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Differentiation of methylbenzylpiperazines (MBPs) and benzoylpiperazine (BNZP) using GC-MS and GC-IRD

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Three-ring substituted methylbenzylpiperazines (MBPs) and their isobaric benzoylpiperazine (BNZP) have equal mass and many common mass spectral fragment ions. The mass spectrum of BNZP yields a unique benzoyl-group containing fragment at *m/z* 122 and an additional major fragment at *m/z* 69 that allows its discrimination from the three MBP regioisomers. 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 (GC-IRD) provides direct confirmatory data for the structural differentiation between the four isomers. The mass spectra in combination with the vapour phase IR spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivatives of these four piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Gas chromatography coupled with time-of-flight mass spectrometry provides an additional means of differentiating between the isobaric MBP and BNZP which have equivalent nominal masses but are different in their elemental composition and exact masses. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: MBPs; methylbenzylpiperazines; GC-IRD; isobarics; GC-MS; benzoylpiperazine

Introduction

Structural modifications of drugs of abuse are well known for the amphetamine-derived designer drugs. A common structural variation is the introduction of a methylenedioxy or dimethoxy moiety into the aromatic ring of amphetamine leading to 3,4-methylenedioxyamphetamine (MDA) or 2,5-dimethoxyamphetamine (2,5-DMA), respectively. In addition to those amphetamines, a series of piperazine-derived compounds have recently entered the illicit drug market and may represent a new group of designer drugs. Many of these compounds are reported to bind to serotonin receptors of the human central nervous system.[1] The 1-aryl-piperazines show good binding affinity to serotonin receptors[1] and the affinity is made more selective with the appropriate aromatic ring substituents.[2,3] It appears that N-benzylpiperazine (BZP) and 3-trifluoromethylphenyl piperazine (3-TFMPP) are currently the most commonly abused compounds of this group.[4] The subjective effects of these piperazine drugs individually and in combination in humans have been described in several recent reports.[5–7] The subjective and physiological effects of BZP/TFMPP combinations resemble those of MDMA and amphetamine. BZP appears to be responsible for the stimulant-like effects while TFMPP exerts its action primarily via serotonergic pathways.[5]

Structural modifications similar to those observed in the amphetamines are already being encountered in the piperazine class of compounds. The methylenedioxy analogue of N-benzylpiperazine, 1-(3,4-methylenedioxybenzyl)-piperazine (3,4-MDBP), is a scheduled drug in the United States[8] and has been described as producing psychoactive effects similar to those of 3,4-methylenedioxymethamphetamine (MDMA).[9–11] A dimethoxybenzylpiperazine derivative, 4-bromo-2,5-dimethoxybenzylpiperazine, was identified in street drug samples seized in Germany in 2006.[12]

Analysis of drugs of abuse in biological and forensic samples has been the focus of many studies over the past years. Gas chromatography-mass spectrometry (GC-MS) is the most widely used technique in the analysis of controlled substances in forensic laboratories.[13–26] A recent report [23] 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 their infrared (IR) transmittance bands. Other reports described GC-IRD and GC-MS studies on the two regioisomeric ring-substituted methylenedioxybenzylpiperazines and their isobaric ring-substituted methoxymethylbenzylpiperazines[24] and ethoxybenzylpiperazines[25] offering methods for discrimination among these compounds. Thus, analytical differentiation among regioisomeric and isobaric substances is an important issue in forensic drug chemistry. The identification of psychoactive drugs in a number of chemical categories is complicated by regioisomeric and isobaric substances related to the target drug.[13–18]

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The methylbenzylpiperazines (MBPs) have an isobaric relationship with benzoylpiperazine (BNZP) since these compounds have the same nominal mass but differ in elemental composition. Substitution of the methyl group on the 2, 3 and 4 positions of the aromatic ring gives three possible ring substituted MBP regioisomers. The 3-methylbenzylpiperazine has recently been reported as a component of clandestine drug samples in Europe.[27] This report will describe GC-IRD and GC-MS studies on the three regioisomeric ring-substituted MBPs and the isobaric substance BNZP in an effort to offer confirmation level discrimination among these compounds.

Experimental

Instrumentation

GC-MS analysis was performed using an Agilent Technologies (Santa Clara, CA, USA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975 C VL Agilent mass selective detector. The GC was operated in splitless mode with a helium (grade 5) flow rate was 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 scan rate was 2.86 scans/s and the source temperature of 230 °C. The GC injector was maintained at 250 °C and the transfer line at 280 °C.

GC-MS chromatographic separation was carried out on a column (30 m × 0.25 mm i.d.) coated with 0.5 μm 100% trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek Corporation (Bellefonte, PA, USA). The separation of the underivatized and pentafluoropropionyl derivatives was performed using a temperature program consisting of an initial hold at 100 °C for 1.0 min, ramped up to 180 °C at a rate of 9 °C/min, held at 180 °C for 2.0 min then increased to 200 °C at a rate of 10 °C/min and held at 200 °C for 5.0 min.

The GC-TOF 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 (Mass Spectrometry Center, Auburn University, Auburn, AL, USA). Chromatographic separation 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) Folsom, CA, USA. 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. The identification was confirmed by elemental composition analysis using accurate mass measurement with an internal calibrant (lockmass 118.9919 m/z, heptacosafluorotributylamine, Sigma Milwaukee, MI, USA) with an acceptable error of less than 5 ppm and by isotope modeling comparing the experimental and theoretical isotope distribution.

GC-IRD studies were carried out on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 autoinjector coupled with an IRD-II detector obtained from Analytical solutions and Providers (ASAP), Covington, KY, USA. The vapour 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 s. The IRD flow cell and transfer line temperatures were 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 column used was a 30 m × 0.25 mm i.d. coated with 0.50 μm 50% phenyl – 50% methyl polysiloxane (Rxi-50) purchased from Restek Corporation (Bellefonte, PA, USA). The temperature programme 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.

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 three regioisomeric MBPs 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 salts using gaseous HCl and purified by recrystallization. The procedure for the synthesis of the BNZP involves the slow addition of benzoyl chloride to a solution of piperazine in dichloromethane in an ice bath. Isolation of the basic fraction gave the corresponding BNZP base, which was converted to the corresponding hydrochloride salt using gaseous HCl and purified by recrystallization. The starting materials for compounds 1, 2 and 3 are 2, 3 and 4-methylbenzaldehyde (o, m and p-tolualdehyde), respectively and the starting material for compound 4 is benzoylchloride, and all are commercially available. 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 from the hydrochloride salts of the piperazines by dissolving approximately 0.3 mg (1.36×10^{-6} mol) of each amine 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). The resulting 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 residue was 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 for analysis.

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 four isomeric piperazines (Compounds 1–4) in this study. The ions of significant relative abundance common to the four isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the four piperazines show fragment ions at *m/z* 148, 134, 105, 85, and 56 as well as other ions of low relative abundance. The proposed structures of these ions are shown in Figures 2 and 3 and are based in part on a previous report describing the fragmentation of unsubstituted benzylpiperazine.[3] The isobaric benzoyl $(C_7H_5O)^+$ fragment has the same nominal mass as the methylbenzyl $(C_8H_9)^+$ cations occurring at *m/z* 105. The mass spectra for the ring substituted MBPs (Compounds 1–3) have almost identical mass spectra to each other

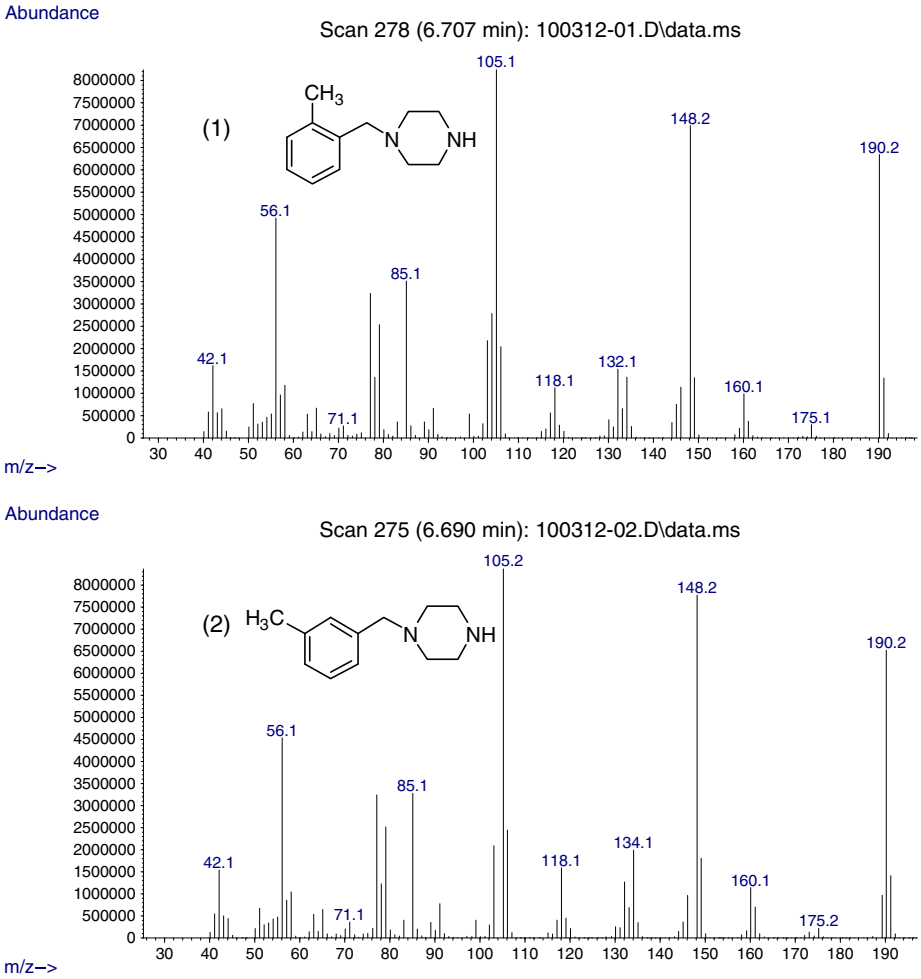

Figure 1. Mass spectra of the four piperazines in this study.

and to the BNZP (Compound 4) except for the characteristic high relative abundance ions at *m/z* 122 and *m/z* 69 which appear to be specific for the BNZP.

The proposed structures for the *m/z* 122 $(C_7H_8NO)^+$ cation and the *m/z* 69 $(C_4H_7N)^{+\cdot}$ Radical cations are shown in the fragmentation scheme in Figure 3. The equivalent ion to the *m/z* 122 fragment has been confirmed by exact mass GC-TOF-MS analysis for the ring substituted methoxybenzoylpiperazines in a previous report from our lab.[26] The suggested structure for the *m/z* 122 ion is the protonated primary benzamide and the structures for this *m/z* 122 ion as well as the *m/z* 69 ion are supported by the mass spectrum of the octa-deutero labelled form of BNZP (benzoyl-d_8-piperazine). This octa-deuterium labeled compound was prepared by slowly adding benzoyl chloride to a solution of d_8-piperazine in dichloromethane in an ice-bath. The mass spectrum for the deuterium labelled form of Compound 4 is shown in Figure 4. The mass spectrum in Figure 4 shows that two deuterium atoms are incorporated into the protonated primary amide in question since the mass increased by 2 Da to *m/z* 124. In addition, the *m/z* 69 ion increased by 7 Da to *m/z* 76 in the octa-deuterium labeled compound which is consistent with C_4H_7N as the elemental composition and the assigned structure in Figure 3.

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 four compounds. Acylation lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectra.[13–15] The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra in this series of substituted piperazines. Figure 5 shows the mass spectrum of the heptafluorobutryl amide of 2-methylbenzylpiperazine as representative of all the perfluoroacylated piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 286, 336, and 386, respectively. The major fragment ion in these spectra occurs at *m/z* 105 and corresponds to the methyl 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 $(M\text{-}105)^+$ cation for each amide. These ions have higher relative abundances in the mass spectra of the derivatized MBPs compared to the mass spectra of the derivatized BNZP. The ion at *m/z* 189 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 major additional marker ions to allow identification of one compound to the exclusion of the others in this series of isomeric piperazine compounds.

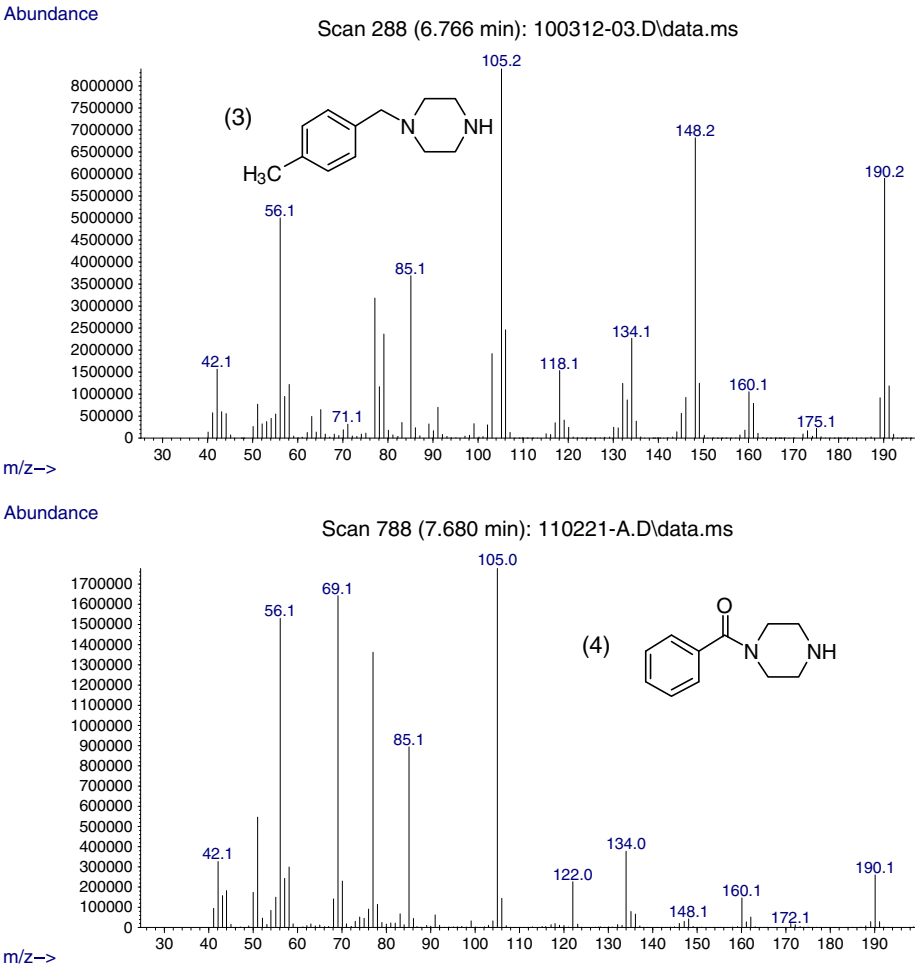

Figure 1. Continued

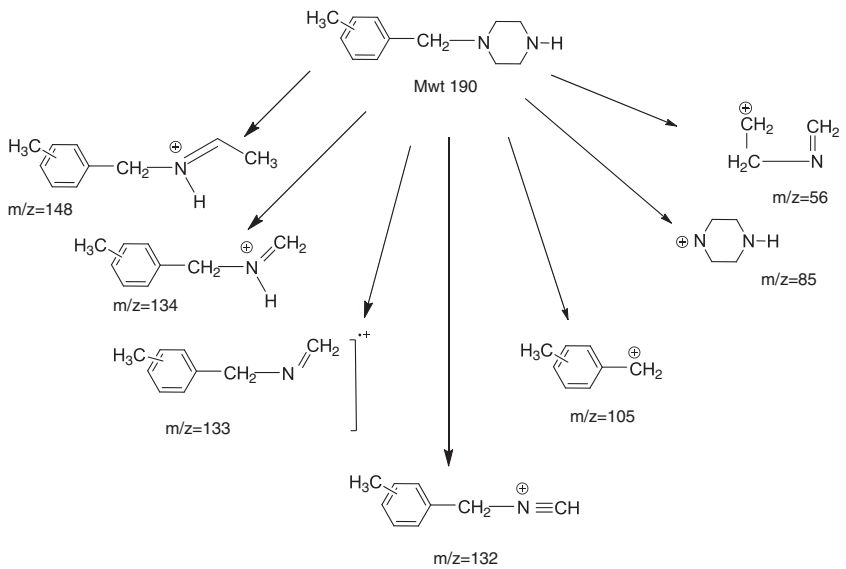

Figure 2. Mass spectral fragmentation products of the methylbenzylpiperazines under EI (70 eV) conditions.

GC-TOF-MS detection provides an excellent means of differentiating between the isobaric MBPs and BNZP which have similar nominal masses but are different in their exact masses. The isobaric benzoyl $(C_7H_5O)^+$ fragment has the same nominal mass as the methylbenzyl $(C_8H_9)^+$ cation occurring at *m/z* 105 but are different in their elemental composition and accordingly different in their calculated masses. Figure 6 shows the GC-TOF-MS exact mass analysis of the 4-methylbenzyl and benzoyl cations

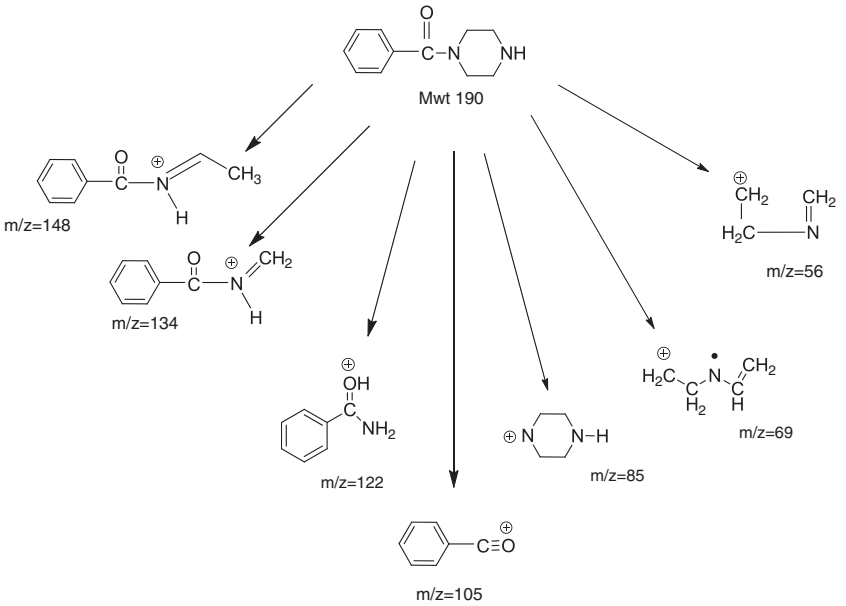

Figure 3. Mass spectral fragmentation products for benzoylpiperazine under EI (70 eV) conditions.

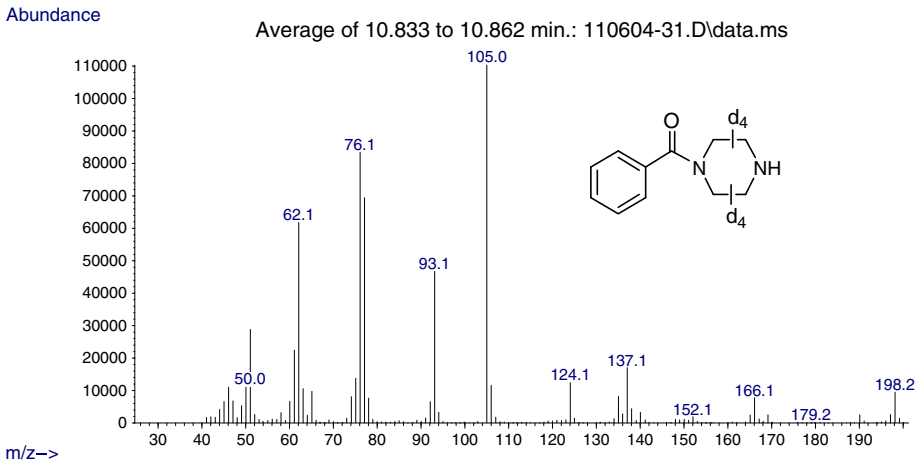

Figure 4. Mass spectrum of benzoyl-d_8-piperazine.

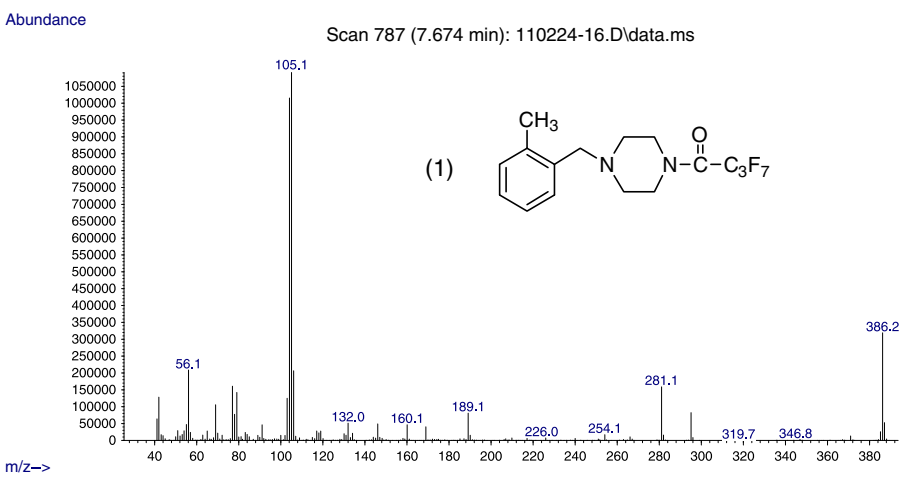

Figure 5. Mass spectrum of the heptafluorobutyryl derivative of 2-methylbenzylpiperazine.

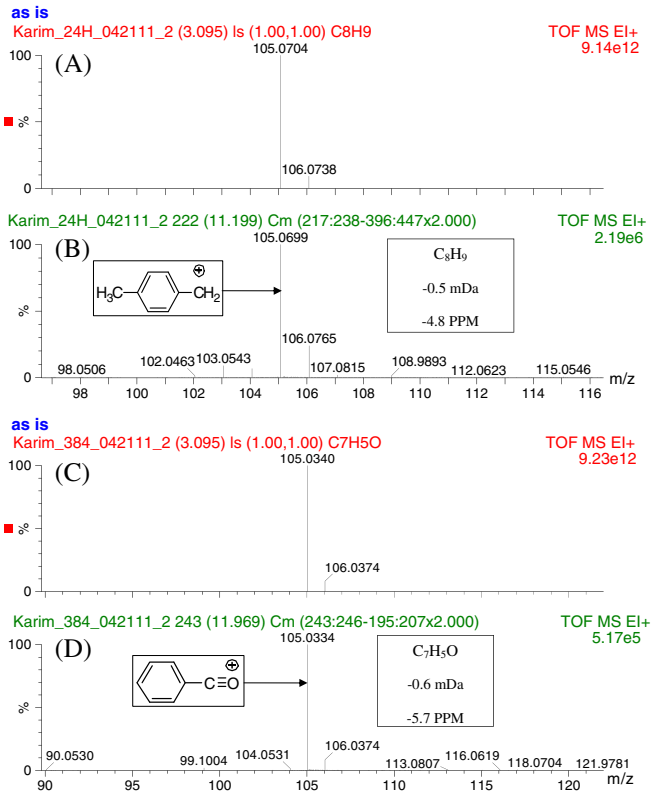

Figure 6. GC-TOF mass spectral analysis of the m/z 105 ion for 4-methylbenzylpiperazine (6A and B) and for benzoylpiperazine (6 C and D). 6A = calculated mass for C_8H_9; 6B = experimental results. 6 C = calculated mass for C_7H_5O; 6D = experimental results

(*m/z* = 105) for compounds 3 and 4, respectively. Figure 6A shows the expected/calculated mass for the C_8H_9 elemental composition. Figure 6B shows the experimental results and the degree of agreement (−4.8 ppm) with the calculated mass. Thus, confirming the *m/z* 105 ion in compound 3 as the elemental composition C_8H_9. These results can be compared to the exact mass analysis for the *m/z* 105 ion (benzoyl cation) in compound 4. Figures 6C and 6D confirm the elemental composition as C_7H_5O with a mass deviation of −5.7 ppm. Thus, exact mass measurements can distinguish between these two isobaric forms of the *m/z* 105 ion.

Vapour-phase IR spectrophotometry

IR spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. GC-IRD was evaluated for differentiation among the four piperazines. IRD should provide compound specificity without the need for chemical modification of the drug molecule. The vapour-phase IR spectra for the four underivatized piperazines are shown in Figure 7. The spectra were generated in the vapour-phase following sample injection into the gas chromatograph and each compound shows a vapour-phase IR spectrum with absorption bands in the regions 700–1700 cm^{-1} and 2700–3100 cm^{-1}. In general, variations in the ring substitution pattern with no change in the side chain composition results in variations in the IR spectrum in the region 700–1700 cm^{-1}.[28] Because the four piperazines share the same side chain (piperazine ring), they share almost the same IR features in the region 2700–3100 cm^{-1}. However, they can be easily

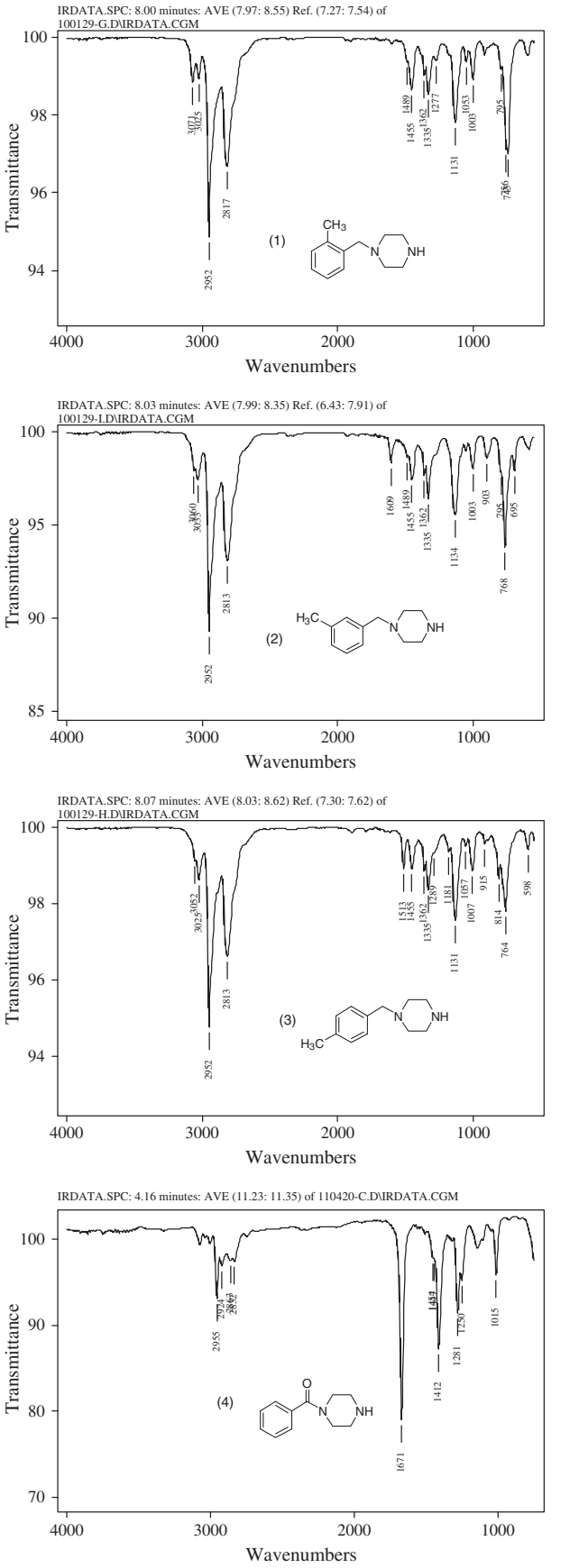

Figure 7. Vapor phase IR spectra of the four piperazine compounds in this study.

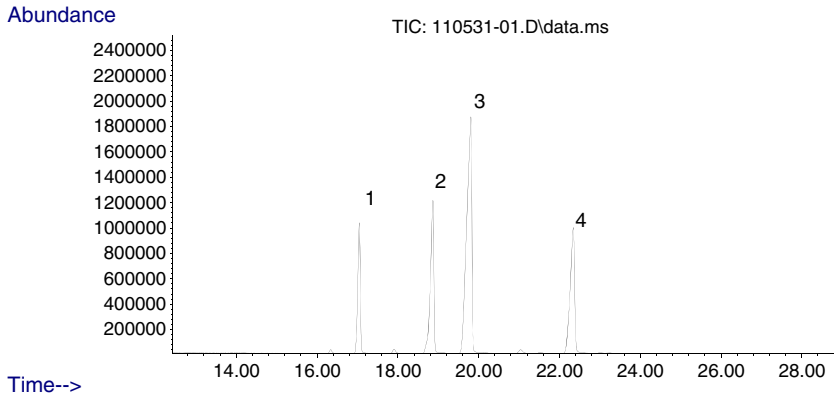

Figure 8. Gas chromatographic separation of the pentafluoropropionyl derivatives of compounds 1–4 using Rtx-200 stationary phase.

differentiated by the positions and intensities of several IR peaks in the region of 750–1620 cm^{-1}.

The BNZP shows a characteristic strong singlet IR band at 1671 cm^{-1} corresponding to the carbonyl group stretching which can distinguish this BNZP from the three ring substituted MBPs. In addition, this compound shows other strong characteristic singlets at 1412 cm^{-1}, 1281 cm^{-1} and 1015 cm^{-1} that are absent in the IR spectra of the three MBPs.

The three ring substituted MBPs 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 medium intensity doublet at 1513 cm^{-1}, 1455 cm^{-1} which is shifted to a weak intensity doublet at 1489 cm^{-1}, 1455 cm^{-1} in compounds 1 and 2. Compound 2 shows a medium peak at 1134 cm^{-1} which is shifted to a peak at 1131 cm^{-1} in both compounds 1 and 3. Compound 2 also has a medium intensity peak at 1609 cm^{-1} which is absent in compounds 1 and 3. These results illustrate the use of vapour phase IR confirmation for the isobaric and regioisomeric compounds in this study. The generated IR spectra show significant differences in the major bands for these four 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 programmes were evaluated and the chromatogram in Figure 8 is representative of the results obtained for all samples on this stationary phase.

In Figure 8, the PFPA derivatives of the three MBPs are less retained than their isobaric BNZP. The three benzylpiperazines eluted in the order of 2, 3, 4- MBPs and the BNZP eluted last in all experiments in this limited series of compounds. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the four isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

Conclusion

The three regioisomeric MBPs have an isobaric relationship to BNZP. These four piperazines yield very similar fragment ions in their mass spectra with only the BNZP showing two unique major fragment ions at *m/z* 69 and *m/z* 122. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. On the other hand the GC-TOF-MS proved to be an excellent discriminatory tool to distinguish between the isobaric forms of the *m/z* 105 base peak in these compounds. GC-IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650 and 1700 cm^{-1}. Additionally, the strong carbonyl absorption bands clearly differentiate the BNZP from the three MBPs. The four piperazines were successfully resolved on the GC stationary phase Rtx-200.

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