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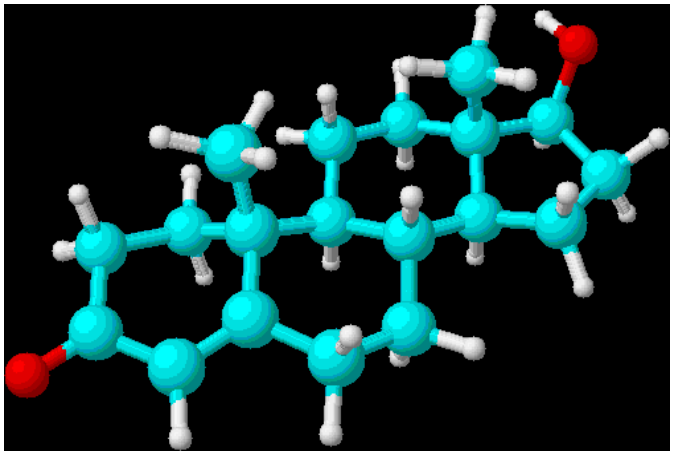

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Contents

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

Synthesis and Identification of N,N-Dimethylcathinone Hydrochloride

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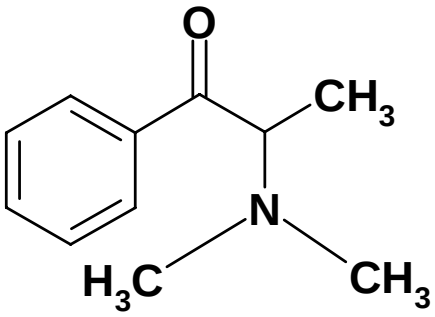
ABSTRACT: The syntheses and analyses of N,N-dimethylcathinone and N-ethylcathinone are presented and discussed.

KEYWORDS: N,N-Dimethylcathinone, N-Ethylcathinone, α-Aminopropiophenones, Synthesis, Analysis, Forensic Chemistry



Dimethylcathinone

Introduction

Although substantial information has been published concerning the analysis and identification of cathinone (2-amino-1-phenyl-1-propanone, α-aminopropiophenone)[a] and methcathinone (2-methylamino-1-phenyl-1-propanone, α-methylaminopropiophenone) [1-6], very little analytical data has been published to assist in the identification of the structural analog N,N-dimethylcathinone hydrochloride (hereafter "dimethylcathinone").[b] A recent seizure of a very large quantity of this drug [7] has spurred interest in its analysis.

While various synthetic aminopropiophenones are popular drugs of abuse in Europe [8,9], with the minor exceptions of methcathinone and methylone (N-methyl-3,4-methylenedioxypropiophenone) [10], this interest has not been matched in the United States (U.S.). The abuse of khat (*Catha edulis*), which contains small amounts of

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[a] "Natural" cathinone (from khat (*Catha edulis*)) is the S enantiomer; however, in order to avoid confusion, "cathinone" is understood to be a generic (common) term for 2-amino-1-phenyl-1-propanone, and the stereochemistry (R, S, or R/S) is specified as appropriate.

[b] *Editor's Note*: Dimethylcathinone is occasionally referred to as "dimethcathinone" (a now superceded common name). Dimethylcathinone is the proper nomenclature.

(2S)-(-)-cathinone [4,11-16], has been endemic in the east African communities within the U.S., but cathinone is virtually never encountered as a clandestinely synthesized or extracted drug, possibly because of its instability in free base form. Under U.S. law, cathinone, khat, and methcathinone are all Schedule I Controlled Substances. Currently (early 2008), dimethylcathinone is not scheduled; however, prosecution of this compound (as a Schedule I drug) would be conducted under the tenets of the Controlled Substances Analogue Enforcement Act.

Quite surprisingly, racemic dimethylcathinone is approximately equipotent with both racemic cathinone and racemic amphetamine, while the (2S)-(-)-enantiomer is nearly equipotent with both (2S)-(-)-cathinone and (2S)-(+)-amphetamine [17,18]. This is in direct contrast with (2S)-(+)-dimethylamphetamine, which has been found in drug discrimination studies to be only approximately one tenth as potent an analeptic agent (CNS stimulant) as (2S)-(+)-amphetamine [17]. This unexpected potency likely explains the recent appearances of dimethylcathinone in clandestine markets.

(2S)-(-)-Dimethylcathinone can be synthesized from (1R,2S)-(-)-N-methylephedrine by oxidation with potassium permanganate [5,19,20] or any of a variety of chromium compounds, most often sodium or potassium dichromate [21-27]. Alternatively, racemic dimethylcathinone can be prepared from 2-bromopropiophenone by reacting with dimethylamine [17,28-32]. Herein, the synthesis and analysis of dimethylcathinone are presented and discussed. For comparative purposes, analytical data for N-ethylcathinone (hereafter "ethylcathinone"), an isomeric structural analog of dimethylcathinone, are also presented.

Experimental

Instrumentation: Solid state Fourier Transform infrared (FTIR) spectra were acquired as a potassium bromide matrix, with a Thermo Nicolet Model 6700 Fourier Transform Infrared Spectrophotometer. Gas phase infrared (IRD) spectra were obtained using a Bio-Rad (now ASAP) IRD II infrared detector interfaced to an Agilent 6890 Gas Chromatograph (GC) with an HP-5 30 m x 0.32 mm x 0.25 m column in splitless mode from 80^0C (2.0 min) at 15^0C/min to 270^0C (2.0 min). Mass spectra were acquired using an Agilent 5973 Mass Selective Detector (MSD) attached to an Agilent 6890 GC. This GC had the same type column as above but used a program from 80^0C (2.0 min) at 20^0C/min to 240^0C (0.5 min) in split mode (100:1). Nuclear magnetic resonance (NMR) spectra were acquired at 400 MHz using a Varian Mercury 400 NMR. The compounds were analyzed as the hydrochloride salts in deuterium oxide (D_2O). Melting points were determined with a Thomas-Hoover "Unimelt" apparatus. Polarimetry on the (2S)-(-)-dimethylcathinone HCl was performed using a Perkin-Elmer Model 241 Polarimeter with a 10 cm (1 decimeter) sample cell. All data were acquired at the DEA North Central Laboratory with the exception of the NMR spectra, which were provided by the DEA Special Testing and Research Laboratory (Dulles, VA).

Syntheses and Melting Points: Racemic dimethylcathinone and racemic ethylcathinone were prepared by reacting 2-bromopropiophenone (Aldrich Chemical Co., Milwaukee, WI) with dimethylamine or ethylamine, respectively, as aqueous free bases at -8^0C (ice-salt bath). (2S)-(-)-Dimethylcathinone HCl was prepared by oxidizing (1R,2S)-(-)-N-methylephedrine HCl (Aldrich) with a sodium dichromate/sulfuric acid solution at -5^0C (ice-salt bath). The hydrochloride salts of all compounds were prepared by the addition of a 5% isopropanol/HCl solution to a chloroform solution of the respective free base.

Racemic dimethylcathinone HCl, mp = 206-206.5^{0}C
Racemic ethylcathinone HCl, mp = 186-188^{0}C
(2S)-(-)-Dimethylcathinone HCl, mp = 197.5-200^{0}C, $[\alpha] = -52.5^O$ (H_2O, 1%), T = 21^0C

Gas Chromatography - Mass Spectrometry (GC/MS): The mass spectra of dimethylcathinone and ethylcathinone were acquired as the free bases in chloroform, prepared by dissolving the HCl salts in water, adding saturated sodium carbonate, and extracting into chloroform. The resulting extracts were then passed through a disposable pipette containing a pledget of glass wool and into a glass vial, then introduced into the GC/MS. The mass

spectra of N-acetylethylcathinone was acquired by adding acetic anhydride to a solution of ethylcathinone in chloroform, and immediately injecting the mixture into the GC/MS (the acetylation occurs in the injection port).

Infrared Spectroscopy (FTIR): The two compounds were analyzed as the hydrochloride salts in a compressed potassium bromide matrix.

Infrared Spectroscopy (IRD): The two compounds were introduced to the IRD as free bases in chloroform through an Agilent 6890 GC.

NMR Spectrometry: The 400 MHz proton NMR spectra were acquired by the DEA Special Testing and Research Laboratory (Dulles, VA). Maleic acid was used as an internal standard (peak at 6.40 ppm). Hydrogen exchange with the D_2O solvent is responsible for the HOD resonance at 4.80 ppm. The 0.00 ppm reference peak is from 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt, present in the D_2O solvent (Aldrich).

Color Tests: Although ethylcathinone HCl is a secondary amine, its response to the secondary amine test is almost imperceptible, with only a slight bluish ring forming in a porcelain spot plate after a short period of time. Both of the cathinone analogs give a dull orange with Chen's reagent. Ethylcathinone starts to respond to the reagent in about 90 seconds, while dimethylcathinone starts to respond in about 180 seconds. Full dissipation of the initial "Robin's Egg Blue" color of the reagent mixture requires 10 minutes or more, giving an orange-brown color. Preparation of the reagents is given in Reference 1.

Results and Discussion

The GC retention times of dimethylcathinone and ethylcathinone are very close using the column and parameters specified in the Instrumentation section (dimethylcathinone 7.07 min; ethylcathinone 7.19 min). The resulting mass spectra are typical of simple phenethylamines (Figures 1a-b). The molecular ions are nearly imperceptible, and a large base peak is observed at m/z = 72, indicative of the respective immonium ions. However, ethylcathinone also has a significant ion at m/z = 44, from loss of ethylene from the m/z = 72 ion [33]. This allows easy differentiation of the two compounds, a distinction that is further enhanced by conversion of ethylcathinone to N-acetylethylcathinone with acetic anhydride. N-Acetylethylcathinone gives a mass spectrum having a very large ion at m/z = 114 (72 + 42) and a small molecular ion at m/z = 219 (177 +42) (Figure 1c). Dimethylcathinone (a tertiary amine) does not react with acetic anhydride. A detailed elucidation of the fragmentation patterns for methamphetamine and related compounds has recently been published [34].

Although GC/MS is the method of choice for identification of many compounds, infrared spectrophotometry may be preferable for dimethylcathinone. The solid state FTIR of a purified sample gives a distinctive spectrum that also allows identification of the salt form (Figures 2a-b). When available, IRD offers the convenience of GC/MS without the sample preparation often required for FTIR. IRD spectra, although lacking the fine structure seen in solid state FTIR spectra, are nonetheless distinct and avoid potential difficulties which may occur with some compounds due to polymorphism (Figures 3a-b).

Proton NMR also gives distinct spectra for dimethylcathinone and ethylcathinone. The spectra are easily distinguished by the resonances for the dimethylamino versus ethylamino groups, between 1.30 and 3.30 ppm (Figures 4a-b).

Color tests (presumptive tests, field tests) are often useful in determining the initial direction of an analysis. In the case of cathinone-based compounds, however, the *beta*-keto group appears to have an adverse effect on several commonly used reagents (e.g., Marquis and secondary amine), by slowing or preventing the color responses typically observed for simple secondary phenethylamines. One test that is somewhat useful is the Chen's Test. When a blank is prepared from the three components comprising the reagent, a "Robin's Egg Blue" precipitate results. This is the initial response of this reagent to the cathinone compounds tested to date. However, when

allowed to sit undisturbed for periods of up to 10 minutes, the blue precipitate dissipates, leaving a clear orange to orange-brown solution. In contrast, the corresponding aminoalcohols typically give an immediate purple response [10].

Melting points are useful in determining if a pure enantiomer or the racemate of dimethylcathinone is present. Enantiomers can be more rigorously identified with polarimetry. However, caution is needed when performing polarimetry on any of the α-aminopropiophenones. Cathinone free base is known to racemize quickly in hydrolytic solvents (methanol, ethanol, etc.), but less rapidly in chloroform or methylene chloride. The propensity for enantiomeric dimethylcathinone to racemize is unknown. The oxalate and hydrochloride salt forms of dimethylcathinone are stable as dry powders.

Acknowledgements

The authors wish to thank Senior Forensic Chemists Patrick A. Hays and Trinette K. Spratley (both of the DEA Special Testing and Research Laboratory, Dulles, VA) for acquiring and providing the NMR spectra.

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* Law Enforcement Restricted Issue.

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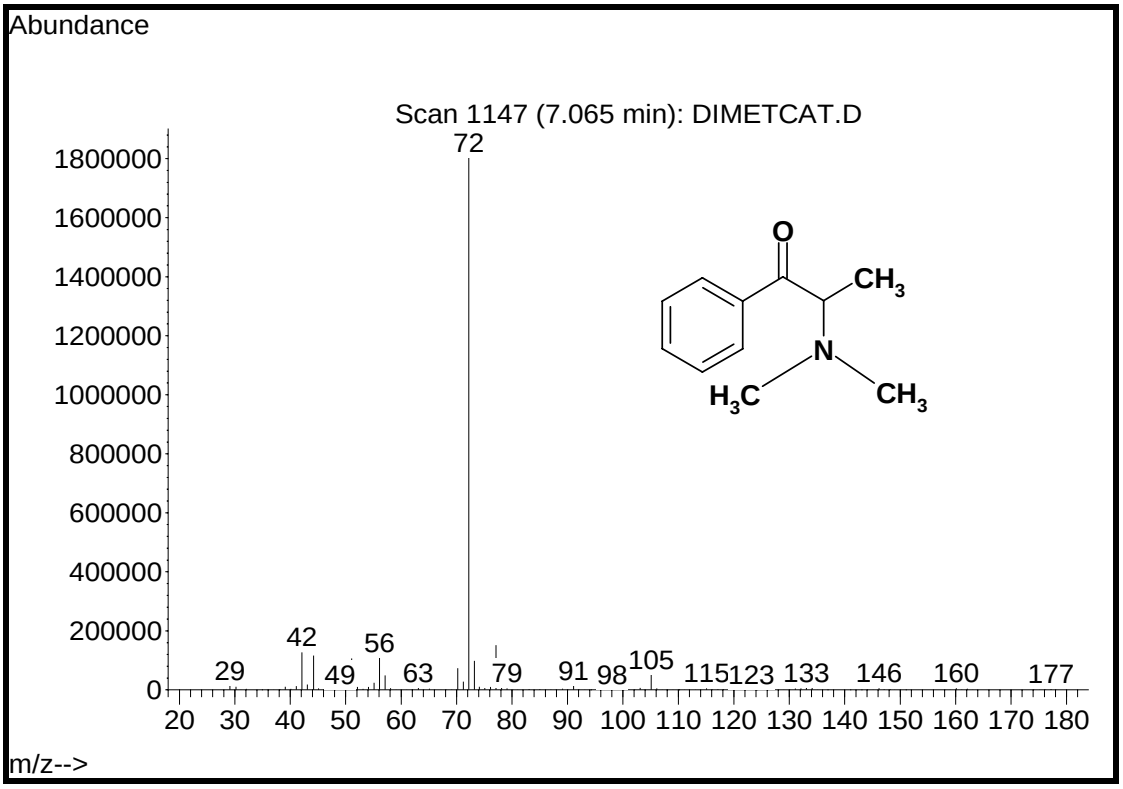

Figure 1a. Mass Spectrum of Dimethylcathinone.

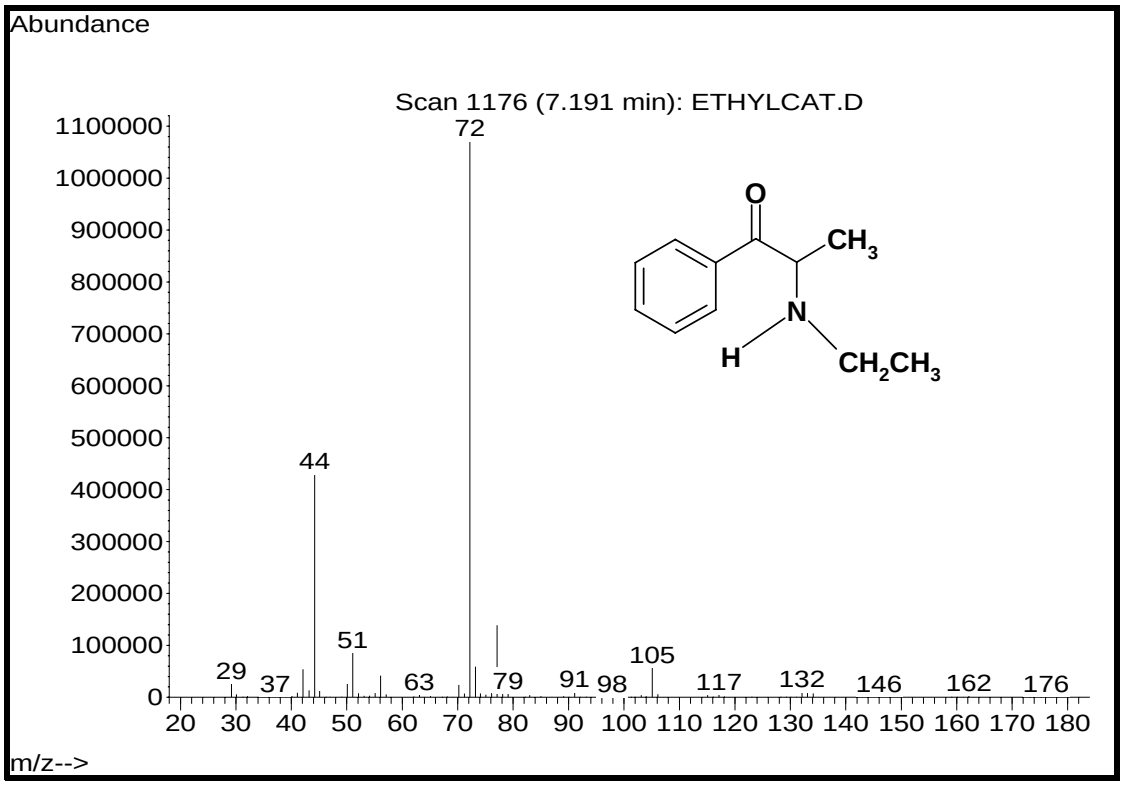

Figure 1b. Mass Spectrum of Ethylcathinone.

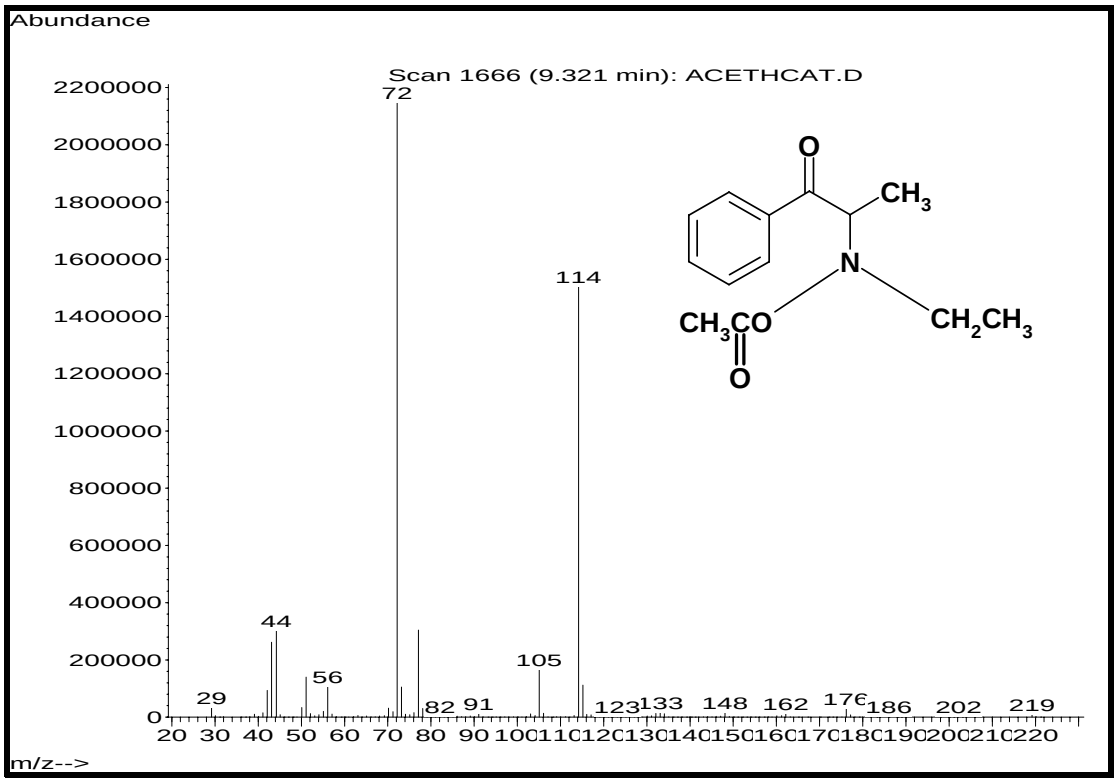

Figure 1c. Mass Spectrum of N-Acetylethylcathinone.

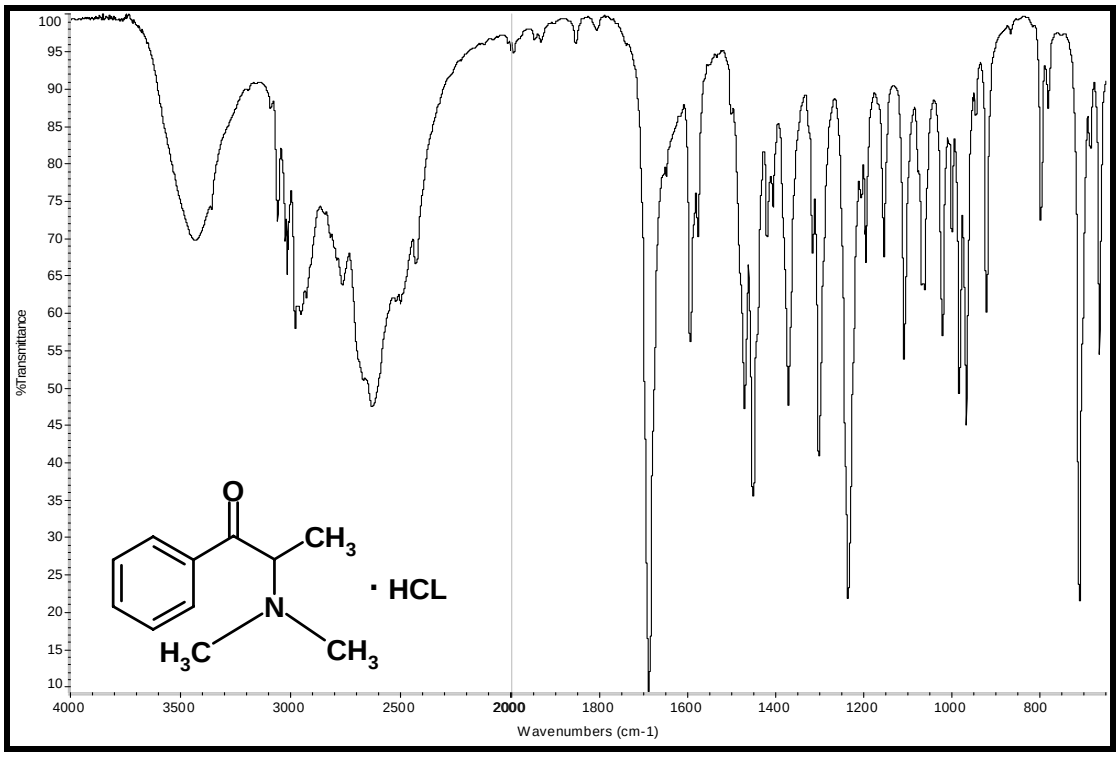

Figure 2a. FTIR of Dimethylcathinone HCl.

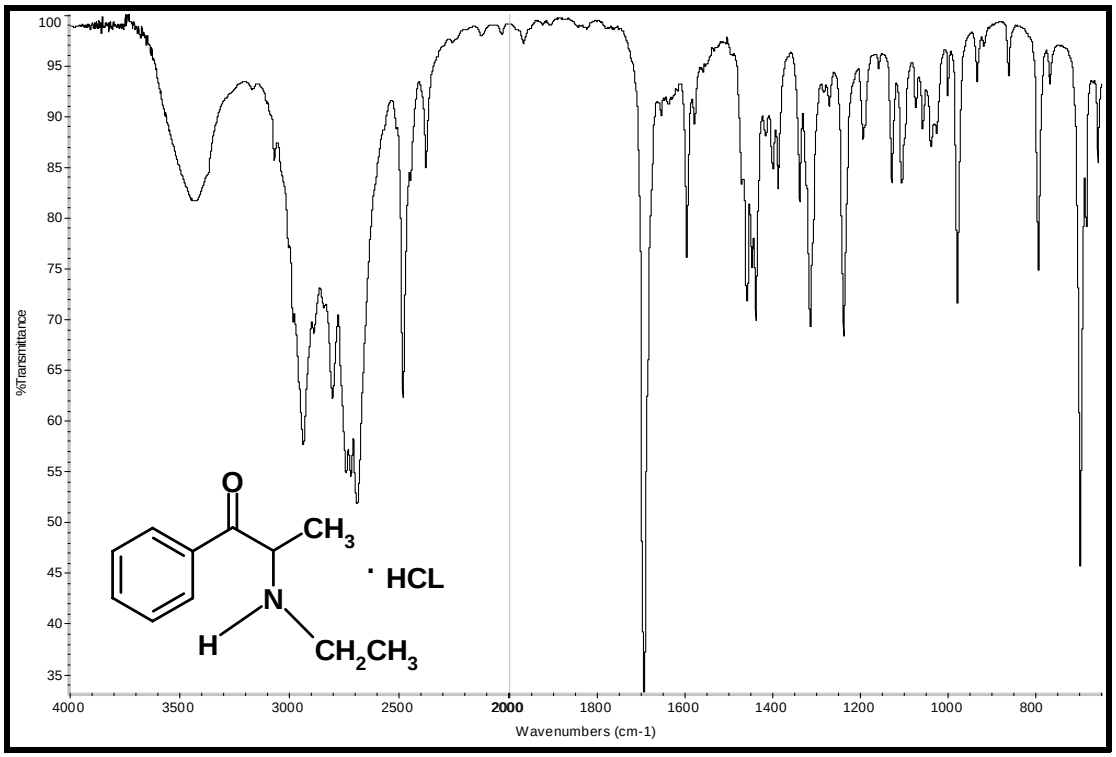

Figure 2b. FTIR of Ethylcathinone HCl.

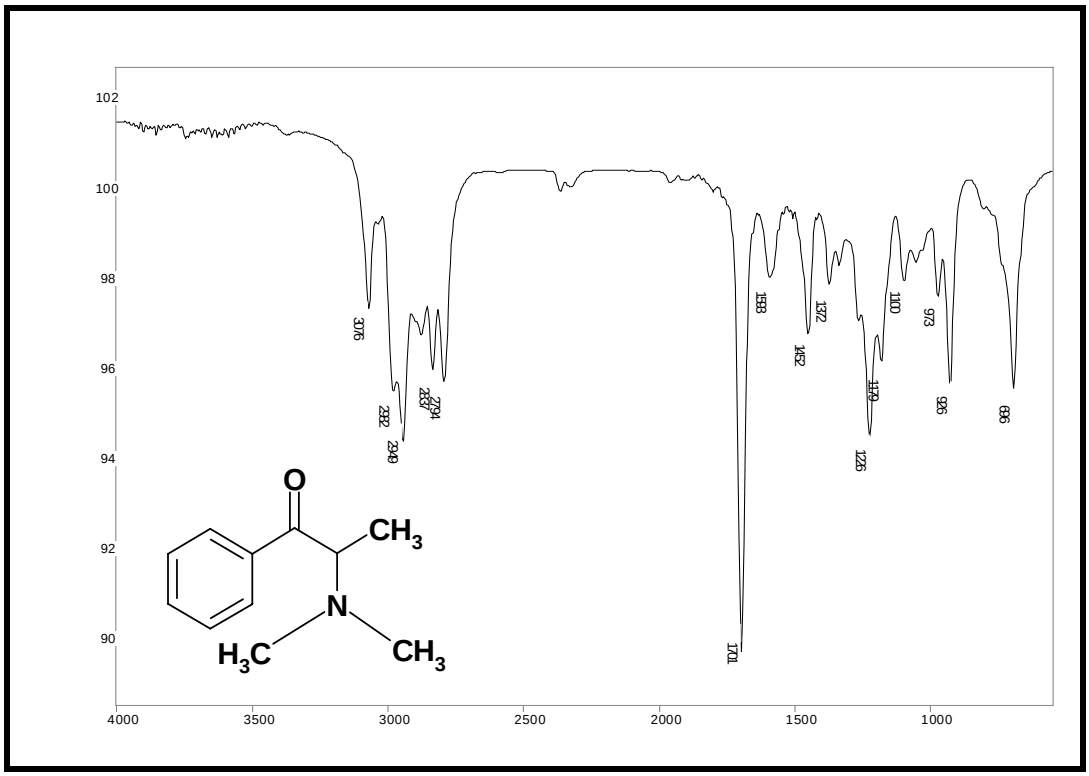

Figure 3a. IRD of Dimethylcathinone.

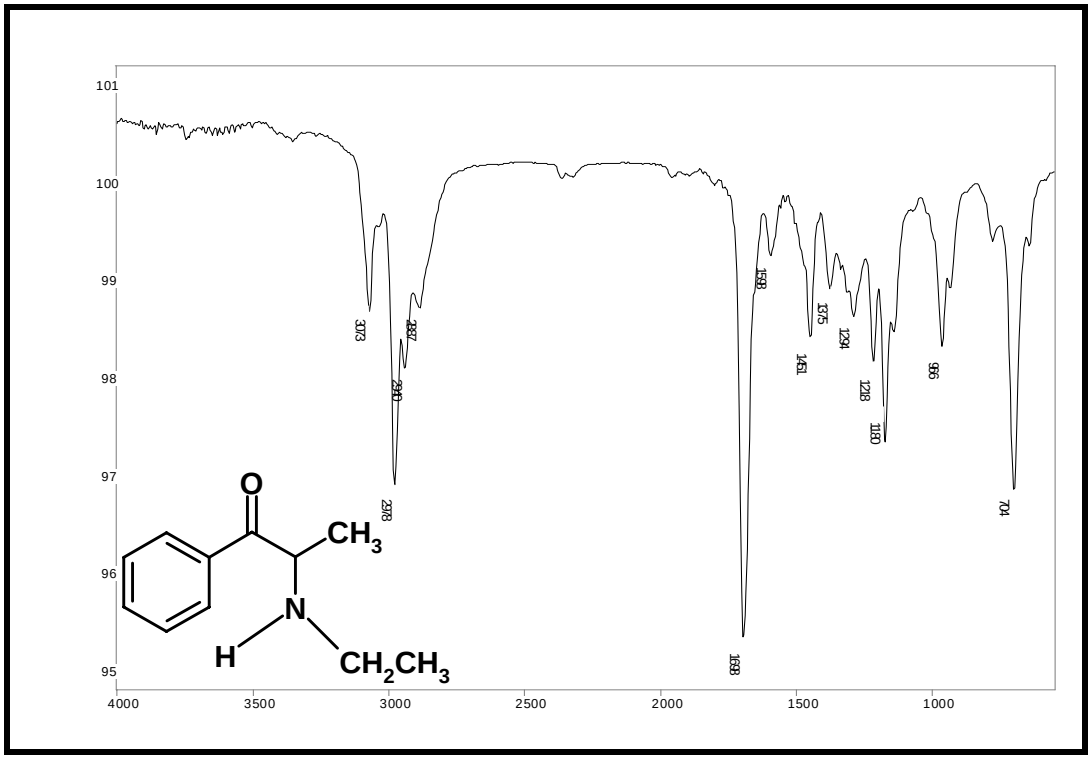

Figure 3b. IRD of Ethylcathinone.

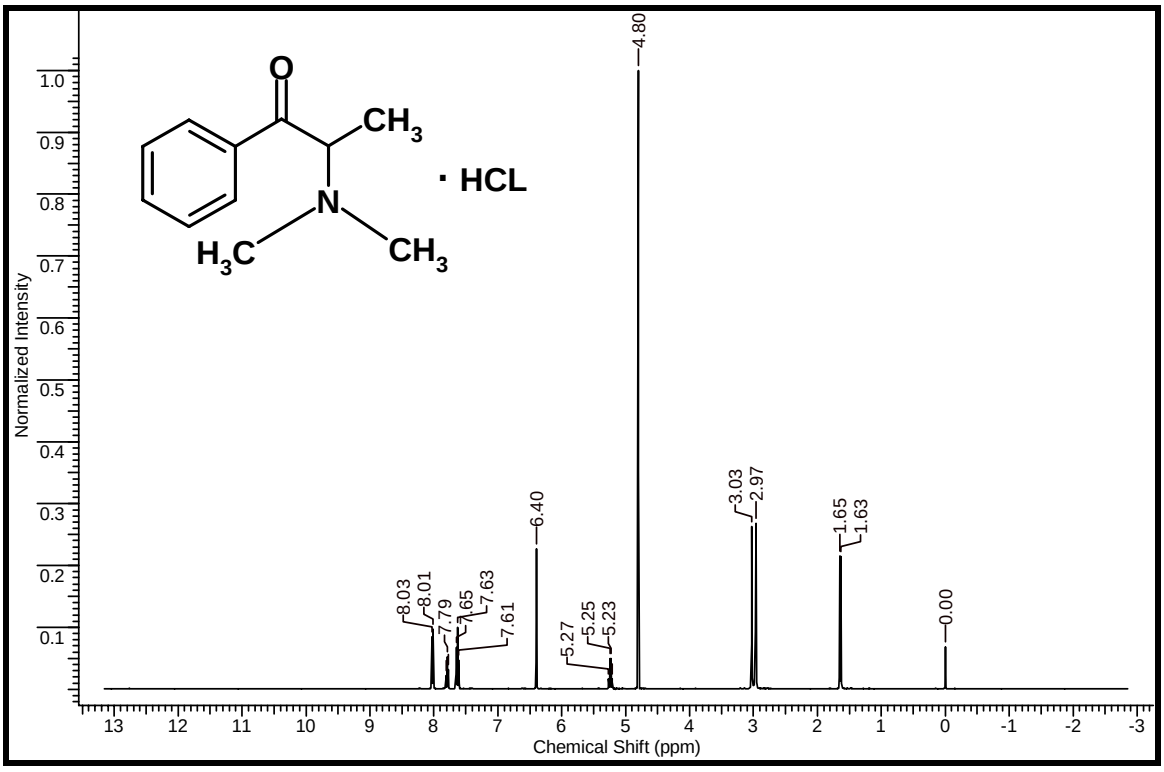

Figure 4a. 400 MHz Proton NMR of Dimethylcathinone HCl in D_2O.

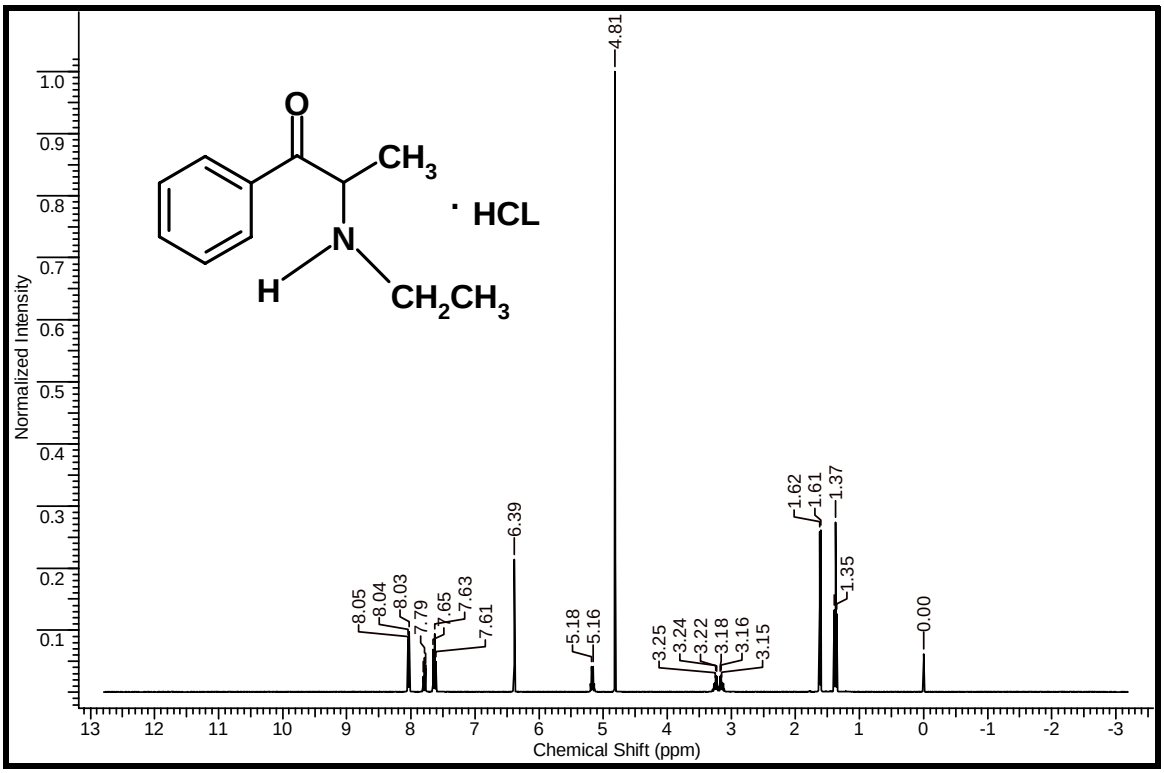

Figure 4b. 400 MHz Proton NMR of Ethylcathinone HCl in D_2O.

Quantitation of the Major Alkaloids in Opium from *Papaver Setigerum* DC

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ABSTRACT: Quantitation of morphine and other major alkaloids in opium gum from specially cultivated *Papaver setigerum* DC ("Wild Poppy") is presented. *Papaver setigerum* plants (n = 14) were grown in an atmosphere containing a slightly elevated level of carbon dioxide (390 ppm). Opium gum collected from the capsules of the mature plants was analyzed for morphine, codeine, thebaine, noscapine, and papaverine, using capillary electrophoresis (CE). Morphine was confirmed at an average of 2 percent by weight. Codeine, noscapine, and papaverine were also detected; however, thebaine was below the limits of quantitation by the employed CE method, and could only be detected by gas chromatography/mass spectrometry.

KEYWORDS: *Papaver setigerum*, *Papaver somniferum*, Opium Poppy, Wild Poppy, Opium, Opium Alkaloids, Quantitation, Forensic Chemistry

Introduction

There are more than one hundred species under the genus *Papaver* that produce alkaloids in the specialized cells called laticifers. However, only two naturally occurring species, *Papaver somniferum* L (Photo 1, next page) and *Papaver setigerum* DC (Photo 2, next page) produce morphine in significant quantities [1]. *Papaver somniferum* is commonly known as opium poppy, and is cultivated around the world for both licit and illicit purposes. *Papaver setigerum*, also known as "wild poppy," is native to the Mediterranean region and Canary Islands. Although several previous reports indicate that *Papaver setigerum* opium contains morphine (*vide infra*), to date it has never been reported to have been used for licit or illicit morphine production.

Scientific publications on *Papaver setigerum* reveal varying opinions amongst scientists in labeling it as a separate species or as the subspecies of *Papaver somniferum*. One of the earliest publications on opium poppies from Fulton [2] suggested a close relationship between *Papaver somniferum* and *Papaver setigerum*. Farmilo *et al.* [3] was the first to report the presence of morphine in the pods, buds, and leaves of *Papaver setigerum*.

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[a] Current Address: Lancaster Laboratories, Inc., 2425 New Holland Pike, Lancaster, PA 17601.

Photo 1. Typical *Papaver Somniferum* Plant. Note the Horizontal Score Marks on the Capsules.

Photo 2. *Papaver Setigerum* Plant Grown for this Study. Note the Smaller, Elongated Capsules (Compare with Photo 1).

Farmilo *et al.* also indicated that the *Papaver setigerum* chromosome count is a tetraploid (n = 22), whereas *Papaver somniferum* is a diploid (n = 11), which suggests that *Papaver somniferum* could not have evolved from *Papaver setigerum* as many scientists had previously suggested. Farmilo's article also compared and contrasted sketches of *Papaver somniferum* and *Papaver setigerum* plants, including drawings of their pods (which differ significantly in appearance). The information supported earlier work by Sugiura [4], who disagreed with the concept of *Papaver setigerum* as a direct ancestor to *Papaver somniferum*, and instead regarded them as separate species, with the possibility of a common ancestor.

One of the earliest (1956) studies on the quantitation of morphine in *Papaver setigerum* opium came from Asahina *et al.*, who reported a morphine content of 5.1% [5]. A detailed publication from the same group in 1957 [6] described two sets of experiments with *Papaver setigerum* plants, reporting morphine values of 5.1% and 7.3% (surprisingly high). The other alkaloids were reported at: Codeine (0.9 and 0.8%); thebaine (2.1 and 1.6%); papaverine (1.9 and 2.6%); and narcotine (also known as noscapine, 0.1 and 0.1%). The paper also reported the relative differences in the alkaloids present in opium from *Papaver somniferum* cultivations in Iran, India, and Turkey versus those present in *Papaver setigerum* opium. A later study from La Valva *et al.* [7] found no morphine, codeine, and thebaine in the *Papaver setigerum* populations from Mediterranean France and southern Italy; however, this latter finding is atypical. Subsequently, Garnock-Jones *et al.* [8] confirmed alkaloids in the air-dried capsules of New Zealand cultivations of *Papaver setigerum* (morphine 0.4%; codeine 0.5%; papaverine 1.6%; narcotine 1.3%; thebaine not reported), but also noted that the alkaloid concentrations were "much lower" in wild plants. Garnock-Jones *et al.* also declared *Papaver setigerum* to be a subspecies of *Papaver somniferum*. Aside from the negative alkaloid values reported by La Valva *et al.*, these reports collectively established *Papaver setigerum* as a separate species (or possibly a rather distantly related subspecies), under the genus *Papaver*, with the potential of producing minor to significant amounts of morphine.

In the era of numerous internet vendors for seeds of both *Papaver somniferum* and *Papaver setigerum*, the forensic science community has a salient interest in the actual alkaloid composition of *Papaver setigerum*, and in the potential use of its opium for illicit purposes. As detailed above, the literature is inconsistent and in some cases is contradictory. The U.S. Controlled Substances Act does not differentiate between species (or subspecies) of *Papaver*. Thus, *Papaver setigerum* is not formally controlled (by name) in the United States; however, opium and related products (such as poppy straw) from *Papaver setigerum* are controlled (Schedule II) if they contain morphine, codeine, and/or thebaine. Of note, opium cultivation laws in many other countries specifically prohibit cultivation of *Papaver setigerum*.

The opium analyzed in this study came from an environmental research project conducted by the U.S. Department of Agriculture, where the primary goal was to study the effects of a slight increase in atmospheric carbon dioxide (CO_2) on *Papaver setigerum* plant growth and alkaloid production [9]. The Mauna Loa Observatory (Hawaii) reports the average ambient atmospheric CO_2 level to be 385 ppm [10]. However, the actual ambient atmospheric CO_2 level is thought to be slightly higher, as was reported by Ziska *et al.* [11]. Therefore, the study was conducted at a 390 ppm CO_2 level, which is believed to provide more valid data on actual alkaloid production in the wild. Fourteen *Papaver setigerum* plants were cultivated in controlled environment grow chambers in an atmosphere containing 390 ppm CO_2. Opium gum was obtained by lancing the mature pods from the plants, in the same manner that opium from *Papaver somniferum* plants is obtained, and was analyzed using a capillary electrophoresis methodology [12].

Experimental

Seeds: Seeds of *Papaver setigerum* DC were obtained from the Institut fur Pflanzengenetik und Kulturpflanzenforschung in Gatersleben, Germany.

Cultivation and Harvesting: The study was conducted using controlled environment chambers (EGC Corporation, Chagrin Falls, OH), with the chamber set at 390 ppm CO_2 for 24 h day-1. The actual average 24 h CO_2 values were 389 +/- 12.1 ppm. The seeds were sown by hand in 2.6 L pots filled with a 4:1:1 mixture of

sphagnum, perlite, and vermiculite. Floral initiation occurred at about 70 days after sowing. There were approximately 8-10 capsules per plant. The scoring of the mature capsules began about two weeks after the loss of the floral petals. Scoring was done using a razor blade, making 2 to 3 one-millimeter deep incisions on the capsule surface. For each capsule, opium gum was collected over a 24 h period on aluminum foil, allowed to air dry for 72 hours, and then weighed. The timing and harvesting techniques match those typically used for *Papaver somniferum*.

Capillary Electrophoresis (CE): Opium alkaloid standards were obtained from the reference collection of the DEA Special Testing and Research Laboratory (Dulles, VA). All CE-grade reagents and run buffer solutions were obtained from Microsolv™ Technology (Eatontown, NJ). High Performance Liquid Chromatography grade methanol was obtained from Burdick and Jackson (Muskegon, MI). High purity, deionized water was obtained from a Millipore Milli-Q-Gradient A10 water system (Bedford, MA). An internal standard stock solution of tetracaine hydrochloride was prepared by weighing 25 mg into a 100 mL volumetric flask and diluted to volume with a 1:11 mixture of methanol and 3.75 mM phosphate buffer (pH 3.2). To obtain the tetracaine internal standard working solution, 6 mL of the tetracaine HCl internal standard stock solution was diluted to volume with 3.75 mM phosphate buffer (pH 3.2) in a 50 mL volumetric flask. Appropriate amounts of morphine, codeine, thebaine, noscapine, and papaverine base standards were weighed into a 100 mL volumetric flask in order to obtain an approximate final concentration of 0.025 mg mL^{-1} for each compound. Ten mL of the internal standard stock solution was pipetted into the above mentioned volumetric flask and diluted to volume with a 1:11 mixture of methanol and 3.75 mM phosphate buffer. Approximately 500 µL of the solution was filtered using 0.45 µm regenerated cellulose Titan filter and transferred to a 1.0 mL polypropylene CE injection vial.

Appropriate amounts of the opium gum samples were weighed into a volumetric flask in order to obtain a concentration of morphine similar to that of the standard. The flask was filled to half volume with methanol and sonicated for 30 minutes at 55^0C to completely extract the alkaloids. The flask was then cooled, and diluted to volume with 3.75 mM phosphate buffer (pH 3.2). 400 µL of the above solution was added to 2.0 mL of the internal standard working solution. Approximately 500 µL of the above solution was filtered using a 0.45 µm regenerated cellulose Titan filter and transferred to a 1.0 mL polypropylene CE injection vial. An Agilent Model HP3DCE capillary electrophoresis system equipped with a diode array detector (Waldbronn, Germany) was used for alkaloid analysis and quantitation, as described by Lurie *et al.* [12]. All experiments were carried out with fused silica 32 cm (23.5 cm to detector window) x 50 µm I.D. pre-made capillaries obtained from Agilent Technologies (Part No: G1600-63211).

Gas Chromatography/Mass Spectrometry (GC/MS): Analyses were conducted using an Agilent (Palo Alto, CA) Model 5973 Quadrupole Mass Selective Detector (MSD) interfaced with an Agilent Model 6890 Gas Chromatograph (GC). The GC contained a J&W Scientific (Rancho Cordova, CA) 30 m x 0.25 mm I.D. fused silica capillary column coated with a film thickness of 0.25 µm DB-1. The injection port was maintained at 280^0C. The oven was programmed with an initial temperature of 90^0C, holding for 2 minutes, then 14^0C per minute increase until 300^0C, holding for another 10 minutes. One mL portions of the methanol extracts of the opium samples were placed into autosampler vials for analysis.

Results and Discussion

Morphine, codeine, noscapine, and papaverine were all detected and quantitated using the CE method described in the Experimental section. Thebaine was detected in the opium using the GC/MS method described in the Experimental section; however, CE quantitation of thebaine was not possible because of its low levels (the TIC indicated thebaine between 0.01 and 0.3%). The low thebaine content was unexpected in view of the value previously reported by Asahina *et al.* [6]. The quantitation results for morphine, codeine, noscapine, and papaverine for the opium samples collected from all 14 plants are presented in Table 1. The amounts of opium obtained from each plant are also presented in Table 1. The average levels were 2% morphine, 3% codeine, 10% noscapine, and 5% papaverine, all by weight of opium. The noscapine quant was stunningly high (5x) relative to

the morphine content, and interestingly, is very similar (on a weight percent basis) to the noscapine content of opium from *Papaver somniferum* cultivations in South and Central America [12].

Studies at the Special Testing and Research Laboratory have shown that the morphine content in *Papaver somniferum* opium varies among growing regions. However, on average, *Papaver somniferum* opium contains about 10 - 13% morphine [13]. These latter results do not derive from controlled cultivations; however, as noted above, it is assumed that these plants were grown at an average ground level CO_2 level of 390 ppm. The electropherograms of typical *Papaver setigerum* and *Papaver somniferum* opiums are shown in Figure 1.

Based on the results of this study, although *Papaver setigerum* opium contains some morphine, it is not a viable source of opium for illicit poppy cultivators. Cultivation and the manual harvesting of opium poppy capsules by lancing and subsequent hand-collection are very time-consuming and labor-intensive processes. *Papaver setigerum* plants are much smaller in size compared to *Papaver somniferum* plants; furthermore, the capsules in *Papaver setigerum* are also very small and elongated in shape (between 10 - 16 millimeters in length and between 4 - 7 millimeters in diameter). In contrast, *Papaver somniferum* capsules are more or less globular in shape and much larger in size (typically between 20 and 40 millimeters in diameter). For these reasons, the total amount of opium that can be obtained from a field of *Papaver setigerum* poppies is much lower than from an equal sized field of *Papaver somniferum* poppies. The much lower morphine content, much lower total opium yield, and the increased time and labor needed to harvest the opium from the small, elongated pods, all explain why *Papaver setigerum* has never received much attention in the areas of either licit or illicit cultivation and morphine production. Based on this study, it would appear that this disinterest is justified.

Acknowledgements

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13. From the compiled opium data obtained from the analyses of thousands of opium samples at the DEA Special Testing and Research Laboratory (Dulles, VA).

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Table 1. Alkaloid Composition of *Papaver setigerum* Opium
(Note: Thebaine was Detected, not Quantified).

Plant Number	Weight of opium obtained from each plant (mg)	Morphine%	Codeine%	Papaverine%	Noscapine%
1	38.1	2.5	3.0	5.2	11.4
2	24.1	2.4	2.9	5.0	10.9
3	38.8	2.3	2.7	4.7	10.3
4	27.6	2.5	2.2	4.8	9.8
5	35.2	3.1	1.8	5.1	10.4
6	34.2	2.1	2.9	5.4	10.8
7	82.6	2.3	2.0	5.5	11.2
8	52.3	2.4	3.0	4.4	9.8
9	67.9	2.3	2.1	5.5	10.5
10	23.1	1.4	3.5	4.6	10.9
11	27.6	2.5	2.6	4.2	9.6
12	21.4	2.0	3.2	4.9	10.1
13	16.9	2.8	2.7	3.3	8.5
14	19.8	1.7	2.4	3.8	9.0
Average	**36.4**	**2.3**	**2.6**	**4.7**	**10.2**
Std Dev	19.132	0.427	0.495	0.660	0.832
Min		1.4	1.8	3.3	8.5
Max		3.1	3.5	5.5	11.4

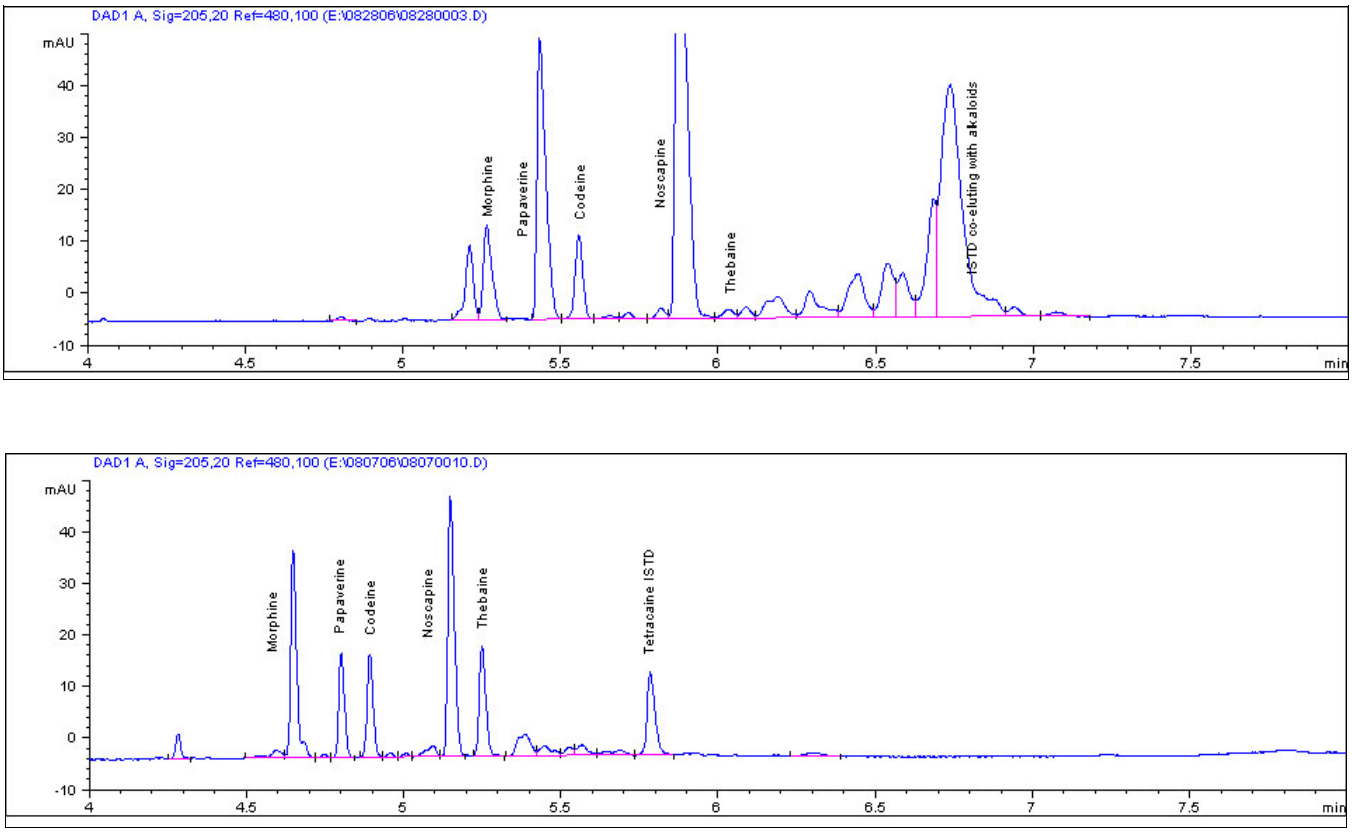

Figure 1. Electropherograms of Typical *Papaver setigerum* (Top Trace) and *Papaver somniferum* (Bottom Trace) Opiums. Time (x-axis) Differs for the Two Runs Because They Were Completed on Distantly Removed Dates (the older the capillary, the longer the retention times; however, the retention order and relative retention times are consistent). The *Papaver somniferum* Opium Electropherogram Presents Data from Opium Poppies Recently Grown in Afghanistan.

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Technical Note

Analysis of Fatty Acids in Marijuana (*Cannabis Sativa* Leaf)

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ABSTRACT: Various fatty acids (palmitic, myristic, oleic, and stearic acids) were identified in 20 marijuana (cannabis leaf) samples recently seized on the illicit market in Rome, Italy. Samples were analyzed by gas chromatography/mass spectrometry to determine delta-9-tetrahydrocannabinol, other minor cannabinoid congeners, and fatty acids. Although cannabis seeds and the oil derived from those seeds are known to be rich in fatty acids, this is believed to be the first study demonstrating the presence of fatty acids in marijuana. The potential value of the results in source determination and comparative analyses is discussed.

KEYWORDS: Marijuana, Fatty Acids, Myristic Acid, Palmitic Acid, Oleic Acid, Stearic Acid, Analysis, GC/MS, Forensic Chemistry

Introduction

Cannabis preparations, especially marijuana (cannabis leaf), are the most widely abused illicit drugs in the world. Because of the significant economic and social impact associated with abuse of marijuana and related products, extensive effort is expended to monitor their production, trafficking, and use. These efforts include source determination (i.e., geographic origin) [e.g., 1-4] and comparative analysis (i.e., sample - sample comparisons) [e.g., 5-8]. Approaches have included classic impurity profiling (including cannabinoid quants and ratios), various DNA-based analyses, and isotope ratio analyses (primarily based on $\delta^{13}C$ and $\delta^{15}N$) [e.g., 9].

Because hemp fiber, seeds, and seed oils all have potential economic value, analysis of *Cannabis sativa* has not been limited to leaf or leaf-derived products. One sub-topic of interest is the presence of fatty acids in the seeds and fruits of cannabis [10-13]. The seeds and seed oils of cannabis contain a wide variety of fatty acids in economically viable amounts. Samir *et al.* [12] reported the concentration of fatty acids and the relative percentage of unsaturated and saturated fatty acids in a number of different samples of cannabis seeds, and noted that climate and growing conditions seemed to influence the composition of these compounds in the different samples. Furthermore, Bagci *et al.* [13] showed that high amounts of individual fatty acids, along with various minor components (tocopherol and tocotrienols) was useful in assessing chemotaxonomic relationships among different varieties of cannabis. Collectively, these results suggest that fatty acids may be of value in source determination and/or comparative analysis of cannabis seeds or seed oils.

However, there do not appear to be any studies reporting the presence of fatty acids in marijuana itself (i.e., cannabis leaf). In this study, a simple gas chromatography/mass spectrometry (GC/MS) method was developed for the determination of fatty acids in marijuana, and was successfully demonstrated on 20 samples seized in Rome, Italy. The technique is sensitive and accurate. The potential value of the results in source determination and comparative analyses is discussed.

Experimental

Materials and Methods: All chemical and reagents employed were of analytical grade. Fatty acid standards were purchased from Sigma-Aldrich. A mixture of the standards, each at a concentration of 0.1 mg/mL, was used for method development. Twenty marijuana samples from the illicit Roman market were analyzed. The samples were stored in the dark in a dry-box prior to analysis. Individual samples (100 milligrams) were extracted with 0.5 mL of chloroform solution at room temperature, and an aliquot injected into the GC/MS.

Instrumentation: GC/MS analyses were performed on a Model Focus-HP Gas Chromatograph fitted with a split-splitless injector (270^{O}C) equipped with a HP-1 capillary column (12 m x 0.2 mm I.D.) coated with 0.3 μm thickness of methylsilicone. The temperature program ramped from 70^{O}C to 280^{O}C at 10^{O}C/min, with a 5 min final hold. Helium was employed as the carrier gas, at a column head pressure of 10 psi. The GC was connected to an HP 5971A Mass Analyzer operating at 70 eV EI over 40-500 a.m.u. in selected ion monitoring (SIM) mode.

Results and Discussion

Cannabis seeds and seed oils have been shown to contain up to 20 fatty acids [10-13]. Analysis of the 20 marijuana samples selected for this study confirmed the (varying) presence of myristic, palmitic, oleic, and/or stearic acids (see Tables 1-3 and Figures 1-4). Table 1 presents the IUPAC names and formulas for the respective acids. Table 2 presents the respective retention times and the ions selected for SIM analysis. Table 3 presents the results by sample (ratio'd against the combined cannabidiol (CBD) and cannabinol (CBN) content). Figures 1-4 display typical SIM chromatograms of the respective acids.

The acids varied dramatically by sample. All four acids were present in only 10 of the 20 samples (1,2,4,5,10,11,13,15,16, and 17). Three acids (myristic, palmitic, and stearic) were present in samples 3 and 18; two acids (myristic and palmitic) were present in samples 6,7,12, and 19; only one acid (oleic) was present in samples 9,14 and 20; and finally, no fatty acids were detected in sample 8. The geographic origin for samples 6 and 7 was alleged to be the Netherlands, and in fact those samples had similar delta-9-tetrahydrocannabinol contents (about 1%) and similar impurity profiles, and were also similar in their fatty acid profile (but with only two acids present and with actual origin unknown, the results are interesting but of only curiosity value). Nonetheless, these results suggest that the fatty acid profile of marijuana may be useful for comparative analyses (sample - sample comparisons). The analysis is easy, quick, and sufficiently sensitive for small sample amounts. Although unlikely [12], the method may also be useful for source determination; however, such an advance would require a much larger database of authentics (samples of known origin).

Additional research is planned for more sensitive determination of fatty acids in marijuana by derivatizing the acids prior to analysis. The results will be the subject of a future report.

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Table 1. IUPAC Names and Formulas.

Common Name	Chemical Name	Formula
Myristic Acid	Tetradecanoic Acid	$C_{14}H_{30}O_2$
Palmitic Acid	Hexadecanoic Acid	$C_{16}H_{34}O_2$
Oleic Acid	9-Octadecenoic Acid[a]	$C_{18}H_{36}O_2$
Stearic Acid	Octadecanoic Acid	$C_{18}H_{38}O_2$

[a] Geometric Isomer (*cis* or *trans*) not determined.

Table 2. Retention Times and Ions Chosen for Selected Ion Monitoring (SIM) Analysis.

SUBSTANCE	*R.T.*	*TARGET ION*
Myristic acid	8:06	228-185
Palmitic acid	10:06	256-213
Oleic acid	11:19	264-282
Stearic acid	11:34	284-241

Table 3. Results Obtained for the Marijuana Samples Analyzed in GC/MS.

SAMPLE	M/(CBD+CBN)	P/(CBD+CBN)	O/(CBD+CBN)	S/(CBD+CBN)
1	0.02	0.30	0.01	0.01
2	0.01	0.50	0.04	0.09
3	0.05	0.20	N	0.02
4	0.05	0.30	0.01	0.01
5	0.03	0.20	0.01	0.03
6	0.02	0.78	N	N
7	0.03	0.8	N	N
8	N	N	N	N
9	N	N	0.01	N
10	0.06	0.20	0.04	0.03
11	0.01	0.48	0.03	0.08
12	0.03	0.70	N	N
13	0.05	0.18	0.03	0.03
14	N	N	0.01	N
15	0.02	0.28	0.01	0.01
16	0.05	0.20	0.04	0.02
17	0.03	0.18	0.01	0.02
18	0.05	0.15	N	0.01
19	0.02	0.6	N	N
20	N	N	0.02	N

M = Myristic Acid
P = Palmitic Acid
O = Oleic Acid
S = Stearic Acid
CBD = Cannabidiol
CBN = Cannabinol
N = None Detected

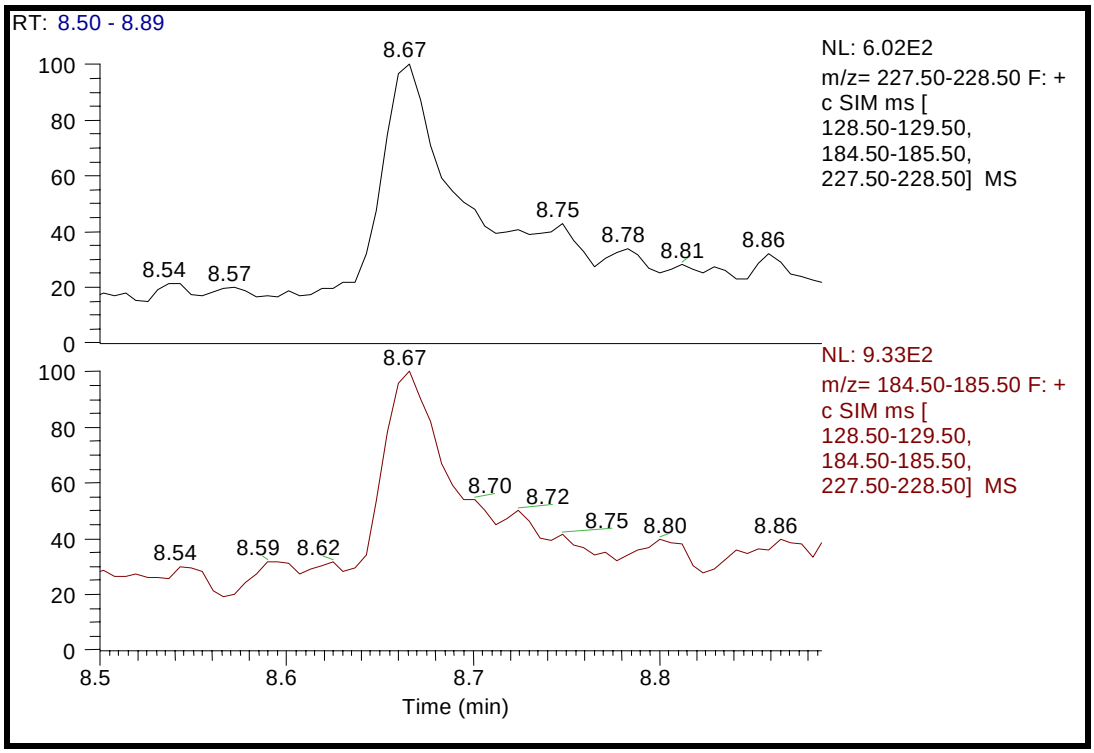

Figure 1. Myristic Acid (GC/MS Analysis in SIM Mode).

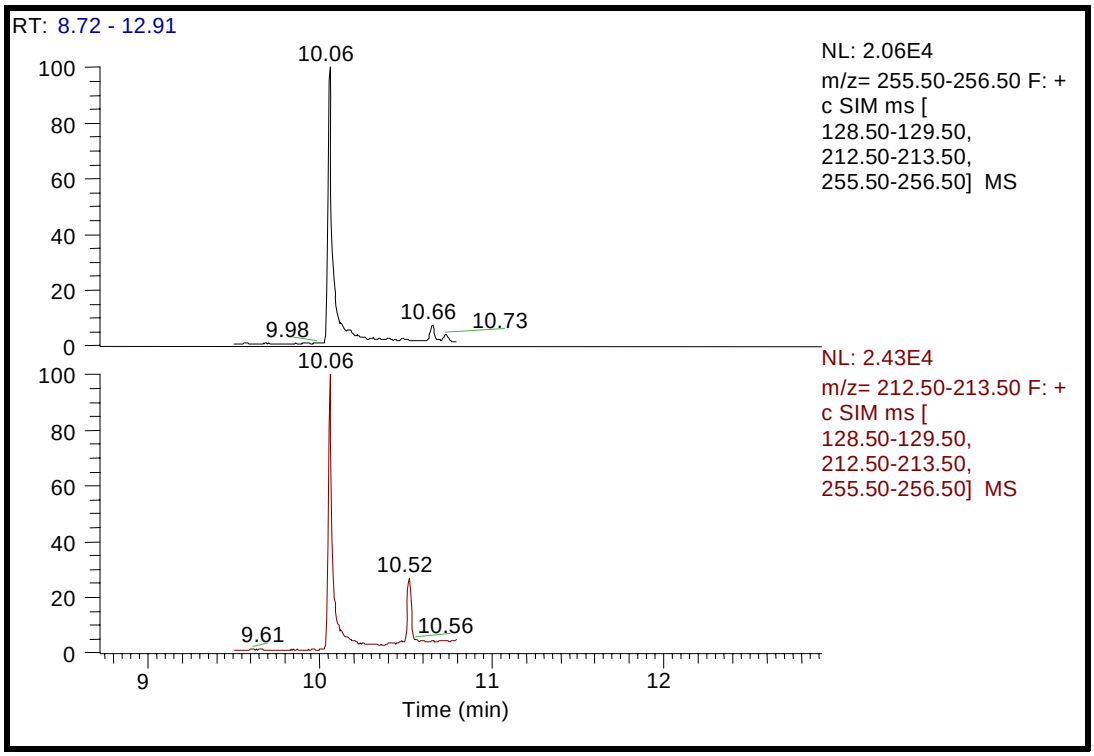

Figure 2. Palmitic Acid (GC/MS Analysis in SIM Mode).

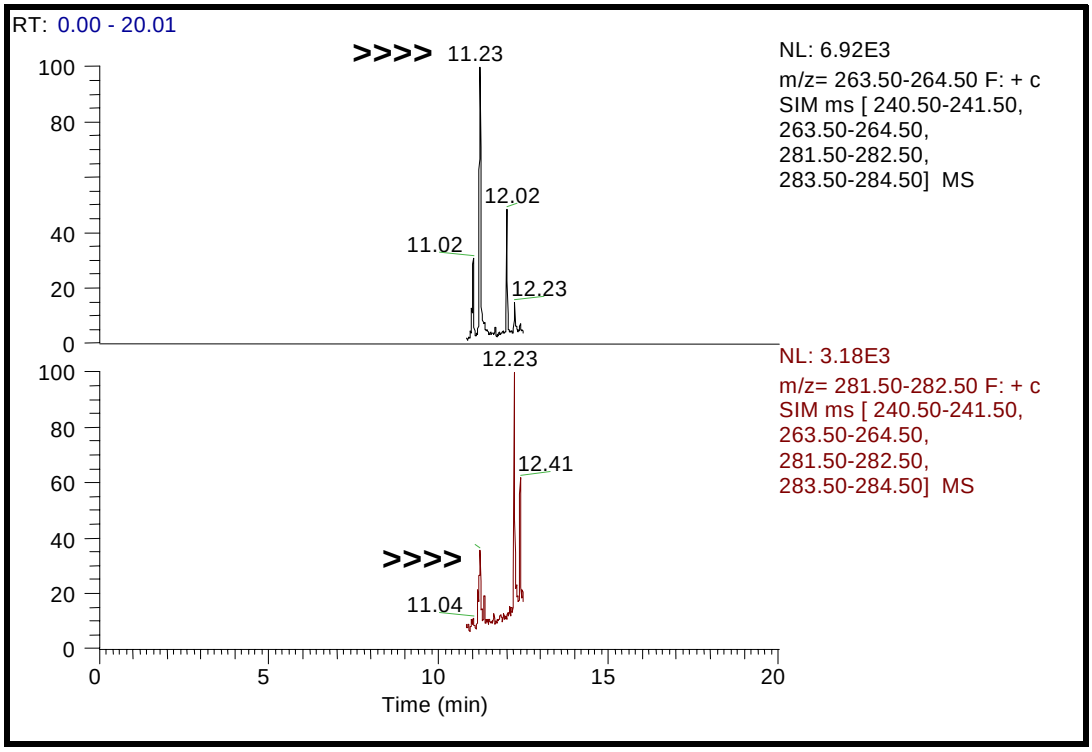

Figure 3. Oleic Acid (GC/MS Analysis in SIM Mode).

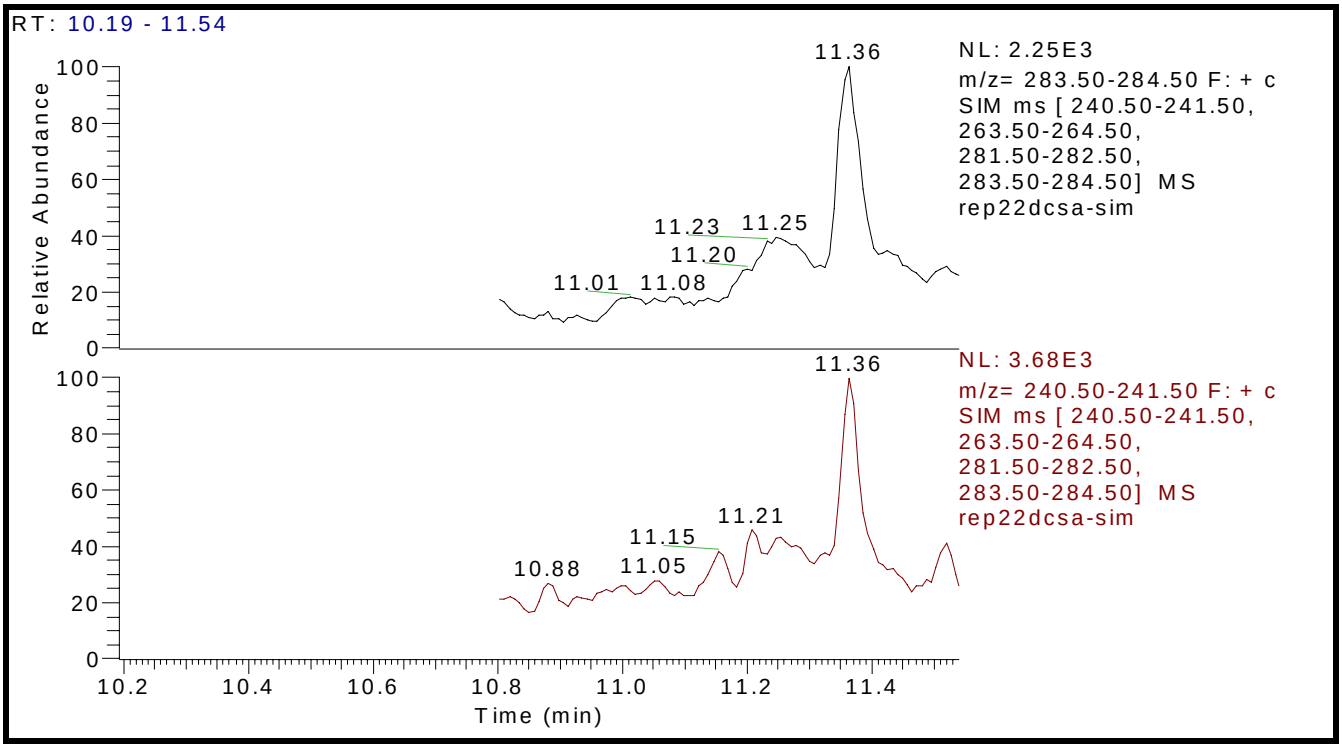

Figure 4. Stearic Acid (GC/MS Analysis in SIM Mode).

Technical Note

The Characterization of Three FLY Compounds (2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY)

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ABSTRACT: The analysis and characterization of 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-*b*;4,5-*b'*]-difuran-4-yl)-2-aminoethane hydrochloride (2C-B-FLY), 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-*b*;4,5-*b'*]-difuran-4-yl)-2-aminopropane hydrochloride (3C-B-FLY), and 1-(8-bromobenzo[1,2-*b*;4,5-*b'*]difuran-4-yl)-2-aminopropane hydrochloride (Bromo-DragonFLY) are presented. Gas chromatography/mass spectra (GC/MS), gas chromatography/infrared spectra (GC/IRD), and solid phase Fourier transform infrared (FTIR) spectra are presented.

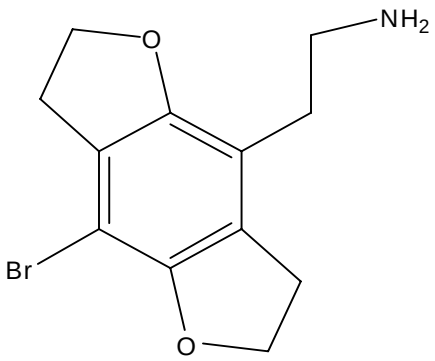
KEYWORDS: 2C-B-FLY, 3C-B-FLY, Bromo-DragonFLY, Phenethylamines, GC/MS, GC/IRD, FTIR, Forensic Chemistry

Introduction

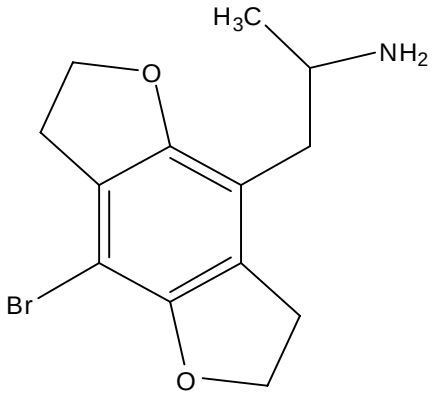
A large number of phenethylamine derivatives are abused for their hallucinogenic properties [1,2]. Recent submissions to various crime laboratories [e.g., 3,4], as well as commentary on various Internet websites and similar venues that are dedicated to drug abuse, indicate an increasing interest in a specific group of phenethylamine analogs referred to as the FLY compounds. Three of the better known FLY compounds are: 1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-*b*;4,5-*b'*]difuran-4-yl)-2-aminoethane hydrochloride (2C-B-FLY), 1-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-*b*;4,5-*b'*]difuran-4-yl)-2-aminopropane hydrochloride (3C-B-FLY, sometimes referred to as Bromo-FLY), and 1-(8-bromobenzo[1,2-*b*;4,5-*b'*]difuran-4-yl)-2-aminopropane hydrochloride (Bromo-DragonFLY) (see Figure 1, next page). Several other FLY compounds are known but are more obscure. The "FLY" designation allegedly derives from the two "wing-like" furan or dihydrofuran rings that are fused on the opposite sides of the central benzene ring, giving an insect-like appearance with the bromo substituent as the head and the ethylamine or isopropylamine substituent as the tail.

At present (early 2008), none of the above FLY compounds are formally controlled in the United States. However, their core structures are highly similar to the Schedule I hallucinogens 4-bromo-2,5-dimethoxy-phenethylamine (2C-B) and 4-bromo-2,5-dimethoxyamphetamine (DOB). For this reason, they could potentially be prosecuted under the tenets of the Controlled Substances Analogue Enforcement Act.

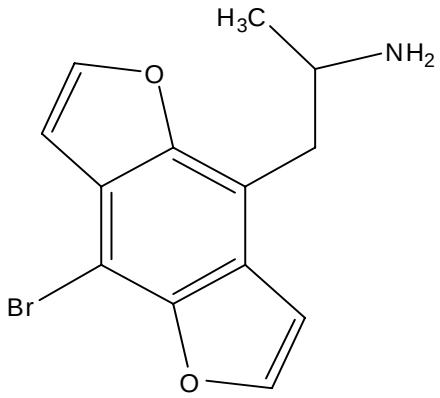
Due to the scarcity of known standards and consequent lack of instrumental data, the identification of these compounds has been hindered. In order to address this issue, mass spectra, gas phase FTIR spectra, and solid phase FTIR spectra are presented for 2C-B-FLY 3C-B-FLY, and Bromo-DragonFLY.



2C-B-FLY



3C-B-FLY



Bromo-DragonFLY

Figure 1. Structures of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY

Experimental

Standards: 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY standards were synthesized and provided by the Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences at Purdue University [5,6].

Gas Chromatography/Mass Spectrometry (GC/MS): Spectra were acquired using an Agilent Model 6890N GC equipped with an Agilent Model 5973 quadrupole mass-selective detector (MSD). The MSD was operated using 70 eV E.I. ionization. The GC was fitted with a 30 m x 0.25 mm I.D. fused silica capillary column coated with 0.52 µm 5% phenylmethyl siloxane (HP-5MS), and was operated using a 50:1 split ratio. The injector port was maintained at 250ºC. The oven temperature program was as follows: Initial temperature 200ºC (2 minutes), ramped to 280ºC at 20ºC per minute (final hold 14 minutes). Helium was used as a purge gas at a rate of 39 cm/second.

Gas Chromatography/Infrared Spectroscopy (GC/IRD): Spectra were acquired using an Agilent Model 6890N GC interfaced with a BioRad Infrared Detector II. The GC was fitted with a 30 m x 0.32 mm I.D. fused silica capillary column coated with 0.52 µm 5% phenylmethyl siloxane (HP-5), and was operated in splitless mode. The injector port temperature was maintained at 250ºC. The oven temperature program was as follows: Initial temperature 55ºC (1 minute), ramped to 275ºC at 25ºC per minute (final hold 6 minutes). The flow cell and transfer line were maintained at 300ºC. Helium was used as a carrier gas at a flow rate of 2 mL/minute.

Fourier Transform Infrared Spectroscopy (FTIR-ATR): Spectra were acquired using a Perkin Elmer Spectrum One Spectrophotometer with a universal attenuated total reflectance (UATR) accessory. Spectra were collected using 4 scans between 4000 cm^{-1} and 500 cm^{-1}.

Results and Discussion

The FLY compounds are alleged to be potent hallucinogens, and they and various other hallucinogenic phenethylamines and tryptamines are often represented to be LSD. The FLY compounds have been submitted to forensic laboratories both in liquid form and on blotter paper. The mass spectra of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY are presented in Figures 2-4. Unlike many simpler phenethylamines, the mass spectrum of each compound displayed a molecular ion peak. Also present are the fragmentation patterns which are characteristic of naturally occurring bromine isotopes. For each compound, *alpha* cleavage is responsible for the base peak. For this reason it is important to ensure that data is collected at a mass range with a minimum below *m/z* = 30. The gas and solid phase FTIR spectra of 2C-B-FLY, 3C-B-FLY, and Bromo-DragonFLY are presented in Figures 5-7 and 7-10, respectively. [Note: References are listed on page 33.]

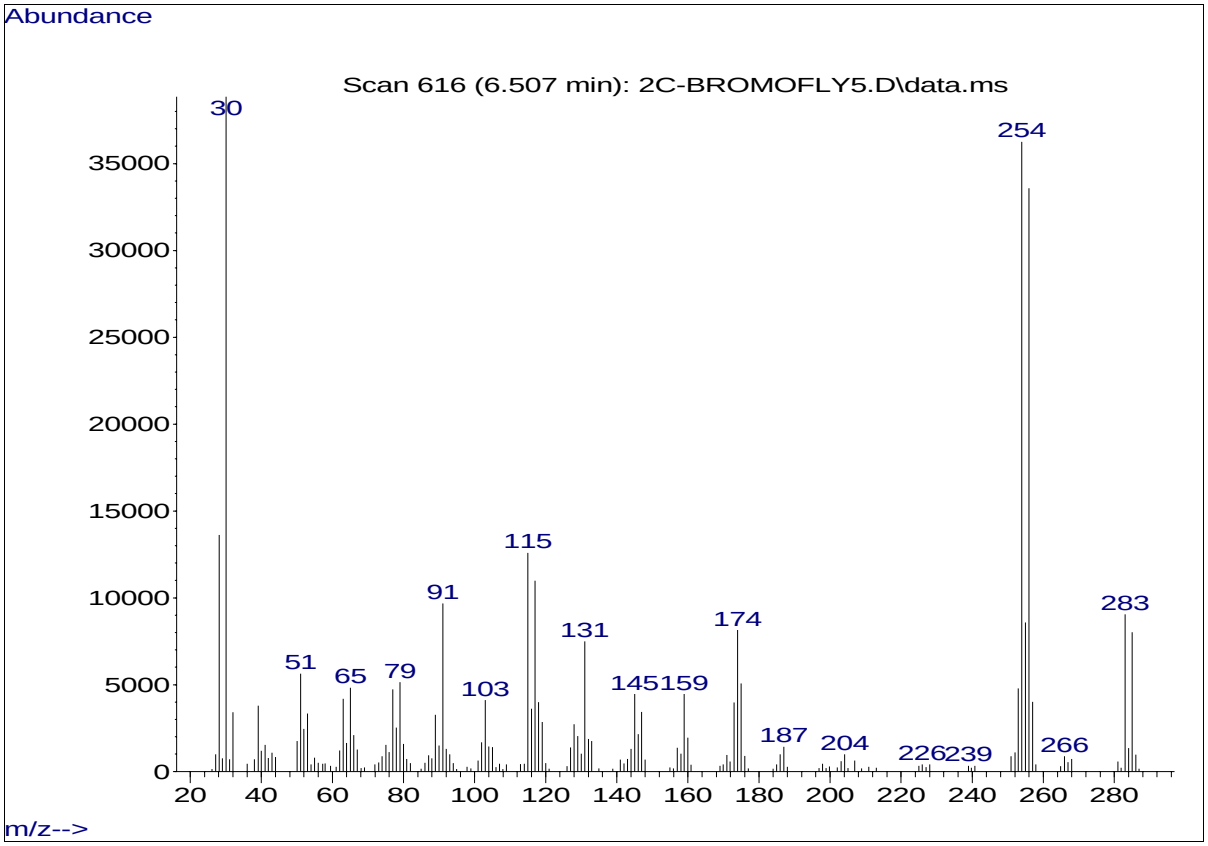


Figure 2. Mass Spectrum of 2C-B-FLY.

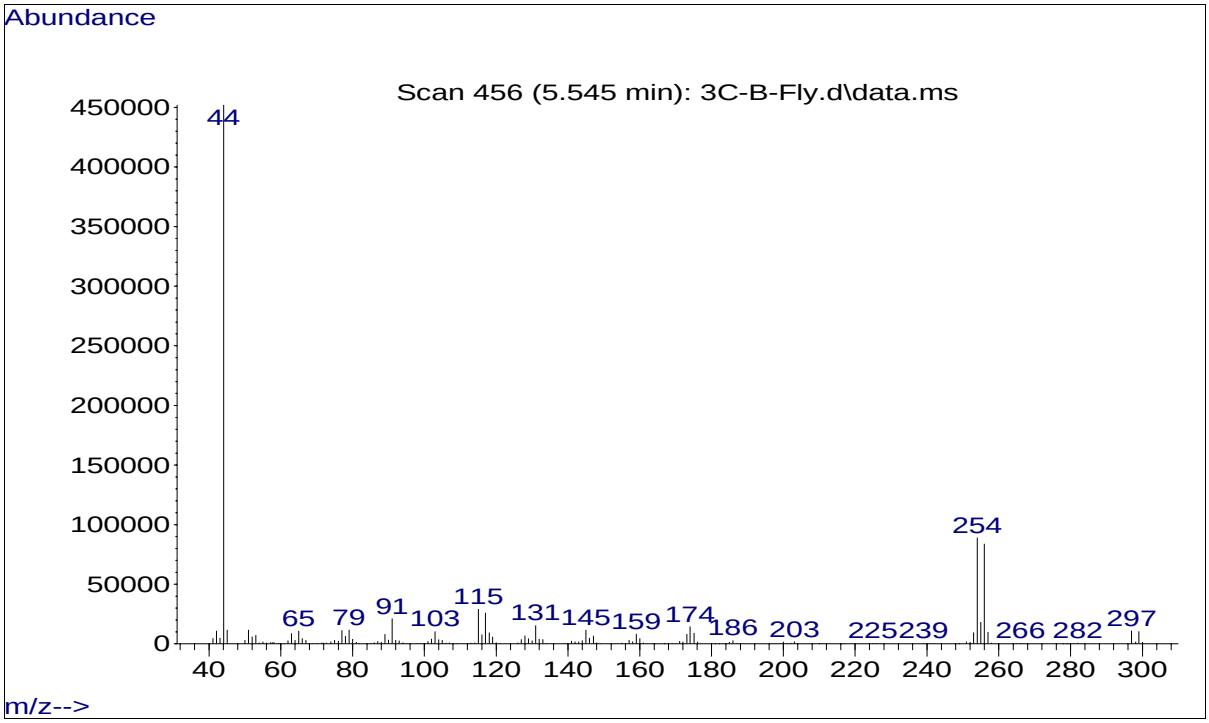


Figure 3a. Mass Spectrum of 3C-B-Fly (Bromo-Fly).

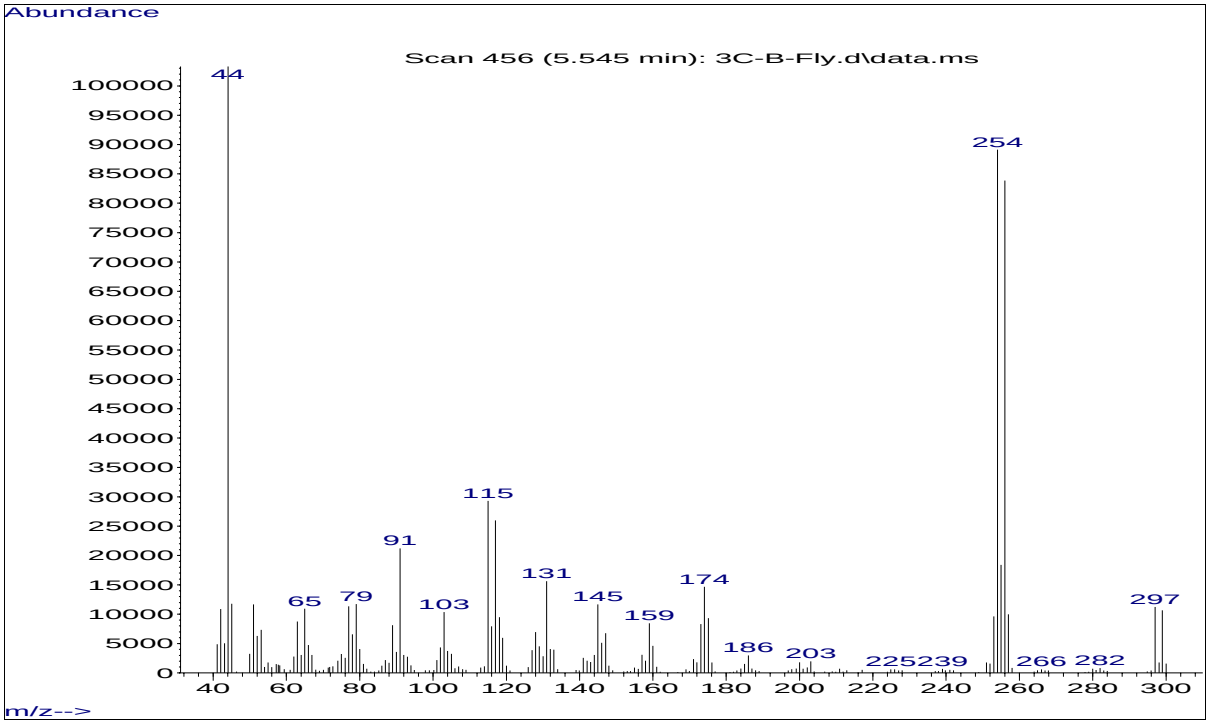


Figure 3b. Mass Spectrum of 3C-B-FLY (Bromo-FLY), Normalized to the 254 ion.

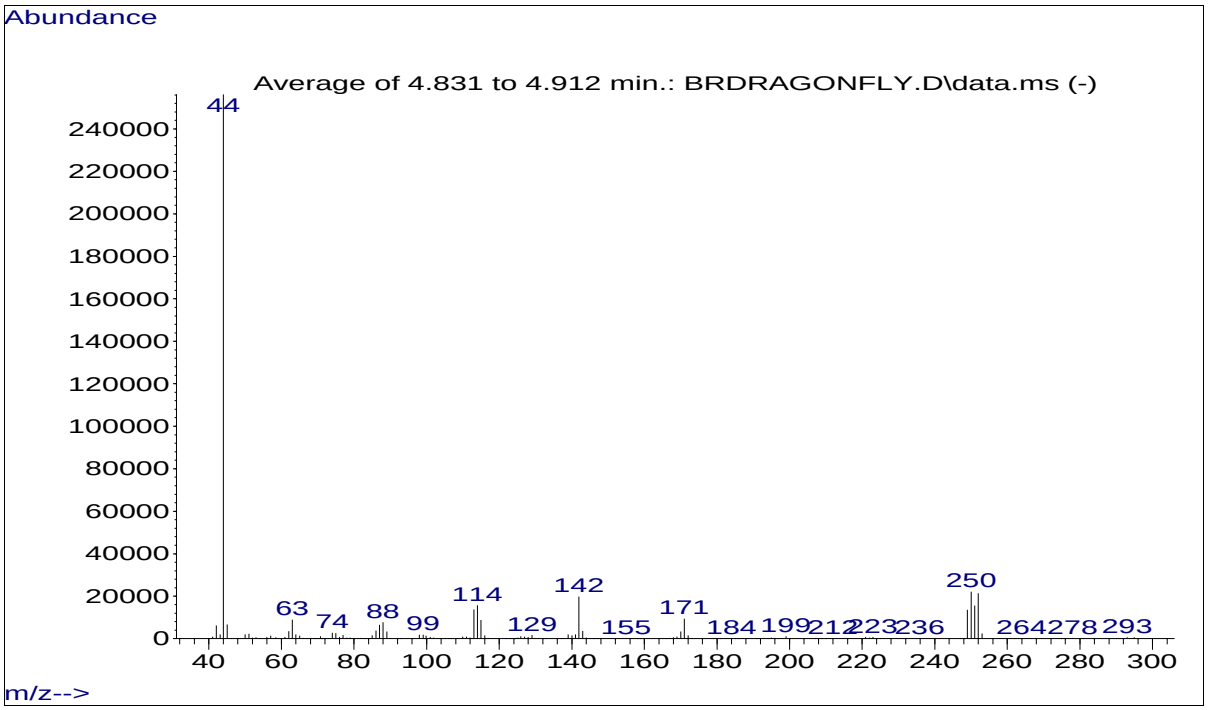


Figure 4a. Mass Spectrum of Bromo-DragonFLY.

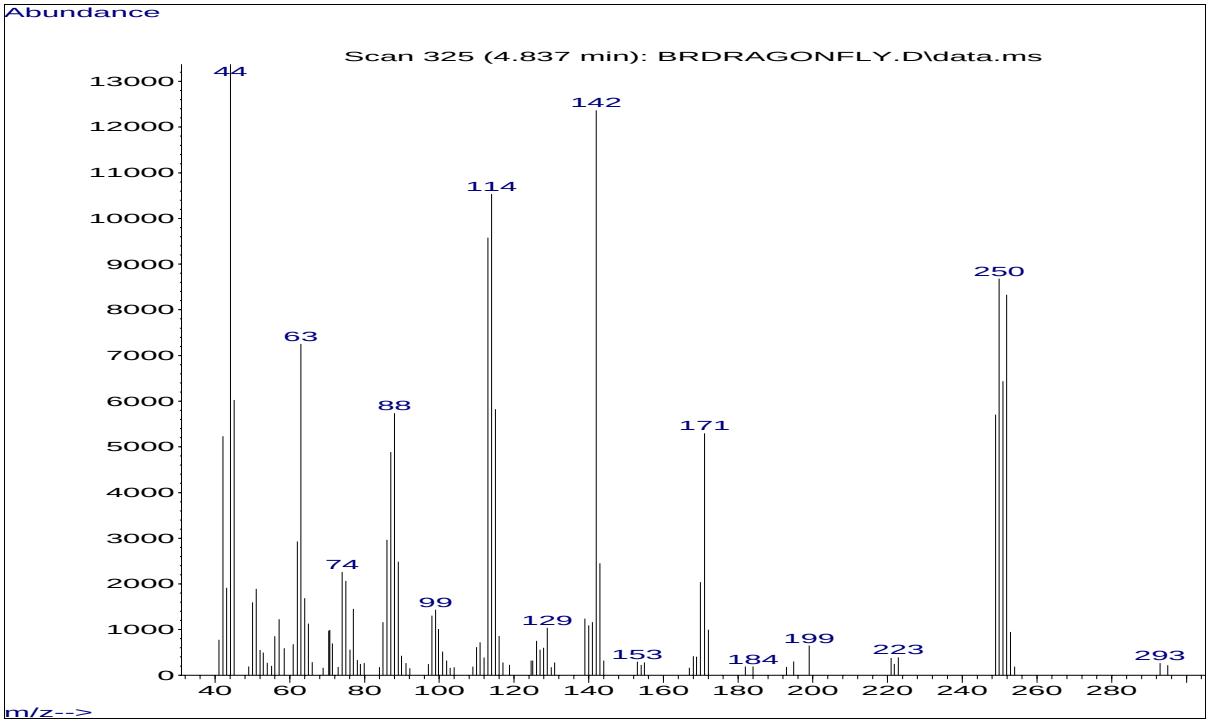


Figure 4b. Mass Spectrum of Bromo-DragonFLY, Normalized to the 142 Ion.

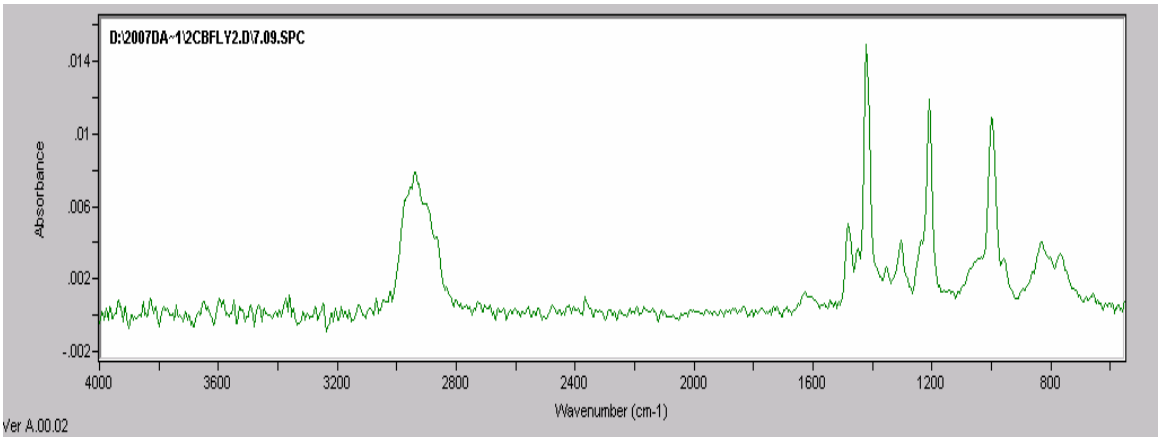


Figure 5. GC/IRD of 2C-B-FLY.

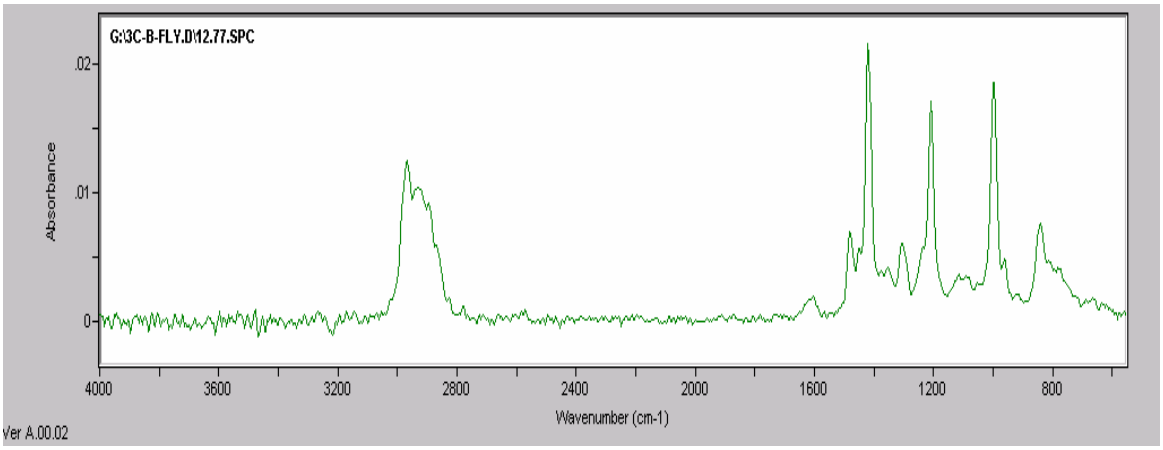


Figure 6. GC/IRD of 3C-B-FLY (Bromo-FLY).

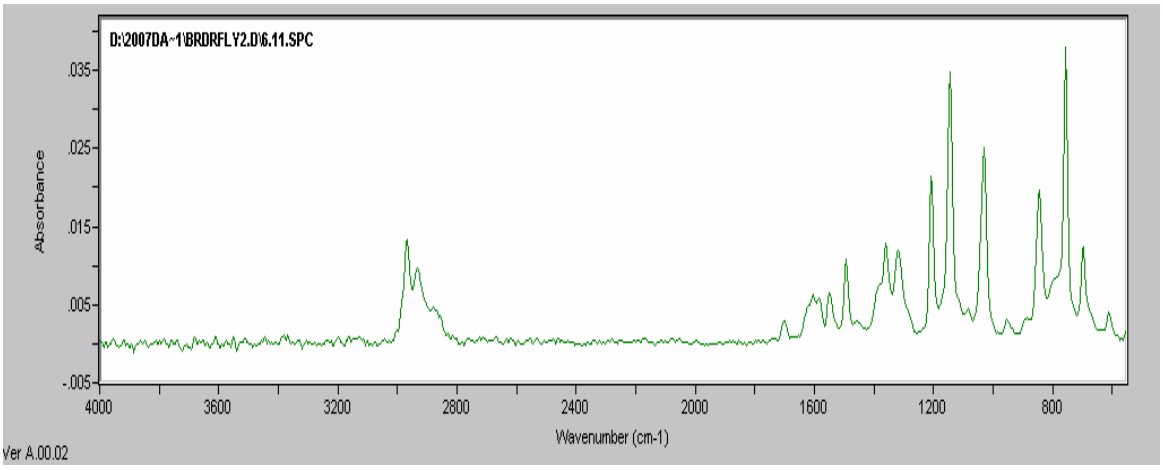


Figure 7. GC/IRD of Bromo-DragonFLY.

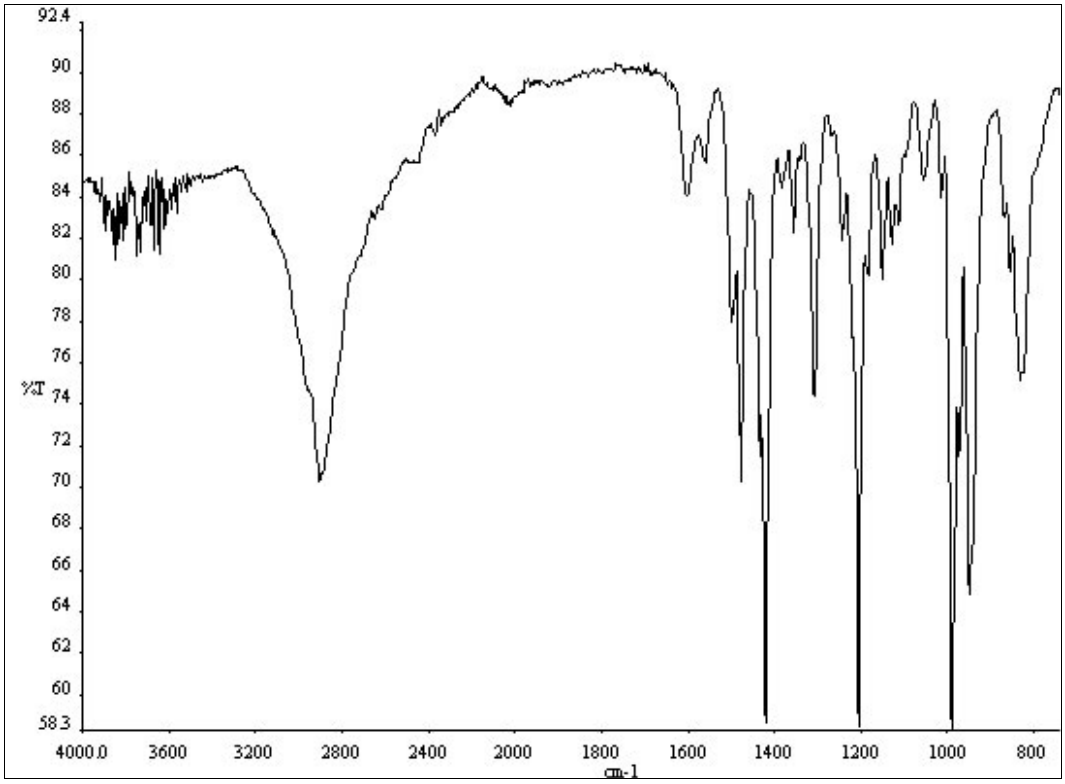


Figure 8. FTIR/ATR Spectrum of 2C-B-FLY.

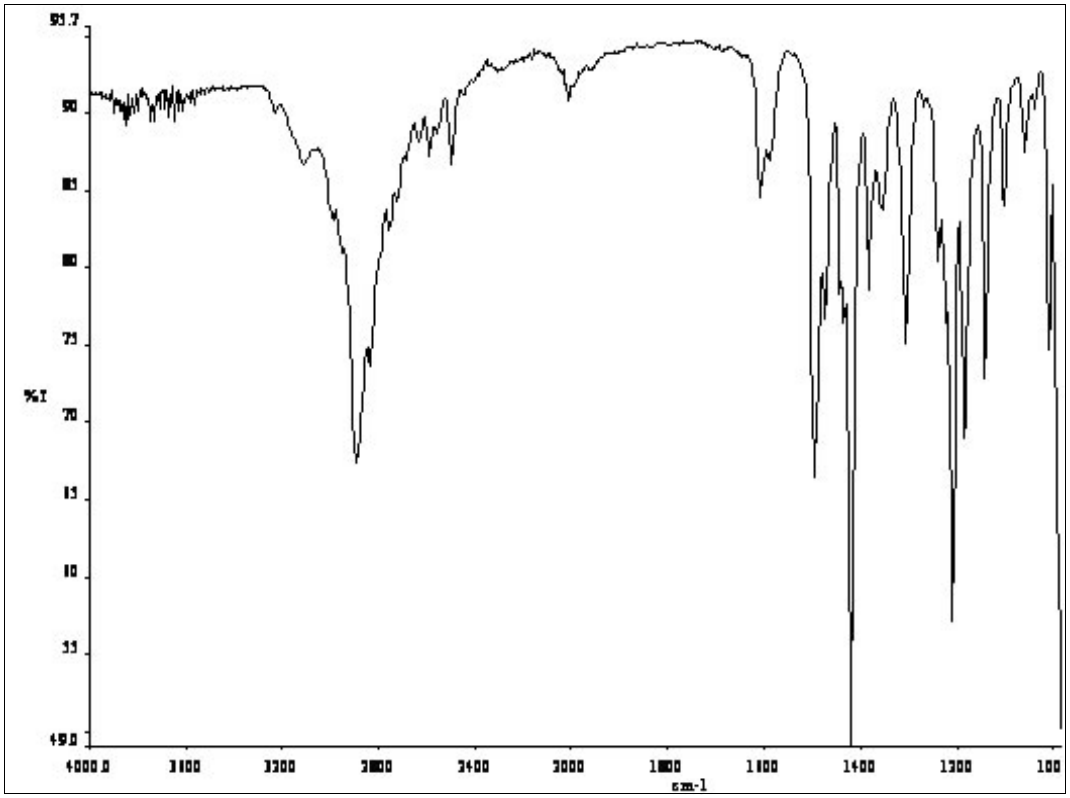


Figure 9. FTIR/ATR Spectrum of 3C-B-FLY (Bromo-FLY).

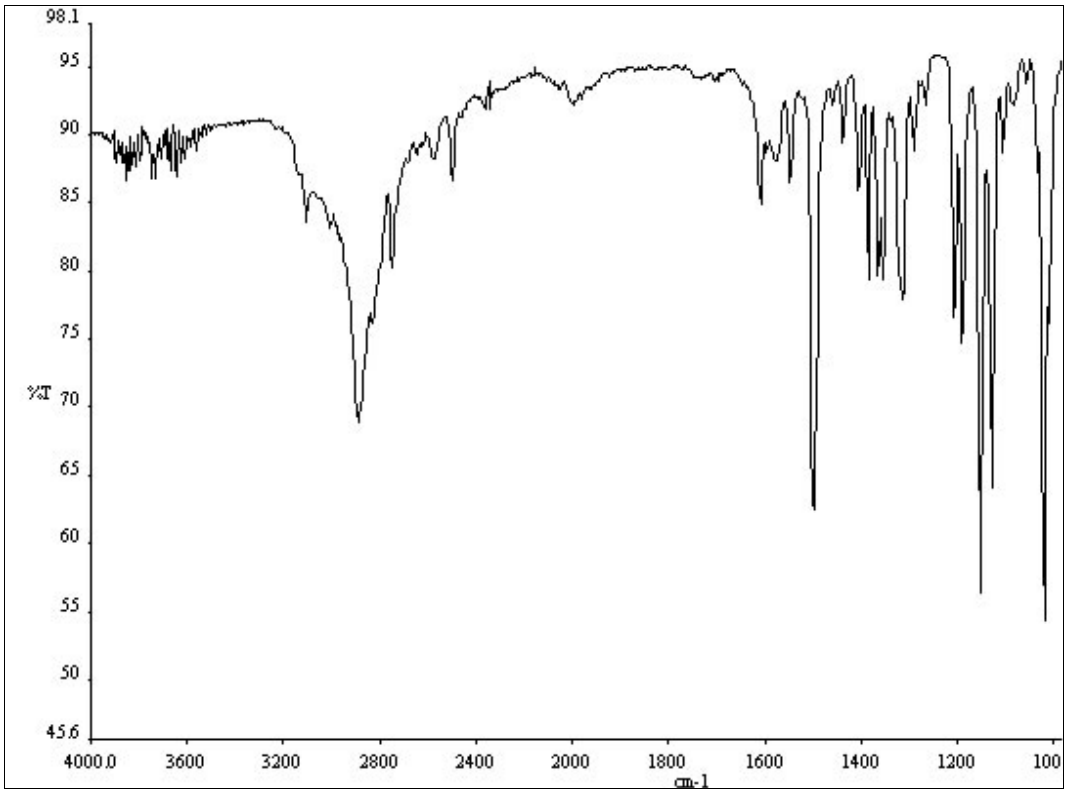


Figure 10. FTIR/ATR Spectrum of Bromo-DragonFLY.

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TECHNICAL NOTE

Comparison of the Novel Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (AccuTOF-DART™) and Signature Analysis for the Identification of Constituents of Refined Illicit Cocaine

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ABSTRACT: The characterization of 25 illicit cocaine samples by a novel application of direct analysis in real time (DART) sample introduction coupled with time-of-flight mass spectrometry (TOF-MS) and cocaine signature analyses is provided. The AccuTOF-DART™ analysis of the cocaine samples resulted in the detection of most analytes, although some compounds were not detected. This new technique is easy, rapid, requires very little sample, and can be used to screen even complex mixtures. Potential applications, including use for signature analyses of controlled substances, are discussed.

KEYWORDS: Cocaine Signature Analyses, DART, TOF-MS, Screening Test, Forensic Chemistry

Introduction

Time-of-flight mass spectrometry (TOF-MS) using exact mass determination has the potential to greatly improve drug screening in forensic laboratories [1-4]. A TOF-DART instrument, which couples a TOF mass spectrometer with a direct analysis in real time (DART) ion source, has been recently introduced. The instrument easily and rapidly screens samples for a wide range of compounds, and requires only minute amounts of sample and little sample preparation. Both sample preparation and sample screening for multiple drug analytes can be completed in minutes with the TOF-DART, whereas conventional cocaine signature analyses or controlled substances screening may take 8 hours or longer. Figure 1 compares the analysis of controlled substances by traditional GC/MS to the novel screening by TOF-DART. The instrument provides sufficient selectivity and accurate elemental composition assignment through exact mass determination, resulting in analytical identification for a wide variety of small molecules, such as drugs and unknown substances (e.g., adulterants, manufacturing solvents, and byproducts), with minimal sample preparation. TOF-DART detects a variety of controlled substances in solid samples or solution preparations [5-6].

In addition to routine sample analysis, AccuTOF-DART™ may have potential as an adjunct technique for signature analyses. While such analyses have become routine in many forensic laboratories, these programs could still benefit from a rapid screening method to identify controlled substances [7]. A procedure with minimal to no

sample preparation would complement existing methods. Determination of complex mixtures of drugs, adulterants, and diluents can help law enforcement track high-level dealers of illicit substances and identify new local or national illicit manufacturing trends. Herein, we provide a direct comparison of cocaine signature and AccuTOF-DART™ analyses of 25 refined illicit cocaine samples.

Experimental

Materials: Twenty-five DEA confiscated cocaine hydrochloride samples were obtained from the National Institute of Drug Abuse's drug supply repository for research (Bethesda, MD). Polyethylene glycol (used as the calibrating reagent) was of reagent-grade quality, and was obtained from Sigma Aldrich Chemical (St. Louis, MO). Cocaine analyte standards were purchased from Cerilliant (Austin, TX) as hydrochloride salt solutions in methanol (cocaine, anhydroecgonine methyl ester, cocaethylene, norcocaine) or acetonitrile (benzoylecgonine), all at 1 mg/mL.

AccuTOF-DART™ Analyses: Analyses were performed at the RTI International's Center for Forensic Sciences using a JEOL USA, Inc. (Peabody, MA) AccuTOF-DART™. The analyses were conducted using positive modes of the DART ion source. The source was operated with a ring lens voltage of 5 V, an orifice 1 voltage of 20 V, and an orifice 2 voltage of 5 V. Electrodes 1 and 2 of the DART source were set to 150 V and 350 V, respectively, while the DART temperature was set to 300^{0}C. The detector was optimized at 2,200 V. The AccuTOF-DART™ was calibrated with polyethylene glycol prior to each sample run. The samples were introduced into the ion source by dipping a glass probe into the sample and passing this through the stream. When available, the mono-isotopic M+H values of the cocaine analytes were verified using certified drug standard solutions.

Cocaine Signature Analyses by Gas Chromatography/Mass Spectrometry (GC/MS): Cocaine signature analyses were conducted by gas chromatography/mass spectrometry, as reported by Casale *et al.* and briefly described herein [7-11]. Analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph (GC). The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a scan range of 34 - 700 mass units, and at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm I.D. fused-silica capillary column coated with 0.25 µm DB-1 (J & W Scientific, Rancho Cordova, CA). The oven temperature was programmed as follows: Initial temperature, 100^{0}C; no hold, program rate, 6^{0}C/min; final temperature, 300^{0}C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and at a temperature of 280^{0}C. The auxiliary transfer line to the MSD was operated at 280^{0}C.

Results and Discussion

Table 1 contains the theoretical M+H values of the target analytes that were detected in the cocaine exhibits by cocaine signature analyses. All values are reported to 0.001 mmu with the exception of petroleum ether (which has a very low mass and thus a larger expected mass error).

The results of the AccuTOF-DART™ analysis of the 25 cocaine samples in comparison to the multi-technique signature analyses are in Table 2. Anhydroecgonine methyl ester (AEME) and cinnamoylcocaine were easily detected in 23 out of the 25 samples, as shown in the AccuTOF-DART™ spectra depicted in Figures 1A - B. In all samples, there was an ion present at *m/z* = 290.139, which is the M+H value of $C_{16}H_{19}NO_4$. This is the molecular formula of the isomeric pair benzoylecgonine (BE) and norcocaine, which have identical (and therefore indistinguishable) masses. Figure 2 shows the presence of the ion at 290.169 in an analyzed sample. The theoretical value of BE and norcocaine is 290.139. Although the difference of 0.030 mmu is not optimal, it may be due to an interferent present at a similar mass, resulting in a skewed *m/z* value. This is a problem that is frequently encountered during TOF-DART analysis. For example, known analytes may be analyzed sequentially and subjected to the same calibration, but while one peak will generate an M+H value 1 or 2 mmu from its

theoretical value, the other will have a difference of more than 10 mmu. Cocaine has a theoretical M+H value of 304.154 and the actual value, as seen in the analysis of a sample in Figure 2, is only 0.002 mmu higher than expected, while this is not the case with the BE/norcocaine isomer. In a recent study, the isomeric pair was analyzed by increasing the orifice 1 voltage to 90, which generated distinguishable ion fragmentation patterns [5]. However, this was done with methanolic standards at a high concentration, and was unsuccessful when analyzing the illicit cocaine samples used in this study.

Tropacocaine and truxillines were present in 5 and 7, respectively, of the cocaine samples (Figures 3A - B), while 3',4',5'-trimethoxycocaine and cocaethylene were undetected. Of the solvents and adulterants/diluents detected by cocaine signature analyses, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) (Figures 4A and B), and dimethylterephthalate (Figure 1A) were all identified by AccuTOF-DART™.

The AccuTOF-DART™ allowed for the rapid introduction and analysis of 25 illicit cocaine samples without the need for sample preparation. However, although this direct analysis resulted in rapid production of data, it also gave inconsistent results. In addition, because the introductions of the powdered samples were done manually, the outcome was analyst dependent (not ideal for signature analyses, where consistency of analysis is critically important). Many samples required multiple analyses to verify the presence or absence of the target analytes. Although analytes such as AEME and cinnamoylcocaine were easily detected in most of the samples, AEME is likely present as an artifact generated from truxillines during analysis. Other analytes such as tropacocaine and 3',4',5'-trimethoxycocaine were minimally detected, if at all.

Conclusions

The AccuTOF-DART™ is a novel approach to forensic analysis; however, its use in the analysis of refined illicit cocaine in this study proved ineffective for detecting the presence of the many compounds that are used to trace a cocaine sample to its geographic origin. In an effort to increase laboratory production, forensic laboratories may wish to utilize AccuTOF-DART™ as a rapid screening test for preliminary sample-to-sample comparison work, which could then be confirmed by more thorough analyses.

Acknowledgments

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Table 1. Theoretical Mono-Isotopic Mass+H of Analytes.

Analyte	Theoretical Mono-Isotopic Mass+H
Anydroecgonine methyl ester	182.117
Benzoylecgonine	290.139
Caffeine	58.958
Cinnamoylcocaine	330.169
Cocaethylene	318.169
Dimethylterephthlate	195.064
Ethyl acetate	89.052
(*Iso-/n-*)Propyl acetate	103.068
Lactose	343.116
Mannitol	303.079
Methyl ethyl ketone	73.064
Methyl isobutyl ketone	101.096
Norcocaine	290.138
Petroleum ether	87-90
Sodium chloride	58.985
3',4',5'-Trimethoxycocaine	393.178
Tropacocaine	246.141
Truxillines	658.325

Table 2. The Number of Samples, out of the Total 25 Analyzed, That Tested Positive for the Various Analytes, Using the AccuTOF-DART™ System and Cocaine Signature Analysis.

Analytes	Cocaine Signature Analyses	AccuTOF-DART™
Anhydroecgonine methyl ester	ND	23
Benzoylecgonine	21	25
Cocaethylene	NA	ND
Cinnamoylcocaine	25	23
Norcocaine	21	25
3',4',5'-Trimethoxycocaine	25	ND
Tropacocaine	25	5
Truxillines	25	7

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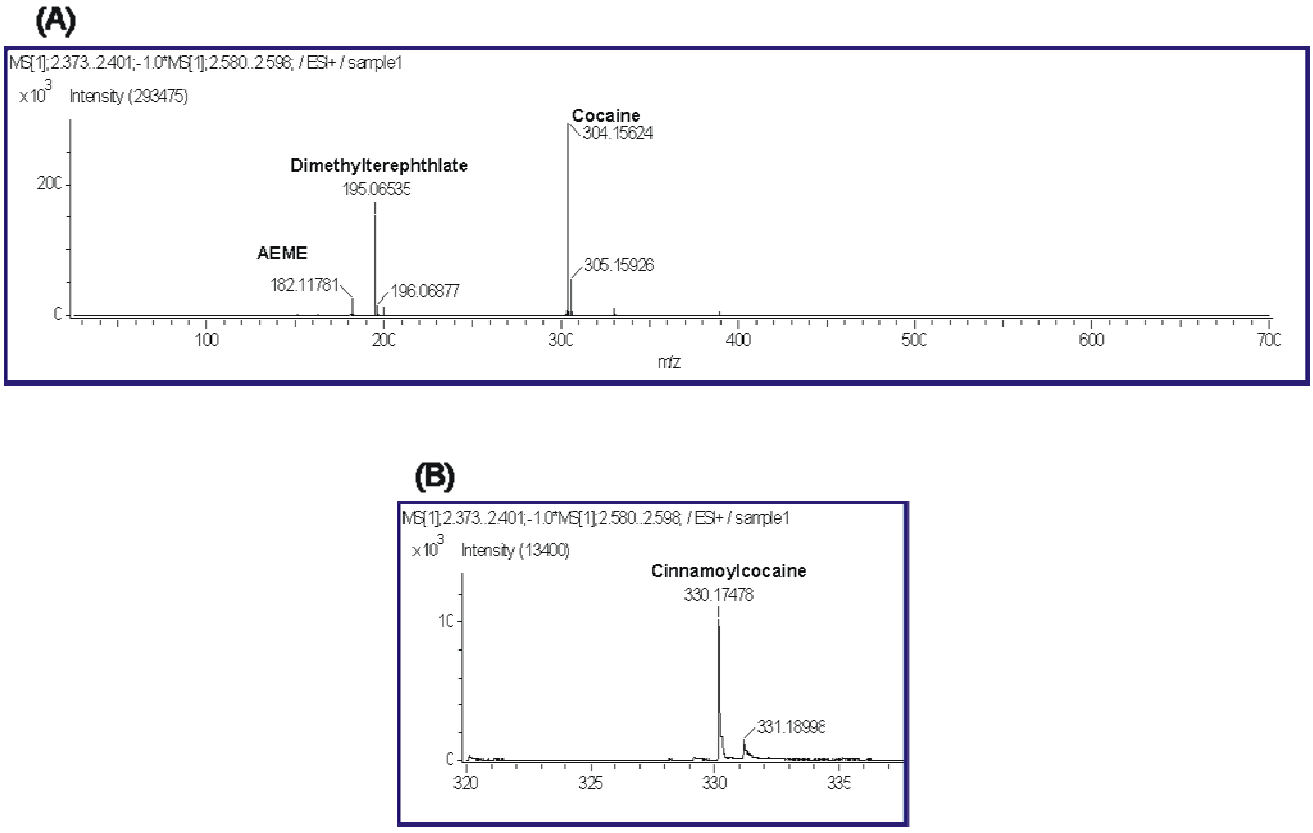

Figure 1. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Cocaine, Anhydroecgonine Methyl Ester (AEME), and Dimethylphthalate; and (B) Cinnamoylcocaines.

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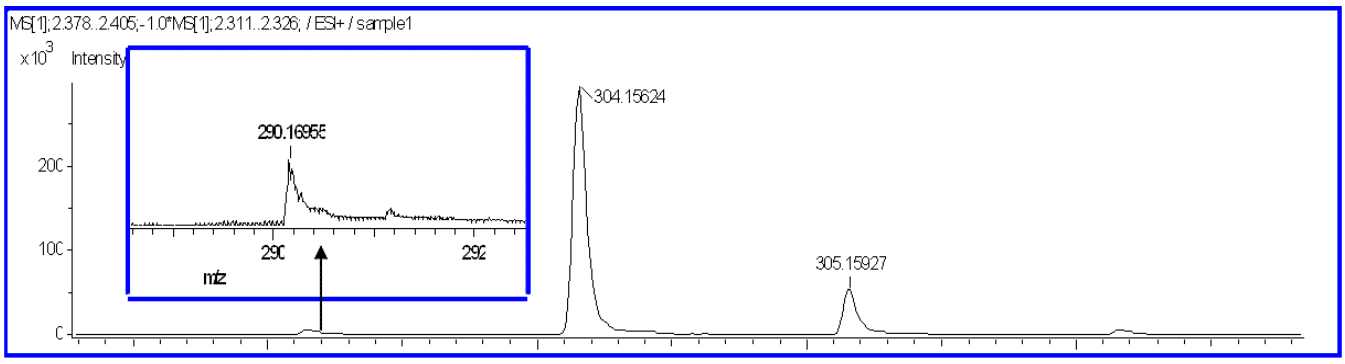

Figure 2. AccuTOF-Dart™ Spectra of an Illicit Cocaine Sample Showing the Presence of Possible Norcocaine and Benzoylecgonine (both at *m/z* = 290.17; see Expansion Window).

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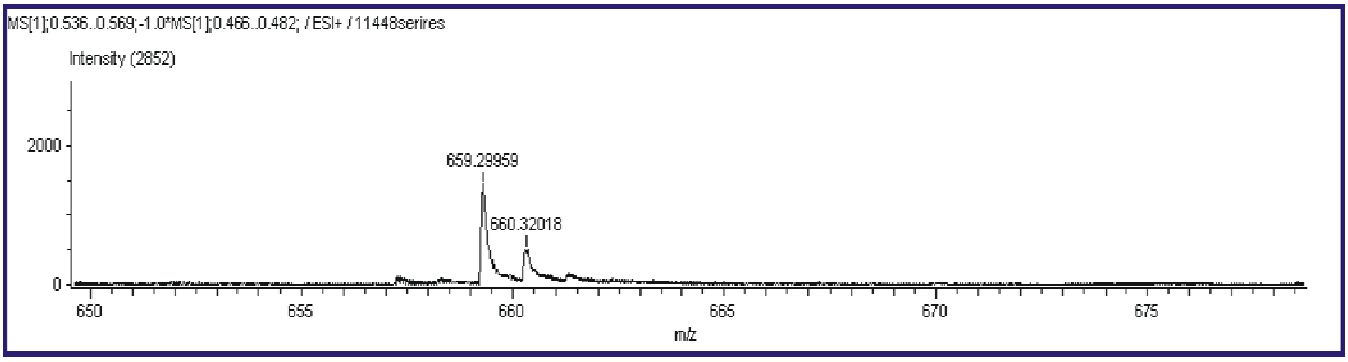

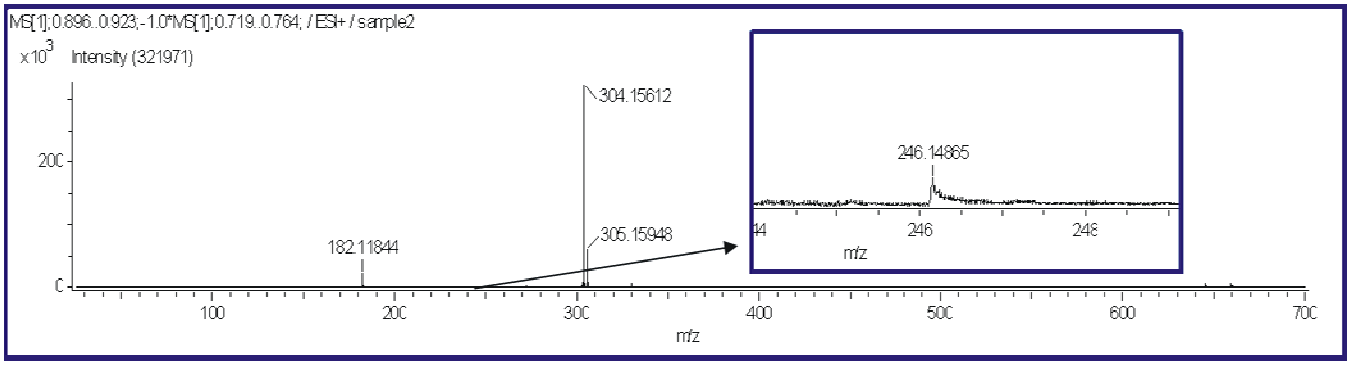

Figure 3. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Truxillines; and (B) Tropacocaine (in Expansion Window).

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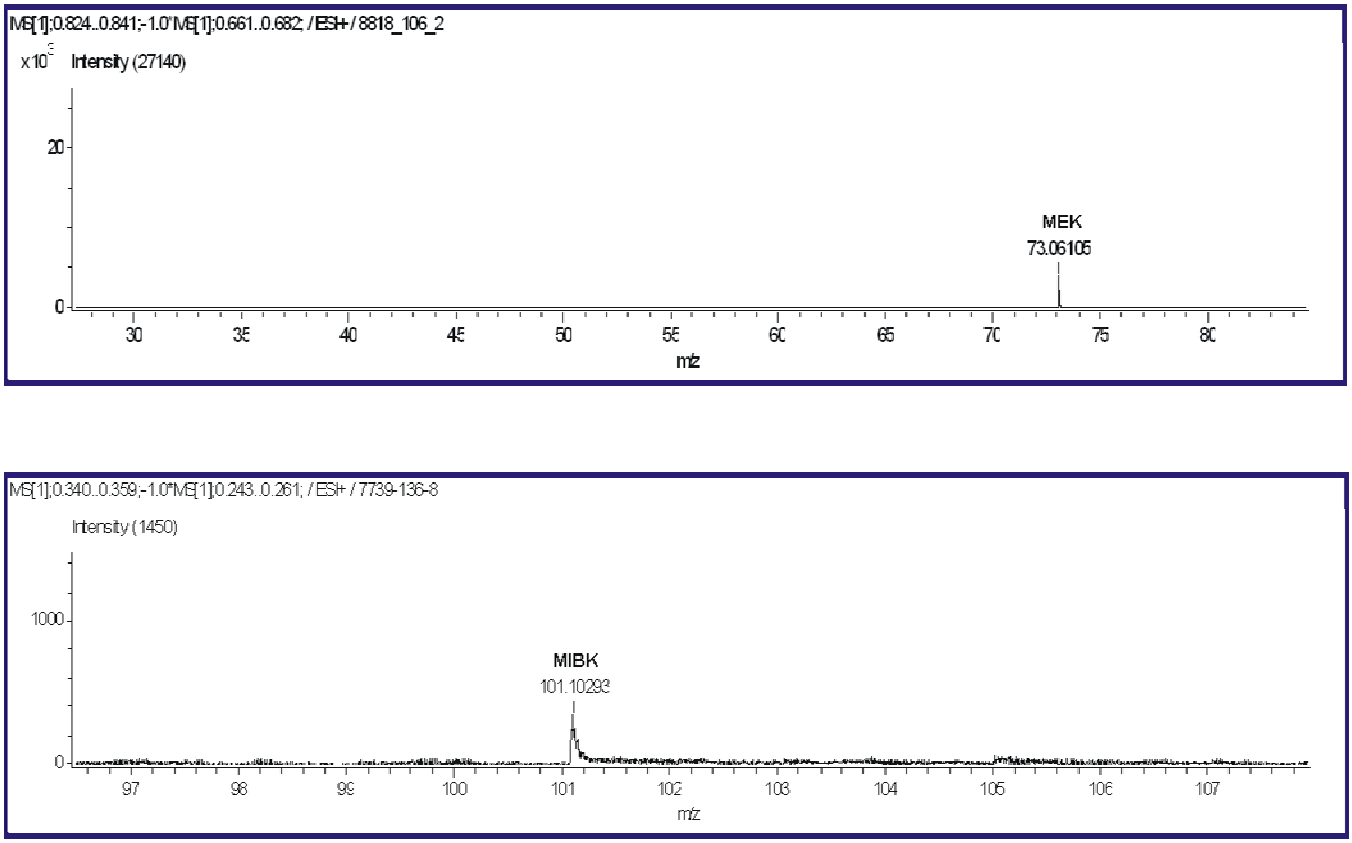

Figure 4. AccuTOF-DART™ Spectra of an Illicit Cocaine Sample Showing the Presence of: (A) Methyl Ethyl Ketone (MEK); and (B) Methyl Isobutyl Ketone (MIBK).

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The Isolation, Identification, and Quantitation of Dimethyltryptamine (DMT) in *Mimosa hostilis*

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ABSTRACT: Dimethyltryptamine (DMT) was extracted from the root bark of *Mimosa hostilis* via three methods, using methanol (direct or via Soxhlet) and acetic acid (direct only), respectively. The product from the direct methanol extraction was used in both qualitative and quantitative analysis, while the product from the acetic acid extraction (isolated in crystal form after workup) was used for qualitative analysis. FTIR/ATR, GC/MS, GC/IRD, ^{1}H-NMR, and HPLC data are presented. Quantitative analysis by ^{1}H-NMR and HPLC indicated 0.9 percent and 0.8 percent DMT, respectively, in the analyzed samples.

KEYWORDS: *Mimosa hostilis*, Dimethyltryptamine, DMT, Extraction, Analysis, Forensic Chemistry

Introduction

Tryptamines are substituted indole compounds which are both naturally occurring and synthetically manufactured. Many tryptamines, including dimethyltryptamine (DMT, Figure 1), have hallucinogen properties, and are therefore listed as Schedule I drugs under the U.S. Controlled Substances Act (21 CFR 1308.11). DMT is present in many plants and their seeds, including in *Mimosa hostilis* and *Psychotria viridis* [1-3], and can be abused by smoking, injection, or ingestion of either these natural materials or their crude or purified extracts, either alone or in combination with other extracts (e.g., Ayahuasca [4].) *Mimosa hostilis* and similar natural plant materials are not formally controlled (by name) in the United States; however, they are controlled (Schedule I) if they are shown to contain DMT or other controlled hallucinogens. Despite their controlled status, a number of DMT-containing natural products, including *Mimosa hostilis*, are openly marketed on the Internet.

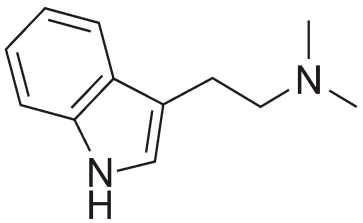

Figure 1. Structure of Dimethyltryptamine (DMT; $C_{12}H_{16}N_2$, m.w. = 188.27).

Clandestine DMT extraction laboratories are occasionally seized by law enforcement agencies [e.g., 5]. The basis of this report was the seizure of an unknown plant material (Photo 1) at a clandestine MDMA (Ecstasy) laboratory in rural Pennsylvania. GC/MS analysis of a methanolic extraction of the material identified DMT. Upon debriefing, the defendant in the case indicated that material was root bark from *Mimosa hostilis*. Similar seizures of this material have been made at other clandestine laboratory sites in the United States, and subsequent analyses of those exhibits confirmed that they also contained DMT.

Photo 1. *Mimosa hostilis* Root Bark Seized at Clandestine Lab in Pennsylvania.

Experimental

Methanol Extraction: The root bark was cut into small pieces then ground in a blender to produce a very fine powder. For direct extraction, methanol was added to the powder, heated to 60^{O}C with stirring for 1 hour, and then filtered. This step was repeated three more times, except the re-extractions were carried out for only 5 - 10 minutes each. The combined extracts were evaporated to a residue over steam, then reconstituted as needed for analysis. For Soxhlet extraction, the powdered material was placed in an extraction thimble, placed in a Soxhlet, and extracted with 50 mL of methanol for approximately 50 volumes. The solvent was evaporated to a residue over steam, then reconstituted as needed for analysis.

Acetic Acid Extraction: The root bark was cut into small pieces then ground in a blender to produce a very fine powder. A 3% acetic acid solution was added to the powder, and the resulting suspension was stirred for approximately two hours. The solution was filtered and transferred to a separatory funnel, made basic with sodium hydroxide, and then extracted with methylene chloride. The methylene chloride solution was isolated, and the aqueous later was re-extracted with a second volume of methylene chloride. The combined extracts were dried over magnesium sulfate, filtered, and evaporated to give a crystalline material.

Fourier Transform Infrared with Attenuated Total Reflectance (FTIR/ATR)
Instrument: Perkin-Elmer Spectrum One FTIR.
Data collection: Four scans were collected between 650 cm^{-1} and 4000 cm^{-1}.
Resolution: 4 cm^{-1}.
Sample: Crystals from the acetic acid extraction.

Gas Chromatograph/Mass Spectrometer (GC/MS)
Instrument: Agilent 6890N GC/Agilent 5973 Mass Selective Detector.
Column: HP-5, 30 m x 0.25 mm x 0.25 μm column.
Temperature program: 90^{O}C - 120^{O}C @ 35^{O}C/min; initial time 1.35 min, then 120^{O}C - 290^{O}C @45^{O}C/min; initial time 0.55 min, final hold time 8.5 min.
Injection port temperature: 300^{O}C.
Transfer line temperature: 280^{O}C.
Ionization source: Electron ionization (EI).
Mass analyzer: Quadrupole.
Scan range: 40 - 525.
Quadrupole temperature: 150^{O}C.
MS source temperature: 230^{O}C.
Sample preparation: Residue from the methanol extraction, reconstituted in methanol.

Gas Chromatograph/Infrared Detector (GC/IRD)
Instrument: Agilent 6890 GC/Varian IRD Detector.
Column: HP-5, 25 m x 320 μm x 0.52 μm column.
Split mode: 5:1.
Temperature program: 100^{O}C for 1.50 min, ramp @ 35^{O}C/min to 120^{O}C, hold for 0.55 min, then ramp @ 40^{O}C/min to 290^{O}C, final hold for 8.13 min.
Inlet temperature: 270^{O}C.
Injection volume: 2 μL.
Constant column flow: 2.0 mL/min.
Transfer line temperature: 280^{O}C.
Flow cell temperature: 280^{O}C.
KBr windows.
Optical resolution: 8.
1.5 scans/sec.
Sample: Residue from the methanol extraction, reconstituted in chloroform.

Proton Nuclear Magnetic Resonance (^{1}H-NMR)
Instrument: Mercury 400 MHz.
Number of transients: 8.
Relaxation delay: 45 seconds.
Pulse: 90^{O}.
Sweep width: 6393.9 Hz.
Temperature: 25^{O}C.
Sample preparation for qualitative analysis: Crystals from the acetic acid extraction, reconstituted in 1 mL CD_3OD.
Sample preparation for quantitative analysis: 5.0 g *Mimosa hostilis* extracted via the methanol extraction procedure, yielding 1.52 g residue. Added 28.0 mg to 1 mL CD_3OD, with 5.544 mg maleic acid added as the internal standard.

High Performance Liquid Chromatography (HPLC)
Instrument: Agilent 1100 Series HPLC.
Column: Phenomonex Partisil 5μm ODS-3 (C-18).
Mobile phase: Phosphate buffer pH 2.5:methanol (90:10).
Injection: 5 μL.
Flow rate: 1.0 mL/min.
Detection: 280 nm.
Run time: 8 minutes.
Sample preparation: 9.9 g *Mimosa hostilis* extracted via methanol extraction procedure, with the residue reconstituted in 100 mL methanol.

Results and Discussion

The extraction of DMT from *Mimosa hostilis* was completed using two different solvents, methanol (direct or via Soxhlet) and acetic acid (direct only). The methanol extraction gave the maximum recovery of DMT for qualitative and quantitative analysis; however, the extract included other soluble plant impurities. The extraction efficiency using methanol was identical whether done directly or via Soxhlet. The acetic acid extraction gave a very clean, pure product, but in lower yield versus the methanol extraction.

FTIR/ATR: The crystals from the acetic acid extraction procedure produced a clean spectrum (Figure 2).

GC/MS: DMT eluted at 6.06 minutes using the described method. The spectra showed a base peak at *m/z* = 58 and the molecular ion at *m/z* = 188, along with smaller peaks at *m/z* = 44, 77, and 130 (Figures 3 and 4).

GC/IRD: DMT eluted at 6.88 minutes using the described method (Figure 5).

^{1}H-NMR (Qualitative): The singlet at 2.35 ppm is due to the two N-methyl groups, the two triplets at 2.70 ppm and 2.95 ppm correspond to the *alpha* and *beta* methylene groups. The multiplet at 7.00 ppm corresponds to protons 2, 5, and 6 on the indole. Finally, the two doublets at 7.25 ppm and 7.50 ppm correspond to protons 4 and 7 on the indole. A slight shift was observed in the extract versus a DMT standard; this was due to pH differences (the spectrum was obtained from DMT acquired using the acetic acid extraction procedure, which involved an acid base workup). (Figure 6). (Quantitative): Using the direct methanol extract, DMT was determined to be 0.9% weight/weight in *Mimosa hostilis* (Figure 7 and Table 1). Using the direct methanol extract, DMT was determined to be 0.9% weight/weight in Mimosa hostilis (Figure 8 and Table 2).

HPLC: DMT eluted in under 3 minutes. Using the methanol extract, DMT was determined to be 0.8% weight/weight in *Mimosa hostilis* (Figure 9 and Table 3).

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* Law Enforcement Restricted Publication.

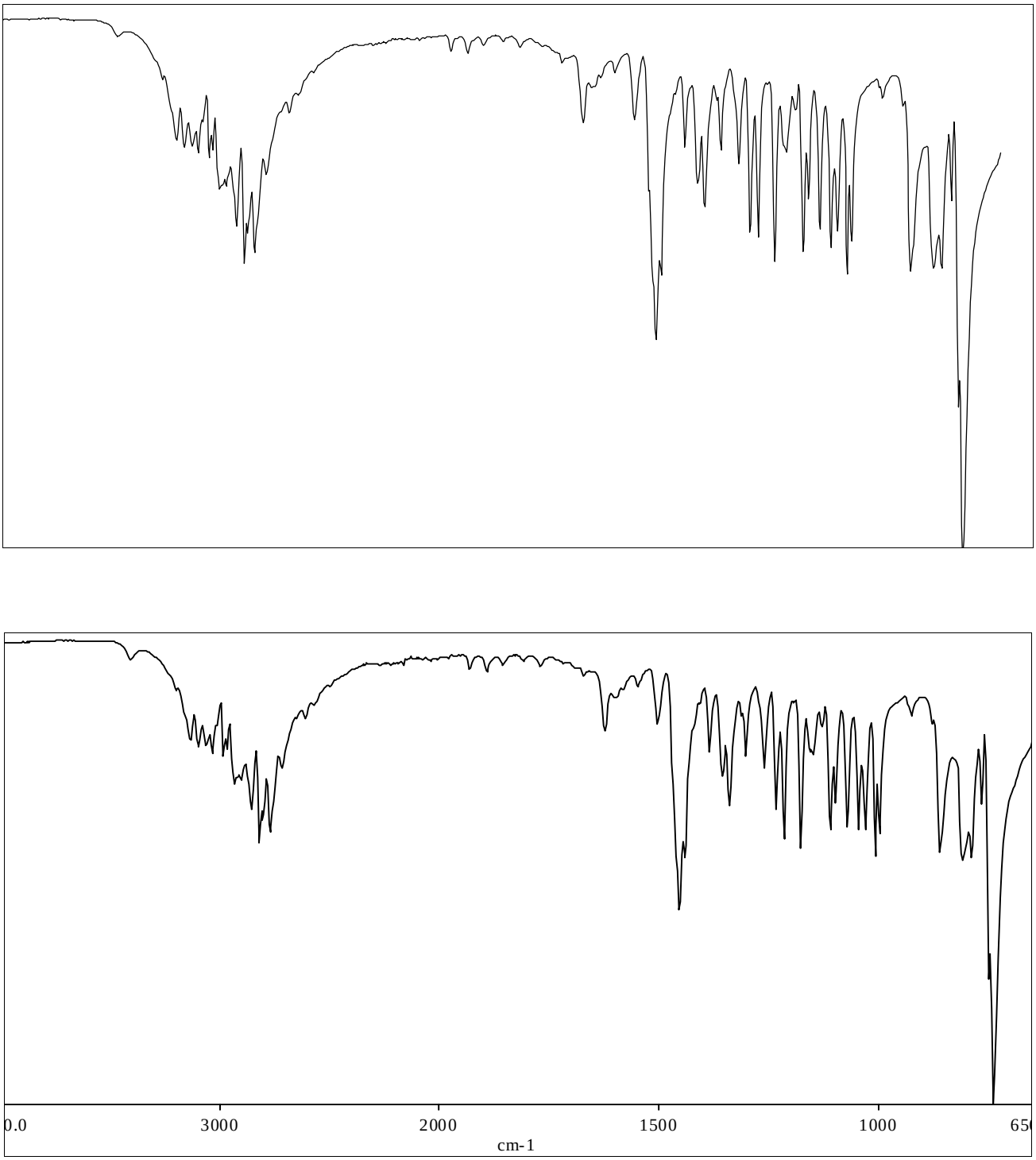

Figure 2. FTIR/ATR of a DMT Standard (Top Trace) and DMT from the Acetic Acid Extraction Procedure (Bottom Trace). [Note: The DMT Standard was Recrystallized from Chloroform.]

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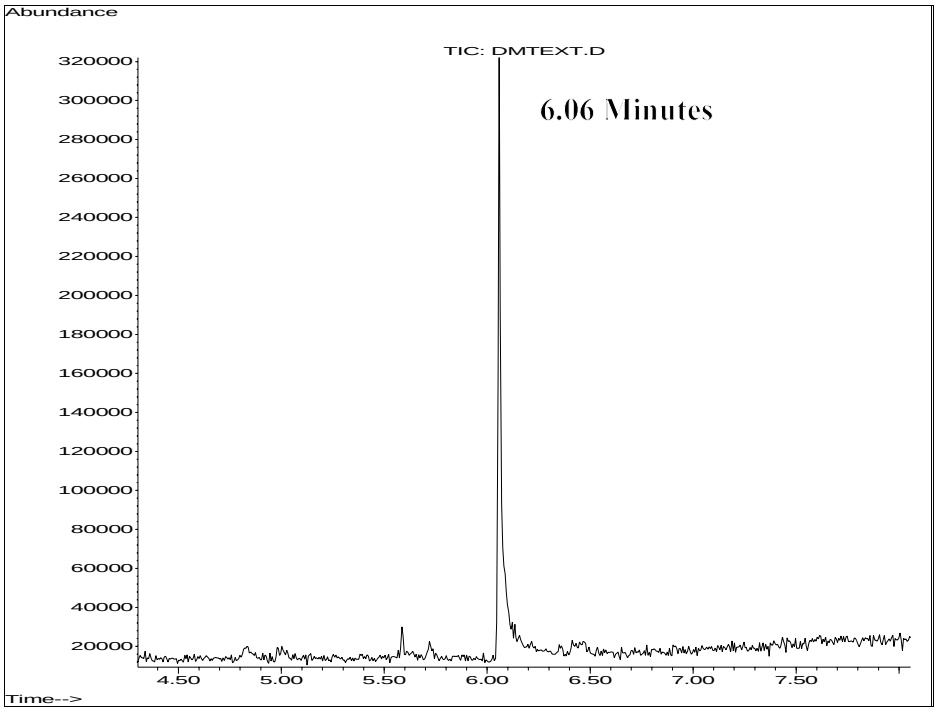


Figure 3. GC/MS Total Ion Chromatogram of DMT (Methanol Extract).

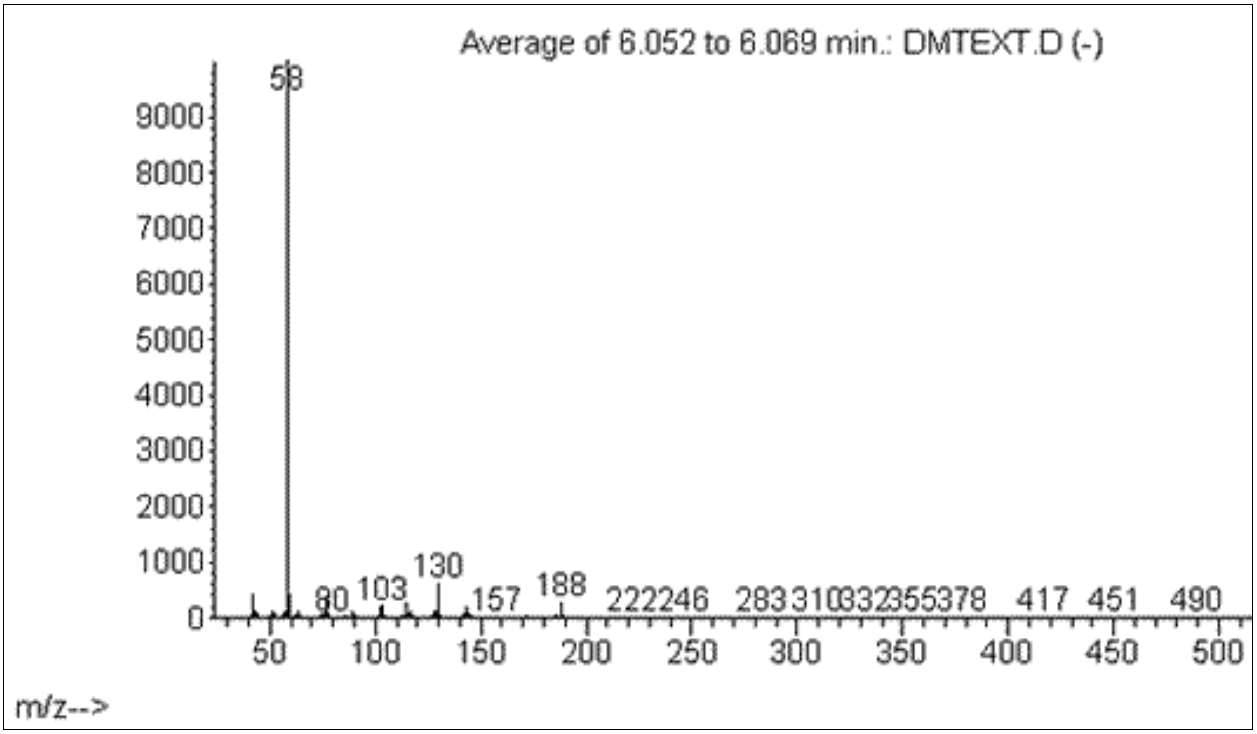
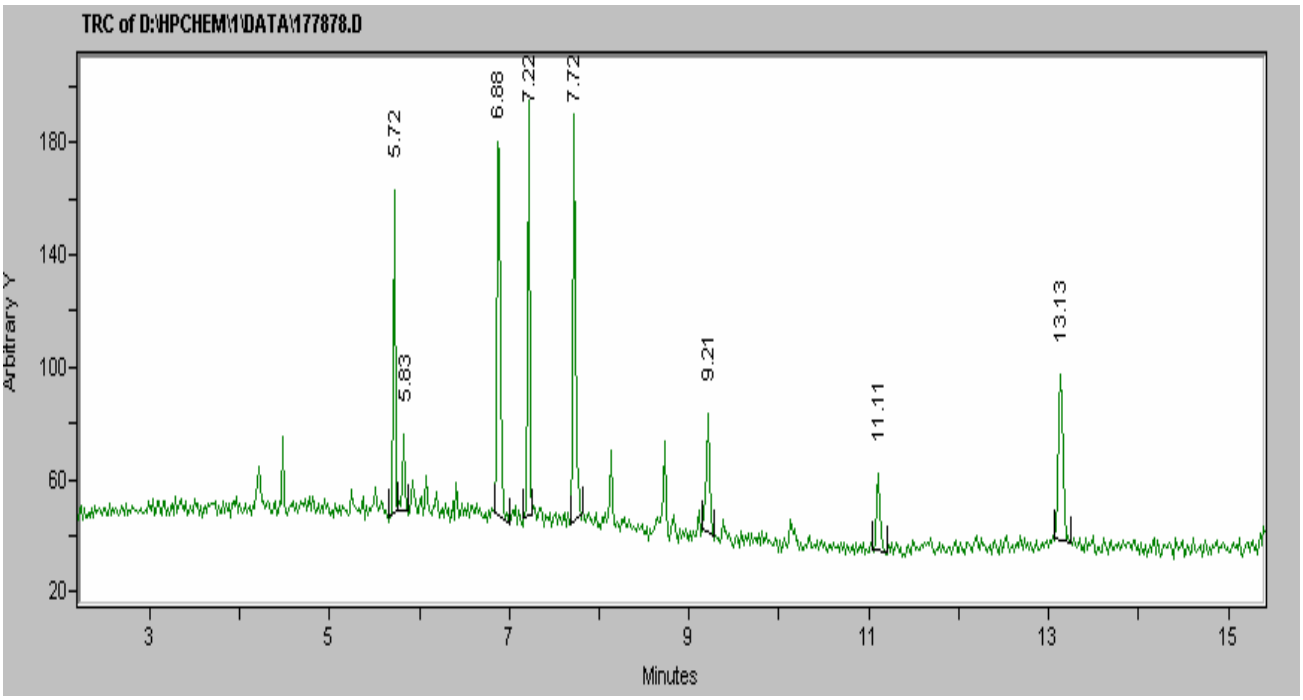


Figure 4. GC/MS Data of DMT (Methanol Extract). [Note: Molecular Ion at *m/z* = 188.]



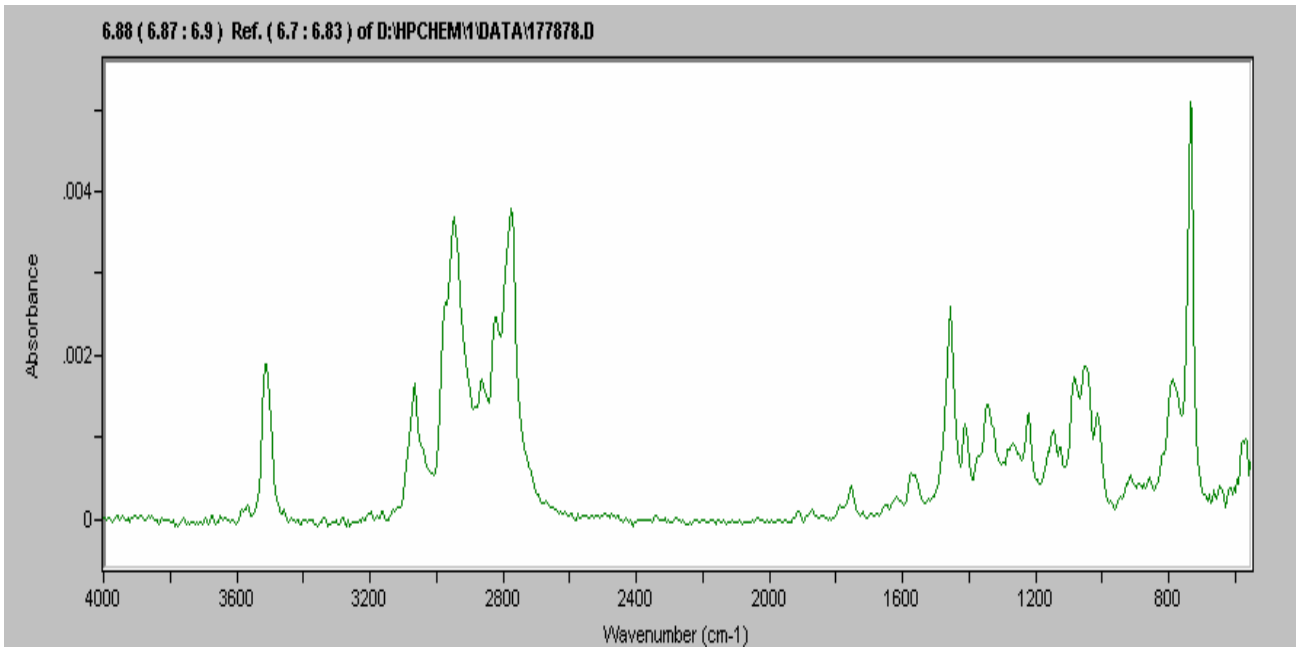


Figure 5. GC/IRD Data of DMT (Methanol Extract). [Note: DMT eluted at 6.88 Minutes.]

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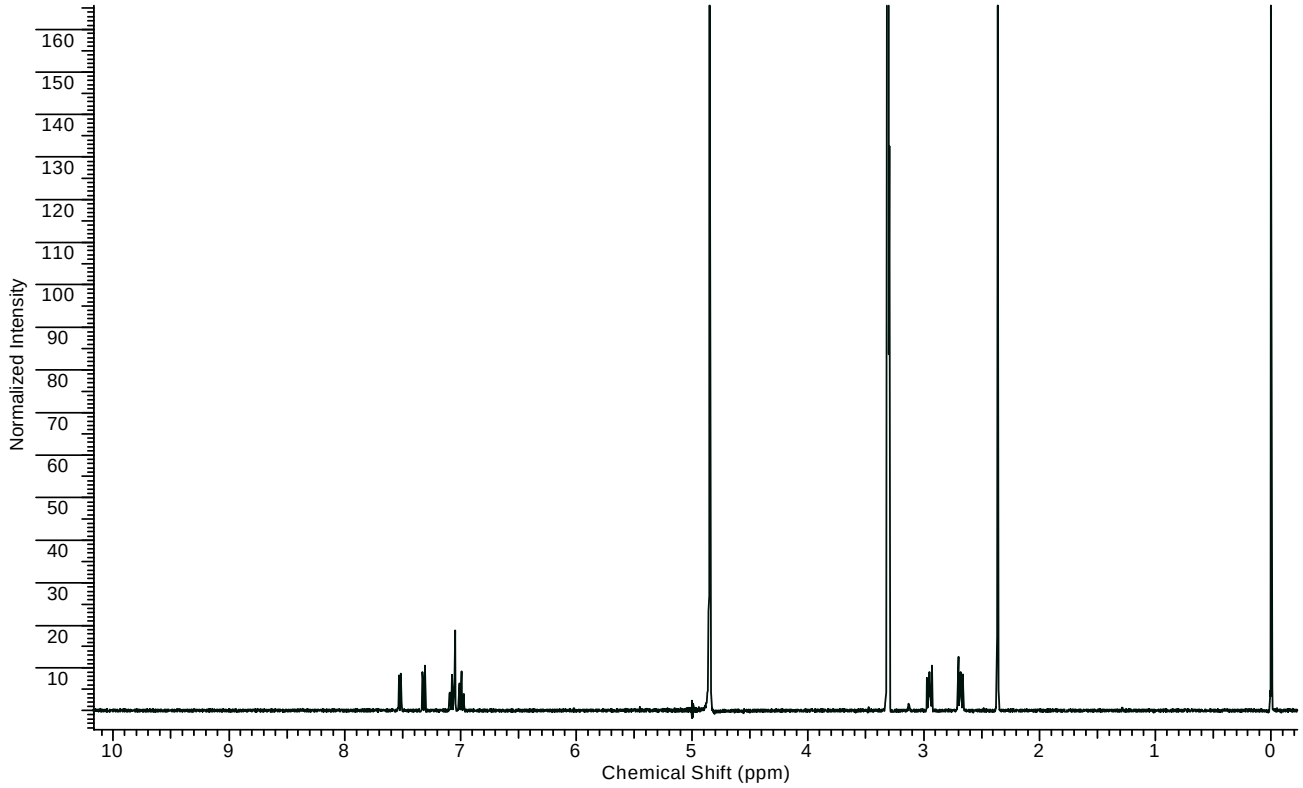

Figure 6a. Full-Scale NMR Data of DMT (Acetic Acid Extract).

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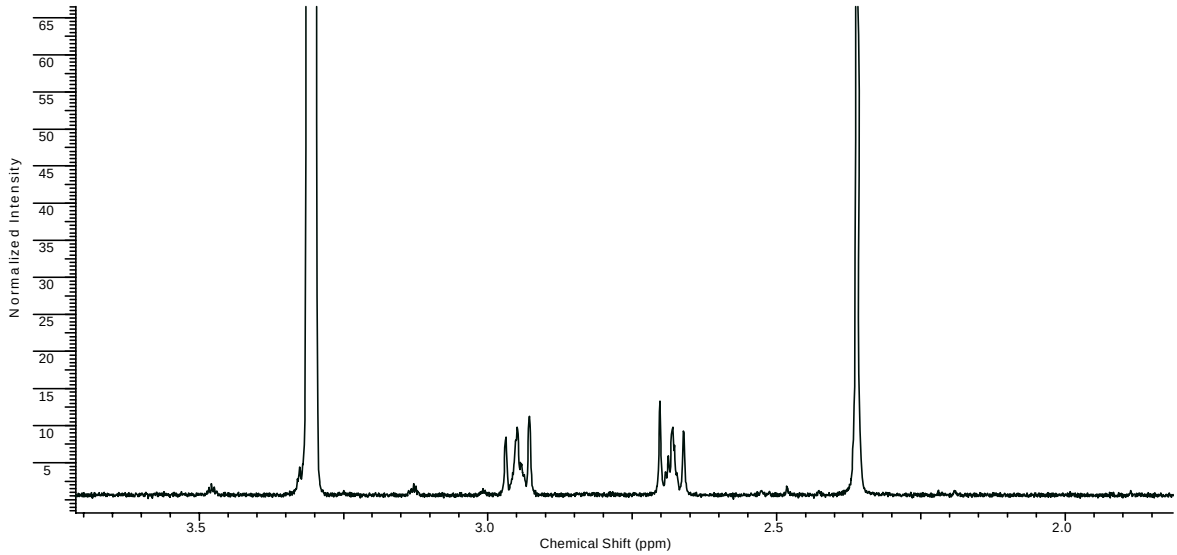

Figure 6b. Expanded Spectrum from 2 to 4 ppm. See Results and Discussion for Peak Assignments.

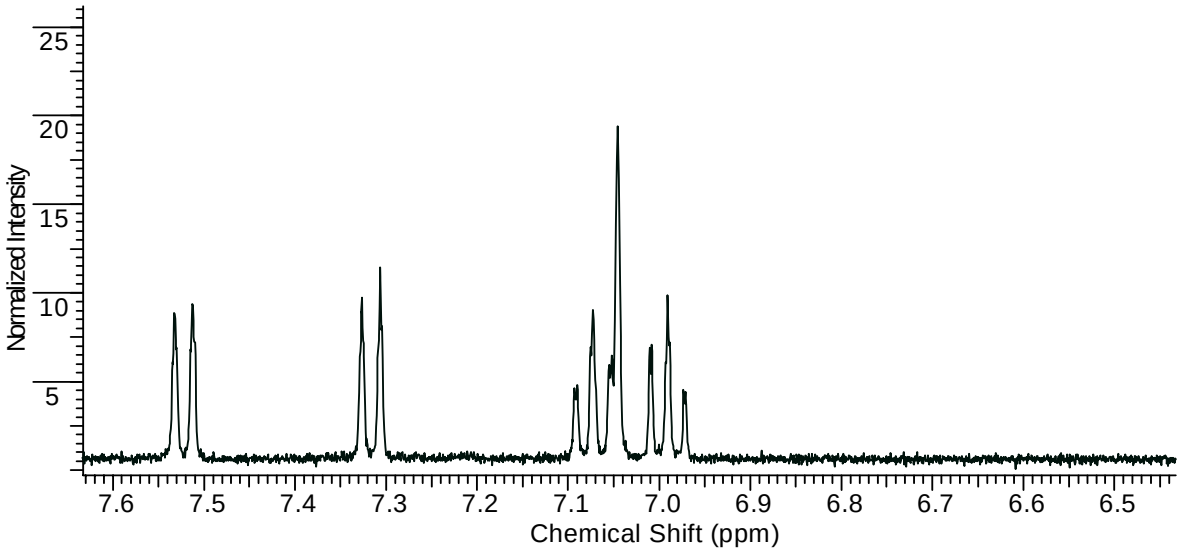

Figure 6c. Expanded Spectrum from 6.5 to 7.6 ppm. See Results and Discussion for Peak Assignments.

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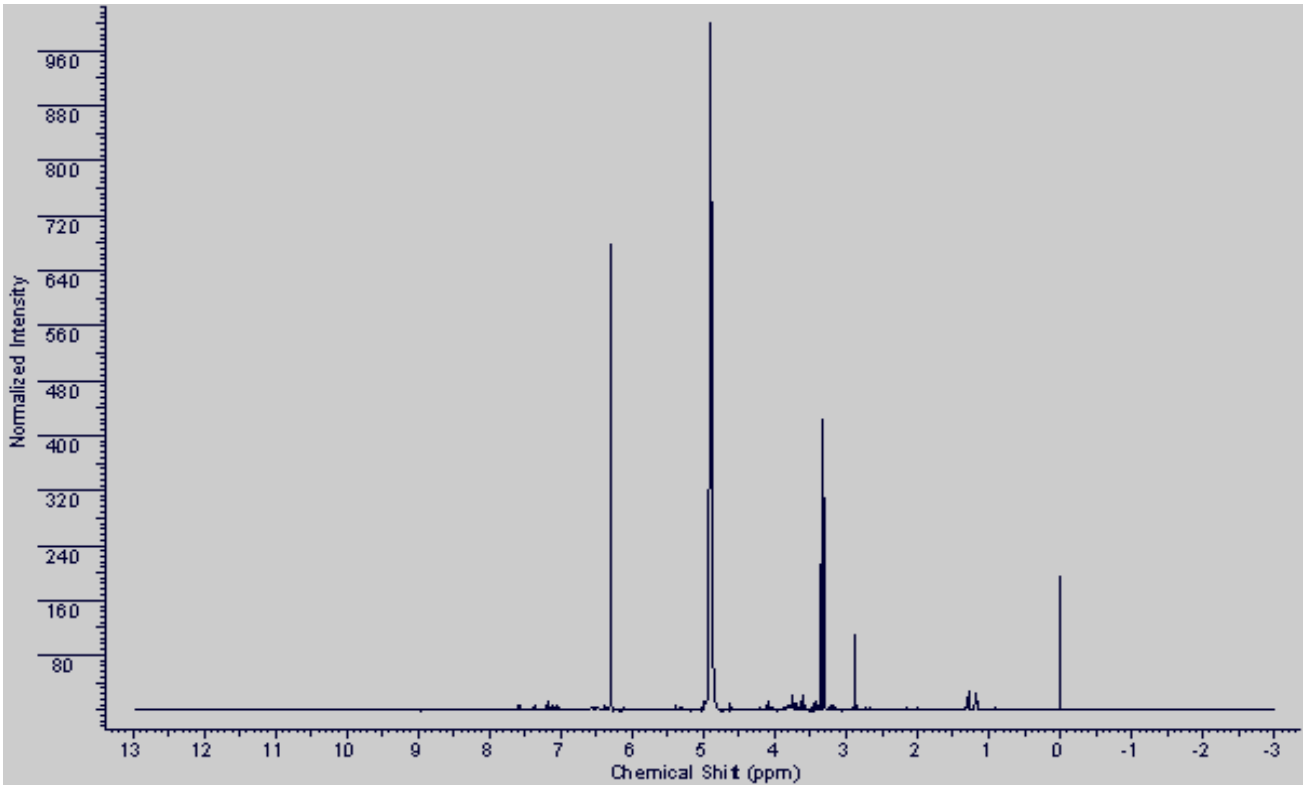

Figure 7a. NMR Quantitation of DMT (Direct Methanol Extract); See Table 1.

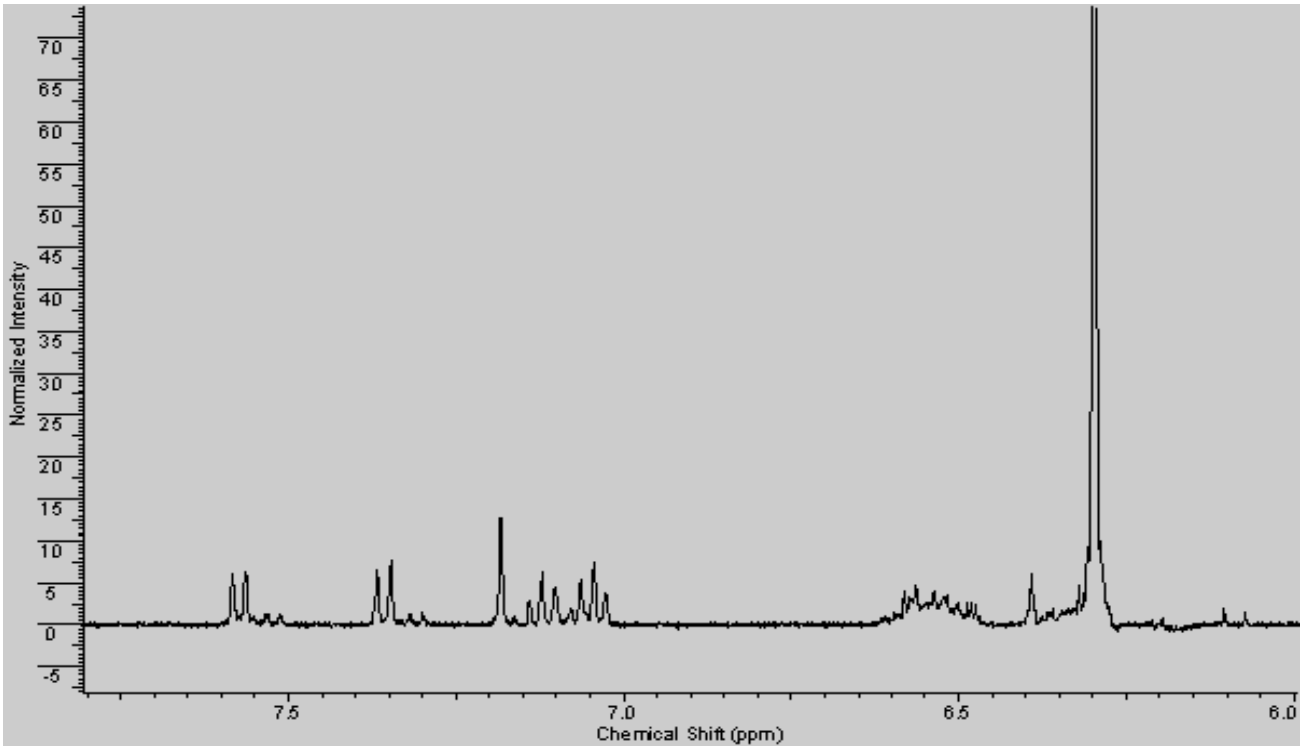

Figure 7b. NMR Quantitation of DMT (Direct Methanol Extract); Expansion; See Table 1.

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Table 1. NMR Quantitation of DMT (Direct Methanol Extract); See Figure 7.					
Original Amount of Plant Material (g)	5.0				
Amount of extraction product (g)	1.52				
Sample Amount (mg)	28.00				
Molecular Weight of Sample	188.3				
Solvent	CD3OD				
Internal Standard (I.S.)	Maleic Acid				
Molecular Weight of I.S.	116.07				
I.S. Amount (mg)	5.544				
Peaks Chemical Shift (ppm)	[6.26..6.34]	[7.02..7.08]	[7.10..7.15]	[7.34..7.42]	[7.54..7.62]
Integral Value	34.12	2.13	1.64	1.39	1.27
Number of protons represented	2	1	1	1	1
Quantitation Value	Internal Standard	4.01%	3.09%	2.61%	2.39%
Average purity of extracted material	3.03%				
Amount of DMT in *Mimosa hostilis*	0.918%				

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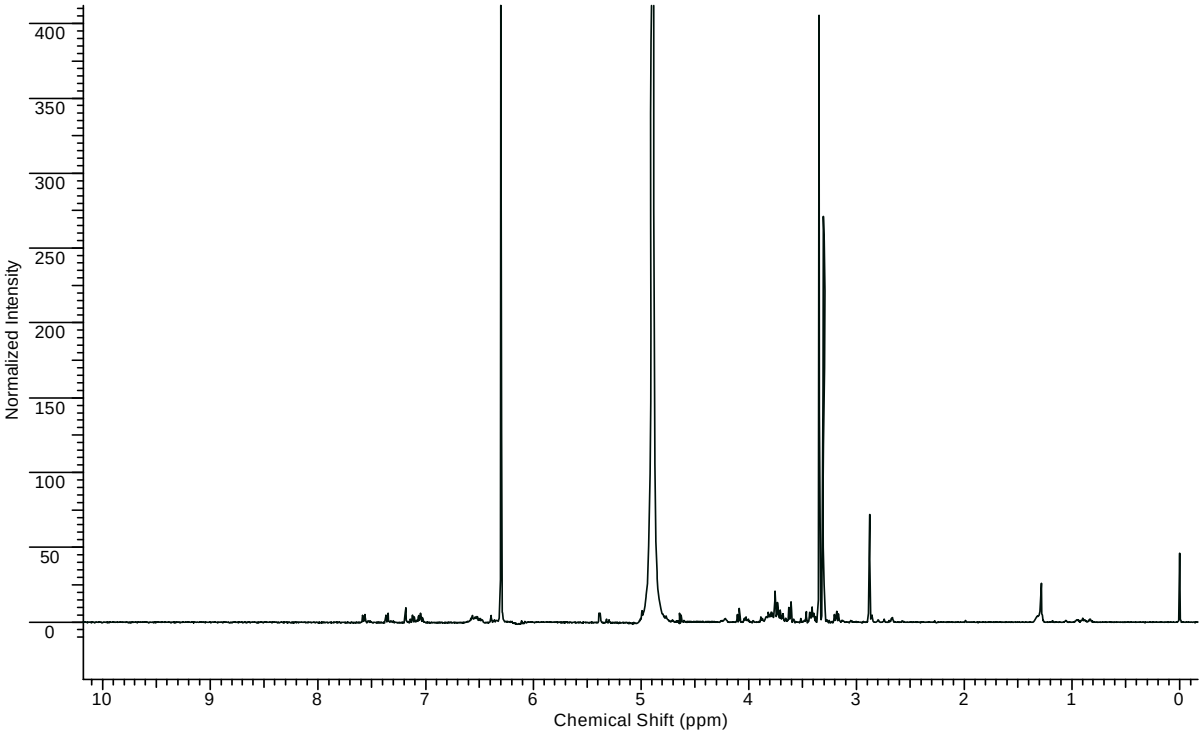

Figure 8a. NMR Quantitation of DMT (Methanol - Soxhlet Extract); See Table 2.

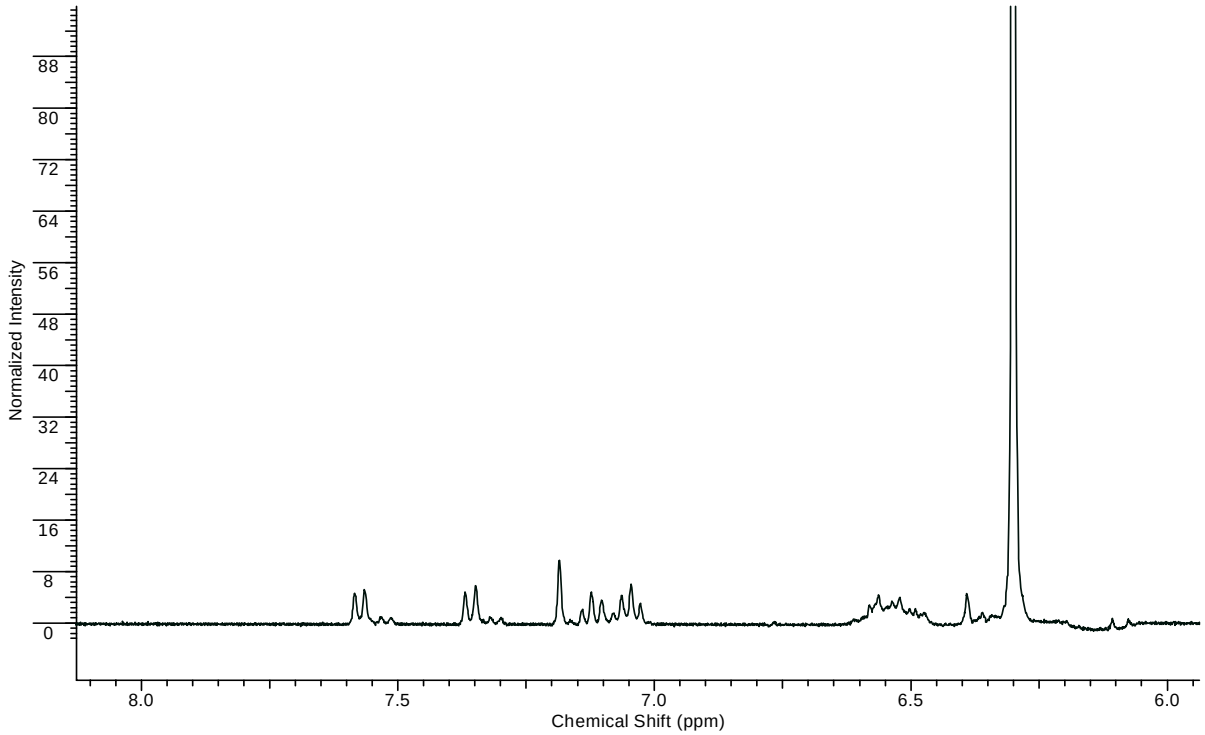

Figure 8b. NMR Quantitation of DMT (Methanol - Soxhlet Extract); Expansion; See Table 2.

Table 2. NMR Quantitation Results of DMT (Soxhlet Extract); See Figure 8				
Sample Amount (mg)	10450.0			
Molecular Weight of Sample	188.3			
Solvent	CD3OD			
Internal Standard (I.S.)	Maleic Acid			
Molecular Weight of I.S.	116.07			
I.S. Amount (mg)	6.118			
Peaks Chemical Shift (ppm)	[6.25..6.33]	[7.01..7.17]	[7.33..7.39]	[7.55..7.61]
Integral Value	32.58	2.575	1.032	0.999
Number of protons represented	2	1	1	1
Quantitation Value	Internal Standard	1.501%	0.601%	0.582%
Amount DMT in *Mimosa hostilis*	0.894%			

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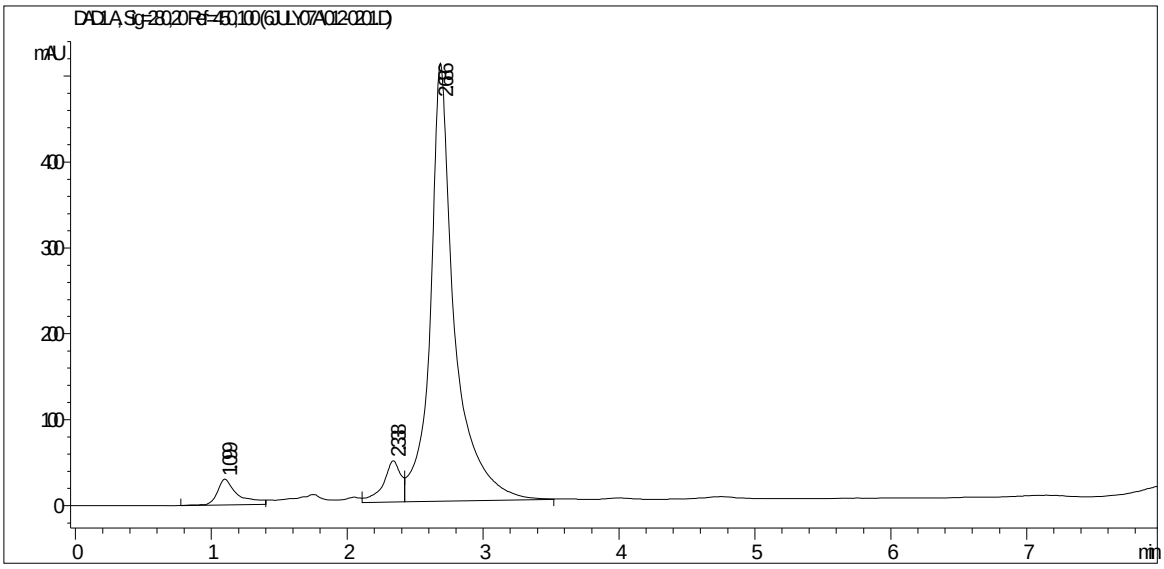

Figure 9. HPLC Quantitation of DMT (Methanol Extract); See Table 2.

Table 3. HPLC Quantitation of DMT (Methanol Extract); See Figure 9.							
	Concentration (mg/ml)	RT 1 (minutes)	RT 2 (minutes)	Area 1	Area 2	Average Area	Quant Value
DMT Standard	0.2924	2.685	2.702	2304.823	2366.984	2335.904	100.00
DMT Extract	99.00	2.686	2.688	6302.684	6339.690	6321.187	0.799

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