitees have been management-level personnel, not bench chemists or analysts. In order to improve the accessibility to this resource, the “Research on Drug Evidence” reviews from the 1995 through 2016 Proceedings are reprinted as the following eight articles. To the author’s knowledge, the only similar long-term series of review articles were those published by Brettell et al. in the biennial “Fundamental and Applied Reviews” issues of Analytical Chemistry from 1983 through 2011 (4a-0). These latter reviews covered all of the major sub-disciplines of forensic science in separate sections, including drug analyses. In addition, two independent reviews that focused primarily on forensic drug analyses were published in 1983 (5) and 2003 (6). In total, these 25 articles provide a nearly comprehensive overview and review of the literature that covers more than 50 years.

Request for Information

Regrettably, INTERPOL did not retain any of the Proceedings prior to the 11th (1995) Symposium (7). In a few cases a summary report was published either in the International Criminal Police Review (8) or as some very brief notes in the next Report of the General Assembly (9). The author’s laboratory has printed or electronic copies of all “Research on Drug Evidence” reports from the 10th (1992) through the 18th (2016) Symposia, all of which were prepared in-house, but nothing prior to the 10th. An extensive online search suggests that the 2nd (1968), 4th (1975), 5th (1978), 6th (1980), and 9th (1989) Proceedings are held in various library archives around the world. At present, the condition, completeness, and accessibility of these holdings are “unknown / to be determined”. There do not appear to be any holdings listed for the 1st (1963), 3rd (1972), 7th (1983), and 8th (1986) Proceedings. It is possible that these latter Proceedings are in un-catalogued library holdings, but that is unlikely. If they exist at all, they would be in the personal collections of the attendees - or (at this point) more likely among the files left at their respective facilities upon their retirements.

Although these first 10 Proceedings have only minimal value as literature reviews, they retain significant historical value as “snap-shots” of the topics of importance in forensic science at those times, as established by the attendees. For this reason, the author requests the assistance of the readership in locating originals or copies of the “Drugs” / “Research on Drug Evidence” reports from the first nine Proceedings. If most or all of these can be located, they and the “Research on Drug Evidence” report from the 10th Proceedings will be reprinted in the 2017 issue of Microgram Journal. The author’s email address is: dea-microgram@usdoj.gov
References

1. See: https://www.interpol.int/INTERPOL-expertise/Forensics/Forensic-Symposium [Date of most recent access: November, 2016.]


3. a) 2010: https://www.interpol.int/content/download/10356/73781/version/2/file/IFFS%202010%20Review%20papers.pdf
   b) 2013: https://www.interpol.int/content/download/21910/206602/version/1/file/IFSMSReviewPapers2013.pdf
   c) 2016: https://www.interpol.int/content/download/33314/426506/version/1/file/INTERPOL%2018th%20IFSMS%20Review%20Papers.pdf [Date of most recent access for all three documents: November, 2016.]

4. All articles were published in Analytical Chemistry; all were titled “Forensic Science”: a) Brettell TA, Saferstein R. 1983;55(5):19R–31R.
   b) Brettell TA, Saferstein R. 1985;57(5):175R-187R.
   c) Brettell TA, Saferstein R. 1987;59(12):162R–174R.
   d) Brettell TA, Saferstein R. 1989;61(12):95R-105R.
   e) Brettell TA, Saferstein R. 1991;63(12):148R-164R.
   f) Brettell TA, Saferstein R. 1993;65(12):293R-310R.
   h) Brettell TA, Saferstein R. 1997;69(12):123-144.
   o) Brettell TA, Butler JM, Almirall JR. 2011;83(12):4539-4556.


7. INTERPOL Staff (several), Personal Communications to the author, 2016.


Note that each of the above provided only minimal details. After the 42nd General Assembly, the Symposia were either barely mentioned, or not at all.
The 1995 “Research on Drug Evidence” Report

[From the 11th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 1995 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

Important Information:


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Citations in this report from Microgram were (and remain) Law Enforcement Restricted. Most citations listed as “Personal Communications” were unpublished reports from “assisting laboratories” provided to the Laboratory Director of the Special Testing and Research Laboratory upon his request; note that this was the last Symposium where such reports were requested for the “Research on Drug Evidence” review.

Page numbering in the bottom center of the first three pages and the upper corners of all subsequent pages are those in the original document, while those in the footers of each page represent the Microgram Journal numbering.

Although not shown in the document, this review was prepared by the author listed above (Klein), and the summary presentation at the Symposium was provided by Richard S. Frank.

This is an image of the best available hard copy. Blank pages in the original document are not duplicated in this reprint.
Research On Drug Evidence

June, 1992 - June, 1995

Prepared by:  U.S. Department of Justice
Drug Enforcement Administration
(Coordinating Laboratory)

Assisted by:  Bundeskriminalamt
Kriminaltechnisches Institut
Wiesbaden, Germany

Australian Government Analytical Laboratories
Canberra, Australia

Victoria Forensic Science Center
Victoria, Australia

National Research Institute of Police Science
Tokyo, Japan

Crime Laboratory
National Bureau of Investigation
Helsinki, Finland

Laboratorio de Criminalistica
Policia de Investigaciones de Chile
Santiago, Chile
Forensic Sciences Service
Stadtpolizei Zurich
Zurich, Switzerland

Forensic Science Laboratory
South African Police Service
Pretoria, Republic of South Africa

Institute of Forensic Science
Sofia, Bulgaria

Eleventh ICPO - INTERPOL
Forensic Sciences Symposium
November, 1995

Lyon, France
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Routine and Improved Analysis of Drug Substances

Problem/Issue:
Standard analytical data are required for previously unknown drugs of abuse and analog (i.e., "designer"-type) drugs. Additionally, improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all drugs of abuse.

Solution:
Illicit drug seizures are constantly monitored to provide a comprehensive overview of new developments. Case reports providing standard analytical data for new drugs are generated for the forensic and enforcement communities. Ongoing research in the forensic community, as well as the general analytical field, constantly provide new and/or improved methods of analysis for routine analysis of seized drugs.

Recent Developments:
In the United States, use of both heroin and LSD are increasing, while use of cocaine has levelled off and shows some signs of decreasing. "Smoking" heroin has increased, due to both increased purity levels and fear of contracting illnesses (AIDS and Hepatitis B) from use of intravenous needles. Designer drugs are sporadically noted; the most prevalent of the new designer drugs is 4-bromo-2,5-dimethoxyphenethylamine (aka: NEXUS or 2-CB). Use of anabolic steroids is steady, with use of human growth hormones or steroid natural production-stimulating drugs (e.g., gamma-hydroxybutyric acid) showing some increases. Among the amphetamines, use of "ice" methamphetamine continues to be only a regional problem, while use of methcathinone (aka: "CAT") has spread slowly. Abuse of flunitrazepam (Rohypnol) has unexpectedly and dramatically increased and throughout the American southwest.

In Europe, use of amphetamines and heroin remains widespread, while use of cocaine and LSD are both growing; "crack" cocaine use is now evident in many of the larger cities. "Record-level" seizures of bulk cocaine shipments (up to metric ton
quantities) have been reported by several nations. Among the "designer drugs," use of
the methylenedioxyamphetamines has rapidly expanded, with several analogs (notably
N-ethyl) and the corresponding methylenedioxyphenyl-2-butanamines also appearing.

In the Far East, Australia and New Zealand report general across-the-board
increases in drug abuse, while methamphetamine use remains ubiquitous in Japan.
Cocaine use is slowly growing throughout the Far East.

Summary:
Since 1992, routine and/or new/improved methods of analysis have been reported
for amphetamines (1-11), Angel Trumpet (12), barbiturates (13-14), benzodiazepines
(15-19) 4-bromo-2,5-dimethoxyphenethylamine (NEXUS) (20), bufotenine (21-24),
cocaine (25-29), dimethprimadine (30-31), dimethylaminorex (32), fenetyline (33),
fentanyl (34-35), 4-fluoroamphetamine and 4-fluoromethamphetamine (36), heroin (37-
42), gamma-hydroxybutyric acid (GHB) (43), N-(2-hydroxyethyl)amphetamine (44),
N-hydroxy-3,4-methylenedioxyamphetamine (45), inhalants (45-47), Jimson Weed (48),
Khat (49-53), LSD (54-60), marijuana (61-63), methylenedioxyamphetamine and
methylenedioxyphenylbutanamines (64-68), methcathinone and cathinone (69-75),
methylmethaqualone (76), N-methyl-1-phenethylamine (77), opium (78-79), PCP and
PCP analogs (80-82), and steroids (83-91).

References:

Amphetamines:
(1) LeBelle, M.J.; Savard, C.; Dawson, B.A.; Black, D.B.; Katyal, L.K.; Zrcek, F.; By,
A.W. "Chiral Identification and Determination of Ephedrine, Pseudoephedrine,
Methamphetamine and Methcathinone by Gas Chromatography and Nuclear
(2) Munro, C.H.; White, P.C. "Evaluation of Diazonium Salts as Visualization Reagents
for the Thin Layer Chromatographic Characterization of Amphetamines,"


Angel Trumpet:

Barbiturates:


Benzodiazepines:


(16) 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.


4-Bromo-2,5-dimethoxyphenethylamine (NEXUS):

Bufotenine:

Cocaine:

Dimethpramide:

Dimethylaminorex:

Fenethylline:

Fentanyl:

4-Fluoroamphetamine/4-Fluoromethamphetamine:

Heroin:


**gamma-Hydroxybutyric Acid (GHB):**


**N-(2-Hydroxyethyl)amphetamine:**


**N-Hydroxy-3,4-methylenedioxymphetamine:**


**Inhalants:**


Jimson Weed:
(48) Koverman, G. "Identification of Scopolamine and Hyoscyamine in Jimson Weed (Datura Stramonium)," Microgram 1993, 26, 122.

Khat (Catha Edulis):

LSD:
(58) Morales, R. "Quantitative Analysis of Lysergic Acid Diethylamide by Gas Liquid


Marijuana:


Methylenedioxyamphetamine and Methylenedioxyphenylbutanamines:


(68) Rosner, P.; Junge, T. "N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine, a Representative of a New Class of Street Drugs," *Microgram* 1994, 27, 411.

**Methcathinone and Cathinone:**


**Methylmethaqualone:**


**N-Methyl-1-phenylethylamine:**

(77) Clark, C.C. "The Identification of N-Methyl-1-phenylethylamine," *Microgram*
1993, 26, 90.

Opium:

PCP (Phencyclidine) and PCP Analogs:

Steroids:
(89) Morley, M.; Matkovich, C. "Screening of Steroids by Thin Layer
Chromatography, Microgram 1993, 26, 214.


II) Novel Syntheses of Illicit Drugs

Problem/Issue:

Forensic chemists must maintain familiarity with current and potential clandestine syntheses of illicit drugs in order to assist enforcement activities, for enhanced safety and effectiveness during enforcement operations and in order to provide expert testimony in Court proceedings.

Solution:

Clandestine laboratory operations are constantly reviewed to provide a comprehensive overview of the field. In cases where new methodologies are in use, case reports are generated for the forensic and enforcement communities.

Recent Developments:

Enforcement efforts and precursor control/monitoring laws have had a fairly dramatic impact on illicit syntheses of amphetamines in the United States, notably on the hydriodic acid/red phosphorus reduction ofephedrine to methamphetamine. Clandestine laboratory operators have responded with new sources of ephedrine and red phosphorus, clandestine syntheses of hydriodic acid and use of alternate phosphorus/iodine reagents. Use of highly reactive active metal reductions of ephedrine (i.e., with lithium or sodium metals in ammonia) is increasing. Use of mobile labs (i.e., motor homes or tractor-trailers) or hotels/short-term rental properties has complicated enforcement efforts; similarly, leaving operating laboratories unattended has become common practice and has increased hazards associated with clandestine laboratory entry. Increasing numbers of "confined space" clandestine laboratories (e.g., buried vehicles, caves, underground chambers or hidden internal compartments in residences, etc.) has necessitated new guidelines for enforcement and forensic personnel entry and disassembly/cleanup. Use of booby-traps in clandestine laboratories and storage sites is increasing.
Summary:
Since 1992, a variety of new methods for synthesis of amphetamine and methamphetamine and their precursors have been reported (1 - 7).

References:

Reference Drug Standards

Problem/Issue:
Reference drug standards are either commercially unavailable or if available are extremely expensive. No reported procedures existed for cleanup and or authentication of reference standards from either synthesized or seized materials.

Solution/Summary:
A systematic procedure for authentication of reference drug standards was developed (1); several reports (2-3) detailed syntheses and/or authentication of specific drug standards.

References:


IV) Comparative Analyses

Problem/Issue:
Comparative analysis (i.e., the systematic application of impurity profiling for determination of commonality of origin) is complicated due to both the high complexity of the data and the extremely large numbers of exhibits. Improved methods are needed for data handling and analysis.

Solution:
In-depth analysis helps identify discriminatory components in impurity profiles. Computer databases, sorting programs and pattern recognition/neural networks provide enhanced data handling and analysis. Case reports of new methodologies are generated for the forensic and enforcement communities.

Recent Developments:
In conjunction with impurity profiling, significant advances in comparative analyses were reported for the amphetamines/methylenedioxyamphetamines and cocaine, especially with respect to data handling. Advances in computer speed and capabilities have allowed direct downloading of data from chromatographic systems and rapid analyses. Improved instrumentation, notably capillary electrophoresis, isotope/ratio-mass spectrometry and deuterium nuclear magnetic resonance spectroscopy, offer new methods for more definitive impurity profiling and comparative analysis. New and dramatic improvements in pattern recognition/neural network programs have immensely improved data handling in cocaine comparative analysis and is expected to find similar application for other comparative analysis problems, especially heroin.

Summary:
Since 1991, impurity profiling has been conducted on amphetamines (5-9), cocaine (10-15), heroin (16-18), marijuana (19-22), methamphetamine (23-25), methylenedioxyamphetamines (26), opium (27), and Source Determination (Ballistics) (28-29).
Comparative Analyses has also been addressed in general terms (1-4).

References:

**General Discussions:**


**Amphetamines:**


Cocaine:


Heroin:


Marijuana:

(19) Robertson, J. (Australian Federal Police, Canberra, Australia); Jagadish, V.;

(20) Court, T.M. (Department of Botany, University of Queensland, St. Lucia, Brisbane, Australia) "Characters in Cannabis Indicative of Provenance," Personal Communication, 1995.


Methamphetamine:


Methylenedioxymethamphetamines:


Opium:

(27) Rembery, B.; Krenn, L.; Kopp, B.; Buchbauer, G.; Nikiforov, A. "Principal Component Analysis (PCA) of Opium Alkaloid Contents for Origin Determina-
Source Determination (Ballistics):


V) Source Determination of Drugs (Impurity Profiling)

Problem/Issue:

Impurity profiling of drugs is important for comparative analysis protocols. However, although certain drugs have been well characterized with respect to their impurity profiles, most have not been properly investigated.

Solution:

High sensitivity analytical techniques (primarily chromatographic) provide detailed impurity profiles. Identification of individual impurities enhance origin identification and comparative analyses and also aid in development of internal standards for improved accuracy and precision of analysis. Case reports are generated for the forensic and enforcement communities.

Recent Developments:

In contrast to most case reports prior to 1992 (which commonly only reported specific impurities noted in individual seized exhibits), there has been a much more systematic effort to identify impurities and establish signature profiles via in-house syntheses, notably with the amphetamines and methylenedioxyamphetamines. Heroin impurity profiling continues in the United States and Germany, with Australia a recent and fast-growing new research investigator in the field. The United States has made significant advances in cocaine impurity profiling, and several European groups have recently initiated new cocaine signature studies. Impurities in precursor chemicals and occluded trace solvents in finished products (notably cocaine and heroin) were both recognized as being increasingly important in impurity profiling.

Summary:

Since 1992, impurity profiling has been conducted on amphetamine (1-4), cocaine (5-17), heroin (18-20), methamphetamine (21-25), methylenedioxyamphetamines (26-32), occluded solvents in drugs (33-34) and drug precursors (35).
References:

Amphetamine:

Cocaine:
(8) Casale, J.F.; Moore, J.M. "3',4',5'-Trimethoxy-Substituted Analogs of Cocaine, cis/trans-Cinnamoylcocaine and Tropacocaine: Characterization and


Heroin:


(20) [Duplicate Citation] Violante, N.; Quaglia, M.G.; Lopez, A.; Caroli, S. 
"Characterization of Cocaine and Heroin samples as a function of their trace 

Methamphetamine:

Synthesized from Ephedrine by Reduction with HI and Red Phosphorus," 


Methylenedioxyamphetamine:


Occluded Solvents:


Precursors:

VI)

Analysis of Adulterants and Diluents

Problem/Issue:

Most "street-level" drugs are "cut" with various adulterants and diluents. Separation and identification of these extraneous materials can be tedious. In addition, new or unusual adulterants and/or diluents are occasionally identified in drug exhibits; standard analytical data are required for these substances. Finally, improved methods of analysis, i.e., faster, more discriminatory, less costly, etc., are needed for all cutting agents.

Solution:

Illicit drug seizures are constantly monitored to provide a comprehensive overview of adulterants and diluents. Case reports providing standard analytical data for new cutting agents are generated for the forensic and enforcement communities. Ongoing research in forensic community provides new and/or improved methods of analysis for routine identification of all adulterants and diluents.

Recent Developments:

Increased computer speed and enhanced search routines enable simultaneous identification of moderate quantities (i.e., 5 - 20 %) of certain cutting agents in cocaine or heroin by FT-IR spectroscopy. The techniques are based on identification of marker peaks for the adulterant or diluent in available "windows" in the infrared spectra of the controlled substance.

Summary:

Since 1992, four new cutting agents were identified and case reports generated (1-4); in addition, several new methodologies for simultaneous identification of common adulterants and diluents via FT-IR techniques were reported (5-6).
References:

**Unusual Adulterants/Diluents:**


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


Problem/Issue:
Gas chromatographic and tandem gas chromatographic techniques are increasingly the method of choice for routine screening and/or identification of illicit drugs. However, use of high-temperature injectors with GC's occasionally results in formation of artifacts due to unimolecular rearrangements of the drug substance(s) (or adulterants or diluents) or reaction(s) of the various components in the exhibit with the injection solvent(s). Such artifacts can severely complicate drug analyses, especially when they involve the controlled substance.

Solution:
Case reports providing information of the appearance and reduction/elimination of analytical artifacts are generated for the forensic community.

Recent Developments:
Anabolic Steroids and N-hydroxylated amphetamine/methylenedioxyamphetamine compounds (all fairly recent arrivals in the forensic arena) are all quite prone to artifact formation in heated injection ports. Use of certain solvents, notably chloroform, methanol or ethanol, have been recognized to be problematic for analyses of certain amine drugs at higher injection port temperatures.

Summary:
Since 1992, four case reports on artifact appearance in GC and/or GC/MS analyses of various controlled substances were reported (1-4).

References:


Problem/Issue:

Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Solution:

Improved/existing and new technologies are reviewed and applied to routine analysis of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

Recent Developments:

Capillary electrophoresis has moved to the forefront in liquid chromatographic analyses of controlled substances. In essence, the technique combines the resolution mechanisms of HPLC and electrophoresis, i.e., a flow system under an electric field, and gives (in many cases) dramatically enhanced resolution and speed of analysis. In particular, use of any of a variety of chiral additives to the run buffer results in chiral differentiation of substrates (a common forensic problem) without use of derivatization or expensive columns. Cleanup time between runs is very fast, and total reagent use and waste materials are far less than in HPLC. Approximately a dozen "routine use" instruments are now commercially offered.

Summary:

Since 1992, a variety of new and/or improved/existing instrumental methods have been utilized for drug analysis, including capillary electrophoresis (and related CE techniques) (1-12), gas chromatography (and tandem GC techniques) (13-18), high performance liquid chromatography (and tandem HPLC techniques) (19-31), thin-layer chromatography (37-39) and several other techniques (32-36).

References:
Capillary Electrophoresis (and Related CE Techniques):


(11) Quang, C.; Khaledi, M.G. "Improved Chiral Separation of Basic Compounds in


**Gas Chromatography (and Tandem GC Techniques):**


(18) Terada, M.; Shinozuka, T.; Yasuda, M.; Yanagida, J.; Wakasugi, C.


**High-Performance Liquid Chromatography (and tandem HPLC techniques):**


HPLC Retention Indices:


**Other General Analytical Methods:**


**Thin-Layer Chromatography:**


IX) Robotics and Computer Programs

Problem/Issue:
Repetitive multi-step analyses and/or data entry is tedious, time wasting and can lead to errors due to fatigue and/or boredom.

Solution:
Repetitive, multi-step analyses have been automated via use of robotics systems. Repetitive data entry/analyses have been improved via development of enhanced computer programs, macros, search routines, etc. In cases where improved performance is observed, case reports are generated for the forensic community.

Recent Developments:
Auto-injectors are now routinely available for virtually all high-quality GC and GC/MS systems, with software already included in the operating systems. Overall capabilities of robotics systems have been greatly enhanced with improved computer support, better "tracking" hardware and software, and new sample-handling protocols (including solid-phase extraction). At least a half-dozen new companies have entered the robotics field.

Summary:
Since 1992, several robotics procedures (1-2) and computer programs (3-7) have been reported.

References:


Sampling Plans

Problem/Issue:
Large drug shipments are almost invariably comprised of multiple units of a standard container size, e.g., several thousand 1 kilogram packages of cocaine. Current U.S. sentencing guidelines require accurate assessment of the makeup of an entire shipment; however, comprehensive analyses of such shipments is a daunting and prodigiously labor intensive task.

Solution/Summary:
Representative sampling plans which permit statistical inferences to be drawn with a pre-established, high degree of confidence were developed.

Recent Developments:
Representative sampling plans have survived numerous Courtroom challenges in the United States.

Summary:
Since 1991, several representative sampling plans have been reported (1-3).

References:

XI) Vapor and Particle Detection (Portable Instrumentation)

Problem/Issue:
New trade agreements and the easing of formally restrictive national and international borders have resulted in dramatic increases in cargo transshipments and personal travel, thereby complicating drug inspection and interdiction efforts at POE's. Discovery and confirmational analysis of suspected drugs in cargo or on individuals is severely hampered by the lack of on-site analytical equipment.

Solution:
Development of portable and highly sensitive vapor and/or particle detectors for drug analyses allows forensic chemists to perform screening type analyses on-site. In those cases where new methodologies have proven effective, case reports are generated for the forensic and enforcement communities.

Recent Developments:
Use of single ion monitoring instruments (such as the Barringer IONSCAN) have become routine in the United States, and has resulted in numerous seizures of controlled substances (primarily cocaine) at POE's, highway monitoring stations, on board marine vessels (both in port and on the high seas), and at individual buildings (both residential and commercial). Other ongoing efforts involve further miniaturization of various GC or GC/MS-type instruments and development of new technologies based on surface-acoustic-wave (SAW), pulsed neutron or biosensor technologies. This field continues to expand very rapidly.

Summary:
Since 1991, a variety of new, portable vapor and/or particle detectors have been reported for drug analyses (1-9).
References:

(1) Sobotka, A.J. (U.S. Drug Enforcement Administration, South Central Laboratory, Dallas, TX, USA) "MAX-2; Field Testing of the Barringer IONSCAN," Personal Communication, 1995.


References (10):


The 1998 “Research on Drug Evidence” Report
[From the 12th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 1998 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

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Research on Drug Evidence

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Office of Forensic Sciences
Special Testing and Research Laboratory

Twelfth ICPO-INTERPOL
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I) Routine and Improved Analysis of Drug Substances

Problem/Issue:

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all drugs of abuse. Additionally, standard analytical data are required for previously unknown drugs of abuse and new analog (i.e., "designer"-type) drugs.

Solution:

Illicit drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as the general analytical field, constantly provide new and/or improved methods of analysis for routine analysis of seized drugs. Case reports providing standard analytical data for new drugs and/or improved analytical protocols for known drugs are generated for the forensic and enforcement communities.

Recent Developments:

In the United States, use of methamphetamine has dramatically increased over the past three years, with concurrent increases in use of amphetamine and other related phenethylamines. Recent increases in both heroin and LSD have leveled off, while use of cocaine has somewhat decreased. Designer drugs - notably the methylenedioxyamphetamine and 4-bromo-2,5-dimethoxyphenethylamine (aka: NEXUS or 2-CB) - are still widely used. Use of anabolic steroids is steady, with use of human growth hormones or steroid natural production-stimulating drugs (e.g., gamma-hydroxybutyrolactone or GHB) continuing to increase. Abuse of flunitrazepam (Rohypnol) as a so-called "date-rape" drug continues. A wide variety of commercial products derived from hemp (cannabis) have been marketed and represent a challenging problem for trace-level analyses of cannabinoids.

In Europe, use of amphetamines, methylenedioxyamphetamine, and heroin
remains widespread, while use of cocaine and LSD continue to grow; "crack" cocaine use is now widespread.

In the Far East, Australia and New Zealand report general across-the-board increases in drug abuse, while methamphetamine use remains ubiquitous in Japan. Cocaine use is growing throughout the Far East.

Use of cocaine, heroin, Mandrax (methaqualone), amphetamine, LSD and methylenedioxyamphetamine all continue to increase in South Africa.

Summary:

Since 1995, routine and/or new/improved methods of analysis have been reported for amphetamines, mono-substituted amphetamines, Amanita Muscaria, Ayahuasca, barbiturates, benzodiazepines, 4-bromo-2,5-dimethoxyphenethylamine (NEXUS) and related compounds, bufotenine, clenbuterol, cocaine, coca tea, dimethpramidile, Dragon’s Blood Incense, fenethylline, fenfluramine, fentanyl, flunitrazepam (Rohypnol), heroin, hydrocodone, gamma-hydroxybutyric acid (or lactone) (GHB), N-(2-hydroxyethyl)-amphetamine, imazalil, inhalants, khat (Catha Edulis) and cathinone, LSD and related ergot alkaloids, marijuana and related cannabinoids, mescaline, methamphetamine, methcathinone, methylenedioxyamphetamine and related compounds, morphine and codeine, opium, opium, morphine and heroin (combined studies), 2-phenylethylamine (phenethylline) and related compounds, poppy tea, psilocybin and psilocin, steroids, and theophylline.

References:

Amphetamines (see also methamphetamine, methylenedioxyamphetamine):


**Mono-Substituted Amphetamines:**


**Amanita Muscaria:**


**Ayahuasca:**


**Barbiturates:**


**Benzodiazepines (see also flunitrazepam):**


4-Bromo-2,5-dimethoxyphenethylamine (NEXUS) and related compounds:


Bufotenine:


Clenbuterol:

Cocaine:


Coca Tea:


Dimethyldimethoamide:


**Dragon's Blood Incense:**

Steiner, R.S., "Dragon's Blood Incense," Microgram, 1997, 30 (11), 258.

**Fenethylline:**


**Fenfluramine:**


**Fentanyls:**


**Flunitrazepam (Rohypnol) (see also benzodiazepines):**

McKibben, T., "Simple and Rapid Color Screening Tests for Flunitrazepam (Rohypnol)," J.


Heroin:


Hydrocodone:


gamma-Hydroxybutyric Acid (or lactone) (GHB):

Wolnik, K.A.; Keitkemper, D.T.; Crowe, J.B.; Barnes, B.S.; Brueggemeyer, T.W., "Application

N-(2-Hydroxyethyl)amphetamine:


Imazalil:


Inhalants:


Khat (Catha Edulis) and Cathinone:


Kalix, P., "Catha edulis, a plant that has amphetamine effects," Pharmacy World and Science, 1996, 18 (2), 69.

LSD and Related Ergot Alkaloids:


Marijuana and related cannabinoids:


Mescaline:


Methamphetamines (see also amphetamines and methylenedioxyamphetamines):


Kuroda, N.; Nomura, R.; Al Dirbashi, O.; Akiyama, S.; Nakashima, K., "Determination of


Methylenedioxyamphetamines and related compounds:

DeRuiter, J.; Holston, P.L.; Clark, C.R.; Noggle, F.T., "Liquid chromatographic and mass
spectral methods of identification for the regioisomeric 2,3- and 3,4-


**Morphine and Codeine:**


Opium:


Opium, Morphine and Heroin (Combined Studies):


2-Phenylethylamine (Phenethylamine) and related compounds:


**Poppy Tea:**


**Psilocybin and Psilocin:**


**Steroids:**


Caerhati, T.; Forgacs, E., "Effect of β-cyclodextrin derivatives on the retention of steroidal


Guiney, L.B., "Quantitation of Testosterone Propionate and Boldenone by 13C - NMR,"
Microgram, 1995, 28 (9), 285.

**Theophylline:**

II) Novel Syntheses of Illicit Drugs, Precursors and Essential Chemicals

Problem/Issue:
Forensic chemists must maintain familiarity with existing and new clandestine syntheses of illicit drugs in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and to provide expert testimony in legal proceedings.

Solution:
Illicit drug seizures and clandestine laboratory operations are continuously monitored to maintain a comprehensive overview of the field. In cases where new drugs are synthesized, or new methodologies are utilized, case reports are generated for the forensic and enforcement communities.

Recent Developments:
Expanding use of the Internet has spread a wide variety of both new and old synthetic procedures for all drugs throughout the world. In the United States, most new syntheses have concentrated on reduction of ephedrine or pseudoephedrine to methamphetamine. Use of active metal reductions (i.e., with lithium or sodium metals in ammonia) continues to increase throughout the midwest, while a wide variety of red phosphorus/hydriodic acid or red phosphorus/iodine based reductions have been seen in the west. New reductions based on hypophosphorous acid have appeared, and some older methods, including reductive aminations of phenylacetone, have reappeared. Use of unusual sources of ephedrine (notably ground ephedra) and pseudoephedrine (primarily commercial tablets) have increased. Use of unusual solvents for salting out procedures, including new refrigerants (freons) and camping stove fuels, has dramatically increased. Counterfeit flunitrazepam (Rohypnol) tablets have appeared. Hydroponics-based marijuana operations continue to spread throughout North America, with hash oil use remaining popular in Canada.
In Europe, most new syntheses have concentrated on production of variants of methylenedioxyamphetamine; however, it remains unclear whether these new analogs are by design or rather unintended errors. In Europe and Southeast Asia, amphetamines and methylenedioxyamphetamine are commonly produced on industrial scales.

In South America, cocaine production continues to be simplified, and a large variety of commercially available farming and industrial products have been used as effective substitutes for "classic" reagents, especially in Colombia. Production of heroin continues to increase.

Summary:

Since 1995, a variety of alternate precursors, unusual substitutes for essential chemicals, and new or modified synthetic methods have been reported.

References:

**Clandestine Laboratory Updates:**


**New or Unusual Drugs and/or Precursors:**


Clandestine Laboratory Appraisals and Safety

Problem/Issue:

Forensic chemists must maintain familiarity with clandestine laboratory procedures, setups and techniques in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and in order to provide expert testimony in Court proceedings.

Solution:

Clandestine laboratory operations are continuously reviewed to provide a comprehensive overview of the field. In cases where new methodologies are noted, or unusual safety concerns are salient, case reports are generated for the forensic and enforcement communities.

Recent Developments:

Expanding use of the InterNet has spread a wide variety of clandestine laboratory methodologies throughout the world, including basic set-up procedures, adaptations of standard consumer products as substitutes for laboratory glassware, equipment, and essential chemicals, concealment techniques, covert surveillance and countersurveillance techniques, and booby trapping.

In the United States, widespread use of active metal reductions of ephedrine (i.e., with lithium or sodium metals in ammonia) represents a serious threat to forensic, law enforcement and fire department personnel. Continuing increases in the numbers of "confined space" clandestine laboratories (e.g., buried vehicles, caves, underground chambers or hidden internal compartments in residences, etc.) has necessitated new guidelines and training for enforcement and forensic personnel entry and disassembly/cleanup. Use of booby-traps in clandestine laboratories and storage sites continues.
Summary:

Since 1995, a number of reports concerning safety in confined space laboratories or unusual hazards associated with certain methamphetamine syntheses have been reported.

References:

Safety Issues - Case Reports:


Confined Space Laboratories:


IV) Reference Drug Standards

Problem/Issue:

Many reference drug standards or structurally related internal standards are either commercially unavailable, or if available are extremely expensive.

Solution:

Controlled substances and their structural or isotopically labelled analogs are synthesized as needed. Internal standards are also prepared as needed. Case reports are published for new or unusual standards or improved synthetic approaches.

Recent Developments:

Increasing use of single ion-monitoring techniques for identification and quantitation of controlled substances and/or precursor compounds and essential chemicals has necessitated the development and use of isotopically labelled analogues or closely related structural isomers.

Summary:

Since 1995, several reports detailing "total syntheses" of various controlled substances have been reported.

References:


V) Comparative Analyses

Problem/Issue:

Comparative analysis (i.e., the systematic application of impurity profiling for determination of commonality of origin) is complicated due to both the high complexity of the data and the large numbers of exhibits. Improved analytical and data handling techniques are needed.

Solution:

In-depth analysis via improved instrumental methodologies help identify discriminatory components in impurity profiles. Computer databases, sorting programs and pattern recognition/neural networks provide enhanced data handling and analysis. Case reports of new methodologies are generated for the forensic and enforcement communities.

Recent Developments:

In conjunction with impurity profiling, a number of comparative analysis protocols were reported.

Summary:

Since 1995, comparative analyses have been conducted on amphetamines, heroin, LSD blotter papers, marijuana, opium, and tablet and capsule logos. Comparative analysis has also been addressed in general terms.

References:

Amphetamines:

Pikkarainen, A.L., "Systematic approach to the profiling analysis of illicit amphetamine," Forensic 

Microgram Journal 2016, Volume 13; Numbers 1-4

**Heroin:**


**LSD:**


**Marijuana:**

Opium:


Source Determination (Ballistics/Toolmarks):


General Discussions:

VII) Source Determination of Drugs (Impurity Profiling)

Problem/Issue:

Impurity profiling of drugs is important for comparative analysis protocols, geo-sourcing and synthetic route determinations. However, although certain drugs have been well characterized with respect to their impurity profiles, most have not been properly investigated.

Solution:

High sensitivity analytical techniques (primarily chromatographic) provide detailed profiles of trace-level impurities, ions, trace metals and stable isotopes. Identification of individual impurities enhance origin identification and comparative analyses and also aid in development of internal standards for improved accuracy and precision of analysis. Case reports are generated for the forensic and enforcement communities.

Recent Developments:

Since 1995, the ongoing and systematic effort to identify impurities and establish signature profiles via in-house syntheses has continued and expanded. Heroin impurity profiling continues in the United States, Germany and Australia. Cocaine impurity profiling continues in the United States and Europe, and has expanded in South America. Analysis of occluded solvents in finished products (notably cocaine, heroin and methamphetamine) continues, and stable isotope analyses have expanded.

Summary:

Since 1995, impurity profiling has been conducted on amphetamine, cocaine, heroin, marijuana, methamphetamine, methylenedioxyamphetamine, and precursors.
References:

Amphetamine:


Cocaine:


Moore, J.M.; Casale, J.F., "Lesser alkaloids of cocaine-bearing plants. Part 1: nicotinoyl-, 2'-pyrroloyl, and 2'- and 3'-furanoyllecgonine methyl ester - Isolation and mass spectral..."


**Heroin:**


**Marijuana:**


Methamphetamine:


Precursors:


General Discussions:


Occluded Solvent Analyses:


VII) Analysis of Adulterants and Diluents

Problem/Issue:

Most "street-level" drugs are "cut" with various adulterants and diluents. Separation and identification of these extraneous materials can be tedious. In addition, new or unusual adulterants and/or diluents are occasionally identified in drug exhibits, and standard analytical data are required for these substances. Finally, improved methods of analysis, i.e., faster, more discriminatory, less costly, etc., are needed for all cutting agents.

Solution:

Illicit drug seizures are continuously monitored to provide a comprehensive overview of adulterants and diluents. Case reports providing standard analytical data for new and/or unusual cutting agents are generated for the forensic and enforcement communities. Ongoing research in forensic community provides new and/or improved methods of analysis for routine identification of all adulterants and diluents.

Recent Developments:

In the United States dimethylsulfone and dimethylphthalate (or its isomers) were commonly identified in methamphetamine and cocaine, respectively. Additional programs for simultaneous identification of moderate quantities (i.e., 5 - 20 %) of certain cutting agents in cocaine or heroin by FT-IR spectroscopy were reported. Several unusual cutting agents were identified. Adulterants and diluents were the focus of several general surveys.

Summary:

Since 1995, several reports detailing common cutting agents were published. In addition, a number of simultaneous determinations of controlled substances and cutting agents were reported.
References:

Adulterants/Diluents:


Simultaneous Analyses of Drugs and Adulterants/Diluents:


Bautista, R.D.; Jimenez, A.I.; Jimenez, F.; Arias, J.J., "Resolution of ternary and quaternary mixtures of drugs in pharmaceutical preparations by use of spectrophotometric data in


VIII) Analytical Artifacts

Problem/Issue:

Gas chromatographic and tandem gas chromatographic techniques are increasingly the method of choice for routine screening and/or identification of illicit drugs. However, use of high-temperature injectors with GC's occasionally results in formation of artifacts due to unimolecular rearrangements of the drug substance(s) (or adulterants or diluents) or reaction(s) of the various components in the exhibit with the injection solvent(s). Such artifacts can severely complicate drug analyses, especially when they involve the controlled substance.

Solution:

Case reports providing information of the appearance and reduction/elimination of analytical artifacts are generated for the forensic community.

Recent Developments:

Nitrites have been recognized to be problematic for analyses of cannabinoids.

Summary:

Since 1995, several case reports on artifact appearance in GC and/or GC/MS analyses of various controlled substances were reported.

References:


IX) New and/or Improved Instrumental Techniques

Problem/Issue:
Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Solution:
Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

Recent Developments:
Capillary electrophoresis and related techniques (electrokinetic chromatography, capillary electrochromatography, and micellar electrokinetic capillary chromatography) have moved to the forefront in liquid chromatographic analyses of controlled substances. Applications have included direct chiral discrimination of optical isomers without derivatization or specialized columns. Specialized injection techniques have enhanced detection limits for a variety of liquid chromatographic techniques. Raman spectroscopy has been investigated for identification of controlled substances. Laser-induced fluorescence has been utilized for ultra-trace level detection of both controlled substances and their impurity profiles.

Summary:
Since 1995, a variety of new and/or improved/existing instrumental methods have been utilized for drug analysis; most have been based on capillary electrophoretic techniques.
References:

Capillary Electrophoresis (and related CE techniques):


Altria, K.D., "Determination of drug-related impurities by capillary electrophoresis," J.


**Gas Chromatography (and GC/MS):**


High-Performance Liquid Chromatography (and tandem HPLC techniques):


Duenas, E.V.; Forero, M.E., "Standardized Methods to Separate and Identify Cocaine, Morphine, Heroin, Codeine, Papaverine, Benzocaine, Procaine, Lidocaine by High-Efficiency, Liquid Chromatography with Diode Array Detector (HPLC-DAD)," *Microgram*, 1996, 29 (8), 207.


Micellar Electrokinetic Capillary Chromatography:


Other General Analytical Methods:


X) Portable Detection and Analytical Instrumentation

Problem/Issue:
New trade agreements and the easing of formally restrictive national and international borders have resulted in dramatic increases in cargo transshipments and personal travel, thereby complicating drug inspection and interdiction efforts at POE's. Discovery and confirmational analysis of suspected drugs in cargo or on individuals is severely hampered by the lack of on-site detection and/or analytical equipment.

Solution:
Development of portable and highly sensitive detectors for drug detection and analyses allows law enforcement personnel and/or forensic chemists to perform screening type analyses on-site. In those cases where new methodologies have proven effective, case reports are generated for the forensic and enforcement communities.

Recent Developments:
Use of ion mobility spectrometers has become routine in the United States, and has resulted in numerous seizures of controlled substances (primarily cocaine) at POE's, highway monitoring stations, on board marine vessels (both in port and on the high seas), and at individual buildings (both residential and commercial). Other ongoing efforts involve further miniaturization of various GC, GC/MS, and ion mobility-type instruments and development of new technologies based on surface-acoustic-wave (SAW), pulsed neutron or biosensor technologies. This field continues to expand very rapidly.

Summary:
Since 1995, a variety of new, portable vapor and/or particle detectors have been reported for drug analyses. Several instruments based on fast neutron analyses have also been reported.
References:


References:


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.


**General Surveys:**

Edited by: Brandenberger, Hans; Maes, Robert A. A. de Gruyter: Berlin, Germany.


The 2001 “Research on Drug Evidence” Report
[From the 13th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 2001 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

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July 1, 1998 - June 30, 2001

Presented by: Joseph P. Bono

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I) **Routine and Improved Analysis of Drug Substances**

**Issue:**

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all drugs of abuse. Additionally, standard analytical data are required for previously unknown drugs of abuse and/or new homolog or analog (i.e., "designer"-type) drugs.

**Solution:**

Illicit drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general field of analytical chemistry, provide new and/or improved methods of analysis for both routine and specialized analyses of seized drugs. Reports providing standard analytical data for new drugs and/or improved analytical protocols for known drugs are generated for the forensic and enforcement communities.

**Recent Developments:**

In the United States, use of methamphetamine continues to increase. Use of amphetamine and other homolog/analog phenethylamines (often sold as methamphetamine) also continue to increase. Use of heroin, cocaine, anabolic steroids, human growth hormones, and LSD have stabilized or decreased. However, use of designer drugs - notably the various methylenedioxyamphetamines (MDA’s) - are rapidly increasing. Use of gamma-hydroxybutyric acid (GHB), gamma-butyrolactone (GBL) and 1,4-butanediol (BD) are also increasing. Abuse of flunitrazepam (Rohypnol) and other benzodiazepines as so-called “date-rape” drugs has decreased concurrent with the increases in the use of the MDA’s, GHB, GBL, and BD. The marketing of commercial products derived from hemp (cannabis) continues to expand. Similarly, the marketing of various “controlled substance mimics” (usually via Internet sales) continues to increase; the majority of these mimics are complex mixtures of non-controlled plants and/or over-the-counter type drugs which are alleged to imitate the physiological effects of marijuana.
In Europe and Russia, use of amphetamines, methylenedioxyamphetamines, and heroin remains widespread, while use of cocaine continues to grow. In the Far East, Australia and New Zealand report general across-the-board increases in drug abuse (especially cocaine and methamphetamine), while methamphetamine use remains ubiquitous in Japan and is rapidly increasing in Cambodia, Thailand, Vietnam, and elsewhere along the Pacific Rim countries, with the spread of so-called “Thai Tabs” (actually primarily produced in Burma/Myanmar) driving the increase. Heroin use in the People's Republic of China is expanding rapidly, especially in the provinces adjoining the Golden Triangle region. Cocaine use is also increasing throughout South America (especially Brazil) and the Far East. Use of cocaine, heroin, Mandrax (methaqualone), amphetamine, LSD and methylenedioxyamphetamine all continue to increase in South Africa.

Summary:

Since 1998, several minor reviews of forensic analysis of drugs of abuse have appeared, and an International Scientific Working Group (SWGDRUG) has begun to formalize standards for forensic laboratories. Routine and/or new/improved methods of analysis have been reported for amphetamines, various substituted amphetamines, barbiturates, benzodiazepines, 4-bromo-2,5-dimethoxyphenethylamine (NEXUS) and related poly-substituted phenethylamines, cocaine, dihydroetorphine and etorphine, etonitazene, fentanyls, flunitrazepam (Rohypnol), heroin, gamma-hydroxybutyric acid (GHB), gamma-butyrolactone (GBL) and 1,4-butanediol (BD), inhalants, ketamine, LSD, marijuana and related cannabinoids, methamphetamines, methaqualone, methcathinone, methylenedioxyamphetamine and related compounds, morphine, codeine, and related opium alkaloids, opiate alkaloids, opium, 2-phenylethylamine (beta-phenethylamine) and related compounds, phenylpropylmethamphetamine, psilocybin, psilocin, and bufotenine, salvia divinorum, sibutramine, steroids, telazol, and terbinafine.

References:

Reviews:


Scientific Working Group for Forensic Analysis of Illicit Drugs:


Amphetamines (see also substituted amphetamines, methamphetamines, methylenedioxyamphetamines):

10. Mancinelli R, Gentili S, Guiducci MS, Macchia T. Simple and reliable high-performance liquid chromatography fluorimetric procedure for the determination of amphetamine-


**Substituted Amphetamines:**


**Barbiturates:**


Benzodiazepines (see also Flunitrazepam):


4-Bromo-2,5-dimethoxyphenethylamine (NEXUS) and related polysubstituted phenethylamines:

**Cocaine:**

37. Airaksinen AJ, Tuppurainen KA, Lotjonen SE, Niemitz M, Yu MX, Vepsalainen JJ,


**Dihydroetorphine and Etorphine:**


**Etonitazene:**


**Fentanyls:**


**Flunitrazepam (Rohypnol) (see also benzodiazepines):**


**Heroin:**


57. United Nations International Drug Control Programme (Scientific Section). Monograph:

**gamma-Hydroxybutyric Acid (GHB), gamma-butyrolactone(GLB) and 1,4-butanediol (BD):**


**Inhalants:**


**Ketamine:**

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**LSD:**


**Marijuana and related cannabinoids:**


89. Ferioli V, Rustichelli C, Pavesi G, Gamberini G. Analytical characterization of hashish


Methamphetamines (see also amphetamines and methylenedioxyamphetamines):


103. 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.


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

**Methaqualone:**

Methcathinone:


Methylenedioxyamphetamines and related compounds:

121. Franzosa ES. MDMA, MDEA, and MBDB tablets seen in the US. Microgram 2000;33(6):121.


133. Clark CR, Noggle FT, Holston PL, DeRuiter J. Methods of differentiation for
regioisomeric 2,3- and 3,4-methylenedioxyphenalkylamines by liquid chromatography and mass spectrometry. Microgram 1998;31(9):244.


Morphine, Codeine and Related Opium Alkaloids:


**Opiate Alkaoids:**

152. Anonymous. Oxycodone (trade names: Tylox, Percodan, Oxycontin). Microgram


Opium:


2-Phenylethylamine (beta-Phenethylamine) and related compounds:


**Phenylpropylmethylamine:**


**Psilocybin, Psilocin, and Bufotenine:**


172. Phelan CP. Identification of psilocin and bufotenine via GC/IRD. Microgram

Salvia Divinorum


Sibutramine:


Steroids:


Telazol:


Terbinafine:

**Miscellaneous:**

II) **Novel Syntheses of Illicit Drugs, Precursors and Essential Chemicals**

**Issue:**

Forensic chemists must maintain familiarity with existing and new clandestine syntheses of illicit drugs in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and to provide expert testimony in legal proceedings.

**Solution:**

Illicit drug seizures and clandestine laboratory operations are continuously monitored to maintain a comprehensive overview of the field. In cases where new drugs are synthesized, or new methodologies are utilized, case reports are generated for the forensic and enforcement communities.

**Recent Developments:**

Continuing use of the Internet has spread a wide variety of both new and old synthetic procedures for all drugs throughout the world. In the United States, the most prevalent synthetic drug is methamphetamine, produced on both large and small ("cottage industry") scales. Large scale operations are centered in Mexico and California (Mexican run), and are based on ephedrine reduction with hydriodic acid. Similarly, most small scale operations have concentrated on reduction of ephedrine or pseudoephedrine to methamphetamine, using a variety of synthetic routes. Use of commercial pseudoephedrine and phenylpropanolamine tablets as precursor sources continue to increase. Use of active metal reductions (i.e., with lithium or sodium metals in ammonia), and iodine-based reductions with hypophosphorous acid, both continue to increase. Use of unusual solvents for salting out procedures, including new refrigerants (Freons), camping stove fuels, and industrial solvents, has dramatically increased. Reductive aminations of phenylacetone continue, but only at a low level.

New designer drugs have also appeared, but are mostly isolated incidents arising from single operations. The only significant exception are the methylenedioxyamphetamines (MDA’s), which are now a worldwide abuse problem. Virtually all MDA’s are produced via reductive aminations of the corresponding ketone; large (industrial-scale) operations are
primarily based in Europe, but similar large scale production laboratories have been identified in South Africa and in Asia. One new analog drug which may become a significant problem is 2,5-dimethoxy-4-\textit{n} -propylthiophenethylamine (2C-T-7), which is one of the hundreds of analog drugs developed by Alexander Shulgin (author of PIHKAL and TIHKAL). In Europe, amphetamines are also commonly produced on industrial scales. In Southeast Asia, illicit production of methamphetamine has exploded, with manufacture of so-called “Thai Tabs” (methamphetamine tablets, commonly also containing caffeine) becoming a major industry in Burma/Myanmar and the People’s Republic of China.

The abuse of \textit{gamma}-hydroxybutyric acid (GHB) and its corresponding cyclic lactone \textit{gamma}-butyrolactone (GBL) have also dramatically increased over the past 5 years, primarily in Europe and the United States. Originally utilized as a steroid substitute and “health food supplement” in body-building circles, GHB became popular as a fast-acting hypnotic/sedative, and rapidly spread via the “rave” party scene. It is clandestinely produced from \textit{gamma}-butyrolactone (GBL), and clandestine chemists soon realized that GBL is in chemical equilibrium with GHB, and could therefore be utilized interchangeably with GHB. This is a significant complication in enforcement efforts against GHB, since GBL is a fairly widely used industrial chemical. In an additional complication, it has been discovered that 1,4-butanediol (BD) and (to a lesser extent) tetrahydrofuran (THF) both convert to GHB in the body, and both of these industrial chemicals are now also being abused as GHB. Several methyl and dimethyl analogs of GHB and GBL have also been reportedly abused.

In southwestern Asia, especially Afghanistan, opium, morphine and heroin production has exploded. In South America, coca cultivation in Bolivia and Peru has dramatically decreased, but cultivation in Colombia has hugely increased. Brazil, Ecuador, and Venezuela are becoming increasingly involved in cocaine production and trafficking. A large variety of commercially available farming and industrial products have been used as effective substitutes for “classic” reagents in cocaine production, especially in Colombia. Industrial production of essential chemicals in Bolivia, Peru and especially Colombia has increased as importation of these same materials has become increasingly restricted. Production of heroin continues to increase in Colombia, and Ecuador and especially Peru are increasingly involved in opium cultivation in support of Colombian heroin production.
Summary:
Since 1998, a variety of alternate precursors, unusual substitutes for essential chemicals, and new or modified synthetic methods have been reported.

References:

Clandestine Laboratory Case Reports:


Clandestine Laboratory Production of New or Unusual Drugs and/or Precursors:


III) Clandestine Laboratory Appraisals and Safety

Issue:

Forensic chemists must maintain familiarity with clandestine laboratory procedures, setups, and techniques in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and in order to provide expert testimony in Court proceedings.

Solution:

Clandestine laboratory operations are continuously reviewed to provide a comprehensive overview of the field. In cases where new methodologies are noted, or unusual safety concerns are salient, reports are generated for the forensic and enforcement communities.

Recent Developments:

Expanding use of the Internet has spread a wide variety of clandestine laboratory methodologies throughout the world, including basic set-up procedures, adaptations of standard consumer products as substitutes for laboratory glassware, equipment, and essential chemicals, concealment techniques, covert surveillance and countersurveillance techniques, and booby trapping. Numerous websites and “chat-lines” are dedicated to illicit drug production and/or use.

Summary:

Since 1998, a number of clandestine laboratory reports have been published.

References:

Clandestine Laboratory Appraisals and Safety:


**Confined Space Laboratories:**


**Safety Issues - Case Reports:**


**Miscellaneous:**

IV) Reference Drug Standards and Total Syntheses

Issue:
Many reference drug standards or structurally related internal standards are either commercially unavailable, or if available are extremely expensive.

Solution:
Controlled substances and their structural or isotopically labelled analogs are synthesized as needed. Internal standards are also prepared as needed. Case reports are published for new or unusual standards or improved synthetic approaches.

Recent Developments:
Increasing use of single ion-monitoring techniques for identification and quantitation of controlled substances and/or precursor compounds and essential chemicals has necessitated the development and use of isotopically labelled analogs, enantiomers, or closely related structural isomers.

Summary:
Since 1998, several reports detailing “total syntheses” of various controlled substances have been reported.

References:


V) **Comparative Analyses**

**Issue:**

Comparative analysis (i.e., the systematic application of impurity profiling for determination of commonality of origin) is complicated due to both the high complexity of the data and the large numbers of exhibits. Improved analytical and data handling techniques are needed.

**Solution:**

In-depth analysis via improved instrumental methodologies help identify discriminatory components in impurity profiles. Computer databases, sorting programs, and pattern recognition/neural networks provide enhanced data handling and analysis. Case reports of new methodologies are generated for the forensic and enforcement communities.

**Recent Developments:**

In conjunction with impurity profiling, a number of comparative analysis protocols were reported.

**Summary:**

Since 1998, comparative analyses have been conducted on heroin, and tablet and capsule logos.

**References:**

**Pattern Recognition:**

**Heroin:**


**Source Determination (Ballistics/Toolmarks):**

VI) **Source Determination of Drugs (Impurity Profiling)**

**Issue:**

Impurity profiling of drugs is important for comparative analysis protocols, geo-sourcing, and synthetic route determinations. However, although certain drugs have been well characterized with respect to their impurity profiles, most have not been properly investigated.

**Solution:**

High sensitivity analytical techniques (primarily chromatographic) provide detailed profiles of trace-level impurities, ions, trace metals, and stable isotopes. Identification of individual impurities enhance origin identification and comparative analyses and also aid in development of internal standards for improved accuracy and precision of analysis. Case reports are generated for the forensic and enforcement communities.

**Recent Developments:**

Since 1998, the ongoing and systematic effort to identify impurities and establish signature profiles via in-house syntheses has continued and expanded. Heroin impurity profiling continues in the United States, Australia, and Germany. Cocaine impurity profiling continues in the United States and Europe, and has expanded in South America. Amphetamine profiling continues in Northern Europe, and methamphetamine profiling is expanding in the United States, Japan, and Australia. Analysis of occluded solvents in finished products (notably cocaine, heroin, and methamphetamine) continues, and stable isotope analyses (notably Isotopic Ratio Mass Spectrometry and Inductively Coupled Plasma/Mass Spectrometry) have expanded.

**Summary:**

Since 1998, impurity profiling has been conducted on amphetamine, cocaine, heroin, marijuana, methamphetamine, 4-methoxyamphetamine, methylenedioxy-amphetamines, opium, and occluded solvents.

**References:**

*Microgram Journal 2016, Volume 13; Numbers 1-4*
General Review:


Amphetamine:


Cocaine:


Heroin:


**Marijuana:**


236. 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.

237. Gigliano GS, Di Finizio A. The *Cannabis Sativa* L. fingerprint as a tool in forensic investigation. Bull Narc 1997/1998;(49(1,2)/50(1,2)):129.
Methamphetamine:


4-Methoxyamphetamine:

Methylenedioxyamphetamines:


Opium:


Occluded Solvent Analyses:


Miscellaneous:

VII) Analysis of Adulterants and Diluents

Issue:

Most "street-level" drugs are "cut" with various adulterants and diluents. Separation and identification of these extraneous materials can be tedious, especially in exhibits which contain many components. In addition, new or unusual adulterants and/or diluents are occasionally identified in drug exhibits, and standard analytical data are required for these substances. Finally, improved methods of analysis, i.e., faster, more discriminatory, less costly, etc., are needed for all cutting agents.

Solution:

Illicit drug seizures are continuously monitored to provide a comprehensive overview of adulterants and diluents. Case reports providing standard analytical data for new and/or unusual cutting agents are generated for the forensic and enforcement communities. Ongoing research in forensic community provides new and/or improved methods of analysis for routine identification of all adulterants and diluents.

Recent Developments:

In the United States, the extensive use of over-the-counter ephedrine or pseudoephedrine containing products for methamphetamine production has resulted in numerous reports on these two precursors. It is increasingly common to identify cocaine in South American heroin, and South American heroin in cocaine. “Thai Tabs” are usually cut with caffeine, and some may contain ketamine as well. “Ecstasy” tablets may contain a mixture of methylenedioxyamphetamine and/or homolog/analog drugs. Use of infrared, Raman, or nuclear magnetic resonance spectroscopy for the simultaneous identification of moderate quantities (i.e., 5 - 20 %) of certain cutting agents in cocaine or heroin is increasing.

Summary:

Since 1998, several reports detailing common cutting agents were published.
References:

Ephedrine and/or Pseudoephedrine:


Other Adulterants/Diluents (may include ephedrine and/or pseudoephedrine):

265. 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
274. McCrossen SD, Bryant DK, Cook BR, Richards JJ. Comparison of LC detection methods in the investigation of non-UV detectable organic impurities in a drug substance.


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


VIII) New and/or Improved Instrumental Techniques

Issue:
Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Solution:
Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

Recent Developments:
Capillary electrophoresis and related techniques have moved to the forefront of liquid chromatographic analyses of controlled substances. Advanced applications have included direct chiral discrimination of optical isomers without derivatization or specialized columns. Specialized injection techniques have enhanced detection limits for a variety of liquid chromatographic and gas chromatographic techniques. Raman spectroscopy has been investigated for identification of controlled substances (and shows great promise for portable instrumentation). Laser-induced fluorescence has been utilized for ultra-trace level detection of both controlled substances and their associated impurities.

Summary:
Since 1998, a variety of new and/or improved/existing instrumental methods have been utilized for drug analysis; most have been based on capillary electrophoretic and Fourier transform infrared and/or Raman techniques.

References:

Capillary Electrophoresis (and related CE techniques):


289. Wallenborg S, Arnold D, Lurie I, Bailey C. On-chip separation of amphetamine and related compounds labeled with 4-fluoro-7-nitrobenzofurazane. Electrophoresis


305. Lurie IS, Conver TS, Ford VL. Simultaneous separation of acidic, basic, and neutral organic compounds, including strong and moderate acids and bases, by capillary electrochromatography. Anal Chem 1998;70:4563.


Gas Chromatography (and GC/MS):


**High-Performance Liquid Chromatography (and tandem HPLC techniques):**


**HPLC Retention Indices:**


**Infrared and Raman Spectroscopy:**


**Nuclear Magnetic Resonance Spectroscopy:**


**Supercritical Fluid Chromatography:**


Miscellaneous:


IX) Portable Detection and Analytical Instrumentation

Issue:

New trade agreements and the easing of formally restrictive national and international borders have resulted in dramatic increases in cargo transshipments and personal travel, thereby complicating drug inspection and interdiction efforts at POE’s. Discovery and confirmational analysis of suspected drugs in cargo or on individuals is severely hampered by the lack of on-site detection and/or analytical equipment.

Solution:

Development of portable and highly sensitive detectors for drug detection and analyses allows law enforcement personnel and/or forensic chemists to perform screening type analyses on-site. In those cases where new methodologies have proven effective, case reports are generated for the forensic and enforcement communities.

Recent Developments:

Use of ion mobility spectrometers has become routine in the United States, and has resulted in numerous seizures of controlled substances (primarily cocaine) at POE’s, highway monitoring stations, on board marine vessels (both in port and on the high seas), and at individual buildings (both residential and commercial). Other ongoing efforts involve further miniaturization of various GC, GC/MS, and ion mobility-type instruments, and development of new technologies based on surface-acoustic-wave (SAW), pulsed neutron or biosensor technologies. This field continues to expand very rapidly; however, most reports are proprietary and are rarely reported in forensic chemistry journals.

Summary:

Since 1998, a variety of new, portable vapor and/or particle detectors have been reported for drug analyses. Several instruments based on fast neutron analyses have also been reported.

References:

Microgram Journal 2016, Volume 13; Numbers 1-4


References:

Analytical Artifacts:


Qualitative Tests:

358. 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.

Sampling Plans:

**General Surveys:**

374. United Nations International Drug Control Programme (International Narcotics Control


Other:

397. Liu SY, Woo SO, Koh HL. HPLC and GC-MS screening of Chinese proprietary medicines for undeclared therapeutic substances. J Pharm Biomed Anal 2001;24(5-


The 2004 “Research on Drug Evidence” Report

[From the 14th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 2004 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

Important Information:


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Citations in this report from the Journal of the Clandestine Laboratory Investigating Chemists Association and Microgram were (and remain) Law Enforcement Restricted. Microgram was split into Microgram Bulletin and Microgram Journal in 2002 and 2003, respectively; except for the 2002 Bulletins, both the Bulletin and Journal were (and are) unclassified.

The “General Overview” (Talking Paper) was removed from this reprint (Editor’s discretion).

This reprint is derived from the original electronic document, and is not an image of the best available hard copy (as was utilized for the 1995 and 1998 reports). For this reason, the pagination in the original document is not retained in this reprint, and some minor reformatting was done to eliminate deadspace.
Research On Drug Evidence
July 1, 2001 - June 30, 2004

Presented by: Robert F.X. Klein


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Fourteenth ICPO - INTERPOL
Forensic Sciences Symposium
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Lyon, France
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Notes:
1. All categories are subdivided by topic or category, then alphabetically by the first author's name.
2. Where appropriate, a short explanatory note is added to the citation to provide additional detail concerning the reference.
3. Note that the following references are law enforcement restricted, and not available to the general public: Microgram and Microgram Bulletin prior to 2003, and the Journal of the Clandestine Laboratory Investigating Chemists Association (all years).
I) Routine and Improved Analysis of Abused Substances

Issue:
Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new homolog or analog (i.e., "designer"-type) drugs.

Solution:
Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general field of analytical chemistry, provide new and/or improved methods of analysis for both routine and specialized analyses of seized drugs. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

References:

Reviews:


**Scientific Working Group for Forensic Analysis of Illicit Drugs:**


**Amphetamine, Methamphetamine, and Dimethylamphetamine (see also Substituted Amphetamines, Phenethylamines, and Methylenedioxyamphetamines):**

10) Brown H, Kirkbride KP, Pigou PE, Walker GS. New developments in SPME, Part 1: The use of vapor-phase deprotonation and on-fiber derivatization with alkylchloroformates in the analysis of preparations containing amphetamines. Journal of Forensic Sciences 2003;48(6):1231. [Presents a method for conversion of solid drug salts to their free bases, capture via SPME, and analysis by GC/MS. The technique can be used for noninvasive recovery from consumer items such as banknotes and garments. Use of on-fiber derivatization with alkylchloroformates improves chromatography and also allows for enantiomer determinations.]


15) Lua AC, Chou TY. Preparation of immunoaffinity columns for direct enantiomeric separation of amphetamine and/or methamphetamine. Journal of Chromatography A 2002;967(2):191. [For direct enantiomeric determination of amphetamine and methamphetamine in urine.]


**para-Substituted Amphetamines:**


Barbiturates:

20) Bartzatt R. Determination of barbituric acid, utilizing a rapid and simple colorimetric assay. Journal of Pharmaceutical and Biomedical Analysis 2002;29(5):909. [Presents three assay methods, which can be utilized on either aqueous or solid samples.]

21) Chang W-T, Smith J, Liu RH. Isotopic analogs as internal standards for quantitative GC/MS analysis - Molecular abundance and retention time differences as interference factors. Journal of Forensic Sciences 2002;47(4):873. [Isotopic analogues of five barbiturates were evaluated as internal standards for GC/MS analyses.]


Benzodiazepines:

25) Aebi B, Sturny-Jungo R, Bernhard W, Blanke R, Hirsch R. Quantitation using GC-TOF-MS: Example of bromazepam. Forensic Science International 2002;128(1-2):84. [Various methods are used to validate the use of GC-TOF-MS for analysis of bromazepam; diazepam and nordiazepam were also studied, but to a lesser extent.]

26) Bakavoli M, Kaykhaii M. Quantitative determination of diazepam, nitrazepam and flunitrazepam in tablets using thin-layer chromatography - densitometry technique. Journal of Pharmaceutical and Biomedical Analysis 2003;31(6):1185. [Also includes
and contrasts HPLC analyses; UV (254 nm) detection was used for both techniques.]


28) Cahours X, Cherkaoui S, Rozing G, Veuthey JL. Microemulsion electrokinetic chromatography versus capillary electrochromatography-UV-mass spectrometry for the analysis of flunitrazepam and its major metabolites. Electrophoresis 2002;23(14):2320. [Flunitrazepam and its three major metabolites (in biological fluids) were separated by the title technique.]

29) Ferreyra CF, Ortiz CS. Simultaneous spectrophotometric determination of phenylpropanolamine HCl, caffeine and diazepam in tablets. Journal of Pharmaceutical and Biomedical Analysis 2002;29(5):811. [UV spectrophotometry and LC methods were used.]

30) Kamande MW, Kapnissi CP, Zhu XF, Akbay C, Warner IM. Open-tubular capillary electrophromatography using a polymeric surfactant coating. Electrophoresis 2003;24(6):945. [The title technique was applied to the analysis of benzodiazepines (not specified in the abstract).]

31) Pirnay S, Ricordel I, Libong D, Bouchonnet S. Sensitive method for the detection of 22 benzodiazepines by gas chromatography - ion trap tandem mass spectrometry. Journal of Chromatography A 2002;954:235. [The title technique method was applied to biological samples.]


33) Suzuki Y, Arakawa H, Maeda M. The capillary electrophoresis separation of benzodiazepine drugs using dextran sulfate and SDS as running buffer. Biomedical Chromatography 2004;18(3):150. [Presents the EKC analysis of 10 benzodiazepines (not specified in abstract). The authors claim that the presented method may also be used for many other pharmaceuticals.]

Dimethoxyphenethylamines:

35) Curtis B, Kemp P, Harty L, Choi C, Christensen D. Postmortem identification and quantitation of 2,5-dimethoxy-4-n-propylthiophenethylamine using GC-MSD and GC-NPD. Journal of Analytical Toxicology 2003;27(7):493. [Primary focus is analysis of biological fluids and tissue samples; however, includes a small scale mass spectra (from GC/MS) of the title compound (i.e., 2C-T-7).]

Chlordiazepoxide:

36) EHEfnawey GB, ElHallag IS, Ghoneim EM, Ghoneim MM. Voltammetric behavior and quantification of the sedative-hypnotic drug chlordiazepoxide in bulk form, pharmaceutical formulation, and human serum at a mercury electrode. Journal of Pharmaceutical and Biomedical Analysis 2004;34(1):75. [Includes comparisons against existing methods.]

Clenbuterol:


Cocaine:

41) Koulis CV, Reffner JA, Bibby AM. Comparison of transmission and internal reflection spectra of cocaine. Journal of Forensic Science 2001;46(4):822. [Study is on cocaine hydrochloride; includes cautionary notes on the use of ATR.]


**Ergot Alkaloids (see also LSD):**


**Fentanyl(s):**

45) DeBoer D, Goemans WPJ, Ghezavat VR, vanOoijen RD, Maes RAA. Seizure of illicitly produced para-fluorofentanyl: Quantitative analysis of the content of capsules and tablets. Journal of Pharmaceutical and Biomedical Analysis 2003;31(3):557. [Presents a GC/MS methodology for the title analysis; HPLC/UV was also used to quantify caffeine being used as an adulterant. The samples derived from an illicit laboratory in the Netherlands.]


47) Van Nimmen NFJ, Veulemans HAF. Development and validation of a highly sensitive
gas chromatographic - mass spectrometric screening method for the simultaneous determination of nanogram levels of fentanyl, sufentanil, and alfentanil in air and surface contamination wipes. Journal of Chromatography A 2004;1035(2):249. [Focus is on sampling for industrial occupational exposure. The technique uses SIM.]

**Flos daturae:**


**Fluoxetine (Prozac):**


**Heroin:**


51) Fitsev IM, Budnikov GK, Blokhin VK, Teslenko PG. Gas chromatographic determination of diacetylmorphine with mass spectrometric detection. Journal of Analytical Chemistry (English translation of Zhurnal Analiticheskoi Khimii) 2003;47(9-12):423. [Appears to be a GC/MS method for analysis of heroin in fluids (not clear in abstract).]

52) Kulikowska J, Celinski R, Soja A, Sybirksa H. Investigations on the quality of home-made poppy straw products ("Compote") at the forensic medicine department in Katowice. Proceedings, 39th Annual TIAFT Meeting, Prague, 2001. [Illicit production of morphine and heroin in Poland (from poppy straw) is reviewed, and the techniques used for analysis of these products are discussed.]


**gamma-Hydroxybutyric Acid (GHB), gamma-Butyrolactone (GBL) and 1,4-Butanediol (BD):**

55) Alston WC, Ng K. Rapid colorimetric screening test for gamma-hydroxybutyric acid (Liquid X) in human urine. Forensic Science International 2002;126(2):114. [Based on the ferric hydroxamate test for ester detection; takes 5 minutes and has a detection limit 0.1 mg/mL for 1 mL samples.]


57) Bravo DT, Harris DO, Parsons SM. Reliable, sensitive, rapid, and quantitative enzyme-based assay for gamma-hydroxybutyric acid (GHB). Journal of Forensic Sciences 2004;49(2):379. [Several assays are presented for detection of GHB in beverages and urine.]


59) Chappell JS, Meyn AW, Ngim KK. The extraction and infrared identification of gamma-hydroxybutyric acid (GHB) from aqueous solutions. Journal of Forensic Sciences 2004;49(1):52. [Presents a liquid-liquid extraction technique for isolating GHB free acid, with analysis by IR.]

60) Chew SL, Meyers JA. Identification and quantitation of gamma-hydroxybutyrate (NaGHB) by nuclear magnetic resonance spectroscopy. Journal of Forensic Sciences 2003;48(2):292. [Presents an NMR technique for identification and quantitation of GHB; the identification of GBL by NMR is also presented.]

61) Ciolino LA, Mesmer MZ, Satzger RD, Machal AC, McCauley HA, Mohrhaus AS. The chemical interconversion of GHB and GBL: Forensic issues and implications. Journal of

63) DeFrancesco JV. An NMR study of the stability of gamma-butyrolactone (GBL) in water. Proceedings of the American Academy of Forensic Sciences 2003;9:32. [Presents a study of the conversion of GBL to GHB over time, starting with different concentrations of GBL.]

64) Duer WC, Byers KL, Martin JV. Application of a convenient extraction procedure to analyze gamma-hydroxybutyric acid in fatalities involving gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol. Journal of Analytical Toxicology 2001;25(7):576.

65) Garcia AD, Catterton AJ. 1,4-Butanediol (BD) - Forensic profile. Microgram Journal 2003;1(1-2):44.

66) Meyers JE, Garcia AD, Almirall JR. The analysis of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in forensic samples using GC/MS and H1-NMR. Proceedings of the American Academy of Forensic Sciences 2003;9:30. [Presents the referenced analyses, and also discusses the interconversion between the two substrates. SPME was utilized to recover the substrates for analysis.]

67) Meyers JE, Almirall JR. The analysis of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in forensic samples using gas chromatography/mass spectrometry (GC/MS) and proton nuclear magnetic resonance (H1-NMR). Proceedings of the American Academy of Forensic Sciences 2004;10:57. [Further investigates the interconversion between GHB and GBL, and presents a procedure for avoiding interconversion prior to analysis.]


70) Smith JV. Method for detection of 4-hydroxybutyric acid and its precursor(s) in fluids. U.S. US 6,617,123 (Cl. 435-19; C12Q1/44), 9 Sep 2003, Appl 607,026, 29 Jun 2000. [Appears to be a detection method for adulterated beverages (not biological fluids).]

71) Sabucedo AJ, Furton KG. Extractionless GC/MS analysis of gamma-hydroxybutyrate and gamma-butyrolactone with trifluoroacetic anhydride and heptafluoro-1-butanol from aqueous samples. Proceedings of the American Academy of Forensic Sciences 2004;10:109. [GHB can be derivatized directly in water solutions, without organic solvent extraction needed. GABA, diethylene glycol, BD, and GBL were analyzed under the same conditions (GBL gave a small response from conversion to GHB).]


74) Witkowski MR, Ciolino LA, DeFrancesco JV. GHB free acid: More on issues of interconversion with isolation and spectroscopic characterization of forensic analysis. Proceedings of the American Academy of Forensic Sciences 2003;9:30. [A forensic profile of the free acid (versus the more commonly encountered base form) is presented and discussed.]


**Ketamine:**


**Khat:**

77) Kite GC, Ismail M, Simmonds MSJ, Houghton PJ. Use of doubly protonated molecules

**LSD:**


**Marijuana and Related Cannabinoids:**


83) Fucci N. Growing cannabis with naphthalene in Rome. Forensic Science International 2003;138(1-3):91. [Presents the analysis of marijuana that was treated with naphthalene as a pesticide in a moderate sized home grow operation (80 plants); naphthalene was found in high concentration in the marijuana.]

85) Gambaro V, Dell'Acqua L, Fare F, Froldi R, Saligari E, Tassoni G. Determination of primary active constituents in cannabis preparations by high-resolution gas chromatography/flame ionization detection and high-performance liquid chromatography/UV detection. Analytica Chimica Acta 2002;468(2):245. [Presents a comparative study between the two title techniques for the complete, quantitative analysis of all the active constituents in cannabis. Validation studies were carried out on hashish.]

86) Gilmore S, Peakall R. Isolation of microsatellite markers in Cannabis sativa L. (marijuana). Molecular Ecology Notes 2003;3(1):105. [Fifteen markers were identified that can characterize genetic diversity in cultivated and natural marijuana populations.]

87) Gilmore S, Peakall R, Robertson J. Short tandem repeat (STR) DNA markers are hypervariable and informative in Cannabis sativa: Implications for forensic investigations. Forensic Science International 2003;131(1):65. [Presents a profiling study of 93 individual cannabis plants of widespread origin, using 5 STR markers. The authors claim that source determination is possible using the presented methods.]


90) Szabady B, Hidvegi E, Nyiredy S. Determination of neutral cannabinoids in hemp samples by overpressured-layer chromatography. Chromatographia 2002;56(Suppl. S):S165. [The overpressured-layer chromatographic separation of neutral cannabinoids (delta(9)-tetrahydrocannabinol, cannabidiol, cannabinol, cannabigerol and cannabichromene) was achieved on amino HPTLC plates, using dichloromethane as the mobile phase.]

91) Wojtasik E, Anyzewska M, Arent I. The optimization of the separation conditions for

**Mescaline/Peyote:**


**Methadone:**


**Methylenedioxymphetamines and Related Compounds:**

94) Aalberg L, DeRuiter J, Noggle FT, Sippola E, Clark CR. Chromatographic and spectroscopic methods of identification for the side-chain regioisomers of 3,4-methylenedioxyphenethylamines related to MDEA, MDMMA, and MBDB. Journal of Chromatographic Science 2003;41(5):227. [Presents the synthesis and GC and GC/MS analyses of ten closely related 3,4-methylenedioxyphenethylamines all having a molecular weight of 207.]


97) Huang YS, Liu JT, Lin LC, Lin CH. Chiral separation of 3,4-methylenedioxymethamphetamine and related compounds in clandestine tablets and urine by capillary electrophoresis/fluorescence spectroscopy. Electrophoresis 2003;24(6):1097. [MDA was also analyzed. Contrasts the title analysis with standard GC/MS methods.]

A study of Ecstasy tablets by TLC, UV, HPLC-DAD, and MS.

99) Piette V, Parmentier F. Analysis of illicit amphetamine seizures by capillary zone electrophoresis. Journal of Chromatography A 2002;979:345. [Presents a CZE methodology for analysis of typical drugs found in Ecstasy tablets]


102) Schneider RC, Kovar KA. Analysis of ecstasy with a monolithic reverse-phase column. Chromatographia 2003;57(5-6):287. [Presents an HPLC method that analyzes for amphetamine, MDMA, MDEA, and N-methyl-1-(3,4-methylenedioxymethylphenyl)-2-butanamine in suspected ecstasy tablets.]

103) Schneider RC, Kovar K-A. Analysis of ecstasy tablets: Comparison of reflectance and transmittance near infrared spectroscopy. Forensic Science International 2003;134(2-3):187. [Presents analyses of mixed composition tablets by the title techniques; transmittance mode was found to be better than reflectance mode.]

Methylphenidate:


Morphine, Codeine, and Related Opium Alkaloids:

106) Baeyens WRG, VanderWeken G, Smet E, GarciaCampana AM, Remon JP. Comparison
of morphine and hydromorphone analysis on reversed phase columns with different diameters. Journal of Pharmaceutical and Biomedical Analysis 2003;32(4-5):913. [Presents the analysis of the title compounds by HPLC on 2, 3, and 4 mm i.d. RP columns with UV detection.]

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


111) Garrido JMPJ, Delerue-Matos C, Borges F, Macedo TRA, Olivera-Brett AM. Electroanalytical determination of codeine in pharmaceutical preparations. Analytical Letters 2002;35(15):2487. [Presents a square wave voltametric (SWV) method and a flow injection analysis system with electrochemical detection (FIA-EC) for determination of codeine in various pharmaceutical preparations. Limitations with certain co-ingredients (e.g., acetaminophen) are discussed.]


120) Sun GX, Wang Y, Sun YQ. The quantitative determinations of glycyrrhizic acid, glycyrrhetic acid, morphine, and sodium benzoate in compound liquorice tablets by HPCE. Journal of Liquid Chromatography and Related Technologies 2003;26(1):43. [Presents a CZE/UV method to perform the title analysis.]


**Opiate Alkaloids:**

122) Kuznetsov PE, Aparkin AM, Zlobin VA, Nazarov GV, Kosterin PV, Lyubun’ EV,


Opium (and Opium Poppies):

124) Lurie IS, Panicker S, Hays PA, Garcia AD, Geer BL. Use of dynamically coated capillaries with added cyclodextrins for the analysis of opium using capillary electrophoresis. Journal of Chromatography A 2003;984(1):109. [Presents a rapid, precise, accurate, and robust method for analysis of the major opium alkaloids in either opium gum or latex. The same conditions may be utilized to analyze LSD exhibits.]

125) Reddy MM, Suresh V, Jayashanker G, Rao BS, Sarin RK. Application of capillary zone electrophoresis in the separation and determination of the principal gum opium alkaloids. Electrophoresis 2003;24(9):1437. [The presented method does not require sample purification or derivatization.]

126) Szucs Z, Szabady B, Szatmary M, Cimpan G, Nyiredy S. High-throughput analytical strategy with combined planar and column liquid chromatography for improvement of the poppy (Papaver somniferum L.) with a high alkaloid content. Chromatographia 2002;56(Suppl. S):S49. [Four different liquid chromatographic methods (multi-layer overpressured-layer chromatography (MLOPLC), normal-phase high-performance thin-layer chromatography (NPHPTLC), rapid reversed-phase high-performance liquid chromatography (RPHPLC), and a second, different RPHPLC method, were used for determination of alkaloid content of over 15,000 poppy capsule samples.]

Overview/Polydrug:

127) Peinhardt G. Identification of illegal drugs in pharmacy laboratories: Combination of thin layer chromatography and immunochemical quick tests. PZ Prisma 2002;9(2):99. [A combination of isolation and analytical methods are presented for detection and determination of cannabis, opiates, heroin, cocaine, amphetamines, designer drugs, and LSD.]
**Pethidine:**


**Phenethylamines (including mixtures of Amphetamines, Methylenedioxy-amphetamines, and Related Compounds):**

129) CampinsFalco P, VerduAndres J, HerraezHernandez R. Separation of the enantiomers of primary and secondary amphetamines by liquid chromatography after derivatization with (−)-1-(9-fluorenyl)ethyl chloroformate. Chromatographia 2003;57(5-6):309. [Analysis of amphetamine, methamphetamine, ephedrine, pseudoephedrine, MDA, MDMA, and MDE are reported. A variety of sample types (not specified in the abstract) were analyzed.]


132) Iwata YT, Kanamori T, Ohmae Y, Tsujikawa K, Inoue H, Kishi T. Chiral analysis of amphetamine-type stimulants using reversed-polarity capillary electrophoresis/positive ion electrospray ionization tandem mass spectrometry. Electrophoresis 2003;24(11):1770. [Presents the specialized CE/MS-MS analyses of a variety of ATS's, ranging from precursor ephedrines to methylenedioxy- substituted drugs.]


**Piperazines:**


136) Kercheval JC. GC/MS analysis of BZP and TFMPP. Mid-Atlantic Association of Forensic Sciences Newsletter 2004;32(2) (no page numbers). [Presents the GC/MS analyses of 1-benzylpiperazine and 1-(3-trifluoromethylphenyl)-piperazine.]


**Polydrug:**

138) Bazylak G, Nagels LJ. Simultaneous high-throughput determination of clenbuterol, ambroxol and bromhexine in pharmaceutical formulations by HPLC with potentiometric detection. Journal of Pharmaceutical and Biomedical Analysis 2003;32(4-5):887. [The title analysis was performed using six different isocratic systems.]

139) Benson AJ, Sabucedo A, Furton KG. Detection and identification of date rape drugs gamma-hydroxybutyrate (GHB), flunitrazepam (Rohypnol), lysergic acid diethylamide (LSD), scopolamine, diphenhydramine, and ketamine by refocused solid phase microextraction high performance liquid chromatography (SPME/HPLC) and solid phase microextraction high performance liquid chromatography mass spectrometry (SPME/HPLC/MS). Proceedings of the American Academy of Forensic Sciences 2003;9:29. [Presents a study of the SPME followed by HPLC and HPLC/MS for analysis of the referenced drugs.]


144) Cherkaoui S, Veuthey JL. Use of negatively charged cyclodextrins for the simultaneous enantioseparation of selected anesthetic drugs by capillary electrophoresis-mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2002; 27(3-4):615. [Presents the enantioseparation of bupivacaine, mepivacaine, ketamine, and prilocaine.]

145) Geraghty E, Wu C, McGann W. Effective screening for "Club Drugs" with dual mode ion trap mobility spectrometry. International Journal for Ion Mobility Spectrometry 2002;5(3):41. [Presents a study on the analysis of various "Rave" drugs by dual mode ITMS, including: Ketamine, GHB, ephedrine, flunitrazepam, methamphetamine, MDA, amphetamine, and MDMA.]


149) Lurie IS. Capillary electrophoresis analysis of a wide variety of seized drugs on the same dynamically coated capillary. Proceedings of the American Academy of Forensic Sciences 2004;10:107. [Drug types include phenethylamines, cocaine, heroin, oxycodone, morphine, LSD, psilocybin, opium, and GHB/GBL; both qualitative and quantitative results are achieved.]


151) Morehead RA. Optimizing HPLC separation of antidepressant drugs through stationary phase selection. Proceedings of the American Academy of Forensic Sciences 2003;9:304. [Includes a discussion of the primary separation mechanisms for 14 drugs; the referenced drugs were not identified.]

152) Pihlainen K, Kostiainen R. Effect of the eluant on enantiomer separation of controlled drugs by liquid chromatography - ultraviolet absorbance detection - electrospray ionisation tandem mass spectrometry using vancomycin and native beta-cyclodextrin chiral stationary phases. Journal of Chromatography A 2004;1033(1):91. [Presents the title study on nine amphetamine derivatives (not specified in abstract), methorphan, and propoxyphene. 14 seized drug samples (not specified in abstract) were analyzed using the optimized methodologies.]

**Propoxyphene:**

153) Magoon T, Ota K, Jakubowski J, Nerozzi M, Werner TC. The use of neutral cyclodextrins as additives in capillary electrophoresis for the separation and identification of propoxyphene enantiomers. Analytical and Bioanalytical Chemistry 2002;373(7):628. [Baseline separation was achieved in approximately 6 minutes.]

**Psilocybin Mushrooms, Psilocybin, and Psilocin:**

154) Linacre A, Cole M, Chun-I Lee J. Identifying the presence of "magic mushrooms" by DNA profiling. Science and Justice 2002;42(1):50. [Presents a minor review of DNA-based analyses of psilocybe and panaeolus mushrooms. The techniques are especially valuable for cases of dry, powdered material where microscopic
characterization is impossible.]


Psilocybe viridis:


Salvia divinorum:


Steroids:

compound has never been commercially marketed, and suggest that a clandestine source may therefore be in operation.]


163) Leinonen A, Kuuranne T, Kostiainen R. Liquid chromatography/mass spectrometry in anabolic steroid analysis-optimization and comparison of three ionization techniques: Electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization. Journal of Mass Spectrometry 2002;37(7):693. [The presented LC/MS/MS technique exhibited high sensitivity and specificity for the detection of various steroids, and may be a suitable technique for screening for the abuse of anabolic steroids.]


(Designer) Tryptamines (see also Psilocybin):


167) Meatherall R, Sharma P. Foxy, a designer tryptamine hallucinogen. Journal of Analytical Toxicology 2003;27(5):313. [Primary focus is analysis of biological fluids; however, includes a small scale mass spectra (from GC/MS) of "Foxy" (5-methoxy-N,N-diisopropyltryptamine).]

Zaleplon:


Zolpidem:

170) ElZeany BA, Moustafa AA, Farid NF. Determination of zolpidem hemitartrate by quantitative HPTLC and LC. Journal of Pharmaceutical and Biomedical Analysis 2003;33(3):393. [Presents the analyses of zolpidem in the presence of its degradation product by TLC-UV densitometry and by HPLC with UV detection.]

Zopiclone:


Miscellaneous:

172) Bartlett V. HPLC analysis of narcotic/acetaminophen admixtures. What to do if a compendium method doesn't work. The Restek Advantage 2002;3:6. [Discusses modifications to established methods for separating admixtures of compounds with similar structures.]
II) **Synthesis and/or Cultivation of Abused Substances, their Precursors, and Essential Chemicals**

**Issue:**
Forensic chemists must maintain familiarity with existing and new clandestine syntheses of abused substances, their precursors, and essential chemicals, and with the cultivation of abused natural products, in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and to provide expert testimony in legal proceedings.

**Solution:**
Illicit drug seizures, clandestine laboratory operations, and illicit grow operations, are continuously monitored to maintain a comprehensive overview of the field. In cases where new drugs are synthesized, or new methodologies are utilized, case reports are generated for the forensic and enforcement communities.

**References:**

**Production of Abused Substances and/or their Precursors and Essential Chemicals:**


of the common illicit syntheses of a variety of hallucinogens.]


III) Clandestine Laboratories - Appraisals and Safety

Issue:
Forensic chemists must maintain familiarity with clandestine laboratory procedures, setups, and techniques in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and in order to provide expert testimony in court proceedings.

Solution:
Clandestine laboratory operations are continuously reviewed to provide a comprehensive overview of the field. In cases where new methodologies are noted, or unusual safety concerns are salient, reports are generated for the forensic and enforcement communities.

References:

Clandestine Laboratory Appraisals and Safety:


dumpsites in Australia.]


**Safety Issues - Case Reports:**


**Miscellaneous:**


destruction).

IV) Reference Drug Standards and Total Syntheses

Issue:
Many reference drug standards or structurally related internal standards are either commercially unavailable, or if available are extremely expensive.

Solution:
Controlled substances and their structural or isotopically labelled analogs are synthesized as needed. Internal standards are also prepared as needed. Case reports are published for new or unusual standards or improved synthetic approaches.

References:


197) Klemenc S. 4-Dimethylaminopyridine as a catalyst in heroin synthesis. Forensic Science International 2002;129(3):194. [Presents a study on the acetylation of morphine using 4-dimethylaminopyridine (4-DMAP) as a catalyst.]


V) **Source Determination of Drugs (Impurity Profiling) and Comparative Analyses**

**Issues:**

Impurity profiling of drugs is important for comparative analysis protocols, geo-sourcing, and synthetic route determinations. However, although certain drugs have been well characterized with respect to their impurity profiles, most have not been properly investigated. Comparative analysis (i.e., the systematic application of impurity profiling for determination of commonality of origin) is complicated due to both the high complexity of the data and the large numbers of exhibits. Improved analytical and data handling techniques are needed.

**Solution:**

High sensitivity analytical techniques (primarily chromatographic) provide detailed profiles of trace-level impurities, ions, trace metals, and stable isotopes. Identification of individual impurities enhance origin identification and comparative analyses and also aid in development of internal standards for improved accuracy and precision of analysis.

In-depth analysis via improved instrumental methodologies help identify discriminatory components in impurity profiles. Computer databases, sorting programs, and pattern recognition/neural networks provide enhanced data handling and analysis, enabling and improving comparative analyses. Case reports are generated for the forensic and enforcement communities.

**References:**

**Amphetamine(s):**


209) Carter JF, Titterton EL, Grant H, Sleeman R. Isotopic changes during the synthesis of amphetamines. Chemical Communications 2002;21:2590. [Presents a study of the variations in C-13 and N-15 during various syntheses of amphetamine. The authors also claim that isotopic characterization can assist in identifying the synthetic origins of illicit MDMA and other amphetamines.]


**Cocaine:**


**Cocaine and Heroin:**

213) Galimov EM, Sevast'yanov VS, Kul'bachevskaya EV, Golyavin AA. Determination of isotopic compositions of carbon and nitrogen by the IRMS method: Implication for the source of narcotic substance origin. Doklady Earth Sciences 2003;393(8):1109. [Presents the title study on cocaine and heroin from different regions.]

**Dimethylamphetamine:**


**Heroin:**

215) Bora T, Merdivan M, Hamamci C. Levels of trace and major elements in illicit heroin. Journal of Forensic Sciences 2002;47(5):959. [Ten elements in 44 illicit heroin samples were determined using electrothermal atomic absorption spectrometry or inductively coupled plasma-atomic emission spectrometry.]


220) Hajdar M, Ruzdic E. Characterisation of heroin samples obtained in the area of the Federation of Bosnia and Herzegovina. Journal of Environmental Protection and Ecology 2003;4(4):873. [Presents the title survey, using GC/FID analysis to detect 8 opium alkaloids and 3 typical adulterants. The number of samples and the date range were not specified in the abstract.]


222) Zhang D, Shi X, Yuan Z, Ju H. Component analysis of illicit heroin samples with GC/MS and its application in source determination. Journal of Forensic Sciences 2004;49(1):81. [Presents a profiling analysis based on both GC and GC/MS. 500 samples were subclassified into nine groups using the presented techniques.]

**Marijuana:**


**Methamphetamine:**

225) Inoue H, Kanamori T, Iwata YT, Ohmae Y, Tsujikawa K, Saitoh S, Kishi T. Methamphetamine impurity profiling using a 0.32 mm i.d. nonpolar capillary column. Forensic Science International 2003;135(1):42. [The presented method allows for determination of 24 different characteristic starting materials and manufacturing byproducts.]


227) Koester CJ, Andresen BD, Grant PM. Optimum methamphetamine profiling with sample preparation by solid-phase microextraction. Journal of Forensic Sciences 2002;47(5):1002. [Volatile and semi-volatile components are recovered from illicit methamphetamine by SPME and analyzed by GC/MS. The method is claimed to be superior for profiling illicit methamphetamine.]

228) Kubicz-Loring E. Illicit methamphetamine profiling. Proceedings of the American Academy of Forensic Sciences 2003;9:30. [The impurity profiles of methamphetamine produced via the HI/red P reduction and Li/NH3 reductions are discussed and contrasted.]


4-Methoxyamphetamine and 4-Methoxymethamphetamine:


235) Waumans D, Bruneel N, Tytgat J. Anise oil as para-methoxyamphetamine (PMA) precursor. Forensic Science International 2003;133(1-2):159. [Presents a study of a large-scale PMA laboratory using anise oil as a precursor source. Includes impurity profiling studies that identified marker compounds for this synthesis.]

236) Waumans D, Bruneel N, Hermans B, Tytgat J. A rapid and simple GC/MS screening method for 4-methoxyphenol in illicitly prepared 4-methoxy-amphetamine (PMA). Microgram Journal 2003;1(3-4):184. [Confirms that 4-methoxyphenol is a marker compound for syntheses of PMA starting from anethole.]

Methylenedioxyamphetamines:

237) Armellin S, Brenna E, Fronza G, Fuganti C, Pincirola M, Serra S. Establishing the synthetic origin of amphetamines by H-2 NMR spectroscopy. Analyst 2004;129(2):130. [The title study was applied to nine samples of N+acetyl-MDA.]

238) Bell SEJ, Barrett LJ, Burns DT, Dennis AC, Speers SJ. Tracking the distribution of "ecstasy" tablets by Raman composition profiling: A large scale feasibility study. Analyst 2003;128(11):1331. [Approximately 1500 tablets (all primarily MDMA) from different seizures in Northern Ireland were analyzed and found to have significant differences in their Raman spectra due to the presence of impurities and the degree of hydration of the MDMA. The results indicated that sample-sample comparisons could be
accomplished using Raman spectroscopy.]

239) Carter JF, Titterton EL, Murray M, Sleeman R. Isotopic characterization of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethylamphetamine (Ecstasy). Analyst 2002;127(6):830. [Via analysis by IRMS and Deuterium NMR.]


242) Gimeno P, Besacier F, Chaudron-Thozet H. Optimization of extraction parameters for the chemical profiling of 3,4-methylenedioxymethamphetamine (MDMA) tablets. Forensic Science International 2003;132(3):182. [Presents an optimized extraction procedure for recovery of impurities from MDMA tablets using diethyl ether extraction from a pH 11.5 buffered solution, followed by GC/MS analysis.]


244) Palhol F, Boyer S, Naulet N, Chabrillat M. Impurity profiling of seized MDMA tablets by capillary gas chromatography. Analytical and Bioanalytical Chemistry 2002;374(2):274. [Presents a study of MDMA tablets seized in France (total number not specified in the abstract). The authors claim that the results suggest that MDP2P is the most commonly used precursor, and that reductive amination is the most common synthetic route used to prepare the MDMA found in the tablets.]

245) Palhol F, Lamoureux C, Naulet N. N-15 Isotopic analyses: A powerful tool to establish links between seized 3,4-methylenedioxymethamphetamine (MDMA) tablets. Analytical and Bioanalytical Chemistry 2003;376(4):486. [Forty-three samples were analyzed by GC-Combustion-IRMS; the authors indicate that the technique can help establish common origins between samples.]

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246) van der Peijl GJQ, van den Boom CPH, Bolck A, Dobney AM. XTC characterisation [sic] using ICPMS. Proceedings of the American Academy of Forensic Sciences 2004;10:53. [Presents the results of an ICPMS study of about 100 ecstasy samples.]

247) Titterton E, Carter J, Murray M, Sleeman R. Characterisation [sic] of ecstasy tablets by isotope ratio mass spectrometry. Proceedings of the 16th Meeting of the International Association of Forensic Sciences, Montpellier, France, September 2-7, 2002, pps 111-115. [MDA- and MDMA-based Ecstasy tablets were analyzed for deuterium, carbon-13, and nitrogen-15 to derive a isotopic fingerprint. Deuterium substitution was also determined via deuterium NMR.]

248) Vohlken BA, Layton SM. Instrumental separation of 3,4-methylenedioxy-amphetamine (MDA) from 1-(3,4-methylenedioxyphenyl)-2-propanol, a co-eluting compound. Microgram Journal 2003;1(1-2):32. [Presents a study of the referenced co-elution problem; includes the mass spectra for the title alcohol.]

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

250) Waddell RJH, NicDaeid N, Littlejohn D. Classification of ecstasy tablets using trace metal analysis with the application of chemometric procedures and artificial neural network algorithms. Analyst 2004;129(3):235. [Presents a study of the practicality of ICP-MS for sample-sample comparisons. Several statistical analyses are evaluated.]

**Opium and Opium Alkaloids:**

251) Al-Amri AM, Smith RM, El-Haj BM, Juma'a MH. The GC-MS detection and characterization of reticuline as a marker of opium use. Forensic Science International 2004;140(2-3):175. [Reticuline was detected as its trimethylsilyl ethers, acetyl esters, and methyl ethers, in opium and in the urine of opium users. The results can be used to differentiate between opium and heroin users.]


253) Kelly SA, Glynn PM, Madden SJ, Grayson DH. Impurities in a morphine sulfate drug product identified as 5-(hydroxymethyl)-2-furfural, 10-hydroxymorphine and
10-oxomorphine. Journal of Pharmaceutical Sciences 2003;92(3):485. [The referenced impurities were isolated by semi-prep HPLC and identified via MS and NMR. The presence of sugars in the drug formulation was implicated in the formation of the impurities.]

**Occluded Solvent Analyses:**

254) Camarasu CC. Unknown residual solvents identification in drug products by headspace solid phase microextraction gas chromatography-mass spectrometry. Chromatographia 2002;56(Suppl. S):S131. [Presents a sensitive headspace SPME method for the extraction of residual solvents from pharmaceutical products (the specific products were not detailed in the abstract). The SPME method appears to be more sensitive than static headspace techniques.]

**Multi-Drug and Miscellaneous:**

255) Binder R, Machata G, Stead H. Analysis of potassium permanganate as a narcotic drug precursor. Archiv fur Kriminologie 2003;211:160. [Thirty-one samples were analyzed for 9 metallic elements using emission spectroscopy and ICP-OES. The results did not allow classification of the samples according to origin.]


258) Palhol F. Contribution of isotopic analyses to the fight against drug trafficking. Actualite Chimique 2003;(8-9):27. [Appears to be an overview of the topic (not clear from the abstract).]


260) Watanabe S, Shibata M, Kataoka K. Comparison of data obtained by various GC
methods for impurity profiling of stimulant drugs. Kanzei Chuo Bunsekishoho 2002;42:73. [Three different GC methods were used for impurity profiling of 10 typical impurities in 12 samples of stimulant drugs (not specified in abstract).]

VI) Analysis of Non-Controlled Pharmaceuticals, Pseudo-Drugs, Adulterants, Diluents, and Precursors

Issue:
Most "street-level" drugs are "cut" with various adulterants and diluents. Many of these cutting agents are pharmaceutical products or precursors. Others are "carry-through" compounds present in precursors (especially in cold remedy products). Separation and identification of these extraneous materials can be tedious, especially in exhibits which contain many components. In addition, new or unusual adulterants and/or diluents are occasionally identified in drug exhibits, and standard analytical data are required for these substances. Finally, improved methods of analysis, i.e., faster, more discriminatory, less costly, etc., are needed for all cutting agents.

Solution:
Reports providing standard analytical data and/or improved analytical protocols for non-controlled pharmaceuticals, pseudo-drugs, adulterants, diluents, and precursors are generated for the forensic and enforcement communities.

References:

Creatine:


263) Dash AK, Sawhney A. A simple LC method with UV detection for the analysis of creatine and creatinine and its application to several creatine formulations. Journal of Pharmaceutical and Biomedical Analysis 2002;29(5):939. [Presents a simple and sensitive LC method for the determination of creatine and creatinine in various creatine supplement formulations.]


265) Wagner SD, Kaufer SW, Sherma J. Quantification of creatine in nutrition supplements by thin layer chromatography-densitometry with thermochemical activation of fluorescence quenching. Journal of Liquid Chromatography and Related Technologies
Ephedra, Ephedrine, and/or Pseudoephedrine and Related Compounds:


alkaloids by liquid chromatography/tandem mass spectrometry. JAOAC International 2003;86(3):471. [Presents an LC-MS/MS methodology for determination of six major ephedra alkaloids in various substrates, ranging from raw ephedra to a high-protein drink mix containing ephedra.]


278) Wedig M, Laug S, Christians T, Thunhorst M, Hozgrabe U. Do we know the mechanism of chiral recognition between cyclodextrins and analytes? Journal of Pharmaceutical and Biomedical Analysis 2002;27(3-4):531. [Chiral separation of ephedrine-type phenethylamines using various cyclodextrins is examined by CE and NMR.]

279) Ye NS, Gu XX, Zou H, Zhu RH. Separation and determination of ephedrine enantiomers by capillary electrophoresis using L-leucine as chiral selector. Chromatographia 2002;56(9-10):637. [The technique was applied to the analysis of ephedra plant extracts.]


281) Zhang JY, Xie JP, Chen XG, Hu ZD. Sensitive determination of ephedrine and pseudoephedrine by capillary electrophoresis with laser-induced fluorescence detection. Analyst 2003;128(4):369. [The title technique was applied to the analysis of ephedra and ephedra preparations.]

282) Zhang JY, Xie JP, Liu JQ, Tian JN, Chen XG, Hu ZD. Microemulsion electrokinetic chromatography with laser-induced fluorescence detection for sensitive determination of
ephedrine and pseudoephedrine. Electrophoresis 2004;25(1):74. [The two substrates were derivatized with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol prior to analysis. The technique was applied to Chinese traditional herbal preparations.]

**Phenylpropanolamine:**


**Other Adulterants/Diluents (including mixtures containing Ephedrine and/or Pseudoephedrine):**


285) Garcia A, Ruperez FJ, Marin A, delaMaza A, Barbas C. Poly(ethyleneglycol) column for the determination of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations. Journal of Chromatography B - Analytical Technologies in the Biomedical and Life Sciences 2003;785(2):237. [Presents a rapid, isocratic HPLC method for determination of the three title compounds in cold medications. UV detection at 215 nm and 310 nm was used.]

286) Geer LC, Hays PA. Letrozole (Femara®) Microgram Journal 2003;1(3-4):190. [Presents analytical data (GC/MS, FTIR, and NMR) for the title compound.]


289) Marin A, Garcia E, Garcia A, Barbas C. Validation of a HPLC quantification of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations: Capsules and sachets. Journal of Pharmaceutical and Biomedical Analysis 2002;29(4):701. [Presents the simultaneous determination and quantitation of the title compounds (and also phenylpropanolamine) in various pharmaceutical formulations.]


292) Qi ML, Wang P, Zhou L, Gu JL, Fu RN. Simultaneous determination of acetaminophen, dextromethorphen [sic] and pseudoephedrine hydrochloride in a new drug formulation for cold treatment by HPLC. Chromatographia 2003;57(3-4):139. [Presents a validated method for the referenced analysis, which is completed in less than 10 minutes per run.]

293) Rothchild R. Identification of a heroin diluent: One- and two-dimensional proton and carbon-13 NMR studies of procaine hydrochloride: Computational studies of procaine and its conjugate acid. Spectroscopy Letters 2003;36(1&2):35. [Presents the isolation (from a street sample of heroin) and identification of the title compound, and also presents ab initio molecular modeling calculations.]


**Theophylline:**

296) Huan L, Kan Q, Wang X, Lui X, Bi K. Simultaneous determination of the contents of
five components in compound theophyllini [sic] tablets by statistical-simulation spectrometry. Huaxue Fenxi Jiliang 2002;11(4):11. [Compounds determined included amidopyrine [sic], phenacetine [sic], theophylline, theobromine, and caffeine.]

VII) New and/or Improved Instrumental Techniques

Issue:
Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Solution:
Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

References:

**Capillary Electrophoresis (and Related Techniques, including Tandem Techniques):**


2001;41(3):203.


**Gas Chromatography (and Tandem GC Techniques):**


310) Dallabetta-Keller T. Trace analysis by GC/MS using pulsed splitless injections. Proceedings - NOBCChE 2001;28:4. [A pressure pulsed injection technique for GC/MS allows for enhanced detection of trace level controlled substances.]

311) Gorecki T, Harynuk J, Panic O. Comprehensive two-dimensional gas chromatography (GC x GC). New Horizons and Challenges in Environmental Analysis and Monitoring [Workshop], Gdansk, Poland, Aug. 18-29, 2003, pps 61-83. [Presented examples include (unspecified) forensic samples.]

High-Performance Liquid Chromatography (and tandem HPLC techniques):

313) Conemans JMH, Van Der Burgt AAM, Van Rooij JML, Pijnenburg CC. The simultaneous determination of illicit drugs with HPLC-DAD. Bull TIAFT 2004;34(1):11. [The presented method is applied to drug powders, various dosage forms, and various biological matrices, in a clinical setting.]


317) Perrin C, Matthijs N, Mangelings D, GranierLoyaux C, Maftouh M, Massart DL, VanderHeyden Y. Screening approach for chiral separation of pharmaceuticals; Part II. Reversed-phase liquid chromatography. Journal of Chromatography A 2002;966(1-2):119. [A screening strategy for the rapid separation of drug enantiomers by reversed-phase liquid chromatography is presented. Results for 37 diverse chiral pharmaceuticals are presented (the specific products were not detailed in the abstract).]


321) Sychev KS, Sychev SN. Application of universal mobile phases in high-effective liquid chromatography for analysis of the objects of food industry, criminology and pharmaceutical chemistry. Zavodskaya Laboratoriya, Diagnostika Materialov 2003;69(9):8. [Various diethylammonium based run buffers are examined for RP-HPLC.]


**Inductive Coupled Plasma- Mass Spectrometry (ICP-MS):**


**Infrared and Raman Spectroscopy:**


tablets (for forensic purposes).]

329) Jarman JL, Seerley SI, Todebush RA, de Haseth JA. Semiautomated depositor for infrared microspectrometry. Applied Spectroscopy 2003;57(9):1078. [Presents a novel method for depositing minute samples for IR microspectrometry (the authors suggest applicability to forensic analyses).]


**Ion Spectroscopy:**


**Mass Spectrometry:**


334) Libong D, Pirmay S, Bruneau C, Rogalewicz F, Ricordel I, Bouchonnet S. Adsorption-desorption effects in ion trap mass spectrometry using in situ ionization. Journal of Chromatography A 2003;1010(1):123. [Quadrupole mass spectrometers were compared for the GC/MS analyses of diazepam, alprazolam, triazolam, LSD, trimethylsilylated LSD, and trimethylsilylated buprenorphine.]


**Microchip Technology:**

Belder D, Deege A, Maass M, Ludwig M. Design and performance of a microchip electrophoresis instrument with sensitive variable-wavelength fluorescence detection. Electrophoresis 2002;23(14):2355. [A modular instrument for high-speed microchip electrophoresis equipped with a sensitive variable-wavelength fluorescence detection system was developed and evaluated using fluorescein isothiocyanate (FITC)-labelled amines, including amphetamine.]

Felton MJ. Lab on a chip: Poised on the brink. Analytical Chemistry 2003;75(23):505A. [A review of the topic, and an overview of the available instrumentation in the field.]

Harris CM. Shrinking the LC landscape. Analytical Chemistry 2003;75(3):65A. [A conversational overview of recent developments in chip-based technologies.]


**Nuclear Magnetic Resonance Spectroscopy:**


**Osmolality:**

Wesley JF. Osmolality - A novel and sensitive tool for detection of tampering of adulterated with ethanol, gamma-butyrolactone, and 1,4-butanediol, and for detection of dilution-tampered demerol syringes. Microgram Journal 2003;1(1-2):8. [Presents the title technique and various real-world applications.]

**Solid Phase Micro-Extraction:**


**Thin Layer Chromatography:**


**X-Ray based Techniques:**


350) Rendle DF. Use of X-rays in the United Kingdom Forensic Science Service. Advances in X-ray Analysis 2003;46:17. [Presents four case studies, including the use of XRD in the analysis of "street drug seizures" (not specified in the abstract)]
VIII) **Portable Detection and Analytical Instrumentation**

**Issue:**
"Free Trade" agreements and the easing of formally restrictive national and international borders have resulted in dramatic increases in cargo transshipments and personal travel, thereby complicating drug inspection and interdiction efforts at POEs. Discovery and confirmational analysis of suspected drugs in cargo or on individuals is severely hampered by the lack of on-site detection and/or analytical equipment.

**Solution:**
Development of portable and highly sensitive detectors for drug detection and analyses allows law enforcement personnel and/or forensic chemists to perform screening type analyses on-site. In those cases where new methodologies have proven effective, case reports are generated for the forensic and enforcement communities.

**References:**


352) Bannister WW, Chen C-C, Curby WA, Chen EB, Damour PL, Morales A. Thermal analysis for detection and identification of explosives and other controlled substances. U.S. US 6406918 B1 18 June 2002. [Includes identification of illicit drugs (i.e., in addition to explosives).]


355) Buryakov IA, Kolomiets YN. Rapid determination of explosives and narcotics using a
multicapillary-column gas chromatograph and an ion-mobility spectrometer. Journal of Analytical Chemistry - Russia (translation of Zhurnal Analiticheskoi Khimii) 2003;58(10):944. [The title technique was applied to detection of heroin, cocaine HCl and cocaine base (crack).]

356) Buryakov IA. Express analysis of explosives, chemical warfare agents, and drugs with multicapillary column gas chromatography and ion mobility increment spectrometry. Journal of Chromatography B - Analytical Technologies in the Biomedical and Life Sciences 2004;800(1-2):75. [The title technique was applied to analysis of heroin, cocaine hydrochloride, and cocaine base.]


361) Harris CM. Raman on the run. Analytical Chemistry 2003;75(3):75A. [A conversational overview of recent developments in portable Raman, including a comparative listing of five commercially available instruments.]

362) Kiraly B, Sanami T, Doczi R, Csikai J. Detection of explosives and illicit drugs using neutrons. Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms 2003;213:452. [Presents a Thermal Neutron Activation technique for the title analyses. The "illicit drugs" were not specified in the abstract.]


367) Smith WD. SAW chip sniffs out cocaine. Analytical Chemistry 2003;75(23):492A. [Presents an overview of the use of surface acoustic wave based devices for detecting cocaine vapor or particulates.]


IX) Miscellaneous

References:

Analytical Artifacts:

371) Varshney K-M. HPTLC study of the stability of heroin in methanol. *Journal of Planar Chromatography* 2002;15(1):46. [Presents the results of a degradation study of heroin in methanol (at room temperature). The results indicate degradation is measurable on Day 2, and is complete in around 38 weeks.]

Chemometrics:


Cocaine:

373) Brachet A, Rudaz S, Mateus L, Christen P, Veuthey J-L. Optimisation [sic] of accelerated solvent extraction of cocaine and benzoylecgonine from coca leaves. *Journal of Separation Science* 2001;24(10-11):865. [A variety of extraction parameters were varied to achieve the optimal results. Analysis was conducted by GC/FID and CE with UV detection.]


Counterfeit Drugs:


Dragon's Blood:

377) Edwards HGM, de Oliveira LFC, Prendergast HDV. Raman spectroscopic analysis of Dragon's Blood resins - Basis for distinguishing between Dracaena (Convallariaceae), Daemonorops (Palmae), and Croton (Euphorbiaceae). Analyst 2004;129(2):134.


Drugs on Currency:


Heroin:

383) Brazier JS, Morris TE, Duerden BI. Heat and acid tolerance of Clostridium novyi Type A spores and their survival prior to preparation of heroin for injection. Anaerobe 2003;9(3):141. [Presents the title study. This study was in followup to the outbreak of clostridium illnesses and deaths in the United Kingdom as a result of the use of contaminated heroin. The results indicate that typical heroin preparation procedures (by abusers) are not adequate to kill the spores.]

Khat:

history, cultivation, and constituents of khat; however, the primary focus is pharmacological.]

**Methamphetamine:**


**Qualitative Tests:**


389) Makarov SA, Simonov EA, Makarov VG, Kozlov AS. Method for determination of narcotic, psychotropic and offensive substances of plant and synthetic origin. Russ. RU 2,205,385 (Cl. G01N21/78) 27 May 2003, Appl. 2,002,103,845, 18 Feb 2002. [Appears to present a narcotics test kit (abstract is not clear).]


*Microgram Journal 2016, Volume 13; Numbers 1-4*

Quality Assurance:


Chang W-T, Smith J, Liu RH. Isotopic analogs as internal standards for quantitative GC/MS analysis - Molecular abundance and retention time differences as interference factors. Journal of Forensic Sciences 2002;47(4):873. [Isotopic analogues of five barbiturates were evaluated as internal standards.]


Hibbert DB. Scientist vs the law. Accreditation and quality assurance. 2003;8(5):179. [Presents an analysis of an Australian court case where convicted clandestine laboratory operators were acquitted on appeal due to alleged shortcomings in the laboratory's standard operating procedures.]


Moeller MR. Forensic conclusiveness and quality assurance of toxicological results. Research in Legal Medicine 2003;30:55. [An overview of the legal consequences of toxicological analyses.]

**Sampling Plans:**


**Surveys and Overviews:**


404) Briellmann TA, Dussy FE, Bovens MG. Forensic analysis of heroin and cocaine seizures. Chimia 2002;56:74. [Presents a survey and overview of seizures in Switzerland (date range not specified in abstract).]


416) Myers S. Forensic science. Nature 2003;421(6925):872. [A minor overview of the development of forensic DNA laboratories; includes some general comments of interest on the "real-life value" of forensic laboratories.]


419) Poon NL, Chong WC. Ecstasy in Hong Kong. Proceedings of the American Academy of
Forensic Sciences 2002;8:60. [An overview of the trends in ecstasy seizures in Hong Kong, including a review of tablet characteristics that might be valuable in source determinations.]


424) van Zundert M. Travel-pills, ecstasy pills, or Grandma's heart-rhythm pills? Pharmaceutisch Weekblad 2002;137(51/52):1825. [Appears to be a conversational overview presenting the use of TLC and GC for the identification of unknowns at a Dutch emergency pill identification lab.]


Other:


428) Bilia AR, Bergonzi MC, Lazari D, Vincieri FF. Characterization of commercial kava-kava herbal drug and herbal drug preparations by means of nuclear magnetic resonance spectroscopy. Journal of Agricultural and Food Chemistry 2002;50(18):5016. [NMR was used to determine the kavalactones in both a finely powdered herbal drug and a commercial extract.]


432) Harris HA, Newman MS, Montreuil RS, Goodrich JT. Comparison of extraction in a drop and solid phase microextraction. Proceedings of the American Academy of Forensic Sciences 2003;9:33. [Explains and compares the two referenced extraction techniques. Drugs utilized include cocaine, phenylpropanolamine, brompheniramine, and dextromethorphan.]


434) Mausolf N. The name of the test. Microgram 2001;34(9):235. [On the Duquenois and related tests for cannabis.]

436) Pitts SJ, Thomson CI. Analysis and classification of common vegetable oils. Journal of Forensic Sciences 2003;48(6):1293. [Presents methods of analysis for canola, corn, olive, peanut, safflower, soybean, and sunflower oils. (Although not stated, this study may also have value in the analysis of preparations of steroids in oils.)]

437) Puschel K, Stein S, Stobbe S, Heinemann A. Analysis of 683 drug packages seized from "body stuffers". Forensic Science International 2004;140(1):109. [Presents a short overview of the practice of internal carrying of controlled substances, with a discussion of packaging and drug types, as observed in Hamburg, Germany.]


The 2007 “Research on Drug Evidence” Report
[From the 15th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 2007 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

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Citations in this report from the Journal of the Clandestine Laboratory Investigating Chemists Association were (and remain) Law Enforcement Restricted.

The “General Overview” (Talking Paper) was removed from this reprint (Editor’s discretion).

This reprint is derived from the original electronic document, and is not an image of the best available hard copy (as was utilized for the 1995 and 1998 reports). For this reason, the pagination in the Proceedings is not retained in this reprint; in addition, minor corrections were made, (where present) "contact information" was removed, and some minor reformatting was done to eliminate deadspace. All widow and orphan lines were left as is. The references in this review were not numbered in the original document. The journal titles may be in complete or abbreviated forms, and the listed page(s) may be only the first page or the entire range (the titles and page(s) duplicate what was provided in the respective abstract).
Research On Drug Evidence
July 1, 2004 - June 30, 2007

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Fifteenth ICPO - INTERPOL
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Lyon, France
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Notes:
1. All categories are subdivided by topic or category, then alphabetically by the first author's last name.
2. Where appropriate, a short explanatory note is added to the citation to provide additional detail concerning the reference.
3. Note that the following reference is law enforcement restricted, and is not available to the general public: The Journal of the Clandestine Laboratory Investigating Chemists Association (all years).
I) **Routine and Improved Analysis of Abused Substances**

**Issue:**

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new homolog or analog (i.e., "designer"-type) drugs.

**Solution:**

Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general field of analytical chemistry, provide new and/or improved methods of analysis for both routine and specialized analyses of seized drugs. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

**References:**

**Reviews:**


Almirall JE. Forensic chemistry education. Analytical Chemistry 2004;77(3):69A. [An overview, including projected future needs.]


Cole M. Drugs of abuse. Crime Scene to Court 2004;293.

survey of unusual designer drugs (tablets) obtained at Dutch "smartshops". Primary drugs included 2C-B, 2C-T-2, and 2C-T-7. Includes social commentary and recommendations.]


Fitsev IM, Blokhin VK, Budnikov GK. Chromatographic techniques in forensic chemical examinations. Journal of Analytical Chemistry (Translation of Zhurnal Analiticheskoi Khimii) 2004;59(12):1171. [A minor review. (Unspecified) psychoactive drugs are discussed.]


**Amphetamine, Methamphetamine, and Dimethylamphetamine (see also Substituted Amphetamines, Phenethylamines, and Methyleneedioxyamphetamines):**


procedure for gas chromatography-mass spectrometry screening and quantitative
determination of amphetamine-type stimulants and related drugs in blood, serum, oral
fluid and urine samples. Journal of Chromatography B - Analytical Technologies in the
Biomedical and Life Sciences 2004;810(1):57.

Quantification of the amphetamine content in seized street samples by Raman
spectroscopy. Journal of Forensic Sciences 2007;52(1):88. [The results were favorably
compared against LC.]

Kato N, Fujita S, Kubo H, Homma H. Fluorescence analysis for p-hydroxymetham-
phetamine in urine by HPLC with post-column reaction. Journal of Liquid

Kawase K, Ogawa Y, Watanabe Y. Component pattern analysis of chemicals using
multispectral THz-imaging system. Proceedings of SPIE - The International Society for
discussion of the application of the technique for the detection of methamphetamine and
MDMA concealed in envelopes.]

Kim JY, Suh SI, In MK, Chung BC. Gas chromatography-high-resolution mass
spectrometric method for determination of methamphetamine and its major metabolite

Kimura H, Matsumoto K, Mukaida M. Rapid and simple quantitation of
methamphetamine by using a homogeneous time-resolved fluoroimmunoassay based on
fluorescence resonance energy transfer from Europium to Cy5. Journal of Analytical

Kishi T, Kanamori T, Tsujikawa K, Iwata YT, Inoue H, Ohtsuru O, Hoshina H, Otani C,
Kawase K. Differentiation of optical active form and racemic form of amphetamine-type

Klette KL, Jamerson MH, MorrisKukoski CL, Kettle AR, Snyder JJ. Rapid simultaneous
determination of amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine,
3,4-methylenedioxyamphetamine, and 3,4-methylenedioxymethylamphetamine in
urine by fast gas chromatography-mass spectrometry. Journal of Analytical Toxicology
Knops LA, Northrop DM, Person EC. Capillary electrophoretic analysis of phosphorus species in clandestine methamphetamine laboratory samples. Journal of Forensic Sciences 2006;51(1):82. [Presents a CE technique that can separate a wide variety of anions (18 listed), including various phosphorus species, in illicitly prepared methamphetamine samples.]


Meng P, Fang N, Wang M, Liu H, Chen DD. Analysis ofamphetamine, methamphetamine and methylenedioxymethamphetamine by micellar capillary electrophoresis using cation-selective exhaustive injection. Electrophoresis 2006;27(16):3210-7. [CSEI is used as an on-line concentration method; in this case, sensitivity was increased 1000-fold versus standard capillary MEKC.]


Moore KA. Amphetamine/sympathomimetic amines. Principles of Forensic Toxicology


Sachs SB, Woo F. A detailed mechanistic fragmentation analysis of methamphetamine and select regioisomers by GC/MS. Journal of Forensic Sciences 2007;52(2):308. [Includes methamphetamine and 7 related compounds.]


Tomaszewski W, Gun'ko VM, Leboda R, Skubiszewska-Zieba J. Interaction of
amphetamine and its N-alkyl-substituted derivatives with micro- and mesoporous adsorbents in polar liquids. Journal of Colloid and Interface Science 2004;282(2):261. [The title technique is used to concentrate amphetamines from "dilute aqueous solutions" (may be biological fluids - not clear in abstract).]


Wu TY, Fuh MR. Determination of amphetamine, methamphetamine, 3,4-methylene-dioxyamphetamine, 3,4-methylenedioxyethylamphetamine, and 3,4-methylenedioxy-methamphetamine in urine by online solid-phase extraction and ion-pairing liquid chromatography with detection by electrospray tandem mass spectrometry. Rapid Communications In Mass Spectrometry 2005;19(6):775.


**Barbiturates:**


Ghanem A. True and false reversal of the elution order of barbiturates on a bonded cellulose-based chiral stationary phase. Journal of Chromatography A 2006;1132(1-2):329. [With "a set of racemic N-alkylated barbiturates" (not specified in the abstract).]

Grove AA, Rohwer ER, Laurens JB, Vorster BC. The analysis of illicit methaqualone


**Benzodiazepines**:


Kratzsch C, Tenberken O, Peters FT, Weber AA, Kraemer T, Maurer HH. Screening, library-assisted identification and validated quantification of 23 benzodiazepines,
flumazenil, zaleplone, zolpidem, and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. Journal of Mass Spectrometry 2004;39(8):856. [The focus is toxicological.]


Rao RN, Parimala P, Khalid S, Alvi SN. Detection of the adulteration of traditional alcoholic beverages by the separation and determination of alprazolam, chloral hydrate, and diazepam using reversed phase high performance liquid chromatography. Analytical Sciences 2004;20(2):383. [200 seized samples were analyzed.]


**Clenbuterol:**


Stefan-van-Staden RI, Lai B. Enantioselective, potentiometric carbon paste electrodes based on C-60 derivatives as chiral selectors for the enantioanalysis of S-clenbuterol. Analytical Letters 2006;39(7):1311. [Using three different electrodes, for analysis of both raw material and serum samples.]


**Cocaine:**


Block R. Cocaine base to soup. Journal of the Clandestine Laboratory Investigating Chemists Association 2006;16(3):21. [Reports on the re-analysis of partially decomposed samples of cocaine base that had been stored in metal paint cans for 6 years.]


**Ergot Alkaloids (see also LSD):**


Lehner AF, Craig M, Fannin N, Bush L, Tobin T. Electrospray [+] tandem quadrupole mass spectrometry in the elucidation of ergot alkaloids chromatographed by HPLC:


Mohamed R, Gremaud E, Richoz-Payot J, Tabet JC, Guy PA. Quantitative determination of five ergot alkaloids in rye flour by liquid chromatography - electrospray ionisation tandem mass spectrometry. Journal of Chromatography A 2006;1114(1):62. [The target alkaloids were ergocristine, ergotamine, ergonovine, ergocornine, and ergokryptine; 15 samples of rye flour were analyzed.]


**Fentanyl(s):**


Drug Enforcement Administration (DEA), U.S. Department of Justice. Control of a
chemical precursor used in the illicit manufacture of fentanyl as a List I chemical. Interim rule with request for comments. Fed Regist 2007;72(77):20039-47.


**Flos daturae:**


Hou SG, Gu XX, Wang SY, Li HX. [Determination of scopolamine and atropine in Flos

**Fluoxetine (Prozac):**


**Heroin:**


standards and illicit samples.]

Beckerleg S, Telfer M, Sadiq A. A rapid assessment of heroin use in Mombasa, Kenya. Substance Use & Misuse 2006;41:1029. [Presents the title survey, done in March, 2004. 496 Heroin users were interviewed.]


Macchia M, Bertini S, Mori C, Orlando C, Papi C, Placanica G. Efficient application of monolithic silica column to determination of illicit heroin street sample by HPLC. Farmaco 2004;59(3):237. [Presents the title analysis (complete in 7 minutes).]

Ren J, Gao J-z, Suo N, Zhao G-h, Yang W, Lue D-y, Sun K-j, Li C-y. Determination of heroin based on analyte pulse perturbation to an oscillating chemical reaction. Chemical Research in Chinese Universities 2004;20(5):534. [For trace level detection of heroin. The application(s) for the technique were not reported in the abstract.]


**gamma-Hydroxybutyric Acid (GHB), gamma-Butyrolactone (GBL) and 1,4-Butanediol (BD):**

Bell SC, Oldfield LS, Shakleya DM, Petersen JL, Mercer JW. Chemical composition and structure of the microcrystals formed between silver(I) and gamma-hydroxybutyric acid


DeFrancesco JV, Witkowski MR, Ciolino LA. GHB free acid: I. Solution formation studies and spectroscopic characterization by 1HNMR and FT-IR. J Forensic Sci 2006;51(2):321-9. [The technique is suitable for analysis of forensic samples containing the free acid, its corresponding salt, and GBL.]

Del Signore AG, McGregor M, Cho BP. 1H NMR analysis of GHB and GBL: Further findings on the interconversion and a preliminary report on the analysis of GHB in serum and urine. Journal of Forensic Sciences 2005;50(1):81. [Spiked samples are included. Focus is toxicological, but the results are pertinent for spiked beverages.]

Elliott S, Burgess V. The presence of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in alcoholic and non-alcoholic beverages. Forensic Science International 2005;151(2-3):289.


Hennessy SA, Moane SM, McDermott SD. The reactivity of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in alcoholic solutions. Journal of Forensic Sciences 2004;49(6):1220. [Presents a study of the formation of esters of GHB, with an emphasis on the formation of the ethyl ester in alcoholic beverages.]

Marinetti LJ, Isenschmid DS, Hepler BR, Kanlueo S. Analysis of GHB and 4-methyl-GHB in postmortem matrices after long-term storage. Journal of Analytical
Matsuda K, Asakawa N, Iwanaga M, Gohda A, Fukushima S, Ishii Y, Yamada H. Conversion of gamma-hydroxybutyric acid to a fluorescent derivative: A method for screening. Forensic Toxicology 2006;24(1):41. [GHB is converted to a fluorescent derivative using 3-bromomethyl-6,7-dimethoxy-1-methyl-1,2-dihydroquinoxaline-2-one. The focus is toxicological, but analysis of powdered and tableted forms of GHB is specifically mentioned in the abstract.]

Mercer JW, Oldfield LS, Hoffman KN, Shakleya DM, Bell SC. Comparative analysis of gamma-hydroxybutyrate and gamma-hydroxyvalerate using GC/MS and HPLC. Journal of Forensic Sciences 2007;52(2):383. [GHB and GHV were derivatized with BSTFA with trimethylchlorosilane prior to GC/MS analyses. UV/Vis detection at 254 nm was used for the HPLC analyses.]


Sabucedo AJ, Furton KG. Extractionless GC/MS analysis of gamma hydroxybutyrate
and gamma butyrolactone with trifluoroacetic anhydride and heptafluoro-1-butanol from aqueous samples. Journal of Separation Science 2004;27(9):703. [Presents a novel technique for the derivatization and analysis of the title compounds directly from dilute aqueous solutions (i.e., beverages).]


Zhang SY, Huang ZP. [A color test for rapid screening of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in drink and urine]. Fa Yi Xue Za Zhi 2006;22(6):424-7.

Ketamine:


Jen HP, Tsai YC, Su HL, Hsieh YZ. On-line preconcentration and determination of ketamine and norketamine by micellar electrokinetic chromatography - Complementary


**Khat:**


**LSD:**


Suzuki S. Lysergic acid diethylamide (LSD). Drugs and Poisons in Humans 2005:225.


Marijuana and Related Cannabinoids:


Caligiani A, Palla G, Bernardelli B. GC-MS analysis of hashish samples: A case of adulteration with colophony. Journal of Forensic Sciences 2006;51(5):1096. [In a sample seized in Italy (colophony is the acidic flux used for soldering).]

Carpentier C, Griffiths P, King LA. An overview of cannabis potency in Europe. Report EMCDDA Insights 2004:1. [Also discusses the results versus the comparable data for the United States and Australia/New Zealand.]


Dussy FE, Hamberg C, Luginbuhl M, Schwerzmann T, Briellmann TA. Isolation of delta(9)-THCA-A from hemp and analytical aspects concerning the determination of


Hanson AJ. Specificity of the Duquenois-Levine and cobalt thiocyanate tests substituting methylene chloride or butyl chloride for chloroform. Microgram Journal 2005;3(3-4):183. [Performs the named tests using methylene chloride or butyl chloride as substitutes for chloroform.]

Hazekamp A, Choi YH, Verpoorte R. Quantitative analysis of cannabinoids from Cannabis sativa using H1 NMR. Chemical and Pharmaceutical Bulletin 2004;52(6):718. [Allows analysis of pure cannabinoids or cannabinoid mixtures from plant material in less than 5 minutes, without pre purification.]


Moore C, Rana S, Coulter C, Feyerherm F, Prest H. Application of two-dimensional gas chromatography with electron capture chemical ionization mass spectrometry to the


Methadone:


Methylenedioxyamphetamine and Related Compounds:

Aalberg L, DeRuiter J, Sippola E, Clark CR. Gas chromatographic optimization study on the side chain and ring regioisomers of methylenedioxymethamphetamine. Journal of Chromatographic Science 2004;42(6):293. [Includes the analysis of 10 isomeric compounds (not specified in the abstract).]

Adamowicz P, Chudzikiewicz E, Lechowicz W. Illicit "Ecstasy" tablets in southern Poland: A two-year review. Z Zagadnien Nauk Sadowych 2004;56:100. [Presents analytical results for 199 tablet seizures submitted over a two year period (time frame not specified in the abstract).]


Concheiro M, de Castro A, Quintela O, Lopez-Rivadulla M, Cruz A. Determination of


Jiang H-p, Ren C-h. Study on DFT of the structure and property of MDMA molecule. Xihua Daxue Xuebao, Ziran Kexueban 2006;25(5):69 6A. [A theoretical study of the structure and properties of MDMA by the "d. functional theory" ("d." was not defined in the abstract). Written in Chinese.]

Kalasinsky KS, Hugel J, Kish SJ. Use of MDA (the "Love Drug") and methamphetamine in Toronto by unsuspecting users of ecstasy. Journal of Forensic Sciences 2004;49(5):1106. [An overview of the use of alleged MDMA tablets containing mixed and/or alternative drugs, focus is biological/toxicological.]


Koelliker S, Oehme M. Structure elucidation of nanogram quantities of unknown designer drugs based on phenylalkylamine derivatives by ion trap multiple mass spectrometry. Analytical and Bioanalytical Chemistry 2004;378(5):1294. [Presents the use of HPLC multiple mass spectrometry on 55 phenylalkylamines (focus is on compounds in European Ecstasy tablets).]


Liu J-T. GC-MS and pentafluoropropionic anhydride derivatization methods for the differentiation of 3,4-methylenedioxymethamphetamine (MDMA) from their regioisomeric 1-(3,4-methylenedioxyphenyl)-2-ethylamines (MDPEAs). Huaxue 2005;63(1):95.


Newton HR. Indanylamphetamine identified. Journal of the Clandestine Laboratory Investigating Chemists Association 2004;14(3):12. [Presents analytical data for 1-(5 indanyl)-2-aminopropane (commonly mis-named as "indanylamphetamine"), a recently encountered designer drug that is an analog of MDA.]

Peters FT, Samyn N, Lamers CTJ, Riedel WJ, Kraemer T, De Boeck G, Maurer HH. Drug testing in blood: Validated negative-ion chemical ionization gas chromatographic-mass spectrometric assay for enantioselective measurement of the designer drugs MDEA, MDMA, and MDA and its application to samples from a controlled study with MDMA. Clinical Chemistry 2005;51(10):1811.


Tanner-Smith EE. Pharmacological content of tablets sold as "Ecstasy": Results from an online testing service. Drug Alcohol Depend 2006;83(3):247-54.


**Methylphenidate:**

Gilbert KM, Skawinski WJ, Misra M, Paris KA, Naik NH, Buono RA, Deutsch HM,

Morphine, Codeine, and Related Opium Alkaloids:


Kuila DK, Lahiri SC. Interactions of morphine and codeine with benzoic acid and substituted benzoic acids. Journal of the Indian Chemical Society 2004;81(11):928. [Investigates the complexes formed by the title compounds. The focus of this study is not clear from the abstract.]


Liu HC, Ho HO, Liu RH, Yeh GC, Lin DL. Urinary excretion of morphine and codeine following the administration of single and multiple doses of opium preparations prescribed in Taiwan as "brown mixture". J Anal Toxicol 2006;30(4):225-31.


Smetkova M, Ondra P, Lemr K. HPLC-MS and CE-MS with atomospheric pressure ionization in analysis of morphine and related compounds. Chemie Listy 2004;98(6):336. [A review and discussion of the title subject. Not clear whether the focus is forensic or toxicological (the latter appears more likely). Written in Czech.]


Zayed MA, Hawash MF, Fahmey MA. Structure investigation of codeine drug using

**Opiate Alkaloids:**


Li SH, He CY, Liu HW, Li K, Liu F. Ionic liquid-based aqueous two-phase system, a sample pretreatment procedure prior to high-performance liquid chromatography of opium alkaloids. Journal of Chromatography B - Analytical Technologies in the
Biomedical and Life Sciences 2005;826(1-2):58.


Qi XH, Mi JQ, Zhang XX, Chang WB. Preparation and application of an immunoaffinity column for direct extraction of morphine and its analogs from opium. Chinese Chemical Letters 2004;15(11):1323. [The presented method uses an IAC for isolation and CE for analysis. The four alkaloids that are selectively isolated are morphine, codeine, dionin, and thebaine.]


Opium (and Opium Poppies):


Lenehan CE, Barnett NW, Lewis SW, Essery KM. Preliminary evaluation of dual acidic potassium permanganate and tris(2,2'-bipyridyl)ruthenium(II) chemiluminescence detection for the HPLC determination of Papaver somniferum alkaloids. Australian


Overview/Polydrug:


Apollonio LG, Pianca DJ, Whittall IR, Maher WA, Kyd JM. A demonstration of the use
of ultra-performance liquid chromatography-mass spectrometry [UPLC/MS] in the
determination of amphetamine-type substances and ketamine for forensic and

Apollonio LG, Whittall IR, Pianca DJ, Kyd JM, Maher WA. Product ion mass spectra of
amphetamine-type substances, designer analogues, and ketamine using ultra-performance
2006;20(15):2259-64.

Bishop SC, McCord BR, Gratza SR, Loeliger JR, Witkowski MR. Simultaneous


Bonato PS, Jabor VAP, de Gaitani CM. Enantioselective analysis of drugs:


Caldwell GW, Yan ZY. Screening for reactive intermediates and toxicity assessment in


Dahlen J, von Eckardstein S. Development of a capillary zone electrophoresis method
including a factorial design and simplex optimisation for analysis of amphetamine,
amphetamine analogues, cocaine, and heroin. Forensic Science International
2006;157(2-3):93.


directly analyzing trace amount analytes in the water-immiscible solution samples.
Xuexiao Huaxue Xuebao 2006;27(5):856. [Abstract specifies cocaine and thebaine. Focus may be toxicological (not clear in abstract). Written in Chinese.]


Laks S, Pelander A, Vuori E, Ali-Toippa E, Sippola E, Ojanpera I. Analysis of street drugs in seized material without primary reference standards. Analytical Chemistry 2004;76(24):7375. [Uses a combination of LC-Time-of-Flight-MS and LC-Chemiluminescence Nitrogen Detection on 21 samples (different drugs). The results were found to be reasonable, with variances from established methods ranging from 4 to 21
percent, and only one apparent false positive.]


Maurer HH. Multi-analyte procedures for screening for and quantification of drugs in blood, plasma, or serum by liquid chromatography-single stage or tandem mass spectrometry (LC-MS or LGMS/MS) relevant to clinical and forensic toxicology. Clinical Biochemistry 2005;38(4):310.

Maurer HH. Position of chromatographic techniques in screening for detection of drugs or poisons in clinical and forensic toxicology and/or doping control. Clinical Chemistry And Laboratory Medicine 2004;42(11):1310.


Nerkis S, Oruc HH. Determination of amounts of the active substance and added substances in cannabis, heroin, and ecstasy tablets used in Bursa and in the Bursa region. Bagimlilik Dergisi 2006;7(1):11. [21 Cannabis, 55 heroin, and 65 Ecstasy tablet exhibits were characterized by GC/MS and FTIR. Written in Turk.]

Nordgren HK, Holmgren P, Liljeberg P, Eriksson N, Beck O. Application of direct urine LC-MS-MS analysis for screening of novel substances in drug abusers. Journal of


**Pethidine:**

**Phenethylamines (including mixtures of Amphetamines, Methylene dioxy-amphetamines, and Related Compounds):**


Habrdova V, Peters FT, Theobald DS, Maurer HH. Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. Journal of Mass Spectrometry


Thigpen A, Deruiter J, Clark CR. GC-MS Studies on the regioisomeric 2,3- and 3,4-methylenedioxyphenethylamines related to MDEA, MDMMA, and MBDB. J Chromatogr Sci 2007;45(5):229-35.


**Piperazines:**


analytical data for BZP and TFMPP.]


Polydrug:

Conemans JMH, Van Der Burgt AAM, Van Rooij JML, Pijnenburg CC. The simultaneous determination of illicit drugs with HPLC-DAD. Bull TIAFT 2004;34(1):11. [The presented method is applied to drug powders, various dosage forms, and various biological matrices, in a clinical setting.]


Magnuson EE, Burnett LJ. Screening system for detection of contraband swallowed narcotics. Applied Magnetic Resonance 2004;25(3-4):567. [Presents a nonimaging, low-frequency NMR technique to detect pellets of heroin or cocaine.]


Propoxyphene:

Microgram Journal 2016, Volume 13; Numbers I-4


Psilocybin Mushrooms, Psilocybin, and Psilocin:


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.


**Salvia divinorum:**


Wolowich WR, Perkins AM, Cienki JJ. Analysis of the psychoactive terpenoid
salvinorin A content in five Salvia divinorum herbal products. Pharmacotherapy 2006;26(9):1268. [Analyses were conducted using HPLC and TLC/GC/MS. The samples were purchased from Internet and "Head Shops." The samples were all subpotent with respect to stated Salvinorin A content, and three also contained unreported adulterants.]

**Scopolamine:**


**Steroids:**


Blackledge RD. The identification of 1-dehydromethandrostenolone. Microgram Journal 2005;3(3-4):186. [A recent steroid seizure was identified by GC/MS as 1-dehydromethandrostenolone, a positional isomer of methyltestosterone.]


MateusAvois L, Mangin P, Saugy M. Use of ion trap gas chromatography-multiple mass


Thevis M, Bommerich U, Opfermann G, Schaenzer W. Characterization of chemically modified steroids for doping control purposes by electrospray ionization tandem mass


Van Thuyne W, Delbeke FT. Validation of a GC-MS screening method for anabolizing agents in solid nutritional supplements. Biomedical Chromatography 2004;18(3):155. [Includes analyses of testosterone, nandralone, stanazolol, metandienone, and various prohormones.]


(Designer) Tryptamines (see also Psilocybin):


Vorce SP, Sklerov JH. A general screening and confirmation approach to the analysis of designer tryptamines and phenethylamines in blood and urine using GC-EI-MS and HPLC-electrospray- MS. Journal of Analytical Toxicology  2004;28(6):407. [Presents the analysis of the pentafluoropropionic derivatives of the title drugs, focus is on biological matrices.]

Wilson JM, McGeorge F, Smolinske S, Meatherall R. A "Foxy" intoxication. Forensic Science International  2005;148(1):31. [Focus is toxicological, but includes mass spectra for the title compound (N,N-diisopropyl-5-methoxytryptamine, also known as "Foxy-Methoxy") and N,N-diisopropyl-5-hydroxytryptamine. Note that there are some nomenclature problems in this article, and the structure and term 5-ethoxy-diisopropyl-tryptamine are incorrectly used in several instances.]


**Zaleplon:**


Zolpidem:

Kelani KM. Selective potentiometric determination of zolpidem hemitartrate in tablets and biological fluids by using polymeric membrane electrodes. Journal of the AOAC International 2004;87(6):1309. [Using four different polymeric membrane sensors.]


Miscellaneous:


Kim SC, Chung H, Lee SK, Park YH, Yoo YC, Yun Y-P. Simultaneous analysis of d-3-methoxy-17-methylmorphinan and l-3-methoxy-17-methylmorphinan by high pressure liquid chromatography equipped with PDA. Forensic Science International 2006;161(2-3):185. [The title compounds are dextromethorphan and levomethorphan. The technique used a chiral column. 32 confiscated samples were analyzed.]

Kuila DK, Muhkopadhyay B, Lahiri SC. Identification and estimation of methaqualone in toffee samples using gas chromatography - mass spectrometry, Fourier transform infrared spectrometry, and high-performance thin-layer chromatography. Forensic Science Communications 2006;8(4): (No Page Numbers). [Presents the analysis of some Indian brand toffee samples suspected to contain adulterants/hypnotic drugs and alcohol. Note that FSC is an on-line journal.]


Neuvonen K, Neuvonen H, Fulop F. Effect of 4-substitution on psychotomimetic activity of 2,5-dimethoxyamphetamines as studied by means of different substituent parameter


Simonov EA, Salomatin VE. Preliminary analysis of substances of unknown origin and complex medicinal formulations. Mikroelementy y Meditsine 2005;6(3):35. [Abstract is unclear as to what technique is used. "Narcotic materials and psychotropic substances" are mentioned. Written in Russian.]


synthetic illicit drugs.


Yuan X, Forman BM. Detection of designer steroids. Nuclear Receptor Signaling 2005;3:(No Page Numbers Listed). [Presents an analytical strategy that detects use of unknown designer steroids "without prior knowledge of their existence". Focus is toxicological (testing of athletes).]
II) Synthesis and/or Cultivation of Abused Substances, their Precursors, and Essential Chemicals

Issue:
Forensic chemists must maintain familiarity with existing and new clandestine syntheses of abused substances, their precursors, and essential chemicals, and with the cultivation of abused natural products, in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and to provide expert testimony in legal proceedings.

Solution:
Illicit drug seizures, clandestine laboratory operations, and illicit grow operations, are continuously monitored to maintain a comprehensive overview of the field. In cases where new drugs are synthesized, or new methodologies are utilized, case reports are generated for the forensic and enforcement communities.

References:

Production of Abused Substances and/or their Precursors and Essential Chemicals:


Brandt SD, Freeman S, McGagh P, Abdul-Halim N, Alder JF. An analytical perspective on favoured synthetic routes to the psychoactive tryptamines. Journal of Pharmaceutical and Biomedical Analysis 2004;36(4):675. [Appears to be a review, focusing on the probable impurities and marker compounds resulting from common illicit syntheses.]

Brandt SD, Freeman S, Fleet IA, Alder JF. Analytical chemistry of synthetic routes to psychoactive tryptamines - Part III. Characterisation of the Speeter and Anthony route to N,N-dialkylated tryptamines using CI-IT-MS-MS. Analyst 2005;130(9):1258.


Karpiesiuk W, Lehner AF, Hughes CG, Tobin T. Preparation and chromatographic characterization of tetrahydrogestrinone, a new "designer" anabolic steroid. Chromatographia 2004;60(5-6):359. [The synthesis of THG from gestrinone is reported.]


Poortman-Van Der Meer A. The synthesis of MDMA with NaBH4 as the reducing agent; the "Cold Method." Journal of the Clandestine Laboratory Investigating Chemists Association 2006;16(3):10. [Details withheld in accordance with Microgram policy.]


Tadeusiak EJ. Synthesis of phosphonic analogues of carnitine and gamma-amino-


Xu YZ, Chen CP. Synthesis of deuterium labeled phenethylamine derivatives. Journal of Labelled Compounds & Radiopharmaceuticals 2006;49(13):1187. [For use as internal standards in GC/MS. Compounds included 2C-B, 2C-C, 2C-I, 2C-T-2, and 2C-T-7.]


Zvilichovsky G, Gbar-Haj-Yahia I. Birch reduction of (-)-ephedrine formation of a new,

---------- Next Section Moved Up to Reduce Deadspace ----------

III) Clandestine Laboratories - Appraisals and Safety

Issue:
Forensic chemists must maintain familiarity with clandestine laboratory procedures, setups, and techniques in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and in order to provide expert testimony in court proceedings.

Solution:
Clandestine laboratory operations are continuously reviewed to provide a comprehensive overview of the field. In cases where new methodologies are noted, or unusual safety concerns are salient, reports are generated for the forensic and enforcement communities.

References:

Clandestine Laboratory Appraisals and Safety:


Safety Issues - Case Reports:

Sudakin DL. Occupational exposure to aluminum phosphide and phosphine gas? A

Miscellaneous:


-------- Next Section Moved Up to Reduce Deadspace --------

IV) Reference Drug Standards and Total Syntheses

Issue:
Many reference drug standards or structurally related internal standards are either commercially unavailable, or if available are extremely expensive.

Solution:
Controlled substances and their structural or isotopically labelled analogs are synthesized as needed. Internal standards are also prepared as needed. Case reports are published for new or unusual standards or improved synthetic approaches.

References:


V) Source Determination of Drugs (Impurity Profiling) and Comparative Analyses

Issues:
Impurity profiling of drugs is important for comparative analysis protocols, geo-sourcing, and synthetic route determinations. However, although certain drugs have been well characterized with respect to their impurity profiles, most have not been properly investigated.

Comparative analysis (i.e., the systematic application of impurity profiling for determination of commonality of origin) is complicated due to both the high complexity of the data and the large numbers of exhibits. Improved analytical and data handling techniques are needed.

Solution:
High sensitivity analytical techniques (primarily chromatographic) provide detailed profiles of trace-level impurities, ions, trace metals, and stable isotopes. Identification of individual impurities enhance origin identification and comparative analyses and also aid in development of internal standards for improved accuracy and precision of analysis.

In-depth analysis via improved instrumental methodologies help identify discriminatory components in impurity profiles. Computer databases, sorting programs, and pattern recognition/neural networks provide enhanced data handling and analysis, enabling and improving comparative analyses. Case reports are generated for the forensic and enforcement communities.

References:

Amphetamine(s):


Goldmann T, Taroni F, Margot P. Analysis of dyes in illicit pills (amphetamine and derivatives). Journal of Forensic Sciences 2004;49(4):716. [Analysis for 14 dyes present in European ecstasy tablets is performed using SPE followed by TLC and/or CEC DAD, the results can be used to link cases.]


Poortman-Van der Meer A, Lock E. Identification of 4-tert-butylamphetamine in clandestine amphetamine samples. Journal of the Clandestine Laboratory Investigating Chemists Association 2006;16(2):23. [The title compound results from the presence of 4-tert-butylphenylaceton as an impurity in phenylacetone possibly produced in eastern Europe.]

**Cocaine:**


**Cocaine and Heroin:**


Morley SR, Hall CJ, Forrest ARW, Galloway JH. Levamisole as a contaminant of illicit cocaine. Journal of the Clandestine Laboratory Investigating Chemists Association 2006;16(4):11. [Focus is on detection in body fluids of cocaine abusers (including six who were deceased) acquired over a 20 week period in the United Kingdom.]


**Heroin:**

Al-Amri AM, Smith RM, El-Haj BM, Juma'a MH. The GC-MS detection and characterization of reticuline as a marker of opium use [Erratum]. Forensic Science International 2004;142(1):59. [Provides a correction to the original article, published 2004;140(2-3):175.]


Casale J, Casale E, Collins M, Morello D, Cathapermal S, Panicker S. Stable isotope analyses of heroin seized from the merchant vessel Pong Su. Forensic Sciences 2006;51(3):603. [See the next citation for the associated lead article on this seizure. The title exhibits were determined to be unique with respect to their origin.]

Collins M, Casale E, Hibbert DB, Panicker S, Robertson J, Vujic S. Chemical profiling of heroin recovered from the North Korean merchant vessel Pong Su. Journal of Forensic Sciences 2006;51(3):597. [The heroin was classified as "unknown" in origin (that is, having a profile that did not resemble any known heroin types).]


Odell LR, Skopec J, McCluskey A. A 'cold synthesis' of heroin and implications in heroin signature analysis utility of trifluoroacetic/acetic anhydride in the acetylation of morphine. Forensic Sci Int 2006;164(2-3):221-9. [Focuses on the impurity profile of heroin produced by this unusual route. Several trifluoroacetyl derivatives were identified, but were also found to be sensitive to typical heroin signature workup and analysis procedures.]

Toske SG, Cooper SD, Morello DR, Hays PA, Casale JF, Casale E. Neutral heroin impurities from tetrahydrobenzylisoquinoline alkaloids. Journal of Forensic Sciences 2006;51(2):308. [Four of the title compounds (laudanosine, reticuline, codamine, and laudanine), all naturally occurring in opium, form 18 detectable neutral impurities under typical heroin processing conditions. These latter impurities were found to be useful for sourcing illicit heroin.]

Zamir A, Cohen Y, Azoury M. DNA profiling from heroin street dose packages. Journal of Forensic Sciences 2007;52(2):389. [DNA could be recovered from fingerprints along the "amorphic" burnt edges of the plastic wrap typically used to package street-level doses of heroin in Israel.]


Zhang ZY, Yang JH, Ouyang H, Li ZJ, Chai ZF, Zhu J, Zhao JZ, Yu ZS, Wang J. Study of trace impurities in heroin by neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry 2004;262(1):295. [62 heroin samples were analyzed for 15 trace elements by NAA. The authors indicate that the results provide origin information.]


**Marijuana:**


Choi YH, Kim HK, Hazekamp A, Erkelens C, Lefeber AWM, Verpoorte R. Metabolomic differentiation of Cannabis sativa cultivars using 1H NMR spectroscopy
and principal component analysis. Journal of Natural Products 2004;67:953. [Cultivars could be differentiated by measurement of delta-9-tetrahydrocannabinolic acid and cannabidiolic acid.]

Datwyler SL, Weiblen GD. Genetic variation in hemp and marijuana (Cannabis sativa L.) according to amplified fragment length polymorphisms. Journal of Forensic Sciences 2006;51(2):371. [The results are useful in linking seizures, for source determination, and for differentiating licit and illicit cultivars of cannabis.]


Hsieh HM, Hou RJ, Chen KF, Tsai LC, Liu SW, Liu KL, Linacre A, Lee JC. Establishing the rDNA IGS structure of Cannabis sativa. Journal of Forensic Sciences 2004;49(3):477. [The authors indicate that the technique can be used to identify and classify samples.]


Toonen M, Ribot S, Thissen J. Yield of illicit indoor cannabis cultivation in the Netherlands. Journal of Forensic Sciences 2006;51(5):1050. [Presents a formula for determining a total expected yield of mature female flower buds (sinsimella) from indoor grow operations, regardless of maturity at the time of seizure.]

**Methamphetamine:**

Armellin S, Brenna E, Frigoli S, Fronza G, Fuganti C, Mussida D. Determination of the synthetic origin of methamphetamine samples by 2H NMR spectroscopy. Analytical Chemistry 2006;78(9):3113. [The results suggest that site specific deuterium NMR can assist in classifying methamphetamine as to precursors and synthetic routes.]


Ishibashi H. Analysis of stable isotope ratio of carbon and nitrogen, as a powerful tool to identify smuggling routes of illegal drugs. Kagaku to Kogyo 2004;57(9):964. [A review of the title topic, including discussion of application to methamphetamine and MDMA. Written in Japanese.]

Iwata YT, Inoue H, Kuwayama K, Kanamori T, Tsujikawa K, Miyaguchi H, Kishi T. Forensic application of chiral separation of amphetamine-type stimulants to impurity analysis of seized methamphetamine by capillary electrophoresis. Forensic Science International 2006;161:92. [A highly sulfated gamma-cyclodextrin was used as the chiral selector.]
Kurashima N, Makino Y, Sekita S, Urano Y, Nagano T. Determination of origin of ephedrine used as precursor for illicit methamphetamine by carbon and nitrogen stable isotope ratio analysis. Analytical Chemistry 2004;76(14):4233. [The title technique could easily differentiate between ephedrine of synthetic versus semi synthetic versus biosynthetic origins, and the differences were found to carry through to the methamphetamine produced from those different origins of ephedrine.]


Qi Y, Evans ID, McCluskey A. Australian Federal Police seizures of illicit crystalline methamphetamine ('ice') 1998-2002: Impurity analysis. Forensic Sci Int 2006;164(2-3):201-10. [19 samples seized at Australian POE's were analyzed by methamphetamine impurity profiling techniques; over 30 characteristic impurities were


Methylenedioxyamphetamine and MDMA:

Aalberg L, Clark CR, DeRuiter J. Chromatographic and mass spectral studies on isobaric and isomeric substances related to 3,4-methylenedioxymethamphetamine. Journal of Chromatographic Science 2004;42(9):464. [Reports on the preparation of a number of compounds that are isobaric or isomeric with MDMA, and comments on the similarities and differences in their mass spectra (actual compounds not reported in the abstract).]


Cox M, Klass G. Synthesis by-products from the Wacker oxidation of safrole in methanol using rho-benzoquinone and palladium chloride. Forensic Sci Int 2006;164(2-3):138-47. [Includes analyses of samples from a clandestine laboratory seized in Australia that was employing this synthesis route.]


Kochana J, Wilamowski J, Parczewski A. SPE-TLC profiling of impurities in 1-(3,4-methylenedioxyphenyl)-2-nitropropene, and intermediate in 3,4-methylenedioxymethamphetamine (MDMA) synthesis. Chromatographia 2004;60(7-8):481. [Appears to be
closely related to a similarly titled article published in the Journal of Liquid Chromatography & Related Techniques 2004;27(15):2463.]


Palhol F, Lamoureux C, Chabrillat M, Naulet N. N15/N14 Isotopic ratio and statistical analysis: An efficient way of linking seized Ecstasy tablets. Analytica Chimica Acta 2004;510(1):1. [Presents the GC/C/IRMS analyses of MDMA from 106 samples. The results can be used for rapid grouping of similar tablets.]


Teng SF, Wu SC, Liu C, Li JH, Chien CS. Characteristics and trends of 3,4-methylenedioxyamphetamine (MDMA) tablets found in Taiwan from 2002 to February 2005. Forensic Sci Int 2006;161(2-3):202-8. [181 tablets were analyzed by GC/MS. Photographs of the tablet logos are shown.]

van Deursen MM, Lock ER, Poortman-van der Meere AJ. Organic impurity profiling of


**Opium and Opium Alkaloids:**


Ziegler J, DiazChavez M, Kramell R, Ammer C, Kutchan TM. Comparative macroarray

Multi-Drug and Miscellaneous:


Bergeron C, Gafner S, Clausen E, Carrier DJ. Comparison of the chemical composition of extracts from Scutellaria lateriflora using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction. Journal of Agricultural and Food Chemistry 2005;53(8):3076.


Daeid NN, Waddell RJH. The analytical and chemometric procedures used to profile illicit drug seizures. Talanta 2005;67(2):280.


Leger MN, Ryder AG. Comparison of derivative preprocessing and automated polynomial baseline correction method for classification and quantification of narcotics in


Meier AW, Liu RH. Forensic applications of isotope ratio mass spectrometry. Advances in Forensic Applications of Mass Spectrometry 2004:149 (Chapter 4). [An overview and review. Appears to focus on biological/toxicological forensic applications (not clear in the abstract).]


Visky D, Jimidar I, VanAel W, Vennekens T, Redlich D, DeSmet M. Capillary


VI) Analysis of Non-Controlled Pharmaceuticals, Pseudo-Drugs, Adulterants, Diluents, and Precursors

Issue:
Most "street-level" drugs are "cut" with various adulterants and diluents. Many of these cutting agents are pharmaceutical products or precursors. Others are "carry-through" compounds present in precursors (especially in cold remedy products). Separation and identification of these extraneous materials can be tedious, especially in exhibits which contain many components. In addition, new or unusual adulterants and/or diluents are occasionally identified in drug exhibits, and standard analytical data are required for these substances. Finally, improved methods of analysis, i.e., faster, more discriminatory, less costly, etc., are needed for all cutting agents.

Solution:
Reports providing standard analytical data and/or improved analytical protocols for non-controlled pharmaceuticals, pseudo-drugs, adulterants, diluents, and precursors are generated for the forensic and enforcement communities.

References:

Creatine:


Ephedra, Ephedrine, and/or Pseudoephedrine and Related Compounds:


An OY, Gao XY, Baeyens WRG, Delanghe JR. Determination of ephedrine and related compounds in pharmaceutical preparations by ion chromatography with direct conductivity detection. Biomedical Chromatography 2005;19(4):266.

Avula B, Khan IA. Separation and determination of ephedrine enantiomers and synephrine by high performance capillary electrophoresis in dietary supplements. Chromatographia 2004;59(1-2):71. [For analyses of E. Sinica and various dietary supplement products. The enantiomers of norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine, N-methylephedrine, and N-methylpseudoephedrine were separated.]


Dinc E, Ozdemir A, Aksoy H, Ustundag O, Baleanu D. Chemometric determination of naproxen sodium and pseudoephedrine hydrochloride in tablets by HPLC. Chem Pharm


N-Trimethylsilyl-trifluoroacetamide. Journal of Chromatography B - Analytical Technologies in the Biomedical and Life Sciences 2004;811(2):201. [Includes analysis of ephedrine, pseudoephedrine, cathine, norephedrine, and methylephedrine. Focus is toxicological.]


Wang W, Li C, Li Y, Hu Z, Chen X. Rapid and ultrasensitive determination of ephedrine and pseudoephedrine derivatized with 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein by


**Phenylpropanolamine:**


**Other Adulterants/Diluents (including mixtures containing Ephedrine and/or Pseudoephedrine):**


Forsdahl G, Gmeiner G. Investigation of the silylation of ephedrines using N-methyl-
N-trimethylsilyl-trifluoroacetamide. Journal of Chromatography B - Analytical Technologies in the Biomedical and Life Sciences 2004;811(2):201. [Includes analysis of ephedrine, pseudoephedrine, cathine, norephedrine, and methylephedrine. Focus is toxicological.]


Haller CA, Duan M, Benowitz NL, Jacob P. Concentrations of ephedra alkaloids and caffeine in commercial dietary supplements. Journal of Analytical Toxicology 2004;28:145. [Presents a novel LC MS/MS technique for performing the title analysis, 35 products were analyzed.]


Lapitskaya MA, Zatonsky GV, Pivnitsky KK. Enantiomeric NMR analysis of chiral epoxides as addition compounds with d-ephedrine. Mendeleev Communications 2005;(5):175.


Miscellaneous:


Cheng YQ, Fan LY, Chen HL, Chen XG, Hu ZD. Method for on-line derivatization and separation of aspartic acid enantiomer in pharmaceuticals application by the coupling of flow injection with micellar electrokinetic chromatography. Journal of Chromatography


analyses.]


Himmelsbach M, Buchberger W, Klampfl CW. Determination of antidepressants in surface and waste water samples by capillary electrophoresis with electrospray ionization mass spectrometric detection after preconcentration using off-line solid-phase extraction. Electrophoresis 2006;27(5-6):1220.


Majumdar TK. Commonly encountered analytical problems and their solutions in liquid chromatography/tandem mass spectrometry (LC/MS/MS) methods used in drug development. Identification and Quantification of Drugs, Metabolites and Metabolizing Enzymes by LC - MS; Progress in Pharmaceutical and Biomedical Analysis 2005;6):35.


Song GX, Deng CH, Wu D, Hu YM. Headspace solid-phase microextraction-gas chromatographic-mass spectrometric analysis of the essential oils of two traditional


VII) New and/or Improved Instrumental Techniques

Issue:
Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Solution:
Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

References:

Capillary Electrophoresis (and Related Techniques, including Tandem Techniques):


Ding Y, Garcia CD. Application of microchip-CE electrophoresis to follow the degradation of phenolic acids by aquatic plants. Electrophoresis 2006;27(24):5119-27.


Liu Z, Pawliszyn J. Microdialysis hollow fiber as a macromolecule trap for on-line coupling of solid phase microextraction and capillary electrophoresis. Analyst


abstract).


Szoko E, Tabi T, Borbas T, Dalmadi B, Tihanyi K, Magyar K. Assessment of the


Xu Y, Gao Y, Wei H, Du Y, Wang E. Field-amplified sample stacking capillary electrophoresis with electrochemiluminescence applied to the determination of illicit drugs on banknotes. Journal of Chromatography A 2006;1115:260. Focus is cocaine and heroin. Baseline resolution was achieved within 6 minutes.]

**Extraction Techniques:**

Bergeron C, Gafner S, Clausen E, Carrier DJ. Comparison of the chemical composition of extracts from Scutellaria lateriflora using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or 70% ethanol extraction. Journal of Agricultural and Food Chemistry 2005;53(8):3076.


**Gas Chromatography (and Tandem GC Techniques):**


Dimandja J-MD. GC x GC. Analytical Chemistry 2004;76(9):167A. [An overview and review of two-dimensional GC techniques.]

Hodjmohammadi MR, Ebrahimi P, Pourmorad F. Quantitative structure-retention relationships (QSRR) of some CNS agents studied on DB-5 and DB-17 phases in gas chromatography. QSAR & Combinatorial Science 2004;23(5):295.


Song SM, Marriott P, Kotsos A, Drummer OH, Wynne P. Comprehensive two-dimensional gas chromatography with time of flight mass spectrometry (GC x GC TOFMS) for drug screening and confirmation. Forensic Science International 2004;143(2 3):87. [78 drugs of interest were analyzed, some forensic samples were also analyzed satisfactorily.]

Song SM, Marriott P, Wynne P. Comprehensive two-dimensional gas chromatography - quadrupole mass spectrometric analysis of drugs. Journal of Chromatography A 2004;1058(1-2):223. [77 underivatized drug standards (not specified in the abstract) were analyzed by the title technique. Appears to be related to a similarly titled article published in Forensic Science International 2004;143(2-3):87 (using TOF-MS).]


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Ali Z, Poole C. Insights into the retention mechanism of neutral organic compounds on

Balogh MP. DESI, IMS, and resurgent challenges to HPLC-MS. LC-GC North America 2006;24(1):46. [An overview.]

Barri T, Jonsson JA. Supported liquid membrane work-up of blood plasma samples coupled on-line to liquid chromatographic determination of basic antidepressant drugs. Chromatographia 2004;59(3-4):161.


Esteve-Romero J, Carda-Broch S, Gil-Agusti M, Capella-Peiro ME, Bose D. Micellar liquid chromatography for the determination of drug materials in pharmaceutical preparations and biological samples. TRAC - Trends In Analytical Chemistry


Lambert W. Pitfalls in LC-MS(-MS) analysis. Bulletin TIAFT 2004;34(2):59. [Discusses the title subject. Includes numerous references.]

Lambert W. Pitfalls in LC-MS(-MS) Analysis. Toxichem und Krimtech 2004;71(2):64. [Language not specified in the abstract (may be in German). A review. Appears to be a re-publication of the article by the same author and title in Bulletin TIAFT 2004;28(6):439.]


Thompson R. A practical guide to HPLC enantioseparations for pharmaceutical compounds. Journal of Liquid Chromatography & Related Technologies


Zhou LZ. Applications of LC/MS in pharmaceutical analysis. Separation Science and Technology 2005;6,499.

**Inductively Coupled Plasma- Mass Spectrometry (ICP-MS, Also ICP-OES):**


Sarin RK, Srivastava S, Srivastava AK, Anil G, Reddy MRP. Multielement
determination in gum opium by microwave digestion and inductively coupled plasma optical emission spectroscopy. Chemical Papers 2004;58(2):101. [Presents the analysis of Indian gum opium by the title technique (13 elements found in quantifiable levels).]


**Infrared and Raman Spectroscopy:**


Bell SEJ, Sirimuthu NMS. Rapid, quantitative analysis of ppm/ppb nicotine using surface-enhanced Raman scattering from polymer-encapsulated Ag nanoparticles (Gel-colls). Analyst 2004;129(11):1032.


Causin V, Marega C, Carresi P, Schiavone S, Marigo A. A quantitative differentiation method for plastic bags by infrared spectroscopy, thickness measurement, and differential scanning calorimetry for tracing the source of illegal drugs. Forensic Science International 2006;164(2-3):148. [50 bags of types typically used for drug packaging were analyzed. The results indicate that even mass-produced bags have a large degree of
variability, and can be differentiated and/or linked.


**Ion Spectroscopy:**


**Mass Spectrometry:**


Pavlic M, Libiseller K, Oberacher H. Combined use of ESI-QqTOF-MS and ESI-QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. Analytical and Bioanalytical Chemistry 2006;386(1):69. [319 drugs (therapeutic and illicit) were analyzed. The resulting spectral library was successfully applied to the characterization of 39 forensic casework samples.]


Rubakhin SS, Jurchen JC, Monroe EB, Sweedler JV. Imaging mass spectrometry:


Shao X, Wang G, Wang S, Su Q. Extraction of mass spectra and chromatographic profiles from overlapping GC/MS signals with background. Analytical Chemistry 2004;76(17):5143. [The authors indicate that the presented methodology is better than the SIMPLISMA technique.]


**Microchip Technology:**


**Nuclear Magnetic Resonance Spectroscopy:**


Osokin DY, Khusnutdinov RR. Two-frequency composite pulses in NQR. Applied Magnetic Resonance 2006;30(1):7. [Use of the title technique for detection of narcotics is specifically mentioned in the abstract (NFI).]

**Solid Phase Micro-Extraction (Headspace Techniques and Solvent Analysis):**


Rearden P, Harrington PB. Rapid screening of precursor and degradation products of chemical warfare agents in soil by solid-phase microextraction ion mobility spectrometry.


**Thin Layer Chromatography:**


**X-Ray based Techniques:**


**Miscellaneous:**


Gartsev NA, Semeikin NP, Sharshin YA, Pomozov VV, Nedorezov AV, Nikiforov AA. Detector for detection of explosives and drugs. RU 2234695 C1 20 Aug 2004. CLASS: ICM: G01N024 00. APPLICATION: RU 2003 106186 6 Mar 2003. [Appears to be based on nuclear quadrupole resonance detection. Drugs not specified. This patent is written in Russian.]

Henry KD, Lovell JS. Stroboscopic system and method for detecting substances, such as explosives and/or drugs, using, in part, short bursts of energy light from a relatively low energy strobe. (Patent) Chemical Abstracts 2006;145:350077u.


Srinivas NR. Simultaneous chiral analyses of multiple analytes: Case studies, implications and method development considerations. Biomedical Chromatography 2004;18(10):759. [A review, includes some illustrative case studies.]


VIII) Portable Detection and Analytical Instrumentation

Issue:
"Free Trade" agreements and the easing of formally restrictive national and international borders have resulted in dramatic increases in cargo transshipments and personal travel, thereby complicating drug inspection and interdiction efforts at POEs. Discovery and confirmational analysis of suspected drugs in cargo or on individuals is severely hampered by the lack of on-site detection and/or analytical equipment.

Solution:
Development of portable and highly sensitive detectors for drug detection and analyses allows law enforcement personnel and/or forensic chemists to perform screening type analyses on-site. In those cases where new methodologies have proven effective, case reports are generated for the forensic and enforcement communities.

References:


Chen Y, Pawliszyn J. Solid-phase microextraction field sampler. Analytical Chemistry


IX) Miscellaneous

References:

Analytical Artifacts:


Chemometrics:


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Hu Y, Liang YZ, Li BY, Li XN, Du YP. Multicomponent spectral correlative


Leger MN, Ryder AG. Comparison of derivative preprocessing and automated polynomial baseline correction method for classification and quantification of narcotics in solid mixtures. Appl Spectrosc 2006;60(2):182-93.


**Cocaine:**

Bowen RAR, George DT, Hortin GL. False-negative result for cocaine metabolites on a


**Counterfeit Drugs:**


deKieffer DE. The Internet and the globalization of counterfeit drugs. Journal of Pharmacy Practice 2006;19:171-177.


Mukhopadhyay R. The hunt for counterfeit medicine. Drugs manufactured by counterfeiters are infiltrating markets worldwide. Investigators are harnessing a variety of analytical techniques to catch as many of the fakes as they can. Anal Chem 2007;79(7):2622-7.


**Dragon's Blood:**


**Drugs on Currency:**


Frederick KA, Pertaub R, Ski Kam NW. Identification of individual drug crystals on paper currency using Raman microspectroscopy. Spectroscopy Letters 2004;37(3):301. [Presents and discusses the title study, using simulated drugs (isoxsuprine and norephedrine) and two common excipients (benzocaine and lidocaine). Fluorescence issues with U.S. currency are discussed.]


**Heroin:**


**Legal Issues:**


**Enantiomer Resolution:**


**Methamphetamine:**


**Qualitative Tests:**

*Microgram Journal 2016, Volume 13; Numbers 1-4*


Zhang S. Kit for combined detection of drugs, its preparation method and blocking agents used for the same. (Patent) Chemical Abstracts 2006;144:144626y.

**Quality Assurance:**


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Sampling Plans:


Horrocks M. Sub-sampling and preparing forensic samples for pollen analysis. Journal of Forensic Sciences 2004;49(5):1024. [The applications include a brief discussion of illicit drugs.]


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The 2010 “Research on Drug Evidence” Report
[From the 16th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 2010 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

Important Information:

Presented at the 16th ICPO / INTERPOL Forensic Science Symposium, Lyon, France, October 5 - 8, 2010.

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Citations in this report from the Journal of the Clandestine Laboratory Investigating Chemists Association were (and remain) Law Enforcement Restricted.

The "General Overview" (Talking Paper) was removed from this reprint (Editor's discretion).

This reprint is derived from the original electronic document, and is not an image of the best available hard copy (as was utilized for the 1995 and 1998 reports). For this reason, the pagination in the Proceedings is not retained in this reprint; in addition, minor corrections were made, (where present) "contact information" was removed, and some minor reformatting was done to eliminate deadspace. All widow and orphan lines were left as is. The references in this review were not numbered in the original document; in addition, in a few cases only the first page of the citation is provided (duplicating what was provided in the respective abstract). Finally, per request by the Symposium organizer, the journal titles were capitalized (and remain so in this reprint).
Research On Drug Evidence
July 1, 2007 - June 30, 2010

Presented by: Jeffrey H. Comparin

Prepared by: Thomas M. Duncan

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Sixteenth ICPO - INTERPOL
Forensic Sciences Symposium
October, 2010

Lyon, France
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Notes:  
1. All categories are subdivided by topic or category, then alphabetically by the first author's last name.  
2. Note that the following reference is law enforcement restricted, and is not available to the general public: The *Journal of the Clandestine Laboratory Investigating Chemists Association* (all years).
I) Routine and Improved Analysis of Abused Substances

Issue:
Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new homolog or analog (i.e., "designer"-type) drugs.

Solution:
Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general field of analytical chemistry, provide new and/or improved methods of analysis for both routine and specialized analyses of seized drugs. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

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Sanderson R. Identification of N-methylbenzylamine hydrochloride, N-ethylbenzylamine


Yohannan M. Detection of phenethylamine, amphetamine, and tryptamine imine by-products from an acetone extraction. Mid-Atlantic Association of Forensic Scientists


**Barbiturates (including Quaaludes):**


**Benzodiazepines:**

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**Methylenedioxyamphetamine and Related Compounds:**


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benzylpiperazines (MDBP) by GC-IRD and GC-MS. FORENSIC SCIENCE INTERNATIONAL 2010;195(1-3):78-85.


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**Salvia Divinorum:**


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Atwood BK, Huffman J, Straiker A, Mackie K. JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB1 receptor agonist. BRITISH JOURNAL OF PHARMACOLOGY 2010;160(3):585-593.


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(Designer) Tryptamines (see also Psilocybin):


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Wolf EU, Raziel A, Katz Y. The "periodic table" of designer drugs in Israel. JOURNAL OF THE CLANDESTINE LABORATORY INVESTIGATING CHEMISTS


Zaleplon:


Zolpidem:


Halasz I, Dinnebier RE. Structural and thermal characterization of Zolpidem hemitartrate hemihydrate (form e) and its decomposition products by laboratory X-ray powder diffraction. JOURNAL OF PHARMACEUTICAL SCIENCES  2010;99(2):871-878.


characterization of process-related substances to the hypnotic agent Zolpidem. ARKIVOC 2009;143-149.


**Zopiclone:**


**Miscellaneous:**


II) Synthesis and/or Cultivation of Abused Substances, their Precursors, and Essential Chemicals

Issue:
Forensic chemists must maintain familiarity with existing and new clandestine syntheses of abused substances, their precursors, and essential chemicals, and with the cultivation of abused natural products, in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and to provide expert testimony in legal proceedings.

Solution:
Illicit drug seizures, clandestine laboratory operations, and illicit grow operations, are continuously monitored to maintain a comprehensive overview of the field. In cases where new drugs are synthesized, or new methodologies are utilized, case reports are generated for the forensic and enforcement communities.

References:

Production of Abused Substances and/or their Precursors and Essential Chemicals:


Bikbulatov RV, Stewart J, Jin WT, Yan F, Roth BL, Ferreira D, Zjawiony JK. Short synthesis of a novel class of salvinorin A analogs with hemiacetalic structure.


Coote SJ, Davies SG, Fletcher AM, Roberts PM, Thomson JE. Enantiospecific stereodivergent synthesis of trans- and cis-N(2),3-dimethyl-4-phenyl-1,2,3,4-tetrahydroisoquinolines. CHEMISTRY-AN ASIAN JOURNAL 2010;5(3):589-604.


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Norman K. The synthesis of amphetamine and methamphetamine: A "big" picture.


Toske SG, Hays PA, Geer BL. The synthesis and identification of


Zhang L, Ding ZY, Shi GY. Asymmetric biosynthesis of (1S,2S)-ephedrine by Morganella morganii CMCC(B)49208. AFRICAN JOURNAL OF BIOTECHNOLOGY 2009;8(4):694-698.
III) Clandestine Laboratories - Appraisals and Safety

Issue:
Forensic chemists must maintain familiarity with clandestine laboratory procedures, setups, and techniques in order to assist enforcement activities, to ensure safety and effectiveness during enforcement operations, and in order to provide expert testimony in court proceedings.

Solution:
Clandestine laboratory operations are continuously reviewed to provide a comprehensive overview of the field. In cases where new methodologies are noted, or unusual safety concerns are salient, reports are generated for the forensic and enforcement communities.

References:


**Clandestine Laboratory Appraisals and Safety:**

Abdullah AFL, Miskelly GM. Recoveries of trace pseudoephedrine and methamphetamine residues from impermeable household surfaces: Implications for sampling methods used during remediation of clandestine methamphetamine laboratories. TALANTA  2010;81(1-2):455-461.


VanDyke M, Erb N, Arbuckle S, Martyny J. A 24-hour study to investigate persistent chemical exposures associated with clandestine methamphetamine laboratories. JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE  2009;6(2):82-89.

**Safety Issues - Case Reports:**

Burge M, Hunsaker JC, Davis GJ. Death of a toddler due to ingestion of sulfuric acid at a clandestine home methamphetamine laboratory. JOURNAL OF BIOLOGICAL CHEMISTRY  2010;285(2):298-301.
IV) Reference Drug Standards and Total Syntheses

Issue:
Many reference drug standards or structurally related internal standards are either commercially unavailable, or if available are extremely expensive.

Solution:
Controlled substances and their structural or isotopically labelled analogs are synthesized as needed. Internal standards are also prepared as needed. Case reports are published for new or unusual standards or improved synthetic approaches.

References:

Microgram Journal 2016, Volume 13; Numbers 1-4

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V) Source Determination of Drugs (Impurity Profiling) and Comparative Analyses

Issues:
Impurity profiling of drugs is important for comparative analysis protocols, geo-sourcing, and synthetic route determinations. However, although certain drugs have been well characterized with respect to their impurity profiles, most have not been properly investigated.

Comparative analysis (i.e., the systematic application of impurity profiling for determination of commonality of origin) is complicated due to both the high complexity of the data and the large numbers of exhibits. Improved analytical and data handling techniques are needed.

Solution:
High sensitivity analytical techniques (primarily chromatographic) provide detailed profiles of trace-level impurities, ions, trace metals, and stable isotopes. Identification of individual impurities enhance origin identification and comparative analyses and also aid in development of internal standards for improved accuracy and precision of analysis.

In-depth analysis via improved instrumental methodologies help identify discriminatory components in impurity profiles. Computer databases, sorting programs, and pattern recognition/neural networks provide enhanced data handling and analysis, enabling and improving comparative analyses. Case reports are generated for the forensic and enforcement communities.

References:

Amphetamine(s):


Cocaine:


**Heroin:**

Cai XL, Wu GP. Preliminary study on identification of heroin from different routes with clustering analysis by Fourier transform infrared spectroscopy. SPECTROSCOPY AND SPECTRAL ANALYSIS 2007;27(12):2441-2444.


Hibbert DB, Blackmore D, Li JF, Ebrahimi D, Collins M, Vujic S, Gavoyannis P. A probabilistic approach to heroin signatures. ANALYTICAL & BIOANALYTICAL


Morello DR, Cooper SD, Panicker S, Casale JF. Signature profiling and classification of illicit heroin by GC-MS analysis of acidic and neutral manufacturing impurities. JOURNAL OF FORENSIC SCIENCES  2010;55(1):42-49.

Nguyen XT, Hoang MH, Do DN, Tran VS. Establishment of the method for analysis of solvent residue in heroin samples to track the origin. TAP CHI DUOC HOC 2007;47(2):34.

Odell LR, Skopec J, McCluskey A. Isolation and identification of unique marker compounds from the Tasmanian poppy Papaver somniferum N. Implications for the identification of illicit heroin of Tasmanian origin. FORENSIC SCIENCE INTERNATIONAL  2009;183(1-3):105-106.


Marijuana:


**Methamphetamine:**


Kunalan V, Daeid NN, Kerr WJ, Buchanan HAS, McPherson AR. Characterization of route specific impurities found in methamphetamine synthesized by the Leuckart and reductive amination methods. ANALYTICAL CHEMISTRY 2009;81(17):7342-7348.


3,4-Methylenedioxymethamphetamine:


Buchanan HAS, Daeid NN, Meier-Augenstein W, Kemp HF, Kerr WJ, Middleditch M. Emerging use of isotope ratio mass spectrometry as a tool for discrimination of 3,4-methylenedioxymethamphetamine by synthetic route. ANALYTICAL CHEMISTRY 2008;80(9):3350-3356.


Cox M, Klass G, Morey S, Pigou P. Chemical markers from the peracid oxidation of


Multi-Drug and Miscellaneous:


VI) Analysis of Non-Controlled Pharmaceuticals, Pseudo-Drugs, Adulterants, Diluents, and Precursors

Issue:

Most "street-level" drugs are "cut" with various adulterants and diluents. Many of these cutting agents are pharmaceutical products or precursors. Others are "carry-through" compounds present in precursors (especially in cold remedy products). Separation and identification of these extraneous materials can be tedious, especially in exhibits which contain many components. In addition, new or unusual adulterants and/or diluents are occasionally identified in drug exhibits, and standard analytical data are required for these substances. Finally, improved methods of analysis, i.e., faster, more discriminatory, less costly, etc., are needed for all cutting agents.

Solution:

Reports providing standard analytical data and/or improved analytical protocols for non-controlled pharmaceuticals, pseudo-drugs, adulterants, diluents, and precursors are generated for the forensic and enforcement communities.

References:

Ephedra, Ephedrine, and/or Pseudoephedrine and Related Compounds:


Hayashi K, Shimura K, Makino T, Mizukami H. Comparison of the contents of kampo decoctions containing ephedra herb when prepared simply or by re-boiling according to the traditional theory. JOURNAL OF NATURAL MEDICINES 2010;64(1):70-74.


**Phenylpropanolamine:**


Other Adulterants/Diluents (including mixtures containing Ephedrine and/or Pseudoephedrine):


Liao CZ, Nicklaus MC. Comparison of nine programs predicting pK(a) values of pharmaceutical substances. JOURNAL OF CHEMICAL INFORMATION AND MODELING 2009;49(12):2801-2812.


Liu Y, Ge H, Zhao K, Yu L. Determination of three chemical components added illegally


**Theophylline:**


**Miscellaneous:**


VII) New and/or Improved Instrumental Techniques

Issue:
Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Solution:
Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

References:

**Capillary Electrophoresis (and Related Techniques, including Tandem Techniques):**

Assuncao NA, Bechara EJH, Simionato AVC, Tavares MFM, Carrilho E. Capillary electrophoresis coupled to mass spectrometry (CE-MS): Twenty years of development. QUIMICA NOVA 2008;31(8):2124-2133.


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Almirall J, Perr J, Guerra P. Method and apparatus for extraction, detection, and


Madej K. Microwave-assisted and cloud-point extraction in determination of drugs and other bioactive compounds. TRAC - TRENDS IN ANALYTICAL CHEMISTRY 2009;28(4):436-446.


Wille SMR, Lambert WEE. Recent developments in extraction procedures relevant to analytical toxicology. ANALYTICAL AND BIOANALYTICAL CHEMISTRY 2007;388(7):1381-1391.

Gas Chromatography (and Tandem GC Techniques):


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High-Performance Liquid Chromatography (and tandem HPLC techniques):


**Inductively Coupled Plasma- Mass Spectrometry (ICP-MS, Also ICP-OES):**


Infrared and Near Infrared Spectroscopy (including Terahertz Spectroscopy):


Clark D, Pysik A. The analysis of pharmaceutical substances and formulated products by vibrational spectroscopy. APPLICATIONS OF VIBRATIONAL SPECTROSCOPY IN PHARMACEUTICAL RESEARCH AND DEVELOPMENT 2007;213-238.


Kazarian SG, Chan KLA. Micro- and macro-attenuated total reflection Fourier transform
infrared spectroscopic imaging. APPLIED SPECTROSCOPY 2010;64(5):135A-152A.


**Ion Mobility Spectrometry:**


**Mass Spectrometry (including Ambient Pressure Techniques and Isotope Ratio):**


Cotte-Rodriguez I, Mulligan CC, Cooks G. Non-proximate detection of small and large molecules by desorption electrospray ionization and desorption atmospheric pressure chemical ionization mass spectrometry: Instrumentation and applications in forensics, chemistry, and biology. ANALYTICAL CHEMISTRY 2007;79(18):7069-7077.

Dove A. Mass spectrometry raises the bar. SCIENCE 2010;328(5980):920-922.


Grange AH. An integrated wipe sample transport/autosampler to maximize for a direct


Kertesz V, Van Berkel GJ. Improved desorption electrospray ionization mass spectrometry performance using edge sampling and a rotational sample stage. RAPID COMMUNICATIONS IN MASS SPECTROMETRY 2008;22(23):3846-3850.

Kertesz V, Van Berkel GJ. Improved imaging resolution in desorption electrospray ionization mass spectrometry. RAPID COMMUNICATIONS IN MASS SPECTROMETRY 2008;22(17):2639-2644.


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Nilles JM, Connell TR, Durst HD. Thermal separation to facilitate direct analysis in real time (DART) of mixtures. ANALYST 2010;135(5):883-886.


Philip RP, Kuder T. The evolution of stable isotope applications in environmental


Talaty N, Mulligan CC, Justes DR, Jackson AU, Noll RJ, Cooks RG. Fabric analysis by ambient mass spectrometry for explosives and drugs. ANALYST 2008;133(11):1532-1540.


Weston DJ. Ambient ionization mass spectrometry: Current understanding of mechanistic theory; analytical performance and application areas. ANALYST 2010;135(4):661-668.


**Microchip Technology:**


**Nuclear Magnetic Resonance Spectroscopy:**


Mo HP, Harwood JS, Raftery D. Receiver gain function: The actual NMR receiver gain. MAGNETIC RESONANCE IN CHEMISTRY 2010;48(3):235-238.


Pantoja-Uceda D, Santoro J. Aliasing in reduced dimensionality NMR spectra: (3,2)D HNHA and (4,2)D HN(COCA)NH experiments as examples. JOURNAL OF BIOMOLECULAR NMR 2009;45(4):351-356.


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Webster GK, Marsden I, Pommerening CA, Tyrakowski CM. Validation of pharmaceutical potency determinations by quantitative nuclear magnetic resonance spectrometry. APPLIED SPECTROSCOPY 2010;64(5):537-542.

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Clelland BL. Forensic applications of Raman microspectroscopy, capillary electrophoresis, chromatography, and mass spectrometry for the analysis of textile fibers, dyes, illicit drugs, and anticoagulant rodenticides. DISSERTATION ABSTRACTS INTERNATIONAL, B 2007;67(9):5041.


Virtanen S, Antikainen O, Yliuruusi J. Determination of the crushing strength of intact...
Solid Phase Micro-Extraction (Headspace Techniques and Solvent Analysis):


Lai H, Corbin I, Almirall JR. Headspace sampling and detection of cocaine, MDMA, and marijuana via volatile markers in the presence of potential interferences by solid phase microextraction-ion mobility spectrometry (SPME-IMS). ANALYTICAL AND BIOANALYTICAL CHEMISTRY 2008;392(1-2):105-113.

Lai HT, Almirall JR. Headspace sampling and detection of cocaine, MDMA, and marijuana via volatile chemical markers; solid phase microextraction-ion mobility spectrometry. 2008 American Academy of Forensic Sciences Annual Meeting.


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Thin Layer Chromatography:


X-Ray based Techniques:


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Miscellaneous:


Ferreira FJO, Crispim VR, Silva AX. Detection of drugs and explosives using neutron computerized tomography and artificial intelligence techniques. RADIATION AND ISOTOPES 2010;68(6):1012-1017.


Silverio FO, Barbosa LCA, Pilo-Veloso D. Pyrolysis as an analytical technique. QUIMICA NOVA 2008;31(6):1543-1552.

VIII) Portable Detection and Analytical Instrumentation

Issue:
"Free Trade" agreements and the easing of formally restrictive national and international borders have resulted in dramatic increases in cargo transshipments and personal travel, thereby complicating drug inspection and interdiction efforts at POEs. Discovery and confirmational analysis of suspected drugs in cargo or on individuals is severely hampered by the lack of on-site detection and/or analytical equipment.

Solution:
Development of portable and highly sensitive detectors for drug detection and analyses allows law enforcement personnel and/or forensic chemists to perform screening type analyses on-site. In those cases where new methodologies have proven effective, case reports are generated for the forensic and enforcement communities.

References:


Carron K, Cox R. Qualitative analysis and the answer box: A perspective on portable Raman spectroscopy. ANALYTICAL CHEMISTRY 2010;82(9):3419-3425.


Grates KM, Ring JG, Savage KA, Denicola TA. Conclusion of validation study of commercially available field test kits for common drugs of abuse. 2008 American Microgram Journal 2016, Volume 13; Numbers 1-4
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Guerra-Diaz P, Gura S, Almirall JR. Dynamic planar solid phase microextraction-ion mobility spectrometry for rapid field air sampling and analysis of illicit drugs and explosives. ANALYTICAL CHEMISTRY 2010;82(7):2826-2835.


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Beverage Analysis:

Clark A. A device for automatically testing whether a drink has been spiked. APPLICATION: GB 2007-5716 24 March 2007.

Loane CJ. Chemical spot test for the detection of drugs of abuse in a beverage. (Patent) CHEMICAL ABSTRACTS 2007;147:501291d.


Chemometrics:


Drabek J. Validation of software for calculating the likelihood ratio for parentage and kinship. FORENSIC SCIENCE INTERNATIONAL-GENETICS 2009;3(2):112-118.


Kafkafi N, Yekutieli D, Elmer GI. A data mining approach to in vivo classification of


Counterfeit Drugs:


Willis RC. Noninvasive testing for counterfeit drugs. ANALYTICAL CHEMISTRY 2007;79(5):1773.


**Dragon's Blood:**

Gupta D, Bleakley B, Gupta RK. Dragon's blood: Botany, chemistry and therapeutic...


**Drugs on Currency:**

Armeta S, de la Guardia M. Analytical methods to determine cocaine contamination of banknotes from around the world. TRAC - TRENDS IN ANALYTICAL CHEMISTRY 2008;27(4):344-351.


**Education:**


Coleman WF. Molecular models of real and mock illicit drugs from a forensic chemistry activity. JOURNAL OF CHEMICAL EDUCATION 2008;85(6):880.

Harmon KJ, Miller LM, Millard JT. Crime scene investigation in the art world: The case


**Legal Issues:**

Bono JP, Siegel JA. Pattern evidence and conformance to the requirements of Daubert. 2008 American Academy of Forensic Sciences Annual Meeting.


**Quality Assurance:**


Hauck WW, Abernethy DR, Williams RL. Metrologic approaches to setting acceptance criteria: Unacceptable and unusual characteristics. JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS 2008;48(3):1042-1045.


**Sampling Plans:**


Mario JR. A probability-based sampling approach for the analysis of drug seizures composed of multiple containers of either cocaine, heroin, or cannabis. FORENSIC SCIENCE INTERNATIONAL 2010;197(1-3):105-113.

**Toolmarks:**


Other:


Wu JJ. In situ test for determining whether items of real or personal property have been exposed to the manuf. of illegal drugs. APPLICATION: US2008-13558 14 January 2008.
The 2013 “Research on Drug Evidence” Report
[From the 17th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 2013 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

Important Information:

Distributed at the 17th ICPO / INTERPOL Forensic Science Symposium, Lyon, France, October 8 - 10, 2013.*

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Citations in this report from the Journal of the Clandestine Laboratory Investigating Chemists Association were (and remain) Law Enforcement Restricted.

The "General Overview" (Talking Paper) was removed from this reprint (Editor's discretion).

This reprint is derived from the original electronic document, and is not an image of the best available hard copy (as was utilized for the 1995 and 1998 reports). For this reason, the pagination in the Proceedings is not retained in this reprint, some minor reformatting was done to eliminate deadspace, and all widow and orphan lines were left as is.

[* Due to travel restrictions in effect in late 2013, this report and the associated "General Overview" (Talking Paper) was not actually presented, but rather the report was only distributed to the attendees.]
Research on Drug Evidence

January 1, 2010 - June 30, 2013

Presented by: Jeffrey H. Comparin
Prepared by: Robert F.X. Klein

U.S. Department of Justice
Drug Enforcement Administration

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Seventeenth ICPO - INTERPOL
Forensic Sciences Symposium
October 2013

Lyon, France
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Preface Notes:

1. With the exception of synthetic cannabinoids and cannabimimetics, all references are subdivided by individual drug, drug group or class, or general topic, then chronologically, and finally alphabetically within each year (first author's last name). Individual synthetic cannabinoids and cannabimimetics are included in that drug group (i.e., not as individual drugs). In addition, and in contrast to past reports from this laboratory, references are organized as much as is practical by specific drug or drug group/class. This change is necessary because of the large numbers of similar types of "designer drugs," most notably the synthetic cannabinoids and cannabimimetics, the cathinones and related amphetamine-type-stimulants, and the methylenedioxyphenethylamines and related hallucinogens.

2. References from January 1, 2010 to June 30, 2010 are included because many were either not cited in the last review (because they had not yet been abstracted or printed), or were cited as "Ahead of Print" (i.e., without volume, issue, or page numbers). Some of the references from January 1, 2013 to June 30, 2013 in this report are similarly cited as "Ahead of Print;" all such references were still in "Ahead of Print" status as of June 30, 2013. Readers should be aware that the year listed with "Ahead of Print" may not reflect the eventual year of publication; however, the article's author(s), article title, and journal should remain the same regardless of the actual year of publication, allowing the full citation to be easily found by Internet searching.

3. Note that the following reference is law enforcement restricted, and is not available to the
general public: *Journal of the Clandestine Laboratory Investigating Chemists Association* (all years). All other references cited in this report were acquired from the "Forensic Chemistry" sections of Chemical Abstracts, and to the author's knowledge are non-restricted. [Please also note that the second quarterly issue of the 2013 *Journal of the Clandestine Laboratory Investigating Chemists Association* (i.e., 2013; 23(2)) had not been published by the reference cutoff date, June 30, 2013.]
1. Routine and Improved Analyses of Abused Substances

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new "designer drugs."

Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general fields of analytical chemistry and toxicology, provide new and/or improved methods of analysis for abused substances. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

1.A - General Reviews and Overviews
1.B - Individual Compounds or Substances
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1.D - Polydrug A: Mixed or Unrelated Named Compounds or Substances

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1.A - General Reviews and Overviews

2010 INTERPOL Triennial Report on forensic science (1); brief overview (2); 2011 Analytical Chemistry biannual review of forensic science (3); brief, conversational overview (4).

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1.B - Individual Compounds or Substances (except individual synthetic cannabinoids and cannabimimetics)

Alprazolam: 2011 analysis by DART-TOF-MS (5);

Amphetamine: 2010 2H and 13C isotope ratios in amphetamine synthesized from benzaldehyde and nitroethane (6); impurity profiling (7); 2011 by Raman and SERS, with spectral analyses by ab initio calculations (8);

1-Benzyl-4-methylpiperazine: 2012 identification by MS, after derivatization with trifluoroacetic anhydride, and by NMR (9);
**Buphedrone (2-(methylamino)-1-phenylbutan-1-one):** 2013 characterization with GC/MS, HPLC-DAD, and LC-MS/MS (10);

**Buprenorphine:** 2011 by GC/MS (11);

**2-(4-Chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe):** 2013 characterization by GC-EI-MS (with and without derivatization with TFAA), LC-ESI-QTOF-MS, FTIR, and NMR (12);

**meta-Chlorophenylpiperazine (m-CPP):** 2011 characterization by easy ambient sonic-spray ionization, XRF, IMS, and NMR (13);

**Citalopram:** 2012 determination by chromatographic and spectrophotometric methods (14);

**Cocaine:** 2010 detection on clothing using Raman (15); transacetylation of benzocaine by acetylsalicylic acid to create N-acetylbenzocaine in cocaine (16); comparison of corona discharge ionization-IMS versus AP-CI-MS for detection of cocaine (17); a 20 year survey of cocaine seized in France (year range not specified in the abstract) (18); detailed evaluation of the mass spectrum of cocaine (19); 2011 detection of cocaine solutions in sealed bottles of (nominal) alcoholic beverages by Raman (20); determination on banknotes using an aptamer-based electrochemiluminescence biosensor (21); detection of 2,6-diisopropynaphthalene as an adulterant in cocaine by GC/MS (22); detection of cocaine solutions in wine bottles by 1H-NMR (23); detection by TLC and cobalt thiocyanate (24); detection based on strand-displacement polymerization and fluorescence resonance energy transfer (25); analysis and classification using GC/IRMS to determine d13C values (26); use of the gold chloride microcrystalline test to identify cocaine and certain adulterants (27); temperature-dependent elimination of benzoic acid during pyrolysis of cocaine (28); analysis by TLC coupled to easy ambient sonic-spray ionization MS (29); use of metastable state nanoparticle-enhanced Raman for highly sensitive detection of cocaine (30); 2012 determination of phenyltetrahydroimidazothiazole enantiomers (present in cocaine) by chiral GC (31); detection by structure-switch aptamer-based CZE (32); determination of the time lag between coca leaf harvest and the seizure and analysis of illicit cocaine (33); analysis using differential mobility spectrometry-MS (34); by electrochemical detection (35); detection using a specialized fluorescence sensor (36); analysis of cocaine smuggled by dissolution in polyvinyl alcohol in a dance pad (37); quantification of binary mixtures of cocaine and adulterants using dispersive Raman, FTIR, and Principal Component Regression (38); analysis of Brazilian "oxi" cocaine (analytical methods not specified in the abstract) (39); 2013 by electrochemical determination (40); by GC/FID (41); detection of hygrine and cuscohygrine as possible markers (to distinguish coca chewing from cocaine abuse) by GC/MS (42); comparative analysis of solvent impurity profiles obtained by HS-GC/MS (43);
Diazepam: 2010 detection in spiked alcoholic beverages by fluorimetry (44);

3,4-Dimethylmethcathinone (3,4-DMMC): 2012 characterization by GC/MS, LC/MS, 1D- and 2D-NMR, IR, and UV (45);

2,5-Dimethoxy-3,4-dimethyl-beta-phenethylamine (2C-G): 2012 by GC-EI/MS (including after derivatization with trifluoroacetic anhydride), LC-ESI/QTOF-MS, LC-ESI/QTOF-MS/MS, FTIR, and 1H- and 13C-NMR (46);

2,5-Dimethoxy-4-nitro-beta-phenethylamine (2C-N): 2012 characterization by GC-EI/MS, LC/ESI-QTOFMS, FTIR, and NMR (including after derivatization with trifluoroacetic anhydride) (47);

2-(Diphenylmethyl)pyrrolidine: 2011 by GC-EI/CI-ion trap-MS and HPLC/DAD-ESI-MS (48);

N-Ethyl-alpha-ethylphenethylamine: 2013 characterization by GC/MS, LC-TOF-MS, and 1D- and 2D-NMR (49);

Ethylphenidate: 2011 characterization by MS, IR, and 1H- and 13C-NMR (50);

Fentanyl: 2012 impurity profiling using UHPLC-MS/MS (51);

Flunitrazepam: 2011 detection using a photocatalytic reaction with ZnO particles with monitoring by UV-Vis (52); 2012 detection in alcoholic beverages by DESI-MS (53);

Glaucine: 2010 detection in "legal highs" (54);

Heroin: 2010 a probabilistic approach to heroin signatures (55); profiling and classification of illicit heroin by GC/MS of acidic and neutral manufacturing impurities (56); by optimized GC/FID (57); analysis by FTIR (58); 2011 identification of levamisole and lidocaine acetylation reaction impurities in heroin (59); rapid and semi-quantitative presumptive testing (60); converting GC/MS heroin profiling to a UHPLC-MS/MS method (61); identification of adulterants and diluents in heroin by IR and/or Raman (62); 2012 analysis of trace elements by ICP-MS (63); comparative evaluation using a simplified clustering analysis (64); impurity profiling by GC (65); by GC (66); analysis of heroin containing aspirin, paracetamol, caffeine, theophylline, codeine, acetyl codeine, and monoacetylmorphine, by GC/MS (67); purification of street samples by prep-HPLC (68); analysis by ICP/MS (69); by reflectance NIR (70); impurity profiling based on the major alkaloids (acetylcodene, 6-monoacetylmorphine, papaverine,
noscapine, codeine, and morphine) (71);

**Human Growth Hormone (HGH): 2010** analysis by CE-ESI-TOF/MS (72);

**Ketamine: 2010** study of the fragmentation pattern of ketamine-heptafluorobutyramide by GC/MS (73); **2012** detection in beverage residues by LC/MS and MS/MS (74); (see also Methoxetamine, below, and Reference # 528);

**Khat (Catha edulis): 2010** preservation of cathinone in khat via drying (75); **2012** qualitative and quantitative analysis of cathinone, cathine, and phenylpropanolamine by GC/MS and GC/FID (76); **2013** analysis by CE (77);

**Kratom: 2012** quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine, and phenylephrine in a kratom cocktail using HPLC (78); by HPLC/ESI-MS (with comparison of 3 different extraction techniques) (79); **2013** by HPLC- DAD (80);

**LSD: 2010** quantitation by HPLC (81); **2012** LSD (and 9,10-dihydro-LSD) - by color testing, TLC, EASI-MS, HPLC-UV (82);

**Marijuana and Marijuana-Derived Cannabinoids: 2010** tracing geographic and temporal trafficking patterns for marijuana in Alaska using stable isotopes (83); differentiation of fibre- and drug type seedlings by GC/MS and chemometrics (84); tracing retail cannabis in the U.S. using hydrogen and carbon isotope ratios to determine geographic origins, cultivation parameters, and trafficking patterns (85,86); evaluation of an experimental indoor hydroponic cannabis grow operation using the Screen of Green method (87); evaluation of an experimental indoor hydroponic Cannabis grow operation, using the "Screen of Green" yield estimation program, THC analysis, and DNA analysis (88); survey of the potency trends of THC and other cannabinoids in marijuana from 1993 to 2008 (89); analysis of marijuana seized in Novi-Sad, Serbia in 2008 (90); determination of THC, CBD, and CBN in edible oils by UHPLC-MS/MS (91); **2011** use of DNA collection cards for in-the-field sampling (92); differentiation of seedlings by GC/MS and Linear Discriminant Analysis, Partial Least Squares Discriminant Analysis, Nearest Neighbor Classification, Learning Vector Quantization, Radial Basis Function Support Vector Machines, Random Forest, and Artificial Neural Networks (93); a survey of cannabinoid ratios in marijuana seized in California from 1996 to 2008 (94); profiling and source determination by GF AAS and ICP OES (95); differentiation of drug and non-drug marijuana using a single nucleotide polymorphism assay (96); analysis of THC in industrial hemp crops in Morocco (97); differentiation of drug-type and fiber-type by multiplex PCR analysis (98); determination of the long term stability of select cannabinoids (method not reported in the abstract) (99); a formula for determining the yield and quality of indoor grow operations (100);
semi-prep scale isolation of tetrahydrocannabinolic acid A (THCA) using two flash chromatography systems (101); 2012 determination of THC by voltammetry (102); investigation of potential interferences by other drugs with the Fast Blue B and Duquenois-Levine color tests (103); a survey of the potency of marijuana grown in Albania (survey range not listed in the abstract) (104); isomerization of CBD and THC under positive ESI conditions (105); an investigation into the hypothesis of transgenic (genetically modified) marijuana (106); a PCR assay for the relative quantification of THCA synthase gene (107); analysis of DNA by CE for geo-sourcing (108); differentiation between very young drug- and hemp-type cannabis seedlings and cuttings by determination of select cannabinoids by HPLC-DAD (109); classification of cultivars based on analysis of cannabinoids and terpenoids (110); preliminary analysis of genetic diversity of hemp cultivars based on ISSR molecular markers (111); use of delta13C isotope ratios for differentiation of samples (112); a study of the effects of electrical lighting power and irradiance on indoor-grown marijuana potency and yield (113); by LC/API-MS and LC/API-MS/MS (114); determination of THC, CBD, and CBN in marijuana grown in northern Thailand, by GC/FID (115); a study of the long-term storage and stability of hash oil (methods not listed in the abstract) (116); a study of the long-term storage and stability of "cannabis resin" (methods not listed in the abstract) (117); identification and characterization of hybrid and/or high potency marijuana (methods not specified in the abstract) (118); a survey of the potency of marijuana seized in Japan in 2010 (methods not listed in the abstract) (119); use of ultrasound for improved extraction of cannabinoids for HPLC analysis (120); evaluation of the uncertainty of THC determined by HPLC (121); 2013 by HPLC-UV following cloud point extraction (122); by DNA analysis (123); by laser-ablation inductively-coupled plasma MS (LA-ICP-MS) - a review, covering many other applications (124); a study of marijuana potency from the 1970s to the 2000s (125); characterization of seeds by DNA analysis (126);

**Mephedrone (4-Methylmethcathinone):** 2010 by color testing, GC/MS, and FTIR (127); by LC (128); 2011 by GC/MS following derivatization with 2,2,2-trichloroethyl chloroformate (129); characterization of 2-, 3- and 4-methylmethcathinone (i.e., mephedrone and its two positional isomers) by GC/MS, NMR, and IR (130); synthesis and characterization (synthetic route and analytical methods not specified in the abstract) (131); an overview and literature review (132); 2012 determination of isotopic fractionation to link precursor to product in the synthesis of (±)-mephedrone (133); a literature review (134); a study of the degradation in alkaline solutions (135); 2013 by SERS with a portable Raman (136);

**Mescaline/Peyote:** 2013 analysis of "peyote tea" by GC/MS and GC/MS/MS in PCI mode (137);

**Methamphetamine:** 2010 enantio-discrimination of methamphetamine by circular dichroism using a porphyrin tweezer (138); an overview of law enforcement efforts against
methamphetamine production in New Zealand (139); isotope fractionation during precipitation (140); recovery and identification of trace methamphetamine and pseudoephedrine on impermeable surfaces in clandestine laboratories (141); identification of three byproducts found in methamphetamine synthesized by the Emde route (142); identification of iodine and red phosphorus using AccuTOF-DART (143); use of phosphorous acid flakes in the reduction of (pseudo)ephedrine to methamphetamine (144); screening of methamphetamine/methyl sulfone exhibits using Raman spectroscopy (145); 2011 analysis by UFLC (Ultra-Fast-LC) (146); an (unsuccessful) attempted synthesis by electrolytic reduction of pseudoephedrine (147); enantioseparation and identification of methamphetamine and the ephedrines using using trifluoroacetic anhydride derivatization and chiral GC/MS (148); analysis using by highly fluorescent polyfluorenes with NH2-terminated side chains (149); chiral analysis by CE with added cyclodextrins (150); a urea - based "one-pot" methamphetamine synthesis (151); chiral separation with CE using dynamically coated capillaries (includes "related compounds") (152); chiral analysis of the enantiomers of ephedrine, pseudoephedrine, chlorinated intermediates, and methamphetamine by derivatization with fluorinated acid anhydrides followed by GC on a cyclodextrin stationary phase, for impurity profiling of methamphetamine synthesized by the Emde method (153); a study of the efficacy of wipe sampling to determine contamination at clandestine laboratories (with analyses by LC/MS or GC/MS) (154); 2012 comparative analysis of impurity profiles from GC/FID (155); the environmental fate of clandestine laboratory waste (156); impurity profiling of Iranian seizures using GC/MS and LC/MS (157); an overview of abuse, treatment, and U.S. law (158); identification of (1S,2S)-1-methylamino-1-phenyl-2-chloropropane as a route specific marker impurity for methamphetamine synthesized from ephedrine via chloroephedrine (159); impurity profiling of methamphetamine synthesised by the Birch method (160); impurity profiling of methamphetamine synthesized using the Nagai method (161); critical evaluation of LLE and SPME methods for impurity profiling (162); detection of trace ephedrine and pseudoephedrine in high-purity methamphetamine by HPLC (163); degradation of 1-(1',4'-cyclohexadienyl)-2-methylaminopropane in soils (164); degradation of methamphetamine production precursors and byproducts in soils (165); chiral analysis of chlorinated intermediates of methamphetamine (from the Emde synthesis) by 1D- and 2D-NMR and GC/MS (166); analysis of a sample cut with diphenylmethane, by GC/MS (167); a study of the effects of synthetic conditions on the d13C, d15N, and d2H isotope ratios of the final product (168); determination of synthetic route via impurity profiling using GC/MS (169); preparation and certification of reference quality material (170); 2013 detection of pharmaceutical impurities in methamphetamine by GC/FID and GC/MS (171); impurity profiling of methamphetamine by CE using a highly sulfated gamma-cyclodextrin as a chiral selector (includes methamphetamine, amphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine) (172); screening of methamphetamine, pseudoephedrine, and ephedrine by a portable lab-on-a-chip instrument (173); evaluation of the use of IMS in remediation of clandestine laboratories (174); influence of precursor solvent extraction on stable isotope signatures of methamphetamine.
prepared from OTC pharmaceuticals using the Moscow and hypophosphorous syntheses (175); impurity profiling of methamphetamine synthesized from P2P prepared from phenylacetic acid (or its esters) (176);

**Methiopropamine: 2011** characterization by IR, MS, and 1H- and 13C-NMR (177); (see also Reference # 250);

**Methorphan: 2012** chiral analysis by GC/MS following derivatization with (-)-menthyl chloroformate (includes MS and NMR analyses of the derivatives) (178);

**Methoxetamine: 2012** by NMR, MS, and IR (with comparisons with ketamine) (179);

**2-(5-Methoxy-1-benzofuran-3-yl)-N,N-dimethylethanamine (5-MeO-BFE) (and its N-ethyl analog): 2012** characterization by MS, NMR, and IR (180);

**4-Methoxyphenylcyclohexylidene: 2011** characterization by MS, IR, and NMR (181);

**4'-Methoxyphenyl-2-propanone: 2012** clandestine synthesis and characterization (182);

**alpha-Methyl-3,4-methylenedioxyphenylpropionamide (MMDPPA): 2013** identified in Australia as an intermediate from helional to MDA (183; see also 184);

**Methylenedioxymethamphetamine (MDA): 2013** from helional (185; see also alpha-methyl-3,4-methylenedioxyphenylpropionamide);

**3,4-Methylenedioxy-N-benzylcathinone (BMDP): 2013** characterization by LC/high res QTOF-MS, ESI-MS, IR, and 1D- and 2D- 1H- and 13C-NMR (186);

**Methylenedioxymethamphetamine (MDMA): 2010** use of stable isotope ratios to differentiate MDMA according to synthetic route (187); identification of some tertiary amines related to MDMA by GC- IRD (188); determination of synthetic route by ICP-MS (189); impurity profiles of MDMA prepared by four different methods (190); **2011** use of impurity profiling, stable isotope analyses, and pattern recognition techniques for characterization and sourcing (191); a historical overview (192); determination of volatile components of MDMA tablets with LC/MS and HS-SPME-GC/MS, for development of canine training aids (193); determination of volatiles by HS-SPME followed by GCxGC and GCxGC-TOFMS (194); by SERS using modified Silver nanoparticles (195); **2012** impurity profiling of MDMA prepared from piperine versus vanillin (196); isolation of MDMA using a specialized SPME cartridge with analysis by GC/MS (197); comparative analysis by GCxGC-TOF-MS (198); **2013** enantiomeric purification by batch
chromatography with a cyclodextrin chiral selector (199); impurity profiling of sassafras oils by GC×GC-TOF-MS (200);

**Methylenedioxypyrovalerone (MDPV): 2010** characterization by GC/MS, NMR, FTIR, and UV (201);

**4-Methylethcathinone (4-MEC): 2013** by GC/MS, HPLC-DAD, and LC-MS/MS (202);

**N-Methylphthalimide:** 2011 characterization by GC/MS, FTIR, and NMR (203);

**4'-Methyl-alpha-pyrrolidinohexanophenone (MPHP): 2011** analysis by GC/MS, HPLC/DAD, and GC/FID (toxicological focus) (204);

**3,4-Methylenedioxypyrovalerone (MDP2P): 2010** differentiation of methoxy methyl phenylacetones related to MDP2P by GC/IRD (205);

**3,4-Methylenedioxypyrovalerone (MDPBP): 2011** characterization by IR, MS, and 1D- and 2D- 1H- and 13C- NMR (206);

**4-Methylthioamphetamine (4-MTA): 2012** impurity profiling of 4-MTA produced by the nitropropene route (207); identification of by-products produced by the Leuckart method, using MS, 1H- and 13C-NMR, IR, and crystallography (208);

**Morphine:** 2012 analysis by FTIR and Raman, with density functional theory (DFT) calculations (209); extraction from poppy seeds, with analysis by GC/MS and GC/FID (210); quantitation in a Chinese traditional medication, by HPLC (211); analysis by cyclic voltammetry, chronoamperometry, and differential pulse voltammetry (212);

**Naphyrone (naphthylpyrovalerone, 1-naphthalen-2-yl-2-pyrrolidin-1-ylpentan-1-one):** 2010 isomer determination by GC- ion trap-EI/CI-MS and 1D/2D NMR spectroscopy (213); 2012 an overview and literature review (214);

**Oxycodone:** 2010 analysis of pyrolysis products by GC and GC/MS (215);

**Phencyclidine (PCP): 2013** false-positive immunoassay caused by MDPV (216);

**Psilocybe Mushrooms:** 2010 comparative analysis of hallucinogenic mushrooms using ATR and transflection IR (217); 2011 by DNA analysis (a review, also including some non-hallucinogenic, poisonous mushrooms) (218);
**alpha-Pyrrolidinopentiophenone**: 2012 by MS, NMR, and IR (219);

**Salvia divinorum**: 2010 thermal degradation products from Salvia divinorum smoke (220); 2012 differentiation from other Salvia species by GC/MS with principal components analysis (221); analysis of "spiked" plant materials by GC/MS (222); 2013 identification of Salvinorin A in Salvia divinorum (but not in 612 related Salvia species) by GC/MS (223); differentiation from marijuana and tobacco by DNA analysis (224);

**Sibutramine**: 2012 by TLC and TLC-densitometry (225); 2013 detection of illicit adulteration of botanical food supplements, by color tests, TLC, HPLC-DAD, MS, and NMR (226);

**Zolpidem**: 2012 by HPLC and MS (includes a degradation study) (227);

**Miscellaneous Drugs**: 2011 characterization of RTI-126 (228).

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1.C - Common Groups or Classes of Compounds or Substances

**Amphetamine-Type Stimulants (ATSs) and Related Phenethylamines (PEAs)**: 2010 analysis of ring and side chain regioisomers of ethoxyphenethylamines related to the controlled substances MDEA, MDMA, and MBDB by GC/MS and GC/IRD (229); methamphetamine, 4-fluoro-, 4-chloro-, 4-bromo-, 4-iodo-, and 4-nitromethamphetamine - analysis by GC/MS following trifluoroacetyl derivatization (230); differentiation of regioisomeric ring-substituted fluorophenethylamines by product ion spectrometry (231); "Fly" and "Dragonfly" Compounds - synthesis and characterization by GC/MS, LC/MS, and LC-MS/MS (232); 2011 GC/MS and GC/IRD studies on the ring isomers of N-methyl-2-methoxyphenyl-3-butanamines (MPBA) related to 3,4-MDMA (233); 4-methylthioamphetamine, 4-fluoroamphetamine, 4-methylamphetamine, 3-trifluoromethylamphetamine, MDA, 2,5-dimethoxyamphetamine, and 2,4,5- and 3,4,5-trimethoxyamphetamines - mass spectrometric properties and identification of some N,N-di-(beta-arylisopropyl)formamides (synthetic impurities) (234); 5- and 6-(2-aminopropyl)-2,3-dihydrobenzofuran - characterization by MS, IR, and NMR (235); amphetamine and methamphetamine - detection by digital image-based colorimetric tests (236); identification of (unspecified) ATSs by GC/MS and GC/FTIR (237); general classification of amphetamines versus non-amphetamines based on GC/FTIR and GC/MS with Principal Component Analysis coupled with Artificial Neural Networks (238); amphetamine, methamphetamine, pseudoephedrine, and five "amphetamine analogs" (not specified in the abstract) - field analysis using the Agilent Bioanalyzer (239); novel syntheses of ATS precursors (240); a review of methods for the chiral determination of ATSs (241); aminooindanes - a review
2012 4- and 5-iodo-2-aminoindan - by MS, NMR, and IR (243); 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylanphetamine - analysis by GC/MS, acetylation, and GC/IRD (244); "amphetamine-type illicit drugs" by a miniaturized gas sensor system using surface ionization (245); DOB and positional isomers - differentiation of various perfluoroacylated derivatives by GC/MS and GC/IRD (246); amphetamine, methamphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine - enantioseparation by CE with contactless conductivity detection (247); a review of the chiral analysis of amphetamine "and related compounds" by CE and NMR (248); 25D-NBOMe, 25E-NBOMe, and 25G-NBOMe - characterization by GC-EI-MS (with and without derivatization with trifluoroacetic anhydride), LC-ESI-QTOF-MS (and MS/MS), FTIR, and NMR (249);

2013 methiopropamine and its 3-thienyl isomer - synthesis and analysis/differentiation by GC (250); o-, m-, p-chloro- and o-, m-, p-fluoro-amphetamine - by CE-LIF, following derivatization with fluorescein isothiocyanate (includes comparisons against CZE-UV, sweeping-MEKC-UV, and LC-Q-TOF-MS) (251); diethylpropion, fenproporex, and sibutramine - in counterfeit tablets, by ATR/FTIR (252); unspecified amphetamines and precursors - by a portable instrument combining miniaturized GC and IR Absorption Spectroscopy (253); 2-, 3-, and 4-methylanphetamine - synthesis and characterization by GC/MS, HR-ESI-MS, NMR, and IR (254); methamphetamine, MDMA, and other unspecified ATSs - by GC/MS after derivatization with iso-Bu chloroformate and SPME (toxicological focus) (255); methamphetamine, MDMA, amphetamine, DMA, and PMA - a review of impurity profiling and syntheses (256);

**Anions: 2010** identification via complexation with meso-octamethylcalix(4)pyrrole and detection using EI-MS (257); **2011** by CE (258, 259);

**Barbiturates: 2010** mephobarbital, pentobarbital, and secobarbital - by MEKC-MS (toxicological focus) (260); **2011** spectrophotometric determination of barbituric acid in pharmaceuticals (261);

**Benzodiazepines: 2011** determination of pK values by potentiometric titration (262); diazepam, estazolam, chlordiazepoxide, and triazolam - analysis by RP-HPLC (263); **2012** clotiazepam, clozapine, and pinazepam - analysis by micellar liquid chromatography (toxicological focus) (264);

**Cathinones: 2010** mephedrone, butylone, 4-methyl-N-ethylcathinone, flephedrone, MDPV, and naphryne - by GC-ion trap-MS (both EI and CI) and NMR (265); mephedrone, methylene, and bk-MBDB - characterization by FTIR, FT-Raman, 1H NMR, 13C NMR, GC/MS, and EI-HRMS (266); **2011** 4-fluoromethcathinone, pentylone, MDPBP, MDPV, and MPPP - by GC-(EI/CI)-MS and NMR (267); 4'-methylethcathinone (4-MEC) and 6 other methcathinone analogs (not specified in the abstract) by LC-MS/MS (268); analysis of isomeric byproducts and related...
impurities in mephedrone and ethylcathinone (269); synthesis and analysis of various methylenedioxyxycathinones, including bk-DMBDB (270); by Raman (271); methylone, bk-MBDB, and bk-MDEA - a review, including analyses by GC/MS, LC/MS, and LC-MS/MS (toxicological focus) (272); 2012 10 homologous and regioisomeric aminoketones related to MDPV - analysis by GC-EI-MS (273); 3,5-difluoromethcathinone and 3,5-dichloromethcathinone - synthesis and characterization by GC/MS, NMR, IR, and GC/IRD (274); the 2,3-isomers of MDPV, butylone, and methylone - synthesis and characterization by GC, IR, GC/MS, and 1H and 13C NMR (275); 4'-methyl-N-ethylcathinone (4-MEC) and 4'-methyl-N-benzylcathinone (4-MBC) - characterization (methods not specified in the abstract (276); buphedrone and pentedrone - synthesis and characterization by FTIR, Raman, 1H- and 13C-NMR, GC/MS, and ESI-HRMS (277); methcathinone, methedrone, and 17 others not specified in the abstract - chiral separation by cyclodextrin-modified CZE (278); cathinone, methcathinone, 4-methylmethcathinone, dimethylcathinone, and 4-methoxymethcathinone - by color testing (281); screening identification of methcathinone and 5 other cathinones by portable ATR/FTIR (282); 4-methylmethcathinone, three positional isomers of fluorocathinones, 4-methoxy-methcathinone, N-ethylcathinone, N,N-dimethylcathinone, buphedrone, and pentedrone - by GC/MS (283); "synthetic cathinones" - detection and screening using a portable ion trap DESI-MS (284); differentiation of isomeric N-alkylated fluorocathinones by GC-MS/MS (285); pentedrone and pentylone - characterization by MS, 1D- and 2D-, 1H- and 13C-NMR, and IR (286); 2013 mephedrone, methylone and MDPV - by ambient ionization MS using arrays of low-temperature plasma probes, and also following injection of trifluoroacetic anhydride directly into the plasma stream for online derivatization (287);

**Ephedrines: 2010** N-acetylpseudoephedrine and N-acetylephedrine - synthesis and characterization by GC-MS, NMR, FTIR, LC-MS, and UPLC-MS (288); 2012 phenylpropanol-amine, cathine, ephedrine, pseudoephedrine, and methylephedrine - analysis by HILIC, with comparison versus RPLC (289); chiral separation of enantiomers of ephedrine and pseudoephedrine in ATSs using achiral modifiers in the gas phase (290); synthesis of alpha-amino-alcohols via the Akabori-Momotani reaction (291); 2013 comparison of RP-UHPLC and HILIC for quantitation, with medium-resolution accurate MS (292);

**Erectile Dysfunction Drugs - Cialis (tadalafil), Levitra (vardenafil), and Viagra (sildenafil): 2010** detection of counterfeits by FTIR, NIR, and Raman (293); identification of (-)-trans-tadalafil, tadalafil, and sildenafil in counterfeit Cialis (294); 2011 development of "classification trees" based on infrared spectroscopic data to discriminate between genuine and counterfeit medicines (295); identification of counterfeits by impurity profiling (296); detection
of counterfeits by Raman (297); 2012 differentiation of legitimate and counterfeit medications by chemometrics and chromatography (298); detection of counterfeits by image processing and statistical analysis (299); analysis of counterfeit Cialis tablets using Raman microscopy and multivariate curve resolution (300); fingerprinting of sildenafil citrate and tadalafil tablets by XRF (301); identification of sildenafil and/or vardenafil using ESI-LC/MS (302); detection of adulteration of capsule shells (a novel and unusual "smuggling" technique) by HPLC-DAD, HPLC/MS, microscopy, and Raman (303); 2013 differentiation between counterfeit and authentic Cialis and Viagra by ATR/FTIR with PCA (304); analysis and profiling by UPLC/MS (305);

**Ergot Alkaloids (see also LSD):** 2012 quantitative analysis using electronic absorption, fluorescence, IR, Raman, CD, ESI-MS, and MALDI-MS (specific compounds not listed in the abstract) (306);

**Fentanyl Derivatives:** 2012 identification of trace level fentanyl derivatives with nonaqueous CE-ESI-MS/MS (307);

**gamma-Hydroxybutyric acid (GHB) and gamma-Butyrolactone (GBL):** 2010 use of IRMS to discriminate between seizures of GBL and for source determination (308); detection of GHB in solutions using a colorimetric sensor array (309); 2011 a study of the spontaneous formation of GHB from GBL in tap water (310); screening for gamma-hydroxybutyrate by ion chromatography (with comparison versus GC/MS) (311); detection of GHB and GBL in adulterated beverages, using 1H-NMR (312); 2012 sodium, potassium, magnesium and calcium salts of gamma-hydroxybutyrate - synthesis and characterization by FTIR, elemental analysis, X-ray powder diffraction analysis, color testing, and microcrystal testing (313); field testing for GHB with a rapid enzymic test (also includes commentary on MDMA, flunitrazepam, and ketamine) (314); 2013 a comprehensive study of the worldwide distribution of GBL using internet monitoring, comparison of packaging, and carbon isotopic measurements (315); in dietary supplements and foods, by GC/MS (using isotopologues for quantitation) (316);

**Methylenedioxyphenethylamines and Related Compounds (note that methylenedioxy-substituted cathinones are categorized under "Cathinones"):** 2010 identification of side chain regioisomers related to MDEA, MDMA, and MBDB (317); 2011 methylenedioxy-2-aminoindans - synthesis and analysis of the 4,5 and 5,6 isomers by GC/MS, ATR/FTIR, and 1H- and 13C-NMR (318); 2012 MDA, alpha-methyl-3,4-methylenedioxy-phenylpropionamide (and 2-chloro-4,5-methylenedioxyamphetamine) - characterization by GC/MS, GC/IRD, ATR/FTIR, and NMR (319);

**Papaver and Opium:** 2010 by cyclodextrin-modified CE following ultrasound-assisted
extraction of Papaver (320); identification of opium poppies using 10 genetic markers (321); 2011 differentiation of P. somniferum, P. rhoas, and P. setigerum by GC/MS and multivariate statistical analyses (322); identification of expressed sequence tag (EST) and simple sequence repeat (SSR) markers (323); determination and analysis of opium alkaloids and crude heroin in complex mixtures by surface-ionization MS (324); 2012 Papaver setigerum by genetic and chemical components analysis (325); opium - determination of 14N and 15N isotopes by proton induced gamma-ray emission (326);

**Piperazines:** 2010 differentiation of methylenedioxybenzylpiperazines by GC/IRD and GC/MS (327); BZP, mCPP, MeBP, MeOPP, MePP, and TFMPP - detection in “Legal Highs” by GC/MS and HPLC-DAD (328); 2011 differentiation of methylenedioxybenzylpiperazines and methoxymethylbenzylpiperazines by GC/IRD and GC/MS (329); BZP and TFMPP - analysis by ATR/FTIR and GC/MS (330); 2012 methoxybenzylpiperazines (OMeBzPs) and methylene-dioxybenzylpiperazines (MDBPs) - differentiation using GC/MS, GC-TOF-MS, and GC/IRD (both underivatized and as perfluoroacylated derivatives (331); 2013 BZP - a review (social focus, but includes “analytical methodologies for the identification of BZP in forensic settings”) (332);

**Plant Materials:** 2010 a review of poisonous plants (includes drugs) (333); 2011 use of cellulose d18O as an index of leaf-to-air vapor pressure difference in tropical plants (334); 2012 analysis of alkaloids from psychoactive plants by nonaqueous CE/MS (specific plants not listed in the abstract) (335); plant DNA fingerprinting - listed applications include “investigation of trade in illicit drugs” (336); 2013 identification of plant materials used as supporting matrices for pharmaceuticals, nutritional supplements, and illicit drugs, by DAD, evaporative light scattering detection, and MS (337); analysis of the plant materials used as support matrices, by DNA analysis, GC/MS, and LC/MS (338; see also Reference Number 352);

**Steroids:** 2010 correlation of the product ion profiles from ESI MS/MS with molecular structures (339); analysis by GC- microchip-AP-photoionization-MS (toxicological focus) (340); identification of anabolic steroids and derivatives using bioassay-guided fractionation and UHPLC/TOFMS analysis (341); 2011 testosterone - IRMS of various black-market products collected in Austria (342); a review of the literature from 2004-2010 (343); analysis by GC/MS using hydrogen as the carrier gas (toxicological focus) (344); 2012 prediction of GC relative retention times of trimethylsilylated derivatives (345); identification of methyltestosterone in counterfeit 4-chlorodehydromethyltestosterone products, by RP-HPLC-ESI-MS (346); elucidation of the m/z 97 ion from androst-4-en-3-one-based steroids by ESI-CID and IRMPD (347); 2013 (primarily) stanozolol, testosterone and nandrolone - a study of authentic and counterfeit products seized in Brazil from 2006 to 2011 (348);
Synthetic Cannabinoids and Cannabimimetics: [Notes: To aid searching for specific compounds, all compounds in this section are listed in alphabetical order within their individual citation (but not within the section). In addition, compounds are listed either by their acronym or full name as was specified in their respective abstract - no effort was made to transcribe acronyms to full chemical names or vice versa. Articles that include both synthetic cannabinoids and/or cannabimimetics with other drugs are detailed in the next section.] 2010 JWH-018 and JWH-073 - by color testing, TLC, GC/MS, and FTIR (349); a survey of synthetic cannabinoids and/or cannabimimetics containing products obtained from June 2008 to September 2009 in Germany/Europe (350); analysis of "Spice Gold" with GC/MS and solid probe MS (351); identification of the plants used as the base materials for products containing synthetic cannabinoids and cannabimimetics (352); JWH-018 - detection by TLC and GC/MS (353); analysis and identification of cannabicyclohexanol, CP-47,497, JWH-018, JWH-073, and oleamide in herbal products by GC/MS and LC/MS (354); an overview of synthetic cannabinoids and cannabimimetics (355); 2011 JWH-203 - characterization by LC/MS, GC/MS, LC with UV detection, NMR, and high-res MS (356); JWH-018, JWH-073, and 9 other unspecified synthetic cannabinoids - a survey of 33 smoking blend products, with analysis by GC/MS (357); JWH-015, JWH-018, JWH-019, JWH-020 JWH-073, JWH-081, JWH 200, JWH-250, WIN 55,212-2 and methanandamide - by LC-MS/MS (toxicological focus) (358); JWH-122 - characterization by NMR, "spectroscopy," and MS (359); JWH-201, JWH-250, and JWH-302 - differentiation by GC/MS fragment ion ratio comparisons (360); an overview and review of synthetic cannabinoids and cannabimimetics, including some GC/MS and LC-MS/MS data (361); (unspecified) analog of a CP 47,497-C8 type compound - by off-line LC-DAD-NMR (362); AM-694, AM-2201, JWH-122, RCS-4, and (2-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone (a positional isomer of RCS-4) - analysis by LC/MS, GC/MS, and NMR (363); AM-694, JWH-019, JWH-122, JWH-210, and (4-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone - analysis by LC/MS, GC/MS MS, and NMR (364); JWH-250 - identification and quantitation by GC/MS, LS/MS, high-res MS, and NMR (365); 1-pentyl-3-(1-naphthoyl)indole, 1-butyln-3-(1-naphthoyl)-indole, 1-hexyl-3-(1-naphthoyl)indole, and 3-[4-(1,1-dimethyloctyl)-2-hydroxyphenyl]-cyclohexan-1-ol - by "chromatography-mass spectrometry" (chromatographic method(s) not specified in the abstract) (366); JWH-018 and JWH-073 - detection by GC/MS (367); JWH-018, JWH-018 N-(2-methylbutyl) isomer, JWH-018 N-(3-methylbutyl) isomer, JWH-201, JWH-250, JWH-302 - isomer differentiation by GC/MS retention times (368); cannabipiperidideanone - identification and characterization by GC/MS, LC/MS, high-res MS, and NMR (369); JWH- 015, JWH-073, JWH-081, JWH-200, JWH-250, JWH-251 - identification and quantitation by GC/MS, LS/MS, high-res MS, and NMR (370); JWH-018 and JWH-073 - detection by GC/MS (371); cannabicyclohexanol (CP-47,497-C8-homolog), JWH-018, JWH-073 - determination by GC/MS (372); 2012 AM2201, JWH-018, and JWH-022 - JWH- 018 and JWH-022 identified as combustion products of AM2201, as determined by GC/MS and Accu-TOF-DART (373); JWH-018 - by DART-TOF-MS (374); JWH-307 - characterization by NMR, GC-HRMS,
ESI-MS/MS, UV, and IR (375); JWH-018 and JWH-073 - purity levels of materials from three different on-line suppliers, as determined by HPLC-UV (376); "synthetic cannabinoids" (specific compounds not listed in the abstract) - analysis by MEKC-DAD (377); AM-694, JWH-018, JWH-019, JWH-073, JWH-081, JWH-210, and JWH-250 - analysis by GC/MS and MALDI-TOF MS (378); AM-679 and 1-pentyl-3-(1-adamantoyl)indole - by LC-UV-MS/MS, LC-TOF-MS, GC/MS, and NMR (379); AM-2201, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-307, and RCS-4 - analysis by LC-ESI-MS/MS (toxicological focus) (380); AM-694, AM-2201, JWH-018, JWH-019, JWH-081, JWH-122, JWH-203, JWH-210, JWH-250, JWH-307, MAM-2201, and RCS-4 - by LC/ESI-MS/MS (toxicological focus) (381); AM-1220 and (N-methylazepan-3-yl)-3-(1-naphthoyl)indole - by TLC, GC/MS, high-res MS, LC-HR-MS/MS, and NMR (382); 3-(1-adamantyl)-1-pentylindole - identification by GC/MS, TLC, NMR, high-res MS, and GC-MS/MS (383); AM-694, AM-2201, CP 47,497 (C=8) (cannabicyclohexanol), JWH-018, JWH-019, JWH-073, JWH-081, JWH-200, JWH-210, JWH-250, RCS-4, and RCS-8 - analysis by TLC, GC/MS, HPLC, and LC-TOF-MS (384); 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone and JWH-412 - separation by flash chromatography and analysis by GC/MS and NMR (385); "synthetic cannabinoids" (five compounds not specified in the abstract) by DART-MS with collision-induced dissociation (386); AM-251 and JWH-015 - analysis by DART-MS (387); color testing for 24 (unspecified) indole-based cannabimimetics (388); an overview (389); naphthoylindoles - by ESI-QTOFMS (390); N-(1-adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA), N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), AM-1220, AM-1241, AM-1248, AM-2233, and CB-13 (CRA-13) - analysis by GC/MS, high-res MS, and NMR (391); 1-butyl-3-(1-(4-methyl)naphthoyl)indole - synthesis and characterization with GC/FID, 1H- and 13C-NMR, DSC, GC/MS, and elemental analysis (392); an overview and review (393); JWH-073 and its 4-methylnaphthoyl analogue - by TLC, NMR, GC/MS, and LC/MS (394); JWH-018, JWH-081, and 10 other (unspecified) "synthetic cannabinoids" - by GC/MS (395); JWH-018 - by GC/MS (396); 2013 JWH-018, JWH-019, JWH-073, and JWH-250 - by GC/MS (397); 5F-UR-144 and UR-144 - by GC/MS, LC-TOF-MS, and 1D- and 2D-NMR (398); AM-2201, JWH-203, JWH-210 and RCS-4 - by LC, high-res MS, LC-QTOF-MS, and NMR (399); 28 (unspecified) "synthetic cannabinoids" - by LC/ESI-MS/MS (toxicological focus) (400); cis- and trans- CP-47,497-C8 (and others not specified in the abstract) - extraction from plant materials by flash chromatography (401); azepane isomers of AM-1220 and AM-2233, AM-2233, and UR-597 - by LC/MS, GC/MS, "accurate MS," and NMR (402); unspecified "cannabimimetics" bearing 2,2,3,3-tetramethylcyclopropene carbonyl moieties - by GC/MS, LC/MS, and NMR (403); JWH-213 - by LC-PDA-MS, GC/MS, high-res MS, and NMR (404); N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide (AB-PINACA) and N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide (AB-FOBICANA) - by LC/MS, GC/MS, high-res MS, and NMR (405); cannabicyclohexanol, JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, JWH-250, and
RCS-4 - by GC/MS, LC-QTOF-MS, and HPLC (406);

**Synthetic Cannabinoids and Cannabimimetics with Other Drugs: 2012**
1-butyl-3-(4-methoxybenzoyl)indole, JWH-018, JWH-073, JWH-122, JWH-250,
1-pentyl-3-(4-methoxybenzoyl)indole, and phenazepam - detection in plant materials (analytical methods not specified in the abstract) (407); 12 "synthetic cannabinoids and cannabimimetics" (not specified in the abstract) and THC - by nano-LC/MS and nano-LC-MS/MS (408); AM-2201, AM-2202, JWH-019, JWH-203, JWH-210, mitragynine (Kratom), (1-(4-pentenyl)-1H-indol-3-yl)(naphthalen-1-yl)methane - analysis by LC/MS, GC/MS, high-res MS, and NMR (409);
2013 AB-001, AM-2232, APINACA, N,5-dimethyl-N-(1-oxo-1-(p-tolyl)butan-2-yl)-2-(N'-(p-tolyl)ureido)benzamide, (4-ethynaphyl)-AM-2201 (EAM-2201), 5-fluoropentyl-3-pyridinoylindole, 5FUR-144 (synonym: XLR11), 4-hydroxy-diethyltryptamine (4-OH-DET), JWH-213, JWH-307, JWH-030, 4-methylbuphedrone, (4-methynaphtyl)-AM-2201 (MAM-2201), (4-methynaphyl)-JWH-022 [synonym: N-(5-fluoropentyl)-JWH-122], N-(4-pentenyl)-JWH-122, UR-144, and URB-754 - detection on plant materials (methods not specified in the abstract) (410); (see also References Numbers 424, 432, 441, 467, 469, and 470);

**Tryptamines (see also Psilocybe Mushrooms): 2010** a review of the analyses of psychoactive N,N-dialkylated tryptamines (411); characterization of the byproducts from the synthesis of DMT by reductive amination, using GC- ion trap-MS (412); profiling psychoactive tryptamine-drug syntheses by MS (to identify route specific impurities) (413); 2011 preparation and analytical characterization of twelve 5-ethoxy-N,N-dialkyl-tryptamines and their deuterated analogues (414); 2012 5-methoxy-2-methyl-N,N-dialkylated tryptamines - synthesis and characterization by 1H and 13C NMR, GC-EI-IT-MS, and CI-IT-MS/MS (415); quantitation of substituted N,N-dimethyl-tryptamines in the presence of natural type XII alkaloids by HPLC, ESI-MS, MS/MS, MALDI-MS, and Raman (416); 2013 AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole) - characterization using 1H- and 13C-NMR, GC-EI/Ci-ion trap-MS, U/HPLC-DAD, and HPLC/MS (417).

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**1.D - Polydrug A: Mixed or Unrelated Named Compounds or Substances**

2010 amphetamines, cocaine, codeine, heroin, and morphine - by CEC-ESI ion trap MS (418); 4-methylmethcathinone, 2-fluoromethamphetamine, alpha-phthalimidopropiophenone, and N-ethylcathinone by GC/MS, NMR, FTIR, and GC/IRD (419); 1,4-benzodiazepines and amfepramone - determination as adulterants in phytotherapeutic formulations by adsorptive cathodic stripping voltammetry (420); separation and detection of seven amphetamines, amphetamine, dextroamphetamine, methamphetamine, and MDMA by CZE with capacitively
coupled contactless conductivity detection (421); hallucinogenic mushrooms and khat by cation exchange LC (422); morphine, morphine HCl, cocaine HCl, codeine phosphate, papaverine HCl, pethidine HCl, and thebaine - differentiation with THz time domain spectroscopy (423); piperazines, phenethylamines (2Cs and FLYs), 4-substituted amphetamines, beta-keto-amphetamines (cathinones), 2,5-dimethoxyamphetamines, pyrrolidinophenones, and synthetic cannabinoids - a review of their analyses (toxicological focus) (424); MDMA, MDA, and methamphetamine in Ecstasy tablets by GC/FID (425); marijuana, cocaine, heroin, MDMA, amphetamine, methamphetamine (and other unspecified drugs) - detection using spectral fluorescence signatures (426); 2011 diazepam, flunitrazepam, and methadone - by FT-NIR (427); cocaine and MDMA - detection on textiles using micro-Raman (428); evaluation of the fragmentation pathways of various drugs of abuse (cannabinoids, ketamine, amphetamine, ATSs, cocaine, and opioids) by LC-QTOF MS/MS and MSE accurate-mass spectra (429); sibutramine, modafinil, ephedrine, norephedrine, metformin, theophylline, caffeine, diethylpropion, and orlistat - identification and quantification in diet aids by UHPLC-DAD (430); cocaine and heroin - an evaluation of impurity profiling for comparative analysis (431); herbal products [khat, Psilocybe mushrooms, opium, and "Spice"], designer drugs in tablet and powder form [e.g., mCPP, 3-fluoromethamphetamine (3-FMA), MDPV, and methylene], and anabolic steroids in oil and tablets - by DAPPI-MS (432); MDMA, ketamine, phenmetrazine, ephedrine, pseudo-ephedrine, caffeine, tramadol (possibly others not listed in the abstract) - analysis of Ecstasy tablets seized in Iran from 2007 to 2008, by physical characterization, color testing, TLC, anion testing, residual solvent analysis, GC/MS, and LC/MS (433); methamphetamine, amphetamine, MDMA, MDEA, MBDB, MDA, and BDB - by GC/MS following derivatization with trifluoroacetic anhydride (434); heroin, dl-methamphetamine, dl-MDMA, and dl-ketamine - application of dispersive liquid-liquid microextraction and CE with UV detection for chiral separation and determination (toxicological focus) (435); cocaine and heroin - analysis of "crack" cocaine in Iran by TLC and GC/MS (proving that most such samples actually contained heroin) (436); benzodiazepines, beta-blockers, angiotensin-converting enzyme inhibitors, phenothiazines, dihydropyridine calcium channel blockers, diuretics, local anesthetics, vasodilators, anti-diabetic, antidepressant, analgesic, and antihistaminic drugs - by LC-MS/MS (toxicological focus) (437); methamphetamine, MDMA, pseudoephedrine, N-formylmethylamphetamine, and 1-benzyl-3-methylnaphthalene - a study of their degradation in soil (438); analysis of "Happy Water" (containing methamphetamine, caffeine, ketamine, and other components) - by GC/MS and GC/FID (439); morphine, codeine, and hydrocodone - by SERS (440); p-fluoroamphetamine, mephedrone, flephedrone, PPP (alpha-pyrrolidinopropiophenone), MDPV, bk-MBDB, pFBT (3-(p-fluorobenzoyl)-tropane), JWH-073, methylene (3,4-methylenedioxymethcathinone), and N-ethylcathinone - by GC/MS, UPLC-QTOF-MS, and NMR (441); m-CPP and MDMA tablets, cocaine, and LSD - by easy ambient sonic-spray ionization MS (442); Ecstasy Tablets - MDMA, methamphetamine, MDEA, MDA, amphetamine, caffeine, and lidocaine - by TLC and EASI-MS (443); methamphetamine, methamphetamine analogs, and MDMA - a theoretical study of the
energetics of the synthesis of various ATS and MDMA (including reactants, products and by-products) (444); cocaine and heroin - a survey of seizures in Luxembourg from 2005 to 2010 (445); bunitrolol, caffeine, cocaine, codeine, diazepam, doxepin, haloperidol, 3,4-methylendioxy-amphetamine, morphine, nicotine, and zolpidem - impact of solvent choice on the analysis of basic drugs by micro-LC/MS (toxicological focus) (446); methamphetamine, MDA, MMDA, and ketamine - detection by 2D THz signatures and spectral dynamics analysis (447); 2012 methandrostenolone, sildenafil, tamoxifen, quinine, clomiphene, dehydroepiandrosterone, anastrazole, clenbuterol, stanozolol, oxandrolone, liothyrone, finasteride, and melatonin in counterfeit drugs and pharmaceutical preparations seized from the black market among bodybuilders - RPLC-DAD and GC/MS (448); antidepressant drugs (sertraline, paroxetine, citalopram, venlafaxine, and fluoxetine) - determination by spectrofluorometry (449); MDA, MDMA, methadone, cocaine, morphine, codeine and 6-monoacetylmorphine - analysis with CZE-TOF-MS (451); MBDB, MMDA-2, and D2PM (and possibly others not specified in the abstract) - enantiomeric separation after derivatization with (R)-(−)-DBD-Py-NCS by UHPLC, with fluorescence and MS detection (452); lidocaine and benzocaine - detection by HPLC with amperometric detection (453); MDMA, ketamine, cocaine, diazepam, phenobarbital, and barbital - analysis using a deep UV/Vis reflected optical fiber sensor (454); cocaine, codeine, nicotine, methadone, phenmetrazine, pentylenetetrazole, niketamide, fencamfamine, and caffeine - by GC/high-res-TOF-MS with a soft ionization source (455); atenolol, salbutamol and cocaine - detection of drug vapors using an ion funnel interface for secondary ESI-MS (456); acetaminophen, phenylephrine, glucose, and caffeine - noninvasive, quantitative analysis of simulated drug mixtures using SORS and multivariate statistical analysis (457); constituents of "legal highs" - MPDV, caffeine, butylone, TFMPP, lidocaine, 4-MEC, mephedrone, pFPP, BZP, and MDPBP - by GC/MS, LC-QTOF-MS, HPLC, and NMR (458); 2013 flunitrazepam, ketamine, and MDMA - detection by IMS (toxicological focus) (459); methoxetamine, 3-methoxyeticyclidine and 3-methoxyphencyclidine - characterization by GC- and CI- MS, NMR, and HPLC-DAD-ESI-MS/MS (toxicological focus) (460); 1,4-benzodiazepines (clonazepam, flurazepam, alprazolam, midazolam, bromazepam, chlordiazepoxide, lorazepam, and diazepam) and antidepressants (bupropion, sertraline, paroxetine, and fluoxetine) - identification as adulterants in phytotherapeutic dieting formulations by voltammetry (461); anorexics (amfepramone, fenproporex, sibutramine), benzodiazepinic anxiolytics (clonazepam, flurazepam, alprazolam, midazolam, medazepam, chlordiazepoxide, diazepam), antidepressants (bupropione, fluoxetine, sertraline, paroxetine), diuretics (hydrochlorothiazide, furosemide, chlortalidone, amiloride, spironolactone), and hypoglycemics (glimepiride, chlorpropamide, glibenclamide) - differentiation by a solid state electrochemical method (462); mephedrone, 5,6-methylenedioxy-2-aminoindane (MDAI), and MDMA - by SERS on copper coins coated with deposited silver (463); Psilocybe mushrooms, 5MeO-DIPT, tryptamine, MDMA and related compounds, and synthetic cannabinoids and cannabimimetics - an overview (464).
2. **Instrument Focus**

Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis.

Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

2.A - Polydrug B: Mixed or Unrelated Groups of Compounds or Substances

**Named Groups of Compounds: 2011** opioids, tranquilizers, stimulants, and hallucinogens - analysis by flow-analysis methods with chemiluminescence or electrochemiluminescence detection (465); a review of the analytical methodologies used to determine adulterants in slimming phytotherapeutic formulations (466); designer cathinones, tryptamines, phencyclidines, and synthetic cannabinoids and cannabimimetics - an overview and review (467); phencyclidine, amphetamine, and tryptamine imine by-products - characterization by GC/MS, IR, and NMR (468); **2012** (unspecified) synthetic cannabinoids, cannabimimetics, and cathinones - by DART-TOF-MS (469); cathinones, pyrrolidinophenones, tryptamines, and synthetic cannabinoids and cannabimimetics - a review of analytical methods (toxicological focus) (470); 24 phenylethylamines (including 8 cathinones), 3 piperazines, and 3 tryptamines (only MDA, MDMA, ethylamphetamine, and AMT were listed in the abstract) - cross-reactivity in immunosorbent assays (471); phenethylamines, tryptamines, piperazines and cathinones - a review of analyses by GC-EI/MS, LC-ESI/QTOF-MS, and (in some cases) by NMR and FTIR (472); **2013** cathinones, phenethylamines, tryptamines, and piperazines - by LC-QQQ-MS/MS in the MRM mode (toxicological focus) (473);

"Ecstasy Tablets": **2010** impurity profiling of tablets seized in Vietnam using GC and GC/MS (474); **2011** variation in likelihood ratios for same- and different-batch comparisons (specific compounds and analytical methods not specified in the abstract) (475); microwave-assisted extraction of tablets for improved impurity profiling (476); chemical profiling by analysis and identification of residual solvents by static headspace (477); **2012** detection of amines in Ecstasy tablets using a fluorogenic probe (478);

Abused Drugs and Pharmaceuticals in Municipal Wastewater Streams: **2010** by isotopic-dilution direct injection RP-LC-MS/MS (location not specified in the abstract) (479); from a wastewater treatment plant located in "the mid-Atlantic U.S.,” by solid phase extraction and GC/MS (480); an overview and review of current methodologies (481); in Paris, France using HPLC-MS/MS after SPE extraction (482); in three Canadian cities (method not specified...
in the abstract) (483); in Zagreb, Croatia using LC-MS/MS (484); 2011 by SPE and LC/MS, including a critical evaluation and verification of methodologies (484); a historical review (486); in Australia (methodologies not specified in the abstract) (487); a sampling strategy for sport villages to monitor doping (488); refining the estimation of illicit drug consumptions from wastewater analysis (489); for estimating total drug consumption in small, semi-enclosed population (methodologies not listed in the abstract) (490); 2012 by Mixed-Mode SPE and LC-QTOF-MS (491); for estimating cocaine consumption in the Brazilian Federal District (492); 2013 a study of the uncertainty associated with the estimation of community illicit drug consumption via analysis of sewage (493); by online-SPE-LC/MS (494);

"Illicit Drugs" - Including "Controlled Substances," "Drugs of Abuse," "Illicit Drugs," "Narcotics," "Seized Drugs" (and similar generic terms): 2010 a sensor for "drugs of abuse" (495); screening for "drugs of abuse" by LC-DAD (496); detection of "drugs" using neutron computerized tomography and artificial intelligence techniques (497); detection of "narcotics" using IMS (498); rapid analyses of "illicit drugs" by FTIR and GC/MS (499); rapid field air sampling and analysis of "illicit drugs" using dynamic planar SPME-IMS (500); determination of "illicit drugs" by UHPLC/MS (501); "illicit drug salt forms" by LC/MS (502); qualitative analysis of "narcotics" using Raman and chemometrics (503); identification of "illicit drugs" by teraHertz spectroscopy (504); detection of "illicit drugs" using a tagged neutron inspection system (505); QSAR study on GC/MS Retention Times of "illicit drugs" (506); 2011 "drugs of abuse" and pharmaceuticals - identification of active ingredients by AP glow discharge MS (507); a review and overview of adulterants in "illicit drugs" and their effects (508); acquiring LC/MS or GC/MS analyses following dissolution of microcrystalline test products from "drugs of abuse" (509); detection of "illicit drugs" on surfaces using DART-TOF-MS (510); detection of drugs by proton exchange reaction MS (511); analysis of "narcotics" by Raman (512); detection of "controlled substances" in tablets by ATR/FTIR (and LC-ESIMS) (513); analysis of "seized drugs" by HILIC (514); analysis of banknotes (Euros) from the Canary Islands for "illicit drugs" by LC and MS (515); analysis of "illicit drugs" by GCxGC (516); detection of packaged or concealed "illicit drugs" by spatially offset Raman (517); detection and identification of "illicit drugs" using neutron based techniques (518); detection of "street drugs" by 3-dimensional Spectral Fluorescent Signatures (519); analysis of "multicomponent illicit drugs" by IMS (520); recovery of "illicit drugs" from surfaces using electrostatic lifting and nanomanipulation, with analysis by nanospray ionization mass spectrometry (521); a review of analysis of "drugs of abuse" by Raman (522); screening for "illicit drugs" on banknotes by LC-MS/MS (523); 2012 a review of hyphenated LC techniques (listed applications include "drugs of abuse in alternative matrices") (524); use of gold-plated Mylar lift films for Raman of "drug residues" (525); 18 (unspecific) "illegal adulterants" in herbal medicines and health foods for male sexual potency - by LC-ESIMS (526); screening of "narcotic drugs" using MECC on a microfluidic device (527); fabrication and use of silver nanoneedles array for SERS and their application in rapid
detection of "narcotics" (stated to be especially sensitive for ketamine) (528); 2013 "forensic drug analysis" by microfluidic devices - an overview (529); an evaluation of the results of impurity profiling of "illicit drugs" from different analytical methods and/or from different laboratories (530); analysis of "seized drugs" by LC-ESI/MS/MS and AP-MALDI-MS/MS, with comparisons of the two techniques (531); an overview of advanced analytical instrumentation and methods for "drugs of abuse" (toxicological focus) (532);

**Pharmaceuticals/Counterfeits (with a focus on differentiation of legitimate versus counterfeit products, or for monitoring quality control for legitimate pharmaceutics): 2010**

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analysis by TLC with AccuTOF-DART MS (569); overview of detection using a portable NIR spectrometer (570); detection and analysis of counterfeit pharmaceutical tablet cores by ATR/FTIR and micro-ATR/FTIR imaging (571); discrimination of illicit tablets by surface granularity (572); identification of the components in drugs by near-infrared hyperspectral unmixing of tablets (573); an overview of counterfeit drugs (574); a review of rapid, noninvasive characterization of pharmaceuticals and counterfeits in packaging or containers using Raman (575, 576); determination of the elemental distributions in tablets by confocal micro-XRF (577); invisible labeling of pharmaceuticals for identification and verification of authenticity (578); a review of chiral analyses of drugs (579); detection of counterfeits by vibrational spectroscopy (580); a review of methods used to detect counterfeits or confirm authenticity (581); overview and review of Raman for analysis of pharmaceuticals (582); an overview and review of counterfeiting (583); analysis of pharmaceuticals with hyperspectral Raman imaging and various chemometric methods (584); analysis of pharmaceuticals by DART-AccuTOF-MS following TLC separation (585); 2012 comparison of handheld to benchtop Raman instruments for the identification of authentic versus counterfeit tablets (586); detection of counterfeit tablets by transmission Raman (587); quality control screening and counterfeit detection using portable Raman (588); evaluation of differently manufactured pharmaceutical tablets (including illicit drugs and counterfeits) Raman hyperspectral images (589); use of laser-induced breakdown spectroscopy and support vector machines for classification of pharmaceuticals and counterfeits (590); by DART-MS - an overview (listed applications include "screening of counterfeit drugs") (591); analysis of "soft" pharmaceuticals and counterfeits (suppositories, etc.) by DART-MS (592); analysis of tablet packaging by Raman microscopy and 2D-correlation spectroscopy (593); monitoring and detection using NIR (594); analysis of residual solvents in counterfeits by GC/MS (595); differentiation of legitimate versus counterfeit drugs by NIR and chemometrics (596); 14 unspecified "sedative-hypnotic drugs" - detection in health foods and traditional Chinese medicines by GC/MS (597); 2013 a review of a paper-based test for screening for counterfeits (598); an overview of chromatographic and spectroscopic detection methods (599); by Raman (600); a review, focusing on HPLC and MS, but also discussing color testing, TLC, GC, Raman, NIR, FTIR, and NMR, using antimalarial drugs and sildenafil (Viagra) as illustrative examples (601); an overview of the use of GC/MS for "forensic substance identification" (602).

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2.B - New and/or Improved Instrumental Techniques

**Atomic Absorption Spectroscopy:** 2012 a review, focusing on pharmaceuticals (listed applications include "forensic") (603);
Capillary Electrophoresis (and Related Techniques, including Tandem Techniques): 2011
CE - a review of the literature from 2006-2010 (focus is "natural products; " listed applications include pharmaceuticals and "toxicological compounds of interest to forensics") (604); 2012 evaluation and optimization of CZE for common drugs of forensic interest in aqueous matrices (605); CE - a review of the literature from 2009 to 2011 (listed focus includes illicit and abused drugs, ions, and small molecules of forensic interest) (606); 2013 a review of recent advances in electrodriven enantioseparations (listed applications include "pharmaceutical" and "forensic") (607);

Gas Chromatography: 2012 a review (listed applications include "bulk drugs") (608);

Infrared Spectroscopy: 2012 ATR/FTIR - a review (includes select chemical, pharmaceutical, and forensic applications) (609); IR of solid-dosage drug substances - an overview (610);

Infrared and Raman Spectroscopy: 2012 in Forensic Science (Reference Text) (611);

Ion Spectroscopy: 2012 IMS with an orthogonal acceleration sector TOF mass analyzer (designed for "forensic applications") (612);

Mass Spectrometry: 2010 identification of active compounds in tablets by flow-injection data-dependent tandem mass spectrometry combined with library searching (613); differentiation of structural isomers of "drug substances" using LC/Q-TOFMS and fragmentation prediction (614); 2011 ESI-MS - use of wooden toothpicks for facile loading and ionization of samples (615); ambient ionization mass spectrometry - an overview and review, including discussions of counterfeit and illicit drugs (616); DART-MS - a review (listed applications include pharmaceuticals and forensics) (617); a review of the applications of DESI-MS (includes "drugs," pharmaceuticals, and "forensics") (618); 2012 ambient desorption/ionization MS (ADI-MS) - an overview and review (listed applications include "forensics") (619); identification of unknowns utilizing accurate MS data and ChemSpider (620); an overview of recent advances (621); identification of unknowns using an API MS/MS library (622); 2013 ambient mass spectrometry - a review, including DESI, DART, and extractive ESI (listed applications include "forensic identification") (623); DESI-MS (listed applications include "illicit drugs") (624);

Microscopy: 2010 an overview (625);

Nuclear Magnetic Resonance Spectroscopy: 2012 high-precision 1H-qNMR - for determination of the purity of standards (626);

Raman: 2010 non-contact, in-the-field analysis of "hazardous materials" by portable Raman
operating in various modes (627); 2011 a review (includes forensic science applications) (628); 2012 multi-wavelength excitation Raman spectrometers and microscopes (listed applications include "narcotics identification") (629);

**Solvent-Microextraction:** 2013 a review (listed applications include forensic and pharmaceutical) (630);

**Stable Isotope Analyses:** 2010 recent advances (includes drugs) (631); position specific 13C analysis for determination of source and the natural attenuation of contaminants (632); a review of the use of stable isotopes in forensic science (633); 2011 an overview of the use of IRMS, proposing a 6-step methodological approach for application to specific forensic issues (634); a general review of the use of stable isotopes to determine source (635); 2012 an overview of the signature value of isotope deltas (636); 2013 a review of inter-laboratory comparability (637); tracking authentic pharmaceuticals by 2H- and 13C-NMR (638);

**Thin Layer Chromatography:** 2011 a review of TLC/MS (639); 2012 quantitative HPTLC-densitometry - converting TLC screening for counterfeit pharmaceuticals to HPTLC (640);

**X-Ray Techniques:** 2012 wavelength-dispersive XRF - for analysis of very small samples (listed applications include "forensic analysis") (641).
3. Miscellaneous Topics

**Clandestine Laboratories - Appraisals and Safety:** 2012 comparison of first responder decontamination procedures (642); testing of fire resistant fabrics after the application of flammable solvents (643); therapeutic detoxification of law enforcement personnel suffering from chronic occupational exposure to methamphetamine (644);

**Education:** 2011 analysis of a simulated drug sample by GC/MS and FTIR (645); analysis of a simulated drug sample by TLC and GC/MS (646); 2013 use of forensic science to teach method development in undergraduate analytical laboratories (647);

**Legal Issues:** 2010 legal issues (648); 2011 legal issues (649); 2012 brief news release concerning counterfeits (650); reference text (651);

**Packaging:** 2011 identification of plastic packaging used by body packers, by IR (652); 2012 a review of the use of SEM/EDS and FTIR to identify counterfeit pharmaceutical packaging (653); analysis of polyethylene cling film (commonly used for packaging illicit drugs) by ATR/FTIR (654);

**Quality Assurance:** 2010 measurement uncertainty in forensic/analytical testing (655); the uncertainty in measurement of the total mass of a substance packaged in numerous containers (656); 2011 comparison of the stability of stock solutions of drugs at freezer, refrigerator, and ambient temperatures (657); measurement uncertainty in sampling and analysis of illicit drugs (658); 2013 use of a software tool ("Drugs WorkBook") for the quantification of illicit drugs (659);

**Sampling Plans:** 2010 an Excel based sampling calculator (660); a probability-based sampling approach for the analysis of multiple containers of cocaine, heroin, or marijuana (661);

**Soil:** 2011 determination of source by XRF (662); 2012 analysis by Raman following oxidative sample preparation (663); an overview of forensic analysis for determining geographical source (664);

**Other:** 2010 an informal classification scheme for "designer drugs" in Israel (665); 2011 an overview of drug production and use in New Zealand (666); synthetic chemist David Nichols discusses his research on psychedelic compounds, commenting on how his products have been abused (667); 2012 Laboratory Information Management System (LIMS) - an overview and review (668).
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(APICA) and N-(1-Adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), and 
Detection of Five Synthetic Cannabinoids, AM-1220, AM-2233, AM-1241, CB-13 
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Structural Elucidation of Four Cannabimimetic Compounds (RCS-4, AM-2201,


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The 2016 “Research on Drug Evidence” Report
[From the 18th ICPO / INTERPOL Forensic Science Symposium]

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ABSTRACT: A reprint of the 2016 “Research on Drug Evidence” Report (a review) is provided.

KEYWORDS: INTERPOL, Illicit Drugs, Controlled Substances, Forensic Chemistry.

Important Information:

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The "General Overview" (Talking Paper) was removed from this reprint (Editor's discretion).

This reprint is derived from the original electronic document, and is not an image of the best available hard copy (as was utilized for the 1995 and 1998 reports). For this reason, the pagination in the Proceedings is not retained in this reprint, some minor reformatting was done to eliminate deadspace, and all widow and orphan lines were left as is.
Research on Drug Evidence

July 1, 2013 - June 30, 2016

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Lyon, France
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Preface Notes:

1. With the exception of synthetic cannabinoids and cannabimimetics, all references are subdivided by individual drug, drug group/class, or general topic, then chronologically (year only) within each subsection, then alphabetically by first author within each year. Synthetic cannabinoids and cannabimimetics are in a separate category (1.D), and are subdivided as individual compounds, groups of compounds, and finally as groups with other drugs.

2. Many citations included in this report are dated prior to July 1, 2013, because they had not yet been abstracted prior to the 2013 report. In addition, many of the references in this report are cited as "Ahead of Print;" because their actual publication citation was never subsequently published in Chemical Abstracts. For this reason, the year listed with "Ahead of Print" may not reflect the actual year of publication; however, the rest of the citation will remain the same, allowing the full citation to be easily found by Internet searching.

3. All citations are formatted in accordance with Uniform Requirements for Manuscripts Submitted to Biomedical Journals, except that journal names are not abbreviated.

4. In contrast to recent reports, no restricted articles are cited in this report.

5. A small number of citations are bolded, reflecting topics judged to be of notable importance. [Bolding removed at the Editor's Discretion]
1. **Routine and Improved Analyses of Abused Substances**

Improved methods of analysis, i.e., faster, more discriminatory, more sensitive, less costly, etc., are needed for all abused substances. Additionally, standard analytical data are required for previously unknown or rarely encountered substances and/or new "designer drugs."

Drug seizures and clandestine laboratory operations are continuously monitored to provide a comprehensive overview of new developments. Ongoing research in the forensic community, as well as in the general fields of analytical chemistry and toxicology, provide new and/or improved methods of analysis for abused substances. Reports providing standard analytical data for new drugs of abuse and/or improved analytical protocols for known drugs of abuse are generated for the forensic and enforcement communities.

1.A - Individual Compounds or Substances
1.B - Individual Natural Products Containing Abused Substances
1.C - Common Groups or Classes of Compounds or Substances
1.D - Synthetic Cannabinoids and Cannabimimetics
1.E - Mixed or Unrelated Individual (Named) Compounds or Substances

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1.A - **Individual Compounds or Substances** (except individual synthetic cannabinoids and cannabimimetics, which are compiled under 1.D)

**Alprazolam:** 2014 by UV/Vis after derivatization with DDQ (1); 2015 as a contaminant in "natural waters" by adsorptive cathodic stripping voltammetry (2);

**2-Amino-1-(4-bromo-2, 5-dimethoxyphenyl)ethan-1-one (bk-2C-B):** 2015 characterization by GC/MS (with and without derivatization with 2,2,2-trichloroethyl chloroformate), LC/HRMS, and NMR (3); synthesis and identification of bk-2C-B by NMR, GC, LC, and HR-MS (4);

**5-(2-Aminopropyl)indole (5-IT):** 2015 an overview (5);

**Amphetamine:** 2013 impurity profile of amphetamine produced from APAAN (6); 2014 identification of 4,6-dimethyl-3,5-diphenylpyridin-2-one as a route specific byproduct for amphetamine synthesized by the APAAN to P2P, Leuckart route (7); 2015 determination of relative enantiomer migration order using racemic amphetamine (8); 2016 impurity profiling of P2P-derived amphetamine; identification and characterization of the by-products from the APAAN and alpha-methylstyrene routes to P2P and their respective impurities following
Leuckart reduction (9);

**Barbital**: 2013 determination by RP-HPLC (10);

**Benzphetamine**: 2014 production and impurity profiling of benzphetamine HCl (11);

**1-Benzylpiperazine (BZP)**: 2013 a review (social focus, but includes "analytical methodologies for the identification of BZP in forensic settings") (12); 2015 determination of the isotopic makeup of BZP synthesized from 3 different sources by IRMS (13);

**4-Bromo-2,5-dimethoxyamphetamine**: 2015 by LC-MS/MS (14);

**2-(4-Bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25B-NBOMe)**: 2015 by HP-TLC (15); a review (16);

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**Buprenorphine**: 2016 abuse and diversion of the buprenorphine transdermal delivery system (18);

**Camfetamine (N-Methyl-3-phenyl-norbornan-2-amine)**: 2014 an overview (19);

**Chloral Hydrate**: 2015 detection of chloral hydrate adulteration in alcoholic beverages (20);

**2-(4-Chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe)**: 2013 characterization by GC-EI-MS (with and without derivatization with TFAA), LC-ESI-QTOF-MS, FTIR, and NMR (21); 2014 an overview (22);

**4-Chloromethcathinone (Clephedrone)**: 2014 characterization by GC/MS, NMR, GC, and CE (23);

**Cocaine**: 2012 rapid separation and characterization of cocaine and various cutting agents by differential mobility spectrometry-MS (24); optical detection using a highly specific triple-fragment aptamer (25); 2013 by electrochemical determination (26); by GC/FID (27); determination on circulated banknotes by CE with UV detection (28); separation of cocaine and phenyltetrahydroimidazothiazole mixtures (29); profiling of cocaine seized in Naples, Italy, by 1H-NMR (30); analysis by GC/MS, ATR/FTIR, and chemometric methods (31); detection of contamination of Brazilian currency by HPLC/UV (32); detection of hygrine and cuscohygrine as
possible markers (to distinguish coca chewing from cocaine abuse) by GC/MS (33); fluorescent sensing of cocaine based on a structure switching aptamer, gold nanoparticles, and graphene oxide (34); comparative analysis of solvent impurity profiles obtained by HS-GC/MS (35); detection by a fluorescent biosensing system (36); 2014 IMS evaluation of cocaine occupational exposure in forensic laboratories (37); electrochemical detection using disposable sensors (38); determination of levamisole and tetramisole in cocaine by enantioselective HPLC with circular dichroism detection (39); the stability of cocaine and its metabolites in municipal wastewater (presents the case for using metabolite consolidation to monitor cocaine utilization) (40); impurity profiling of cocaine seized by the Brazilian Federal Police in 2009-2012 (41); determination of cocaine, benzoic acid, benzoylecgonine, caffeine, lidocaine, phenacetin, benzocaine, and diltiazem by HPLC/DAD (42); analysis of "crack" by Scotts color testing, TLC, GC/FID, and GC/MS (43); quantification by IR and PLSR (44); detection by microfluidic paper sensors (45); determination of the isomeric truxillines in illicit cocaine via CGC/FID and their use and implication in the determination of cocaine origin and trafficking routes (46); a bio-inspired solid phase extraction sorbent material for cocaine (47); radiographic (CT) features of intracorporeally smuggled (body-carried) liquid cocaine versus solid cocaine (48); determination of cocaine, its metabolites, and its pyrolytic products by LC-MS using a chemometric approach (49); determination by diffuse reflectance measurements in the near IR (50); colorimetric detection with aptamer-gold nanoparticle conjugates coupled to an android-based color analysis (51); qualitative analysis by DESI-MS (52); the evaluation of trace cocaine on banknotes (53); novel optical fibre-based cocaine sensors (54); a study of the inclusion complex between p-sulfonated calix[4]arene with cocaine HCl by fluorescence and 1H NMR (55); 2015 determination of cocaine on Brazilian banknotes (analytical methodology not identified in the abstract) (56); multicriteria FTIR/ATR wavenumber selection to differentiate cocaine base versus HCl (57); an electroanalytical method for the quantification of aminopyrine in cocaine (58); chemical profiling of cocaine seizures in Finland by GC/MS (59); comparison of canine detection of methyl benzoate released from 4 different species of snapdragon versus actual cocaine (60); differentiation of South American crack and domestic (US-produced) crack cocaine via HS-GC/MS (61); the influence of medium and elicitors on the production of cocaine, amino acids, and phytohormones by Erythroxylum coca calli (62); a study of the inclusion behavior of p-sulfonated calix[4,6,8]arene with cocaine HCl by fluorescence and 1H NMR (63); a discussion of levamisole in cocaine preparations (64); quantification of cocaine and adulterants by IR and PLSR (65); determination of cocaine in creek water via SPE with subsequent analyses by either HPLC or GC (66); quantification of cocaine, caffeine, 4-dimethylaminoantipyrine, levamisole, lidocaine, and phenacetin by GC/NPD (67); copper thiocyanato complexes and cocaine (a case of "black cocaine") (68); chemical profiling of cocaine in Brazil from 2010 to 2013, a discussion of the increase in aminopyrine in cocaine (analytical methodology not identified in the abstract) (69); HS-GC-MS analysis of South American commercial solvents to monitor their use in the illicit conversion of cocaine base to HCl (70); profiling cocaine and some
common adulterants by FTIR/ATR (71); a review of nanomaterial-based cocaine aptasensors (72); profiling of cocaine by FTIR/ATR, GC/MS, and HS-GC/MS determination of minor alkaloids and residual solvents (73); ultra-high frequency piezoelectric aptasensor for the label-free detection of cocaine (74); identification of different forms of cocaine and substances used in adulteration using NIR Raman spectroscopy and infrared absorption spectroscopy (75); determination of cocaine, its main metabolites, and its pyrolytic products by HPLC-UV-CAD (76); voltammetric determination of cocaine using carbon screen printed electrodes chemically modified with uranyl Schiff base films (77); optical fibre fluorescent chemical probes for the detection of cocaine (78); 2016 detection and unambiguous identification of traces of cocaine on Euro banknotes using FAPA-MS (79); analysis of cocaine and its adulterants by TLC coupled to paper spray ionization MS (80); fast on-site screening of cocaine with a wearable fingertip sensor based on voltammetry (81); geographically sourcing cocaine’s origin by delineation of 19 major coca growing regions in South America (82); determination of cocaine, diltiazem, benzocaine, levamisole, caffeine, phenacetin, lidocaine, and dipyrone by LC/DAD (83); use of a small-molecule-dependent split aptamer assembly for detection of cocaine (84); detection by a fluorescence immunoassay (85); use of a key aptamer structure-switching mechanism for the ultrahigh frequency detection of cocaine (86); the stability of cocaine impurity profiles during 12 months of storage, by GC/MS and HS-GC-MS (87); removal of benzoylecgonine in water matrices by UV254/H2O2 processing using a flow microcapillary film array photoreactor (88); determination of procaine in cocaine by a paper-based device coupling electrochemical sample pretreatment and colorimetric detection (89); polarographic determination of the stability constant of the complex formed between cocaine and cobalt thiocyanate (90); detection by an electrochemical aptasensor (91); a fluorescent aptasensor for cocaine based on a G-quadruplex and ruthenium polypyridyl complex molecular light switch (92);  

**Clobazam (7-chloro-1-methyl-5-phenyl-1,5-dihydrobenzo[1,4]diazepine-2,4-dione): 2015**  
the dynamic behavior of clobazam on HPLC chiral stationary phases (93); 2016 spectroscopic and quantum chemical studies of the molecular geometry, frontier molecular orbital, NLO, and NBO analysis of clobazam (94);  

**Codeine: 2013** detection using a label-free electrochemical biosensor based on a DNA aptamer (95); 2014 a rapid colorimetric method for the detection of codeine sulphate using unmodified gold nanoprobe (96); analysis of codeine phosphate sustained release capsules by HPLC (97); 2015 development of an abuse- and alcohol-resistant formulation of codeine phosphate (98); 2016 photocatalytic degradation of codeine by UV-irradiated TiO2 (99);  

**Deschloroketamine (2-Methylamino-2-phenylcyclohexanone): 2016** characterization of deschloroketamine by GC/MS, LC/HRMS, MS/MS, and NMR (100);
**Desomorphine ("Krokodil"):** 2014 a review (101); 2015 an overview and review (102); analysis by TLC, UV/Vis, 1H NMR, and FTIR (103);

**Diazepam:** 2015 differentiation of licit and illicit diazepam tablets by DSC (104); 2016 determination of the compatibility between diazepam and tablet excipients by DSC, thermogravimetry, and IR (105);

**3,4-Dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide (U-47700):** 2016 the first reported fatality associated with U-47700 (and implications for forensic analysis) (106);

**1-(2,3-Dihydro-1H-inden-5-yl)-2-phenyl-2-(pyrrolidin-1-yl)-ethanone ("Indapyrphenidone"):** 2015 characterization by GC/MS, LC-HRMS, NMR, and X-ray crystallography (107);

**Diltiazem:** 2015 analytical characterization of two new related impurities of diltiazem (2-(4-methoxyphenyl)-5-methyl-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4]thiazepin-3-yl acetate and 2-(4-methoxyphenyl)-4-oxo-5-vinyl-2,3,4,5 -tetrahydrobenzo[b][1,4]thiazepin-3-yl acetate) by HRMS and NMR (108);

**1-(3,4-Dimethoxyphenyl)-2-(ethylamino)pentan-1-one (DL-4662):** 2015 characterization by NMR, GC/MS, and HPLC (109);

**4,4'-Dimethylaminorex (4,4'-DMAR):** 2015 chemistry, pharmacology, and toxicology (110); an overview (111);

**1,3-Dimethylamylamine (DMAA):** 2014 determination by 1H NMR (112); 2015 identification by DART-QTOF-MS (113);

**1,3-Dimethylbutylamine (DMBA):** 2015 identification in dietary supplements by UHPLC/MS (114);

**N,N-Dimethyltryptamine (DMT):** 2014 conformational, spectroscopic and nonlinear optical properties (a theoretical study) (115); a review (also presenting the results of a global survey) (116);

**Eszopiclone:** 2013 determination by UHPLC and HPLC (117);

**N-Ethyl-alpha-ethylphenethylamine:** 2013 characterization by GC/MS, LC-TOFMS, and 1D- and 2D-NMR (118);
2-(Ethylamino)-1-(4-methylphenyl)-1-pentanone (4-MEAP): 2015 analysis by GC/MS, NMR, and LC/EIS (119);

Ethylone (3,4-Methylenedioxy-N-ethylcathinone): 2015 synthesis and characterization of two conformational polymorphs of ethylone HCl by FTIR, FT-Raman, powder XRD, GC-MS, ESI-MS/MS and NMR (13C CPMAS, 1H, 13C) (120);

Etizolam: 2014 synthesis (121);

Fenethylline: 2016 a review (122);

Fentanyl: 2012 impurity profiling of illicit fentanyl using UHPLC-MS/MS (123); 2015 discussion of a case of abuse via extn. of fentanyl from transdermal patches (124); organic and inorganic impurity profiling of fentanyl produced by 6 different methods, using GC-MS, LC-MS, and ICP-MS (125); 2016 impurity profiling using multivariate statistical analysis of orthogonal mass spectral data (includes GC/MS, LC-MS/MS-TOF, and ICPMS) (126);

Flephedrone: 2015 characterization by 1H, 13C, 15N HMBC, and 19F NMR (127);

Flubromazepam: 2013 characterization by NMR, GC/MS, LC-MS/MS, and LC-QTOF-MS (128);

Flunitrazepam: 2013 electroanalytical sensing using screen-printed graphite electrodes (129); 2014 electroanalytical sensing using electrogenerated chemiluminescence (130); 2015 novel reductive-reductive mode electrochemical detection by HPLC with dual electrode detection (131); 2016 detection in beverages using portable Raman (132);

6-Fluoro-3,4-methylenedioxyamphetamine: 2015 crystal structure (133);

4'-Fluoro-α-pyrrolidinobutyrophenone (4F-PBP): 2015 structural characterization by 1H, 13C, 19F NMR, and MS (134);

Heroin: 2012 a review of crystal water in heroin HCl standard (135); 2013 high resolution impurity profiling by UHPLC (136); determination of heroin, morphine, 6-MAM, codeine, and 6-acetylcodine drug samples using HPLC with “parallel segmented flow,” which enables the simultaneous use of UV-absorbance, tris(2,2'-bipyridine)ruthenium(III) chemiluminescence, and permanganate chemiluminescence (137); 2014 determination of heroin, 6-acetylmorphine, acetylcodine, morphine, noscapine, papaverine, caffeine, acetaminophen, lactose, lidocaine, mannitol, and piracetam by 1H NMR and 2D DOSY 1H NMR (138); comparison of quantitation
of illicit heroin HCl samples obtained by quantitative NMR versus results obtained by CE (139); an overview of the detection of heroin (140); inorganic impurity profiling and classification of illicit heroin by ICP-MS (141); 2015 acetaminophen, caffeine, diazepam, phenobarbital, and alprazolam in heroin by GC/MS (142); characterization and origin of the 'B' and 'C' compounds in the acid/neutral forensic signatures of heroin (143); classification of illicit heroin by UPLC-Q-TOF analysis of acidic and neutral manufacturing impurities (144); 2016 site-and species-specific hydrolysis rates of heroin to the mono-acetylmorphines (145);

**Human Growth Hormone (HGH) (and related substances):** 2014 identification of the growth hormone-releasing hormone analogue [Pro1, Val14]-hGHRH in a confiscated product (146); identification and quantification of GHRP-2 by NMR and MS (147); 2015 quantification of HGH by isotope dilution-HPLC/MS (148);

**Hydrocodone:** 2014 synthesis from thebaine in six steps (149); 2015 wastewater effluent hydrocodone concentrations as an indicator of a drug disposal program success (analytical methodology not identified in the abstract) (150);

**Hydromorphone:** 2016 two orthorhombic polymorphs of hydromorphone (151);

**gamma-Hydroxybutyric Acid (GHB) (also gamma-Butyrolactone (GBL), 1,4-Butanediol (BD), and Tetrahydrofuran (THF)):** 2013 a comprehensive study of the worldwide distribution of GBL using internet monitoring, comparison of packaging, and carbon isotopic measurements (152); detection of GHB, GBL, and BD in dietary supplements and foods, by GC/MS (using isotopologues for quantitation) (153); development of a fluorescent sensor for GBL (154); 2014 a review of the relative risks of GHB and GBL (155); development of a fluorescent sensor for GHB (156); 2015 analysis of GBL and 1,4-BD by chemical ionization-ion trap-GC/MS (157); 2016 comparative study of GHB and other derivative compounds (GBL, butyric acid, and succinic acid) by spectroelectrochemistry Raman on platinum surface (158); detection of BD in spiked drinks (analytical methodology not provided in the abstract) (159);

**Ibogaine:** 2013 determination by GC-MS/MS (160);

**2-(4-Iodo-2,5-dimethoxyphenyl)-N-[(2,3-methylenedioxyphenyl)methyl]ethanamine (25I-NBMD):** 2013 characterization by LC, ESI-QTOFMS, GC/MS, and MS/MS (161);

**Ketamine:** 2012 a simple color testing reagent for screening (162); 2013 screening in orange juice by TLC (163); a review of O-chlorophenyl cyclopentyl ketone (the precursor for ketamine) (164); 2014 wearable devices based on ionic liquid-based SPME for the environmental monitoring of ketamine (165); estimation by UV/Vis (166); electroanalytical sensing using
electrogenerated chemiluminescence (167); a review (168);

**Lisdexamfetamine Dimesylate**: 2012 synthesis and characterization by FT-IR, NMR, ESI-TOF/MS, GC-MS, and HPLC (169);

**Lysergic Acid Diethylamide (LSD)**: 2014 determination by adsorptive stripping voltammetry (170);

**Mephedrone (4-Methylmethcathinone)**: 2013 by SERS with a portable Raman (171); 2014 a study of phase transformations (to minimize transitions between polymorphic forms during storage) (172); use of mephedrone as a exemplar in an interpretative spectroscopy exercise in a second-year bioscience program (173); analysis of purity and cutting agents in street-level samples from South Wales collected between Nov. 2011 and March 2013, by FTIR (4-fluoromethcathinone and 4-methylethcathinone were also found) (174); structures of mephedrone hydrogen sulfate and its polymorphs under ambient and high pressure conditions (175); 2015 computational studies on molecular structure and interpretation of vibrational spectra, thermodynamical and HOMO-LUMO analyses of mephedrone using density functional theory and ab initio methods (176); spectrophotometric determination (177); identification of 1,2,3,5-tetramethyl-4-(4-methylphenyl)-1H-imidazol-3-ium salt (TMMPI), formed during the synthesis of mephedrone (analysis by GC/MS, LC/MS, NMR, and crystal structure determination (178); 2016 detection via an anthracene molecular probe (by NMR) (179);

**Methamphetamine**: 2012 analysis of the enantiomeric makeup of methamphetamine in OTC inhalers (also includes a toxicology study) (180); fates of precursors and byproducts in soil from the Leuckardt, Nagai, and dissolving metal reductive syntheses of methamphetamine (181); evaluation of the effects of synthesis conditions on the delta13C, delta15N, and delta2H stable isotope ratio values of methamphetamine (182); 2013 detection of pharmaceutical impurities in methamphetamine by GC/FID and GC/MS (183); rapid quantitation of methamphetamine by FTIR/ATR and Chemometrics (184); impurity profiling by CE using a highly sulfated gamma-cyclodextrin as a chiral selector (includes methamphetamine, amphetamine, ephedrine, pseudoephedrine, norephedrine, and norpseudoephedrine) (185); screening of methamphetamine, pseudoephedrine, and ephedrine by a portable lab-on-a-chip instrument (186); quantitation of airborne methamphetamine by SPME and GC/MS (187); detection in indoor air using dynamic SPME followed by GC/MS (188); elemental profiling of methamphetamine using ICPMS (189); influence of precursor solvent extraction on stable isotope signatures of methamphetamine prepared from OTC pharmaceuticals using the Moscow and hypophosphorous syntheses (190); stable isotope analysis of methamphetamine, to help determine precursors (191); molecular fluorescence spectroscopy of methamphetamine in methanol (192); rapid, nondestructive screening test for methamphetamine in clandestine laboratory liquids by Raman (193); impurity
profiling of methamphetamine synthesized from P2P prepared from phenylacetic acid or its esters (194); terahertz spectra of methamphetamine HCl (195); 2014 differentiation of ephedrine and pseudoephedrine based methamphetamine samples by 2D-HPLC (196); determination of methamphetamine in sewers using a Polar Organic Chemical Integrative Sampler followed by HPLC-MS/MS (197); real time quantitative (Simon) colourimetric test for methamphetamine detection using digital and mobile phone technology (198); a review of methamphetamine profiling (199); use of IRMS for methamphetamine profiling (comparison of ephedrine and pseudoephedrine-based samples to P2P-based samples) (200); use of 10-ethylacridine-2-sulfonyl chloride for detection of methamphetamine (201); 2015 "amine-rich carbon nanodots" as a fluorescence probe for methamphetamine precursors (202); photocatalytic degradation of methamphetamine in wastewater by UV/TiO2 (203); use of methamphetamine impurity profiling for intelligence gathering (204); detection by a fluorescence nanosensor (with comparison with HPLC) (205); identification of trans-N-methyl-4-methyl-5-phenyl-4-penten-2-amine HCl as an impurity in methamphetamine synthesized via reductive amination of P2P made from phenylacetic acid/lead (II) acetate (206); enantiomeric profiling of methamphetamine by LC-MS-MS (207); 2016 determination of the synthetic routes of methamphetamine using GC-MS and multivariate analysis (208); demethylation of methamphetamine by UV treatment at wastewater treatment plants (209); detection of trace methamphetamine by dual-mode plasmonic naked-eye colorimetry and a SERS sensor with a handheld Raman spectrometer (210);

**Methaqualone:** 2013 simultaneous determination of methaqualone, saccharin, paracetamol, and phenacetin in illicit drug samples by HPLC (211);

**Methcathinone:** 2012 detection by HPLC (212); 2013 qualitative and quantitative analysis by LC/MS/MS (213); quantitative analysis by GC/MS (214);

**Methiopropamine:** 2015 indirect electrochemical detection of methiopropamine (MPA) and 2-aminoindane (2-AI) by Raman spectroscopy, presumptive (color) testing, HPLC, and electrochemical analysis (this mixture was referred to as "synthacaine") (215); by selective reagent ionisation-TOF-MS for analysis of a mixture of methiopropamine and benzocaine (also referred to as "synthacaine") (216);

**Methoxetamine:** 2013 by GC-MS and 1H- and 13C-NMR (217); 2014 a review (218);

**2-Methoxydiphenidine (2-MXP):** 2015 synthesis and characterization (includes the positional isomers; toxicological focus) (219);

**para-Methyl-4-methylaninorex:** 2014 an overview of deaths from use (220);
3,4-Methylenedioxy-N-benzyl catinone (BMDP): 2013 characterization by LC/high res QTOF-MS, EI-MS, IR, and 1D- and 2D- 1H- and 13C-NMR (221);

3,4-Methylenedioxymethamphetamine (MDMA): 2013 enantiomeric purification by batch chromatography with a cyclodextrin chiral selector (222); use of organic and inorganic impurities in MDMA for comparative analyses (223); impurity profiles of MDMA synthesized by different routes or by variations in the same routes, by GC/MS and GCxGC-TOF-MS (224); 2014 the effects of extn. procedure and GC temp. programming on MDMA impurity profiles (225); by voltammetry (226); 2015 analysis by direct laser ablation with TOFMS (227); compression studies (228); impurity profiling of MDMA synthesised from catechol (229); chemiluminescence detection of MDMA in street drug samples (230);

3,4-Methylenedioxymethamphetamine-4-methylaminorex (MDMAR): 2015 synthesis of the cis- and trans-isomers, with characterization by "chromatographic, spectroscopic, mass spectrometry, and crystal structure analysis" (231);

Methylenedioxypyrovalerone (MDPV): 2013 injection of MDPV among needle exchange program participants in Hungary (232); 2014 a review, including sepn. and analysis by TLC, GC/MS, HPLC, and LC/MS (233); analysis by GC/MS and LC/MS (234); a review (235); see also phencyclidine (below) for a related citation;

4-Methylethcathinone (4-MEC): 2013 by GC/MS, HPLC-DAD, and LC-MS/MS (236);

Methylhexaneamine: 2013 by GC/HR-TOFMS with soft ionization (237);

β-Methylphenylethylamine (BMPEA): 2015 by LC-QTOF-MS (238);

4-Methylthioamphetamine (4-MTA): 2012 identification of common impurities found in 4-MTA produced by the reductive amination and nitropropene routes (239); identification and synthesis of by-products found in 4-MTA produced by the Leuckart method (240);

Mianserin (a psychoactive tetracyclic antidepressant): 2012 by TLC, color testing, and UV (241);

Midazolam: 2015 a review of published, validated methods for determination of midazolam in pharmaceuticals (242);

Morphine: 2013 evaluation of stationary phases based on silica hydride, using morphine as the model compound (243); determination in compound liquorice tablets by HPLC with online SPE
2014 detection using electroactive polymers (245); 271 highly sensitive detection based on molecular imprinting polymers using surface plasmon resonance (246); determination in pharmaceutical samples by kinetic spectrophotometry (247); conformational complexity of morphine and morphinum in the gas phase and in water (a DFT and MP2 study) (248); degradation of morphine in opium poppy processing waste composting (249); 2015 "fingerprinting" using chromatographic purity profiling and multivariate data analysis (250); a study of the stability of morphine sulfate orally disintegrating tablets (analytical methodology not identified in the abstract) (251); a review of sugar derivatives of morphine (252); 2016 a structural and computational study (to determine morphine's mechanism of action as an antioxidant) (253); photostability of 6-MAM and morphine exposed to controlled UV irradiation in water and methanol (254); characterization and origin differentiation of morphine base, HCl, and sulfate (and other unspecified "morphine derivatives") by DSC/TG and FTIR (255); detection using cathodically electropolymerized, molecularly imprinted poly(p-aminostyrene) films (256); determination in pharmaceutical products by on-line SPE and HPLC (257);

**Oripavine:** 2014 a review of the chemistry of oripavine and its derivatives (258);

**Oxycodone:** 2013 analysis of oxycodone/acetaminophen tablets by HPLC (259); a study on the effectiveness of reformulated (abuse deterrent) oxycodone tablets (260); 2014 a review (261); the impact of a reformulation of extended-release oxycodone designed to deter abuse in a group of prescription opioid abusers (262); reductions in reported deaths following the introduction of extended-release oxycodone with an abuse-deterrent formulation (263); 2015 impact of the introduction of an abuse-deterrent sustained-release formulation in Australia (264); an overview of the level and methods of tampering with a tamper-resistant formulation (265); 2016 evaluation of the tamper-resistant properties of biphasic immediate-release / extended-release oxycodone/acetaminophen tablets (266);

**Phenazepam:** 2012 analysis of phenazepam by GC/MS and LC-MS/MS (267);

**Phencyclidine (PCP):** 2013 false-positive PCP immunoassay caused by MDPV (268);

**Phenobarbital:** 2014 detection by an electrochemical sensor based on molecular imprinted polymer (269); detection by an electrochemical sensor based on molecular imprinted technique and electropolymerization membrane (270); characterization of the monosolvates between phenobarbital and acetonitrile, nitromethane, dichloromethane, and 1,4-dioxane by single-crystal and powder X-ray diffraction, thermoanal. methods, FTIR, Raman, and solid-state NMR (271); 2015 simultaneous determination of phenobarbital and aspirin by HPLC (272); 2016 a study of polymorphism of phenobarbital by structural, thermal, and VT-Raman spectroscopy (273);
Phenyl Acetyl Carbinol (L-PAC and R-PAC): 2014 isolation/selection of the best yeast culture and its metabolic control for the biotransformation of benzaldehyde to 1-hydroxy-1-phenyl-2-propanone (274); use of substituted benzaldehydes for the manuf. of substituted L-PAC analogs (which were subjected to reductive amination to give the corresponding substituted pseudoephedrine/ephedrine analog, which were then either reduced or oxidized to produce the corresponding methamphetamine or methcathinone analogs) (275); 2015 biosynthesis of R-PAC in [BMIM][PF6]/aqueous biphasic system using Saccharomyces cerevisiae (276);

Phenyl-2-propanone (P2P, Phenylacetone): 2016 a detailed analysis of the impurities formed when P2P is synthesized via an aldol condensation of benzaldehyde and Me Et ketone (MEK), followed by a Baeyer-Villiger reaction, followed by ester hydrolysis (route specific markers for this synthesis include 3-methyl-4-phenyl-3-buten-2-one, 2-methyl-1,5-diphenylpenta-1,4-diene-3-one, 2-(methylamino)-3-methyl-4-phenyl-3-buten-2-one, 2-(methylamino)-3-methyl-4-phenyl-3-butene, 2-(methylamino)-3-methyl-4-phenylbutane, and 1-(methylamino)-2-methyl-1,5-diphenylpenta-4-ene-3-one) (277);

Pregabalin: 2016 a literature review (278);

Pyrazolam (8-Bromo-1-methyl-6-pyridin-2-yl-4H-[1,2,4]triazolo[4,3-a][1,4]benzo-diazepine): 2013 characterization by GC/MS, LC-MS/MS, LC-QTOFMS, and NMR (also includes a toxicology study) (279);

alpha-Pyrrolidinopentiophenone (alpha-PVP): 2013 thermal degradation during GC/MS analysis (280); 2016 structure by crystallography (281);

Scopolamine: 2013 detection in spiked samples by portable CE with contactless conductivity detection (282);

Sibutramine: 2012 quantitative determination in adulterated herbal slimming formulations by TLC-image analysis and TLC-densitometry (Dragendorff reagent was used for spot detection) (283); 2013 detection of illicit adulteration of botanical food supplements, by color tests, TLC, HPLC-DAD, MS, and NMR (284); 2015 detection and quantitation in herbal medicines by NIR (285);

Testosterone: 2014 stable carbon isotope ratio profiling of illicit preparations (by GC-IRMS) (286); 2016 screening for in aquatic environments by DART-MS (287);

Tianeptine: 2016 identification by "a multi-pronged analysis approach" (not detailed in the abstract) (288);
Tramadol: 2014 a survey of abuse of tramadol in the U.K. (289);

1-(3-(Trifluoromethyl)phenyl)piperazine (TFMPP): 2014 an FTIR, FT-Raman, UV/Vis, and DFT quantum chemical study (290);

Zolpidem: 2014 development of modified-release tablets of zolpidem tartrate (291);

Zopiclone (see also Eszopiclone): 2015 quantitative determination of zopiclone and its impurity by four different spectrophotometric methods (292); quantitative determination of zopiclone and its impurity by HPTLC (293).

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1.B - Individual Natural Products Containing Abused Substances (except natural products laced with synthetic cannabinoids and/or cannabimimetics)

Overviews and/or Reviews: 2013 an overview of the hallucinogenic plant and fungal species naturally growing in Mediterranean countries (including Phalaris aquatica, Peganum harmala, Mandragora officinarum, Hyoscyamus niger, Atropa belladonna, Datura stramonium, Cannabis sativa, Psilocybe semilanceata, and Amanita muscaria) (294); 2014 natural products as lead structures for the synthesis of "smart" and "recreational" drugs (295); comprehensive comparison of different MS techniques for the detection, identification, and characterization of bioactive substances in herbal materials, including saponins, alkaloid, tropeine alkaloids, lycopodium alkaloids, phenethylisoquinoline alkaloids, benzyltetrahydroisoquinolines, morphine, berberine, dauricine, quinolines, flavonoids, flavones, flavanols, anthocyanidins, etc. (296); a review, covering kava, kratom, Salvia divinorum, bufotenine, glaucine, betel, pituri, lettuce opium, and kanna (297);

Ayahuasca: 2015 quantitative determination of the alkaloids in Tetrapterys mucronata (a plant occasionally used in Ayahuasca preparation) by HPLC-ESIMS/MS (bufotenine, 5-methoxy-N-methyltryptamine, 5-methoxybufotenine, and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline were identified) (298); 2016 analysis by DART-HRMS (299);

Betel (Piper betle Linn): 2013 an overview of its phytochemistry, pharmacological profile, and therapeutic uses (300);

Coca (Erythroxylum): 2012 identification using DNA analysis (301); 2014 chemosystematic identification of 15 new cocaine-bearing Erythroxylum cultigens grown in Colombia for illicit cocaine production (302); selection and validation of reference genes for quantitative gene
expression studies (303);

**Damiana** (*Turnera diffusa*): **2013** identification and discrimination of damiana in herbal blends by GCxGC (304);

**Datura stramonium (Jimson weed, Angel Trumpet):** **2013** isolation of (3R,5R,7Z)-3-hydroxy-5-dec-7-enolide, (R)-tuberolactone, daturadiol, monolinoleyl glycerol, linoleic acid, and lutein from Datura stramonium (analytical methodology not identified in the abstract) (305); a review, including testing methods for Flos daturae (306); **2014** a review of the use of Datura for poisoning (307); **2015** analysis of phytochemical alkaloids in Datura stramonium by GC/MS (308); DNA molecular identification of Datura medicinal plants using ITS2 barcode sequence (309); determination of hyoscyamine and scopolamine in Datura stramonium by HPLC (310); fingerprint analysis of Daturae flos using rapid resolution LC-ESI-MS (311);

**Ephedra:** **2013** determination of ephedrine and pseudoephedrine in Herba ephedrae from different habitats and species by HPLC (312); a review and overview, covering the past 10 years (313); optimum conditions for extracting ephedrine from Ephedra sinica by response surface methodology (based on HPLC analyses) (314); **2014** correlation between the main alkaloid contents and the powder fractions of pulverized Ephedra sinica (analysis by HPLC) (315); **2015** determination of the total alkaloids content, total phenolics content, and total flavonoids content, and determine their relationship in dry herb of Ephedra major, Ephedra distachya subsp. helvetica, Ephedra monosperma, Ephedra fragilis, Ephedra foeminea, Ephedra alata, Ephedra altissima, and Ephedra foliata, by UHPLC/UV (316); the influence of genetic factors on the ephedrine alkaloid composition ratio in ephedra (317); identification and determination of biogenic amines in Ephedrae herba by RP-HPLC with precolumn derivatization (318);

**Hawaiian Baby Woodrose** (*Argyreia nervosa*): **2015** determination of its alkaloid composition (319);

**Khat** (*Catha edulis*): **2012** determination of of cathinone, cathine, and phenylpropanolamine in khat by GC/MS and GC/FID (320); **2013** evaluation of the effect of various drying techniques on the levels of cathinone in khat (321); optimized GC analysis for cathine, phenylpropanolamine, and cathinone in khat following derivatization with MSTFA (322); analysis by CE (323); **2015** isolation of kaempferol, quercetin, and myricetin skeletons from khat, with structural analysis by 1H and 13C NMR, and UV (sugars determined by TLC after acid hydrolysis) (324); use of cation-exchange solid-phase and liquid-liquid extraction for the determination of khat alkaloids by reversed phase HPLC-DAD (325); rapid differentiation of khat using single point and imaging vibrational spectroscopy (326); use of a (-)-norephedrine-based molecularly imprinted polymer for the solid-phase extraction of psychoactive phenylpropylamino alkaloids from khat (327); a
review (328); a review (329);

**Kratom (Mitragynine speciosa):** 2013 by microscopy, TLC, and HPLC (330); by HPLC/DAD (331); 2014 by DART-MS (332); quantification of mitragynine in Kratom by an indirect competitive enzyme-linked immunosorbent assay (333); identification of mitragynine and O-desmethyltramadol in kratom (analytical method not identified in the abstract) (334); comparison of GC/MS, SFC with DAD, and HPLC with MS and DAD for detection of mitragynine and other indole and oxindole alkaloids in kratom (335); 2015 identification and characterization of indole and oxindole alkaloids in kratom using LC-accurate-QTOF-MS (336); a review (337); a review of its phytochemistry (338); detection of mitragynine and its analogs (analytical method not identified in the abstract) (339); a review of the chemistry of mitragyna alkaloids (340); the chemistry of the mitragynines (341); an overview and review (342); an overview of the physicochemical properties of mitragynine (includes UV and HPLC analyses) (343); 2016 monitoring the misuse of kratom in sports (344); a review (345); extraction of mitragynine from kratom (346);

**Marijuana and Hemp (Cannabis sativa) and associated Phytocannabinoids:** 2012 comparison of bulk and compound-specific δ13C isotope ratio analyses for the discrimination of marijuana samples (347); effects of electrical lighting power and irradiance on indoor-grown marijuana potency and yield (348); of THC in marijuana, by HPLC (349); 2013 effects of cultural conditions on the hemp fibres (350); of marijuana extracts by HPLC/UV following cloud point extraction (351); chemical profiling of different hashish seizures by GC/MS and statistical methodology (7 cannabinoids were profiled; analytical methodology not identified in the abstract) (352); production, characterization, and application of hemp essential oil (353); optimisation and characterisation of marijuana extracts obtained by supercritical fluid extraction, focused ultrasound extraction, and retention time locking GC/MS (354); by laser-ablation-ICPMS - a review, covering many other applications (355); a study of marijuana potency from the 1970s to the 2000s (356); supercritical CO2 extraction of cannabis seed oil (and its fatty acid composition analysis) (357); use of ultrasound to extract flavanoids from cannabis (with analysis by UV) (358); determination of cannabinol in "hemp food" by UHPLC-MS/MS (359); potency survey in the Venice, Italy area from 2010-2012 (360); 2014 identification and quantification of cannabinoids in cannabis by HPLC/MS (361); cold pressing and supercritical CO2 extraction of hemp seed oil (362); simultaneous quantification of THC, THC-Acid-A, CBN, and CBD in seized drugs by HPLC/DAD (363); a surface plasmon resonance-based method for detection and determination of cannabinoids (THC, CBD, and CBN) in hashish, using silver nanoparticles (364); variation in mineral composition in the leaves, bark and core of 5 fibre hemp cultivars (365); comparison of 2 different conventional working electrodes for detection of THC using square-wave voltammetry (366); Bayesian classification criterion for discriminating between drug type (illegal) and fiber type (legal) cannabis at an early stage of the growth (367); analysis of
marijuana samples of varying age by the Duquenois-Levine color test (368); variation in preliminary phytochemical screening of cannabis leaf, stem and root (369); separation of aroma compounds from industrial hemp by supercritical CO2 extraction and on-line fractionation (370); fast fingerprinting of cannabinoid markers by laser desorption ionization using silica plate extraction (371); elucidation of the Duquenois-Levine chromophore (372); the kinetics and thermodynamics of hempseed oil extraction by n-hexane (373); evaluation of fatty acid profile, antioxidant content and metabolic content of cannabinoid-free cannabis grown in the Po valley, Italy (374); identification of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane as a volatile marker of hashish (375); analytical and phytochemical characterization of the unsaponifiable fraction of cannabis seed oil (376); resolution of co-eluting compounds of cannabis comprehensive 2D-GC/MS with Multivariate Curve Resolution-Alternating Least Squares (377); metals and organic compounds in the biosynthesis of cannabinoids - a chemometric approach to correlating the metal content in the different parts of cannabis with the soild where plants were cultivated (and with their cannabinoids content) (378); synthesis of all 4 stereoisomers of THC (379); understanding cultivar-specificity and soil determinants of the cannabis microbiome (includes descriptions of the endorhiza-, rhizosphere-, and bulk soil-associated microbiome of 5 distinct cannabis cultivars) (380); cannabis potency in the Venice area (Italy) (2013 update) (381); extraction of flavonoids from cannabis by ultrasound (and its scavenging activity towards the DPPH radical) (382); 2015 minor oxygenated cannabinoids (9α-hydroxyhexahydrocannabinol, 7-oxo-9α-hydroxyhexahydrocannabinol, 10α-hydroxyhexahydrocannabinol, 10a-Rhydroxyhexahydrocannabinol, Δ9-THC aldehyde A, 8-oxo-Δ9-THC, 10α-hydroxy-10-oxo-Δ8-THC, 9α-hydroxy-10-oxo-Δ6a,10α-THC, and 1’S-hydroxycannabinol) from high potency cannabis (structural elucidation was accomplished by 1D and 2D NMR, HRMS, and GC/MS) (383); supercritical CO2 extraction of hemp seed oil (384); ab initio quantum mechanical calculations on THC (385); fatty acid composition, and oxidation stability of the hempseed oil from 4 cannabis cultivars (386); determination of the conformation of THC by linear and nonlinear CD (387); using compact mass spectrometry for detection and quantification of cannabinoids in cannabis (388); potential oil yield, evaluation of elemental profiling methods, including laser-induced breakdown spectroscopy, ICP-MS, LA-ICP-MS, and μXRF for the differentiation of cannabis grown in different nutrient solutions (389); quality analysis of cannabis seed oils extracted by the hot-pressing method, the cold-pressing method, or by an aq. enzymic method (390); analysis of cannabinoids and terpenes in cannabis by HPLC/DAD and GC/FID (391); synthesis of THC and related derivatives via a Diels-Alder route (392); isobaric drug analyses of THC and CBD by DART and hydrogen/deuterium exchange (393); molecular imaging of cannabis leaf tissue with MeV-SIMS (394); analysis of marijuana by LC techniques (a literature survey 1990 - 2015) (395); review of marijuana testing rules in Colorado, methods used for testing, and test results (396); increasing sample throughput of cannabis analyses by using a highly selective stationary phase combined with superficially porous particle technol. for HPLC and LC-MS/MS (includes comparison versus UHPLC) (397); screening of cannabinoids in
industrial-grade hemp using 2D-LC with chemiluminescence detection (398); use of 1H NMR and HPLC/DAD to determine cannabis chemotype, extract profiling, and specification (399); the relationship between cannabinoid content and composition of fatty acids in hempseed oils (400); characterization of the smell of marijuana by SPME with multidimensional GC/MS (401); analysis of residual solvents in cannabis extracts by GC (402); an overview of recent improvements in chromatography for analysis of marijuana (403); determination of the relative percentage distribution of THCA and Δ9-THC in herbal cannabis seized in Austria - impact of different storage temperatures on stability (404); feasibility of facile quantification of cannabinoid content in cannabis to discriminate drug- from fiber-type cannabis in the field (405); cannabinoid dose and label accuracy in marijuana edibles (406); determination of THC, CBD, and CBN by GC/MS (focus on athletic doping) (407); differences in the extraction of THC, THCA, and CBN from cannabis by long-lasting liq. extn. in a Soxhlet app. versus pressurized liq. extn. (408); improving quality control methods for extracting cannabis by flash chromatography (409); determination of selected metals in leaves of cannabis by flame AA (410); simultaneous extraction of total flavonoids and total phenolic compounds from hemp (411); 2016 evolution of 8 cannabinoids and 23 terpenes during the growth of cannabis plants from different chemotypes (412); comparison of new and traditional fiber hemp cultivars (stem, bark, and core yield, and chemical composition) (413); heated headspace SPME of marijuana for chemical testing (414); rapid quantitative chemical analysis of cannabinoids in seized cannabis using heated HS-SPME and GC/MS (415); qual. and quant. detn. of CBDA, CBD, CBN, THC and THC-A in "cannabis-based medicinal exts." by HPLC/UV and HPLC-ESI-QTOF-MS (416); report from a Colorado private laboratory on regional cannabis potency (THC, CBD, CBN, THCA, CBDA, THCV, CBDV, CBG, and CBC) by UHPLC analysis (417); potency trends in confiscated cannabis (includes analytical methods; time frame not indicated in the abstract) (418); changes in cannabis potency (focusing on THC and CBD) over the last 2 decades (1995 - 2014) (419); a discussion of the chem. diversity, biosynthesis, and biol. activity of the various compds. in cannabis, and how these compds. can be used to chem. classify cannabis cultivars (420); analytical testing for the cannabis industry (consumer safety vs. regulatory requirements) - an overview of current protocols for testing for the active phytochem. constituents (i.e., cannabinoids and terpenes), but also for potential contaminants including heavy metals, residual solvents, pesticides, mycotoxins, and microbiol. contaminants (421); use of flash chromatography for rapid extraction of cannabinoids from marijuana edibles (422); analysis of cannabis grown in eastern Oregon for THC, THC-A, CBD, and CBN (edibles, concentrates, and waxes were also tested) (423); comparison of fiber and seed productivity of 14 com. hemp cultivars were tested in 4 contrasting environments (Latvia, the Czech Republic, France, and Italy) (424); the influences of cultivation setting on the lipid distributions, concentrations, and carbon isotope ratios in cannabis (these lipids can currently be used to trace cultivation methods of cannabis and may become a more powerful marker in the future, once the mechanism(s) behind the patterns is uncovered) (425); detection of Δ9-THC and Δ8-THC (and also CBD and CBN) by HPLC/UV
Marijuana (Genetic and/or Proteomic Analyses): 2012 investigations into transgenic marijuana (428); 2013 extraction of high quality DNA from seized Moroccan hashish (429); analysis of THCA Synthase gene expression by real-time quantitative PCR (430); chemotype and genotype of cannabinoids in hemp (431); by DNA analysis (432); polymorphism of DNA and accumulation of cannabinoids by cultivated and wild hemp (433); characterization of seeds by DNA analysis (434); 2014 a simple and efficient method for high quality genomic DNA isolation from cannabis containing high amount of polyphenols (435); diversity analysis in cannabis based on large-scale development of expressed sequence tag-derived simple sequence repeat markers (436); application of DNA barcoding in cannabis identification (437); a PCR marker linked to a THCA synthesize polymorphism is a reliable tool to discriminate potentially THC-rich plants of cannabis (438); nomenclature proposal and SNPSTR haplotypes for 7 new cannabis STR loci (439); characterization of 15 STR cannabis loci - nomenclature proposal and SNPSTR haplotypes (440); 2015 the phytoremediation potential of hemp - identification and characterization of heavy metals responsive genes (441); genetic structure of 5 dioecious industrial hemp varieties (442); genetic identification of cannabis using chloroplast trnL-F gene (443); genetic resources of cannabis in the gene bank at INF&MP in Poznan (which holds about 150 accessions from various regions of the world) (444); cold acclimation induces distinctive changes in the chromatin state and transcript levels of COR genes in 9 cannabis varieties with contrasting cold acclimation capacities (445); sequence heterogeneity of cannabidiolic- and tetrahydrocannabinolic acid-synthase in cannabis and its relationship with chemical phenotype (446); the genetic structure of marijuana and hemp (447); gene duplication and divergence affecting drug content in cannabis (448); characterisation of cannabinoid composition in a diverse cannabis germplasm collection (449); 2016 proteomic characterization of hempseed (450); the inheritance of chemical phenotype in cannabis (regulation of the propyl-/pentyl cannabinoid ratio, and completion of a genetic model) (451); monitoring metabolite profiles of cannabis trichomes during flowering period using 1H NMR-based metabolomics and real-time PCR (452); use of embryos extracted from individual cannabis seeds for genetic studies and forensic applications (a unique profile for each individual was obtained, and a clear differentiation between hemp and marijuana varieties was observed) (453); identification and characterization of the hemp WRKY transcription factors in response to abiotic stresses (454);

Marijuana - Miscellaneous Topics: 2014 the effects of photoperiod on phenological development and yields of industrial hemp (455); detection of pesticides in seized illegal cannabis plants by UPLC/MS-MS in pos. ESI mode using MRM and GC/MS using scan mode (456); 2015 germination characteristics of hemp seeds under single NaCl treatments of varying concentrations (457); method development towards quantifying marijuana consumption using sewage based drug epidemiology (458); medical marijuana’s public health lessons - implications
for retail marijuana in Colorado (459); determination of herbicides paraquat, glyphosate, and aminomethylphosphonic acid in marijuana samples by CE (460); an overview of the occupational hazards for employees working in the state-permitted marijuana industries (461); issues with retail promotion of marijuana edibles (462); method development towards quantifying marijuana consumption using sewage based drug epidemiology (463); a series of editorials (published in Nature) concerning various aspects of state-permitted marijuana (464); an overview of health and safety issues for state-permitted marijuana businesses (465); a review on the ingredients in and safety of "hemp seed food" (466); 2016 the appropriateness of applying ISO/IEC 17025 standards to cannabis testing laboratories (467); quantification of THC-COOH in wastewater from a residential treatment plant as a tracer of cannabis use, using LC-MS/MS (468); oral cannabidiol does not alter the subjective, reinforcing, or cardiovascular effects of smoked cannabis (469); an overview of the changing regulations and rules of the state-permitted cannabis industry (470); the effects of ethephon (a plant growth regulator) on changes in the amount of many terpenoid compounds in cannabis, including THC, CBD, chlorophyll, carotenoids, α-tocopherol, and pyruvate (471); an overview of the American Herbal Product Assocn.'s (AHPA) industry guidelines on manufacturing, producing, dispensing, and lab. operation standards as they apply to state-permitted cannabis (including the American Herbal Pharmacopeia's (AHP) cannabis monograph) (472); an overview on preserving personal cultivation rights while regulating commercial cultivation as agriculture (focusing on the excessive energy, water, and other resources needed for cannabis cultivation) (473); evaluation of three multiresidue methods for the determination of 61 pesticides on marijuana by LC-MS/MS (474); an overview of the establishment of the cannabis subdivision of the American Chemical Society (475); use of "cannavaping" as a means for administering "medical marijuana" (476); antifungal activity of the volatiles of high potency cannabis against Cryptococcus neoformans (477); quantification of THC-COOH in wastewater to assess cannabis consumption in Washington state (478);

[Marijuana ("Synthetic Marijuana") - See "Synthetic Cannabinoids and Cannabinomimetics" (Subsection 1.D)]

**Mimosa: 2013** characterization and purity of DMT isolated from Mimosa tenuiflora inner barks (479);

**Mushrooms (including Psilocybe mushrooms): 2013**: simultaneous determination of mushroom toxins by LC-TOF-MS (480); **2014** analysis of mushrooms by Fluorescent Random Amplified Microsatellites (F-RAMS) (15 samples of Amanita rubescens and 22 samples of other hallucinogenic and nonhallucinogenic mushrooms of the genera Amanita and Psilocybe were profiled) (481); **2015** identification of psilocybin, psilocin, baecystin, norbaeocystin, and aeruginascin in Pholiotina cyanopus by LC/MS (482); genetic identification of hallucinogenic and other poisonous mushrooms (483); **2016** DNA-based taxonomic identification of...
basidiospores in hallucinogenic mushrooms in "grow-kits" (including LC-UV quali-/quantitative determination of psilocybin and psilocin) (484);

Opium / Opium Poppy / Poppy Seeds (see also Papaver below, and Opiates in Subsection 1.C): 2013 the effects of potassium, boron, and strontium on poppy cultivation (such enhancements may impact impurity profiling studies based on elemental analysis) (485); 2014 simultaneous detn. of morphine, codeine, thebaine, oripavine, papaverine, and noscapine in poppy straw by 2 HILIC methods (486); a review of cold pressed poppy seed oils (487); unambiguous characterization of analytical markers in 4 opium samples using an ion mobility trace detector-mass spectrometer (488); physicochemical properties of opium marc (a waste product from commercial opium processing) (489); management of opium marc as a hazardous waste (490); results from an effort to detect opium fields from a Hyperion image covering a study area in Southwest Afghanistan (491); 2015 comparative analysis of volatile flavor compounds of poppy seed oil extracted by two different methods via GC/MS (492); analysis of alkaloids in poppy straw by HPLC (493); 2016 analysis of opium poppy by 2D-HPLC (494); analysis of poppy seeds (intended for use as food) that had been adulterated with poppy straw (i.e., containing morphine and codeine) by IRMS (495);

Papaver (other species): 2016 measurement of some benzylisoquinoline alkaloids in Papaver bracteatum (496); developmental accumulation of thebaine and some gene transcripts in different organs of Papaver bracteatum (497);

Papaver (Genetic and/or Proteomic Analyses): 2011 characterization of SSR markers in opium poppies (498); 2014 a review of benzylisoquinoline alkaloid biosynthesis in opium poppy (499); development of genomic simple sequence repeat markers in opium poppy by next-generation sequencing (500); comparative analysis of Papaver somniferum genotypes having contrasting latex and alkaloid profiles (501); transcriptome profiling of alkaloid biosynthesis in elicitor induced opium poppy (502); recessive loci Pps-1 and OM differentially regulate PISTILLATA-1 and APETALA3-1 expression for sepal and petal development in Papaver somniferum (503); variation in fatty acid composition of three Turkish opium poppy lines (504); 2015 regulation of the alkaloid biosynthesis by miRNA in opium poppy (505); comparative study for stability and adaptability through different models in developed high thebaine lines of opium poppy (506); 2016 molecular genetic diversity and association mapping of morphine content and agronomic traits in Turkish opium poppy germplasm (507);

Peyote (and other mescaline-containing cacti): 2013 analysis of "peyote tea" by GC/MS and GC/MS/MS in PCI mode (508); 2014 phytochemical study of Echinopsis peruviana (509);

Plant Materials (Multiple Plants in Single Studies): 2013 identification of plant materials
used as supporting matrices for pharmaceuticals, nutritional supplements, and illicit drugs, by DAD, evaporative light scattering detection, and MS (510); a review of chromatographic herbal fingerprints (the "herbs" and the chromatographic method(s) were not identified in the abstract) (511); isotopic analyses to discriminate between organic and "conventional" plants (512); the effects of 11 elements (Co, Mo, Zn, W, Cr, Cu, B, Fe, V, Mn, Ni plus Ca for second species) on the formation and accumulation of indoles and isoquinolines in seedlings of Catharanthus roseus L. and Papaver somniferum L. (513); analysis of the plant materials used as support matrices, by DNA analysis, GC/MS, and LC/MS (514); an overview and review of the application of 2D-IR for determining the composition, origin, and authenticity of herbal medications (515); 2014 evaluation of mycotoxins, mycobiota, and toxigenic fungi in opium poppy, licorice root, Indian rennet, and others (516); the study of elemental profile of some important medicinal plants by Flame AA (the study included Papaver somniferum) (517); comparison of plant DNA extraction kits for plants identification in forensic botany (the plant species were not identified in the abstract) (518); determination of metabolites in finely powdered plant material by Direct Laser Desorption Ionization MS (519); chemotaxonomical classification of the Solanaceae Atropa belladonna, Datura stramonium, Hyoscyamus niger, Solanum dulcamara, and Duboisia by FTIR/ATR in combination with cluster anal. (520); use of hyperspectral data for detection of cannabis and poppy sites, including those mixed with masking vegetation (521); 2015 transcriptome profiling of Catha edulis and Ephedra sinica identifies genes potentially involved in amphetamine-type alkaloid biosynthesis (522); phytoaccumulation of heavy metals in natural vegetation, including cannabis (523); application of chemometrics for identification of psychoactive plants (Salvia divinorum, Mitragyna speciosa, Psychotria viridis, and Calea zacatechichi) using GC/MS, AAS, and ICP/MS (524); the chemical properties of cold-pressed vegetable oils from seeds of hemp (Cannabis sativa L.), blue poppy (Papaver somniferum L.), and several other plants (525); biosynthesis of amphetamine-like alkaloids in Catha edulis and Ephedra spp. (526); profile of toxic metals in 12 different plant materials, including marijuana, by AA (527); use of EILC/MS with supersonic molecular beams for analysis, including cannabis (528); 2016 determination of Mn, Ni, Rb, and Sr in powdered stimulant plants (ginseng, guarana, and others) using high-resolution continuum source AA followed by chemometric classification (529); phytochemical profiling of plants using GC/MS (including cannabis) (530); use of high-throughput DART-HR-TOFMS to screen plant-based drugs of abuse for psychotropic alkaloids and adulterants (plants not identified in the abstract) (531); analysis of Datura spp. seeds, kratom powder, kava powder, Salvia divinorum leaves, Kanna crushed leaf material, Mimosa hostilis, Banasteriopsis caapi, and Morning Glory seeds by DART-HRMS (532);

**Psychotria viridis (and related species):** 2015 examination of Psychotria viridis (DMT was identified by TLC and HPLC) (533); 2016 structural characterization of dimeric indole alkaloids (brachybotryne, its N-oxide deriv., along with bufotenine) from Psychotria brachybotrya by NMR spectroscopy and theoretical calculations (534);
**Salvia divinorum:** 2013 differentiation of Salvia divinorum from marijuana and tobacco by DNA analysis (535); 2014 quantitative determination of salvinorin A in Salvia divinorum (analytical methodology not identified in the abstract) (536); analysis of "legal high" products containing Salvia divinorum for Salvinorins A, B, C, and D (analytical methodology not identified in the abstract) (537); 2015 determination of salvinorin A in commercial products available in Mexico, using HPLC (538); 2016 an overview of the chem. and pharmacol. of Salvia divinorum and salvinorin A (539).

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**1.C - Common Groups or Classes of Compounds or Substances (except Synthetic Cannabinoids and Cannabimimetics)**

**(2-Aminopropyl)indoles:** 2013 2-, 3-, 4-, 5-, 6- and 7-(2-aminopropyl)indole - analyses by GC/MS and LC/MS (540);

**Amphetamine-Type Stimulants (ATSs) and Related Phenethylamines (PEAs):** 2011 impurity profiling of various ATSs by physical characterization, qualitative and quantitative analyses, and identification of adulterants, byproducts, and precursors, using GC, GC/MS, and cluster analyses (541); 2012 analysis of 2-, 3-, and 4-methylmethamphetamine and 2-, 3-, and 4-methylethamphetamine, by GC/MS and GC/IRD (542); analysis of methamphetamine, amphetamine, and ecstasy by insideneedle adsorption trap based on molecularly imprinted polymer followed by GC/FID (543); 2013 analysis of 4-bromo-2,5-beta-trimethoxyphenethylamine (BOB), 4-methyl-2,5-beta-trimethoxyphenethylamine (BOD), 3,4-methyleneedioxy-beta-methoxyphenethylamine (BOH), and 4-methyl-2,5-dimethoxy-beta-hydroxyphenethylamine (BOHD), by LC-MS/MS (toxicological focus) (544); differentiation of stimulant amphetamines, hallucinogenic amphetamines, and nonamphetamine (none specified in the abstract) by GC/FTIR and cluster analysis (545); determination of ephedrine, methamphetamine, and amphetamine by SERS (546); analysis of amphetamine and methamphetamine by GC-MS after propylchloroformate derivatization (547); determination of diethylpropion, fenproporex, and sibutramine in counterfeit tablets, by FTIR/ATR (548); determination of amphetamines and precursors by a portable instrument combining miniaturized GC and IR Absorption Spectroscopy (549); determination of (unspecified) amphetamines by GC/FTIR (550); synthesis and characterization of 2-, 3-, and 4-methylethamphetamine by GC/MS, HR-ESI-MS, NMR, and IR (551); a chemometric system for the automated detection of 159 ATSs, using GC/FTIR (552); a review of the 2C series of PEAs (553); analysis of methamphetamine, MDMA, and other ATSs by GC/MS after derivatization with iso-Bu chloroformate and SPME (toxicological focus) (554); detection of volatile compounds that could indicate an ATS by SPME-GC/MS (P2P was detected in every stimulant sample, and 1-phenyl-1,2-propanedione was detected in some stimulant
samples) (555); determination of (unspecified) amphetamines by GC/FTIR (556); a review of
impurity profiling and syntheses of methamphetamine, MDMA, amphetamine, DMA, and PMA
(557); identification of phenethylamine, ephedrine, and MDMA by Raman, SERS, and DFT
(558); analysis of six (unspecified) isomers of mono-methoxyethylamphetamines and
mono-methoxydimethylamphetamines (MeO-DMAs) by GC-EI-MS/MS (559); 2014 detection of
amphetamines by cluster analysis (560); determination of N-ethyl-α-ethyl-phenethylamine
(ETH), N,N-diethylphenethylamine, and phenethylamine in dietary supplements by LC-MS/MS
(561); synthesis and SARs of N-benzyl phenethylamines as 5-HT2A/2C agonists (562); potential
interferences in the GC/MS analyses of methiopropamine, 4-fluoroamphetamine,
4-fluoromethamphetamine, and 4-methylamphetamine (563); synthesis of [13C6]-labeled
amphetamine, methamphetamine, MDA, MDMA, MDEA, PMA, PMMA, 3,5-dimethoxy-
phenethylamine, 4-bromo-2,5-dimethoxyphenethylamine, and 2,5-dimethoxy-4-iodophenethyl-
amine (564); enantioselective hydrogenation of α,β-disubstituted nitroalkenes to synthesize
chiral amphetamines (565); synthesis of phenethylamine via anti-Markovnikov hydroamination
of alkenes catalyzed by a two-component organic photoredox system (566); simultaneous
enantioselective separation of methamphetamine, ephedrine, pseudoephedrine, and the chloro-
intermediates formed during the Emde method, after derivatization with trifluoroacetic anhydride
(567); detection of amine-based stimulants by a novel fluorescent sensor (568); chiral separation
cartonone and amphetamine derivatives by HPLC/UV using sulfated β-cyclodextrin as a chiral
mobile phase additive (569); 2015 comparisons of chiral analyses of 10 cathinone and
amphetamine-derivatives by CEC, SFC, and 3 different LC methods (570); simultaneous
voltammetric detection of MDMA and PMA (571); analysis of ATSs by DSC (572);
enantioselective synthesis of ephedrine, amphetamine, and their analogues via two stereocentered
Co(III)-catalyzed hydrolytic kinetic resolution of racemic syn-benzyloxy epoxide (573); analysis
of amphetamine, methamphetamine, norephedrine, norpseudoephedrine, ephedrine, pseudo-
ephedrine, dimethylamphetamine, and methylephedrine by chiral CE/MS (574); determination of
MDMA, methamphetamine, MDA, and MDEA by by portable CE with contactless conductivity
detection (575); fast separation of 11 cathinones and 4 phenylethylamines by SFC-positive-ESI-
triple-quad-MS (576); "novel" sympathomimetics in supplements actually recapitulate the work
of synthetic chemists at pharmaceutical firms during the 1930s and 1940s (577); characterization
of N-(ortho-methoxybenzyl)-3,4-dimethoxyamphetamine, N-(ortho-methoxybenzyl)-4-ethyl-
amphetamine, N-(ortho-methoxybenzyl)-4-methylmethamphetamine, and N-(ortho-methoxy-
benzyl)-5-(2-aminopropyl)benzofuran by MS, IR, and NMR (578); 2016 electrochemilumines-
cent detection of methamphetamine and amphetamine (579);

**Barbiturates:** 2013 analysis of barbital, phenobarbital, pentobarbital, amobarbital, secobarbital,
butilbital, pentothal, and butabarbital by IR and and Raman (580); 2014 by colorimetric sensing
(581); computing the acidities of barbituric and thiobarbituric acid (582); a theoretical study on
the isomerization and tautomerism of 16 isomers of barbituric acid, using MP2 and B3LYP
an overview of the polymorphism and tautomerism of barbituric acid (584); a review of the chem. of barbituric acids employed in the design and synthesis of different types of compds (585);

**Benzodiazepines: 2013** cross reactivity of 3-hydroxyflunitrazepam, 7-aminonitrazepam, brotizolam, delorazepam, pinazepam, and α-hydroxy-midazolam with a commercial immuno-assay test (includes LC-MS/MS analyses) (586); analysis of 11 different benzodiazepines and metabolites by SERS (benzodiazepines not identified in the abstract) (587); an FTIR/ATR spectral library of benzodiazepines (588); analysis of nitrazepam, clonazepam, lorazepam, chlordiazepoxide, alprazolam, clozapine, and diazepam by HPTLC with densitometric measurement and UV scanning (toxicological focus) (589); 2014 quantum chemical study of some benzodiazepines by density functional theory (590); determination of clonazepam and its related substances in pharmaceutical formulations by HPLC (591); determination of bromazepam, clonazepam, and diazepam in the Guanda River, Brazil (analytical methodology not identified in the abstract) (592); detection of diazepam, flunitrazepam, and temazepam in spiked drinks by GC/MS (593); a review of the analysis of benzodiazepines by LC with electrochem. detn. (since 2006, with earlier reports given in summary) (594); analysis of diazepam, alprazolam, clorazepate, temazepam, and bromazepam by confocal Raman microscopy (595); differentiation of benzodiazepines by Raman (596); low temperature separation of the inter-converting enantiomers of diazepam, flunitrazepam, prazepam, and tetrazepam by dynamic HPLC on chiral stationary phases (597); detection of benzodiazepines in drinks by electrophoretic fingerprinting (598); 2015 use of supported liquid extraction for the analysis of benzodiazepines by SERS (599); characterization of clonazolam, deschloroetizolam, flubromazolam, and meclonazepam by NMR, GC-EI-MS, LC-MS/MS, LC-QTOF-MS, and IR (600); determination of diazepam, clonazepam, and alprazolam in dietary supplements by UHPLC-HR-Quad-MS (601); predictive modelling of the toxicity of benzodiazepines using descriptor-based QSTR, group-based QSTR, and 3D-toxicophore mapping (602); a study of the mechanism of mass spectral fragmentation of benzodiazepines (603); analysis of chlordiazepoxide, midazolam, nitrazepam, estazolam, oxazepam, lorazepam and alprazolam by HPLC with UV or DAD detection (604); 2016 analysis of benzodiazepines by chip-based electrochromatography coupled to ESI-MS detection (605); determination of chlordiazepoxide; lorazepam; diazepam; oxazepam; medazepam in an alc. "grappa" drink by packed sorbent (MEPS)-UHPLC-UV (606);

**Benzofurans: 2015** pharmacological profile of 5-APB, 5-APDB, 6-APB, 6-APDB, 4-APB, 7-APB, 5-EAPB, 5-MAPDB, and the benzodifuran 2C-B-FLY (607);

**Bromo-, Chloro-, and Fluoro- Amphetamines and Methamphetamines: 2013** analysis of 2-, 3-, and 4-chloro- and 2-, 3-, and 4-fluoro- amphetamines by CE-LIF, following derivatization
with fluorescein isothiocyanate (includes comparisons against CZE-UV, sweeping-MEKC-UV, and LC-Q-TOF-MS) (608); synthesis and characterization of fluoroamphetamine and fluoro-methamphetamines by GC/MS and LC-MS/MS, before and after derivatization with various reagents (compounds not specified in the abstract) (609); 2015 discrimination of 2-, 3-, and 4-fluoromphetamine by Raman (610); differentiation of ring-substituted bromoamphetamine analogs by GC/MS (611); identification of the regioisomers of the chloroamphetamine and chloromethamphetamine by GC-MS/MS (612);

**Cathinones**: 2012 mass spectral fragmentation of 25 cathinones (not identified in the abstract) by GC-HR-TOF-MS using a soft ionization source (613); analysis of 4-MMC, 4-, 3-, or 2-fluoromethcathinone, 4-methoxymethcathinone, N-ethylcathinone, and N,N-dimethylcathinone by GC/MS (includes a stability study) (614); 2013 characterization of 31 synthetic cathinones (not identified in the abstract) by GC/MS, IR, and NMR (615); analysis of mephedrone, methylone, and MDPV by ambient ionization MS using arrays of low-temperature plasma probes, and also following injection of trifluoroacetic anhydride directly into the plasma stream for online derivatization (616); analysis of BMDP, butylene, MDPBP, MDPV, methylone, and pentylene by HPLC-HR-QTOF-MS (617); analysis of 38 cathinones (not specified in the abstract) by hybrid Q-TOF-MS and LC/MS/MS (618); an overview and review (619); analysis of (unspecified) "bath salt" cathinones by DART-MS (620); an overview and review of synthetic cathinones (621); analysis of 16 cathinones using "presumptive testing" (not specified in the abstract), TLC, and GC/MS (622); an overview of "bath salts" (including mephedrone, MDPV, and possibly others) (623); characterization of mephedrone and pentedrone by single-crystal X-ray diffraction (624); analysis of 4-methylmethcathinone, three positional isomers of fluoromethcathinones, 4-methoxymethcathinone, N-ethylcathinone, N,N-dimethylcathinone, buphedrone, and pentedrone by GC/MS (625); a review of mephedrone, MDPV (and possibly others) (626); 2014 enantiomeric analysis of 10 new cathinones by CEC on a chiral stationary phase (627); identification of trace levels of synthetic cathinones using Raman (cathinones not identified in the abstract) (628); analysis of 13 synthetic cathinones and associated psychoactive substances by ESI-high performance-IMS (629); identification of MDPV, 3,4-methylenedioxy-α-pyrrolidinobutihenone (MDPBP), 4-fluoromethcathinone (4-FMC), butylene, mephedrone, naphyrone, 4-methylmethcathinone (4-MEC), ethcathinone, α-pyrrolidinopentiophenone (α-PVP), and 3-methyl-α-pyrrolidinopropiophenone (3-MPPP) by GC/FID and GC/MS (630); screening and comparative analysis of synthetic cathinones by portable microchip electrophoresis (631); chiral separation of 12 cathinones by cyclodextrin-assisted CE with UV and MS detection (632); use of DART-MS in-source collision induced dissociation and high mass accuracy for determination of new psychoactive cathinones (633); screening for 16 cathinones by "presumptive testing", TLC, and GC/MS (634); electrochemical detection of (±)-methcathinone, (±)-mephedrone, and (±)-4'-methyl-N-ethylcathinone (635); electroanalytical sensing of mephedrone and methylethcathinone (636); synthesis and characterization of 9 new derivs. of
cathinone (obtained by modifying the carbonyl group to create cyclic ketals and thiketals, oximes, and hydrazones of cathinone and of cathinone phthalimide; analytical methodologies not identified in the abstract) (637); QSAR modelling of 4-methylbuphedrone and 4-methoxy-N,N-dimethylcathinone, with comparison to methylenedioxypyrovalerone (638); characterization of 4-fluoromethcathinone, ethcathinone, buphedrone, methedrone, pentedrone, 3,4-dimethylmethcathinone, 4-methylethcathinone, and others by FTIR, GC/MS, 1HNMR, and wavelength dispersive XRF (639); 2015 analytical and synthetic studies on substituted cathinones (no details provided in the abstract) (640); analysis of methcathinone, 3,4-methylenedioxymethcathinone, 3,4-methylenedioxypropyvalerone, and 4'-methyl-α-pyrrolidinopropiophenone by LC/MS (641); isotopic profiling of cathinones for comparative analyses (642); identification and characterization of α-PVT, α-PBT, and their bromothienyl analogs (643); a review of the R- and S- isomers of cathinones, focusing on MDPV (644); an overview and review of the neurotoxicity of the cathinones (645); identification and characterization of 4-fluoro-PV9 and α-PHP by HPLC, HPLC/DAD, ESI-Ion-Trap-MS in MS2 and MS3 modes, GC/MS, thermogravimetric anal., DSC, FTIR, UV/Vis, and NMR (646); compatibility of highly sulfated cyclodextrin with ESI at low nanoliter/minute flow rates and its application to CE-ESI/MS analysis of cathinone derivatives (647); the electrochemical detection of mephedrone (4-MMC) and 4'-methyl-N-ethylcathinone (4-MEC) (648); a study of the decomposition of the HCl salts of 8 cathinone derivatives in air (649); crystal structures of two forms of MDPV HCl and one form of ethylene HCl (650); preparation and characterization of the tertiary catinones N,N-dimethylcathinone, N,N-diethylcathinone, and 2-(1-pyrrolidinyl)-propiophenone by NMR and MS (the enantiomers were also prepared and identified by HPLC and CD) (651); analysis of (±)-4'-methylmethylcathinone and (±)-4'-methyl-N-ethylmethcathinone by HPLC/UV and amperometric detection ("NRG-2" is a focus) (652); 2016 differentiation of cyclic tertiary amine cathinone derivatives (the cyclic amines azetidine, pyrrolidine, piperidine, and azepane were incorporated into a series of cathinones related to MDPV) by product ion-EI-MS and MS/MS (653); thermal degradation of 4-ethylmethcathinone, 4-methylethcathinone, buphedrone, butylone, ethcathinone, ethylene, flephedrone, 3,4-methylenedioxy-α-pyrrolidinobutiophenone, 3,4-methylenedioxypropyvalerone, mephedrone, methcathinone, methedrone, methylone, 4-methyl-α-pyrrolidinobutiophenone, naphyrone, pentedrone, pentylene and pyrovalerone under GC/MS conditions (654); identification of methylone and pentedrone by NMR, IR, UV/Vis, MS/MS, and HR-TOF-MS (655); identification and characterization of iso-4-BMC, β-TH-naphyrone, mexedrone, and 4-MDMC by LCQTOF-MS, GC/MS, and NMR (656); chiral separation of new cathinones on chiral ion-exchange type stationary phases (657);

"Ecstasy Tablets" (that is, Tablets or Powders specified in their Titles or Abstracts as Ecstasy - these may in fact contain MDMA, a mixture of MDMA with one or more other Drugs, or only one or more non-MDMA drugs): 2013 elemental analysis of Ecstasy tablets by graphite furnace atomic absorption, for comparative analysis (abstract indicates Cu, Mg, Ba, Ni,
Cr, and Pb) (658); 2014 determination of metals (Zn, Al, Ca, Mg, K, Na, Ba, Fe, B, Cu, and Pt) in Ecstasy tablets using ICP-OES and XRF (659); 2015 a discussion of "luminescent" Ecstasy tablets (a marketing ploy) (660); detection of MDMA, methamphetamine, and 20 other substances in Ecstasy tablets, including caffeine, 2C-B, piperazines, amphetamines, and phencyclidine, by GC/MS (661); 2016 comparison of the purity and adulteration of the crystalline (powder) samples versus tablets in the Spanish Ecstasy market 2000-2014, by TLC, GC/MS, and UV (662);

**Ephedrines:** 2012 interconversion of ephedrine and pseudoephedrine during heptafluorobutyric anhydride derivatization (663); 2013 comparison of RP-UHPLC and HILIC for quantitation, with medium-resolution accurate MS (664); 2014 identification of ephedrine by use of charge-transfer complexes (with analysis of the complexes by elemental anal., IR, Raman, 1H NMR, and UV-Vis (665);

**Ergot Alkaloids:** 2014 a review of the biosynthetic pathways of ergot alkaloids (666); detection of ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine) in rye and triticale grains (analytical methodologies not identified in the abstract) (667); determination of ergotamine tartrate in tablets using LC with fluorimetric and UV detection (668); an overview of the biosynthesis of the ergot alkaloids (669); identification of ergot alkaloid in two Argyraea nervosa "legal high" products by HPLC-HRMS/MS (670); a review of the detection of ergot alkaloid derivatives by TLC (671); aptamer-based extraction of ergot alkaloids from ergot contaminated rye feed (672); 2015 determination of ergot alkaloids in grain products by LC-ion trap-MS (673); an evaluation of fast dissolving tablets of ergotamine tartrate (674); determination of ergovaline in tall fescue seed and straw using a QuEChERS extraction method by HPLC with fluorescence detection (675); 2016 an overview and review (676); quantitative and qualitative transcriptome analysis of four industrial strains of Claviceps purpurea with respect to ergot alkaloid production (677); determination of ergot alkaloids in Morning Glory cultivars by LC-Q-TOF-MS (678); screening for total ergot alkaloids in rye flour by planar SPE-fluorescence detection and MS (679);

**Fentanyl Derivatives:** 2014 analysis of the inclusion complexes between cyclodextrins and fentanyl by NMR and computational studies (680); an efficient, optimized synthesis of fentanyl and related analogs (681); 2015 improved and optimized syntheses of fentanyl and related analogs (682);

**2-, 3-, and 4-Fluorophenmetrazines:** 2016 synthesis, characterization, and differentiation of the fluorophenmetrazine isomers (683);

**"FLY" Compounds:** 2014 synthesis of labeled 2C-B-FLY and Bromo-DragonFLY for use as
internal standards (684);

**Methiopropamine (and its 3-thienyl isomer): 2013** synthesis and analysis/differentiation by GC (685);

**NBOMe Compounds: 2013** characterization of 25D-NBOMe [2-(2,5-dimethoxy-4-methylphenyl)-N-(2-methoxybenzyl)ethanamine], 25E-NBOMe [2-(4-ethyl-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine], and 25G-NBOMe [2-(2,5-dimethoxy-3,4-dimethylphenyl)-N-(2-methoxybenzyl)ethanamine (686); 2014 an overview and review (687); 2015 a review (688); detection of NBOMEs (and other NPSs) on blotter papers by direct ATR-FTIR (689); analysis of 25I-NBOMe, 25BNBOMe, 25C-NBOMe and other dimethoxyphenyl-N-[(2-methoxyphenyl)methyl]ethanamine derivatives on blotter paper by DART-Accu-TOF-MS and HPLC-triple quadrupole-MS (690); an overview (691);

**Opiates: 2012** determination of morphine and codeine by HPLC-quadrupole mass selective detection (may be a toxicological study) (692); 2013 analysis of morphine and codeine by TLC and densitometry (693); 2014 some insights into hydrate formation and stability of morphinanapes by powder X-ray diffraction, IR, DSC, and isothermal calorimetry (694); isomerization of codeine and morphine into hydrocodone and hydromorphone using a water-sol. rhodium complex formed from com. available [Rh(COD)(CH3CN)2]BF4 and 1,3,5-triaza-7-phosphaadamantane (695); a review of the TLC of morphine analogs (compounds not identified in the abstract) (696); 2015 potential use of oriental poppy hairy roots for producing thebaine, morphine, and codeine (697); a review covering the synthesis of buprenorphine, naltrexone, naloxone, and naltuphine from naturally occurring opiates such as thebaine and oripavine (698); the stereochemistry and spectral assignment of thebaine derivatives based on a 1D NOESY NMR study (699); degradation of morphine and codeine by gamma radiation in methanol (700); radiation induced destruction of thebaine, papaverine, and noscapine in methanol (701); a review of AH-7921 (702); separation of morphine, hydromorphone, and norcodeine using ESI and paper spray coupled to high-field asymmetric waveform IMS (703);

**Opiates (Bio-Engineered): 2014** use of a microbial biomanufacturing platform for natural and semisynthetic opioids, using Saccharomyces cerevisiae (704); 2015 heroin from bio-engineered yeast (705); heroin from bio-engineered yeast (706); failure of an attempted large-scale effort to produce thebaine using home-brew type conditions (707); synthesis of morphinan alkaloids from norlaudanosoline using Saccharomyces cerevisiae (708); a feasibility study for production of thebaine and hydrocodone from sugar by bio-engineered yeast (709); a review, detailing the current status of microbial benzylisoquinoline alkaloid synthesis and derivatization (710); a call to regulate the synthesis of morphine by bio-engineered yeasts (711); 2016 metabolic engineering for the production of plant isoquinoline alkaloids (712); complete biosynthesis of opioids
(thebaine) by yeast (713); a review of the production of thebaine and hydrocodone from D-glucose by fermentation (714); total biosynthesis of opiates (thebaine) by stepwise fermentation using engineered E. coli (715);

**1-(1-Phenylcyclohexyl)piperidine (PCP) and 1-(1-phenylcyclohexyl)pyrrolidine (PCPy) analogues:** 2014 characterization by GC-ion trap-EI-, CI-, and HR-MS, LC-ESI-triple-quadrupole linear ion trap-MS/MS, IR, DAD, and 1H and 13C NMR (716);

**Phenothiazines:** 2013 separation and identification of prochlorperazine, promethazine, chlorpromazine, and trifluromoperazine (717);

**Phosphodiesterase-5 Inhibitors - Cialis (tadalafil), Levitra (vardenafil), Viagra (sildenafil), and similar drugs:** 2013 a multivariate-based wavenumber selection method for classifying Cialis and Viagra into authentic or counterfeit classes by ATR/FTIR (718); analysis for residual solvents in counterfeit tablets and capsules of Cialis and Viagra (analytical method not indicated in the abstract) (719); simultaneous qualitative and quantitative analysis of counterfeit Cialis by Raman (720); analysis of 38 compounds (sildenafil, tadalafil, vardenafil and their analogs) in illicit erectile dysfunction products by LC-ESI-MS/MS (721); differentiation between counterfeit and authentic Cialis and Viagra by ATR/FTIR with PCA (722); analysis and profiling by UPLC/MS (723); characterization of sildenafil citrate tablets from different sources by NIR chemical imaging and chemometric tools (724); 2014 profiling authentic and counterfeit Viagra and Cialis using XRF, direct infusion ESIMS, UPLC-MS, and ATR-FTIR (725); simultaneous determination of of sildenafil, tadalafil, vardenafil and acetildenafil in health-care foodstuffs by UHPLC/MS (726); qualitative and quantitative analysis of sildenafil in traditional medicines and dietary supplements by HPLC/UV and IR (727); 2015 isolation and structural characterization of chloropropanoylpretadalafil in a dietary supplement by HPLC-UV, GC/FT-IR/MS, and HRMS (728); detection of sildenafil citrate in herbal formulations by UV/Vis (729); differentiating genuine and counterfeit Viagra tablets by dynamic thermal analysis (730); 2016 use of transmission-mode desorption electrospray MSMS to screen for synthetic phosphodiesterase-5 inhibitors in samples of adulterated herbal dietary supplements (731); analysis of dietary supplements containing phosphodiesterase type-5 (PDE-5) inhibitors by LC/MS and HPLC/UV (732);

**Piperazines:** 2012 differentiation of methylenedioxybenzylpiperazines and ethoxybenzylpiperazines by GC/IRD and GC/MS (733); 2013 characterization of six ring regioisomeric dimethoxybenzylpiperazines (DMBzPs) by GC/MS and GC/IRD (734); analysis of the six-ring regioisomeric dimethoxybenzyl-N-methylpiperazines (DMBMPs) by GC/MS (735); a presumptive color spot test method for the detection of benzylpiperazine and piperazine analogues (736); determination of chlorophenylpiperazine isomers by CE (737); analysis of phenyl and benzyl piperazines by HPLC with chemiluminescence detection (738); 2014 six ring
regionisomeric dimethoxybenzoyl-N-methylpiperazines (DMBzMPs) by GC/MS and IR (739); analysis of regioisomeric bromodimethoxy benzyl piperazines related to 4-bromo-2,5-dimethoxy-benzylpiperazine by GC/MS and FTIR (740); differentiation of the 1-(methylenedioxyphenyl)-2-piperazinopropanes and 1-(methoxyphenyl)-2-piperazinopropanones by GC/IRD and GC/MS (741); 2015 analysis of six ring regioisomeric dimethoxyphenylpiperazines (DOMePPs) by GC/MS and IR (742); analysis of 23 benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) containing tablets by HPLC and IRMS (743); an overview of 1-cyclohexyl-4-(1,2-diphenylethyl)piperazine (MT-45) (744);

**Steroids:** 2013 determination of tetrahydrogestrinone and related anabolic androgenic steroids by MEKC (745); a study of authentic and counterfeit products (primarily stanozolol, testosterone, and nandrolone) seized in Brazil from 2006 to 2011 (746); analysis of methandienone and methyltestosterone in tablets by color testing and GC/MS (747); a review of the bioanalytical challenges in detecting unknown anabolic androgenic steroids (in doping control analysis) (748); screening for steroids in traditional medicine and nutraceutical products using electrospun cellulose acetate nanofibers as thin layer chromatographic media (749); 2015 analysis of anabolic steroids by GC-EI/MS, GC-EI/MS/MS, LC-ESI/MS/MS, LCAg+CIS/MS/MS, and GC-ESI/MS/MS (for doping control) (750); determination of anabolic-androgenic steroid adulterants in counterfeit drugs by UHPLC-MS/MS (751); identification and quantification of anabolic steroid esters by DART-HRMS (752); an overview and review of the anabolic androgenic steroids in supplements (753); determination of anabolic agents in dietary supplements by LC-HRMS (754); a summary of the designer steroids that are most commonly sold in dietary supplements (as of April 2014) (755); 2016 improved detection of steroids and evidence for their regiospecific decompositions using anion attachment MS (756); analysis of steroids in dietary supplements by non-targeted mass spectrometry (757); analysis of anabolic steroids by GC-CI-TQuad-MS (758);

**Tryptamines (see also Mushrooms):** 2013 characterization of AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole) by 1H- and 13C-NMR, GCEI/CI-ion trap-MS, U/HPLC-DAD, and HPLC/MS (759); simultaneous determination of tryptamine analogues in designer drugs using GC/MS and LC-MS/MS (only 5-methoxy-N,N-diethyltryptamine and 5-methoxy-N-methyl-N-isopropyltryptamine were identified in the abstract, among many more) (760); 2015 a review of the use, analysis, and toxicity of tryptamines (only DMT is specifically noted in the abstract) (761); 2016 synthesis of psilocin, bufotenin, serotonin, and various homologues and branched tryptamine derivatives (762); characterization of N,N-diallyltryptamine (DALT), and 2-phenyl-, 4-acetoxy-, 4-hydroxy-, 4,5-ethylenedioxy-, 5-methyl-, 5-methoxy-, 5-methoxy-2-methyl-, 5-ethoxy-, 5-fluoro-, 5-fluoro-2-methyl-, 5-chloro-, 5-bromo-, 5,6-methylenedioxy-, 6-fluoro-, 7-Me, and 7-ethyl DALTs, by NMR, GC/MS, EI/MS, low and high mass accuracy MS/MS, PDA, and GC solid-state IR (763).
1.D - Synthetic Cannabinoids and Cannabimimetics [Notes: Compounds are listed either by their acronym or full name as was specified in their respective abstract - no effort was made to transcribe acronyms to full chemical names or vice versa. Articles that include both synthetic cannabinoids and/or cannabimimetics with other drugs are detailed separately.]

**Individual Synthetic Cannabinoids and Cannabimimetics: 2013**
Identification of (1-(cyclohexylmethyl)-1H-indol-3-yl)(4-methoxynaphthalen-1-yl)methanone by LC/MS and NMR (764); purification and characterization of 3-methyl-6-[3-(trifluoromethyl)-phenyl]-1,2,4-triazolo[4,3-b]pyridazine (CL 218872) by MS, IR, and NMR (765); characterization of JWH-213 by LC-PDA-MS, GC/MS, high-res MS, and NMR (766); analysis of N-[3-(2-methoxyethyl)-4,5-dimethyl-2(3H)-thiazolylidene]-2,2,3,3-tetramethylcyclopropanecarboxamide (A-836339) by LC/MS, GC/MS, highres MS, NMR, and X-ray crystallography (767); identification of [1-(tetrahydropyran-4-ylmethyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone (A-834,735) by LC-ESI-QTOFMS, GC/MS, 1D- and 2D-NMR, and FTIR (768); 2014 an outbreak of exposure to a novel synthetic cannabinoid (abstract not available) (769); analysis of methyl 2-[[1-(5-fluoropentyl)-3-methyl-1H-indol-3-ylcarbonyl]amino]butyrate (770); structural elucidation of a new open chain isomer of the cannabimimetic cyclopropoylindole A-796,260 by NMR and MS (771); determination of HU-210 by HPLC (772); identification of JWH-018 by LC-MS/MS (773);

**2015** isolation and identification of AB-FUBINACA (774); structural elucidation of N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-pyrazole-5-carboxamide (a homolog of AZ-037) by NMR and MS (775); characterization of naphth-1-yl 1-(5-fluoropentyl)-1H-indole-3-carboxylate (CBL-2201) by 1H, 13C, and 15N NMR, FTIR, and GC/MS (776); new monoclonal antibodies specific for 1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole (AM694) (777); identification of N,N-bis(1-pentyl-indol-3-yl-carboxy)naphthylamine (BiPICANA) by LC/MS, HRMS, NMR, and X-ray crystallography (778); analysis of AB-CHFUPYCA [N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrrole-5-carboxamide] by GC/MS, LC/MS, GC/HRMS, and NMR (779); 2016 determination of the absolute configuration of MDMB-CHMICA by vibrational and electronic CD spectroscopy, X-ray crystallog., and HPLC (780); separation and structural characterization of JWH-018-cyclohexyl methyl derivative (NE-CHMIMO) by flash chromatography, GC/MS, IR, and NMR (781); analysis of 3-benzyl-5-[1-(2-pyrrolidin-1-ylethyl)-1H-indol-3-yl]-1,2,4-oxadiazole by GC/MS, GC/HRMS, UHPLC/HRMS2, FTIR, and 1H and 13C NMR (782);

**Multiple Synthetic Cannabinoids and Cannabimimetics:**

[Note: Each year in this subsection is separated by a line space.]
2012 separation and structural characterization of JWH-412 and 1-[5-(fluoropentyl)-1H-indol-3-yl]-(4-methylnaphthalen-1-yl)methanone using GC/MS, NMR, and flash chromatography (783); analysis of cannabinoids by IR, GC/MS, LC/MS, and 1H NMR (784); analysis of CP-47,497-C8 JWH-250, and RCS-4 by TLC, GC/MS, light optical microscopy, and "phytochemical reactions" (785);

2013 analysis of JWH-018, JWH-019, JWH-073, and JWH-250 by GC/MS (786); analysis of 5F-UR-144 and UR-144 by GC/MS, LC-TOF-MS, and 1D-and 2D-NMR (787); an overview of synthetic cannabinoids in South Korea from 2009 to June 2013 (788); analysis of AM-2201, JWH-203, JWH-210 and RCS-4 by LC, high-res MS, LCQTOF-MS, and NMR (789); correlated results from the analyses of synthetic cannabinoids in Turkey from 2010 to 2012 (790); analysis of JWH-019, JWH-081, JWH-203, and JWH-250 by UHPLC-QTOF-MS (791); analysis of 28 (unspecified) "synthetic cannabinoids" by LC/ESI-MS/MS (toxicological focus) (792); isolation of cis- and trans- CP-47,497-C8 (and others not specified in the abstract) - extraction from plant materials by flash chromatography (793); analysis of azepane isomers of AM-1220 and AM-2233, AM-2233, and URB-597 by LC/MS, GC/MS, "accurate MS," and NMR (794); isolation and analysis of 1-butyl-3-(2-methoxybenzoyl)indole and the 2-methoxy isomer of RCS-4 by column chromatography and prep-HPLC, followed by GC/MS, ESI-TOFMS, and 1D- and 2D-NMR (795); a review of the analysis of synthetic cannabinoids on botanical materials (796); analysis of (unspecified) "cannabimimetics" bearing 2,2,3,3-tetramethylcyclopropane-carbonyl moieties by GC/MS, LC/MS, and NMR (797); characterization of some synthetic cannabinoids, derivatives of indole-3-carboxylic acid, by GC-HRMS, UHPLC-HRMS, NMR, and FTIR (798); detection of AB-001, AM-2232, APINACA, N,5-dimethyl-N-(1-oxo-1-(ptolyl)butan-2-yl)-2-(N'-(p-tolyl)ureido)benzamid, (4-ethynaphthyl)-AM-2201 (EAM-2201), 5-fluoropentyl-3-pyridinoylindole, 5FUR-144 (synonym: XLR11), 4-hydroxydiethyltryptamine (4-OH-DET), JWH-213, JWH-307, JWH-030, 4-methylbuphedrone, (4-methynaphthyl)-AM-2201 (MAM-2201), (4-methynaphthyl)-JWH-022 [synonym: N-(5-fluoropentyl)-JWH-122], N-(4-penteny)-JWH-122, UR-144, and URB-754 on plant materials (methods not specified in the abstract) (799); analysis of N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide (AB-PINACA) and N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indazole-3-carboxamide (AB-FUBINACA) by LC/MS, GC/MS, high-res MS, and NMR (800); a pharmacological study of the structural features of synthetic cannabinoids and their in vivo cannabimimetic activity (801); simultaneous determination of JWH-018 and JWH-073 by UFLC (Ultra-Fast LC) (802); analysis of cannabicyclohexanol, JWH-018, JWH-073, JWH-081, JWH-122, JWH-210, JWH-250, and RCS-4 by GC/MS, LC-QTOF-MS, and HPLC (803);

2014 an overview of the emergence, identification, legislation and metabolic characterization of synthetic cannabinoids in herbal incense products (804); chromatographic and mass spectral studies on 6 1-pentyl-acylindoles (regioisomeric synthetic cannabinoids) (805); analysis and
differentiation of substituted 1-alkyl-3-acylindoles (isomeric synthetic cannabinoids) by GC-MS, IR, and some exact mass GC-TOF-MS (806); differentiation of 1-alkyl-3-acylindoles and 1-acyl-3-alkylindoles (isomeric synthetic cannabinoids) by GC MS, and IR (807); a review (808); differences in the GC-EI-MS spectra of JWH-250, JWH-302, and JWH-201 (809); presumptive color-testing of synthetic cannabimimetics by Duquenois-Levine, van Urk, and 2,4-DNPH (810); analysis of AM-2201, JWH-122, JWH-203, JWH-210, and RCS-4 by DART-MS (811); identification and quantification of synthetic cannabinoids by GC/MS and GC/ECD (812); synthesis and biological activities of synthetic cannabinoids (813); structural elucidation, analytical characterization, and identification of [1-(5-fluoropentyl)-1H-indazol-3-yl-(naphthalen-1-yl)methanone, naphthalen-1-yl(1-pentyl-1H-benzo[d]imidazol-2-yl)methanone, and 1-(5-fluoropentyl)-1H-benzo[d]imidazol-2-yl(naphthalen-1-yl)methanone by GC/MS, GC/HR-MS, UHPLC-HR-MS, NMR, and FT-IR (814); identification and analysis of indol-3-carboxylate series and indazole-3-carboxylates (novel cannabinoids) by GC/MS, GC-HRMS, UHPLC-HRMS, NMR, and FTIR (815); analysis of the 6 benzoyl-substituted 1-pentylindoles (isomeric synthetic cannabinoids) by GC/MS and FTIR (816); simultaneous determination of 10 synthetic cannabinoids by HPLC (817);

2015 a retrospective survey of synthetic cannabimimetics in Bulgaria 2010-2013 (818); synthesis and SARs of RCS-4 and its regioisomers and C4 homologue (819); identification of 8-quinolinyl 4-methyl-3-(1-piperidinylsulfonyl)benzoate (QMPSB), MAM-1220, and CHM-081 by GC/MS, LC/MS, and NMR (820); synthesis and spectroscopic analysis of analogues of 1H-indol-3-yl-(2,2,3,3-tetramethylcyclopropyl)methanone and 1H-indol-3-yl(adamantan-1-yl)methanone by NMR, MS, FTIR, and GC-FTIR (821); quantitation of 32 synthetic cannabinoids (dibenzopyrans, cyclohexylphenols, naphthoylindoles, benzoylindoles, phenylacetoylindoles, tetramethylcyclopropylindoles) on plant materials by a validated HPLC/UV method (822); QSARs of 43 cannabimimetic aminoalkylindole derivatives and their metabolites (823); qualitative and quantitative analysis of 2 fluorine containing cannabinoids (XLR-11 and AM-2201) by 19F-NMR, with comparison against GC/MS (824); the variability of active ingredients in Spice within Alaska as an indicator mechanism for manufacture and distribution (825); rapid screening and quantification of synthetic cannabinoids in herbal products with COSY and TOCSY NMR (826); separation of cannabinoids on 3 different mixed-mode columns (827); an overview and review of synthetic cannabinoids (828); differentiation of the positional isomers of JWH-081 by GC-EI-MS and GC-MS/MS (829); identification and quantification of 5-fluoro-AB-PINACA, AB-CHMINACA, AB-FUBINACA, 5-fluoro-PB-22, 5-fluoro-AMB, MDMB-CHMICA, EAM-2201, and STS-135 by GC/MS (830); identification of synthetic cannabinoids by UHPLC-TOFMS and GC/MS (among 32 solutes, only JWH-018 and CP47,497 are identified in the abstract) (831); synthesis and characterization of N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide (3,5-AB-CHMFUPPYCA) and differentiation from its 5,3-regioisomer (832); analysis of ADB-BINACA, AB-FUBICA,
ADB-FUBICA, and AB-BICA by LC-HRMS, GC/MS, and NMR (833); identification and analytical characteristics of 5 new synthetic cannabinoids with an indazole-3-carboxamide structure bearing an N-1-methoxy carbonylalkyl group by GC/MS, GC/HRMS, UHPLC-HRMS/MS, and 1H and 13C NMR (834); a review of synthetic cannabinoids (835); analysis of 1-n-pentyl-3-(1-naphthoyl)indole (JWH-018), three deuterium-labeled analogues, and the inverse isomer 1-naphthoyl-3-n-pentylindole by MS (836); analysis of JWH-018 and its 5 regioisomers by GC/MS (837); separation and detection of cannabicyclohexanol (CCH: cis-isomer), trans-CCH, 5-(1,1-dimethylheptyl)-2-[(1R,3S)-3-hydroxycyclohexyl]-phenol (CP-47497), 5-(1,1-dimethylheptyl)-2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl]-phenol (CP-55940), 3-(1,1′-dimethylheptyl)-6aR,7,10,10aR-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol (HU-210), 2-[1R-3-methyl-6R-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenedi ol (CBD), (1-pentyl-1H-indol-3-yl)-1-naphthalenyl-methanone (JWH-018), (1-butyl-1H-indol-3-yl)-1-naphthalenyl-methane none (JWH-073) and 1-(1-pentyl-1H-indol-3-yl)-2-(2-methoxyphenyl)-ethanone (JWH-250) by SFC/MS (838); an overview of illnesses and deaths from abuse of synthetic cannabinoids (839); syntheses and analytical characterizations of 15 N-alkyl-arylcyclohexylamines by GC and HPLC coupled to multiple forms of mass spectrometry, as well as NMR, UV/DAD, and IR (840); characterization of 2 thiazolylindoles and a benzimidazole (potential cannabinoids) by MS, IR, and NMR (841); a review of bioisosteric fluorine in the clandestine design of synthetic cannabinoids (842); identification and quantitation of 5-fluoro-ADBPINACA and MAB-CHMINACA by HRMS, GC/MS, and LC-MS/MS (843); a study on the fragmentation pathways of JWH-018 and JWH-073 (844); determination and identification of synthetic cannabinoids and their metabolites in different matrices by chromatographic, spectroscopic, and spectrometric methods (845);

2016 differentiation of the 6 regioisomeric dimethoxybenzoyl-1-pentylindoles by EIMS and FT-IR (846); a study of the fragmentation of 21 synthetic cannabinoids with an iso-Pr group or a tert-Bu group by EI-Quad-MS and positive ESI-TOF-MS (847); analysis of 22 synthetic cannabinoids, and separately of JWH018 and 9 of its positional isomers, by ultra high performance SFC (848); variation in commercial "smoking mixtures" containing third-generation synthetic cannabinoids (849); identification of 6 synthetic cannabinoids by DART-LTQ ORBITRAP (850); identification of APINACA 2H-indazole analogue, AMPPPCA, and 5F-AMPPPCA by LC-QTOF-MS, GC-TOF-MS, and NMR (851); differentiation of JWH-122 and JWH-210 by GC-EI-MS/MS (852); analysis of 5F-AMB and PX-3 by 1H and 13C NMR, HR-MS/MS, and Raman (853); an overview and review of recent international trends in Spice use (854); analysis of the 2-alkyl-2H-indazole regioisomers of synthetic cannabinoids AB-CHMINACA, AB-FUBINACA, AB-PINACA, and 5F-AB-PINACA (possible manufacturing impurities with cannabimimetic activities) by 1H and 13C NMR, GC/MS, and UV/Vis (855); rapid identification of 10 synthetic cannabinoids by DART-MS and NMR (856); use of a QSAR model to determine the affinity of synthetic cannabinoids to the CB1 receptor
identification and characterization of ADB-BICA, NNL-1, NNL-2, and PPA(N)-2201 by LC-QTOF-MS, GC/MS, FTIR, and NMR (858); determination of 8 synthetic cannabinoids by heat assisted sample introduction and dielectric barrier discharge ionization MS (859); Synthetic Cannabinoids and Cannabimimetics with Other Drugs (except when a minor part of a larger study): 2012 identification of atropine, scopolamine, lysergamide mitragynine, 4-methoxymethcathinone, 3-fluoromethcathinone, JWH-073, JWH-081, JWH-0250, and JWH-0251 in "herbal products" purchased via the Internet in 2009 and 2010 by LC/PDA/MS and GC/MS (860); analysis of CP-47,497, CP-47,497-C8, JWH-018, JWH-073, JWH-200, MDPV, mephedrone, and methylone by UHPLC/TOFMS (861); 2013 a review, including a comparison of the natural and synthetic cannabinoid materials (862); identification of ADB-FUBINACA, ADBICA, AM-2201 4-methoxynaphthyl analog, APICA N-(5-fluoropentyl) analog, APINACA N-(5-fluoropentyl) analog, JWH-122 N-(5-chloropentyl) analog, QUPIC, QUCHIC, and UR-144; N-(5-chloropentyl) analog (alpha-pyrrolidinovalerothiophenone (alpha-PVT) and 3,4-dichloro-N-((1-(dimethylamino)cyclohexyl)methyl)benzamide (AH-7921) also identified) (863); an overview of Psilocybe mushrooms, 5-MeO-DIPT, tryptamine, MDMA and related compounds, synthetic cannabinoids, and cannabimimetics (864); 2014 analysis of piperazine derivatives (BZP, MPMP, TFMPP), cathinone derivatives (N-ethylcathinone, bathylone, ethylone, methylone, buphedrone, flephedrone), pyrovalerone derivatives (MDPV, naphyrone), and synthetic cannabinoids (AM-694, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-250), by GC-EI-MS (865); determination of AM-2201, JWH-018, JWH-022 JWH-073, JWH-122, JWH-203, JWH-210, JWH-250, HU-210, RCS-4, THC, and various metabolites by UHPLCMS/MS (866); analysis of cocaine, methylone, 4’-methylethcathinone, 3,4-MDPV, JWH-210, JWH-250, and JWH-203 by ion mobility-TOF-MS (867); analysis of a mixture of diphenidine and 5-fluoro-AB-PINACA (868); 2015 an overview of cannabis vs. synthetic cannabinoids (869); an overview of synthetic cathinones and cannabinoids (870); a review of a major researcher's 50 years of research on cannabinoids, with future-looking comments (871); analysis of synthetic cathinones and cannabimimetic agents by MS, LC/MS, LC-MS/MS, NMR, IR, and DART-MS (872).

1.E - Polydrug A: Mixed or Unrelated Individually Named Compounds or Substances

[Note: Each year in this subsection is separated by a line space.] 2012 analysis of cocaine, heroin, and MDMA by spectral fluorescence (873); use of a modified multiwall carbon nanotubes paste electrode for simultaneous voltammetric determination of morphine and diclofenac in biological and pharmaceutical samples (874); an extended overview

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and review of "date-rape" drugs (GHB, MDMA, flunitrazepam, and ketamine) (875);

2013 detection of flunitrazepam, ketamine, and MDMA by IMS (toxicological focus) (876); analysis of methoxetamine, 3-methoxyeticyclidine, and 3-methoxyphencyclidine by GC- and CI-MS, NMR, and HPLC-DAD-ESI-MS/MS (toxicological focus) (877); identification of 1,4-benzobenzodiazepines (clonazepam, flurazepam, alprazolam, midazolam, bromazepam, chlordiazepoxide, lorazepam, and diazepam) and antidepressants (bupropion, sertraline, paroxetine, and fluoxetine) as adulterants in phytotherapeutic dieting formulations by voltammetry (878); differentiation of anorexics (amfepramone, fenproporex, sibutramine), benzodiazepinic anxiolytics (clonazepam, flurazepam, alprazolam, midazolam, medazepam, chlordiazepoxide, diazepam), antidepressants (bupropion, fluoxetine, sertraline, paroxetine), diuretics (hydrochlorothiazide, furosemide, chlortalidone, amiloride, spironolactone), and hypoglycemics (glimepiride, chlorpropamide, glibenclamide) by a solid state electrochemical method (879); analysis of tramadol and morphine by spectrofluorimetry and spectrophotometry (880); determination of morphine, nalbuphine, and "naltrexone drugs" in bulk and pharmaceutical formulations by a kinetic spectrophotometric method (881); determination of tramadol, morphine, nalbuphine and naltrexone analgesic drugs using potassium permanganate and spectrophotometry (882); determination of 13 sedative-hypnotics in health foods (including phenobarbital, estazolam, and diazepam) by HPLC-MS/MS (883); detection of lidocaine, diazepam, and ketamine as adulteration in foodstuffs and beverages by HPLC (884); analysis of methaqualone, saccharin, paracetamol, and phenacetin in illicit drugs by HPLC (885); an overview of the analyses of BZP, mephedrone, JWH-018, TFMPP, sage poet, kratom, fly agaric, kava-kava, and others (886); determination of 4 cathinones (mephedrone, butylone, 4-Me-PPP, and 4-MEC) and 5 tryptamines (5-EtO-DPT, 5-EtO-DALT, 5-EtO-MIPT, 5-EtO-ALCHT, and 5-EtO-2-MALET by ESI-AP-Ion Mobility-TOF-MS (887); identification of kratom, 2C-C-NBOMe, 25I-NBOMe, RH-34 and UR-144, 2-(2,3-dimethoxyphenyl)-N-(3,4,5-tri-methoxybenzyl)ethanamine (DMA-NBTOMe), acetylated 25I-NBOMe, acetylated DMA-NBTOMe by GC/MS and NMR (888); analysis of barbital, clozapine, chlordiazepoxide, midazolam maleate, phenobarbital, perphenazine, promethazine HCl, chlorormezanone, nitrazepam, amobarbital, oxazepam, secobarbital sodium, estazolam, lorazepam, clonazepam, alprazolam, diazepam, and triazolam by UHPLC with PDA detection (889); analysis of alprazolam, estazolam, clonazepam, diazepam, phenobarbital, midazolam maleate, triazolam, nitrazepam, barbital, secobarbital, chlordiazepoxide, lorazepam, amobarbital, and oxazepam by UHPLC with PDA detection (890); analysis of mephedrone, 5,6-methylenedioxy-2-aminoindane (MDAI), and MDMA by SERS on copper coins coated with deposited silver (891); detection of 6 chemical constitutes illegally added into health foods for dieting by UPLC-MS/MS (only sibutramine HCl and phenolphthalein were identified in the abstract) (892); identification of undeclared synthetic drugs (ranitidine, orphenadrine citrate, piroxicam, and dexamethasone) in medicines illegally sold as phytotherapies by diffusion-ordered NMR spectroscopy and

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HPLC-UV-SPE-NMR (893); the longterm stability of 4-MEC, MDAI, methoxetamine, 5-MeO-DALT, 6-APB, MPA, 5-IAI, MDAT, 2-AI, AMT, 25C-NBOMe, AH-7921, 5-MAPB in blood and plasma, as determined by HPLC/DAD, LC-MS/MS, and UHPLC-Q-TOF-MS (894); determination of dextromethorphan and levomethorphan in heroin by enantioselective HPLC and electronic CD (895); identification of sibutramine HCl, fenfluramine HCl, phenolphthalein, strychnine, ephedrine HCl, and hydrochlorothiazide in health foods with weight reducing properties by TLC and HPLC-MS/MS (896); a survey of 449 "legal highs" seized in Poland between mid-2008 and mid-2011 (including MPDV, caffeine, butylone, TFMPP, lidocaine, 4-MEC, mephedrone, pFPP, BZP, and MDPBP, and others) (897);

2014 analysis of 4-fluoroamphetamine, methiopropamine, ethcathinone, 4-methylethcathinone, N-ethylbuphedrone, ethylphenidate, 5-MeO-DALT, dimethocaine, 5-(2-aminopropyl)benzofuran, and nitracaine by a Selective Reagent Ionisation-TOFMS (898); trends in Irish street-level heroin and cocaine 2010-2012 (899); terahertz detection of ketamine and ATSs (900); an overview of the presence of mephedrone, 4-methylethcathinone, BZP, MDPV, TFMPP, methoxetamine, 4-fluoromethcathinone, 4-methylamphetamine, PMA, methylene, PMMA, napryrone, alpha-methyltryptamine, butylone, MDAI, desoxypipradrol, D2PM, MPA, synthetic cannabinoids, 2-AI, 5-IAI, 5-MeODALT, MDPBP, 5/6-APB, pentedrone, and pentyline in post-mortem and criminal casework (toxicological focus) (901); analysis of 2-aminopropylbenzofuran with 4 potential positional isomers, methiopropamine, and 2-(ethylamino)-1-(4-methylphenyl)pentan-1-one by GC/MS and NMR (902); analysis of alprazolam and fluoxetine by UV/Vis (903); a review on detecting residues of chlorpromazine and diazepam in foods (904); identification of ephedrine, caffeine, furosemide, fenfluramine, phenolphthalein, sibutramine, N-desmethyl sibutramine, and N-didesmethyl sibutramine in weight controlling health food by UHPLC/DAD (905); analysis of bromazepam, flunitrazepam, fluoxetine hydrochloride, clozapine, and risperidone by TLC (906); analysis of cocaine, LSD, levamisole, papaverine, and others by MALDI-HRMS, HPLC/DAD, and Quad-MS (907); analysis of cocaine, heroin, methamphetamine, oxycodone, and amphetamine on currency by LC/MS (908); syntheses, characterization, and in vitro metabolism of nitracaine, methoxypiperamide and meptetramine (909); analysis of MDMA and mCPP by CE (910); determination of the stability in solution of 4-MEC, MDAI, methoxetamine, 5-MeO-DALT, 6-APB, MPA, 5-IAI, MDAT, 2-AI, AMT, 25C-NBO Me, AH-7921, and 5-MAPB by HPLC-DAD, LC-MS/MS, and UHPLC-Q-TOF-MS (911); analysis of amphetamine, methamphetamine, MDMA, N,N-dimethylamphetamine, PMA, PMMA, BZP, TFMPP, mCPP, and MeOP by DESI-MS (912); analysis of 3-methylmethcathinone, methylone, butylone, 4-methylethcathinone, flephedrone, methylenedioxypyrovalerone, pentedrone, methoxetamine, APINACA, AKB48, benzydamine, meta-chlorophenylpiperazine, 5-MeO-DALT, 5-MeOMIPT, 6-APB, 4-APB, diphenidine, and others, by single quadrupole GC/MS, positive ESI-LC/HRMS, and NMR (913); determination of lidocaine, ketamine, and diazepam in foodstuffs using micellar LC (914);
analysis of amphetamine, methamphetamine, caffeine, paracetamol, and theophylline by HPLC (915); identification of the piperazine derivative MT-45 (I-C6), the synthetic peptide Noopept (GVS-111), the synthetic cannabinoid A-834735, 4-methoxy-α-PVP, and 4-methylbuphedrine (analytical methodologies not provided in the abstract) (916); analysis of FUB-PB-22, 5-fluoro-NNEI indazole analog (5-fluoro-MN-18), AM-2201 indazole analog (THJ-2201), XLR-12, 5-fluoro-AB-PINACA, 5-chloro-AB-PINACA, AB-CHMINACA, and 5-fluoro-AMB; DL-4662, α-PHP, 4-methoxy-α-POP, 4-methoxy-α-PHP, and 4-fluoro-α-PHP; 2-(2-ethylaminopropyl)benzofuran (2-EAPB), nitracaine, diclofensine, diphenidine, 1-benzylpiperidine, and acetyl-fentanyl (analytical methodologies not identified in the abstract) (917); analysis of mixtures of methamphetamine, MDMA, and ketamine by GC/MS and GC/FID (918); 2015 analysis of dextromethorphan, 2-aminoindane, and lidocaine using handheld NIR, Raman, and FTIR/ATR instruments (919); examination of "third hand smoke" from cocaine and methamphetamine as a source of recoverable trace evidence (920); detection of cocaine and ketamine by paper microfluidic devices (921); qualitative, quantitative, and temporal study of cutting agents for cocaine and heroin confiscated in western Switzerland from 2006 to 2014 (analytical methodologies not identified in the abstract) (922); detection of cocaine, phytocannabinoids, nicotine, caffeine, and others in the air by collection on filters with analysis by GC/MSD (923); detection of nicotine, caffeine, cocaine, cannabinol, cannabidiol, and THC on particulates in indoor air (analytical methodology not identified in the abstract) (924); trends from 2002 to 2013 in the diversion and abuse of oxycodone, hydrocodone, hydromorphone, fentanyl, morphine, and tramadol (925); validation of a GC/FID for the quantitation of cocaine and heroin (926); analysis of various NPSs, including "Synthacaine" (purported to be a mixt. of methiopropamine (MPA) and dimethocaine, but instead containing MPA and benzocaine), two positional isomers of (2-aminopropyl)-benzofuran (5-APB and 6-APB), 2-amino-1-(4-bromo-2,5-dimethoxyphenethyl)ethaneone (bk-2C-B), and 2-(ethylamino)-1-(4-methylphenyl)-pentan-1-one (MEAP) (analytical methodologies not identified in the abstract) (927); analysis of two component mixts. of morphine-papaverine and acridine-papaverine by TLC-IMS (928); identification of brodifacoum, black tar heroin and its impurities (morphine, codeine, noscapine, papaverine, and monoacetyl-morphine), crack cocaine, and 1-methylaminanthraquinone by an atmospheric solid analysis probe interfaced to a linear ion trap-MS (929); analysis of 25H-NBOMe, 25D-NBOMe, 25E-NBOMe, 25I-NBMD, RH34, escaline, 5-DBFPV, 3,4-MDPHP, 3,4-dimethyl-NEB, 3,4-dimethyl-α-ethylaminopentiophenone, 3,4-dimethyl-α-PVP, 4F-α-ethylaminopentiophenone, bk-IVP, bk-IBP, MMXE, 25INBOMe, ADB-CHMINACA, 5F-ADB, and butane-1,4-diol by GC/MS, HRMS, and NMR (930); determination of amphetamine, cocaine, methadone, diazepam, methylphenidate, oxazepam, tramadol, morphine, buprenorphine, and 6-monoacetyl-morphine by SERS (931); determination of 22 drugs of abuse and transformation products in airborne particulate matter by pressurized liquid extraction followed by LC-MS/MS (cannabinol, cocaine, and methamphetamine were the most abundant cmpds; the other 18 cmpds were not identified in the abstract) (932); detection of THC, methamphetamine, and amphetamine at low
ppb level in air using a field asymmetric IMS microchip sensor (933); use of paper microfluidic devices for presumptive identification of cocaine, opiates, ketamine, various phenethylamines, and others (934); detection of phytocannabinoids, cocaine, lidocaine, and nicotine by ESI-FT-ICR-MS (with comparison against the fast blue B colorimetric test (935); use of fluorescent d10 metal complexes for the presumptive identification of cocaine, PCP, diphenhydramine, and benzylpiperazine (936); determination of benzodiazepines and zolpidem in water samples (using polypropylene tubes as single-use and low-cost sorptive extraction materials) (937); determination of phentermine, phendimetrazine, phenmetrazine, fenfluramine, benfluorex, mephentermine, fencanfamine, sibutramine, sildenafil, vardenafil, and tadalafil in food supplements by LC-HR-MS (938); analysis of venlafaxine, escitalopram, fluoxetine, candesartan, risperidone, trihexyphenidyl, thioridazine, aripiprazole, and trifluoperazine by UHPLC (939); a review of the published voltammetric and potentiometric methods developed for determination of dextromethorphan and diphenhydramine (940); analysis of FDU-NNEI, AB-CHMINACA, MN-18, N-OHEDMA, dimethoxy-α-PHP (analytical methodologies not identified in the abstract) (941); analysis of methamphetamine, morphine, and codeine by a probe ESI-MS with a discontinuous atmospheric pressure interface (942); determination of barbital, phenobarbital, chloromezanone, amobarbital, zopiclone, melatonin, chlorphenamine maleate, clozapine, zaleplon, zolpidem tartrate, oxazepam, nitrazepam, triazolam, clonazepam, midazolam maleate, diazepam, and olanzapine in traditional Chinese medicines and health foods by HPLC (943); determination of carbamazepine, doxepin, diazepam, lorazepam, amitriptyline, temazepam, oxazepam, and alprazolam in an urban water system (analytical method not identified in the abstract) (944);

2016 use of screen-printed electrodes for quantification of cocaine and THC (945); the persistence of illicit drug smoke residues from cocaine and methamphetamine and their recovery from common household surfaces (946); chemical profiling of cocaine and heroin as a tool to decipher the structure and organisation of illicit drug markets (947); a review of the cutting of cocaine and heroin (948); analysis of mephedrone and MDAI by microcrystalline testing and Raman microspectroscopy (949); use of a fluorescence probe for ketamine and methamphetamine detection without pretreatment (950); IMS response of cocaine, heroin, methamphetamine, MDMA, and THC against environmental background levels (951); the vaporization enthalpy and vapor pressure of fenpropidin and phencyclidine (PCP) at T/K = 298.15 by correlation GC (952); simultaneous determination of morphine and naltrexone by HPLC (953); analysis of 5-MAPDB, 5-AEDB, MDMA methylene homolog, 6-Br-MDMA, and 5-APB-NBOMe by LC-QTOF-MS, GC/MS, and NMR (954); sorption of ionized pharmaceutical and illicit drugs to a mixed-mode coated microsampler, including amphetamine, amitriptyline, promazine, chlorpromazine, trifluromazine, difenzoquat, 8 basic pharmaceutical and illicit drugs (MDMA, atenolol, alprenolol, metoprolol, morphine, nicotine, tramadol, verapamil, 3 neutral benzodiazepines (diazepam, temazepam, and oxazepam), and diclofenac (955); analysis of a mixt. of cocaine, MDA, and MDMA by single analyzer precursor scanning using an ion trap (956); analysis of the
phenethylamine derivative 2-(4-iodo-2,5-dimethoxyphenyl)-N-[(3,4-methylenedioxyphenyl)-methyl]ethanamine (25I-NB34MD) and the piperazine derivative 1-(3,4-difluoromethylene-dioxybenzyl)piperazine (DF-MDBP) by LC/MS, GC/MS, HRMS, and NMR (957).

------- Next Section Moved Up to Reduce Deadspace -------

2. Instrument Focus

Forensic Chemists must maintain familiarity with updates in current instrumental techniques and become versant in new, improved methods of analysis. Improved/existing and new technologies are reviewed and applied to both routine and specialized analyses of drugs. In cases where improved performance is observed, case reports are generated for the forensic community.

2.A - Polydrug B: Mixed or Unrelated Groups of Compounds or Substances Named Groups of Compounds: 2013 analysis of 277 "selected" synthetic cannabinoids and cathinones, amphetamines, natural cannabinoids, opioids, cocaine and other "important drugs of abuse" by UHPLC-HR-TOFMS (toxicological focus) (958); analysis of cathinones, phenethylamines, tryptamines, and piperazines by LCQQQ-MS/MS in the MRM mode (toxicological focus) (959); 2014 analysis of various phenethylamines, cathinones, synthetic cannabinoids, and tryptamines by IMS (compounds not identified in the abstract) (960); an overview and literature review of synthetic cannabinoids and synthetic cathinones (961); a review of the analysis of (unspecified) "psychostimulants" by TLC (962); qualitative analysis of 34 synthetic cannabinoids and synthetic cathinones by GC-triple quadrupole-MS/MS (963); screening and identification of cathinones, synthetic cannabinoids/cannabimimetics, and phenethylamines by UHPLC with DAD and MS detection (964); a review of the analysis of of (unspecified) "anesthetics" by TLC (965); identification of 61 different psychoactive substances (predominantly substituted phenethylamines, cathinones, tryptamines and synthetic cannabinoids) by LC-chemiluminescence-nitrogen detection (966); analysis of morphine and a series of adrenergic phenolic amines (not identified in the abstract) by chemiluminescence detection on 3D-printed and CNC milled flow-cells (967); crossreactivity of 24 phenylethylamines (including 8 cathinone derivatives), 3 piperazines, and 3 tryptamines in commercial enzyme-linked immunosorbent assays (968); chiral analysis of seven benzo-furys, four cathinones, two diphenidines, ethylphenidate, methiopropamine, and thiothinone by CE (969); an overview of the appearance and evolution of cannabimimetics and cathinones (970); 2015 characterization of 25INB2OMe, 25I-NB3OMe, 25I-NB4OMe, 25I-NB2B, 25I-NB3B, 25I-NB4B, their 5-methoxytryptamine counterparts, and 6 meta-substituted N-benzyl derivs. of 5-methoxytryptamine (CF3, F, CH3, Cl, I, SCH3), by GC/ion trap-MS in both EI and CI modes, LC/DAD, IR, ESI-QTOF-MS/MS, and Triple-Quad-MS/MS (971); analysis of 11 phenethylamines and cathinones by 1H-NMR, COSY, TOCSY, and DOSY.
(972); an overview of synthetic cannabinoids and designer cathinones (973); regioisomeric and enantiomeric analyses of 24 designer cathinones and phenethylamines using UHPLC and CE with added cyclodextrins (compounds not identified in the abstract) (974); a review of the detection methods (including covering colorimetric detection, immunochem. assays, GC/MS analyses, and LC/MS) for synthetic cannabinoids and cathinones (975); cross-reactivity of 2,5-dimethoxyamphetamine, “2C” cmpds (2,5-dimethoxyphenethylamines), β-keto amphetamines, substituted amphetamines, piperazines, α-pyrrolidinopropiophenones, tryptamines and PCP analogs on five commercial immunoassay screening kits (976); 2016 separations of barbiturates, sulfonamides, nucleic bases, and nucleosides on polymethacrylate zwitterionic monolithic micro-columns in 2D-LC (977);

Abused Substances Illegally Added to Licit Pharmaceuticals, Herbal Medications, Health Supplements, and Foodstuffs (Notes: A) Specific, named compounds are compiled in their individual categories above; B) There are many dozens/hundreds of highly repetitive articles pertaining to adulteration of Chinese foods, food seasonings, health care supplements, sexual enhancement aids, Chinese Traditional Medicines, etc.; only a select six of these are included below): 2012 analysis of for anorexigenic, benzodiazepinic, and antidepressant drugs in phytopharmaceuticals by GC/MS (978); 2013 detection of undeclared synthetic drugs in traditional herbal medicines, using LC-MS/MS, GC-MS/MS, and similar techniques (979); standardless 1H-NMR determination of a "wide range of" pharmacologically active substances in dietary supplements and medicines (only mesterolone is specifically mentioned in the abstract) (980); 2014 detection of 35 illegally added steroid compounds in foods and dietary supplements by LC-MS/MS (981); detection of 29 weight loss compounds in foods and dietary supplements by LC-MS/MS (982); a review of the determination of pharmacologic adulterants in herbal-based pharmaceuticals by CE (983); rapid identification of 22 drugs illegally added into sleep-improving health foods by UHPLC-TOF-MS (984); 2015 simultaneous analysis of 28 narcotic adulterants (not identified in the abstract) used in dietary supplements by LC-MS/MS (985); analysis of 24 sedative-hypnotic drugs (not identified in the abstract) illegally added into health foods, by UPLC-ESI-QTOF/ MS (986); screening of 24 sedative hypnotics illegally added to "improving sleep" health foods by HPLC-ion trap-MS (987); determination of 36 chemicals added into traditional Chinese medicines and health care products by UPLC-MS/MS (988); substitute reference substance and secondary mass spectral libraries for rapid screening of sedative hypnotic drugs illegally added to Chinese drugs and health products by HPLC-DAD and HPLC-MS/MS (989); an overview and review of alkaloids in foods (990); determination of caffeine and adrenergic stimulants in food supplements by HPLC/DAD (991); identification of chemical substances illegally adulterated in traditional Chinese medicines and health foods by physico-chem. anal., TLC, HPLC, LC/MS, GC/MS, CE, ion mobility chromatog., IR, NIR, Raman, and LC-MS/MS (992); 2016 a comprehensive strategy to detect the fraudulent adulteration of herbs by FTIR and chemometrics, as well as LC-HRMS
Abused Drugs and Pharmaceuticals in Surface Waters and Municipal Wastewater Streams:

[Note: Each year in this subsection is separated by a line space.]

2012 analysis of sewage in the Brazilian Federal District as a means for estimating cocaine consumption (analytical method not specified in the abstract) (998);

2013 a study of the uncertainty associated with the estimation of community illicit drug consumption via analysis of sewage (999); analysis for mephedrone, methylene, MDPV, BZP, TFMPP, methcathinone, and MDMA in sewage in Adelaide, Australia, by SPE-LC-MS/MS (1000); a review of drugs of abuse in waters and wastewaters: occurrence, analysis, and forensic applications (1001); detection of of pharmaceuticals and "food additives" in sewage by SPE-LC-MS/MS (1002); by online-SPE-LC/MS (1003); analysis for 25 different drugs in wastewater by solid phase extraction and GC/MS (1004); detection of of illicit drugs in wetlands water by LC/MS (1005); analysis of wastewater in Finland for abused drugs and opioids, using SPE and LC-MS/MS (1006);

2014 identification and quantification of trace concns. of pharmaceuticals (caffeine, prazosin, enalapril, carbamazepine, nifedipine, levonorgestrel, simvastatin, hydrochlorothiazide, gliclazide, diclofenac-Na, and mfenamic acid) in surface waters, by LC-TOF/MS (1007); removal efficiencies of cocaine, amphetamine, methamphetamine, THC-COOH, benzylecgonine, MDMA, ketamine, heroin, and other drugs at a wastewater treatment plant (analytical methodology not identified in the abstract) (1008); determination of stimulants, hallucinogens and their metabolites, opioids, morphine derivs., benzodiazepines, antidepressants, and others in wastewaters in England (analytical methodologies not identified in the abstract) (1009); population normalization using ammonium in wastewater-based epidemiology, and its application to illicit drug monitoring (benzoyl ecoinone, THC-COOH, cocaine, and 4-hydroxy-3-methoxymethamphetamine are named in the abstract) (1010); use of a cavitand-grafted silicon microcantilever as a universal probe for illicit and designer drugs in water (1011); determination of amphetamines, MDMA, cocaine, opioids, cannabis, and ketamine, and their major metabolites, in urban wastewaters by UHPLC-MS/MS (1012); determination of 1525 micro-pollutants and transformation products in wastewater by LC-QTOF-MS with an accurate-mass
database (1013); survey of the occurrence of pharmaceuticals in Spanish drinking waters (1014); highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by LC-MS/MS (1015); screening of illicit and licit drugs in waters in Colombia by LC-QTOF-MS (1016); determination of 21 acidic pharmaceuticals and personal care products in the Turia River Basin, Spain by LC/MS-MS/ESI-NI (1017); a selection of papers from the first international multidisciplinary conference on detecting illicit drugs in wastewater (1018); evaluation of illicit and licit drug consumption based on wastewater analysis in Fort de France urban area (Martinique, Caribbean) (1019); determination of nalbuphine, naltrexone, morphine, and tramadol by a bromatometric assay (1020); a review of the analysis of chiral pharmaceuticals in the environment (wastewater) by chiral chromatography coupled with mass spectrometry (1021); a sampling method for detecting analgesics, psycholeptics, antidepressants, and illicit drugs in aquatic environments in the Czech Republic (1022); quantification of (unspecified) target drugs in different wastewater samples by a validated SPE/LC-MS/MS method (1023); the ecotoxicity and contribution to the environmental hazard of pharmaceuticals in hospital wastewater (1024); use of columns containing sand and undisturbed fine-grained sediments to simulate injection of wastewater contg. caffeine, methamphetamine, and acetaminophen into a septic system, leaky sewer, or landfill (1025); a review of the determination of pharmaceuticals and illicit drugs in waters by LC-HRMS (1026); communal assessment of drugs of abuse and identification of their transformation products by analysis of sewage/wastewater by online SPE-LC-HRMS (1027); estimation of illicit and pharmaceutical drug consumption estimated via wastewater analysis (1028); a review of the occurrence, effects, and methods for detection of antibiotics and illicit drugs in the environment (1029); determination of cocaine, benzoylcegonine, ecgonine methylester, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 6-monoacetylmorphine, amphetamine, methamphetamine, ecstasy, mephedrone, methylenedioxypyrovalerone, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, ketamine, and norketamine in sewage (analytical method not identified in the abstract) (1030); an overview of international management trend of pharmaceuticals and personal care products in water environments (1031); the transformation products of illicit drugs in the aquatic environment (1032); estimation of amphetamine and methamphetamine use through sewage-based analysis (1033); determination of pharmaceuticals and personal care products in a mesoscale subtropical watershed and their application as sewage markers (1034); comparison of illicit drug use in three selected towns in Slovakia by wastewater analysis (1035); identification of contaminants in water by UHPLC-QTOF-MS (1036); analysis for ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona, Spain (1037); an overview of organic contaminants in surface water and groundwater in Italy (1038); determination of amphetamines in wastewater by LC-MS/MS (1039); a discussion of the need to develop ethical guidelines for researchers using sewage epidemiol. to monitor drug use in the general population and in specific precincts, including prisons, schools, and workplaces (1040); determination of benzodiazepines, related pharmaceuticals, and metabolites in water by SPE and
LC-MS/MS (1041); using biomarkers in wastewater to monitor community drug use (focus on NPSs) (1042); determination of over 400 priority and emerging pollutants in water and wastewater by SPE and LCTOF-MS (1043); removal efficiencies of amphetamine-type stimulants, cocaine and benzoylecgonine, opioids, codeine, MDA, fentanyl, dihydrocodeine, and heroin at each point of wastewater treatment (analytical methodology not identified in the abstract) (1044); determination of cocaine, benzoylecgonine, propranolol, diclofenac, amitriptyline, carbamazepine, carbamazepine-epoxide, citalopram, metoprolol, carisoprolol, and sertraline in urban streams in Brazil (analytical methodology not identified in the abstract) (1045); systematic screening for common wastewater marking pharmaceuticals in urban aquatic environments (1046);

2015 occurrence and in-stream attenuation of wastewater-derived pharmaceuticals in Iberian rivers, Spain (1047); determination of 4 benzodiazepines (bromazepam, carbamazepine, diazepam, and nordiazepam) and 4 barbiturates (barbital, pentobarbital, phenobarbital, and secobarbital) in river water and wastewater using SPE followed by LC-(ESI)MS/MS (1048); screening for pharmaceuticals and illicit drugs in wastewater and surface waters of Spain and Italy by UHPLC-QTOF-MS and LC-LTQ-Orbitrap-MS (1049); determination of heroin and methadone in wastewater in Lausanne, Switzerland (analytical methodology not identified in the abstract) (1050); fast determination of 40 drugs in water (10 effluent wastewater and 10 surface water samples) using large volume direct injection LC-MS/MS (1051); determination of 10 synthetic cannabinoids, cathinones, piperazines and pyrrolidophenones in wastewater by LC-MS/MS (1052); determination of ketamine and mephedrone in wastewater in 17 cities in Italy, by SPE-LC-MS/MS (1053); methamphetamine and ketamine (analytical methodology not identified in the abstract) (1054); an overview and review of determination of contaminants in water by UHPLC/MS (1055); detection of cocaine and benzoylecgonine (and other drugs) in samples collected from three sewage treatment plants in Cyprus by off-line solid phase extn. followed by LC-MS/MS (1056); screening for more than 1,000 licit and illicit drugs and their metabolites in wastewater and surface waters from the Bogota, Colombia area by SPE followed by UHPLC-QTOF-MS (1057); detection of amphetamines, opioids, cocainics [sic], cannabinoids, lysergics, and their corresponding metabolites by SPE-LC-HR-MS (1058); advances towards a universal screening for organic pollutants in waters, by GC-QTOF-MS and LC-QTOF-MS (1059 and 1060); detection of illicit drugs in raw sewage influents by HRMS (1061); chemometric application of pharmaco-signatures in different aquatic systems (1062); determination of methoxetamine, butylone, ethylone, methylene, methiopropamine, PMMA, and PMA in sewage by LC-ESI-MS/MS (1063); the systematic and day-today effects of chemical-derived population estimates on wastewater-based drug epidemiology (1064); use of a Fenton-like reaction to remove illicit drugs and pharmaceuticals from wastewater (emphasis on methamphetamine and tramadol) (1065); determination of amphetamine and methamphetamine at 10 wastewater treatment plants by LC-HR-MS/MS (1066); detection of methamphetamine,
amphetamine, and codeine in wastewater (analytical methodology not identified in the abstract) (1067); analysis of pharmacologically active compounds in the environment by chiral LC-MS/MS (1068); detection of 4'-methyl-α-pyrrolidinohexanophenone (MPHP), 2-[4-(ethyl-sulfanyl)-2,5-dimethoxyphenyl]ethanamine (2C-T-2; Rosy), 4-methyl-5-phenyl-4,5-dihydro-1,3-oxazol-2-amine (4-MAR), and 1-(4-methoxyphenyl)-2-propanamine (PMA) in raw sewage by HR-MS (location not identified in the abstract) (1069); linking drugs of abuse in wastewater to contamination of surface and drinking water (17 drugs of abuse, including cocaine, several amphetamines, opioid drugs, and 2 metabolites, benzoylecgonine, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (a metabolite of methadone) were investigated; analytical methodology not identified in the abstract) (1070); determination of alcohol and cocaine co-consumption in 2 European cities as assessed by wastewater analysis using LC-MS/MS (1071); wastewater-based epidemiology of stimulant drugs based on analysis of sewage samples from 42 European cities collected daily for one week in March, 2013 (1072); determination of 25 synthetic psychoactive compds., including amphetamine, sympathomimetic substituted amphetamines, synthetic cathinones, and ketamine, in raw wastewater, secondary effluent, and river water by SPE followed by LC-MS/MS (1073); comparison of wastewater analysis and population surveys for use of methamphetamine, MDMA, and cocaine (1074); determination of cocaine and benzoylecgonine in the Esmeraldas watershed in Ecuador (analytical methodology not identified in the abstract) (1075); comparison of population surveys with wastewater analysis for monitoring illicit drug consumption in Italy from 2010-2014 (1076); identification of "a wide range of suspected and unknown compds. in environmental samples" by LC-HRMS (1077); determination of the occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse, and related metabolites in offshore seawater by SPME and LC-MS/MS (1078); determination of pharmaceuticals in coastal systems using SPE and UPLC-MS/MS (1079); use of wastewater-based epidemiology to estimate consumption of methamphetamine, benzoylecgonine, MDMA, methadone, oxycodone, and hydrocodone (analytical methodology not identified in the abstract) (1080); analysis of illicit drugs in wastewater to assess the market share held by criminal groups (1081); determination of amphetamine, methamphetamine, MDMA, and cocaine in 7 locations in Belgium over 2011-2015 (analytical methodology not identified in the abstract) (1082); validation and uncertainties evaluation of an isotope dilution-SPELC-MS/MS for the quantification of drug residues in surface waters (including diazepam and MDMA) (1083); determination of cocaine and benzoylecgonine in environmental samples by newly developed sorbent materials (1084); detection of opioid analgesics, amphetamines, cocaine, heroin, stimulants, anesthetics, sedatives, anxiolytics, designer drugs, phosphodiesterase-5 inhibitors, and amphetamine and methamphetamine drug precursors in wastewaters by LC-MS/MS (1085); quantitative analysis of morphine, oxymorphone, hydromorphone, oxycodone, hydrocodone, and THC-COOH in river and wastewater by UHPLCMSMS with an API/ESI source (1086); source discrimination of drug
residues in wastewater by chiral LC-MS-MS (a case study) (1087); determination of drugs of abuse and alcohol consumption through sewage-based epidemiology among different groups of population on the Greek Island of Lesvos (1088); screening for drugs of abuse in the wastewater in a small college town in Southern Arkansas by GC/MS, GC/FID, and HPLC/MS (1089); screening for NPSs in urban wastewater using HRMS (1090); evaluation of sampling plans for cocaine, methamphetamine, MDMA, and methadone in wastewater (1091); wastewater based epidemiology in Finland (samples analyzed by UHPLC-MS/MS) (1092); estimation of drug abuse in 9 Polish cities by wastewater analysis by HPLC-MS/MS (1093); determination of heroin, cocaine, amphetamine, MDMA, methamphetamine, cannabis, codeine, and methadone in 6 Croatian cities (analytical methodology not identified in the abstract) (1094); correlated results from an Australia-wide wastewater monitoring of cocaine/benzoylecgonine, methamphetamine, and MDMA (analysis by LC-MS/MS) (1095); determination of cocaine, MDMA, and methamphetamine residues in wastewater by LC/MS (1096); common illicit drugs (primarily methamphetamine and ketamine and their metabolites in surface waters) (analytical methodology not identified in the abstract) (1097); removal of psychoactive pharmaceuticals and illicit drugs from wastewaters by zerovalent iron and iron(VI) (1098); determination of cocaine, benzoylecgonine, ephedrine, MDMA, methadone and its metabolite EDDP in Spanish river basins by online SPE-LC-ESI-MS/MS (1099); a review on the stability of illicit drugs in sewers and wastewater samples (1100); analyses for 48 emerging pollutants, including 25 drugs of abuse and metabolites, 17 cytostatic drugs, and 6 iodinated contrast media, in tap water in Madrid, Spain by SPE and LCMS/ MS (1101); effects of time delay between sample collection and extraction of wastewater samples for amphetamine and opioid analysis (1102); quant. analysis of 33 cmpds in a Brazilian coastal zone., including cocaine and benzoylecgonine, by LC-MS/MS (1103); detection and quantification of various opioid compounds (primarily heroin and morphine) in urban wastewater in Cookeville, Tennessee by LC-MS/MS (1104); determination of cocaine, methamphetamine, MDMA, amphetamine, codeine, morphine, heroin, fentanyl, oxycodone, methadone, BZP, TFMPP, methcathinone, methylone, mephedrone, MDPV, alpha-PVP, PMA, 25C-NBOMe, 25B-NBOMe, 25I-NBOMe, and cannabis in Adelaide, Australia for up to 4 years between Dec. 2011 and Dec. 2015 (analytical methodology not identified in the abstract) (1105); determination of metabolites of methamphetamine, cocaine, THC, and heroin by LC/MS (1106); determination of amphetamine-like cmpds., ketamine, cocaine, and opioids in North China (analytical methodology not identified in the abstract) (1107); detection of cocaine in wastewater with DNA-directed immobilization aptamer sensors (1108);

"Novel Psychoactive Substances" (NPSs): 2013 a review of 1320 cases containing one or more of 26 synthetic cannabinoids, 12 designer stimulants, and 5 hallucinogenic-like drugs (1109); an overview of the New Zealand approach to regulated NPSs (1110); 2014 a study of the prevalence and correlates of NPS use amongst a group of regular Ecstasy users in Australia (1111); a review (1112); a review (1113); a report from the European Drug Emergencies
Network on their efforts to improve the knowledge of acute drug toxicity of recreational drugs and NPS (1114); an overview of the effects and risks associated with NPSs (toxicological focus) (1115); an overview of legislation against NPSs in Ireland (1116); identification of NPSs by FTIR, Raman, and GC-IR (1117); an overview of the emerging trends in the abuse of NPSs (1118); an overview (1119); detection and presumptive identification of NPSs by a portable NIR spectrometer (1120); the impact of new retail restrictions and product licensing on the regulated legal market for NPS products in New Zealand (1121); an overview of the high variability of active ingredients concentration, mislabelled preparations, and presence of multiple psychoactive substances in NPS products (1122); 2015 wide-range screening of NPSs by FIA-HRMS (1123); detection and characterization of NPSs by IMS (1124); a review (1125); a brief overview of recent trends (1126); rapid screening of 35 NPSs by IMS and DART-QTOF-MS (1127); a study on the prediction of bioactivity of NPSs (1128); detection of NPSs by SERS (1129); an overview of recent developments in the analysis of NPSs (1130); an overview of NPSs and their impact on forensic science (1131); an overview and review, covering years 2013-2015 (1132); 2016 a proposal for a new categorization of NPSs based on neurobiol. mechanisms of action (1133); an overview of how NPSs are studied, produced, marketed, and controlled (1134); screening for 221 NPSs by infrared and Raman (1135); a review on the screening for NPSs by LC coupled with low-and high-resoln. MS (covering PubMed-listed studies from Jan. 2014 to Jan. 2016) (1136); detection of NPSs in street samples by NIR and chemometrics (1137); an update on New Zealand's legal market for NPSs (1138); an overview of the pharmacology of stimulant and hallucinogen NPSs (1139); a brief overview of the NPS situation in Japan (1140); a brief overview of the analytical challenges posed by NPSs (1141);

"Hallucinogens", "Hypnotics" (and similar generic terms): 2013 editorial remarks against the global prohibition of psychoactive drugs (1142); 2014 a review of the non-medical use of dissociative drugs (1143); a review of the determination of of anxiolytics and sedatives by TLC (1144); 2015 sedative-hypnotic and anxiolytic effects of "lotus leaf alkaloid extract" (the exact species of lotus - there are many - was not identified in the abstract) (1145);

"Illicit Drugs" (including "Controlled Substances," "Drugs of Abuse," "Illicit Drugs," "Narcotics," "Seized Drugs," and similar generic terms): 2012 "drugs of abuse" by Raman (1146); use of spatially offset Raman to detect "illicit drugs" through opaque plastic containers, colored glass bottles, paper envelopes, and clothes (1147); a review on THz time-domain spectroscopy (including THz spectra for "drugs of abuse") (1148); detection of "drugs of abuse" using SERS (1149); application of handheld FTIR and Raman spectrometers for detection of "drugs of abuse" (1150); a review of the analysis of "seized drugs" by UHPLC and UHPLC-MS (1151); a short review of recent advances in analysis of "drugs" (and other substrates) by MS (1152); 2013 an evaluation of the results of impurity profiling of "illicit drugs" from different analytical methods and/or from different laboratories (1153); detection of trace amounts of "illicit
drugs” on surfaces by direct analyteprobed nanoextraction coupled to nanospray ionization-mass spectrometry (1154); detection of "drugs" concealed inside diffusely scattering packaging, including plastic, paper, and cloth, by spatially offset Raman (1155); analysis of "illicit drugs" by ambient pressure thermal desorption ionization MS (1156); rapid screening for 73 "toxic and harmful substances" in foods by UHPLC/MS, with sample cleanup using the QuEChERS system (1157); an overview and review of the analysis of "illegal drug products" (1158); the effects of solvents on the analysis of "drugs" by ESI-MS (1159); a review of the analysis of "law-evading and illegal drugs" using liq.-liq. extn. and GC/MS (1160); a review of CE and CEC methods used for analysis of "drugs" in biological matrices (1161); use of a supramolecular sensor array with two fluorescent receptors to detect "addictive OTC drugs" (1162); analysis of "seized drugs" by LC-ESI/MS/MS and AP-MALDI-MS/MS, with comparisons of the two techniques (1163); detection of "illicit substances" and pharmaceutical counterfeits by nuclear quadrupole and magnetic resonance (1164); annual review of "banned substances" (sports doping focus) (1165); an overview of advanced analytical instrumentation and methods for "drugs of abuse" (toxicological focus) (1166); 2014 screening of textiles for "contraband drugs" using portable Raman spectroscopy and chemometrics (1167); an evaluation of the effectiveness of MS, IR, and portable Raman to analyze commonly encountered drug mixts., as well as "legal highs" (1168); screening of "drugs of abuse" using a commercial paper spray system (1169); detection of "abused drugs" by HPLC (1170); an overview of recent trends in the analysis of "emerging drugs of abuse" (1171); analysis of designer drugs ("bath salts") by Raman and SERS (1172); a review of new designer "drugs of abuse" (1173); a quantitative structure-toxicity relationship of the aquatic toxicity for various "narcotic pollutants" using the norm indexes (1174); 2015 a comprehensive review of the pyrolysis of "drugs of abuse" (1175); analysis of "drugs of abuse" (naturally occurring psychotropic drugs and new designer drugs) by DART-MS (1176); use of diazonium ions for the presumptive testing of "narcotics" containing an activated aromatic ring (1177); screening for "illicit drugs" by direct-heating HSSPME with GC/MS (1178); identification of "abused drugs" by GC/FTIR (1179); an overview and review of "drugs of abuse" and their detection methodologies (1180); determination of "illicit drugs" and their metabolites on banknotes by methanol extn. followed by LC-MS/MS (1181); a review of alkylsilyl derivatization techniques in the analysis of "illicit drugs" by GC (GHB, amphetamines, opiates, and cannabinoids were mentioned in the abstract) (1182); indirect chiral separation of "new recreational drugs" by GC/MS using trifluoroacetyl-l-prolyl chloride (1183); 2016 results of the Trans European Drug Information (TEDI) project (results for cocaine, ecstasy, and amphetamine, plus comments on NPSs detected between 2008 and 2013) (1184); rapid identification of "seized controlled substances" and related compounds by MS/MS without chromatography (1185); screening of "drugs of abuse" using DART-MS (1186); "forensic drug" analysis by chemical derivatization followed by GC/MS and LC/MS (1187); a survey of the qual. distribution of "drugs of abuse" (mostly NPSs) confiscated in Italy between 2013 and 2015 (1188);
**Pharmaceuticals/Counterfeits (with a focus on differentiation of legitimate versus counterfeit products, or for monitoring quality control for legitimate pharmaceutics; see also a significant number of citations concerning counterfeits under Phosphodiestrase-5 Inhibitors, above):**

2012 a review of the detection of counterfeit medications by Raman (1189); 2013 a review of a paper-based test for screening for counterfeits (1190); a general overview of the chromatographic techniques used to characterize counterfeit and illegal pharmaceuticals (1191); an overview of chromatographic and spectroscopic counterfeit detection methods (1192); examination of tablet surfaces by Multimodal DESI-MS imaging to detect counterfeits (1193); detection of counterfeit medications with portable Raman (1194); analysis of pharmaceuticals by Raman (1195); a review on the detection of counterfeit medications, focusing on HPLC and MS, but also discussing color testing, TLC, GC, Raman, NIR, FTIR, and NMR, using antimalarial drugs and sildenafil as illustrative examples (1196); 2014 detection of counterfeit medications with Raman and NIR (1197); confirmational identification of pharmaceuticals via DART-TOF-MS (1198); an overview of pharmaceutical process validation of solid dosage form (1199); 2015 systematic chemical and packaging analysis of counterfeit medications to derive useful intelligence (1200); 2016 an analytical strategy for rapid identification of counterfeit medications (1201); a review of the identification of counterfeit medicines by chemometrics (1202); a comprehensive review on prevalence, detection, and prevention of counterfeit drugs (1203).

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**2.B - Instrument Focus**

**General Overviews and Reviews (and articles covering multiple techniques):**

2014 an overview of forensic drug analyses, including an analytical road-map (1204); an overview of drug testing, covering chem. testing, chromatog., spectroscopy, CE, immunoassay, and IMS (1205); 2015 a review of miniaturized separation techniques for forensic drugs analysis (including CE, CEC, and nano-LC) (1206);

**Color Testing:**

2014 the effect of benzene ring substituents on the mechanism of Duquenois Levine test for (phyto-)cannabinoid detection (1207); detection of pharmaceuticals using paper analytical devices (embedded with various color-testing reagents) (1208); 2015 use of presumptive color tests for NPSs (abstract not available) (1209); the modernization of physical appearance and solution color tests using quantitative tristimulus colorimetry (1210);

**Computerized Tomography (CT):**

2015 dual-energy CT behavior of heroin, cocaine, and typical adulterants (1211); use of CT (and X-ray, ultrasound, and MRI) to detect body packing (1212);

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Electrophoresis (and Related Techniques): 2013 determination of active ingredients and preservatives in pharmaceuticals by CZE (1213); a review of recent advances in electrodriven enantioseparations (listed applications include "pharmaceutical" and "forensic") (1214); 2014 separation of acidic drugs by CEC using both chlorinated and nonchlorinated polysaccharide-based selectors (1215); a comprehensive overview and review (1216); a review of the application of CE techniques in toxicological analysis (1217); a review of recent method developments and applications of CE/DAD to pharmaceuticals (1218); 2015 a review of electromigrative sepn. techniques in forensic toxicol. (1219);

Gas Chromatography: 2013 forensic applications of GC (1220); 2016 a review of the forensic potential of comprehensive 2D-GC (1221); cleanup of complex matrices (containing drugs) by QuEChERS followed by GC analysis (1222);

Hyperspectral Imaging: 2013 development of a handheld widefield hyperspectral imaging (HSI) sensor for standoff detection of explosive, chemical, and narcotic residues (stated applications include "locating production facilities of illegal drugs") (1223);

Infrared Spectroscopy: 2013 use of IR spectral imaging for drug quality control (1224); 2014 analysis of varied substrates by FTIR spectroscopic imaging (1225); use of a handheld near IR spectrometer for the classification of 140 different substances, including cocaine, heroin, oxycodone, diazepam, synthetic cathinones, and synthetic cannabinoids (1226);

Ion Chromatography: 2012 ion chromatographic analysis of pharmaceuticals to determine authenticity and adulteration (listed applications include "forensic analysis") (1227); 2014 a review of ion chromatography-mass spectrometry (1228);

Ion Mobility Spectroscopy: 2014 use of DESI-AP-IMS for drug detection (1229);

"Lab-on-a-Chip" (Microfluidics): 2011 the use of microfluidic platforms for solid form screening of pharmaceuticals by Raman (1230); 2013 an overview of "forensic drug analysis" by microfluidic devices (1231); 2014 enhancement of chemiluminescent detection in microfluidic systems, for anal. of a wide range of compds., including illicit drugs and pharmaceuticals (1232);

Liquid Chromatography: 2012 an overview of good laboratory practices for HPLC (1233); an overview of some of the most recent applications of hyphenated LC techniques for forensic analyses (1234); 2013 quantitative structure-retention relationships models for prediction of HPLC retention time of small molecules (1235); 2014 use of immobilized polysaccharide-based stationary phases for enantioseparation in normal versus reversed phase HPLC (1236); a review of the use of chiral supercritical fluid chromatography for analysis of pharmaceuticals and drugs
of abuse (1237); a review of HILIC, discussing the development, basic sepn. mechanisms, stationary and mobile phases, and summarizing its applications in several research fields (1238); 2015 a chemometric approach to improve the accuracy and precision of quantitation in 2D-LC with dual detectors and multivariate curve resolution (1239); simultaneous determination of hydrophobicity and dissociation constant for 161 drugs by gradient RP-HPLC/MS (1240); HPLC method development using structure-based database search, physico-chemical prediction, and chromatographic simulation (1241); 2016 automated screening of reversed-phase stationary phases for small-molecule separations using LC/MS (emphasis on LC) (1242); simulation of elution profiles under gradient elution conditions, with mismatched injection and mobile phase solvents (includes simulated sepn. of selected amphetamines) (1243);

**Mass Spectrometry:** 2012 the mass spectra of designer drugs (reference text) (1244); 2013 use of Desorption Electro-Flow Focusing Ionization of explosives and narcotics for ambient pressure mass spectrometry (the "narcotics" included cocaine; no others were listed in the abstract) (1245); a review of DART-MS (1246); a review of ultrasensitive MS of organic molecules (listed applications include "forensics") (1247); a review of ambient MS, including DESI, DART, and extractive ESI (listed applications include "forensic identification") (1248); an evaluation of standardized software for processing GC/MS data from different instruments (1249); the application of ultra-fast triple quadrupole LC-MS/MS for forensic analysis of "abused drugs" (1250); a review of DESI-MS (listed applications include "illicit drugs") (1251); evaluation and testing of an alternative search algorithm for compound identification using the Wiley Registry of Tandem Mass Spectral Data, MSforID (1252); mass spectrometry using Matrix Assisted Ionization in vacuum (1253); 2014 recent advances in forensic drug analysis by DART-MS (1254); 2015 a review of surface-assisted laser desorption ionization (SALDI) MS for forensic analysis (1255); a review of forensic mass spectrometry (1256); a wide use target screening system for GC/MS (1257); a new quant. contained-electrospray process for ESI-MS (1258); the use of the partial least squares method to model the positive ESI response produced by small pharmaceutical molecules (1259); a review of the characterization of synthetic and natural product pharmaceuticals by functional group analysis using ESI-ion trap-MS (1260); the use of online chemistry databases to facilitate structure identification (1261); a review of identification criteria and complicating factors for drug confirmation by mass spectrometry (1262); 2016 a review of the applications of ambient mass spectrometry to forensic chemistry (1263); a review of DART-MS (1264); determination of trace palladium in chemical bulk drug by ICP-MS (1265); a review of DART-MS (1266);

**Microextraction Techniques:** 2013 a review of liquid phase micro-extraction (LPME) techniques used in analysis of Chinese traditional medicines (1267); a review (listed applications include forensic and pharmaceutical) (1268); 2015 a review of SPME techniques (1269); 2016 a review of coupling SPME with ambient MS (1270); a review of microextraction in forensic
toxicology (1271); an overview of microextraction techniques for illicit drug testing (1272);

**Microscopy and Microscopic Instrumental Techniques:** 2013 comparison between microcrystalline tests performed on microscope slides versus flat capillary tubes (1273); 2014 a review of developments in applications of FTIR microspectroscopy, covering 2005 to 2013 (1274); 2015 use of an FTIR/ATR microscope for detecting analytes in high-interfering matrixes and in products with unknown ingredients (illicit tablets, counterfeit tablets, and unknown powders) (1275);

**Nuclear Magnetic Resonance Spectroscopy:** 2013 tracking authentic pharmaceuticals by 2H- and 13C-NMR (1276); 2015 cocaine, MDMA, and "metilona" (possibly methylone?) by "No-D NMR" (i.e., without the use of deuterated solvents) (1277); an overview of a "crime-scene NMR laboratory" (1278); 2016 improving the performance of high-precision qNMR measurements by a double integration procedure (1279); a review of the use of quant. 1H NMR spectroscopy in drug discovery and development (including a review of the pertinent literature between 1963 and 2015) (1280);

**Raman:** 2013 Use of THz-Raman accessing molecular structure with Raman spectroscopy for enhanced chemical identification, analysis, and monitoring (especially for discrimination of polymorphs) (1281); 2014 deep Raman detection with 2D correlation analysis for elucidation of a subsurface component under thick powder or packed contents in a bottle (1282); 2016 a review of the applications of SERS in forensic science (1283);

**Spectrophotometry:** 2014 methods for evaluating the visual limits of color perception (a proposal to create common rules for constructing color test scales for visual colorimetric assays) (1284); the molecular electron ionization cross-section and $\lambda_{\text{max}}$ in the studies of activities of alkaloids (1285); 2015 defining optimal conditions of colors in 3D space in dependence on gamma values, illumination, and background color (1286); 2016 a review of derivative UV-Vis spectrophotometry (1287);

**Stable Isotopes:** 2011 forensic applications (reference text) (1288); 2012 a review of the forensic applications of IRMS (1289); 2013 a review of inter-laboratory comparability of stable isotope data (1290); an extensive review of the isotopic analogies of molecules and minerals (1291); the use of carbon stable isotope ratios in drugs characterization (by IRMS) (1292); global isoscapes for $\delta^{18}$O and $\delta^{2}$H in precipitation (1293); 2014 spatial, seasonal, and source variability in the stable oxygen and hydrogen isotopic composition of tap waters throughout the U.S. (1294); 2015 precipitation isotope ($\delta^{18}$O) zones revealed in time series modeling across Canada and northern U.S. (1295); simple spreadsheet templates for the determination of the measurement uncertainty of stable isotope ratio delta values (1296); a review of IRMS for source
determination (1297);

**Supercritical Fluid Chromatography:** 2016 an evaluation of innovative stationary phase ligand chemistries and analytical conditions for the analysis of basic drugs by SFC (1298);

**Thin Layer Chromatography (and similar Planar Chromatographic Methods):** 2013 an overview, including "forensic applications" (1299);

"Vibrational Spectroscopy" (Raman, mid-, near- and far-IR, and THz Spectroscopy):
2012 a review of the use of IR spectroscopy, terahertz spectroscopy and Raman spectroscopy in forensic sciences (1300); a review of sampling techniques for Raman, mid-, near- and far-IR, and THz spectroscopy (1301);

**X-Ray Techniques:** 2013 the use of energy dispersive X-ray diffraction (ED-XRD) spectra of drugs (and explosives) to detect "body packing" (1302);

**Other:** 2012 trace determination of metals (copper, zinc, nickel, cobalt, iron, arsenic, antimony, bismuth, vanadium, molybdenum, selenium, and lead) in drugs and pharmaceuticals as N-phenyl[1,2 methane fullerene C60]C61 complexes (1303); 2013 the use of gamma detectors in explosives and narcotics detection systems (1304); a review of microfluidic paper-based analytical devices and micro total analysis systems (1305); the utility of cyclodextrins in analytical chemistry (1306); 2014 a review of the use of acidic potassium permanganate as a chemiluminescence reagent (1307); the use of a chiral diffraction grating to measure the enantiomeric excess of a chiral compound (1308); the application of UV laserinduced solid-state fluorescence spectroscopy for characterization of solid dosage forms (1309); 2015 a review of miniaturized separation techniques (1310); a review of the pyrolysis of drugs of abuse (1311); an overview of emerging hyphenated SEMEDX (scanning electron microscopy with energy dispersive X-ray spectroscopy) and Raman spectroscopy systems (1312); a review of capacitively coupled contactless conductivity detection (1313); 2016 a review of enhanced performance separations, covering papers published in *Analytical Chemistry* from late 2014 through May 2016 (1314).
3. Miscellaneous Topics

**Abuse Deterrent Formulations (see also numerous, specific examples under oxycodone and opiates):** 2013 an overview of prescription drug abuse and the need for abuse deterrent formulations (1315); 2014 development and impact of prescription opioid abuse deterrent formulation technologies (1316); the use of prescription opioids with abuse-deterrent technology to address opioid abuse (1317); a review of extended release hydrocodone (1318); the U.S. FDA draft guidance for developing abuse-deterrent opioid analgesics (1319); an overview of anti-drug-abuse measures, including abuse-deterrent formulations (1320); an overview of methods used to reduce abuse potential of commonly abused pharmaceuticals (1321); 2015 an overview of the advance in the R&D of abuse-deterrent opioid analgesics (1322); an overview of abuse-deterrent formulations in countering opioid misuse and abuse (1323); an overview and review of abuse-deterrent formulations (1324); 2016 a comparison of the effectiveness of abuse-deterrent formulations of oxymorphone and oxycodone extended-release drugs (1325); a review and assessment of the potential impact of abuse-deterrent formulations of prescription opioid analgesics (1326); an overview of prodrug technology and its application for developing abuse-deterrent opioids (1327); a review (1328); an assessment of extended release abuse deterrent formulations (1329);

**Anions and Cations:** 2016 a review of the simultaneous separation of cations and anions by CE (1330);

**Bacteria:** 2014 recovery and identification of bacterial DNA from heroin and methamphetamine (1331); 2016 a discussion of a recent increase in drug abusers in Scotland who have presented with Staphylococcus aureus bacteremia with life-threatening complications due to their injection of NPSs (1332); the use of microbe analyses for forensic and criminal investigations (1333);

**Canines:** 2014 the efficacy of drug detection by fully-trained police dogs varies by breed, training level, type of drug and search environment (1334); treatment and prevention of acute poisoning of drug dogs caused by exposure to methamphetamine, ketamine, and MDMA (1335); 2015 a review of the advances in the use of odor as forensic evidence through optimizing and standardizing instruments and canines (1336);

**Clandestine Laboratories - Appraisals and Safety:** 2014 an update on the hazards and health effects assocd. with clandestine drug laboratories (1337); an evaluation of the acute and chronic environmental effects of clandestine methamphetamine waste (1338); adsorption and desorption characteristics of methamphetamine, MDMA, and pseudoephedrine in soils (1339); an overview and discussion of home preparations of abused substances (1340); 2015 vehicle-mounted portable mass spectrometry for covert detection of clandestine methamphetamine laboratories.
(1341); **2016** decontamination of personal protective equipment and related materials contaminated with toxic chemicals (1342);

**Degradation of Drugs and Pharmaceuticals:** **2014** a review of forced degradation and stability indicating studies of drugs (1343); determination of pharmaceutical impurities and degradation products by NMR (1344); **2015** analysis of degradation products from drugs by a rapid resolution LC-collision energy correlated-MS (1345); **2016** a stability-indicating UPLC-MS/MS assay for 1960's era pharmaceuticals in dosage forms (1346);

**Education:** **2013** use of forensic science to teach method development in undergraduate analytical laboratories (1347); the use of paper-based diagnostics with high school students to model forensic investigation and colorimetric analysis (1348); **2014** the use of forensic science and simulated crimes in a one-week long "Criminal Camp" to teach the theory and practice of basic concepts in chem., physics, medicine, and biol. (1349); using education to combat "chemophobia" (1350); a course for non-science majors at a college that looks at the chem. behind the crime itself, and the chem. behind the anal. of evidence from the crime (1351); a discussion for the need for forensic science programs to develop job-related skills in their students (1352); an overview of forensic science (1353); initiation and evolution of a forensic chemistry program (1354); the use of forensic chem.-themed activities to introduce fundamental concepts, such as the scientific method, to middle and high school students (1355); an overview of the chemistry behind forensic science (1356); use of presumptive and confirmatory tests using analogs of illicit drugs as an undergraduate instrumental methods exercise (using multiple color tests, GC-MS, and ATR-FTIR) (1357); utilizing the "CSI Effect" in chemistry instruction (1358); a discussion for the need, development, and implementation of an effective continuing forensic science education program (1359); a discussion of the need for robust and rigorous scientific research in academia based on need-based input from forensic practitioners who see the day-to-day issues in their laboratories (1360); an overview of the Forensic Science Education Programs Accreditation Commission's (FEPAC) accreditation program, the FEPAC stds., and the process involved in seeking FEPAC accreditation (1361); careers in forensic chemistry (1362); using The Poisoner's Handbook in conjunction with teaching a first-term general / organic / biochemistry course (1363); **2015** a universal internet-based prevention program for ecstasy and NPSs (for teenaged students) (1364); a discussion of the efforts to develop integrated forensic platforms that allow for the forensic investigation of human biol. traces, identification of illicit drugs, and the study of digital evidence (1365); a case study review of a problem based learning approach used to educate and train young forensic scientists through the use of six sigma investigative tools (using hydrolysis of cocaine to benzoylecgonine at various pHs as the teaching example) (1366); **2016** a performance task case study (misconduct) for teaching data analysis and critical thinking (1367); using a "Drug of the Week" approach to educate chemistry students about prescription drugs and their abuse (1368); use of a variety of small scenes using doll house
furniture to educate criminal justice majors (1369);

**Immunoassays:** 2014 a review of the practical aspects of immunoassays and their application in clin. chem. for anal. of medicines and drugs of abuse (1370); the use cheminformatics to predict cross reactivity of "designer drugs" to their currently available immunoassays (1371);

**Impurities and Impurity Profiling:** 2012 a review of detection techniques for trace pharmaceutical impurities (1372); 2013 an overview (1373); a review of impurities in pharmaceuticals (1374); comparison of CCC and prep-HPLC for separating minor impurities in drugs (1375); 2014 analysis of impurities in drugs by LC-MS (1376); an overview of impurity profiling of pharmaceuticals (1377); a compendium of techniques for the analysis of pharmaceutical impurities, including TLC, HPTLC, HPLC, UPLC, GC., flash chromatog., SFC, CE, MECC, UV/Vis, IR, NMR, MS, LC/MS, LC-MS/MS, LC/NMR, HPLC/DAD-MS, HPLC/DAD/NMR-MS, UHPLC-MS, UHPLC-MS/MS, and chemometrics (1378); analysis and impurity identification in pharmaceuticals (1379); an overview of the impurity profiling methods for pharmaceuticals per current U.S. Pharmacopoeia guidelines (1380); 2015 method development for impurity profiling using SFC and comparing 6 different stationary phases (1381); an overview and review of recent advances in pharmaceutical impurity profiling (1382); a review of impurity profiling of drugs since 2010 (1383); development of an achiral SFC method with UV and MS detection for impurity profiling of drugs (1384 and 1385); an overview and review of impurity profiling of pharmaceuticals (1386); a review of impurity profiling, covering TLC, HPLC, HPTLC, LC-MS, LC-NMR, LC-NMR-MS, GC-MS, and LC-MS (1387); a review of impurity profiling of drugs (1388); 2016 an analysis of ionic interactions when characterizing 9 different stationary phases for drug impurity profiling with SFC (1389);

**Inhalants:** 2015 an overview of the abuse of nitrous oxide (1390);

**Labelling and Packaging:** 2012 examination of counterfeit labels on pharmaceuticals by IR and Raman (1391); 2013 a study on the effects of common drug packaging materials on nondestructive detection of contents by Raman spectrometry (1392); 2014 quantitative analysis of torn and cut duct tape physical end matching (1393); detection of counterfeit blister packaging by FTIR and chemometric methods (1394); a review of the identification of stamp impressions, including by microscopy, computer-assisted artificial identification, and anal. methods (UV-Vis, fluorometry, IR spectroscopy, Raman spectroscopy, TLC, LC, GC, and MS) (1395); 2015 evaluation of drug packaging by DSC (1396); determination of ethylene dichloride in drug packaging material made of polyethylene dichloride by headspace GC/ECD (1397);

**Legal Issues:** 2014 a regulatory perspective on the abuse potential evaluation of novel stimulant drugs in the U.S. (1398); an effort to develop objective scientific methods to quantify and define
the important "substantially similar" structural parameter used in several laws (1399); 2015 a proposal for objective scientifically-derived measures of molecular structural similarity (1400);

**Precursors:** 2013 impurity profiling of sassafras oils by GC×GC-TOF-MS (1401); 2014 a brief overview of the precursors for drugs of abuse (1402); determination of safrole in ethanol extract of nutmeg (Myristica fragrans Houtt) using RP-HPLC (1403);

**Quality Assurance:** 2013 use of a software tool ("Drugs WorkBook") for the quantification of illicit drugs (1404);

**Sampling Plans:** 2013 a study of particle size of amphetamine, heroin, cocaine, and herbal cannabis and its influence on mass reduction (1405); a general sampling plan for the quant. instrumental anal. of heroin, cocaine, amphetamine, cannabis resin, MDMA tablets, and herbal cannabis (1406); a new sampling plan which focuses on sample heterogeneity (from ENFSI) (1407);

**Sensors (Biological and Instrumental):** 2013 a review of biological organisms as volatile compound detectors (stated applications include illicit drugs) (1408); 2014 assessing the potential of metal oxide semiconducting gas sensors for illicit drug detection markers (1409); use of a parasitic wasp as a biosensor for cocaine (1410); a review of biosensors in forensic analysis (1411); 2015 detection of illicit drugs by trained honeybees (1412);

**Soil:** 2012 forensic examination (reference text) (1413); 2014 by elemenatal analysis (1414); use of visible microspectrophotometry and FTIR/ATR for examination of soils for trace evidence (1415); 2016 protocols for soil examinations (1416);

**Surveys of Drug Use:** 2014 a comparative evaluation of whether computer survey technology improve reports on alcohol and illicit drug use in the general population (1417); an overview of 4 different systems utilized in Australia for monitoring drug use (1418); the use of internet snapshot surveys to enhance understanding of the availability of 2 NPSs (4-methylaminorex and 4,4'-dimethylaminorex) (1419); a survey of pharmacological cognitive enhancement among university students in the UK and Ireland who were abusing "smart drugs" (modafinil, methylphenidate, or Adderall) (1420); 2015 a measure of the "interest" in MDPV, methylone, 4-MEC, 4-HO-MET, MXE, 6-APB, AH-7921, and 3-MMC before and after its scheduling in Sweden (1421); an update on the Pistoia Alliance Controlled Substance Compliance Service Project (1422); a discussion and evaluation of the contents, the destinations, and the sources of 960 postal items seized by Swiss customs at the Swiss border between 2013 and 2014 (1423);

**Other:** 2013 collection of trace chemicals from diverse surfaces by use of strippable coatings
the practical relevance of pattern uniqueness in forensic science (1425); 2014 a review of resolution by fractional crystallization of diastereomeric salts (1426); determination of water in active pharmaceutical ingredients using ionic liquid HS-GC and two different detection protocols (not identified in the abstract) (1427); reducing the complexity of an agent-based local heroin market model (1428); the examination of trace physical evidence and artificial materials (1429); use of an online database of chemical compounds for the purpose of structure identification (1430); 2015 a discussion of the comparison processes and evaluation systems that form a forensic intelligence framework, advocating scientific decision criteria and a structured but flexible and dynamic architecture (1431); an overview of ingestion of illicit drugs by "parachuting" (1432); the use of DNA sequencing analyses of the fungal diversity found in dust samples for geo-sourcing (1433); an assessment of the toxicity of the refill liquids for electronic cigarettes (based on the presence of microorganisms, diethylene glycol, ethylene glycol, hydrocarbons, ethanol, aldehydes, tobacco-specific nitrosamines, and solvents) (1434).

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Analysis of Drug Trafficking Ledgers

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ABSTRACT: Drug trafficking operations commonly document their activities in ledgers. The complexity of these ledgers is usually intended to help secrete the recorded information, making their interpretation challenging. The Federal Bureau of Investigation's Cryptanalysis and Racketeering Records Unit recently conducted a study of ledgers from 165 drug cases that spanned 15 years. The following characteristics were analyzed: Type of drug or drugs; use of slang, codewords, and weight indicators; the use of redenomination of numbers, pricing, and dates; and duplicate entries. The results illustrate the frequency with which these characteristics appear within ledgers. An emphasis is placed on the value of duplication, which is observed when critical information is recorded in multiple places within one or more ledgers.

KEYWORDS: Drug Ledgers, Record Analysis, Code, Duplication, Slang.

Introduction

According to statistics recently published on the Federal Bureau of Prisons website, 82,109 federal inmates were convicted as a result of drug offenses - 46.4% of all federal inmates [1]. The next largest criminal offense - weapons, explosives, and arson - accounted for only 29,834 inmates in prison - just over one third of the number of inmates incarcerated for drug-related offenses. The investigations and cases corresponding to drug-related crimes in the U.S. are handled by multiple levels of law enforcement, including local, state, and federal agencies. A major element of a number of these investigations is the analysis of drug ledgers. In an attempt to conceal the true nature of their illicit activities and/or for the sake of brevity, the authors of these ledgers often record data in (apparently) incomplete and/or cryptic manners. Careful review and interpretation of the ledgers, however, can reveal information about the business' inventory and distribution, the size and scope of the operation, the number and roles of participants, how long the business has operated, when transactions occurred, which drug or drugs were distributed, and cash flow and profit calculations [2]. Such information is extremely valuable in prosecuting and sentencing the individuals involved in the operation.

The Cryptanalysis and Racketeering Records Unit (CRRU) of the Federal Bureau of Investigation (FBI) Laboratory has a team of forensic examiners trained to analyze and decrypt drug ledgers. The CRRU analyzed ledgers from 165 cases spanning 15 years to determine their commonalities and differences. [In this study, a case refers to all ledgers from a single investigation.] The examined cases were geographically diverse, covering 27 U.S. states, Washington D.C., Puerto
Rico, and the U.S. Virgin Islands. The size and scope of the businesses varied significantly. One of the smaller operations documented less than 40 grams of heroin being sold predominantly at $10 per 0.1 gram to approximately 24 accounts, for a total of $3,705. In contrast, one of the larger operations documented a minimum of 272 kilograms of heroin priced between $45,000 and $54,000 each, for a minimum total value of $12,727,000. The latter operation also distributed 325 kilograms of cocaine, priced between $22,500 and $27,000 each, for a minimum total value of $8,160,300. The collective results highlight multiple characteristics within drug ledgers that have the potential to aid law enforcement agencies.

Experimental

The 165 cases in this study were worked from 2000 to 2015. Other cases from this time frame lacked sufficient data in one or more areas in question, and therefore were not included. A database was created to record and organize the data and characteristics from each selected case, including: The presence of one or more drugs; the inclusion of slang, abbreviations, and codewords; the presence of redenomination of numbers, pricing, and dates; and duplication (each of these terms is defined and described more fully below).

Results and Discussion

Table 1 illustrates the frequency with which each of the primary characteristics was identified in ledgers with respect to the different types of drugs involved.

Drug Type

The drug(s) represented in each ledger were as follows: Cocaine 37.0% (61 cases), heroin 7.3% (12), marijuana 37.6% (62), methamphetamine 12.1% (20), and unidentified drugs 40.6% (67). Even when the records clearly documented the operations of an illicit drug business, however, in some cases the identity of one or more of the drugs was not definitively clear. This was due

<table>
<thead>
<tr>
<th>Drug</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>61</td>
<td>24</td>
<td>44</td>
<td>29</td>
<td>61</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>Heroin</td>
<td>12</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Marijuana</td>
<td>62</td>
<td>28</td>
<td>55</td>
<td>25</td>
<td>59</td>
<td>51</td>
<td>58</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>20</td>
<td>12</td>
<td>18</td>
<td>9</td>
<td>20</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Unidentified</td>
<td>67</td>
<td>19</td>
<td>53</td>
<td>26</td>
<td>61</td>
<td>47</td>
<td>52</td>
</tr>
</tbody>
</table>

A - Number of Cases
B - Cases Involving Multiple Drugs
C - Slang, Abbreviations, and/or Codewords Identified
D - Redenomination of Numbers Identified
E - Pricing Identified
F - Dates Identified
G - Duplication Identified
either to the drug’s price not being present, or the price being consistent with the known prices of more than one drug. For example, a price of $1,000 per unit could be consistent with either pounds of marijuana or ounces of cocaine, at different time periods and/or locations. In such cases, the examiner would need to further examine the ledger to conclusively identify (if possible) which drug was actually being represented. It was determined that at least 24% of the cases in this study dealt with multi-drug businesses - which explains why the preceding summation of case numbers exceeds 165 and the percentages totaled more than 100%. For this reason, it is important that the determination of which drug or drugs is represented is made using the totality of the information in the ledgers, including elements such as pricing and slang.

Slang, Abbreviations, and Codewords
Identifying slang within ledgers usually provides useful clues in determining which drug is represented. The authors often use slang, abbreviations, and/or codewords when referring to drugs, unit quantities, and/or weights. The terminology typically varies based on the organization, geographic location, and time of occurrence, but still can be useful for identifying the drug(s). Figure 1 depicts a section of a drug ledger that refers to methamphetamine as "ventana" (Spanish for "window") and cocaine as "nieve" (Spanish for "snow"), which are common slang terms for those drugs [3]. In some cases, slang and abbreviations are quite likely used simply for the purpose of brevity. In these instances, the terms are understood in context. In other cases, however, codewords are specifically used as a means to disguise the true

![Figure 1 - Example of the Use of Slang in a Ledger.](image)
nature of the records. This can make the writing cryptic, as it is intended (by design) to be fully understood only by the author or a small group within the operation [4,5]. However, one man's slang can be another man's codeword - and vice-versa. Over time, words that were once considered to be codewords may become common slang - and (again) vice-versa. For this reason, there are many cases where it cannot be determined from the ledger(s) if the author intended to make their writing cryptic, or if their verbiage was used for brevity, or if what was once intended to be cryptic became familiar simply due to long-term usage. In the present study, 77.0% of the cases were found to include slang, abbreviations, and/or codewords.

**Weight Indicators**

Weight indicators are the units in which a drug is measured, such as ounces (oz), pounds (lb), grams (g), and kilograms (kg). They are often referred to using slang terms such as "quarters", "grandes", "pieces", "cuadros", etc. These weight indicators can also be useful for identifying the drug in question. For example, if an examiner discovers the word "smack" in a ledger (historically used as a codeword for heroin), finding the notation "g" or "grams" - the most common weight indicator noted in the heroin cases in this study - helps to strengthen the conclusion that the ledger is a record of a heroin business. Knowledge of such correlations can help an examiner to recognize which drug(s) are represented in the ledger(s).

**Redenomination of Numbers**

Another measure used to disguise the information in ledgers is redenomination of numbers. This occurs when the author, again as a means for making the writing cryptic or for brevity, either moves the decimal point within a number or drops the zeroes off the end of a figure. In most instances, redenomination of numbers was utilized when recording unit prices. For example, if an operation is selling kilograms of cocaine for $27,000 each, the author writes $27 or 27 as the price per unit. This practice adds to the complexity of interpreting the payments and units.

Redenomination of numbers was found in 41.2% of the cases in this study. Figure 2 provides an example, where it is clear that three zeroes are omitted from each number since the phrase “1 Thousand balance” [balance] is written at the end. An effective technique to confirm redenomination of numbers is to verify the math on other documents where the author wrote out the same calculations in their entirety.

![Figure 2 - An Example of Redenomination of Numbers.](image)
Pricing
Inclusion of pricing is observed in 94.6% of the drug ledgers in this study. Examiners can make more informed decisions about which drug or drugs are represented by knowing the drug prices in the area where the seizure was made. These prices can then be compared to the numbers found in the ledgers. In this study, in the 76.9% of the times that an examiner identified pricing, the specific drug or drugs present was also conclusively identified. Additionally, since the price of a drug is often date dependent, finding both the dates of distribution (or acquisition) and the prices per unit can be useful information. For example, Figure 3 was identified as a marijuana bale list. This shows 10 bales with a gross weight of 199 pounds and 70 ounces. The weight of the wrapping is listed to the right of each bale, which actually totals 76 ounces, not 74 (the author of the list made a math error). The incorrectly calculated wrapping weight of 74 ounces was subtracted from the gross weight producing a total of 198 pounds and 12 ounces. The record also contains a total amount of $136,143.75, yielding a price of $685 per pound, which was consistent with marijuana prices for the date/location of the seizure. The pricing information in combination with the subtraction of the wrapping weight (which is only done with marijuana records), along with the format of the list, allows the examiner to testify that this is a marijuana bale list.

Figure 3 - A Marijuana Bale List.
Dates
Dates also aid in sorting and totaling amounts of money and drugs. Dates can appear in a variety of formats (e.g., omitting month, day, and/or year), and in this study were present in 74.6% of the records. Recording the dates enables the author to document the business' activity more accurately. Successfully identifying dates in a ledger is critical in determining when actual transactions occurred. They are also critical for determining if calculations and transactions were duplicated across different pages, ledgers, or seizure locations.

Duplication
When the same transactions or unit quantities are recorded in two or more locations within drug ledgers, duplication is said to be present. This occurs as a result of multiple people within the business maintaining their own records, or as a result of a single author repeating notes within multiple ledgers. Figure 4 depicts an example of evidence from two different seizure locations that document an identical transaction of pounds of marijuana. Duplication was noted in 80% of the ledgers in this study. Figure 5 (next page) shows the number of records containing duplication with respect to each specific drug. Duplication in marijuana ledgers stands out at 93.6%, while the other drug records varied between 75 and 82%. As mentioned previously, many cases contained records of a multi-drug operation, which explains why the sum of the "total cases" portion of Figure 5 is greater than 165.

Figure 4 - An Example of Duplication.
Conservative Presumption

When sifting through the often massive quantities of data in drug ledgers, tallying every pricing figure as a new payment, or summing every unit of drugs as a unique quantity, can lead to potentially inflated (and therefore incorrect) conclusions. To avoid double or triple counting when totaling monies or drugs, CRRU examiners utilize a practice called conservative presumption, which involves comparing entries and calculations against one another in order to ensure that each transaction or unit is only counted once. If an examiner determines or even suspects that a set of calculations are duplicative, the numbers are only counted once in the report. While this may understate the totals, this more conservative approach ensures that the results are not exaggerated [4,5]. Conducting examinations without this caution introduces the possibility of inflation of unit totals, thus risking the inclusion of erroneous information in reports and testimony. Identification and discounting even minor duplication is important, as sentencing is sometimes based not on the amount of the drug seizure, but rather on the quantities documented in the ledgers.

Figure 5
Conclusions

This study highlights the typical characteristics found within drug ledgers. These characteristics can be used by law enforcement to conduct analyses of drug ledgers. A more complete understanding of an illicit drug trafficking operation can be elucidated through meticulous review of its ledgers. Identification of specific characteristics, such as slang, abbreviations, and codewords; the presence of redenomination of numbers, pricing, and dates; and the recognition of duplicate entries, are crucial in producing a wholly accurate report.

Disclaimers

This is publication 16-08 of the Laboratory Division of the FBI. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the FBI or the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. 105 provides that "copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. 101 defines a United States Government work as a work prepared by an employee of the United States Government as a part of that person's official duties.

References


Analysis of “Marijuana Edibles” – Food Products Containing Marijuana or Marijuana Extracts – An Overview, Review, and Literature Survey

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ABSTRACT: An overview and review of the analysis of food products containing marijuana or marijuana extracts, as reported in the scientific literature through 2016, is presented.

KEYWORDS: Marijuana Edibles, Marijuana Concentrates, Cannabis, Hemp, Tetrahydrocannabinol, THC, Analysis, Chromatography, Forensic Chemistry.

Introduction

Although still illegal under Federal and many state statutes, food products containing marijuana (Cannabis sativa L.) or marijuana extracts are currently common in states that either permit or decline to prosecute “medical” or “recreational” marijuana, and are increasingly being submitted to forensic laboratories for analysis – especially in neighboring states where marijuana statutes are still being enforced. Such products, generally referred to as “marijuana edibles,” range from beverages to candies to baked goods, and can contain herbal cannabis ranging from entire leaves down to very finely ground material; semi-refined cannabis preparations such as hashish, sinsemilla, or cannabis resin; or moderately to highly refined cannabis extracts and concentrates such as hash oil, “butane honey oil” (BHO), or similar preparations. Due to the range of THC-containing adulterants, and the variability and complexity of their edible “support matrices,” the qualitative and quantitative analyses of such products range from facile to significantly challenging (1,2,3). An overview and review of this topic, with an emphasis on methods published from 2005 through 2016, is presented herein. To the author’s knowledge, the analyses of marijuana edibles has not been previously reviewed or surveyed (4).

Search Details

Searches were conducted using the Chemical Abstracts Service’s Scientific & Technical Information Network (STN), Google, PubMed, by reading select forensic journals (notably the entire run of Microgram, Microgram Bulletin, and Microgram Bulletin LE 1967 to 2016), and/or by reviewing the reference citation lists of pertinent

1 Utilized herein as a generic term for marijuana concentrates obtained via extraction using butane, supercritical CO2, or an equivalent low polarity solvent or supercritical fluid.

2 Including “budder”, “erl”, “marijuana rosin tech”, “shatter”, “wax”, and other highly viscous or semi-solid, high THC concentrates. [Note that such slang / street names change constantly.]
articles or pertinent chapters of select reference texts. In general, on-line searches were conducted using four linked terms, one each from: A) Chromatography, electrochromatography, electrophoresis, spectrometry, or spectroscopy; B) marijuana or an equivalent term (cannabinoids, cannabis, hash oil, hashish, hempseed, marihuana, phytocannabinoids, tetrahydrocannabinol, tetrahydrocannabinolic acid, THC or THCA\(^3\) – but no slang terms); C) food, foodstuffs, or a specific term (baked goods, beer, beverage(s), candy/ies, edible(s), liquor, milk, seed oil, tea, or wine); and D) analysis, analytical, or forensic. Followup searches were conducted as the results suggested. The STN and PubMed searches were limited from 1990 to 2016, while only the top 100 “hits” on Google were checked. No mass media sources (i.e., newspapers, magazines, radio, television, or their Internet equivalents) are cited.

An issue of note while conducting searches using Google was the significant number of pertinent, on-line “application notes,” “infomercials,” and similar reports. Nearly all of these have appeared in the past five years. With the exception of a few application notes that were re-published in LC-GC or American Laboratory, and two “cannabis industry” reports summarizing the salient issues with preparing marijuana edibles with accurate and consistent potency levels (vide infra), these are not included. While there are no reasons to doubt the validity of the presented information, virtually all of these reports are either from scientific instrumentation companies touting the capabilities of one of their instruments or from commercial analytical laboratories offering for-fee testing services, and (in the author’s judgment) therefore are not appropriate for this review.

\(^3\) THCA = Tetrahydrocannabinolic Acid (not 11-nor-9-Carboxy-THC). In this review, THCA is utilized to represent both THCA isomers (THCA-A and THCA-B).

The Development of Marijuana Edibles

Marijuana edibles can be arbitrarily divided into three generations. “First Generation Marijuana Edibles” are products that were illicitly produced for personal consumption or for small-scale sale on the black market, long before the advent of state-permitted/non-prosecuted medical or recreational marijuana (or even the term marijuana edibles).\(^4\) These products enabled marijuana use without smoking, thereby reducing its detectability and/or providing an alternate consumption mechanism for users who were either adverse to smoking or who preferred the effects of orally consumed marijuana (5,6,7,8,9,10,11,12,13,14,15, 16,17; see also: 18). While already widespread – albeit low level – among marijuana users in the 1960s, the first such exhibit (cannabis resin smeared on bread) was not reported to Microgram until 1970 (19), suggesting only minimal interest among law enforcement personnel or forensic chemists. Until around 2000, most products of this type consisted of herbal cannabis, hashish, or cannabis resin in home-made baked goods such as brownies, cookies, fudge, and similar dessert-type items (e.g., 20,21,22,23,24).

“Second Generation Marijuana Edibles” started to appear soon after California legalized use of medical marijuana in 1996 (25); these products included various types of candies and other packaged foods. Many of these were provided in zip-lock plastic bags with homemade labels, while others were professionally packaged and labelled with names that mimicked well-known consumer products, e.g., “Stoners” (mimicking Snickers® candy bars) (26,27), “Buddafingas” (Butterfinger® candy bars) (28), “Splif” (Jif® peanut butter) (29), and “Budtella” (Nutella® hazelnut-chocolate spread) (30). Additional items included THC

\(^4\) The first citations for marijuana edibles in PubMed appeared in 2013.
lollipops (31,32,33), THC candies (34,35,36,37), “pot butter” (or “ganja butter”) (38,39,40,41), chewing gum (42,43), “pot shots” (hard liquor containing suspended herbal cannabis) (44; see also: 45), and others (46,47). The majority of these latter products contained a marijuana extract (i.e., hash oil or BHO) or concentrate, with the remainder containing plant material (i.e., herbal cannabis, sinsemilla, or hashish); many also included a small marijuana leaf logo on their labelling or packaging.

“Third Generation Marijuana Edibles” refer to the current crop of state-permitted/non-prosecuted products. The passage of Amendment 64 in Colorado (48) and Initiative 502 in Washington (49), both in 2012, may be regarded as the break point between the second and third generations, as it marked the transition of marijuana edibles from a widespread cottage industry to large-scale, commercial production. While many of the products are highly similar to Second Generation Marijuana Edibles, their variety, quantities, THC potency levels, and marketing are unprecedented. In addition, based on an informal survey (by the author) of recipes and cannabis industry information, as of December, 2016 nearly all of the large-scale manufacturers of these items are utilizing liquid marijuana concentrates – not herbal cannabis – as the THC source in their products.

“Hemp Food Edibles”

A peripheral but pertinent subset of marijuana edibles are “hemp food edibles,” i.e., foodstuffs containing the seeds, oil (from pressing the seeds), and/or the flour (from grinding the seeds) obtained from “industrial hemp” (henceforth hemp), a cultivar of Cannabis sativa L. that (usually) contain only trace to very low amounts of THC and THCA. Despite their deliberately innocuous names, however, hemp and hemp food edibles are legally suspect under Federal law; to wit, hemp and hemp food edibles that contain any detectable amounts of THC are still considered to be Schedule I materials under the U.S. Controlled Substances Act; i.e., they are in fact marijuana and marijuana edibles, albeit low potency (50).

Currently, hemp is a “niche” crop grown primarily in China, North Korea, Canada, a moderate number of European Union (EU) nations, and in lesser amounts elsewhere, including (with quite stringent restrictions, 51) in the U.S. (52,53,54,55,56).

The seeds, oil, and flour from hemp are touted (sometimes to excess) for their health benefits – especially the oil, a rich source of highly valued omega-3 fatty acids (57,58,59,60,61; see also: 62). Hemp food edibles (and numerous other non-edible, hemp-derived consumer products) began to appear in greater numbers in the early to mid-1990s, as hemp cultivation was allowed, encouraged, and/or increased especially in Canada and the EU; they were initially popular, not for their potential health benefits or nutritional value, but rather for their novelty or shock impact (which has since faded, for obvious reasons).

Not surprisingly, the initial wave of hemp food edibles were often contaminated with phytocannabinoids. Although many of these products did in fact contain only trace to minor amounts of THC, some contained enough to result in positive drug tests (primarily urinalyses) for marijuana.

5 Including soaps, shampoos, cosmetics, and biofuels made with hempseed oil, as well as paper, clothing, and other textiles made with hemp fiber (which is one of the strongest and most versatile plant-derived fibers known); these are not further addressed in this review (see References 52-56 for extensive information).

6 A few others were inadvertently (or in some cases deliberately) produced with seeds, oil, or flour from marijuana instead of industrial hemp.
This resulted in numerous claims that positive tests for marijuana use were actually from consumption of hemp food edibles – even when those tests indicated THC metabolite levels several orders of magnitude higher than those that could possibly be caused by such products. Such claims in turn resulted in numerous articles either proving or disproving the likelihood of a positive test from consuming various products (not detailed in this review; see: 63). It was subsequently determined that inadequate cleansing of the seeds left residual cannabis resin on the seed exteriors, which would carry through to the hemp food edibles (64). These findings resulted in increasingly tighter regulations on acceptable THC levels on the seeds, forcing hemp cultivators to switch to cultivars with even lower native THC levels, and hemp processors to more thoroughly wash their seed stocks, significantly reducing the problem. The EU cutoff limit for THC in hemp is currently 0.2% (65), and the cultivars that meet this standard are published annually (66); most other hemp-growing nations have similar – though not as strict – regulations on domestically produced hemp and hemp-derived products.  

The analyses of hemp food edibles for THC was addressed in depth in multiple articles from 2000 to 2008 (67,68,69,70,71,72,73,74). Collectively, these studies provided useful insight into the subsequent analyses of marijuana edibles – in some cases, the only published workup procedures for certain products are those that were originally developed for hemp food edibles.

(Unadulterated) Food, Hemp Food Edible, or Marijuana Edible?

A disturbing consequence to the rapid increase in marijuana edibles is the concurrent increase in their accidental consumption (especially by children or pets) as unadulterated food products or less commonly as hemp food edibles. A number of overviews (75,76,77,78,79,80,81,82,83,84) and case reports (85,86,87,88,89,90,91) have been published in the scientific, medical, and veterinary literature, a few of which included the analyses of the suspect items.

Analysis of Marijuana Edibles – An Overview

The analyses of alkaloids (and other plant constituents, additives, and contaminants) in foodstuffs is a very heavily researched topic (see, e.g.: 92, 93,94,95,96,97). As of December 2016, however, a universal, validated method for comprehensive, quantitative analysis for phytocannabinoids in marijuana edibles has not been published. This is not surprising, given the wide range and still increasing variety of such products; the broad array of ingredients in most prepared foods; the variety of THC sources being utilized in their preparation (as well as the heterogeneity of the plant material when that is used as the source [98, 99,100,101; see also: 102,103]); the thermal lability of THCA and the other acidic phytocannabinoids (104,105,106,107,108,109); the high affinity of the lipophilic phytocannabinoids for the fats and oils present in most foods; and the significant representative sampling challenges resulting from the inherent heterogeneity of most solid food products (compounded by the varied and sometimes amateurish marijuana edible preparation practices in current use [110, 111]).

In lieu of a universal method, a variety of

7 The current USDA limit for THC in U.S. produced industrial hemp is 0.3% (51).

8 A much larger number of examples have been reported in various mass media sources; these are not included in this review.

9 In December, 2016 a PubMed search on "analysis of alkaloids in foods" returned over 6,500 citations.
procedures have been reported for specific sub-
types of products (e.g., beverages); to date, how-
ever, in the majority of these studies the analytical
methodology is presented for a single exhibit, a
small set of virtually identical exhibits, or a small
set of highly similar exhibits.

In the simplest case – i.e., a product that contains
sizable/recoverable pieces of visible cannabis, but
little or no other plant material(s) (112) – a
physical separation and standard marijuana
analysis may be conducted (i.e., microscopy, color
testing, GC/FID, and/or GC/MS); however, this
can be quite tedious and may give an ambiguous
result or a false negative if the THC, THCA, and
other major phytocannabinoids were de facto
extracted from the plant material by the food
matrix or by its preparation – which would be
expected if the ingredients included significant
amounts of ethanol or any lipophilic ingredient
(butter, lard, oil, etc.), especially if typical baking
temperatures were utilized. In such cases, addi-
tional workup of the “support matrix” would be
required to confirm THC, THCA, CBD, etc.

For exhibits where cannabis is not visibly present
– or is present but is not practically recoverable –
sample prep is nearly always designed to obtain
an extract for analysis. Liquids (including oils)
are typically subjected to one or more liquid-
liquid and/or solid phase extractions (LLEs or
SPEs). Water-soluble solid samples (e.g., a sugar-
based, hard or gummy candy) are either dissolved
in water and extracted, or finely ground and
triturated. More complex, solid samples are first
homogenized and triturated, or mixed with a
sorbent and homogenized, then triturated. The
triturates are then isolated by filtering or centri-
fuging. Alternately, samples may be subjected to elution on a short column or a Soxhlet extractor.
Problematic semi-solid or viscous samples may be
extracted directly, or frozen at dry ice or liquid
nitrogen temperatures prior to homogenization
and workup. Vortexing or (with care) sonication
can improve extraction or trituration efficiency.
Derivatization, while advantageous for some anal-
eses, at present is only occasionally employed.

Proper solvent selection is a critical aspect of the
workup (113). Use of low polarity solvents
usually result in reasonably clean triturates/extracts, but suffer from low recoveries, especially
of the polar phytocannabinoids (most notably
THCA, CBD, and CBDA). In contrast, use of
high polarity solvents give good recoveries of the
phytocannabinoids, but the triturates/extracts also
contain a rich array of components from the
support matrix. Back LLEs, SPEs, use of solvents
or mixed solvents of intermediate polarity, and/or
evaporation of extracts and reconstitution of the
resulting residues in different solvents, are
available options, but take additional time and
resources. In general, if the intent of the analysis
is merely to qualitatively prove the presence or
absence of THC, the workup and analysis is
usually facile; however, if a quantitative analysis
of multiple phytocannabinoids is needed, then the
optimal workup will likely vary for every different
type of marijuana edible.10,11

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10 Even (superficially) “identical” edible
matrices may actually be quite different. Consider,
e.g., two “nut brownies”, one made using lard,
cashews, and dark corn syrup, and the other made
using butter, peanuts, and cane sugar – but otherwise
prepared as similarly as possible with respect to the
other ingredients, amounts, baking time, temperature,
etc. Even if an identical amount of the same BHO
concentrate was used in their preparation, and both
exhibits were worked up by the same procedure, their
dissimilar extraction characteristics (from the
different sugars, fats, and oils present) and diverse
array of matrix-derived contaminants would result in
slightly to moderately differing quantitative results.

11 A complete analysis would also
determine pesticides, herbicides, fungicides, heavy
metals, mycotoxins, residual solvents, etc.; however,
these are not addressed in this review.
Analyses of the triturates/extracts or reconstituted residues are typically conducted by GC/FID, GC/MS, HPLC with UV, PDA, or LIF detection, or by a more sophisticated method, e.g., UHPLC-MS/MS. Of significant concern, if analyses are conducted on GC-based instrumentation, “dilute-and-shoot” injections of crude triturates/extracts (i.e., those obtained with high polarity solvents, especially those from substrates that contained high amounts of sugars) can result in fouling of injection ports, liners, and columns,\(^\text{12}\) decomposition and loss of thermally labile phytocannabinoids, and poor chromatographic performance \((114)\). In contrast, most LC-based methods are far more tolerant of such triturates/extracts, and are also much better able to handle sensitive components \((115)\).

Finally, concentrated residues obtained from low polarity solvents (which therefore are reasonably clean) may be reconstituted in a deuterated solvent for NMR analysis, or even (for exhibits containing at least moderate amounts of THC) submitted to color testing and/or TLC analyses.

**A Survey of Reported Analyses**

In each case, the edible matrix, the focus of the analysis (i.e., THC, THC/THCA, THC/CBD, all major phytocannabinoids, etc.), the workup procedure, the analytical methodology/ies, and the reference citation, are specified. Where significantly different matrices with varying workup procedures are included in one article (e.g., a beverage and a baked good), where possible each matrix is detailed separately. Where multiple references for the same matrix (e.g., hempseeds) are cited, the presented order is chronological/most recent first. Peripherally pertinent references (i.e., that include some analytical details) are cited as “See also”. Additional comments are provided in the reference citations as appropriate.

**Aqueous and Alcoholic Exhibits**

* **Aqueous Extracts and Alcohol Tinctures** – These are traditional forms of “medicinal” cannabis preparations, that are still occasionally submitted to forensic laboratories as unusual marijuana exhibits or as topical medications \((116)\).

\* Prepared Ethanolic Extracts; THC, THCA, CBN, CBD, CBDA, CBG, CBGA, cannflavin A/B, and total phenolics; herbal cannabis was extracted with 20%, 40%, or 80% ethanol/water, filtered, and analyzed by HPLC/DAD \((117)\).

\* Prepared Cold and Hot Water Extracts; THC and THCA; the aqueous solutions were filtered, extracted with hexane, and the extracts dried to residues and reconstituted in CDCl\(_3\) for NMR analyses. Alternately, a hot water extract was freeze-dried, reconstituted in 80% aqueous methanol, and an aliquot was mixed with D\(_2\)O and analyzed by NMR. The NMR analyses included 1D and 2D (DOSY and NOESY) experiments with solvent peak suppression \((118)\).

\* Prepared Ethanolic Extracts; THC and THCA; herbal cannabis was extracted with 20%, 40%, or 80% ethanol, filtered, the respective filtrates evaporated to dryness, reconstituted with CHCl\(_3\), methanol, or water, and an aliquot was mixed with D\(_2\)O and analyzed by NMR. The NMR analyses included 1D and 2D (DOSY and NOESY) experiments with solvent peak suppression \((119)\).

**Beverages** – Of note, a growing number of commercially produced, marijuana-based alcoholic beverages (beers, wines, and hard liquors) are...
being marketed as of December, 2016.

“Sodas” (carbonated); spiked THC, CBD, and CBN (and 35 spiked pesticides); an aliquot was degassed by sonication, added to 1:99 acetic acid/acetonitrile, the mixture added to a specialized mixture of “extraction salts” (the so-called QuEChERS technique (120)), vortexed, centrifuged, and the supernatant analyzed by LC-MS/MS (121).

* “Hemp Products” (beverages, including beer, tea, and vodka); trace THC; the solution was mixed with methanolic KOH, extracted with hexane, acidified with HCl, extracted with 1:9 ethyl acetate/hexane with vigorous mixing and centrifuging. The organic layer was evaporated to dryness under nitrogen, derivatized with BSTFA, and an aliquot analyzed by GC/MS (122).

* “Hempen Ale” – THC and 11-nor-9-carboxy-THC; the ale was subjected to SPE, derivatized with BSTFA, and analyzed both by standard GC/MS and GC/MS in SIM mode (123).

* See also: “Beverages” (124); “Hempen Ale” (125).

**Milk** – Milk is an unusually challenging matrix due to its high fat content. Although “marijuana milk” (usually prepared by boiling herbal cannabis in whole milk) has been reported (126), as of December, 2016 there are no reports of its analysis (however, see: 127). Trace-level analyses have been conducted on human breast milk obtained from lactating mothers who had been using marijuana (128,129,130), or on milk from lactating animals that had been foraging on wild cannabis/hemp or that had THC or marijuana extracts administered to them for study purposes.

* Human Breast Milk; ultra-trace THC, CBD, and CBN; the milk was saponified with methanolic NaOH, centrifuged, and the supernatant subjected to SPE. Qualitative analysis by Isotope Dilution UPLC-MS/MS (131).

* Human Breast Milk; trace THC, 11-hydroxy-THC, 11-nor-9-carboxy-THC; the milk was pasteurized, diluted 1:1 with methanol, centrifuged, and the supernatant subjected to SPE. Analysis by LC-MS/MS (132).

* Ewe’s Milk; trace C-14-labelled THC; the milk was freeze-dried, extracted with ethanol, the extracts centrifuged, the supernatant was cooled (to precipitate some lipids), then isolated and evaporated to dryness under vacuum, reconstituted in water, then extracted with pet ether and then with diethyl ether. Qualitative analysis by radio-quantitation (scintillation counting) and separately by TLC (133).

* See also: Buffalo Milk (134); Human Breast Milk (135,136); Rat Milk (137); and Squirrel Monkey Milk (138).

**Tea** (i.e., Cannabis Tea) – Typically prepared by boiling herbal cannabis in water – is a simple but variable matrix due to the differing extraction efficiencies and solubilities of the phytocannabinoids in hot water (THC is poorly soluble even in boiling water), potentially complicated by the decarboxylation of THCA, CBDA, and several other acidic phytocannabinoids under extended heating conditions.

* Cannabis Tea; focus is on THC and THCA, but additional phytocannabinoids were observed in the chromatograms; the tea was freeze-dried, reconstituted in ethanol, and analyzed by HPLC/UV (139).

* Cannabis Tea; THC, THCA; an aliquot of the tea was diluted with methanol and analyzed by HPLC with UV and fluorescence detection (140).
Lipophilic (Oil) Exhibits

Oils are also an unusually challenging matrix due to the lipophilicity of the less polar phytocannabinoids (THC, CBN, etc.)

**Hempseed Oil (cannabis oil, hemp oil)** – Due to the very large number of studies on this product, only references from 2000 through 2016 are cited.

* Hempseed Oil (commercial-grade foodstuff); THC, CBD, CBN; the oil was homogenized, added to acetonitrile, sonicated, cooled to -15°C, and an aliquot of the acetonitrile layer analyzed by GC/MS (141).

* “Edible Vegetable Oil”; trace THC; the oil was extracted with methanol, submitted to SPE, and the eluant analyzed by UPLC-negative ESI-MS/MS (142).

* “Edible Oil” (commercial-grade hempseed oil); THC, CBD, CBN; the oil was extracted with methanol, submitted to SPE, and the eluant analyzed by UPLC-MS/MS (143).

* “Hemp Products” (44 different oils); trace THC; the oil was mixed with methanolic KOH, extracted with hexane, acidified with HCl, extracted with 1:9 ethyl acetate/hexane with vigorous mixing and centrifuging. The organic layer was evaporated to dryness under nitrogen, derivatized with BSTFA, and an aliquot analyzed by GC/MS (144).

* “Cannabis Oil” (commercial-grade hempseed oil); THC, CBD, CBN, CBC; the oil was added to n-hexane and extracted several times with acetonitrile, the combined extracts washed with 2% aqueous NaCl, then with hexane. The acetonitrile was dried under nitrogen, reconstituted in an unspecified solvent (presumably acetonitrile), and analyzed by HPTLC and GC/MS (145).

* “Hemp Oils” (several different products); THC, CBD, CBN; the sample was extracted 3 times with methanol with sonication, the extracts isolated and evaporated to dryness under nitrogen, derivatized with MSTFA, and analyzed by GC/MS (146).

* Hempseed Oil (health supplements); THC; the oil was added to acetonitrile, mixed thoroughly, cooled to -70°C, centrifuged, the acetonitrile layer isolated, dried under nitrogen, derivatized with MSTFA, centrifuged again, and the supernatant analyzed by GC/MS. Alternately, the oil was added to acetonitrile, mixed thoroughly, an aliquot of the acetonitrile layer removed and dried under nitrogen, the residue reconstituted in hexane and submitted to SPE. The eluant was dried under nitrogen, reconstituted in 20% ethyl acetate/hexane, and analyzed by GC/MS (147).

* Hempseed Oil; THC, THCA; an aliquot of the oil was diluted with methanol and analyzed by HPLC with UV and fluorescence detection (148).

* See also: Hempseed Oil (149).

**Hemp seeds (cannabis seeds)** – As previously noted (vide supra), virtually all of the THC and other phytocannabinoids “in” hemp seeds is actually due to cannabis resin adhering to the exteriors of the seeds; however, trace levels of phytocannabinoids have been identified within the seeds (vide infra). Due to the very large number of studies on this product, only references from 2000 through 2016 are cited.

* “Hemp Nuts” (containing cannabis seeds); trace THC, CBD, CBN; the nuts were extracted with 60% isopropanol, and the extracts were analyzed by HPLC-MS/MS (150; see also: 151).

* “Drug and Fiber Type Cannabis Seeds; trace THC; the seeds were added to 99:1 chloroform/
methanol, homogenized, centrifuged, and the supernatant was separated and evaporated to dryness. The residue was reconstituted in methanol, centrifuged, and the supernatant mixed with 1N KOH in methanol and 9:1 hexane/ethyl acetate and vortex mixed. The upper layer was isolated, evaporated to dryness, reconstituted in hexane and submitted to a short silica gel column. The appropriate fraction of the eluant was analyzed by GC/MS (152).

* Hempseeds; THC, THCA; the seeds were homogenized, extracted with 9:1 methanol/methylene chloride with sonication, an aliquot of the supernatant diluted with methanol and analyzed by HPLC with UV and fluorescence detection (153).

**Pharmaceuticals** – Includes Federally approved pharmaceuticals only. Although these are not marijuana edibles, they are included due to their close similarity to hemp oil samples and other oil-based supplements containing significant amounts of phytocannabinoids.

* Dronabinol Capsules (synthetic THC in sesame oil); THC; the oil was removed from the capsule, diluted 9:1 chloroform/methanol and further with 9:1 trichloroethane/methanol, and an aliquot analyzed by HPLC/UV (154).

* Dronabinol Capsules (synthetic THC in sesame oil; includes solutions in vials); THC; the oil was removed from the capsule (or vial), diluted with absolute ethanol, and aliquots analyzed: (a) by TLC with confirmation with Fast Blue BB after development; or (b) by HPLC/UV (155).

* Dronabinol Capsules (synthetic THC in sesame oil); THC, CBN; the oil was removed from the capsule, diluted with absolute ethanol, and an aliquot analyzed: (a) by HPLC with variable wavelength UV or PDA; or (b) by GC/FID (156).

* In different pharmaceutical “vehicles” (support agents); THC; the sample was diluted with an “appropriate solvent” containing an internal standard, and analyzed by HPLC (157).

**Solid, Complex Exhibits**

* Brownies (prepared using many different consumer mixes); stability study on spiked THC and CBD; after preparation (baking and cooling), a small portion of the brownie was added to methanol, thoroughly mixed, centrifuged, and an aliquot of the supernatant was analyzed by UPLC-MS/MS (158).

* Marijuana Edibles (hard candies, chocolates, “gummies”, “cookie and cream bar”, brownies, oils; spiked THC, CBD, and CBN (and 35 spiked pesticides); the sample was mixed with water, then mixed with 1:99 acetic acid/acetonitrile, the mixture added to a specialized mixture of “extraction salts” (QuEChERS), vortexed (shaken with the assistance of metal balls if necessary), centrifuged, and the supernatant analyzed by LC-MS/MS (159).

* “Hemp Foods” (unspecified products); trace “characteristic cannabinol”; the sample was extracted with methanol, the extract concentrated and submitted to SPE, the eluant evaporated to near dryness under nitrogen, reconstituted in 77:23 methanol/water, and analyzed by UHPLC-MS/MS (160).

* “Baked Goods” (a brownie and a cookie); THC, CBD, CBN; a small portion of the brownie or cookie was added to methanol, thoroughly mixed, filtered, the eluant centrifuged, the supernatant isolated and filtered again, and an aliquot of the filtrate analyzed by UHPLC/MS (161; includes multiple references).

* “Hemp Products” (solid products, many
different types); trace THC; the solid was mixed with methanolic KOH, homogenized, extracted with hexane, acidified with HCl, extracted with 1:9 ethyl acetate/hexane with vigorous mixing and centrifuging. The organic layer was evaporated to dryness under nitrogen, derivatized with BSTFA, and an aliquot analyzed by GC/MS (162).

* “Biscuits” (the British term for cookies – several types); THC, THCA; a portion of the biscuit was homogenized, extracted with 9:1 methanol/methylene chloride with vigorous mixing, filtered, an aliquot of the supernatant diluted with methanol and analyzed by HPLC with UV and fluorescence detection (163).

See also: “Edibles” (Gummies, Chocolate, Brownies, Oil, Caramels) and “Topical Lotions” (164); “Edibles” (165); and “Edible Medical Cannabis Products” (Baked Goods, Candies, and Chocolates) (166).

**Multiple Matrices** (studies that provide general procedures for workup and analysis)

* “Cannabis-Based Products” (20 different products, including oral supplements, vapes, topicals, and veterinary items, with 3 duplicates for repeat analyses); THC, CBD, THCA, CBDA; the product was extracted with 99.5% ethanol, vortexed, sonicated, filtered, and an aliquot evaporated and screened by IMS; those products that tested positive had aliquots analyzed by UPLC-QTOF-HRMS (167).

* “Hemp Products” (included 9 solid foods and 16 beverages); trace to low-level THC; solid products were homogenized, extracted with methanol, the extracts were filtered, concentrated, reconstituted in methanol and screened by immunoassay (EMIT-II). Samples that tested positive were analyzed by GC/MS in SIM mode. Liquids were screened (undiluted) by immunoassay (EMIT-II). Samples that tested positive were subjected to SPE, with analysis by GC/MS in SIM mode (169).

* “Hemp Food Products” (included 30 different liquid and solid products); THC, CBD, CBN; Method 1 (HS-SPME) – the sample was homogenized, hydrolyzed with a mixture of aqueous sodium hydroxide and sodium carbonate, heated with vigorous agitation, and the resulting mixture was subjected to HS-SPME, derivatized with MSTFA, and analyzed by GC/MS. Method 2 (LLE, done for comparison against Method 1) – the sample was added to an equal amount of 9:1 hexane/ethyl acetate, homogenized with sonication, centrifuged, and the organic layer isolated, evaporated to dryness under nitrogen, derivatized with MSTFA, and analyzed by GC/MS. Method 1 was determined to be superior (170).

**A Note Concerning Ongoing Developments**

The intent of this review was to provide a “snapshot” of the analyses of marijuana edibles as of December, 2016 – not to make any specific recommendations for such analyses. As is typical with reviews of dynamic topics, it will be rapidly superseded by ongoing research – as well as by ongoing developments in the cannabis industry (especially the recent surge in cannabis-based oral supplements). Of note, the American Chemical Society (ACS) initiated a Cannabis Chemistry Subdivision in 2015 (171), and approximately three dozen cannabis-related presentations were made at the 2015 and 2016 ACS Annual Meetings (172); few of these, however, presented analyses
of any marijuana edibles. The AOAC International solicited for standard methods for analyses of marijuana and marijuana edibles in 2016, at the 130th AOAC Annual Meeting and Exposition (173). The U.S. Food and Drug Administration (FDA) has analyzed cannabis-based products for THC and/or CBD (174), and several publications providing broadly applicable methods are in preparation (175). In short, the next five years should see significant advances in this field.

Acknowledgments

The assistance of DEA Librarians Kristin Carr and Rose Russo in acquiring numerous references, and Laura Ciolino, U.S. FDA, for valuable discussions, are gratefully acknowledged.

References and Additional Notes

[Note: In order to minimize the odd spacings created by the use of fully justified columns for references, they and the author’s associated notes are provided in full page, left-justified format.]

1. Halford B. Analyzing cannabis. Chemical & Engineering News 2013;91(49):32-33. [Note: There are numerous mass media reports (many easily found on-line) concerning the various issues with marijuana edibles, including discussions of wide potency variations, contamination by pesticides, heavy metals, and molds, decomposition, accidental consumption by children and pets, overdoses, and more. Although dating from 2013, the above C&EN article was selected as a more scientific overview of this dynamic and rapidly evolving situation.]


19. Anonymous. “The One.” Microgram 1970;3(8):200. [Notes: It was stated that this material could also be smoked in a pipe. No workup procedures or analytical results were provided; this is typical of Microgram Intelligence Alerts from this era. All issues of Microgram (1967 through March, 2002) and the first nine issues of Microgram Bulletin (April through December, 2002) are permanently law enforcement restricted publications. From 1967 through mid-1973, Microgram was published by BDAC and then BNDD. Starting in mid-1973, Microgram and its successors (Microgram Bulletin and Microgram Bulletin LE) have been published by DEA.]


22. Anonymous. Dark chocolates with a marijuana odor. Microgram 1992;25(4):75. [Note: Hexane extracts were found to contain THC.]


35. See: Anonymous, Reference #32.


48. Colorado Constitution, Article 18 (Miscellaneous), Section 16 (Personal Use and Regulation of Marijuana). Proclamation by the Governor, December 12, 2012.


50. Clarification of the New Drug Code (7350) for Marijuana Extract; see: https://www.deadiversion.usdoj.gov/schedules/marijuana/m_extract_7350.html [Date of Most Recent Access: December, 2016.]


54. Carus M, Sarmento L. The European Hemp Industry: Cultivation, processing and applications for fibres, shivs, seeds and flowers. EIHA 2016-05. Posted at:


63. Lachenmeier DW. Hempoil food products – A problem? Deutsche Lebensmittel-Rundschau: Zeitschrift für Lebensmittelkunde und Lebensmittelrecht 2004;100(12):481-490. [Note: Written in German.]


65. EU Regulation 1308/2013.


67. Zoller O, Rhyn P, Zimmerli B. High-performance liquid chromatographic determination of delta-9-tetrahydrocannabinol and the corresponding acid in hemp containing foods with special

68. See: Lachenmeier, Reference #63.


75. This issue is covered in some depth in Barrus, Capogrossi, et al., Reference #4, pps. 5-9.


80. Weiss S. Edibles: For experts only? Ingesting marijuana, as opposed to smoking it, has come a long way since the days of homemade pot brownies. State Legislatures 2015;41(3):23. [Note: A one page news alert/editorial.]

82. Nolen RS. Bad medicine or natural remedy? States' legalization of marijuana has implications for veterinary medicine. Journal of the American Veterinary Medical Association 2014;245(7):726-750.


90. See: Uges, Reference #24.


110. For two overviews, see: (a) Martin RW. “The trouble with edibles. (The trouble with producing cannabis-infused edible products.)” Posted at:
Even when present, content labelling must be regarded with skepticism; see: (a) Vandrey R, Raber JC, Raber ME, Douglass B, Miller C, Bonn-Miller MO. Cannabinoid dose and label accuracy in edible medical cannabis products. Journal of the American Medical Association 2015;313(24):2491-2493. [Notes: In this study (which was widely cited in mass media reports), 75 marijuana edibles acquired from dispensaries located in Los Angeles and San Francisco, California and Seattle, Washington were analyzed to determine the accuracy of their labelling with respect to their THC and (where included) their CBD contents. The results were striking: Only 17% were accurately labelled with respect to their THC contents; 23% were underlabelled, and 60% were overlabelled. Some products were found to have only negligible amounts of THC. Similar findings were obtained for their CBD contents.] (b) Ruth AC, Gryniewicz-Ruzicka CM, Trehy ML, Kornspan N, Coody G. Consistency of label claims of internet-purchased hemp oil and cannabis products as determined using IMS and LC-MS: A marketplace survey. Journal of Regulatory Science 2016;4(3):1-6. [Notes: In this study, 20 “hemp oil products” (and 3 duplicates) were analyzed; 18 tested positive for at least one cannabinoid (three at less than 0.01%), but four labelled as containing CBD contained none, and three others contained CBD below their labelled contents.]

See, however: Kuwayama K, Yamamuro T, Tsujikawa K, Miyaguchi H, Kanamori T, Iwata YT, Inoue H. Utilization of matrix-assisted laser desorption/ionization imaging mass spectrometry to search for cannabis in herb mixtures. Analytical and Bioanalytical Chemistry 2014;406(19):4789-4794. [Notes: In this study, cannabis in herbal mixtures is found by spreading the mixture of plant material on an adhesive tape and scanning the tape with MALDI/IMS. It is certainly possible that this methodology – or a variation thereof – could be utilized to analyze solid food products containing herbal cannabis and/or other plant material(s).]

For an good overview of the extraction of cannabinoids (a review with numerous citations), see: Raharjo and Verpoorte, Reference #109.

See: Raharjo and Verpoorte, Reference #109.


See: Anonymous, Reference #44; and Anonymous, Reference #45.

See: Peschel W. Quality control of traditional cannabis tinctures, Reference #104. [Author’s comment: This is a well-done, quite extensive study of cannabis tinctures.]

119. See Politi, Peschel, et al., Reference #118.

120. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. Journal of the AOAC International 2003;86(2):412-431. [Note: This uses the QuEChERS technique.]


122. See: Holler, Bosy, et al., Reference #74.

123. Gibson CR, Williams RD, Browder RO. Analysis of Hempen Ale for cannabinoids. Journal of Analytical Toxicology 1998;22(2):179. [Note: This is a one page “Letter to the Editor”.]

124. See Vandrey, Raber, et al., Reference #111a. [Notes: Included multiple, unspecified products; THC and CBD were the primary focus, but other cannabinoids were also determined; two samples of each respective product were combined and analyzed by HPLC (the extracting solvent and workup procedure were not identified).]


127. Giroud C, Menetrey A, Augsburger M, Buclin T, Sanchez-Mazas P, Mangin P. Hemp tea versus hemp milk: Behavioural, physiological effects, blood, urine, saliva, and sweat cannabinoids levels following ingestion by 2 groups of 6 healthy volunteers. Z Zagadnien Nauk Sadowych (Problems of Forensic Sciences) 2000;42:102-110. [Notes: The authors reported that 1.6 mg and 23.2 mg of THC were recovered from 2 dL of water and milk, respectively – but provided no information on how this was determined. The much higher THC content in the milk was attributed to its lipophilic character.]


134. Ahmad GR, Ahmad N. Passive consumption of marijuana through milk: A low level chronic exposure to delta-9-tetrahydrocannabinol (THC). Journal of Toxicology – Clinical Toxicology 1990;28(2):255-260. [Notes: This is an ambiguous study. The analytical focus is on detection of trace 11-nor-delta-9-THC-9-carboxylic acid (i.e., the primary metabolite from THC) in buffalo milk and urine; however, the writeup implies several times that the THC in the milk was also determined – though not reported. The THC and deuterium-labelled THC (IS) were extracted by an (unspecified) organic solvent after alkaline hydrolysis, derivatized by bis-trimethyltrifluoroacetamide, and analyzed by GC/MS. Due to the ambiguity and lack of experimental details, this reference is included as “pertinent background” only.]


136. Perez-Reyes M, Wall ME. Presence of Δ9-tetrahydrocannabinol in human milk. New England Journal of Medicine 1982;307(13):819-820. [Notes: This was a “Letter to the Editor”, not a full article. Analysis by GC/MS (reported erroneously by Friguls, Joya, et al., Reference #129, as by LC/MS). No workup details were provided; therefore, this reference is included as “pertinent background” only.]


141. See: Petrovic, Debeljak, et al., Reference #58.


144. See: Holler, Bosy, et al., Reference #74.


146. See: Lachenmeier, Kroener, et al., Reference #69.


149. Zhang G, Guo J, Bi K. Study on the extraction process for cannabinoids in hemp seed oil by orthogonal design. Zhong Yao Cai 2005;28(5):417-418. [Notes: Determined that the “best” procedure for extracting cannabinoids from hempseed oil was two extractions with methanol, 15 minutes each. Not clear (from the abstract) how the extracts were analyzed. Written in Chinese.]

150. Chang CW, Tung CW, Tsai CC, Wu YT, Hsu MC. Determination of cannabinoids in hemp nut products in Taiwan by HPLC-MS/MS coupled with chemometric analysis: Quality evaluation and a pilot human study. Drug Testing and Analysis 2017;9(6):888-897. [Note: Although dated 2017, this article was actually posted on-line in late 2016.]

151. Zhou W-J, Song J-Z, Fu W-W, Tan H-S, Bian Z-X, Xu H-X. Chemical comparison of two dosage forms of hemp seed pills by UHPLC-Q-ToF-MS/MS and multivariate statistical techniques. Journal of Pharmaceutical and Biomedical Analysis 2013;84:59-68. [Notes: Although this article presents the analysis of (apparently) the same type “hemp nut” products as referenced by Chang, Tung, et al. (Reference #150), no cannabinoids were identified among the constituents – possibly because the products were only one component in a six herb mixture, and as a result the target phytocannabinoids were below the lower detection limit. Therefore, although the presented analytical procedures may be useful, this reference is included as “pertinent background” only.]

152. See: Ross, Mehmedic , et al., Reference #64. [Note: This article includes numerous pre-2000 citations regarding the analysis of hempseeds.]


155. USP Monographs for Bulk Drug Substances and Other Ingredients; 2016 edition (USP 40-NF 35); Dronabinol (#3907) and Dronabinol Capsules (#3908).


158. Wolf CE, Poklis JL, Poklis A. Stability of tetrahydrocannabinol and cannabidiol in prepared quality control medible brownies. Journal of Analytical Toxicology 2017;41(2):153-157. [Notes: The authors used a modification of the procedures by Jiang, Stenzel, et al. (Reference #161; see below). The study indicated that THC and CBD were not affected by the matrix or the baking temperatures (300°C); however, while a valuable contribution, in this author’s opinion the study would have been more insightful if THCA and CBD standards (which are thermally labile) had been included. Although dated 2017, this article was actually posted on-line in late 2016.]

159. See: Wang and Fanning, Reference #121.

160. Wang Q-l, Zhang A-z. UHPLC-MS/MS determination of characteristic cannabinoid in hemp food. Lihua Jianyan, Huaxue Fence 2013;49(6):720-724. [Notes: Not clear from the abstract what products were analyzed, or whether “characteristic cannabinoid” actually was CBN, or if THC was intended – the authors’ other articles indicated THC and other phytocannabinoids (see Zhang and Wang, References #s 142 and 143). Written in Chinese.]


162. See: Holler, Bosy, et al., Reference #74.


166. See: Vandrey, Raber, et al., Reference #111a. [Notes: Included multiple, unspecified products; THC and CBD were the primary focus, but other cannabinoids were also determined; two samples of each respective product were homogenized and analyzed by HPLC (the extracting solvent and workup procedure were not identified).]


168. See: Pellegrini, Marchei, et al., Reference #73. [Note: The presented method was subjected to a limited validation study.]

169. See: Below, Rosenstock, et al., Reference #70.

170. See: Lachenmeier, Kroener, et al., Reference #69. [Note: Method 1 was subjected to a limited validation study.]


172. For a selection of the more pertinent presentations (citations only) see: Klein RFX. The 2016 “Research on Drug Evidence” Report [From the 18th ICPO / INTERPOL Forensic Science Symposium]. Microgram Journal 2016;13(1-4):609-817. [Note: There is an extensive section in this triennial review that covers marijuana.]


174. See: (a) Ruth, Grynewicz-Ruzicka, et al., Reference #111b. (b) U.S. Food and Drug Administration. Warning Letters and Test Results for Cannabidiol-Related Products. Posted at: https://www.fda.gov/newsevents/publichealthfocus/ucm484109.htm [Date of Most Recent Access: December, 2016.]

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Synthesis and Characterization of Benzoylfentanyl and Benzoylbenzylfentanyl

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ABSTRACT: The synthesis and characterization of benzoylfentanyl and benzoylbenzylfentanyl via gas chromatography-mass spectrometry, Fourier transform infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy were conducted to confirm the identification of two seized exhibits.

KEYWORDS: Fentanyls, Fentanyl-Related Compounds, Illicit Drugs, Analysis, Forensic Chemistry.

Introduction

As of December 2017, fentanyl and 29 other fentanyl-related compounds (FRCs) have been identified in DEA casework [1]. Only a limited number of these compounds have been reported in the literature, however [1-5]. The syntheses and characterization of two new FRCs, benzoylfentanyl and benzoylbenzylfentanyl, are presented herein (Figures 1a,b). At this laboratory

Figure 1a. Benzoylfentanyl
[C_{26}H_{28}N_{2}O; mw = 384.5]

Figure 1b. Benzoylbenzylfentanyl
[C_{25}H_{26}N_{2}O; mw = 370.5]
(STRL), the structures of FRCs are typically confirmed by proton nuclear magnetic resonance spectroscopy (1H-NMR); however, the majority of forensic laboratories do not have NMR spectrometers, and therefore rely on gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR) for the identification of drug exhibits. In many cases, however, GC-MS and/or FTIR do not produce unambiguous identification and differentiation of closely related compounds; therefore, standards are needed for direct comparisons of retention data and spectra. Herein, we report the syntheses of benzoylfentanyl and benzoylbenzylfentanyl and their characterization by GC-MS, FTIR, and 1H-NMR. The GC-MS results allow for the unambiguous identification of these two FRCs in sample analysis.

**Experimental**

**Chemicals, Reagents, and Materials**

All solvents (except the NMR solvents) were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). NMR solvents were from Cambridge Isotopes (Tewksbury, MA). All other chemicals were reagent-grade quality and products of Sigma-Aldrich Corporation (Milwaukee, WI).

**Synthesis of Benzoylfentanyl and Benzoylbenzylfentanyl**

Both syntheses were conducted at this laboratory. In accordance with Journal policy, exact experimental details are not provided, but are outlined in Figures 2 and 3 (see next page). Briefly, 4-ANPP was reacted with benzoyle chloride to give benzoylfentanyl, which was converted to its HCl ion-pair in 80% overall yield. 1-Benzyl-4-piperidone was reacted with aniline to give 1-benzyl-4-anilinopiperidone, which was then reacted with benzoyl chloride to give benzoylbenzylfentanyl, which in turn was converted to its HCl ion-pair in 37% overall yield.

**Gas Chromatography-Mass Spectrometry**

Mass spectra were obtained on two different instruments, as detailed below. GC-MS Method #1 was used at this laboratory for the analyses of the benzoylbenzylfentanyl exhibit and reference standard, while GC-MS Method #2 was used at the Mid-Atlantic Laboratory for the analyses of the benzoylfentanyl exhibit and reference standard.

Method #1: Mass spectra were obtained on an Agilent Model 5975C quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 7890A gas chromatograph (GC) (Palo Alto, CA). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34-600 amu, and a scan rate of 2.59 scans/s. 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°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 source was operated at 230°C.

Method #2: Mass spectra were obtained on an Agilent Model 5977A quadrupole MSD that was interfaced with an Agilent Model 7890B GC. The MSD was operated in the EI mode with an ionization potential of 70 eV, a scan range of 40-500 amu, and a scan rate of 3.2 scans/s. The GC was fitted with a 15 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-5MSUI (J & W Scientific). The oven temperature was programmed as follows: Initial temperature, 145°C; initial hold,
Figure 2. Synthesis of Benzoylfentanyl.

1.0 min; program rate, 20°C/min to 280°C, hold for 0.25 min; program rate, 45°C/min to 295°C, hold for 3.167 min. The injector was operated in the split mode (20 : 1) at 280°C. The MSD source was operated at 230°C.

Fourier Transform Infrared Spectroscopy / Attenuated Total Reflectance
Infrared spectra were obtained on a Thermo-Nicolet Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory (Madison, Wisconsin). Instrument parameters were: Resolution = 4 cm⁻¹; gain = 8;

Figure 3. Synthesis of Benzoylbenzylfentanyl.

optical velocity = 0.4747; aperture = 150; and scans/sample = 16.

Proton Nuclear Magnetic Resonance Spectroscopy
1H-NMR spectra were obtained on an Agilent 600MR-DD2 600 MHz NMR equipped with a 5
mm OneNMR pulse field gradient probe (Palo Alto, CA). The sample temperature was maintained at 25°C. Standard Agilent pulse sequences were used to obtain proton, carbon-13 (proton decoupled), HSQC, HMBC (C13 and N15), COSY, H2BC, and NOESY spectra for structural elucidation (however, only the proton spectra are presented herein). Samples were dissolved in 1 mL deuterated chloroform (CDCl₃) containing 0.03% v/v tetramethylsilane (TMS) as the 0 ppm reference.

Results and Discussion

Case #1 (approximately 36 grams of white powder seized in West Virginia in July, 2017; exact location and details sensitive): The exhibit was submitted to the DEA Mid-Atlantic Laboratory. GC-MS (Method #2) analysis (Figure 4, upper) identified benzoic acid, α-PVP, caffeine, U-47700, alprazolam, and an unknown, late-eluting compound at approximately 14.6 minutes. The unknown produced a mass spectrum with a base peak at m/z 105 and other major ions at m/z 77, 197, and 293 (Figure 5, next page, upper). The apparent molecular ion (i.e., M-2H) was at m/z 382. The unknown was therefore suspected to be benzoylfentanyl based on the fragment ions and the late retention time. The Mid-Atlantic Laboratory requested that the Fentanyl Signature Profiling Program of this laboratory

![Figure 4. Reconstructed Ion Chromatograms of (Upper) Case #1 and (Lower) Case #2.](image)
(FSPP) synthesize a reference standard of this compound. GC-MS comparison of the synthesized reference material with the unknown peak gave an identical retention time and mass spectrum, thus confirming the identification. The concentration of benzoylfentanyl in the powder was determined to be approximately 1%.

Case #2 (approximately 48 grams of white powder seized in California in August, 2017; exact location and details sensitive): The exhibit was submitted to the DEA Western Laboratory. GC-MS (Method #1) analysis (Figure 4, lower) identified benzylfentanyl and an unknown, late-eluting compound at approximately 32.5 minutes. The unknown produced a mass spectrum with a base peak at $m/z$ 91 and other major ions at $m/z$ 77, 82, 105, 146, 172, and 265 (Figure 5, lower). The apparent molecular ion was at $m/z$ 370. The unknown was therefore suspected to be benzoylbenzylfentanyl based on the fragment ions and the late retention time. The Western Laboratory requested that the FSPP synthesize a reference standard of this compound. GC-MS comparison of the synthesized reference material with the unknown peak gave an identical retention time and mass spectrum, thus confirming the identification. The concentration of benzoylbenzylfentanyl in the powder was determined to be approximately 64%.
The FTIR/ATR and 1H-NMR spectra for the benzoylfentanyl HCl and benzoylbenzylfentanyl HCl reference standards, as acquired at this laboratory, are illustrated in Figures 6 and 7, respectively (next two pages); Figure 7 includes expansion plots. [The actual case exhibits were not submitted to FTIR or NMR analyses at this laboratory.]

Cautionary Note

The relative potencies of these two FRCs are unknown; for this reason, analysts should exercise appropriate care with exhibits suspected to contain either one of these compounds – or any other FRCs [6].

References

[1] Song K, de Armas AM. The analytical profile of fluorobutyryl fentanyl isomers. Abstracts, American Academy of Forensic Sciences 70th Annual Meeting, Seattle, Washington, February 19 - 24, 2018:B189. [Note: According to the Advanced Program, 15 presentations on fentanyl and/or fentanyl-related compounds will be made at this meeting; see: https://www.aafs.org/wp-content/uploads/CompleteAP.pdf [Date of most recent access: January 24, 2018.]]


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[Figures 6 and 7 Follow.]

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Figure 6. FTIR/ATR (Upper) of Benzoylfentanyl HCl and (Lower) of Benzoylbenzylfentanyl HCl.
Figure 7. 1H NMR Spectra of (A) Benzoylfentanyl HCl and (B) Benzoylevbenzylfentanyl HCl. [Note: Compound preparation solvent impurities are marked with an asterisk (*) above them.]

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