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Differentiation of methoxybenzoylpiperazines (OMeBzPs) and methylenedioxybenzylpiperazines (MDBPs) By GC-IRD and GC-MS

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The designer drug 3,4-methylenedioxybenzylpiperazine (3,4-MDBP), its positional isomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) and three regioisomeric ring-substituted methoxybenzoylpiperazines (OMeBzPs) have identical elemental composition and no marked differences in their mass spectra with only the three methoxybenzoylpiperazine regioisomers showing one unique major fragment ion at *m/z* 152. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in the relative abundance of some fragment ions but did not alter the fragmentation pathway to provide unique ions for discrimination among these isomers. Exact mass determination using gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS) did not provide any discrimination among these compounds since the main fragment ions are of identical elemental composition.

Gas chromatography coupled to infrared detection (GC-IRD) provides direct confirmatory data for the identification of the carbonyl containing compounds and the differentiation of the psychoactive designer drug 3,4-MDBP from its direct (2,3-MDBP) and indirect (OMeBzPs) regioisomers. The mass spectra in combination with the vapour phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivative forms of the five piperazines involved in this study were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Keywords: methylenedioxybenzylpiperazine; MDBPs; methoxybenzoylpiperazines; OMeBzPs; GC-IRD; regioisomers; GC-MS; perfluoroacylation; GC-TOF-MS

Introduction

A series of piperazine-derived compounds have recently entered the illicit drug market and represent a new group of designer drugs. Several compounds of the 1-arylpiperazine type are known to have good binding affinity for serotonin receptors of the human central nervous system.[1] This affinity is made more selective with the appropriate aromatic ring substituents.[2] The most commonly abused compounds of this group are reported to be N-benzylpiperazine and 3-trifluoromethylphenyl piperazine (3-TFMPP).[3] Both N-benzylpiperazine and 3-TFMPP were placed in Schedule 1 of the United States Controlled Substance Act in September 2002.[4] Recently, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP) has been described as producing psychoactive effects similar to those of 3,4-methylenedioxymethamphetamine (MDMA).[5–7] Some of these piperazine compounds are commercially available and are not yet under specific legal control.[8]

The 3,4-methylenedioxybenzylpiperazine (3,4-MDBP; piperonylpiperazine) has been reported as a potential drug of abuse while 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.[9–12] 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 to infrared detection (GC-IRD) provided discrimination between these two compounds based on differences in position and intensity in their IR transmittance bands. Other reports[14,15] described GC-IRD and gas chromatography-mass spectrometry (GC-MS) studies on the two regioisomeric ring substituted methylenedioxybenzylpiperazines and their isobaric ring-substituted methoxymethylbenzylpiperazines and ethoxybenzylpiperazines offering methods for discrimination among these compounds.

GC-MS is the most commonly employed technique in the analysis of controlled substances in forensic laboratories.[16–21] The identification of psychoactive drugs in a number of chemical categories is complicated by the existence of regioisomeric and isobaric substances related to the target drug.[9–17] These isomeric substances are a challenge to forensic analyses that must depend heavily on mass spectrometry for confirmation level data. These regioisomeric and isobaric substances have the same nominal mass and yield essentially identical mass spectra. Previous studies[10,17] have shown that chemical derivatization methods (primarily perfluoroacylation) can be successfully applied to discriminate among many isomerically related compounds. Derivatization can alter major fragmentation pathways often providing additional structural information about an individual isomer as well as altered chromatographic properties.[10–12,16,17] However in some cases, derivatization does not provide characteristic mass spectral fragment ions for individual isomers.[12]

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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-IRD is characterized by scanning quickly enough to obtain vapour phase IR spectra of compounds eluting from the capillary GC column. This technique has been successfully used in the identification of amphetamine isomers[22] as well as side chain regioisomers related to methamphetamine and phentermine.[23] Recently, GC-IRD studies have been described for the differentiation of ring- and side-chain-substituted ethoxyphenethylamines, methoxymethcathinones and methylenedioxymethamphetamines.[24]

The three ring-substituted methoxybenzoylpiperazines have an indirect regioisomeric relationship with 2,3 and 3,4-MDBP. Indirect regioisomers are those with the same nominal and exact masses but have different arrangements of the atoms in their chemical structures. Substitution of the methoxy group at the 2, 3, and 4 positions of the aromatic ring gives three possible ring substituted benzoyl piperazine compounds. The methoxybenzoyl ($C_8H_7O_2$) substituted piperazines have an indirect regioisomeric relationship to that of the methylenedioxybenzyl ($C_8H_7O_2$) substituted piperazines. The lack of analytical reference standards for these compounds makes their identification and discrimination a challenge to forensic drug chemistry.

This report will describe GC-IRD and GC-MS studies on the two regioisomeric ring substituted methylenedioxybenzylpiperazines and their indirect regioisomeric ring-substituted methoxybenzoylpiperazines in an effort to offer discrimination methods among all these compounds.

Experimental

Instrumentation

GC-MS analysis was performed using a 7890A gas chromatograph with a 7683B auto injector coupled with a 5975 C VL mass selective detector purchased from Agilent Technologies (Santa Clara, CA, USA). 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 100% trifluoropropyl methyl polysiloxane (Rtx-200). The separation of the pentafluoropropionyl and heptafluorobutyryl derivatives was

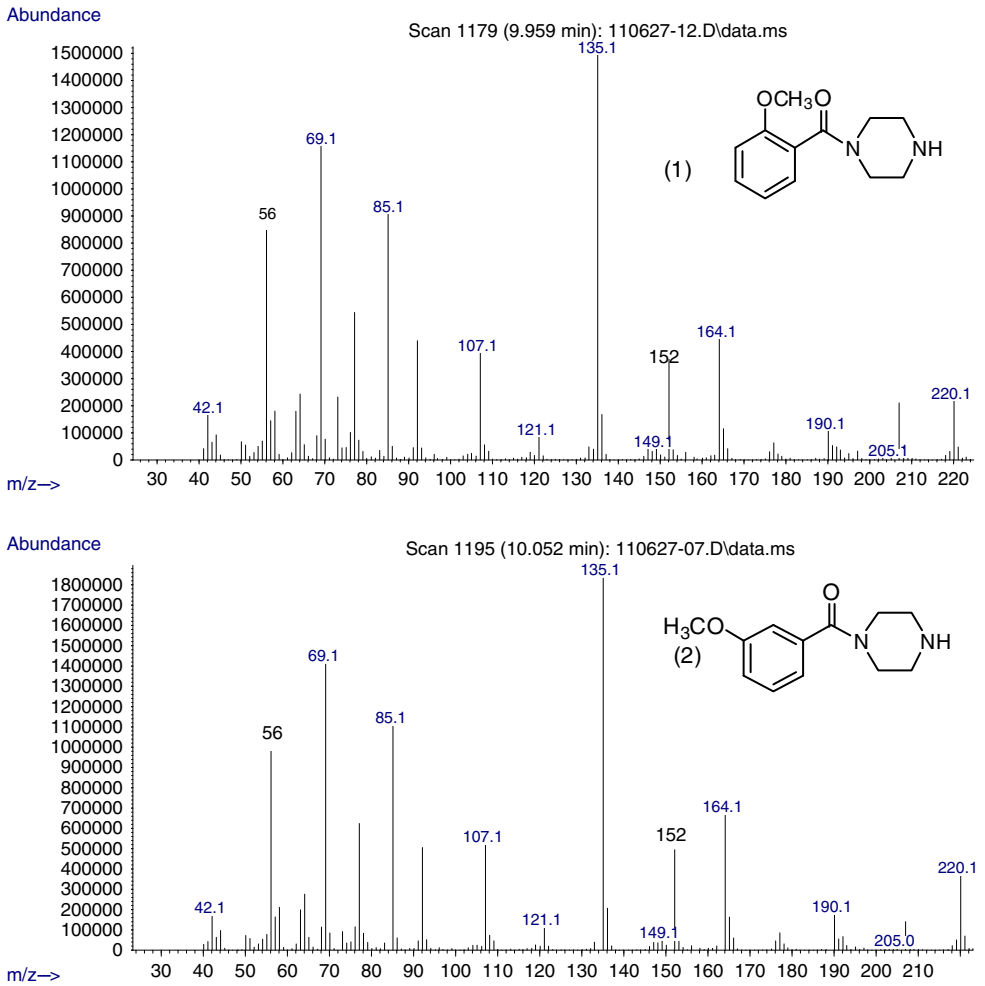

Figure 1. Mass spectra of the underivatized methoxybenzoylpiperazines and methylenedioxybenzylpiperazines in this study.

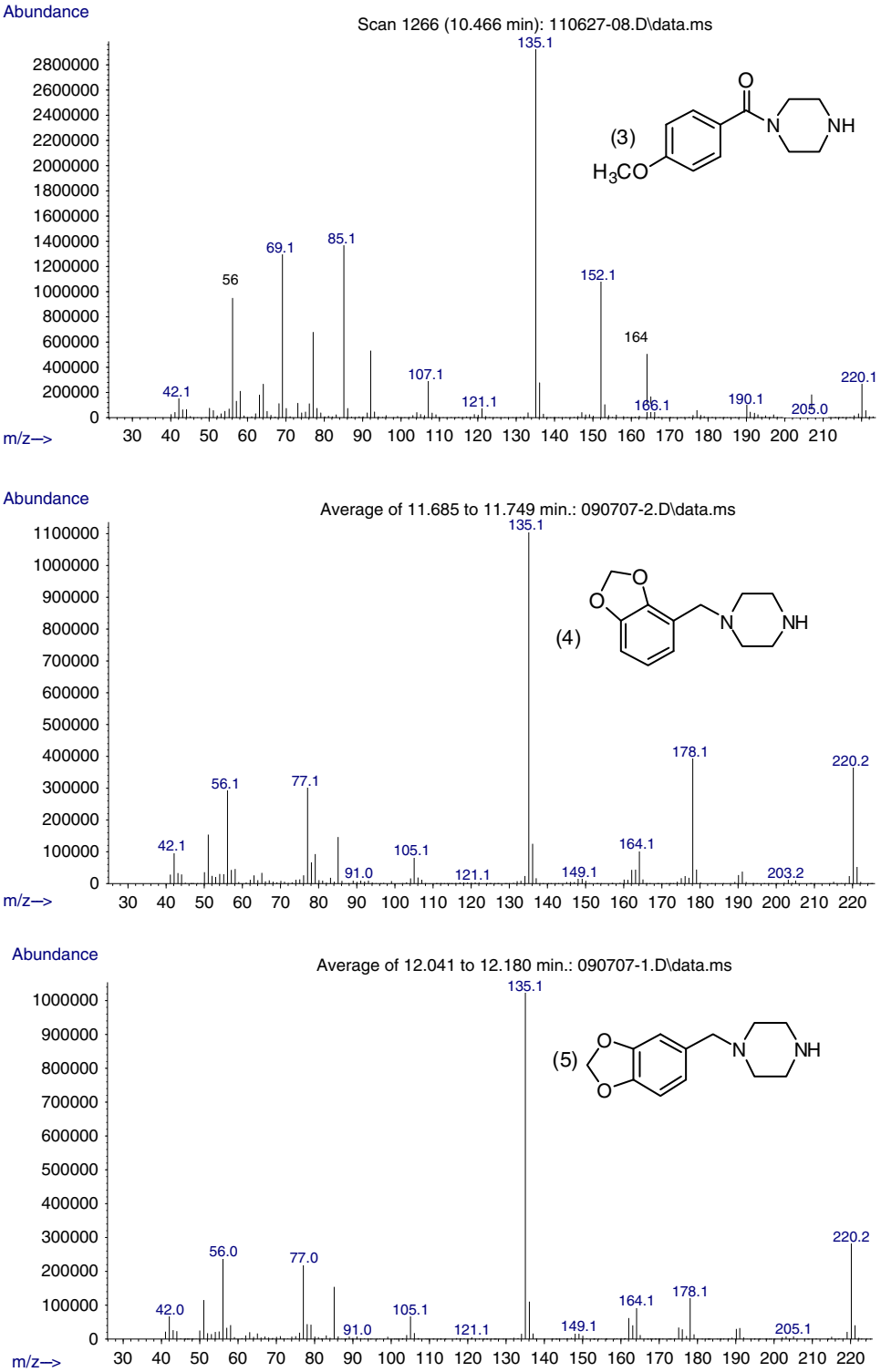

Figure 1. (*Continued*).

performed using a temperature program consisting 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.

The GC-TOF analysis was done at the Mass Spectrometry Center, Auburn University. The analysis utilized a 6890 N gas chromatograph with a 7683B auto injector purchased from Agilent Technologies (Santa Clara, CA, USA) coupled to a Waters GCT Premier benchtop orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer. Chromatographic analysis was carried out using a capillary column 30 m × 0.25 mm i.d. coated with a stationary phase film thickness of 0.50 μm DB5-MS column (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/min followed by a hold at 250 °C for

7 min. The identification was confirmed by elemental composition analysis using accurate mass measurement with an internal calibrant (lockmass 118.9919 *m/z*, heptacosafluorotributylamine, Sigma) with an acceptable error of less than 5 ppm and by isotope modelling comparing the experimental and theoretical isotope distribution.

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, USA). The vapour phase infrared spectra were recorded in the range of 4000–650 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans per second. 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% methyl polysiloxane (Rxi-50). The temperature programme involved in this study consisted of initial temperature of 70 °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. The capillary column used in these studies was purchased from Restek Corporation (Bellefonte, PA, USA).

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

Drugs and reagents

The general procedure for the synthesis of the two regioisomeric benzylpiperazines involves the reductive amination of the appropriately substituted benzaldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride

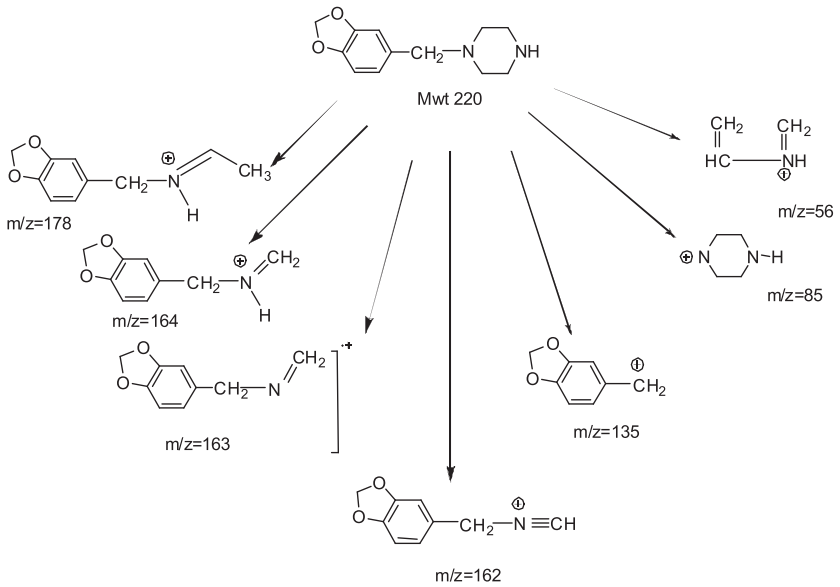

Figure 2. Mass spectral fragmentation pattern of the underivatized 3,4-methylenedioxybenzylpiperazine under EI (70 eV) conditions.

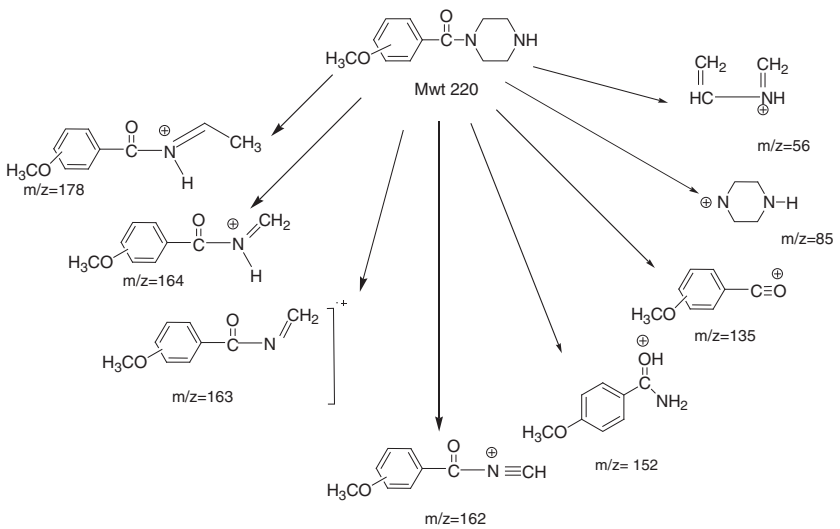

Figure 3. Mass spectral fragmentation pattern of the underivatized methoxybenzoylpiperazines under EI (70 eV) conditions.

salts using gaseous HCl and purified by recrystallization. The general procedure for the synthesis of the three regioisomeric methoxybenzoylpiperazines involves the slow addition of the appropriately substituted benzoyl chloride to a solution of piperazine in dichloromethane in an ice bath. Isolation of the basic fraction gave the corresponding benzoylpiperazine 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-methoxy benzoylchloride, 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 previously reported.[25,26] All laboratory reagents and solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) or Fisher Scientific (Atlanta, GA, USA). The derivatizing reagents trifluoroacetic anhydride (TFA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were purchased from Sigma-Aldrich, Inc. (Milwaukee, WI, USA).

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 or 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

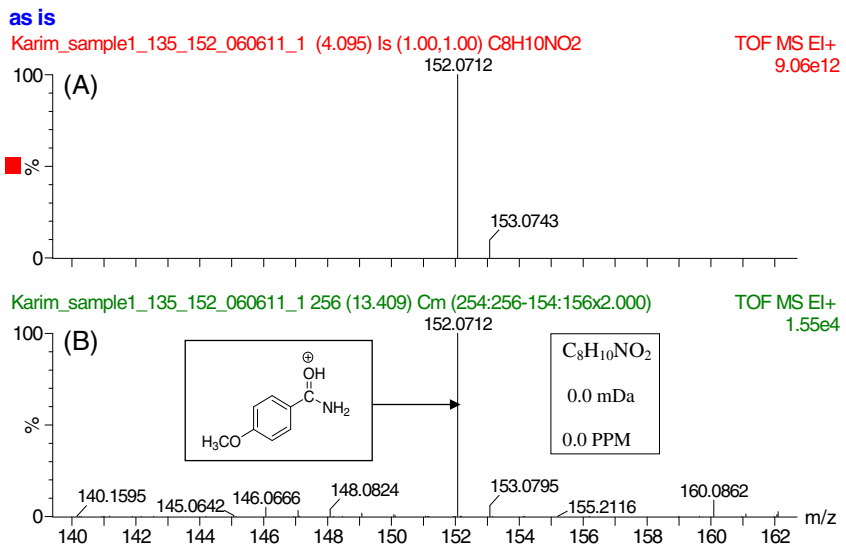

Figure 4. GC-TOF mass spectral analysis of the *m/z* 152 ion for 4-methoxybenzoylpiperazine. (A) calculated mass for $C_8H_{10}NO_2$; (B) experimental results.

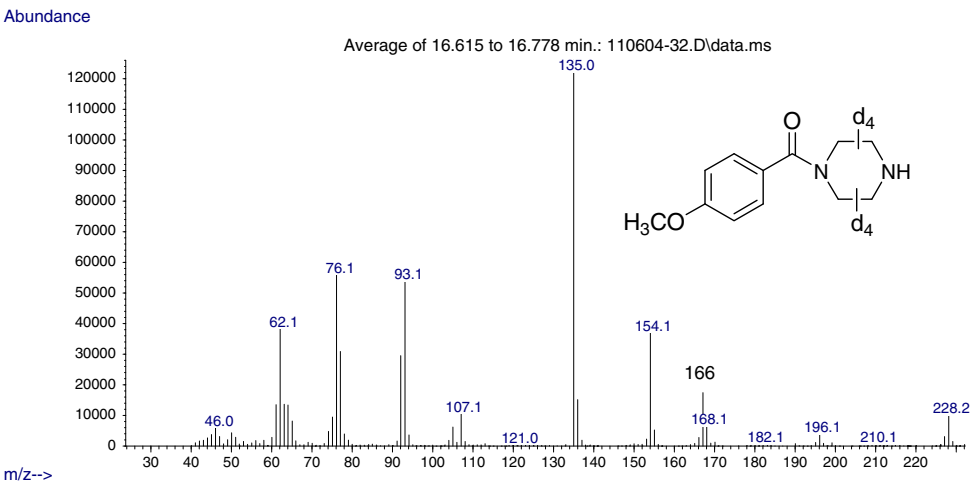

Figure 5. Mass spectrum of the 4-methoxybenzoyl-d_8-piperazine.

spectra of all five isomeric piperazines (compounds 1–5). The ions of significant relative abundance common to the five isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the five piperazines show the fragment ions at *m/z* 178, 164, 135, 85, and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figures 2 and 3 and are based on a previous report describing the fragmentation of the unsubstituted benzylpiperazines.[8] The regioisomeric methoxybenzoyl $(C_8H_7O_2)^+$ fragments have the same nominal and exact masses as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cations occurring at *m/z* 135. The mass spectra for the ring substituted methoxybenzoylpiperazines (compounds 1–3) have almost identical mass spectra to each other and to the methylenedioxybenzylpiperazine isomers

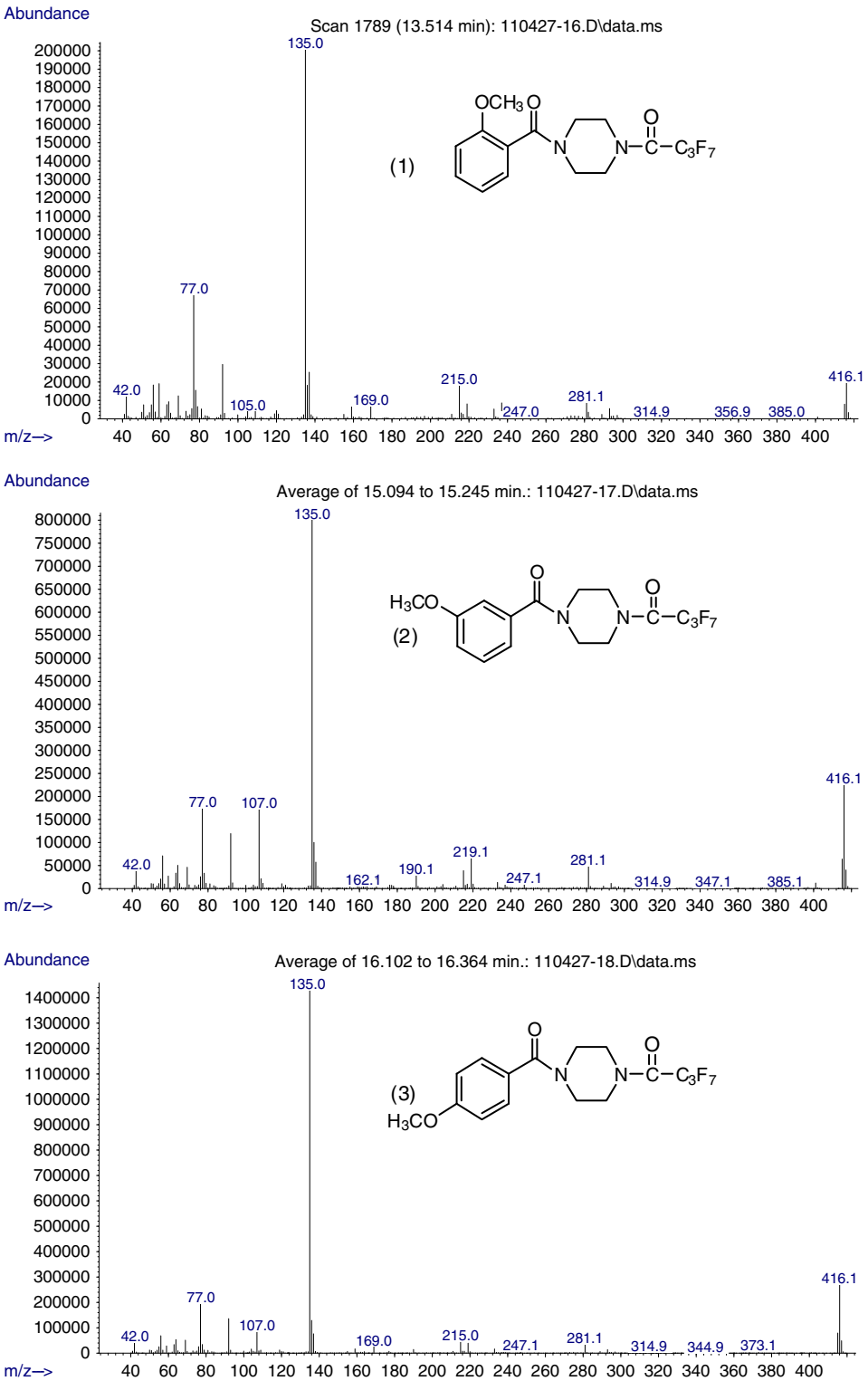

Figure 6. Mass spectra of the heptafluorobutyryl derivatives of the three methoxybenzoylpiperazines in this study.

(compounds 4 and 5) except for the characteristic high relative abundance ion at *m/z* 152 which appears to be specific for the three regioisomeric methoxybenzoylpiperazines.

Exact mass analysis using GC-TOF-MS confirmed the unique *m/z* 152 ion in the regioisomeric benzoylpiperazines (compounds 1–3) as the elemental composition $C_8H_{10}NO_2$. Figure 4 shows the exact mass measurement results for the *m/z* 152 ion in the 4-methoxybenzoylpiperazine. The upper panel (A) shows the expected/calculated mass for the $C_8H_{10}NO_2$ elemental composition and the lower panel (B) shows the experimental results along with the degree of agreement (0.0 mDa, 0.0 ppm) between the calculated and experimental results.

The proposed structure for the *m/z* 152 $C_8H_{10}NO_2$ ion is shown in Figure 4. The suggested structure for this fragment involves the formation of the protonated methoxybenzamide. The proposed structure for the *m/z* 152 ion is supported by the mass spectrum of the octa-deutero labeled form of 4-methoxybenzoylpiperazine (4-methoxybenzoyl-d_8-piperazine). This octa-deuterium labelled compound was prepared by slowly adding 4-methoxybenzoyl chloride to a solution of d_8-piperazine in dichloromethane in an ice-bath. The mass spectrum for the deuterium labelled form of compound 3 is shown in Figure 5 and indicates that two deuterium atoms remain as a part of the ion in question since the mass increased by 2 Da to *m/z* 154 in this case.

The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric piperazines, in an effort to individualize their mass spectra and identify additional unique marker ions for differentiation among these five compounds. Acylation lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectra.[10–12]

The trifluoroacetyl, pentafluoropropionyl, and heptafluorobutyryl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 6 shows the mass spectra of the heptafluorobutyryl amides of the three methoxybenzoylpiperazines as representatives of all the perfluoroacylated piperazines evaluated in this study. The spectra for the HFBA derivatives of compounds 4 and 5 have been reported previously.[13] 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 or benzoyl cations. 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

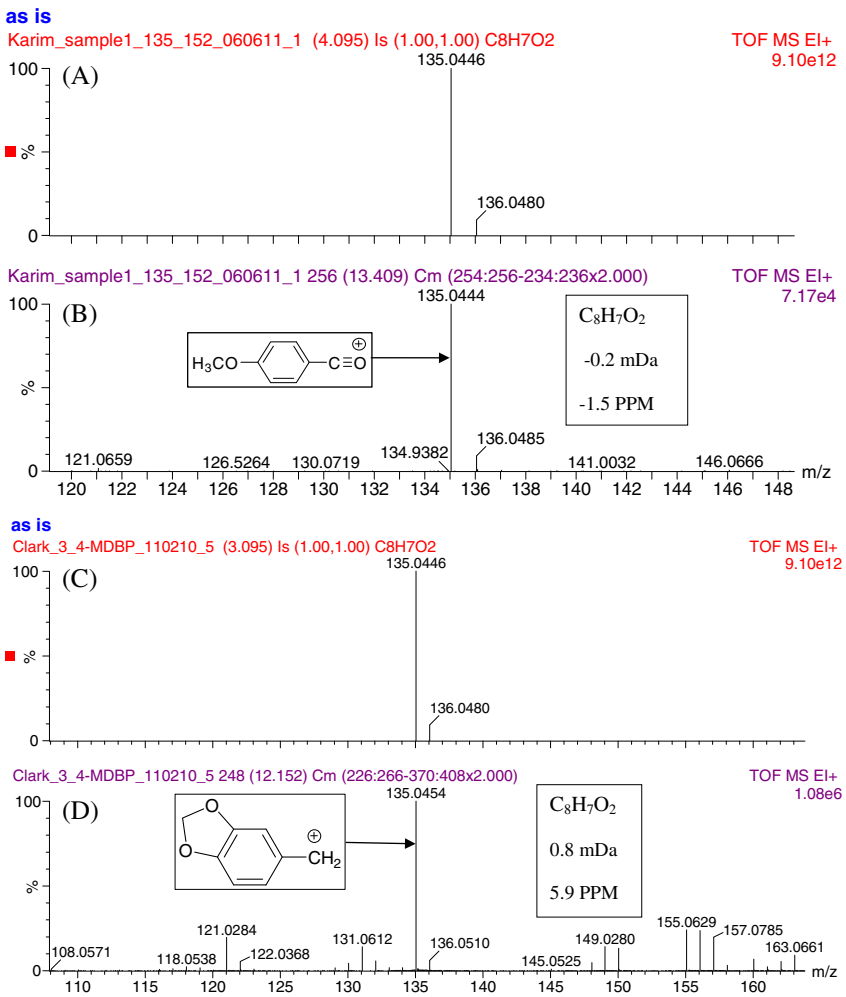

Figure 7. GC-TOF mass spectral analysis of the m/z 135 ion for 4-methoxybenzoylpiperazine and 3,4-methylenedioxybenzylpiperazine. (A) calculated mass for $C_8H_7O_2$; (B) experimental results. (C) calculated mass for $C_8H_7O_2$; (D) experimental results.

$(M\text{-}135)^+$ ion for each amide. These ions have higher relative abundances in the mass spectra of the derivatized methylenedioxybenzylpiperazines[13] compared to the mass spectra of the methoxybenzoylpiperazines. The ion at *m/z* 219 was observed in the spectra of all derivatives and is likely formed by the elimination of the perfluoroacyl moiety. Those ions occurring at *m/z* 69, 119, and 169 are the perfluoroalkyl cations trifluoromethyl, pentafluoroethyl or heptafluoropropyl from the appropriate amides. These studies show that chemical derivatization (perfluoroacylation) does not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others in this set of compounds.

The isomeric methoxybenzoyl $(C_8H_7O_2)^+$ fragments have the same nominal and exact masses as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at *m/z* 135. Figure 7 shows the GC-TOF-MS exact mass analysis of the 4-methoxybenzoyl and 3,4-methylenedioxybenzyl cation (*m/z* = 135) for compounds 3 and 5, respectively. The upper panel (A) shows the expected/calculated mass for the $C_8H_7O_2$ elemental composition. The lower panel (B) shows the experimental results and the degree of agreement (−0.2 mDa) with the calculated mass. Thus, confirming the *m/z* 135 ion in compound 3 as the elemental composition $C_8H_7O_2$. These results can be compared to the exact mass analysis for the *m/z* 135 ion (3,4-methylenedioxybenzyl cation) in

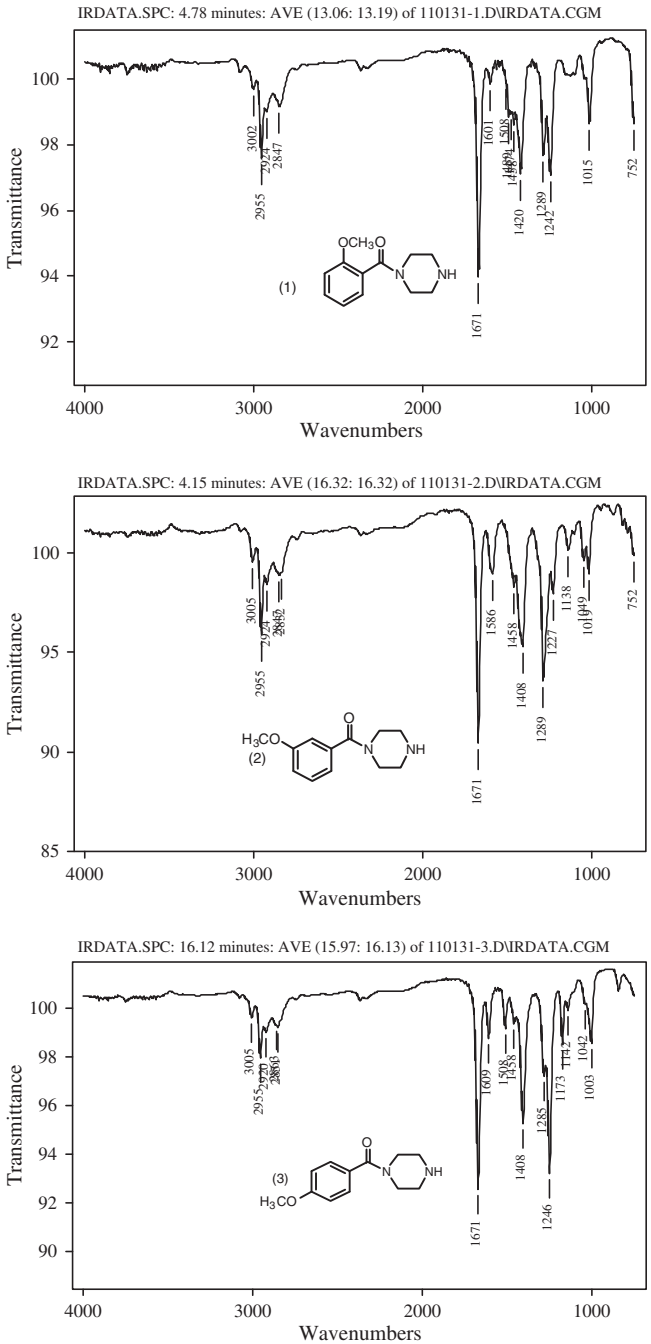

Figure 8. Vapour phase IR spectra of the five piperazines involved in this study.

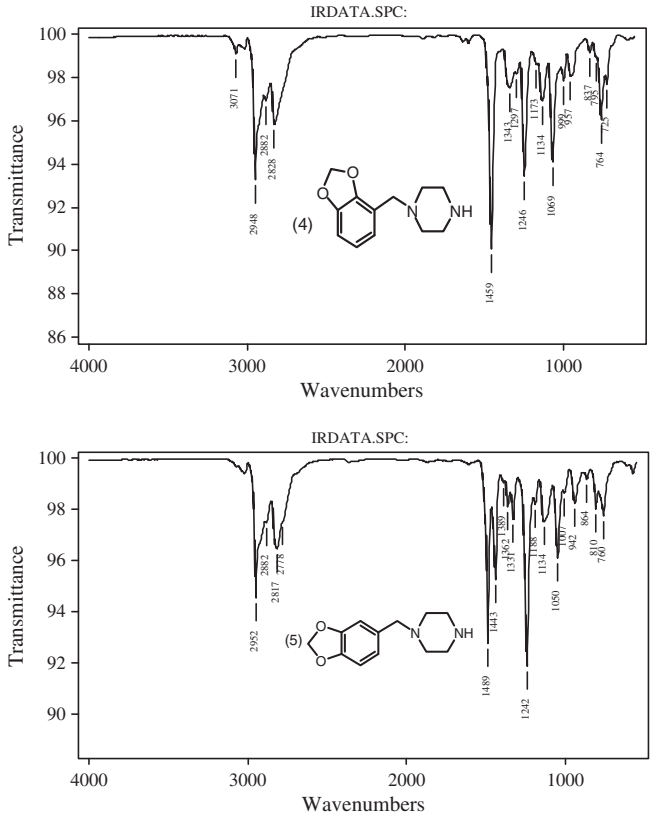

Figure 8. (*Continued*).

compound 5. Figures 7C and 7D confirm the elemental composition as $C_8H_7O_2$ with a mass deviation of 0.8 mDa. Thus, exact mass measurement does not distinguish between these indirectly regioisomeric forms of the $(C_8H_7O_2)^+$ *m/z* 135 ion and did not provide any discriminatory advantage over the conventional GC-MS technique.

Vapour-phase infrared spectroscopy

Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. GC-IRD was evaluated for differentiation among the five isomeric piperazines. The vapour phase infrared spectra for the five piperazines are shown in Figure 8. The spectra were generated in the vapour phase following sample injection into the gas chromatograph and each compound shows transmittance bands in the regions of 650–1700 cm^{-1} and 2700–3100 cm^{-1}. In general, variations in the substitution pattern on the aromatic ring result in variations in the IR region from 650–1700 cm^{-1}.[23] However, variations in the side-chain pattern often lead to variations in the 2700–1700 cm^{-1} region.[23] Since the five piperazines share the same degree of nitrogen substitution, i.e. the same side chain, they have almost identical IR spectra in the region 2700–3100 cm^{-1}. However, they can be easily differentiated by the positions and intensities of several IR peaks in the region of 650–1700 cm^{-1}.

The three regioisomeric methoxybenzoylpiperazines share a characteristic strong singlet IR band at 1671 cm^{-1} corresponding to the carbonyl group stretching which can distinguish these three benzoylpiperazines from the two methylenedioxybenzylpiperazines. The three ring-substituted benzoylpiperazines share almost the same IR features in the region of 2700–3100 cm^{-1}. However, they can be differentiated by the positions and intensities of several IR peaks in the region of 650–1700 cm^{-1}. Compound 3 shows a strong peak at 1246 cm^{-1} which is shifted to a medium intensity doublet at 1289 cm^{-1}, 1242 cm^{-1} in compound 1 and a strong singlet at 1289 cm^{-1} in compound 2. Compound 1 shows a strong peak at 1420 cm^{-1} which is shifted to a peak at 1408 cm^{-1} in both compounds 2 and 3. Compound 3 also has a medium intensity peak at 1003 cm^{-1} which is shifted to a peak at 1015 cm^{-1} in compound 1 and a weak singlet at 1019 cm^{-1} in compound 2.

The 2,3-MDBP regioisomer (compound 4) is characterized by the medium-intensity band at 764 cm^{-1} which is split into doublet peaks of weak and equal intensity at 760 and 810 cm^{-1} in the 3,4-MDBP regioisomer (compound 5). Additionally, 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 1069 cm^{-1} which is shifted to a medium intensity peak at 1050 cm^{-1} in the 3,4-regioisomer. The vapour 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 is the peak of strong intensity at 1242 cm^{-1} compared to the peak of intermediate intensity at 1246 cm^{-1} in the 2,3-isomer. The second is the doublet absorption peak of weak intensity at 1331 and 1362 cm^{-1} which appears as a very weak doublet at 1297 and 1343 cm^{-1} in the 2,3-isomer. The third is

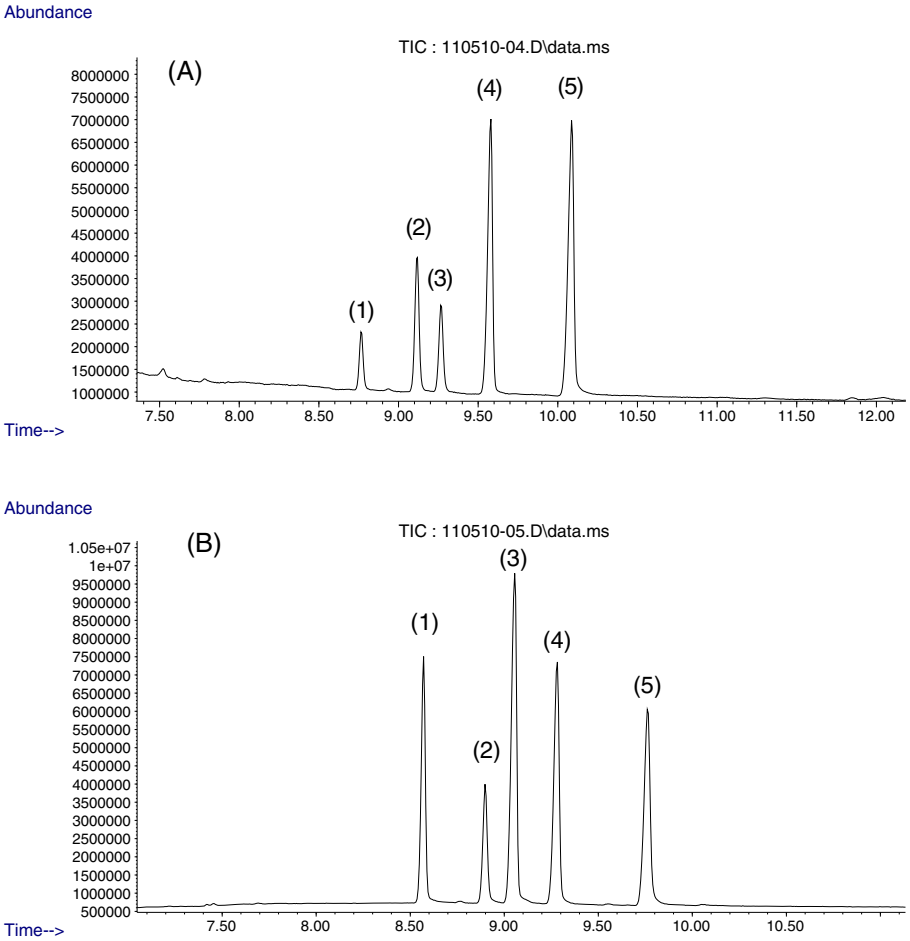

Figure 9. Gas chromatographic separation of the (A) pentafluoropropionyl derivatives and (B) heptafluorobutyryl derivatives of the five piperazines using Rtx-200 column.

the strong doublet peak for 3,4-MDBP appearing at 1443 and 1489 cm^{-1}. The former is of nearly half the intensity of the latter. This was equivalent to the very strong singlet appearing at 1459 cm^{-1} in the 2,3-regioisomer with no equivalent band at 1443 cm^{-1}.

These results provide an excellent illustration of the value of vapour phase IR confirmation for the regioisomeric substances in this study. The generated IR spectra show significant differences in the major bands for these five compounds.

Gas chromatography

Gas chromatographic separation of the underivatized and derivatized piperazines was accomplished on a capillary column of dimensions 30 m × 0.25 mm and 0.5-μm film depth of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatograms in Figure 9 are representative of the results obtained for all samples on this stationary phase.

In Figures 9A and 9B, the PFPA and HFBA derivatives of the three methoxybenzoylpiperazines are less retained than their regioisomeric methylenedioxybenzylpiperazines. The three benzoylpiperazines eluted in the order of 2, 3, 4-methoxybenzoylpiperazine. The controlled substance 3,4-MDBP eluted last in all experiments in this limited series of compounds. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the five isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

Conclusion

The three methoxybenzoylpiperazines have an indirect regioisomeric relationship to the controlled substance 3,4-MDBP and its regioisomer 2,3-MDBP. The five regioisomeric piperazines yield very similar fragment ions in their mass spectra with only the three methoxybenzoylpiperazine regioisomers showing one unique major fragment ion at *m/z* 152. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. In addition, the GC-TOF exact mass measurements did not provide any discrimination among these compounds. GC-IRD offered unique and characteristic IR spectra that allowed discrimination among these compounds using the region between 650 and 1700 cm^{-1}. Additionally, the strong carbonyl absorption bands clearly differentiate the methoxybenzoylpiperazines from the methylenedioxybenzylpiperazines. The five PFPA and HFBA derivatives were successfully resolved on the stationary phase Rtx-200.

Acknowledgements

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