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Article

Differentiation of Methylenedioxybenzylpiperazines and Ethoxybenzylpiperazines by GC–IRD and GC–MS

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The substituted benzylpiperazines, 3,4-methylenedioxybenzylpiperazine, its regioisomer 2,3-methylenedioxybenzylpiperazine and three isobaric ring substituted ethoxybenzylpiperazines have equal mass and many common mass spectral fragment ions. The mass spectra of the three ethoxybenzylpiperazines yield a unique fragment at *m/z* 107 that allows the discrimination of the three ring substituted ethoxybenzylpiperazines from the two methylenedioxy isomers. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions, but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

Gas chromatography coupled with infrared detection provides direct confirmatory data for the structural differentiation between the five isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The perfluoroacyl derivatives of the ring substituted benzylpiperazines were resolved on a stationary phase of 50% phenyl and 50% methylpolysiloxane.

Gas chromatography coupled with time-of-flight mass spectrometric detection provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazine and 4-ethoxybenzylpiperazine, which have similar nominal masses but are different in their calculated exact masses.

Introduction

The 1-arylpiperazine group of compounds represent a new category of drugs of abuse (1, 2). This class of potential designer drugs includes *N*-benzylpiperazine, 1-(3,4-methylenedioxybenzyl)-piperazine (3,4-MDBP) and phenylpiperazines such as 1-(3-trifluoromethyl-phenyl)piperazine, 1-(3-chlorophenyl)piperazine and 1-(4-methoxyphenyl)piperazine (3). Both *N*-benzylpiperazine and 3-trifluoromethylphenyl piperazine (3-TFMPP) were placed into Schedule 1 of the United States Controlled Substance Act in September of 2002 (4). Recently, 3,4-MDBP has been described as producing psychoactive effects similar to those of 3,4-methylenedioxymethamphetamine (MDMA) (5–7).

3,4-MDBP has been reported (8) as a potential drug of abuse, although the pharmacology of its 2,3 regioisomer has not yet been reported. Indeed the analysis of 3,4-MDBP in biological and forensic samples has been the focus of several studies in recent years (8–12). A recent report (13) showed that 3,4-MDBP cannot be differentiated from its 2,3 regioisomer using mass spectrometry even after chemical derivatization. However, gas chromatography coupled with infrared detection (GC–IRD) provided discrimination between these two compounds based on differences in position and intensity in several IR absorbance bands. Another report (14) described GC–IRD and GC–mass spectrometry (MS) studies on the two regioisomeric ring substituted methylenedioxybenzylpiperazines and their isobaric ring substituted methoxymethylbenzylpiperazines.

GC–MS is the most commonly employed technique in the analysis of controlled substances in forensic laboratories (15–20). The identification of psychoactive drugs in many chemical categories is complicated by the existence of regioisomeric and isobaric substances related to the target drug (8, 10–16). Many of these isomeric substances have equivalent mass spectral fragments and are a challenge to forensic analyses that depend heavily on mass spectrometry for confirmation level data.

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–IRD is characterized by scanning quickly enough to obtain vapor phase IR spectra of compounds eluting from the capillary GC columns. This technique has been successfully used in the identification of amphetamine isomers (21) as well as side chain regioisomers related to methamphetamine and phentermine (22). Recently, GC–IRD studies have been described for the differentiation of ring and side chain substituted ethoxyphenethylamines, methoxymethcathinones and methylenedioxymethamphetamines (23).

The ethoxybenzylpiperazines have an isobaric relationship with 2,3 and 3,4-MDBP. Isobaric compounds are those with the same nominal mass but with different elemental composition. Substitution of the ethoxy group on the 2, 3 and 4 positions of the aromatic ring gives three possible ring substituted ethoxybenzyl piperazine isomers of equivalent nominal mass to that of the methylenedioxybenzylpiperazines.

This report will describe GC–IRD and GC–MS studies on the two regioisomeric ring substituted methylenedioxybenzylpiperazines and their isobaric ring substituted ethoxybenzylpiperazines in an effort to offer confirmation level discrimination among these compounds.

Experimental

Instrumentation

GC–MS analysis was performed using a 7890A gas chromatograph with a 7683B auto injector coupled with a 5975C VL

mass selective detector purchased from Agilent Technologies (Santa Clara, CA). The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless mode with a helium (grade 5) flow rate at 0.7 mL/min and the column head pressure was 10 psi. The MS was operated in the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230°C. The GC injector was maintained at 250°C and the transfer line at 280°C.

Chromatographic separation was carried out using a capillary column 30 m × 0.25 mm i.d. coated with 0.50 μm of 50% phenyl−50% methylpolysiloxane (Rxi-50). The temperature program consisted of an initial temperature of 70°C for 1 min, ramped up to 250°C at a rate of 30°C per min, followed by a hold at 250°C for 15 min.

The GC−time-of-flight (TOF) analysis utilized a 6890N gas chromatograph with a 7683B auto injector purchased from Agilent Technologies (Santa Clara, CA) coupled to a Waters GCT Premier benchtop orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer. Chromatographic separation was carried out using a DB5-MS capillary column 30 m × 0.25 mm i.d. coated with a stationary phase film thickness of 0.50 μm (J&W Scientific). The temperature program consisted of an initial temperature of 70°C for 1 min, ramped up to 250°C at a rate of 15°C per min, followed by a hold at 250°C for 7 min. Elemental composition determination of fragment ions was accomplished using accurate mass measurement and isotope modeling analysis comparing the experimental and theoretical isotope distribution. The internal calibrant was heptacosafluorotributylamine (m/z 118.9919, Sigma Chemical Co.) and the experimental results were within 5 ppm of the theoretical value.

GC−IRD studies were carried out using a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 auto-injector coupled with an IRD-II infrared detector (IRD-II) obtained from Analytical Solutions and Providers (ASAP, Covington, KY). The vapor phase infrared spectra were recorded in the range of 4,000−650 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans per s. The IRD flow cell and transfer line temperatures were maintained at 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 and a column head pressure of 10 psi. The capillary column used was a 30 m × 0.25 mm i.d. coated with 0.50 μm of 50% phenyl−50% Rxi-50. The temperature program involved in this study consisted of 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. Both capillary columns used in these studies were purchased from Restek (Bellefonte PA).

In GC−MS, GC−TOF and GC−IRD analyses, samples were dissolved and diluted in HPLC-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced, individually and in physical mixtures, via the auto injectors using an injection volume of 1 μL in both the GC−MS and GC−IRD studies and 2 μL in the GC−TOF analyses.

Drugs and Reagents

The general procedure for the synthesis of these five isomeric benzylpiperazines involves the reductive amination of the appropriately substituted benzaldehyde and piperazine in the presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for Compounds 1, 2 and 3 are 2, 3 and 4-ethoxy benzaldehyde, respectively, and the starting material for Compound 5 is 3,4-methylenedioxybenzaldehyde (piperonal), and all are commercially available. 2,3-Methylenedioxybenzaldehyde is the starting material for 2,3-MDBP (Compound 4) and its preparation has been reported (24, 25). All laboratory reagents and solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Atlanta, GA). The derivatizing reagents trifluoroacetic anhydride (TFA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were purchased from Sigma-Aldrich. (Milwaukee, WI).

Derivatization procedure

Each perfluoroamide was prepared individually by dissolving approximately 0.3 mg (1.36×10^{-6} mol) of each amine hydrochloride salt in 50 μL of ethyl acetate, followed by addition of a large excess (250 μL) of the appropriate derivatizing agent (TFA, PFPA or HFBA), and the 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.

Results and Discussion

Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 1 shows the EI mass spectra of all five isomeric substituted benzylpiperazines (Compounds 1−5). The ions of significant relative abundance common to the five isomers likely arise from fragmentation of the piperazine ring. These spectra show fragment ions at m/z 178, 164, and 135 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 2 and are based on a previous report describing the fragmentation of the unsubstituted benzylpiperazines (9). The ethoxy benzyl $(C_9H_{11}O)^+$ fragments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. However, the relative abundances for the ions in the spectra for the five isomeric benzylpiperazines are slightly different. The mass spectra for the ring substituted ethoxybenzylpiperazines (Compounds 1−3) have almost identical mass spectra to the methylenedioxy isobars (Compounds 4 and 5), except for the unique ion at m/z 107. This ion at m/z 107 represents the loss of 28 mass units (ethylene, C_2H_4) from the ethoxybenzyl cation at m/z 135, as presented in Figure 3 (19). The relative abundance of this marker ion at m/z 107 is highest in the mass spectrum of the 4-ethoxy isomer, likely due to the conjugation of the 1,4-ring substituents. Thus, these mass spectra provided one unique fragment ion yielding some

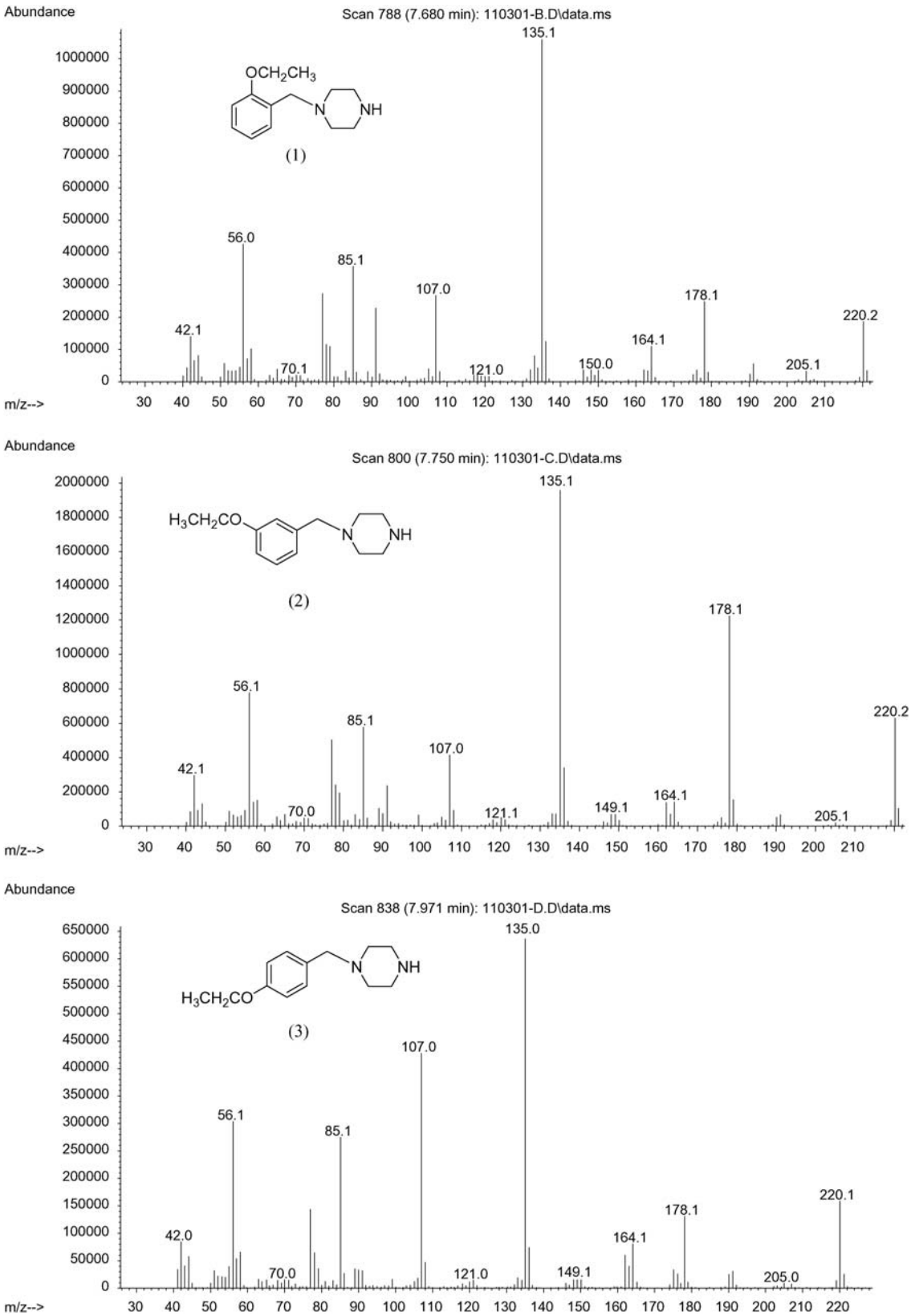
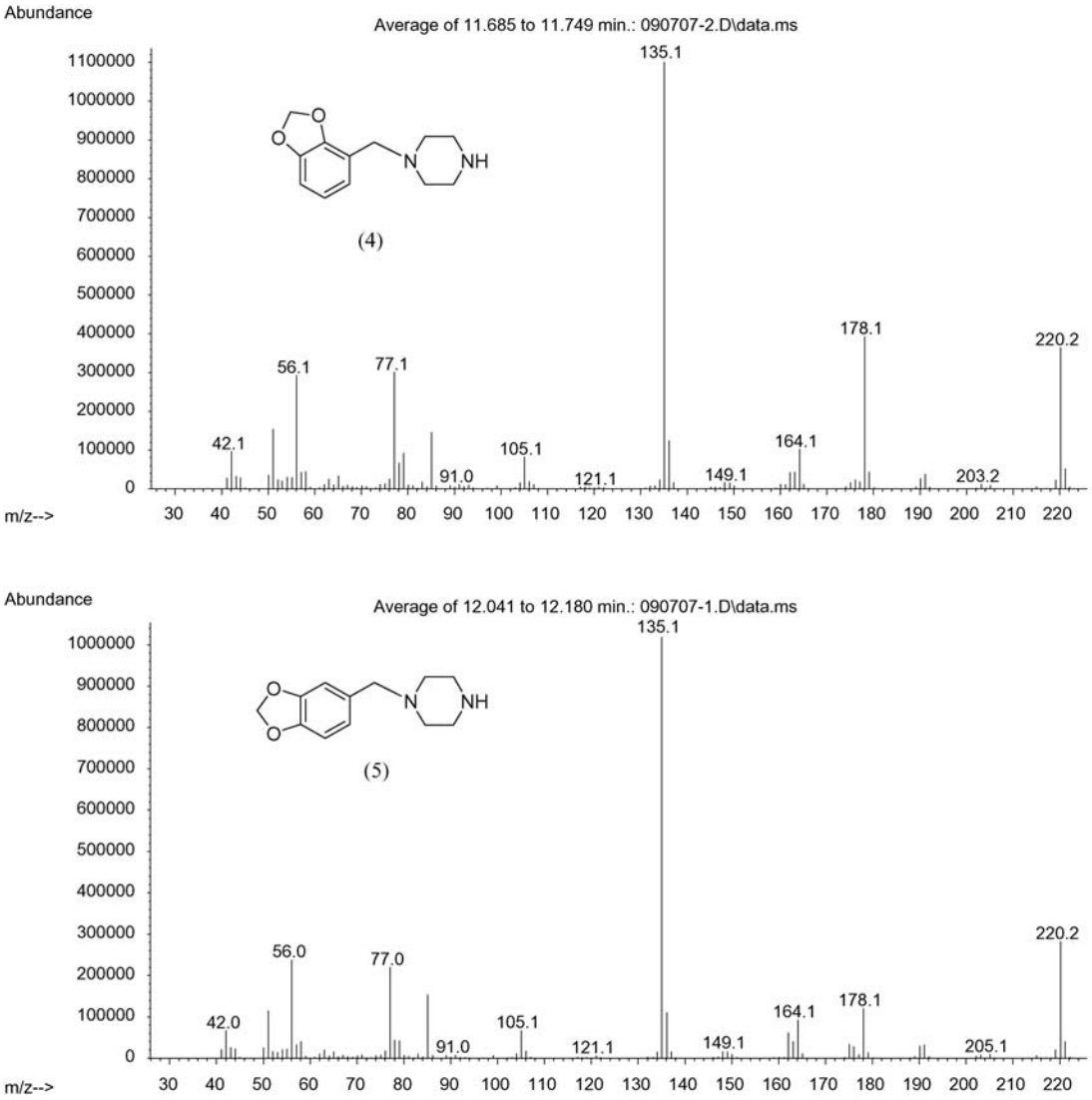


Figure 1. Mass spectra of the methylenedioxy and ethoxybenzylpiperazines in this study.



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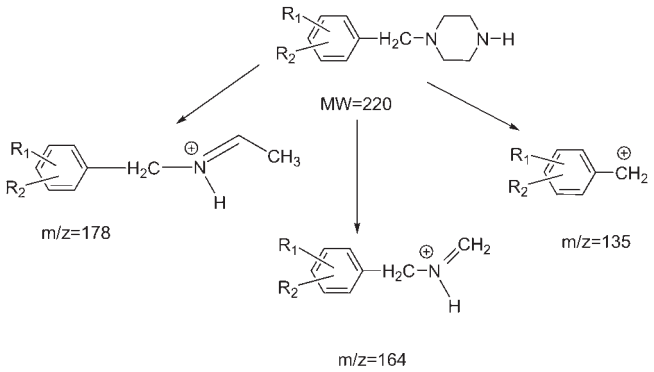

$R_1 = OCH_2CH_3$, R_2 = H in case of EBP

R_1, R_2 = methylenedioxy in case of MDBP

Figure 2. Mass spectral fragmentation pattern (EI, 70 eV) of the underivatized methylenedioxypiperazines and ethoxybenzylpiperazines.

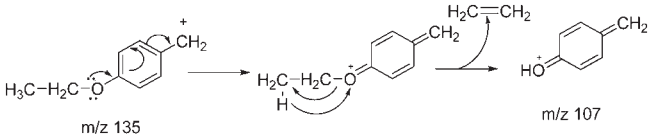

Figure 3. Mass spectral fragmentation of the ethoxybenzylpiperazines yielding the fragment cation at m/z 107.

discrimination of the ethoxybenzylpiperazines from the isobaric methylenedioxybenzyl compounds.

The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric benzylpiperazines, in an effort to individualize their mass spectra and identify additional unique marker ions for these five compounds. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary nitrogen were evaluated for their ability to individualize the mass spectra of this series of

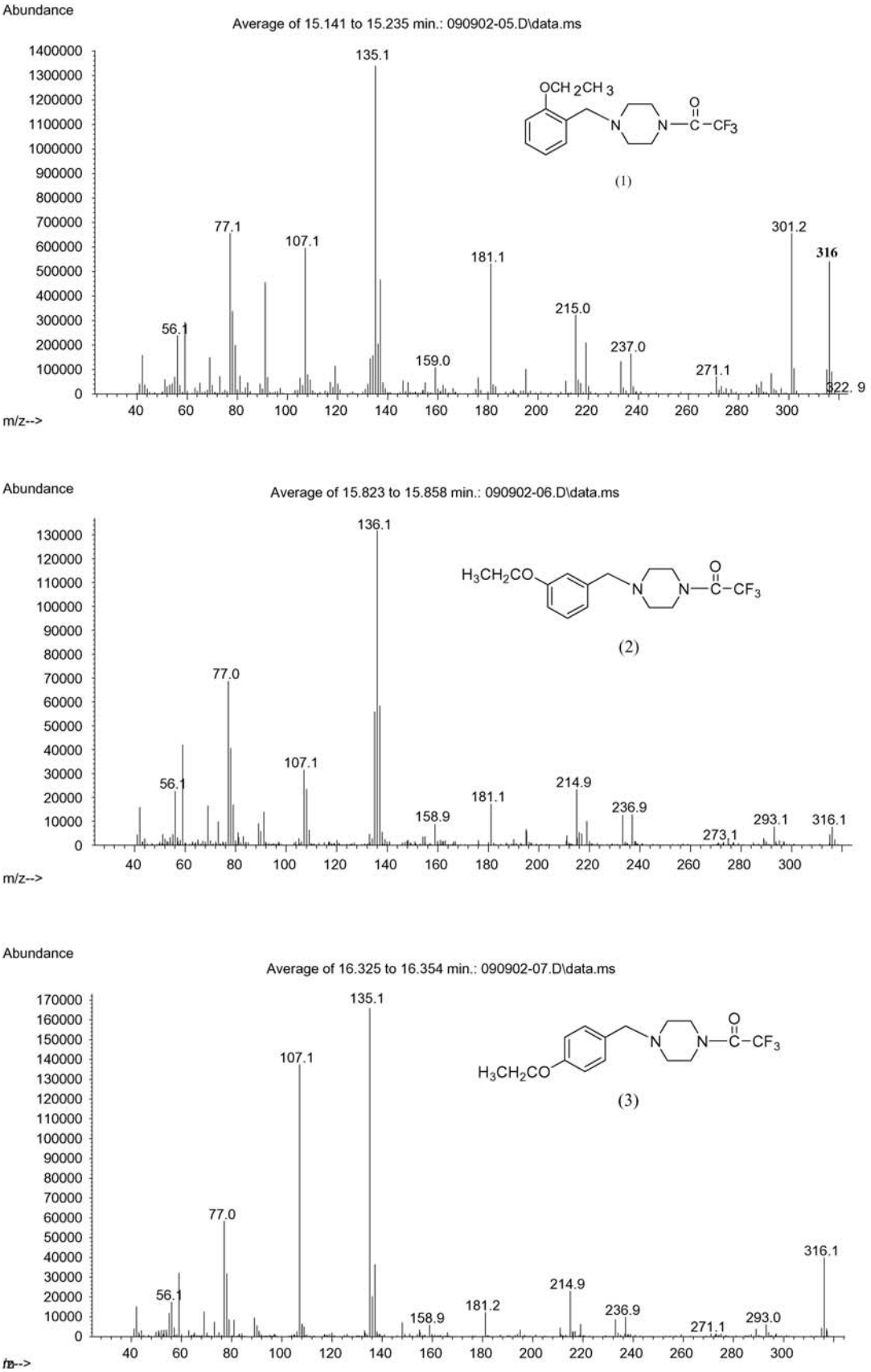

Figure 4. Mass spectra of the trifluoroacetyl derivatives of the five piperazine compounds in this study.

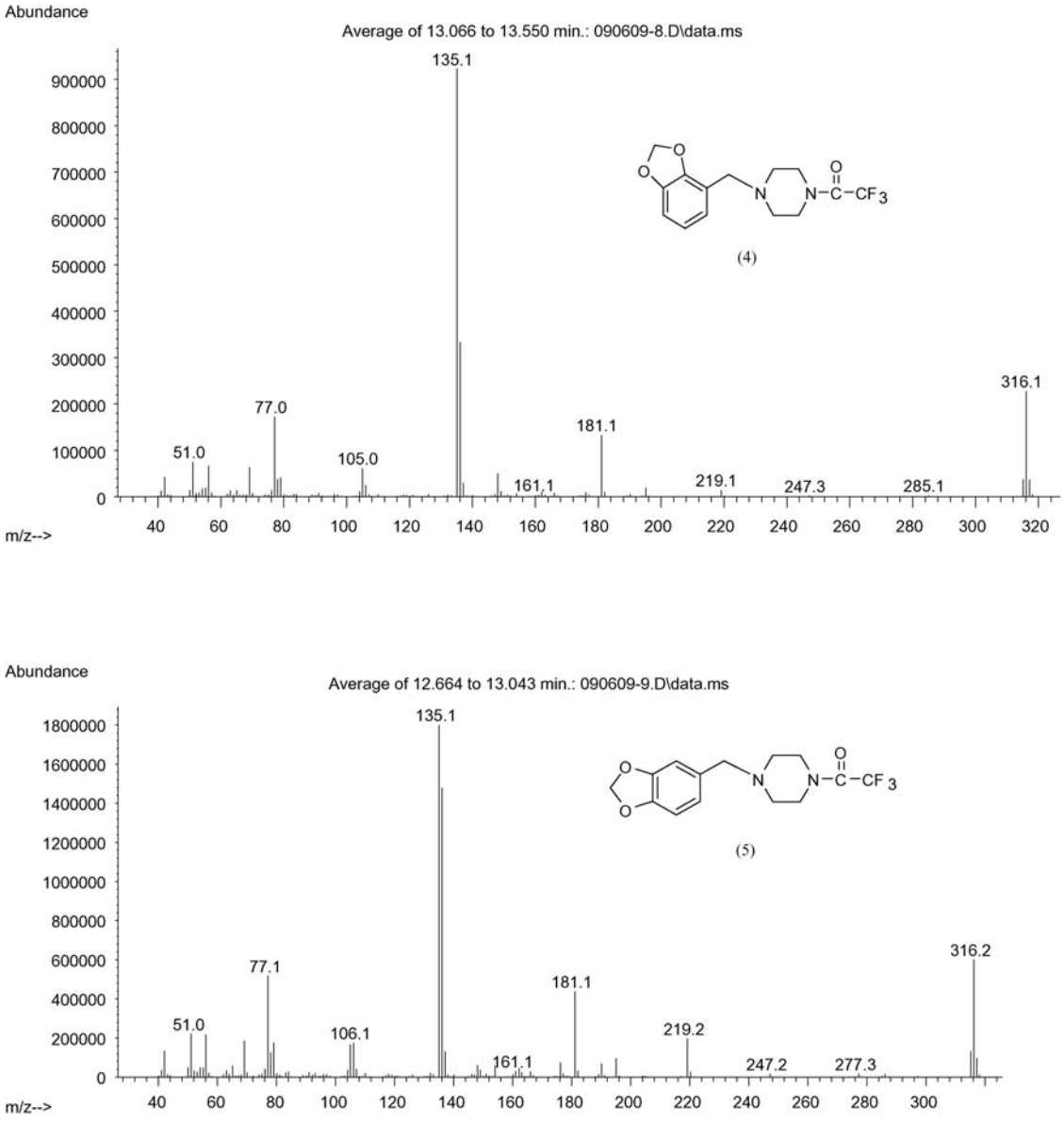

Figure 4 (Continued)

substituted benzylpiperazines. Figure 4 shows the mass spectra of the trifluoroacetyl amides of the five studied compounds as representative spectra for all the perfluoroacyl piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at m/z 316, 366 and 416, respectively. The major fragment ion in these spectra occurs at m/z 135 and corresponds to the ring substituted benzyl cation. Furthermore, an additional fragment ion series occurring at m/z 181, 231 and 281 for the TFA, PFPA and HFBA amides respectively corresponds to the $(M\text{-}135)^+$ ion for each amide. The ion at m/z 219 was observed in the spectra of all derivatives and is likely formed by the elimination of the acyl moiety. Those ions occurring at m/z 69, 119 and 169 are the perfluoroalkyl cations trifluoromethyl, pentafluoroethyl or heptafluoropropyl from the appropriate amides. The mass spectra for the perfluoroamides of the ring substituted ethoxybenzylpiperazines (Compounds 1–3) continued to show the unique ion at m/z 107 with the highest relative abundance in the mass spectrum of the 4-ethoxy isomer. In addition, the fragment cations at $[M\text{-}15]^+$ appeared at m/z 301, 351 and 401 in the mass spectra of the TFA, PFPA and HFBA derivatives of the 2-ethoxy isomer, respectively. Thus, these studies show that chemical derivatization (perfluoroacylation) does not offer any additional unique marker ions to allow further differentiation of one compound to the exclusion of the others in this study.

GC–TOF provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazine and 4-ethoxybenzylpiperazine, which have similar nominal masses but are different in their calculated exact masses. The ethoxybenzyl $(C_9H_{11}O)^+$ fragments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135, but are different in their elemental

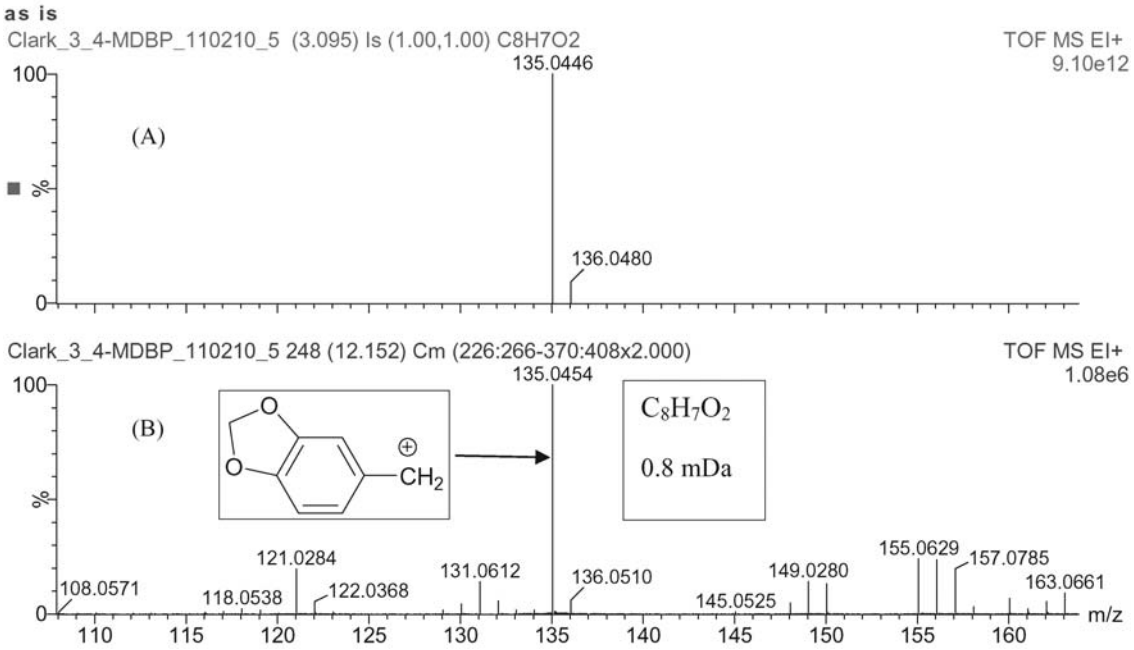

Figure 5. GC–TOF mass spectral analysis of the m/z 135 ion for 3,4-methylenedioxybenzylpiperazine: calculated mass for $C_8H_7O_2$ (A); experimental results (B).

composition and accordingly different in their calculated masses. Figure 5 shows the GC–TOF-MS exact mass analysis of the 3,4-methylenedioxybenzyl cation ($m/z = 135$) for Compound 5. Figure 5A shows the expected/calculated mass for the $C_8H_7O_2$ elemental composition. Figure 5B shows the experimental results and the degree of agreement (0.8 mDa) with the calculated mass. Thus, the m/z 135 ion in Compound 5 can be confirmed as the elemental composition $C_8H_7O_2$. These results can be compared to the exact mass analysis for the m/z 135 ion (4-ethoxybenzyl) in Compound 3. Figures 6A and 6B confirms the elemental composition as $C_9H_{11}O$ with a mass deviation of 0.1 mDa. Thus, exact mass measurements distinguish between these isobaric forms of the m/z 135 ion. Figures 6C and 6D confirm the elemental composition C_7H_7O for the unique rearrangement ion at m/z 107 visible in the ethoxybenzylpiperazine compounds.

Vapor-phase infrared spectroscopy

Infrared spectrometry is often used as a confirmatory method for compound identification in forensic drug analysis. GC–IRD was evaluated for differentiation among the five isomeric benzylpiperazines. Infrared detection should provide compound specificity without the need for chemical modification of the parent molecule. The vapor phase infrared spectra for the five benzylpiperazines are shown in Figure 7. The spectra were generated in the vapor phase following sample injection into the gas chromatograph. Each compound shows a vapor phase IR spectrum with transmittance bands in the regions 650–1,700 cm^{-1} and 2,700–3,100 cm^{-1}. In general, variations in the substitution pattern on the aromatic ring result in variations in the IR transmittance in the region 650–1,700 cm^{-1} region of the IR spectrum (22). Because the five piperazines share the same degree of nitrogen substitution, i.e., the same side chain, they have almost identical IR spectra in the 2,700–3,100 cm^{-1} region. However, they can be easily differentiated by the positions and intensities of several IR bands in the region of 650–1,700 cm^{-1}. The spectra in Figure 7 show only the useful region for differentiation in this series of compounds.

The 2,3-MDBP regioisomer (Compound 4) is characterized by the medium intensity band at 764 cm^{-1} that is split into doublet peaks of weak and equal intensity at 760 and 810 cm^{-1} in the 3,4-MDBP regioisomers (Compound 5). Also, the IR spectrum of the 2,3-isomer shows other weak doublet peaks at 957 and 999 cm^{-1}, which are shifted to a singlet at 942 cm^{-1} for 3,4-MDBP. The 2,3-MDBP regioisomer has a relatively strong IR band at 1,069 cm^{-1} that is shifted to a medium intensity peak at 1,050 cm^{-1} in the 3,4-regioisomer. The vapor phase IR spectrum of the 3,4-MDBP regioisomer can be distinguished from that of the 2,3-regioisomer by at least three IR bands of varying intensities. The first of these is the peak of strong intensity appearing at 1,242 cm^{-1} compared to the peak of intermediate intensity at 1,246 cm^{-1} in the 2,3-isomer. The second is the doublet absorption peak of weak intensity at 1,331 and 1,362 cm^{-1}, which appears as a very weak doublet at 1,297 and 1,343 cm^{-1} in the 2,3-isomer. The third is the strong doublet peak for 3,4-MDBP appearing at 1,443 and 1,489 cm^{-1}. The former is of nearly half the intensity of the latter. This was equivalent to the very strong singlet appearing at 1,459 cm^{-1} in the 2,3-regioisomer with no equivalent band at 1,443 cm^{-1}.

The three regioisomeric ethoxybenzylpiperazines share almost the same IR features in the region of 2,700–3,100 cm^{-1}. However, they can be differentiated by the positions and intensities of several IR peaks in the region of 650–1,610 cm^{-1}. Compound 3 shows a strong peak at 1,509 cm^{-1} that is shifted to two medium intensity doublets at 1,480, 1,455 and 1,486, 1,447 cm^{-1} in Compounds 1 and 2, respectively. Compound 2 shows a strong peak at 1,258 cm^{-1} that is shifted to peaks at

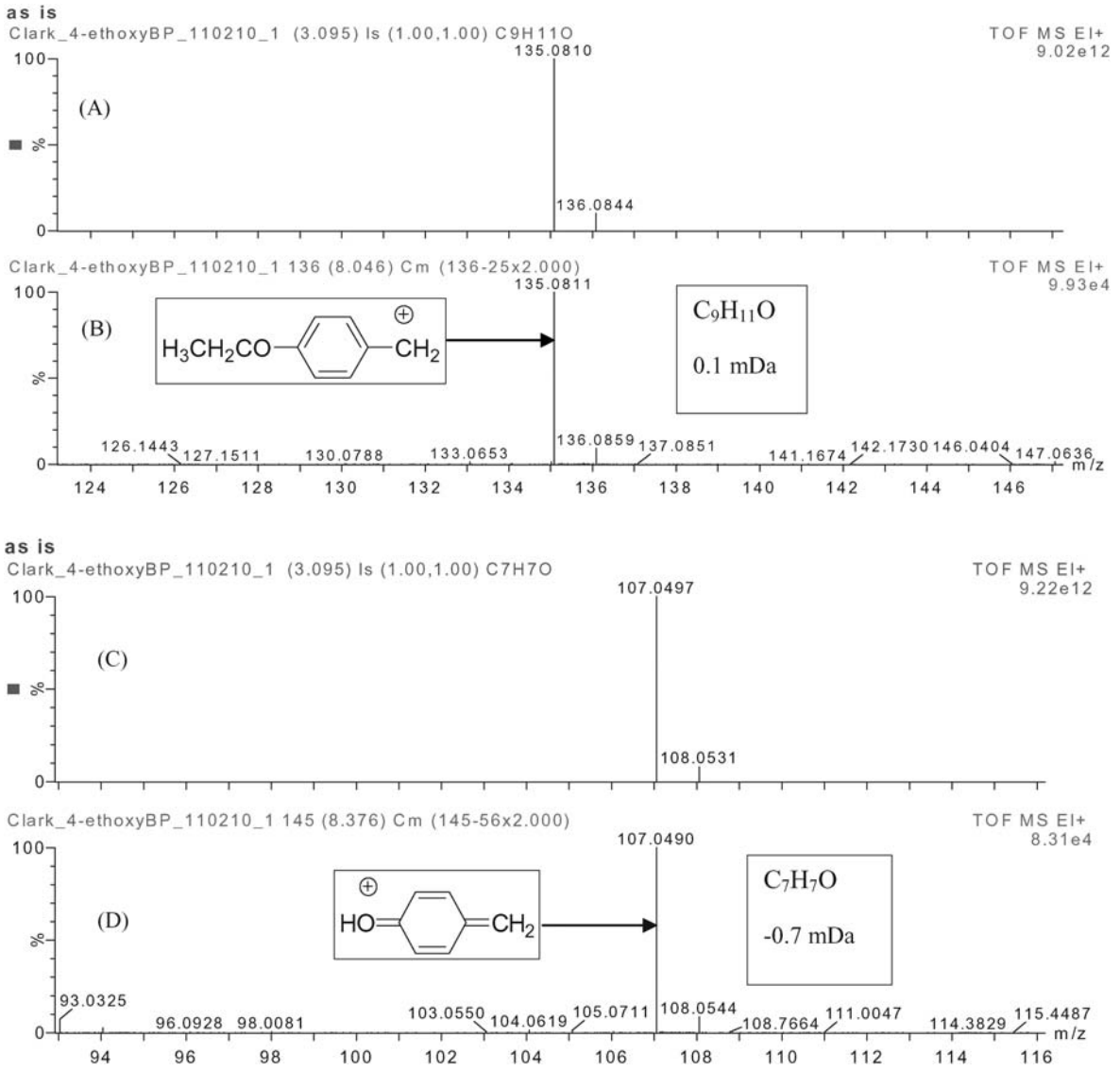

Figure 6. GC–TOF mass spectral analysis of the *m/z* 135 and *m/z* 107 ions for 4-ethoxybenzylpiperazine: calculated mass for $C_9H_{11}O$ (A); experimental results (B); calculated mass for C_7H_7O (C); experimental results (D).

1,235 and 1,239 cm^{-1} in Compounds 1 and 3, respectively. Compound 2 also has a characteristic peak at 1,601 cm^{-1} that is almost absent in Compound 1 and shifted to a weak singlet at 1,609 cm^{-1} in the IR spectrum of Compound 3.

These results provide an excellent illustration of the value of vapor phase IR confirmation for isobaric substances where the generated IR spectra show significant differences in the major bands for these five compounds. Furthermore, vapor phase infrared spectra provide distinguishing and characteristic information to determine the aromatic ring substitution pattern in the substituted piperazine regioisomers included in this study.

Gas chromatography

Gas chromatographic separation of the derivatized piperazines was accomplished on a capillary column of 50% phenyl–50% Rxi-50. Several temperature programs were evaluated and the chromatogram in Figure 8 is representative of the results obtained for all samples on this stationary phase. Figure 8 shows the GC separation of the HFBA derivatives of the ethoxy and methylenedioxybenzylpiperazines. The elution order is the same for all perfluoroacylated derivatives (TFA, PFPA and HFBA), with the three ethoxybenzylpiperazine amides eluting before the methylenedioxybenzylpiperazine compounds. The 2-ethoxybenzylpiperazine derivative eluted first, followed by the 3-isomer and then the 4-ethoxy isomer. The drug substance 3,4-MDBP eluted last in all experiments in this limited series of compounds.

Conclusion

The three ethoxybenzylpiperazines have an isobaric relationship to the controlled substance 3,4-MDBP and its regioisomer 2,3-MDBP. The three regioisomeric ethoxy compounds yield a

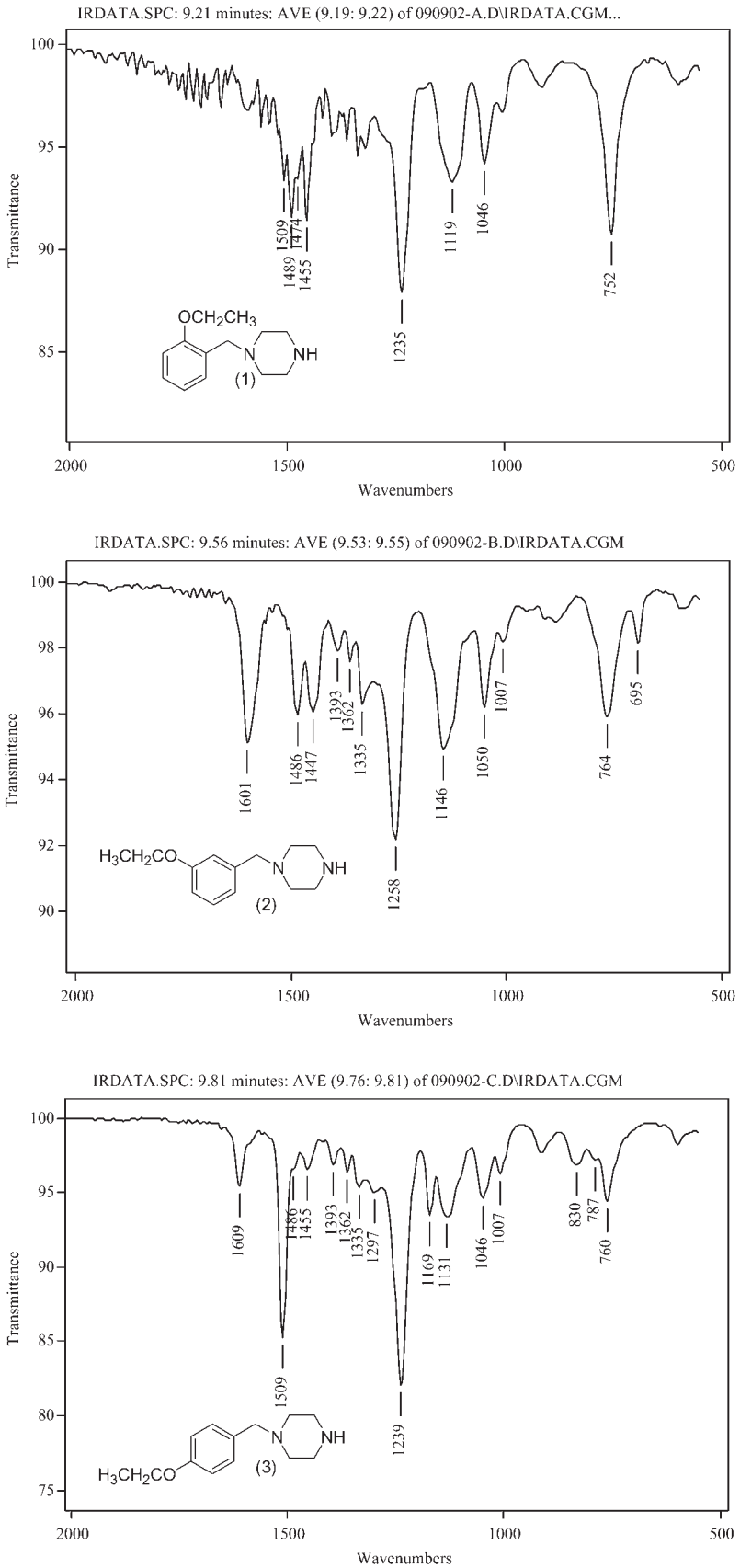

Figure 7. Vapor phase IR spectra of the five methylenedioxypiperazines and ethoxybenzylpiperazines.

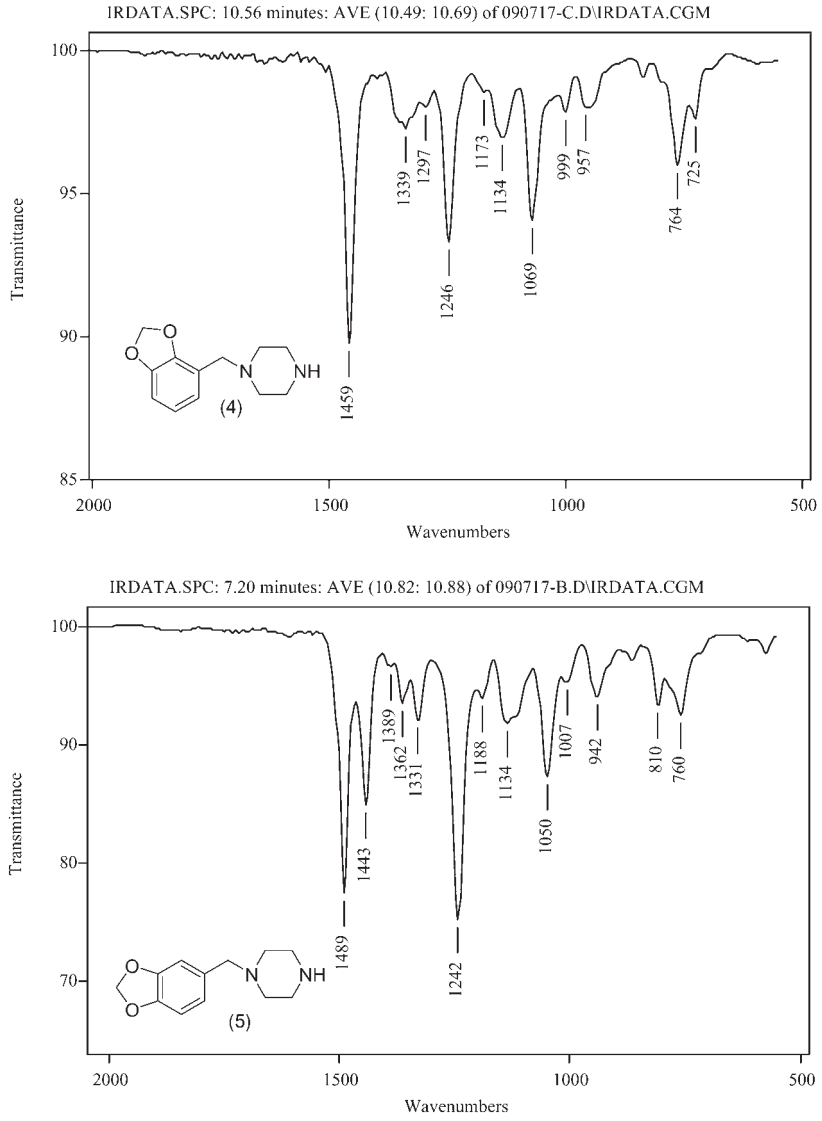

Figure 7. (Continued)

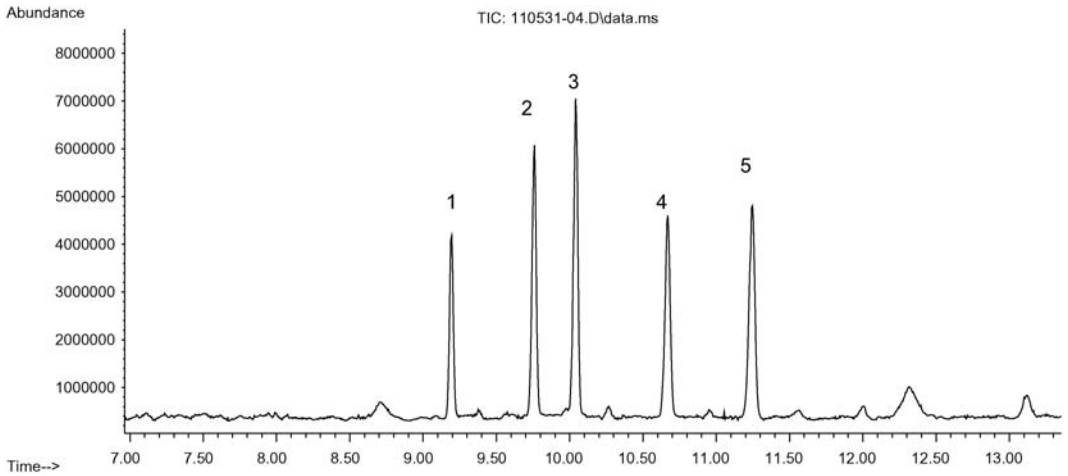

Figure 8. GC–MS separation of the HFBA derivatives of the five methylenedioxypiperazines and ethoxybenzylpiperazines using the Rxi-50 column.

unique fragment ion at m/z 107 in their EI mass spectra, which allowed for discriminating them from the isobaric methylenedioxy compounds.

Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound. GC–IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650–1,700 cm^{-1}. The five HFBA derivatives were successfully resolved on the stationary phase Rxi-50.

Gas chromatography coupled with time-of-flight mass spectrometric detection provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazines and 4-ethoxybenzylpiperazines, which have similar nominal masses but are different in their calculated masses. However, exact mass techniques do not provide any additional data for differentiation among regioisomeric fragments of the same elemental composition.

Acknowledgments

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