

GC–MS Studies on the Regioisomeric 2,3- and 3,4-Methylenedioxyphenethylamines Related to MDEA, MDMMA, and MBDB

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Abstract

Three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equal mass have been reported as drugs of abuse in forensic studies in recent years. These compounds are 3,4-methylenedioxy-*N*-ethylamphetamine (MDEA), 3,4-methylenedioxy-*N,N*-dimethylamphetamine (MDMMA), and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). The mass spectra of the regioisomers (2,3-methylenedioxyphenethylamines) are essentially equal to the three compounds reported as drugs of abuse. This paper reports the synthesis, mass spectral characterization, and chromatographic analysis of these six regioisomeric amines. The six regioisomeric methylenedioxyphenethylamines are synthesized from commercially available starting materials. The electron impact mass spectra of these regioisomers show some variation in the relative intensity of the major ions with only a couple of minor ions that may indicate side chain specific fragments. Differentiation by mass spectrometry is only possible after the formation of the perfluoroacyl derivatives, pentafluoropropionylamides (PFPA) and heptafluorobutrylamides (HFBA). Gas chromatographic separation on non-polar stationary phases (Rtx-1 and Rtx-5) is not successful at resolving the three 3,4-methylenedioxyphenethylamines from the three 2,3-methylenedioxyphenethylamines as the underivatized amines. The six underivatized amines are resolved on the more polar trifluoropropylmethyl polysiloxane Rtx-200 stationary phase as well as a permethylated beta-cyclodextran Rtx- β DEX stationary phase. Gas chromatographic separation is successful at resolving the four PFPA and the four HFBA derivatives on the Rtx-200 stationary phase as well as the permethylated beta-cyclodextran stationary phase. The 2,3-methylenedioxyphenethylamine derivatives (compounds 4 and 6) eluted before the 3,4-methylenedioxyphenethylamine derivatives (compounds 1 and 3) as both the PFPA and HFBA derivatives.

Introduction

The methylenedioxyamphetamines, such as 3,4-methylene-

dioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA), are all psychoactive compounds with structural similarities to amphetamines and mescaline (a psychedelic phenethylamine). MDA, MDMA, and MDEA have all been shown to produce very similar peripheral and central effects in humans, with slight differences in potency, time of onset, and duration of action (1). Some of the effects on the central nervous system have been described as a heightened sense of awareness, increased tactile sensations, and a strong desire to be with people, without significant distortion of perception and/or hallucinations (2). Also, the homologous primary amine, 3,4-methylenedioxyphenylbutanamine (BDB), has both hallucinogenic and stimulant effects (3). Monomethylated derivatives of BDB, *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), are less potent than BDB, and the *N,N*-dimethylated derivatives (MMBDB) are behaviorally inactive. However, MBDB has been reported to have novel central nervous system effects with neither stimulant nor hallucinogenic properties (4). In that particular study, MBDB and MDMA were found to be generally similar in effect with two exceptions. First, the onset of action in MBDB was slower and gentler than for MDMA. Second, MBDB seemed to produce euphoria and stimulant properties in lesser amounts than MDMA. Additionally, the rewarding properties of MBDB appeared to be smaller than those of MDMA, as suggested by a 2.5 times weaker potency in the conditioned place preference test in rats (5).

It has been suggested that 3,4-methylenedioxyphenylalkylamines may represent a novel class of pharmacological agents, labeled entactogens (4). These compounds, which include MDA, MDMA, MDEA, and *N*-methyl-3,4-methylenedioxyphenylbutanamine (MBDB), do not fit the pharmacological profile of either phenethylamine hallucinogens (i.e., mescaline) or psychomotor stimulants (i.e., amphetamines). The term entactogen is derived from Greek roots meaning “a touching within”, in reference to the ability of the drugs to promote inward reflection and positive self-assessment.

Regioisomer differentiation is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (6–8). Three of the six substances in this study have

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already appeared in street drug samples. Some of these compounds may be pharmacologically inactive, and the pharmacological properties of others are unknown, but all have the strong possibility to be identified as MDEA, MDMMA, and/or MBDB by mass spectrometry (MS). In this project, all six regioisomers, including MDEA, MDMMA, and MBDB are compared by chromatographic and spectroscopic techniques and methods for their differentiation and resolution were explored.

The ability to distinguish between these regioisomers directly increases the specificity of the analysis for the target drug of abuse. The mass spectrum is usually the confirmatory piece of evidence for the identification of drugs of abuse in forensic laboratories. There are many compounds with essentially equal mass spectra as the drugs of abuse, which makes it very difficult to differentiate in a forensic setting. For major drugs of abuse, such as the amphetamines and MDMA, there are many positional isomers (regioisomers) in the alkyl side chain or in the aromatic ring substitution pattern that can yield nearly an identical mass spectrum. While nuclear magnetic resonance (NMR) can be a useful method for differentiation of these regioisomers, it is not a technique with direct application for all areas of forensic drug chemistry and not always readily available in most laboratories. Thus, the analysis of these "street drug" samples must depend heavily on chromatographic methods as well as MS.

When other compounds exist with the ability to produce nearly identical mass spectra as the drug of interest, identification by gas chromatography (GC)–mass spectrometry (MS) must focus on the ability of the chromatographic system to separate the "non-drug" regioisomers from the drug of interest. The regioisomers that coelute with the drugs of interest in chromatographic separations could be mistaken for the drug of abuse itself. Without the appropriate standards, thorough method validation is not possible, and, thus, coelution of the regioisomer (the non-drug) with the drug remains a possibility.

The targets of this study were six methylenedioxyphenethylamines (see Figure 1) with a molecular weight of 207 and the potential to produce mass spectra with major fragment ions at m/z 72 for the imine and m/z 135/136 for the benzyl fragment. Therefore, analysis of the underivatized regioisomers by electron ionization MS alone does not provide significant data for the

specific differentiation of one of these regioisomers to the exclusion of the other five isomers. The specific identification must be based on a combination of mass spectral data as well as chromatographic resolution of the regioisomeric substances.

Experimental

GC–MS analysis was performed with an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). The MS was operated in the electron impact (EI) mode using ionization voltage of 70 eV and a source temperature of 230°C. Samples were dissolved in high-performance liquid chromatography (HPLC)-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and manually introduced (1 μ L), individually and in a physical mixture, using a 10- μ L Hamilton syringe (Hamilton Co., Reno, NV).

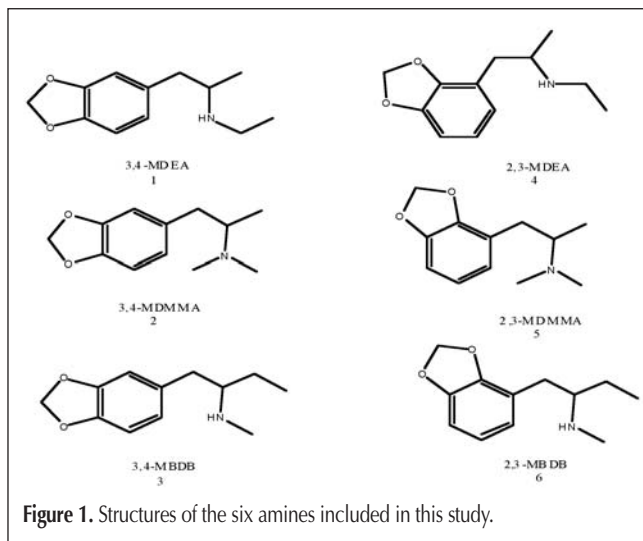
The separation was carried out on a 30 m \times 0.25-mm i.d. column coated with 0.25- μ m 100% dimethyl polysiloxane (Rtx-1), a 30 m \times 0.25-mm i.d. column coated with 0.25- μ m 95% dimethyl-5% diphenyl polysiloxane (Rtx-5), a 30 m \times 0.25-mm i.d. column coated with 0.25- μ m trifluoropropylmethyl polysiloxane (Rtx-200), and a 30 m \times 0.25-mm i.d. column coated with 0.25- μ m 86% dimethyl polysiloxane and 14% cyanopropylphenyl (Rtx- β DEXcst), all obtained from Restek corporation (Bellefonte, PA).

The retention data was generated using two temperature programs. Program 1 consisted of an initial hold at 100°C for 1.00 min, ramped up to 180°C at a rate of 9°C/min and held at 180°C for 2.00 min, then ramped to 200°C at a rate of 10°C/min and held at 200°C for 5.00 min. Program 2 consisted of the exact same temperature conditions with the only difference being between the temperatures of the injector and the detector. For program 1 the injector temperature was 250°C and the detector temperature was 280°C. For program 2 the injector temperature and the detector temperatures were the same, both being 200°C.

Synthesis of methylenedioxyphenethylamines

The synthesis of the regioisomers in this study begins with the appropriately substituted benzaldehyde, 2,3-methylenedioxybenzaldehyde, or 3,4-methylenedioxybenzaldehyde (piperonal). Piperonal was obtained from Aldrich Chemical Company (Milwaukee, WI) and 2,3-methylenedioxybenzaldehyde was made from 2,3-dihydroxybenzaldehyde (Aldrich Chemical Company) according to previously reported methods (8). A sample of 2,3-dihydroxybenzaldehyde was added to a potassium carbonate solution of dimethylformamide (DMF) followed by methylene bromide and copper oxide. The solution was refluxed, water was added, and the mixture was extracted with methylene chloride, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure.

The appropriately substituted methylenedioxybenzaldehyde was added to a solution of *n*-butylamine in benzene. The mixture was refluxed using a Dean-Stark trap and the solvent evaporated to dryness under reduced pressure. The appropriate nitroalkane (nitroethane or nitropropane) was added, and the mixture was refluxed for 1 h followed by addition of acetic acid, water, and ice.



The resulting mixture was acidified with concentrated hydrochloric acid and extracted into methylene chloride, and the organic phase was washed with water and dried over sodium sulfate. The solution was filtered and evaporated to dryness under reduced pressure, and the resulting nitrostyrene product was added to a mixture of toluene, water, and concentrated hydrochloric acid containing ferric chloride and iron. The mixture was refluxed and filtered, and the toluene layer was isolated and extracted with concentrated hydrochloric acid, washed with water, then with saturated sodium bicarbonate, and finally again with water. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The resulting ketone was purified using Kugelrohr distillation.

The appropriate ketone was added to a methanol solution of the appropriately substituted amine hydrochloride and sodium cyanoborohydride. Upon completion, the methanol was evaporated to dryness under reduced pressure. The residue was suspended in water and extracted with methylene chloride; the organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The resulting basic fraction gave light yellow oils which were converted to the hydrochloride salts by dissolving in dry ether followed by gaseous hydrochloric acid.

Derivatization procedure

Each perfluoroamide was prepared individually from the hydrochloride salts by dissolving approximately 0.3 mg (1.5×10^{-6} moles) of each amine in 50 μL of ethyl acetate followed by the addition of a large excess (250 μL) of the appropriate derivatizing agent (pentafluoropropionic anhydride or heptafluorobutyric anhydride). The derivatization reaction mixtures were incubated in capped vials at 70°C for 20 min. Following incubation, each sample was evaporated to dryness under a stream of air at 55°C and reconstituted with 200 μL of ethyl acetate and 50 μL of pyridine. A portion of this solution was further diluted with HPLC-grade acetonitrile, and volumes of 1 μL of the resulting solutions were injected into the GC-MS for analysis.

Results and Discussion

Preparation of the regioisomers

The general methods for the preparation of the six regioisomeric 2,3- and 3,4-methylenedioxyphenethylamines is outlined in Figure 2, and the structures of all six compounds prepared and evaluated in this study are shown in Figure 1. The synthesis for these compounds begins with 2,3- and 3,4-methylenedioxybenzaldehyde (piperonal) as starting materials. The method of preparation for 2,3-methylenedioxybenzaldehyde has been described previously from our laboratory (8), and piperonal is a commercially available material.

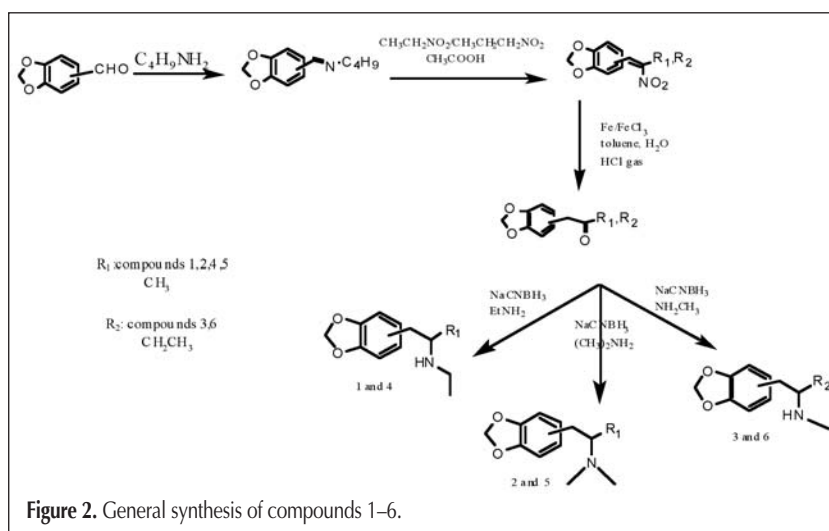
Preparation begins by combining the appropriate methylenedioxybenzaldehyde (2,3- or 3,4-) with *n*-butyl amine and benzene and

allowing the mixture to reflux overnight. The resulting *n*-butylimine is allowed to react with the appropriate nitroalkane (nitropropane or nitroethane). Reduction of the resulting 2-nitroalkene is done with iron and ferric chloride in refluxing toluene. Initial reduction occurs at the nitro group to yield the enamine which, upon hydrolysis, yields the desired ketone. The ketones were purified by Kugelrohr distillation, then reacted with the appropriate amine hydrochloride (dimethyl, methyl, or ethyl) and sodium cyanoborohydride in methanol. This reductive amination yields the desired amines, which were converted into the hydrochloride salts using gaseous hydrochloric acid. Combination of the appropriate aldehyde, nitroalkane, and alkyl amine allowed for the synthesis of the six amines shown in Figure 1 (labeled as compounds 1–6).

Mass spectral studies of the regioisomers

Figure 3 shows the EI mass spectra for the regioisomeric 2,3- and 3,4-methylenedioxyphenethylamines 1–6 included in this study. MS is the primary method for confirming the identity of drugs of abuse in forensic samples. These spectra indicate that very little structural information is available for differentiation among these regioisomers because the major fragment ions occur at equal masses. The mass spectra are characterized by a base peak formed by an alpha cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. The alpha cleavage reaction yields the regioisomeric butylimine fragments at m/z 72, the 2,3- or 3,4-methylenedioxybenzyl cation fragment at m/z 135, and the radical cation at m/z 136. The major fragmentation patterns are shown in Figure 4. These regioisomers all have essentially the same mass spectra, which represent a significant challenge for analytical drug chemistry. This is especially significant in this series of amines since compounds 1, 2, and 3 have all been reported in recent years as components of clandestine drug samples (2,9).

The m/z 44 ion in the spectra for compounds 1 and 4 occurs from the loss of ethylene from the *N*-ethyl group of the base peak (m/z 72). However, this low mass ion is present in the mass spectra for many substituted phenethylamines as well as other compounds and does not provide enough diagnostic information to



individualize these mass spectra. These spectra also contain ions of much lower relative abundances, which do not provide sufficient information for differentiation among these regioisomers.

In the next part of this study, various perfluoroacylated derivatives of the 2,3- and 3,4-methylenedioxyphenethylamines were prepared and evaluated in an effort to individualize the mass spectra and maintain or improve chromatographic resolution. Acylation of the amines greatly lowers the basicity of the nitrogen, and this often allows other fragmentation pathways to play a more prominent role in the mass spectrum (10). The pentafluoropropionyl (PFPA) and heptafluorobutryl (HFBA) derivatives of the methylenedioxyphenethylamines were evaluated for their ability to individualize the mass spectra and provide unique ions for compound identification and differentiation. The mass spectra for the four pentafluoropropionyl and the four heptafluorobutryl amides of the secondary amines (the tertiary amines do not form stable acylation products) are shown in Figures 5 and 6. For the PFPA and HFBA derivatives, the spectra show a common base peak at 218 and 268, which corresponds to the loss of 135 mass units from the molecular ions at 353 and 403, respectively. The ions at m/z 218 and 268 are the PFPA and HFBA imine species likely formed from the alpha cleavage reaction of the amide

nitrogen. Thus, the m/z 218 and 268 ions in the mass spectrum of the PFPA and HFBA amides are analogous to the m/z 72 in the underivatized species. Figure 7 shows the structures of the major fragment ions for the perfluoroacyl derivatives of 1–6.

There are two major diagnostic pathways in the mass spectrum of the PFPA and HFBA derivatives, which allows differentiation of the side chain regioisomers. The first of these is the alkene fragment observed at m/z 162 and m/z 176; these ions occur in the spectra for both the PFPA and HFBA derivatives, indicating that the perfluoroacyl moiety is not a component of these ions. The m/z 162 ion occurs in the mass spectrum for the PFPA and HFBA derivatives of compounds 1 and 4, the *N*-ethyl MDAs. This alkene fragment is the radical cation (see Figure 7) resulting from cleavage of the bond between nitrogen and the alkyl carbon of the hydrocarbon side chain. This bond cleavage occurs following an initial hydrogen rearrangement yielding the radical cation species at m/z 162. Thus, the m/z 162 ion is indicative of the C3 alkene chain attached to the aromatic ring. The analogous fragmentation pathway for the PFPA and HFBA derivatives of compounds 3 and 6 yields a fragment ion at m/z 176, indicating a C4 side chain attached directly to the methylenedioxyphenyl ring.

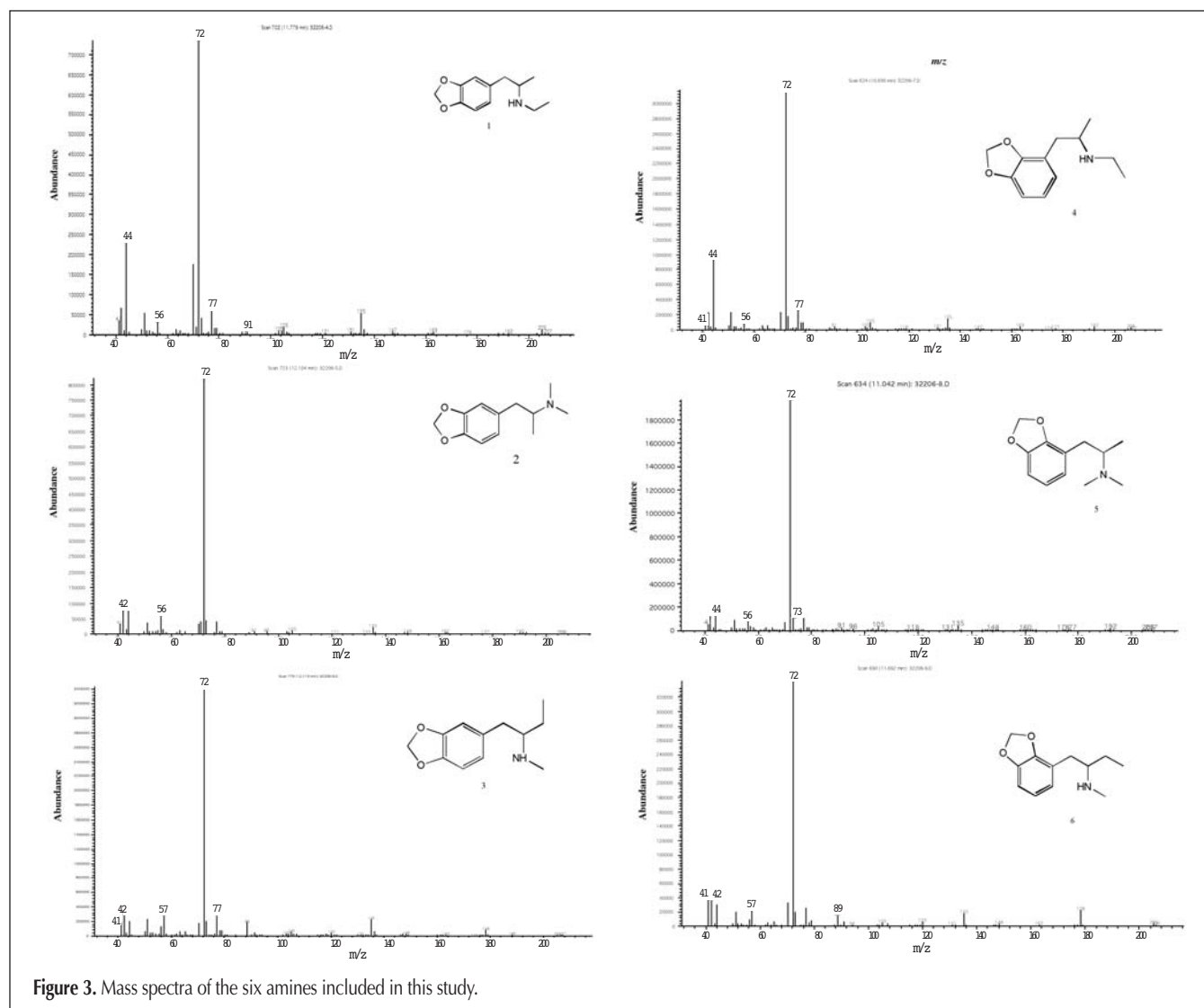
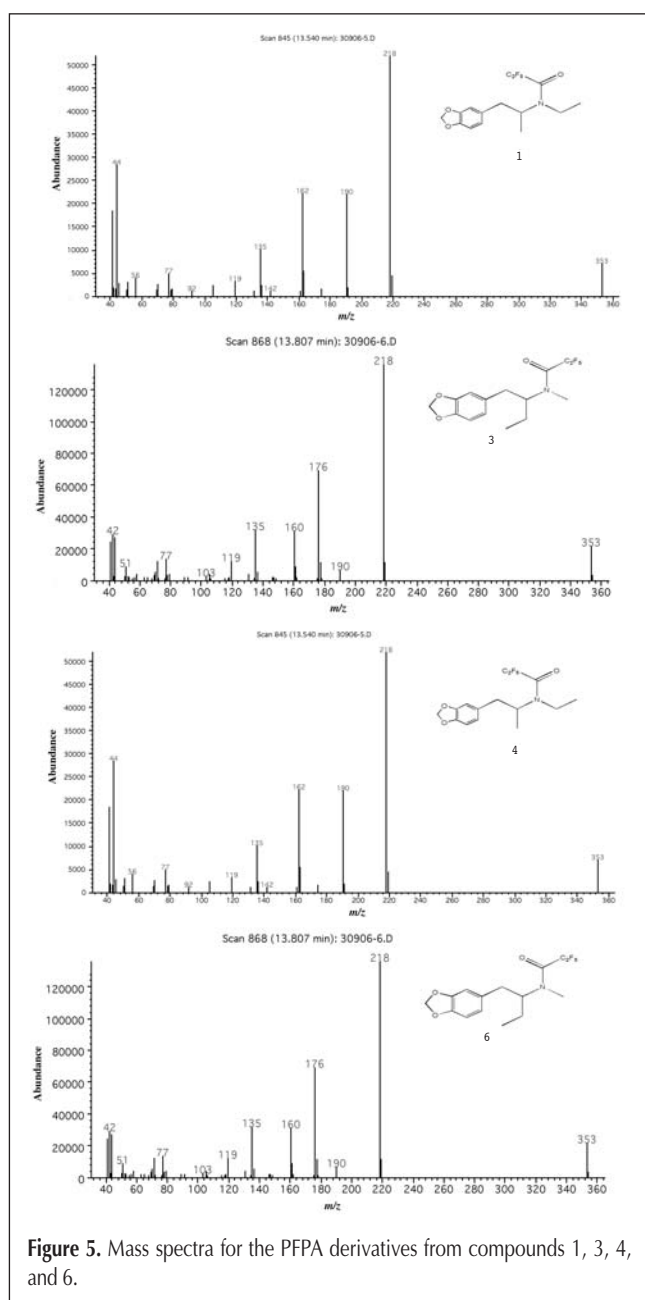
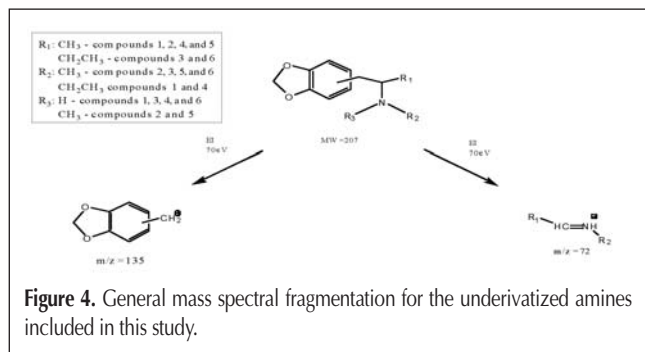
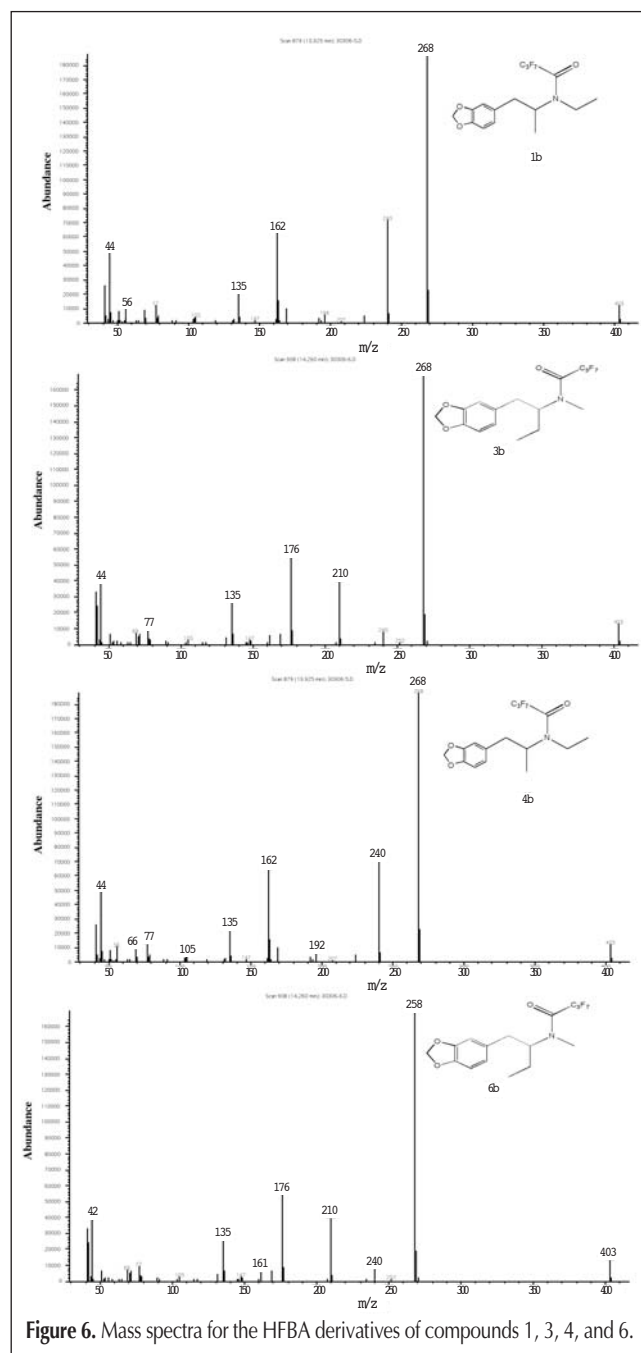


Figure 3. Mass spectra of the six amines included in this study.

The second diagnostic fragmentation pathway does contain the perfluoracyl group or a portion of the perfluoroacyl groups and therefore appears at different masses for the PFPA and HFBA derivatives. The loss of mass 28 (the *N*-ethyl group lost as ethylene)



lene) from the base peak in the mass spectrum of compounds 1 and 4 appears at m/z 190 and m/z 240 for the PFPA and HFBA derivatives, respectively. While these ions can occur in the *C*-ethyl regioisomers (compounds 3 and 6), the loss of ethylene from the *C*-ethyl amine derivatives is a much less prominent ion. When the side chain consists of an *N*-methyl group (compounds 3 and 6), the base peak undergoes a rearrangement fragmentation reaction to yield m/z 160 and m/z 210 for the PFPA and HFBA derivatives, respectively, (Figure 7). The structure of this ion has been confirmed by deuterium labeling experiments in other series of *N*-methyl-phenethylamines (6,10). Thus, the mass spectra of these perfluoroacyl derivatives allow identification of the carbon side chain attached directly to the aromatic ring and identification of the alkyl group bonded to nitrogen.

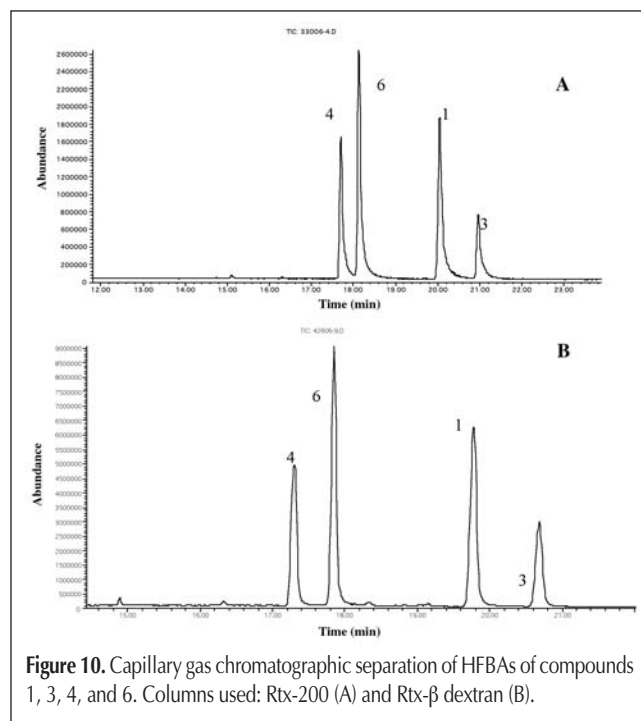
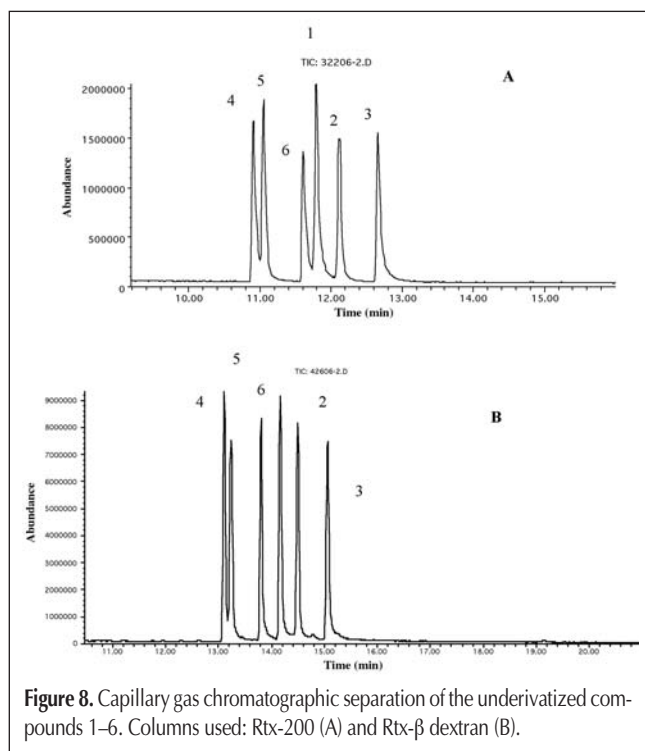
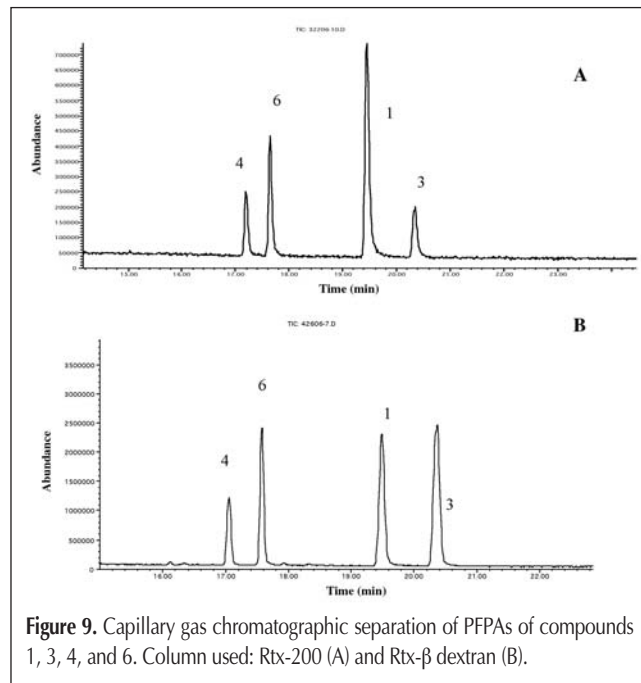
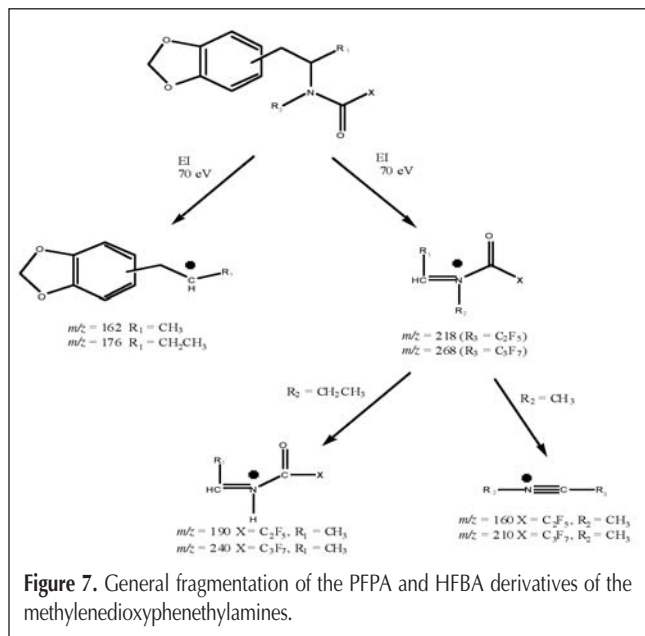


Gas chromatography of the regioisomers

When other compounds exist that have the potential to produce nearly identical mass spectra as the drug of interest, the separation of the “nondrug regioisomers” from the actual drug is a critical issue. MS alone does not provide enough information to distinguish between the 6 regioisomers in this study as the underivatized species. Therefore, the identification by GC–MS must depend heavily on the ability of the chromatographic system to separate the drug molecules from the nondrug regioisomers.

The gas chromatographic separation of amines 1–6 is shown in Figure 8. This separation was obtained using a 30 m × 0.25-mm i.d. column with a 0.25- μ m film of trifluoropropylmethyl

polysiloxane, Rtx-200 (Figure 8A), and a permethylated cyclodextran, Rtx- β dextran (Figure 8B), column of the same dimensions. The retention times are greater on the Rtx- β DDEX stationary phase, and the resolution and peak shape are also better on the Rtx- β DDEX stationary phase. The chromatograms in Figure 8 show that the three 2,3-methylenedioxyphenethylamines, compounds 4, 5, and 6, elute before any of the three 3,4-methylenedioxyphenethylamines, compounds 1, 2, and 3. The order of side chain elution within the individual ring substitution patterns is *N*-ethyl followed by *N,N*-dimethyl in the arylpropylamine series. The *N*-methyl derivative of the arylbutanamine elutes third within each ring substitution series. Thus, the longest contin-



uous hydrocarbon side chain (C4) shows the greatest retention on the Rtx-200 and Rtx- β dextran phases. The retention properties of these amines were also compared on a more nonpolar (100%) dimethyl polysiloxane column (Rtx-1) and 95% dimethyl-5% diphenyl polysiloxane (Rtx-5) of the same column dimensions and film thickness as described earlier. The observed elution order of the side chain regioisomers and ring regioisomers was the same as that obtained for the more polar phase, Rtx-200. However, in our experiments using the same temperature programs as used in Figure 8, complete resolution of all six compounds was not obtained. Compounds 1 and 6 coelute under these conditions.

The gas chromatographic properties of the PFPA and HFBA derivatives of the four regioisomers were compared on four columns: a dimethyl polysiloxane (Rtx-1), dimethyl-diphenyl polysiloxane (Rtx-5), trifluoropropylmethyl polysiloxane (Rtx-200), and permethylated cyclodextran (Rtx- β DEX). The PFPA and HFBA derivatives showed improved resolution when compared to the underivatized amines. Several temperature programs were evaluated, and the best compromises between resolution and analysis time were used to generate the data in the chromatograms in Figures 9 and 10. The chromatogram for the PFPA derivatives in Figure 9 were generated using the Rtx-200 column and Rtx- β DEX with fairly high temperature programs. In this case, the derivatized compound 4 elutes first, closely followed by compound 6. The third compound to elute is compound 1 followed by compound 3. The elution order is exactly the same for the PFPA derivatives as well. In both cases, compound 3 showed the greatest retention. The resulting data shows that the same elution order was observed in both the derivatized and the underivatized compounds. Resolution of the derivatives was excellent on both phases.

Conclusion

In summary, three regioisomeric 3,4-methylenedioxyphenethylamines having equal molecular weight and major mass spectral fragments of equivalent mass have been reported as components of clandestine drug samples in recent years. These drugs of abuse (MDEA, MDMMA, and MBDB) are a subset of a total of six methylenedioxyphenethylamines of molecular weight 207 yielding regioisomeric fragment ions of equal mass (m/z 72 and 135/136) in the electron impact mass spectra. The results of this study show that the traditional EI mass spectrum provides little structural information for differentiating among these 6 compounds. Because of the unique similarity of these compounds by mass spectrometry, the specific identification of a compound such as 3,4-MBDB requires methods to eliminate the other five isomers. Thus, the ultimate identification of any one of

these amines with the elimination of the other five regioisomeric substances depends heavily on chromatographic methods.

Derivatization of these amines with various perfluoroacylating agents yields amides with improved resolution compared with the underivatized amines by gas chromatography. These perfluoroacyl derivatives significantly individualize the mass spectra. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes; however, the HFBA derivatives offer more unique fragment ions for additional mass spectral discrimination among these regioisomers. Although columns Rtx-1, Rtx-5, Rtx-200, and Rtx- β DEX were all used to generate retention/resolution data in this study, the Rtx-200 and the Rtx- β DEX stationary phases gave the best resolution of the underivatized amines and the derivatized amides.

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