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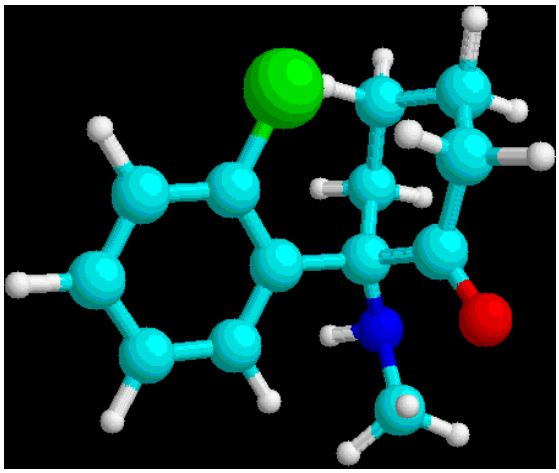

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Cover Art: "Ball and Stick" Model of Ketamine (Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).

Characterization of the "Indanylamphetamines"

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Presented in part at the Clandestine Laboratory Investigating Chemists Association 14th Annual Technical Training Seminar, Portland, Oregon, September 7 - 12, 2004.

ABSTRACT: Spectroscopic and chromatographic data are provided for 5-(2-aminopropyl)-2,3-dihydro-1H-indene **1** (the indane analog of 3,4-methylenedioxyamphetamine **2**), 4-(2-aminopropyl)-2,3-dihydro-1H-indene **3** (the aromatic ring positional isomer of **1**), and their respective synthetic intermediates. The data allow the identification and differentiation of **1** and **2** in illicit drug exhibits.

KEYWORDS: Indanylamphetamine, Amphetamine Analogs, Designer Drugs, Chemical Analysis, Forensic Chemistry.

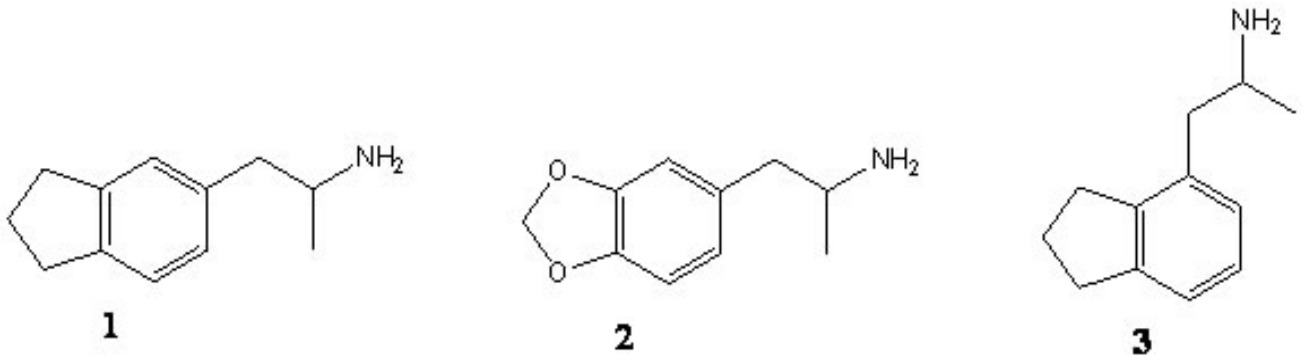


Figure 1. Structural Formulas

Introduction

Clandestine laboratory operators have synthesized so-called "designer" or "analog" drugs for many years in efforts to avoid prosecution under existing statutes, and/or to produce more powerful drugs or drugs with alternate central nervous system (CNS) and/or psychoactive properties. The production (and use) of such compounds are the focus of a wide variety of texts, literature articles, and websites. The best known texts in this field, including extensive syntheses of designer/analog drugs along with detailed reports of their CNS and/or psychoactive activity levels based on self-experimentation, are PIHKAL (Phenethylamines I Have Known And Loved) and TIHKAL (Tryptamines I Have Known And Loved) by Shulgin and Shulgin [1,2].

Currently, the methylenedioxyamphetamines (3,4-methylenedioxyamphetamine (MDA, **2**), 3,4-methylenedioxy-methamphetamine (MDMA), etc.) are the most popular and widely used CNS-active, psychoactive drugs on the illicit markets. Virtually all of the common MDA's are controlled under U.S. and international statutes, encouraging the production and use of designer/analog drugs.

Additional encouragement occurred in late 2000, when the seizure of the world's largest-ever lysergic acid diethylamide (LSD) synthesis laboratory, and the disruption of its associated distribution network [3], resulted in a major decline in LSD supplies worldwide, and an elevated demand for alternate hallucinogens. These have included traditional and well known substances such as psilocybin mushrooms, but also some unusual substances such as *Salvia divinorum* and many of the psychoactive phenethylamines and tryptamines featured in PIHKAL and TIHKAL.

Since about 2003, the indanyl analog of MDA, that is, 5-(2-aminopropyl)-2,3-dihydro-1H-indene **1** (also known as 1-(5-indanyl)-2-aminopropane, commonly abbreviated as 5-IAP or IAP (Figure 1)) has been submitted to forensic laboratories in the U.S., usually as suspected ecstasy (MDMA). 5-IAP is also commonly - but incorrectly - referred to as "indanylamphetamine" (probably a misinterpretation of the meaning of "IAP"). 5-IAP was first reported by the Nichols group in 1993 [4], and again in 1998 [5], in two studies focusing on its pharmacological activity.

Although the Nichols group does not so state, the synthesis of 5-IAP invariably produces a lesser quantity of its aromatic ring positional isomer, 4-(2-aminopropyl)-2,3-dihydro-1H-indene (4-IAP) **3**. Although 4-IAP is not known (or expected) to have significant CNS stimulant activity (and therefore has minimal abuse potential), its close structural similarity to 5-IAP, and its likely presence in exhibits containing illicitly prepared 5-IAP, merits detailed spectroscopic and chromatographic delineation of the two compounds.

Experimental

Chemicals and Reagents

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

Instrumentation

Gas Chromatography/Mass Spectrometry (GC/MS) - Mass spectra were obtained on an Agilent Model 5973 quadrupole mass-selective detector (MSD) that was interfaced with an Agilent Model 6890 gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV and a scan range of 34-700 amu at 1.34 scans/second. The GC was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with 0.25 µm DB-1 (J & W Scientific, Rancho Cordova, CA, USA). The oven temperature was programmed as follows: initial temperature, 100 ^{O}C; initial hold, 0.0 min; program rate, 6 ^{O}C/min; final temperature, 300 ^{O}C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) and a temperature of 280 ^{O}C. The auxiliary transfer line to the MSD was operated at 280 ^{O}C.

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

Nuclear Magnetic Resonance Spectroscopy (NMR) - Proton (^{1}H), carbon (^{13}C), and 2-dimensional NMR spectra were obtained on a Varian Inova 600 MHz NMR using a 5 mm Varian Nalorac Z-Spec broadband variable temperature, pulse field gradient probe (Varian, Palo Alto, CA). All compounds were dissolved in deuterochloroform ($CDCl_3$) containing 0.03 percent v/v tetramethylsilane (TMS) as the 0 ppm reference compound. The sample temperature was maintained at 25 ^{O}C. Standard Varian pulse sequences were used to acquire proton, proton-decoupled carbon, and gradient versions of COSY, HSQC, and HMBC. Data processing was performed using software from Varian and Applied Chemistry Development (ACD/Labs, Toronto, Canada). Prediction of proton and carbon spectra was accomplished using ACD/Labs HNMR and CNMR Predictors.

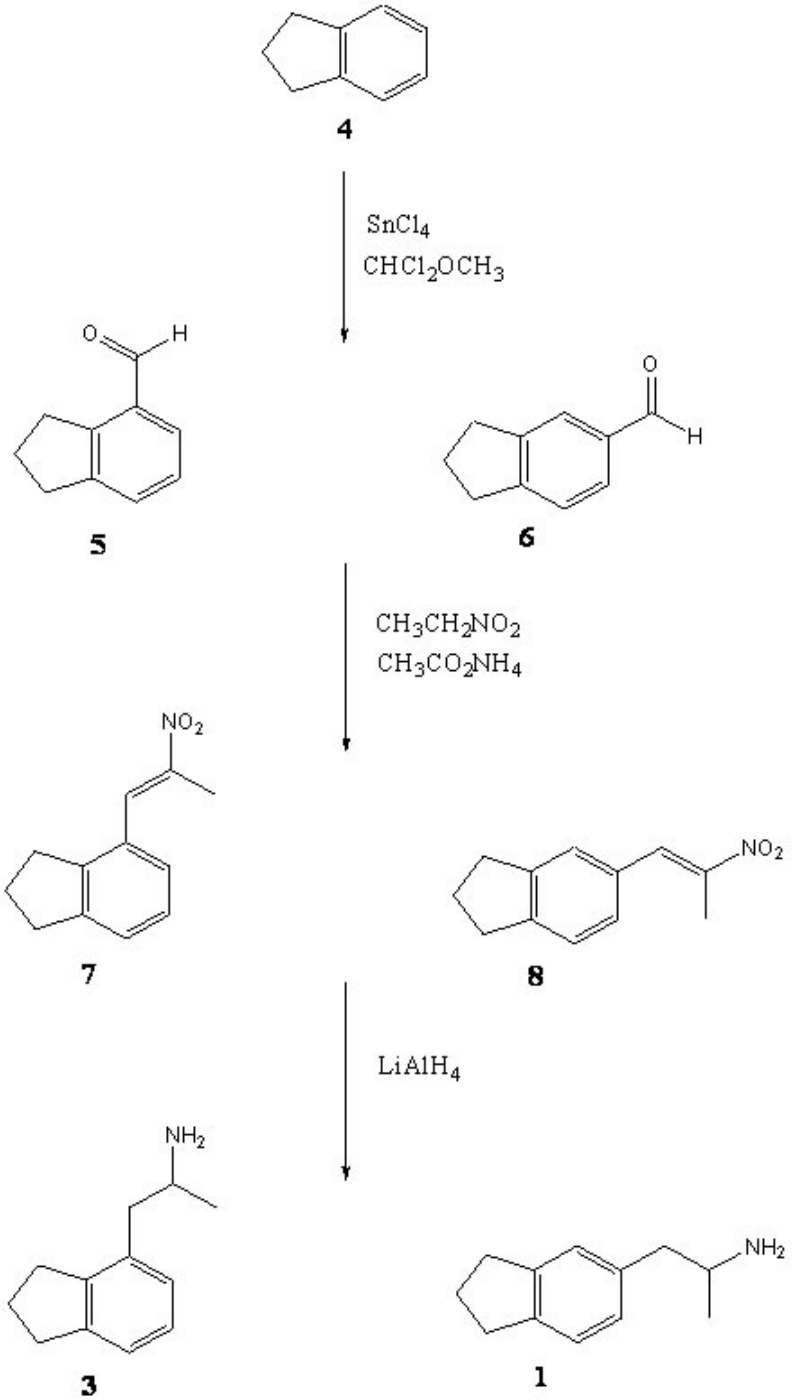
Syntheses

The procedure of Nichols *et al.* [4] was followed for the preparation of 5-IAP **1** and its intermediates. A modification of the same procedure was utilized to prepare 4-IAP **3** and its intermediates. Due to the sensitive nature of this subject, exact experimental details and yields are not reported.

Results and Discussion

The synthetic procedure described by Nichols *et al.* is the most convenient route to 5-IAP, but as previously noted it produces both 4-IAP and 5-IAP (see Figure 2). To summarize, indane **4** is formylated with $SnCl_4$ and dichloromethyl methyl ether to give a mixture of the aldehydes **5** and **6** in about a 15:85 ratio. If desired, the aldehydes can be separated via alumina column chromatography. Condensation of the aldehydes with nitroethane gave the nitropropenes **7** and **8**. Nitropropene **8** can be isolated from **7** by recrystallization from *n*-hexane at -76 ^{O}C. 4-IAP **3** and 5-IAP **1** are obtained from their respective nitropropenes **7** and **8** via $LiAlH_4$ reduction. If the intermediate products are not purified, the resulting final product will contain both 4-IAP **3** and 5-IAP **1** in about a 15:85 ratio.

Figure 2.



GC retention time data for the respective compounds are presented in Table 1. The amines were injected as their free bases since the hydrochloride ion-pairs of some phenethylamines undergo thermally induced degradation and chromatograph poorly [6]. 4-IAP and 5-IAP (15:85) are baseline resolved under the chromatographic conditions utilized (Figure 3).

Table 1: Gas Chromatographic Retention Times (min) for the "Indanylamphetamines" and their Synthetic Precursors. [a]

Compound	Retention Time
1	9.60
2	9.00
3	9.40
4	2.97
5	6.67
6	7.16
7	14.59
8	15.80

[a] Conditions given in Experimental Section.

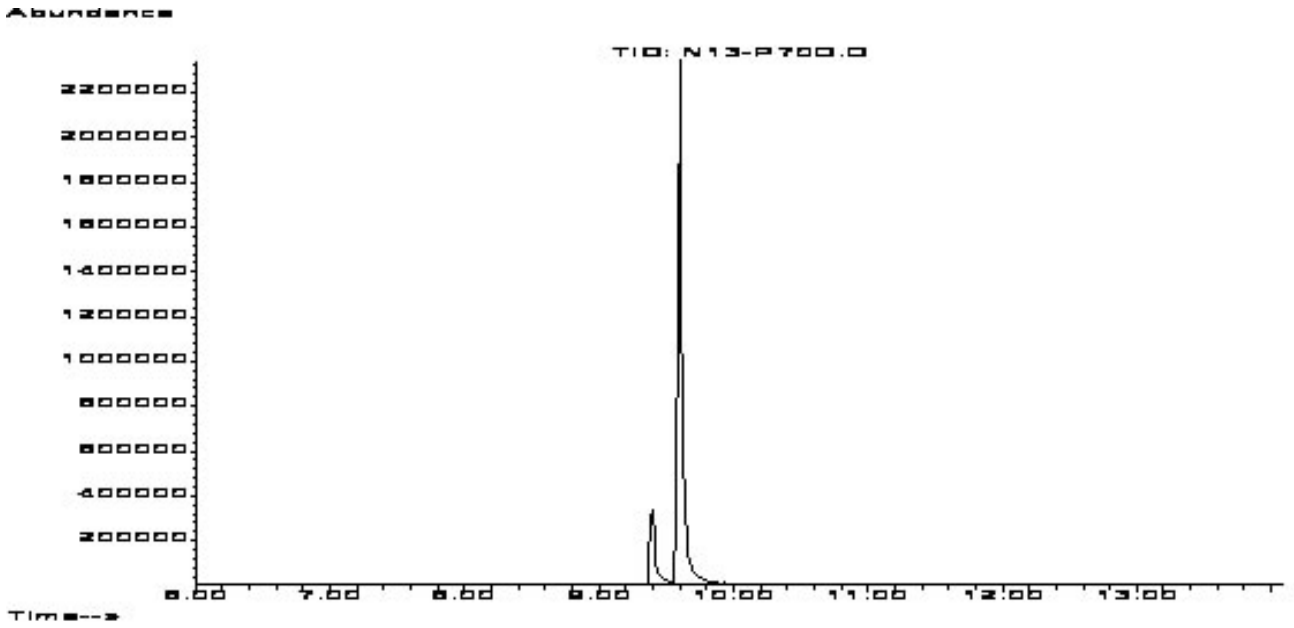


Figure 3. Partial Reconstructed Total Ion Chromatogram of a Mixture of 4-IAP and 5-IAP. Peaks: 1 = 4-IAP; and 2 = 5-IAP

The IR spectra for 4-IAP and 5-IAP are illustrated in Figure 4. Comparison of the hydrochloride ion pairs reveals similar absorption patterns with the most prominent, yet subtle, differences in the C-H out-of-plane bending frequencies between 700 - 900 cm^{-1}. However, since the spectra are quite similar, additional or supplementary spectroscopic methods should be utilized for definitive identification.

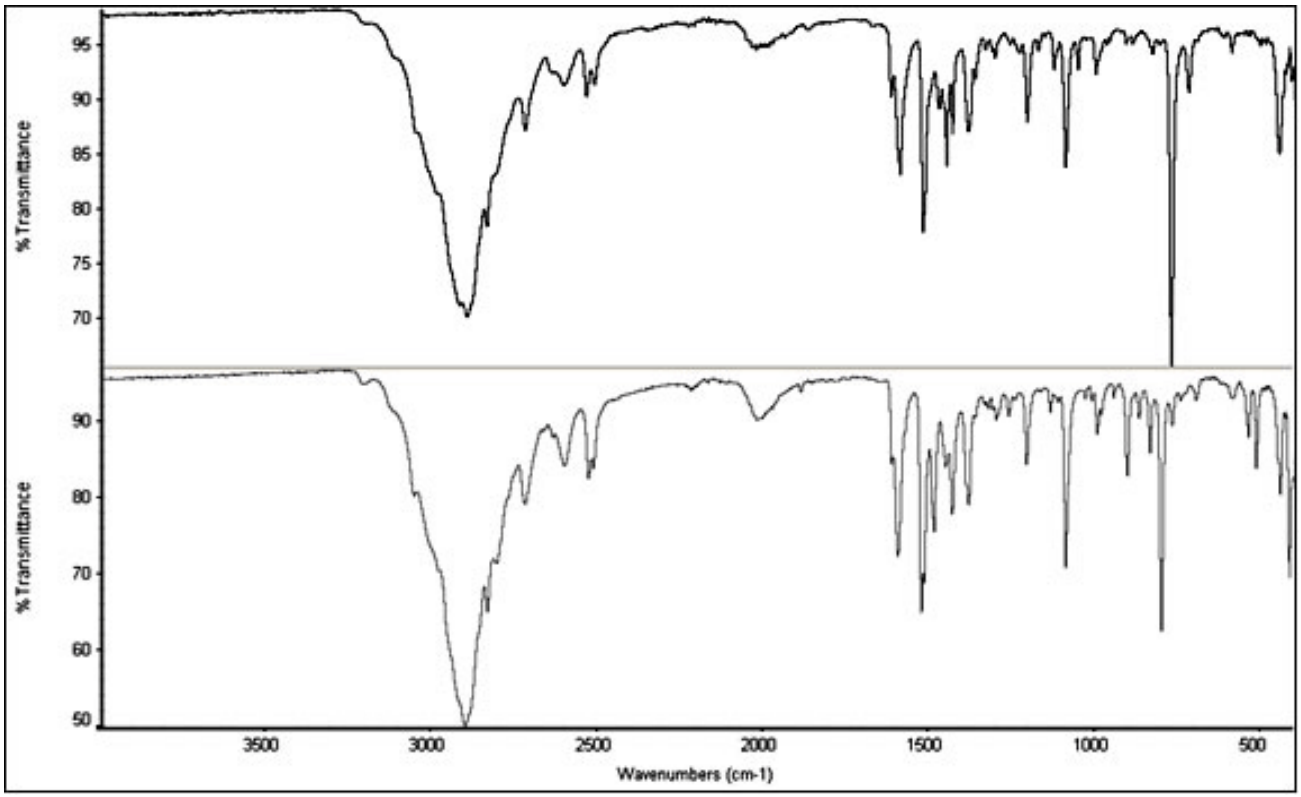


Figure 4. Infrared Spectra (FTIR-ATR) of 4-IAP HCl (upper) and 5-IAP HCl (lower).

Mass spectra for 4-IAP and 5-IAP, nitropropenes **7** and **8**, and aldehydes **5** and **6**, are presented in Figures 5 - 7, respectively (top and bottom traces). 4-IAP and 5-IAP each gave a base peak at *m/z* 44, but were easily distinguished by the relative abundances of ions at *m/z* 115 and 117 and also at *m/z* 128 and 131 (Figure 5). Both gave weak fragment ions as well as a weak molecule ion at *m/z* 175. The nitropropene intermediates **7** and **8** each gave base peak at *m/z* 115, but were easily distinguished by the relative abundances of ions at *m/z* 115 and 117 and also at *m/z* 141 and 145 (Figure 6). The aldehyde intermediates **5** and **6** each gave base peak at *m/z* 146, and were easily distinguished by the relative abundances of ions at *m/z* 145 and 146 (Figure 7).

The proton and carbon chemical shifts and splitting patterns for 4-IAP, 5-IAP, and their respective intermediates are presented in Tables 2 and 3, respectively. Assignments were based on proton and carbon chemical shift values, proton splitting patterns and coupling constants, and correlations between proton and carbon using the HSQC (directly bonded carbon-to-proton) and HMBC (2, 3, or 4 bond correlations between carbon and proton) experiments. The proton and carbon spectra for each structure were predicted using ACD/Labs HNMR and CNMR Predictors as an additional check. The substituent position on the indane ring was very easily determined using the aromatic proton splitting patterns. Substitution at carbon 4 resulted in 3 adjacent protons, giving a doublet, triplet, and doublet splitting pattern for the 5, 6, and 7 hydrogens, respectively. Substitution at carbon 5 resulted in a broad singlet (H-4) and two broad doublets (H-6 and H-7). The broadness of the singlet and the H-6 doublet is caused by a coupling constant less than one Hertz, typical of *meta* protons.

Conclusions

Analytical data is presented to assist delineating 4-IAP from 5-IAP, as well as their respective synthetic intermediates. Characterization is best achieved by GC/MS or NMR. Due to their similarities, the FTIR spectra should be supplemented with another spectroscopic method for definitive identification.

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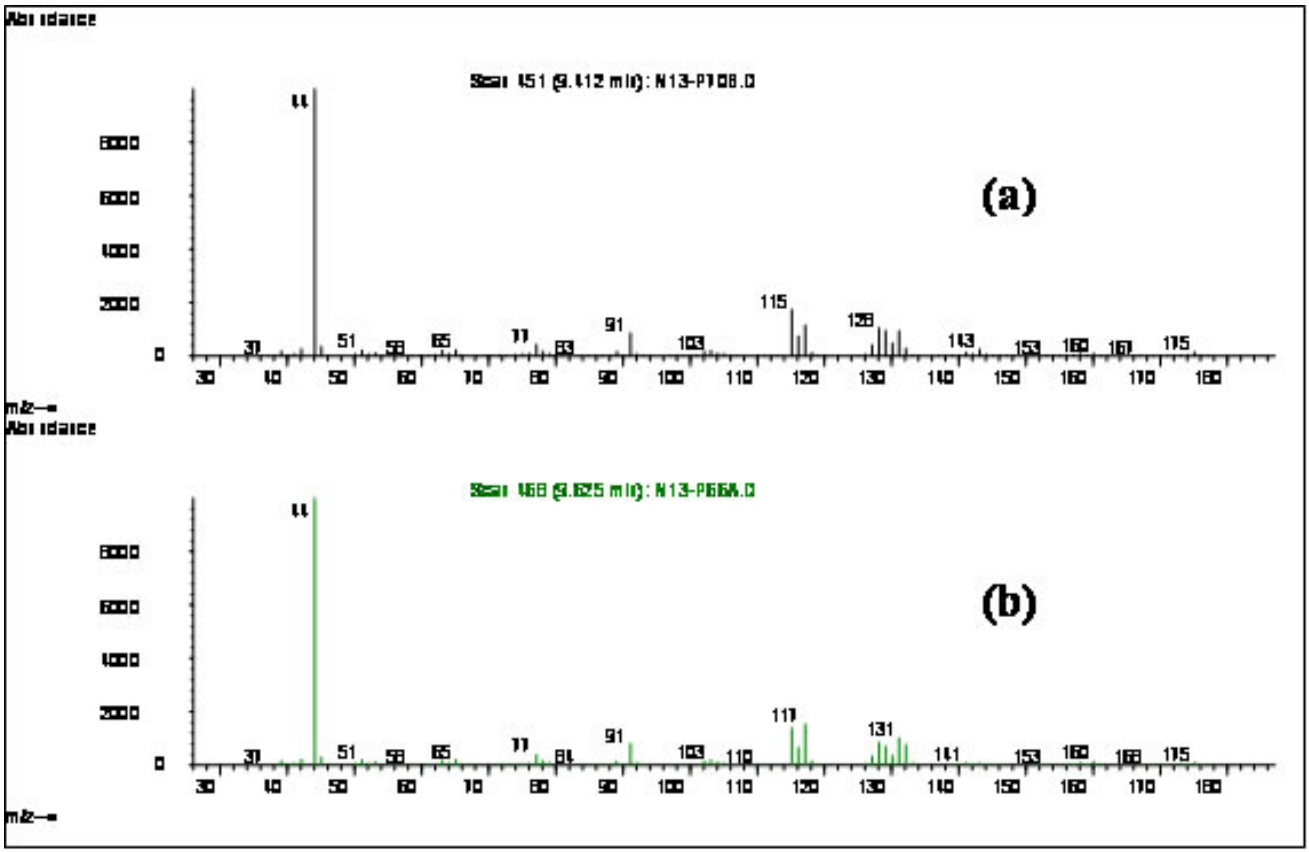

Figure 5. Electron Ionization Mass Spectra of (a) 4-IAP HCl and (b) 5-IAP HCl.

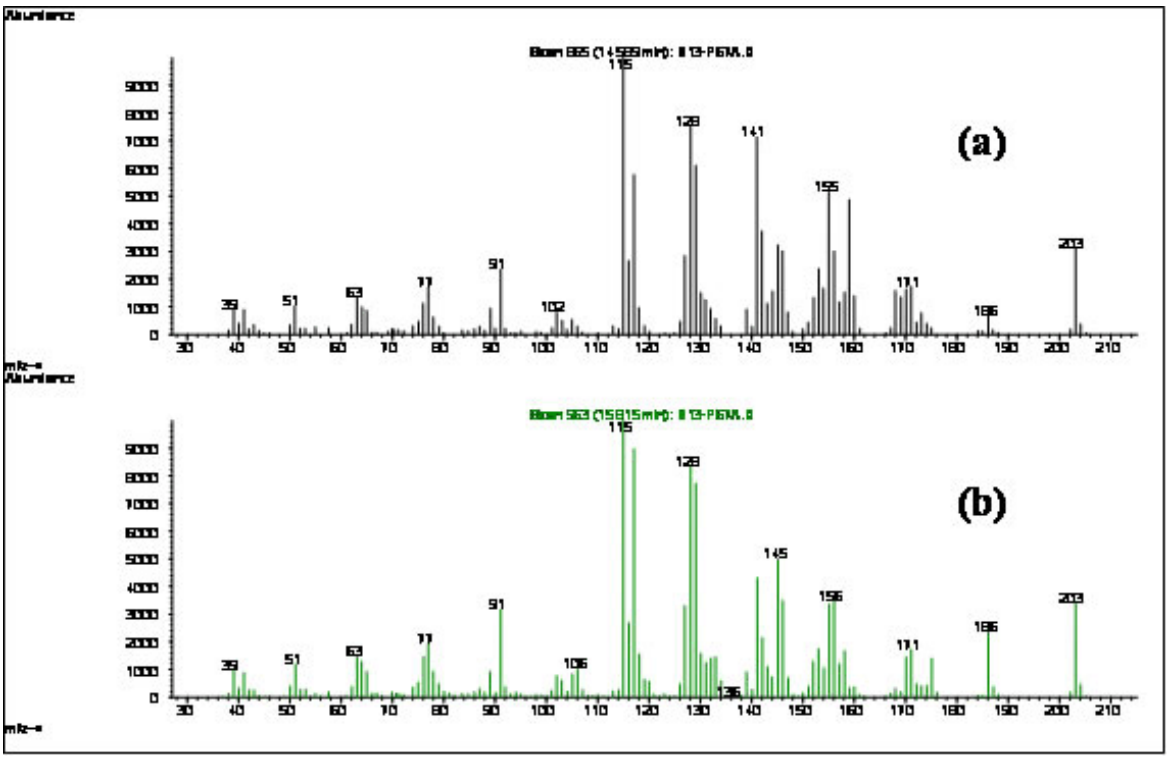

Figure 6. Electron Ionization Mass Spectra of (a) 4-[1-(nitropropenyl)]-2,3-dihydro-1H-indene **7** and (b) 5-[1-(nitropropenyl)]-2,3-dihydro-1H-indene **8**.

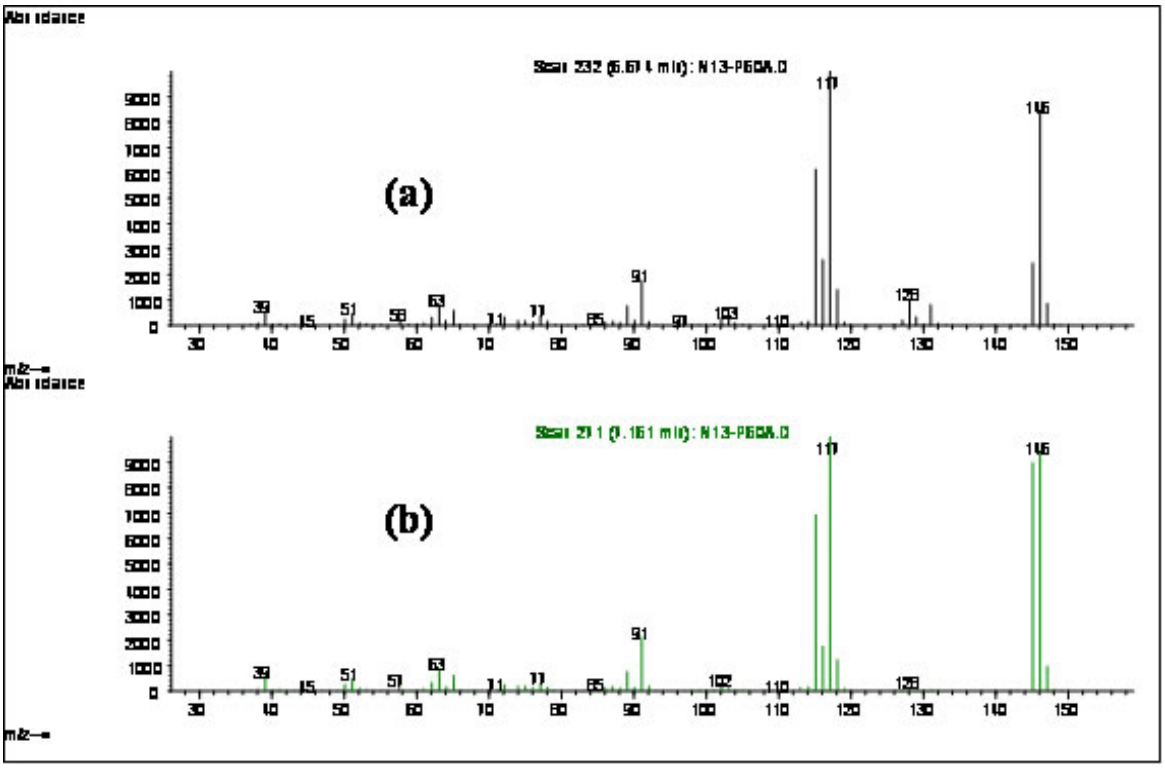

Figure 7. Electron Ionization Mass Spectra of (a) 2,3-dihydro-1H-indene-4-carboxaldehyde **5** and (b) 2,3-dihydro-1H-indene-5-carboxaldehyde **6**.

Table 2: NMR Proton Chemical Shifts (in ppm) and Splitting Patterns of 4-IAP HCl, 5-IAP HCl, and Related Compounds. Samples Run in $CDCl_3$ with TMS as the Reference Compound for 0 ppm.

Proton(s)	**1**	**3**	**5**	**6**	**7**	**8**
1	2.87 t	2.89-2.97 m	2.06 p	2.97 t	2.93 t	2.95 t
2	2.05 p	2.06 p	2.91 t	2.13 p	2.13 p	2.12 p
3	2.86 t	2.89-2.97 m	3.29 t	2.97 t	2.98 t	2.95 t
4	7.07 bs	--	--	7.73 bs	--	7.30 s
5	--	6.99 d	7.63 d	--	7.17 d	--
6	6.96 bd	7.09 t	7.32 t	7.66 bd	7.23 t	7.21 dd
7	7.14 d	7.13 d	7.47 d	7.36 d	7.30 d	7.29 d
	--	--	aldehyde 10.15 s	aldehyde 9.96 s	alkene 8.12 s	alkene 8.08 bs
CH3	1.38 d	1.40 d	--	--	2.40 s	2.46 s
CH2	2.82 dd 3.22 dd	2.86 dd 3.24 dd	--	--	--	--
CH	3.54 m	3.58 m	--	--	--	--
NH3+	8.46 bs	8.52 s	--	--	--	--

bd = broad doublet, bs = broad singlet, d = doublet, dd = doublet of doublets, m = multiplet, p = pentet, s = singlet, t = triplet.

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Table 3: NMR Carbon Chemical Shifts (in ppm) of 4-IAP HCl, 5-IAP HCl, and Related Compounds. Samples Run in $CDCl_3$ with TMS as the Reference Compound for 0 ppm.

Carbon	**1**	**3**	**5**	**6**	**7**	**8**
1	32.78	33.08	33.08	33.41	31.80	32.92
2	25.44	25.06	25.45	25.59	24.82	25.37
3	32.50	31.55	31.97	32.61	32.91	32.74
3a	145.03	143.2	146.60	145.51	145.1	145.17
4	125.35	131.64	132.8 **	125.40	128.68	125.95
5	133.48	127.22	129.42	135.49	125.91	130.33
6	127.16	126.71	126.9	129.13	126.57	128.47
7	124.62	123.42	130.14	125.03	126.12	124.84
7a	143.21	145.0	152.80	152.28	145.1	147.04
aldehyde	--	--	192.98	192.54	--	--
alkene CH	--	--	--	--	131.91	134.33
alkene quaternary	--	--	--	--	147.86	146.77
CH3	18.13	18.29	--	--	14.08	14.18
CH2	40.99	38.94	--	--	--	--
CH	50.03	48.89	--	--	--	--
** = chemical shift determined using HMBC experiment. Peak not visible in direct carbon experiment.						

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Laboratory Analysis of the Conversion of Pseudoephedrine to Methamphetamine From Over-the-Counter Products

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Presented in part at the American Pharmacists Association Annual Meeting, Orlando, Florida, April 1-5, 2005.

ABSTRACT: Two approaches to convert pseudoephedrine (PSE) to methamphetamine from over-the-counter (OTC) PSE products were examined. The first approach was two-step, and involved PSE extraction followed by conversion using the Birch method. Multiple-active products containing PSE and 2 - 4 actives were tested, including caplet, tablet, liquid, and liquid-filled softgel forms. The extent of conversion to methamphetamine varied among the extracts, and was up to 30.7 percent of the PSE present in the starting product. PSE extract conversion to methamphetamine was realized regardless of dosage form (i.e., whether solids, liquids, or liquid-filled softgels were used). The second approach involved direct conversion of PSE to methamphetamine using the Birch method. Materials tested included pure PSE powder, and also a combination of PSE plus an analgesic as either a powder mixture or as an OTC caplet. The extent of conversion to methamphetamine ranged from 54.1 to 67.7 percent of the PSE present in the starting material. These results provide scientific proof that PSE from solid and liquid OTC products can be converted to methamphetamine using either extraction or direct approaches (both employed by small clandestine laboratory operators). The ease and extent of PSE conversion from extracts appears to be independent of the PSE starting quantity, dosage forms, and presence of other actives.

KEYWORDS: Acetaminophen, Birch Method, Dextromethorphan, Extraction, Guaifenesin, Methamphetamine, Nazi Method, OTC PSE Products, Pseudoephedrine, Forensic Chemistry

Introduction

Methamphetamine abuse has reached widespread proportions in the United States, causing serious social, economic, and environmental problems for communities, and draining scarce law enforcement resources (1-4). Currently, thousands of small toxic laboratories (STLs) are seized annually throughout the country, especially in the Midwest and western states. With very few exceptions, these laboratories are not operated by professional chemists, but rather by “cooks” who have learned from other “cooks”, the Internet (5), or from underground publications (6). Production scales are typically one ounce or less, and are intended for personal use and/or limited distribution. It is estimated that approximately 35 percent of the methamphetamine used in the United States comes from small-scale laboratories (7).

At present, the most popular precursor used for the clandestine manufacture of methamphetamine is pseudoephedrine (PSE) contained in over-the-counter (OTC) sinus and cold preparations (8). The preferred

starting material has been single-ingredient tablets (i.e., containing no other active ingredients). The PSE in these products are extracted with alcohol, filtered, and converted to methamphetamine via the Birch reduction method (Nazi method) or one of the red phosphorus methods (9,10). The first step in this study was therefore to determine the efficiency of extracting PSE from a variety of dosage forms, with subsequent conversion to methamphetamine using the Birch reduction method, which is currently the most popular among STL "cooks." The second approach was to directly convert PSE or PSE-containing OTC products to methamphetamine, without the preliminary extraction step, again using the Birch reduction method.

Experimental

The described experiments were conducted by National Medical Services, Willow Grove, Pennsylvania, an independent forensic laboratory accredited by the American Society of Crime Laboratory Directors – Laboratory Accreditation Board (ASCLD-LAB).

Pseudoephedrine Extraction Followed by Birch Method Conversion

A simple extraction process was performed on three multiple-active ingredient OTC products, each of which contained PSE, the pain reliever acetaminophen, and up to two other active ingredients (Table 1). The process involved grinding the tablets or caplets (modified for liquid-filled softgels), dissolving the resulting powder or liquid in denatured ethanol, filtering to isolate the solution, evaporating it to a small volume, adding acetone to precipitate the PSE, and collecting the precipitate by filtration. The procedure was conducted on a large scale (equivalent to 7.5 grams of PSE (i.e., 250 tablets/caplets or 100 liquid-filled softgels)) to simulate a typical small-scale illicit methamphetamine synthesis. The recovered PSE was directly submitted to the Birch reduction method. The method involves dissolution of the PSE in anhydrous ammonia, and then adding lithium metal (9). Quantitative analysis of the recovered PSE (and other active ingredients) from the first step, and of the methamphetamine and unreacted PSE from the second step, were performed using liquid chromatography/tandem mass spectrometry (LC/MS/MS).

Because illicit laboratories are known to employ additional extraction techniques (in addition to the method described above), five multiple-active OTC products, each of which contained PSE, acetaminophen, and up to 2 additional active ingredients (Table 1), were submitted to a more complex extraction process. This latter procedure involved dissolution of the sample in dilute hydrochloric acid, washing with a naphtha-based organic solvent to remove polymers, waxes, and other inert ingredients, alkalinization to form PSE base, two extractions with toluene to isolate the PSE base, and then conversion of the PSE base back to the HCl salt. The complex extraction was also conducted on a 7.5 gram PSE scale (250 caplets, 100 softgels, or 600 mL of liquid). quantitative analysis of the extracts and synthesized methamphetamine was again conducted by LC/MS/MS.

Direct Birch Method Conversion

Pure PSE powder, a mixture of PSE and acetaminophen powder, and ground caplets containing PSE and acetaminophen were directly subjected to the Birch reduction method, and the resulting products were subjected to quantitative analysis by LC/MS/MS. The quantity of PSE in each of the starting materials was 0.2 grams, 0.2 grams, and 0.18 grams, respectively.

Results and Discussion

Pseudoephedrine Extraction Followed by Birch Method Reduction

The results demonstrated PSE conversion to methamphetamine from all of the PSE precipitates subjected to the Birch method reduction (Table 2). The percentage of PSE in the starting OTC product that was converted to methamphetamine ranged from 0.3 to 30.7 percent. The conversion efficiency was comparable for PSE solid forms (ranging from 3.0 to 100 percent of the PSE in the extract being converted to methamphetamine) and PSE liquid forms (ranging from 37.5 to 100 percent of PSE in the extract being converted to methamphetamine). The starting quantity of PSE and the presence of other ingredients did not appear to affect the extent of conversion.

Direct Birch Method Conversion

Direct conversion of pure PSE powder was performed as a baseline reference. A second, powdered mixture of PSE (0.2 g) and acetaminophen (1.0 g) was used to assess the potential for interference from acetaminophen in the conversion process (since many of the OTC medications contain a high proportion of acetaminophen). A third experiment using an OTC tablet product containing PSE and acetaminophen was used to further verify the potential of direct conversion (6 tablets containing 0.18 g PSE and 3.0 g acetaminophen were ground using a mortar and pestle). The results confirmed PSE conversion to methamphetamine from all PSE forms, with 54.1 to 67.7 percent conversion of PSE to methamphetamine (Table 2). The results for PSE plus acetaminophen (i.e., powder and caplet) were comparable to those of PSE powder alone. Based on the results from the PSE extraction, it would be expected that direct conversion would also succeed for liquid and soft-gel forms.

Conclusions

The results of this study demonstrated that OTC sinus and cold preparations containing PSE can be converted to methamphetamine either directly or following a PSE extraction procedure, from both single- and multiple-active ingredient formulations. This study provides scientific proof that virtually any OTC product containing PSE can be used to manufacture methamphetamine (11,12). That is, the potential for methamphetamine production from PSE-containing OTC products is independent of dosage form (solid, liquid, or liquid-filled softgel), presence of actives, and formulation variations.

Acknowledgements

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Table 1. Active Ingredients Per Dosage Unit Included in Tested OTC PSE Products.

Product Identifier	Dosage Form	Decongestant (mg)	Analgesic (mg)	Antihistamine (mg)	Antitussive (mg)	Expectorant (mg)
PSE Simple Extraction Followed by Birch Method Conversion						
1	Caplet	PSE (30)	APAP (500)	-	-	-
2	Tablet	PSE (30)	APAP (325)	-	-	GUA (200)
3	Liquid Filled Softgel	PSE (30)	APAP (250)	-	DXM (10)	GUA (100)
PSE Complex Extraction Followed by Birch Method Conversion						
1	Caplet	PSE (30)	APAP (500)	-	-	-
4	Caplet	PSE (30)	APAP (500)	CLR (2)	-	-
5	Caplet	PSE (30)	APAP (325)	-	DXM (15)	GUA (200)
3	Liquid Filled Softgel	PSE (30)	APAP (250)	-	DXM (10)	GUA (100)
6	Liquid	PSE (30)	APAP (500)	DOX (6.25)	DXM (15)	-

APAP - Acetaminophen; CLR - Chlorpheniramine; DOX - Doxylamine; DXM - Dextromethorphan; GUA - Guaifenesin; OTC - Over-the-Counter; PSE - Pseudoephedrine.

Table 2. Results of Methamphetamine Conversion of OTC PSE Products.

Product Identifier	Dosage Form	Quantity of PSE in Starting Product/ Material (grams)	Percent PSE in Extract from Product	Methamphetamine Conversion	
				Percent PSE in Extract Converted to Methamphetamine	Percent PSE in Starting Products/ Material Converted to Methamphetamine
PSE Simple Extraction Followed by Birch Method Conversion					
1	Caplet	7.5	11.0	3.0	0.3
2	Tablet	7.5	6.5	7.7	0.5
3	Liquid Filled Softgel	3.0	14.8	37.5	5.6
PSE Complex Extraction Followed by Birch Method Conversion					
1	Caplet	7.5	30.7	100.0	30.7
4	Caplet	7.5	25.2	61.4	15.5
5	Caplet	7.5	41.1	51.4	21.1
3	Liquid Softgel	3.0	5.5	84.6	4.7
6	Liquid	1.2	5.4	100.0	5.4
PSE Conversion by Direct Birch Method (No PSE Extraction Step)					
PSE HCl Powder		0.2			67.7
PSE HCl + APAP Powder		0.2			65.5
PSE HCl + APAP Caplet		0.18			54.1

APAP - Acetaminophen; OTC - Over-the-Counter; PSE - Pseudoephedrine.

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Spectral Characterization of 2,4-Dimethoxy-3-methylphenethylamine, and Comparison to 2,5-Dimethoxy-4-methylphenethylamine ("2C-D")

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ABSTRACT: Synthesis and analytical data for 2,4-dimethoxy-3-methylphenethylamine (**2**) and its hydrochloride salt (**3**) are described. **2** was synthesized from 2,4-dimethoxy-3-methylbenzaldehyde via *trans*-2,4-dimethoxy-3-methyl-β-nitrostyrene (**1**). The compounds were characterized by ^{1}H NMR, ^{13}C NMR, GC/MS, and FTIR. The data was compared to 2,5-dimethoxy-4-methylphenethylamine (2C-D).

KEYWORDS: Designer Drugs, Dimethoxyphenethylamines, Synthesis, Isomescaline, 2C-D, Desoxy, TIM, Forensic Chemistry

Introduction

A large number of phenethylamines derivatives are known, many of which have been reported to have CNS-stimulant and/or psychoactive properties.[1] As a result, many phenethylamines compounds are listed as controlled substances. Notably, for each of these controlled substances are various possible isomers differing only in the positioning of the phenyl substituents. These positional isomers and analogues are (with few exceptions) not formally controlled; however, they may be prosecuted under the Analogue Statute of the Controlled Substances Act.

Examples of positional isomers that have circulated in the chemical underground are 2,5-dimethoxy-4-methylphenethylamine HCl (also known as "2C-D") and 3,5-dimethoxy-4-methylphenethylamine HCl (also known as "DESOXY").[1] Recently, an exhibit containing 2C-D was received at this laboratory. Interestingly, the ^{1}H NMR spectrum of 2C-D displays two singlets in the aromatic region that could potentially be confused for a doublet, albeit with a suspiciously large vicinal coupling constant (10 Hz). Trisubstituted phenethylamines may only form vicinally-derived doublets in the aromatic region if the phenyl substituents are arranged such that the two aromatic protons are *alpha* to each other.

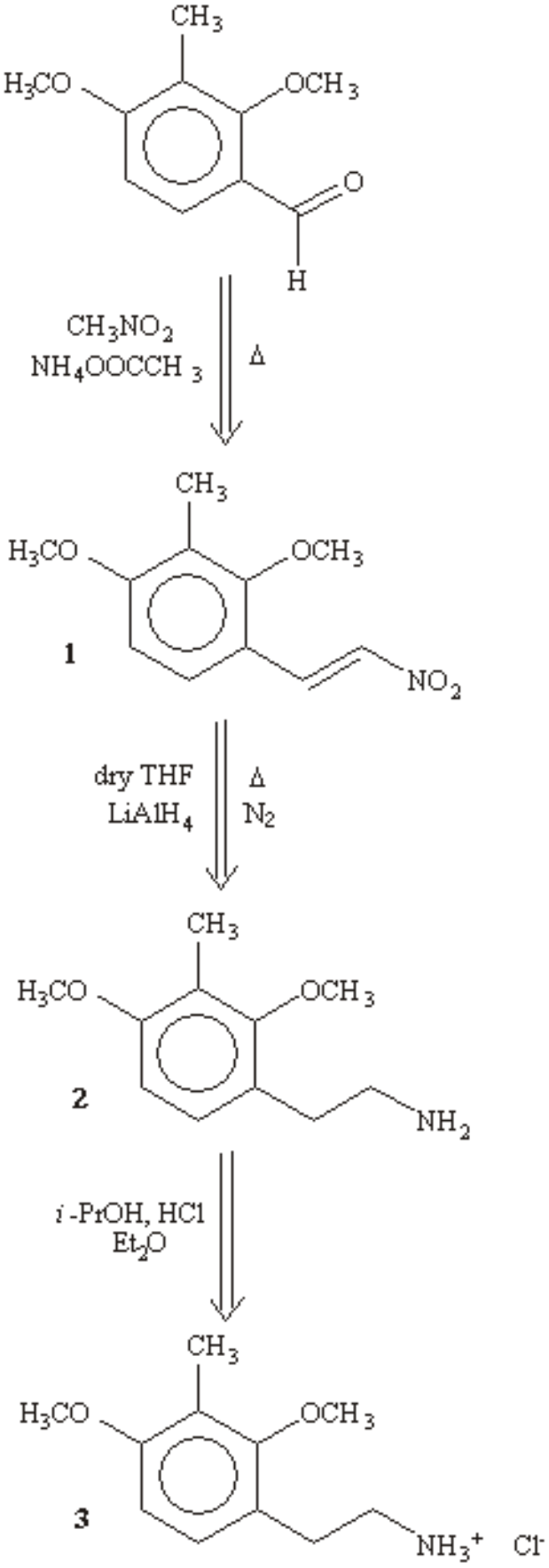
An example of an isomer of 2C-D having adjacent phenyl protons is 2,4-dimethoxy-3-methylphenethylamine HCl (**3**).[2] While NMR spectral differences between **3** and 2C-D can be predicted, it was preferable to demonstrate these differences from actual data.

The synthesis of 2,4-dimethoxy-3-methyl-β-nitrostyrene (**1**), 2,4-dimethoxy-3-methylphenethylamine (**2**), and **3** was originally reported by Merchant, *et al*.[2] and is provided herein along with new spectroscopic data (Scheme 1). In addition, the analytical results are compared to those of the recently received 2C-D exhibit.

Experimental

Reagents: All reagents and solvents were obtained from commercial sources and unless otherwise noted were used as received. Tetrahydrofuran was dried with Na/benzophenone and distilled under nitrogen prior to use.

Scheme 1.


CH3
H3CO
OCH3
O
H
CH3NO2
NH4OOCCH3
Δ
CH3
H3CO
OCH3
1
NO2
dry THF
LiAlH4
Δ
N2
CH3
H3CO
OCH3
2
NH2
i-PrOH, HCl
Et2O
CH3
H3CO
OCH3
3
NH3+
Cl-

Instrumentation: FTIR spectra were recorded on a Nexus 470 FTIR Spectrometer fitted (where noted) with a 3-bounce diamond ATR from SensIR Technologies. ^{1}H and $^{13}C\{^{1}H\}$ (proton-decoupled) NMR spectra were recorded at 24(±1) ^{O}C on a Varian Mercury 400 NMR Spectrometer. Chemical shifts (in ppm) are referenced to the residual solvent peak ($CHCl_3$, ^{1}H: δ 7.24 (singlet); (CHD_2OD, ^{1}H: 3.30 (quintet); $CDCl_3$, $^{13}C\{^{1}H\}$: 77.0 (triplet); CD_3OD, $^{13}C\{^{1}H\}$: δ 49.0 (septet) ppm). Mass spectral data were obtained from an Agilent 6890 Gas Chromatograph equipped with a ZB-1 column of 30 m x 0.25 mm with a film thickness of 0.25 μm, and equipped with an Agilent 5973N Mass Selective Detector in electron impact mode. The GC had an injector temperature of 250 ^{O}C, and was oven programmed with initial temperature of 100 ^{O}C increased at 35 ^{O}C per minute to 295 ^{O}C (held 6.43 min). The mass spectrum was scanned from *m/z* 34 to 500.

2,4-Dimethoxy-3-methyl-β-nitrostyrene (1-(2,4-dimethoxy-3-methylphenyl)-2-nitroethene) (1): To a nitromethane solution (30 mL) of anhydrous ammonium acetate (1.0 g, 13 mmol) was added 2,4-dimethoxy-3-methylbenzaldehyde (8.0 g, 44 mmol). The resulting mixture was stirred and heated for 20 minutes at light reflux. The solvent was then removed under reduced pressure (via rotary evaporator) while warming. The resulting orange solid was recrystallized from isopropanol, collected by vacuum filtration, and dried under vacuum (8.3 g, 85% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12 (d, J=13.7 Hz, 1H), 7.72 (d, J=13.7 Hz, 1H), 7.34 (d, J=8.8 Hz, 1H), 6.69 (d, J=8.6 Hz, 1H), 3.87 (s, 3H), 3.74 (s, 3H), 2.15 (s, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100.6 MHz): δ 162.4, 159.8, 136.0, 135.5, 129.1, 121.0, 116.3, 106.8, 61.3, 55.9, 9.0 (11 signals expected and observed) ppm. FTIR (KBr, cm^{-1}): 1621 ($\nu_{C=C}$ str), 1597 ($\nu_{aromatic\ C=C}$ str), 1334 (ν_{NO2} sym str), 1109 (ν_{C-O-C} sym str). FTIR (ATR, cm^{-1}): 1622 ($\nu_{C=C}$ str), 1591 ($\nu_{aromatic\ C=C}$ str), 1336 (ν_{NO2} sym str), 1107 (ν_{C-O-C} sym str). GC/MS: Rel. Rt: 2.00 (relative to methamphetamine), *m/z* (assignment): 223 (M^{+}), 176 (base peak).

2,4-Dimethoxy-3-methylphenethylamine (2): To a 500 mL round bottom flask was added 2.1 g $LiAlH_4$ (56 mmol) and 70 mL dry THF. Under a nitrogen atmosphere was slowly added (via an addition funnel) 2.5 g **1** (11 mmol) dissolved in 60 mL dry THF. The resulting solution was heated at reflux with stirring under a nitrogen atmosphere for 7 hours. After cooling the reaction mixture to ambient temperature, an equal volume of water (130 mL) was added, with the initial addition being done drop wise to minimize the vigorous reaction. The reaction mixture was extracted with EtOAc (4 x 90 mL); each extract was dried with Na_2SO_4, filtered, and combined. Removal of the solvent under reduced pressure resulted in a pale yellow oil as the crude product. This oil was redissolved in 10 mL CH_2Cl_2 and extracted with several fractions (3 - 4 mL each) of aqueous HCl (pH 2-3) until the pH of the final aqueous fraction did not increase (the latter was discarded). The combined aqueous fractions were base extracted with 2 M NaOH and CH_2Cl_2. The organic layer was collected and removal of the solvent under reduced pressure yielded 1.3 g of a clear oil (58% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.95 (d, J=8.2 Hz, 2H), 6.57 (d, J=8.4 Hz, 2H), 3.77 (s, 3H), 3.68 (s, 3H), 2.89 (t, J=7.0 Hz, 2H), 2.69 (t, J=7.0 Hz, 2H), 2.13 (s, 3H), 1.8 (br-s, N-H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100.6 MHz): δ 157.5, 157.2, 127.2, 124.5, 119.6, 106.0, 60.6, 55.5, 43.1, 34.0, 9.1 (11 signals expected and observed) ppm. FTIR (neat/NaCl, cm^{-1}): 3366 (ν_{NH} str), 3296 (ν_{N-H} str), 1602 (ν_{N-H} bend), ~1590 (sh, $\nu_{aromatic\ C=C}$ str), 1268 (ν_{C-N} str), 1108 (ν_{C-O-C} sym str). FTIR (ATR, cm^{-1}): 3371 (ν_{N-H} str), 3289 (ν_{N-H} str), 1601 (ν_{N-H} bend), ~1590 (sh, $\nu_{aromatic\ C=C}$ str), 1266 (ν_{C-N} str), 1103 (ν_{C-O-C} sym str). GC/MS: Rel. Rt: 1.57 (relative to methamphetamine), *m/z* (assignment): 195 (M^{+}), 166 (base peak).

2,4-Dimethoxy-3-methylphenethylamine HCl (3). To a test tube of 0.34 g **2** (1.8 mmol) dissolved in ~6 mL isopropanol was added 5-6 drops of concentrated HCl and mixed well. Crystallization was induced by addition of 0.5 mL Et_2O and cooling to ~2 ^{O}C for 2 hours. The resulting mixture was decanted, and the resulting crystalline solid was rinsed with diethyl ether and dried under vacuum, yielding 0.25 g of a white crystalline solid (63% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.32 (br-s, N-H, 3H), 7.00 (d, 8.4 J=8.4 Hz, 1H), 6.57 (d, J=8.2 Hz, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.22 (br-m, 2H), 3.01 (t, J=7.1 Hz, 2H), 2.11 (s, 3H); ^{1}H NMR (CD_3OD, 400 MHz): δ 7.05 (d, J=8.4 Hz, 1H), 6.71 (d, J=8.4 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.11 (t, J=7.6 Hz, 2H), 2.91 (t, J=7.6 Hz, 2H), 2.13 (s, 3H); $^{13}C\{^{1}H\}$ NMR (CD_3OD, 100.6 MHz): δ 159.7, 158.8, 128.9, 122.6, 120.9, 107.6, 61.3, 56.1, 41.6, 29.4, 9.4 (11 signals expected and observed) ppm. FTIR (KBr, not assigned). FTIR (ATR, not assigned).

Results and Discussion

Synthesis of **1** involved a condensation/dehydration of the precursor 2,4-dimethoxy-3-methylbenzaldehyde with nitromethane in the presence of ammonium acetate (Scheme 1). Recrystallization from isopropanol provided yellow crystals of **1** in good yield. The mass spectrum of **1** (Figure 1) is consistent with its structure.

The ^{1}H NMR spectrum of **1** (Figure 2) is consistent with formation of the expected, more stable *trans* isomer as evidenced by downfield chemical shifts and relatively large vicinal coupling constants compared to those typically found in the *cis* counterparts. The coupling constants for the alkene protons are slightly depressed with respect to comparable *trans* compounds due to the added electron-withdrawing effect of the nitro group.

The IR (Figures 3 and 4) spectral assignments also support the *trans* isomer of **1** based upon the work of By *et al*.[3] (wherein related β-methyl-β-nitrostyrenes were compared and characterized by IR/Raman spectroscopy). Notably, the lower frequency for the ethylenic C=C stretching mode of **1**, compared to the β-methyl-β-nitrostyrenes, can be accounted for by increased conjugation with the aromatic ring in the absence of the sterically hindering β-methyl group, allowing for a more planar conformation. On the other hand, the higher frequency observed for the symmetric NO_2 stretching band of **1** can be explained by the absence of the electron donating β-methyl group.

The free base form of **2** was obtained from reduction of **1** with $LiAlH_4$ in dry THF under an inert atmosphere (Scheme 1). The crude oily product obtained after work up of the reaction mixture was shown to contain minor amounts of impurities. Surmising that the desired product might have differing pKa value(s) from those of the impurities, **2** was successfully isolated by acid extraction with careful control of pH, followed by basic extraction. The MS, FTIR, ^{1}H NMR spectra (Figures 5, 6, and 7, respectively) were consistent with the formation of **2**.

Conversion of **2** to its hydrochloride salt was done from an isopropanolic solution mixed with a small amount of concentrated hydrochloric acid and diethyl ether, yielding a white, crystalline solid (Scheme 1) of **3**. The IR spectra of **3** (Figures 8 and 9) are complicated by the broad and numerous bands displayed, particularly in the region between 3500 - 2000 cm^{-1}, as is expected for hydrated primary amine salts.[4]

The ^{1}H NMR spectrum (Figure 10) exhibits a broad peak for the protonated amine at 8.32 ppm. Despite extensive drying of the crystalline material under vacuum, a water peak is still observed at ~1.7 ppm, likely due to the inclusion of a hydrogen bonded water molecule in the crystalline lattice of **3**, suggesting the formation of a hydrate complex upon crystallization. Addition of 1 - 2 drops CD_3OD to a $CDCl_3$ solution of **3** results in a shift of the H_2O peak downfield ~1.5 ppm as CD_3OH is formed. In CD_3OD, the ^{1}H NMR spectrum (Figure 11) of **3** lacks peaks for the exchangeable amino and water protons. Due to a reduced solubility relative to **2** in chloroform solution, the ^{13}C NMR spectrum of **3** was obtained in deuterated methanol.

Not surprisingly, the FTIR and mass spectra of **3** and 2C-D are fairly similar. However, differences in the substitution patterns on the phenyl ring make these compounds readily distinguishable by ^{1}H NMR, as displayed in the spectrum (Figure 12) of the 2C-D exhibit received into this lab. The most distinguishing features are the two singlets of the phenyl protons in the spectrum of 2C-D, at 6.69 and 6.66 ppm, whereas **3** displays two doublets at 7.00 and 6.57 ppm, respectively.

It should be noted that closely related analogues such as 2,3,4-trimethoxyphenethylamine (also known as "isomescaline") and 2,4-dimethoxy-3-thiomethylphenethylamine (also known as "TIM") have been reported to be "non-active" (that is, having no noticeable pharmacological effects on the user.[1]) The isostructural nature of **3** with these pharmacologically inactive compounds suggests that it is likewise inactive. However, because other dimethoxy/methyl-substituted phenethyamine isomers of **3** (e.g., 2C-D and DESOXY) are psychoactive, the situation is unclear. Regardless, these and other possible isomers can be readily distinguishable by NMR.

Acknowledgments

Notably, the concept for this project originated from former DEA Southeast Laboratory Director William D. Beazley. The author would like to thank Senior Forensic Chemist Patrick A. Hays, DEA Special Testing and Research Lab (Dulles, Virginia) for helpful information regarding the ^{1}H NMR spectrum of 2C-D. Also, appreciation goes to DEA Librarian Rosemary Russo (Arlington, Virginia) for searching for and providing literature references.

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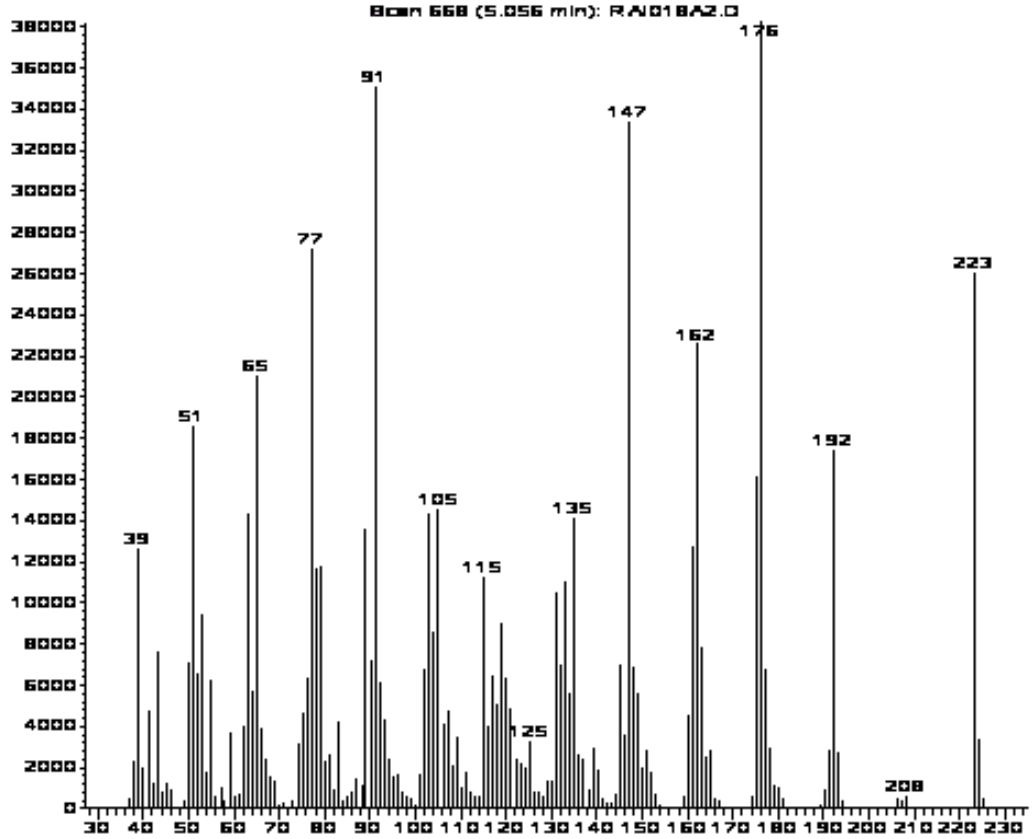

Figure 1. Mass Spectrum of **1**.

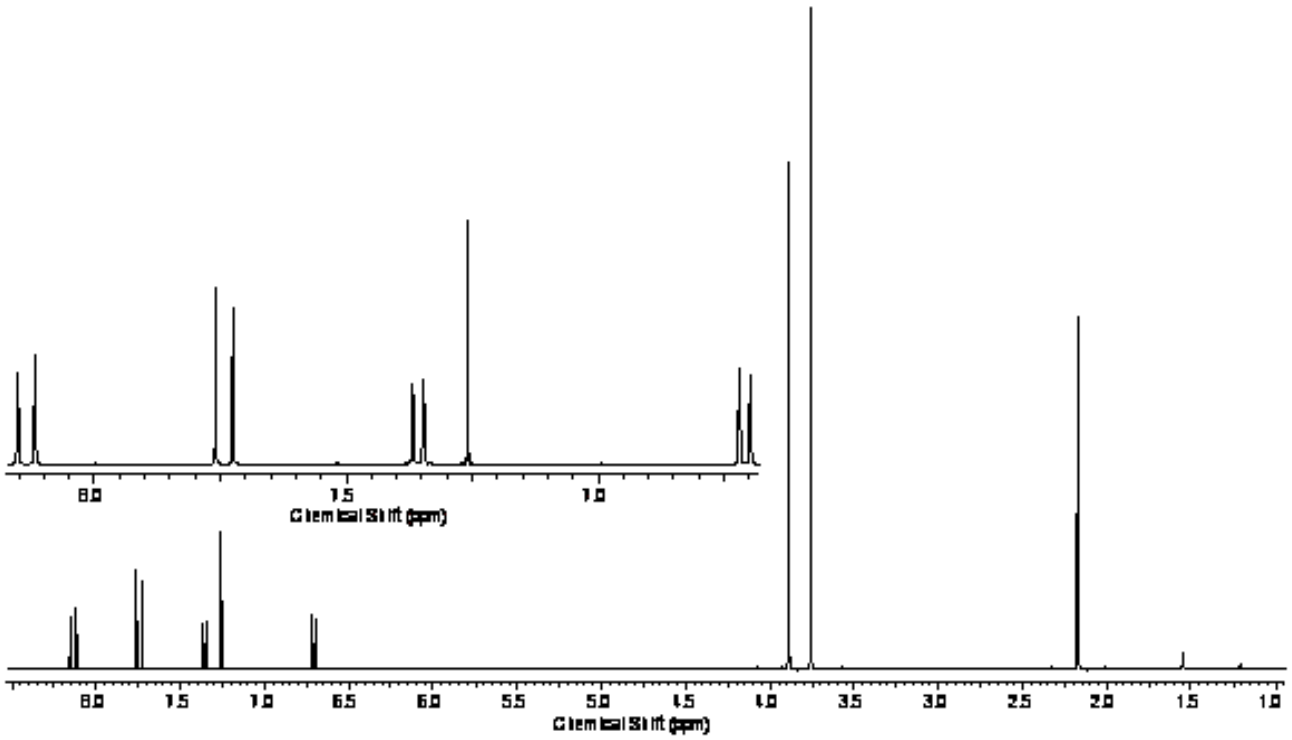

Figure 2. ^{1}H NMR Spectrum of **1** in $CDCl_3$.

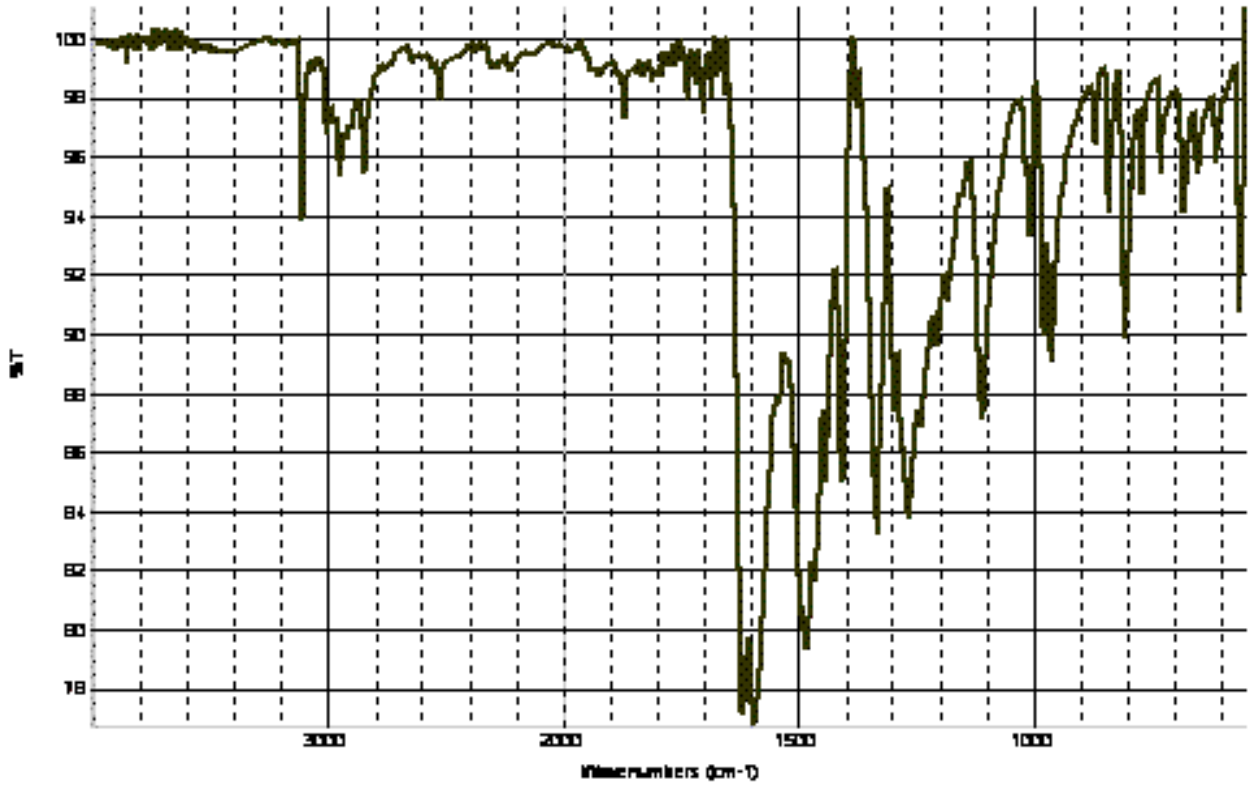

Figure 3. FTIR (KBr) Spectrum of **1**.

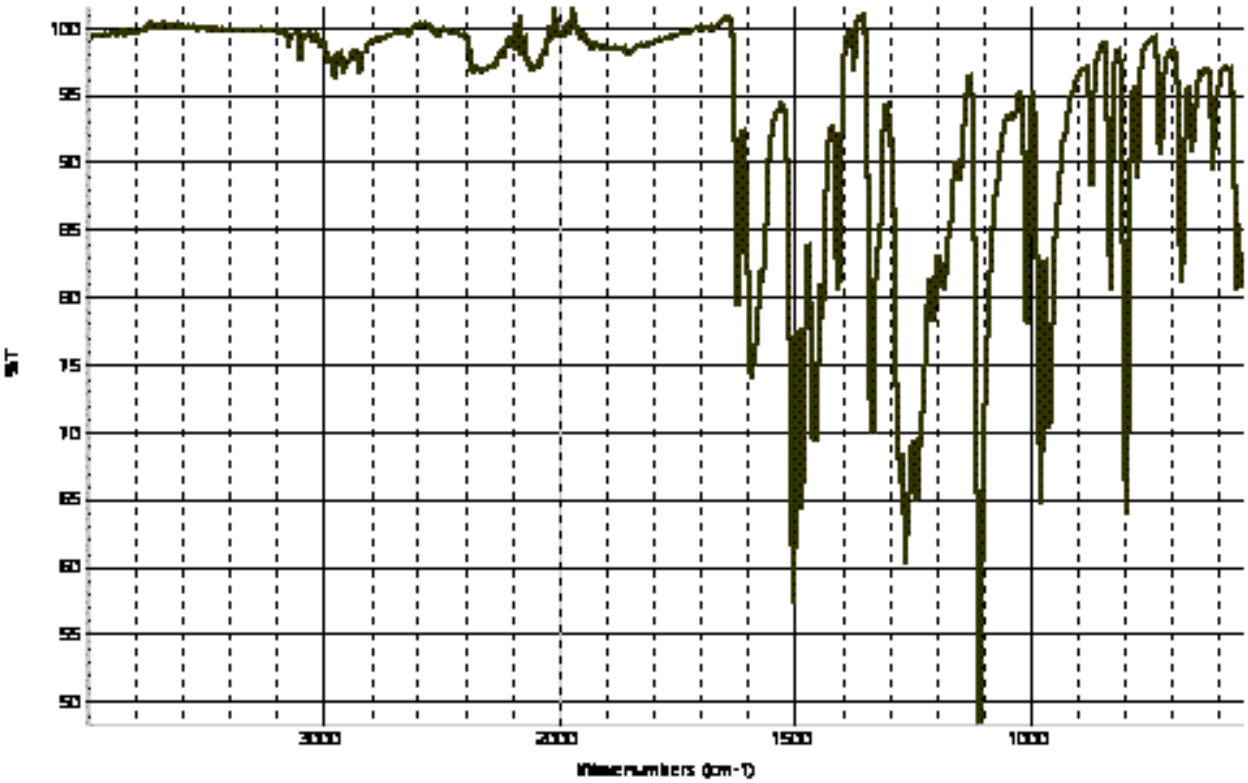

Figure 4. FTIR (ATR) Spectrum of **1**.

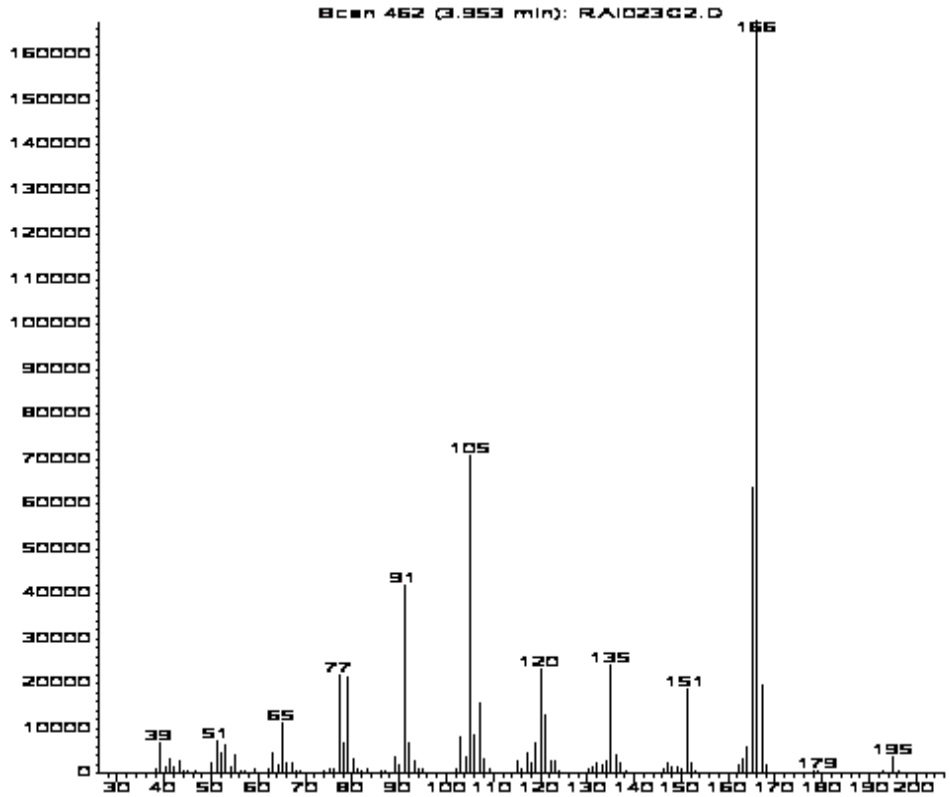

Figure 5. Mass Spectrum of **2**.

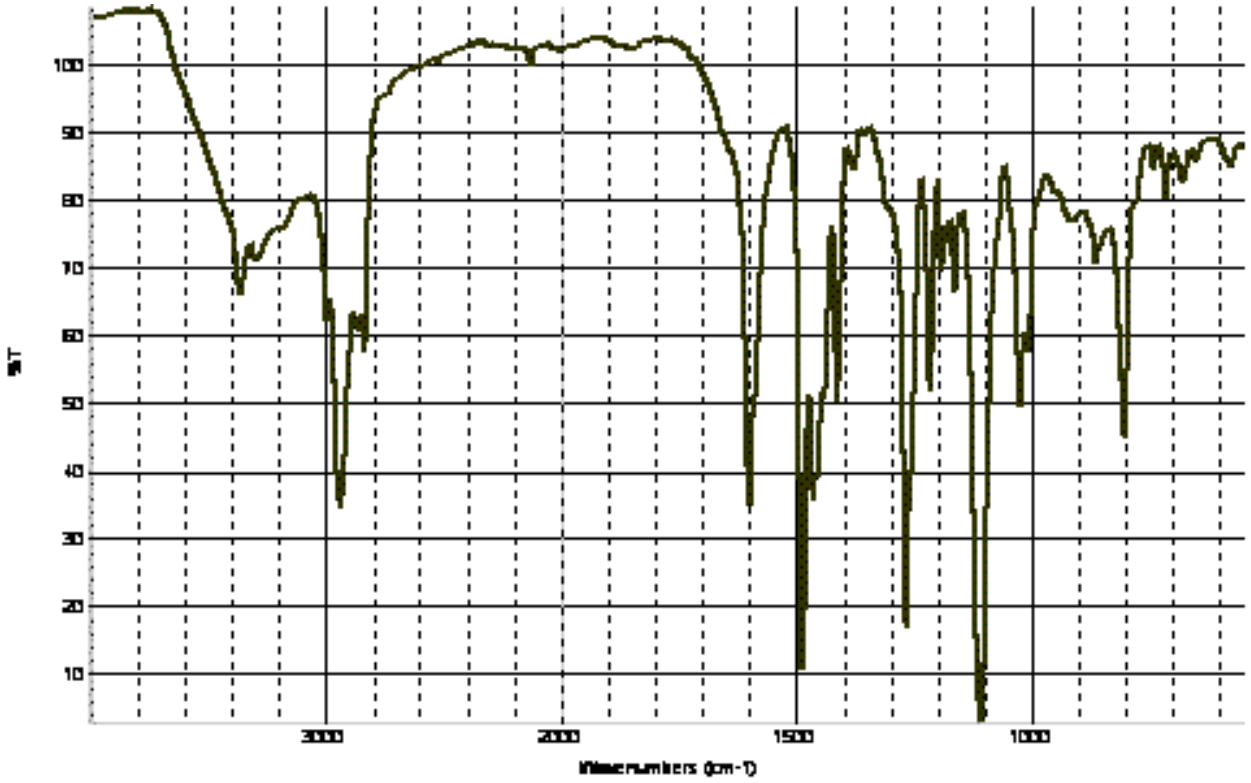

Figure 6. FTIR (Neat, NaCl) Spectrum of **2**.

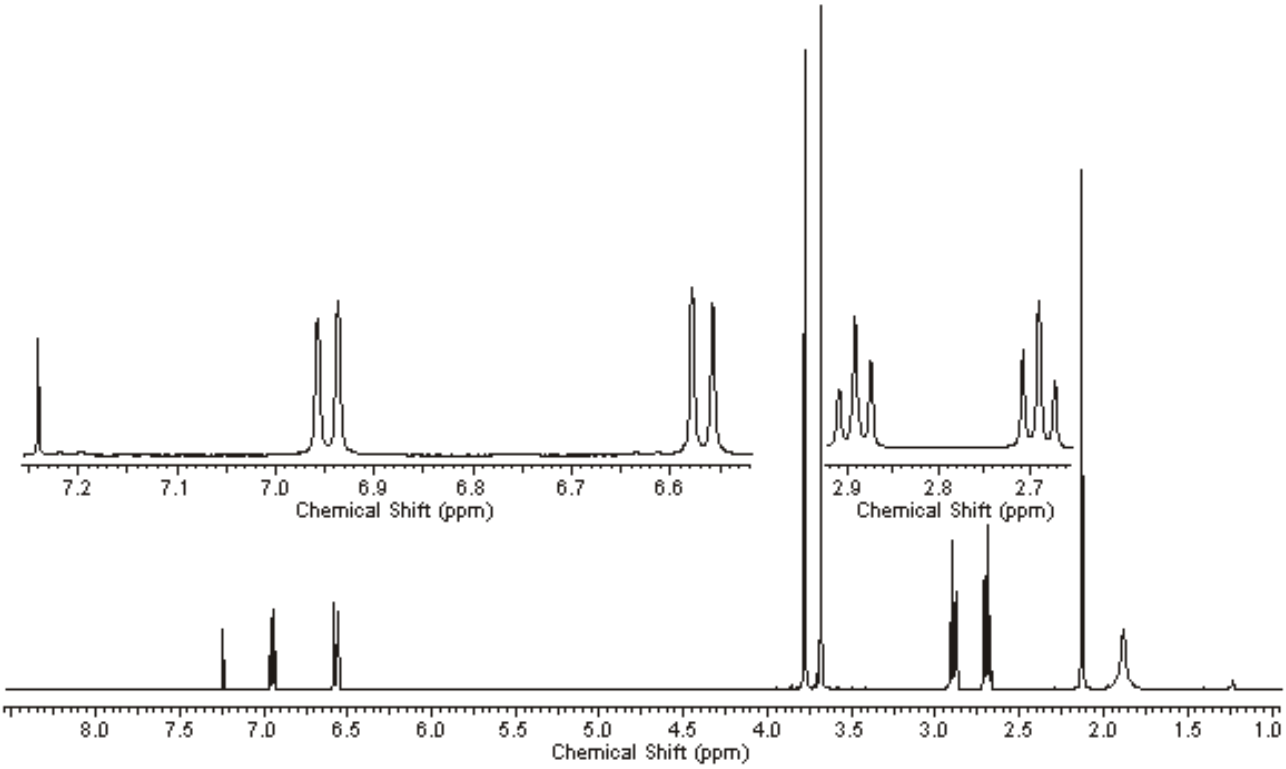

Figure 7. ^{1}H NMR Spectrum of **2** in $CDCl_3$.

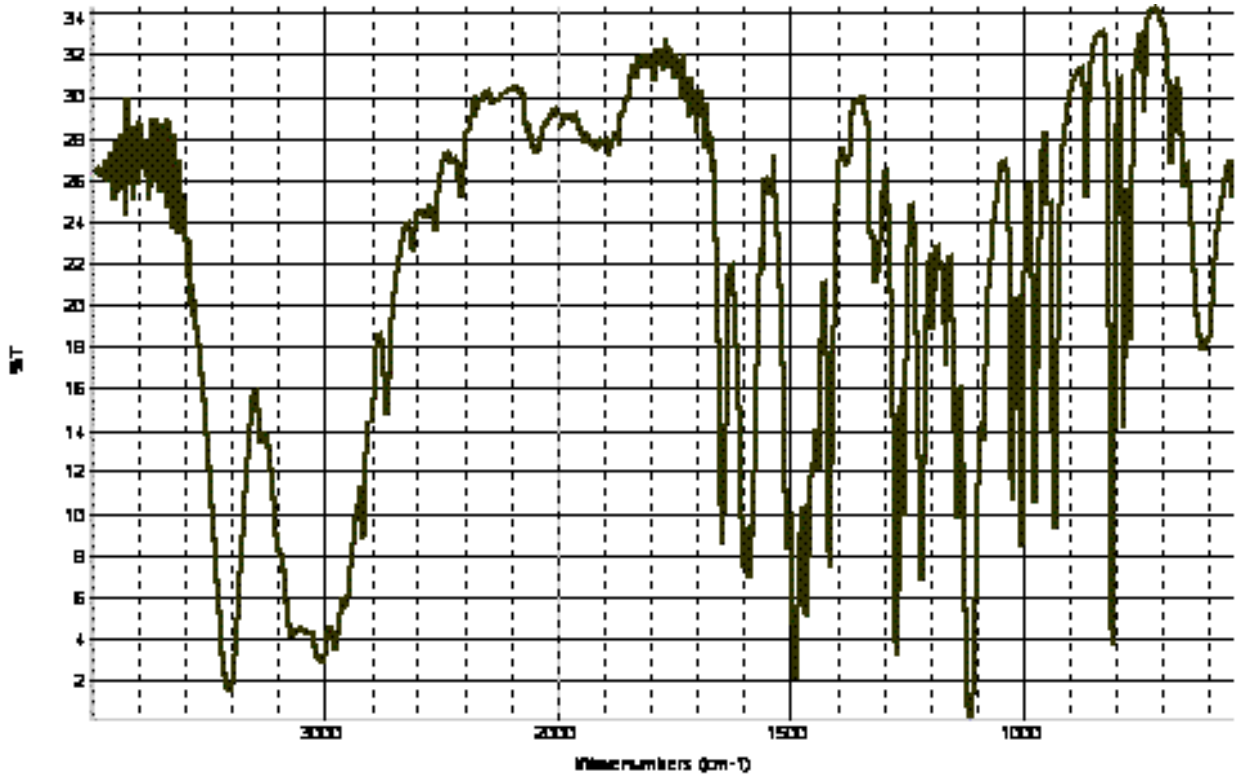

Figure 8. FTIR (KBr) Spectrum of **3**.

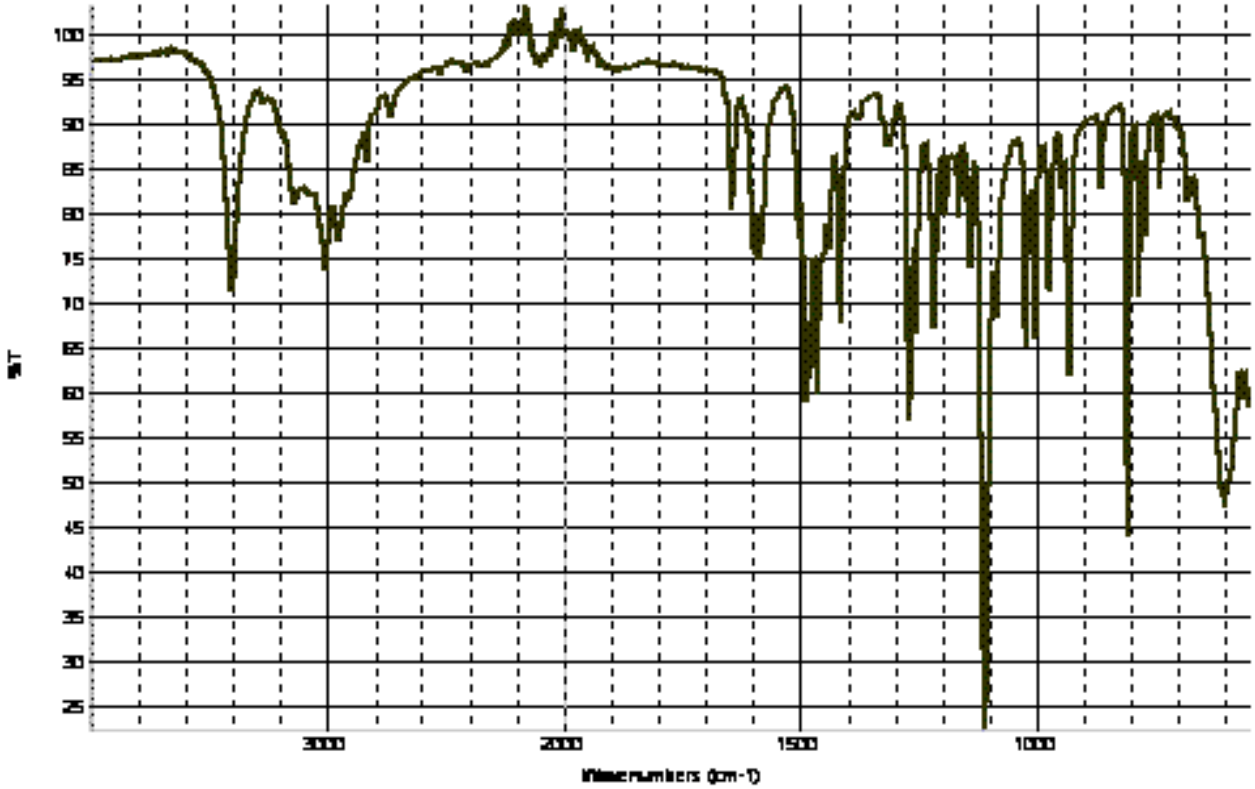

Figure 9. FTIR (ATR) Spectrum of **3**.

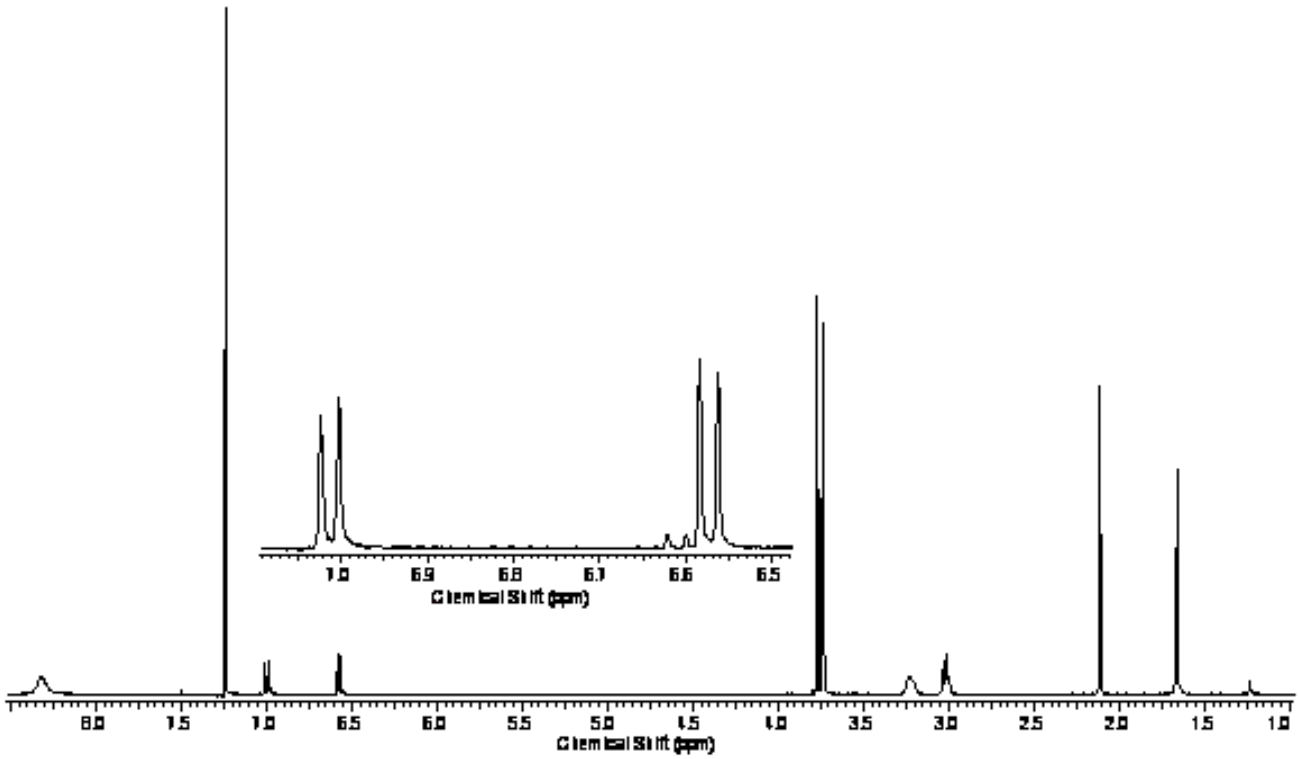

Figure 10. ^{1}H NMR Spectrum of **3** in $CDCl_3$.

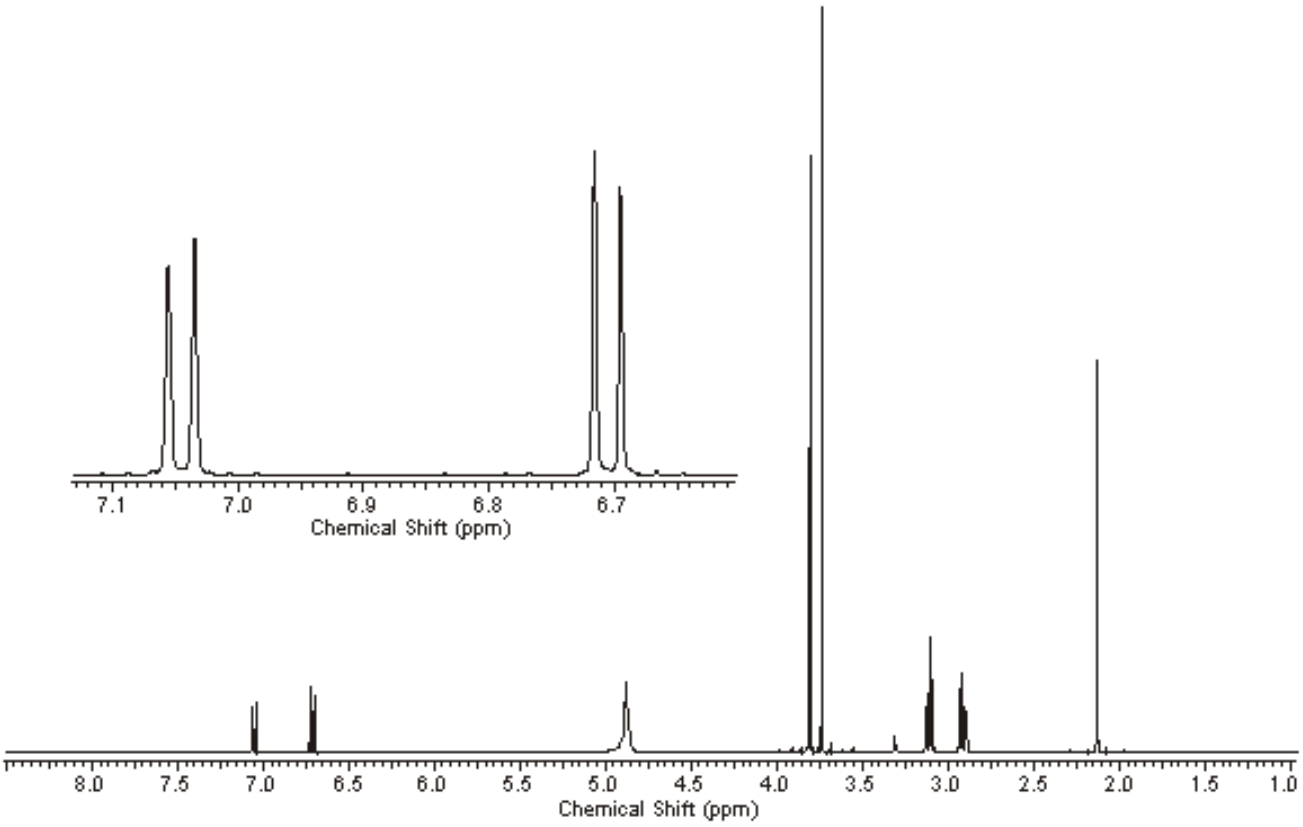

Figure 11. ^{1}H NMR Spectrum of **3** in CD_3OD.

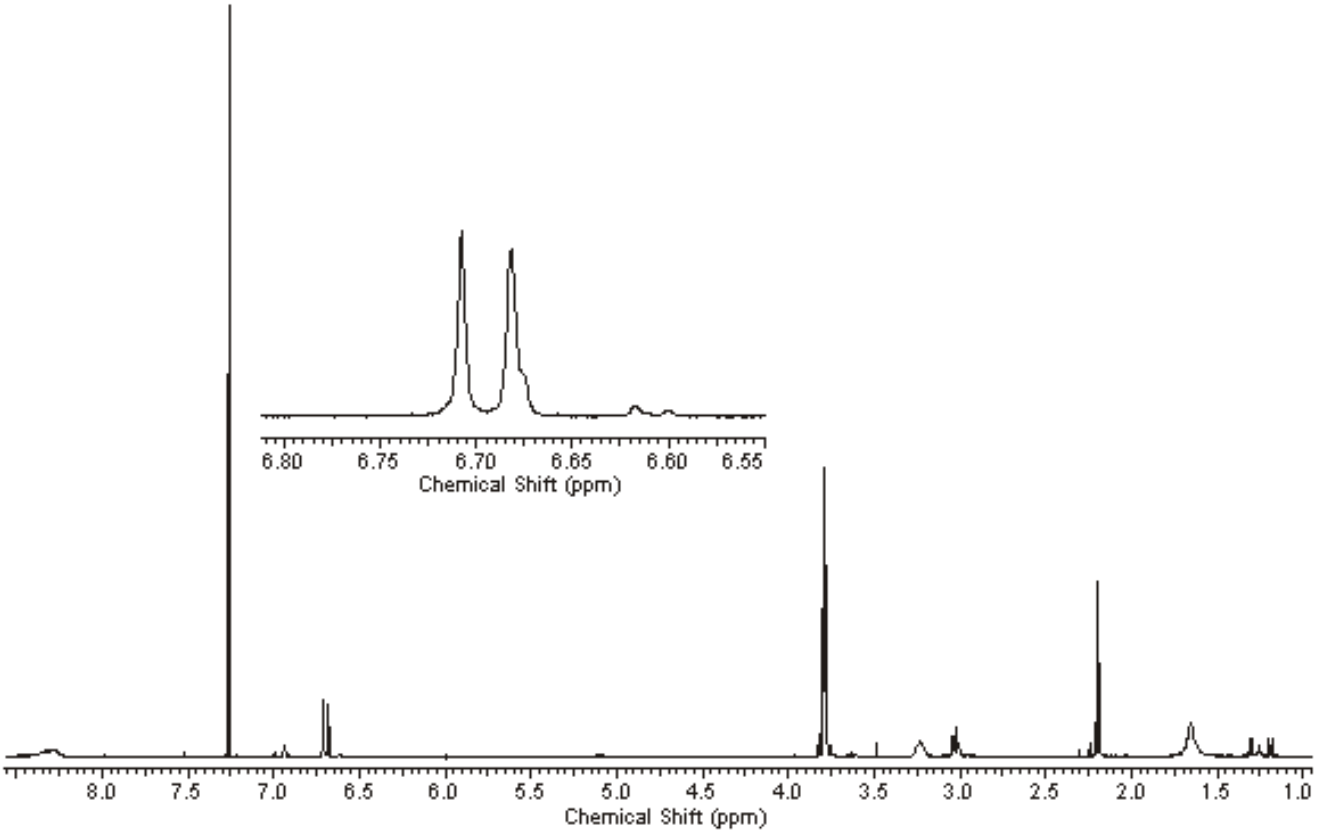

Figure 12. ^{1}H NMR Spectrum of a 2C-D Exhibit in $CDCl_3$.

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Technical Note

Analytical Profile of Modafinil

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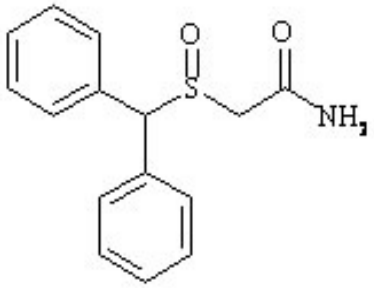
ABSTRACT: Analytical data (color tests, GC/MS, and FTIR) are reported for modafinil.

KEYWORDS: Modafinil, Provigil, Color Testing, GC/MS, FTIR, Forensic Chemistry.

Figure 1

Modafinil: 2-[(Diphenylmethyl)sulfinyl]acetamide;
$C_{15}H_{15}NO_2S$; mw = 273.36



Introduction

Modafinil (Figure 1), the active constituent of Provigil® tablets, became a Schedule IV controlled substance in January 1999. According to the manufacturer, modafinil is a CNS stimulant which possesses, "wake-promoting actions like sympathomimetic agents including amphetamine and methylphenidate, although the pharmacologic profile is not identical to that of sympathomimetic amines" [1].

Presumptive testing and instrumental data were collected to assist in the identification of submissions of modafinil tablets.

Experimental

Standard and Reagents
A reference standard of modafinil (Lot# 084K4633) was obtained from Sigma. Potassium bromide (IR grade, lot# 035261) and methylene chloride (ACS grade, lot# 040933) were obtained from Fisher. The derivatizing agent BSTFA+TMCS (99:1, lot# LA90822) was obtained from Supelco.

Methods and Instrumentation
Presumptive Color Tests: Portions of modafinil were placed in reagent wells followed by the addition of various presumptive color test reagents.

Tablet Extraction: A Provigil tablet extraction procedure was obtained from Cephalon [2]. A single tablet was ground and placed in a separatory funnel followed by the addition of 50-mL de-ionized water and 50-mL methylene chloride. The mixture was shook for approximately one minute with venting. A portion of the lower layer was drained, filtered, and evaporated to dryness, leaving a white powder residue.

Derivatization: A small portion of modafinil reference standard was placed in an autosampler vial followed by ~1 mL de-ionized water and ~0.5 mL BSTFA-TMCS derivatizing agent. The vial was capped tightly, mixed well, and incubated at ~70 ºC for 30 minutes.

GC/MS Analysis: Analysis was performed with a HP 6890 GC equipped with a DB-35MS column (15 m x 0.25 mm ID, and film thickness 0.25 μm) and coupled to a HP 5973 Mass Selective Detector. The temperature during the analysis run increased from 90 ºC to 300 ºC at 20 ºC/minute, held for 5 minutes, increased up to 310 ºC at 30 ºC/minute, and held for 0.5 minute. The temperatures of the injection port and transfer line were 250 ºC and 280 ºC, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/minute. The MSD was operated in the Electron Ionization mode. Mass spectra were recorded at 70 eV, with a scanning range of *m/z* 40 - 400.

FTIR Analysis: FTIR analysis was performed using a Perkin Elmer Spectrum 1000 spectrometer. Samples were analyzed in KBr and scanned 16 times from 4000 - 400 cm^{-1} at a resolution of 4 cm^{-1}.

Results and Discussion

The results of five presumptive color tests are summarized in Table 1. Based on the results, only the Marquis and Liebermann's reagents give a positive test (however, neither is very specific or definitive).

Table 1.

Reagent	**Resulting Color**
Marquis	Yellow/Orange ⇒ Brown
Liebermann's	Darkening Orange
Sodium Nitroprusside	No Color
Cobalt Thiocyanate	No Color
Ehrlich's	No Color

Underivatized modafinil (reference standard) severely degraded during GC/MS analysis, displaying five primary peaks under the specified conditions. The latest eluting compound (Rt = 11.13 minutes) had a base ion at *m/z* = 167 (likely a rearranged ion derived from the diphenylmethinyl fragment). Derivatizing with BSTFA resulted in a substantially more abundant peak at 11.15 minutes. The mass spectrum of this peak is shown in Figure 2. Figure 2 also suggests that the ion at *m/z* = 167 is the expected ion for either derivatized or underivatized modafinil. While the TMS derivative shows some degradation, the derivative is much more stable than modafinil under under GC conditions, and is therefore more suitable for GC/MS analysis.

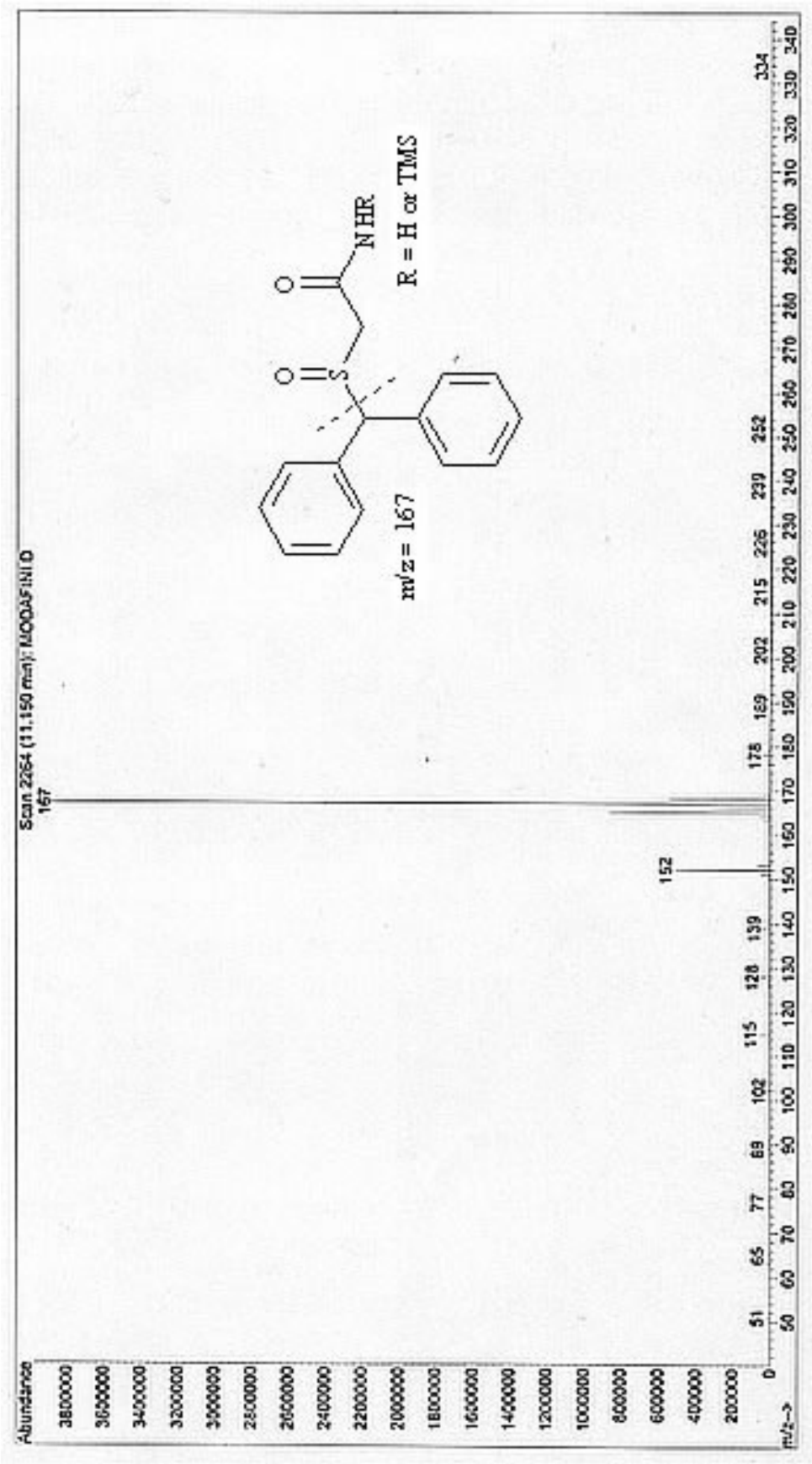
The FTIR spectrum of modafinil is presented in Figure 3. Discussions with technical staff at Cephalon indicate that the spectrum changes when extracted into methylene chloride and evaporated down, suggesting a hydrated form and an anhydrous form, or polymorphism. Figure 4 depicts the latter spectrum; comparison between the two spectra reveal minor differences in the range of 4000-3000 cm^{-1}, indicating that polymorphism is more likely.

References

1. Provigil® Patient Information Leaflet.

2. Cephalon, Inc. Analytical Test Method, MET-0023-1538, Version 1.

Figure 2 – Mass Spectrum of Modafinil-TMS



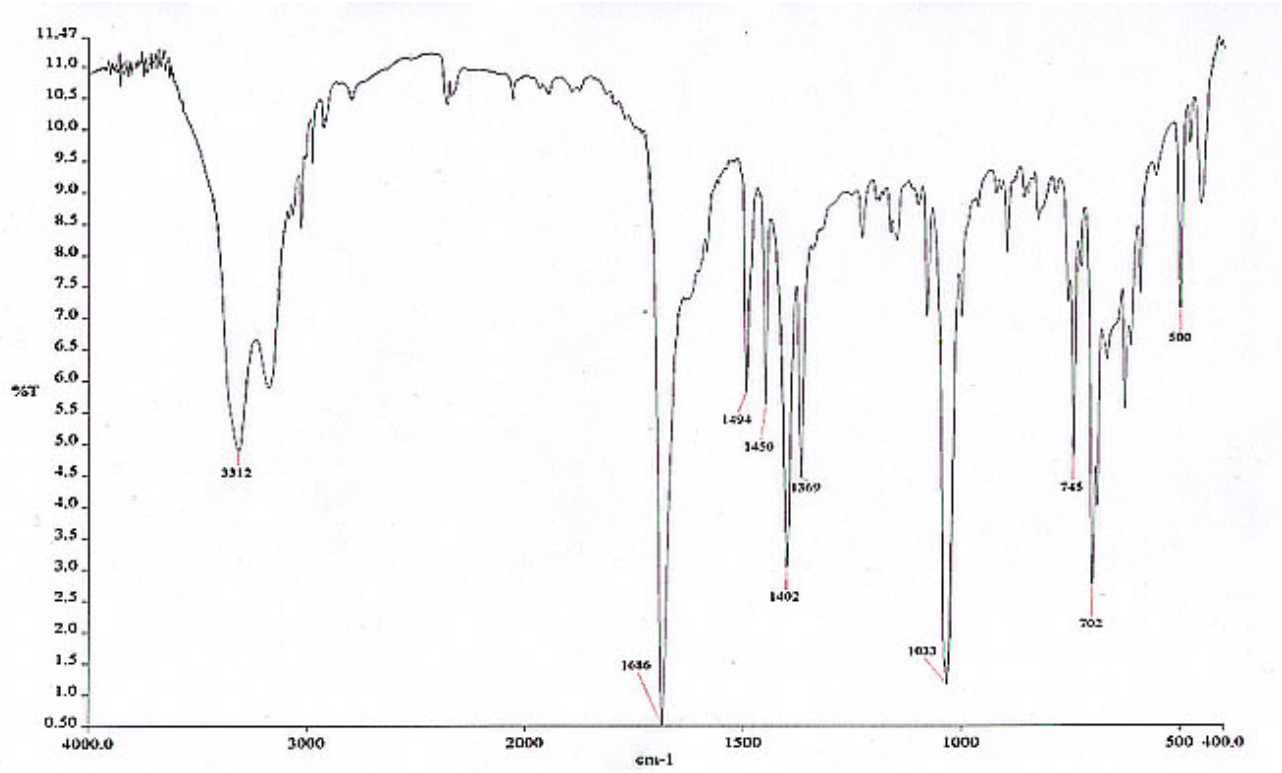


Figure 3 – Infrared Spectrum of Neat Modafinil

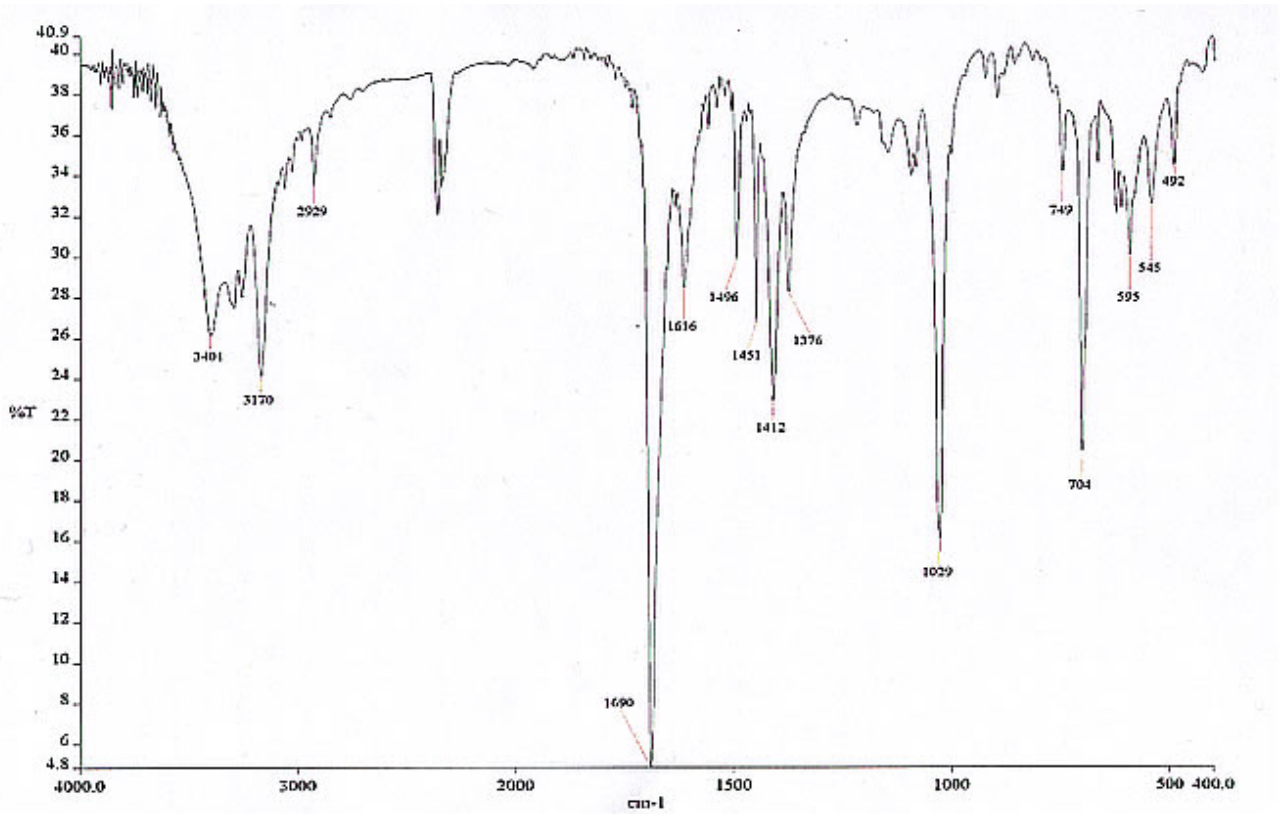


Figure 4 – Infrared Spectrum of Extracted Modafinil

Quantitation and Enantiomeric Determination of Propoxyphene Using Capillary Zone Electrophoresis

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ABSTRACT: Validated Methods for the quantitation of d-propoxyphene HCl and d-propoxyphene napsylate were developed using capillary zone electrophoresis, using an uncoated capillary, a lithium phosphate buffer, and using thiamine HCl as the internal standard. The addition of a small amount of acetonitrile to the injection solvent facilitated the solubilization of d-propoxyphene napsylate. The analytes' responses were reproducible, provided accurate recovery values, and were linear within the experimental concentration range. A chiral analysis was also conducted, using the same capillary but with 2-hydroxypropyl-β-cyclodextrin added to the run buffer. The methods were specifically developed for the analysis of pharmaceutical tablets containing d-propoxyphene HCl or d-propoxyphene napsylate, which typically are adulterated only with caffeine, aspirin, and/or acetaminophen; however, the method is applicable to analysis of a wide variety of other drugs.

KEYWORDS: Capillary Zone Electrophoresis, CZE, d-Propoxyphene HCl, d-Propoxyphene Napsylate, Chiral Analysis, Forensic Chemistry

Introduction

d-Propoxyphene is a mild narcotic analgesic found in various pharmaceutical preparations, usually as the hydrochloride or napsylate salt. These preparations typically also contain large amounts of acetaminophen, aspirin, or caffeine. This drug is prescribed for pain relief; however, it is also abused for its euphoric side effects [1], and it is therefore commonly diverted into the illicit drug trade. Currently, d-propoxyphene is a Schedule IV controlled substance in the United States; however l-propoxyphene is not controlled. This requires enantiomeric determination for all samples containing propoxyphene.

Propoxyphene is thermally labile, and will break down on a gas chromatograph. Therefore, most of the literature procedures for its analysis are based on liquid chromatographic techniques, more recently including capillary electrophoretic methods [1,2]. The analysis of controlled substances with CE, especially using specialized capillary coatings and/or run buffers, have been shown to produce highly accurate and reproducible results [3-5]. However, some of these techniques are relatively costly and complicated. The described methodologies are simple, inexpensive, and can also be utilized for a wide variety of other drugs, including the phenethylamines [6].

The first CE method for quantitation and enantiomeric determination of propoxyphene was reported in 1994 [7]. However, the methodology required the use of fairly long capillaries, resulting in long analysis times. In addition, because d-propoxyphene napsylate has a solubility limit of approximately 1.0 mg/mL in 0.01 N HCl, the quantitation samples were prepared at concentrations less than 0.6 mg/mL, and had to be sonicated for several hours prior to analysis.

Solubility problems in CE can be addressed by the use of an organic modifier in the injection solvent and/or run buffer. For d-propoxyphene napsylate, acetonitrile was determined to be an appropriate modifier. Thiamine HCl

was selected as the method internal standard, as it is commercially available, inexpensive, and not found in typical pharmaceutical preparations or in most illicit drug samples.

Experimental

Preparation of Internal Standard Stock Solution

Thiamine HCl (Sigma, St. Louis, MO) was dissolved in 0.01 N HCl, for a concentration of 1 mg/mL. The deionized water used to produce the 0.01 N HCl was obtained from a Milli Q® Gradient 10A purification system (Millipore, Bedford, MA).

Preparation of the Achiral Buffer

A 100 mM solution of phosphoric acid was prepared using deionized water and ≥85% reagent grade phosphoric acid (J.T. Baker, Phillipsburg, NJ). The solution was then titrated to a pH of 2.30 ±0.02 with solid lithium hydroxide (Sigma, St. Louis, MO). (Precise pH control is very important in CE, as it affects both migration times and selectivity.) The buffer was filtered prior to use through a 0.45 µm filter, using an Agilent (Wilmington, DE) Solvent Filter/Degasser. Because this buffer contains no preservatives, it was stored at 7 ºC, and was replaced every 6 to 8 weeks.

Preparation of the Chiral Buffer

2-Hydroxypropyl-β-cyclodextrin (Sigma, St. Louis, MO) was added to the achiral buffer such that its concentration was 20 mM. The buffer was filtered prior to use through a 0.45 µm filter.

Preparation of Capillaries

The capillary was prepared in-house, using a 50 µm ± 3 µm ID with a 363 µm ± 10 µm OD flexible polyamide-coated fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ). The capillary was manually cut to a nominal length of 34 cm ± 0.5 cm using a CE column cutter equipped with a diamond blade. Both ends of the capillary were inspected under a microscope to ensure that the glass edge was straight and perpendicular to the length of the capillary tubing, and also was free of debris and defects. The detector window was produced by removing the polyamide coating using a standard window maker equipped with a 7 mm heating module (MICROSOLV® , Long Branch, NJ). The coating on each end of the capillary was removed using a 2 mm heating module. The new capillary was initially conditioned at 40 ºC by flushing it with 1.0 N NaOH (5 minutes), 0.1 N NaOH (10 minutes), deionized water (5 minutes), and 100 mM lithium phosphate buffer (10 minutes). Subsequently, capillaries were conditioned once every 24 hours at 15 ºC by flushing them with 1.0 N NaOH (1 minutes), 0.1 N NaOH (2 minutes), deionized water (1 minutes), and 100 mM lithium phosphate buffer (2 minutes).

Sample and Standard Preparation for d-Propoxyphene Hydrochloride

d-Propoxyphene HCl standard (Sigma, St. Louis, MO) or a d-propoxyphene HCl-containing sample was accurately weighed and placed in a volumetric flask with an appropriate aliquot of thiamine HCl stock solution (1:5), and the solution was diluted to final volume with 0.01 N HCl. The concentration of the standard or sample in each solution varied between 0.2 - 0.5 mg/mL. Each solution was filtered prior to injection through a syringe equipped with a 0.45 µm filter (Acrodisc®). For the linearity studies, eight solutions of the d-propoxyphene HCl were prepared at concentrations ranging from 0.05 to 1.3 mg/mL, with the internal standard concentration constant at 0.2 mg/mL. The standard and sample solutions were diluted with 0.01 N HCl to approximately 0.05 to 0.10 mg/mL for the chiral analyses. Enantiomers were determined using the chiral buffer.

Sample and Standard Preparation for d-Propoxyphene Napsylate

d-Propoxyphene napsylate standard or a d-propoxyphene napsylate-containing sample was accurately weighed and placed in a 100 mL volumetric flask with 4 mL acetonitrile and sonicated of approximately 5.0 minutes. An appropriate aliquot of thiamine HCl stock solution (1:5) was added, and the solution was diluted to final volume with 0.01 N HCl. The concentration of the standard or sample in each solution varied between 0.2 - 0.5 mg/mL (the amount of acetonitrile in the final solutions was approximately 4%). Each solution was filtered prior to

injection through a syringe equipped with a 0.45 µm filter (Acrodisc®). For the linearity studies, eight solutions of the d-propoxyphene napsylate were prepared at concentrations from 0.05 to 1.2 mg/mL, containing varying amounts of acetonitrile (0.2% to 4.8%). Additional solutions containing d-propoxyphene napsylate were also prepared from 0.569 to 0.737 mg/mL, containing acetonitrile ranging from 6% to 14%. The appropriate aliquot of thiamine HCl stock solution (1:5) was added, and the solution were diluted to final volume with 0.01 N HCl. The standard and the sample solutions were diluted to approximately 0.05 to 0.10 mg/mL with 0.01 N HCl for the chiral analyses. Enantiomers were determined using the chiral (20 mM 2-hydroxypropyl-β-cyclodextrin) buffer.

Capillary Electrophoresis

Experiments were performed using a Hewlett Packard 3DCE capillary electrophoresis system (Agilent Technologies, Wilmington, DE), equipped with a diode array detector set at a wavelength of 207 nm with a bandwidth of 7 nm. For quantitations, the capillary was flushed with buffer for 2.5 minutes between injections for the HCl, and for 1.0 minutes with 0.1 N NaOH and 2.0 minutes with the achiral buffer for the napsylate. The buffer was replenished after six injections to prevent depletion of electrolytes and charge. The capillary temperature was maintained at 15 ºC. The hydrodynamic injection time was 2.5 seconds at 50 mBar. The applied voltage was 14.5 kV, which was determined empirically to maintain current below 60 µA, thereby limiting Joule heating while also optimizing analysis time.

For the enantiomer determination, the capillary was flushed with buffer for 2.5 minutes between injections for both the HCl and the napsylate salts. The standard and the sample solutions were diluted to approximately 0.2 mg/mL with 0.01 N HCl. The enantiomers were determined using the chiral buffer, using the same applied voltage (14.5 kV). The d,l-propoxyphene standard and sample injection was set at 50 mBar of pressure for 1.5 seconds, followed by a second co-injection of 0.01 N HCl at 20 mBar for 1.0 second. The samples and standards of d-propoxyphene and l-propoxyphene were injected at 50 mBar of pressure for 1.5 seconds, followed by a second co-injection of the d,l-propoxyphene standard at 20 mBar for 1.0 second. The buffer was not replenished after six injections, but rather was utilized until depleted.

Results and Discussion

The objective of the study was to accurately and rapidly quantitate d-propoxyphene HCl and d-propoxyphene napsylate by CZE without interferences from adulterants or diluents. CZE permits direct analysis without requiring extractions. The use of a simple aqueous buffer for the HCl salt reduces analysis cost and allows for simple disposal; the use of acetonitrile as an organic modifier for the napsylate salt is nearly as convenient. Because neutral compounds migrate at the rate comparable to the electroosmotic flow (EOF), while negatively charged (acidic) compounds migrate at a rate slower than the EOF, these species are not detected using the presented method. Therefore, pharmaceutical tablets that contain adulterants such as caffeine, aspirin, or acetaminophen do not interfere. For example, the analysis of a 50 mg d-propoxyphene napsylate tablet containing 325 mg of acetaminophen displays no peak(s) for the acetaminophen (Figure 1).

These methods were specifically developed for pharmaceutical tablets containing d-propoxyphene HCl or d-propoxyphene napsylate, which typically are adulterated only with caffeine, aspirin, and/or acetaminophen. As noted above, these adulterants do not interfere with achiral quantitation; therefore, a selectivity study was not conducted (or required for this application). In the unlikely event that counterfeit pharmaceuticals containing additional adulterants were encountered, the alternative adulterants would have to be identified by spectroscopic means, after which the sample would have to be evaluated to ensure that the selectivity requirements were met, before proceeding with CZE analysis.

The linearity study demonstrated that the calculated errors were less than five percent, and the correlation coefficients were greater than 0.998, within the specified linear range (see Table I). The linearity studies were conducted both with the method using a 1.0 minute flush with the 0.1 N NaOH followed by a 2.0 minute flush of the achiral buffer, and with the method using a 2.5 minute flush with the achiral buffer. It was determined that the use of a 0.1 N NaOH flush resulted in a higher correlation coefficient.

The precision was determined by injecting two concentrations of analyte at the lower and upper ends of the established linear range. The %RSDs for the two concentrations did not exceed 3% for the HCl or the napsylate. Furthermore, an additional five solutions ranging from 0.569 to 0.737 mg/mL of d-propoxyphene napsylate containing varying amounts of acetonitrile (6, 8, 10, 12, or 14%) gave equivalent %RSD values. The precision was determined both with the 1.0 minute flush with the 0.1 N NaOH followed by a 2.0 minute flush of the achiral buffer, and with the 2.5 minute flush with the achiral buffer. Again, it was determined that the use of a 0.1 N NaOH flush resulted in a lower, more consistent %RSDs (Tables II and III).

The accuracy (recovery) was determined by preparing three different concentrations of the analyte with each of the following adulterants: Acetaminophen, aspirin, and caffeine. The concentrations of the analytes represented the lower, middle, and upper linear ranges (i.e., 10%, 50%, and 80%), and contained the appropriate amount of the internal standard. The samples were prepared by sonicating for 5 minutes, and were also compared to non-sonicated samples. The CZE results were compared to the actual values, and did not exceed a 5.0% difference (Table IV). However, samples that were sonicated gave lower errors.

Conclusions

CZE is an effective technique for the quantitation of pharmaceutical preparations containing d-propoxyphene HCl or d-propoxyphene napsylate. Quantitative results were shown to be accurate, reproducible, and precise, and allowed analyses to be accomplished in less than 6 minutes. The use of thiamine HCl as the internal standard was convenient, did not interfere with any known controlled adulterant, and is commercially available at low cost. Chiral separations are conveniently accomplished on the same system with the use of 2-hydroxypropyl-β-cyclodextrin in the run buffer. The described system offers an approach for routine analysis that is simple, robust, practical, and inexpensive. The methodology has been applied to a broader range of illicit drugs, including synthetic opiates and phenethylamines, under the same/similar operating conditions with equal success, and has been used to analyze a large number seized exhibits over the past two years.

Acknowledgments

The author expresses appreciation to the following staff employed at the DEA Southwest Laboratory: Senior Forensic Chemist Harry F. Skinner, and Forensic Chemists Alan Randa, Dean A. Kirby, Nathan Salazar, and Nicole C. Payne-King, for their technical contributions. The author would also like to thank Librarian Rose Mary Russo of the DEA Library (Arlington, Virginia) for her assistance in acquiring references.

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* * * * *

Table I: Linearity Study, Data for d-Propoxyphene HCl and Napsylate.

d-Propoxyphene	Linear Range (mg/mL)	Range of % Error	Correlation Coefficient	Y Intercept	Slope
Hydrochloride	0.0513 to 1.23	0.581 to 3.98	0.99991	0.042	19.30
Napsylate [1] (with 0.1N NaOH flush)	0.0509 to 1.22	0.11 to 2.72	0.99995	0.032	12.7813
Napsylate [1,2] (without 0.1N NaOH flush)	0.101-1.22	0.239 to 2.05	0.99981	-0.0023	12.5327

[1] Standards sonicated for 5 minutes in acetonitrile before the addition of 0.01 N HCl and Thiamine HCl internal standard. Percent acetonitrile varied as follows: 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, 4.0, and 4.8, respectively.

[2] The percent error was 7.6 at a concentration of 0.0509 mg/mL.

Table II: Data for Repeatability Study for d-Propoxyphene Napsylate.

Propoxyphene Napsylate Repeatability (Sonicated)							
Percent Acetonitrile	0.8%	3.2%	6%	8%	10%	12%	14%
Concentration of napsylate (mg/mL)	0.203	0.815	0.737	0.569	0.729	0.714	0.602
%RSD (with 0.1N NaOH flush)	1.46	1.67	1.46	0.801	0.833	2.19	1.74
%RSD (without 0.1N NaOH flush)	1.46	1.13	2.49	2.22	0.756	1.01	0.755

Table III: Data for Repeatability Study for d-Prropoxyphene HCl.

d-Propoxyphene HCl		
Concentration (mg/mL)	0.205	0.822
%RSD	0.372	0.987

* * * * *

Table IV: Data for Recovery Study for d-Propoxyphene Napsylate.

d-Propoxyphene Napsylate Recovery at 4% Acetonitrile (Sonicated)			
Adulterant	Actual percentage d-propoxyphene napsylate (% PXP) and Calculated percent error (% error)		
Acetaminophen	%PXP= 11.28 % error= 0.576	%PXP= 45.17 % error= 1.55	%PXP= 78.18 % error= 0.147
Aspirin	%PXP= 8.81 % error= 1.59	%PXP= 49.21 % error= 1.61	%PXP= 78.7 % error= 1.00
Caffeine	%PXP= 8.59 % error= 1.97	%PXP= 48.32 % error= 0.166	%PXP= 80.42 % error= 2.77

* * * * *

Table V: Data for Recovery Study for d-Propoxyphene Napsylate.

d-Propoxyphene Napsylate Recovery at 4% Acetonitrile (Non-Sonicated)			
Adulterant	Actual percentage d-propoxyphene napsylate (%PXP) and Calculated percent error (% error)		
Aspirin	* %PXP= N/D * % error= N/D	%PXP= 45.82 % error= 3.09	%PXP= 85.02 % error= 4.16
Caffeine	* %PXP= N/D * % error= N/D	%PXP= 52.17 % error= 4.69	%PXP= 85.90 % error= 4.91
* N/D = not determined			

Table VI: Data for Recovery Study for d-Propoxyphene HCl.

d-Propoxyphene HCl Recovery			
Adulterant	Actual Percentage d-Propoxyphene HCl (%PXP) and Calculated Percent Error (% Error)		
Acetaminophen	%PXP= 11.27 % error= 0.621	%PXP= 45.81 % error= 0.185	%PXP= 79.37 % error= 0.460
Aspirin	%PXP= 10.85 % error= 0.645	%PXP= 50.71 % error= 0.424	%PXP= 77.15 % error= 0.972
Caffeine	%PXP= 9.23 % error= 1.89	%PXP= 51.18 % error= 0.840	%PXP= 83.11 % error= 0.0120

* * * * *

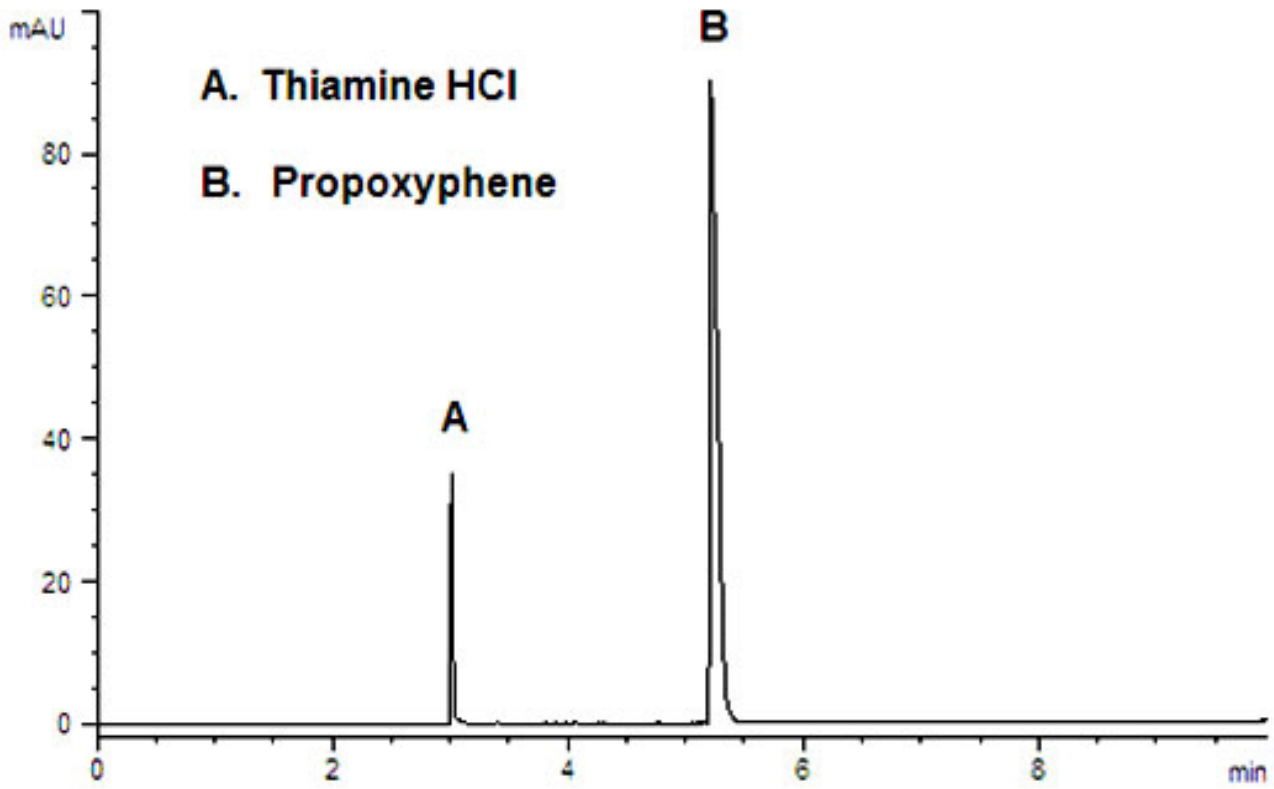

Figure 1: Electropherogram of Thiamine and Propoxyphene.

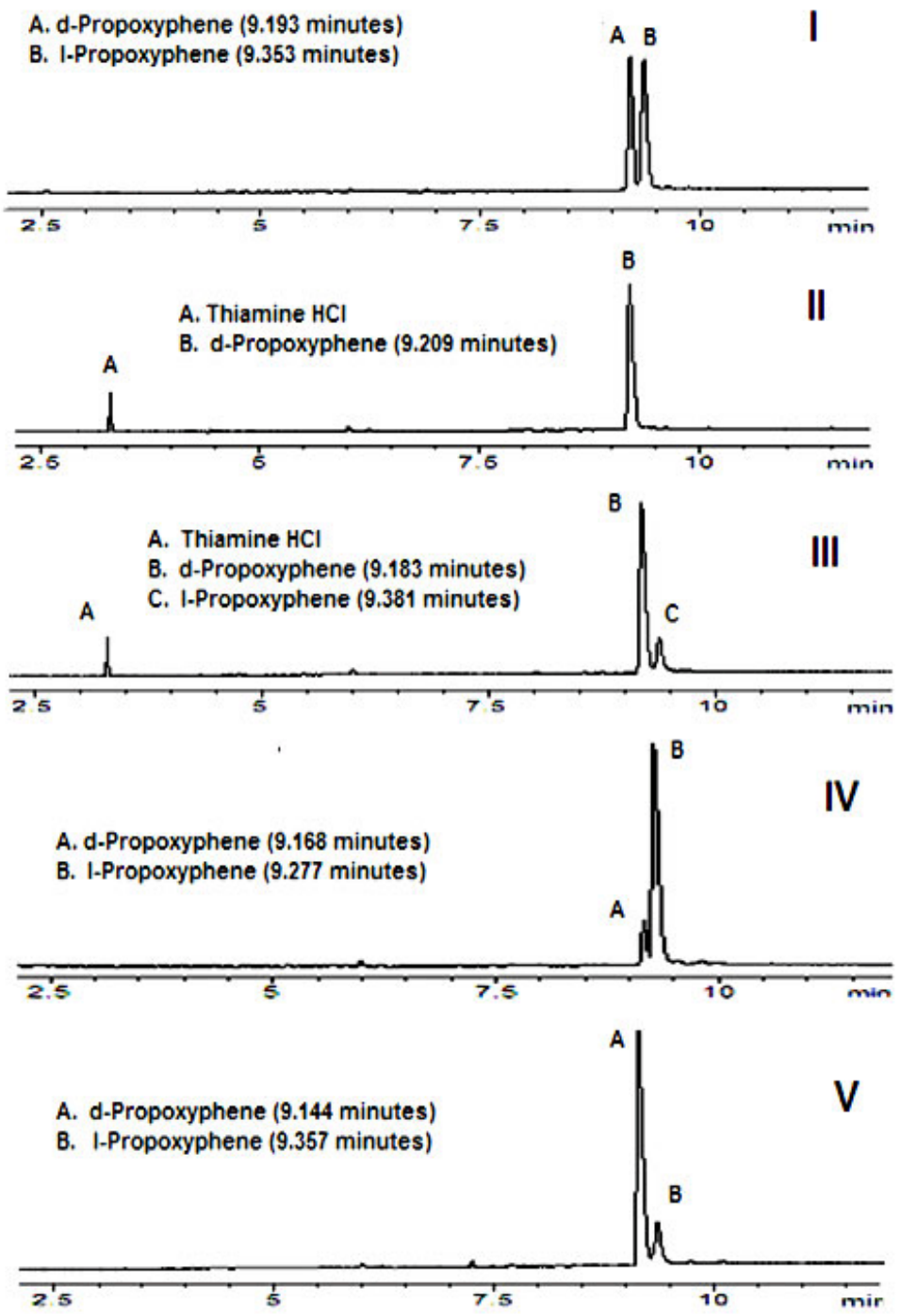

Figure 2: The Electropherograms for the Chiral Analysis of Propoxyphene Napsylate:

I. The racemic separation of the d,l-propoxyphene napsylate standard.
II. The chiral analysis of a sample.
III. The sample co-injected with the d,l-propoxyphene napsylate standard.
IV. The standard l-propoxyphene nasylate with the d,l-propoxyphene napsylate standard.
V. The standard d- propoxyphene nasylate with the d,l-propoxyphene napsylate standard.

Identification and Quantitation of Hydromorphone Hydrochloride in Palladone® (Extended Time-Release) Capsules

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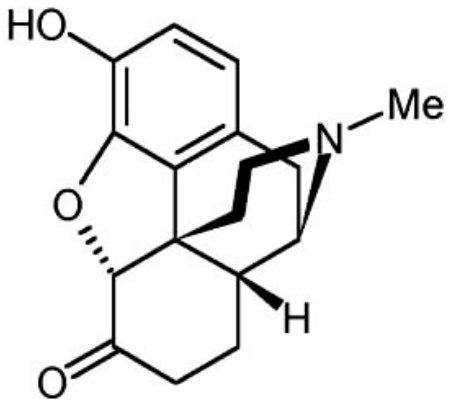
ABSTRACT: Palladone® is an extended time-release formulation of hydromorphone hydrochloride. The time-release matrix presents some unusual analytical challenges (especially for quantitation). FTIR (GC/IRD), 400 MHz ^{1}H-NMR, GC/MS, and CE (DAD) data are presented, enabling qualitative and quantitative analyses of Palladone® formulations.

KEYWORDS: Palladone®, Hydromorphone, Time-Release, Synthetic Opiate, Forensic Chemistry



Hydromorphone

Introduction

Hydromorphone is a synthetic opiate derived from morphine. It is a controlled substance (Schedule II) under the U.S. Controlled Substances Act. Palladone® is an extended time-release formulation of hydromorphone hydrochloride produced by Purdue Pharma (1). Controlled release formulations are usually solid dosage forms (capsules or tablets) that contain individual pellets that, when administered orally, slowly release the drug over a longer time frame (that is, different types of pellets in the formulation dissolve at different rates, thereby giving a lower but much longer lasting, steady-state concentration of the drug). Currently, Palladone® capsules are available in the following concentrations: 8, 12, 16, and 32 milligrams/capsule.

Synthetic opiates are popular among drug abusers, and are therefore occasionally submitted for analysis to forensic laboratories. Controlled release formulations present challenges for both qualitative and (especially) quantitative analysis. In order to accurately quantitate the drug, it must be possible to separate the drug from the matrix in a quantitative manner. This study presents both qualitative and quantitative methodologies for the analyses of Palladone® capsules.

Experimental

Chemicals and Reagents

Buffers and solutions were products of MicroSolv Technology (Eatontown, NJ). Chloroform, methanol, and acetone were products of Burdick and Jackson Laboratories (Muskegon, MI). $CDCl_3$, D_2O, and TMS were products of Sigma-Aldrich (Milwaukee, WI).

Qualitative Analyses

Vapor Phase Infrared Spectroscopy

The FTIR spectrum was obtained on a Nicolet 6700 FTIR GC-IRD (Figure 1).

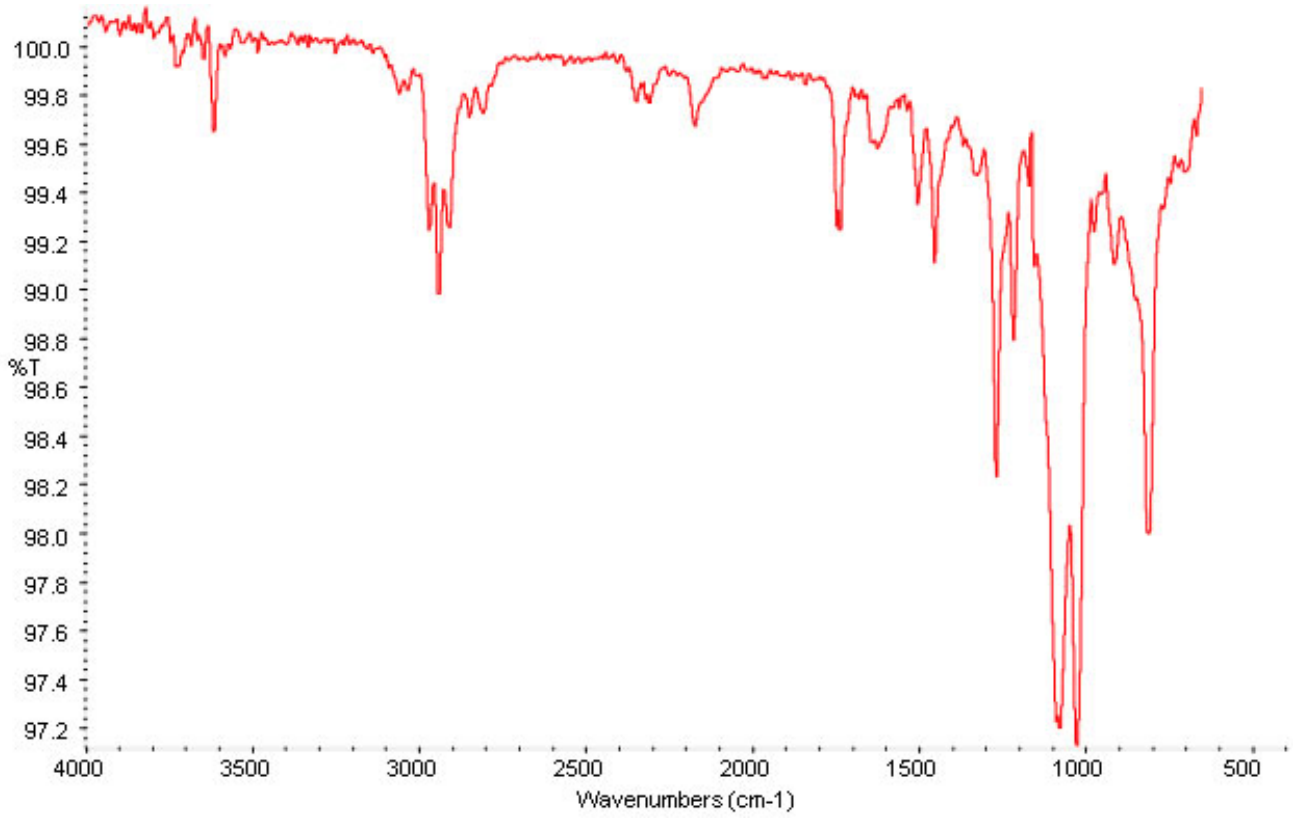


Figure 1. Vapor Phase FTIR of Hydromorphone.

Nuclear Magnetic Resonance Spectroscopy

One dimensional NMR analyses of hydromorphone and Palladone® were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe.

Hydromorphone hydrochloride has four isomeric forms (two keto [Ia,Ib] and two enol [IIa,IIb] forms (Figure 2)), each exhibiting two possible N-methyl orientations) under acidic conditions in either D_2O or CD_3OD. The relative proportions of these forms in solution depends on the solvent and the solution pH. Use of NMR for identification and quantitation of hydromorphone hydrochloride is therefore not recommended, because great care would be required to identify and properly integrate the signals generated by the four keto-enol forms. However, by utilizing a basic extraction with sodium bicarbonate into $CDCl_3$, only one form of hydromorphone base is observed (Figure 3).

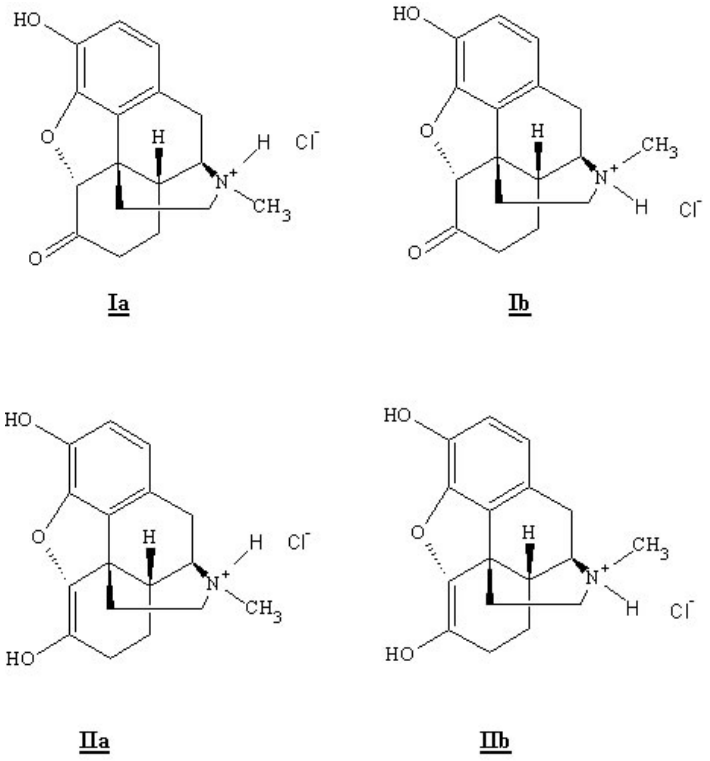


Figure 2. Keto-Enol Structures of Hydromorphone Hydrochloride.

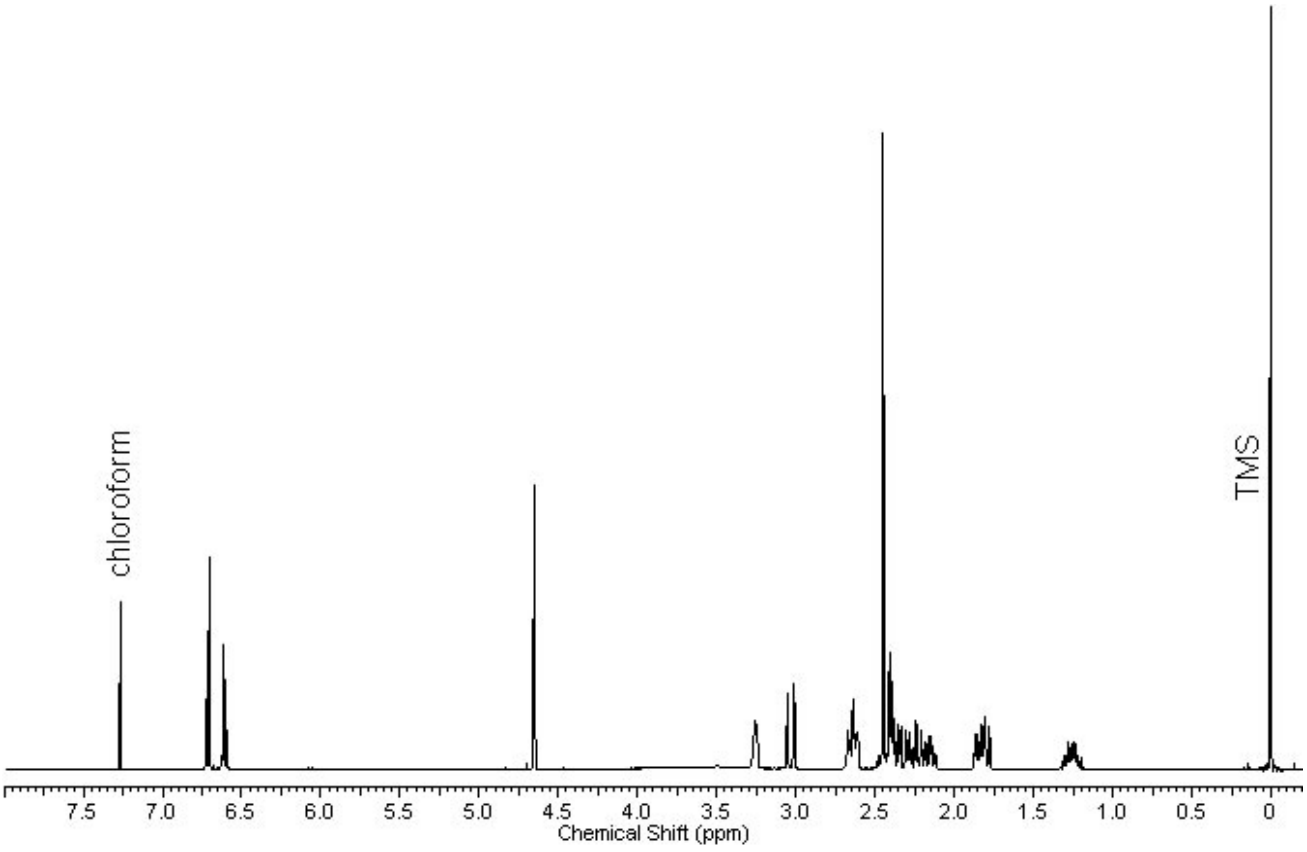


Figure 3. Hydromorphone Base in $CDCl_3$.

Workup of Palladone® for NMR Analysis: Weigh 20 milligrams of Palladone® into a 15 mL centrifuge tube. Add 0.5 mL saturated sodium bicarbonate in D_2O and 1 mL $CDCl_3$ containing 0.03% TMS, and sonicate for 15 minutes. This produces a white emulsion, which is then centrifuged. The $CDCl_3$ (lower) layer is then isolated and dried over anhydrous sodium sulfate, filtered, and transferred to an NMR tube. The resulting spectrum (Figure 4) displays both the hydromorphone peaks and the peaks from the capsule matrix material(s) (compare with Figure 3).

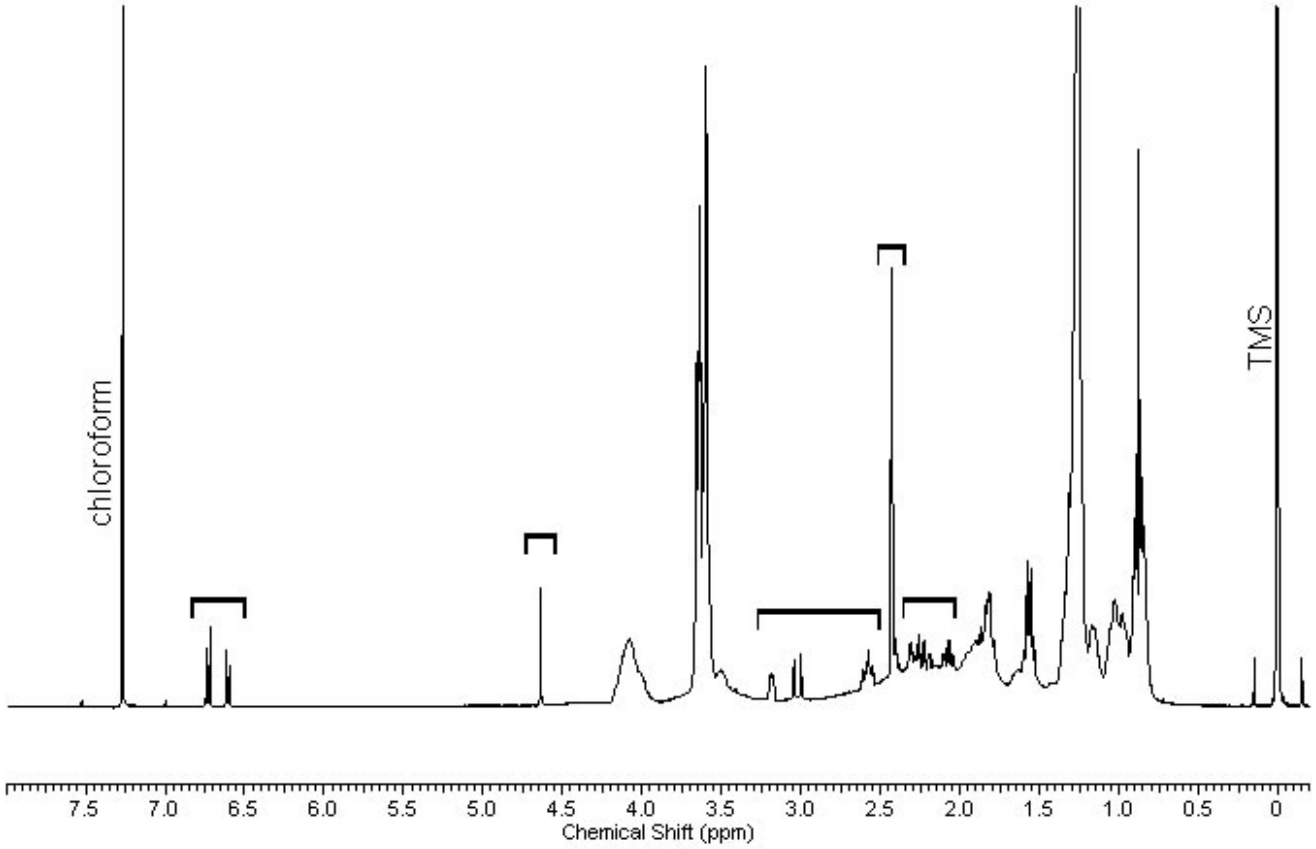


Figure 4. Palladone® (basified) in $CDCl_3$ (Note: Brackets indicate location of hydromorphone base peaks for identification).

Gas Chromatography/Mass Spectrometry

Instrument:	Agilent 6890N with an Agilent 5973 MSD
Column:	DB-1, 30 m x 0.25 mm x 0.25 µm film thickness
Injector Temperature:	280 ^{O}C
Oven Temperature:	90 ^{O}C for 2 minutes, 14 ^{O}C/minute to 280 ^{O}C
Carrier Gas:	Helium with split ratio = 25:1
Scan Range:	34 - 550 amu
Electron Ionization:	70 eV

Hydromorphone has a chemical formula of $C_{17}H_{19}NO_3$, and a molecular weight of 285.34. The mass spectrum shows the molecular ion (which is also the base peak) at *m/z* 285 (Figure 5).

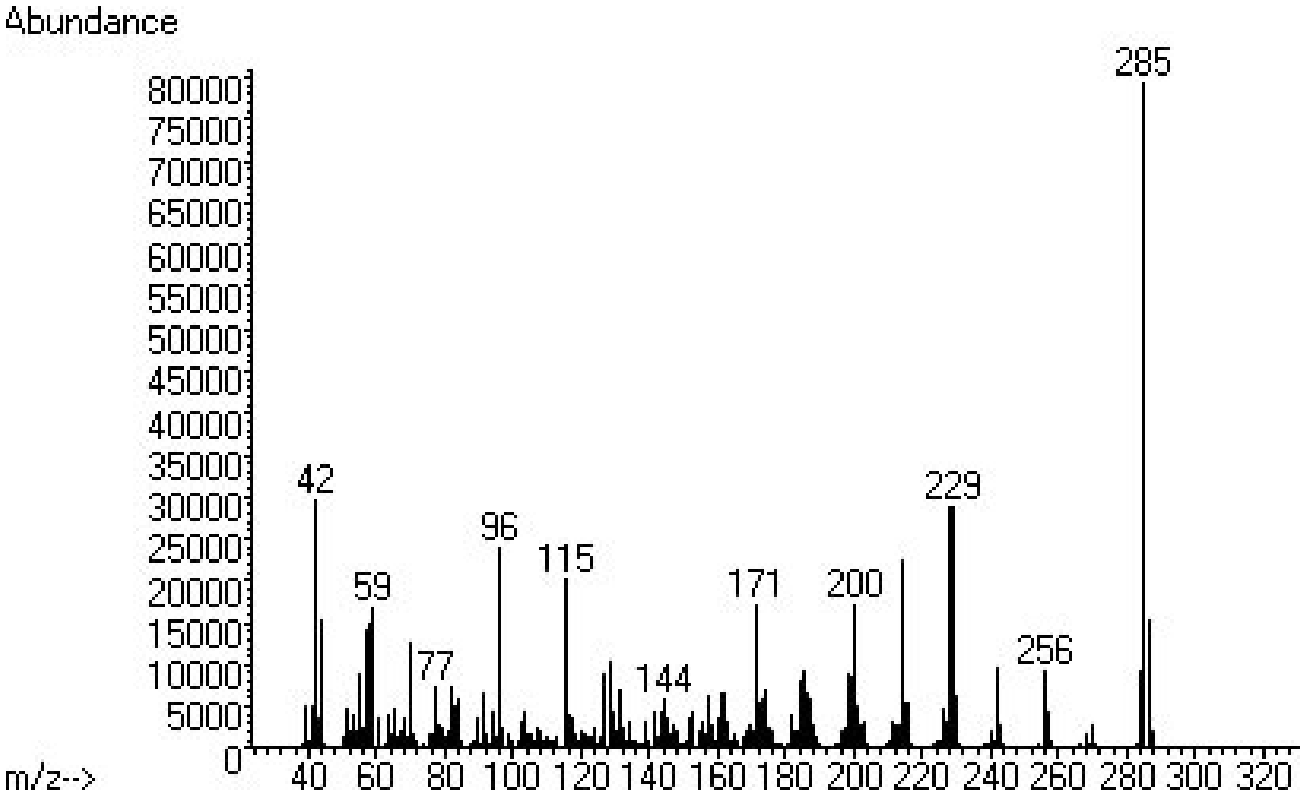


Figure 5. Mass Spectrum of Hydromorphone.

Quantitative (Capillary Electrophoresis) Procedures

As noted above, Palladone® presents some challenges for quantitative analysis. When utilizing GC, HPLC, or many modes of CE, the peak shapes are broad and non-symmetrical, causing difficulties for quantitation. In this study capillary electrophoresis (CE) was employed utilizing a dynamic coating with a chiral run buffer. Previous studies have shown that this method has improved peak shape over dynamic coatings without chiral additives (2). As shown, this approach resulted in excellent peak shape (Figure 6).

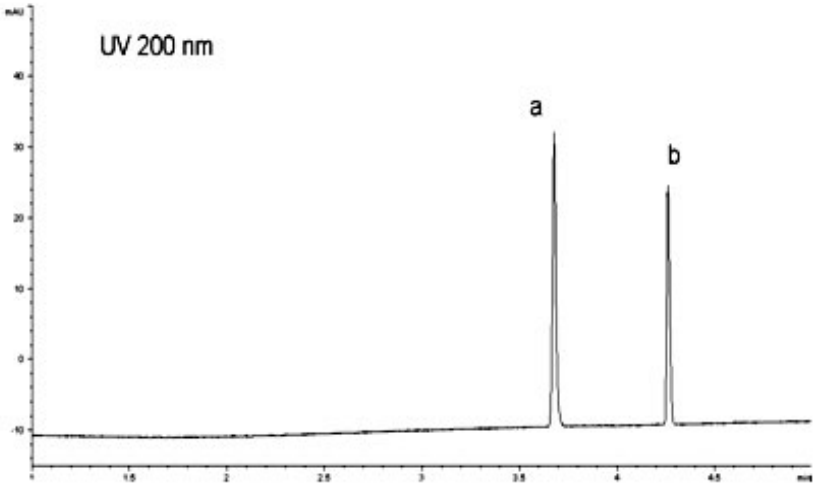


Figure 6. CE of a Palladone® Tablet; Peak Identities: (a) Hydromorphone; and (b) Procaine (IS).

An additional study was conducted to determine a method for quantitative recovery of hydromorphone hydrochloride from Palladone® capsules. The recovery study showed that using a mixture of 20% methanol : 80% injection solvent to prepare the sample gives a recovery of greater than 96 percent from any of the four Palladone® concentrations (Figure 7).

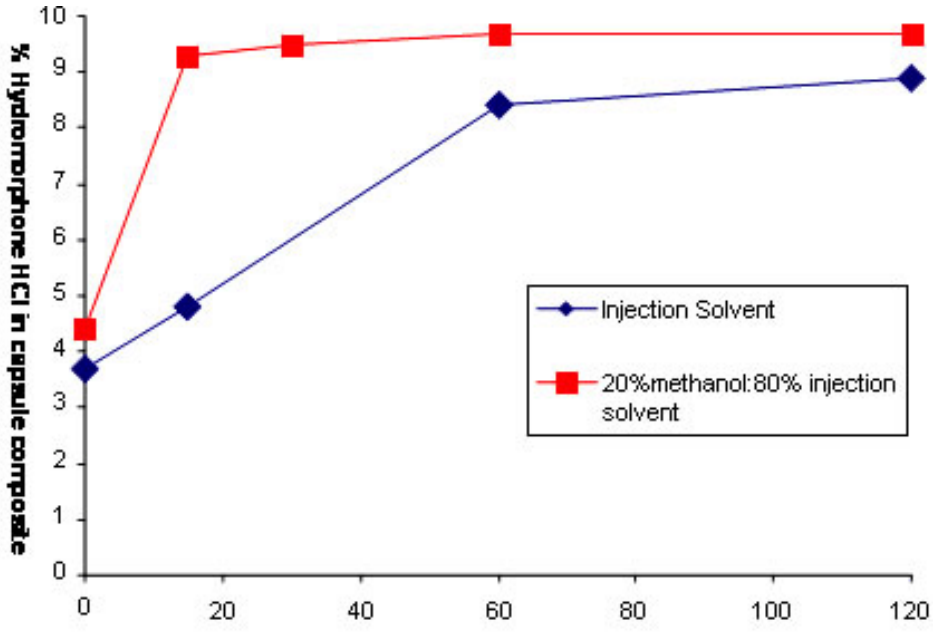


Figure 7. Recovery of Hydromorphone Hydrochloride from Palladone®.

Capillary Electrophoresis Procedures

Run Buffer: CElixir accelerator solution B (pH 2.5) + 50 mM 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) (Sigma). Weigh 1576 mg of HP-β-CD into a 50 mL Erlenmeyer flask. Pipette 20.0 mL of CElixir accelerator solution B (pH 2.5) and shake vigorously. Filter into 22 mL Teflon PVA vials (Cole Parmer) using a 0.45 µm, 25 mm regenerated cellulose filter.

Injection Solvent: Weigh 1,034 milligrams of sodium phosphate monobasic into a 100 mL volumetric flask. Dilute to volume with HPLC grade water. Adjust to approximately pH 2.6 using phosphoric acid added dropwise. Transfer contents into a 2000 mL volumetric flask with the aid of HPLC grade water. Dilute to volume with HPLC grade water. This final solution contains 3.75 mM phosphate (pH 3.2).

Internal Standard Stock Solution (ISSS): Weigh an appropriate amount of procaine hydrochloride into a volumetric flask to obtain a final concentration of approximately 1.0 mg/mL. Dilute to volume with 20% methanol : 80% injection solvent.

Standard Solution: Weigh an appropriate amount of standard hydromorphone hydrochloride into a volumetric flask to obtain a final concentration of approximately 0.10 mg/mL. Pipette an appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/mL. Dilute to volume with 20% methanol : 80% injection solvent. Filter approximately 1.0 mL of solution with a 0.45 µm, 25 mm regenerated cellulose filter into a 2.0 mL glass vial (Agilent part # 5182-0567). Care should be taken to ensure that there are no air bubbles on the bottom of the glass vial. Cap the vial with a polypropylene cap (Agilent part # 5182-9697).

Sample Preparation: Weigh an appropriate amount of sample into a volumetric flask so that the final hydromorphone hydrochloride concentration is approximately equal to that of the standard solution. Pipette an appropriate amount of internal standard solution to obtain a final concentration of 0.1 mg/mL. Dilute to volume with 20% methanol : 80% injection solvent. If the sample is a time-release preparation, it should be sonicated for

at least one hour. Filter approximately 1.0 mL of sample solution with a 0.45 μm, 25 mm regenerated cellulose filter into a 2.0 mL glass vial (Agilent part # 5182-0567). Once again, care should be taken to ensure that there are no air bubbles on the bottom of the glass vial. Cap the vial with a polypropylene cap (Agilent part # 5182-9697).

Instrumental Conditions:

Capillary Electrophoresis:	HP 3D instrument operated in CE mode
Capillary:	50 μm i.d. x 32.2 cm (23.7 cm length to detector)
Capillary Temperature:	15 ^{O}C
Conditioning:	0.1 N NaOH; 1 minute H_2O CElixir Reagent A (MicroSolv CE); 2 minutes CElixir Reagent B, pH 2.5 (MicroSolv CE)
Run Buffer:	CElixir Reagent B, pH 2.5 (MicroSolv CE) + 7.88% (w/v) HP-β-CD (hydroxypropyl-β-cyclodextrin)
Voltage:	20 kV
Injection:	Sample – 50mbar x 2 seconds followed by water at 35 mbar x 1 second
Total run time/sample:	6 minutes

Validation Criteria:

Linearity Range:	0.0217 mg/mL – 0.9774 mg/mL
Repeatability:	RSD < 0.81 %
Accuracy:	E % < 3.9 %
Correlation Coefficient (R^2):	0.99995

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gamma-Hydroxybutyrate, Silver Salt (AgGHB): Identification of *gamma*-Hydroxybutyrate (GHB) via Conversion to the Silver Salt

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ABSTRACT: A practical method for the identification of *gamma*-hydroxybutyrate (GHB) via infrared analysis of the corresponding silver salt is presented. The method is facile and robust, and complements the GC/MS analysis of GHB derivatives.

KEYWORDS: *gamma*-Hydroxybutyrate, *gamma*-Hydroxybutyric Acid, GHB, Sodium Oxybate, Infrared Spectrophotometry, IR, Silver Nitrate, Precipitation, Derivatization, GC/MS, Forensic Chemistry

Introduction

The continuing abuse of *gamma*-hydroxybutyric acid (GHB) and *gamma*-hydroxybutyrate (also commonly abbreviated as "GHB") has prompted the forensic community to develop an array of analytical methodologies to identify it in its various forms (1). As new salt forms of GHB have been encountered, forensic chemists have relied primarily on infrared spectrophotometry (IR) for identification (2-5). In conjunction with IR, derivatization of GHB (usually via silylation) with subsequent analysis by Gas Chromatography/Mass Spectrometry (GC/MS) has served as a complementary means of identification of the organic ligand (2).

The silver nitrate test has been a staple in the analytical laboratory for many years; however, its use is typically limited to the presumptive identification of simple halides and commonly encountered polyatomic ions by precipitation, often followed by a solubility test for the precipitate. This study was initiated to investigate the use the silver nitrate test as a fast and easy method to presumptively identify GHB in the field. However, the resulting silver salt precipitate (i.e., AgGHB) has proven to be quite valuable for more rigorous laboratory identification.

There are several advantages to converting GHB from a group I or II metal salt (e.g., LiGHB, NaGHB, KGHB, or $Ca(GHB)_2$) into AgGHB. The most immediate and practical advantage is increased stability with respect to water absorption. The silver salt is far less hygroscopic than any of its group I or II metal counterparts. This property gives the analyst a much longer time frame in which to conduct further analyses. Thus, the preparation and direct characterization of the AgGHB salt directly by IR, or by subsequent derivatization followed by GC/MS analysis, increases the specificity and accuracy of the analysis.

Experimental

Intrumentation

The IR spectra were collected by two instruments: A Nicolet 6700 FT-IR equipped with a single-bounce diamond ATR accessory, and (for KBr windows) with an ATI Mattson Genesis Series FT-IR. The GC/MS data were obtained using a Agilent 6890 GC equipped with a 5873 MSD (EI, 70 eV) and HP-5MS column (30 m long x 0.32 mm ID x 0.25 μm thickness) heated from 90 ^{O}C to 280 ^{O}C at 10 ^{O}C/minute.

Precipitation of AgGHB from Samples

The following methodology effectively yields AgGHB in acceptable purity. The procedure first precipitates all ions by addition of silver nitrate, then isolates AgGHB from the other silver salts based on solubility.

1. Place five drops of an aqueous sample or a small amount (100 – 200 mg) of a solid sample in a test tube. Add 5 drops of deionized (DI) water and 5 drops of 1 N $AgNO_3$ (aq) and mix well.

2. If no precipitate is formed, then there are probably no interfering anions (proceed to step 3). If a precipitate is formed, add 1 mL of DI water to test the solubility of the precipitate. If the precipitate dissolves with this additional water, then proceed to step 3. (Note that at high concentrations, AgGHB forms a precipitate that readily dissolves in excess water.) If the precipitate does not dissolve with excess water, separate the precipitate by decantation, centrifugation, or filtration. This (non-dissolving) precipitate is most likely the silver salt of a halide or a polyatomic anion. Collect the remaining (clear) liquid. Add one more drop of $AgNO_3$ solution to ensure that all remaining interfering ions are precipitated. If a second crop precipitate forms, repeat the above process until no further precipitation occurs. Proceed to step 3.

3. Add an equal volume of alcohol (methanol, ethanol, or isopropanol) to the recovered solution to precipitate any AgGHB. If a precipitate forms, collect it by centrifugation, and dry it under an air or nitrogen purge at ~70 ^{O}C. If no precipitate forms, add additional alcohol (you may need to add up to 2 - 3 equivalent volumes of alcohol). In solution, the AgGHB precipitate is finely divided and moves like a lyotropic liquid crystal (opalescent). A convenient mechanism to remove residual alcohol, water, and remaining organic impurities is to add ether, mix, and then decant and discard the ether. The precipitate forms initially as a white powder, but may darken over time due to heat and exposure to air. This degradation (to perhaps a silver oxide, carbonate, etc.) does not appear to affect the IR spectrum - a testament to the robustness of the technique.

4. Obtain an IR spectrum. If the spectrum appears to contain excess water (broadened bands) during a KBr pellet analysis, allow the chamber to purge with nitrogen for about 15 minutes. If the same effect is observed during an ATR analysis, allow the material to simply air dry. Both purging with nitrogen and air drying will remove the water and considerably sharpen the spectral bands.

5. An optional test to perform is direct silylation of AgGHB followed by GC/MS analysis. Derivatization can be accomplished with bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing a small amount of trimethychlorosilane (TMCS), followed by heating. To guard against the introduction of silver metal ions onto the GC column, the derivatization solution should be passed over solid NaCl to capture residual silver metal ion in the form of AgCl. The resulting solution is then analyzed by GC/MS.

Results and Discussion

Analysis of AgGHB by IR

Group I and II metal salts of GHB are notoriously hygroscopic, as evidenced by the IR spectral bands which become severely blurred upon absorption of atmospheric moisture. One of the major advantages of converting these salts into AgGHB is that the silver salt is considerably less hygroscopic than any of the common Group I and II metal salts of GHB. This property is demonstrated in Figure 1, which shows the IR spectrum of a wet AgGHB sample isolated from an actual exhibit containing both a GHB salt and GBL. Several of the bands which initially appeared blurry became much sharper as the IR chamber was purged with nitrogen (which effectively drove off the residual water). In Figure 2, the transmission IR spectrum of AgGHB (i.e., acquired as a KBr window) is compared to that of the ATR spectrum. The five step procedure described above was successfully used to convert the Na, Li, K, and Ca salts of GHB into AgGHB (see Figure 3 for the IR spectra of these four salts).

Interfering Components

There are numerous components that can potentially affect the purity of AgGHB. However, few interferences were observed. Halides and other common polyatomic anions are removed by precipitation with excess $AgNO_3$, prior to recovery of AgGHB (i.e., while the solution is still aqueous). Sugar, a common component in liquid GHB exhibits, had no effect on the purity of the AgGHB in controlled experiments. This absence of carry-over was most likely due to sugar's solubility in the lower alcohols. The same is true for $AgNO_3$, which remains in solution upon addition of the alcohol.

As noted earlier, excessive heating and prolonged exposure to air can cause minor degradation of the AgGHB. However, this darkening does not appear to affect the IR spectrum. This is demonstrated in Figure 4, which shows that the IR spectrum of a nearly four year old sample of AgGHB is indistinguishable from that of a freshly prepared sample.

Further Use of AgGHB

Further attempts to characterize AgGHB versus the Na, Li, K, and Ca GHB salts proved to be fruitless. Techniques such as HPLC and proton NMR cannot differentiate AgGHB from other GHB salts.

Conclusions

The precipitation method described above is an effective and selective method for removal of GHB salts from solution. Interferences by halide and common polyatomic anions and components such as $AgNO_3$ and sugar are minimized via filtration of unwanted precipitates and washings of the target precipitate with selected solvents at key points in the isolation scheme. The major advantage of forming the silver salt of GHB is its increased stability, which in turn affords the chemist greater opportunity for further testing. Perhaps the primary benefit of this work is the increased specificity. The conversion to and characterization of GHB as the silver salt, followed by secondary derivatization and characterization with a silylating reagent, significantly increases the specificity of the analysis, and thus yields a more in-depth identification.

Acknowledgements

The author would like to thank Forensic Chemist Scott J. Tschaekofske of the Minnesota Department of Public Safety, Bureau of Criminal Apprehension, for extensive discussions concerning this topic.

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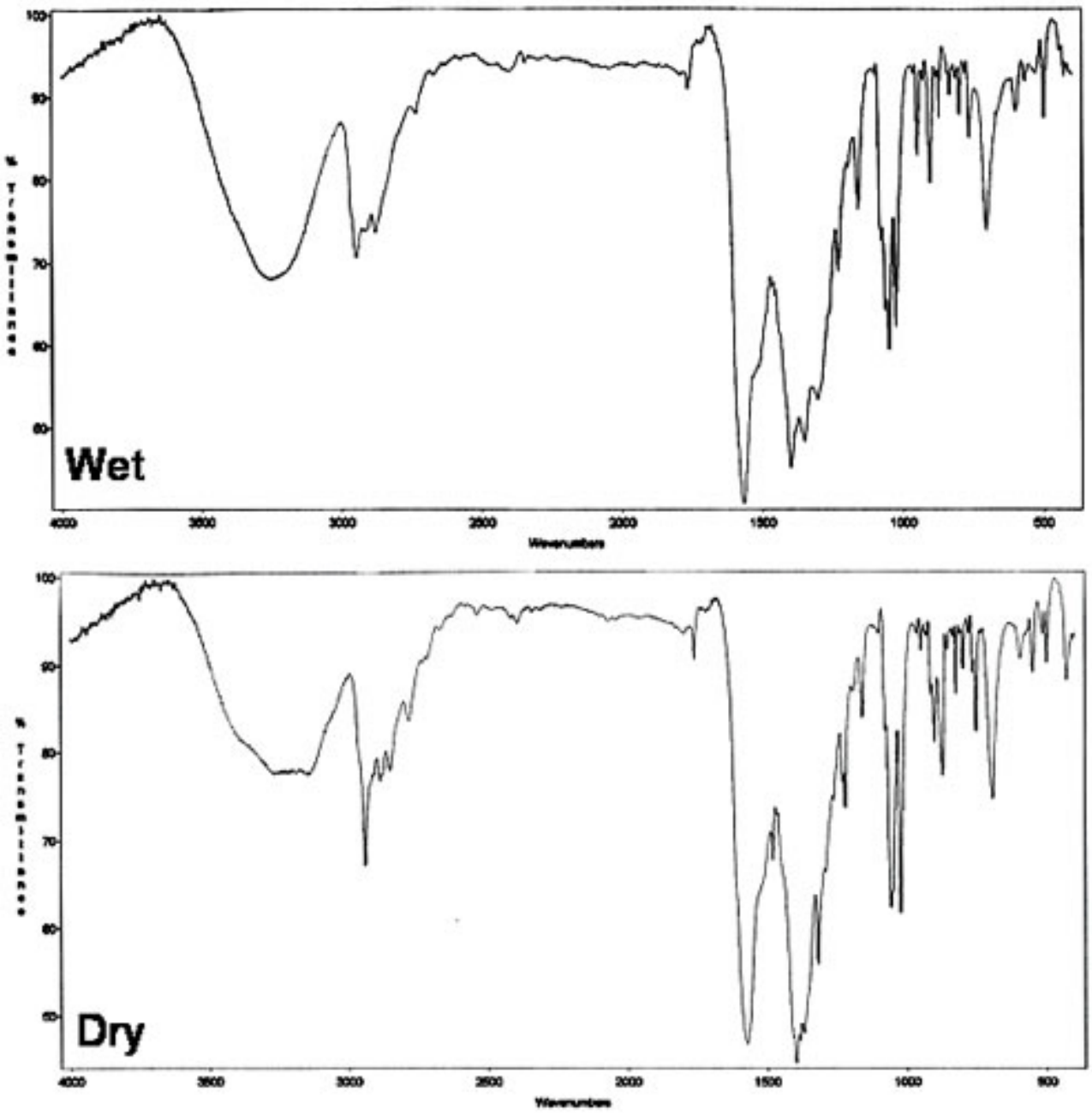

Figure 1: Effect of Moisture on IR Spectrum of AgGHB (Transmission).

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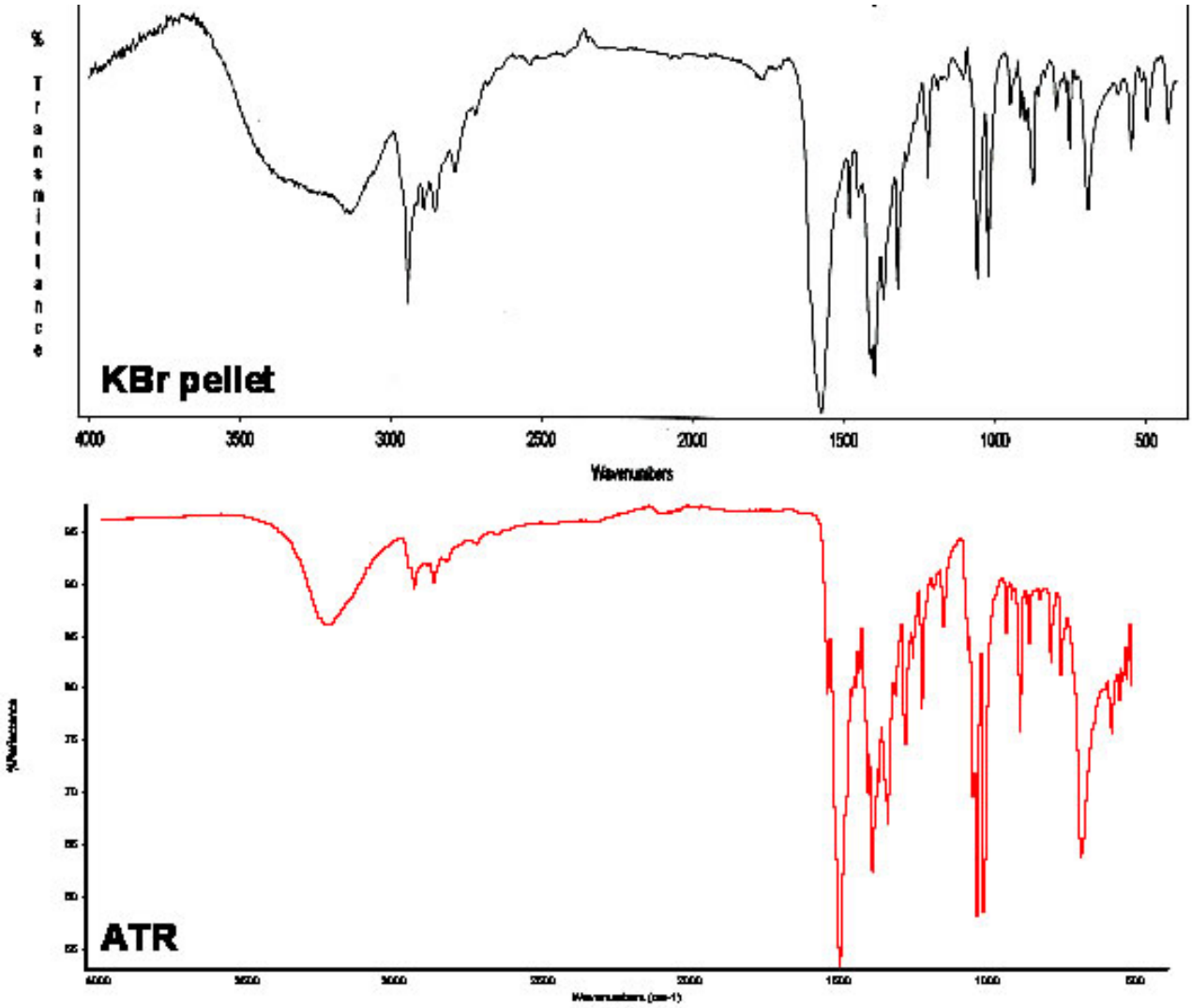

Figure 2: Transmission and Reflectance IR Spectra of AgGHB.

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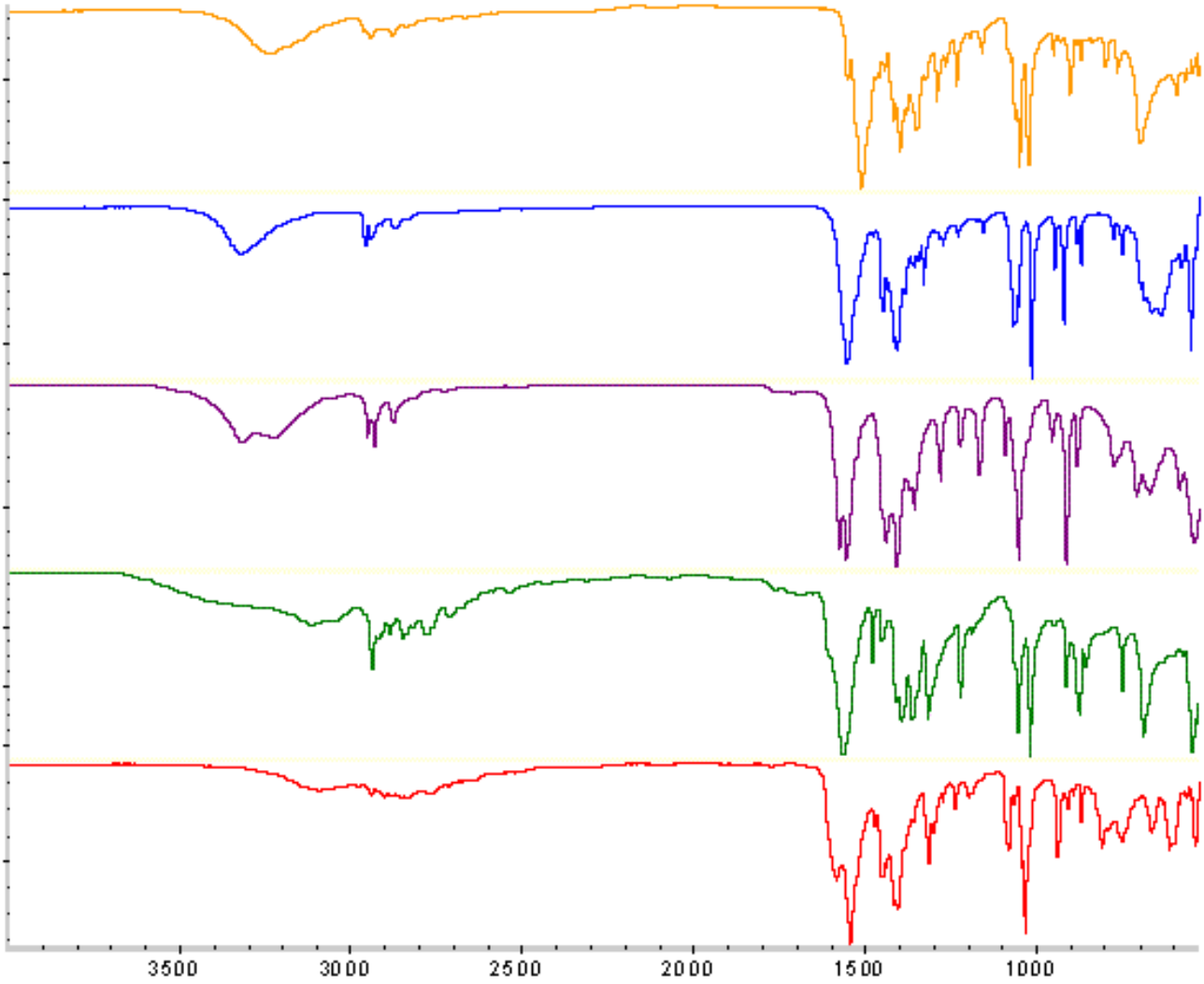

Figure 3a: IR/ATR Spectra of AgGHB (top), NaGHB, LiGHB, KGHB, and $Ca(GHB)_2$.

Table 1: Frequencies for IR/ATR Spectral Bands of AgGHB, NaGHB, LiGHB, KGHB, and $Ca(GHB)_2$ (in cm^{-1}).

AgGHB	NaGHB	LiGHB	KGHB	$Ca(GHB)_2$
3241	3318	3318	3113	3091
2945	2959	3227	2945	2941
2877	2942	2954	2886	1587
1552	2870	2932	2845	1544
1512	1555	2877	2777	1451
1418	1451	1574	2714	1417
1398	1405	1555	1564	1407

(Continued Next Page)

(Table 1, Continued)

AgGHB	NaGHB	LiGHB	KGHB	$Ca(GHB)_2$
1349	1329	1438	1480	1312
1292	1272	1409	1452	1238
1232	1229	1357	1393	1082
1162	1156	1282	1361	1032
1049	1066	1222	1318	936
1026	1015	1167	1220	910
952	946	1092	1056	868
904	920	1055	1019	810
870	881	954	914	752
800	867	914	874	664
763	774	881	859	613
698	753	778	752	600
592	666	711	687	537
567	635	672	548	
	576	581		
	550	540		

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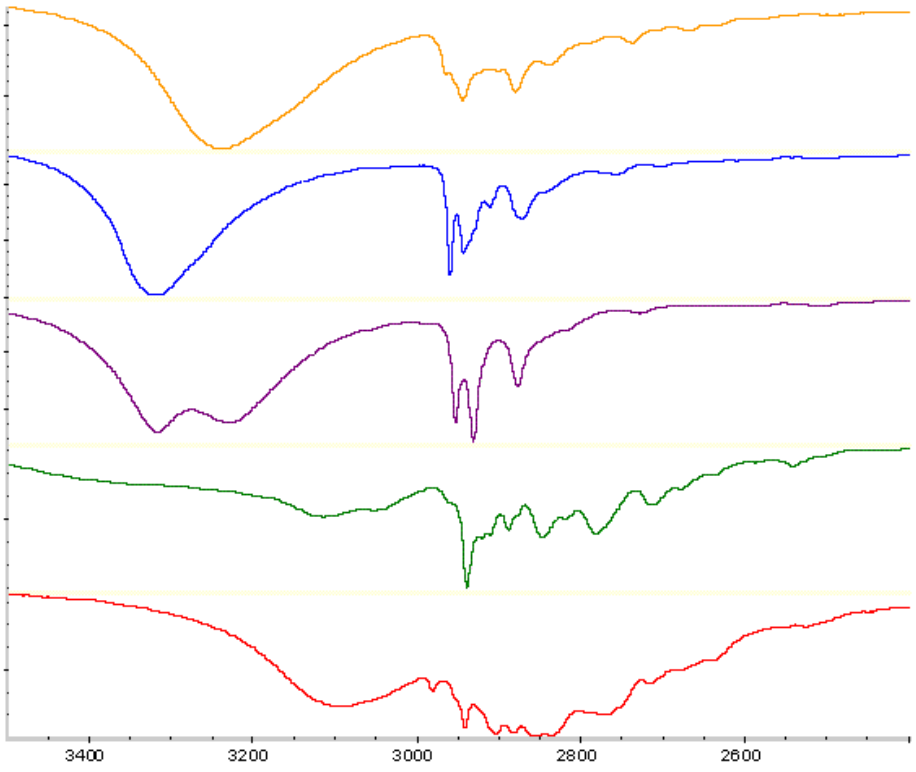

Figure 3b: IR/ATR Spectra of AgGHB (top), NaGHB, LiGHB, KGHB, and $Ca(GHB)_2$ (Expanded View of 3500 - 2400 cm^{-1}).

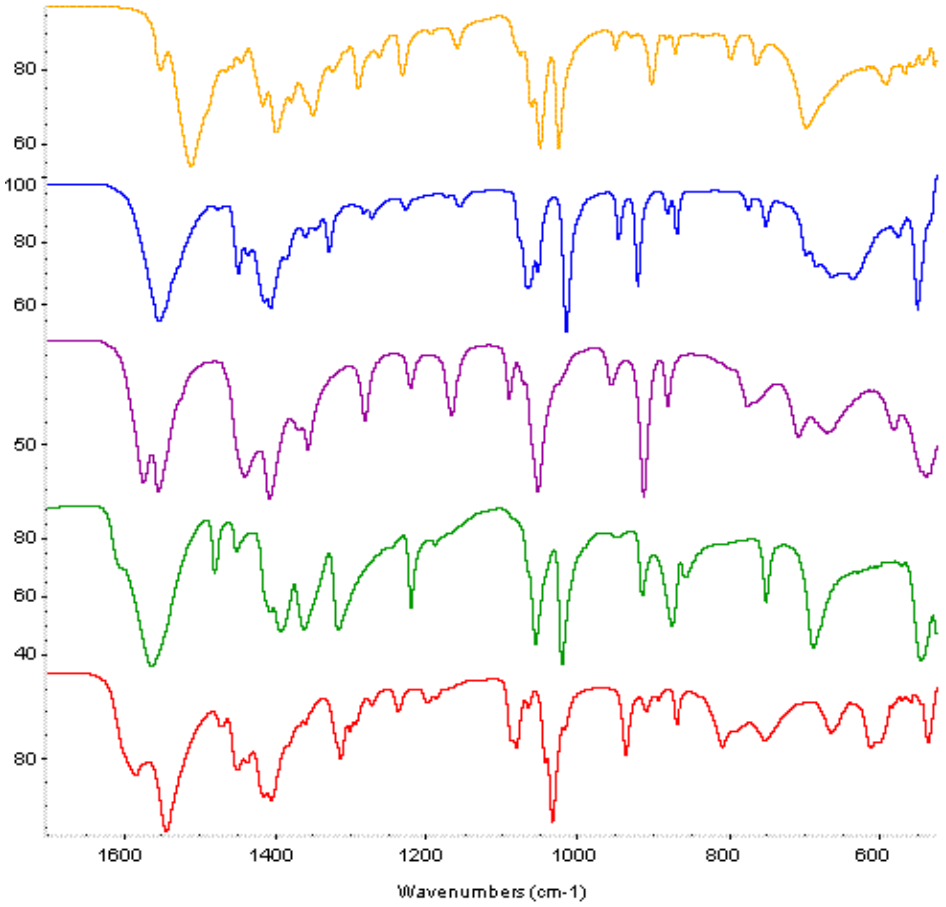

Figure 3c: IR/ATR Spectra of AgGHB (top), NaGHB, LiGHB, KGHB, and $Ca(GHB)_2$ (Expanded View of 1700 - 530 cm^{-1}).

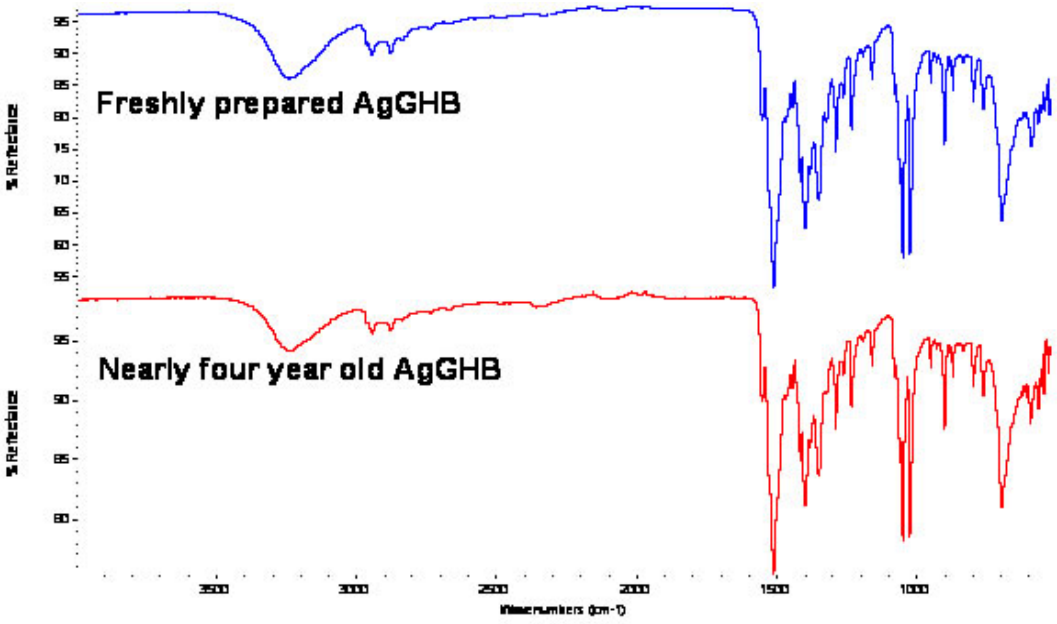

Figure 4: IR/ATR Spectra of Freshly Prepared and Aged AgGHB.

Analytical Profiles for Five "Designer" Tryptamines

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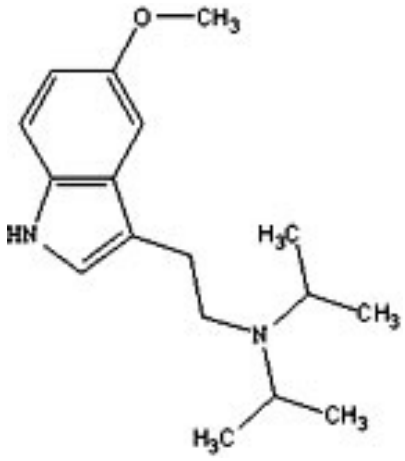
ABSTRACT: Analytical data (Color Tests, GC/FID, GC/MS, FTIR/ATR, ^{1}H-NMR, and HPLC) for five hallucinogenic "designer" (synthetic) tryptamines is reported. The compounds (5-methoxy-N,N-diisopropyl-tryptamine hydrochloride (5-MeO-DIPT); 5-methoxy-N-methyl-N-isopropyltryptamine base (5-MeO-MIPT), 5-methoxy-α-methyltryptamine hydrochloride (5-MeO-AMT), N,N-dipropyltryptamine hydrochloride (DPT), and 5-methoxy-N,N-dimethyltryptamine base (5-MeO-DMT)) are increasingly encountered in forensic, crime, and toxicology laboratories.

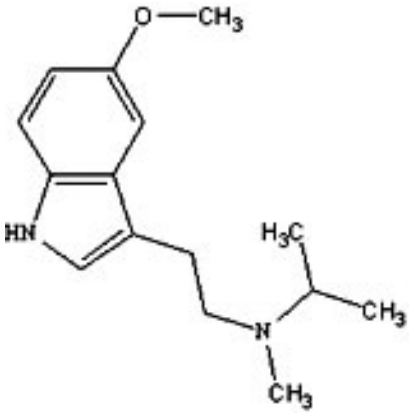
KEYWORDS: Tryptamines, Analogues, Hallucinogens, Color Testing, GC/MS, ^{1}H-NMR, FTIR/ATR, HPLC, Forensic Chemistry

Introduction

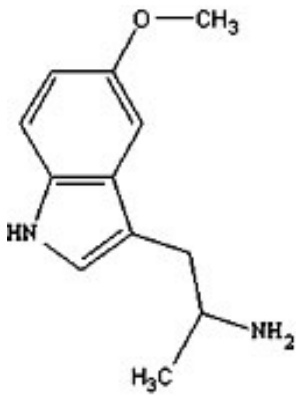
Over the past six months, this laboratory has received over 40 referral drug samples suspected of containing hallucinogenic "designer" tryptamines. Some hallucinogenic tryptamines (e.g., N,N-dimethyltryptamine (DMT), psilocybin, bufotenine, etc.) are naturally produced in fungi, plants, and animals, but these "designer" tryptamines are non-naturally occurring compounds that are produced in laboratories [1]. 5-Methoxy-N,N-diisopropyltryptamine hydrochloride (5-MeO-DIPT), 5-methoxy-N-methyl-N-isopropyltryptamine base (5-MeO-MIPT), 5-methoxy-α-methyltryptamine hydrochloride (5-MeO-AMT), N,N-dipropyltryptamine hydrochloride (DPT), and 5-methoxy-N,N-dimethyltryptamine base (5-MeO-DMT) (Figure 1) are all synthetically produced analogues of known hallucinogenic tryptamines, and have been submitted with increasing frequency to federal, state, and local forensic, crime, and toxicology laboratories throughout the United States. On September 29, 2004, 5-MeO-DIPT (also known by its street names of "Foxy" and "Foxy-Methoxy") became



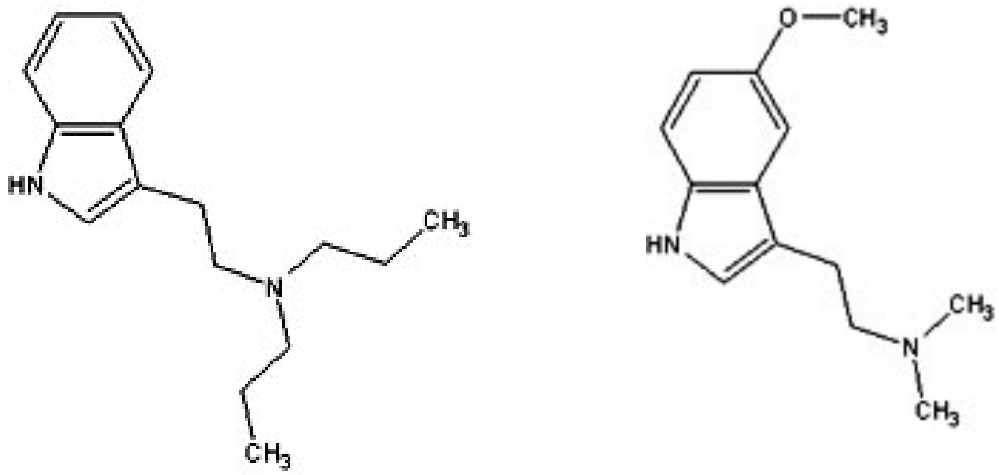
5-MeO-DIPT



5-MeO-MIPT



5-MeO-AMT



DPT **5-MeO-DMT**

Figure 1: Structures of Tryptamine Analogues

federally regulated as a Schedule I Controlled Substance [2]. As of the submission date of this article (April, 2005), 5-MeO-AMT, 5-MeO-MIPT, DPT, and 5-MeO-DMT are not yet specifically listed in the Controlled Substance Act (CSA); however, individuals trafficking in these substances can be prosecuted under the Analogue Statute of the Controlled Substances Act [3]. Herein, we report analytical data (Color Tests, GC/FID, GC/MS, FTIR, NMR, and HPLC) for these five tryptamines.

Experimental

Color Test Reagents

Ehrlich's Reagent: 0.5 g of *para*-dimethylaminobenzaldehyde (p-DMAB) in a mixture containing 50 mL of ethyl alcohol and 50 mL concentrated hydrochloric acid [5]. *Marquis Reagent*: 100 mL formaldehyde in 1000 mL concentrated sulfuric acid [5].

Fourier Transfer Infrared Spectroscopy/Attenuated Total Reflectance (FTIR/ATR)

FTIR spectra were collected on a Thermo Nicolet Nexus 670 FTIR with a potassium bromide (KBr) beam splitter and a deuterated triglycine sulfate (DTGS) KBr detector, equipped with a single bounce Durascope Attenuated Total Reflectance (ATR) accessory. Thirty-two (32) scans were collected between 4000 cm^{-1} and 400 cm^{-1}, with a resolution of 4.0 cm^{-1}.

Gas Chromatography/Flame Ionization Detector (GC/FID)

GC analyses were performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector, using a J & W Scientific DB-1 column with a 30 m x 0.25 mm ID and 0.25 µm film thickness. Instrumental parameters include an injector temperature of 280 ^{O}C, hydrogen carrier gas with a flow rate of 1.1 mL/minute, a split ratio of 25:1, and nitrogen make-up gas. The detector temperature was 280 ^{O}C. The oven temperature was initially held at 100 ^{O}C for 1 minute, then ramped at 12 ^{O}C/minute to 280 ^{O}C and held for 9 minutes. The concentration for each of the tryptamine analogues was 4 mg/mL in chloroform with a 1 µL injection.

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS spectra were collected on an Agilent 6890N GC interfaced with an Agilent 5973N Mass Selective Detector (MSD) using a scan acquisition from 34 to 550 amu. A J & W Scientific DB-1 column with a 30 m x 0.25 mm ID and 0.25 µm film thickness was utilized. The injection port temperature was set at 280 ^{O}C. The

carrier gas was Helium with a split ratio of 25:1 and constant flow of 1 mL/minute. The oven temperature was initially held at 100 ^{O}C for 1 minute, then ramped at 12 ^{O}C/ minute to 300 ^{O}C and held for 7 minutes. A volume of 1 μL containing a concentration of 4 mg/mL of each tryptamine analogue in chloroform was injected.

High Performance Liquid Chromatography (HPLC)
HPLC analyses were performed on a Hewlett Packard (HP) Series 1100 HPLC equipped with an ultraviolet lamp and diode array detector (DAD). A volume of 20 μL containing a concentration of 0.4 mg/mL of each tryptamine analogue diluted in phosphate buffer was injected onto a Whatman Partisil 5 ODS 3, 3.2 x 125 mm column, and scanned from 220 nm – 340 nm with a threshold of 1.0 mAU. An HPLC gradient program was utilized with an initial 20 minute ramp from 95:5 phosphate buffer/methanol to 70:30 phosphate buffer/methanol. This was held for 6 minutes. This was followed by a 10 minute ramp from 70:30 phosphate buffer/methanol to 20:80 phosphate buffer/methanol and held for 4 minutes. The pump flow was 0.76 mL/minute with a total run time of 45 minutes.

Nuclear Magnetic Resonance (^{1}H-NMR)
Proton NMR analyses were performed on a Varian Mercury 400 MHz NMR using a 5 mm Nalorac Indirect Detection probe, or on a Varian Unity 500 MHz NMR with a 5 mm Varian Indirect Detection probe. The samples were prepared at 10 - 30 mg/mL in deuterium oxide (D_2O) containing 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt (TSP) as the reference at 0 ppm (Aldrich Chemical Co., Milwaukee, WI). The proton spectra were obtained with 8 scans using a 45 second delay and 90^{O} pulse.

Results and Discussion

Color Testing
Testing each of the tryptamine analogues with the Ehrlich's reagent produced the same change in color from purple to blue, except for DPT HCl, which produced a violet color change and 5-MeO-MIPT which changed from purple to a faint blue. In the presence of the Marquis reagent, each tryptamine analogue produced the same color change from yellow to black, except for DPT HCl which gave a yellow color only, as shown in Table 1.

Table 1: Results of Color Testing.

"Designer" Tryptamine	Ehrlich's Reagent	Marquis Reagent
N,N-DPT HCl	violet	yellow
5-MeO-DMT	purple to blue	yellow to black
5-MeO-MIPT	purple to faint blue	yellow to black
5-MeO-DIPT HCl	purple to blue	yellow to black
5-MeO-AMT HCl	purple to blue	yellow to black

GC/FID
The tryptamine analogues were first injected separately to establish an absolute retention time, followed by an injection of a mixture containing the tryptamine analogue and tryptamine itself (as an internal standard) to establish a relative retention time. Based upon the relative retention times, each tryptamine was fully resolved, as shown in Table 2.

HPLC
The HPLC chromatograms show that each tryptamine has the same ultraviolet spectra (UV) and molar absorptivity due to the UV detection of identical chromophores. The retention time is utilized to distinguish each tryptamine, noting that in aqueous acid, each one has a λmax at 276 nm. DPT HCl has a λmax at 280 nm, as shown in Table 3.

Table 2: Results of GC/FID Analyses.

"Designer" Tryptamine	Absolute Retention Time	Relative Retention Time vs. Tryptamine (10.547 minutes)
5-MeO-AMT	12.732 minutes	1.207
5-MeO-DMT	12.946 minutes	1.227
DPT	13.625 minutes	1.292
5-MeO-MIPT	14.272 minutes	1.353
5-MeO-DIPT	15.195 minutes	1.441

Table 3: Results of HPLC Analyses.

"Designer" Tryptamines	Retention Time	λmax in Aqueous Acid
5-MeO-AMT	9.325 minutes	276 nm
5-MeO-DMT	10.280 minutes	276 nm
5-MeO-MIPT	11.961 minutes	276 nm
5-MeO-DIPT	17.051 minutes	276 nm
DPT	17.016 minutes	280 nm

FTIR/ATR

The infrared spectra of 5-MeO-AMT HCl (Figure 2) is a primary amine salt which shows N-H stretching in the region of 3256 cm^{-1}. 5-MeO-DIPT HCl (Figure 3) and DPT HCl (Figure 5) are tertiary amine hydrochlorides which exhibit a N-H stretch in the region of 3156 cm^{-1} to 3186 cm^{-1}. In the region of 3000 cm^{-1} to 3035 cm-1, 5-MeO-MIPT (Figure 4) and 5-MeO-DMT (Figure 6) exhibit an aromatic C-H stretch.

GC/MS

The mass spectra are displayed in Figures 7 - 11. Each of the base peaks are attributed to *alpha* cleavage of the amine side chain, with the exception of 5-MeO-AMT (Figure 7). 5-MeO-AMT produces a base peak at *m/z* 161 due to *alpha* cleavage and proton transfer to the indole moiety. 5-MeO-AMT has a molecular ion at *m/z* 204 and a prominent peak at *m/z* 44. 5-MeO-MIPT (Figure 8) gives a molecular ion at *m/z* 246 with a base peak at *m/z* 86. The ion at *m/z* 160 suggests the loss of the methyl-isopropylamine side chain ($C_5H_{12}N$). 5-MeO-DIPT (Figure 9) gives a molecular ion at *m/z* 274 with a base peak at *m/z* 114 ($C_7H_{16}N^+$). The fragmentation at *m/z* 160 suggests the loss of 114 from the molecular ion. DPT (Figure 10) gives a molecular ion at *m/z* 244 and a base peak at *m/z* 114, with fragments at *m/z* 130 due to a loss of $C_7H_{16}N$ and *m/z* 144 due to the loss of $C_6H_{14}N$ from the molecular ion. 5-MeO-DMT (Figure 11) gives a molecular ion at *m/z* 218, with a fragmentation at *m/z* 160 due to the loss of the base peak at *m/z* 58 ($C_3H_8N^+$). Each mass spectrum has a different molecular ion and a different base peak, except for 5-MeO-DIPT (Figure 9) and DPT (Figure 10). These compounds have a base peak at *m/z* 114. *Alpha* cleavage is a dominant reaction of amines which produces the base peak in N-alkylamines and α-substituted primary amines, leading to the loss of the largest alkyl group [4].

NMR

The NMR spectra are displayed in Figures 12 -16. All five compounds were easily distinguishable by proton NMR. The 5-methoxy substituted tryptamines have the same peak patterns and very similar chemical shifts in the aromatic region and methoxy region: 4 aromatic protons (2 doublets, a singlet, and a doublet of doublets) and 3 protons at 3.9 ppm (singlet for the methoxy group). DPT is not substituted at position 5, giving a different aromatic peak pattern for 5 protons (2 doublets, 2 triplets, and one singlet), and these signals have different chemical shifts from the 5-methoxy compounds, as shown in Figure 1.

All five compounds have unique and easily interpretable peak patterns. A singlet at 2.8 - 2.9 ppm indicates an N-CH_3; integration will determine if the peak represents a mono- or di-methyl group. Doublets at 1.2 - 1.3 ppm indicate methyls bonded to a methine that are *beta* to the amine nitrogen (Figures 12-14). Integration of these doublets and their associated methines will determine if the group is a diisopropylamine, monoisopropylamine, or a simple N-CHR-CH_3. The spectrum of DPT (Figure 15) contains a triplet at 0.9 ppm integrating to 6 protons, indicating 2 methyls bonded to 2 methylenes (multiplets at 1.6-1.7 ppm) bonded to 2 more methylenes (multiplet at 3.1 ppm); i.e., an N,N-dipropyl group. Ethyl amine protons are found as triplets or multiplets above 3 ppm.

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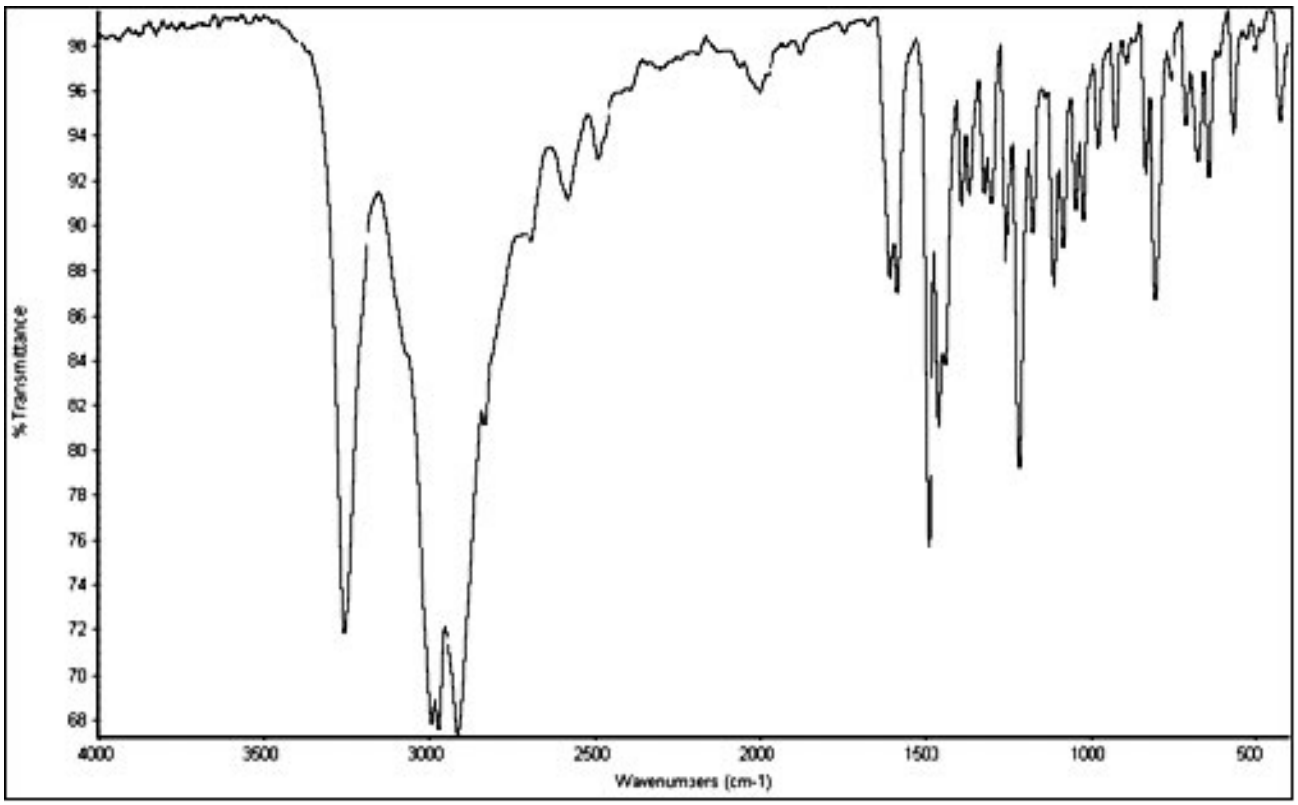

Figure 2: FTIR Spectrum of 5-Methoxy-α-methyltryptamine HCl.

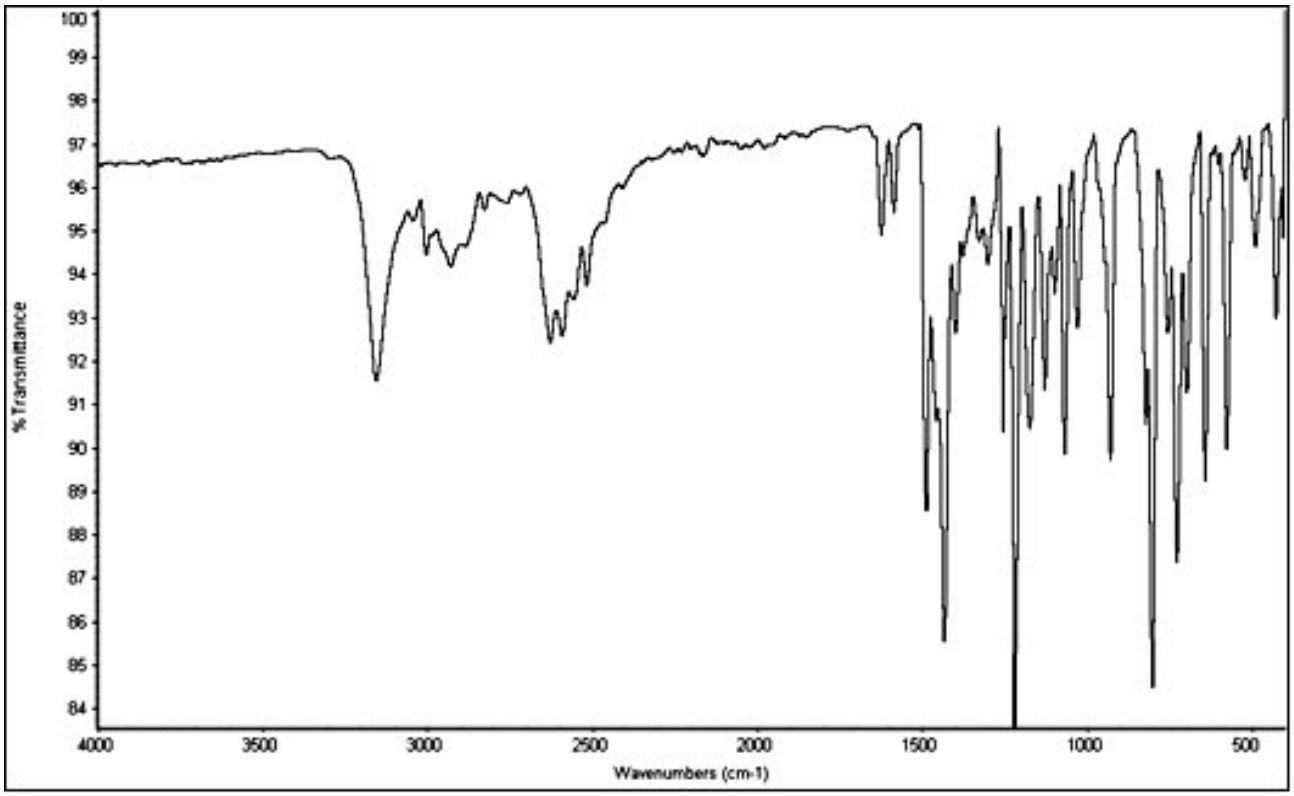

Figure 3: FTIR Spectrum of 5-Methoxy-N,N-diisopropyltryptamine HCl.

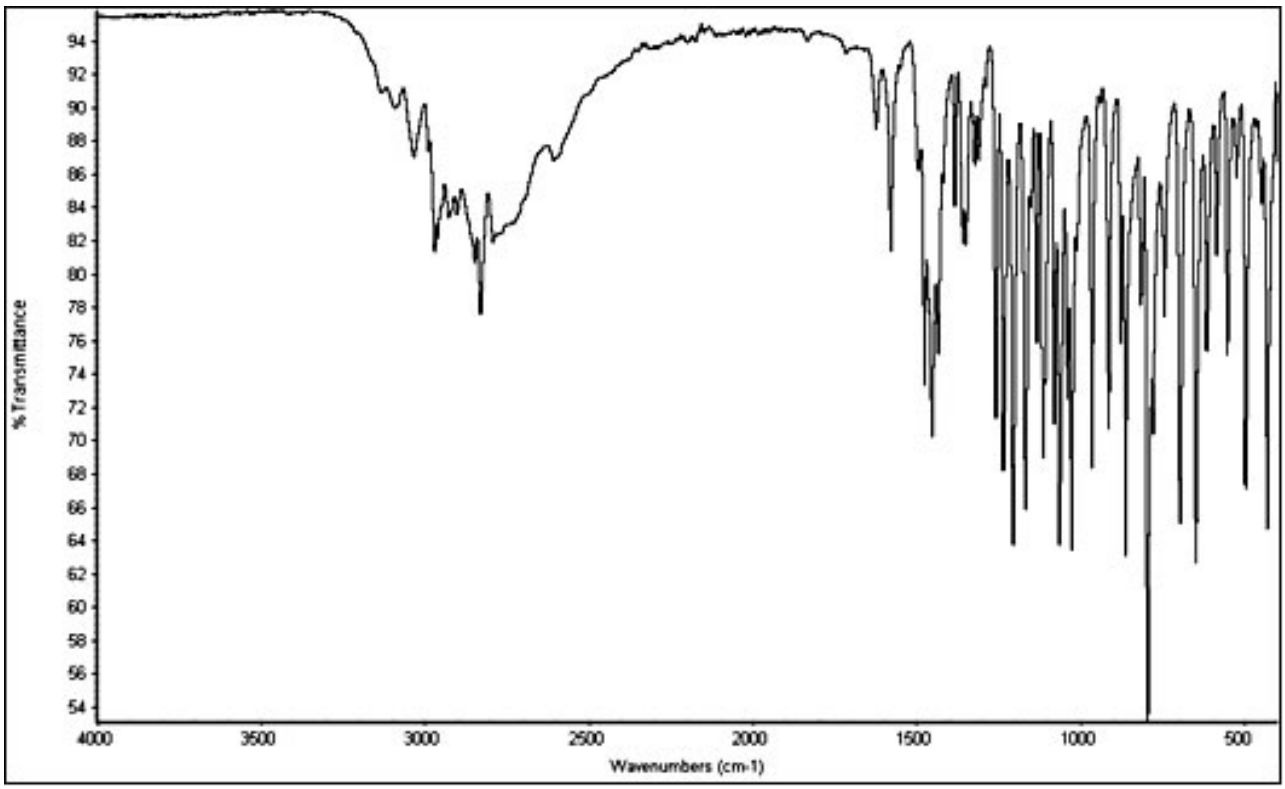

Figure 4: FTIR Spectrum of 5-Methoxy-N-methyl-N-isopropyltryptamine Base.

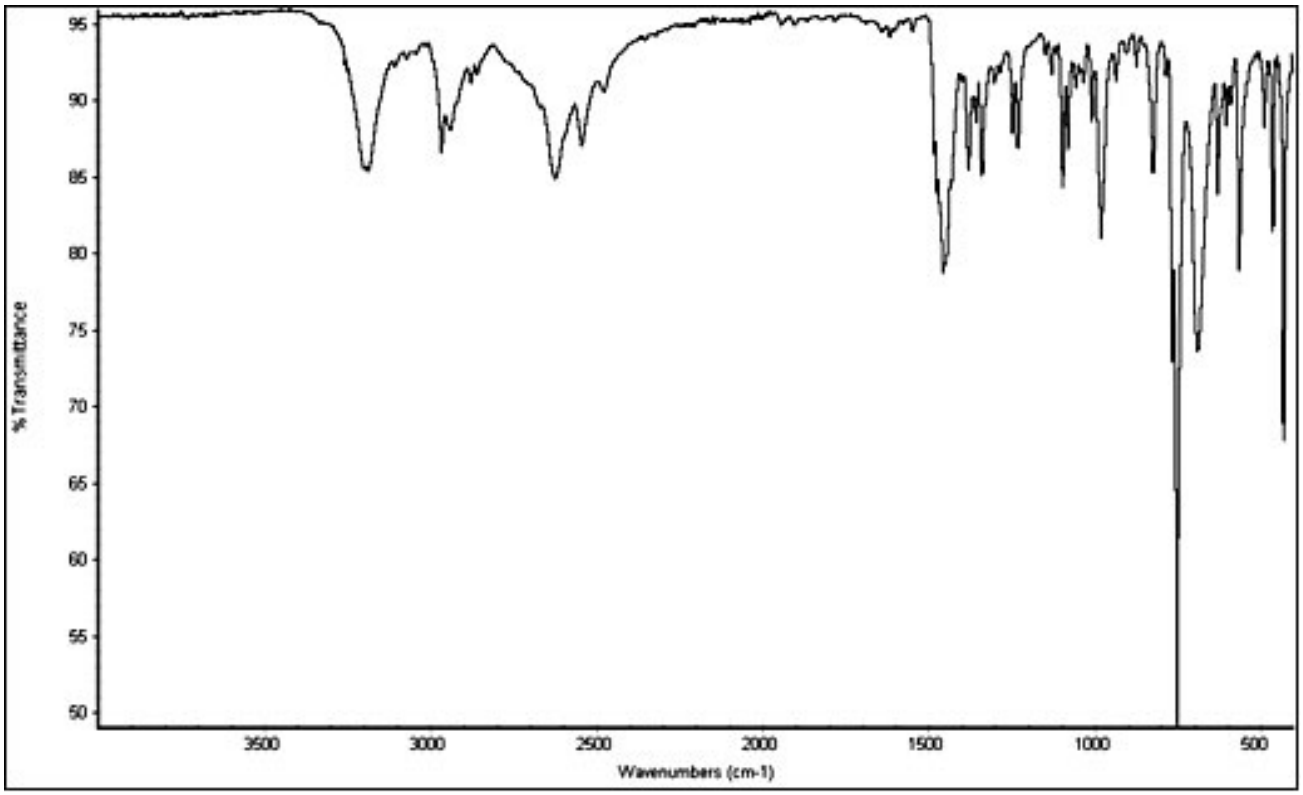

Figure 5: FTIR Spectrum of N,N-Dipropyltryptamine HCl.

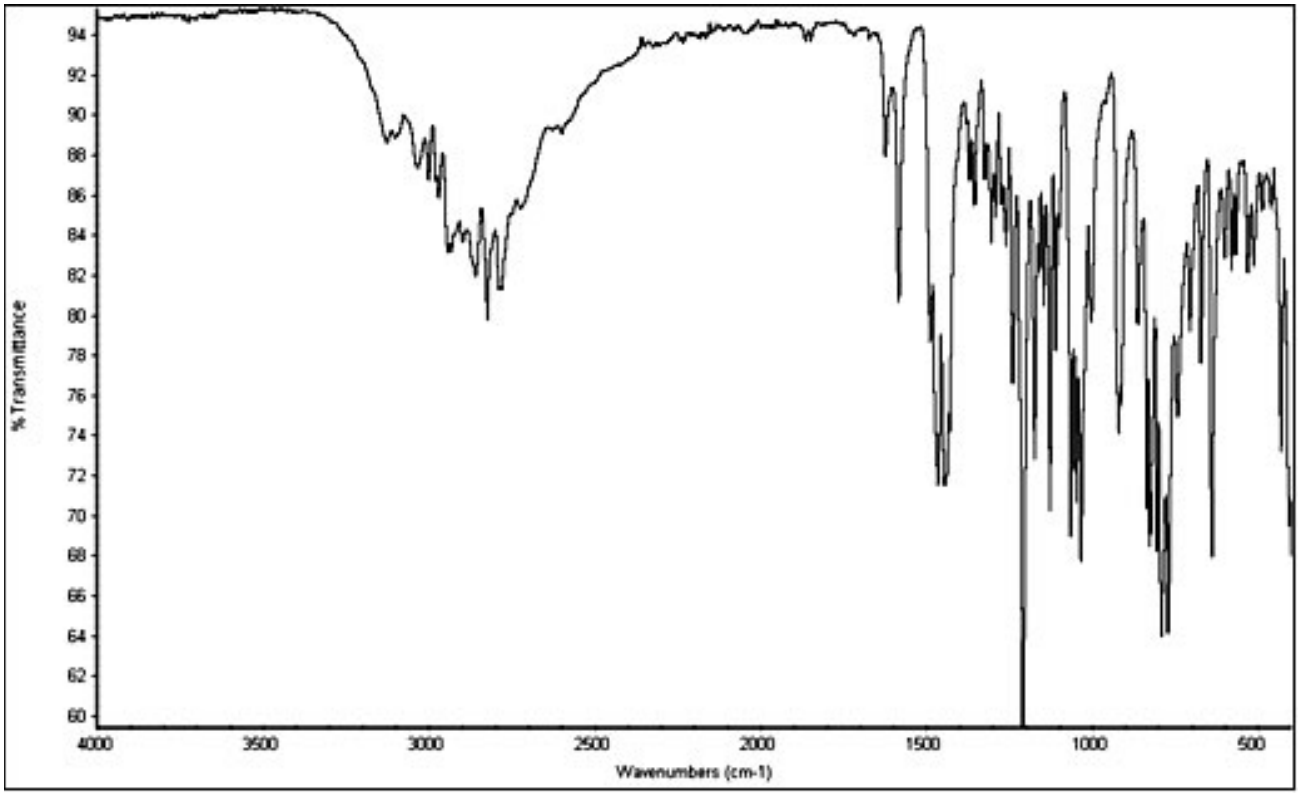

Figure 6: FTIR Spectrum of 5-Methoxy-N,N-dimethyltryptamine Base.

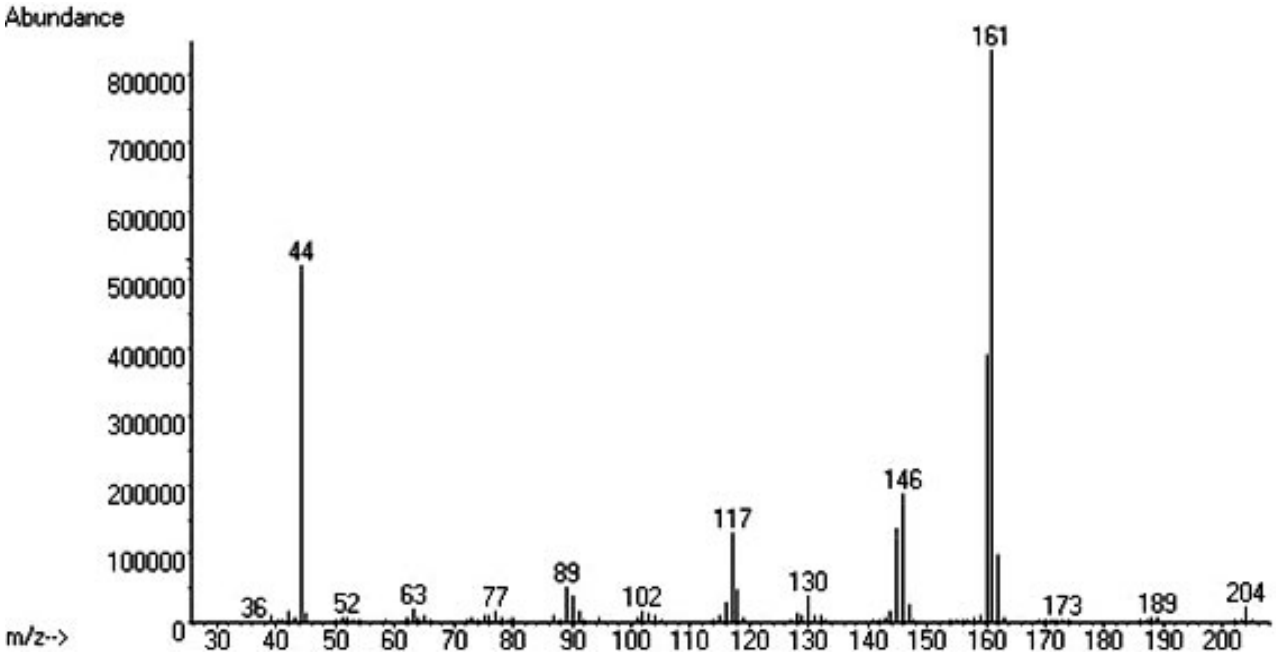

Figure 7: Mass Spectrum of 5-Methoxy-α-methyltryptamine.

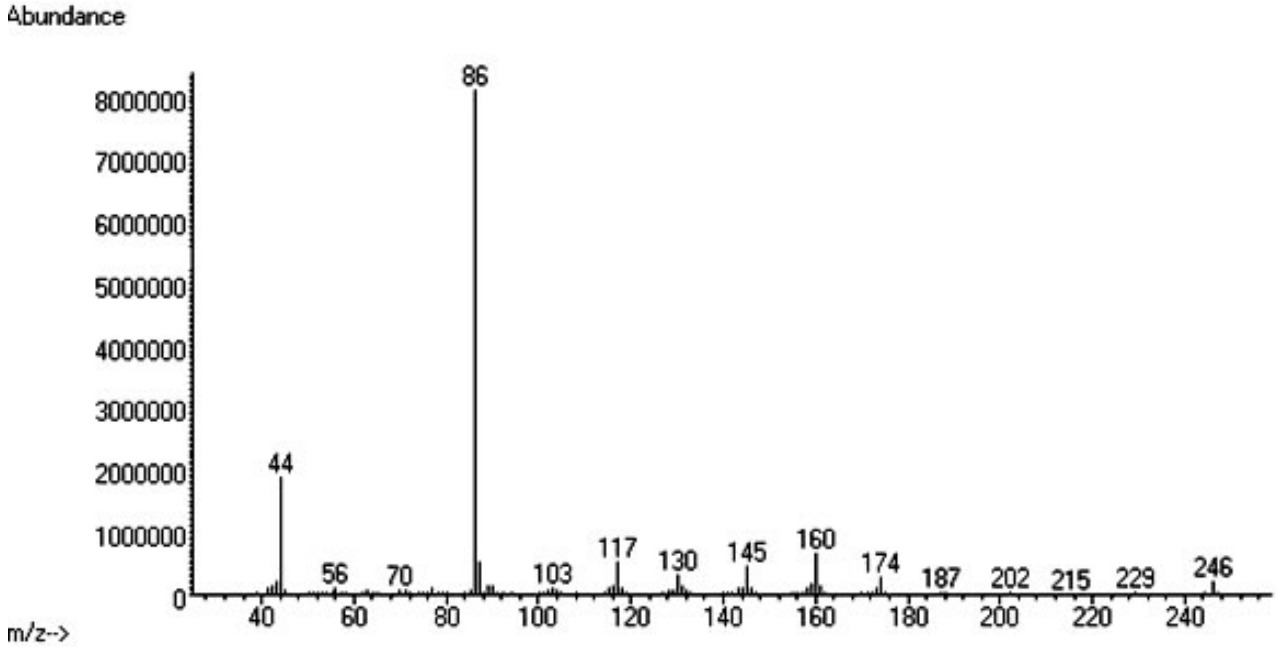

Figure 8: Mass Spectrum of 5-Methoxy-N-methyl-N-isopropyltryptamine.

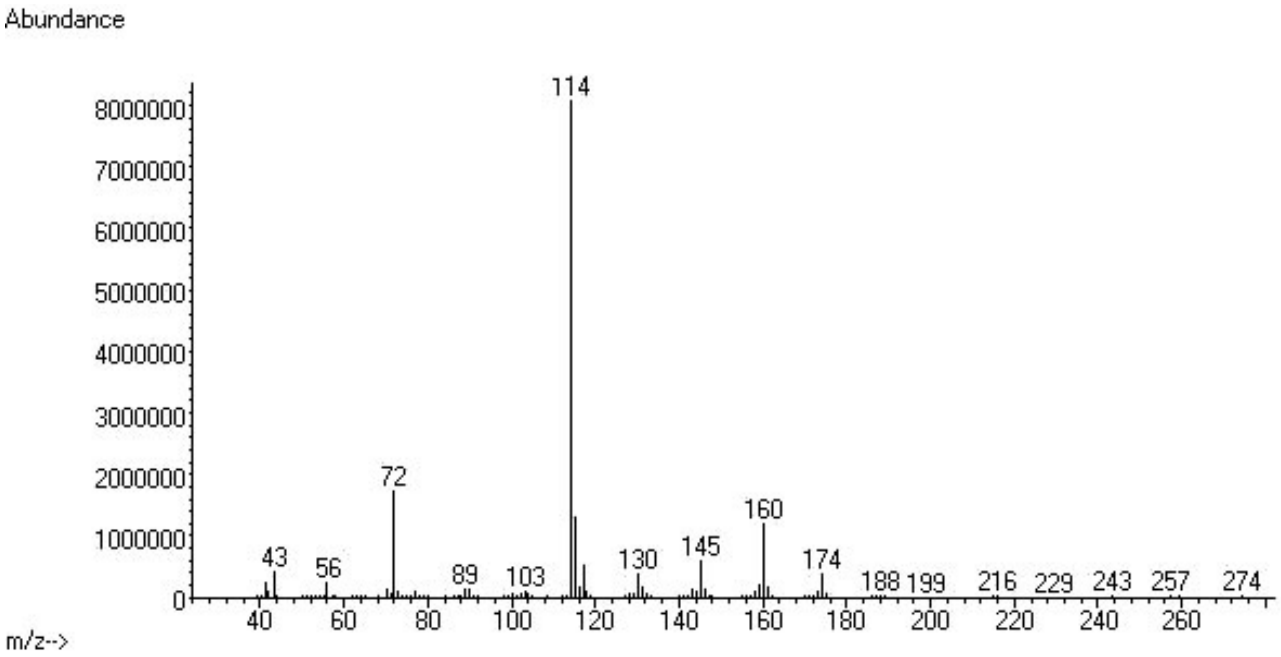

Figure 9: Mass Spectrum of 5-Methoxy-N,N-diisopropyltryptamine.

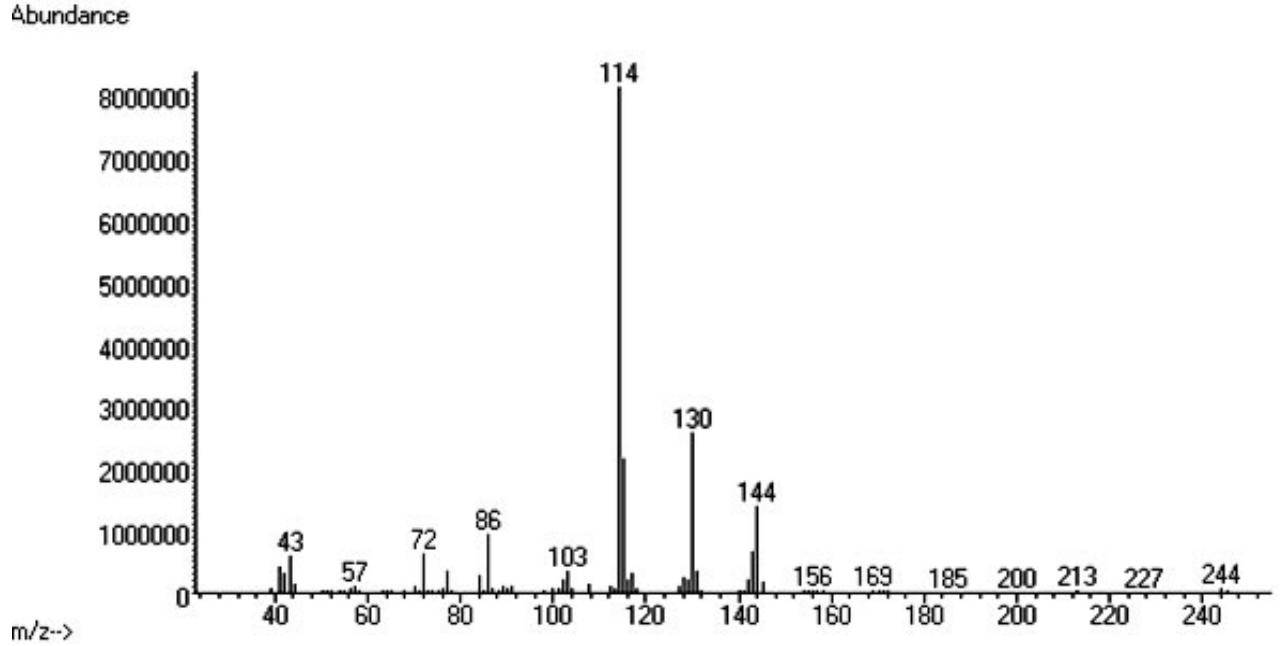

Figure 10: Mass Spectrum of N,N-Dipropyltryptamine.

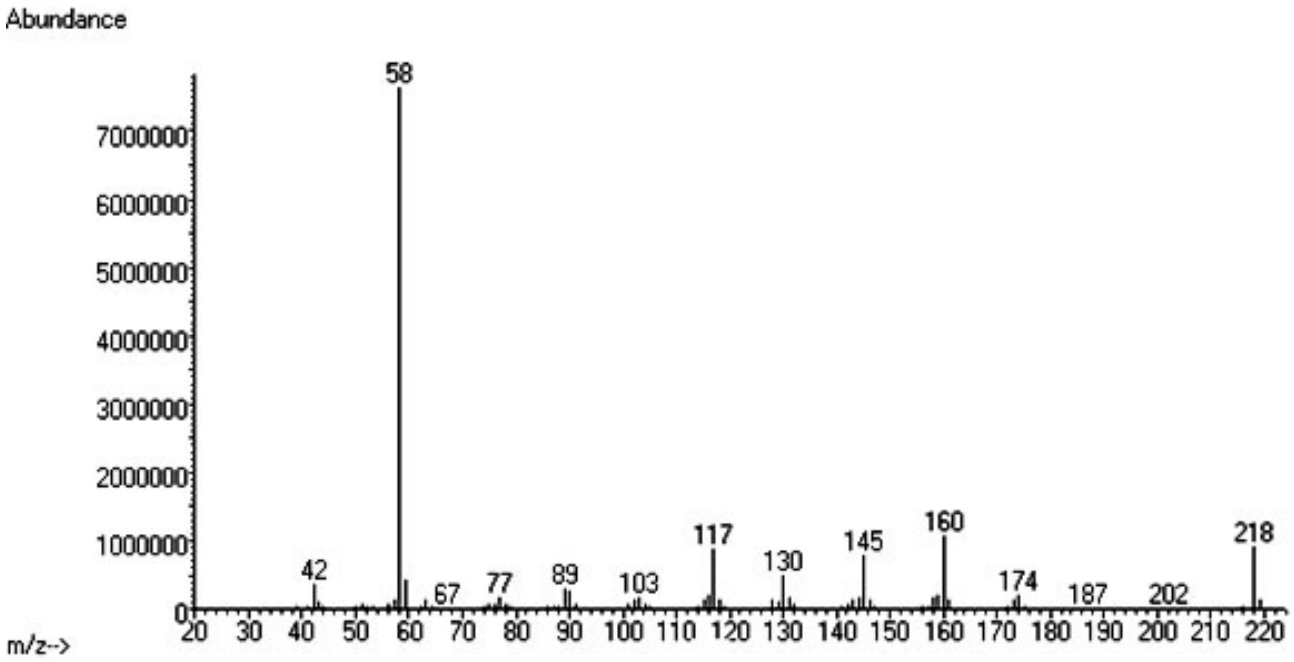

Figure 11: Mass Spectrum of 5-Methoxy-N,N-dimethyltryptamine.

[Figures 12 - 16 Follow]

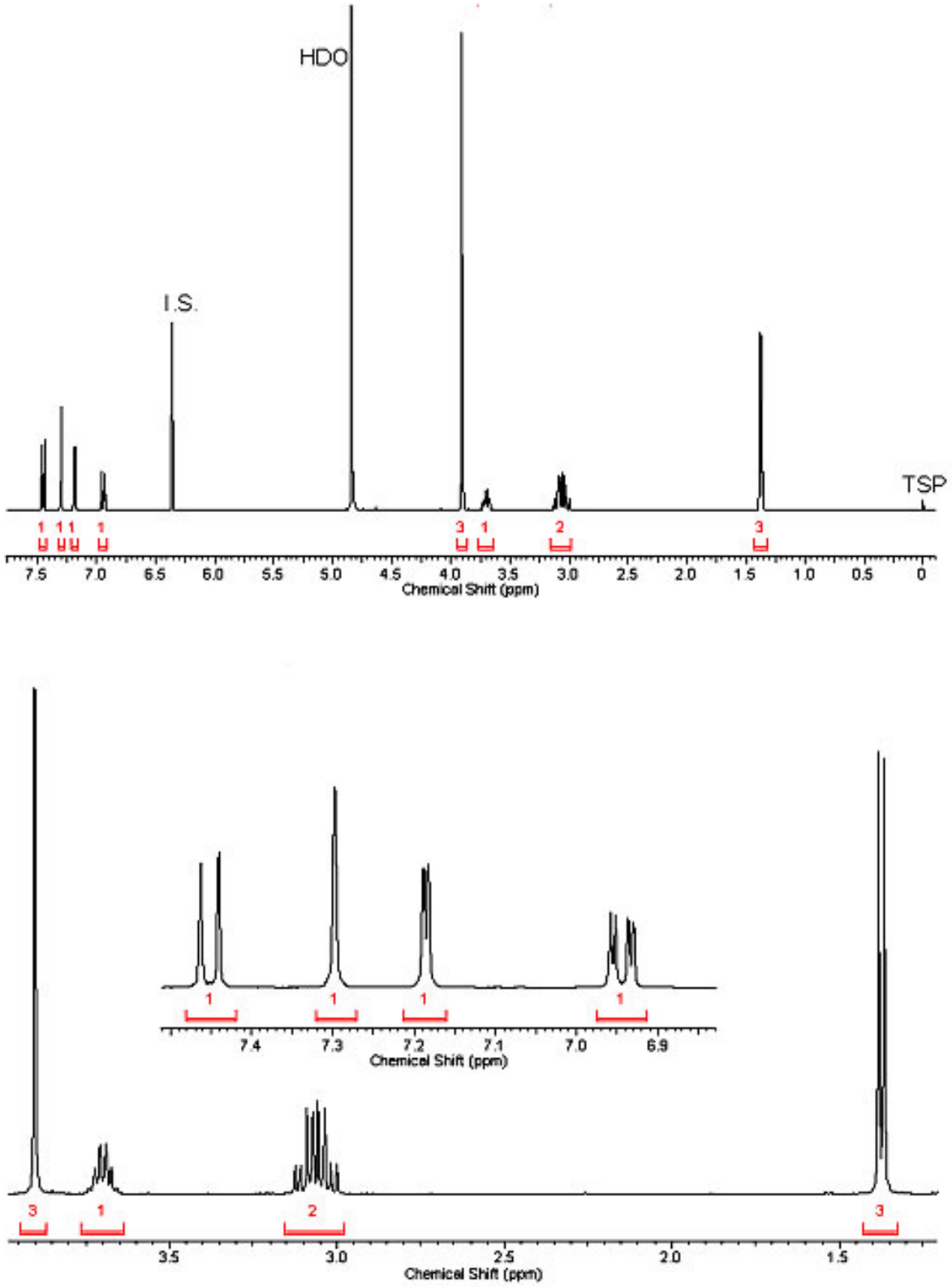

Figure 12: Proton NMR (400 MHz) of 5-Methoxy-α-methyltryptamine (with Insets).

^{1}H NMR (400 MHz, D_2O) δ ppm 7.45 (d, J=8.90 Hz, 1 H) 7.30 (s, 1 H) 7.18 (d, J=2.45 Hz, 1 H) 6.94 (dd, J=8.90, 2.45 Hz, 1 H) 3.91 (s, 3 H) 3.65 - 3.74 (m, J=7.50, 6.65 (x3) 6.50 Hz, 1 H) 3.10 (dd, J=14.80, 6.50 Hz, 1 H) 3.03 (dd, J=14.80, 7.50 Hz, 1 H) 1.38 (d, J=6.65 Hz, 3 H).

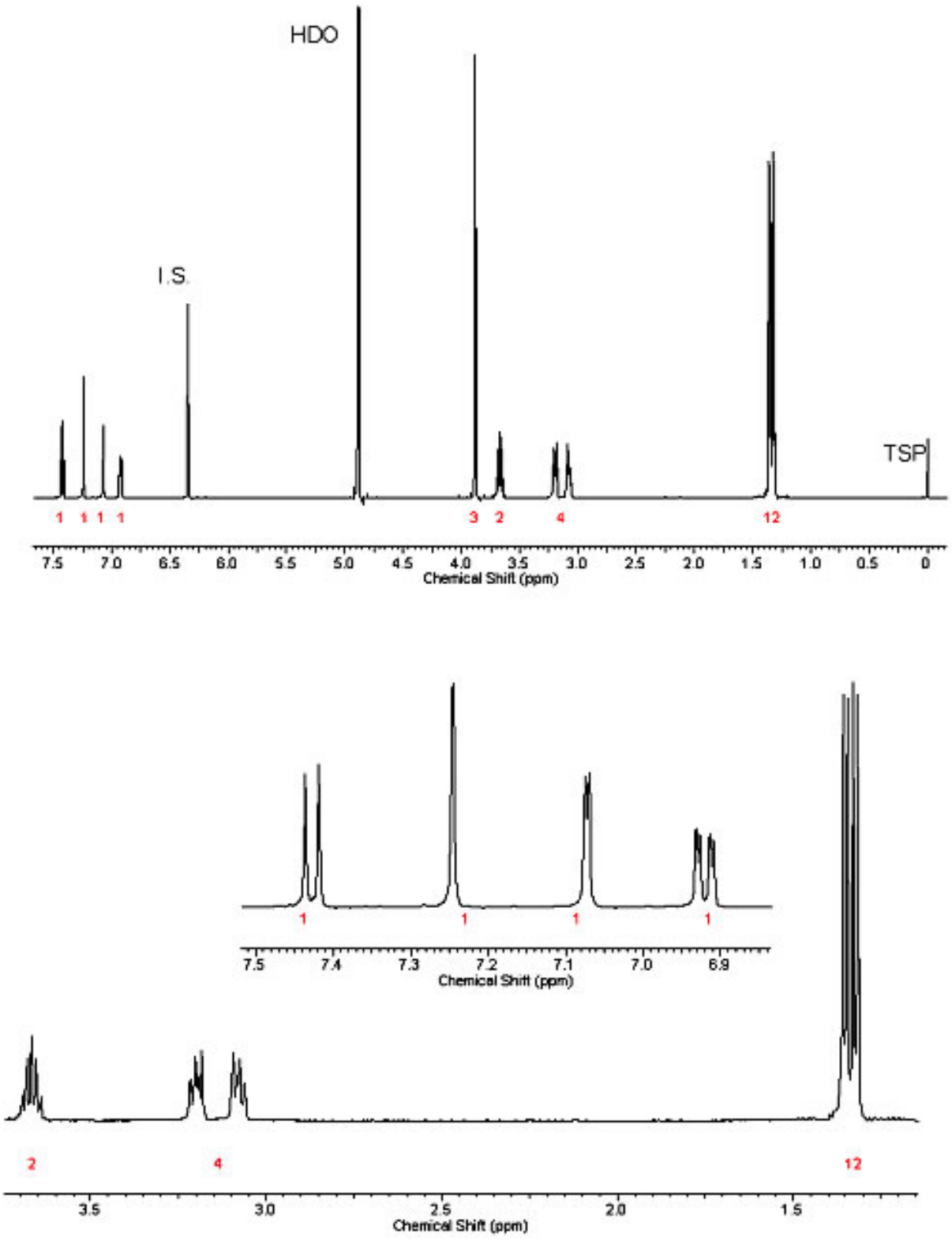

Figure 13: Proton NMR (500 MHz) of 5-Methoxy-N,N-diisopropyltryptamine (with Insets).

^{1}H NMR (500 MHz, D_2O) δ ppm 7.43 (d, J=8.80 Hz, 1 H) 7.25 (s, 1 H) 7.07 (d, J=2.42 Hz, 1 H) 6.92 (dd, J=8.88, 2.42 Hz, 1 H) 3.88 (s, 3 H) 3.53 - 3.77 (m, J=6.40 (x6) Hz, 1 H) 3.20 (dd, J=10.50, 6.00 Hz, 2 H) 3.08 (dd, J=10.50, 6.00 Hz, 2 H) 1.35 (d, J=6.64 Hz, 6 H) 1.32 (d, J=6.55 Hz, 6 H).

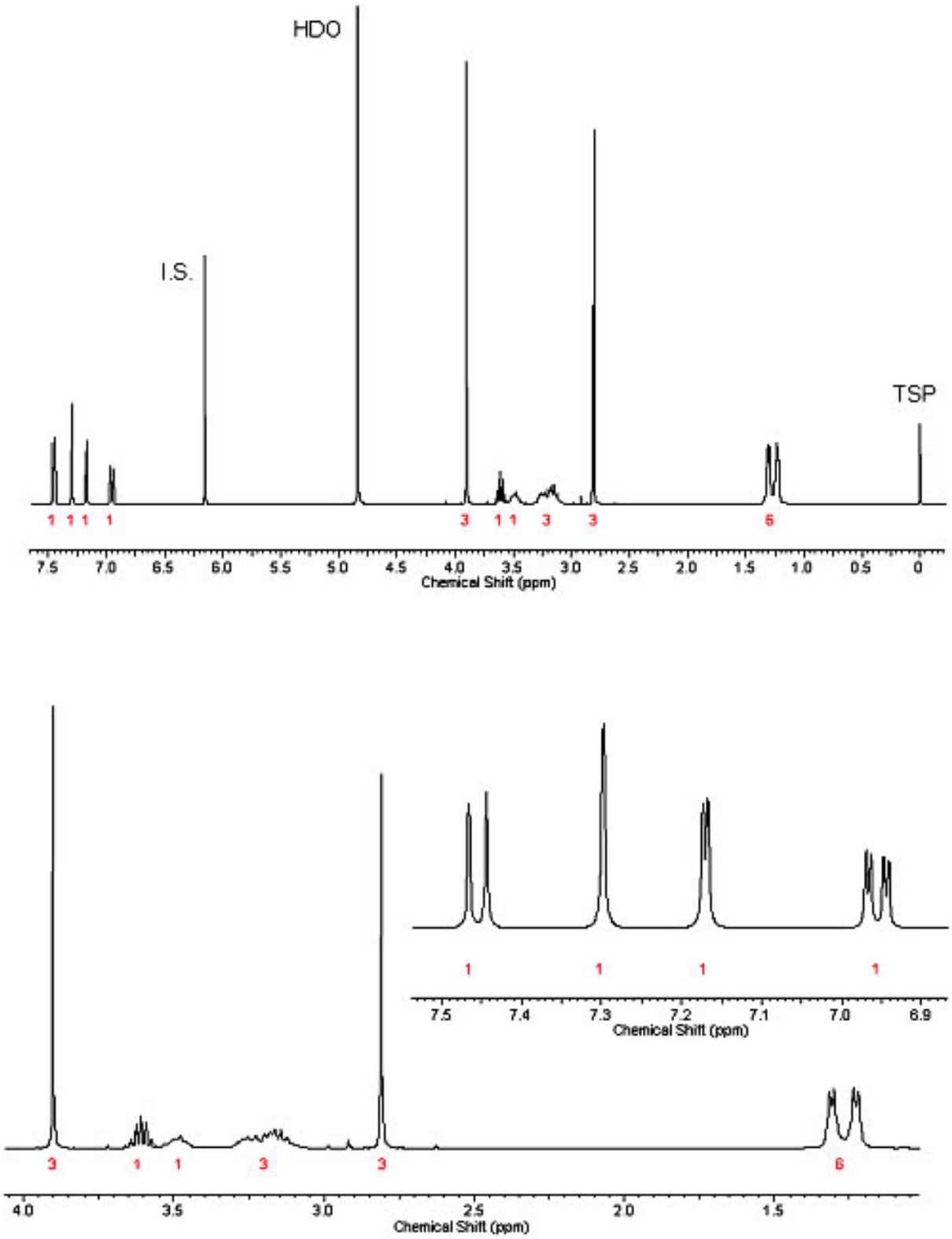

Figure 14: Proton NMR (400 MHz) of 5-Methoxy-N-methyl-N-isopropyltryptamine (with Insets).

^{1}H NMR (400 MHz, D_2O) δ ppm 7.45 (d, J=8.90 Hz, 1 H) 7.30 (s, 1 H) 7.17 (d, J=2.45 Hz, 1 H) 6.96 (dd, J=8.80, 2.45 Hz, 1 H) 3.90 (s, 3 H) 3.55 - 3.69 (m, J=6.70 (x6) Hz, 1 H) 3.37 - 3.55 (m, 1 H) 3.05 - 3.33 (m, 3 H) 2.81 (s, 3 H) 1.31 (d, J=6.46 Hz, 3 H) 1.23 (d, J=6.26 Hz, 3 H).

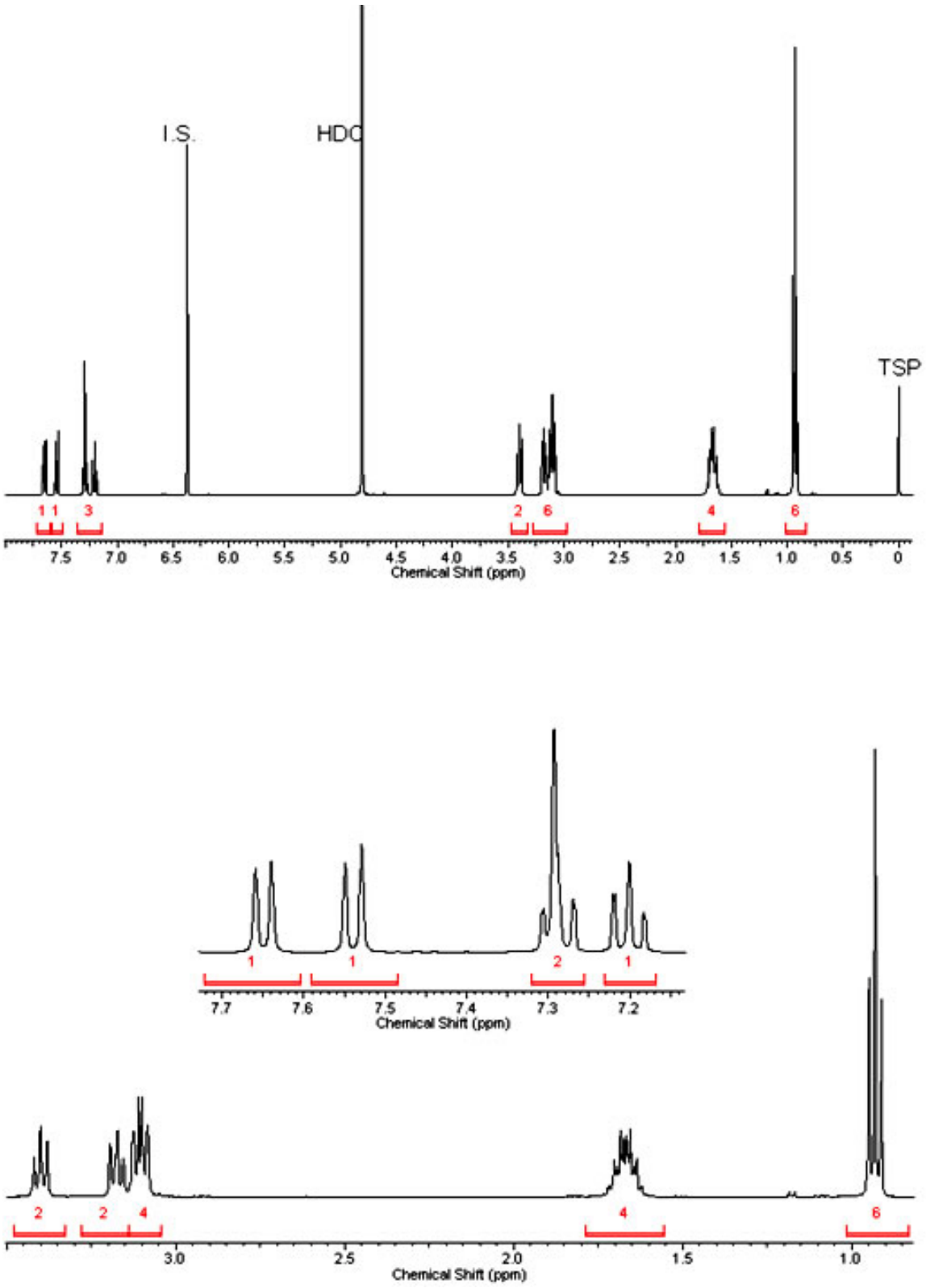

Figure 15: Proton NMR (400 MHz) of N,N-Dipropyltryptamine (with Insets).

^{1}H NMR (400 MHz, D_2O) δ ppm 7.65 (d, J=7.83 Hz, 1 H) 7.54 (d, J=8.12 Hz, 1 H) 7.29 (s, 1 H) 7.29 (ddd, J=8.14, 7.07, 1.03 Hz, 1 H) 7.20 (ddd, J=7.73, 7.29, 0.93 Hz, 1 H) 3.40 (dd, J=8.31, 7.14 Hz, 2 H) 3.17 (dd, J=8.22, 7.14 Hz, 2 H) 3.07 - 3.14 (m, 4 H) 1.60 - 1.74 (m, 4 H) 0.93 (t, J=7.38 Hz, 6 H).

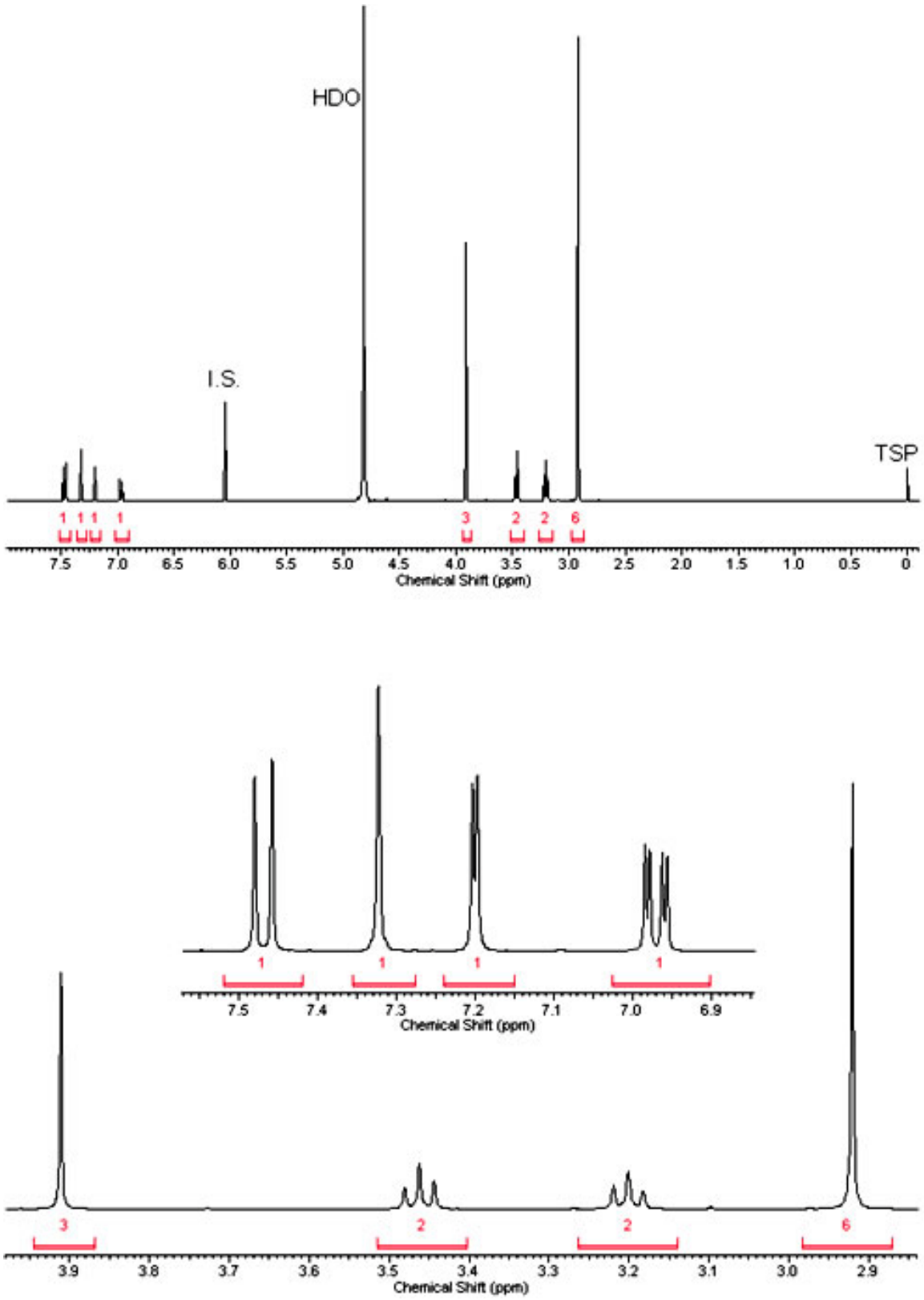

Figure 16: Proton NMR (400 MHz) of 5-Methoxy-N,N-dimethyltryptamine (with Insets).

^{1}H NMR (400 MHz, D_2O) δ ppm 7.47 (d, J=8.90 Hz, 1 H) 7.32 (s, 1 H) 7.20 (d, J=2.45 Hz, 1 H) 6.97 (dd, J=8.90, 2.45 Hz, 1 H) 3.91 (s, 3 H) 3.46 (t, J=7.43 Hz, 2 H) 3.20 (t, J=7.43 Hz, 2 H) 2.92 (s, 6 H).

Desloratadine: The Reaction Byproduct of the Reduction of Cold Tablets Containing Loratadine with Hydriodic Acid/Red Phosphorus

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ABSTRACT: Production of methamphetamine via hydriodic acid/red phosphorus reduction of over-the-counter pseudoephedrine products that contain other active co-ingredients will generate products that are contaminated with those co-ingredients and/or their reduction byproducts. In the case of pseudoephedrine products containing loratadine, the final product will contain desloratadine. Identification of desloratadine in methamphetamine therefore provides an indication of the commercial product used as the precursor in the synthesis.

KEYWORDS: Desloratadine, Loratadine, Pseudoephedrine, Methamphetamine, Hydriodic Acid, Red Phosphorus, Reduction, Trace Analysis, Impurity Profiling, Forensic Chemistry

Introduction

Clandestine methamphetamine laboratories are prevalent in the United States. One of the primary synthetic methods encountered is the reduction of ephedrine or pseudoephedrine with hydriodic acid/red phosphorus.[1] When first encountered and for many years thereafter, commercial hydriodic acid and red phosphorus were used in the reduction. In recent years, however, hydriodic acid and red phosphorus purchases have been restricted by law, forcing the clandestine laboratory operators to search for alternative sources. Red phosphorus is commonly obtained by the use of matchbooks and flares whereas hydriodic acid needs to be synthesized by the clandestine laboratory operator. The synthetic methods to generate hydriodic acid use iodine and either red phosphorus or other reactive phosphorous compounds such as hypophosphorous acid or phosphorous acid.[2]

Due to the increased restrictions on obtaining pure precursor ephedrine or pseudoephedrine, most clandestine laboratory operators are utilizing common cold tablet preparations.[3,4] These cold tablet preparations contain either ephedrine or pseudoephedrine, and often other ingredients such as cough suppressants, analgesics, expectorants, or antihistamines. Common co-ingredients include acetaminophen, brompheniramine, chlorpheniramine, dextromethorphan, diphenhydramine, doxylamine, guaifenesin, and triprolidine. When these compounds are present in the ephedrine/pseudoephedrine reduction mixtures, they will be carried through the reaction sequence unchanged, or will produce characteristic byproducts that are identifiable by GC/MS.[5,6]

The identification of these compounds or their byproducts in clandestinely produced methamphetamine can assist the analyst in determining which cold tablet preparation was used as the precursor source. The ratios of these byproducts relative to methamphetamine are usually very low in the final product. However, they can be easily extracted and identified.[7] As new cold products become available on the market, clandestine laboratory operators will use them to obtain pseudoephedrine and manufacture methamphetamine. Any new co-products contained in

these cold tablets have the potential to produce impurities not previously encountered by the forensic analyst. A recent example is tablets containing loratadine, which are already being used in methamphetamine production. Loratadine is the active ingredient in Claritin®, which recently changed from a prescription to an over-the-counter medication.

Experimental

Reactions

Loratadine, red phosphorus and 30 mL of 57 % hydriodic acid were refluxed (boiling point = 120 ^{O}C) in a round bottom flask fitted with a condenser. The reactions were monitored by removal of aliquots with subsequent analysis. The aliquots were sampled initially, once the mixture began to reflux, and then at every hour. The progress of each reaction was monitored as a decrease of the precursor and the formation of desloratadine. The progress was also monitored by detection of intermediates and byproducts.

Gas Chromatography

These analyses were performed using an Agilent Technologies 6890N Gas Chromatograph equipped with electronic pneumatic control and a flame ionization detector. A 10.0 m x 0.32 mm i.d. fused-silica capillary column coated with 0.52 µm DB-5 (Agilent Technologies) was employed. Hydrogen was the carrier gas, with an average linear velocity of 40 cm/sec (constant flow). The injection port and detector were maintained at 280 ^{O}C. The samples were extracted into ether, and one µL of each sample was injected in split mode (25:1). The oven temperature was programmed as follows: Initial temperature 130 ^{O}C, hold for 1.0 minute, then increase temperature 25 ^{O}C per minute to 280 ^{O}C, hold for 1.0 minute (total run time = 10.0 minutes).

Gas Chromatography/Mass Spectrometry

The electron impact (EI) mass spectra were obtained using an Agilent Technologies Mass Spectrometer. The spectrometer was equipped with a 5973 mass selective detector and 6890N gas chromatograph. A 30.0 m x 0.25 mm i.d. fused-silica capillary column coated with 0.25 µm HP5-MS (Agilent Technologies) was employed. Helium was the carrier gas with an average linear velocity of 40 cm/sec (constant flow). The injection port and ion sources were set at 240 ^{O}C and 180 ^{O}C, respectively. One µL from each of the samples was injected in split mode (30:1). The oven temperature was programmed as follows: Initial temperature 100 ^{O}C for 1.0 minute, then increase temperature 20 ^{O}C per minute to 280 ^{O}C, hold for 10.0 minutes (total run time = 20 minutes). The mass spectrometer was scanned over an *m/z* range of 40 - 500. The transfer lines were maintained at 280 ^{O}C.

Infrared Spectrophotometry

The infrared spectra were obtained in potassium bromide on a Nicolet Magna 560 Fourier Transform Infrared (FTIR) Spectrophotometer. The infrared spectra were also obtained by attenuated total reflectance (ATR) on an Avatar 370 FTIR Spectrophotometer.

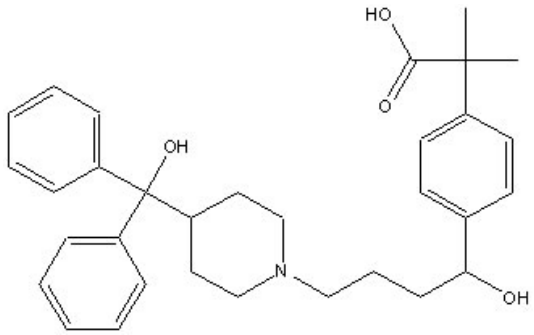
Results and Discussion

The intermediates and byproducts in the synthesis of methamphetamine utilizing ephedrine/pseudoephedrine via HI/red P are well documented.[1, 7-11] For example, if chlorpheniramine or 1-(4-methylphenyl)-1-(2-pyridyl)-3-pyrolidinopropane (commonly referred to as “reduced triprolidine”) are present in a methamphetamine sample, then the precursor source of the pseudoephedrine also certainly contained chlorpheniramine or triprolidine, respectively.[5] Whenever a new compound shows up in the finished product, this indicates that a new, previously unused cold tablet preparation has probably been used as the precursor source.

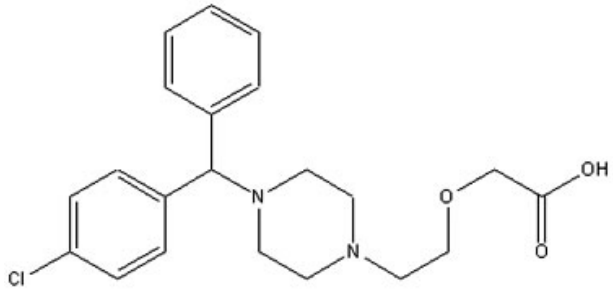
Recently, a methamphetamine sample was analyzed in this laboratory and was found to contain a new compound not seen in previous exhibits. The compound was in very low concentration in comparison to the methamphetamine, indicating it was probably not added as a cutting agent. The gas chromatogram is shown in Figure 1. The compound eluted on the gas chromatograph in the same general area of other “byproduct amines”

produced from the previously discussed pseudoephedrine pharmaceutical preparations.[5-7] The mass spectrum was easily obtained by employing an extraction technique to enhance these compounds.[7] That is, the methamphetamine sample (50 to 100 mg) was dissolved in 2-3 mL of water and made pH 8/9 basic with sodium bicarbonate. The basic solution was then extracted with 2 mL of hexane. The majority of the methamphetamine remained in the aqueous solution and did not extract into the hexane. Neutrals and "byproduct amines" are enhanced over the methamphetamine and also over the most common cutting agent dimethylsulfone. The extracted solution was further enhanced by evaporating it to dryness on a hot plate at 90º C, using a stream of air for about two minutes. Phenyl-2-propanone, methamphetamine, and residual dimethylsulfone are more volatile and evaporate, leaving only the suspected "byproduct amine." In the present case, after the compound was isolated, the mass spectrum was easily obtained. The mass spectrum of the compound gave a base peak of 280 amu and parent ion of 310 amu, as shown in Figure 2.

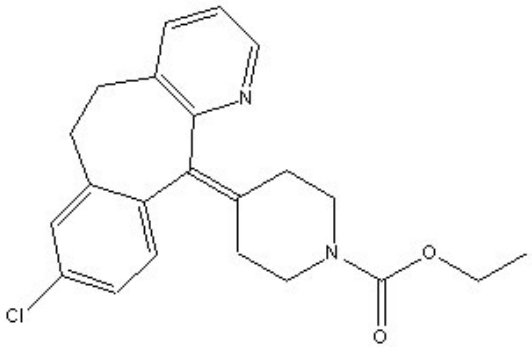
Several new products containing pseudoephedrine and previously unreported antihistamines including fexofenadine, cetirizine, and loratadine are now commercially available. The molecular weights are as follows: Fexofenadine (mw: 501.7), cetirizine (mw: 388.9) and loratadine (mw: 382.9). A "D" at the end of the proprietary name of the antihistamine product denotes that the product also contains the decongestant pseudoephedrine. The ratio of fexofenadine HCl to pseudoephedrine HCl is 60:120 mg/tablet. The ratio of cetirizine HCl to pseudoephedrine HCl is 5:120 mg/tablet. The ratio of loratadine to pseudoephedrine sulfate is 5:120 or 10:240 mg/tablet. The loratadine containing product has recently been converted from a prescription to



Fexofenadine



Cetirizine



Loratadine

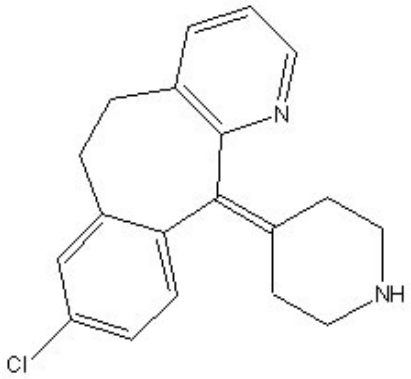
an over-the-counter product, making it much easier to obtain than the other two products. Based on the ratio of the "byproduct amine" to methamphetamine in the methamphetamine exhibits, and the mass spectrum of the new compound, the loratadine product with pseudoephedrine was suspected to be the source of the new byproduct. The structures of fexofenadine, cetirizine, and loratadine are shown above.

The chemical name of loratadine is ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cycloheptal[1,2-b]pyridine-11-ylidene-1-piperidine carboxylate. Loratadine is also known as 8-chloro-5,6-dihydro-11H-benzo[5,6]cycloheptal-[1,2-b]pyridine-11-ylidene-1-piperidine carboxylic acid ethyl ester. Based on the structure of loratadine, the reaction with HI was suspected to cleave the amide group and reduce the double bond. Both of these structural changes are consistent with the cleavage of the amide/ester groups and the reduction of the double bond, as previously observed with triprolidine.[5] The expected products would have molecular weights of 310.8 and 312.8 amu, respectively.

Loratadine was obtained by extracting commercial tablets containing only loratadine as the active ingredient. The ground tablets were extracted with chloroform, recrystallized with acetonitrile/ether, and air-dried on a hot plate. The mass spectrum and infrared spectrum of the solid matched a standard of loratadine (USP Cat. #137020). The mass spectrum and infrared spectra (KBr and ATR) are shown in Figures 3 - 5.

The isolated loratadine was refluxed with the same ratio of hydriodic acid and red phosphorus often used in methamphetamine manufacture, and the reaction monitored by gas chromatography. The retention times for the reaction product and loratadine were 7.12 and 9.24 minutes, respectively. The reaction product formed as soon the reaction mixture was heated. The gas chromatograph retention time and the mass spectrum of this reaction product are the same as the unknown compound encountered in the methamphetamine exhibits. Loratadine was also refluxed with only hydrochloric acid, and this mixture produced the same reaction product.

The unknown compound had to be an acid cleaved amide product with no reduction of the double bond, since hydrochloric acid is not capable of reducing the double bond. The identity of the unknown compound is 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cycloheptal[1,2-b]pyridine-11-ylidene-1-piperidine, or more simply desloratadine. The identity was confirmed by comparison with extracted desloratadine from pharmaceutical tablets containing desloratadine. The methamphetamine precursor therefore had to be tablets containing loratadine and pseudoephedrine, since commercial tablets containing desloratadine do not contain pseudoephedrine. The mass spectrum for desloratadine is shown in Figure 6. The structure of desloratadine is shown below.



Desloratadine

The main byproduct formed when manufacturing methamphetamine using loratadine-containing pseudoephedrine tablets is desloratadine. However, in some of the methamphetamine samples containing desloratadine another "byproduct amine" was also detected. The compound elutes just after desloratadine. The mass spectrometer

total ion chromatogram is shown in Figure 7. This compound has a base peak of 82 amu with an ion at 267 amu and a suspected parent peak of 310 amu. The mass spectrum is shown in Figure 8. This compound has not yet been identified, and will be addressed in future studies.

Conclusions

Use of cold tablet preparations as the source of pseudoephedrine has presented challenges in the identification of the trace amounts of the "byproduct amines" in methamphetamine samples. These challenges will continue as new pharmaceutical combinations with pseudoephedrine are made available. The identification of these byproducts in clandestinely produced methamphetamine can help analysts in determining which cold tablet preparations were used as the precursor source, and to link specific exhibits and cases, or both.

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[Figures 1 - 8 Follow.]

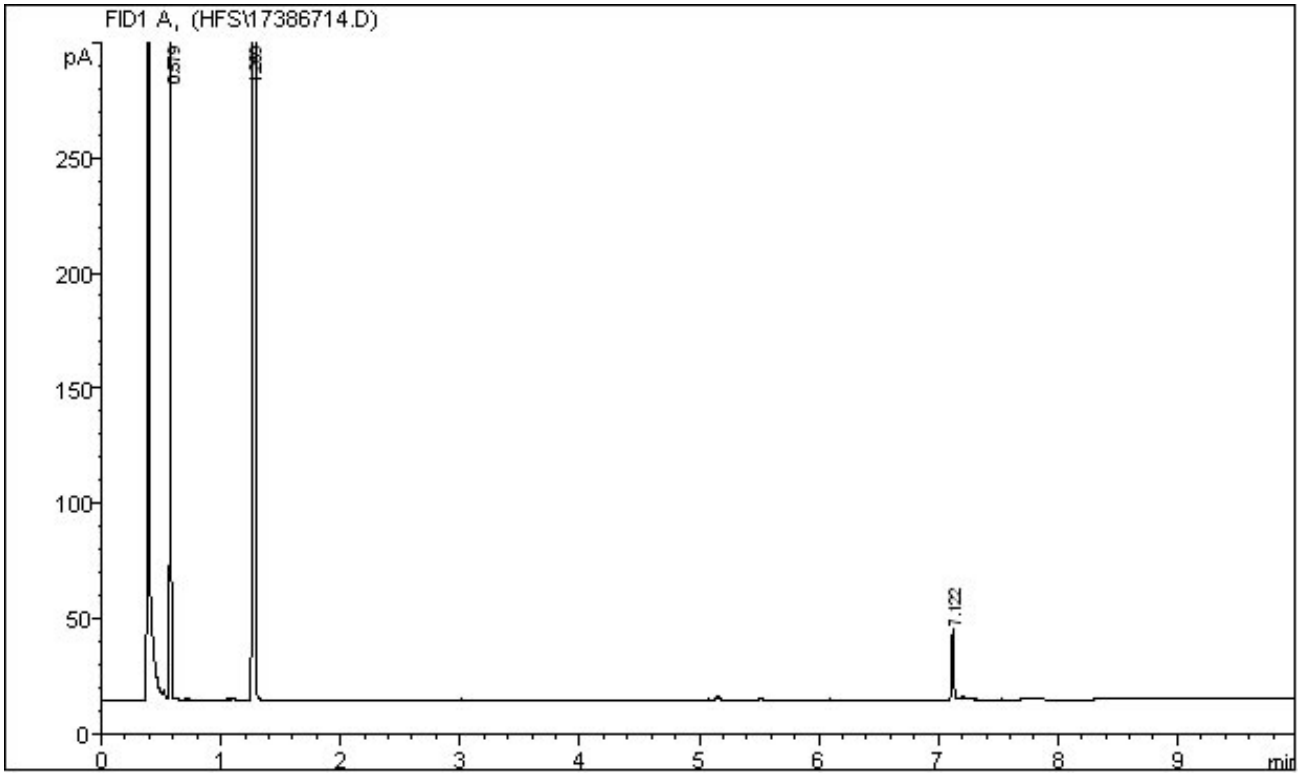

Figure 1. Gas Chromatogram of a Methamphetamine Sample Containing Dimethylsulfone and the Unknown "Byproduct Amine." Retention times: Dimethylsulfone: 0.579 Minutes; Methamphetamine: 1.289 Minutes; and Unknown "Byproduct Amine": 7.122 Minutes.

* * * * *

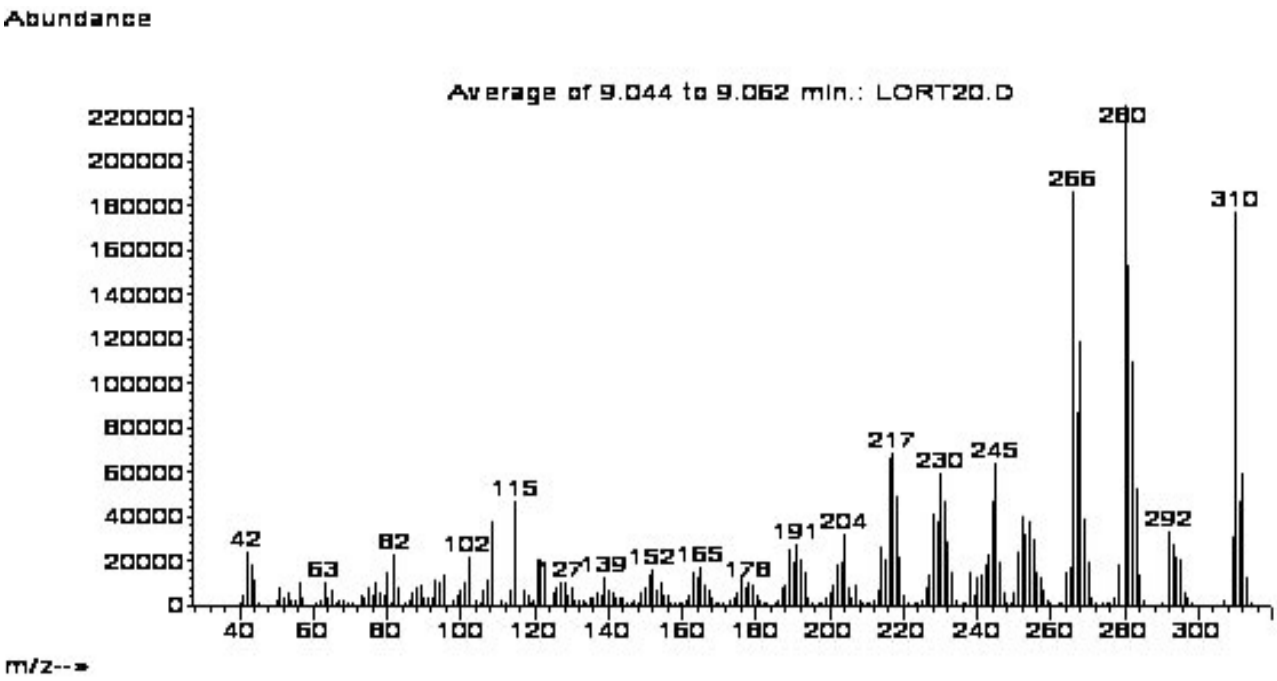

Figure 2. Mass Spectrum of the Previously Unseen "Byproduct Amine."

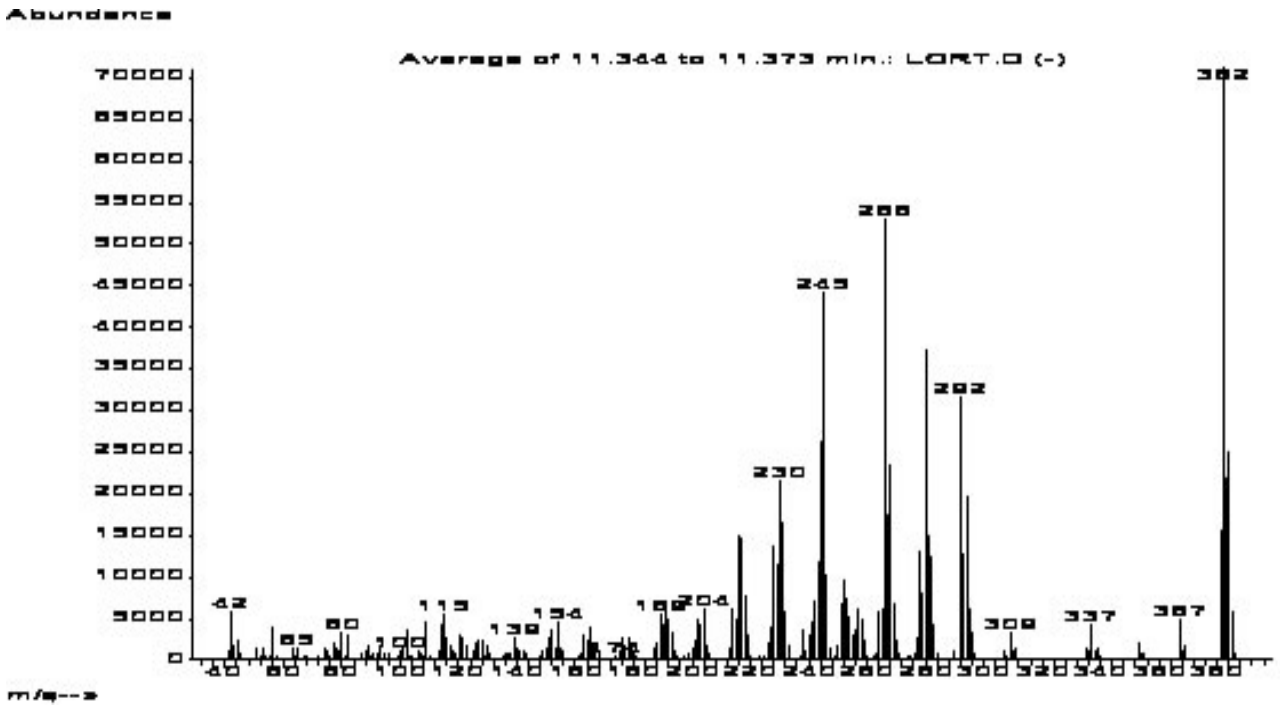

Figure 3. Mass Spectrum of Loratadine.

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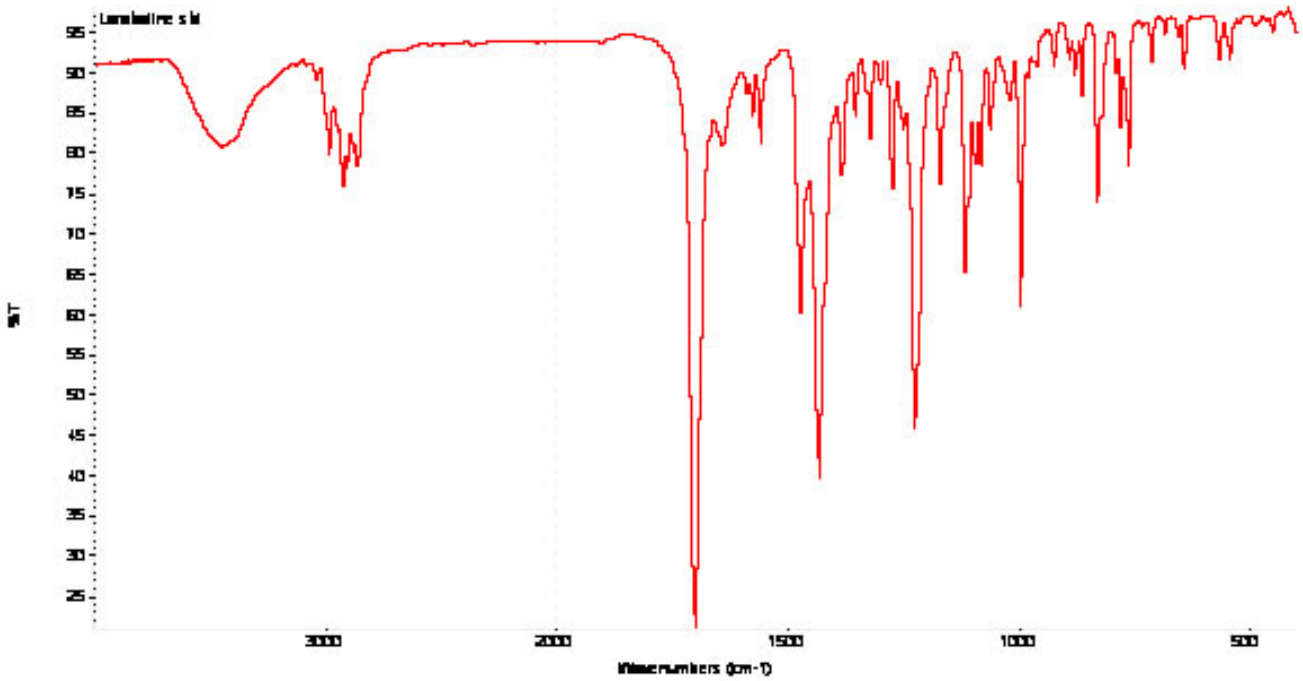

Figure 4. Infrared Spectrum of Loratadine in KBr.

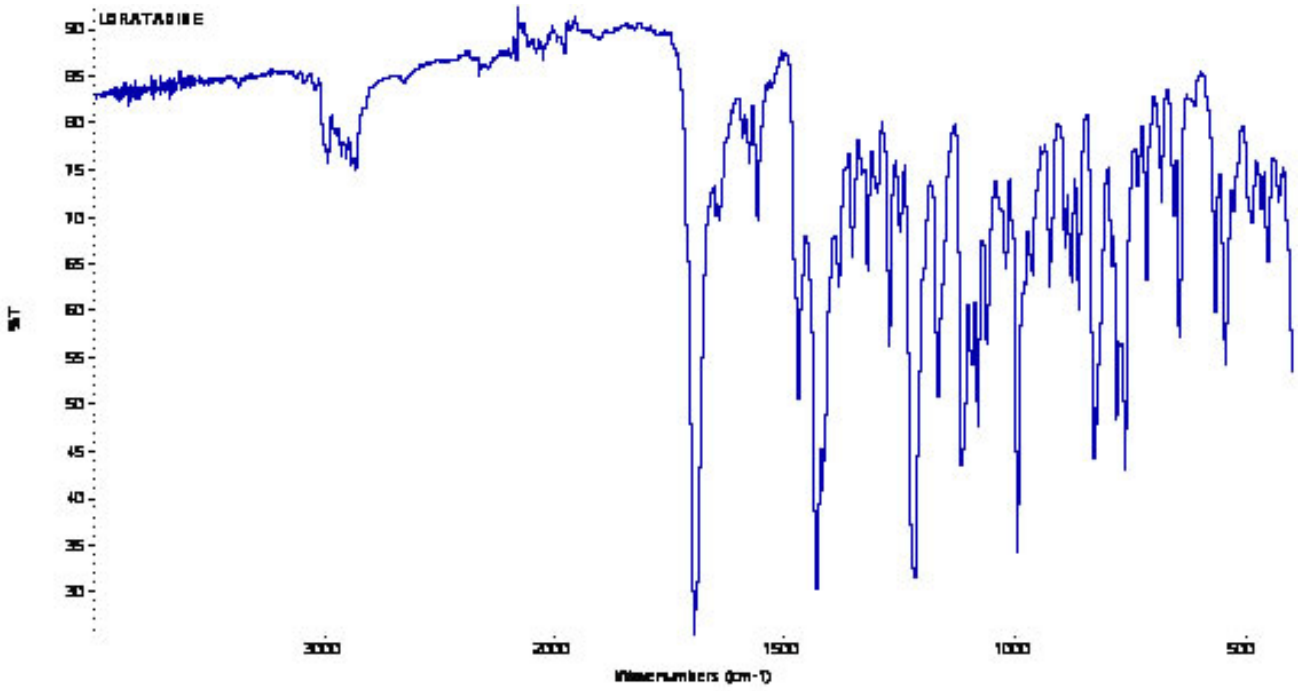

Figure 5. Infrared Spectrum of Loratadine by ATR.

* * * * *

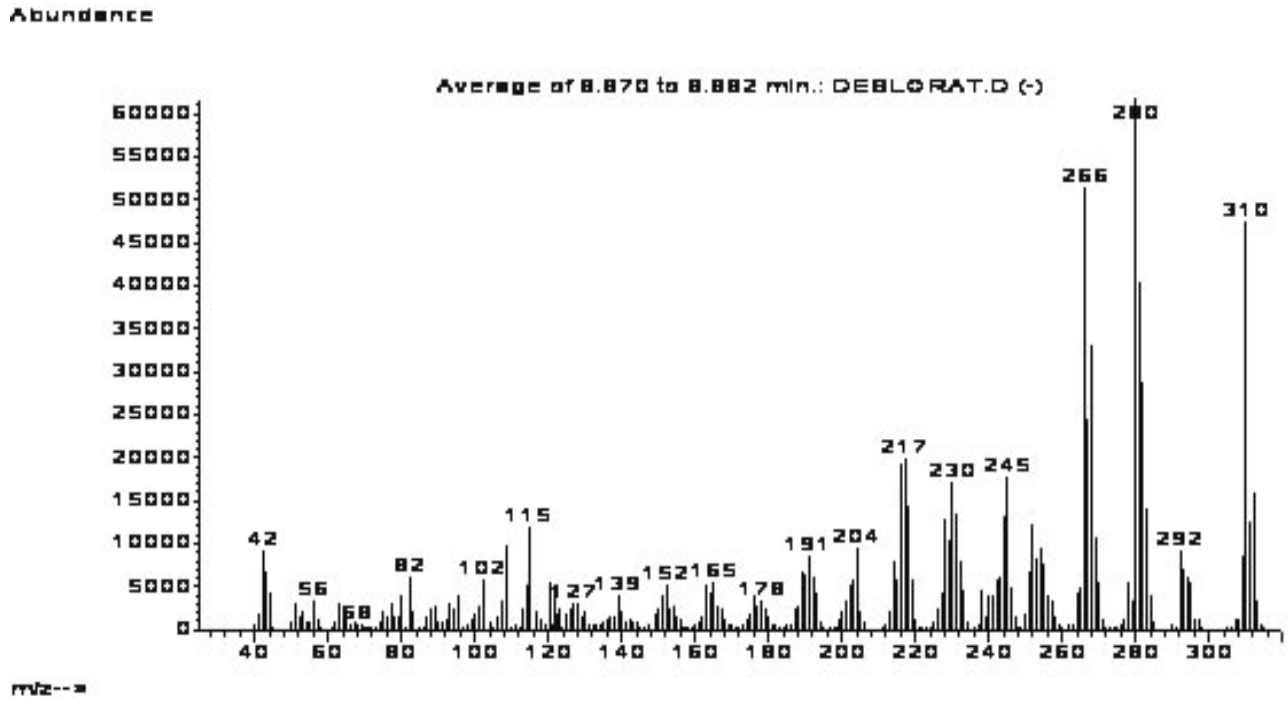

Figure 6. Mass Spectrum of Desloratadine.

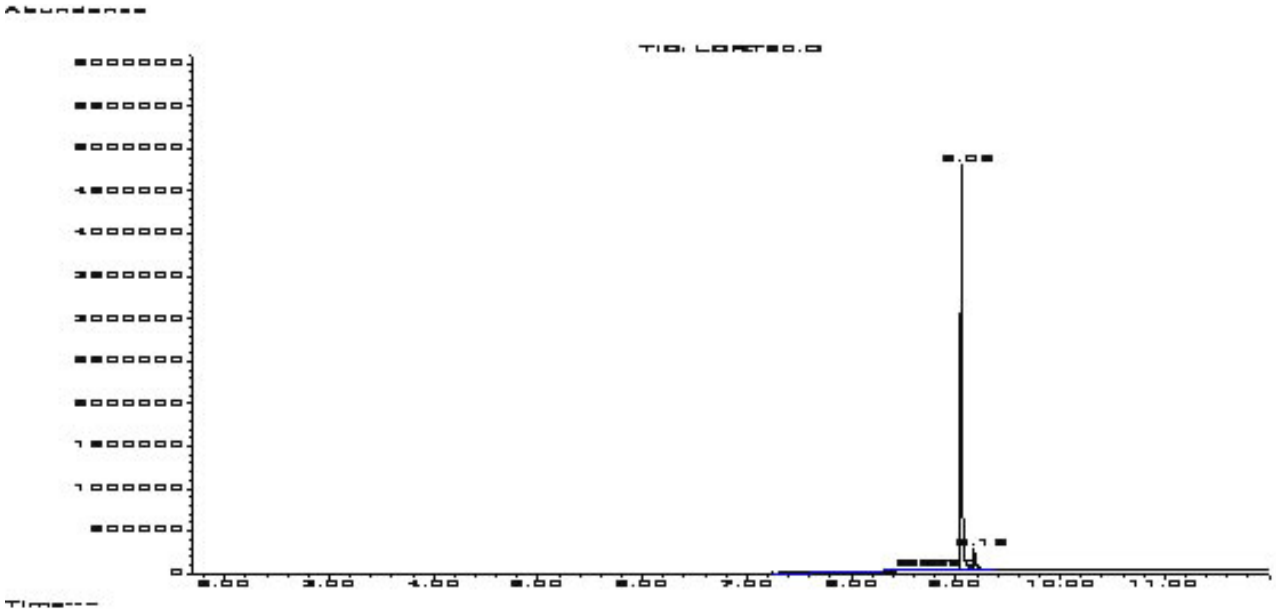

Figure 7. Total Ion Chromatogram of Desloratadine from Reaction of Loratadine with Hydriodic Acid/Red Phosphorus.

* * * * *

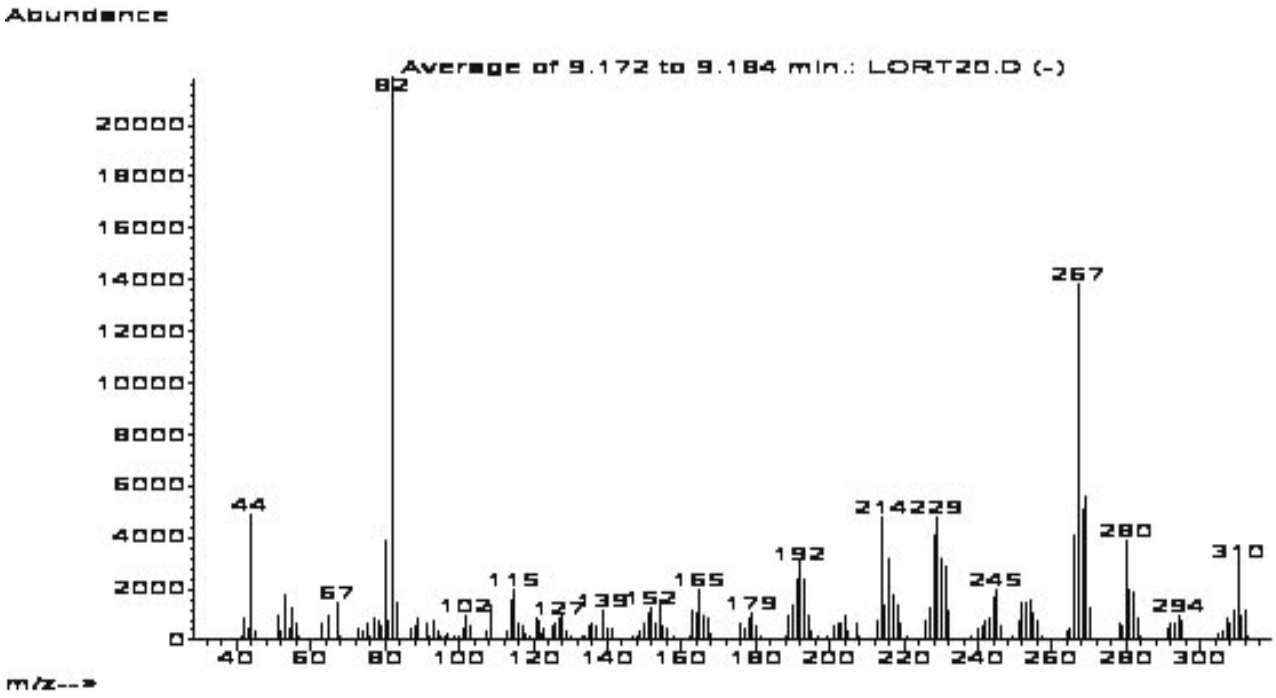

Figure 8. Mass Spectrum of Secondary Unknown Product.

Identification of Phenethylamines and Methylenedioxyamphetamines Using Liquid Chromatography Atmospheric Pressure Electrospray Ionization Mass Spectrometry

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ABSTRACT: A liquid chromatography - mass spectrometric (LC/MS) procedure utilizing atmospheric pressure electrospray ionization (API-ES) was developed for the identification of phenethylamines, methylenedioxy-amphetamine analogs, and other related compounds of forensic interest. An evaluation of three Phenomenex Synergi C-18 columns (Hydro-RP, Polar-RP, Fusion-RP) was performed using 22 compounds of interest to determine optimum selectivity. The method utilizes an isocratic buffered system of 10 mM ammonium formate pH 3.7 - acetonitrile along with diode array detection at 280 nm and 210 nm. Ionization is effected via electrospray in positive mode, resulting in a protonated pseudomolecular ion for the compounds of interest. Electrospray parameters were optimized via flow injection analysis and collision induced dissociation experiments were performed to optimize fragmentation of the compounds of interest. Sample preparation was minimal, and there was no need to derivatize.

KEYWORDS: Phenethylamines, Methylenedioxyamphetamine Analogs, LC/MS, Electrospray, Collision Induced Dissociation, Forensic Chemistry

Introduction

Gas chromatography/mass spectrometry (GC/MS) is considered the standard technique for the identification of sympathomimetic amines such as phenethylamines and structurally related substituted compounds [1-5]. However, many of these compounds exhibit mass spectra with a very predominant base peak and very low molecular and fragment ions, which can make the identification challenging. Software normalization techniques have been employed in discriminating mass spectra of amines by removing the dominant base peak and normalizing the spectrum to a lower residual ion [5]. Derivatization techniques utilizing perfluorinated anhydrides such as heptafluorobutyryl (HFB), pentafluoropropionyl (PFP), or trifluoroacetyl (TFA), or silylating derivatives such as BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) and MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide), have also been widely employed, and both improve specificity and produce more readily identifiable mass spectra [6-7].

A liquid chromatograph coupled with atmospheric pressure ionization (LC-API), in either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) mode, provides an alternative technique to GC/MS for the identification of illicit drugs. LC/MS has been used successfully to analyze a broad range of compounds, with applications in forensic and clinical toxicology [8-12], anti-doping testing [13], and therapeutic drug monitoring [14]. LC/MS is ideal for thermolabile, low molecular weight compounds, non-volatile compounds, and/or highly polar drugs, eliminating the need for derivatization.

Atmospheric pressure ionization (API) is a relatively soft ionization technique generating either protonated $[M+H]^+$ or deprotonated pseudomolecular ions $[M-H]^-$, or multiply charged ions; these are formed through ion

evaporation in ESI, and through gas-phase chemical ionization in APCI. API sources yield low fragmentation, which would normally preclude the use of single quadrupole instruments. However, in-source collision dissociation (CID) can take place in the ion source, allowing for fragmentation of analyte ions via collisions with neutral molecules from residual solvent and gas molecules. This results in bond cleavages and rearrangements that are representative of the molecular structure of the molecule.

This study presents an LC/MS method using electrospray ionization (ESI) for the separation and confirmation of phenethylamines (PEAs), methylenedioxyamphetamines (MDAs), and other compounds routinely encountered in illicit drug seizures. HPLC separations were optimized using three different C-18 stationary phases, and CID experiments were performed in order to obtain mass spectra with fragmentations characteristic for each compound examined.

Experimental

LC/MS Methodology

Analyses were performed using an Agilent Technologies 1100 series high pressure liquid chromatograph (HPLC), including a quarternary pump, vacuum degasser, autosampler, thermostatted column compartment, diode array detector, and coupled to an Agilent Technologies 1100 series Model SL single quadrupole mass spectrometer equipped with an electrospray ionization interface. Nitrogen drying gas was generated using a nitrogen generator (Agilent Technologies 5183-2003) coupled to a Jun Air air compressor.

Chromatographic separations were evaluated using three different Phenomenex Synergi columns (15 cm x 3.0 mm, 4 µ 80 A) - Hydro-RP, Polar-RP and Fusion-RP. A mobile phase of 10 mM ammonium formate pH 3.7: Acetonitrile (88:12) delivered at a flow rate of 0.5 mL/minute was used to elute the compounds of interest. The column temperature was thermostatically controlled at 40 ^{O}C. An injection volume of 2 µL was used.

Mass analyses were performed in scan mode from a mass range *m/z* 50 - 350 measuring positive pseudomolecular ions. The fragmentor was set at 150 V for all compounds except phenethylamine which was run at 70 V in order to observe the pseudomolecular ion. Spray chamber parameters were as follows: 12.0 L/minute drying gas, 350 ^{O}C drying gas temperature, 40 psig nebulizer, 4000 V capillary voltage.

All illicit samples examined were dissolved in the mobile phase and filtered thru a 0.45 µ nylon membrane filter prior to analysis. Flow injection analysis was performed on all compounds examined by varying the fragmentor voltage from 100 - 240 V in increments of 20 V. Nebulizer pressure, capillary voltage, and drying gas flow were operated as per manufacturer's specifications. Complete system control and data evaluation was carried out using the Agilent Chemstation for LC/MS.

Reagents

All drug standards were obtained from the reference collection of the DEA Northeast Laboratory. Standards were prepared at a concentration of 0.05 mg/mL diluted in 10 mM ammonium formate pH 3.7. Ammonium formate (99.995+ %), formic acid (95 - 97 %), and acetonitrile (LC/MS Chromasolv grade) were obtained from Sigma Aldrich, St. Louis, MO. Ultrapure water from a Millipore Gradient 10-Elix 3 system (Billerica, MA) was used to prepare all buffers in the study.

Results and Discussion

Optimization of the HPLC conditions was performed using an ammonium formate buffer at pH 3.7 and acetonitrile as the organic modifier. Acetonitrile was chosen as opposed to methanol because it gave more symmetrically shaped peaks and efficiently resolved the compounds of interest. PEAs and MDAs are ideal candidates for positive ion ESI because low pH buffers completely ionize basic compounds (i.e., resulting in protonated species). A total of 22 compounds including structurally similar sympathomimetic amines, MDAs,

and adulterants routinely encountered in illicit seizures were evaluated on three Phenomenex Synergi C-18 columns (Hydro-RP, Polar-RP and Fusion-RP).

Selectivity data for each of the three columns evaluated are listed in Tables 1 - 3. All three columns gave similar selectivities for the 12 most commonly encountered substrates (phenylpropanolamine, phenethylamine, ephedrine, pseudoephedrine, methylephedrine, amphetamine, dimethylamphetamine, methamphetamine, phentermine, 3,4-MDA, 3,4-MDMA, 3,4-MDEA, and MBDB) (see Tables 1 - 3 and Figures 1 - 3). The Polar-RP column (an ether linked phenyl phase with hydrophilic endcapping) exhibited slightly more retentiveness for the N-substituted MDA analogs MDEA, MBDB, and N,N-dimethyl-MDA, along with dimethylamphetamine and ketamine. The adulterant caffeine, commonly encountered in MDMA seizures submitted to our laboratory, was retained on the Polar-RP column, but co-eluted with MDMA on the Hydro-RP and Fusion-RP columns (Tables 1 - 3). Case submissions containing both illicit tablets and powders were analyzed using the established LC/MS procedure (see Figures 13 - 14). Results were verified using a GC/MS method. Both the Hydro-RP and Polar-RP columns have been used interchangeably for routine case submissions, with good success.

Figures 4 - 10 show the mass spectra of 17 PEAs and MDAs examined under the ESI conditions specified. All compounds examined (except phenethylamine) exhibited a protonated pseudomolecular ion using a fragmentor of 150 V. Decreasing the voltage to 70 V revealed the protonated pseudomolecular ion for phenethylamine.

Methamphetamine and phentermine exhibit similar GC/MS fragmentation patterns, but are readily differentiated under ESI conditions. Both compounds are easily resolved on the Hydro-RP and Fusion-RP columns (see Figure 3). These isomers are more closely resolved on the Polar-RP column with a resolution of 1.55 (HPLC) and 1.22 (MS) using the half-width method calculation. Methamphetamine and phentermine both exhibit a pseudomolecular ion of *m/z* 150, but are easily differentiated by their characteristic fragment ions, with methamphetamine exhibiting a *m/z* 119 product ion and phentermine exhibiting a *m/z* 133 product ion (see Figure 4). The ability to resolve these isomeric pairs chromatographically and differentiate them by their mass spectral fragmentation patterns allows for facile identification of these two compounds. Phentermine and 4-methoxymethamphetamine are unresolved on the three columns tested - but this combination has never been encountered at our laboratory (see Figure 3).

The MDAs were resolved on all three columns (see Figure 1). N-hydroxy-3,4-MDA was strongly retained on the Hydro-RP, and eluted in 44 minutes. However, the Polar-RP and Fusion-RP columns offered a more efficient elution, with retention times less than eleven minutes. N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) is readily resolved from its regioisomeric MDA derivatives (i.e., 3,4-MDEA, and N,N-dimethyl-3,4-MDA) on all three columns. All three compounds exhibit a protonated pseudomolecular ion at *m/z* 208. 3,4-MDEA and N,N-dimethyl-3,4-MDA exhibit indistinguishable ESI mass spectra (Figures 7). 3,4-MDEA and MBDB are readily differentiated by their product ions, with MBDB exhibiting product ions at *m/z* 177 and 135 while MDEA exhibits product ions at *m/z* 163 and 133 (see Figure 7).

3,4-MDA exhibits a pseudomolecular ion at *m/z* 180, with product ions at *m/z* 163, 133, and 105, while 3,4-MDMA exhibits a pseudomolecular ion and base peak at *m/z* 194, with similar product ions (see Figure 5). All the substituted MDA analogs examined (except 3,4-MDEA and N,N-dimethyl-3,4-MDA) are readily distinguishable by their pseudomolecular ions. In addition, all of the MDAs are resolved on all three columns, thereby allowing for differentiation even of 3,4-MDEA and N,N-dimethyl-3,4-MDA via retention time matching.

In the present study, a fragmentor voltage of 150 V was chosen in order to observe a protonated pseudomolecular ion and sufficient fragmentation product ions that would allow for conclusive identification of each compound examined. Table 4 provides a summary of the relative abundances of the five major ions for each compound examined. The protonated molecular ion was the base peak for 12 of the 22 compounds examined.

Flow injection analysis allows for direct sample injection into the mass spectrometer and was used in order to optimize API-MS parameters. This allowed for rapid method development. CID experiments via flow injection analysis were performed on all compounds by varying the fragmentor voltages from 100 - 240 V.

Varying the fragmentor voltages had the greatest impact on the rate of fragmentation as observed during the CID of 3,4-MDMA (Figure 12). The protonated pseudomolecular ion *m/z* 194 is most abundant at 100 V and gradually decreases as the fragmentor voltage is ramped to 240 V. At 100 V, the pseudomolecular ion is the base peak, and there are minimal fragment product ions. As the fragmentor voltage is increased, characteristic product ions are observed, and increase in intensity (Figure 11).

Fragmentor voltages were optimized for each respective compound (see Table 5). The sensitivity of higher mass ions was higher at lower fragmentor voltages, while the sensitivity of lower mass ions increased at higher fragmentor voltages. Area response sensitivity gradually decreased for compounds examined as a function of increasing fragmentor voltage (see Figure 15).

In-source CID has been shown to produce similar fragmentations as conventional CID in the collision cell of a tandem mass spectrometer (MS-MS) - but not necessarily of the same intensities. A requirement for in-source CID is a complete separation of the compounds being studied, as opposed to conventional CID using a tandem MS, where a precursor ion is specifically selected, followed by fragmentation [15,17,18]. A disadvantage of in-source CID is that since all ions are fragmented there is no mechanism to elucidate which product ions originated from which precursor ion [18]. In addition, no commercial in-source CID mass spectral libraries exist at present, requiring the user to create an in-house CID mass spectral library for the compounds of interest [16].

In conclusion, in-source fragmentation using a single quadrupole mass spectrometer allows for the positive identification of PEAs and MDAs. The instrumentation is user friendly, provides the ability to perform rapid method development using in-source CID, and offers extracted ion monitoring to deconvolute complex chromatograms. The LC/MS method has been implemented at our laboratory, and has been instrumental in confirming the presence of PEAs, MDAs, and common adulterants found in complex illicit mixtures. This provides a complementary and/or alternative means of identification of PEAs and MDAs.

Acknowledgments

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[Table 1 - 5 and Figures 1 - 15 Follow.]

Table 1. Selectivity of Compounds Examined on Phenomenex Synergi Hydro-RP.

Compound	*MS Rt (minutes)*	*RRt (Methamphetamine)*
Niacinamide	**1.93**	**0.32**
Phenylpropanolamine	**2.87**	**0.47**
Acetaminophen	**2.90**	**0.47**
Phenethylamine	**2.99**	**0.49**
Ephedrine	**3.72**	**0.61**
Pseudoephedrine	**3.73**	**0.61**
Benzylpiperazine	**4.08**	**0.67**
Methylephedrine	**4.22**	**0.69**
Amphetamine	**4.97**	**0.81**
Caffeine	**5.06**	**0.83**
3,4-MDA	**5.57**	**0.91**
Methamphetamine	***6.12***	***1.00***
3,4-MDMA	**6.66**	**1.09**
Dimethylamphetamine	**7.06**	**1.15**
Phentermine	**7.36**	**1.20**
N,N-Dimethyl-3,4-MDA	**7.62**	**1.25**
4-Methoxymethamphetamine	**7.64**	**1.25**
Ethylamphetamine	**8.50**	**1.39**
3,4-MDEA	**9.30**	**1.52**
Ketamine	**11.01**	**1.80**
MBDB	**12.30**	**2.01**
N-Hydroxy-3,4-MDA	**44.24**	**7.23**

* * * * *

Table 2. Selectivity of Compounds Examined on Phenomenex Synergi Polar-RP.

Compound	*MS Rt (minutes)*	*RRt (Methamphetamine)*
Niacinamide	**2.39**	**0.37**
Phenylpropanolamine	**3.15**	**0.49**
Phenethylamine	**3.22**	**0.50**
Acetaminophen	**3.65**	**0.57**
Ephedrine	**4.07**	**0.63**
Pseudoephedrine	**4.19**	**0.65**
Methylephedrine	**4.83**	**0.75**
Benzylpiperazine	**4.86**	**0.75**
Amphetamine	**5.05**	**0.78**
3,4-MDA	**6.30**	**0.98**
Methamphetamine	***6.44***	***1.00***
Phentermine	**6.84**	**1.06**
Caffeine	**7.88**	**1.22**
3,4-MDMA	**7.99**	**1.24**
Dimethylamphetamine	**8.20**	**1.27**
4-Methoxymethamphetamine	**8.34**	**1.30**
Ethylamphetamine	**8.79**	**1.36**
N,N-Dimethyl-3,4-MDA	**10.33**	**1.60**
N-Hydroxy-3,4-MDA	**10.59**	**1.64**
3,4-MDEA	**11.12**	**1.73**
Ketamine	**12.31**	**1.91**
MBDB	**13.10**	**2.03**

* * * * *

Table 3. Selectivity of Compounds Examined on Phenomenex Synergi Fusion-RP.

Compound	*MS Rt (minutes)*	*RRt (Methamphetamine)*
Niacinamide	**2.35**	**0.39**
Phenylpropanolamine	**3.52**	**0.52**
Phenethylamine	**3.58**	**0.53**
Ephedrine	**3.80**	**0.63**
Pseudoephedrine	**3.84**	**0.64**
Acetaminophen	**3.94**	**0.66**
Benzylpiperazine	**4.28**	**0.71**
Methylephedrine	**4.94**	**0.73**
Amphetamine	**4.98**	**0.83**
3,4-MDA	**5.76**	**0.96**
Methamphetamine	***6.00***	***1.00***
Caffeine	**6.04**	**1.01**
3,4-MDMA	**6.77**	**1.13**
Dimethylamphetamine	**6.78**	**1.13**
Phentermine	**7.19**	**1.20**
4-Methoxymethamphetamine	**7.44**	**1.24**
N,N-Dimethyl-3,4-MDA	**7.64**	**1.27**
Ethylamphetamine	**8.13**	**1.36**
3,4-MDEA	**9.32**	**1.55**
Ketamine	**10.70**	**1.78**
N-Hydroxy-3,4-MDA	**10.93**	**1.82**
MBDB	**11.96**	**1.99**

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Table 4. Mass Ion Abundances and % Relative Intensity of Mass Ion Abundances at 150 V for compounds examined.

Compound	*Ion#1(m/z)*	*Ion#2(m/z)*	*Ion#3(m/z)*	*Ion#4(m/z)*	*Ion#5(m/z)*
Niacinamide	**123(100%)**	124(7.4%)	80 (3.3%)	50(2.4%)	78(1.3%)
Acetaminophen	**152(100%)**	110(18.2%)	102(14.7%)	153(9.3)	174(3.3%)
Phenethylamine	105(100%)	79(5.7%)	103(4.6%)		
Methylephedrine	**180(100%)**	162(21.0%)	181(12.6%)	135(5.9%)	163(2.5%)
Phenylpropanolamine	134(100%)	135(13.6%)	117(7.1%)	**152(6.7%)**	
Ephedrine	148(100%)	**166(33%)**	149(10.9%)	135(4.3%)	167(4.0%)
Pseudoephedrine	148(100%)	**166(13.9%)**	149(11.9%)	133(3.5%)	167(1.8%)
Benzylpiperazine	**177(100%)**	178(12.2%)	91(4.9%)	85(1.2%)	
Amphetamine	91(100%)	119(59.7%)	**136(9.0%)**	120(6.3%)	65(0.9%)
Caffeine	**195(100%)**	196(10.3%)	138(4.3%)		
3,4-MDA	163(100%)	164(11.4%)	**180(9.1%)**	135(7.8%)	133(6.9%)
Methamphetamine	**150(100%)**	91(82.8%)	119(70.5%)	151(11.7%)	92(6.8%)
3,4-MDMA	163(100%)	**194(73.3)**	164(11.0%)	195(9.5%)	135(6.7%)
Dimethylamphetamine	**164(100%)**	119(15.9%)	91(15.4%)	165(13.2%)	92(1.2%)
Phentermine	133(100%)	91(63.4%)	**150(11.9%)**	134(10.9%)	105(7.9%)
N,N-Dimethyl-3,4-MDA	**208(100%)**	163(35.3%)	209(13.6%)	164(4.0%)	135(2.6%)
4-Methoxymethamphetamine	149(100%)	**180(37.6%)**	121(14.1%)	150(11.6%)	181(4.6%)
Ethylamphetamine	**164(100%)**	91(32.8%)	119(32.7)	165(13.0%)	120(3.2%)
3,4-MDEA	**208(100%)**	163(70.4%)	209(14.1%)	135(5.1%)	133(4.7%)
Ketamine	**238(100%)**	240(32.3%)	239(14.5%)	207(12.7%)	179(6.9%)
MBDB	**208(100%)**	135(50.1%)	177(40.3%)	209(14.3%)	147(6.0%)
N-Hydroxy-3,4-MDA	163(100%)	**196(17.8%)**	164(10.9%)	105(7.1%)	135(6.3%)

* * * * *

Table 5. Fragmentor Voltage Optimization of Mass Ions for Compounds Examined.

Compound	*Ion#1 (Voltage)*	*Ion#2*	*Ion#3*	*Ion#4*	*Ion#5*
Niacinamide	123(120V)	124 (120)	80 (200)	50 (200)	78 (200)
Acetaminophen	152(100)	110(180)	93(200)	153(120)	174(3.3%)
Phenethylamine	122 (70)	105(120)	79(180)		
Methylephedrine	180(100)	162(180)	181(100)	135(160)	163(180)
Phenylpropanolamine	134(140)	135(140)	117(180)	152(100)	
Ephedrine	148(160)	166(100)	149(160)	135(140)	167(100)
Pseudoephedrine	148(160)	166(100)	149(160)	133(200)	167(100)
Benzylpiperazine	177(100)	178(100)	91(200)	85(180)	
Amphetamine	91(180)	119(140)	136(100)	120(140)	65(220)
Caffeine	195(140)	196(140)	138(200)		
3,4-MDA	163(140)	164(140)	180(120)	135(200)	133(180)
Methamphetamine	150(100)	91(200)	119(140)	151(100)	92(180)
3,4-MDMA	163(160)	194(100)	164(160)	195(100)	135(200)
Dimethylamphetamine	164(100)	119(160)	91(200)	165(100)	92(200)
Phentermine	133(140)	91(180)	150(100)	134(140)	105(180)
N,N-Dimethyl-3,4-MDA	208(120)	163(180)	209(120)	164(180)	135(220)
4-Methoxymethamphetamine	149(160)	180(100)	121(200)	150(160)	181(100)
Ethylamphetamine	164(100)	91(200)	119(140)	165(100)	120(140)
3,4-MDEA	208(100)	163(180)	209(100)	135(220)	133(180)
Ketamine	238(120)	240(100)	239(100)	207(180)	179(180)
MBDB	208(100)	135(200)	177(160)	209(100)	147(180)
N-Hydroxy-3,4-MDA	163(160)	196(100)	164(140)	105(220)	135(200)

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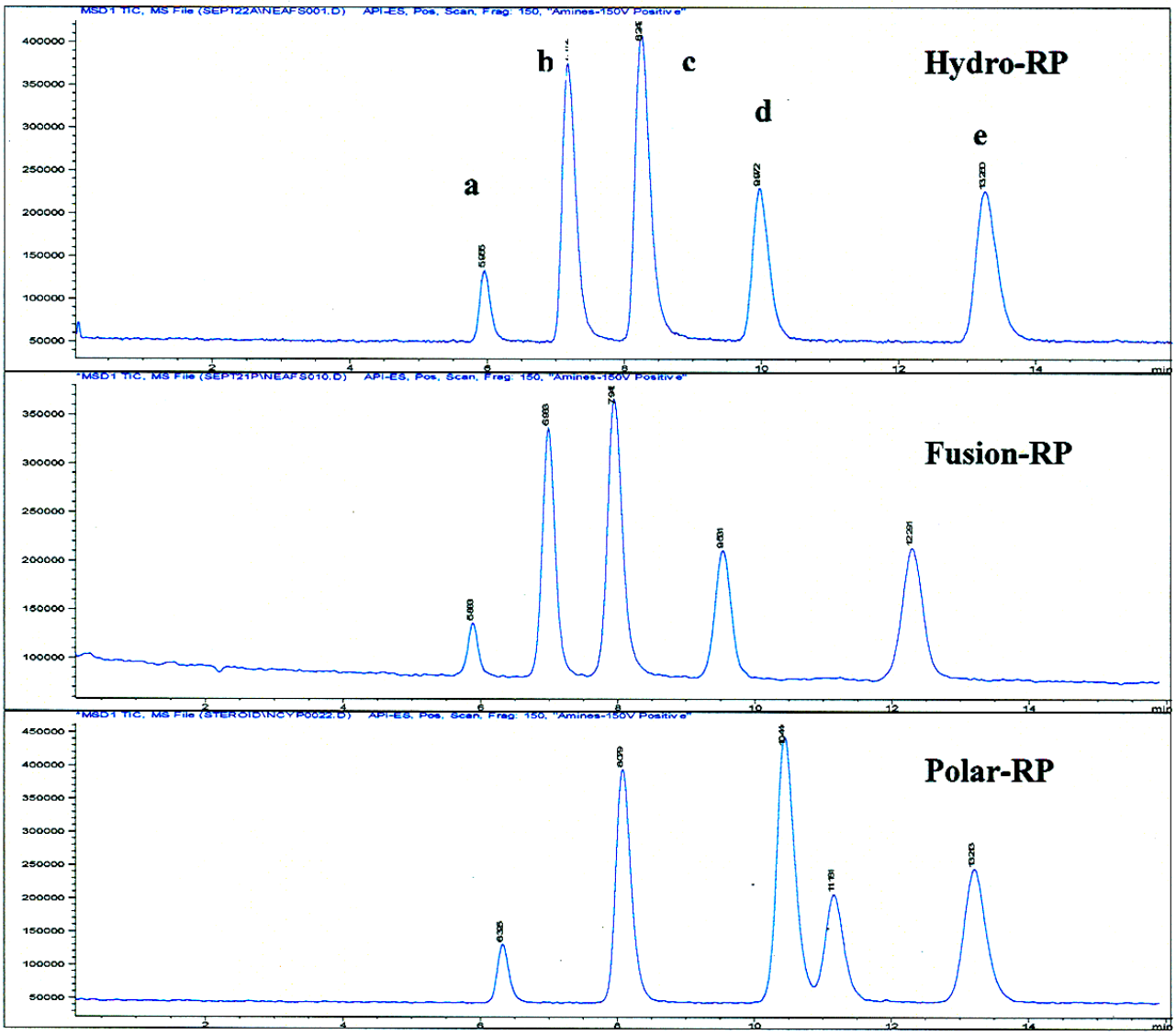

Figure 1. Total Ion Chromatogram of 5 Components Mixture of 3,4-MDA Analogs. (a) 3,4-MDA, (b) 3,4-MDMA, (c) N,N-Dimethyl-3,4-MDA, (d) 3,4-MDEA, (e) MBDB.

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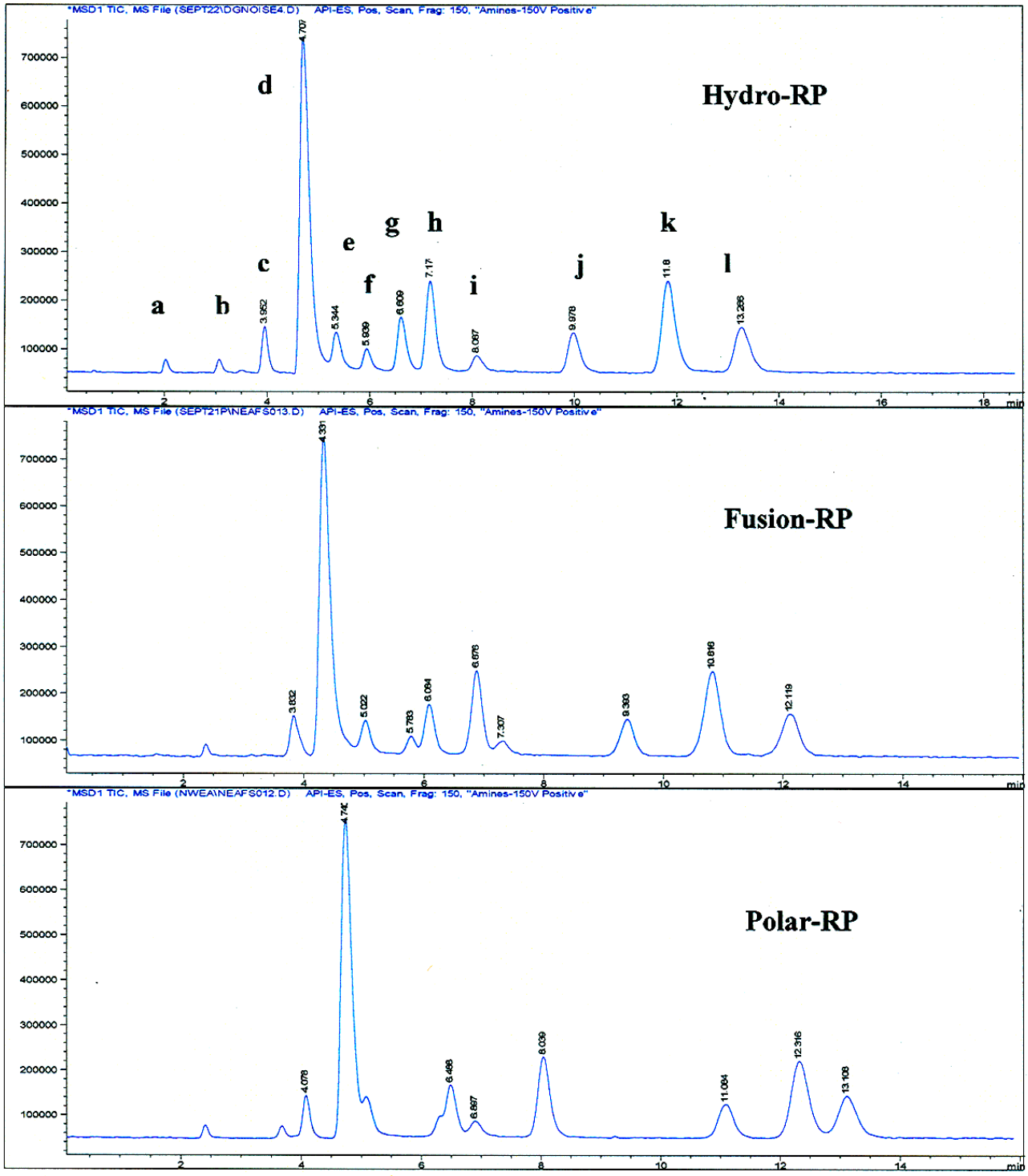

Figure 2. Total Ion Chromatogram of 12 Component Drug Mixture. (a) Niacinamide, (b) Acetaminophen, (c) Ephedrine, (d) Benzylpiperazine, (e) Amphetamine, (f) 3,4-MDA, (g) Methamphetamine, (h) 3,4-MDMA, (i) Phentermine, (j) 3,4-MDEA, (k) Ketamine, (l) MBDB.

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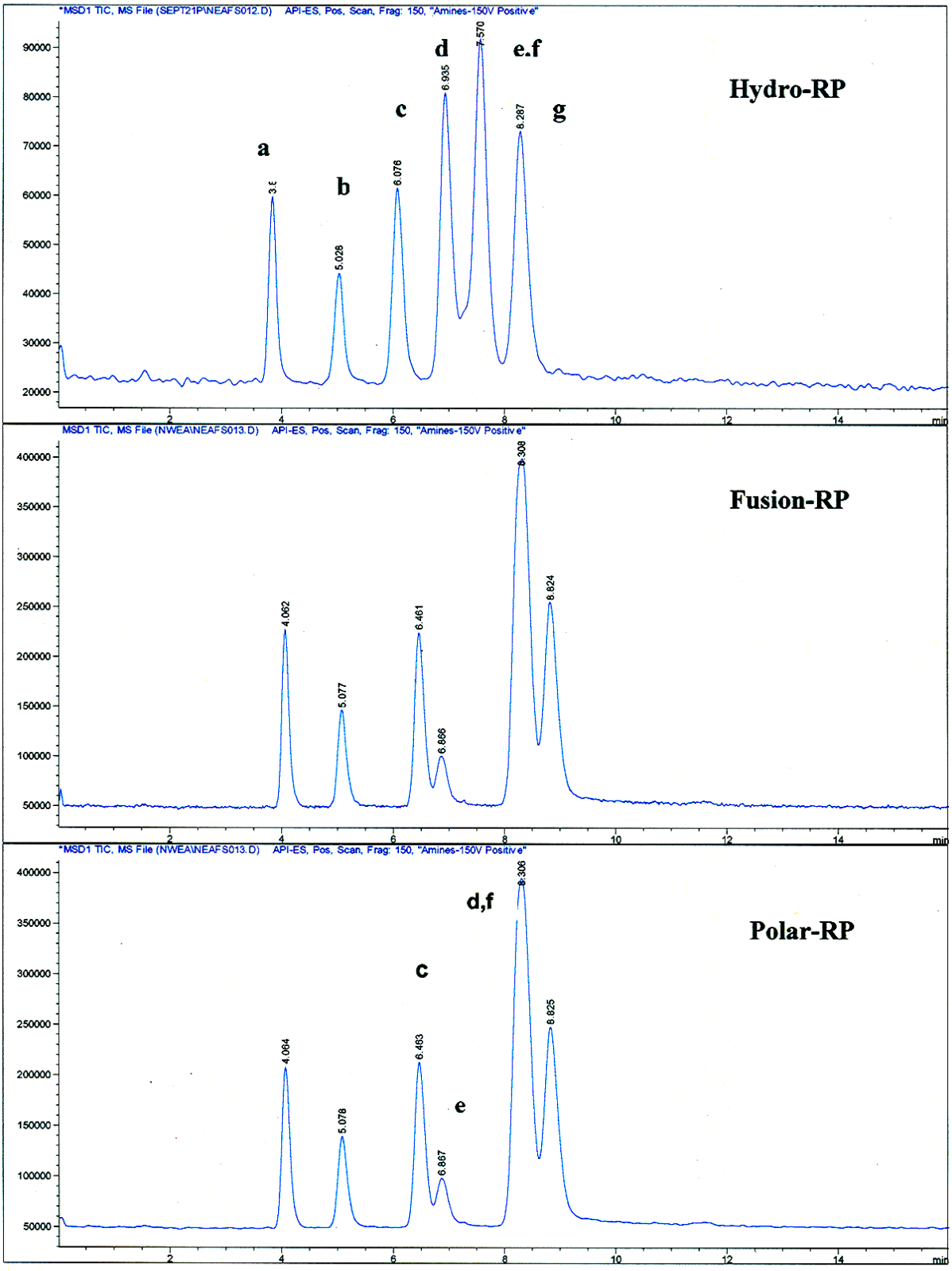

Figure 3. Total Ion Chromatogram of Mixture of 7 amines of interest. (a) Ephedrine, (b) Amphetamine, (c) Methamphetamine, (d) Dimethylamphetamine, (e) Phentermine, (F) 4-Methoxymethamphetamine, (g) Ethylamphetamine.

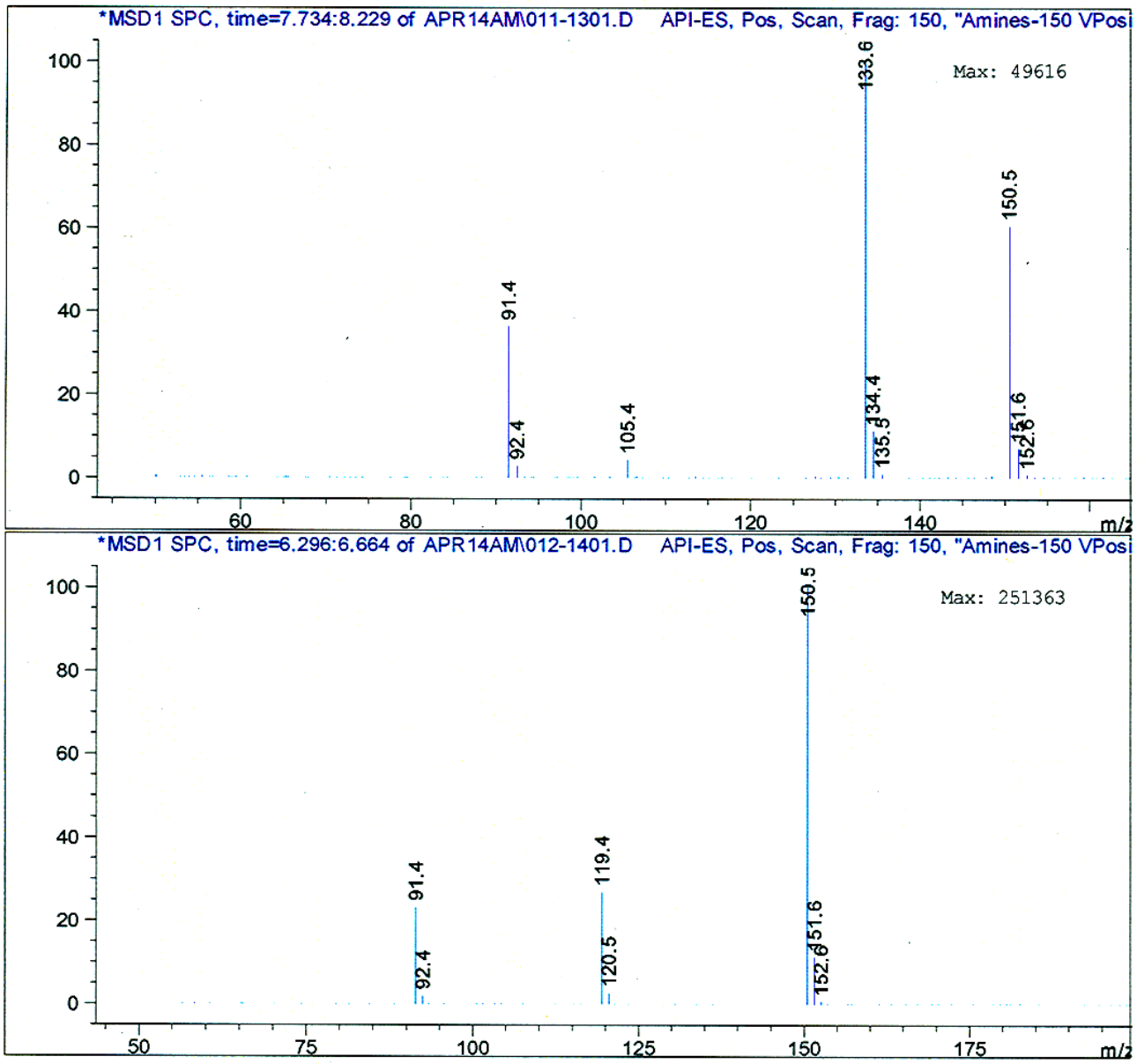

Figure 4. ESI Mass Spectra of Methamphetamine (top) and Phentermine (bottom).

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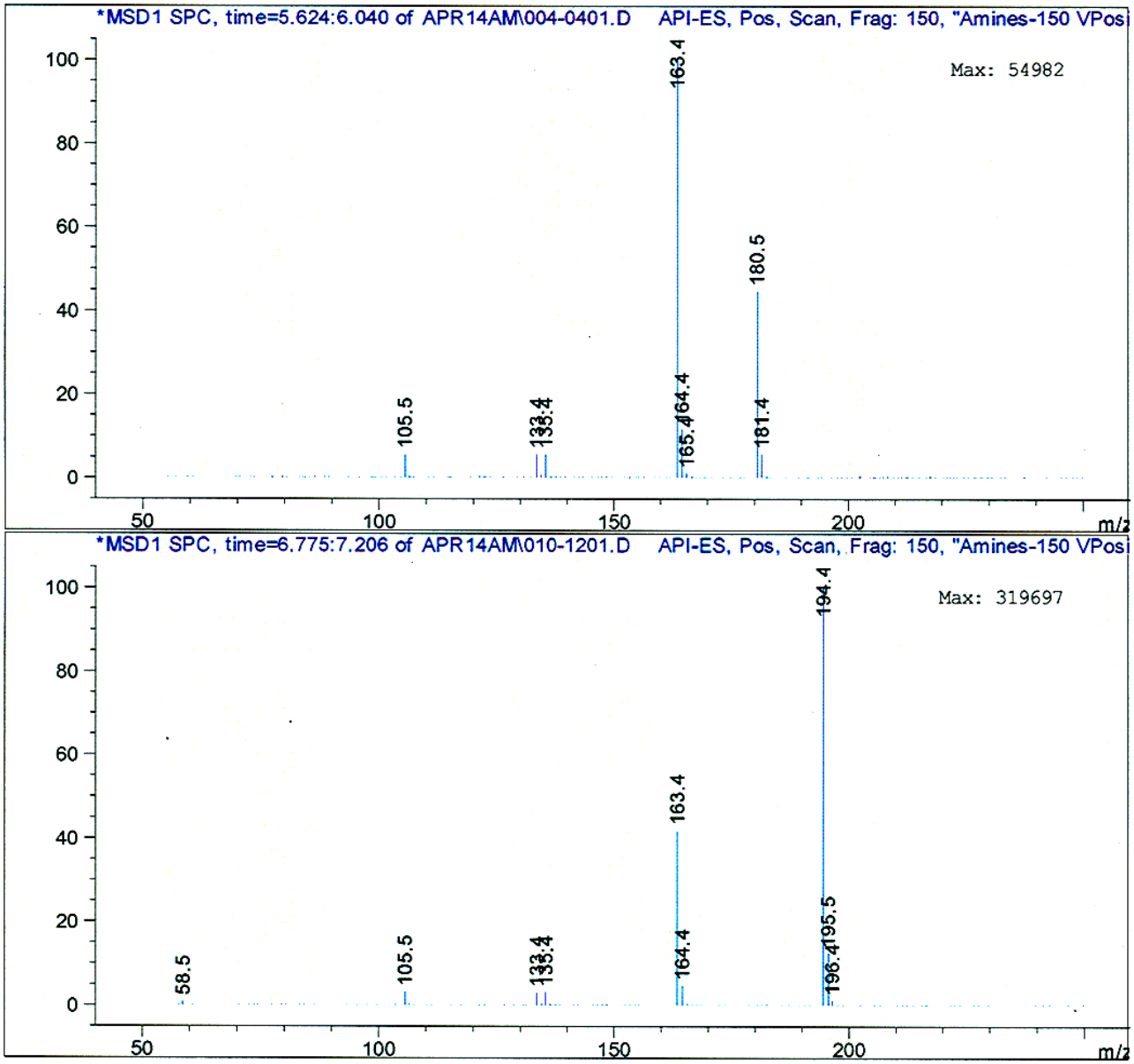

Figure 5. ESI Mass Spectra of 3,4-MDA (top) and 3,4-MDMA (bottom).

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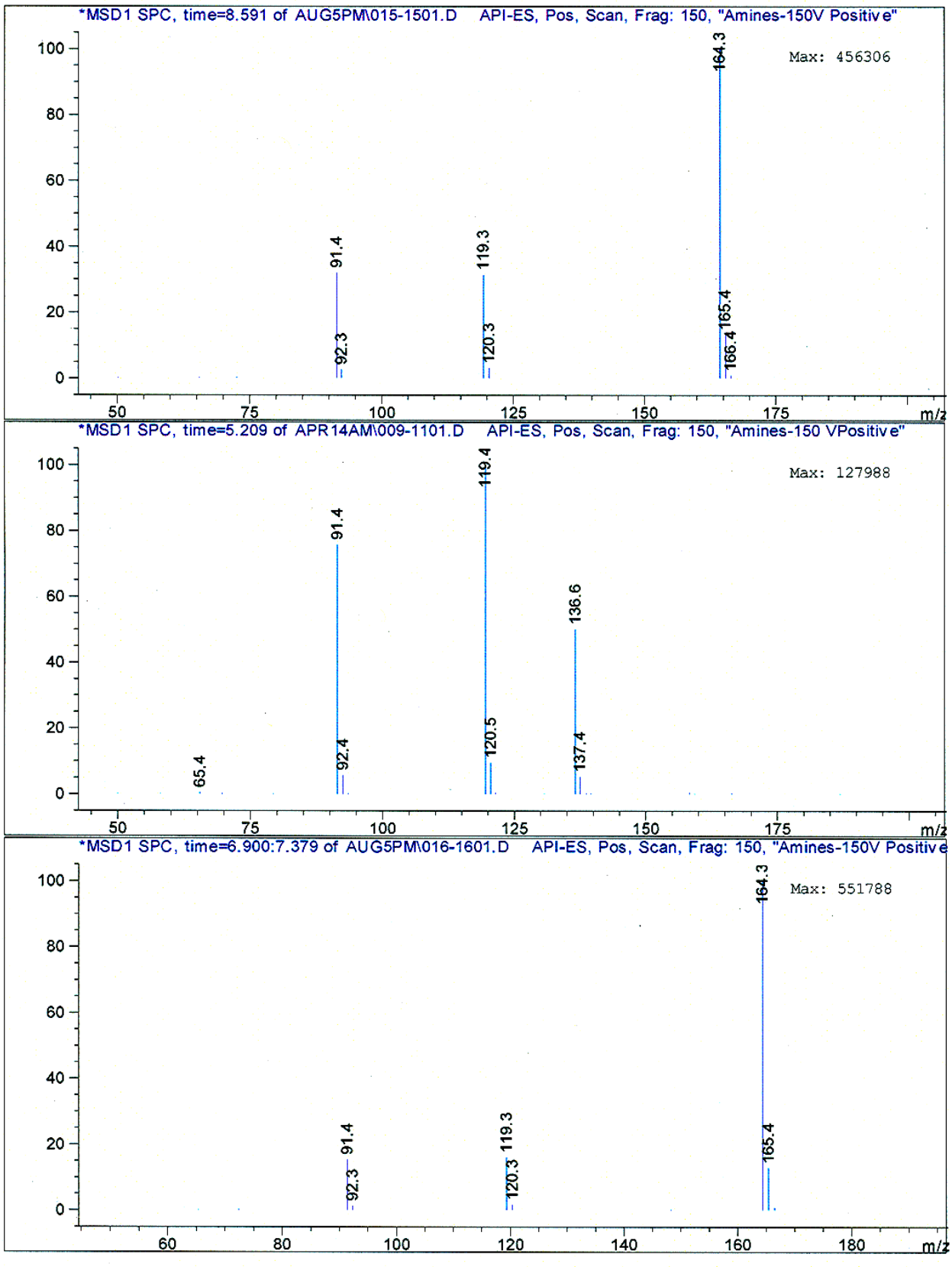

Figure 6. ESI Mass Spectra of Ethylamphetamine (top), Amphetamine (middle), and Dimethylamphetamine (bottom).

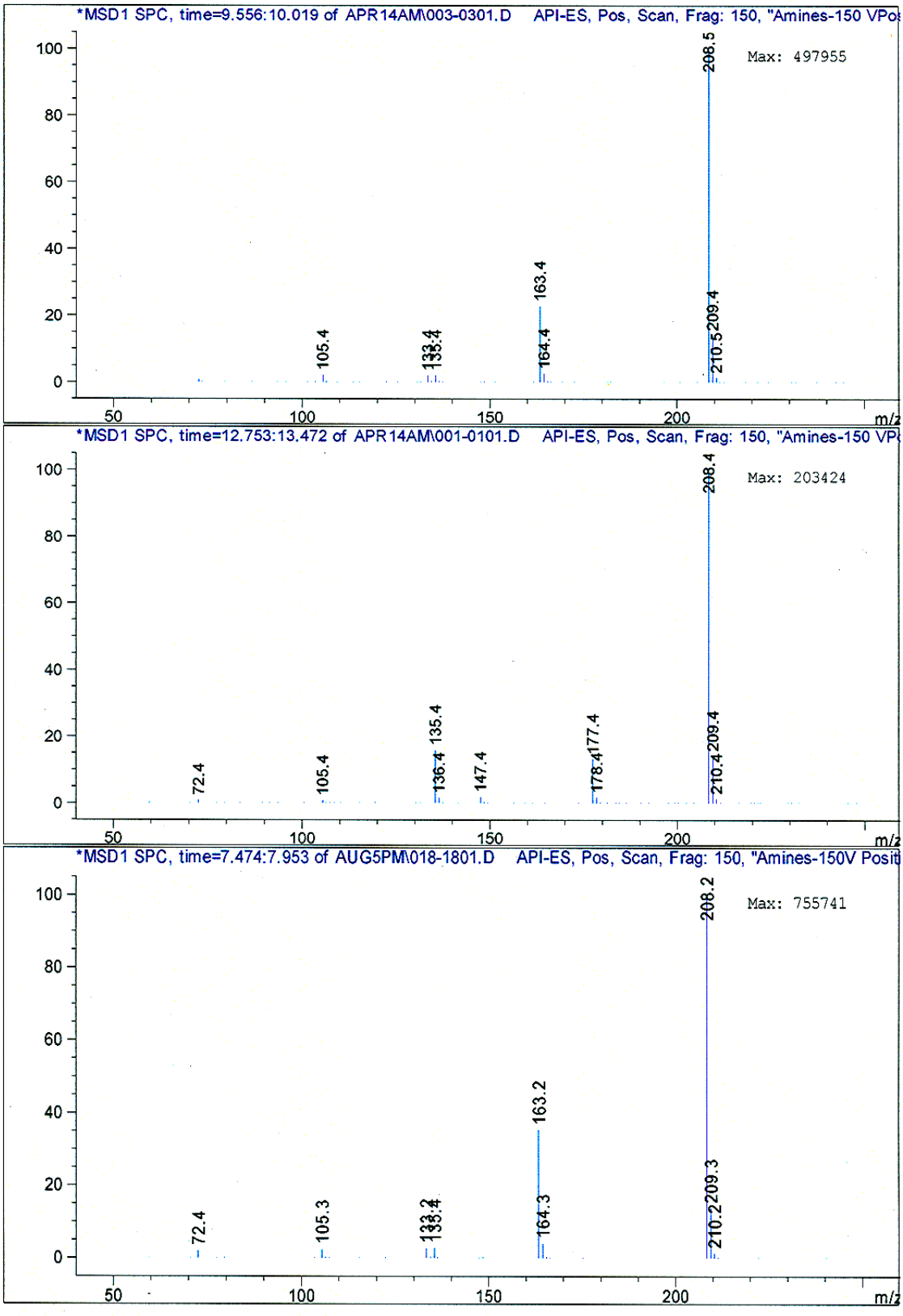

Figure 7. ESI Mass Spectra of 3,4-MDEA (top), MBDB (middle), and N,N-Dimethyl-3,4-MDA (bottom).

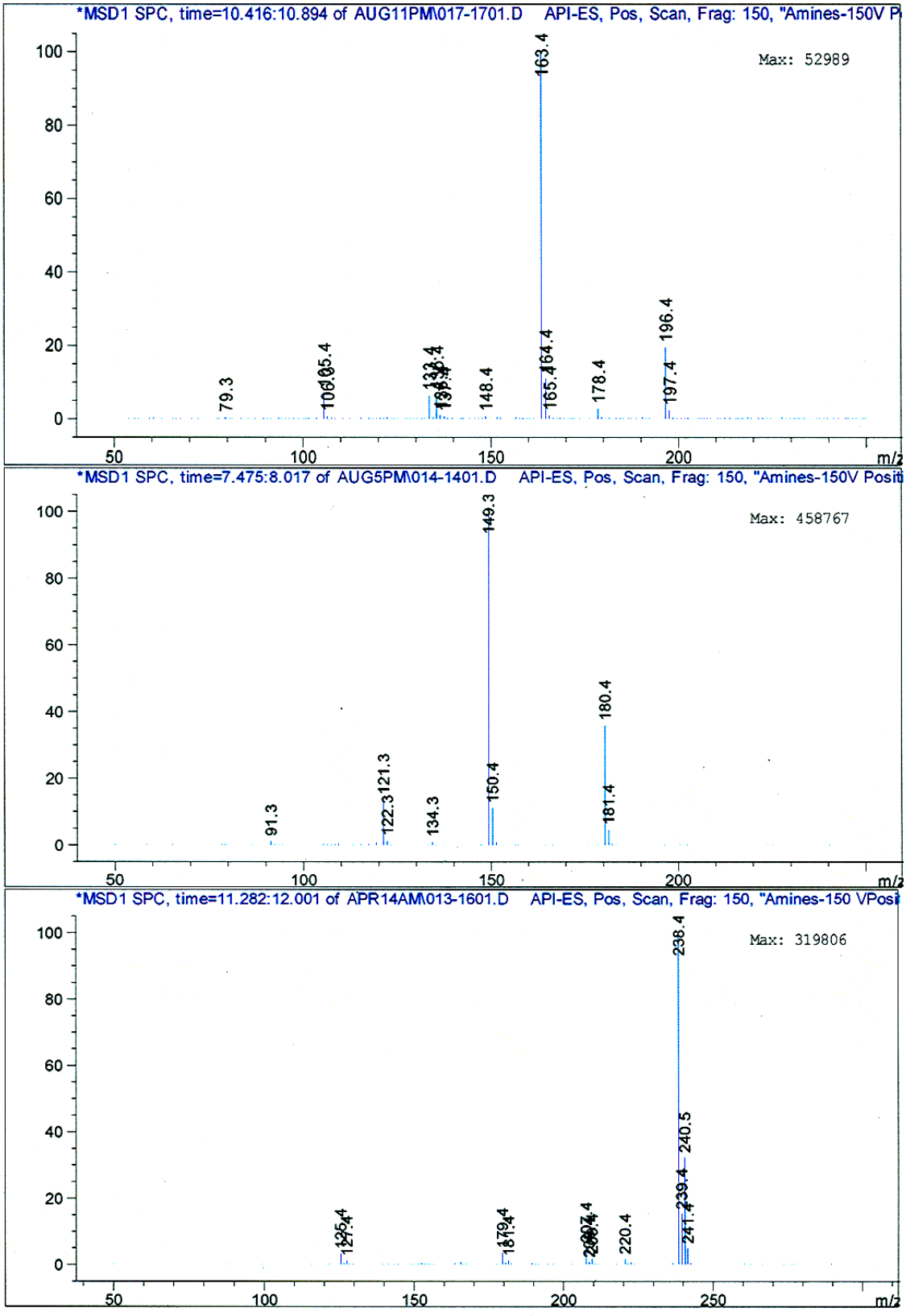

Figure 8. ESI Mass Spectra of N-Hydroxy-3,4-MDA (top), 4-Methoxymethamphetamine (middle), and Ketamine (bottom).

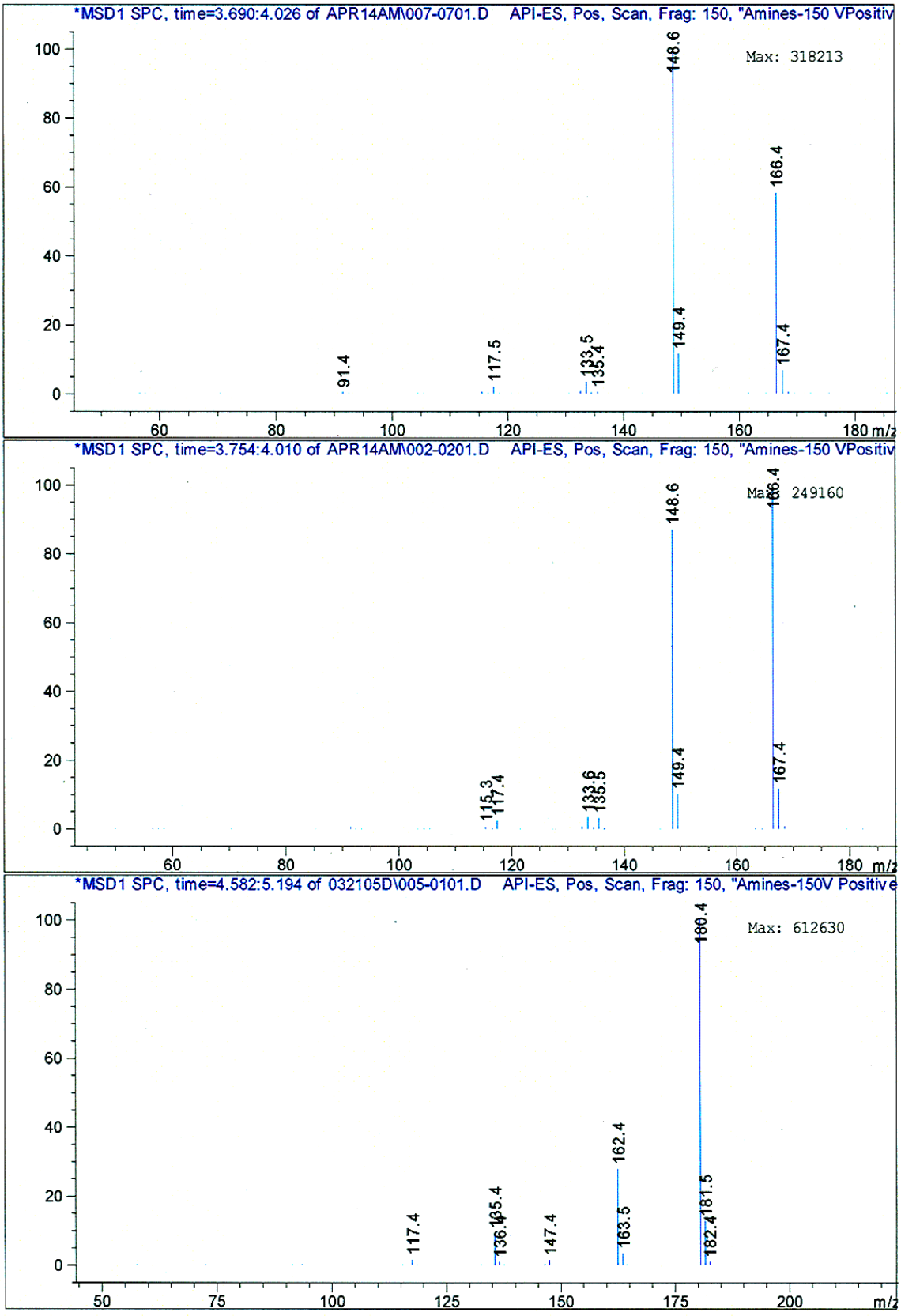

Figure 9. ESI Mass Spectra of Pseudoephedrine (top), Ephedrine (middle), and Methylephedrine (bottom).

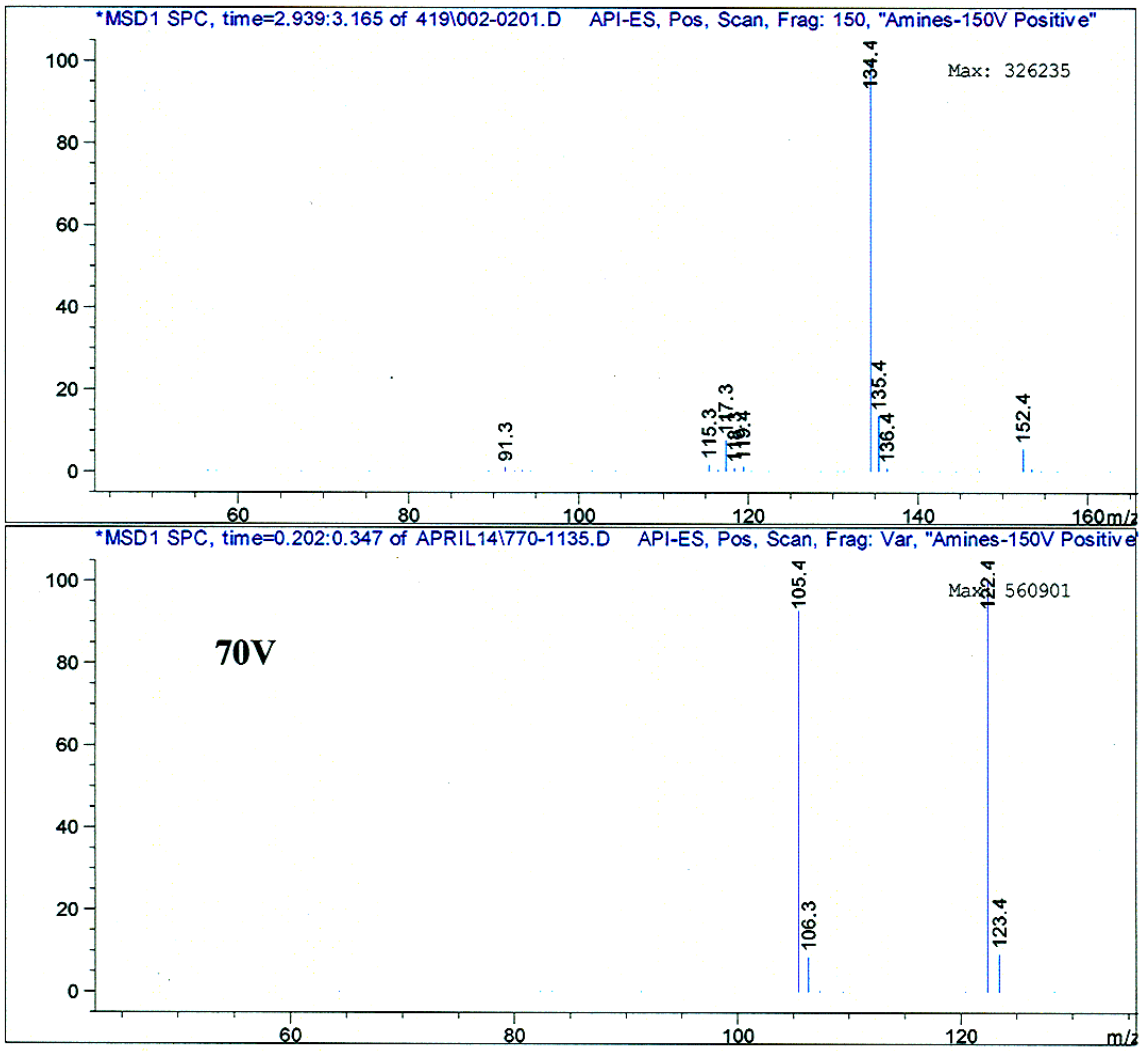

Figure 10. ESI Mass Spectra of Phenylpropanolamine (top) and Phenethyamine (bottom).

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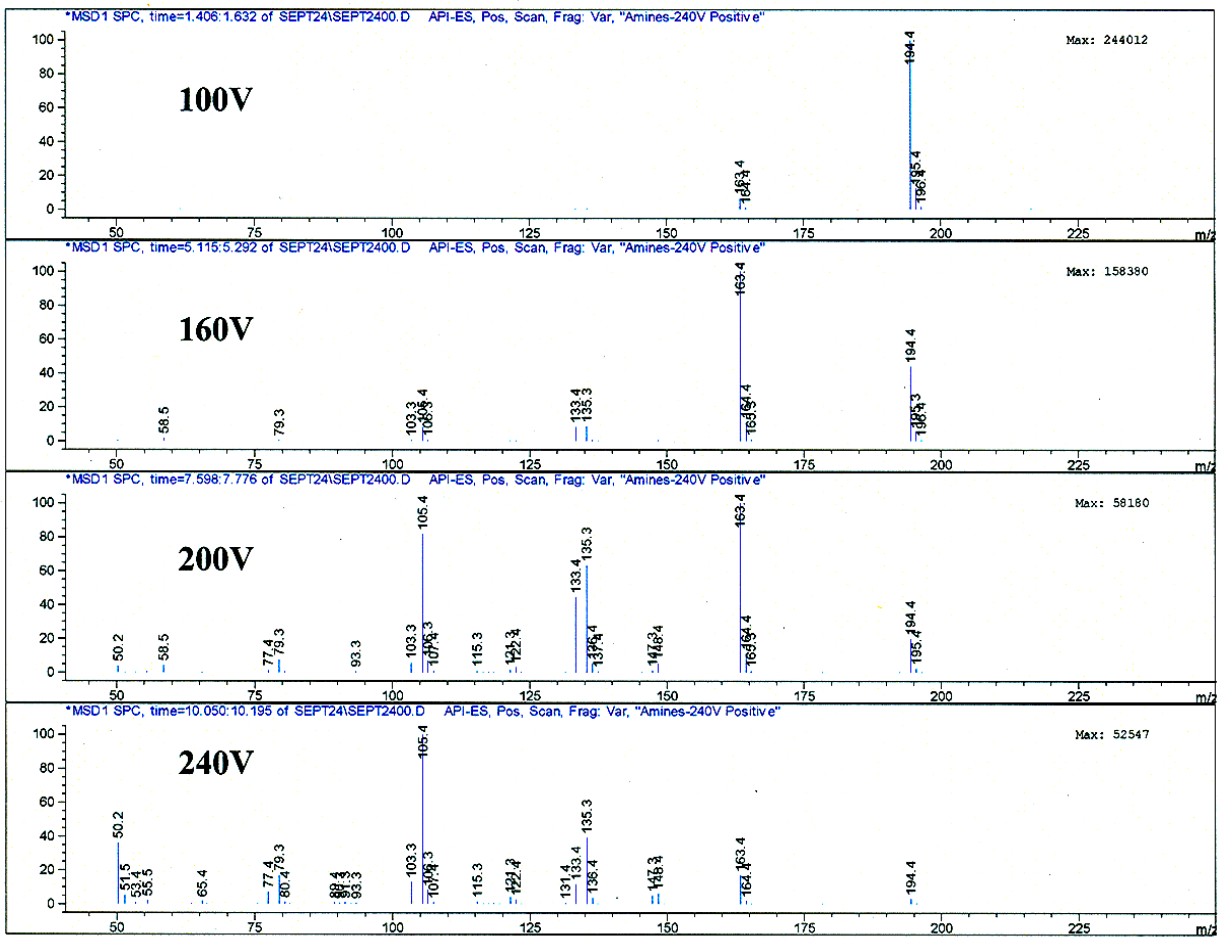

Figure 11. Collision Induced Dissociation of 3,4-MDMA.

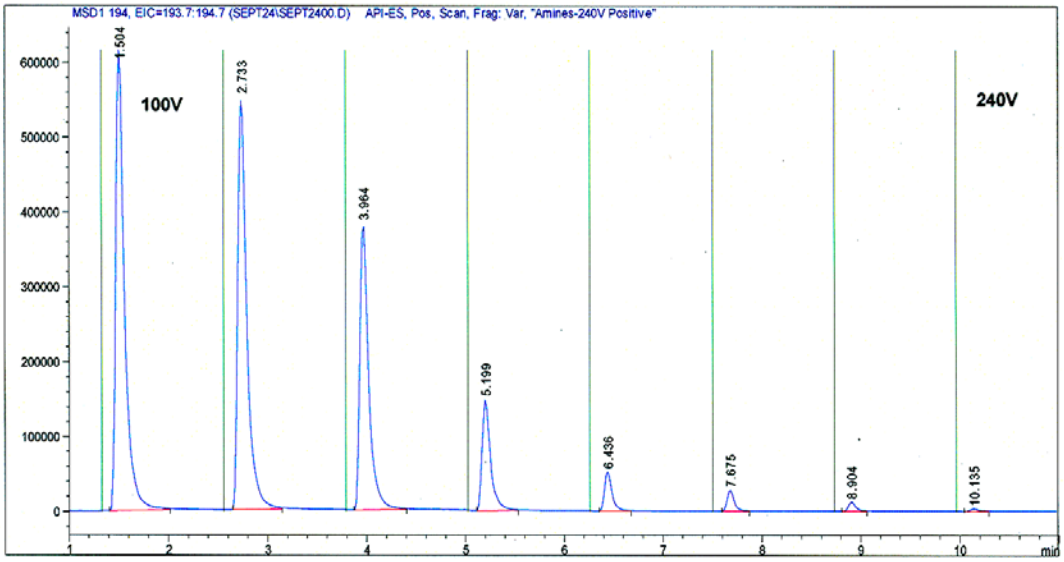

Figure 12. Flow Injection Analysis of 3,4-MDMA Monitored at *m/z* 194. Fragmentor Ramped from 100 V - 240 V at 20 V Increments.

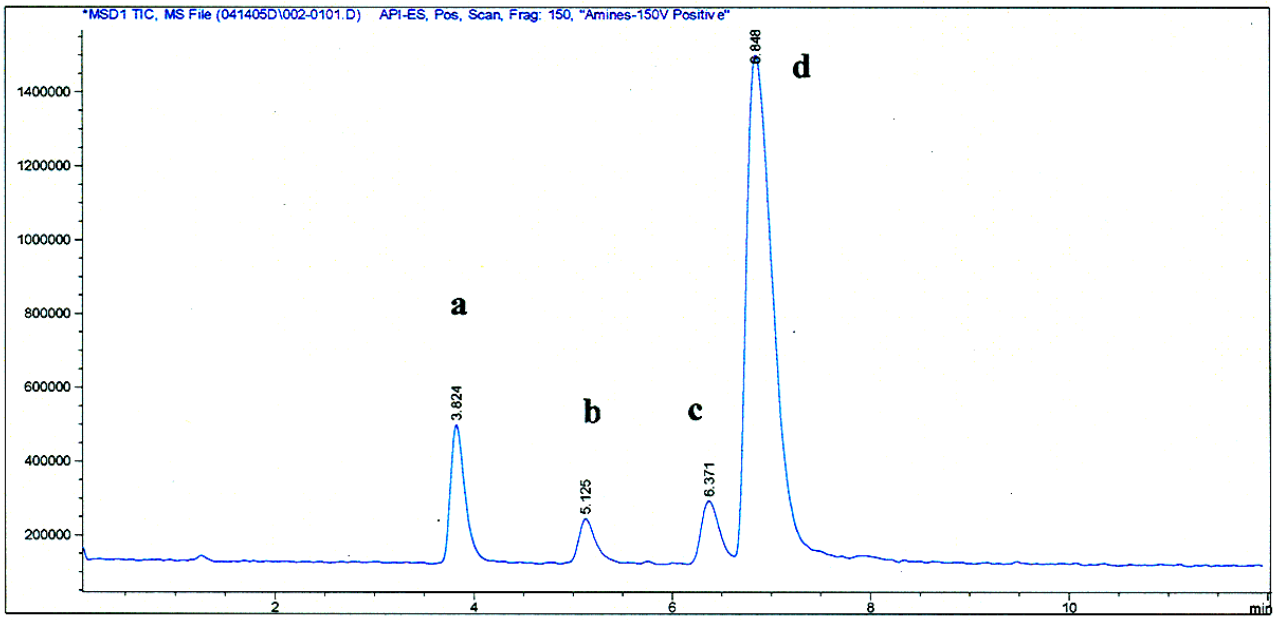

Figure 13. Total Ion Chromatogram of Illicit Tablets Containing (A) Ephedrine, (B) Caffeine, (C) Methamphetamine, and (d) 3,4-MDMA, on a Hydro-RP Column.

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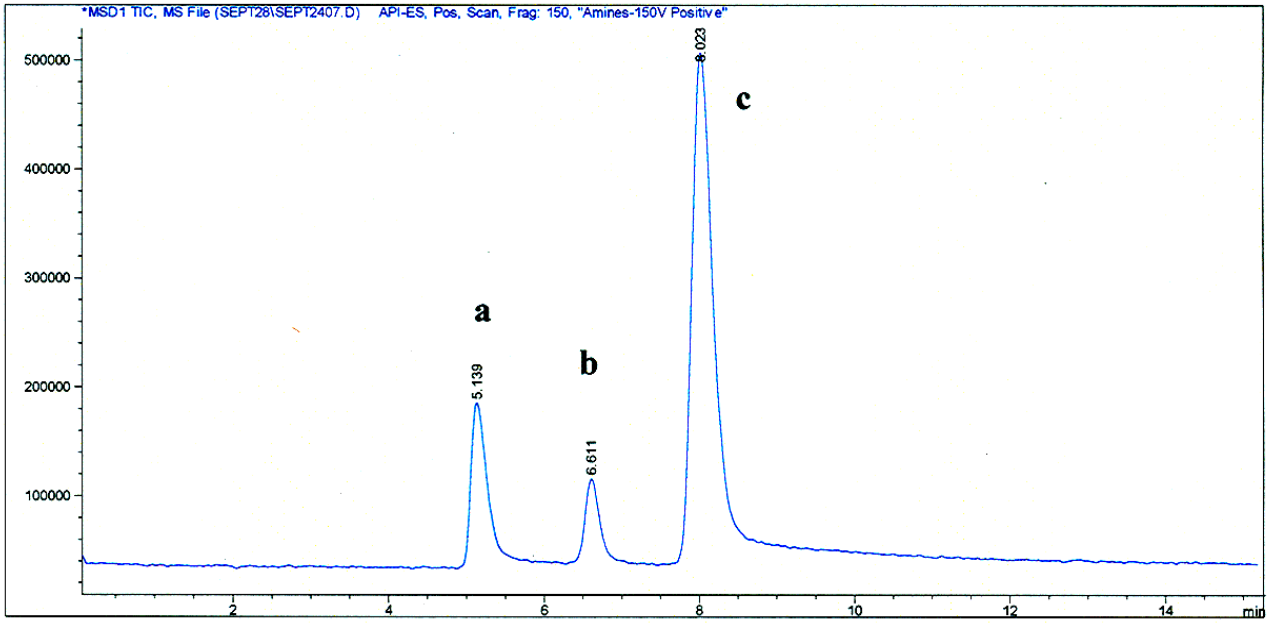

Figure 14. Total Ion Chromatogram of Illicit Powder Containing (A) Amphetamine, (B) Methamphetamine, and (C) Caffeine, on a Polar-RP Column.

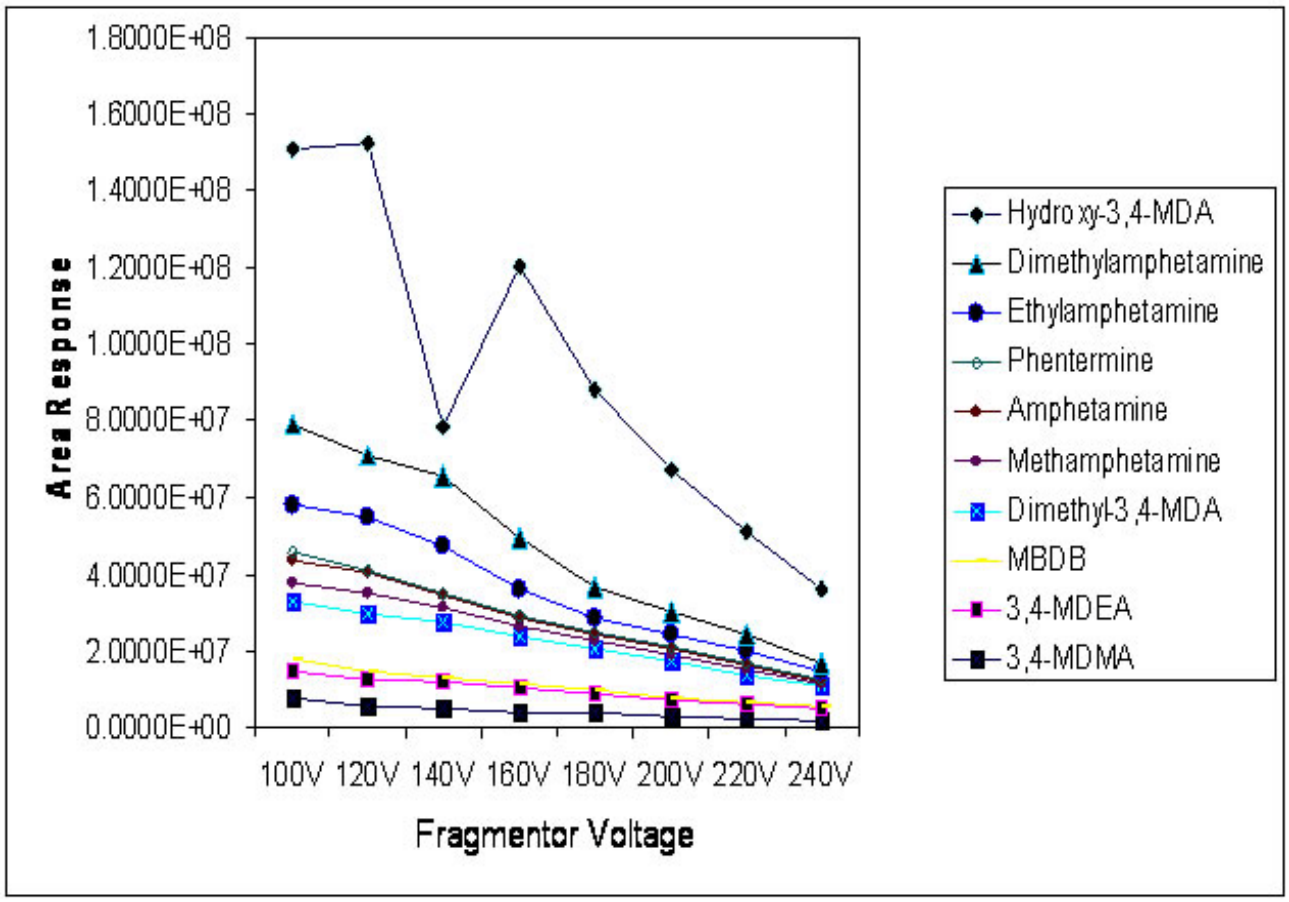

Figure 15. Fragmentor Voltage Optimization for Compounds of Interest.

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