Liquid Chromatographic and Mass Spectral Methods of Identification for the Regioisomeric 2,3- and 3,4-Methylenedioxyphenalkylamines

Jack DeRuiter, Pamela L. Holston, and **C. Randall Clark***
Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn, AL 36849

F. Taylor Noggle
Alabama Department of Forensic Sciences, Wire Road, Auburn, AL 36830

Abstract

A series of ring and side-chain regioisomers of 3,4-methylenedioxymethamphetamine are compared by chromatographic and spectroscopic methods. Regioisomerism at the aromatic ring and the alkyl side-chain in the methylenedioxyphenalkylamines produces a variety of compounds that have very similar analytical properties. The specific identification of one of these compounds in a forensic drug sample depends on the analyst's ability to eliminate other regioisomers as possible interfering substances. The 2,3- and 3,4-regioisomers of methylenedioxymethamphetamine, *N*-ethyl-methylenedioxyamphetamine, 1-methylenedioxyphenyl-2-butanamine, and *N*-methyl-1-methylenedioxyphenyl-2-butanamine are synthesized from commercially available precursor chemicals. The mass spectra for the underivatized amines are very similar and do not provide sufficient information to differentiate among the side-chain or ring regioisomers. Preparation of the pentafluoropropionamides of the amines produces derivatives that show mass spectral fragmentation identifying the substituent attached to nitrogen and the number of carbons attached directly to the aromatic ring. The regioisomeric amines are well-resolved in a reversed-phase chromatographic system using a Hypersil-Elite C_{18} stationary phase and acidic hydroorganic mobile phases.

Introduction

The major focus of designer drug activity in recent years has been in the area of ring-substituted phenalkylamines. A number of clandestinely produced drugs of abuse from this category have appeared as street drugs, including 3,4-methylenedioxymethamphetamine (1), *N*-hydroxy-3,4-methylenedioxyamphetamine (2), *N*-methyl-3,4-methylenedioxy-2-butanamine (3), and 4-bromo-2,5-dimethoxyphenethylamine (4). The recent appearance of 4-bromo-2,5-dimethoxyphenethylamine (Nexus) as a street drug has brought a renewed interest in the development of definitive methods for the identification of individual regioisomers of substituted phenethylamines (5). Nuclear magnetic resonance (NMR) is a very useful method for regioisomer differentiation; however, it is not a technique with direct application for all areas of forensic drug chemistry. The analysis of street drug samples and analytical toxicology must depend heavily on chromatographic methods as well as mass spectrometry.

The ability to distinguish between regioisomers directly enhances the specificity of the analysis for the target drugs of abuse. The mass spectrum is often the confirmatory piece of evidence in the identification of drugs of abuse in the forensic laboratory. Though the mass spectrum is often considered a specific "fingerprint" for an individual compound, there are many substances that produce very similar or almost identical mass spectra. Many of these compounds, which yield the same mass spectrum, are positional isomers in the alkyl side-chain or the aromatic ring substitution pattern (regioisomers).

When other existing compounds have the potential to produce the same mass spectrum as the drug of interest, the identification by gas chromatography–mass spectrometry (GC–MS) must be based entirely on the ability of the chromatographic system to separate the "counterfeit substances" from the actual drug of abuse. The substances that coelute with the drug of abuse will be misidentified. If the forensic scientist has never analyzed the counterfeit substances, the ultimate concern is how he or she can be sure that these compounds would not coelute with the drug of abuse. The significance of this question is related to many factors; chief among them are the efficiency of the chromatographic system and the number of possible counterfeit substances. In this project, a series of ring and side-chain regioisomers of 3,4-methylenedioxymetham-

* Author to whom correspondence should be addressed.

phetamine (see Figure 1) were compared by chromatographic and spectroscopic methods, and methods for their differentiation were explored.

Experimental

Instrumentation and methods

GC–MS analyses were performed using a Hewlett-Packard 5970B Mass Selective Detector (Palo Alto, CA). The mass spectrometer was operated in the electron-impact (EI) mode with an ionization voltage of 70 eV and a source temperature of 220°C. The samples were dissolved in methanol (1 mg/mL), and 0.5 µL was introduced into the MS via a GC 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 to 275°C at a rate of 12°C/min with a hold time of 6 min. The split ratio for the GC was 10:1, and the injector port temperature was 230°C. The carrier gas was ultrapure helium.

Liquid chromatographic (LC) analyses were conducted using a Waters model 590 pump (Milford, MA), a Laboratory Data Control 3000 Spectromonitor UV detector (Riveria Beach, FL), a Rheodyne 7125 injector (Cotati, CA), and a Linear model LR 93125 recorder. The analytical column was 15 cm × 4.6-mm i.d. Hypersil Elite C_{18} (Shandon HPLC, Cheshire, UK). The mobile phase consisted of pH 3.0 phosphate buffer and methanol (70:30) for Figure 5 and pH 3 phosphate buffer and acetonitrile (90:10) for Figure 6. The pH 3.0 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 UV absorbance detector was operated at 280 nm and 0.05 absorbance units full scale (AUFS). The mobile phase flow rate was 1 mL/min, the compounds were prepared as methanol solutions, and volumes of 2–10 µL were injected.

Synthesis of the 3,4-methylenedioxy compounds

The 3,4-methylenedioxyamphetamines (3,4-MDMA and 3,4-MDEA) and butanamines (3,4-BDB and 3,4-MBDB) were prepared by reductive amination of 3,4-methylenedioxyphenyl-2-propanone and 3,4-methylenedioxyphenyl-2-butanone with the appropriate amines as reported previously (6,7).

Synthesis of the 2,3-methylenedioxy compounds

The 2,3-methylenedioxyamphetamines (2,3-MDMA and 2,3-MDEA) were prepared from 2,3-dihydroxybenzaldehyde using the method of Casale et al.(8). The butanamines (2,3-BDB and 2,3-MBDB) were prepared from 2,3-dihydroxybenzaldehyde via the 2,3-methylenedioxyphenyl-2-nitrobutene intermediate (6,7).

PFPA derivatization

The pentafluoropropionylamide (PFPA) derivatives were prepared by extracting a 0.5-mg/mL solution of each amine in 0.5N NaOH (4 mL) with *n*-hexane (10 mL), adding pentafluoropropionic anhydride (50 µL) to the extracted solutions, and heating them at 120°C for 10 min. The hexane solutions were evaporated to dryness, the residue was dissolved in *n*-hexane, and 1 µL was injected into the GC.

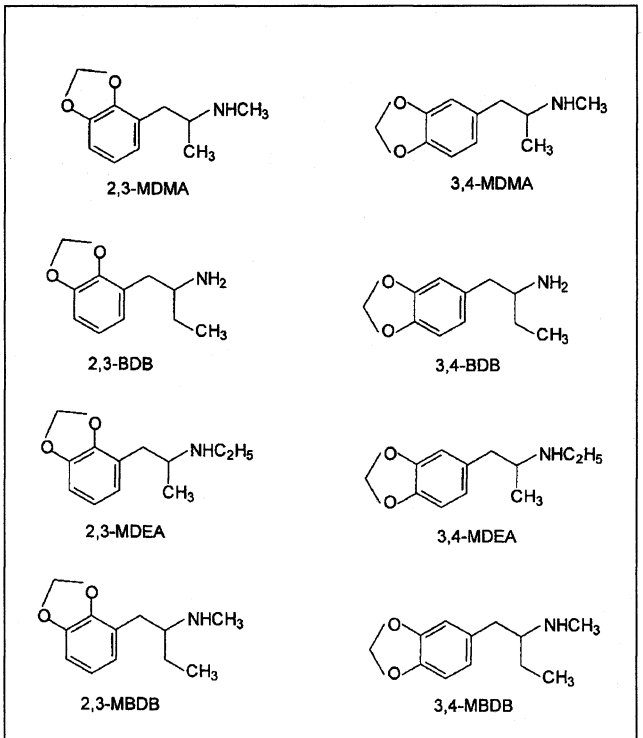

Figure 1. Structures of the 2,3- and 3,4-methylenedioxyphenalkylamines in this study.

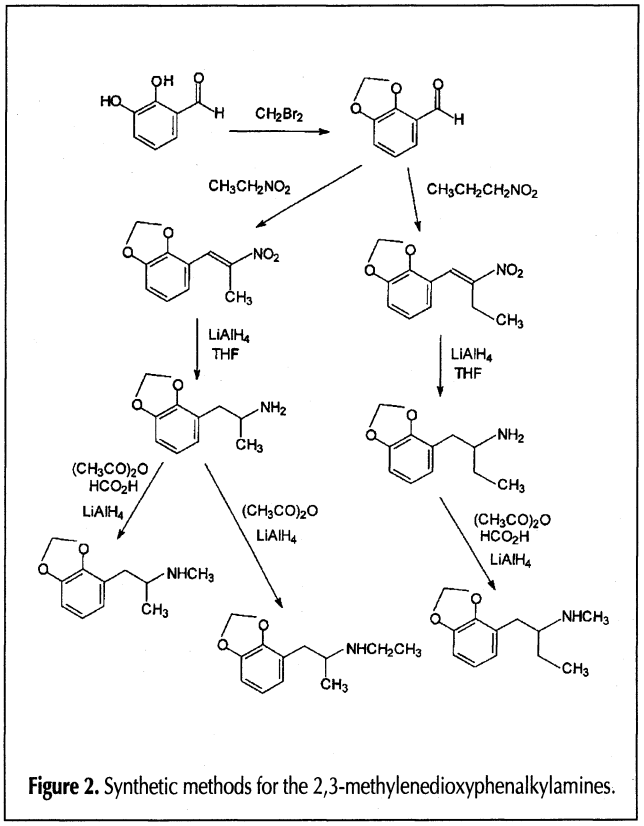

Figure 2. Synthetic methods for the 2,3-methylenedioxyphenalkylamines.

Results and Discussion

The compounds examined in this study represent a group of closely related substances whose individual identification is of considerable significance in forensic drug chemistry. Regioisomerism at the aromatic ring and the alkyl side-chain in the methylenedioxyphenalkylamines produces a variety of compounds that have very similar analytical properties. The specific identification of one of these compounds in a forensic drug sample depends on the analyst's ability to eliminate other regioisomers as possible interfering substances. The methylenedioxy ring can be fused to the aromatic ring in a 2,3- or 3,4- pattern, yielding regioisomerism of the aromatic portion of the molecule. Alkyl side-chain regioisomerism is most significant when imine fragments of equivalent mass and similar abundance appear in the mass spectra of these compounds. The major fragmentation process in EI mass spectrometry of phenethylamines is the homolytic cleavage of the alpha and beta carbons of the side-chain to yield the benzyl fragment and the imine fragment. Thus, regioisomerism within the side-chain or aromatic ring will yield mass spectra of significant similarity. There are a number of examples of the practical significance of regioisomerism in forensic drug chemistry, including the differentiation of 3,4-methylenedioxymethamphetamine (MDMA) from 3,4-methylenedioxyphenyl-2-butanamine (BDB) and that of *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA) from *N*-methyl-3,4-methylenedioxyphenyl-2-butanamine (MBDB).

In this study, the 2,3- and 3,4-methylenedioxyphenyl ring substitution regioisomers of MDMA, BDB, MDEA, and MBDB were prepared, and chromatographic and spectral methods were evaluated for their differentiation. The methods for the preparation of the 3,4-methylenedioxy regioisomers have been described in previous reports (6–8). The general procedure for synthesis of the 2,3-MDAs and BDBs is outlined in Figure 2. The 2,3-methylenedioxybenzaldehyde was prepared by treating 2,3-dihydroxybenzaldehyde with CH_2Br_2. Condensation of the aldehyde with a nitroalkane under basic conditions yields the 2-nitroalkenes, which, upon reduction with lithium aluminium hydride (LAH), yields the primary amines.

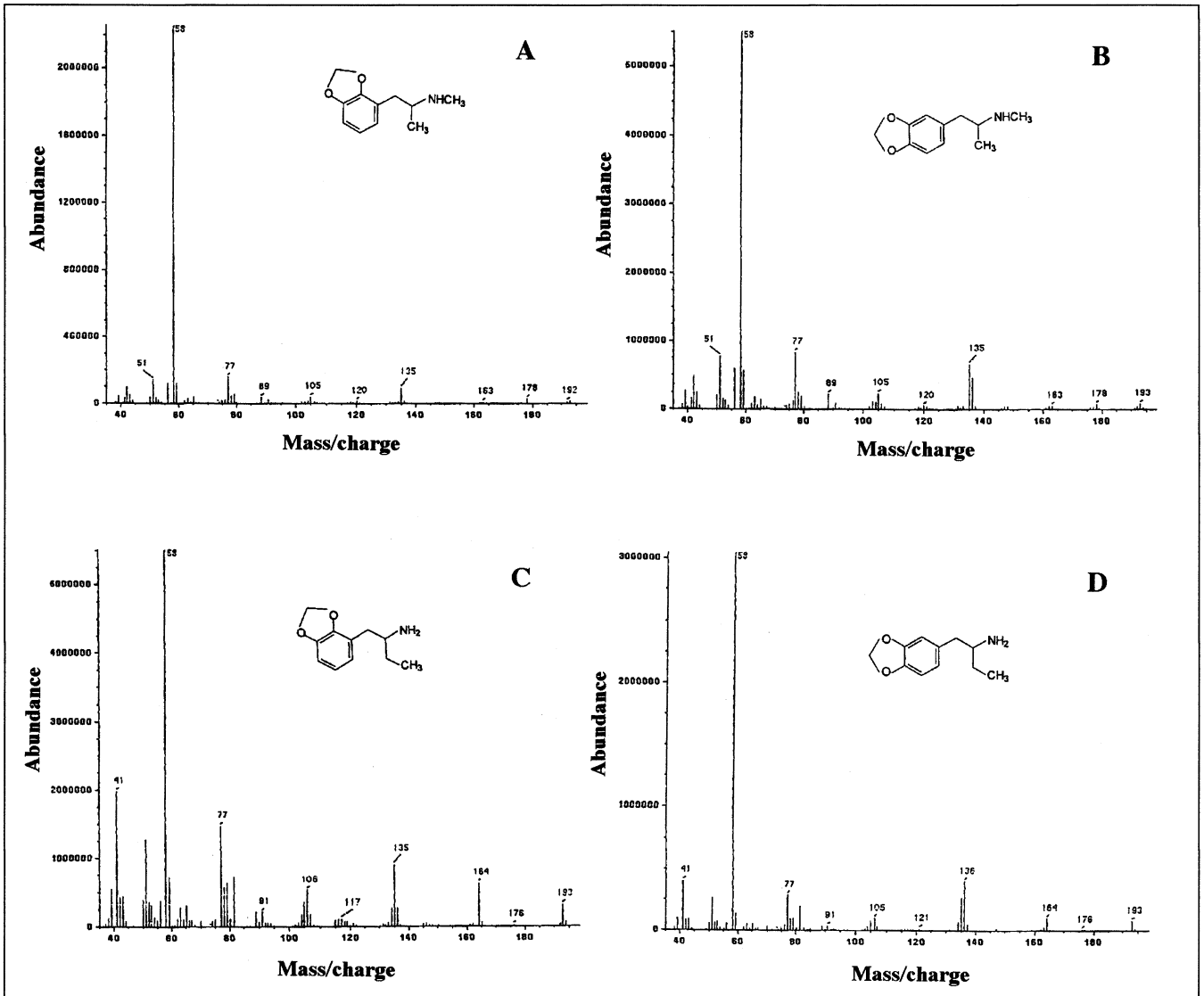

Figure 3. Mass spectra for (A) 2,3-MDMA, (B) 3,4-MDMA, (C) 2,3-BDB, and (D) 3,4-BDB.

The *N*-methyl and *N*-ethyl analogues were prepared from the primary amines by acylation followed by LAH reduction.

The eight compounds synthesized in this study (see Figure 1) can be subdivided into two groups of four regioisomeric amines: 2,3- and 3,4-MDMA and 2,3- and 3,4-BDB make up one group, and 2,3- and 3,4-MBDB and 2,3- and 3,4-MDEA make up another. The four MDMA and BDB (molecular weight [MW], 193) isomers each yielded major fragments of identical mass in the EI mass spectra, *m/z* 135 for the methylenedioxybenzyl fragment and *m/z* 58 for the propylimine. The four MBDB and MDEA (MW, 207) isomers yielded the same *m/z* 135 ion and the butylimine fragment of *m/z* 72. Figure 3 shows the EI mass spectra for the four compounds yielding the propylimine fragment (*m/z* 58) and the methylenedioxybenzyl fragment (*m/z* 135). These spectra indicate that very little structural information is available for the specific differentiation among these regioisomeric amines. The spectra in Figures 3A and 3B were obtained from 2,3- and 3,4-MDMA, and the spectra in Figures 3C and 3D were obtained from 2,3- and 3,4-BDB. Comparison of the mass spectra of the regioisomeric ring substituents (2,3- versus 3,4-methylenedioxyphenyl) shows fragments of the same mass with only slight differences in relative intensity. Comparison of the side-chain regioisomers in Figure 3 (MDMA versus BDB) shows the high mass ion at *m/z* 164 in the BDB isomers and the *m/z* 178 fragment in the MDMA isomers. These fragments result from the loss of the less favored alpha-alkyl group in the side-chain, an ethyl group (M-29, *m/z* 164) and a methyl group (M-15, *m/z* 178) for BDB and MDMA, respectively.

In previous studies (9), methods for the differentiation between side-chain regioisomers in phenethylamine-type compounds based on mass spectral fragmentation of the pentafluoropropionylamide derivatives has been successful. The fragmentation of the bond between the alkyl side-chain and the nitrogen in the PFPA-derivatized amines yielded prominent ions that identified the nature of the hydrocarbon chain attached directly to the aromatic ring. Figures 4A and 4B show the mass spectra for the PFPA derivatives of 2,3- and 3,4-MDMA. These spectra are essentially identical; each shows a significant molecular ion at *m/z* 339 and a base peak at *m/z*

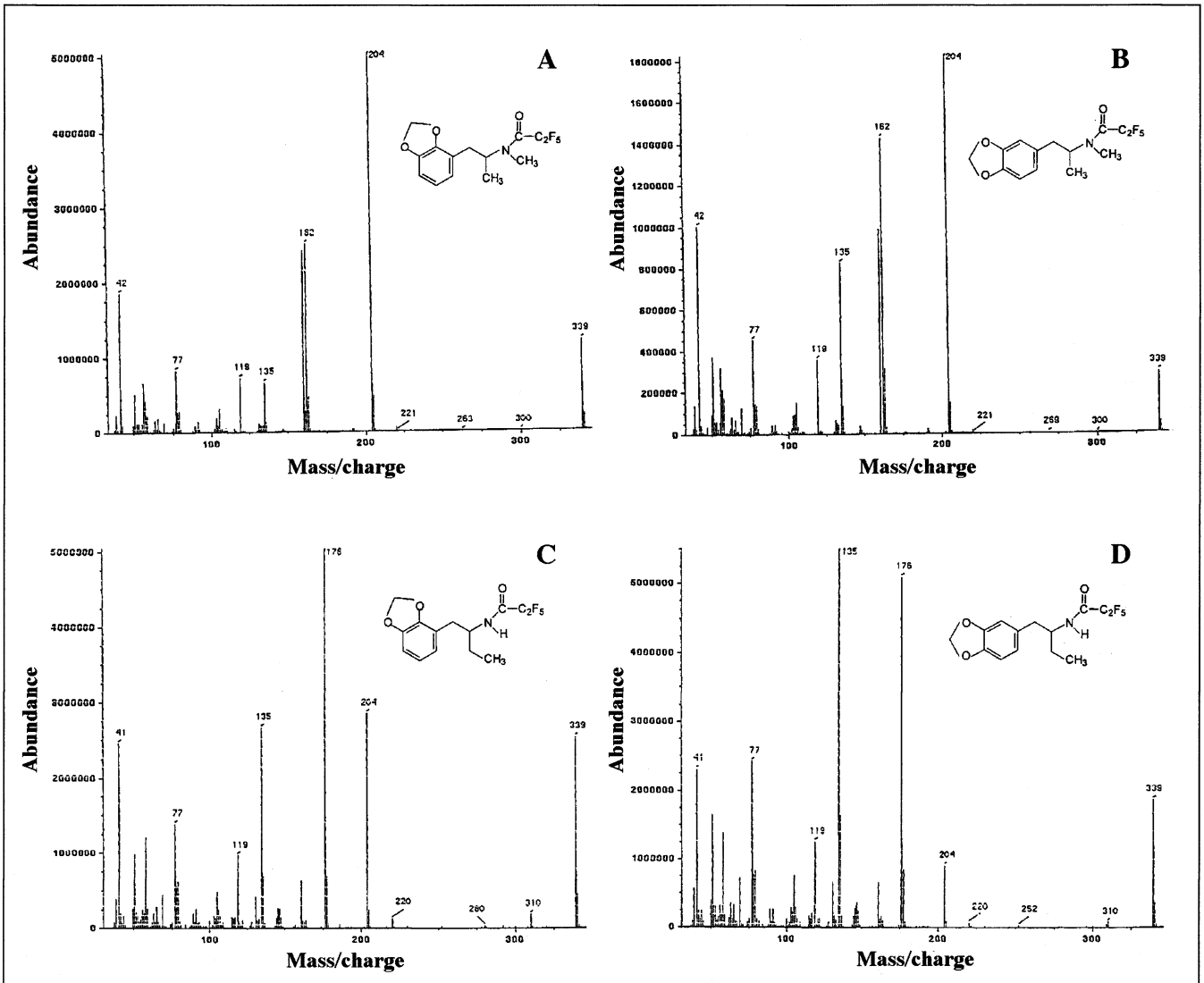

Figure 4. Mass spectra for (A) 2,3-MDMA–PFPA, (B) 3,4-MDMA–PFPA, (C) 2,3-BDB–PFPA, and (D) 3,4-BDB–PFPA.

204 resulting from the loss of the methylenedioxybenzyl radical from the molecular ion. The *m/z* 204 ion is the PFPA-derivatized imine fragment that appears at *m/z* 58 for the underivatized amines (see Figure 3). The mass spectra for the PFPA derivatives of 2,3- and 3,4-BDB in Figures 4C and 4D show these same fragments; however the base peak for the BDB derivatives occurred at *m/z* 176. The *m/z* 176 is a diagnostic ion that identifies the alkyl chain attached directly to the aromatic ring in these BDB derivatives as a butyl group. This ion is the result of bond fragmentation that occurs between the amide nitrogen and the adjacent alkyl carbon of the side-chain. The analogous alkene ion for the MDMA derivatives (Figures 4A and 4B) occurred at *m/z* 162, indicating a propyl group attached directly to the aromatic ring. These ions of major abundance allow for the differentiation of the MDMA isomers from the BDB isomers as PFPA derivatives. The *m/z* 160 ion is a four-centered rearrangement product common to the PFPA derivatives of *N*-methylphenethylamines. Deuterium labeling experiments (9) have been used to confirm the structure of the analogous *m/z* 160 ion in the mass spectrum of methamphetamine–PFPA. The fragmentation reactions for the PFPA derivatives of the regioisomers in this study are summarized in Figure 5.

The underivatized mass spectra for the second group of four regioisomeric amines (2,3- and 3,4-MDEA and 2,3- and 3,4-MBDB) are shown in Figure 6. The base peak in all four spectra was the *m/z* 72 imine ion, and the major difference in these spectra was the greater abundance of the *m/z* 44 ion in the MDEA isomers. This *m/z* 44 ion was due to a loss of ethylene from the *N*-ethyl group of the imine (*m/z* 72), and this fragmentation occurred to a greater extent than the loss of ethylene from the *C*-ethyl group of the regioisomeric *m/z* 72 ion obtained from the MBDB isomers.

The PFPA-derivatized amines produced mass spectra (Figure 7) with a strong molecular ion (*m/z* 353) and the acylated imine base peak at *m/z* 218. The 2,3- and 3,4-MDEA derivatives (Figures 7A and 7B) showed a strong *m/z* 162 ion for the methylenedioxyphenylpropene fragment characteristic of the C_3 hydrocarbon chain attached directly to the aromatic ring. The *m/z* 190 ion was due to a loss of ethylene from the *N*-ethyl group analogous to the fragmentation seen for the underivatized imine. The MBDB derivatives in Figures 7C and 7D showed the ion at *m/z* 176 characteristic of the C_4 hydrocarbon chain attached directly to the aromatic ring and the *m/z* 160 rearrangement ion associated with the *N*-methyl derivatives.

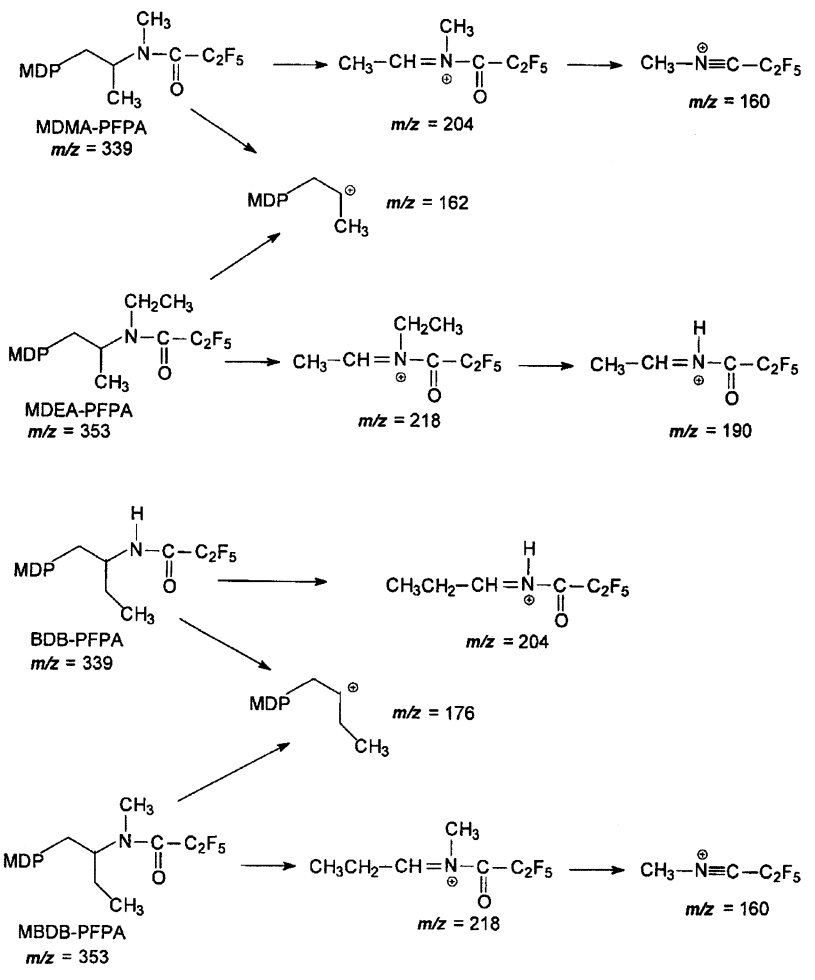

Figure 5. Mass spectral fragmentation products for the methylenedioxyphenalkylamines. MDP = methylenedioxyphenyl.

In all cases, the PFPA-derivatized amines yielded mass spectra that readily identified the side-chain regioisomers. The pentafluoropropionylamides sufficiently weakened the bond between the nitrogen and the alkyl carbon to produce arylalkene fragments that identified the size of the alkyl group attached directly to the aromatic ring in the parent amines. Further fragmentation of the acylimine base peak in the PFPA derivatives produced ions that identified the *N*-substituent of the parent amine. Though the mass spectra of the PFPA derivatives allowed differentiation of side-chain regioisomerism in these compounds, very little differences were observed for the 2,3- versus 3,4- regioisomerism of the methylenedioxy ring substitution. Chemical ionization MS using methane as the source gas did not provide any additional information regarding regioisomerism of the ring substituents. In previous reports, Casale et al. (8) and Soine et al. (10) have described the relative abundance of the 135/136 ions in the parent amines as the major points of differentiation between the 2,3- and 3,4-regioisomers of MDA, MDMA, and related amines. These authors have further pointed out some differences in the infrared (IR) spectra between 2,3- and 3,4-regioisomers (8,10). The only reported color test information (10) indicated that 2,3-MDA produces a rose or pink color in concentrated sulfuric acid, whereas 3,4-

MDA yields a deep purple color under identical conditions.

The regioisomeric MDMA and BDBs were separated in a reversed-phase LC system consisting of a C_{18} stationary phase (Hypersil Elite C_{18}) and a mobile phase of 30% methanol in pH 3 phosphate buffer. The chromatogram in Figure 8 shows the separation of these four compounds and illustrates the sensitivity of reversed-phase methods for the slight differences in nonpolar surface area among these regioisomers. The compound showing the greatest polarity and eluting first in this system was the 3,4-MDMA followed by the 2,3-MDMA (peak 2) regioisomer. Thus both isomers having the C_3 side-chain directly attached to the methylenedioxyphenyl ring eluted before the isomers with the C_4 side-chain. Among the C_4 isomers, the 3,4-BDB regioisomer (peak 3) eluted before the 2,3- regioisomer (peak 4); thus in this chromatogram, the 2,3-substitution pattern for the methylenedioxy group was more lipophilic than the corresponding 3,4- substitution pattern. The chromatogram in Figure 8 shows these regioisomeric amines to be well-resolved with excellent peak shape using an acidic mobile phase. Under these conditions, the amines existed as the protonated species and were chromatographed as the conjugate acid species. The Hypersil Elite C_{18} stationary phase is a high-surface-coverage material and does not require silanol masking agents such as triethylamine to prevent peak tailing for amines. The four compounds shown in Figure 8 were analyzed by GC on a methylsilicone stationary phase under temperature-programmed conditions (described in the Experimental section), and these compounds eluted over a 0.6-min time window from 6.7 to 7.3 min.

The chromatogram in Figure 9 shows the reversed-phase separation for the four regioisomeric amines with the *m/z* 72 imine fragment. These compounds required an acidic acetonitrile mobile phase in order to maximize the isocratic separation. The effects of structure on retention properties among these compounds was the same as those observed in the previous series. The two compounds with the C_3 alkyl chain attached to the aromatic ring eluted first; the 3,4-MDEA eluted before the 2,3-MDEA (peak 2), and the two compounds with the C_4 alkyl group attached directly to the aromatic ring showed greater lipophilicity. The 3,4-MBDB (peak 3) eluted before 2,3-MBDB (peak 4) with excellent resolution of all four compounds in this chromatographic system. The GC separation of these

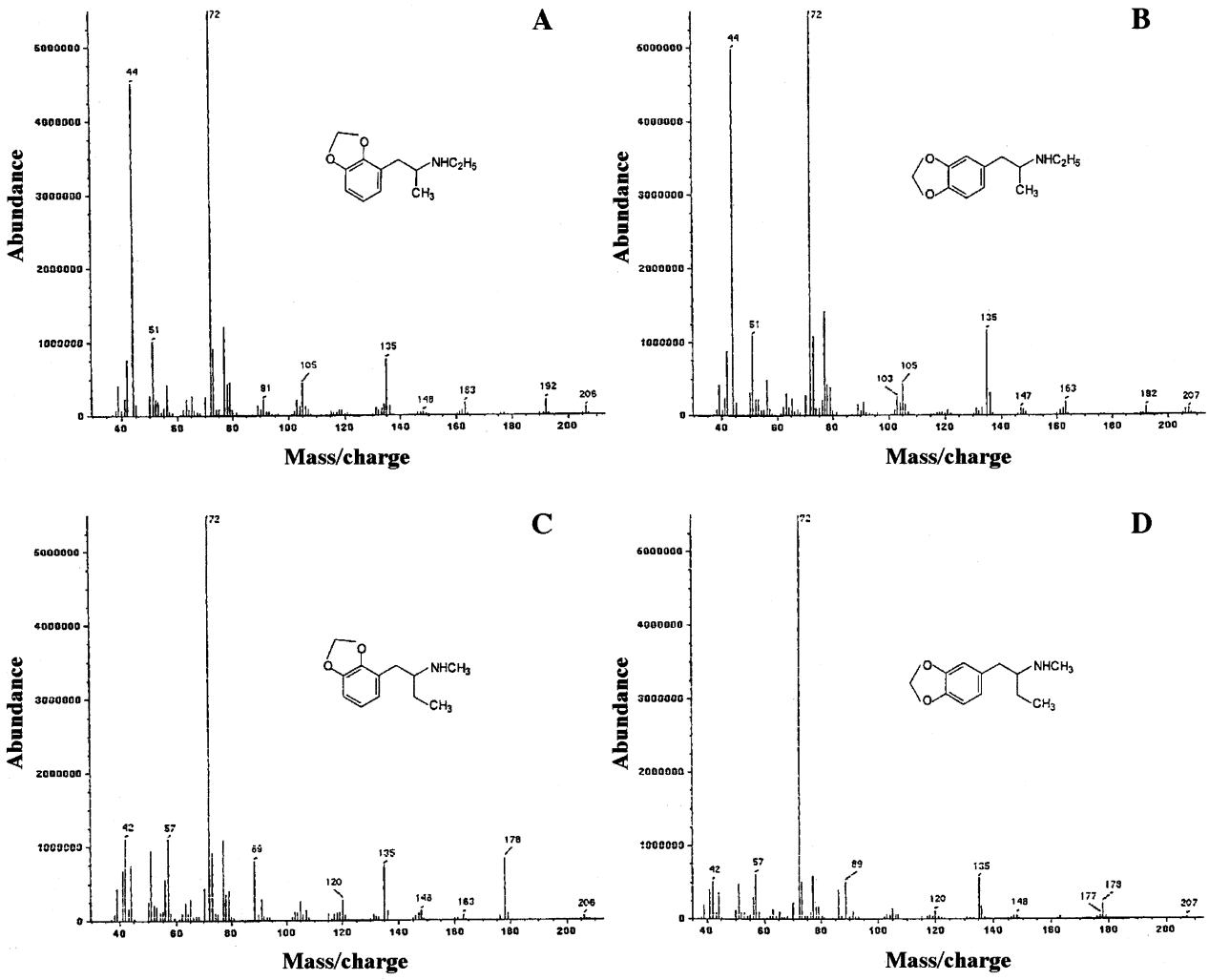

Figure 6. Mass spectra for (A) 2,3-MDEA, (B) 3,4-MDEA, (C) 2,3-MBDB, and (D) 3,4-MBDB.

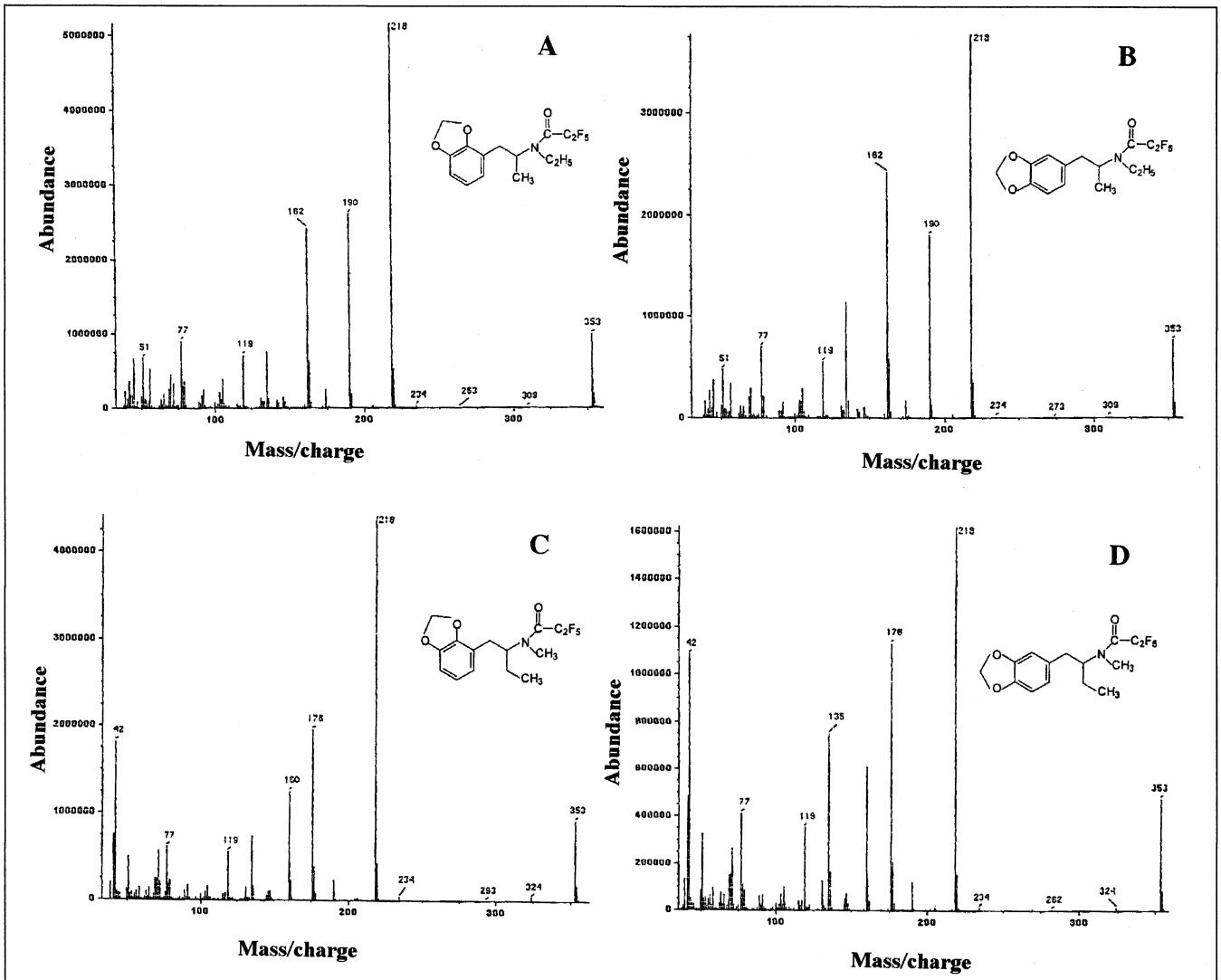

Figure 7. Mass spectra for (A) 2,3-MDEA–PFPA, (B) 3,4-MDEA–PFPA, (C) 2,3-MBDB–PFPA, and (D) 3,4-MBDB–PFPA.

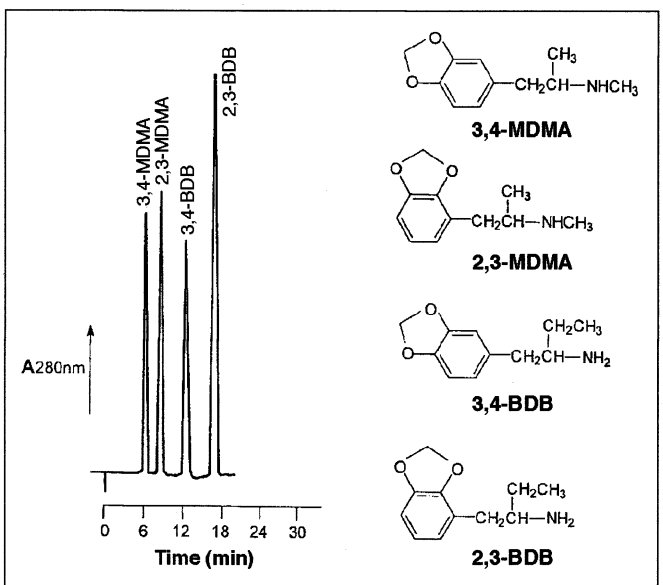

Figure 8. HPLC separation of 3,4- and 2,3-MDMA and 3,4- and 2,3-BDB. A Hypersil Elite C_{18} column was used with 70% 0.05M phosphate (pH 3) and 30% methanol (1.0 mL/min, 280 nm, and 0.05 AUFS).

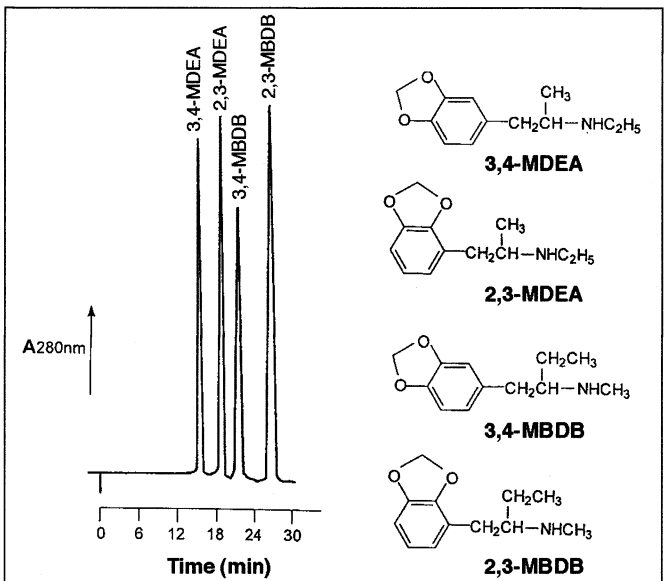

Figure 9. HPLC separation of 3,4- and 2,3-MDEA and 3,4- and 2,3-MBDB. A Hypersil Elite C_{18} column (150 x 4.6 mm) was used with 90% 0.05M phosphate (pH 3) and 10% acetonitrile (1.0 mL/min, 280 nm, and 0.05 AUFS).

four compounds produced an elution range of 0.5 min (7.0–7.5 min) on the methylsilicone stationary phase under temperature-programmed conditions.

The change in organic modifier composition from methanol to acetonitrile in Figures 8 and 9 did not alter the effects of structure on retention in these compounds. The observed elution order was the same for several C_{18} stationary phases in both methanol- and acetonitrile-modified acidic aqueous systems. The chromatograms in Figures 8 and 9 represent the systems providing maximium resolution for these two groups of regioisomers and provide an excellent example of the sensitivity of reversed-phase LC to slight differences in the relative polarity of solute molecules.

Conclusion

A series of regioisomeric amines related to the methylenedioxyphenethylamine drugs of abuse were prepared, and methods for their specific identification were evaluated. The underivatized mass spectra of these compounds did not allow clear differentiation of side-chain or ring regioisomerism. The parent amines showed ions of identical mass and only slight differences in relative abundance in their EI mass spectra. The PFPA-derivatized amines showed prominent ions that allowed for the identification of the alkyl side-chain attached directly to the aromatic ring and the nature of the nitrogen substituent in the parent amine. These diagnostic ions were the base peaks in the mass spectra of some of the derivatives and had high relative intensities (50% or greater) in the others. Reversed-phase LC was very sensitive to the small differences in hydrophobicity among these regioisomeric substances and produced excellent resolution of these otherwise very similar compounds. The regioisomers with the C_3 side-chain directly attached to the methylenedioxyphenyl ring eluted before the isomers with the C_4 side-chain. Within each side-chain length, the 2,3-substitution pattern for the methylenedioxy group was more lipophilic than the corresponding 3,4- substitution pattern.

References

1. R.M. Braun. New variety of street drugs poses growing problem. *Chemical and Engineering News*, September 9,1985, pp. 7–16.
2. U. Braun, A.T. Shulgin, and G. Braun. Centrally active *N*-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine). *J. Pharm. Sci.* **69:** 192–95 (1980).
3. Anon. Fido dido appears in Florida. *Microgram* **28:** 101 (1995).
4. *Federal Register*, 21 CFR Part 1308, Schedules of Controlled Substances, Placement of 4-Bromo-2,5-dimethoxyphenethylamine into Schedule I, Vol. 60, No. 106, June 2, 1995, pp. 28718–19.
5. J. DeRuiter, C.R. Clark, and F.T. Noggle. LC and GC–MS analysis of 4-bromo-2,5-dimethoxyphenethylamine (Nexus) and 2-propanamine and 2-butanamine analogues. *J. Chromatogr. Sci.* **33:** 583–90 (1995).
6. F.T. Noggle, C.R. Clark, A.K. Valaer, and J. DeRuiter. Liquid chromatographic and mass spectral analysis of *N*-substituted analogues of 3,4-methylenedioxyamphetamine. *J. Chromatogr. Sci.* **26:** 410–15 (1988).
7. C.R. Clark, J. DeRuiter, A.K. Valaer, and F.T. Noggle. Gas chromatographic–mass spectrometric and liquid chromatographic analysis of designer butanamines related to MDMA. *J. Chromatogr. Sci.* **33:** 328–37 (1995).
8. J.F. Casale, P.A. Hays, and R.F.X. Klein. Synthesis and characterization of the 2,3-methylenedioxyamphetamines. *J. Forensic Sci.* **40:** 391–400 (1995).
9. C.R. Clark, A.K. Valaer, F.T. Noggle, and J. DeRuiter. GC–MS analysis of acylated derivatives of methamphetamine and regioisomeric phenethylamines. *J. Chromatogr. Sci.* **33:** 485–92 (1995).
10. W.H. Soine, R.E. Shark, and D.T. Agee. Differentiation of 2,3-methylenedioxyamphetamine from 3,4-methylenedioxyamphetamine. *J. Forensic Sci.* **28:** 386–90 (1983).

Manuscript accepted January 13, 1998.