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Analytical and Synthetic Studies on Designer Drugs of the Piperazine Class

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Analytical and Synthetic Studies on Designer Drugs of the Piperazine Class

Abstract:

This project has addressed issues of resolution and discriminatory capabilities in controlled substance analysis with the goal of providing additional reliability and selectivity for forensic evidence and analytical data on analytes of the piperazine class. A number of piperazine-containing compounds have appeared on the illicit drug market in recent years including N-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (3-TFMPP), 1-(3-chlorophenyl)piperazine (mCPP), 1-(3,4-methylenedioxybenzyl)piperazine (3,4- MDBP) and 1-(4-bromo-2,5-dimethoxybenzyl)-piperazine (BrDMBP). While some of these piperazines are still commercially available others are designer analogues that can be synthesized in clandestine labs.

Exploration and designer development in the piperazine drugs using models based on substituted amphetamines and related phenethylamines is likely to continue for many years. Current clandestine recipes/procedures used for amphetamine-type molecules can be applied directly for piperazine synthesis. Thus, clandestine labs will not need to learn any new synthetic techniques. Restricting the availability of piperazine would require placing dozens of substances from commercial sources around the globe under federal control. Therefore, legal control of the key precursor substance, piperazine, will not prevent the further clandestine/designer exploration of this group of compounds. The forensic chemist must identify the compound in order to know if it is an analogue of a controlled substance.

The overall goal of this project was a comprehensive analytical study of those benzylpiperazines, phenylpiperazines, benzoylpiperazines and designer phenethylpiperazines of significance in forensic drug chemistry. The initial phase of this work was the organic synthesis of regioisomeric piperazines and more than 100 substituted piperazines of potential forensic interest were evaluated. Chemical characterization has included tools common to forensic science labs such as MS and IR. The chromatographic separation for each series of isomers has been accomplished and structure-retention relationship in the regioisomers and isobaric piperazines has been evaluated.

The general structural categories evaluated in this study are shown in Figure 1. The availability of all the necessary compounds to establish and prove the structure-retention, structure-fragmentation and other structure-property analytical experiments is the first step in this research. The project has completed the following: **1)** Synthesis of aromatic ring substituted benzylpiperazines (Series I in Figure 1) focusing on those aromatic ring substituents (Aromatic Groups A and B) commonly found in ring substituted phenethylamines drugs of abuse and the most significant substituents of isobaric equivalence (such as methoxy, dimethoxy, methylenedioxy, methoxymethyl, ethoxy, trifluoromethyl, methyl, chloro, bromo, fluoro, and bromodimethoxy). **2)** Synthesis of aromatic ring substituted phenylpiperazines (Series II, Figure 1) following the same general protocol. **3)** Synthesis of substituted benzoyl-piperazines (Series III, Figure 1) and focusing on aromatic ring substituents of designer interest (Aromatic Group B and C in Figure 1). **4)** Synthesis of selected phenethyl-piperazines (Series IV, Figure 1). **5)** GC-MS evaluation of the regioisomeric and isobaric piperazines. **6)** Evaluation of GC methods for the separation of all isomers producing equivalent mass spectra.

7) Evaluation of chemical derivatives of these isomers for differential mass spectral properties.

8) Evaluation of GC-MS and IR data for specific differentiation of all isomers producing equivalent mass spectra.

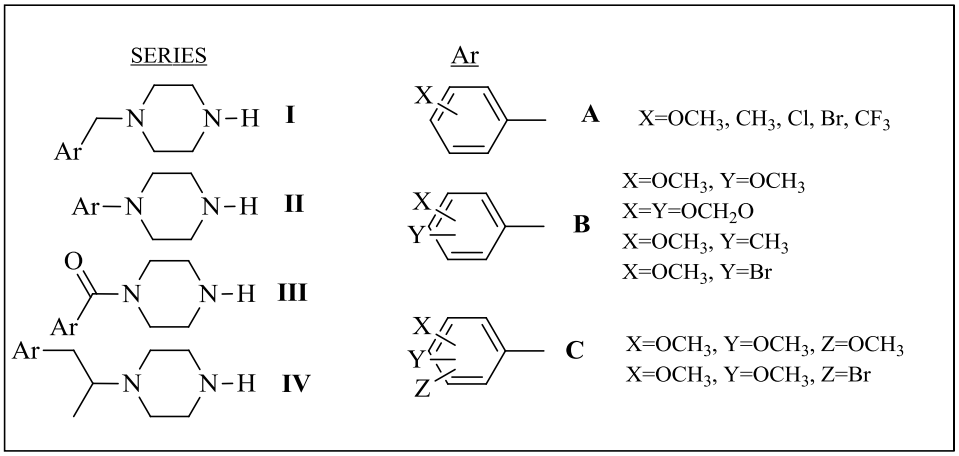


Figure 1. General structures for the piperazines in this project.

Additionally, the commercial availability of N-methylpiperazine has allowed us to investigate some tertiary amine piperazines from Series I and III in which the NH is replaced by N-methyl. These substances would be logical designer extensions of known piperazine related compounds. A number of isotope labeling studies were done which required the synthesis of deuterium and carbon-13 labeled substances. These studies included labeling the aromatic side chain groups as well as the piperazine ring. This report details the results of those studies.

Summary:

The purpose of this project was to develop regioisomer specific analytical methods for the identification of ring substituted benzyl-, phenyl-, benzoyl- and designer phenethyl-piperazines. This was accomplished by the chemical synthesis of all regioisomeric forms of selected aromatic ring substituted piperizines; generation of MS and IR analytical profiles; chromatographic studies on all regioisomeric piperazines having overlapping analytical profiles; design and validation of confirmation level methods to identify each compound to the exclusion of other regioisomeric forms. A number of piperazine-containing compounds have appeared on the illicit drug market in recent years and these compounds may represent a new class of designer drugs.

Piperazine and piperazine derivatives are synthetic substances and do not occur naturally. Benzylpiperazine (BZP) has been investigated previously as a potential antidepressant drug however it was never marketed due to its' amphetamine-like stimulant side effects. A mixture of BZP and other piperazine derivatives have been described as yielding pharmacological effects similar to that of MDMA although these effects require higher doses than MDMA. BZP appears to exert effects primarily on the dopamine receptor system and serves to potentiate the effects of other piperazines such as TFMPP acting primarily on the serotonergic system. Thus, the combination dosages of BZP and TFMPP yield behavioral effects similar to MDMA.

The initial phase of this work involved the organic synthesis of over 100 isomeric piperazine derivatives and related compounds. We prepared compounds from four structural categories which included phenylpiperazines, benzylpiperazines, benzoylpiperazines and phenethyl-piperazines. All these secondary amines were also studied as the various perfluoroacyl derivatives (TFA, PFPA and/or HFBA). In addition to the secondary amine piperazines, several series of 4-N-methyl piperazines of the benzyl and benzoyl categories were synthesized from the commercially available precursor substance, N-methylpiperazine. Piperazine is also a commercially available substrate as are some of the more likely ring substituted benzene substrates (benzaldehydes, benzoic acids and anilines). We focused on those aromatic ring substituents which have already been used as designer modifications in other series of drugs of abuse such as the substituted amphetamine/phenethylamine, cathinone (bath salt), N-BOMe, cannabinoids and perhaps others. When all the synthetic precursor substances are commercially available, the preparation involves one synthetic step. This is the case for numerous examples in the benzyl and benzoyl series of compounds in this project. We evaluated the phenylpiperazines and the benzylpiperazines since some examples of these series have already appeared in clandestine samples and designer modifications are likely to continue. We examined the benzoylpiperazine series as logical next generation designer modifications based on the structural similarity to the benzylpiperazine series as well as similarity with the bk-type substances related to the cathinone/bath salt materials. The phenethylpiperazines are also potential extensions based on the popularity of amphetamine related substances as well as the commercially availability of piperazine as a substitute amine in some of the more common synthetic pathways.

The detailed portion of this report is divided into four major sections for each of the four structural categories in this project: phenylpiperazines, benzylpiperazines, benzoylpiperazines and phenethylpiperazines. Each of the four sections then contains individual chapters based on the aromatic ring substituents for each structural category. For example, in the phenylpiperazine section the reader will find a chapter on the dimethoxyphenylpiperazines, etc. Each chapter will give the analytical details and include the collected spectra for this group of compounds. The spectra include EI MS of the parent piperazine as well as derivatives, IR and chromatographic separation of each set of regioisomers. A discussion of the details of the analytical properties can be found in each chapter and includes an evaluation of the EI MS fragmentation processes, comparison of the IR spectra and a chromatographic separation of the regioisomers in the series. In many of the chapters, unique experiments are described that were carried out in order to validate MS fragmentation processes and other structure-property relationships.

In addition to the four structural categories we proposed in the application for this project, we indicated a fifth category for validating observed structure-analytical property relationships. In this category a number of stable isotope labelled compounds were prepared to add support to the proposed MS fragmentation products and mechanisms. This work included the synthesis of numerous deuterium labelled piperazine derivatives and a few examples of carbon-13 labelled isomers. The studies are often conducted in concert with GC-TOF-MS analysis for exact mass measurements on the EI fragments to confirm the structural assignments and mechanisms.

The benzylpiperazine series includes a number of monosubstituted aromatic ring products; the three regioisomeric methyl-, fluoro-, chloro-, bromo-, trifluoromethyl-, and methoxy-substituted piperazines. For the disubstituted aromatic ring derivatives the six dimethoxy and the two methylenedioxypiperazines were evaluated. We prepared all seven of the trisubstituted bromo-dimethoxybenzylpiperazines. One of these isomers, 4-bromo-2,5-dimethoxybenzylpiperazine has already appeared in a clandestine street drug sample. However, the other regioisomeric derivatives have not been reported or evaluated as yet. In this project all seven possible isomers were prepared in order to evaluate analytical specificity for this series. The 4-bromo-2,5-dimethoxybenzyl-piperazine has been called 2C-B-BZP in some forensic drug data bases and drug websites. The 2C-B-BZP drug has been reported as a component of street drug samples in Europe. In addition to the above isomers we have prepared the 4-N-methyl derivatives of the monomethoxy and dimethoxybenzylpiperazines and a few other example compounds.

The EI mass spectra were collected for each of the benzylpiperazines prepared in this project and they are presented in the specific chapters in this report. The major mass spectral fragments are identified and their mechanisms of formation described. These fragmentation studies make use of deuterium and carbon-13 labeling experiments as well as exact mass fragment analysis and some product ion spectra. The fragmentation process generally yields ions for the substituted benzyl portion of the structure as well as ions common for the piperazine ring at m/z 85 and m/z 56. The mass spectra are similar for each of the substituted aromatic ring regioisomeric series. Once the compound is

identified by mass spectrometry as a member of a specific regioisomeric series, infrared studies allow us to differentiate among the individual isomers to provide a specific identification. In some cases derivatization provides some additional structural information for these piperazines, however, derivatization is less helpful in the piperazines than it is in other series of compounds such as the phenethylamines. The significant intramolecular distance between the secondary amine nitrogen site of derivatization (acylation) and the regioisomeric variations in these structures diminishes the likelihood of unique fragmentation among the acylated regioisomeric derivatives. The gas chromatographic peak shapes for the acylated derivatives of each set of regioisomeric compounds were significantly better than the underivatized seconday amines. The underivatized parent secondary amine molecules showed significant GC peak tailing on a number of stationary phases.

The substituted phenylpiperazine series of isomers were prepared in a synthetic process which involves the formation of the piperazine ring as the last step in the procedure. The source for the substituted phenyl groups in these compounds as well as the N-1 nitrogen of the piperazine ring are the substituted anilines and a number of these are commercially available. The synthetic formation of the remaining components of the piperazine occurs by dual alkylation of the primary aniline nitrogen to yield the cyclic final product. We focused our efforts in this series on those commercially available aniline isomers likely to be incorporated into the continued designer exploration of this series of compounds. We prepared the monosubstituted chloro- methyl and methoxy-phenylpiperazines and the disubstituted methylenedioxy-, dimethyl- and dimethoxy-phenylpiperazines. A number of

other regioisomeric substituted phenylpiperazines such as TFMPP have already appeared in street drug samples and have been described in the forensic drug analysis literature.

The EI mass spectra for this series of phenylpiperazine compounds are characterized by a much higher relative abundance of the molecular ions. The substituted phenyl cations are much less stable than the benzyl cations and thus do not fragment as readily. A number of unique ions characteristic of fragmentation within the piperazine ring have a high relative abundance in these spectra. Ions occurring at $(M-42)^+$ indicate the loss of C_2H_4N from the molecular ion. Exact mass analysis confirmed this species as occurring from cleavage within the piperazine ring. The mass spectra are similar for each of the substituted aromatic ring regioisomeric series. Once the compound is identified by mass spectrometry as a member of a specific regioisomeric series, infrared studies allow us to differentiate among the individual isomers to provide a specific identification. In some cases we have ATR generated FT-IR spectra and in other cases the spectra are vapor phase GC-IR generated. The acylated sets of regioisomeric equivalents were resolved by GC and the relative elution order described in some cases based on structural features.

The benzoylpiperazines can be viewed as more closely related to the carbonyl containing amines such as the cathinone or bath salts type compounds. While the amide structural feature in these benzoylpiperazines eliminates basicity for the N-1 nitrogen the N-4 nitrogen remains unmodified and allows these molecules to continue to show appropriate basicity. This series of compounds are prepared via monoacylation of piperazine with a reactive carboxylic acid equivalent such as an ester or acylhalide. In this work the

benzoylpiperazines were synthesized from piperazine and a ring substituted benzoyl chloride. Many of these benzoyl chlorides are commercially available and others can be prepared from appropriately ring substituted benzoic acids, aldehydes or benzyl alcohols. The benzoylpiperazine series includes a number of monosubstituted aromatic ring products; the three regioisomeric methyl-, trifluoromethyl-, and methoxy-substituted piperazines. For the disubstituted aromatic ring derivatives the six dimethoxy and the two methylenedioxypiperazines were evaluated. In addition we prepared a series of tertiary amines, the N-1-benzoyl-N-4-methylpiperazines, based on the commercial availability of the precursor, N-methylpiperazine. The secondary amines were also evaluated as the perfluoroacyl derivatives however the tertiary amines do not form stable acylated products.

The additional site for ionization at the carbonyl oxygen in the EI fragmentation for these compounds adds some uniqueness to the mass spectra for this series. A number of significant fragments occur as a result of bond migration and rearrangements across the piperazine ring from the N-4 nitrogen to the carbonyl oxygen of the amide group. These ions and the potential mechanisms for their formation were determined by deuterium labeling of multiple sites within the molecules. The benzoyl cation and the protonated primary amide are characteristic fragments in this series. The specific mass for these fragments depends on the nature of the aromatic ring substituents. The primary amide occurs via multiple hydrogen migrations from the piperazine ring to the amide functional group. This series of compounds also shows unique IR bands due to the carbonyl feature of the amides for these benzoylpiperazines. The acylated sets of regioisomeric

equivalents were resolved by GC and the relative elution order described in some cases based on structural features.

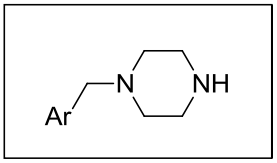
The phenethylpiperazines contain a combination of molecular features and can be viewed as derivatives of either piperazine or the more common phenethylamines. Essentially this is a series of compounds in which the amino group of a phenethylamine has been replaced by a piperazine moiety. The synthesis of compounds in this series essentially follows the various methods for amphetamine/phenethylamine compounds with piperazine or N-methylpiperazine serving as the source for the amino group. This is series IV in Figure 1 and we have prepared and evaluated a series of the general phenethylamine structural backbone and a series of the aminoketone cathinone-like compounds. The aromatic ring substituents include methyl-, fluoro-, chloro-, bromo-, trifluoromethyl-, methoxy- and methylenedioxy-groups. The necessary molecular framework for the phenethyl group was prepared based on available precursor materials prior to the introduction of the piperazine groups.

The EI mass spectra for this group of compounds is dominated by the immonium cation containing the piperazine ring with both nitrogen atoms and occurring at an odd mass since two nitrogens are a part of the structure. These immonium cations result from loss of the substituted aromatic ring species as either the benzyl or benzoyl radical depending on the structural features of the parent molecule. As is the case for most phenethylamines, the immonium cation species is by far the most abundant ion in the EI mass spectrum.

The IR spectra clearly differentiate the carbonyl containing aminoketones from the classical phenethylamines.

The overall goal of this project was a comprehensive analytical study of those benzylpiperazines, phenylpiperazines, benzoylpiperazines and designer phenethylpiperazines of significance in forensic drug chemistry. The availability of all the necessary compounds to establish and validate the structure-retention, structure-fragmentation and other structure-property analytical experiments was the first step in this research. In this work over 100 compounds belonging to these four series were synthesized and evaluated. The following individual chapters provide the analytical profiles and discuss the correlations of these structural features with the analytical results. Many of the spectra generated in this project will be available in electronic format at the website http://forendex.southernforensic.org/.

SECTION I Benzylpiperazines



The benzylpiperazine series includes a number of monosubstituted aromatic ring products; the three regioisomeric methyl-, fluoro-, chloro-, bromo-, trifluoromethyl-, and methoxy-substituted piperazines. For the disubstituted aromatic ring derivatives the six dimethoxy and the two methylenedioxypiperazines were evaluated. We prepared all seven of the trisubstituted bromo-dimethoxybenzylpiperazines. One of these isomers, 4-bromo-2,5-dimethoxybenzylpiperazine has already appeared in a clandestine street drug sample. However, the other regioisomeric derivatives have not been reported or evaluated as yet. In this project all seven possible isomers were prepared in order to evaluate analytical specificity for this series. The 4-bromo-2,5-dimethoxybenzyl-piperazine has been called 2C-B-BZP in some forensic drug data bases and drug websites. The 2C-B-BZP drug has been reported as a component of street drug samples in Europe. In addition to the above isomers we have prepared the 4-N-methyl derivatives of the monomethoxy and dimethoxybenzylpiperazines and a few other example compounds.

The EI mass spectra were collected for each of the benzylpiperazines prepared in this project and they are presented in the specific chapters in this report. The major mass spectral fragments are identified and their mechanisms of formation described. These fragmentation studies make use of deuterium and carbon-13 labeling experiments as well as exact mass fragment analysis and some product ion spectra. The fragmentation process

generally yields ions for the substituted benzyl portion of the structure as well as ions common for the piperazine ring at m/z 85 and m/z 56. The mass spectra are similar for each of the substituted aromatic ring regioisomeric series. Once the compound is identified by mass spectrometry as a member of a specific regioisomeric series, infrared studies allow us to differentiate among the individual isomers to provide a specific identification. In some cases derivatization provides some additional structural information for these piperazines, however, derivatization is less helpful in the piperazines than it is in other series of compounds such as the phenethylamines. The significant intramolecular distance between the secondary amine nitrogen site of derivatization (acylation) and the regioisomeric variations in these structures diminishes the likelihood of unique fragmentation among the acylated regioisomeric derivatives. The gas chromatographic peak shapes for the acylated derivatives of each set of regioisomeric compounds were significantly better than the underivatized seconday amines. The underivatized parent secondary aminemolecules showed significant GC peak tailing on a number of stationary phases.

Chapter 1

Differentiation of Methylenedioxybenzylpiperazines (MDBPs) By GC-IRD and GC-MS

The substituted benzylpiperazine, 3,4-methylenedioxybenzylpiperazine and its regioisomer 2,3-methylenedioxybenzylpiperazine 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-meter capillary column containing an Rxi-50 stationary phase.

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylenedioxybenzylpiperazines

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 1-1 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 methylenedioxybenzyl-piperazines 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 Figure 1-2 and are based on the work of de Boer et al [de Boer et al, 2001]. This previous work described the fragmentation of the unsubstituted benzylpiperazine [de Boer et al, 2001] and the structures for the fragment ions in the two methylenedioxybenzyl- regioisomers are 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 methylenedioxybenzyl piperazines, 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 [Peters et al, 2003]. However, acylation of the secondary nitrogen in the piperazine ring does not alter the basicity of the tertiary amine nitrogen.

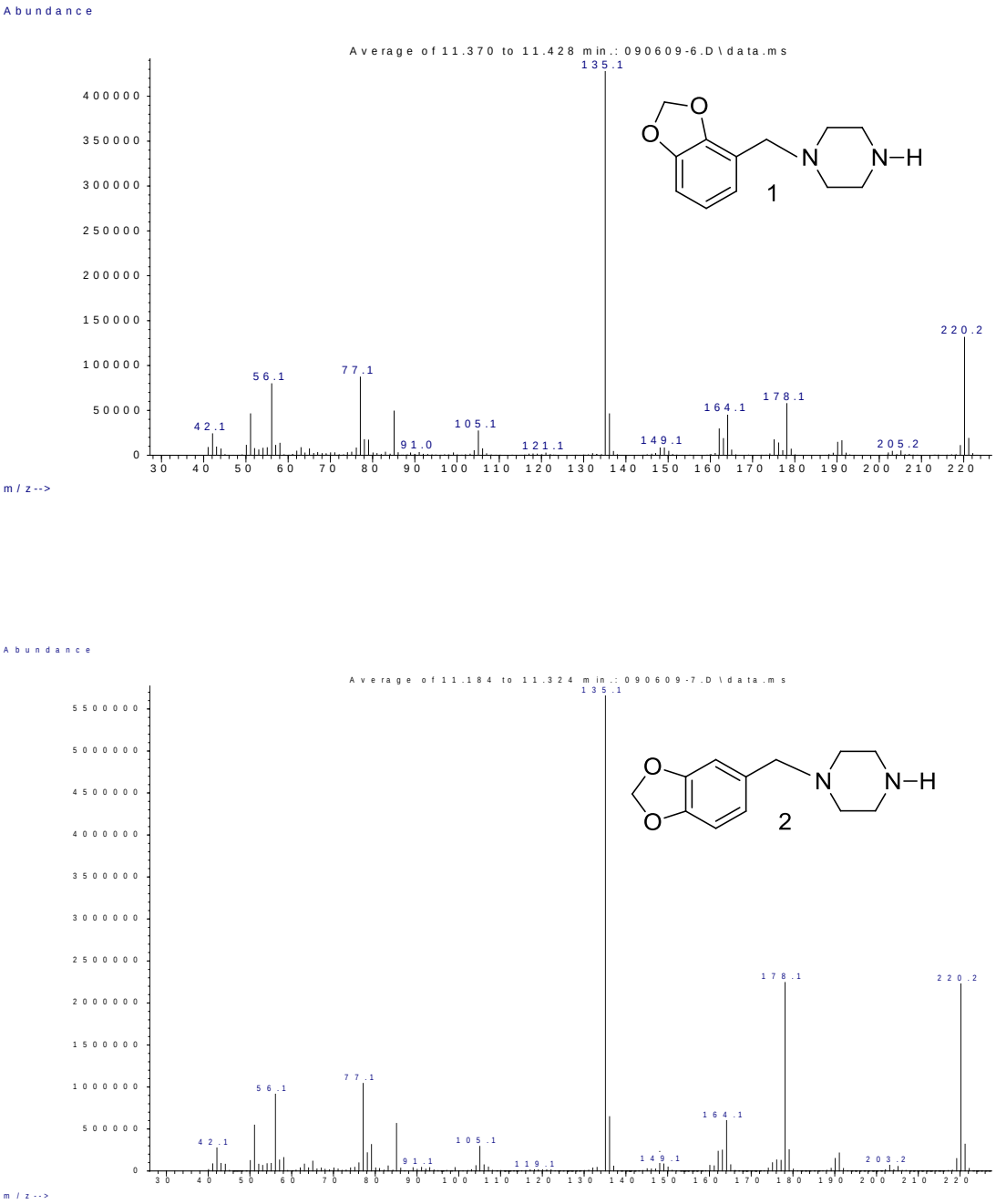


Fig. 1-1: EI Mass spectra of the two methylenedioxybenzylpiperazines.

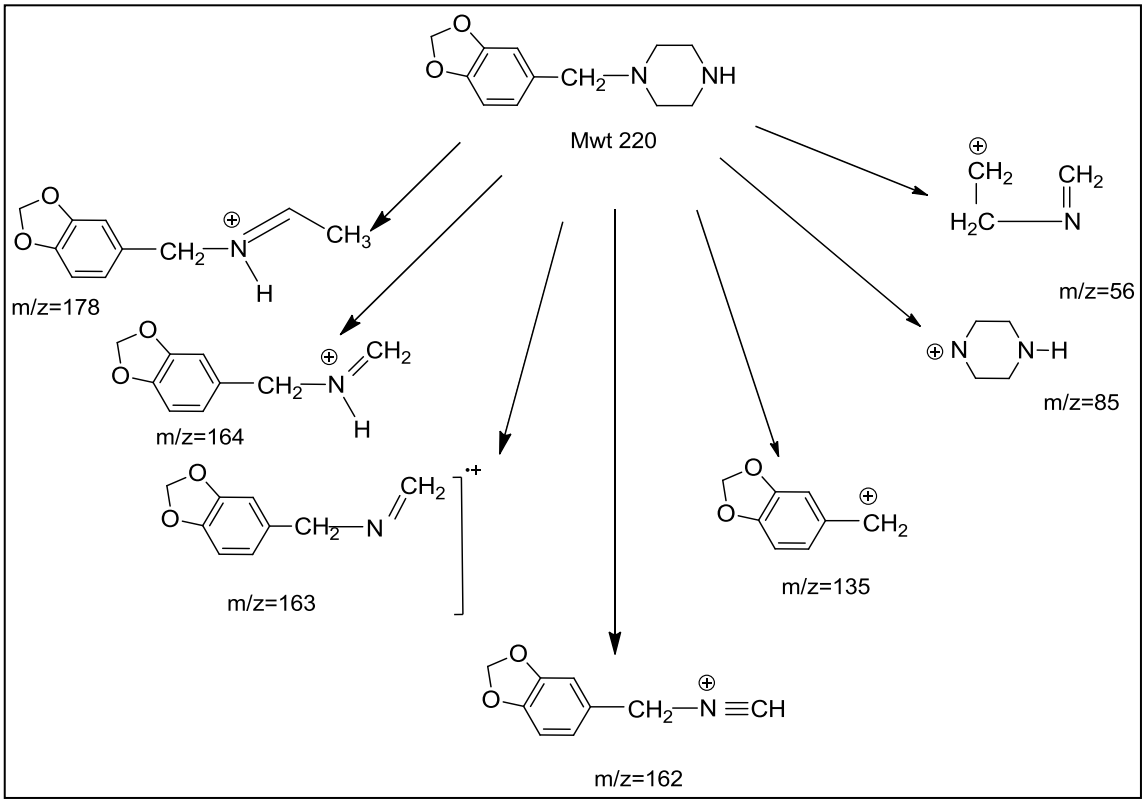


Fig. 1-2: Mass spectral fragmentation pattern of the underivatized 3,4-methylenedioxybenzylpiperazine under EI (70eV) conditions.

The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl 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 Figures 1-3, 1-4 and 1-5. 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 $(M-135)^+$ 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.

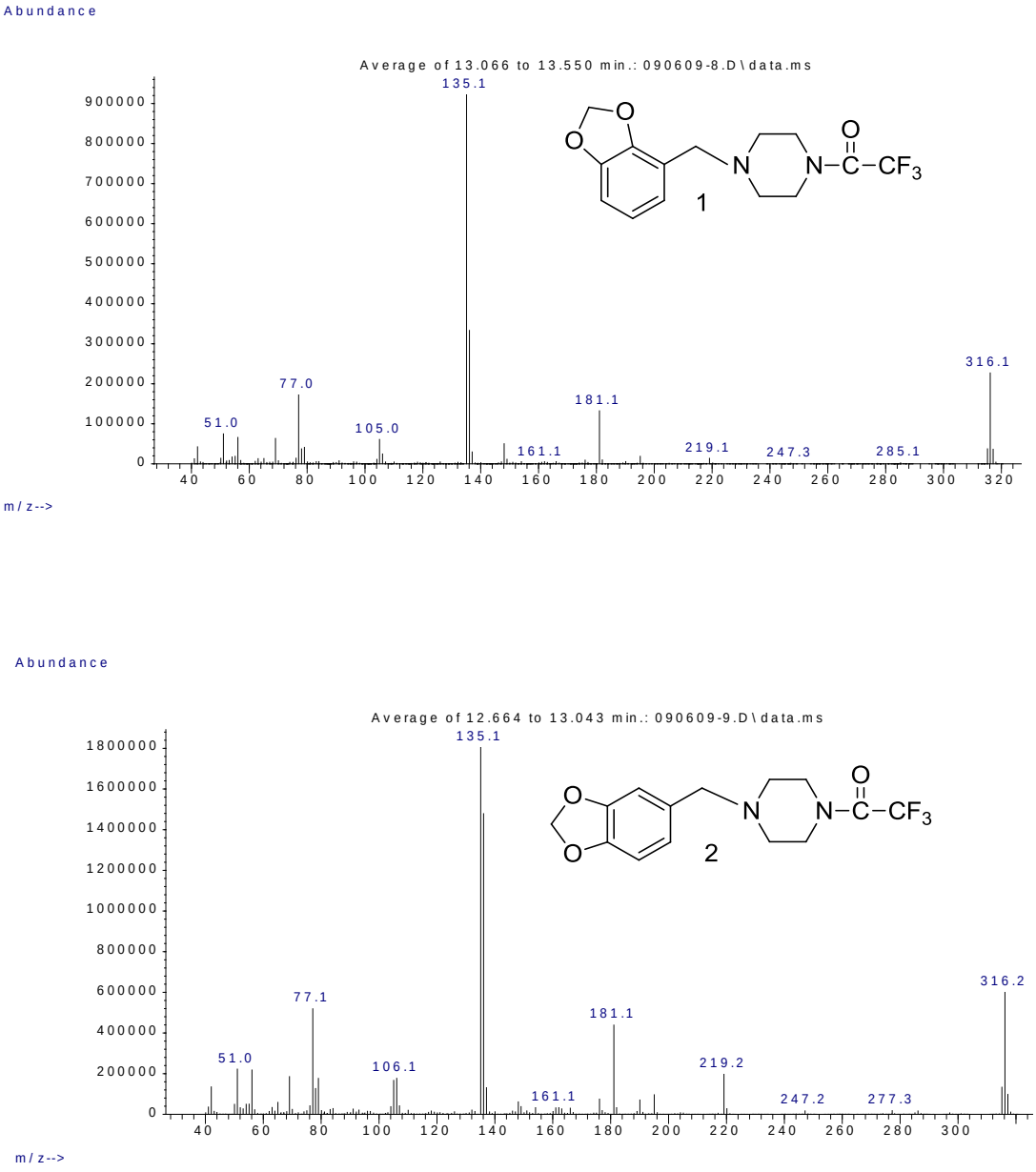


Fig. 1-3: MS spectra of trifluoroacetyl derivatives of the two piperazine compounds.

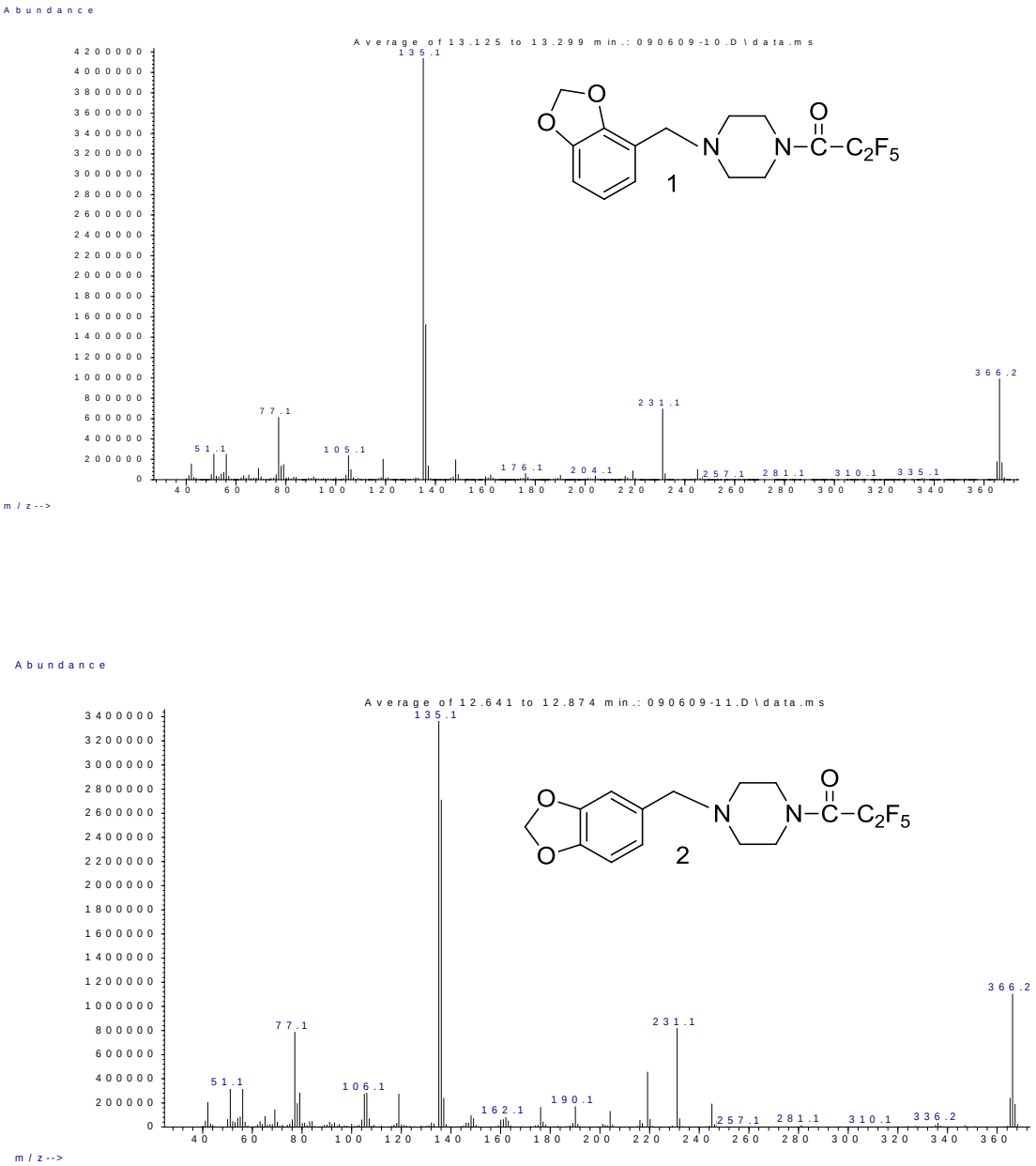


Fig. 1-4: MS spectra of pentafluoropropionyl derivatives of the two piperazine compounds.

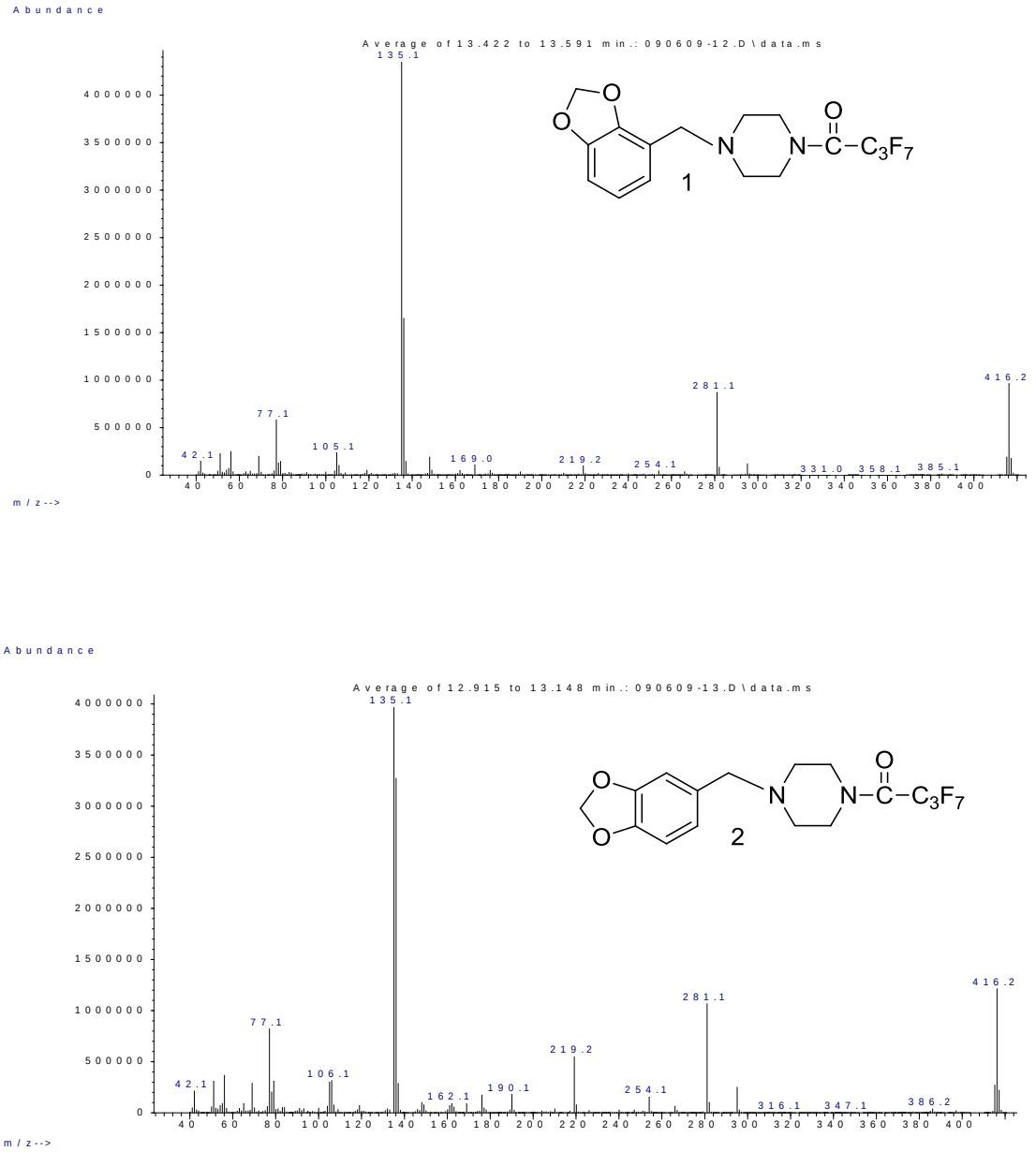


Fig. 1-5: MS spectra of heptafluorobutyryl derivatives of the two piperazine compounds.

Vapor-phase Infra-Red Spectrophotometric Studies of the Methylenedioxybenzylpiperazines

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 are shown in Fig. 1-6. 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} [Awad et al, 2009]. 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-

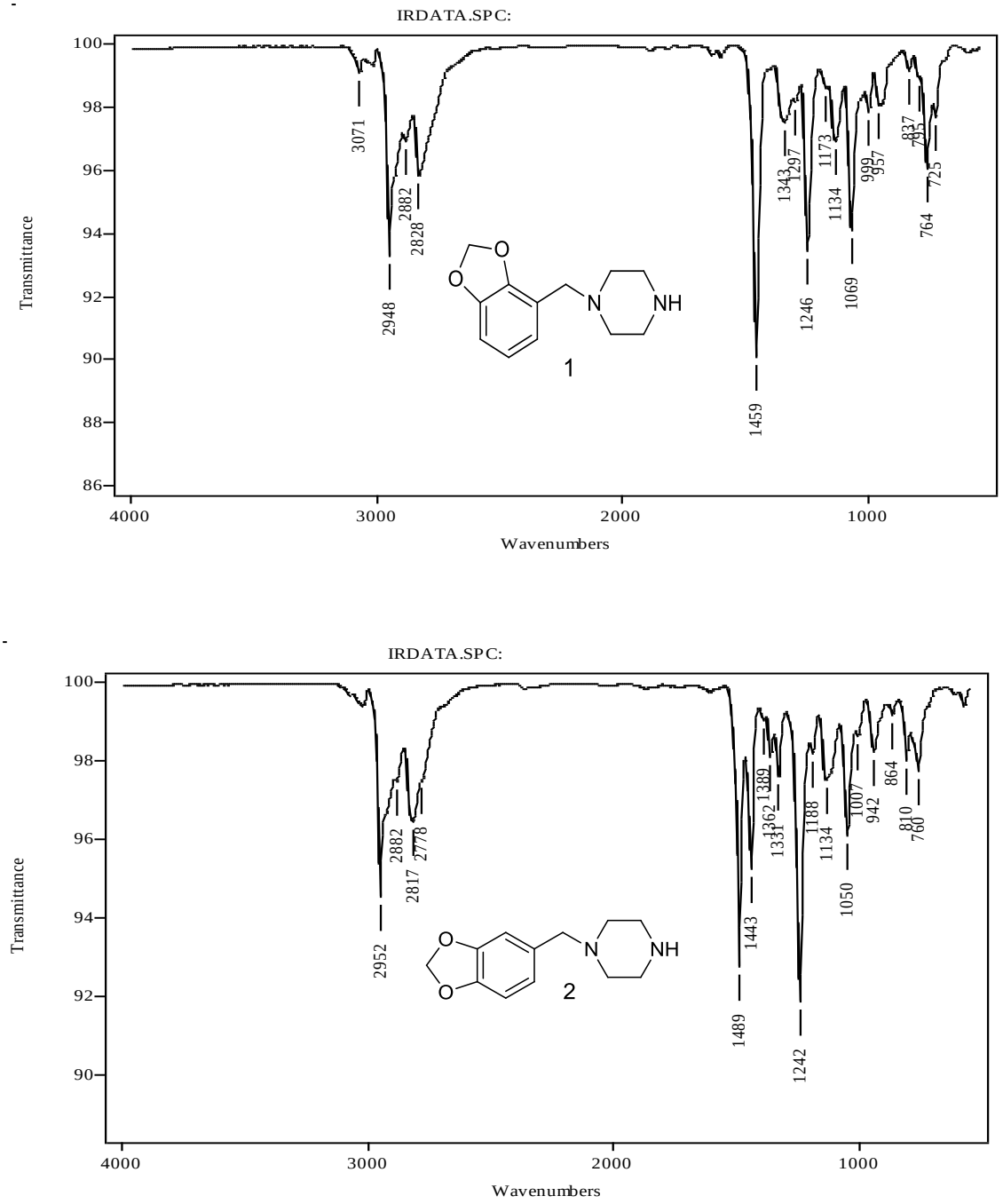


Fig. 1-6: Vapor phase IR spectra of (1); 2,3-methylenedioxybenzyl piperazine and (2); 3,4-methylenedioxybenzyl piperazine.

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- vs 3,4-MDBP) for the methylenedioxy-group in these substituted piperazine regioisomers.

Gas Chromatographic Separation of the Methylenedioxybenzylpiperazines

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 minute, ramped up to 230°C at a rate of 20°C per minute followed by a hold at 230°C for 15 minutes. 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).

The chromatograms in Figures 1-7 and 1-8 are representative of the results obtained for all samples on the two columns. The chromatograms in Figure 1-7 show the separation of the piperazines on the Rtx-200 and the Rxi-50 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 Figure 1-8 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.

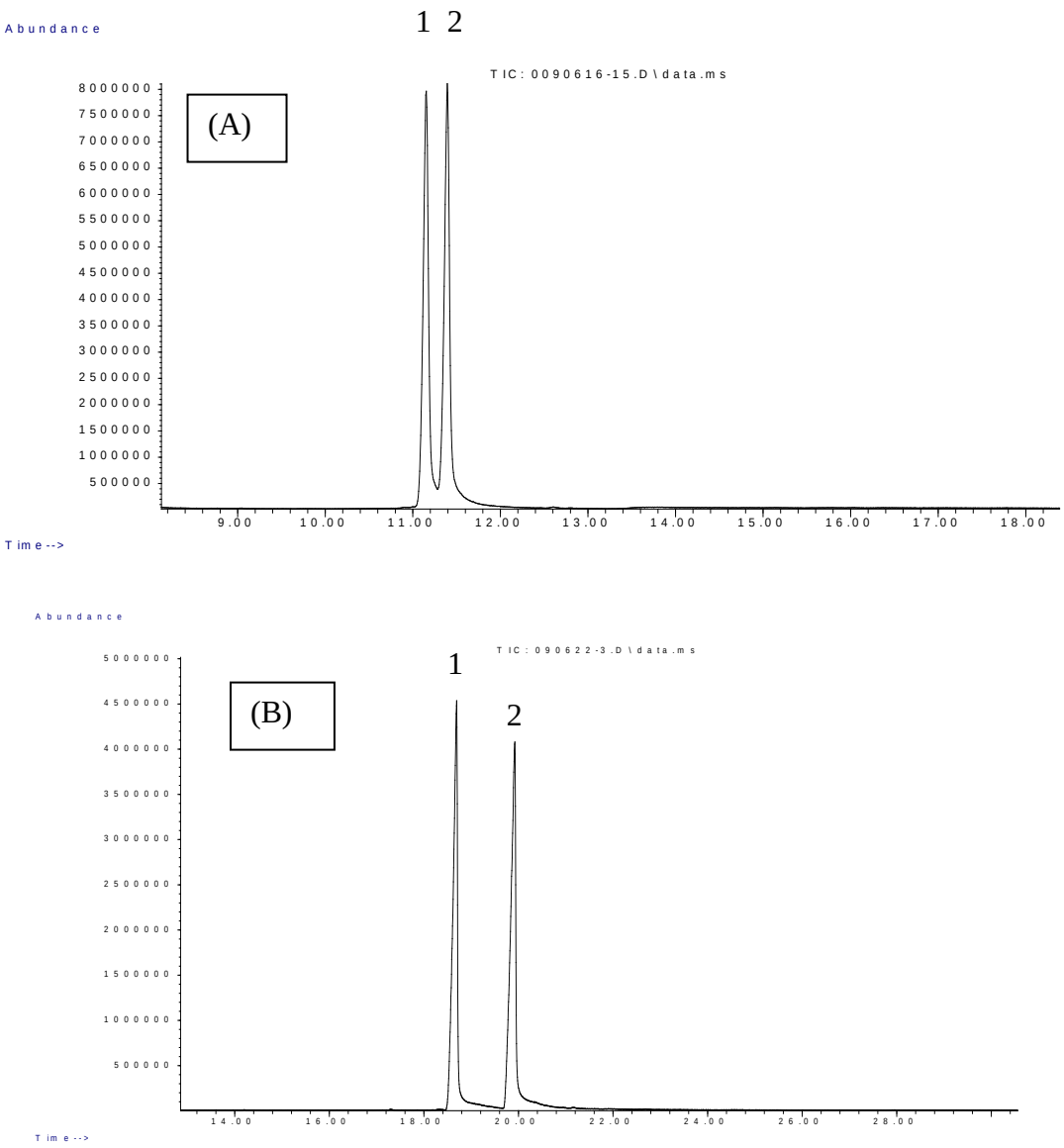


Fig. 1-7: Gas chromatographic separation of (1) 2,3-methylenedioxybenzyl piperazine and (2) 3,4-methylenedioxybenzylpiperazine. Columns: Rxi-50 (A) and Rtx-200 (B).

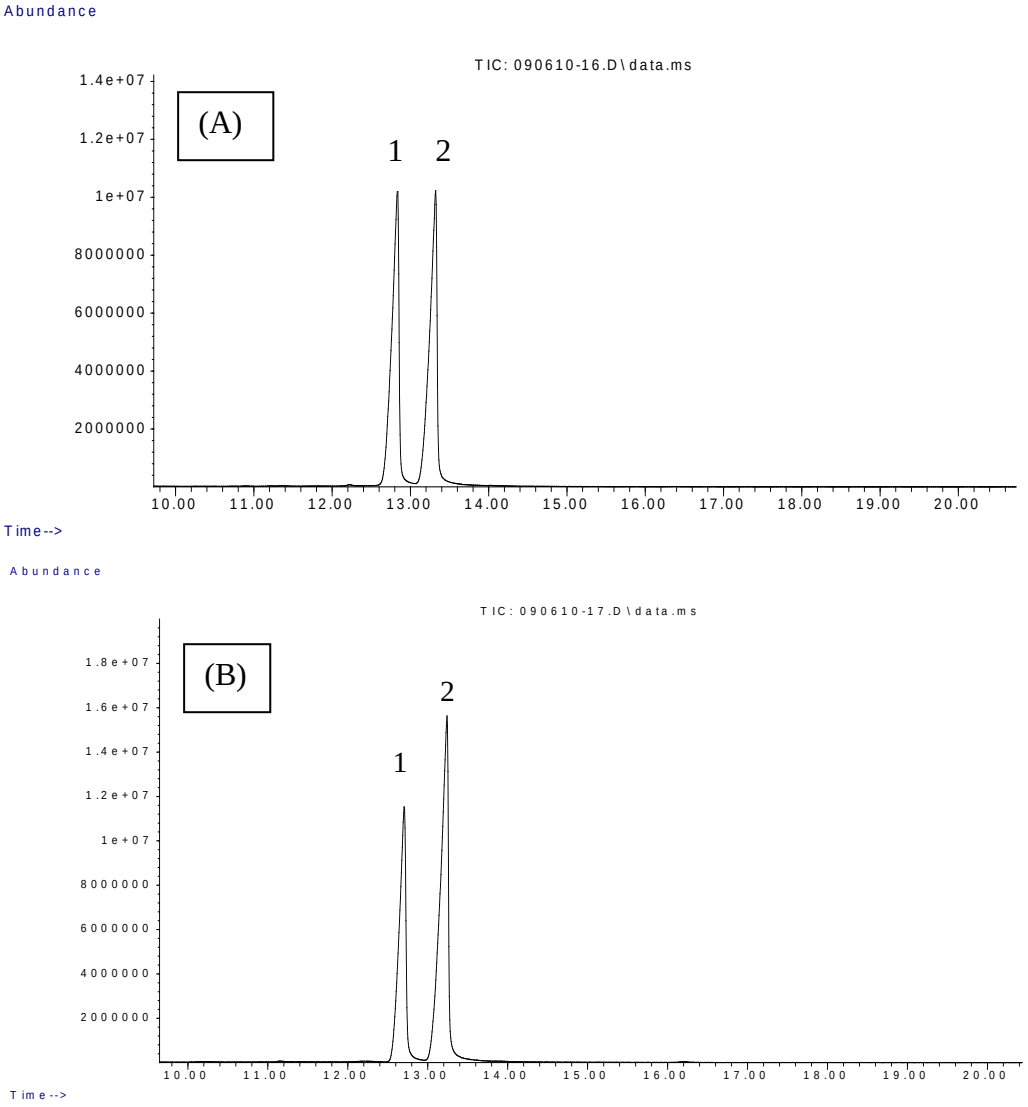


Fig. 1-8: Gas chromatographic separation of the trifluoroacetyl (A) and pentafluoropropionyl (B) derivatives using Rxi-50 column. (1) 2,3-methylenedioxybenzylpiperazine and (2) 3,4-methylenedioxybenzylpiperazine.

Conclusion

The two regioisomeric methylenedioxybenzyl piperazines 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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T. Awad, T. Belal, J. DeRuiter, K. Kramer and 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.

Chapter 2

Differentiation of Methylenedioxybenzylpiperazines (MDBPs) and Ethoxybenzylpiperazines (EBPs) by GC-IRD and GC-MS

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

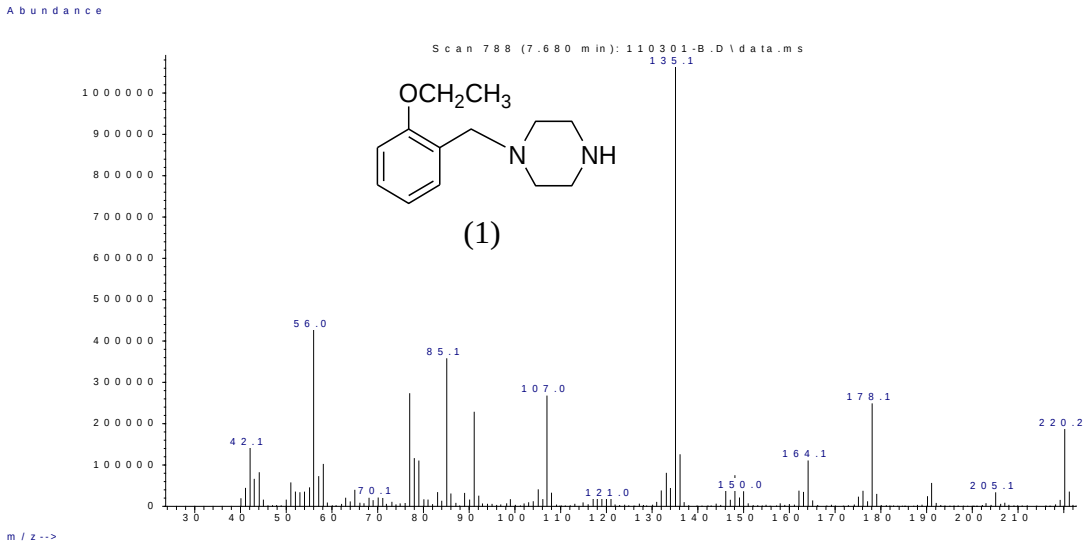
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the five isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The perfluoroacyl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

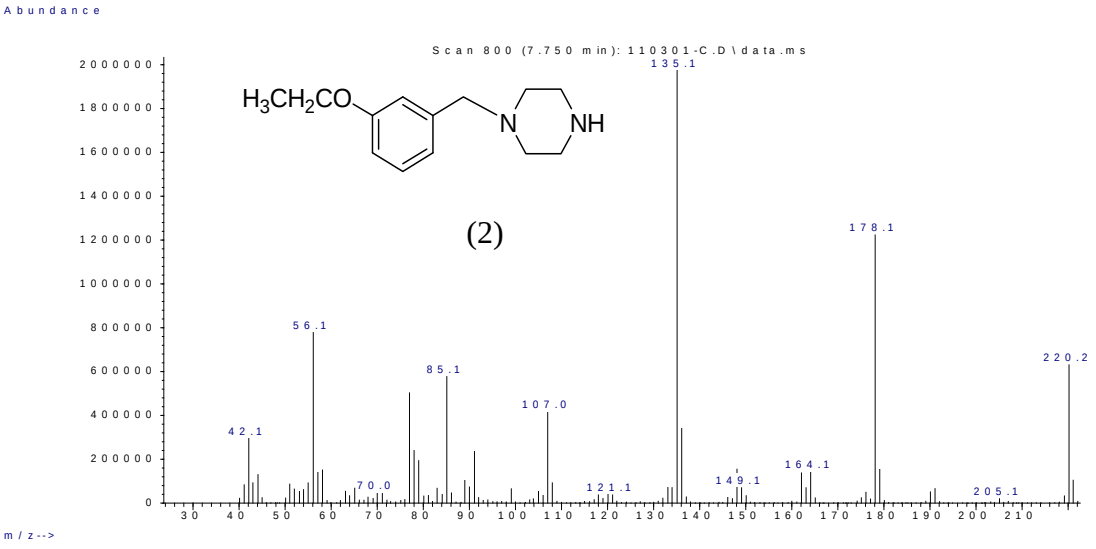
Gas chromatography coupled with time-of-flight mass spectrometric detection provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazine and 4-ethoxybenzylpiperazine which have similar nominal masses but are different in their calculated exact masses.

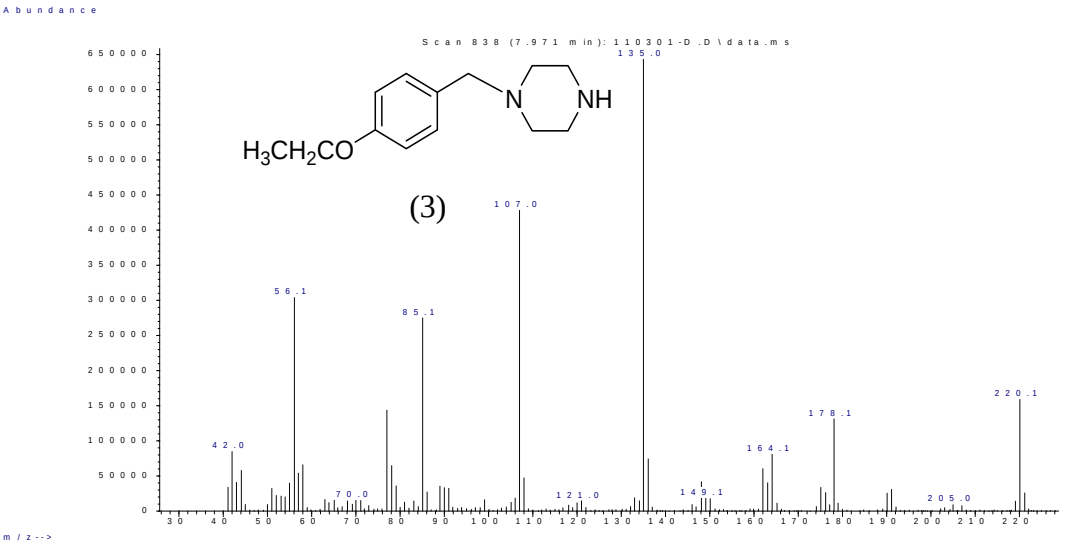
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylenedioxybenzylpiperazines (MDBPs) and Ethoxybenzylpiperazines (EBPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 2-1 shows the EI mass spectra of all five isomeric substituted benzylpiperazines (Compounds 1-5). These spectra show fragment ions at m/z 178, 164, and 135 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 2-2 and are based in part on a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. The isobaric ethoxy benzyl $(C_9H_{11}O)^+$ fragments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. However, the relative abundances for the ions in the spectra for the five isomeric benzylpiperazines are slightly different. The mass spectra for the ring substituted ethoxybenzylpiperazines (Compounds 1-3) have almost identical mass spectra compared to the methylenedioxy isobars (Compounds 4 and 5) except for the unique ion at m/z 107. This ion at m/z 107 represents the loss of 28 mass units (ethylene, C_2H_4) from the ethoxybenzyl cation at m/z 135 as presented in Figure 2-3 [Awad *et al*, 2007]. The relative abundance of this marker ion at m/z 107 is highest in the mass spectrum of the 4-ethoxy isomer likely due to the conjugation of the 1,4-ring substituents. Thus, these mass spectra provided some

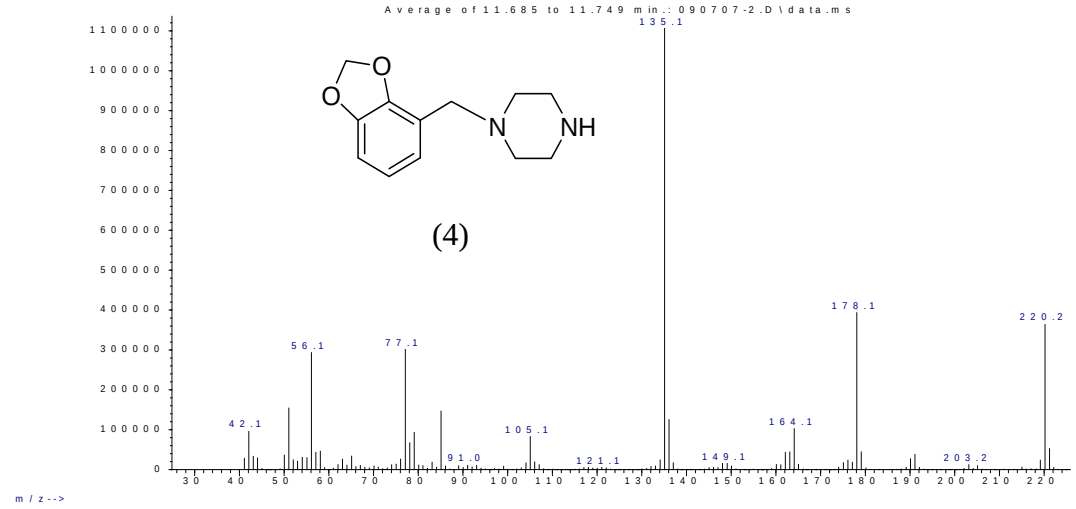
discrimination of the ethoxybenzylpiperazines from their isobaric methylenedioxy compounds.







(3)



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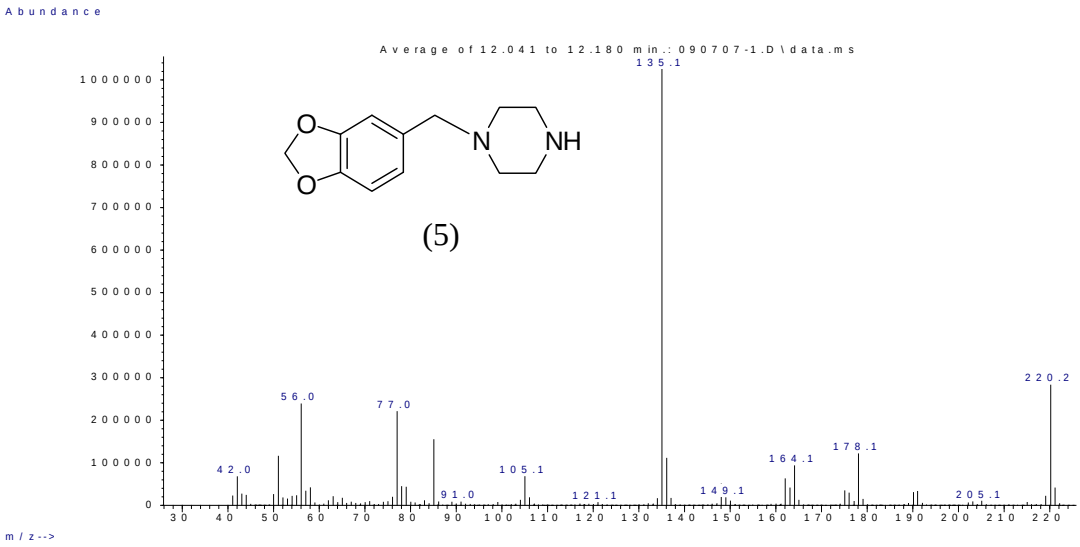
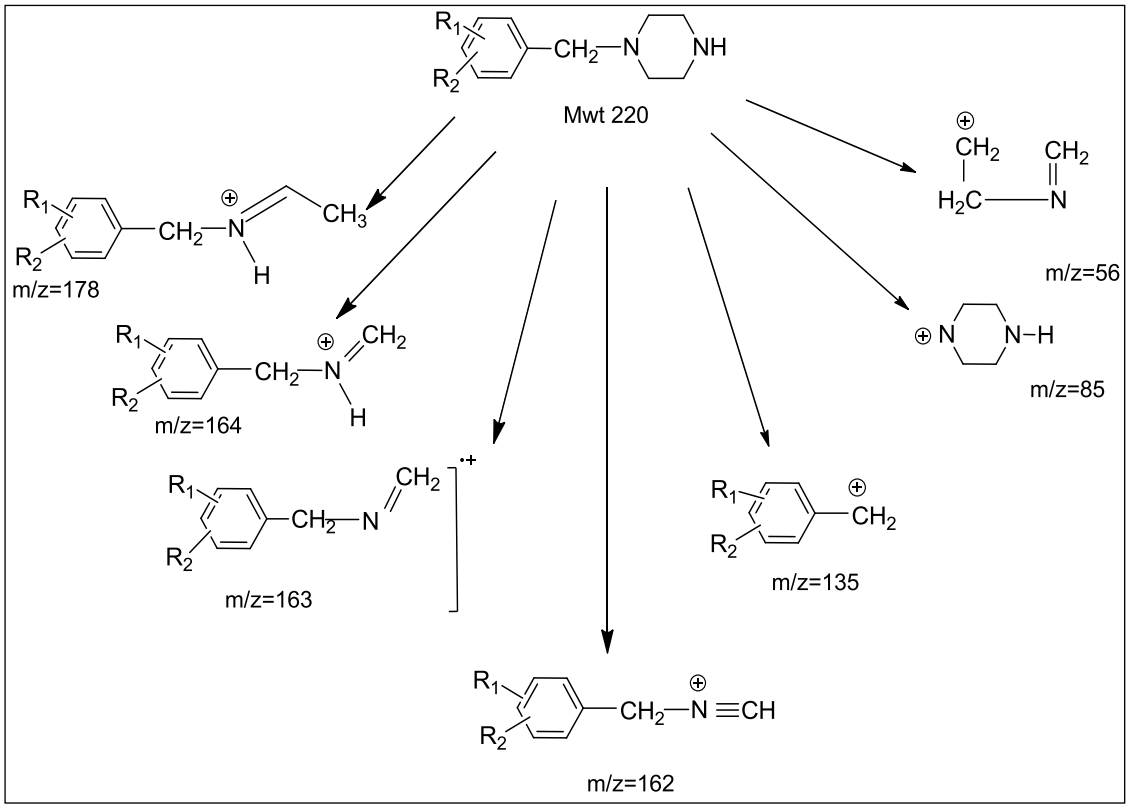


Fig. 2-1: EI mass spectra of the methylenedioxy and ethoxybenzylpiperazines in this study.



R_1 = OCH_2CH_3, R_2 = H in case of EBP

R_1, R_2 = dioxymethylene in case of MDBP

Fig. 2-2: Mass spectral fragmentation pattern of the underivatized methylenedioxy and ethoxybenzylpiperazines under EI of 70eV.

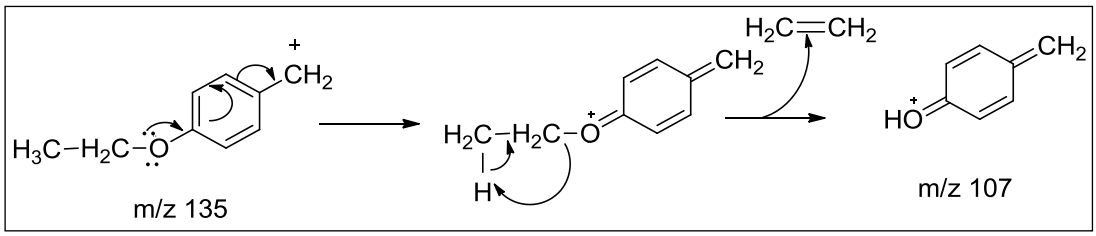
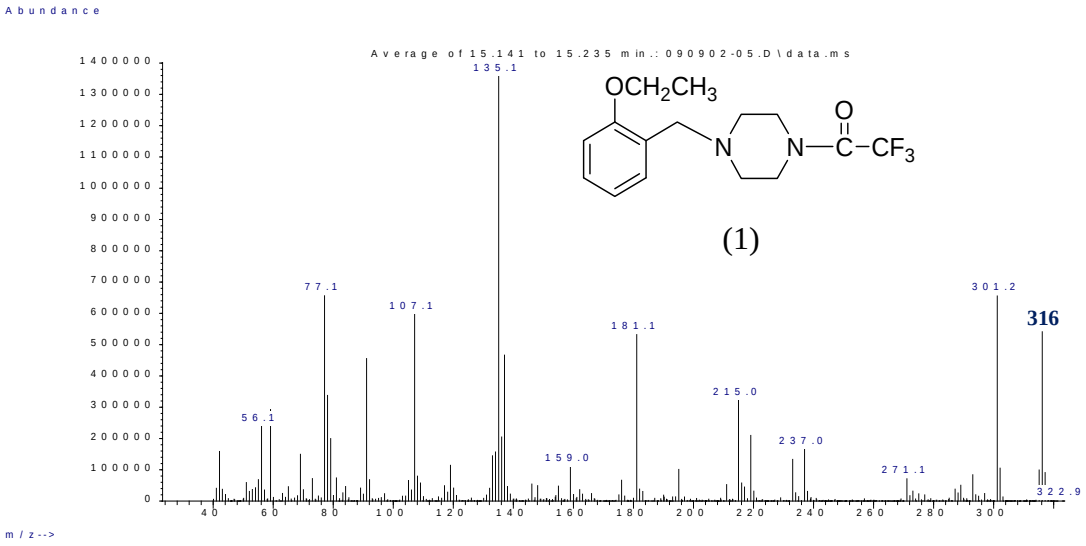


Fig. 2-3: Mass spectral fragmentation of the ethoxybenzylpiperazines yielding the fragment cation at m/z 107.

The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric benzylpiperazines, in an effort to individualize their mass spectra and identify additional unique marker ions for these five compounds.

The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary nitrogen were evaluated for their ability to individualize the mass spectra of this series of substituted benzylpiperazines. Figure 2-4 shows the mass spectra of the trifluoroacetyl amides of the five studied compounds as representative spectra for all the perfluoroacyl piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 316, 366 and 416, respectively. The major fragment ion in these spectra occurs at *m/z* 135 and corresponds to the ring substituted benzyl cation. Furthermore, an additional fragment ion series occurring at *m/z* 181, 231 and 281 for the TFA, PFPA and HFBA amides, respectively corresponds to the $(M-135)^+$ ion for each amide. The ion at *m/z* 219 was observed in the spectra of all derivatives and is likely formed by the elimination of the acyl moiety. Those ions occurring at *m/z* 69, 119 and 169 are the perfluoroalkyl cations trifluoromethyl, pentafluoroethyl or heptafluoropropyl from the appropriate amides. The mass spectra for the perfluoroamides of the ring substituted ethoxybenzylpiperazines (Compounds 1-3) continued to show the unique ion at m/z 107 with the highest relative abundance in the mass spectrum of the 4-ethoxy isomer. In addition, the fragment cations at $[M-15]^+$ appeared at m/z 301, 351 and 401 in the mass spectra of the TFA, PFPA and HFBA derivatives of the 2-ethoxy isomer, respectively. 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 study.



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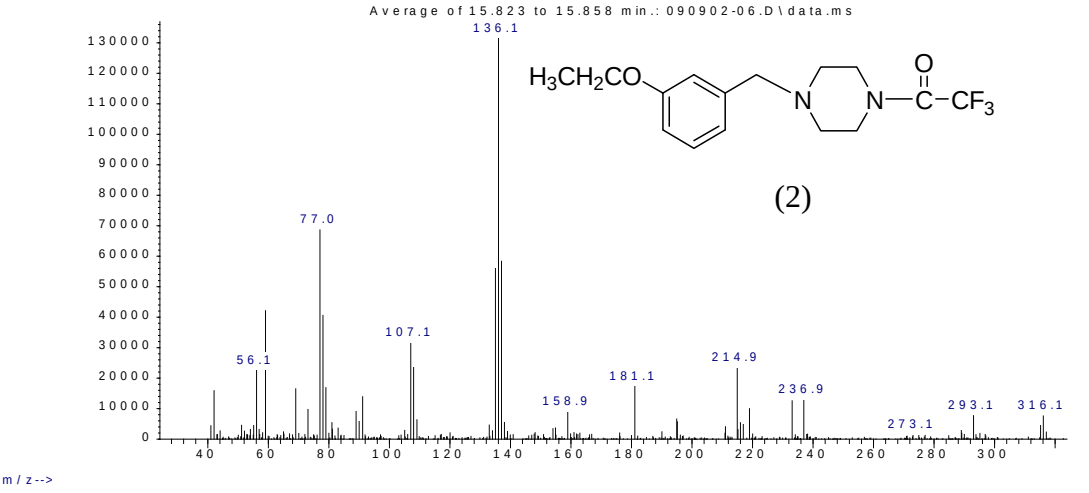

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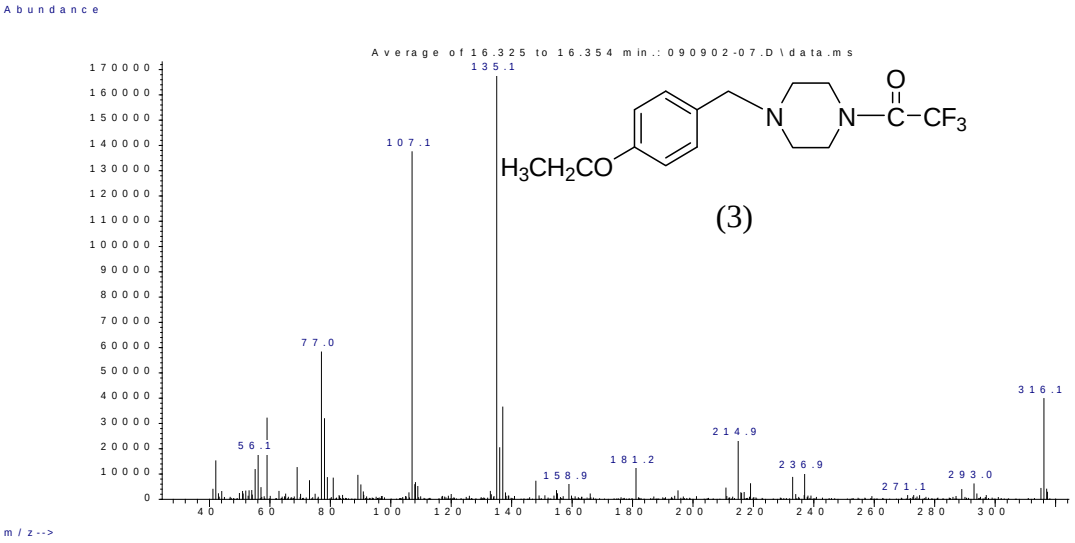

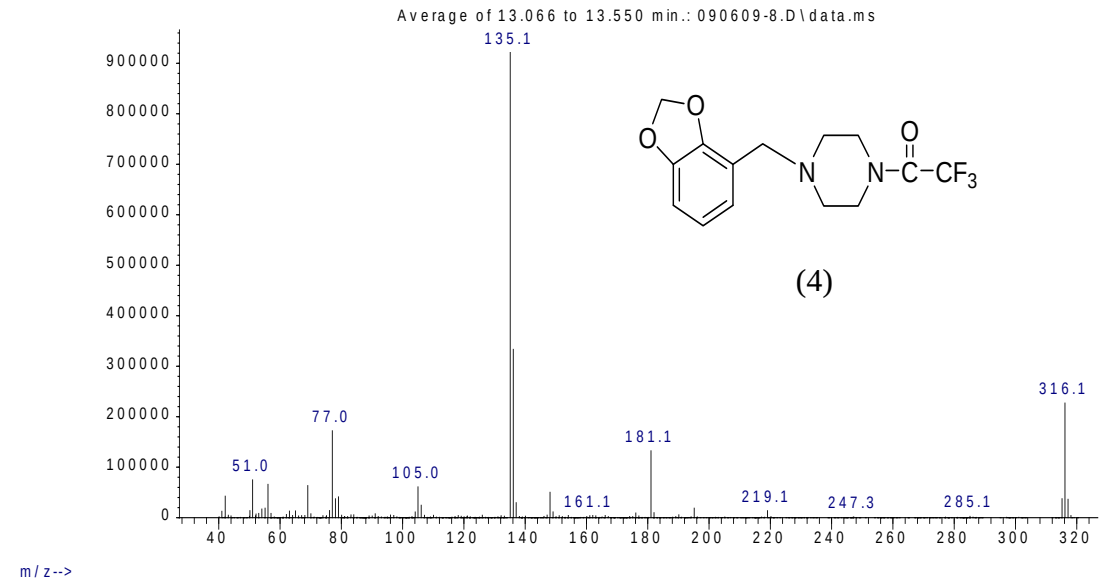

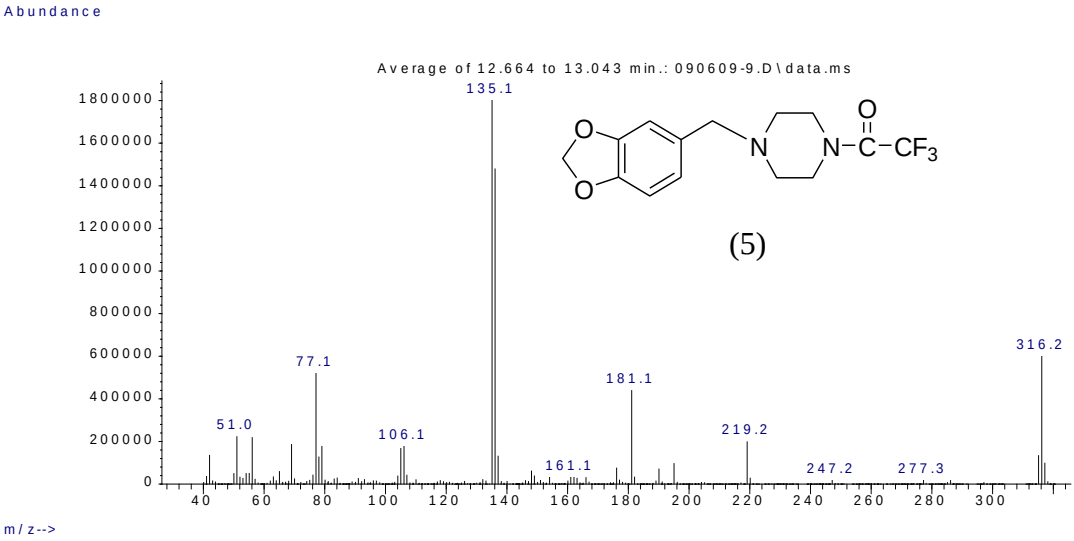

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Fig. 2-4: Mass spectra of the trifluoroacetyl derivatives of the five piperazine compounds in this study.

Gas chromatography coupled with time-of-flight mass spectrometric detection provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazine and 4-ethoxybenzylpiperazine which have similar nominal masses but are different in their calculated exact masses. The ethoxybenzyl $(C_9H_{11}O)^+$ fragments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135 but are different in their elemental composition and accordingly different in their calculated masses. Figure 2-5 shows the GC-TOF-MS exact mass analysis of the 3,4-methylenedioxybenzyl cation (m/z=135) for compound 5. The upper panel (5A) shows the expected/calculated mass for the $C_8H_7O_2$ elemental composition. The lower panel (5B) shows the experimental results and the degree of agreement (0.8 mDa, 5.9 ppm) with the calculated mass. Thus, confirming the m/z 135 ion in compound 5 as the elemental composition $C_8H_7O_2$. These results can be compared to the exact mass analysis for the m/z 135 ion (4-ethoxybenzyl) in compound 3. Figure 6A and 6B confirms the elemental composition as $C_9H_{11}O$ with a mass deviation of 0.1 mDa (0.7 ppm). Thus, exact mass measurements distinguish between these isobaric forms of the m/z 135 ion. Panels C and D in Figure 2-6 confirm the elemental composition C_7H_7O for the unique rearrangement ion at m/z 107 seen in the ethoxybenzylpiperazine compounds.

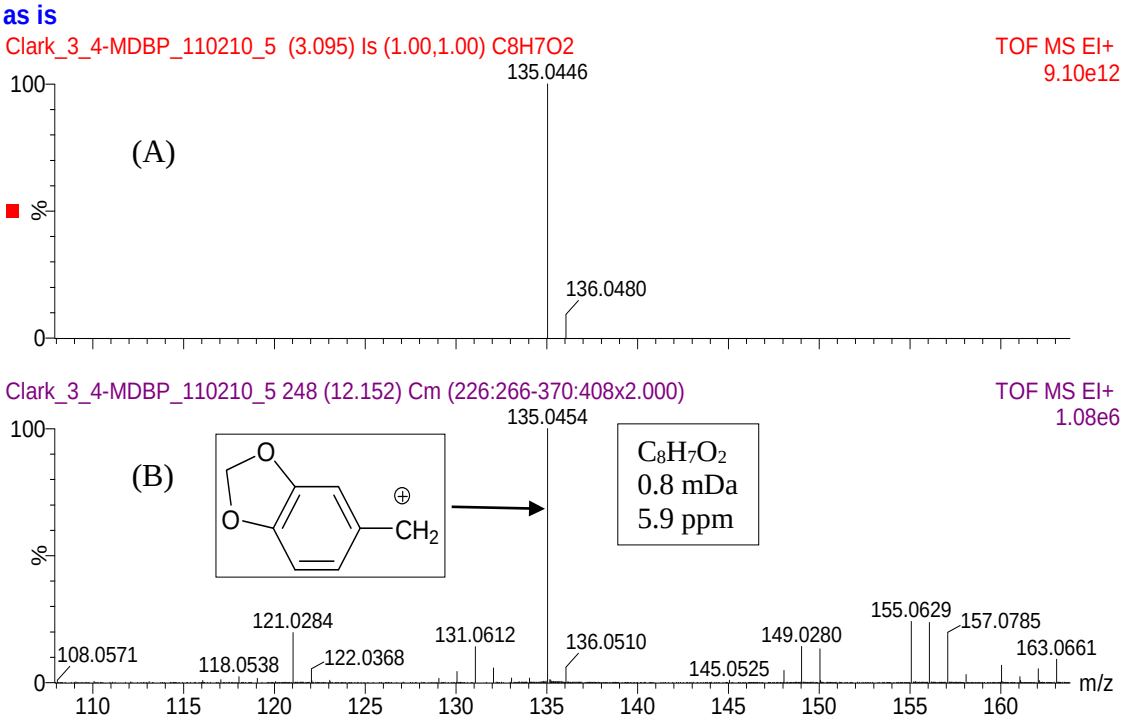


Fig. 2-5: GC-TOF mass spectral analysis of the m/z 135 ion for 3,4-methylenedioxybenzylpiperazine. 5A= calculated mass for $C_8H_7O_2$; 5B= experimental results.

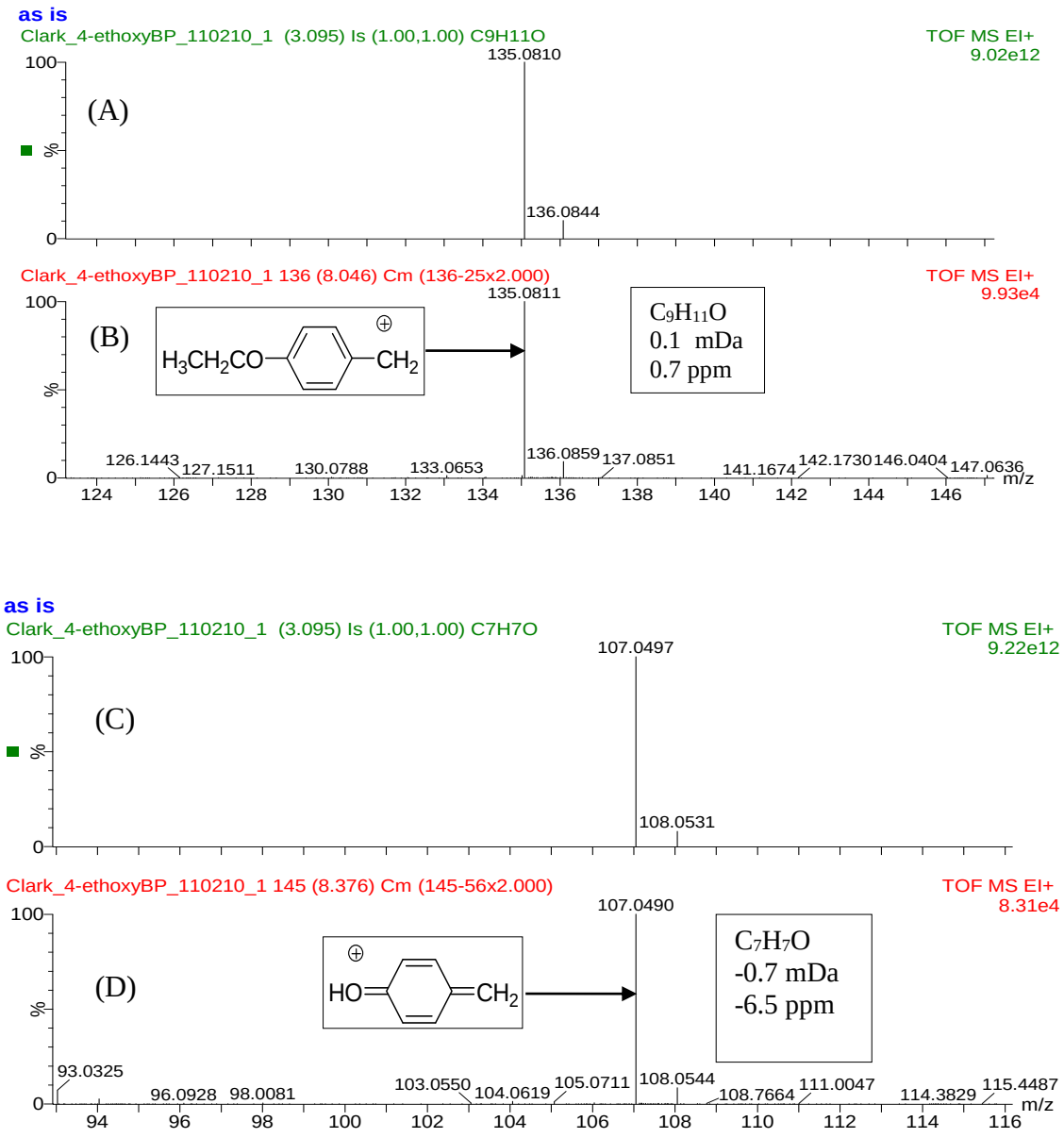
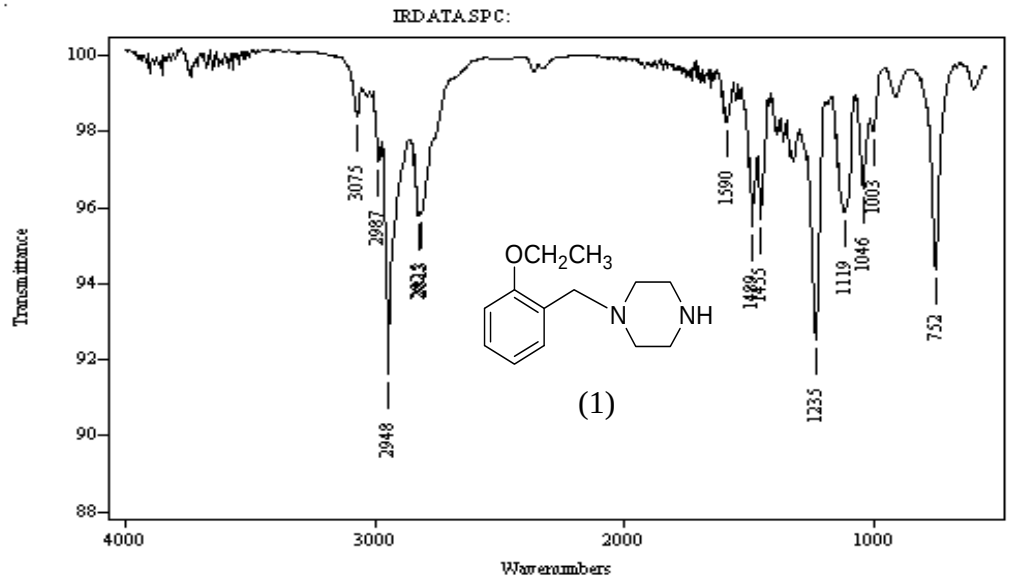


Fig. 2-6: GC-TOF mass spectral analysis of the m/z 135 and m/z 107 ions for 4-ethoxybenzylpiperazine. 6A= calculated mass for $C_9H_{11}O$; 6B= experimental results. 6C= calculated mass for C_7H_7O; 6D= experimental results.

Vapor-phase Infra-Red Spectrophotometric Studies of the Methylenedioxybenzylpiperazines (MDBPs) and Ethoxybenzylpiperazines (EBPs)

Infrared spectrometry is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the five isomeric benzylpiperazines. Infrared detection should provide compound specificity without the need for chemical modification of the parent molecule. The vapor phase infrared spectra for the five benzylpiperazines are shown in Figure 2-7. The spectra were generated in the vapor phase following sample injection into the gas chromatograph. Each compound shows a vapor phase IR spectrum with transmittance bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 cm^{-1}. In general, variations in the substitution pattern on the aromatic ring results in variations in the IR transmittance in the region 650 – 1700 cm^{-1} [Awad *et al*, 2009]. 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 infrared spectra and results for the two MDBPs have been previously discussed in details in Chapter 1.



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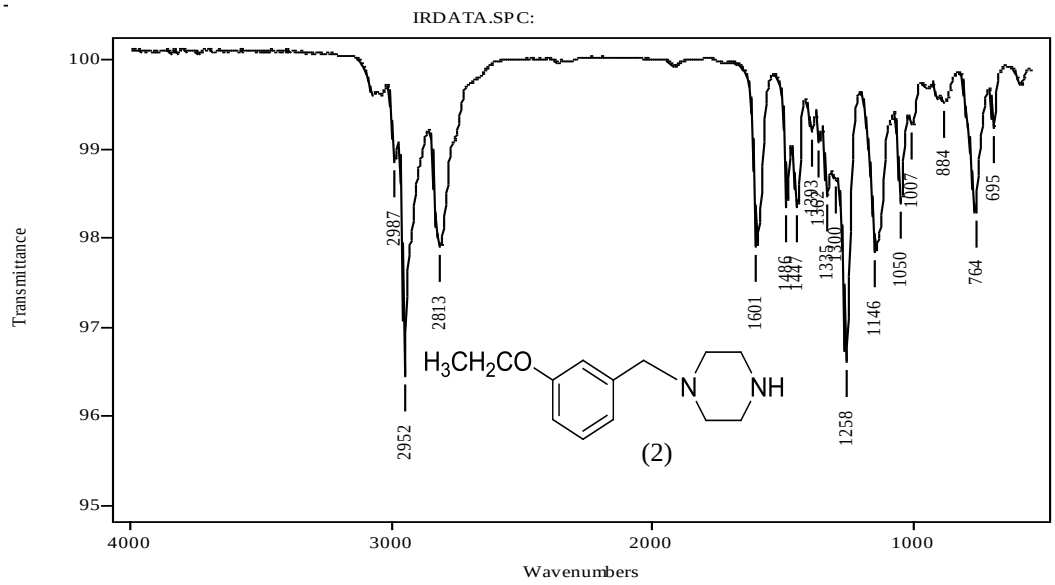

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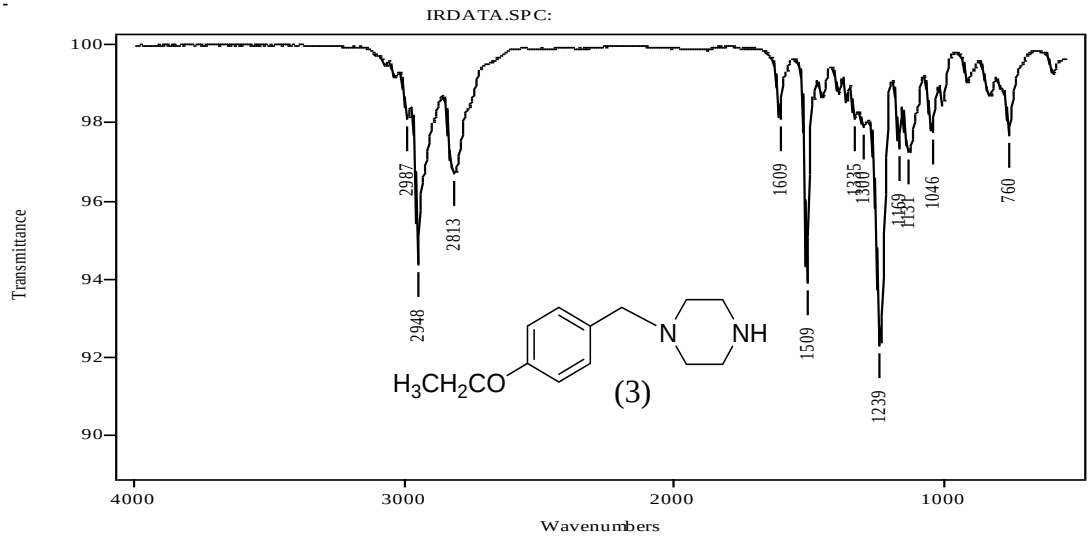

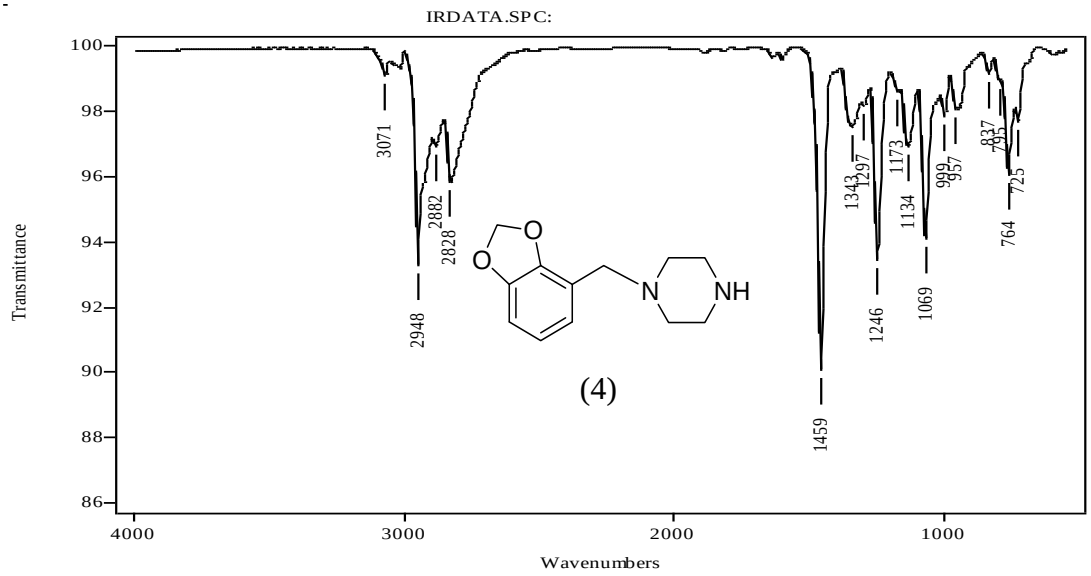

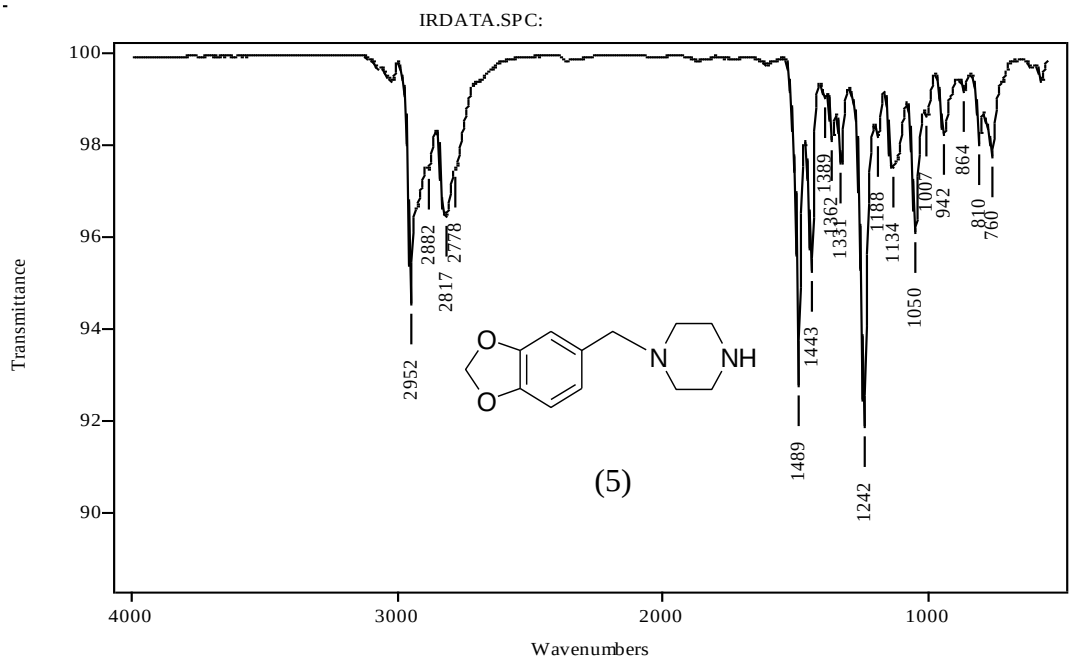

Fig. 2-7: Vapor phase IR spectra of the five methylenedioxy and ethoxybenzylpiperazines.

The three regioisomeric ethoxybenzylpiperazines 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 – 1610 cm^{-1}. Compound 3 shows a strong peak at 1509 cm^{-1} which is shifted to two medium intensity doublets at 1480 cm^{-1} , 1455 cm^{-1} and at 1486 cm^{-1}, 1447 cm^{-1} in compounds 1 and 2, respectively. Compound 2 shows a strong peak at 1258 cm^{-1} which is shifted to peaks at 1235 cm^{-1} and 1239 cm^{-1} in compounds 1 and 3, respectively. Compound 2 also has a characteristic peak at 1601 cm^{-1} which is almost absent in compound 1 and shifted to a weak singlet at 1609 cm^{-1} in the IR spectrum of compound 3.

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

Gas Chromatographic Separation of the Methylenedioxybenzylpiperazines (MDBPs) and Ethoxybenzylpiperazines (EBPs)

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

Several temperature programs were evaluated and the chromatogram in Figure 2-8 is a representative of the results obtained for all samples on this stationary phase. In Figure 2-8 the HFBA derivatives of the ethoxybenzylpiperazines are less retained than their isobaric methylenedioxybenzylpiperazines. 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 in addition to no advantage in chromatographic resolution.

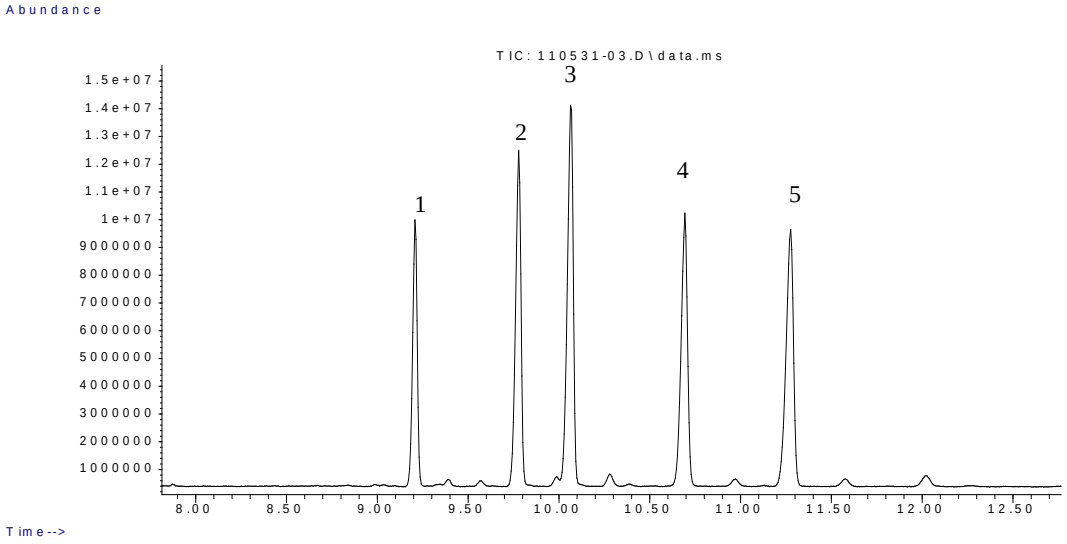

Fig. 2-8: Gas chromatographic separation of the heptafluorobutyryl derivatives of the five piperazine isomers using Rxi-50 column. The number over the peak corresponds to the compound number.

Conclusion

The three ethoxybenzylpiperazines have an isobaric relationship to the controlled substance 3,4-MDBP and its regioisomer 2,3-MDBP. The three regioisomeric ethoxy compounds yield a unique fragment ion at m/z 107 in their EI mass spectra which allowed for discriminating them from the isobaric methylenedioxy compounds.

Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound. GC-IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650-1700 cm^{-1}. The five TFA and PFPA derivatives were successfully resolved on the stationary phase Rxi-50.

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

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Awad, T., Clark, C.R., DeRuiter, J. 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.

Awad, T., Belal, T., DeRuiter, J., Kramer, K. and Clark, C. R. Comparison of GC-MS and GC-IRD methods for the differentiation of methamphetamine and regioisomeric substances, Forensic Science International 185 (2009) 67-77.

Chapter 3

Differentiation of Methylenedioxybenzylpiperazines (MDBPs) and their corresponding ring substituted Methoxymethylbenzylpiperazines (MMBPs) "at 2,3 and 3,4 positions" by GC-IRD and GC-MS

The substituted benzylpiperazines, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP), its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) and four isobaric ring substituted methoxymethylbenzylpiperazines (MMBPs) have almost identical mass spectra. Perfluoroacylation of the secondary amine nitrogen of these isomeric 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 isomer to the exclusion of the other compounds.

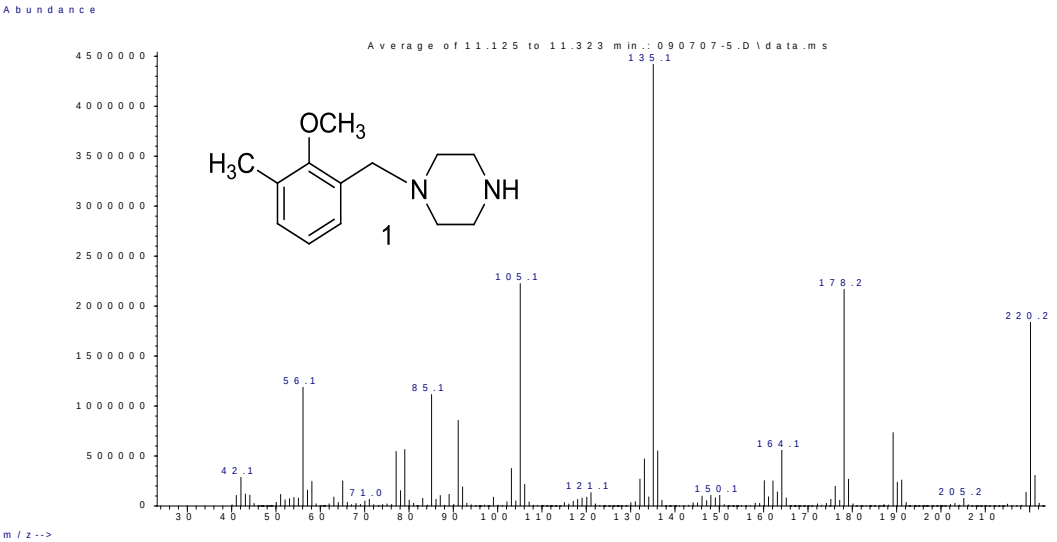
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the six isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivative forms of the ring substituted benzylpiperazines were resolved on the polar stationary phase Rtx-200.

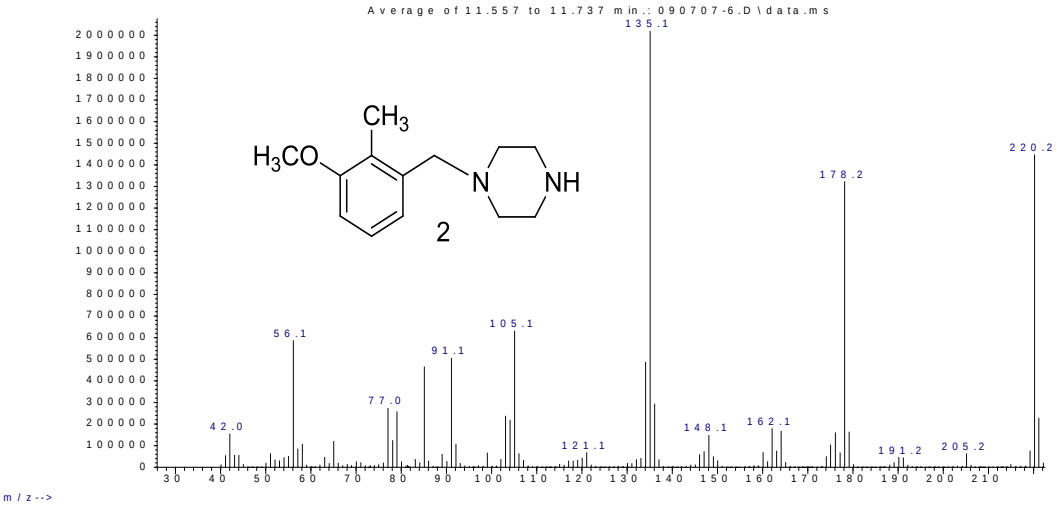
Gas chromatography coupled with time-of-flight mass spectrometric detection provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazine and 4-methoxy-3-methylbenzylpiperazine which have similar nominal masses but are different in their calculated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

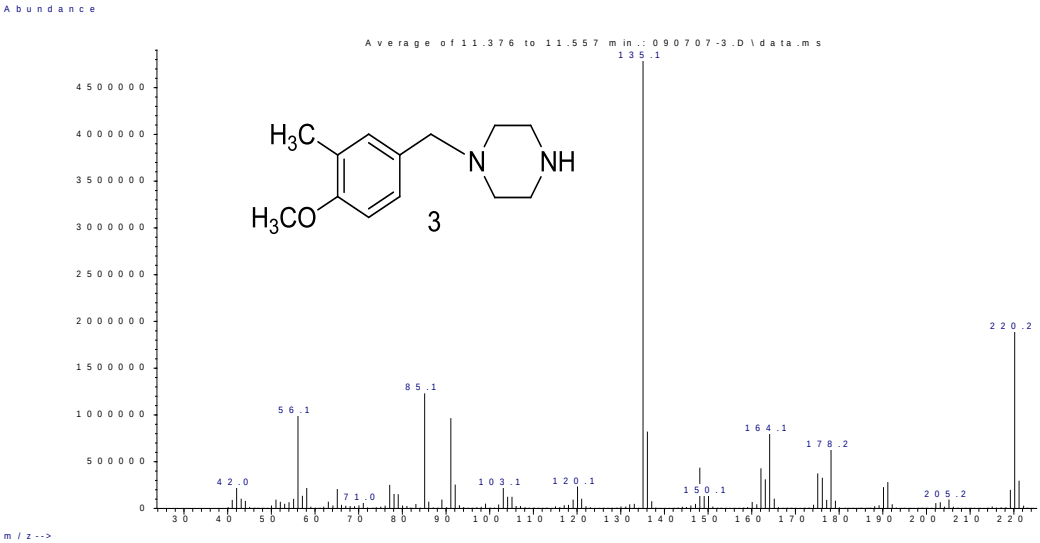
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylenedioxybenzylpiperazines (MDBPs) and their corresponding ring substituted Methoxymethylbenzylpiperazines (MMBPs) "at 2,3 and 3,4 positions"

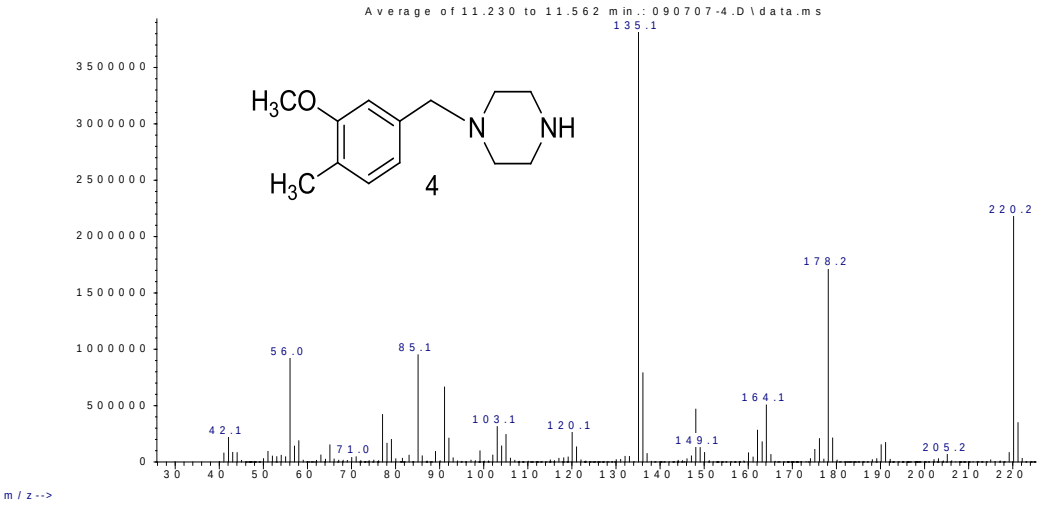
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 3-1 shows the EI mass spectra of all six isomeric benzylpiperazines (Compounds 1-6). The base peak in all these spectra occurs at m/z 135 and this ion corresponds to the mass equivalent regioisomeric/isobaric ring substituted benzyl cations. The additional high mass ions of significant relative abundance common to the six isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the six benzylpiperazines show fragment ions at m/z 178, 164, and 135 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 3-2 and are based in part on a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. However, the relative abundances for the ions in the spectra for the six isomeric benzylpiperazines are slightly different and these results indicate that very little specific structural information is available for differentiation among these isomers. Compounds 3 and 6 even show very similar relative abundance pattern for the major fragment ions. Thus, the mass spectra alone do not provide specific confirmation of identity for any individual isomer to the exclusion of the others.

An additional fragmentation pathway which is characteristic for all the ortho-methoxy ring substituted compounds is described in Figure 3-3. Those methoxymethylbenzylpiperazines with the methoxy group in the ortho position relative to









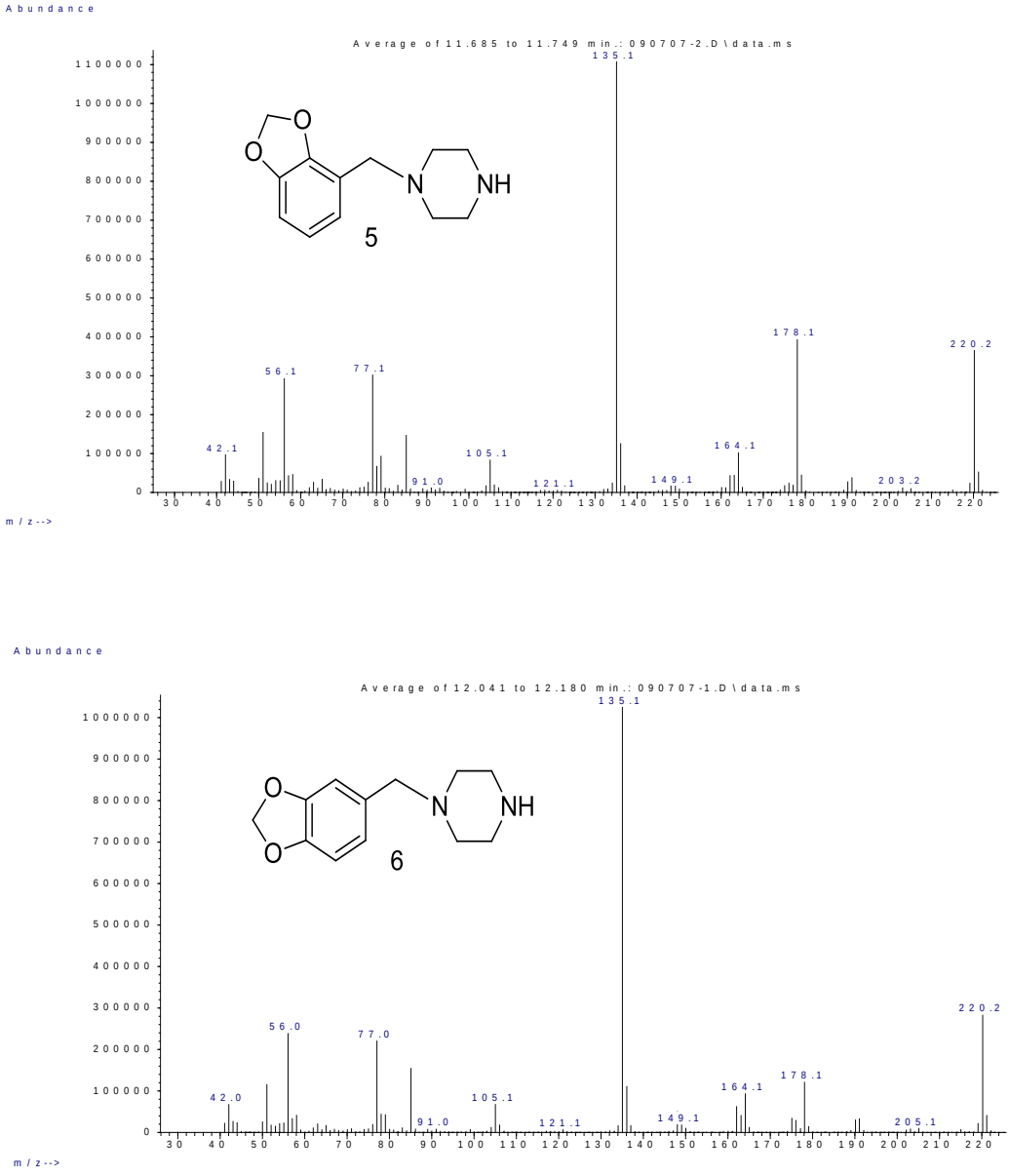
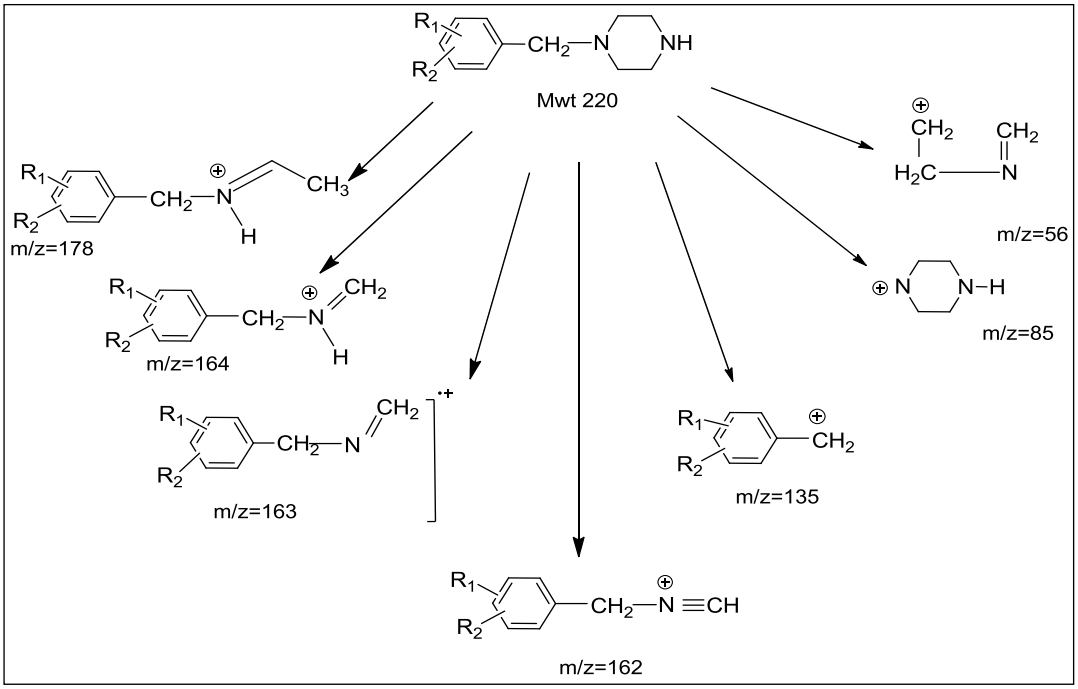


Fig. 3-1: EI mass spectra of the six methylenedioxy and methoxymethylbenzylpiperazines.



R_1 = OCH_3, R_2 = CH_3 for the MMBPs
R_1, R_2 = methylenedioxy for the MDBPs

Fig. 3-2: EI mass spectral fragmentation pattern of the methylenedioxy and methoxymethylbenzylpiperazines.

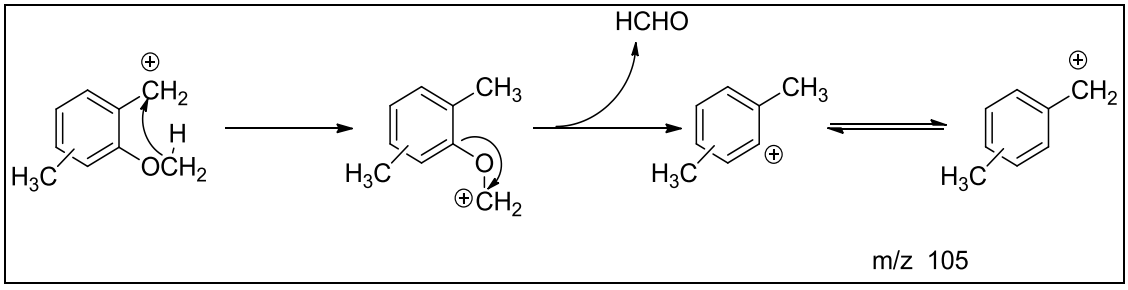
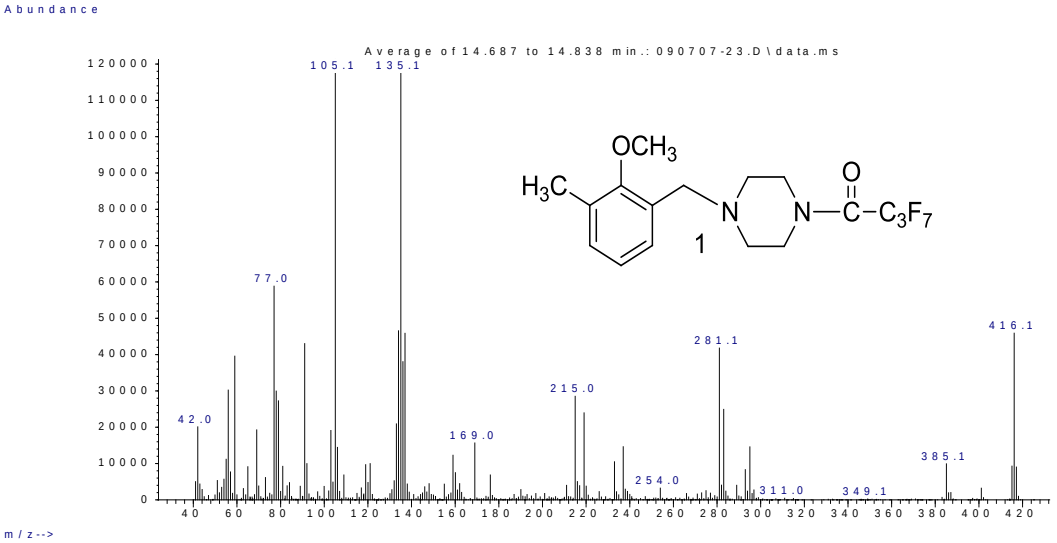


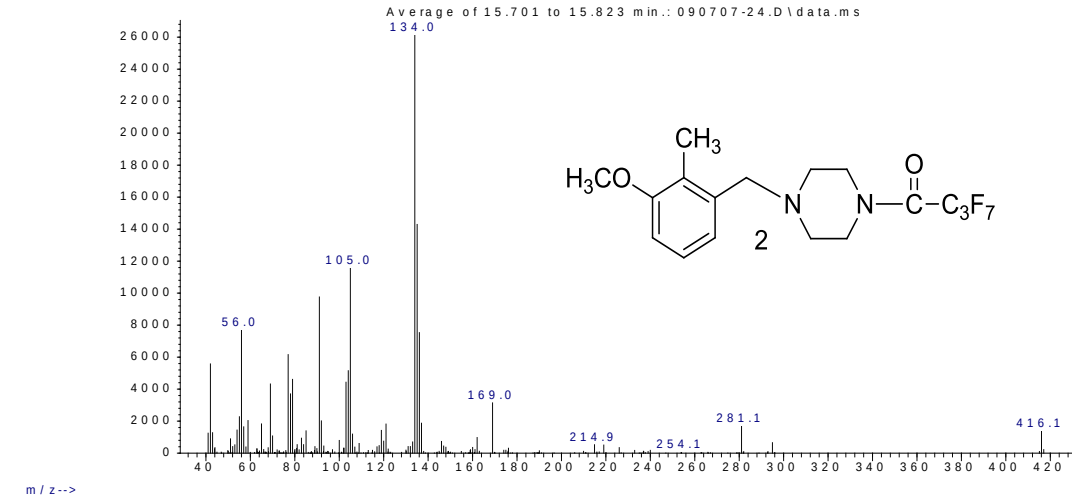
Figure 3-3: Mechanism for the formation of the m/z 105 ion in the mass spectra of the underivatized and derivatized 2-methoxy regioisomers of the methoxymethylbenzylpiperazines.

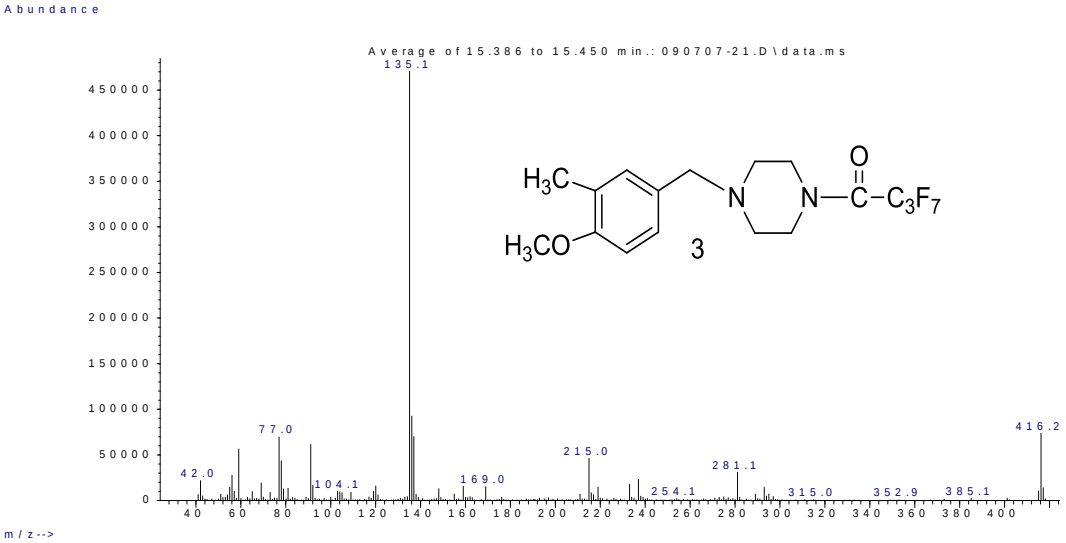
the side chain are characterized by a significant m/z 105 ion. This ion likely arises from the loss of mass 30 (CH_2O) from the initial methoxymethylbenzylic cation at m/z 135. The m/z 105 ion is a significant fragment only when the methoxy group is ortho to the piperazine side chain and therefore the site of initial benzylic cation formation as in Compound 1. This m/z 105 ion can be formed by 1,6-hydride shift (ortho effect) from a hydrogen of the ortho-methoxy group to the benzyl cation followed by the loss of formaldehyde as in Figure 3-3. This fragment occurs in all the mass spectra of the underivatized and TFA, PFPA and HFBA derivatives of the ortho-methoxy MMBPs. This suggested mechanism for the loss of CH_2O from the ortho-methoxy benzyl cations was previously described from our lab [Awad *et al*, 2007].

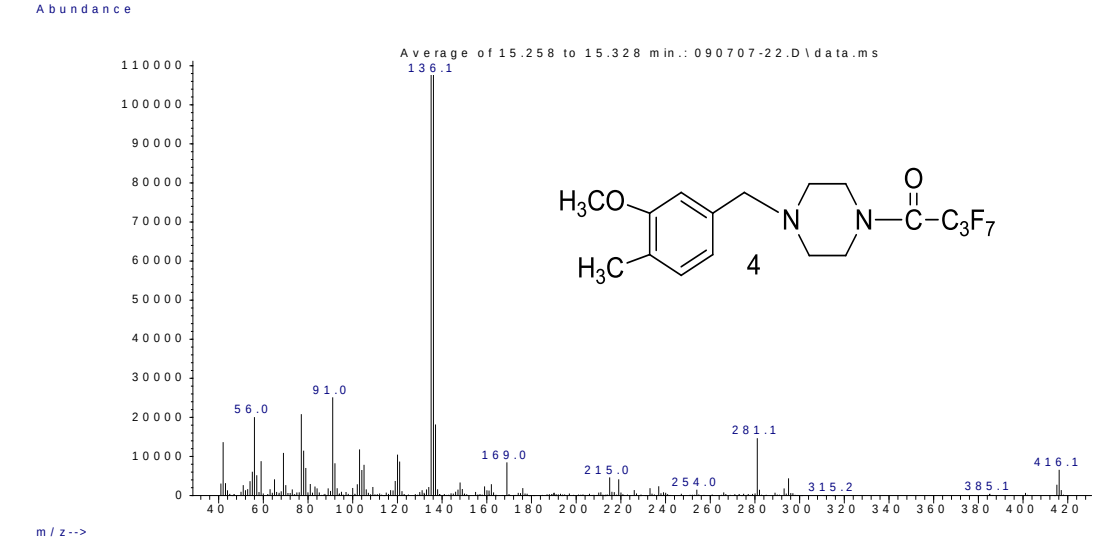
The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric ring substituted benzylpiperazines, in an effort to individualize their mass spectra and identify unique marker ions that would allow discrimination between these six compounds. Acylation of an amine lowers the basicity and can often allow other fragmentation pathways to play a more prominent role in the resulting mass spectra.

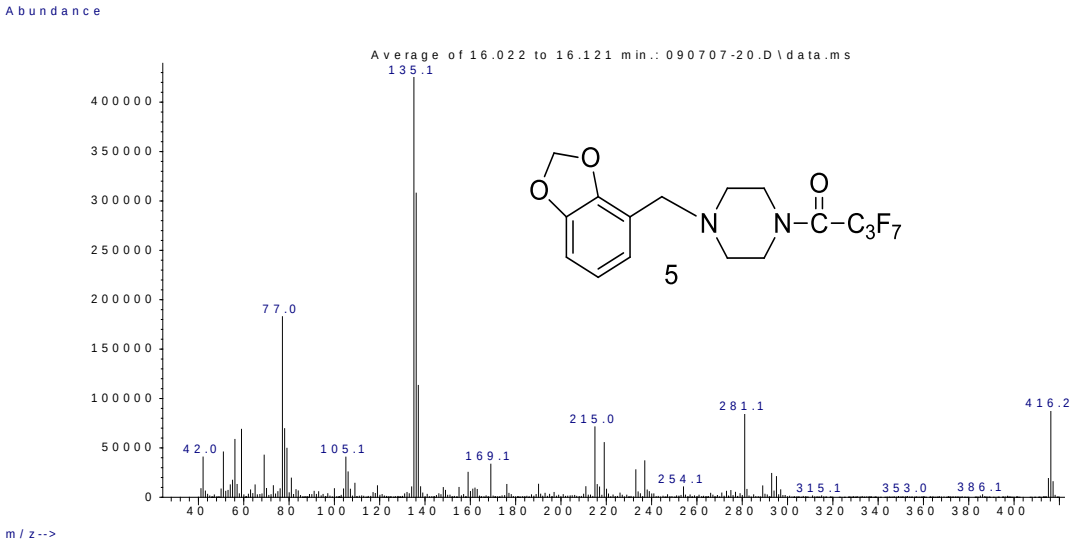
The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra and provide data for the individualization of the isomers. Figure 3-4 shows the mass spectra of the heptafluorobutyryl amides of the six studied compounds as representative spectra for all the perfluoroacyl amides. 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 aromatic ring











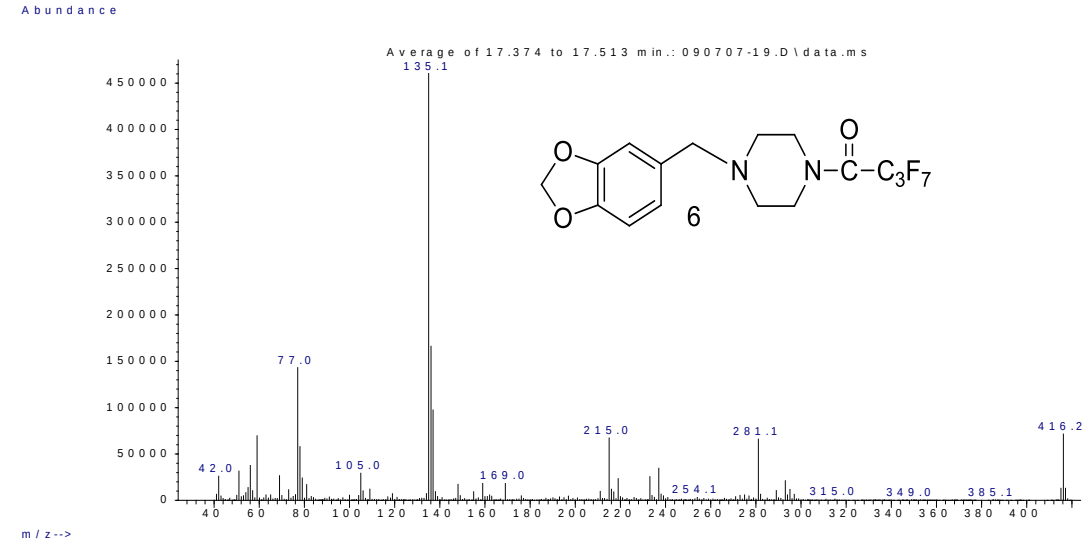


Fig. 3-4: EI mass spectra of the heptafluorobutyrylamides for the six substituted benzylpiperazines in this study.

substituted benzyl cation. Furthermore, an additional fragment ion series occurring at *m/z* 181, 231 and 281 for the TFA, PFPA and HFBA amides, respectively corresponds to the $(M-135)^+$ 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 six isomers. The HFBA derivative of compound 3 again shows essentially the same fragment ions in a very similar pattern of relative abundance with that of the HFBA derivative of 3,4-MDBP, compound 6.

Gas chromatography coupled with time-of-flight mass spectrometric detection provides an additional means of differentiating between the isobaric compounds 3,4-methylenedioxybenzylpiperazine and 4-methoxy-3-methylbenzylpiperazine which have similar nominal masses but are different in their calculated exact masses. The methoxymethylbenzyl $(C_9H_{11}O)^+$ fragments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135 but are different in their elemental composition and accordingly different in their calculated masses. Figure 3-5 shows the GC-TOF-MS exact mass analysis of the 3,4-methylenedioxybenzyl cation (m/z=135) for compound 6. 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 (0.8 mDa, 5.9 ppm) with the calculated mass. Thus, confirming the m/z 135 ion in compound 6 as the elemental composition $C_8H_7O_2$. These results can be compared to the exact mass analysis for the m/z 135 ion (4-methoxy-3-

methylbenzyl) in compound 3. Figure 6A and 6B confirms the elemental composition as $C_9H_{11}O$ with a mass deviation of -3.1 mDa (-22.9 ppm). Thus, exact mass measurements distinguish between these isobaric forms of the m/z 135 ion.

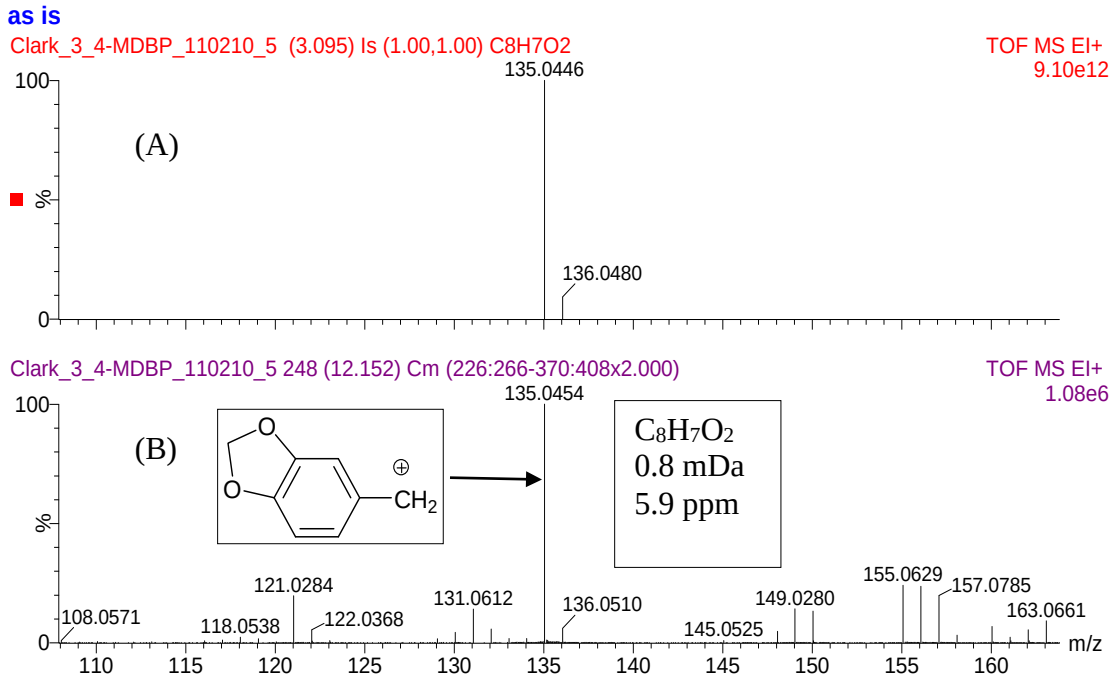


Fig. 3-5: GC-TOF mass spectral analysis of the m/z 135 ion for 3,4-methylenedioxybenzylpiperazine. A= calculated mass for $C_8H_7O_2$; B= experimental results.

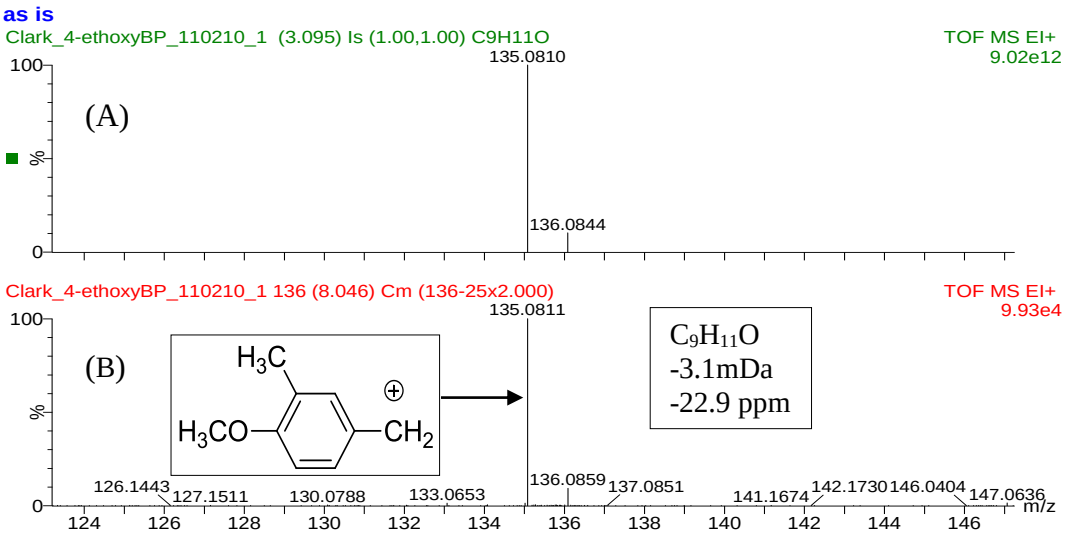
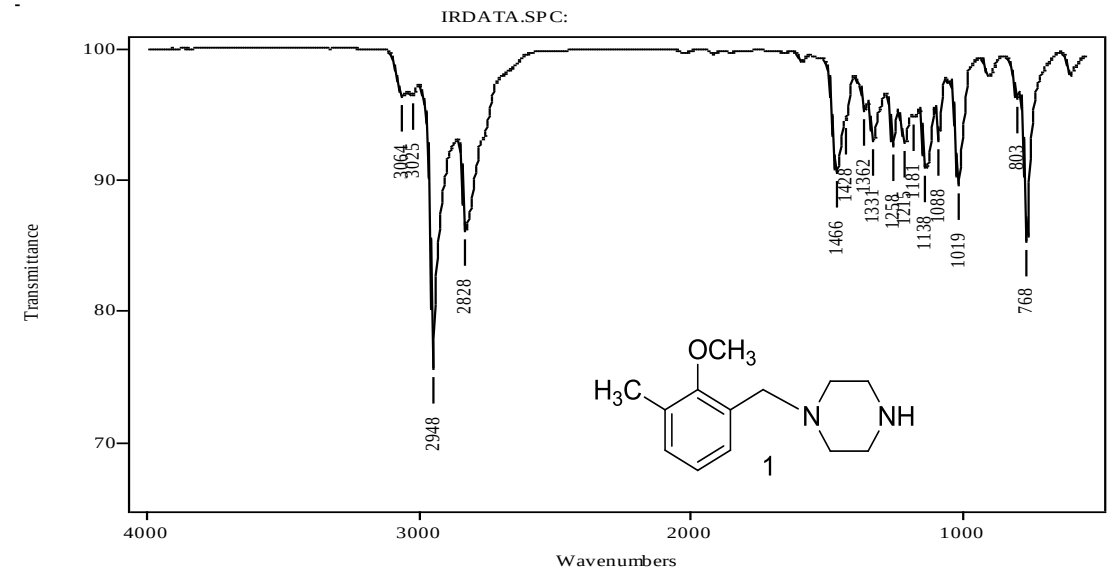


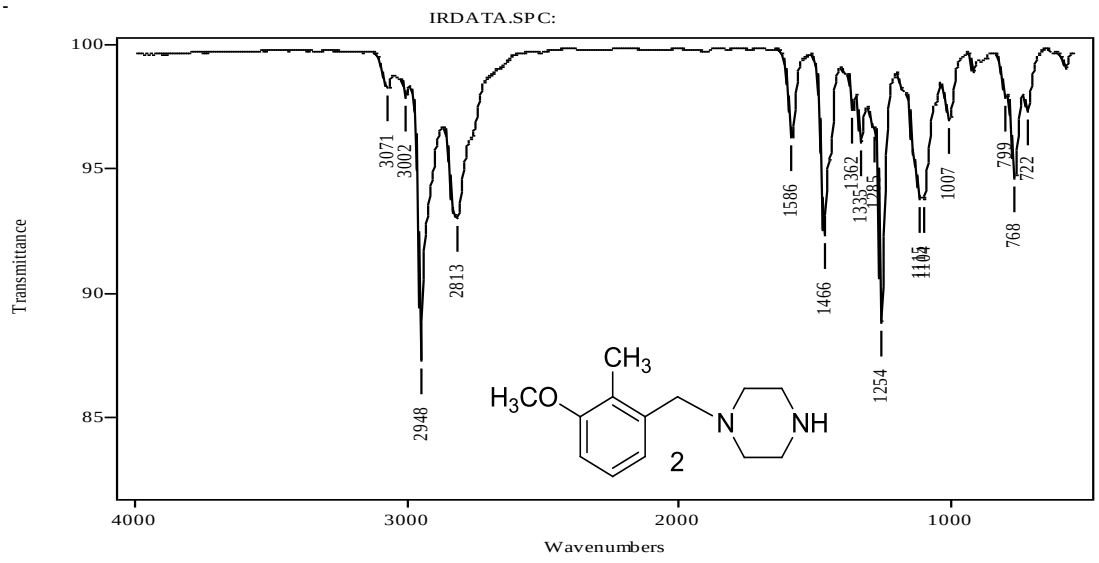
Fig. 3-6: GC-TOF mass spectral analysis of the m/z 135 ion for 4-methoxy3-methylbenzylpiperazine. A= calculated mass for $C_9H_{11}O$; B= experimental results.

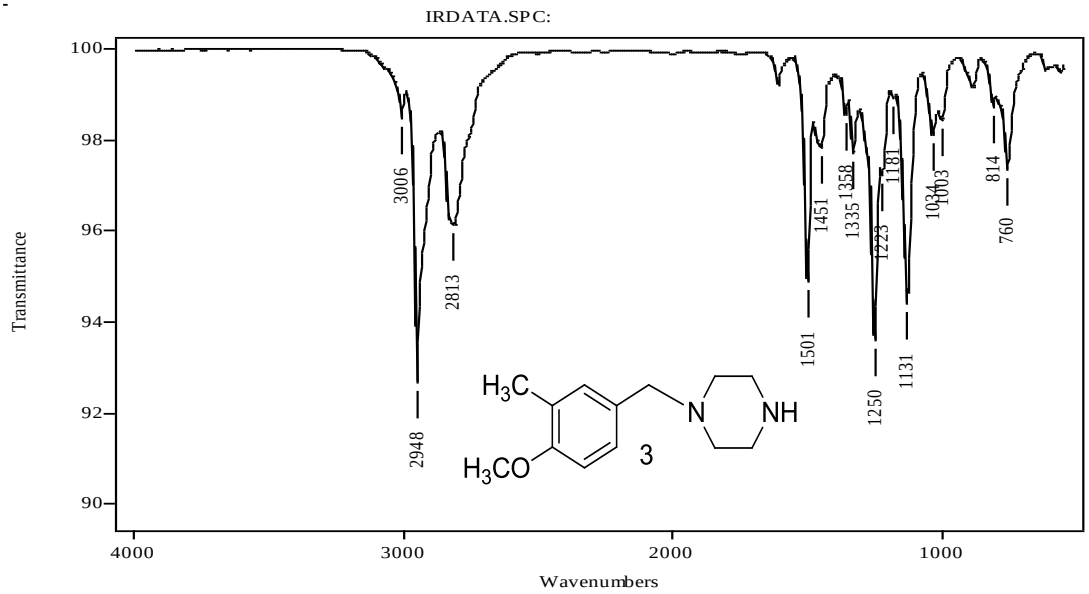
Vapor-phase Infra-Red Spectrophotometric Studies of the Methylenedioxybenzylpiperazines (MDBPs) and their corresponding ring substituted Methoxymethylbenzylpiperazines (MMBPs) "at 2,3 and 3,4 positions"

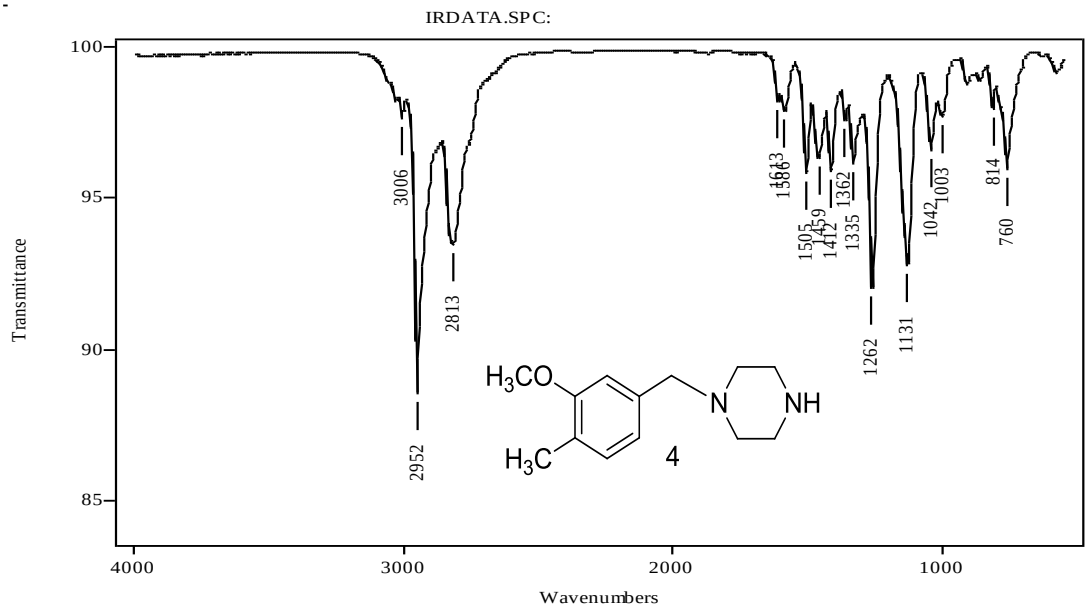
Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the six isomeric substituted benzylpiperazines. Infrared analysis should provide compound specificity without the need for chemical modification of the parent molecule. The vapor phase infrared spectra for the six benzylpiperazines are shown in Figure 3-7. The spectra were generated in the vapor phase following sample injection into the gas chromatograph. Each compound shows a vapor phase IR spectrum with bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 cm^{-1}. In general, variations in the substitution pattern on the aromatic ring results in variations in the IR spectra in the region 650 – 1700 cm^{-1}. Since the six piperazines share the same degree of nitrogen substitution, i.e. the same side chain, they have almost identical IR bands in the 2700 – 3100 cm^{-1} region. However, these compounds can be easily differentiated by the positions and intensities of several IR peaks in the region of 650 – 1700 cm^{-1}.

The infrared spectra and results for the two MDBPs have been previously discussed in details in Chapter 1.









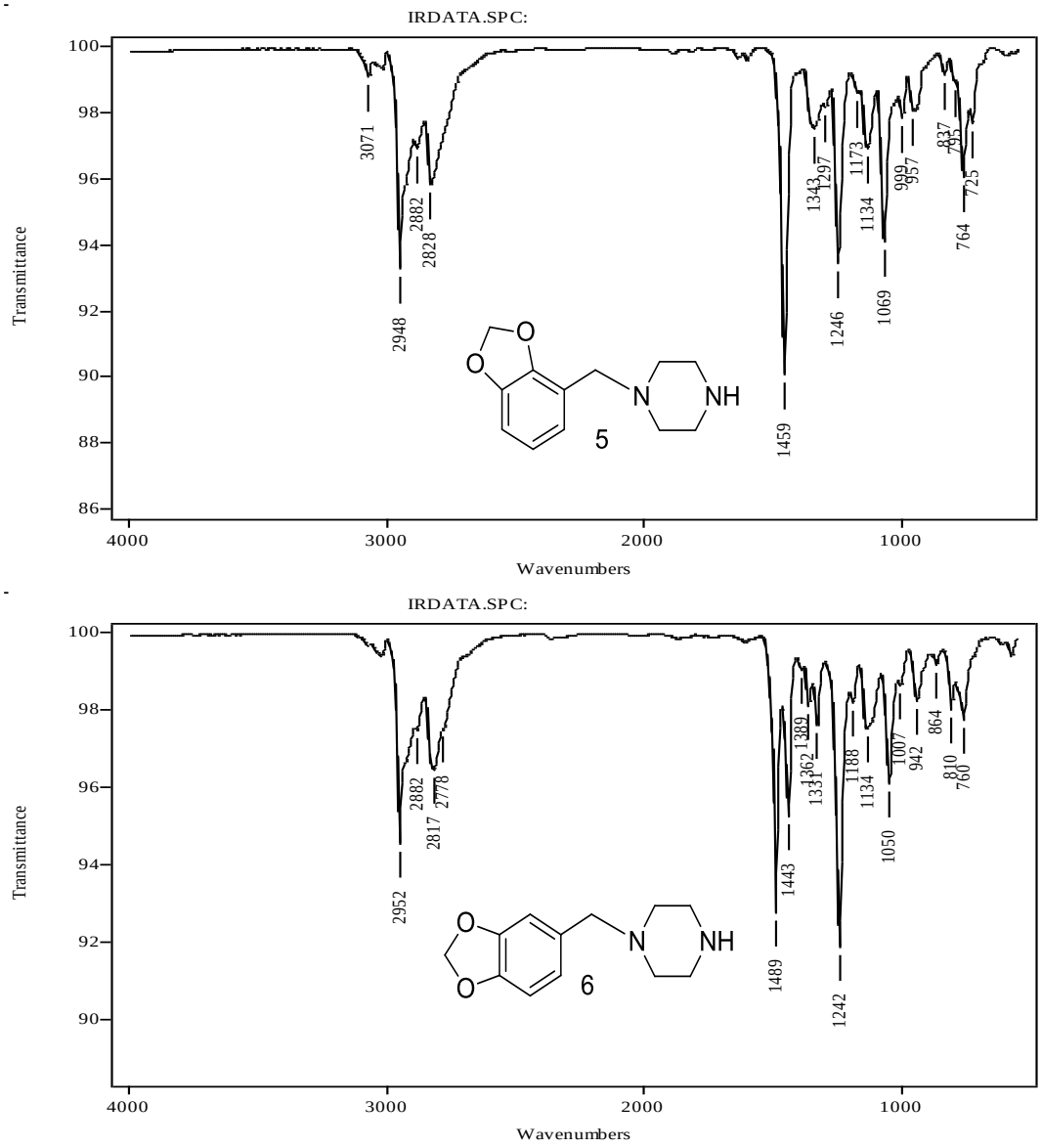


Fig. 3-7: Vapor phase IR spectra of the six underivatized methylenedioxy and methoxymethylbenzylpiperazines.

The four regioisomeric methoxymethylbenzylpiperazines share almost the same IR features in the region of 2700 – 3100 cm^{-1} and can be differentiated by the positions and intensities of several IR peaks in the region of 650 – 1610 cm^{-1}. Compound 3 shows a strong peak at 1501 cm^{-1} which is shifted to a weak one at 1505 cm^{-1} in compound 4 and to a medium peak at 1466 cm^{-1} in both 1 and 2. Compound 4 shows strong peaks at 1262 cm^{-1} and 1131 cm^{-1} which are shifted to peaks at 1250 cm^{-1} and 1131 cm^{-1} of nearly equal intensity in compound 3, very weak peaks at 1258 cm^{-1} and 1138 cm^{-1} in compound 1 and a singlet at 1254 cm^{-1} in compound 2. The six isomers share a medium intensity peak in the 760 cm^{-1} range with all three of the 3,4-substituted isomers showing the absorption band at 760 cm^{-1} and this band shifts to slightly higher values at 764 and 768 cm^{-1} for the 2,3-substituted isomers.

Figure 3-8 provides an excellent illustration of the value of vapor phase IR confirmation for isobaric substances. The region from 1800 to 650 cm^{-1} is compared for 3,4-MDBP (compound 6) and 4-methoxy-3-methylbenzylpiperazine (compound 3) showing significant differences in the major bands for these two compounds. Thus, these two compounds which yield almost identical mass spectra in the underivatized and derivatized forms show significant differences in their vapor phase IR spectra in this expanded region of their spectra. Furthermore, vapor phase infrared spectra provide distinguishing and characteristic information to determine the aromatic ring substitution pattern in the substituted piperazine regioisomers included in this study.

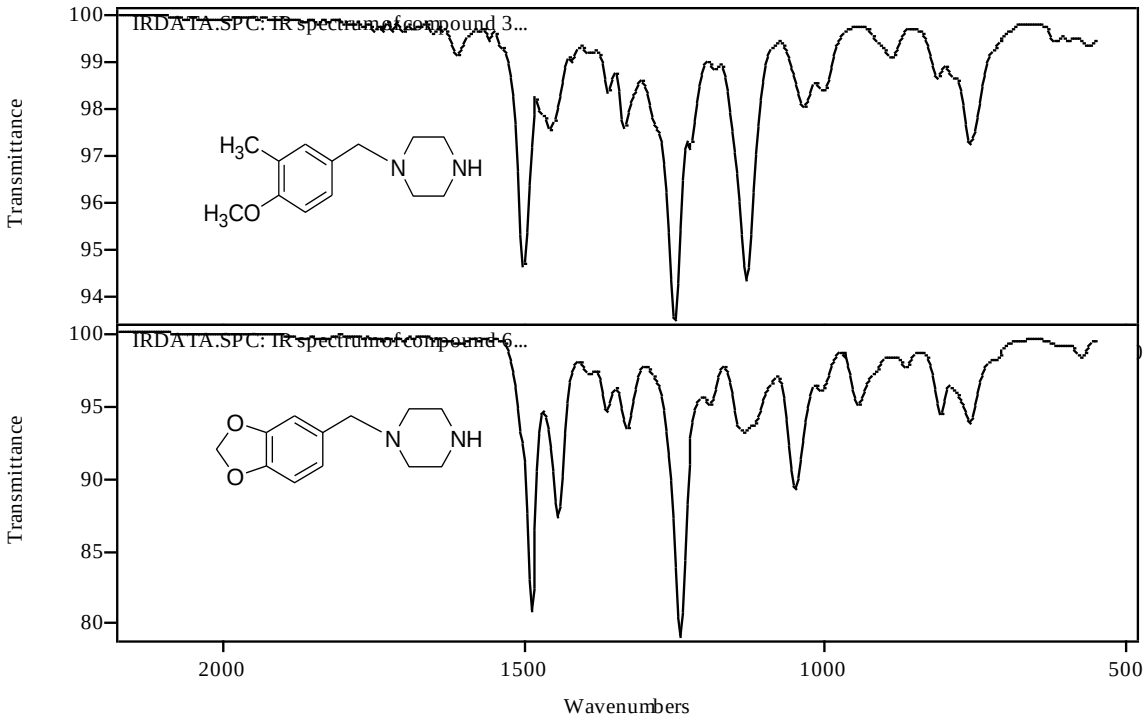


Fig. 3-8: Vapor phase IR spectra of compounds 3 and 6 in the region between 650 – 1800 cm^{-1}.

Gas Chromatographic Separation of the Methylenedioxybenzylpiperazines (MDBPs) and their corresponding ring substituted Methoxymethylbenzylpiperazines (MMBPs) "at 2,3 and 3,4 positions"

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 the relatively polar stationary phase, 100% trifluoropropyl methyl polysiloxane (Rtx-200). The temperature program consisted of an initial temperature of 100°C for 1 minute, ramped up to 180°C at a rate of 12°C per minute followed by a hold at 180°C for 2 minutes then ramped up to 200°C at a rate of 10°C/min and held at 200°C for 5.0 min. The chromatogram in Figure 3-9 is a representative of the results obtained for all samples on this stationary phase.

In Figure 3-9 the methoxymethylbenzylpiperazines are less retained than their isobaric methylenedioxybenzylpiperazines. The drug substance 3,4-MDBP eluted last in this limited series of compounds in all chromatographic experiments. The TFA derivatives of the six isomers were resolved on the same stationary phase. However, in the case of PFPA and HFBA derivatives compounds 2 and 5 coeluting even with fairly long analysis times in the 30 to 40 minutes range. Thus, perfluoroacylation did not provide any additional mass spectral discrimination among the six isomers in addition to no advantage in chromatographic resolution.

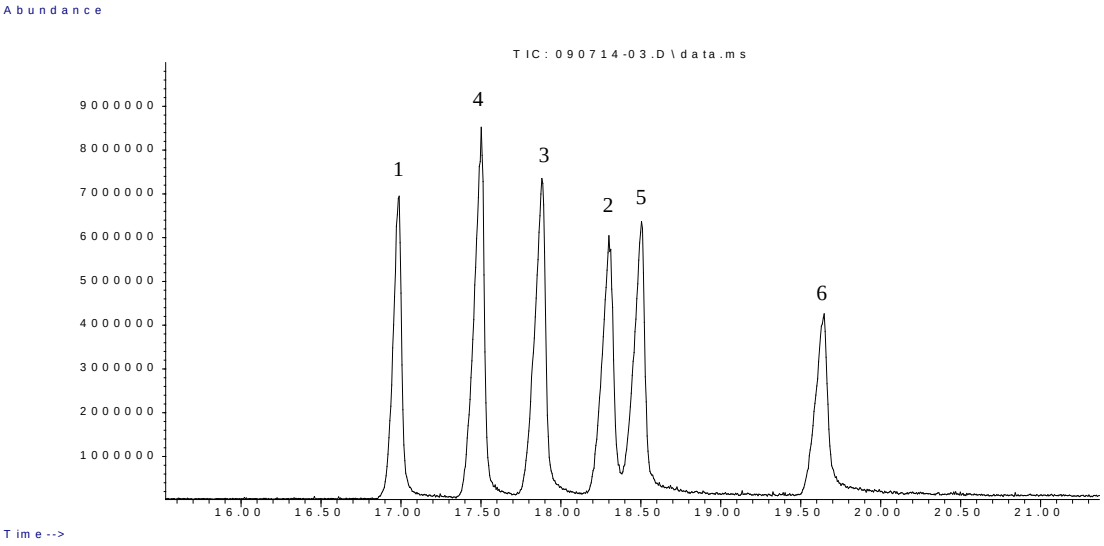


Fig. 3-9: Gas chromatographic separation of the six underivatized benzylpiperazines on Rtx-200 column. The numbers over the peaks correspond to the compound numbers.

Conclusion

The four methoxymethylbenzylpiperazines have an isobaric relationship to the potential drug of abuse 3,4-MDBP and its regioisomer 2,3-MDBP. All six compounds show the same fragment ions in their EI mass spectra. Chemical derivatization (perfluoroacylation) did not offer any unique marker ion to allow identification of one compound to the exclusion of the others. GC-IRD offered unique and characteristic IR spectra that allowed for discrimination among these compounds in the region between 650-1700 cm^{-1}. The six underivatized isomers were successfully resolved by gas chromatography on the polar stationary phase Rtx-200.

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

References

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Awad, T., DeRuiter, J., Clark, C.R. Gas Chromatography-Mass Spectrometry Analysis of Regioisomeric Ring Substituted Methoxy Methyl Phenylacetones, J. Chromatogr. Sci. 45 (2007) 458-465.

Chapter 4

Differentiation of Methylenedioxybenzylpiperazines (MDBPs) and Methoxymethylbenzylpiperazines (MMBPs) by GC-IRD and GC-MS

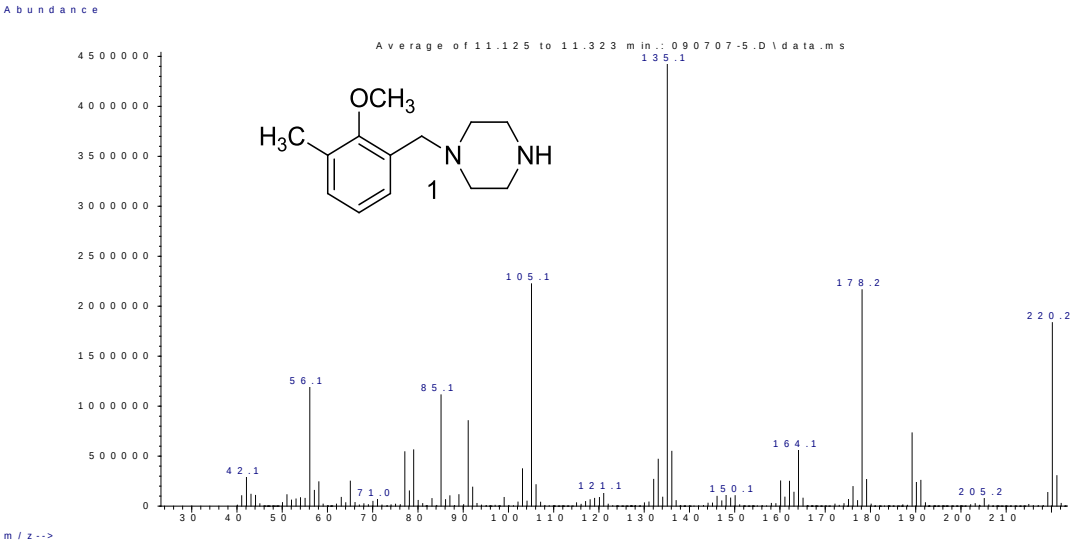
The substituted benzylpiperazines, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP), its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) and all ten possible isobaric ring substituted methoxymethylbenzylpiperazines (MMBPs) have almost identical mass spectra. Perfluoroacylation of the secondary amine nitrogen of these isomeric 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 isomer to the exclusion of the other compounds.

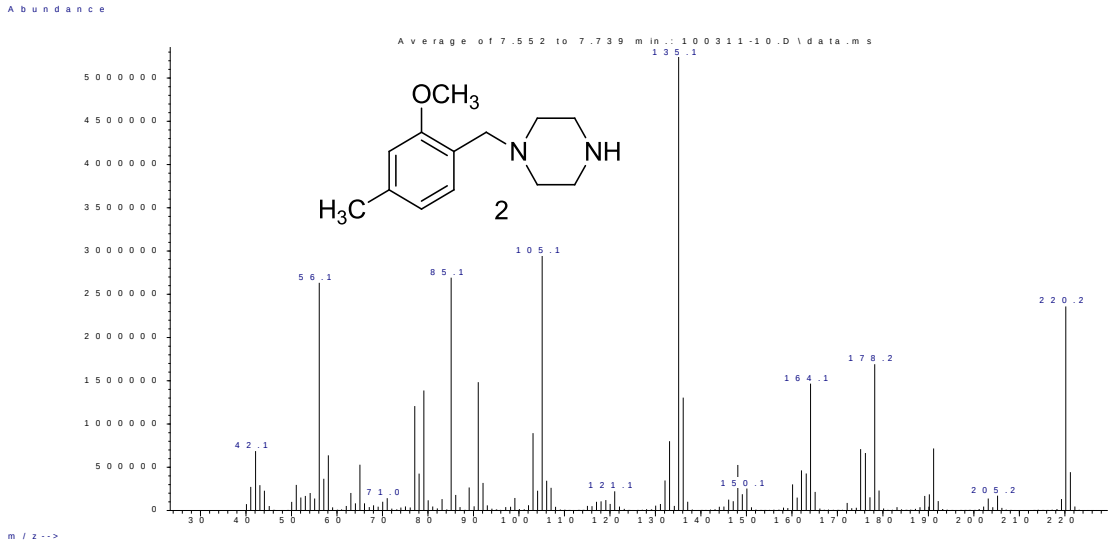
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the twelve isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivative forms of the ring substituted benzylpiperazines were resolved on the polar stationary phase Rtx-35.

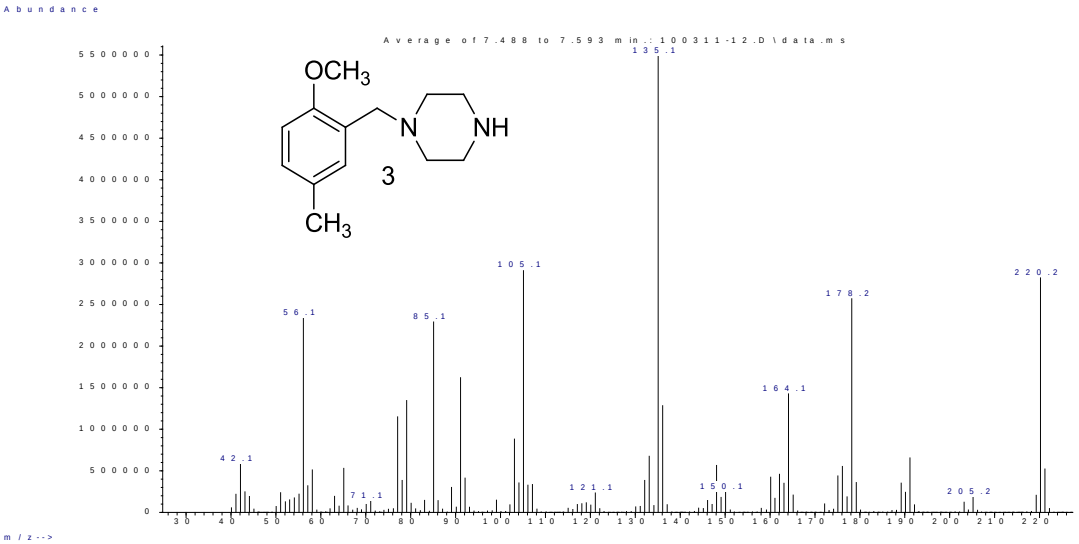
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylenedioxybenzylpiperazines (MDBPs) and Methoxymethylbenzylpiperazines (MMBPs)

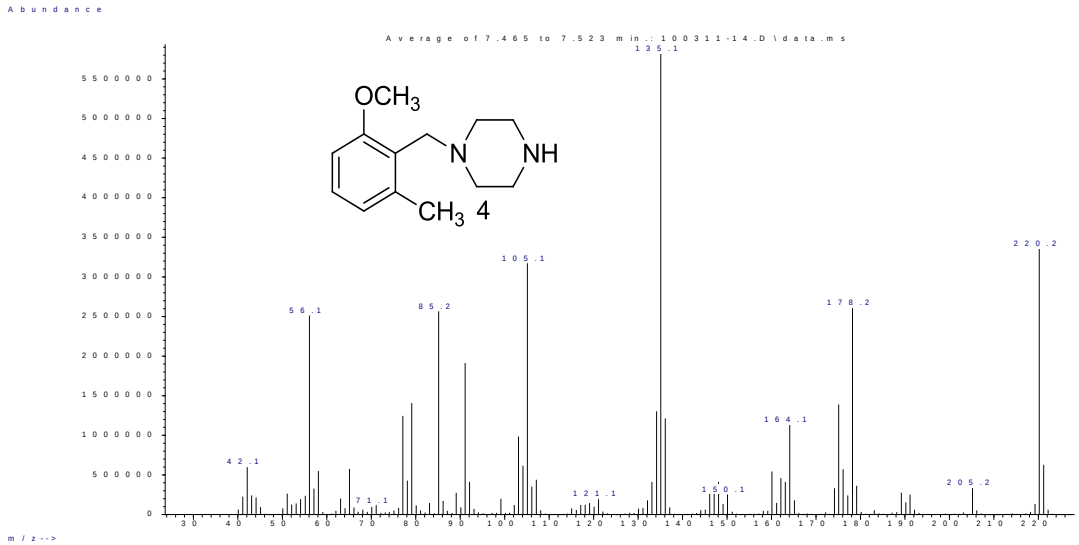
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 4-1 shows the EI mass spectra of all twelve isomeric benzylpiperazines (Compounds 1-12). The mass spectra of all of the compounds are almost identical to each other and produce the same fragments described in the previous chapter. The base peak in all these spectra occurs at m/z 135 and this ion corresponds to the mass equivalent regioisomeric/isobaric ring substituted benzyl cations. The additional high mass ions of significant relative abundance common to the twelve isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the twelve benzylpiperazines show fragment ions at m/z 178, 164, and 135 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 3-2 and are related to a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. However, the relative abundances for the ions in the spectra for the twelve isomeric benzylpiperazines are slightly different and these results indicate that very little specific structural information is available for differentiation among these isomers.

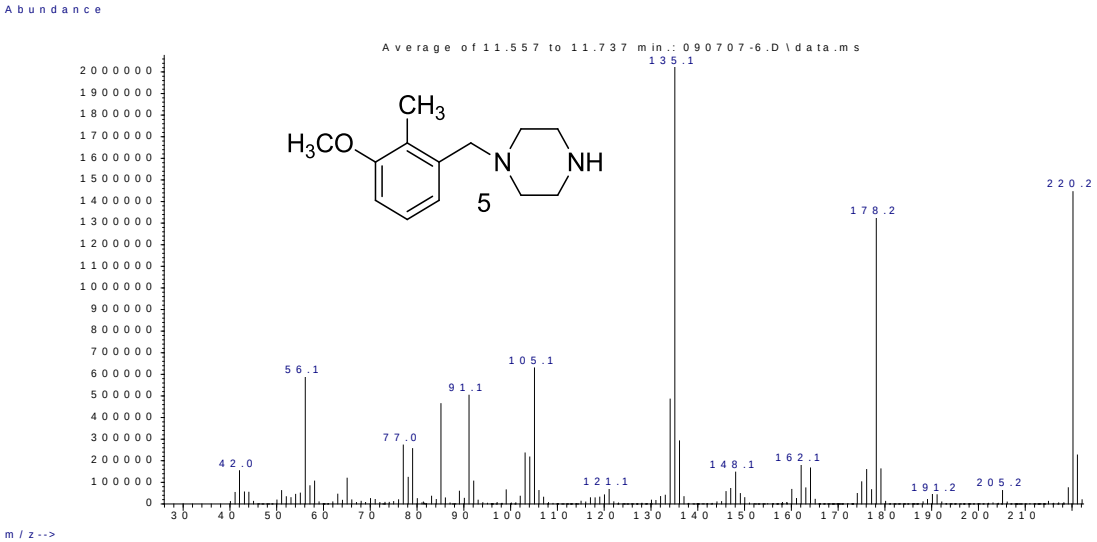
The fragmentation pathway that was discussed in chapter 3 which is characteristic for all the ortho-methoxy ring substituted compounds and was described in Figure 3-3 before is still occurring in those methoxymethylbenzylpiperazines with the methoxy group in the ortho position relative to the side chain. Those compounds are characterized by a significant m/z 105 ion. This ion likely arises from the loss of mass 30 (CH_2O) from the initial methoxymethylbenzylic cation at m/z 135. The m/z 105 ion is a significant

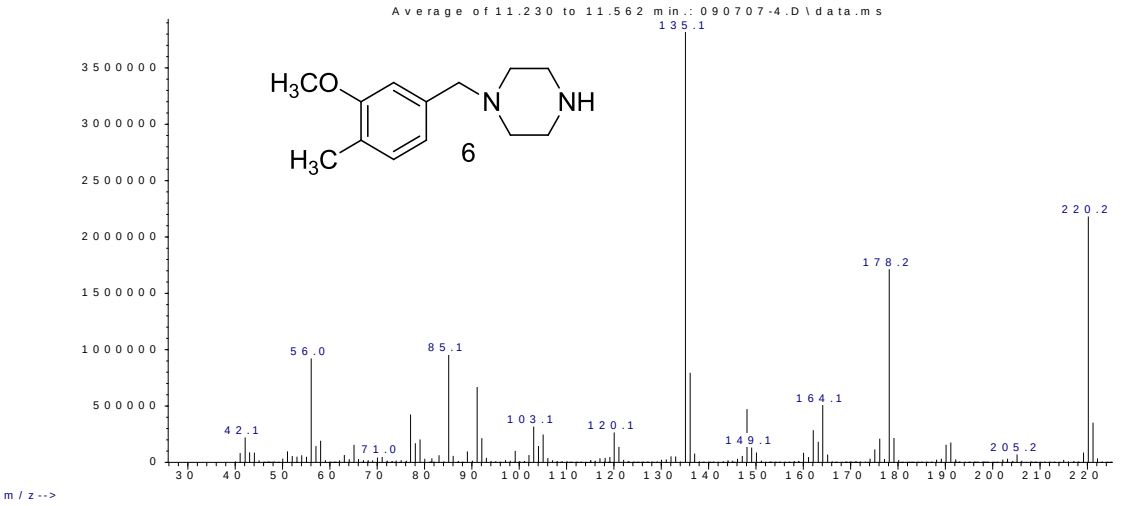


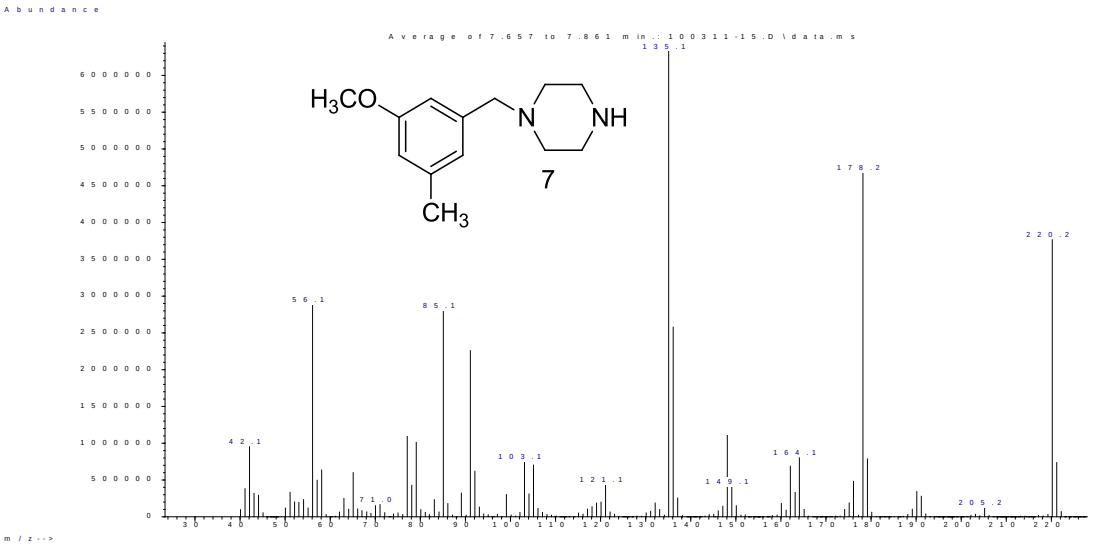


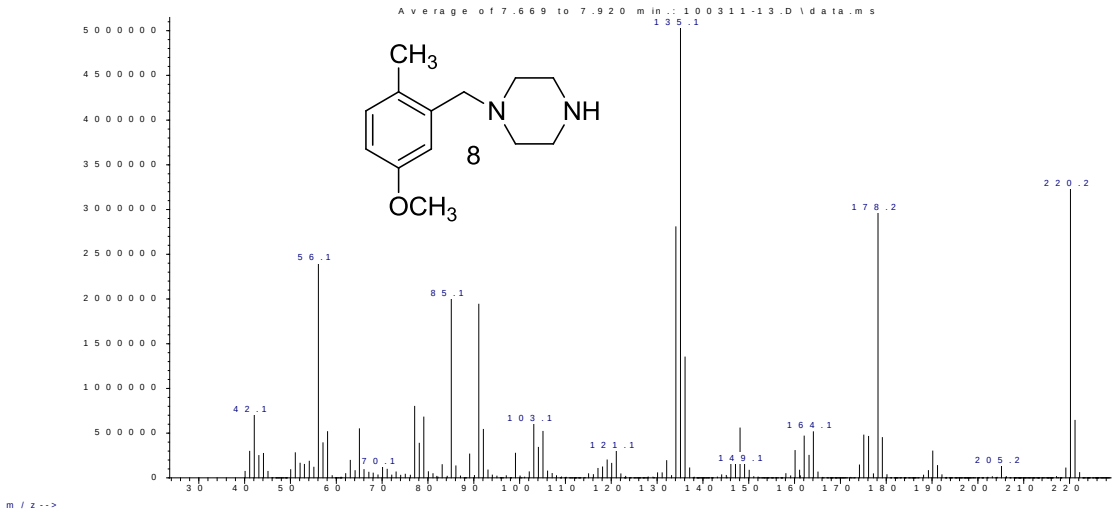


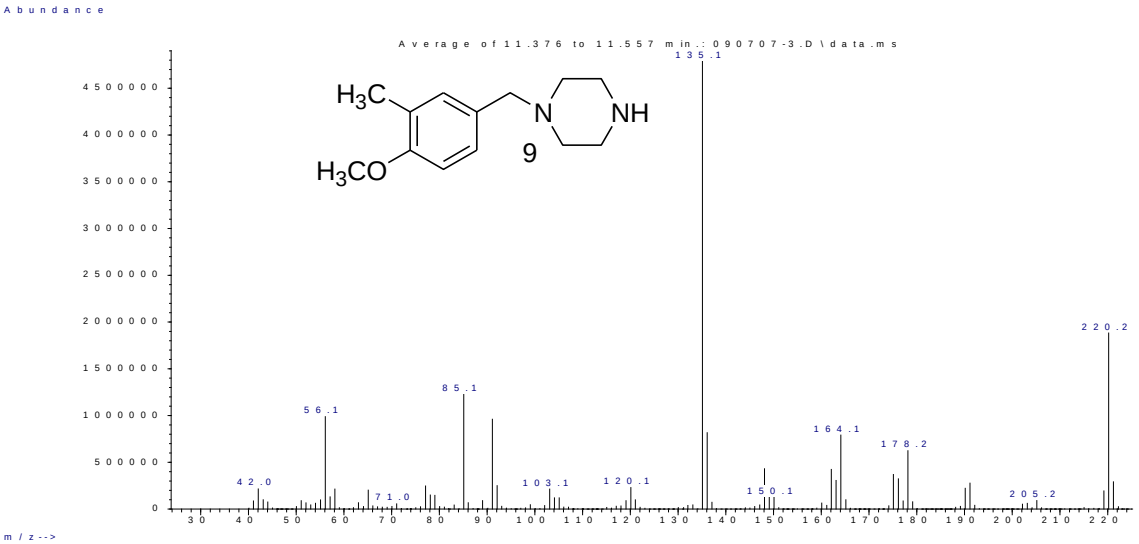


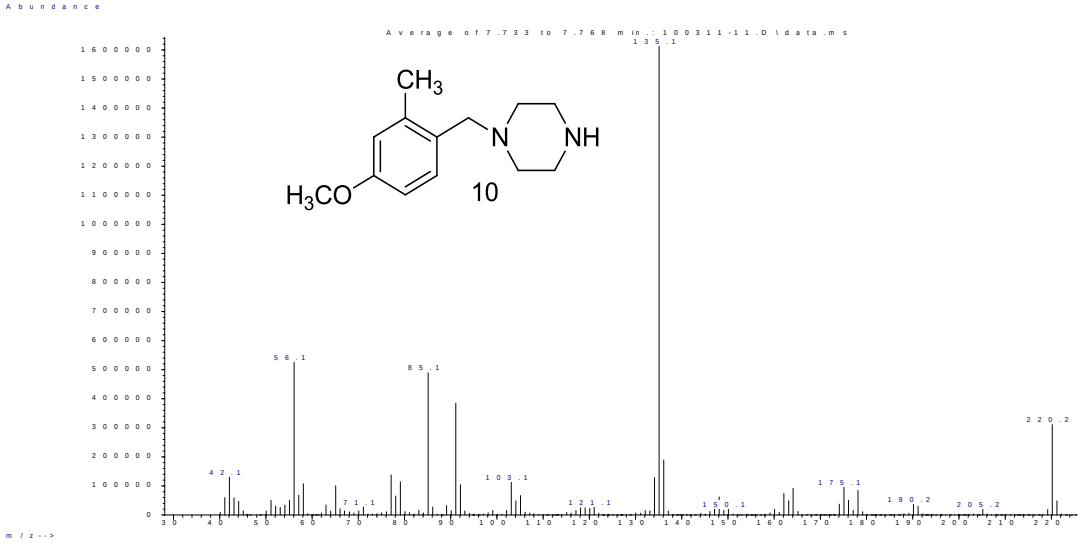












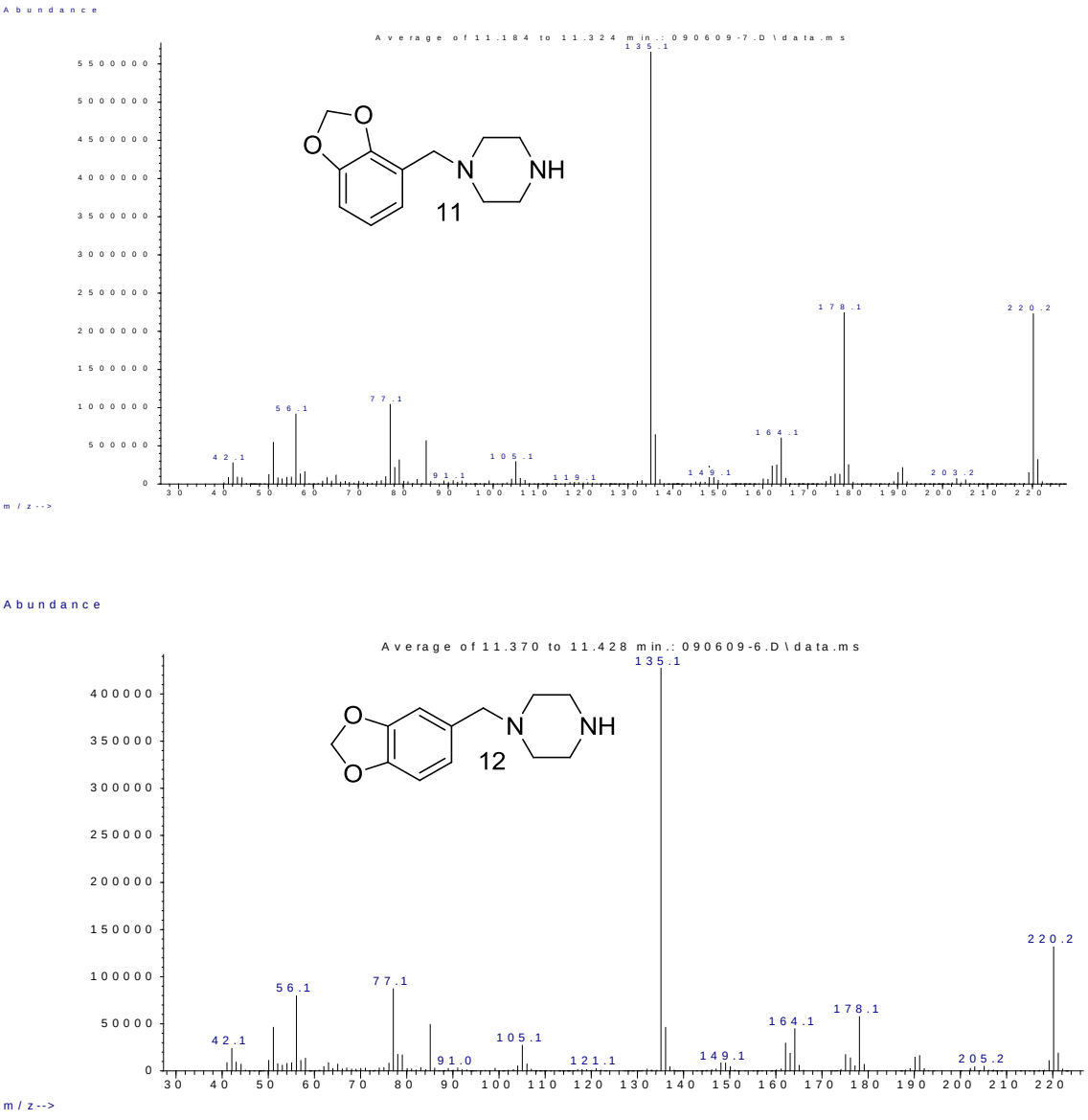
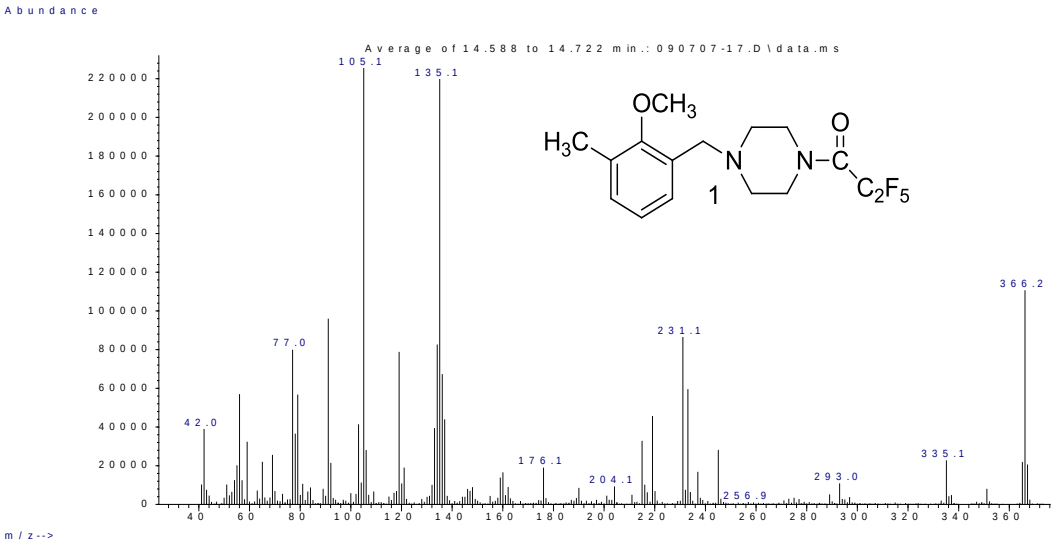
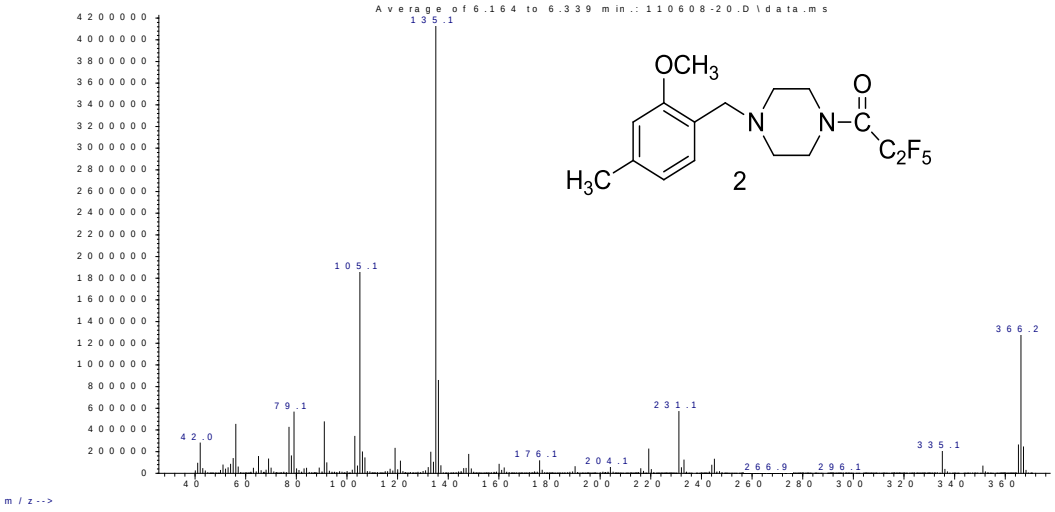


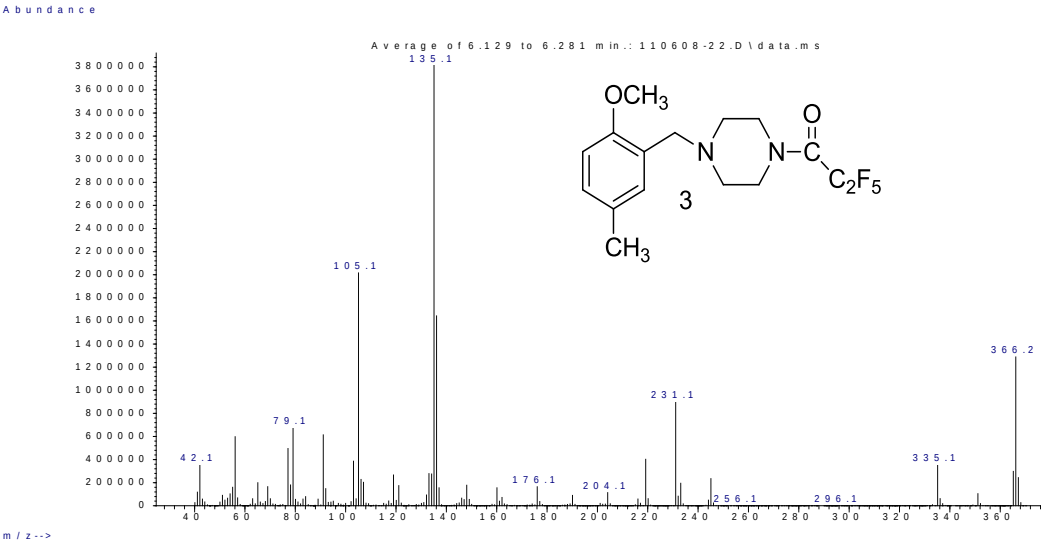
Fig. 4-1: EI mass spectra of the 12 benzylpiperazines in this study.

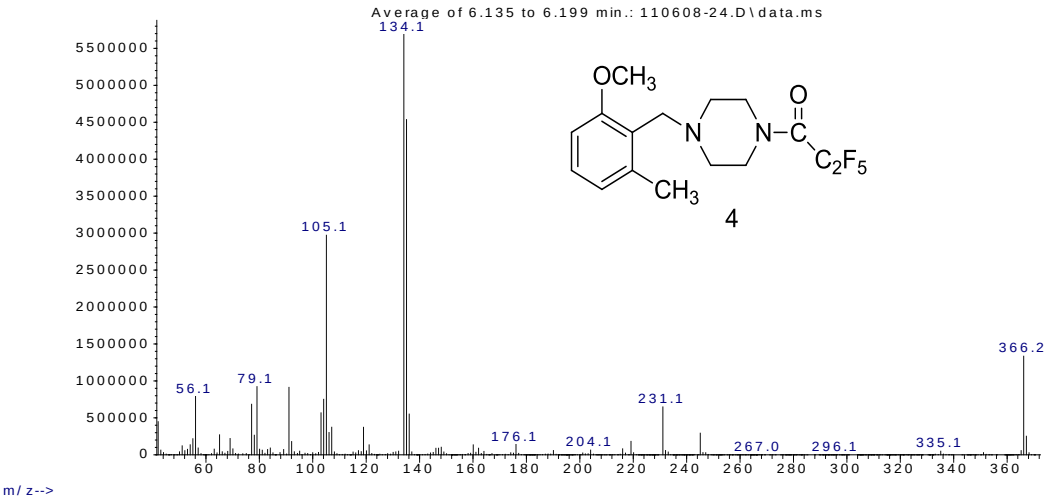
fragment only when the methoxy group is ortho to the piperazine side chain and therefore the site of initial benzylic cation formation as in Compounds 1, 2, 3 and 4 as previously discussed in chapter 3.

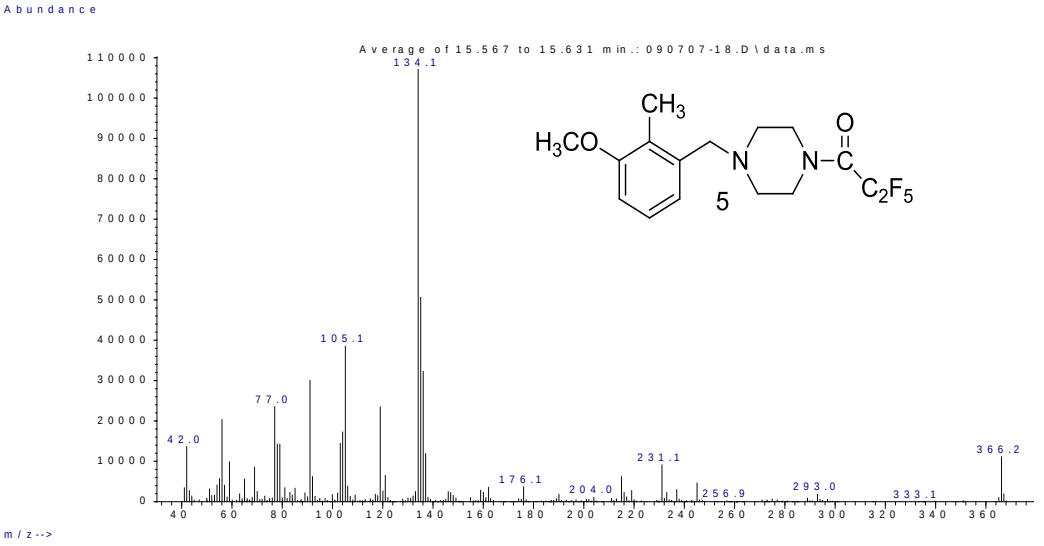
The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric ring substituted benzylpiperazines, in an effort to individualize their mass spectra and identify unique marker ions that would allow discrimination between these twelve compounds. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectrum of 3,4-MDBP and provide data for the exclusion of the other eleven isomers. Figure 4-2 shows the mass spectra of the pentafluoropropionyl amides of the twelve studied compounds as representative spectra for all the perfluoroacyl amides. 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 aromatic ring substituted benzyl cation. Furthermore, an additional fragment ion series occurring at *m/z* 181, 231 and 281 for the TFA, PFPA and HFBA amides, respectively corresponds to the $(M-135)^+$ 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 twelve isomers.

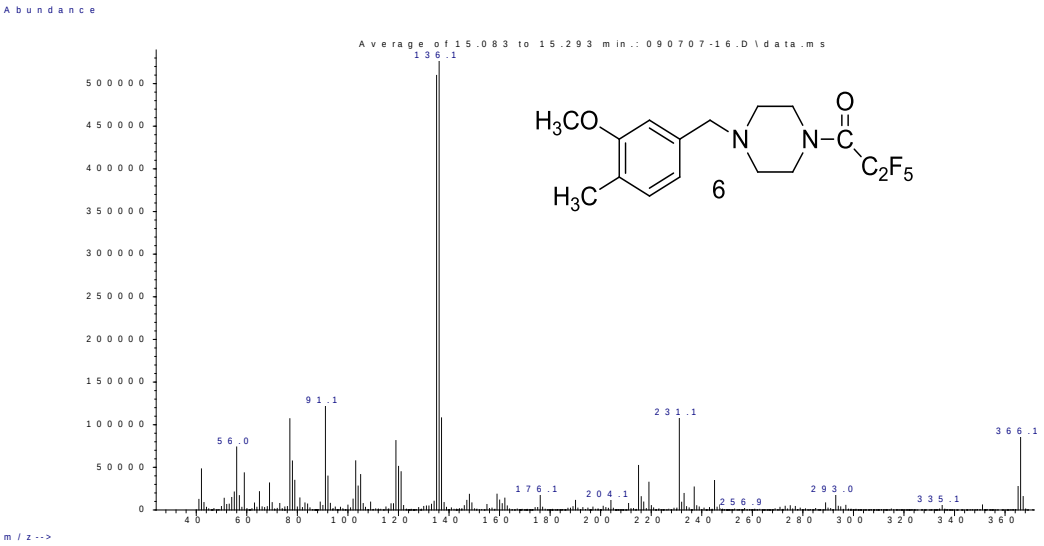


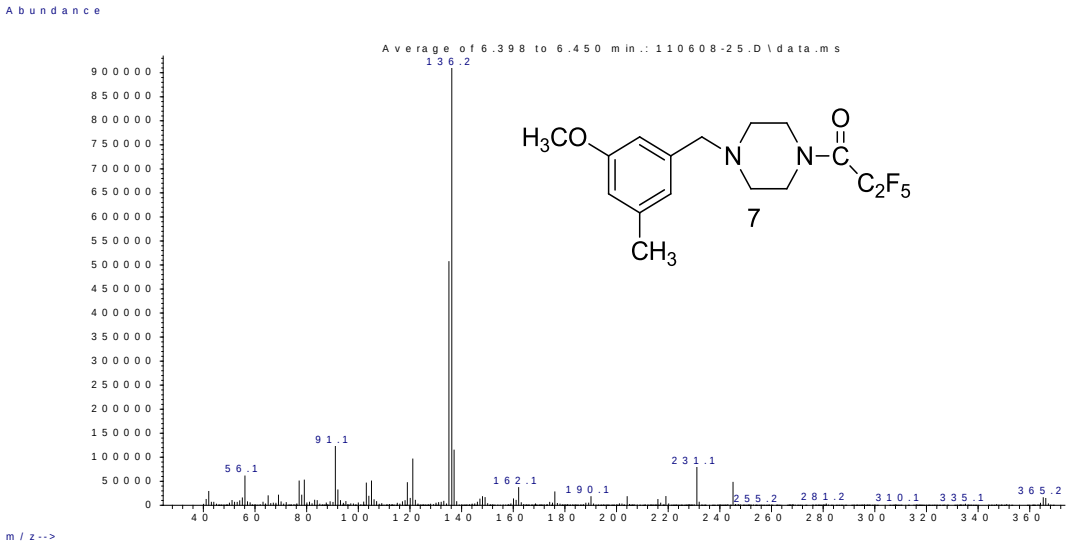












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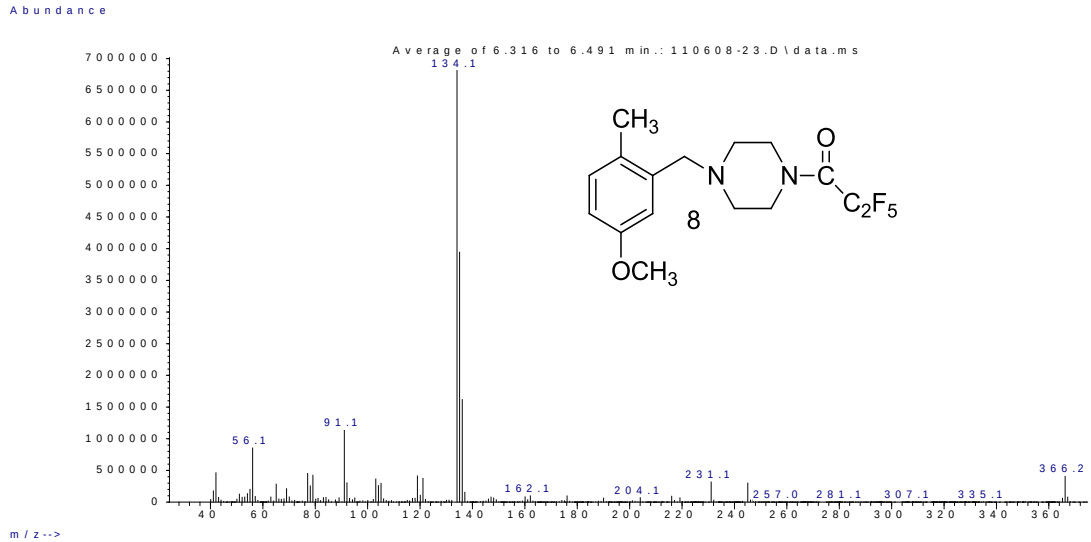

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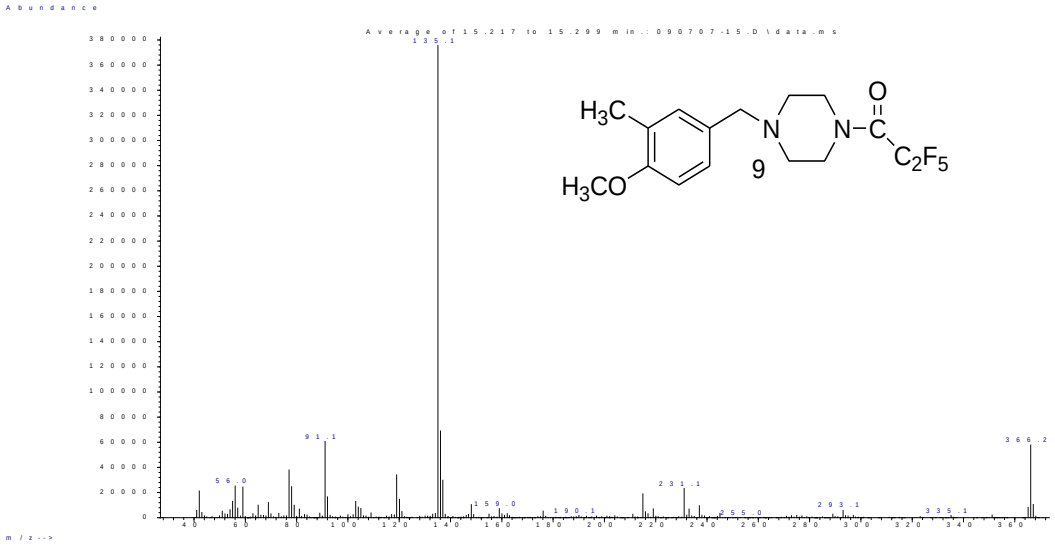

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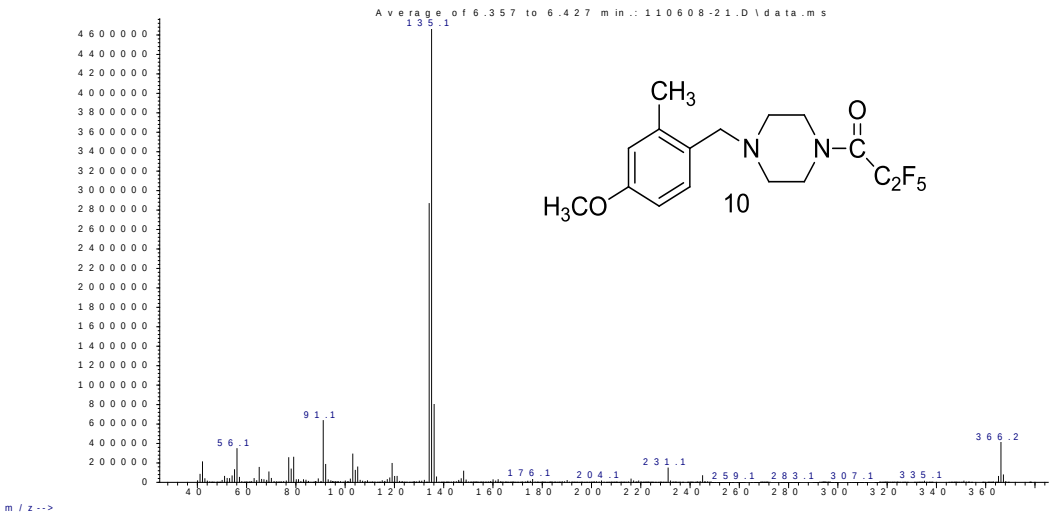

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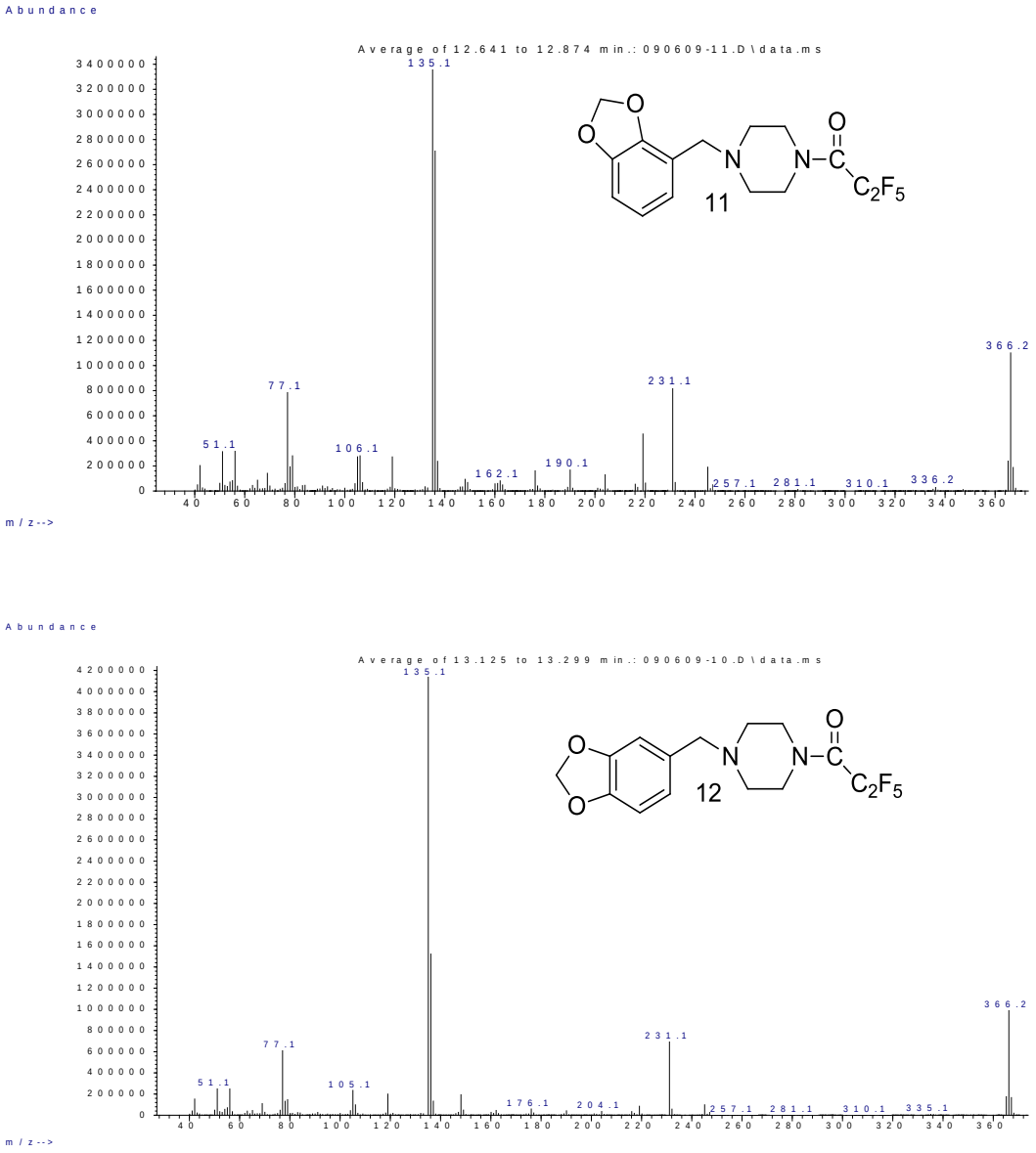
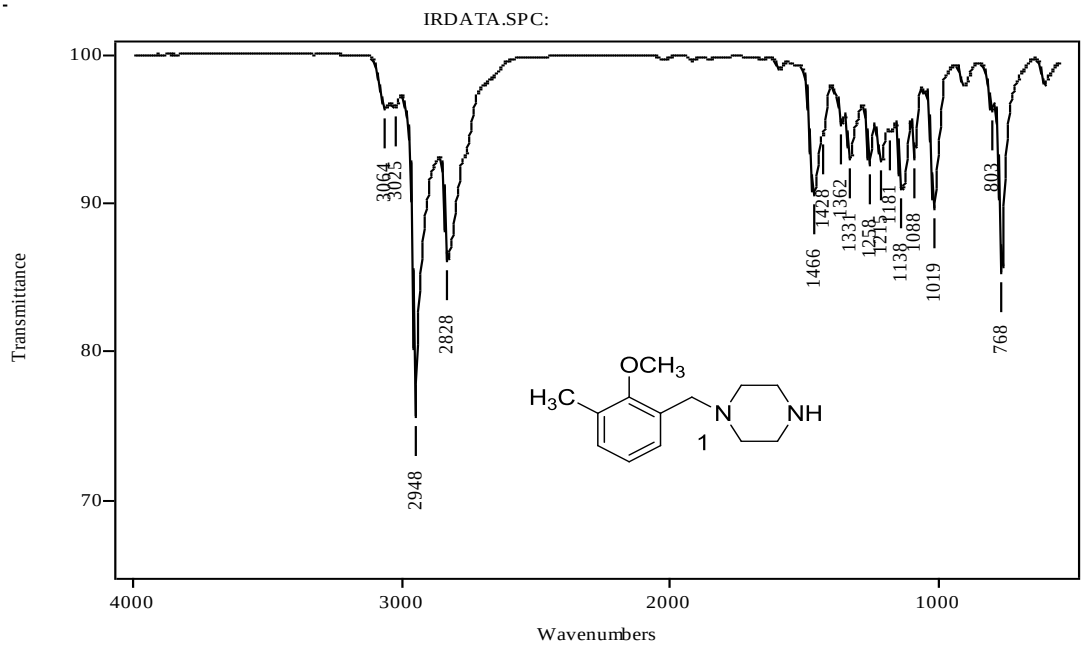


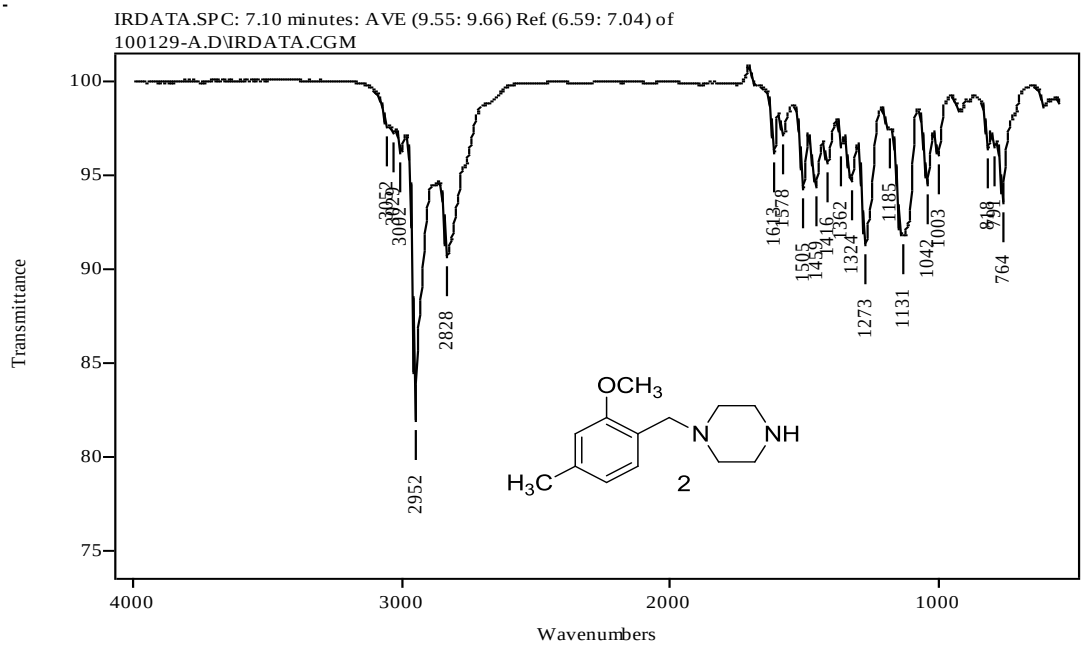
Fig. 4-2: EI mass spectra of the pentafluoropropionylderivatives of the 12 benzylpiperazines in this study.

Vapor-phase Infra-Red Spectrophotometric Studies of the Methylenedioxybenzylpiperazines (MDBPs) and Methoxymethylbenzylpiperazines (MMBPs)

Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the twelve isomeric substituted benzylpiperazines. Infrared analysis should provide compound specificity without the need for chemical modification of the parent molecule. The vapor phase infrared spectra for the twelve benzylpiperazines are shown in Figure 4-3. The spectra were generated in the vapor phase following sample injection into the gas chromatograph. Each compound shows a vapor phase IR spectrum with bands in the regions 650 –1700 cm^{-1} and 2700 – 3100 cm^{-1}. In general, variations in the substitution pattern on the aromatic ring results in variations in the IR spectra in the region 650 – 1700 cm^{-1}. Since the twelve piperazines share the same degree of nitrogen substitution, i.e. the same side chain, they have almost identical IR bands in the 2700 – 3100 cm^{-1} region. However, these compounds can be easily differentiated by the positions and intensities of several IR peaks in the region of 650 – 1700 cm^{-1}. In the preceding chapter, we have already discussed the differentiation of compounds 11 and 12 from compounds 1, 5, 6 and 9. Now we will discuss the differentiation of compounds 11 and 12 from the other 6 compounds.

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-





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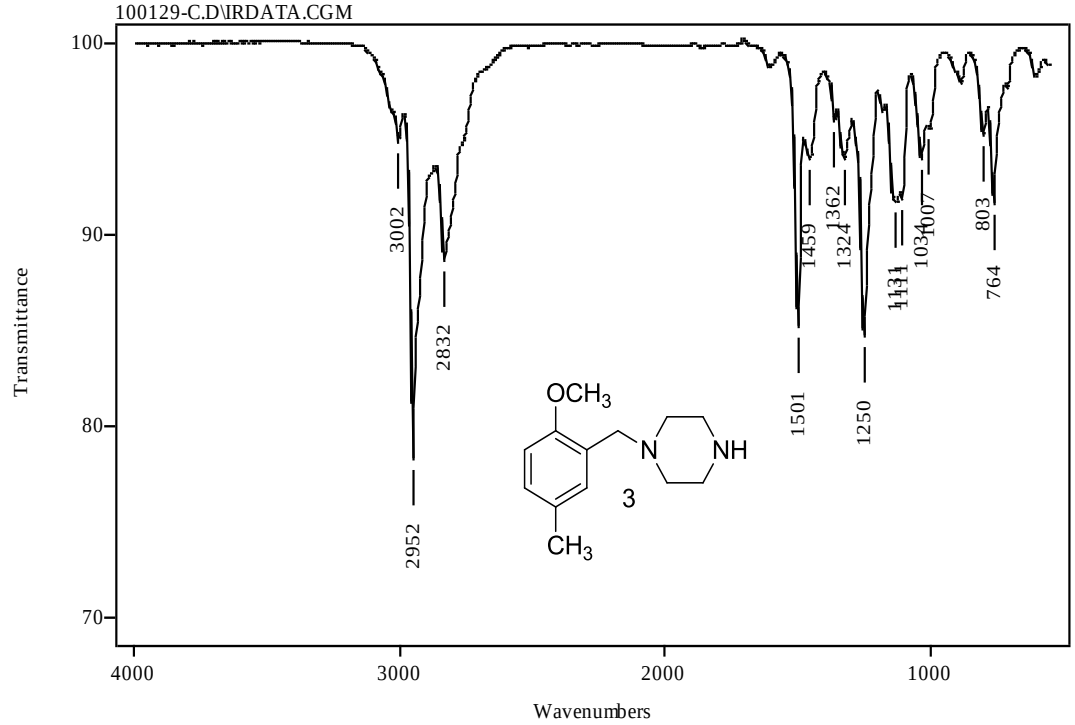

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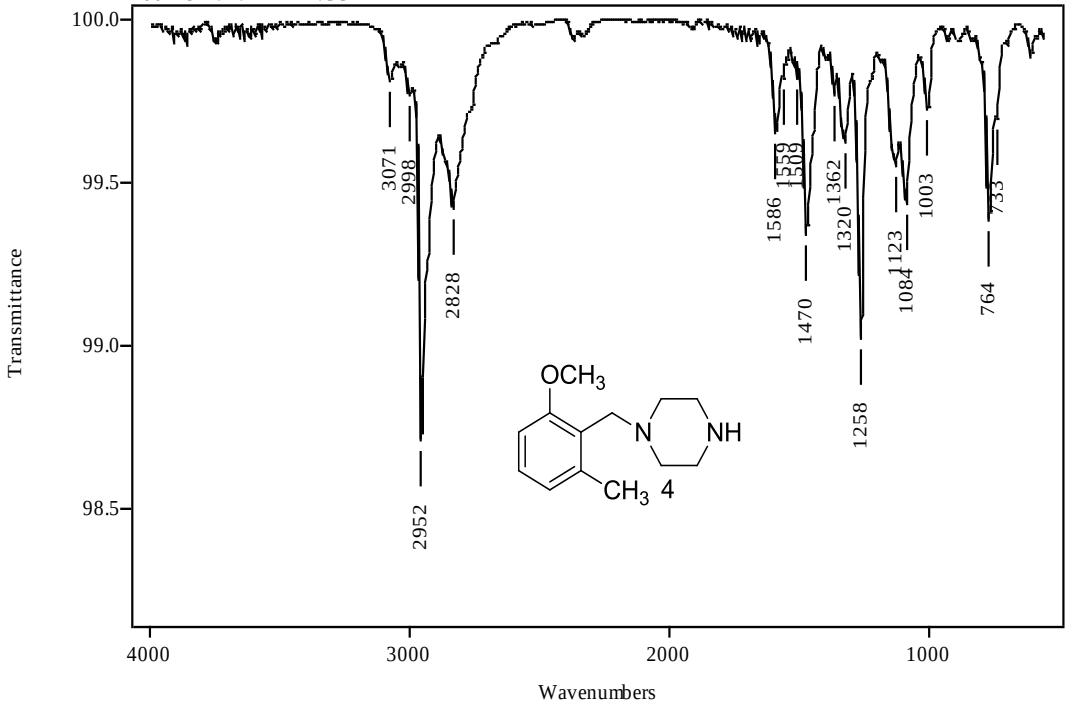

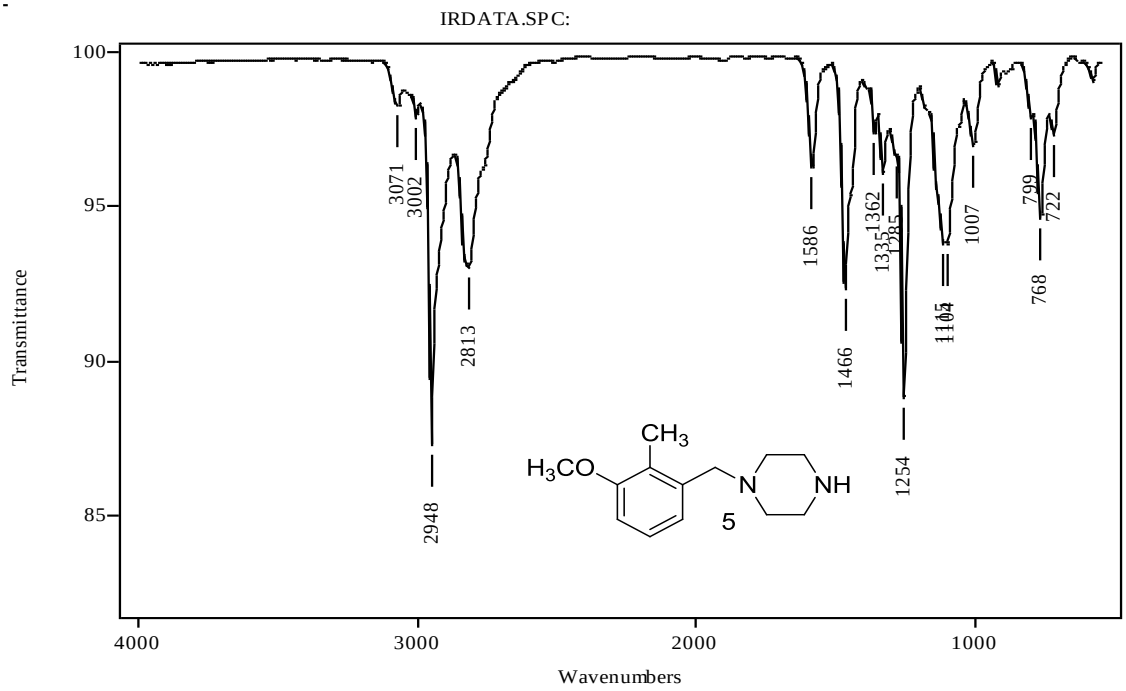

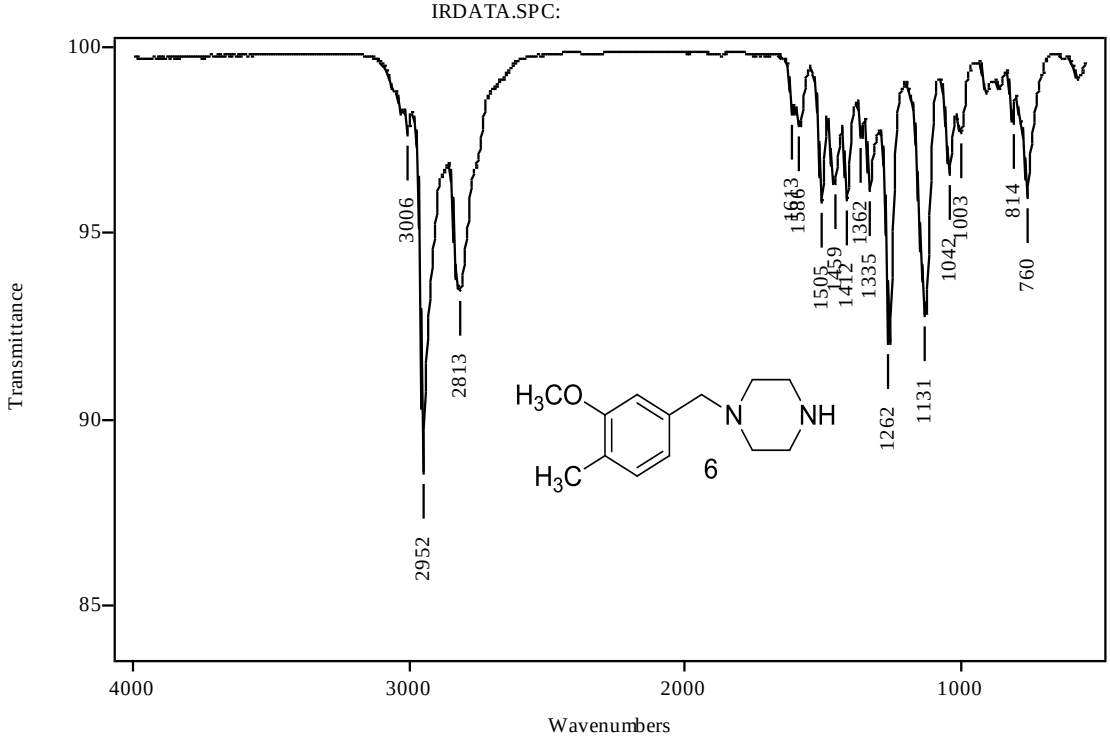

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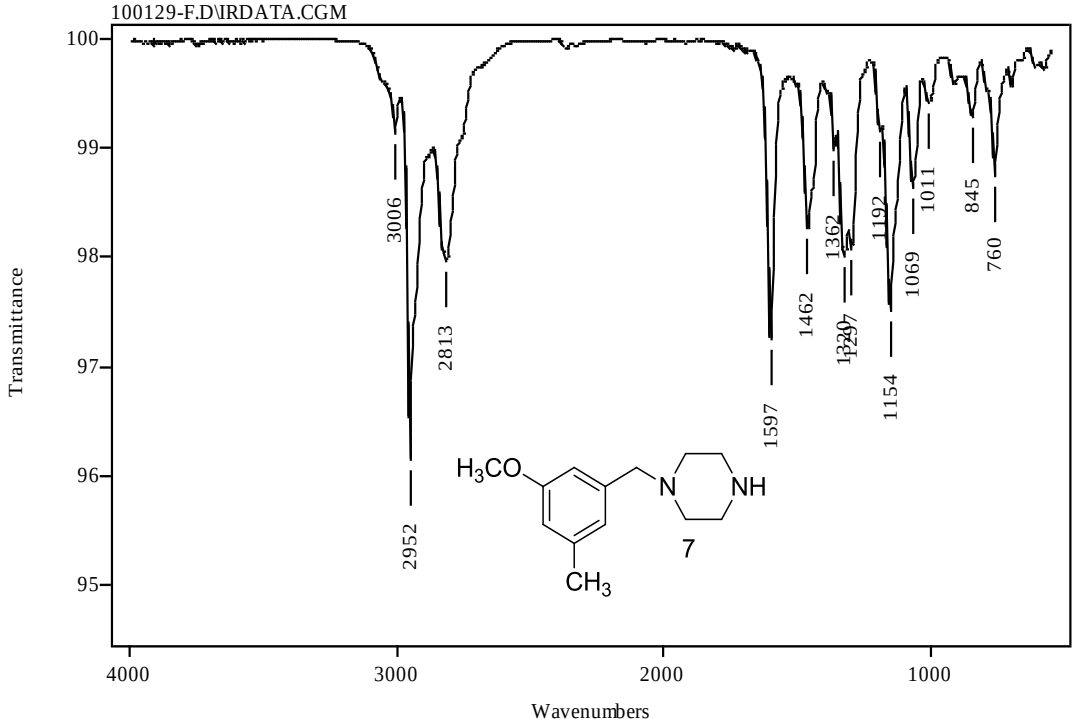

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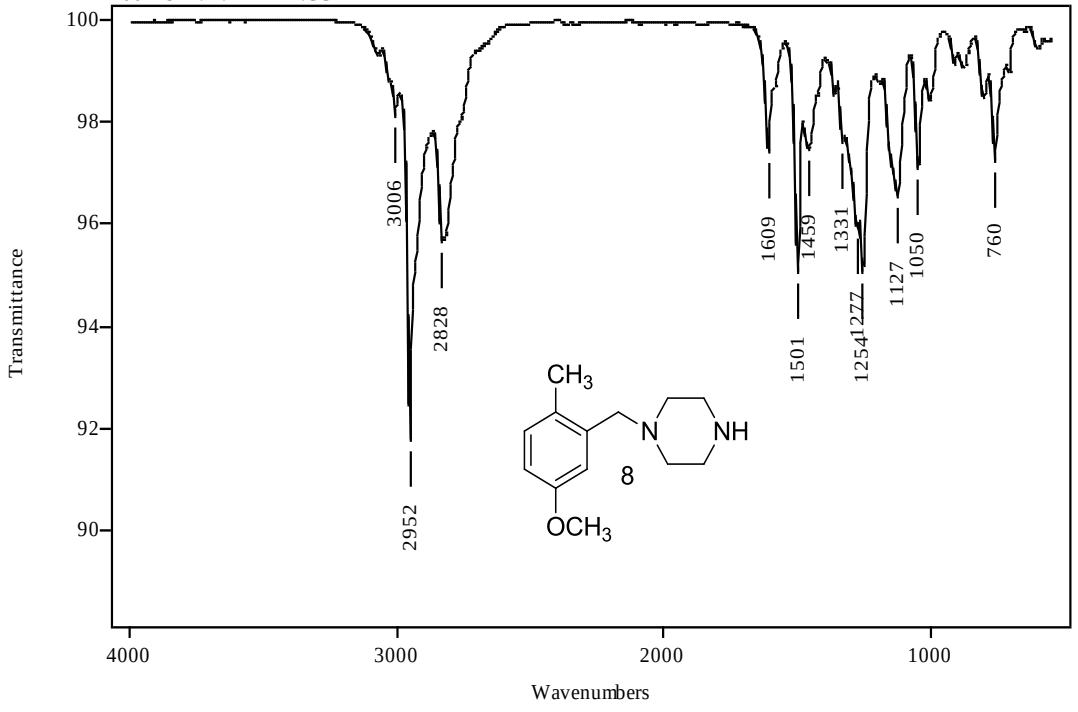

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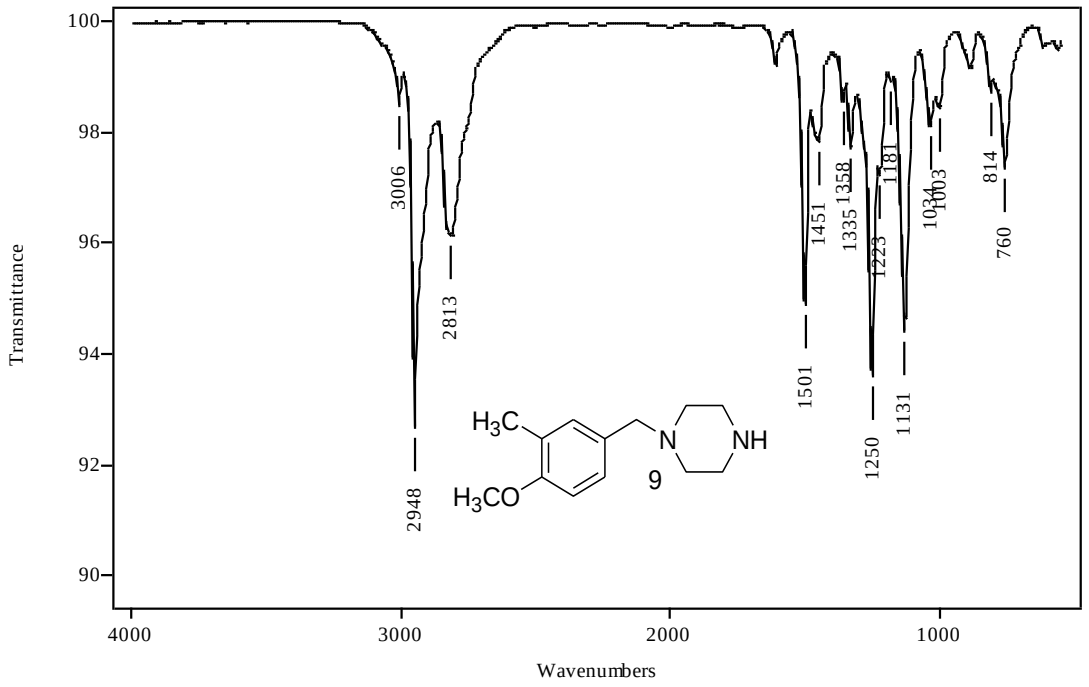

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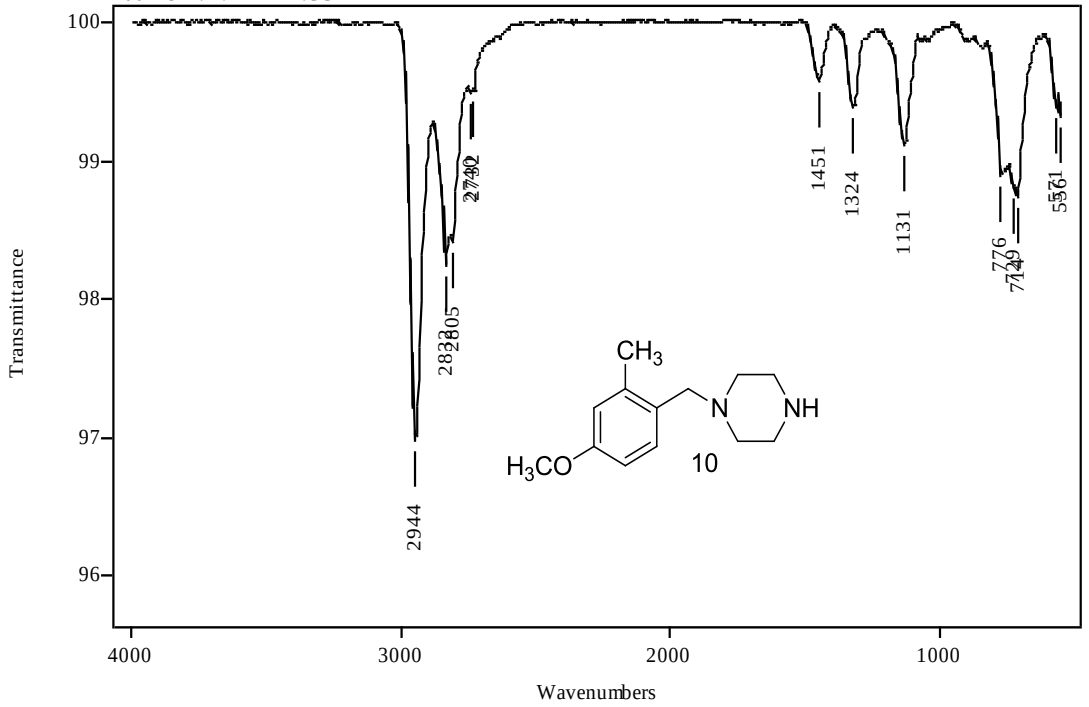


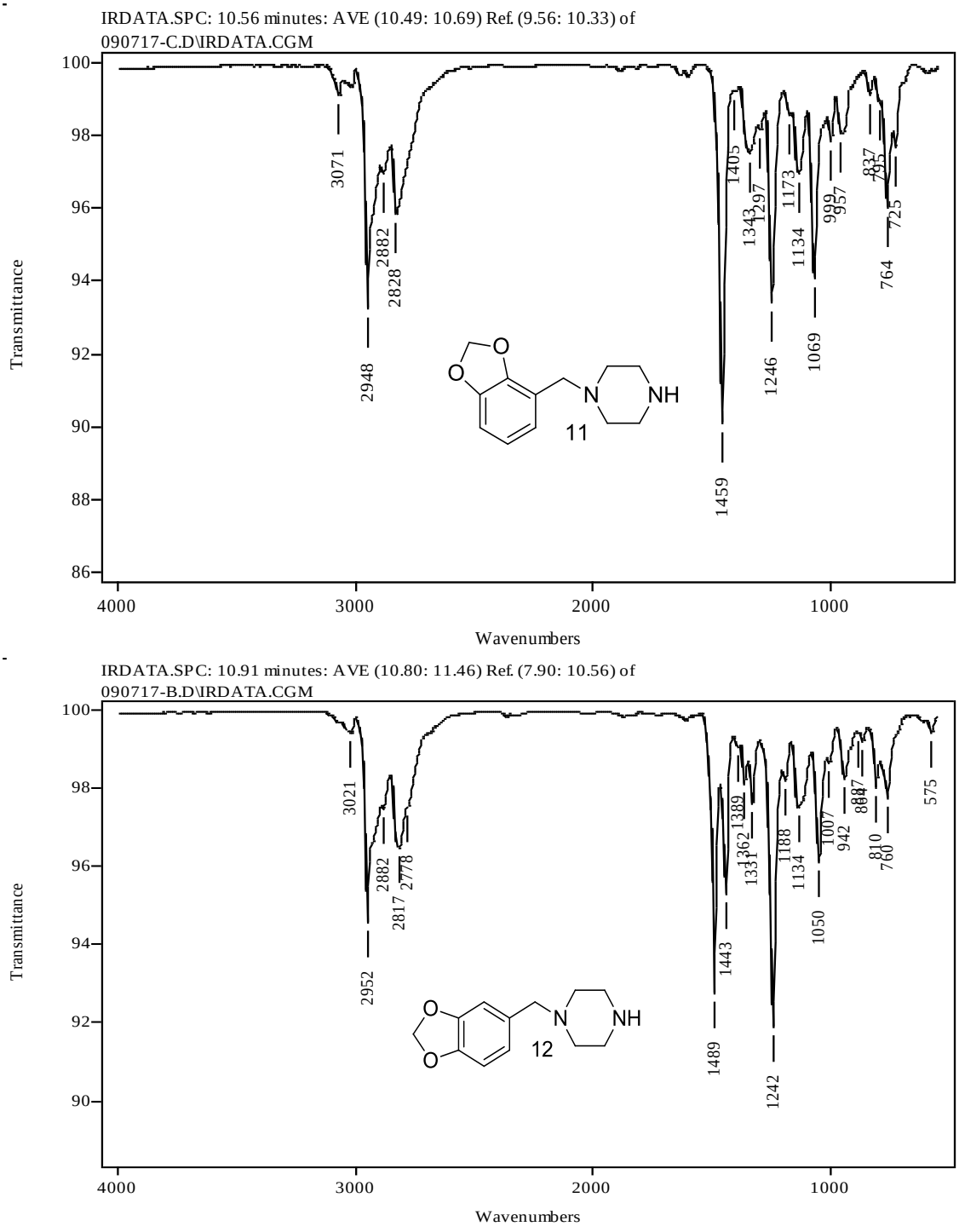


Fig. 4-3: Vapor phase IR spectra of the 12 underivatized methylenedioxy and methoxymethylbenzylpiperazines.

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-regioisomer 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} .

The ten regioisomeric methoxymethylbenzylpiperazines share almost the same IR features in the region of 2700 – 3100 cm^{-1} and can be differentiated by the positions and intensities of several IR peaks in the region of 650 – 1610 cm^{-1}. Compound 3 shows a strong peak at 1501 cm^{-1} which is shifted to a weak one at 1586 cm^{-1} in compound 4 and to a strong peak at 1597 cm^{-1} in compound 7. Compound 8 shows a medium peak at 1254 cm^{-1} which is shifted to 1154 cm^{-1} in compound 7, strong peak at 1250 cm^{-1} in compound 3 and a singlet at 1258 cm^{-1} in compound 4. The twelve isomers share a medium intensity peak in the 760 cm^{-1} range with all three of the 3,4-substituted isomers showing the absorption band at 760 cm^{-1} and this band shifts to slightly higher values at 764 and 768 cm^{-1} for the 2,3-substituted isomers.

Gas Chromatographic Separation of the Methylenedioxybenzylpiperazines (MDBPs) Methoxymethylbenzylpiperazines (MMBPs)

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 the relatively polar stationary phase, 35% diphenyl/65% dimethylpolysiloxane (Rtx-35). The temperature program consisted of an initial temperature of 70ºC for 1 minute, ramped up to 150ºC at a rate of 7.5ºC per minute followed by a hold at 150ºC for 2 minutes then ramped up to 250°C at a rate of 10°C/min and held at 250°C for 15 min. The chromatograms in Figures 4-4 – 4-7 are representatives of the results obtained for all samples on this stationary phase.

In Figure 4-4 the ten methoxymethylbenzylpiperazines are less retained than their isobaric methylenedioxybenzylpiperazines. The drug substance 3,4-MDBP eluted last in this limited series of compounds in all chromatographic experiments. Those isomers having the methoxy group in the ortho position relative to the side chain (compounds 1, 2, 3 and 4) were the first to elute out of the column. As we kept on trying different temperature programs and stationary phases, we could not resolve compounds 5, 8 and 9 from each other as illustrated in Figure 4-4. Therefore, the ten regioisomeric methoxymethylbenzylpiperazines were divided into three subgroups based on the position of the methoxy group relative to the side chain. The first subgroup includes those compounds with the ortho methoxy group (compounds 1-4) in addition to compounds 11 and 12 and their chromatographic separation is in Figure 4-5. The second subgroup includes those compounds with the meta methoxy group (compounds 5-8) in addition to

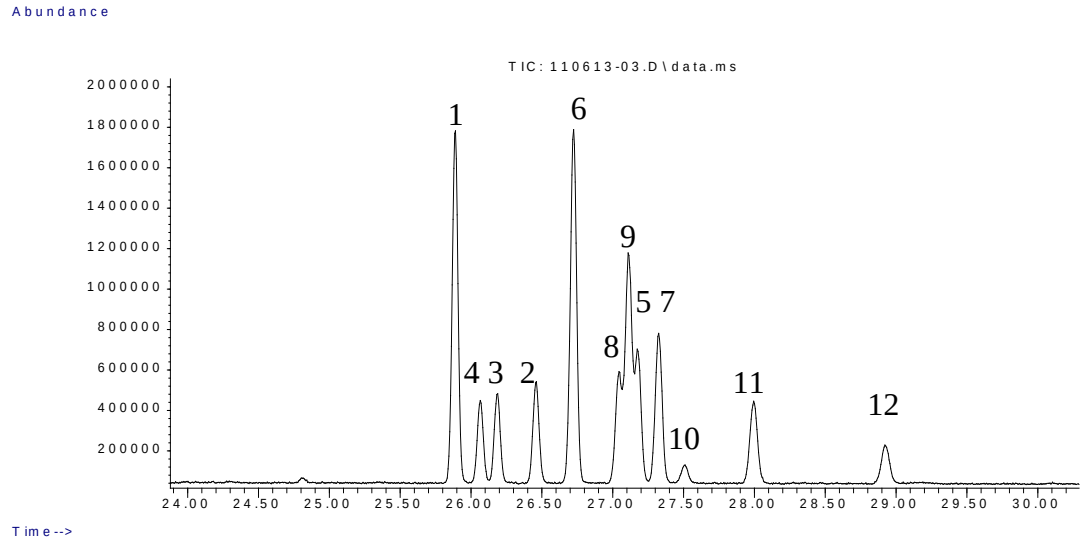


Fig. 4-4: Gas chromatographic separation of the pentafluoropropionyl derivatives of the 12 piperazine isomers using Rtx-35 column. The number over the peak corresponds to the compound number.

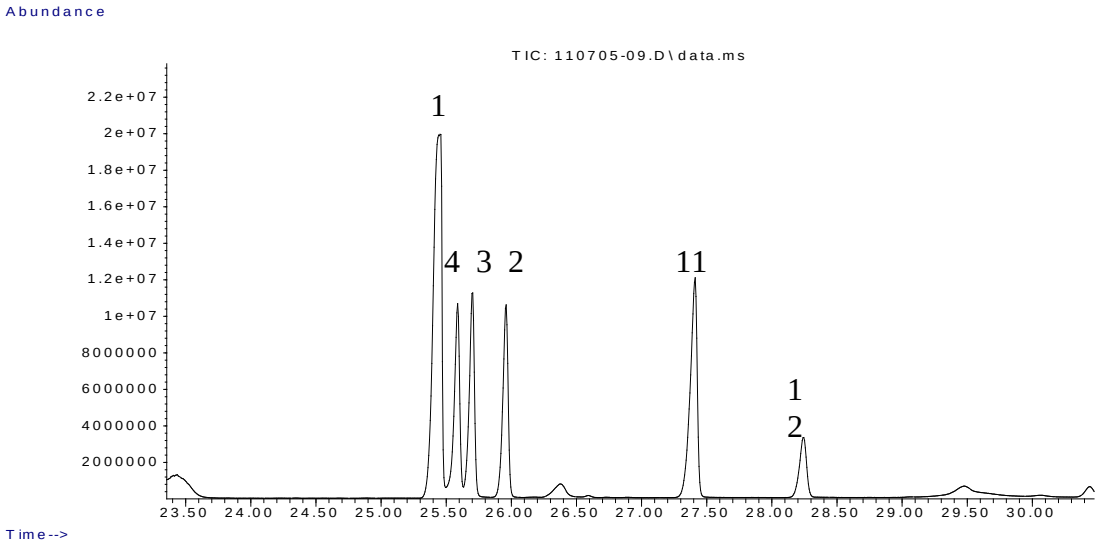


Fig. 4-5: Gas chromatographic separation of the pentafluoropropionyl derivatives of the MMBPs with o-methoxy group and methylenedioxypiperazines using Rtx-35 column. The number over the peak corresponds to the compound number.

compounds 11 and 12 and their chromatographic separation is in Figure 4-6. Finally, the third subgroup includes those compounds with the para methoxy group (compounds 9 and 10) in addition to compounds 11 and 12 and their chromatographic separation is in Figure 4-7.

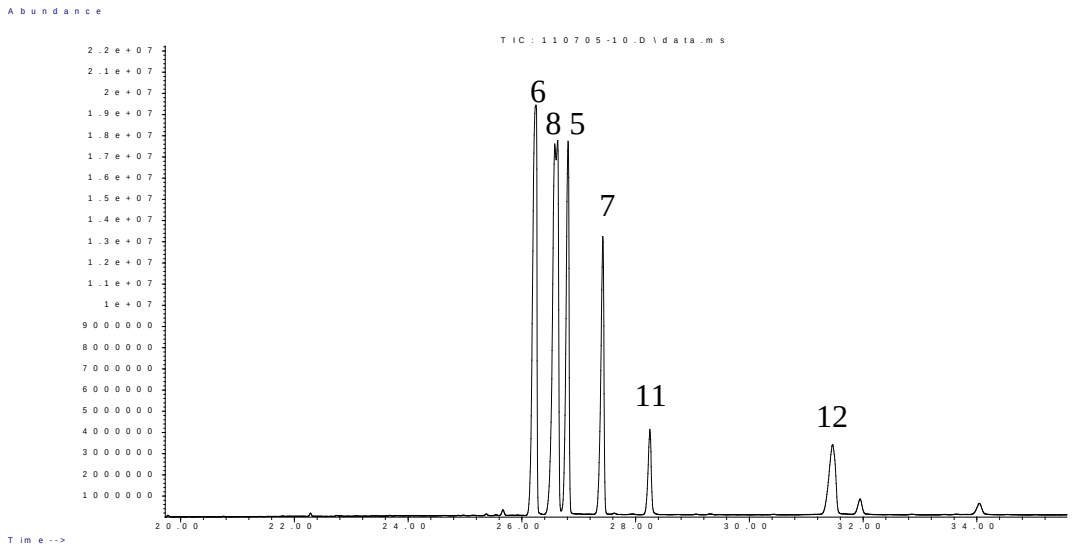


Fig. 4-6: Gas chromatographic separation of the pentafluoropropionyl derivatives of the MMBPs with m-methoxy group and methylenedioxypiperazines using Rtx-35 column. The number over the peak corresponds to the compound number.

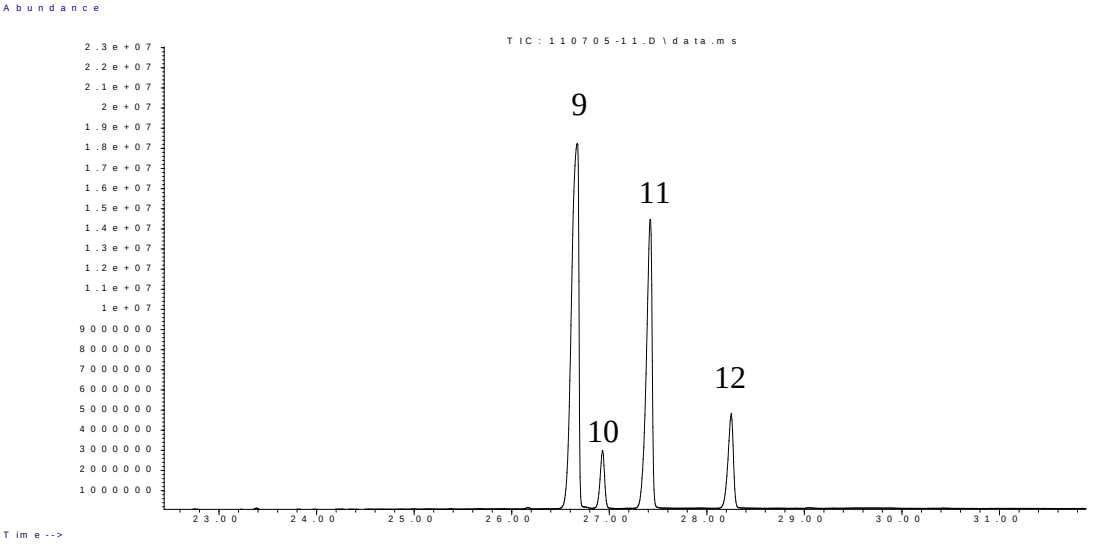


Fig. 4-7: Gas chromatographic separation of the pentafluoropropionyl derivatives of the MMBPs with p-methoxy group and methylenedioxypiperazines using Rtx-35 column. The number over the peak corresponds to the compound number.

Conclusion

The ten methoxymethylbenzylpiperazines have an isobaric relationship to the potential drug of abuse 3,4-MDBP and its regioisomer 2,3-MDBP. All twelve compounds show almost the same fragment ions in their EI mass spectra. Chemical derivatization (perfluoroacylation) did not offer any unique marker ion to allow identification of one compound to the exclusion of the others. GC-IRD offered unique and characteristic IR spectra that allowed for discrimination among these compounds in the region between 650-1700 cm^{-1}. The twelve pentafluoropropionyl isomers were partially resolved by gas chromatography on the polar stationary phase Rtx-35.

References

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 Science International 121 (2001) 47-56.

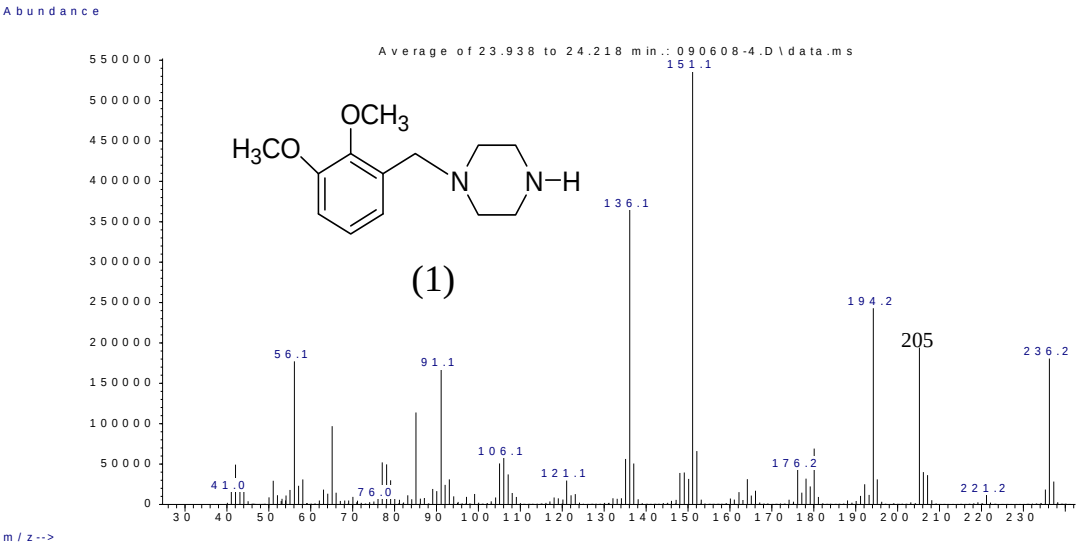
Chapter 5

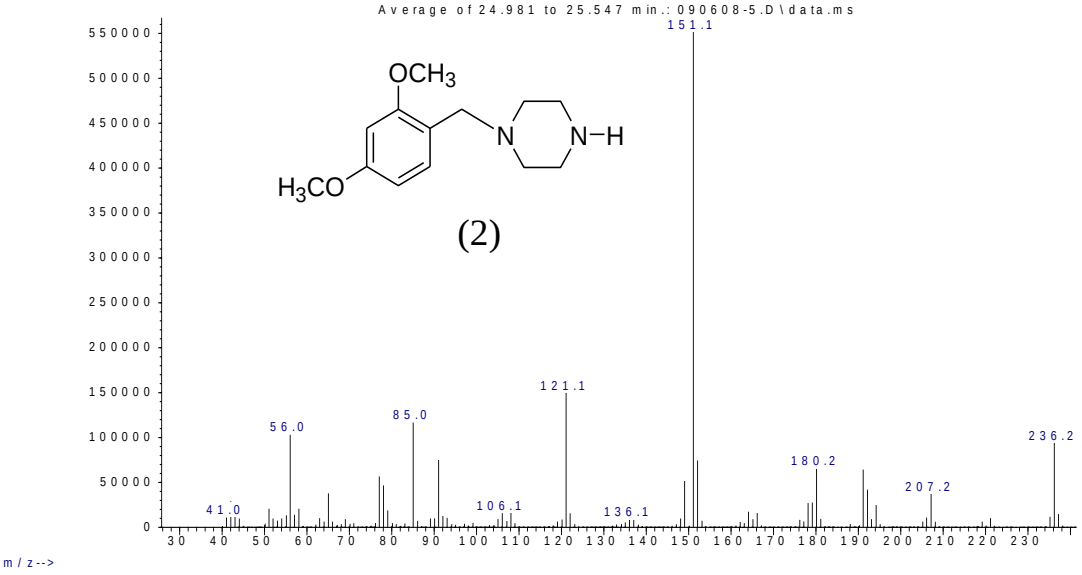
GC-MS and GC-IRD Studies on the Six Ring Regioisomeric Dimethoxybenzylpiperazines (DMBPs)

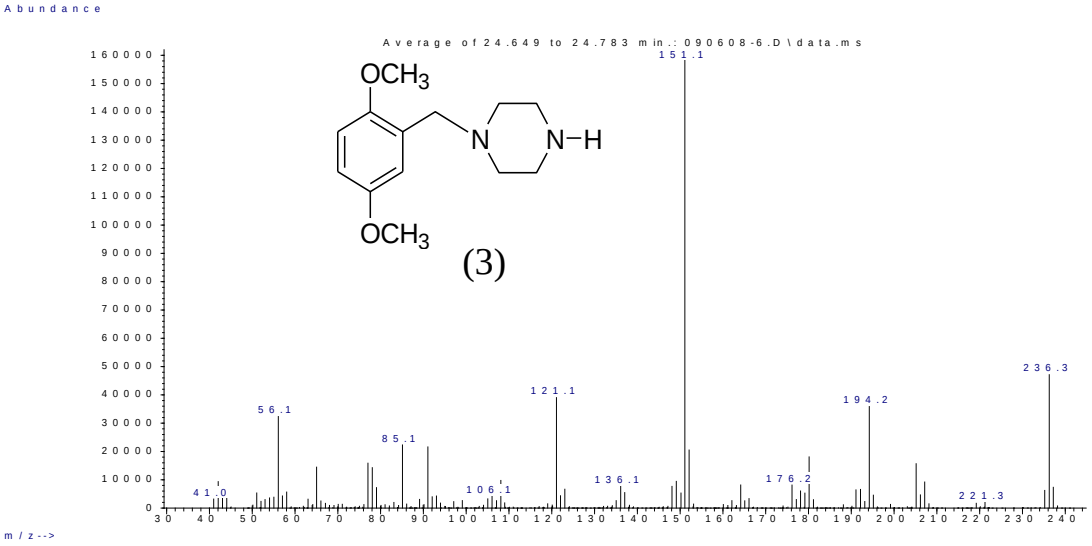
Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the differentiation between the six regioisomeric dimethoxybenzylpiperazines. These six regioisomeric substances are resolved by GC and the vapor phase infrared spectra clearly differentiate among the six dimethoxybenzyl substitution patterns. The mass spectra for the six regioisomeric dimethoxybenzylpiperazines are almost identical. With only the 2,3-dimethoxy isomer showing one unique major fragment ion at m/z 136. Thus mass spectrometry does not provide for the confirmation of identity of any one of the six isomers to the exclusion of the other compounds. Perfluoroacylation of the secondary amine nitrogen for each of the six 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.

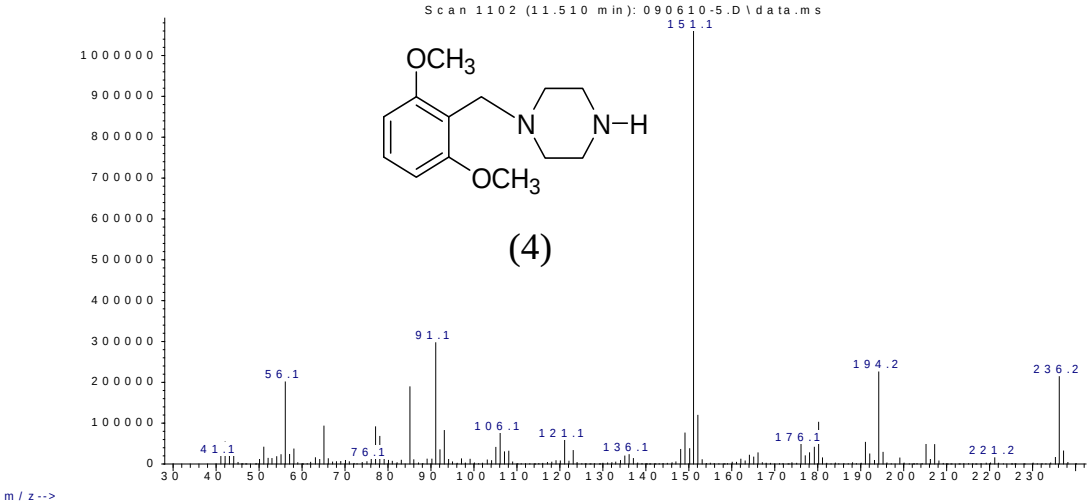
Mass spectral studies of the underivatized and perfluoroacylated derivatives of the Dimethoxybenzylpiperazines (DMBPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 5-1 shows the EI mass spectra of the six regioisomeric dimethoxybenzylpiperazines (Compounds 1-6). The mass spectra in Figure 5-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. The common fragment ions observed for the regioisomeric dimethoxy groups substituted on the aromatic ring likely indicate that the piperazine ring is the initial source for most of the fragmentation. The dimethoxybenzyl cation m/z 151 is the base peak in all these spectra. The structures for the fragment ions in the unsubstituted aromatic ring for benzylpiperazine BZP have been described by [de Boer *et al*, 2001]. Applying these fragmentation pathways to dimethoxybenzylpiperazines (DMBPs) yield the fragment ions at m/z 194, 180, 179, 178, 151, 121, 85 and 56 as shown in Figure 5-2. The structures for the fragmentation in the six DMBP regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual DMBP regioisomer except for the characteristic high relative abundance ion at m/z 136 which appears to be specific for the 2,3-regioisomer.









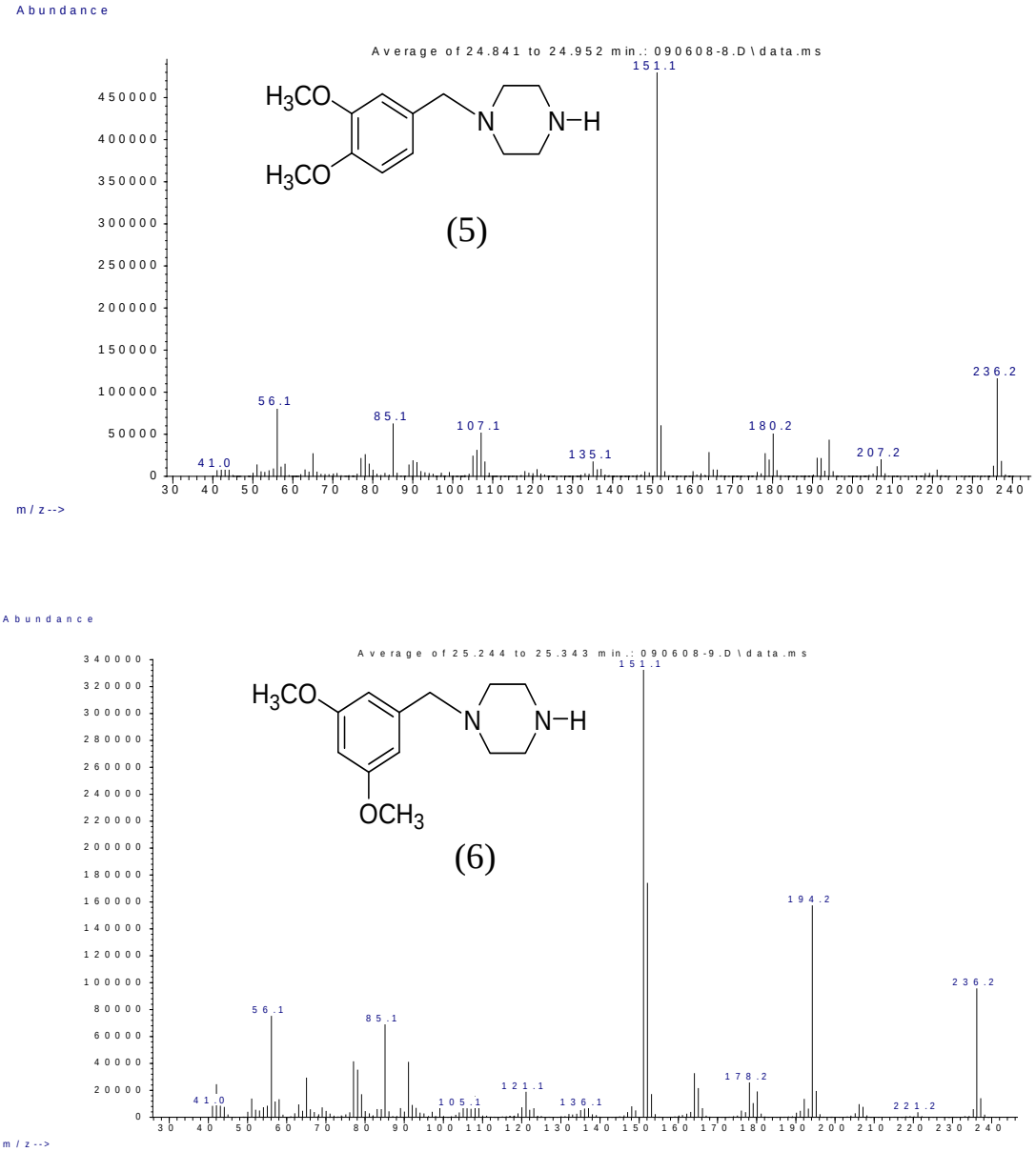
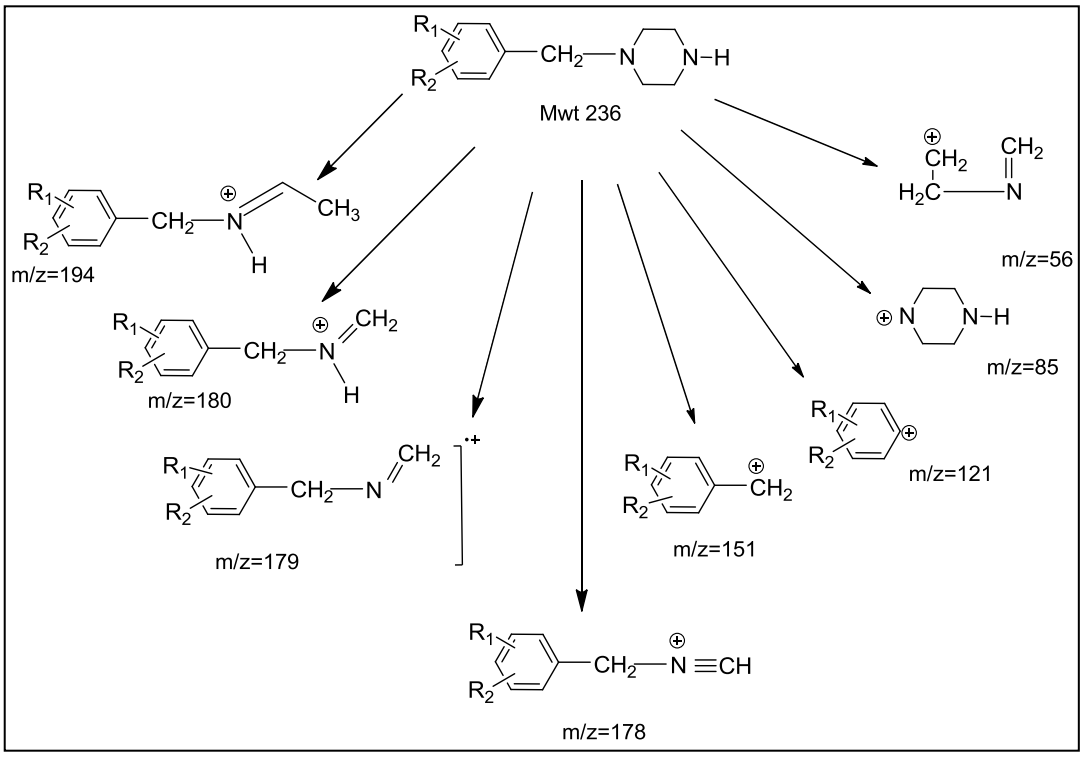


Fig. 5-1: EI mass spectra of the six dimethoxybenzylpiperazines.



$R_1 = R_2 = OCH_3$

Fig. 5-2: EI mass spectral fragmentation pattern of the underivatized dimethoxybenzylpiperazines.

Exact mass analysis using GC-TOF-MS confirmed the m/z 136 ion as the elemental composition $C_8H_8O_2$. Figure 5-3 shows the exact mass measurement results for the m/z 136 ion in the 2,3-isomer. The upper panel (A) shows the expected/calculated mass for the $C_8H_8O_2$ elemental composition and the lower panel (B) shows the experimental results along with the degree of agreement (0.1 Da, 0.7 ppm) between the calculated and experimental results.

The proposed structure and mechanism for the formation of the m/z 136 $C_8H_8O_2$ ion is shown in Figure 5-4. The suggested structure for this fragment involves loss of a methyl group from one of the methoxy-substituents of this crowded 1,2,3-trisubstituted aromatic ring. The proposed structure for the m/z 136 ion is supported by the mass spectra of the mono-, tri-, and hexa-deutero labeled forms of this compound.

The mono-deuterium labeled compound was prepared by reducing the imine formed between 2,3-dimethoxybenzaldehyde and piperazine with sodium cyanoboro-deuteride. The precursor aldehydes for the d_6- and d_3-forms of Compound 1 were prepared by treating 2,3-dihydroxybenzaldehyde and 2-hydroxy-3-methoxybenzaldehyde respectively with d_3-methyliodide in the presence of potassium carbonate. The resulting deuterium labeled aldehydes were reductively aminated with piperazine in the presence of sodium cyanoborohydride.

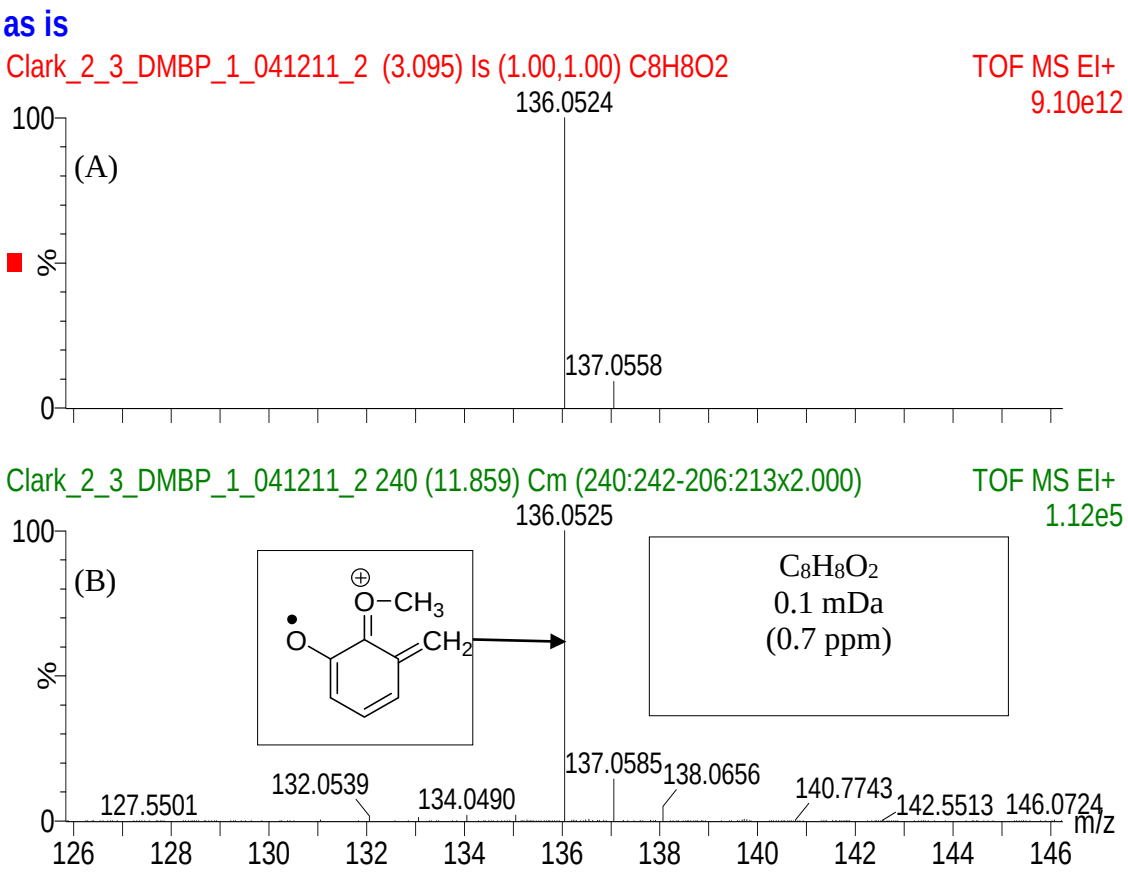


Fig. 5-3: GC-TOF mass spectral analysis of the m/z 136 ion for 2,3-dimethoxybenzylpiperazine.

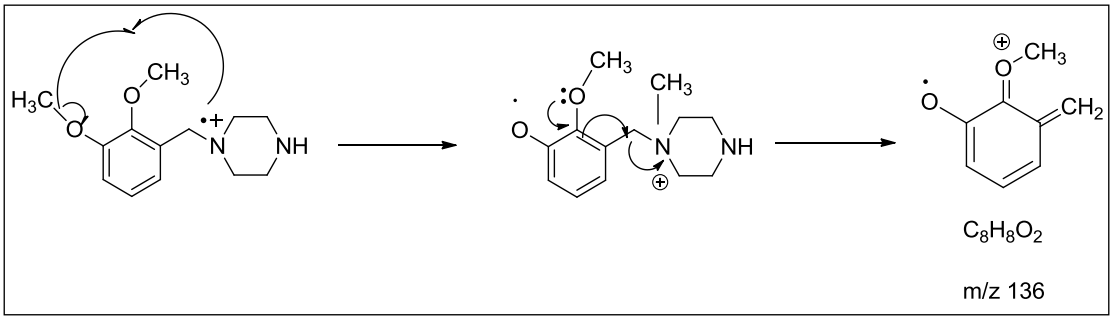


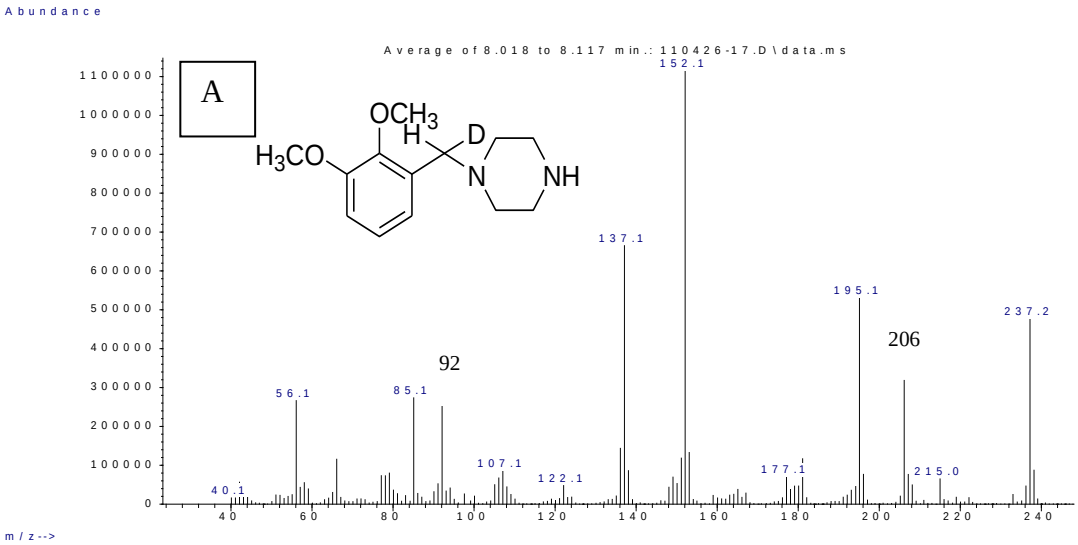
Fig. 5-4: Proposed mechanism for the formation of the m/z 136 ion in the mass spectrum of 2,3- dimethoxybenzylpiperazine.

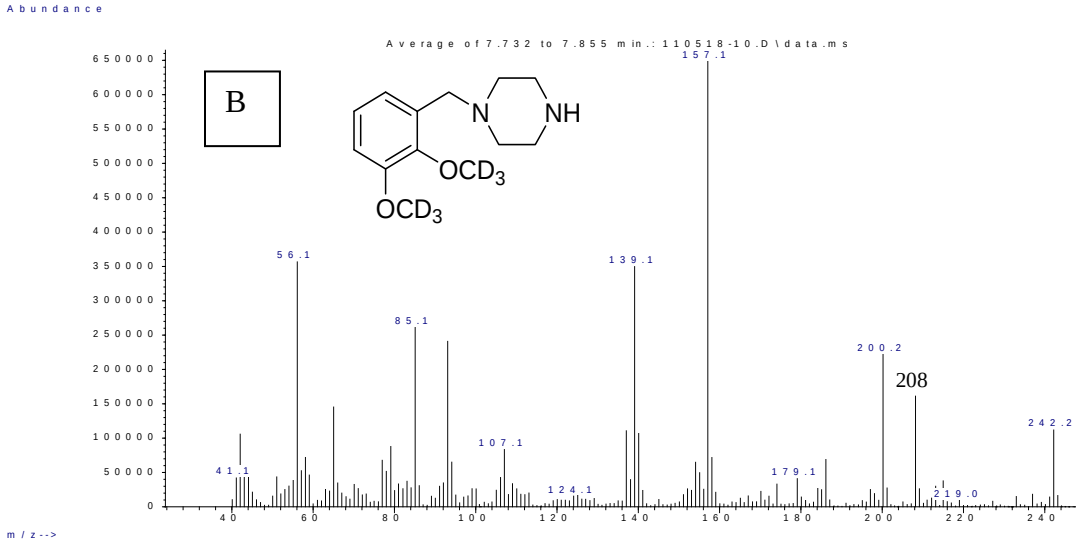
The mass spectra for the three deuterium labeled forms of Compound 1 are shown in Figure 5-5. The spectrum in Figure 5-5.A shows that the single deuterium label at the benzylic position remains a part of the ion in question since the mass increased by 1 Da to m/z 137 in this example. These results confirm that the benzylic carbon is a component of the m/z 136 ion observed in the mass spectrum for Compound 1. These results indicate that one methyl group from the 2- and 3-methoxy substituents must be eliminated in order to reach the $C_8H_8O_2$ elemental composition.

The proposed loss of a methyl group from one of the crowded methoxy-groups is confirmed by the mass spectrum for the d_6-form of Compound 1 (Figure 5-5.B). The methyl groups of both methoxy substituents are labeled with deuterium (di-OCD_3) in this form of Compound 1. The spectrum in Figure 5-5.B shows the ion in question now at m/z 139, indicating the presence of three deuterium species in the resulting fragment. Furthermore, these data confirm the loss of a CD_3 moiety to yield the m/z 139 from the d_6-form of Compound 1.

The mass spectrum in Figure 5-5.C provides information which suggests 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 Figure 5-4. The mass spectrum in Figure 5-5.C is for the 2-trideuteromethoxy-3-methoxybenzylpiperazine and indicates the majority of the label remains in the ion in question yielding the m/z 137, 138, 139 cluster of fragment masses. This cluster of ions is likely the result of some scrambling of hydrogen/deuterium between the adjacent methoxy groups before the fragmentation process is complete. The fragmentation mechanism suggested in Figure 5-4 shows the

migration of the methyl group from the 3-methoxy substituent to the piperazine nitrogen to yield a quaternary





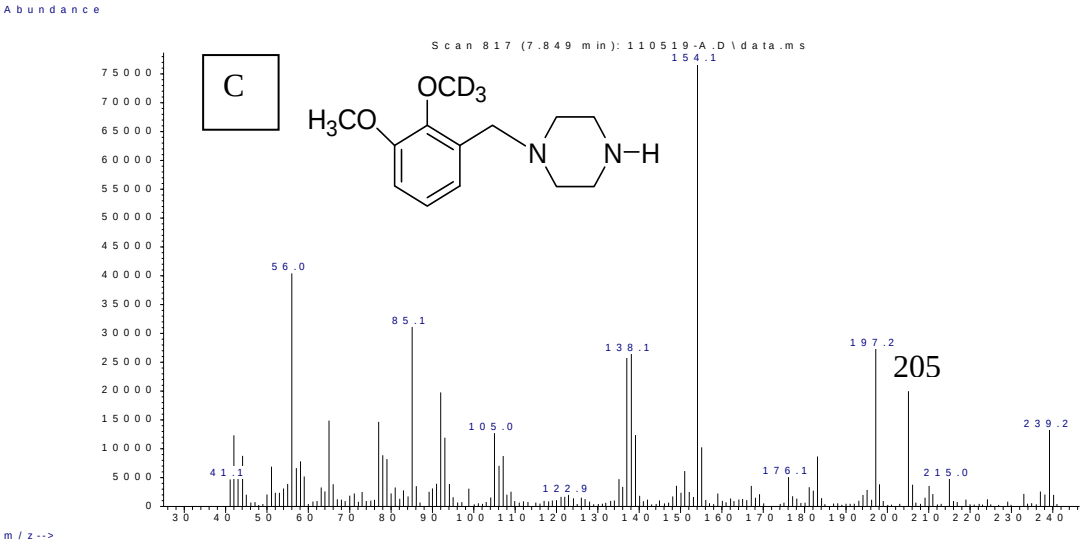


Fig. 5-5. Mass spectra for d_1, d_6, and d_3-2,3-dimethoxybenzylpiperazine.

nitrogen as the initial step in the process. Thus, the electron donating 2-methoxy group is in conjugation with the side-chain to participate in the elimination step yielding the m/z 136 ion. If, on the other hand, the methyl group from the 2-substituent migrates in the first step to yield the quaternary nitrogen then the remaining 3-methoxy group is not capable of efficiently participating in the elimination step.

As a further proof to this mechanism, we prepared the ortho ^{13}C- isotope of compound 1. We prepared the precursor aldehyde for this ^{13}C- isotope by treating 2-hydroxy-3-methoxybenzaldehyde with ^{13}C-methyliodide in the presence of potassium carbonate. The resulting ^{13}C-labeled aldehyde was reductively aminated with piperazine in the presence of sodium cyanoborohydride. The mass spectrum in Figure 5-6 provides confirmation 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 Figure 5-4. The mass spectrum in Figure 5-6 is for the 2-^{13}Cmethoxy-3-methoxybenzylpiperazine and indicates that all the ^{13}C label remains in the ion in question yielding the m/z 137 fragment.

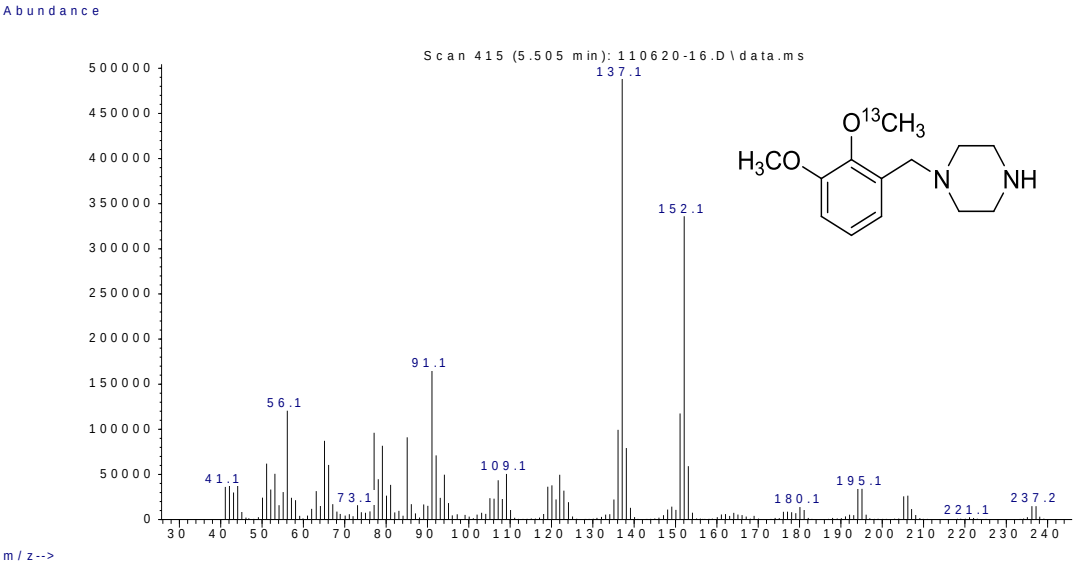


Fig. 5-6. Mass spectrum for the 2-^{13}Cmethoxy-3-methoxybenzylpiperazine.

The 2,3-dimethoxybenzylpiperazine (Figure 5-1) shows a second unique ion at m/z 205 (M-31)+ likely from the direct loss of a methoxy group from the molecular ion. The mass spectra in Figures 5-5.B and 5-5.C for the deuterated forms of Compound 1 confirm that the m/z 205 specifically results from loss of the methoxy group at the 2-position.

An additional fragmentation pathway which is characteristic for all the ortho-methoxy ring substituted compounds is described in Figure 5-7. Those dimethoxybenzylpiperazines with the methoxy group in the ortho position relative to the side chain are characterized by a significant m/z 121 ion. This ion likely arises from the loss of mass 30 (CH_2O) from the initial dimethoxybenzylic cation at m/z 151. The m/z 121 ion is a significant fragment only when the methoxy group is ortho to the piperazine side chain and therefore the site of initial benzylic cation formation as in Compounds 1-4.

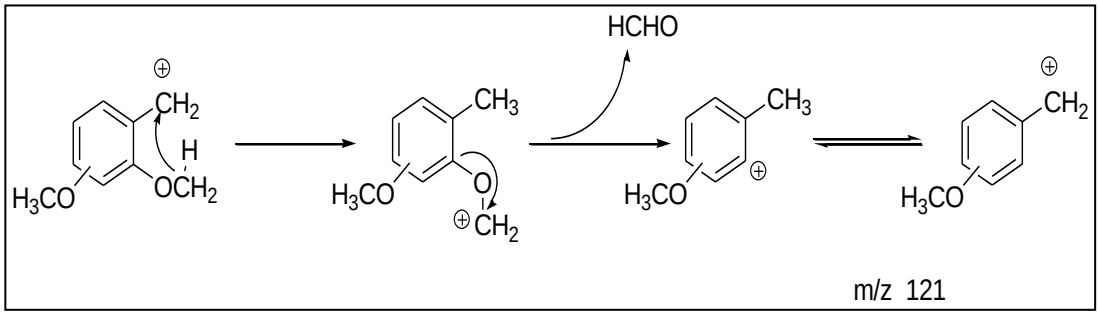
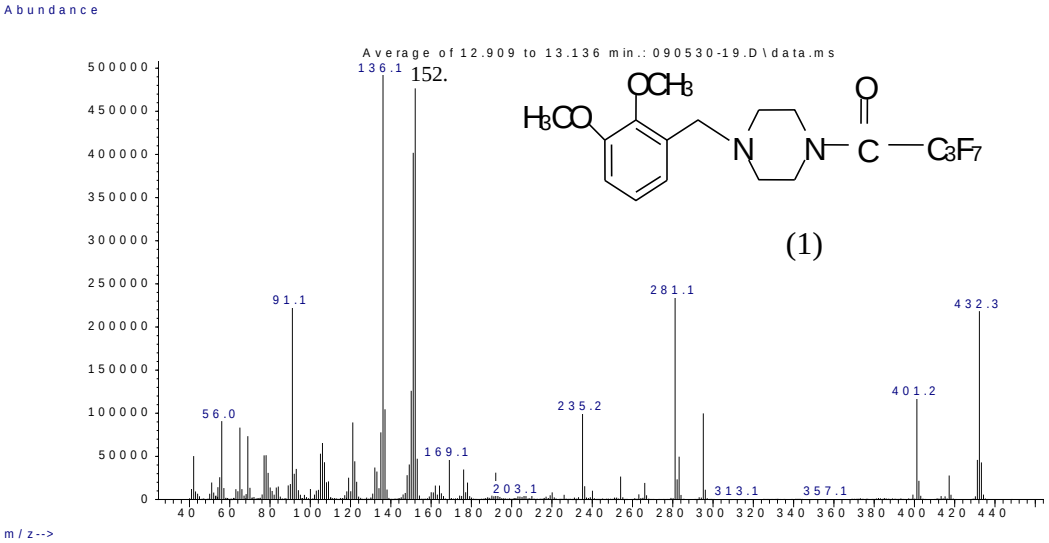


Figure 5-7. Mechanism for the formation of the m/z 121 ion in the mass spectra of the underivatized and derivatized 2-methoxy regioisomers of the dimethoxybenzylpiperazines.

This m/z 121 ion can be formed by 1,6-hydride shift (ortho effect) from a hydrogen of the ortho-methoxy group to the benzyl cation followed by the loss of formaldehyde as in Figure 5-7. This fragment occurs in all the mass spectra of the underivatized and TFA, PFPA and HFBA derivatives of the ortho-methoxy DMBPs. This suggested mechanism for the loss of CH_2O from the ortho-methoxy benzyl cations was previously discussed [Awad *et al*, 2007 and Maher *et al*, 2009].

The mass spectra for the six heptafluorobutyryl amides are shown in Figure 5-8 as representatives of the perfluoroacylated piperazines. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives were all evaluated for their ability to individualize the mass spectra of each regioisomer to the exclusion of the other regioisomeric compounds. From these spectra, a common peak with high relative abundance occurs at m/z 332, 382 and 432, which corresponds to the molecular ions for TFA, PFPA and HFBA amides, respectively. Fragment ions occurring at *m/z* 194, 181, 121 and 56 seen in all mass spectra of the piperazine amides are due to different patterns of cleavage reactions in the piperazine ring as previously described for acyl derivatives of BZP. Fragment ions at m/z 235 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety from the corresponding derivative. A similar pathway yields ions at $(M-151)^+$ from loss of the dimethoxybenzyl fragment from the other piperazine nitrogen. Those ions occurring at m/z 69, 119 and 169 are formed as a result of the formation of trifluoromethyl, pentafluoroethyl or heptafluoropropyl cations from the TFA, PFPA and HFBA amides, respectively. There is no significant difference between the spectra of the six compounds except for the characteristic high relative abundance ion at m/z 136 specific for the perfluoroacylated 2,3-DMBP. Thus, even acylation of the six piperazines does not give characteristic fragments that help to discriminate among the six regioisomers.



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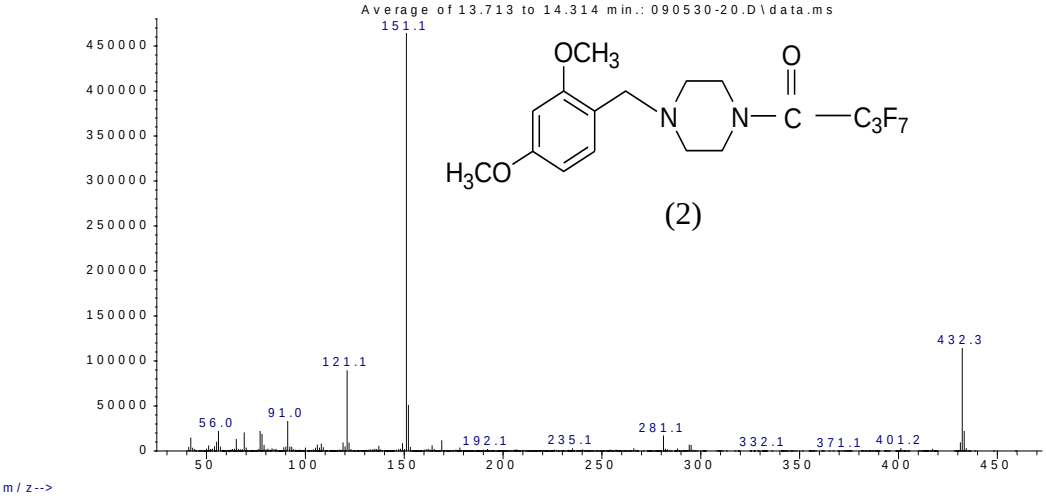

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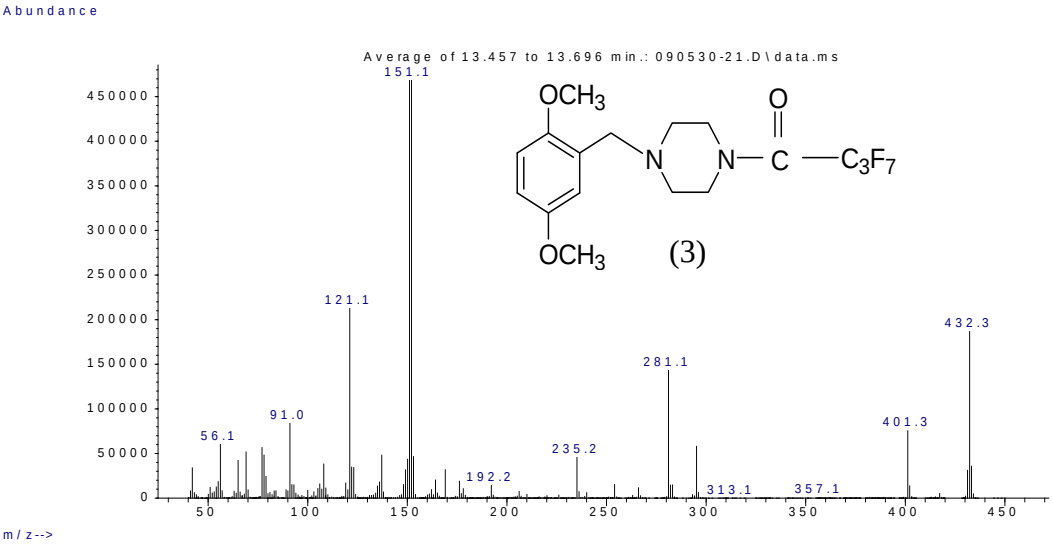
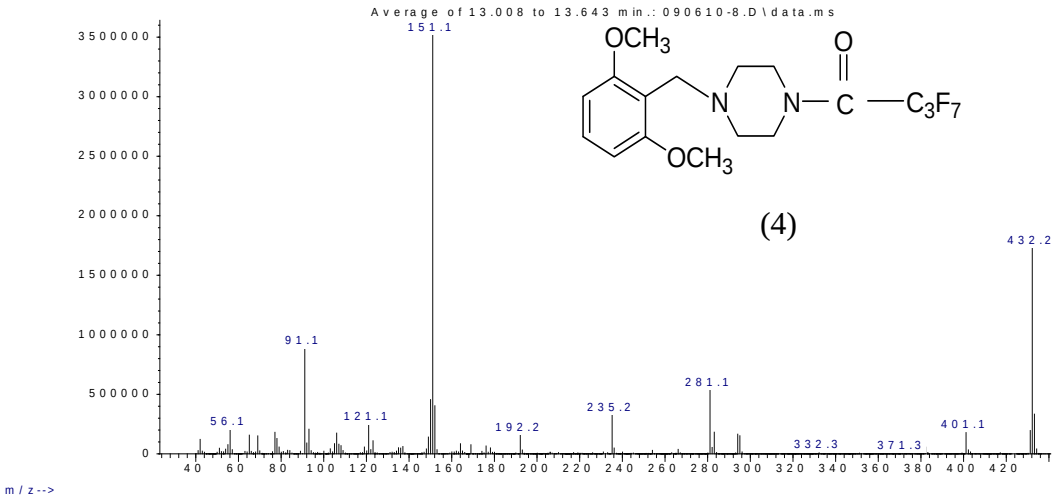
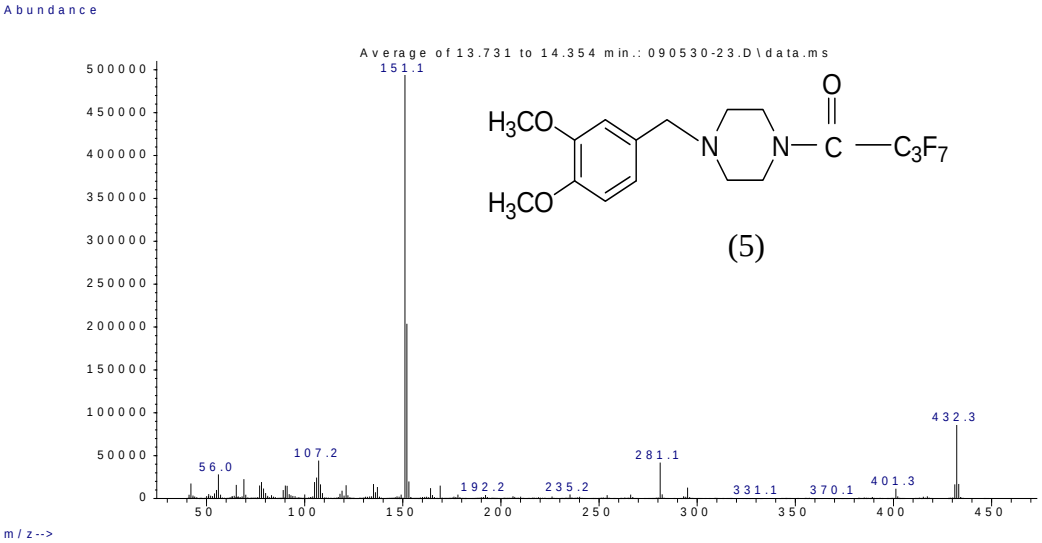






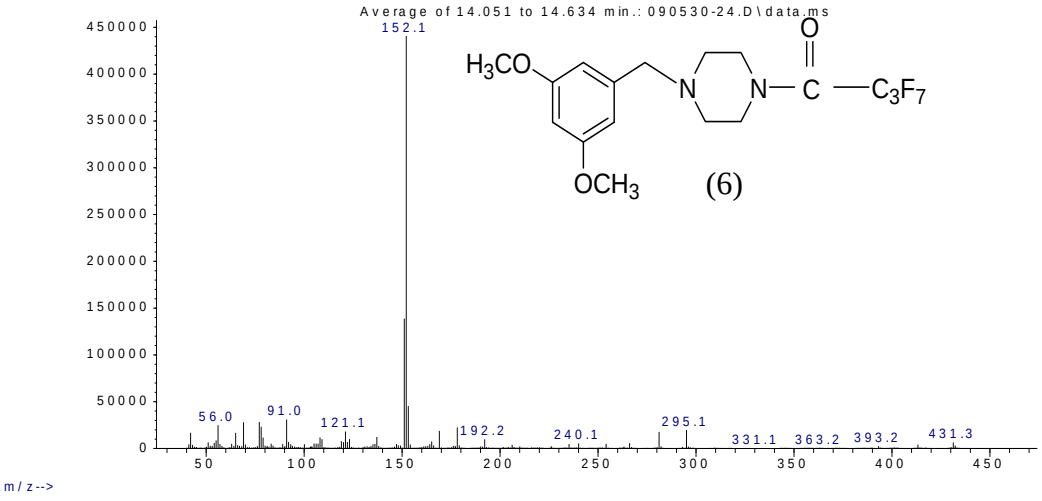
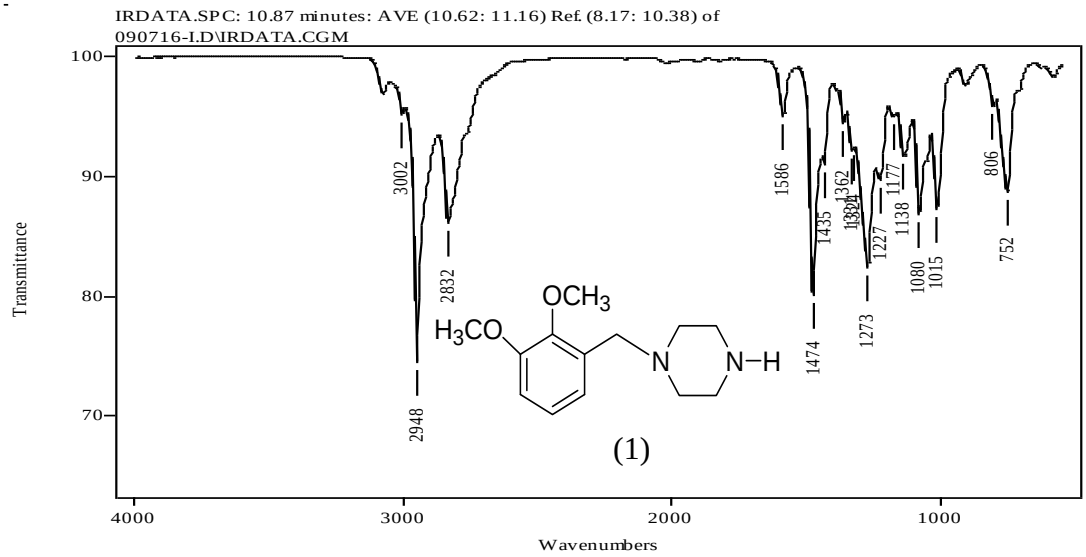


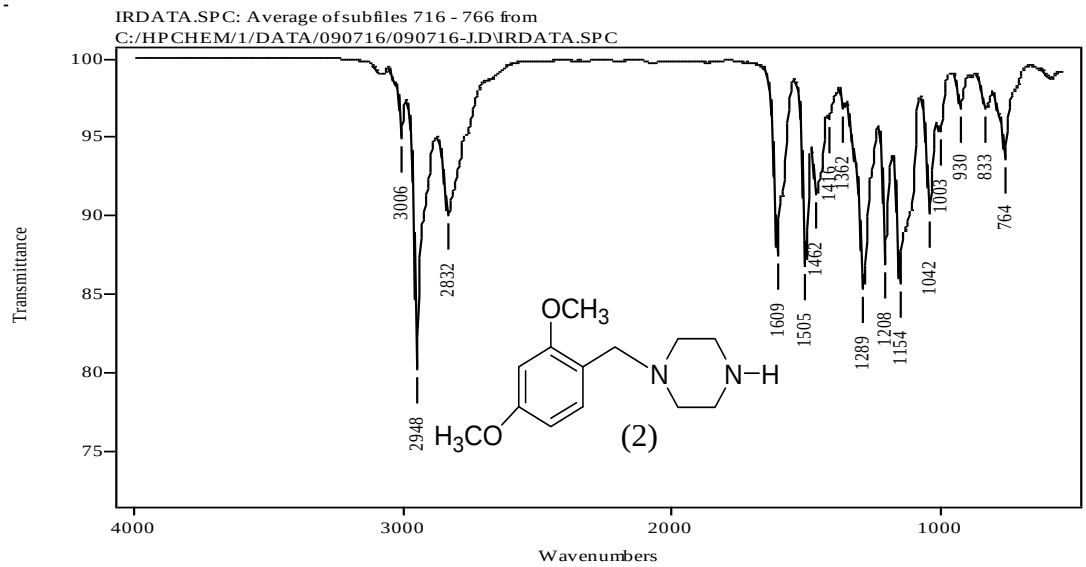
Fig. 5-8. MS spectra of heptafluorobutyryl derivatives of the six dimethoxybenzylpiperazine compounds.

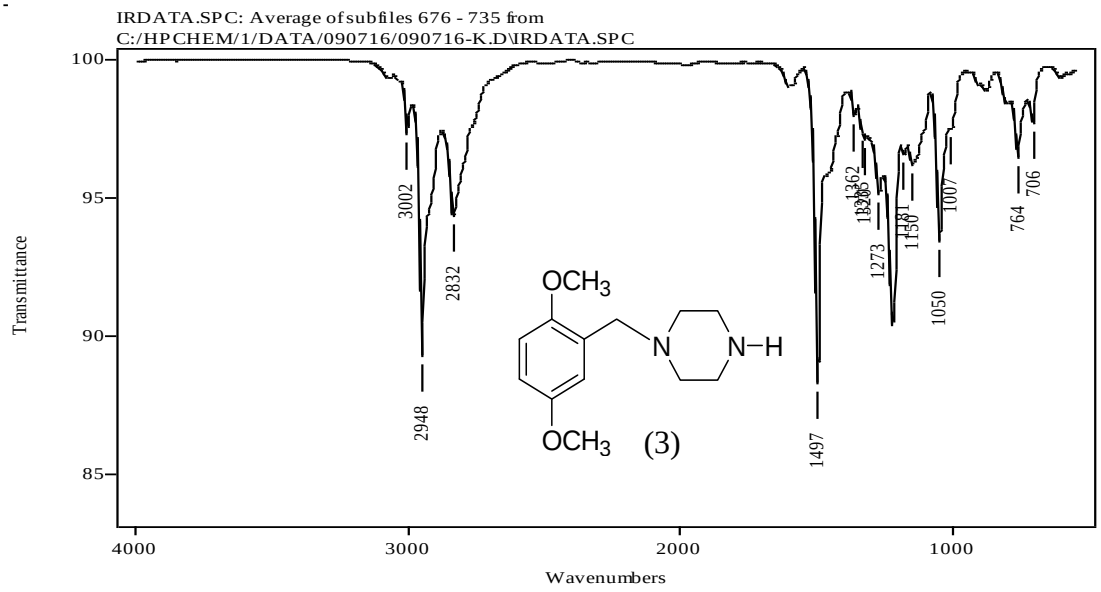
Vapor-phase Infra-Red Spectrophotometric Studies of the Dimethoxybenzylpiperazines (DMBPs)

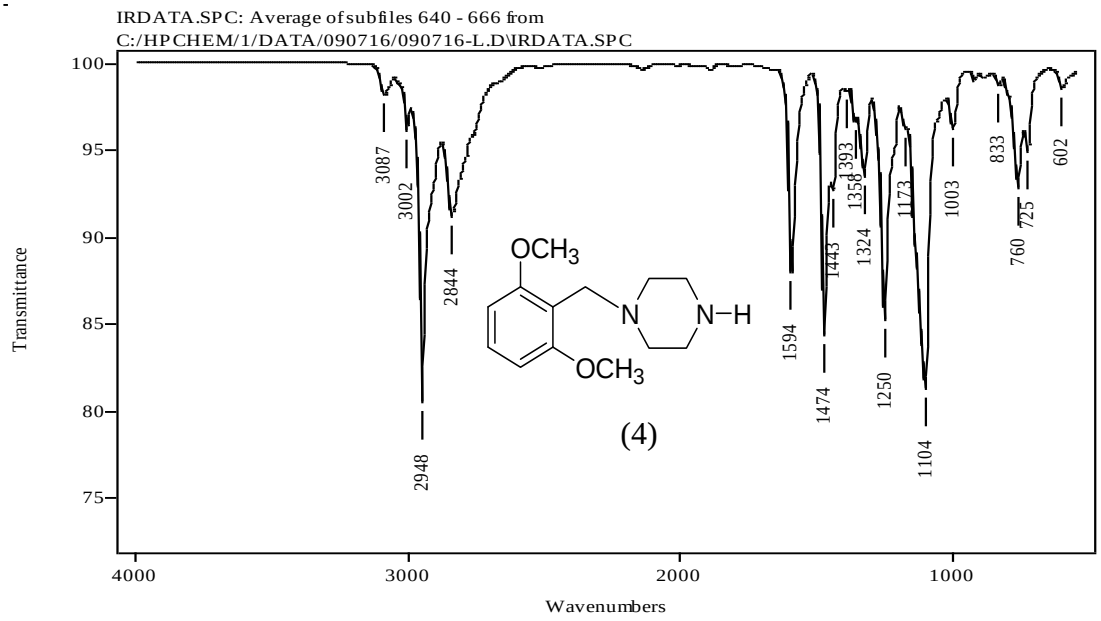
The vapor-phase infrared spectra for the six underivatized piperazines are shown in Figure 5-9. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph. Each compound shows a vapor-phase IR spectrum with absorption bands in the regions 700 – 1700 cm^{-1} and 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}. Because the six 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 IR peaks in the region of 750 – 1620 cm^{-1}.

The 2,3-DMBP regioisomer is characterized by the medium intensity band at 1474 cm^{-1} which is split into doublet peaks of medium and equal intensity at 1609 and 1505 cm^{-1} in the 2,4-DMBP regioisomer. This isomer also has another medium intensity band at 1273 cm^{-1} shifted to a medium singlet at 1289 cm^{-1} in the IR spectrum of the 2,4 isomer. Finally, the IR spectrum of 2,3-DMBP shows a weak doublet peak at 1015 and 1080 cm^{-1} which is shifted to a doublet at 1154 cm^{-1} and 1208 cm^{-1} in 2,4-DMBP. The 3,5-DMBP regioisomer can be distinguished by the relatively strong IR band at 1597 cm^{-1} which is shifted to a strong intensity peak at 1509 cm^{-1} in the 3,4-regioisomer, a strong intensity peak at 1497 cm^{-1} in the 2,5-regioisomer and a medium intensity doublet at 1474 and 1594 cm^{-1} in the 2,6-regioisomer The vapor-phase IR spectrum of the 3,4-DMBP regioisomer can be distinguished by a singlet of strong intensity appearing at 1273 cm^{-1} co compared to a peak of strong intensity at 1154 cm^{-1} in the 3,5-isomer, a









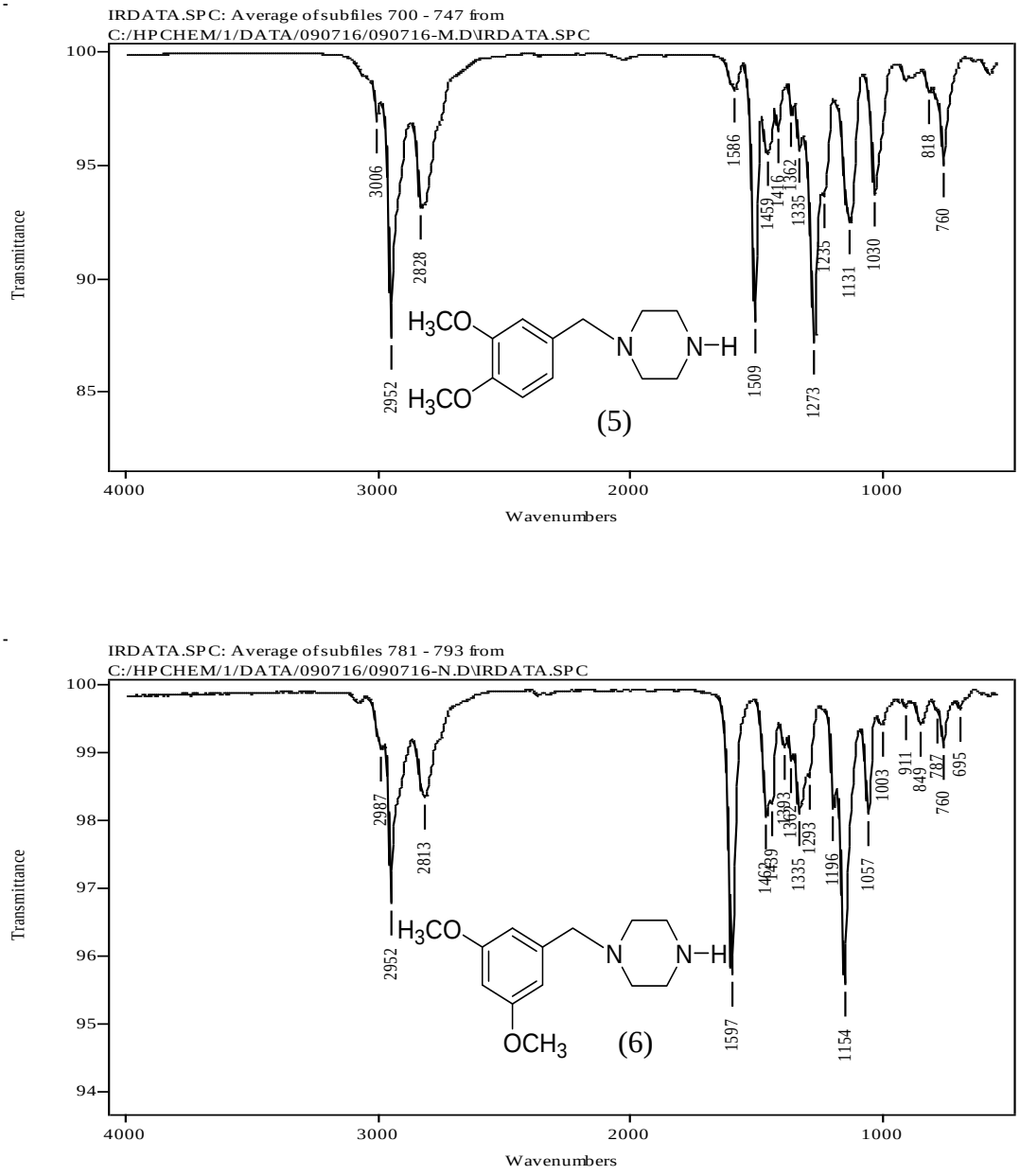


Fig. 5-9. Vapor phase IR spectra of the six dimethoxybenzylpiperazines.

strong singlet at 1240 cm^{-1} in the 2,5 isomer and a doublet of medium intensity at 1104 and 1250 cm^{-1} in the 2,6-isomer.

This study shows that vapor phase 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 to individualize the regioisomers in this set of uniquely similar compounds. Thus, GC-IRD readily discriminates between the members of this limited set of regioisomeric dimethoxybenzylpiperazine compounds

Gas Chromatographic Separation of the Dimethoxybenzylpiperazines (DMBPs)

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). 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 ramped to 200°C at a rate of 10°C/min and held at 200°C for 5.0 min. The separation of the trifluoroacetyl and heptafluorobutyryl derivatives was performed using a temperature program consisting of an initial hold at 70°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 15.0 min.

The representative chromatogram in Figure 5-10 shows the separation of the PFPA derivatives of the dimethoxybenzylpiperazines. This separation requires an analysis time of over fifty minutes and the elution order appears related to the degree of substituent crowding on the aromatic ring. Compounds 1 and 4 elute first and these two isomers contain substituents arranged in a 1,2,3-pattern on the aromatic ring. Three isomers (Compounds 2, 3 and 5) have two groups substituted 1,2 with one isolated substituent. The 1,3,5-trisubstituted pattern in Compound 6 provides minimum intramolecular crowding and elutes last in this group of compounds. The elution order was the same for the underivatized and all derivatized dimethoxybenzylpiperazines evaluated in this project.

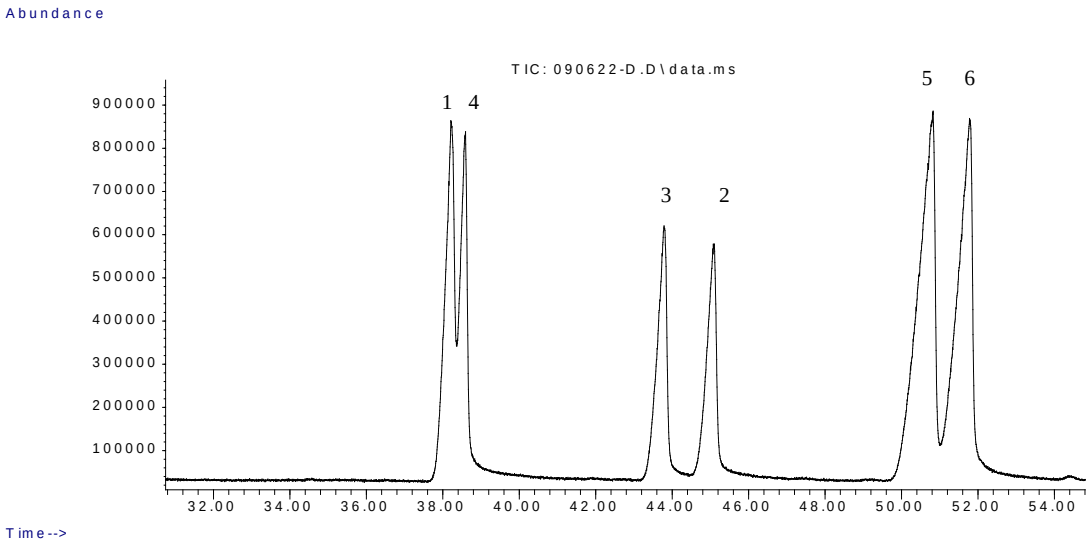


Fig. 5-10. Gas chromatographic separation of the pentafluoropropionyl derivatives of the DMBPs using Rtx-200 column. The number over the peak corresponds to the compound number.

Conclusion

The six regioisomeric dimethoxybenzylpiperazines yield the same fragment ions in their mass spectra even after perfluoroacylation. GC-IRD analysis yields unique and characteristic vapor phase infrared spectra for these six regioisomeric piperazines. These spectra allow discrimination among the six regioisomeric compounds included in this study. This differentiation was accomplished without the need for chemical derivatization. Mixtures of the six piperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions.

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Awad, T., DeRuiter, J., Clark, C.R. Gas Chromatography-Mass Spectrometry Analysis of Regioisomeric Ring Substituted Methoxy Methyl Phenylacetones, J. Chromatogr. Sci. 45 (2007) 458-465.

Maher, H.M., Awad, T., DeRuiter, J. Clark, C.R. GC-MS and GC-IRD studies on Dimethoxyamphetamines (DMA): Regioisomers Related to 2,5-DMA, Forensic Sci. Int. 192 (2009) 115-125.

Chapter 6

GC-MS Analysis of the Six Ring Regioisomeric Dimethoxybenzyl-N-methylpiperazines (DMBMPs)

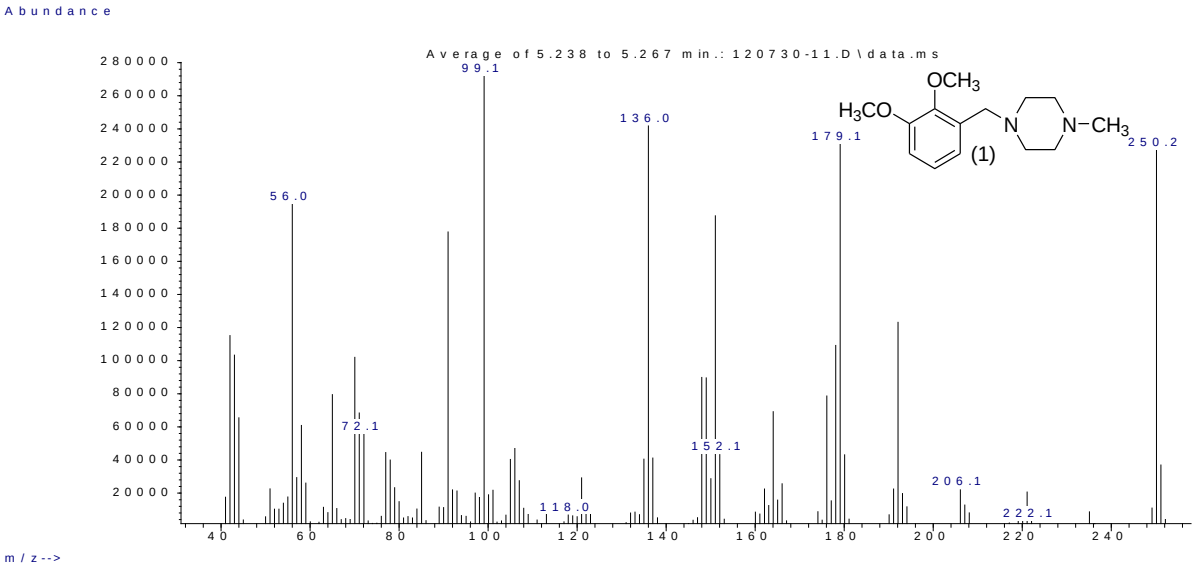
The complete series of regioisomeric dimethoxybenzyl-N-methylpiperazines were synthesized and evaluated in GC-MS studies. The EI mass spectra show fragment ions characteristic of both the dimethoxybenzyl and the N-methylpiperazine portions of the molecules. These characteristic fragments include the dimethoxybenzyl cation and radical cation at m/z 151 and m/z 152 as well as the m/z 99 N-methylpiperazine cation and the low mass ion at m/z 56 for the $C_3H_6N^+$ seen in almost all piperazine EI spectra. The 2,3-dimethoxybenzyl-N-methylpiperazine yields a regioisomer specific fragment at m/z 136 and the elemental composition for this radical cation $C_8H_8O_2$ was confirmed by exact mass analysis. Deuterium labeling studies provided evidence for the proposed structures for a number of the major fragment ions in the mass spectra of these regioisomeric compounds. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and retention appears related to the degree of steric crowding of the aromatic ring substituents. The most crowded patterns of substitution elute first while the more symmetrical 1,3,5-substitution pattern has the highest retention time.

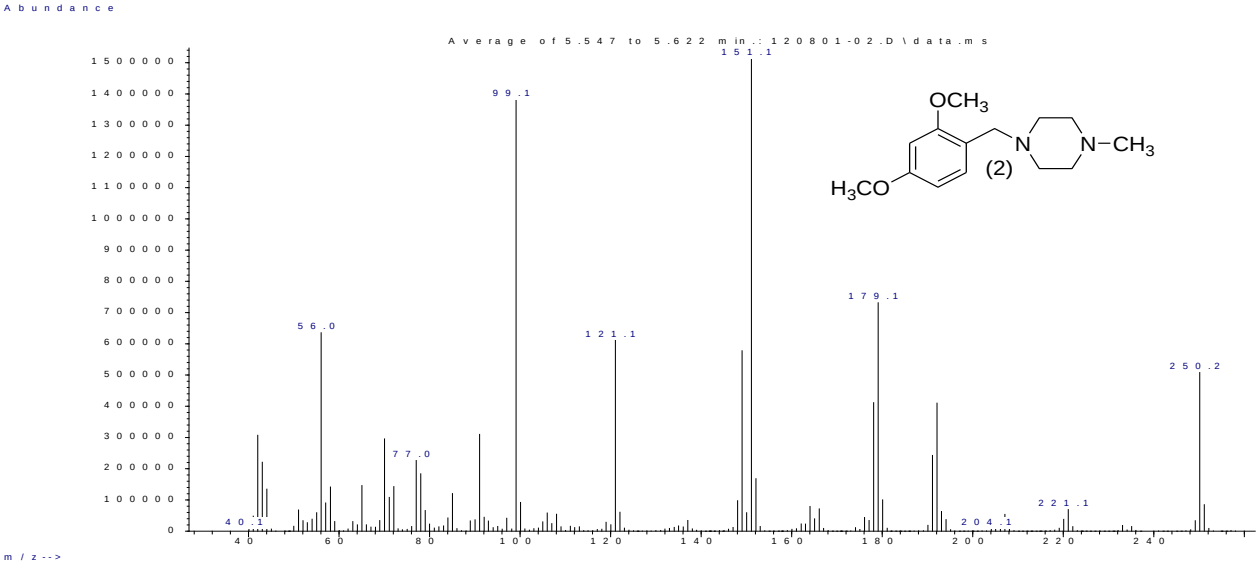
Mass spectral studies of the Dimethoxybenzyl-N-methylpiperazines (DMBMPs)

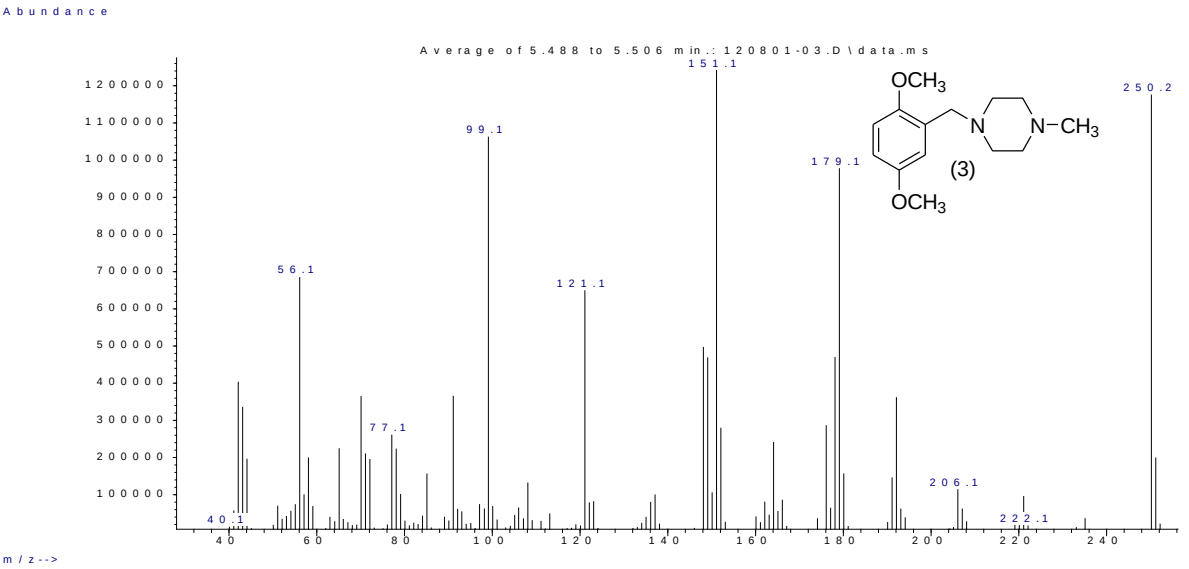
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 6-1 shows the EI mass spectra of the six regioisomeric dimethoxybenzyl-N-methylpiperazines (Compounds 1-6) in this study. All six regioisomers show molecular ions at m/z 250 and these ions are of significant relative intensity. Major fragment ions of equivalent mass occur in the spectra for these six compounds with only the relative intensity of the individual ions as the major spectral variation. Fragmentation of the bond between the benzylic carbon and the adjacent piperazine nitrogen provides the base peak in all six spectra. The dimethoxybenzyl cation at m/z 151 is the base peak for compounds 2, 3, 4, and 5 while the dimethoxybenzyl radical cation (m/z 152) resulting from a hydrogen migration rearrangement pathway is the base peak for compound 6. Fragmentation of the same bond between the benzylic carbon and the adjacent piperazine nitrogen with charge retention on the N-methylpiperazine group yields the cation at m/z 99, the base peak for compound 1. The structures for the base peaks in the mass spectra for these regioisomeric dimethoxybenzyl-N-methylpiperazines are shown in Figure 6-2.

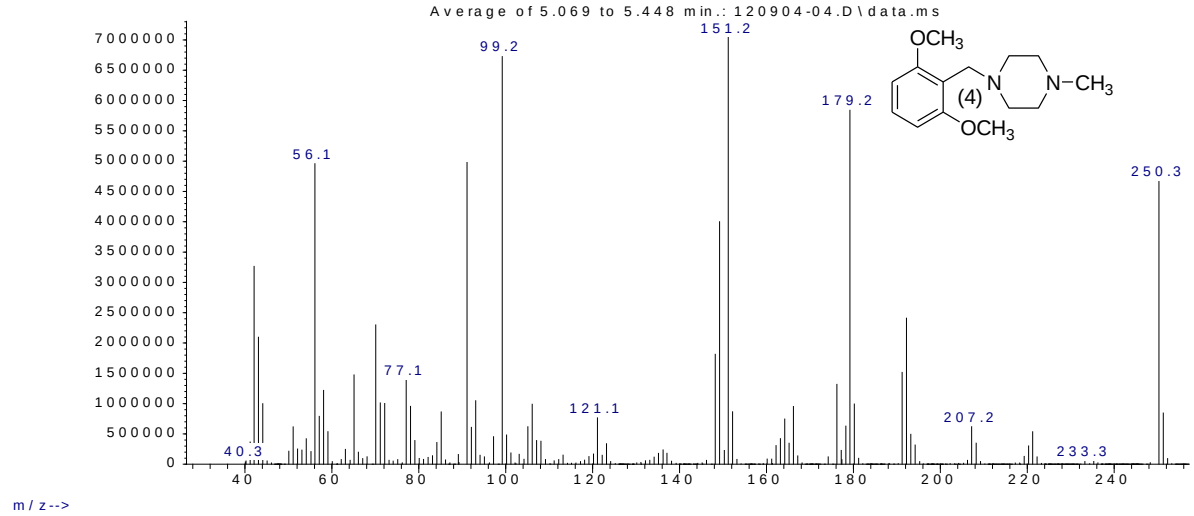
The internal fragmentation within the piperazine ring produces a number of unique ions in the mass spectra of these dimethoxybenzyl-N-methylpiperazines. The low mass ion of highest relative abundance in these spectra is the m/z 56 cation. This ion is a characteristic fragment for the piperazine ring and is almost universal in all N-1 monosubstituted piperazines (N-4 substituent is hydrogen). This m/z 56 ion was shown

by exact mass measurements to have an elemental composition $C_3H_6N^+$ in previous studies [Abdel-Hay *et al*, 2013]. Furthermore this ion yielded a mass shift of +6 Da









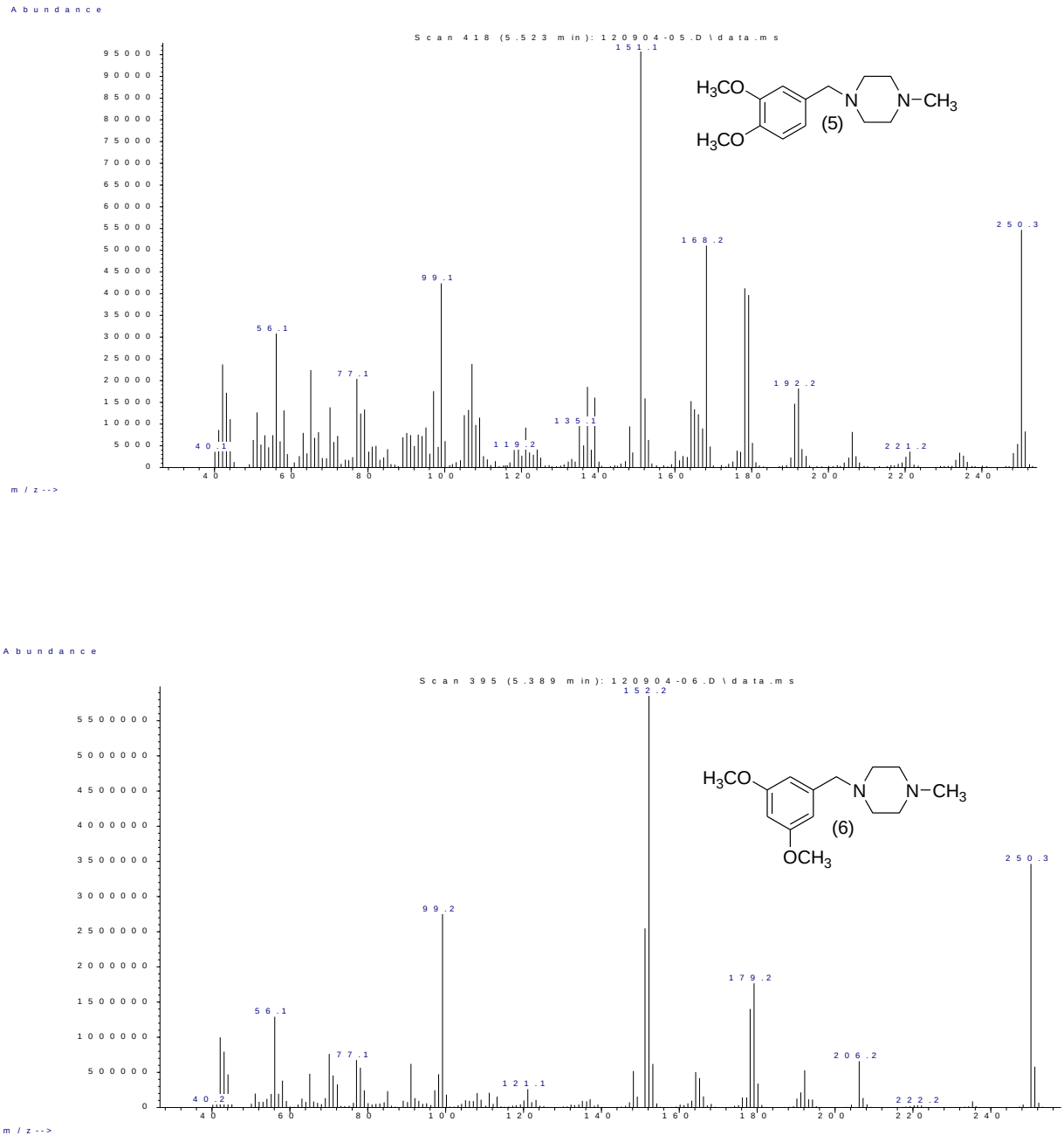


Fig. 6-1: Mass Spectra for the six regioisomeric dimethoxybenzyl-N-methylpiperazines.

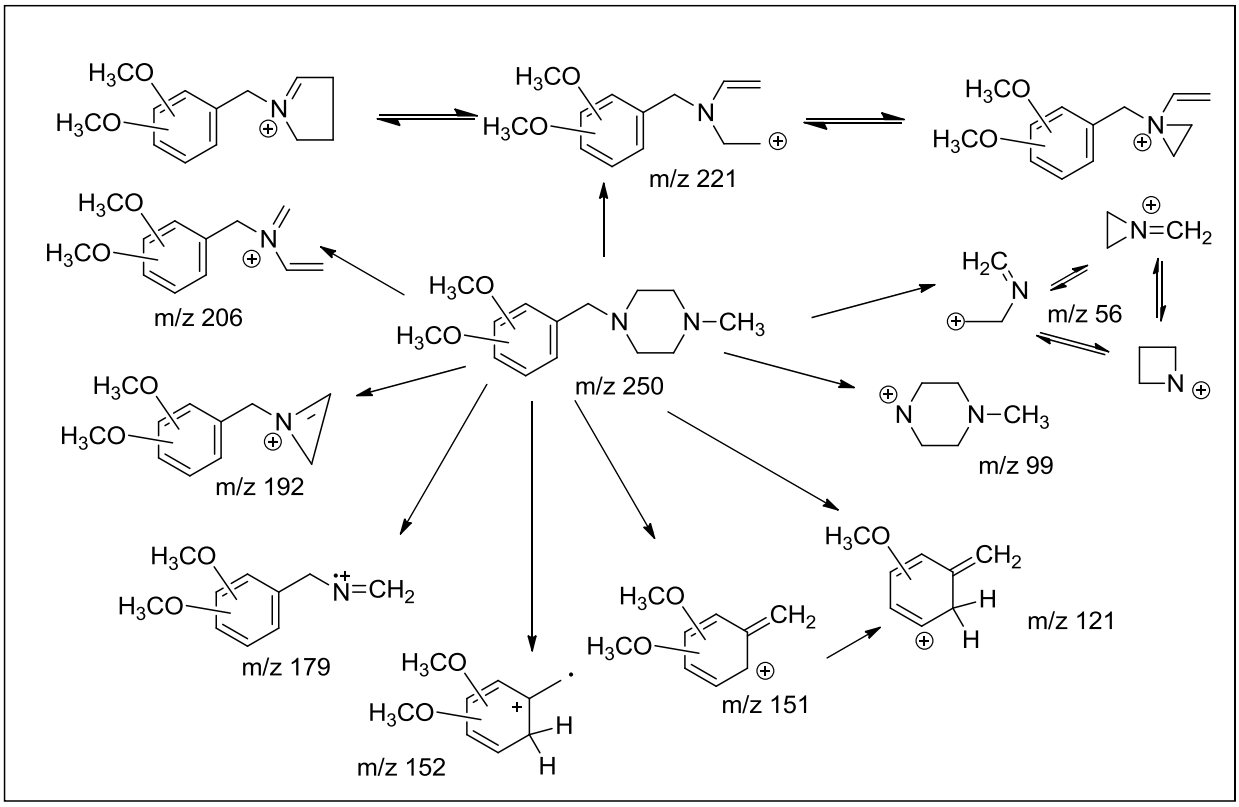


Fig. 6-2: Mass spectral fragmentation for the six regioisomeric dimethoxybenzyl-N-methylpiperazines.

when d_8-piperazine was used to label all the four carbons of the piperazine ring. Thus, the m/z 56 ion was confirmed to contain six hydrogen atoms and three of the carbons from the original piperazine portion of these molecules, this confirmation was based on studies in the dimethoxybenzylpiperazines (N-4=H). The formation of the m/z 56 ion in the previous series of dimethoxybenzylpiperazine (N-4=H) regioisomers involved an initial migration of the hydrogen on nitrogen at N-4 to the tertiary N-1 nitrogen followed by a radical site alpha cleavage initiated by the N-4 nitrogen to break the carbon-carbon bond of the piperazine ring. The last step in the formation of the m/z 56 ion is the heterolytic breaking of the N-1 to carbon bond to yield the $C_3H_6N^+$ cation. The equivalent mechanistic pathway is illustrated for the N-4=CH_3 compounds in this study in Figure 6-3.

The formation of the $C_3H_6N^+$ m/z 56 ion for the N-4=CH_3 series of regioisomers involves methyl group migration from N-4 to N-1 of the piperazine ring. The N-4=CD_3 labeled form of 2,3-dimethoxybenzyl-N-methylpiperazine was prepared by reacting the monosubstituted dimethoxybenzylpiperazine (N-4=H) with CD_3I. The mass spectrum for this labeled compound is presented in Figure 6.4.A and shows that the m/z 56 species did not undergo a mass shift as a result of the N-CD_3 group. Thus, the N4 methyl group is not a part of the m/z 56 ion and indicates that the methyl group migration is consistent with the N-H migration in the monosubstituted series [Abdel-Hay *et al*, 2013]. The use of piperazine-D_8 in the monosubstituted series produced a mass shift of +6 Da for the ion in question yielding the $C_3D_6N^+$ cation at m/z 62. The mass spectrum for 2,3-dimethoxybenzylpiperazine-D_8 is shown in Figure 6-4.B. When the N4-methyl (CH_3) group is added to the 2,3-dimethoxybenzylpiperazine-D_8 the resulting mass spectrum

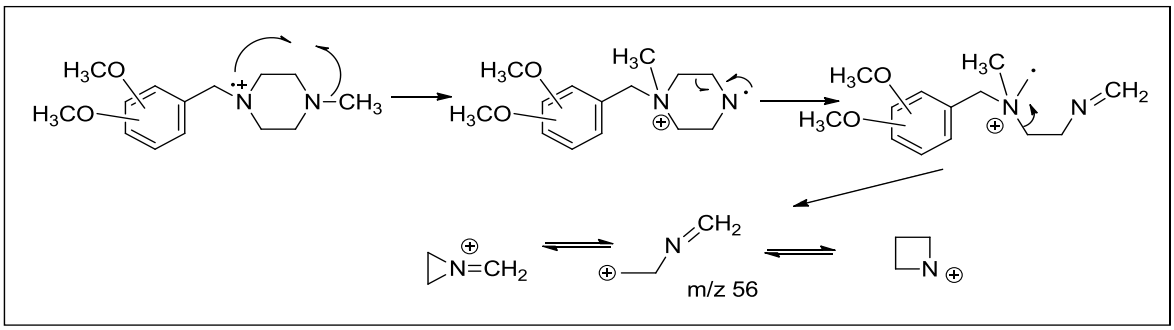
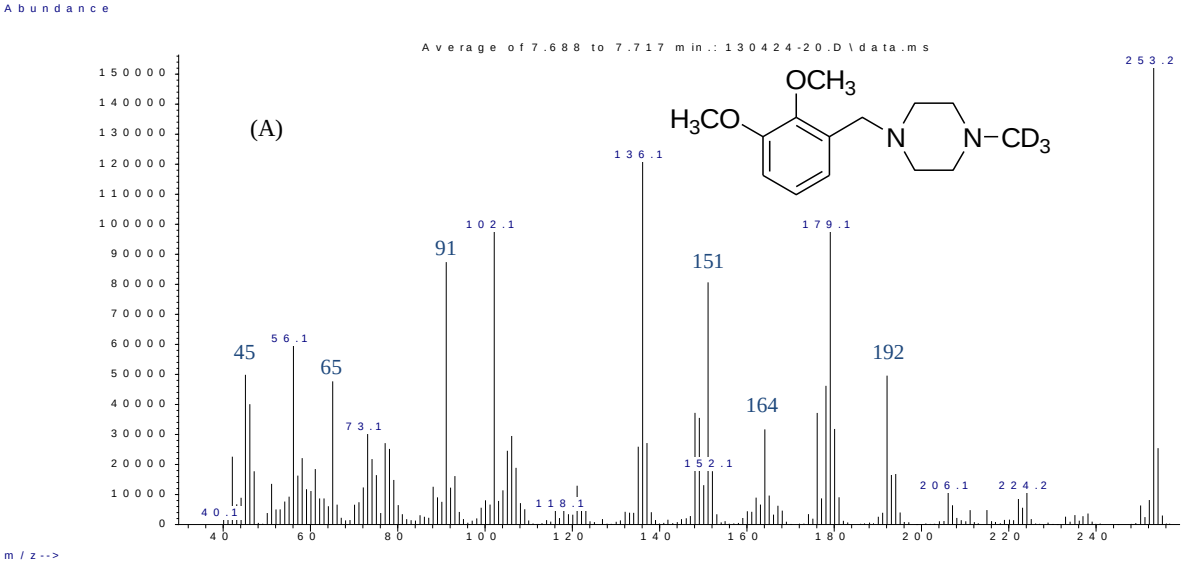
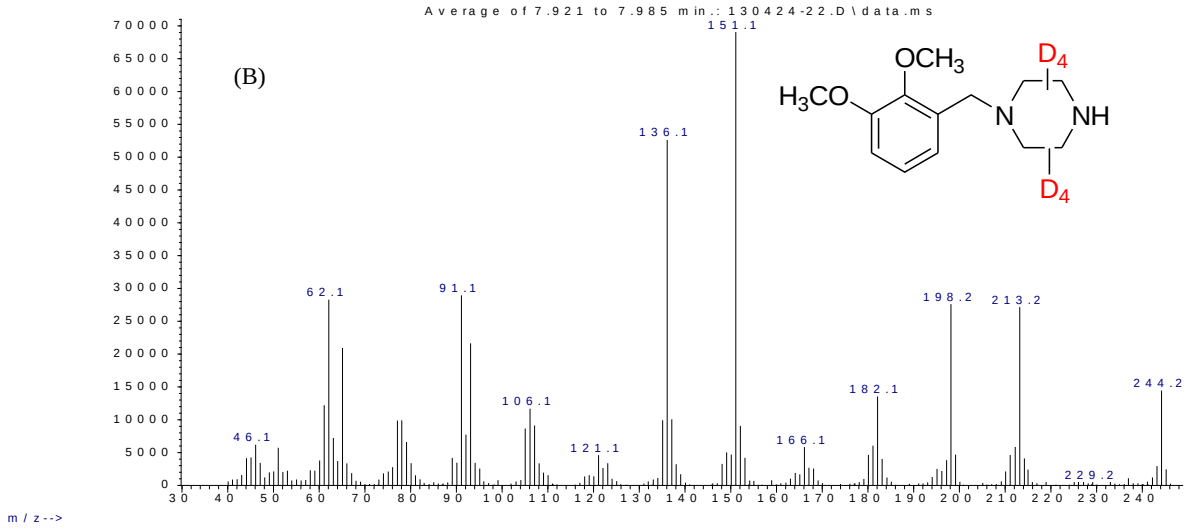


Fig. 6-3. Mechanism for the formation of the m/z 56 cation in the six regioisomeric dimethoxybenzyl-N-methylpiperazines.





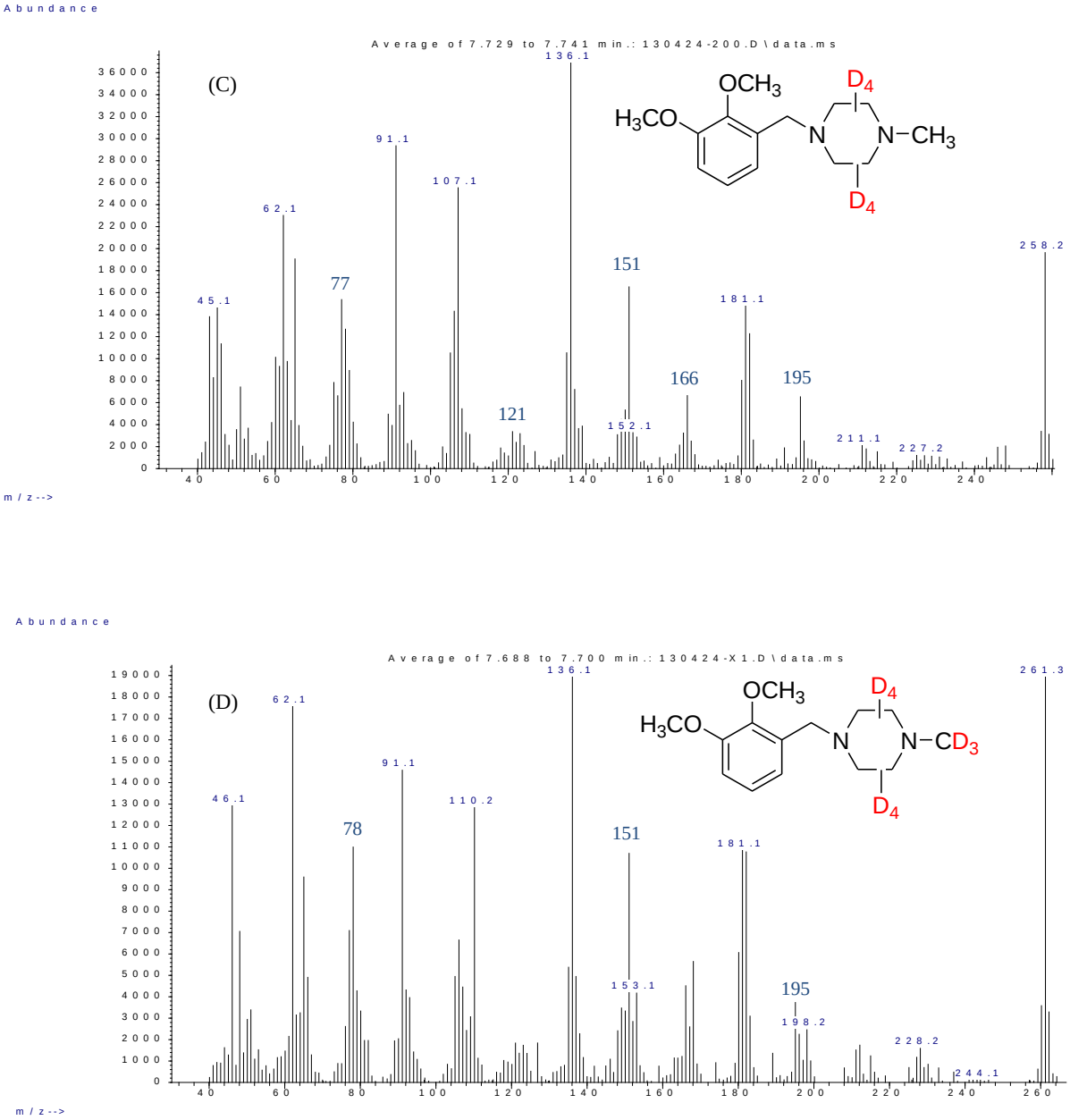


Fig. 6-4. Mass spectra for deuterium labeled 2,3-dimethoxybenzyl-N-methylpiperazines.

(Figure 6-4.C) continues to show a mass shift of +6 Da for the $C_3D_6N^+$ cation at m/z 62. The mass spectrum for the 2,3-dimethoxybenzyl-N-CD_3-piperazine-D_8 in Figure 6.4.D also shows the +6 Da mass shift for this ion further confirming the structure for this characteristic fragment. The mass shifts for the molecular ions in Figure 6-4.A-D provide validation of the structures of the labeled species. Furthermore, the fragment ions for the individual piperazine species at m/z 102, 93, 107 and 110 in Figures 6-4.A-D respectively confirm the position of the deuterium labels in the model compounds.

The mass spectrum for the 2,3-dimethoxybenzyl-N-methylpiperazine regioisomer in Figure 6-1 as well as all the deuterium labeled derivatives of this compound in Figure 6-4 show a unique fragment at m/z 136. This ion does not undergo any deuterium initiated mass shifts thus this fragment does not come from the piperazine portion of the molecule. Exact mass analysis shows this ion to be $C_8H_8O_2$, a radical cation species. This ion essentially represents the molecular equivalent of a methyl group loss from the m/z 151 cation to form a peak of intensity approximately equal to the base peak for 2,3-dimethoxybenzyl regioisomer. Previous deuterium labeling studies in the monosubstituted (N-4=H) dimethoxybenzylpiperazines [Abdel-Hay *et al*, 2013] showed that the methyl group lost to form the m/z 136 species is from the methoxy group at the 3-position of the aromatic ring. The resonance stabilizing effects of the 2-methoxy group favor the loss of the methyl group from the 3-position. A possible pathway for the formation of the m/z 136 ion is shown in Figure 6-5. The mass spectrum in Figure 6-6 provides support that the fragmentation pathway for these N-methylpiperazines in this study is equivalent to the previously reported monosubstituted dimethoxybenzylpiperazines. The mass spectrum in Figure 6-6 is for the deuterium

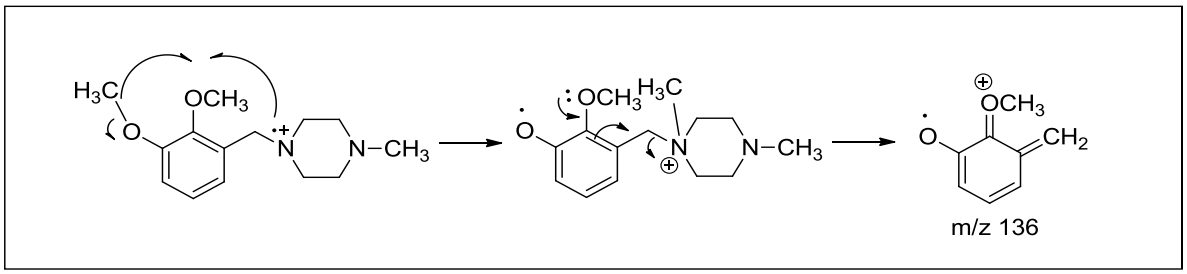


Fig.6-5: Mechanism for the formation of the m/z 136 ion in 2,3-dimethoxybenzyl-N-methylpiperazine.

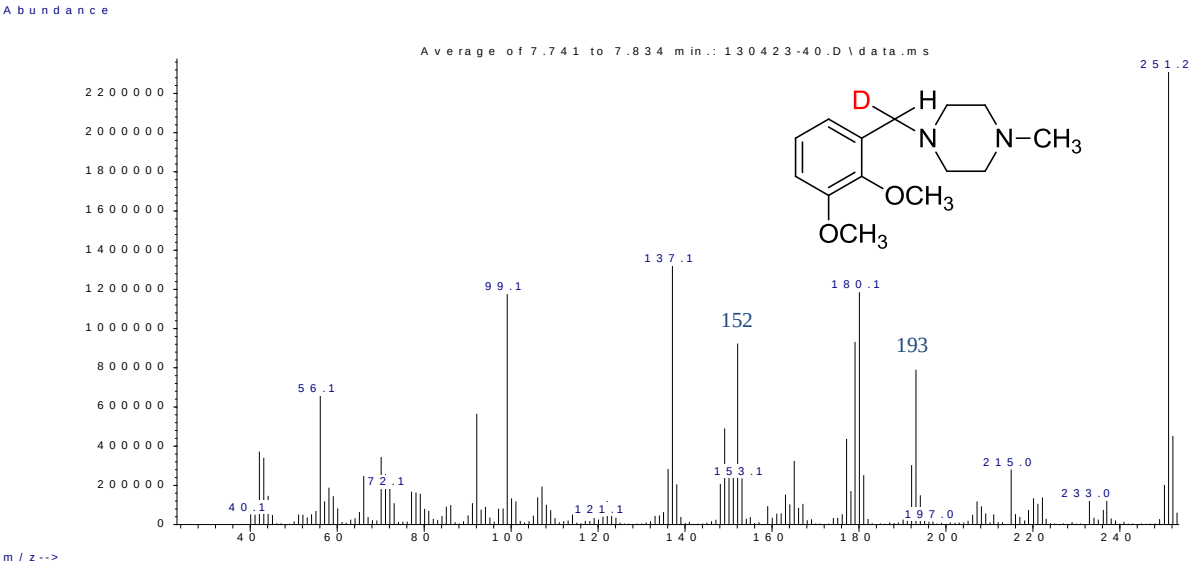


Fig. 6-6: Mass spectrum the benzylic carbon deuterium labeled 2,3-dimethoxybenzyl-N-methylpiperazines.

labeled benzylic carbon of 2,3-dimethoxybenzyl-N-methylpiperazine and the results show a mass shift of +1 Da at m/z 137. These data indicate that the benzylic carbon remains a part of the ion in question and leaves only the methyl group from one of the two methoxy ring substituents as the source for the elimination fragmentation. Thus the formation of the m/z 136 radical cation in this N-methylpiperazine series is the same as the previously reported [Abdel-Hay *et al*, 2013] 2,3-dimethoxybenzylpiperazine series. The deuterium labeling of the benzylic carbon followed the synthetic method for the six N-methylpiperazines using sodium cyanoborodeuteride ($NaBD_3CN$) as the reducing reagent.

The spectra for these six regioisomeric dimethoxybenzyl-N-methylpiperazines in Figure 6-1 show two major high mass fragment ions at m/z 179 and m/z 192 of variable relative abundance. A comparison of the fully deuterium labeled 2,3- dimethoxybenzyl-N-methylpiperazine-D_{11} in Figure 6-4.D with the spectrum for the equivalent unlabeled isomer in Figure 6-1 supports the proposed structures for the m/z 179 and m/z 192 fragments in Figure 6-2. Furthermore, the spectrum in Figure 6-4.D for the D_{11} derivative supports the proposed structures for the minor high mass ions at m/z 206 and m/z 221 observed in many of the spectra in Figure 6-1.

Gas Chromatographic Separation of the Dimethoxybenzyl-N-methylpiperazines (DMBMPs)

Gas chromatographic separation of the dimethoxybenzyl-N-methylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 6-7. The separations of the six regioisomers 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. This chromatogram shows the separation of the six dimethoxybenzyl-N-methylpiperazine regioisomers in this study. The elution order appears related to the degree of substituent crowding on the aromatic ring. Compounds 1 and 4 elute first and these two isomers contain substituents arranged in a 1,2,3-pattern on the aromatic ring. Three isomers (Compounds 2, 3 and 5) have two groups substituted 1,2 with one isolated substituent. The 1,3,5-trisubstituted pattern in Compound 6 provides minimum intramolecular crowding and elutes last in this group of compounds. The two compounds with maximum crowding substituted in a 1,2,3 manner on the aromatic ring show the 2,3-dimethoxy substitution pattern to elute first followed by the 2,6-dimethoxy isomer eluting second. The relative position of the methoxy groups appears to determine the elution order in the three compounds having two groups substituted in a 1,2 pattern. Within this group of three compounds the first to elute is the 1,4-relationship for the two methoxy groups in

compound 3. This is followed by the 1,3-pattern for compound 2 and lastly the 1,2-pattern for compound 5.

In previous studies on the chromatographic properties of the monosubstituted piperazines (N4=H), these secondary amines showed severe chromatographic tailing on a number of stationary phases. This issue was overcome by acylation of the secondary amine nitrogen with perfluoroacyl groups such as the pentafluoropropionyl group and others. Adequate peak shape and resolution were obtained for these tertiary amines (compounds 1-6) in this study.

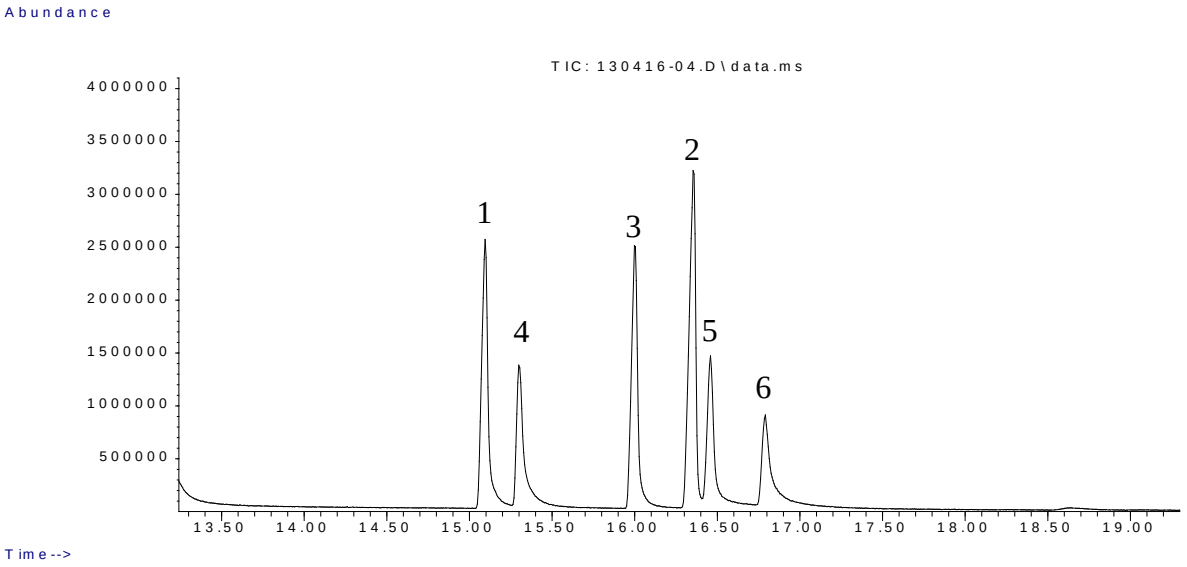


Fig. 6-7: Gas chromatographic separation of the six regioisomeric dimethoxybenzyl-N-methylpiperazines.

Conclusion

The six regioisomeric dimethoxybenzyl-N-methylpiperazines yield the same fragment ions in their mass spectra. Deuterium labeling was successful in confirming the elemental composition of the characteristic fragments in the mass spectra of all the dimethoxybenzyl-N-methylpiperazines. Mixtures of the dimethoxybenzyl-N-methylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order appears related to the degree of substituent crowding on the aromatic ring with the most crowded 1,2,3 substitution patterns eluting first and the highest retention for the compound with minimum intramolecular crowding (the 1,3,5-trisubstitution pattern).

References

Abdel-Hay, K.M., DeRuiter, J. and Clark, C. GC-MS and GC-IRD Studies on the Six Ring Regioisomeric Dimethoxybenzylpiperazines (DMBPs). Drug Testing and Analysis, 5 (2013) 560-572. DOI:10.1002/dta.1417

Chapter 7

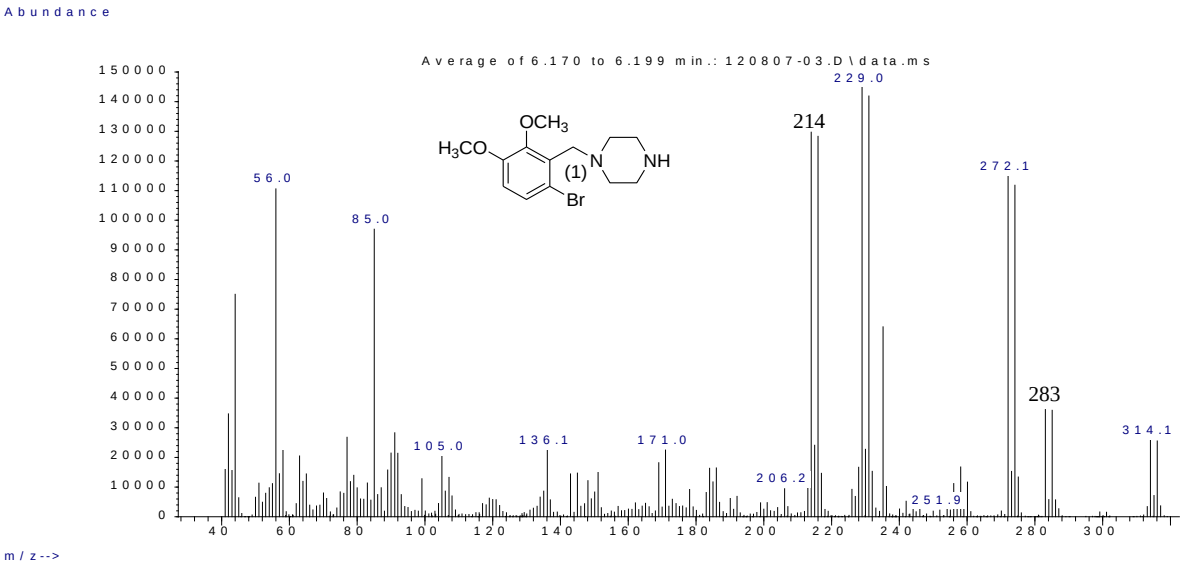
Regioisomeric Bromodimethoxybenzylpiperazines Related to the Designer Substance 4-Bromo-2,5-dimethoxybenzylpiperazine:GC-MS and FTIR Analysis

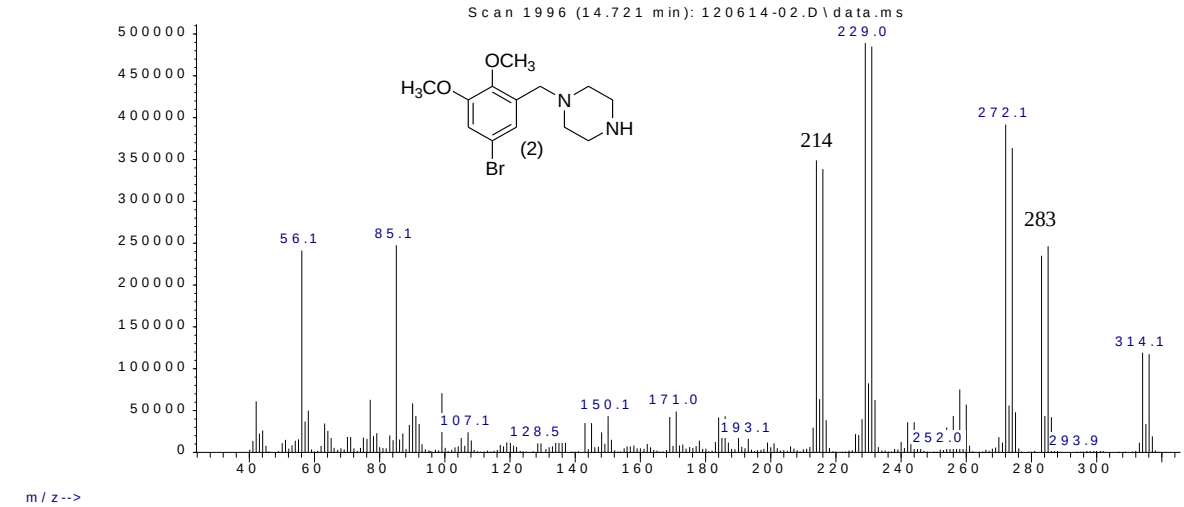
A series of seven regioisomeric bromodimethoxybenzylpiperazines including the designer benzylpiperazine (4-bromo-2,5-dimethoxybenzylpiperazine) were synthesized and their analytical profiles evaluated using GC-MS and FT-IR. The mass spectra for the seven regioisomeric bromodimethoxybenzylpiperazines 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 the differentiation between the seven regioisomeric aromatic ring substituted bromodimethoxybenzylpiperazines. Mixtures of the seven piperazine PFPA derivatives were successfully resolved via capillary gas chromatography using the relatively polar stationary phase composed of 100% trifluoropropyl methyl polysiloxane.

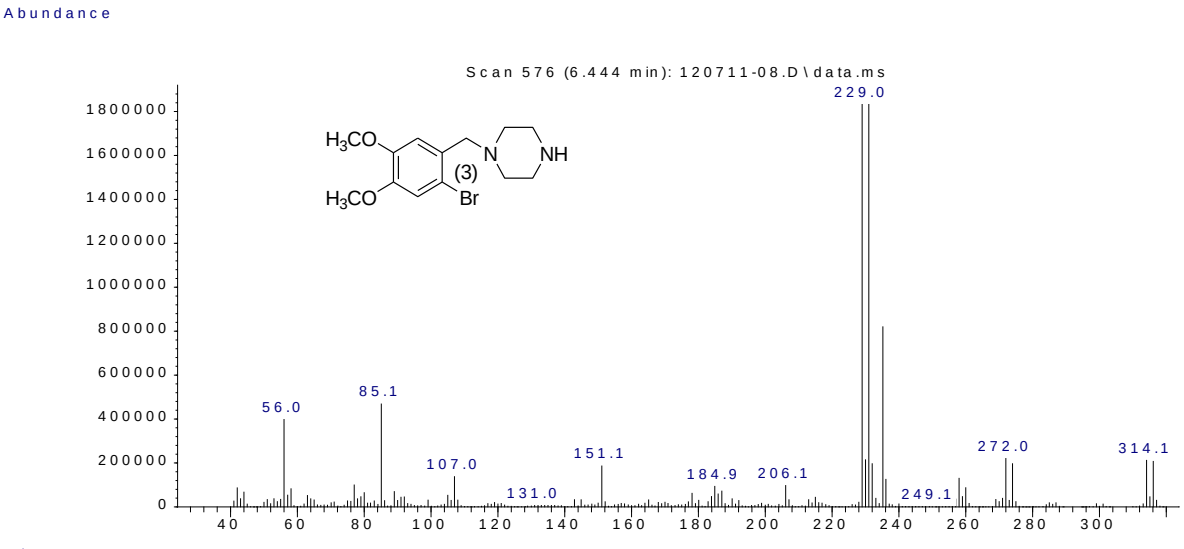
Mass spectral studies of the Bromodimethoxybenzylpiperazines

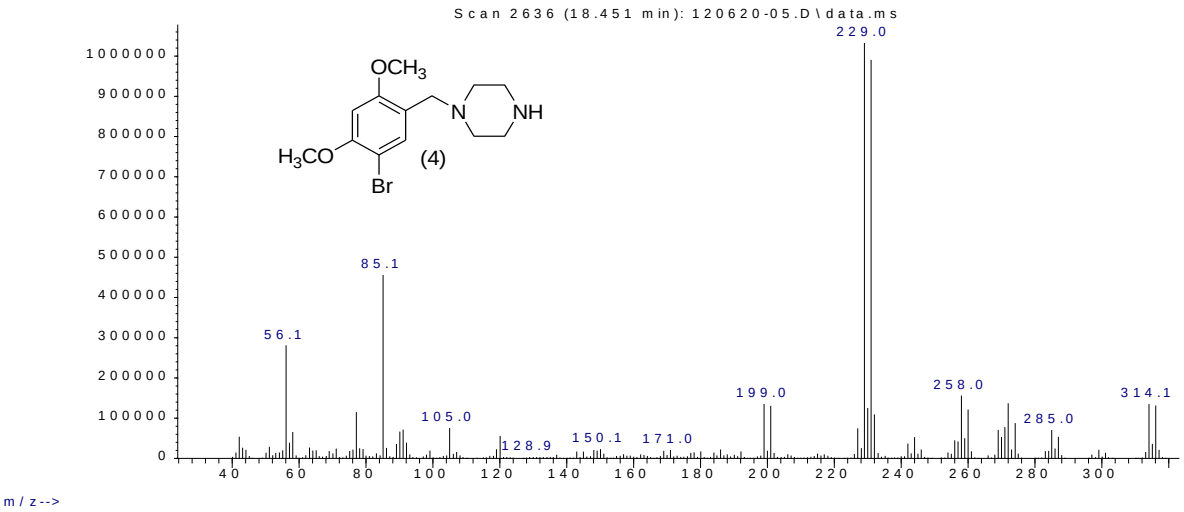
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 7-1 shows the EI mass spectra of the seven regioisomeric bromodimethoxybenzylpiperazines (Compounds 1-7). The mass spectra in Figure 7-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. 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 [de Boer *et al*, 2001] and labeling experiments using d_8-piperazine have provided additional confirmation for fragment ion structures [Abdel-Hay *et al*, 2012]. Equivalent fragmentation pathways for the bromodimethoxybenzylpiperazines (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 Figures 7-1 and 7-2. 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).

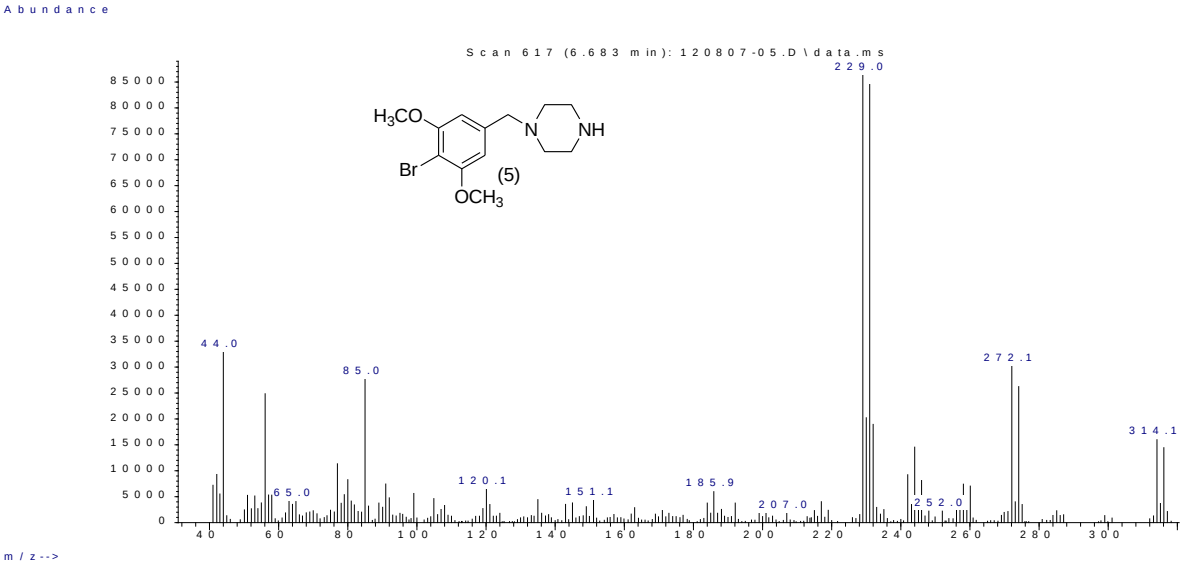
The proposed structure and mechanism for the formation of the unique m/z 214/216 C_8H_7BrO2 ion is shown in Figure 7-3. The suggested structure for this fragment

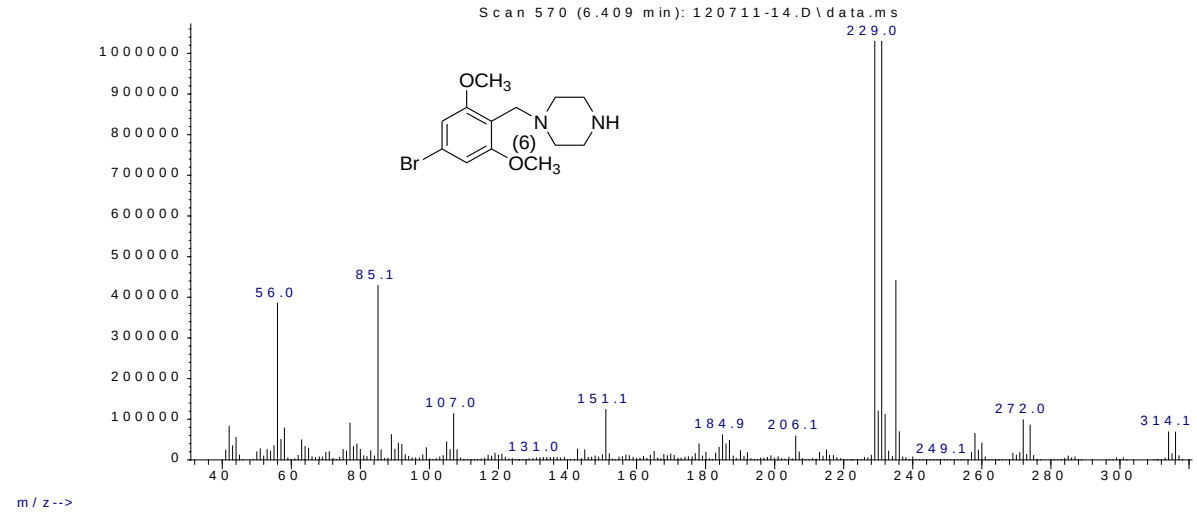












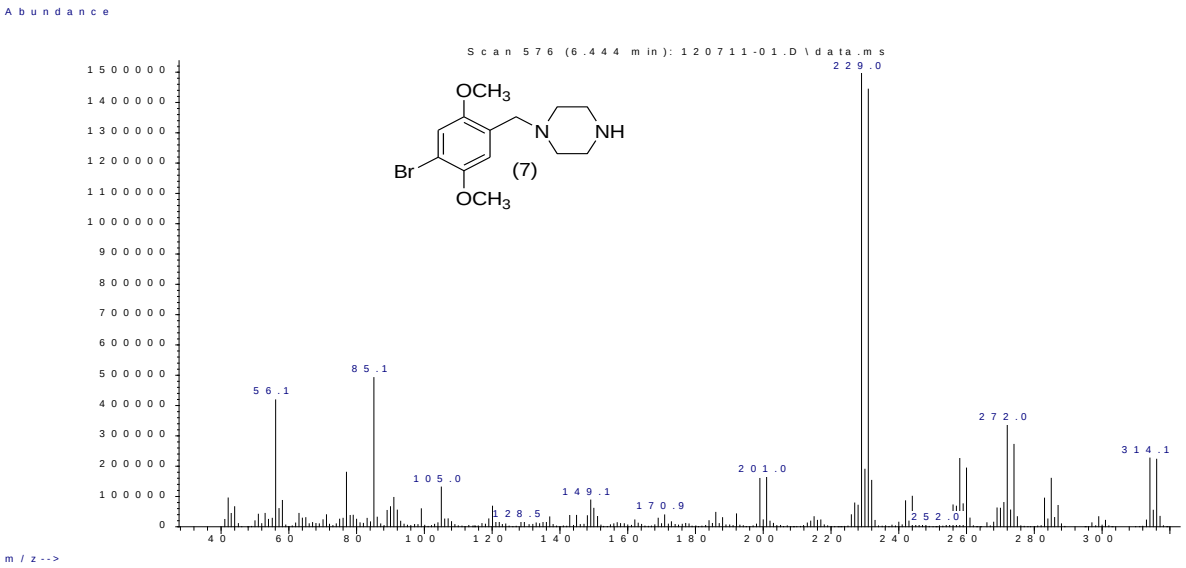


Fig. 7-1: EI mass spectra of the seven bromodimethoxybenzylpiperazines.

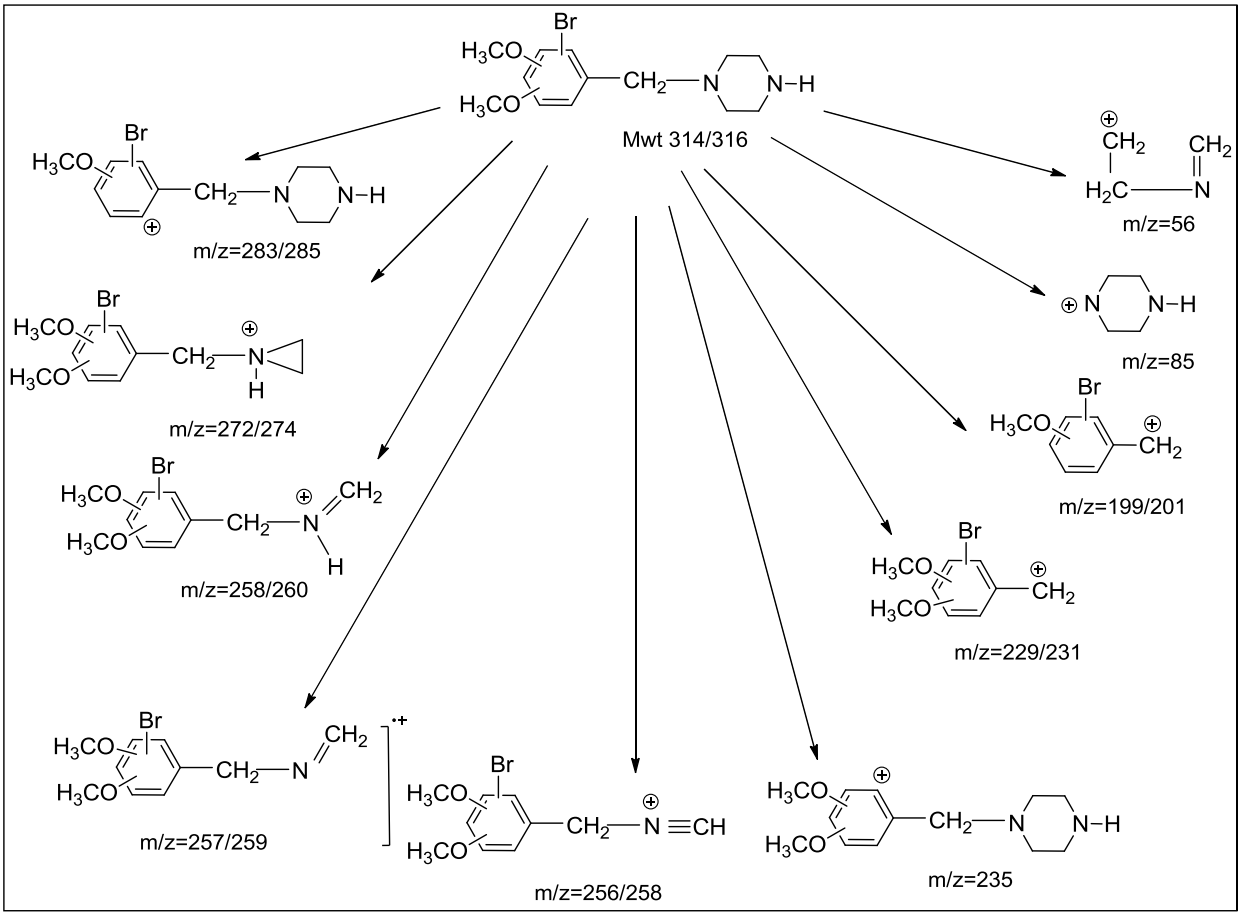


Fig. 7-2: EI mass spectral fragmentation pattern of the underivatized bromodimethoxybenzylpiperazines.

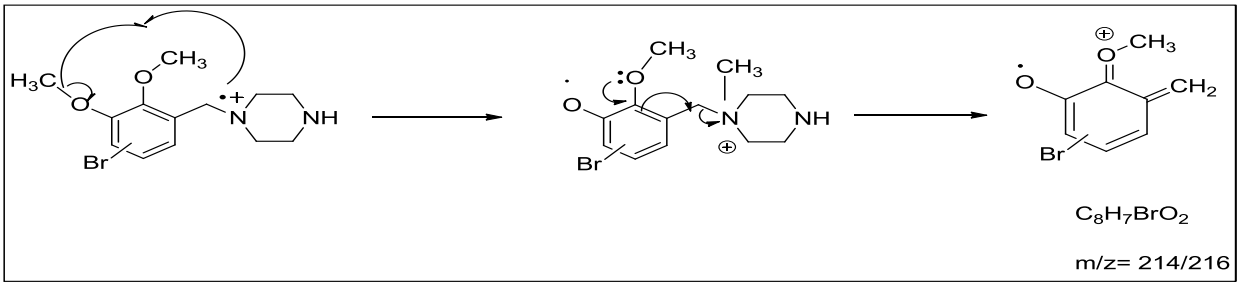


Fig. 7-3: Proposed mechanism for the formation of the m/z 214/216 ion in the mass spectra of the brominated 2,3-dimethoxybenzylpiperazines (compounds 1 and 2).

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 [Abdel-Hay *et al*, 2013]. 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 Figure 7-3 [Abdel-Hay *et al*, 2013].

An additional fragmentation pathway which is observed for some of the ortho-methoxy ring substituted compounds is described in Figure 7-4. Those bromodimethoxybenzylpiperazines 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 Figure 7-4. This fragment occurs in all the mass spectra of the underivatized and PFPA derivatives of the ortho-

methoxy BrDMBPs. This suggested mechanism for the loss of CH_2O from the ortho-methoxy benzyl cations was previously discussed [Maher *et al*, 2009].

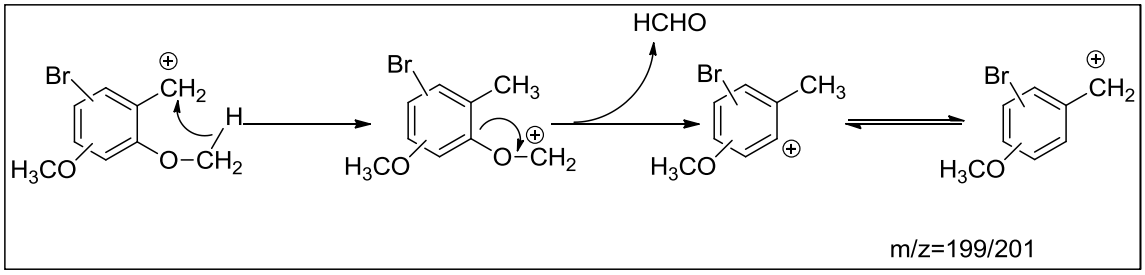
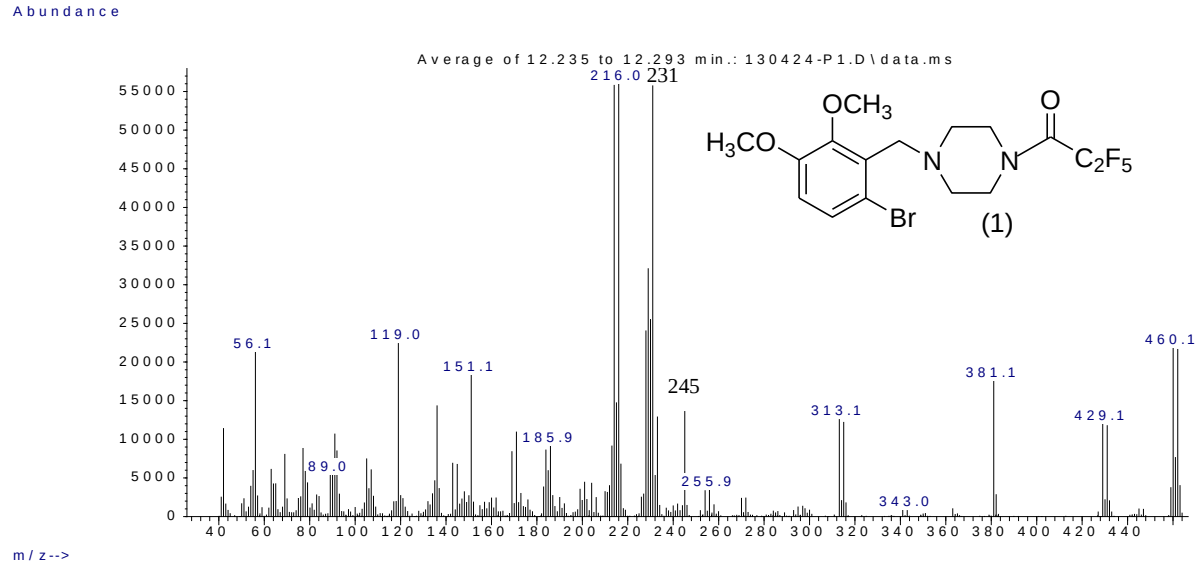
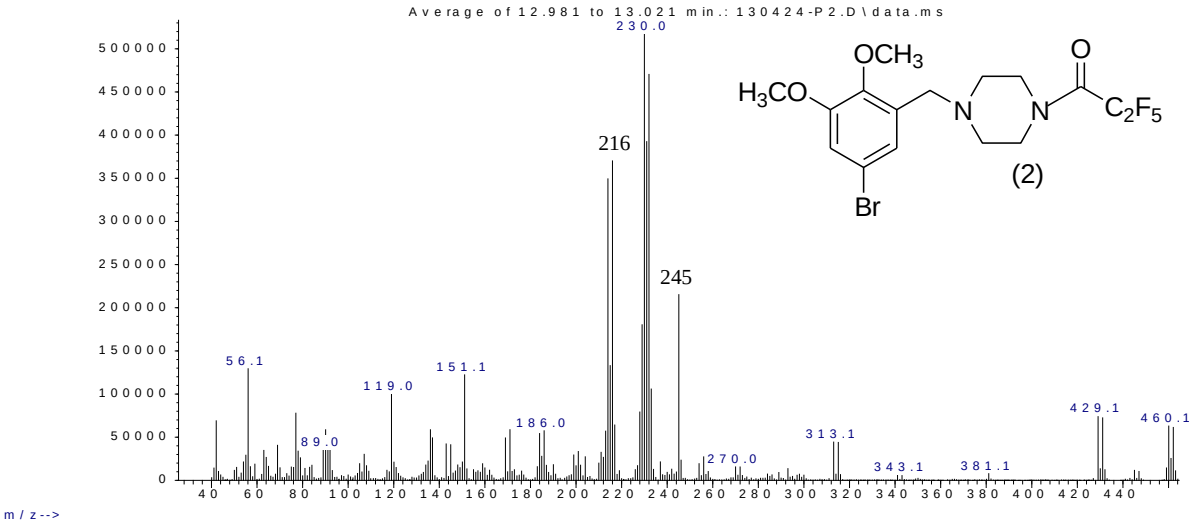


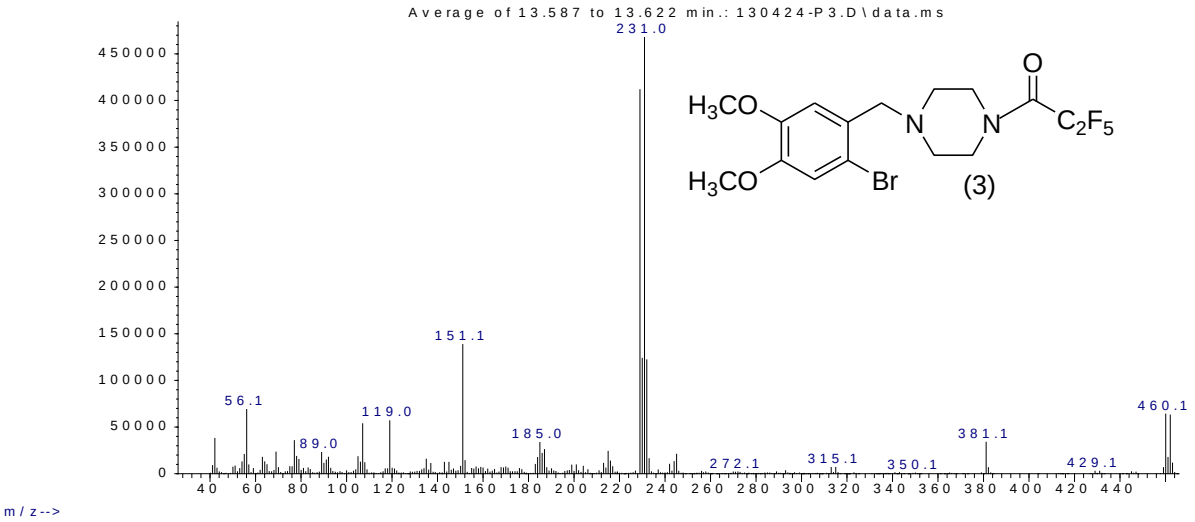
Fig. 7-4: Mechanism for the formation of the m/z 199/201 ion in the mass spectra of the underivatized and derivatized 2-methoxy regioisomers of the bromodimethoxybenzylpiperazines.

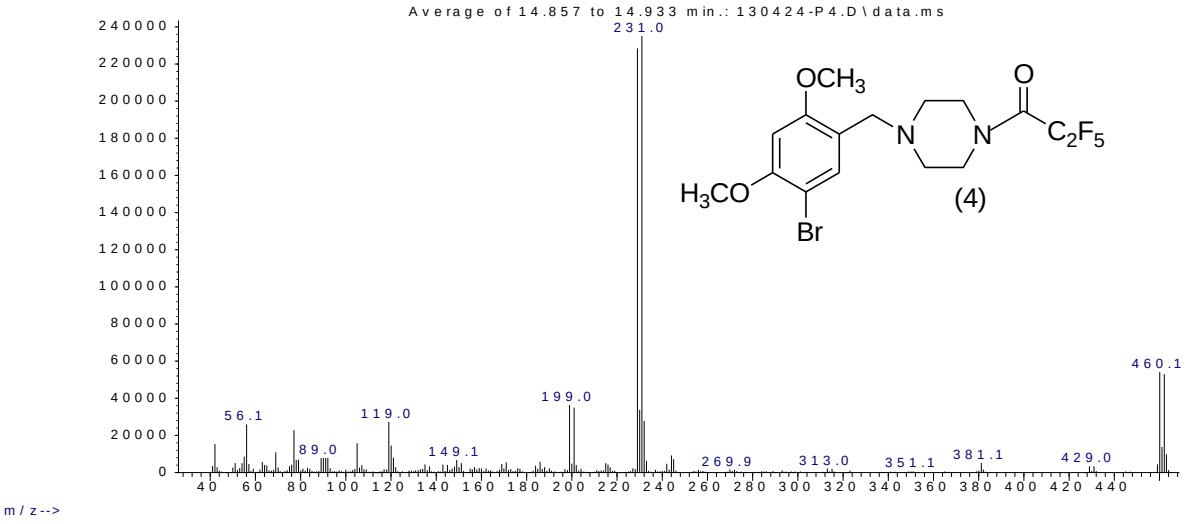
The second phase of this study involved the preparation and evaluation of acylated derivatives of the seven regioisomeric bromodimethoxybenzylpiperazines in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds.

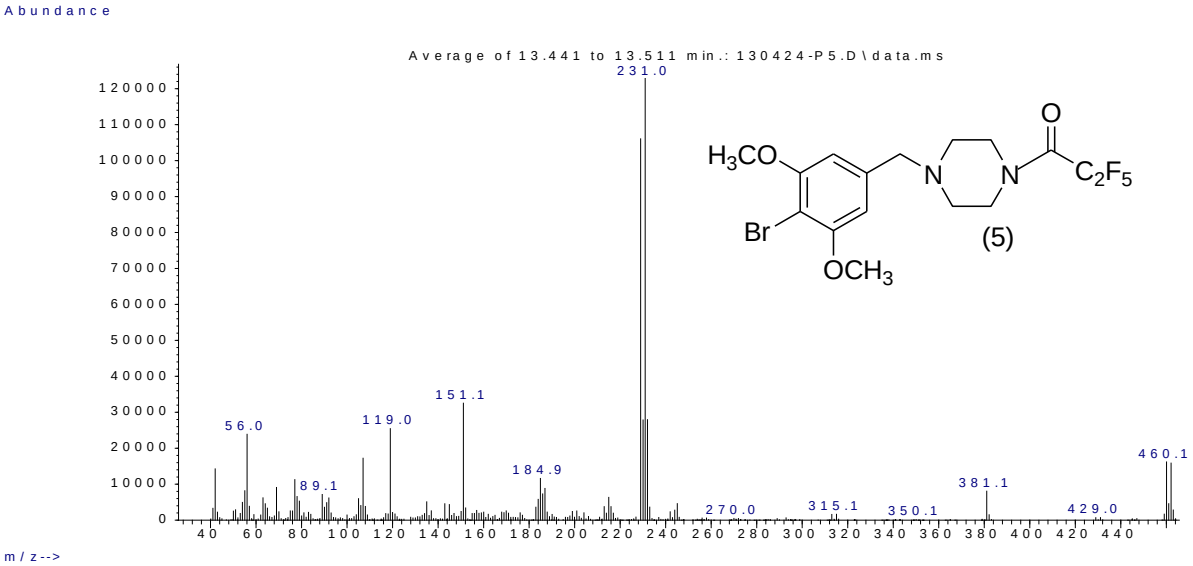
The mass spectra for the seven pentafluoropropionyl amides are shown in Figure 7-5. The pentafluoropropionyl derivatives were all evaluated for their ability to individualize the mass spectra of each regioisomer to the exclusion of the other regioisomeric compounds. The proposed fragmentation pathways for the PFPA derivatives of the seven bromodimethoxybenzylpiperazines (BrDMBPs) are shown in Figure 7-6. From these spectra, a common peak with high relative abundance occurs at m/z 460/462, which corresponds to the molecular ions for these PFPA 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 Figure 7-5. 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 PFPA derivatives [Abdel-Hay *et al*, 2013]. 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 PFPA 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 immonium cation fragment is present in a subset of these compounds but not apparently

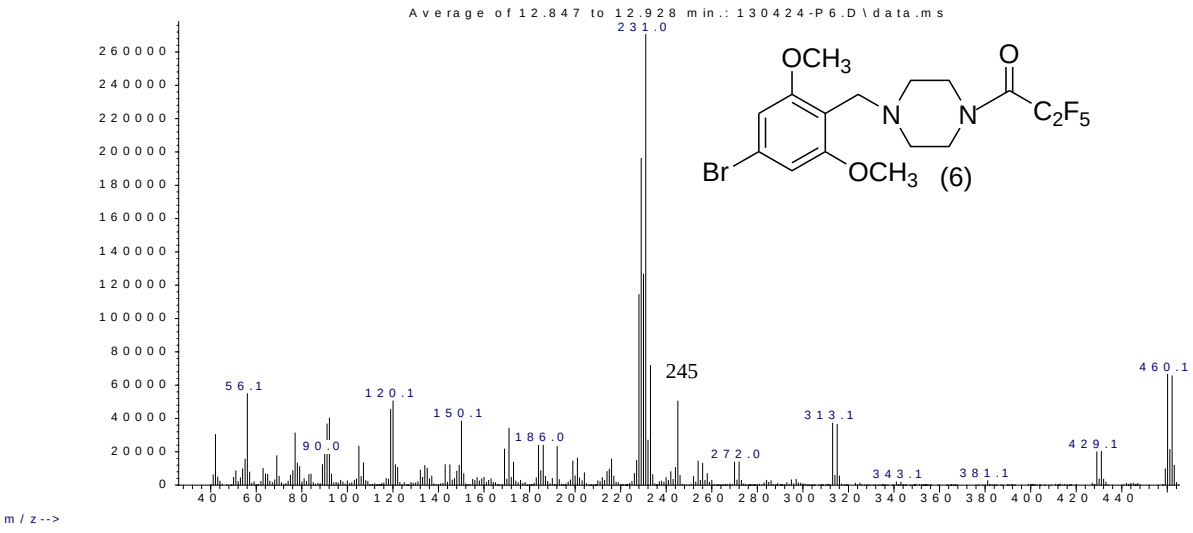












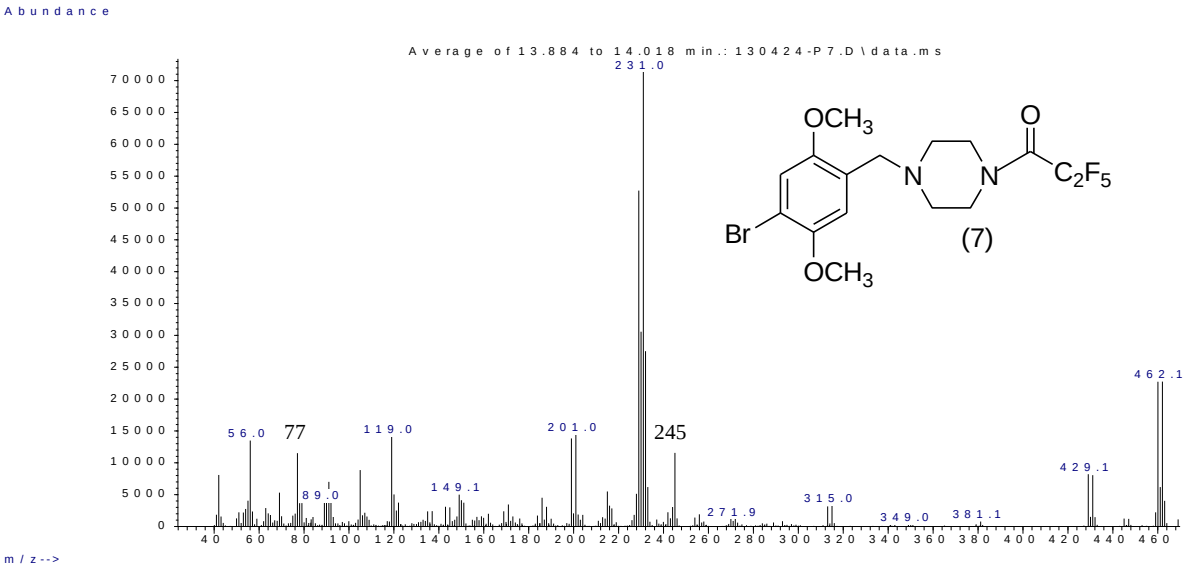


Fig. 7-5: MS spectra of pentafluoropropionyl derivatives of the seven piperazine compounds.

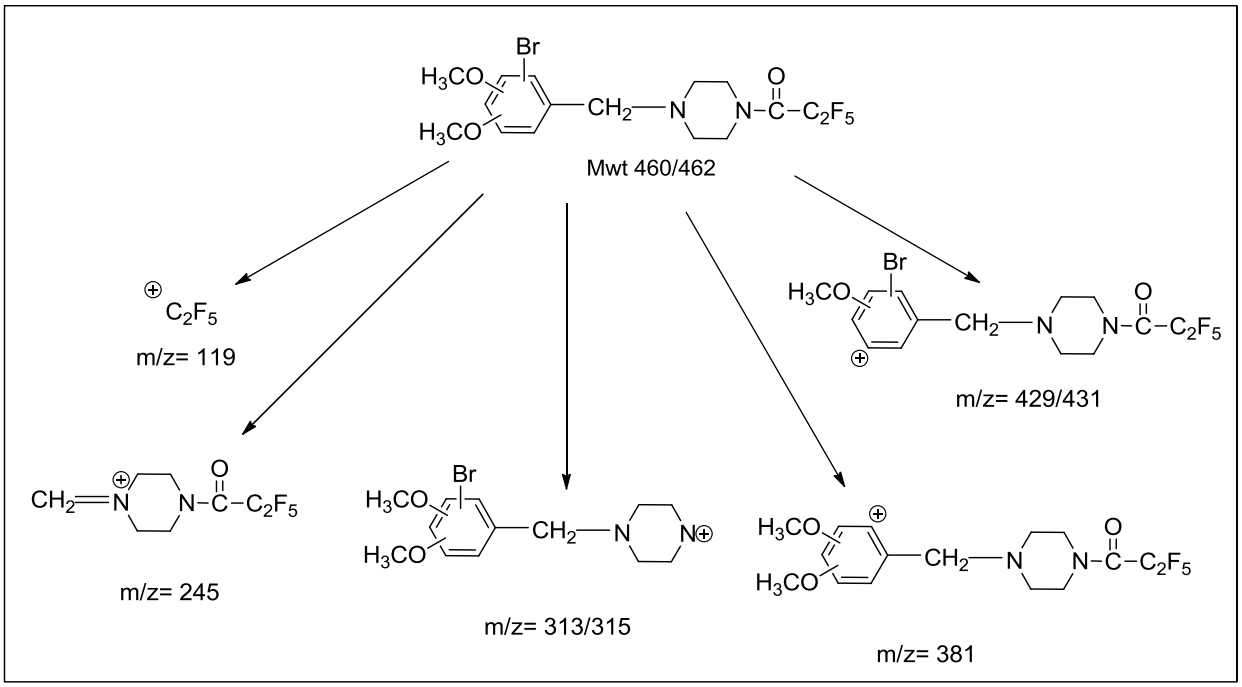


Fig. 7-6: EI mass spectral fragmentation pattern of the PFPA derivatives of the bromodimethoxybenzylpiperazines.

correlated with a specific structural feature. Thus, even acylation of the seven piperazines does not give characteristic fragments that help to discriminate among the seven regioisomers.

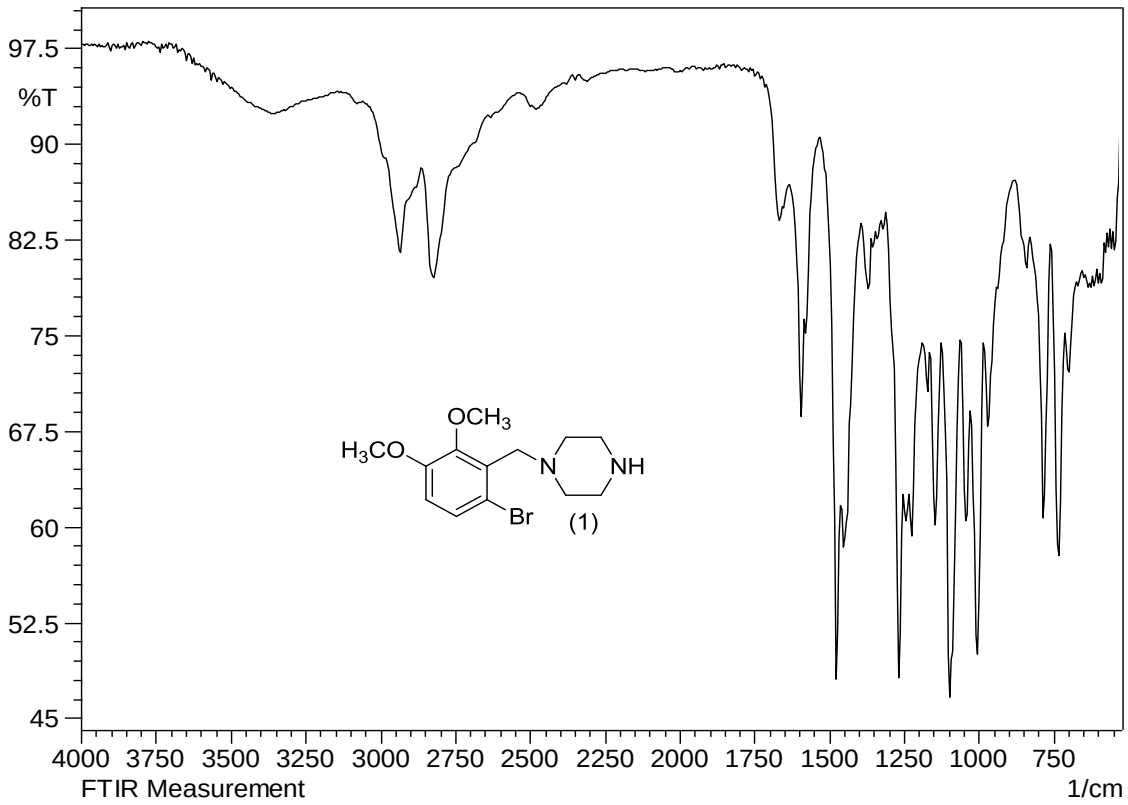
FTIR Spectroscopic Study of the Bromodimethoxybenzylpiperazine

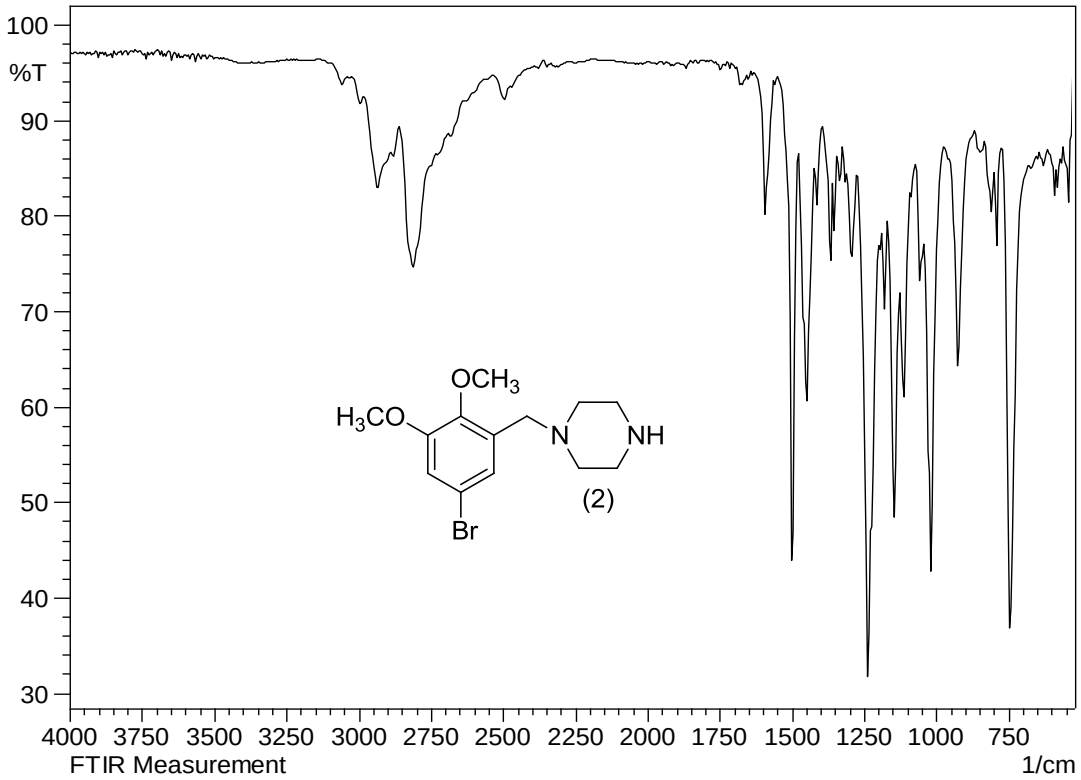
Attenuated total reflection fourier transform infrared spectroscopy (IR) was evaluated for differentiation among the seven regioisomeric BrDMBPs. This method has the possibility of yielding compound specificity without the need for chemical modification of the drug molecule. The IRs for the seven underivatized piperazines are shown in Figure 7-7. 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} [Kempfert, 1988]. 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}.

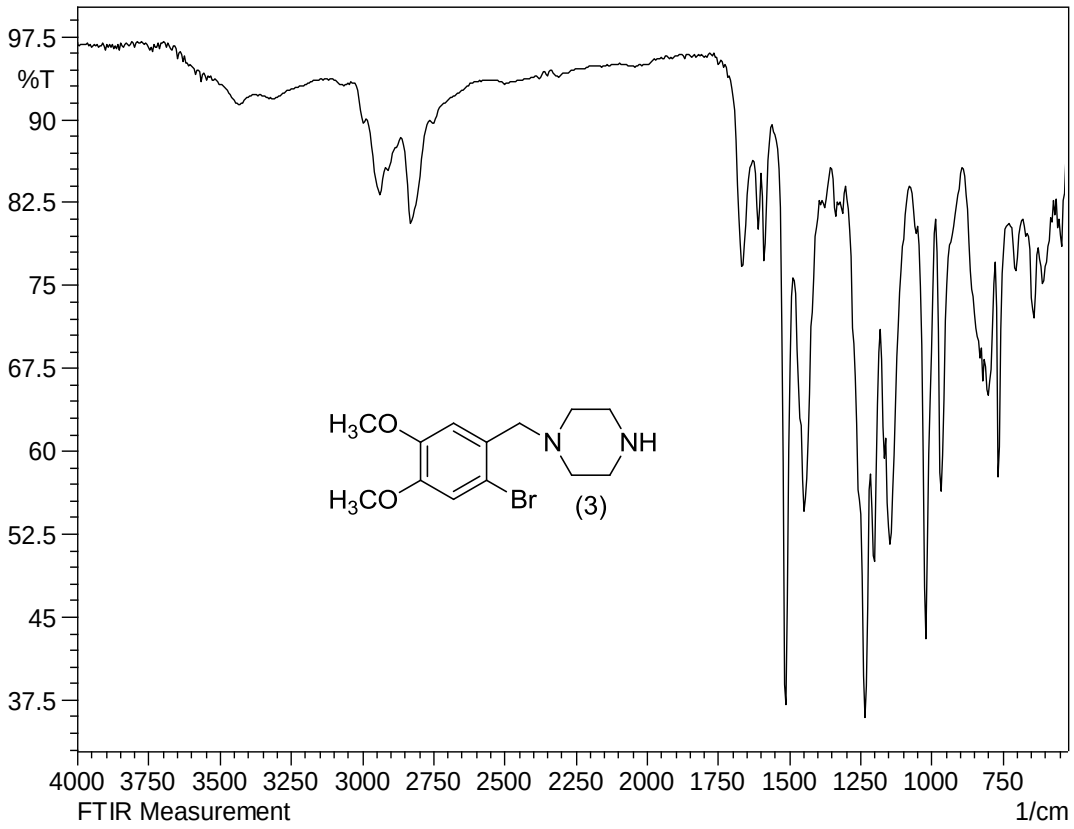
Compound 1 is characterized by the strong intensity band at 1471 cm^{-1} which is split into peaks of equal intensity at 1593 and 1456 cm^{-1} in compound 2. This isomer also has another medium intensity doublet at 1286 and 1273 cm^{-1} shifted to a singlet at 1249 cm^{-1} in the IR spectrum of compound 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

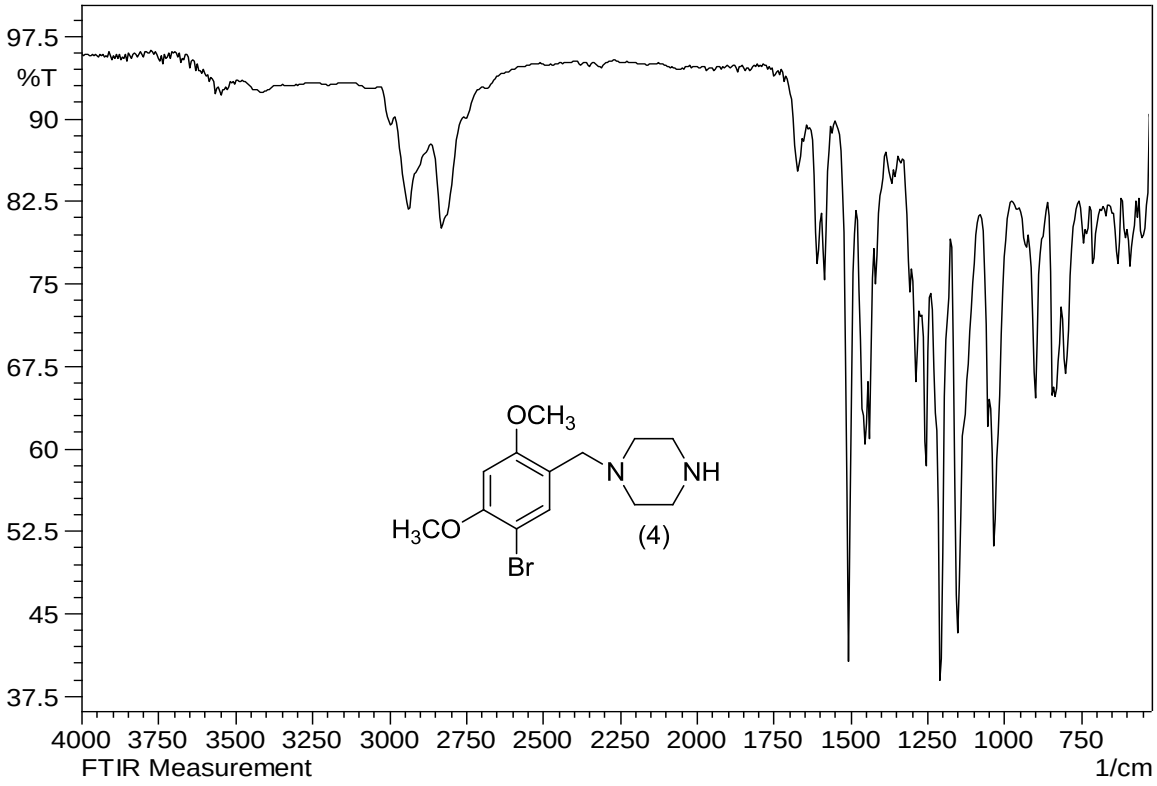
1519 cm^{-1} which is shifted to a strong intensity peak at 1486 cm^{-1} in compound 6, a strong doublet at 1506 and 1471 cm^{-1} in compound 5. The IR spectrum of compound 5 can be distinguished by a singlet of strong intensity appearing at 1127 cm^{-1} compared to a peak of strong intensity at 1035 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.

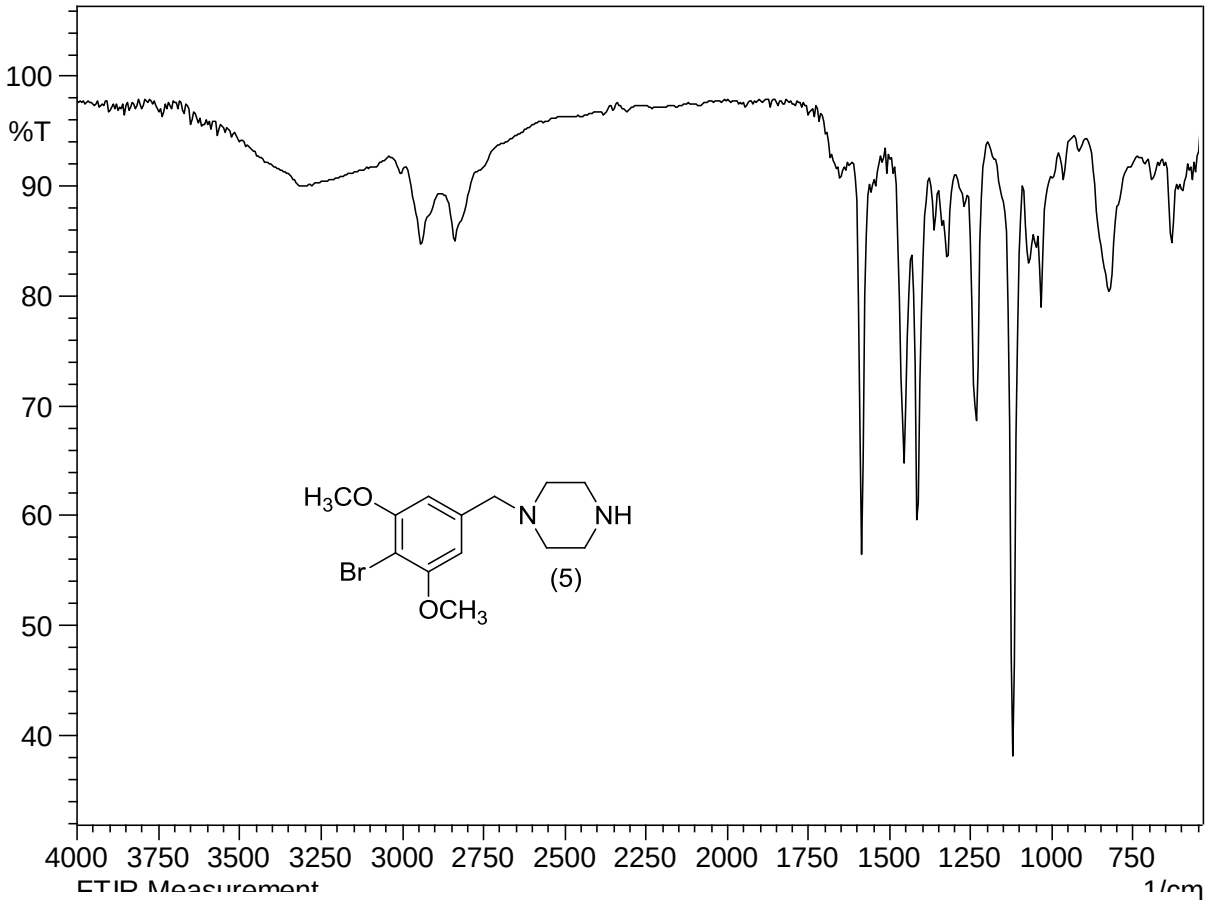
This study shows that infrared spectra provide useful data for differentiation among these regioisomeric piperazines of mass spectral equivalence. Mass spectrometry establishes these compounds as having an isomeric relationship of equal molecular weight and equivalent major fragment ions. Infrared absorption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

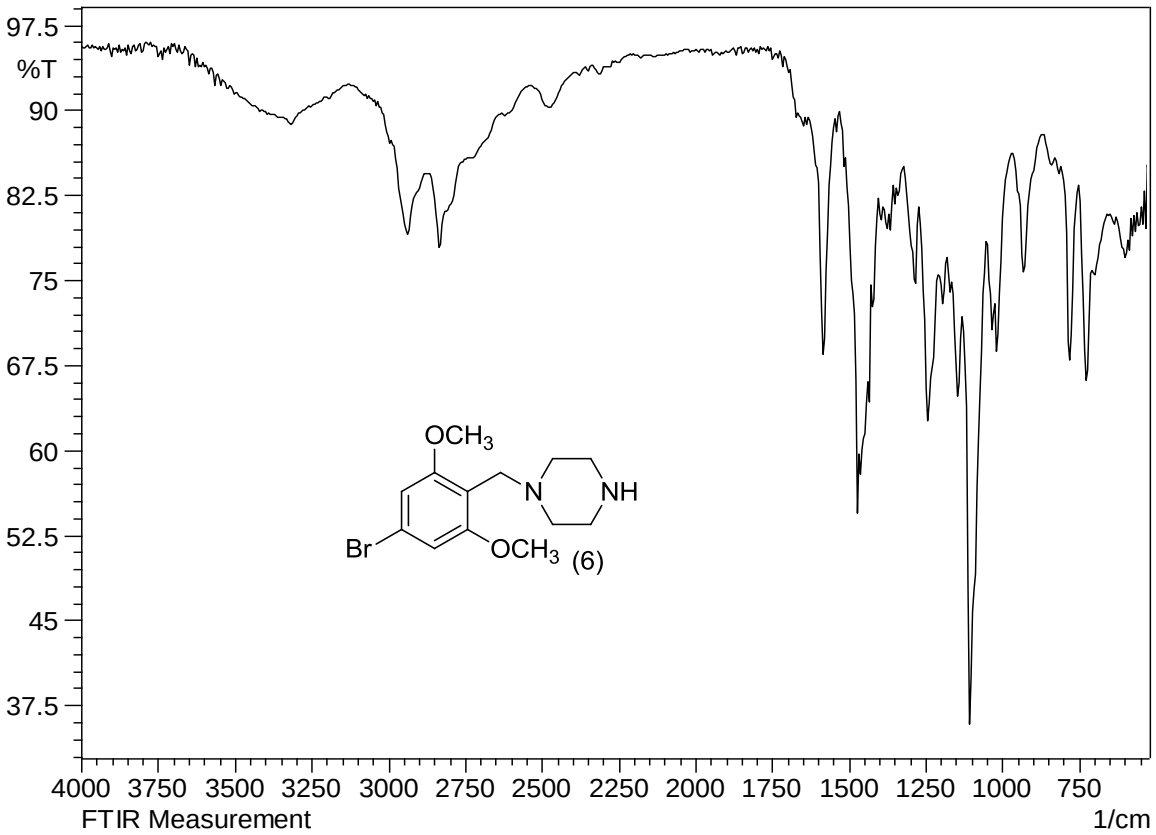












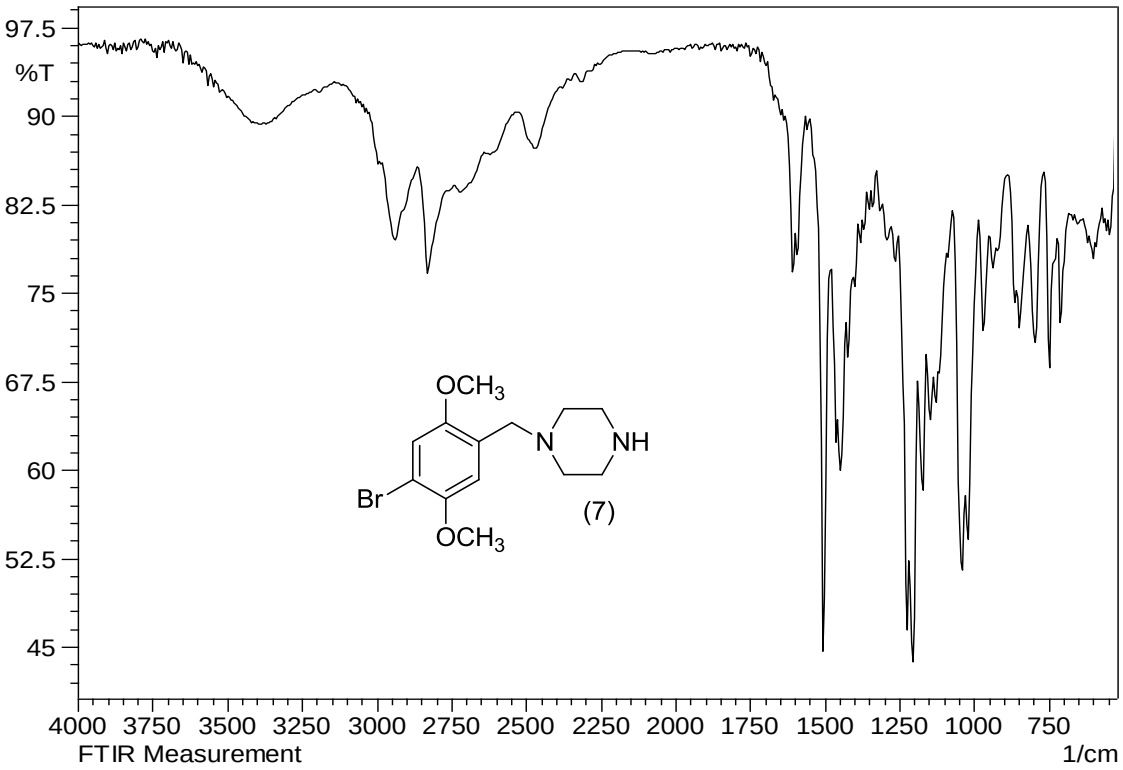


Fig. 7-7: ATR FTIR spectra of the seven bromodimethoxybenzylpiperazines.

Gas Chromatographic Separation of the Bromodimethoxybenzylpiperazines

Gas chromatographic separation of the derivatized piperazines was accomplished on an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase using a capillary column (30m × 0.25mm, 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 Figure 7-8. This chromatogram shows the separation of the PFPA derivatives of the seven bromodimethoxybenzylpiperazines. 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 two compounds (4 and 7) and these latest eluents have in common the bromine ortho to a methoxy group or 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 PFPA forms of these two compounds show some ions which could be used for differentiation. Compound 7 shows a higher relative abundance for $(M-31)^+$ 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.

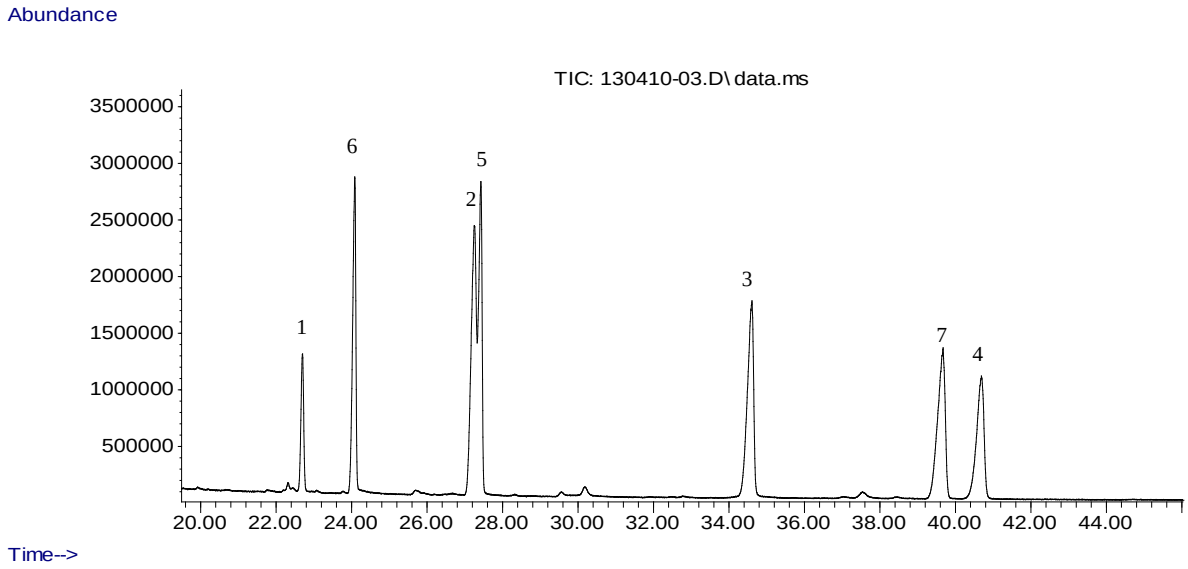


Fig. 7-8: Gas chromatographic separation of the pentafluoropropionyl derivatives of the bromodimethoxybenzylpiperazines using Rtx-200 column.

Conclusion

The seven regioisomeric bromodimethoxybenzylpiperazines 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. ATR 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.

References

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 Science International 121 (2001) 47-56.

Abdel-Hay, K.M., DeRuiter, J. and Clark, C. GC-MS and GC-IRD Studies on the Six Ring Regioisomeric Dimethoxybenzylpiperazines (DMBPs). Drug Testing and Analysis, 5 (2013) 560-572. DOI:10.1002/dta.1417

Abdel-Hay, K.M., DeRuiter, J. and Clark, C. Differentiation of methylbenzylpiperazines (MBPs) and benzoylpiperazine (BNZP) using GC-MS and GC-IRD. Drug Testing and Analysis 4(6) (2012) 441-448.

Maher, H.M., Awad, T., DeRuiter, J. Clark, C.R. GC-MS and GC-IRD studies on Dimethoxyamphetamines (DMA): Regioisomers Related to 2,5-DMA, Forensic Sci. Int. 192 (2009) 115-125.

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Chapter 8

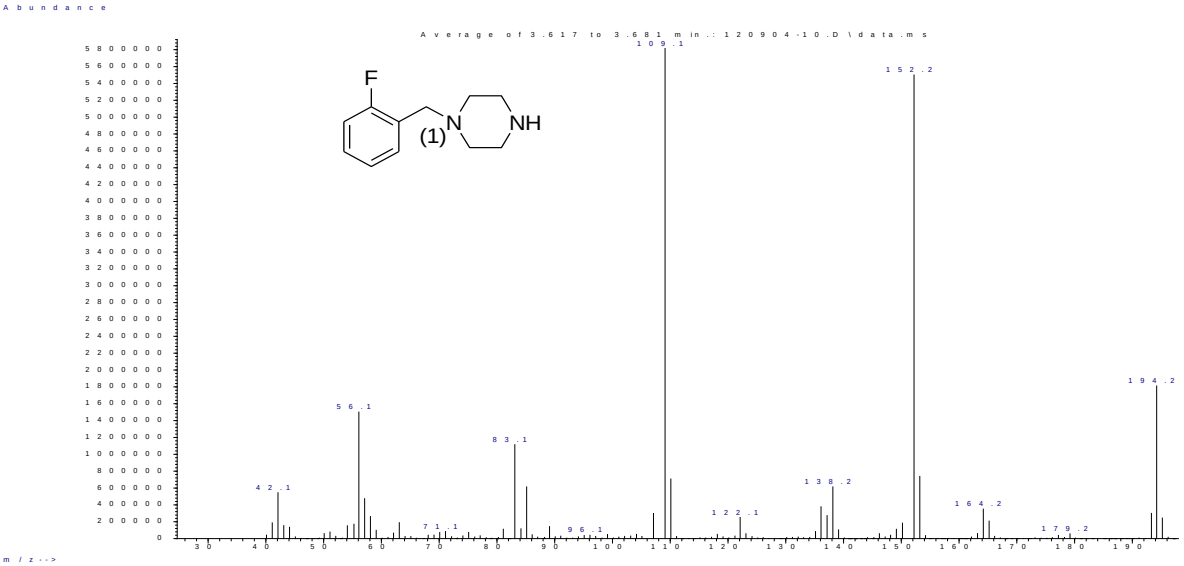
GC-MS Studies on the Ring Regioiomeric Fluorobenzylpiperazines (FBPs)

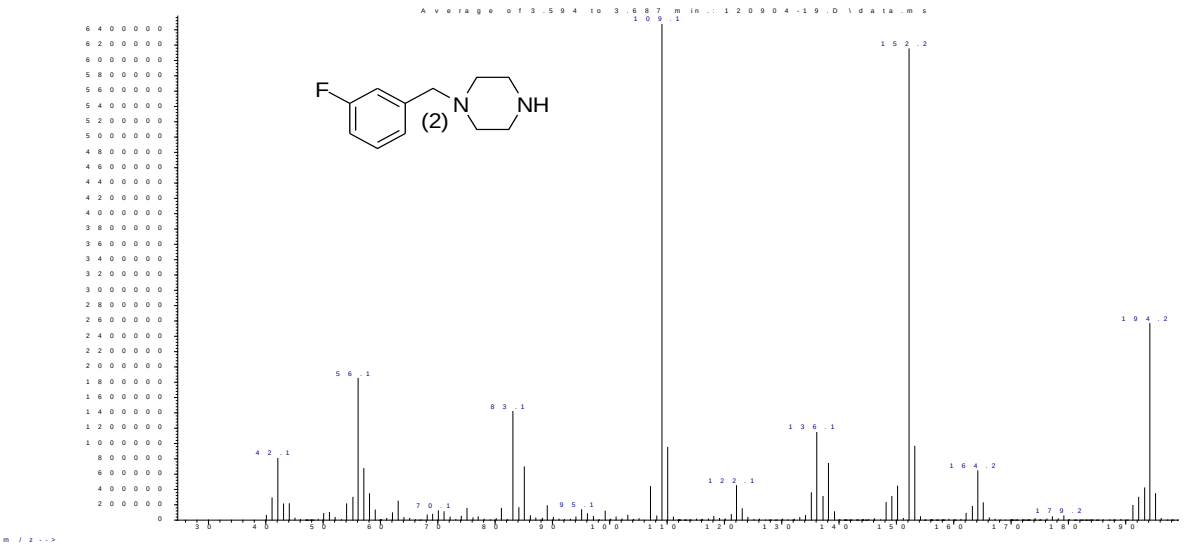
Three ring substituted fluorobenzylpiperazines (FBPs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass Spectral Studies of the Fluorobenzylpiperazines (FBPs)

Figure 8-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3) in this study. The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the three piperazines show fragment ions at m/z 152, 138, 109, 85 and 56 as well as other ions of low relative abundance. The proposed structures of these ions are shown in Figure 8-2 and are based in part on a previous report describing the fragmentation of unsubstituted benzylpiperazine [de Boer *et al*, 2001]. The mass spectra for the ring substituted fluorobenzylpiperazines (Compounds 1-3)





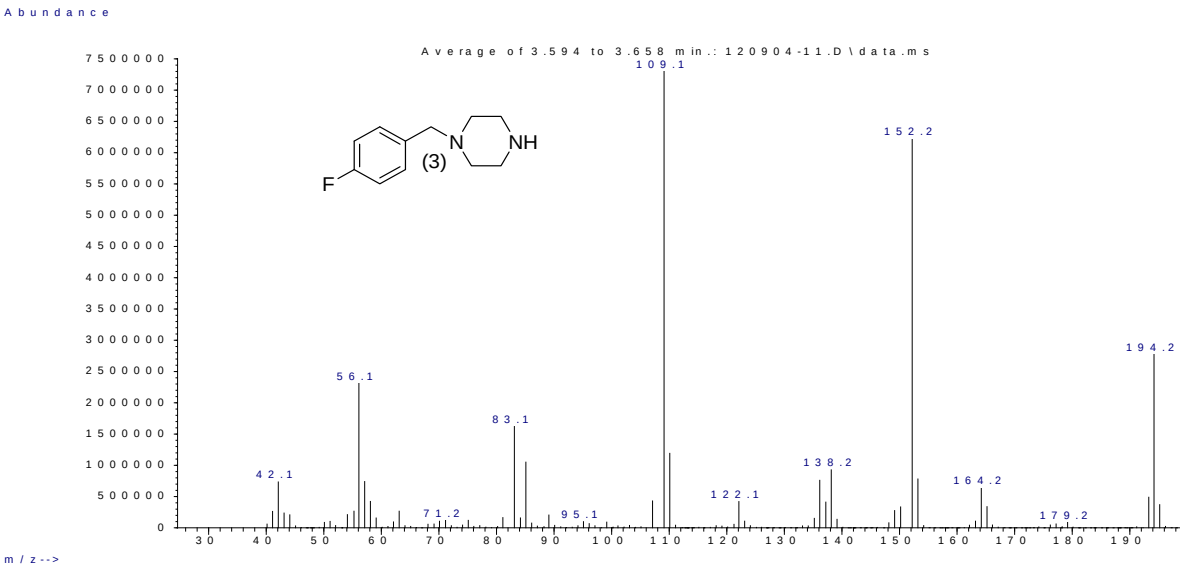


Fig. 8-1: EI mass spectra of the three fluorobenzylpiperazines.

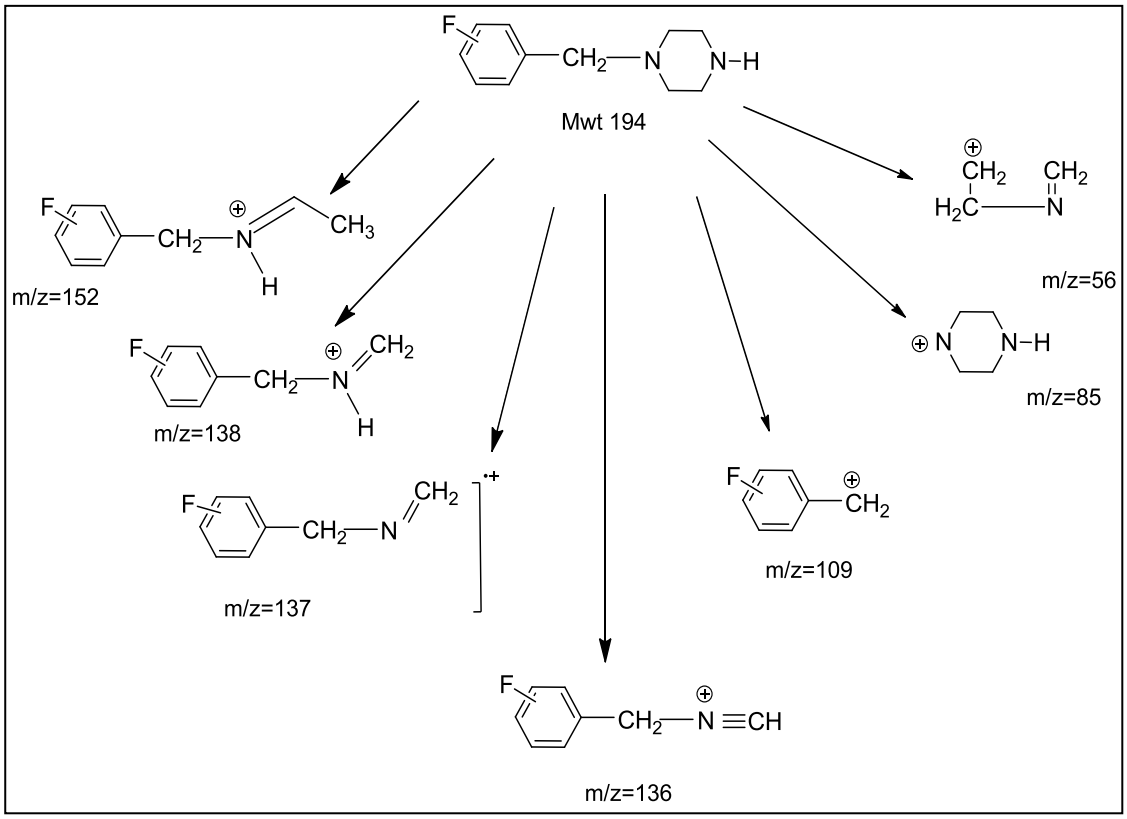
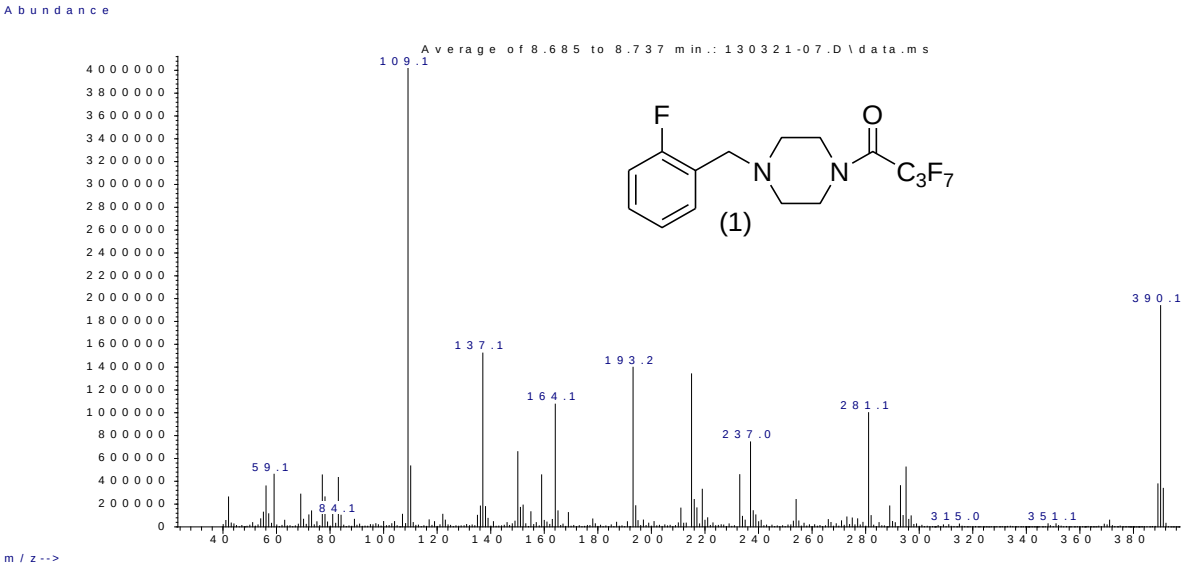
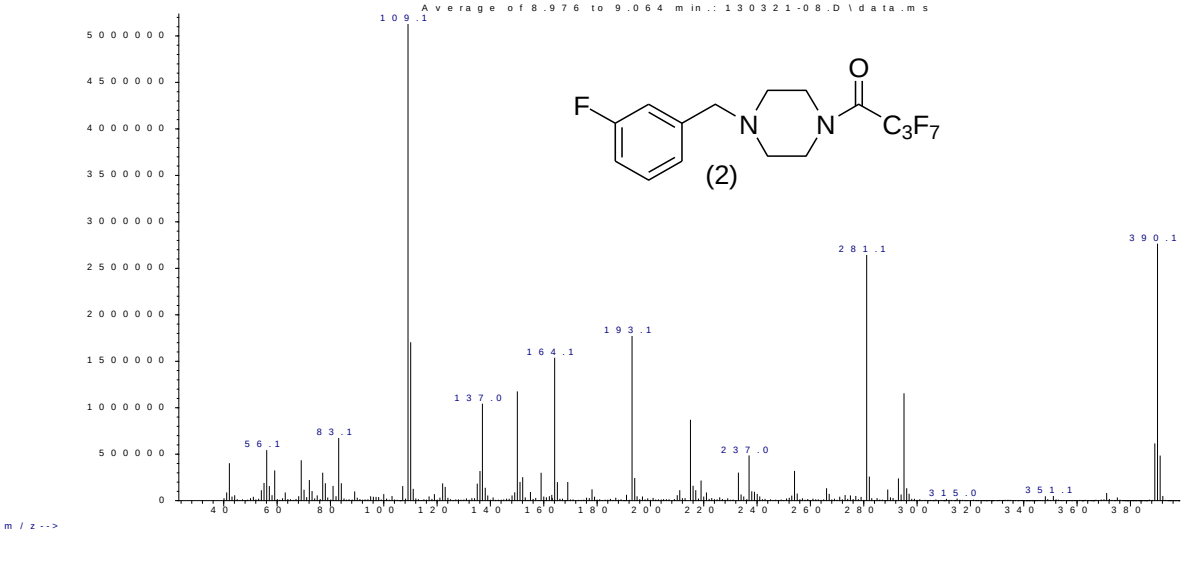


Fig. 8-2: EI mass spectral fragmentation pattern of the underivatized fluorobenzylpiperazines.

are essentially identical. 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 8-3 shows the mass spectra of the heptafluorobutryl amides of the three compounds as representatives of all the perfluoroacylated piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 290, 340 and 390, respectively. The major fragment ion in these spectra occurs at *m/z* 109 and corresponds to the fluoro substituted benzyl 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 $(M\text{-}109)^+$ ion for each amide. The ion at *m/z* 193 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 other in this series of isomeric piperazine compounds.



(1)



(2)

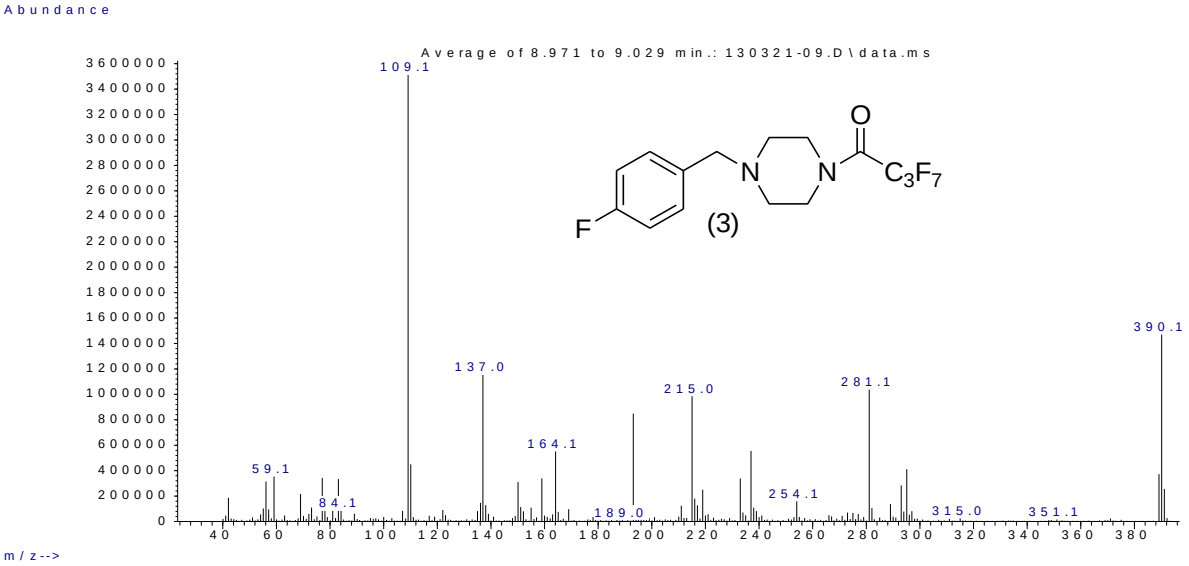


Fig. 8-3: Mass spectra of heptafluorobutryl derivatives of the three fluorobenzylpiperazine compounds.

Gas Chromatographic Separation of the Fluorobenzylpiperazines (FBPs)

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). The separation of the HFBA 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 12°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. The chromatogram in Figures 8-4 is a representatives of the results obtained for all samples on this stationary phase.

In Figure 8-4 the HFBA derivatives of the three fluorobenzylpiperazines eluted in the order of 2, 3, 4-fluorobenzylpiperazine. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

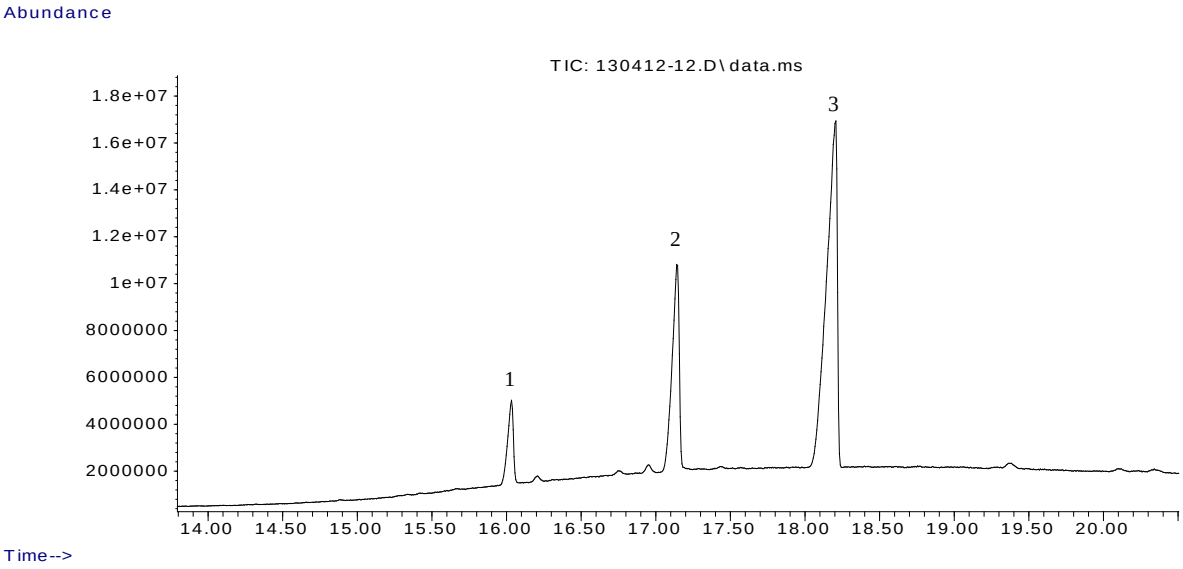


Fig. 8-4: Gas chromatographic separation of the heptafluorobutyryl derivatives of FBPs using Rtx-200 column. The number over the peak represents the compound number.

Conclusion

The three regioisomeric fluorobenzylpiperazines have a regioisomeric relationship to each other. These three piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. The three piperazines were successfully resolved on the GC stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Chapter 9

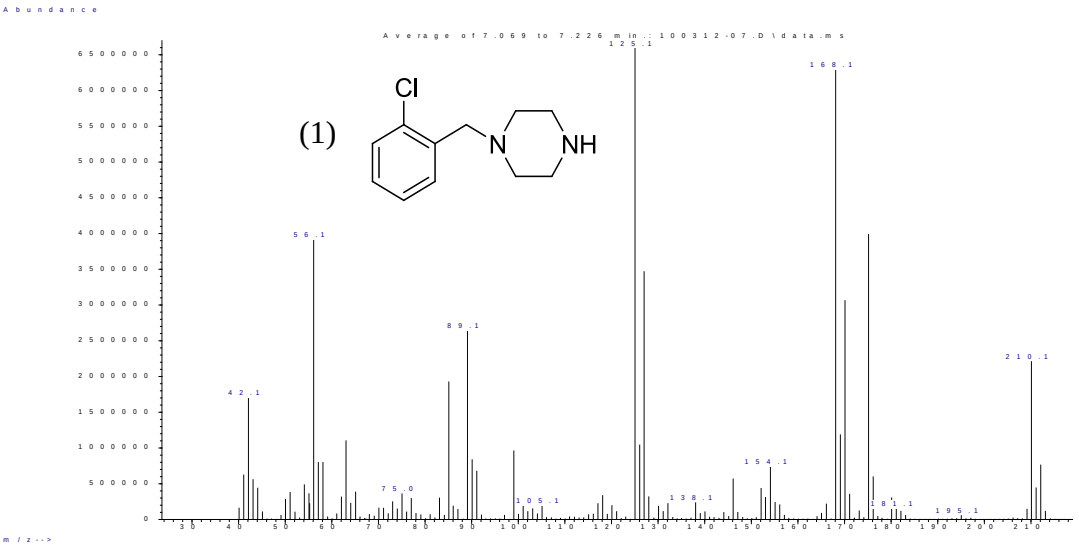
Differentiation of the Chlorobenzylpiperazines (ClBPs) by GC-IRD and GC-MS

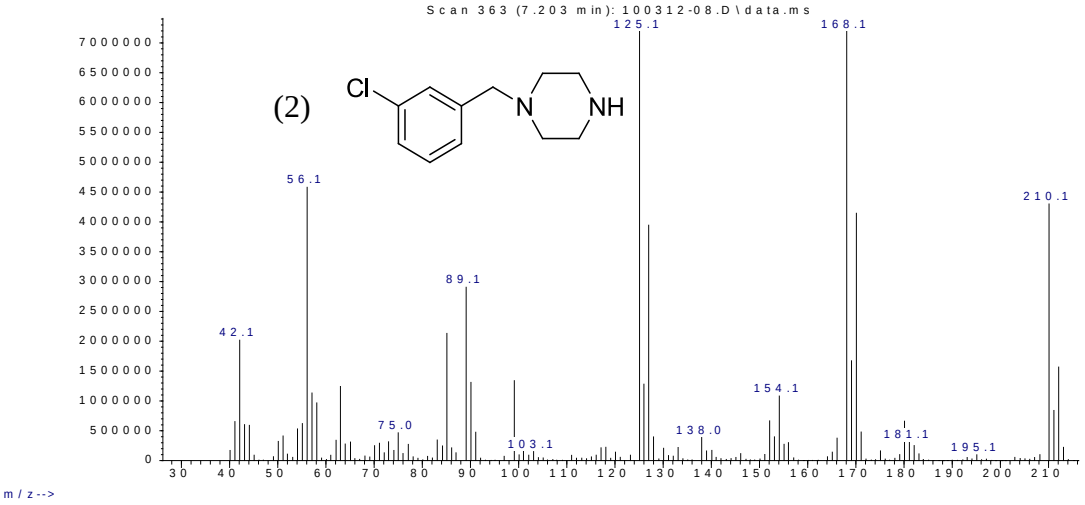
Three ring substituted chlorobenzylpiperazines (ClBPs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter 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 three isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass Spectral Studies of the Chlorobenzylpiperazines (ClBPs)

Figure 9-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3) in this study. The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the three piperazines show fragment ions at m/z 168, 154, 125, 85 and 56 as well as other ions of low relative abundance. The proposed structures of these ions are shown in Figure 9-2 and are based in part on a previous report describing the fragmentation of unsubstituted benzylpiperazine [de Boer *et al*, 2001]. The mass spectra for the ring substituted chlorobenzylpiperazines (Compounds 1-3)





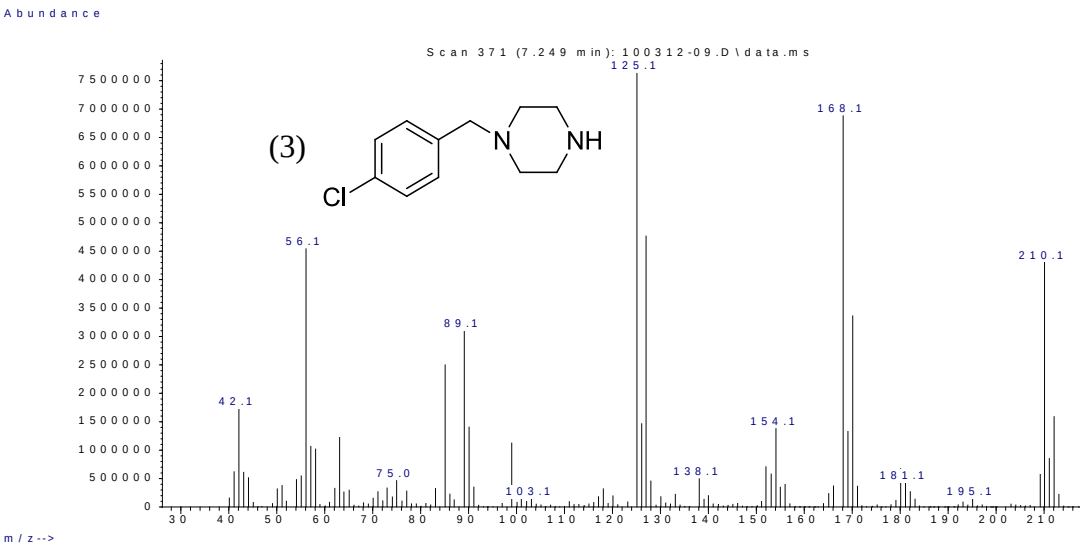


Fig. 9-1: EI mass spectra of the three chlorobenzylpiperazines.

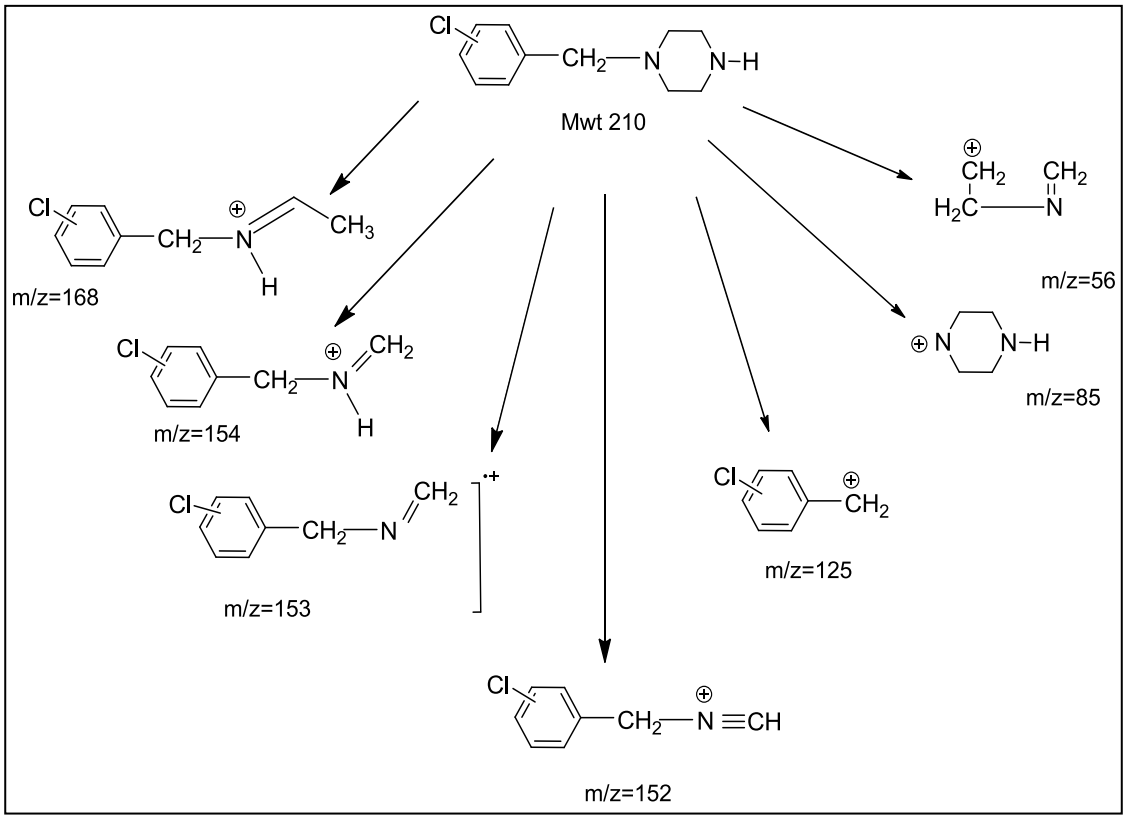
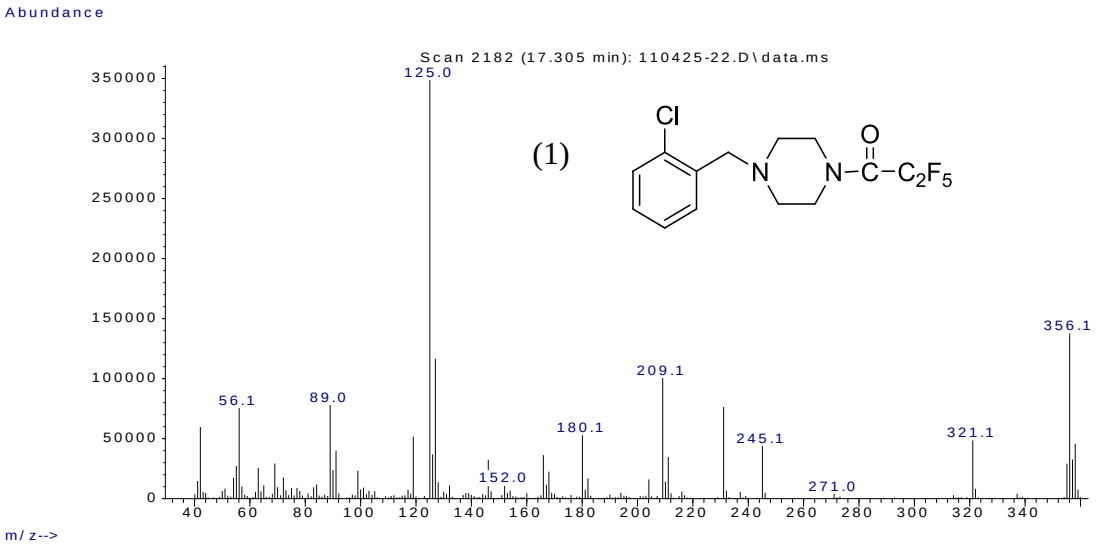
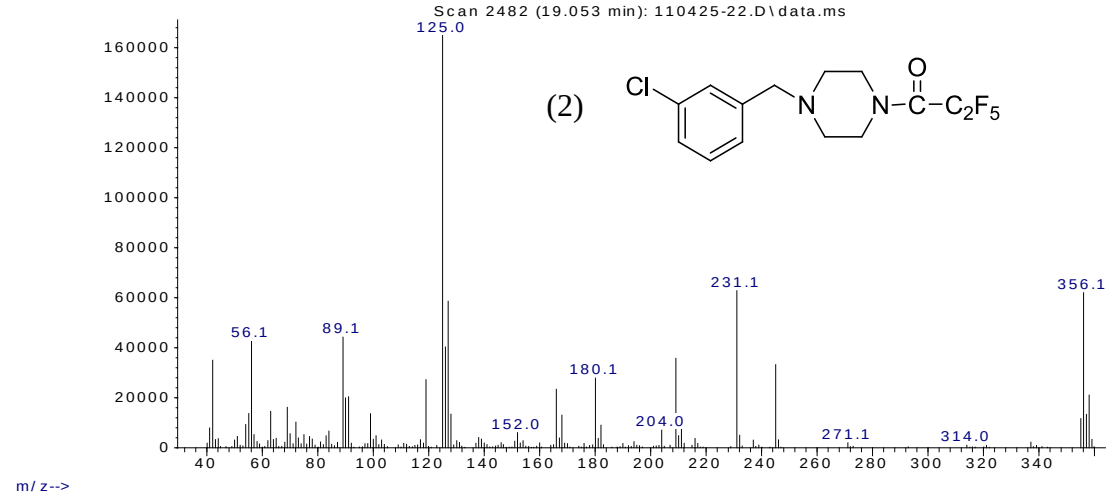


Fig. 9-2: EI mass spectral fragmentation pattern of the underivatized chlorobenzylpiperazines.

are essentially identical. 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 9-3 shows the mass spectra of the pentafluoropropionyl amides of the three compounds as representatives of all the perfluoroacylated piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 306, 356 and 406, respectively. The major fragment ion in these spectra occurs at *m/z* 125 and corresponds to the chloro substituted benzyl 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 $(M-125)^+$ ion for each amide. The ion at *m/z* 209 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 other in this series of isomeric piperazine compounds.





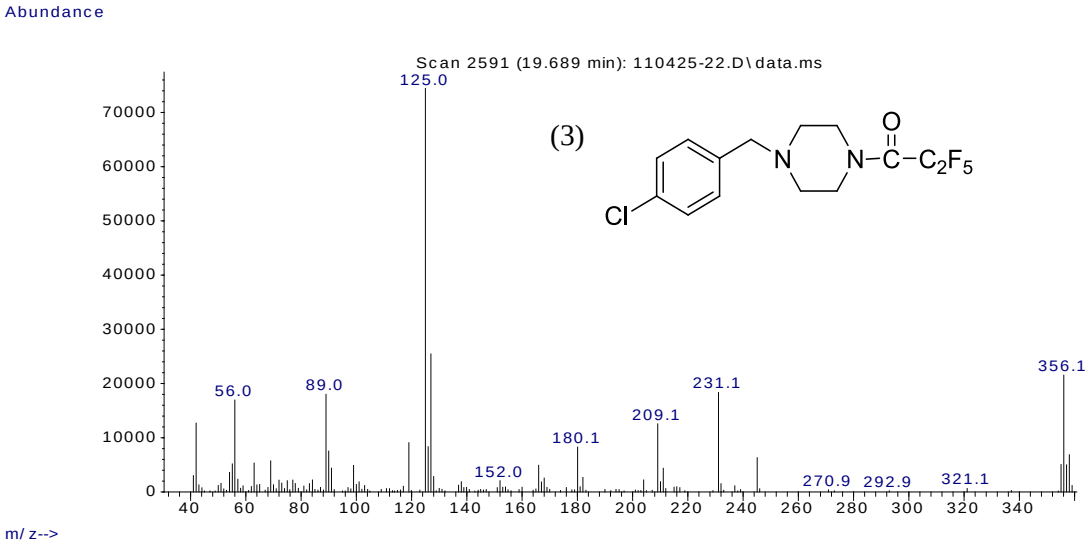


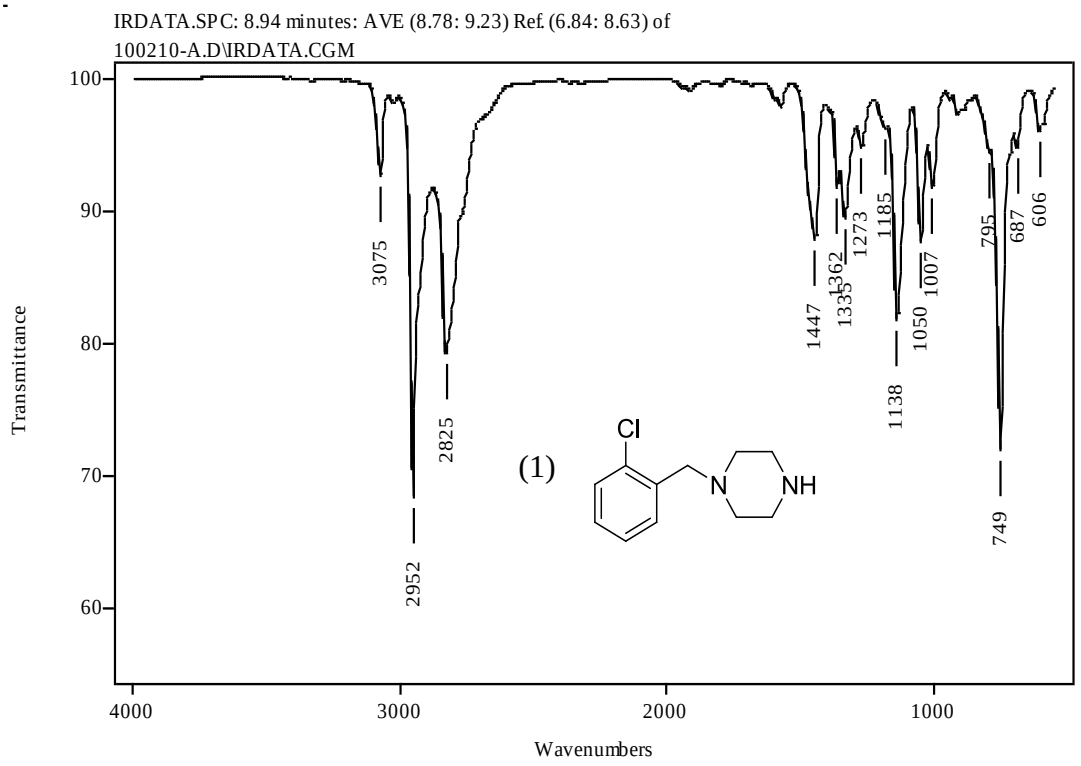
Fig. 9-3: Mass spectra of pentafluoropropionyl derivatives of the three chlorobenzylpiperazine compounds.

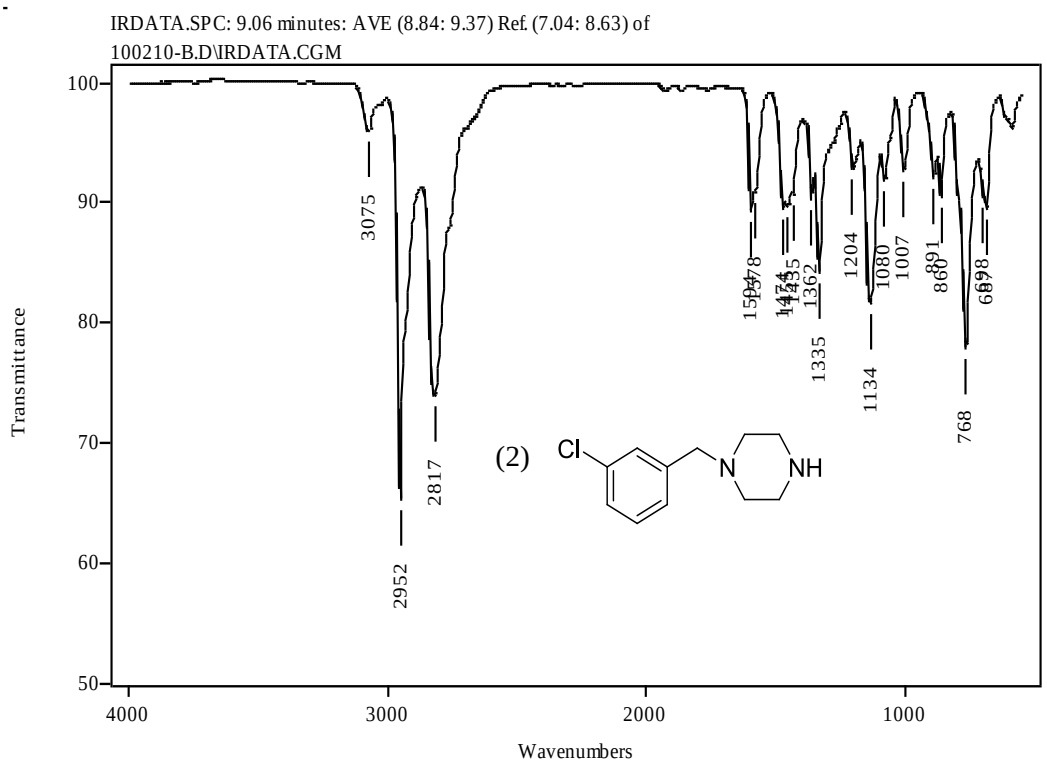
Vapor-phase Infra-Red Spectrophotometry of the Chlorobenzylpiperazines (ClBPs)

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 three piperazines. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the three underivatized piperazines are shown in Figure 9-4. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph and each compound shows a vapor-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}. 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 differentiated by the positions and intensities of several IR peaks in the region of 750 – 1620 cm^{-1}.

The three ring substituted chlorobenzylpiperazines 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 singlet at 1493 cm^{-1} which is shifted to a weak intensity singlet at 1447 cm^{-1} in compound 1. Compound 2 shows a medium peak at 1134 cm^{-1} which is shifted to a peak at 1138 cm^{-1} in compound 1 and to a doublet at 1131, 1096 cm^{-1} in compound 3. These results provide an excellent illustration of the value of vapor 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 three compounds.





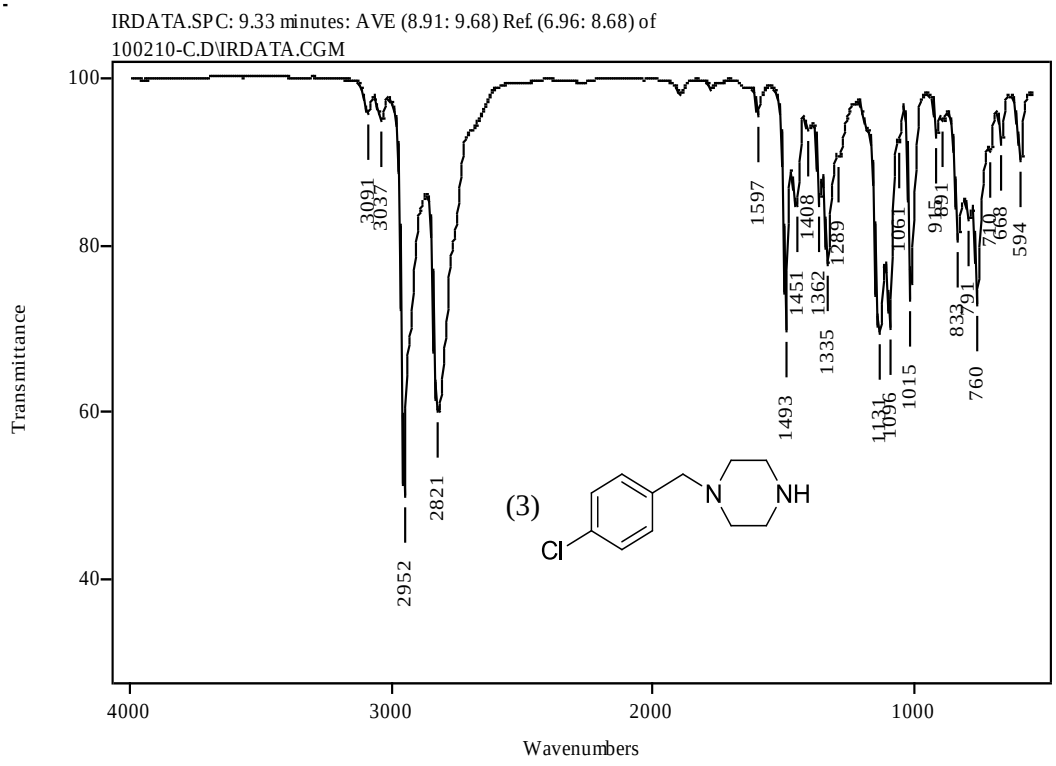


Fig. 9-4: Vapor phase IR spectra of the three chlorobenzyl piperazines.

Gas Chromatographic Separation of the Chlorobenzylpiperazines (ClBPs)

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). The separation of the TFA and PFPA 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 12°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. The chromatograms in Figures 9-5 and 9-6 are representatives of the results obtained for all samples on this stationary phase.

In Figures 9-5 and 9-6 the TFA and PFPA derivatives of the three chlorobenzylpiperazines eluted in the order of 2, 3, 4-chlorobenzylpiperazine. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

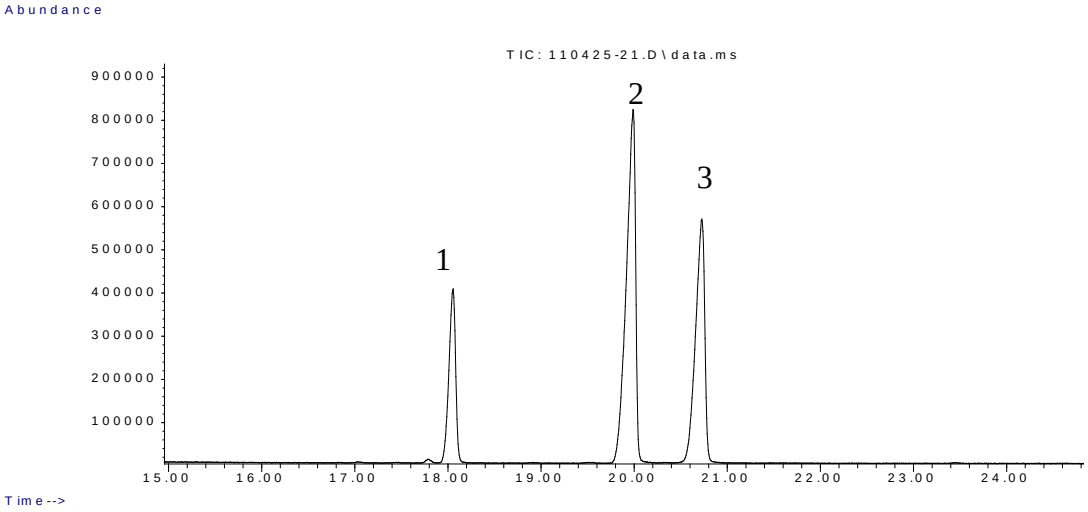


Fig. 9-5: Gas chromatographic separation of the trifluoroacetyl derivatives of ClBPs using Rtx-200 column. The number over the peak represents the compound number.

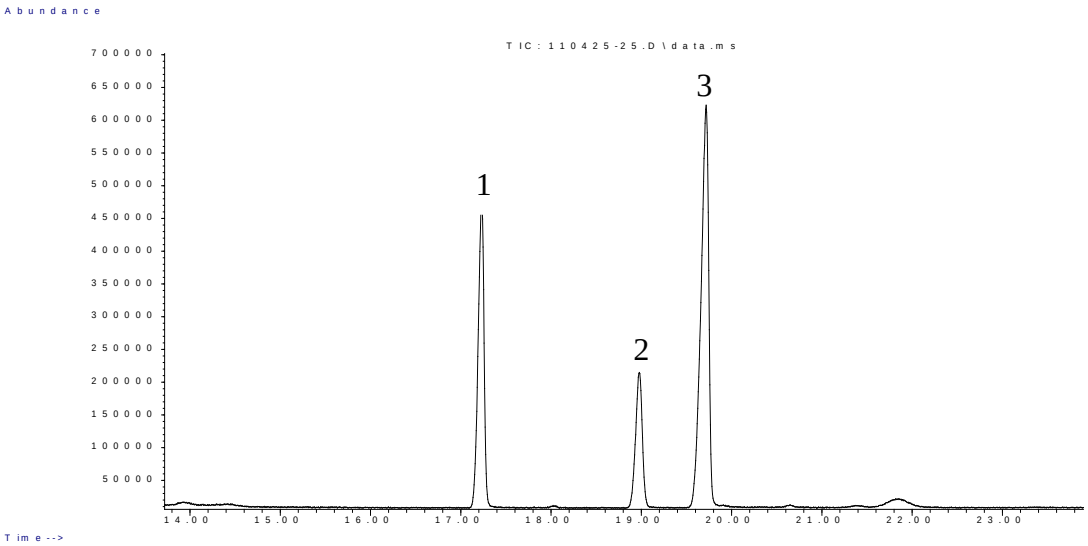


Fig. 9-6: Gas chromatographic separation of the pentafluoropropionyl derivatives of ClBPs using Rtx-200 column. The number over the peak represents the compound number.

Conclusion

The three regioisomeric chlorobenzylpiperazines have a regioisomeric relationship to each other. These three piperazines yield very similar fragment ions in their mass spectra Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. GC-IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650-1700 cm^{-1}. The three piperazines were successfully resolved on the GC stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Chapter 10

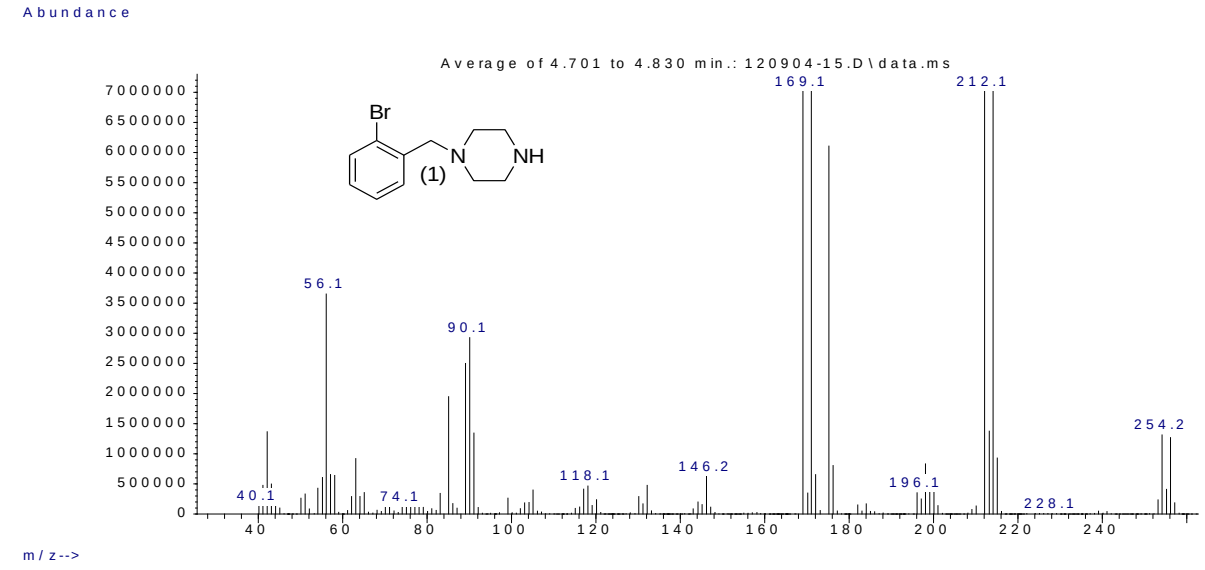
GC-MS Studies on the Ring Regioiomeric Bromobenzylpiperazines (BrBPs)

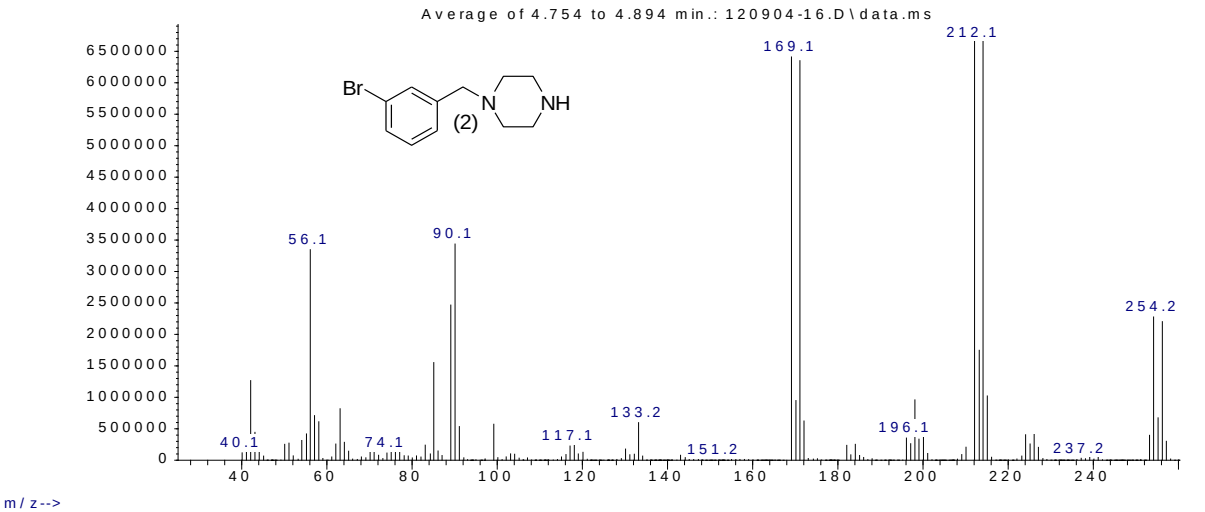
Three ring substituted bromobenzylpiperazines (BrBPs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass Spectral Studies of the Bromobenzylpiperazines (BrBPs)

Figure 10-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3) in this study. The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the three piperazines show fragment ions at m/z 212/214, 198/200, 169/171, 85 and 56 as well as other ions of low relative abundance. The proposed structures of these ions are shown in Figure 10-2 and are based in part on a previous report describing the fragmentation of unsubstituted benzylpiperazine [de Boer *et al*, 2001]. The mass spectra for the ring substituted bromobenzylpiperazines (Compounds 1-3)





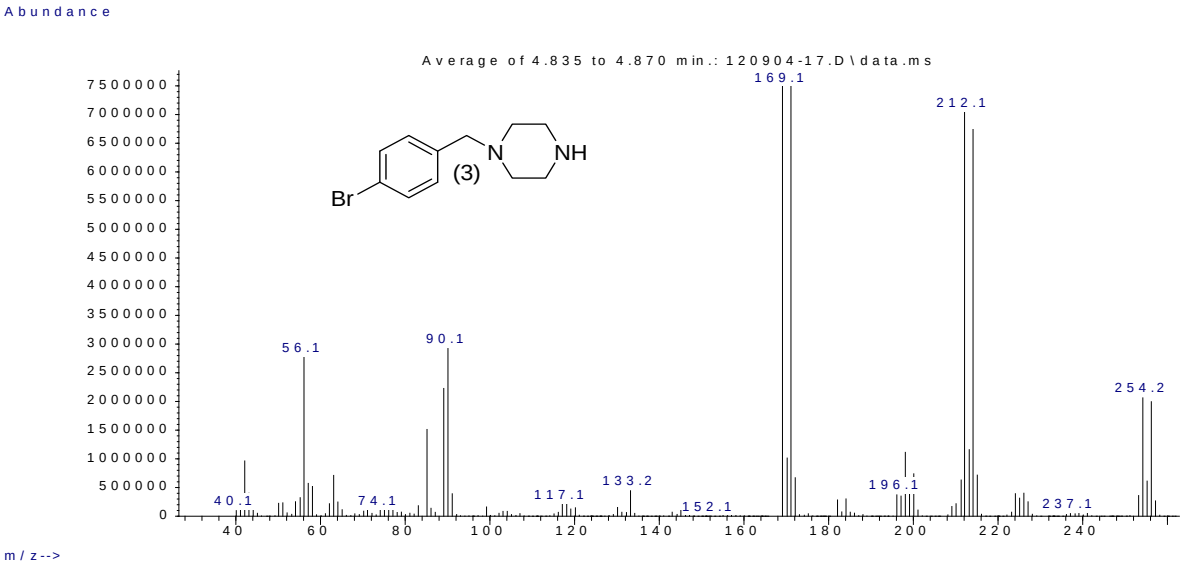


Fig. 10-1: EI mass spectra of the three bromobenzylpiperazines.

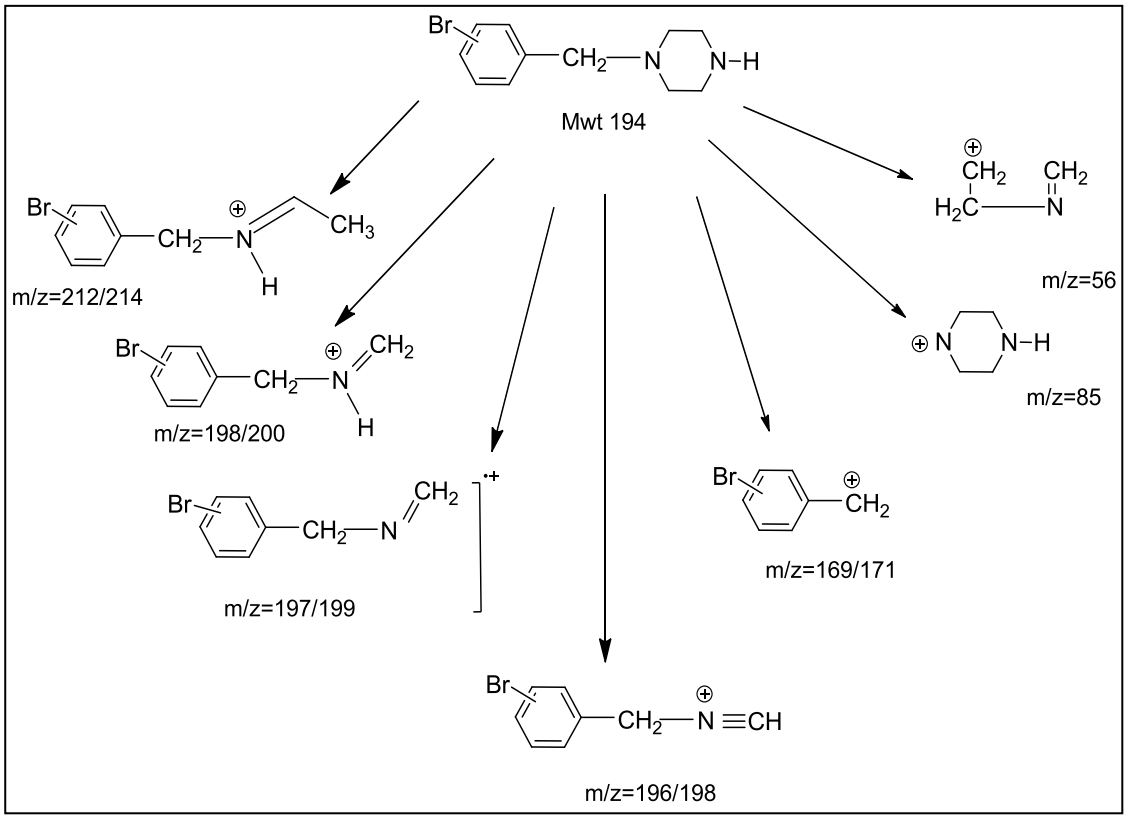
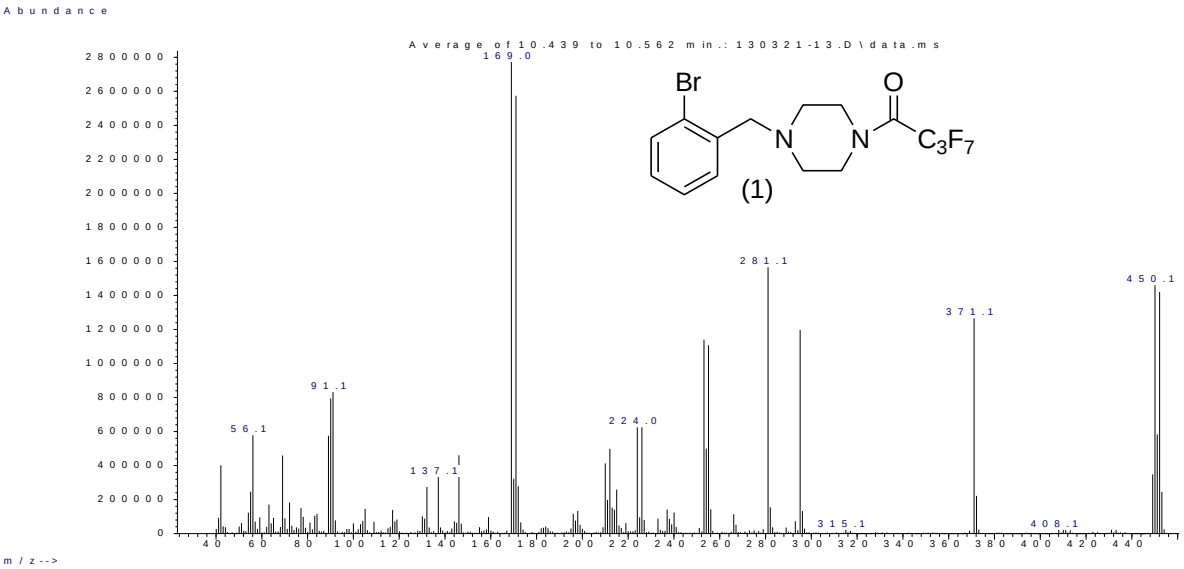
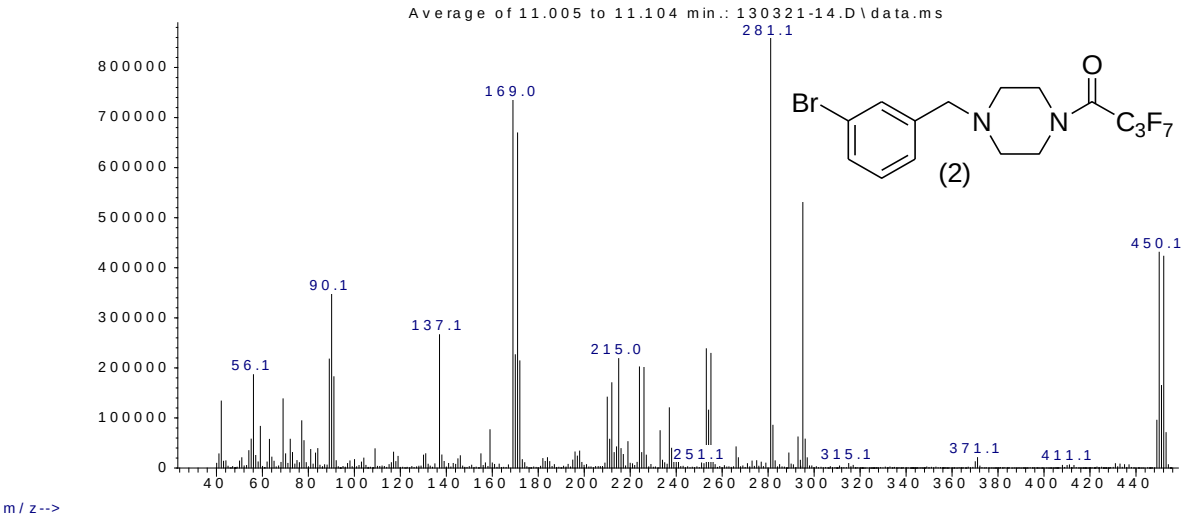


Fig. 10-2: EI mass spectral fragmentation pattern of the underivatized bromobenzylpiperazines.

are essentially identical. 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 10-3 shows the mass spectra of the heptafluorobutryl amides of the three compounds as representatives of all the perfluoroacylated piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 350, 400 and 450, respectively. The major fragment ion in these spectra occurs at *m/z* 169/171 and corresponds to the bromo substituted benzyl 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 $(M\text{-}169)^+$ ion for each amide. The ion at *m/z* 253 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 other in this series of isomeric piperazine compounds.





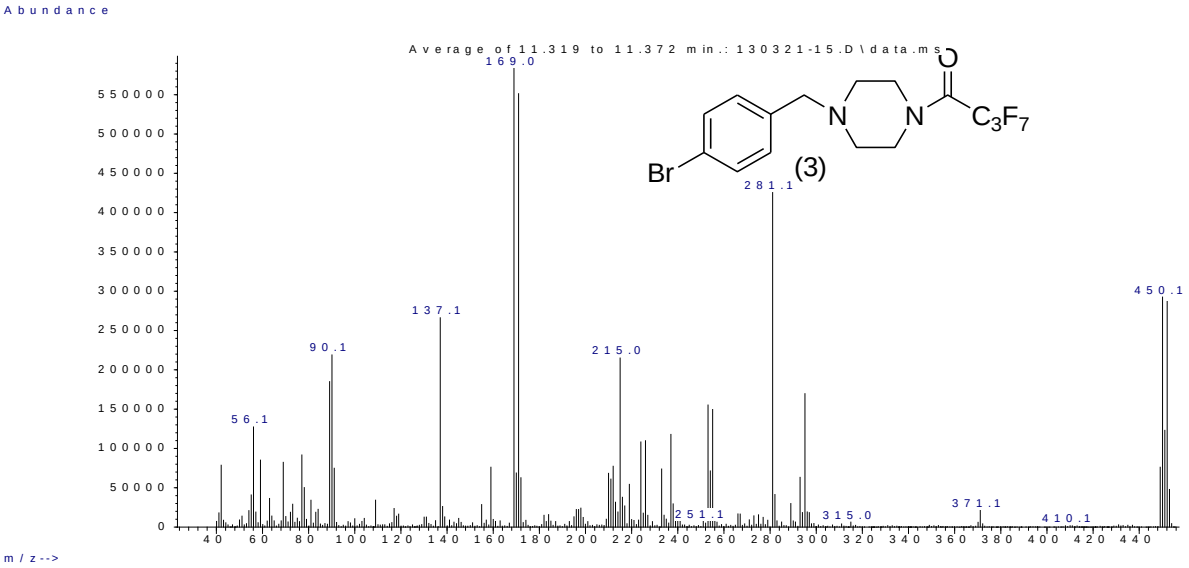


Fig. 10-3: Mass spectra of heptafluorobutryl derivatives of the three bromobenzylpiperazine compounds.

Gas Chromatographic Separation of the Bromobenzylpiperazines (BrBPs)

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). The separation of the HFBA 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 12°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. The chromatogram in Figures 10-4 is a representatives of the results obtained for all samples on this stationary phase.

In Figure 10-4 the HFBA derivatives of the three bromobenzylpiperazines eluted in the order of 2, 3, 4-bromobenzylpiperazine. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

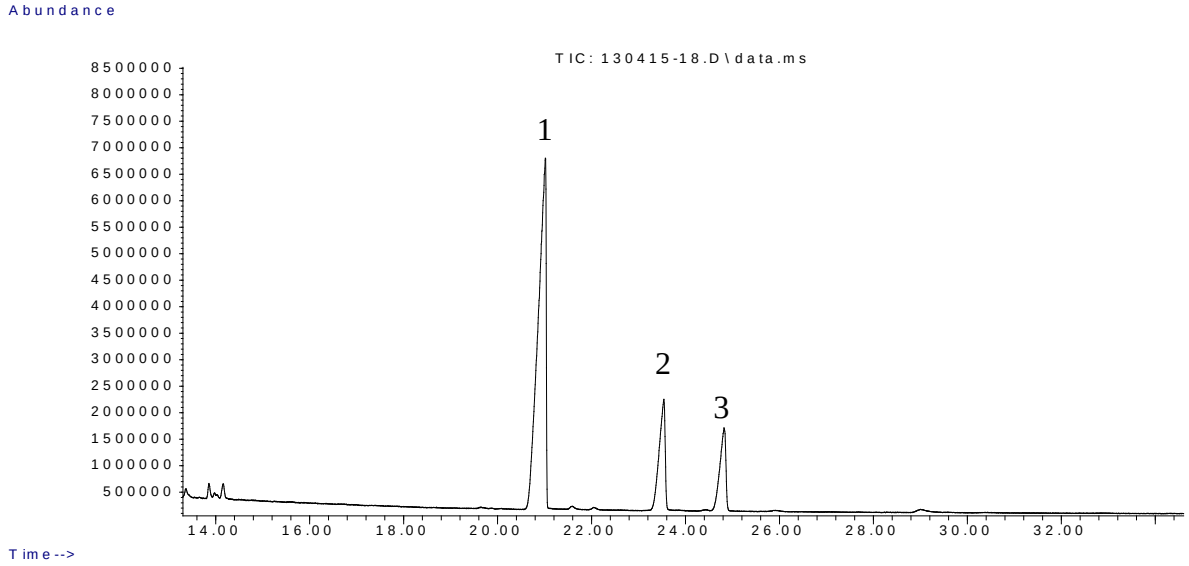


Fig. 10-4: Gas chromatographic separation of the heptafluorobutyryl derivatives of BrBPs using Rtx-200 column. The number over the peak represents the compound number.

Conclusion

The three regioisomeric bromobenzylpiperazines have a regioisomeric relationship to each other. These three piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. The three piperazines were successfully resolved on the GC stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Chapter 11

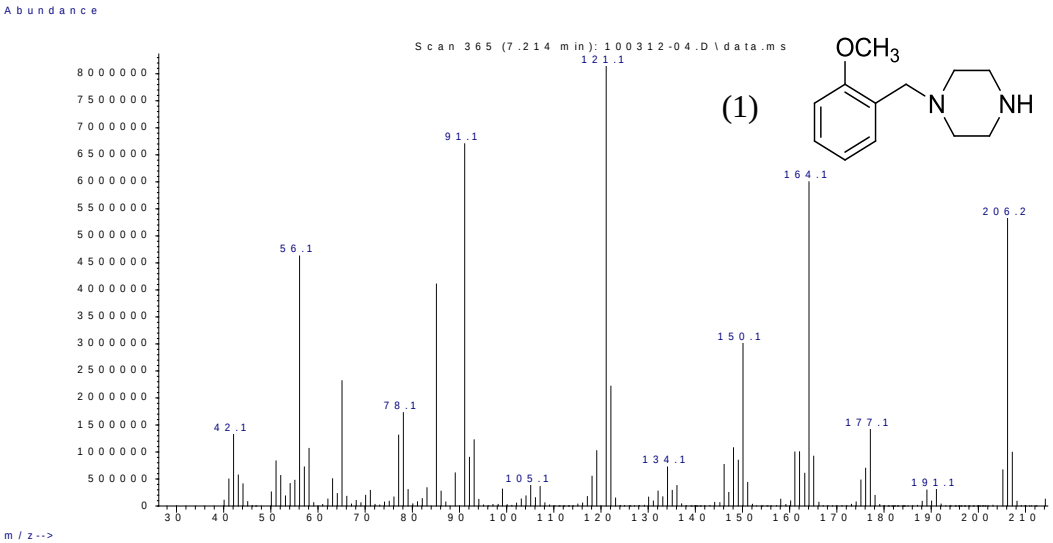
Differentiation of the Methoxybenzylpiperazines (OMeBPs) by GC-IRD and GC-MS

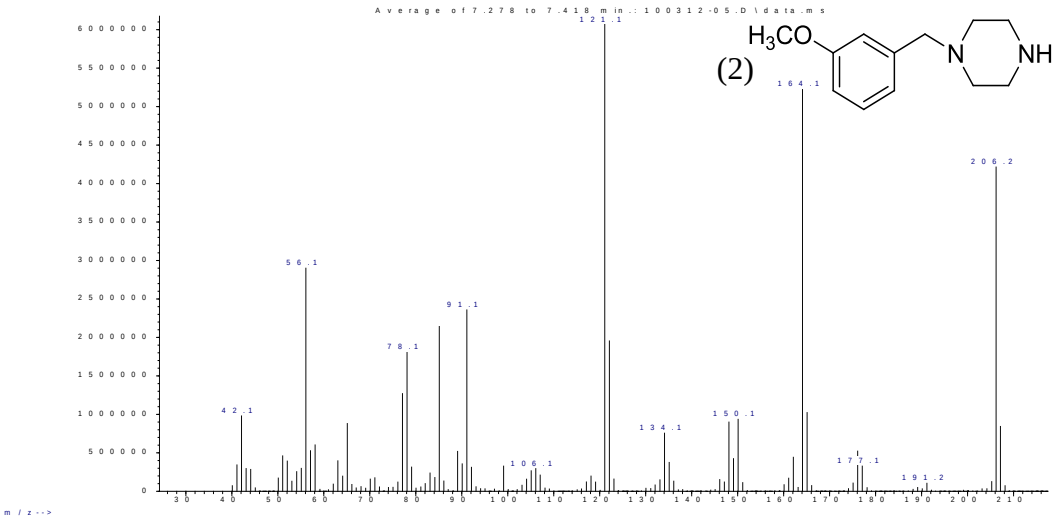
Three ring substituted methoxybenzylpiperazines (OMeBPs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not 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 three isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methoxybenzylpiperazines (OMeBPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 11-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3) in this study. The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the three piperazines show fragment ions at m/z 164, 150, 121, 91, 85 and 56 as well as other ions of low relative abundance. The proposed structures of these ions are shown in Figure 11-2 and are similar to a previous report describing the fragmentation of unsubstituted benzylpiperazine [de Boer *et al*, 2001]. The mass spectra for the ring substituted methoxybenzylpiperazines (Compounds 1-3) have almost identical mass spectra to each other. An additional fragmentation pathway similar to that described before in chapter 3 which is characteristic for the ortho-methoxy ring substituted compound (compound 1) is described in Figure 11-3. This compound with the methoxy group in the ortho position relative to the side chain is characterized by a significant m/z 91 ion. This ion likely arises from the loss of mass 30 (CH_2O) from the initial methoxybenzylic cation at m/z 121. The m/z 91 ion is most abundant when the methoxy group is ortho to the piperazine side chain and therefore the site of initial benzylic cation formation in Compound 1. This m/z 91 ion can be formed by 1,6-hydride shift (ortho effect) from a hydrogen of the ortho-methoxy group to the benzyl cation followed by the loss of formaldehyde as in Figure 11-3.





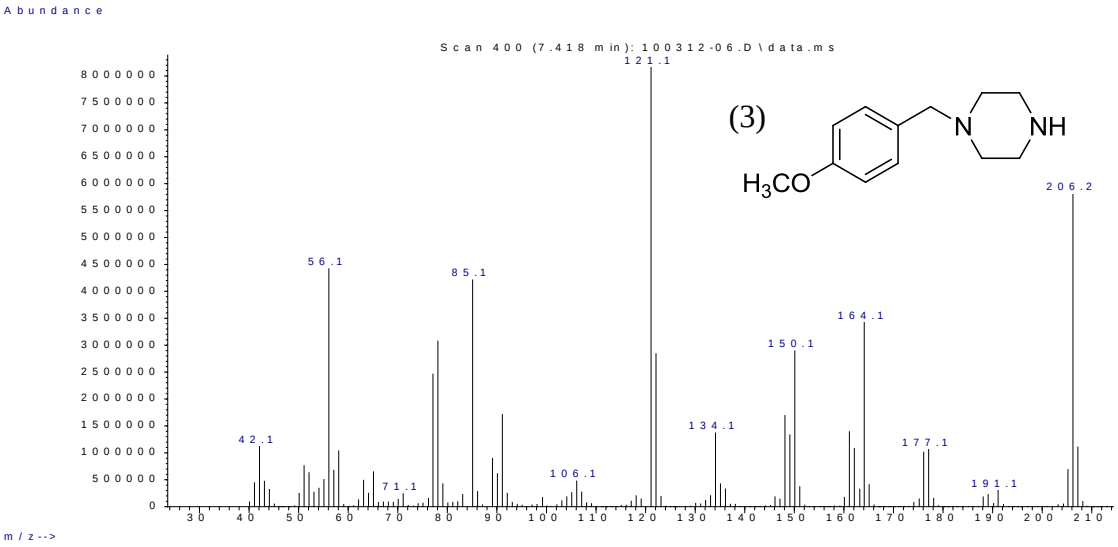


Fig. 11-1: EI mass spectra of the three methoxybenzylpiperazines.

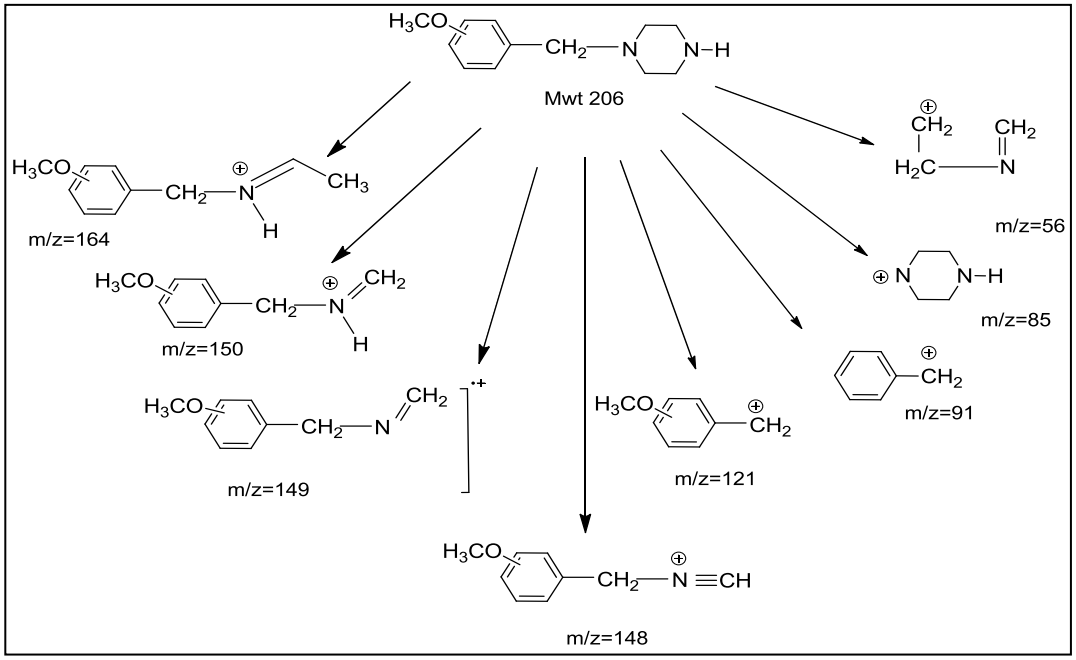


Fig. 11-2: EI mass spectral fragmentation pattern of the underivatized methoxybenzylpiperazines.

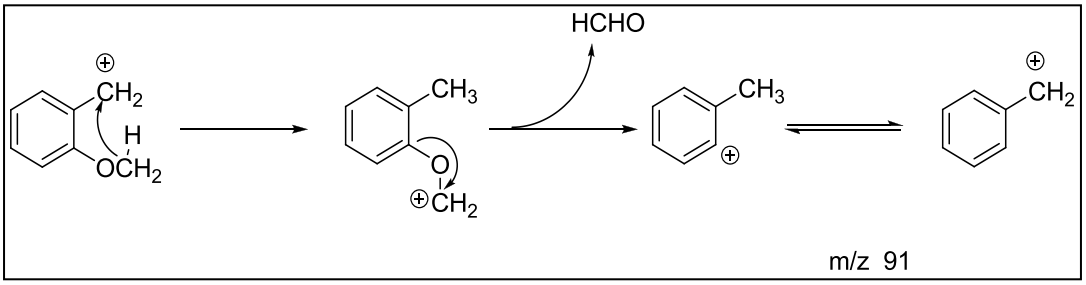
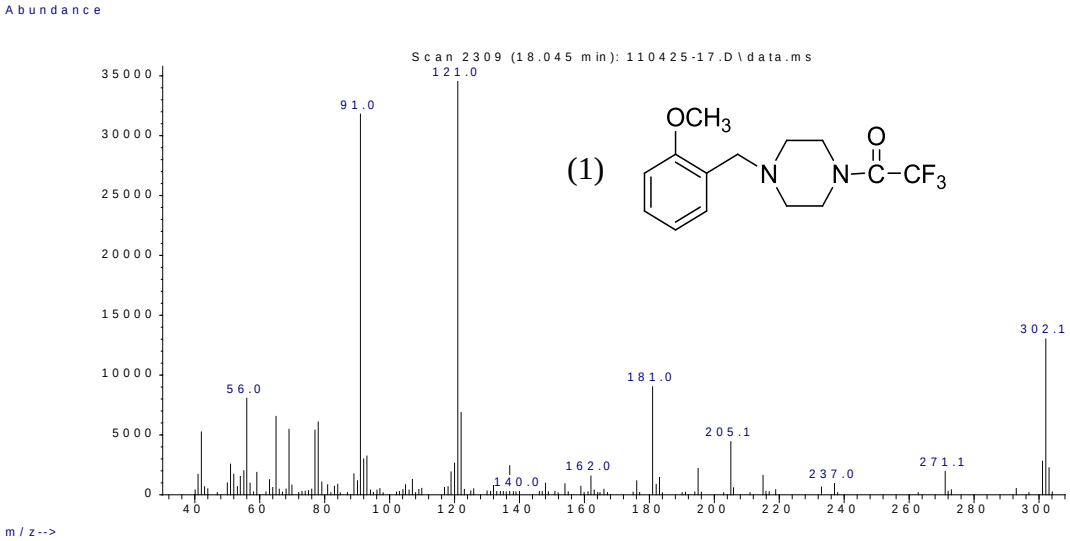


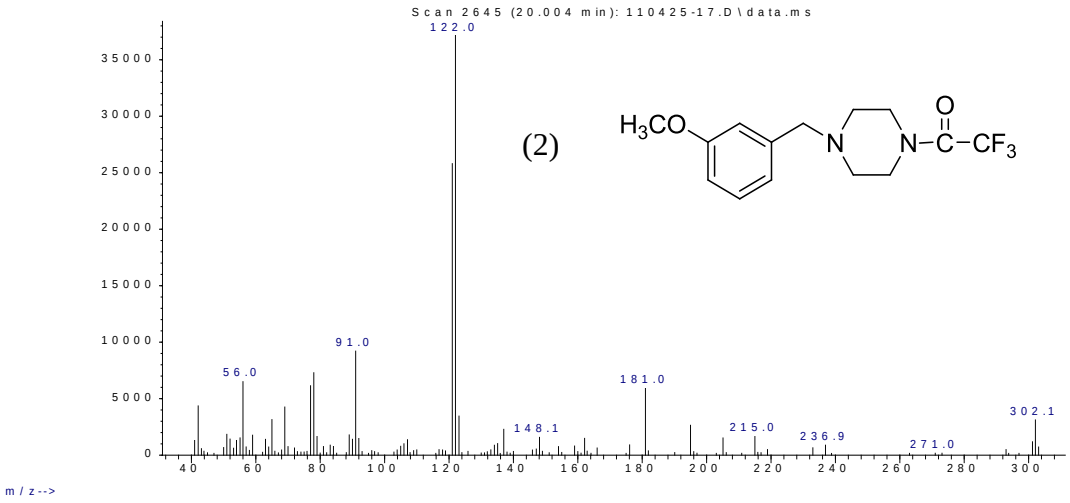
Fig. 11-3: Mechanism for the formation of the m/z 91 ion in the mass spectra of the regioisomers of the methoxybenzylpiperazines

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 three compounds. 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 11-4 shows the mass spectra of the trifluoroacetyl amides of the three compounds as representatives of all the perfluoroacylated piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 302, 352 and 402, respectively. The major fragment ion in these spectra occurs at *m/z* 121 and corresponds to the methoxy substituted benzyl 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 $(M\text{-}121)^+$ ion for each amide. The ion at *m/z* 205 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 other in this series of isomeric piperazine compounds.



(1)



(2)

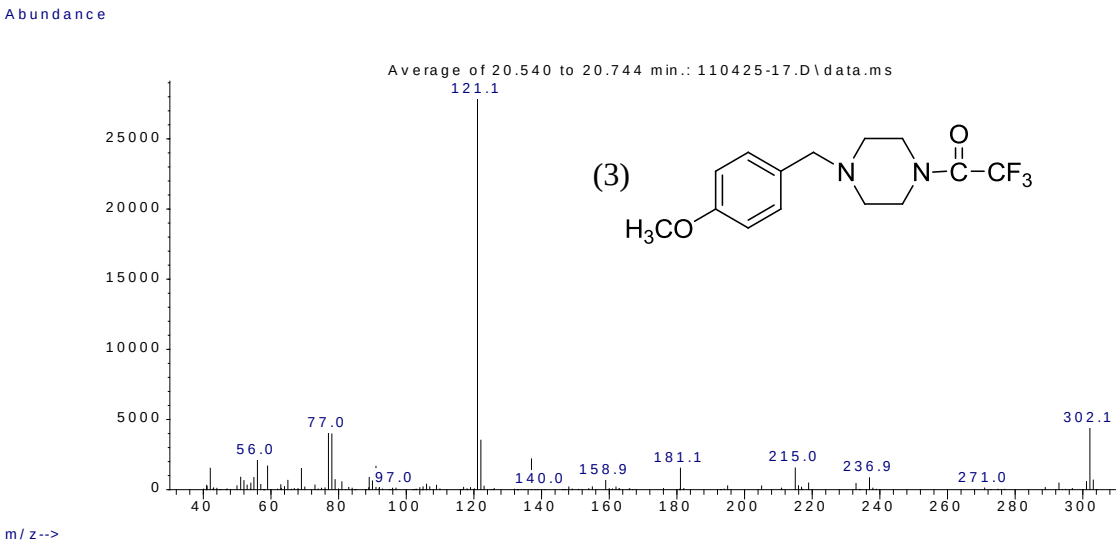


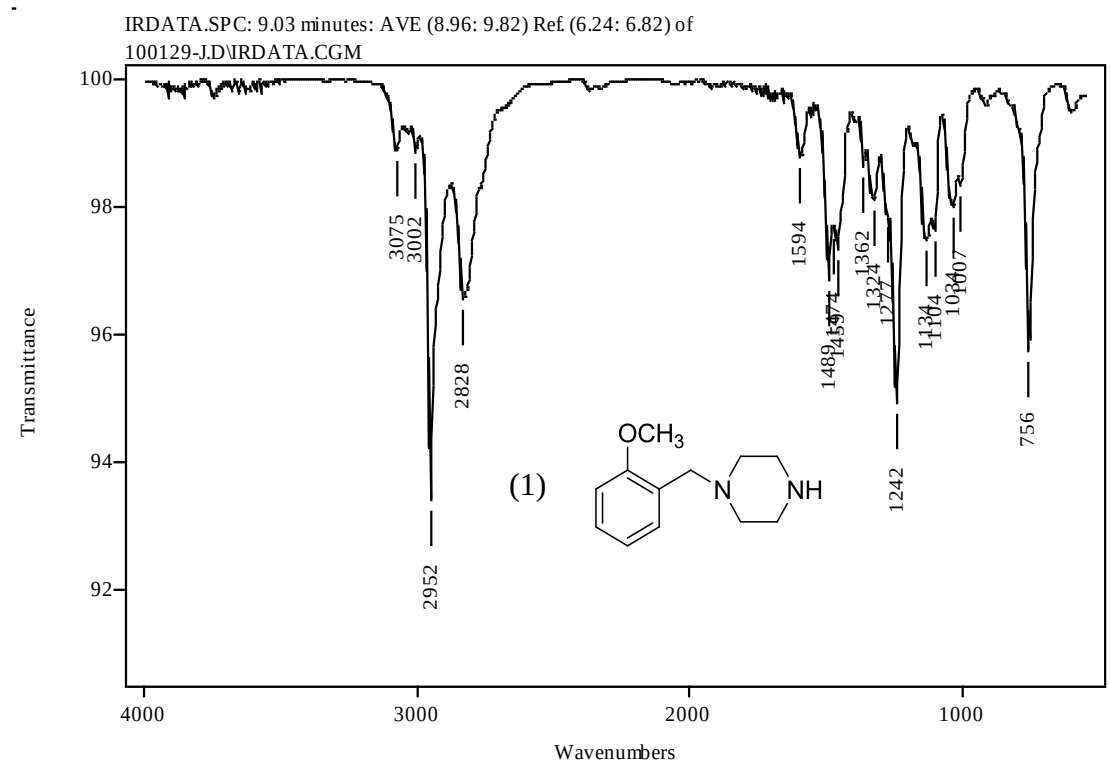
Fig. 11-4: Mass spectra of trifluoroacetyl derivatives of the three piperazine compounds.

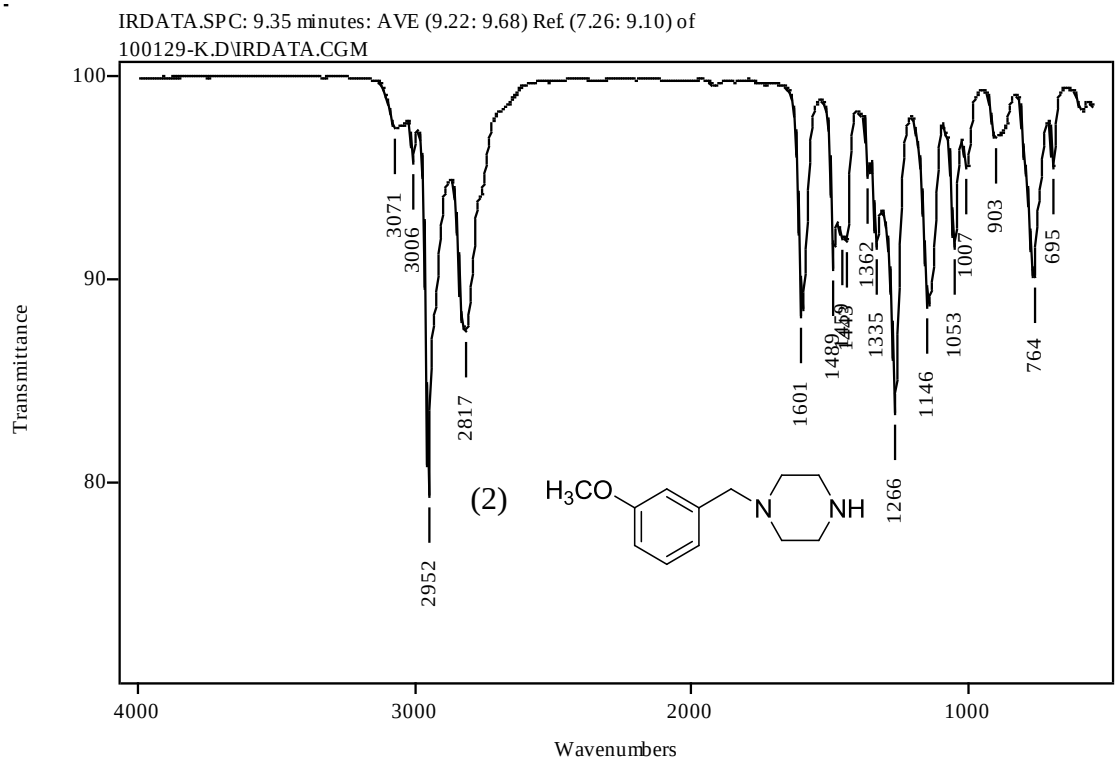
Vapor-phase Infra-Red Spectrophotometric Studies of the the Methoxybenzylpiperazines (OMeBPs)

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 three piperazines. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the three underivatized piperazines are shown in Figure 11-5. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph and each compound shows a vapor-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}. Because the three 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 differentiated by the positions and intensities of several IR peaks in the region of 750 – 1620 cm^{-1}.

Compound 3 shows a strong singlet at 1513 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 1164 cm^{-1} which is shifted to a peak at 1134 cm^{-1} in compound 1 and to peak at 1173 cm^{-1} in compound 3. Compound 2 also has a medium intensity peak at 1609 cm^{-1} which is absent in compounds 1 and 3. These results provide an excellent illustration of the value of vapor phase IR confirmation for the three regioisomeric compounds in this

study. The generated IR spectra show significant differences in the major bands for these three compounds.





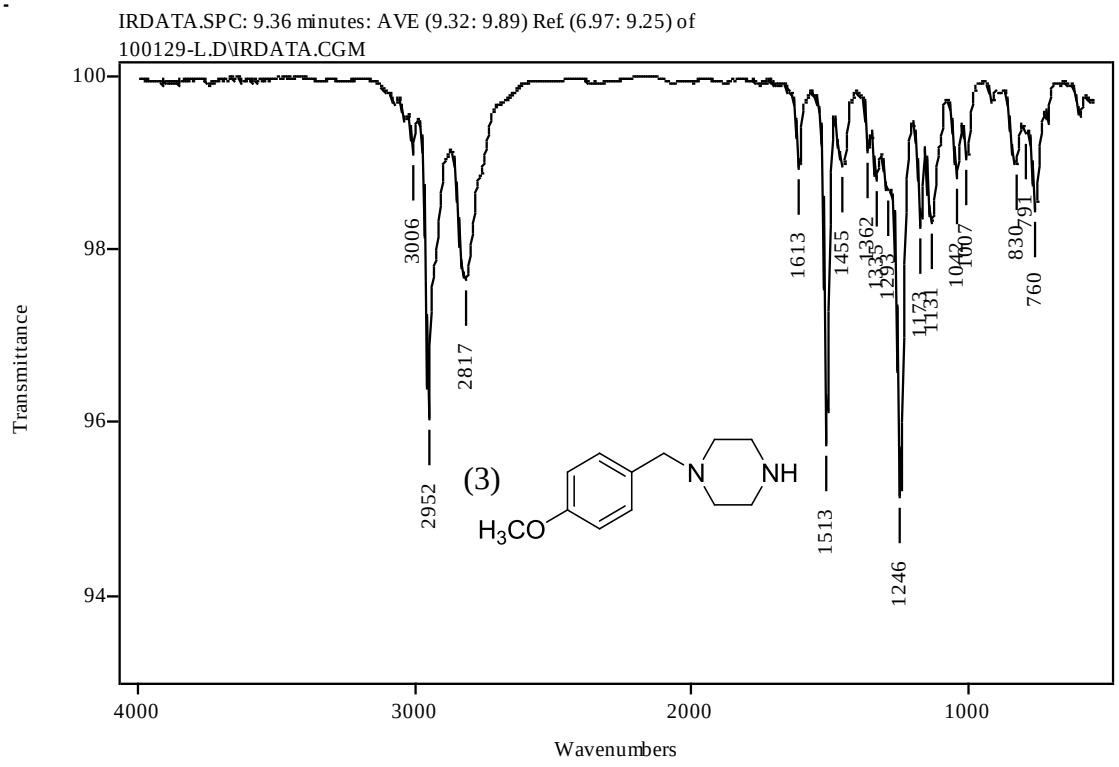


Fig. 11-5: Vapor phase IR spectra of the three methoxybenzylpiperazines.

Gas Chromatographic Separation of the Methoxybenzylpiperazines (OMeBPs)

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). The separation of the TFA and PFPA 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 12°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.

In Figures 11-6 and 11-7 the TFA and PFPA derivatives of the three methoxybenzylpiperazines eluted in the order of 2, 3, 4-methoxybenzylpiperazine. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

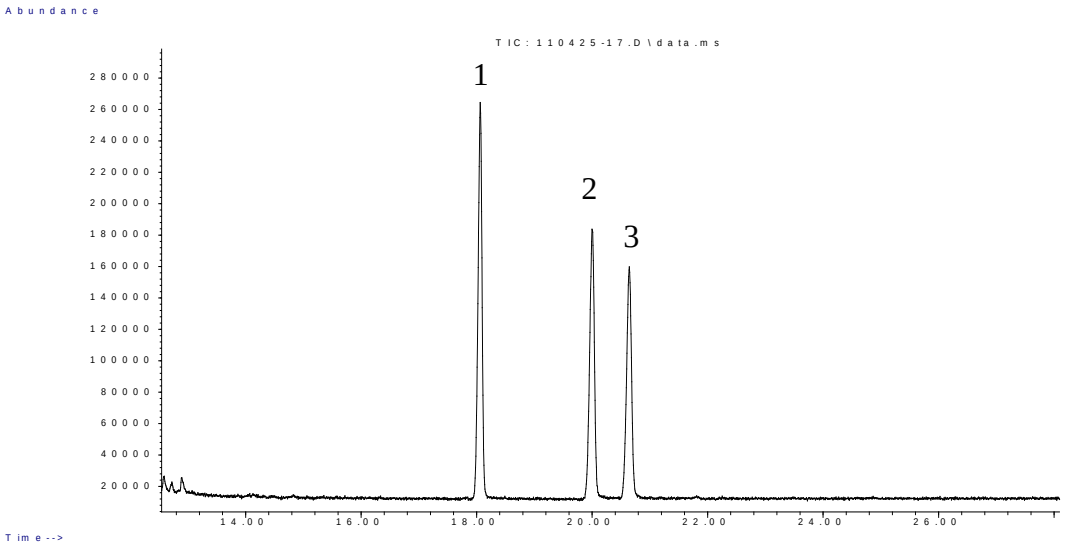


Fig. 11-6: Gas chromatographic separation of the trifluoroacetyl derivatives of the OMeBPs using Rtx-200 column. The number over the peak corresponds to the compound number.

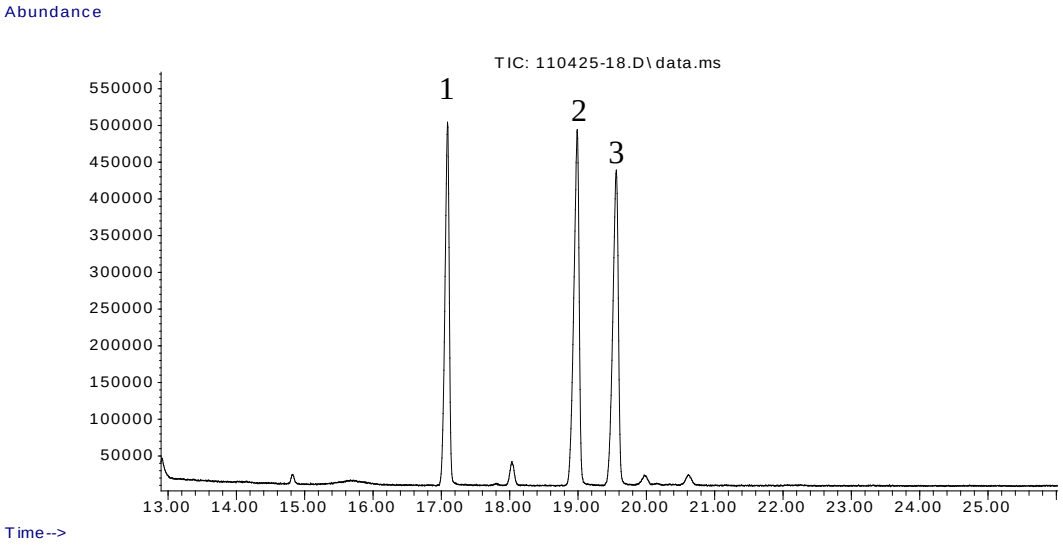


Fig. 11-7: Gas chromatographic separation of the pentafluoropropionyl derivatives of the OMeBPs using Rtx-200 column. The number over the peak corresponds to the compound number

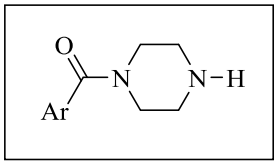
Conclusion

The three regioisomeric methoxybenzylpiperazines have a regioisomeric relationship to each other. These three piperazines yield very similar fragment ions in their mass spectra Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. GC-IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650-1700 cm^{-1}. The three piperazines were successfully resolved on the GC stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

SECTION II Benzoylpiperazines



The benzoylpiperazines can be viewed as more closely related to the carbonyl containing amines such as the cathinone or bath salts type compounds. While the amide structural feature in these benzoylpiperazines eliminates basicity for the N-1 nitrogen the N-4 nitrogen remains unmodified and allows these molecules to continue to show appropriate basicity. This series of compounds are prepared via monoacylation of piperazine with a reactive carboxylic acid equivalent such as an ester or acylhalide. In this work the benzoylpiperazines were synthesized from piperazine and a ring substituted benzoyl chloride. Many of these benzoyl chlorides are commercially available and others can be prepared from appropriately ring substituted benzoic acids, aldehydes or benzyl alcohols. The benzoylpiperazine series includes a number of monosubstituted aromatic ring products; the three regioisomeric methyl-, trifluoromethyl-, and methoxy-substituted piperazines. For the disubstituted aromatic ring derivatives the six dimethoxy and the two methylenedioxypiperazines were evaluated. In addition we prepared a series of tertiary amines, the N-1-benzoyl-N-4-methylpiperazines, based on the commercial availability of the precursor, N-methylpiperazine. The secondary amines were also evaluated as the perfluoroacyl derivatives however the tertiary amines do not form stable acylated products.

The additional site for ionization at the carbonyl oxygen in the EI fragmentation for these compounds adds some uniqueness to the mass spectra for this series. A number of significant fragments occur as a result of bond migration and rearrangements across the piperazine ring from the N-4 nitrogen to the carbonyl oxygen of the amide group. These ions and the potential mechanisms for their formation were determined by deuterium labeling of multiple sites within the molecules. The benzoyl cation and the protonated primary amide are characteristic fragments in this series. The specific mass for these fragments depends on the nature of the aromatic ring substituents. The primary amide occurs via multiple hydrogen migrations from the piperazine ring to the amide functional group. This series of compounds also shows unique IR bands due to the carbonyl feature of the amides for these benzoylpiperazines. The acylated sets of regioisomeric equivalents were resolved by GC and the relative elution order described in some cases based on structural features.

Chapter 12

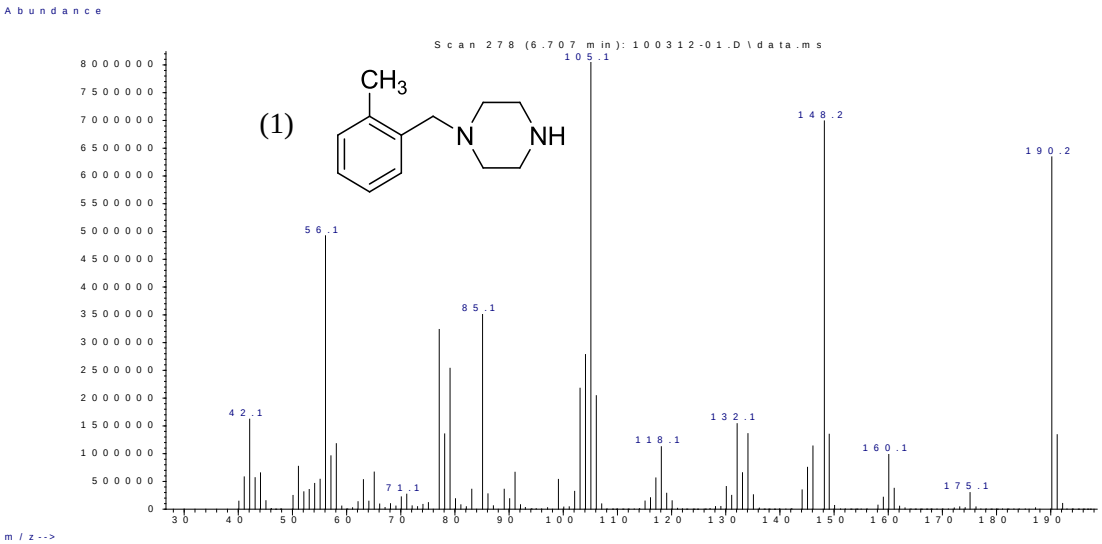
Differentiation of Methylbenzylpiperazines (MBPs) and Benzoylpiperazine (BNZP) using GC-IRD and GC-MS

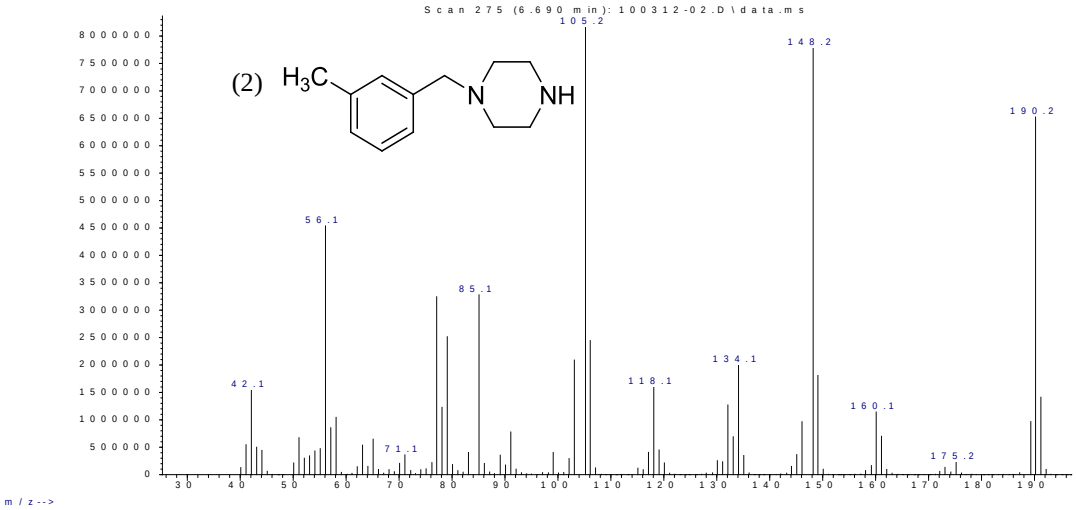
Three ring substituted methylbenzylpiperazines (MBPs) and their isobaric benzoylpiperazine (BNZP) have equal mass and many common mass spectral fragment ions. The mass spectrum of the benzoylpiperazine yields a unique fragment at m/z 122 that allows its discrimination from the three methylbenzylpiperazine 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 vapor phase infrared 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 methylbenzylpiperazine and benzoylpiperazine which have equivalent nominal masses but are different in their elemental composition and exact masses.

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylbenzylpiperazines (MBPs) and Benzoylpiperazine (BNZP)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 12-1 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 12-2 and 12-3 and are related in part on a previous report describing the fragmentation of unsubstituted benzylpiperazine [de Boer *et al*, 2001]. 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 methylbenzylpiperazines (Compounds 1-3) have almost identical mass spectra to each other and to the benzoylpiperazine (Compound 4) except for the characteristic high relative abundance ion at m/z 122 which appears to be specific for the benzoylpiperazine. In addition to that, the mass spectrum of the benzoylpiperazine shows high relative abundance of the fragment ion m/z 69.





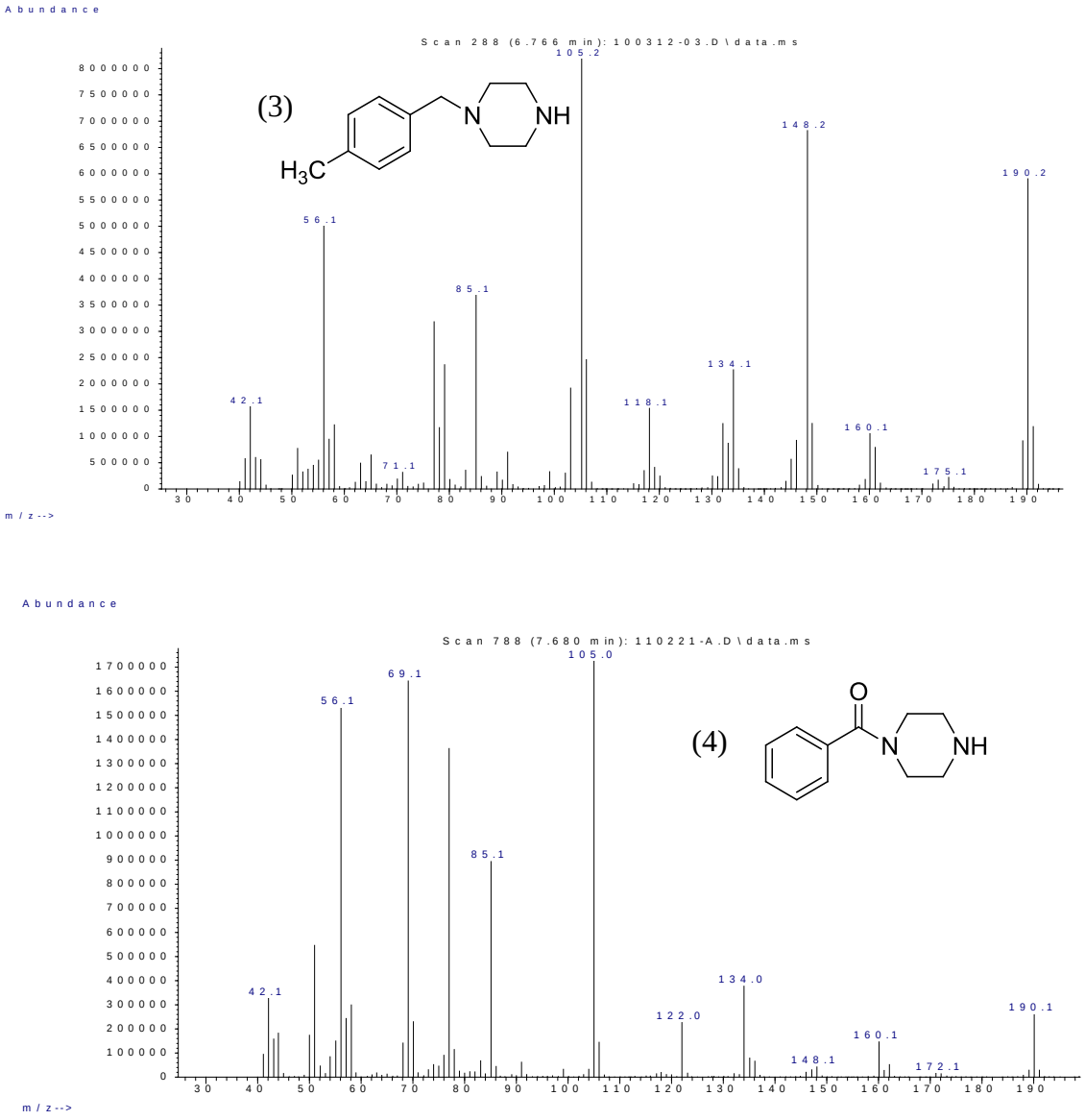


Fig. 12-1: Mass spectra of the four underivatized piperazines in this study.

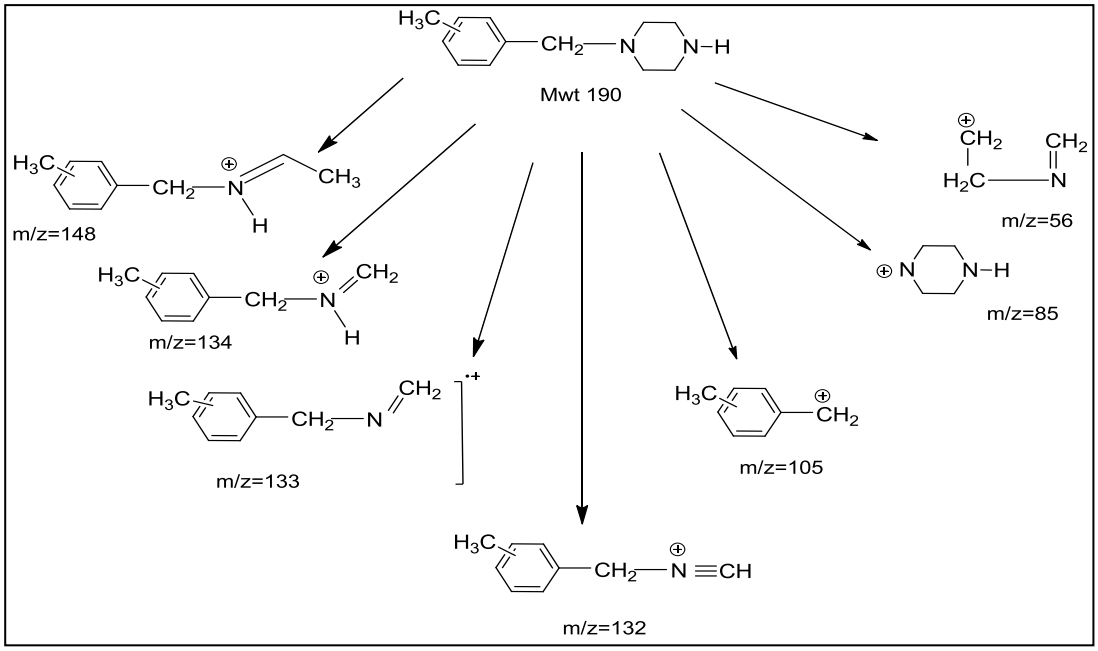


Fig. 12-2: Mass spectral fragmentation pattern of the underivatized methylbenzylpiperazines under EI (70eV) conditions.

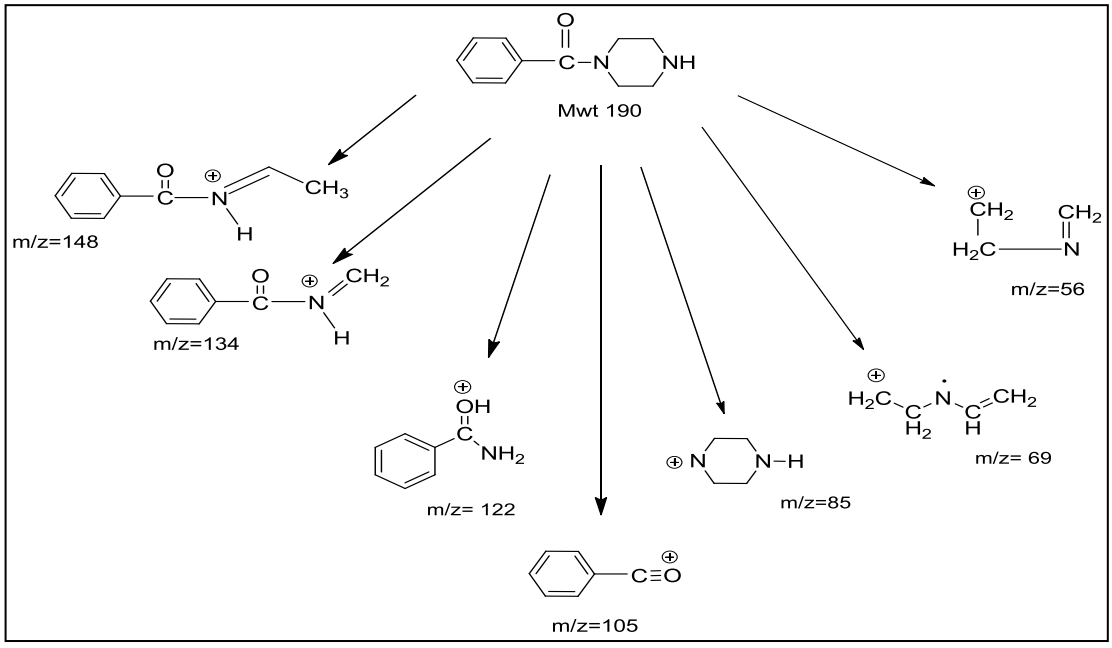


Fig. 12-3: Mass spectral fragmentation pattern of the underivatized benzoylpiperazine under EI (70eV) conditions.

The proposed structure for the m/z 122 $(C_7H_8NO)^+$ ion is shown in the fragmentation scheme in Figure 12-3 and the equivalent ion has been confirmed by exact mass GC-TOF-MS analysis for the ring substituted methoxybenzoylpiperazines [Abdel-Hay *et al*, 2012]. The suggested structure for this fragment involves the formation of the protonated primary benzamide and the structure for this m/z 122 ion is supported by the mass spectrum of the octa-deutero labeled form of benzoylpiperazine (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 labeled form of Compound 4 is shown in Figure 12-4. The mass spectrum in Figure 12-4 shows that two deuterium atoms remain as a part of the ion in question since the mass increased by 2 Da to m/z 124 in this case. The structure of this characteristic fragment was also confirmed by the mass spectrum of the penta-deutero labeled benzoylpiperazine (d5-BNZP). The protonated benzamide ion mass increased by 5 Da to m/z 127 as shown in Figure 12-5.

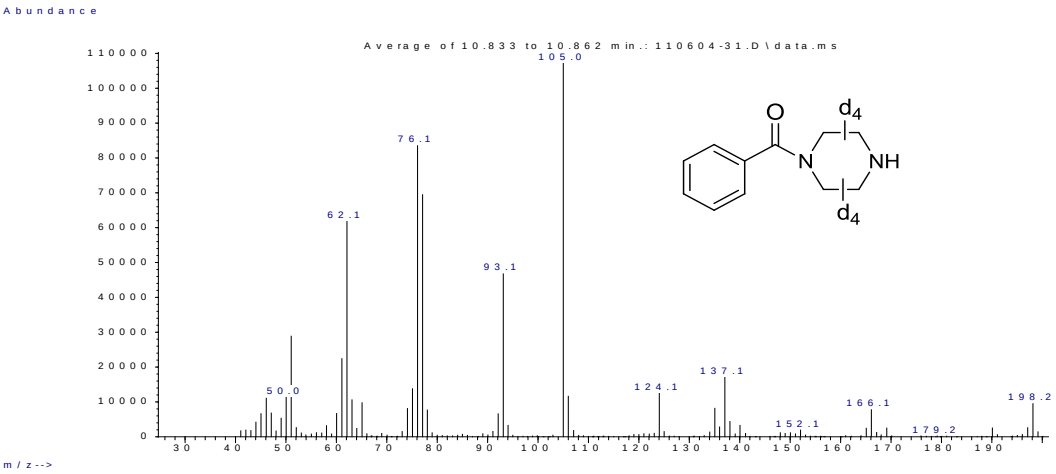


Fig. 12-4: Mass spectrum of the benzoyl-d_8-piperazine.

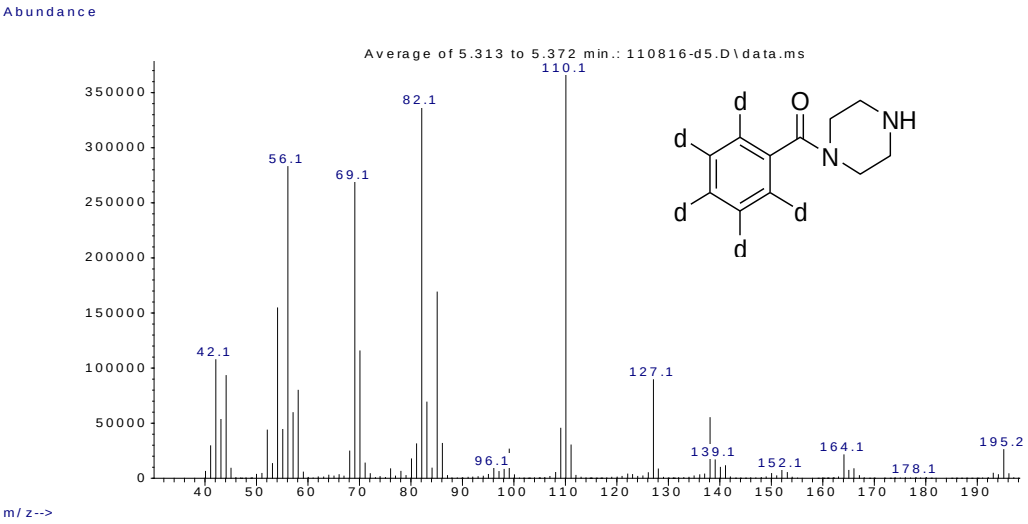


Fig. 12-5: Mass spectrum of the d5-benzoylpiperazine.

The mechanism of formation of the characteristic ions at m/z 122 and m/z 69 in the benzoylpiperazine is illustrated in Figure 12-6. It starts with a migration of the piperazine proton to the carbonyl oxygen followed by a 1,4-hydride shift to form the hydrogen rearranged molecular ion in Figure 12-6 which can either transform to the protonated benzamide ion at m/z 122 or the fragment ion at m/z 69. This mechanism can also be confirmed by the mass spectrum of N-methylbenzoylpiperazine (Figure 12-7). This compound is prepared using the same procedure used to prepare benzoylpiperazine using 1-methylpiperazine instead of piperazine. The mass spectrum of N-methylbenzoylpiperazine does not show the fragment ion at m/z 122 which indicates that substituting the piperazine proton on nitrogen with a bulkier group prevented the proposed hydrogen migration from happening and forming the protonated benzamide ion. The chemical structure of the m/z 69 ion (C_4H_7N) is further confirmed by the mass spectrum of the benzoyl-d_8-piperazine in Figure 12-4 as the corresponding fragment is shifted 7 Da higher to become m/z 76.

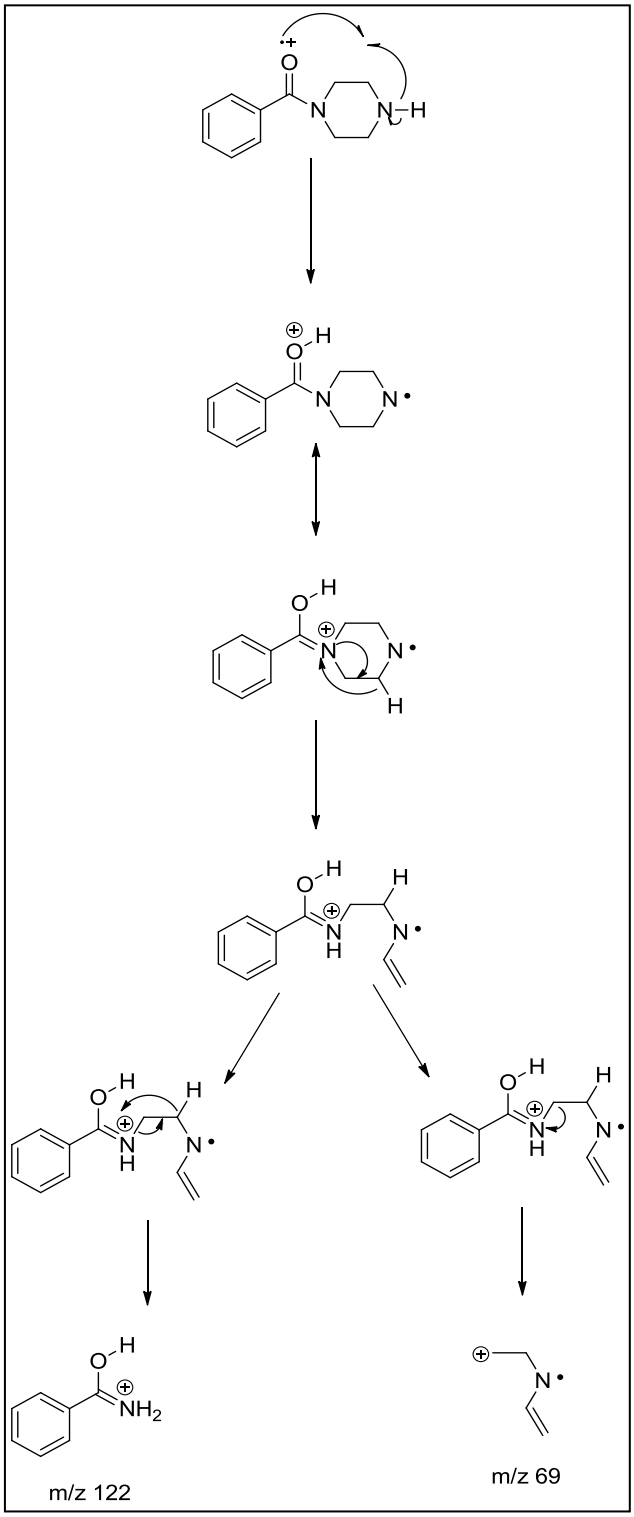


Fig. 12-6: Mechanism of the formation of the m/z 122 and m/z 69 ions in the benzoylpiperazine (BNZP).

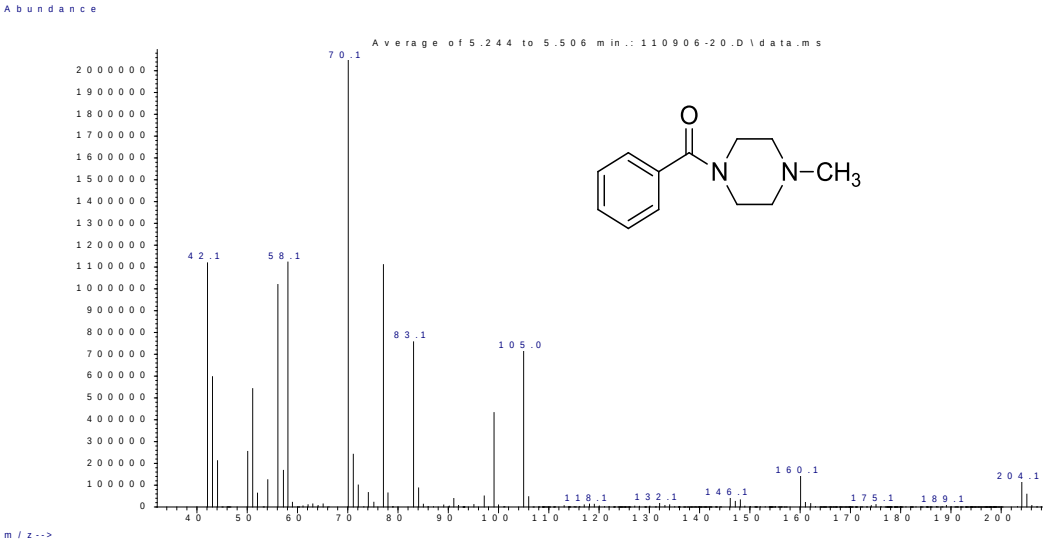
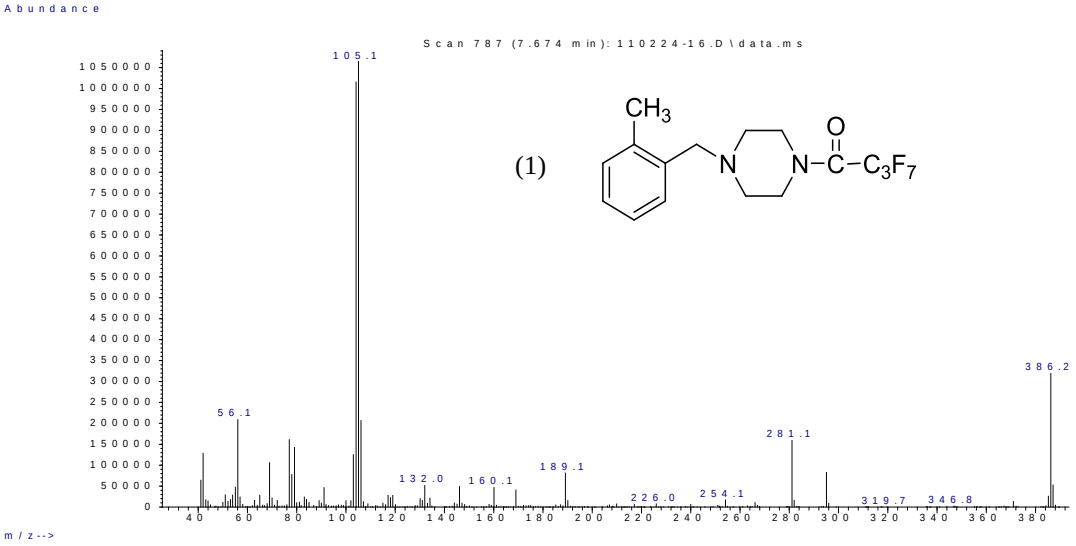


Fig. 12-7: Mass spectrum of the N-methylbenzoylpiperazine.

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. 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 12-8 shows the mass spectra of the heptafluorobutryl amides of the four compounds as representatives 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 $(M-105)^+$ ion for each amide. These ions have higher relative abundances in the mass spectra of the derivatized methylbenzylpiperazines compared to the mass spectra of the derivatized benzoylpiperazine. 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 other in this series of isomeric piperazine compounds.



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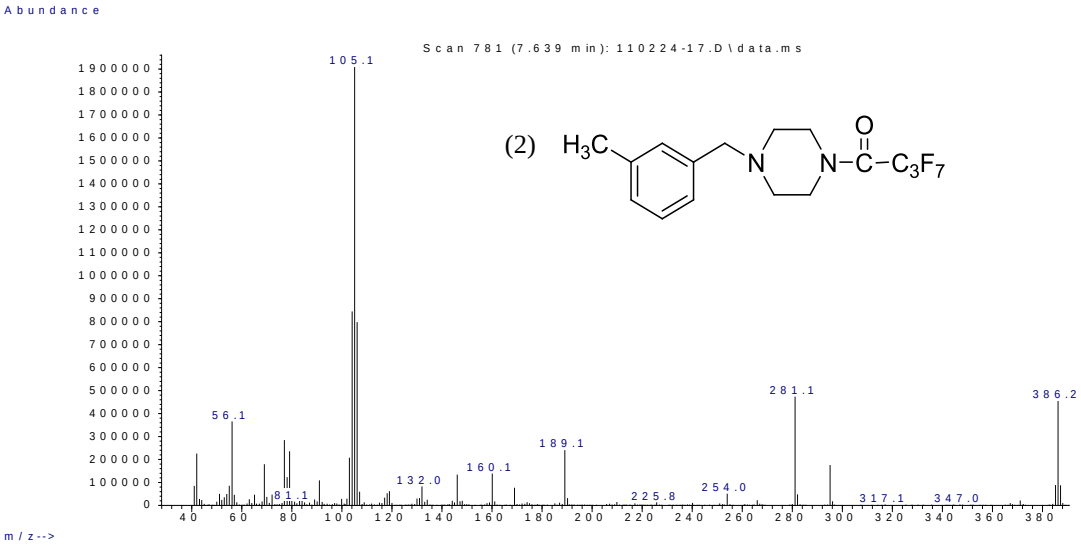

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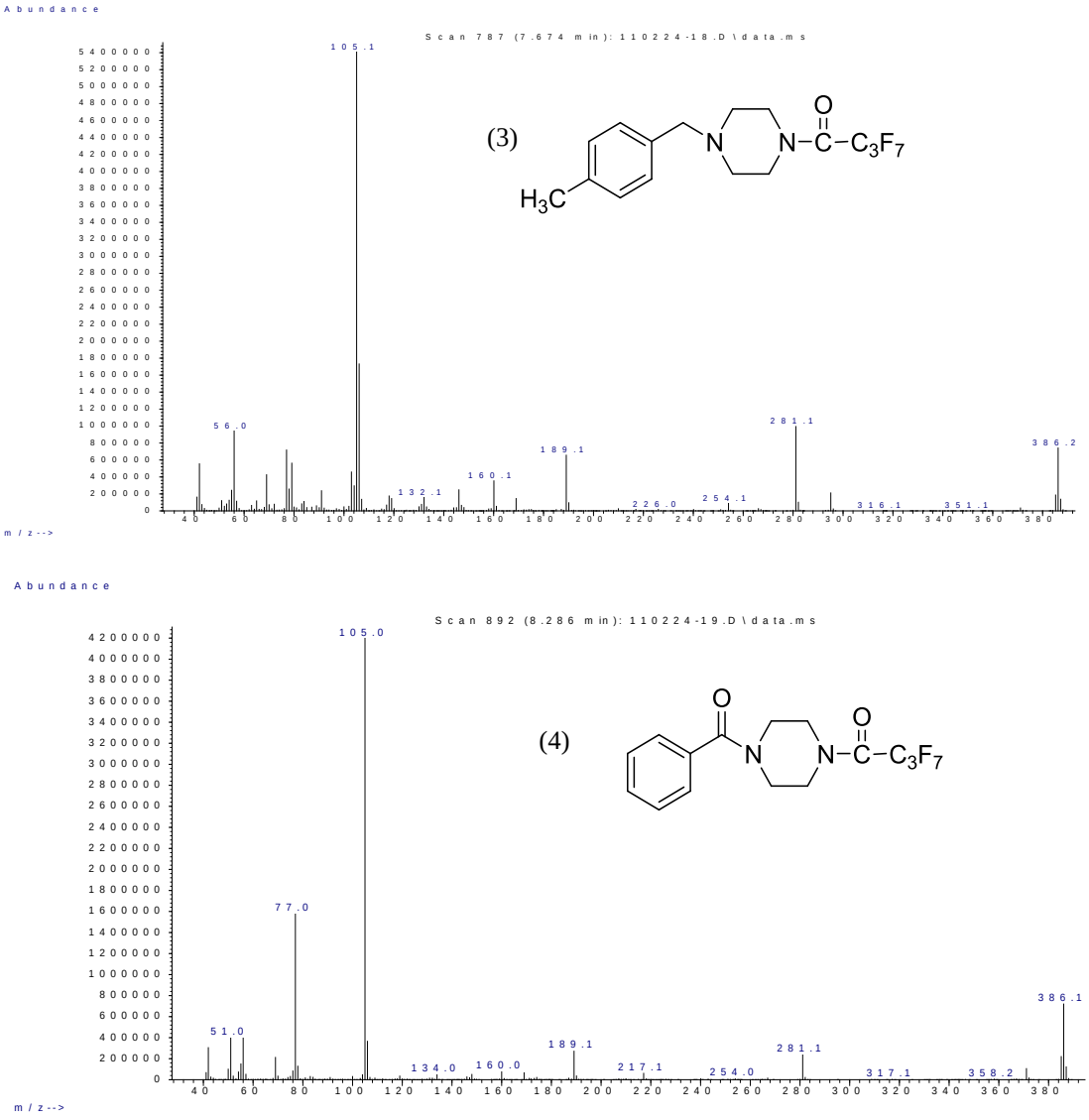


Fig. 12-8: Mass spectra of heptafluorobutyryl derivatives of the four piperazine compounds in this study.

Gas chromatography coupled with time-of-flight mass spectrometric detection provides an excellent means of differentiating between the isobaric methylbenzylpiperazines and benzoylpiperazine 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 12-9 shows the GC-TOF-MS exact mass analysis of the 4-methylbenzyl and benzoyl cations (m/z=105) for compounds 3 and 4, respectively. The upper panel (A) shows the expected/calculated mass for the C_8H_9 elemental composition. The lower panel (B) shows the experimental results and the degree of agreement (-0.5 mDa, -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. Panels C and D confirm the elemental composition as C_7H_5O with a mass deviation of (-0.6 mDa, -5.7 ppm). Thus, exact mass measurements can distinguish between these two isobaric forms of the m/z 105 ion.

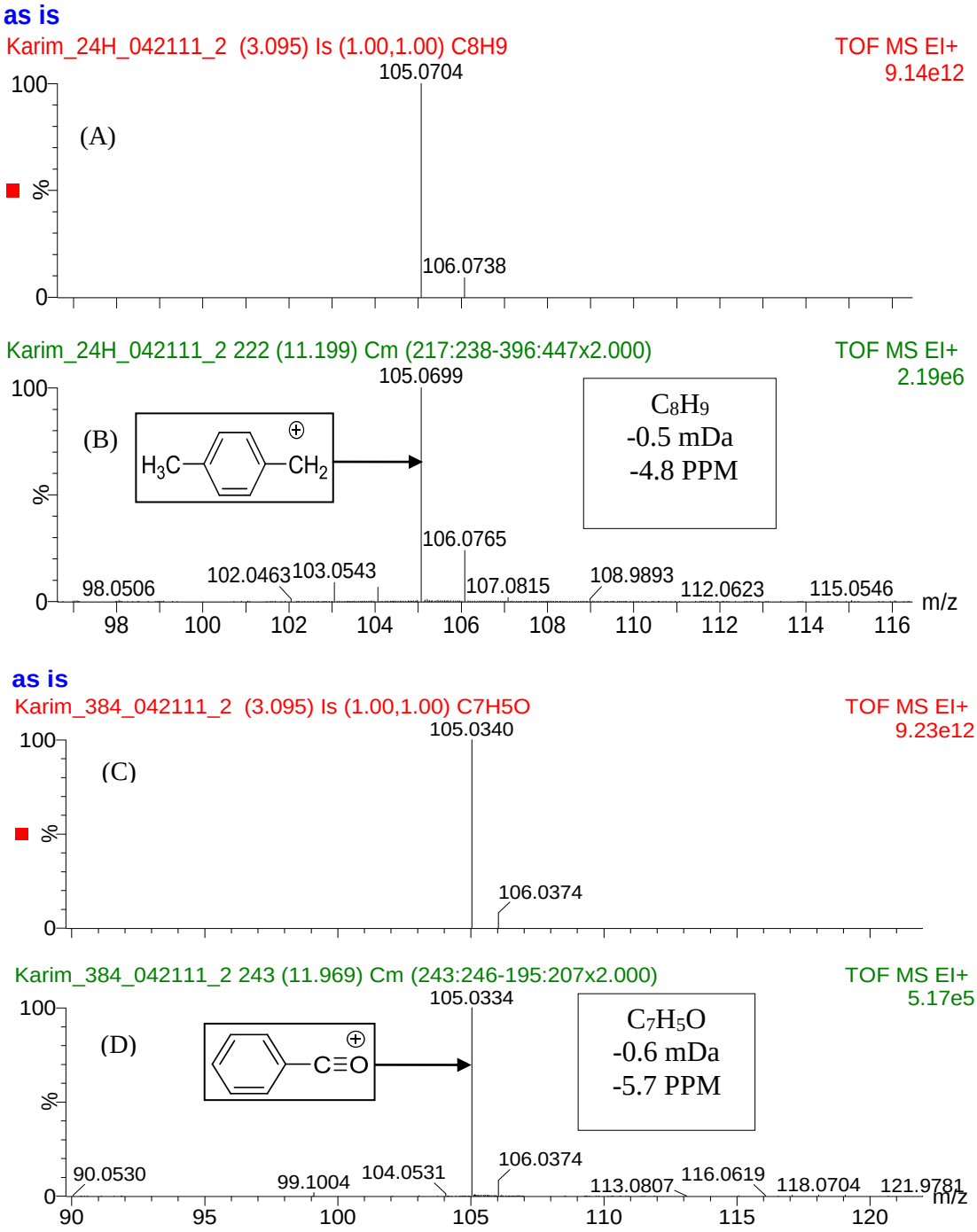


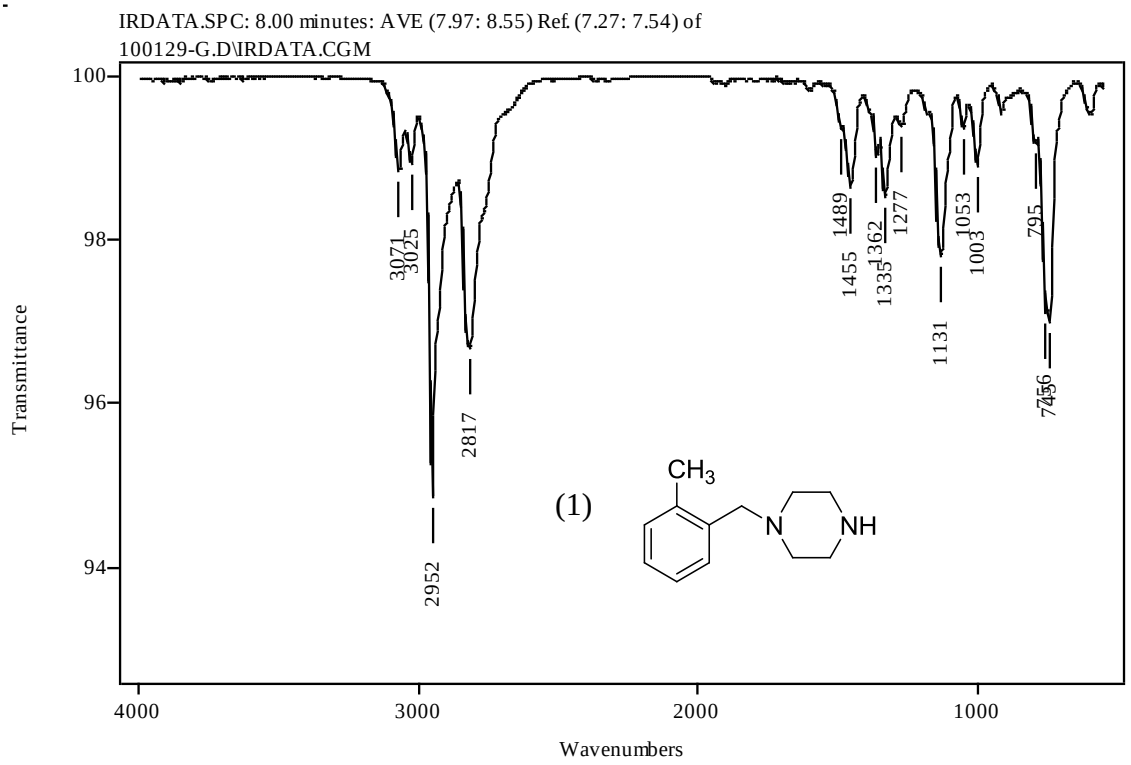
Fig. 12-9: GC-TOF mass spectral analysis of the m/z 105 ion for 4-methylbenzylpiperazine and for benzoylpiperazine. A= calculated mass for C_8H_9; B= experimental results. C= calculated mass for C_7H_5O; D= experimental results.

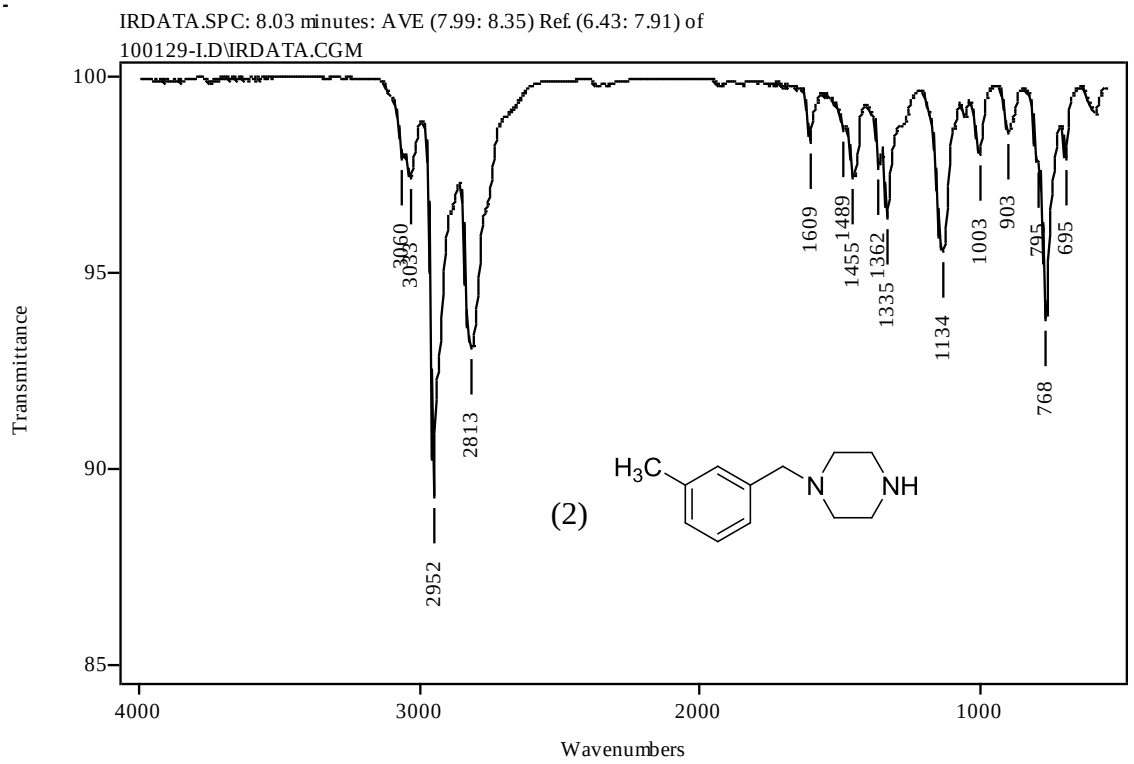
Vapor-phase Infra-Red Spectrophotometric Studies of the the Methylbenzylpiperazines (MBPs) and Benzoylpiperazine (BNZP)

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 four piperazines. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the four underivatized piperazines are shown in Figure 12-10. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph and each compound shows a vapor-phase IR spectrum with absorption bands in the regions 700 – 1700 cm^{-1} and 2700 – 3100 cm^{-1}. 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 differentiated by the positions and intensities of several IR peaks in the region of 750 – 1620 cm^{-1}.

The benzoylpiperazine shows a characteristic strong singlet IR band at 1671 cm^{-1} corresponding to the carbonyl group stretching which can distinguish this benzoylpiperazine from the three ring substituted methylbenzylpiperazines. 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 methylbenzylpiperazines.

The three ring substituted methylbenzylpiperazines 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.





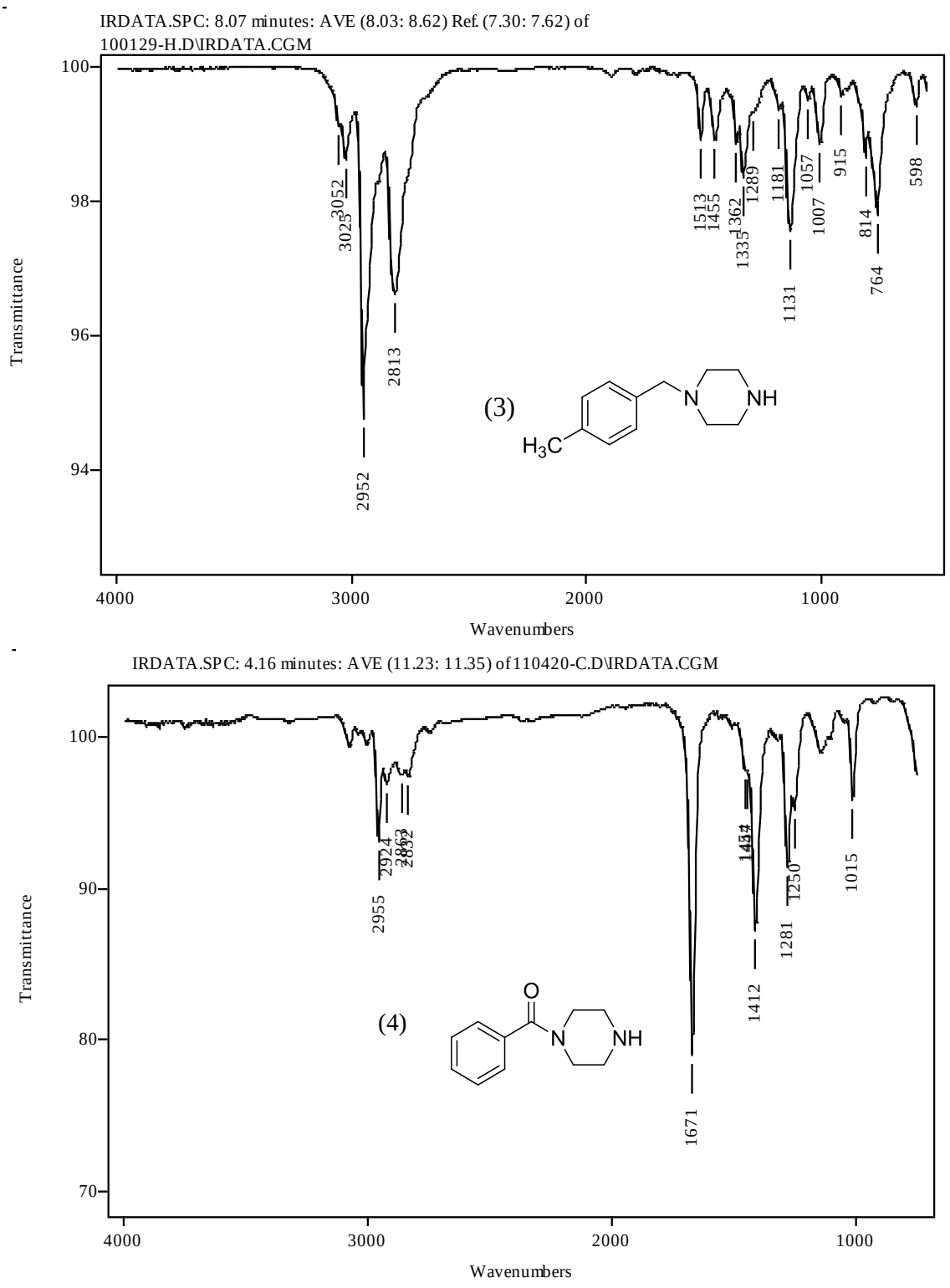


Fig. 12-10: Vapor phase IR spectra of the four piperazine compounds in this study.

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 provide an excellent illustration of the value of vapor 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 Chromatographic Separation of the Methylbenzylpiperazines (MBPs) and Benzoylpiperazine (BNZP)

Gas chromatographic separation 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). 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 ramped to 200°C at a rate of 10°C/min and held at 200°C for 5.0 min. and the chromatogram in Figure 12-11 is representative of the results obtained for all samples on this stationary phase.

In Figure 12-11 the PFPA derivatives of the three methylbenzylpiperazines are less retained than their isobaric benzoylpiperazine. The three benzylpiperazines eluted in the order of 2, 3, 4-methylbenzylpiperazine and the benzoylpiperazine 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.

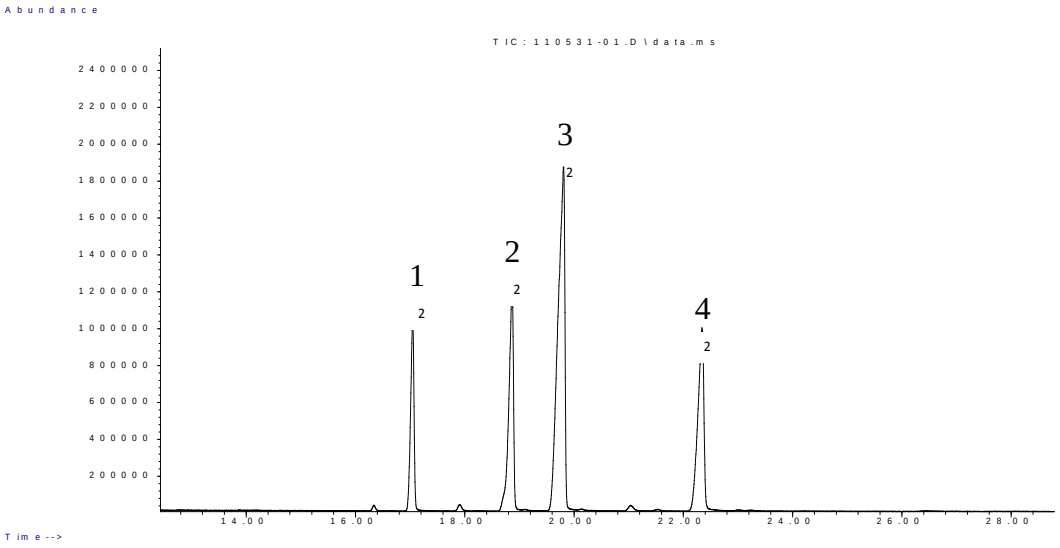


Fig. 12-11: Gas chromatographic separation of the four pentafluoropropionyl derivatives using Rtx-200 column. The number over the peak corresponds to the compound number.

Conclusion

The three regioisomeric methylbenzylpiperazines have an isobaric relationship to benzoylpiperazine. These four piperazines yield very similar fragment ions in their mass spectra with only the benzoylpiperazine showing one unique major fragment ion at 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 differentiate 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 650-1700 cm^{-1}. Additionally, the strong carbonyl absorption bands clearly differentiate the benzoylpiperazine from the three methylbenzylpiperazines. The four piperazines were successfully resolved on the GC stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Abdel-Hay, K. M., DeRuiter, J. and Clark, C. R. Differentiation of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs) by GC-IRD and GC-MS. Drug Testing and Analyis, 4(6) (2012) 430-440. DOI: 10.1002/dta.348

Chapter 13

Differentiation of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs) by GC-IRD and GC-MS

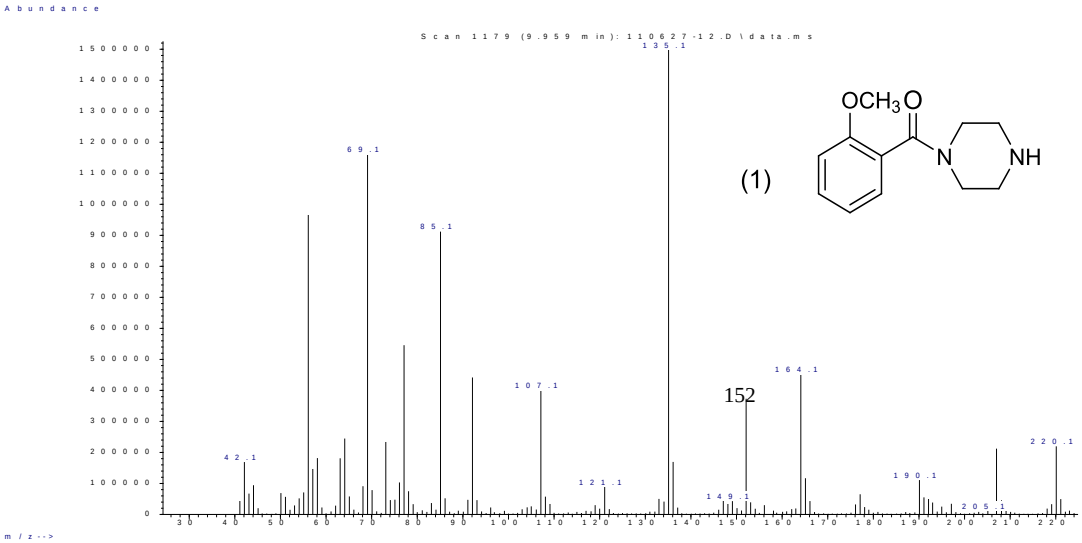
The designer drug 3,4-methylenedioxybenzylpiperazine (3,4-MDBP), its positional isomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) and three regioisomeric ring substituted methoxybenzoylpiperazines (OMeBzPs) have identical elemental composition and no marked differences in their mass spectra with only the three methoxybenzoylpiperazine regioisomers showing one unique major high mass 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 did not provide any discrimination among these compounds since the main fragment ions are of identical elemental composition.

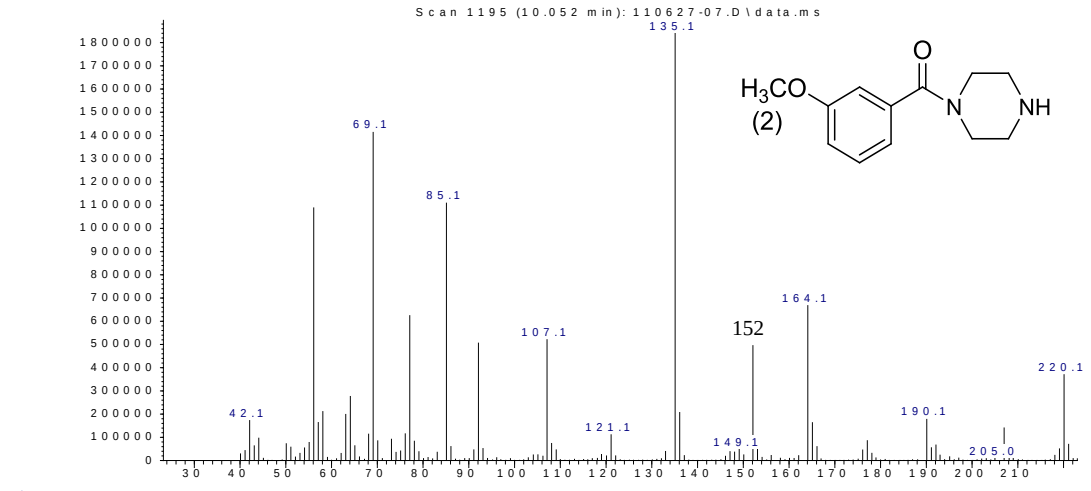
Gas chromatography coupled to infrared detection (GC-IRD) provides direct confirmatory data for the identification of the carbonyl containing compounds and the differentiation of the psychoactive designer drug 3,4-MDBP from its direct (2,3-MDBP) and indirect (OMeBzPs) regioisomers. The mass spectra in combination with the vapor 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).

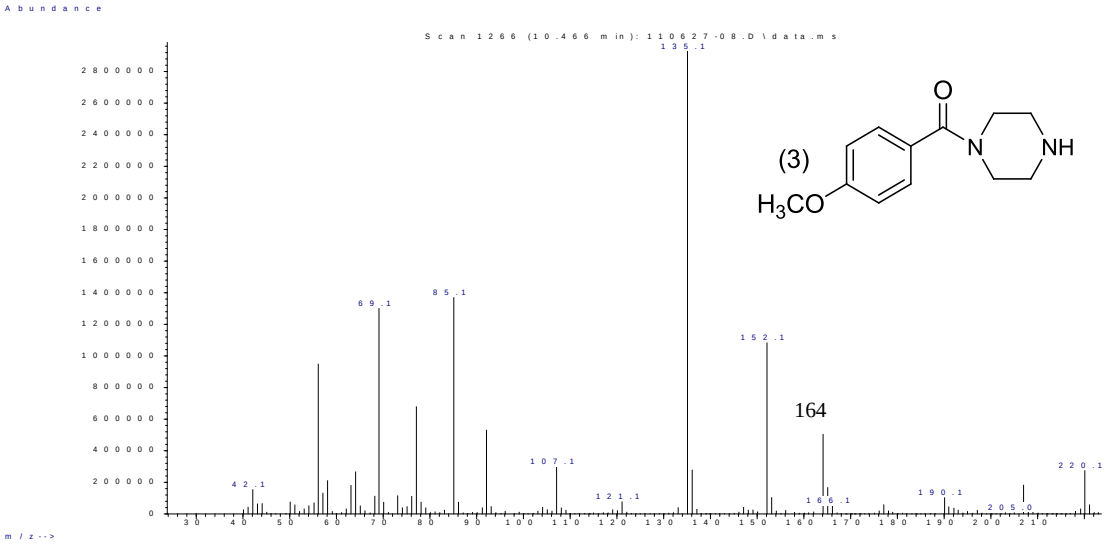
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs)

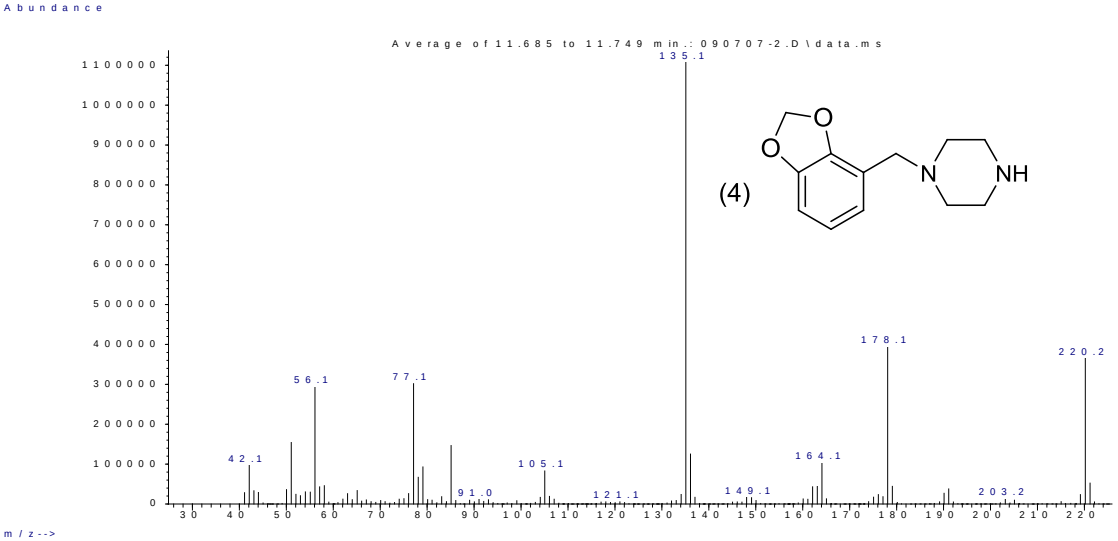
Figure 13-1 shows the EI mass spectra of all five isomeric piperazines (Compounds 1-5). The ions of significant relative abundance common to the five isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the five piperazines show the fragment ions at m/z 178, 164, 135, 85 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figures 1-2 and 13-3 and are related to a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. 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 (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. In addition to that, the mass spectra of the three methoxybenzoylpiperazines show high relative abundance of the fragment ion m/z 69.

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 13-3 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









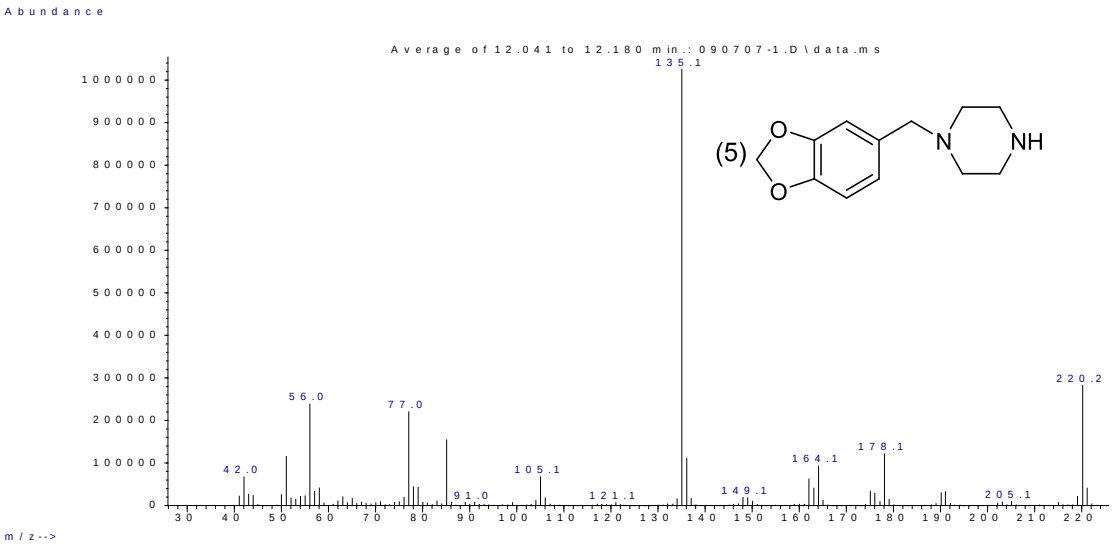


Fig.13-1: Mass spectra of the underivatized methylenedioxybenzylpiperazines and methoxybenzoylpiperazines in this study.

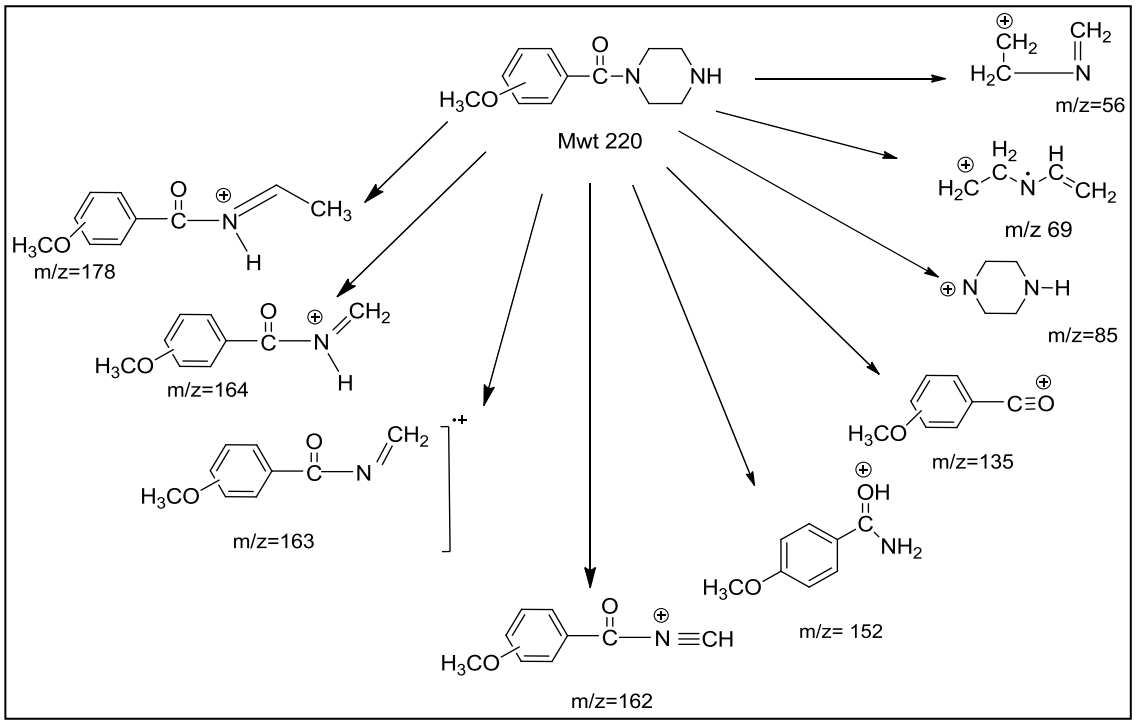


Fig. 13-2: Mass spectral fragmentation pattern of the underivatized methoxybenzoylpiperazines under EI (70eV) conditions.

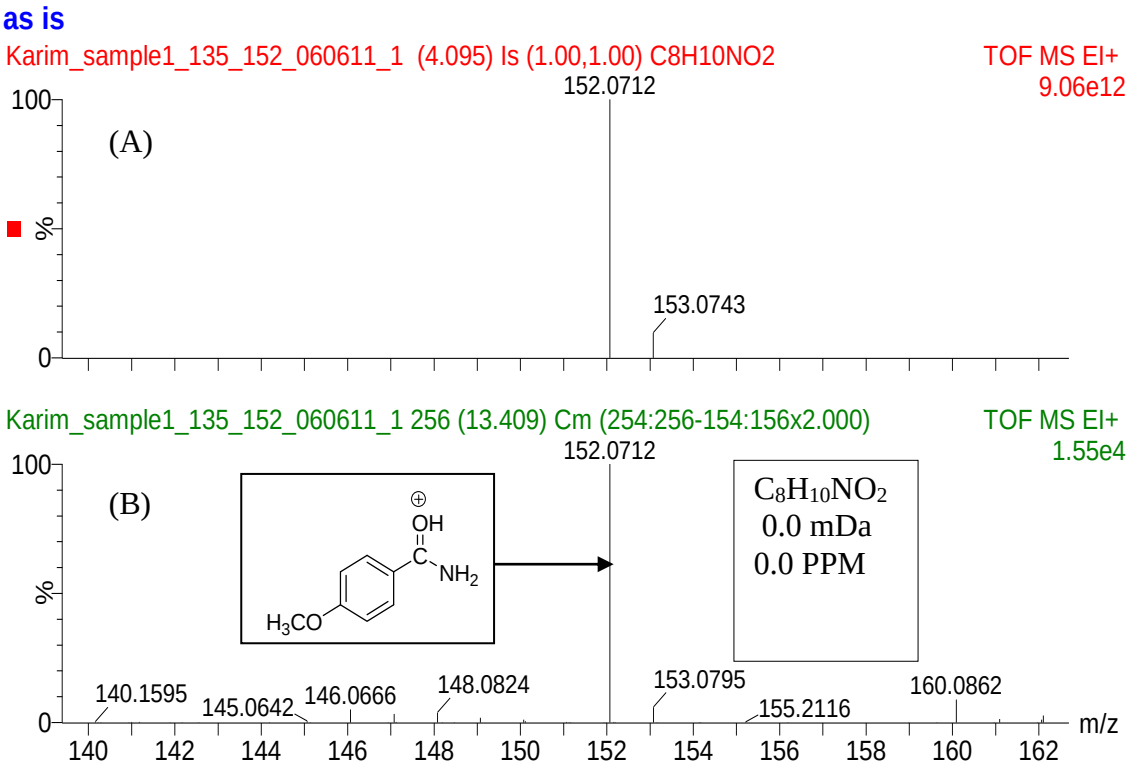


Fig. 13-3: GC-TOF mass spectral analysis of the m/z 152 ion for 4-methoxybenzoylpiperazine. A= calculated mass for C8H10NO2; B= experimental results.

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 13-3. The protonated primary methoxybenzamide (m/z 152) is supported by the mass spectrum of the octa-deutero labeled form of 4-methoxybenzoylpiperazine (4-methoxybenzoyl-d_8-piperazine). This octa-deuterium labeled 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 labeled form of Compound 3 is shown in Figure 13-4. The mass spectrum in Figure 13-4 shows 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 mechanism of formation of the characteristic ions at m/z 152 and m/z 69 in the methoxybenzoylpiperazine is similar to that illustrated for benzoylpiperazine in Figure 12-6. It starts with a migration of the piperazine proton to the carbonyl oxygen followed by a 1,4-hydride shift to form the hydrogen rearranged molecular ion which can either transform to the protonated methoxybenzamide ion at m/z 152 or the fragment ion at m/z 69. The chemical structure of the m/z 69 ion (C_4H_7N) is further confirmed by the mass spectrum of the 4-methoxybenzoyl-d_8-piperazine in Figure 13-4 as the corresponding fragment is shifted 7 Da higher to become m/z 76.

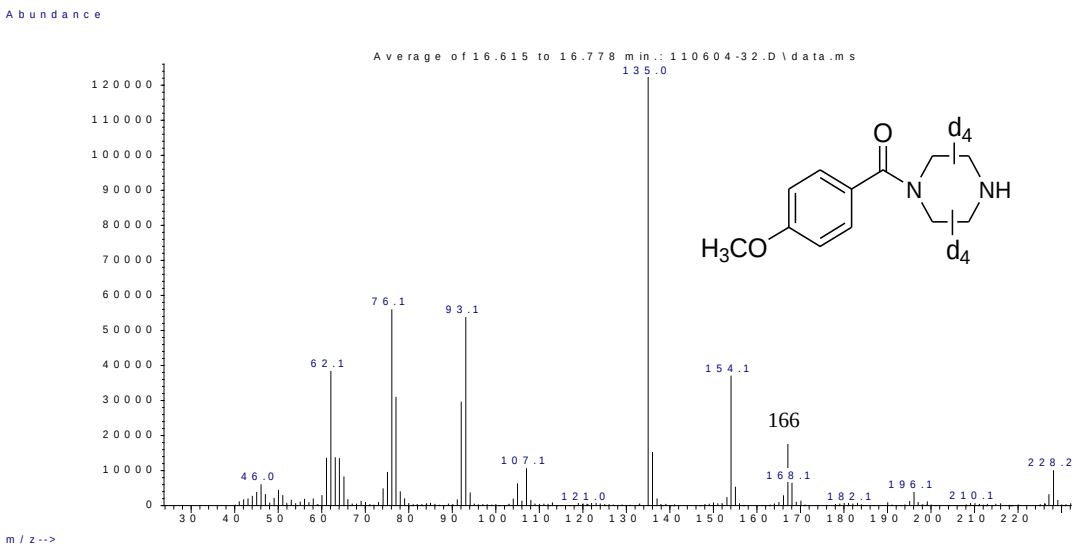
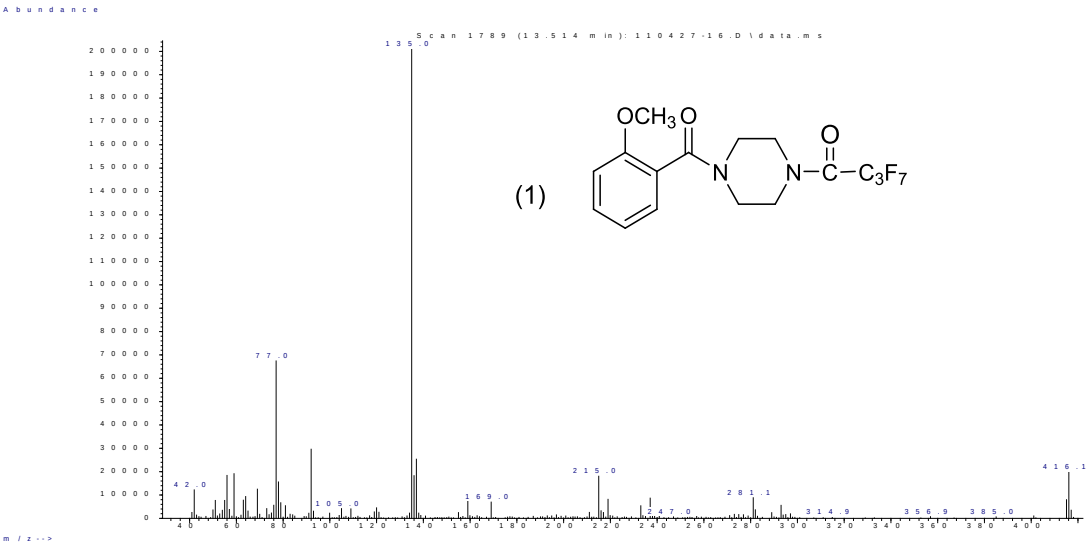


Fig. 13-4: Mass spectrum of the 4-methoxybenzoyl-d_8-piperazine.

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.

The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 13-5 shows the mass spectra of the heptafluorobutryl amides of the five studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ions for TFA, PFPA and HFBA amides yield peaks of high relative abundance at *m/z* 316, 366 and 416, respectively. The major fragment ion in these spectra occurs at *m/z* 135 and corresponds to the ring substituted benzyl 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 $(M\text{-}135)^+$ ion for each amide. These ions have higher relative abundances in the mass spectra of the derivatized methylenedioxybenzylpiperazines 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 other in this set of compounds.



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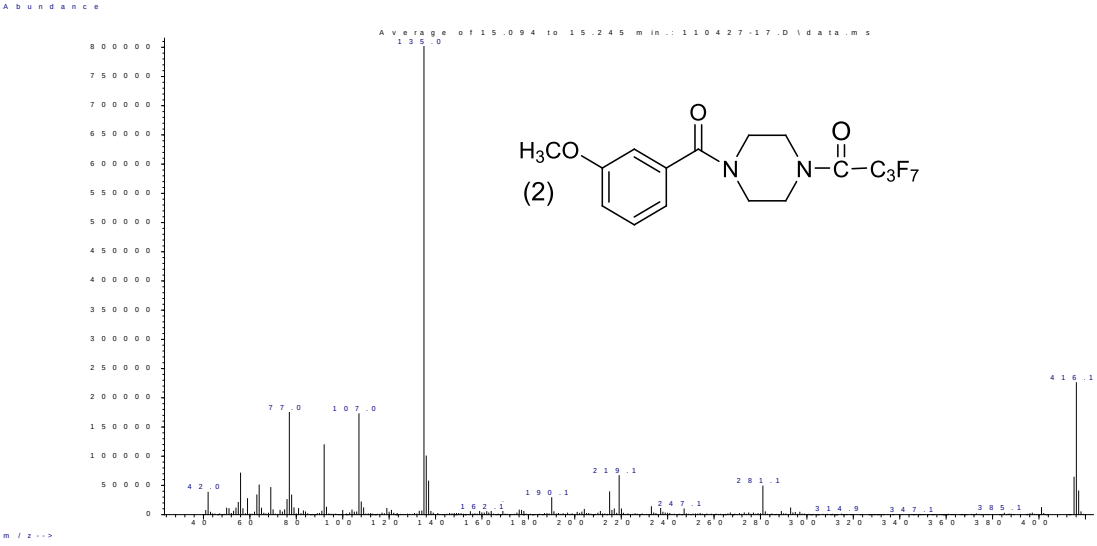

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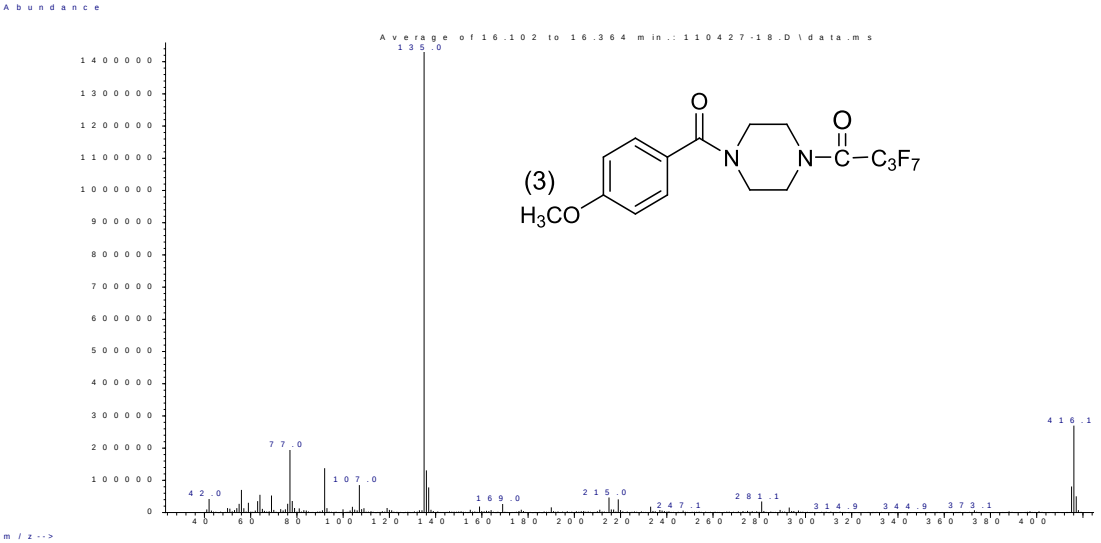

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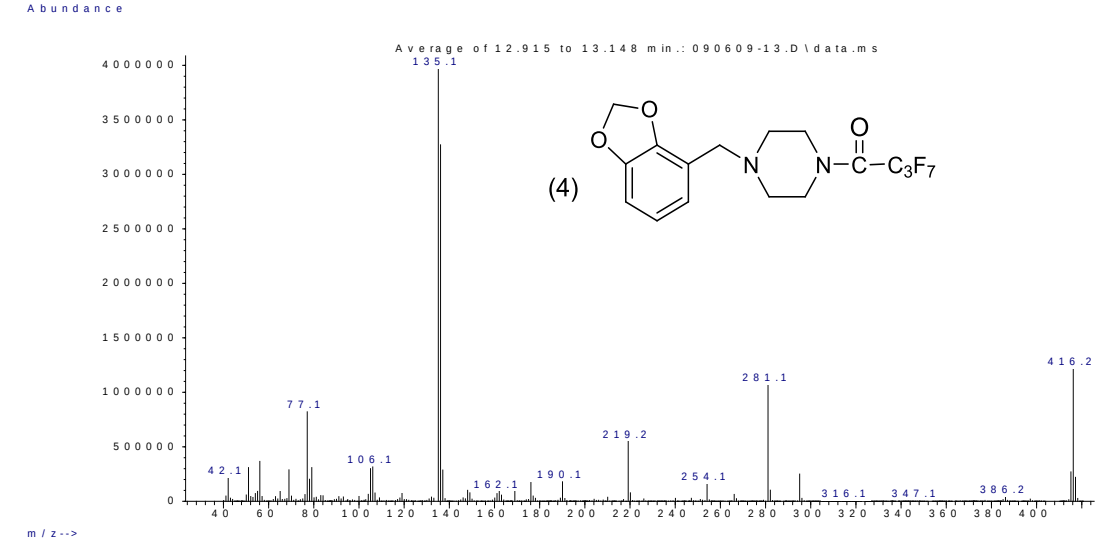

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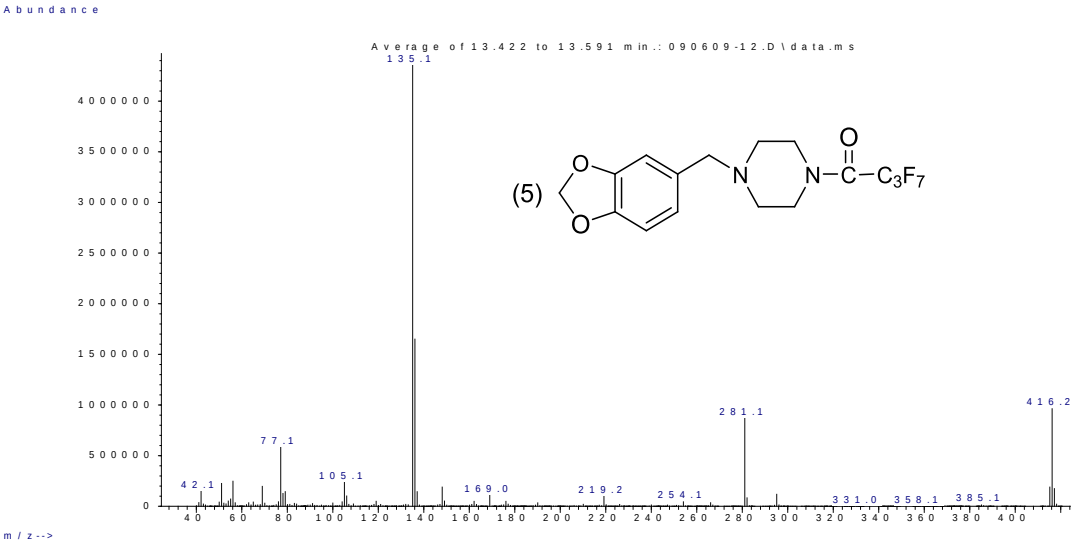


(5)

Fig. 13-5: Mass spectra of the heptafluorobutyryl derivatives of the five piperazine compounds in this study.

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 13-6 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, -1.5 ppm) 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 compound 5. Figures 13-6C and 13-6D confirm the elemental composition as $C_8H_7O_2$ with a mass deviation of 0.8 mDa or 5.9 ppm. 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.

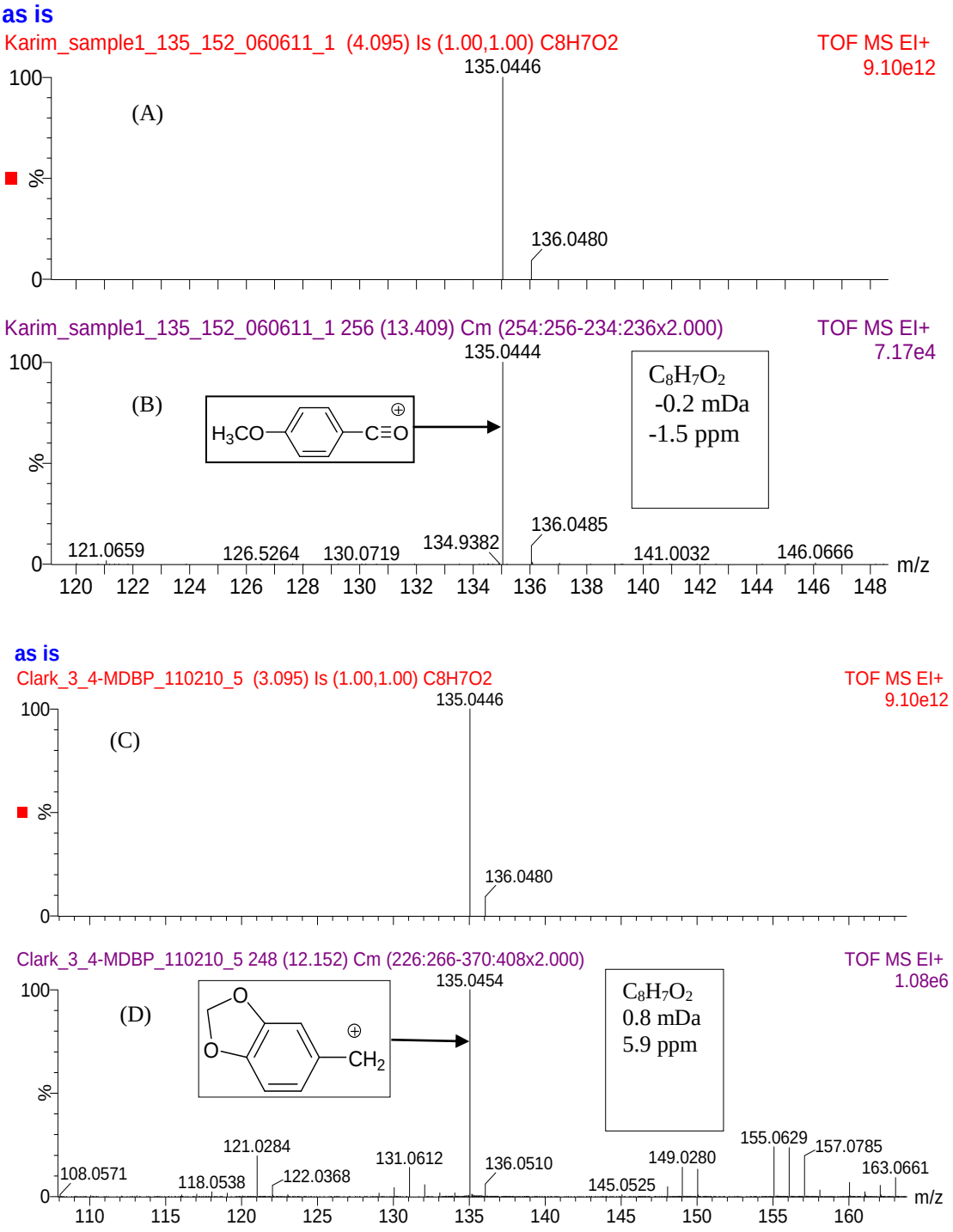


Fig. 13-6: GC-TOF mass spectral analysis of the m/z 135 ion for 4-methoxybenzoylpiperazine and 3,4-methylenedioxybenzylpiperazine. A= calculated mass for C8H7O2; B= experimental results. C= calculated mass for C8H7O2; D= experimental results.

Vapor-phase Infra-Red Spectrophotometric Studies of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs)

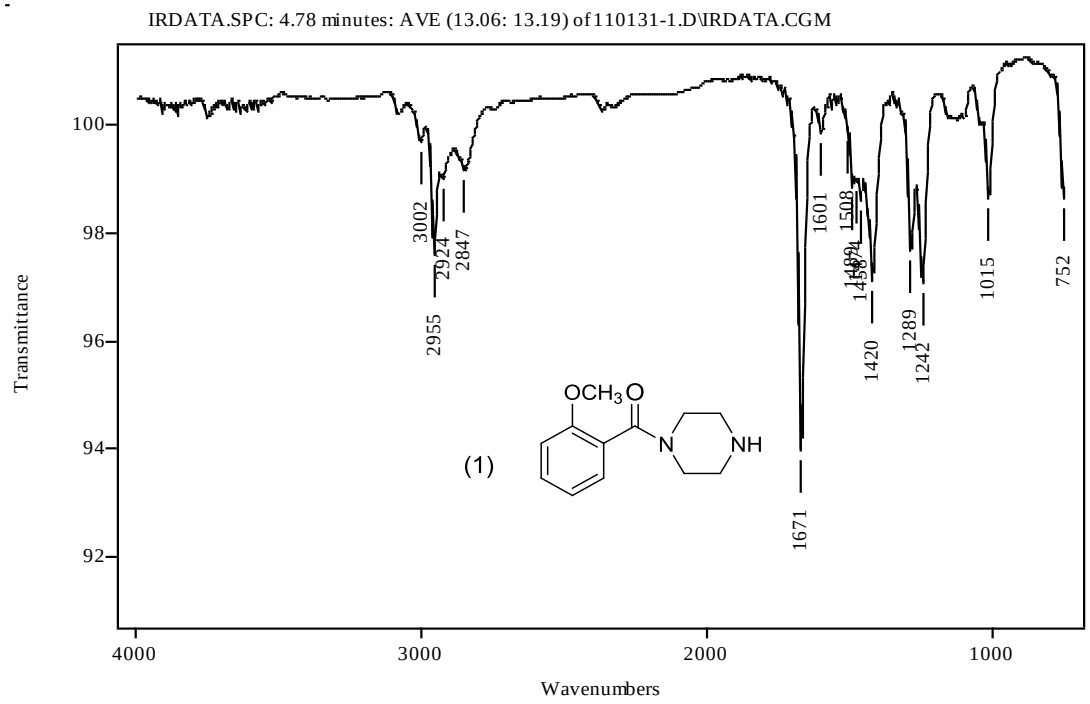
Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the five isomeric piperazines. The vapor phase infrared spectra for the five piperazines are shown in Figure 13-7. The spectra were generated in the vapor phase following sample injection into the gas chromatograph and each compound shows transmittance bands in the regions 650 – 1700 cm^{-1} and 2700 – 3100 cm^{-1}. In general, variations in the substitution pattern on the aromatic ring results in variations in the IR region from 650 – 1700 cm^{-1}. However, variations in the side chain composition leads to variations in the 2700 – 1700 cm^{-1} region. 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}.

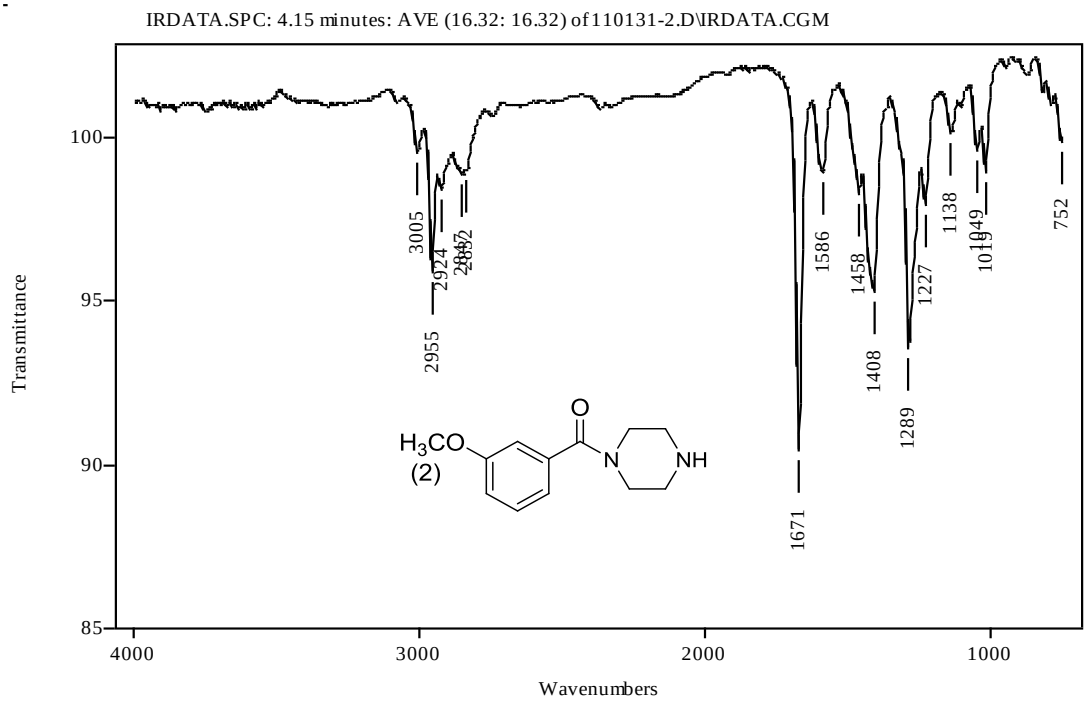
The three regioisomeric methoxybenzoylpiperazines share a characteristic strong singlet IR band at 1671 cm^{-1} corresponding to the carbonyl group stretching which can distinguish these three benzoylpiperazines from the two methylenedioxybenzylpiperazines. The three ring substituted benzoylpiperazines 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

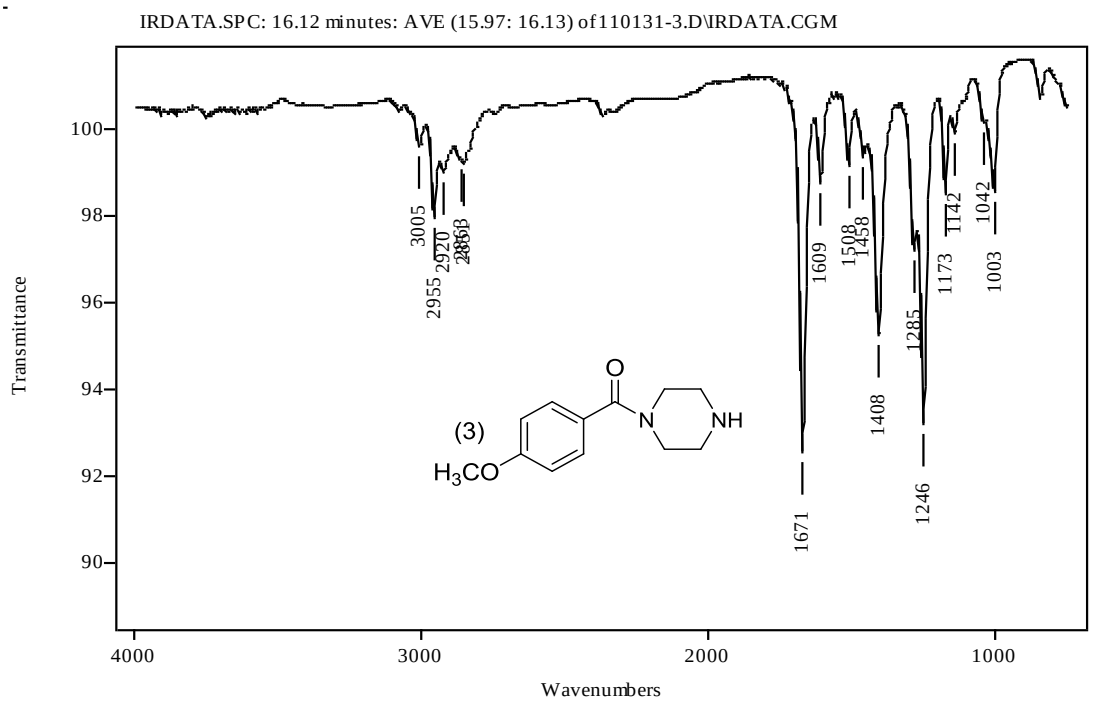
cm^{-1} in compound 2. Compound 1 shows a strong peak at 1420 cm^{-1} which is shifted to a peak at 1408 cm^{-1} in both compounds 2 and 3. Compound 3 also has a medium intensity peak at 1003 cm^{-1} which is shifted to a peak at 1015 cm^{-1} in compound 1 and a weak singlet at 1019 cm^{-1} in compound 2.

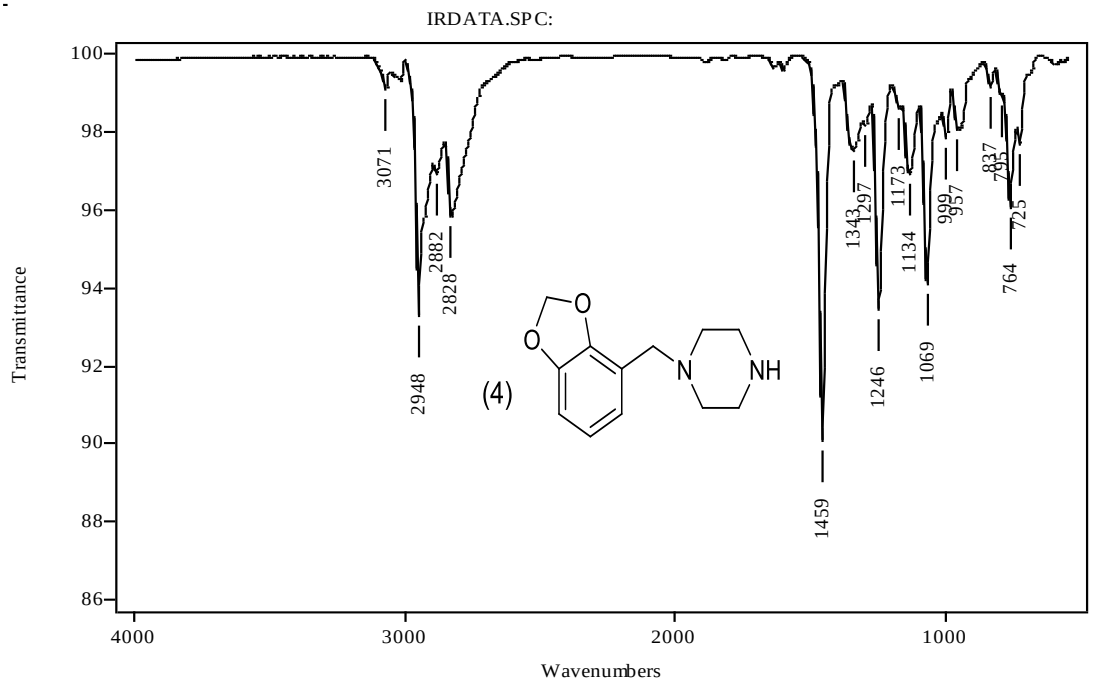
The infrared spectra and results for the two MDBPs have been previously discussed in details in Chapter 1.

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









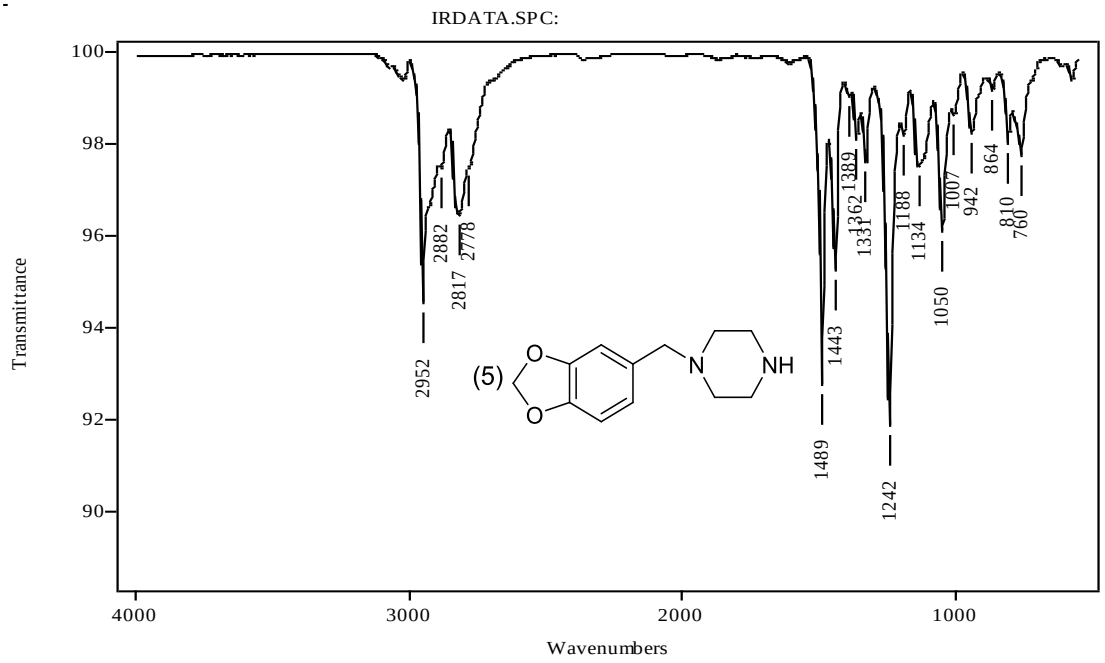


Fig. 13-7: Vapor phase IR spectra of the five piperazines involved in this study.

Gas Chromatographic Separation of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs)

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 performed using a temperature program consisting of an initial temperature of 70°C for 1 minute, ramped up to 250°C at a rate of 30°C per minute followed by a hold at 250°C for 15 minutes.

The chromatograms in Figure 13-8 are representatives of the results obtained for all samples on this stationary phase. In Figure 13-8A and 13-8B 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.

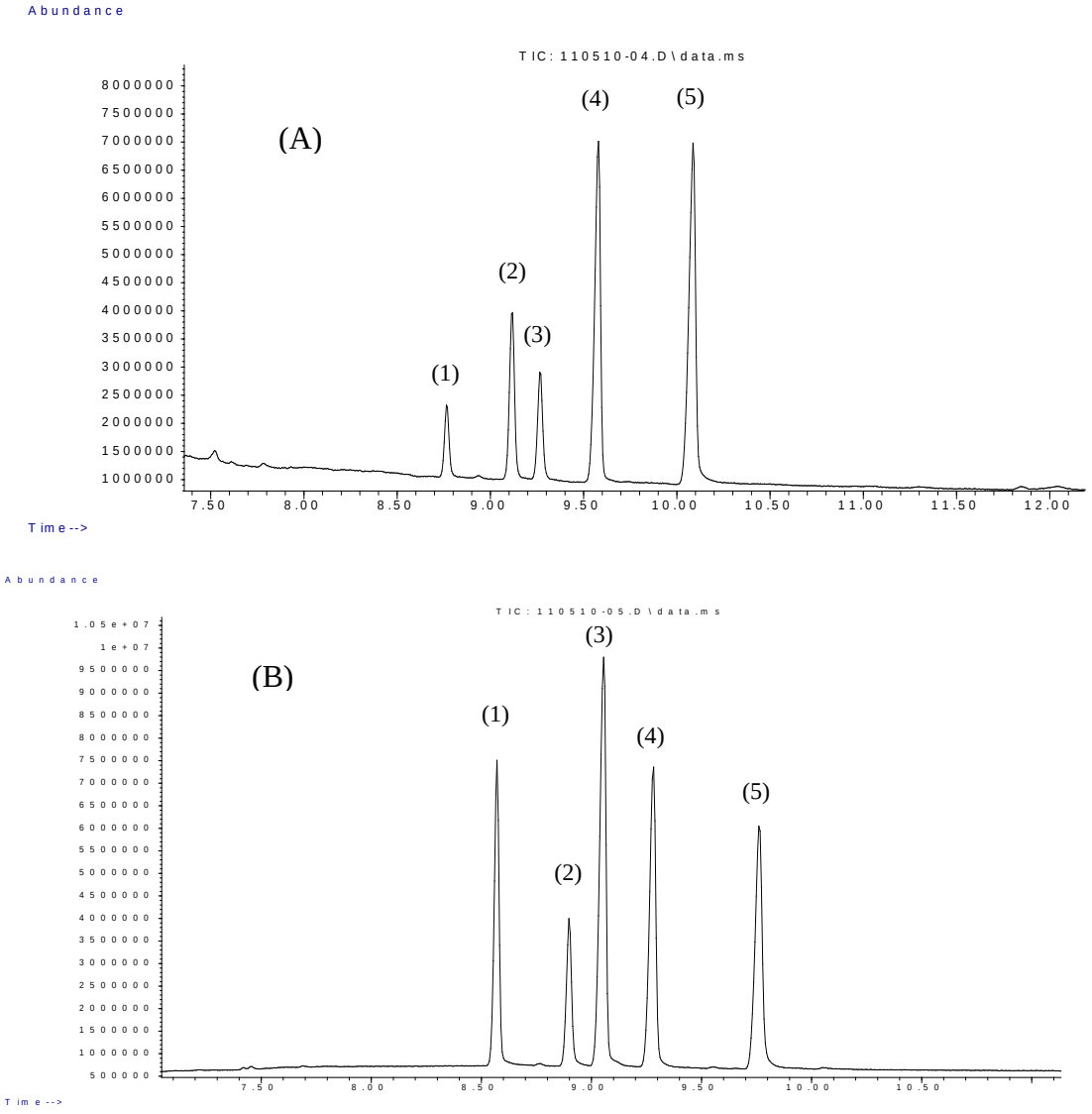


Fig. 13-8: Gas chromatographic separation of the (A) pentafluoropropionyl derivatives and (B) heptafluorobutyryl derivatives of the five piperazines using Rtx-200 column. The number over the peak represents the compound number.

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 high mass 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 did not offer any discrimination among these compounds. GC-IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650-1700 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.

References

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 Science International 121 (2001) 47-56.

Chapter 14

Differentiation of Trifluoromethylbenzylpiperazines (TFMBZPs) and Trifluoromethylbenzoylpiperazines (TFMBOPs) by GC-MS

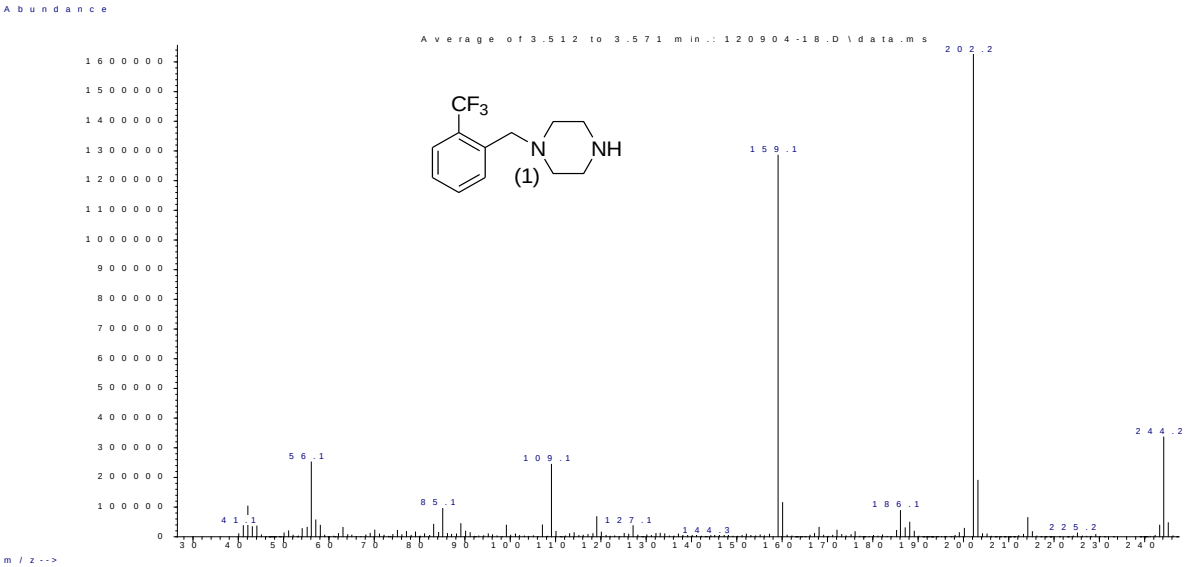
Two series of regioisomers – the TFMBZPs and the TMFBOPs - were synthesized and analyzed as potential "hybrid" derivatives of the BZP and TMFPP piperazine drugs of abuse. The TFMBZPs are readily differentiated from TMFBOPs by their mass spectra based differences in their mass, the base peaks in their mass spectra as well as several other unique fragment ions. However the mass spectra of each regioisomer in each of these two series have fragment ions of identical mass and thus cannot be differentiated by this analytical method alone. Furthermore, chemical derivatization by perfluoroacylation did not offer any additional unique marker fragment ions in the mass spectrum to allow identification of one regioisomer in a series to the exclusion of the other two regioisomers. The perfluoroacylamides of the regioisomers in the TFMBZP series and the regioisomers in the TMFBOP series were readily separated by GC on the stationary phase Rtx-200 and eluted in an order similar to other perfluoroacyl-derivatives of other benzyl- and benzoylpiperazine compounds reported earlier.

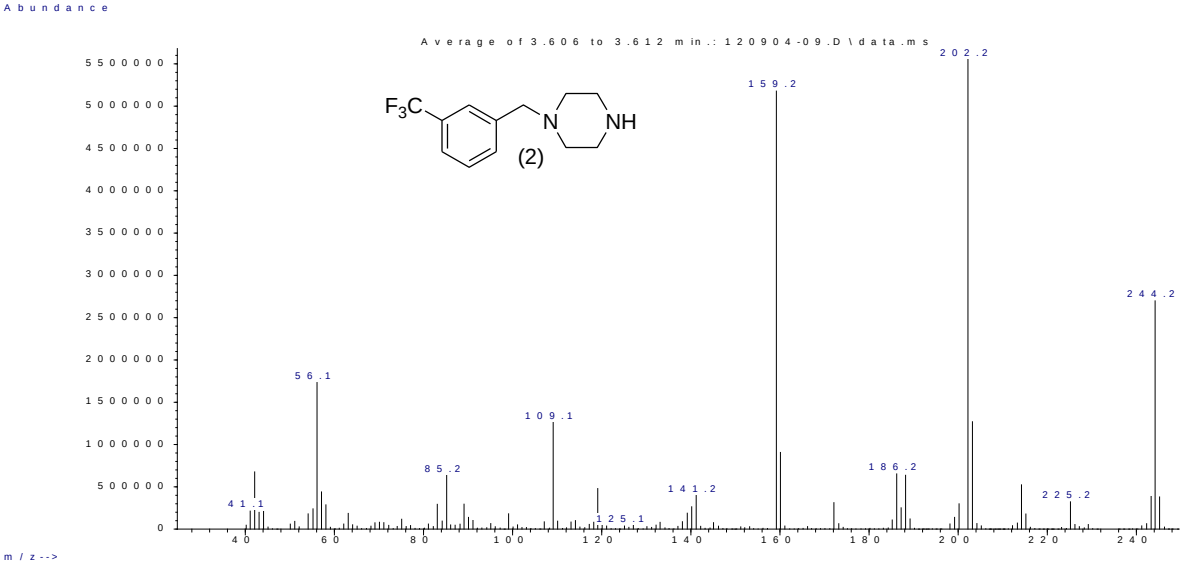
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Trifluoromethylbenzylpiperazines (TFMBZPs) and Trifluoromethylbenzoylpiperazines (TFMBOPs)

Mass spectrometry remains the primary method for confirming the identity of drugs in forensic samples. The mass spectra of the three regioisomeric trifluoromethylbenzylpiperazines in this study are shown in Figure 14-1. All three of these compounds show fragment ions at m/z 202 (M-42, ethylene-trifluoromethylbenzylamine), 159 (M-85, trifluoromethylbenzyl), 85 (piperazine) and 56 (methylene, ethyleneamine). These are the same major fragment ions observed in the mass spectra of the methylbenzylpiperazines and other ring substituted benzylpiperazines reported earlier [Abdel-Hay *et al*, 2012]. The fragmentation pathways for the trifluoromethylbenzylpiperazines and the corresponding methylbenzylpiperazines are outlined in Figure 14-2 for comparison (mass spectra for the methylbenzylpiperazines not shown). The spectra in Figures 14-1 also show several fragment ions unique to the trifluoromethyl substituent including ions at m/z 225 from loss of a single fluorine from the molecular ion, a m/z 140 from loss of fluorine from the trifluoromethylbenzyl fragment (m/z 159), and m/z 109 from loss of CF_2 from the trifluorobenzyl fragment. However, all three of the trifluoromethylbenzylpiperazines have nearly identical mass spectra, and thus cannot be differentiated using this methodology.

In an effort to individualize the mass spectra and identify additional unique marker ions for differentiation among the three isomeric trifluoromethylbenzylpiperazines, the heptafluorobutyryl (HFBA) amide derivative of each isomer was prepared and analyzed.

Acylation lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectra. Figure 14-3 shows the mass





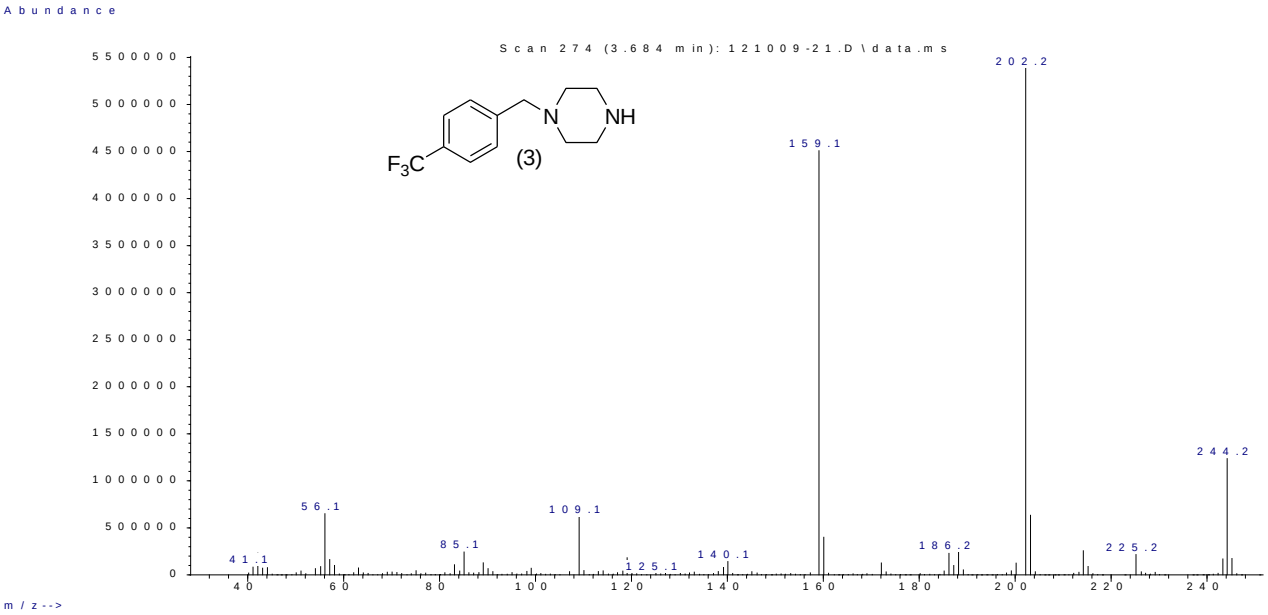


Fig. 14-1: Mass spectra of the TMFBZP regioisomers.

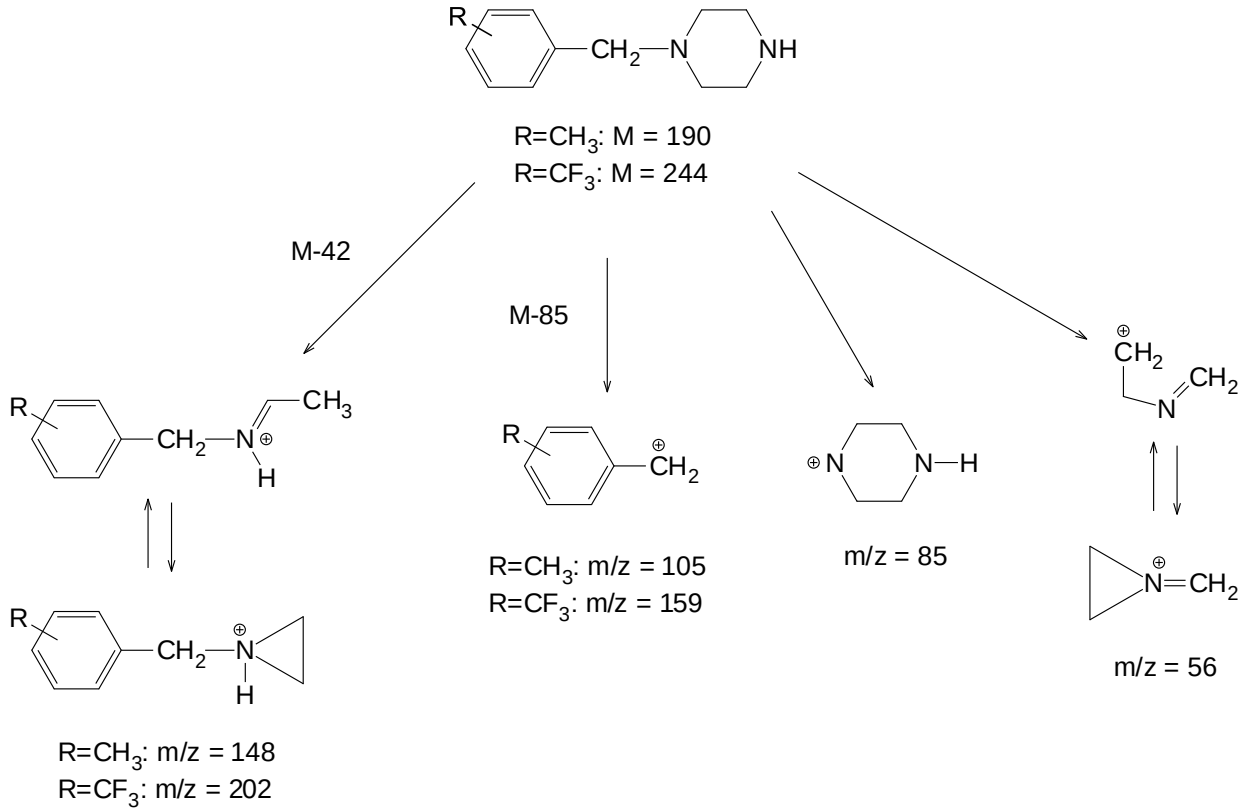
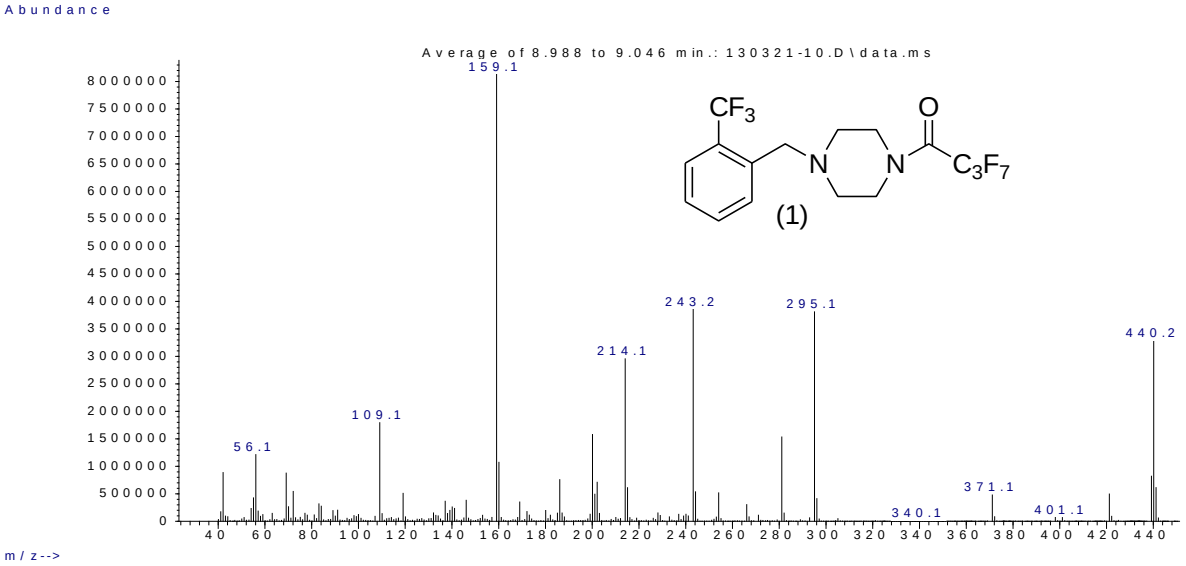
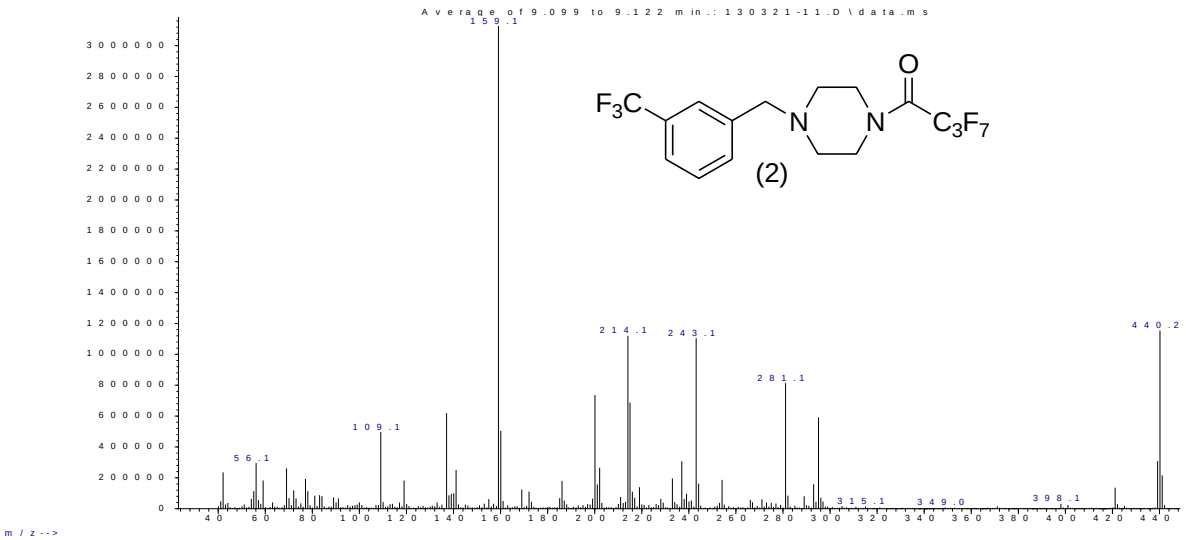


Fig. 14-2: Mass spectral fragmentation pattern of the TMFBZP regioisomers under EI (70 eV) conditions.





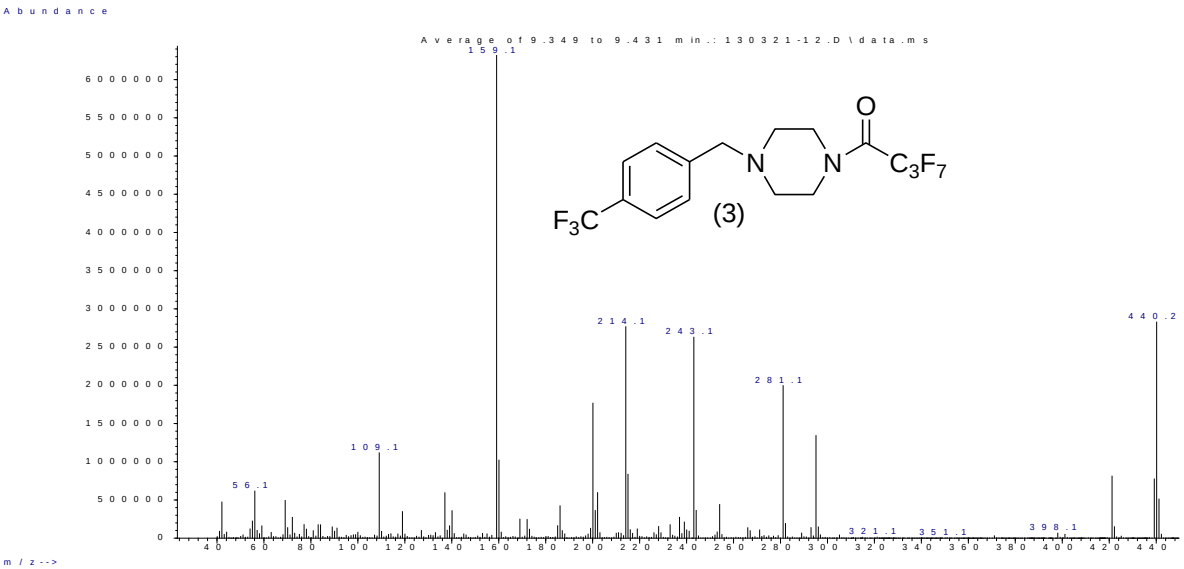


Fig. 14-3: Mass spectra of the TMFBZP-HFBA regioisomers.

spectra of the HFBA amides of the three trifluoromethylbenzylpiperazines. The molecular ions for these amides yield peaks of high relative abundance at m/z 440, and a major fragment ion at m/z 159 which corresponds to the trifluoromethylbenzyl cation (Figure 14-4). Additional significant fragment ions in the spectra of all three regioisomers occurred at m/z 295 for the methylene-HFBA-piperazine ion, m/z 281 for the HFBA-piperazine ion, m/z 243 for the trifluoromethylbenzylpiperazine ion, m/z 214 for the trifluoromethylbenzylpiperazine ring fragmented ion, and m/z 109 for the fluorobenzyl ion, presumably resulting from loss of CF_2 from the trifluoromethylbenzyl cation. However, since these ions are present in the mass spectra of all three regioisomeric HFBA-trifluoromethylbenylpiperazines, chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one regioisomer the exclusion of the others in this set of compounds.

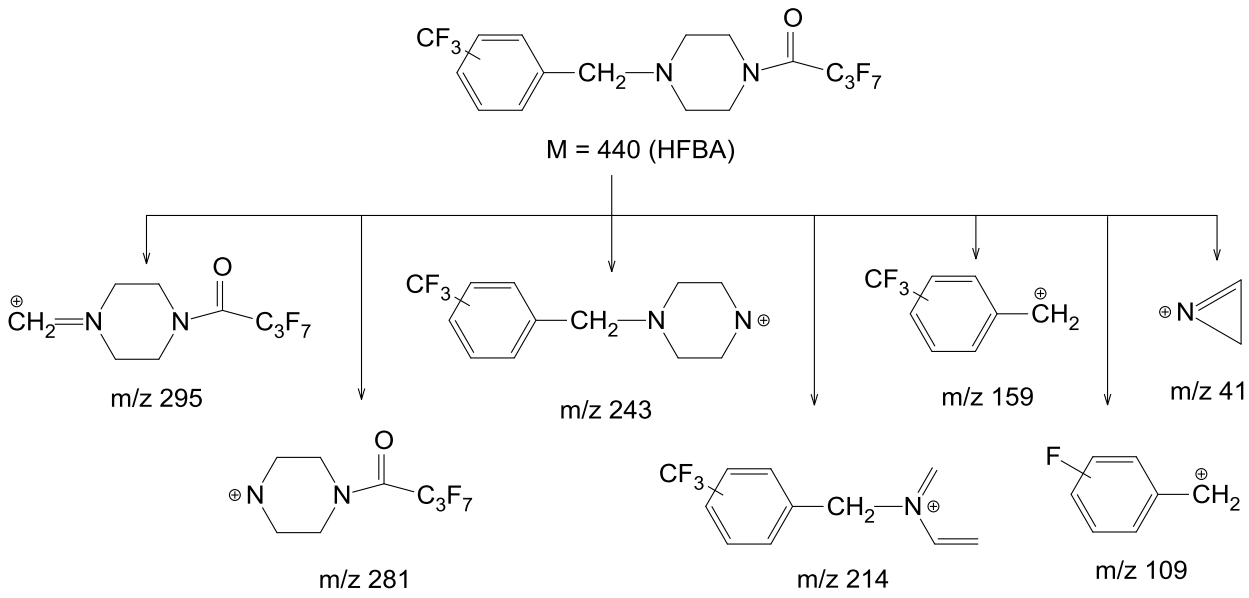
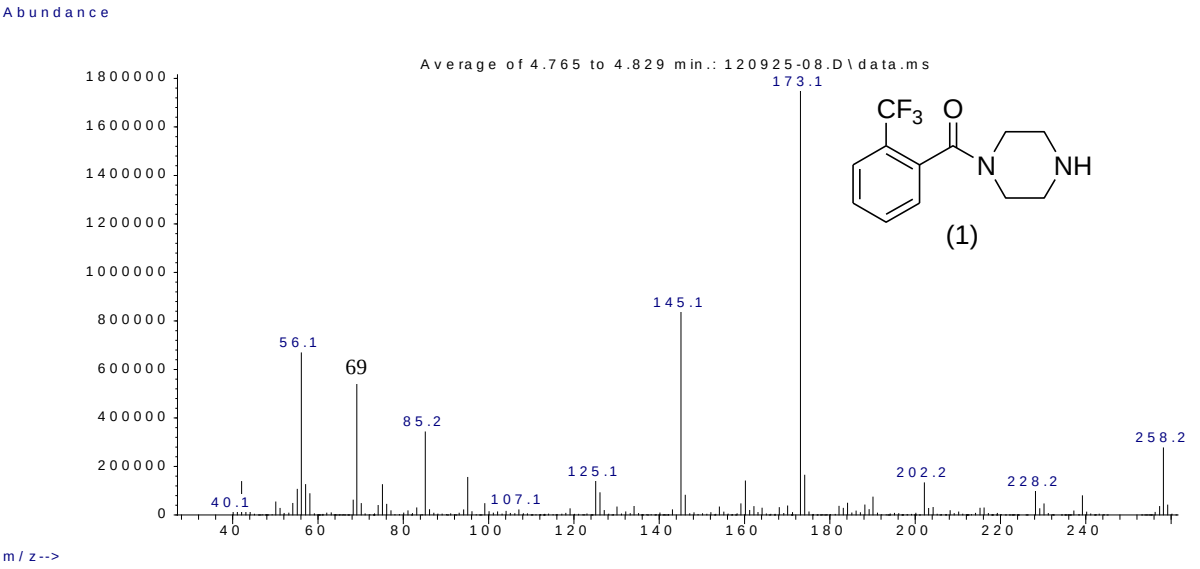
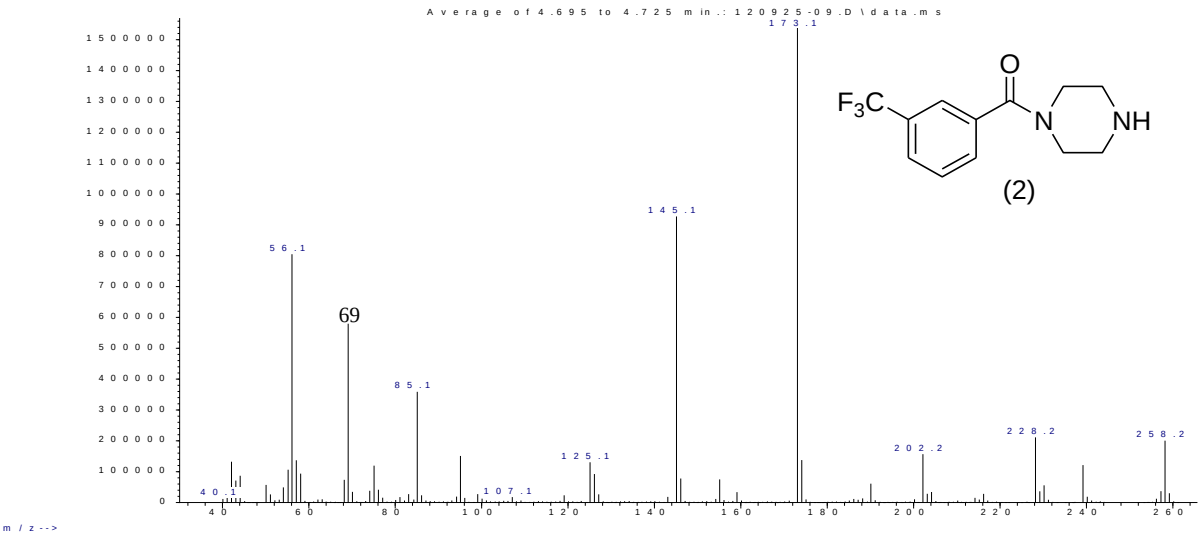


Fig. 14-4: Mass spectral fragmentation pattern of the TMFBZP-HFBA regioisomers under EI (70 eV) conditions.

The mass spectra of the regioisomeric trifluoromethylbenzoyl piperazines are shown in Figure 14-5. The ions of significant relative abundance common to all of the benzoyl piperazines appear to arise from fragmentation of the piperazine ring as shown in Figure 14-6. These include the methylene-ethylene ion at m/z 228 (M-30), the methylene ion at m/z 202 (M-56), the protonated amide ion at m/z 190 (M-68), the benzoyl ions at m/z 173 (M-85) and the aromatic ion at m/z 145 (M-113). The spectra for all three trifluoromethyl benzoyl piperazines also include three piperazine fragments of relatively high abundance at m/z 85, 69 and 56. This fragmentation pattern is consistent with results obtained in our earlier studies with benzoyl-, methoxybenzoyl, dimethoxybenzoyl- and methylenedioxybenzoylpiperazines, as shown in Figure14-6 [Abdel-Hay *et al*, 2012]. In these earlier studies the identity of the primary amide (M-68) and benzoyl (M-85) ions were confirmed by exact mass analysis using GC-TOF-MS and deuterium labeling studies [Abdel-Hay *et al*, 2012]. The trifluoromethylbenzoyl piperazines do contain several fragments in their mass spectra not present in the methylbenzoylpiperazine series due to the loss of fluorine from the molecular ion (m/z 239) and apparent loss of HF (m/z 125) or F_2 (m/z 107) from the benzoyl ion (m/z 145). However, all three of the trifluoromethylbenzoylpiperazines have nearly identical mass spectra, and thus cannot be differentiated using this methodology.

As was done in the trifluoromethylbenzylpiperazine series described above, the pentafluoropropionyl (PFPA) amide derivative of each trifluoromethylbenzoylpiperazine isomer was prepared and analyzed in an effort to individualize the mass spectra and





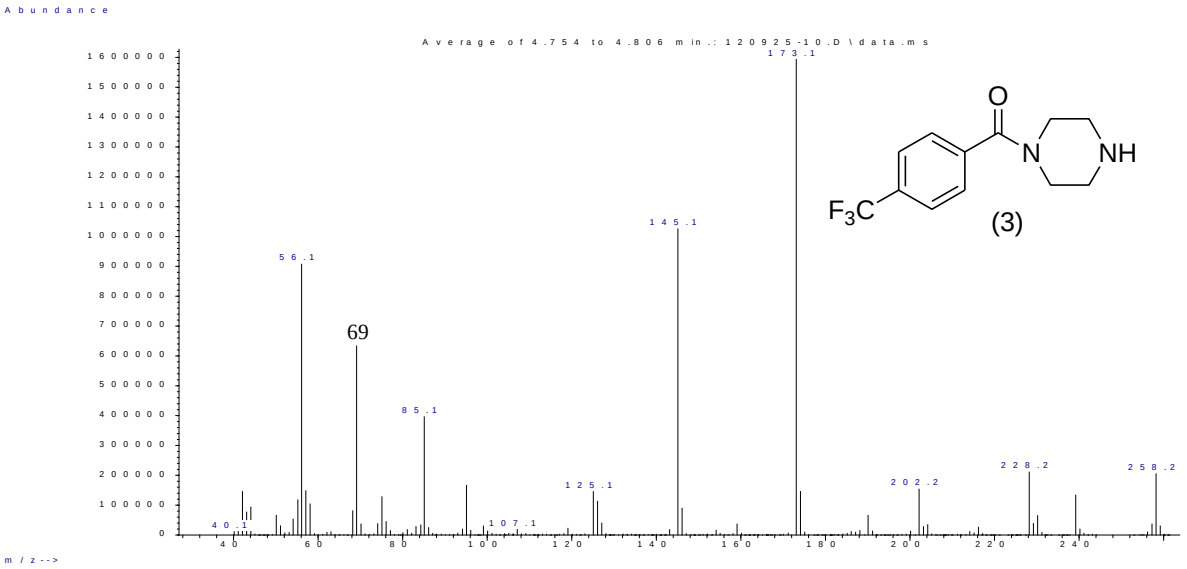


Fig. 14-5 Mass spectra of the TMFBOP regioisomers.

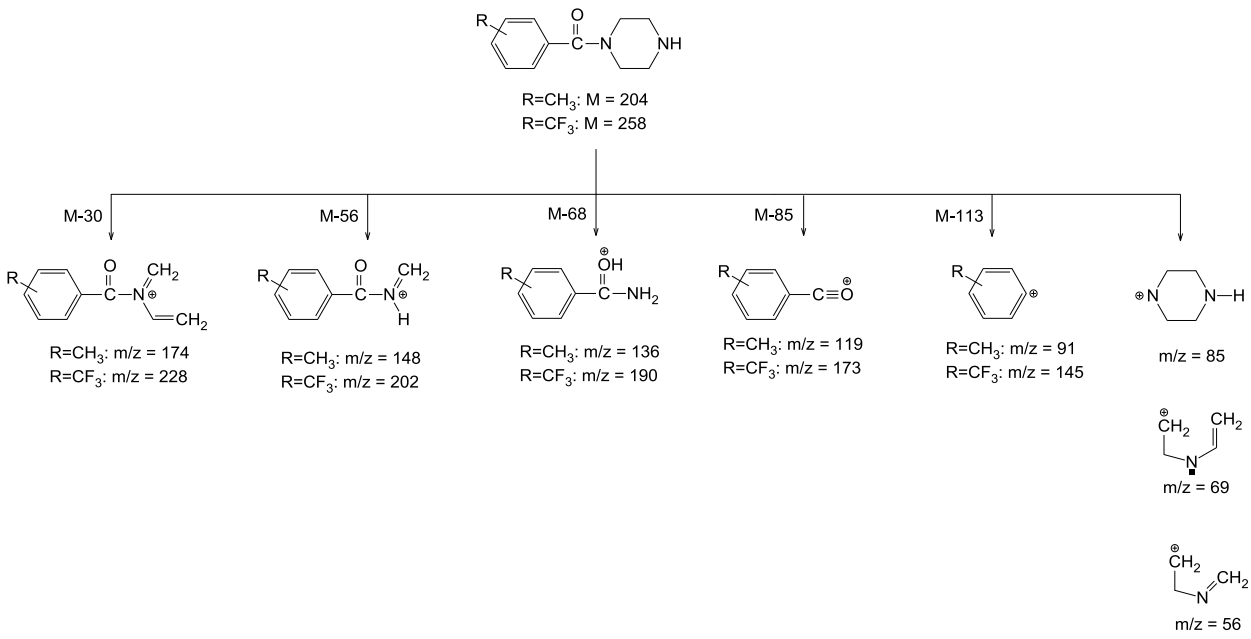
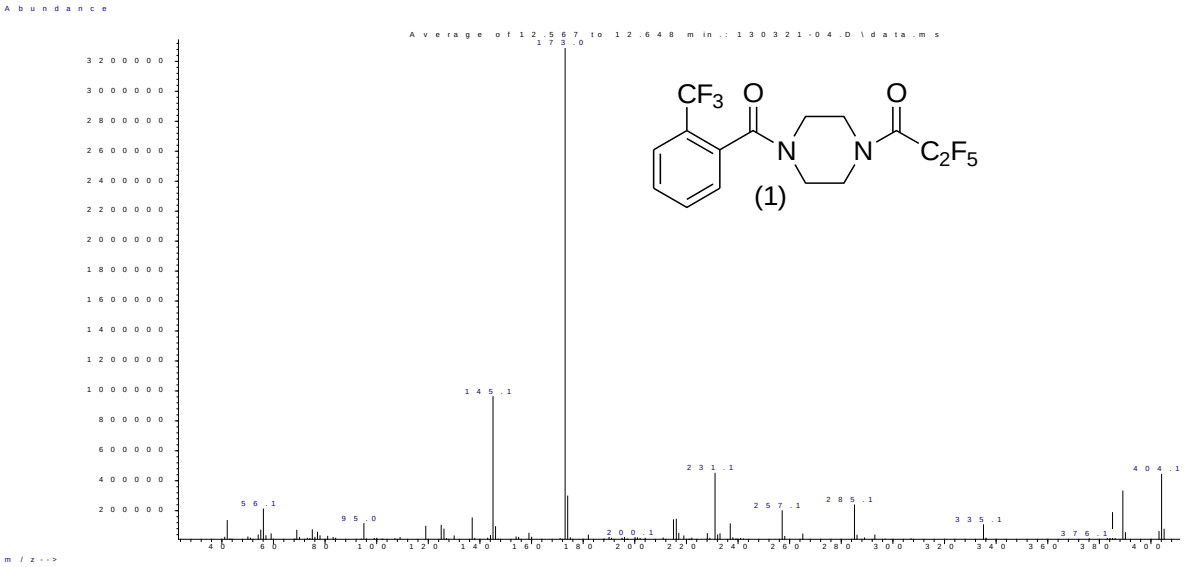


Fig. 14-6: Mass spectral fragmentation pattern of the TMFBOP regioisomers under EI (70 eV) conditions.

identify additional unique marker ions for differentiation among these three regioisomers (Figure 14-7). The molecular ions for these three amides yield peaks of high relative abundance at m/z 404, and a major fragment ion at m/z 173 which corresponds to the trifluoromethylbenzoyl cation (Figure 14-8). Additional significant fragment ions in the spectra of all three regioisomers occurred at m/z 285 (M-119) for the trifluorobenzoylpiperazinylcarbonyl cation, m/z 257 (M-147) for the trifluoromethylbenzoylpiperazinyl cation, and m/z 145 (M-259) for the trifluoromethylphenyl cation. Unfortunately since these ions are present in the mass spectra of all three regioisomeric PFPA-trifluoromethylbenzoylpiperazines, chemical derivatization did not offer any additional unique marker ions to allow identification of one regioisomer the exclusion of the others in this set of compounds.



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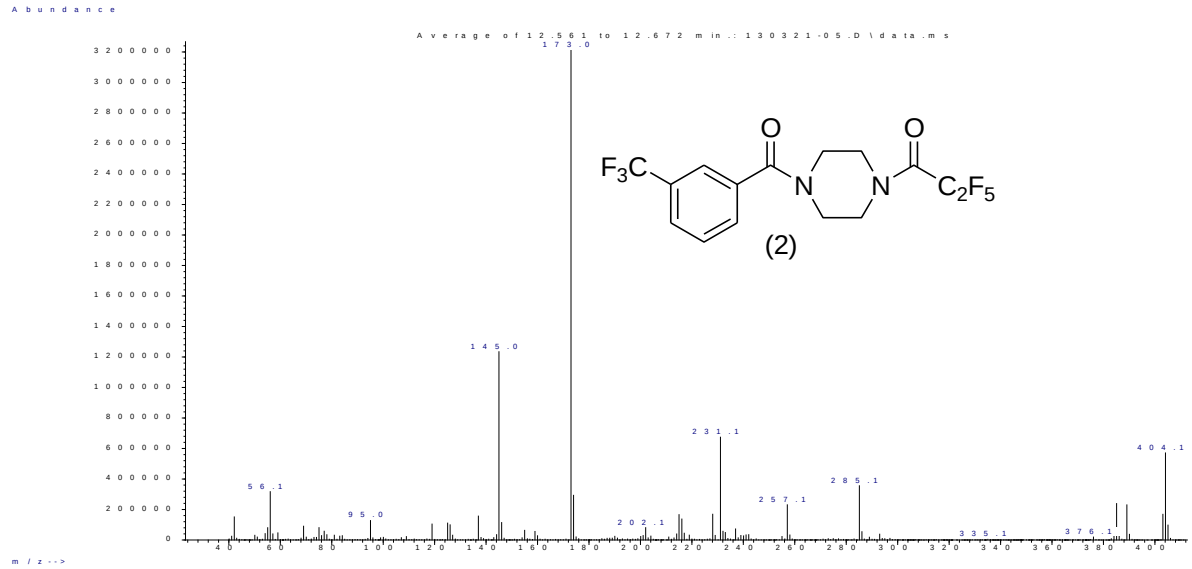

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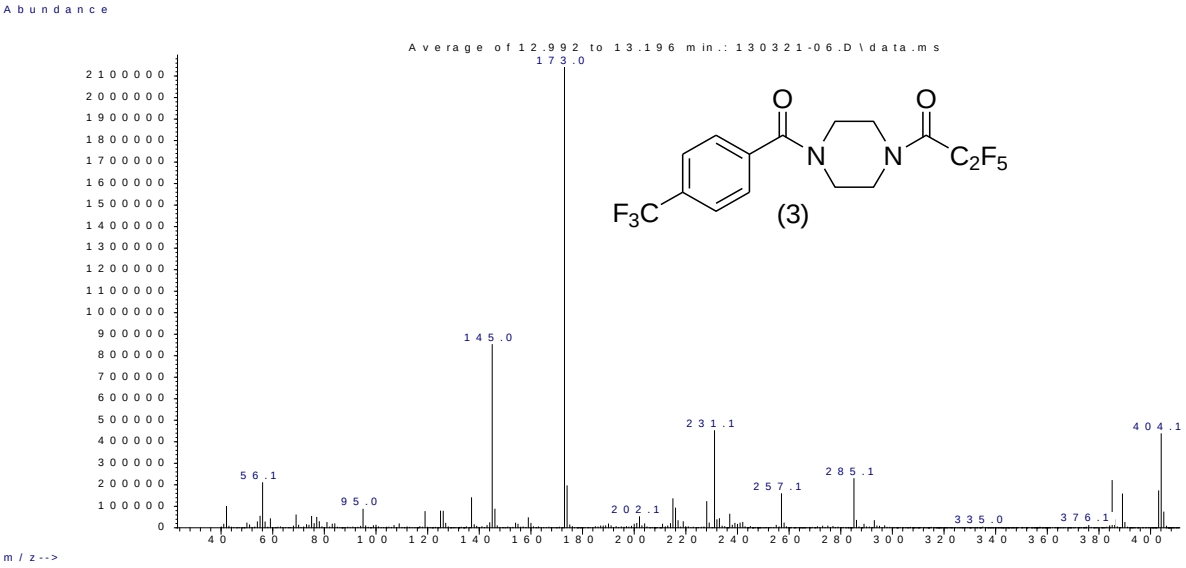


Fig. 14-7: Mass spectra of the TMFBOP-PFPA regioisomers.

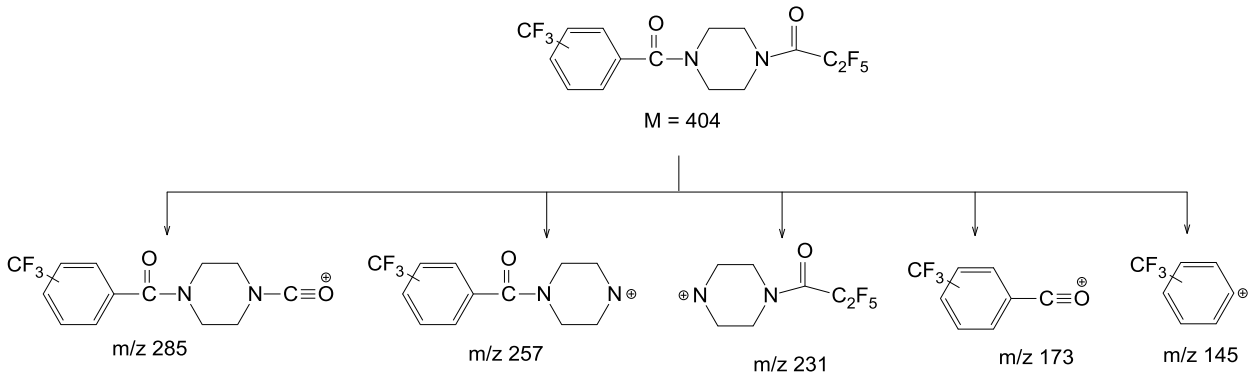


Fig. 14-8: Mass spectral fragmentation pattern of the TMFBOP-PFPA regioisomers under EI (70 eV) conditions.

Gas Chromatographic Separation of Trifluoromethylbenzylpiperazines (TFMBZPs) and Trifluoromethylbenzoylpiperazines (TFMBOPs)

Gas chromatographic separation of the perfluoroacyl-derivatized piperazine series was accomplished on a capillary column of dimensions 30 m 0.25 mm and 0.5-mm film depth of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatograms in Figure 14-9 and 14-10 are representative of the results obtained for all samples on this stationary phase. The separation of the pentafluoropropionyl and heptafluorobutyryl derivatives was performed using a temperature program consisting of 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 60.00 min. Under these conditions, the three regiosiomeric heptafluorobutyryl-TFMBZPs were readily resolved and eluted in the order of the 2-regioisomer followed by the 3-regioisomer and lastly the 4-regioisomer (Figure 14-9). The pentafluoroproprionyl-TFMBOPs were also well resolved under these conditions and gave the same elution order for the regioisomers (2 < 3 < 4) (Figure 14-10). Furthermore, these elution orders are consistent with results obtained with other benzylpiperazines and benzoylpiperazines reported earlier [Abdel-Hay *et al*, 2012]. Thus while the perrfluoroacylated derivatives did not provide any additional mass spectral discrimination among the regioisomers in each series, it did offer a method for marked improvement in the chromatographic resolution compared to the underivatized piperazines and for specific regioisomer discrimination. However, specific discrimination would require reference materials in order to confirm the complete chromatographic resolution for all the regioisomers in each series.

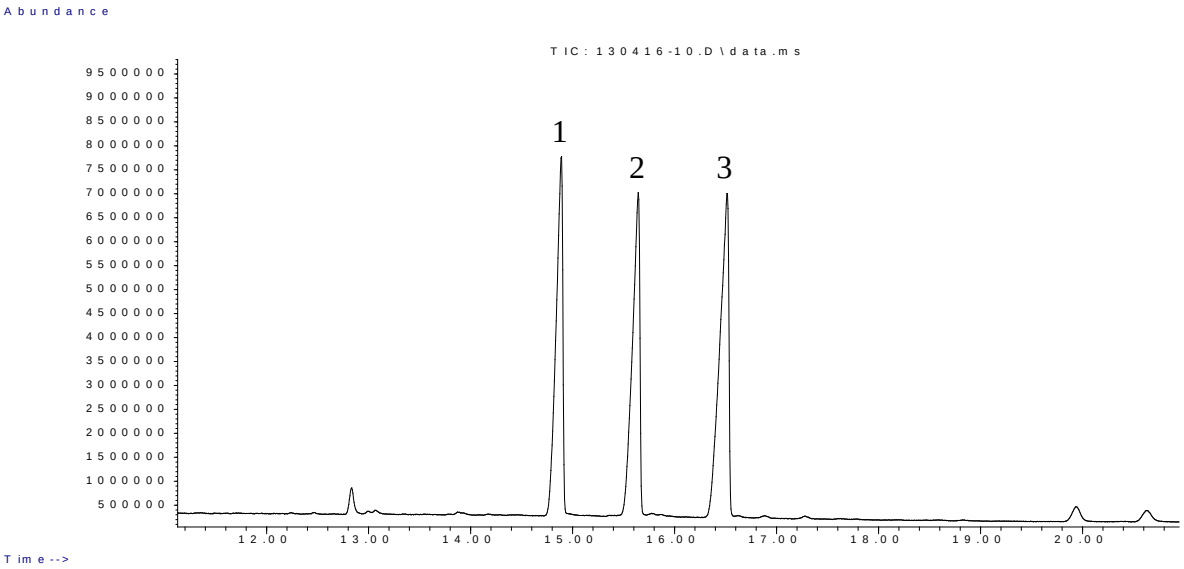


Fig. 14-9: Gas chromatographic separation of the TMFBZP-HFBA regioisomers using a Rtx-200 column.

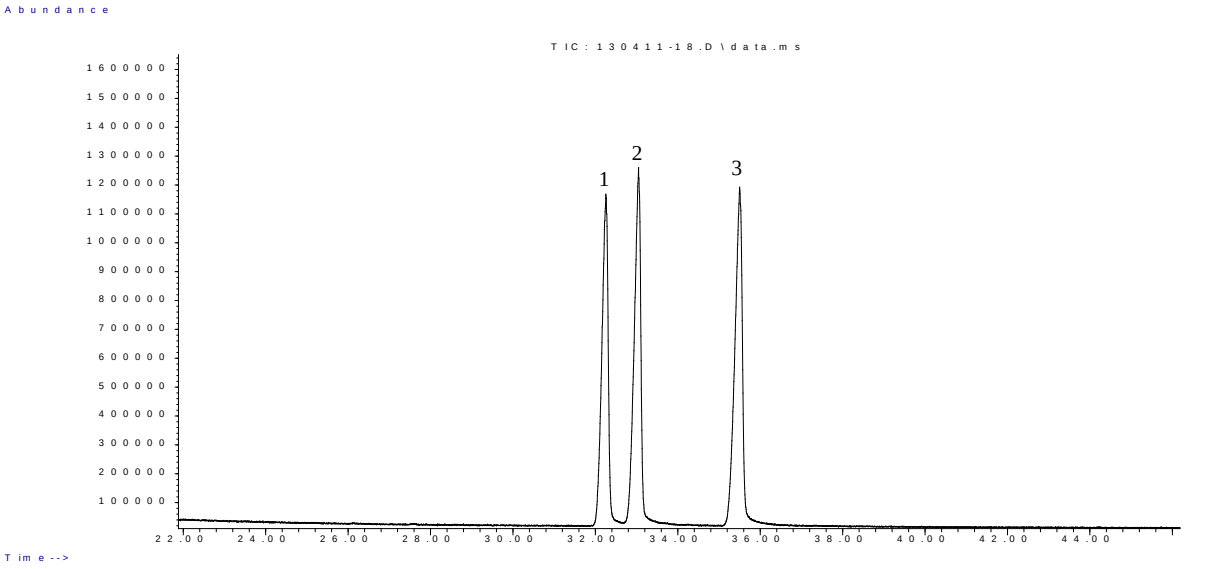


Fig. 14-10: Gas chromatographic separation of the TMFBOP-PFPA regioisomers using a Rtx-200 column.

Conclusion

Two series of regioisomers – the TFMBZPs and the TMFBOPs were synthesized and analyzed as potential "hydrid" derivatives of the BZP and TMFPP piperazine drugs of abuse. The TFMBZPs are readily differentiated from TMFBOPs by their mass spectra based differences in their mass, the base peaks in their mass spectra as well as several unique fragmentation ions. However the mass spectra of the regioisomers in each of these two series have virtually identical fragment ions and thus cannot be differentiated by this analytical method. Furthermore, chemical derivatization by perfluoroacylation did not offer any additional unique marker fragment ions in the mass spectrum to allow identification of one regioisomer in a series to the exclusion of the other two regioisomers. The perfluoroacylamides of the regioisomers in the TFMBZP series and the regioisomers in the TMFBOP series were readily separated by GC on the stationary phase Rtx-200 and eluted in an order similar to other perfluoroacyl-derivatives of other monosubstituted benzyl- and benzoylpiperazines.

References

Karim M.Abdel-Hay, Jack DeRuiter and C. Randall Clark, "Differentiation of Methylbenzylpiperazines (MBPs) and Benzoylpiperazine (BNZP) using GC-MS and GC-IRD," Drug Testing and Analysis, 4, 441-448 (2012).

Chapter 15

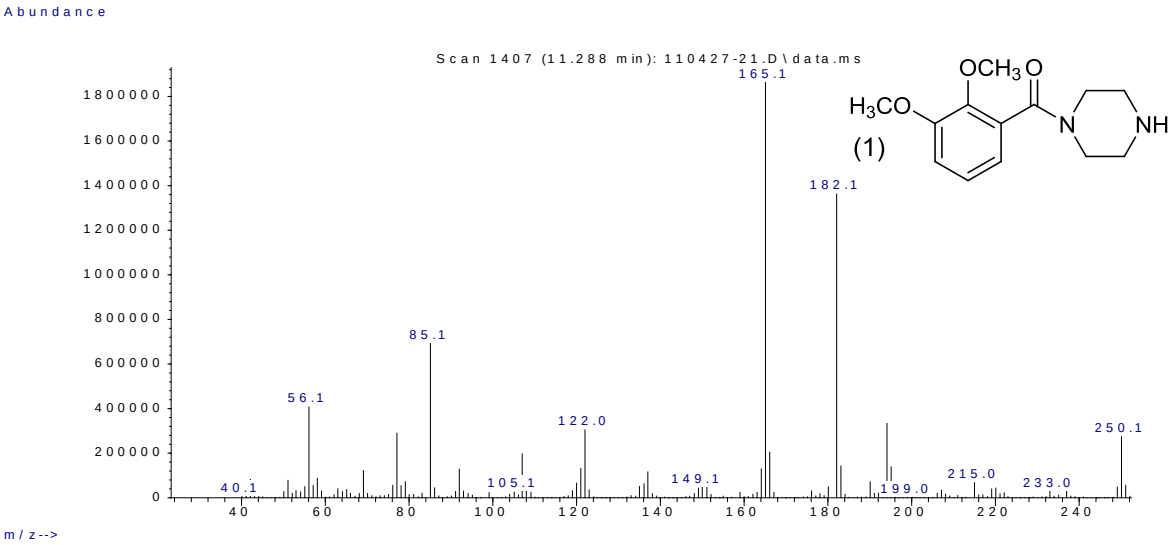
GC-MS and GC-IRD Studies on the Six Ring Regioisomeric Dimethoxybenzoylpiperazines (DMBzPs)

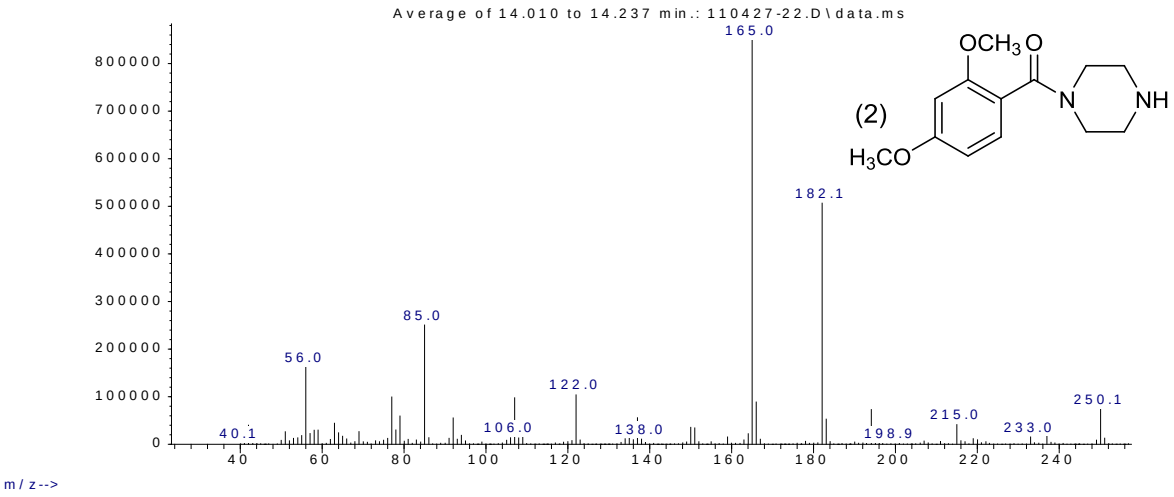
Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the differentiation between the regioisomeric dimethoxybenzoylpiperazines. These six regioisomeric substances are well resolved by GC and the vapor phase infrared spectra clearly differentiate among the six dimethoxybenzoyl substitution patterns. However, the mass spectra for these regioisomeric dimethoxybenzoylpiperazines are almost identical. Thus, gas chromatography with mass spectrometry detection (GC-MS) does not provide for the confirmation of identity of any one of the isomers to the exclusion of the other compounds. Perfluoroacyl derivatives of the six regioisomers were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation of structure.

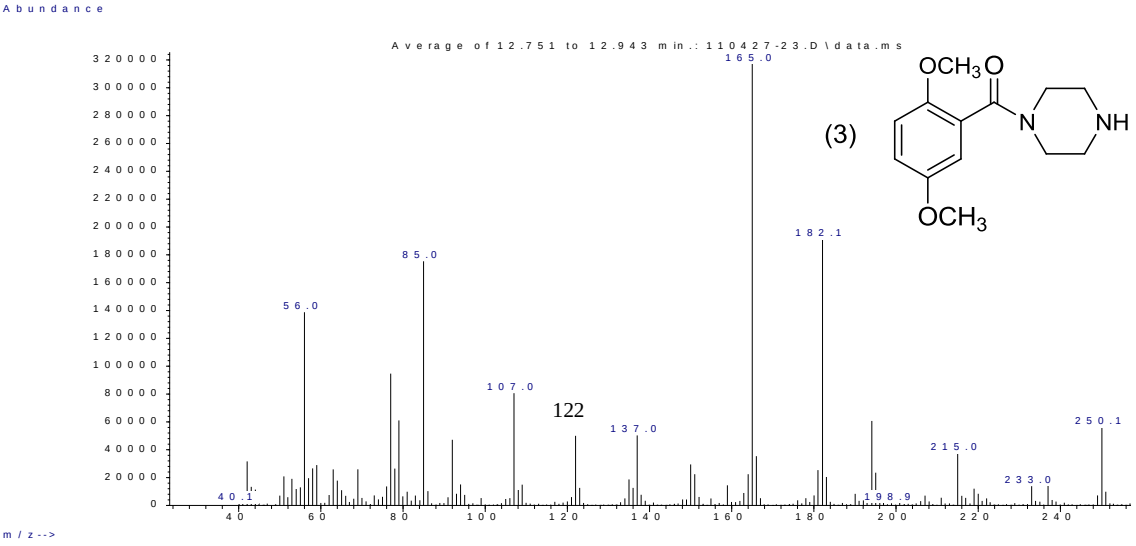
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Dimethoxybenzoylpiperazines (DMBzPs)

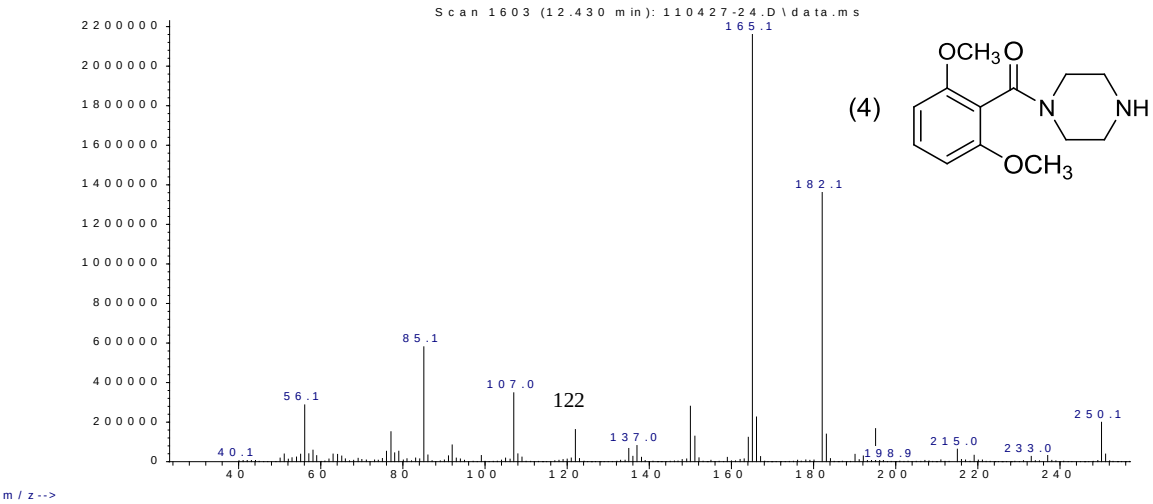
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 15-1 shows the EI mass spectra of the six regioisomeric dimethoxybenzoylpiperazines (Compounds 1-6). The mass spectra in Figure 15-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. These common fragment ions are from the regioisomeric dimethoxy group substitution on the aromatic ring as well as fragment ions generated by the piperazine ring. The base peak in all these mass spectra is the dimethoxy benzoyl cation occurring at m/z 165. The structures for the major fragment ions in these isomers are shown in Figure 15-2. The low mass ions at m/z 85, 69 and 56 come from fragmentation of the piperazine ring while the ions at m/z 122 and 137 represent fragments of the dimethoxybenzene portion of these isomeric [de Boer *et al*, 2001]. While the relative abundances are slightly different for the fragment ions in these compounds, mass spectrometry does not provide confirmation of identity for an individual DMBzP regioisomer based on unique ions present only for a single compound.

These benzoylpiperazines show a common ion for the protonated primary benzamide and this ion occurs at m/z 182 for these dimethoxybenzoylpiperazines. The proposed structure for the m/z 182 is shown in Figure 15-2. The m/z 182 ion involves the formation of the protonated primary amide, dimethoxybenzamide and this process has been described for the monomethoxybenzoylpiperazines in a previous report from our lab [Abdel-Hay *et al*, 2012]. The proposed structure for the m/z 182 ion is supported by the









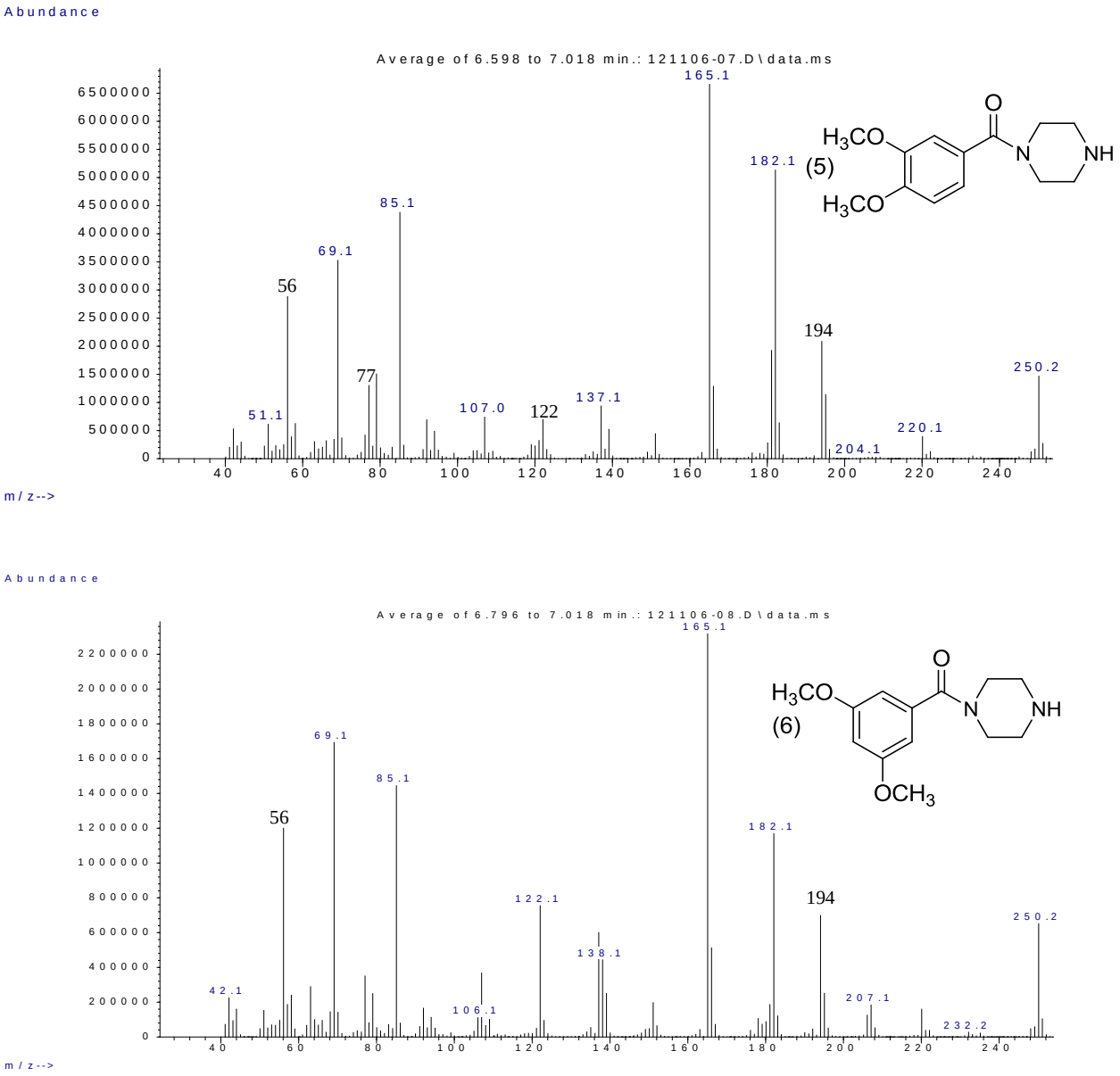


Fig. 15-1: Mass spectra of the underivatized six dimethoxybenzoylpiperazines.

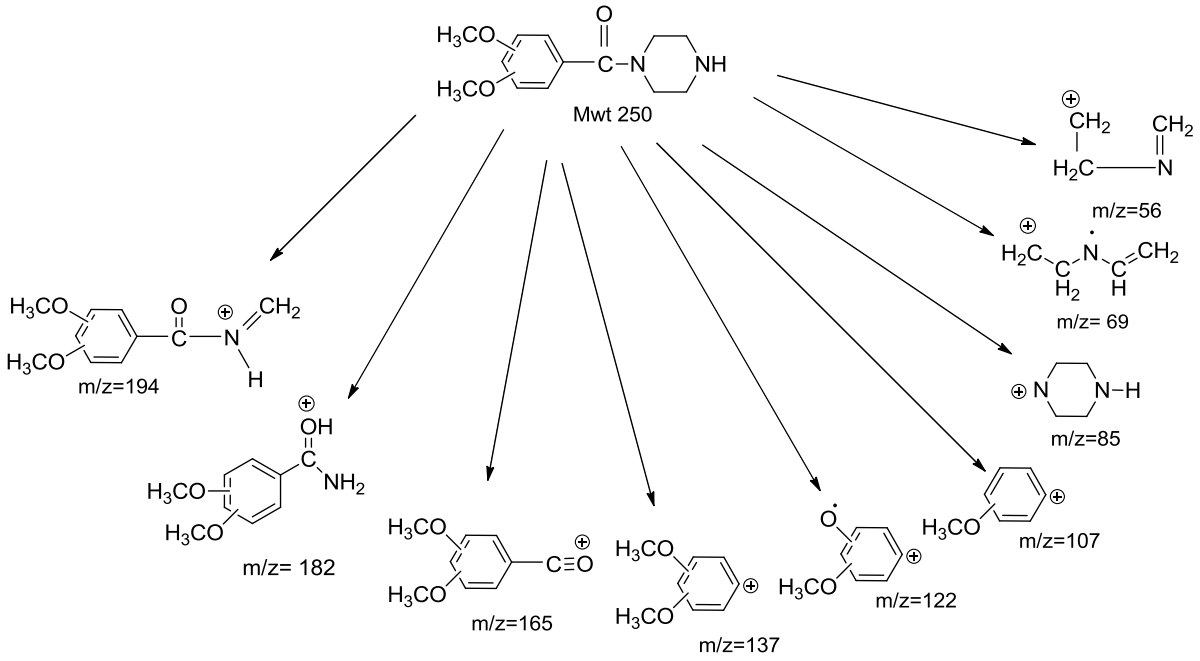


Fig. 15-2: Mass spectral fragmentation pattern of the underivatized dimethoxybenzoylpiperazines under EI (70eV) conditions.

mass spectrum of the corresponding octa-deutero labeled form of 3,4-dimethoxybenzoylpiperazine (3,4- dimethoxybenzoyl-d_8-piperazine). This octa-deuterium labeled compound was prepared by slowly adding 3,4-dimethoxybenzoyl chloride to a solution of d_8-piperazine in dichloromethane solvent cooled in an ice-bath. The mass spectrum for the deuterium labeled form of Compound 5 is shown in Figure 15-3. The mass spectrum in Figure 15-3 shows that two deuterium atoms are a part of the ion in question since the mass increased by 2 Da to m/z 184 in this case.

The mass spectrum in Figure 15-3 also supports the structural assignments for the low mass ions shown in Figure 15-2. The m/z 56 ion shifts to m/z 62 in the spectrum of the d_8-labelled derivative indicating six deuterium atoms remaining in the m/z 62 ion. Similarly the shift of m/z 69 to m/z 76 shows seven deuterium atoms in this ion and the m/z 85 to m/z 93 for all eight deuterium atoms in the intact piperazine cation. The ions at m/z 122, 137 and 165 show no mass shifts in the spectrum in Figure 15-3 as expected based on their formation pathways.

The other higher mass fragment in Figure 15-3 occurs at m/z 197 and this ion represents a mass shift of 3 Da from the m/z 194 ion for the unlabelled isomer. This mass data also supports the assigned structure for this ion shown in Figure 15-2. A similar structure was described by de Boer et al [de Boer *et al*, 2001] for the benzylpiperazines.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the six regioisomeric dimethoxybenzoylpiperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these isomeric compounds.

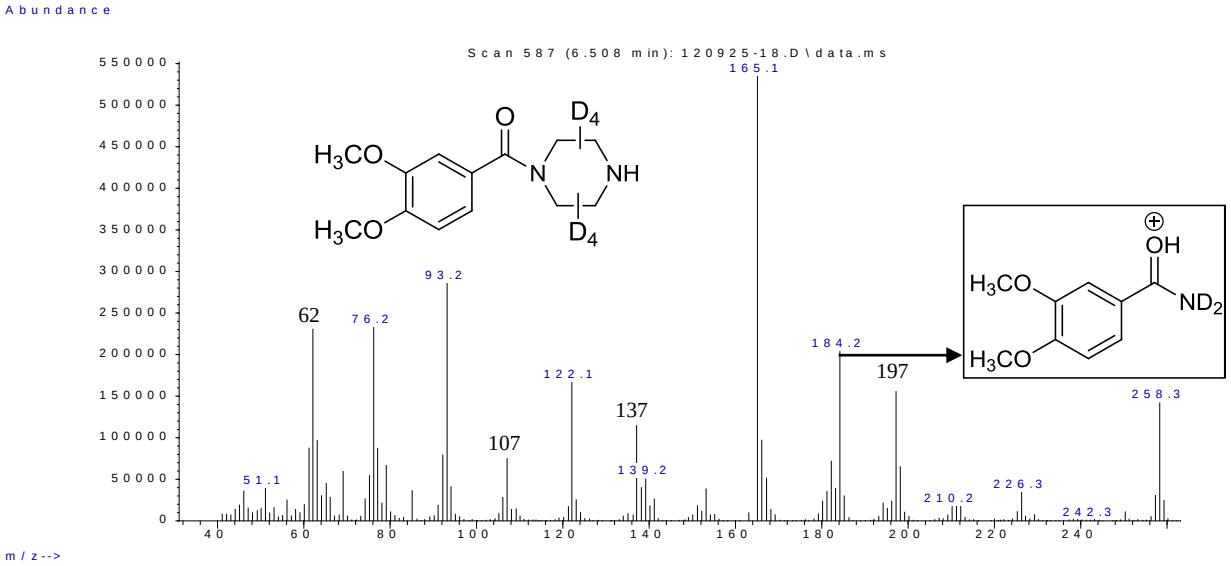
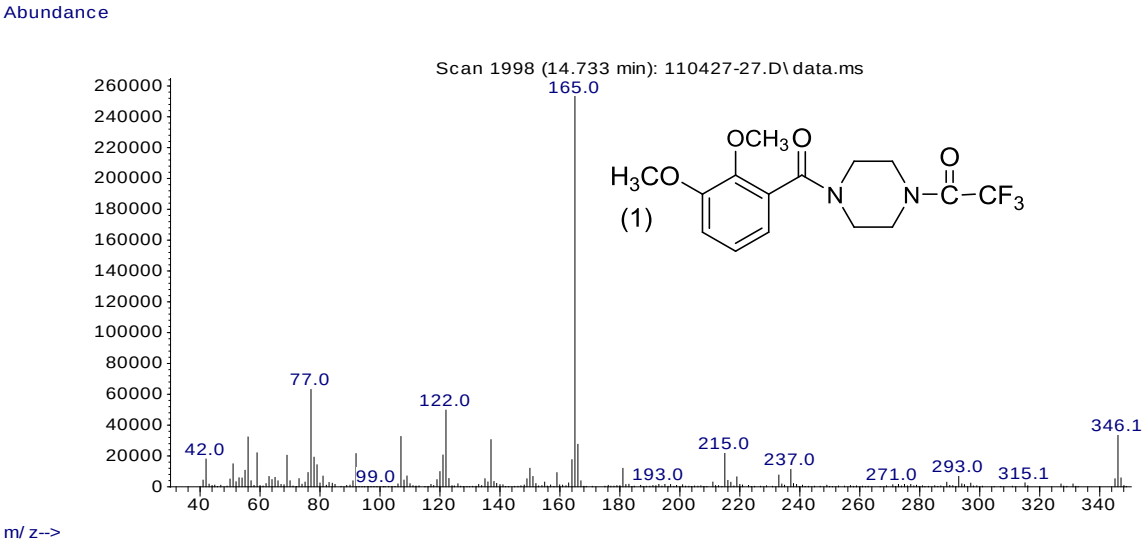
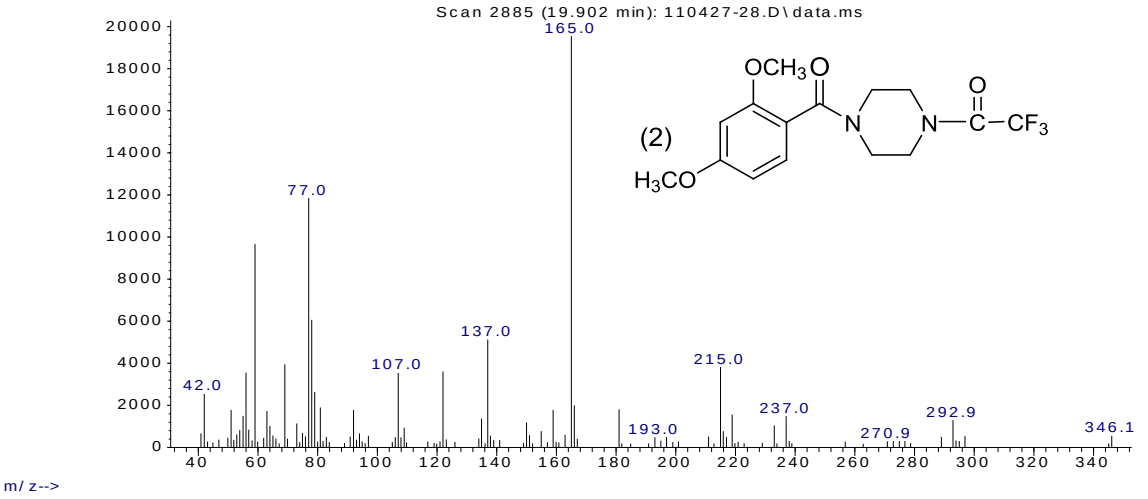
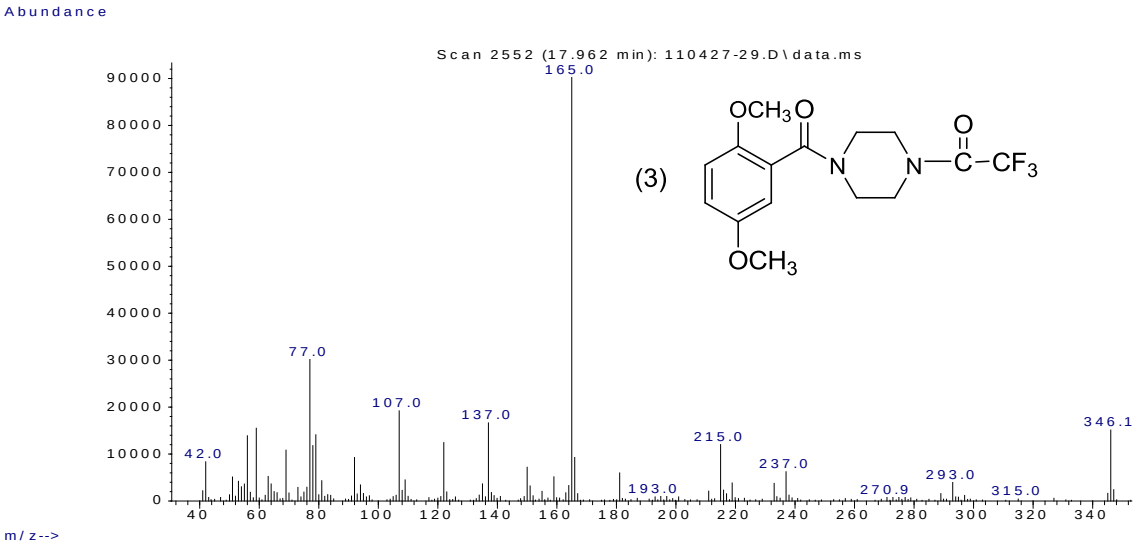


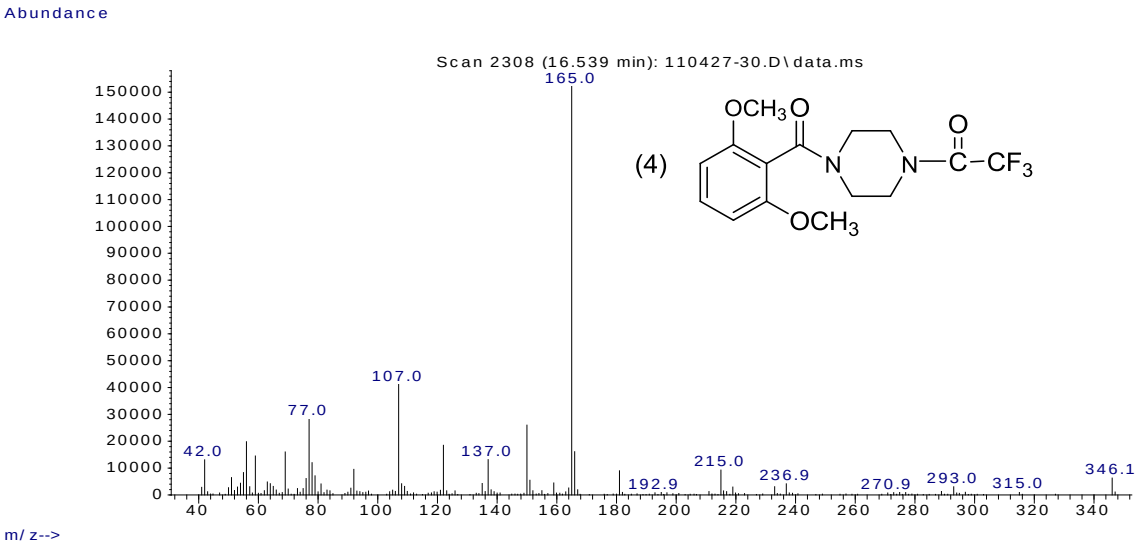
Fig. 15-3: Mass spectrum of the 3,4-dimethoxybenzoyl-d_8-piperazine

The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra of each regioisomer to the exclusion of the other regioisomeric compounds. The mass spectra for the six trifluoroacetyl amides are shown in Figure 15-4 as representatives of the mass spectra of all the perfluoroamides. From these spectra, a common peak with high relative abundance occurs at m/z 346, 396 and 446, which corresponds to the molecular ions for TFA, PFPA and HFBA amides, respectively. Those ions occurring at *m/ z* 69, 119 and 169 are formed as a result of the elimination of trifluoromethyl, pentafluoroethyl or heptafluoropropyl moiety from the TFA, PFPA and HFBA amides, respectively. There are no unique fragment ions providing significant difference between the MS spectra of the six compounds. Thus, even acylation of the six piperazines does not give characteristic fragments that help to discriminate among the six regioisomers.









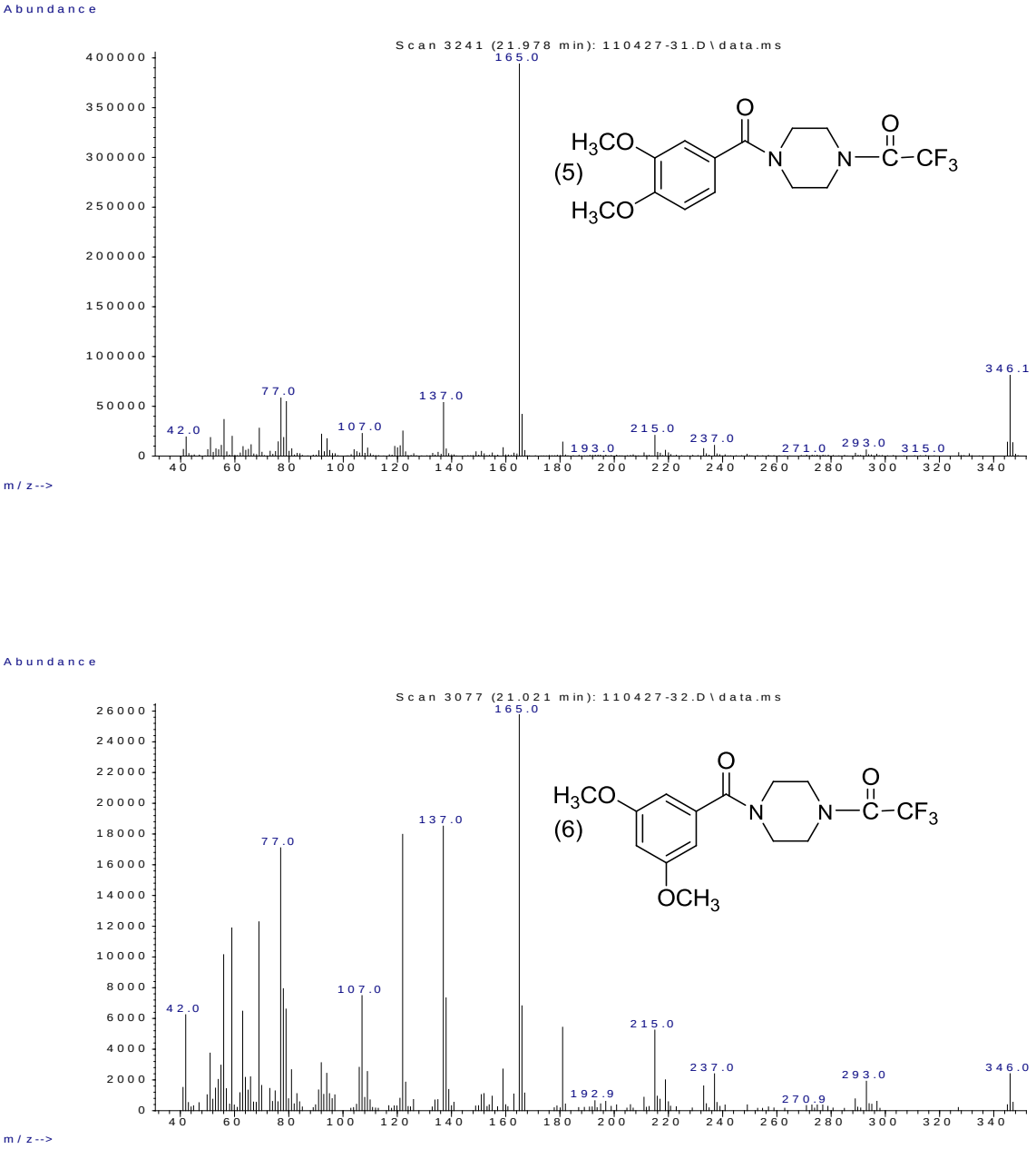
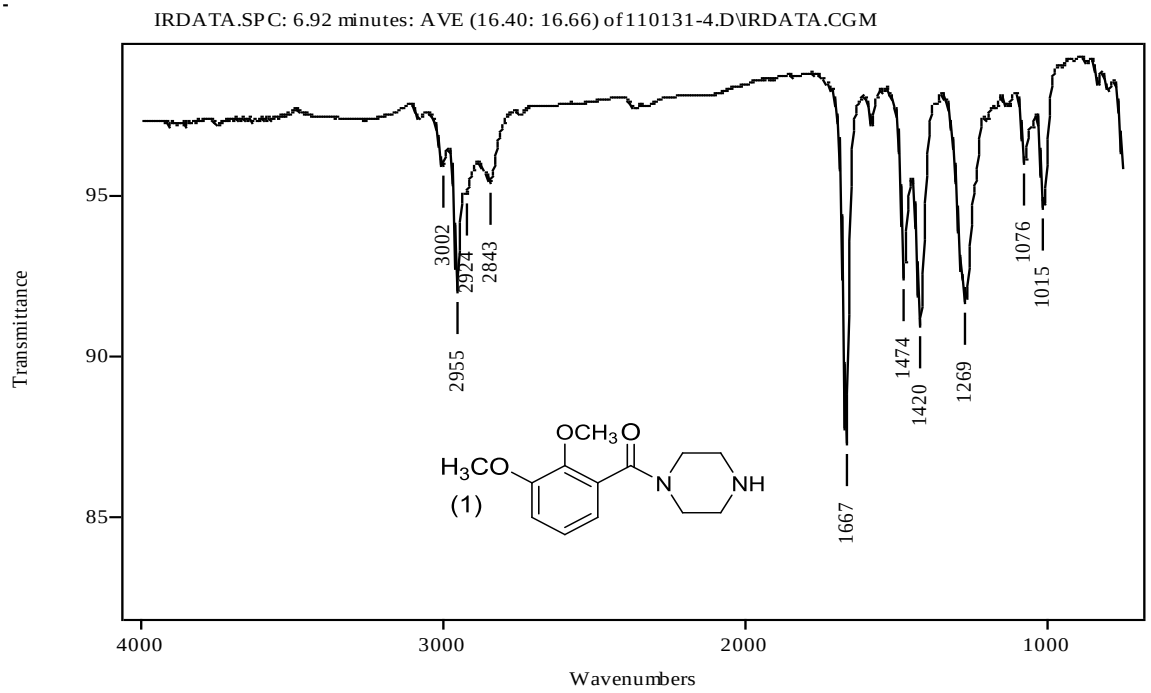


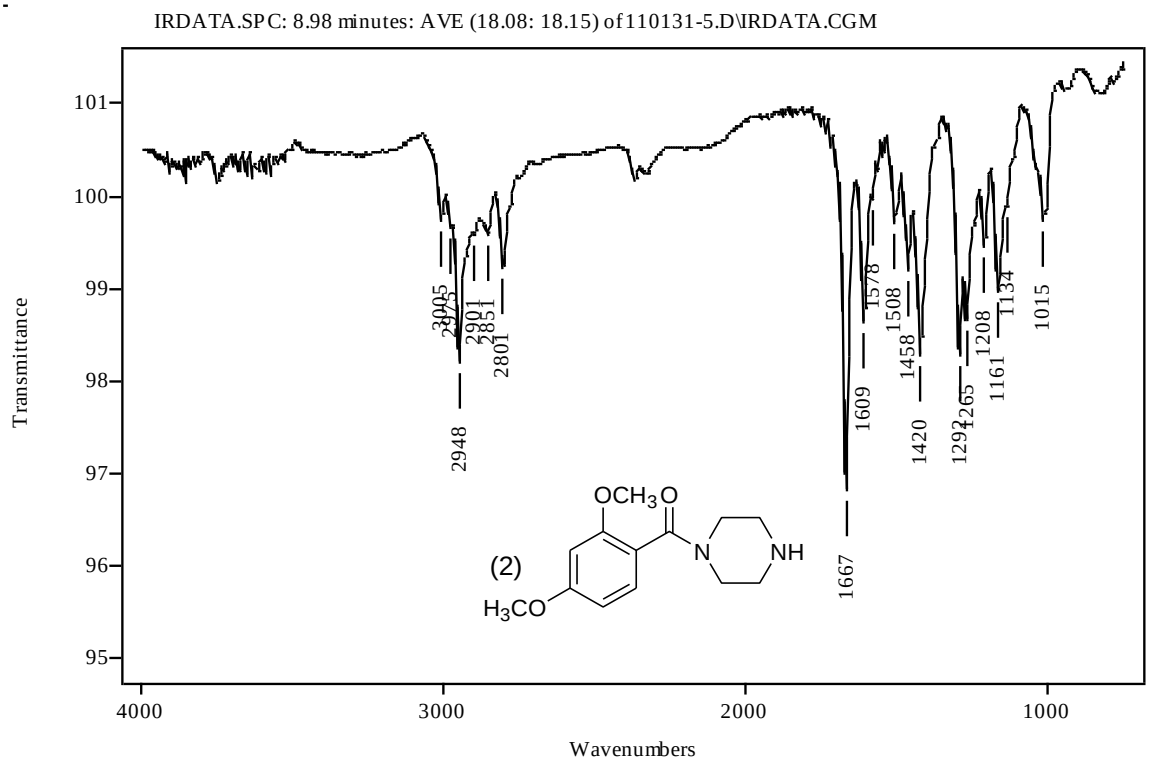
Fig. 15-4: Mass spectra of the trifluoroacetyl derivatives of the six piperazine compounds.

Vapor Phase Infrared Spectrophotometric Studies of the Dimethoxybenzoylpiperazines (DMBzPs)

Infrared spectrometry is often used as a confirmatory method for compound identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the six regioisomeric DMBzPs. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the six underivatized piperazines are shown in Figure 15-5. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph. Thus the sampling of a chromatographic peak assures a high level of compound purity for the IR spectrum. Each compound shows a vapor-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}. Because the six 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 IR peaks in the region of 750 – 1620 cm^{-1}.

The IR spectra of the six regioisomers share a common IR absorption band at 1667 cm^{-1} corresponding to the carbonyl group stretching of the tertiary amide. The 2,3-DMBzP regioisomer is characterized by the medium intensity band at 1269 cm^{-1} which is split into doublet peaks of medium and equal intensity at 1292 and 1265 cm^{-1} in the 2,4-DMBzP regioisomer. This isomer also has another medium intensity band at 1420 cm^{-1} shifted to a strong singlet at 1427 cm^{-1} in the IR spectrum of the 2,5 isomer. The 3,5-





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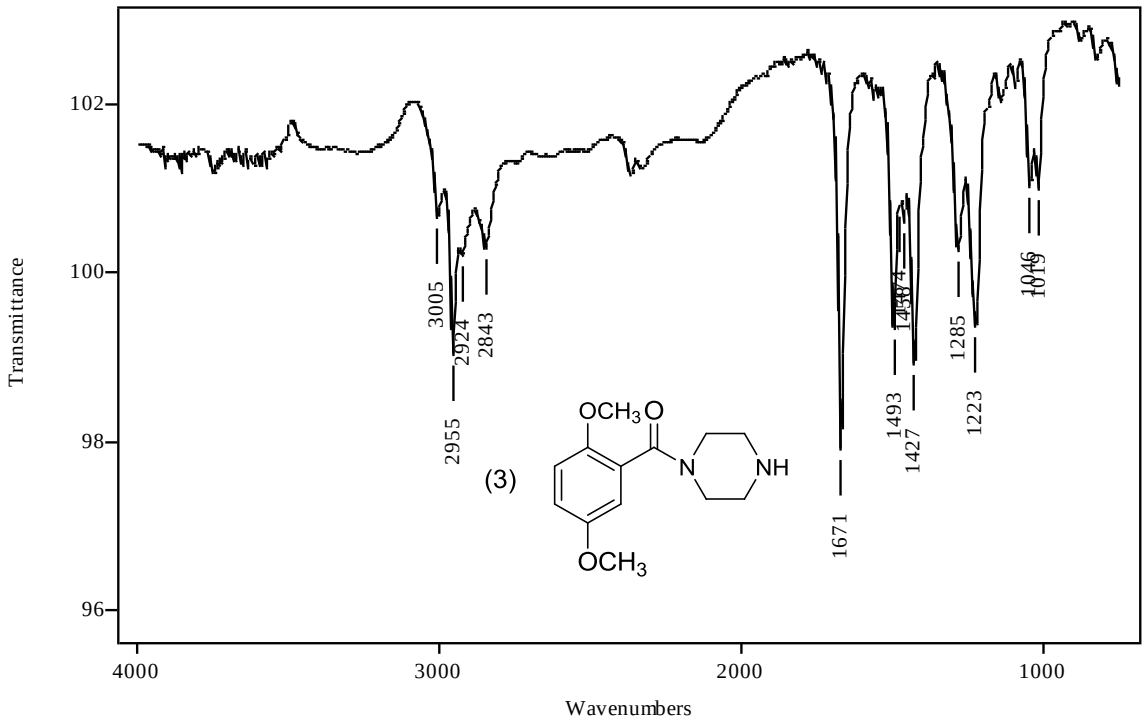

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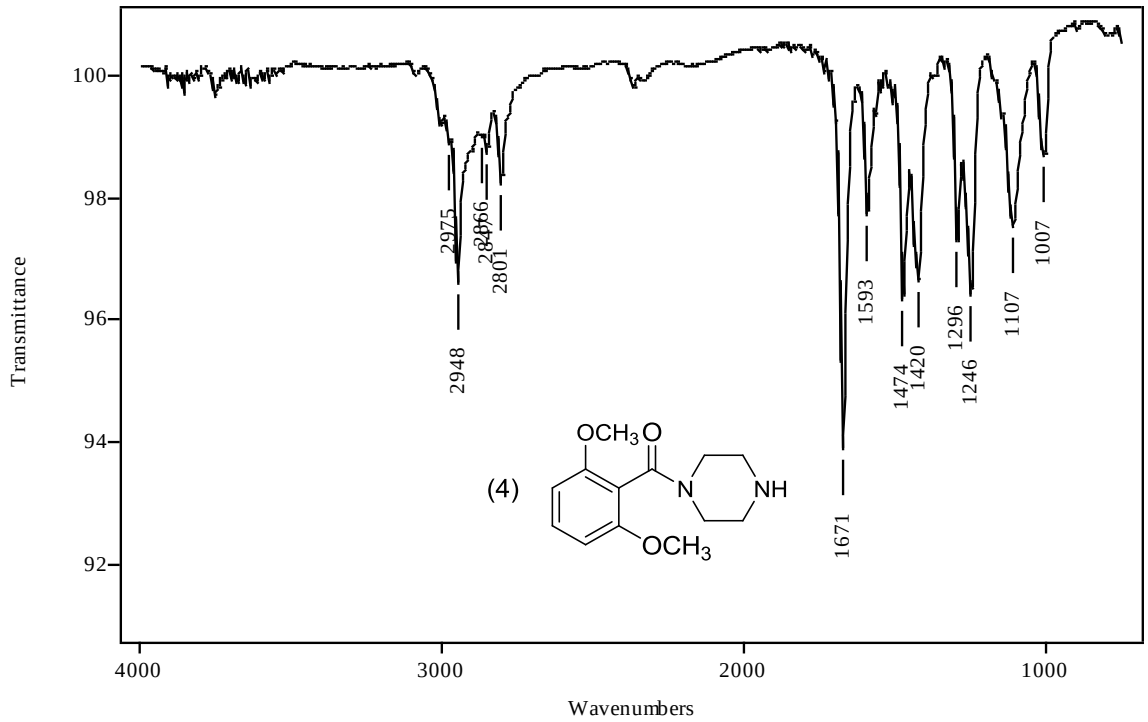

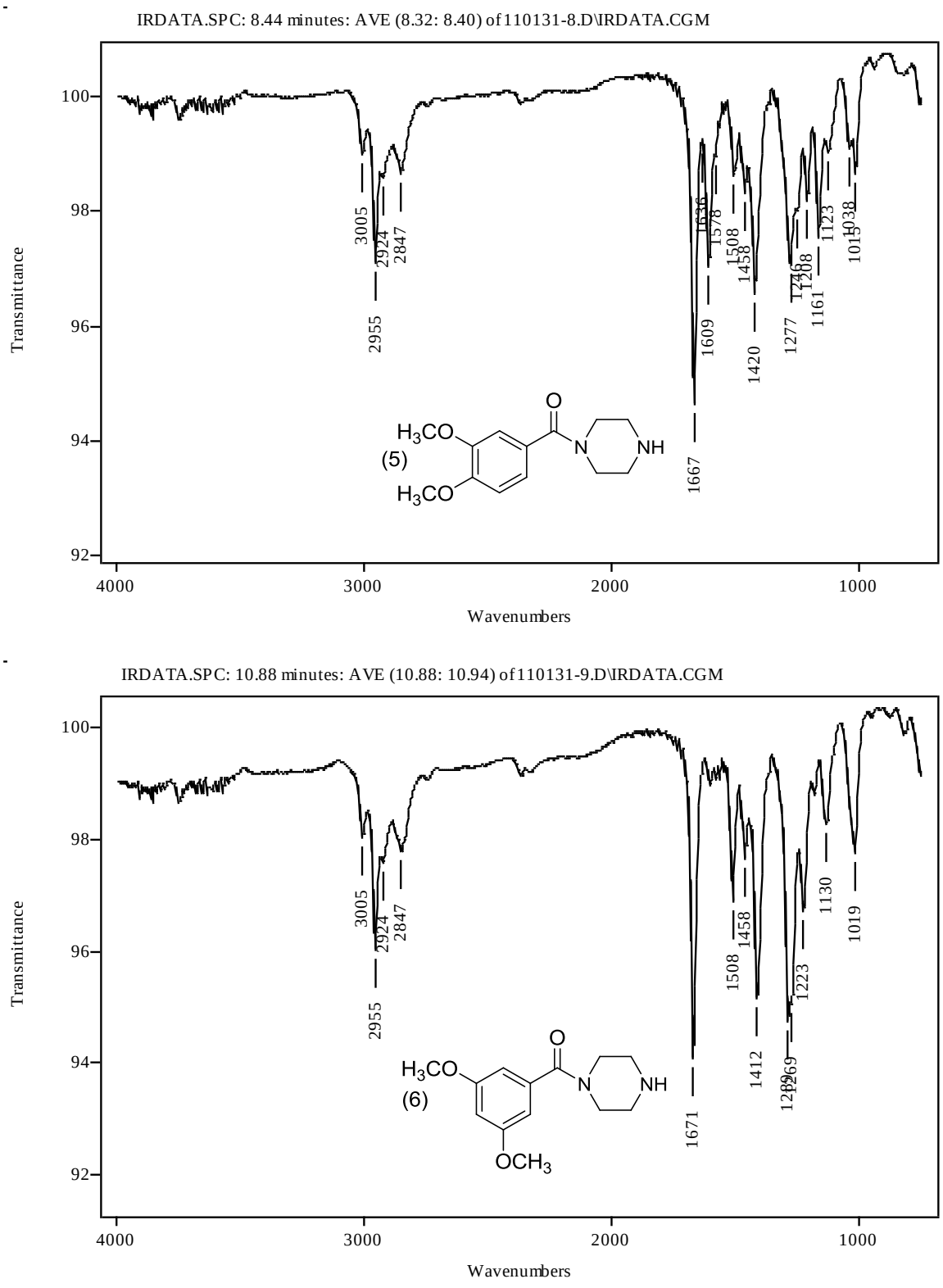

Fig. 15-5: Vapor phase IR spectra of the six dimethoxybenzoyl piperazines.

DMBzP regioisomer can be distinguished by the relatively strong IR band at 1289 cm^{-1} which is shifted to a medium intensity peak at 1277 cm^{-1} in the 3,4-regioisomer, a strong intensity peak at 1223 cm^{-1} in the 2,5-regioisomer and a medium intensity doublet at 1296 and 1248 cm^{-1} in the 2,6-regioisomer The vapor-phase IR spectrum of the 2,6-DMBzP regioisomer can be distinguished by a singlet of medium intensity appearing at 1107 cm^{-1} compared to a doublet of weak intensity at 1046, 1019 cm^{-1} in the 2,5-isomer, a weak singlet at 1019 cm^{-1} in both the 3,4 and 3,5 isomers.

These results show that vapor phase 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 spectral data provide distinguishing and characteristic information to individualize the regioisomers in this set of uniquely similar compounds. Thus, GC-IRD readily discriminates between the members of this set of regioisomeric dimethoxybenzoylpiperazine compounds.

Gas Chromatographic Separation of the Dimethoxybenzoylpiperazines (DMBzPs)

Gas chromatographic separation of the derivatized piperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-μm film thickness. Several temperature programs were evaluated and the representative chromatogram in Figure 15-6 shows the separation of the PFPA derivatives of the dimethoxybenzoylpiperazines. The separation of the pentafluoropropionyl derivatives was performed using a temperature program consisting of an initial hold at 70°C for 1.0 min, ramped up to 250°C at a rate of 30°C/min then held at 250°C for 20 min.

The elution order appears related to the degree of substituent crowding on the aromatic ring. Compounds 1 and 4 elute first and these two isomers contain substituents arranged in a 1,2,3-pattern on the aromatic ring. Three isomers (Compounds 2, 3 and 5) have two groups substituted 1,2 with one isolated substituent. The 1,3,5-trisubstituted pattern in Compound 6 provides minimum intramolecular crowding and elutes last in this group of compounds. The elution order was the same for the underivatized and all derivatized dimethoxybenzoylpiperazines evaluated in this project.

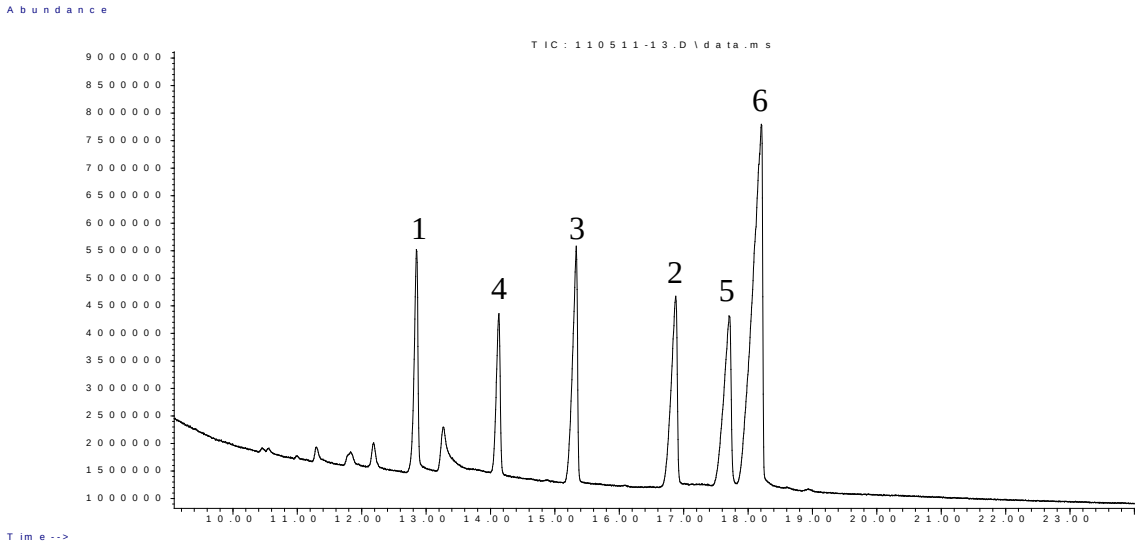

Fig. 15-6: Gas chromatographic separation of the pentafluoropropionyl derivatives using Rtx-200 column.

Conclusion

The six regioisomeric dimethoxybenzoylpiperazines yield the same fragment ions in their mass spectra even after perfluoroacylation. GC-IRD analysis yields unique and characteristic vapor phase infrared spectra for these six regioisomeric piperazines. These spectra allow discrimination among the six regioisomeric compounds included in this study. This differentiation was accomplished without the need for chemical derivatization. Mixtures of the six piperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions.

References

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Abdel-Hay, K. M., DeRuiter, J., Clark, C.R. Differentiation of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs) By GC-IRD and GC-MS, Drug Testing and Analysis. 4(6) (2012) 430-440.

Chapter 16

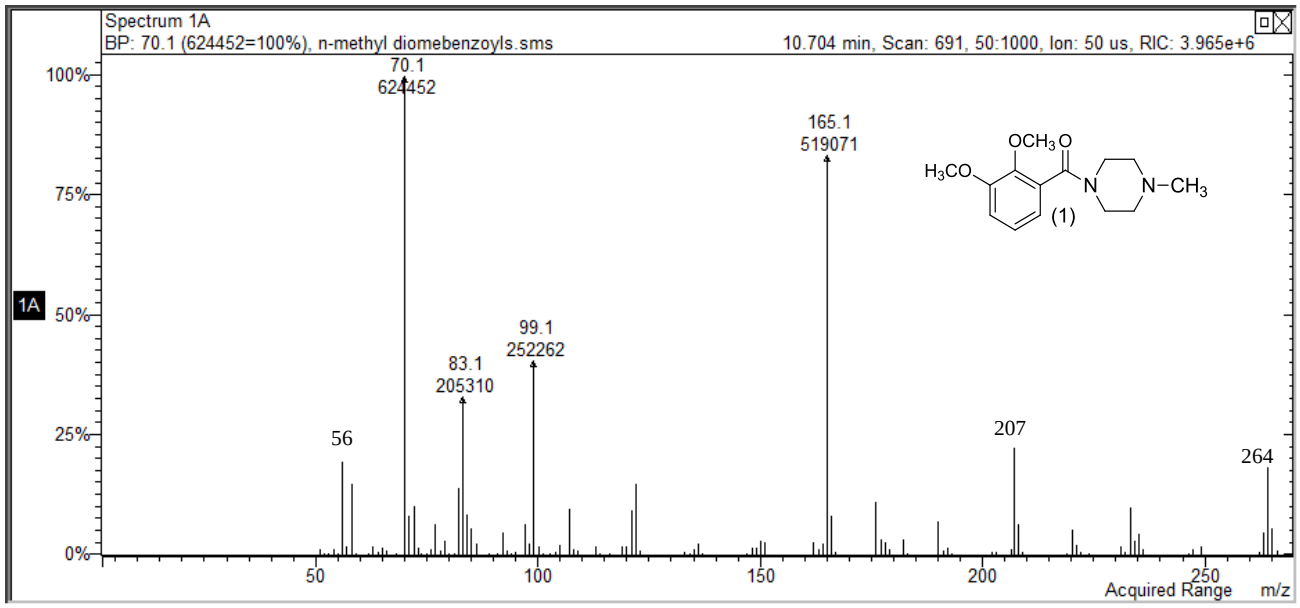
GC-MS and FTIR Analysis of the Six Ring Regioisomeric Dimethoxybenzoyl-N-methylpiperazines (DMBzMPs)

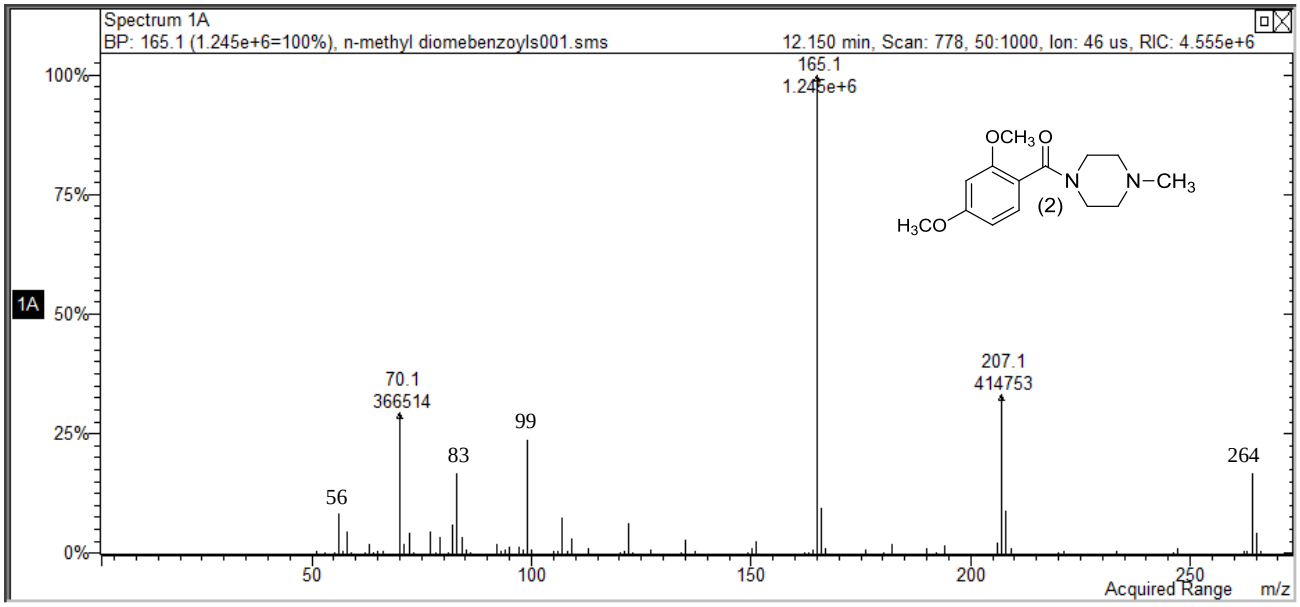
The complete series of regioisomeric dimethoxybenzoyl-N-methylpiperazines were synthesized and evaluated in GC-MS and FTIR studies. The EI mass spectra show fragment ions characteristic of both the dimethoxybenzoyl and the N-methylpiperazine portions of the molecules. These characteristic fragments include the dimethoxybenzoyl cation at m/z 165 as well as the m/z 99 N-methylpiperazine cation and the low mass ion at m/z 56 for the $C_3H_6N^+$ seen in almost all piperazine EI spectra. Attenuated total reflection infrared spectroscopy provides direct confirmatory data for the differentiation between the six regioisomeric aromatic ring substituted dimethoxybenzoyl-N-methylpiperazines. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and retention appears related to the degree of steric crowding of the aromatic ring substituents. The most crowded patterns of substitution elute first while the more symmetrical 1,3,5-substitution pattern has the highest retention time.

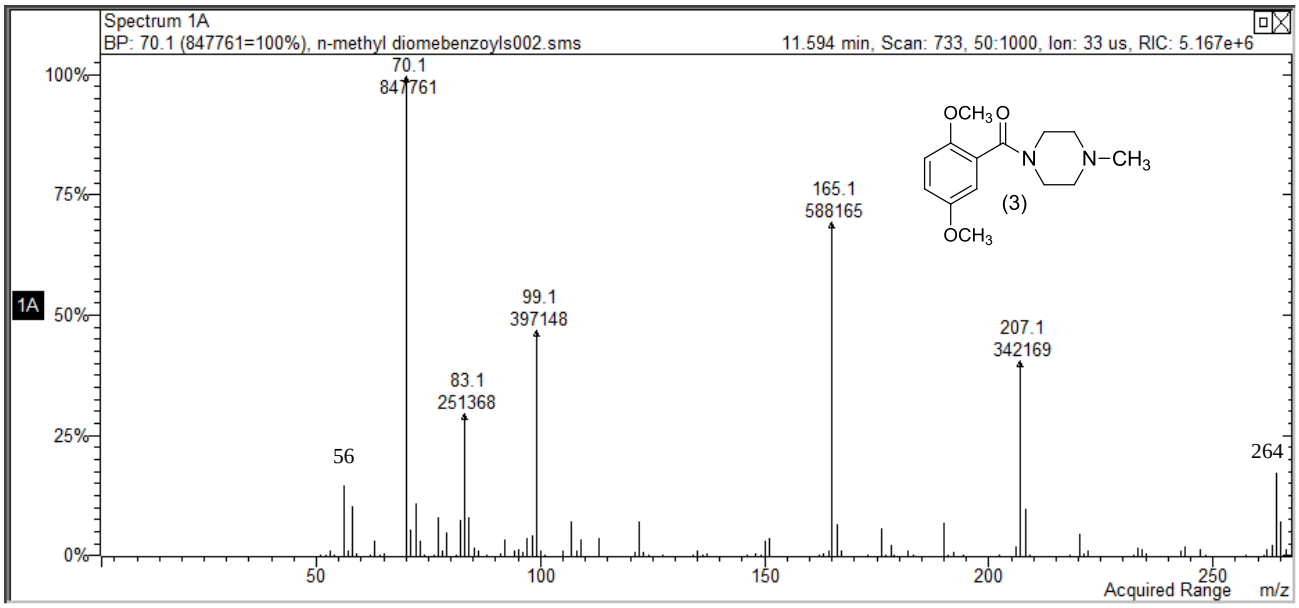
Mass spectral studies of the Dimethoxybenzoyl-N-methylpiperazines (DMBzMPs)

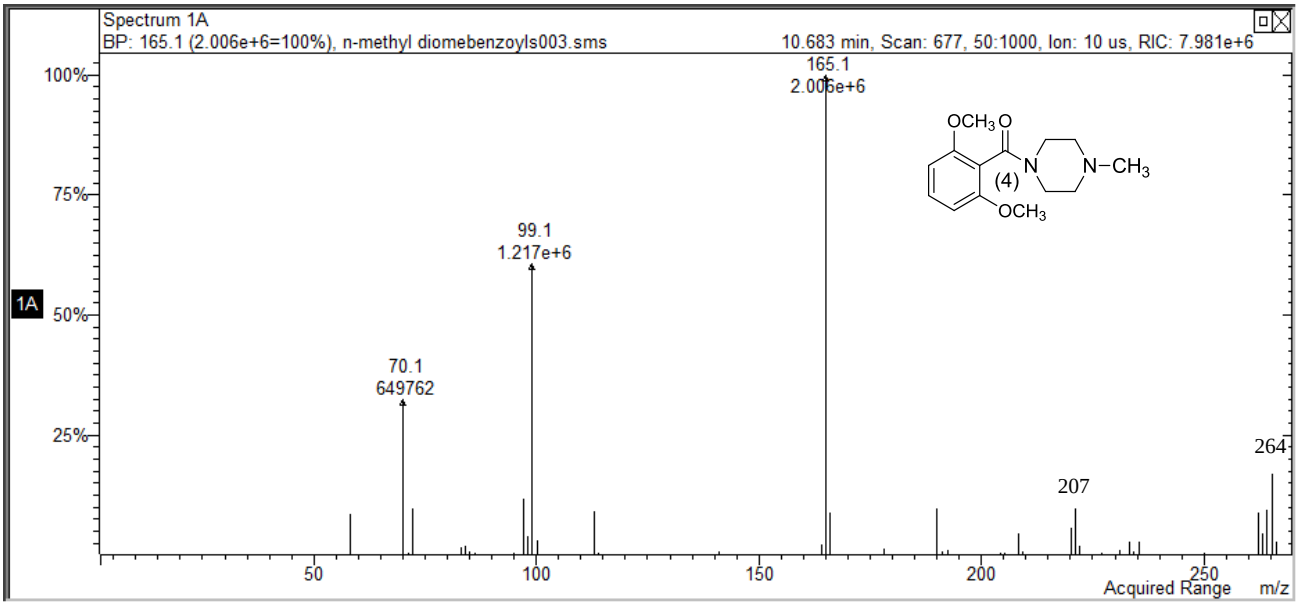
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 16-1 shows the EI mass spectra of the six regioisomeric dimethoxybenzoyl-N-methylpiperazines (Compounds 1-6) in this study. All six regioisomers show molecular ions at m/z 264 and these ions are of significant relative intensity. Major fragment ions of equivalent mass occur in the spectra for these six compounds with only the relative intensity of the individual ions as the major spectral variation. Fragmentation of the bond between the benzoylic carbon and the adjacent piperazine nitrogen provides the base peak in all six spectra. The dimethoxybenzoyl cation at m/z 165 is the base peak for compounds 2, 3, 4, and 5. Fragmentation of the same bond between the benzoylic carbon and the adjacent piperazine nitrogen with charge retention on the N-methylpiperazine group yields the cation at m/z 99. The structures for the base peaks in the mass spectra for these regioisomeric dimethoxybenzoyl-N-methylpiperazines are shown in Figure 16-2.

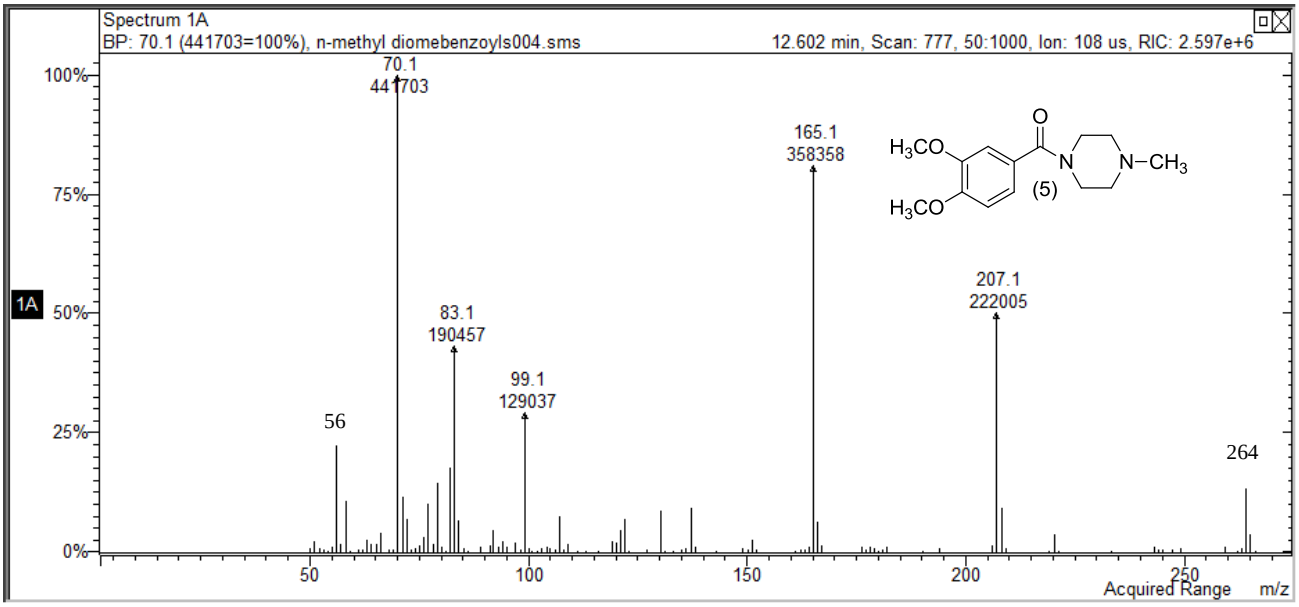
The internal fragmentation within the piperazine ring produces a number of unique ions in the mass spectra of these dimethoxybenzoyl-N-methylpiperazines. The low mass ion of highest relative abundance in these spectra is the m/z 56 cation. This m/z 56 ion was shown by exact mass measurements to have an elemental composition $C_3H_6N^+$ in previous studies [Abdel-Hay *et al*, 2013]. Furthermore this ion yielded a mass shift of +6 Da











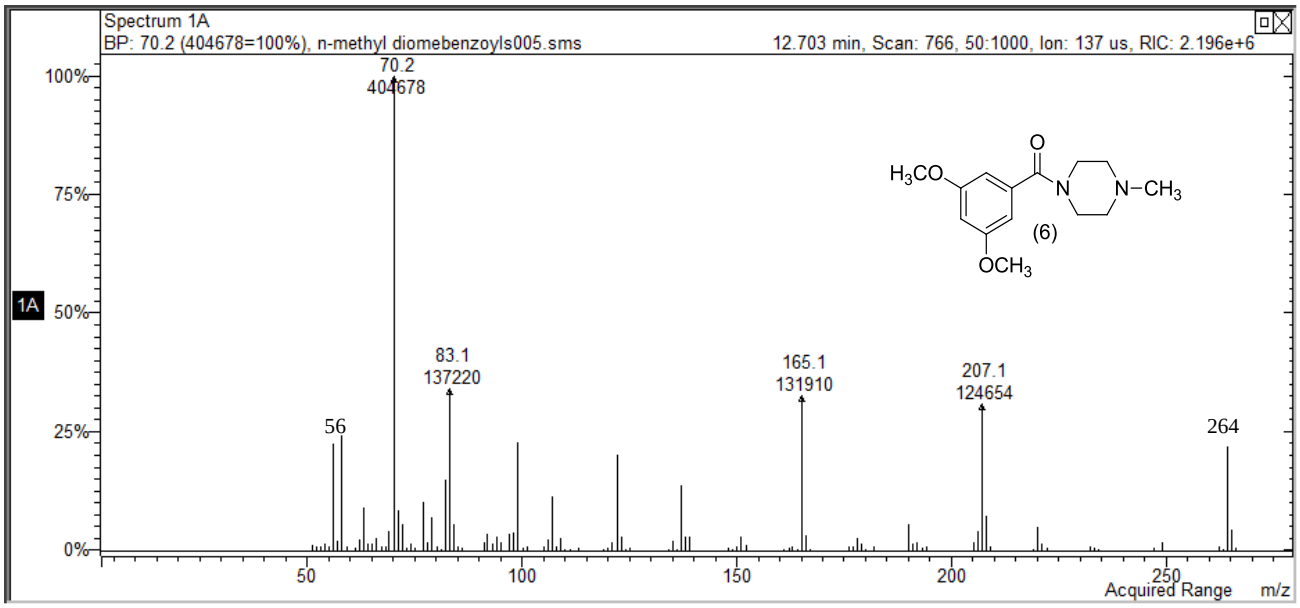


Fig. 16-1: Mass Spectra for the six regioisomeric dimethoxybenzoyl-N-methylpiperazines.

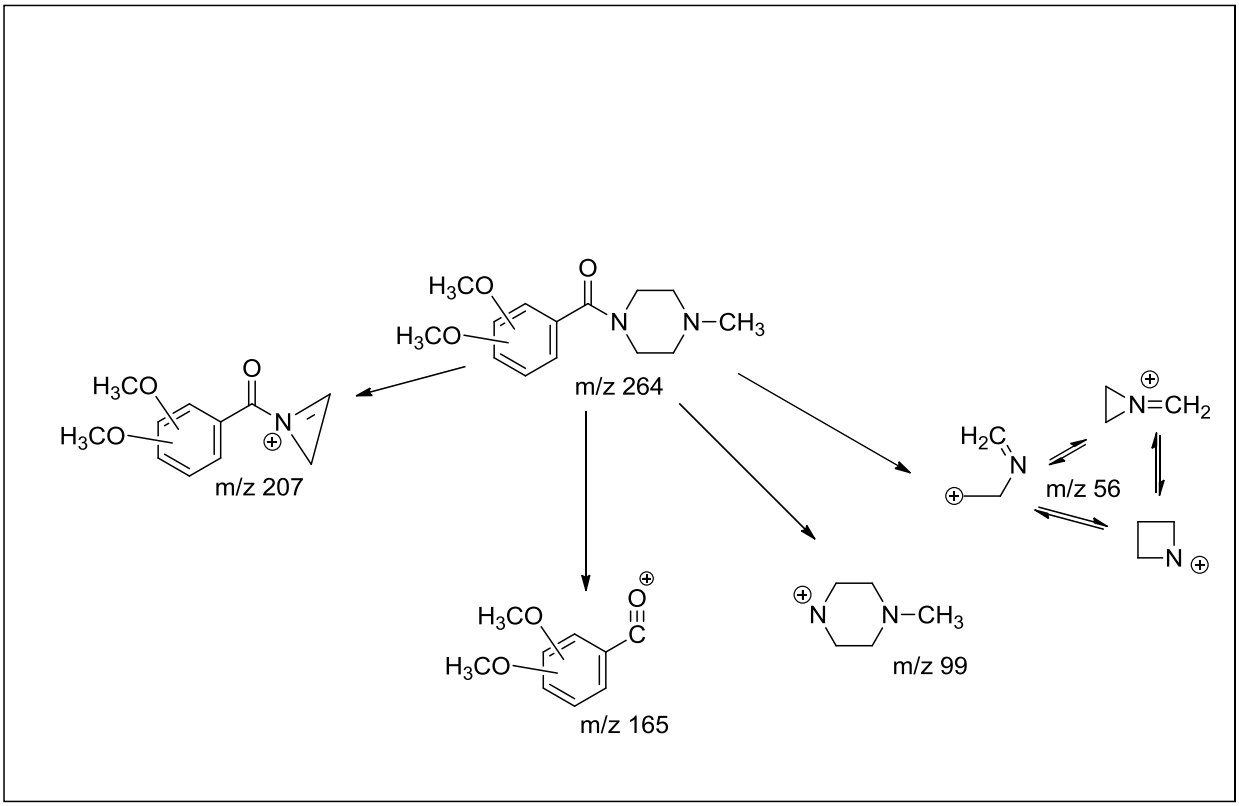


Fig. 16-2: Mass spectral fragmentation for the six regioisomeric dimethoxybenzoyl-N-methylpiperazines.

when d_8-piperazine was used to label all the four carbons of the piperazine ring. Thus, the m/z 56 ion was confirmed to contain six hydrogen atoms and three of the carbons from the original piperazine portion of these molecules, this confirmation was based on studies in the dimethoxybenzylpiperazines (N-4=H). The formation of the m/z 56 ion in the previous series of dimethoxybenzylpiperazine (N-4=H) regioisomers involved an initial migration of the hydrogen on nitrogen at N-4 to the tertiary N-1 nitrogen followed by a radical site alpha cleavage initiated by the N-4 nitrogen to break the carbon-carbon bond of the piperazine ring. The last step in the formation of the m/z 56 ion is the heterolytic breaking of the N-1 to carbon bond to yield the $C_3H_6N^+$ cation. The equivalent mechanistic pathway is illustrated for the N-4=CH_3 compounds in this study in Figure 16-3.

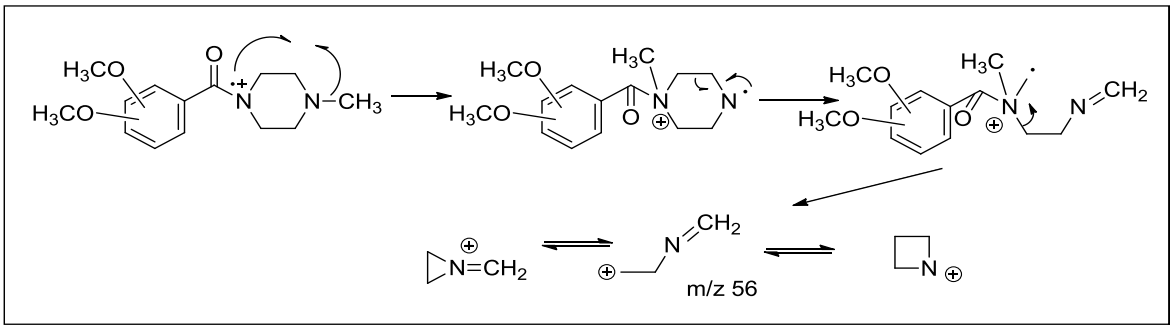


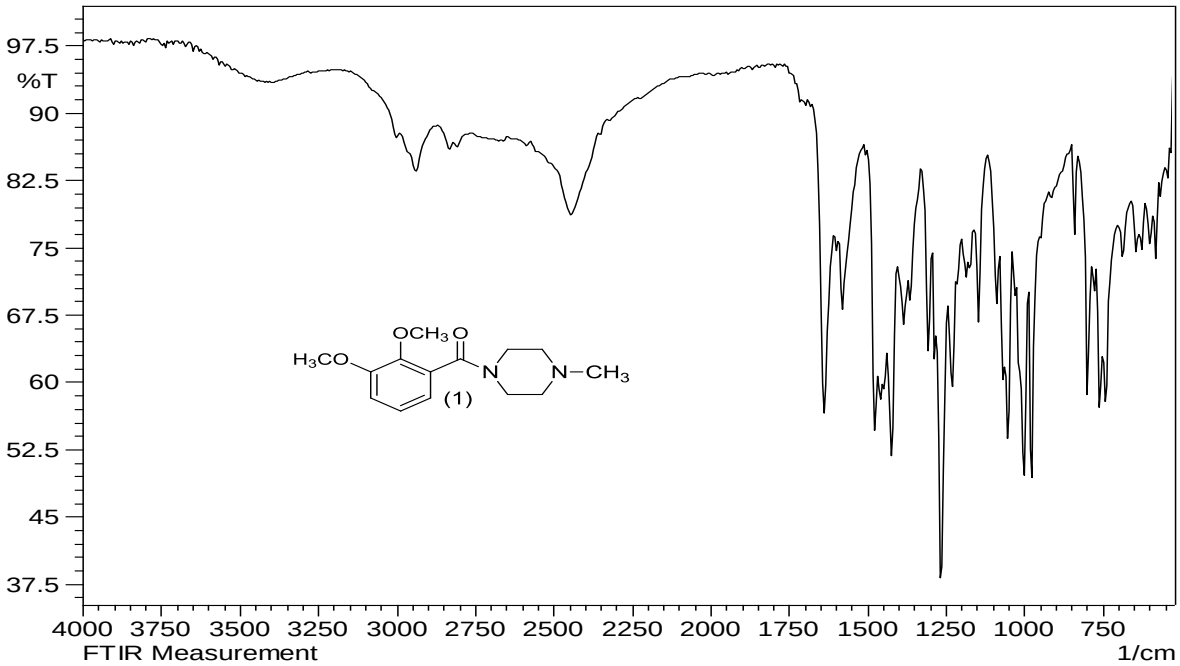
Fig. 16-3. Mechanism for the formation of the m/z 56 cation in the six regioisomeric dimethoxybenzoyl-N-methylpiperazines.

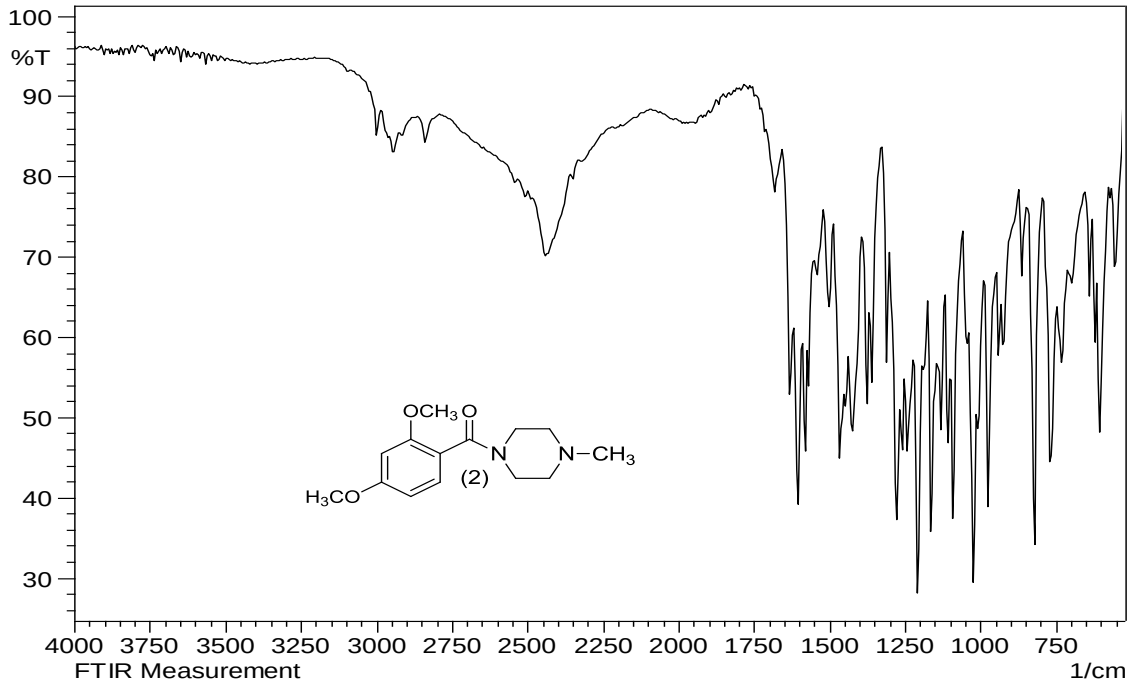
FTIR Spectroscopic Study of the Dimethoxybenzoyl-N-methylpiperazines (DMBzMPs)

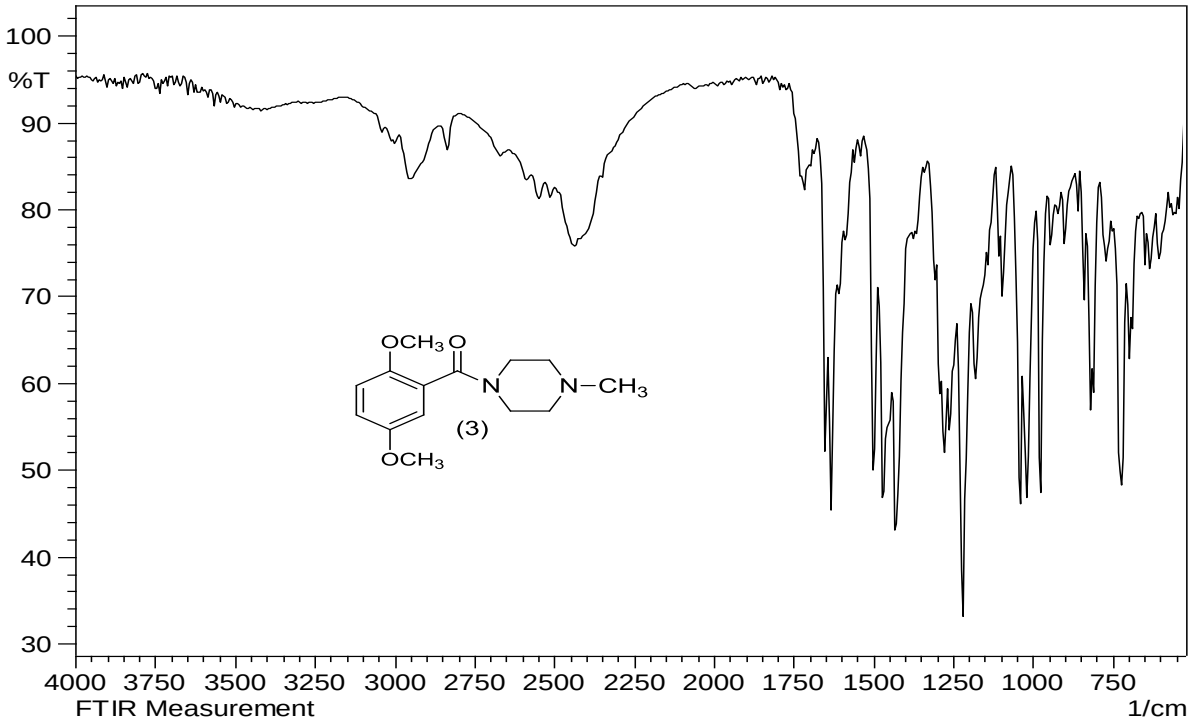
Attenuated total reflection fourier transform infrared spectroscopy (IR) was evaluated for differentiation among the six regioisomeric DMBzMPs. This method has the possibility of yielding compound specificity without the need for chemical modification of the drug molecule. The IRs for the six underivatized piperazines are shown in Figure 16-4. The IR spectra of the six regioisomers share a common IR absorption band at 1667 cm^{-1} corresponding to the carbonyl group stretching of the tertiary amide. The 2,3-DMBzMP regioisomer is characterized by the medium intensity band at 1265 cm^{-1} which is split into doublet peaks of medium and equal intensity at 1278 and 1259 cm^{-1} in the 2,4-DMBzMP regioisomer. This isomer also has another medium intensity band at 1420 cm^{-1} shifted to a strong singlet at 1431 cm^{-1} in the IR spectrum of the 2,5 isomer. The 3,5-DMBzMP regioisomer can be distinguished by the relatively strong IR band at 1292 cm^{-1} which is shifted to a medium intensity peak at 1277 cm^{-1} in the 3,4-regioisomer, a strong intensity peak at 1223 cm^{-1} in the 2,5-regioisomer and a medium intensity doublet at 1296 and 1248 cm^{-1} in the 2,6-regioisomer The FTIR spectrum of the 2,6-DMBzMP regioisomer can be distinguished by a singlet of medium intensity appearing at 1105 cm^{-1} compared to a doublet of weak intensity at 1046, 1019 cm^{-1} in the 2,5-isomer, a weak singlet at 1019 cm^{-1} in both the 3,4 and 3,5 isomers.

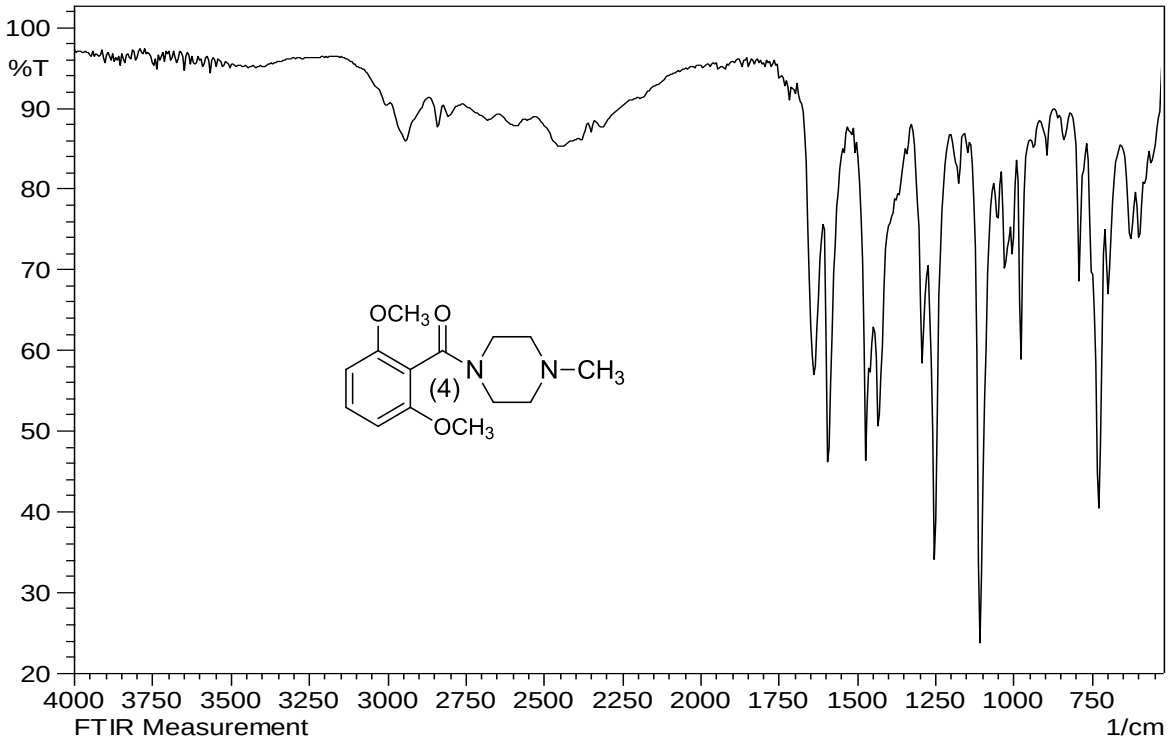
These results show that FTIR spectra provide useful data for differentiation among these regioisomeric piperazines of mass spectral equivalence. Mass spectrometry establishes these compounds as having an isomeric relationship of equal molecular weight and equivalent major fragment ions. Infrared absorption spectral data provide

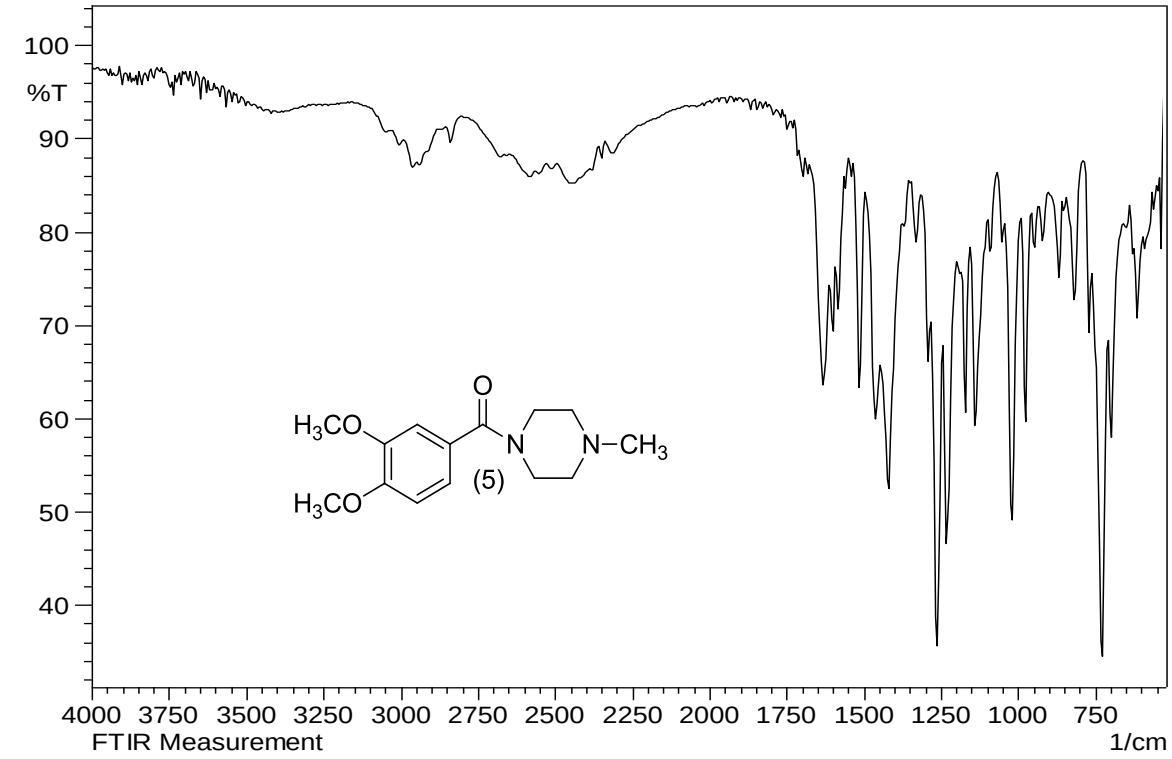
distinguishing and characteristic information to individualize the regioisomers in this set of uniquely similar compounds.











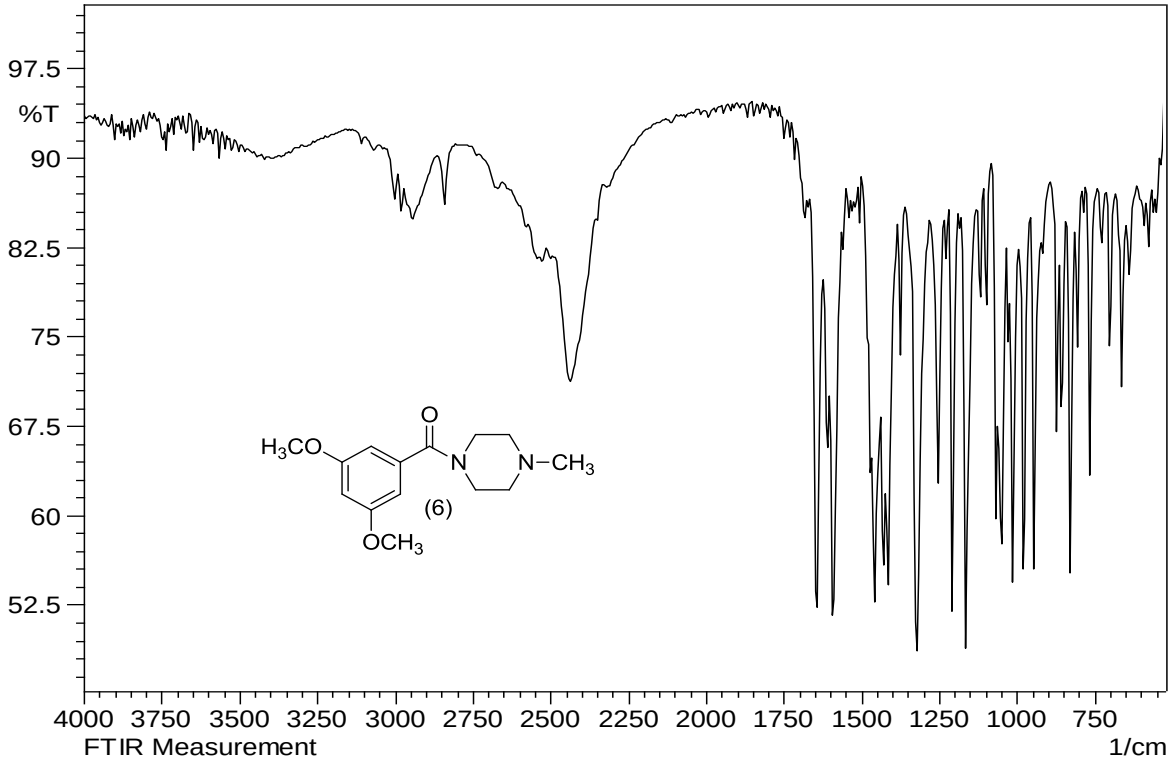


Fig. 16-4: ATR FTIR spectra of the six DMBzMPs.

Gas Chromatographic Separation of the Dimethoxybenzoyl-N-methylpiperazines (DMBzMPs)

Gas chromatographic separation of the dimethoxybenzoyl-N-methylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 16-5. The separations of the six regioisomers 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 25.0 min. This chromatogram shows the separation of the six dimethoxybenzoyl-N-methylpiperazine regioisomers in this study. The elution order appears related to the degree of substituent crowding on the aromatic ring. Compounds 1 and 4 elute first and these two isomers contain substituents arranged in a 1,2,3-pattern on the aromatic ring. Three isomers (Compounds 2, 3 and 5) have two groups substituted 1,2 with one isolated substituent. The 1,3,5-trisubstituted pattern in Compound 6 provides minimum intramolecular crowding and elutes last in this group of compounds. The two compounds with maximum crowding substituted in a 1,2,3 manner on the aromatic ring show the 2,3-dimethoxy substitution pattern to elute first followed by the 2,6-dimethoxy isomer eluting second. The relative position of the methoxy groups appears to determine the elution order in the three compounds having two groups substituted in a 1,2 pattern. Within this group of three compounds the first to elute is the 1,4-relationship for the two methoxy groups in

compound 3. This is followed by the 1,3-pattern for compound 2 and lastly the 1,2-pattern for compound 5.

In previous studies on the chromatographic properties of the monosubstituted piperazines (N4=H), these secondary amines showed severe chromatographic tailing on a number of stationary phases. This issue was overcome by acylation of the secondary amine nitrogen with perfluoroacyl groups such as the pentafluoropropionyl group and others. Adequate peak shape and resolution were obtained for these tertiary amines (compounds 1-6) in this study.

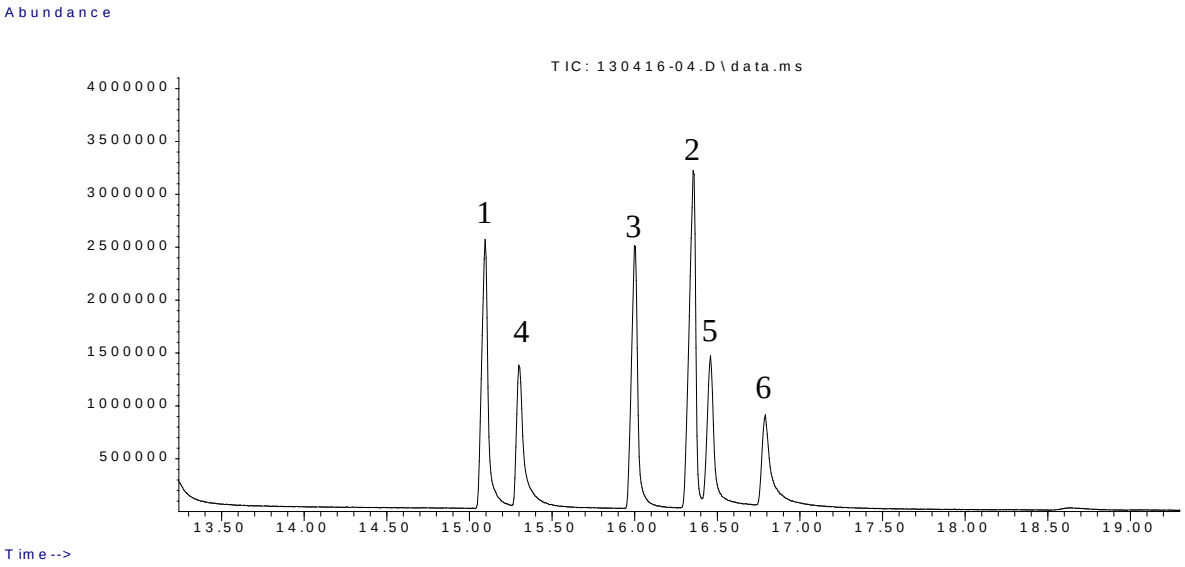


Fig. 16-5: Gas chromatographic separation of the six regioisomeric dimethoxybenzoyl-N-methylpiperazines.

Conclusion

The six regioisomeric dimethoxybenzoyl-N-methylpiperazines yield the same fragment ions in their mass spectra. ATR FTIR analysis yields unique and characteristic infrared spectra for these regioisomeric piperazines. These spectra allow discrimination among the six regioisomeric compounds included in this study. This differentiation was accomplished without the need for chemical derivatization. Mixtures of the dimethoxybenzoyl-N-methylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order appears related to the degree of substituent crowding on the aromatic ring with the most crowded 1,2,3 substitution patterns eluting first and the highest retention for the compound with minimum intramolecular crowding (the 1,3,5-trisubstitution pattern).

References

Abdel-Hay, K.M., DeRuiter, J. and Clark, C. GC-MS and GC-IRD Studies on the Six Ring Regioisomeric Dimethoxybenzylpiperazines (DMBPs). Drug Testing and Analysis, 5 (2013) 560-572. DOI:10.1002/dta.1417

Chapter 17

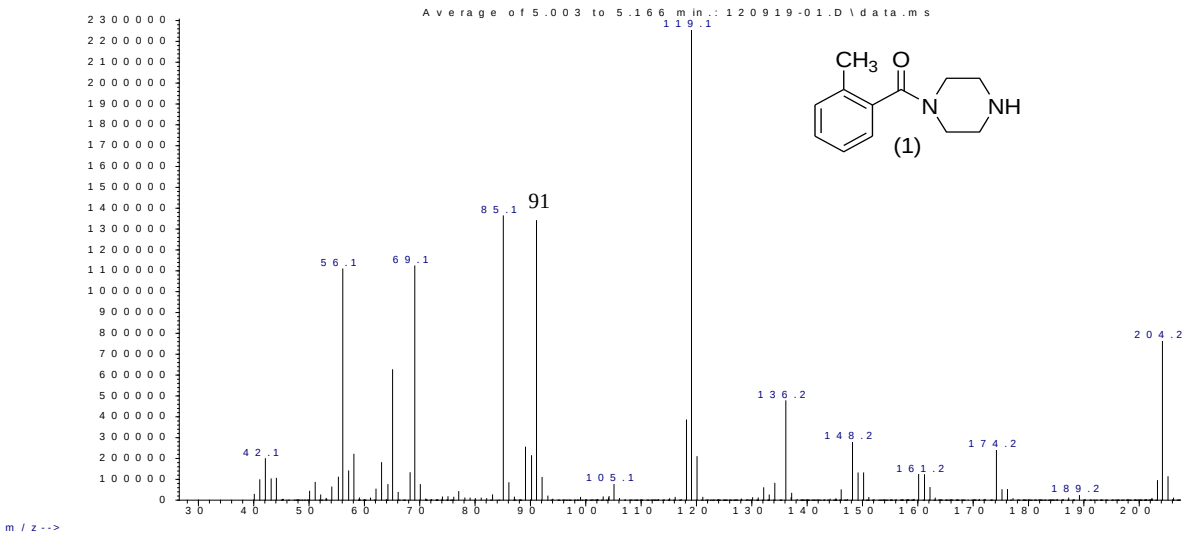
Differentiation of Methylbenzoylpiperazines (MBOPs) by GC-MS

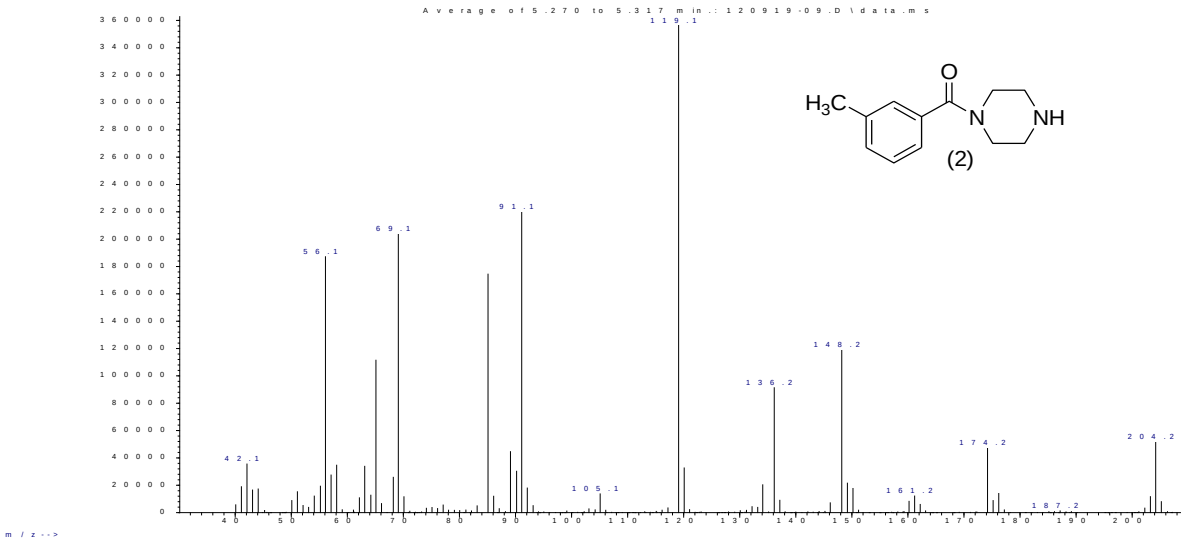
A series of regioisomeric methylbenzoylpiperazines were synthesized and analyzed as potential piperazine drugs of abuse. The mass spectra of each regioisomer in this series have fragment ions of identical mass and thus cannot be differentiated by this analytical method alone. Furthermore, chemical derivatization by perfluoroacylation did not offer any additional unique marker fragment ions in the mass spectrum to allow identification of one regioisomer in a series to the exclusion of the other two regioisomers. The perfluoroacylamides of the regioisomers in the MBOPs series were readily separated by GC on the stationary phase Rtx-200 and eluted in an order similar to other perfluoroacyl-derivatives of other benzoylpiperazine compounds reported earlier.

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylbenzoylpiperazines (MBOPs)

Mass spectrometry remains the primary method for confirming the identity of drugs in forensic samples. The mass spectra of the regioisomeric methylbenzoyl piperazines are shown in Figure 17-1. The ions of significant relative abundance common to all of the benzoyl piperazines appear to arise from fragmentation of the piperazine ring as shown in Figure 17-2. These include the methylene-ethylene ion at m/z 174 (M-30), the methylene ion at m/z 148 (M-56), the protonated amide ion at m/z 136 (M-68), the benzoyl ions at m/z 119 (M-85) and the aromatic ion at m/z 91 (M-113). The spectra for all three methylbenzoyl piperazines also include three piperazine fragments of relatively high abundance at m/z 85, 69 and 56. This fragmentation pattern is consistent with results obtained in our earlier studies with benzoyl-, methoxybenzoyl, dimethoxybenzoyl- and methylenedioxybenzoylpiperazines, as shown in Figure17-2 [Abdel-Hay *et al*, 2012]. In these earlier studies the identity of the primary amide (M-68) and benzoyl (M-85) ions were confirmed by exact mass analysis using GC-TOF-MS and deuterium labeling studies [Abdel-Hay *et al*, 2012]. All three of the methylbenzoylpiperazines have nearly identical mass spectra, and thus cannot be differentiated using this methodology.

The pentafluoropropionyl (PFPA) amide derivative of each methylbenzoylpiperazine isomer was prepared and analyzed in an effort to individualize the mass spectra and





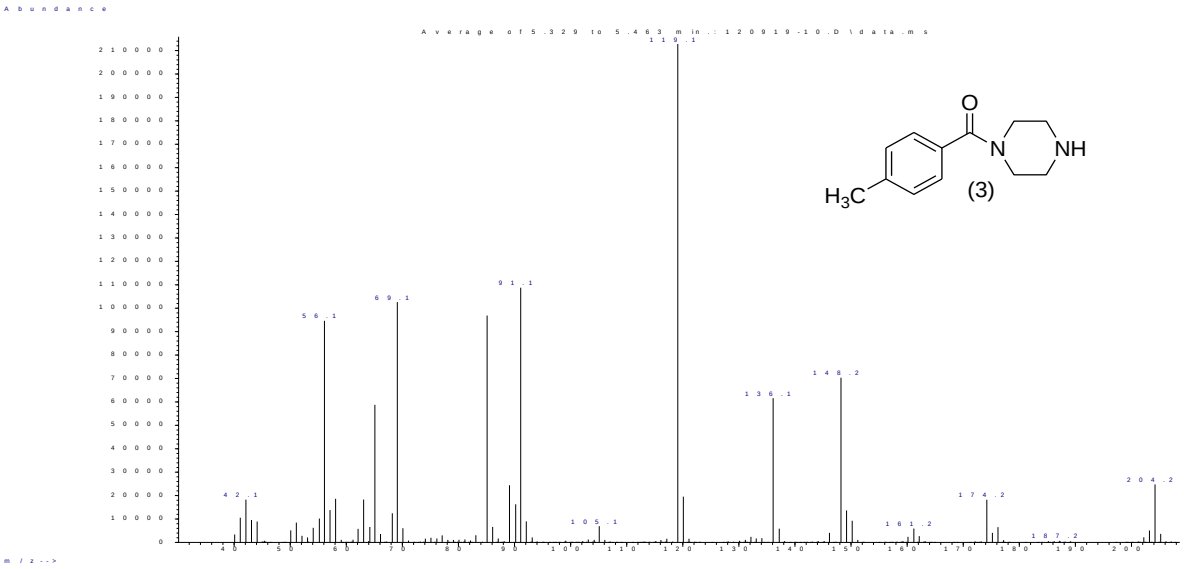


Fig. 17-1: Mass spectra of the MBOP regioisomers.

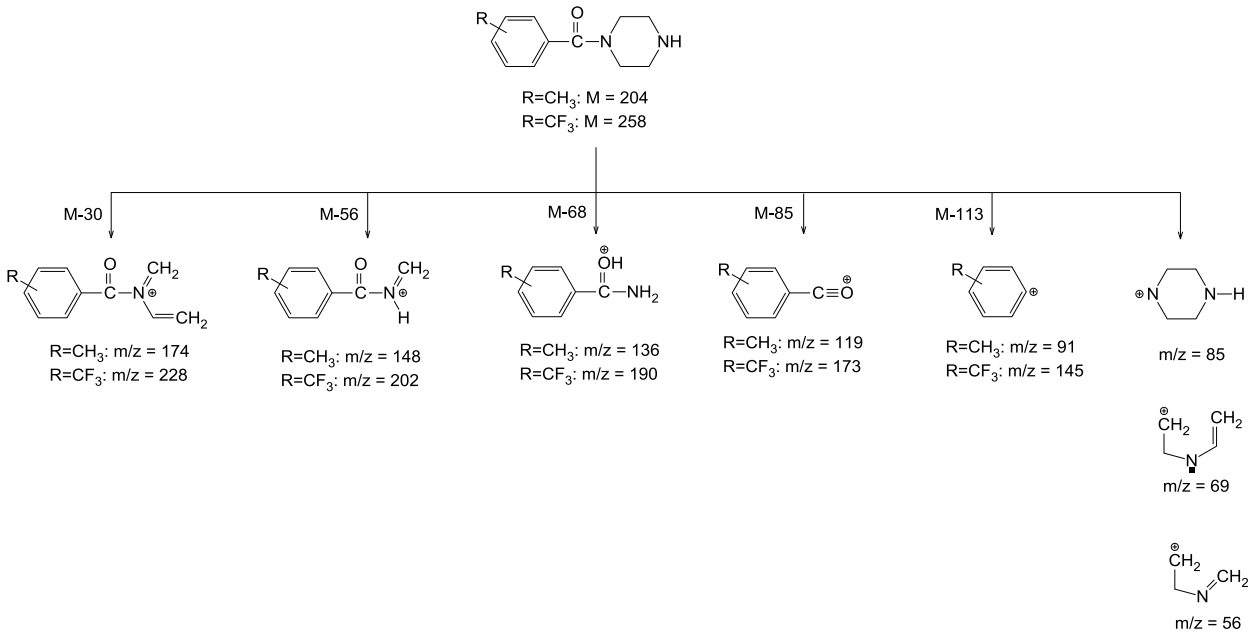
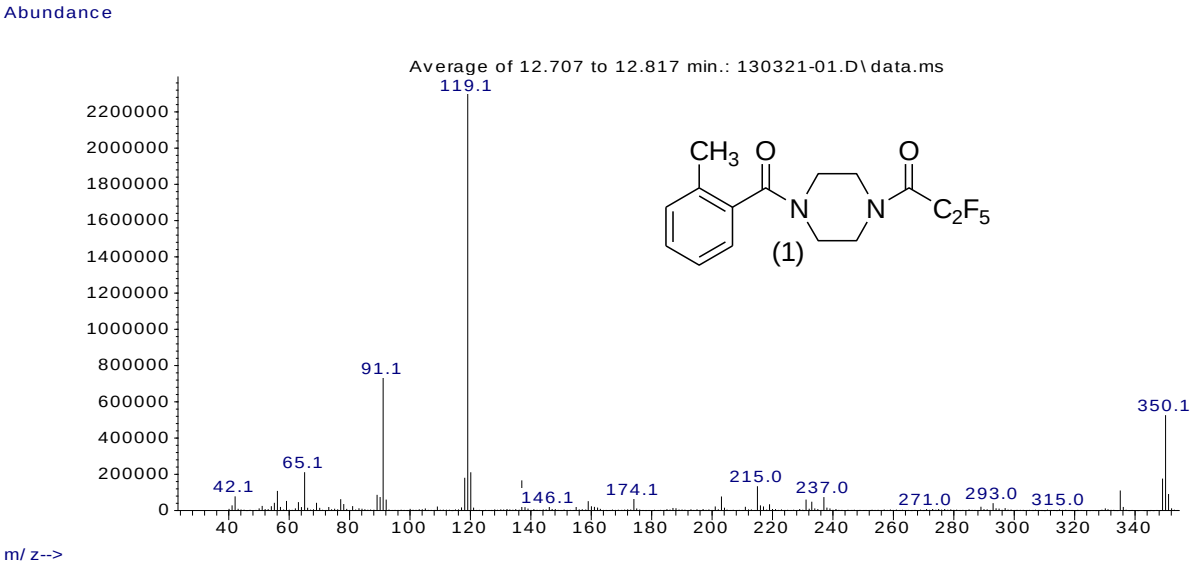


Fig. 17-2: Mass spectral fragmentation pattern of the MBOP regioisomers under EI (70 eV) conditions.

identify additional unique marker ions for differentiation among these three regioisomers (Figure 17-3). The molecular ions for these three amides yield peaks of high relative abundance at m/z 350, and a major fragment ion at m/z 119 which corresponds to the methylbenzoyl cation (Figure 17-3). Additional significant fragment ions in the spectra of all three regioisomers occurred at m/z 231 (M-119) for the benzoylpiperazinylcarbonyl cation, m/z 203 (M-147) for the methylbenzoylpiperazinyl cation, and m/z 91 (M-259) for the methylphenyl cation. Unfortunately since these ions are present in the mass spectra of all three regioisomeric PFPA-methylbenzoylpiperazines, chemical derivatization did not offer any additional unique marker ions to allow identification of one regioisomer the exclusion of the others in this set of compounds.



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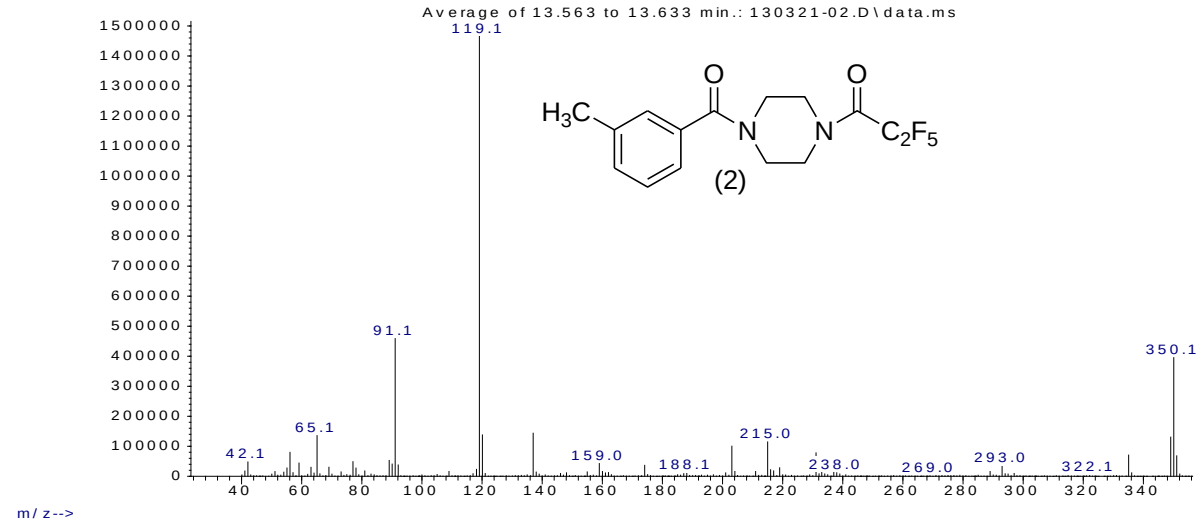


(2)

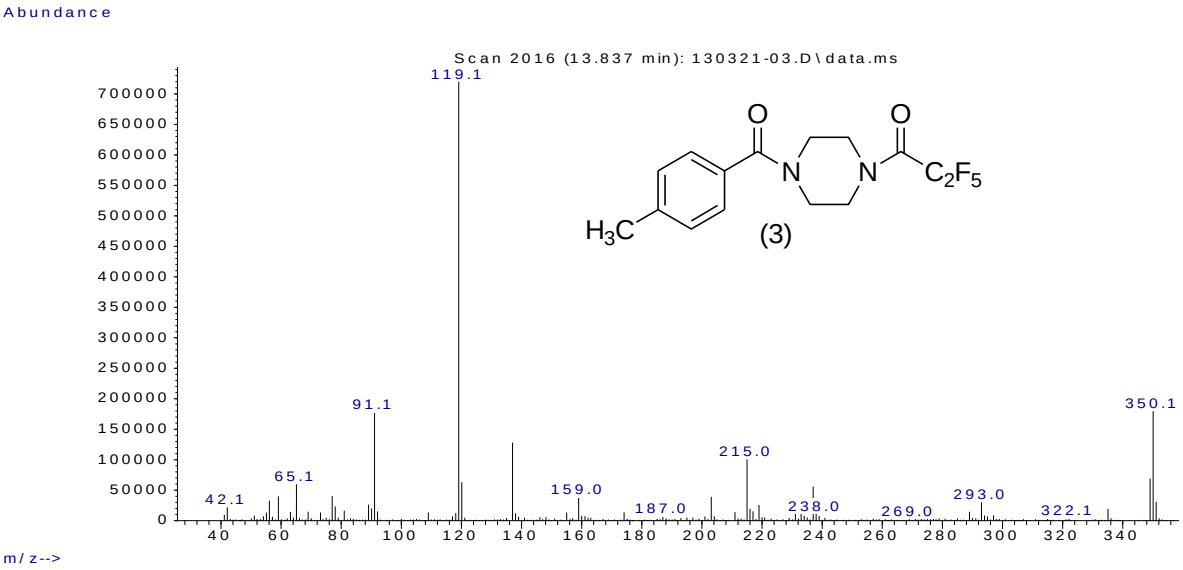


Fig. 17-3: Mass spectra of the MBOP-PFPA regioisomers.

Gas Chromatographic Separation of the Methylbenzoylpiperazines (MBOPs)

Gas chromatographic separation of the perfluoroacyl-derivatized piperazine series was accomplished on a capillary column of dimensions 30 m 0.25 mm and 0.5-mm film depth of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatogram in Figure 17-4 is a representative of the results obtained for all samples on this stationary phase. The separation of the pentafluoropropionyl derivatives was performed using a temperature program consisting of 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 60.00 min. Under these conditions, the pentafluoroproprionyl-MBOPs were well resolved and gave same elution order for the regioisomers (2 < 3 < 4) (Figure 17-4). Furthermore, these elution orders are consistent with results obtained with other benzylpiperazines and benzoylpiperazines reported earlier [Abdel-Hay *et al*, 2012]. Thus while the perrfluoroacylated derivatives did not provide any additional mass spectral discrimination among the regioisomers in each series, it did offer a method for marked improvement in the chromatographic resolution compared to the underivatized piperazines and for specific regioisomer discrimination. However, specific discrimination would require reference materials in order to confirm the complete chromatographic resolution for all the regioisomers in each series.

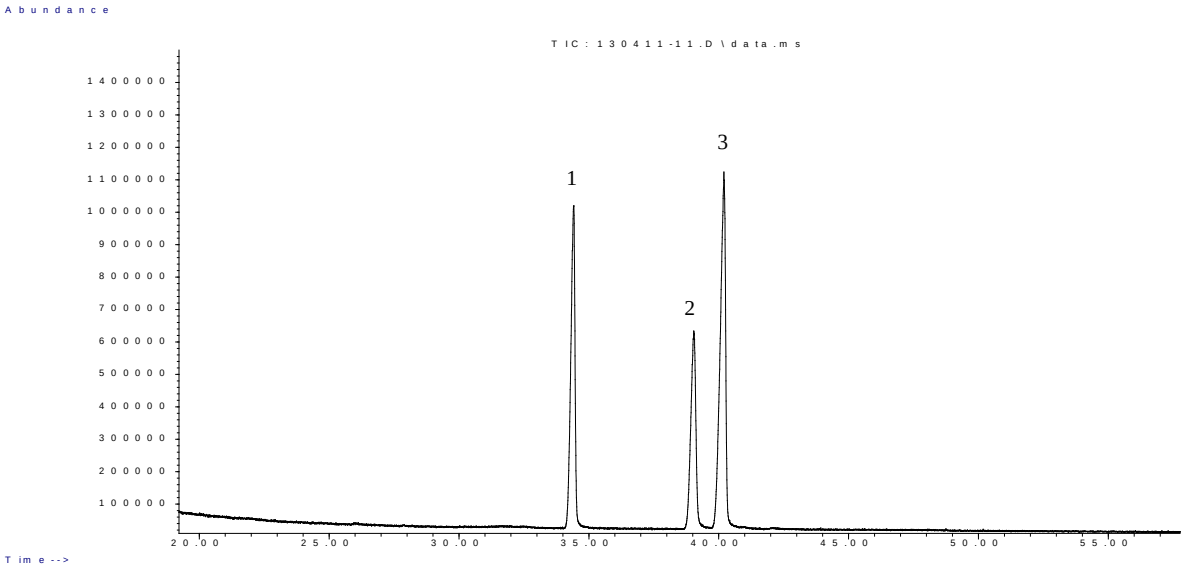


Fig. 17-4: Gas chromatographic separation of the MBOP-PFPA regioisomers using an Rtx-200 column.

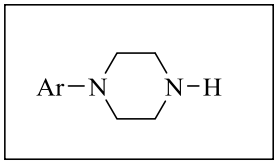
Conclusion

The series of regioisomeric methylbenzoylpiperazines were synthesized and analyzed as potential piperazine drugs of abuse. The mass spectra of each regioisomer in this series have fragment ions of identical mass and thus cannot be differentiated by this analytical method alone. Furthermore, chemical derivatization by perfluoroacylation did not offer any additional unique marker fragment ions in the mass spectrum to allow identification of one regioisomer in a series to the exclusion of the other two regioisomers. The perfluoroacylamides of the regioisomers in the MBOPs series were readily separated by GC on the stationary phase Rtx-200 and eluted in an order similar to other perfluoroacyl-derivatives of other benzoylpiperazine compounds reported earlier.

References

Abdel-Hay, K. M., DeRuiter, J., Clark, C.R. Differentiation of Methoxybenzoylpiperazines (OMeBzPs) and Methylenedioxybenzylpiperazines (MDBPs) By GC-IRD and GC-MS, Drug Testing and Analysis. 4(6) (2012) 430-440.

SECTION III Phenylpiperazines



The substituted phenylpiperazine series of isomers were prepared in a synthetic process which involves the formation of the piperazine ring as the last step in the procedure. The source for the substituted phenyl groups in these compounds as well as the N-1 nitrogen of the piperazine ring are the substituted anilines and a number of these are commercially available. The synthetic formation of the remaining components of the piperazine occurs by dual alkylation of the primary aniline nitrogen to yield the cyclic final product. We focused our efforts in this series on those commercially available aniline isomers likely to be incorporated into the continued designer exploration of this series of compounds. We prepared the monosubstituted chloro- methyl and methoxy-phenylpiperazines and the disubstituted methylenedioxy-, dimethyl- and dimethoxy-phenylpiperazines. A number of other regioisomeric substituted phenylpiperazines such as TFMPP have already appeared in street drug samples and have been described in the forensic drug analysis literature.

The EI mass spectra for this series of phenylpiperazine compounds are characterized by a much higher relative abundance of the molecular ions. The substituted phenyl cations are much less stable than the benzyl cations and thus do not fragment as readily. A number of unique ions characteristic of fragmentation within the piperazine ring have a high relative abundance in these spectra. Ions occurring at $(M\text{-}42)^+$ indicate the loss of C_2H_4N from the

molecular ion. Exact mass analysis confirmed this species as occurring from cleavage within the piperazine ring. The mass spectra are similar for each of the substituted aromatic ring regioisomeric series. Once the compound is identified by mass spectrometry as a member of a specific regioisomeric series, infrared studies allow us to differentiate among the individual isomers to provide a specific identification. In some cases we have ATR generated FT-IR spectra and in other cases the spectra are vapor phase GC-IR generated. The acylated sets of regioisomeric equivalents were resolved by GC and the relative elution order described in some cases based on structural features.

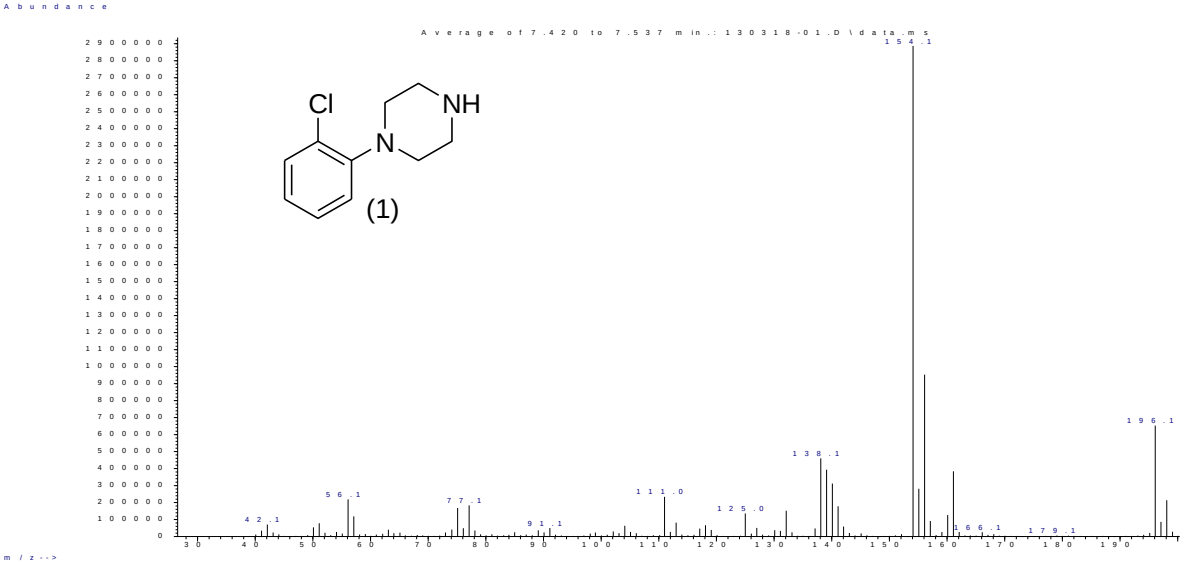
Chapter 18

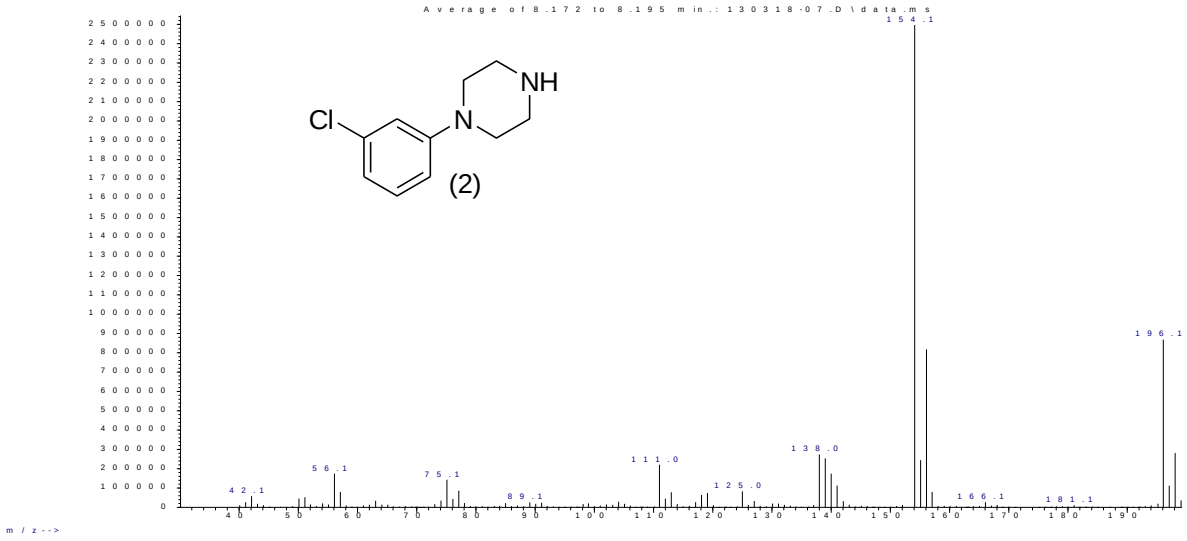
GC-MS Studies on the Three Ring Regioisomeric Chlorophenylpiperazines (ClPPs)

The complete series of regioisomeric chlorophenylpiperazines were synthesized and evaluated using GC-MS. The EI mass spectra show fragment ions characteristic of both the chlorophenyl and the piperazine portions of the molecules. These characteristic fragments include the chlorophenyl aziridinium cation at m/z 154 and chlorophenyl cation at m/z 111 as well as the m/z 138, 139 and 140 frgament cluster. In addition to that the low mass ion at m/z 56 for the $C_3H_6N^+$ was observed in all piperazine EI spectra. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and the elution was in the order of ortho, meta followed by para ClPPs.

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Chlorophenylpiperazines (ClPPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 18-1 shows the EI mass spectra of the three regioisomeric chlorophenylpiperazines (Compounds 1-3). The mass spectra in Figure 18-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. The common fragment ions observed for the regioisomeric chloro group substitution on the aromatic ring likely indicate that the piperazine ring is the source for most of the fragmentation. The structures for the fragmentation product ions are summarized in Figure 18-2. The ions of significant relative abundance common to all three regioisomers likely arise from fragmentation of the piperazine ring. The chlorophenyl aziridinium cation at m/z 154 is the base peak in all these spectra. The structures for the fragment ions in the unsubstituted aromatic ring for phenylpiperazine have been described by de Boer et al [de Boer *et al*, 2001]. Equivalent fragmentation pathways for the chlorophenylpiperazines (ClPPs) yield the fragment ions at m/z 154, 140, 139, 138, 111 and 56 as shown in Figures 18-1 and 18-2. The structures for the fragments in the three ClPPs regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual ClPP regioisomers.





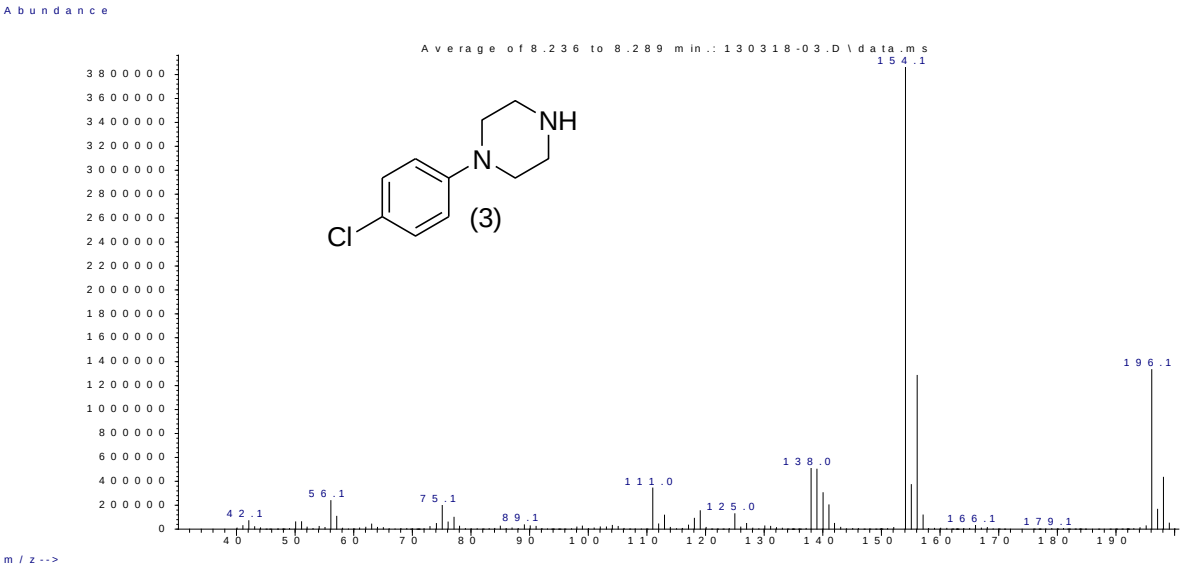


Fig. 18-1: EI mass spectra of the three chlorophenylpiperazines in this study.

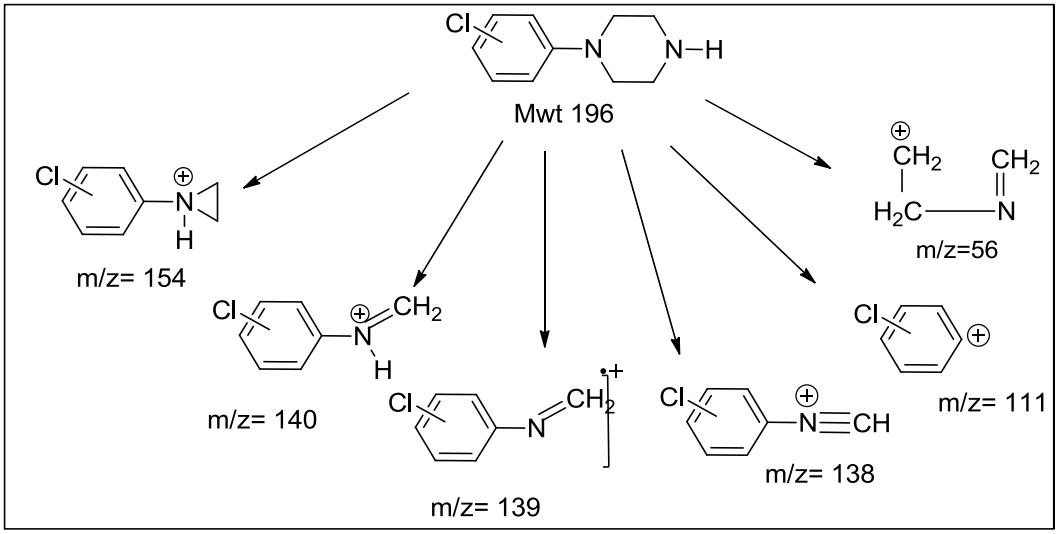
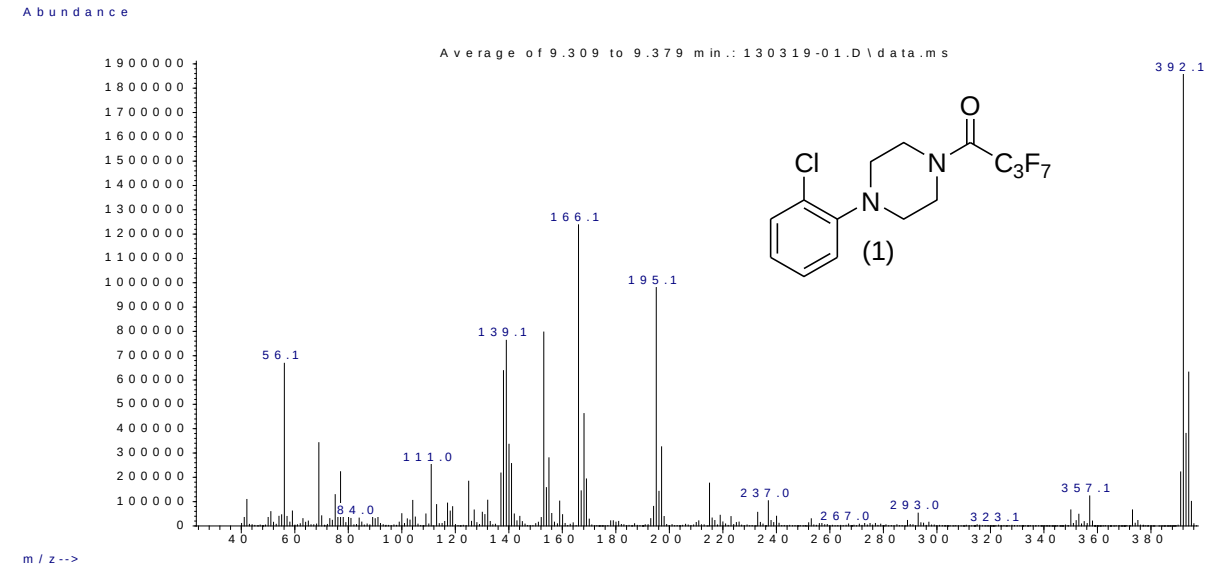
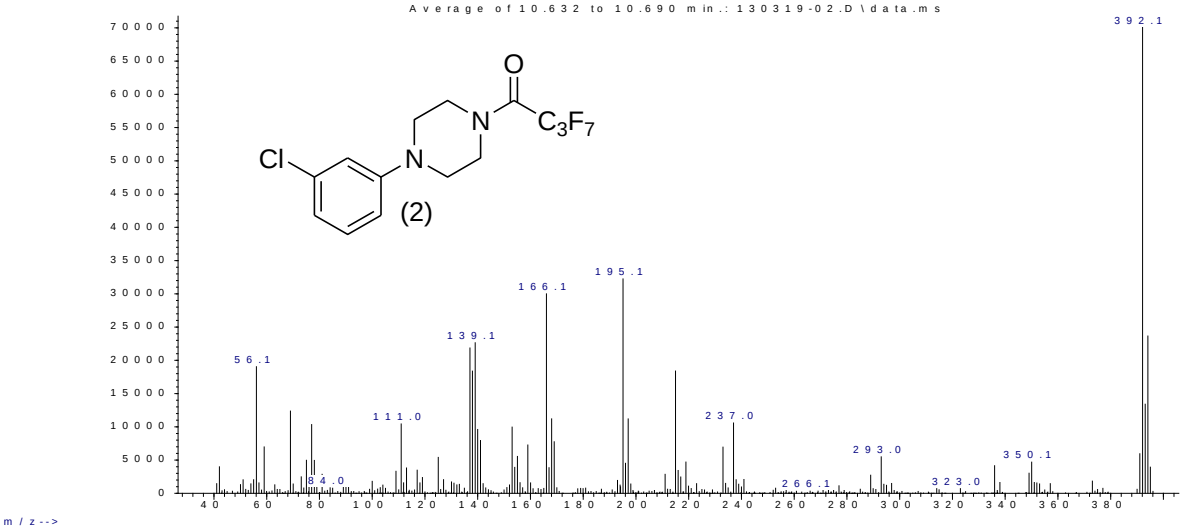


Fig. 18-2: EI mass spectral fragmentation pattern of the underivatized chlorophenylpiperazines.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the regioisomeric chlorophenyl piperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds. The heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra of ClPPs. The mass spectra for the three heptaflourobutyryl amides are shown in Figure 18-3. From these spectra, a common peak with high relative abundance occurs at *m/ z* 392 which corresponds to the molecular ions for HFBA amides. Fragment ions occurring at *m/ z* 140, 139, 138, 111 and 56seen in all MS spectra of piperazine acyl amides are due to different patterns of cleavage reactions in the piperazine ring, analogous to those found in the underivatized compounds. Fragment ions at *m/ z* 195 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety from the corresponding derivative. Those occurring at *m/ z* 169 are formed as a result of the elimination of heptafluoropropyl moiety from the HFBA amides. There is no significant difference between the mass spectra of the three compounds. Thus, even acylation of the three piperazines does not give characteristic fragments that help to discriminate among these regioisomeric compounds.





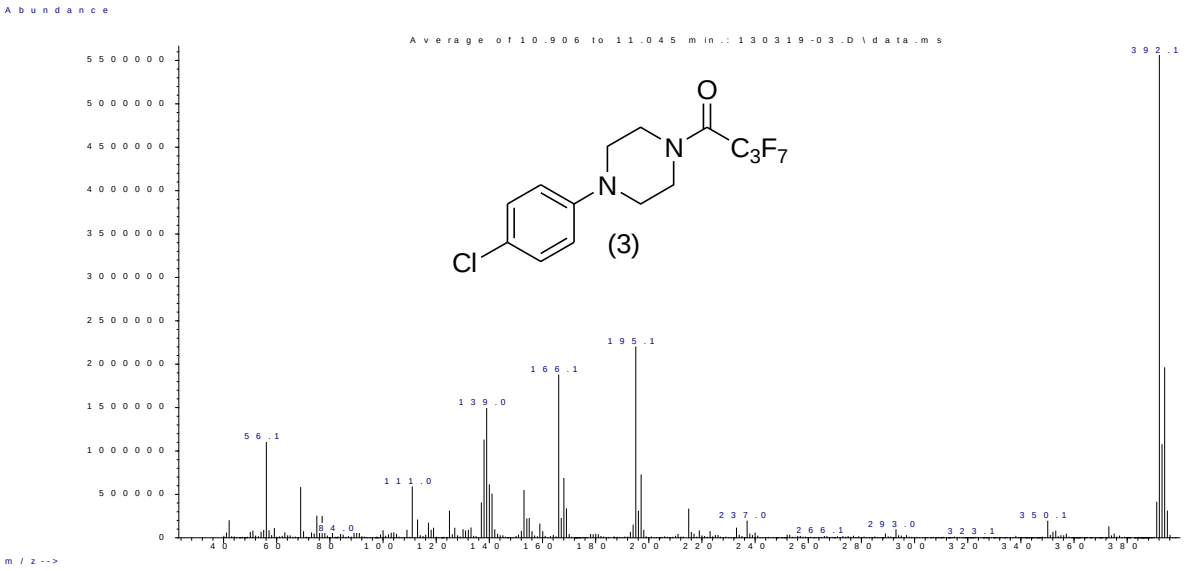


Fig. 18-3: MS spectra of heptafluorobutyryl derivatives of the three chlorophenylpiperazines.

Gas Chromatographic Separation of the Chlorophenylpiperazines (ClPPs)

Gas chromatographic separation of the HFBA derivatives of the three chlorophenylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 18-4. The separation of the heptafluorobutyryl 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 ramped up to 200°C at a rate of 10°C/min, held at 200°C for 25.0 min

This chromatogram shows the separation of the three regioisomers in this study. The elution order was ortho followed by meta then para chlorophenylpiperazine (compounds 1 then 2 then 3).

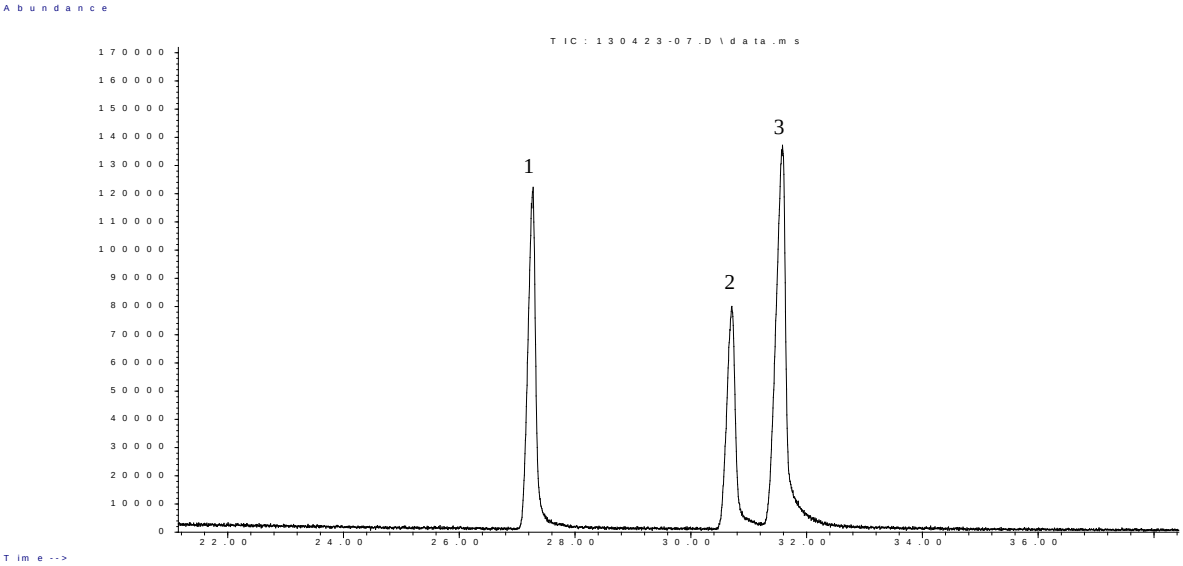


Fig. 18-4: Gas chromatographic separation of the heptafluorobutyryl derivatives of the three chlorophenylpiperazines using Rtx-200 column.

Conclusion

The three regioisomeric chlorophenylpiperazines yield the same fragment ions in their mass spectra. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Mixtures of the chlorophenylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order was in the order of ortho, meta then para regioisomers.

References

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 Science International 121 (2001) 47-56.

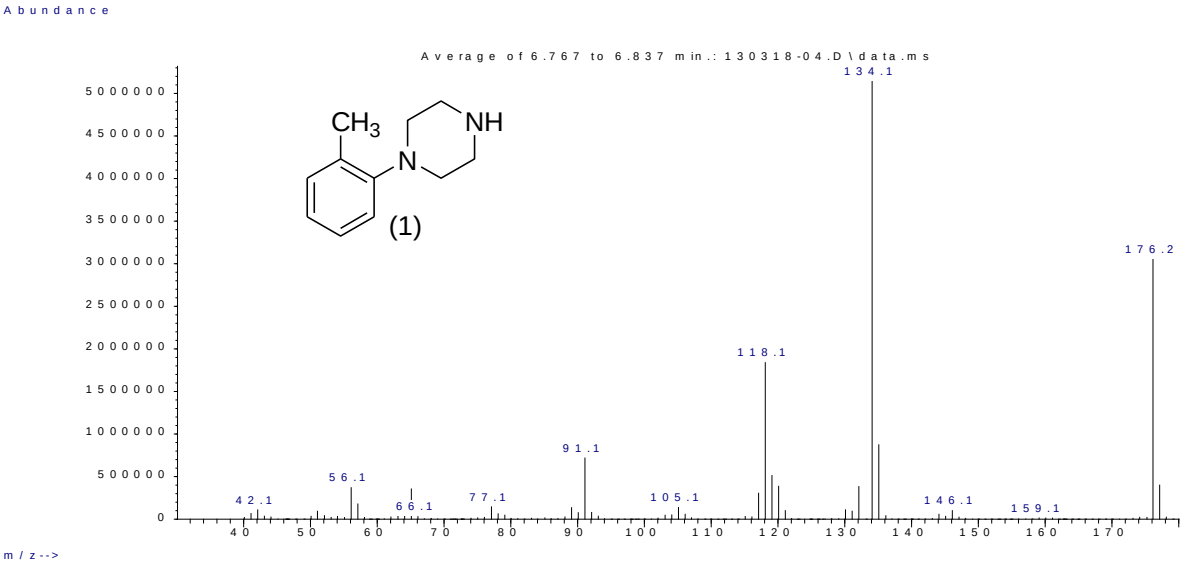
Chapter 19

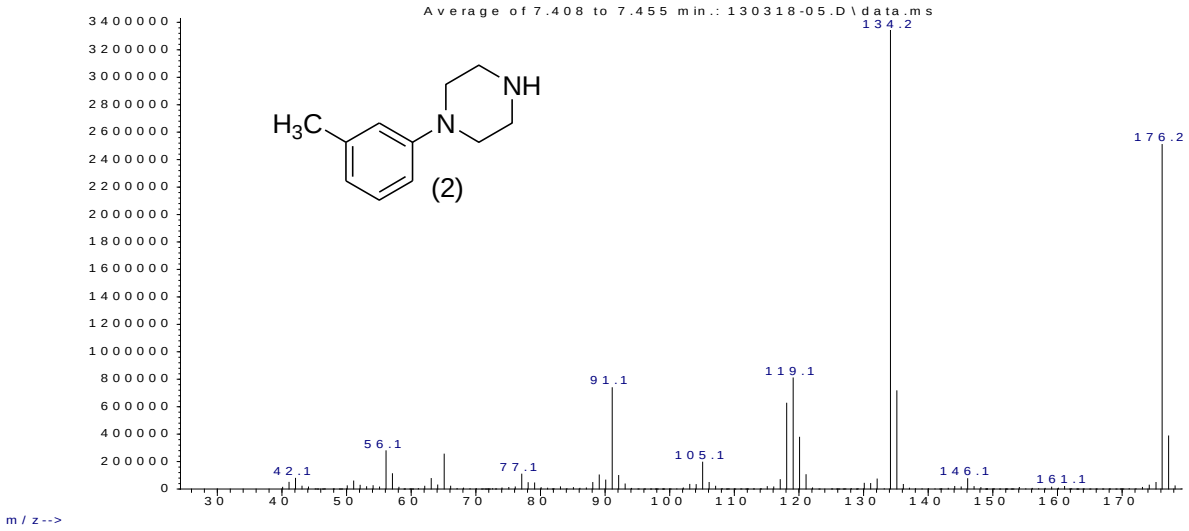
GC-MS Studies on the Three Ring Regioisomeric Methylphenylpiperazines (MPPs)

The complete series of regioisomeric methylphenylpiperazines were synthesized and evaluated using GC-MS. The EI mass spectra show fragment ions characteristic of both the methylphenyl and the piperazine portions of the molecules. These characteristic fragments include the methylphenyl aziridinium cation at m/z 134 and methylphenyl cation at m/z 91 as well as the m/z 118, 119 and 120 frgament cluster. In addition to that the low mass ion at m/z 56 for the $C_3H_6N^+$ was observed in all piperazine EI spectra. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and the elution was in the order of ortho, meta followed by para MPPs.

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methylphenylpiperazines (MPPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 19-1 shows the EI mass spectra of the three regioisomeric methylphenylpiperazines (Compounds 1-3). The mass spectra in Figure 19-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. The common fragment ions observed for the regioisomeric methyl group substitution on the aromatic ring likely indicate that the piperazine ring is the source for most of the fragmentation. The structures for the fragmentation product ions are summarized in Figure 19-2. The ions of significant relative abundance common to all three regioisomers likely arise from fragmentation of the piperazine ring. The methylphenyl aziridinium cation at m/z 134 is the base peak in all these spectra. The structures for the fragment ions in the unsubstituted aromatic ring for phenylpiperazine have been described by de Boer et al [de Boer *et al*, 2001]. Equivalent fragmentation pathways for the methylphenylpiperazines (MPPs) yield the fragment ions at m/z 134, 120, 119, 118, 91 and 56 as shown in Figures 19-1 and 19-2. The structures for the fragments in the three MPPs regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual MPP regioisomer.





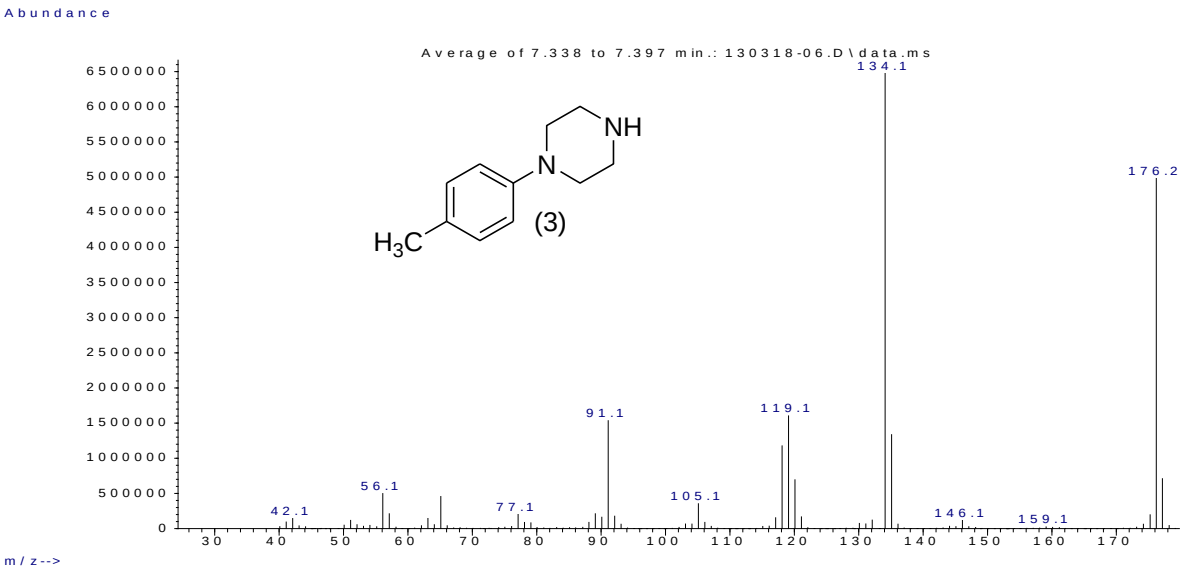


Fig. 19-1: EI mass spectra of the three methylphenylpiperazines in this study.

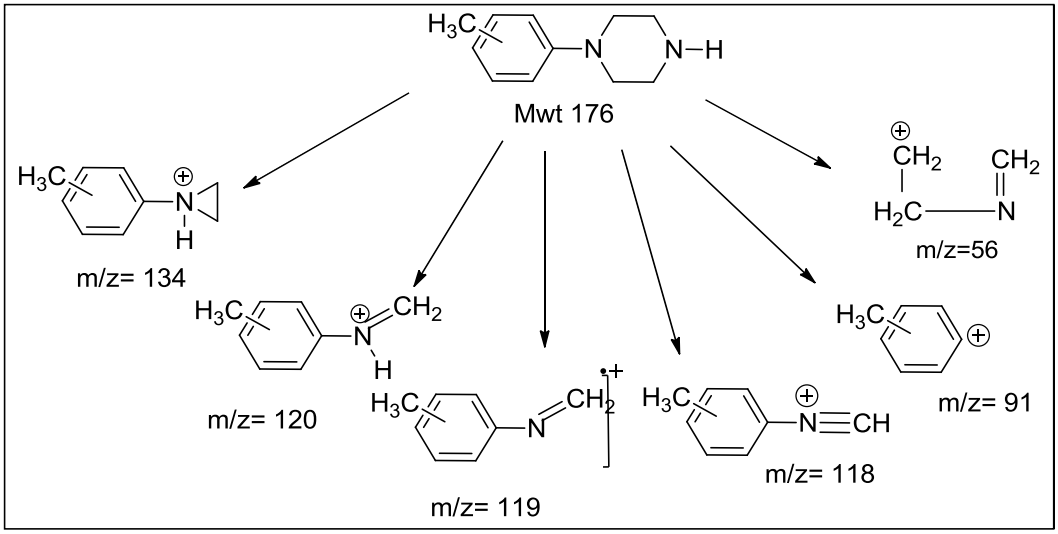
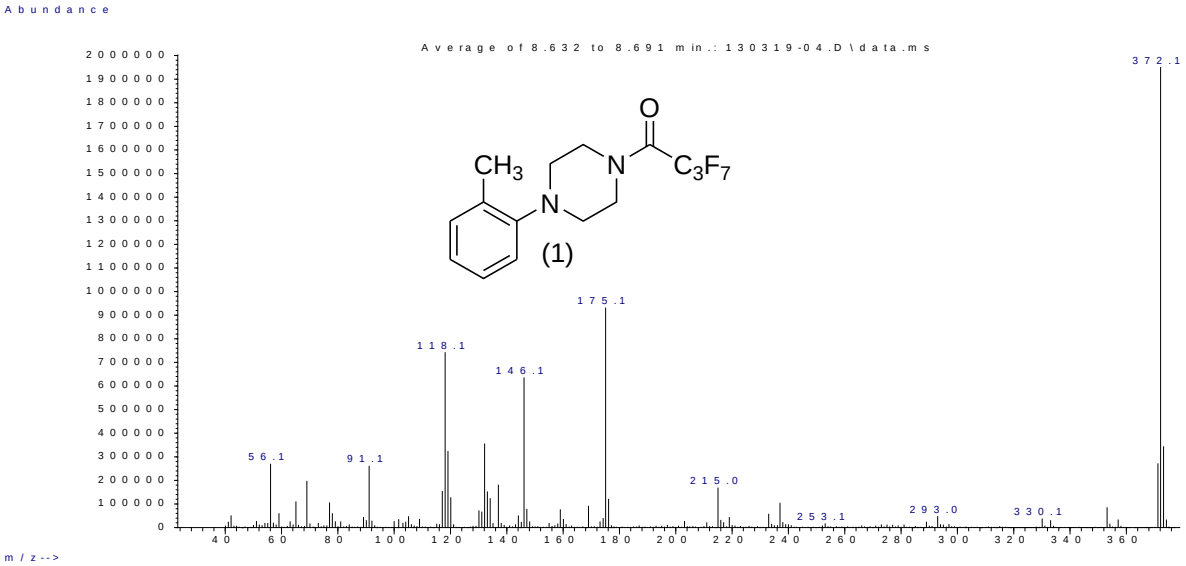
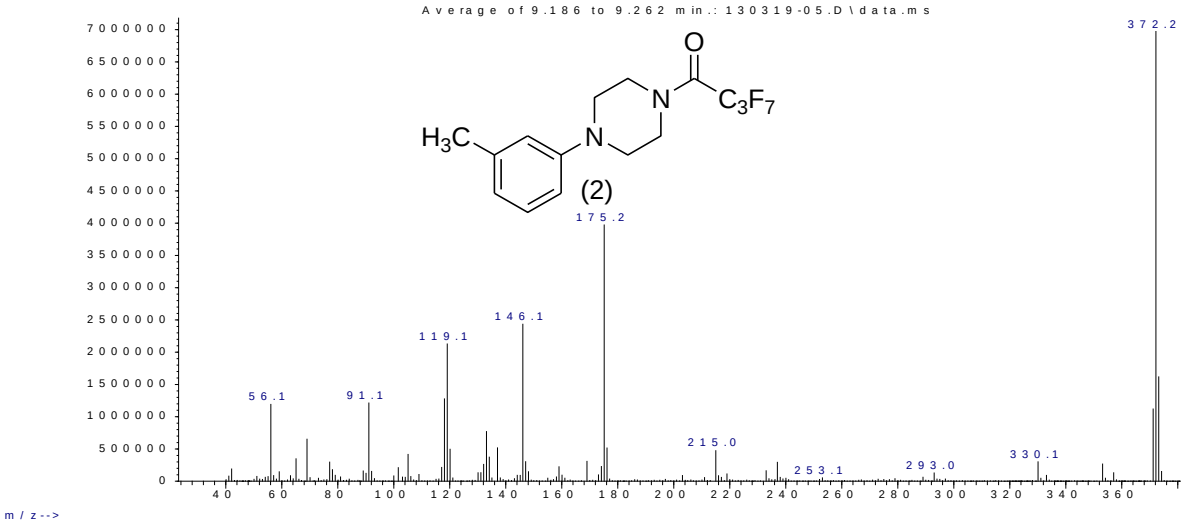


Fig. 19-2: EI mass spectral fragmentation pattern of the underivatized methylphenylpiperazines.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the regioisomeric methylphenyl piperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds. The heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra of MPPs. The mass spectra for the three heptaflourobutyryl amides are shown in Figure 19-3. From these spectra, a common peak with high relative abundance occurs at *m/ z* 372 which corresponds to the molecular ions for HFBA amides. Fragment ions occurring at *m/ z* 120, 119, 118, 91 and 56 seen in all MS spectra of piperazine acyl amides are due to different patterns of cleavage reactions in the piperazine ring, analogous to those found in the underivatized compounds. Fragment ions at *m/ z* 175 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety from the corresponding derivative. Those occurring at *m/ z* 169 are formed as a result of the elimination of heptafluoropropyl moiety from the HFBA amides. There is no significant difference between the mass spectra of the three compounds. Thus, even acylation of the three piperazines does not give characteristic fragments that help to discriminate among these regioisomeric compounds.





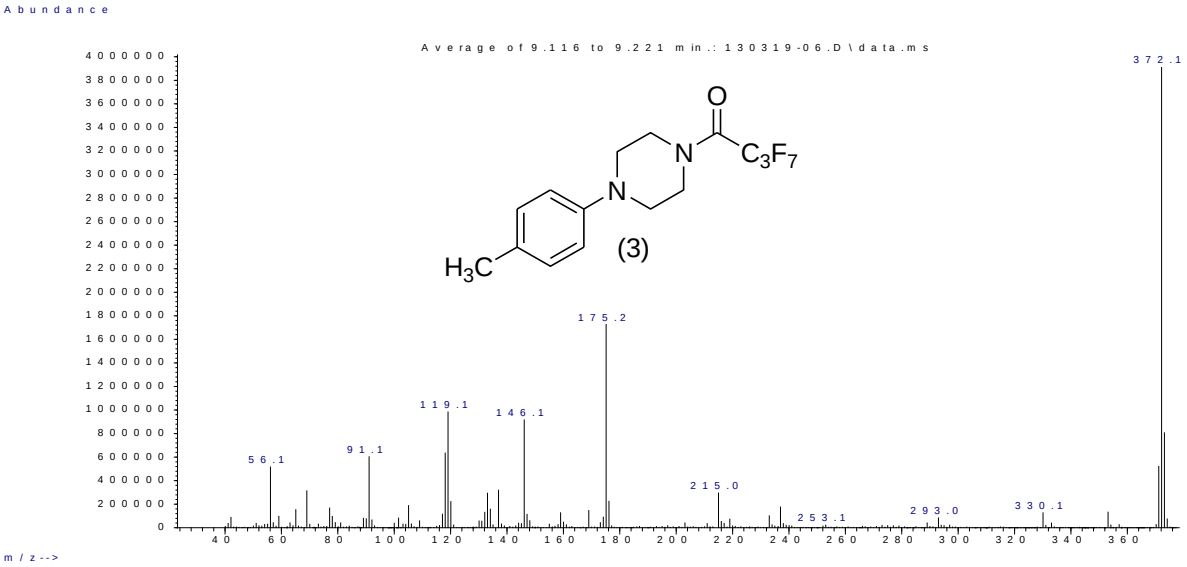


Fig. 19-3: MS spectra of heptafluorobutyryl derivatives of the three methylphenylpiperazines.

Gas Chromatographic Separation of the Methylphenylpiperazines (MPPs)

Gas chromatographic separation of the HFBA derivatives of the three methylphenylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 19-4. The separation of the heptafluorobutyryl 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 12°C/min, held at 180°C for 2.0 min, then ramped up to 200°C at a rate of 10°C/min, held at 200°C for 20.0 min

This chromatogram shows the separation of the three regioisomers in this study. The elution order was ortho followed by meta then para chlorophenylpiperazine (compounds 1 then 2 then 3).

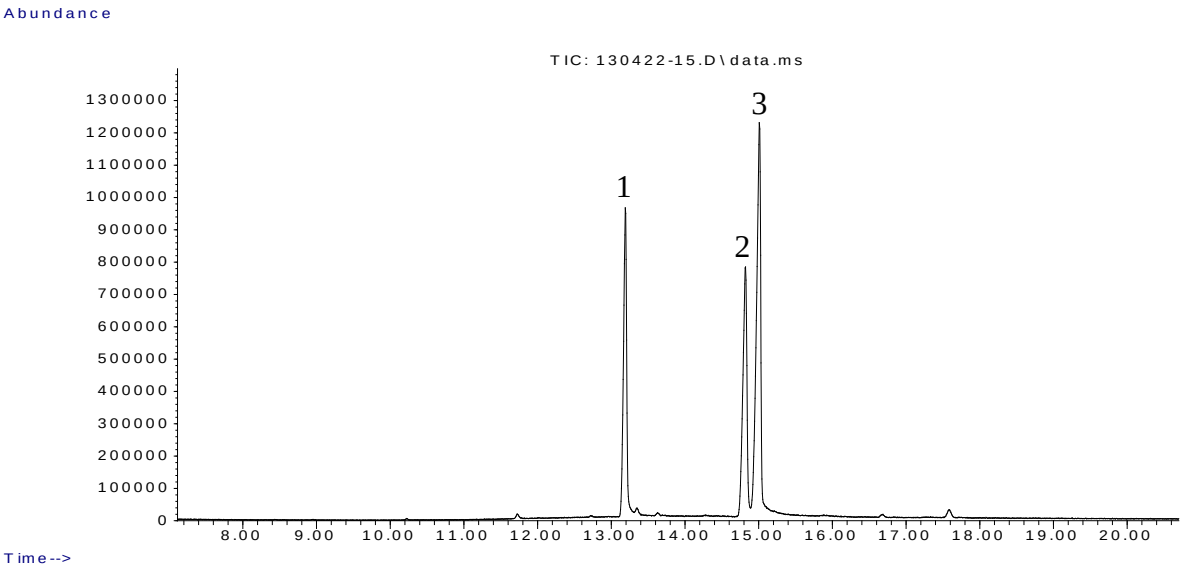


Fig. 19-4: Gas chromatographic separation of the heptafluorobutyryl derivatives of the three methylphenylpiperazines using Rtx-200 column.

Conclusion

The three regioisomeric methylphenylpiperazines yield the same fragment ions in their mass spectra. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Mixtures of the methylphenylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order was in the order of ortho, meta then para regioisomers.

References

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 Science International 121 (2001) 47-56.

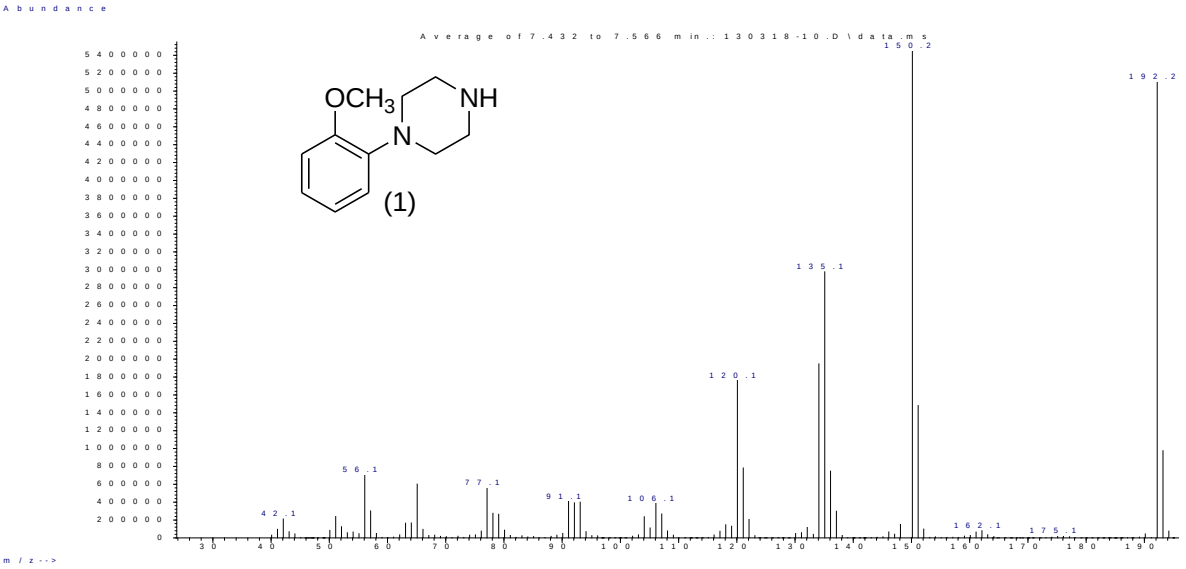
Chapter 20

GC-MS Studies on the Three Ring Regioisomeric Methoxyphenylpiperazines (OMePPs)

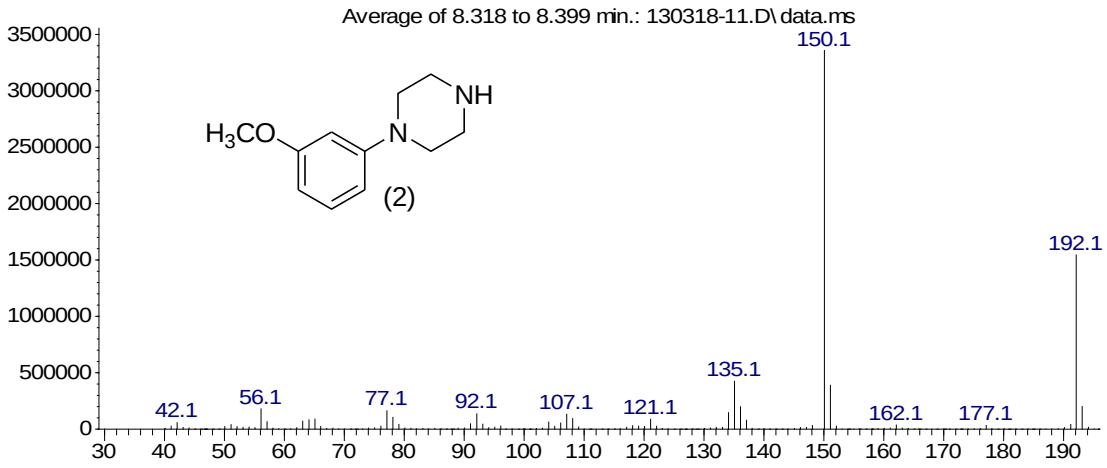
The complete series of regioisomeric methoxyphenylpiperazines were synthesized and evaluated using GC-MS. The EI mass spectra show fragment ions characteristic of both the methoxyphenyl and the piperazine portions of the molecules. These characteristic fragments include the methoxyphenyl aziridinium cation at m/z 150 and methoxyphenyl cation at m/z 107 as well as the m/z 134, 135 and 136 frgament cluster. In addition to that the low mass ion at m/z 56 for the $C_3H_6N^+$ was observed in all piperazine EI spectra. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and the elution was in the order of ortho, meta followed by para OMePPs.

Mass spectral studies of the underivatized and perfluoroacylated derivatives of Methoxyphenylpiperazines (OMePPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 20-1 shows the EI mass spectra of the three regioisomeric methoxyphenylpiperazines (Compounds 1-3). The mass spectra in Figure 20-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. The common fragment ions observed for the regioisomeric methyoxy group substitution on the aromatic ring likely indicate that the piperazine ring is the source for most of the fragmentation. The structures for the fragmentation product ions are summarized in Figure 20-2. The ions of significant relative abundance common to all three regioisomers likely arise from fragmentation of the piperazine ring. The methoxyphenyl aziridinium cation at m/z 150 is the base peak in all these spectra. The structures for the fragment ions in the unsubstituted aromatic ring for phenylpiperazine have been described by de Boer et al [de Boer *et al*, 2001]. Equivalent fragmentation pathways for the methoxyphenylpiperazines (OMePPs) yield the fragment ions at m/z 150, 136, 135, 134, 107 and 56 as shown in Figures 20-1 and 20-2. The structures for the fragments in the three OMePPs regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual OMePP regioisomer.



Abundance



m/z-->

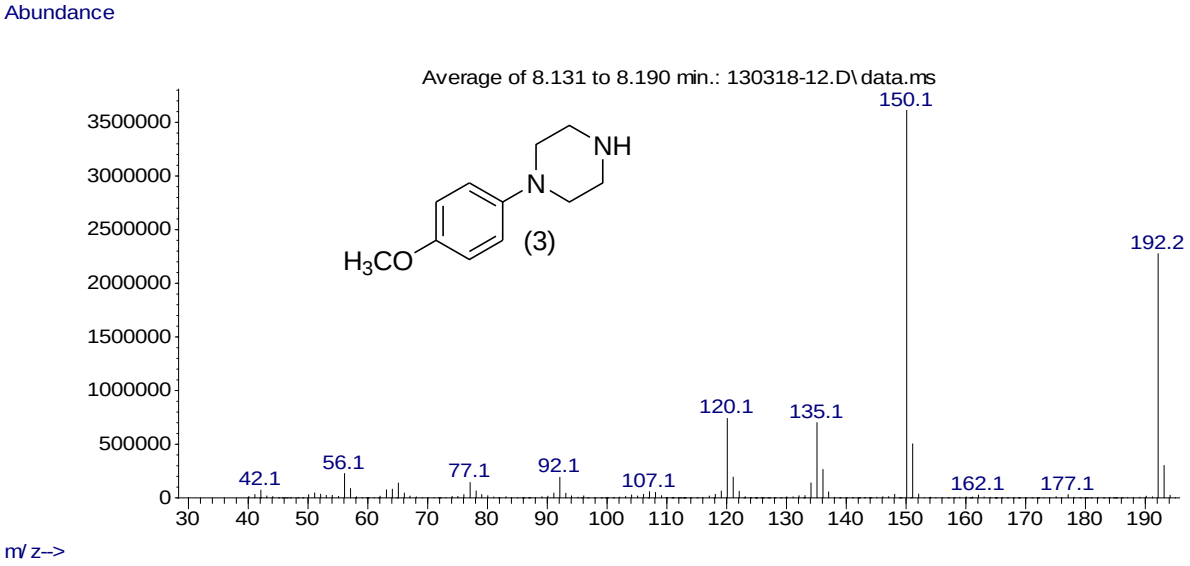


Fig. 20-1: EI mass spectra of the three methoxyphenylpiperazines in this study.

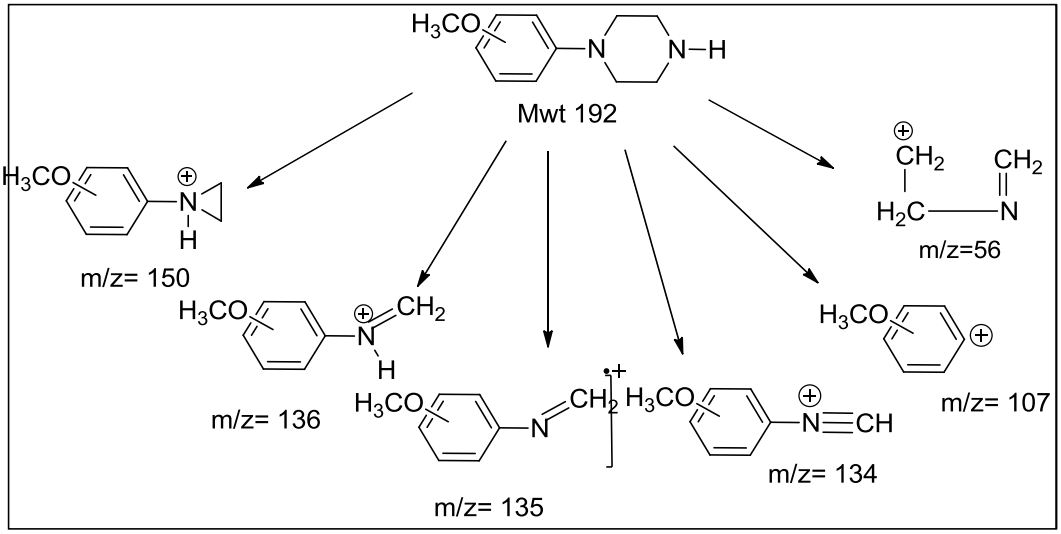
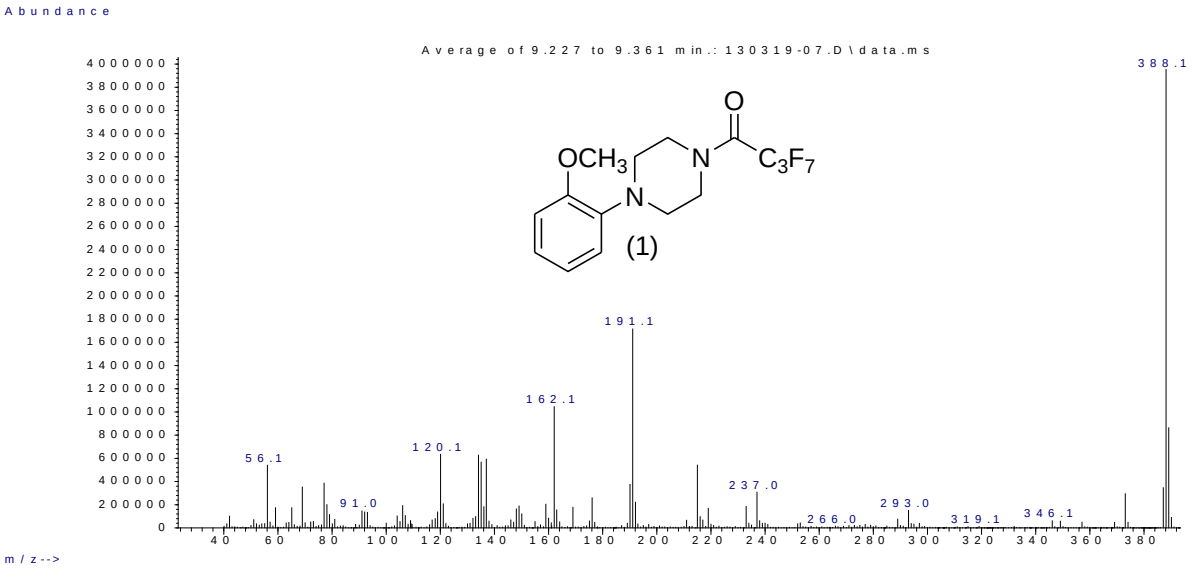
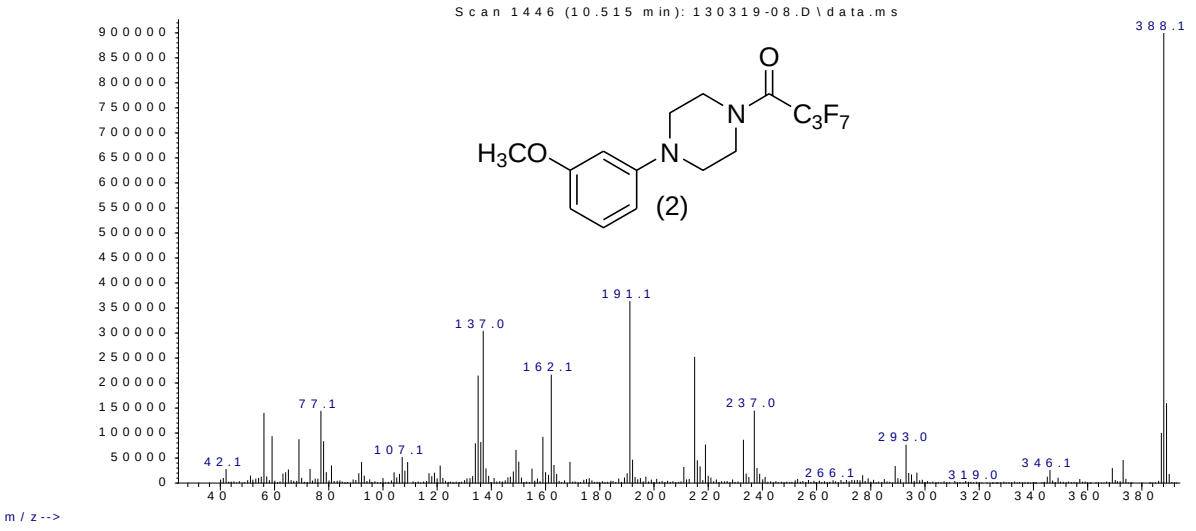


Fig. 20-2: EI mass spectral fragmentation pattern of the underivatized metoxylphenylpiperazines.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the regioisomeric methoxyphenyl piperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds. The heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra of OMePPs. The mass spectra for the three heptaflourobutyryl amides are shown in Figure 20-3. From these spectra, a common peak with high relative abundance occurs at *m/ z* 388 which corresponds to the molecular ions for HFBA amides. Fragment ions occurring at *m/ z* 136, 135, 134, 107 and 56 seen in all MS spectra of piperazine acyl amides are due to different patterns of cleavage reactions in the piperazine ring, analogous to those found in the underivatized compounds. Fragment ions at *m/ z* 191 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety from the corresponding derivative. Those occurring at *m/ z* 169 are formed as a result of the elimination of heptafluoropropyl moiety from the HFBA amides. There is no significant difference between the mass spectra of the three compounds. Thus, even acylation of the three piperazines does not give characteristic fragments that help to discriminate among these regioisomeric compounds.





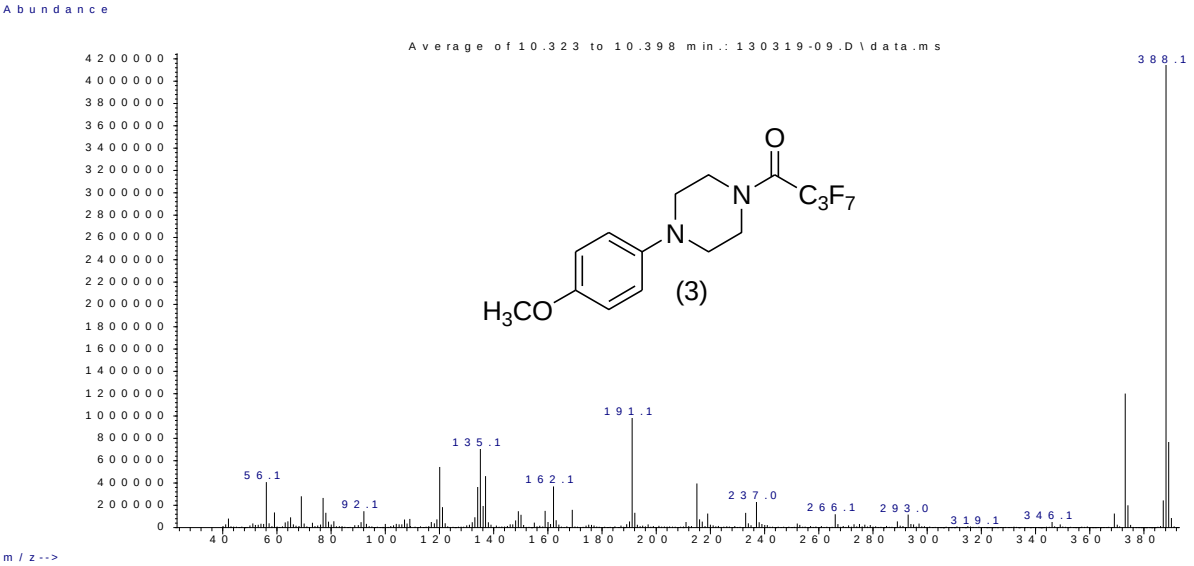


Fig. 20-3: MS spectra of heptafluorobutyryl derivatives of the three methoxyphenylpiperazines.

Gas Chromatographic Separation of the Methoxyphenylpiperazines (OMePPs)

Gas chromatographic separation of the HFBA derivatives of the three methoxyphenylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 20-4. The separation of the heptafluorobutyryl 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 12°C/min, held at 180°C for 2.0 min, then ramped up to 200°C at a rate of 10°C/min, held at 200°C for 20.0 min

This chromatogram shows the separation of the three regioisomers in this study. The elution order was ortho followed by meta then para chlorophenylpiperazine (compounds 1 then 2 then 3).

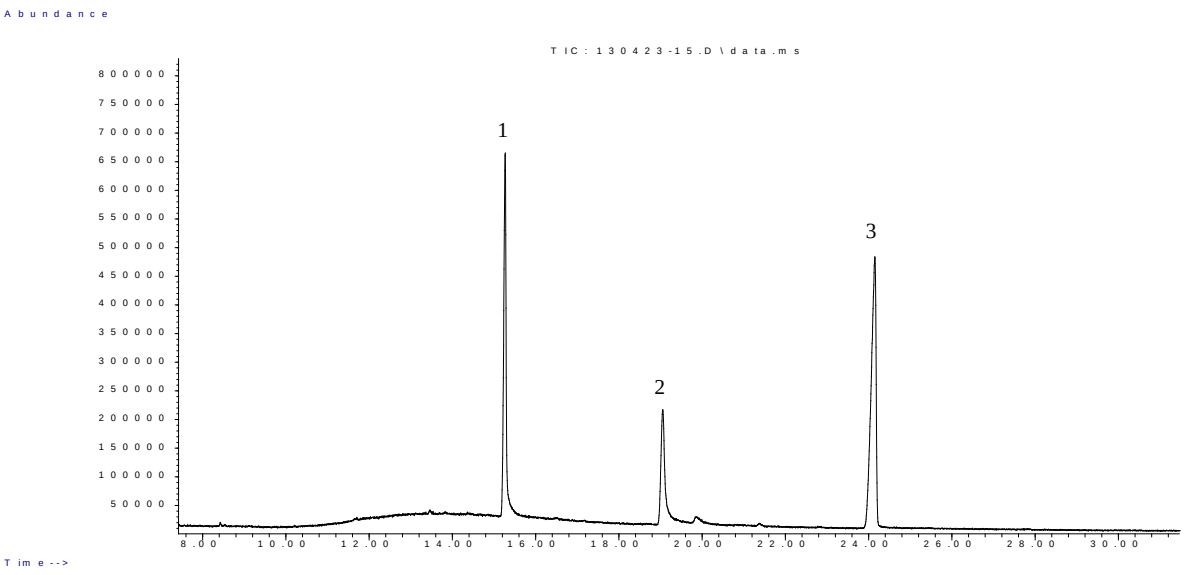


Fig. 20-4: Gas chromatographic separation of the heptafluorobutyryl derivatives of the three methoxyphenylpiperazines using Rtx-200 column.

Conclusion

The three regioisomeric methoxyphenylpiperazines yield the same fragment ions in their mass spectra. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Mixtures of the methoxyphenylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order was in the order of ortho, meta then para regioisomers.

References

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 Science International 121 (2001) 47-56.

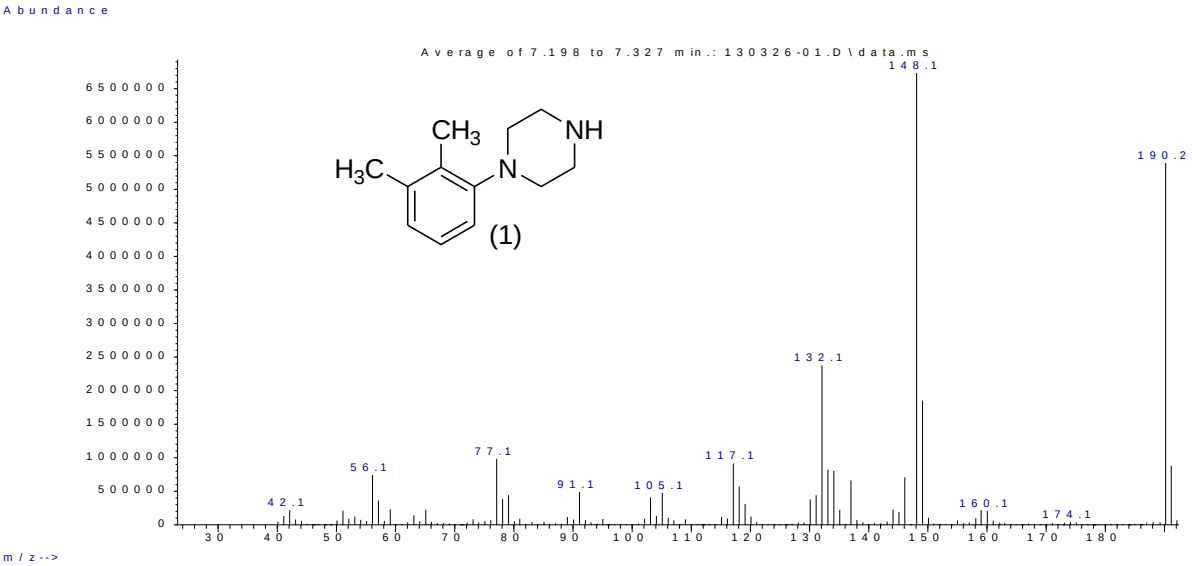
Chapter 21

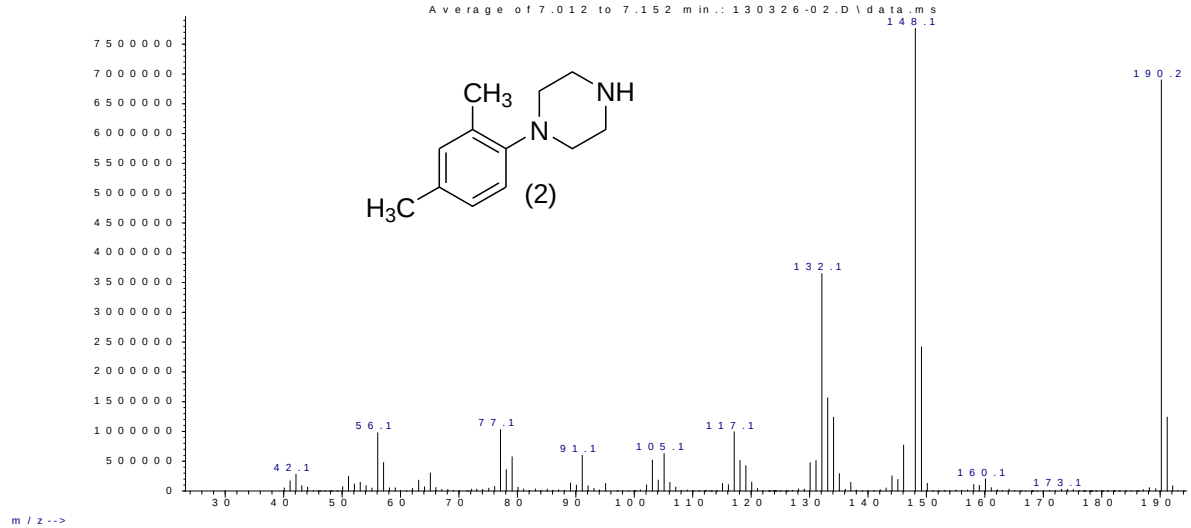
GC-MS and ATR FTIR Studies on the Six Ring Regioisomeric Dimethylphenylpiperazines (DMPPs)

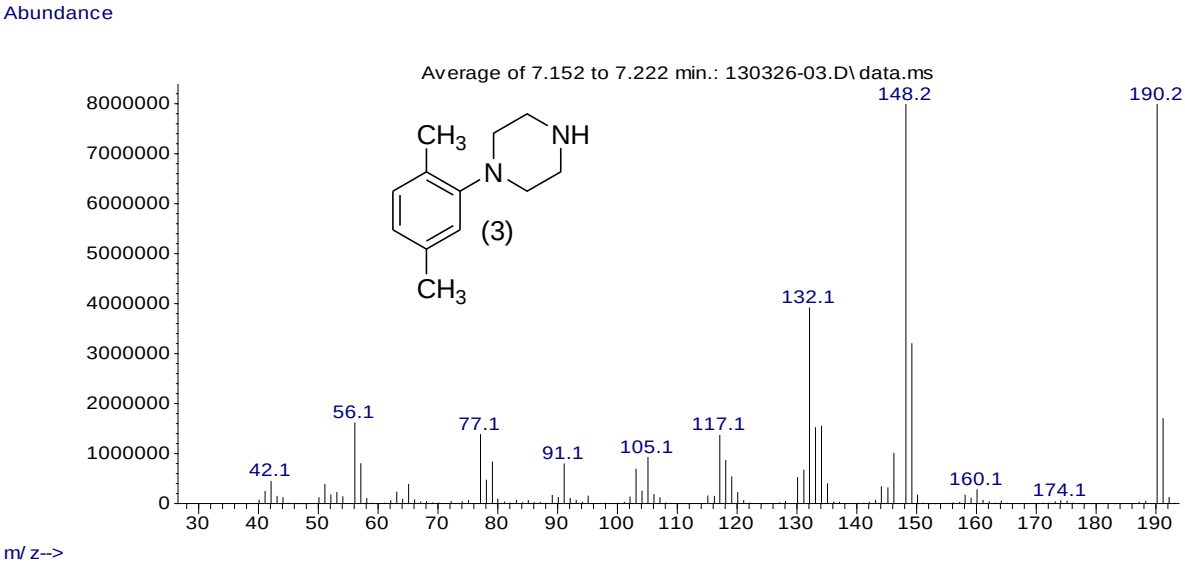
The complete series of regioisomeric dimethylphenylpiperazines were synthesized and evaluated using GC-MS and FT-IR. The EI mass spectra show fragment ions characteristic of both the dimethylphenyl and the piperazine portions of the molecules. These characteristic fragments include the dimethylphenyl aziridinium cation at m/z 148 and dimethylphenyl cation at m/z 105 as well as the m/z 132, 133 and 134 frgament cluster. In addition to that the low mass ion at m/z 56 for the $C_3H_6N^+$ was observed in all piperazine EI spectra. Perfluoroacylation of the secondary amine nitrogen for each of the six regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Attenuated total reflection Fourier transform infrared spectroscopy (ATR FTIR) provides direct confirmatory data for the differentiation between the six regioisomeric aromatic ring substituted dimethylphenylpiperazines. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and retention appears related to the degree of steric crowding of the aromatic ring substituents. The most crowded patterns of substitution elute first while the more symmetrical 1,3,5-substitution pattern has the highest retention time.

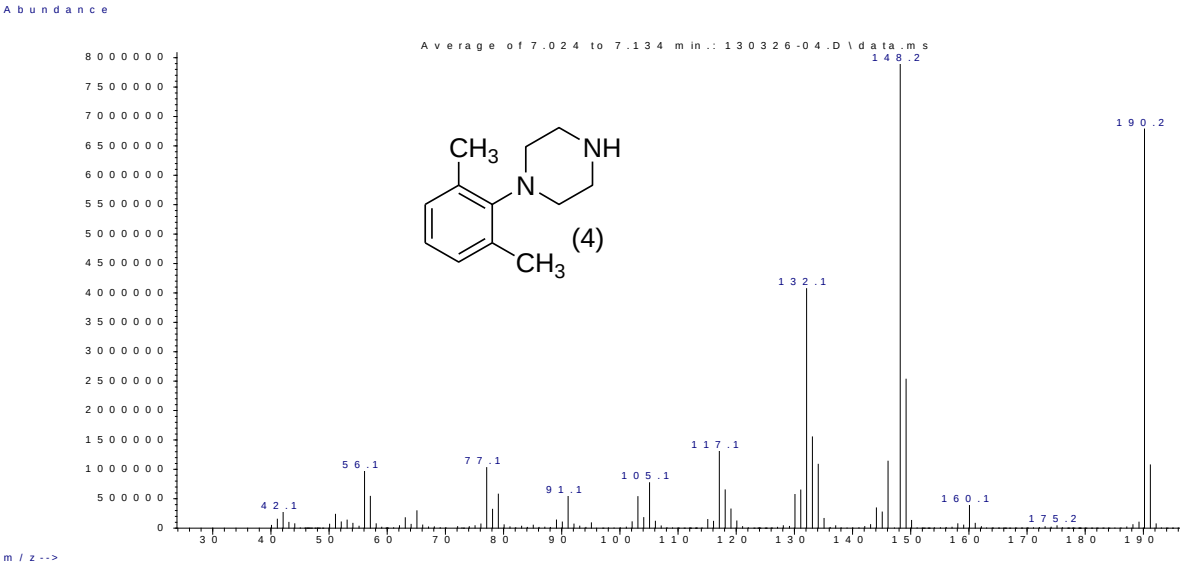
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Dimethylphenylpiperazines (DMPPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 21-1 shows the EI mass spectra of the six regioisomeric dimethylphenylpiperazines (Compounds 1-6). The mass spectra in Figure 21-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. The common fragment ions observed for the regioisomeric dimethyl group substitution on the aromatic ring likely indicate that the piperazine ring is the source for most of the fragmentation. The structures for the fragmentation product ions are summarized in Figure 21-2. The ions of significant relative abundance common to all three regioisomers likely arise from fragmentation of the piperazine ring. The dimethylphenyl aziridinium cation at m/z 148 is the base peak in all these spectra. The structures for the fragment ions in the unsubstituted aromatic ring for phenylpiperazine have been described by de Boer et al [de Boer *et al*, 2001]. Equivalent fragmentation pathways for the dimethylphenylpiperazines (DMPPs) yield the fragment ions at m/z 148, 134, 133, 132, 105 and 56 as shown in Figures 21-1 and 21-2. The structures for the fragments in the six DMPPs regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual DMPP regioisomers.









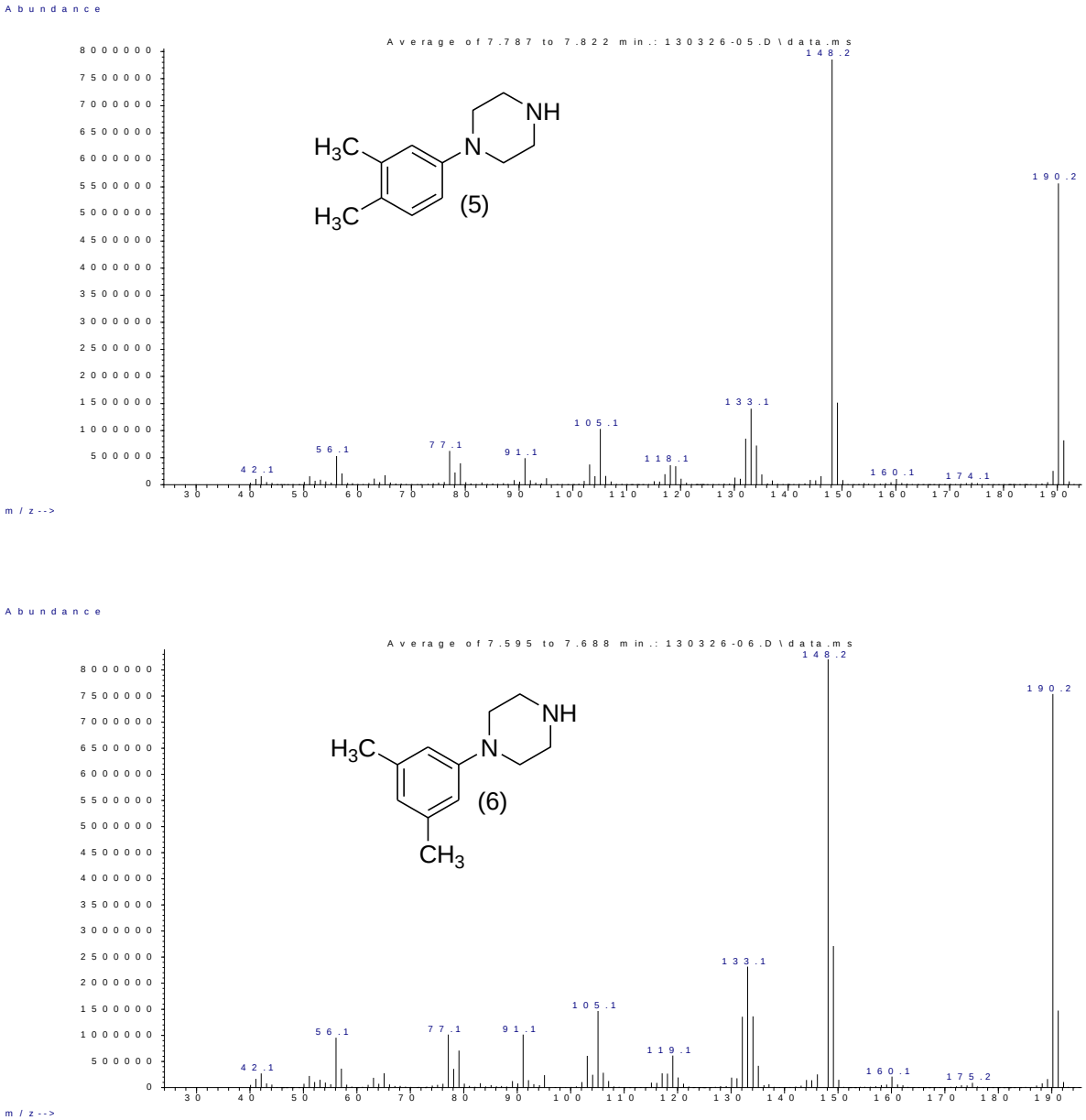


Fig. 21-1: EI mass spectra of the six dimethylphenylpiperazines in this study.

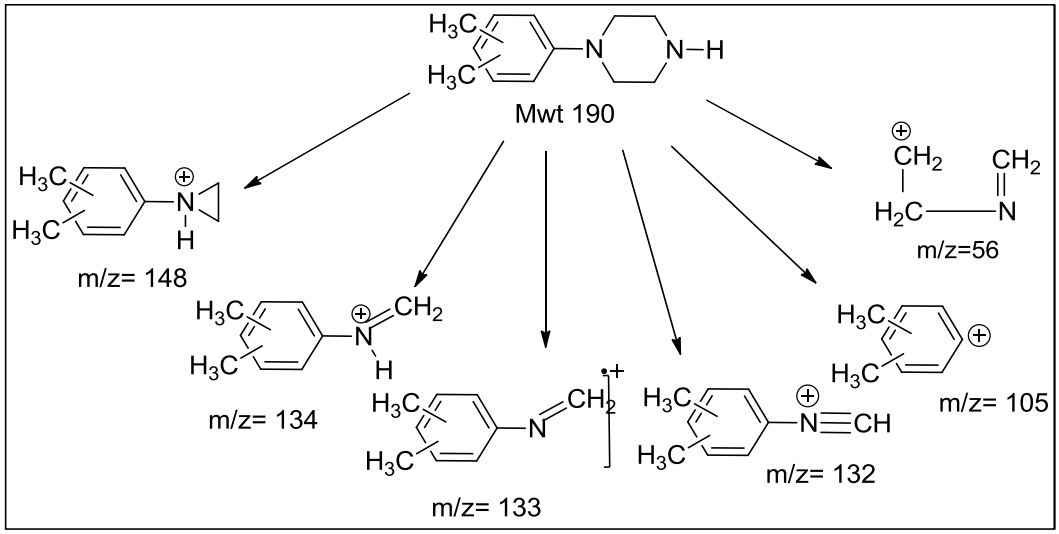
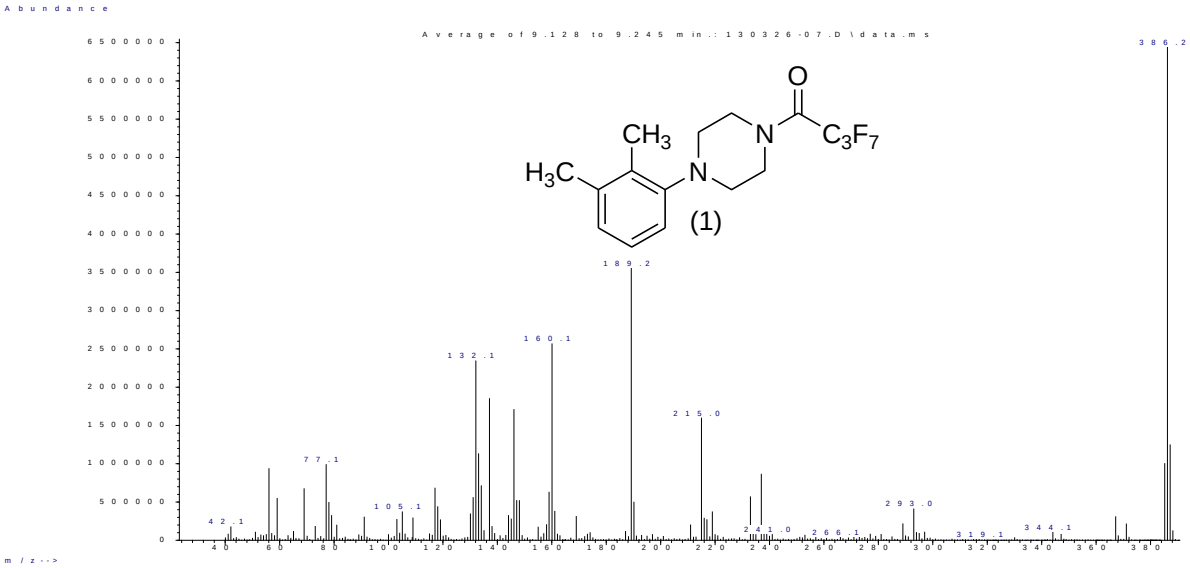
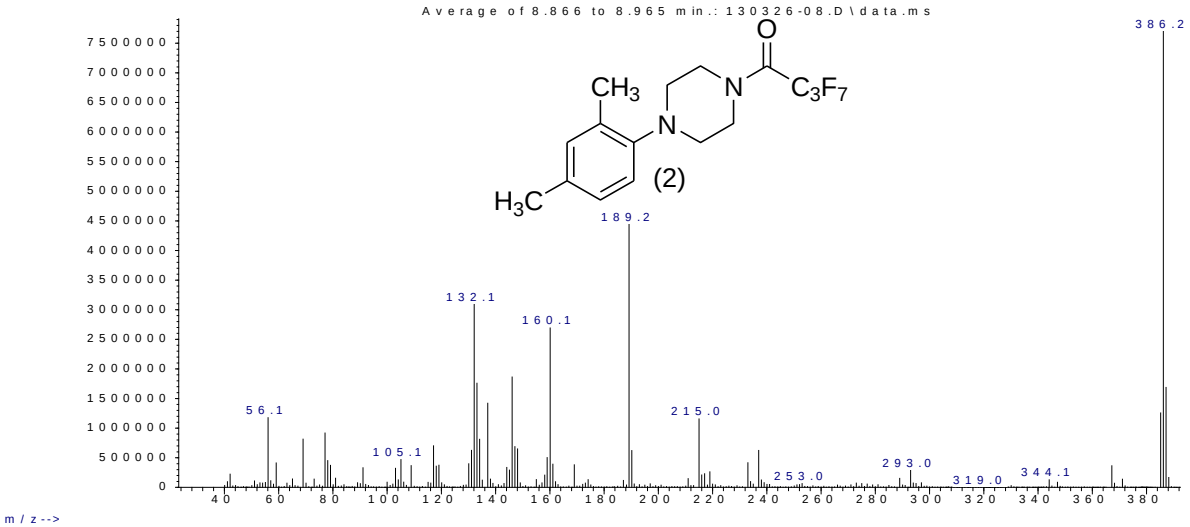
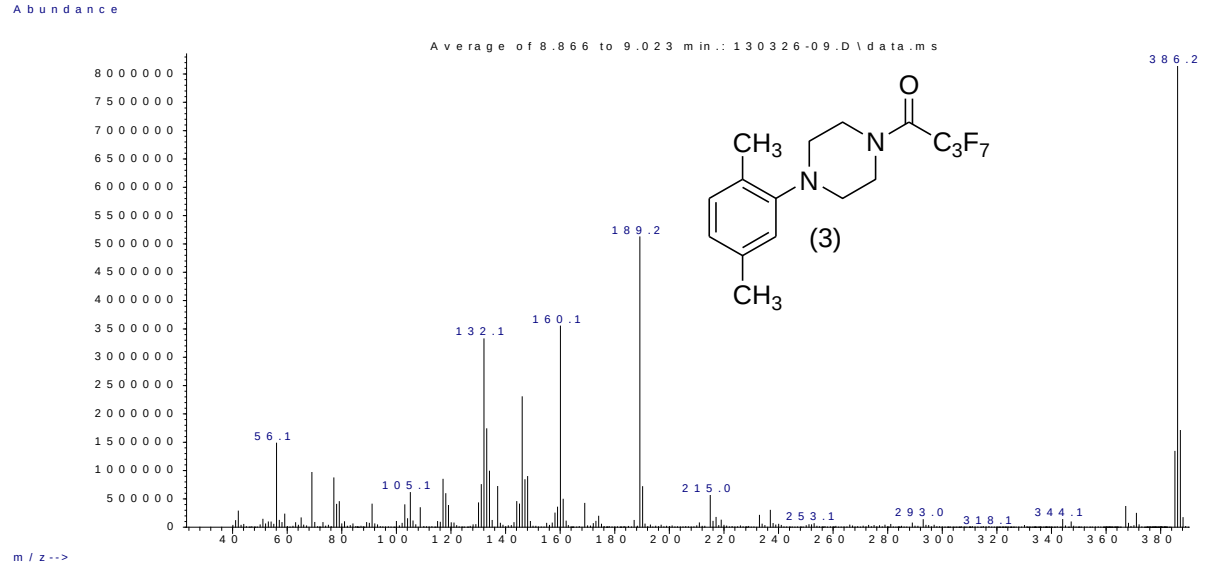


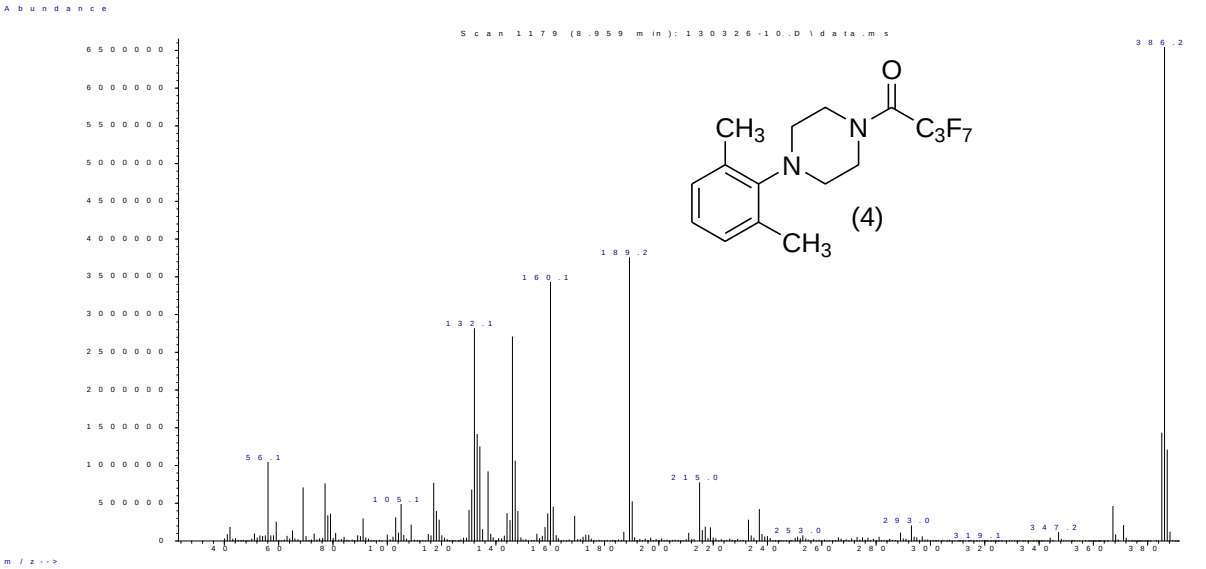
Fig. 21-2: EI mass spectral fragmentation pattern of the underivatized dimethylphenylpiperazines.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the regioisomeric dimethylphenyl piperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds. The heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra of DMPPs. The mass spectra for the six heptaflourobutyryl amides are shown in Figure 21-3. From these spectra, a common peak with high relative abundance occurs at *m/ z* 386 which corresponds to the molecular ions for HFBA amides. Fragment ions occurring at *m/ z* 134, 133, 132, 105 and 56seen in all MS spectra of piperazine acyl amides are due to different patterns of cleavage reactions in the piperazine ring, analogous to those found in the underivatized compounds. Fragment ions at *m/ z* 189 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety from the corresponding derivative. Those occurring at *m/ z* 169 are formed as a result of the elimination of heptafluoropropyl moiety from the HFBA amides. There is no significant difference between the mass spectra of the six compounds. Thus, even acylation of the six piperazines does not give characteristic fragments that help to discriminate among these regioisomeric compounds.









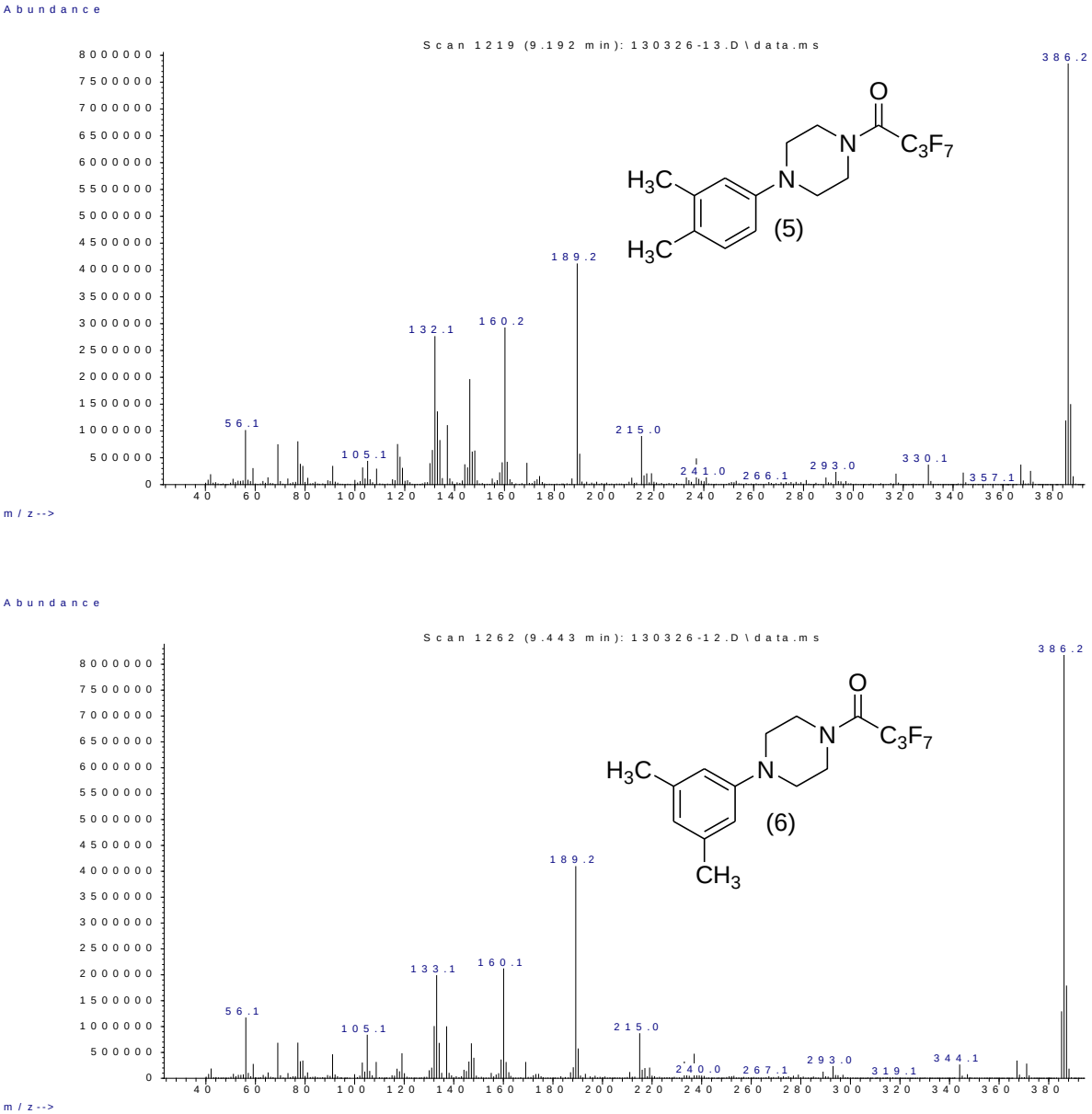


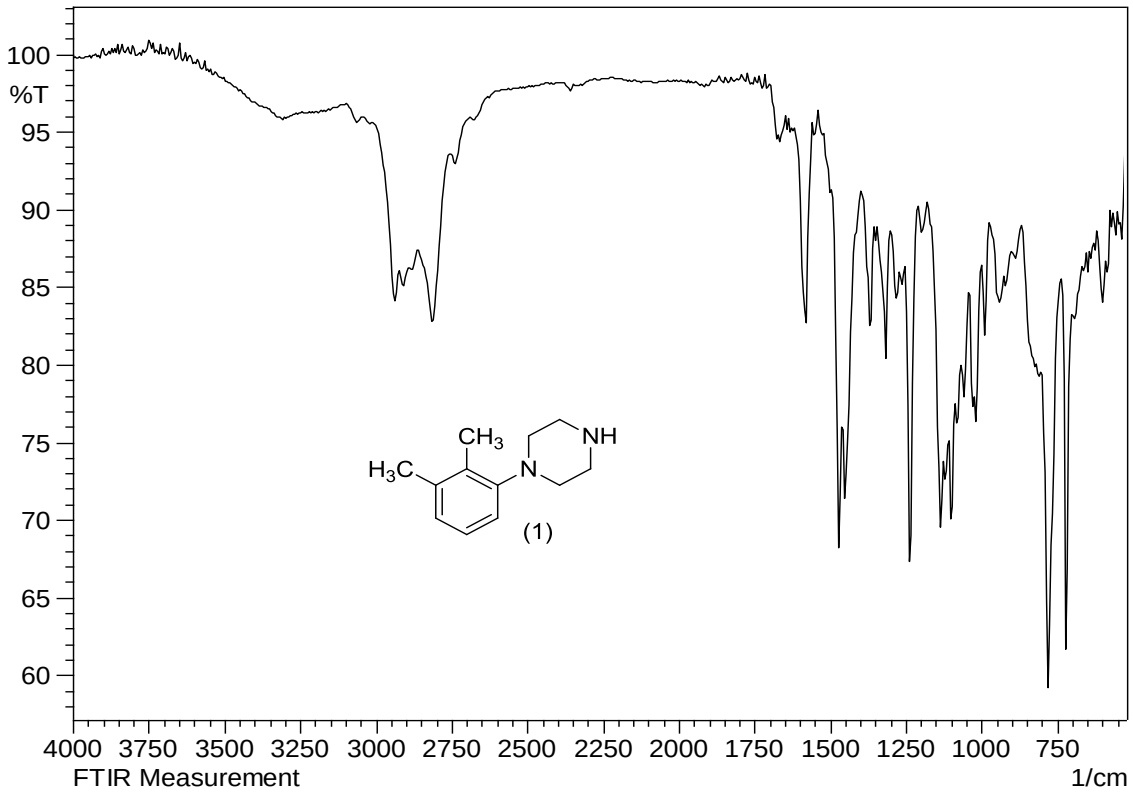
Fig. 21-3: MS spectra of heptafluorobutyryl derivatives of the six dimethylphenylpiperazines.

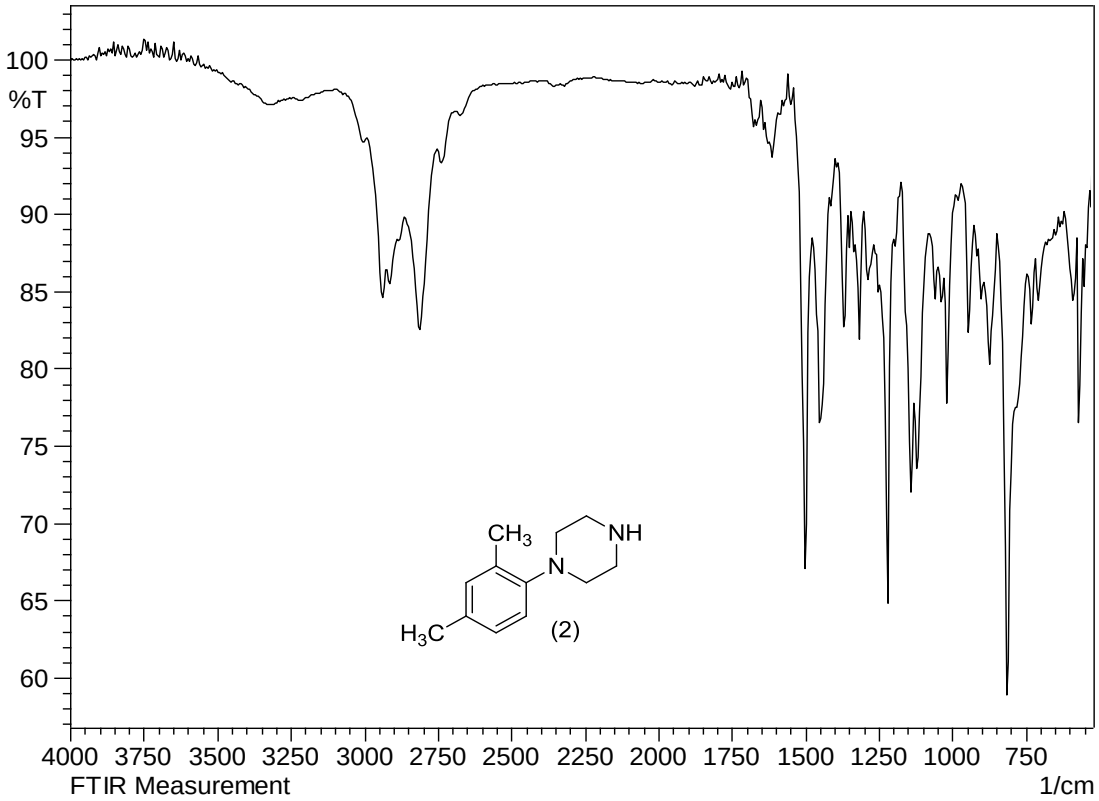
FTIR Spectroscopic Study of the Dimethylphenylpiperazines (DMPPs)

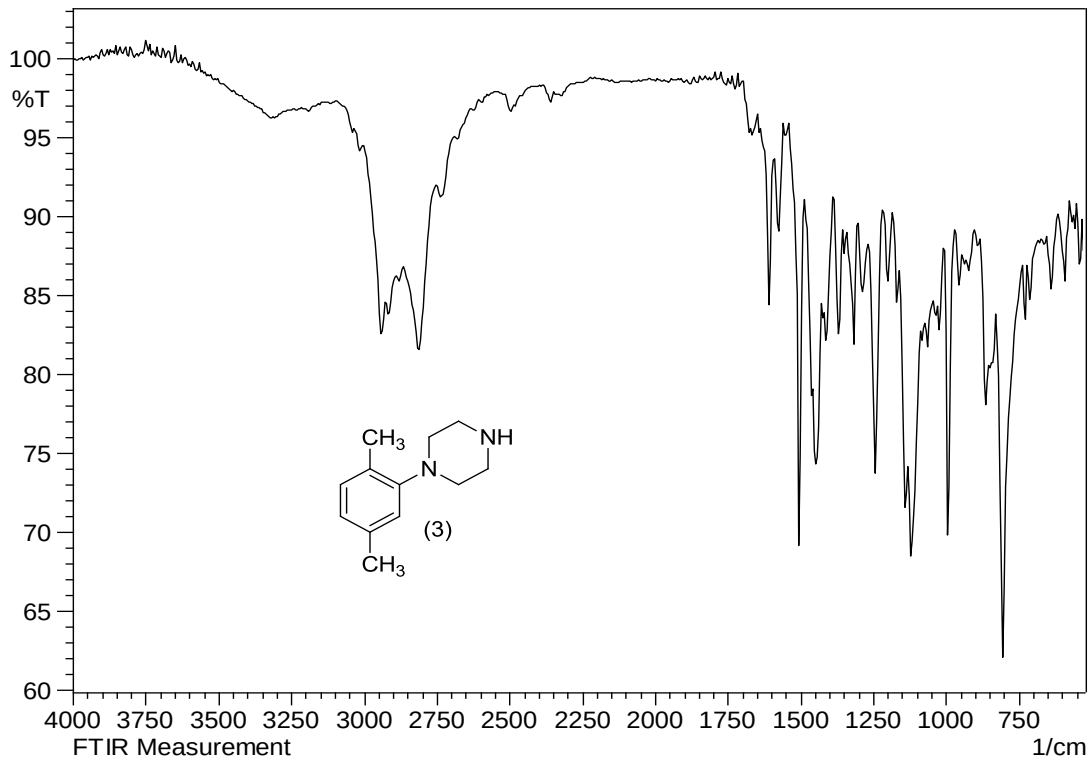
An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. Attenuated total reflection Fourier transform infrared spectroscopy (ATR FTIR) was evaluated for differentiation among the six regioisomeric dimethylphenylpiperazines. Infrared detection could provide compound specificity without the need for chemical modification of the drug molecule. The Attenuated total reflection Fourier transform infrared spectra for the six underivatized piperazines are shown in Figure 21-4.

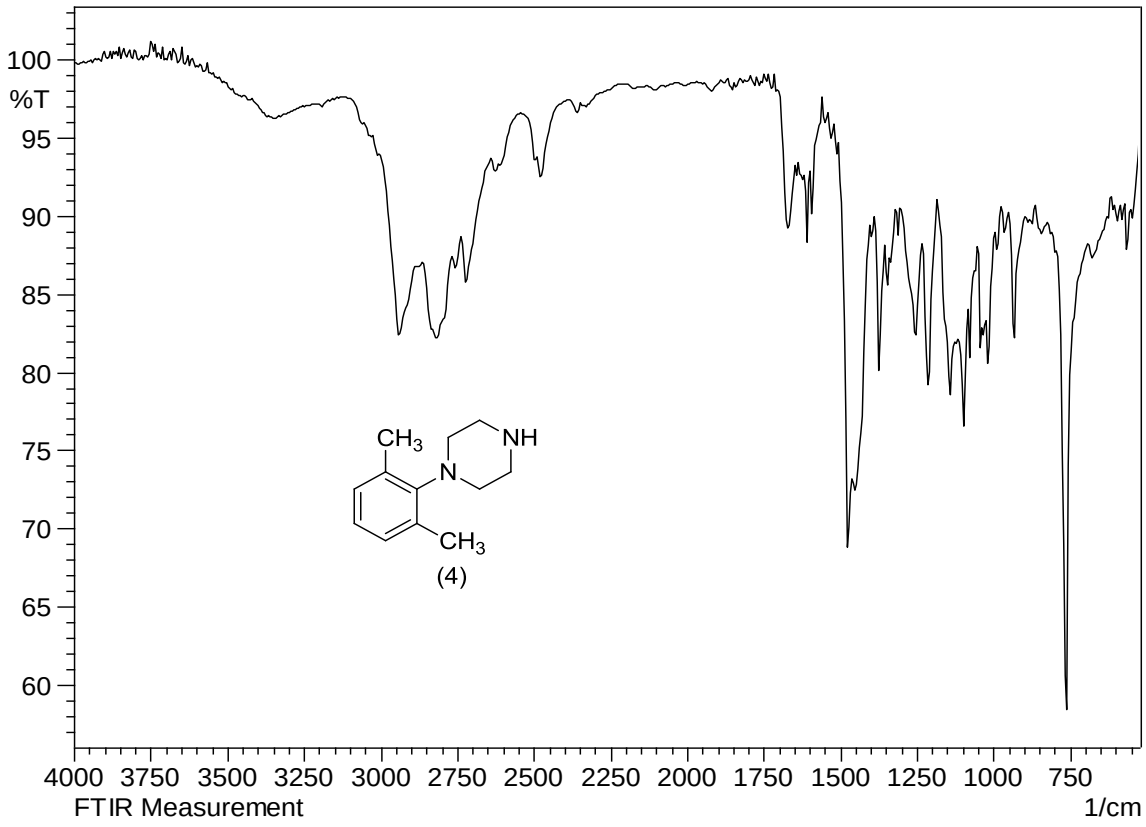
The 2,3-DMPP regioisomer is characterized by the medium intensity band at 1579 cm^{-1} which is shifted to 1504 cm^{-1} in the 2,5-DMPP regioisomer. This isomer also has a doublet at 1471 and 1452 cm^{-1} shifted to a doublet at 1498 and 1450 cm^{-1} in the IR spectrum of the 2,4 isomer. Finally, the IR spectrum of 2,3-DMPP shows a strong band at 1236 [1] which is shifted to 1220 cm^{-1} and 1139 cm^{-1} in the 2,4-DMPP and 2,5-DMPP, respectively. The 3,5-DMPP regioisomer can be distinguished by the relatively strong IR band at 1593 cm^{-1} which is shifted to a strong intensity doublet at 1504 and 1481 cm^{-1} in the 3,4-regioisomer, a strong intensity doublet at 1504 and 1446 cm^{-1} in the 2,5-regioisomer and a strong intensity doublet at 1593 and 1473 cm^{-1} in the 2,6-regioisomer The vapor-phase IR spectrum of the 3,4-DMPP regioisomer can be distinguished by a singlet of strong intensity appearing at 1240 cm^{-1} compared to a peak of strong intensity at 1263 cm^{-1} in the 3,5-isomer, a strong singlet at 1139 cm^{-1} in the 2,5 isomer and two bands of weak intensity at 1253 and 1211 cm^{-1} in the 2,6-isomer.

This study shows that Fourier transform 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 to individualize the regioisomers in this set of uniquely similar compounds. Thus, FTIR readily discriminates between the members of this limited set of regioisomeric dimethylphenylpiperazine compounds.









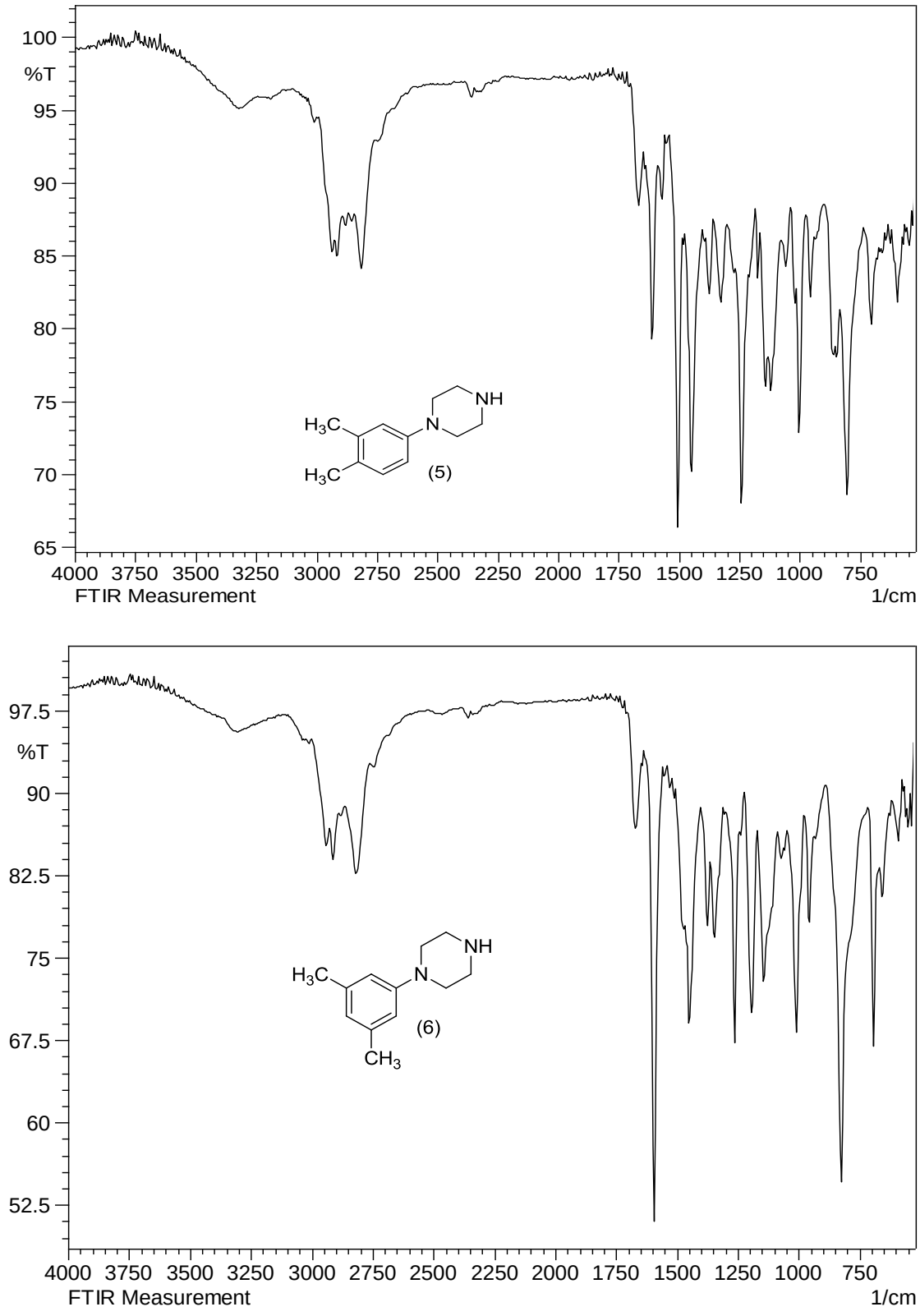


Fig. 21-4: FTIR spectra of the six dimethylphenylpiperazines in this study.

Gas Chromatographic Separation of the Dimethylphenylpiperazines (DMPPs)

Gas chromatographic separation of the HFBA derivatives of the six dimethylphenylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 21-5. The separation of the heptafluorobutyryl derivatives was performed using a temperature program consisting of an initial hold at 70°C for 1.0 min, ramped up to 250°C at a rate of 30°C/min, held at 250°C for 15.0 min

This chromatogram shows the separation of the six regioisomers in this study. The elution order appears related to the degree of substituent crowding on the aromatic ring. Compounds 1 and 4 elute first and these two isomers contain substituents arranged in a 1,2,3-pattern on the aromatic ring. Three isomers (Compounds 2, 3 and 5) have two groups substituted 1,2 with one isolated substituent. The 1,3,5-trisubstituted pattern in Compound 6 provides minimum intramolecular crowding and elutes last in this group of compounds. The two compounds with maximum crowding substituted in a 1,2,3 manner on the aromatic ring show the 2,3-dimethyl substitution pattern to elute first followed by the 2,6-dimethyl isomer eluting second. The relative position of the methyl groups appears to determine the elution order in the three compounds having two groups substituted in a 1,2 pattern. Within this group of three compounds the first to elute is the 1,4-relationship for the two methyl groups in compound 3. This is followed by the 1,3-pattern for compound 2 and lastly the 1,2-pattern for compound 5.

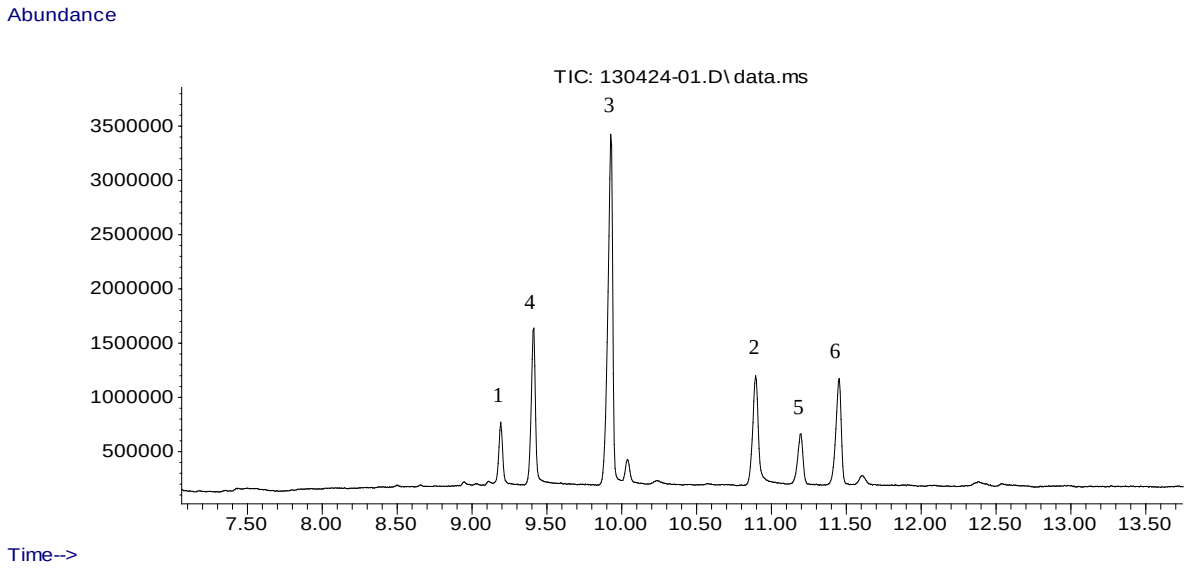


Fig. 21-5: Gas chromatographic separation of the heptafluorobutyryl derivatives of the six dimethylphenylpiperazines using Rtx-200 column.

Conclusion

The six regioisomeric dimethylphenylpiperazines yield the same fragment ions in their mass spectra. Perfluoroacylation of the secondary amine nitrogen for each of the six regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. ATR FTIR analysis yields unique and characteristic infrared spectra for these regioisomeric piperazines. These spectra allow discrimination among the six regioisomeric compounds included in this study. This differentiation was accomplished without the need for chemical derivatization. Mixtures of the dimethylphenylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order appears related to the degree of substituent crowding on the aromatic ring with the most crowded 1,2,3 substitution patterns eluting first and the highest retention for the compound with minimum intramolecular crowding (the 1,3,5-trisubstitution pattern).

References

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 Science International 121 (2001) 47-56.

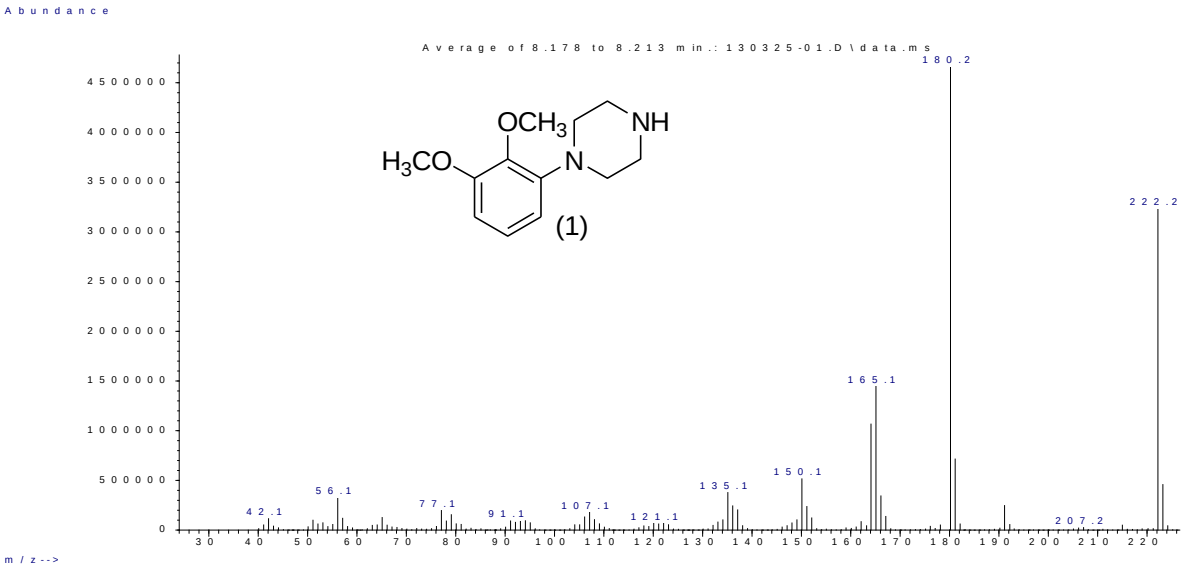
Chapter 22

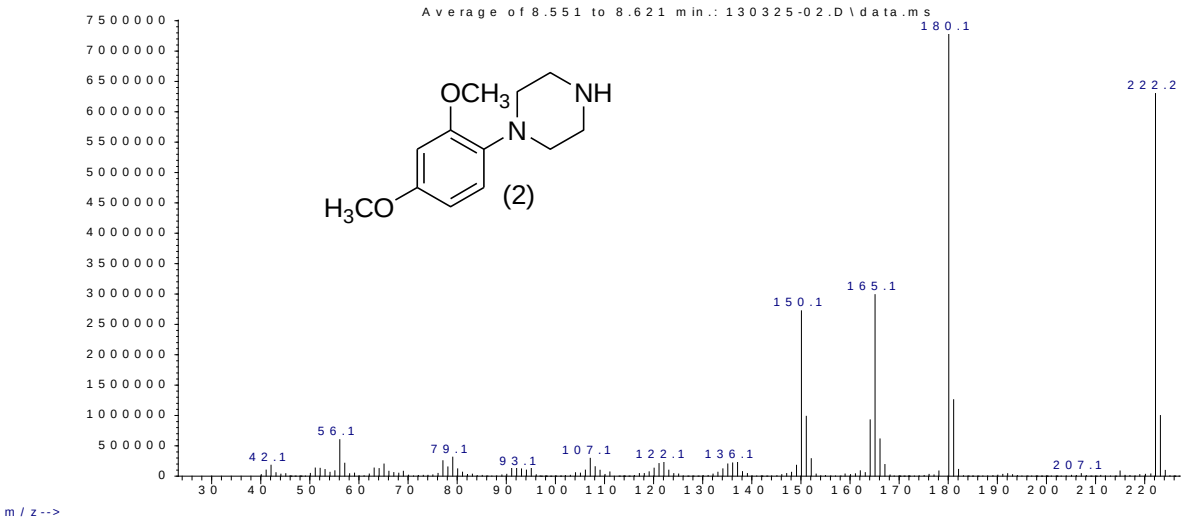
GC-MS and ATR FTIR Studies on the Six Ring Regioisomeric Dimethoxyphenylpiperazines (DOMePPs)

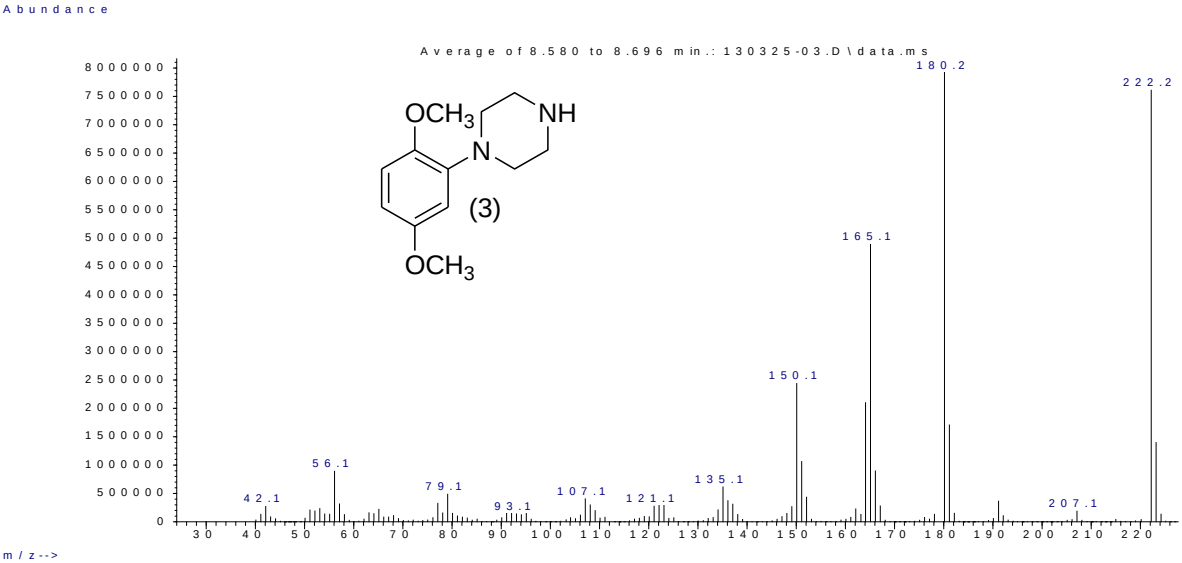
The complete series of regioisomeric dimethoxyphenylpiperazines were synthesized and evaluated using GC-MS and FT-IR. The EI mass spectra show fragment ions characteristic of both the dimethoxyphenyl and the piperazine portions of the molecules. These characteristic fragments include the dimethoxyphenyl aziridinium cation at m/z 180 and dimethoxyphenyl cation at m/z 137 as well as the m/z 164, 165 and 166 frgament cluster. In addition to that the low mass ion at m/z 56 for the $C_3H_6N^+$ was observed in all piperazine EI spectra. Perfluoroacylation of the secondary amine nitrogen for each of the six regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. Attenuated total reflection Fourier transform infrared spectroscopy (ATR FTIR) provides direct confirmatory data for the differentiation between the six regioisomeric aromatic ring substituted dimethoxyphenylpiperazines. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and retention appears related to the degree of steric crowding of the aromatic ring substituents. The most crowded patterns of substitution elute first while the more symmetrical 1,3,5-substitution pattern has the highest retention time.

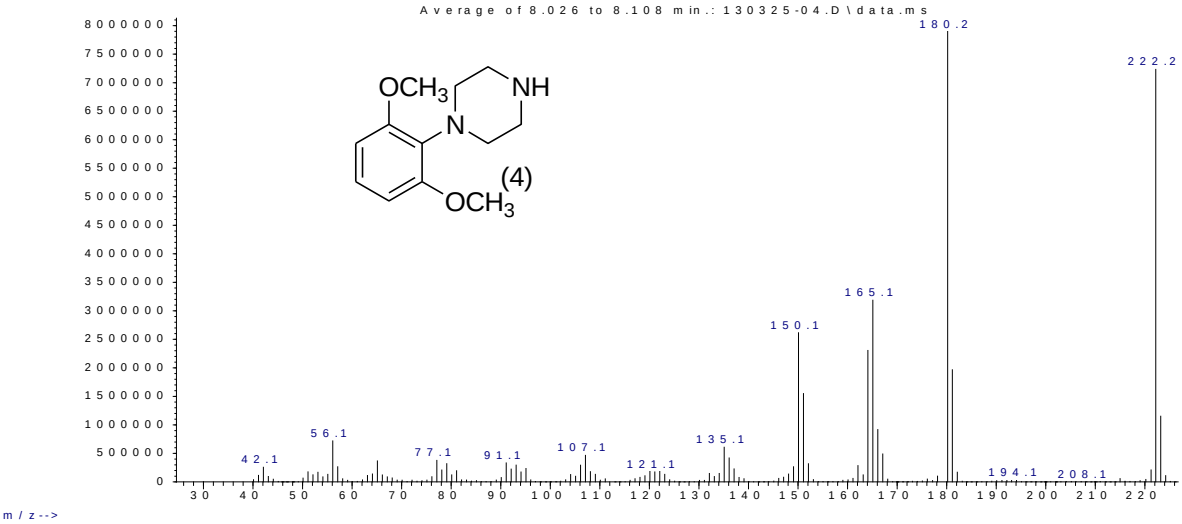
Mass spectral studies of the underivatized and perfluoroacylated derivatives of Dimethoxyphenylpiperazines (DOMePPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 22-1 shows the EI mass spectra of the six regioisomeric dimethylphenylpiperazines (Compounds 1-6). The mass spectra in Figure 22-1 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses. The common fragment ions observed for the regioisomeric dimethoxy group substitution on the aromatic ring likely indicate that the piperazine ring is the source for most of the fragmentation. The structures for the fragmentation product ions are summarized in Figure 22-2. The ions of significant relative abundance common to all three regioisomers likely arise from fragmentation of the piperazine ring. The dimethoxyphenyl aziridinium cation at m/z 180 is the base peak in all these spectra. The structures for the fragment ions in the unsubstituted aromatic ring for phenylpiperazine have been described by de Boer et al [de Boer *et al*, 2001]. Equivalent fragmentation pathways for the dimethoxyphenylpiperazines (DMPPs) yield the fragment ions at m/z 180, 166, 165, 164, 137 and 56 as shown in Figures 22-1 and 22-2. The structures for the fragments in the six DOMePPs regioisomers are likely equivalent. These data indicate that mass spectrometry does not provide confirmation of identity for an individual DOMePP regioisomers.









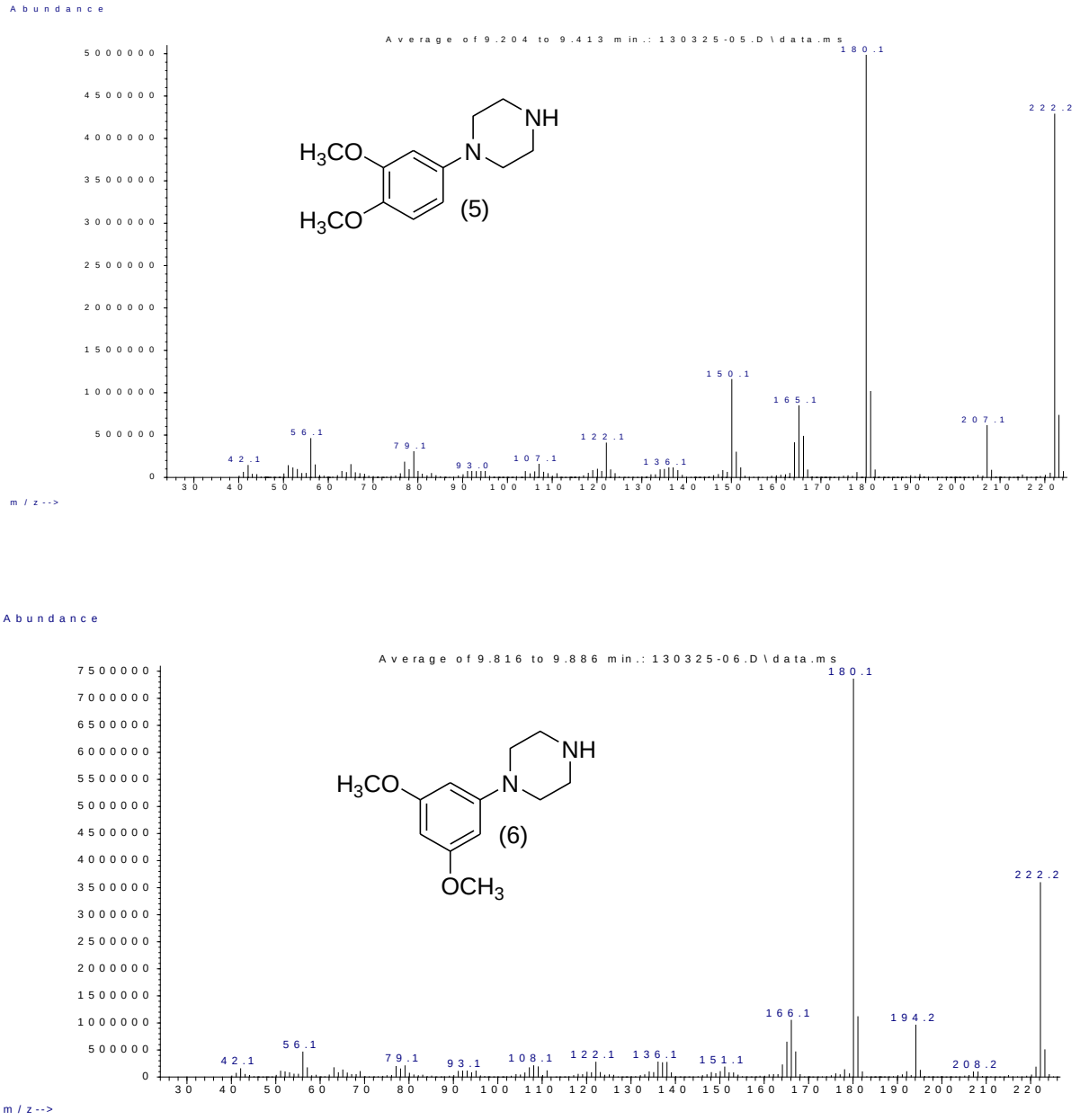


Fig. 22-1: EI mass spectra of the six dimethoxyphenylpiperazines in this study.

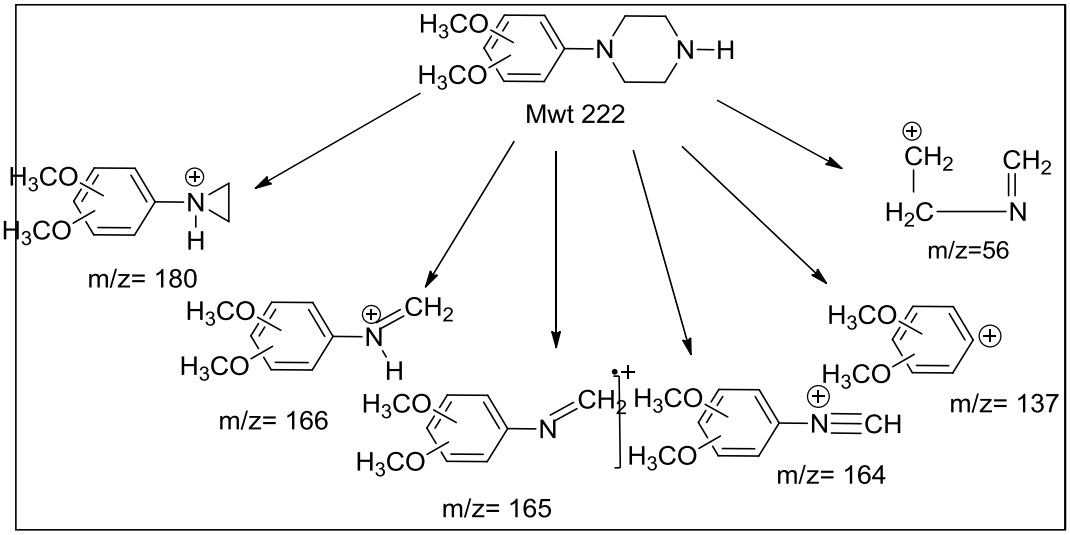
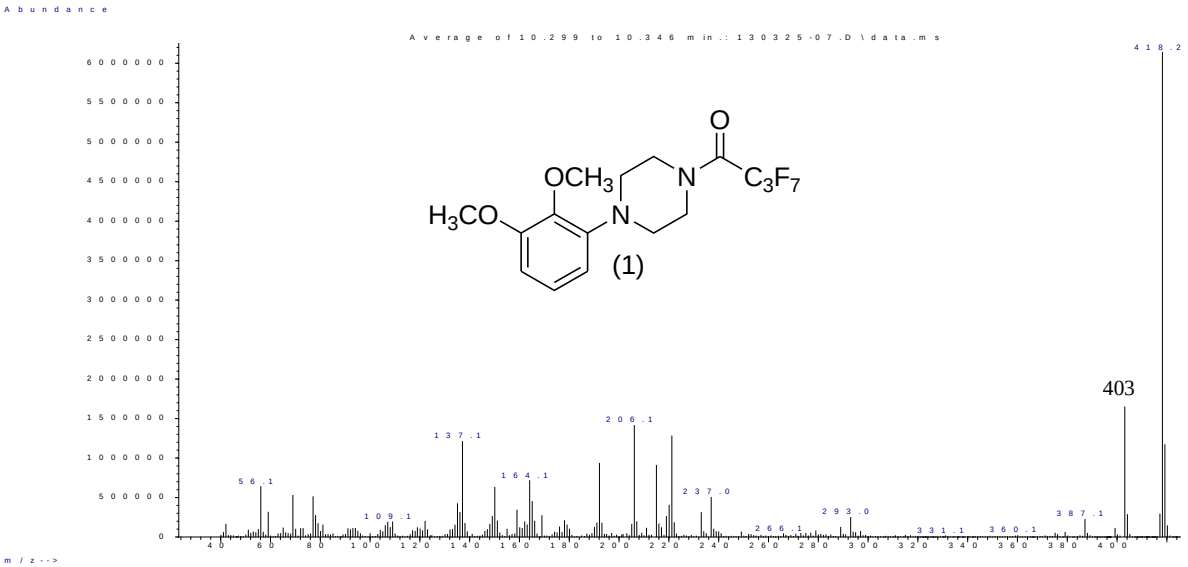
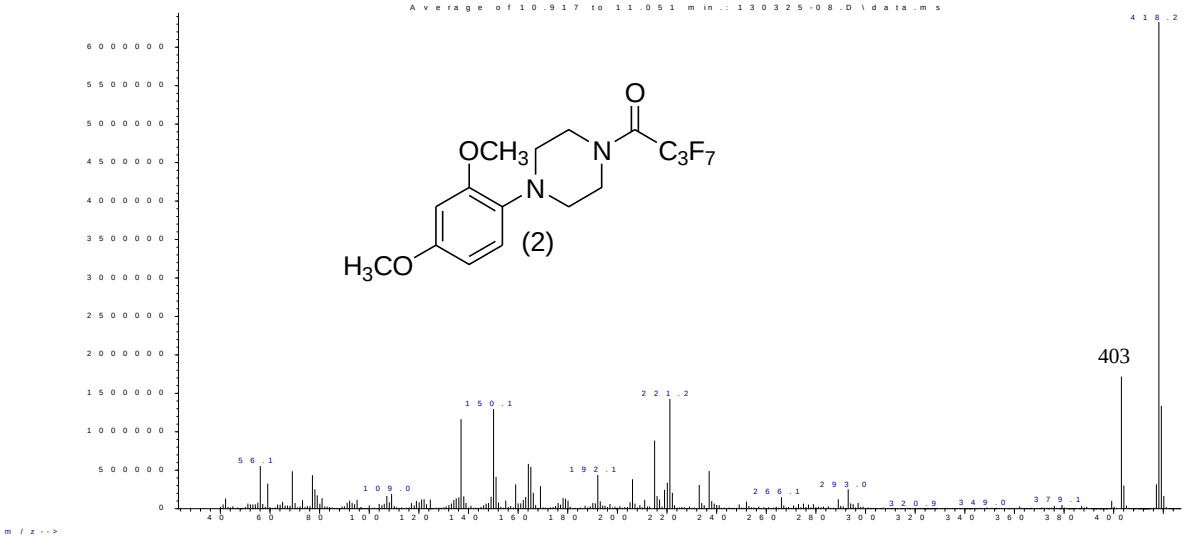
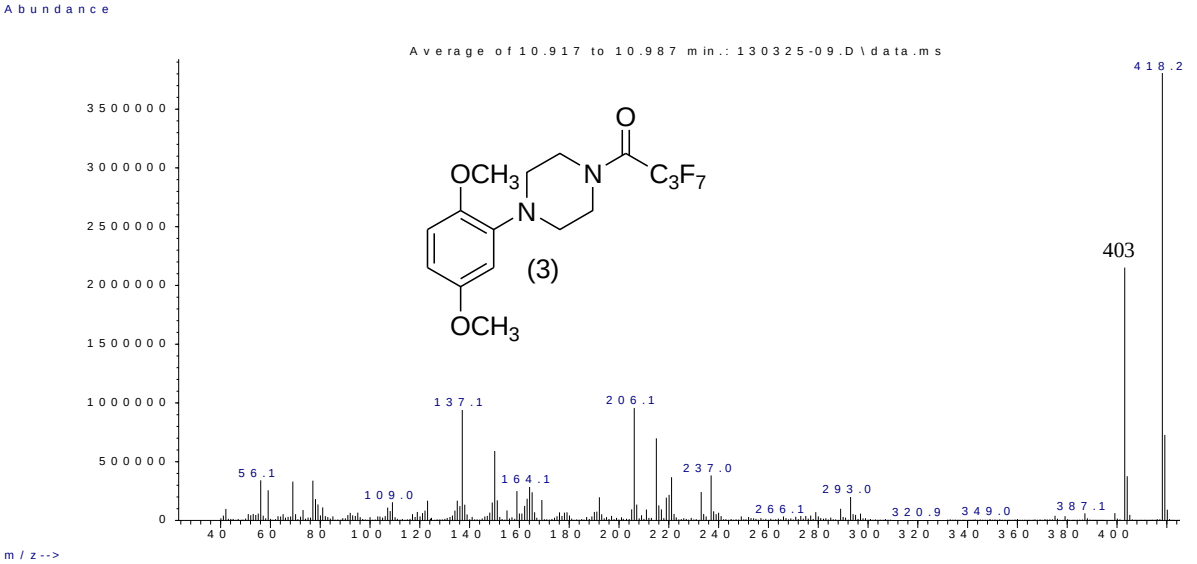


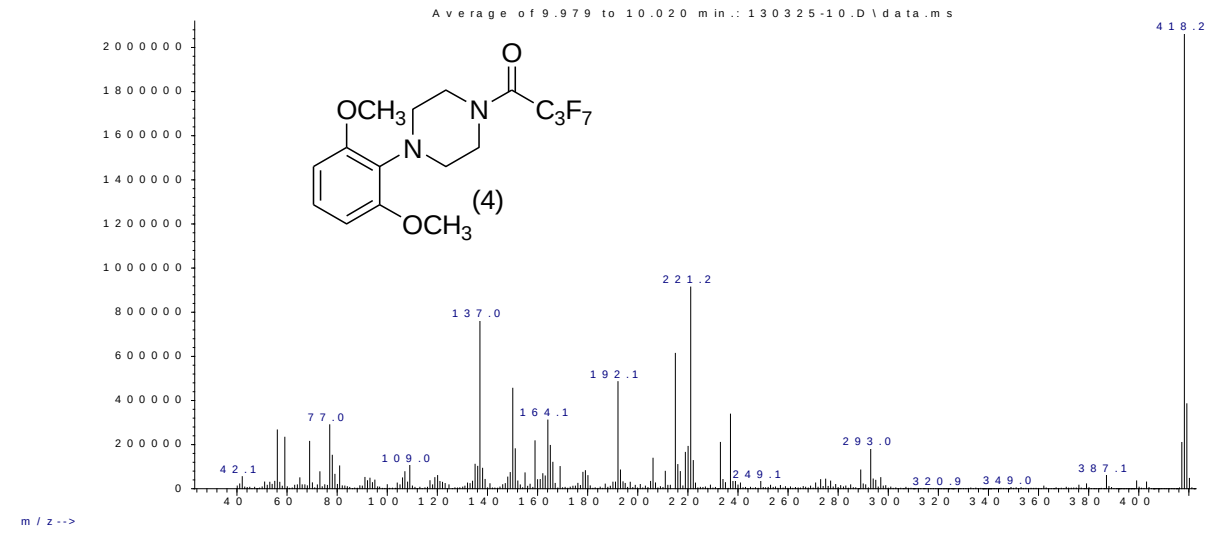
Fig. 22-2: EI mass spectral fragmentation pattern of the underivatized dimethoxyphenylpiperazines.

The second phase of this study involved the preparation and evaluation of acylated derivatives of the regioisomeric dimethoxyphenyl piperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these compounds. The heptafluorobutryl derivatives were evaluated for their ability to individualize the mass spectra of DOMePPs. The mass spectra for the six heptaflourobutyryl amides are shown in Figure 22-3. From these spectra, a common peak with high relative abundance occurs at *m/ z* 418 which corresponds to the molecular ions for HFBA amides. Fragment ions occurring at *m/ z* 166, 165, 164, 137 and 56 seen in all MS spectra of piperazine acyl amides are due to different patterns of cleavage reactions in the piperazine ring, analogous to those found in the underivatized compounds. Fragment ions at *m/ z* 221 seen in all derivatized spectra are likely formed by the elimination of the acyl moiety from the corresponding derivative. Those occurring at *m/ z* 169 are formed as a result of the elimination of heptafluoropropyl moiety from the HFBA amides. There is no significant difference between the mass spectra of the six compounds. Thus, even acylation of the six piperazines does not give characteristic fragments that help to discriminate among these regioisomeric compounds.









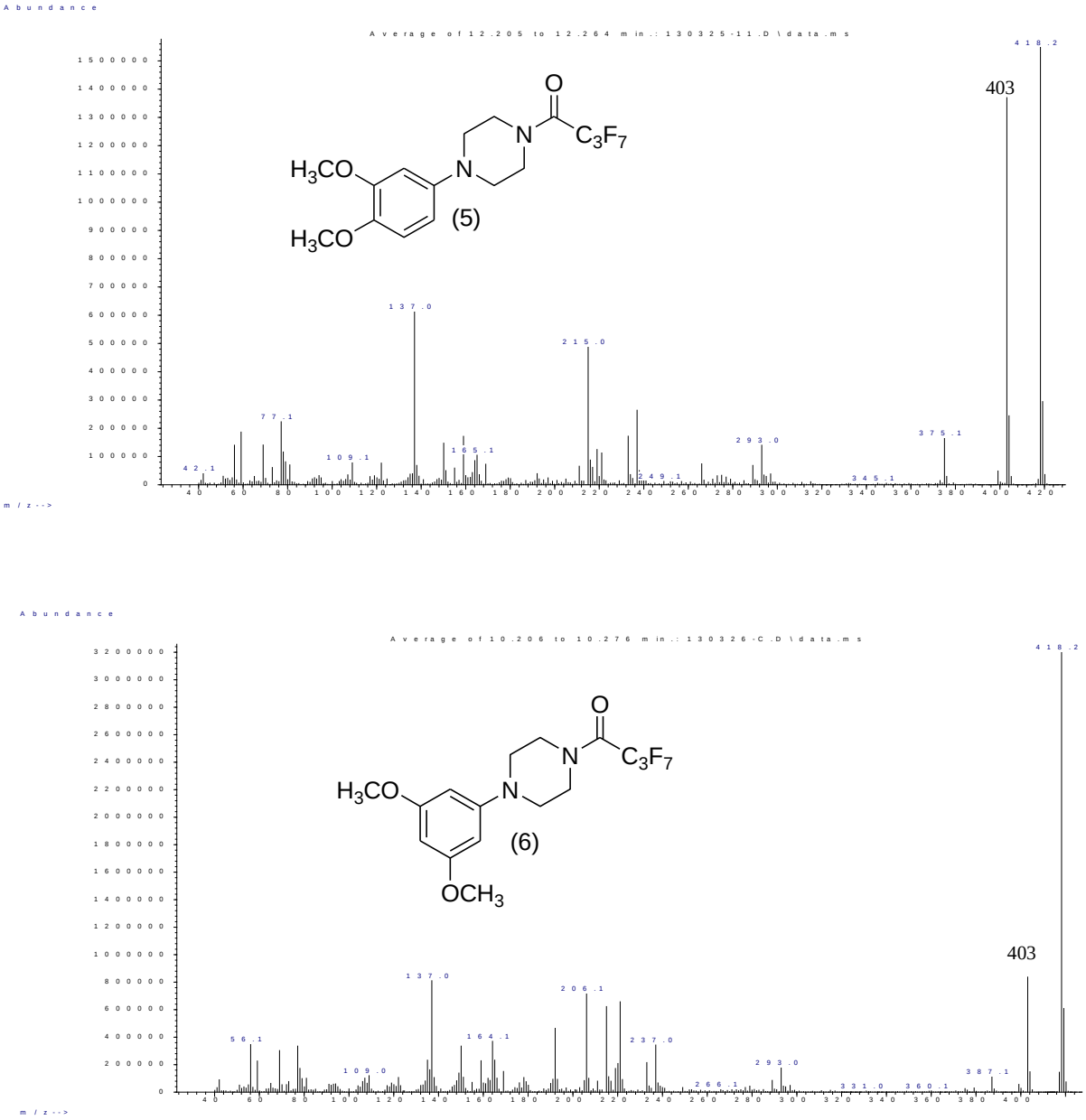


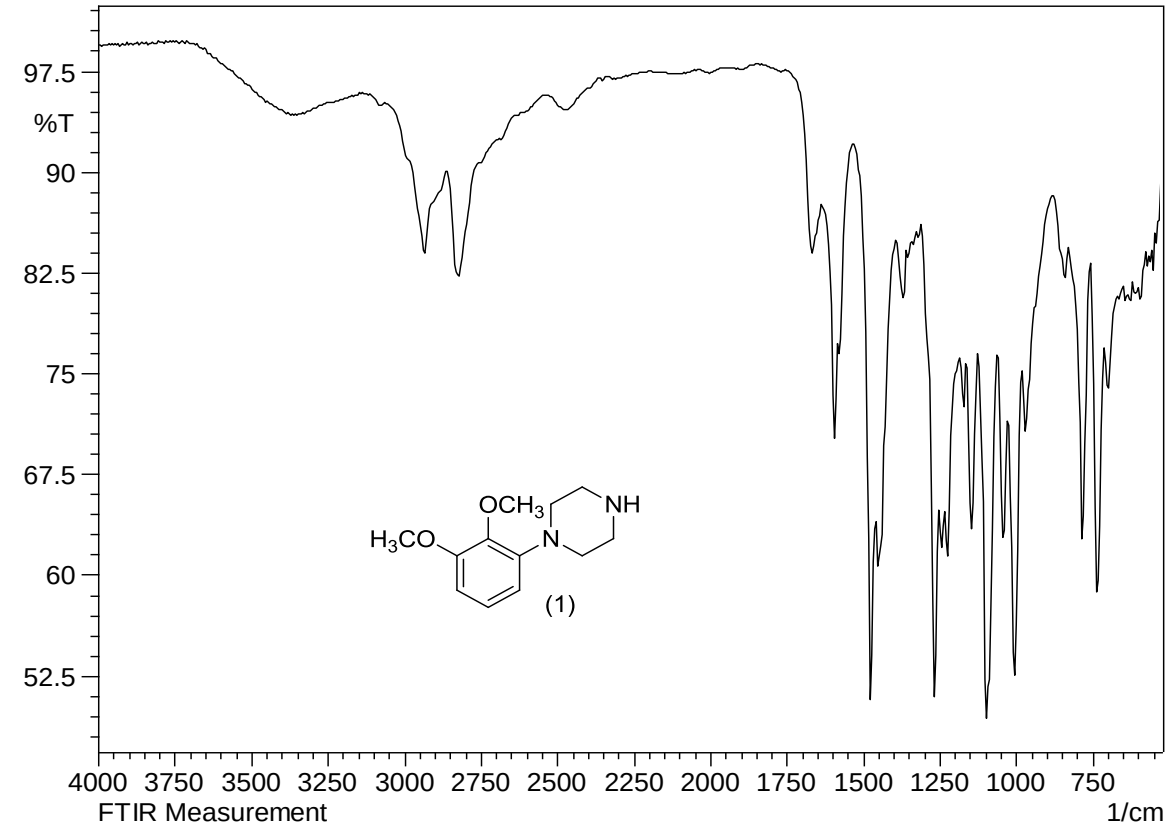
Fig. 22-3: MS spectra of heptafluorobutyryl derivatives of the six dimethoxyphenylpiperazines.

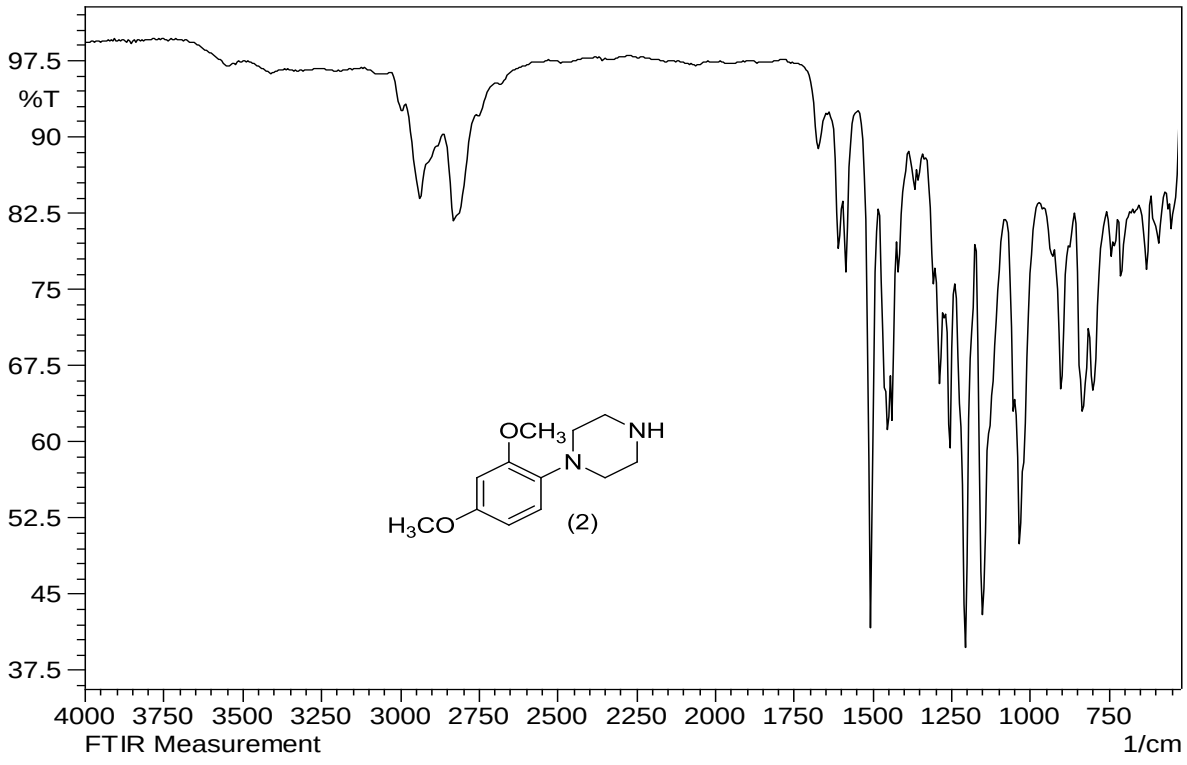
FTIR Spectroscopic Study of the Dimethoxyphenylpiperazines (DOMePPs)

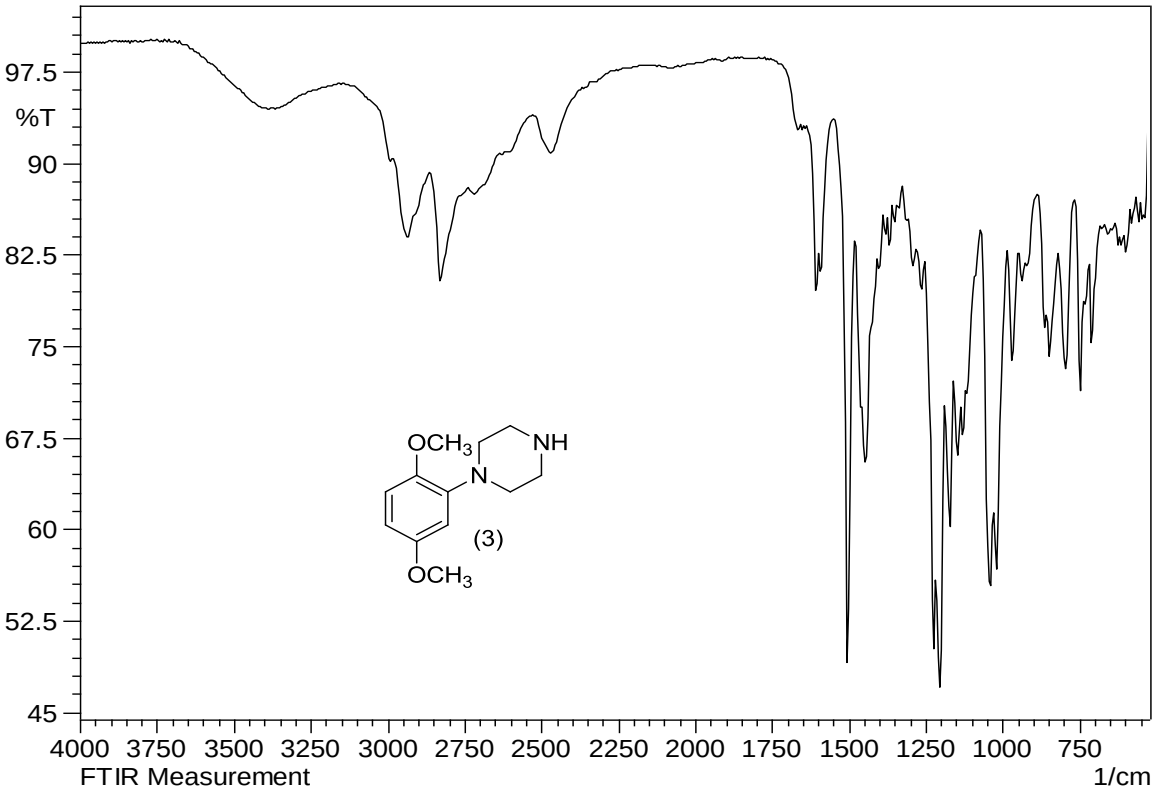
An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. Attenuated total reflection Fourier transform infrared spectroscopy (ATR FTIR) was evaluated for differentiation among the six regioisomeric dimethoxyphenylpiperazines. Infrared detection could provide compound specificity without the need for chemical modification of the drug molecule. The Attenuated total reflection Fourier transform infrared spectra for the six underivatized piperazines are shown in Figure 22-4.

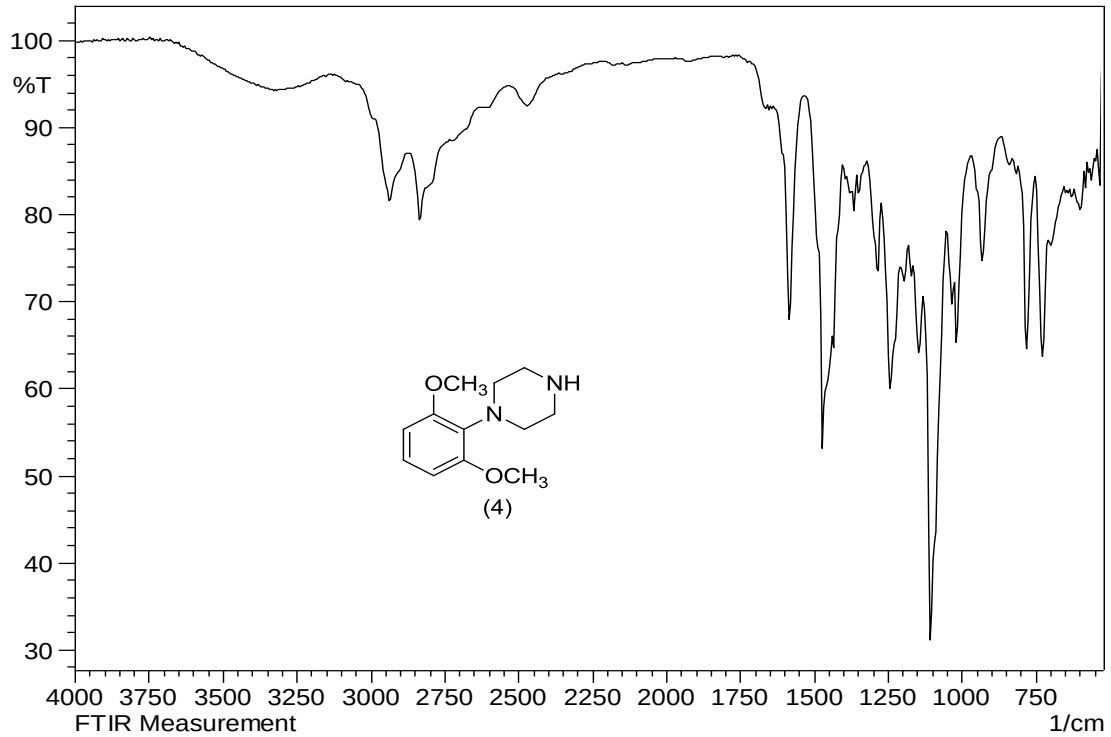
The 2,3-DOMePP regioisomer is characterized by the medium intensity band at 1579 cm^{-1} which is shifted to 1504 cm^{-1} in the 2,5-DOMePP regioisomer. This isomer also has a doublet at 1471 and 1452 cm^{-1} shifted to a doublet at 1498 and 1450 cm^{-1} in the IR spectrum of the 2,4 isomer. Finally, the IR spectrum of 2,3-DOMePP shows a strong band at 1236 [1] which is shifted to 1220 cm^{-1} and 1139 cm^{-1} in the 2,4-DOMePP and 2,5-DOMePP, respectively. The 3,5-DOMePP regioisomer can be distinguished by the relatively strong IR band at 1593 cm^{-1} which is shifted to a strong intensity doublet at 1504 and 1481 cm^{-1} in the 3,4-regioisomer, a strong intensity doublet at 1504 and 1446 cm^{-1} in the 2,5-regioisomer and a strong intensity doublet at 1593 and 1473 cm^{-1} in the 2,6-regioisomer The vapor-phase IR spectrum of the 3,4-DOMePP regioisomer can be distinguished by a singlet of strong intensity appearing at 1240 cm^{-1} compared to a peak of strong intensity at 1263 cm^{-1} in the 3,5-isomer, a strong singlet at 1139 cm^{-1} in the 2,5 isomer and two bands of weak intensity at 1253 and 1211 cm^{-1} in the 2,6-isomer.

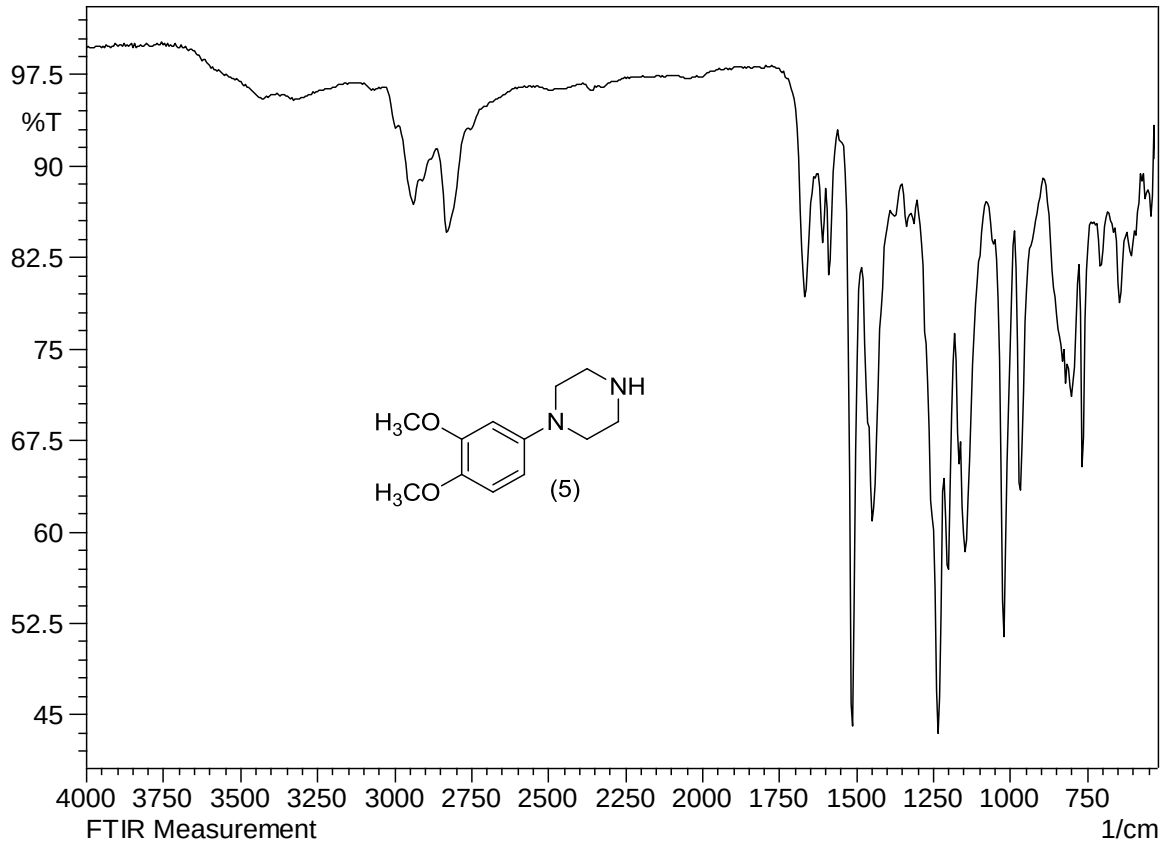
This study shows that Fourier transform infrared spectra provide useful data for differentiation among these regioisomeric piperazines of mass spectral equivalence. Mass spectrometry establishes these compounds as having an isomeric relationship of equal molecular weight and equivalent major fragment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











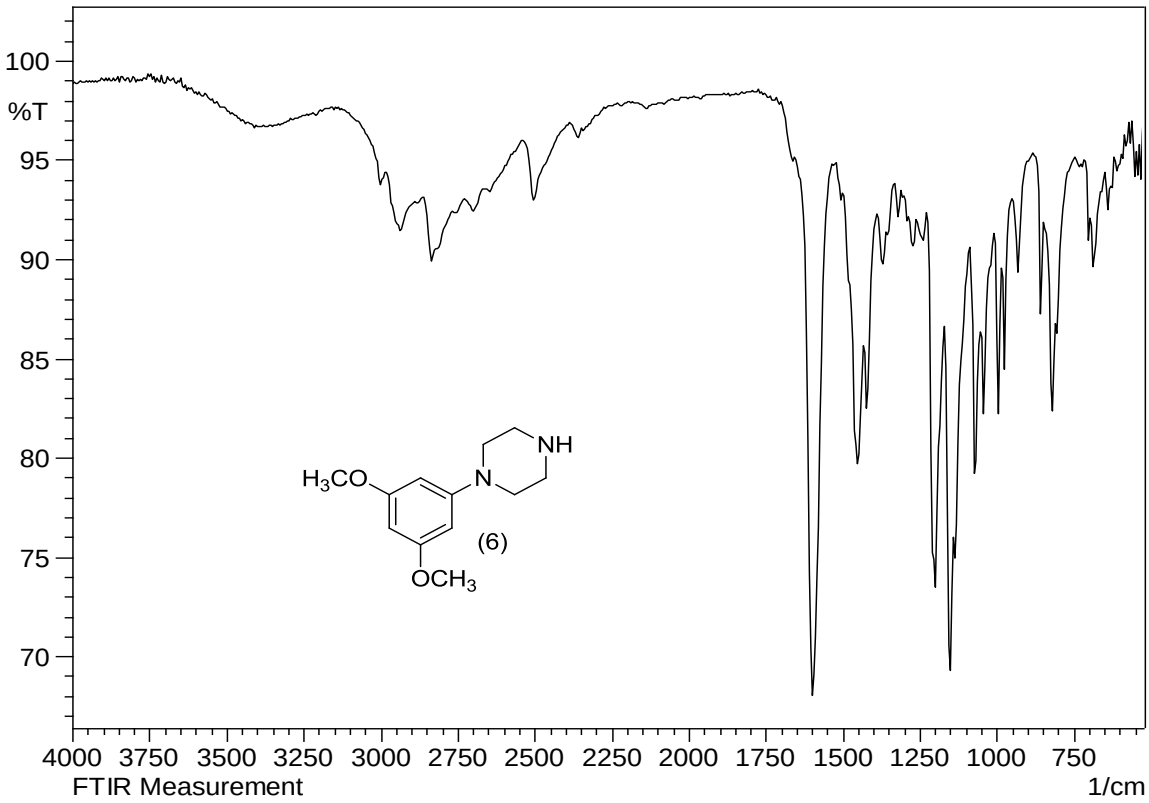


Fig. 21-4: FTIR spectra of the six dimethoxyphenylpiperazines in this study.

Gas Chromatographic Separation of the Dimethoxyphenylpiperazines (DOMePPs)

Gas chromatographic separation of the HFBA derivatives of the six dimethoxyphenylpiperazines was accomplished using an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase in a capillary column (30m × 0.25mm) of 0.5-µm film thickness. Several temperature programs were evaluated and the most efficient program was used to generate the representative chromatogram in Figure 21-5. The separation of the heptafluorobutyryl derivatives was performed using a temperature program consisting of an initial hold at 70°C for 1.0 min, ramped up to 150°C at a rate of 7.5°C/min, held at 150°C for 2.0 min then ramped up to 250°C at a rate of 10°C/min, held at 250°C for 25.0 min

This chromatogram shows the separation of the six regioisomers in this study. The elution order appears related to the degree of substituent crowding on the aromatic ring. Compounds 1 and 4 elute first and these two isomers contain substituents arranged in a 1,2,3-pattern on the aromatic ring. Three isomers (Compounds 2, 3 and 5) have two groups substituted 1,2 with one isolated substituent. The 1,3,5-trisubstituted pattern in Compound 6 provides minimum intramolecular crowding and elutes last in this group of compounds. The two compounds with maximum crowding substituted in a 1,2,3 manner on the aromatic ring show the 2,3-dimethoxy substitution pattern to elute first followed by the 2,6-dimethoxy isomer eluting second. The relative position of the methoxy groups appears to determine the elution order in the three compounds having two groups substituted in a 1,2 pattern. Within this group of three compounds the first to elute is the 1,4-relationship for the two methoxy groups in compound 3. This is followed by the 1,3-pattern for compound 2 and lastly the 1,2-pattern for compound 5.

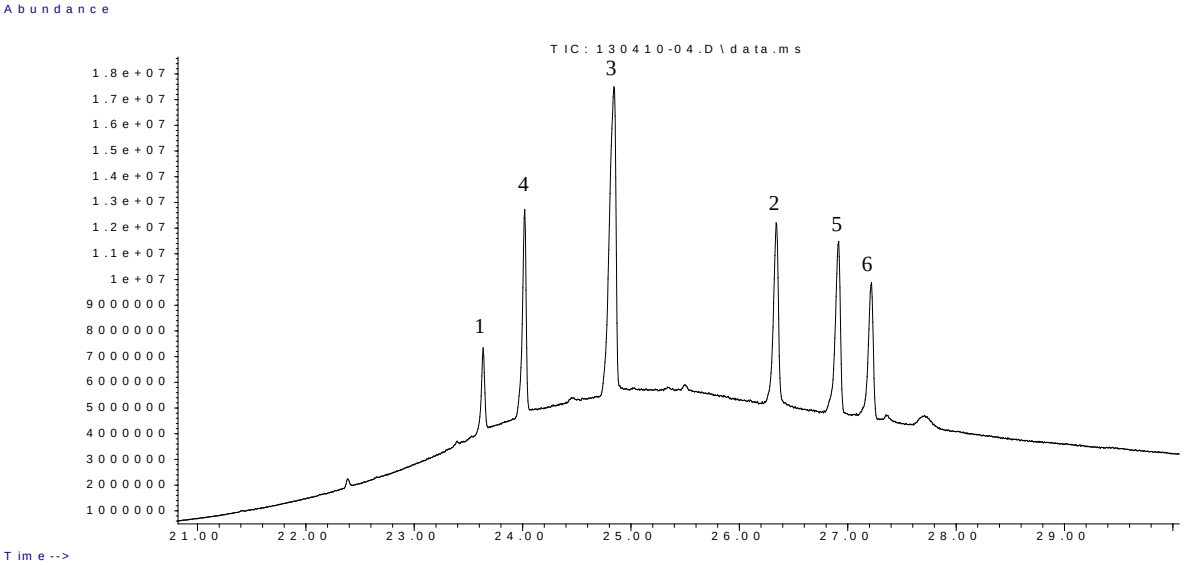


Fig. 22-5: Gas chromatographic separation of the heptafluorobutyryl derivatives of the six dimethoxyphenylpiperazines using Rtx-200 column.

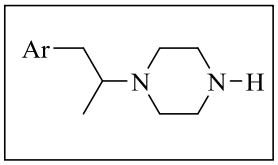
Conclusion

The six regioisomeric dimethoxyphenylpiperazines yield the same fragment ions in their mass spectra. Perfluoroacylation of the secondary amine nitrogen for each of the six regioisomers was done in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation. ATR FTIR analysis yields unique and characteristic infrared spectra for these regioisomeric piperazines. These spectra allow discrimination among the six regioisomeric compounds included in this study. This differentiation was accomplished without the need for chemical derivatization. Mixtures of the dimethoxyphenylpiperazines were successfully resolved via capillary gas chromatography using a relatively polar stationary phase and temperature programming conditions. The elution order appears related to the degree of substituent crowding on the aromatic ring with the most crowded 1,2,3 substitution patterns eluting first and the highest retention for the compound with minimum intramolecular crowding (the 1,3,5-trisubstitution pattern).

References

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 Science International 121 (2001) 47-56.

SECTION IV Phenethylpiperazines



The phenethylpiperazines contain a combination of molecular features and can be viewed as derivatives of either piperazine or the more common phenethylamines. Essentially this is a series of compounds in which the amino group of a phenethylamine has been replaced by a piperazine moiety. The synthesis of compounds in this series essentially follows the various methods for amphetamine/phenethylamine compounds with piperazine or N-methylpiperazine serving as the source for the amino group. This is series IV in Figure 1 and we have prepared and evaluated a series of the general phenethylamine structural backbone and a series of the aminoketone cathinone-like compounds. The aromatic ring substituents include methyl-, fluoro-, chloro-, bromo-, trifluoromethyl-, methoxy- and methylenedioxy-groups. The necessary molecular framework for the phenethyl group was prepared based on available precursor materials prior to the introduction of the piperazine groups.

The EI mass spectra for this group of compounds is dominated by the immonium cation containing the piperazine ring with both nitrogen atoms and occurring at an odd mass since two nitrogens are a part of the structure. These immonium cations result from loss of the substituted aromatic ring species as either the benzyl or benzoyl radical depending

on the structural features of the parent molecule. As is the case for most phenethylamines, the immonium cation species is by far the most abundant ion in the EI mass spectrum. The IR spectra clearly differentiate the carbonyl containing aminoketones from the classical phenethylamines.

Chapter 23

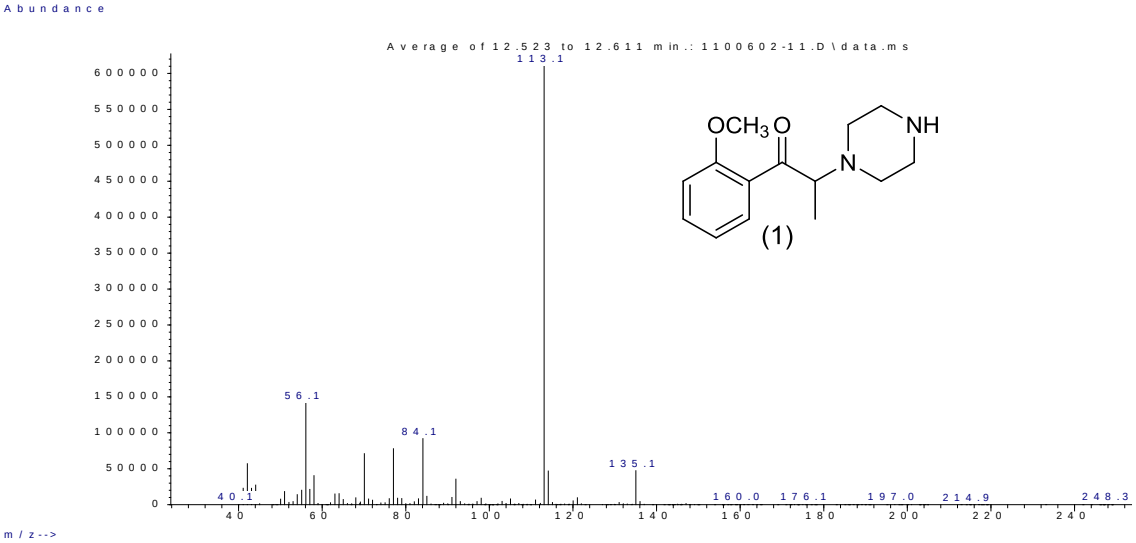
Differentiation of the 1-(methylenedioxyphenyl)-2-piperazinopropanes (MDPPPs) and 1-(monomethoxyphenyl)-2-piperazinopropanones (OMePPPOs) By GC-IRD and GC-MS

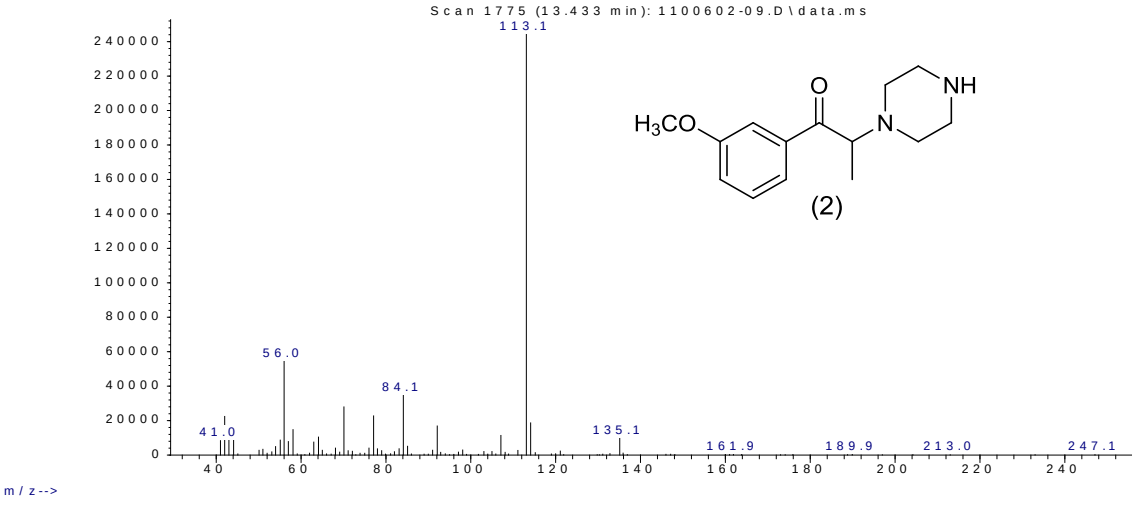
Two amphetamine-like piperazine containing compounds, 1-(3,4-methylenedioxyphenyl)-2-piperazinopropane (3,4-MDPPP), its positional isomer 1-(2,3-methylenedioxyphenyl)-2-piperazinopropane (2,3-MDPPP) and three methcathinone-like piperazine containing regioisomeric ring substituted 1-(methoxyphenyl)-2-piperazinopropanones (OMePPPOs) have identical elemental composition and no marked differences in their mass spectra. 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.

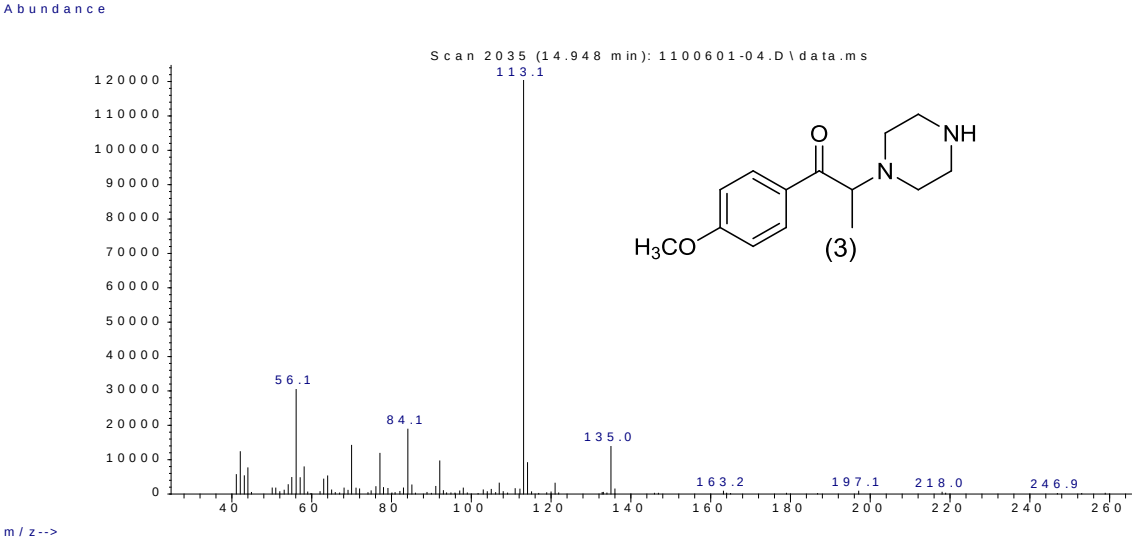
Gas chromatography coupled to infrared detection (GC-IRD) provides direct confirmatory data for the identification of the carbonyl containing compounds and the differentiation of the 3,4-MDPPP from its direct (2,3-MDPPP) and indirect (OMePPPOs) regioisomers. The vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The perfluoroacyl derivative forms of the five piperazines involved in this study were resolved on two stationary phases, the first is composed of 100% dimethyl polysiloxane (Rtx-1) and the second of 5% diphenyl and 95% dimethyl polysiloxane (Rtx-5).

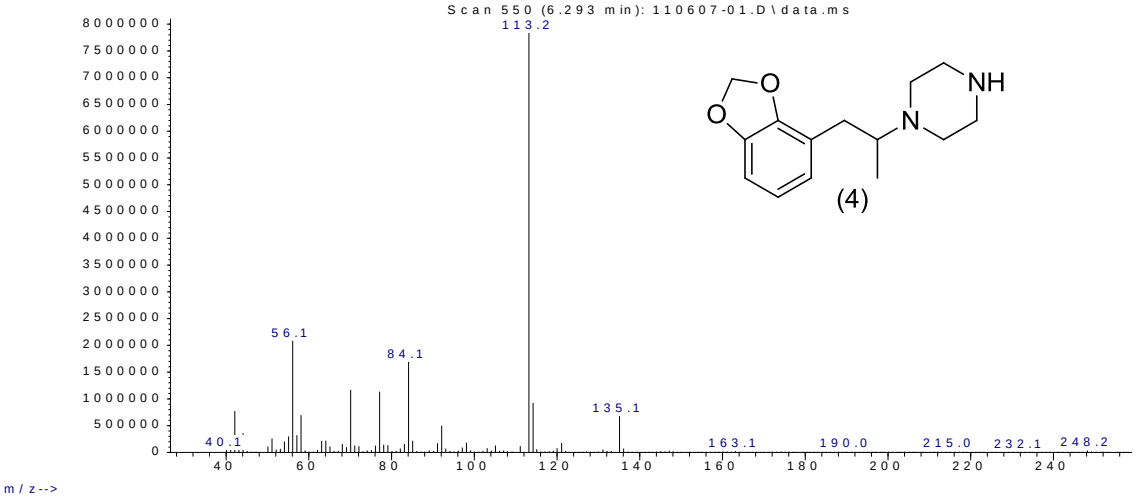
Mass spectral studies of the underivatized and perfluoroacylated derivatives of 1-(methylenedioxyphenyl)-2-piperazinopropanes (MDPPPs) and 1-(monomethoxyphenyl)-2-piperazinopropanones (OMePPPOs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 23-1 shows the EI mass spectra of all five isomeric piperazines (Compounds 1-5). The mass spectra of the five piperazines show fragment ions at m/z 135, 113, 84 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figures 23-2 and 23-3 and these are similar to a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. The mass spectra of the five piperazines did not show any molecular ion peak. The base peak in the mass spectra of all the five compounds is the fragment ion at m/z 113 resulting from the nitrogen initiated alpha cleavage of the molecular ion. The regioisomeric methoxybenzoyl $(C_8H_7O_2)^+$ fragments have the same nominal and exact masses as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cations and occurs at m/z 135. The mass spectra for the ring substituted methoxyphenylpiperazinopropanones (Compounds 1-3) have almost identical mass spectra to each other and to the methylenedioxyphenylpiperazinopropane isomers (Compounds 4 and 5).









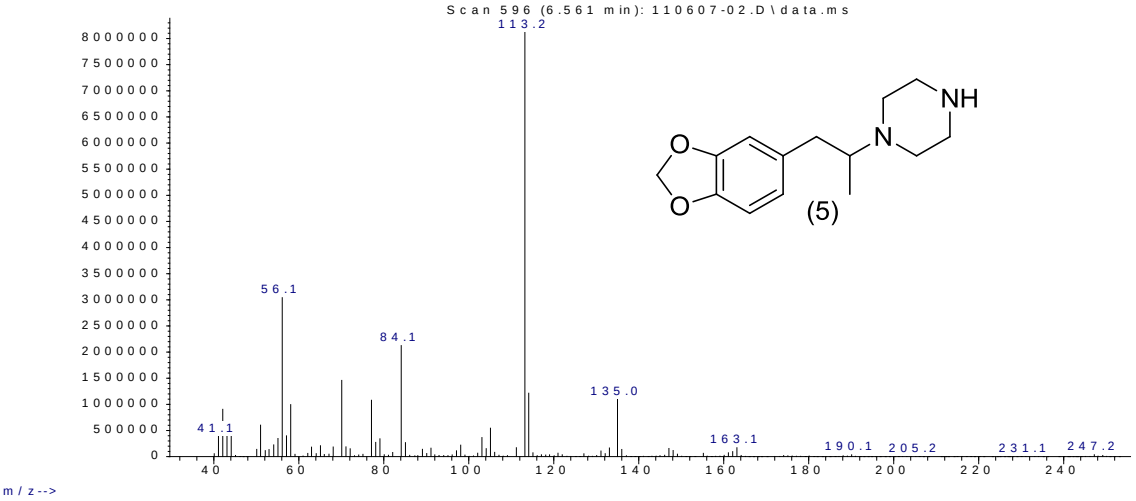


Fig. 23-1: Mass spectra of the five underivatized piperazines in this study.

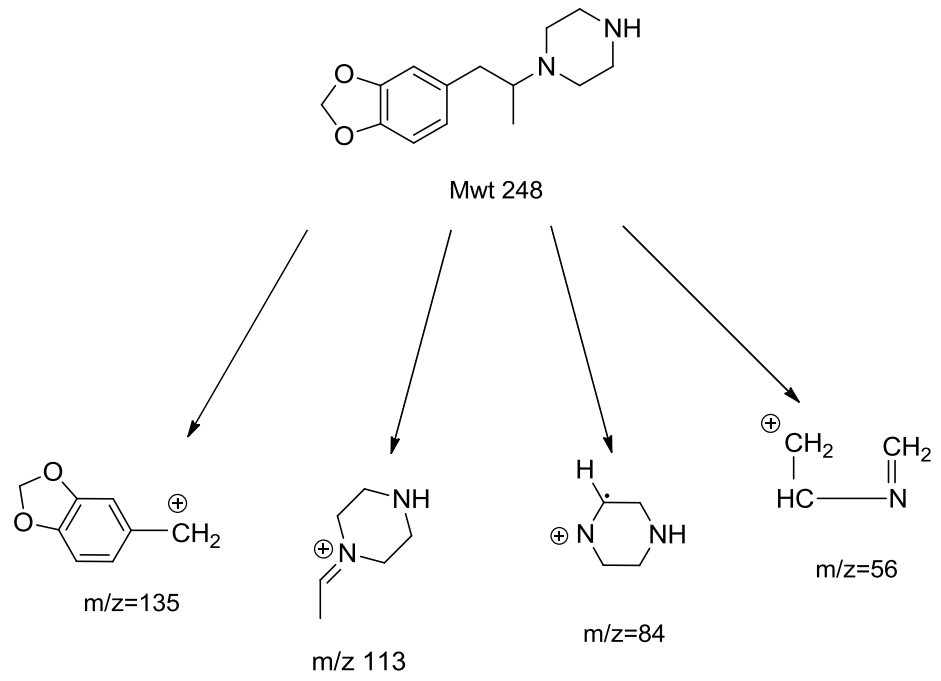


Fig. 23-2: Mass spectral fragmentation pattern of the underivatized 1-(3,4-methylenedioxyphenyl)-2-piperazinopropane (3,4-MDPPP) under EI (70eV) conditions.

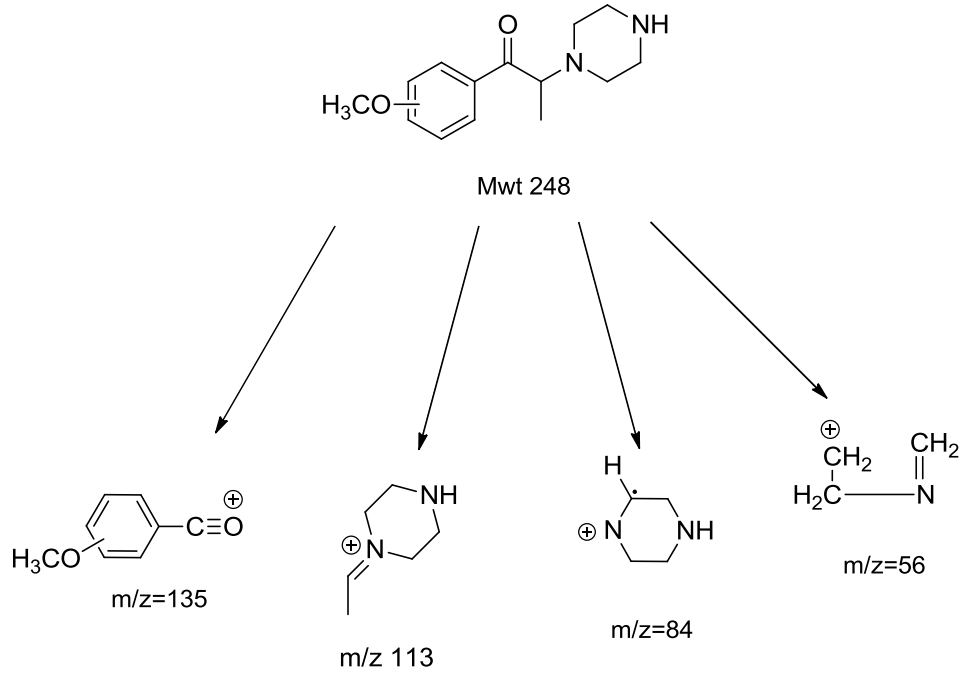
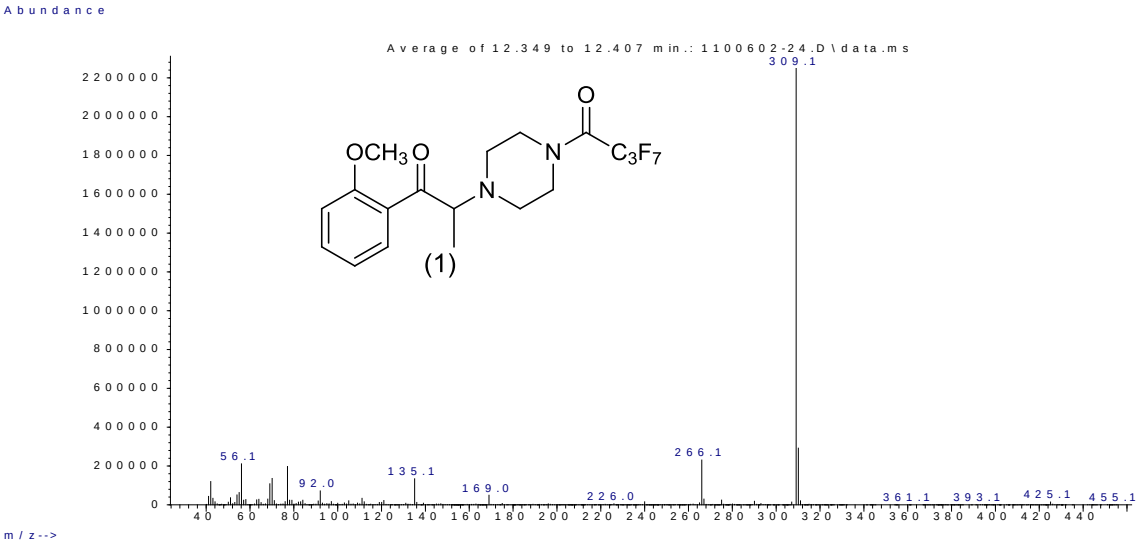
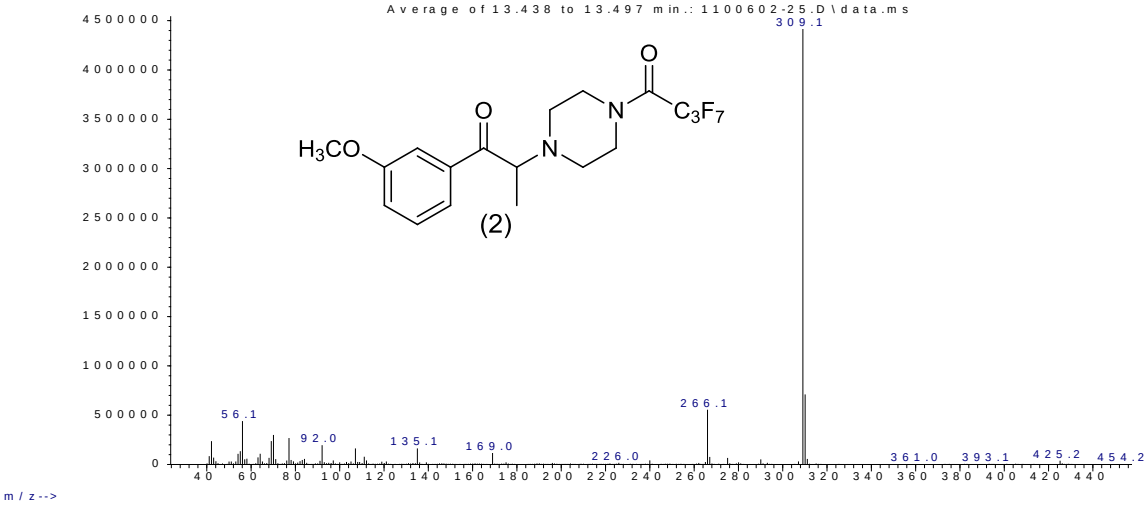


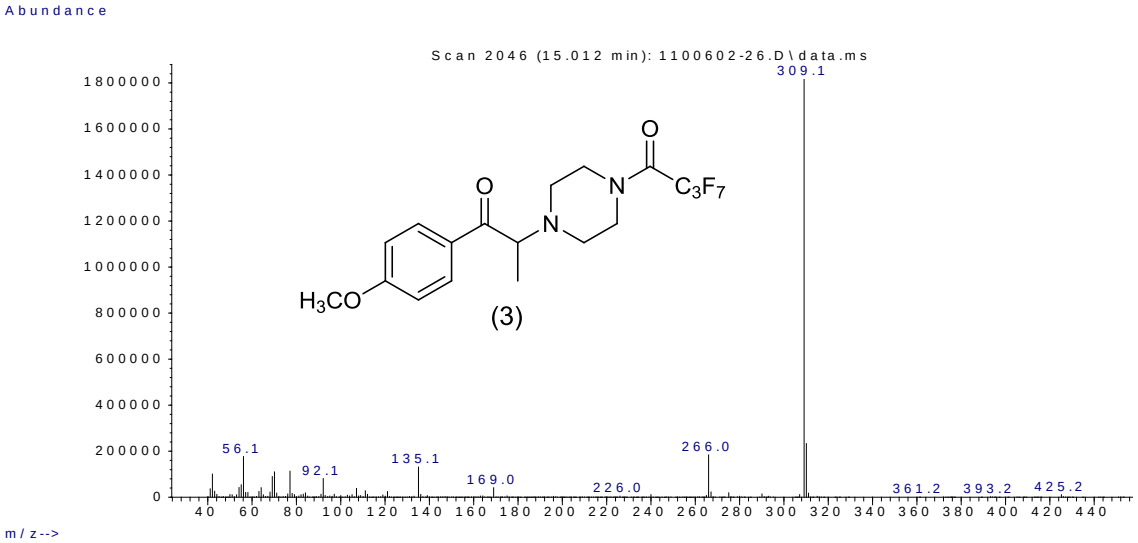
Fig. 23-3: Mass spectral fragmentation pattern of the underivatized 1-(methoxyphenyl)-2-piperazinopropanones (OMePPPOs) under EI (70eV) conditions.

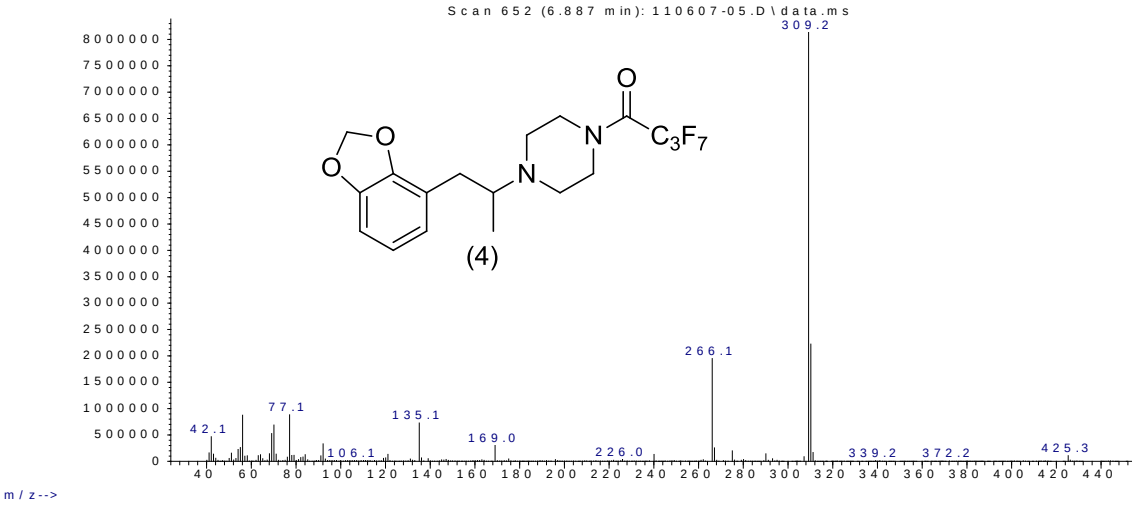
The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric piperazines in an effort to individualize their mass spectra and identify additional unique marker ions for differentiation among these five compounds. The pentafluoropropionyl and heptafluorobutryl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 23-4 shows the mass spectra of the heptafluorobutryl amides of the five studied compounds as representatives of all the perfluoroacylated piperazines evaluated in this study. The molecular ion peaks for the five PFPA and HFBA amides were absent and the major fragment ions occur at *m/z* 259 and 309 for the PFPA and HFBA amides, respectively and correspond to the (M-135) alpha cleavage piperazine-containing fragment. Furthermore, an additional characteristic fragment ion series occurring at *m/z* 216 and 266 for PFPA and HFBA amides, respectively corresponds to the $(M-178)^+$ ion for each amide. The proposed structure and mechanism for the formation of the m/z 266 ion in the mass spectra of the heptafluorobutyryl derivatives of the five piperazines in this study is shown in Figure 23-5. The suggested structure for this fragment involves a migration of a hydrogen atom from the piperazine ring to the piperazine tertiary nitrogen atom followed by an alpha cleavage then the loss of the phenethylamine radical to form the m/z 266 ion. The proposed structure for the m/z 266 ion is supported by the mass spectra of the unsubstituted, mono-deutero unsubstituted and octa-deutero unsubstiuted phenylpiperazinopropane and their corresponding three heptafluorobutyryl derivatives.

The unsubstituted phenylpiperazinopropane was prepared by reducing the imine formed between phenylacetone and piperazine with sodium cyanoborohydride. The









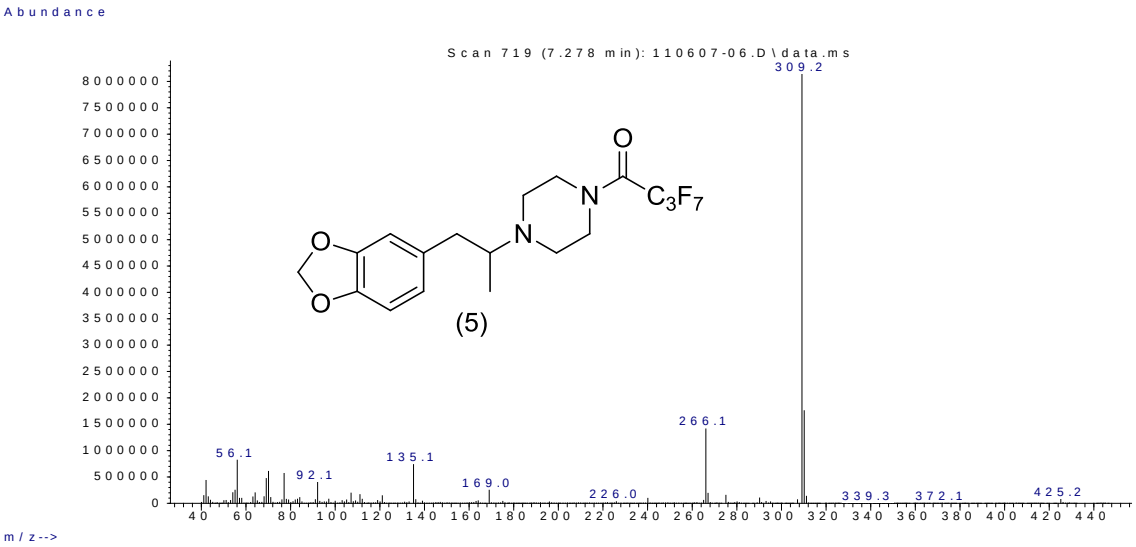


Fig. 23-4: Mass spectra of the heptafluorobutyryl derivatives of the five piperazines in this study

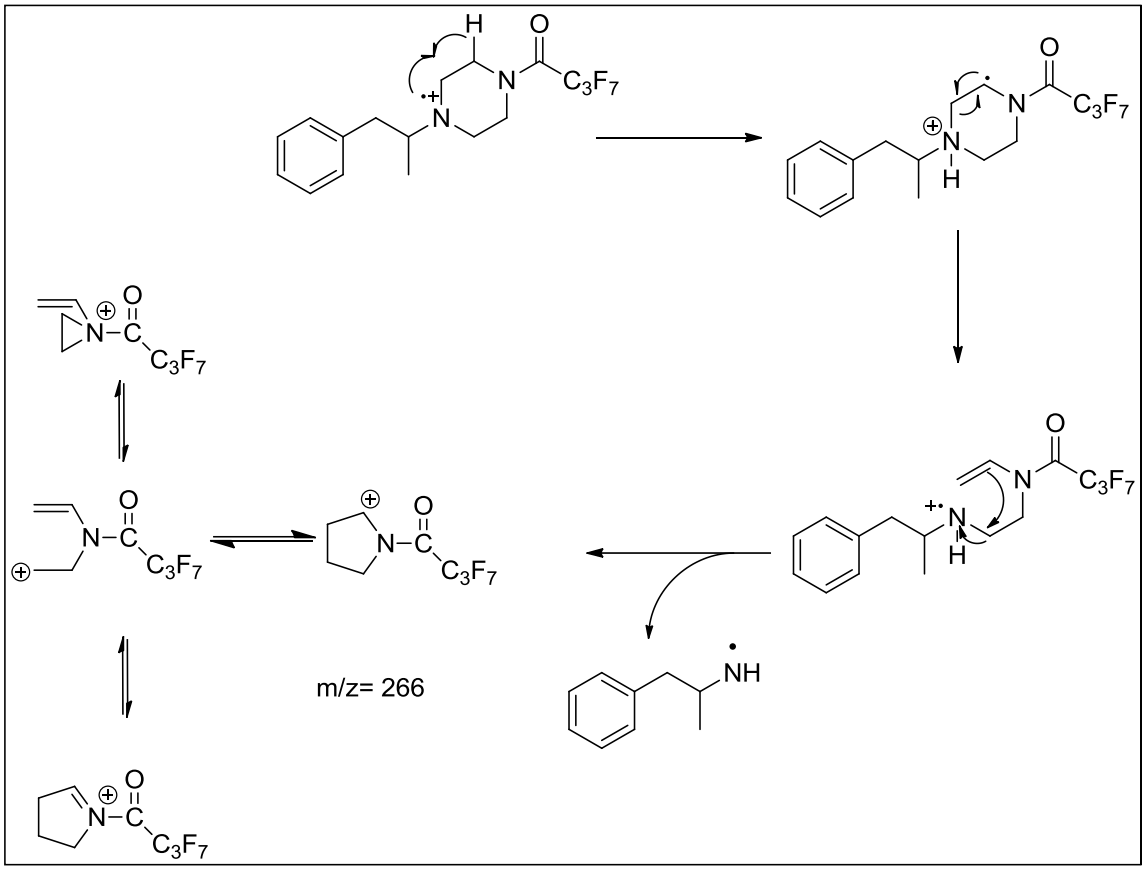


Fig. 23-5: Proposed mechanism for the formation of the m/z= 266 ion in the mass spectra of the heptafluorobutyryl derivatives of the five piperazines in this study.

mono-deuterium labeled compound was prepared by reducing the imine formed between phenylacetone and piperazine with sodium cyanoborodeuteride. The octa-deuterium labeled compound was prepared by reducing the imine formed between phenylacetone and d_8-piperazine with sodium cyanoborohydride.

The mass spectra of the unsubstituted, mono-deutero unsubstituted and octa-deutero unsubstiuted phenylpiperazinopropane and their corresponding three heptafluorobutyryl derivatives are shown in figures 23-6, 23-7 and 23-8. The spectrum in Figure 23-6A shows the base peak at m/z 113 resulting from the alpha cleavage of the molecular ion for phenylpiperazinopropane. Figure 23-6B shows the mass spectrum of the heptafluorobutryl amide of the unsubstiuted phenylpiperazinopropane. The molecular ion peak for the HFBA amide was absent and the major fragment ion in its' spectrum occurs at m/z 309 and corresponds to the alpha cleavage piperazine-containing fragment in addition to the fragment ion at m/z 266.

The spectra in Figures 23-7A and 23-7B show that the single deuterium label remains as a part of the base peaks resulting from the alpha cleavage of the molecular ions of the underivatized and perfluoroacylated derivative of the mono-deuterated phenylpiperazinopropane. The observed masses for these ions increased by 1 Da to m/z 114 and m/z 310 in the spectra of the underivatized and the perfluoroamide, respectively. On the other hand, the $(M-178)^+$ ion remained at m/z 266 which confirms that the phenethylamine part of the structure is lost during the formation of this characteristic ion.

The spectra in Figures 23-8A and 23-8B show that the octa deuterium label remains as a part of the base peaks resulting from the alpha cleavage of the molecular ions of the underivatized and perfluoroacylated derivative of the octa-deuterated

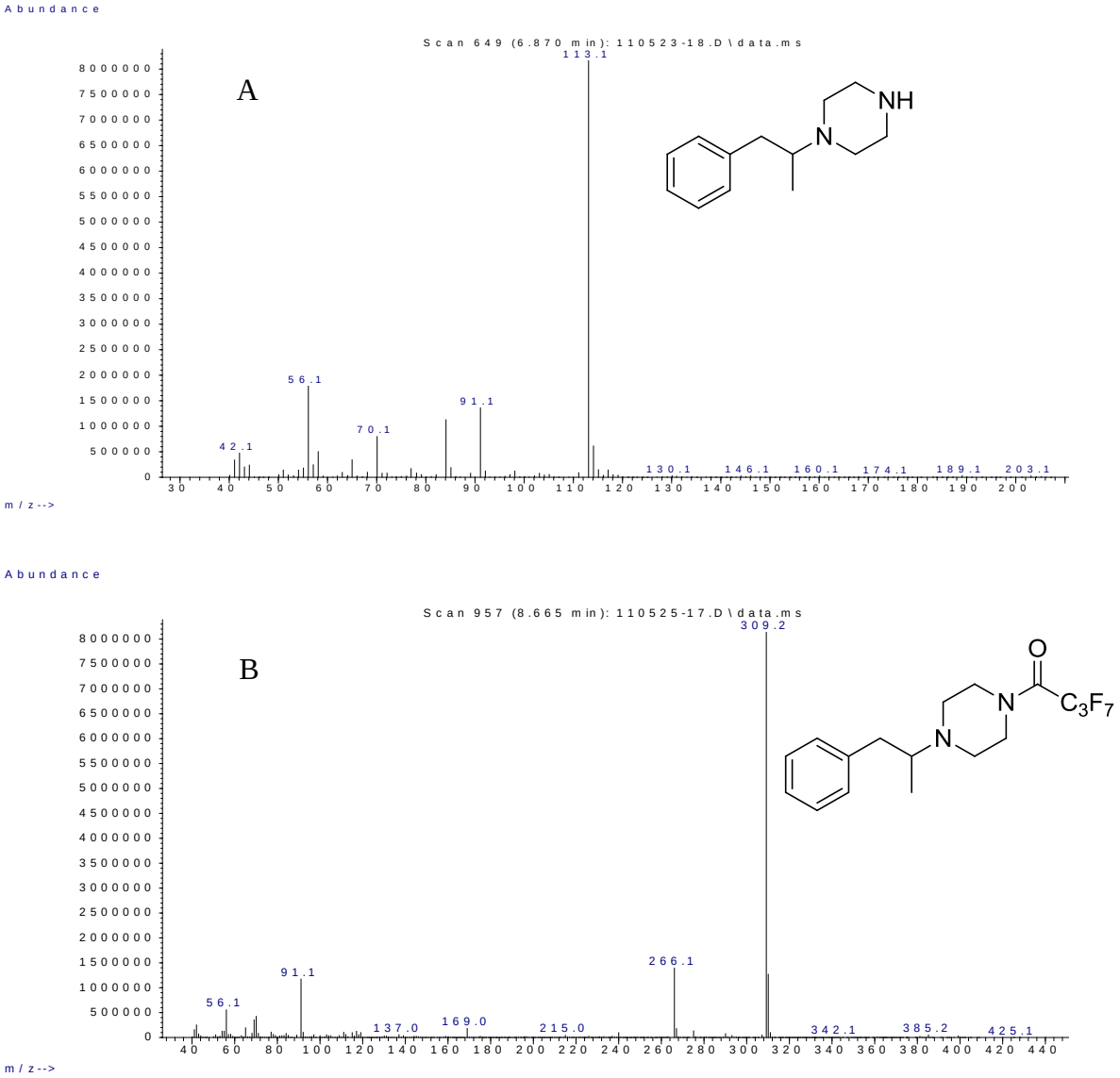


Fig. 23-6: Mass spectra of the (A) unsubstituted phenylpiperazinopropane and (B) its heptafluorobutyryl derivative.

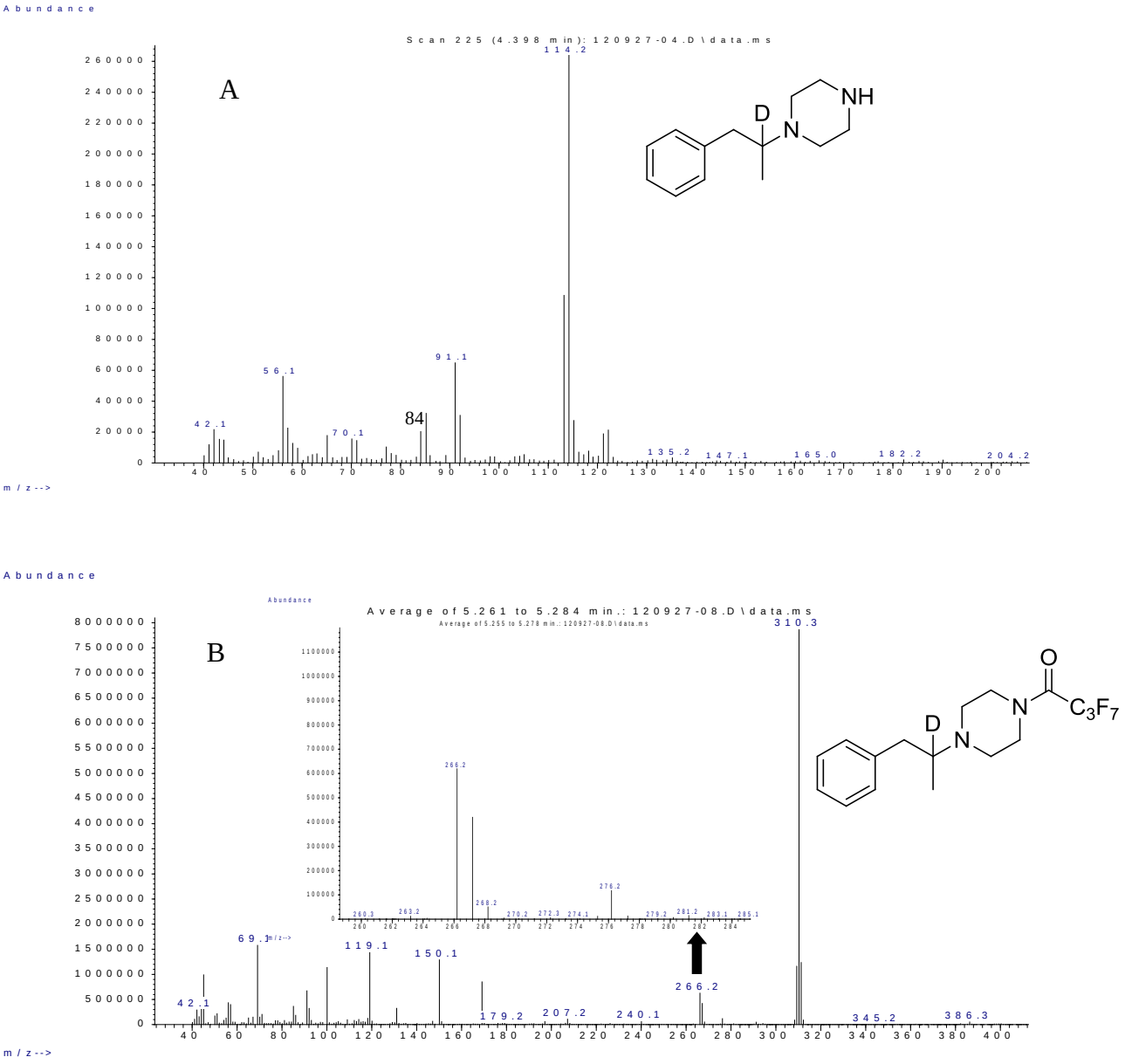


Fig. 23-7: Mass spectra of the (A) mono-deuterated phenylpiperazinopropane and (B) its heptafluorobutyryl derivative.

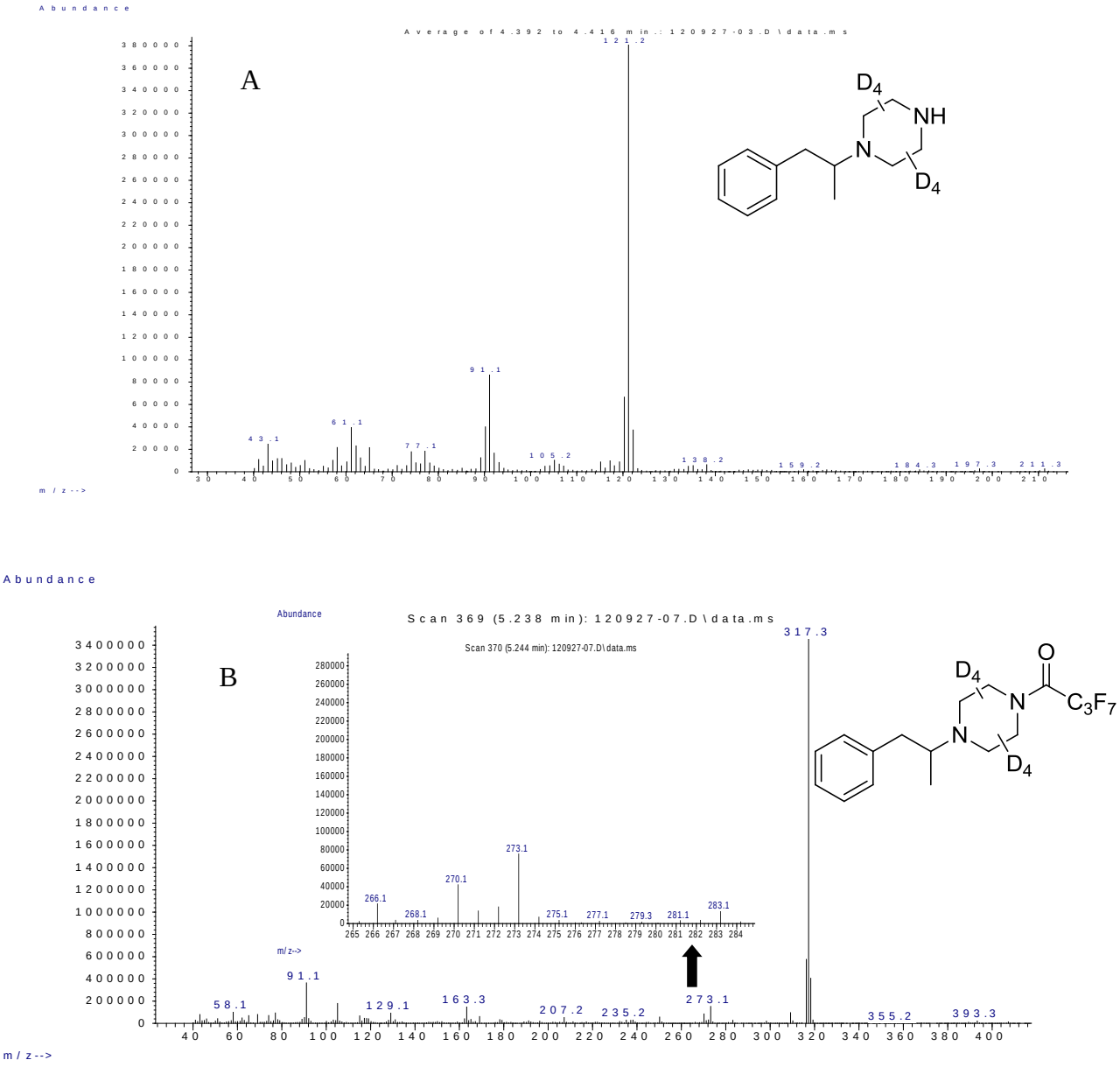


Fig. 23-8: Mass spectra of the (A) octa-deuterated phenylpiperazinopropane and (B) its heptafluorobutyryl derivative.

phenylpiperazinopropane since their masses increased by 8 Da to m/z 121 and m/z 317 in the spectra of the underivatized and the perfluoroamide, respectively. The proposed migration of a hydrogen atom from the piperazine ring to the piperazine tertiary nitrogen atom and the subsequent loss of the phenethylamine part is further confirmed by the spectrum of the heptafluoroamide of the octa-deuterated phenylpiperazinopropane. The characteristic ion at m/z 266 in the HFBA derivative of the undeuterated phenylpiperazinopropane has increased by 7 Da in the observed spectrum of the octa-deuterated compound yielding the m/z 273 fragment. This confirms that one deuterium atom has migrated from the piperazine ring to the piperazine tertiary nitrogen atom and then subsequently lost with the phenethylamine part of the structure leaving seven deuterium atoms in the structure of this characteristic $(M\text{-}178)^+$ ion in the spectra of the heptafluoroamides of the studied compounds.

The ion at *m/z* 135 was observed in the spectra of all derivatives and corresponds to the ring substituted benzyl or benzoyl fragments. Those ions occurring at m/z 119 and 169 are the perfluoroalkyl cations 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.

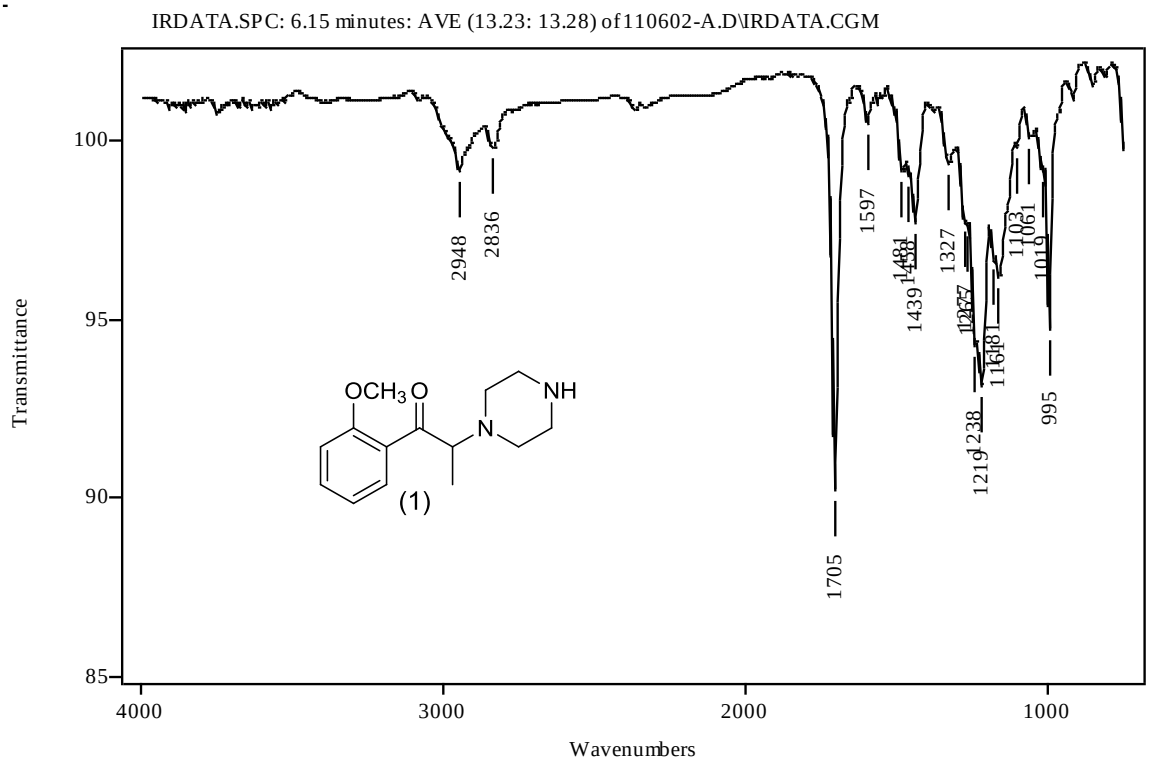
Vapor-phase Infra-Red Spectrophotometric Studies of 1-(methylenedioxyphenyl)-2-piperazinopropanes (MDPPPs) and 1-(monomethoxyphenyl)-2-piperazinopropanones (OMePPPOs)

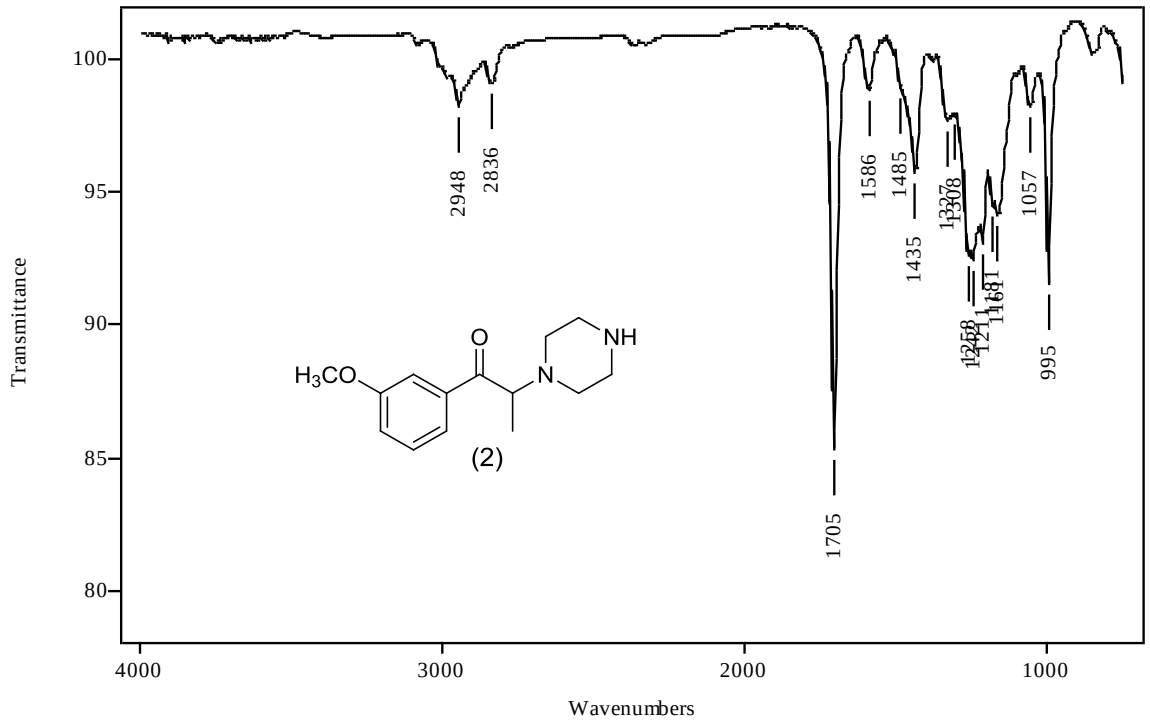
Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the five isomeric piperazines. The vapor phase infrared spectra for the five piperazines are shown in Figure 23-9. The spectra were generated in the vapor phase following sample injection into the gas chromatograph and each compound shows transmittance bands in the regions 650 – 1700 cm^{-1} and 2700 – 3100 cm^{-1}. 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}.

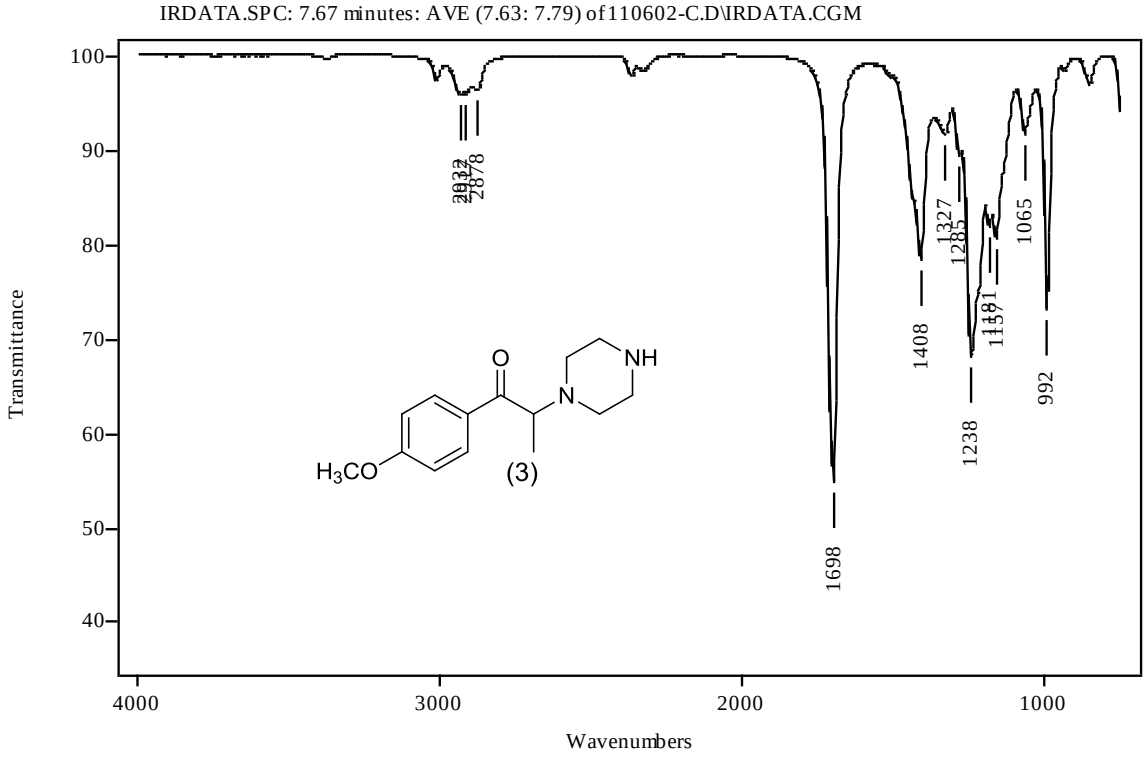
The three regioisomeric OMePPPOs share a characteristic strong singlet IR band at 1705 cm^{-1} in compounds 1 and 2 and at 1698 cm^{-1} in compound 3 corresponding to the carbonyl group stretching which can distinguish these three OMePPPOs from the two MDPPPs. The three ring substituted OMePPPOs can be differentiated by the positions and intensities of several IR peaks in the region of 650 – 1700 cm^{-1}. Compound 1 shows a strong peak at 1219 cm^{-1} which is shifted to a medium intensity doublet at 1258 cm^{-1}, 1242 cm^{-1} in compound 2 and a strong singlet at 1238 cm^{-1} in compound 3. Compound 3 shows a strong peak at 1408 cm^{-1} which is shifted to a weak peak at 1435 cm^{-1} in compound 2 and at 1439 cm^{-1} in compound 1. Compound 3 also has a strong intensity peak at 992 cm^{-1} which is shifted to a peak at 995 cm^{-1} in both compounds 1 and 2.

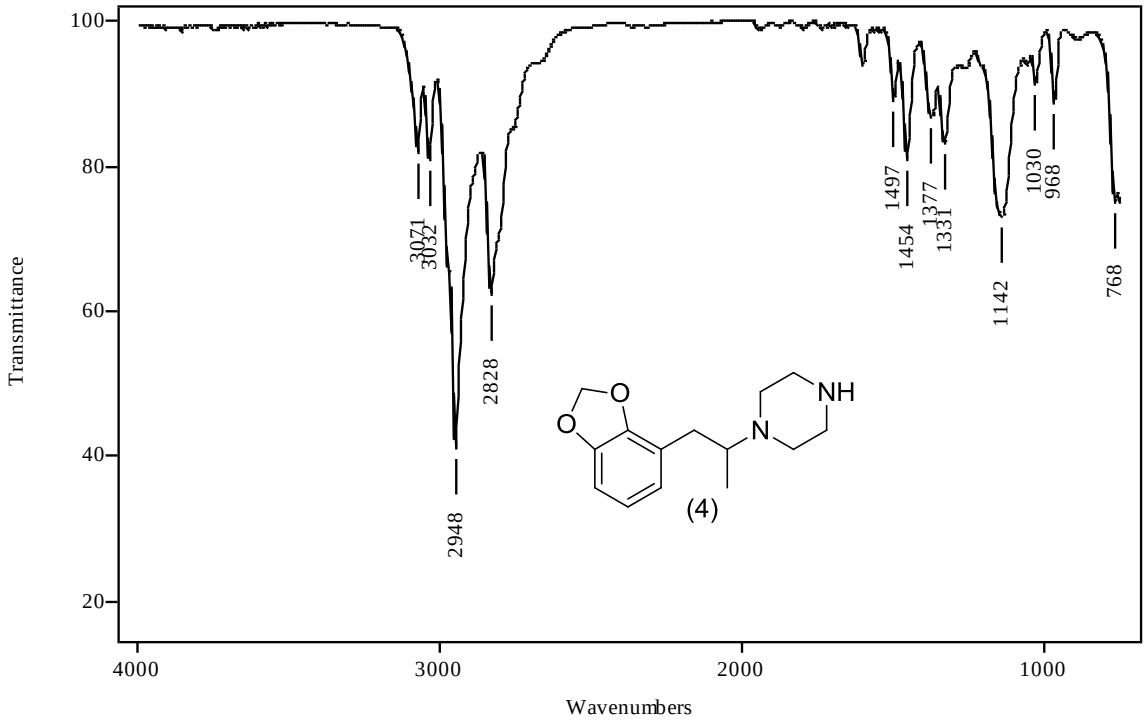
The 3,4-MDPPP regioisomer (Compound 5) is characterized by the strong intensity band at 1489 cm^{-1} which is shifted to a medium singlet at 1454 cm^{-1} in the 2,3-MDPPP regioisomer (Compound 4). Also the IR spectrum of the 3,4-isomer shows a singlet at 1246 cm^{-1} which is shifted to a broad singlet at 1142 cm^{-1} in the 2,3-MDPPP. The 2,3-MDPPP regioisomer has a medium doublet at 1377, 1331 cm^{-1} which is shifted to a very weak doublet at 1362, 1355 cm^{-1} in the 3,4-regioisomer.

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









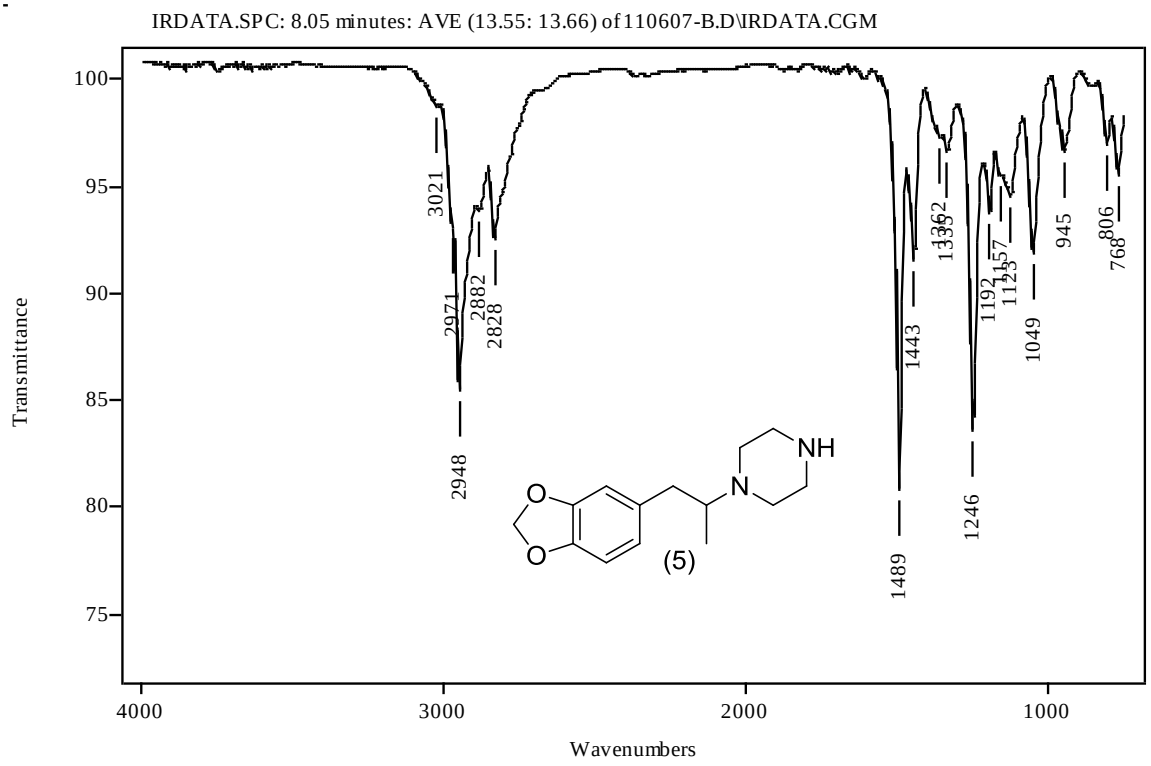


Fig. 23-9: Vapor phase IR spectra of the five piperazines involved in this study.

Gas Chromatographic Separation of 1-(methylenedioxyphenyl)-2-piperazinopropanes (MDPPPs) and 1-(monomethoxyphenyl)-2-piperazinopropanones (OMePPPOs)

Gas chromatographic separation of the derivatized piperazines was accomplished on a capillary column 30 m × 0.25 mm i.d. coated with 0.50 µm of 100% dimethyl polysiloxane (Rtx-1) and a capillary column 30 m × 0.25 mm i.d. coated with 0.50 µm of 5% diphenyl and 95% dimethyl polysiloxane (Rtx-5). Several temperature programs were evaluated and the chromatograms in Figure 23-10 are representative of the results obtained for all samples on these two stationary phases. The separation of the heptafluorobutyryl derivatives was performed using a temperature program consisting of an initial temperature of 70^{o}C for 1 minute, ramped up to 250^{o}C at a rate of 30^{o}C per minute followed by a hold at 250^{o}C for 15 minutes.

In Figure 23-10A and 23-10B the HFBA derivatives of the three methoxyphenylpiperazinopropanones are less retained than their regioisomeric methylenedioxyphenylpiperazinopropanes. The three OMePPPOs eluted in the order of 2, 3, 4-methoxyphenylpiperazinopropanone. The 3,4-MDPPP 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.

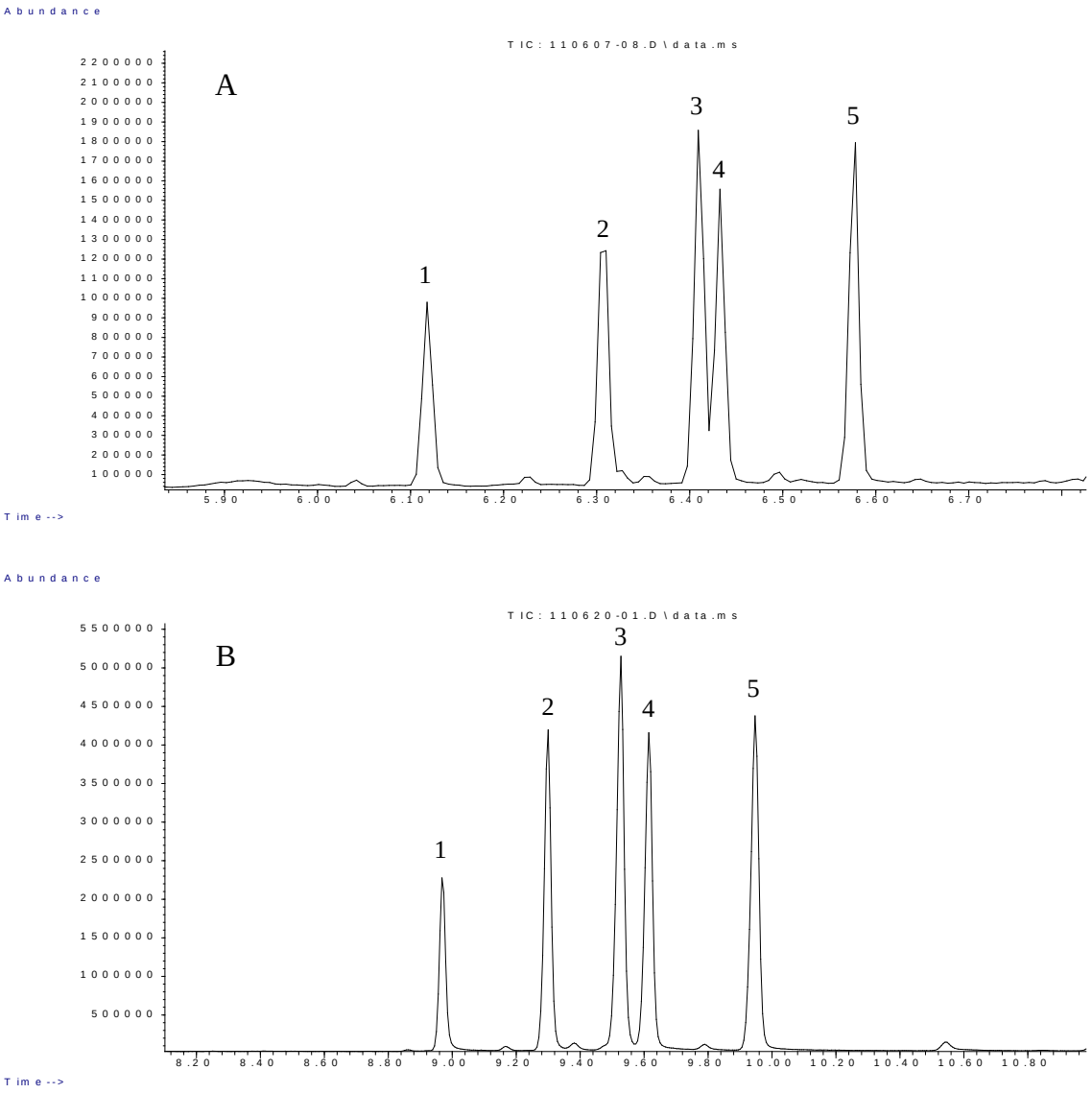


Fig. 23-10: Gas chromatographic separation of the heptafluorobutyryl derivatives of the five piperazines using (A) Rtx-1 and (B) Rtx-5 column. The number over the peak corresponds to the compound number.

Conclusion

The three methoxyphenylpiperazinopropanones have an indirect regioisomeric relationship to 3,4-MDPPP and its regioisomer 2,3-MDPPP. The five regioisomeric piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. GC-IRD offered unique and characteristic IR spectra that allowed discrimination among these compounds in the region between 650-1700 cm^{-1}. Additionally, the strong carbonyl absorption bands clearly differentiate the methoxyphenylpiperazinopropanones from the methylenedioxyphenylpiperazinopropanes. The five HFBA derivatives were successfully resolved on the stationary phases Rtx-1 and Rtx-5.

References

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 Science International 121 (2001) 47-56.

Chapter 24

Differentiation of the 1-(methoxyphenyl)-2-piperazinopropanes (OMePPPs) by GC-IRD and GC-MS

Three ring substituted methoxyphenylpiperazinopropanes (OMePPPs) have equal mass and many common mass spectral fragment ions. 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.

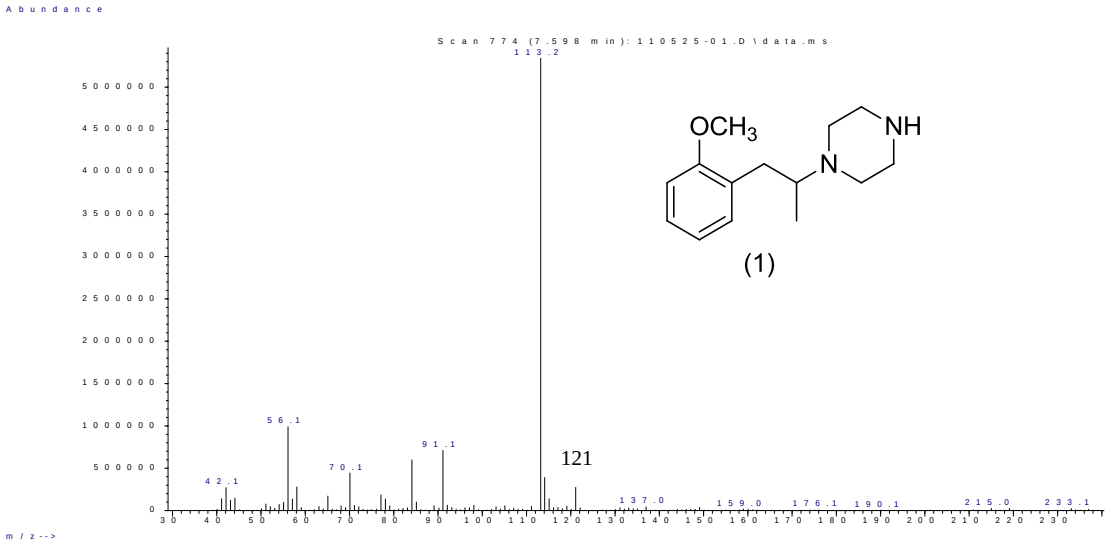
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the three isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

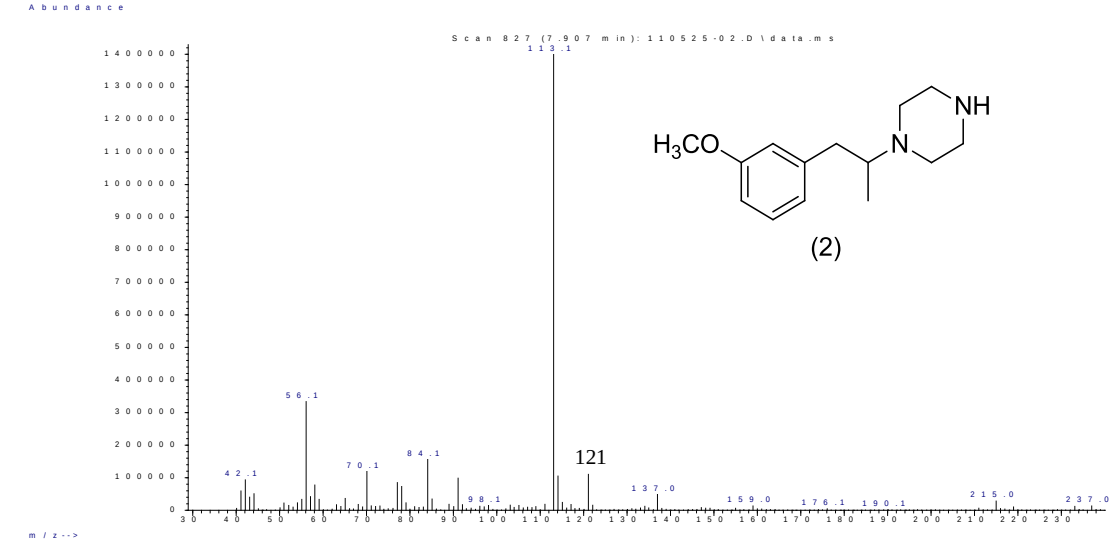
Mass spectral studies of the underivatized and perfluoroacylated derivatives of 1-(methoxyphenyl)-2-piperazinopropanes (OMePPPs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 24-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3) in this study. The mass spectra of the three piperazines did not show any molecular ion peak. The base peak in the mass spectra of all the three compounds is the fragment ion at m/z 113 resulting from the alpha cleavage of the molecular ion. The proposed structures of these ions are shown in Figure 24-2.

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.

The pentafluoropropionyl and heptafluorobutryl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 24-3 shows the mass spectra of the heptafluorobutryl amides of the three studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ion peaks for the three PFPA and HFBA amides were absent in their mass spectra. The major fragment ion in these spectra occurs at *m/z* 259 and 309 for the PFPA and HFBA amides, respectively and corresponds to the alpha cleavage piperazine-containing fragment. Furthermore, an additional fragment ion series occurring at *m/z* 216 and 266 for PFPA and HFBA amides respectively corresponds to the $(M-178)^+$ ion for each amide. The ion at *m/z* 121 was observed in the spectra of all derivatives and corresponds to the ring substituted methoxybenzyl fragments. Those ions occurring at





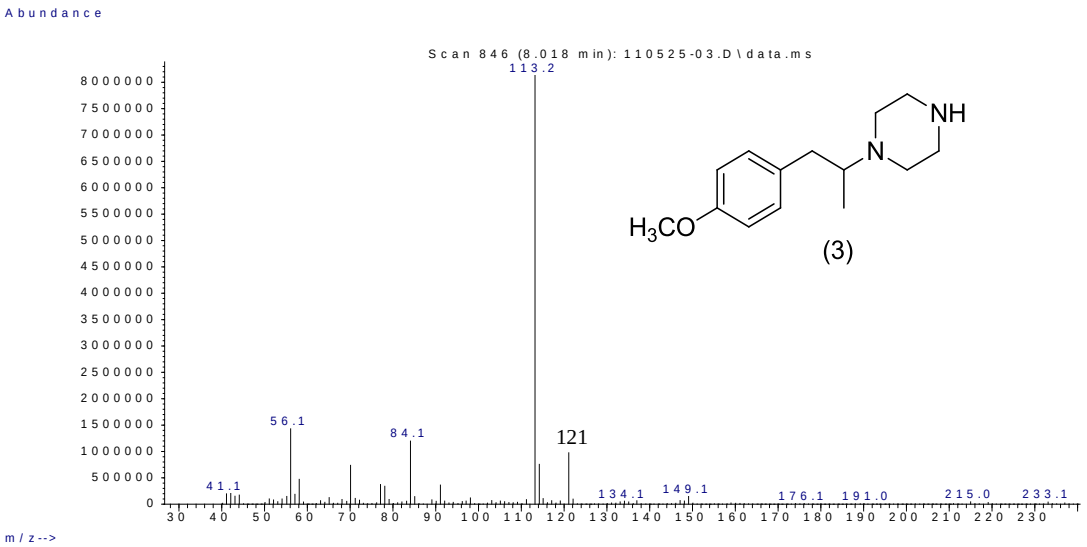


Fig. 24-1: Mass spectra of the underivatized piperazines in this study.

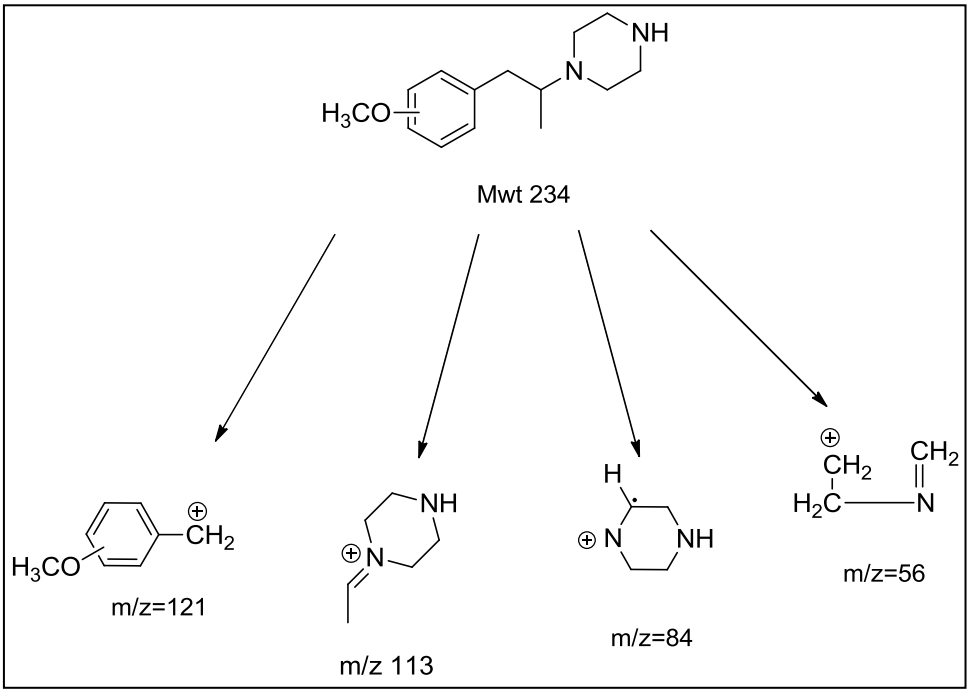
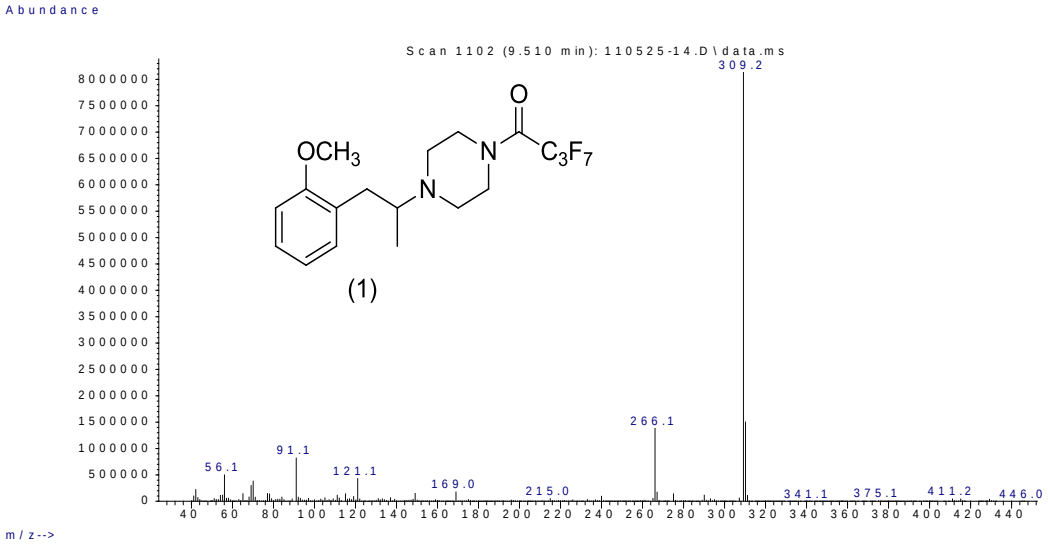
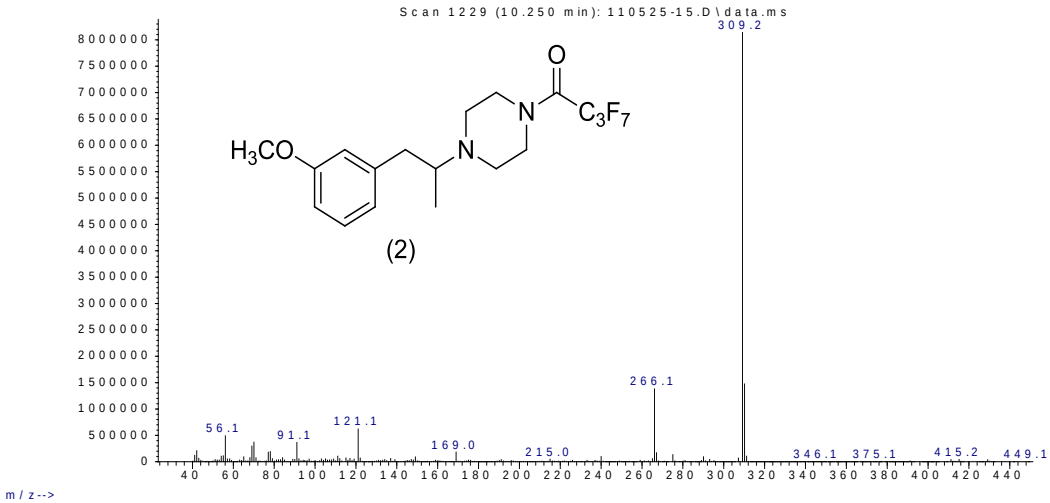


Fig. 24-2: Mass spectral fragmentation pattern of the underivatized 1-(methoxyphenyl)-2-piperazinopropanes (OMePPPs) under EI (70eV) conditions.





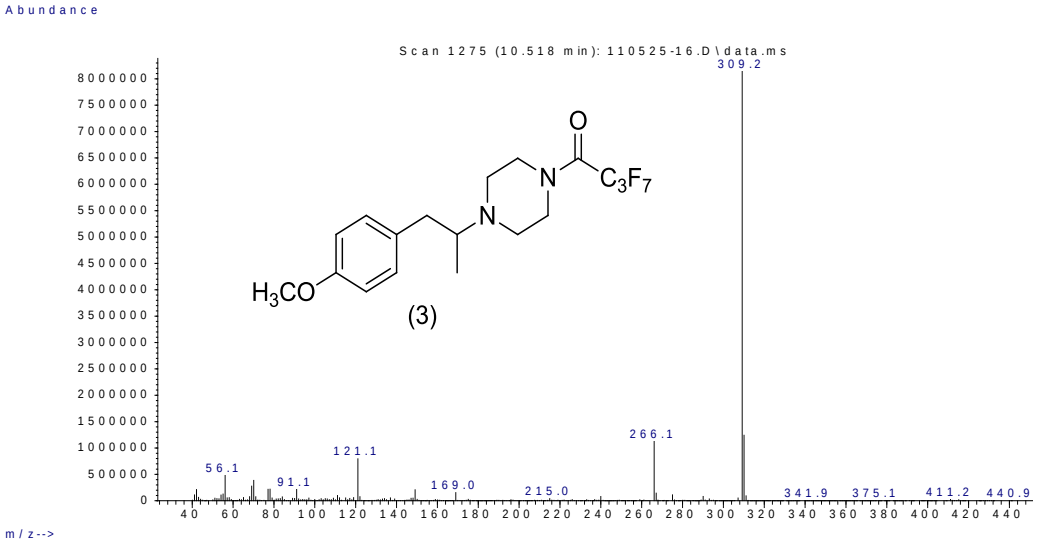


Fig. 24-3: Mass spectra of the heptafluorobutyryl derivatives of the piperazine compounds in this study.

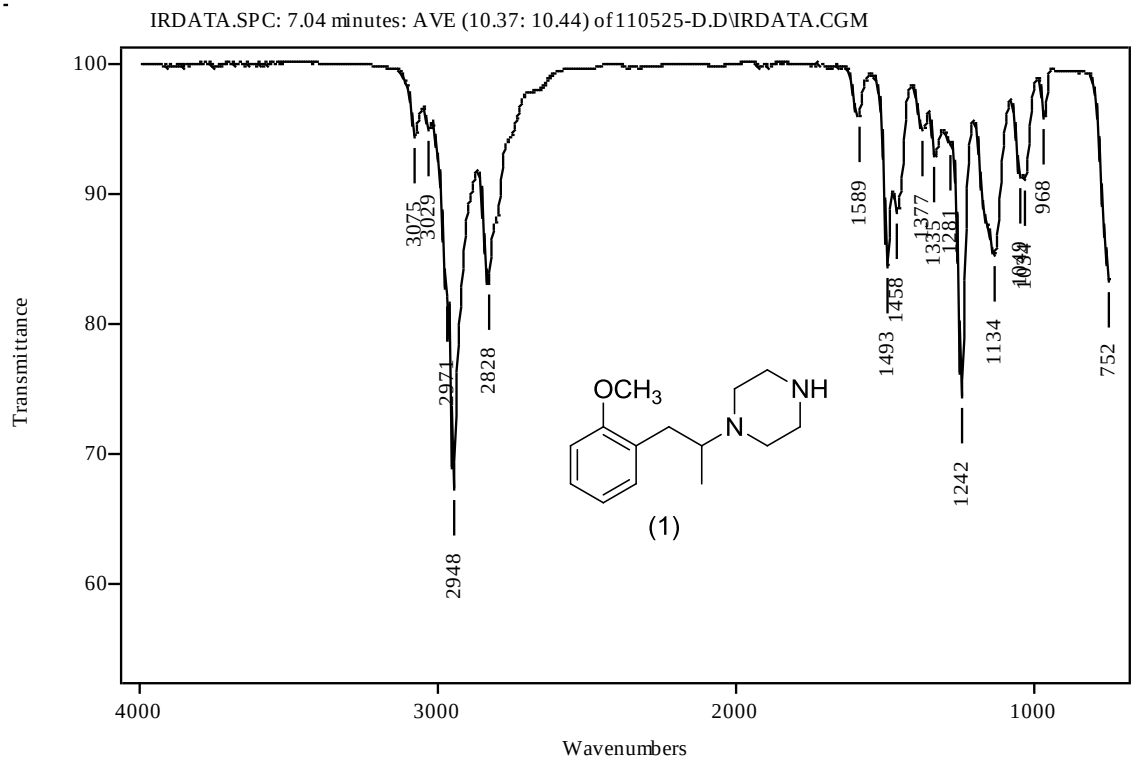
m/z 119 and 169 are the perfluoroalkyl cations pentafluoroethyl or heptafluoropropyl from the appropriate amides. These studies show that chemical derivatization (perfluoroacylation) does not offer any additional unique marker ions to allow identification of one compound to the exclusion of the other in this set of compounds.

Vapor-phase Infra-Red Spectrophotometric Studies of the 1-(methoxyphenyl)-2-piperazinopropanes (OMePPPs)

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 three piperazines. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the three underivatized piperazines are shown in Figure 24-4. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph and each compound shows a vapor-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}. 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 differentiated by the positions and intensities of several IR peaks in the region of 750 – 1620 cm^{-1}.

The three ring substituted methoxyphenylpiperazinopropanes 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 singlet at 1512 cm^{-1} which is shifted to a medium intensity doublet at 1489 cm^{-1}, 1454 cm^{-1} in compound 2 and to a singlet at 1493 cm^{-1} in compound 1. Compound 2 shows a medium peak at 1154 cm^{-1} which is shifted to a peak at 1134 cm^{-1} in compound 1 and to peak at 1173 cm^{-1} in compound 3. Compound 2 also has a strong intensity peak at 1262 cm^{-1} which is shifted to 1246 cm^{-1} in compound 3 and to 1242 cm^{-1}

in compound 1. These results provide an excellent illustration of the value of vapor phase IR confirmation for the three regioisomeric compounds in this study. The generated IR spectra show significant differences in the major bands for these three compounds.



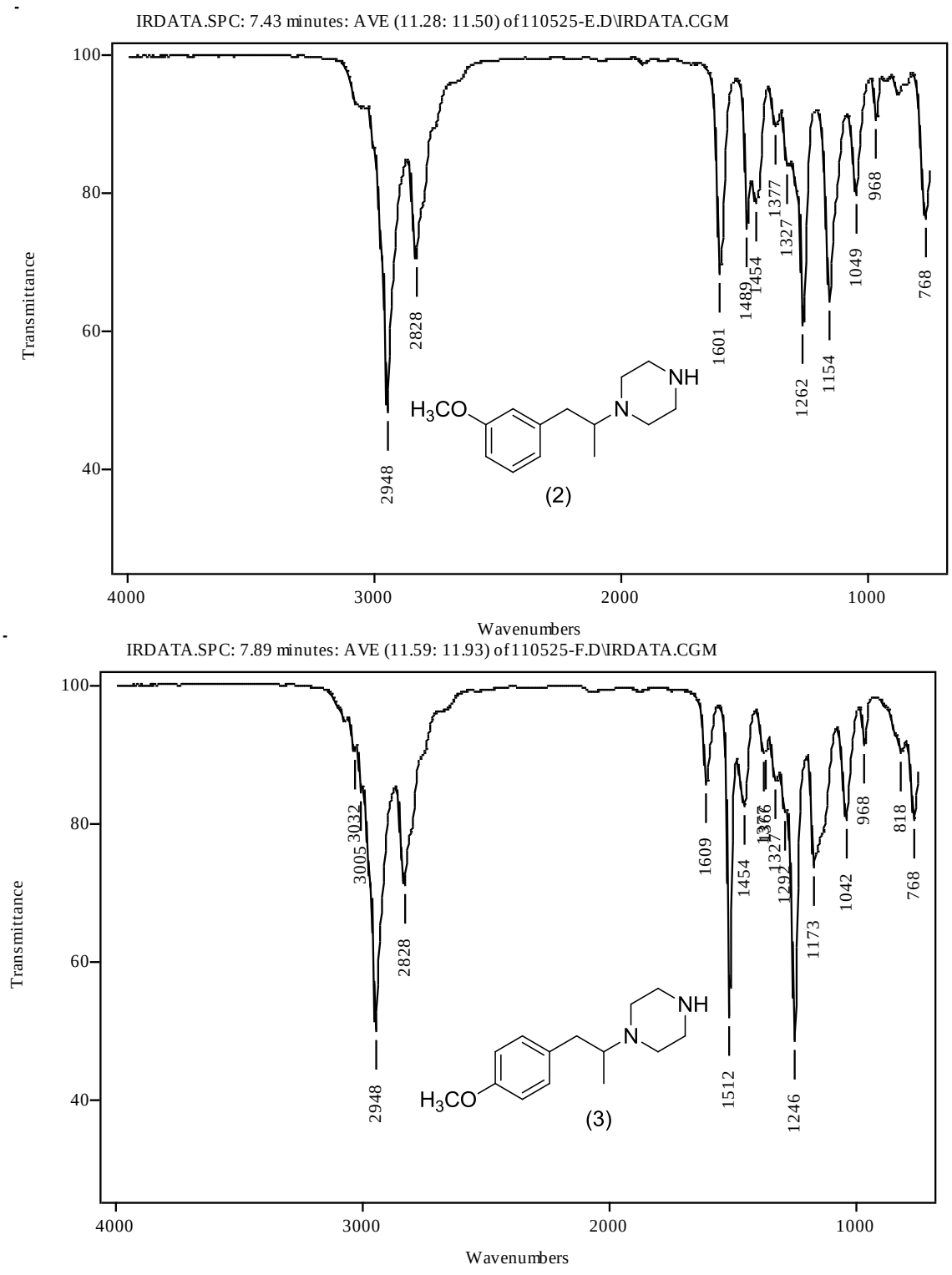


Fig. 24-4: Vapor phase IR spectra of the piperazines involved in this study.

Gas Chromatographic Separation of the 1-(methoxyphenyl)-2-piperazinopropanes (OMePPPs)

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). The separation of the PFPA derivatives was performed using a temperature program consisting of an initial hold at 70°C for 1.0 min, ramped up to 250°C at a rate of 30°C/min and holding the temperature for 10 minutes. The chromatograms in Figures 24-5 is a representative of the results obtained for all samples on this stationary phase.

In Figure 24-5 the PFPA derivatives of the three methoxyphenylpiperazinopropanes eluted in the order of 2, 3, 4-methoxyphenylpiperazinopropane. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

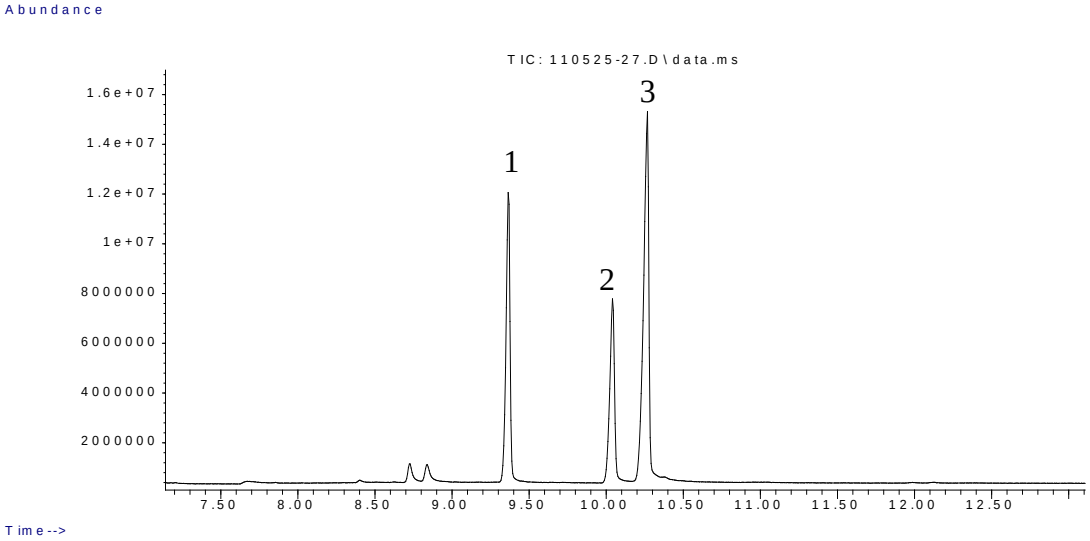


Fig. 24-5: Gas chromatographic separation of the pentafluoropropionyl derivatives using Rtx-200 column. The number over the peak represents the compound number

Conclusion

The three regioisomeric methoxyphenylpiperazinopropanes have a regioisomeric relationship to each other. These three piperazines yield very similar fragment ions in their mass spectral and chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. GC-IRD offered unique and characteristic IR spectra that allowed the discrimination among these compounds in the region between 650-1700 cm^{-1}. The three piperazines were successfully resolved on the GC stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Chapter 25

Differentiation of the 1-(monofluorophenyl)-2-piperazinopropanones (FPPPOs) By GC-MS

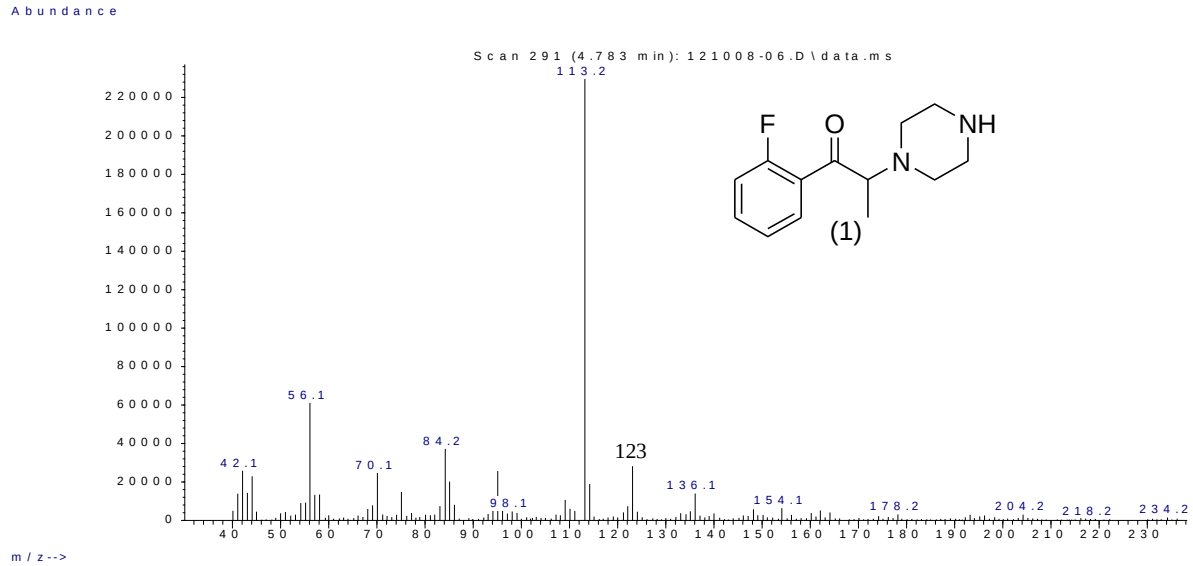
Three ring substituted fluorophenylpiperazinopropanones (FPPPOs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

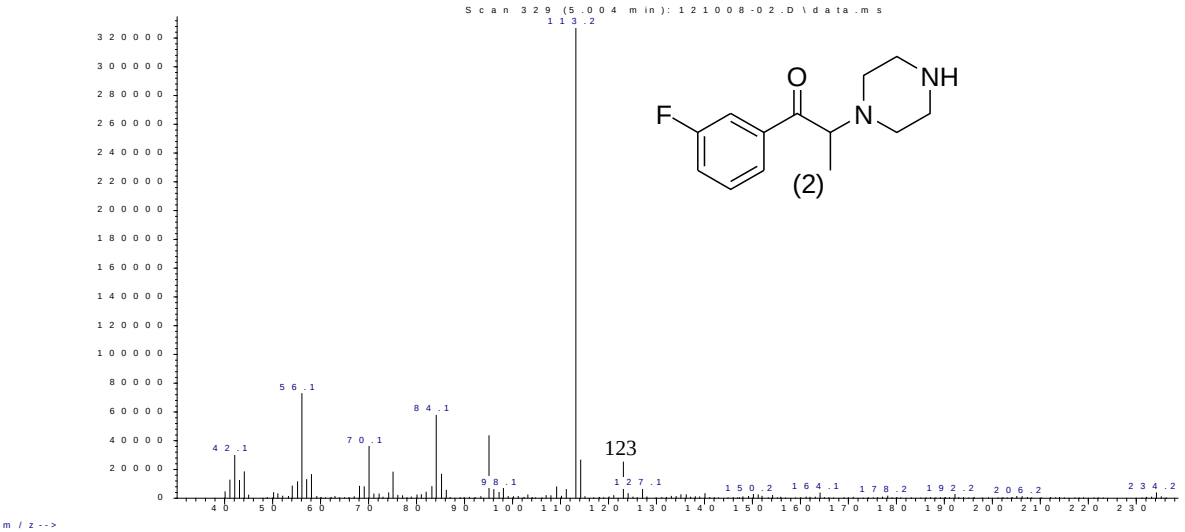
The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass spectral studies of the underivatized and perfluoroacylated derivatives of 1-(monofluorophenyl)-2-piperazinopropanones (FPPPOs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 25-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3). The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring in addition to the alpha cleavage (α-cleavage) products. The mass spectra of the three piperazines show the fragment ions at m/z 123, 113, 84 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 23-2 and are based on a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. The mass spectra of the three piperazines did not show any molecular ion peak. The base peak in the mass spectra of all the three compounds is the fragment ion at m/z 113 resulting from the alpha cleavage of the molecular ion. The regioisomeric fluorobenzoyl $(C_7H_4FO)^+$ fragments have the same nominal and exact masses. The mass spectra for the ring substituted fluorophenylpiperazinopropanones (Compounds 1-3) have almost identical mass spectra to each other.

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 three compounds.





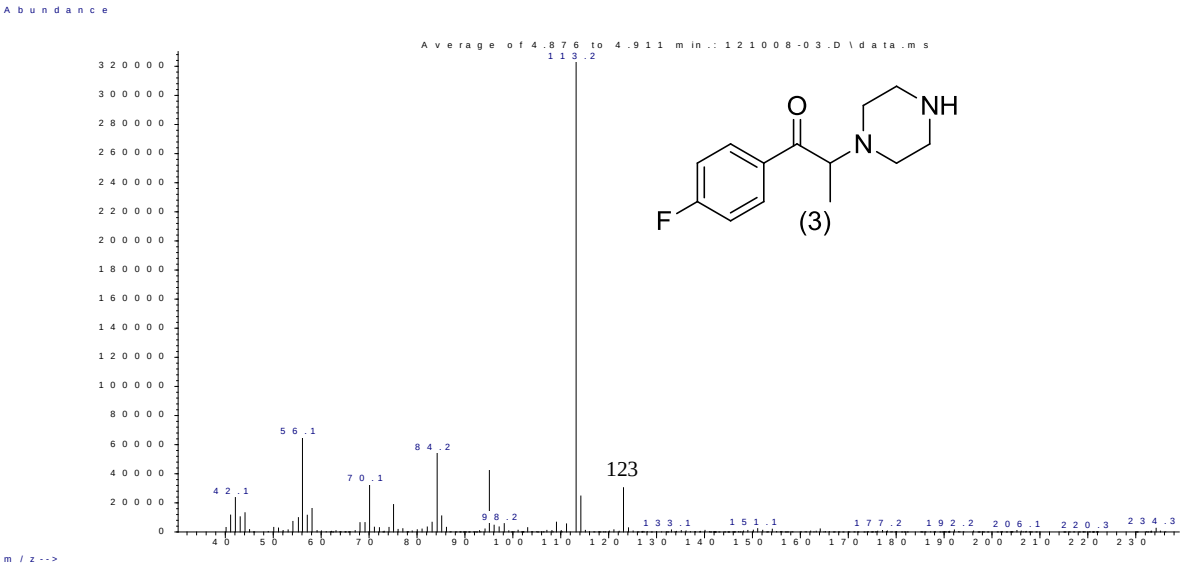


Fig. 25-1: Mass spectra of the three underivatized piperazines in this study

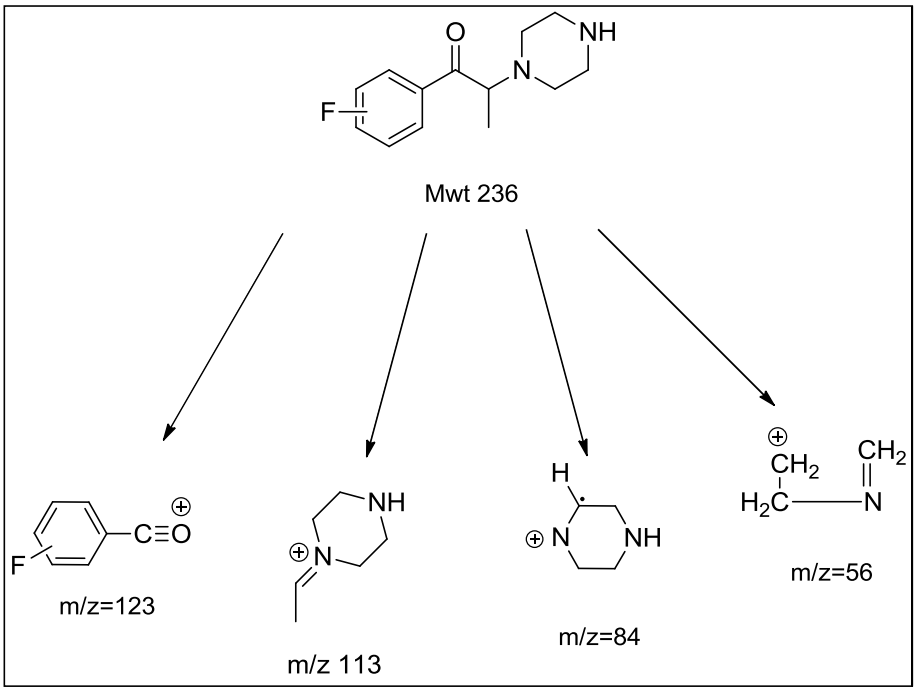
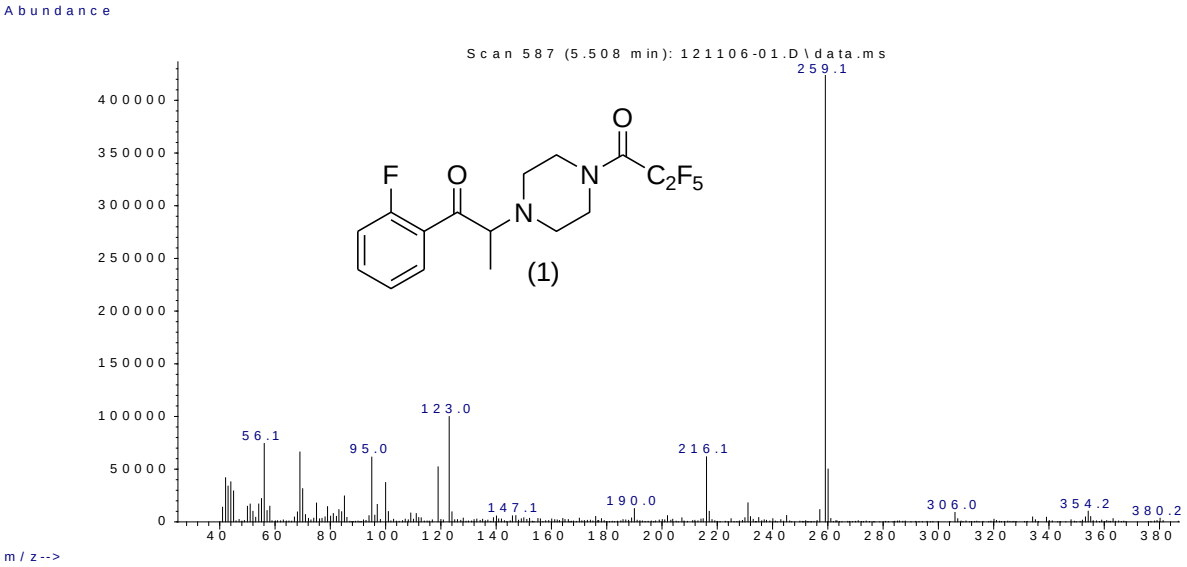


Fig. 25-2: Mass spectral fragmentation pattern of the underivatized 1-(fluorophenyl)-2-piperazinopropanones (FPPPOs) under EI (70eV) conditions.

The pentafluoropropionyl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 25-3 shows the mass spectra of the pentafluoropropionyl amides of the three studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ion peaks for the three PFPA amides were absent in their mass spectra. The major fragment ion in these spectra occurs at *m/z* 259 and 309 for the PFPA and HFBA amides, respectively and corresponds to the alpha cleavage piperazine-containing fragment. Furthermore, an additional characteristic fragment ion series occurring at *m/z* 216 and 266 for PFPA and HFBA amides, respectively corresponds to the $(M-178)^+$ ion for each amide. The proposed structure and mechanism for the formation of the m/z 216 ion in the mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study was previously discussed in details in Chapter 23.

The ion at *m/z* 123 was observed in the spectra of all derivatives and corresponds to the ring substituted benzoyl fragments. Those ions occurring at m/z 119 and 169 are the perfluoroalkyl cations 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 other in this set of compounds.



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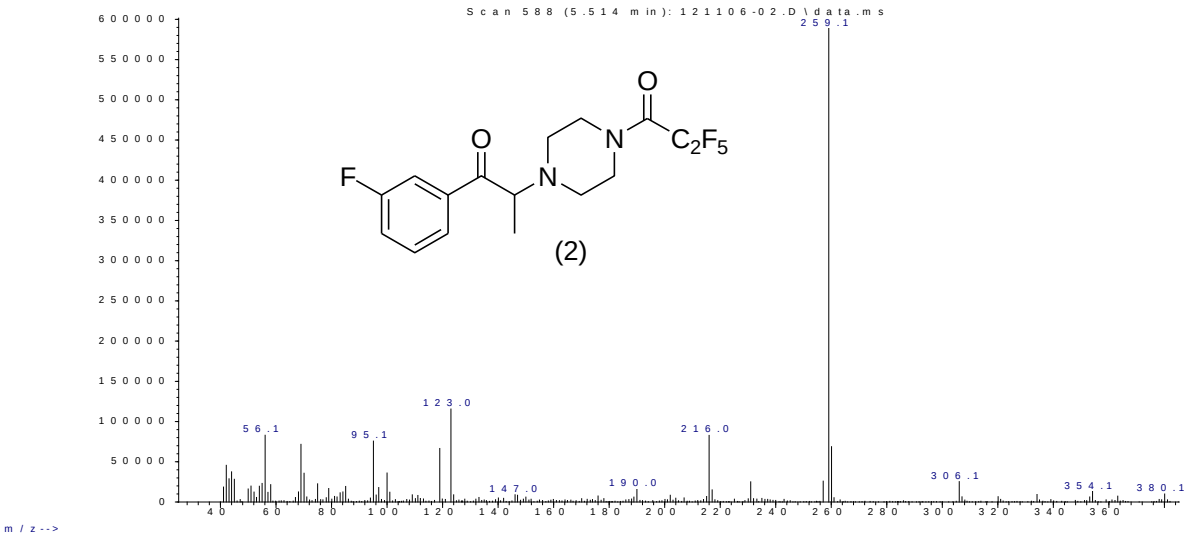

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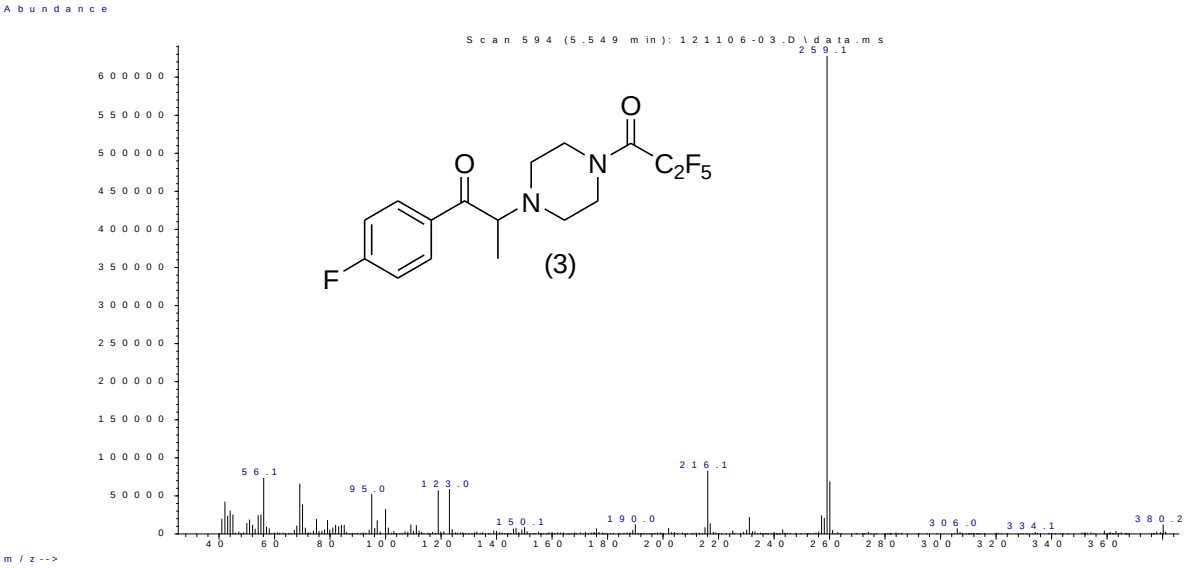


Fig. 25-3: Mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study

Gas Chromatographic Separation of the 1-(monofluorophenyl)-2-piperazinopropanones (FPPPOs)

Gas chromatographic separation of the derivatized piperazines was accomplished on a capillary column 30 m × 0.25 mm i.d. coated with 0.50 µm of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatogram in Figure 25-4 is a representative of the results obtained for all samples on this stationary phase. 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 30.0 min.

In Figure 25-4 the PFPA derivatives of the three fluorophenylpiperazinopropanones are eluted in the order of 2, 3, 4-fluorophenylpiperazinopropanone. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

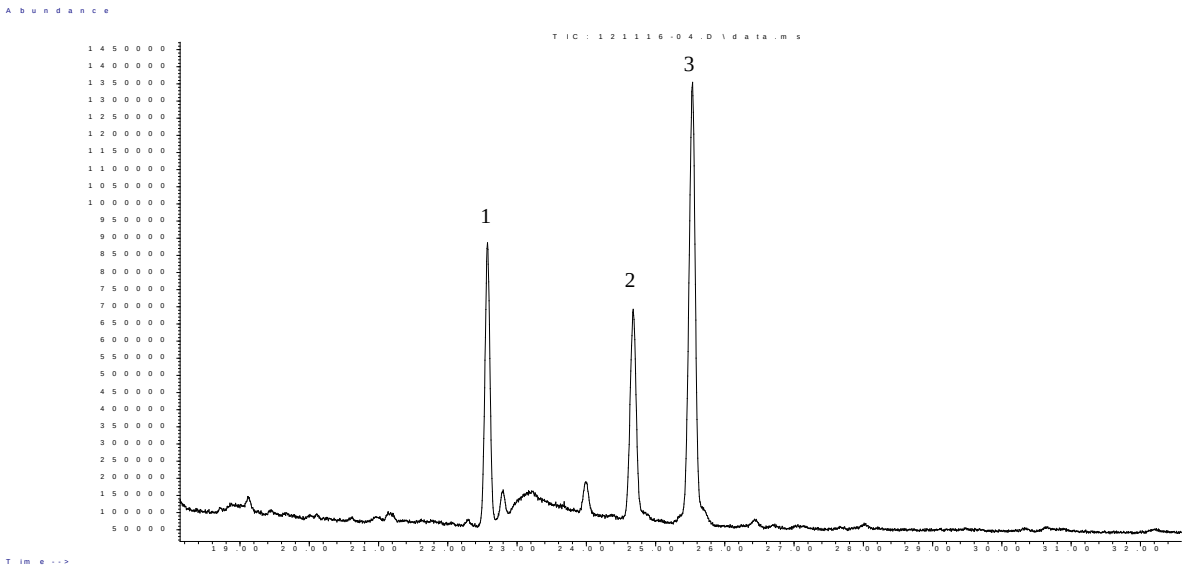


Fig. 25-4: Gas chromatographic separation of the pentafluoropropionyl derivatives of the three piperazines using Rtx-200 column. The number over the peak corresponds to the compound number.

Conclusion

The three fluorophenylpiperazinopropanones have a direct regioisomeric relationship to each other. The three regioisomeric piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. The three PFPA derivatives were successfully resolved on the stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Chapter 26

Differentiation of the 1-(monochlorophenyl)-2-piperazinopropanones (ClPPPOs) By GC-MS

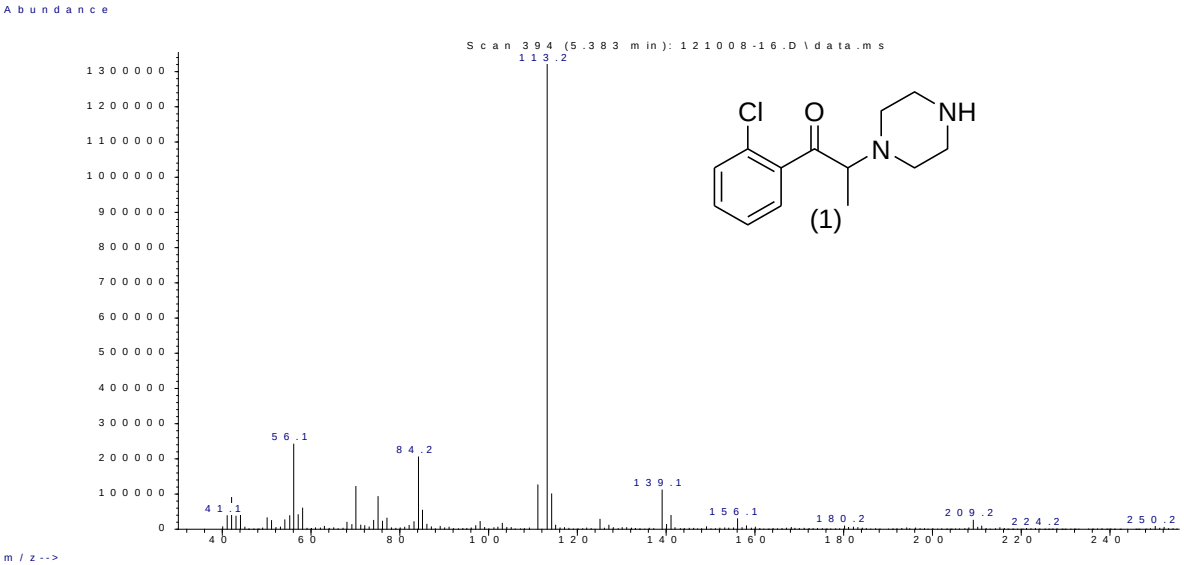
Three ring substituted chlorophenylpiperazinopropanones (ClPPPOs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass spectral studies of the underivatized and perfluoroacylated derivatives of 1-(monochlorophenyl)-2-piperazinopropanones (ClPPPOs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 26-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3). The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring in addition to the alpha cleavage (α-cleavage) products. The mass spectra of the three piperazines show the fragment ions at m/z 139, 113, 84 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 26-2 and are based on a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. The mass spectra of the three piperazines did not show any molecular ion peak. The base peak in the mass spectra of all the three compounds is the fragment ion at m/z 113 resulting from the alpha cleavage of the molecular ion. The regioisomeric chlorobenzoyl $(C_7H_4ClO)^+$ fragments have the same nominal and exact masses. The mass spectra for the ring substituted chlorophenylpiperazinopropanones (Compounds 1-3) have almost identical mass spectra to each other.

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 three compounds.



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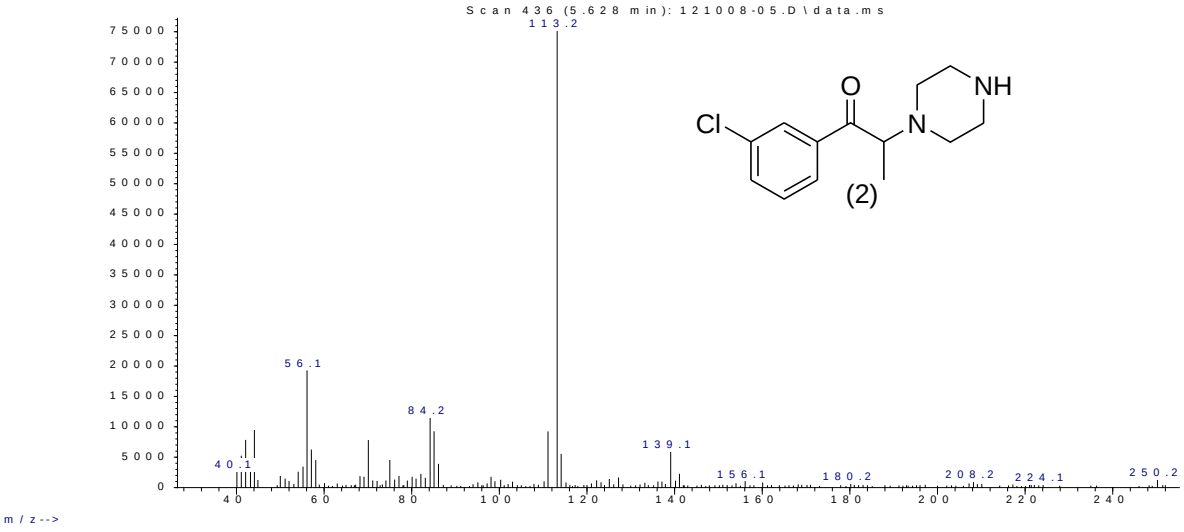

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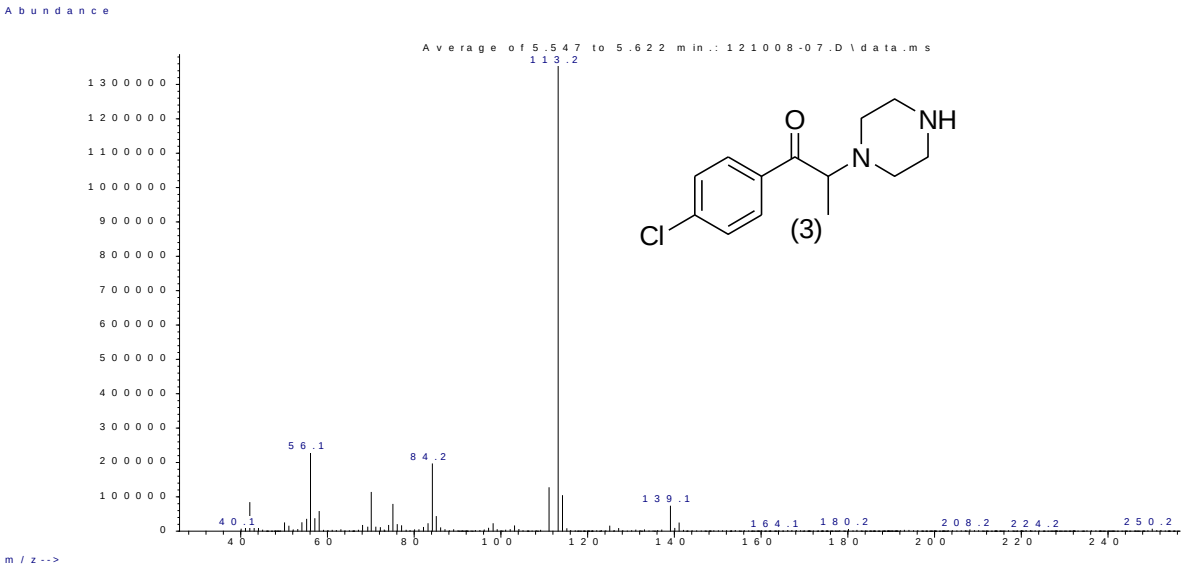


Fig. 26-1: Mass spectra of the three underivatized piperazines in this study

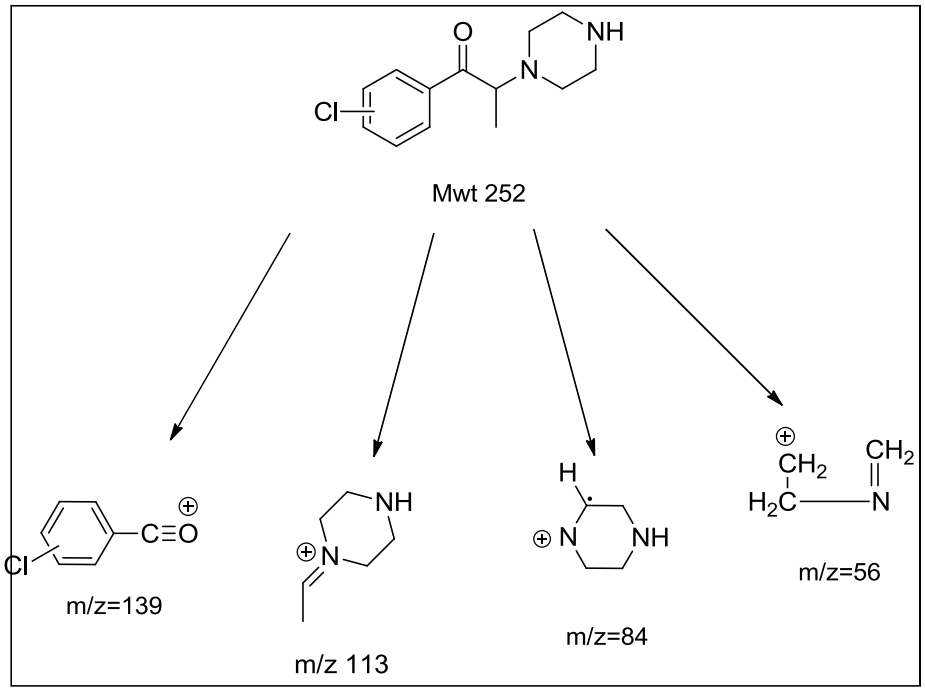
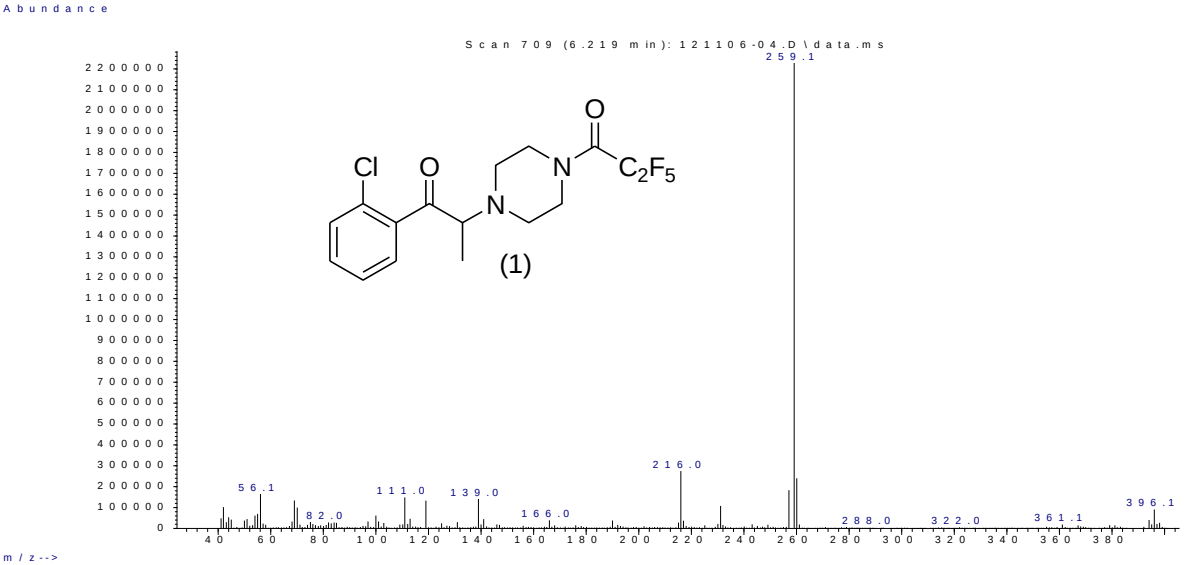


Fig. 26-2: Mass spectral fragmentation pattern of the underivatized 1-(chlorophenyl)-2-piperazinopropanones (ClPPPOs) under EI (70eV) conditions.

The pentafluoropropionyl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 26-3 shows the mass spectra of the pentafluoropropionyl amides of the three studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ion peaks for the three PFPA amides were absent in their mass spectra. The major fragment ion in these spectra occurs at *m/z* 259 and 309 for the PFPA and HFBA amides, respectively and corresponds to the alpha cleavage piperazine-containing fragment. Furthermore, an additional characteristic fragment ion series occurring at *m/z* 216 and 266 for PFPA and HFBA amides, respectively corresponds to the $(M-178)^+$ ion for each amide. The proposed structure and mechanism for the formation of the m/z 216 ion in the mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study was previously discussed in details in Chapter 23.

The ion at *m/z* 139 was observed in the spectra of all derivatives and corresponds to the ring substituted benzoyl fragments. Those ions occurring at m/z 119 and 169 are the perfluoroalkyl cations 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 other in this set of compounds.



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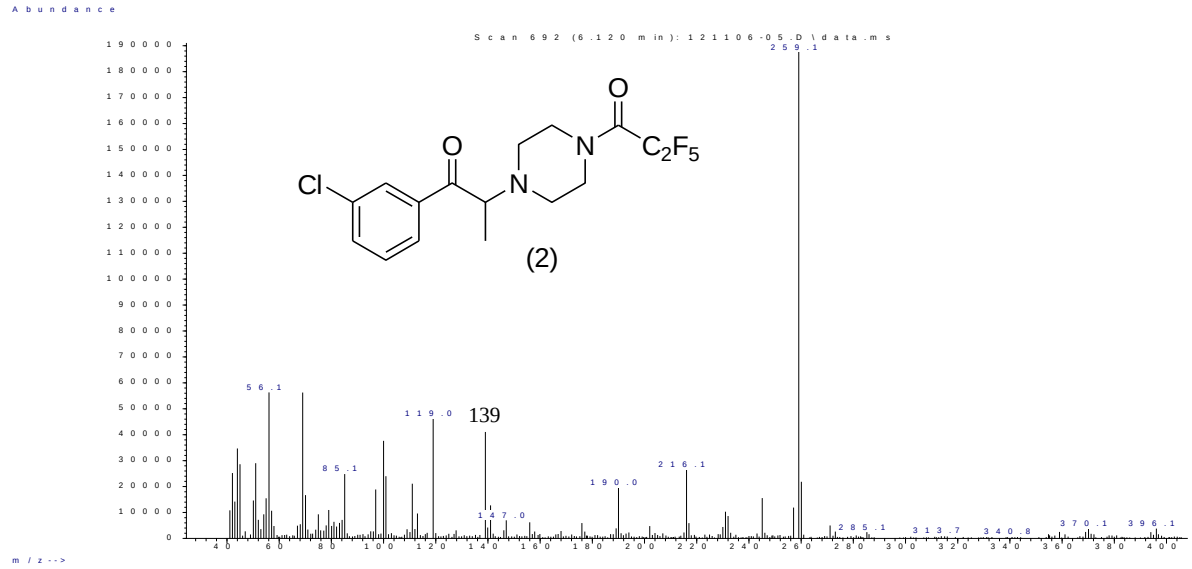

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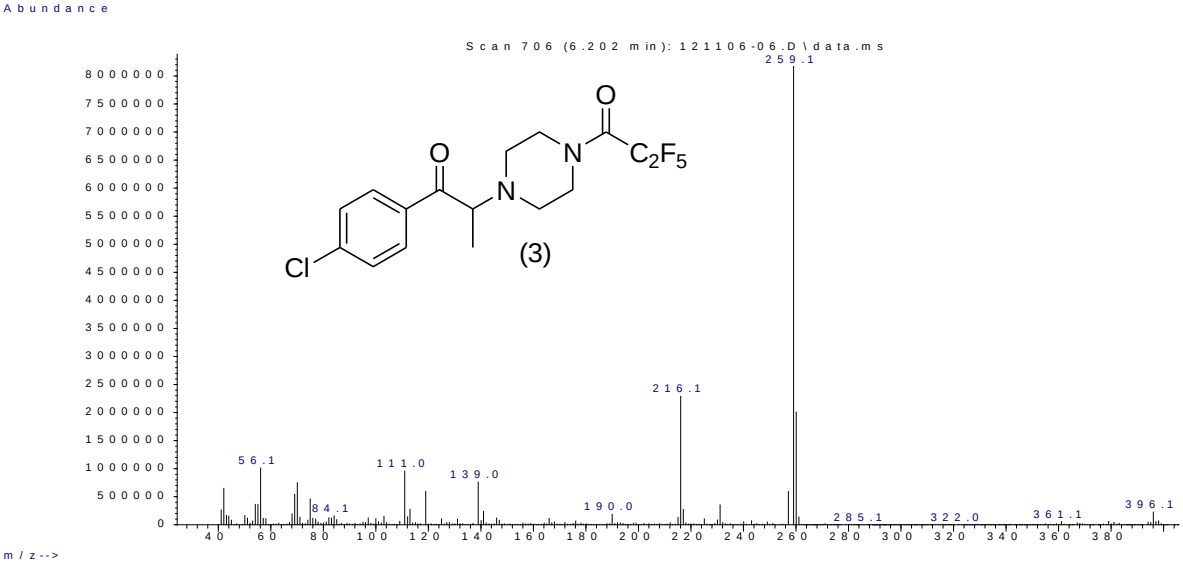


Fig. 26-3: Mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study

Gas Chromatographic Separation of the 1-(monochlorophenyl)-2-piperazinopropanones (ClPPPOs)

Gas chromatographic separation of the derivatized piperazines was accomplished on a capillary column 30 m × 0.25 mm i.d. coated with 0.50 µm of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatogram in Figure 26-4 is a representative of the results obtained for all samples on this stationary phase. The separation of the pentafluoropropionyl derivatives was performed using a temperature program consisting of an initial hold at 70°C for 1.0 min, ramped up to 150°C at a rate of 7.5 °C/min, held at 180°C for 2.0 min then ramped to 250°C at a rate of 10°C/min and held at 200°C for 20.0 min.

In Figure 26-4 the PFPA derivatives of the three chlorophenylpiperazinopropanones are eluted in the order of 2, 3, 4-chlorophenylpiperazinopropanone. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

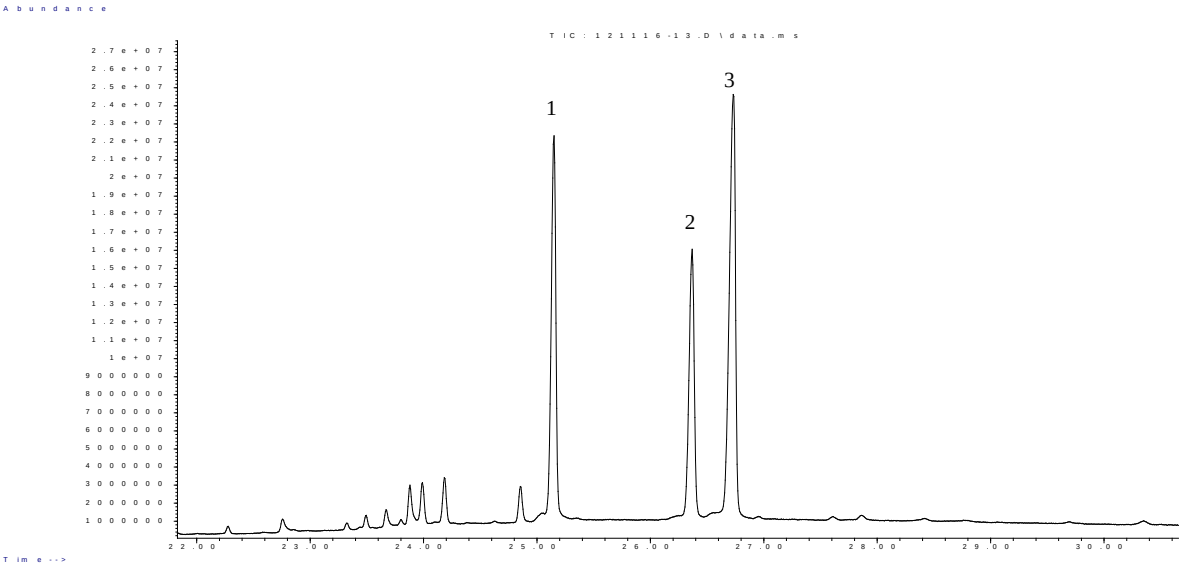


Fig. 26-4: Gas chromatographic separation of the pentafluoropropionyl derivatives of the three piperazines using Rtx-200 column. The number over the peak corresponds to the compound number.

Conclusion

The three chlorophenylpiperazinopropanones have a direct regioisomeric relationship to each other. The three regioisomeric piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. The three PFPA derivatives were successfully resolved on the stationary phase Rtx-200.

References

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 Science International 121 (2001) 47-56.

Chapter 27

Differentiation of the 1-(monobromophenyl)-2-piperazinopropanones (BrPPPOs) By GC-MS

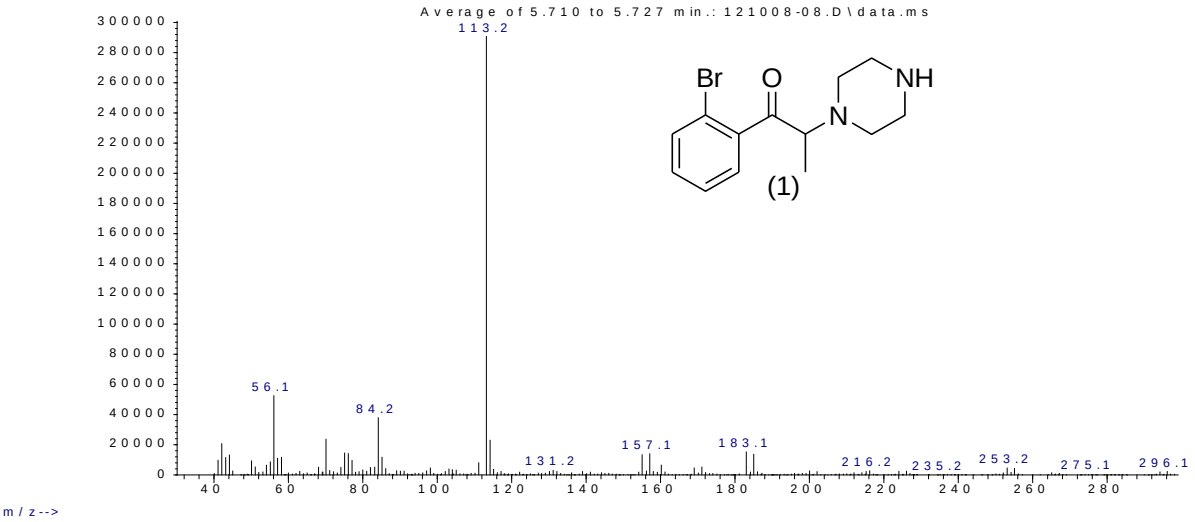
Three ring substituted bromophenylpiperazinopropanones (BrPPPOs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

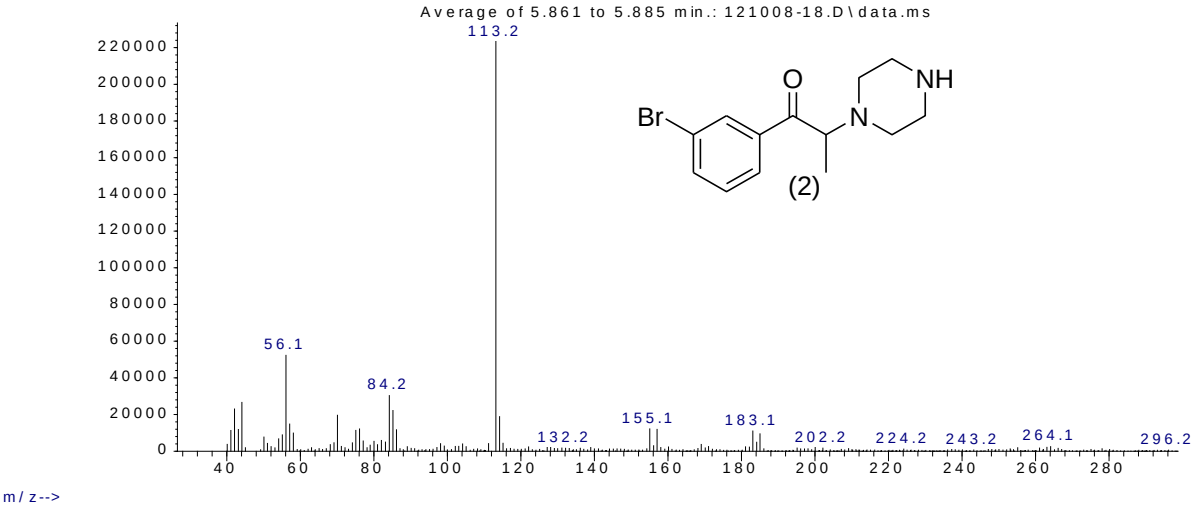
The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass spectral studies of the underivatized and perfluoroacylated derivatives of 1-(monobromophenyl)-2-piperazinopropanones (BrPPPOs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 27-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3). The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring in addition to the alpha cleavage (α-cleavage) products. The mass spectra of the three piperazines show the fragment ions at m/z 183/185, 113, 84 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 27-2 and are based on a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001].]. The mass spectra of the three piperazines did not show any molecular ion peak. The base peak in the mass spectra of all the three compounds is the fragment ion at m/z 113 resulting from the alpha cleavage of the molecular ion. The regioisomeric bromobenzoyl $(C_7H_4BrO)^+$ fragments have the same nominal and exact masses. The mass spectra for the ring substituted bromophenylpiperazinopropanones (Compounds 1-3) have almost identical mass spectra to each other.

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 three compounds.





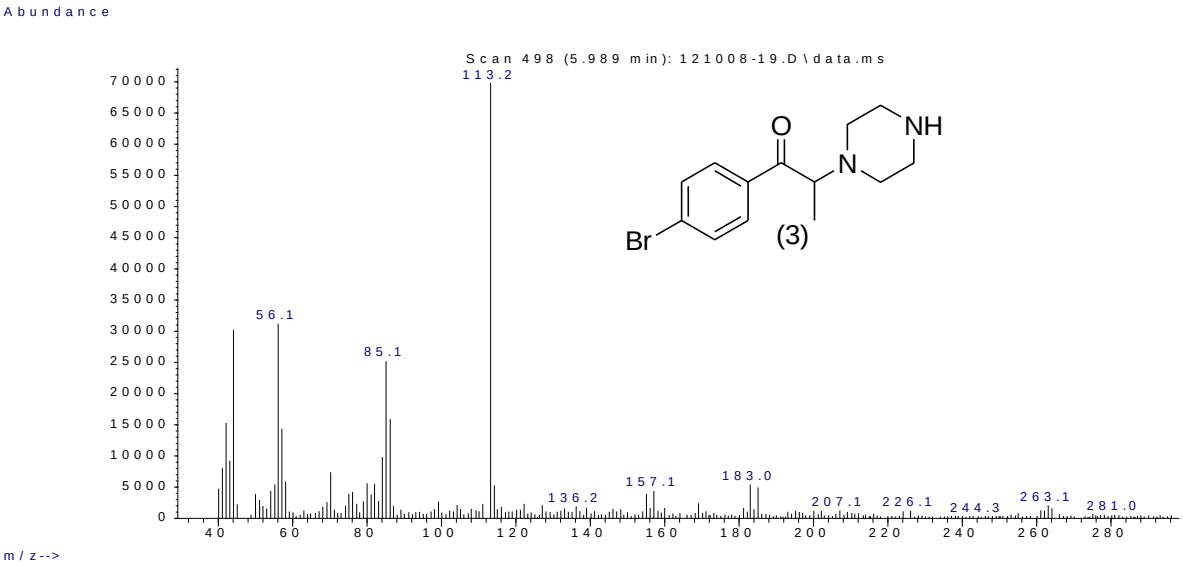


Fig. 27-1: Mass spectra of the three underivatized piperazines in this study

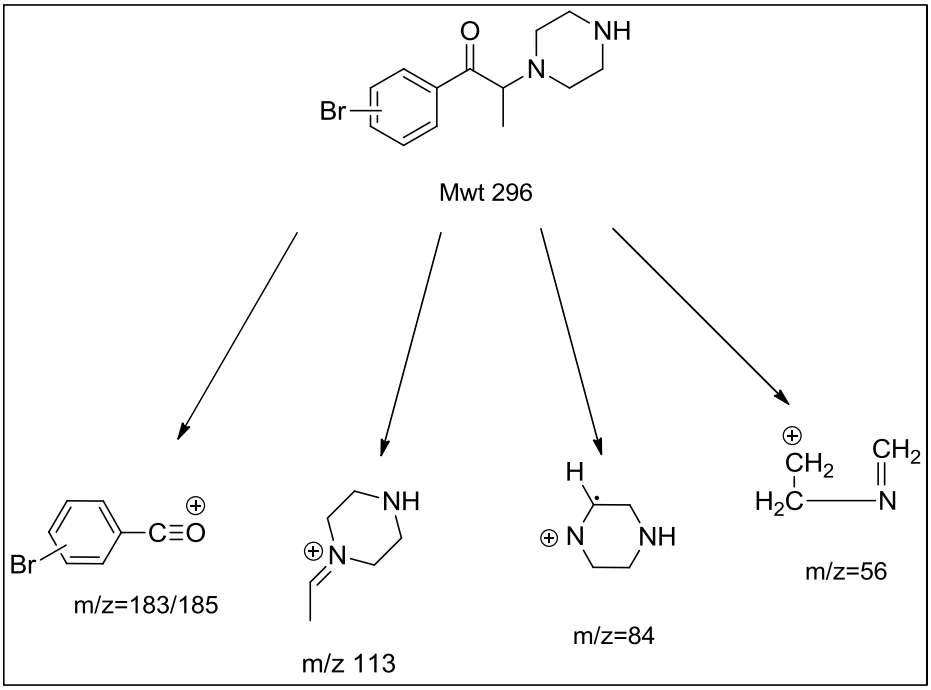
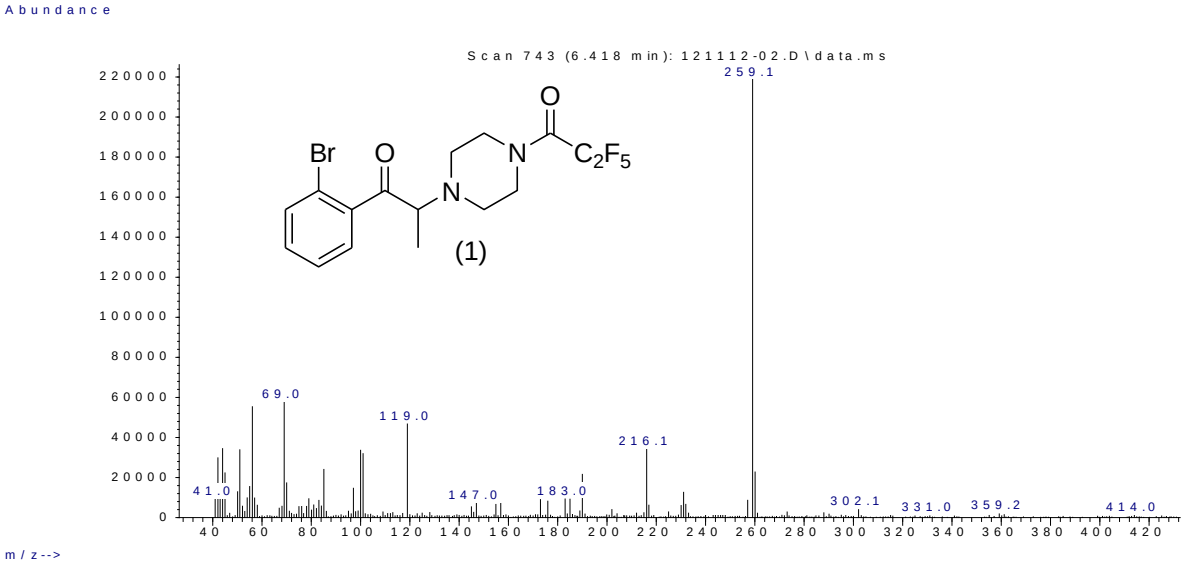
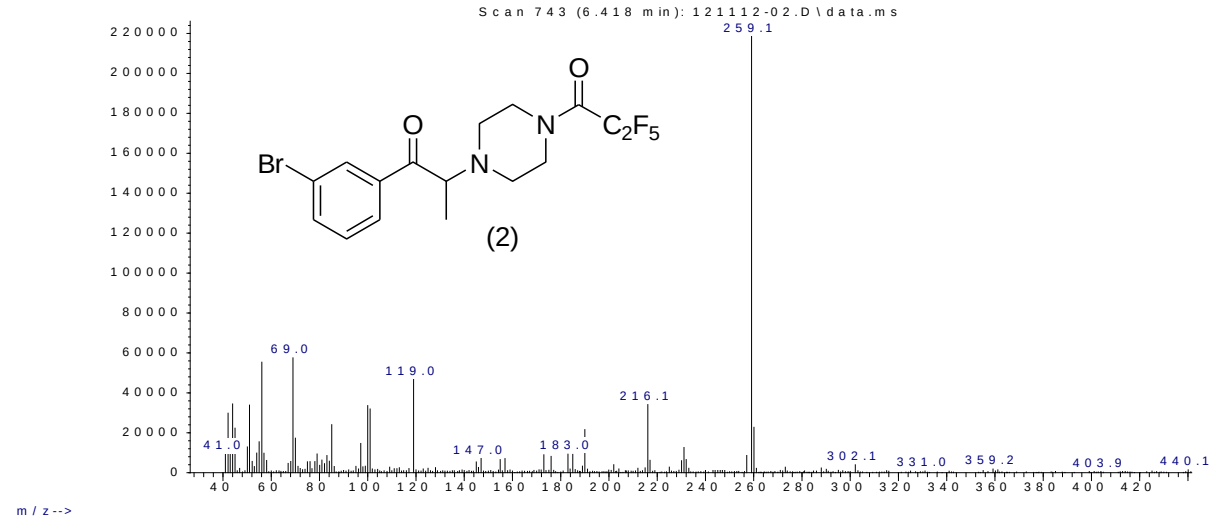


Fig. 27-2: Mass spectral fragmentation pattern of the underivatized 1-(bromophenyl)-2-piperazinopropanones (BrPPPOs) under EI (70eV) conditions.

The pentafluoropropionyl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 27-3 shows the mass spectra of the pentafluoropropionyl amides of the three studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ion peaks for the three PFPA amides were absent in their mass spectra. The major fragment ion in these spectra occurs at *m/z* 259 and 309 for the PFPA and HFBA amides, respectively and corresponds to the alpha cleavage piperazine-containing fragment. Furthermore, an additional characteristic fragment ion series occurring at *m/z* 216 and 266 for PFPA and HFBA amides, respectively corresponds to the $(M-178)^+$ ion for each amide. The proposed structure and mechanism for the formation of the m/z 216 ion in the mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study was previously discussed in details in Chapter 23.

The ion at *m/z* 183/185 was observed in the spectra of all derivatives and corresponds to the ring substituted benzoyl fragments. Those ions occurring at m/z 119 and 169 are the perfluoroalkyl cations 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 other in this set of compounds.





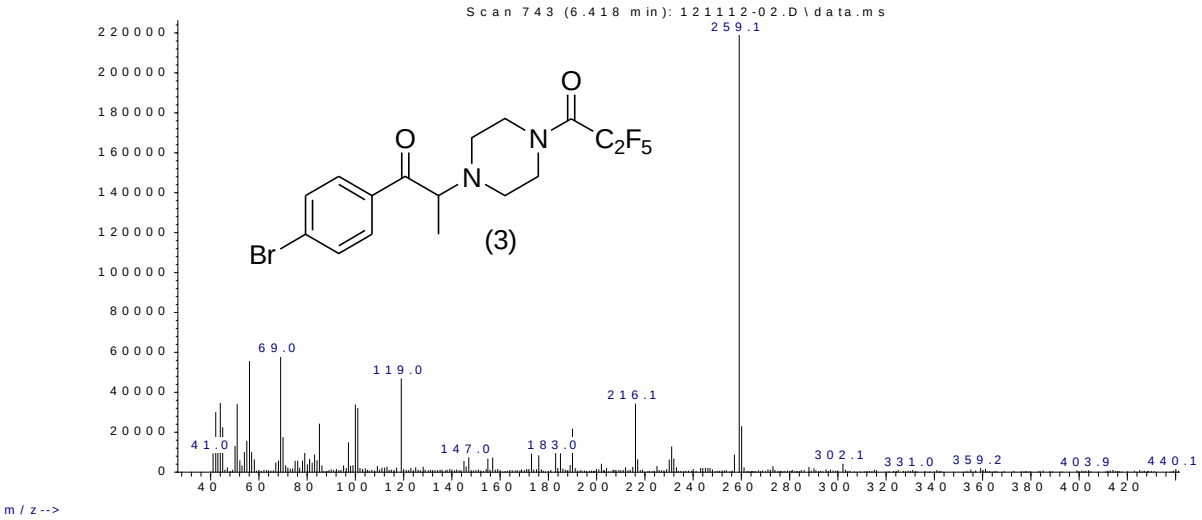


Fig. 27-3: Mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study

Gas Chromatographic Separation of the 1-(monobromophenyl)-2-piperazinopropanones (BrPPPOs)

Gas chromatographic separation of the derivatized piperazines was accomplished on a capillary column 30 m × 0.25 mm i.d. coated with 0.50 µm of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatogram in Figure 27-4 is a representative of the results obtained for all samples on this stationary phase. The separation of the pentafluoropropionyl derivatives was performed using a temperature program consisting of an initial hold at 70°C for 1.0 min, ramped up to 150°C at a rate of 7.5 °C/min, held at 180°C for 2.0 min then ramped to 250°C at a rate of 10°C/min and held at 200°C for 20.0 min.

In Figure 27-4 the PFPA derivatives of the three bromophenylpiperazinopropanones are eluted in the order of 2, 3, 4-bromophenylpiperazinopropanones. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

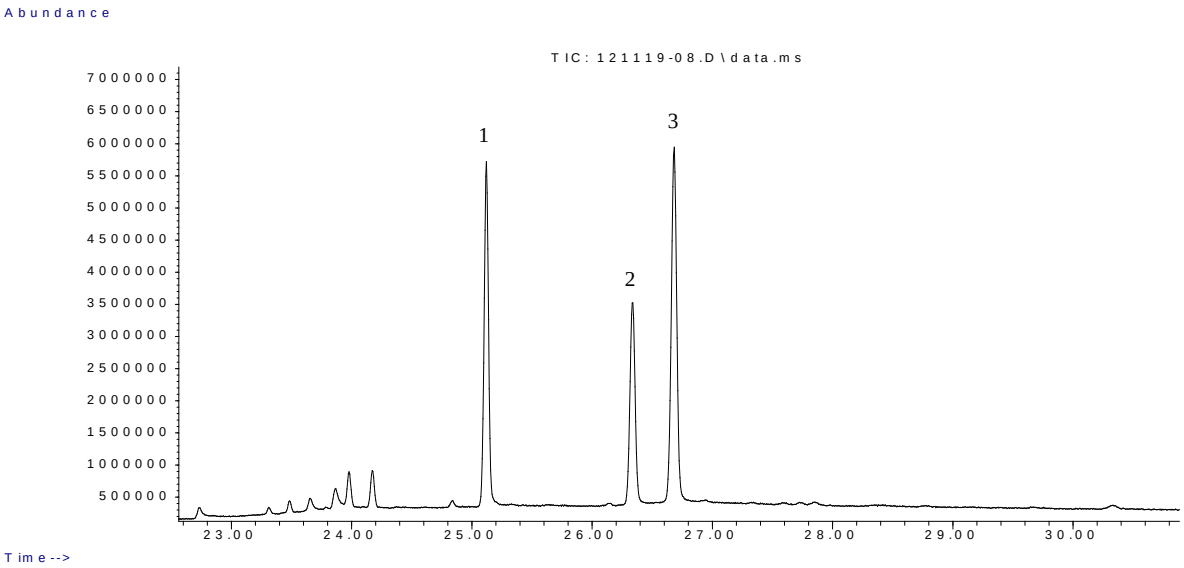


Fig. 27-4: Gas chromatographic separation of the pentafluoropropionyl derivatives of the three piperazines using Rtx-200 column. The number over the peak corresponds to the compound number.

Conclusion

The three bromophenylpiperazinopropanones have a direct regioisomeric relationship to each other. The three regioisomeric piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. The three PFPA derivatives were successfully resolved on the stationary phase Rtx-200.

References

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Chapter 28

Differentiation of the 1-(trifluoromethylphenyl)-2-piperazinopropanones (CF_3PPPOs) By GC-MS

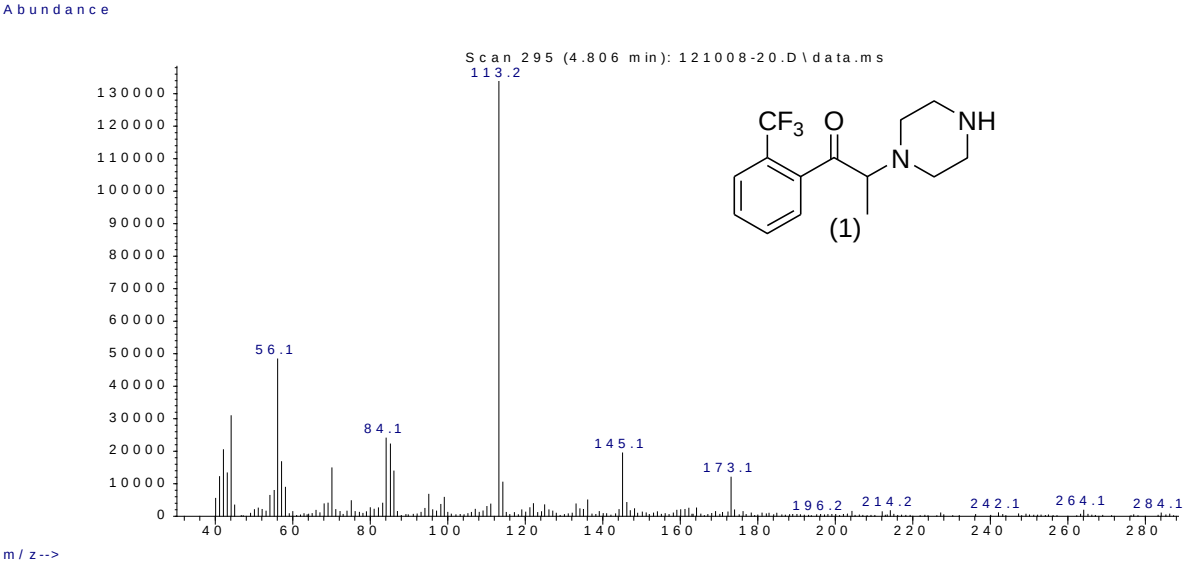
Three ring substituted trifluoromethylphenylpiperazinopropanones (CF_3PPPOs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.

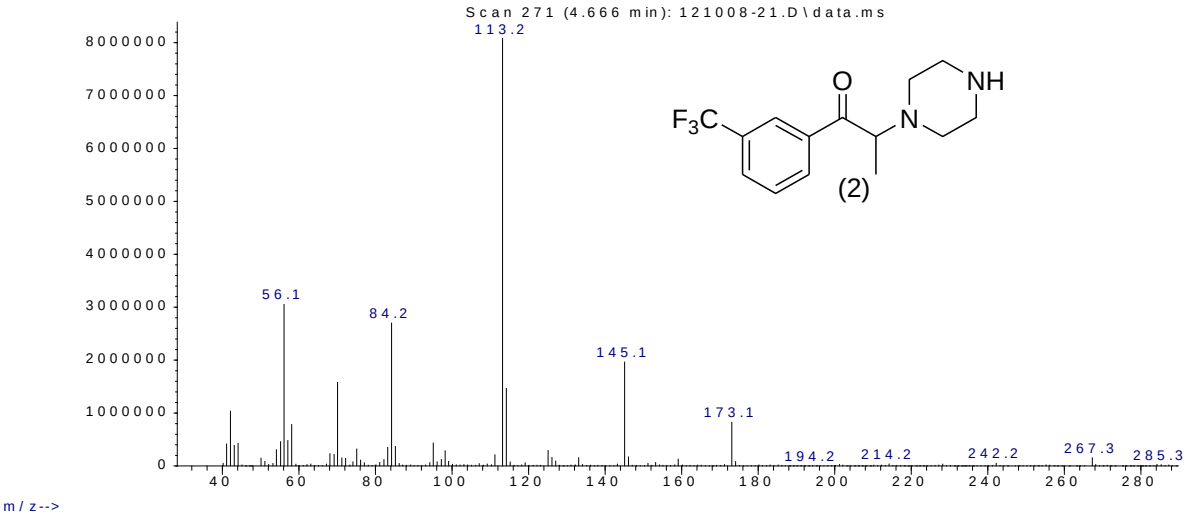
The underivatized and perfluoroacyl derivatives of these three piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200).

Mass spectral studies of the underivatized and perfluoroacylated derivatives of 1-(trifluoromethylphenyl)-2-piperazinopropanones (CF_3PPPOs)

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 28-1 shows the EI mass spectra of all three isomeric piperazines (Compounds 1-3). The ions of significant relative abundance common to the three isomers likely arise from fragmentation of the piperazine ring in addition to the alpha cleavage (α-cleavage) products. The mass spectra of the three piperazines show the fragment ions at m/z 173, 113, 84 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Figure 28-2 and are based on a previous report describing the fragmentation of the unsubstituted benzylpiperazines [de Boer *et al*, 2001]. The mass spectra of the three piperazines did not show any molecular ion peak. The base peak in the mass spectra of all the three compounds is the fragment ion at m/z 113 resulting from the alpha cleavage of the molecular ion. The regioisomeric trifluoromethylbenzoyl $(C_8H_4F_3O)^+$ fragments have the same nominal and exact masses. The mass spectra for the ring substituted trifluoromethylphenylpiperazinopropanones (Compounds 1-3) have almost identical mass spectra to each other.

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 three compounds.





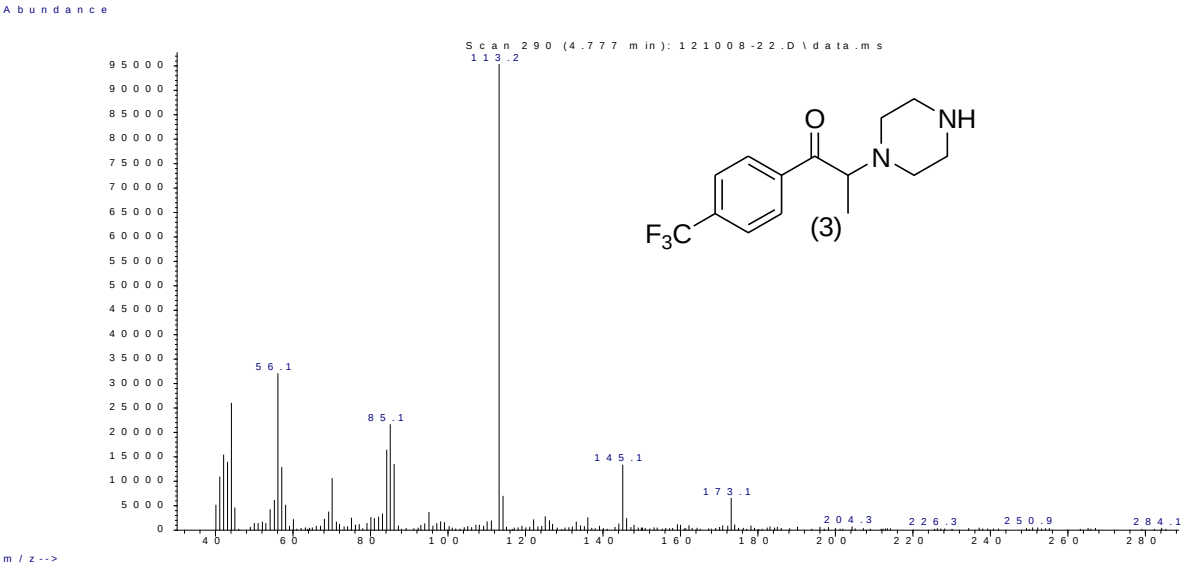


Fig. 28-1: Mass spectra of the three underivatized piperazines in this study

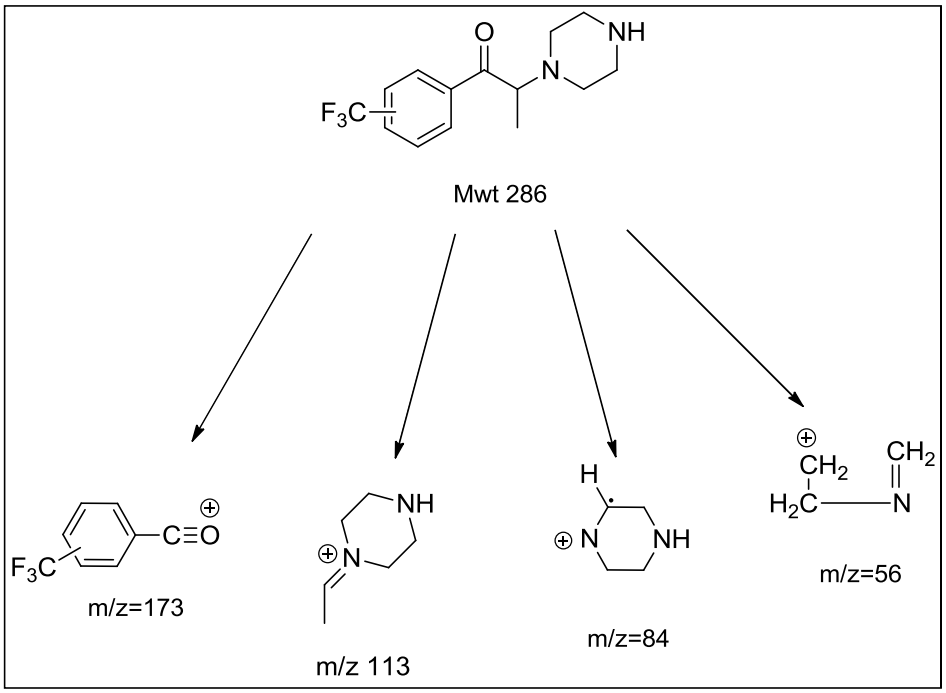
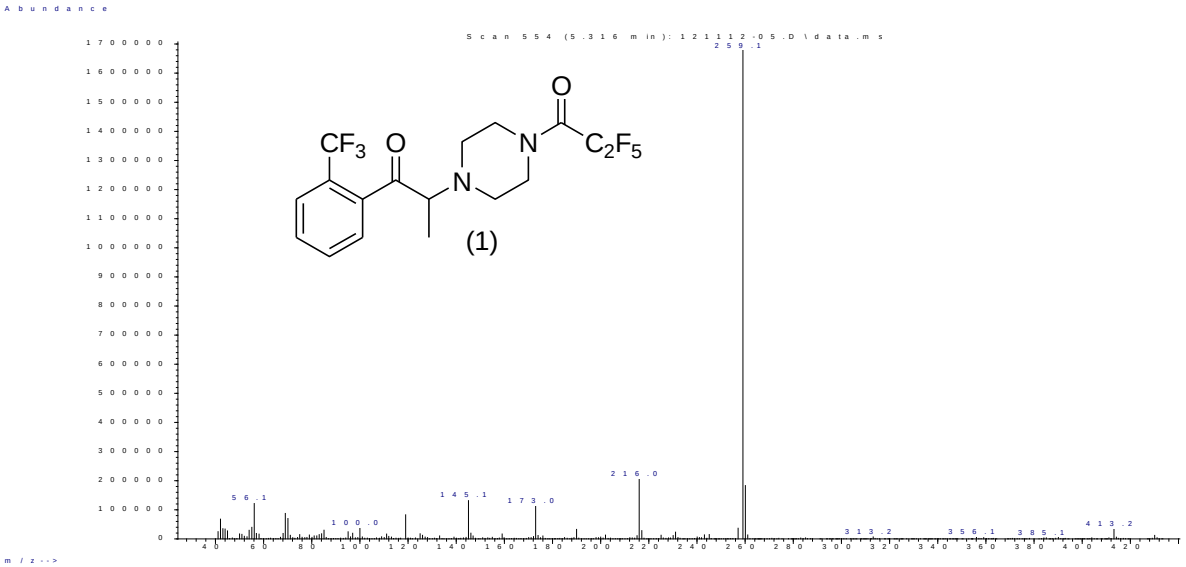


Fig. 28-2: Mass spectral fragmentation pattern of the underivatized 1-(trifluoromethylphenyl)-2-piperazinopropanones (CF_3PPPOs) under EI (70eV) conditions.

The pentafluoropropionyl derivatives of the secondary nitrogen were all evaluated for their ability to individualize the mass spectra of this series of substituted piperazines. Figure 28-3 shows the mass spectra of the pentafluoropropionyl amides of the three studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ion peaks for the three PFPA amides were absent in their mass spectra. The major fragment ion in these spectra occurs at *m/z* 259 and 309 for the PFPA and HFBA amides, respectively and corresponds to the alpha cleavage piperazine-containing fragment. Furthermore, an additional characteristic fragment ion series occurring at *m/z* 216 and 266 for PFPA and HFBA amides, respectively corresponds to the $(M-178)^+$ ion for each amide. The proposed structure and mechanism for the formation of the m/z 216 ion in the mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study was previously discussed in details in Chapter 23.

The ion at *m/z* 173 was observed in the spectra of all derivatives and corresponds to the ring substituted benzoyl fragments. Those ions occurring at m/z 119 and 169 are the perfluoroalkyl cations 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 other in this set of compounds.



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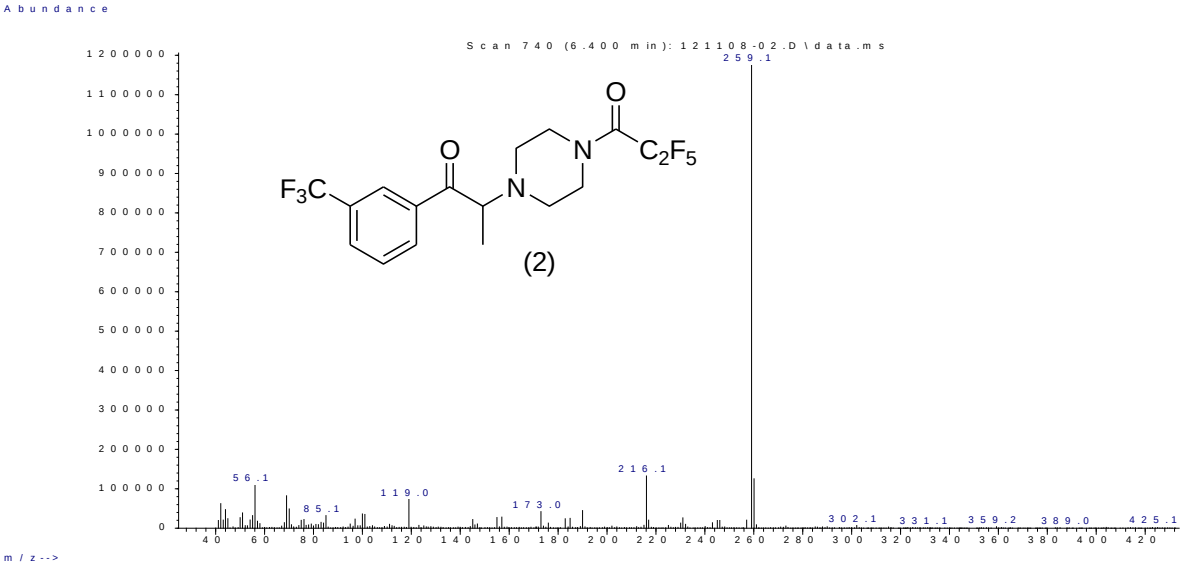

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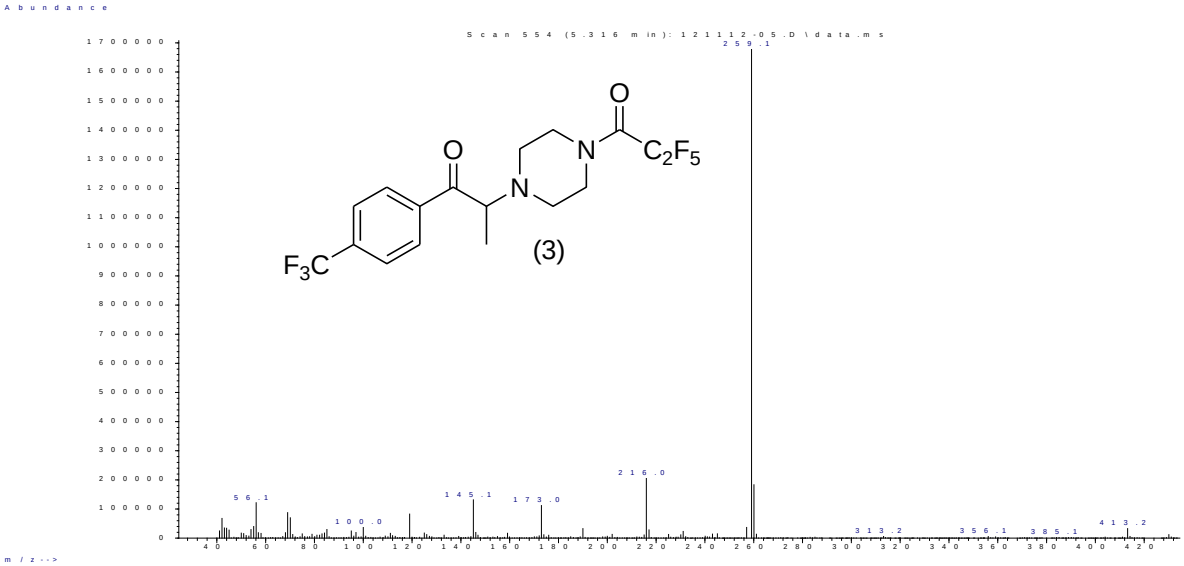

Fig. 28-3: Mass spectra of the pentafluoropropionyl derivatives of the three piperazines in this study

Gas Chromatographic Separation of the 1-(trifluoromethylphenyl)-2-piperazinopropanones (CF_3PPPOs)

Gas chromatographic separation of the derivatized piperazines was accomplished on a capillary column 30 m × 0.25 mm i.d. coated with 0.50 µm of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and the chromatogram in Figure 28-4 is a representative of the results obtained for all samples on this stationary phase. 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 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 30.0 min.

In Figure 28-4 the PFPA derivatives of the three trifluoromethylphenylpiperazinopropanones are eluted in the order of 2, 3, 4-trifluoromethylphenylpiperazinopropanones. The perfluoroacylated derivatives did not provide any additional mass spectral discrimination among the three isomers. However, perfluoroacylation offered marked improvement in the chromatographic resolution compared to the underivatized piperazines.

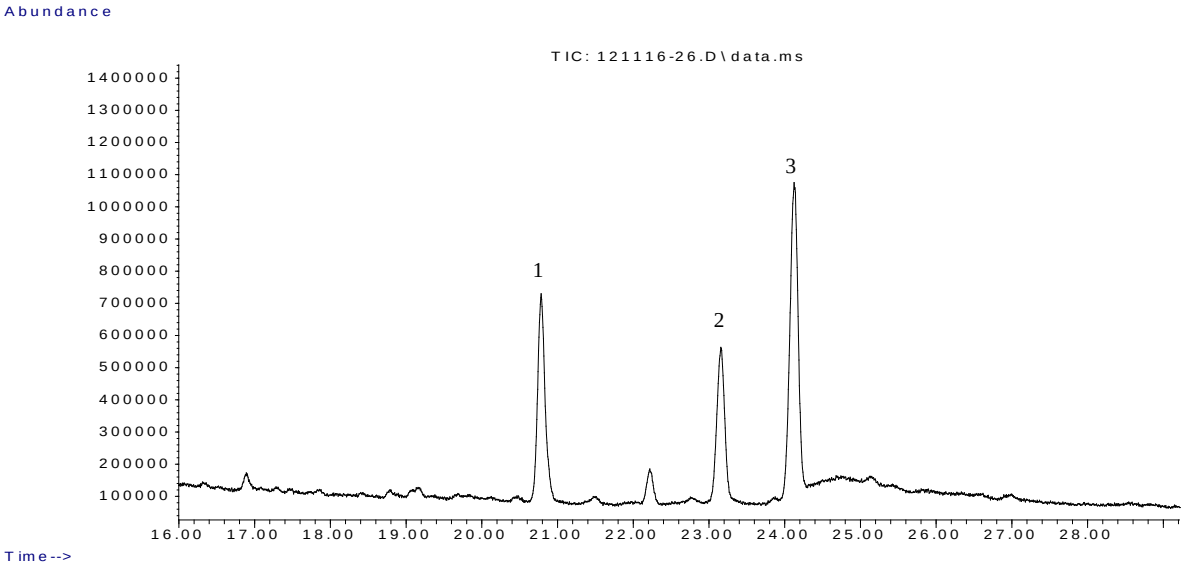

Fig. 28-4: Gas chromatographic separation of the pentafluoropropionyl derivatives of the three piperazines using Rtx-200 column. The number over the peak corresponds to the compound number.

Conclusion

The three trifluoromethylphenylpiperazinopropanones have a direct regioisomeric relationship to each other. The three regioisomeric piperazines yield very similar fragment ions in their mass spectra. Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one compound to the exclusion of the others. The three PFPA derivatives were successfully resolved on the stationary phase Rtx-200.

References

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Experimental

1. Materials, Instruments, GC-Columns and Temperature Programs

1.1. Materials

The majority of the synthetic starting materials were obtained from Aldrich chemical company (Milwaukee, WI, USA).

Piperazine, N-methylpiperazine 3,4-Methylenedioxyphenylacetone, piperonal, 2,3-dihydroxybenzaldehyde, o-toluealdehyde, m-toluealdehyde, p-toluealdehyde o-anisaldehyde, m-anisaldehyde, p-anisaldehyde, 2-hydroxypropiophenone, m-methoxyproiophenone, p-methoxypropiophenone, 2,3-dimethyl anisole, 3-methyl salicylic acid, 4-methyl salicylic acid, 2-methoxy-phenylacetone,4-methoxy-2-methyl benzaldehyde, 4-methoxy-3-methyl benzaldehyde, benzaldehyde, dibromomethane, thionyl chloride, copper(II) oxide, sodium cyanoborohydride, sodium cyanobordeuteride, d_5-benzoyl chloride, d_8-piperazine, 2-ethoxybenzaldehyde, 3-ethoxybenzaldehyde, 4-ethoxybenzaldehyde, 2-fluorobenzaldehyde, 3-fluorobenzaldehyde, 4-fluorobenzaldehyde, 2-chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2-bromobenzaldehyde, 3-bromobenzaldehyde, 4-bromobenzaldehyde, 2-trifluoromethylbenzaldehyde, 3-trifluoromethylbenzaldehyde, 4-trifluoromethylbenzaldehyde bromodimethoxybenzaldehydes, 2,3-dimethoxybenzaldehyde, 2,4-dimethoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, 2,6-dimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,5-dimethoxybenzaldehyde, benzoyl chloride, 2,3-dimethoxybenzoylchloride, 2,4-dimethoxybenzoylchloride, 2,4-dimethoxybenzoyl chloride, 2,5-dimethoxybenzoylchloride, 2,6-dimethoxybenzoylchloride, 3,4-dimethoxybenzoylchloride, 3,5-dimethoxybenzoic acid,

2-methoxybenzoylchloride, 3-methoxybenzoylchloride, 4-methoxybenzoylchloride, 2-trifluoromethylbenzoylchloride, 3-trifluoromethylbenzoylchloride, 4-trifluoromethylbenzoylchloride, aniline, 2-chloroaniline, 3-chloroaniline, 4-chloroaniline, 2-methylaniline, 3- methylaniline, 4- methylaniline, 2-methoxyaniline, 3-methoxyaniline, 4- methoxyaniline, 2,3-dimethylaniline, 2,4- dimethylaniline, 2,5-dimethylaniline, 2,6- dimethylaniline, 3,4- dimethylaniline, 3,5- dimethylaniline, 2,3-dimethoxyaniline, 2,4-dimethoxyaniline, 2,4- dimethoxyaniline, 2,5- dimethoxyaniline, 2,6- dimethoxyaniline, 3,4- dimethoxyaniline, 3,5- dimethoxyaniline, propiophenone, 2-fluoropropiophenone, 3-fluoropropiophenone, 4-fluoropropiophenone, 2-chloropropiophenone, 3-chloropropiophenone, 4-chloropropiophenone, 2-bromopropiophenone, 3-bromopropiophenone, 4-bromopropiophenone, 2-trifluoromethylpropiophenone, 3-trifluoromethylpropiophenone, 4-trifluoromethylpropiophenone, phenylacetone, o-methoxyphenylacetone, m-methoxyphenylacetone, p-methoxyphenylacetone, Sodium bis(2- methoxyethoxy) aluminum hydride (Red-Al) in toluene, 2-methylfuran, potassium carbonate, pyridine, trifluoroacetic anhydride, pentafluropropionic anhydride and heptaflurobutyric anhydride were purchased from UCT (Bristol, PA, USA). 2-methoxy-5-methyl benzaldehyde was purchased Trans World Chemicals (Rockville, MD, USA). 3-methoxy-4-methyl benzoic acid methyl ester was purchased from TCI America (Portland, OR, USA). Methyl iodide was purchased from Acros Organics (Morris Plains, NJ, USA).

HPLC grade acetonitrile, methylenechloride, methanol, toluene, tetrahydrofuran and ferric chloride were purchased from Fisher Scientific, (Atlanta, GA, USA). Diethyl ether,

2-propanol, methylene chloride, carbon tetrachloride, benzene, tetrahydrofuran (THF) and chloroform were purchased from Fisher Scientific (Fair Lawn, N.J., USA).

1.2. Instruments

GC–MS analysis was performed using a 7890A gas chromatograph with a 7683B auto injector coupled with a 5975C VL mass selective detector purchased from Agilent Technologies (Santa Clara, CA). The mass spectral scan rate was 2.86 scans /s. The GC was operated in splitless mode with a helium (grade 5) flow rate at 0.7 mL/min and the column head pressure was 10 psi. The MS was operated in the electron impact (EI) mode using an ionization voltage of 70 eV and a source of temperature of 230°C. The GC injector was maintained at 250°C and the transfer line at 280°C.

The GC-TOF analysis was done at the Mass Spec Center, Auburn University. The analysis utilized a 6890N gas chromatograph with a 7683B auto injector purchased from Agilent Technologies (Santa Clara, CA) coupled to a Waters GCT Premier benchtop orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer. 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 modeling 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. The vapor 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 280ºC 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.

Attenuated total reflection 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 per second. 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.

1.3. GC-Columns

Different capillary GC columns were evaluated throughout the course of this work, however only the columns that showed good compromises between resolution and analysis time are illustrated in Table 1. All columns used were purchased from Restek Corporation (Bellefonte PA, USA) and have the same dimensions, 30m x 0.25mm-i.d. coated with 0.25 µm fd. Inlet pressure was converted according to the constant flow mode and the total flow was 60 ml/min. The injection was in the split mode with an injector temperature at 250°C.

Table 1. List of columns used and their composition

Column name	Column composition
Rtx-1	100% Dimethyl polysiloxane
Rtx-5	95% dimethyl-5%diphenyl polysiloxane
Rtx-35	65% dimethyl-35%diphenyl polysiloxane
Rtx-200	100% trifluoropropyl methyl polysiloxane
Rxi-50	50% phenyl–50% methyl polysiloxane

1.4. Temperature Programs

Different temperature programs were evaluated throughout the course of this work, however only programs showing the best compromises between resolution and analysis time are illustrated in Table 2.

Table 2. List of temperature programs use

Temperature program name	Injector temperature °C	Detector temperature °C	Program setup
TP-1	250	280	Hold column temperature at 70°C for 1 minute then the temperature was ramped up to 250°C at a rate of 30°C / minute and set at 250°C for 5 min
TP-2	250	280	Hold column temperature at 100°C for 1 minute then the temperature was ramped up to 180°C at a rate of 12°C /minute. Column temperature was held at 180°C for 2 minutes then was ramped up to 200°C at a rate of 1°C / minute and set at 200°C for 5 minutes

TP-3	250	280	Hold column temperature at 100°C for 1 minute then the temperature was ramped up to 180°C at a rate of 9°C /minute. Column temperature was held at 180°C for 2 minutes then was ramped up to 200°C at a rate of 10°C / minute and set at 200°C for 5 minutes
TP-4	250	250	Hold column temperature at 70°C for 1 minute then the temperature was ramped up to 150°C at a rate of 7.5°C /minute. Column temperature was held at 150°C for 2 minutes then was ramped up to 250°C at a rate of 10°C / minute and set at 250°C for 15 minutes

2. Synthesis of the Regioisomeric and Isobaric Piperazines

2.1. Synthesis of the ring substituted benzylpiperazines

2.1.1. Synthesis of the methylenedioxybenzylpiperazines (MDBPs)

2,3-Dihydroxybenzaldehyde (5.0 g, 0.03 mol) and potassium carbonate (18.75 g, 0.136mol) were dissolved in 50 ml of DMF. Dibromomethane (18.9 g, 7.6 ml, 0.10mol) was added dropwise at room temperature, followed by addition of copper (II) oxide (0.010 g). The reaction mixture was refluxed for 2 hours and additional dibromomethane (18.9 g, 7.6 ml, 0.10mol) was added. The mixture was allowed to reflux overnight. The mixture was first vacuum filtered and then DMF was removed by Kugelrohr distillation. The obtained brown oil was suspended with water and extracted with dichloromethane (3x 30 ml). The combined organic extract was washed with 5% potassium hydroxide

solution, brine and 2N hydrochloric acid. The methylene chloride was evaporated and the obtained oil was distilled by Kugelrohr apparatus (100°C/ 3 mmHg), which gave 2,3-methylenedioxybenzaldehyde (3.2 g, 0.021mol, 59%) as light yellow oil.

The mixture of either 2,3-methylenedioxybenzaldehyde or piperonal (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2,3-methylenedioxybenzylpiperazine (2.6g, 0.0118 mol, 71.6%) or 3,4-methylenedioxybenzylpiperazine (2.64g, 0.012 mol, 72.2%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2. Synthesis of the methoxymethylbenzylpiperazines (MMBPs)

2.1.2.1. Synthesis of the 2-methoxy-5-methylbenzylpiperazine, 4-methoxy-3-methylbenzylpiperazine and 4-methoxy-2-methylbenzylpiperazine

The mixture of either 2-methoxy-5-methylbenzaldehyde (2.5g, 0.0165 mol) or 4-methoxy-3-methylbenzaldehyde (2.5g, 0.0165 mol) or 4-methoxy-2-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous

magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-methoxy-5-methylbenzylpiperazine (2.6g, 0.0118 mol, 71.6%), 4-methoxy-3-benzylpiperazine (2.64g, 0.012 mol, 72.2%) and 4-methoxy-2-methylbenzylpiperazine or 3,4-methylenedioxybenzylpiperazine (2.64g, 0.012 mol, 72.2%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2.2. Synthesis of the 2-methoxy-3-methylbenzylpiperazine and 2-methoxy-4-methylbenzylpiperazine

Methyl iodide (20 g, 0.503mol) and potassium carbonate (60g, 0.434mol) were added to a solution of 3-methyl salicylic acid or 4-methyl salicylic acid (25.5g, 0.168mol) in dry acetone (300ml) and the reaction mixture was refluxed for one week. The mixture was gravity filtered and the residue was washed with acetone (3 x 30 ml) and the combined filtrate was evaporated under reduced pressure to yield 2-methoxy-3-methyl benzoic acid methyl ester or 2-methoxy-4-methyl benzoic acid methyl ester

Red-Al (77ml) was added to a solution of 2-methoxy-3-methyl benzoic acid methyl ester or 2-methoxy-4-methyl benzoic acid methyl ester (22.36g, 0.135mol) in benzene (200ml) under nitrogen atmosphere. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml). The organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100ml) and the organic layer was washed with water (3x30ml). The methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 2-methoxy-3-methyl benzyl alcohol or 2-methoxy-4-methyl benzyl alcohol.

Pyridinium chlorochromate (64.6g, 0.298mol) and celite (64.6g) were added to a solution of 2-methoxy-3-methylbenzyl alcohol or 2-methoxy-4-methyl benzyl alcohol. (23g, 0.169mol) in methylene chloride (500ml) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with ether (200ml) and stirred for 30 minutes then filtered over a pad of silica gel (200-400mesh) and the residue was washed with ether (3x30ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 2-methoxy-3-methylbenzaldehyde or 2-methoxy-4-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 2-methoxy-3-methylbenzaldehyde or 2-methoxy-4-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-methoxy-3-methylbenzylpiperazine (2.0g, 0.01 mol, 80%), 3-methoxy-4-methylbenzylpiperazine (2.0g, 0.01 mol, 80%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2.3. Synthesis of the 2-methoxy-6-methylbenzylpiperazine

A solution of copper sulfate pentahydrate (18.04g, 0.0723mol) and potassium persulfate (60.55g, 0.22mol) in water (250 ml) was added dropwise to a solution of 2,3-

dimethyl anisole (9.82, 0.073mol) in acetonitrile (250ml) and the reaction mixture was refluxed for 45 minutes. The reaction mixture was cooled to room temperature, solvent volume was reduced under reduced pressure and 2-methoxy-6-methylbenzaldehyde was extracted using methylene chloride (4x 35 ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford crude 2-methoxy-6-methylbenzaldehyde.

A mixture of 2-methoxy-6-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-methoxy-6-methylbenzylpiperazine (2.1g, 0.01 mol, 84%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2.4. Synthesis of the 3-methoxy-2-methylbenzylpiperazine

Methyl iodide (37.36g, 0.26mol) and potassium carbonate (36.45g, 0.264mol) were added to a solution of 3-hydroxy-2-methyl benzoic acid (10.0g, 0.066mol) in dry acetone (200 ml) and the reaction mixture was refluxed overnight. The mixture was gravity filtered and the residue was washed with acetone (3x 30 ml) and the combined organic filtrate was evaporated under reduced pressure to give 3-methoxy-2- methylbenzoic acid methyl ester as orange crystals (7.8g, 65.7%).

Red-Al (77ml) was added to a solution of 3-methoxy-2-methyl benzoic acid methyl ester (22.36g, 0.135mol) in benzene (200ml) under nitrogen atmosphere. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml). The organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100ml) and the organic layer was washed with water (3x30ml). The methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 3-methoxy-2-methyl benzyl alcohol. Pyridinium chlorochromate (64.6g, 0.298mol) and celite (64.6g) were added to a solution of 3-methoxy-2-methyl benzyl alcohol. (23g, 0.169mol) in methylene chloride (500ml) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with ether (200ml) and stirred for 30 minutes then filtered over a pad of silica gel (200-400mesh) and the residue was washed with ether (3x30ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 3-methoxy-2-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 3-methoxy-2-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 3-

methoxy-2-methylbenzylpiperazine (2.0g, 0.01 mol, 80%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2.5. Synthesis of the 3-methoxy-4-methylbenzylpiperazine

Red-Al (11.2 gm, 0.55 mol) was added to a solution of 3-methoxy-4-methyl benzoic acid methyl ester (22.36g, 0.135mol) in benzene (200ml) under nitrogen atmosphere. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml). The organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100ml) and the organic layer was washed with water (3x30ml). The methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 3-methoxy-2-methyl benzyl alcohol.

Pyridinium chlorochromate (64.6g, 0.298mol) and celite (64.6g) were added to a solution of 3-methoxy-4-methyl benzyl alcohol. (23g, 0.169mol) in methylene chloride (500ml) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with ether (200ml) and stirred for 30 minutes then filtered over a pad of silica gel (200-400mesh) and the residue was washed with ether (3x30ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 3-methoxy-4-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 3-methoxy-4-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30

ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 3-methoxy-4-methylbenzylpiperazine (1.5, 0.007 mol, 60%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2.6. Synthesis of the 3-methoxy-5-methylbenzylpiperazine

Sodium metal (25.5g, 1.1mol) was added in 250 mg portions over 2.5 hours to absolute ethanol (500ml) in an ice cooled dry three neck flask under nitrogen and the mixture was stirred overnight. Acetone (58.93g, 1.0 mol) and diethyl oxalate (146.27g) were then added dropwise over 3 hours and the resulting thick yellow mixture was stirred for additional 2 hours. The resulting ethyl sodium acetopyrovate was collected by vacuum filtration was dried over night.

Ethyl sodium acetopyrovate (164.3g, 0.912 mol)was dissolved in water (155 ml) followed by the addition of glacial acetic acid (155 ml, 1.06 mol) and the reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then poured on ice (200g) followed by the addition of concentrated sulfuric acid (35 ml). 3-Acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester was formed as yellow solid and was collected by vacuum filtration , washed with water and dried under vacuum over night.

Magnesium oxide (45.3g, 1.12 mol) was added in three portion to a suspension of 3-Acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester (85.5g, 0.277 mol) in water (1540 ml) and the reaction mixture was refluxed for 2 hours. The

reaction mixture was then filtered under vacuum and the residue was washed with hot water. The filtrate volume was reduced under reduced pressure and it was then cooled to room temperature. Hydrochloric acid gas was then bubbled to afford 3-hydroxy-5-methyl benzoic acid that was isolated by gravity filtration and dried overnight.

Methyl iodide (37.36g, 0.26mol) and potassium carbonate (36.45g, 0.264mol) were added to a solution of 3-hydroxy-5-methyl benzoic acid (10.0g, 0.066mol) in dry acetone (200 ml). Excess methyl iodide was added and the reaction mixture was refluxed overnight. GC-MS analysis of the reaction mixture showed only two peaks of m/z 180/149 and 194/149 indicating the formation of methyl-3-methoxy-5-methyl benzoate and ethyl-3-methoxy-5-methyl benzoate, respectively. The mixture was gravity filtered and the residue was washed with acetone (3x 30 ml) and the combined organic filtrate was evaporated under reduced pressure to afford a mixture of methyl-3-methoxy-5-methyl benzoate and ethyl-3-methoxy-5-methyl benzoate.

Red-Al (60 ml) was added to a solution of the crude methyl-3-methoxy-5-methyl benzoate and ethyl-3-methoxy-5-methyl benzoate (23g) in benzene (200ml) under nitrogen atmosphere. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml). The organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100ml) and the organic layer was washed with water (3x30ml). The methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 3-methoxy-5-methyl benzyl alcohol.

Pyridinium chlorochromate (64.6g, 0.298mol) and celite (64.6g) were added to a solution of 3-methoxy-5-methyl benzyl alcohol. (23g, 0.169mol) in methylene chloride (500ml) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with ether (200ml) and stirred for 30 minutes then filtered over a pad of silica gel (200-400mesh) and the residue was washed with ether (3x30ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 3-methoxy-5-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 3-methoxy-5-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 3-methoxy-5-methylbenzylpiperazine (2.0g, 0.01 mol, 80%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.2.7. Synthesis of the 5-methoxy-2-methylbenzylpiperazine

A solution of 2-methylfuran (6.56g, 0.08mol) in methylene chloride (30.0 ml) was added dropwise to a solution of ethyl propiolate (7.84g, 0.08mol) and anhydrous aluminum chloride (10.64g, 0.0596mol) in methylene chloride (120ml) and the resulting reaction mixture was stirred at room temperature for 30 minutes. The reaction mixture was then shaken vigorously with water and the organic layer was separated. The organic

layer was extracted with 5% sodium hydroxide solution (3x 30 ml) and the combined aqueous basic layer was acidified with concentrated hydrochloric acid. The acidified aqueous layer was extracted with ethyl acetate (4 x30 ml) and the combined organic layer was dried over anhydrous sodium sulfate. The organic layer was then filtered and evaporated under reduced pressure to afford ethyl 5-hydroxy-2-methylbenzoate.

Methyl iodide (37.36g, 0.26mol) and potassium carbonate (36.45g, 0.264mol) were added to a solution of ethyl 5-hydroxy-2-methylbenzoate (10.0g, 0.066mol) in dry acetone (200 ml) and the reaction mixture was refluxed overnight. The mixture was gravity filtered and the residue was washed with acetone (3x 30 ml) and the combined organic filtrate was evaporated under reduced pressure. GC-MS monitoring of the reaction showed 2 peaks of m/z 180/149 and 194/149 methyl 5-methoxy-2-methyl benzoate and ethyl 5-methoxy-2-methyl benzoate, respectively.

Red-Al (60 ml) was added to a solution of crude methyl-5-methoxy-2-methyl benzoate and ethyl -5-methoxy-2-methyl benzoate (10.32g) in benzene (200 ml) under nitrogen atmosphere. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml). The organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100ml) and the organic layer was washed with water (3x30ml). The methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 5-methoxy-2-methylbenzyl alcohol (6.7g, 0.44mol).

Pyridinium chlorochromate (64.6g, 0.298mol) and celite (64.6g) were added to a solution of 5-methoxy-2-methyl benzyl alcohol. (6.7g, 0.44mol) in methylene chloride

(500ml) and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with ether (200ml) and stirred for 30 minutes then filtered over a pad of silica gel (200-400mesh) and the residue was washed with ether (3x30ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 5-methoxy-2-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 5-methoxy-2-methylbenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 5-methoxy-2-methylbenzylpiperazine (2.0g, 0.01 mol, 80%) were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.3. Synthesis of the ethoxybenzylpiperazines (EBPs)

A mixture of either 2-ethoxybenzaldehyde or 3-ethoxybenzaldehyde or 4-ethoxybenzaldehyde (2.5g, 0.0165 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil

was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-ethoxybenzylpiperazine or 3-ethoxybenzylpiperazine or 4-ethoxybenzylpiperazine were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.1.4. Synthesis of the methylbenzylpiperazines (MBPs)

A mixture of either 2-methylbenzaldehyde (o-tolualdehyde) or 3-methylbenzaldehyde (m-tolualdehyde) or 4-methylbenzaldehyde (p-tolualdehyde) (1 g, 0.01 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-methylbenzylpiperazine or 3-methylbenzylpiperazine or 4-methylbenzylpiperazine were obtained by filtration. MS, molecular weight 190, m/z 105 [100%].

2.1.5. Synthesis of the methoxybenzylpiperazines (OMeBPs)

A mixture of either 2-methoxybenzaldehyde (o-anisaldehyde) or 3-methoxybenzaldehyde (m-anisaldehyde) or 4-methoxybenzaldehyde (p-anisaldehyde) (1 g, 0.007 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was

allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-methoxybenzylpiperazine or 3-methoxybenzylpiperazine or 4-methoxybenzylpiperazine were obtained by filtration. MS, molecular weight 206, m/z 121 [100%].

2.1.6. Synthesis of the fluorobenzylpiperazines (FBPs)

A mixture of either 2-fluorobenzaldehyde or 3-fluorobenzaldehyde or 4-fluorobenzaldehyde (1 g, 0.007 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-fluorobenzylpiperazine or 3-fluorobenzylpiperazine or 4-fluorobenzylpiperazine were obtained by filtration. MS, molecular weight 194, m/z 109 [100%].

2.1.7. Synthesis of the chlorobenzylpiperazines (ClBPs)

A mixture of either 2-chlorobenzaldehyde or 3-chlorobenzaldehyde or 4-chlorobenzaldehyde (1 g, 0.007 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-chlorobenzylpiperazine or 3-chlorobenzylpiperazine or 4-chlorobenzylpiperazine were obtained by filtration. MS, molecular weight 210, m/z 125 [100%].

2.1.8. Synthesis of the bromobenzylpiperazines (BrBPs)

A mixture of either 2-bromobenzaldehyde or 3-bromobenzaldehyde or 4-bromobenzaldehyde (1 g, 0.005 mol) and piperazine (1.43g, 0.0165 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2-bromobenzylpiperazine or 3-

bromobenzylpiperazine or 4-bromobenzylpiperazine were obtained by filtration. MS, molecular weight 254, m/z 169 [100%].

2.1.9. Synthesis of the dimethoxybenzylpiperazines (DMBPs)

A mixture of either of 2,3-dimethoxybenzaldehyde, 2,4-dimethoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, 2,6-dimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,5-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and piperazine (1.72g, 0.02 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.48g, 0.04 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzylpiperazine product were obtained by filtration. MS, molecular weight 236, m/z 151 [100%].

2.1.10. Synthesis of the dimethoxybenzyl-N-methylpiperazines (DMBMPs)

A mixture of either of 2,3-dimethoxybenzaldehyde, 2,4-dimethoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, 2,6-dimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,5-dimethoxybenzaldehyde (3.3 g, 0.02 mol) and N-methylpiperazine (2.0 g, 0.02 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.48g, 0.04 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by

extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzylpiperazine product were obtained by filtration. MS, molecular weight 236, m/z 151 [100%].

2.1.11. Synthesis of the bromodimethoxybenzylpiperazines (BrDMBPs)

A mixture of the appropriate bromodimethoxybenzaldehyde (4 g, 0.02 mol) and piperazine (1.72g, 0.02 mol) in methanol was stirred for half an hour. Then sodium cyanoborohydride (2.48g, 0.04 mol) was added and the mixture was allowed to stir for half an hour. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzylpiperazine product were obtained by filtration. MS, molecular weight 314, m/z 229/231 [100%].

2.2. Synthesis of the ring substituted benzoylpiperazines

2.2.1. Synthesis of the unsubstituted benzoylpiperazine

(2.6 g, 0.03 mol) of piperazine were dissolved in 50 ml of dichloromethane in a round bottom flask and the flask was placed in an ice bath for 15 minutes. Benzoyl chloride (1.4 g, 0.01 mol) was dissolved in dichloromethane and the solution was dripped slowly over

the piperazine solution over 10 minutes. The mixture was allowed to stir for 15 minutes. The solution was evaporated under reduced pressure to yield light yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the benzoylpiperazine products were obtained by filtration. MS, molecular weight 190, m/z 105 [100%].

2.2.2. Synthesis of the monomethoxybenzoylpiperazines

(2.6 g, 0.03 mol) of piperazine were dissolved in 50 ml of dichloromethane in a round bottom flask and the flask was placed in an ice bath for 15 minutes. 2-methoxybenzoylchloride or 3-methoxybenzoylchloride or 4-methoxybenzoylchloride (1.7 g, 0.01 mol) was dissolved in dichloromethane and the solution was dripped slowly over the piperazine solution over 10 minutes. The mixture was allowed to stir for 15 minutes. The solutions were evaporated under reduced pressure to yield light yellow oils. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzoylpiperazine products were obtained by filtration. MS, molecular weight 220, m/z 135 [100%].

2.2.3. Preparation of 3,5-dimethoxybenzoylchloride

Thionyl chloride (2.58 g, 0.0217 mol) was added dropwise to 3,5-dimethoxybenzoic acid (3.64 g, 0.02 mol) in 50 ml of chloroform. The mixture was refluxed over three hours. Chloroform was evaporated under reduced pressure and benzene was added to the residue twice and evaporated under reduced pressure. The

residue obtained was distilled by Kugelrohr apparatus, which gave 3,5-dimethoxybenzoyl chloride (2.8 g, 0.014 mol, 70%) as a colorless liquid.

2.2.4. Synthesis of the dimethoxybenzoylpiperazines

(2.6 g, 0.03 mol) of piperazine were dissolved in 50 ml of dichloromethane in a round bottom flask and the flask was placed in an ice bath for 15 minutes. 2,3-dimethoxybenzoylchloride or 2,4-dimethoxybenzoyl chloride or 2,5-dimethoxybenzoylchloride or 2,6-dimethoxybenzoylchloride or 3,4-dimethoxybenzoylchloride or 3,5-dimethoxybenzoylchloride (2 g, 0.01 mol) were dissolved in dichloromethane and the solution was dripped slowly over the piperazine solution over 10 minutes. The mixture was allowed to stir for 15 minutes. The solutions were evaporated under reduced pressure to yield light yellow oils. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzoylpiperazine products were obtained by filtration. MS, molecular weight 250, m/z 165 [100%].

2.2.5. Synthesis of the dimethoxybenzoyl-N-methylpiperazines

(3.0 g, 0.03 mol) of n-methylpiperazine were dissolved in 50 ml of dichloromethane in a round bottom flask and the flask was placed in an ice bath for 15 minutes. 2,3-dimethoxybenzoylchloride or 2,4-dimethoxybenzoyl chloride or 2,5-dimethoxybenzoylchloride or 2,6-dimethoxybenzoylchloride or 3,4-dimethoxybenzoylchloride or 3,5-dimethoxybenzoylchloride (2 g, 0.01 mol) were dissolved in dichloromethane and the solution was dripped slowly over the piperazine solution over 10 minutes. The mixture was allowed to stir for 15 minutes. The solutions

were evaporated under reduced pressure to yield light yellow oils. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzoylpiperazine products were obtained by filtration. MS, molecular weight 264, m/z 165 [100%].

2.2.6. Synthesis of the trifluoromethylbenzoylpiperazines

(2.6 g, 0.03 mol) of piperazine were dissolved in 50 ml of dichloromethane in a round bottom flask and the flask was placed in an ice bath for 15 minutes. 2-trifluoromethylbenzoylchloride or 3-trifluoromethylbenzoylchloride or 4-trifluoromethylbenzoylchloride (2.1 g, 0.01 mol) were dissolved in dichloromethane and the solution was dripped slowly over the piperazine solution over 10 minutes. The mixture was allowed to stir for 15 minutes. The solutions were evaporated under reduced pressure to yield light yellow oils. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding benzoylpiperazine products were obtained by filtration. MS, molecular weight 258, m/z 173 [100%].

2.3. Synthesis of the ring substituted phenylpiperazines

The general procedure for the synthesis of the ring substituted phenylpiperazines begins with the appropriately substituted aniline as starting material. The desired regioisomer phenylpiperazine was synthesized by adding solution of bis (2-chloroethyl) amine hydrochloride (2.3g, 13.1 mmol), anhydrous potassium carbonate (1.8 g), the appropriately substituted aniline and diglyme (7.5 mL) was heated at reflux for 24 hours.

The reaction mixture was then allowed to cool to room temperature and water added (50 mL). The aqueous mixture was made acidic (pH 1) by careful additional of conc. HCl. The resultant acidic solution was washed with dichloromethane (2 X 50 mL) to remove non-basic impurities. The acidic solution was then cooled and made alkaline (pH 12) by careful addition of KOH pellets and this alkaline solution extracted with dichloromethane (2 X 75 mL). The combined dichloromethane was washed with water (100 mL) and then dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the dichloromethane solvent was evaporated under reduced pressure to yield the product as oil. This oil was converted to the corresponding hydrochloride salt using gaseous HCl.

2.4. Synthesis of the ring substituted 1-phenyl-2-piperazinopropanes and 1-phenyl-2- piperazinopropanones

2.4.1. Synthesis of the 1-(methylenedioxyphenyl)-2-piperazinopropanes (MDPPPs)

2,3-Dihydroxybenzaldehyde (5.0 g, 0.03 mol) and potassium carbonate (18.75 g, 0.136mol) were dissolved in 50 ml of DMF. Dibromomethane (18.9 g, 7.6 ml, 0.10mol) was added dropwise at room temperature, followed by addition of copper (II) oxide (0.010 g). The reaction mixture was refluxed for 2 hours and additional dibromomethane (18.9 g, 7.6 ml, 0.10mol) was added. The mixture was allowed to reflux overnight. The mixture was first vacuum filtered and then DMF was removed by Kugelrohr distillation. The obtained brown oil was suspended with water and extracted with dichloromethane (3x 30 ml). The combined organic extract was washed with 5% potassium hydroxide solution, brine and 2N hydrochloric acid. The methylene chloride was evaporated and the

obtained oil was distilled by Kugelrohr apparatus (100°C/ 3 mmHg), which gave 2,3-methylenedioxybenzaldehyde (3.2 g, 0.021mol, 59%) as light yellow oil.

The mixture of 2,3-methylenedioxybenzaldehyde (3.8 g, 0.025mol) and n- butylamine (10.4 g, 0.142mol) in benzene (120 ml) was refluxed over one day with water removed by a Dean Stark trap. The benzene was evaporated under reduced pressure. The crude imine was dissolved in glacial acetic acid (7.5 ml) and nitroethane (1.88 g, 0.025mol) was added. The reaction mixture was allowed to reflux over one hour. It was poured over crushed ice and acidified to pH 1 with conc. hydrochloric acid. Yellow brown crystals developed, which were isolated by filtration and washed with water. The crystals of 2,3-methylenedioxyphenyl-2-nitropropene (3.1 g, 0.015mol, 60%) were air dried.

2,3-Methylenedioxyphenyl-2-nitropropene (3.1 g, 0.015 mmol) was dissolved in toluene (15 ml) and 15 ml of water. The resulting solution was mixed with powdered iron (4.49 g, 0.088mol), ferric chloride (0.90 g, 0.006mol) and concentrated hydrochloride acid (6 ml). The mixture was stirred vigorously and refluxed over a day. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5 N hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 2,3-methylenedioxyphenyl-2-propanone (2,3-methylenedioxyphenylacetone) (1.12 g, 0.0063 mol, 42%) as a yellow oil.

The mixture of either 2,3-methylenedioxyphenylacetone or 3,4-methylenedioxyphenylacetone (1.12 g, 0.006 mol) and piperazine (1.43g, 0.0165 mol) in

methanol was stirred overnight. Then sodium cyanoborohydride (2.1g, 0.033 mol) was added and the mixture was allowed to stir for 6 hours. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 1-(2,3-methylenedioxyphenyl)-2-piperazinopropane or 1-(3,4-methylenedioxyphenyl)-2-piperazinopropane were obtained by filtration. MS, m/z 113 [100%].

2.4.2. Synthesis of the 1-(monomethoxyphenyl)-2-piperazinopropanes (OMePPPs)

The mixture of either o-, m-, or p-methoxyphenylacetone (2.46 g, 0.015 mol) and piperazine (1.72 g, 0.02 mol) in methanol was stirred overnight. Then sodium cyanoborohydride (2.48g, 0.04 mol) was added and the mixture was allowed to stir for 6 hours. The reaction was quenched by adding ice/water mixture and stirring the mixture for 20 min followed by extracting the final product using dichloromethane (3x30 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 1-(methoxyphenyl)-2-piperazinopropane were obtained by filtration. MS, m/z 113 [100%].

2.4.3. Synthesis of the 1-(monomethoxyphenyl)-2-piperazinopropanones (OMePPPOs)

Methyl iodide (37.36g, 0.26mol) and potassium carbonate (36.45g, 0.264mol) were added to a solution of 2`-hydroxypropiophenone (15 g, 0.1 mol) in dry acetone (200 ml) and the reaction mixture was refluxed overnight. The mixture was gravity filtered and the residue was washed with acetone (3x 30 ml) and the combined organic filtrate was evaporated under reduced pressure to give 2`-methoxypropiophenone.

2` or 3` or 4`-methoxypropiophenone (1 g, 0.006 mol) was dissolved in acetic acid in a round bottom flask. Bromine (1.2 g, 0.007 mol) was dripped slowly and the solution was stirred for an hour. The reaction mixture was poured into cold water and then extracted with dichloromethane (30x3 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil of the alpha brominated propiophenone.

Piperazine (0.6 g, 0.007 mol) was dissolved in dichloromethane in around bottom flask. A solution of the brominated methoxypropiophenone (1.4 g, 0.006 mol) in dichloromethane was slowly added to the piperazine solution and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the methylenechloride was evaporated under vacuum to yield the oily 1-methoxyphenyl)-2-piperazinopropanone product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 1-(methoxyphenyl)-2-piperazinopropanone were obtained by filtration. MS, m/z 113 [100%].

2.4.4. Synthesis of the 1-(monofluorophenyl)-2-piperazinopropanones (FPPPOs)

2` or 3` or 4`-fluoropropiophenone (1.1 g, 0.006 mol) was dissolved in acetic acid in a round bottom flask. Bromine (1.2 g, 0.007 mol) was dripped slowly and the solution was stirred for an hour. The reaction mixture was poured into cold water and then extracted with dichloromethane (30x3 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil of the alpha brominated propiophenone.

Piperazine (0.6 g, 0.007 mol) was dissolved in dichloromethane in around bottom flask. A solution of the brominated fluoropropiophenone (1.4 g, 0.006 mol) in dichloromethane was slowly added to the piperazine solution and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the methylenechloride was evaporated under vacuum to yield the oily 1-(fluorophenyl)-2-piperazinopropanone product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 1-(fluorophenyl)-2-piperazinopropanone were obtained by filtration. MS, m/z 113 [100%].

2.4.5. Synthesis of the 1-(monochlorophenyl)-2-piperazinopropanones (ClPPPOs)

2` or 3` or 4`-chloropropiophenone (1.4 g, 0.006 mol) was dissolved in acetic acid in a round bottom flask. Bromine (1.2 g, 0.007 mol) was dripped slowly and the solution was stirred for an hour. The reaction mixture was poured into cold water and then extracted with dichloromethane (30x3 ml). The combined organic extract was dried with anhydrous

magnesium sulfate, filtered and evaporated to yield yellow oil of the alpha brominated propiophenone.

Piperazine (0.6 g, 0.007 mol) was dissolved in dichloromethane in around bottom flask. A solution of the brominated chloropropiophenone (1.4 g, 0.006 mol) in dichloromethane was slowly added to the piperazine solution and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the methylenechloride was evaporated under vacuum to yield the oily 1-(chlorophenyl)-2-piperazinopropanone product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 1-(chlorophenyl)-2-piperazinopropanone were obtained by filtration. MS, m/z 113 [100%].

2.4.6. Synthesis of the 1-(monobromophenyl)-2-piperazinopropanones (BrPPPOs)

2` or 3` or 4`-bromopropiophenone (1.7 g, 0.006 mol) was dissolved in acetic acid in a round bottom flask. Bromine (1.2 g, 0.007 mol) was dripped slowly and the solution was stirred for an hour. The reaction mixture was poured into cold water and then extracted with dichloromethane (30x3 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil of the alpha brominated propiophenone.

Piperazine (0.6 g, 0.007 mol) was dissolved in dichloromethane in around bottom flask. A solution of the brominated bromopropiophenone (1.4 g, 0.006 mol) in dichloromethane was slowly added to the piperazine solution and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the

methylenechloride was evaporated under vacuum to yield the oily 1-(bromophenyl)-2-piperazinopropanone product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 1-(bromophenyl)-2-piperazinopropanone were obtained by filtration. MS, m/z 113 [100%].

2.4.7. Synthesis of the 1-(trifluoromethylphenyl)-2-piperazinopropanones (CF_3PPPOs)

2` or 3` or 4`-trifluoromethylpropiophenone (1.5 g, 0.006 mol) was dissolved in acetic acid in a round bottom flask. Bromine (1.2 g, 0.007 mol) was dripped slowly and the solution was stirred for an hour. The reaction mixture was poured into cold water and then extracted with dichloromethane (30x3 ml). The combined organic extract was dried with anhydrous magnesium sulfate, filtered and evaporated to yield yellow oil of the alpha brominated propiophenone.

Piperazine (0.6 g, 0.007 mol) was dissolved in dichloromethane in around bottom flask. A solution of the brominated trifluoromethylpropiophenone (1.4 g, 0.006 mol) in dichloromethane was slowly added to the piperazine solution and the mixture was refluxed for 2 hours. The reaction mixture was cooled to room temperature and the methylenechloride was evaporated under vacuum to yield the oily 1-(trifluoromethylphenyl)-2-piperazinopropanone product. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of the corresponding 1-(trifluoromethylphenyl)-2-piperazinopropanone were obtained by filtration. MS, m/z 113 [100%].

3. Preparation of the Perfluoroacyl Derivatives

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.

4. Preparation of Pyridinium chlorochromate

Chromium trioxide (100.0g, 1mol) was added rapidly with stirring to a 6M hydrochloric acid solution (184.0 ml). After 5 minutes, the homogenous solution was cooled to 0^{o}C and pyridine (79.1g, 1mol) was carefully added over 10 minutes. Recooling the mixture to 0^{o}C gave a yellow orange solid which was collected on a sintered glass funnel and dried for one hour under vaccum [Corey and Suggs, 1975].