



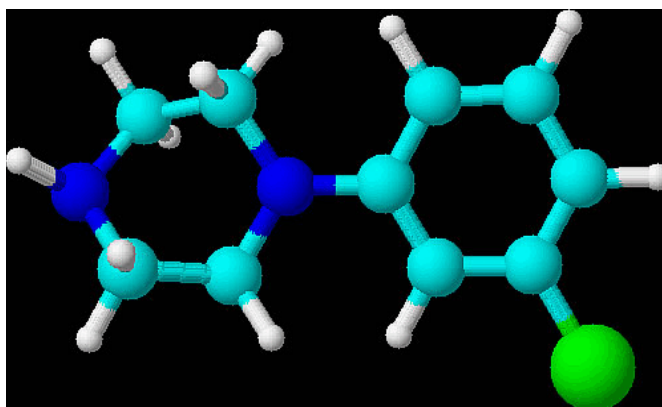
# Microgram

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

Cover Art: “Ball and Stick” Model of 3-Chlorophenylpiperazine (mCPP; Courtesy of Patrick A. Hays, DEA Special Testing and Research Laboratory, Dulles, VA).

# The Use of Dipropionylmorphine as a Structurally-Related Internal Standard for Gas Chromatographic Quantitation of Heroin

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**ABSTRACT:** Dipropionylmorphine is utilized as a structurally similar internal standard for quantitation of illicit heroin via gas chromatography with flame ionization detection. The described method has excellent selectivity, precision, and accuracy, with a relative standard deviation of less than 0.2 percent and a correlation coefficient of 0.99999. The quantitative results were in excellent agreement with other quantitative methods. The synthesis of high-purity dipropionylmorphine from morphine is detailed.

**KEYWORDS:** Dipropionylmorphine, Synthesis, Gas Chromatography, Flame Ionization Detection, Heroin, Quantitation, Internal Standard, Forensic Chemistry.

## Introduction

The quantitative determination of illicit heroin (Figure 1) and related opium alkaloids is important for forensic, toxicological, and judicial purposes. Many methods have been published for this purpose, including using, for example, high pressure liquid chromatography (HPLC) [1], capillary electrophoresis (CE) [2], nuclear magnetic resonance (NMR) [3], and gas chromatography (GC) [4-6].

Internal standards are routinely incorporated in quantitative methods. Structurally similar internal standards are preferred because they improve method accuracy and precision. However, most analytical methods that employ internal standards use non-structurally related hydrocarbons such as *n*-tetracosane [4], *n*-octacosane [4], and *n*-triacontane [5] because of their wide availability, high purity, high stability, and relative ease in handling. In contrast, methods using structurally related internal standards are uncommon.

Specifically looking at gas chromatography analysis with flame ionization detection (GC/FID), an ideal internal standard should have similar chemical and physical properties as well as comparable FID responses to the analyte of interest. As such, the structurally related internal standard would maximize precision and accuracy [6] and minimize issues related to reactivity, absorption, solubility, and inlet/on-column degradation [4,7].

Diacetylnalorphine has been previously utilized as a structurally similar internal standard for heroin quantitation [8], and satisfies the above criteria. However, it is not an ideal compound for routine use because of the relatively high cost of its precursor (nalorphine), and its moderate instability in solution (three months at 4°C).

To date, only diacetylnalorphine has been reported for use as a structurally similar internal standard for heroin quantitation. However, dipropionylmorphine (Figure 1) was previously utilized as a target compound for quantitation of morphine in toxicological samples [9] (in this study, propionic anhydride was utilized as a derivatization reagent). *A priori*, dipropionylmorphine would be an ideal internal standard for heroin quantitation by GC/FID, providing it is non-coincident with any other opium alkaloids or typical adulterants and diluents. Herein we report the facile synthesis and successful use of dipropionylmorphine as a structurally similar internal standard for GC/FID analysis of heroin.

## Experimental

**Reagents:** All solvents were obtained from Burdick and Jackson Laboratories (Muskegon, MI). Diethylamine, propionylchloride, ammonium hydroxide, alumina, and activated carbon were obtained from Sigma Aldrich Inc. (St. Louis, MO). Heroin hydrochloride and morphine hydrochloride standards were obtained from this laboratory's reference collection.

**Synthesis of Dipropionylmorphine:** Morphine hydrochloride monohydrate (40.0 g, 0.124 mol) was combined with 800 mL of acetonitrile and propionyl chloride (51.1 g, 0.552 mol) in a 2-liter round-bottom flask fitted with a water-cooled condenser. The solution was refluxed gently, with stirring, for 22.5 hours. Upon cooling, the reaction mixture was split into four 200 mL aliquots. Each portion was added slowly with stirring to a mixture of isooctane (1.0 liter) and diethyl ether (800 mL), causing crude dipropionylmorphine hydrochloride to precipitate from solution. The crystals were captured via suction filtration and washed with anhydrous diethyl ether (200 mL). All four crops of crude material were then dissolved into 400 mL of water and filtered. The filtrate was washed with isooctane (500 mL), then with anhydrous diethyl ether (400 mL). The solution was adjusted to pH 9 with concentrated ammonium hydroxide and extracted with methylene chloride (2 x 300 mL). The extracts were combined, dried over anhydrous sodium sulfate, filtered, and treated with activated carbon (2 g). The carbon was removed by filtering through a celite pad. The filtrate was evaporated *in vacuo* to provide approximately 40 grams of 96 percent dipropionylmorphine base. The material was chromatographed on a basic alumina column (600 g containing 4 percent H<sub>2</sub>O) using methylene chloride. The first 800 mL of eluate was collected and evaporated *in vacuo* to a clear oil. The oil was dissolved into a minimal volume of anhydrous diethyl ether in a flask, and the flask was scratched to crystallize dipropionylmorphine base as a chromatographically pure (99+ percent) white powder (34.2 g, 70 percent yield).

**Gas Chromatography - Flame Ionization Detection (GC/FID):** An Agilent 6890N GC with a DB-5 column (30 m x 0.25 mm I.D., 0.25 µm film thickness) was utilized. The oven temperature program began at 205°C (1 minute hold), ramped to 240°C at 12°C/minute (5 minute hold), ramped at 4°C/minute to 275°C (1 minute hold), and then ramped at 15°C/minute to 285°C (2.33 minute hold). The carrier gas was hydrogen (99.999 percent UHP) at a flow rate of 0.9 mL/minute, with a split ratio of 25:1. The injector and detector temperatures were maintained at 280°C.

**Gas Chromatography - Mass Spectrometry (GC/MS):** An Agilent 6890N GC/MSD with a DB-1 column (30 m x 0.25 mm I.D., 0.25 µm film thickness) was utilized. The oven temperature program began at 90°C (2 minute hold), ramped to 300°C at 14.0°C/minute (10.0 minute hold). The carrier gas was ultra high purity Helium at a flow of 1.0 mL/minute, with a split ratio of 25:1. The injector and detector temperatures were maintained at 280°C. The mass spectrum of dipropionylmorphine is presented in Figure 2.

**Internal Standard Stock Solution:** A stock solution of dipropionylmorphine was prepared at 1 mg/mL in chloroform. The solution was used at room temperature and can be stored at 4°C for up to two years without detectable degradation.

**Standard and Sample Preparation:** Approximately 18 - 20 mg of heroin hydrochloride standard was accurately weighed into a 50 mL Erlenmeyer flask. 5.00 mL of the internal standard stock solution and 20 mL of chloroform (containing 50 µL of diethylamine) were added to the Erlenmeyer flask and the solution was allowed to sit for 5 minutes. Samples were prepared in the same manner with slight modifications in sample weight to maintain sample concentration within the linear range of the method (see below). Aliquots of both standard and sample were transferred to separate autosampler vials for analysis.

**Linearity and Precision:** Nine individual concentrations of heroin hydrochloride were prepared (at 0.106, 0.207, 0.418, 0.611, 0.757, 0.807, 1.011, 1.632, and 2.039 mg/mL) with the internal standard, as described above. All nine concentrations were utilized to calculate the method linearity and precision.

## ***Results and Discussion***

High purity dipropionylmorphine is easily prepared from morphine hydrochloride. As expected, heroin and dipropionylmorphine give highly similar FID responses. The selectivity of dipropionylmorphine on a DB-5 column was excellent, with no interferences with any of the opium alkaloids, adulterants, and diluents typically present in illicit heroin (see Figure 3 and Table 1). The stock solution was stable for over 2 years at 4°C (no detectable degradation or hydrolysis, and consistent FID peak area and height counts (see Figure 4)).

The method linearity was determined over the concentration range stated in the Experimental section. The calculated correlation coefficient ( $R^2$ ) was 0.99999 (see Figure 5). The method precision was determined using all nine of the solutions listed in the Experimental section, with seven replicate injections per solution. The Relative Standard Deviations (RSDs) ranged from 0.04 to 0.17 percent. The method accuracy was determined by quantitating 11 illicit samples that had previously been analyzed in this laboratory via proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) and capillary electrophoresis (CE). The average difference for the three methods was determined to be 2.6 percent absolute (see Table 2).

Finally, the GC/FIDs of four different types of heroin are presented in Figure 6. Highly refined samples, such as Southeast Asian (SEA/4) and South American (SA) heroin, as well as crudely refined samples, such as Southwest Asian (SWA/A) and Mexican black tar (MEX) heroin, can all be routinely quantitated utilizing this method.

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9. Zezulak BA, Snyder JJ, Needleman SB. Simultaneous analysis of codeine, morphine, and heroin after  $\beta$ -Glucuronidase hydrolysis. *Journal of Forensic Sciences* 1993;38(6):1275-1285.

\* Law Enforcement Restricted Publication.

**Table 1.** Relative Retention Times (RRT) of Some Common Adulterants and Alkaloids.

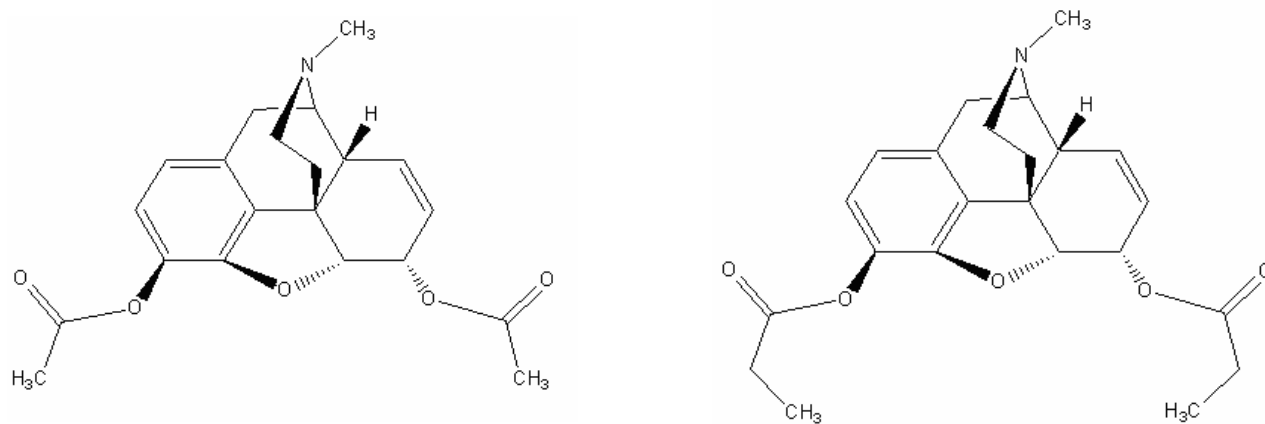
<u>Compound</u>	<u>RRT (minute)</u>
Acetaminophen	0.16
Phenacetin	0.17
Caffeine	0.22
Diphenhydramine	0.23
Theophylline	0.27
Procaine	0.30
Cocaine	0.39
Codeine	0.51
Morphine	0.54
Acetylcodeine	0.61
O6-Monoacetylmorphine	0.62
Heroin	0.75
Quinine	0.97
<b>Dipropionylmorphine</b>	<b>1.00</b>
Papaverine	1.03
Noscapine	1.53

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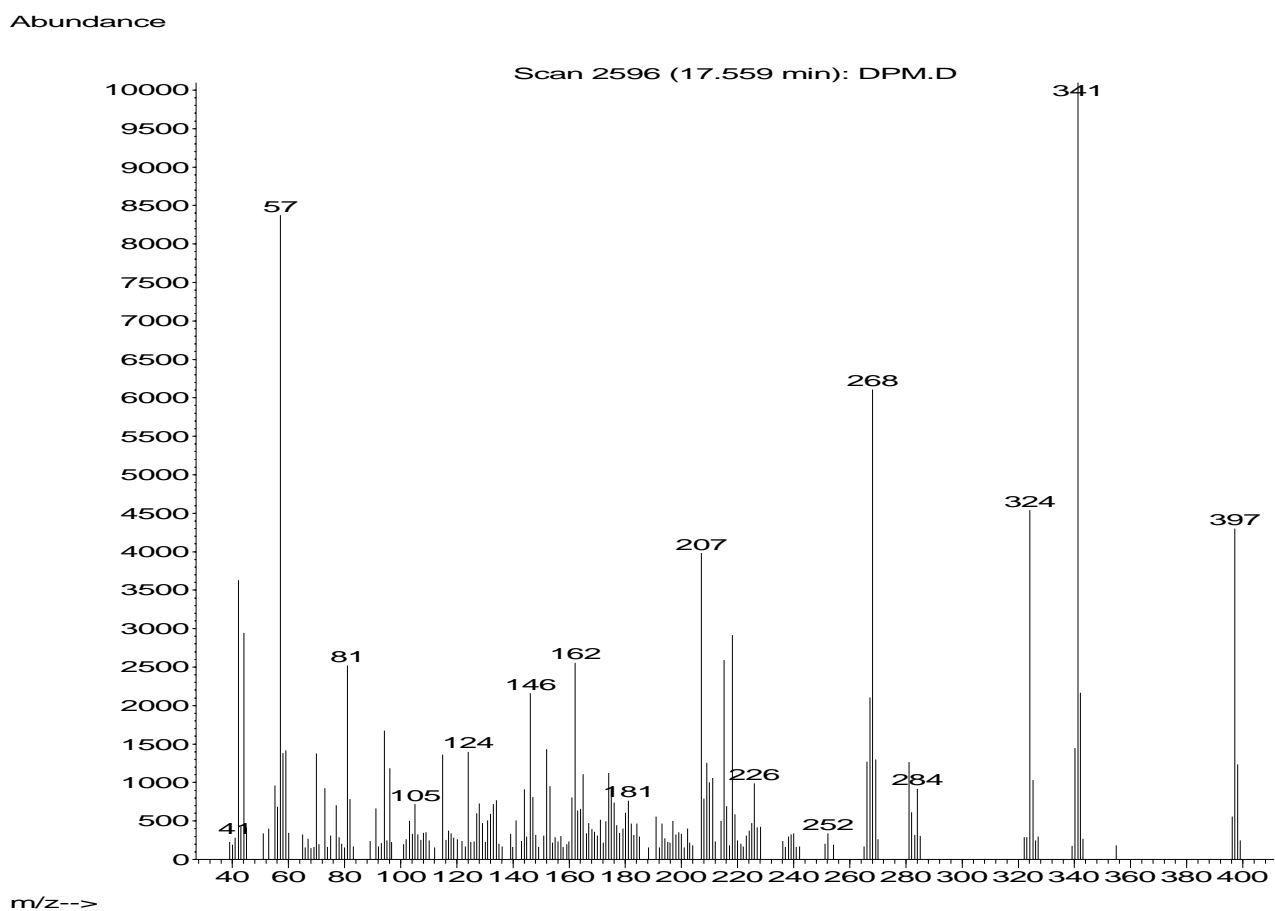
**Table 2.** Comparison of 11 Samples Quantitated by Different Methods.

<b>Sample</b>	<b>% by CE</b>	<b>% by NMR</b>	<b>% by GC/FID using Dipropionylmorphine</b>
1	11.7	N/A	10.9
2	83.6	N/A	81.2
3	74.2	72.2	71.8
4	86.3	N/A	86.3
5	93.3	93.2	89.4
6	86.0	N/A	86.1
7	80.9	78.0	77.8
8	47.6	N/A	45.8
9	56.5	58.0	55.2
10	11.2	10.9	10.7
11	10.2	11.9	9.7

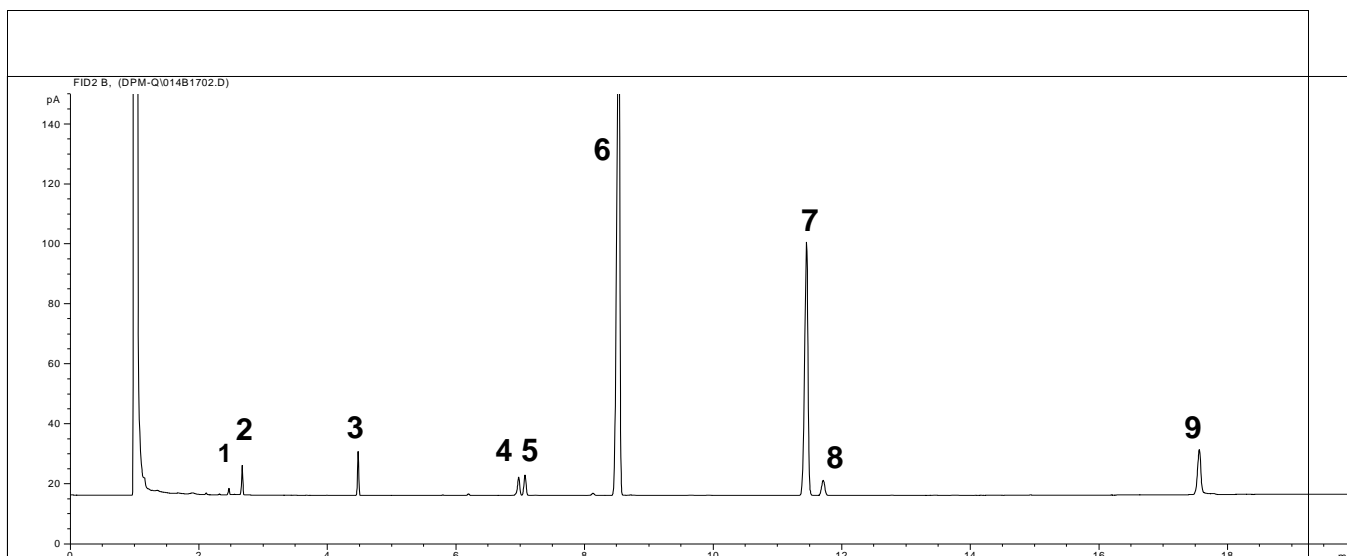
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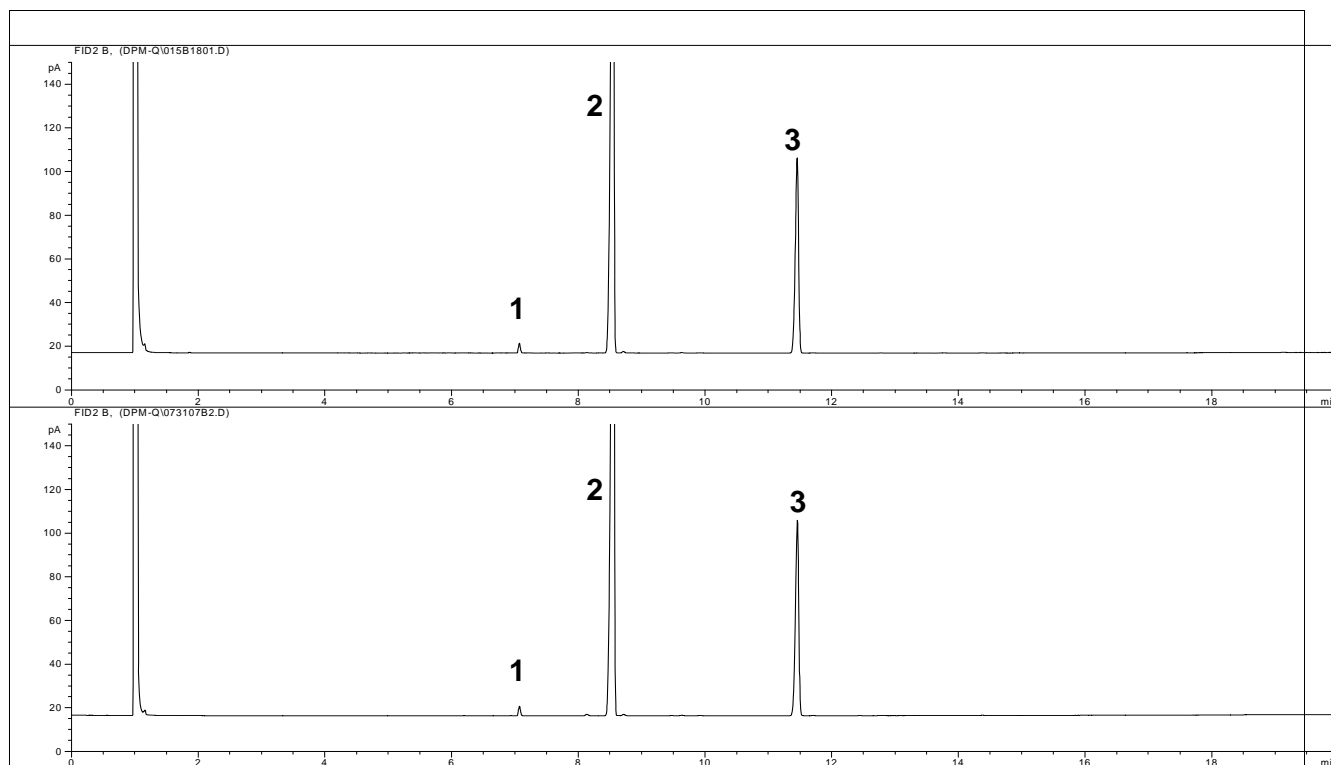
**Figure 1.** Structures of Heroin (Left) and Dipropionylmorphine (Right).



**Figure 2.** Mass Spectrum of Dipropionylmorphine.

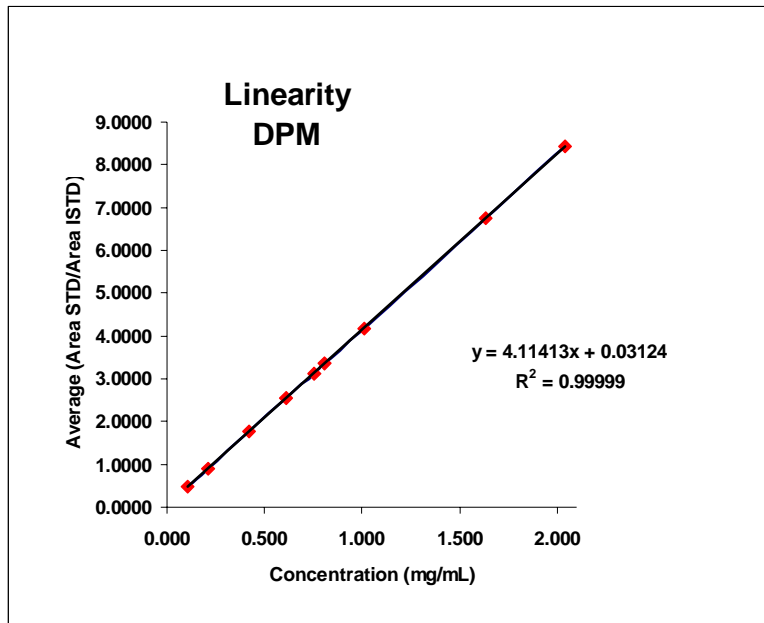


**Figure 3.** Capillary GC/FID Profile of a Typical Street Heroin Sample Containing Several Adulterants. Peak Identification: 1 = Caffeine, 2 = Lidocaine, 3 = Cocaine, 4 = Acetylcodeine, 5 = O6-Monoacetylmorphine, 6 = Heroin, 7 = Internal Standard, 8 = Papaverine, 9 = Noscapine.

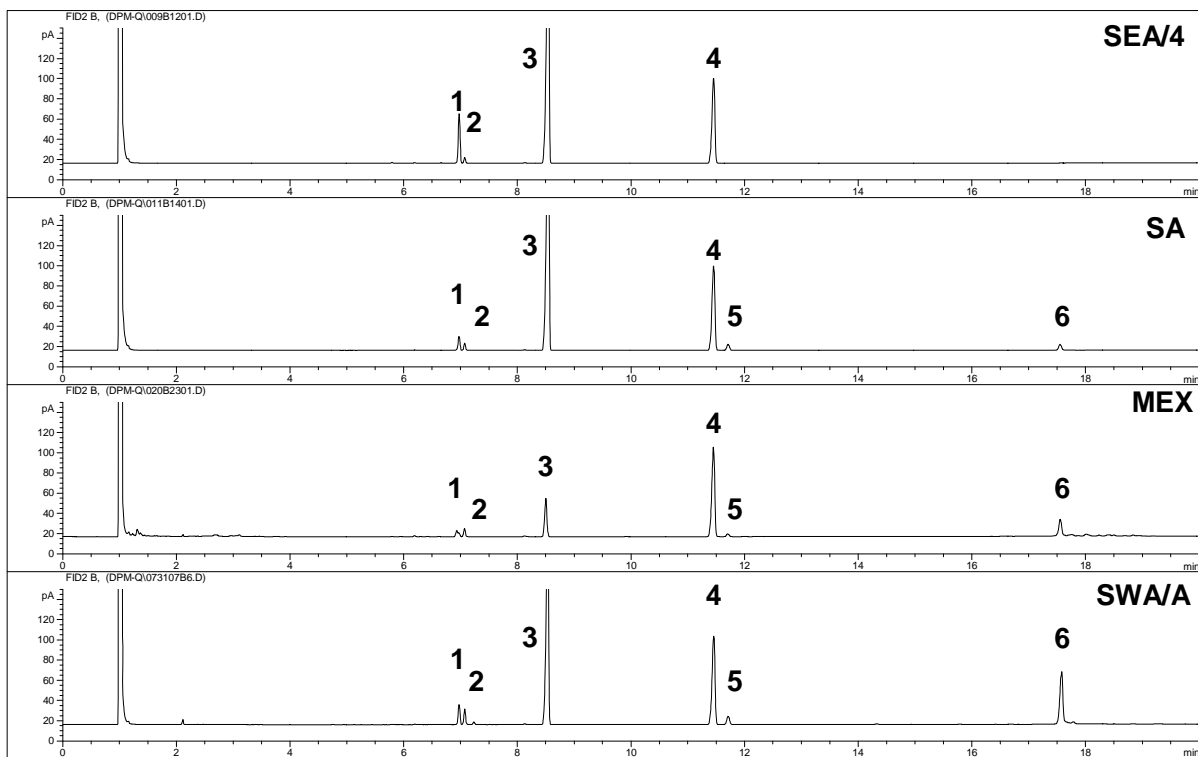


**Figure 4.** GC/FID Comparison of Internal Standard Prepared on May 14, 2004 (Upper) and Internal Standard Prepared on November 6, 2007 (Lower); No Loss in Peak Area Detected. Peak Identification: 1 = O6-Monoacetylmorphine, 2 = Heroin Standard (contains a small amount of O6-Monoacetylmorphine), 3 = Dipropionylmorphine Internal Standard.





**Figure 5.** Linearity for Heroin with Dipropionylmorphine Internal Standard.



**Figure 6.** GC/FID Comparison of Four Types of Heroin. Southeast Asian (SEA/4) 86.3 % Heroin Hydrochloride, South American (SA) 86.1 % Heroin Hydrochloride, Mexican (MEX) 9.8 % Heroin as Hydrochloride, and Southwest Asian (SWA/A) 57.6 % Heroin Base. Peak Identification: 1 = Acetylcodeine, 2 = O6-Monoacetylmorphine, 3 = Heroin, 4 = Dipropionylmorphine Internal Standard, 5 = Papaverine, 6 = Noscapine.

# Rapid Screening of Seized Drug Exhibits Using Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

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[Presented in Part at the 2007 American Society for Mass Spectrometry (ASMS) Fall Workshop - The Art of Open Air Ionization on Surfaces, Philadelphia, PA (November 9, 2007).]

**ABSTRACT:** Desorption electrospray ionization mass spectrometry (DESI-MS), an extension of the electrospray ionization technique, is utilized for the rapid screening and/or pre-analysis of multi-unit exhibits. Multiple real-case analyses are presented, including hydrocodone tablets, Ecstasy tablets containing MDMA/methamphetamine mixtures, prednisone tablets, alprazolam tablets, marijuana, counterfeit sildenafil citrate tablets containing sildenafil base, counterfeit oxycodone tablets containing fentanyl, breath mints containing  $\Delta^9$ -tetrahydrocannabinol, a chocolate-coated opium bar, and an unknown liquid containing a mixture of *gamma*-butyrolactone and *gamma*-hydroxybutyric acid. Analyses were conducted directly on the samples, in most cases with no sample preparation, and the results were obtained in less than 30 seconds per sample. Additionally, the use of DESI-MS/MS enabled identification of the controlled substances and adulterants in the samples.

**KEYWORDS:** Desorption Electrospray Ionization Mass Spectrometry, DESI-MS, DESI-MS/MS, Controlled Substances, Screening, Analysis, Forensic Chemistry.

## ***Introduction***

Forensic laboratories with large caseloads need high-throughput sampling and identification techniques in order to reduce turn-around times while fulfilling the requirements of the judicial system. For exhibits composed of multiple units, even if the units are visually indistinguishable, the standard analytical protocol requires a pre-analysis (screening) on each individual or selected unit to determine composition, before either combination of the units into a homogeneous composite, or separation of the units into several mutually exclusive subgroups, based on the results. For decades, screening was done with a combination of visual inspection, color tests, microcrystal tests, thin-layer chromatography, microscopy, and similar techniques. However, most such techniques are only presumptive, and evolving laboratory policy and procedures mandate the identification of all controlled substances in each individual unit prior to composition.

The capabilities of analytical instruments (including screening instruments) have been greatly extended with the addition of powerful detectors, autosampling devices, data handling computers, and library searching abilities. However, increasing sophistication usually equates to longer analyses and the generation of very large amounts of data, increasing turnaround times. Furthermore, sample preparation, extractions, and chemical derivatization steps are still often necessary in order to conclusively identify active ingredient(s). Currently, gas chromatography - mass spectrometry (GC/MS) is the most commonly used technique for the screening and preliminary identification of forensic exhibits. However, depending on instrument parameters, even short GC

analyses can take five minutes between injections. For a case with 25 samples, this already translates into more than two hours of analysis, not including sample preparation time and data processing. Standard GC and GC/MS analyses usually take considerably longer. Pre-analyses/screening of individual units can also be done with infrared (FTIR) or Raman spectroscopy; however, these techniques are best suited to pure or uncut samples, and are usually not very fast. For these reasons, there is a continuing need for instrumental techniques that can very rapidly analyze large numbers of samples, including samples with multiple components.

A rapid, confirmational screening technique for the analysis of benzodiazepines in drinks using direct electrospray probe / mass spectrometry has been reported [1]. However, although able to identify unknown samples during rapid screening, an extraction procedure was required prior to analysis. Fast analysis of multiple drugs of abuse in urine and hair using liquid chromatography - mass spectrometry techniques have been reported [2,3]. More recently, Cooks *et al.* [4] reported the development of a new, ambient sampling technique, desorption electrospray ionization (DESI). This technique allows for the rapid analyses of samples under ambient conditions and without any type of sample preparation, using mass spectrometry as the detector. Also recently, Cody *et al.* developed another technique for rapid atmospheric sampling using mass spectrometry [5]. Direct analysis in real time (DART) interfaced with time-of-flight mass spectrometry also allows the analysis of samples without the need for sample preparation or extraction procedures. The introduction of these techniques represents a significant step forward in the development of high-throughput analyses. Their application to the analysis of forensic samples could have a great impact on the quantity and quality of data generated by forensic chemists, especially for the rapid confirmatory screening of controlled substances.

During DESI, charged solvent droplets are directed towards the surface of a sample, resulting in the formation of singly and/or multiply charged species similar to those observed under normal electrospray conditions [6,7]. The samples can be analyzed either directly or after deposition onto a non-conducting surface. The interface of the DESI technique with a mass spectrometer (DESI-MS) or a tandem mass spectrometer (DESI-MS/MS) allows for the formal identification of the charged species. It has been proposed that the ion formation process during DESI occurs via at least three mechanisms [4]. The first involves a molecule-pick-up process resulting from the impact of charged solvent droplets onto the sample surface. This mechanism is believed to be responsible for the ESI-like spectra generated during DESI. The second involves a charge and momentum transfer leading to desorption of the sample molecules as ions. Indirect evidence for this mechanism is obtained by the generation of DESI spectra for carotenoid compounds, which otherwise are not ionized using ESI. The third involves the volatilization or desorption of neutral species followed by gas-phase ionization via ion/molecule reactions.

Herein, multiple applications of DESI-MS and DESI-MS/MS for the screening and analysis of various controlled substances are presented. Our previous studies are also summarized here, as they further demonstrate the application of this recently developed technique for forensic analyses [8]. Samples of licit as well as illicit origin were investigated, and the rapid identification of the controlled active ingredient(s) was/were achieved. Analyses were conducted directly on the samples, in most cases with no sample preparation, and were completed in less than 30 seconds per sample.

### ***Experimental***

Experiments were performed using a Thermo Fisher Scientific LCQ Advantage MAX quadrupole ion-trap mass spectrometer equipped with an IonMAX atmospheric pressure ionization source and an ESI probe (San Jose, CA). This system was interfaced to a Thermo Fisher Scientific Surveyor HPLC system. For the DESI experiments, the desorbing solvent was delivered by the built-in syringe pump on the mass spectrometer or by the solvent delivery pump of the HPLC system. These two options allowed for delivery of solvent at flow rates between 2 and 100  $\mu\text{L}$  per minute. Depending on experimental needs, the desorbing solvent was a mixture of methanol, deionized water (0.1 percent formic acid), and/or acetonitrile (0.1 percent formic acid). The solvent was directed to the ESI interface without further modification.

The atmospheric pressure ionization chamber was kept opened to the laboratory atmosphere and the automatic high voltage shut-off of the IonMAX chamber was disabled in order to allow sampling. Once constant ESI solvent, voltage, and current were achieved, direct sampling was performed. As during routine electrospray operation, the droplet desolvation process was aided using Nitrogen (99 percent;  $100 \pm 20$  psi) as both the sheath and auxiliary gas, operated at 50 and 20 units, respectively. The ESI source transfer capillary was maintained at a temperature of  $250^{\circ}\text{C}$ , while the capillary and tube lens were kept at 30 and 15 V, respectively.

Instrument control, data collection and analysis were performed using the Xcalibur software (version 1.4) provided by the instrument manufacturer. Mass spectrometry data were collected in the positive ion mode using either the real-time view provided by the Tune Plus program module or through the Sequence module provided by the software. By setting up the mass analyzer to collect both full-scan and  $\text{MS}^n$  data, molecular weight and structural information was obtained. Collision-induced dissociation experiments were performed after optimization of the collision energy (typically 25-35 percent eV) for the analyte. Helium (99.999 percent;  $40 \pm 10$  psi) was used as both the trapping and collision gas. Under these conditions, the fragmentation data obtained from DESI-generated ions can be directly compared to the laboratory-generated standard spectral library previously developed using ESI-generated ions.

Samples were obtained from seized exhibits submitted to the laboratory for forensic analysis, and were analyzed as received (i.e., with no preparation or derivatization steps prior to the DESI-MS and/or DESI-MS/MS analyses). Sampling was performed by positioning a flat portion of the material in question, using non-conducting (Teflon®) tweezers, slightly below the entrance to the mass spectrometer, within the electrospray plume region (see Photo 1). The samples were positioned to obtain a 45 degree angle (approximately) between the flat surface to be analyzed and the electrospray emitter (optimal sample positioning has been addressed by Cooks *et al.* [4]). Samples were analyzed for 1 to 5 seconds, and sufficient solvent-only collection time (approximately 1 minute) was allowed between samples in order for the signal background to return to low levels.

### **Results and Discussion, I - Previous Studies**

Previously, this laboratory used DESI-MS and DESI-MS/MS for the rapid analyses of various tablets and marijuana [8]. The objectives and results of those studies (summarized below) demonstrated the use of these techniques for pre-analysis/screening purposes.

**Objective 1:** *To demonstrate that the technique can detect the presence of one or more controlled substances in the presence of other major matrix components.* Analyses were conducted on authentic Vicodin® tablets and on illicit Ecstasy-type tablets suspected to contain 3,4-methylenedioxymethamphetamine (MDMA). Each Vicodin® tablet contained 5 mg of hydrocodone bitartrate, 500 mg of acetaminophen, and various tablet binding components [9]. Figure 1 shows the ambient sampling of five of these tablets. The total-ion-current (TIC) (upper) trace shows the real-time sampling of the tablets (completed in less than 6 minutes), with each peak representing a different tablet. The bottom five panels display the full-scan mass spectra for each tablet. The molecular ions at  $m/z$  152 and 325 correspond to protonated acetaminophen (MW = 151 Da) and the sodium-bound dimer of acetaminophen ( $2\text{M}+\text{Na}^+$ )<sup>+</sup>, respectively. The peak at  $m/z$  300 is due to protonated hydrocodone (MW = 299 Da). It is noteworthy that the presence of acetaminophen does not interfere with the detection of hydrocodone, even though the concentration of the latter is 100 times lower. The observed relative intensities of the two components vary from sample to sample due to the variable (non-reproducible) positioning of the tablets within the ESI plume.

Figure 2 shows the ambient sampling of five suspected Ecstasy tablets. The full-scan mass spectral data shows a major component at  $m/z$  194, consistent with MDMA (MW = 193 Da), and additional peaks at  $m/z$  163 and 150. The peak at  $m/z$  163 corresponds to a fragment commonly observed during analysis of the methylenedioxyphenethylamines. The peak at  $m/z$  150 suggests the presence of methamphetamine (MW = 149

Da). Further chemical analysis confirmed the presence of MDMA hydrochloride and methamphetamine hydrochloride at concentrations of 57.0 and 9.5 milligrams/tablet, respectively.

**Objective 2:** *To demonstrate that the methodology is free of interferences and/or cross-contamination (carry-over) from previously tested samples.* Analyses were conducted on authentic prednisone and Vicodin® tablets. The first and third tablets were prednisone (MW = 358 Da), while the second and fourth tablets were Vicodin® (same as above). Figure 3 shows the results from the alternating sampling of the four tablets. The prednisone tablets (left panels) display a molecular ion at  $m/z$  359. The Vicodin® tablets (right panels) again display the molecular ions for acetaminophen and hydrocodone. No interferences or cross-contamination (carry-over) are observed in any of the spectra.

**Objective 3:** *To demonstrate that the methodology can identify controlled substances.* DESI-MS/MS analyses were conducted on 23 authentic Xanax® tablets; each tablet contained 2 milligrams of alprazolam (MW = 308 Da). Figure 4 shows the ambient sampling for four of these tablets. The fragmentation data contains the major fragment ions generated upon dissociation of the  $m/z$  309 ion; comparison with a previously generated library standard confirmed alprazolam. For a total tablet weight of 260 milligrams, this represents detection of the active ingredient at a concentration of 0.7 percent. Of note, the MS/MS data obtained using DESI can be directly compared with the laboratory-generated ESI library using standard MS/MS conditions. Thus, the development of a DESI-MS/MS specific library is not necessary.

**Objective 4:** *To demonstrate that the methodology can analyze plant material.* Cooks and co-workers previously performed DESI-MS analyses on various natural products, including tomatoes and hibiscus flowers [4]. Their experiments confirmed the ability of DESI-MS to detect some of the main components in these substances, regardless of interferences generated by the complicated plant matrices. Figure 5 displays the DESI-MS data from the ambient sampling of three dried marijuana leaves. The spectrum included one major molecular ion at  $m/z$  315 and minor peaks at  $m/z$  311, 327, 341, and 359. The peak at 311 corresponds to protonated cannabinol (MW = 310 Da), while the peak at  $m/z$  315 is due to *delta*-9-tetrahydrocannabinol (THC; MW = 314 Da). The peaks at  $m/z$  341 and 359 are probably due to other minor cannabinoids, likely including the carboxylic acid of THC (MW = 358 Da). The identification of THC was confirmed by performing MS/MS experiments, which matched the previously generated library standard.

## ***Results and Discussion, II - Recent Applications***

Additional forensic applications of DESI-MS and DESI-MS/MS continue to be developed at this laboratory. The most recent applications (summarized below) include analyses of counterfeit pharmaceuticals, “medical” marijuana exhibits, *gamma*-butyrolactone (GBL) and *gamma*-hydroxybutyrate (GHB), and disguised opium formulations.

*Counterfeit Pharmaceuticals* - Counterfeit tablets and liquids are commonly submitted to this laboratory, usually pursuant to diversion investigations targeting the commercial black market. For example, the laboratory has recently received multiple exhibits of white tablets suspected to be counterfeit Viagra® (see Figure 6). These tablets all bore the characteristic logos; however, legitimate tablets are blue and contain sildenafil citrate. Analysis by DESI-MS and DESI-MS/MS confirmed sildenafil (MW = 474 Da); however, citrate (MW = 190) was not present (see Figure 6, lower panel), confirming that the tablets were counterfeits.

In another recent case, the laboratory received 9,463 round, concave, green tablets bearing “OC” and “80” inscriptions, suspected to be counterfeit or mimic Oxycontin® (see Figure 7). Again, the tablets all bore the correct logos; however, the tablets were slightly smaller than the legitimate product, and also were green throughout (the legitimate product is a compressed white powder with a colored coating). Analysis of nearly ten thousand tablets would be a daunting task for any forensic laboratory; however, the DEA evidence sampling plan allows for preliminary analysis of 29 randomly selected tablets, and if the results are consistent in all 29 tablets, formulation of a final composite for full characterization and purity determination [10]. Even screening of 29

tablets would be a tasking usually requiring many hours; however, the DESI-MS/MS analyses were completed in less than 20 minutes. The tablets actually contained very low levels of fentanyl (MW = 336 Da), not oxycodone (MW = 315 Da), confirming that they were mimics.

*“Medical” Marijuana* - The recent seizures of “medical” marijuana concoctions at dispensaries in California have resulted in numerous submissions of previously unseen materials suspected to contain THC. Analysis of such exhibits can be challenging, due to their wide variety and often highly complex matrices (typically foods and candies). One such case included 14 multiple colored flat squares described as “THC Breath Mints” (see Figure 8). DESI-MS/MS confirmed THC in all 14 samples, in less than 10 minutes.

*Opium* - Forensic laboratories occasionally receive controlled substances concealed inside food items, typically candy bars. A recent such case included 15 chocolate-covered opium bars (see Figure 9). In this case, DESI-MS analysis of the surface of the bars (i.e., the chocolate) would not indicate any controlled substance. However, analysis of a shaved piece of the suspected opium confirmed the five primary opium alkaloids: Morphine (MW = 285), codeine (MW = 299), thebaine (MW = 311), papaverine (MW = 339), and noscapine (MW = 413). The lower panel in Figure 9 shows the mass spectrum for bar #13, displaying the expected five protonated ions. The analysis of all 15 samples was completed in less than 6 minutes.

*GHB/GBL Mixtures* - DESI/MS analyses can also be conducted on liquids. A recent such submission consisted of a clear liquid suspected to contain the “date-rape” drug *gamma*-hydroxybutyrate (GHB; MW = 104 Da). Analysis was accomplished by placing a drop of the sample onto a glass slide and positioning it within the ESI plume, giving four major ions at  $m/z$  87, 105, 173, and 191. The smaller ions are indicative of protonated GBL (MW = 86 Da) and GHB, respectively, while the two larger ions at  $m/z$  173 and 191 correspond to the GBL homo-dimer [(2GBL+H<sup>+</sup>)<sup>+</sup>] and the hetero-dimer [(GBL+GHB+H<sup>+</sup>)<sup>+</sup>], respectively (see Figure 10).

## Conclusions

DESI-MS and DESI-MS/MS experiments are ideal for the rapid screening of multiple-unit exhibits. The technique provides reproducible data with a high degree of sensitivity. DESI-MS experiments will provide molecular weight information and therefore a presumptive or preliminary identification (this will need to be confirmed with a second technique like GC/MS, FTIR, or NMR). DESI-MS/MS further provides structural information via fragmentation data that can be directly compared with standard reference spectra. Analyses are accomplished in less than 30 seconds per sample, without sample preparation or extraction procedures.

## Acknowledgments

The author thanks Forensic Chemists Jason A. Bordelon, Michael M. Brousseau, and Alan M. Randa (all at this laboratory) for contributions with DESI-MS data collection, and Supervisory Forensic Chemist Esther W. Chege (this laboratory) for review of the manuscript.

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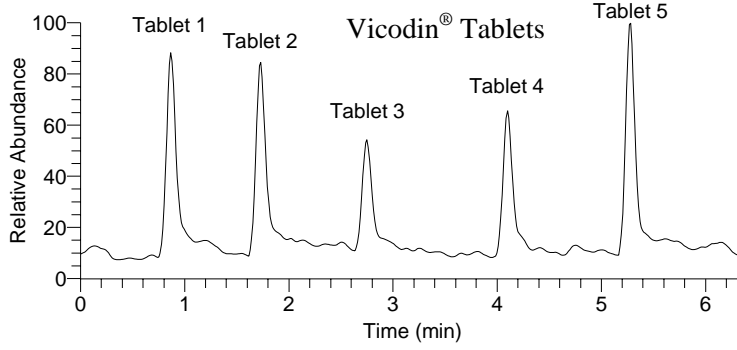
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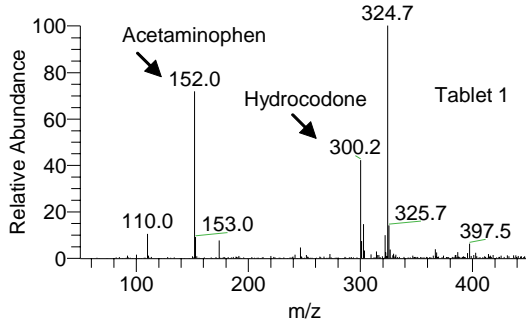
**Photo 1.** Sampling of a Tablet for DESI Analysis.

RT: 0.00 - 6.38 SM: 15G

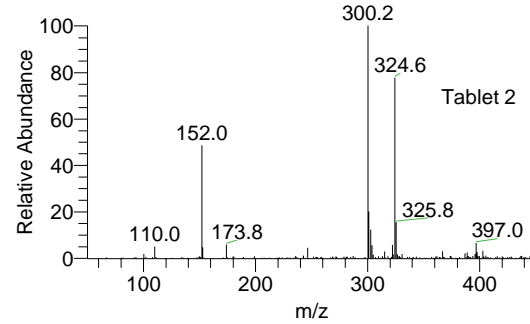


NL: 1.83E6  
Base Peak m/z=  
50.0-600.0 F: + c  
ESI Full ms [  
50.00-600.00] MS  
Apr252005\_009

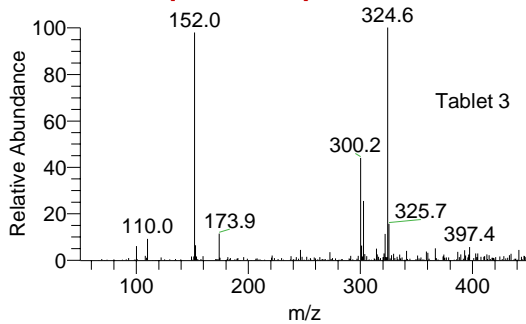
Apr252005\_009 #64-76 RT: 0.80-0.95 AV: 13 SB: 53  
F: + c ESI Full ms [ 50.00-600.00]



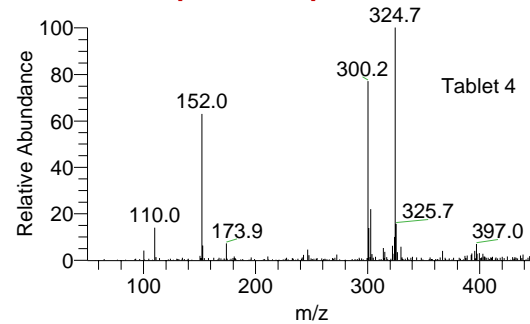
Apr252005\_009 #132-143 RT: 1.67-1.81 AV: 12 SB: 51  
F: + c ESI Full ms [ 50.00-600.00]



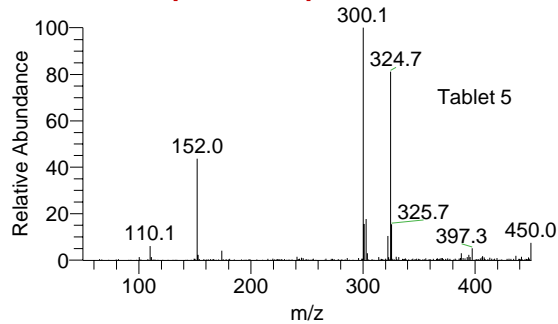
Apr252005\_009 #213-223 RT: 2.70-2.83 AV: 11 SB: 52  
F: + c ESI Full ms [ 50.00-600.00]



Apr252005\_009 #318-329 RT: 4.04-4.18 AV: 12 SB: 58  
F: + c ESI Full ms [ 50.00-600.00]



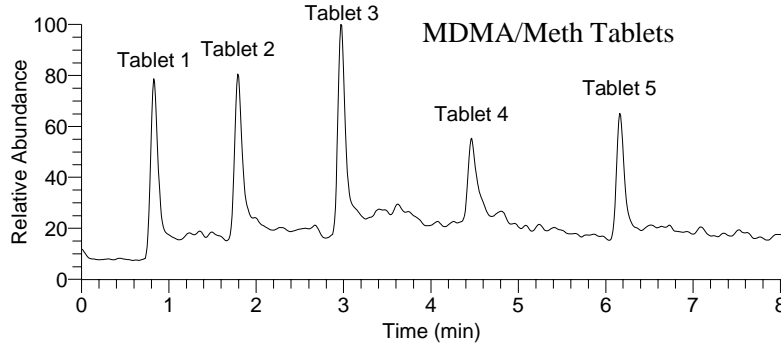
Apr252005\_009 #410-419 RT: 5.21-5.32 AV: 10 SB: 54  
F: + c ESI Full ms [ 50.00-600.00]



**Figure 1.** Rapid Sampling of Vicodin® Tablets using DESI-MS.

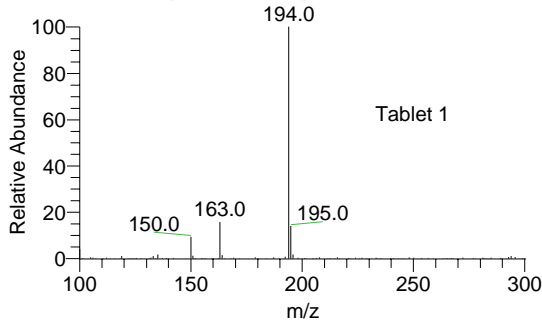


RT: 0.00 - 8.01 SM: 15G

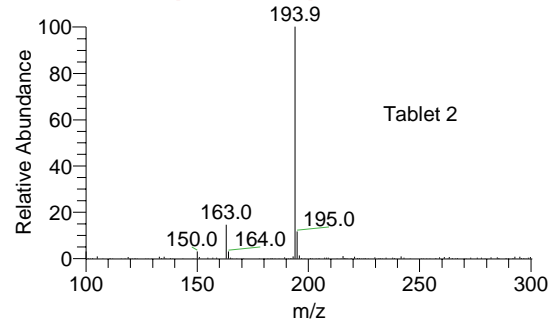


NL:  
5.35E6  
Base Peak F: + c  
ESI Full ms [ 50.00-400.00]  
MS  
Apr212005\_005

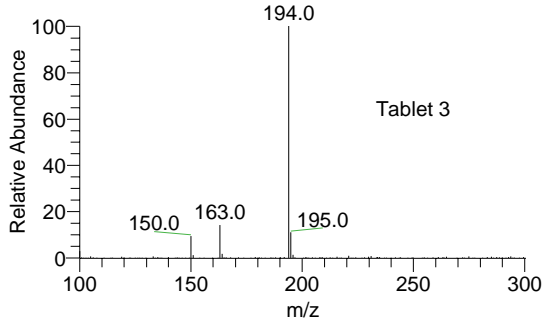
Apr212005\_005 #71-83 RT: 0.77-0.90 AV: 13 SB: 47  
F: + c ESI Full ms [ 50.00-400.00]



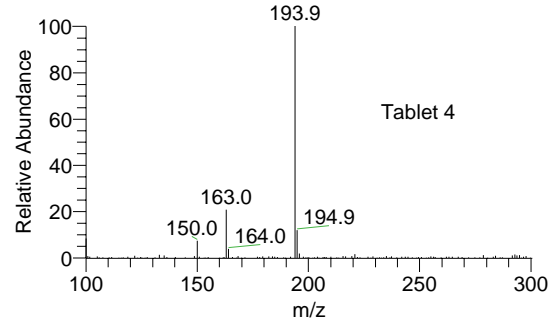
Apr212005\_005 #158-171 RT: 1.72-1.86 AV: 14 SB: 53  
F: + c ESI Full ms [ 50.00-400.00]



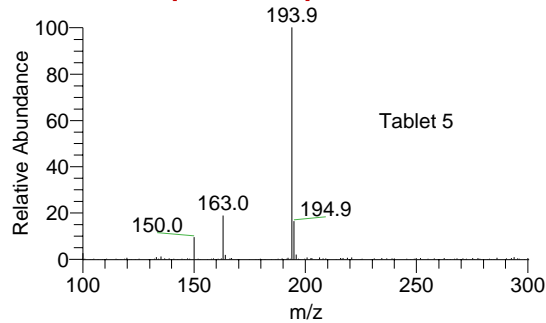
Apr212005\_005 #267-279 RT: 2.91-3.05 AV: 13 SB: 58  
F: + c ESI Full ms [ 50.00-400.00]



Apr212005\_005 #404-416 RT: 4.41-4.54 AV: 13 SB: 48  
F: + c ESI Full ms [ 50.00-400.00]

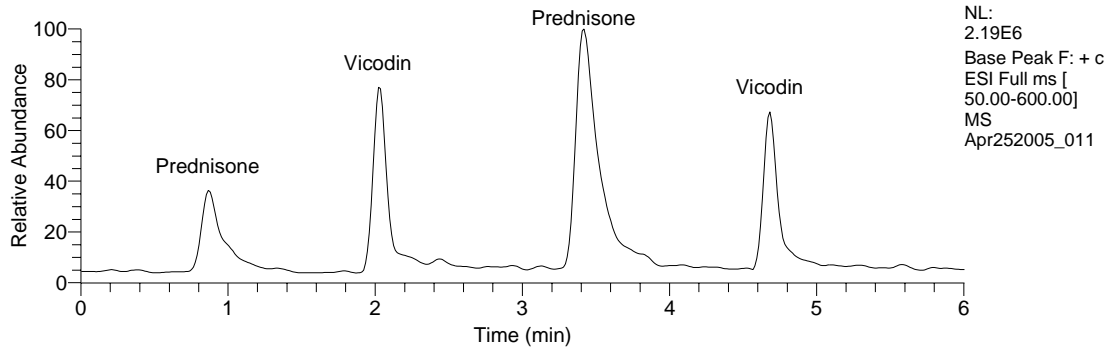


Apr212005\_005 #559-571 RT: 6.11-6.24 AV: 13 SB: 52  
F: + c ESI Full ms [ 50.00-400.00]

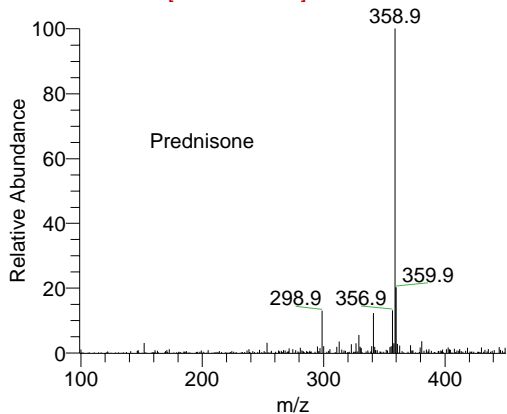


**Figure 2.** Rapid Sampling of MDMA/Methamphetamine Tablets using DESI-MS.

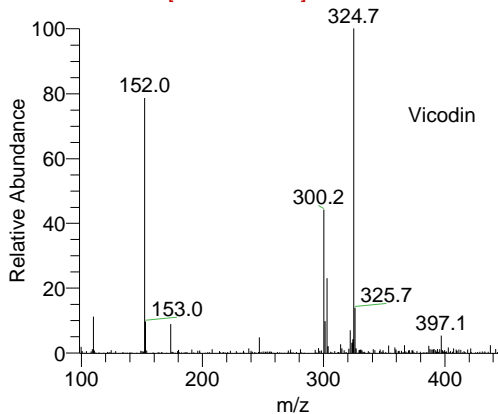
RT: 0.00 - 6.00 SM: 15G



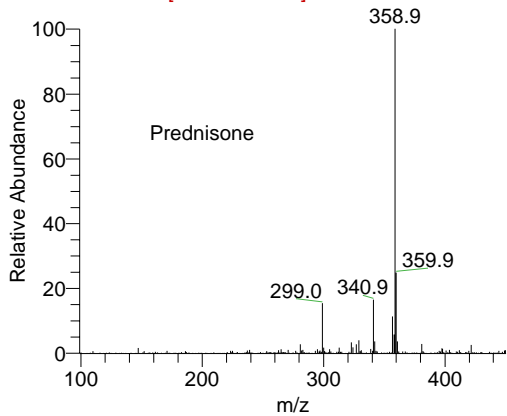
Apr252005\_011 #64-77 RT: 0.80-0.97 AV: 14 SB: 50  
F: + c ESI Full ms [ 50.00-600.00]



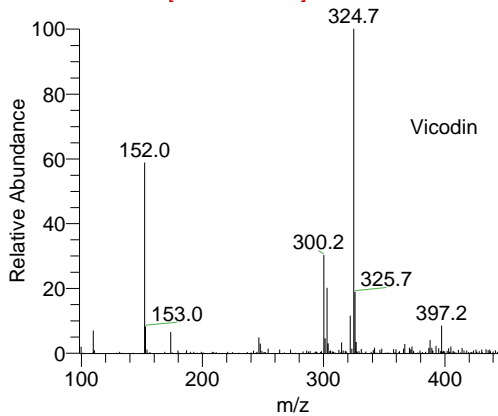
Apr252005\_011 #156-166 RT: 1.97-2.10 AV: 11 SB:  
F: + c ESI Full ms [ 50.00-600.00]



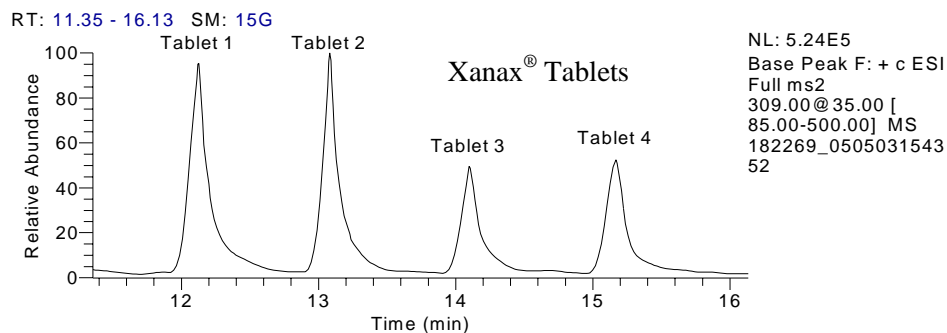
Apr252005\_011 #265-278 RT: 3.36-3.52 AV: 14 SB:  
F: + c ESI Full ms [ 50.00-600.00]



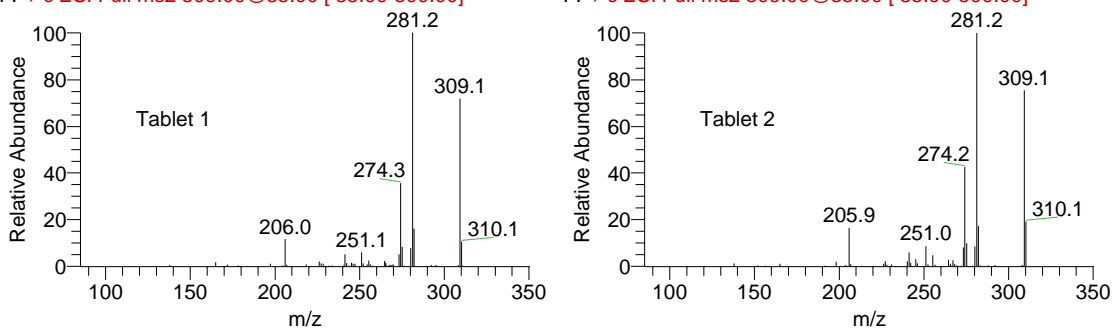
Apr252005\_011 #364-374 RT: 4.62-4.75 AV: 11 SB:  
F: + c ESI Full ms [ 50.00-600.00]



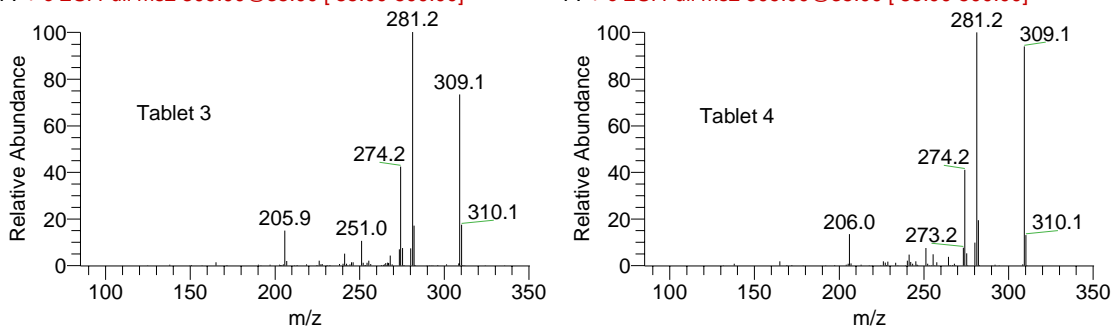
**Figure 3.** Rapid Alternating Sampling of Prednisone and Vicodin® Tablets using DESI-MS.



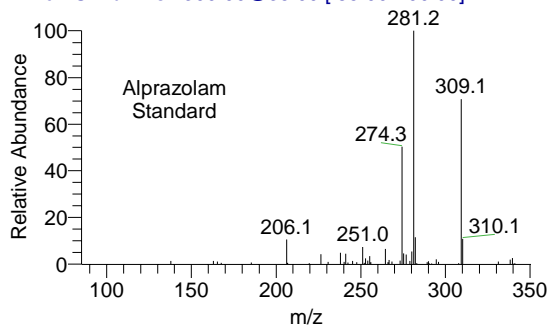
182269\_050503154352 #589-601 RT: 12.03-12.20 AV: 13182269\_050503154352 #641-654 RT: 13.01-13.19 AV: 14  
 F: + c ESI Full ms2 309.00@35.00 [ 85.00-500.00] F: + c ESI Full ms2 309.00@35.00 [ 85.00-500.00]



182269\_050503154352 #694-706 RT: 14.01-14.18 AV: 13182269\_050503154352 #751-755 RT: 15.13-15.19 AV: 5  
 F: + c ESI Full ms2 309.00@35.00 [ 85.00-500.00] F: + c ESI Full ms2 309.00@35.00 [ 85.00-500.00]

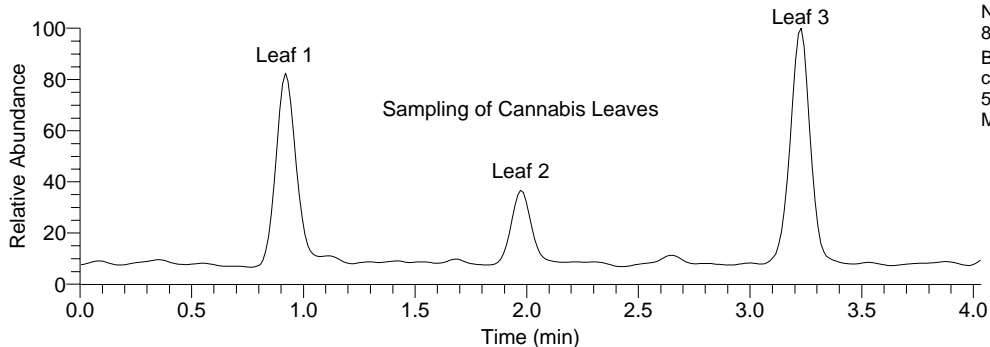


Alprazolam\_MSMS35 #59-66 RT: 1.22-1.36 AV: 8 NL:  
 T: + c ESI Full ms2 309.00@35.00 [ 85.00-400.00]



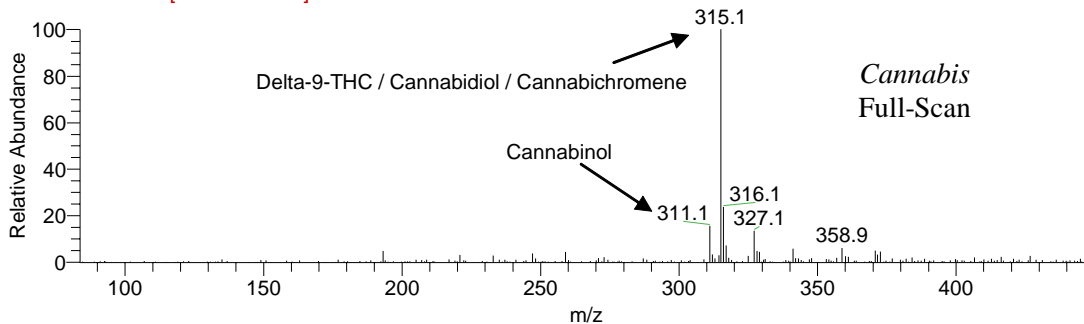
**Figure 4.** Fragmentation Spectra ( $m/z$  309) Obtained During the DESI-MS/MS Sampling of 4 Xanax® Tablets. Also shown is the MS/MS Spectrum of the Alprazolam Standard.

RT: 0.00 - 4.03 SM: 15G

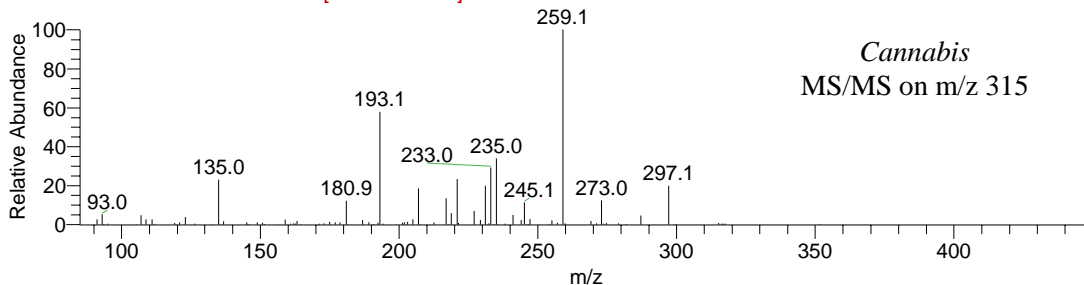


NL:  
8.72E5  
Base Peak F: +  
c ESI Full ms [  
50.00-600.00]  
MS Cannabis4

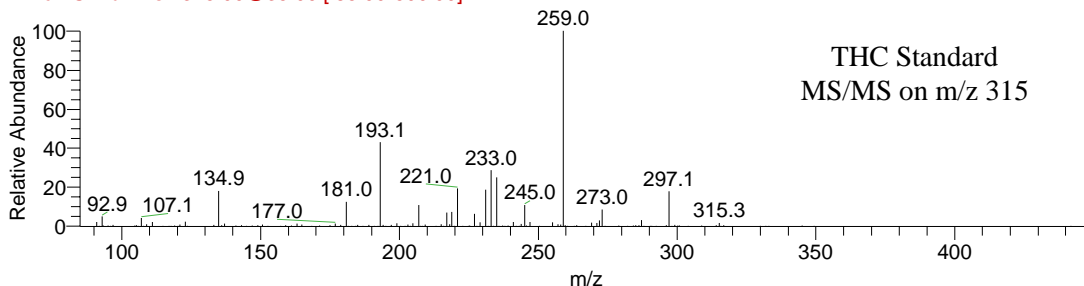
Cannabis4 #252-263 RT: 3.15-3.29 AV: 12 SB: 45 2.84-3.04, 3.38-3.72 NL: 5.60E5  
F: + c ESI Full ms [ 50.00-600.00]



Cannabis4 #421-432 RT: 6.12-6.31 AV: 12 NL: 7.57E4  
F: + c ESI Full ms2 315.00@35.00 [ 85.00-600.00]



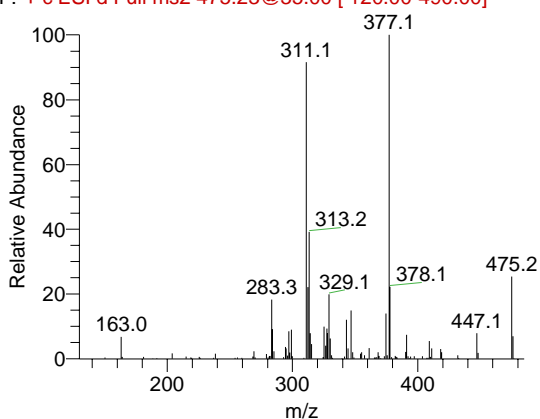
Delta9THC #746-769 RT: 11.51-11.83 AV: 24 SB: 32 7.33-8.00 NL: 1.43E5  
F: + c ESI Full ms2 315.00@35.00 [ 85.00-600.00]



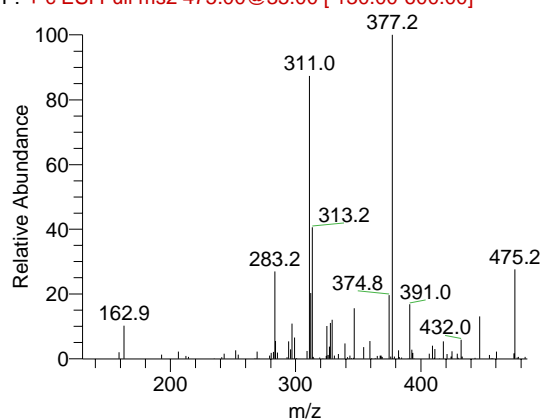
**Figure 5.** DESI-MS and DESI-MS/MS Analysis of Cannabis Leaves. Also Shown is the MS/MS Spectrum for the THC Standard.



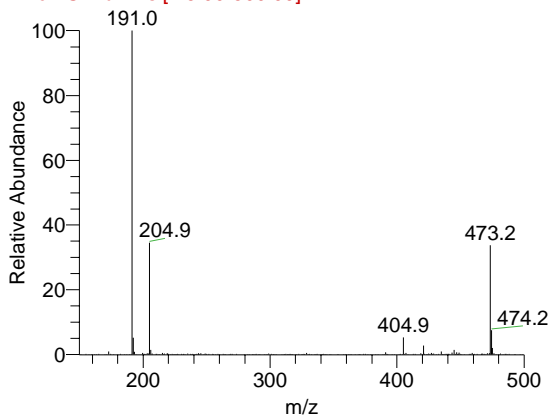
Sildenafil\_MSMS35 #29-56 RT: 0.32-0.53 AV: 14 SB: 4  
 F: + c ESI d Full ms2 475.23@35.00 [ 120.00-490.00]



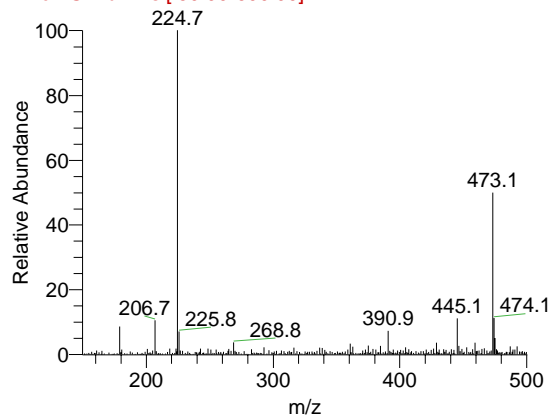
186304\_010 #470-484 RT: 6.91-7.04 AV: 15 NL: 3.35E5  
 F: + c ESI Full ms2 475.00@35.00 [ 130.00-600.00]



Viagra\_Neg #153-169 RT: 1.71-1.89 AV: 17 NL: 7.06E5  
 F: - c ESI Full ms [ 75.00-500.00]



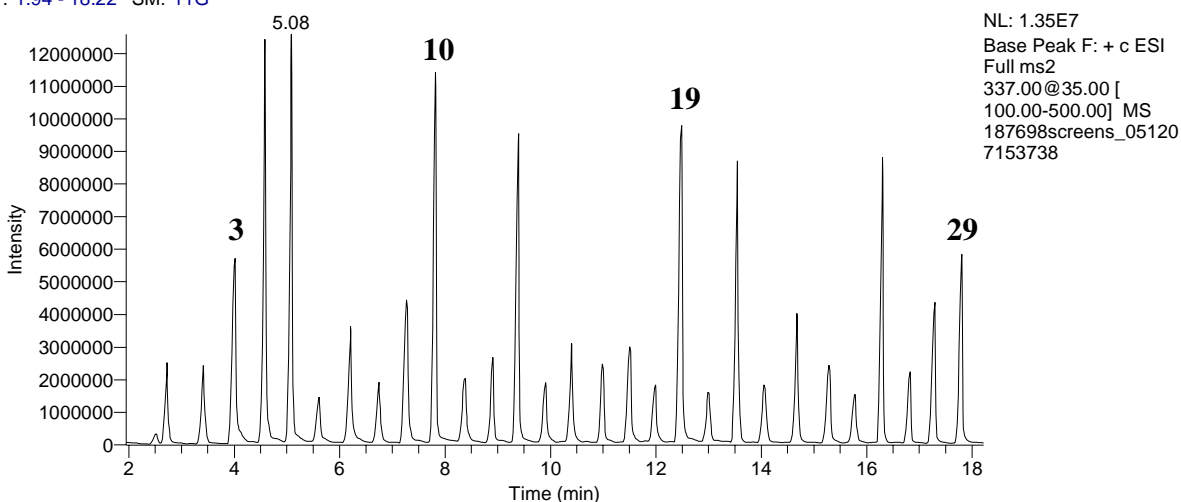
186304\_008 #543-552 RT: 6.85-6.97 AV: 10 NL: 5.09E5  
 F: - c ESI Full ms [ 50.00-600.00]



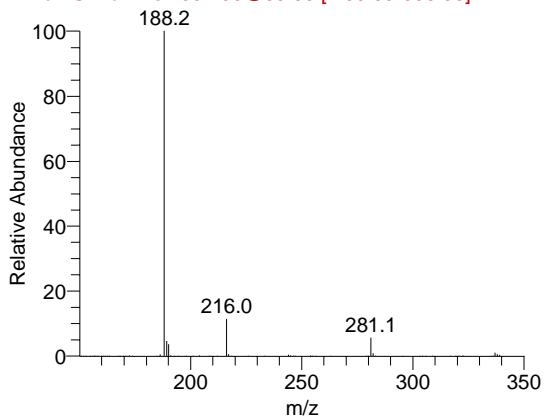
**Figure 6.** Upper Panel: Counterfeit Viagra® Tablets. Middle Panel: DESI-MS/MS Data (Fragmentation of  $m/z$  475) in the Positive Ion Mode for Legitimate (Left) and Counterfeit (Right) Tablets. Lower Panel: DESI-MS Data (Full Scan) in the Negative Ion Mode for Legitimate (Left) and Counterfeit (Right) Tablets.



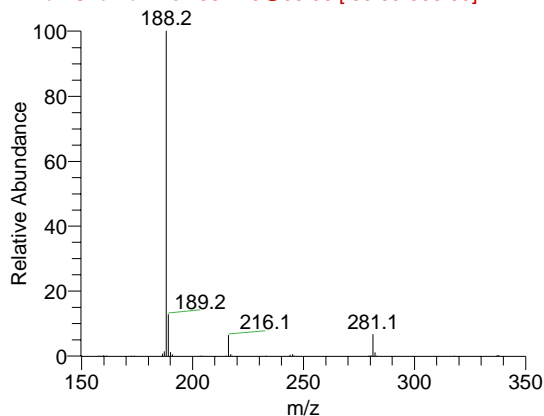
RT: 1.94 - 18.22 SM: 11G



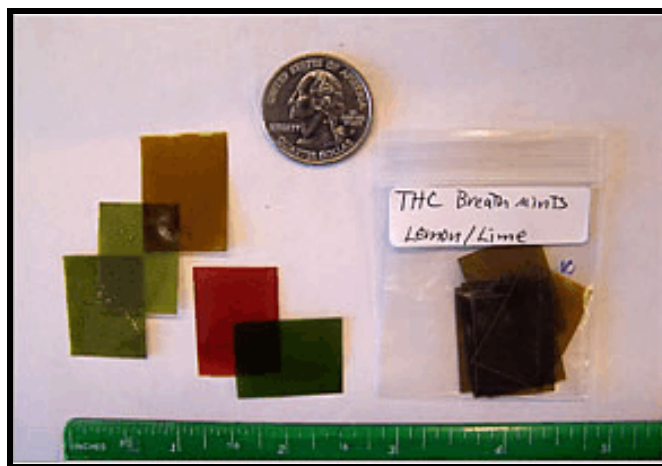
187698screens\_051207153738 #212-223 RT: 3.94-4.06  
F: + c ESI Full ms2 337.00@35.00 [ 100.00-500.00]



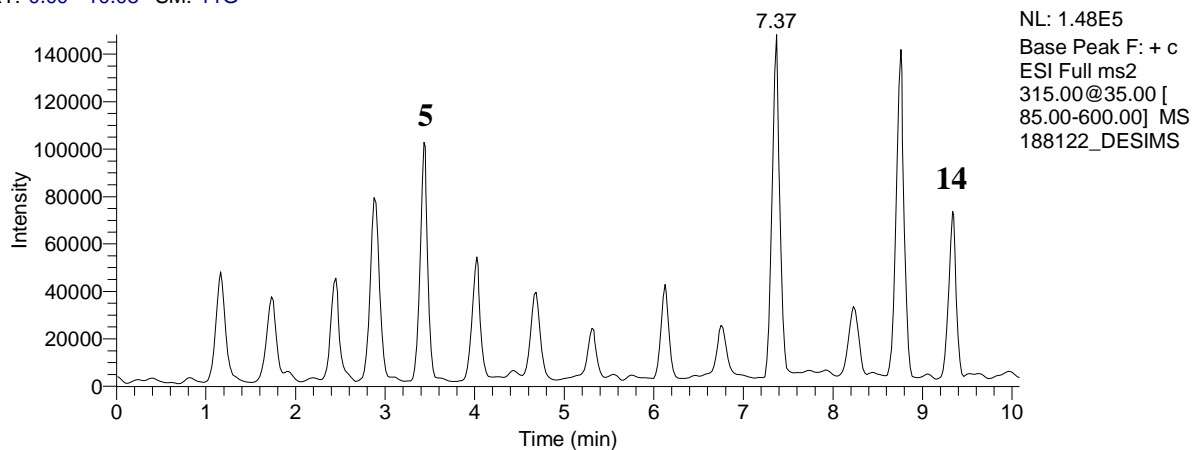
FentanylCitrate\_MSMS #28-52 RT: 0.34-0.57 AV: 12 NL:  
F: + c ESI d Full ms2 337.26@35.00 [ 80.00-350.00]



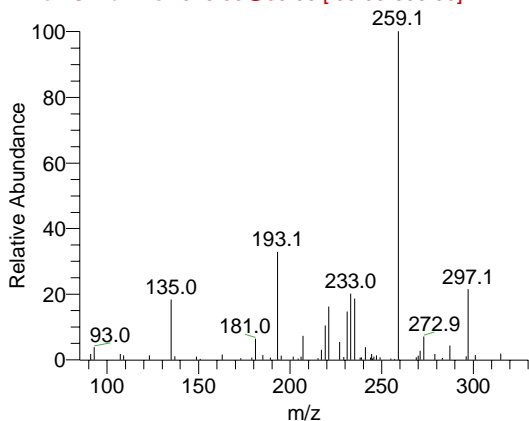
**Figure 7.** Upper Panel: Counterfeit Oxycontin® Tablets. Middle Panel: DESI-MS Sampling of 29 Unknown Tablets. Lower Panel: DESI-MS/MS Data (Fragmentation of  $m/z$  337) for Unknown Tablet #3 (Left) and Fentanyl Standard (Right).



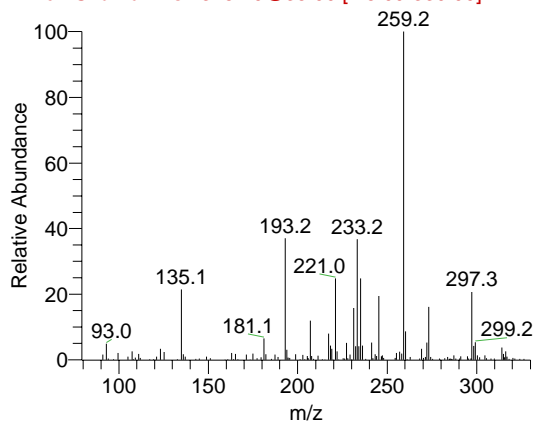
RT: 0.00 - 10.08 SM: 11G



188122\_DESIMS #220-227 RT: 4.60-4.75 AV: 8 NL:  
F: + c ESI Full ms2 315.00@35.00 [ 85.00-600.00]



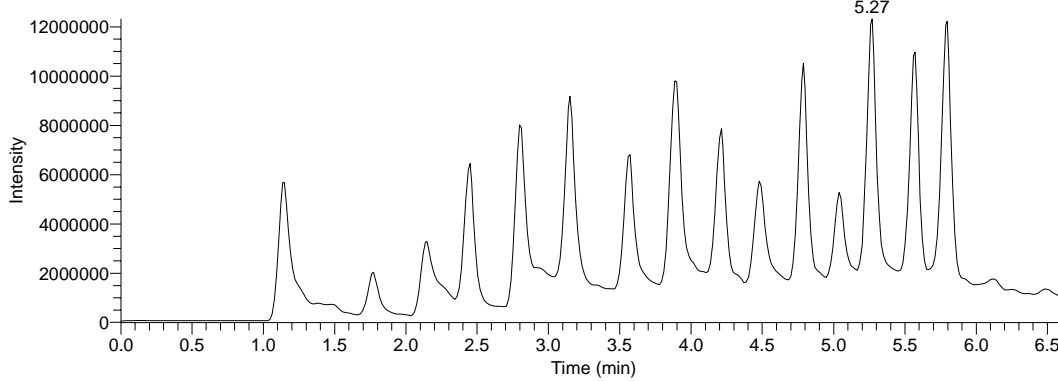
D9THC\_01 #31-52 RT: 0.37-0.57 AV: 9 NL: 3.70E5  
F: + c ESI d Full ms2 315.40@35.00 [ 75.00-330.00]



**Figure 8.** Upper Panel: “Medical” Marijuana Breath Mints. Middle Panel: DESI-MS/MS Sampling of 14 Breath Mints. Lower Panel: DESI-MS/MS Data (Fragmentation of  $m/z$  315) for Breath Mint #7 (Left) and MS/MS Spectrum for the THC Standard (right).

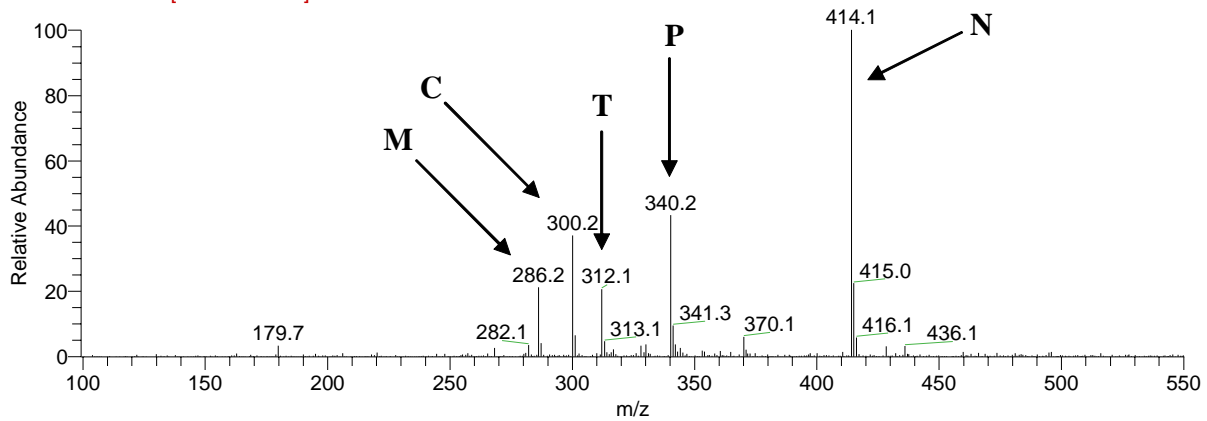


RT: 0.00 - 6.63 SM: 11G



NL:  
1.23E7  
Base Peak F: +  
c ESI Full ms [  
50.00-600.00]  
MS  
196870\_screen

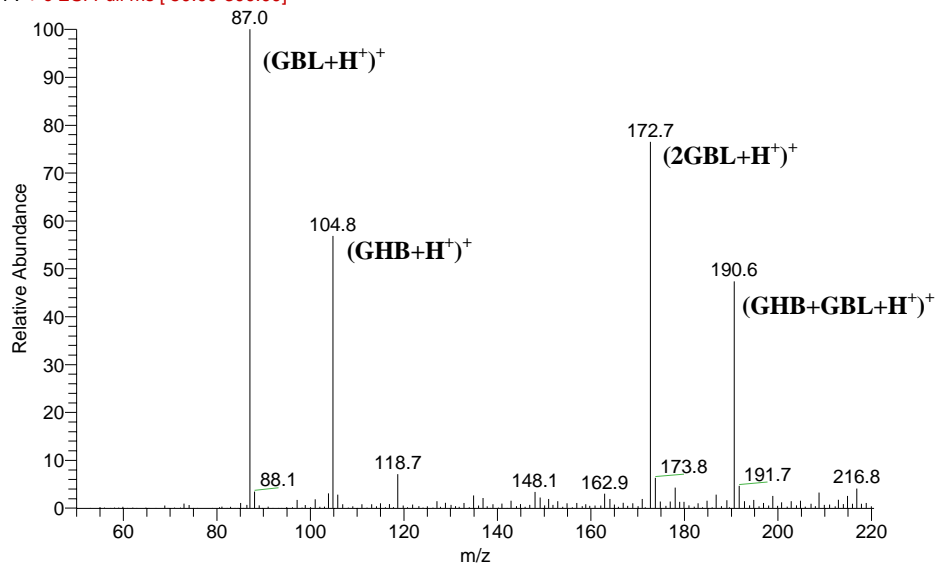
196870\_screen #349-355 RT: 5.23-5.31 AV: 7 NL: 1.06E7  
F: + c ESI Full ms [ 50.00-600.00]



**Figure 9.** Upper Panel: Chocolate-Covered Opium Bars. Middle Panel: DESI-MS Sampling of 15 Opium Bars. Lower Panel: DESI-MS Spectrum of Bar #13 Containing the Expected Molecular Ions for the Five Main Opium Alkaloids (Morphine, Codeine, Thebaine, Papaverine, and Noscapine).



192920\_06 #2-11 RT: 0.02-0.11 AV: 10 NL: 3.30E6  
F: + c ESI Full ms [ 50.00-300.00]



**Figure 10.** DESI-MS Spectrum Obtained from Analysis of Clear Liquid Found to Contain *gamma*-Butyrolactone (GBL; MW = 86) and *gamma*-Hydroxybutyric Acid (GHB; MW = 104).

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\* \* \* \* \*

# Discovery of an Interesting Temperature Effect on the Sensitivity of the Cobalt Thiocyanate Test for Cocaine

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Troop E Laboratory  
122 South Ellis Street  
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**ABSTRACT:** During investigation of the mechanism and specificity of the Scott's (cobalt(II) thiocyanate) test for cocaine, it was discovered that the ambient temperature affected the equilibrium between the pink (negative) and the blue (positive) test results. At 4°C (~39°F) the sensitivity of the test was doubled versus room temperature (22°C (~72°F)), while temperatures in excess of 40°C (~104°F) decreased the sensitivity of the test more than twofold versus room temperature. These findings can impact the storage, use, and interpretation of commercially available cocaine test kits in typical field settings that are experiencing very cold or (especially) very hot ambient temperatures. A number of recommendations are offered to minimize the effects of hot temperatures on the test kits.

**KEYWORDS:** Cocaine, Scott's Test, Cobalt Thiocyanate, Presumptive Test, Color Test, Sensitivity, Temperature, Forensic Chemistry.

## *Introduction*

The use of presumptive color tests in forensic and analytical laboratories to screen drug submissions is common [1]. Because of their ease of use and interpretation, a number of presumptive color tests for commonly submitted drugs have been incorporated into portable test kits for use by law enforcement personnel in field settings.<sup>2</sup> These test kits are mass-produced by a number of commercial manufacturers, and typically consist of one or more small ampoules of reagents in self-contained pouches that are reasonably priced, convenient, and safe to use. The results are easily interpreted, and the used kits are easily disposed of in accordance with hazardous waste statutes. Typically, a suspected controlled substance is placed into a tube or a pouch prior to breaking a glass ampoule containing a solution of a test reagent, agitating the mixture, and observing the results (usually an obvious color change). In more complex kits, a series of ampoules is broken in sequence, and the intermediate results at each

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<sup>1</sup> Current Address: Custom Sensors and Technology, 531 Axminister Drive, Fenton, MO 63026.

<sup>2</sup> Most such kits are based on chemical tests; more recently, a number of kits based on immunoassay testing have been produced (the latter are not further addressed in this study).

step dictate whether to continue to completion. Virtually all such kits come with instructions and color charts that show the expected color(s) for positive test results, and law enforcement personnel are well trained in their use.

A positive test result is considered to be a presumptive identification for the controlled substance that was being tested for, and it would be submitted to the laboratory as such. In addition to being a preliminary identification for laboratory analysis, a positive field test is also valuable as probable cause for an arrest, a further search incident to an arrest, and/or a search warrant. Furthermore, positive field tests ease the pressure on the judicial system, as defendants very commonly plead out during preliminary hearings when faced with presumptive identifications.

However, because of the wide variety of illicit drugs with similar appearances, virtually any suspect material would almost certainly be submitted (as an unknown/suspected controlled substance) even if the field test gave a negative or inconclusive result - especially if the appearance or packaging of the material, or the circumstances of the seizure, suggested that it was a controlled substance. However, a negative or inconclusive field test would likely result in a rush analysis request to the laboratory (especially likely if suspects were being detained pending the results), which is disruptive to laboratory operations. In addition, negative/inconclusive tests can encourage guilty defendants ("guilty" in this context meaning they are fully aware of the actual identity of the suspect material) to vigorously contest judicial proceedings until the results of analysis are returned from the laboratory. Thus, reliable and accurate tests are critical.

### *Cobalt Thiocyanate Test for Cocaine*

The cobalt thiocyanate test for cocaine was first introduced by Young in 1931 [2]. The original test employed a two percent aqueous solution of cobalt(II) thiocyanate and tin(II) chloride in an aqueous hydrochloric acid solution. A positive test displays a blue color, while a negative test remains pink (i.e., the color of the test reagent). Even from its introduction, Young and others recognized that this test, while useful for rapid presumptive testing of cocaine, was not specific [3]; i.e., various other compounds gave "positive" results when subjected to this test. A number of variations of the test have subsequently been reported, virtually all focused on improving its specificity and/or sensitivity. Accounts of the evolution of the various versions of this test, with their relative advantages and disadvantages, are well-documented in the literature [4].

The most commonly employed current incarnation of this test, known as the Scott's test [4e], employs a three-stage sequence: 1) A 1:1 water/glycerine solution of cobalt thiocyanate is added to the suspect substance (resulting in a blue precipitate and a blue solution); 2) Concentrated hydrochloric acid is added (the blue precipitate dissolves and the liquid turns pink); and 3) Chloroform is added (the upper (aqueous) layer remains pink, while the lower (chloroform) layer turns blue). A positive result at each stage is required in order to qualify as a positive test for cocaine. This sequence is not only more specific for cocaine, but can also detect both cocaine base and cocaine hydrochloride. However, despite its significantly improved specificity versus the original (Young) test, the Scott's test is still subject to false positive and false negative results, which have inspired continuing modifications and alternative tests. Nonetheless, its convenience and utility as a presumptive test in the hands of trained personnel have made it a mainstay in the arsenal of qualitative forensic reagents, and it is the basis for most (if not all) chemistry-based field test kits for cocaine.

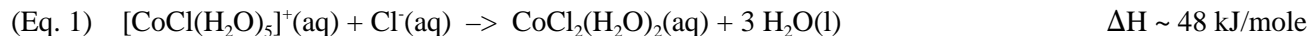
Not surprisingly, the Scott's test is one of the most frequently performed field tests. As noted above, cocaine test kits that are based on the Scott's test are commercially produced by several different manufacturers; however, all are similar in their design and use. Typically, a qualitatively prescribed amount of the suspected cocaine is placed inside a thick transparent plastic pouch containing three secured ampoules, and the pouch is sealed. The first ampoule, containing the cobalt thiocyanate solution, is broken, the mixture is agitated, and the color and precipitate (if any) are noted. The second ampoule, containing the hydrochloric acid solution, is broken, the mixture is further agitated, and the color is again noted. Finally, the third ampoule, containing chloroform, is broken, again with agitation and observation of the colors in the two layers [5]. A positive result at each of the three stages is considered to be a presumptive identification of cocaine. Anything less than a positive result at any stage is considered to be inconclusive or negative.

However, a positive test does not confirm cocaine, and a negative test does not mean that cocaine is not present. As noted above, a number of other compounds give a “positive” test result (thus giving what is typically referred to as a “false positive”). Still other compounds can interfere with a positive result (giving a “false negative”). In addition, the test is sensitive to the quantity of test material used - both insufficient and excessive quantities of cocaine are documented to produce false negatives [4e]. The potential for chemical interference(s), and the sensitivity of the test to the quantity of cocaine present, are especially important considering the fact that illicit cocaine is almost always cut with other substances at the retail and wholesale levels, and (increasingly) even at the production level (currently (2008), it is common for cocaine kilogram bricks produced in South America to be adulterated with small to moderate percentages of diltiazem, hydroxyzine, or levamisole [6]). Adulterants and diluents can not only interfere with the test, but also decrease the actual amount of cocaine placed in the test kit.

### *Mechanistic Studies of the Cobalt Thiocyanate Test*

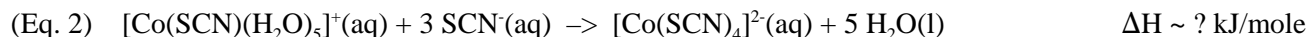
Efforts to better understand the cobalt thiocyanate test and its limitations have been aimed at two primary areas of study: 1) Elucidation of the mechanism of the test [7]; and 2) Empirical documentation of compounds giving false positive or interfering tests. While an abundance of useful information exists in the forensic literature for each of these two areas of study, a rigorous explanation of the mechanism of the test remains elusive.

As noted above, a positive test displays a blue color in Step 1, while a negative test retains the pink color of the test reagent. Although various hypotheses have been published, the exact structures of the blue and pink complexes are unknown. It is known that octahedral cobalt(II) complexes, such as  $[\text{Co}(\text{H}_2\text{O})_6]^{2+}$ , are typically pink, whereas tetrahedral cobalt(II) complexes like  $[\text{CoCl}_4]^{2-}$  are typically an intense blue [8]. Ligand field theory explains these phenomena quite well. Critically, these changes in geometry are reversible and are often accompanied by enthalpy changes. For example, the equilibrium of octahedral and tetrahedral cobalt complex ions in the presence of chloride anion may be expressed as follows [9]:



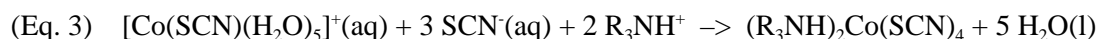
Since the forward reaction is endothermic (i.e., it requires heat as a reactant), raising the temperature favors the formation of the blue tetrahedral complex, while lowering the temperature favors the formation of the pink octahedral complex. This phenomenon is an excellent demonstration of Le Chatelier’s principle [10].

A reasonable inference would be that a similar change in cobalt geometry occurs in the Scott’s test. The basic equation for this analogous equilibrium (i.e., substituting thiocyanate for chloride) can be expressed as follows:



However, in this case the exact structure of the pink octahedral and the blue tetrahedral species are unknown, and it is likely that gradual replacement of aqua with thiocyanato ligands gives a range of pink and blue colored intermediate species.

In the absence of cocaine, the equilibrium would be expected to lie to the left in an aqueous solution (where the excess water competes for the coordination sites on the cobalt). However, if cocaine is present, the substitution of two bulky, relatively hydrophobic protonated cocaine cations in the coordination sphere lends stability to the complex, rendering it more soluble in organic solvents (such as chloroform), favoring the right side of the equilibrium. That is, the cocaine serves to partly or fully exclude water from the coordination sphere, causing the equilibrium to shift to the right. Thus, the blue coordination compound responsible for a positive Scott’s test might be  $(\text{R}_3\text{NH})_2\text{Co}(\text{SCN})_4$ , where  $\text{R}_3\text{NH}^+$  represents the protonated cocaine molecule [7], as follows:



Surprisingly, no studies examining the effect(s) of temperature on this equilibrium have been reported in the forensic literature. Le Chatelier's principle predicts that applying thermal stresses to the equilibrium system will affect its position, favoring either the reactants or the products, and thereby altering the sensitivity of the test. Thus far, published studies of the sensitivity and specificity of the Scott's test have focused on the presence of interfering analytes giving false positive or false negative results, on substituting different acids and organic solvents in the test, or on the quantities of cocaine used in the test kits [4], but none have considered temperature.

Furthermore, the various manufacturers of the test kits do not specifically address this issue in their product literature. One of the test kit manufacturers does state (in a newsletter) that "a cold test will simply show the color reactions slower than a room temperature test" [11]. However, the same newsletter also states that "[f]ield tests can be stored without concern in any container (desks, briefcases, cabinets, glove compartments, or vehicle trunks)." In view of the thermodynamics of the cobalt complexes, these assertions appear dubious.

Test kits are typically stored in vehicles prior to use, where (depending on locale) temperatures can fall below freezing during winter months and can exceed 140°F (60°C) during summer months. Two critical questions are: 1) Do the temperatures commonly achieved inside the cabin or trunk of a vehicle during winter or summer months significantly affect the sensitivity of the Scott's test?; and 2) Can temperature-controlled storage be used to enhance the sensitivity of the Scott's test?

### ***Experimental***

*[Editor's Notes: Because publication in Microgram Journal could be interpreted as an endorsement or a counter-endorsement by the U.S. Drug Enforcement Administration, the names of the test-kit manufacturers and the names of their test kits have been redacted from this article. The results apply equally to virtually any Scott's test-based test kit.]*

Cobalt(II) thiocyanate was purchased from Sigma-Aldrich. Concentrated hydrochloric acid and chloroform were purchased from Fisher Scientific. Reference standard cocaine was purchased from Sigma-Aldrich and was stored and used in the drug chemistry section of the Missouri State Highway Patrol Troop E Satellite Laboratory. Cocaine test kits used for this study were purchased from a well known manufacturer of narcotics field test kits, and were stated to be applicable for testing both cocaine salts and cocaine base. The provided instructions for use were followed, including a printed qualitative sample size indicator. Reduced temperature studies were conducted using a standard portable cooler with ice as the cooling agent. Elevated temperature studies were conducted using a standard laboratory oven.

### ***Stock Solution Studies***

A preliminary test was done to confirm that the equilibrium reaction operating in the Scott's test for cocaine could in fact be manipulated by temperature. For economy, a stock solution of cobalt thiocyanate was prepared to closely match the concentration of the solution contained in the test kit ampoules. A series of calibration solutions was prepared such that the concentration of the test kit solution was within the concentration range of the calibration set, as judged by visual inspection. The actual absorbances of the solutions - measuring 0.05, 0.10, and 0.15 M in concentration - were formally measured at 517 nanometers using a Beckman DB-G UV-Vis spectrophotometer.

Using the resulting calibration curve and measuring the absorbance of the test kit solution from the ampoule, it was determined that the test kit solution was approximately 0.11 M cobalt(II) thiocyanate. A stock solution at this concentration was prepared and used in preliminary tests with various quantities of cocaine and at room, reduced, and elevated temperatures.

### *Reduced-Temperature Studies (with Test Kits)*

A number of test kits were cooled to approximately 4°C in a small insulated cooler. While these pouches were being cooled, a benchmark test was performed using a kit at room temperature, following the manufacturer's instructions printed on the product box. The quantity of cocaine prescribed by the circle printed on the box was measured visually and then weighed using a laboratory balance (mass = 0.5 mg). This sample was then placed into the control test kit, and the series of ampoules was broken in sequence with agitation and observation in accord with the instructions. This test result was noted and was the basis of comparison for all subsequent tests.

The quantity of cocaine (0.5 mg) and the procedures used for the benchmark test were then duplicated for a test pouch cooled to 4°C (39°F). Following this test, a second reduced-temperature test was performed using a quantity of cocaine approximately one-half the size of the recommended amount used in the initial test (0.2 mg). The quantity of cocaine was cut in half again, to approximately one-fourth the size recommended by the manufacturer (0.1 mg), and the test procedure was repeated. Duplicate tests were run to confirm each result. Trials were also performed to compare the effect of pre-cooling the test kits prior to introduction of the cocaine samples versus attempting to cause a positive or negative result to be reversed by cooling the test kits after a result was obtained at room temperature for a given sample.

### *Elevated-Temperature Studies (with Test Kits)*

A number of test kits were warmed to either 45°C (113°F) or 60°C (140°F) in a standard laboratory oven. Another benchmark test was performed at room temperature and was used as a reference in the elevated-temperature study. The quantity of cocaine (0.5 mg) and the procedures for the benchmark test were then duplicated for test pouches warmed to 45°C and 60°C. Following each of these tests, a second set of elevated-temperature tests was performed using a quantity of cocaine approximately double the size of the recommended amount used in the initial test (1 mg).

## **Results and Discussion**

### *Stock Solution Studies*

Initial investigations revealed that 1 milligram of cocaine, combined in a test tube with one drop of concentrated hydrochloric acid and two drops of 0.11 M cobalt thiocyanate stock solution, resulted in the formation of blue flakes and a blue solution - a positive test. Based on literature data for the aqua complexes of cobalt(II) chloride, it was expected that decreasing the temperature of this positive test solution would shift the equilibrium toward the pink octahedral complex responsible for a negative test, while increasing the temperature would have the opposite effect. In actuality, however, cooling the blue solution to 4°C (39°F) had no significant impact on the color, while raising the temperature resulted in a change from blue to pink. At only 30°C (86°F), the solution began changing from blue to pink with blue specks. Continued heating to 60°C (140°F) resulted in a pink solution with no blue specks.

Next, the mass of cocaine was decreased to less than 0.5 milligram. This quantity was insufficient to produce a blue color in the test tube at room temperature. Raising the temperature of this solution gradually to 60°C (140°F) did not produce any significant changes in its appearance. However, cooling the solution to 4°C (39°F) resulted in a change in color from pink to blue - a positive test. Collectively, these results suggest that the reaction responsible for the change from pink to blue color in the Scott's test for cocaine is exothermic with a  $\Delta H$  on the same order of magnitude (but of opposite sign) as that for the endothermic reaction involving cobalt(II) chloride (shown in Eq. 1). The results also indicate that the temperature of a field test kit for cocaine will significantly impact the sensitivity and accuracy of its response.

### *Reduced-Temperature Studies (with Test Kits)*

Results are summarized in Table 1. Interestingly, in one trial, the benchmark test, using 0.5 milligram of cocaine at room temperature, yielded a negative result (in that Step 3 failed to produce the requisite blue lower chloroform layer). This indicates that there is little tolerance for error in the manufacturer's instructions for this particular test kit. In actual practice, this result would likely prompt re-testing with a larger quantity. However, cooling the negative test pouch to 4°C (39°F) resulted in a blue coloration in the chloroform layer, indicating a positive test for cocaine. Furthermore, cooling a new test kit pouch to 4°C prior to re-testing at the 0.5 milligram level resulted in a noticeably stronger positive test result at all three stages, further confirming enhanced sensitivity at lower temperatures.

To further study this finding, the quantity of cocaine was cut to approximately 0.2 mg. Testing this amount with a pouch that had been pre-cooled to 4°C again resulted in a positive result at all three stages. However, when the sample size was reduced to 0.1 mg, Steps 1 and 2 gave positive tests, but the chloroform layer did not develop a blue color in Step 3.

These results stand in contrast to the statement made in the manufacturer newsletter [11], and they confirm that storing the test kit at low temperatures - either incidentally due to weather conditions or intentionally in a portable cooler - increases its sensitivity by more than a factor of two. The results also show that cooling a test pouch that was positive at Steps 1 and 2 but negative at Step 3 is not as effective as pre-cooling it to 4°C prior to testing.

### *Elevated-Temperature Studies (with Test Kits)*

Results are summarized in Table 2. Since the benchmark test was already at the limit for a repeatable positive test, it was expected that a relatively small temperature increase would result in a negative test - and in fact pre-warming the test kits to 45°C (113°F) gave negative results at all three stages. Not surprisingly, increasing the temperature to 60°C (140°F) also gave negative results. These temperatures are routinely attained inside a parked vehicle during warm weather, especially if the vehicle is exposed to the open sun, and even if the windows are slightly opened for ventilation.

To attempt to compensate for the reduced sensitivity observed at higher temperatures, the sample size was increased from 0.5 mg to 1 mg. In the 45°C (113°F) trials, Step 1 yielded a pink solution with no blue flakes. Continuing the test procedure through Step 3 (despite the test instructions dictating that the test be terminated after a negative result for Step 1), a faint blue chloroform layer was observed. Anyone following the test instructions, however, would never have reached this stage. Again not surprisingly, increasing the temperature to 60°C (140°F) still gave negative results with 1 mg. This confirms that elevated temperatures decrease the sensitivity of the Scott's field test at least twofold.

### *Transient False Positives at Elevated Temperatures*

Elevated temperatures, in addition to producing false negatives, had a further complicating factor. At both 45°C and at 60°C, a transient blue solution was observed at Step 2, which persisted for a few seconds after mixing and agitating. This transient coloration is also often observed at room temperature, just after breaking the second ampoule, even in the absence of cocaine. Presumably it is due to a temporary and localized high concentration of chloride ions prior to complete mixing of the pouch contents, but at room temperature it dissipates very rapidly with agitation. At 45 and 60°C, however, due to the thermodynamic equilibrium of the cobalt(II) chloride reaction (Eq. 1), this color is more intense and persists for a much longer time. In fact, at 60°C, even up to one minute after the initial intense blue color dissipates, the color of the solution can best be described as pink to lavender, rather than pink. This is presumably due to a sufficient concentration of the blue tetrahedral chloro complex, or of one or more of the intermediate (i.e., partially chlorinated) complexes, imparting a blue tint to the otherwise pink solution. This result is obviously directly attributable to the temperature of the pouch when used, and again could potentially lead to false positive or inconclusive tests.

### *Safety Issues at Elevated Temperatures*

In addition to the loss of sensitivity and accuracy at elevated temperatures, two safety considerations were noted: First, the plastic clip that was used to seal the pouches (intended to prevent leakage of the reagents during agitation) can be deformed by elevated temperatures (45 - 60°C), potentially allowing the pouch to open accidentally or leak during agitation, spilling hazardous chemicals. A second concern is that chloroform boils at 61°C. Therefore, breaking the third ampoule while the kit is over 60°C will (at a minimum) result in pressurization of the pouch, and possibly cause a hazardous chemical aerosol spray to be emitted from an improperly sealed pouch. The authors are unaware of any literature reports of such problems, but the potential clearly exists.

### ***Conclusions (and Recommendations)***

The findings of this study suggest that better guidelines can and should be implemented for the storage and use of cocaine field test kits. The National Institute of Justice sets a generic standard for color test reagents and kits that recommends an ambient test temperature between 10°C and 40°C (50°F and 104°F) [12]. Based on the results of the presented study, the high temperature limit of 40°C is clearly too high for cocaine field test kits, and can result in false negatives. Furthermore, although not as critical an issue, the low temperature limit of 10°C is also too high, and needlessly sacrifices sensitivity.

Although the test kits can be stored and used at room temperature, storage at 4°C (39°F) is recommended to both enhance the sensitivity of the test and reduce the likelihood of false negatives due to low sample purity or user error. From a practical viewpoint, a single test kit can be cooled to close to this recommended temperature in approximately 10 minutes by clipping it to the front of a vehicle's dashboard vent while running the air conditioner at maximum cooling capacity (assuming the vehicle's A/C unit is properly operating). Maintaining a test kit clipped in this position at all times while on call would ensure the ready availability of a cooled test kit when needed, and is recommended in scenarios where law enforcement personnel have a reasonable but minor expectation of need. In cases where a larger volume of testing is anticipated, such as a planned search of a home, building, vehicle, boat, ship, or aircraft, etc., or where operational circumstances otherwise preclude using a vehicle's air conditioner, test kits may be maintained at 4°C by storing them in a small, portable cooler with ice or cold-packs, or in a 12-volt powered portable thermoelectric cooler maintained at 39°F (4°C). In testing, it was determined that a pair of test kits can be cooled from 100°F (38°C) to 4°C in 20 minutes in a typical thermoelectric cooler (i.e., intended for use in a vehicle), and can be maintained at that temperature indefinitely if the power supply is maintained. If necessary, large quantities of test kits could be stored in a designated chemical refrigerator at an appropriate facility, to ensure a constant supply of pre-cooled test kits that can be transferred as needed to portable thermoelectric cooler in vehicles.

Finally, besides yielding recommendations for the storage and transportation of cocaine field test kits, these findings also suggest the prudence of scrutinizing other field tests (i.e., for other illicit substances) to determine whether they have similar temperature sensitivity issues.

Future studies at the authors' laboratories include examination and quantitation of the effects of temperature on the response of known interferences in the Scott's test for cocaine; i.e., does use at 4°C both increase the sensitivity of the test to cocaine but also to other substances that are already known to give false positives? Or (as may well be) does it further improve the specificity of the test for cocaine?

### **Acknowledgments**

The authors wish to acknowledge the contributions of Pamela Johnson (Criminalist Supervisor) and Amie Nix (Criminalist / Drug Chemist) (both of the Missouri State Highway Patrol, Troop E Laboratory), and Dr. Bruce



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\* \* \* \* \*

Temperature	Mass	Step 1	Step 2	Step 3
22°C (72°F)	0.5 mg	pink solution blue flakes  (+)	pink solution  (+)	<u>pink upper layer</u> colorless lower  (-)
22°C (72°F)	0 mg  (-) control	pink solution no blue flakes  (-)	pink solution  (+)	<u>pink upper layer</u> colorless lower  (-)
4°C (39°F)	0.5 mg	pink solution blue flakes  (+)	pink solution  (+)	<u>pink upper layer</u> blue lower  (+)
	0.2 mg	pink solution blue flakes  (+)	pink solution  (+)	<u>pink upper layer</u> blue lower  (+)
	0.1 mg	pink solution blue flakes  (+)	pink solution  (+)	<u>pink upper layer</u> colorless lower  (-)
	0 mg  (-) control	pink solution no blue flakes  (-)	pink solution  (+)	<u>pink upper layer</u> colorless lower  (-)

**Table 1.** Results of Reduced-Temperature Study. Results are noted along with a (+) or (-) symbol to indicate a positive or negative inference. Test instructions dictate that the test ends after the first negative result. Procedure: Step 1: The first ampoule, containing the cobalt thiocyanate solution, is broken, the mixture is agitated, and the color is noted. A blue color in the solution or oily blue flakes indicates the possible presence of cocaine and permits advancement to the next step; Step 2: The second ampoule, containing the hydrochloric acid solution, is broken, the mixture is further agitated, and the color is again noted. A pink colored solution is observed but is not particularly diagnostic for cocaine; Step 3: The third ampoule, containing chloroform, is broken, again with agitation and observation of the resultant colored layers. A pink upper layer and a blue lower layer indicate the possible presence of cocaine.

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Temperature	Mass	Step 1	Step 2	Step 3
22°C (72°F)	0.5 mg	pink solution blue flakes (+)	pink solution (+)	<u>pink upper layer</u> faint blue lower (+)
45°C (113°F)	0 mg (-) control	pink solution no blue flakes (-)	pink solution (+)	<u>pink upper layer</u> colorless lower (-)
60°C (140°F)	0 mg (-) control	pink solution no blue flakes (-)	pink ~ lavender solution (?)	<u>pink upper layer</u> colorless lower (-)
45°C (113°F)	0.5 mg	pink solution no blue flakes (-)	pink solution (+)	<u>pink upper layer</u> colorless lower (-)
60°C (140°F)	0.5 mg	pink solution no blue flakes (-)	pink ~ lavender solution (?)	<u>pink upper layer</u> colorless lower (-)
45°C (113°F)	1 mg	pink solution no blue flakes (-)	pink solution (+)	<u>pink upper layer</u> faint blue lower (+)
60°C (140°F)	1 mg	pink solution no blue flakes (-)	pink ~ lavender solution (?)	<u>pink upper layer</u> colorless lower (-)

**Table 2.** Results of Elevated-Temperature Study. Results are noted along with a (+) or (-) symbol to indicate a positive or negative inference. A (?) symbol indicates an unpredicted observation that may be confusing or inconclusive to the field analyst. Test instructions dictate that the test ends after the first negative result. Procedure: See Table 1.

\* \* \* \* \*

# Identification of N-Methylbenzylamine Hydrochloride, N-Ethylbenzylamine Hydrochloride, and N-Isopropylbenzylamine Hydrochloride

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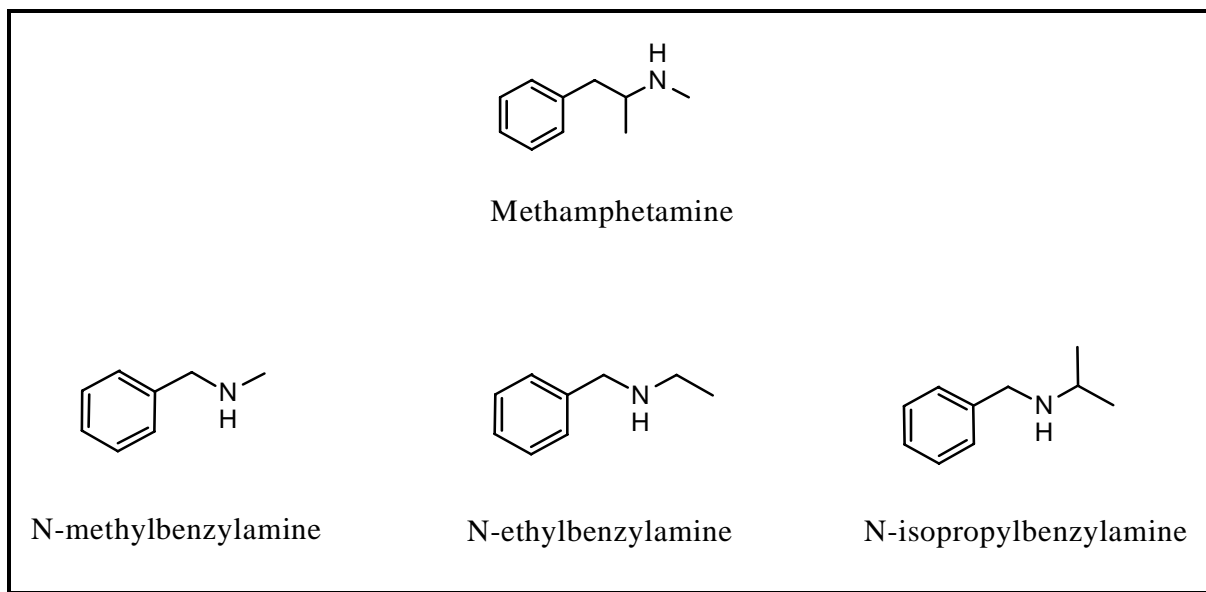
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**ABSTRACT:** N-Methylbenzylamine hydrochloride, N-ethylbenzylamine hydrochloride, and N-isopropylbenzylamine hydrochloride have recently been utilized to adulterate or mimic illicit methamphetamine hydrochloride (especially “Ice” methamphetamine). The characterizations of these three alkylbenzylamines by color testing, melting point determination, GC/MS, FTIR/ATR, and <sup>1</sup>H-NMR are presented.

**KEYWORDS:** N-Methylbenzylamine, N-Ethylbenzylamine, N-Isopropylbenzylamine, “Ice” Methamphetamine, GC/MS, FTIR/ATR, <sup>1</sup>H-NMR, Forensic Chemistry

## Introduction

Over the past 18 months, DEA and other forensic laboratories have received increasing numbers of suspected or purported high purity bulk methamphetamine hydrochloride exhibits that subsequent analyses showed to actually be a high purity alkylbenzylamine or less commonly, methamphetamine adulterated with an alkylbenzylamine [1-3]. Most of these exhibits were seized along or near the southwest border, or along the usual trafficking routes in the American southwest. In some cases, the alkylbenzylamine or methamphetamine/alkylbenzylamine mixtures



**Figure 1.** Structures of Methamphetamine and the N-Alkylbenzylamines.

were further diluted with dimethyl sulfone (a common methamphetamine “cut”). The first of these compounds encountered at this laboratory, N-methylbenzylamine hydrochloride, was submitted in early 2007. N-Ethylbenzylamine hydrochloride began to appear during the summer of 2007, and N-isopropylbenzylamine hydrochloride began to appear in late 2007 [Figure 1]. In 2008, to date (and for reasons unknown), N-isopropylbenzylamine hydrochloride appears to have become the dominant alkylbenzylamine among these submissions.

In most cases, the alkylbenzylamines were crystalline shards or crystalline powders that visually resembled “Ice” or “crystal” methamphetamine (e.g., see Photos 1 - 3). In addition, bulk exhibits (i.e., more than 2 kilograms) were packaged similarly to what is typically encountered for bulk methamphetamine (e.g., in plastic food-storage containers wrapped in cellophane and tape or in large ziplock plastic bags, etc. (e.g., see Photo 4)). And further, the bulk exhibits were smuggled similarly to other illicit drugs - and in some cases were co-smuggled with packages of other (actual) illicit drugs. For these reasons, it is widely accepted that they are being used as methamphetamine mimics (that is, as “rip-off”/sham narcotics), as opposed to “decoys” intended to divert law enforcement attention. In fact, all three alkylbenzylamines have been identified in retail (street-level) samples.



Photo 1 - Typical Exhibit of N-Methylbenzylamine HCl [1]



Photo 2 - Typical Exhibit of N-Ethylbenzylamine HCl



Photo 3 - Typical Exhibit of N-Isopropylbenzylamine HCl [2]



Photo 4 - N-Isopropylbenzylamine HCl Exhibits in Food-Storage Containers [2]

Not surprisingly, the analytical characteristics of the alkylbenzylamines are both similar and dissimilar to the simple phenethylamine drugs. One significant issue is that the various spectra may or may not be included in the libraries installed in the instruments present at most forensic laboratories. The characterization of N-methylbenzylamine, N-ethylbenzylamine, and N-isopropylbenzylamine by melting point, color testing, GC/MS, FTIR/ATR, and <sup>1</sup>H-NMR are presented herein.

## ***Experimental***

### Reagents

*Alkylbenzylamines:* N-Methylbenzylamine base, N-ethylbenzylamine base, and N-isopropylbenzylamine base were obtained from Sigma-Aldrich (St. Louis, MO). The respective hydrochloride salts were prepared by dissolving the free bases in acetone and adding concentrated hydrochloric acid. The resulting crystals were filtered, washed multiple times with acetone/ether (50/50), and air dried.

*Other Reagents:* Methamphetamine hydrochloride, amphetamine sulfate, phenethylamine sulfate, and dimethylsulfone were all obtained from this laboratory's reference collection.

*Test Solutions:* Two test solutions were prepared for GC/MS Analyses: (A) Test Solution A contained approximately 0.5 mg/mL each of dimethylsulfone and the respective bases of the three alkylbenzylamines, methamphetamine, phenethylamine, and amphetamine, in diethyl ether (prepared from their respective salts by basification with 1 M NaOH followed by extraction with diethyl ether). (B) Test Solution B contained approximately 0.5 mg/mL each of dimethylsulfone and the respective salts of N-methylbenzylamine, N-ethylbenzylamine, methamphetamine, phenethylamine, and amphetamine, in methanol.

### Instrumentation

*Melting Points:* Melting points for the respective hydrochloric salts were determined using an Stanford Research Systems Opti - Melt Model MPA-100 melting apparatus (Sunnyvale, CA), and are reported in Table 1.

*GC/MS:* Mass spectra (70 eV EI) were obtained using a 5975B Agilent Technologies Inert Mass Selective Detector equipped with a 6890N Gas Chromatograph. Two different columns were used: (a) An Agilent Technologies DB5-MS, 15 m, 0.25 mm i.d., fused-silica capillary column with 0.25  $\mu\text{m}$  film thickness; or (b) An Agilent Technologies HP5-MS, 30 m, 0.25 mm i.d., fused-silica capillary column with 0.25  $\mu\text{m}$  film thickness. Helium was used as the carrier gas with an average linear velocity of 45 cm/sec (constant flow). The injection port and ion sources were set at 280°C and 230°C, respectively. For analysis, 1  $\mu\text{L}$  of the respective Test Solution was injected in split mode (50:1). The oven temperature was programmed as follows: 90°C for 1 minute, ramped at 30°C per minute to 150°C, then held there for 2.0 minutes (total run time = 5.00 minutes). The spectra were obtained by scanning over an  $m/z$  range of 40 - 500.

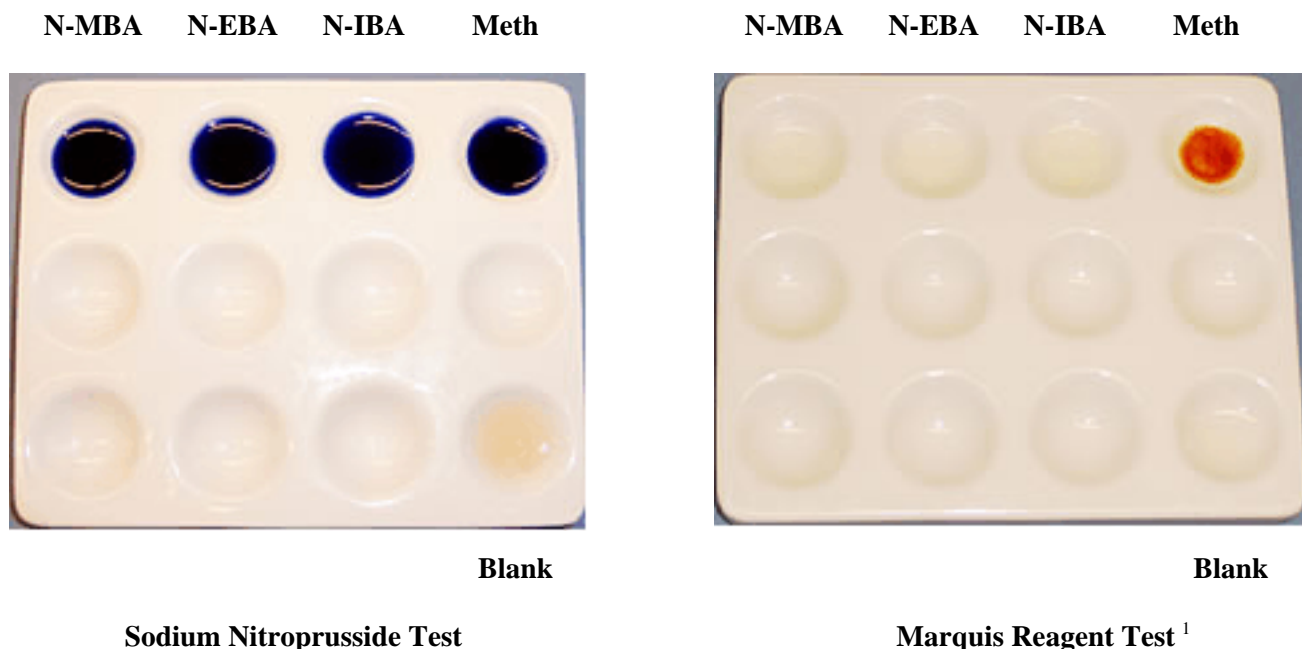
*FTIR/ATR:* Spectra were obtained using a Nicolet Avatar 370 FTIR Spectrophotometer operated in the ATR mode. Sixteen scans were collected at a resolution of 4.0  $\text{cm}^{-1}$ .

*<sup>1</sup>H-NMR:* Spectra were obtained using a Varian Mercury 400 MHz NMR. The compounds were analyzed as the hydrochloride salts in D<sub>2</sub>O (approximately 30 mg/mL) containing TSP as the 0 ppm reference (Note that the spectra are incorrectly labelled with "TMS" - TSP was actually used). Eight scans were collected, using a 90° pulse and a 2 second relaxation delay. Spectra were processed using 1.0 Hz line broadening.

## ***Results and Discussion***

The high purity and clean appearance of the various alkylbenzylamine submissions suggest that they were industrially (not clandestinely) produced. However, the large crystal forms of some submissions (especially N-isopropylbenzylamine hydrochloride) indicates that clandestine operators are recrystallizing them in order to better mimic large "Ice" methamphetamine crystals. All three alkylbenzylamines have melting points slightly higher than methamphetamine (see Table 1). Interestingly, the DEA Western Laboratory (San Francisco, CA) reported that the N-isopropylbenzylamine hydrochloride crystals in one bulk submission crushed noticeably more easily than typical "Ice" methamphetamine crystals [2]. A similar propensity was noted during this study;

however, it is unknown if that finding is universal for all three alkylbenzylamines, or instead is an anomaly for recrystallized N-isopropylbenzylamine hydrochloride from one clandestine source. Color testing by sodium nitroprusside gave positive (blue) results for all three alkylbenzylamines; however, testing with the Marquis reagent gave negative results (see below).



-----  
<sup>1</sup> Caution: Depending on the concentration and the age of Marquis reagent, the alkylbenzylamines can give a presumably "false" or weak positive after approximately 30 seconds (a positive Marquis test usually takes less than 10 seconds).  
-----

The total ion chromatogram (TIC) for the analysis of Test Solution A on the 15 m DB-5 column is shown in Figure 2a. All compounds were baseline separated with the exception of amphetamine and N-ethylbenzylamine. The TIC for the analysis of Test Solution B on the 30 m HP-5 column is shown in Figure 2b; the 30 m HP-5 column was able to partially resolve amphetamine and N-ethylbenzylamine. Figures 3 through 5 show the mass (GC/MS), infrared (FTIR/ATR), and nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra, respectively, for N-methylbenzylamine hydrochloride, N-ethylbenzylamine hydrochloride, N-isopropylbenzylamine hydrochloride.

The simple alkylbenzylamines are traditionally used as intermediates in organic syntheses. None of the three alkylbenzylamines are controlled, and none are believed to have appreciable CNS stimulant effects at typical methamphetamine dosage levels. The pharmacological effects of high dosages on humans are unknown [4].

### Acknowledgments

The author thanks the following personnel (all of this laboratory): Jason A. Bordelon (Senior Forensic Chemist) for mentoring and valuable contributions; James L. Jacobs (Forensic Chemist, who was the first to identify N-methylbenzylamine hydrochloride at the laboratory) for standard preparations; and Michael M. Brousseau (Forensic Chemist) for acquiring the <sup>1</sup>H-NMR spectra.

[Note: References are posted on Page 43.]

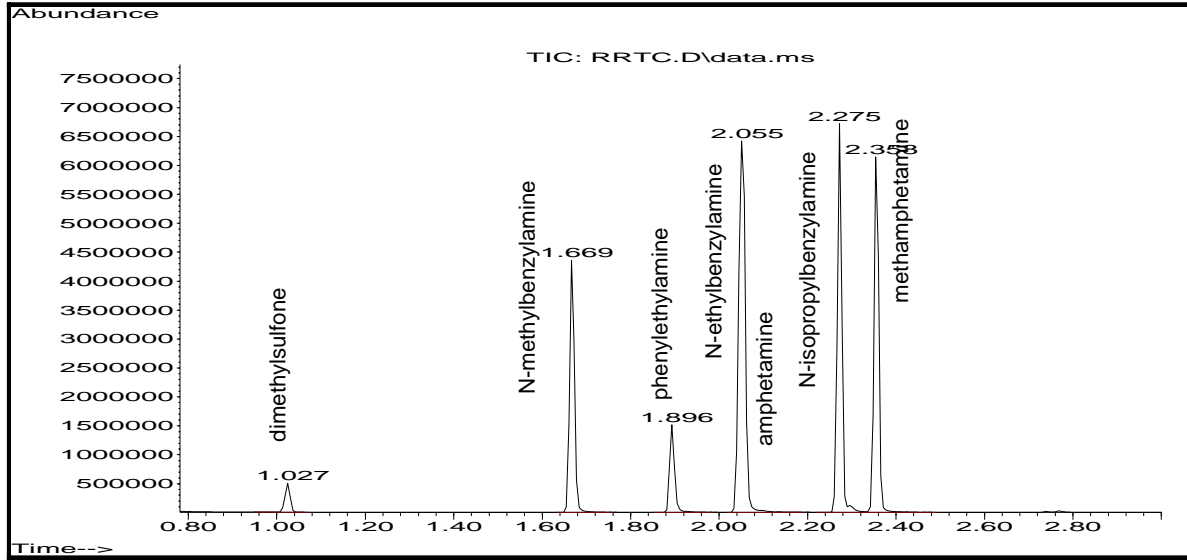
**Table 1. Melting Points.**

N-Methylbenzylamine hydrochloride, mp = 180.1 - 181.4°C

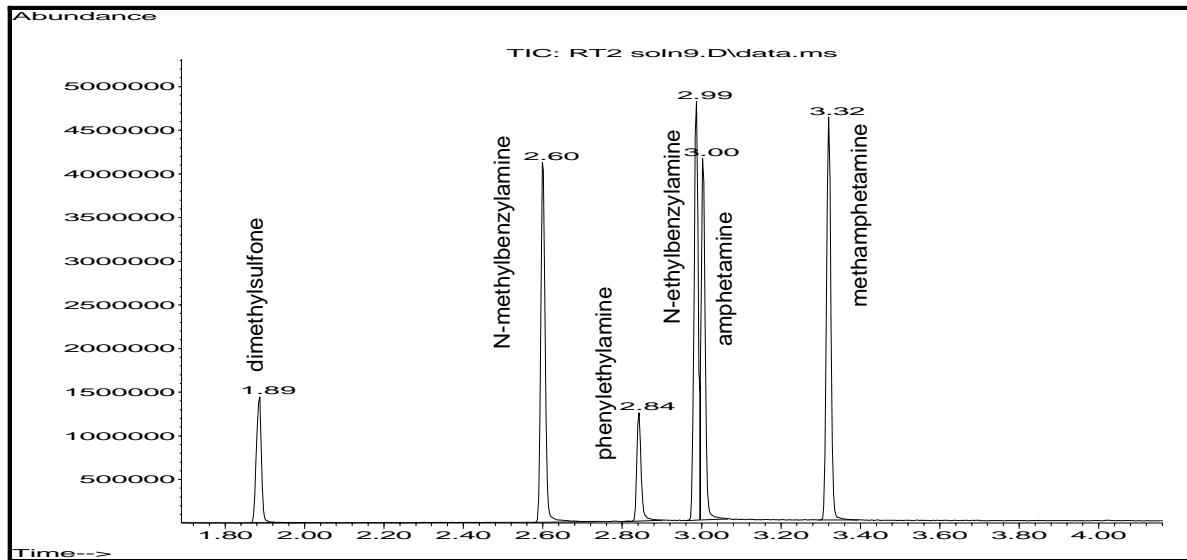
N-Ethylbenzylamine hydrochloride, mp = 183.7 - 184.5°C

N-Isopropylbenzylamine hydrochloride, mp = 192.0 - 193.3°C

[Note: *d*-Methamphetamine hydrochloride, mp = 172 - 174°C]

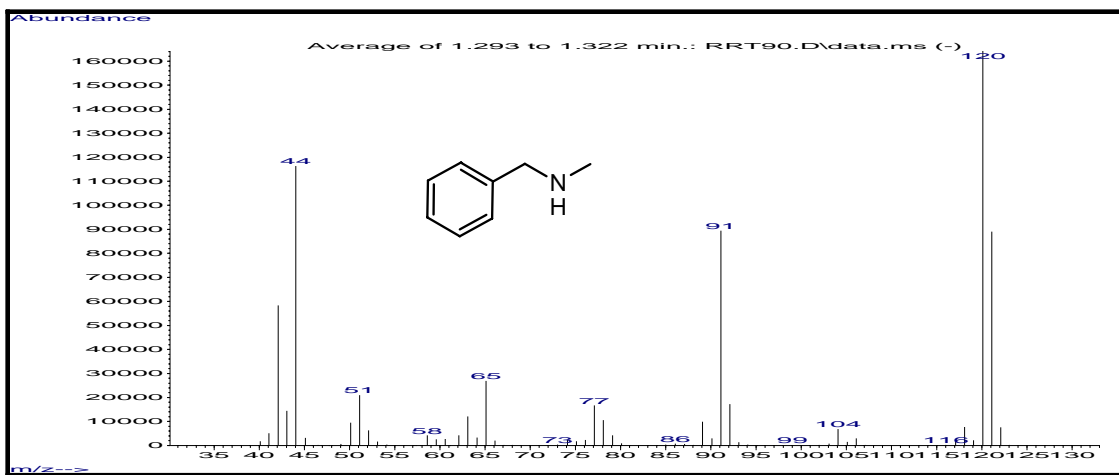


**Figure 2a.** GC/MS Total Ion Chromatogram (TIC) of the Sample Mixture A.

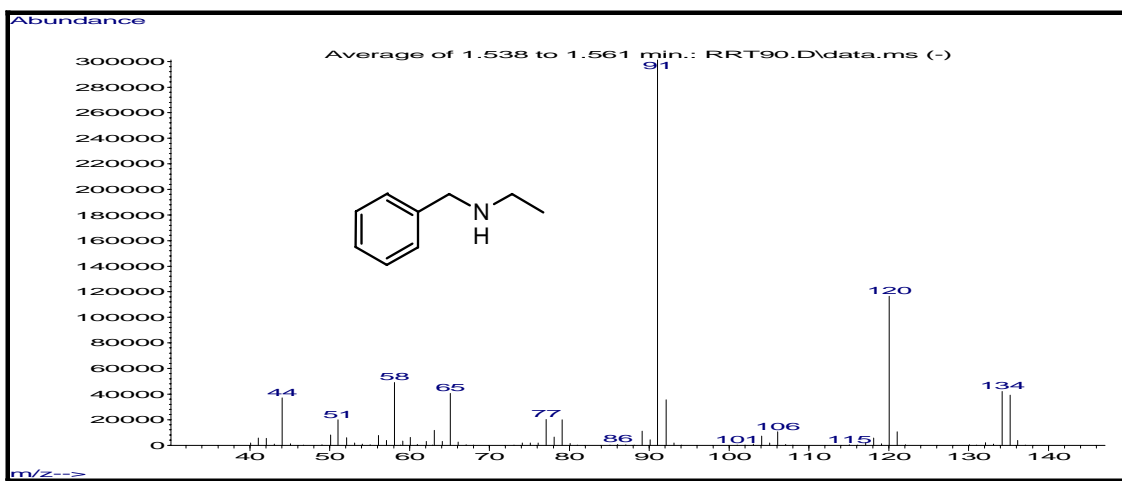


**Figure 2b.** GC/MS Total Ion Chromatogram (TIC) of the Sample Mixture B.

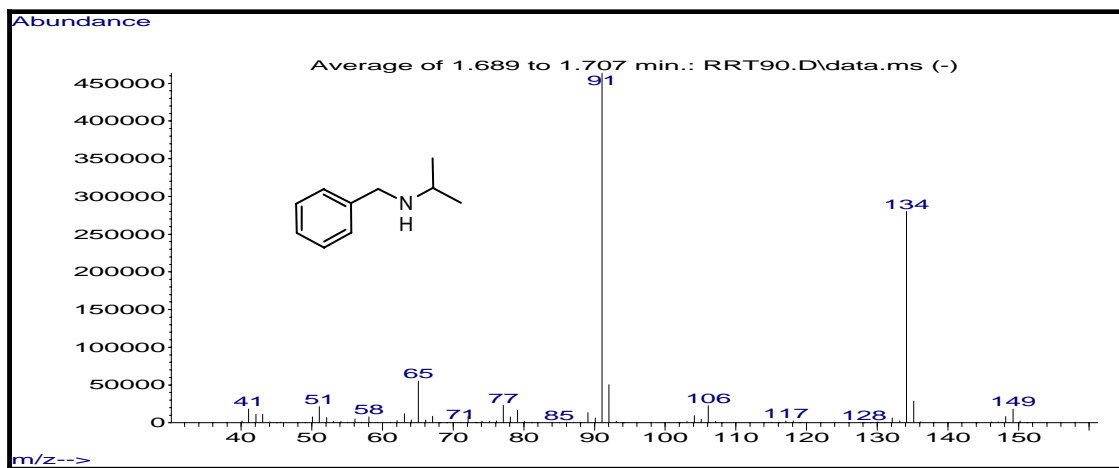




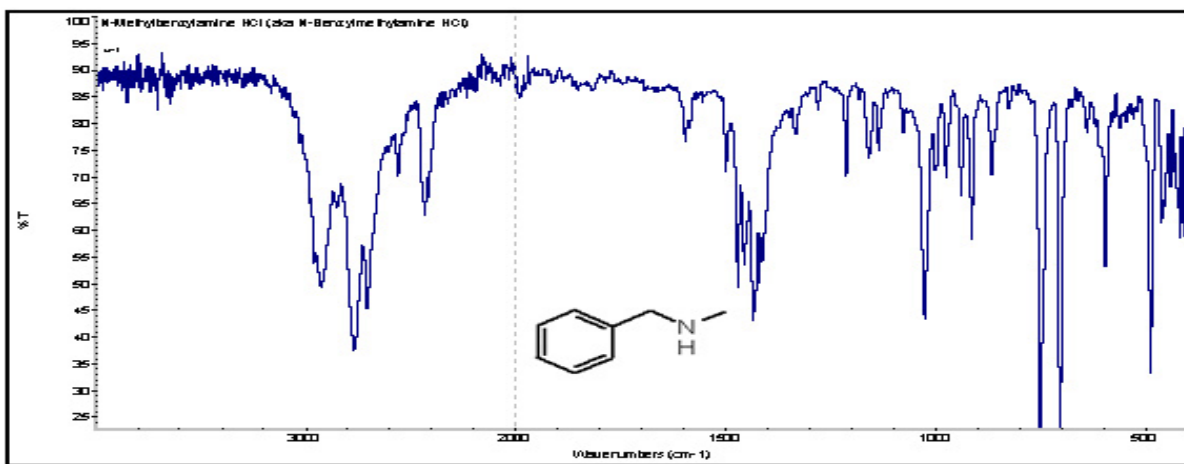
**Figure 3a.** Mass Spectrum of N-Methylbenzylamine.



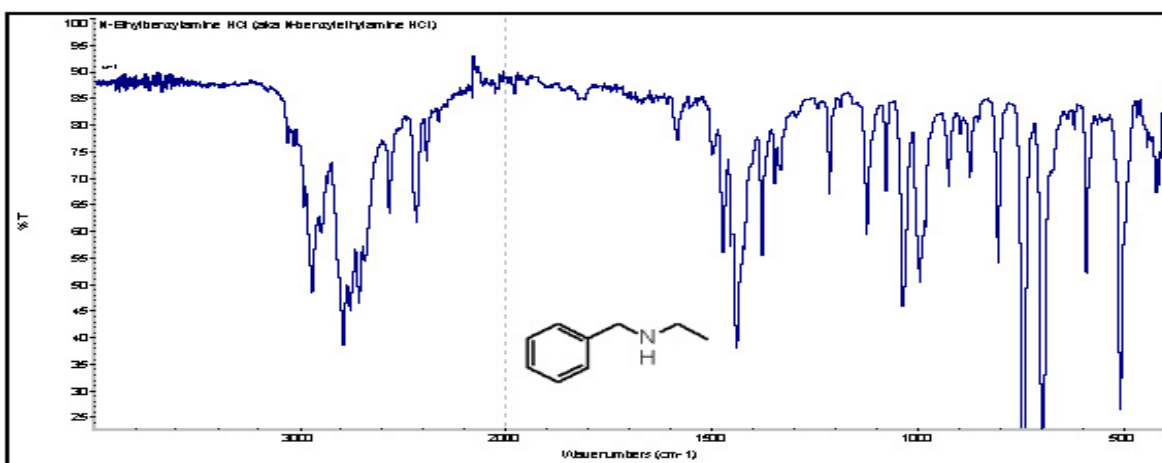
**Figure 3b.** Mass Spectrum of N-Ethylbenzylamine.



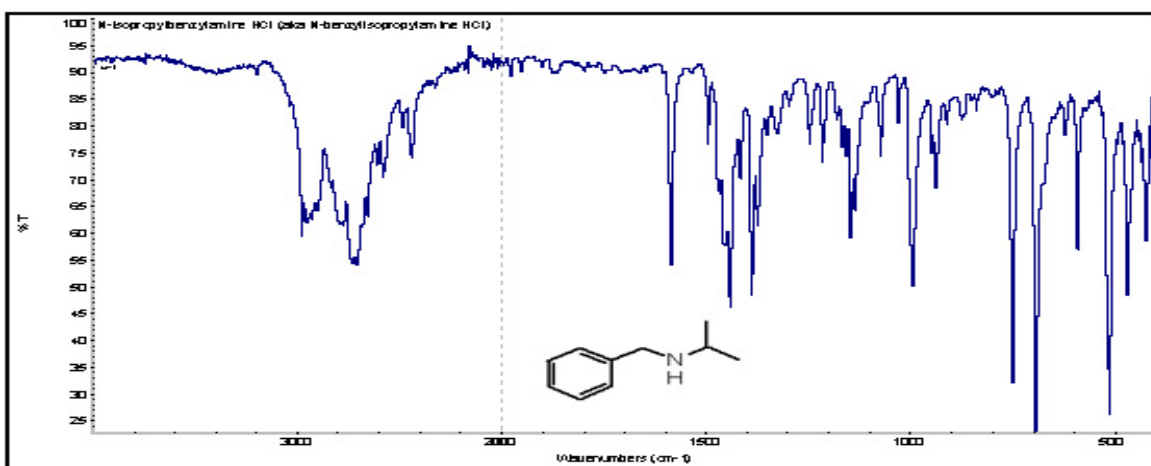
**Figure 3c.** Mass Spectrum of N-Isopropylbenzylamine.



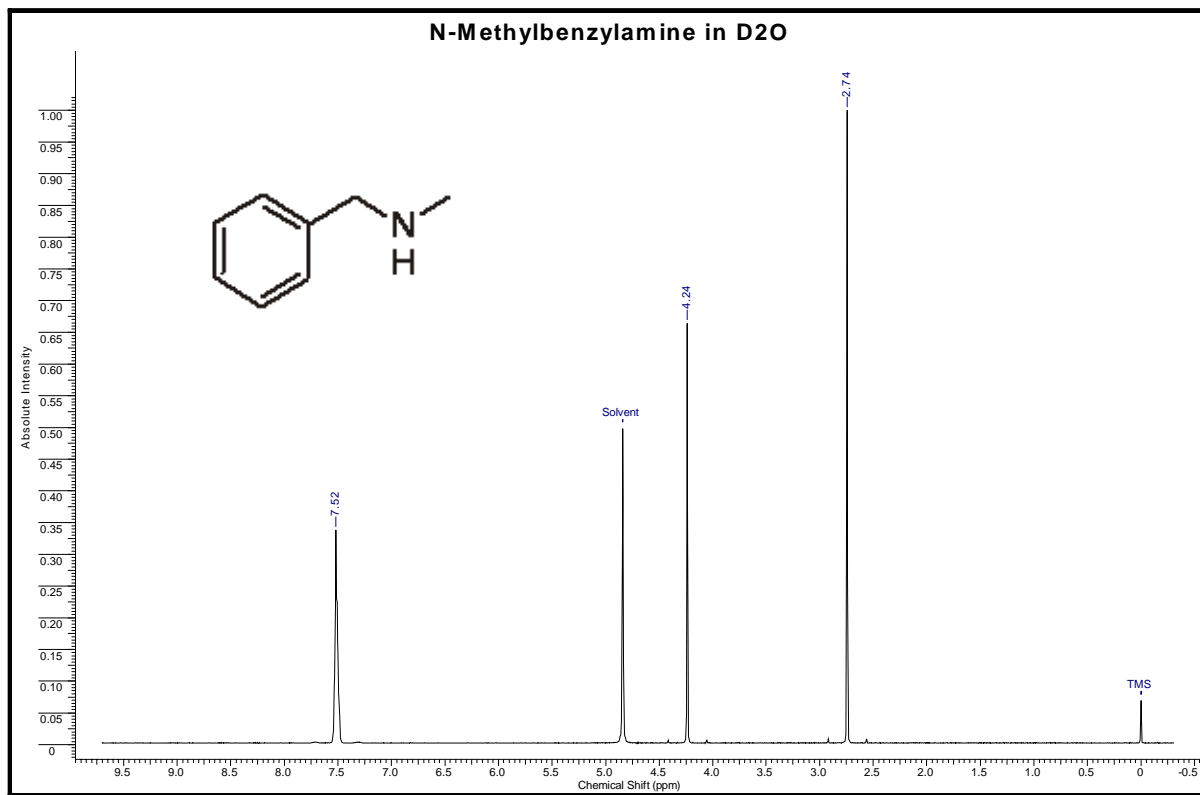
**Figure 4a.** FTIR/ATR of N-Methylbenzylamine Hydrochloride.



**Figure 4b.** FTIR/ATR of N-Ethylbenzylamine Hydrochloride.



**Figure 4c.** FTIR/ATR of N-Isopropylbenzylamine Hydrochloride.



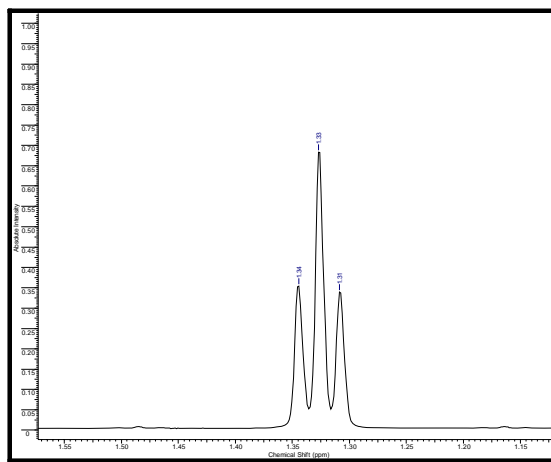
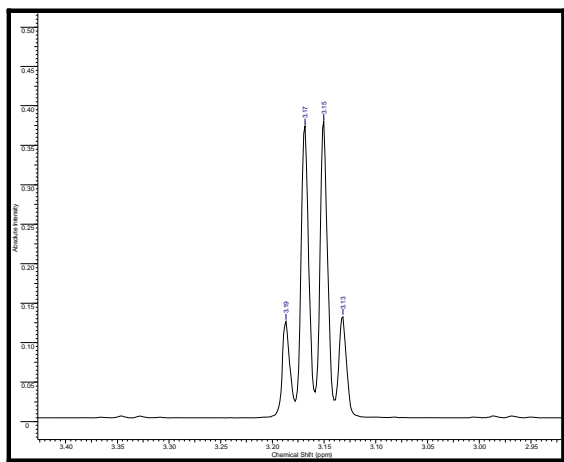
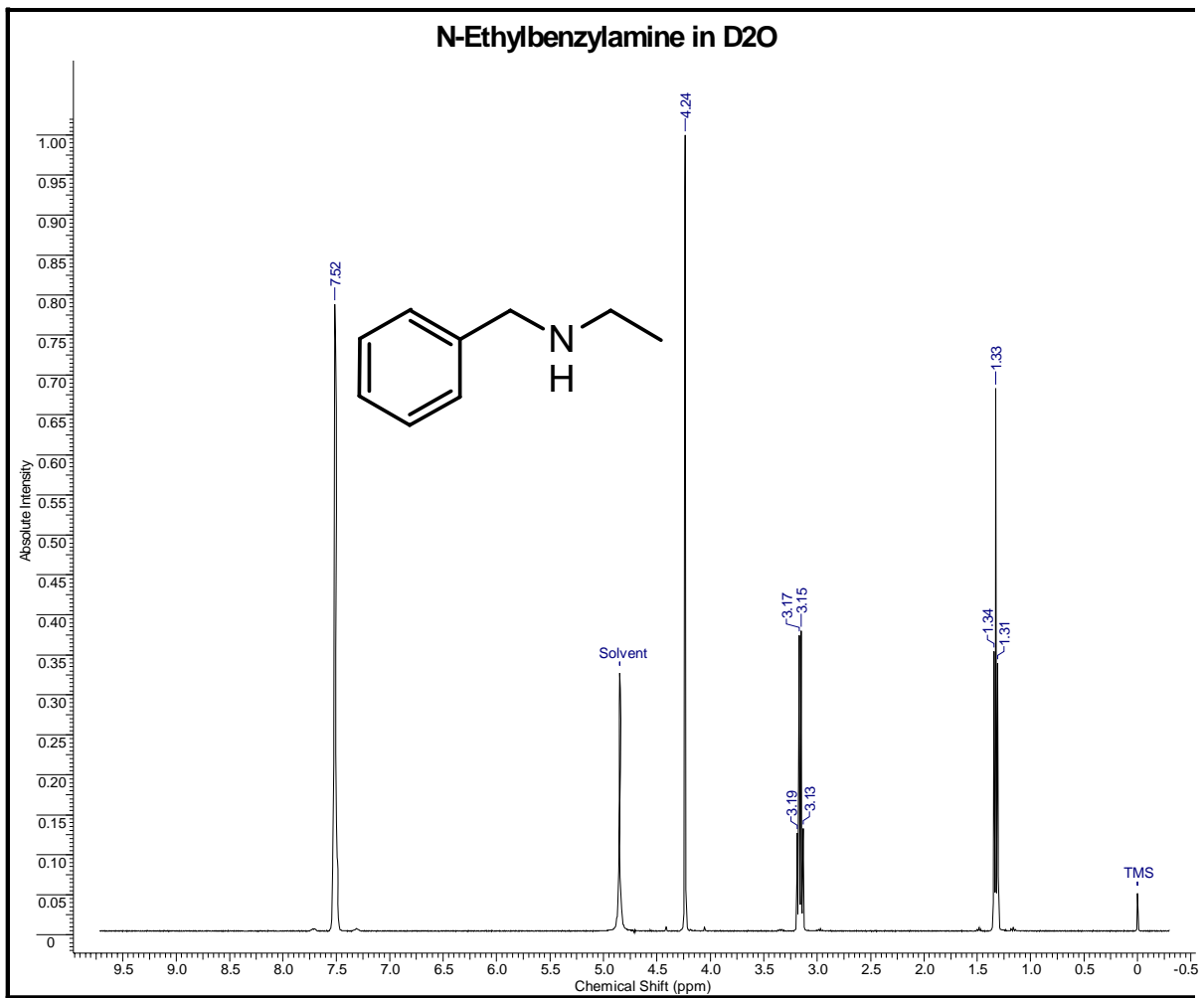
**Figure 5a.** 400 MHz <sup>1</sup>H-NMR Spectrum of N-Methylbenzylamine Hydrochloride in D<sub>2</sub>O.

[Figures 5b and 5c Follow (Next 2 Pages).]

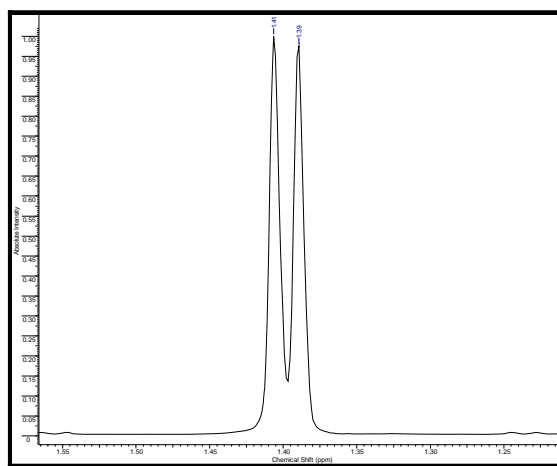
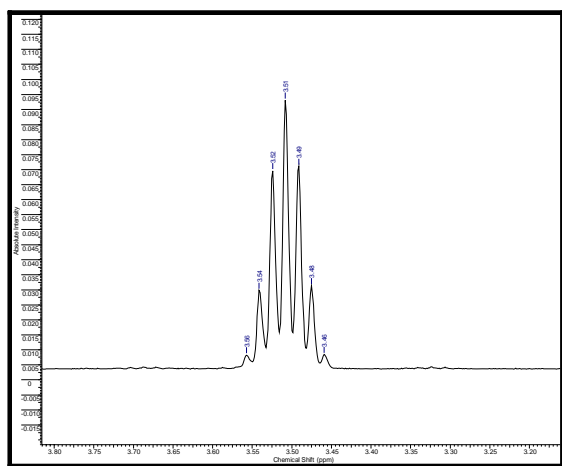
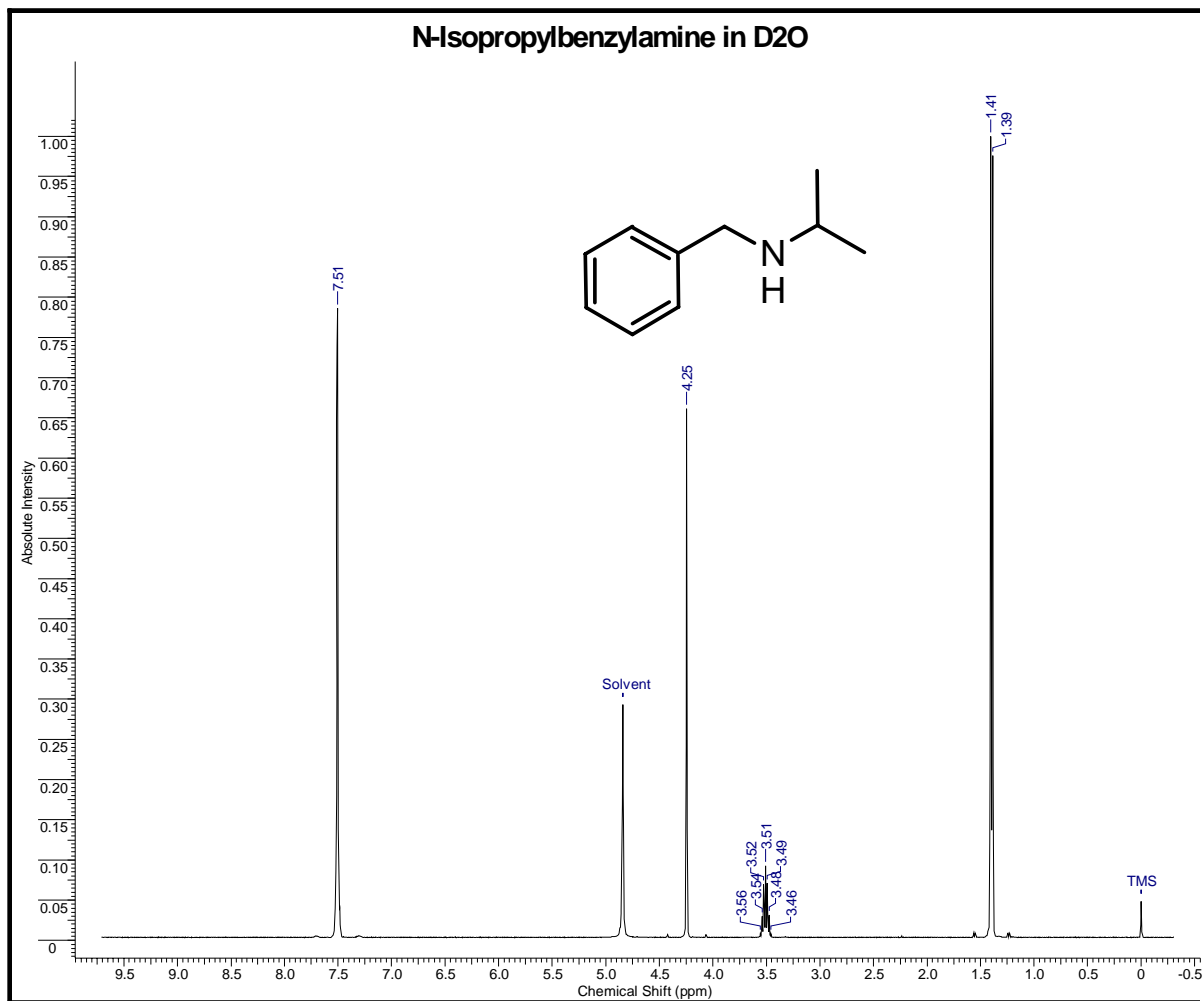
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**Figure 5b.** 400 MHz <sup>1</sup>H-NMR Spectrum of N-Ethylbenzylamine Hydrochloride in D<sub>2</sub>O.



**Figure 5c.** 400 MHz <sup>1</sup>H-NMR Spectrum of N-Isopropylbenzylamine Hydrochloride in D<sub>2</sub>O.

# Isolation of Methamphetamine from 1-(1',4'-Cyclohexadienyl)-2-methylaminopropane (CMP) Using Potassium Permanganate

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**ABSTRACT:** Methamphetamine illicitly prepared via active metal/ammonia (Birch) reduction of ephedrine or pseudoephedrine is commonly contaminated with 1-(1',4'-cyclohexadienyl)-2-methylaminopropane (CMP), often in significant amounts. Large percentages of CMP in methamphetamine samples result in poor quality (mixed) FTIR spectra. Preliminary treatment/cleanup of CMP-contaminated samples with potassium permanganate gives "clean" methamphetamine for FTIR analysis.

**KEYWORDS:** Methamphetamine, 1-(1',4'-Cyclohexadienyl)-2-methylaminopropane, Ephedrine, Pseudoephedrine, Birch Reduction, Potassium Permanganate, Forensic Chemistry

## Introduction

One of the primary methods of clandestine methamphetamine synthesis is the reduction of ephedrine or pseudoephedrine utilizing an alkali metal such as lithium or sodium, and liquefied ammonia. During a typical reduction of (pseudo)ephedrine, only the hydroxyl group is reduced, producing methamphetamine. With excess alkali metal, and in the presence of an additional proton source [1-8], the aromatic ring is additionally reduced to form a cyclohexadiene (Figure 1). This product is readily generated and is consistent with Birch (Na, EtOH, NH<sub>3</sub>) type reactions. The product produced in this reaction is known as 1-(1',4'-cyclohexadienyl)-2-methylaminopropane (CMP), or more simply, the Birch reduction product. On occasion, the CMP to methamphetamine ratio is very high in the final product of this synthesis, which can yield an undesirable, mixed infra-red spectrum (Figure 2 - second pane). Separation and identification of methamphetamine and CMP is easily accomplished by gas chromatography/mass spectroscopy, but some scientists prefer infrared spectroscopy, as it provides easy differentiation of the various phenethylamines. This paper will describe a quick, qualitative method for the elimination of CMP commonly found with methamphetamine manufactured from (pseudo)ephedrine using the lithium - ammonia reduction method [9-11].

## Experimental

### Reagents and Solutions:

- \* A 2% solution of potassium permanganate was prepared by dissolving 0.5 grams KMnO<sub>4</sub> in 25 mL water (use caution, potassium permanganate is a moderately strong oxidizing corrosive).
- \* Aqueous base (sodium hydroxide, sodium bicarbonate, etc.).

- \* Organic solvent (hexane, diethyl ether, or similar).
- \* Hydrogen chloride (HCl).
- \* Mixture of Methamphetamine - CMP (60:40).

*Instrumentation:*

- \* Nicolet Avatar 370 DTGS-Thermo Electron Corporation. Smart Golden Gate Diamond ATR with KRS-5 Lenses. Number of scans 16, resolution  $4\text{ cm}^{-1}$ , and range  $400 - 4000\text{ cm}^{-1}$ .
- \* Agilent 5973 GC-MSD quadrupole electron impact mass spectrometer system with a 30 m HP-5 MS, 0.25 mm, 0.25  $\mu\text{m}$  column. Carrier gas is ultra pure Helium. Instrument parameters: Temperature  $90^{\circ}\text{C}$  to  $300^{\circ}\text{C}$  at  $30^{\circ}\text{C}/\text{minute}$ , initial time 1 minute, final hold time 6 minutes, injection port temperature  $260^{\circ}\text{C}$ , transfer line  $280^{\circ}\text{C}$ .
- \* LCQ Advantage Max ThermoFinnigan quadrupole ion-trap mass spectrometer equipped with an electrospray ionization source (ESI) and interfaced to a Surveyor HPLC system. Phenomenex Luna column C18 -  $2.0 \times 30\text{ mm} \times 3\ \mu\text{m}$ . Gradient flow of 95:5 to 5:95 Solvent A/Solvent B over a 10 minutes run. Solvent A is  $\text{H}_2\text{O}$  with 0.1% (v/v) formic acid, while Solvent B is acetonitrile with 0.1% (v/v) formic acid. The flow rate was  $200\ \mu\text{L}/\text{minute}$ . Samples were prepared using Solvent A. Mass spectrometry data were collected in the positive ion mode using the full-scan mode in order to provide molecular weight information.

*Procedure:*

1. Place 25 mg of the sample (methamphetamine/CMP) in a test tube.
2. Dissolve the sample in 3 mL of water and add 0.5 mL of 2%  $\text{KMnO}_4$  solution, then agitate with vortex.
3. Add aqueous base (e.g., sodium hydroxide, sodium bicarbonate, or similar) to the test tube to make a basic solution ( $\text{pH} > 12$ ).
4. Add organic solvent (3 mL hexane) to the test tube, shake, and isolate the organic layer in a new, clean vessel.
5. Bubble HCl gas through the organic extract.
6. Isolate the precipitate (filtration, evaporation, or similar), dry, and obtain an IR spectrum.

***Results and Discussion***

The potassium permanganate reaction was performed on a mixed (60:40) sample of methamphetamine and CMP. Prior to performing the potassium permanganate reaction, this sample was analyzed by mass spectrometry for confirmation of sample components (Figures 3, 4A, and 4B). Potassium permanganate was then reacted with the mixture. When CMP is reacted with potassium permanganate, the double bonds on CMP are hydroxylated. By applying this technique with an aqueous base/organic solvent extraction, the CMP sodium salt formed remained in the aqueous phase while methamphetamine passed into the organic phase, where it was isolated by precipitation as the hydrochloride salt form. The final product was then sufficiently pure to be identified by infrared spectroscopy (Figure 2). Again a mass spectrometer was used to determine the effectiveness of the reaction, and the analysis confirmed that methamphetamine had been fully isolated from CMP (Figure 5 and 6).

To verify the hydroxylation of CMP and to show that no methamphetamine is produced by this reaction, a pure sample of CMP was reacted with potassium permanganate using the described technique and then analyzed by LC/MS (Figure 7). The presence of the 186 and 220 fragments in the mass spectrum obtained indicate that a mixture of dihydroxylated and tetrahydroxylated derivatives of CMP are produced by reaction with aqueous potassium permanganate ( $\text{pH} > 8$ ) [12]. There is no indication in the mass spectrum that CMP is converted to methamphetamine (no significant molecular ion at  $m/z$  150). Methamphetamine is left unaffected when reacted with potassium permanganate (Figure 8).

## Conclusions

In mixtures where the ratio of CMP to methamphetamine is high, the isolation of methamphetamine can be achieved by reacting CMP with potassium permanganate and an aqueous base. The procedure facilitates the isolation of methamphetamine from its primary by-product associated with the lithium - ammonia method of methamphetamine synthesis. It is rapid and straightforward, with few steps, and allows for convenient identification of methamphetamine using infrared spectroscopy.

## Acknowledgments

The authors acknowledge the contributions and assistance of Supervisory Chemist David W. Love; Senior Forensic Chemist Sandra E. Rodriguez-Cruz, Ph.D.; and Laboratory Worker Donald G. Smith (all at this laboratory).

## References

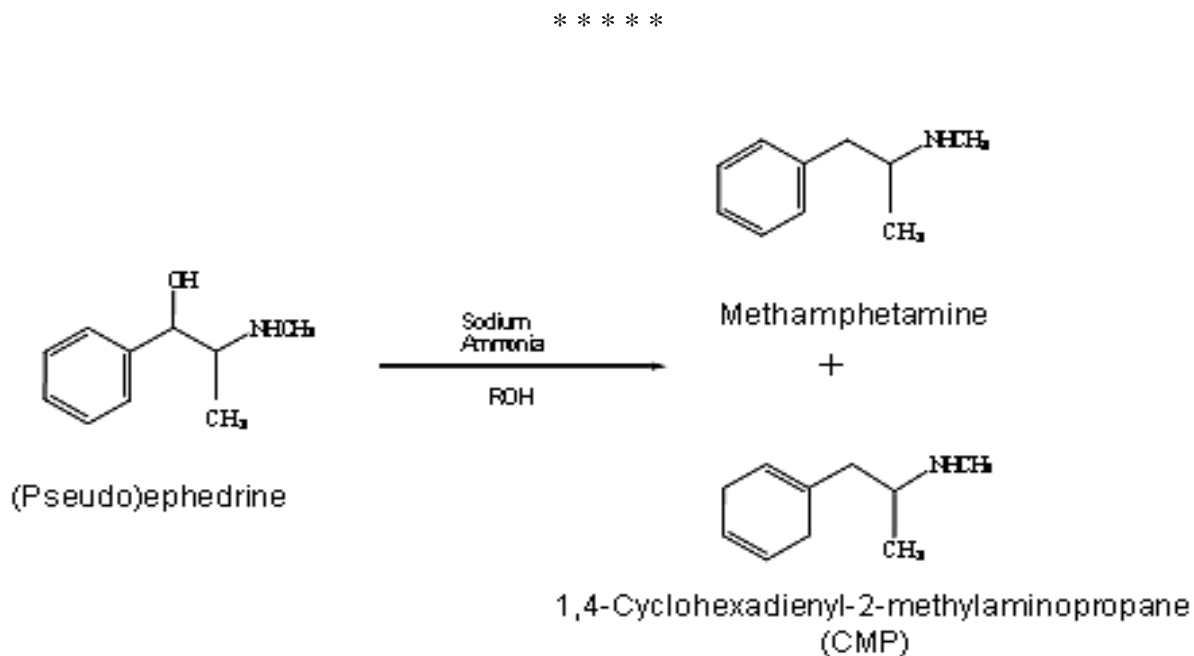
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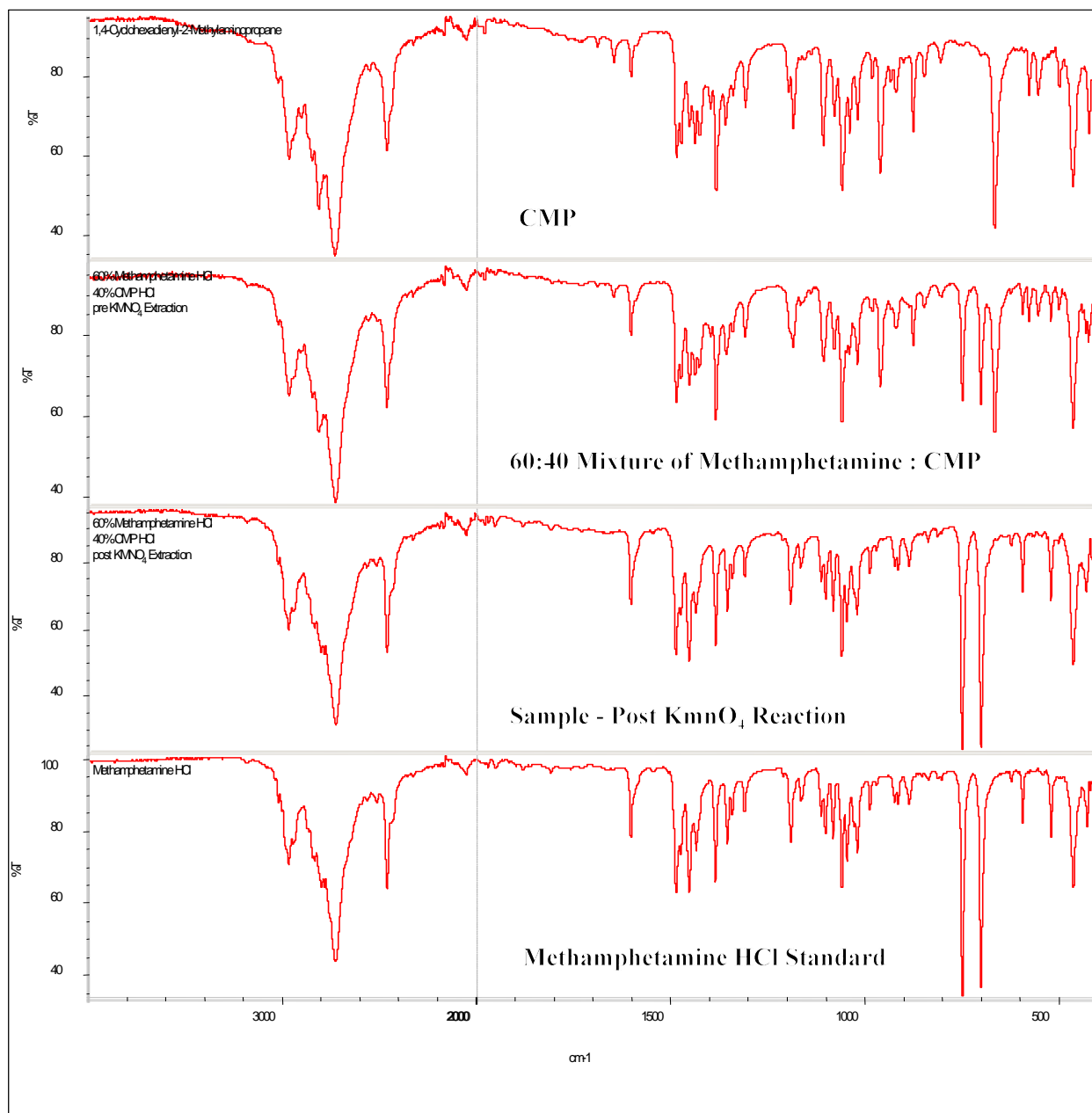
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\* Law Enforcement Restricted Publication.



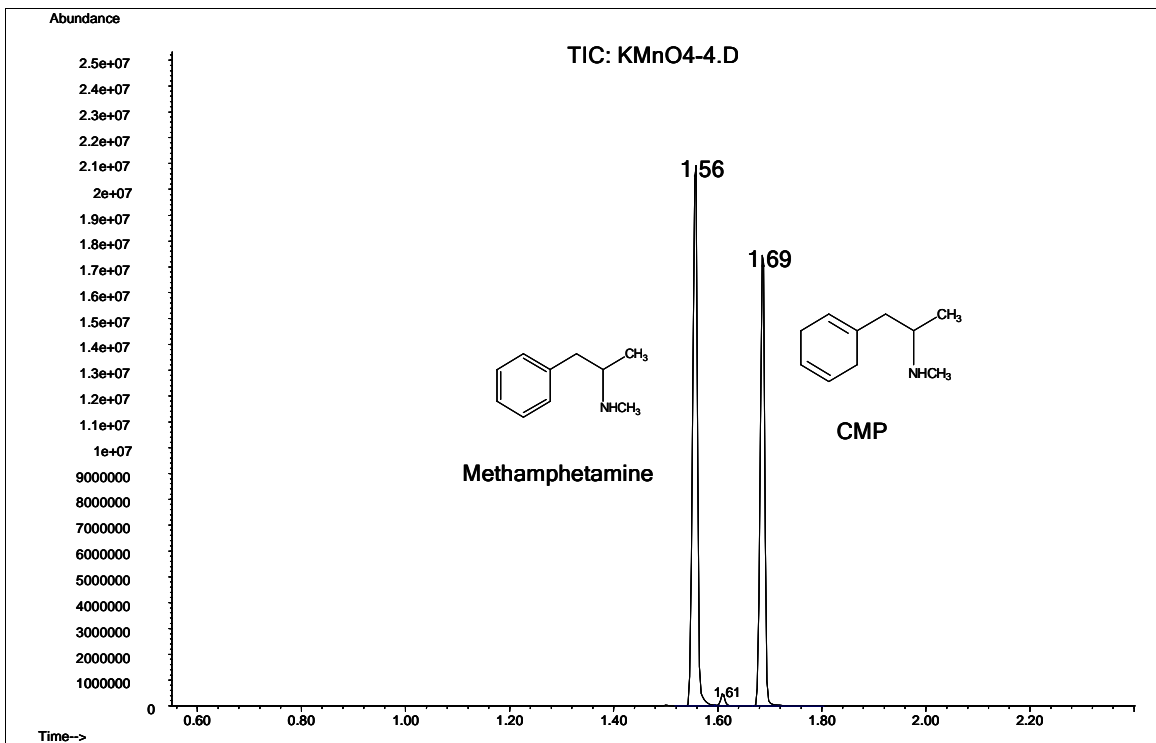
**Figure 1.** Classic Birch Route of Production with Excess Alkali Metal and Additional Proton Source.

[Figures 2 - 8 Follow.]

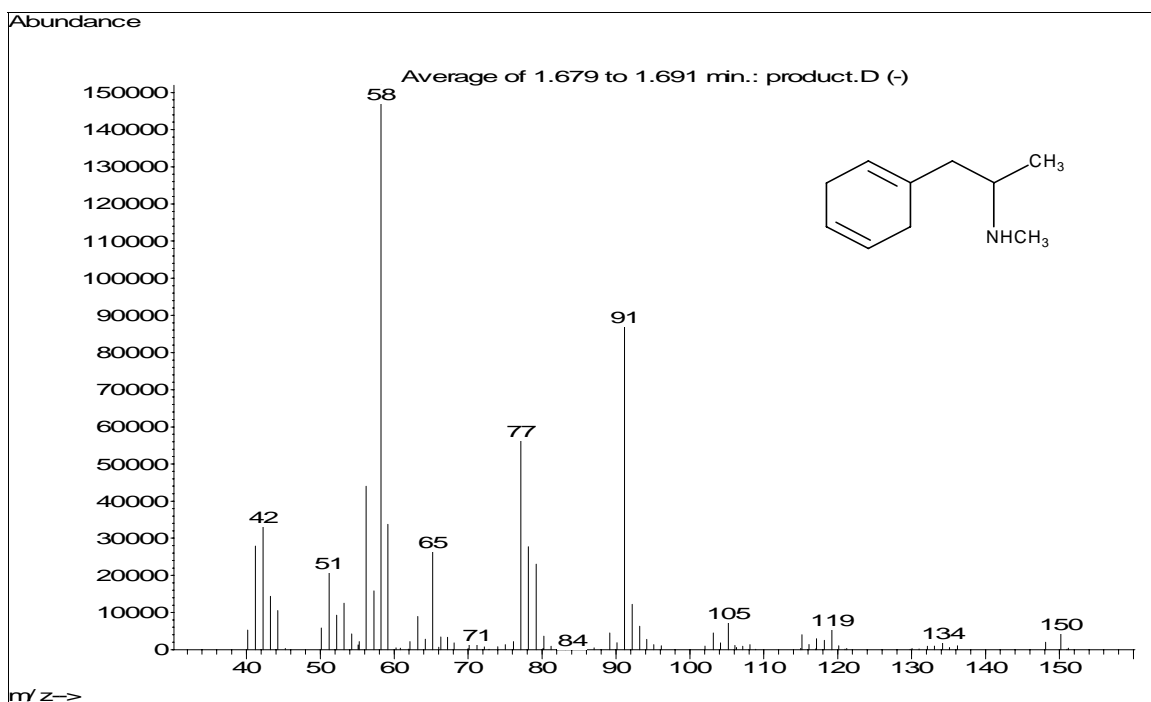


**Figure 2.** IR Spectra - Pre and Post Potassium Permanganate Reaction Versus Reference Standards.

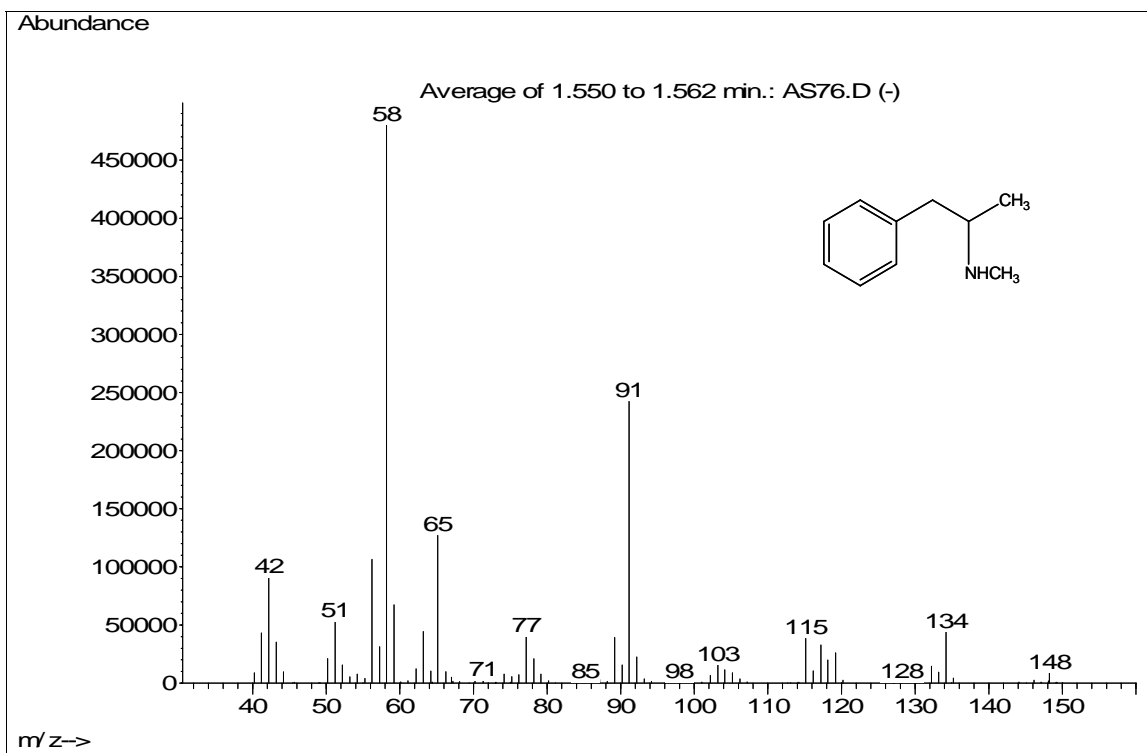
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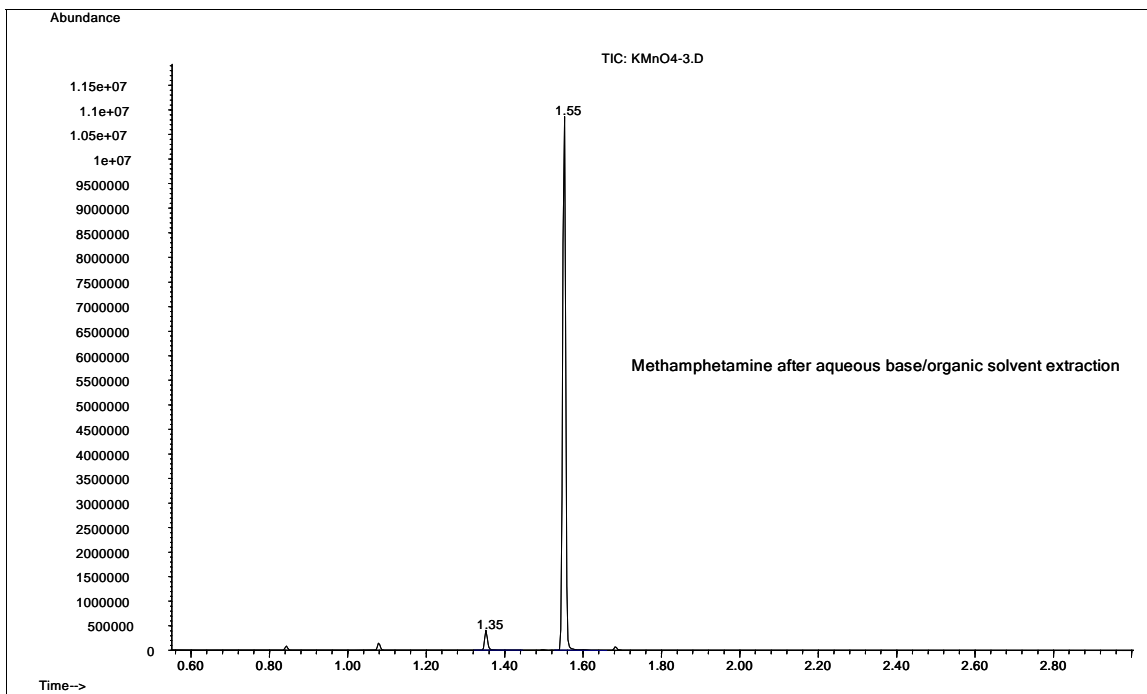
**Figure 3.** Mass Spectrometer TIC Post Adding Methamphetamine Standard to CMP for a 60:40 Mixture.



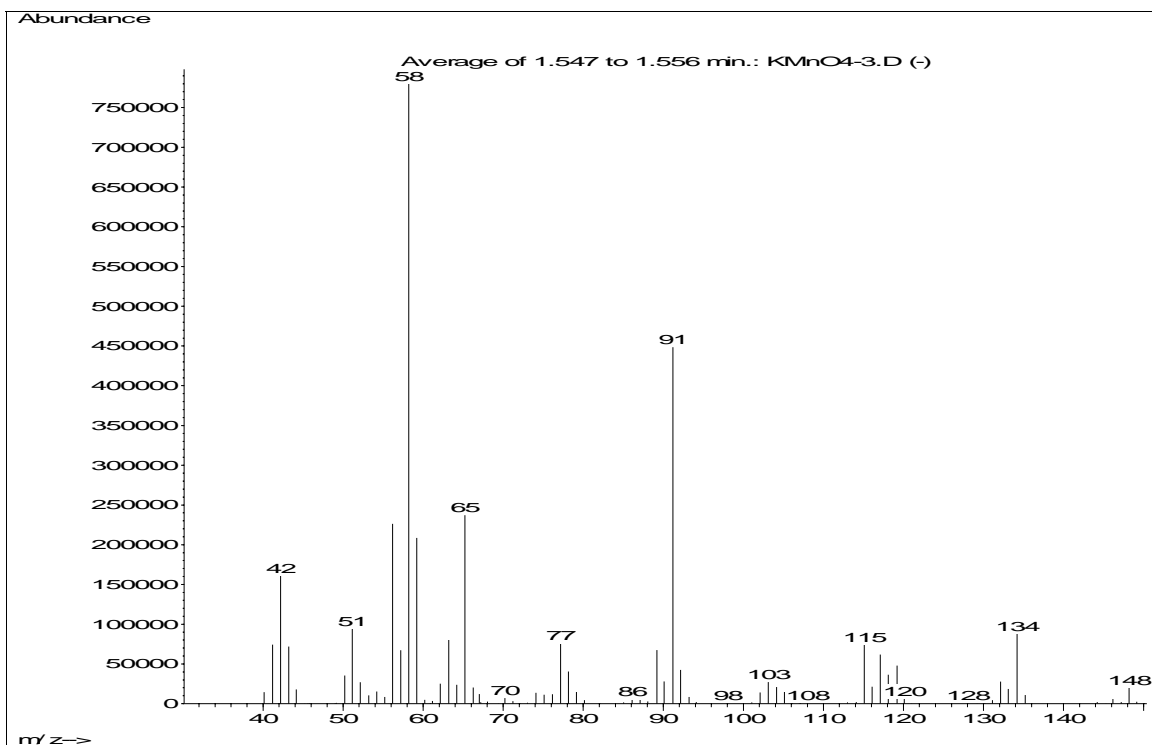
**Figure 4A.** Mass Spectrum of CMP.



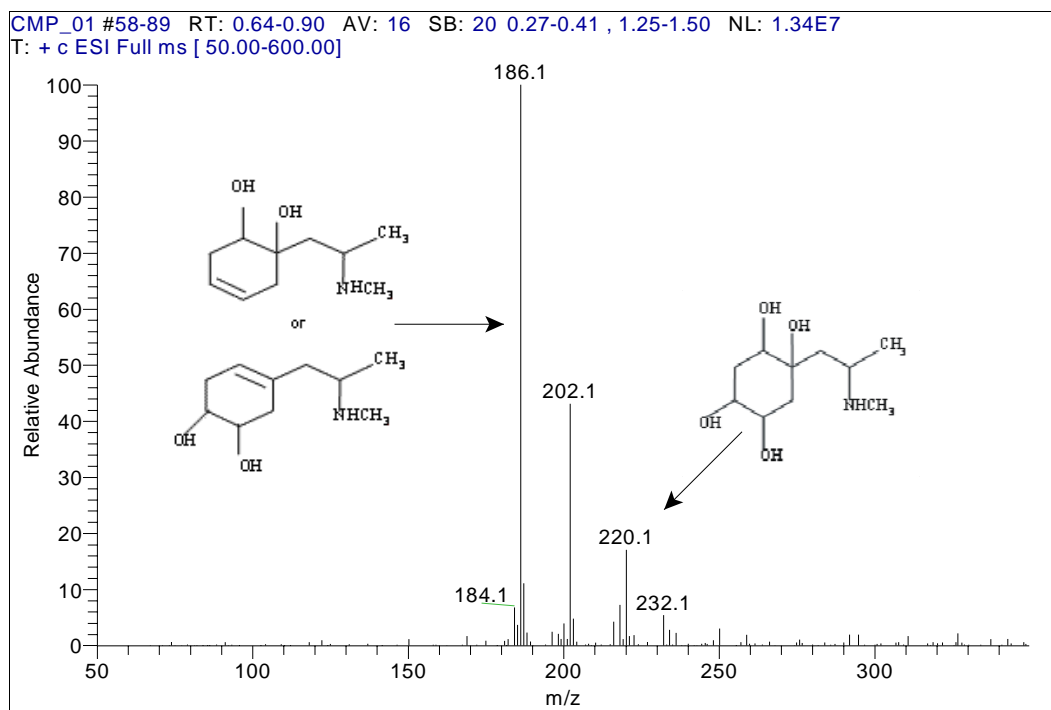
**Figure 4B.** Mass Spectrum of Methamphetamine.



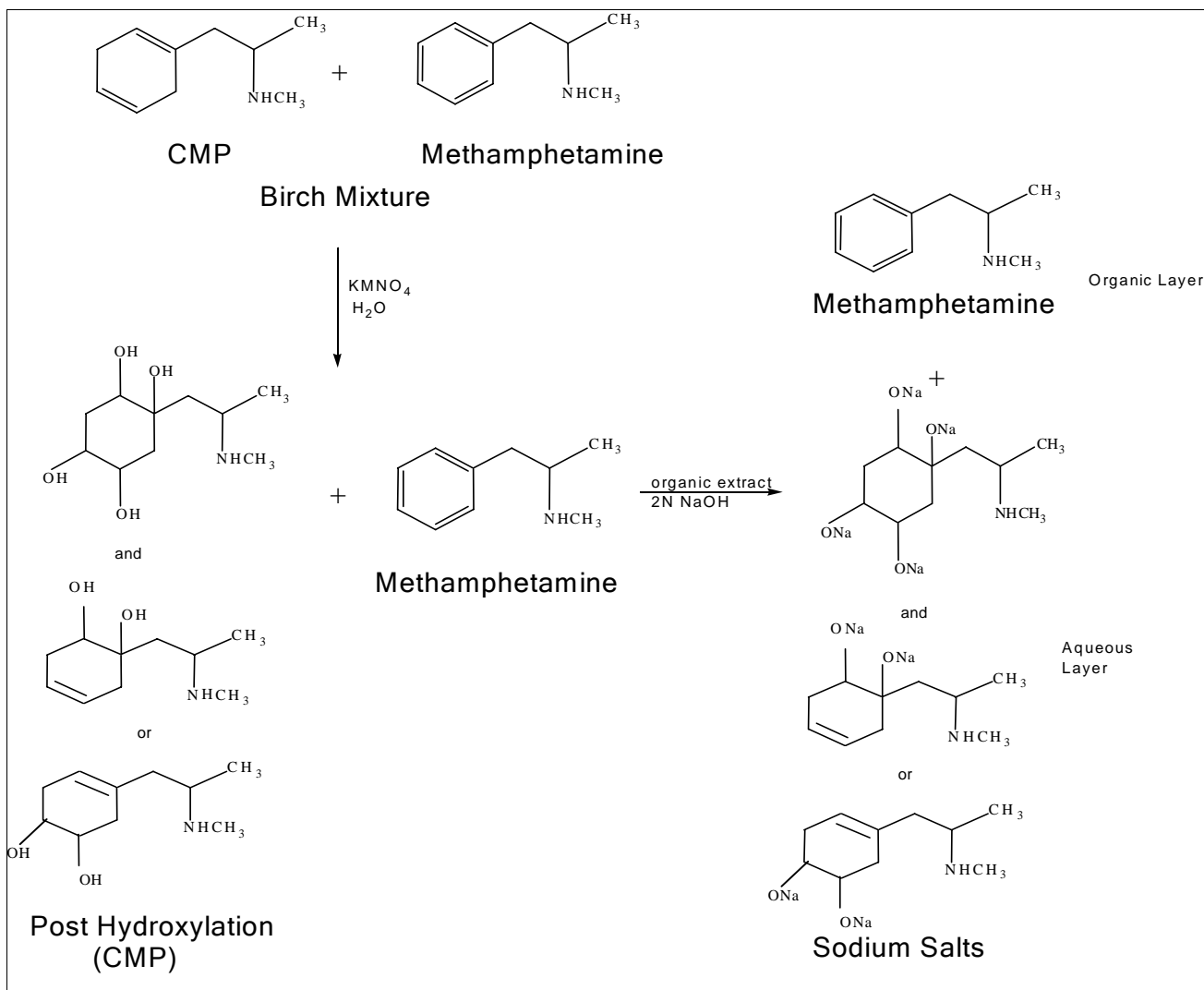
**Figure 5.** TIC Post Potassium Permanganate Reaction.



**Figure 6.** Mass Spectrum of Methamphetamine Post Potassium Permanganate Reaction.



**Figure 7.** ESI-MS Spectrum Indicating the Presence of both Dihydroxylated and Tetrahydroxylated Derivatives of CMP.



**Figure 8.** Proposed Potassium Permanganate Reaction.

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\* \* \* \* \*

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As noted above, the email notification indicates which issue has been posted, provides the *Microgram* URL, and additional information as appropriate. Note that *Microgram* e-notices will NEVER include any attachments, or any hyperlink other than the *Microgram* URL. **This is important, because the *Microgram* email address is routinely hijacked and used to send spam, very commonly including malicious attachments.** For this reason, all subscribers are urged to have current anti-viral, anti-spyware, and firewall programs in operation. However, in order to ensure that the email notifications are not filtered as spam, the [DEA-Microgram-2008 -at- mailsnare](mailto:DEA-Microgram-2008-at-mailsnare.net) email address must be "whitelisted" by the Office's ISP.

## Costs

Access to *Microgram Journal* is free.

## Submissions to *Microgram Journal*

Manuscripts are accepted both from within and outside of DEA, and reviewers are both internal (from within DEA) and external.

All submissions must be in English. All submissions should, whenever possible, be submitted electronically, as straight email or as an IBM® PC-compatible Corel WordPerfect® or Microsoft Word® attachment, to: [DEA-Microgram-2007-at-mailsnare.net](mailto:DEA-Microgram-2007-at-mailsnare.net) Current versions of Corel WordPerfect® or Microsoft Word® (defined as having release dates less than 5 years old) should be utilized. If electronic (email) submission is not possible, submissions may be mailed to: *Microgram* Editor, Drug Enforcement Administration, Office of Forensic Sciences, 8701 Morrissette Drive, Springfield, VA 22152. Hard-copy manuscripts should be submitted in triplicate, and should also be accompanied by an electronic version (written in either Corel WordPerfect® or Microsoft Word®) on a 3 ½ inch IBM® PC-compatible diskette, 100 or 250 MB Iomega® zip diskette, or an IBM® PC-compatible CD. **Note that diskettes should be mailed in an irradiation-proof protective sleeve, and the mailing envelope should be marked: “Warning - Contains Electronic Media - Do Not Irradiate”.** Hard-copy manuscripts should be printed in black ink using a laser or ink jet printer, double-spaced, on one side of 8 1/2" x 11" or A4 high quality white bond paper. A Times New Roman/12-point font is preferred for all submissions (electronic or hard copy). Each page, including illustrations, should have a one-inch (25 mm) margin on all sides. All photos and figures should also be submitted as stand-alone attachments, not only embedded in the manuscript. The pages should be numbered, but not stapled together.

Note that mailed submissions may be subject to lengthy handling delays beyond the control of the Office of Forensic Sciences, and electronic media sent through the mail may be destroyed *en route* by sanitizing procedures, despite protective measures and written warnings. All submissions should include the following **Contact Information:** The Full Name and Address of Submitting Laboratory or Office, and the Full Name, Phone Number, FAX Number, and Preferred email Address of the Submitting Individual.

**Scientific Research Articles** are formal, full length reports detailing original research in the detection and analysis of suspected controlled substances for forensic/law enforcement purposes, excluding in post-ingestion human/animal biological matrices (blood, urine, meconium, sweat, hair, etc.) **Technical Notes** are shorter communications concentrating on a specific drug (or drug class), unusual case, novel or unusual procedure or method, or minor original research, again excluding in post-ingestion human/animal biological matrices. Each article/note should be a “stand-alone” work; serial publications will not be considered. Similarly, articles/notes which essentially duplicate existing literature will not be considered unless the presented data reflect significant advances in instrumentation made since the original publication(s) (however, see: Dual Publications, below). All submissions will be subjected to peer review, and authors will be notified of the results of the review(s) within three months after the manuscript is *received* by the Office of Forensic Sciences.

The following guidelines should be used for all Articles (Technical Notes may follow an abbreviated version as appropriate):

**Cover Letter** - Provide the standard contact information and pertinent correspondence (if any) for the Editor.

**Title** - Should be specific and amenable to indexing; they should not include acronyms or abbreviations except for very common instrumental technique acronyms (e.g., GC/MS or HPLC) and/or very common drug acronyms (e.g., MDMA or PCP). Titles should be sufficiently informative that the readership should not have to read the Abstract or the Introduction to understand the focus of the article. If the



manuscript reflects work previously presented at a scientific meeting, a statement detailing that presentation should be included as a footnote to the Title.

**Author(s)/Affiliation(s)** - The author's full name (including middle initial(s)) and title, and the full name and address of the laboratory or office should immediately follow the title. The author's degree level may be included if desired, but is not required (however, multiple authors should all include or all exclude this information). If there are several authors from two or more laboratories or offices, each set of authors should be listed separately, followed by their corresponding laboratory name and address (that is, Authors I, Laboratory I, Authors II, Laboratory II, etc.) Excessive authorship should be avoided. If there is more than one author, the primary author should be indicated with a superscripted asterisk. The name, phone numbers (Voice and FAX), preferred email address, and (if different from the laboratory or office address) the full mailing address of the contact person should be included on the title page.

**Abstract** - State the purpose, procedures, and principal findings of the paper, in 120 words or less. Avoid the use of abbreviations, and use only common acronyms as defined under "Titles". Note that the abstract will be provided to *Chemical Abstracts*.

**Keyword List** - A minimum of five (maximum ten) abstracting keywords should be included. Unless inappropriate, the last keyword pair should always be "Forensic Chemistry."

**Introduction** - Briefly state the issue or problem. Detail existing practice in the topic area, and explain the shortcomings (if any) in what has been previously reported and/or what is being currently done in the field; that is, compare and contrast the selected methodology with previous and/or existing methods. Provide theoretical and practical background for novel or rarely utilized experimental or instrumental methods. Include pertinent references (avoid "Personal Communications").

**Experimental** (Chemicals, Instrumentation, Procedures) - Detail the chemicals, instruments, and procedures utilized (including experimental parameters). However, **USE CAUTION IN DETAILING SYNTHESSES OF CONTROLLED OR ABUSED SUBSTANCES**, especially novel syntheses to known controlled substances, or syntheses of novel substances that may be subject to abuse, that are not yet well known in the scientific and/or underground literature. [In such cases, a simple statement should be included to the effect that: "Experimental details on this synthesis are not provided, in accordance with *Journal* policy."]

**Results and Discussion** - Present findings in a logical, easily followed sequence. Describe what was done, and where appropriate what conclusions can be drawn. Compare and contrast the findings with previous studies and/or current practice. Discuss any problems and/or unresolved issues.

**Conclusions** - Optional - Summarized results should be included only for complex articles. Conclusions should not merely duplicate the Abstract or the summary paragraph in the Results and Discussion section.

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