



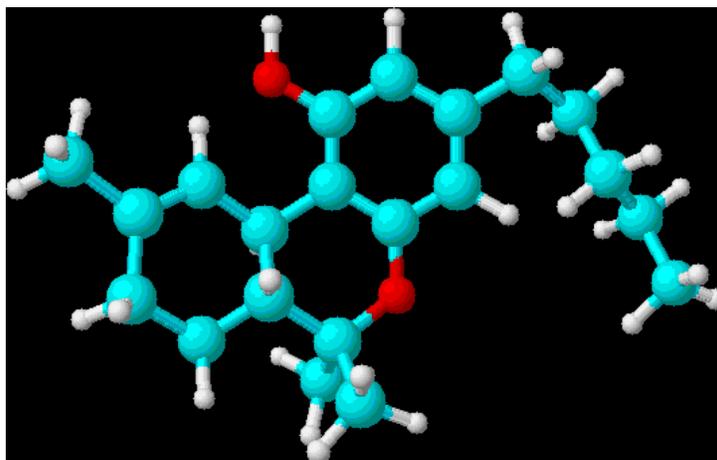
Microgram

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Note: In order to prevent automated theft of email addresses off the Internet postings of *Microgram Journal*, all email addresses reported in the *Journal* have had the “@” character replaced by “-at-”.

Cover Art: “Ball and Stick” Model of Δ^9 -Tetrahydrocannabinol (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).

Technical Note

Identification of Bufotenine in Yopo Seeds via GC/IRD

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ABSTRACT: The analysis of seeds from yopo (*Anadenanthera peregrina*) by GC/IRD and GC/MS is presented. The GC/IRD technique is easily able to discriminate between bufotenine (present in yopo seeds) and its positional isomer psilocin.

KEYWORDS: Yopo, *Anadenanthera Peregrina*, Bufonenine, Psilocin, Tryptamines, GC/IRD, GC/MS, Forensic Chemistry

Introduction

Yopo (*Anadenanthera peregrina*) is a tree that is native to the open plains of South America (1,2). Its leaves, bark, and seeds (sometime called “beans”) reportedly contain bufotenine (5-hydroxydimethyltryptamine), dimethyltryptamine (DMT), and 5-methoxydimethyltryptamine (5-MeO-DMT) (1-6). The seeds are ground with a mortar and pestle into a snuff-like powder that is used by indigenous peoples in various religious rituals. Because the various tryptamines that are present in yopo are hallucinogenic, the seeds are also subject to abuse, and so are irregularly encountered in forensic laboratories (3).

Recently, this laboratory (NCIS - RFL - San Diego) received a zip-lock plastic bag that contained approximately 20 suspected yopo seeds (see Photo 1, next page). The exhibit had been confiscated from a U.S. Navy member in Japan (no further details).

Analysis of any substrate containing bufotenine by GC/MS is complicated by the similarity of its mass spectrum with that of its positional isomer psilocin (4-hydroxydimethyltryptamine). Bufotenine and psilocin are both controlled under Schedule I of the U.S. Controlled Substances Act, but bufotenine-containing substrates are

¹ The NCIS San Diego Laboratory ceased operations in early 2006.



Photo 1

submitted far less commonly to forensic laboratories than psilocin-containing substrates. Analysis and discrimination of the isomers is usually accomplished using a combination of GC and GC/MS, with confirmation (if needed) by additional GC/MS analysis of their respective N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives (7,8). However, GC/IRD is both simpler and gives distinct and easily distinguished spectra (8). Herein, we report the analysis of yopo seeds using a combination of GC/IRD and GC/MS, and compare and contrast the respective spectra for bufotenine and psilocin.

Experimental

Standard Preparation: Bufotenine monooxalate and psilocin standards (Sigma, St. Louis, MO) were provided by the DEA Southwest Laboratory. For GC/MS and GC/IRD analyses, a small amount (not weighed) of these standards were placed in glass vials and dissolved in a few drops of methanol.

Sample Preparation: Using a scalpel blade, the thin hard dark brown outer coating was removed from one of the seeds. The inside, uniform, light brownish-yellow material was placed in a mortar, covered with saturated sodium bicarbonate, and macerated with a pestle. After sitting for several minutes, the resulting solution was transferred to a separatory funnel and extracted with a small amount of chloroform. The extract was filtered through a cotton plug in a disposable Pasteur pipette. After concentrating via evaporation, the extract was analyzed by GC/MS at the NCIS - RFL - San Diego, and also by both GC and GC/IRD at the DEA Southwest Laboratory.

Gas Chromatography: An Agilent Technology 6890N GC equipped with a flame ionization detector was used. The GC was fitted with a 10 m x 0.10 mm i.d. capillary column coated with 0.34 μm 5 % phenylmethyl siloxane (J&W DB-5). The GC was operated in a split mode of approximately 50:1. The injector port and detector temperatures were maintained at 280 $^{\circ}\text{C}$. The oven temperature program was as follows: Initial temperature, 100 $^{\circ}\text{C}$ for 1 minute, ramped up to 280 $^{\circ}\text{C}$ at 25 $^{\circ}\text{C}$ per minute, with a final hold of 1.5 minutes. Hydrogen was used at an average velocity of 99 cm/second.

Gas Chromatography/Mass Spectrometry: An Agilent Technology 6890 GC interfaced to an Agilent Technology 5972A Mass Selective Detector was used. The GC was fitted with a 30 m x 0.25 mm i.d. capillary column with 0.25 μm 5 % polyphenylmethyl siloxane (J & W DB-5MS). The GC was operated in a split mode of 50:1. Helium was used as a carrier gas at a column flow rate of 28 cm/second. The injection port temperature was maintained at 250 $^{\circ}\text{C}$. The oven temperature program was as follows: Initial temperature, 70 $^{\circ}\text{C}$ for 2

minutes, ramped up to 300 °C at 20 °C per minute, with a final hold of 15 minutes. The MSD transfer line was maintained at 280 °C. The MSD was operated at 70 eV.

Gas Chromatography/Infrared Spectroscopy: A Varian/Digilab GC/IRD was used. The GC was fitted with a 25 m x 0.32 mm i.d. capillary column with 0.52 µm 5 % phenylmethyl siloxane (HP-5). The GC was operated in a splitless mode, with a purge delay time of 0.50 minute. Helium was used as the carrier gas at a column flow rate of 36 cm/second. The injector port temperature was maintained at 275 °C. The oven temperature program was as follows: Initial temperature, 70 °C for 1 minute; ramped up to 300 °C at 25 °C per minute, with a final hold of 3 minutes. The flow cell and transfer line temperatures were maintained at 250 °C.

Results and Discussion

The workup procedure gave a chloroform extract that was surprisingly clean, and that contained a significant amount of bufotenine based on the intensities of the GC, GC/MS, and GC/IRD signals. Clearly, even less than one seed would have provided sufficient sample for analysis and identification. The psilocin eluted prior to the bufotenine on all three instrument systems, and the elution time for the yopo seed extract matched the bufotenine standard (the seed extract's greater concentration caused some peak broadening). Figures 1 through 6 show, respectively, the GC, GC/MS, and GC/IRD instrumental results for the seed extract, the psilocin standard, and the bufotenine standard.

Although others have reported that the seeds (or beans) contain DMT and 5-MeO-DMT in addition to bufotenine (1-6), in fact only bufotenine was found in the seeds in this case. A portion of these seeds were sent to James S. Miller, Ph.D., Curator and Director at the William L. Brown Center for Plant Genetic Resources, Missouri Botanical Garden [P.O. Box 299, St. Louis, MO 63166-0299]; Dr. Miller confirmed that the seeds were from *Anadenanthera peregrina*, "Yopo."

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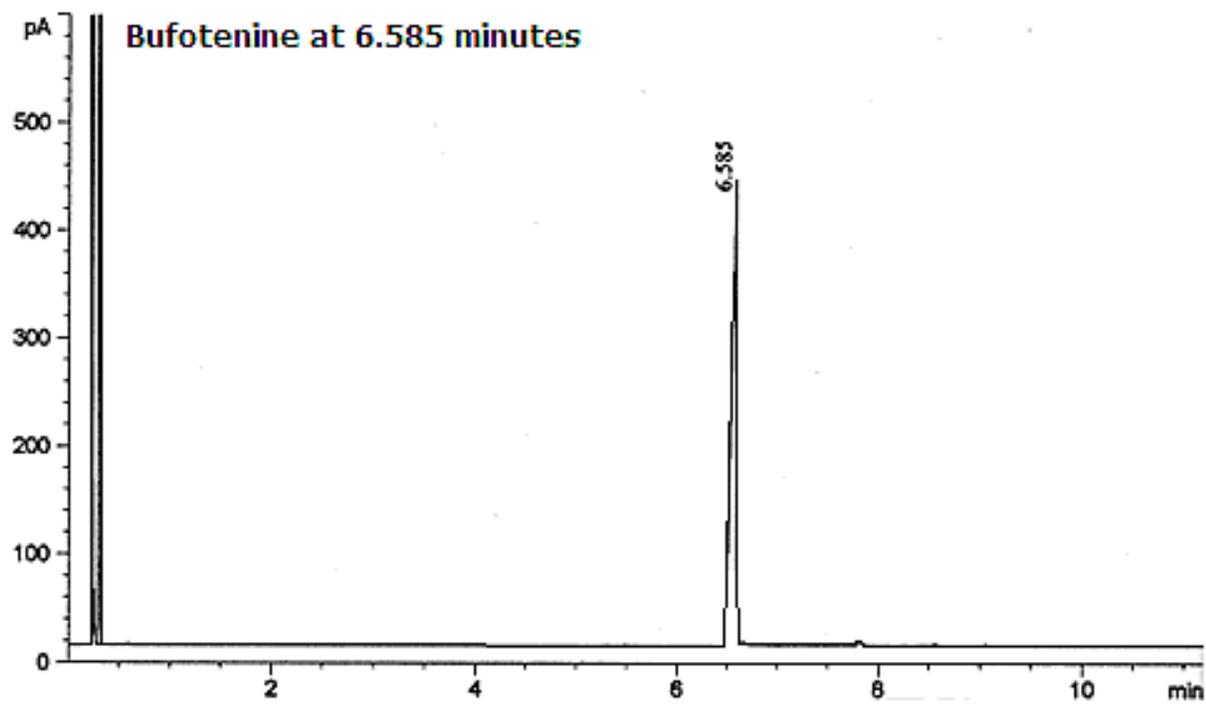
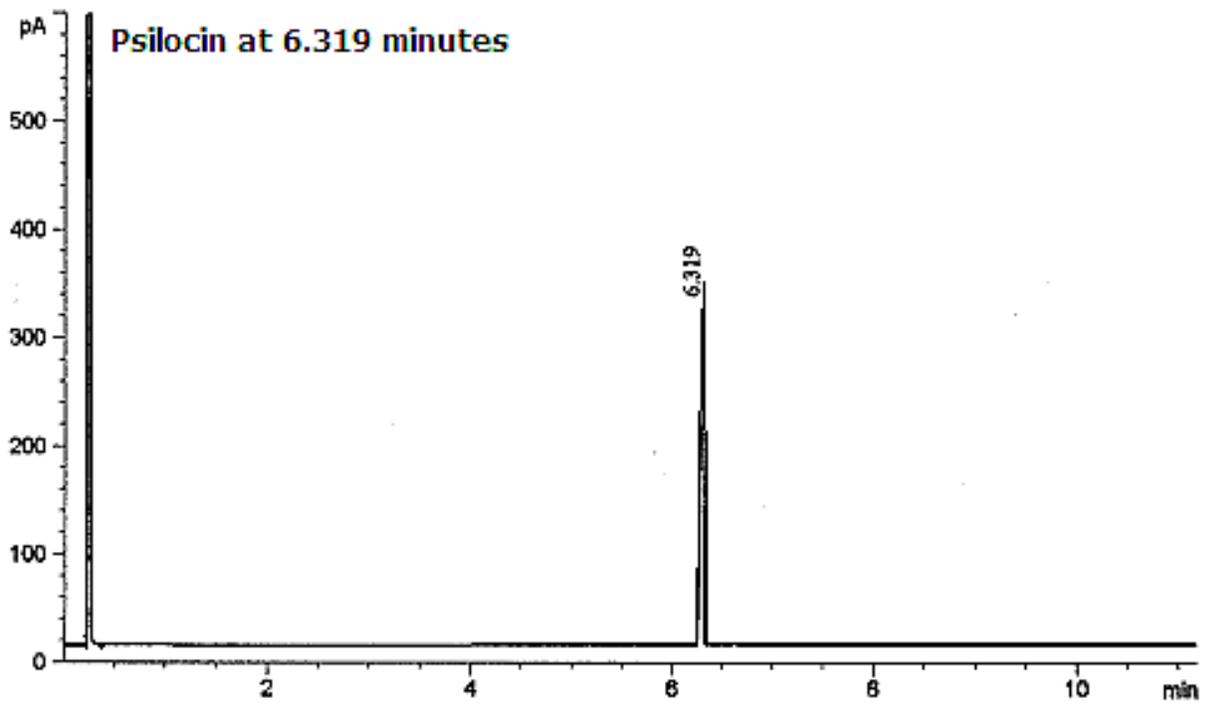


Figure 1. Chromatographs of Psilocin and Bufotenine.

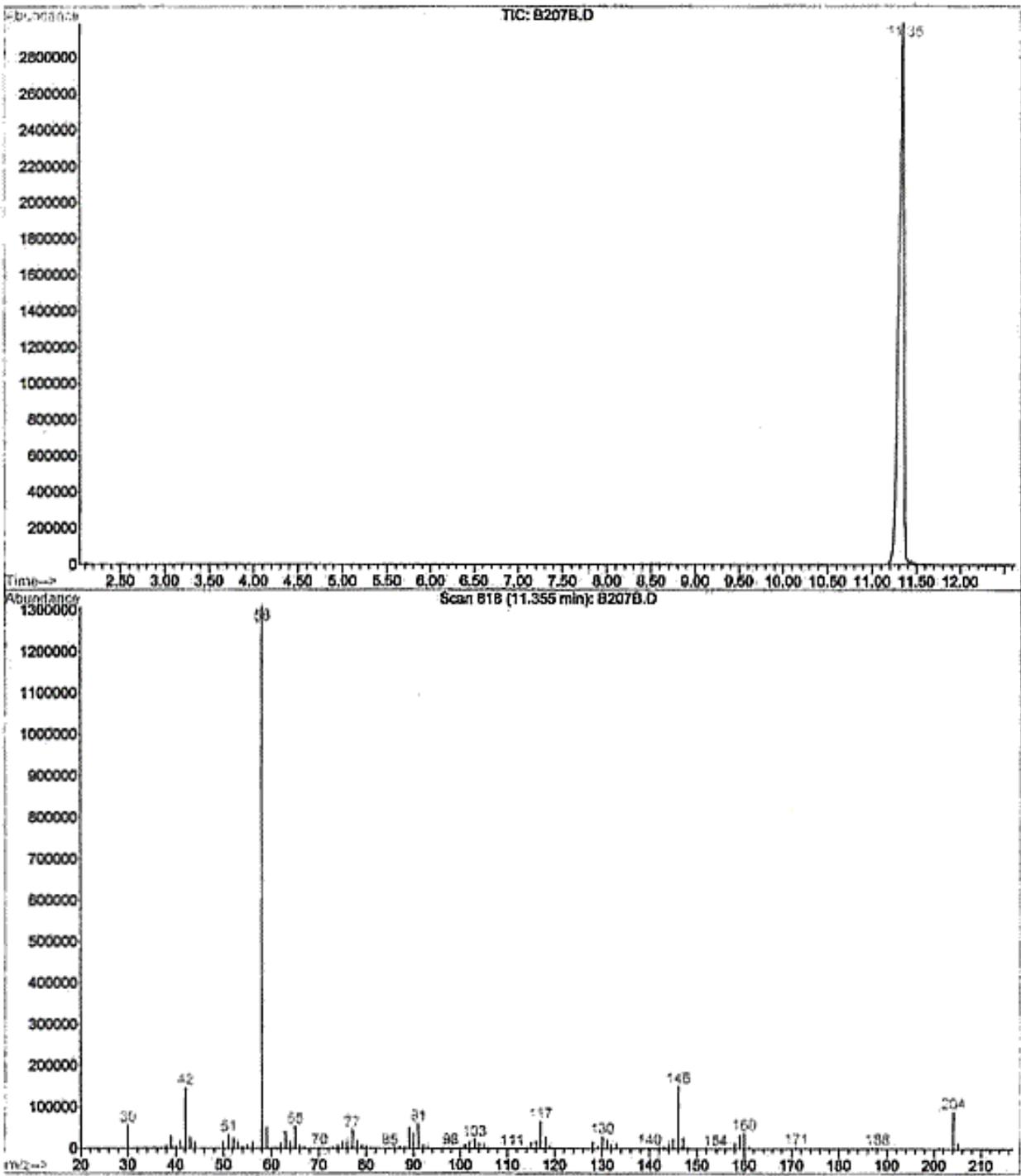


Figure 2. TIC and Mass Spectrum of Yopo Seed Extract.

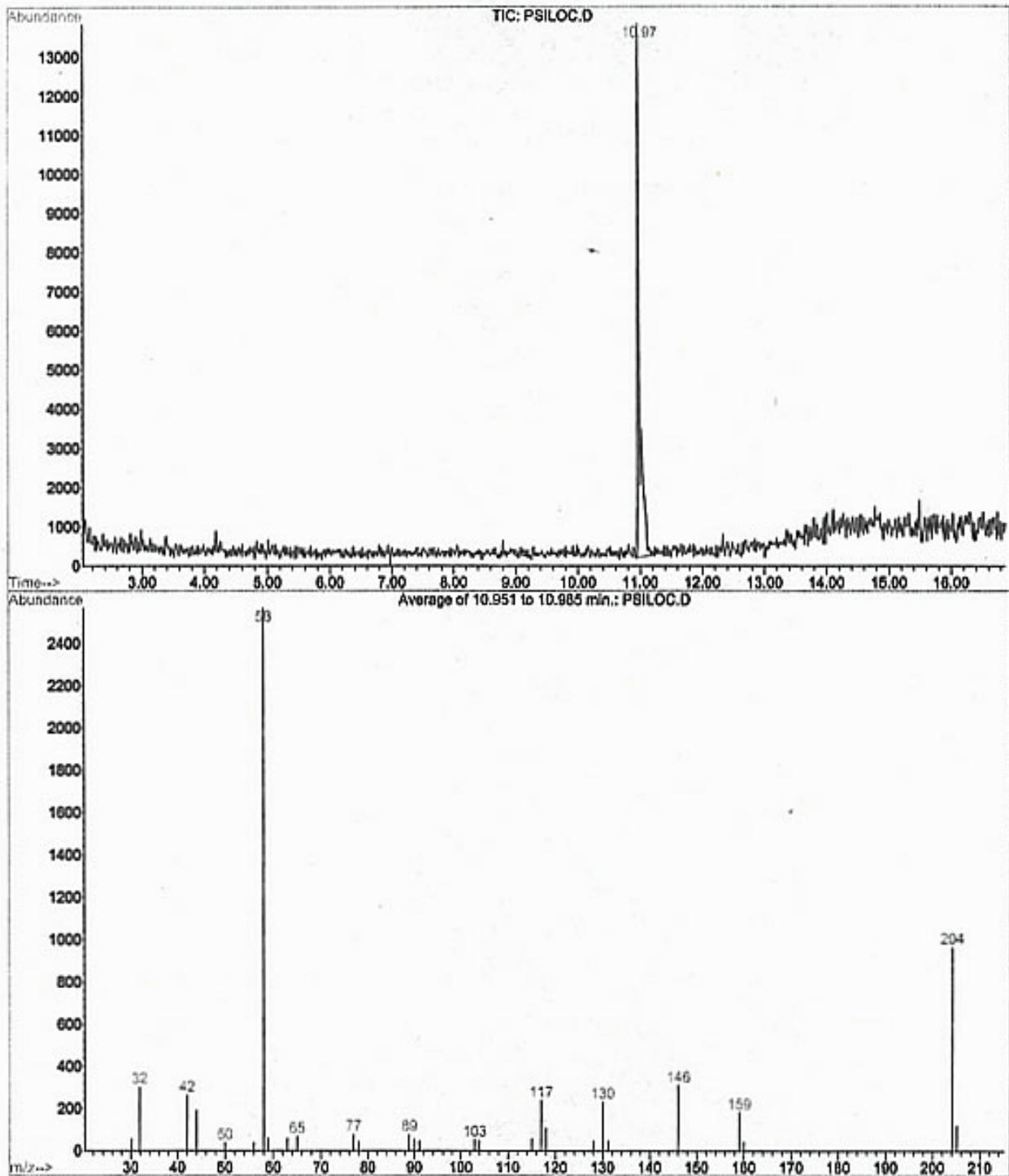


Figure 3. TIC and Mass Spectrum of Psilocin Standard.

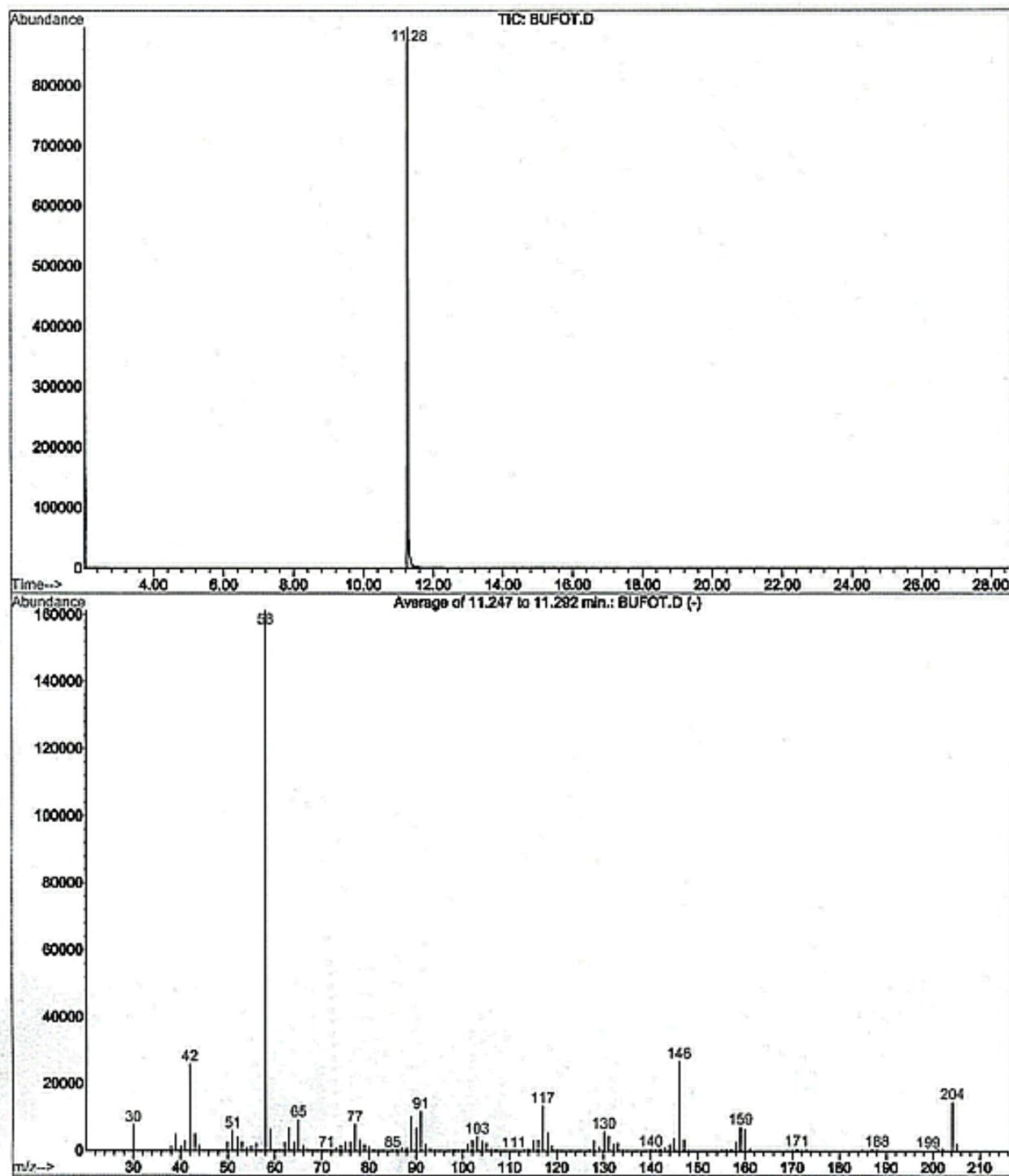


Figure 4. TIC and Mass Spectrum of Bufotenine Standard.

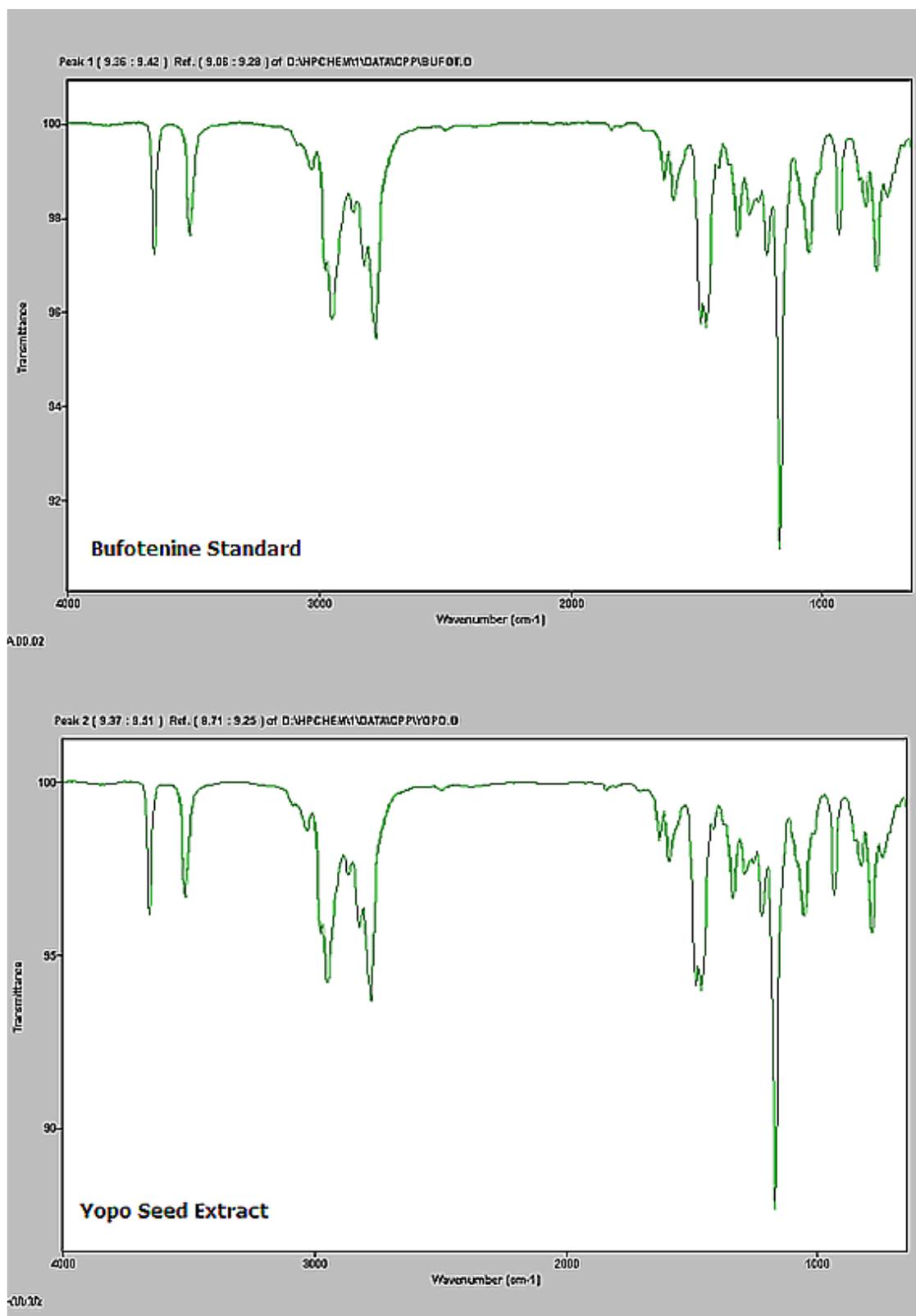


Figure 5. GC/IRD Spectra of Bufotenine Standard and Yopo Seed Extract.

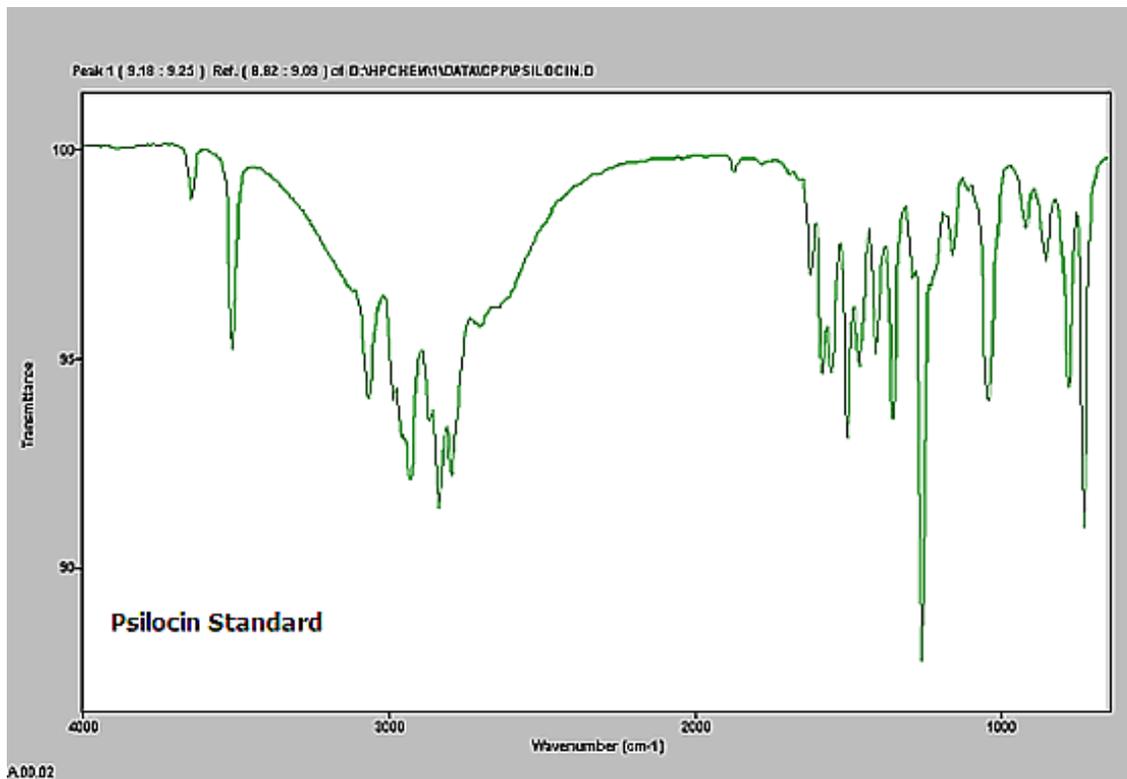


Figure 6. GC/IRD Spectra of Psilocin Standard.

* * * * *

Analytical Profiles for 3,4,5-, 2,4,5-, and 2,4,6-Trimethoxyamphetamine

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ABSTRACT: Analytical profiles (Marquis color testing, infrared spectroscopy, nuclear magnetic resonance, thin layer chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry) are presented for 3,4,5-trimethoxyamphetamine, 2,4,5-trimethoxyamphetamine, and 2,4,6-trimethoxyamphetamine. The data allows identification and differentiation of these positional isomers.

KEYWORDS: 3,4,5-Trimethoxyamphetamine, 2,4,5-Trimethoxyamphetamine, 2,4,6-Trimethoxyamphetamine, TMA, Positional Isomers, Marquis, IR, NMR, TLC, HPLC, GC/MS, Forensic Chemistry

Introduction

Most of the trimethoxyamphetamines (TMAs) are hallucinogens (1). There are six different positional isomers, that differ only in the respective positions of the three methoxy groups on the benzene ring (see Figure 1, next page). Of the six isomers, 3,4,5-trimethoxyamphetamine (TMA-1), 2,4,5-trimethoxyamphetamine (TMA-2), and 2,4,6-trimethoxyamphetamine (TMA-6) are more important than other three isomers, both from the perspective of their legal status and their circulation in Japanese drug markets. Unlike in the United States, positional isomers of hallucinogenic phenethylamines are not automatically controlled under Japanese statutes. Thus, TMA-1 is controlled by the Narcotics and Psychotropics Control Law in Japan, while TMA-2 is currently uncontrolled (but is anticipated to be scheduled in the near future), and TMA-6 is currently uncontrolled. In Japan, TMA-1 is usually sold as a solid, while TMA-2 and TMA-6 are more commonly sold in liquid forms, usually mixed with pigments, flavors, and sometimes other psychoactive compounds. Currently, abuse of 2,3,4-trimethoxyamphetamine (TMA-3), 2,3,5-trimethoxyamphetamine (TMA-4), and 2,3,6-trimethoxyamphetamine (TMA-5) have not been reported in Japan.

Because the legal status of the TMAs vary by structure in Japan, it is important to be able to identify and differentiate between (at least) TMA-1, TMA-2, and TMA-6. To our knowledge, no methods have been reported for such differentiation. Herein, we present analytical data (color testing, infrared spectroscopy (IR), nuclear magnetic resonance (NMR), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography/mass spectrometry (GC/MS)) for TMA-1, TMA-2, and TMA-6.

Experimental

Syntheses: Authentic standards of hydrochloride salts of TMA-1, TMA-2, and TMA-6 were synthesized in our laboratory using previously reported procedures (1). All other chemicals used were of analytical grade.

Color Testing: Marquis reagent was prepared by adding one drop of formaldehyde to 1 mL of concentrated sulfuric acid (2). The sample was placed in a depression of spot plates, and 3 drops of the reagent were added. [The TLC spray reagents are reported below.]

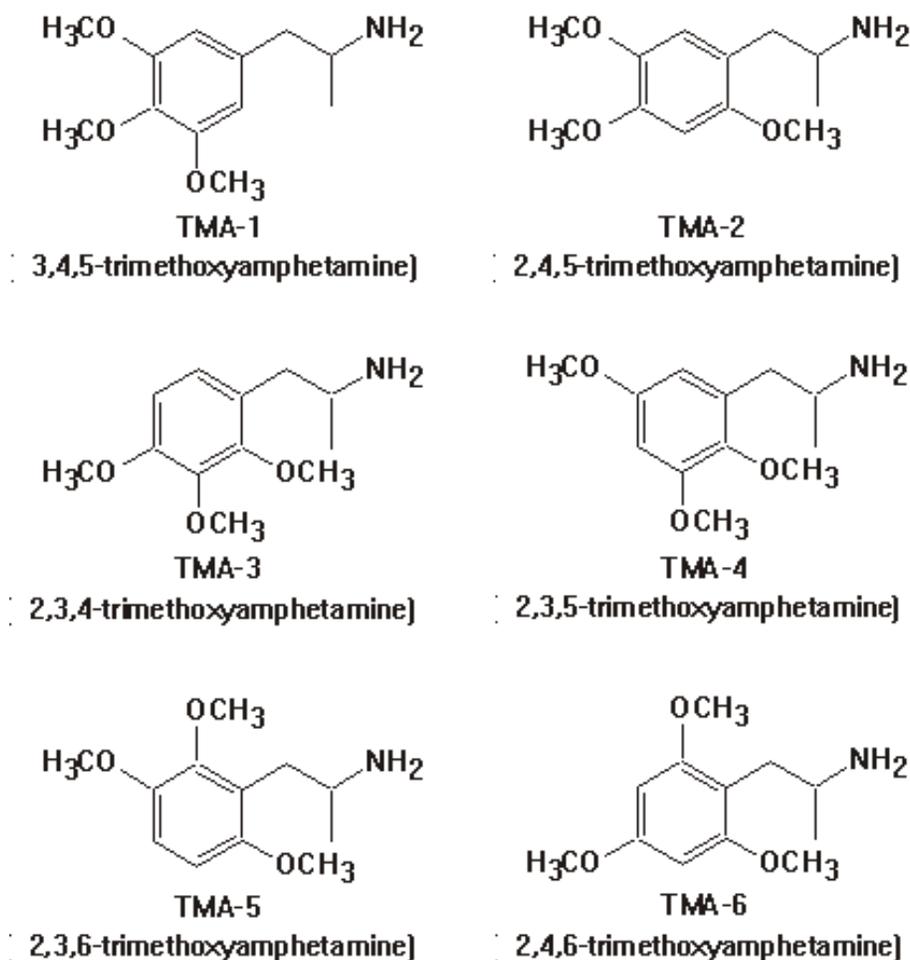


Figure 1. The TMA Positional Isomers.

FTIR: A Shimadzu FTIR-8900 Fourier Transform Infrared Spectrophotometer was used. The substrates were analyzed using the standard potassium bromide method. Thirty-two scans were collected between 4000 and 450 cm^{-1} , with a resolution of 4.0 cm^{-1} .

NMR: Proton NMR analyses were performed on a JEOL JNM-ECP600 NMR spectrometer. The samples were prepared at approximately 10 mg/mL in methanol- d_4 (CD_3OD), using added tetramethylsilane (TMS) as the 0.0 ppm reference.

TLC: TLC analyses were performed using the method of Takahashi *et al.* (3), with a minor modification. The analyses were carried out on silica gel plates (10 x 10 cm) containing a fluorescent indicator (254 nm) on glass support (Merck, Darmstadt, Germany). The respective hydrochlorides of each TMA were dissolved in methanol at concentrations of 10, 1, and 0.1 mg/mL. These were applied manually on the plates with a microsyringe. A solvent mixture of chloroform/methanol/25 % aqueous ammonia (75:25:3 v/v/v) was used as the mobile phase. After development and evaporation of the mobile phase, the compounds were detected by UV (254 nm) and by spraying with Dragendorff or fluorescamine reagents (prepared as follows):

Dragendorff reagent: Bismuth hydroxide (0.9 g) was dissolved in concentrated hydrochloric acid (2 mL), and potassium iodine (3 g) dissolved in water (3 mL) and 70 % aqueous acetic acid (45 mL) were then added (4).

Fluorescamine reagent: Fluorescamine (0.5 mg) was dissolved in acetone (1 mL) (2). The spots were observed under UV (365 nm).

HPLC: HPLC analyses were performed using the method of Kikura-Hanajiri *et al.* (5), with a minor modification. A Shimadzu LC-10ADvp series equipped with an SPD-M10Avp diode array detector set at 230 nm was used. The column was a Symmetry C18 column (Waters, 150 mm x 2.1 mm i.d., 3.5 μ m) protected by an OptiGuard C18 guard column (Optimize technology), and was operated at 40 °C. The mobile phase, delivered at a flow rate of 0.2 mL/min, was a gradient of a mixture of acetonitrile-methanol (7:3 v/v) (B) in 10 mM ammonium formate (pH 3.5) (A): 0-1 min, 10 % B; 1-24 min, from 10 % to 33 % linear gradient of B in A. Sample Prep: A volume of 20 μ L containing 10 μ g/mL of each trimethoxyamphetamine hydrochloride dissolved in distilled water was injected.

GC/MS: GC/MS analyses were performed using a GCMS-QP5050A (Shimadzu) equipped with a DB-5MS capillary column (Agilent technologies, 30 m x 0.25 mm i.d., 0.25 μ m film thickness). The temperature of the injector and the interface was set at 250 °C. The oven temperature was held at 50 °C for 1 min, then raised to 300 °C at 15 °C/min, then held for 3 min. Helium was used as the carrier gas (head pressure 52.8 kPa, column flow 1.0 mL/min at 50 °C, constant pressure). The mass spectrometer was operated under electron ionization (EI) mode. One microliter samples were injected in the splitless mode. Sample Prep: For the free bases, the respective hydrochloride salts (200 μ g) were dissolved in 1 mL of distilled water, basified to pH 12 with 1 M sodium hydroxide, and extracted with 1 mL of ethyl acetate. The extract was transferred to a GC vial. For the trifluoroacetylated derivatives, 100 μ L of trifluoroacetic anhydride and 100 μ L of ethyl acetate was added to 50 μ g of the respective hydrochloride salt, and the mixture heated at 55 °C for 20 min. After evaporation of excess reagents, the residue was redissolved in 1 mL of ethyl acetate, and transferred to a GC vial.

Results and Discussion

Color Testing: The Marquis reagent reacted the three TMAs to give the following colors: TMA-1: Red; TMA-2: Pale yellow; and TMA-6: Orange. Different ring substitution patterns are known to give different colors with the Marquis reagent (6); however, the color differences between TMA-1, TMA-2, and TMA-6 were distinct and (somewhat) unexpected.

IR: The IR spectra of the three TMA hydrochloride salts are shown in Figure 2. The spectral patterns in the fingerprint region ($< 1500 \text{ cm}^{-1}$) were completely different, and could therefore be used to unambiguously identify and differentiate the compounds.

NMR: The Proton NMR spectra are shown in Figure 3. The splitting patterns in the aromatic region were different for TMA-2 (two singlet peaks) versus TMA-1 and TMA-6 (one singlet peak). TMA-2 has two chemically nonequivalent protons, while TMA-1 and TMA-6 have two chemically equivalent protons. TMA-1 and TMA-6 could be distinguished by chemical shifts of their aromatic protons. The respective values for TMA-1 and TMA-6 were 6.56 ppm and 6.25 ppm. These values did not agree with those predicted from the empirical rule (7) (6.13 ppm for TMA-1 and 6.00 ppm for TMA-6), but the relative difference was consistent.

TLC: The R_f values of TMA-1, TMA-2, and TMA-6 using the described system were 0.69, 0.65, and 0.59, respectively. Although the spots were very close, they could be differentiated from one another. Table 1 (next page) shows the detection limits by the UV (254 nm) and various detection reagents. The sensitivities of the reagents were in decreasing order: Fluorescamine reagent, Marquis reagent, and Dragendorff reagent. However, the fluorescamine and Dragendorff reagents gave minimal color differences between the three isomers (green fluorescence under UV (365 nm) for the fluorescamine reagent, and orange for the Dragendorff reagent). On the other hand, spraying with Marquis reagent gave different colors, as follows: TMA-1: Orange but immediately fading; TMA-2: yellow; and TMA-6: Orange then changing to purple-red.

Table 1. Detection Limits (milligrams) of the TMAs.

Compound	Detection			
	UV (254 nm)	Dragendorff	Fluorescamine	Marquis
TMA-1	2	10	0.05	5
TMA-2	1	5	0.2	0.05
TMA-6	2	10	0.2	0.5

HPLC: Figure 4 shows the HPLC chromatogram of a mixture of the three TMAs.

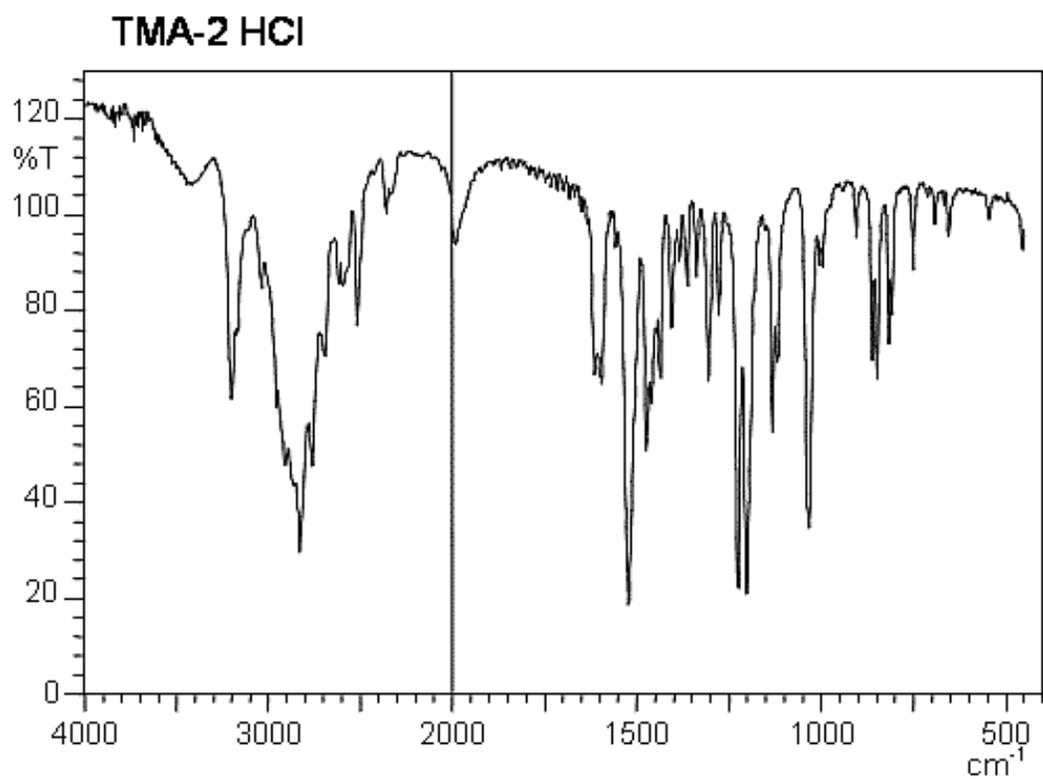
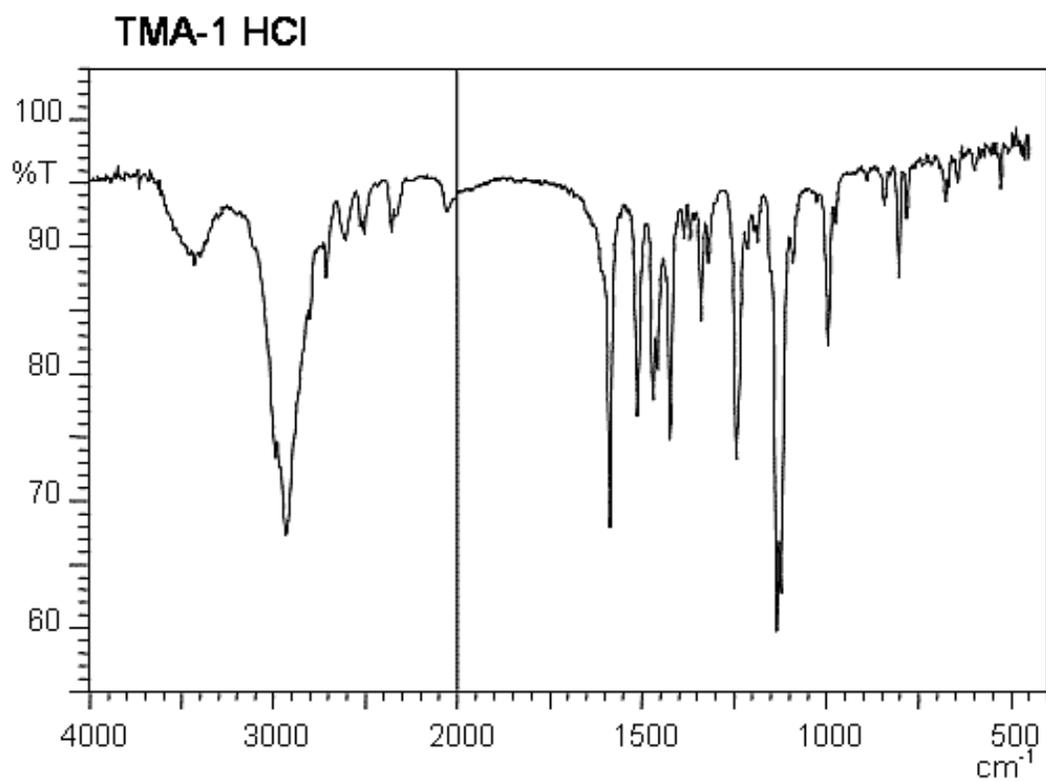
GC/MS: Figure 5 shows the total ion chromatograms (TICs) of the nonderivatized and trifluoroacetylated (TFA-derivatized) TMAs. The nonderivatized TMAs all displayed tailing, and TMA-1 and TMA-2 were not baseline resolved. However, the TFA-derivatives displayed improved peak shapes and enhanced separation between TMA-1 and TMA-2.

Figure 6 shows the EI mass spectra. The spectra of nonderivatized TMAs were similar, and it was especially difficult to discriminate between TMA-1 and TMA-2. However, the TFA-derivatives (though also similar) were sufficiently different for differentiation.

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[Figures 2 - 6 Follow (Note: Figure 4 is Between Figures 2c and 3a in Order to Improve Layout).]



Figures 2a-b. IR Spectra of the Hydrochloride Salts of TMA-1 and TMA-2.

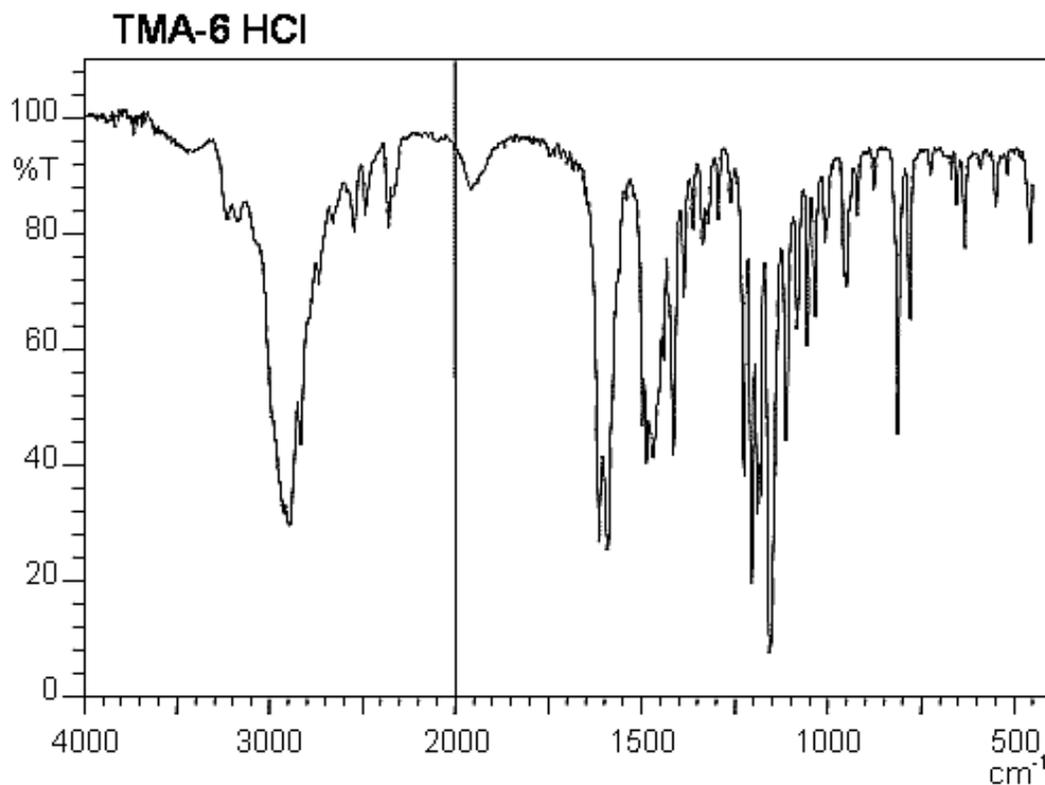


Figure 2c. IR Spectra of the Hydrochloride Salt of TMA-6.

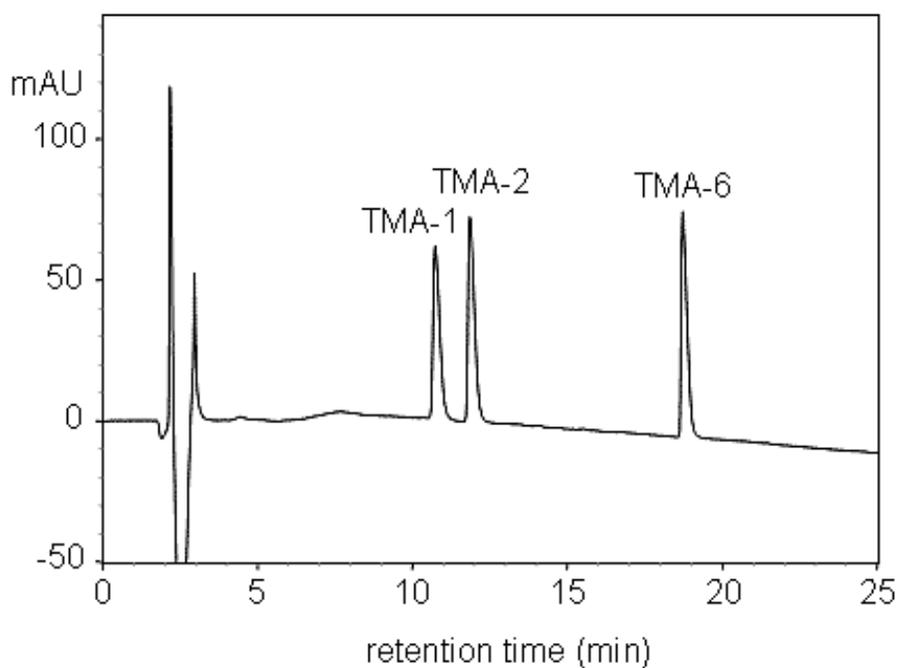


Figure 4. HPLC Chromatogram of a Mixture of the Three TMAs (Detection: UV 230 nm). Retention times (Minutes): TMA-1 - 10.7, TMA-2 - 11.9, and TMA-6 - 18.7.

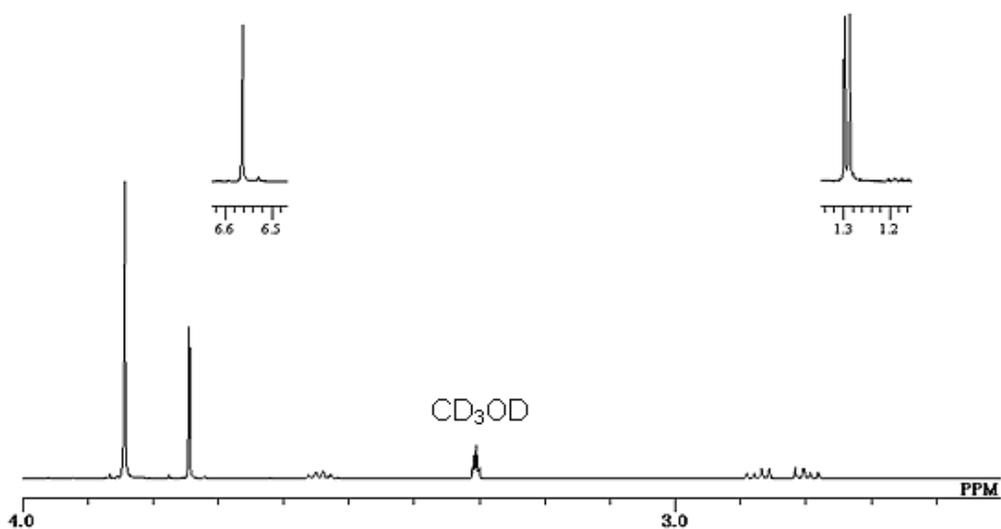
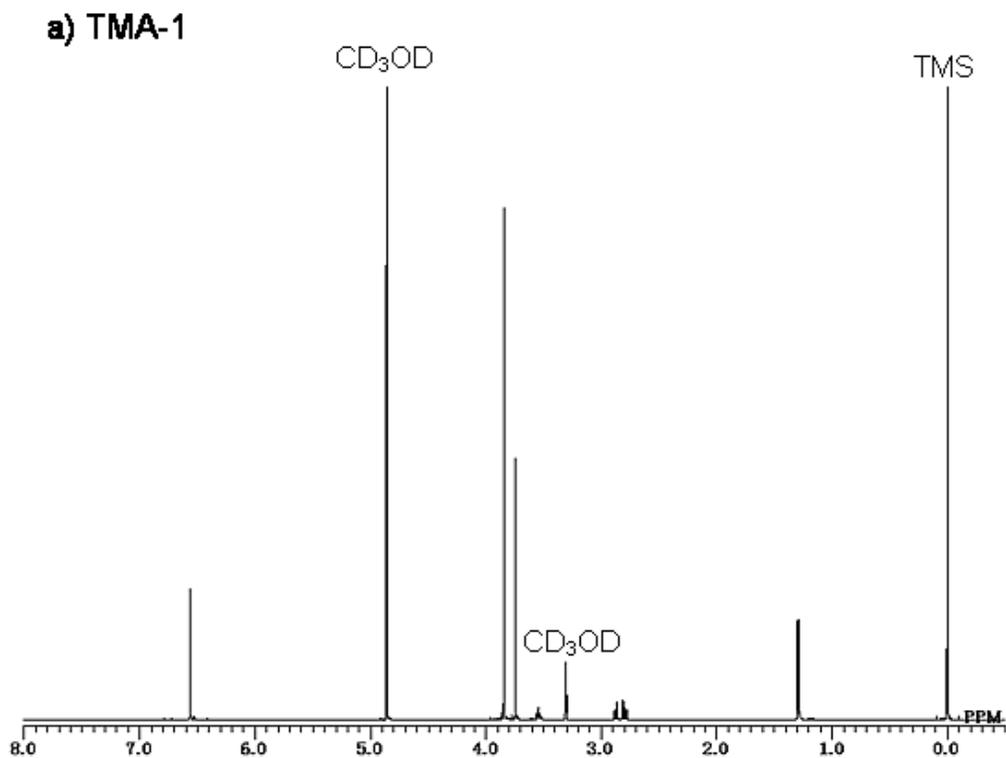


Figure 3a. Proton NMR (600 MHz) of TMA-1: ¹H-NMR (CD₃OD) δ: 6.56 (2H, s), 3.84 (6H, s), 3.74 (3H, s), 3.58-3.51 (1H, m), 2.87 (1H, dd, *J* = 13.7, 7.1 Hz), 2.80 (1H, dd, *J* = 13.5, 7.4 Hz), 1.29 (3H, d, *J* = 6.6 Hz).

b) TMA-2

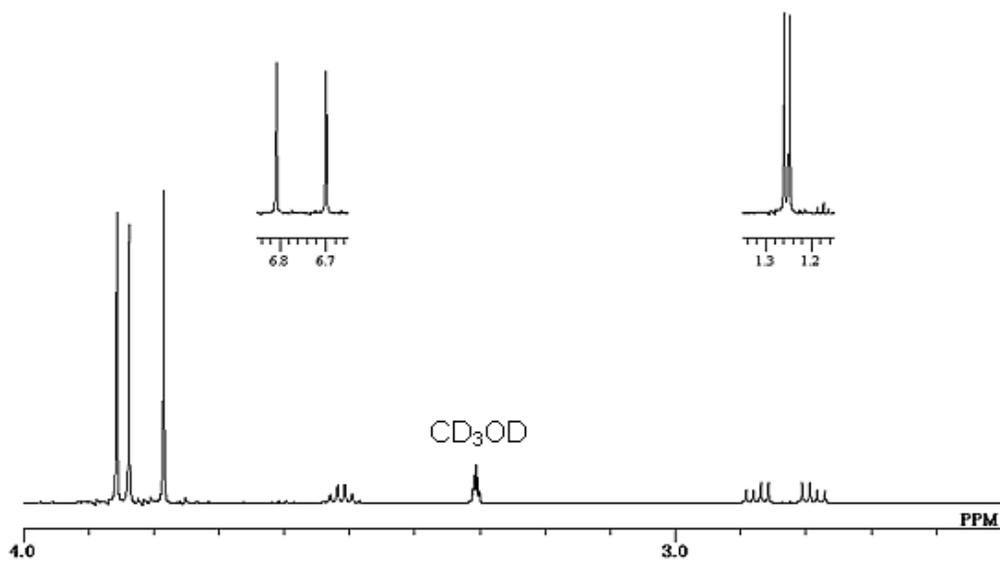
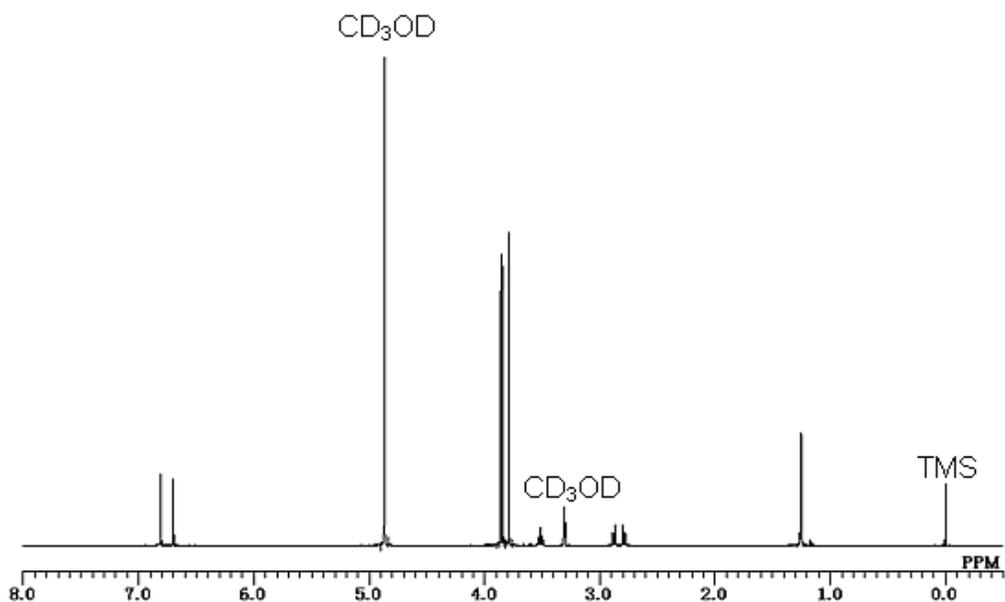


Figure 3b. Proton NMR (600 MHz) of TMA-2: ¹H-NMR (CD₃OD) δ: 6.81 (1H, s), 6.70 (1H, s), 3.86 (3H, s), 3.84 (3H, s), 3.78 (3H, s), 3.54-3.48 (1H, m), 2.87 (1H, dd, *J* = 13.6, 6.9 Hz), 2.79 (1H, dd, *J* = 13.7, 6.9 Hz), 1.25 (3H, d, *J* = 6.7 Hz).

c) TMA-6

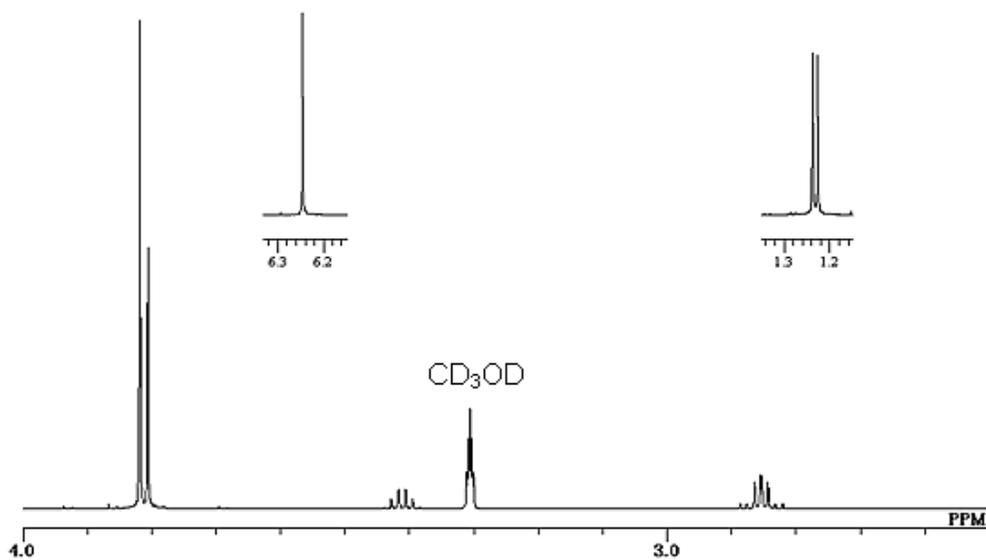
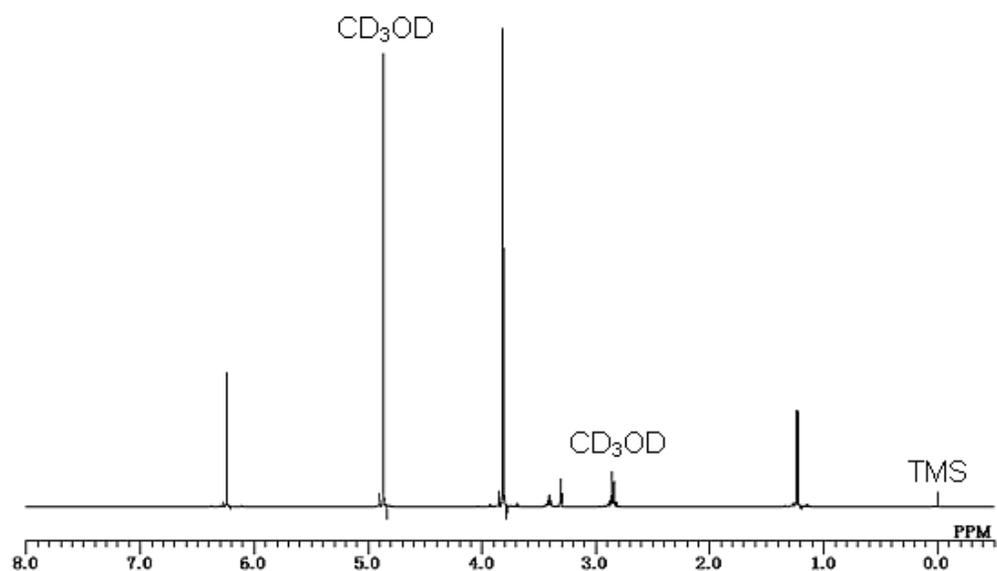


Figure 3c. Proton NMR (600 MHz) of TMA-6: $^1\text{H-NMR}$ (CD_3OD) δ : 6.25 (2H, s), 3.82 (6H, s), 3.81 (3H, s), 3.44-3.38 (1H, m), 2.87 (1H, dd, $J = 13.0, 6.0$ Hz), 2.84 (1H, dd, $J = 13.3, 6.8$ Hz), 1.23 (3H, d, $J = 6.7$ Hz).

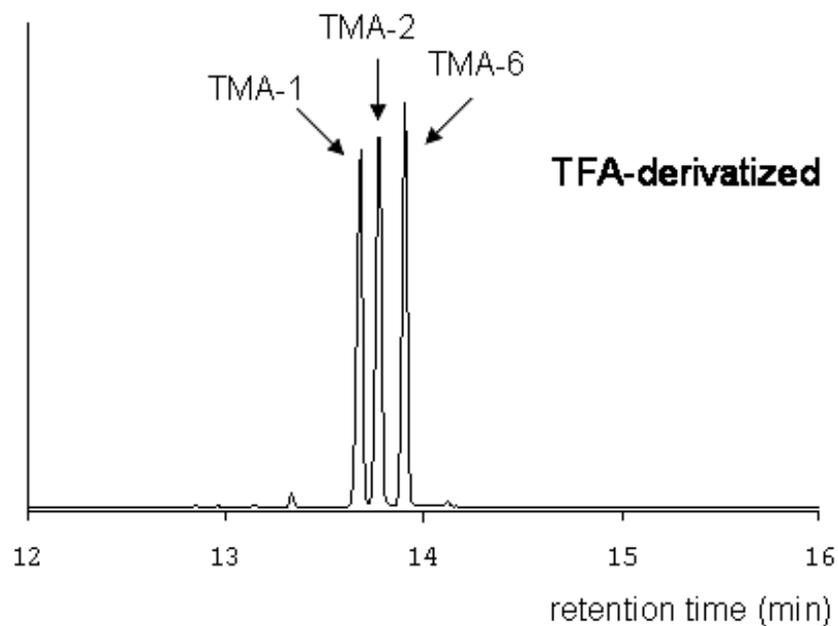
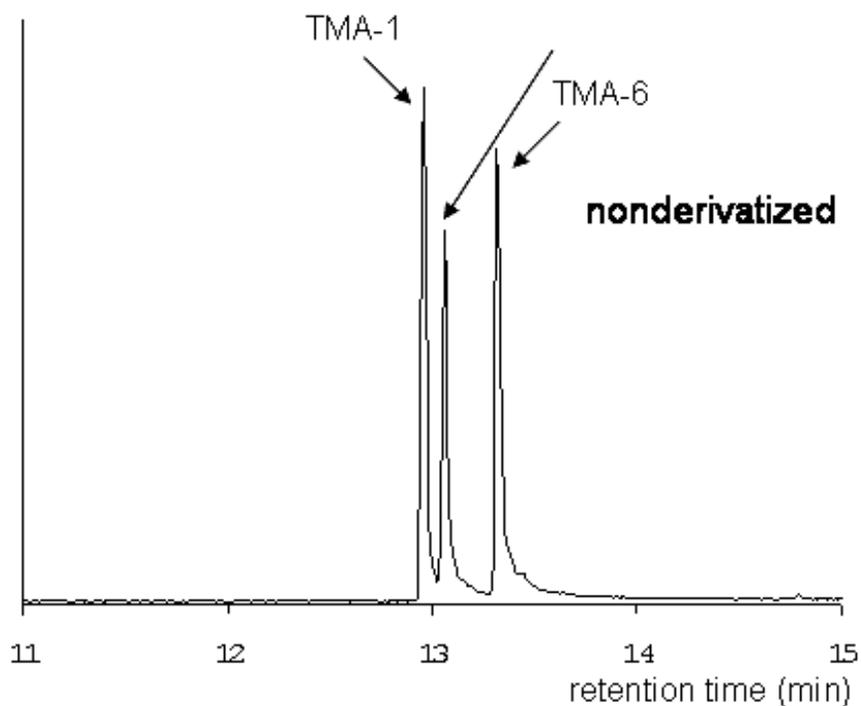


Figure 5. Total Ion Chromatograms of the Three Non-Derivatized and TFA-Derivatized TMAs.

[Retention Indices: Non-Derivatized: TMA-1 - 1724; TMA-2 - 1739; TMA-6 - 1771;
 TFA-Derivatized: TMA-1 - 1814; TMA-2 - 1830; TMA-6 - 1849.]

nonderivatized

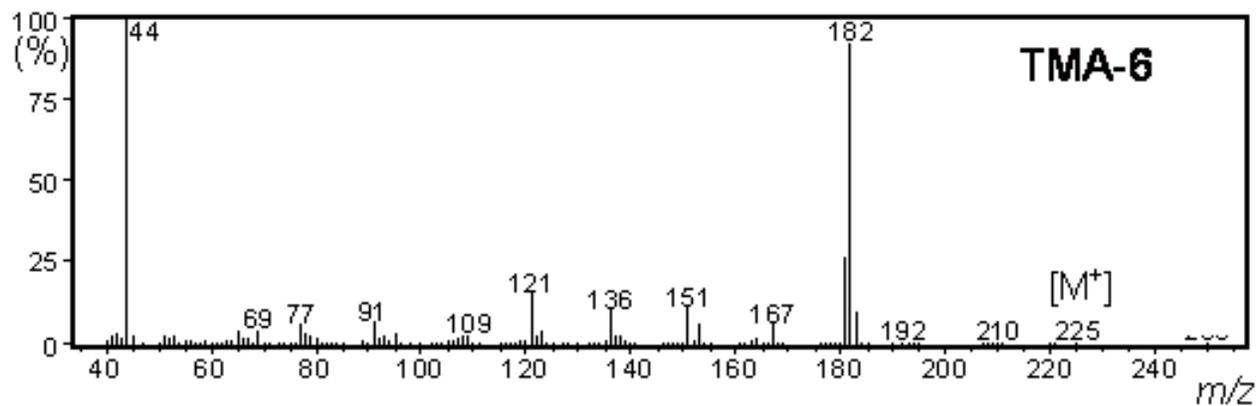
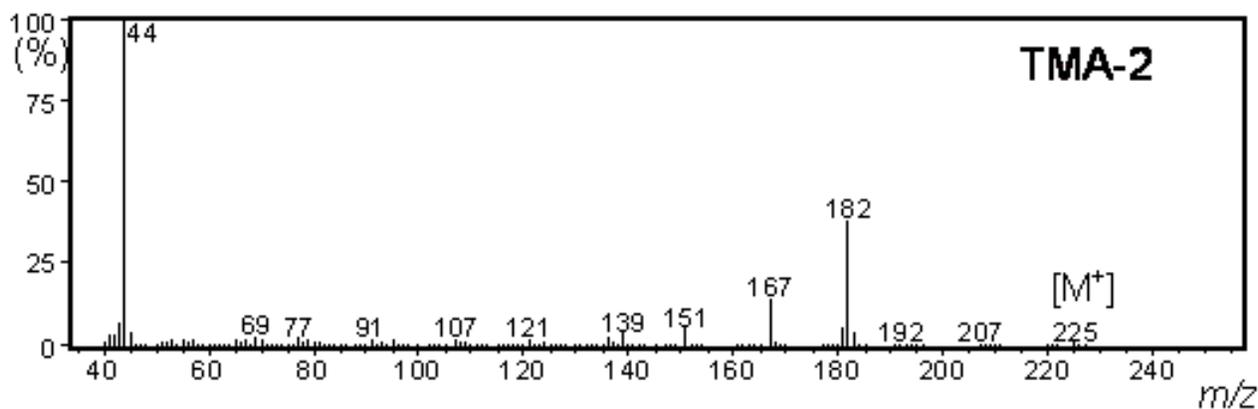
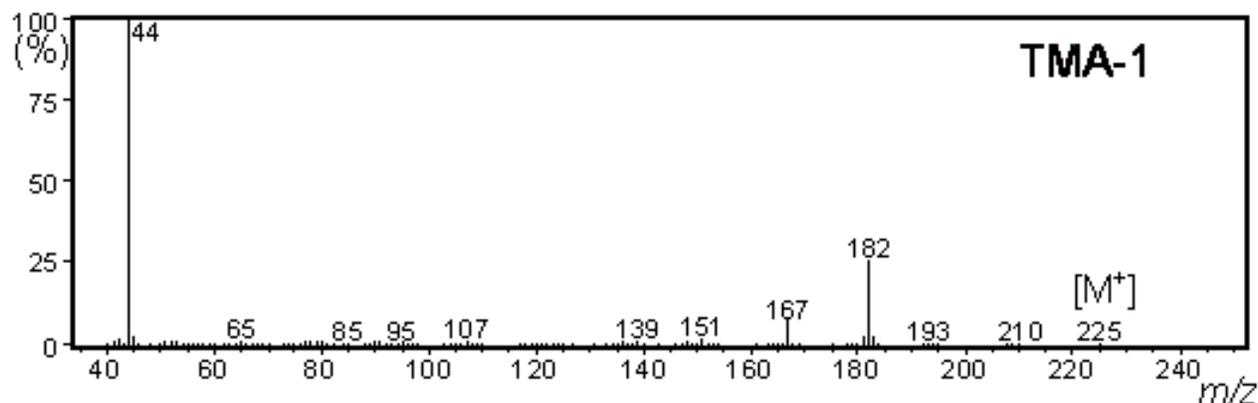


Figure 6a. EI Mass Spectra of the Three Non-Derivatized TMAs.

TFA-derivatized

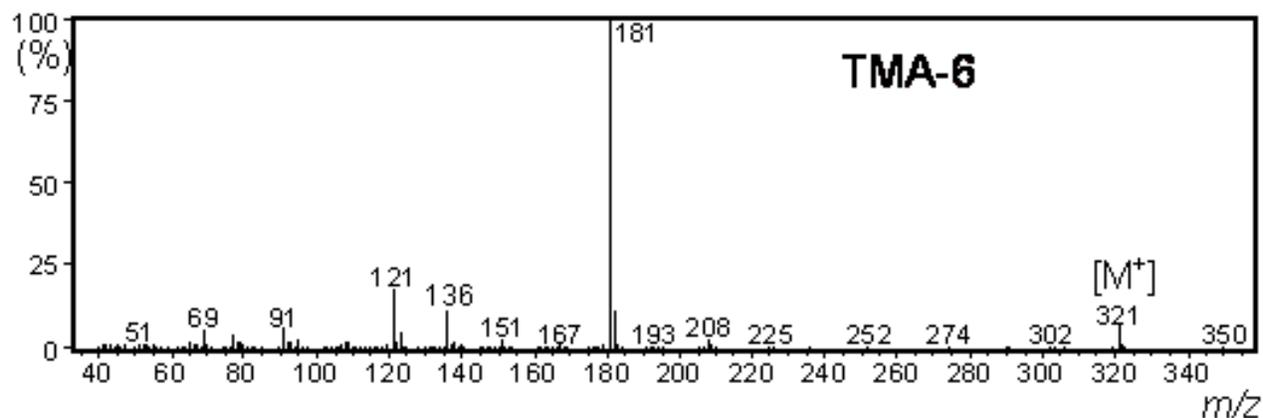
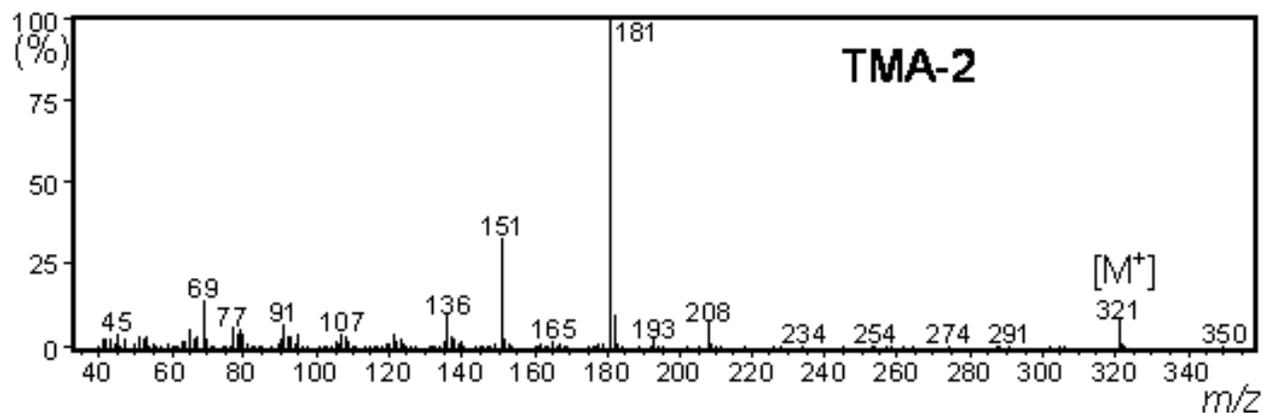
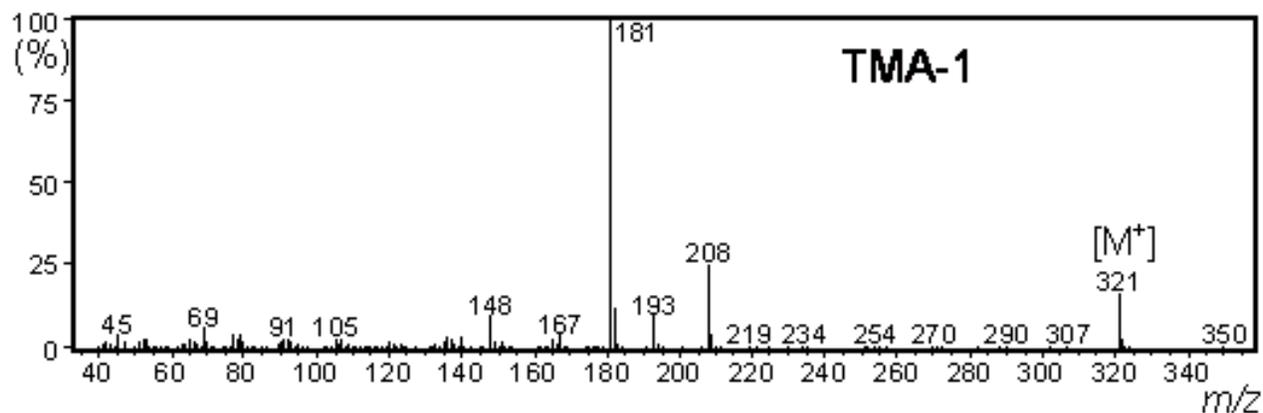


Figure 6b. EI Mass Spectra of the Three TFA-Derivatized TMAs.

Technical Note

A New, Highly Specific Color Test for Ketamine

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ABSTRACT: A new color test for the screening/presumptive identification of ketamine is reported. Treatment of ketamine with alkaline gold bromide produces a deep purple color within approximately one minute that changes to dark, blackish-purple within approximately two minutes. The color, color change, and time frames constitutes a highly specific screening test for ketamine. Of particular note, the test is negative for amphetamine, methamphetamine, MDA, MDMA, and phencyclidine (PCP), all of which are occasionally encountered in combination with ketamine.

KEYWORDS: Ketamine, Gold Bromide, Color Test, Screening Test, Forensic Chemistry

Introduction

Ketamine (Figure 1) is a medical and veterinary anesthetic and a controlled substance (Schedule III in the United States). Due to its anesthetic and hallucinogenic properties, ketamine is increasingly abused (1-3). Because its synthesis is challenging, its presence in illicit drug markets is almost universally due to diversion of pharmaceutical stocks. It is available in powder, liquid, and solid dosage forms, and for abuse purposes is smoked, snorted, injected, or taken orally. More recently, ketamine is being increasingly encountered as an added component in Ecstasy-type tablets. Other controlled substances that are occasionally encountered mixed with ketamine in Ecstasy-type tablets include (but are not limited to): Amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and phencyclidine (PCP).

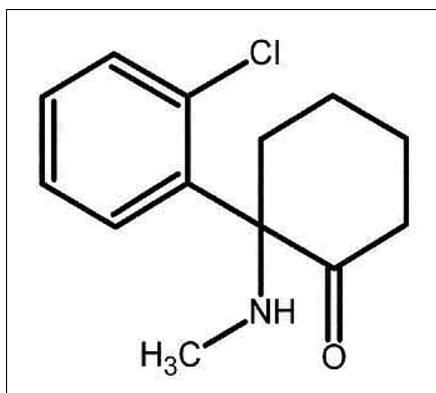


Figure 1. **Ketamine** ((+/-)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone);
 $C_{13}H_{16}ClNO$; m.w. (Base) = 237.7, (HCl) = 274.2.

There are numerous analytical methods for the identification of ketamine in forensic and toxicological laboratories (4-13); however, until recently only one color test (the Janovsky reagent (11,14)) was available for screening purposes. Unfortunately, although moderately specific the Janovsky reagent (alkaline *meta*-dinitrobenzene) is rather insensitive (detection limit about 1.25 milligrams) and is therefore infrequently used for screening of mixed samples or solutions. In early 2007, Morris reported a modified cobalt thiocyanate color test for ketamine that is highly specific (15); however, it is also rather insensitive (detection limit also about 1.25 milligrams). Herein, a new presumptive color test for the preliminary screening of ketamine is reported. The test is simple, easy to perform, nearly twice as sensitive as the Janovsky and Morris tests, and highly specific.

Experimental

Materials: All standards used were from Sigma, Alltech, and Matheson. Gold bromide and sodium hydroxide were both analytical grade.

Reagents: A 0.5 % solution of gold bromide was prepared in deionized water, resulting in a brownish yellow colored solution. A 0.2 M solution of NaOH was also prepared in deionized water.

Method: One drop of 0.5 % gold bromide solution and one drop of the 0.2 M NaOH solution were combined in a spot plate well. A small amount of the substrate was added to the spot well and mixed, and the color monitored over approximately the next 2 minutes.

Results and Discussion

A literature search indicates that gold bromide has not been previously reported for color testing; however, acidified gold bromide has been used in a rather obscure microcrystal test for caffeine (16-18).

As noted in the Experimental section, the alkaline gold bromide test reagent is brownish-yellow in color. Upon treatment with the test reagent, ketamine gives a deep purple color within approximately one minute, that turns to a dark, blackish-purple color within approximately two minutes. A blank does not produce any color changes. Forty-seven other compounds (illicit drugs, adulterants, and diluents) which are frequently encountered in forensic laboratories were also tested (see Table 1, next page). A few of these compounds produced the same purple color as ketamine, but in all such cases there was a major time difference for the development of the color. A few compounds having hydroxyl or phenolic groups (acetaminophen, ascorbic acid, lactose, mannitol, morphine, and sucrose) gives the purple color almost instantaneously. Similarly, heroin (both standard and street-level) also gives the purple color almost instantaneously (the observation that heroin standard gives a positive test confirms that the positive test for street-level heroin was not just due to the presence of morphine or sugars). However, none of the tested compounds gave the color and color change like ketamine over the two minute time frame. In addition, none of the other amine drugs tested gave a positive test, including those most commonly encountered in combination with ketamine in illicit samples. Ketamine is not commonly encountered in combination with heroin, morphine, or the other tested diluents that do give an instantaneous color development - nonetheless, if a nearly instantaneous color change is observed, the test cannot be used for presumptive identification of ketamine.

As noted in the Introduction, the Janovsky and Morris tests have limits of detection of approximately 1.25 milligrams, in both cases requiring additional efforts to maximize sensitivity. The limit of detection for the presented test, with no special efforts to maximize sensitivity, was 0.8 milligrams - nearly twice as sensitive.

The initial purple color may be due to the formation of a complex between the gold and the ketamine. The cause for the change of color from purple to dark blackish-purple is unknown; however, it may be due to a redox reaction that produces a small amount of colloidal gold.

Table 1. Color Testing Results.

Compound	Observation of Color	Compound	Observation of Color
Ketamine	+	Acetaminophen	+
Methamphetamine	-	Alprazolam	-
Amphetamine	-	Ascorbic acid	(+)
Starch	-	Benzocaine	-
Diazepam	-	Bromazepam	-
Flunitrazepam	-	Butalbital	-
Phenobarbital	-	Caffeine	-
Heroin	+	Clonazepam	-
Diphenhydramine	-	α -Lactose	+
Sucrose	+	Lidocaine	-
Codeine Base	-	Lorazepam	-
MDMA	-	Manitol	+
Cocaine base	-	Sodium Chloride	-
Clonazepam	-	Nicotinamide	-
Morphine	+	Nitrazepam	-
MDA	-	Oxazepam	-
PCP	-	Pentazocine	-
Butalbital	-	Quinine	-
Cocaine HCl	-	Stearic acid	-
Fentanyl Citrate	-	Temazepam	-
Phentermine	-	Triazolam	-
Quinine HCl	-	Calcium Carbonate	-
Codeine HCl	-	Butalbarbital	-
Morphine Sulfate	+	Sodium Carbonate	-

Conclusions

The presented color test can be used either as a standalone screen or in combination with either or both the Janovsky reagent or the new, modified cobalt thiocyanate test by Morris. The three tests are highly complementary in that only a few (and different) compounds interfere with each test; therefore, use of any two and certainly all three would constitute a uniquely specific screen and presumptive identification of ketamine, either alone or in combination with other controlled substances and/or adulterants, so long as at least 1.25 milligrams of ketamine is present in each test sample.

Acknowledgements

I am greatly indebted to the Forensic Science Center at Chicago, Illinois (USA) for providing access to the facilities and necessary materials to conduct this research. The assistance of Art Weathers (Forensic Scientist III, Forensic Science Center at Chicago) in conducting this work is highly appreciated. The assistance of Imran Majeed (Assistant Research Officer, Center of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan) in preparing this manuscript is also highly appreciated.

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Eszopiclone (Lunesta™): An Analytical Profile

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ABSTRACT: Eszopiclone (Lunesta™) is a nonbenzodiazepine hypnotic/sedative prescribed for treatment of insomnia. Analytical data (gas chromatography, mass spectrometry, infrared spectroscopy, ultra performance liquid chromatography, and proton and carbon-13 nuclear magnetic resonance spectroscopy) for eszopiclone are presented.

KEYWORDS: Lunesta™, Eszopiclone, Hypnotic, Sedative, Forensic Chemistry

Introduction

The DEA Special Testing and Research Laboratory recently received a sample of eszopiclone (Figure 1) to add to its reference standards collection. Eszopiclone (the active “S” enantiomer of zopiclone) is a nonbenzodiazepine hypnotic/sedative prescribed for treatment of chronic (long-term) insomnia. It is currently marketed in tablet form as Lunesta™, in concentrations of 1, 2, or 3 milligrams per tablet (see Photo 1) (1). Although it has a reduced potential for abuse versus classic benzodiazepine hypnotic/sedatives, it is a Schedule IV controlled substance, and federal law restricts it to prescription use. Based upon its potential for abuse, and the limited literature available concerning its analysis, herein are provided GC, GC/MS, FTIR-ATR, UPLC, and ¹H- and ¹³C-NMR data for eszopiclone.

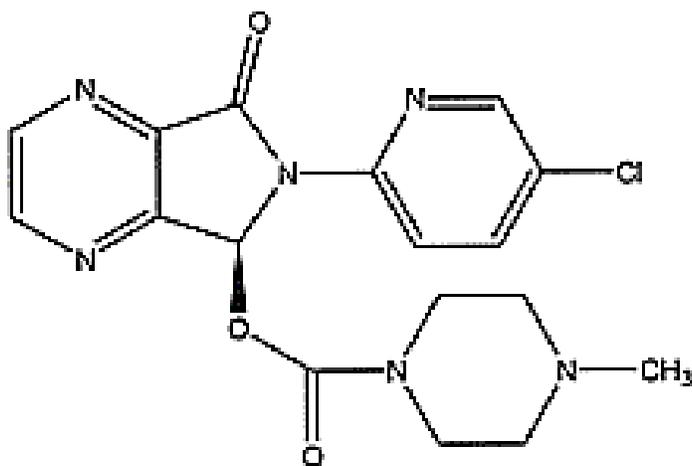


Figure 1. Eszopiclone.



Photo 1. 3 Milligram Tablet
(Note: Diameter is 6.4 Millimeters).

Experimental and Discussion

Eszopiclone

Source: Sepracor Canada, LTD. (Windsor, Nova Scotia, Canada)

Lot Number / Purity: 029-0014 RS / 99.9 %

Chemical Formula / CAS Number: C₁₇H₁₇ClN₆O₃ / [138729-47-2]

Molecular Weight: 388.81 amu

Melting Point: 202 - 203 °C (2)

Solubility: [Chloroform: Soluble; Methanol: Somewhat Soluble; Deionized H₂O: Somewhat Soluble]

Gas Chromatography (GC)

Instrument: Agilent 6890 equipped with a Flame Ionization Detector (FID)

Column: DB-1, 30 m x 0.25 mm I.D., 0.25 µm film thickness

Injector Temperature: 280 °C

Oven Temperature: 100 °C for 1 minute, 12 °C/minute to 280 °C, 7 minute hold

Carrier Gas: Hydrogen at 1.1 mL/minute, split ratio = 25:1

Approximately 8.95 milligrams and 8.63 milligrams of eszopiclone were added to 2 mL of methanol and 2 mL of chloroform, respectively, and vortexed until dissolved; eszopiclone took longer to dissolve in methanol than in chloroform. Utilizing the described experimental parameters, both solutions displayed a major chromatographic peak at 21.14 minutes. In addition, both solutions displayed the same chromatographic pattern with minor early eluting peaks - possibly due to eszopiclone breakdown. The chromatogram of the chloroform solution is illustrated in Figure 2.

Gas Chromatography/Mass Spectrometry (GC/MS)

Instrument: Agilent 6890 Gas Chromatograph equipped with an Agilent 5973 Mass Selective Detector (MSD)

Column: DB-1, 30 m x 0.25 mm I.D., 0.25 µm film thickness

Injector Temperature: 280 °C

Oven Temperature: 100 °C for 2 minutes, 14 °C/minute to 300 °C, 10 minute hold

Carrier Gas: Helium at 1.0 mL/minute, split ratio = 25:1

Scan Range: 34-550 amu

Electron Ionization: 70 eV

In methanol, one major peak at approximately 18 minutes was observed in the Total Ion Chromatogram (TIC) (Figure 3), with minor early eluting peaks as noted above. The fragmentation pattern shows a base peak at *m/z* 143 with other mass fragments at *m/z* 245, 99, 112, 217, 139, and 56 (Figure 4). The molecular ion was not detected.

Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR)

Instrument: Thermo-Nicolet Nexus 670 FTIR Spectrometer equipped with SensIR Dura-Scope Attenuated Total Reflectance (ATR) Accessory (1-Bounce Diamond/KRS-5 Focusing)

The eszopiclone standard was analyzed directly without preparation. Figure 5 (full spectrum) and Figure 6 (fingerprint region) illustrate the baseline-corrected spectra. The following is a list of assignments and corresponding wavenumbers (cm⁻¹): Aromatic C-H stretch (3077), aliphatic C-H stretch (2941, 2837, 2789), ester carbonyl stretch (1730), amide carbonyl stretch (1713), CH₂ bend (1462), CH₃ bend (1417), tertiary aromatic amine (1370), aliphatic C-N (1290, 1238, 1140), C-O stretch (1086), and C-Cl stretch (848) (3).

Ultra Performance Liquid Chromatography (UPLC)

Instrument: Waters ACQUITY Ultra Performance Liquid Chromatograph (UPLC) equipped with Waters 2996 Photo Diode Array (PDA) Detector
Column: 2.1 mm x 100 mm Waters ACQUITY UPLC BEH C18, 1.7 μ m
Mobile Phase: A: 100 mM Phosphate buffer, pH 1.8; B: Acetonitrile
Flow Rate: 0.43 mL/minute
Linear Gradient: 98 % to 35 % A over 10 minutes, 35 % A for 2 minutes

A 100 mM phosphate buffer, pH 1.8, was added to 2.32 milligrams of eszopiclone until a 25.0 mL final volume was obtained. The solution was then sonicated for 15 minutes. Utilizing the above parameters, one peak at a retention time of 3.73 minutes was observed (Figure 7). Figure 8 illustrates the UV spectrum between the wavelengths 220 - 340 nm. The maximum UV absorbance is 301 nm.

Nuclear Magnetic Resonance (NMR) Spectroscopy

^1H - and ^{13}C -NMR spectra (see Figures 9 and 10, respectively) were acquired on a Varian Mercury *Plus* 400 MHz instrument using a Nalorac 5 mm indirect detect pulse field gradient (PFG) probe at 25 $^{\circ}\text{C}$. (^1H parameters: Number of scans (nt) = 8, pulse width (pw) = 45 $^{\circ}$, relaxation delay (d1) = 5 s, acquisition time (at) = 2.5 s; ^{13}C parameters: nt = 4098, pw = 45 $^{\circ}$, d1 = 1 s, at = 1.3 s, complete proton decoupling). Spectra were processed using ACD's *SpecManager* software (Applied Chemistry Development Inc.©, Toronto, Canada). Eszopiclone was prepared in CDCl_3 containing 0.05 % v/v tetramethylsilane (TMS; Aldrich Chemical Co., Milwaukee, WI) at 16.84 mg/mL. Chemical shifts (δ) are reported in parts per million (ppm) using TMS (0.0 ppm) as the reference standard. ^1H data are reported as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant, number of protons present. ^1H -NMR (400 MHz, CDCl_3) δ 8.90 (d, J = 2.5 Hz, 1H), 8.85 (d, J = 2.5 Hz, 1H), 8.52 (d, J = 8.9 Hz, 1H), 8.40 (d, J = 2.4 Hz, 1H), 8.02 (s, 1H), 7.80 (dod, J = 8.9, 2.5 Hz, 1H), 3.65 (br m, 1H), 3.54 (br m, 1H), 3.25 (br s, 2H), 2.42 (br m, 2H), 2.26 (s, 3H), 2.22 (br m, 1H), 2.05 (br m, 1H). ^{13}C -NMR (100 MHz, CDCl_3) δ 165.4, 162.9, 155.6, 153.4, 148.4, 147.8, 146.8, 143.9, 138.1, 133.4, 128.3, 116.1, 79.1, 54.5, 52.3, 46.1, 44.1.

Acknowledgements

The authors wish to thank Senior Forensic Chemist Patrick A. Hays (this laboratory), for his time and NMR expertise, and Senior Forensic Chemist Dr. Edward S. Franzosa (this laboratory), for his time and help with imaging the tablets.

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[Figures 2 - 10 Follow.]

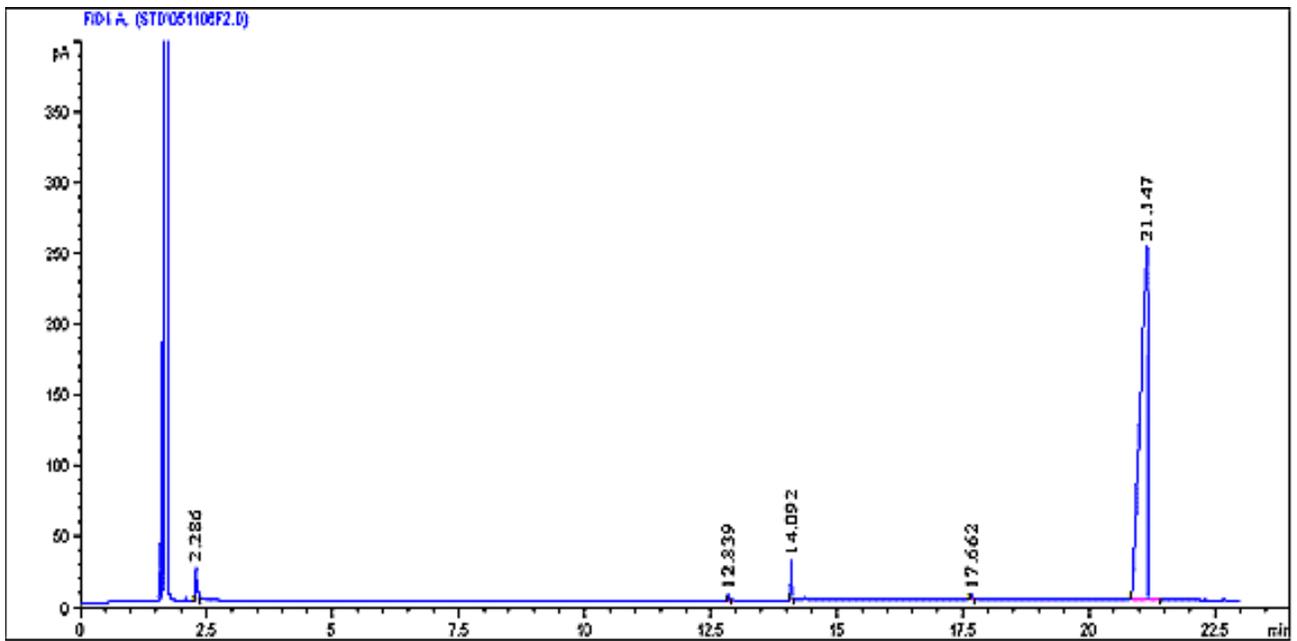


Figure 2. Gas Chromatogram of Eszopiclone in Chloroform.

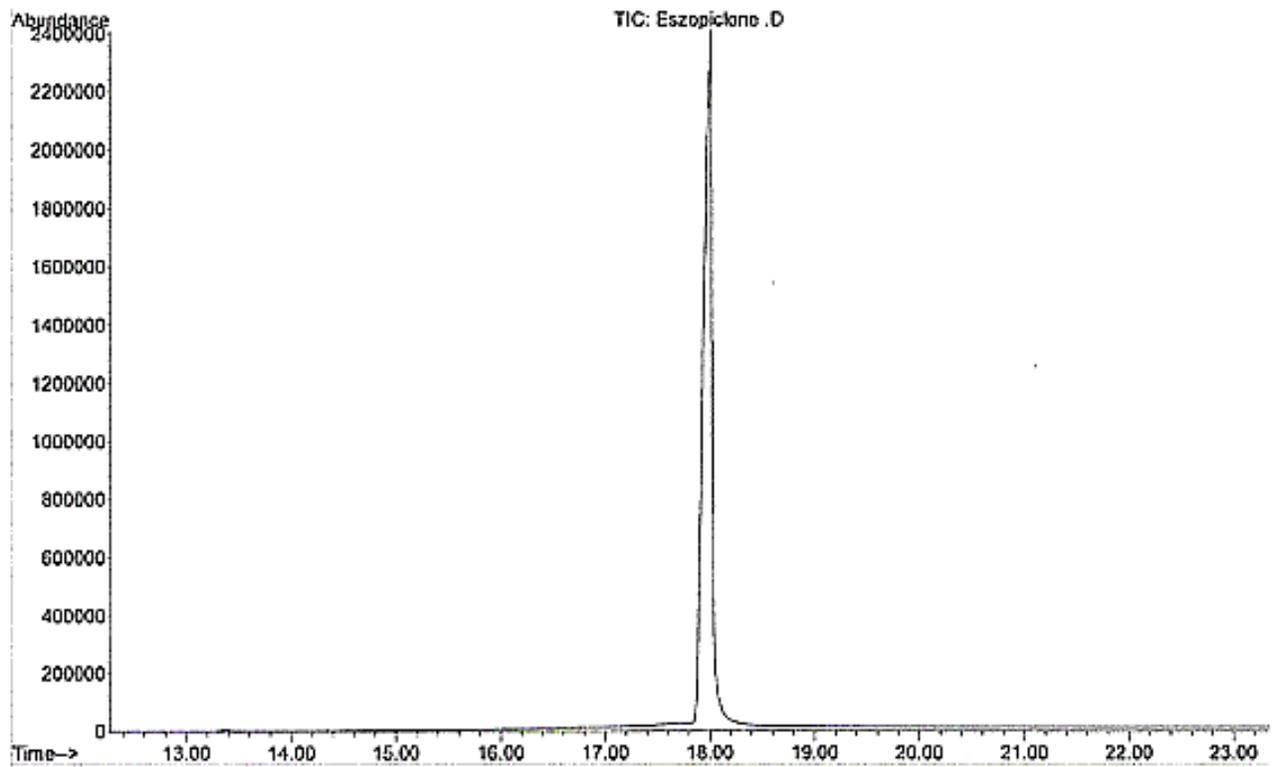


Figure 3. GC/MSD Total Ion Chromatogram of Eszopiclone.

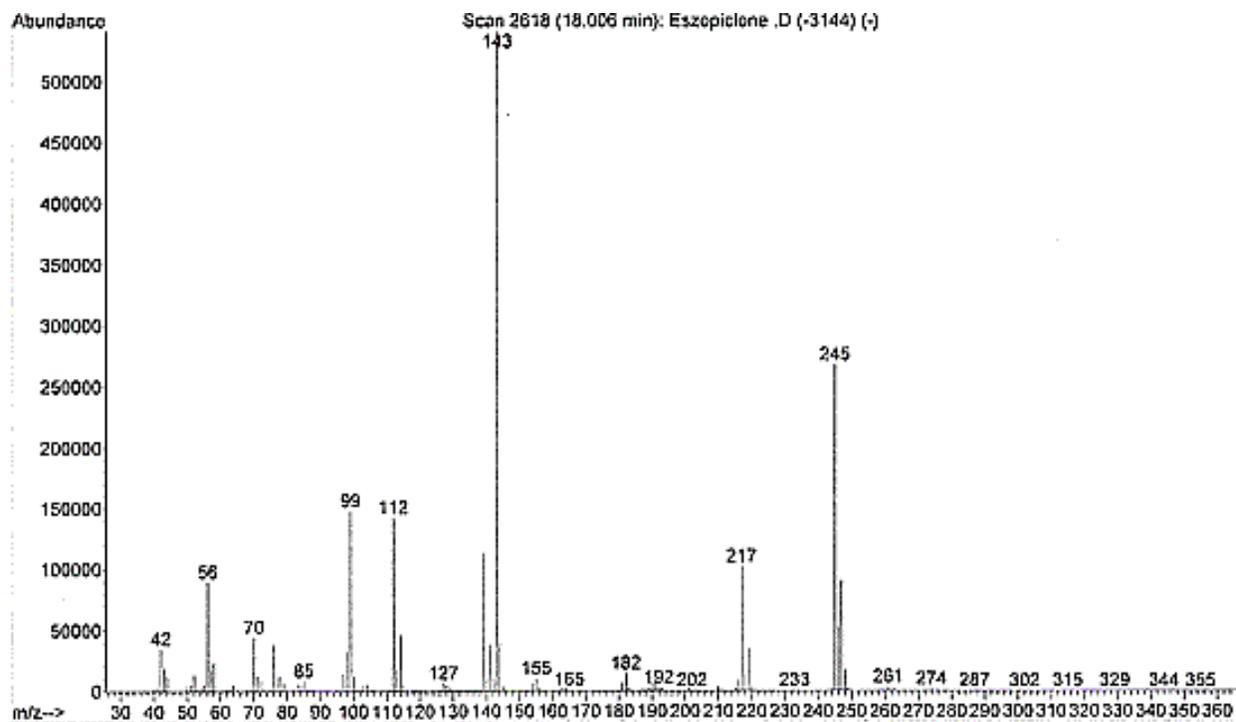


Figure 4. Electron Ionization Mass Spectrum of Eszopiclone.

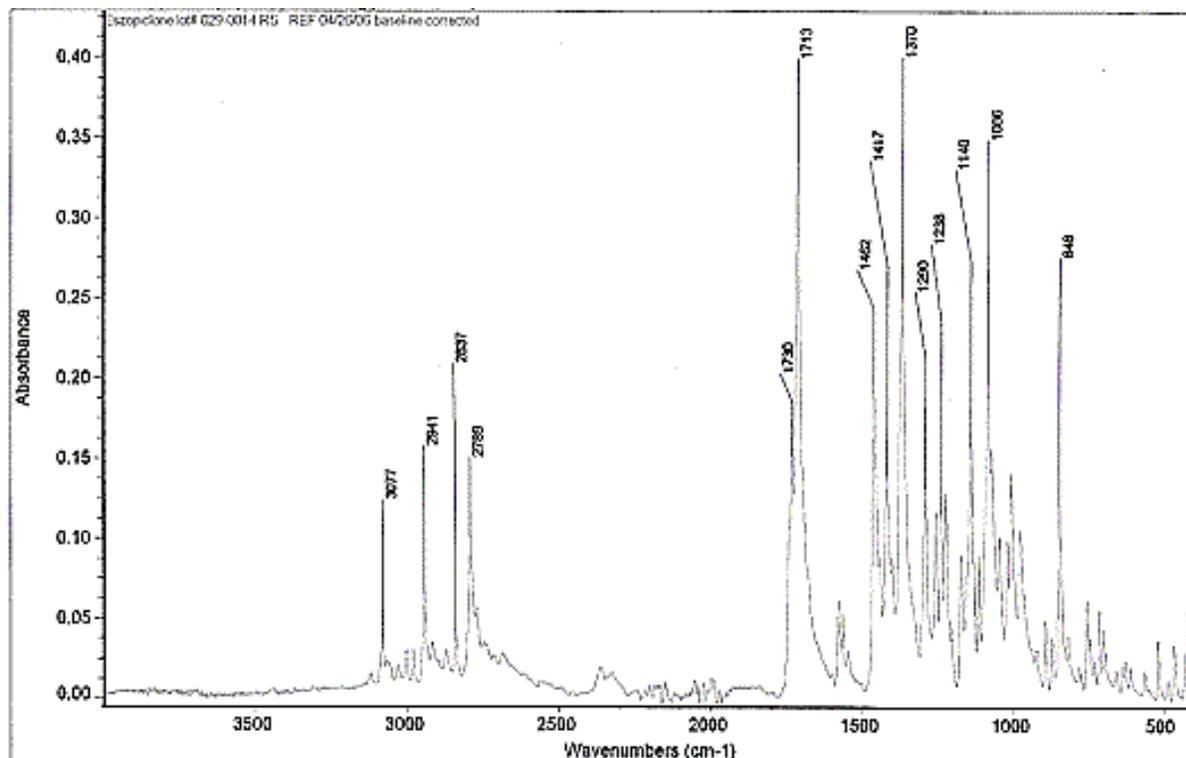


Figure 5. FTIR-ATR Spectrum of Eszopiclone.

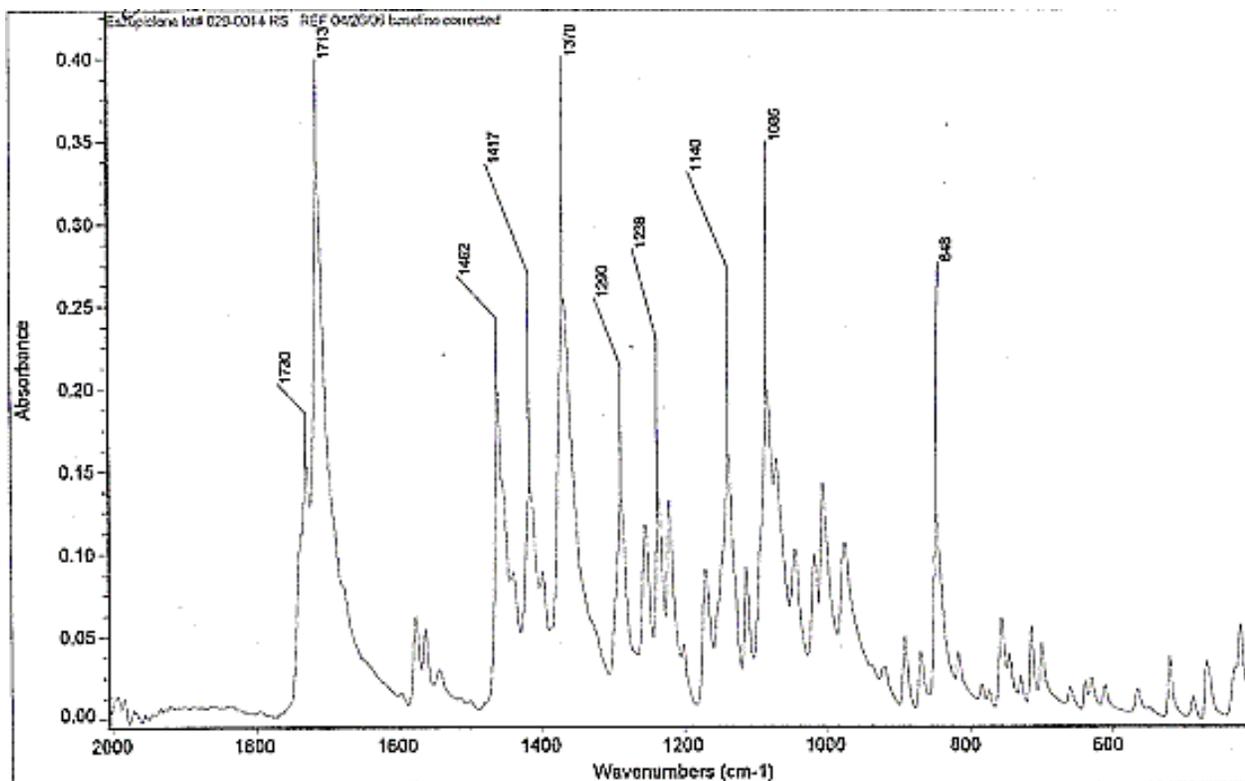


Figure 6. FTIR-ATR Spectrum of Eszopiclone, Fingerprint Region.

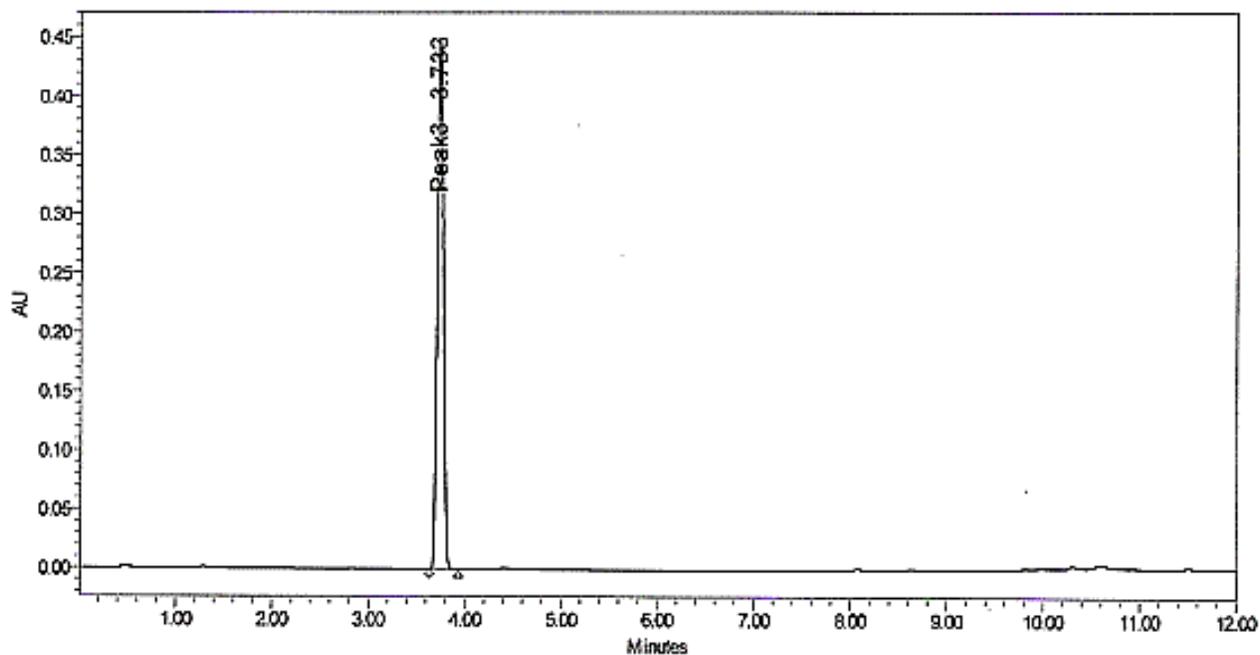


Figure 7. Ultra Performance Liquid Chromatogram of Eszopiclone.

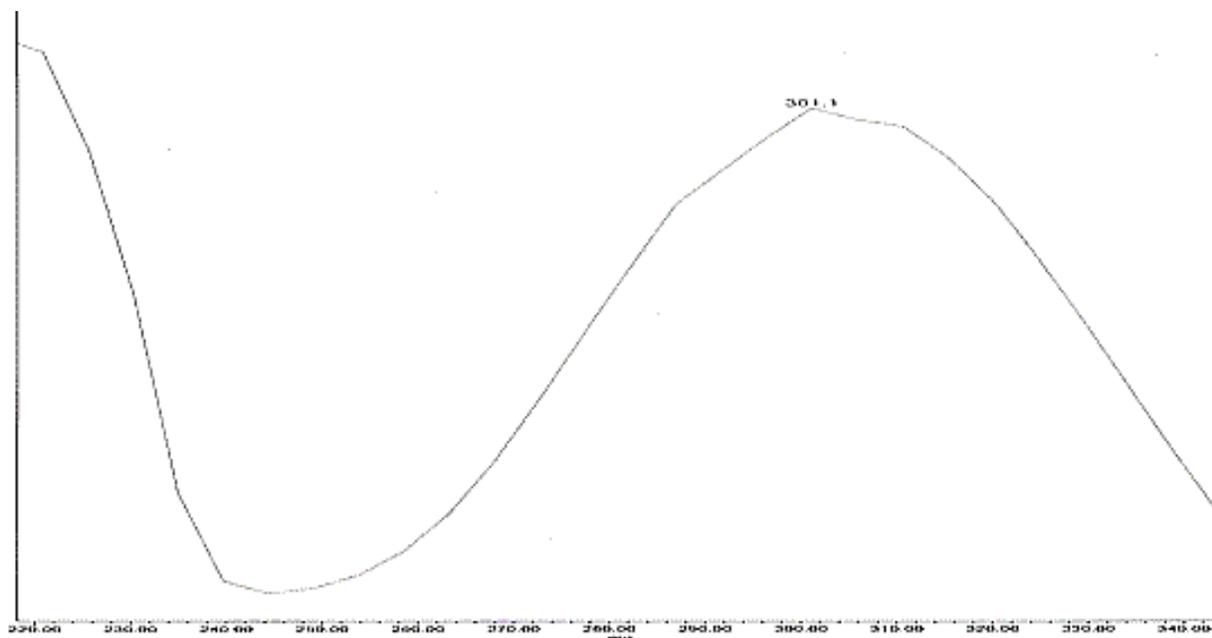


Figure 8. 220 - 340 nm UV Spectrum of Eszopiclone from UPLC. UV max = 301 nm.

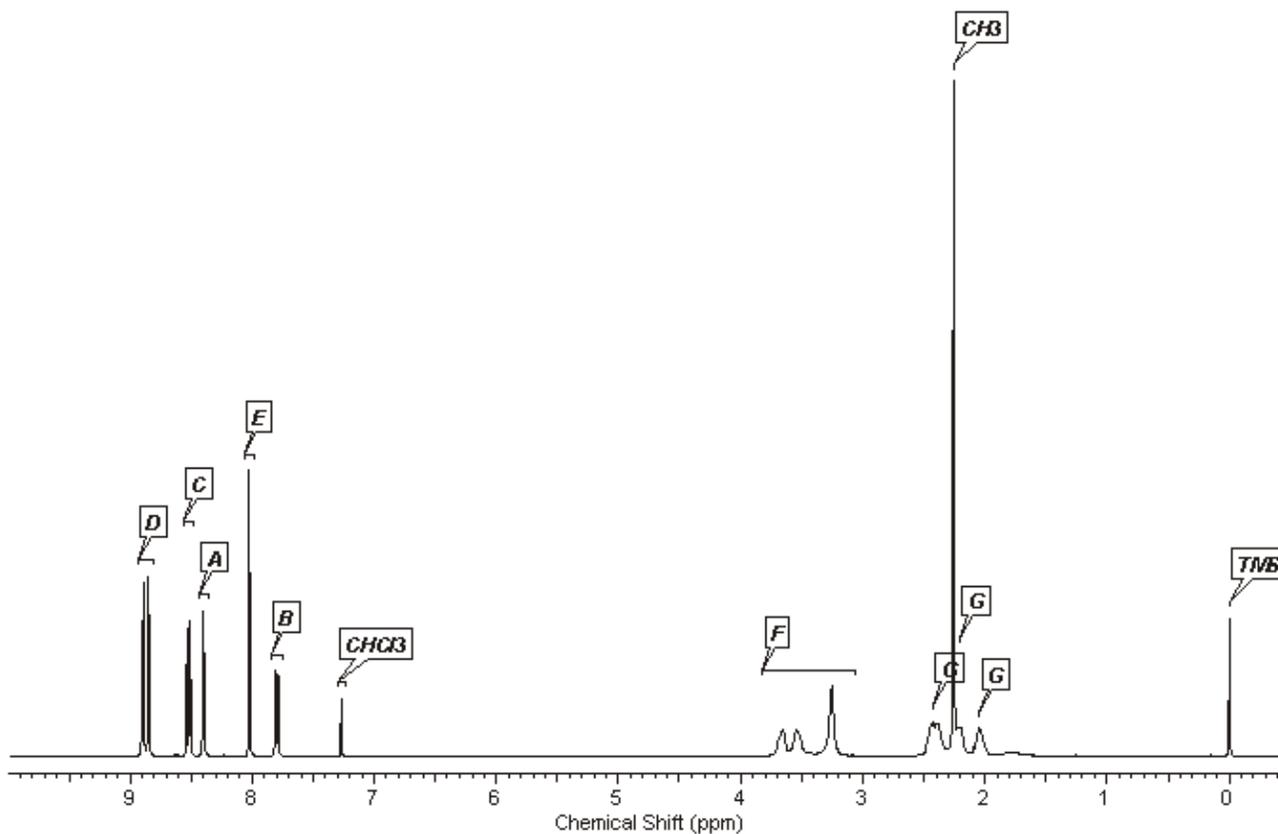


Figure 9a. 400 MHz ^1H -NMR Spectrum of Eszopiclone in CDCl_3 (See Next Page for Assignments).

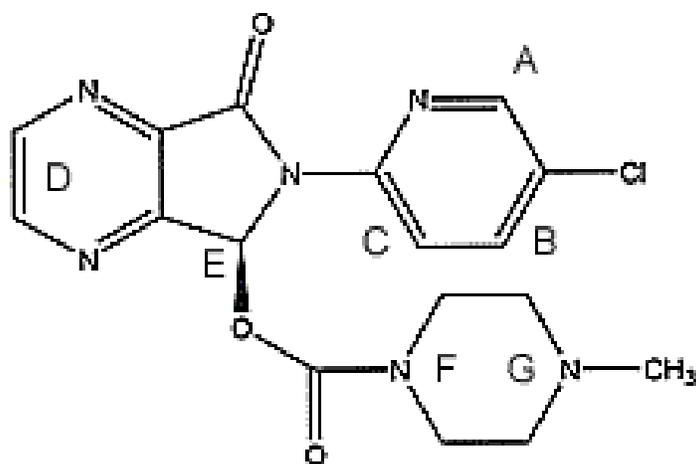


Figure 9b. Assignments of Protons for Eszopiclone (See Figure 9a for the Spectrum).

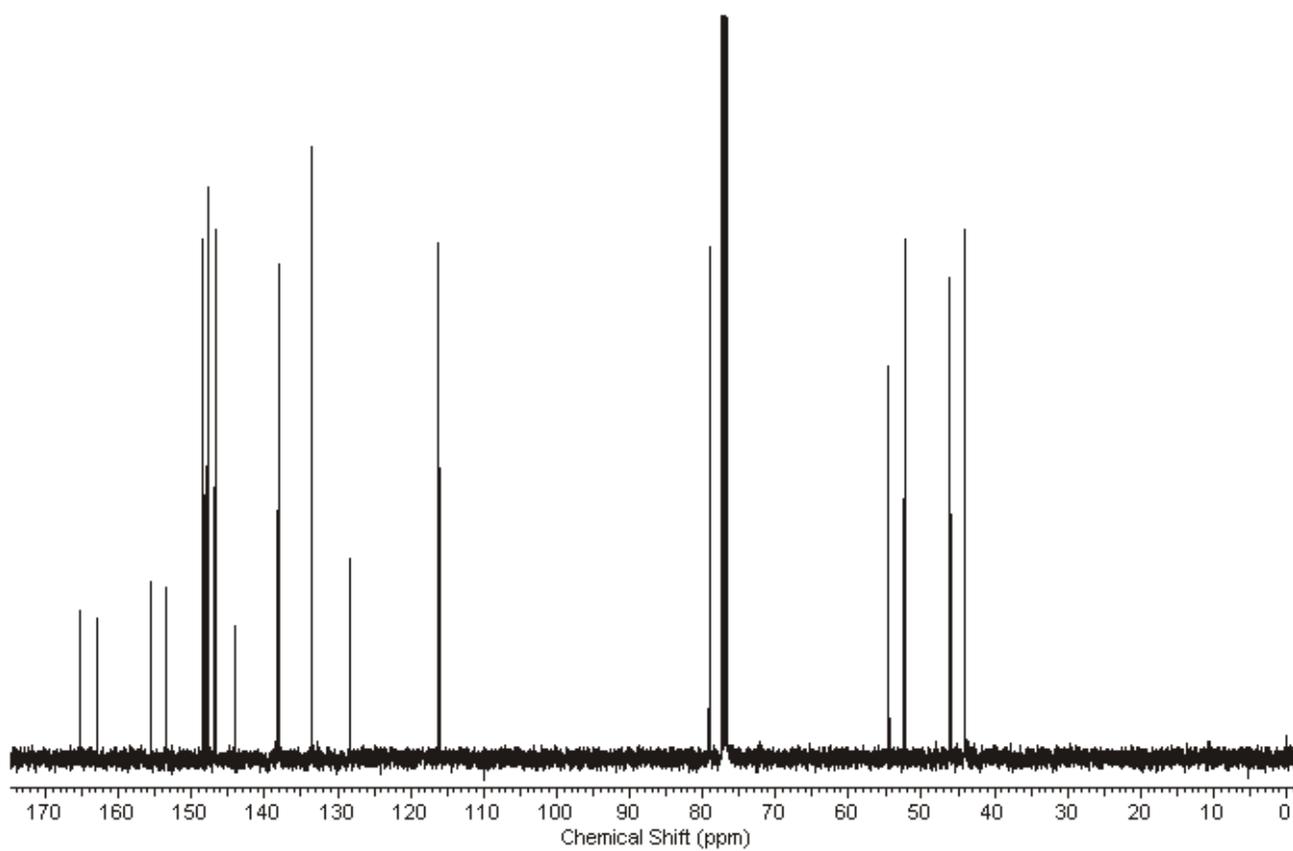


Figure 10. 100 MHz ^{13}C -NMR Spectrum of Eszopiclone in CDCl_3 .

* * * * *

Technical Note

Isolation of *cis*-Cinnamoylcocaine from Crude Illicit Cocaine via Alumina Column Chromatography

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ABSTRACT: The isolation procedure of gram quantities of *cis*-cinnamoylcocaine from crude cocaine base is provided. Isolation was achieved through classical alumina column chromatography and recrystallization. The procedure will enable forensic scientists to obtain a standard of *cis*-cinnamoylcocaine for cocaine signature analyses and related research.

KEYWORDS: *cis*-Cinnamoylcocaine, Column Chromatography, Isolation, Cocaine Signature Analyses, Forensic Chemistry

Introduction

Cocaine signature analyses have become routine in many forensic laboratories. These analyses are intended for both sample-to-sample comparison work (tactical intelligence) and geographic origin classification (strategic intelligence). Several chromatographic methods have been published over the past 15 years which utilize *cis*-cinnamoylcocaine (Figure 1), a naturally occurring product in coca, as one of the target compounds (1-6). Since *cis*-cinnamoylcocaine is not commercially available, standard material must be either synthesized or isolated from illicit cocaine. However, the synthesis procedure with followup purification by preparative high performance liquid chromatographic (HPLC), as reported by By, Lodge, and Sy (7), is problematic for forensic laboratories not staffed for synthetic work or equipped with a preparative HPLC. Similarly, the isolation from

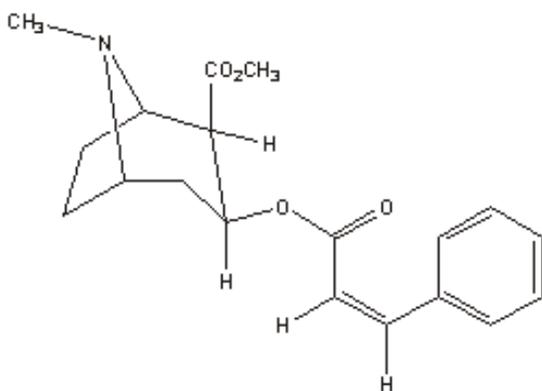


Figure 1. *cis*-Cinnamoylcocaine.

illicit cocaine utilizing ion-pair chromatography, as reported by Moore (8), yields only milligram quantities and cannot be scaled up. Herein, we provide a simple chromatographic procedure for the isolation of gram quantities of *cis*-cinnamoylcocaine from crude cocaine base.

Experimental

Materials: A crude cocaine base exhibit containing approximately 13 percent *cis*-cinnamoylcocaine was acquired from the research collection of this laboratory. All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were of reagent-grade quality and were products of Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4 percent (w/w).

Gas Chromatography/Mass Spectrometry (GC/MS): Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100 °C; no hold, program rate, 6 °C/min; final temperature, 300 °C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280 °C. The auxiliary transfer line to the MSD was operated at 280 °C.

Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR): Spectra were obtained on a Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Spectra were collected using 32 scans between 4000 cm^{-1} and 400 cm^{-1} at a resolution of 4 cm^{-1} .

Proton Nuclear Magnetic Resonance Spectroscopy ($^1\text{H-NMR}$): Spectra were obtained on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac indirect detection, variable temperature, pulse field gradient probe with PulseTune® (Varian, Palo Alto, CA). The compound was dissolved in deuteriochloroform (CDCl_3) containing 0.03 percent v/v tetramethylsilane (TMS) as the 0 ppm reference. The temperature of the sample was maintained at 25 °C. Standard Varian pulse sequences were used to acquire the proton spectra. Processing of data was performed using software from Applied Chemistry Development (ACD/Labs, Toronto, Canada).

Isolation of *cis*-Cinnamoylcocaine: Crude cocaine base (170 grams containing approximately 13 percent *cis*-cinnamoylcocaine) was dissolved into one liter of warm diethyl ether/hexane (1:1) and eluted on a glass chromatographic column (100 cm x 5.5 cm ID) containing 1.0 kilogram of basic alumina (150 mesh). The column was then eluted with 1.0 liter of diethyl ether, followed by 1.0 liter of diethyl ether/chloroform (1:1). The bulk of the *cis*-cinnamoylcocaine was contained in the diethyl ether fractions. The combined diethyl ether fractions were evaporated *in vacuo* to an oil (34 grams of 55 percent *cis*-cinnamoylcocaine), which was chromatographed again on 1.0 kilogram of basic alumina (same size column) using the following series of solvents: 500 mL diethyl ether/hexane (1:2), 500 mL diethyl ether/hexane (1:1), 500 mL diethyl ether/hexane (2:1), 500 mL diethyl ether/hexane (5:1), and 1500 mL diethyl ether. The diethyl ether/hexane (5:1) and 1500 mL diethyl ether fractions were then combined and evaporated *in vacuo* to a light yellow oil (9.9 grams of 88 percent *cis*-cinnamoylcocaine). The resulting oil was chromatographed again on 1.0 kilogram of basic alumina (same size column) using 500 mL diethyl ether/hexane (1:2), 500 mL diethyl ether/hexane (1:1), 500 mL diethyl ether/hexane (2:1), 500 mL diethyl ether/hexane (5:1), and 2000 mL diethyl ether. The first 750 mL of the diethyl ether fractions were combined and evaporated *in vacuo* to a clear oil (7.0 grams of 96 percent *cis*-cinnamoylcocaine) which crystallized slowly upon standing. The product was recrystallized from diethyl ether/petroleum ether (20 - 40 °C) to give 6.17 grams of 99 percent pure *cis*-cinnamoylcocaine as a white solid (28 percent recovery).

Results and Discussion

Crude cocaine base contains (mostly) cocaine, lesser amounts of both *cis*- and *trans*- cinnamoylcocaine, numerous other tropane alkaloids, and various processing impurities and byproducts. Under the described chromatographic procedures, cocaine and *trans*-cinnamoylcocaine predominate in the hexane/diethyl ether fractions and elute prior to *cis*-cinnamoylcocaine. Although cocaine and *trans*-cinnamoylcocaine have some carryover, *cis*-cinnamoylcocaine is enriched significantly in the diethyl ether fractions. More polar cocaine impurities such as norcocaine, ecgonine, and benzoylecgonine are retained by the alumina column. Two additional alumina column passes of the enriched *cis*-cinnamoylcocaine, followed by recrystallization, were sufficient to give an analytically pure (99 percent or better) sample. The FTIR-ATR, ¹H-NMR, and GC/MS spectra are illustrated in Figures 2 - 4, respectively. The reported procedure can be utilized to isolate *cis*-cinnamoylcocaine even from refined illicit cocaine exhibits containing as little as 3 percent *cis*-cinnamoylcocaine. Samples of *cis*-cinnamoylcocaine should be stored in amber glass bottles or in a dark location, as observations suggest that isomerization to *trans*-cinnamoylcocaine occurs over extended periods of time.

Acknowledgments

The authors are indebted to Senior Forensic Chemist Patrick A. Hays (this laboratory) for his assistance in acquiring the NMR data.

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[Figures 2 - 4 Follow.]

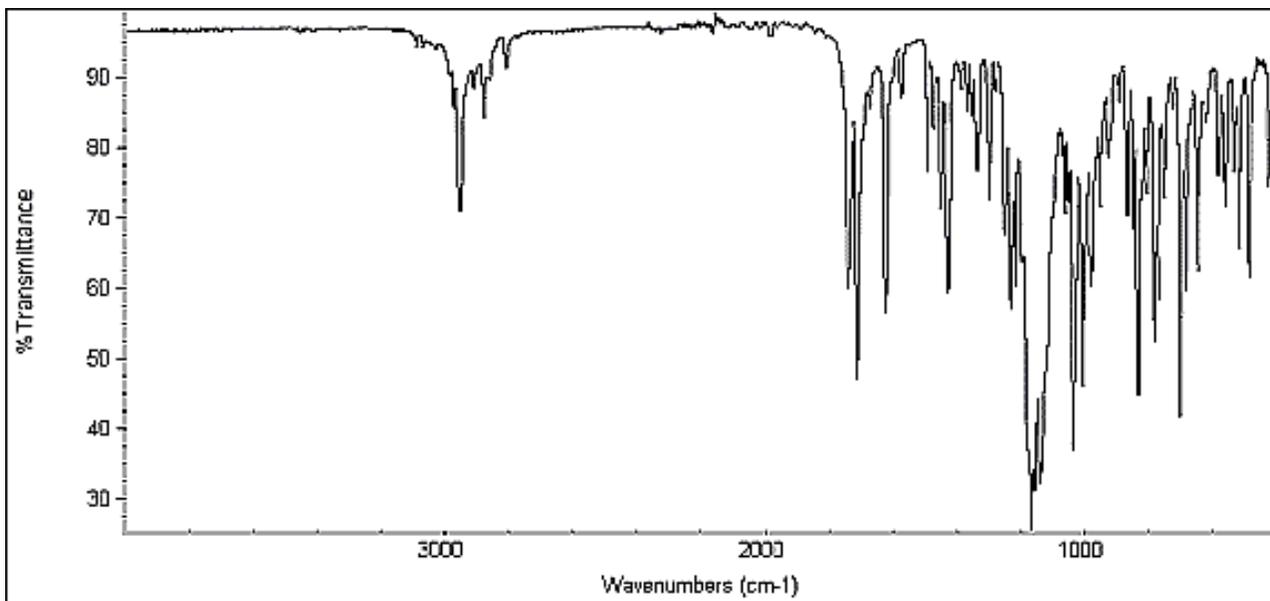


Figure 2. Infrared Spectrum (FTIR-ATR) of *cis*-Cinnamoylcocaine.

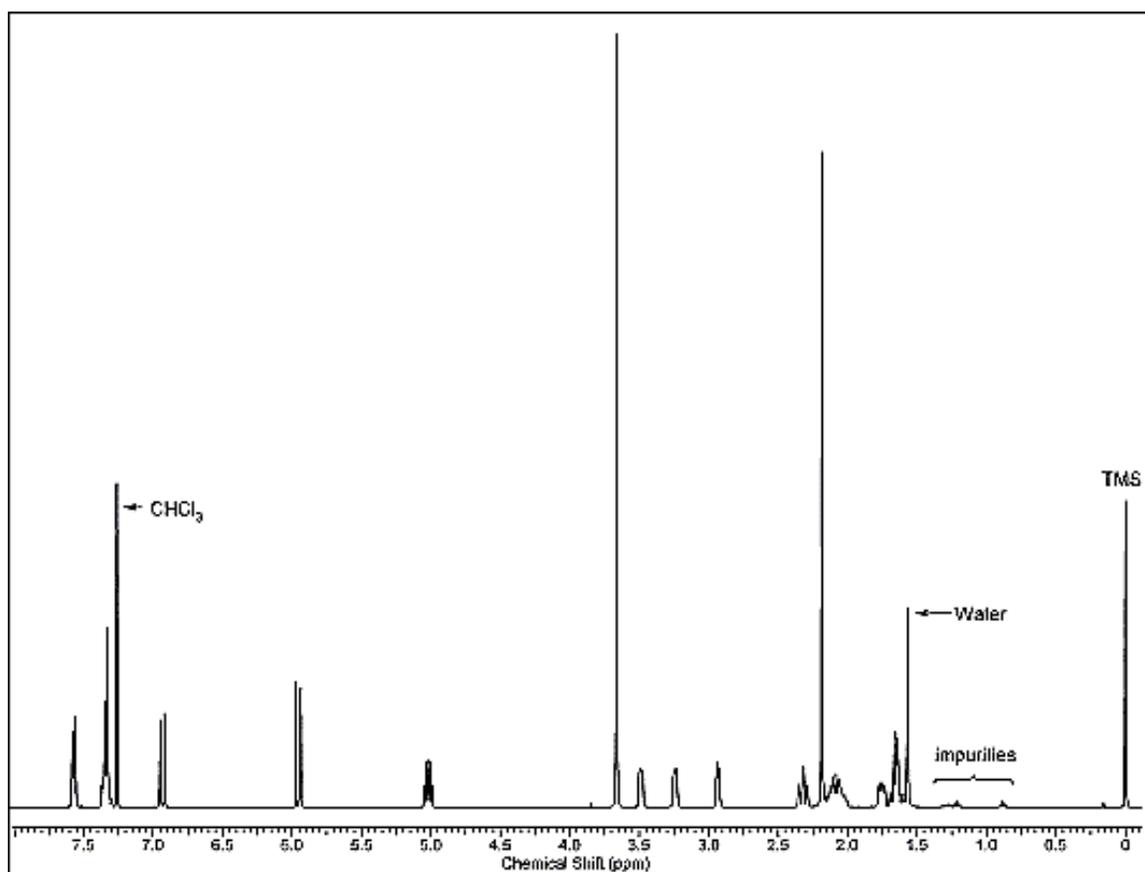


Figure 3. Proton NMR Spectrum of *cis*-Cinnamoylcocaine.

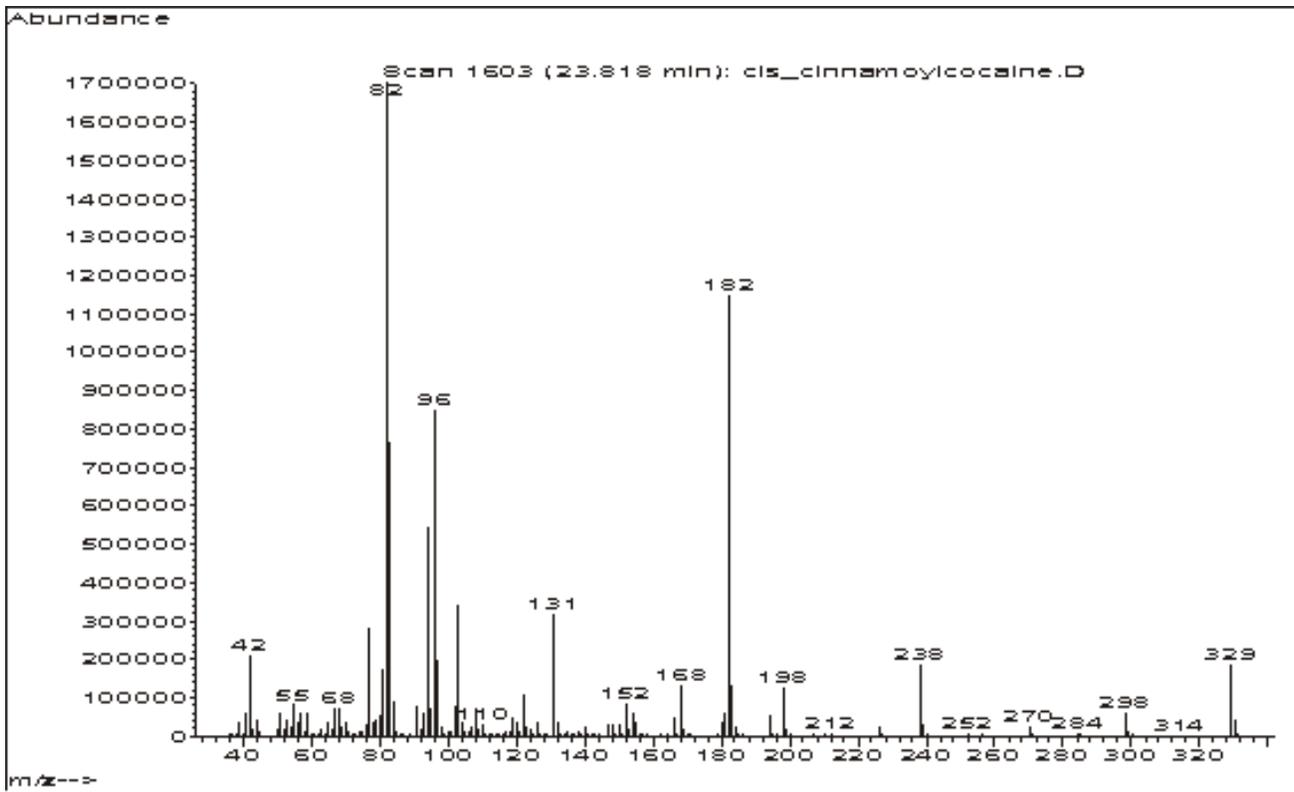


Figure 4. Gas Chromatography/Mass Spectra (70 eV EI) of *cis*-Cinnamoylcocaine.

Technical Note

The Characterization of 4-Methoxy-N-ethylamphetamine Hydrochloride

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ABSTRACT: The synthesis, analysis, and characterization of 4-methoxy-N-ethylamphetamine hydrochloride is presented. Analytical data (gas chromatography/mass spectrometry, Fourier transform infrared spectroscopy, and proton nuclear magnetic resonance spectroscopy) are presented.

KEYWORDS: 4-Methoxy-N-ethylamphetamine, Phenethylamine, Synthesis, Analysis, Forensic Chemistry

Introduction

In late 2004, this laboratory received a white crystalline substance submitted as an unknown suspected phenethylamine (seizure exhibit) for identification and characterization. It was thought that this compound might be one of the many esoteric phenethylamine “designer drugs” described in PIHKAL (1). Preliminary screening indicated that the sample contained a single component and was relatively pure. Utilizing proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectroscopy and a computerized structural elucidation program, the compound was tentatively identified as 4-methoxy-N-ethylamphetamine hydrochloride (Figure 1). Surprisingly, this compound is not detailed in PIHKAL, and furthermore has few literature citations, including on websites dedicated to drug abuse. It therefore constitutes a new phenethylamine-type “designer drug.” For this reason, and also to confirm the tentative identification via direct spectral comparisons, it was synthesized and fully characterized via gas chromatography/mass spectrometry (GC/MS), Fourier transform infrared spectroscopy (FTIR), and $^1\text{H-NMR}$ spectroscopy.

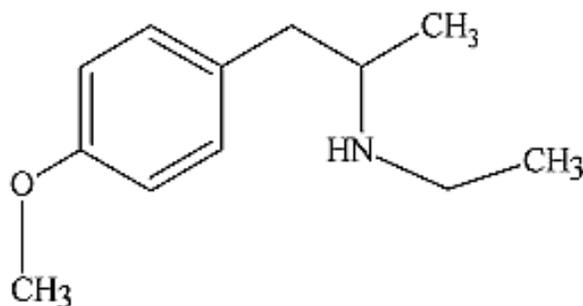


Figure 1. 4-Methoxy-N-ethylamphetamine.

Experimental

Chemicals, Reagents, and Materials: All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). All other chemicals were of reagent-grade quality and products of Aldrich Chemical (Milwaukee, WI). 4-Methoxyamphetamine HCl (the starting material for the synthesis) was acquired from the reference collection of this laboratory.

Gas Chromatography/Mass Spectrometry (GC/MS): Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent (Palo Alto, CA) Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 μm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100 $^{\circ}\text{C}$; no hold, program rate, 6 $^{\circ}\text{C}/\text{min}$; final temperature, 300 $^{\circ}\text{C}$; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280 $^{\circ}\text{C}$. The auxiliary transfer line to the MSD was operated at 280 $^{\circ}\text{C}$.

Infrared Spectroscopy (FTIR-ATR): Spectra were obtained on a Nexus 670 FTIR equipped with a single bounce attenuated total reflectance (ATR) accessory. Spectra were collected using 32 scans between 4000 cm^{-1} and 400 cm^{-1} at a resolution of 4 cm^{-1} .

Proton Nuclear Magnetic Resonance Spectroscopy ($^1\text{H-NMR}$): Spectra were obtained on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac indirect detection, variable temperature, pulse field gradient probe with PulseTune[®] (Varian, Palo Alto, CA). The compound was dissolved in deuterium oxide (D_2O) containing 0.05 percent (by weight) 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid, sodium salt (TSP) as a 0 ppm reference and 5 mg/mL maleic acid as the quantitative internal standard. The temperature of the sample was maintained at 25 $^{\circ}\text{C}$. Standard Varian pulse sequences were used to acquire proton, proton-decoupled carbon, and gradient versions of COSY, HSQC, and HMBC. Processing of data was performed using software from Varian and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Structural elucidation was performed manually and by using ACD/Labs Structure Elucidator[®] software.

Syntheses:

4-Methoxy-N-acetylamphetamine: 4-Methoxyamphetamine HCl (5.00 grams, 24.8 mmol) was dissolved into 25 mL of water in a 500-mL Erlenmeyer flask, followed by addition of 250 mL of saturated aqueous sodium bicarbonate, with stirring for several minutes. Acetic anhydride (21.6 grams, 211 mmol) was then added slowly and stirred for 2 hours at room temperature. The reaction was extracted with methylene chloride (3 x 100 mL). The extracts were combined, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give 4-methoxy-N-acetylamphetamine as a light yellow oil (5.0 grams, 99 percent purity, 97.5 percent yield).

4-Methoxy-N-ethylamphetamine HCl: A flame-dried 1-liter round bottom flask fitted with an addition funnel and water-cooled condenser was charged with 100 mL diethyl ether containing 1.0 M LiAlH_4 (100 mmol). Approximately 75 mL of anhydrous diethyl ether containing 4-methoxy-N-acetylamphetamine (5.0 grams, 24.2 mmol) was added dropwise over 30 minutes, followed by an additional 125 mL of anhydrous diethyl ether, and the mixture was refluxed overnight. The reaction was quenched slowly, in sequence, with 4.0 mL of water, 4.0 mL of 15 percent aqueous NaOH, and 12 mL of water, and was then stirred for approximately 30 minutes. The lithium and aluminum salts were removed via suction filtration through a Celite pad, which was washed with an additional 100 mL diethyl ether. The filtrate was dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give a clear oil. The oil was reconstituted in 35 mL isopropanol, treated with isopropanolic HCl until pH 5, and then diluted to approximately 800 mL with diethyl ether. The resulting precipitate was collected via suction filtration, washed with additional diethyl ether to remove traces of excess HCl, and desiccated under vacuum to remove residual solvent to give 4-methoxy-N-ethylamphetamine HCl as a white crystalline powder (3.22 grams, 58 percent yield).

Results and Discussion

Independent synthesis, spectral characterization, and comparison of authentic 4-methoxy-N-ethylamphetamine HCl to the submitted unknown confirmed its identity. The infrared spectrum (Figure 2) displays an absorbance pattern that is consistent with a secondary amine halogen (HCl) ion-pair and a *para* disubstituted aromatic ring. The mass spectrum (Figure 3) gives fragments at m/z 72 (base peak), 121, and 192, all consistent with a methoxy-substituted-N-ethylamphetamine. The $^1\text{H-NMR}$ spectrum (Figure 4) exhibited two doublets at 7.0 and 7.3 ppm, integrating to 2 protons each, typical of a *para* substituted benzene. The singlet at 3.8 ppm integrates to 3 protons and corresponds to the methoxy group (supported by $^{13}\text{C-NMR}$ (not shown)). The multiplet at 3.5 ppm integrates to 1 proton and corresponds to the methine (which is bonded to the methyl group (a doublet at 1.2 ppm integrating to 3 protons), the methylene group (2 doublet of doublets at 2.8 and 3.1 ppm integrating to 1 proton each), and the nitrogen). The methylene proton chemical shifts (2.8 and 3.1 ppm) confirm that they are bonded to the benzene ring. The remaining proton peaks (the multiplet at 3.2 ppm integrating to 2 protons and the triplet at 1.3 ppm integrating to 3 protons) are of the N-ethyl group. The spectrum peaks below 3.6 ppm are, as expected, nearly identical to those of MDEA (Figure 5).

The starting material used in this synthesis (4-methoxyamphetamine, also known as “PMA”) is itself a controlled substance that is abused worldwide (especially in North America and Europe); therefore, it is quite unlikely that the synthetic procedure described herein was utilized by the original clandestine chemist - nor is it likely to be utilized in the future by any clandestine chemists. The choice of this synthetic route was based on convenience, since 4-methoxyamphetamine was available from this laboratory’s reference collection. Although not investigated, the clandestine chemist in this case probably started his synthesis from 4-methoxyphenyl-2-propanone. It is doubtful whether he intended to make 4-methoxy-N-ethylamphetamine, as comments made in PIHKAL concerning the homologous compound 4-methoxy-N-methylamphetamine (also known as 4-methoxymethamphetamine, PMMA) would suggest that 4-methoxy-N-ethylamphetamine is only a moderate stimulant with minimal (if any) hallucinogenic properties. In addition, both PMA and PMMA are toxic compounds that have been implicated in numerous deaths over the past four decades, and it is likely that 4-methoxy-N-ethylamphetamine would display similar toxicity.

Conclusions

The gas chromatography and infrared and mass spectra of 4-methoxy-N-ethylamphetamine are expected to be similar to its 2- and 3-methoxy substituted analogs. $^1\text{H-NMR}$ provides unequivocal identification. Although quite unlikely, if this compound becomes more common in illicit markets, the acronym “PMEA” is an obvious choice.

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[Figures 2 - 5 Follow.]

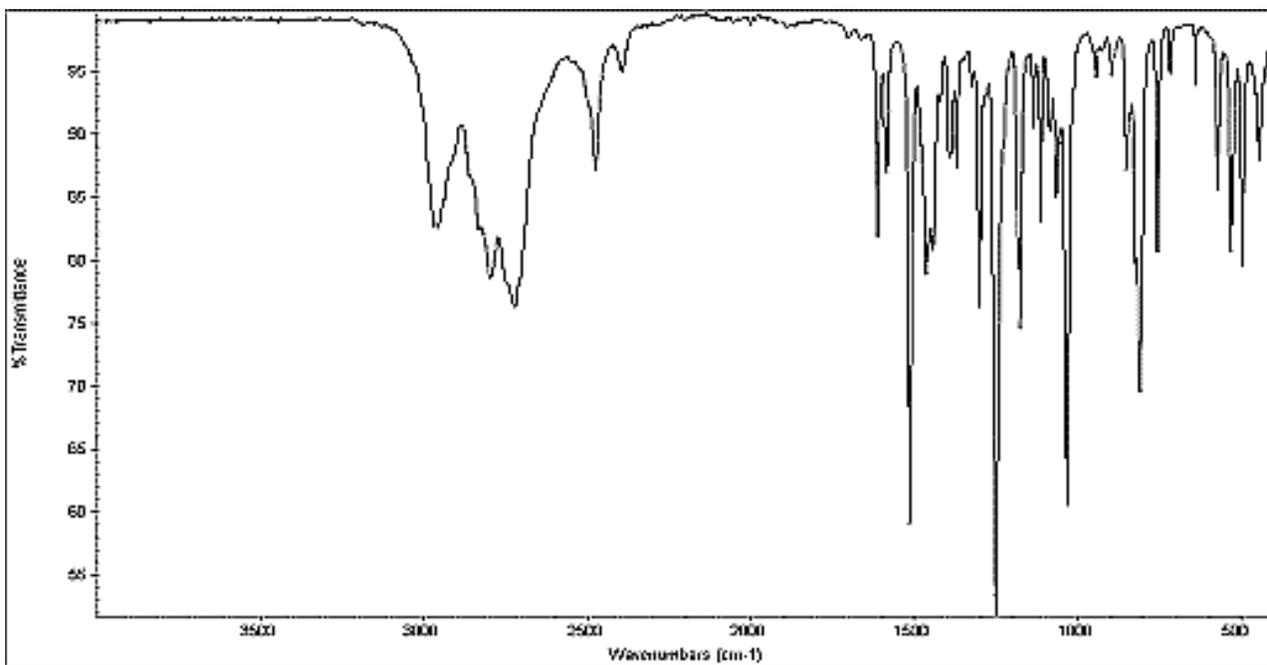


Figure 2. Infrared Spectrum (FTIR-ATR) of 4-Methoxy-N-ethylamphetamine HCl.

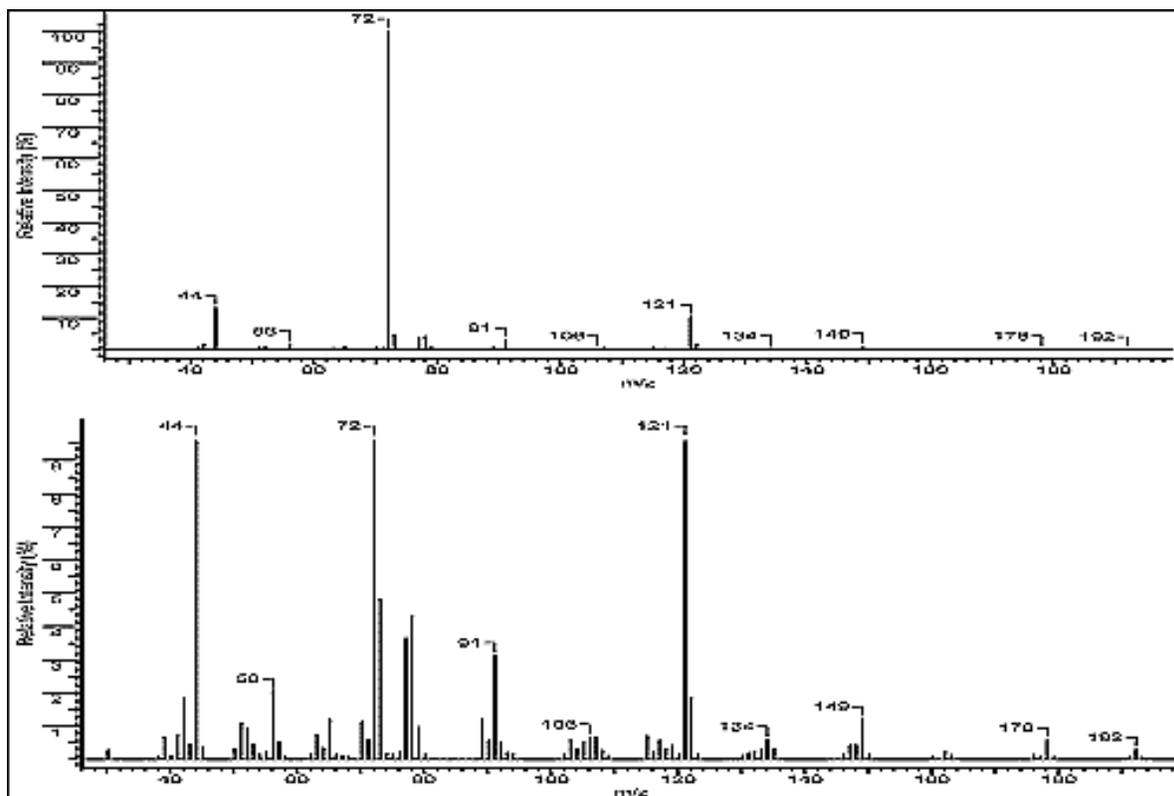


Figure 3. Gas Chromatography/Mass Spectra of 4-Methoxy-N-ethylamphetamine; Normalized (Upper Spectrum) and Enhanced 10x (Lower Spectrum).

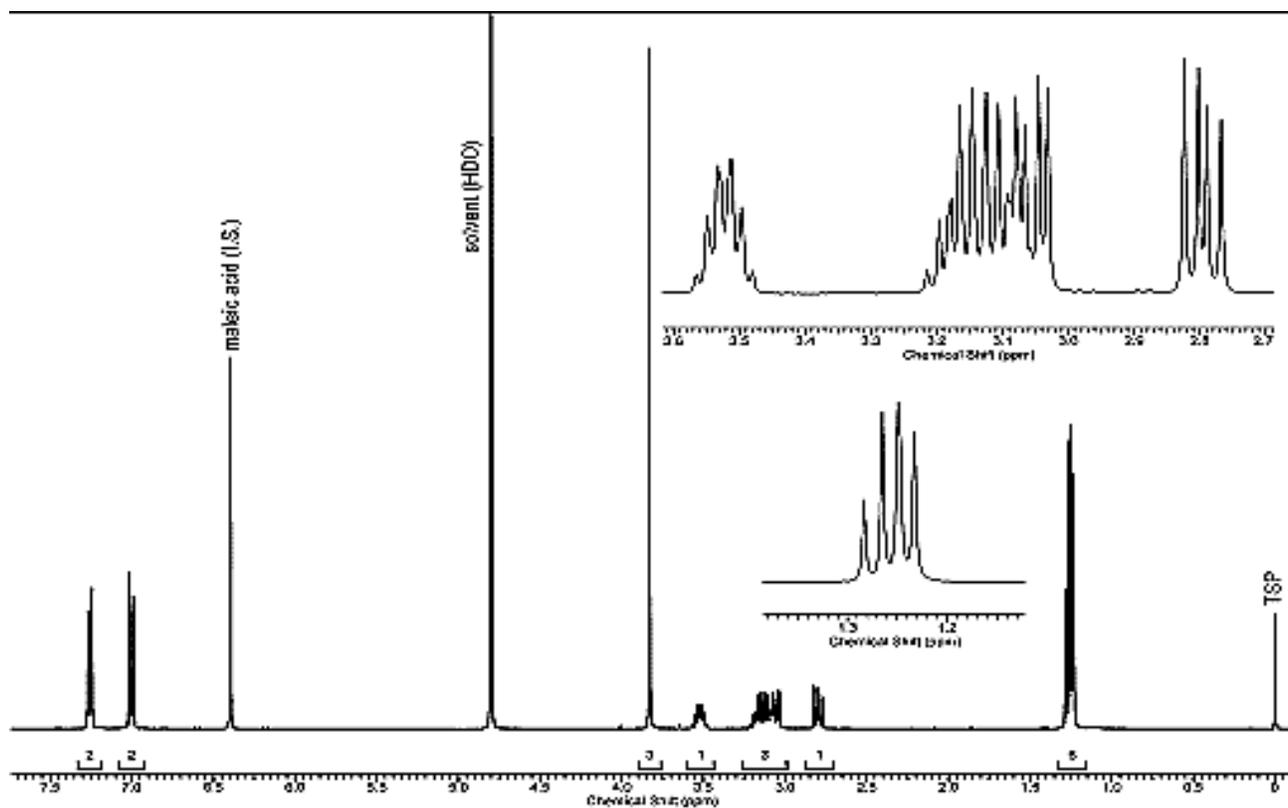


Figure 4. ¹H-NMR Spectrum of 4-Methoxy-N-ethylamphetamine HCl.

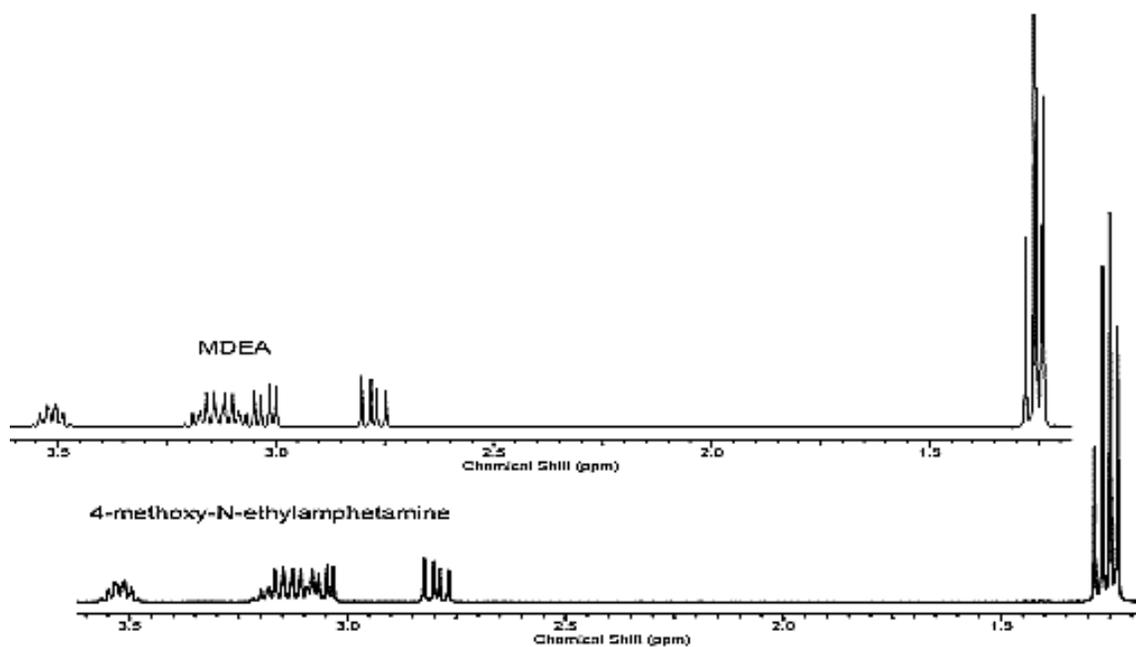


Figure 5. ¹H-NMR Spectrum of 4-Methoxy-N-ethylamphetamine HCl and 3,4-Methylenedioxy-N-ethylamphetamine (MDEA).

Quantitation of Cocaine by Gas Chromatography-Flame Ionization Detection Utilizing Isopropylcocaine as a Structurally Related Internal Standard

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ABSTRACT: The quantitation of cocaine by gas chromatography-flame ionization detection using isopropylcocaine as a structurally related internal standard is presented. The selectivity, precision, and accuracy of the method are detailed. The facile, multi-gram synthesis of isopropylcocaine standard from cocaine (via two different routes) is described.

KEYWORDS: Isopropylcocaine, Synthesis, Gas Chromatography, Flame Ionization Detection, Cocaine Quantitation, Internal Standard, Forensic Chemistry

Introduction

The analysis of cocaine exhibits has been a major task in forensic, crime, and toxicological laboratories over the past 20 - 25 years. Federal Sentencing Guidelines (1), as well as some state criminal statutes, require quantitative analysis of cocaine exhibits. In addition, the accurate assay of cocaine is also a critical element for laboratories that are conducting cocaine signature analyses (2-3). In the DEA's Cocaine Signature Program (CSP), target alkaloids are quantitated relative to the amount of cocaine present, not to the total sample weight. Therefore, even samples that are cut, either with an adulterant or a diluent, can still be analyzed for signature purposes as though they were uncut. However, this technique requires highly accurate quantitations of all of the target alkaloids.

Several gas chromatographic methods have been developed and validated for cocaine quantitation (4-8). These methods utilize an internal standard (ISTD) to improve the precision of the quantitative analysis; however, the ISTDs utilized in these studies (tetraphenylethylene, morphine HCl, cyclobenzapine HCl, methylpalmitate, or eicosane) are not structurally related to cocaine, and in fact in most instances have dissimilar chemical and physical properties. Thus, the presence of impurities, possible acid hydrolysis of cocaine (9), dirty injection ports, and the formation of artifacts (10), can decrease the accuracy of the assay (11). The use of a structurally related internal standard (SR-ISTD) minimize the factors that affect the resulting analyte signal (in this case cocaine), since the SR-ISTD will have virtually the same response to the detector (3). The gas chromatographic method presented herein employs isopropyl cocaine as the SR-ISTD. Isopropylcocaine is not commercially available; however, also as described herein it can be synthesized from cocaine and commercially available reagents (see Figure 1, next page).

Experimental

Materials: Pharmaceutical cocaine base and hydrochloride were obtained from Merck Chemical (Rahway, NJ). Chloroform was a distilled-in-glass product of Burdick and Jackson Laboratories (Muskegon, MI). All other

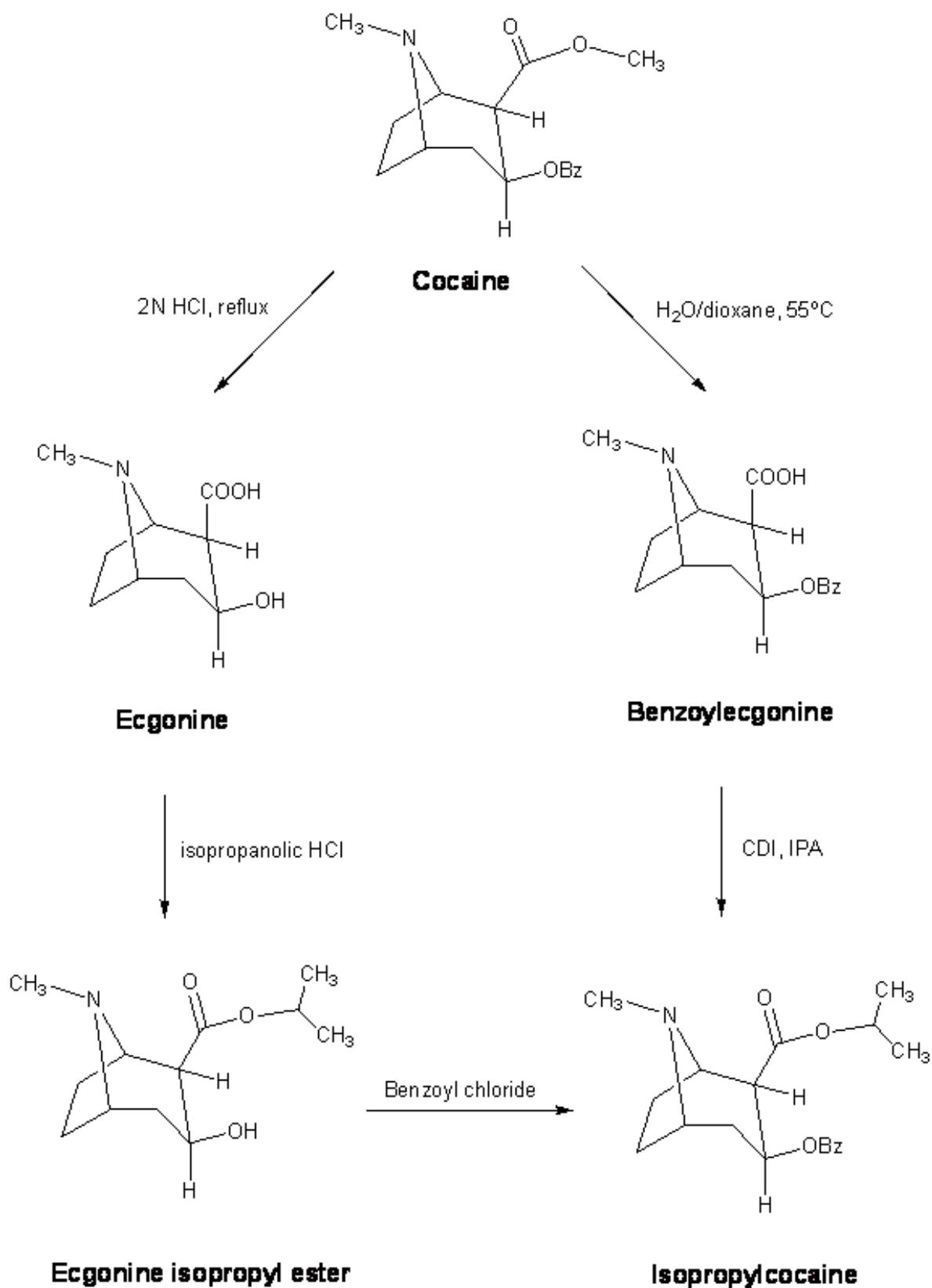


Figure 1. Synthetic Routes to Isopropylcocaine.

reagents and chemicals were reagent-grade quality products of the Sigma-Aldrich Chemical Company (Milwaukee, WI). Illicit refined cocaine HCl was acquired from the reference collection of this laboratory.

Syntheses (Acid Hydrolysis Route):

Ecgonine HCl: Refined illicit cocaine HCl (250 grams, 0.736 mol) was combined with water (500 mL) and concentrated hydrochloric acid (25.0 mL) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was gently refluxed, with stirring, for 6 days. Benzoic acid precipitated from the solution upon cooling. The reaction mixture was extracted with chloroform (5 x 500 mL) to remove benzoic acid and methyl benzoate. The aqueous phase was then added slowly, with stirring, to acetone (7.2 liters) to precipitate ecgonine HCl. The precipitate was captured via suction filtration, washed with additional acetone (1.5 liters), then dried to provide ecgonine HCl as a white powder (107 grams, 65 percent yield).

Ecgonine Isopropyl Ester HCl: Ecgonine HCl (40.0 grams, 0.18 mol) was combined with isopropanolic HCl (2.0 liters, 0.14 grams/mL) in a 5-liter round-bottom flask fitted with a water-cooled condenser. The solution was gently refluxed, with stirring, for 3 days. The isopropanol was evaporated *in vacuo* to an oil. The oil was dissolved in water (500 mL), adjusted to pH 10 with concentrated NaOH, and extracted with methylene chloride (3 x 200 mL). The combined extracts were washed with water (3 x 400 mL) and brine (200 mL), then dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to a clear oil (42.0 grams). The oil was dissolved in anhydrous diethyl ether (500 mL), and isopropyl ecgonine HCl was precipitated by adding ethereal HCl (0.05 grams/mL) until a pH of 4 was achieved. The ether was decanted from the crystalline product, and acetone (400 mL) added with stirring. The product was captured via suction filtration, washed with additional acetone (400 mL) and diethyl ether (400 mL), then dried to provide ecgonine isopropyl ester HCl as a white powder (32.0 grams, 67 percent yield).

Isopropylcocaine: Ecgonine isopropyl ester HCl (31.1 grams, 0.118 mol) was combined with pyridine (200 mL) and benzoyl chloride (19.8 grams, 0.142 mol) in a 1-liter round-bottom flask fitted with a drying tube. After stirring for 1 hour, acetone (400 mL) was added to precipitate isopropylcocaine HCl. The product was captured via suction filtration, washed with additional acetone (2 x 200 mL) and diethyl ether (200 mL), then dried to provide isopropylcocaine HCl as a white powder containing a small amount of pyridine HCl. The product was dissolved in water (100 mL), adjusted to pH 9 with solid sodium carbonate, then extracted with methylene chloride (3 x 200 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to provide isopropylcocaine HCl as a white crystalline powder (31.6 grams, 81 percent yield, 99+ percent purity).

Syntheses (Base Hydrolysis Route):

Benzoylecgonine: Pharmaceutical cocaine base (70.6 grams, 0.233 mol) was combined with water (250 mL) and dioxane (350 mL) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was heated at 55 °C for 9 days. The reaction mixture was evaporated *in vacuo* to provide crude benzoylecgonine tetrahydrate as a white powder. The powder was washed with diethyl ether (2 x 400 mL) to remove any remaining cocaine base, then dried to give 67.5 grams of benzoylecgonine tetrahydrate. The product was dissolved in boiling acetone (750 mL), cooled to room temperature, diluted with diethyl ether (2.25 liters), and allowed to stand overnight at 5 °C. The resulting crystalline product was captured via suction filtration, washed with additional diethyl ether (600 mL), then dried to provide anhydrous benzoylecgonine as a white powder (53.5 grams, 79 percent yield).

Isopropylcocaine: Anhydrous benzoylecgonine (30.7 grams, 0.106 mol) was combined with methylene chloride (500 mL) and 1',1'-carbonyldiimidazole (17.2 grams, 0.106 mol) in a 1-liter round-bottom flask fitted with a drying tube, and stirred overnight. Isopropanol (26.8 grams, 0.447 mol) was added, and the solution was stirred for 6 days. The reaction was extracted with 3 N HCl (2 x 200 mL). The combined aqueous extracts were washed with methylene chloride (200 mL), adjusted to pH 9 with concentrated ammonium hydroxide, and extracted with methylene chloride (3 x 200 mL). The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give a clear oil (30.0 grams). The oil was dissolved in petroleum

ether (20 - 40 °C boiling range, 300 mL) and allowed to stand overnight, resulting in precipitation of the imidazole by-product. The solution was suction filtered to remove this byproduct, and the filtrate evaporated *in vacuo* to give a clear oil which crystallized upon standing. This was recrystallized from petroleum ether, then dried to provide isopropylcocaine base as a white powder (23.3 grams, 66 percent yield, 99+ percent purity).

Gas Chromatography - Flame Ionization Detection (GC-FID): Analyses were performed with an Agilent (Palo Alto, CA) Model 6890N gas chromatograph. One mL of the prepared solutions was placed into an autosampler vial for analysis under the following conditions: A 30 m x 0.25 mm ID fused-silica column coated with 0.25 µm HP-1 (Agilent) was used. Hydrogen (99.999 percent UHP) was the carrier gas at a flow rate of 1.1 mL/minute. The injection port and flame ionization detector were maintained at 280 °C. Samples (2 µL) were injected in the split mode (25:1) by an Agilent 7683 Series Auto Injector. The oven temperature was programmed isothermally at 250 °C for 7.00 minutes. Nitrogen was used as the auxiliary make-up gas for the detector.

Structurally Related Internal Standard Stock Solution: Isopropylcocaine base was dissolved into chloroform at a concentration of 0.9 mg/mL (equivalent to 1.0 mg/mL isopropylcocaine hydrochloride). The solution was stored at 4 °C when not in use. Solutions can be stored for one year at 4 °C without detectable degradation. The solution should be warmed to room temperature before use.

Standard Solutions for Linearity Study and Calibration: Individual solutions containing 0.038, 0.087, 0.23, 0.44, 0.63, 0.83, 1.00, 1.53, and 2.03 mg/mL of cocaine base in chloroform were prepared. Each also contained 0.18 mg/mL of the SR-ISTD.

Standard and Sample Preparation: About 18 to 20 mg of cocaine hydrochloride (or 16 to 18 mg for cocaine base) was accurately weighed (to the nearest 0.01 mg) into a 50 mL Erlenmeyer flask, and 5.0 mL of the SR-ISTD stock solution and 20 mL of chloroform containing 50 µL of diethylamine were added. The solutions were allowed to sit for 5 minutes. Aliquots of standard and sample solutions were transferred to autosampler vials for analyses.

Results and Discussion

The synthesis of isopropylcocaine is relatively simple, and can be performed on a large scale with common glassware and reagents. The mass spectrum of isopropylcocaine is illustrated in Figure 2. Isopropylcocaine was selected as the SR-ISTD for several reasons. Its close structural similarity to cocaine means it will have a similar FID response. Second, it has excellent chromatographic properties, and does not interfere with any other coca alkaloids or commonly encountered diluents and adulterants (see Figure 3 for chromatographic profiles of illicit cocaine base and illicit cocaine HCl). Third, only a small amount (about 5 mg) is needed for each analysis. Fourth and finally, it was found to be very stable. A stock solution stored for up to one year at 4 °C in chloroform yielded no detectable hydrolysis or degradation products, and produced the same number of integrated area counts over that entire time frame.

The linearity of the method was confirmed over the concentration range listed in the Experimental Section, and linear regression analysis determined the correlation coefficient (R^2) as 0.9999 (Figure 4). The average error difference between the known concentrations and the predicted concentrations was +/- 0.75 percent between 0.44 mg/mL and 1.53 mg/mL. For routine analyses, a single point calibration of approximately 0.75 mg/mL was used. Method selectivity was excellent; the identities and retention times of some common adulterants and diluents using the presented methodology are shown in Table 1 (reported retention times are relative to cocaine). Other coca alkaloids and common cutting agents do not interfere with cocaine or isopropylcocaine. The precision of the method was determined using the nine linearity concentrations, with five replicate injections per concentration. The resulting calculated Relative Standard Deviation (RSD) for each concentration was less than 0.21 percent, and in some instances was as low as 0.02 percent. The accuracy of the method was tested over a 14 month period by having eleven different chemists quantitate a secondary cocaine standard (having a known cocaine

concentration of 84.6 percent) against the pharmaceutical cocaine standard during routine casework. Over that time period, 188 quantitative observations for the secondary standard were recorded. The average value obtained was 84.7 percent, with a range of 83.4 - 86.0 percent. The RSD for all 188 observations was found to be 0.58 percent. The overall absolute error of the assay was determined to be less than 1 percent.

Acknowledgments

The authors wish to thank Supervisory Forensic Chemist Valerie Colley (this laboratory) for providing assistance with the analytical data.

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[Table 1 and Figures 2 - 4 Follow.]

Table 1. Relative Retention Times (RRT) of Some Common Adulterants/Diluents and Coca Alkaloids.

<u>Compound</u>	<u>RRT (min)</u>
Ecgonine methyl ester	0.48
Benzocaine	0.49
Acetaminophen	0.52
Caffeine	0.61
Lidocaine	0.65
Procaine	0.76
Cocaine	1.00
Isopropylcocaine	1.14
cis-Cinnamoylcocaine	1.36
trans-Cinnamoylcocaine	1.78
Benzoyllecgonine	1.89

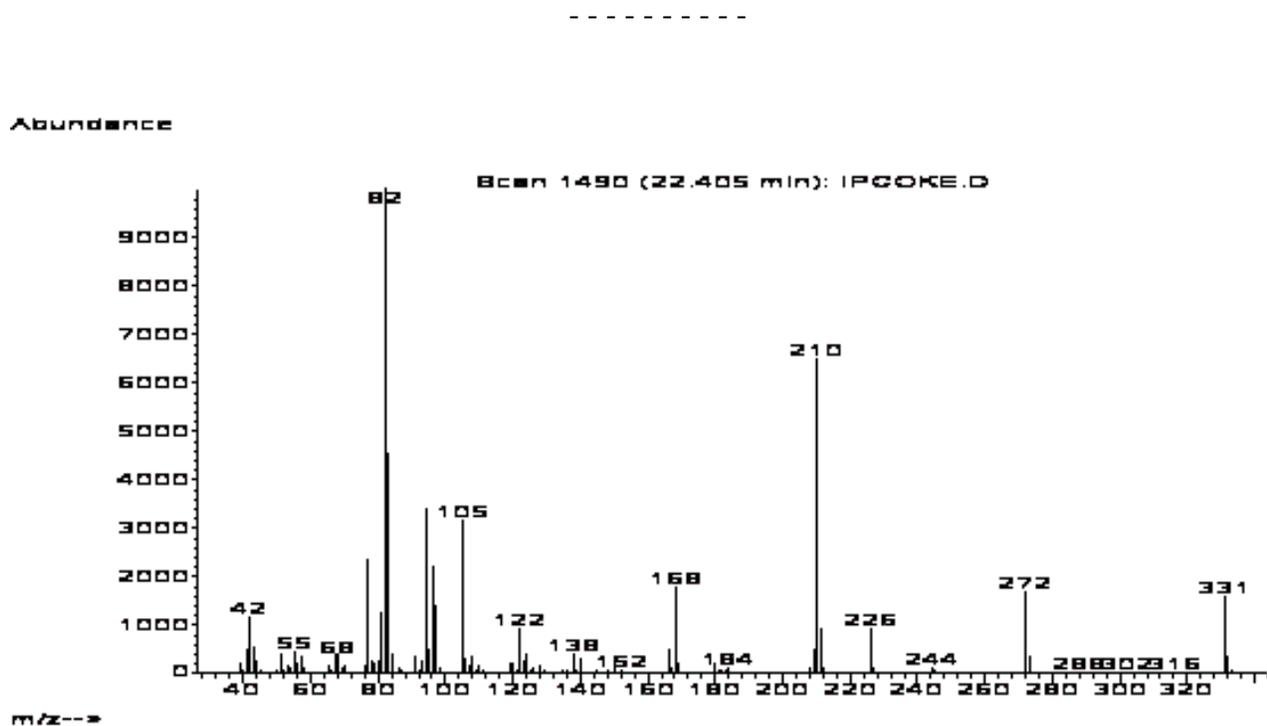


Figure 2. Electron Ionization Mass Spectrum of Isopropylcocaine.

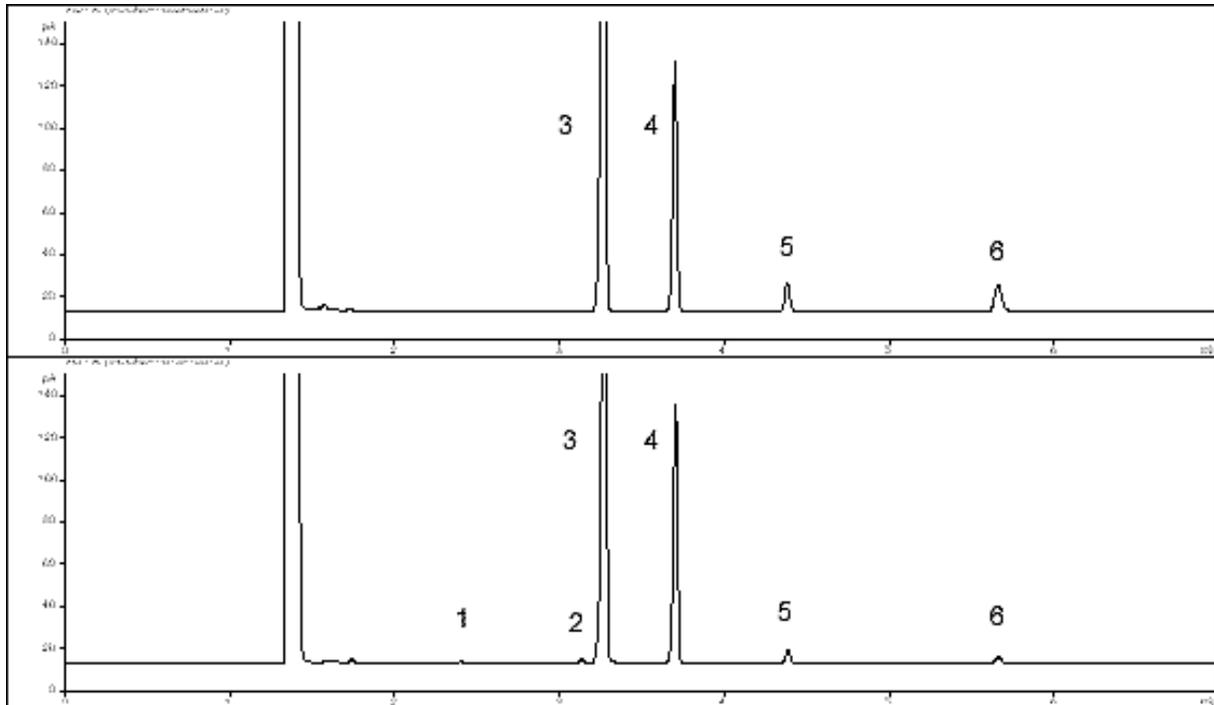


Figure 3. Capillary Gas Chromatographic Profiles of (Upper) 86.1 Percent Illicit Cocaine Base Exhibit and (Lower) 86.2 Percent Illicit Cocaine HCl Exhibit. Peak Identification: 1 = Tropacocaine, 2 = Norcocaine, 3 = Cocaine, 4 = Isopropylcocaine (SR-ISTD), 5 = *cis*-Cinnamoylcocaine, and 6 = *trans*-Cinamoylcocaine.

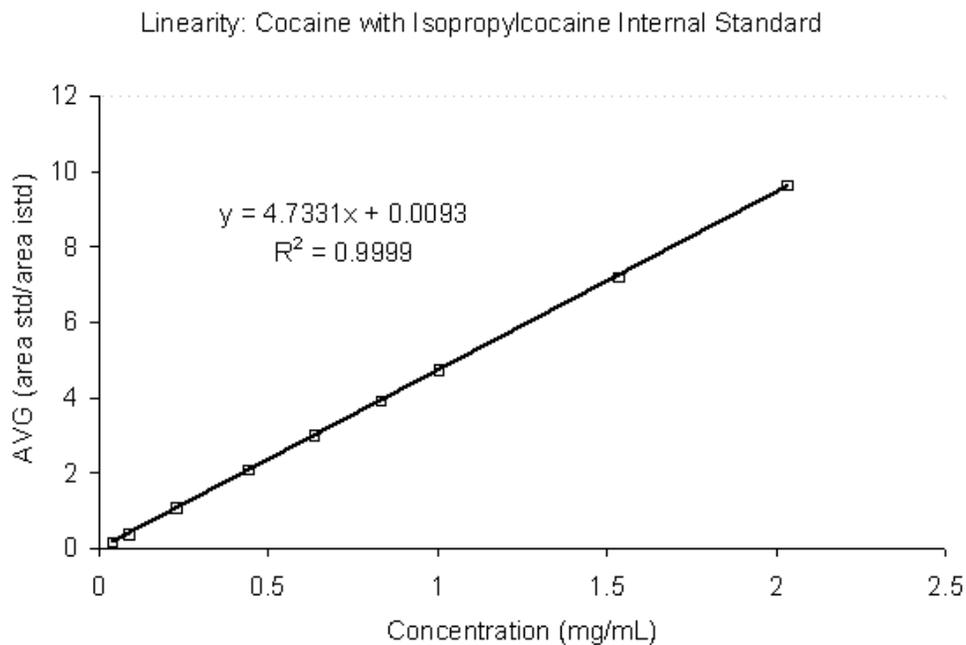


Figure 4. Calibration Curve for the Isopropylcocaine Internal Standard Methodology.

Dehydrochlormethyltestosterone: An Analytical Profile

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[Previously Reported in Part as an Intelligence Alert; see: *Microgram Bulletin* 2006;39(7):87.]

ABSTRACT: Analytical data (GC, GC/MS, FTIR, HPLC, ^1H - and ^{13}C - NMR) for the analysis and identification of dehydrochlormethyltestosterone ((17 β)-4-chloro-17-hydroxy-17-methylandrosta-1,4-dien-3-one) is presented. Historical background is also included.

KEYWORDS: Dehydrochlormethyltestosterone, Chlorodehydromethyltestosterone, Turanabol, Turinabol, Anabolic Steroid, Controlled Substance, Analysis, Forensic Chemistry

Introduction

The Drug Enforcement Administration Mid-Atlantic Laboratory recently received a submission of steroids and steroid-related exhibits that were seized during a consent search of a residence in Winchester, Virginia. The exhibits included 15 bottles, each labeled "Turanabol," "Chlorodehydromethyltestosterone," and "Golden Triangle Pharmaceuticals" (see Photo 1). Despite identical appearances (same bottle type, labeling, lot number, and number of tablets (100)), six of the bottles contained nondescript orange capsules while nine bottles contained nondescript yellow capsules (see Photo 2, next page). Subsequent analyses confirmed that the orange capsules contained dehydrochlormethyltestosterone as the only active ingredient, while the yellow capsules contained primarily dehydrochlormethyltestosterone with minor amounts of stanozolol and methandrostenolone (see structures, next page). This is believed to be the first submission of dehydrochlormethyltestosterone to the DEA laboratory system (1).

Dehydrochlormethyltestosterone is a Schedule III controlled substance in the United States and is also listed in the 2006 Prohibited List/World Anti-Doping Code. It gained notoriety as a result of the East German Olympic doping scandals that were fully exposed after the fall of the Berlin wall (2). Data from



Photo 1

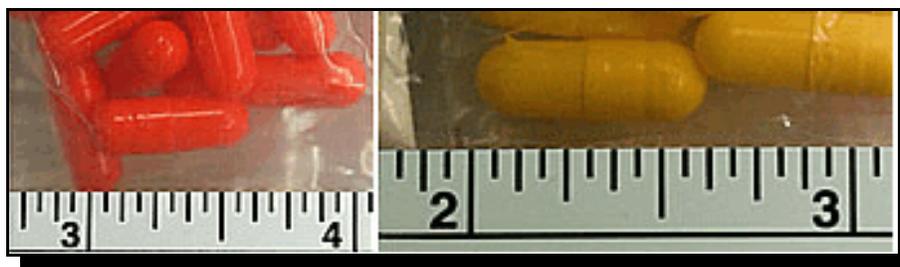
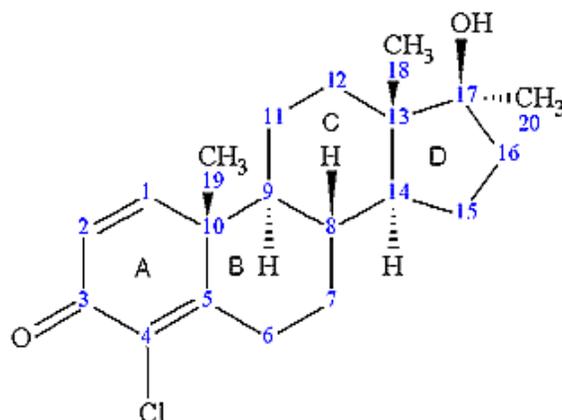
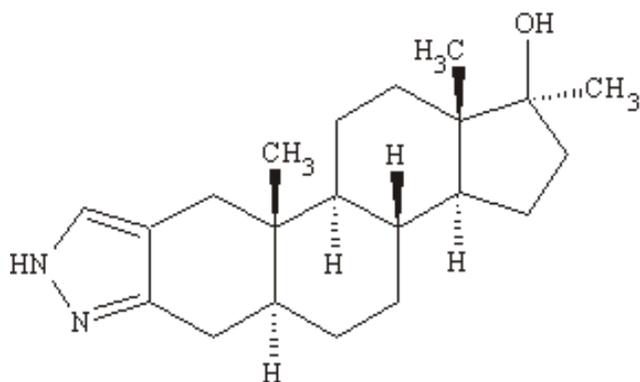


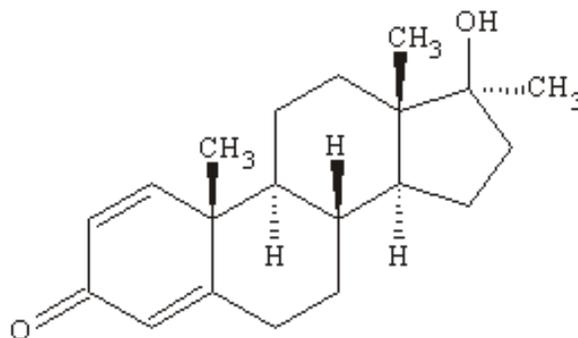
Photo 2 (Note that Both Types of Capsules are the Same Size)



Dehydrochloromethyltestosterone (with Labeling of Rings (A-D) and IUPAC Numbering of Carbons).



Stanozolol



Methandrostenolone

East German medical personnel involved in the doping indicated that dehydrochloromethyltestosterone produced dramatic increases in speed and strength, but with detrimental side effects such as deepening of the voice, increased acne, and body hair growth. Other, long term side-effects ranged from liver damage to severe gynecological disorders (2). This steroid is no longer legitimately produced, and appears to be available only as an illicitly-prepared product on the black market.

As with nearly all anabolic steroids, dehydrochlormethyltestosterone has multiple name variations, including (but not limited to): Dehydrochloromethyltestosterone, chlordehydromethyltestosterone, chlorodehydromethyltestosterone, 4-chlorodehydromethyltestosterone, 4-chloromethandienone, 4-chlor-1-dehydro-17 α -methyltestosterone, 1,4-androstadien-4-chloro-17 β -ol-3-one, and 4-chloro-17 β -hydroxy-17 α -methyl-androst-1,4-dien-3-one. The most common trade name for dehydrochlormethyltestosterone, Oral-Turinabol®, is often abbreviated as “OT” in both the scientific literature and on internet websites dedicated to anabolic steroid use/abuse (2,3).

Not surprisingly, most of the scientific literature dedicated to the analysis of dehydrochlormethyltestosterone has a toxicological focus (that is, analysis of biological fluids for dehydrochlormethyltestosterone metabolites for detection of doping (4,5). Although there are a number of reports of submissions of dehydrochlormethyltestosterone to forensic and crime laboratories (6), complete forensic analysis of dehydrochlormethyltestosterone has not been previously reported, and even standard reference texts in the field (e.g., 7,8,9) do not contain data for this compound. Herein, we report analytical data (GC, GC/MS, FTIR-ATR, HPLC, and ¹H- and ¹³C- NMR) for the analysis and identification of the title steroid. In addition, because this is the first comprehensive report for this steroid, an in-depth analysis of the NMR data is presented.

Experimental

Standard: A reference standard of dehydrochlormethyltestosterone was obtained from Steraloids (Newport, RI).

Gas Chromatography (GC): GC screening was conducted using an Agilent 6890N (Waldbronn, Germany) equipped with flame ionization detector (FID). The sample was dissolved in methanol and injected into the instrument using the parameters below.

Instrument	Agilent 6890N
Column	HP-5 (5 % phenyl/95 % methyl silicone); 12 m x 0.2 mm i.d. x 0.33 μ m thickness
Carrier Gas	Helium at 1.0 mL/min
Temperatures	Injector: 270 °C Detector: 280 °C Oven Program: 175 °C for 1 min 15 °C/min to 280 °C Hold at 280 °C for 4 min
Injection Parameters	Split ratio = 60:1, 1 mL injected

Gas Chromatography/Mass Spectrometry (GC/MS): An Agilent 6890N gas chromatograph equipped with an Agilent 5973 Mass Selection Detector (MSD) (Waldbronn, Germany) was used in the electron ionization (EI) mode to obtain mass spectra of samples and standards. Instrumental parameters are listed below. Agilent’s MS Interpreter (Version 0.9) was used to derive the relative abundances of the molecular ion cluster.

Instrument	Agilent 6890N with Agilent 5973 Mass Selection Detector
Column	HP-5MS (5 % phenyl/95 % methyl silicone); 15 m x 0.25 mm x 0.25 μ m thickness
Carrier Gas	Helium at 1.0 mL/min
Temperatures	Injector: 280 °C Oven Program: 150 °C for 0.5 min 30 °C/min to 300 °C Hold at 300 °C for 1.5 min
Injection Parameters	Split ratio = 75:1, 1 mL injected

Detector	Quadrupole Mass Detector
Temperatures	Transfer Line: 280 °C MS Quad: 150 °C MS Source: 230 °C
Acquisition Mode	Scan
Solvent Delay Time	0.5 minutes
Scan Parameters	Mass Range: 40 - 450 amu Sample #: 3 (2n = 8 samples taken at each mass) Resulting Scan Rate = 1.84 scans/sec

Fourier Transform Infrared Spectrometer - Attenuated Total Reflectance (FTIR-ATR): Infrared spectroscopy was performed using a Thermo Nicolet Nexus 670 Fourier Transform Infrared Spectrometer (FTIR) (Madison, WI) equipped with a Golden Gate Attenuated Total Reflectance (ATR) detector. The sample was prepared by extraction of the capsule matrix with methanol followed by evaporation. The IR spectrum was collected by averaging 24 scans with a resolution of 4.0 wavenumbers (cm⁻¹).

High Performance Liquid Chromatography (HPLC): HPLC was conducted using an Agilent 1100 Series instrument (Waldbronn, Germany) using ultraviolet (UV) detection. The sample was dissolved in methanol and injected into the instrument using the parameters below (10).

Instrument	Agilent 1100 Series
Column	Waters Xterra RP18 (4.6 x 150 mm, 3.5 mm)
Mobile Phase	80 % Water (W): 20 % Acetonitrile (A) hold for 3 min Ramp to 55 % W: 45 % A for 2 min and hold for 8 min Ramp to 35 % W: 65 % A for 3 min and hold for 10 min Ramp to 10 % W: 90 % A for 5 min and hold for 9 min
Temperature	45 °C
Detection Wavelength	225 nm
Injection Volume	5 mL
Injection Solvent	Methanol

Nuclear Magnetic Resonance (NMR) Spectroscopy: One and two dimensional (1D and 2D) NMR experiments were performed on a Varian Mercury 400 MHz NMR using a 5 mm Varian Nalorac pulse field gradient (PFG) indirection detection probe (Varian Inc., Palo Alto, CA). Standard Varian pulse sequences were employed. The sample and standard were prepared in deuterated methanol (CD₃OD) with tetramethylsilane (TMS) added (approximately 0.05 % v/v) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, WI). The ¹H-NMR spectrum of the standard was obtained with 8 scans using a 45 second delay, 90 ° pulse, 2 second acquisition time, and oversampling of 6. The ¹³C-NMR spectrum of the standard was obtained with proton decoupling; 2,000 scans were acquired, using a 1 second delay, 45 ° pulse, 1.2 second acquisition time, and oversampling of 3. Samples were maintained at 25 °C. Standard Varian gradient versions of 2D NMR experiments were performed to help make assignments, including homonuclear COSY (2 - 4 bond proton-proton through bond correlations), NOESY (proton - proton spatial nearness correlations for protons < 4 angstroms apart), heteronuclear HSQC (proton to directly bonded carbon correlations), and HMBC (2, 3, or 4 bond proton to carbon correlations). Structural elucidation was performed utilizing Applied Chemistry Developments (ACD/Labs, Toronto, Canada) software (HNMR Predictor, CNMR predictor, and Structure Elucidator).

Results and Discussion

Gas Chromatography (GC): The chromatogram is not shown. Methandrostenolone, dehydrochlormethyltestosterone, and stanozolol eluted at 7.96, 9.32, and 10.29 minutes, respectively. The peak shape for stanozolol was broad in comparison to the other steroids. The mixture in the yellow tablets was not formally quantitated, but was estimated as roughly 100 : 5 : 2.5 dehydrochlormethyltestosterone : methandrostenolone : stanozolol. Table 1 lists the relative retention times for cocaine, heroin, and six other steroids with similar chromatography.

Table 1. GC Relative Retention Times.

Drug (GC)	RRt
Cocaine	0.580
Mesterolone	0.813
Testosterone	0.822
Heroin	0.829
Methyltestosterone	0.836
Methandrostenolone	0.854
Testosterone Acetate	0.882
Fluoxymesterone	0.990
Dehydrochlormethyltestosterone	1.000
Stanozolol	1.103
Testosterone Isocaproate	1.188

Gas Chromatography/Mass Spectrometry (GC/MS): The mass spectra of dehydrochlormethyltestosterone, methandrostenolone, and stanozolol are shown in Figures 1 - 3, respectively. Dehydrochlormethyltestosterone displayed a molecular ion at m/z 334. Analysis of the molecular ion cluster (i.e., for $C_{20}H_{27}O_2Cl$) revealed close agreement with the theoretical values obtained from the MS Interpreter program, confirming the molecular formula and the presence of a chlorine atom (Table 2). Of note, the spectra did not give a satisfactory match with any compound in the instrument's database, indicating both that the compound is not a different steroid and that dehydrochlormethyltestosterone is not entered.

Table 2. Theoretical versus Actual Values for the Relative Abundances for the Molecular Ion Cluster (i.e., for $C_{20}H_{27}O_2Cl$).

Mass (amu)	Theoretical (Relative Abundance)	Experimental (Relative Abundance)
334	100.00	100.00
335	22.73	22.87
336	34.84	34.92
337	7.53	7.41
338	0.93	0.83

Fourier Transform Infrared Spectrometer - Attenuated Total Reflectance (FTIR-ATR): The IR spectrum of the reference standard is shown in Figure 4. The spectrum displayed major absorbances for O-H (3485 cm^{-1}), C-H (2947 cm^{-1}) and C=O (1655 cm^{-1}). Comparison of the reference standard with the sample is shown in Figure 5. The direct comparison did not show a high quality match; it is suspected that either polymorphism or the presence of other soluble capsule materials in the extract caused the differences in the spectra. Again, neither spectrum gave a satisfactory match with any compound in the instrument's database, indicating both that the compound is not a different steroid and that dehydrochlormethyltestosterone is not entered.

High Performance Liquid Chromatography (HPLC): The chromatograms for dehydrochlormethyltestosterone standard and a mixture of dehydrochlormethyltestosterone and stanozolol standards (roughly 5 : 95) are shown in Figure 6. The two peaks resulting from the dehydrochlormethyltestosterone - stanozolol mixture did not resolve using this method (inset in Figure 6). However, they are resolved by GC or GC/MS, enabling each to be identified. Table 3 lists the relative retention times of a series of similarly sized steroids. Figure 7 shows the UV spectrum of dehydrochlormethyltestosterone.

Table 3. HPLC Relative Retention Times (Asterisks denote steroids analyzed at an earlier date; the retention times were adjusted relative to the dehydrochlormethyltestosterone).

Drug (LC)	RRt
Fluoxymesterone	0.64
Boldenone*	0.70
Nandrolone*	0.73
Methandrostenolone	0.75
Testosterone	0.79
Methyltestosterone	0.87
Dehydrochlormethyltestosterone	1.00
Stanozolol	1.02
Testosterone Acetate	1.37
Methenolone Acetate*	1.46
Nandrolone Propionate*	1.52
Testosterone Propionate*	1.77
Nandralone Phenylpropionate*	1.86
Testosterone Phenylpropionate*	1.98
Testosterone Isocaproate*	2.14
Testosterone Cypionate*	2.34
Methenolone Enanthate*	2.40
Nandralone Decanoate*	2.58
Testosterone Decanoate*	2.62
Testosterone Undecylanate*	2.68

Nuclear Magnetic Resonance (NMR) Spectroscopy: The ¹H-NMR spectrum of the reference standard are shown in Figures 8a - b. Spectral assignments are summarized in Table 4 (next page). The proton, carbon, and HSQC experiments showed that the unknown molecule contained 20 carbons and 26 non-exchangeable hydrogens. There were 6 quaternary, 5 methine, 6 methylene, and 3 methyl carbons. Adding the carbons (20), non-labile protons (26), oxygens (2), and chlorine (1 based on the MS data), gives a molecular weight of 333 Daltons. The remaining mass (1 Dalton) is due to an exchangeable proton. Using the HMBC NMR data, it was determined that there is one carbonyl carbon (180 ppm), 1 - 3 bonds from 4 alkene carbons at 126.5, 128.6, 158.9, and 166.1 ppm, two of which are protonated, with the hydrogens (6.32 and 7.33 ppm) coupled to each other ($J = 10.1$ Hz). This corresponds well to a doubly conjugated ketone on ring "A" with the carbonyl at position 3, protonated alkene carbons at positions 1 and 2, and quaternary alkene carbons at positions 4 and 5 (meaning position 4 has a substituent, presumably the chlorine). Far removed is a quaternary carbon at 82.0 ppm, indicating that it is bonded to oxygen (likely bonded to the exchangeable proton). Assuming that this is a common steroid ring structure, placement of the carbon (82 ppm) bonded to oxygen would be at the 17 position. This accounts for all but the methyl group, and since the 82 ppm carbon is a quaternary carbon, the methyl group is attached at the 17 position. Comparison of the experimental data with that predicted with the ACD software showed very good agreement. In addition, comparison of the ¹H-NMR spectrum of the unknown to methandrostenolone showed they were nearly identical below 2.0 ppm, indicating that the B, C, and D rings are the same.

Table 4. NMR Data and Assignments.

Position	Carbon ppm	Proton			
		ppm	#H	Type	Coupling Constants (<i>J</i>) (Hz)
1	158.9	7.33	1	bd	10.1
2	126.5	6.32	1	d	10.1
3	180.2	-	-	-	-
4	128.6	-	-	-	-
5	166.1	-	-	-	-
6a	30.1	2.4	1	td	13.6(x2), 5.2
6b	30.1	3.27	1	dt	13.6, 3.2(x2)
7a	33.5	0.99	1	m	-
7b	33.5	2.02	1	abdq	13.6, ~3.9, ~3.9, 3.2
8	37.5	1.84	1	m	-
9	54.9	1.06	1	m	-
10	48.1	-	-	-	-
11a (or 15)	24.1	1.33	1	m	-
11b (or 15)	24.1	1.81	1	m	-
12a	32.7	1.31	1	m	-
12b	32.7	1.61	1	m	-
13	47.0	-	-	-	-
14	51.0	1.23	1	m	-
15a (or 11)	24.4	1.39	1	m	-
15b (or 11)	24.4	1.63	1	m	-
16a	39.1	1.68	1	m	-
16b	39.1	1.87	1	m	-
17	82.0	-	-	-	-
18	14.7	0.94	3	s	-
19	19.6	1.35	3	s	-
20	26.1	1.16	3	s	-

b = Broad, d = Doublet, m = Multiplet, abdq = Broad Doublet of Quartets, s = Singlet, and t = Triplet. Many coupling constants could not be determined due to the complexity of the ¹H-NMR spectrum.

Final confirmation of the compound's identity was achieved via comparison of mass spectral fragmentation patterns, GC retention times, and proton and carbon NMR spectra, with the reference standard.

Acknowledgements

The authors gratefully acknowledge Forensic Chemist Esther Chege and Senior Forensic Chemist Charles Matkovich, both of the Mid-Atlantic Laboratory, for their assistance running NMR experiments.

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[References Continued on Page 65.]

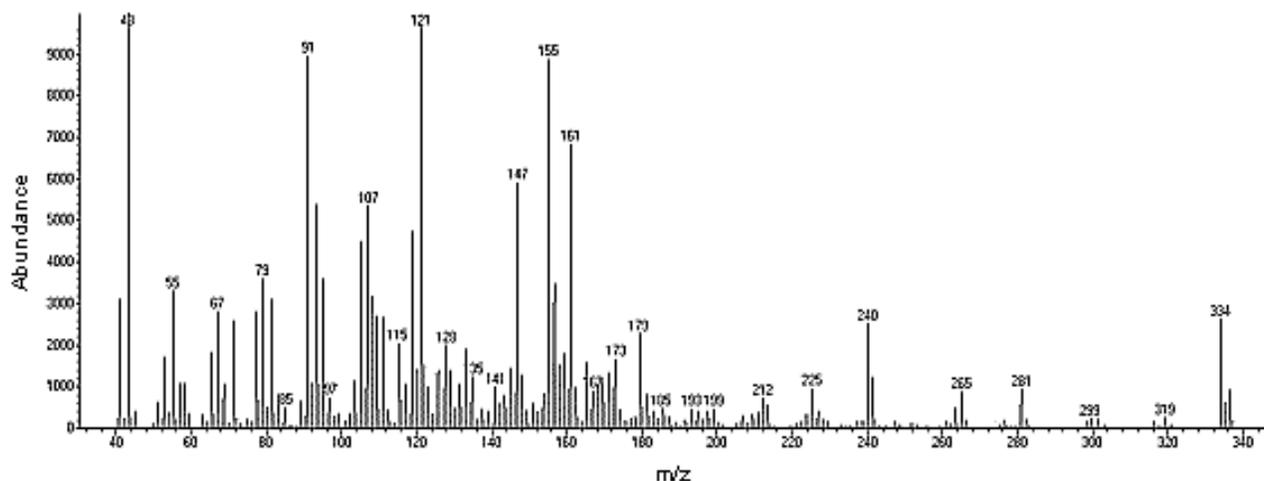


Figure 1. Mass Spectrum of Dehydrochlormethyltestosterone.

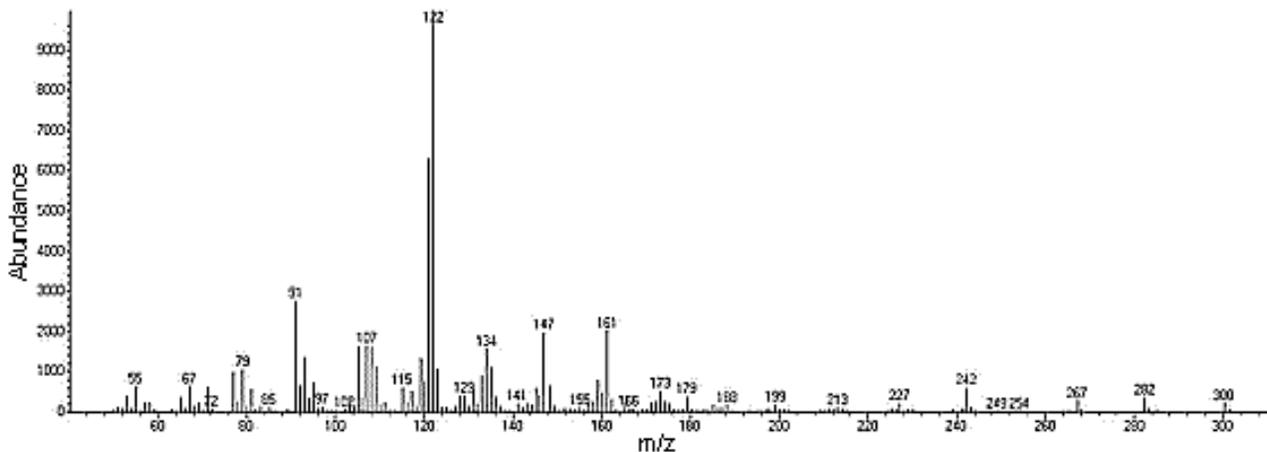


Figure 2. Mass Spectrum of Methandrostenolone.

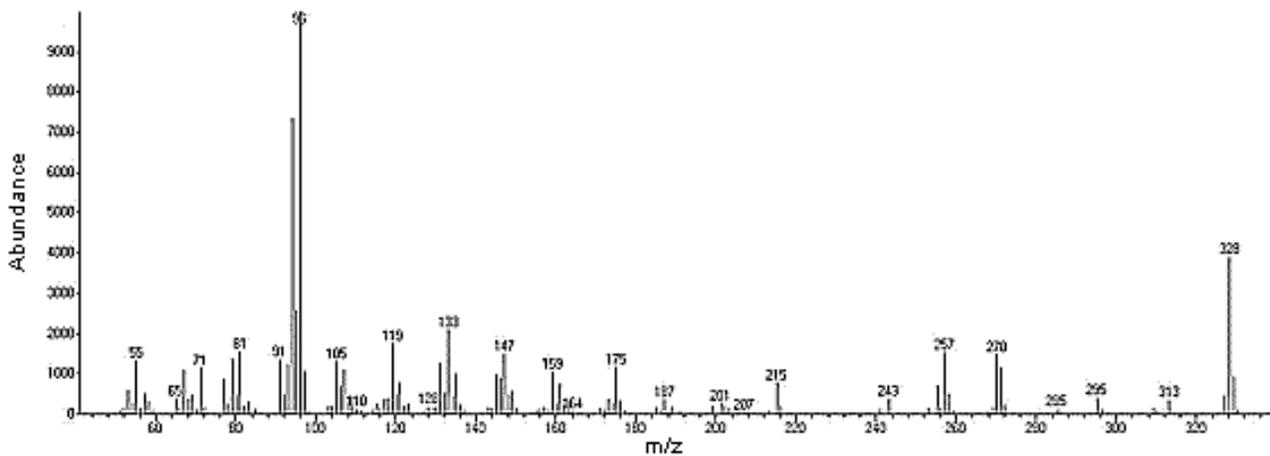


Figure 3. Mass Spectrum of Stanozolol.

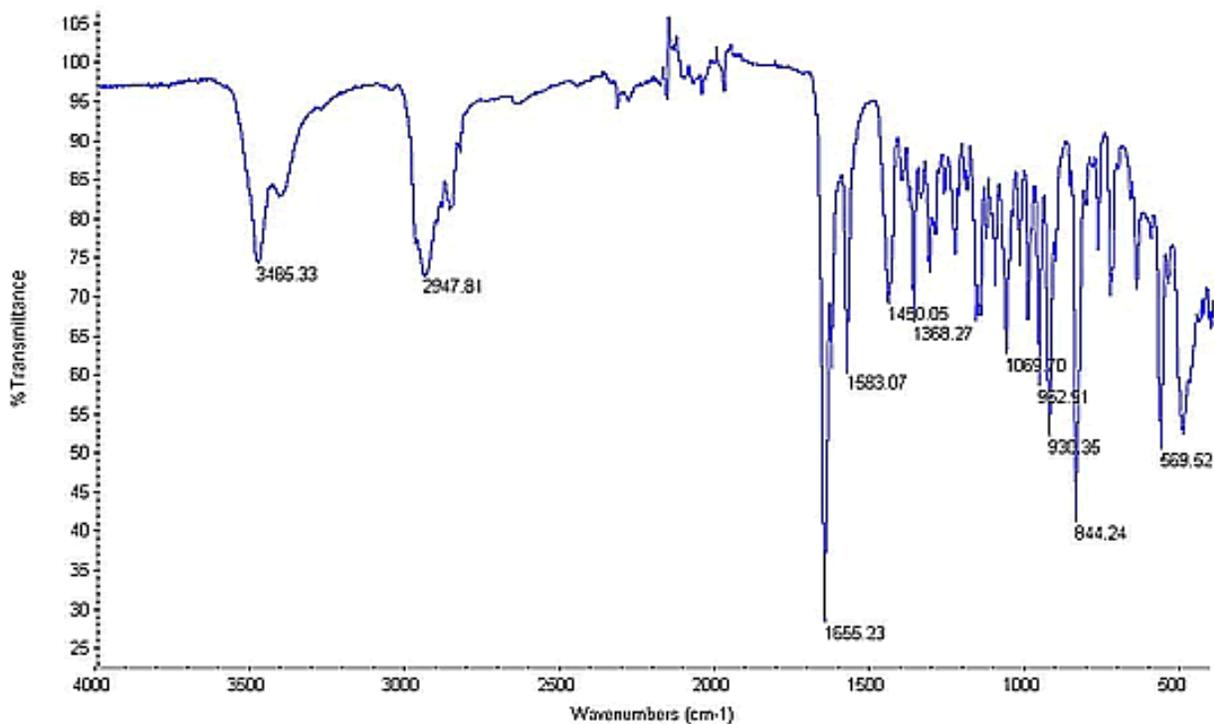


Figure 4. The Infrared Spectrum (FTIR-ATR) of Dehydrochloromethyltestosterone Reference Standard.

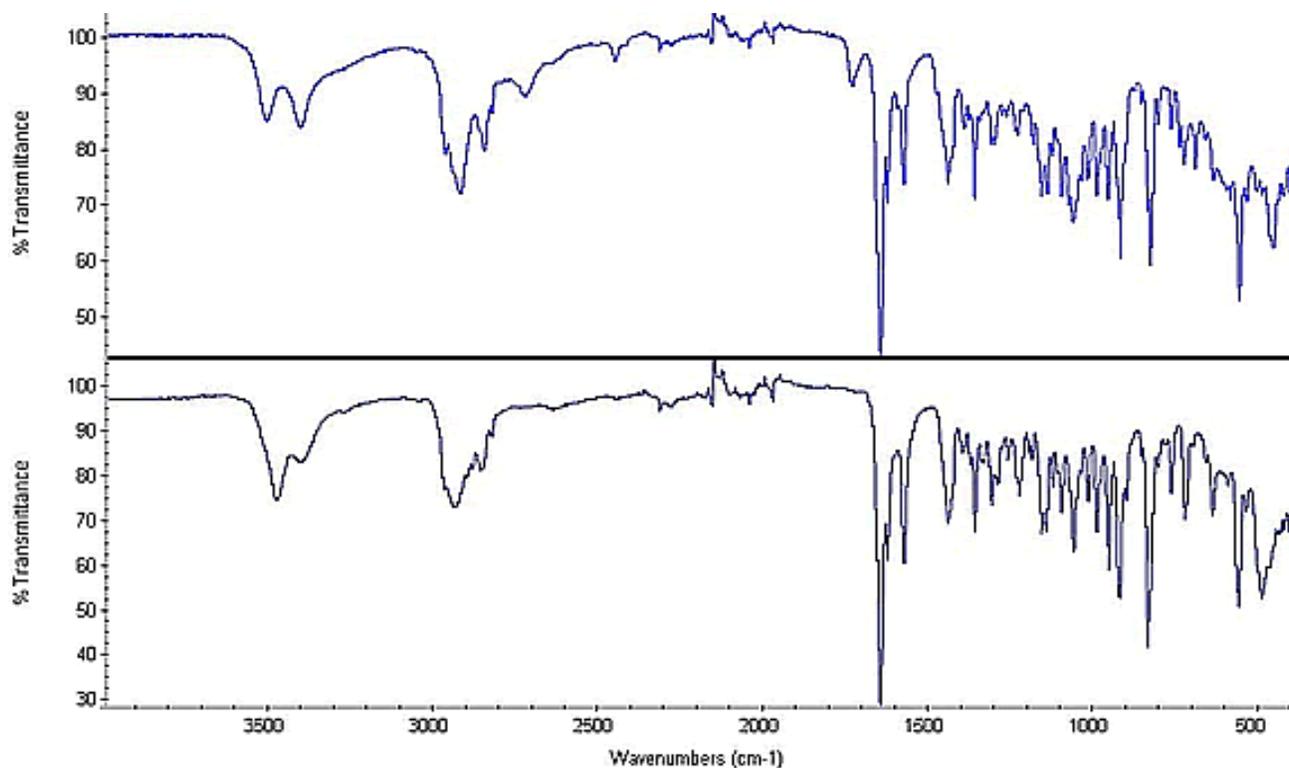


Figure 5. Infrared Spectrum (FTIR-ATR) of Sample of Orange Colored Capsule's Methanol Soluble Materials (Upper Trace) Compared to the Reference Standard (Lower Trace).

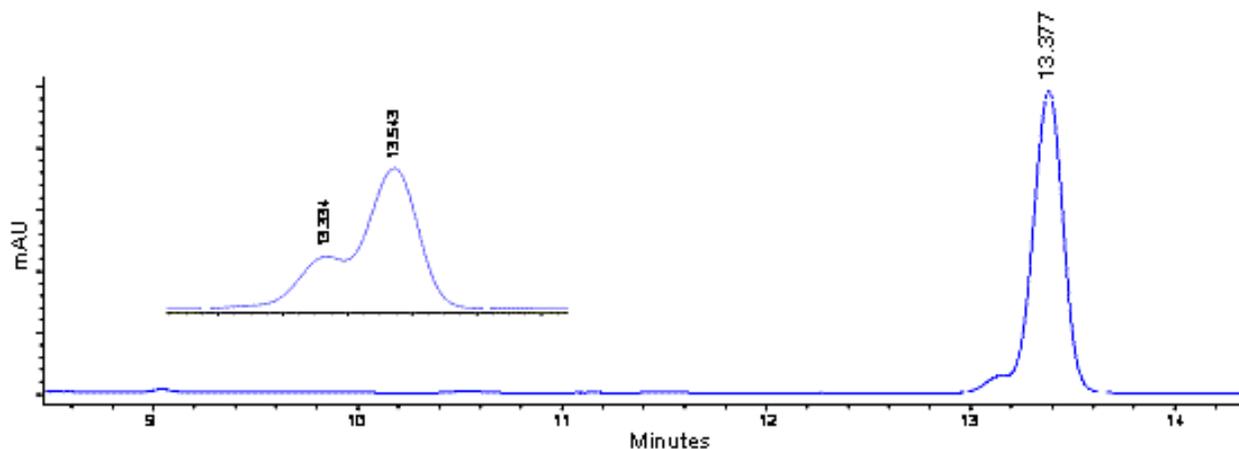


Figure 6. The HPLC UV Chromatogram (225 nm Detection) for Dehydrochloromethyltestosterone Standard (13.377 Minutes). The Inset Shows the Chromatogram for the 5 : 95 Mixture of Dehydrochloromethyltestosterone (13.334 minutes) and Stanozolol (13.543 minutes) Standards.

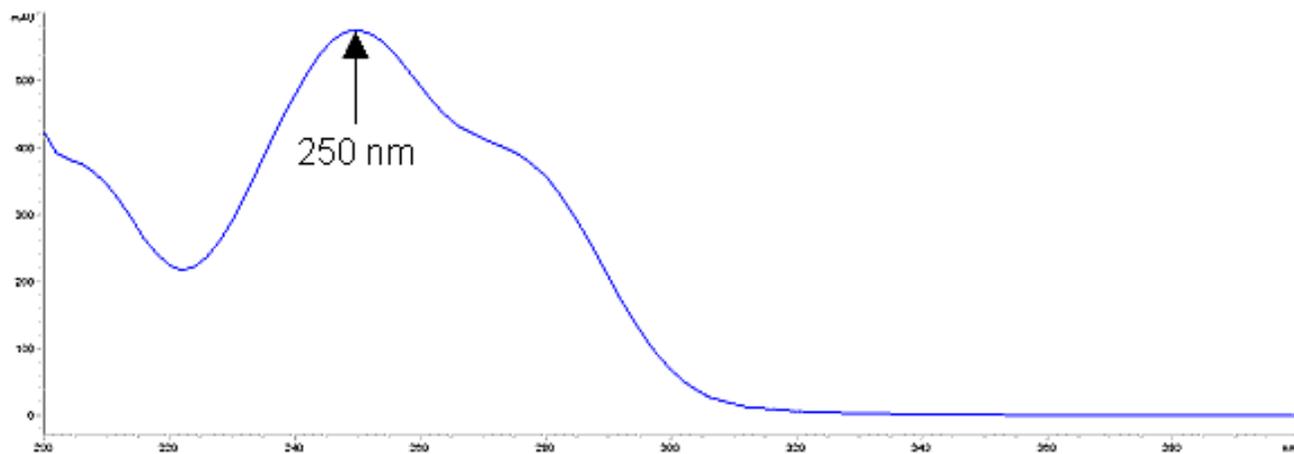


Figure 7. HPLC UV Spectrum of Dehydrochloromethyltestosterone in Methanol.

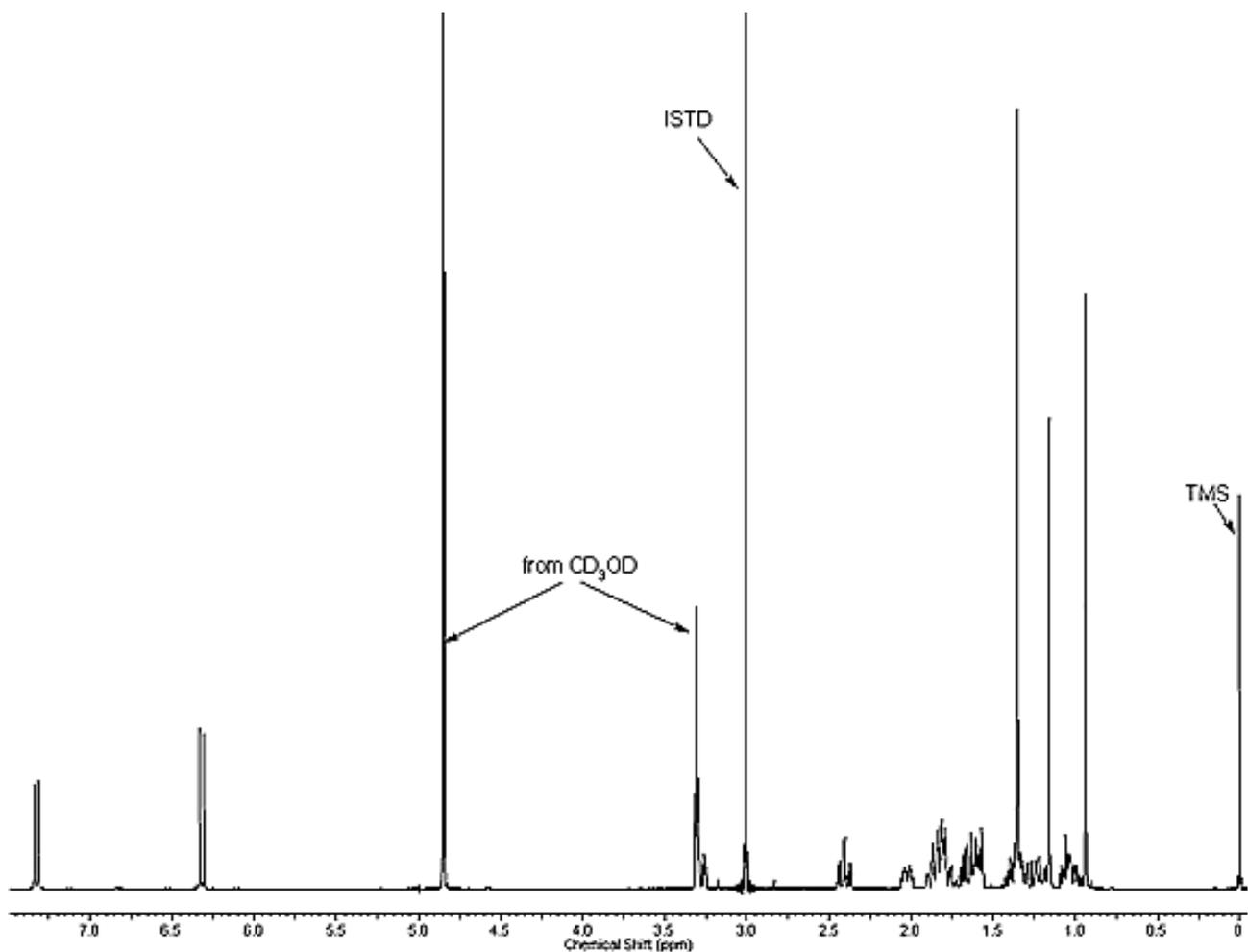


Figure 8a. $^1\text{H-NMR}$ of Dehydrochloromethyltestosterone Reference Standard in CD_3OD . Dimethylsulfoxone (Listed as ISTD) was used to Quantitate the Standard.

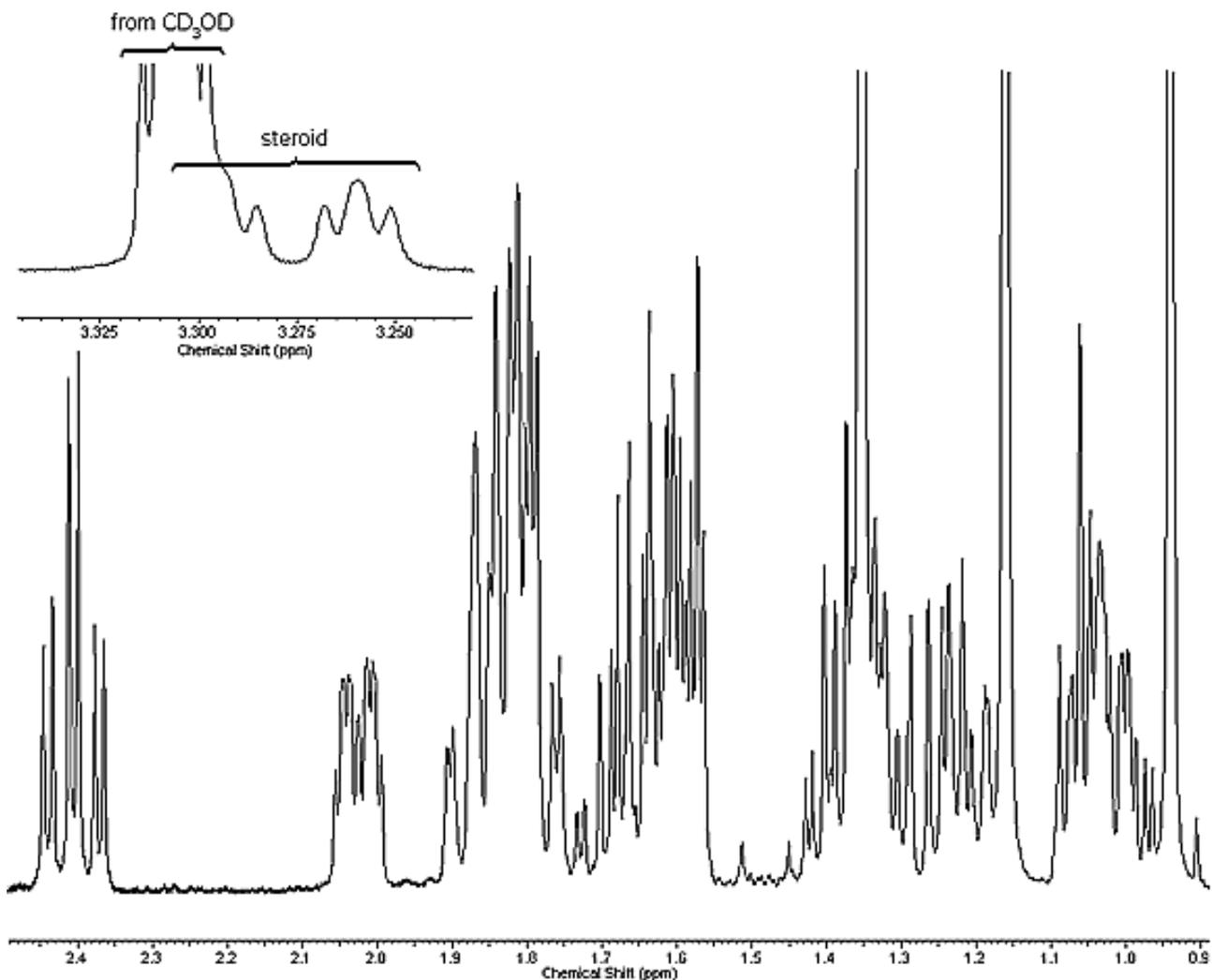


Figure 8b. Alkyl Region of Proton Spectrum of Figure 8a, Expanded to Show Peak Splitting Patterns.

References (Continued from Page 61)

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10. Method developed by DEA Senior Forensic Chemist David Rees of the Mid-Atlantic Laboratory.

Technical Note

Qualitative and Quantitative Analysis of Ionamin 30 Capsules (Containing a Time-Release Formulation of Phentermine)

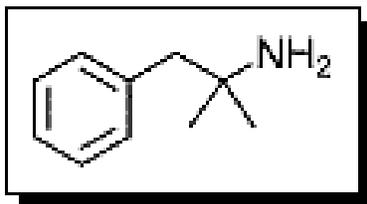
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ABSTRACT: Analysis of a time-release formulation of phentermine required sonication in water for 60 minutes, in order to release the active compound from the matrix.

KEYWORDS: Phentermine, Ionamin 30, Time-Release Formulation, Analysis, HPLC, ¹H-NMR, Sonication, Forensic Chemistry



Phentermine

Introduction

The Mid-Atlantic Laboratory recently received a large submission of multiple exhibits allegedly containing various forms of phentermine. The exhibits were seized in Laurel, Maryland (no further details). One exhibit included 1,494 yellow capsules (14 x 5 millimeters), each labelled as “Ionamin 30” and containing brown resin beads and white powder (see Photos 1 and 2, next page). Ionamin 30 is a time-release formulation of phentermine containing 30 milligrams of phentermine in a cationic exchange resin complex (1). However, preliminary analyses of methanol and chloroform extracts of the capsule contents using GC, GC/MS, and NMR indicated no controlled substances. Further research on drug-resin complexes revealed that the time-release mechanism in these capsules involves a water-permeable/acid insoluble barrier that allows the substance to be slowly released into the body. Herein, a method for the analysis of this type of formulation is presented. The method may be useful for other time-release formulations.

Experimental

Chemicals and Reagents: Phentermine standard was acquired from this laboratory’s reference collection. All other chemicals were of reagent-grade quality or better.



Photo 1



Photo 2

HPLC: Analyses were performed using a Agilent 1100 Series High Performance Liquid Chromatograph. Acquisition Parameters are summarized below:

Column:	RP18 Waters Symmetry Shield; 3.5 μm particle size, 150 mm x 4.6 mm
Detector:	Diode Array (Detection at 210 nm)
Temperature:	30 $^{\circ}\text{C}$
Flow Rate:	1.0 mL/minute
Injection Volume:	3 μL
Buffer:	4000 mL HPLC grade water, 9.6 grams sodium phosphate monobasic, adjusted to pH 2.3 with phosphoric acid, 8.0 mL hexylamine, and 50 milligrams sodium azide
Mobile Phase:	2.3 pH buffer:acetonitrile (85:15)

Standard Solution: A standard solution of phentermine was prepared at approximately 0.5 mg/mL with 0.3 mg/mL resorcinol in 95:5 buffer:acetonitrile.

Sample Solution: A portion of sample was accurately weighed into a volumetric flask, a small amount of room temperature water added, and the mixture was sonicated for at least 60 minutes. The resulting solution was diluted with additional water to give an estimated phentermine concentration of approximately 0.5 mg/mL. The diluted solution was filtered through a 0.2 micron filter before injection onto the HPLC.

Quantitative Procedure: Inject 3 μL of the solution onto the HPLC. The preferred wavelength for phentermine is 210 nm with a bandwidth of 10 nm.

$^1\text{H-NMR}$: Analyses were performed using a Varian Mercury-Plus 400 MHz NMR using a 5 mm Varian Nalorac indirect detection, variable temperature, pulse field gradient probe with PulseTune® (Varian, Palo Alto, CA). The compound was dissolved in deuterated water (D_2O) containing 1 percent (w/w) 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt as the reference compound. The temperature of the sample was maintained at about 21 $^{\circ}\text{C}$. Standard Varian (vNMR Version 6.1) pulse sequences were used to acquire the proton spectra. Eight scans were acquired for each spectrum. Processing of data was performed using software from Applied Chemistry Development Laboratory, Version 8 (Toronto, Canada).

Results and Discussion

Phentermine is an appetite suppressant (anorectic) used in the management of obesity (1,2). Because it is also a stimulant that is subject to abuse, phentermine is a Schedule IV controlled substance under the U.S. Controlled Substances Act.

Analyses of standard preparations of phentermine is straightforward (3,4). However, time-release formulations of pharmaceuticals require preliminary workup to release the active ingredient from the matrix. In the present study, attempted dissolution of the contents of an Ionamin 30 time-release formulation of phentermine in either methanol or chloroform was ineffective. The resin used in the Ionamin formulation is water-permeable, and it was found that sonication in water was sufficient to release the trapped phentermine. The release was time-dependent; detectable amounts of phentermine (sufficient for a qualitative determination) were released after 10 minutes of sonication, but complete release (required for accurate quantitation) required 60 minutes of sonication (see Figures 1 and 2). The sonicated solution can also be dried down and reconstituted in chloroform/methanol for GC and/or GC/MS analysis, or in deuterated water for NMR analysis (Figures 3a and b).

The white powder in the capsules was not identified, but according to the literature it is a mixture of lactose, magnesium stearate, and titanium dioxide (1).

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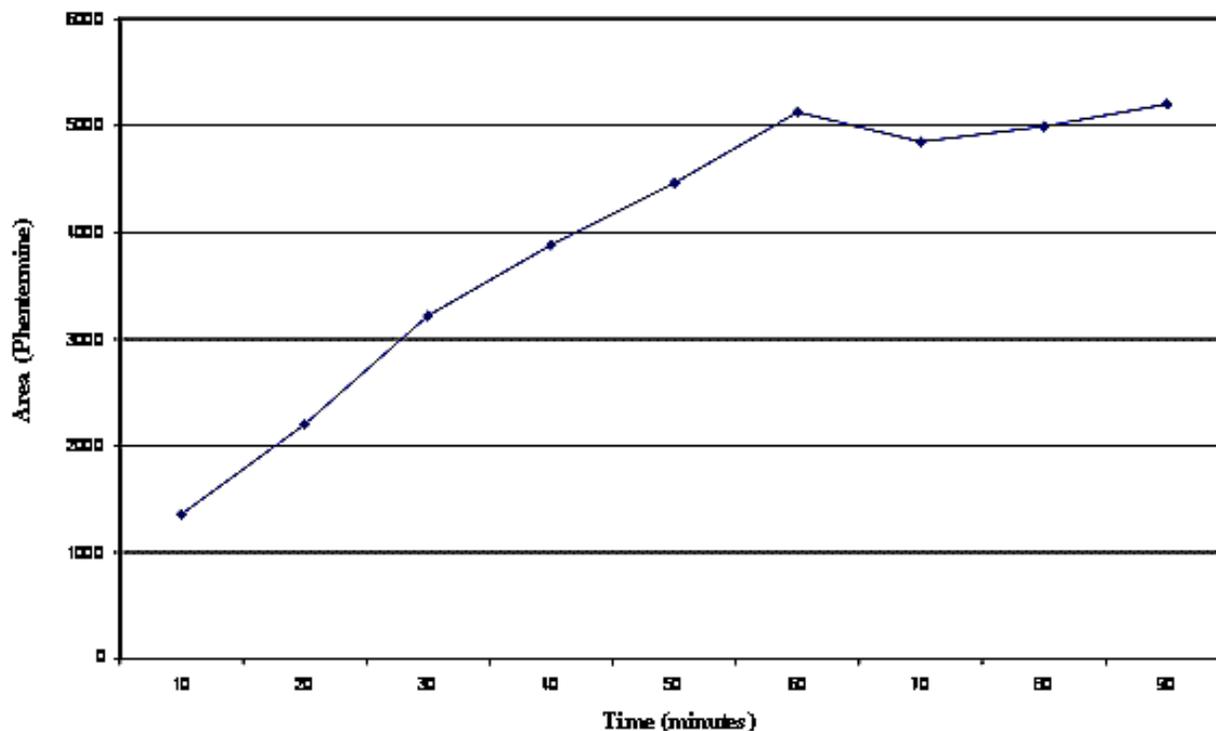


Figure 1. Ten Minute HPLC Qualitative Interval Study of 30 mg Phentermine Capsule.

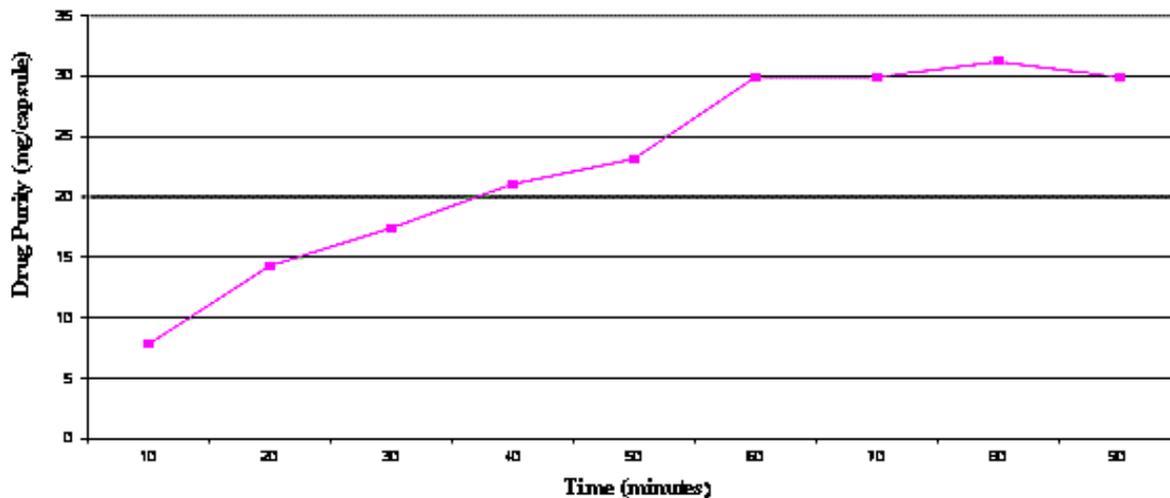


Figure 2. Ten Minute HPLC Quantitation Interval Study of 30 mg Phentermine Capsule.

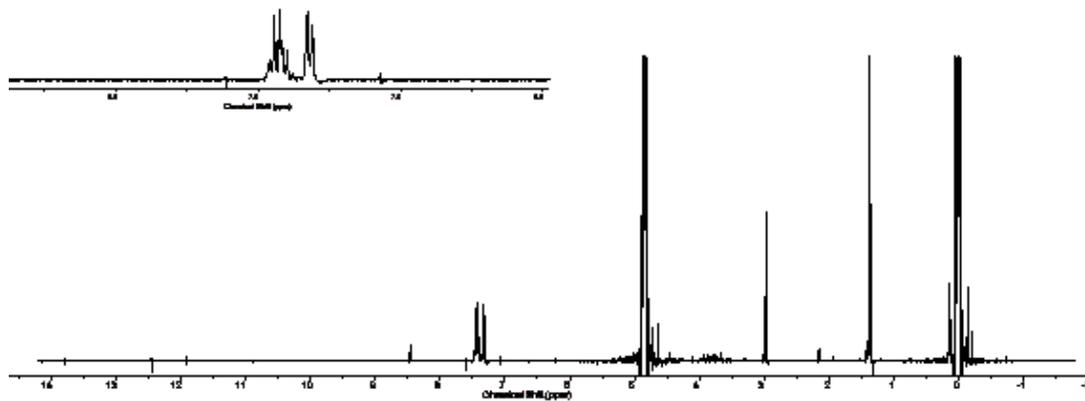


Figure 3a. ¹H-NMR Spectrum of Ionamin 30 Capsule in Deuterated Water.

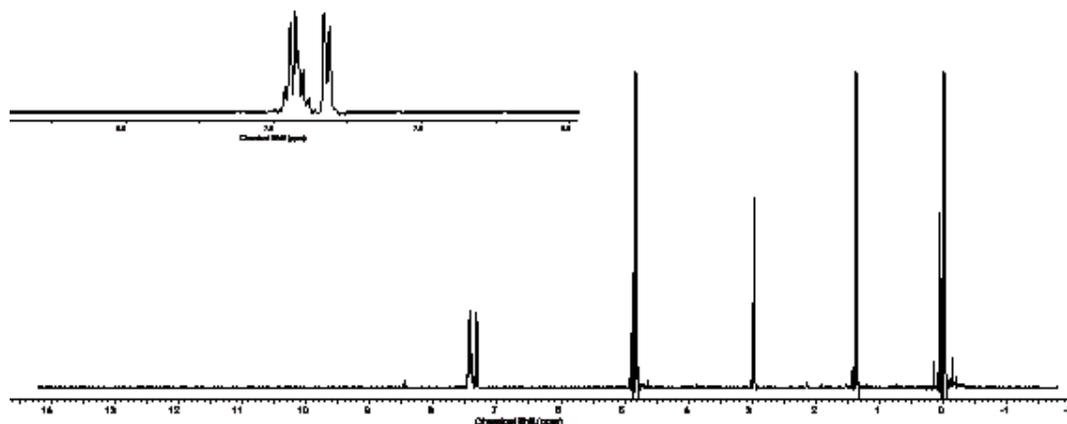


Figure 3b. ¹H-NMR Spectrum of Phentermine Hydrochloride Standard in Deuterated Water.

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