

GC–MS and GC–IRD Studies on the Ring Isomers of *N*-Methyl-2-Methoxyphenyl-3-Butanamines (MPBA) Related to 3,4-MDMA

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Abstract

The mass spectra of the controlled substance 3,4-MDMA and its regioisomer 2,3-MDMA are characterized by an imine fragment base peak at m/z 58 and additional fragments at m/z 135/136 for the methylenedioxybenzyl cation and radical cation, respectively. Three positional ring methoxy isomers of *N*-methyl-2-(methoxyphenyl)-3-butanamine (MPBA) have an isobaric relationship to 2,3- and 3,4-MDMA. All five compounds have the same molecular weight and produce similar EI mass spectra. This lack of mass spectral specificity for the isomers in addition to the possibility of chromatographic co-elution could result in misidentification. The lack of reference materials for the potential imposter molecules constitutes a significant analytical challenge. Perfluoroacylation of the amine group reduced the nitrogen basicity and provided individual fragmentation pathways for discrimination among these compounds based on unique fragment ions and the relative abundance of common ions. Studies using gas chromatography with infrared detection provided additional structure-IR spectra relationships. The underivatized amines and the perfluoroacylated derivatives (PFPA and HFBA) were resolved by capillary gas chromatography on a 100% dimethylpolysiloxane stationary phase. The perfluoroacylated derivatives showed better resolution on a cyclodextrin modified stationary phase.

Introduction

Regioisomeric and isobaric substances are considered a significant challenge for the analytical techniques used to identify specific substances. This is extremely important when some of these molecules are legally controlled drugs of abuse or controlled precursor substances (1–10). While the mass spectrum is often considered a specific “fingerprint” for an individual compound, there are other substances that produce very similar or almost identical mass spectra. Many of these compounds that yield the same mass spectrum are of an isobaric relationship to the drugs of abuse. Such compounds having mass spectral equivalency and similar elution properties, perhaps co-elution, represent a

serious analytical challenge. In these cases, identification by gas chromatography (GC)–mass spectrometry (MS) must be based primarily upon the chromatographic system’s ability to separate the entire set of substances. Those substances co-eluting in the chromatographic system could be misidentified. A complete set of standards must be available for a thorough method validation study and to exclude the possibility of co-elution of combinations of the regioisomeric and/or isobaric molecules. Furthermore, the ability to distinguish among these regioisomers directly enhances the specificity of the analysis for the target molecules.

Previous reports (1–10) in this series have described the analytical properties of a group of compounds that have unique regioisomeric or isobaric equivalence to the drug of abuse 3,4-methylenedioxyamphetamine (3,4-MDMA) or its regioisomer 2,3-methylenedioxyamphetamine (2,3-MDMA). These compounds are likely to produce major mass spectral fragment ions of equivalent mass to 3,4-MDMA and provide a significant challenge for chromatographic resolution. Differentiation of regioisomers and isobaric substances is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (1–10). Methods for molecular individualization without the need for reference standards of every regioisomeric/isobaric compound have significant advantages in forensic drug chemistry.

Three ring methoxy isomers of *N*-methyl-2-(methoxyphenyl)-3-butanamines have isobaric relationship to 2,3- and 3,4-MDMA with the potential to produce almost identical mass spectra as 3,4-MDMA (Figure 1). They have the strong possibility of being misidentified as 3,4-MDMA by some commonly used analytical methods. These MPBA isomers would possibly appear as clandestine drug samples only by direct chemical synthesis and are not likely to occur as impurities of common synthetic routes for the preparation of MDMA. In this project, the perfluoroacylated derivatives of the five amines (Figure 1, compounds 1–5) were prepared and evaluated for their ability to individualize the GC–MS properties of the studied compounds. Previous studies on some regioisomeric substances related to phenethylamines showed that derivatization can provide additional structural fragmentation information as well as improvement in chromatographic properties (4–10).

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Infrared spectroscopy is considered a confirmation method for the identification of organic compounds due to the uniqueness of infrared spectra for very similar organic molecules. GC–FTIR spectroscopy is characterized by scanning quickly enough to obtain IR spectra of peaks eluting from the capillary columns. Thus this technique combines the separation power of GC with the identification power of IR. The GC–FTIR has been successfully applied to the confirmation of drug identification in forensic drug chemistry (11,12). GC–infrared detection (IRD) has been applied in previous studies to the differentiation of ring and side chain substituted regioisomeric phenethylamines related to the drug of abuse, 3,4-MDMA (12,13).

The aim of this study is to evaluate GC with mass spectral or IRD for the discrimination and characterization of the three positional ring methoxy isomers of *N*-methyl-2-(methoxyphenyl)-3-butanamines in relation to 2,3- and 3,4-MDMA.

Experimental

Instrumentation

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 ionization (EI) mode using electron energy of 70 eV. The splitless injector liner was 4 mm i.d. \times 6.5 mm o.d. and 78.5 mm in length. The injector temperature was set at 230°C, and the transfer line was maintained at 280°C. Samples were dissolved in high-performance liquid chromatography (HPLC)-grade acetonitrile (Fisher

Scientific, Fairlawn, NJ) and manually introduced (1 μ L) individually and in a physical mixture using a 10- μ L Hamilton syringe (Hamilton Co., Reno, NV).

All separations were generated using two temperature programs. Program 1 consisted of an initial hold at 70°C for 1 min, ramped up to 150°C at a rate of 5°C/min and held at 150°C for 2 min, then ramped to 250°C at a rate of 10°C/min and held at 250°C for 20 min. This program was used to separate the underivatized and the perfluoroacyl derivatives of the studied compounds on 30 m \times 0.25 mm i.d. column coated with 0.25 μ m 100% dimethyl polysiloxane (Rtx-1). Program 2 was used to separate the pentafluoropropionylamides (PFPA) and heptafluorobutylamides (HFBA) on a 30 m \times 0.25 mm i.d. column coated with 0.25 μ m 14% cyanopropyl phenyl–86% dimethylpolysiloxane doped with a proprietary cyclodextrin material (Rt β DEXcst). This program had an initial hold at 100°C for 1 min, ramped up to 180°C at a rate of 9.0°C/min, held at 180°C for 2 min, and finally ramped to 200°C at a rate of 10°C/min then held at 200°C for 20 min. Both columns were purchased from Restek Corporation (Bellefonte, PA).

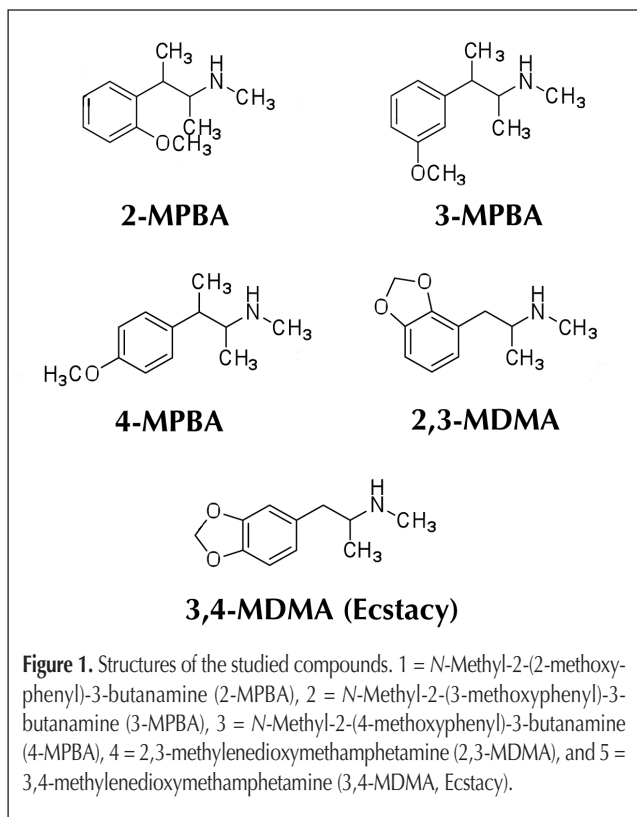
GC–IRD studies were carried out on a Hewlett-Packard 5890 Series II GC and a Hewlett-Packard 7673 auto-injector coupled with an IRD-II infrared detector (Analytical Solutions and Providers, Covington, Kentucky). The vapor phase infrared detector (IRD) spectra were recorded in the range of 4000–550 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans per second. The IRD flow cell temperature as well as the transfer line was 280°C, and the GC was operated in the splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min. The column used was a 30 m \times 0.25 mm i.d. coated with 0.50 μ m 50% phenyl–50% methyl polysiloxane (Rxi-50) purchased from Restek Corporation (Bellefonte PA). The temperature program involved in this study consisted of an initial temperature of 100°C for 1 min, ramped up to 230°C at a rate of 20°C per min followed by a hold at 230°C for 15 min. Samples were dissolved and diluted in HPLC-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced via the autoinjector using an injection volume of 1 μ L.

Drugs and reagents

Samples of 2,3- and 3,4-MDMA and the three positional ring methoxy isomers of *N*-methyl-2-(methoxyphenyl)-3-butanamines were synthesized as previously described (1,3,6–8). All laboratory reagents and chemicals were obtained from Aldrich Chemical Co. or Fisher Scientific (Atlanta, GA). Pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were purchased from Sigma-Aldrich, Inc. (Milwaukee, WI).

Derivatization Procedure

Each perfluoroamide was prepared individually from the hydrochloride salts of the regioisomeric and isobaric compounds by dissolving approximately 0.3 mg (1.55×10^{-6} mol) of each amine in 50 μ L of ethyl acetate, followed by addition of large excess (250 μ L) of the appropriate derivatizing agent (PFPA or HFBA), and the derivatization reaction mixtures were incubated in capped tubes 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 each final solution (50 μL) was diluted with HPLC grade acetonitrile (200 μL) to give the working solutions. A volume of 1 μL of each working solution was injected into the GC.

General synthetic methods

Compounds 1–3 were prepared by the same general procedure using the appropriately substituted methoxyphenylacetone. Dry tetrahydrofuran (THF) was added dropwise to 60% sodium hydride in mineral oil, followed by dropwise addition of the methoxyphenylacetone and methyl iodide in THF. The reaction mixture was refluxed and quenched with aqueous THF. The solvent was evaporated to yield yellow oil 2-(methoxyphenyl)-3-butanone purified by vacuum distillation. The ketones were converted to the desired amines via reductive amination (3).

The methods for the preparation of the 2,3- and 3,4-MDMA have been described in previous reports (1,3). The general procedure for the synthesis of these compounds begins with the appropriate aldehyde, 2,3-methylenedioxybenzaldehyde and 3,4-methylenedioxy-benzaldehyde (piperonal), as starting materials. The preparation of 2,3-methylenedioxybenzaldehyde has been reported previously (1,8). Condensation of the appropriate aldehyde with nitroethane under basic conditions yields the 2-nitroalkenes, which can be reduced to the primary amines or hydrolyzed to the corresponding ketones and reductively aminated.

Results and Discussion

Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 2 shows two example EI mass spectra for this group of compounds *N*-methyl-2-(3-methoxyphenyl)-3-butanamine (Compound 2) and 2,3-MDMA (Compounds 4). The spectra in Figure 2 indicate that very little structural information is available for differentiation among these isomers because the major fragment ions occur at equal masses. The mass spectra of the five studied compounds including 3,4-MDMA are characterized by a base peak at m/z 58 formed by α -cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and amine. In all of the five compounds (molecular weight = 193), the α -cleavage reaction yields the substituted imine fragment at m/z 58 and the 2,3-, 3,4-methylenedioxybenzyl fragment or the isobaric methoxy phenyl ethyl fragment at mass 135 for the cation fragment and 136 for the corresponding radical cation. Thus, the mass spectra for the five compounds contains major ions at m/z 58 and 135/136 as well as other ions of low relative abundance (1–10). This lack of mass spectral specificity in addition to the possibility of chromatographic co-elution with 2,3- and/or 3,4-MDMA could result in misidentification of the target drug. Furthermore, the lack of available reference samples for the three isobaric *N*-methyl-2-(methoxyphenyl)-3-butanamines complicates the individual identification of any one of these substances. This constitutes a significant analytical challenge, where

the specific identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate the regioisomeric/isobaric nondrug from the actual drug of interest. Additionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

In the present study, various perfluoroacylated derivatives of the regioisomeric secondary amines were prepared and evaluated in an effort to individualize their mass spectra and maintain or improve chromatographic resolution. Acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the mass spectra (4–10). The pentafluoropropionyl and heptafluorobutyryl derivatives of the studied compounds were evaluated for their ability to individualize the mass spectra of 2,3- and 3,4-MDMA and the three methoxy isomers of *N*-methyl-2-(methoxyphenyl)-3-butanamines.

The mass spectra for the five pentafluoropropionyl and heptafluorobutyryl amides are shown in Figures 3 and 4, respectively. From these spectra, a common peak occurs at m/z 204 and 254, which corresponds to the loss of 135 Da from the molecular ions at 339 and 389 for PFPA and HFPA amides, respectively. These ions at m/z 204 and 254 are the PFPA and HFPA imine species, likely formed from the α -cleavage of the amide nitrogen to eliminate the corresponding methoxyphenyl ethyl radical (Compounds 1–3) or 2,3-, 3,4-methylenedioxybenzyl radical (Compounds 4 and 5). Thus the m/z 204 and 254 in PFPA and HFPA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M-135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Figure 5. The corresponding substituted benzyl cation at m/z 135 is a fragment common to all the spectra in Figures 3 and 4.

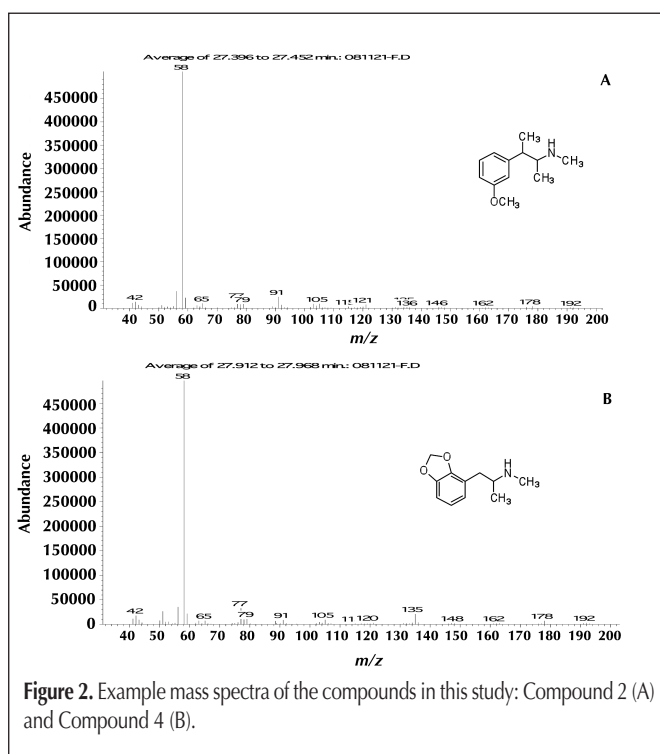


Figure 2. Example mass spectra of the compounds in this study: Compound 2 (A) and Compound 4 (B).

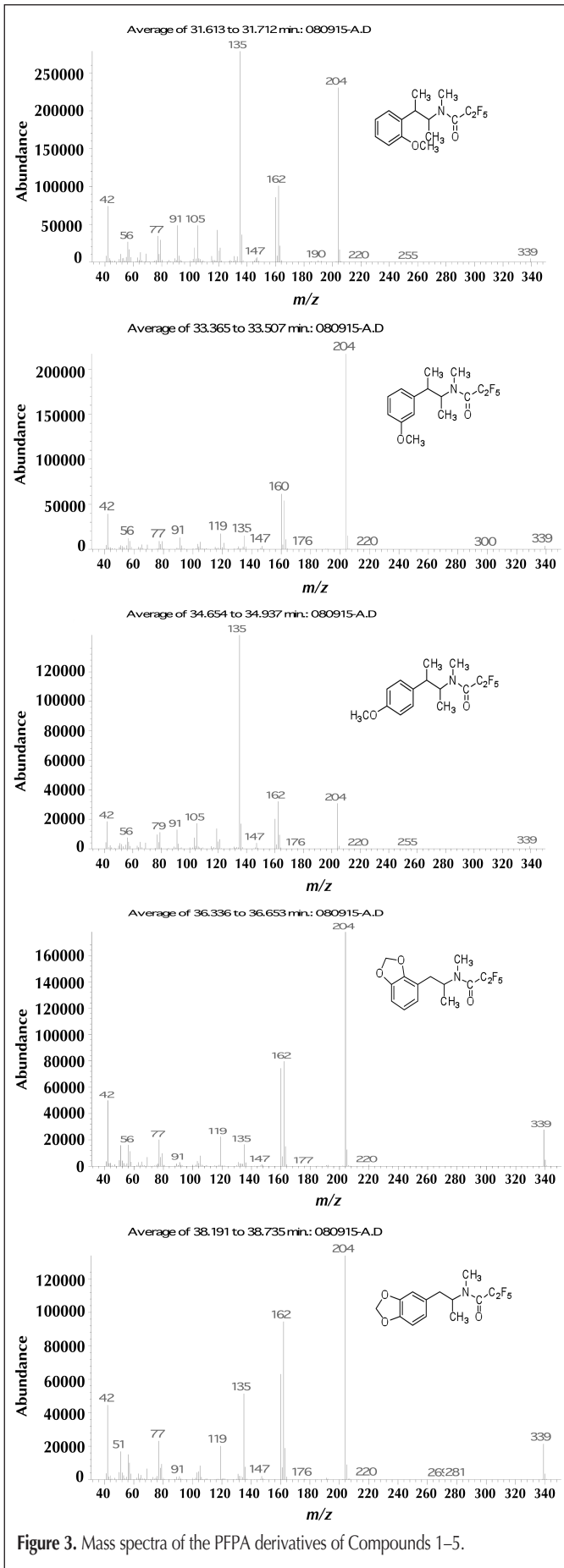


Figure 3. Mass spectra of the PFA derivatives of Compounds 1–5.

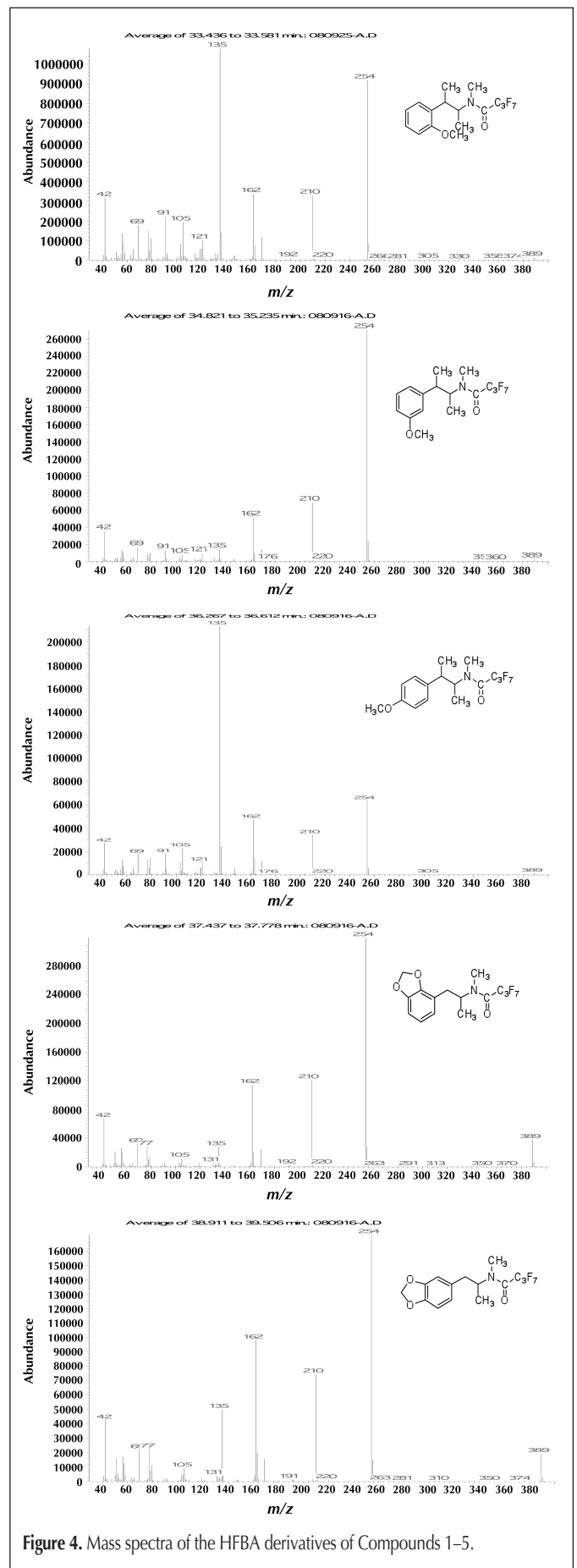
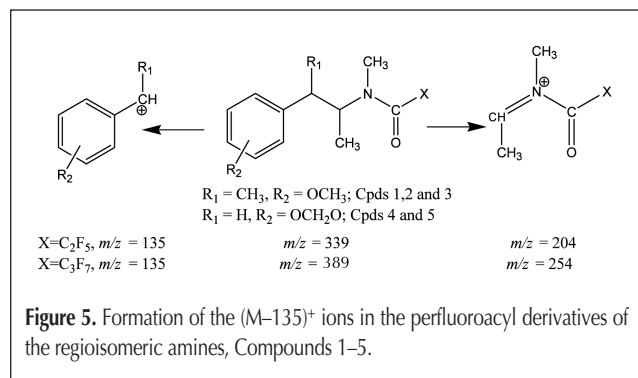


Figure 4. Mass spectra of the HFBA derivatives of Compounds 1–5.

The decreased role for α -cleavage reactions in the fragmentation of these amides allows the formation of ions more diagnostic of each individual isomer. Acylation, and in particular the perfluoroacylation, weakens the bond between nitrogen and the α -carbon of the substituted phenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. The alkene fragment observed at m/z 162 occurs in the spectra of both the PFFA and HFBA derivatives, indicating that the perfluoroacyl moiety is not a component of these ions. This alkene fragment, which is methylenedioxyphenylpropene or its isobaric methoxyphenylbutene, is the radical cation 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 likely from the benzylic carbons to the carbonyl oxygen. Thus the m/z 162 is indicative of the number of carbon in the side chain attached directly to the aromatic ring (see Figure 6).

A comparison of the PFFA derivatives for the five studied compounds with the HFBA derivatives (Figures 3 and 4, respectively) indicates unique ions at m/z 160 and 210. This difference of 50 Da (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7 , respectively. Thus, an analysis of the masses of the components, which make up the fragment at m/z 160, include for example C_2F_5 (119 Da) and CH_3 (15 Da), leaving only a mass of 26 available for the total of 160 Da. The mass 26 would correspond to CN and the proposed mechanism for the formation of $(\text{C}_2\text{F}_5\text{CNCH}_3)^+$ is shown in Figure 7. An equivalent fragmentation pathway has been previously reported (4–10) for other substituted *N*-methylphenethylamines.

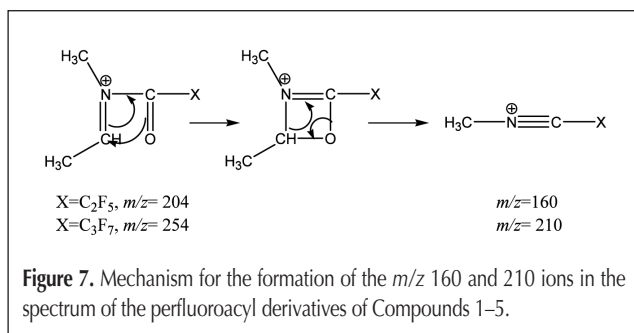
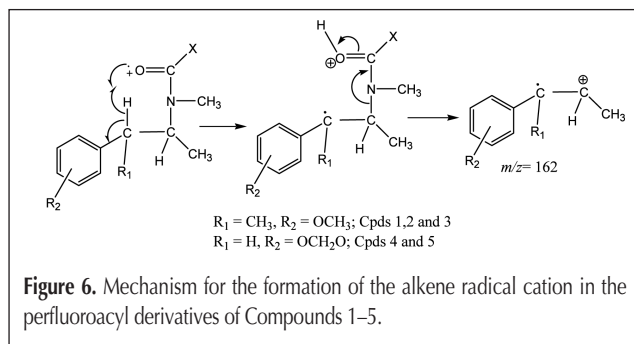
Figures 3 and 4 show that both PFFA and HFBA derivatives of the five studied compounds show the same fragments, and the only difference is in their relative abundance. Thus, the GC–MS analysis of the derivatized isomers served to divide the compounds into two groups; the reference 2,3- and 3,4-MDMA and the isobaric methoxyphenyl-alkylamine isomers. The mass spectra of 2,3- and 3,4-MDMA were significantly different from those of the studied isobaric isomers in terms of relative abundances of fragment ions. The significant relative abundance of the m/z 135 ions distinguishes 2- and 4-MPBA from the MDMA and the 3-MPBA isomer. The m/z 135 base peak for the 2- and 4-MPBAs is due to the resonance stabilization by the ortho- and para- methoxy groups and this resonance stabilization is not available when the methoxy group is in the meta or 3-position. However, the m/z 204, 254 ions are the base peaks for MDMA and the 3-MPBA perfluoroacyl derivatives, respectively, and



these ions are less significant in the 2- and 4-methoxy isomers. Also the 3-MPBA can be differentiated from the MDMA based on the relative abundance of the molecular ion peak at m/z 339, 389 for PFFA and HFBA, respectively, which is much more pronounced in the MDMA relative to the isobaric 3-MPBA isomer. The relative abundance of the fragment ions at m/z 204, 254 can be used to differentiate between the two 2- and 4-MPBA derivatives. These ions are of much greater abundance in the 2-methoxy regioisomer. The two MDMA can be differentiated depending on the relative abundance of the m/z 135, which is much greater in 3,4-MDMA relative to 2,3-MDMA.

Vapor-phase IR spectroscopy

Infrared spectroscopy is often used as a confirmatory method for drug identification in forensic drug analysis. GC–IRD was evaluated for differentiation among the two reference drugs; 2,3- and 3,4-MDMA and their isobaric ring regioisomers of *N*-methyl-2-(methoxyphenyl)-3-butanamines. The vapor phase infrared spectra could provide compound specificity without the need for chemical modification of the parent molecule. The vapor-phase infrared spectra for the five studied compounds are shown in Figure 8. The spectra were generated in the vapor-phase following sample injection into the GC. Each compound shows a vapor-phase IR spectrum with absorption bands in the regions 700–1700 cm^{-1} and 2700–3100 cm^{-1} . Both regions are useful in the identification and differentiation among the compounds in this study. It was previously noticed that variations in the side chain composition results in variations in the IR spectrum in both regions (13). On the other hand, if the side chain composition is kept constant and only the ring substitution is changed, this results in different IR bands in the region 700–1700 cm^{-1} . Having different chemical composition of the side chain, the two MDMA could be significantly distinguished from their isobaric MPBA isomers. A great difference in the IR spectra of both groups at IR regions 700–1700 cm^{-1} and 2700–3100 cm^{-1} could



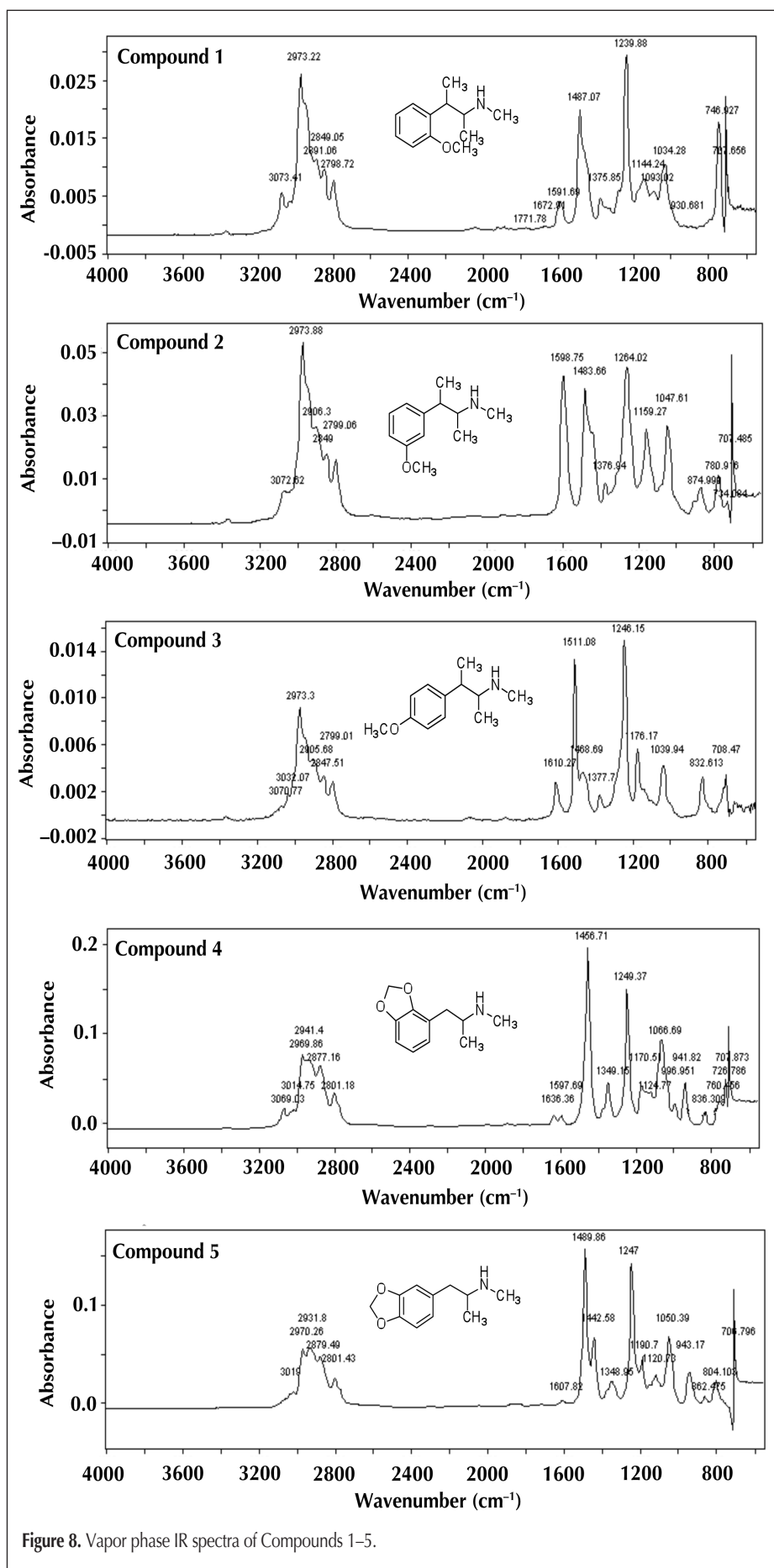


Figure 8. Vapor phase IR spectra of Compounds 1–5.

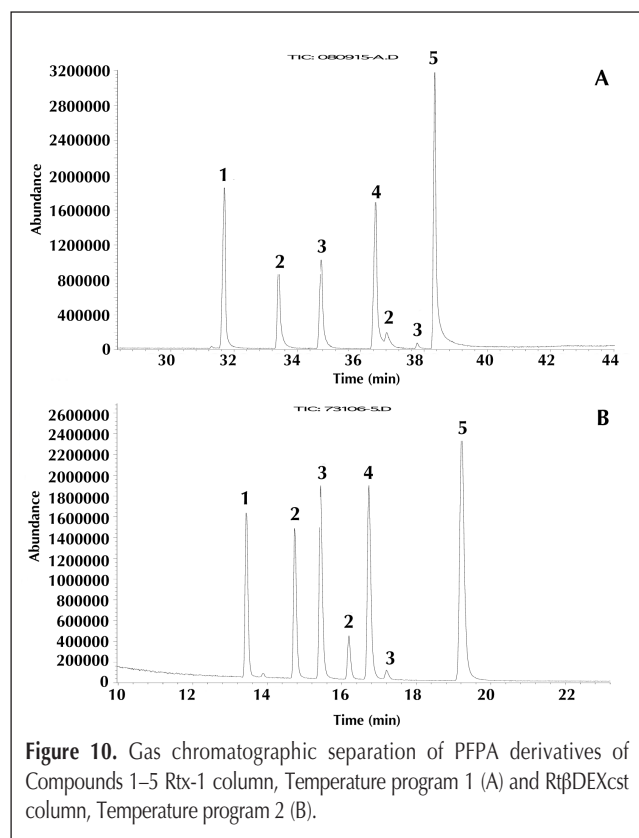
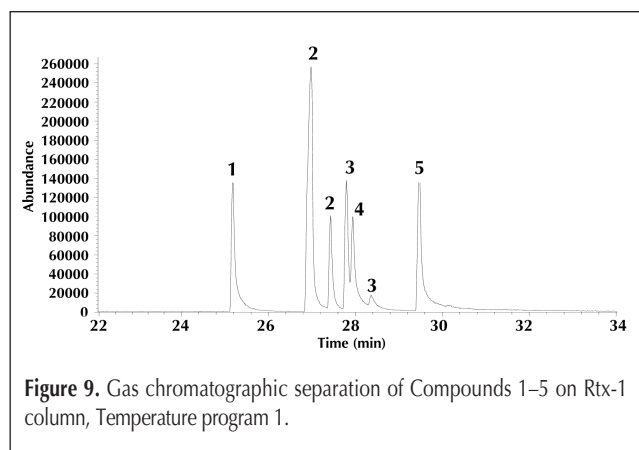
be observed (Figure 8). The three isomeric MPBA compounds showed sharp strong absorption bands at 2973 cm^{-1} compared with the two MDMA that had relatively weaker absorption at 2970 cm^{-1} . Also great differences were observed in the positions and intensities of the absorption bands in the IR region $700\text{--}1700\text{ cm}^{-1}$. Moreover, the IR spectra could enable the discrimination among different regioisomeric MPBA and also between 2,3- and 3,4-MDMA.

Compounds 1–3 yield almost identical IR features in the region $2800\text{--}3100\text{ cm}^{-1}$. However, they can be easily differentiated by the positions and intensities of several absorption bands in the region $700\text{--}1610\text{ cm}^{-1}$. The 2-methoxy regioisomer is characterized by the strong absorption band at 746 cm^{-1} , which is missing in the other 2 regioisomers. Another sharp band at approximately 1239 cm^{-1} is also characteristic for this regioisomer. This band is still sharp but shifted to 1264 and 1246 cm^{-1} in the case of the 3- and 4-methoxy regioisomers, respectively. The 3-methoxy isomer can be distinguished by at least 3 IR bands of medium to strong absorption. The first is the two characteristic peaks of equal intensity at 1047 and 1159 cm^{-1} , which are shifted to 1034 , 1144 and 1039 , 1176 cm^{-1} in the case of the 2- and 4-methoxy regioisomers, respectively. The second is the strong absorption band at 1483 cm^{-1} , which remained within the same intensity but shifted to higher wave number, 1487 cm^{-1} , for the 2-methoxy regioisomer while it is absent in the 4-methoxy isomer. The third is the strong band at about 1599 cm^{-1} , which is converted to small bands at 1591 and 1610 cm^{-1} for 2- and 4-methoxy regioisomers, respectively. Finally, the 4-methoxy regioisomer can be identified by the strong absorbance at 1511 cm^{-1} while the other two regioisomers do not show this peak.

Concerning the reference compounds: 2,3- and 3,4-MDMA, both compounds have methamphetamine side chain composition; therefore they show similar IR absorption to other methamphetamine compounds in the region $2800\text{--}3100\text{ cm}^{-1}$. The two MDMA regioisomers can be differentiated using two IR bands (13). Because these two MDMA share the same side chain composition but differ in the ring substitution pattern, they

show significant changes only in the region 700–1700 cm^{-1} . The 2,3-MDMA shows a medium intensity peak at 1066 cm^{-1} while the 3,4-MDMA shows a similar peak at 1050 cm^{-1} . Another more evident IR band to differentiate between these two compounds can be seen in the region 1440–1490 cm^{-1} where the 2,3-MDMA shows a strong band at 1456 cm^{-1} and the 3,4-MDMA has two adjacent peaks in this region at 1442 and 1489 cm^{-1} of which the latter is a strong absorption band.

These studies indicate that vapor phase infrared spectra provide useful data for differentiation among these regioisomeric and isobaric amines of mass spectral equivalence. Infrared absorption bands provide distinguishing and characteristic information to individualize the amines in this set of uniquely similar compounds which cannot be obtained from their MS spectra except after derivatization.

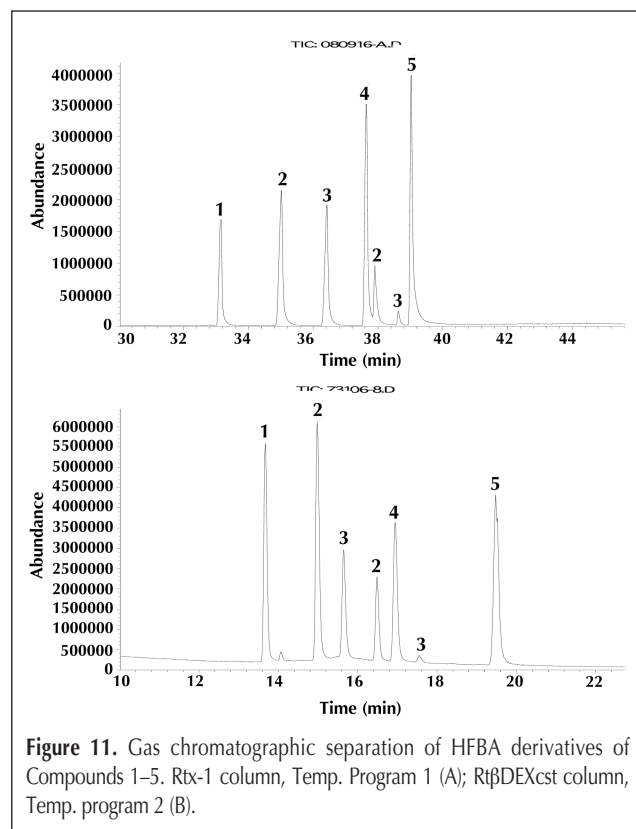


Gas chromatography

Mass spectrometry alone may not provide enough information to distinguish among the underivatized isomers in this study. Therefore the identification by GC–MS must depend heavily on the ability of the chromatographic system to separate these isomeric substances.

The GC properties of the PFPA and HFBA derivatives of the 2,3- and 3,4-MDMAs together with the three regioisomeric *N*-methyl-2-(methoxyphenyl)-3-butanamines were compared on two stationary phases of similar column dimensions (30 m × 0.25 mm). The stationary phases compared in this study were the relatively nonpolar phases, 100% dimethyl polysiloxane (Rtx-1) and 14% cyanopropyl phenyl–86% dimethylpolysiloxane doped with a proprietary cyclodextrin material (RtβDEXcst). Several temperature programs were evaluated, and the best compromises between resolution and analysis time were used to generate the final chromatograms in Figures 9–11. The chromatographic separation of the underivatized compounds on Rtx-1 is shown in Figure 9. It was observed that both 3- and 4-MPBA (compounds 2 and 3) showed an additional peak of identical mass spectrum, which corresponds to the diastereomeric compound. These diastereomeric compounds were verified by examining the mass spectra of the derivatized compounds. Compound 1 (2-MPBA) did not show a peak for the diastereomeric isomer and this may be the result of either very low concentration of the minor diastereomer compared with the major diastereomer, lack of formation during the synthetic procedure, or coelution. The perfluoroacyl derivatives showed improved chromatographic resolution compared to the underivatized species.

The cyclodextrin containing stationary phase (RtβDEXcst)



column provided better resolution when compared with (Rtx-1) as seen in Figures 10 and 11. This stationary phase consists of a polysiloxane polymer containing a modified β -cyclodextrin. Cyclodextrins have been used extensively in separation science because they have shown the ability to discriminate among positional isomers, functional groups, homologues, and enantiomers. Cyclodextrins are capable of forming inclusion compounds with a wide range of hydrophobic molecules and entrapment inclusion occurs without the formation of formal chemical bonds (14,15). Functionalized cyclodextrins form viscous oils suitable for GC stationary-phase coatings and have been used either neat or diluted in polysiloxane polymer as chiral stationary phases for GC applications (16,17). Retention in these systems has been described by a pseudophase model in gas-liquid chromatography in which the two-phase component (pseudophase) exists in the stationary phase (18).

Regarding the elution order in this limited set of compounds and in all cases, the controlled substance 3,4-MDMA elutes last. For the major diastereomeric component of the MPBAs, the elution order is as follows; 2-MPBA elutes first followed by 3-MPBA and 4-MPBA (Figures 9–11).

Conclusion

Differentiation of the controlled 3,4-MDMA and its regioisomer 2,3-MDMA from the three positional ring methoxy isomers of *N*-methyl-2-(methoxyphenyl)-3-butanamines, which have an isobaric relationship to 2,3- and 3,4-MDMA, was accomplished using a combination of gas chromatography and mass or infrared spectrometry. All five compounds have the same nominal mass and produce similar EI mass spectra under common chromatographic conditions. Thus, the traditional electron ionization mass spectrum provides little structural information for differentiating among these compounds. Because of the unique similarity of these compounds by mass spectrometry, the specific identification of a compound such as 3,4-MDMA requires the use of reference standards of each of the other amines in addition to their chromatographic resolution.

The compounds were successfully resolved on two different stationary phases. Derivatization of the amines with perfluoroacylating agents yields amides that significantly individualize the mass spectra and allow for unambiguous identification. GC-IRD studies provided additional structure-IR spectra relationships that allowed the discrimination of the two MDMA from their isobaric MPBA without the need of chemical derivatization.

Acknowledgements

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