Journal of Chromatographic Science 2012;50:1–9
doi:10.1093/chromsci/bmr013

Article

GC–MS and GC–IRD Studies on Dimethoxyphenethylamines (DMPEA): Regioisomers Related to 2,5-DMPEA

Hadir M. Maher[1], Tamer Awad[2,3], Jack DeRuiter[3] and C. Randall Clark[3*]

[1]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt, [2]Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismaillia 41552, Egypt, and [3]Division of Medicinal Chemistry, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849

*Author to whom correspondence should be addressed: email: clarkcr@auburn.edu

Received 26 August 2010; Revised 12 January 2011

A series of regioisomeric dimethoxyphenethylamines have a mass spectra essentially equivalent to the drug substance 2,5-dimethoxyphenethylamine (2,5-DMPEA). These substances have a molecular weight of 181, and major fragment ions in their electron ionization mass spectra at *m/z* 151/152. The trifluoroacetyl, pentafluoropropionyl, and heptafluorobutryl derivatives of these primary amines were prepared and evaluated by gas chromatography with mass spectrometry detection (GC–MS). The mass spectra for these derivatives do not show unique fragment ions to allow the specific identification of a particular isomer. Thus, GC–MS does not provide for the confirmation of identity of any one of the six isomers to the exclusion of the other five compounds. However, GC–MS does divide the compounds into two groups depending on the mass of the base peak. GC with infrared detection provides direct confirmatory data for the identification of 2,5-DMPEA from the other regioisomers involved in the study. Perfluoroacylated derivatives of the six regioisomeric dimethoxyphenethylamines were successfully resolved via capillary GC on a non-polar stationary phase consisting of 50% phenyl and 50% methyl polysiloxane (Rxi-50).

Introduction

In recent years, the popularity of synthetic drugs, sometimes referred to as psychoactive designer or rave drugs, has been increasing due to their stimulant and hallucinogenic effects. The phenethylamine (PEA) related compounds with structural similarities to both amphetamine and the psychedelic PEA, mescaline (1) are common drugs of abuse. Among these types of PEA analogues are 2,5-dimethoxyamphetamine (2,5-DMA), 2,5-dimethoxyphenethylamine (2,5-DMPEA), 2,5-dimethoxy-4-ethylthiophenethyl-amine (2CT-2), 2,5-dimethoxy-4-n-propylthiophenethylamine (2C-T-7), 4-chloro-2,5-dimethoxyphenethylamine (2C-C), 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and 2,5-dimethoxy-4-iodophenethylamine (2C-I) (2).

The increased availability of the two carbon (2C)-series of PEA derivatives on the illicit market is a serious concern (3). The 2C designation has been used to indicate an un-branched 2C linkage between the phenyl and amine groups. Analytical data have been reported for the identification of 2,5-DMPEA and related analogues by measuring their proton nuclear magnetic resonance spectra, infrared spectra, and mass spectra (2). Many separation methods, such as HPLC-UV (4), capillary electrophoresis (CE) (5), and thin-layer chromatography (TLC) (2) are reported for the qualitative analysis of PEA analogues. Furthermore, gas chromatography–mass spectrometry (GC–MS) (2, 6–9), liquid chromatography with photodiode array spectrophotometry (10, 11) and LC–MS (12–15) have been used for preliminary study and screening to discriminate PEA compounds from other hallucinogens. Determination of some members of the 2C-series of PEA designer drugs by means of CE separation with native fluorescence and light emitting diode induced fluorescence detection has been reported (3). A method for the determination of 11 hallucinogenic PEAs after fluorescence labeling has been performed by ultra-fast liquid chromatography (16).

Mass spectrometry is usually the confirmatory piece of evidence for the identification of drugs in forensic and other regulatory laboratories. While the mass spectrum is considered a fingerprint for an individual compound, there are many compounds with essentially equal mass spectra among the substituted PEA. Analysis of these designer drugs is often hindered by the lack of primary reference standards that are required in conventional techniques, where identification is based on the comparison of chromatographic retention, as well as confirmatory spectra.

When other compounds exist with the ability to produce nearly identical mass spectra as the drug of interest, the identification by GC–MS must focus on the ability of the chromatographic system to separate the imposter molecules from the drug of interest. Differentiation of regioisomers is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (17–20). There are an additional five ring regioisomeric PEAs related to 2,5-DMPEA, and the structures of this set of compounds are shown in Figure 1. It is noteworthy that only the 2,5-dimethoxyphenethylamine was described as a potential drug for abuse (2), while the hallucinogenic effect for the regioisomers was not reported. Thus, analytical differentiation among these six regioisomeric substances is an important issue in forensic drug chemistry. This is especially significant since all six precursor isomeric dimethoxybenzaldehydes are commercially available.

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. The GC-Fourier transform infrared spectroscopy technique has been successfully used as a confirmatory spectroscopic method of identification in forensic drug chemistry (21). GC with

infrared detection (IRD) has been applied for the differentiation of some ring and side chain substituted regioisomeric PEAs related to the drug of abuse, 3,4-MDMA (22, 23). It has also been successfully used to discriminate among the regioisomeric 2, 3, and 4-trifluoromethylphenylpiperazines (24), as well as DMPEAs (25).

This report will focus on a comparison of GC–MS and GC–IRD methods for the differentiation of the six regioisomeric DMPEAs. Furthermore, this work will illustrate the value of vapor phase infrared detection for direct differentiation among regioisomeric compounds having mass spectral equivalence.

Experimental

Instrumentation

GC–MS analysis was performed using 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 an ionization voltage of 70 eV. The GC was operated in splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi. The injector temperature was set at 250°C, and the transfer line was maintained at 280°C. Samples were dissolved in HPLC-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and manually introduced (1 μL) individually and in a physical mixture using a 10-μL Hamilton syringe (Hamilton Co., Reno, NV).

The separation of trifluoroacetyl (TFA) derivatives was performed using a temperature program consisting of an initial hold at 100°C for 4 min, ramped up to 170°C at a rate of 1.3°C/min and held at 170°C for 10 min, then ramped to 250°C at a rate of 25°C/min and held at 250°C for 5 min. The pentafluoropropionyl (PFPA) and heptafluorobutryl (HFBA) derivatives of the six compounds were separated using a temperature program consisting 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 3 min, then ramped to 200°C at a rate of 2.5°C/min and held at 200°C for 10 min.

The GC–IRD studies were carried out on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 auto-injector, coupled with an IRD-II infrared detector obtained from ASAP Analytical (Analytical Solutions and Providers, Covington, Kentucky). The vapor phase infrared 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 s. 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), with a flow rate of 1.0 mL/min and a column head pressure of 12 psi. The temperature program involved in this study consisted of an initial temperature of 70°C for 1 min, ramped up to 250°C at a rate of 30°C/min, followed by a hold at 250°C for 15 min. Samples were dissolved and diluted in HPLC-grade acetonitrile and introduced via the auto-injector using an injection volume of 1 μL. The column used in both GC–MS and GC–IRD studies was a 30 m × 0.25 mm i.d. column coated with 0.50 μm 50% phenyl–50% methyl polysiloxane (Rxi-50) purchased from Restek Corporation (Bellefonte, PA).

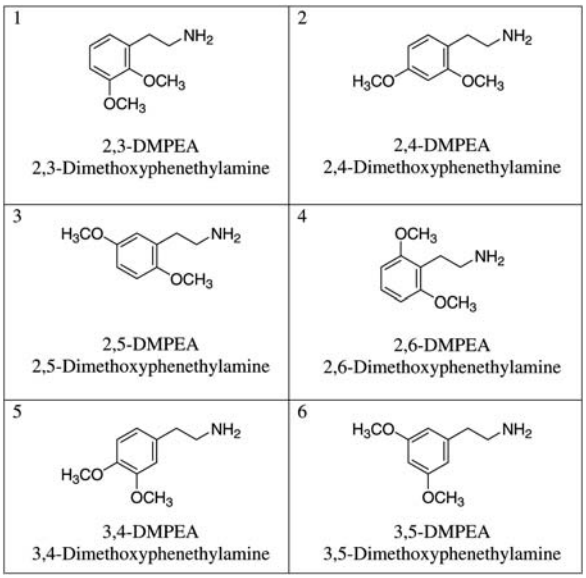

Figure 1. Structures of the six regioisomeric dimethoxyphenethylamines in this study.

Drugs and reagents

The six regioisomeric DMPEAs (Figure 1) used in this study were synthesized in the laboratory. All reagents and chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Atlanta, GA). The derivatizing agents (TFA, PFPA, and HFBA) were purchased from Sigma-Aldrich, Inc. (Milwaukee, WI).

Derivatization procedure

The perfluoroamides were prepared individually from each of the six regioisomers by dissolving approximately 0.3 mg (1.66×10^{-6} mol) of each amine in 50 μL of ethyl acetate, followed by the addition of a large excess (250 μL) of the appropriate derivatizing agent (TFA, PFPA, or HFBA). The derivatization reaction mixtures were incubated in capped tubes at 70°C for 20 min, then evaporated to dryness under a stream of air at 55°C. The resulting residue was reconstituted with 200 μL of ethyl acetate and 50 μL of pyridine, and a portion of each solution (50 μL) was diluted with HPLC-grade acetonitrile (200 μL) to give the working solutions.

General synthetic methods

The six regioisomeric DMPEAs were prepared by the same general procedure using the appropriately substituted dimethoxybenzaldehyde. Condensation of the aldehyde with n-butylamine under dehydrating conditions gave the corresponding imine. The imine was then reacted with nitromethane under acidic conditions to yield the appropriate nitroethene. The latter was then reduced to the corresponding amine using lithium aluminum hydride. The amines were then converted to hydrochloride salts using gaseous HCl. The synthesis of the analytical sample of 2-trideuteromethoxy-3-methoxy-amphetamine was reported previously (25).

Results and Discussion

Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 2 shows the EI mass spectra of the six regioisomeric DMPEAs (Compounds 1–6). The dimethoxybenzyl radical cation at m/z 152 is the base peak for five of the six regioisomeric compounds. The corresponding dimethoxybenzyl cation at m/z 151 is a significant peak for compounds 2 and 5. Other high mass fragments at m/z 137 and 121 are observed for individual regioisomers in this group. The structures for these fragments are shown within the individual spectra in Figure 2. It should be pointed out that the immonium cation commonly observed as the base peak for PEAs would occur at m/z 30 for this unbranched/unsubstituted ethylamine series of compounds. The lower mass range limit for the scan range in Figure 2 was mass 40, thus the spectra do not show a peak for the immonium cation fragment.

The only unique fragment in the spectra in Figure 2 is the m/z 150 ion observed as the base peak for Compound 1, 2,3-dimethoxyphenethylamine. This ion at m/z 150 represents the loss of 31 mass units from the molecular ion at m/z 181 and likely occurs via direct loss of a methoxy group (CH_3O) from the molecular ion. Since the m/z 150 ion only occurs in the 2,3-DMPEA regioisomer, a direct displacement of the methoxy group by the nitrogen atom of the amine side chain is perhaps the best explanation for this unique fragmentation pathway. The steric crowding in this 1,2,3-trisubstituted ring system could promote cyclization and methoxy group displacement in this isomer.

Evidence to support this cyclization with displacement of the methoxy group ortho to the ethylamine side chain can be found in Figure 3, which shows the analogous region of the mass spectrum 2,3-dimethoxyamphetamine (Figure 3A) and 2-trideutero-methoxy-3-methoxyamphetamine (Figure 3B). The addition of the methyl branch in the side chain adds an additional 14 mass units, and the cyclized cationic amine occurs at m/z 164. The mass spectrum in Figure 3A shows the benzylic radical cation at m/z 152 as expected along with the cyclized cationic amine at m/z 164 resulting from loss of the ortho methoxy group (M-31). The fragments in Figure 3B confirm the proposed pathway for this crowded 1,2,3-trisubstituted ring system. The benzylic radical cation in Figure 3B occurs at m/z 155, due to an increase of three mass units due to the 2-substituted OCD3 group. However, the cyclic amine cation remains at m/z 164, showing that it is specifically the 2-substituted (ortho) OCD3 group displaced during this characteristic fragmentation pathway.

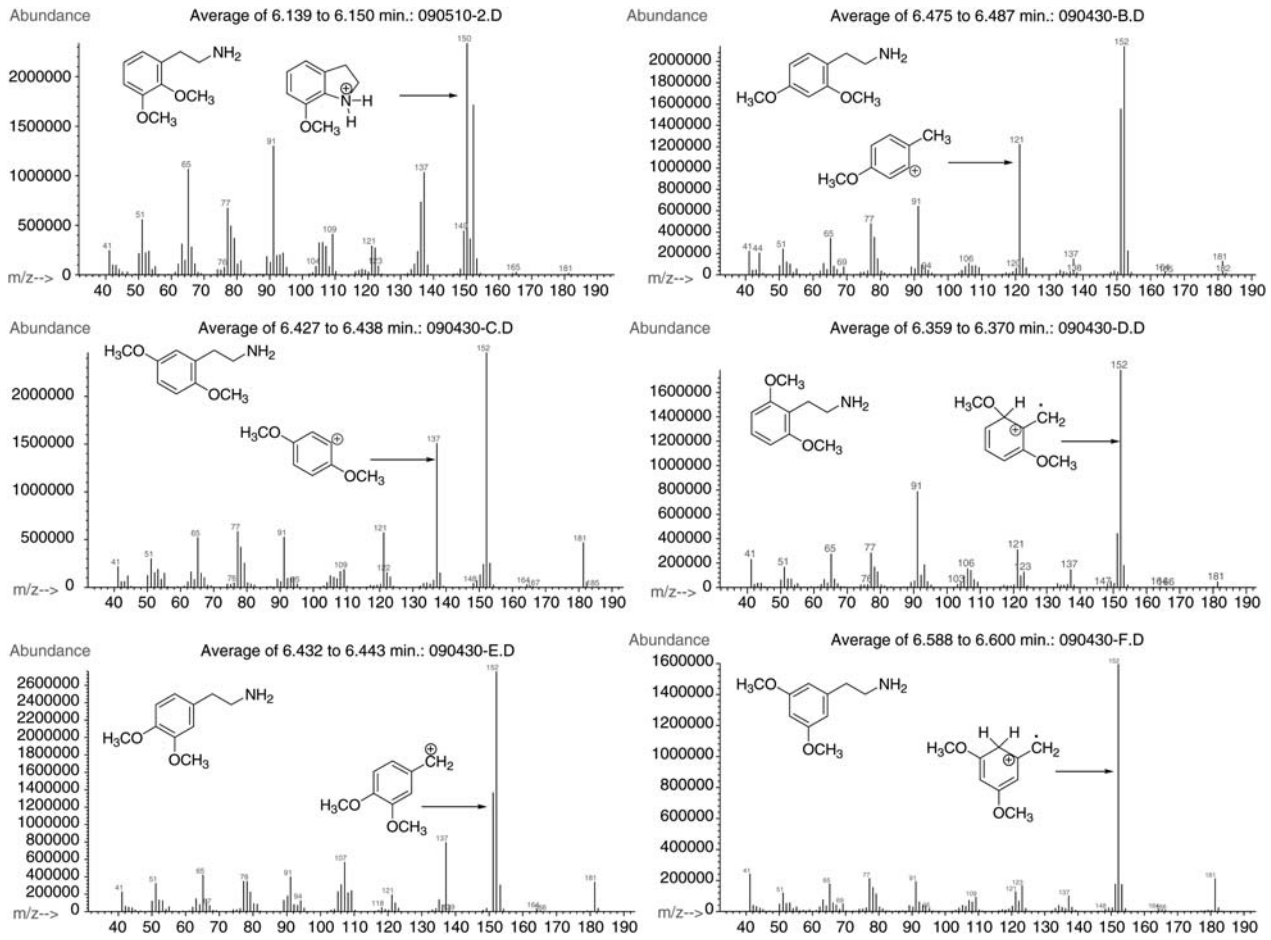

Figure 2. Mass spetra of the six regioisomeric dimethoxyphenethylamines.

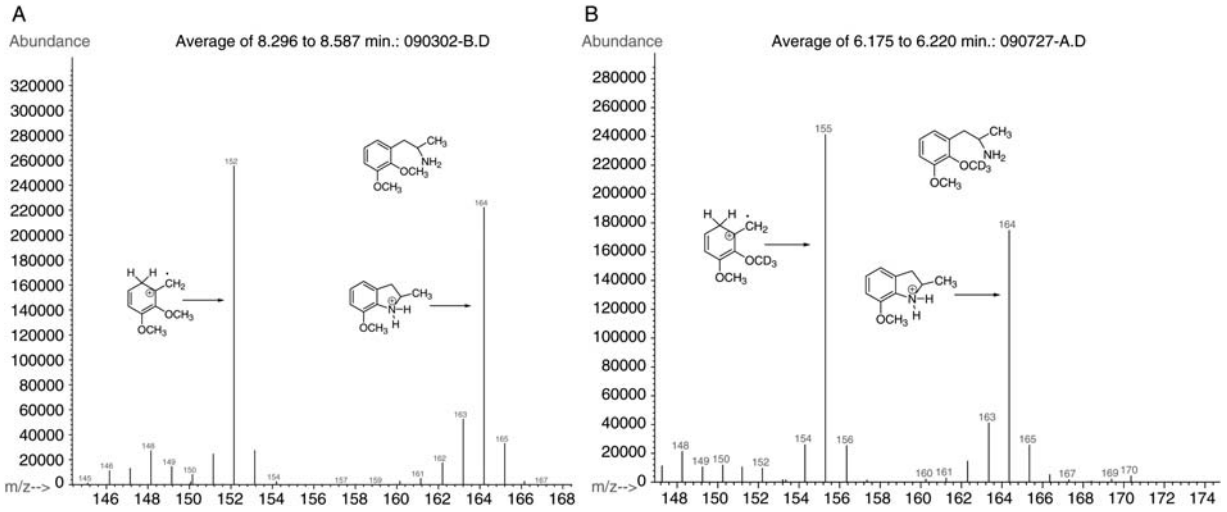

Figure 3. (A) Selected high mass region from the mass spectrum of 2,3-dimethoxyamphetamine. (B) Selected high mass region from the mass spectrum of 2-trideuteromethoxy-3-methoxyamhetamine.

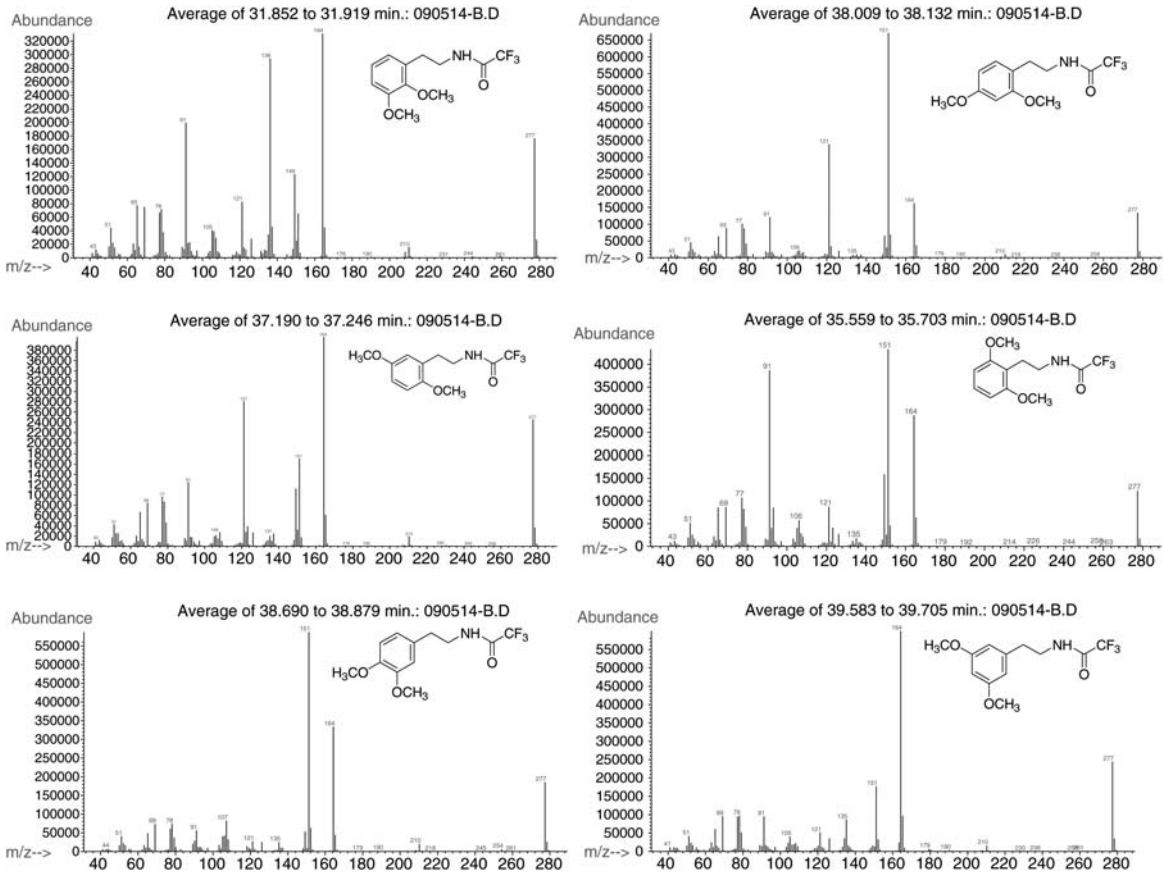

Figure 4. Mass spectra of the TFA derivatives of the regioisomeric dimethoxyphenethylamines.

The d_3-amphetamine sample used for the experiment in Figure 3B was prepared by treating a commercially available sample of 2-hydroxy-3-methoxybenzaldehyde with d3-methyliodide (CD_3I). Subsequent treatment of the aldehyde with nitroethane, followed by an $LiAlH_4$ reduction, gave the desired sample of 2-trideuteromethoxy-3-methoxy-amphetamine (25).

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 spectrum (17–20). The

TFA, PFPA, and HFBA derivatives of the studied compounds were evaluated for their ability to individualize the mass spectra of each compound in this series of regioisomeric DMPEAs. The mass spectra for the six TFA amides are shown in Figure 4 and are representative of all the perfluoroacyl derivatives (TFA, PFPA, and HFBA) examined in this study. The alkene fragment observed at m/z 164 occurs in the spectra of all the TFA, PFPA, and HFBA derivatives, indicating that the perfluoroacyl moiety is not a component of this ion. This alkene fragment, which is dimethoxyphenylethene, is the radical cation resulting from cleavage of the bond between nitrogen and the alkyl carbon of the ethyl side chain (Figure 5). This bond cleavage occurs following an initial hydrogen rearrangement likely from the benzylic carbon to the carbonyl oxygen. Thus, the m/z 164 ion is indicative of the two carbon chain attached directly to the dimethoxyphenyl ring.

Those DMPEAs with the methoxy group in the ortho position are characterized by a significant m/z 121 peak. This ion likely arises from the loss of mass 30 (CH_2O), from the initial dimethoxybenzyl cation at m/z 151 (Figure 6). The m/z 121 ion is a significant fragment only when the methoxy-group is ortho to the alkyl side chain and therefore the site of benzylic cation formation. This m/z 121 ion can be formed by 1,6-hydride shift (ortho-effect) from a hydrogen atom of the methoxy group to the benzyl cation followed by loss of formaldehyde. This fragment (m/z 121) occurs in all spectra of TFA, PFPA, and HFBA of ortho methoxy DMPEA, indicating that the structure does not contain the perfluoroacyl moiety. The suggested mechanism for the loss of CH_2O from the ortho-methoxy benzyl cations was previously discussed (18). A major fragment at m/z 136 occurs in the mass spectra of the three perfluoroacyl derivatives of 2,3-DMPEA. This fragment corresponding to $C_8H_8O_2$ could be attributed to the loss of the ethyl side chain from the corresponding alkene radical cation at m/z 164 (Figure 7). This can occur by hydrogen migration from the methoxy group to the benzyl position followed by loss of ethene.

These six regioisomers can be divided into two groups depending on the base peak. The first group is comprised of three compounds: 2,3-, 2,5- and 3,5-DMPEA, showing the alkene radical cation at m/z 164 as the base peak. The second group includes 2,4-, 2,6-, and 3,4-DMPEA and these compounds show the fragment at m/z 151 as the base peak. Additionally, members within the same group show only differences in the relative abundance of some major peaks.

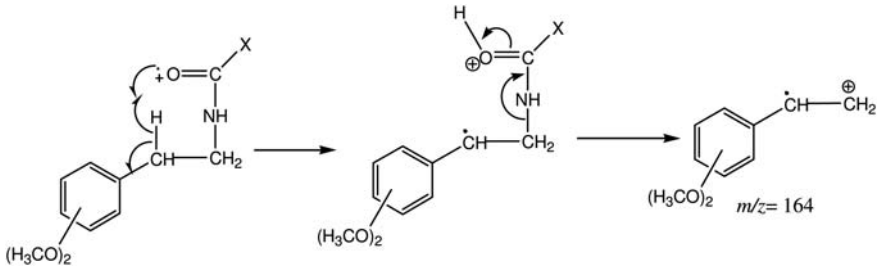

Figure 5. Mechanism for the formation of the alkene radical cation at m/z 164.

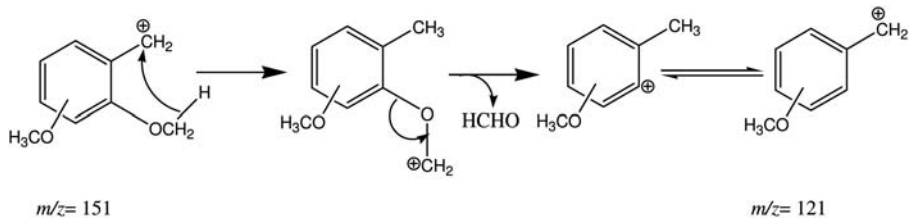

Figure 6. Mechanism for the formation of the m/z 121 ions in the spectra of the perfluoroacyl derivatives of the 2-methoxy regioisomers.

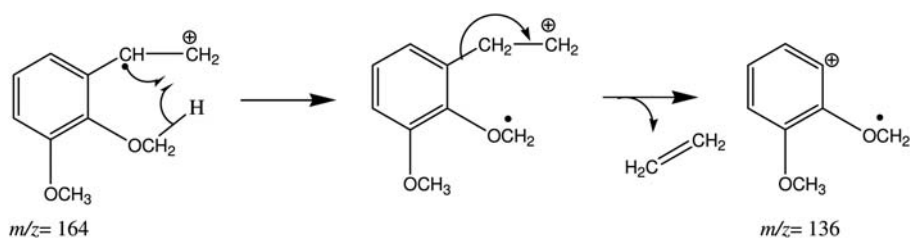

Figure 7. Mechanism for the formation of the m/z 136 ions in the spectra of the perfluoroacyl derivatives of the 2,3-DMPEA.

Mass spectrometry establishes these compounds as having an isomeric relationship of equal molecular weight and equivalent major fragment ions. Perfluoroacylation of these compounds did not provide unique fragments (except for m/z 136 in 2, 3-DMPEA) to individualize the regioisomers in this set of regioisomeric compounds. However, the perfluoroacylation did serve to divide the isomers into two groups of three compounds each, based on the mass of the base peak.

Vapor-phase infrared spectroscopy

GC–MS studies on the perfluoroacylated derivatives of the six regioisomeric dimethoxyphenethylamines showed that the compounds could be divided into two main groups depending on their base peak. Infrared spectroscopy is often used as a confirmatory method for drug identification in forensic drug analysis. In the next phase of this study, GC–IRD was evaluated for differentiation of these six regioisomeric amines. The vapor-phase infrared spectra for the two groups of compounds (Group 1: 2,3-, 2,5- and 3,5-DMPEA; and Group 2: 2,4, 2,6- and 3,4-DMPEA) are shown in Figures 8 and 9, respectively. The spectra are generated in the vapor-phase following sample injection into the gas chromatograph. Examining the IR spectra within each group reveals that significant differences exist among the spectra distinguishing these regioisomeric compounds. Although all six regioisomers

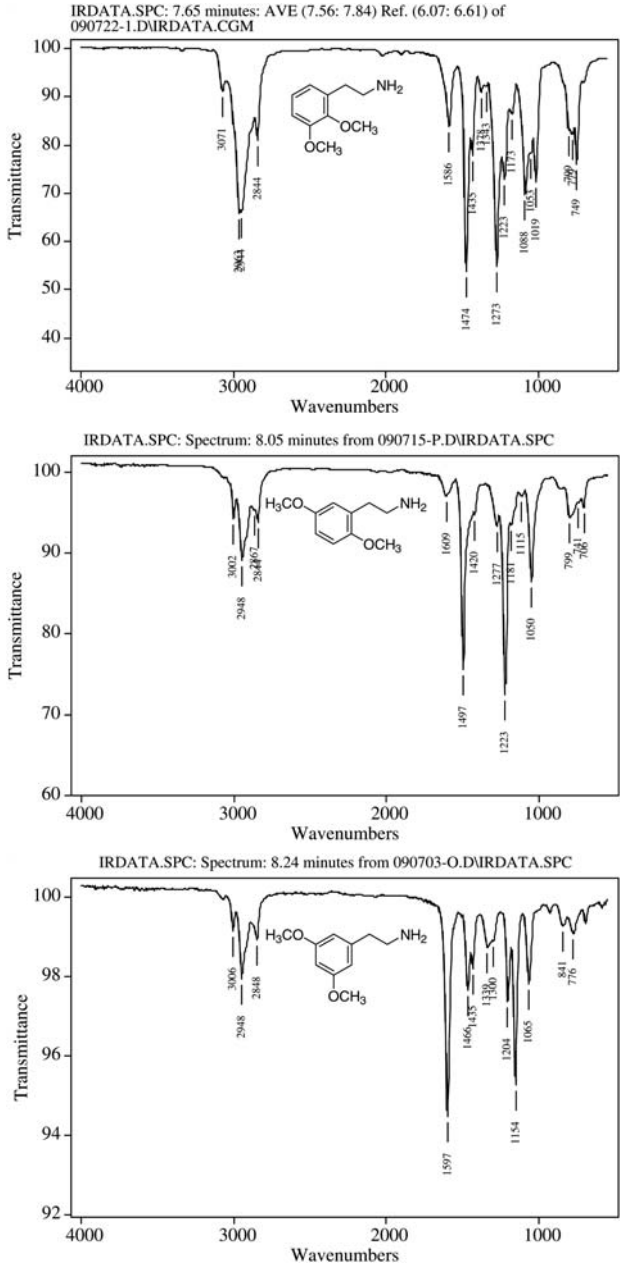

Figure 8. Vapor phase IR spectra of the DMPEA having a base peak at m/z 164 in their mass spectra (Group 1); compound 1: 2,3 = DMPEA, compound 3: 2,5-DMPEA and compound 6: 3,5-DMPEA.

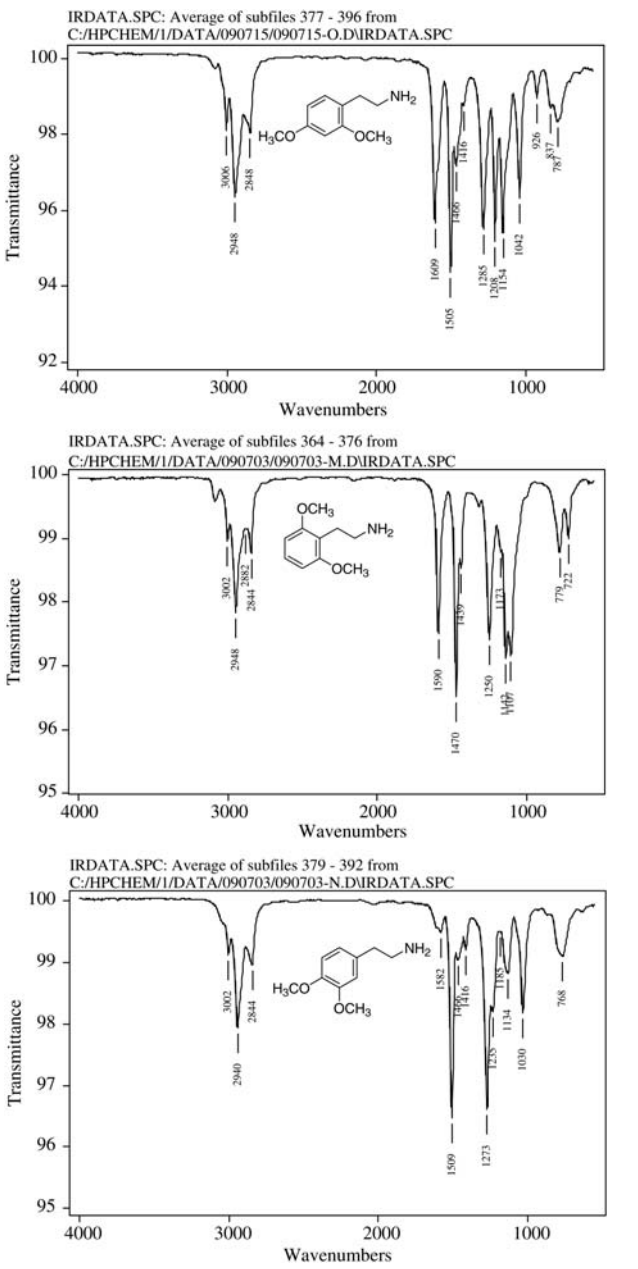

Figure 9. Vapor phase IR spectra of the DMPEA having a base peak at m/z 151 in their mass spectra (Group 2) compound 2; 2,4-DMPEA, compound 4; 2,6-DMPEA and compound 6; 3,4-DMPEA.

show similar absorption bands in the IR region 2800–3100 cm^{-1}, they can be easily differentiated by several IR absorption bands of medium to strong intensity in the region 700–1700 cm^{-1}. Among members of the first group (Figure 8), at least six regions in their IR spectra can be used for the characterization of these compounds. The first is the peak of intermediate intensity at 749 cm^{-1}, which is characteristic to 2,3-DMPEA, and this peak is not present in the other two isomers. The second is the two adjacent peaks, appearing in the IR specrum of 2,3-DMPEA at about 1019 and 1088 cm^{-1}, which appears as a singlet at 1050 and 1065 cm^{-1} for 2,5- and 3,5-DMPEA, respectively. The third region involves the strong IR peak at 1223 cm^{-1} with a following weaker peak at 1277 cm^{-1} for the 2,5-DMPEA. Similar peaks at almost the same wave numbers can be found in the spectrum of the 2,3-DMPEA with the higher intensity peak at 1273 cm^{-1}. On the other hand, these peaks are shifted to shorter wave numbers at 1154, 1204 cm^{-1} for 3,5-DMPEA. The fourth is the broad band, appearing at 1339 cm^{-1} in the IR spectrum of 3,5-DMPEA, which is characteristic for this particular isomer because no equivalent band occurs in the other two regioisomers. The fifth is the strong peak appearing at 1474 cm^{-1} in the IR spectrum of 2,3-DMPEA, which is shifted to 1497 cm^{-1} for 2,5-DMPEA, while a much weaker peak can be found at 1466 cm^{-1} in the IR spectrum of 3,5-DMPEA. The last is the strong singlet peak at 1597 cm^{-1}, which distinguishes the IR spectrum of 3,5-DMPEA. This band is present as much smaller peaks at 1586 and 1609 cm^{-1} for 2,3- and 2,5-DMPEA, respectively.

Similarly, members of the second group (Figure 9) could be differentiated by several absorption bands in the 700–1700 cm^{-1} IR region. The first IR bands are in the 700 cm^{-1}

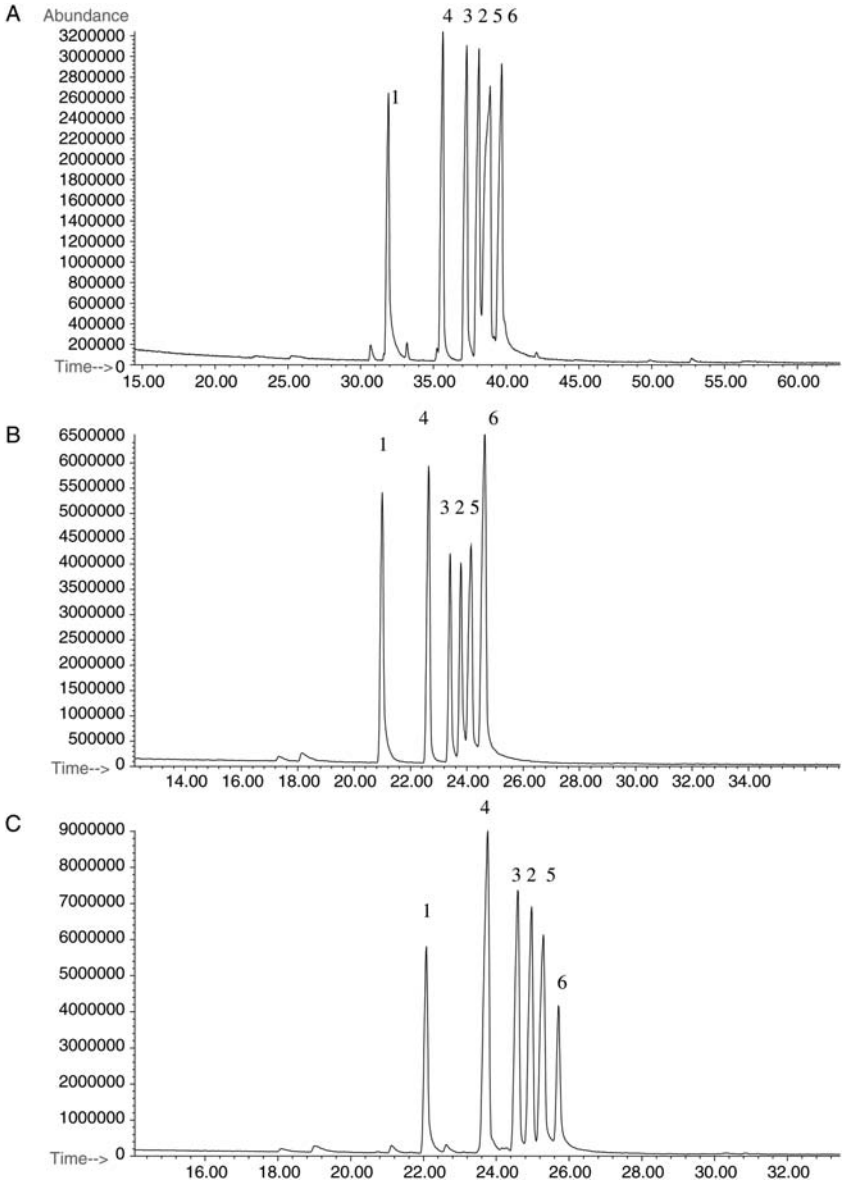

Figure 10. GC chromatograms of A; TFA, B; PFPA, and C; HFBA derivatives of compounds 1–6.

region where the 2,6- isomer shows two adjoining medium intensity peaks at 722 and 779 cm^{-1} while the 2,4- and 3,4-isomers each show only one peak in the same region at 787 and 768 cm^{-1}, respectively. The second IR band is in the 900 cm^{-1} region where only the 2,4- isomer shows a medium intensity peak at 926 cm^{-1} while similar peaks in the same region are lacking in the other two isomers. The absorption band at 1042 cm^{-1} appearing in the IR spectrum of 2,4-DMPEA is shifted to 1030 cm^{-1} for 3,4-DMPEA and not present in the spectrum of 2,6-DMPEA. The doublet peak observed in the IR spectrum of 2,4-DMPEA at 1154 and 1208 cm^{-1} remains as a doublet peak but shifted to shorter wavenumbers at 1107 and 1142 cm^{-1} for 2,6-DMPEA and a broad peak at 1134 cm^{-1} for 3,4-DMPEA. The sharp peak appearing at 1285 cm-1 in the IR spectrum of 2,4-DMPEA, shifted to 1250 cm^{-1} in 2,6-DMPEA while it is present as two adjacent peaks at 1235 and 1273 cm^{-1} for 3,4-DMPEA. The strong peak at 1470 cm^{-1} with a preceding weaker one at 1439 cm^{-1} is characteristic for the 2,6-DMPEA. The other two isomers in this group are quite different and show a relatively low intensity IR peak at 1466 cm^{-1} in addition to a stronger one close to 1500 cm^{-1}. Finally, there is a strong absorption peak appearing at 1609 and 1590 cm^{-1} for 2,4- and 2,6-DMPEA, respectively, and this peak appears as a small broad band at 1582 cm^{-1} in the IR spectrum of 3,4-DMPEA.

These results show that vapor phase infrared spectra can provide useful data for differentiation among these regioisomeric dimethoxyphenethylamines. Mass spectrometry establishes these compounds as having an isomeric relationship of equal molecular weight and equivalent major fragment ions. Mass spectra of the perfluoroacylated derivatives of these compounds do not provide fragment ions characteristic for a particular isomer. However, GC–IRD readily discriminates the drug of abuse; 2,5-DMPEA from its other five structurally related regioisomers.

Gas chromatography

The TFA, PFPA, and HFBA derivatives of the six regioisomeric dimethoxyphenethylamines, were compared on a 50% phenyl–50% methyl polysiloxane (Rxi-50) stationary phase. Several temperature programs were evaluated, and two different programs were used to generate the final chromatograms in Figure 10. The chromatograms show that, regardless of the derivative type, 2,3-DMPEA elutes first, followed by 2,6-DMPEA, 2,5-DMPEA, 2,4-DMPEA, 3,4-DMPEA, and then 3,5-DMPEA, which is the last eluting compound. This elution order parallels the degree of potential intramolecular interactions for the substituent groups attached to the aromatic ring. The first two compounds to elute, the 2,3- and 2,6-isomers, represent the two compounds in which all three ring substituents are crowded in a 1, 2, 3-ring substitution pattern. The next three compounds have only two groups substituted in a 1,2-crowded pattern. While the last compound to elute, the 3,5-DMPEA isomer has the most symmetrical structure with substituents arranged to minimize interactions between groups.

A direct comparison of the separation of the three derivative types on Rxi-50 column indicates that mixtures of both PFPA and HFBA derivatives could be resolved within 26 min. However, at least 40 min were needed to resolve the TFA derivatives since the shorter analysis time resulted in coelution of the peaks for 2,4-DMPEA and 3,4-DMPEA.

Conclusion

The six regioisomeric dimethoxyphenethylamines yield similar fragment ions in their mass spectra even after perfluoroacylation, except for some unique fragment ions observed for 2,3-DMPEA. GC–MS studies showed that the perfluoroacylated derivatives served to divide the compounds into two main groups based on the mass of the base peak in the spectrum. GC-IRD analysis yields unique and characteristic vapor phase infrared spectra to differentiate among members within each group. These spectra allow discrimination among all the regioisomeric compounds included in this study without the need for chemical derivatization. Mixtures of perfluoroacylated regioisomers were successfully resolved via capillary gas chromatography using relatively nonpolar stationary phases and temperature programming conditions.

Acknowledgement

This project was supported by cooperative agreement 2006-DN-BX-K016, U.S. Department of Justice, Office of Justice Programs, National Institute of Justice. The opinions contained herein are those of the author(s) and do not necessarily represent the official position of the U.S. Department of Justice. The Auburn University Laboratory thanks Analytical Solutions and Providers LLC of Covington, Kentucky for the use of the GC–IRD equipment.

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