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Differentiation of methylenedioxybenzylpiperazines (MDBP) by GC–IRD and GC–MS

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ABSTRACT

The substituted benzylpiperazine, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP) and its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) have almost identical mass spectra. Perfluoroacylation of the secondary amine nitrogen of these regioisomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions. However the spectra did not yield any unique fragments for specific identification of one regioisomer to the exclusion of the other compound.

Gas chromatographic separation coupled with infrared detection (GC–IRD) provides direct confirmatory data for structural differentiation between the two regioisomers. The mass spectrum in combination with the vapor-phase infrared spectrum provides for specific confirmation of each of the regioisomeric piperazines. The underivatized and perfluoroacyl derivative forms of the ring substituted benzylpiperazines were resolved on a 30-m capillary column containing an Rxi-50 stationary phase.

1. Introduction

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]. This new class of potential designer drugs includes a variety of benzylpiperazine substitution patterns such as N-benzylpiperazine, 1-(3,4-methylenedioxybenzyl)-piperazine and phenylpiperazines such as 1-(3-trifluoromethyl-phenyl)piperazine, 1-(3-chlorophenyl)piperazine and 1-(4-methoxyphenyl)piperazine [3]. The most commonly abused compounds of this group are reported to be N-benzylpiperazine and 3-trifluoromethylphenylpiperazine (3-TFMPP) [3]. Recently, 3,4-MDBP has been described as producing psychoactive effects similar to those of 3,4-methylenedioxymethamphetamine (MDMA) [4–6]. Some of the piperazine compounds are commercially available and are not yet under specific legal control [7].

Analysis of 3,4-MDBP in biological and forensic samples has been the focus of several studies in recent years [8–11]. Gas chromatography-mass spectrometry (GC–MS) is the most commonly employed technique in the analysis of controlled substances in forensic laboratories [12–17].

The 3,4-methylenedioxybenzylpiperazine has been reported as a potential drug while the pharmacological properties of its 2,3-regioisomer have not been described. These two compounds have the same nominal mass (MW = 220) and yield almost identical EI mass spectra. Without reference standards and with a possibility of chromatographic co-elution the discrimination between these two isomers presents a challenge to forensic drug chemistry. 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 [8–13]. These isomeric substances are a challenge to forensic analyses that must depend heavily on mass spectrometry for confirmation level data. Many of these regioisomeric and isobaric substances have the same nominal mass and yield essentially identical mass spectra. Previous studies [9,13] 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 [9–13]. However in some cases, derivatization did not yield characteristic mass spectral fragment ions for individual isomers [11].

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. Gas chromatography with infrared detection (GC–IRD) is characterized by scanning quickly enough to obtain IR spectra of peaks eluting from the

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capillary GC columns. This technique has been successfully used in the identification of amphetamine isomers [18] as well as side chain regioisomers of methamphetamine and phentermine [19]. Recently, GC–IRD studies have been described for the differentiation of ring and side chain substituted ethoxyphenethylamines, methoxymethcathinones and methylenedioxymethamphetamines [20].

This report will describe GC–IRD and GC–MS discrimination studies on the regioisomeric ring substituted methylenedioxybenzylpiperazines.

2. Experimental

2.1. Instrumentation

GC–MS analysis was performed using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto-injector coupled with a 5975C VL Agilent mass selective detector. 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.

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) obtained from Analytical Solutions and Providers (ASAP), Covington, KY. The vapor-phase infrared spectra were recorded in the range of 4000–700 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans/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) at a flow rate of 0.7 mL/min and a column head pressure of 10 psi.

Chromatographic separations were carried out using two stationary phases. Column one was a 30 m × 0.25 mm i.d. capillary coated with 0.50 μm of 50% phenyl–50% methyl polysiloxane (Rxi-50). The temperature program consisted of an initial temperature of 100 °C for 1 min, ramped up to 230 °C at a rate of 20 °C/min followed by a hold at 230 °C for 15 min. Column two was a 30 m × 0.25 mm i.d. capillary coated with 0.5 μm of 100% trifluoropropyl methyl polysiloxane (Rtx-200). The separation 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 ramped to 200 °C at a rate of 10 °C/min and held at 200 °C for 5.0 min. Both GC capillary columns used in this study were purchased from Restek Corporation (Bellefonte, PA).

In both GC–MS and GC–IRD analyses, samples were dissolved and diluted in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fairlawn, NJ) and introduced, individually and in physical mixtures, via the auto-injector using an injection volume of 1 μL.

2.2. Drugs and reagents

The general procedure for the synthesis of these two regioisomeric methylenedioxybenzylpiperazines utilize 2,3-methylenedioxybenzaldehyde and 3,4-methylenedioxybenzaldehyde (piperonal), as starting materials. The preparation of 2,3-methylenedioxybenzaldehyde has been reported previously [21,22]. The two regioisomers were prepared by the reductive amination of the appropriate aldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding methylenedioxybenzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. All laboratory reagents and solvents were

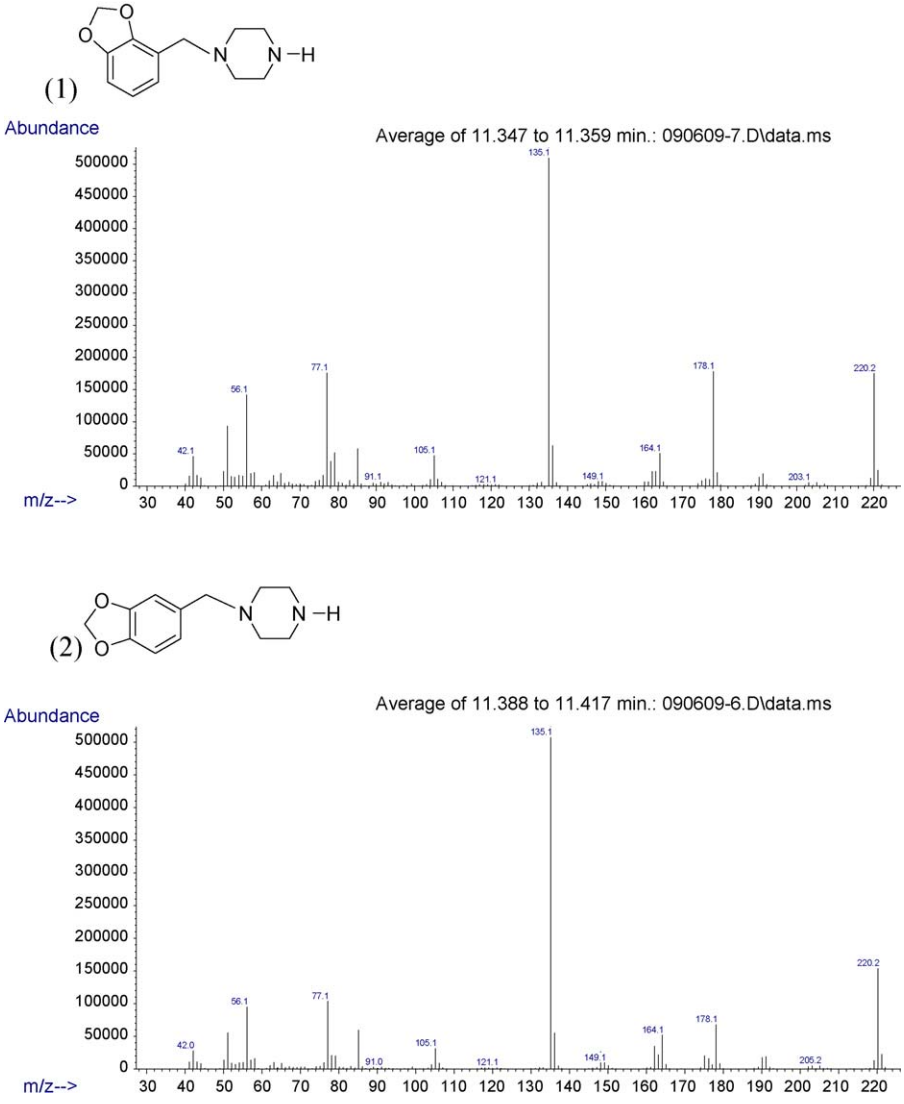

Fig. 1. Mass spectra of the methylenedioxybenzylpiperazines.

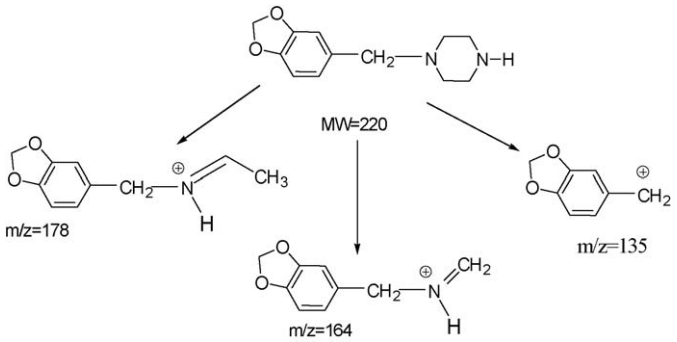

Fig. 2. Mass spectral fragments for 3,4-methylenedioxybenzylpiperazine.

obtained from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Atlanta, GA). The derivatizing agents trifluoroacetic anhydride (TFA), pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) were purchased from Sigma–Aldrich, Inc. (Milwaukee, WI).

2.3. 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.

3. Results and discussion

3.1. Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Fig. 1 shows the EI mass spectra of the two regioisomeric methylenedioxybenzylpiperazines (Compounds 1 and 2). The ions of significant relative abundance common to the two regioisomers likely arise from fragmentation of the piperazine ring. The mass spectra of both regioisomeric methylenedioxybenzylpiperazines show the 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 Fig. 2 and are based on the work of de Boer et al. [7]. The previous work described the fragmentation of the unsubstituted benzylpiperazine [7] and the structures for the fragment ions in the two methylenedioxybenzyl regioisomers are

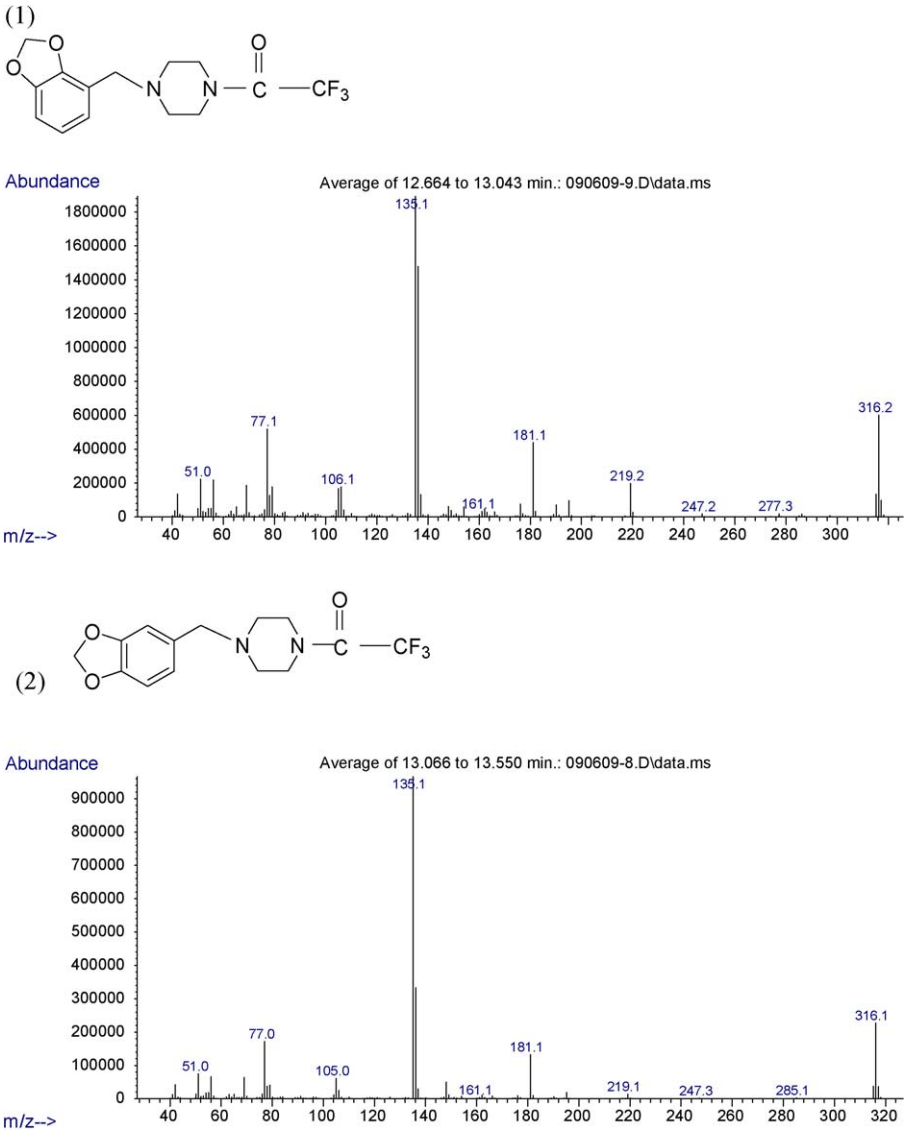

Fig. 3. Mass spectra of the trifluoroacetylpiperazine regioisomers.

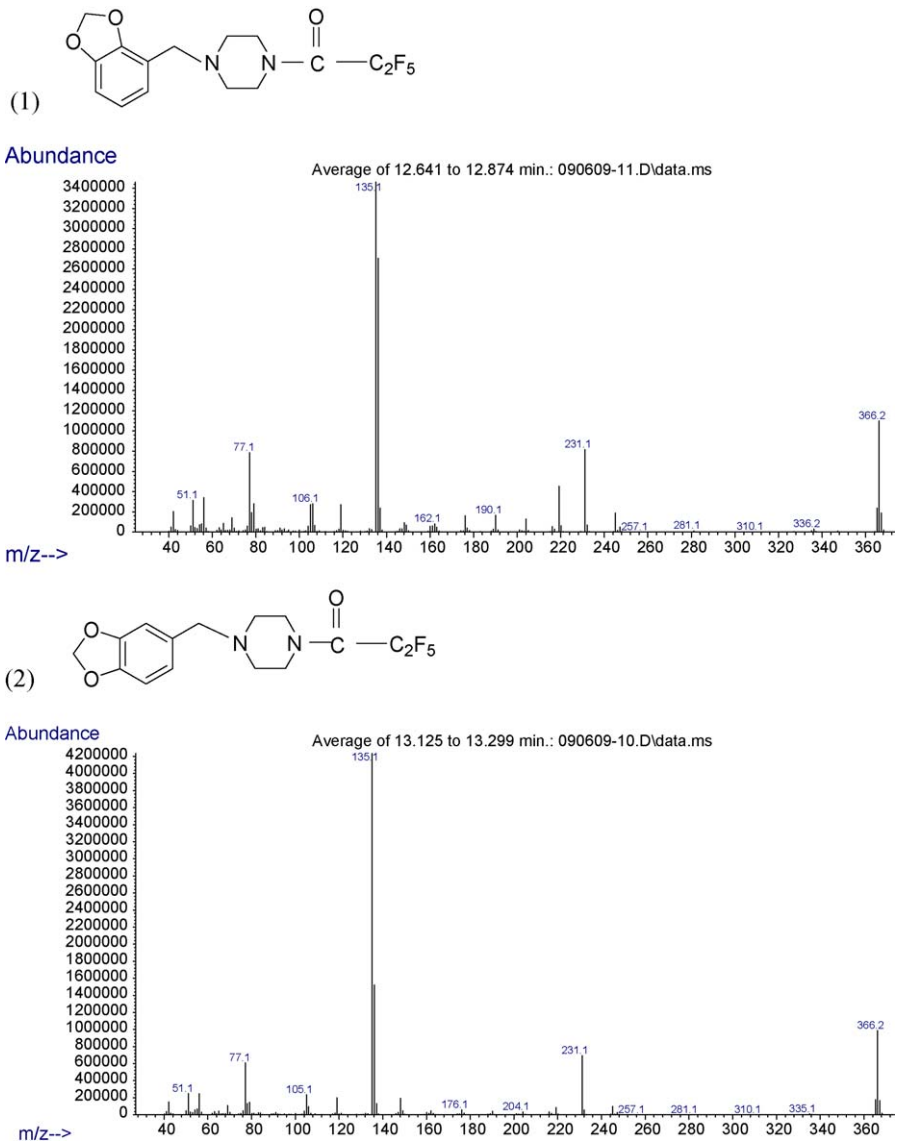

Fig. 4. Mass spectra of the pentafluoropropionylpiperazine regioisomers.

likely equivalent. The relative abundances for the ions in the spectra for the two regioisomeric MDBPs are also equivalent. These results indicate that very little structural information is available for differentiation among these isomers. Thus, the mass spectra alone do not provide specific identity confirmation for the individual isomers.

The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the regioisomeric methylenedioxybenzylpiperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these two compounds. Acylation lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum [9–12]. However, acylation of the secondary nitrogen in the piperazine ring does not alter the basicity of the tertiary amine nitrogen.

The trifluoroacetyl, pentafluoropropionyl and heptafluorobutyryl derivatives were evaluated for their ability to individualize the mass spectrum of 3,4-MDBP to the exclusion of the 2,3-regioisomer. The mass spectra of the perfluoroacyl amides of the two compounds are shown in Figs. 3–5. 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 methylenedioxybenzyl 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. These studies further indicate that no ions of significance were found to differentiate between the two regioisomers.

3.2. Vapor-phase infrared spectrophotometry

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas chromatography with infrared detection (GC–IRD) was evaluated for differentiation among the two regioisomeric MDBPs. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the two methylenedioxybenzylpiperazines

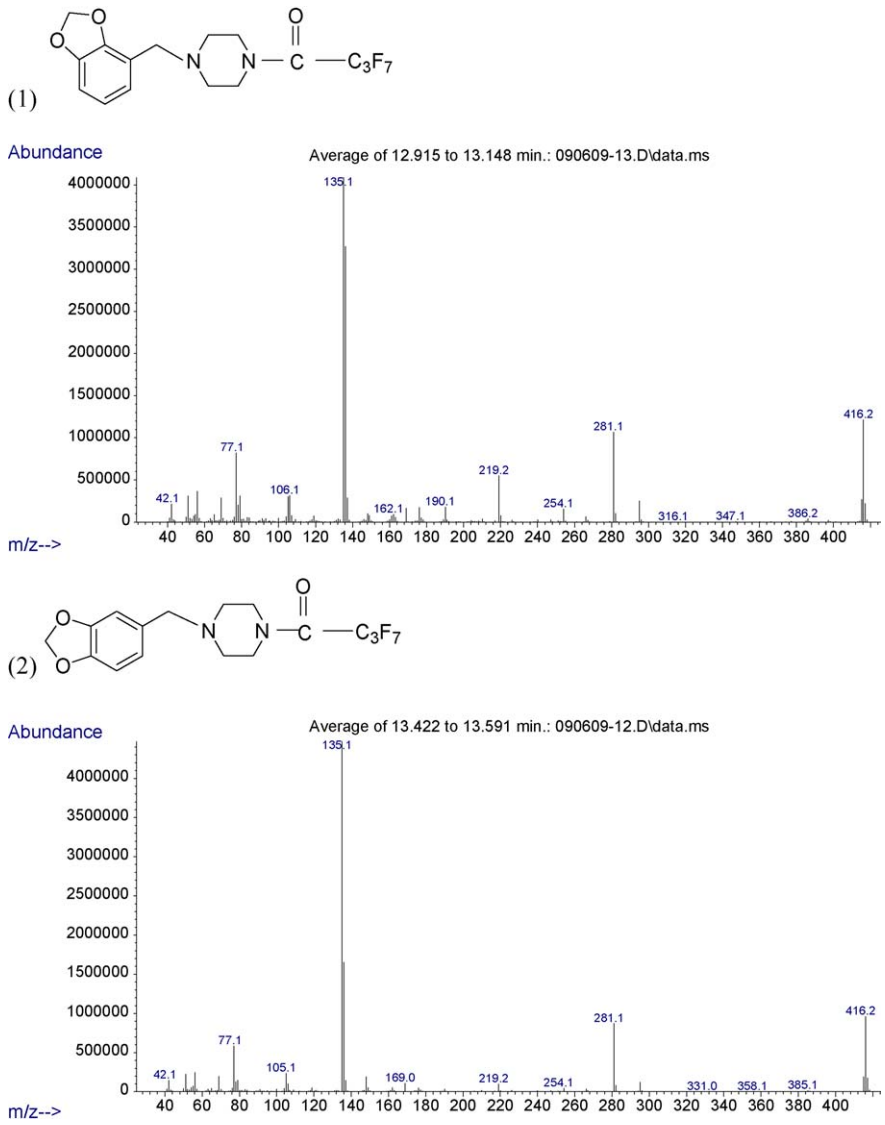

Fig. 5. Mass spectra of the heptafluorobutyrylpiperazine regioisomers.

are shown in Fig. 6. 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 700–1700 cm^{-1} and 2700–3100 cm^{-1}. In general, variations in the position of the methylenedioxy-group on the aromatic ring results in variations in the IR transmittance in the region 700–1700 cm^{-1} [19]. Since the two piperazines share the same degree of nitrogen substitution, they have almost identical IR transmittance 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 750–1620 cm^{-1}.

The 2,3-MDBP regioisomer 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 regioisomers. 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 1069 cm^{-1} which is shifted to a medium intensity peak at 1050 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-regioisomers by at least three IR bands of varying intensities. The first of which is the peak of strong intensity appearing 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 the strong doublet absorption 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}.

In summary, vapor-phase infrared spectra provide distinguishing and characteristic information to determine the position of ring attachment (2,3-MDBP vs. 3,4-MDBP) for the methylenedioxy-group in these substituted piperazine regioisomers.

3.3. Gas chromatography

Gas chromatographic separation of the underivatized and derivatized piperazines was accomplished on two stationary phases using capillary columns of the same dimensions (30 m × 0.25 mm, 0.5-μm film thickness). The stationary phases compared in this study were the relatively polar phase, 100% trifluoropropyl methyl polysiloxane (Rtx-200) and 50% phenyl–50% methyl polysiloxane

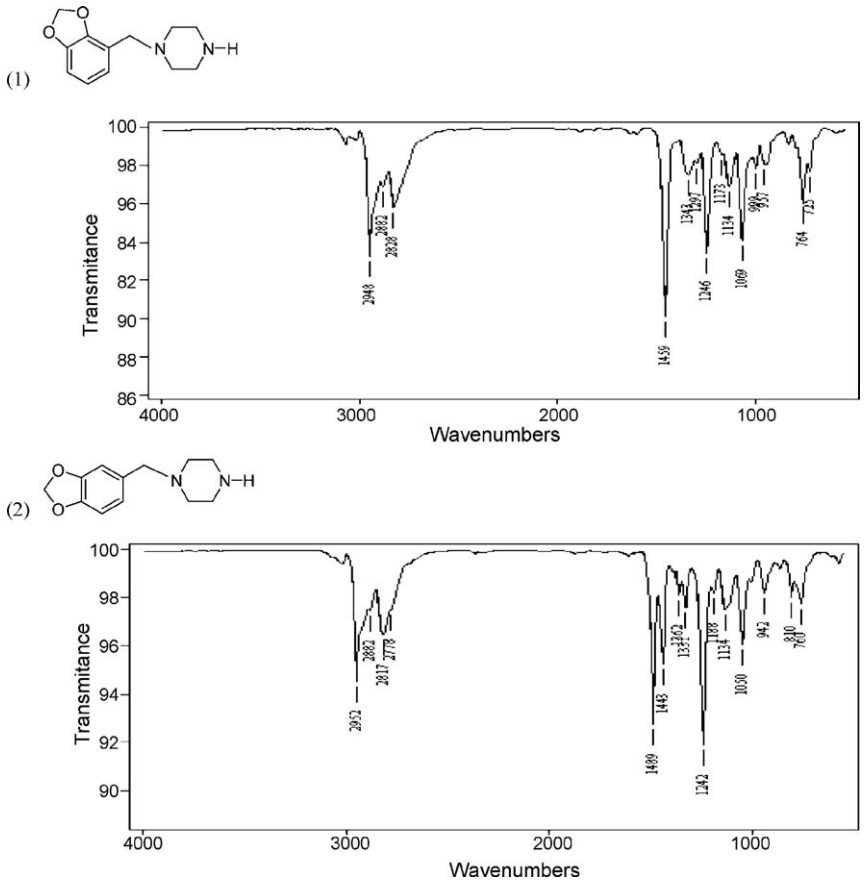

Fig. 6. Vapor-phase IR spectra of the methylenedioxybenzylpiperazines.

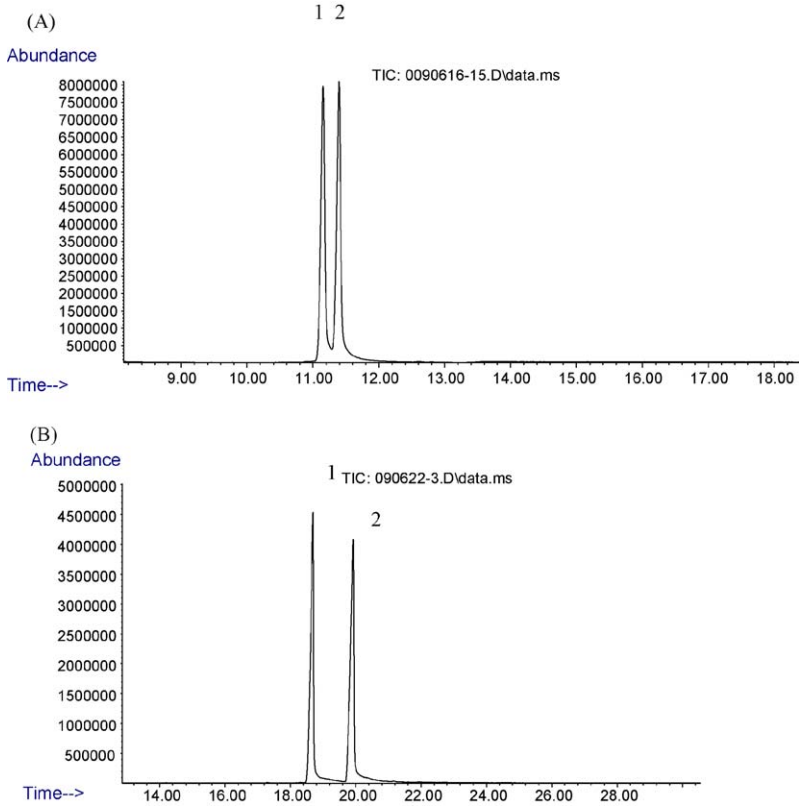

Fig. 7. Gas chromatographic separation of (1) 2,3-methylenedioxybenzylpiperazine and (2) 3,4-methylenedioxybenzylpiperazine. Columns: Rxi-50 (A) and Rtx-200 (B).

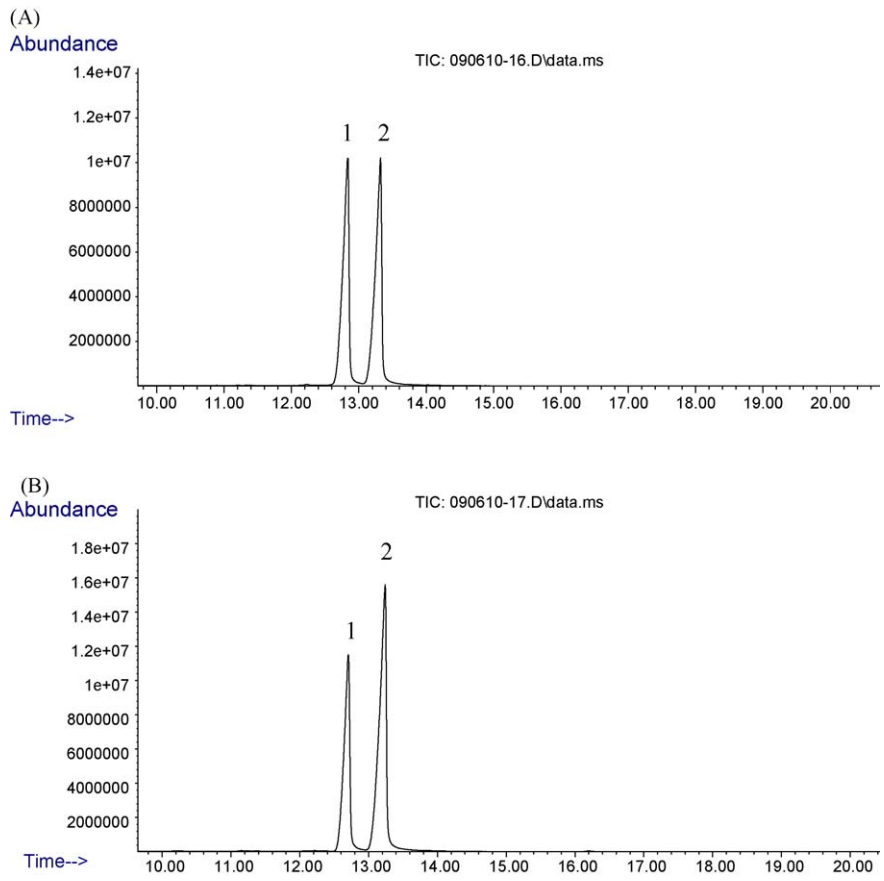

Fig. 8. Gas chromatographic separation of the trifluoroacetyl (A) and pentafluoropropionyl (B) derivatives using Rxi-50 column. (1) 2,3-Methylenedioxybenzylpiperazine and (2) 3,4-methylenedioxybenzylpiperazine.

(Rxi-50). Several temperature programs were evaluated and the chromatograms in Figs. 7 and 8 are representative of the results obtained for all samples on the two columns. The chromatograms in Fig. 7 show the separation of the piperazines on the Rxi-50 and Rtx-200 stationary phases. The two isomers are well resolved and 2,3-MDBP elutes before the 3,4-isomer on both columns. The separations shown in Fig. 8 are representative of the results obtained for all the perfluoroacylpiperazines evaluated in this study. The TFA, PFPA, and HFBA derivatives yielded similar chromatograms with the 2,3-isomer eluting first in every case.

4. Conclusion

The two regioisomeric methylenedioxybenzylpiperazines have the same molecular formula and nominal mass and yield the same fragment ions in their EI mass spectra. Perfluoroacylation did not offer any unique marker ions to allow differentiation between these isomers. GC–IRD analysis yields unique and characteristic vapor-phase infrared spectra for these two regioisomeric piperazines allowing discrimination between them. This differentiation was accomplished without the need for chemical derivatization. The two piperazines as well as their perfluoroacyl derivatives were successfully resolved via capillary gas chromatography on two stationary phases.

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References

[1] R.A. Glennon, R.M. Slusher, R.A. Lyon, M. Titeler, J.D. McKenney, 5-HT_1 and 5-HT_2 binding characteristics of some quipazine analogues, J. Med. Chem. 29 (1986) 2375–2380.
[2] R.A. Glennon, Central serotonin receptors as targets for drug research, J. Med. Chem. 30 (1987) 1–12.
[3] R.F. Staack, L.D. Paul, D. Schmid, G. Roider, B. Rolf, Proof of a 1-(3-chlorophenyl)piperazine (mCPP) intake—use as adulterant of cocaine resulting in drug–drug interactions? J. Chromatogr. B 855 (2007) 127–133.
[4] H. Tsutsumi, M. Katagi, A. Miki, N. Shima, T. Kamata, M. Nishikawa, K. Nakajima, H. Tsuchihashi, Development of simultaneous gas chromatography–mass spectrometric and liquid chromatography–electrospray ionization mass spectrometric determination method for the new designer drugs, *N*-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and their main metabolites in urine, J. Chromatogr. B 819 (2005) 315–322.
[5] R. Haroz, M.I. Greenberg, New drugs of abuse in North America, Clin. Lab. Med. 26 (2006) 147–164.
[6] H.L. Yarosh, E.B. Katz, A. Coop, W.E. Fantegrossi, MDMA-like behavioral effects of N-substituted piperazines in the mouse, Pharmacol. Biochem. Behav. 88 (2007) 18–27.
[7] D. de Boer, I.J. Bosman, E. Hidvégi, C. Manzoni, A.A. Benkö, L.J.A.L. dos Reys, R.A.A. Maes, Piperazine-like compounds: a new group of designer drug-of-abuse on the European Market, Forensic Sci. Int. 121 (2001) 47–56.
[8] R.F. Staack, H.H. Maurer, New designer drug 1-(3,4-methylenedioxybenzyl)piperazine (MDBP): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry, J. Mass Spectrom. 39 (2004) 255–261.
[9] F.T. Peters, S. Schaefer, R.F. Staack, T. Kraemer, H.H. Maurer, Screening for and validated quantification of amphetamines as well as of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry, J. Mass Spectrom. 38 (2003) 659–676.
[10] S.P. Vorce, P. Shawn, J.M. Holler, B. Levine, M.R. Past, Detection of 1-benzylpiperazine and 1-(3-trifluoromethylphenyl)-piperazine in urine analysis specimens using GC-MS and LC-ESI-MS, J. Anal. Toxicol. 32 (2008) 444–450.
[11] H.M. Maher, T. Awad, C.R. Clark, Differentiation of the regioisomeric 2, 3, and 4-trifluoromethylphenylpiperazines (TFMPP) by GC-IRD and GC-MS, Forensic Sci. Int. 188 (2009) 31–39.

[12] L. Aalberg, J. DeRuiter, E. Sippola, C.R. Clark, Gas chromatographic optimization studies on the side chain and ring regioisomers of methylenedioxymeth-amphetamine, J. Chromatogr. Sci. 42 (2004) 293–298.
[13] T. Awad, J. DeRuiter, C.R. Clark, GC-MS analysis of acylated derivatives of the side chain and ring regioisomers of methylenedioxymethamphetamine, J. Chromatogr. Sci. 43 (2005) 296–303.
[14] T. Awad, C.R. Clark, J. DeRuiter, Chromatographic and mass spectral studies on methoxymethcathinones related to 3,4-methylenedioxymethamphetamine, J. Chromatogr. Sci. 44 (2006) 155–161.
[15] T. Awad, C.R. Clark, J. DeRuiter, Chromatographic and mass spectral studies on methoxy methyl methamphetamines related to 3,4-methylenedioxymeth-amphetamine, J. Chromatogr. Sci. 45 (2007) 468–476.
[16] T. Awad, C.R. Clark, J. DeRuiter, GC-MS analysis of acylated derivatives of the side chain regioisomers of 4-methoxy-3-methyl phenethylamines related to methylenedioxymethamphetamine, J. Chromatogr. Sci. 45 (2007) 477–485.
[17] T. Awad, J. DeRuiter, C.R. Clark, GC-MS analysis of the ring and side chain regioisomers of ethoxyphenethylamines, J. Chromatogr. Sci. 46 (2008) 671–679.
[18] W. Duncan, W.H. Soine, Identification of amphetamine isomers by GC/IR/MS, J. Chromatogr. Sci. 26 (1988) 521–526.
[19] T. Awad, T. Belal, J. DeRuiter, K. Kramer, C.R. Clark, Comparison of GC-MS and GC-IRD methods for the differentiation of methamphetamine and regioisomeric substances, Forensic Sci. Int. 185 (2009) 67–77.
[20] T. Belal, T. Awad, J. DeRuiter, C.R. Clark, GC-IRD methods for the identification of isomeric ethoxyphenethylamines and methoxymethcathinones, Forensic Sci. Int. 184 (2009) 54–63.
[21] L. Aalberg, J. DeRuiter, F.T. Noggle, E. Sippola, C.R. Clark, Chromatographic and mass spectral methods of identification for the side chain and ring regioisomers of methylenedioxymethamphetamine, J. Chromatogr. Sci. 38 (2000) 329–337.
[22] J.F. Casale, P.A. Hays, R.F.X. Klein, Synthesis and characterization of the 2,3-methylenedioxyamphetamines, J. Forensic Sci. 40 (1995) 391–400.