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Regioisomeric bromodimethoxy benzyl piperazines related to the designer substance 4-bromo-2,5-dimethoxybenzylpiperazine: GC–MS and FTIR analysis

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ABSTRACT

A series of seven regioisomeric bromodimethoxy benzyl piperazines including the designer benzylpiperazine (4-bromo-2,5-dimethoxybenzylpiperazine) were synthesized and their analytical profiles evaluated using GC–MS and FT-IR. The mass spectra for the seven regioisomeric bromodimethoxy benzyl piperazines are almost identical with only the two 2,3-dimethoxy isomers showing one unique major fragment ion at *m/z* 214/216. Thus, mass spectrometry alone does not provide for the confirmation of identity of any one of the seven compounds to the exclusion of the other isomers. Perfluoroacylation of the secondary amine nitrogen for each of the seven regioisomers gave mass spectra showing some differences in the relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation of structure. Attenuated total reflection infrared spectroscopy provides direct confirmatory data for differentiation between the seven regioisomeric aromatic ring substituted bromodimethoxy benzyl piperazines. Mixtures of the seven piperazine PFP derivatives were successfully resolved via capillary gas chromatography using a relatively polar stationary phase composed of 100% trifluoropropyl methyl polysiloxane.

1. Introduction

Structural modifications of drugs of abuse are well known for the amphetamine-derived designer drugs. The most common variations involve the introduction of a methylenedioxy, dimethoxy or bromo moiety into the aromatic ring of amphetamine leading to compounds such as 3,4-methylenedioxyamphetamine (MDA), 2,5-dimethoxyamphetamine (2,5-DMA) and 4-bromo-2,5-dimethoxyamphetamine (DOB), repectively. In addition to the amphetamines, a series of piperazine-derived compounds have recently entered the illicit drug market and represent a new group of designer drugs. Many of these piperazines 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]. It appears that *N*-benzylpiperazine and 3-trifluoromethylphenyl piperazine (3-TFMPP) are among the most commonly abused compounds of this group [3].

Structural modifications similar to those found in the amphetamines are also encountered in the piperazine compounds. 1-(3,4-methylenedioxybenzyl)-piperazine (3,4-MDBP) is the methylenedioxy analogue of *N*-benzylpiperazine (BZP), a scheduled compound in the USA [4]. Recently, 3,4-MDBP has been described as producing psychoactive effects similar to those of 3,4-methylenedioxymethamphetamine (MDMA) [5–7]. Furthermore, 4-bromo-2,5-dimethoxy-benzylpiperazine (2C-B-BZP) is a psychoactive compound of the piperazine chemical class which has been sold as a "designer drug" [8,9] and is reported to produce stimulant effects similar to those of benzylpiperazine (BZP) [9]. A clandestine sample of 4-bromo-2,5-dimethoxybenzylpiperazine was identified in a street drug sample in Germany in 2006 [8]. The analytical structure elucidation and differentiation of 4-bromo-2,5-dimethoxybenzylpiperazine and one of its regioisomers 2-bromo-4,5-dimethoxybenzylpiperazine was reported using gas chromatography–mass spectroscopy (GC–MS), product ion spectroscopy (GC–MS/MS), and nuclear magnetic resonance (NMR) spectroscopy [8].

The most likely method for the synthesis of (2C-B-BZP) is via the reductive amination of piperazine with 4-bromo-2,5-

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dimethoxybenzaldehyde under reducing conditions. Several regioisomeric bromodimethoxybenzaldehydes are commercially available and uncontrolled, thus a number of monobrominated dimethoxybenzylpiperazines can be prepared by the same synthetic methodology from readily available precursors. The pharmacological effects for the other regioisomers have not been extensively described. Thus, analytical differentiation among the brominated derivatives of these regioisomeric dimethoxybenzylpiperazines is an important issue in forensic drug chemistry.

Gas chromatography–mass spectrometry (GC–MS) is the most widely used technique in the analysis of controlled substances in forensic laboratories [10–21]. The regioisomer issue is extremely important when some of these molecules are legally controlled drugs or controlled precursor substances [10–15]. This study is concerned with the differentiation of the seven monobrominated products resulting from the six possible regioisomeric dimethoxybenzylpiperazines. Such compounds have mass spectral equivalency and similar chromatographic elution properties. Those substances co-eluting in the chromatographic system and having common mass spectra could be misidentified. The ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target molecules.

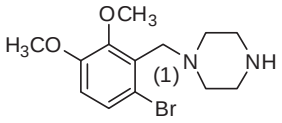
Previous studies [11–14] have shown that chemical derivatization methods (primarily acylation) can be used to add analytical specificity to the analysis of regioisomeric primary and secondary amines of varying side-chain structure. Derivatization can alter major fragmentation pathways often providing additional structural information about an individual isomer as well as altered chromatographic properties [11–14]. However, amine acylation is less successful for the identification of regioisomeric aromatic ring substitution patterns and is not useful for tertiary amines that do not form stable acylation products.

This group of seven compounds represents the complete set of monobromonated products of all possible regioisomeric dimethoxybenzylpiperazines. Analytical differentiation of the regioisomeric bromodimethoxy benzyl piperazines (compounds 1–7 in Fig. 1) can be a significant issue since all these isomers represent likely designer analogues in this series and methods to differentiate them have not been reported. The aim of this study is to evaluate analytical methods using GC–MS and infrared spectroscopy (FTIR) to characterize and differentiate among this set of ring regioisomeric compounds.

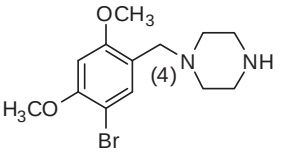
2. Experimental

2.1. Instrumentation

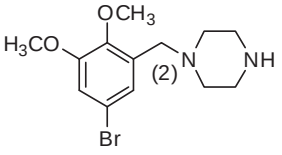
GC–MS analysis was performed using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless mode with a helium (grade 5) flow rate of 0.7 mL/min and a column head pressure of 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.



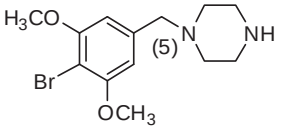
(1) 6-Br-2,3-DMBP
6-Bromo-2,3-Dimethoxybenzylpiperazine



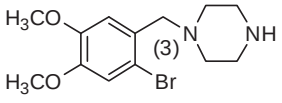
(4) 5-Br-2,4-DMBP
5-Bromo-2,4-Dimethoxybenzylpiperazine



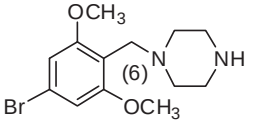
(2) 5-Br-2,3-DMBP
5-Bromo-2,3-Dimethoxybenzylpiperazine



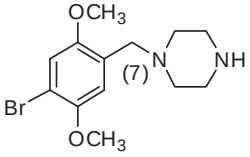
(5) 4-Br-3,5-DMBP
4-Bromo-3,5-Dimethoxybenzylpiperazine



(3) 2-Br-4,5-DMBP
2-Bromo-4,5-Dimethoxybenzylpiperazine



(6) 4-Br-2,6-DMBP
4-Bromo-2,6-Dimethoxybenzylpiperazine



(7) 4-Br-2,5-DMBP
4-Bromo-2,5-Dimethoxybenzylpiperazine
(2C-B-BZP)

Fig. 1. Structures of the seven bromodimethoxy benzyl piperazines in this study.

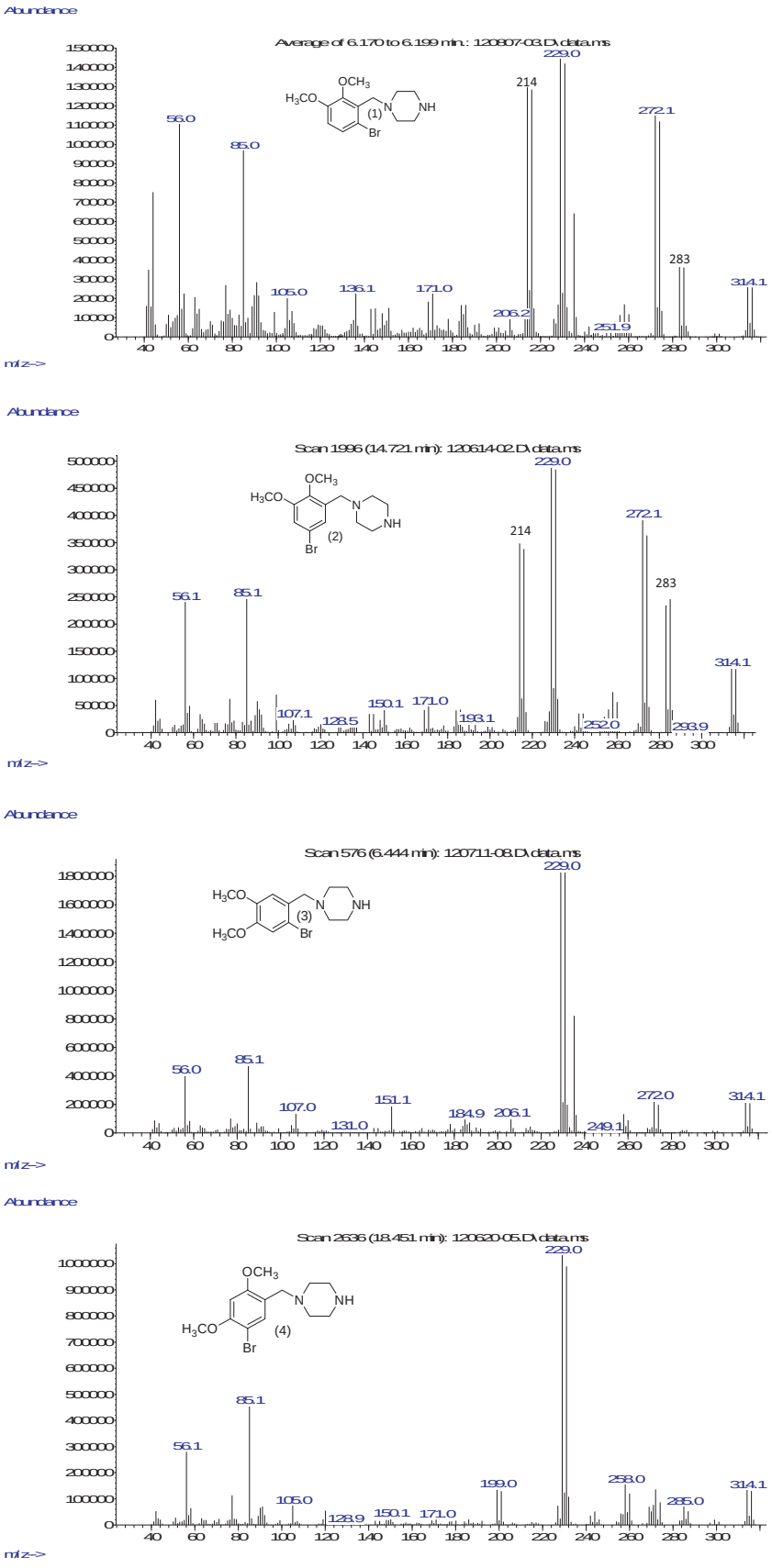


Fig. 2. EI mass spectra of the seven bromodimethoxy benzyl piperazines.

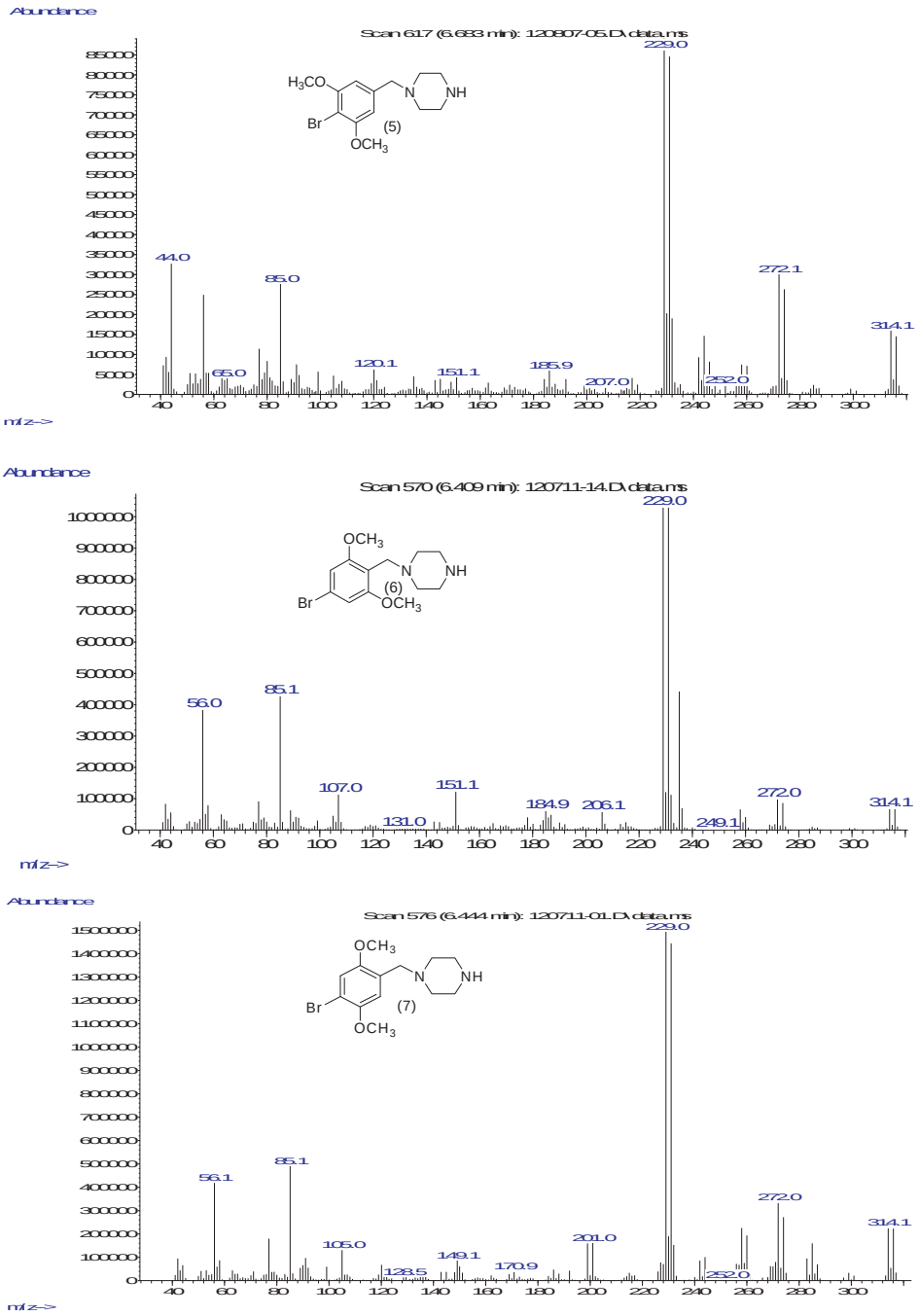


Fig. 2. (Continued)

GC–MS chromatographic separations were carried out on a column (30 m × 0.25 mm i.d.) coated with 0.5 μm film of 100% trifluoropropyl methyl polysiloxane (Rtx-200) purchased from Restek Corporation (Bellefonte, PA). The separation of the 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 7.5 °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 60.00 min.

Attenuated total reflection fourier transform infrared (ATR-FTIR) spectra were obtained on a Shimadzu IRAffinity-1 fourier transform infrared spectrophotometer (Kyoto, Japan) equipped with a DLATGS detector with temperature control system at a resolution of 4 cm^{-1} with an aperture of 3.5 mm and scan rate of 10 scans/s. The FTIR spectrophotometer was equipped with MIRacle single reflection horizontal ATR accessory (Pike Technologies, WI). The single-reflection sampling plate of the accessory has a 1.8 mm round crystal surface allowing reliable analysis of small samples. FTIR spectra were recorded in the range of 4000–520 cm^{-1}. The samples were prepared by dissolving the solid or oily compounds in acetonitrile and introducing the resulting solutions in small volumes to the center of the single-reflection sampling plate.

2.2. Drugs and reagents

The general procedure for the synthesis of these seven regioisomeric bromodimethoxy benzyl piperazines (BrDMBPs) begins with the appropriate bromodimethoxybenzadehyde. The starting aldehydes for compounds 1, 2, 3, 4, 5 and 7 are 6-bromo-2,3-dimethoxybenzaldehyde, 5-bromo-2,3-dimethoxybenzaldehyde, 6-

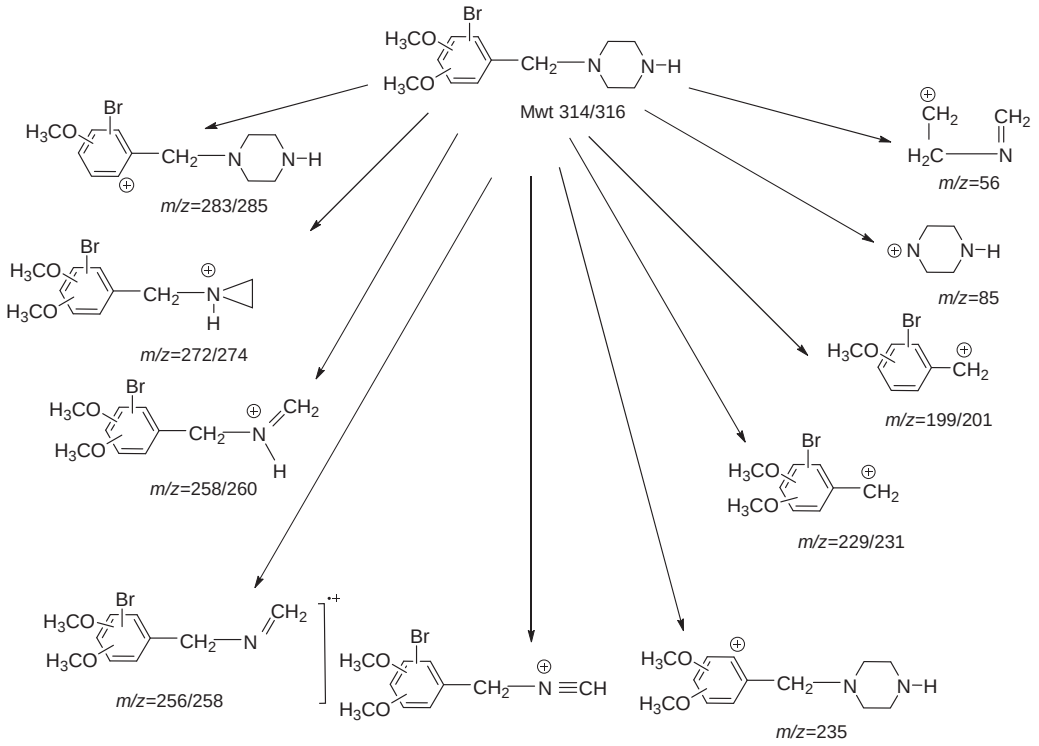


Fig. 3. EI mass spectral fragmentation pattern of the underivatized bromodimethoxy benzyl piperazines.

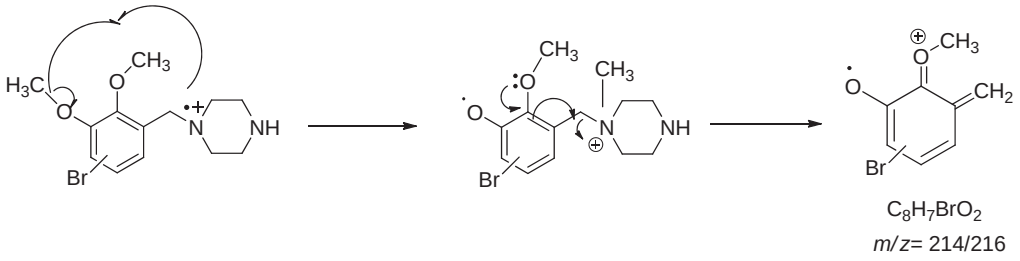


Fig. 4. Proposed mechanism for the formation of the *m*/*z* 214/216 ion in the mass spectra of the brominated 2,3-dimethoxybenzylpiperazines (compounds 1 and 2).

bromo-3,4-dimethoxybenzaldehyde (6-bromoveratraldehyde), 5-bromo-2,4-dimethoxybenzaldehyde, 4-bromo-3,5-dimethoxybenzaldehyde and 4-bromo-2,5-dimethoxybenzaldehyde, respectively and all are commercially available. The starting material for compound 6 is 4-bromo-2,6-dimethoxybenzaldehyde and it was prepared by treating a cold solution of 2,6-dimethoxybenzaldehyde in glacial acetic acid with bromine in glacial acetic acid. The seven regioisomeric BrDMBPs were synthesized by stirring a solution of the appropriate aldehyde in methanol with piperazine and sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding BrDMBPs, which were converted to the hydrochloride salts using gaseous HCl. All laboratory reagents and chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Fisher Scientific (Atlanta, GA). The derivatizing agent pentafluoropropionic anhydride (PFPA) was purchased from Sigma–Aldrich, Inc. (Milwaukee, WI).

2.3. Derivatization procedure

Each perfluoroamide was prepared individually from the hydrochloride salt of each regioisomer by dissolving

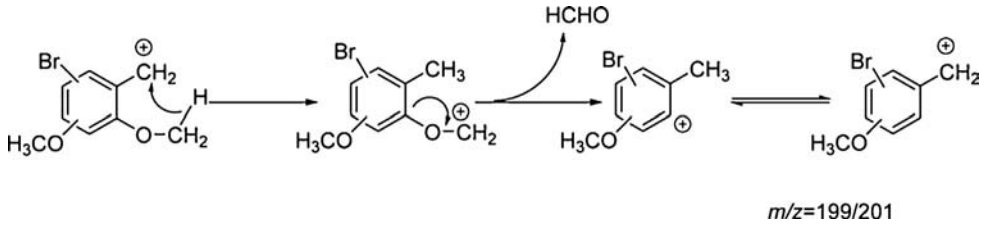


Fig. 5. Mechanism for the formation of the *m*/*z* 199/201 ion in the mass spectra of the underivatized and derivatized 2-methoxy regioisomers of the bromodimethoxy benzyl piperazines.

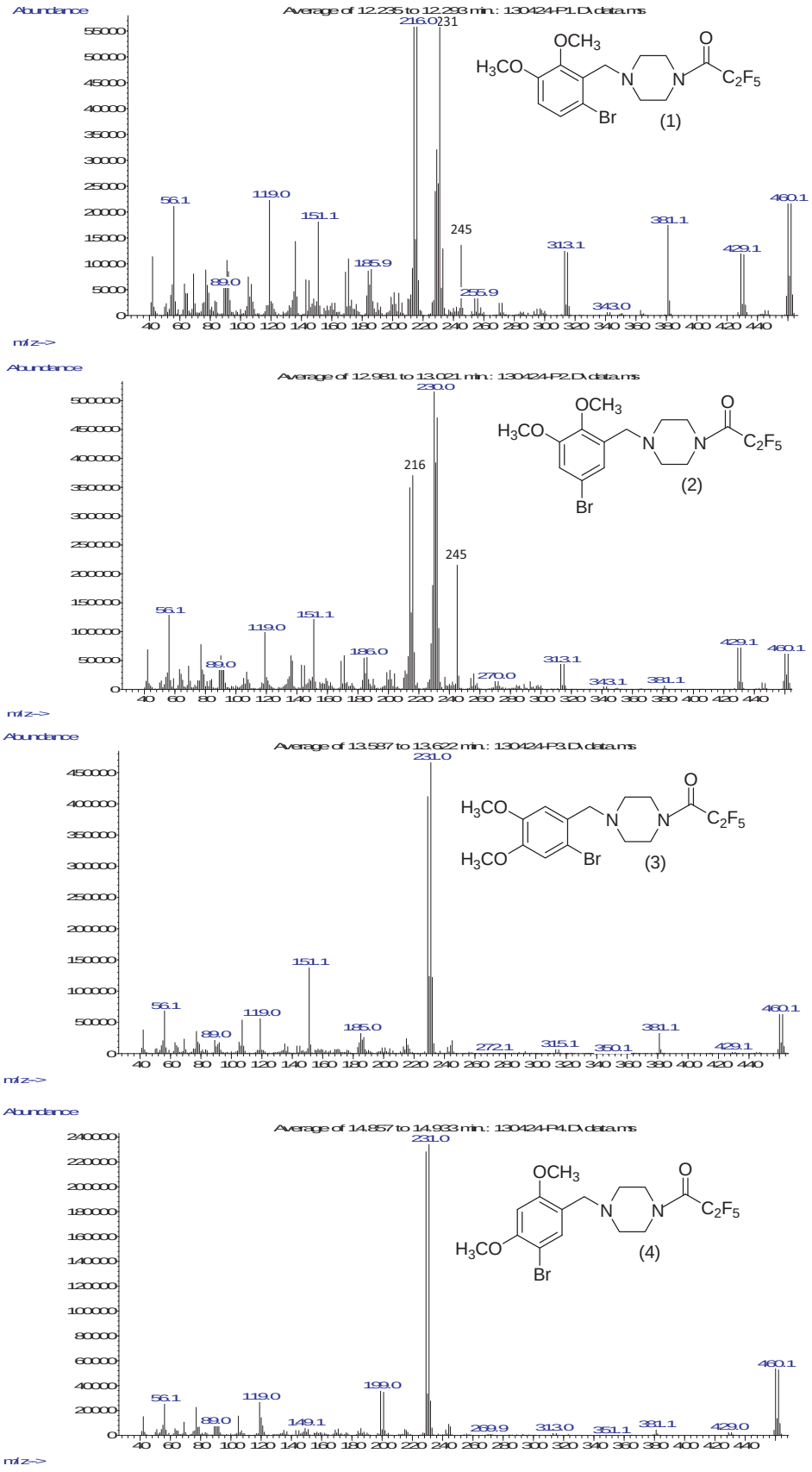


Fig. 6. EI mass spectra of the pentafluoropropionyl derivatives of the seven piperazine compounds.

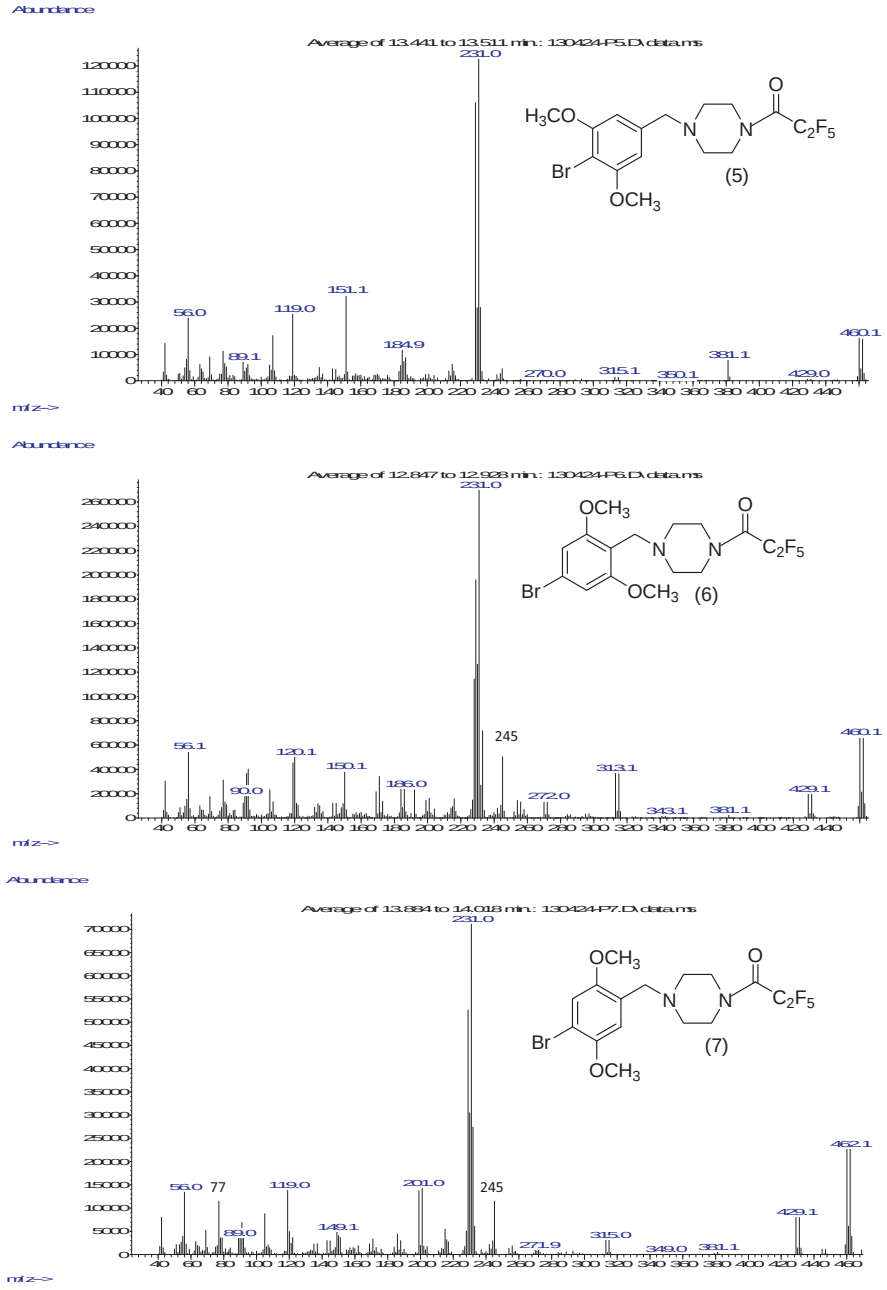


Fig. 6. (Continued)

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 derivatizing agent pentafluoropropionic anhydride (PFPA). The derivatization 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. 2 shows the EI mass spectra of the seven regioisomeric BrDMBPs (compounds 1–7). The mass spectra in Fig. 2 indicate that all the major fragment ions occur at equal masses for these isomeric compounds, however the spectra do provide some direction for differentiation among this group of piperazines. The common fragment ions observed for the regioisomeric bromodimethoxy groups substituted on the aromatic ring likely indicate that the piperazine ring is the initial source for most of the fragmentation. The bromodimethoxybenzyl cation *m/z* 229/231 is the base peak in all these spectra. The structures for some of the fragment ions in the unsubstituted aromatic ring for benzylpiperazine BZP have been reported [22] and labeling experiments using d_8-piperazine have provided additional confirmation for fragment ion structures [23]. Equivalent fragmentation pathways for the bromodimethoxy benzyl piperazines (BrDMBPs) yield the fragment ions at *m/z* 272/274, 258/260, 257/259, 256/258, 229/231, 199/201, 85 and 56 as shown

in Figs. 2 and 3. The structures for the fragments in the seven BrDMBPs regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual BrDMBP regioisomer except for the characteristic high relative abundance ion at *m/z* 214/216 which appears to be specific for the two 2,3-dimethoxy regioisomers (compounds 1 and 2).

The proposed structure and mechanism for the formation of the unique *m/z* 214/216, $C_8H_7BrO_2$ ion is shown in Fig. 4. The suggested structure for this fragment involves loss of a methyl group from the meta methoxy-substituent of this crowded 1, 2, 3-trisubstituted aromatic ring. This characteristic fragment ion is equivalent to the *m/z* 136 ion in the mass spectrum of 2,3-dimethoxybenzylpiperazine. The proposed structure and mechanism of formation of the characteristic *m/z* 136 ion was described in detail in a previous report from our laboratory [24]. The proposed structure for the *m/z* 136 ion was supported by the mass spectra of the mono-, tri-, and hexa-deutero labeled forms of this compound in addition to the exact mass analysis using GC–TOF–MS. The ortho ^{13}C-labeled form of the 2,3-DMBP confirmed that the methyl group loss to yield the *m/z* 136 species is from the methoxy substituent at the 3-position as described in the fragmentation scheme in Fig. 4 [24].

An additional fragmentation pathway which is observed for some of the ortho-methoxy ring substituted compounds is described in Fig. 5. Those BrDMBPs with the methoxy group in the ortho position relative to the side chain often show a significant *m/z* 199/201 ion. This ion likely arises from the loss of mass 30 (CH_2O) from the initial bromodimethoxybenzylic cation at *m/z* 229/231. The *m/z* 199/201 ion only occurs in isomers having the methoxy group ortho to the piperazine side chain and therefore the site of initial benzylic cation formation. These ions are most prominent for compounds 4 and 7. This *m/z* 199/201 ion can be formed by 1,6-hydride shift (ortho effect) from a hydrogen of the ortho-methoxy group to the benzylic cation followed by the loss of formaldehyde as in Fig. 5. This fragment occurs in all the mass spectra of the underivatized and PFP derivatives of the ortho-methoxy BrDMBPs. This suggested mechanism for the loss of CH_2O from the ortho-methoxy benzyl cations was previously discussed [24–26].

The spectra in Fig. 2 provide some direction for differentiation among these isomeric compounds. For example, compounds 1 and 2 show a unique and characteristic *m/z* 214/216 fragment not seen in any of the other five compounds. Furthermore, compound 1 has significant fragments at *m/z* 44 and *m/z* 235 and these ions are not seen for compound 2. Compound 3 and 6 have a significant fragment at *m/z* 235 and do not show the ions at *m/z* 214/216. This differentiates compounds 3 and 6 from 1 and 2 yet does not allow differentiation between 3 and 6. The remaining group consisting of three compounds (4, 5 and 7) shows very similar mass spectra and only some differences in relative abundance of some ions. Thus, compounds 4 and 5 have very similar mass spectra to that of 2C-B-BZP, compound 7.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the seven regioisomeric BrDMBPs in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds. Acylation lowers the basicity of nitrogen and in some cases can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum [11–14].

The mass spectra for the seven pentafluoropropionyl amides are shown in Fig. 6. The pentafluoropropionyl derivatives were all evaluated for their ability to individualize the mass spectra of each regioisomer to the exclusion of the other regioisomeric compounds. The proposed fragmentation pathways for the PFP derivatives of the seven bromodimethoxy benzyl piperazines (BrDMBPs) are shown in Fig. 7. From these spectra, a common peak with high relative abundance occurs at *m/z* 460/462, which corresponds to the molecular ions for these PFP amides. Fragment ions occurring at *m/z* 429/431 from the loss of a methoxy group from the molecular ion and the $(M\text{-}Br)^+$ ion at *m/z* 381 are seen in many of the spectra in Fig. 6. The ions at *m/z* 229/31, 199/201 and 56 seen in the mass spectra of the parent piperazine species are also present in these PFP derivatives [24]. Fragment ions at *m/z* 313/315 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety $(M\text{-}C_2F_5CO)^+$ from the corresponding derivative. A similar pathway yields ions at $(M\text{-}229)^+$ from loss of the bromodimethoxybenzyl fragment from the other piperazine nitrogen. Those ions occurring at *m/z* 119 are formed as a result of the formation of pentafluoroethyl cations from the PFP amides. The characteristic high relative abundance ion at *m/z* 214/216 specific for the perfluoroacylated brominated 2,3-DMBPs continue to be characteristic for this ring substitution pattern. Furthermore, the *m/z* 245 iminium cation fragment is present in a subset of these compounds and is a peak of significant relative abundance for compounds 1, 2, 6 and 7. While not apparently correlated with a specific ring substitution feature, this observation does allow

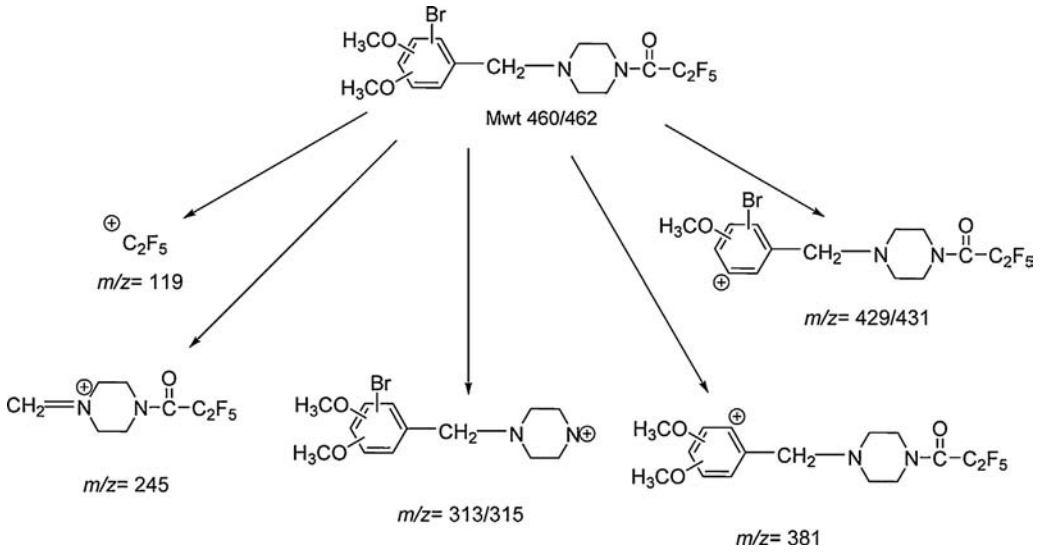


Fig. 7. EI mass spectral fragmentation pattern of the PFP derivatives of the bromodimethoxy benzyl piperazines.

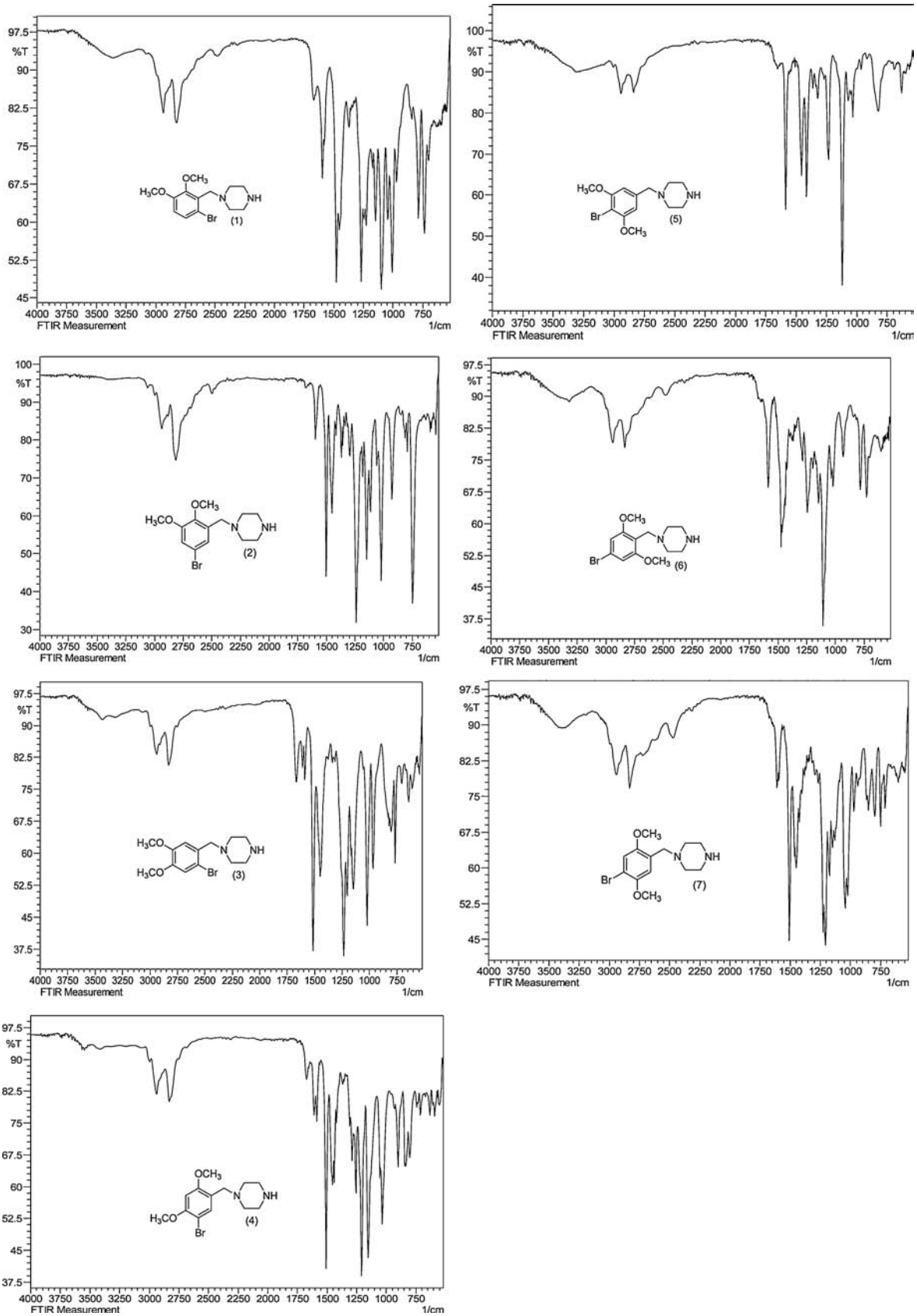


Fig. 8. ATR FTIR spectra of the seven bromodimethoxy benzyl piperazines.

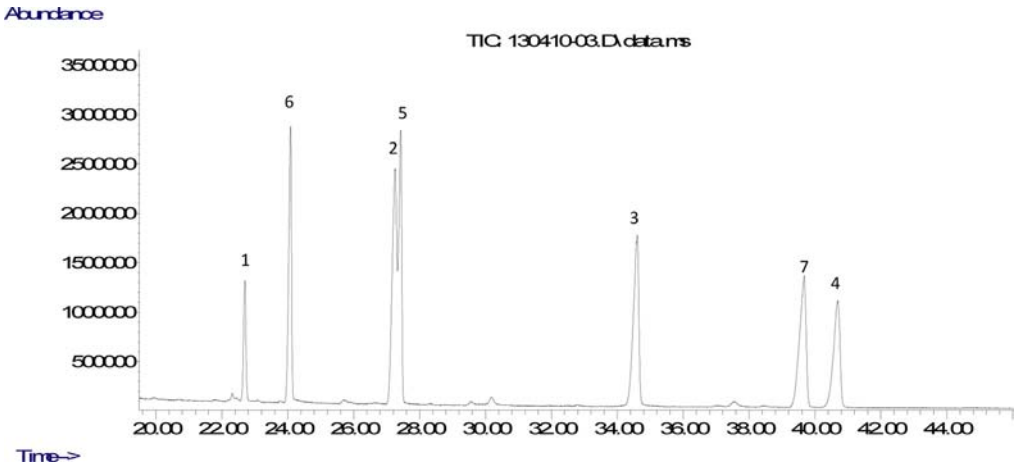


Fig. 9. Gas chromatographic separation of the pentafluoropropionyl derivatives using Rtx-200 column. The numbers over the peaks correspond to the compound numbers in Fig. 1.

differentiation between 2C-B-BZP and compounds 4 and 5. This set of three compounds showed the most similar mass spectra in the underivatized spectra. Thus, a combination of the underivatized and acylated spectra of the seven piperazines does provide some direction for discrimination among the seven regioisomers.

3.2. Infrared spectroscopy

Attenuated total reflection fourier transform infrared spectroscopy (IR) was evaluated for differentiation among the seven regioisomeric BrDMBPs. This method has the possibility of yielding compound specificity without the need for chemical modification of the drug molecule. The IRs for the seven underivatized piperazines are shown in Fig. 8. Each compound shows an 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} [27]. Because the seven piperazines share the same side chain, they share almost the same IR features in the region 2700–3100 cm^{-1}. However, they can be easily differentiated by the positions and intensities of several absorption bands in the region of 750–1750 cm^{-1}.

Compound 1 is characterized by the strong intensity band at 1471 cm^{-1} which is split into peaks of equal intensity at 1593 and 1456 cm^{-1} in compound 2. This isomer also has another medium intensity doublet at 1286 and 1273 cm^{-1} shifted to a singlet at 1249 cm^{-1} in the IR spectrum of compound 2. Finally, the IR spectrum of compound 1 shows a strong band at 1128 cm^{-1} which is shifted to 1028 cm^{-1} in compound 3 and to 1035 cm^{-1} in compound 4. Compound 7 can be distinguished by the relatively strong IR band at 1519 cm^{-1} which is shifted to a strong intensity peak at 1486 cm^{-1} in compound 6, a strong doublet at 1506 and 1471 cm^{-1} in compound 5. The IR spectrum of compound 5 can be distinguished by a singlet of strong intensity appearing at 1127 cm^{-1} compared to a peak of strong intensity at 1035 cm^{-1} in compound 4, a strong singlet at 1185 cm^{-1} in compound 6 and a band of medium intensity at 1190 cm^{-1} in compound 7.

This study shows that infrared spectra provide useful data for differentiation among these regioisomeric piperazines of mass spectral equivalence. Mass spectrometry establishes these compounds as having an isomeric relationship of equal molecular weight and equivalent major fragment ions. Infrared absorption bands provide distinguishing and characteristic information for the regioisomers in this set of uniquely similar compounds. Thus, FTIR readily discriminates between the members of this limited set of regioisomeric bromodimethoxy benzyl piperazine compounds.

3.3. Gas chromatographic separation

Gas chromatographic separation of the derivatized piperazines was accomplished on an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase using a capillary column (30 m × 0.25 mm, 0.5 μm film thickness). Several temperature programs were evaluated, and the best compromise between resolution and analysis time was used to generate the final chromatogram in Fig. 9. This chromatogram shows the separation of the PFP derivatives of the seven bromodimethoxy benzyl piperazines. This separation required an analysis time of over forty minutes. The elution order of the seven bromopiperazines is related to the degree of substituent crowding on the aromatic ring. Compound 1 elutes first and this regioisomer has the most crowded ring with the four substituents arranged in a 1, 2, 3, 4 pattern on the ring. Three isomers (compounds 2, 5 and 6) have three groups substituted 1, 2, 3 with one isolated substituent. Both compounds 2 and 6 have bromine as the isolated substituent and elute after compound 1. Compound 5 follows compounds 1, 6 and 2 in the elution order and has the piperazine-containing side chain as the isolated substituent. The 1, 2, 4, 5-substituted pattern in compounds 3, 4 and 7 provides minimum intramolecular crowding and elute last in this group of compounds. In this final group, compound 3 elutes earlier than the other two compounds (4 and 7) and these latest eluents have in common the bromine ortho to a methoxy group on the aromatic ring. Compound 4 shows chromatographic elution properties most similar to the known street drug of abuse; compound 7. Under some experimental conditions these compounds could display very similar retention (i.e. coelution). The mass spectra for the PFP forms of these two compounds show some ions which could be used for differentiation. Compound 7 shows a higher relative abundance for $(M\text{-}31)^+$ loss of a methoxy group as well as a much more significant ion at $m/z = 245$ for the immonium cation. These ions are not observed in appreciable abundance for compound 4 as well as the next closest eluting isomer; compound 3. Additionally, the infrared spectra provide a number of significant absorption bands to distinguish among these more closely eluting isomers.

4. Conclusion

The seven regioisomeric bromodimethoxy benzyl piperazines yield the same fragment ions in their mass spectra even after perfluoroacylation with only the two 2,3-dimethoxy isomers showing one unique major fragment ion at *m*/*z* 214/216. Perfluoroacylation of the secondary amine nitrogen for each of the seven regioisomers gave mass spectra showing some differences in the relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation of structure. FTIR analysis yields unique and characteristic infrared spectra for these regioisomeric piperazines. These spectra allow discrimination among the seven regioisomeric compounds included in this study. This differentiation was accomplished without the need for chemical derivatization. Mixtures of the seven piperazines were successfully resolved via capillary gas chromatography using the relatively polar stationary phase (Rtx-200) composed of 100% trifluoropropyl methyl polysiloxane.

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