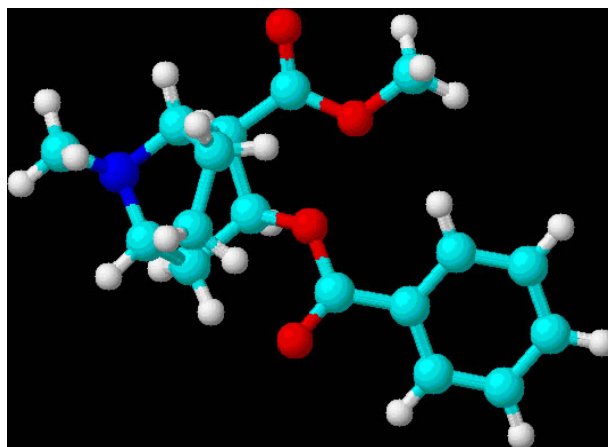

Microgram

Journal

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

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

Letter to the Editor regarding: Vohlken BA, Layton SM. Instrumental Separation of 3,4-Methylenedioxyamphetamine (MDA) from 1-(3,4-Methylenedioxyphenyl)-2-propanol, a Co-Eluting Compound. *Microgram Journal* 2003(1-2):32.

Sir:

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

Peter Ausili
DEA North Central Laboratory, Chicago, Illinois

* * * * *

Technical Note

The Identification of 5-Methoxy-*alpha*-methyltryptamine (5-MeO-AMT)

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

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

Summary

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

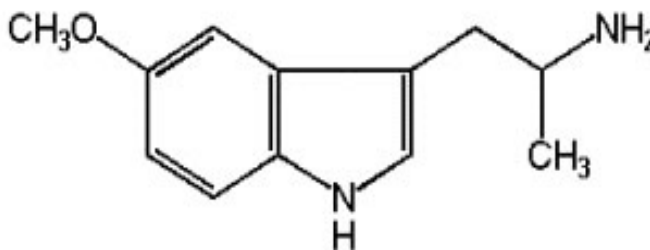


Figure 1: Structure of 5-Methoxy-*alpha*-methyltryptamine (C₁₂H₁₆N₂O; mw = 204.27)

Experimental

Color Tests

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

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

Tryptamine	Sodium Nitroprusside Test
α -Methyltryptamine	Cherry Red
<i>N</i> -Methyltryptamine	Purple
5-Methoxy- α -methyltryptamine	Cherry Red

Table 1: Sodium Nitroprusside Reagent Test Results

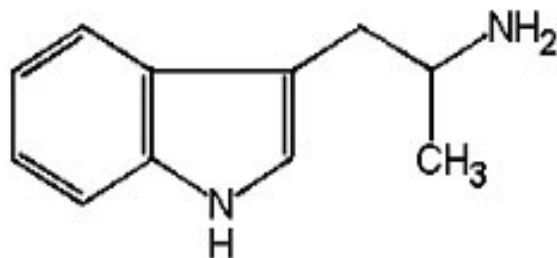


Figure 2: Structure of *alpha*-Methyltryptamine ($C_{11}H_{14}N_2$; mw = 174.24)

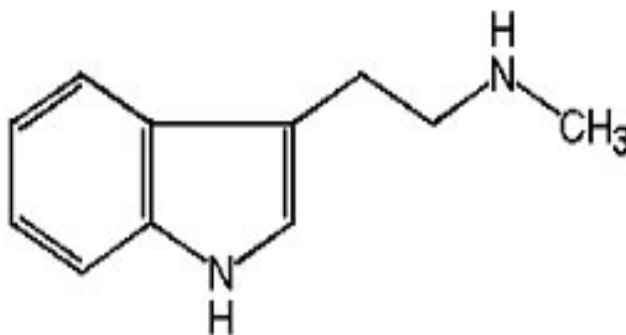


Figure 3: Structure of *N*-Methyltryptamine ($C_{11}H_{14}N_2$; mw = 174.24)

Gas Chromatography / Mass Spectrometry

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

Tryptamine	Retention Time (Minutes)
α -Methyltryptamine (AMT)	3.54
<i>N</i> -Methyltryptamine (NMT)	3.65
5-Methoxy- α -methyltryptamine (5-MeO-AMT)	4.67
5-Methoxy- <i>N,N</i> -dimethyltryptamine (5-MeO-DMT)	4.84

Table 2: Retention Times for Tryptamine Standards

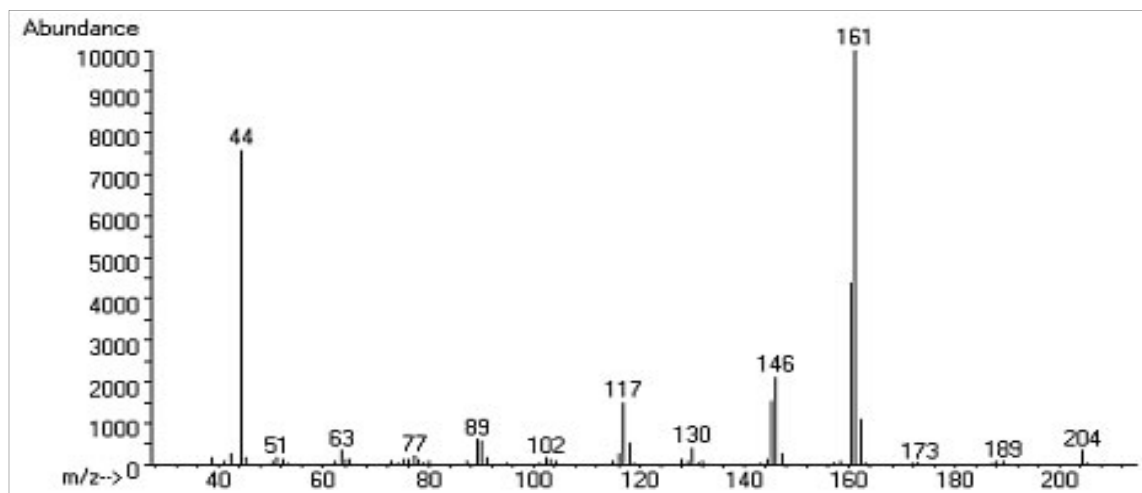


Figure 4: Mass Spectrum of 5-Methoxy-*alpha*-methyltryptamine

Results and Discussion

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

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

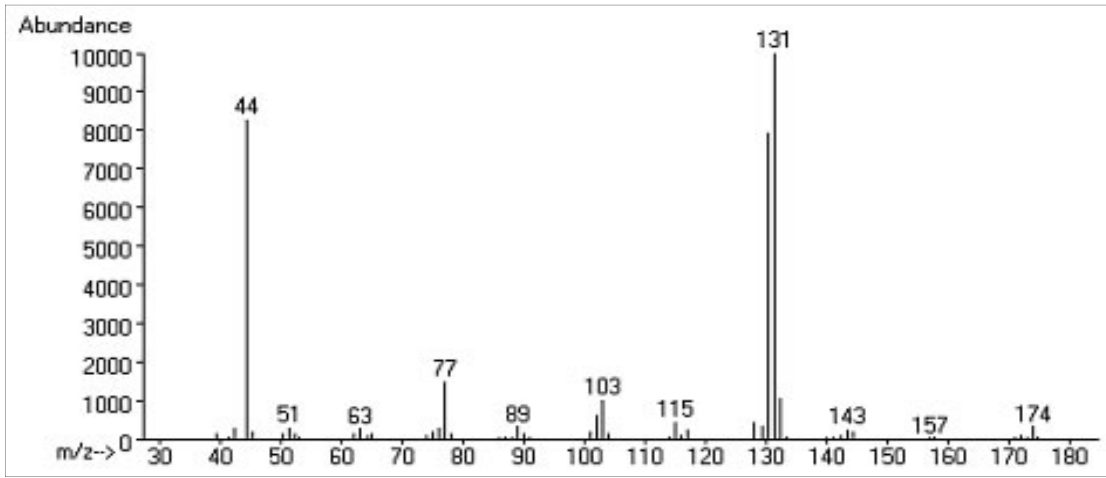


Figure 5a: Mass Spectrum of *N*-Methyltryptamine

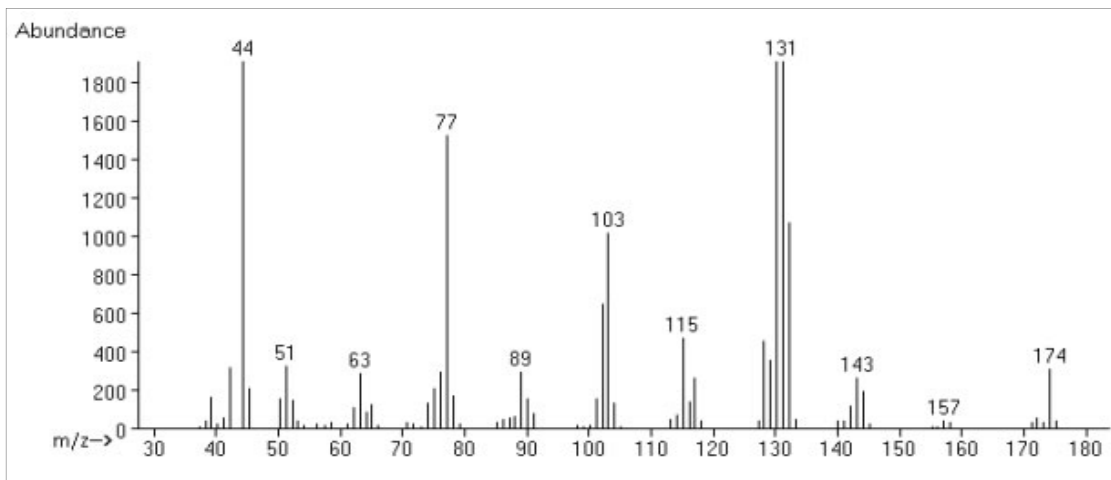


Figure 5b. Expanded Mass Spectrum of *N*-Methyltryptamine

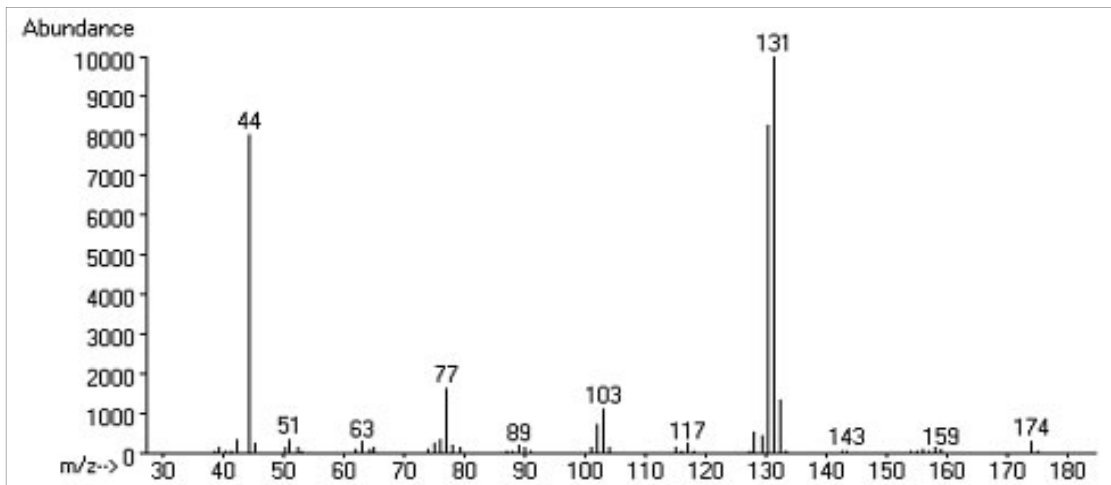


Figure 6a: Mass Spectrum of α -Methyltryptamine

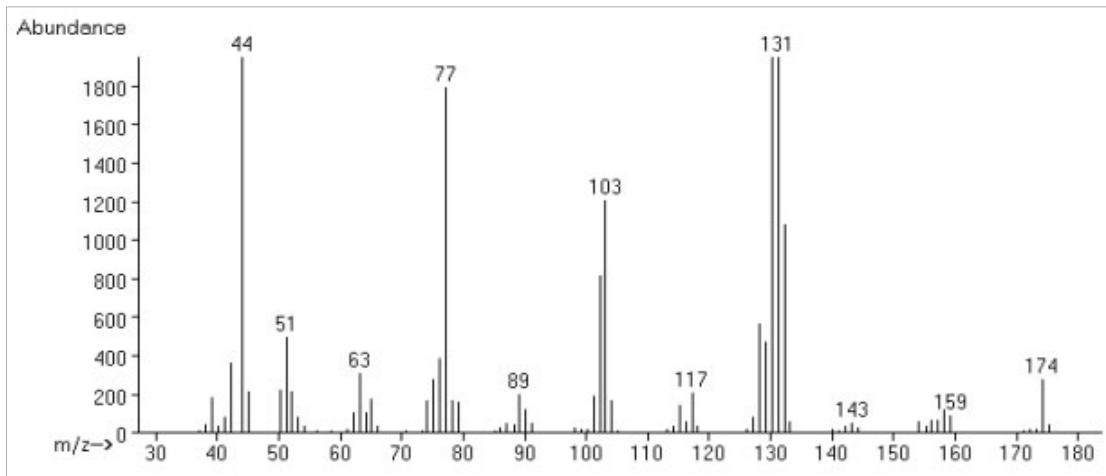


Figure 6b. Expanded Mass Spectrum of *alpha*-Methyltryptamine

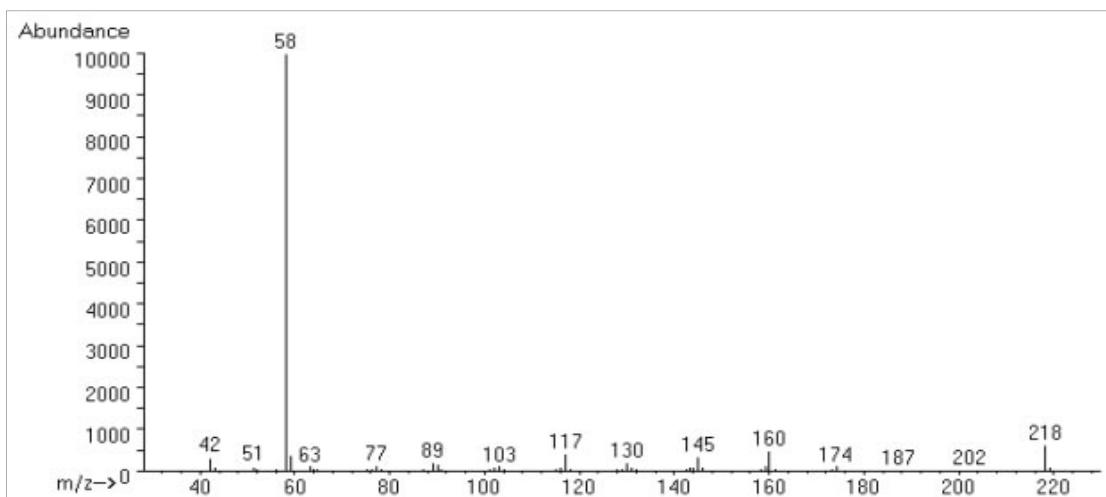


Figure 7. Mass Spectrum of 5-Methoxy-*N,N*-dimethyltryptamine

Acknowledgements

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

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Technical Note

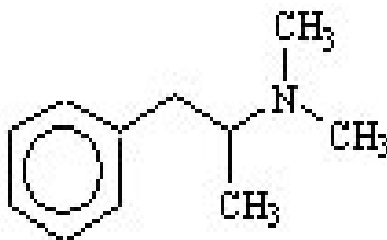
The Identification of *d-N,N*-Dimethylamphetamine (DMA) in an Exhibit in Malaysia

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

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



Introduction

This laboratory recently received an exhibit consisting of approximately 200 grams of a white crystalline substance that was suspected to be methamphetamine hydrochloride. Crystalline methamphetamine hydrochloride (known locally by the street name of “syabu”) is very frequently encountered by the Central Laboratory and its nine branch laboratories. However, in this case the substance was instead determined to be *d-N,N*-dimethylamphetamine hydrochloride with traces of methamphetamine. There have been occasional literature reports of dimethylamphetamine in the United States, some of which included analytical data (*vide infra*); however, those reports were in a law enforcement restricted periodical (*Microgram*), and so are not generally available. More recently, crystalline dimethylamphetamine was reported to be a low prevalence drug of abuse in Japan, making its first appearance there in 1998¹. To our knowledge, this is the first report of dimethylamphetamine in Malaysia. Dimethylamphetamine is not currently designated as a controlled substance or “dangerous drug” in Malaysia (that is, like amphetamine or methamphetamine). This paper presents a brief of our analytical findings.

Experimental

Color Test and Reagents

Marquis Reagent and Simon’s Regent: These were prepared from analytical grade reagents according to the standard formulations given in the literature².

GC/MS

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

HPLC

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

FTIR

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

Melting Point

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

Polarimetry

The optical rotation of two solutions containing 0.048 grams/mL and 0.024 grams/mL of sample in distilled water were measured with a Bellingham & Stanley (London) polarimeter³. The accuracy of the instrument was checked by determining the specific optical rotation of a sucrose standard solution (9.78 grams/100mL) and comparing with the literature value⁴. An analytical grade sucrose from Mayer & Baker was used.

Results and Discussion

Color Tests

Treating the sample with the Marquis reagent gave a color change from orange to brown. A faint blue color developed slowly with the Simon's reagent. As a tertiary amine, DMA should not produce a color change with Simon's reagent⁵; therefore, this result suggested the low-level presence of a secondary amine (such as methamphetamine) or some other contaminant.

GC/MS

The GC/MS chromatogram showed two peaks – a small peak preceding a much larger one. The mass spectrum of the large peak (Figure 1) was typical of phenethylamines in that it had a dominant parent ion (at $m/z = 72$) but otherwise only small fragment ions. The spectrum of the primary component was found to be similar to the literature DMA spectra^{6,7,8,9}, while the small peak was identified as methamphetamine from its mass spectrum and retention time. The low level presence of methamphetamine was consistent with the findings from the color tests.

HPLC

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

FTIR

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

Melting Point

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

Polarimetry

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

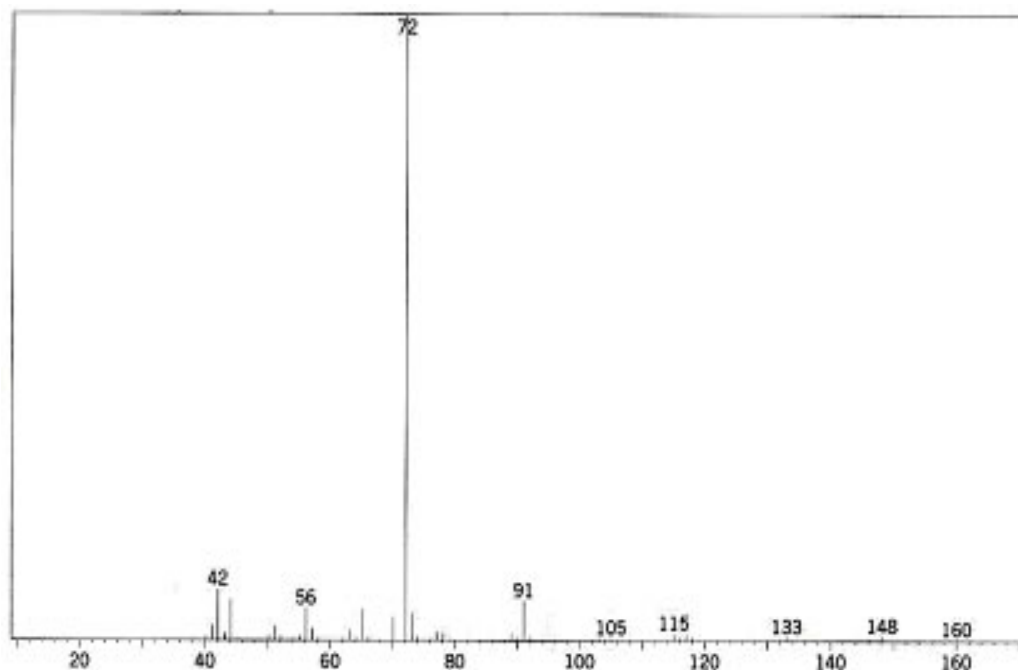


Figure 1. Mass Spectrum of Sample (*N,N*-Dimethylamphetamine)

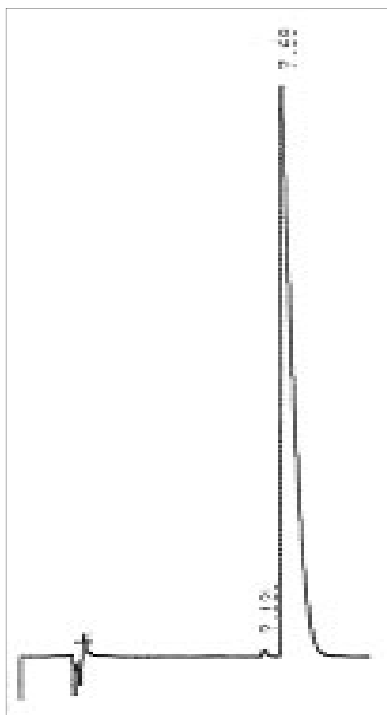


Figure 2. HPLC of Sample (Methamphetamine = 7.12 minutes;
N,N-Dimethylamphetamine = 7.68 Minutes)

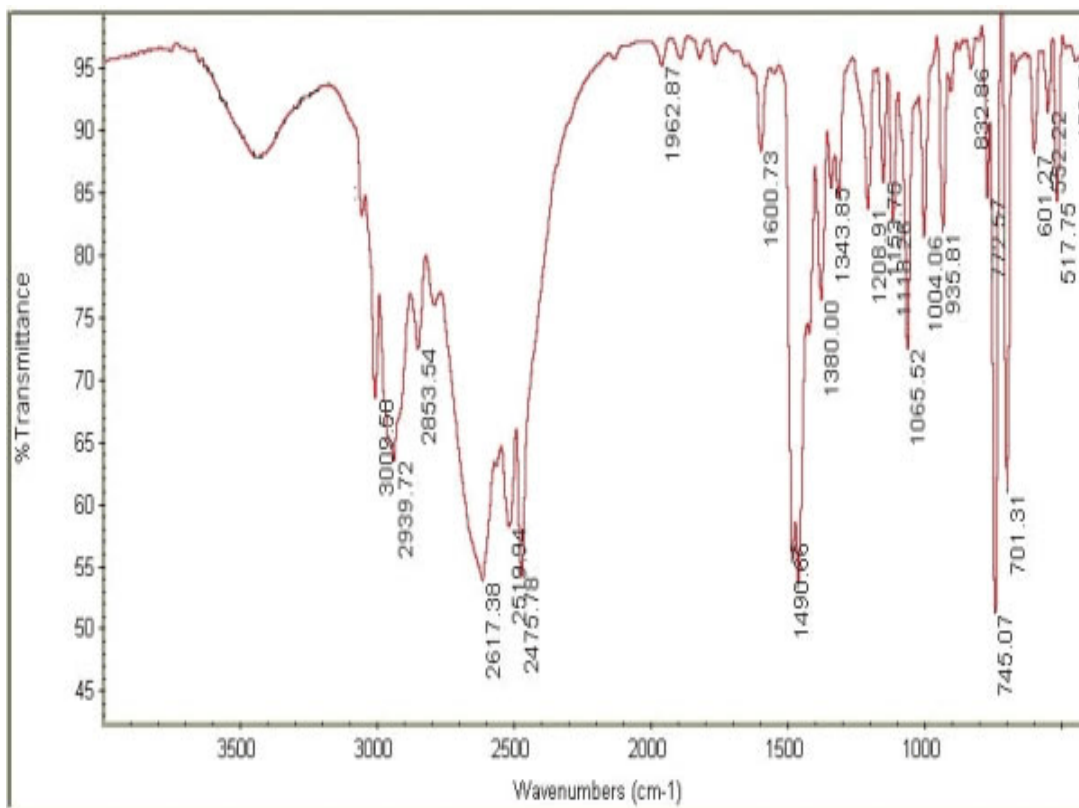


Figure 3: FTIR Spectrum of Sample (98% *N,N*-Dimethylamphetamine Hydrochloride)

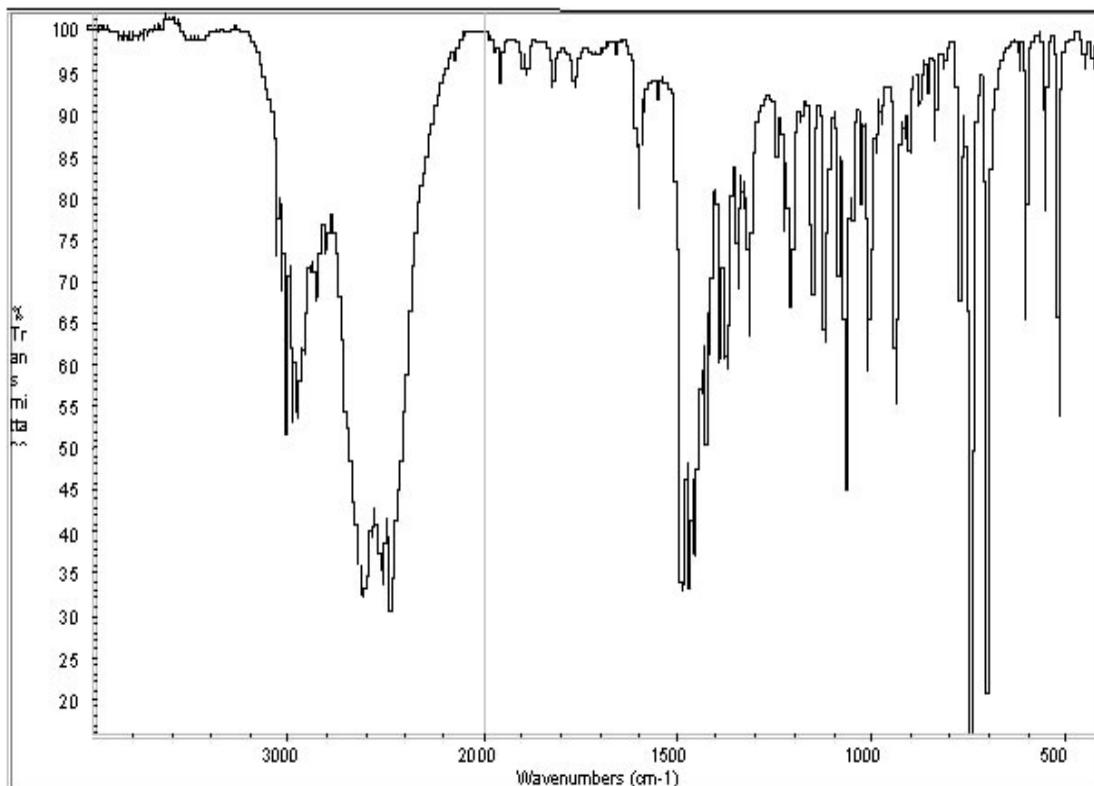


Figure 4: IR Spectrum of Reference Grade *N,N*-Dimethylamphetamine Hydrochloride (Source: Forensic Science Laboratory of the Osaka Prefectural Police Headquarters)

Conclusions

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

Acknowledgements

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

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

* * * * *

Profiling of Ecstasy Tablets Seized in Japan

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

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

Introduction

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

Experimental

Chemicals

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

Physical Characteristics

Fifteen parameters (logo, cleavage, coat, vertical view, horizontal view, diameter, thickness, weight, smell, outside color, inside color, color, toughness, capping, and appearance of logo) were measured to establish the

physical characteristics of each tablet. The tablets were also photographed with a digital camera. An identification number was applied to each tablet, consisting of a logo number, color number, and serial number.

Extraction Procedure

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

GC/MS Analysis

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

HPLC Analysis

A Shiseido Nanospace HPLC, equipped with a UV detector linked to a data system (S-MC, Shiseido, Tokyo, Japan), was used for qualitative and quantitative analysis of the components in the tablets. Chromatographic separation was achieved using an ODS-type semi-microcolumn (CAPCELL PAK C18 UG 120 S5, 250 mm x 1.5 mm i.d.). The mobile phase used for ephedrine, MDA, MDMA, MDEA, methamphetamine, and ketamine was 5 mmol/L SDS in 20 mmol/L KH₂PO₄-CH₃CN (65:35). The mobile phase used for caffeine was H₂O-methanol (7:3). The flow rate was maintained at 0.1 mL/minute. Separation was carried out at 50 °C for ephedrine, MDA, MDMA, MDEA, methamphetamine, and ketamine, and 35 °C for caffeine. The monitoring wavelength was 210 nm for ephedrine, MDA, MDMA, MDEA, methamphetamine, and ketamine, and 254 nm for caffeine. Good linearity for this quantitative analysis was confirmed over the concentration range of 0.1 – 0.8 mg/mL ($r^2 = 0.9993 - 0.9997$ for six compounds).

Results and Discussion

Physical Characteristics

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

















Chemical Characteristics





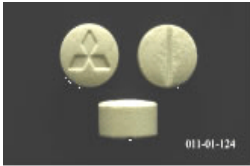















The active ingredients in each tablet were identified by GC/MS and HPLC. The detected components were MDMA, MDA, ephedrine, caffeine, ketamine, and methamphetamine. Thirty-five tablets contained MDMA as the sole active ingredient. The content range (calculated as MDMA hydrochloride) was 37 - 160 mg/tablet.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

















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

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

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

<p>Tulip</p>  <p>MDMA 160 mg/tab</p>	<p>B29</p>  <p>MDMA 157 mg/tab</p>	<p>B29</p>  <p>MDMA 151 mg/tab</p>	<p>XL</p>  <p>MDMA 146 mg/tab</p>
<p>Mitsubishi</p>  <p>MDMA 103 mg/tab</p>	<p>cK</p>  <p>MDMA 98 mg/tab</p>	<p>XL</p>  <p>MDMA 98mg/tab</p>	<p>Bird (Fry)</p>  <p>MDMA 89 mg/tab</p>
<p>Mickey Mouse</p>  <p>MDMA 88 mg/tab</p>	<p>Crocodile</p>  <p>MDMA 87 mg/tab</p>	<p>CU</p>  <p>MDMA 87 mg/tab</p>	<p>FF</p>  <p>MDMA 80 mg/tab</p>
<p>Star</p>  <p>MDMA 77 mg/tab</p>	<p>007</p>  <p>MDMA 70 mg/tab</p>	<p>Smiley</p>  <p>MDMA 69 mg/tab</p>	<p>(No Logo)</p>  <p>MDMA 66 mg/tab</p>

<p>Mitsubishi</p>  <p>MDMA 64 mg/tab</p>	<p>!</p>  <p>MDMA 64 mg/tab</p>	<p>Mitsubishi</p>  <p>MDMA 64 mg/tab</p>	<p>Butterfly</p>  <p>MDMA 63 mg/tab</p>
<p>Mitsubishi</p>  <p>MDMA 61 mg/tab</p>	<p>Lozenge</p>  <p>MDMA 61 mg/tab</p>	<p>Superman</p>  <p>MDMA 60 mg/tab</p>	<p>Armani</p>  <p>MDMA 59 mg/tab</p>
<p>Lozenge</p>  <p>MDMA 57 mg/tab</p>	<p>Propeller</p>  <p>MDMA 57 mg/tab</p>	<p>Cellular Phone</p>  <p>MDMA 56 mg/tab</p>	<p>Smurf</p>  <p>MDMA 54 mg/tab</p>
<p>Motorola</p>  <p>MDMA 51 mg/tab</p>	<p>007</p>  <p>MDMA 50 mg/tab</p>	<p>Smiley</p>  <p>MDMA 46 mg/tab</p>	<p>Fish</p>  <p>MDMA 42 mg/tab</p>
<p>Hole</p>  <p>MDMA 42 mg/tab</p>	<p>Smiley</p>  <p>MDMA 40 mg/tab</p>	<p>Mercedes</p>  <p>MDMA 37 mg/tab</p>	<p>Tower</p>  <p>MDMA 106 mg/tab Caffeine 35 mg/tab</p>

<p style="text-align: center;">Tower</p>  <p style="text-align: center;">MDMA 98 mg/tab Caffeine 29 mg/tab</p>	<p style="text-align: center;">Popeye</p>  <p style="text-align: center;">MDMA 98 mg/tab Caffeine 40 mg/tab</p>	<p style="text-align: center;">Bearded Face</p>  <p style="text-align: center;">MDMA 88 mg/tab Caffeine 28 mg/tab</p>	<p style="text-align: center;">Butterfly</p>  <p style="text-align: center;">MDMA 80 mg/tab Caffeine 27 mg/tab</p>
<p style="text-align: center;">RN</p>  <p style="text-align: center;">MDMA 53 mg/tab Caffeine 6 mg/tab</p>	<p style="text-align: center;">Yin-Yang</p>  <p style="text-align: center;">MDMA 38 mg/tab Caffeine 62 mg/tab</p>	<p style="text-align: center;">(No Logo)</p>  <p style="text-align: center;">MDMA 100 mg/tab MA 4 mg/tab Ketamine - Trace</p>	<p style="text-align: center;">Crown (Cross)</p>  <p style="text-align: center;">MDMA 86 mg/tab MA 2 mg/tab Ketamine 5 mg/tab</p>
<p style="text-align: center;">SX</p>  <p style="text-align: center;">MDMA 84 mg/tab MA 3 mg/tab Ketamine 4 mg/tab</p>	<p style="text-align: center;">SX</p>  <p style="text-align: center;">MDMA 72 mg/tab MA 3 mg/tab Ketamine 4 mg/tab</p>	<p style="text-align: center;">Spider</p>  <p style="text-align: center;">MDMA 64 mg/tab MA 21 mg/tab Ketamine 43 mg/tab</p>	<p style="text-align: center;">Crown (Ruff)</p>  <p style="text-align: center;">MDMA 64 mg/tab MA 21 mg/tab Ketamine 43 mg/tab</p>
<p style="text-align: center;">V2K</p>  <p style="text-align: center;">MDMA 43 mg/tab MA 10 mg/tab Ketamine 64 mg/tab</p>	<p style="text-align: center;">Y2K</p>  <p style="text-align: center;">MDMA 38 mg/tab MA 16 mg/tab Ketamine 48 mg/tab</p>	<p style="text-align: center;">Y2K</p>  <p style="text-align: center;">MDMA 37 mg/tab MA 16 mg/tab Ketamine 52 mg/tab</p>	<p style="text-align: center;">Mitsubishi</p>  <p style="text-align: center;">MDMA 5 mg/tab MA 2 mg/tab Ketamine - trace</p>



888  MDA 75 mg/tab	Two Hearts  MDA 54 mg/tab Ephedrine 40 mg/tab	*****	*****
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Figure Abbreviations: MA - Methamphetamine; MDA - 3,4-Methylenedioxyamphetamine; MDMA - 3,4-Methylenedioxymethamphetamine.

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

ID No.	Logo	Diameter	Thickness (mm)	Weight (mg)	Outside color	Active Ingredients (Percent per Tablet)
101	"B29"	8.3	4.7	287	blue	MDMA (55 %)
102	"B29"	8.3	4.5	285	brown	MDMA (53 %)
103	"!"	8.3	3.3	205	yellow	MDMA (31 %)
104	Bird (Fry)	7.1	3.0	140	blue	MDMA (64 %)
105	Mickey Mouse	7.1	4.3	225	brown	MDMA (39 %)
106	Motorola	8.5	3.6	140	brown	MDMA (36 %)
107	Mitsubishi	9.3	4.4	297	grey	MDMA (22 %)
108	Tower	8.2	5.0	325	yellow	MDMA (30 %) caffeine (9 %)
109	Smiley	7.1	3.5	168	orange	MDMA (24 %)
110	"SX"	8.1	4.3	218	orange	MDMA (33 %) methamphetamine (1 %) ketamine (2 %)
111	"007"	7.2	4.7	224	brown	MDMA (22 %)
112	Propeller	10.1	4.4	305	white	MDMA (19 %)
113	Crocodile	8.1	4.6	227	grey	MDMA (38 %)
114	Smiley	7.2	4.2	202	green	MDMA (34 %)
115	Fish	7.2	3.0	139	blue	MDMA (30 %)
116	"FF"	7.1	5.2	256	yellow	MDMA (31 %)
117	"RN"	8.1	3.9	216	green	MDMA (25 %) caffeine (3 %)
118	Bearded Face	8.2	5.2	309	yellow	MDMA (28 %) caffeine (9 %)
119	Yin-Yang	8.2	4.3	259	white	MDMA (15 %) caffeine (24 %)
120	"Y2K"	9.1	3.2	207	green	MDMA (18 %) methamphetamine (8 %) ketamine (23 %)
121	"Y2K"	9.2	3.4	209	green	MDMA (18 %) methamphetamine (8 %) ketamine (25 %)
122	Popeye	8.6	4.2	261	white	MDMA (38 %) caffeine (15 %)

123	Two Hearts	8.1	5.1	226	purple	MDA (24 %) ephedrine (18 %)
124	Mitsubishi	8.2	5.4	311	white	MDMA (20 %)
125	Armani	8.9	4.7	315	white	MDMA (19 %)
126	"cK"	8.2	4.9	304	grey	MDMA (32 %)
127	Hole	8.5	4.5	282	green	MDMA (15 %)
128	Crown (Ruff)	8.8	5.8	204	white	MDMA (25 %) methamphetamine (5 %) ketamine (10 %)
129	"XL"	8.2	4.9	294	orange	MDMA (33 %)
130	Crown (Cross)	9.1	4.1	221	brown	MDMA (39 %) methamphetamine (1 %) ketamine (2 %)
131	"CU"	8.1	4.6	262	green	MDMA (33 %)
132	Cellular Phone	9.2	4.3	274	white	MDMA (20 %)
133	Spider	9.2	7.0	260	blue	MDMA (25 %) methamphetamine (8 %) ketamine (17 %)
134	"SX"	8.1	4.2	234	brown	MDMA (36 %) methamphetamine (1 %) ketamine (2 %)
135	Tulip	8.4	6.5	348	brown	MDMA (46 %)
136	"XL"	8.2	4.8	265	orange	MDMA (55 %)
137	Star	8.6	6.2	328	green	MDMA (23 %)
138	Mercedes	7.1	2.6	105	white	MDMA (35 %)
139	Lozenge	7.2	3.3	161	white	MDMA (35 %)
140	Superman	9.2	3.6	281	white	MDMA (21 %)
141	Smurf	9.1	3.8	280	white	MDMA (19 %)
142	"007"	8.1	5.0	311	red	MDMA (23 %)
143	no logo	7.3	4.5	179	orange	MDMA (37 %)
144	Butterfly	8.0	5.0	307	yellow	MDMA (21 %)
145	Lozenge	7.1	3.3	165	white	MDMA (37 %)
146	"V2K"	9.2	3.0	234	orange	MDMA (18 %) methamphetamine (4 %) ketamine (27 %)
147	Mitsubishi	8.0	5.0	304	yellow	MDMA (34 %)
148	no logo	8.1	4.3	254	green	MDMA (39 %) methamphetamine (2 %) Ketamine trace
149	"888"	8.2	4.3	217	rose	MDA (35 %)
150	Mitsubishi	9.0	5.0	319	white	MDMA (20 %)
151	Butterfly	9.2	4.4	326	green	MDMA (25 %)
152	Mitsubishi	9.4	5.0	306	green	MDMA (2 %) methamphetamine (1 %) Ketamine trace
153	Smiley	7.1	3.2	180	orange	MDMA (26 %)
154	Tower	8.1	5.0	333	yellow	MDMA (32 %) caffeine (11 %)

Acknowledgments

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Technical Note

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

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

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

Introduction

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

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

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

The most common analytical techniques reported in the literature for analysis of hallucinogenic mushrooms are all based on methanol extraction. In the most common procedure, the mushrooms are simply soaked in methanol overnight, and the resulting extracts condensed to near dryness and then analyzed using TLC and GC/MS (7-8). A more rapid technique involves placing the mushrooms in a closed vial with methanol, heating for a half an hour, then heating to dryness; the resulting residue is taken into 0.1 N sodium hydroxide, then extracted with

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

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

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

Materials and Methods

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

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

Procedure for Pure Mushrooms:

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

8. The biphasic solution is centrifuged, and the chloroform layer collected in a shell vial.
9. The chloroform extract is concentrated under air, transferred to a micro vial, and analyzed on the GC/MS.

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

Procedure for Mushroom/Chocolate Concoctions:

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

The results are shown in Figure 2.

Results and Discussion

The presented acetic acid facilitated extraction of psilocyn from mushrooms is more rapid and convenient versus traditional methanolic extraction procedures, which require long time frames or potentially destructive heating. In addition, the use of sodium bicarbonate as a neutralization agent keeps the pH below 8.5, thereby avoiding base-facilitated destruction of psilocyn. The Total Ion Chromatogram (TIC) of the mushroom only sample shows a clean psilocyn peak (Figure1). Analysis by TLC also shows only psilocyn. No psilocybin was detected - this is perhaps due to the activity of the phosphatase enzymes present in the mushrooms, which can dephosphorylate psilocybin to psilocyn in aqueous medium (19).

Analysis of mushroom/chocolate concoctions requires additional cleanup steps. Analysis of the chloroform extract at Step 12 (that is, before the extract was washed with sodium bicarbonate) showed three peaks in the TIC (Figure 2). The small peak at 4.223 minutes is due to caffeine, the broad peak at 4.50 minutes is due to theobromine, and the sharp peak at 4.837 minutes is due to psilocyn. Caffeine and theobromine (both purine alkaloids) result from the chocolate; theobromine is the main alkaloid in chocolate (2.8 - 3.5 % in cocoa), and caffeine is another major alkaloid (0.1 - 0.4 %) (20). After washing the chloroform extract (at Step 12) with 2 % sodium bicarbonate solution, the amounts of theobromine and caffeine are very low (see Figure 3), resulting in a nearly clean TIC showing only psilocyn. However, the mass spectrum of psilocyn depicted in Figure 3 showed an extraneous ion 109, probably resulting from trace theobromine. When the chloroform extract was washed with

water only, the peak due to theobromine nearly disappears, and there is no extraneous 109 fragment, but the peak due to caffeine is still present (see Figure 4).

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

The mass spectra acquired in this study for caffeine, theobromine, and psilocyn are presented in Figures 5 -7.

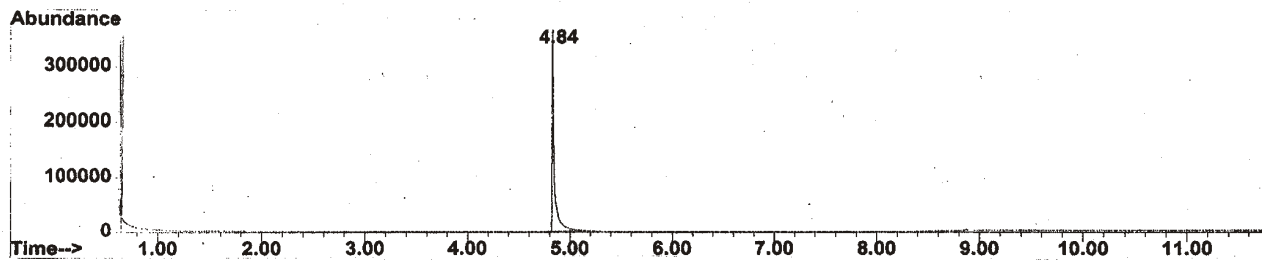


Figure 1. Total Ion Chromatogram of Mushrooms (Only) using Acetic Acid and Sodium Bicarbonate (4.84 Minutes = Psilocyn).

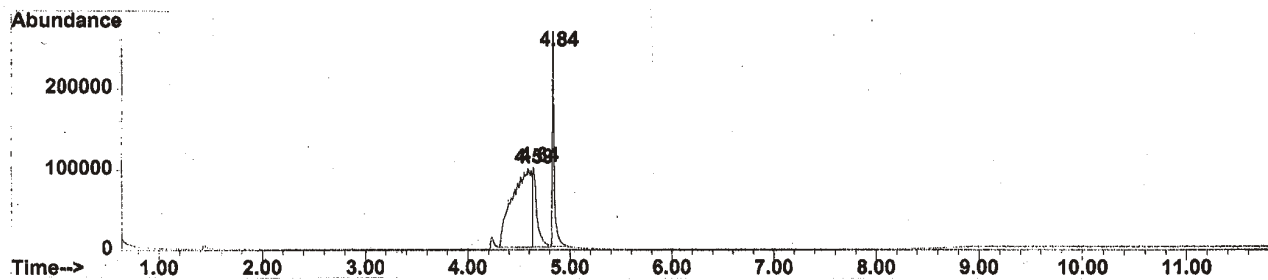


Figure 2. Total Ion Chromatogram of Mushroom/Chocolate Concoctions using Acetic Acid and Sodium Bicarbonate (4.22 Minutes = Caffeine; 4.50 Minutes = Theobromine; and 4.84 Minutes = Psilocyn).

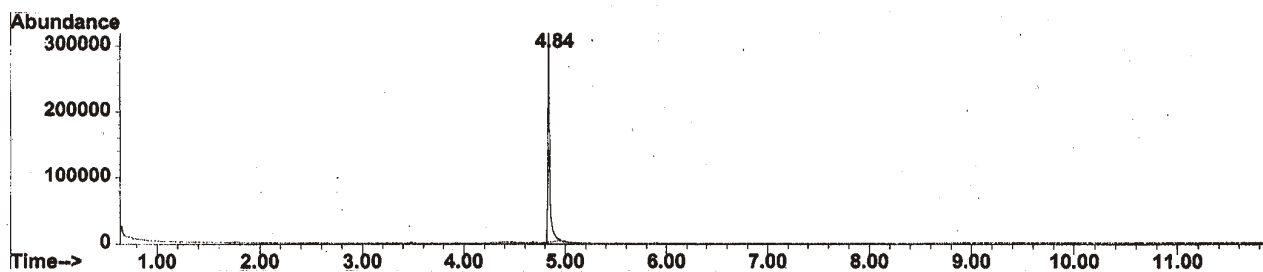


Figure 3. Total Ion Chromatogram of Mushroom/Chocolate Concoctions when the Chloroform Extract was Washed with 2 Percent Aqueous Sodium Bicarbonate.

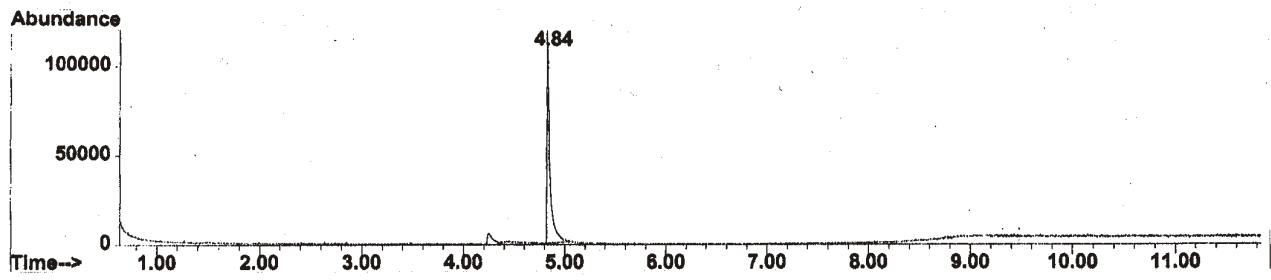


Figure 4. Total Ion Chromatogram of Mushroom/Chocolate Concoctions when the Chloroform Extract was Washed with Water.

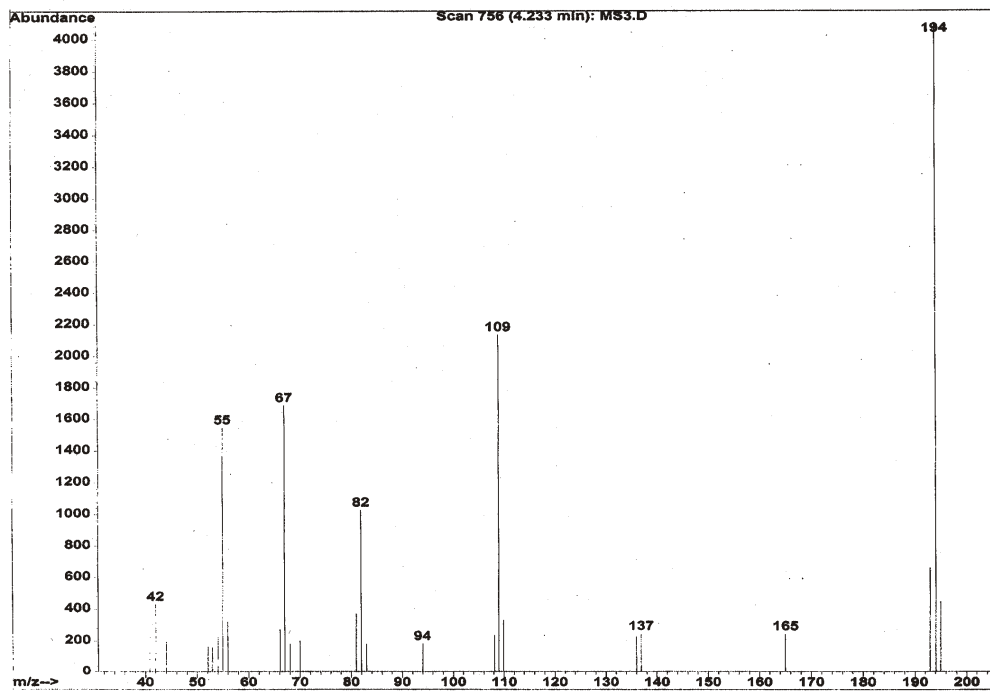


Figure 5. Mass Spectrum of Caffeine.

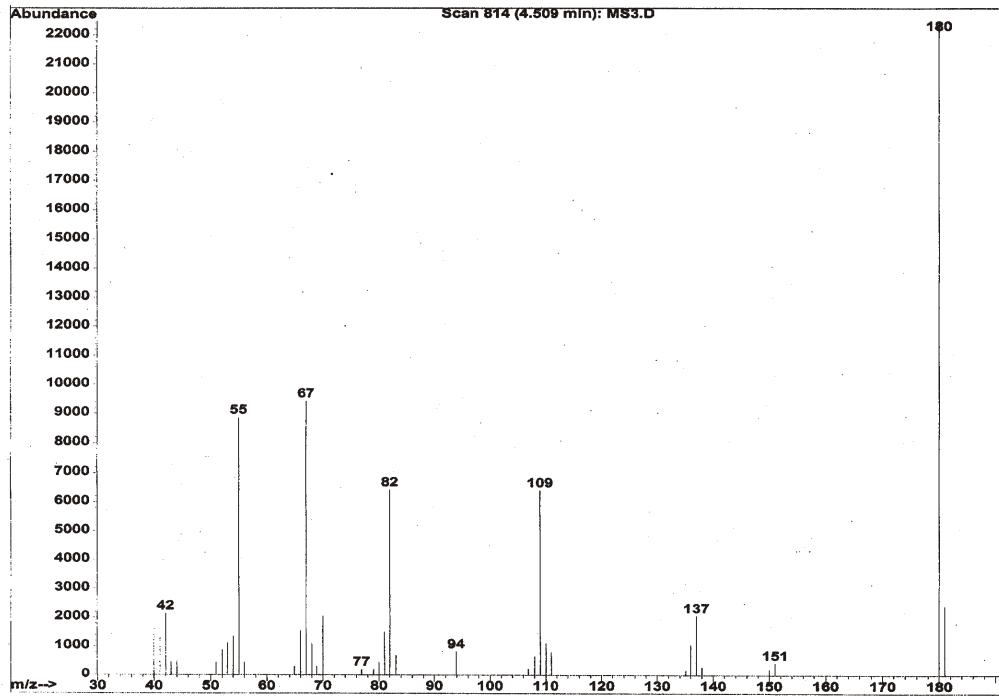


Figure 6. Mass Spectrum of Theobromine

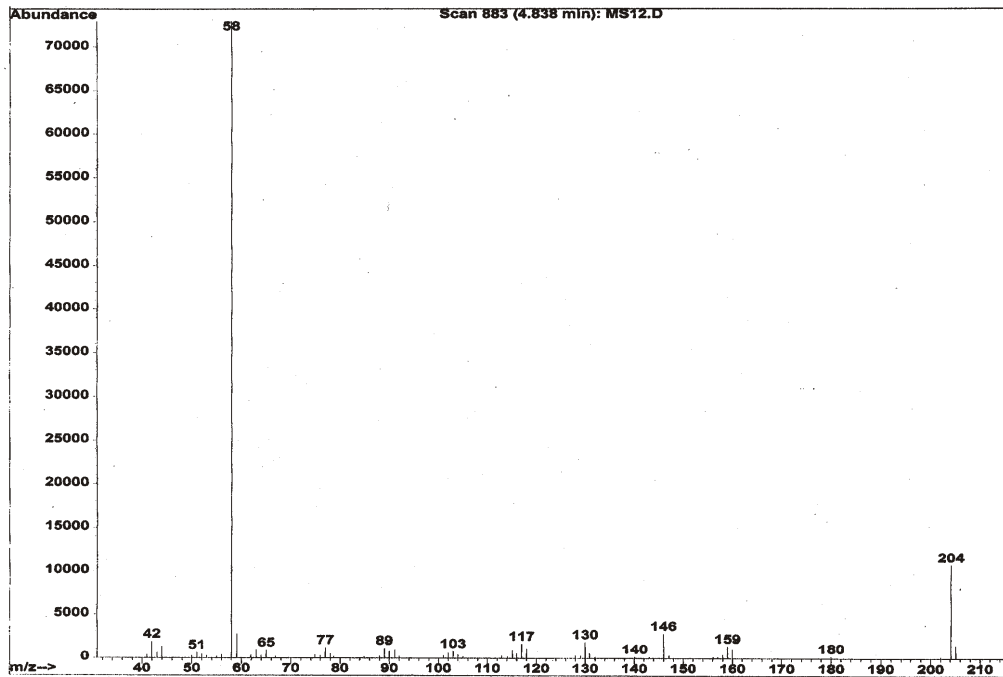


Figure 7. Mass Spectrum of Psilocyn.

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

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

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

Introduction

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

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

4-Methoxyamphetamine (*para*-methoxyamphetamine, PMA) is a much older designer drug, which after many years of very low occurrence, has been encountered in increased frequency during the past few years - and has also been linked with a number of user deaths. However, in contrast to 4-MTA or 2C-I, PMA was already a legally restricted substance, because of its previous appearances. Due to its known high toxicity, its resurgence is somewhat surprising. PMA is usually illicitly produced via a multi-step synthesis from either anisaldehyde (4-methoxybenzaldehyde) or anethole (1-methoxy-4-(1-propenyl)benzene, or *para*-propenylanisole). Anethole is a major component of star anise, anise, and fennel essential oils [1]; these oils are used in vast quantities in the food and pharmaceutical industry, and so are widely available. This latter fact makes them particularly attractive precursors for clandestine chemists.

Forensic chemists are sometimes requested to determine the precursor or the synthetic route used to prepare a seized drug preparation; this information can reveal valuable information for law enforcement agencies. Analytical information for detection of Leuckart-specific impurities in PMA has been previously reported [2,3]. However, those reports focused only on impurities that confirmed the clandestine chemist's use of the Leuckart reaction, and not on the determination of the original precursor (that is, anisaldehyde versus anethole).

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

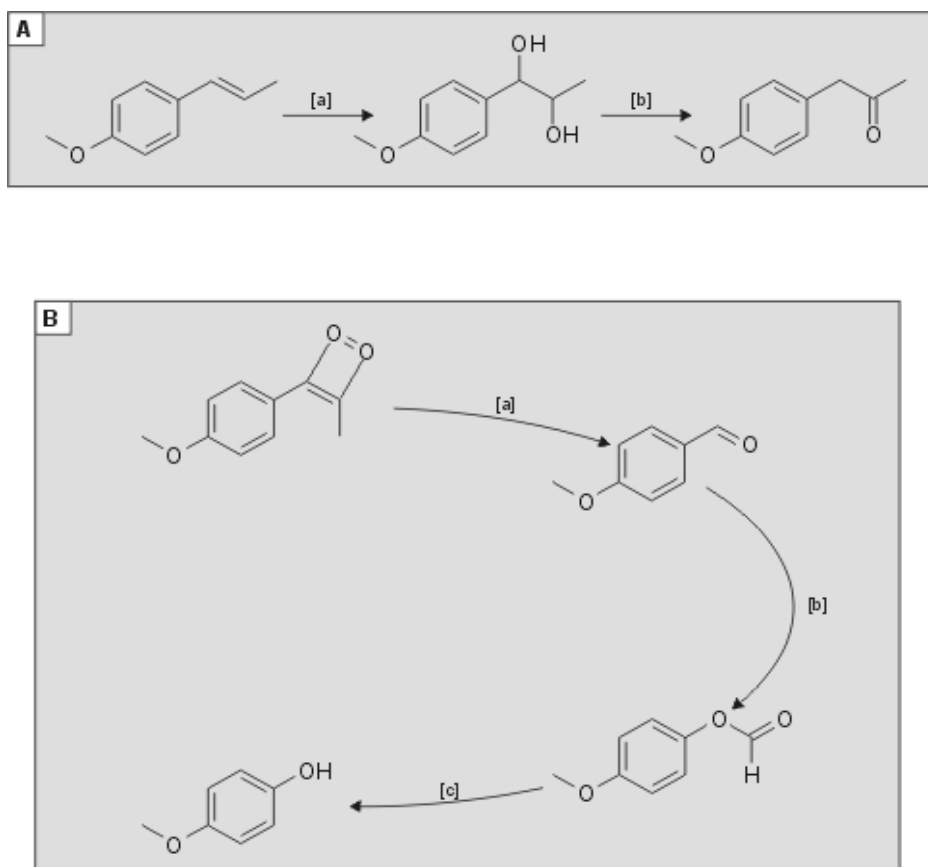


Figure 1:

- A: Scheme of the peracid oxidation reaction of anethole. Anethole is reacted with a peracid ([a], typically performic or peracetic acid), after which the obtained glycol is converted to 4-methoxyphenyl-2-propanone by refluxing in a sulfuric acid/methanol mixture.
- B: Overview of the 4-methoxyphenol impurity formation. The propenyl double bond of anethole is oxidatively cleaved ([a]) to yield 4-methoxybenzaldehyde. This substance is further reacted to *O*-formyl-4-methoxyphenol due to the presence of peracids in the reaction mixture ([b]). This compound will then be hydrolyzed to yield 4-methoxyphenol ([c]).

We have previously reported the use of headspace solid-phase microextraction - GC/MS (GC-HSPME/MS) technique for detection of 4-methoxyphenol in illicitly prepared PMA [4]; however, this methodology is not yet generally available in forensic laboratories. Herein we report a more simple and rapid technique for extraction and identification of 4-methoxyphenol.

Experimental

Chemicals

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

Instrumentation

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

Performic Acid Oxidation of Anethole

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

Sample Preparation

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

Results and Discussion

4-Methoxyphenol as Specific Peracid Oxidation Marker

We have previously analyzed star anise oil, anise oil, and fennel oil (all natural sources of anethole), and determined that 4-methoxyphenol is not naturally present in any of these essential oils. In addition, there are no literature reports of 4-methoxyphenol being present in these oils. Our analysis of commercial anisaldehyde

(Merck, Acros Organics) also confirmed that 4-methoxyphenol was not present. Furthermore, production of PMA from anisaldehyde (starting with the Henry condensation route (anisaldehyde and nitroethane) used by clandestine chemists) did not produce 4-methoxyphenol at any stage. These results confirm that the presence of 4-methoxyphenol is not due to natural contamination, or produced as a synthetic by-product in the illicit synthesis of PMA from anisaldehyde.

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

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

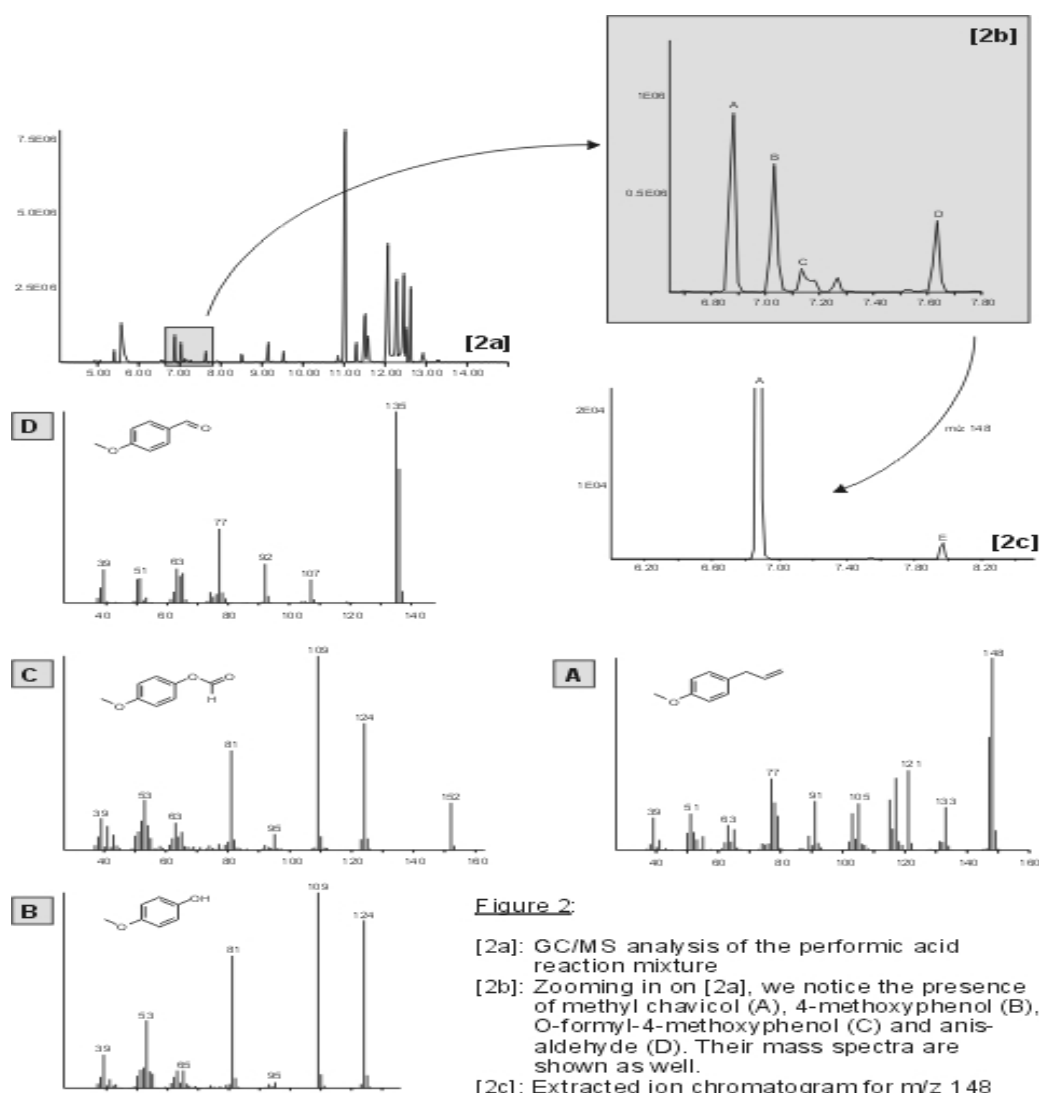


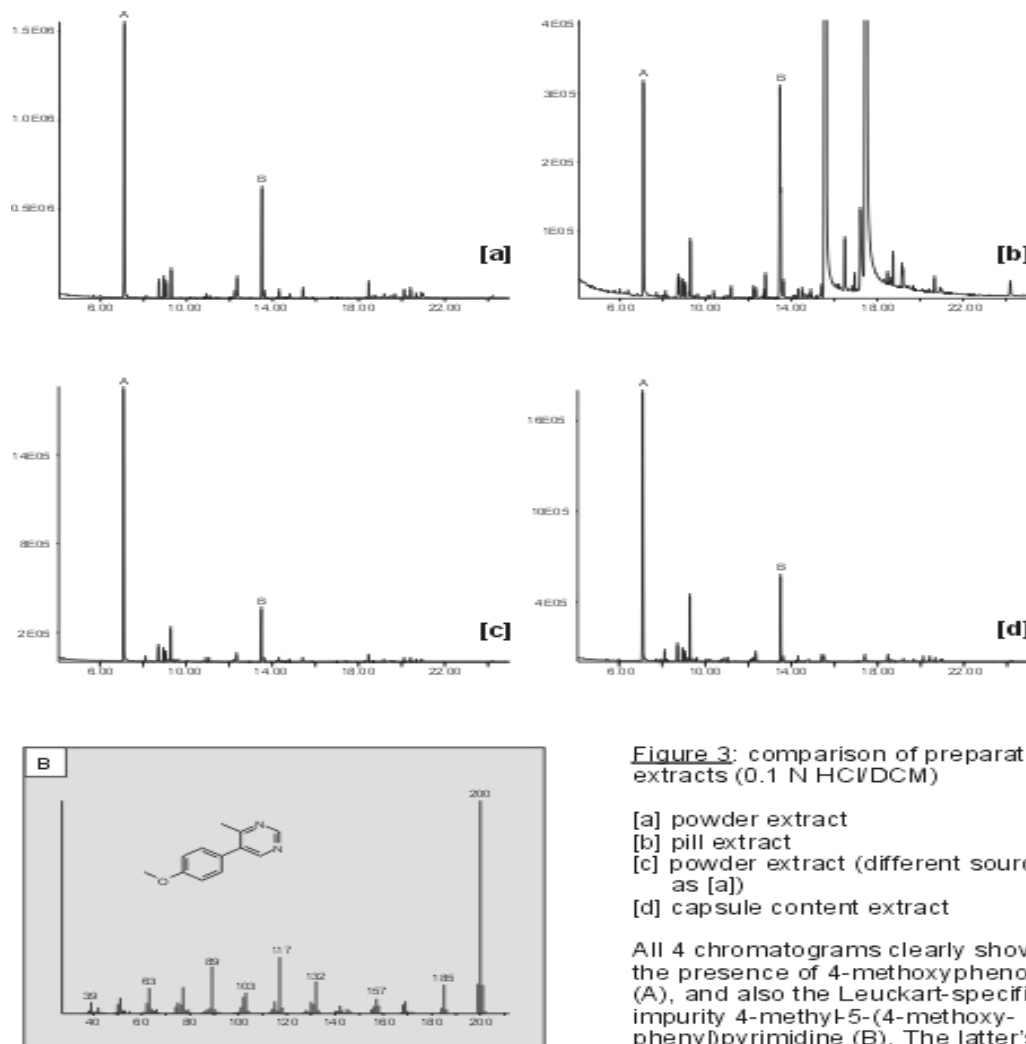
Figure 2:

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

The Occurrence of 4-Methoxyphenol in Illicitly Prepared PMA

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

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



Conclusions

4-Methoxyphenol is a synthetic by-product formed during the peracid oxidation of anethole, and thus serves as a marker compound for PMA prepared using anethole as the original precursor. The presented extraction and identification techniques are rapid and simple. Based on our work, 4-methoxyphenol would *probably* not be

present if the clandestine chemist performed a distillation to purify the intermediate 4-methoxyphenyl-2-propanone; however, few clandestine chemists perform such steps. In this study, the four illicitly prepared samples of PMA contained impurities from both the peracid oxidation and the Leuckart reductive amination reaction steps.

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Letrozole (Femara[®])

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

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

Introduction

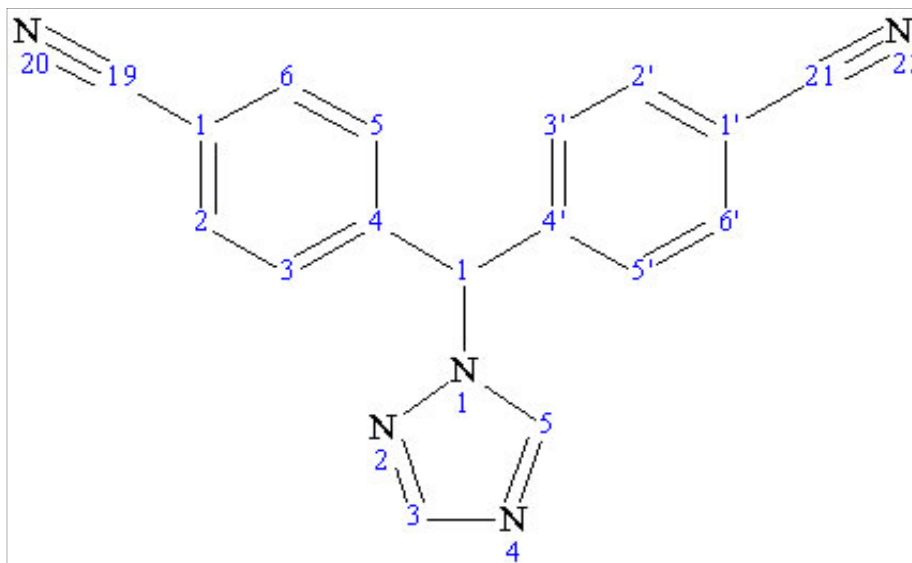
This laboratory occasionally receives unusual unknowns that were seized as suspected controlled substances. Recently, we received 4.46 grams of a flocculent white powder (see Photo 1), that had been included in a group of steroids submitted from a U.S. Customs seizure in Anchorage, Alaska. The steroids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



Photo 1

Preliminary analyses by Gas Chromatography/Mass Spectrometry (GC/MS, Figure 1) and Fourier Transform Infrared Spectroscopy (FTIR, Figure 2) gave no matches when searched in reference databases. The infrared spectrum showed an unusually strong peak in the region 2230 cm⁻¹, suggesting strong carbon-nitrogen triple bond (nitrile) stretching. Based on the GC/MS, the molecular weight was 285 atomic mass units (amu); this was confirmed by Liquid Chromatography/Mass Spectrometry (LC/MS) with chemical ionization. Further analysis by proton and carbon-13 Nuclear Magnetic Resonance (NMR) Spectroscopy (Figures 3 and 4) with quantitative

analysis suggested a molecule with the chemical formula $C_{17}H_{11}N_5$. An internet search for compounds with that formula returned letrozole as a possibility. Letrozole is an anti-cancer drug (Femara[®]) produced by Novartis Pharmaceuticals Corporation (East Hanover, NJ). Further analysis and comparison to spectral data and reference standard material (provided by Novartis) confirmed that the sample was letrozole (see structural formula below).



Letrozole

Chemical Name: 4,4'-(1H-1,2,4-Triazol-1-ylmethylene)dibenzonitrile

Empirical Formula: $C_{17}H_{11}N_5$

Molecular Weight: 285.31 amu

Melting Range: 184 - 185 °C

Therapeutic Category: Anti-cancer

Solubility: Freely soluble in chloroform, slightly soluble in methanol, practically insoluble in water.

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



Photo 2

Experimental

GC/MS

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

FTIR

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

NMR

One dimensional NMR analyses were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe. The sample was prepared at 17 mg/mL in deuterated chloroform (CDCl_3) containing TMS (tetramethylsilane) as the reference standard (Aldrich Chemical Co., Milwaukee, WI). The proton spectrum of the standard was obtained with 8 scans using a 45 second delay, 90° pulse, 2 second acquisition time, and oversampling by a factor of 6. The carbon spectrum of the standard was obtained with 256 scans using a 1 second delay, 45° pulse, 1.2 second acquisition time, and oversampling by a factor of 3. The sample was maintained at 25 °C. Gradient versions of the 2-Dimensional NMR experiments HSQC (correlation of hydrogens directly bonded to carbons) and HMBC (correlation of hydrogens 2, 3, or 4 bonds from carbons) were performed to make assignments (listed in Table 1). Prior to the arrival of the reference standard, structural elucidation was performed utilizing Applied Chemistry Developments (ACD, Toronto, Canada) software (HNMR Predictor, CNMR predictor, and Structure Elucidator).

Results and Discussion

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

References

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

[Figures 1 - 4 and Table 1 Follow.]

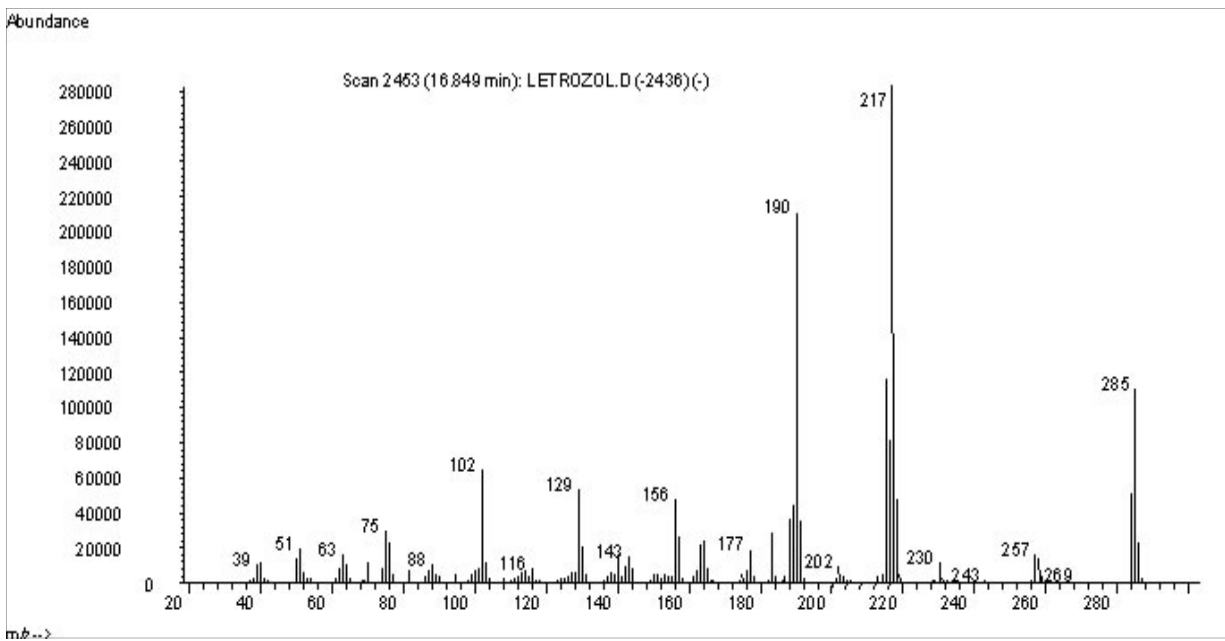


Figure 1. Mass Spectrum of Letrozole.

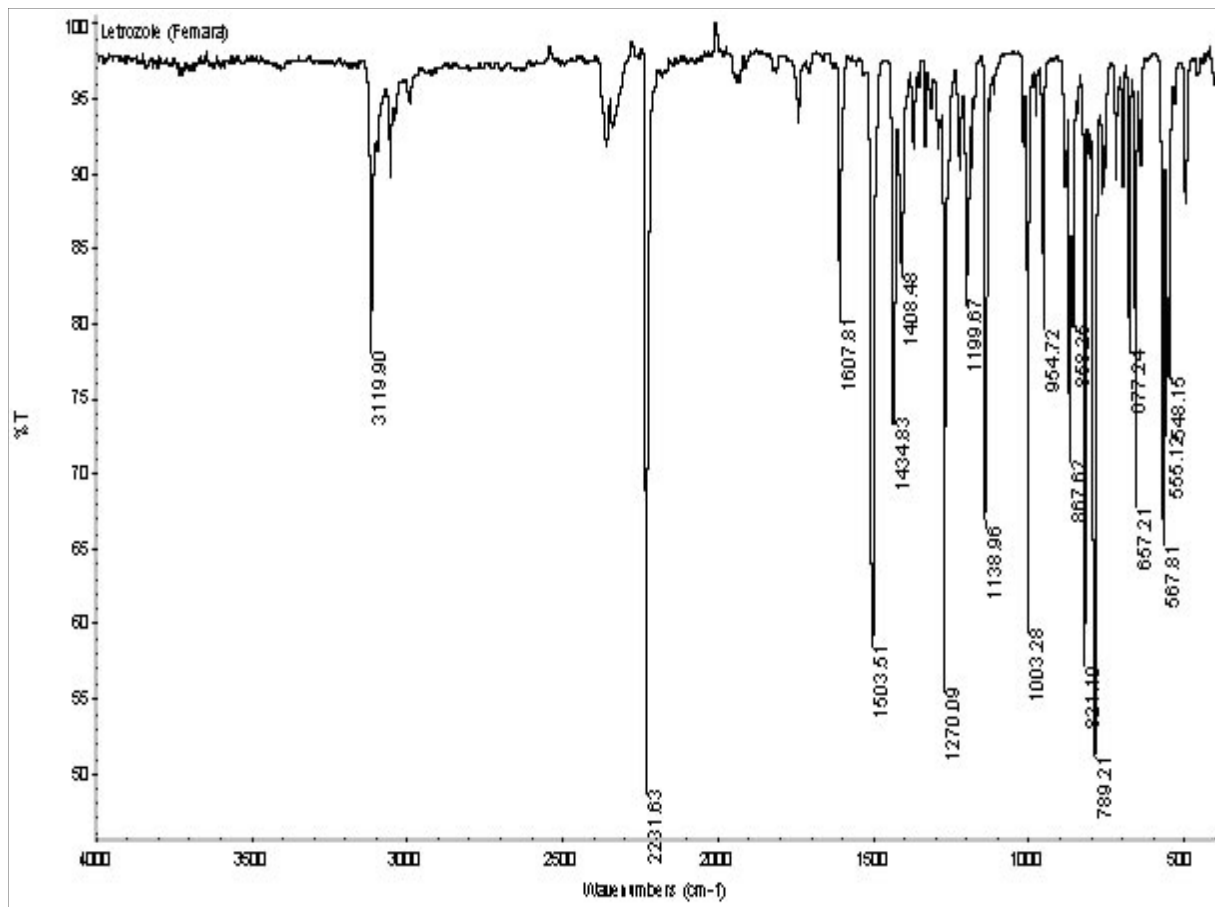


Figure 2. FTIR of Letrozole.

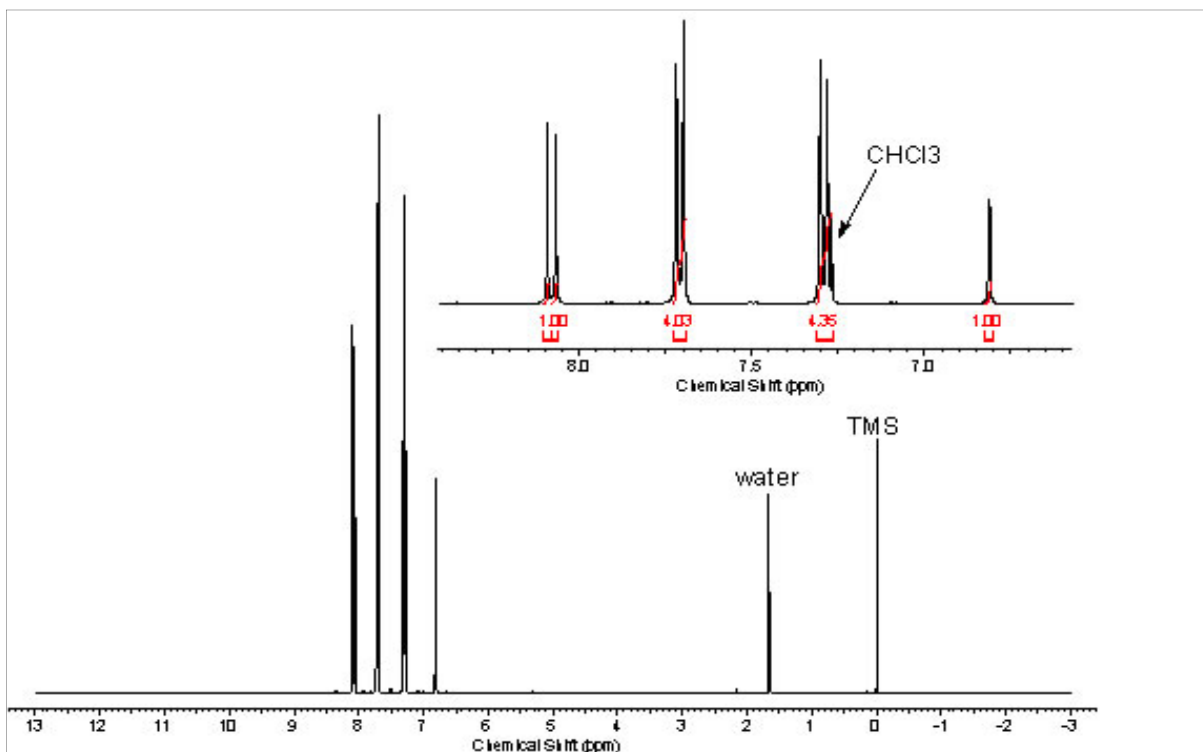


Figure 3. ^1H -NMR Spectrum of Letrozole (400.2 MHz).

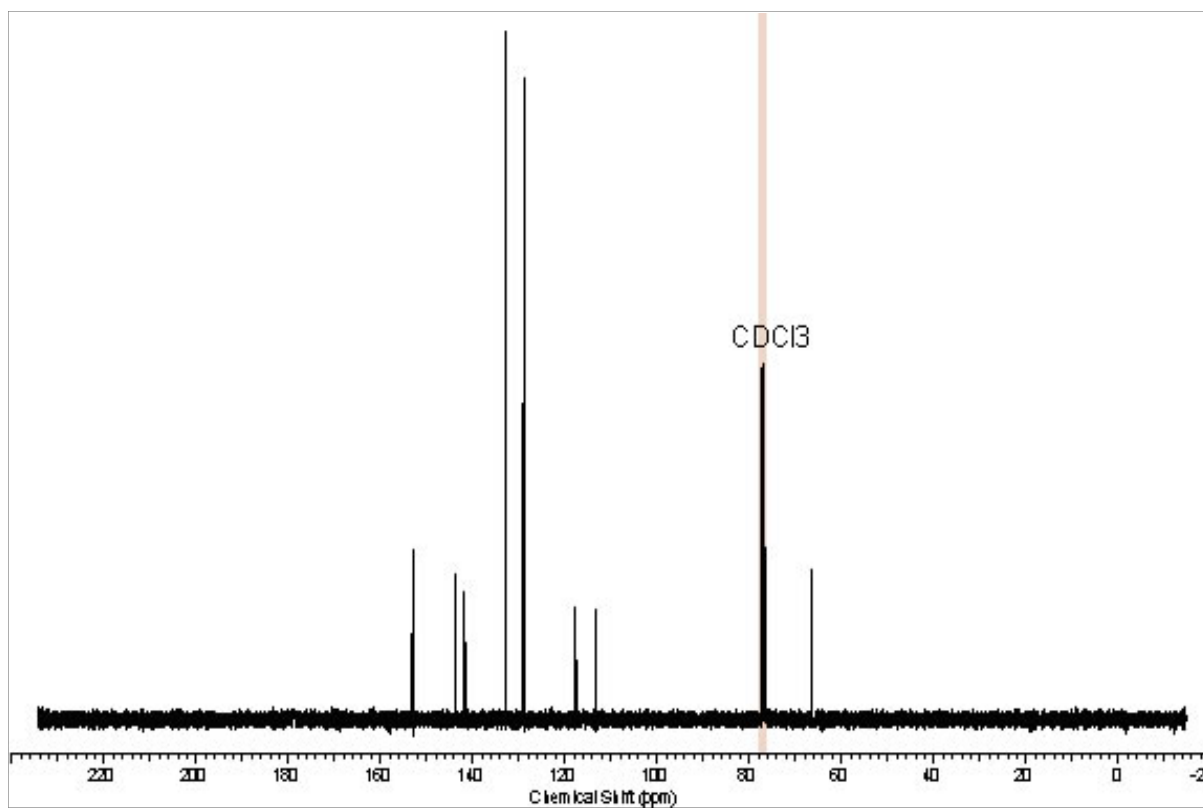


Figure 4. ^{13}C -NMR Spectrum of Letrozole (100.6 MHz).

Table 1. NMR Assignments of Protons and Carbons for Letrozole.

Carbon Chemical Shift (ppm)	Proton Chemical Shift (ppm)	Atom Number and Notes
66.36	6.81 (singlet)	1 (alkyl methine)
113.29		1,1' (benzene rings)
117.82		19, 21 (benzene rings)
128.9	7.70, 7.72 (doublet)	3, 5, 3', 5' (benzene rings)
132.91	7.28, 7.30 (doublet)	2, 6, 2', 6' (benzene rings)
141.76		4, 4' (benzene rings)
143.68	8.09 (singlet)	5 (triazole ring)
153.05	8.07 (singlet)	3 (triazole ring)

* * * * *

An Overview of DNA Methods for the Identification and Individualization of Marijuana

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ABSTRACT: The purpose of this review is to summarize the status of DNA-based methods for the identification and individualization of marijuana. In forensics, both identification of a substance as marijuana and the subsequent individualization of a sample may be desired for casework. Marijuana identification methods in the United States primarily include biochemical tests and, less frequently, DNA-based tests. Under special circumstances, DNA-based tests can be useful. For example, if the quantity of seized marijuana is extremely small and/or biochemical tests do not detect any Δ 9-tetrahydrocannabinol (THC), DNA identification of plant material as *Cannabis* is still possible. This circumstance can arise when seeds, trace residue, tiny leaf fragments, or fine roots need to be analyzed. Methods for the individualization of marijuana include Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), and Short Tandem Repeat (STR) techniques that link an evidentiary sample to a source. Marijuana growers propagate their plants either by seed or by cloning. Seed-generated marijuana plants are expected to have unique DNA profiles analogous to a human population. Cloned marijuana plants, however, exhibit identical DNA profiles that allow for tracking of plant material derived from a common genetic lineage. The authors have validated the AFLP method for marijuana samples and are constructing a comparative database of marijuana seizure samples to estimate the expected frequency of a DNA profile match between unrelated plants. Continued development of DNA-based methods for plants can be useful for marijuana and other types of plant evidence in forensics.

KEYWORDS: *Cannabis*; Polymorphism (Genetics); Polymorphism; Restriction Fragment Length; Random Amplified Polymorphic DNA Technique; Tandem Repeat Sequences.

Cannabis sativa (marijuana) has existed since ancient times and is widely used as a fiber source, a food source, a medicine and a euphoriant (1-4). Marijuana has been used to treat a variety of ailments including glaucoma, pain, nausea, asthma, depression, neuralgia and insomnia (5). Like many crops (e.g. wheat, corn), marijuana was originally a naturally occurring weed species. It was bred and cultivated into a significant cash crop for a multi-billion dollar illicit industry. The hallucinogenic properties of marijuana are derived from Δ 9-tetrahydrocannabinol (THC) (6) and potent (high-THC level) marijuana cultivars are sought by the discerning marijuana users (7,8). Before the mid-1970's, the majority of U.S. marijuana was imported from Mexico. When the U.S. and Mexico co-operated in marijuana eradication programs, a domestic growing industry began in the United States. Most of the highly prized United States cultivars originated from a few breeding stocks from the West Coast (5). Now, seeds for marijuana growers are accessible worldwide via the Internet; seed catalogues are posted with extensive descriptions and price lists (e.g. www.cannabisseeds.org). The extent of genetic variation in marijuana populations is unknown due to the illicit nature of this substance and because growers propagate plants secretively. A survey of marijuana seizure samples using DNA profiling methods could be used to assess levels of genetic diversity within this crop.

There are two main steps in most forensic classification schemes that can be applied to marijuana seizure samples. The first step involves identification of a sample. For marijuana, both biochemical (9,10) and DNA tests (11-12) are available to identify a substance as *Cannabis*. The second step is individualization (source attribution). For marijuana, several DNA-based methods are under development and will be described in later sections. Biochemical methods to establish geographic origin of a plant have met with variable success (13-17). However, contaminants (18) and packaging (17) have shown a correlation with marijuana source. Biochemical profiling has also successfully differentiated between resinous and textile *Cannabis* (19), drug subgroups (marijuana, sinsemilla, Thai sticks, ditchweed) (7) and plant gender (20).

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

Marijuana Drug Facts

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

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

In 1977, regional narcotics enforcement squads were replaced by a Statewide Narcotics Task Force in Connecticut (27). The Task Force is authorized to enforce the state laws concerning the manufacture, distribution, sale and possession of narcotics and controlled substances. In addition to enforcement, the Connecticut Statewide Narcotics Task Force collects and provides information regarding drug seizures for Connecticut on an annual basis (25,27). Both indoor and outdoor marijuana grow operations have been identified in Connecticut (25,27). The outdoor grow season in Connecticut begins in April and continues until harvest time in mid-September. The indoor grow season is year-round. The Statewide Narcotics Task Force, in conjunction with the Drug Enforcement Administration (DEA), sponsors and coordinates the Domestic Cannabis Eradication/Suppression Program in Connecticut (25,27). For the first nine months of 2002, statistics for the Statewide Narcotics Task Force domestic *Cannabis* eradication program indicated while greater numbers of outdoor plots were identified compared to indoor grow operations, the number of plants seized were comparable between indoor and outdoor cultivation plots (Table 1) (27). Indoor grow operations may be increasing in number and scale or are more easily

detected based on a comparison of data from the years 1999-2002 (Table 1) (27). According to Connecticut statistics for 2002, marijuana distribution and consumption has steadily increased and the demand for high quality hydroponically grown marijuana has also increased despite the greater cost to the consumer (25,27). Marijuana cultivated in Connecticut represents a small fraction of the amount consumed by its state residents. The majority of consumed marijuana is imported from California, Texas and Mexico (25,27).

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

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

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

Marijuana Identification

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

Biochemical Tests

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

cases where the plants have been harvested but the roots have been left at the crime scene. In these situations, DNA testing can provide a means for marijuana identification that would otherwise not be possible.

DNA Tests

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

Marijuana Individualization

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

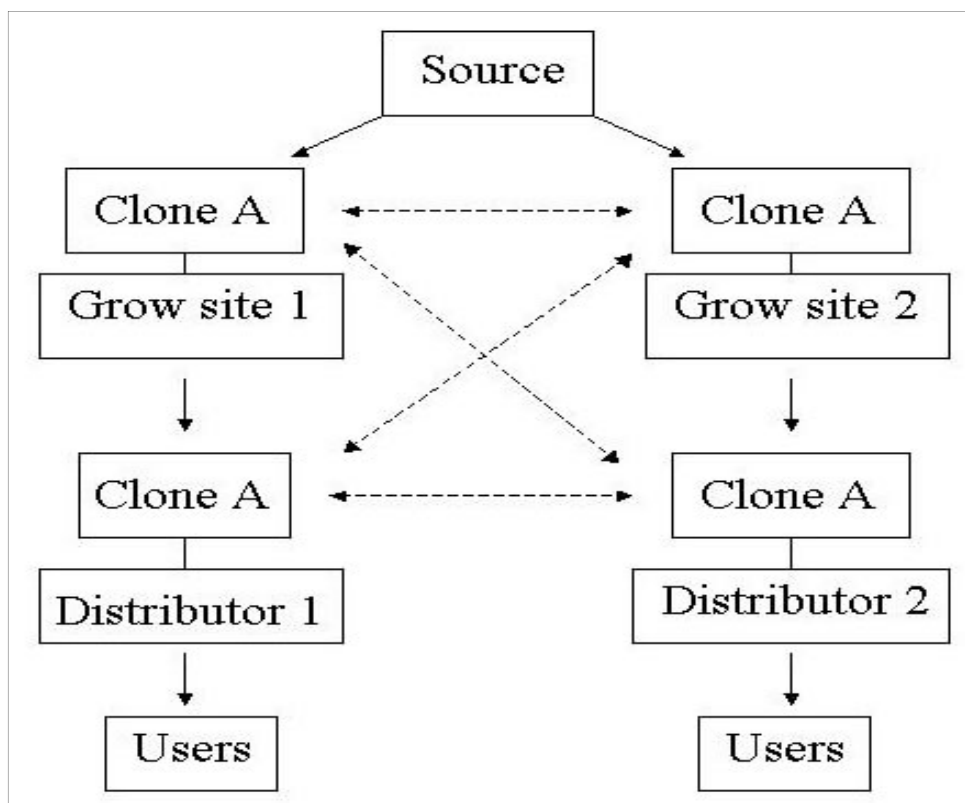


Figure 1. Potential forensic linkages based on individualized marijuana sample information. Growers sharing cloned plants can be associated based on identical , Amplified Fragment Length Polymorphisms (AFLP) profiles. Growers may be linked to major marijuana distributors and marijuana user seizure material may be traced back to a common distributor of clonal marijuana.

Table 2. A comparison of three DNA methods for the individualization of plant samples.*

<u>Method</u>	<u>Discrimination Power</u>	<u>Relative Cost</u>	<u>Input DNA</u>
RAPDs	moderate ¹	low	1-10 nanograms
AFLPs	high	moderate-high	1-10 nanograms
STRs	high	moderate-high	1-10 nanograms

* RAPD = Randomly Amplified Polymorphic DNAs; AFLP = Amplified Fragment Length Polymorphisms; STR = Short Tandem Repeats.

¹ Ability to distinguish between unrelated individuals.

Randomly Amplified Polymorphic DNAs (RAPDs)

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

Amplified Fragment Length Polymorphisms (AFLPs)

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

Fragment Length Polymorphisms database generation for comparative purposes. Validation of the Amplified Fragment Length Polymorphisms method for marijuana samples is complete (21,22) and our analyses of cloned marijuana (courtesy of Dr. Gary Shutler, Royal Canadian Mounted Police) has shown that clonal Amplified Fragment Length Polymorphisms profiles are highly reproducible (Figure 2). In contrast, Amplified Fragment Length Polymorphisms profiles from unrelated marijuana plants are easily distinguishable from each other using this method (Figure 3).

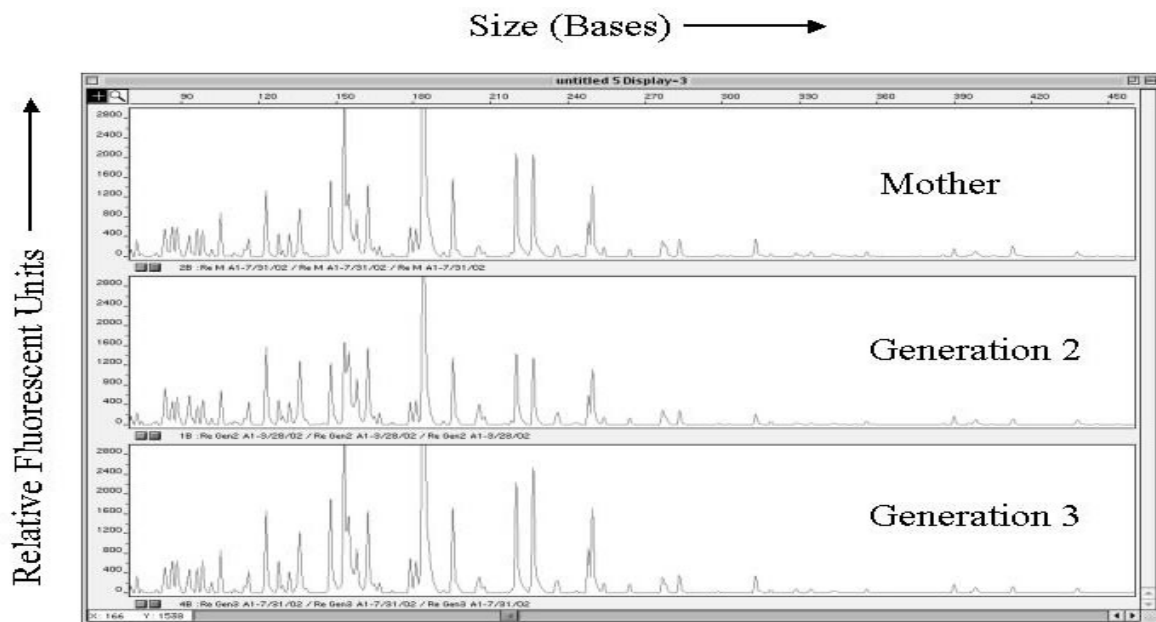


Figure 2. Amplified Fragment Length Polymorphism (AFLP) analysis of known clonal generations exhibit identical DNA profiles. Known clonal marijuana generations were propagated by the Royal Canadian Mounted Police (RCMP, Winnipeg) and were generously provided through collaboration with Dr. Gary Shutler.

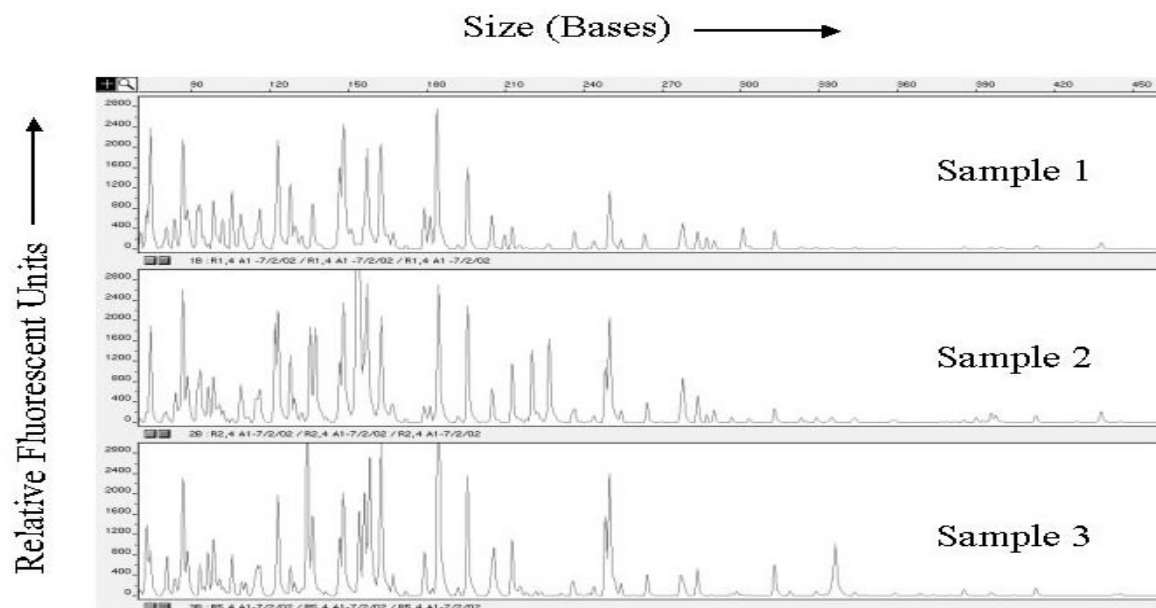


Figure 3. Marijuana samples from unrelated cases have distinct Amplified Fragment Length Polymorphism (AFLP) profile differences. Samples #1-3 were generously provided from adjudicated cases by Dr. Eric Buel (Vermont Crime Laboratory).

Short Tandem Repeats (STRs)

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

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

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

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

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

Comparative Databases

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

Conclusion

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

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

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Report of the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Conference (Montreal)

Montreal, Canada; October 7 - 9, 2003

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The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) is an International Working Group dedicated to the development and implementation of minimum standards for the identification of drug exhibits in forensic science laboratories. The primary objectives for the Group are:

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

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

Susan Ballou (National Institute of Standards and Technology, Gaithersburg, Maryland)

Joseph P. Bono (Drug Enforcement Administration, Office of Forensic Sciences, Arlington, Virginia)

Dr. Bob Bramley (Forensic Science Service, Trident Court, Birmingham, England)

Gary Chasteen (California Association of Criminalists, Los Angeles County Sheriff's Laboratory, Downey, California)

Dr. Maria Eugenia Forero (National Institute of Legal Medicine and Forensic Science, Bogotá, Colombia)

Richard Gervasoni (American Society of Crime Laboratory Directors, Montgomery County Police Department Laboratory, Rockville, Maryland)

Linda Jackson (Mid-Atlantic Association of Forensic Scientists, Virginia Division of Forensic Sciences, Richmond, Virginia)

Thomas J. Janovsky (Drug Enforcement Administration, Office of Forensic Sciences, Arlington, Virginia)

Dr. Tohru Kishi (National Research Institute of Police Science, Chiba, Japan)

Richard Laing (Health Canada, Burnaby, British Columbia, Canada)

Jack Mario (Northeastern Association of Forensic Scientists and ASTM, Suffolk County Crime Laboratory, Hauppauge, New York)

Jerry Massetti (Northwestern Association of Forensic Scientists, California Department of Justice, California Criminalistics Institute, Sacramento, California)

Richard Paulas (Mid-Western Association of Forensic Scientists, Illinois State Police, Chicago, Illinois)

Catherine Quinn (Victoria Forensic Science Center, McCleod, Australia)

Dr. Conrad Roberson (South Eastern Association of Forensic Scientists, Georgia Bureau of Investigation, Decatur, Georgia)

Dr. Charles "Chris" Tindall (Metropolitan State College Academia, Denver, Colorado)

Eileen Waninger (Federal Bureau of Investigation, Quantico, Virginia)

Dr. Udo Zerell (Bundeskriminalamt, Wiesbaden, Germany)

Etienne van Zyl (South African State Police, Pretoria, South Africa)

Two core committee members were unable to attend the Fall 2003 conference:

Dr. Erkki Sippola (European Network of Forensic Science Institutes (ENFSI), National Bureau of Investigation, Crime Laboratory, Vantaa, Finland)

Dr. Howard Stead (Division for Policy Analysis and Public Affairs, United Nations Office on Drugs and Crime, Vienna, Austria)

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

Accomplishments

The accomplishments of the Montreal Conference included:

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

"For exhibits of cannabis that lack sufficient observable macroscopic and microscopic botanical detail (e.g., extracts or residues), the Δ^9 -tetrahydrocannabinol (THC) or other cannabinoids must be identified using the principles set forth in sections 3.1 and 3.2."
2. After review by forensic drug examiners, the core committee approved a recommendation entitled: **Code of Professional Practice for Drug Analysts.**

3. After review by forensic drug examiners the core committee approved a recommendation entitled: **Validation of Analytical Techniques.**
4. Review of document entitled: **Sampling Seized Drugs for Qualitative Analysis.** This document has been returned to the subcommittee for reformatting and will be re-submitted to the forensic community for comment in the near future.

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

SWGDRUG Publication (Part 2)

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

- Part I **Code of Professional Practice**
- Part II **Education and Training**
- Part III **Methods of Analysis**
 - A. Sampling for qualitative analysis (Currently under revision for additional comments)
 - B. Drug Identification
- Part IV **Quality Assurance**
 - A. General Practices (Currently being updated)
 - B. Validation of Analytical Techniques

Issues to Be Addressed at the Next SWGDRUG Conference:

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

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PART I - A CODE OF PROFESSIONAL PRACTICE FOR DRUG ANALYSTS

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

SECTION 1: INTRODUCTION

- 1.1 A Code of Professional Practice is intended to provide the framework of ethical values and scientific and legal obligations within which the analyst should operate. Details are also usually provided on how alleged breaches of the Code will be investigated, what sanctions are available and how appeals should be pursued.
- 1.2 A Code of Professional Practice is essential to analysts and their managers in helping them carry out their duties in a proper manner and in making appropriate decisions when questions of ethics arise.
- 1.3 A Code of Professional Practice that is enforced and publicly available is also a powerful means of demonstrating the professional expectations of analysts and the reliability of their findings to others in the criminal justice system and the public at large.
- 1.4 SWGDRUG recommends that all employers of analysts develop a Code of Professional Practice and the means of dealing with breaches of the Code.
- 1.5 SWGDRUG further recommends that all Codes of Professional Practice for analysts should include, as a minimum, provisions relating to their professional conduct, their casework and the reporting of their results, as provided in Section 2.

SECTION 2: CODE OF PROFESSIONAL PRACTICE

2.1 Professional Conduct

Analysts should:

- 2.1.1 Act with honesty, integrity and objectivity;
- 2.1.2 Work only within the bounds of their professional competence;
- 2.1.3 Take reasonable steps to maintain their competence;
- 2.1.4 Recognize that their overriding duty is to criminal justice;
- 2.1.5 Declare to their employer any prior contact or personal involvement, which may give rise to conflict of interest, real or perceived;
- 2.1.6 Declare to their employer or other appropriate authority any pressure intended to influence the result of an examination.

2.2 Casework

Analysts should:

- 2.2.1 Ensure and be able to demonstrate that the integrity and security of evidential materials and the information derived from their analysis have been maintained while in their possession;
- 2.2.2 Ensure that they have a clear understanding of what the customer needs and all the necessary information, relevant evidential materials and facilities available to reach a meaningful conclusion in an appropriate timeframe;
- 2.2.3 Employ an appropriate analytical approach, using the facilities available;
- 2.2.4 Make and retain full, contemporaneous, clear and accurate records of all examinations and tests conducted, and conclusions drawn, in sufficient detail to allow meaningful review and assessment of the conclusions by an independent person competent in the field;
- 2.2.5 Accept responsibility for all casework done by themselves and under their direction;
- 2.2.6 Conduct all professional activities in a way that protects the health and safety of themselves, co-workers, the public and the environment.

2.3 Reporting

Analysts should:

- 2.3.1 Present advice and testimony, whether written or oral, in a clear and objective manner;
- 2.3.2 Be prepared to reconsider and, if necessary, change their conclusions, advice or testimony in light of new information or developments, and take the initiative in informing their employer and customers promptly of any such changes that need to be made;
- 2.3.3 Take appropriate action if there is potential for, or there has been, a miscarriage of justice due to new circumstances that have come to light, incompetent practice or malpractice;
- 2.3.4 Preserve customer confidentiality unless officially authorized to do otherwise.

APPENDIX

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

Casework

- 2.2.1 To ensure and be able to demonstrate that the integrity and security of evidential materials and the information derived from their analysis have been maintained while in their possession:
 - Keeping a record of the chain of custody;
 - Making special note of the security of sealing and packaging of the evidential materials as received;
 - Preserving the evidential materials from contamination, adulteration, deterioration, loss or theft by use of appropriate working practices and utilization of suitable storage facilities with controlled access;
 - Using a unique identifier for the evidential materials, any sub-sample taken from them and any accompanying documentation, that will minimize the risk of misidentification;
 - Keeping the evidential materials in their original condition for future reference, insofar as this is possible;
 - Securely repackaging and resealing the evidential materials after their examination;

Preserving and returning all original packaging, with original seals intact, where this is possible;
Ensuring that access to the evidential materials and all documentation relating to these, before and after their examination, is restricted to authorized personnel.

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

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

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

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

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

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

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

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

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

Being aware of and complying at all times with current health and safety legislation;
Ensuring that all relevant risk assessments have been carried out and safe systems of work are in place and being followed;
Ensuring that others in the vicinity of their work are aware of their activities, particularly where these involve the investigation of clandestine laboratories, potential exposure to controlled drugs,

especially from bulk seizures, the use of other hazardous materials or the destruction/disposal of drugs and other hazardous materials.

Reporting

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

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

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

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

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

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

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

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PART II - EDUCATION AND TRAINING

SECTION 1: INTRODUCTION

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

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

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

SECTION 2: EDUCATION AND EXPERIENCE FOR ANALYSTS

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

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

or

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

SECTION 3: CONTINUING PROFESSIONAL DEVELOPMENT

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

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

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

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

3.1.1.2 Training completed must be documented.

3.1.1.3 Training can be provided from a variety of sources, including, but not limited to the following:

- 3.1.1.3.1 Chemistry or instrumental courses taught at the post-secondary educational level
- 3.1.1.3.2 Instrument operation or maintenance courses taught by vendors
- 3.1.1.3.3 In-service classes conducted by the employer
- 3.1.1.3.4 In-service training taught by external providers
- 3.1.1.3.5 Participation in relevant scientific meetings or conferences (e.g., presenting a paper, attending a workshop, providing reports on conferences).

SECTION 4: INITIAL TRAINING REQUIREMENTS

- 4.1 These minimum requirements allow individual laboratories to structure their training program to meet their needs as it relates to type of casework encountered, analytical techniques, available instrumentation and level of preparedness of trainees.
- 4.2 There must be a documented training program, approved by laboratory management, that focuses on the development of theoretical and practical knowledge, skills and abilities necessary to examine seized drug samples and related materials. The training program must include the following:
 - 4.2.1 Documented standards of performance and a plan for assessing theoretical and practical competency against these standards (e.g., written and oral examinations, critical reviews, analysis of unknown samples and mock casework per topic area)
 - 4.2.2 A training syllabus providing descriptions of the required knowledge and skills in specific topic areas in which the analyst is to be trained, milestones of achievement, and methods of testing or evaluating competency
 - 4.2.3 A period of supervised casework representative of the type the analyst will be required to perform
 - 4.2.4 A verification document demonstrating that the analyst has achieved the required competence
- 4.3 Topic areas in the training program will include, as a minimum, the following:
 - 4.3.1 Relevant background information on drugs of abuse (e.g., status of control and chemical and physical characteristics)
 - 4.3.2 Techniques, methodologies and instrumentation utilized in the examination of seized drug samples and related materials
 - 4.3.3 Quality assurance
 - 4.3.4 Expert/Court testimony and legal requirements
 - 4.3.5 Laboratory policy and procedures (such as sampling, evidence handling, safety and security) as they relate to the examination of seized drug samples and related materials.
- 4.4 An individual qualified to provide instruction must have demonstrated competence in the subject area and in the delivery of training.

SECTION 5: REFERENCES AND DOCUMENTS

- 5.1 The following references and documents must be available and accessible to analysts:
- 5.1.1 College/university level textbooks for reference to theory and practice in key subject areas, e.g., general chemistry, organic chemistry and analytical chemistry
 - 5.1.2 Reference literature containing physical, chemical and analytical data. Such references include the Merck Index, Clarke's Analysis of Drugs and Poisons, laboratory manuals of the United Nations Drug Control Program, in-house produced spectra and published standard spectra, (e.g., Mills And Roberson's Instrumental Data For Drug Analysis, or compendiums from Pfleger or Wiley)
 - 5.1.3 Operation and maintenance manuals for each analytical instrument
 - 5.1.4 Relevant periodicals (e.g., Journal of Forensic Sciences, Forensic Science International, Microgram, Journal of Canadian Society of Forensic Science, Journal of Japanese Association of Science and Technology of Identification and Analytical Chemistry)
 - 5.1.5 Laboratory quality manual, standard operating procedures, and method validation and verification documents
 - 5.1.6 Relevant jurisdictional legislation (e.g., statutes and case law relating to controlled substances, and health and safety legislation).

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PART III B - METHODS OF ANALYSIS/DRUG IDENTIFICATION

SECTION 1: INTRODUCTION

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

SECTION 2: CATEGORIZING ANALYTICAL TECHNIQUES

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

Table 1: Categories of Analytical Techniques

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectroscopy	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Raman Spectroscopy	Liquid Chromatography	Melting Point
	Microcrystalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	
	Cannabis Only:	
	Macroscopic Examination	
	Microscopic Examination	

SECTION 3: IDENTIFICATION CRITERIA

SWGDRUG recommends that laboratories adhere to the following minimum standards:

- 3.1 When a validated Category A technique is incorporated into an analytical scheme, then at least one other technique (from either Category A, B or C) must be used.
- 3.1.1 This combination must identify the specific drug present and must preclude a false positive identification.

- 3.1.2 When sample size allows, the second technique should be applied on a separate sampling for quality assurance reasons. When sample size is limited, additional measures should be taken to assure that the results correspond to the correct sample.
- 3.1.3 All Category A techniques must have data that are reviewable.
- 3.2 When a Category A technique is not used, then at least three different validated methods must be employed.
 - 3.2.1 These in combination must demonstrate the identity of the specific drug present and must preclude a false positive identification.
 - 3.2.2 Two of the three methods must be based on uncorrelated techniques from Category B.
 - 3.2.3 A minimum of two separate samplings should be used in these three tests. When sample size is limited, additional measures should be taken to assure that the results correspond to the correct sample.
 - 3.3.4 All Category B techniques must have reviewable data.
- 3.3 For the use of any method to be considered of value, test results must be considered “positive.” While “negative” test results provide useful information for ruling out the presence of a particular drug or drug class, these results have no value toward establishing the forensic identification of a drug.
- 3.4 In cases where hyphenated techniques are used (e.g., gas chromatography-mass spectrometry, liquid chromatography-diode array ultraviolet spectroscopy), they will be considered as separate techniques provided that the results from each are used.
- 3.5 Cannabis exhibits tend to have characteristics that are visually recognizable. Macroscopic and microscopic examinations of cannabis will be considered, exceptionally, as uncorrelated techniques from Category B when observations include documented details of botanical features. Additional testing must follow the scheme outlined in sections 3.1 or 3.2.
 - 3.5.1 For exhibits of cannabis that lack sufficient observable macroscopic and microscopic botanical detail (e.g., extracts or residues), Δ^9 -tetrahydrocannabinol (THC) or other cannabinoids must be identified utilizing the principles set forth in sections 3.1 and 3.2.
- 3.6 Examples of reviewable data are:
 - 3.6.1 Printed spectra, chromatograms and photographs or photocopies of TLC plates
 - 3.6.2 Contemporaneous documented peer review for microcrystalline tests
 - 3.6.3 Recording of detailed descriptions of morphological characteristics for cannabis (only)
 - 3.6.4 Reference to published data for pharmaceutical identifiers.

SECTION 4: COMMENT

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

PART IV A - QUALITY ASSURANCE/GENERAL PRACTICES

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

SECTION 1: INTRODUCTION

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

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

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

1.1 QUALITY MANAGEMENT SYSTEM

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

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

SECTION 2: PERSONNEL

2.1 JOB DESCRIPTION

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

2.2 DESIGNATED PERSONNEL AND RESPONSIBILITIES

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

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

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

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

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

2.2.5 Analyst: A designated person who

- 2.2.5.1 Examines and analyzes seized drugs or related materials, or directs such examinations to be done
- 2.2.5.2 Independently has access to unsealed evidence in order to remove samples from the evidentiary material for examination AND
- 2.2.5.3 As a consequence of such examinations, signs reports for court or other purposes.
- 2.2.6 Supervisor: A designated person who has the overall responsibility and authority for the technical operations of the drug analysis section. Technical operations include, but are not limited to protocols, analytical methodology, and technical review of reports.

2.3 QUALIFICATIONS/EDUCATION

2.3.1 Technical Support Personnel will

- 2.3.1.1 Have education, skills and abilities commensurate with their responsibilities AND
- 2.3.1.2 Have on-the-job training specific to their position.

2.3.2 Technicians/Assistant Analysts will

- 2.3.2.1 Have education, skills and abilities commensurate with their responsibilities AND
- 2.3.2.2 Have on-the-job training specific to their position.

2.3.3 Analysts will have

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

or

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

2.3.4 Supervisors will

- 2.3.4.1 Meet all the requirements of an analyst (2.3.3),
- 2.3.4.2 Have a minimum of two (2) years of experience as an analyst in the forensic analysis of drugs and
- 2.3.4.3 Demonstrate knowledge necessary to evaluate analytical results and conclusions.

2.4 INITIAL TRAINING REQUIREMENTS

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

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

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

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

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

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

2.5 MAINTAINING COMPETENCE

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

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

2.5.1.2 Training completed must be documented.

SECTION 3: PHYSICAL PLANT

3.1 PHYSICAL PLANT REQUIREMENTS

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

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

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

3.1.4 A laboratory cleaning schedule must be established and implemented.

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

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

SECTION 4: EVIDENCE CONTROL

Laboratories shall have and follow a documented evidence control system to ensure the integrity of physical

evidence.

4.1 RECEIVING AND IDENTIFYING EVIDENCE

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

4.1.1 Submission documents or copies

4.1.2 Identity of party requesting analysis and the date of request

4.1.3 Description of items of evidence submitted for analysis

4.1.4 Identity of the person who delivers the evidence, along with date of submission

4.1.4.1 For evidence not delivered in person, descriptive information regarding mode of delivery and tracking information

4.1.5 Chain of custody record

4.1.6 Unique case identifier

4.2 INTEGRITY OF EVIDENCE

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

4.3 STORAGE OF EVIDENCE

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

4.4 DISPOSITION OF EVIDENCE

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

4.5 DOCUMENTATION PROCEDURES

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

SECTION 5: ANALYTICAL PROCEDURES

5.1 ANALYTICAL PROCEDURES FOR DRUG ANALYSIS

5.1.1 The laboratory shall have and follow written analytical procedures.

- 5.1.2 The laboratory shall have in place protocols for the sampling of evidence.
- 5.1.3 Work practices shall be established to prevent contamination of evidence during analysis.
- 5.1.4 The laboratory shall monitor the analytical processes using appropriate controls and traceable standards.
- 5.1.5 The laboratory shall have and follow written guidelines for the acceptance and interpretation of data.
- 5.1.6 Analytical procedures must be validated in compliance with Section 10.
- 5.1.7 The analyst shall determine the identity of a drug in a sample, and be assured that the result relates to the right submission. This is best established by the use of at least two appropriate techniques based on different principles and two independent samplings.

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

- 5.2.1 The identity of certified reference materials should be verified prior to their first use.
- 5.2.2 The identity of uncertified reference materials must be authenticated prior to use by methods such as mixed melting point determination, Mass Spectrometry, Infrared Spectroscopy, or Nuclear Magnetic Resonance Spectrometry.
- 5.2.3 Verification must be performed on each new drug lot.
- 5.2.4 All verification testing must be documented to include the name of the individual who performed the identification, date of verification, verification test data, and reference identification.

SECTION 6: INSTRUMENT/EQUIPMENT PERFORMANCE

6.1 **INSTRUMENT PERFORMANCE**

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

- 6.1.1 Monitoring should include the use of reference standards, test mixtures, calibration standards, blanks, etc.
- 6.1.2 Instrumentation performance monitoring must be documented.

6.2 **EQUIPMENT**

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

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

SECTION 7: CHEMICALS AND REAGENTS

7.1 **CHEMICALS AND REAGENTS**

- 7.1.1 Chemicals and reagents used in drug testing must be of the appropriate grade for the tests performed.
- 7.1.2 There must be written formulations for all chemical reagents produced within the laboratory.
- 7.1.3 Documentation for reagents prepared within the laboratory must include identity, concentration (when appropriate), date of preparation, identity of the individual preparing the reagents and the expiration date (if appropriate).
- 7.1.4 The efficacy of all test reagents must be checked prior to their use in casework. The results of these tests should be documented.
- 7.1.5 Chemical and reagent containers should be dated and initialed when received and also when first opened.

SECTION 8: CASEWORK DOCUMENTATION, REPORT WRITING AND REVIEW

8.1 CASEWORK

- 8.1.1 Documentation must contain sufficient information to allow a peer to evaluate case notes and interpret the data.
- 8.1.2 Evidence handling documentation should include chain of custody, the initial weight/count of evidence to be examined (upon receipt by the analyst), information regarding the packaging of the evidence upon receipt, a description of the evidence and communications regarding the case.
- 8.1.3 Analytical documentation should include procedures, standards, blanks, observations, results of the tests, and supporting documentation including charts, graphs, and spectra generated during an examination.
- 8.1.4 Casework documentation must be preserved according to written laboratory policy.

8.2 REPORT WRITING

- 8.2.1 Reports issued by the laboratory must meet the requirements of the jurisdiction served. These may include:
 - 8.2.1.1 Identity of the examining laboratory
 - 8.2.1.2 Case identifier
 - 8.2.1.3 Identity of the contributor
 - 8.2.1.4 Date of receipt
 - 8.2.1.5 Date of report
 - 8.2.1.6 Descriptive list of submitted evidence
 - 8.2.1.7 Identity of analyst
 - 8.2.1.8 Results/Conclusions

8.2.1.9 Analytical techniques employed

8.3 CASE REVIEW

- 8.3.1 The laboratory must have a written policy establishing the protocols for technical and administrative case review.
- 8.3.2 The laboratory must have a written policy to determine the course of action should an analyst and reviewer fail to agree.

SECTION 9: PROFICIENCY AND COMPETENCY TESTING

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

9.1 PROFICIENCY TESTING

- 9.1.1 Laboratories shall perform proficiency testing in order to verify the laboratory's performance in comparison to other laboratories. The frequency of the proficiency testing should be at least annually.
- 9.1.2 The proficiency testing samples should be representative of the laboratory's normal casework.
- 9.1.3 The analytical scheme should be in concert with the normal laboratory analysis procedures.

9.2 COMPETENCY TESTING

- 9.2.1 Laboratories will monitor the competency of their analysts. They should do so at least once a year. One of the ways of doing this is by participating in competency tests.
- 9.2.2 Competency testing samples should be representative of the laboratory's normal casework.
- 9.2.3 The analytical scheme should be in concert with the normal laboratory analysis procedures.

SECTION 10: VALIDATION AND VERIFICATION

- 10.1 Method validation is required to demonstrate that the method is suitable for its intended purpose.
 - 10.1.1 For qualitative analysis the parameters that need to be checked are specificity, limit of detection, and reproducibility.
 - 10.1.2 Minimum acceptability criteria should be described along with means for demonstrating compliance.
 - 10.1.3 Validation documentation is required.
- 10.2 Laboratories adopting methods validated elsewhere should determine their own limit of detection and reproducibility.

SECTION 11: LABORATORY AUDITS

- 11.1 Audits of laboratory operations should be conducted at least once a year.
- 11.2 Records of each audit must be maintained and should include the scope, date of the audit, name of the person conducting the audit, findings, and corrective actions taken, if necessary.

SECTION 12: DEFICIENCY OF ANALYSIS

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

- 12.1 This policy must include the following:
 - 12.1.1 A definition of a deficiency as any erroneous analytical result or interpretation, or any unapproved deviation* from an established policy or procedure in an analysis.
 - * Deviations from established policy must have documented management approval.
 - 12.1.2 A requirement for immediate cessation of the activity or work of the individual involved, if warranted by the seriousness of the deficiency, as defined in the written policy.
 - 12.1.3 A requirement for administrative review of the activity or work of the individual involved.
 - 12.1.4 A requirement for evaluation of the impact this THAT deficiency may have had on other activities of the individual(S) or other analysts.
 - 12.1.5 A requirement for documentation of the follow-up action taken as a result of the review.
 - 12.1.6 A requirement for communication to appropriate employees of any confirmed deficiency which may have implications for their work.

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

SECTION 13: HEALTH AND SAFETY

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

- 13.1 HEALTH AND SAFETY REQUIREMENTS
 - 13.1.1 All personnel should receive appropriate health and safety training.
 - 13.1.2 The drug analysis laboratory shall operate in accordance with laboratory policy and comply with any relevant statutory regulations.

- 13.1.3 Laboratory health, and safety manual(s) shall be readily available to all laboratory personnel.
- 13.1.4 Material Safety Data Sheets (MSDS) shall be readily available to all laboratory personnel.
- 13.1.5 All chemicals, biohazards and supplies must be stored and disposed of according to applicable government regulations and laboratory policy.
- 13.1.6 Safety hazards such as syringes, items with sharp edges or noxious substances should be so labeled.

SECTION 14: DOCUMENTATION

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

- 14.1 Test methods/procedures for drug analysis.
- 14.2 Reference standards (including source and verification).
- 14.3 Preparation and testing of reagents.
- 14.4 Evidence handling protocols.
- 14.5 Equipment calibration and maintenance.
- 14.6 Equipment inventory (e.g., manufacturer, model, serial number, acquisition date).
- 14.7 Proficiency testing.
- 14.8 Personnel training and qualification.
- 14.9 Quality assurance protocols and audits.
- 14.10 Health, safety and security protocols.
- 14.11 Validation data and results.

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PART IV B - QUALITY ASSURANCE/VALIDATION OF ANALYTICAL METHODS

SECTION 1. INTRODUCTION

1.1 Definition and purpose of Validation

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

1.2 Analytical scheme

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

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

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

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

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

1.3 Individual laboratory responsibility

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

1.4 Operational environment

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

1.5 Documentation

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

Personnel involved

Dates

Observations from the process

A statement of conclusions and/or recommendations

Authorization approval signature

1.6 Recommendation

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

SECTION 2. GENERAL VALIDATION PLAN

2.1 Purpose/Scope

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

2.1.1 Performance Specification

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

2.1.2 Process Review

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

2.2 Analytical Method

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

Visual characterization (e.g., macroscopic examination)
Determination of quantity of sample, which may include:

- Weight
- Volume
- Item count

Sampling (representative or random, dry, homogenized, etc.)

Sample preparation

- Extraction method
- Dissolution
- Derivatization
- Crystallization

- Techniques for introducing the sample into the instrumentation

Instrumental parameters and specifications

- A list of the instruments and equipment (e.g., balance and glassware) utilized
- Instrument conditions

Software applications

Calculations

- Equation(s) to be used
- Unit specification
- Number of measurements required
- Reference values
- Significant figure conventions
- Conditions for data rejection
- Uncertainty determination

2.3 Reference materials in validation

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

2.4 Performance Characteristics

2.4.1 Selectivity

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

2.4.2 Matrix effects

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

2.4.3 Recovery

May be determined for quantitative analysis.

2.4.4 Accuracy

2.4.4.1 Precision (repeatability/reproducibility)

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

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

2.4.4.2 Trueness

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

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

2.4.5 Range

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

2.4.5.1 Limit of detection (LOD)

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

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

2.4.5.2 Limit of quantitation (LOQ)

Limit of Quantitation (LOQ) must be determined for all quantitative methods. Determine

the lowest concentration that has an acceptable level of uncertainty.

2.4.5.3 Linearity

Linearity must be determined for all quantitative methods.

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

2.4.6 Robustness

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

2.4.7 Ruggedness

Ruggedness may be determined for qualitative or quantitative methods.

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

2.5 Uncertainty

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

3. Quality Control

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

4. References

A) The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, EURACHEM Guide, 1998.

B) Federal Register, Part VIII, Department of Health and Human Services, March 1995, pp 11259-62.

C) "Validating Analytical Chemistry Methods", Enigma Analytical Training Course (Version 2000-1), Breckenridge, CO, 2000, pp 8-4, 8-5.

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SWGDRUG GLOSSARY

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

- Accreditation** Procedure by which an accreditation body formally recognizes that a laboratory or person is competent to carry out specific tasks.
- Accreditation Body** Independent science-based organization that has the authority to grant accreditation.
- Accuracy** Trueness and precision compose accuracy.
- Analysis** Technical operation to determine one or more characteristics of, or to evaluate the performance of, a given product, material, equipment, physical phenomenon, process, or service according to a specified procedure.
- Analyst** A designated person who:

Examines and analyzes seized drugs or related materials, or directs such examinations to be done. Independently has access to "open" (unsealed) evidence in order to remove samples from the evidence for examination.
As a consequence of such examinations, signs reports for court or other purposes.
- Audit** A review conducted to compare the various aspects of the laboratory's performance with a standard for that performance.
- Blank** Specimen or sample not containing the analyte.
- Calibration** Set of operations that establishes, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding known values of a measurand.
- Certified Reference Material (CRM)** A reference material, one or more of whose property values have been certified by a technical procedure, accompanied by or traceable to a certificate or other documentation that has been issued by a certifying body.
- Certifying Body** Independent science-based organization that has the competence to grant certification.
- Chain of Custody** Procedures and documents that account for the integrity of a sample by tracking its handling and storage from its point of collection to its final disposition.
- Controls** Samples used to determine the validity of the calibration, that is, the linearity and stability of a quantitative test or determination over time. Controls are either prepared from the reference material (separately from the calibrators, that is, weighed or measured separately), purchased, or obtained from a pool of previously analyzed samples. Where possible, controls should be matrix-matched to samples and calibrators.
- Control Sample** A standard of comparison for verifying or checking the finding of an experiment.
- Correlated Techniques** Correlated techniques are those that have the same fundamental mechanism of characterization. For example, this would prevent the choice of two gas chromatographic tests both based on a partition mechanism (e.g., methylsiloxane

and phenylmethylsiloxane) or two thin layer chromatographic systems both based on an adsorption mechanism.

- Deficiency of Analysis** Any erroneous analytical result or interpretation, or any unapproved deviation from an established policy or procedure in an analysis.
- False Positive** Test result that states that a drug is present when, in fact, such a drug is not present in an amount less than a threshold or designated cut-off concentration.
- Health & Safety Manager** A designated person who is responsible for maintaining the laboratory health and safety program (including an annual review of the program) and who monitors compliance with the program.
- Independent Test Result** Result obtained in a manner not influenced by any previous results on the same or similar material.
- Laboratory** Facilities where analyses are performed by qualified personnel using adequate equipment.
- Limit of Detection:** Limit of detection (LOD) is the smallest measured content from which it is possible to deduce the presence of the analyte with reasonable statistical certainty.
- Limit of Quantitation:** The limit of quantitation (LoQ) is the lowest concentration of analyte that can be determined with an acceptable level of precision and trueness.
- Linearity:** Defines the ability of the method to obtain test results proportional to the concentration of analyte.
- Method** Detailed, defined procedure for performing an analysis. See Procedure.
- Procedure** Specified, documented way to perform an activity.
- Proficiency Testing** Ongoing process in which a series of proficiency samples, the characteristics of which are not known to the participants, are sent to laboratories on a regular basis. Each laboratory is tested for its accuracy in identifying the presence (or concentration) of the drug using its usual procedures.
- Qualitative Analysis** Test that determines the presence or absence of specific drugs in the sample.
- Qualitative Test** See **Qualitative Analysis**
- Quality Assurance (QA)** System of activities whose purpose is to provide, to the producer or user of a product or a service, the assurance that it meets defined standards of quality with a stated level of confidence.
- Quality Assurance Manager** A designated person who is responsible for maintaining the quality management system and who monitors compliance with the program.
- Quality Management** That aspect of the overall management function that determines and implements the quality policy.
- Quality Manual** Document stating the general quality policies, procedures and practices of an organization.

Quantitative Analysis	Procedure to determine the quantity of drug present in a sample.
Quantitative Test	See Quantitative Analysis
Range	Set of concentrations of analyte in which the error of a method is intended to lie within specified limits.
Reference Material	Material or substance one or more properties of which are sufficiently well established to be used for calibrating an apparatus, assessing a measurement method, or assigning values to materials.
Repeatability	Closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement.
Report	Document containing a formal statement of results of tests carried out by a laboratory.
Representative Sample	Statistically, a sample that is similar to the population from which it was drawn. When a sample is representative, it can be used to make inferences about the population. The most effective way to get a representative sample is to use random methods to draw it. Analytically, it is a sample that is a portion of the original material selected in such a way that is possible to relate the analytical results obtained from it to the properties of the original material.
Reproducibility	Closeness of agreement between the results of successive measurements of the same analyte in identical material made by the same method under different conditions, e.g., different operators and different laboratories and considerably separated in time.
Robustness	The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
Sample	A portion of the whole material to be tested. Statistically, it is a set of data obtained from a population.
Sampling	Analytically, the whole set of operations needed to obtain a sample, including planning, collecting, recording, labeling, sealing, shipping, etc. Statistically, it is the process of determining properties of the whole population by collecting and analyzing data from a representative segment of it.
Selectivity	Extent to which a method can determine particular analyte(s) in a mixture without interference from the other components in the mixture. A method that is perfectly selective for an analyte or group of analytes is said to be specific.
Specificity	See Selectivity .
Standard Operating Procedures (SOPs)	A written document which details the method of an operation, analysis, or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.
Supervisory Chemist	A designated person who has the overall responsibility and authority for the technical operations of the drug analysis section.

- Technical/Assistant Analyst** A person who analyses evidence, but does not issue reports for court purposes.
- Technical Support Personnel** A person who performs basic laboratory duties, but does not analyze evidence.
- Test** See **Analysis**
- Traceable** Ability to trace the history, application, or location of an entity by means of recorded identification. See also **Chain of Custody**.
- Traceability** The property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.
- Trueness:** The closeness of agreement between the average value obtained from a large set of test results and an accepted reference value.
- Uncertainty:** Parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand.
- Validation** Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled.
- Verification** Confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. (Method works in your lab as well as where it was validated.)

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