Chromatographic and Mass Spectrometric Methods for the Differentiation of *N*-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine from Regioisomeric Derivatives

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Abstract

Methods are described for the gas chromatographic–mass spectrometric identification of the street drug *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB or MDP-2-MB) and its differentiation from two uniquely isomeric drugs, *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA) and *N,N*-dimethyl-3,4-methylenedioxyamphetamine (MDMMA). These positional isomers have the same molecular weight (MW = 207) and fragment by a common mechanism under electron impact mass spectrometric conditions to yield a base peak of the same mass (*m/z* 72). Derivatization of the two secondary amines (MBDB and MDEA) with pentafluoropropionic anhydride (PFPA) yields amides with fragment ions which individualize their EI mass spectra. The PFPA derivative of MBDB yields diagnostic ions at *m/z* 160 and 176, whereas the PFPA derivative of MDEA produces ions at *m/z* 162 and 190. This EI spectra individualization for MBDB and MDEA is particularly significant since these two compounds have similar retention properties in the PFPA-derivatized and underivatized forms and since both are known street drugs.

Introduction

Over the past several decades, there has been significant pharmacological and forensic interest in methylenedioxyamphetamine (1-[3,4-methylenedioxyphenyl]-2-propanamine, MDA) and several *N*-substituted derivatives of MDA (1–4). The popularity of compounds of this structural class in the clandestine market appears to be related to their ability to elicit central nervous system (CNS) effects beyond the stimulant and hallucinogenic effects characteristic of many other drugs of abuse (e.g., methamphetamine, cocaine, LSD). Most notable of these are their "entactogenic" properties, which include the ability to decrease anxiety, increase self-awareness, and enhance empathy.

Pharmacological studies have demonstrated that the entactogenic potential of MDA derivatives is a function of *N*-substitution. For example, the parent primary amine (MDA) displays CNS stimulant (methamphetamine-like) and hallucinogenic (LSD-like) activities, as well as some entactogenic properties. The *N*-methyl derivative, MDMA, also displays some stimulant and entactogenic activity, but it has little hallucinogenic activity, and the *N*-ethyl analogue, MDEA, exhibits only entactogenic activity. Continued synthetic and pharmacologic studies led to the discovery of *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (*N*-methyl-1-[1,3-benzodioxol-5-yl]-2-butanamine, MBDB), a side chain homologue of MDMA that is reported to possess primarily entactogenic activity.

In the past, only MDA and several *N*-substituted derivatives including *N*-methyl-MDA (MDMA), *N*-ethyl-MDA (MDEA), *N,N*-dimethyl-MDA (MDMMA), and *N*-hydroxy-MDA (*N*-OHMDA) analogues (Scheme 1) have been identified in clandestine drug samples. Recently, however, there have been several reports from the United States (2) and abroad (3) of the appearance of MBDB in street drug samples.

MBDB is a regioisomer of the MDA derivatives, *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA) and *N,N*-dimethyl-3,4-methylenedioxyamphetamine (MDMMA), and the MDMA homologue, *N*-methyl-1-(3,4-methylenedioxyphenyl)-3-butanamine (HMDMA) (Scheme 1). All of these compounds have the same molecular weight and yield similar mass spectra characterized by molecular ions of low abundance and base peaks at *m/z* 72 resulting from amine-dominated cleavage. Although these isomers clearly yield different proton magnetic resonance spectra, this is not a common technique used for the small amounts of samples often found in forensic and drug screening samples.

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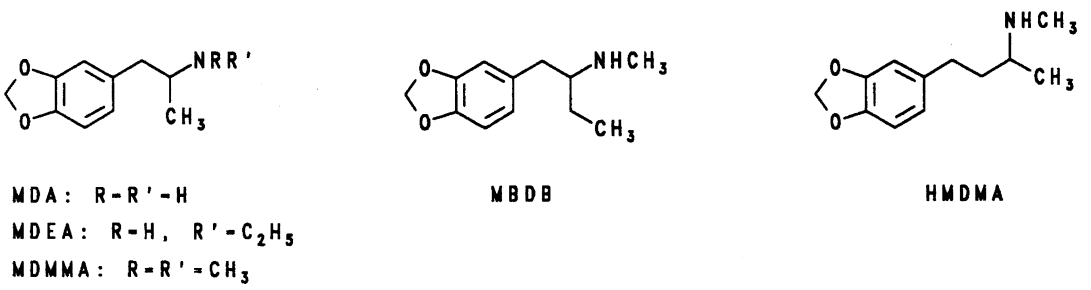


Scheme 1. Structures of MBDB, MDA, MDEA, MMDMA and HMDMA.

NMR methods would not be directly useful for the analysis of drugs from biological samples. In an earlier study, Rosner and Junge (3) reported differences in the mass spectra of these three uniquely isomeric compounds to lie in the daughter ion spectra for the base peak (*m/z* 72) (3). However, without the aid of this MS–MS technique, mass spectrometry does not provide significant data for differentiation among these three compounds. In this paper, alternative methods involving derivatization are described to differentiate MBDB from its isomeric analogues.

Experimental

Instrumentation and methods

GC–MS analyses were performed using a Hewlett-Packard 5970B mass selective detector (Palo Alto, CA). The ionization voltage was 70 eV, and the source temperature was 220°C. The mass range was 35–500, and the scan rate was 1.38. The samples were dissolved in methanol (0.5 mg/mL), and 1.0 µL was introduced into the mass spectrometer via a gas chromatograph equipped with a 12-m × 0.20-mm i.d. fused-silica column with a 0.33-µm film thickness of methylsilicone (HP-1). The column temperature was held at 70°C for 2.5 min and programmed to 170°C at a rate of 25°C/min and from 170°C to 275°C at a rate of 12°C/min with a hold time of 6 min. The split ratio for the GC was 20:1, and the injector port temperature was 230°C.

The samples derivatized with PFPA were prepared by extracting a 0.5 mg/mL solution of each amine in 0.5N NaOH (4 mL) with *n*-hexane (10 mL) and adding PFPA (50 µL) to the extract solutions. The solutions were heated at 120°C for 10 min. The hexane solutions were evaporated to dryness, and the residue was dissolved in *n*-hexane. One microliter was injected into the GC.

Liquid chromatographic analyses were conducted using a Laboratory Data Control Constametric 3000 pump, 3100 Spectromonitor ultraviolet detector, and CI 4100 Integrator (Riveria Beach, FL) and a Rheodyne 7125 injector (Cotati, CA). The analytical column had dimensions of 30 cm × 3.9-mm i.d. and was packed with Bondclone C_{18} (Phenomenex Inc., Torrance, CA). The analytical column was preceded by a Direct Connect guard column (Alltech; State College, PA) packed with CO:Pell ODS (Whatman). The mobile phase consisted of a phosphate buffer (pH 3.0), acetonitrile, and triethylamine (600:100:1) at a flow rate of 1.25 mL/min. The phosphate buffer was prepared by mixing 9.2 g of monobasic sodium phosphate in 1 L of double-distilled water and adjusting the pH to 3.0 with H_3PO_4. The ultraviolet absorbance detector was operated at 280 nm and 0.2 AUFS. A 15-µL aliquot of a methanol solution (1 mg/mL) of each compound was injected.

Synthesis

N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA), *N,N*-dimethyl-3,4-methylenedioxyamphetamine (MDMMA), and *N*-methyl-1-(3,4-methylenedioxyphenyl)-3-butanamine (homo MDMA, HMDMA) were synthesized as reported previously (4,5).

Results and Discussion

The mass spectra for the isomeric amines (MBDB, MDEA and MDMMA) are shown in Figure 1 and the fragmentation pattern that yields the base peak is illustrated in Scheme 2. The base peak at *m/z* 72 for each of these amines is the C_4-iminium species resulting from the loss of the 3,4-methylenedioxybenzyl radical from the molecular ion. Although the precise structure for the base peak varies among these compounds, as illustrated in Scheme 2, the mass of the ion remains constant. Figure 1A shows the mass spectrum for MBDB with the base peak at *m/z* 72 and minor ions at 135 and 136 for the methylenedioxybenzyl radical and rearranged radical cation, respectively. The *m/z* 44 ion in the spectrum of MDEA (Figure 1B) is a predominant fragment. This ion occurs through the loss of ethylene from the *N*-ethyl group of the base peak C_4-iminium species. Thus, one of the major differences in the spectra in Figure 1A and 1B is the relative intensity of the *m/z* 44 ion. Similarly, Rosner and Junge (3) reported that the major differences in the mass spectra for these three uniquely isomeric compounds are related to the daughter ion spectra for the base peak (*m/z* 72). Without the aid of this MS–MS technique, mass spectrometry cannot provide significant data for

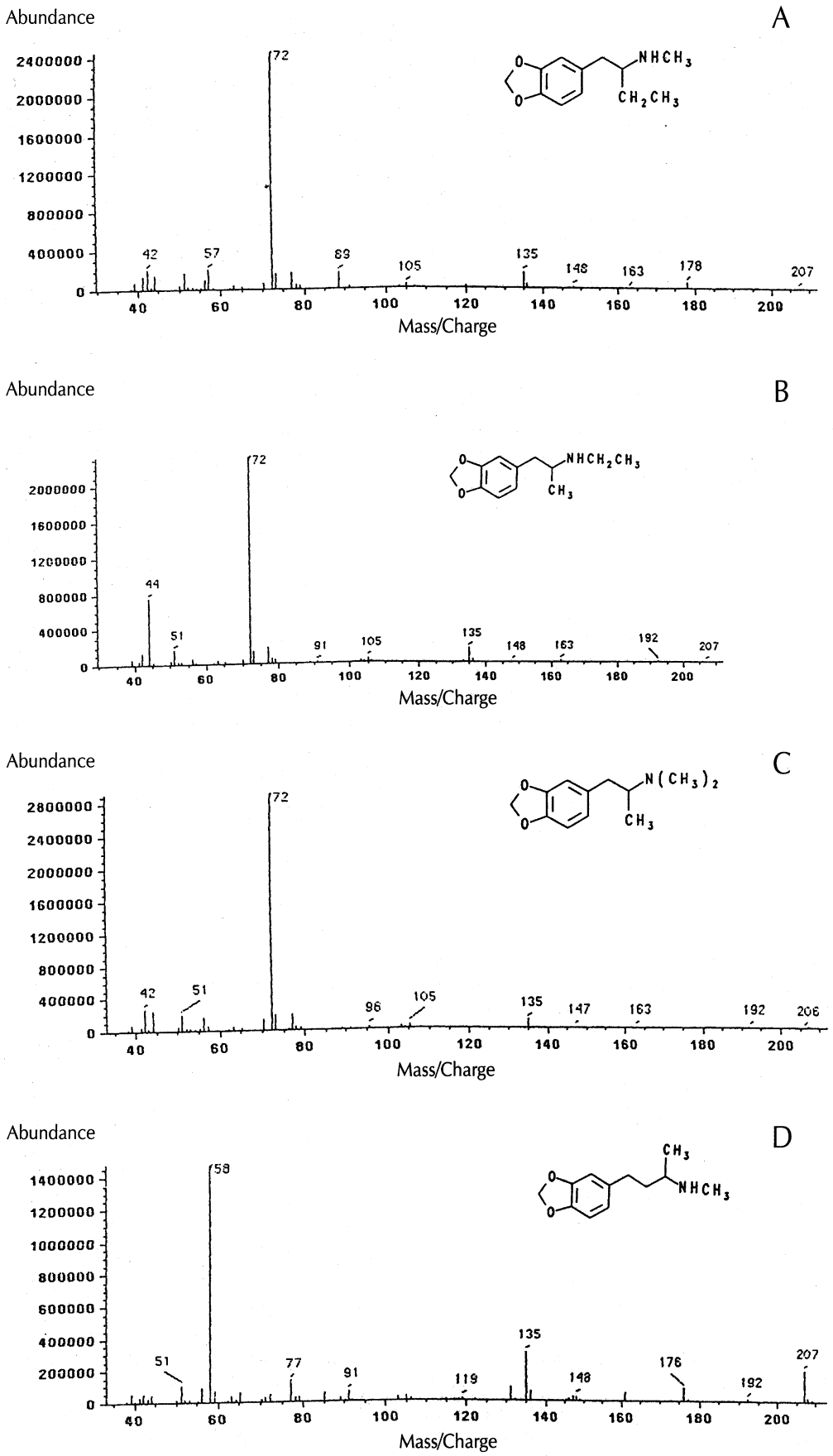
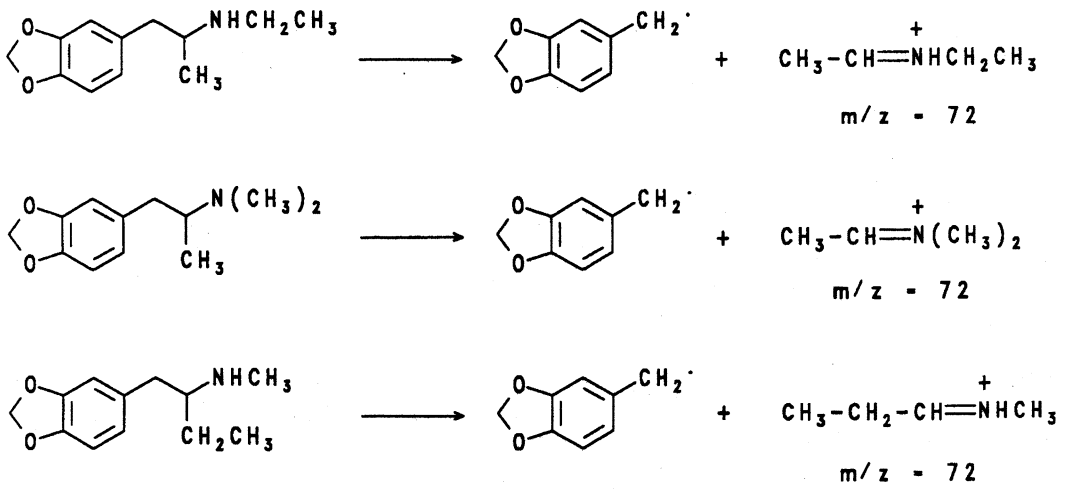


Figure 1. Mass spectra of MBDB (A), MDEA (B), MDMMA (C), and HMDMA (D).



Scheme 2. Amine-dominated MS fragmentation of MDEA, MMDMA and MBDB.

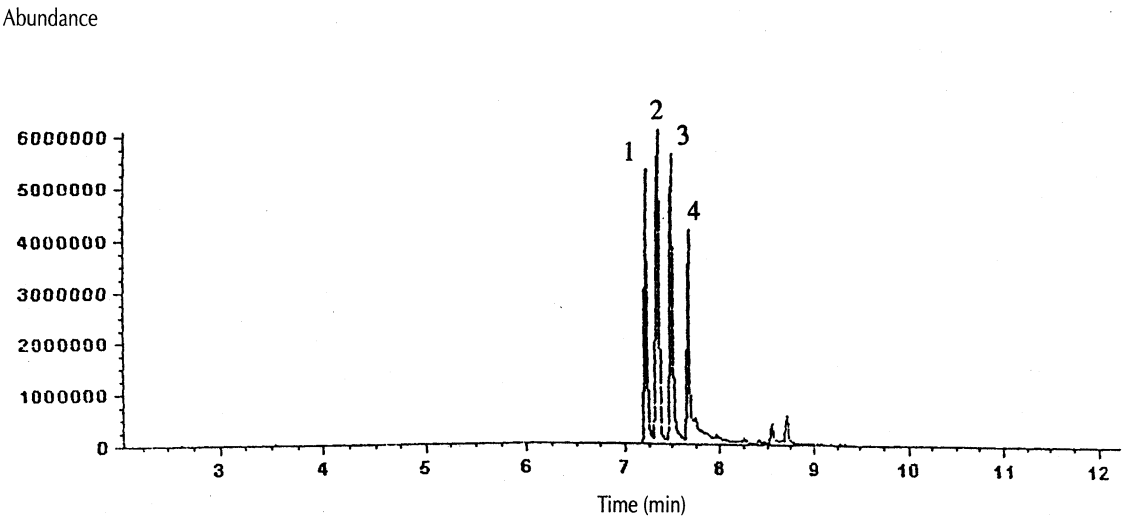


Figure 2. Gas chromatographic separation of MDEA (peak 1, 7.23 min), MDMMA (peak 2, 7.35 min), MBDB (peak 3, 7.50 min), and HMDMA (peak 4, 7.68 min).

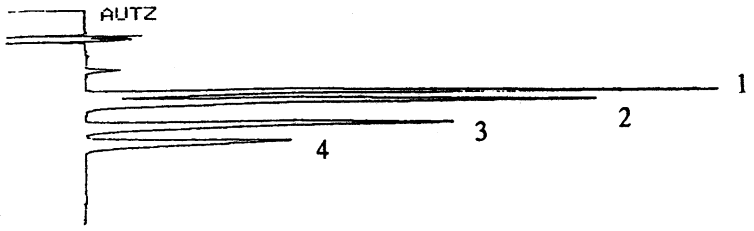


Figure 3. Liquid chromatographic separation of MDMMA (peak 1, 7.93 min), MDEA (peak 2, 8.77 min), MBDB (peak 3, 10.91 min) and HMDMA (peak 4, 12.60 min).

differentiation among these three compounds.

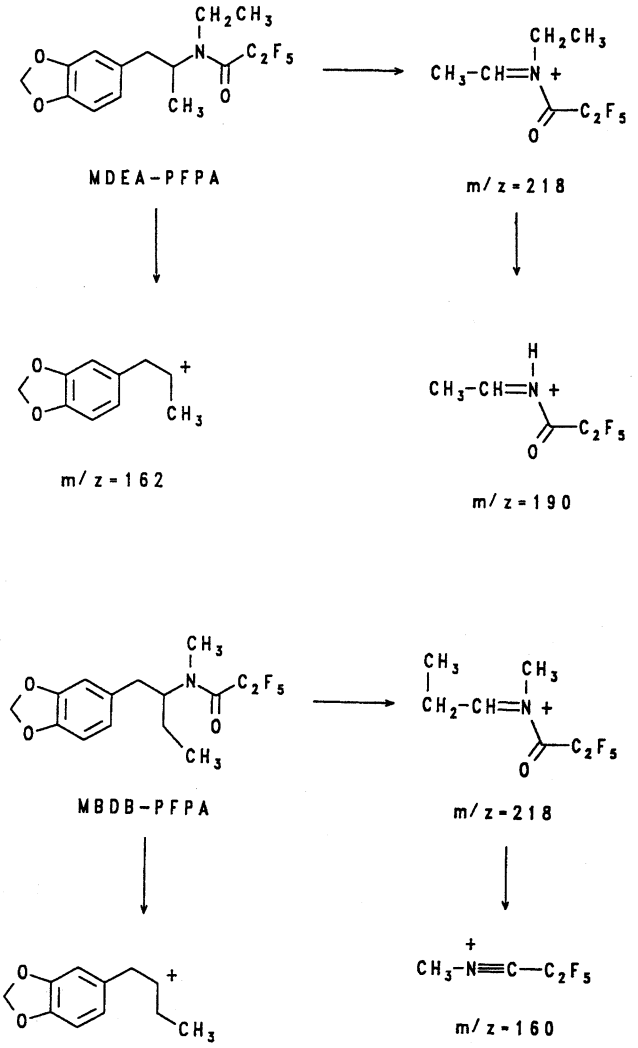
The mass spectrum for MDMMA is shown in Figure 1C. Its major fragment ions are similar to those in Figure 1A and 1B. The primary differentiation among these compounds must be made based on chromatographic resolution since their mass spectra are very similar. Thus the specific identification of one of these uniquely isomeric amines would require a complete set of ref-

erence standards in order to validate the ability of the chromatographic system to resolve these three compounds. This differentiation is especially critical since both secondary amines (MDEA and MBDB) are known drugs of abuse.

The mass spectrum in Figure 1D was obtained for *N*-methyl-1-(3,4-methylene-dioxyphenyl)-3-butanamine (homo MDMA, HMDMA). This compound has the same molecular weight as the other three amines, but a methylamino group is substituted at the 3-position of the butanamine side chain. This difference produces a base peak of lower mass (*m/z* 58) and allows this isomeric amine to be easily distinguished from the other three amines of the same molecular weight whose mass spectra are shown in Figure 1A–1C.

The GC separation of the four compounds is shown in Figure 2. This separation was obtained with a 12-m × 0.20-mm i.d. column containing a 0.33-µm thickness of methylsilicone (HP-1). The GC was operated at 70°C for 2.5 min and programmed to 170°C at a rate of 25°C/min and then to 275°C at a rate of 12°C/min with a final hold time of 6 min. The four compounds eluted over a 0.5-min window under these conditions. HMDMA has the highest retention time (peak 4, 7.68 min). The three compounds that have a molecular weight of 207 and a base peak at *m/z* 72 elute over approximately 0.25 min with the two MDA derivatives, MDEA (peak 1, 7.23 min) and MDMMA (peak 2, 7.35 min) eluting before MBDB (peak 3, 7.50 min). Due to the similar retention properties of these three uniquely isomeric amines, reference standards are necessary to make a specific identification based on direct GC–MS data from the underivatized amines.

The liquid chromatographic separation of these compounds is shown in Figure 3. The slight variation in hydrophobic surface area allows for efficient resolution of these isomers in a reversed-phase system. The system consisted of a silica-based C_{18} stationary phase and an acidic mobile phase of phosphate buffer (pH 3.0), acetonitrile, and triethylamine (600:100:1). The compound eluting first (peak 1, 7.93 min) is the tertiary amine MDMMA followed by MDEA (peak 2, 8.77 min). The two propanamines with the C_3 carbon chain attached to the aromatic ring elute before either of the compounds that have an aryl-C_4 structure. The two aryl-C_4 butanamines are more retained; MBDB (peak 3, 10.91 min) elutes before HMDMA (peak 4, 12.60 min). However, the specific identification of MBDB, MDEA, and MDMMA would still require reference standards of all of these compounds to ensure that the chromatographic system can resolve these uniquely isomeric compounds.



Scheme 3. MS fragmentation of the PFPA derivatives of MDEA and MBDB.

In previous work (6,7), methamphetamine and four isomeric amines with a molecular weight of 149 and a base peak at *m/z* 58 yielded individualized mass spectra following formation of the pentafluoropropionylamide (PFPA) derivatives. The individualization results from loss of the amide group to yield a significant arylhydrocarbon fragment. The PFPA derivatives of MBDB, MDEA, and HMDMA were prepared in an effort to gain additional information from the mass spectra of these isomers. Tertiary amines such as MDMMA do not form stable acylated derivatives. Thus a mass spectrum that remains unchanged following treatment with acylation reagents such as pentafluoropropionic anhydride could indicate the presence of a tertiary amine. The mass spectra in Figure 4 show significant major fragments that can be used to differentiate among the most similar of these amines, MBDB and MDEA. All three spectra in Figure 4 show a molecular ion at *m/z* 353 and a major fragment at *m/z* 135 for the 3,4-methylenedioxybenzyl carbocation. The base peak in the spectra for the PFPA derivatives of MBDB and MDEA occurs at *m/z* 218 and is the result of loss of the 3,4-methylenedioxybenzyl radical from the molecular ion $(M\text{-}135)^+$. The PFPA group sufficiently weakens the bond between nitrogen and the aliphatic carbon to produce a significant arylhydrocarbon fragment, arylpropene, from MDEA (*m/z* 162, Figure 4A) and the fragment, arylbutene, from MBDB (*m/z* 176, Figure 4B).

The spectrum of the PFPA derivative of MDEA (Figure 4A) shows an additional characteristic

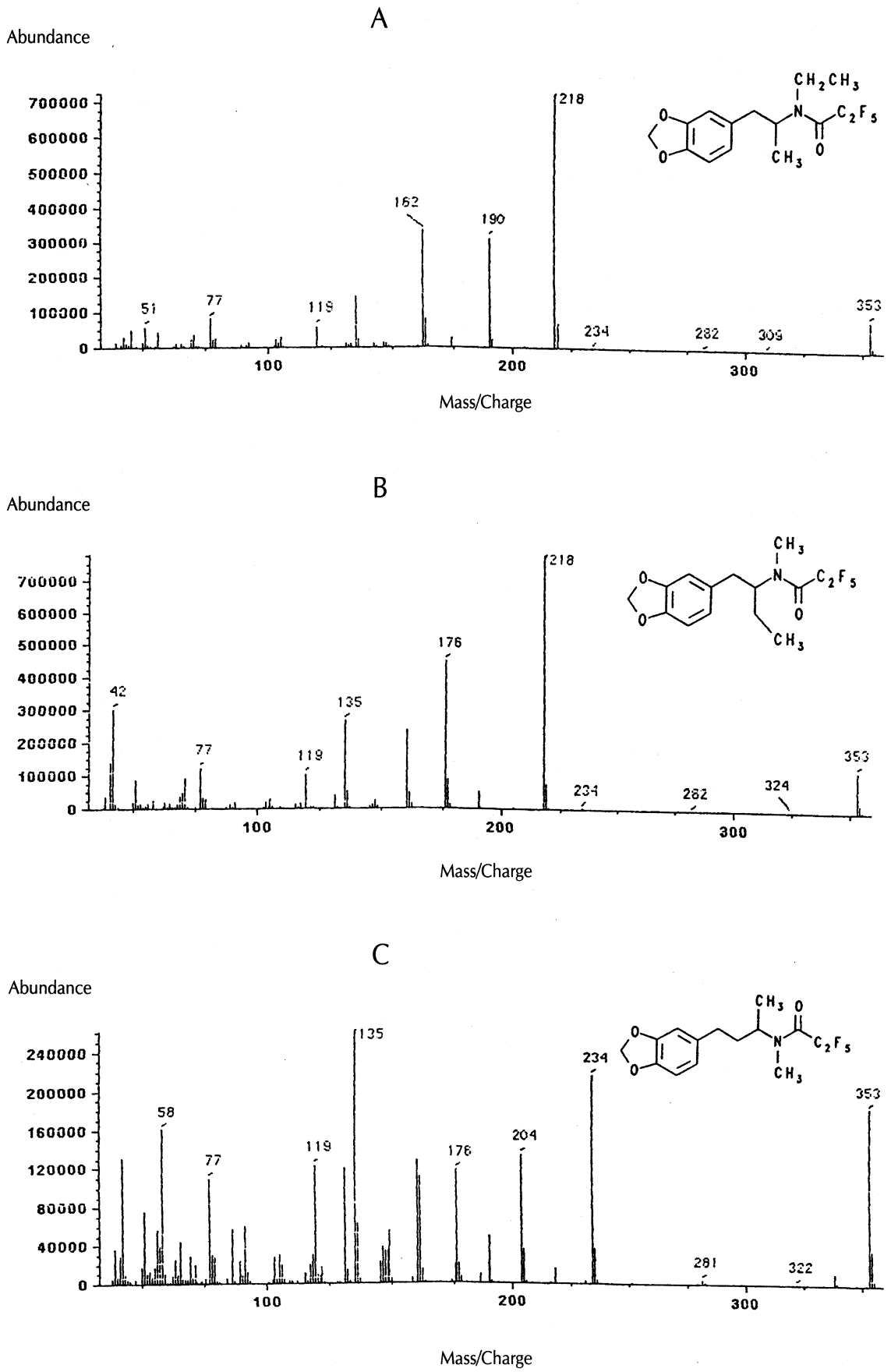
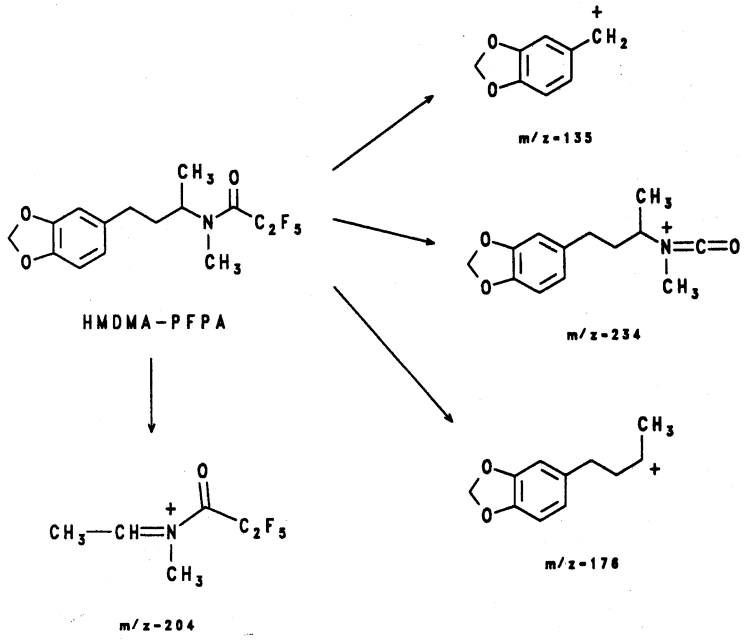


Figure 4. Mass spectra of the PFPA derivatives of MDEA (A) MBDB (B), and HMDMA (C).

fragment ion at *m/z* 190. This ion is the result of a rearrangement of the *N*-ethyl group of the base peak to lose ethylene (Scheme 3). The second unique ion in the spectrum of the PFPA derivative of MBDB (Figure 4B) occurs at *m/z* 160. The structure for this fragment is shown in Scheme 3. This ion is the result of a four-centered addition/decomposition reaction of the base peak. The result is the loss of a molecule of propionaldehyde (or equivalent) from the *m/z* 218 ion. This rearrangement reaction appears to be unique to the acylated *N*-methyl imine fragment such as the *m/z* 218 fragment for the PFPA derivative of MBDB. This fragment has been observed (6–8) in the mass spectra of various acylated derivatives of methamphetamine, MDMA, ephedrine and other related compounds. The structure of the *m/z* 160 fragment in the mass spectrum for the PFPA derivative of methamphetamine was confirmed by deuterium labeling experiments in a previous study (6,7).

The PFPA derivative of HMDMA shown in Figure 4C is easily differentiated from the two other spectra since the base peak occurs at *m/z* 135. This spectrum shows the *m/z* 176 fragment for 3,4-methylenedioxyphenylbutene radical cation and a major fragment at *m/z* 204 for the PFPA-propylimine, 14 mass units less than the PFPA-butylimine base peaks in the mass spectra of MDEA–PFPA and MBDB–PFPA. The significant fragment at *m/z* 234 in Figure 4C is the loss of 119 mass units from the molecular ion and most likely is the loss of C_2F_5 from the molecular ion (Scheme 4).

The GC separation of the three PFPA-derivatized amines is shown in Figure 5. The amide derivatives show slightly higher retention than the underivatized amines and elute in the same relative order. The PFPA derivatives of MDEA and MBDB show the most similar elution properties and actually show poorer resolution than the underivatized amines under these conditions. The chromatograms in Figures 2 and 5 were obtained under identical chromatographic conditions. However, a comparison of the spectra in Figure 4A and 4B shows fragment ions of significant relative abundance which can be used to differentiate between the uniquely isomeric compounds, MBDB and MDEA. The PFPA derivative of MDEA shows major fragments at *m/z* 162 and 190, and the mass spectrum for the PFPA derivative of MBDB shows characteristic ions at *m/z* 176 and 160. The PFPA derivatization of these unique isomers results in sufficient individualization of their mass spectra to allow for specific identification. Thus, PFPA derivatization is an economical alternative to the MS–MS techniques for the identification and differentiation of these two drugs of abuse.



Scheme 4. MS fragmentation of the PFPA derivative of HMDMA.

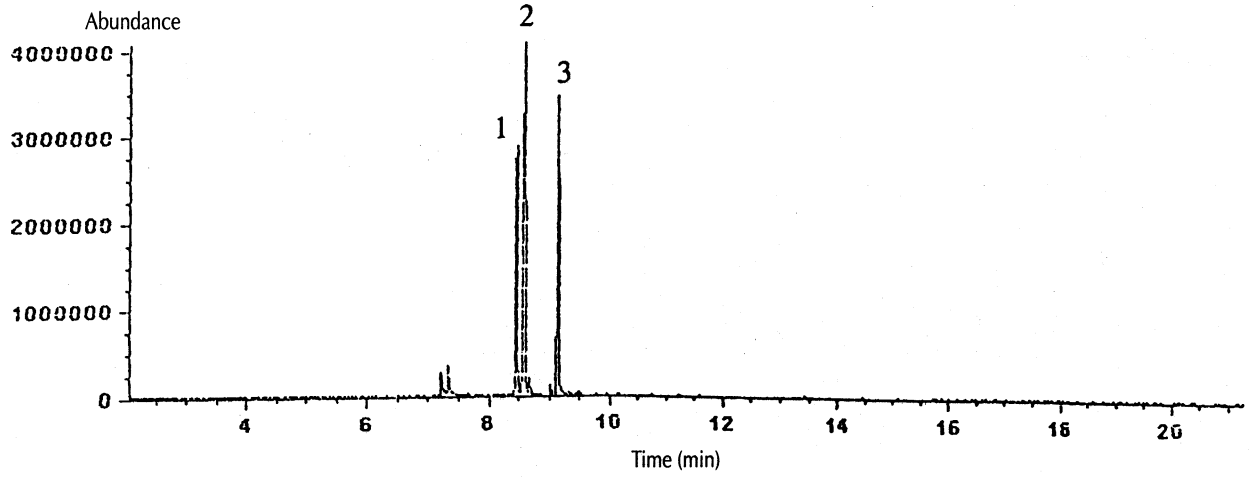


Figure 5. Gas chromatographic separation of PFPA-MDEA (peak 1, 8.46 min), PFPA-MBDB (peak 2, 8.59 min) and PFPA-HMDMA (peak 3, 9.14 min).

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