Mass Spectral, Infrared and Chromatographic Studies on Designer Drugs of the Piperazine Class

by

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Abstract

The controlled drug 3,4-methylenedioxybenzylpiperazine (3,4-MDBP) has regioisomeric and isobaric substances of mass equivalence, which have similar analytical properties and thus the potential for misidentification. The direct regioisomers of 3,4-MDBP include the 2,3-methylenedioxy substitution pattern and the indirect regioisomers include the three ring substituted methoxybenzoylpiperazines. The ethoxy and methoxymethyl ring substituted benzylpiperazines constitute the major category of isobaric substances evaluated in this study.

The direct and indirect regioisomers of 3,4-MDBP and also isobaric substances related to MDBP were synthesized and compared to 3,4-MDBP by using gas chromatographic and spectrophotometric techniques. The GC-MS studies of the direct regioisomers and isobaric substances of 3,4-MDBP indicated that they can not be easily differentiated by mass spectrometry. The synthesized compounds were converted to their perfluoroacyl derivatives, trifluoroacetyl (TFA), pentafluoropropionyl amides (PFPA) and heptafluorobutryl amides (HFBA), in an effort to individualize their mass spectra and to improve chromatographic resolution. Derivatized 3,4-MDBP was not distingushed from its derivatized regioisomers or isobars using mass spectrometry. No unique fragment ions were observed for the various regioisomeric and the isobaric compounds. Gas chromatographic studies indicated that the optimum separation of regioisomers and isobaric compounds of 3,4-MDBP was obtained when a 100% trifluoropropyl methyl polysiloxane column was used at gradient temperature program

rates. Exact mass determination techniques such as gas chromatography coupled to time of fight mass spectrometric detection (GC-TOF-MS) was used and proved to be successful in discriminating among isobaric compounds that have the same nominal mass but are different in their elemental composition and hence their exact masses. On the other hand, GC-TOF-MS is not a successful tool to differentiate between regioisomers that have both the same nominal and exact masses.

In addition, the gas chromatography coupled to infrared detection (GC-IRD) proved to be an excellent tool in differentiating 3,4-MDBP from all of its regioisomers and isobars. Other ring substituted benzylpiperazines such as chloro, methoxy and methylbenzylpiperazines were prepared and their analytical properties were studied in this dissertation.

Other chemical classes of piperazines were also synthesized and evaluated during this study. Some ring substituted benzoylpiperazines, 1-(phenyl)-2-piperazinopropanes (PPPs) that have amphetamine-like side chain and 1-(phenyl)-2-piperazinopropanones which have cathinone-like side chain (PPPOs) were synthesized in the lab. Their mass spectral, infrared and gas chromatographic separation and properties were studied too.

In addition to that, isotope labeling experiments such as deuterium (D) and carbon 13 (^{13}C) labeling were used to confirm mass spectrometric fragmentation mechanisms that result in the formation of some key fragment ions or to confirm the elemental composition of these fragment ions.

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In the name of Allah, the Most Beneficent, the Most Merciful I begin. First and foremost I want to say “Alhamdolellah” thanks god for your help and giving me the power to achieve this humble piece of work. During the preparation of this dissertation many people, both in USA and Egypt have given me support. I am so grateful to them all. Especially I would like to thank:

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List of Abbreviations

µl	Micro liter
µm	Micrometer
°C	Degree centigrade
2,3-MDBP	2,3-Methylenedioxybenzylpiperazine
3,4-MDBP	3,4-Methylenedioxybenzylpiperazine
bk	Benzylketo
BZP	Benzylpiperazine
BNZP	Benzoylpiperazine
ClBPs	Chlorobenzylpiperazin
d3-CH_3I	trideuterated methyliodide
Da	Dalton
DMBPs	Dimethoxybenzylpiperazines
DMBzPs	Dimethoxybenzoylpiperazines
DMF	Dimethylformamide
EBPs	Ethoxybenzylpiperazines
EI	Electron impact
ev	Electron volt
f.d.	Film depth
GC	Gas chromatography

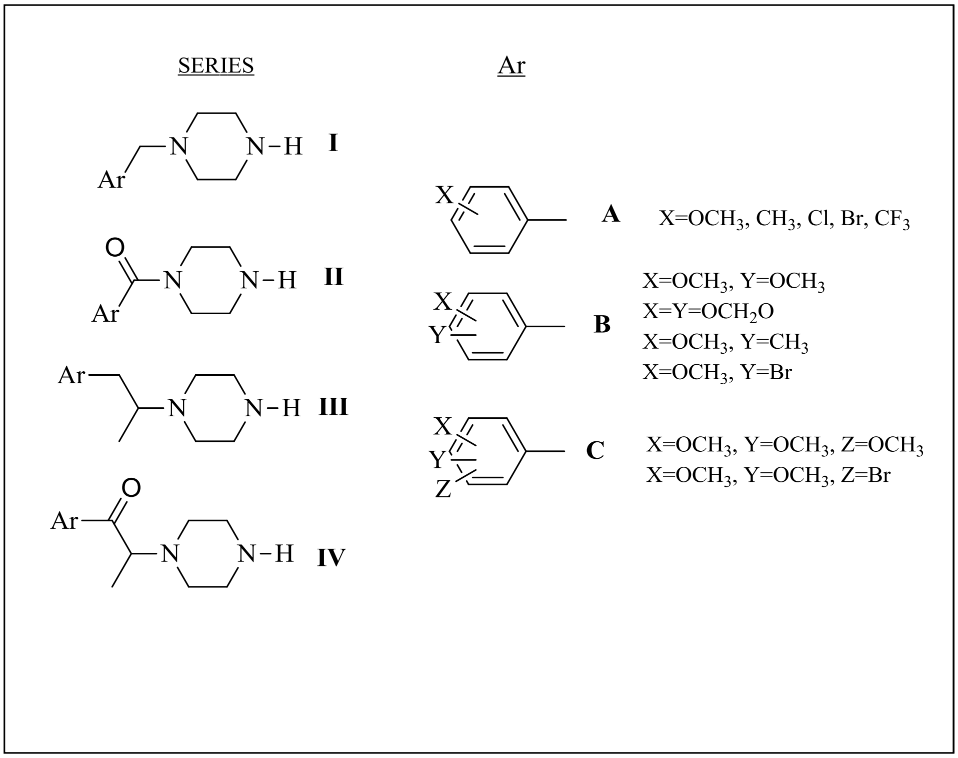
GC-IRD	Gas chromatography coupled to infrared detection
GC-MS	Gas chromatography- mass spectrometry
GC-TOF-MS	Gas chromatography with time of flight mass spectrometry
HFBA	Heptafluorobutyric anhydride
HPLC	High performance liquid chromatography
i.d.	Internal diameter
IR	Infrared
m	Meter
m-	Meta
MBPs	Methylbenzylpiperazines
mDa	Millidalton
MDBPs	Methylenedioxybenzylpiperazines
MDPPPs	1-(methylenedioxyphenyl)-2-piperazinopropanes
min	Minute
ml	Milliliter
mm	Millimeter
MMBPs	Methoxymethylbenzylpiperazines
MS	Mass spectrometry
nm	Nanometer
o-	Ortho
OMeBPs	Methoxybenzylpiperazines
OMeBzPs	Methoxybenzoylpiperazines
OMePPPOs	1-(methoxyphenyl)-2-piperazinopropanones

OMePPPs	1-(methoxyphenyl)-2-piperazinopropanes
p-	Para
PCC	Pyridinium chlorochromate
PFPA	Pentafluoropropionic anhydride
ppm	Part per million
PPPOs	1-(phenyl)-2-piperazinopropanones
PPPs	1-(phenyl)-2-piperazinopropanes
Red Al	Sodium bis(2-methoxyethoxy) aluminum hydride
RT	Room temperature
TFA	Trifluoroacetic anhydride
TFMPP	Trifluoromethylphenylpiperazine

1. Literature Review

1.1. Introduction

A series of piperazine-derived compounds have recently entered the illicit drug market and represent a new group of “designer drugs”. Several compounds of the 1-arylpiperazine type are known to have good binding affinity for serotonin receptors of the human central nervous system [Glennon *et al,* 1986]. This affinity is made more selective with the appropriate aromatic ring substituents [Glennon *et al*, 1987]. These compounds can be classified according to the chemical structure into benzylpiperazines (I), benzoylpiperazines (II), 1-(phenyl)-2-piperazinopropanes (III), and 1-(phenyl)-2-piperazinopropanones (IV). The general structures for these piperazines are shown in Scheme 1.



Scheme 1. General structures for the piperazines in this study.

The most commonly abused compounds of this group are reported to be N-benzylpiperazine (BZP) and 3-trifluoromethylphenylpiperazine (3-TFMPP) [Staack *et al*, 2007]. 1-Benzylpiperazine (BZP), the first piperazine encountered in the clandestine market is a central nervous system (CNS) stimulant with around 10% of the potency of d-amphetamine [Bye *et al*, 1973]. Alternative chemical names for BZP include 1-benzyl-1,4-diazacyclohexane, *N*-benzylpiperazine and, less precisely, benzylpiperazine. Street names have included A2, Legal X and Pep X. TFMPP is mentioned as an active hallucinogen in scene books [Shulgin, 1991]. Recently, BZP and TFMPP had the reputation of producing amphetamine-like stimulant effects [Campbell *et al*, 1973] and the psychoactive effects of methylenedioxymethamphetamine (MDMA) [Herndon *et al*, 1992], respectively.

Structural modifications similar to those known for the amphetamines can likewise be found in this class of compounds. 1-(3,4-methylenedioxybenzyl)piperazine (3,4-MDBP or 1-piperonylpiperazine) is the corresponding methylenedioxy derivative of N-benzylpiperazine (BZP). In the recent years a number of other piperazine derivatives have been reported in clandestine drug samples including 1-(4-methoxyphenyl)piperazine (pMeOPP), 1-(4-methylphenyl)-piperazine (pMPP), 1-(4-fluorophenyl)piperazine (pFPP), 1-(3,4-methylenedioxybenzyl)piperazine (MDBP) and 1-(4-bromo-2,5-dimethoxybenzyl)-piperazine (BrDMBP) [Westphal *et al*, 2009]. The later two compounds are products of "designer drug" modification and clandestine laboratory synthesis.

Tablets or capsules or pills are the most common dosage forms and these are administered orally. However powder forms also can be snorted, smoked as an alternative to amphetamine-derived designer drugs [Staack *et al*, 2003].

The goal of clandestine manufacturers is often to prepare substances with pharmacological profiles that are sought after by the user population. Clandestine manufacturers are also driven by the desire to create substances that circumvent existing laws. In Europe, as a result of the substance-by-substance scheduling approach, the appearance of new substances cannot be immediately categorized as illicit drugs. This offers room for clandestine experimentation into individual substances within a class of drugs with similar pharmacological profiles, perhaps yielding substances of increased potency. In the USA, continued designer exploration has resulted in legislation (Controlled Substances Analog Act) to upgrade the penalties associated with clandestine use of all compounds of a series. Thus, identification of new piperazine derivatives and other designer drugs is essential and a significant task for forensic laboratories.

1.2. History

A variety of piperazine derivatives have been identified in illicit drug samples over the past several years. The large-scale misuse of certain piperazine derivatives, often referred to as "party pills", was first reported in New Zealand and became common in Europe in the early 2000s [de Boer *et al*, 2001]. Despite claims by some tablet and capsule suppliers that they are herbal products, piperazine and its derivatives are synthetic substances that do not occur naturally [Alansari *et al*, 2006]. BZP and TFMPP had the reputation of producing amphetamine-like stimulant effects [Campbell *et al*, 1973] and the psychoactive effects of methylenedioxymethamphetamine (MDMA) [Herndon *et al*, 1992], respectively. In addition, their use was not controlled at that time. This caused the rapid spread of the abuse of these drugs as club-drugs or recreational drugs among the youth in the United States. In the last decade, such abuse has been even reported in Japan and Europe, and the fatal intoxication by benzylpiperazine has also been reported [Balmelli et al, 2001]. In 2002, the increasing abuse of BZP and TFMPP in the USA led to the temporary placement of these two compounds into Schedule I of the Controlled substance Act (CSA) [Staack *et al*, 2003] followed by the final placement of BZP into Schedule 1 in 2004 [DEA, Fed. Regist. 2004]. Japan's authorities also controlled these substances under the Narcotics and Psychotropics Control Law in 2003, as the encountering of BZP and TFMPP was increasingly reported even in Japan [Tsutsumi *et al*, 2005].

As countries across the globe moved to control the distribution and use of BZP in the mid 2000s, the use of another piperazine, 1-(3-chlorophenyl)piperazine (mCPP), became more widespread. It is estimated that almost 10% of illicit tablets sold in Europe over this time period, as part of the illicit ecstasy market, contained mCPP [Europol–EMCDDA, 2009]. Furthermore, mCPP was found in more cases in more countries and in larger amounts than any other psychoactive substance reported through the Early Warning System since the monitoring process started in 1997 [Europol–EMCDDA, 2009]. Other mixtures of piperazine derivatives became common during 2008, but most consisted of variations of BZP, TFMPP, mCPP and 1,4-dibenzylpiperazine (DBZP); the blends of up to four different piperazines have been named "X4". Sometimes the piperazines are mixed with other substances such as amphetamine, cocaine, ketamine and MDMA. Since the end of the 1990s, piperazines are often proffered as a legal and safe alternative to amphetamine-derived drugs.

In the recent years a number of other piperazine derivatives have been reported in clandestine drug samples including 1-(4-methoxyphenyl)piperazine (pMeOPP), 1-(4-methylphenyl)-piperazine (pMPP), 1-(4-fluorophenyl)piperazine (pFPP),1-(3,4-methylenedioxybenzyl)piperazine (MDBP) and 1-(4-bromo-2,5-dimethoxybenzyl)-piperazine (BrDMBP) [Westphal *et al*, 2009]. The later two compounds are products of "designer drug" modification and clandestine laboratory synthesis.

A number of ring substituted 1-benzyl- and 1-aryl piperazines have been investigated as CNS-active drugs and vasodilators, but no piperazine of this structural type is licensed for medicinal use. The piperazine drugs of abuse are not chemically similar to any of the more common substances of misuse, but have a more distant connection with phencyclidine and with 1-phenylethylamine and its derivatives. The suggestion that BZP and other piperazine derivatives are extracted from the pepper plant may arise from confusion with the unrelated substance piperine, a constituent of black pepper (Piper nigrum) [Gee *et al*, 2007]. Also, BZP and other 1-benzyl and 1-arylpiperazines have no current human or veterinary pharmaceutical use in any country. BZP is sometimes erroneously described as a "worming agent or anthelminthic drug" although piperazine itself is used as an anthelminthic drug [Gee *et al*, 2007].

BZP was initially developed by Burroughs Wellcome as a potential antidepressant drug, but was never developed commercially because it produced amphetamine-like effects, although the relative potency was only 10% [Campbell *et al*, 1973]. After a dose of 50–100 mg in human volunteers, BZP was found to increase pulse rate, blood pressure (systolic and diastolic) and pupillary dilation. Physical problems reported were (in order of frequency): poor appetite, hot/cold flushes, heavy sweating, stomach pains/nausea, headaches and tremors/shakes. Psychological problems experienced were (in order of frequency): trouble sleeping, loss of energy, strange thoughts, mood swings, confusion and irritability. In both New Zealand and Europe, case reports note an association of BZP with grand mal seizures [Wood et al, 2007]. There have been a few instances of fatalities involving BZP, but in none was BZP the immediate cause of death, and all of these instances involved other drugs [Elliott, 2008, Gee *et al*, 2010].

The widespread use of piperazines with only a low record of toxicity is stated by activists as proof of safety for legalization of these drugs. However, currently available toxicological data do not support such claims. In view of the similar pharmacological modes of action of benzylpiperazines and amphetamines, similar toxicities are anticipated and reported in some case studies and cases of piperazine poisonings. Furthermore, serotonin syndrome has been observed in a clinical study with 1-phenylpiperazines [Klaassen *et al*, 1998]. Estimation of safety is also complicated by the fact that piperazines, amphetamines and methylenedioxyamphetamines are similarly marketed, consumed by the same population, and show similar or overlapping pharmacological symptoms. Therefore a piperazine poisoning can easily be wrongly diagnosed as poisoning by another drug. Furthermore, piperazines are not detected by routinely used immunochemical screening procedures for drugs of abuse, but require an appropriate toxicological analysis (eg, by gas-chromatography mass-spectrometry).

1.3. Pharmacology

1.3.1. Pharmacodynamics

BZP has been shown to have a mixed mechanism of action, acting on the serotonergic and dopaminergic receptor systems in a similar fashion to MDMA [Baumann *et al*, 2004]. BZP has amphetamine-like actions on the serotonin reuptake transporter, which increase serotonin concentrations in the extracellular fluids surrounding the cell and thereby increasing activation of the surrounding serotonin receptors [Tekes *et al*, 1987 and Lyon *et al*, 1986]. BZP has a lower inhibitory effect on the noradrenaline reuptake transporter and the dopamine reuptake transporter [Baumann *et al*, 2004]. BZP has a high affinity action at the alpha2-adrenoreceptor, it is an antagonist at this receptor, like yohimbine, which inhibits negative feedback, causing an increase in released noradrenaline.

BZP also acts as a non-selective serotonin receptor agonist on a wide variety of serotonin receptors [Tekes *et al*, 1987]. Binding to $5HT_{2A}$ receptors may explain its mild hallucinogenic effects at high doses, while partial agonist or antagonist effects at the $5HT_{2B}$ receptors may explain some of BZPs peripheral side effects, as this receptor is expressed very densely in the gut, and binding to $5HT_3$ receptors may explain the common side effect of headaches, as this receptor is known to be involved in the development of migraine headaches.

1.3.2. Subjective effects

The effects of BZP are largely similar to amphetamines [Fantegrossi *et al*, 2005] with one study finding that former amphetamine addicts were unable to distinguish between dextroamphetamine and BZP administered intravenously [Campbell *et al*, 1973]. Users report alertness, euphoria and a general feeling of well being. The perception of certain sensations such as taste, colour or music may be subjectively enhanced. The average duration of effects is longer than that of dextroamphetamine, typically lasting 4–6 hours with reports as long as 8 hours depending on the dose. A recent study has shown that mixtures of BZP with other piperazine drugs such as TFMPP share certain pharmacodynamic traits with MDMA [Baumann *et al*, 2005].

Upon ingestion of between 50 mg and 200 mg of BZP, the user may experience any or all of the following:

Initial Effects [Nicholson *et al*, 2006]

- Feelings of euphoria, wonder, amazement, well-being, energy and elation
- Rapid mood elevation
- Enhanced sociability
- Enhanced appreciation of music
- Increased desire to move, also slight increase in stereotypy
- Skin tingling
- Decreased appetite
- Repetitive thought patterns
- Actual and perceived changes in body temperature
- Mild jaw clenching/bruxism
- Increased heart rate
- Dilation of pupils
- Flushing
- Mild xerostomia (dry mouth)
- Slight urinary incontinence, often described as "leaking" a small amount of urine after urinating (not due to loss of bladder control)

Later Effects [Nicholson *et al*, 2006]

- Mild headache
- Nausea
- Hangover-like symptoms (common with high doses)
- Fatigue
- Indigestion (similar to acid indigestion/heartburn)
- Increased hunger (and sometimes thirst)
- Insomnia
- Confusion
- Depression (particularly with frequent/heavy use)

Animal studies have demonstrated that BZP stimulates the release and inhibits the reuptake of dopamine, serotonin and norepinephrine, similar to methamphetamine. On the other hand, mixtures of BZP and TFMPP [Baumann *et al*, 2005] appear to mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). 1-Phenylpiperazines such as TFMPP have been regarded as a full (though non-selective) 5-HT2C agonist, but the binding profile of TFMPP at various serotonin receptors is complex. It has also been demonstrated that TFMPP functions as a 5-HT releaser in hippocampal slices, a property shared by MDMA, which may explain the presence of TFMPP in some illegitimate "ecstasy" tablets.

The negative effects of mCPP, often typical of a serotonin syndrome, include anxiety, dizziness, confusion, shivering, sensitivity to light and noise, fear of losing control, migraine and panic attacks. No fatal poisonings from mCPP have been reported. The subjective effects of mCPP and MDMA are somewhat comparable, but unlike MDMA and BZP, mCPP has little effect on the dopaminergic system. Like TFMPP and other 1-arylpiperazines, mCPP appears to be a ligand for a variety of serotonin receptors (agonist at the 5HT2C receptor, and antagonists at the 5HT2B receptor). mCPP has been widely used as a probe of serotonin function in psychiatric research [Klaassen *et al*, 1998].

1.3.3. Toxic effects

As with most sympathomimetic stimulants there appear to be significant side effects associated with BZP use. BZP reportedly produces insomnia and a mild to severe hangover after the drug effect wears off [Nicholson *et al*, 2006] however, some manufacturers in New Zealand have started including recovery pills which contain 5-HTP and vitamins which allegedly ease these hangovers.

The major side effects include dilated pupils, blurred vision, dryness of the mouth, extreme alertness, pruritus, confusion, agitation, tremors, extrapyramidal symptoms (dystonia, akathisia), headache, dizziness, anxiety, insomnia, vomiting, chest pain, hallucinations, paresthesia, tachycardia, hypertension, palpitations, collapse, hyperventilation, sweating, hyperthermia, and problems with urine retention [Theron *et al*, 2007]. The more severe toxic effects include psychosis or adverse psychiatric events [Mohandas *et al*, 2008] renal toxicity [Alansari *et al*, 2006], respiratory failure, hyperthermia, serotonin syndrome [Schep *et al*, 20011] rhabdomyolysis [Gee *et al*, 2010] and seizures [Nicholson *et al*, 2006]. Blood benzylpiperazine concentrations have been measured either to confirm clinical intoxication or as part of a medicolegal death investigation [Baselt *et al*, 2008].

1.3.4. Christchurch study

The majority of the toxic effects information came from a study conducted between 1 April 2005 to 1 September 2005. The study recorded all presentations associated with party pill use at the Emergency Department of Christchurch Hospital, New Zealand by recording them on a prospective data collection form. The aim was to study the patterns of human toxicity related to the use of benzylpiperazine-based 'party pills'. 61 patients presented on 80 occasions. Patients with mild to moderate toxicity experienced symptoms such as insomnia, anxiety, nausea, vomiting, palpitations, dystonia, and urinary retention. Significantly, fourteen toxic seizures were recorded with two patients suffering life-threatening toxicity with status epilepticus and severe respiratory and metabolic acidosis. It was concluded that BZP appears to induce toxic seizures in neurologically normal subjects [Gee *et al*, 2005]. The results of this study and others like it [Theron *et al*, 2007] showed that BZP can cause unpredictable and serious toxicity in some individuals, but the data and dosage collection were reliant on self reporting by drug users, which may result in under-reporting, and there were complicating factors like the frequent presence of alcohol and other drugs.

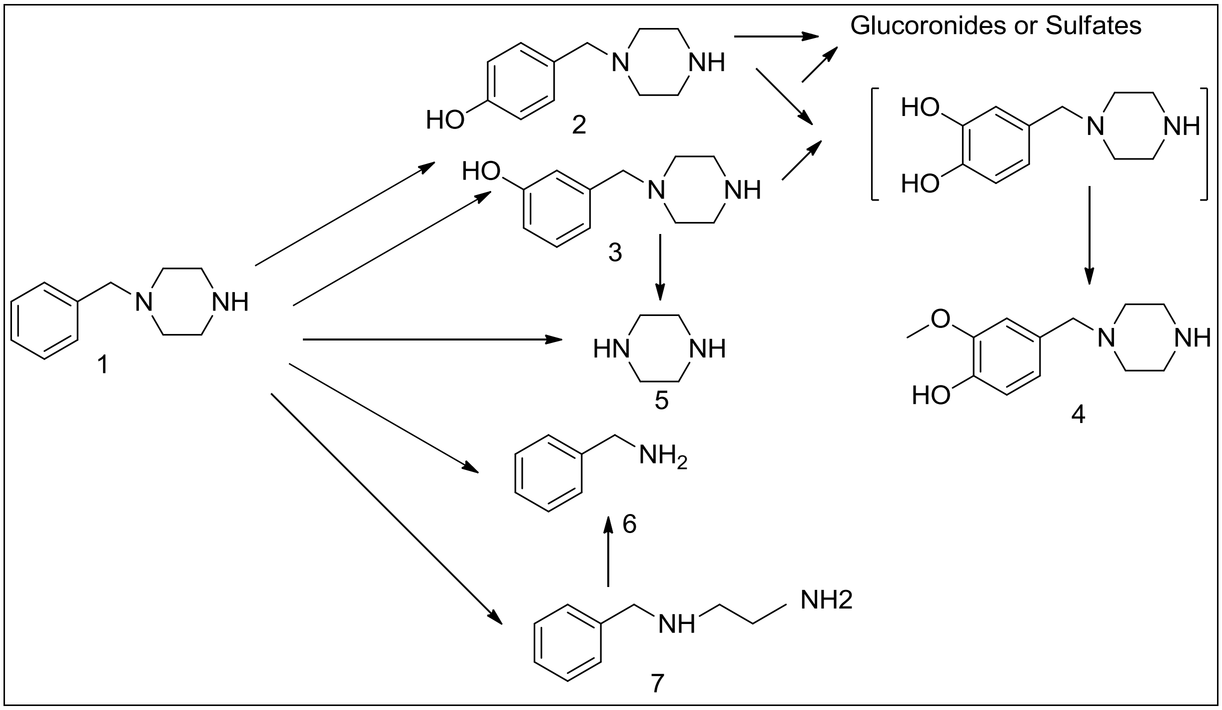
1.4. Metabolism

In contrast to new medicaments, which are extensively studied in controlled clinical studies concerning metabolism, including cytochrome P450 (CYP) isoenzyme differentiation, and further pharmacokinetics, designer drugs are consumed without any safety testing. The corresponding data on the metabolism is mostly incomplete or even not available at all [Staack et al, 2005].

However, data on the metabolism is strongly required. It is a prerequisite for developing toxicological screening procedures, especially in urine, and for toxicological risk assessment. Furthermore variations in the formation of pharmacologically active metabolites, formation of toxic metabolites, or interactions with other medicaments (drug abusers often are polytoxicomaniacs) may have consequences for the assessment of analytical results in clinical or forensic toxicology as well as in doping control. Furthermore, there is good evidence that drug metabolism by genetically variable CYPs can influence the risk of drug dependence, the amount of drug consumed by dependent individuals and some of the toxicities associated with drug taking-behavior [Howard et al, 1991].

1.4.1. Metabolism of BZP

The metabolism of BZP (Compound 1 in Scheme 2) was studied in male Wistar rats and the identified metabolites were confirmed as human metabolites by analysis of human urine after intake of this designer drug [Staack *et al*, 2002]. BZP was not extensively metabolized and mainly excreted as unchanged parent compound. Three metabolic targets could be identified for BZP: the aromatic ring, the benzylic carbon and the piperazine ring. The aromatic ring was metabolically altered by single (Compounds 2 and 3 in Scheme 2) or double hydroxylation followed by catechol-O-methyl-transferase (COMT) catalyzed methylation to N-(4-hydroxy-3-methoxy-benzyl)piperazine (Compound 4 in Scheme 2). As a mixture of glucuronidase and arylsulfatase was used for conjugate cleavage, formation of the corresponding glucuronides and/or sulfates was postulated. N-Dealkylation at the benzyl carbon led to the liberation of piperazine (Compound 5 in Scheme 2). Formation of benzoic acid, as described for other BZP derivatives could not unequivocally be confirmed as it is ubiquitous in urine due to its use as food preservative [Liebich *et al*, 1990]. The piperazine heterocycle was degraded by double N-dealkylation leading either to the formation of benzylamine (Compound 6 in scheme 2) or to N-benzylethylenediamine (Compound 7 in Scheme 2), depending on the positions of these metabolic reactions. The proposed scheme for the metabolism of BZP in male Wistar rats and in humans is shown in Scheme 2.



Scheme 2. Proposed scheme for the metabolism of BZP in male Wistar rats and in humans [Staack *et al*, 2002].

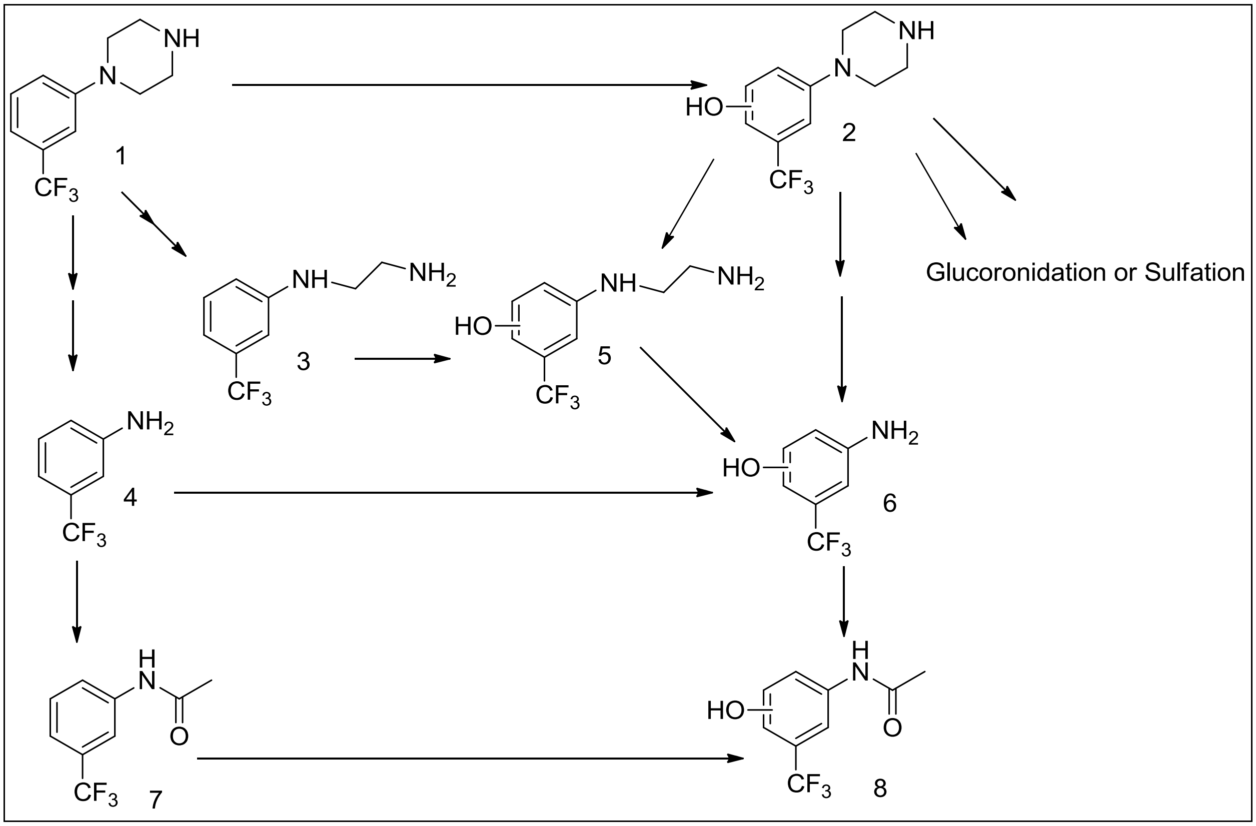
1.4.2. Metabolism of 3,4-MDBP

The metabolism of MDBP was studied in male Wistar rats [Staack *et al*, 2004]. This BZP derivative underwent similar, partially overlapping, metabolic pathways with BZP. As described for BZP, MDBP was mainly excreted as unchanged parent compound. Metabolic alteration of the aromatic moiety, the benzyl carbon and the piperazine heterocycle was also described for MDBP. Demethylenation of the 3,4-methylenedioxy moiety to the corresponding catechol and further methylation to N-(4-hydroxy-3-methoxy-benzyl)piperazine followed by partial glucuronidation or sulfation led to formation of metabolites common with BZP. Likewise, N-dealkylation at the benzylic carbon, leading to piperazine, was described. Degradation of the piperazine heterocycle by double N-dealkylation led to the corresponding N-benzylethylenediamine and benzylamine derivatives N-(3,4-methylenedioxybenzyl) ethylenediamine and 3,4-methylenedioxybenzylamine.

Demethylenation of methylenedioxy compounds and subsequent methylation to the corresponding hydroxy-methoxy metabolites are known for several methylenedioxy compounds [Ensslin *et al*, 1996, Maurer *et al*, 1996].

1.4.3. Metabolism of TFMPP

In contrast to BZP and 3,4-MDBP, TFMPP (Compound 1 in Scheme 3) is extensively metabolized and almost exclusively excreted as metabolites. The major metabolic reaction was aromatic hydroxylation to hydroxyl TFMPP (Compound 2 in Scheme 3) followed by partial glucoronidation or sulfation [Staack *et al*, 2003]. Degradation of the piperazine heterocycle by double N-dealkylation could be observed for the parent compound TFMPP, leading to the formation of N-(3-trifluoromethylphenyl)-ethylenediamine (Compound 3 in Scheme 3) or to 3-trifluoromethylaniline (Compound 4 in Scheme 3), as well as for its hydroxylated metabolite hydroxy TFMPP leading to the formation of N-(hydroxy-3-trifluoromethylphenyl)-ethylenediamine (Compound 5 in Scheme 3) or to hydroxy-3-trifluoromethylaniline (Compound 6 in Scheme 3). Partial N-acetylation was reported as phase II reaction of the aniline derivatives (Compounds 7 and 8 in Scheme 3).



Scheme 3. Proposed scheme for the metabolism of TFMPP in male Wistar rats [Staack *et al*, 2003].

1.4.4. Metabolism of mCPP

In a first study, the metabolism of mCPP was investigated in male Sprague-Dawley rats, which were administered radio-labeled mCPP [Mayol *et al*, 1994]. Extensive metabolism was reported and no unchanged mCPP could be detected in the urine samples. Para-hydroxy mCPP was identified as the major metabolite, which could not be detected unconjugated. Glucuronidation and sulfation were postulated as the conjugation reactions as shown by studies with glucuronidase, with and without the glucuronidase inhibitor saccharic acid-1, 4-lactone, or arylsulfatase.

This working group mentioned the formation of further, not structurally characterized metabolites. Reinvestigation of the metabolism by using male Wistar rats confirmed the finding of extensive metabolism of mCPP and the formation of hydroxy mCPP as the major urinary metabolite [Staack *et al*, 2003]. In contrast to the first study, small amounts of unmetabolized mCPP were detected in urine and hydroxy mCPP could also be found unconjugated. In studies on the mCPP precursor drug trazodone, the formation of mCPP N-glucuronides was reported [Caccia *et al*, 1982].

Moreover, further metabolites were identified. In addition to the aromatic hydroxylation, degradation of the piperazine heterocycle by double N-dealkylation of mCPP to N-(3-chlorophenyl)ethylenediamine or to 3-chloroaniline. Hydroxy-3-chloroaniline was the only metabolite resulting from degradation of the piperazine moiety of hydroxy mCPP. The corresponding ethylenediamine derivative was not detected, in contrast to the metabolism of TFMPP. The aniline metabolites were partially N-acetylated.

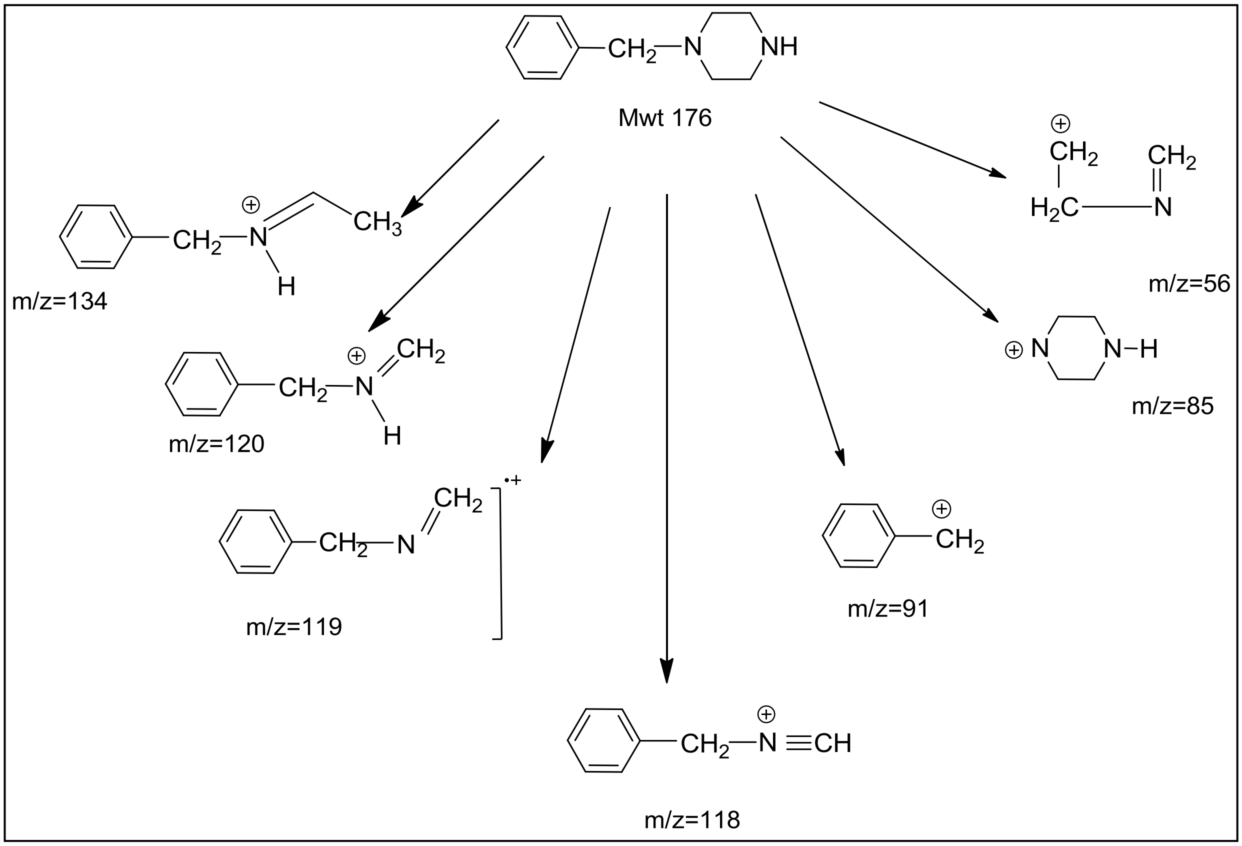
1.4.5. Metabolism of p-(OMePP)

The metabolism of MeOPP was studied in male Wistar rats [Staack et al, 2004]. As the two other phenylpiperazines, MeOPP was extensively metabolized. O-Demethylation of the methoxy moiety was the major metabolic step, as known from the methoxy-substituted amphetamine derivatives PMA and PMMA. The formed metabolite hydroxyphenylpiperazine was subsequently conjugated by partial glucuronidation or sulfation as concluded from studies with a mixture of glucuronidase and arylsulfatase. N-(4-Methoxyphenyl) ethylenediamine and 4-methoxyaniline were formed by degradation of the piperazine moiety of MeOPP and 4-hydroxyaniline could be detected as the piperazine-degraded metabolite of hydroxyphenylpiperazine. As described for mCPP, the corresponding ethylenediamine metabolite of the major metabolite could not be detected, in contrast to the findings of the metabolism of TFMPP. 4-hydroxyaniline was found to be partially glucuronidated or sulfated. Furthermore, it could be shown to be N-acetylated to N-acetyl-4-hydroxyaniline, which actually is the analgesic drug acetaminophen (paracetamol).

1.5. Analytical methods used to separate and identify piperazines

1.5.1. Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is the main tool used for the detection and identification of unknown drugs in forensic and other drug screening laboratories. [de Boer *et al*, 2001] reported the electron impact (EI) mass spectrometric fragmentation pathway for some underivatized and acetylated benzyl and phenylpiperazines. The ions of significant relative abundance common to the BZP likely arise from fragmentation of the piperazine ring and are shown in Scheme 4. The mass spectrum of BZP shows the fragment ions at m/z 134, 120, and 91 as well as other ions of low relative abundance. The structures of these fragment ions as proposed by de Boer *et al* are shown in Scheme 4 [de Boer *et al*, 2001].



Scheme 4. EI-MS fragmentation pathway for BZP as proposed by de Boer *et al.*

In addition, studies on the metabolism of 3,4-MDBP and TFMPP and their toxicological data in rat urine using GC-MS were reported [Staack *et al*, 2003 and Staack *et al*, 2004].

1.5.2. Gas chromatography with infrared detection (GC-IRD)

The absorption of IR radiation is also considered one of the non-destructive techniques that can be used for the identification of organic molecules. The region from 1250 to 600 cm^{-1} is generally classified as the "fingerprint region" and is usually a result of bending and rotational energy changes of the molecule as a whole. However since the clandestine samples are usually impure, overlapping absorptions of different molecules present in the sample becomes a possibility. Hence, this region is not useful for identifying functional groups, but can be useful for determining whether or not samples are chemically identical.

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 regioisomeric trifluoromethylphenylpiperazines (TFMPP) [Maher *et al*, 2009]. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule.

1.5.3. Nuclear magnetic resonance (NMR)

NMR is a nondestructive flexible technique that can be used for the simultaneous identification of pure compounds and even mixtures of compounds in one sample. Its advantages, compared to GC-MS techniques, include stereochemical differentiation and the capability to analyze nonvolatile compounds. However, the lack of use in forensic laboratories can be attributed to the high cost of instrumentation and the poor sensitivity of NMR. Solid state NMR also can be used for analytical purposes in much the same way as solution NMR. The observed chemical shifts however differ in the solution and solid states because of conformational freezing and packing effects.

NMR was utilized in the analytical structure elucidation of a new designer benzylpiperazine (4-bromo-2,5-dimethoxybenzylpiperazine) that was seized in Germany in 2006 [Westphal *et al*, 2009].

1.5.4. Liquid chromatography- electrospray ionization mass spectrometric detection (LC-MS) and liquid chromatography- ultraviolet detection (HPLC-UV)

LC-MS is a non-destructive exact mass determination technique. It utilizes chemical ionization to identify the molecular ion of drugs or their metabolites. Analytical aspects and profiles of some designer piperazine-derived drugs of abuse.e.g. 1-benzylpiperazine, 1-[4-methoxyphenyl]piperazine and TFMPP using both Liquid chromatography- electrospray ionization mass spectrometric detection (LC-MS) and liquid chromatography- ultraviolet detection (HPLC-UV) have been reported by [de Boer *et al*, 2001].

Development of simultaneous gas chromatography-mass spectrometric and liquid chromatographic-electrospray ionization mass spectrometric determination method for the new designer drugs, N-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and their main metabolites in urine was also reported [Tsutsumi *et al*, 2005]

1.6. Project rational

Several aromatic ring substituted benzyl and phenylpiperazines have already appeared in forensic drug samples in the U.S. According to statistics compiled by the Alabama Department of Forensic Sciences 907 piperazine-containing case items have been worked during the 2009 calendar year (there were 558 cases involving MDA/MDMA in 2009 reported by ALDFS) [ALDFS, 2009]. This can be compared to 1 case item involving a piperazine drug sample in 2007 and a total of 300 piperazine cases in 2008. Similar trends likely exist throughout the entire country. The most commonly encountered drugs from this category are N-benzylpiperazine, 1-(3-chlorophenyl)piperazine (m-CPP) and 1-(3-trifluoromethyl-phenyl)piperazine (3-TFMPP) additionally these compounds often appear in combination in clandestine dosage forms. Substitution at the 3-position (meta) is the most common among clandestine piperazine samples. The regioisomeric 2- and 4-positions of the same substituent should yield identical mass spectra. Indeed, the mass spectra for all 3 regioisomeric TFMPPs are identical as reported from our lab [Maher *et al*, 2009]. It is apparent that simply matching a library mass spectrum does not provide confirmatory data to differentiate among these three isomers since the three spectra are identical. In this specific case all three TFMPP isomers are commercially available and if the analyst has reference samples of all three the issue can be readily solved via chromatographic separation and retention time matching. However, the ultimate concern is "if the laboratory has never analyzed the counterfeit/imposter substances and does not have reference data or reference samples, how can the analyst be sure that these compounds would not co-elute with the target drug substance?"

Designer piperazine drugs will continue to appear in clandestine drug samples and the development of these compounds is likely to parallel designer modifications in the amphetamine-type molecules. Indeed, reports have already appeared indicating clandestine synthesis and street availability for 3,4-methylenedioxybenzylpiperazine and 4-bromo-2,5-dimethoxybenzylpiperazine [Westphal *et al*, 2009]. Additional designer development based on substituted amphetamine/phenethylamine and benzylketone-type (bk-type) models [Zaitsu *et al*, 2009] are likely to occur in the near future. Issues of designer piperazine identification as well as regioisomer/isobaric relationships in these designer molecules will continue to be significant in forensic drug analysis.

Perhaps the U. S. Controlled Substance Analog Act would make all these “isomers” controlled substances. Therefore, it could be argued that isomer differentiation is not necessary in forensic drug science. However, the compound must be specifically identified in order to even know if it falls into the category as an analog of a controlled substance.

Restricting the availability of piperazine would require placing dozens of substances from commercial sources around the globe under federal control. While piperazine is not known to occur naturally, it can be obtained commercially (a host of free base and salt forms are available from suppliers worldwide) or from any of a variety of synthetic methods [Dinsmore *et al*, 2002]. Most reported synthetic methods involve either direct cyclization of ethylenediamine in the presence of a catalyst (i.e. zeolite HZSM-5) at high temperature, or by cyclodehydration of N-(2-hydroxyethyl)-ethylenediamine. Both of these starting materials are available. Alternatively, piperazine can be obtained by hydrolysis of a variety of commercially available 1-amido- or 1-carbamate substituted piperazines (i.e. 1-carbethoxypiperazine, 1-formylpiperazine, etc.). Thus, legal control of the key precursor substance, piperazine, is not likely to prevent the further clandestine/designer exploration of this group of compounds.

This project will investigate the forensic analytical chemistry of these future designer substances. The resulting analytical data as well as methods for reference standard synthesis represent important advancements in forensic drug chemistry. The goal of this work is to have the data and methods for differentiation among these designer regioisomeric and isobaric substances available to assist in drug identification.

1.7. Statement of research objectives

The purpose of this project is to develop regioisomer and isobar specific methods for the forensic identification of ring substituted benzylpiperazines, benzoylpiperazines and designer 1-phenyl-2-piperazinopropanes and propanones. The general structures for these compounds are shown in Scheme 1 (Section 1.1 p.g.2). This will be accomplished by:

1) The chemical synthesis of all regioisomeric and isobaric forms of selected ring substituted piperizines.

2) Generation of a complete analytical profile for each compound using the following analytical

techniques: GC/MS, GC/IRD, GC-TOF-MS.

3) Chromatographic studies to separate/resolve all regioisomeric and isobaric piperazines having overlapping analytical profiles.

4) Design and validation of confirmation level methods to identify each compound to the exclusion of other regioisomeric and isobaric forms.

The appearance of increasingly structurally diverse piperazine derivatives in clandestine samples highlights several key issues of immediate forensic significance. First, most of these compounds have regioisomeric analogues which would not be readily differentiated and specifically identified using standard forensic analytic methodology. For example, while 3-TFMPP has been reported as a hallucinogen, the pharmacologic activity of its 2-trifluoromethyl and 4-trifluoromethyl regioisomers has not been reported. All three of these compounds are commercially available or can be easily prepared by published synthetic methods. Also, few analytical studies have been reported for the differentiation of the complete set of trifluoromethylphenylpiperazine regioisomers, or regiosiomers of other ring substituted phenyl and benzyl piperazines such as 3,4-methylenedioxybenylpiperazine and its 2,3-regioisomer.

The second key issue of forensic importance among the piperazine compounds involves designer drug development. As mentioned before, 3,4-MDBP and BrDMBP have been reported recently as designer derivatives of BZP, prepared in clandestine laboratories. These compounds contain aromatic substituents known to enhance hallucinogenic or entactogenic activity in the phenylalkylamine (amphetamine and methylenedioxy-amphetamine) drugs of abuse series. As regulatory controls tighten with respect to commercially available piperazines, designer derivative exploration and synthesis is expected to continue. This is particularly important in the case of benzylpiperazine series since the syntheses are relatively straight-forward and many starting materials are readily accessible. Further designer exploration would likely parallel that observed in the phenylalkylamine series of drugs and involve methoxy, dimethoxy, bromo-dimethoxy, methylenedioxy, trifluoromethyl, chloro, methyl substituents, and regiosiomers of these substitution patterns.

Additionally carbonyl containing benzoyl piperazines are likely extensions in this series based on similarity of clandestine drug development in cathinone, methcathinone, methalone and other benzylketophenethylamines (referred to in some recent literature as bk-phenethylamines, i.e. bk-MBDB has been reported from some jurisdictions). Designer modification and exploration in this bk-series is a major issue in forensic drug chemistry at the present time [Archer *et al*, 2009].

Phenalkylpiperazines in which the amine portion of the phenethylamines is replaced by a piperazines group is a likely future designer modification (Scheme 1). Such compounds could be synthesized by some "clandestine chemistry recipes" used in the synthesis of phenethylamines.

Scheme 1 shows the general structures for the compounds to be prepared and evaluated in this project. There are three regioisomeric variants for each monosubstituted aromatic ring (ortho-, meta-, para- or 2-, 3-, 4-substituted), six regioisomers for equivalent disubstitution, and ten ring substitution patterns for disubstituted nonequivalent groups. In this project we propose to evaluate four series of piperazine containing molecules: benzyl piperazines (I), benzoyl piperazines (II), phenalkylpiperazines (III) and phenylpiperazinoketones (IV).

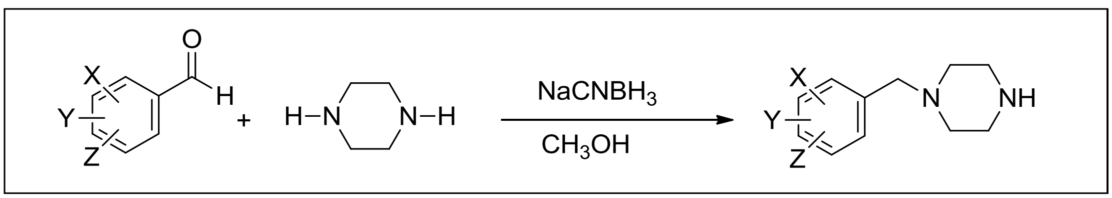
In the first series (I) (benzylpiperazines) the research will focus on the complete set of regioisomeric ring substituted piperazines that have already appeared in clandestine drug samples. These are primarily the monosubstituted aromatic ring compounds shown in Scheme 1 (Aromatic Group A and some in Group B). Some of these compounds are commercially available and we need only make those regioisomers or isobars not commercially available in order to have a complete series for analytical studies. For the other three categories (II-IV) initial efforts will focus on evaluating those likely designer target substitution patterns based on model drugs of abuse such as methcathinone, bk-phenethylamines and methylenedioxyphenylamines. The ring substituents in aromatic ring categories B and C are the most likely designer targets and the recent street appearance in Europe of 4-bromo-2,5-dimethoxybenzylpiperazine [Westphal *et al*, 2009] confirms the general direction of clandestine exploration of these compounds.

2. Synthesis of the Regioisomeric and Isobaric Piperazines

Regioisomeric and isobaric substances are considered a significant challenge for the analytical techniques used to identify specific molecules. This is considered extremely important when some of these molecules are legally controlled drugs and others may be uncontrolled, non-drug species or imposter molecules. Methylenedioxybenzylpiperazines have direct and indirect regioisomers as well as isobaric substances of equal molecular weight and fragmentation products of identical mass. The direct regioisomers are those substances containing the methylenedioxybenzyl ring system which yields the methylenedioxybenzyl carbocation fragment as the base peak ($C_8H_7O_2^+$, m/z 135) in the mass spectrum. The indirect regioisomers of MDBP consist of those substances which do not contain the methylenedioxybenzyl system yet contain the equivalent empirical formula $C_8H_7O_2^+$ yielding m/z 135, the methoxybenzoylpiperazines are perhaps the most likely possibility. The ten methoxymethylbenzylpiperazines, the three ethoxybenzylpiperazines yield the corresponding mass equivalent substituted benzyl carbocation ($C_9H_{11}O^+$, m/z 135) are the isobaric substances evaluated in this study. In this chapter the synthesis of these direct and indirect regioisomeric and isobaric substances related to 3,4-MDBP or other types of piperazine compounds are described while their analytical properties including chromatographic separations are discussed in chapter 3.

2.1. Synthesis of the ring substituted benzylpiperazines

The synthesis of the ring substituted benzylpiperazines involves the condensation of piperazine with substituted benzaldehydes as illustrated in Scheme 5. Treatment of the substituted benzaldehydes with piperazine under reductive amination conditions in methanol will yield the intermediate imine which can be reduced by sodium cyanoborohydride, an imine selective reducing agent. Reductive amination or similar methods using other substituted benzaldehydes will yield other desired benzylpiperazines in this study. The benzylpiperazine products are isolated by simple extraction and recrystallization of the product salts. While some of the substituted benzaldehydes are commercially available, others are not. Those not available can be obtained by synthetic methods routinely employed in our laboratories and described later in this section.



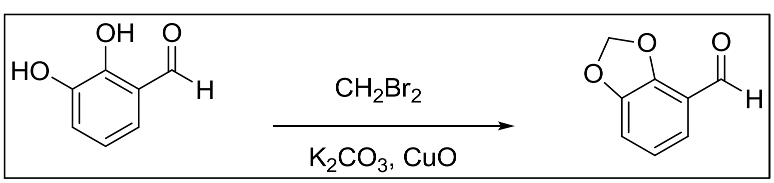
Scheme 5. General synthesis for the 1-benzylpiperazines.

2.1.1. Synthesis of the methylenedioxybenzylpiperazines (MDBPs)

The general procedure for the synthesis of these two regioisomeric methylenedioxybenzyl piperazines utilize 2,3-methylenedioxybenzaldehyde and 3,4- methylenedioxybenzaldehyde (piperonal), as starting materials. Piperonal is commercially available while the 2,3-methylenedioxybenzaldehyde was prepared in our lab. The preparation of 2,3-methylenedioxybenzaldehyde has been reported previously [Soine *et al*.,1983 and Casale *et al*.,1995] and is outlined in Scheme 6.

2,3-Dihydroxybenzaldehyde was converted to 2,3-methylenedioxybenzaldehyde by adding dibromomethane to a solution of 2,3-dihydroxybenzaldehyde and potassium carbonate in dimethylformamide (DMF), followed by the addition of copper(II)oxide and the resulting mixture was heated at reflux overnight. Solvent extraction followed by Kugelrohr distillation produced the pure 2,3-methylenedioxybenzaldehyde (2,3-piperonal).

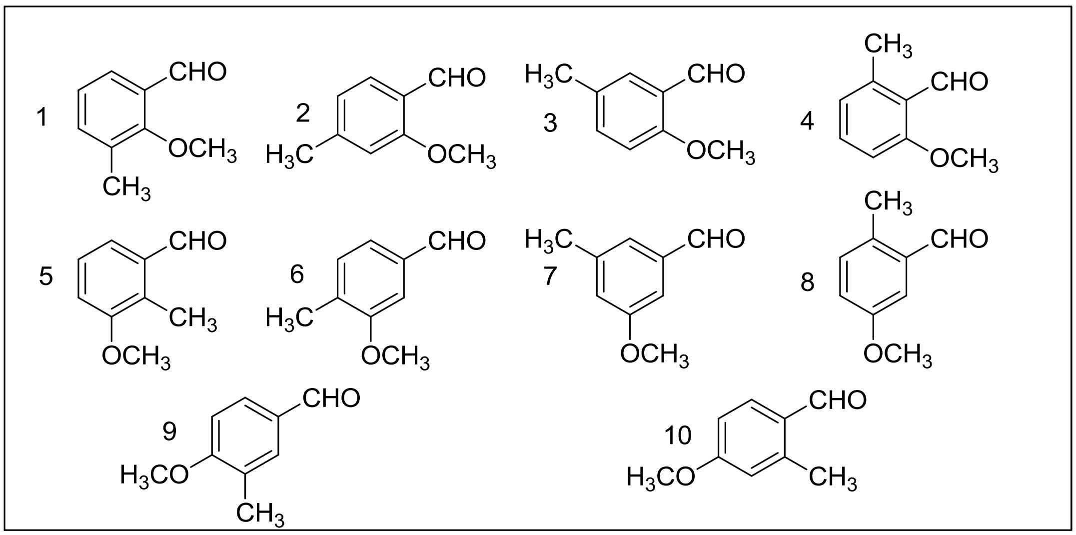
The two regioisomers were prepared by the reductive amination of the appropriate aldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding methylenedioxybenzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization.



Scheme 6. Synthesis of 2,3-methylenedioxybenzaldehyde

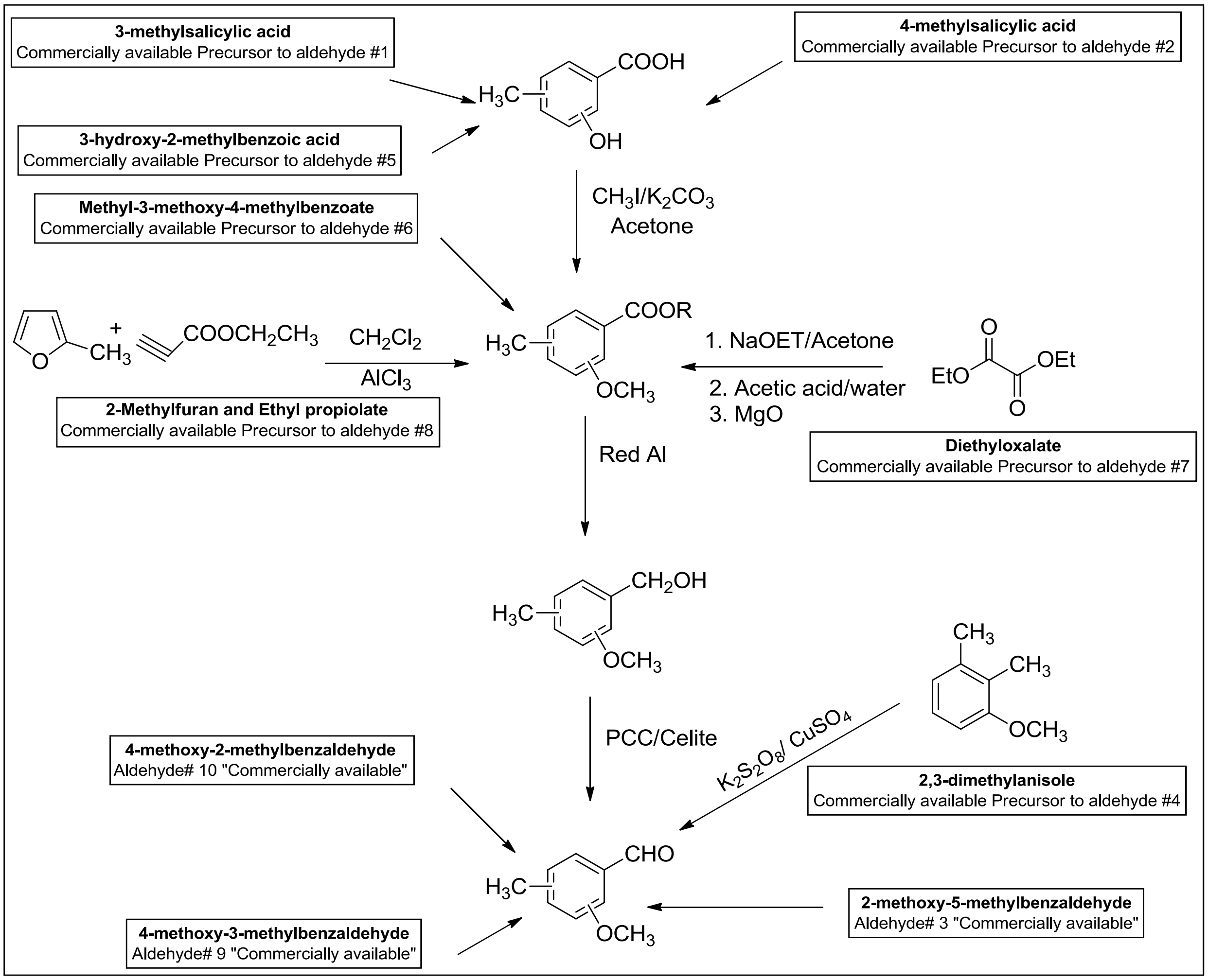
2.1.2. Synthesis of the methoxymethylbenzylpiperazines (MMBPs)

The general procedure for the synthesis of these ten ring regioisomeric benzylpiperazines involves the reductive amination of the appropriate methoxymethylbenzaldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the ten compounds are shown in Scheme 7.



Scheme 7. Structures for the ten regioisomeric methoxymethylbenzaldehydes.

There are only three commercially available benzaldehydes, 2-methoxy-5-methylbenzaldehyde (Aldehyde 3), 4-methoxy-3-methylbenzaldehyde (Aldehyde 9) and 4-methoxy-2-methylbenzaldehyde (Aldehyde 10). Three other substitution patterns were synthesized from commercially available hydroymethyl benzoic acids, 3-methyl salicylic acid, 4-methyl salicylic acid and 3- hydroxy-2-methyl benzoic acid. One substitution pattern was prepared from commercially available methyl ester of 3-methoxy-4-methyl benzoic acid and another from 2,3-dimethylanisole. Thus a total of eight of the required methoxymethylbenzaldehydes were available as the appropriately substituted aromatic system. The remaining two aldehydes were prepared by selective synthetic methods which formed the desired substitution patterns. The synthetic routes are summarized in Scheme 8 and described individually in the next sections.

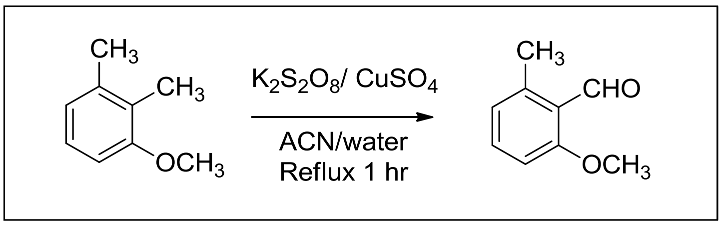


Scheme 8. Summary of the methods used in synthesizing the ten substituted methoxymethyl benzaldehydes

2.1.2.1. Synthesis of 2-methoxy-6-methylbenzaldehyde

Hauser and Ellenberger reported that the ortho- methyl group in 2,3- dimethylanisole could be selectively oxidized by refluxing with potassium persulfate and copper sulfate pentahydrate in 50:50 acetonitrile:water (Scheme 9) to yield 2-methoxy-6-methylbenzaldehyde [Hauser and Ellenberger, 1987].

The resulting 2-methoxy-6-methylbenzaldehyde was converted to 2-methoxy-6-benzylpiperazine using the same procedures illustrated in Scheme 5.

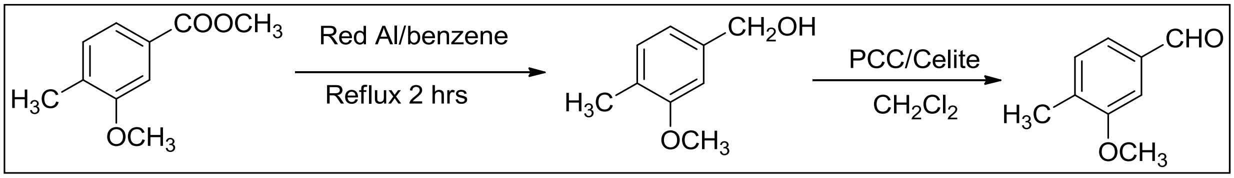


Scheme 9. Selective oxidation of 2,3-dimethylanisole to yield aldehyde 4.

2.1.2.2. Synthesis of 3-methoxy-4-methylbenzaldehyde

3-methoxy-4-methylbenzaldehyde was synthesized from the commercially available methyl-3-methoxy-4-methyl benzoate. The ester was selectively reduced to the corresponding alcohol, 3-methoxy-4-methyl benzyl alcohol by refluxing with the organic solvent soluble hydride reducing agent sodium bis-2-methoxy-ethoxy-aluminium hydride (Red Al) for two hours. The resulting alcohol was selectively oxidized to 3-methoxy-4-methylbenzaldehyde by overnight stirring with pyridinium chlorochromate (PCC) and celite in methylene chloride (Scheme 10).

The resulting benzaldehyde was converted to 3-methoxy-4-methylbenzylpiperazine by following the same procedures outlined in Scheme 5.



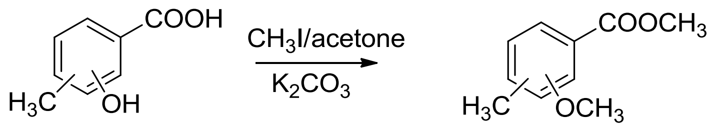
Scheme 10. Synthesis of 3-methoxy-4- methylbenzaldehyde.

2.1.2.3. Synthesis of 2-methoxy-3-methylbenzaldehyde, 2-methoxy-4-methylbenzaldehyde and 3-methoxy-2-methylbenzaldehyde

Commercially available 3-methyl salicylic acid, 4-methyl salicylic acid and 3-hydroxy-2-methyl benzoic acid were converted to the corresponding methoxymethylbenzaldehydes, 2-methoxy-3-methylbenzaldehyde, 2-methoxy-4-methylbenzaldehyde and 3-methoxy-2-methylbenzaldehyde, respectively, through methylation of the hydroxyl group accompanied by esterification of the carboxylic acid followed by reduction of the resulting esters to the corresponding alcohols which in turn was selectively oxidized to yield the appropriately substituted methoxymethylbenzaldehydes.

The acids, 3-methyl salicylic acid, 4-methyl salicylic acid and 3-hydroxy-2-methyl benzoic acid were individually stirred with excess methyl iodide in dry acetone and in the presence of potassium carbonate at room temperature for three days yielding methyl- 2-methoxy-3-methylbenzoate, methyl- 2-methoxy-4-methylbenzoate and methyl-3-methoxy-2-methylbenzoate, respectively (Scheme 11).

The resulting esters were selectively reduced to the corresponding benzyl alcohols, 2-methoxy-3-methylbenzyl alcohol, 2-methoxy-4-methylbenzeyl alcohol and 3- methoxy-2-methylbenzyl alcohol, followed by selective oxidation to the corresponding benzaldehydes, 2-methoxy-3-methylbenzaldehyde, 2-methoxy-4-methylbenzaldehyde and 3-methoxy-2-methylbenzaldehyde using the same procedure described in Scheme 10.

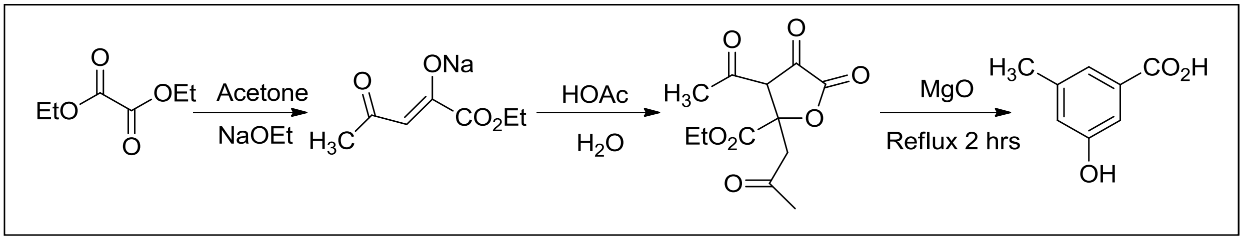


Scheme 11. Synthesis of methyl- ring substituted methoxymethylbenzoate from the corresponding hydroxyl methyl benzoic acids.

The resulting benzaldehydes were converted to the corresponding methoxymethylbenzylpiperazines, 2-methoxy-3-methylbenzylpiperazine, 2-methoxy-4-methylbenzylpiperazine and 3-methoxy-2-methylbenzylpiperazine using the same procedures outlined in Scheme 5.

2.1.2.4. Synthesis of 3-methoxy-5-methylbenzaldehyde

Overnight condensation of acetone and diethyl oxalate in presence of sodium ethoxide under nitrogen atmosphere yielded ethyl sodium acetopyrovate which was converted to 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester by stirring in a mixture of water and glacial acetic acid (1:1 v/v) for two hours at room temperature. 3-Hydroxy-5-methyl benzoic acid was obtained from 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester by refluxing with magnesium oxide in water for two hours [Turner and Gearien, 1952] (Scheme 12).

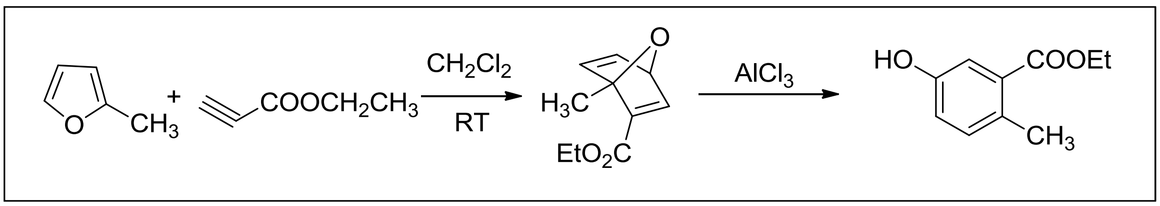


Scheme 12. Synthesis of 3-hydroxy-5-methyl benzoic acid.

3-hydroxy-5-methyl benzoic acid was converted to methyl-3-methoxy-5-methylbenzoate using the same procedures outlined in Scheme 11, however GC-MS analysis of the reaction mixture showed two peaks of m/z 180/149 and 194/149 which indicated a mixture of methyl-3-methoxy-5-methyl benzoate and ethyl-3-methoxy-5-methyl benzoate, respectively. These data suggest that during the reaction of 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester with magnesium oxide, a mixture of both 5-hydroxy-3-methylbenzoic acid and ethyl-5- hydroxy-3-methyl benzoate was formed. No attempt at separation of the two products was carried out since the following step includes selective reduction of the ester functional groups following the procedures outlined in Scheme 10 and both compounds yielded one alcohol, 3-methoxy-5-methyl benzyl alcohol. Finally, 3-methoxy-5-methyl benzyl alcohol was selectively oxidized to the corresponding benzaldehyde using the same procedures described in Scheme 10. The resulting benzaldehyde was converted to the corresponding benzylpiperazine using the same reaction procedure in Scheme 5.

2.1.2.5. Synthesis of 5-methoxy-2-methylbenzaldehyde

The Diels Alder type condensation between 2-methylfuran and ethyl propiolate in the presence of anhydrous aluminum chloride gave ethyl-5-hydroxy-2-methylbenzoate [Randad and Erickson, 1999]. The reaction procedure is outlined in Scheme 13. A solution of 2-methylfuran in methylene chloride was added dropwise to a solution of ethyl propiolate and anhydrous aluminum chloride in methylene chloride and the resulting reaction mixture was stirred at room temperature for 30 minutes. The reaction was terminated by addition of water and ethyl-5-methoxy-2-methylbenzoate was synthesized by refluxing 5-hydroxy-2-methyl benzoic acid ethyl ester with methyl iodide and potassium carbonate in dry acetone over night. The procedures are outlined in Scheme 11.



Scheme 13. Synthesis of ethyl-5-hydroxy-2-methylbenzoate

Synthesis of 5-methoxy-2-methyl benzaldehyde was carried out using the same procedures outlines in Scheme 10, where selective reduction of ethyl-5-methoxy-2-methylbenzoate using Red-Al yielded 5-methoxy-2-methyl benzyl alcohol which was then selectively oxidized using pyridinium chlorochromate and celite in methylene chloride to yield the desired methoxy methyl substituted benzaldehyde. The initial cycloaddtion reaction is reported to be regiospecific yielding only 1-methyl-2-carbethoxy-7-oxobicyclo [2.2.1] heptadiene intermediate shown in Scheme 13. If the other intermediate had formed (i.e.1-methyl-3-carbethoxy-7-oxobicyclo [2.2.1] heptadiene) the resulting aldehyde would be 2-methoxy-5-methyl benzaldehyde. 5-Methoxy-2-

methyl benzaldehyde was converted to 5-methoxy-2-methylbenzylpiperazine using the same procedure outlined in Scheme 5.

2.1.3. Synthesis of the ethoxybenzylpiperazines (EBPs)

The general procedure for the synthesis of these three ring regioisomeric benzylpiperazines involves the reductive amination of the ethoxybenzaldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the three compounds are 2, 3 and 4-ethoxy benzaldehyde, respectively and all are commercially available.

2.1.4. Synthesis of the methylbenzylpiperazines (MBPs)

The general procedure for the synthesis of the three regioisomeric methylbenzylpiperazines involves the reductive amination of the appropriately substituted benzaldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the three compounds are 2, 3 and 4-methylbenzaldehyde (o, m and p-toluealdehyde), respectively and all are commercially available.

2.1.5. Synthesis of the monomethoxybenzylpiperazines (OMeBPs)

The general procedure for the synthesis of the three regioisomeric methoxybenzylpiperazines involves the reductive amination of the appropriately substituted benzaldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the three compounds are 2, 3 and 4-methoxybenzaldehyde, respectively and all are commercially available.

2.1.6. Synthesis of the dimethoxybenzylpiperazines (DMBPs)

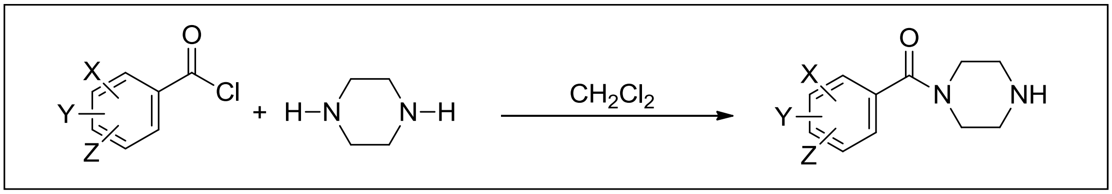
The general procedure for the synthesis of these six regioisomeric dimethoxybenzylpiperazines begins with the appropriate aldehyde, 2,3- , 2,4- , 2,5-, 2,6- , 3,4- and 3,5-dimethoxybenzaldehyde, as starting materials and all are commercially available. The six regioisomers were synthesized by stirring a solution of the appropriate aldehyde in methanol with piperazine and sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding dimethoxybenzylpiperazines, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization.

2.1.7. Synthesis of the chlorobenzylpiperazines (ClBPs)

The general procedure for the synthesis of the three regioisomeric chlorobenzylpiperazines involves the reductive amination of the appropriately substituted benzaldehyde and piperazine in presence of sodium cyanoborohydride. Isolation of the basic fraction gave the corresponding benzylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the three compounds are 2, 3 and 4-chlorobenzaldehyde, respectively and all are commercially available.

2.2. Synthesis of the ring substituted benzoylpiperazines

The ring substituted benzoylpiperazines can be prepared by classic acid chloride-amine displacement chemistry as shown in Scheme 14 below. Treatment of the appropriately substituted benzoyl chlorides with piperazine in dichloromethane as organic solvent will yield the desired products directly.



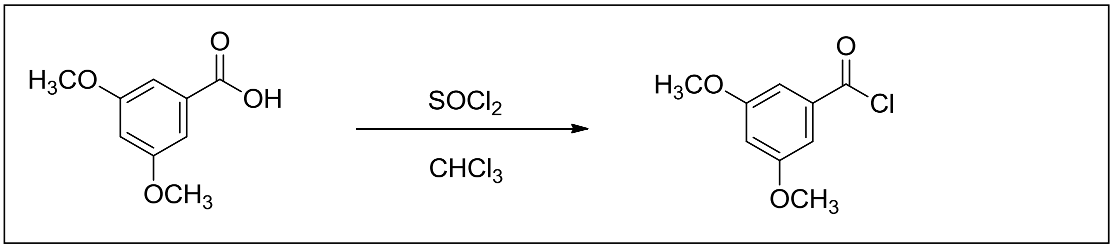
Scheme 14. General synthesis for the 1-benzoylpiperazines.

2.2.1. Synthesis of the monomethoxybenzoylpiperazines

The general procedure for the synthesis of the three regioisomeric methoxybenzoylpiperazines involves the slow addition of the appropriately substituted benzoyl chloride to a solution of piperazine in dichloromethane in an ice bath. Isolation of the basic fraction gave the corresponding benzoylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the three compounds are 2, 3 and 4-methoxybenzoylchloride, respectively and all are commercially available.

2.2.2. Synthesis of the six ring regioisomeric dimethoxybenzoylpiperazines

The general procedure for the synthesis of these six regioisomeric dimethoxybenzoylpiperazines involves the slow addition of the appropriately substituted benzoyl chloride to a solution of piperazine in dichloromethane in an ice bath. Isolation of the basic fraction gave the corresponding benzoylpiperazine bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials are 2,3, 2,4, 2,5, 2,6 and 3,4-dimethoxybenzoylchloride, respectively and all are commercially available. 3,5-dimethoxybenzoylchloride is prepared from 3,5-dimethoxybenzoic acid by treatment with thionyl chloride in chloroform as shown in Scheme 15 below.

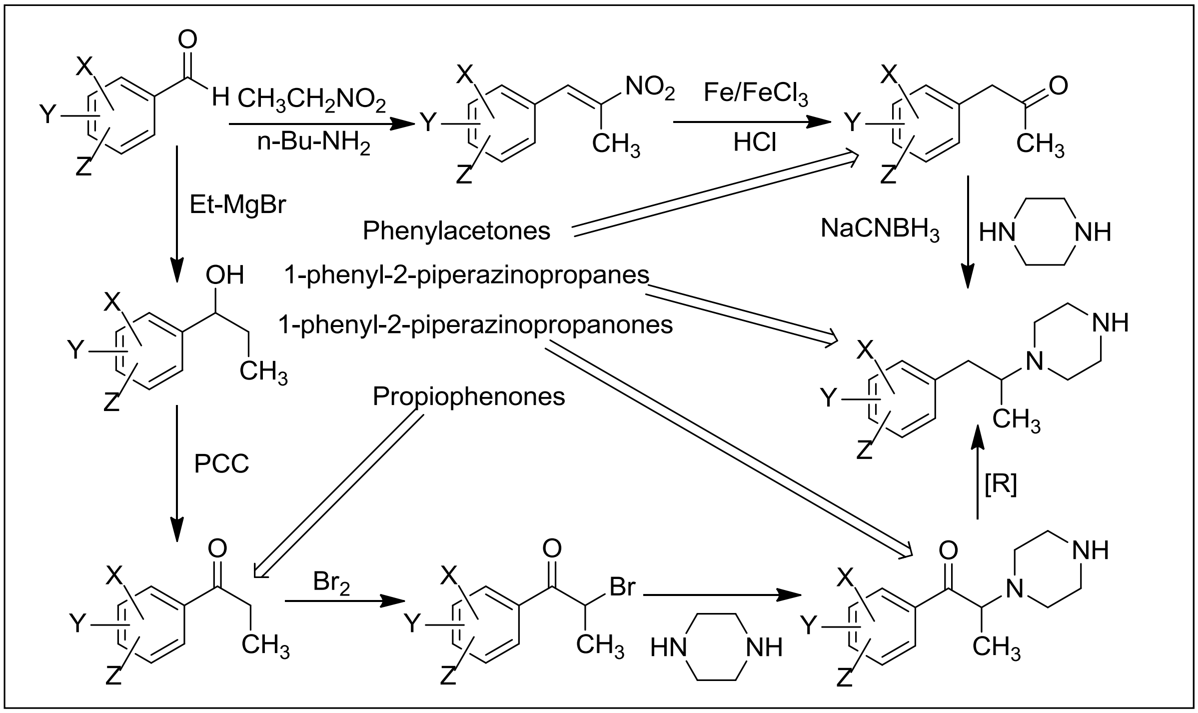


Scheme 15. Preparation of 3,5-dimethoxybenzoylchloride

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

The 1-phenyl-2-piperazinopropanes can be prepared as outlined in the Scheme 16 below. Laboratory prepared or commercially available ring substituted benzaldehydes can be treated with nitroethane under dehydration conditions to prepare the intermediate phenylnitropropenes. Reductive hydrolysis of these nitropropenes will yield the corresponding substituted phenylacetones which can be directly subjected to reductive amination with piperazine using cyanoborohydride in methanol as solvent to yield the desired 1-phenyl-2-piperazinopropane products. The aldehydes can also be reacted with ethylmagnesium bromide (EtMgBr) to yield the corresponding benzyl alcohol which is oxidized to the corresponding propiophenone. The substituted propiophenones can be brominated with bromine in acetic acid to give the alpha-bromoketone intermediates. Reaction of these bromoketones with piperazine will provide the 1-phenyl-2-piperazinopropanones via direct displacement. These piperazino-ketones are potential designer drugs based on the cathinone/methcathinone and the benzylketo-type (bk-type) compounds. Finally reduction of the ketone moiety will also yield the desired 1-phenyl-2-

piperazinopropane compounds.



Scheme 16. General synthesis for the 1-phenyl-2-piperazinopropanes and 1-phenyl-2-piperazinopropanones

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

The general procedure for the synthesis of the two regioisomeric MDPPPs involves the reductive amination of the appropriately substituted phenylacetone and piperazine in presence of sodium cyanoborohydride overnight as in Scheme 16. Isolation of the basic fraction gave the corresponding 1-(methylenedioxyphenyl)-2-piperazinopropane bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting material for the 3,4-regioisomer is 3,4-methylenedioxyphenylacetone and is commercially available.

2,3-methylenedioxyphenylacetone is the starting material for the synthesis of the 2,3-regioisomer and is not commercially available. Its preparation is shown in Scheme 16. It is prepared by reacting 2,3-methylenedioxybenzaldehyde with nitroethane to yield 2,3-methylenedioxynitropropene. The n-butylimine derivative is first formed from 2,3-piperonal and n-butylamine followed by the introduction of nitroethane. The reaction mixture was heated at reflux for an hour and 2,3-methylenedioxynitropropene was obtained after purification.

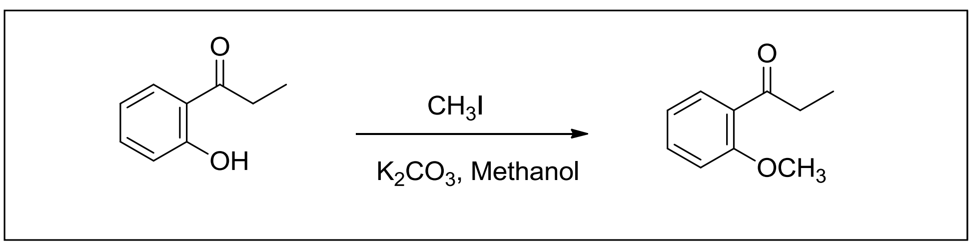
The conversion of 2,3-methylenedioxynitropropene to the corresponding phenylacetone was accomplished by reacting nitropropene with iron and hydrochloric acid in the presence of a catalytic amount of ferric chloride in a two phase solvent system, toluene and water. The reaction mixture was stirred vigorously at reflux for 24 hours. During the reaction the nitro group was first reduced to form 1-(2,3-methylenedioxyphenyl)-2-aminopropene, which tautomerized to the imine and then hydrolyzed to the desired phenylacetone. The preparation of 2,3-piperonal was previously discussed in section 2.1.1 and is shown in Scheme 6.

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

The general procedure for the synthesis of the three regioisomeric OMePPPs involves the reductive amination of the appropriately substituted phenylacetone and piperazine in presence of sodium cyanoborohydride overnight as shown in Scheme 16. Isolation of the basic fraction gave the corresponding 1-(monomethoxy)-2-piperazinopropane bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the o-, m- and p-isomers are 2`, 3` and 4`-methoxyphenylacetone, respectively and all are commercially available.

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

The general procedure (Scheme 16) for the synthesis of the three regioisomeric OMePPPOs involves the bromination of the appropriately substituted propiophenone using bromine in glacial acetic acid at room temperature to yield the corresponding bromopropiophenone. This is followed by the slow addition of the appropriately substituted bromopropiophenone to a solution of piperazine in dichloromethane and refluxing the mixture for two hours. Isolation of the basic fraction gave the corresponding 1-(monomethoxyphenyl)-2-piperazinopropanones bases, which were converted to the corresponding hydrochloride salts using gaseous HCl and purified by recrystallization. The starting materials for the m- and p-isomers are 3\` and 4\`-methoxypropiophenone, respectively and both are commercially available. The starting material for the ortho isomer is 2\`-methoxypropiophenone and is prepared by methylating 2\`-hydroxypropiophenone using iodomethane and potassium carbonate in methanol as solvent as shown in Scheme 17.



Scheme 17. Preparation of 2\`-methoxypropiophenone.

3. Analytical Studies and Isotope Labeling of the Regioisomeric and Isobaric Piperazines

The ability of the analytical method to distinguish between regioisomers and isobars directly enhances the specificity of the analysis for the target drugs of abuse. The mass spectrum is often the confirmatory piece of evidence for the identification of drugs of abuse in the forensic laboratory. While the mass spectrum is often considered a specific “fingerprint” for an individual compound, there may be other substances, not necessarily having any known pharmacological activity, capable of producing very similar or almost identical mass spectra. These imposter substances provide the possibility for misidentification as the drug of abuse itself. In the case of the piperazines, there may be many positional isomers, direct or indirect regioisomers, as well as isobaric compounds which yield a similar mass spectrum. A compound co-eluting with the controlled drug and having the same mass spectrum as the drug of abuse would represent a significant analytical challenge. The ultimate concern then is “if the forensic scientist has never analyzed all the non-drug substances, how can she/he be sure that any of these compounds would not co-elute with the drug of abuse?” The significance of this question is related to many factors, chief among these is the chromatographic system separation efficiency and the number of possible counterfeit substances. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of abuse.

NMR is a nondestructive flexible technique that can be used for the simultaneous identification of pure compounds and even mixtures of compounds in one sample. Its advantages, compared to GC-MS techniques, include stereochemical differentiation and the capability to analyze nonvolatile compounds. However, the lack of use in forensic laboratories can be attributed to the high cost of instrumentation and the poor sensitivity of NMR. In addition, NMR requires pure samples which are hard to satisfy in the case of biological samples.

Infrared spectroscopy is considered a confirmation method for the identification of organic compounds due to the uniqueness of infrared spectra for very similar organic molecules. Gas chromatography with infrared detection (GC-IRD) is characterized by scanning quickly enough to obtain vapor phase IR spectra of compounds eluting from the capillary GC columns.

All the regioisomers, direct and indirect as well as isobaric compounds have a strong possibility to be misidentified as the controlled drug, by some analytical methods especially mass spectrometry. In this chapter, all direct ring regioisomers, and some indirect regioisomers as well as a group of isobaric compounds related to 3,4-MDBP and other potential piperazine-derived drugs of abuse are compared by chromatographic and spectroscopic techniques, and methods for their differentiation are explored. Exact mass determination techniques such as gas chromatography coupled to time of flight mass spectrometric detection (GC-TOF-MS) will be utilized to differentiate between isobaric compounds that have essentially the same nominal masses but are different in their elemental composition and accordingly have different exact masses. Isotope labeling such as deuterium (D) and carbon 13 (^{13}C) labeling will be used to confirm mass spectrometric fragmentation mechanisms that result in the formation of some key fragment ions or to confirm the elemental composition of these fragment ions.

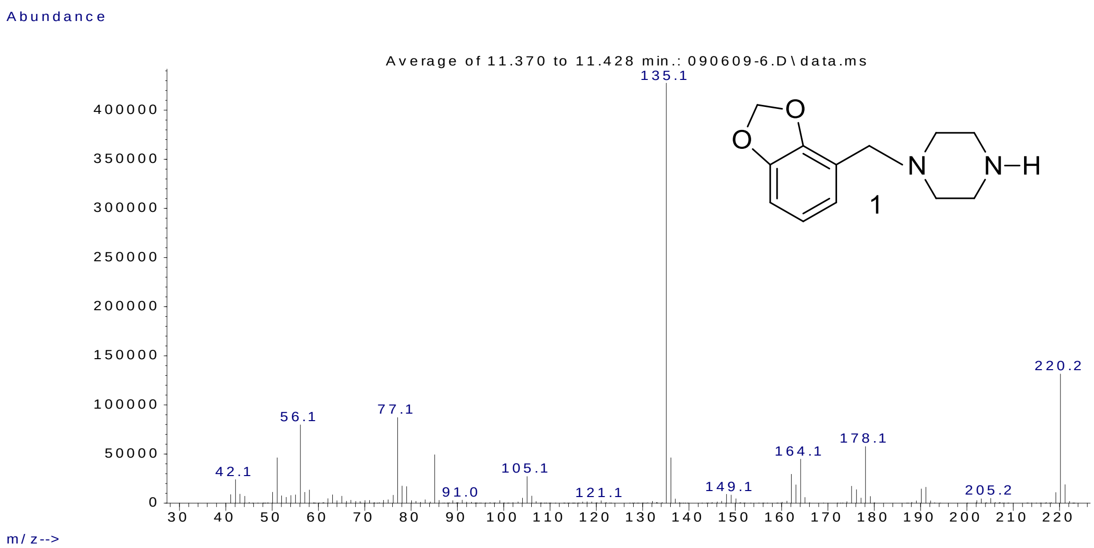
3.1. Differentiation of Methylenedioxybenzylpiperazines (MDBPs) by GC-IRD and GC-MS

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

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

3.1.1. Mass spectral studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 1 shows the EI mass spectra of the two regioisomeric methylenedioxybenzylpiperazines (Compounds 1 and 2). The mass spectra of both regioisomeric methylenedioxybenzylpiperazines show the fragment ions at m/z 178, 164, and 135 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Scheme 18 and are based on the work of [de Boer *et al*, 2001].



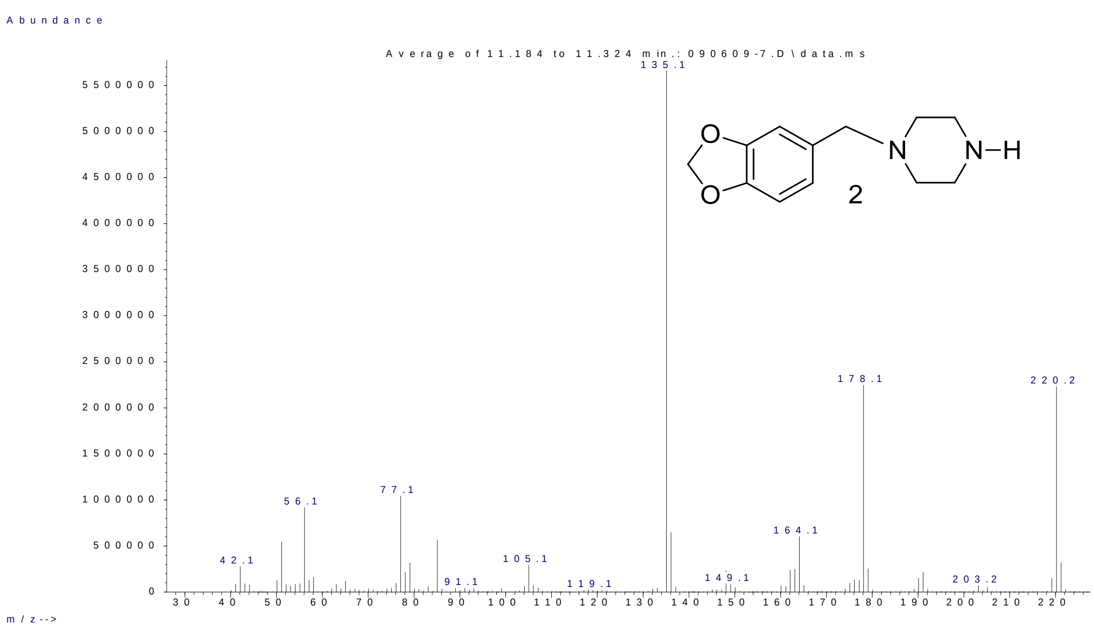
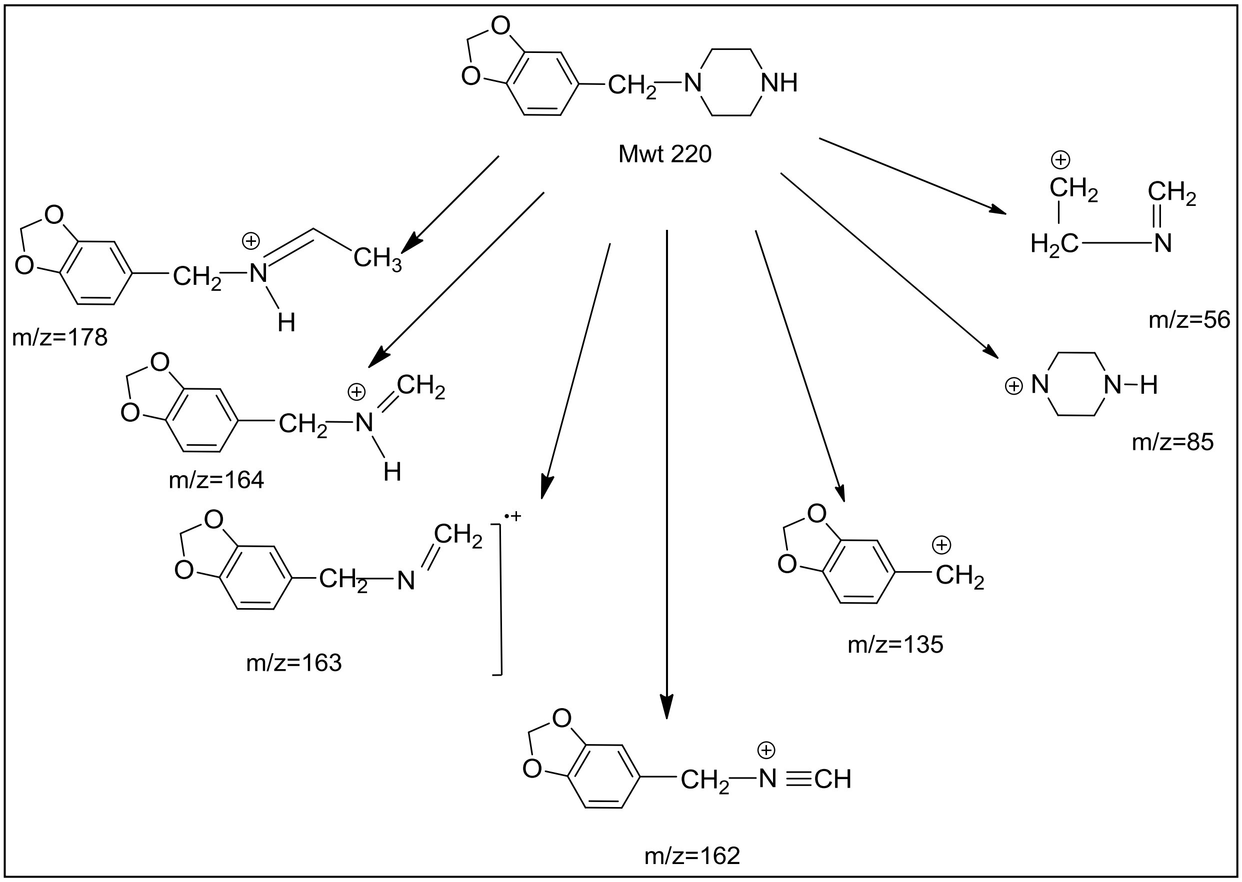


Fig. 1. EI Mass spectra of the two methylenedioxybenzylpiperazines.

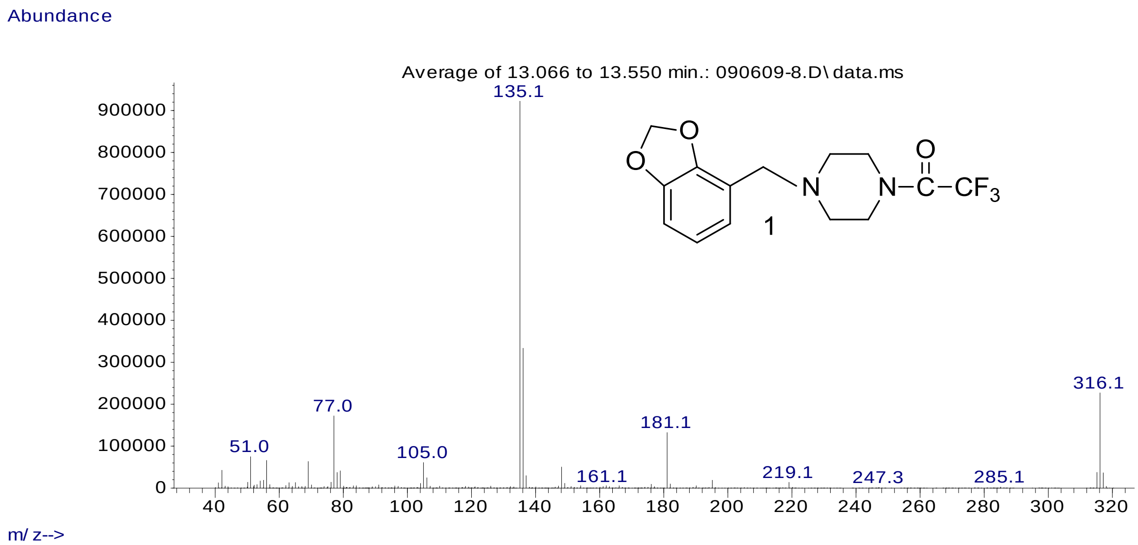


Scheme 18. Mass spectral fragmentation pattern of the underivatized 3,4-methylenedioxybenzylpiperazine under EI (70eV) conditions.

The previous work described the fragmentation of the unsubstituted benzylpiperazine 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 from their mass spectra for differentiation among these isomers. Thus, the mass spectra alone do not provide specific identity confirmation for the individual isomers.

The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the regioisomeric methylenedioxybenzylpiperazines, in an effort to individualize their mass spectra and identify marker ions that would allow discrimination between these two compounds. Acylation lowers the basicity of the acylated nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum [Peters *et al*, 2003 and Aalberg, *et al*, 2004]. However, acylation of the secondary nitrogen in the piperazine ring does not alter the basicity of the tertiary amine nitrogen.

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 2, 3 and 4. 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.



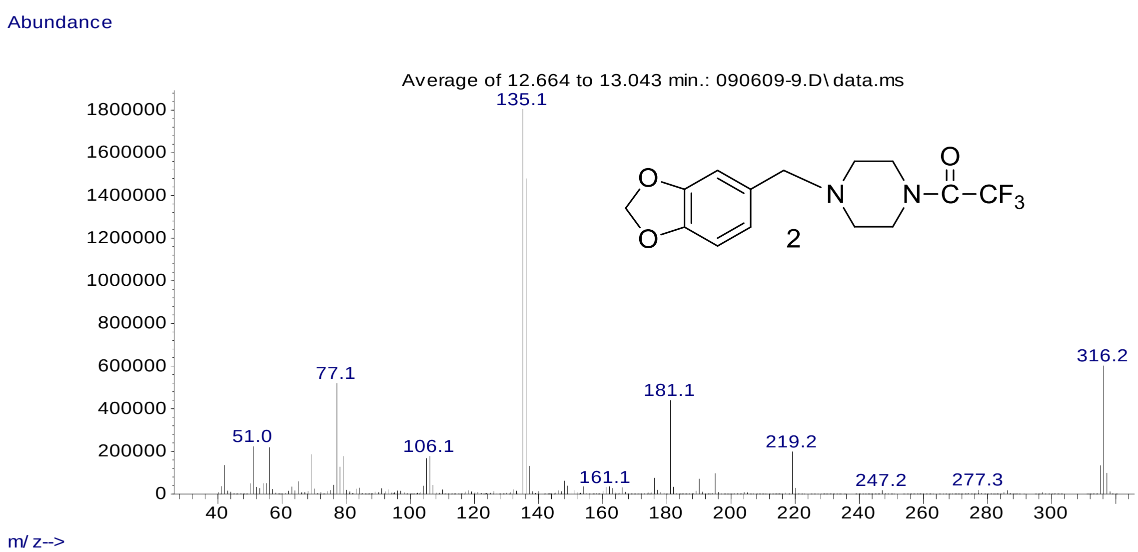
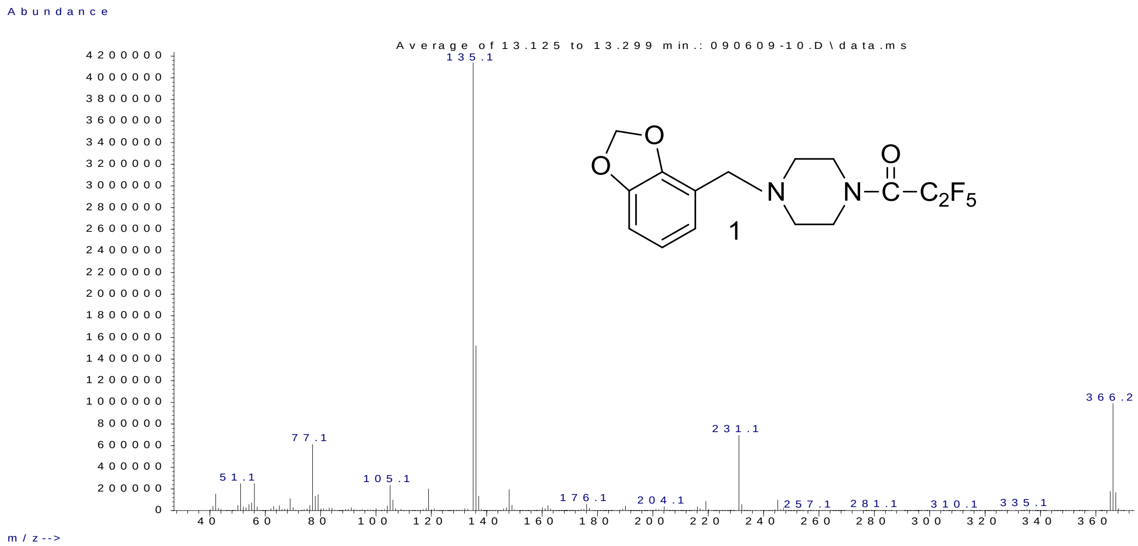


Fig. 2. Mass spectra of the trifluoroacetyl derivatives of the MDBP regioisomers.



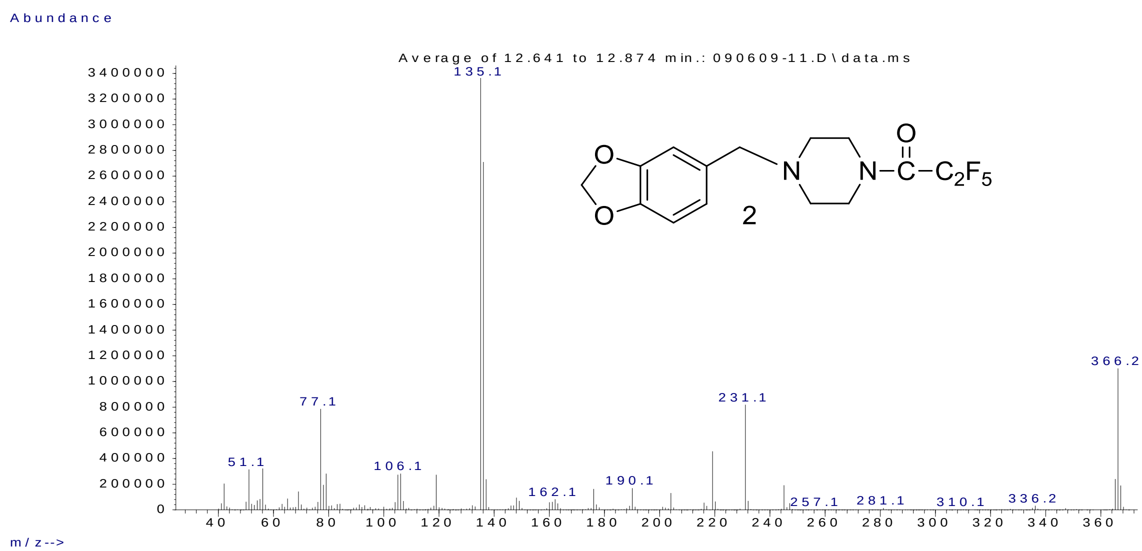
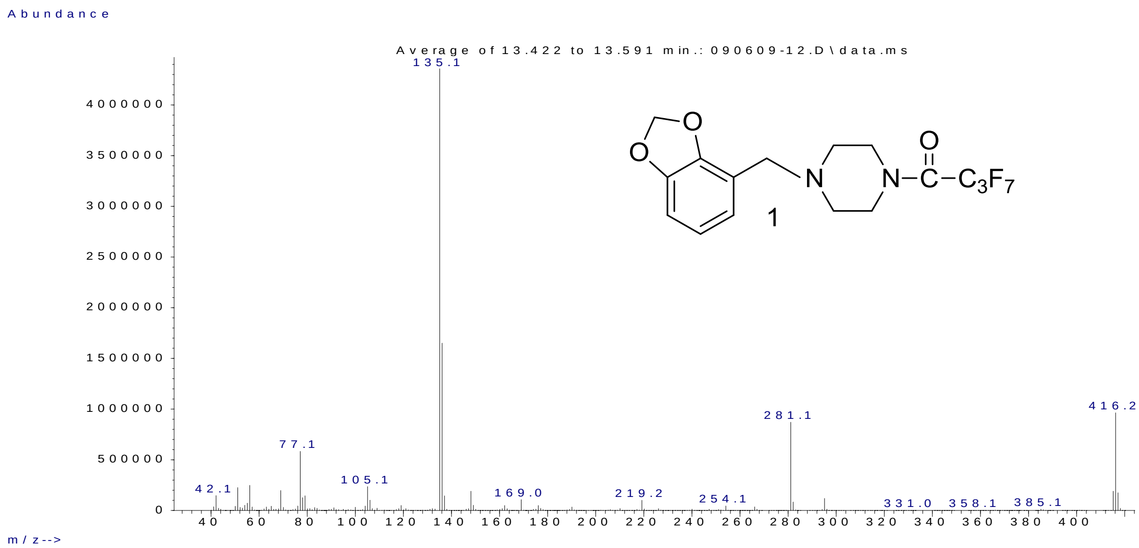


Fig. 3. Mass spectra of the pentafluoropropionyl derivatives of the MDBP regioisomers.



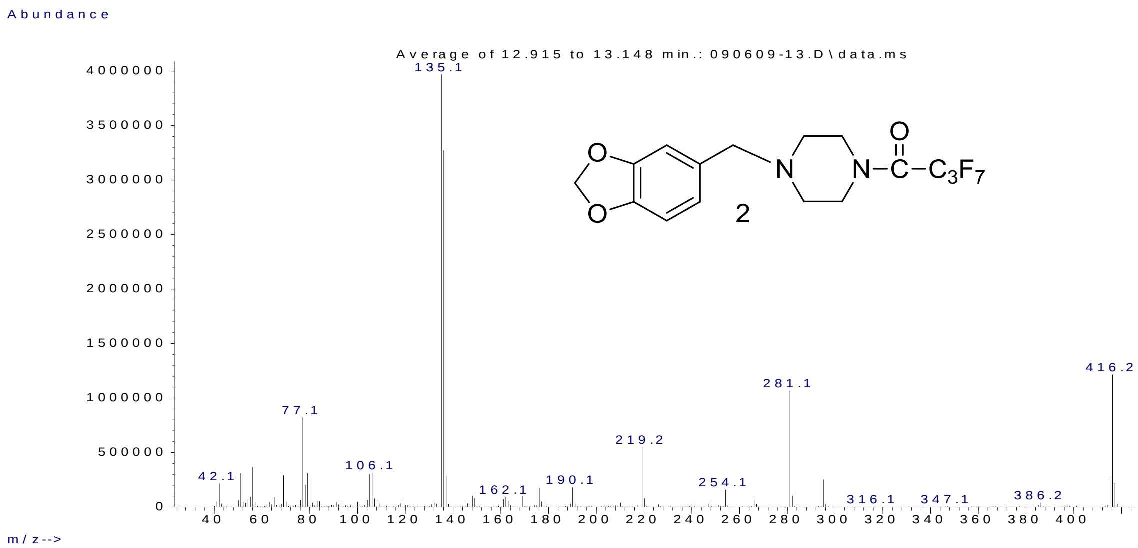
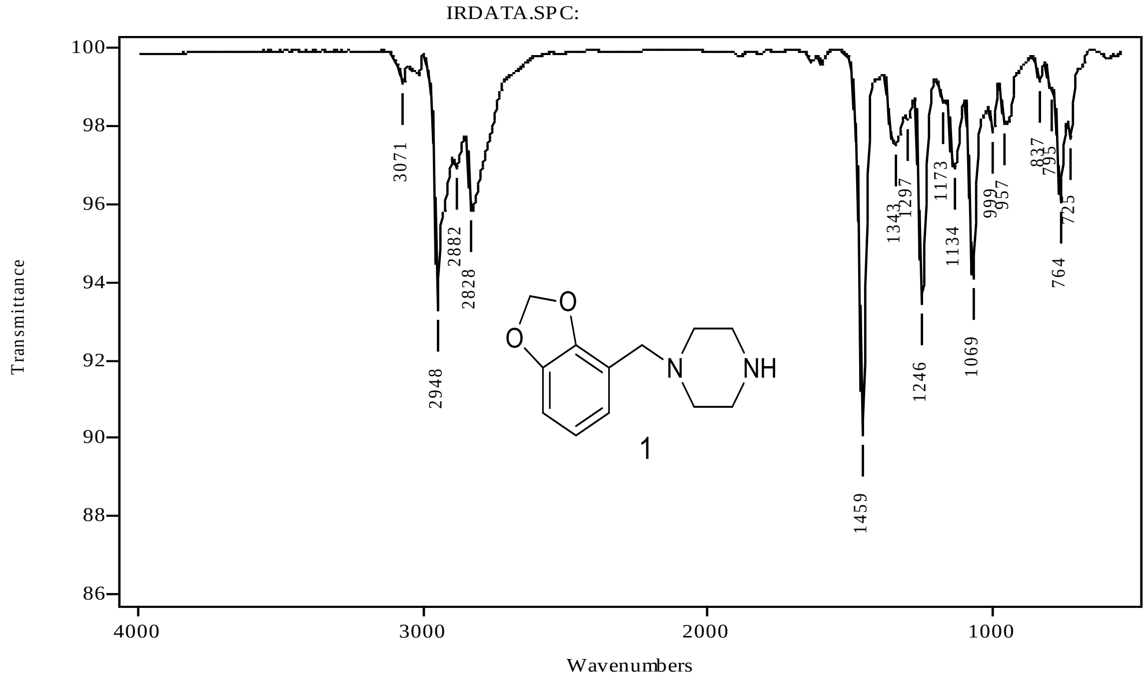


Fig. 4. Mass spectra of the heptafluorobutyryl derivatives of the MDBP regioisomers.

3.1.2. Vapor-phase Infra-Red Spectrophotometry

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the two regioisomeric MDBPs. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the two methylenedioxybenzylpiperazines are shown in Fig. 5. 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-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



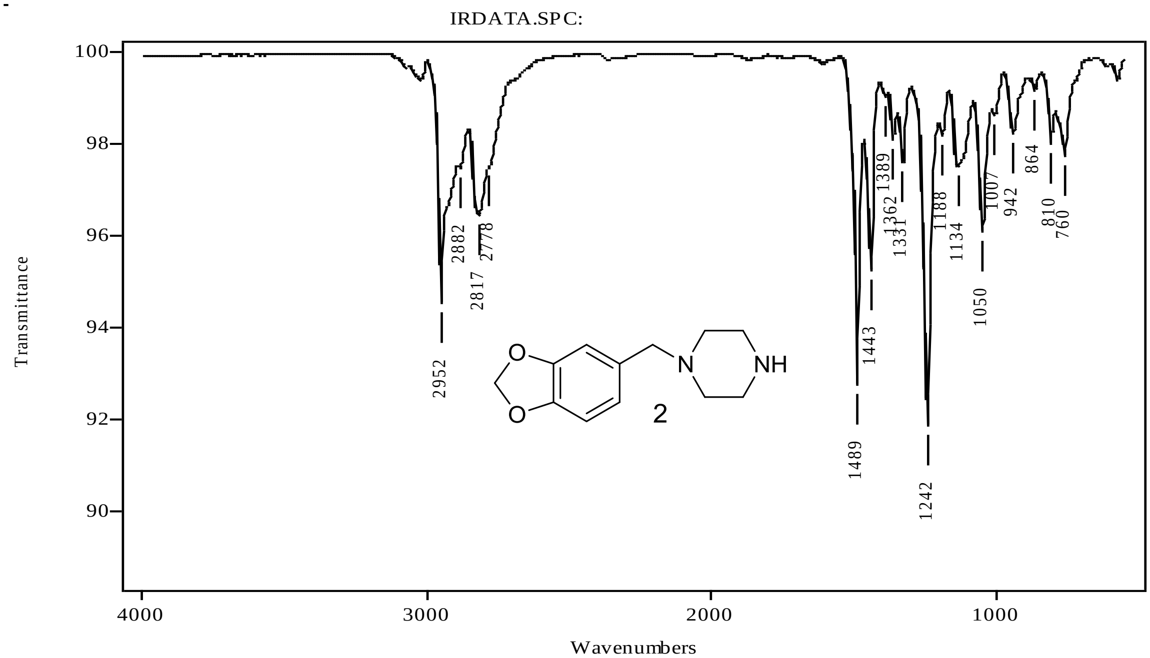


Fig. 5. Vapor phase IR spectra of the two regioisomeric methylenedioxybenzylpiperazines.

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.

3.1.3. Gas Chromatographic Separation

Gas chromatographic separation of the underivatized and derivatized piperazines was 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^{o}C for 1 minute, ramped up to 230^{o}C at a rate of 20^{o}C per minute followed by a hold at 230^{o}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.

Several temperature programs were evaluated and the chromatograms in Figures 6 and 7 are representative of the results obtained for all samples on the two columns. The chromatograms in Figure 6 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 7 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.

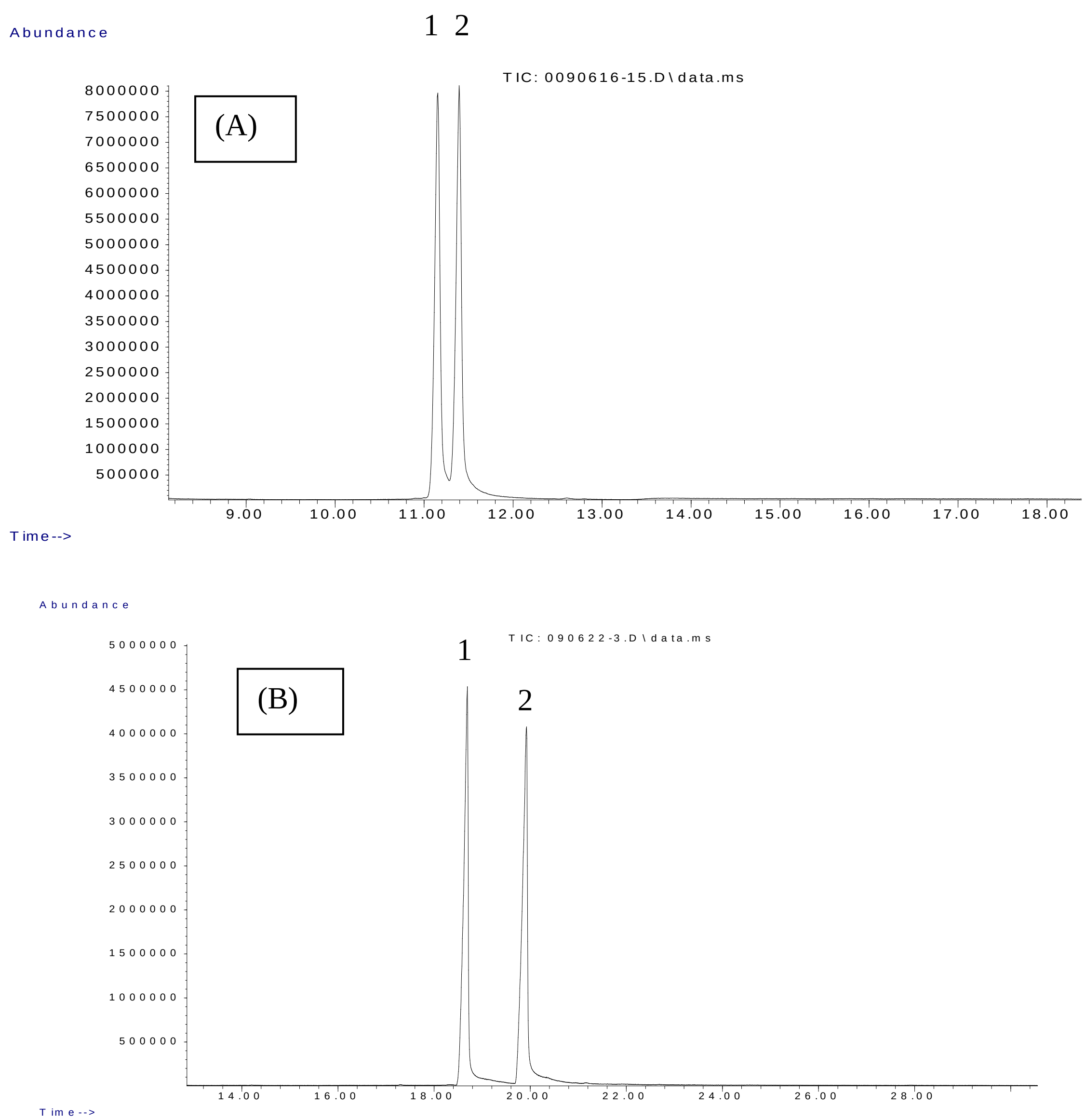


Fig. 6. Gas chromatographic separation of (1) 2,3-methylenedioxybenzyl piperazine and (2) 3,4-methylenedioxybenzylpiperazine. Columns: Rxi-50 (A) and Rtx-200 (B).

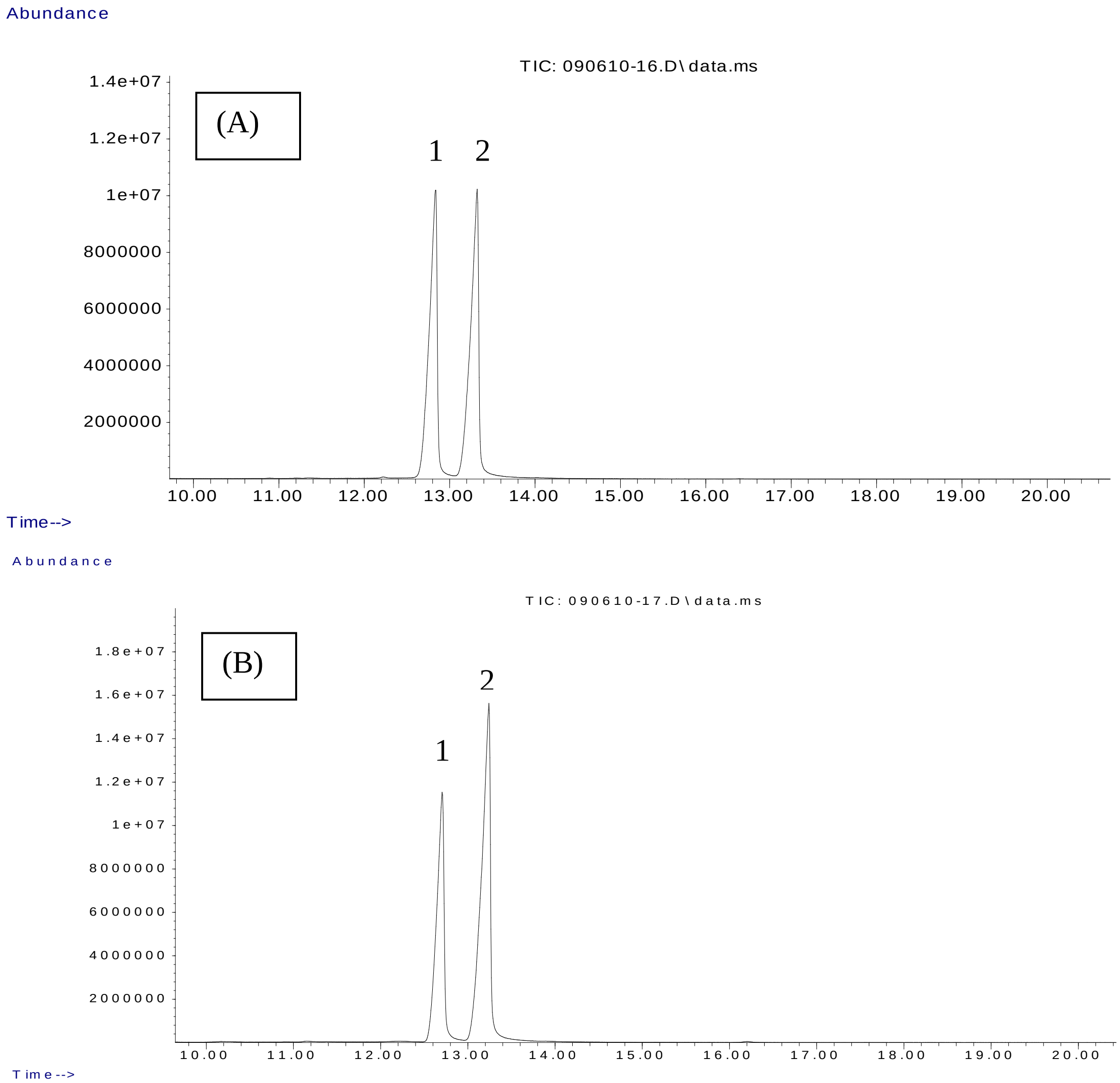


Fig. 7. Gas chromatographic separation of the trifluoroacetyl (A) and pentafluoropropionyl (B) derivatives using Rxi-50 column. (1) 2,3-methylenedioxybenzylpiperazine and (2) 3,4-methylenedioxybenzylpiperazine.

3.1.4. 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.

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

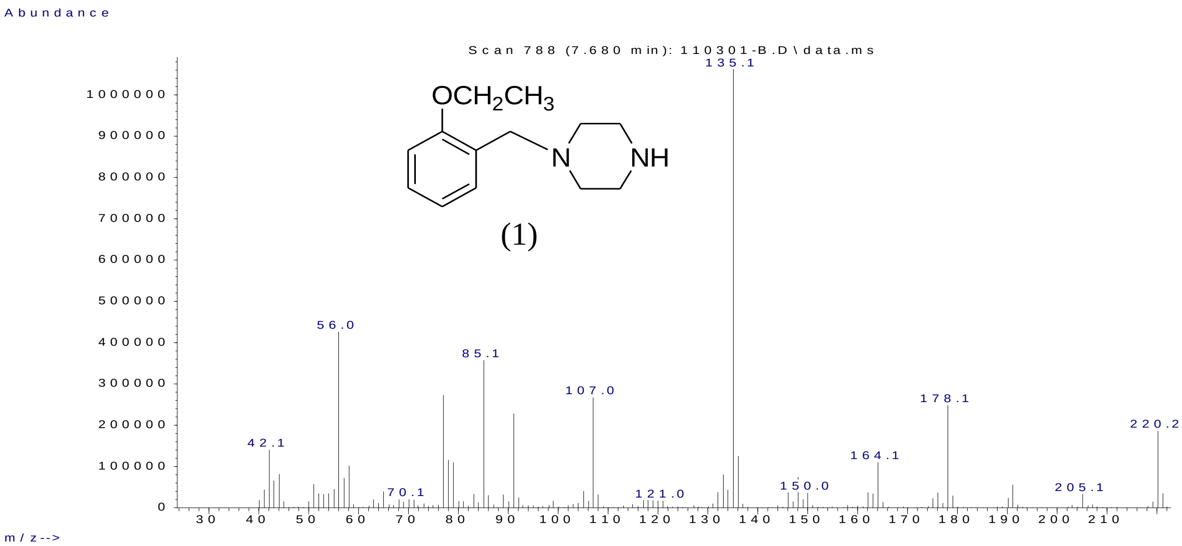
The substituted benzylpiperazines, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP), its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) and three isobaric ring substituted ethoxybenzylpiperazines (EBPs) have equal mass and many common mass spectral fragment ions. The mass spectra of the three 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.

3.2.1. Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 8 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 Scheme 19 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 Scheme 20 [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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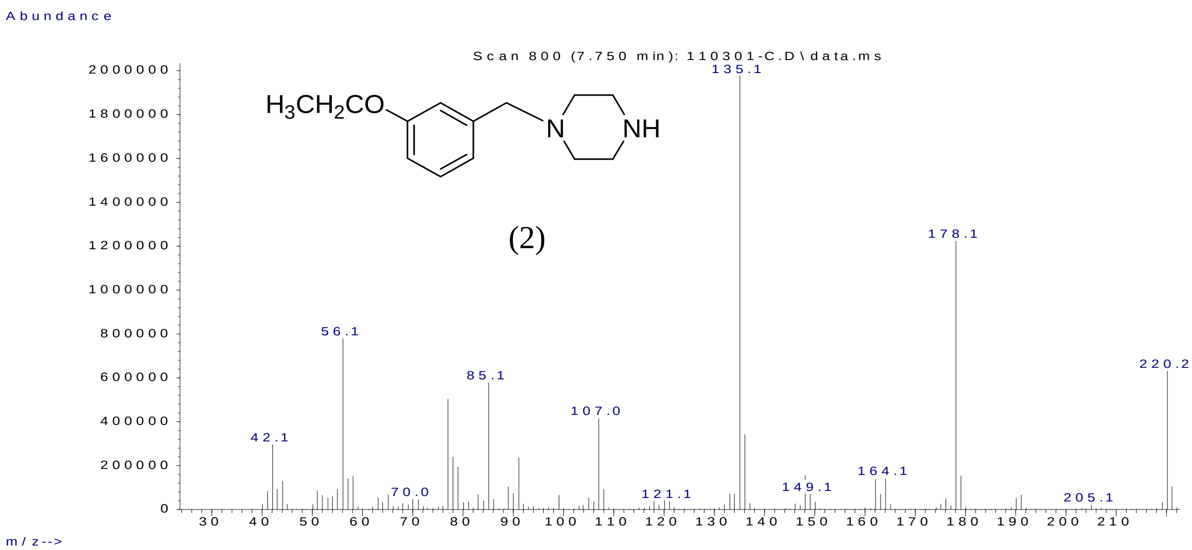
NH
(1)
135.1
56.0
85.1
107.0
178.1
220.2
42.1
164.1
70.1
121.0
150.0
205.1
m/z-->


Abundance
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H3CH2CO
N
NH
(2)
135.1
178.1
56.1
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107.0
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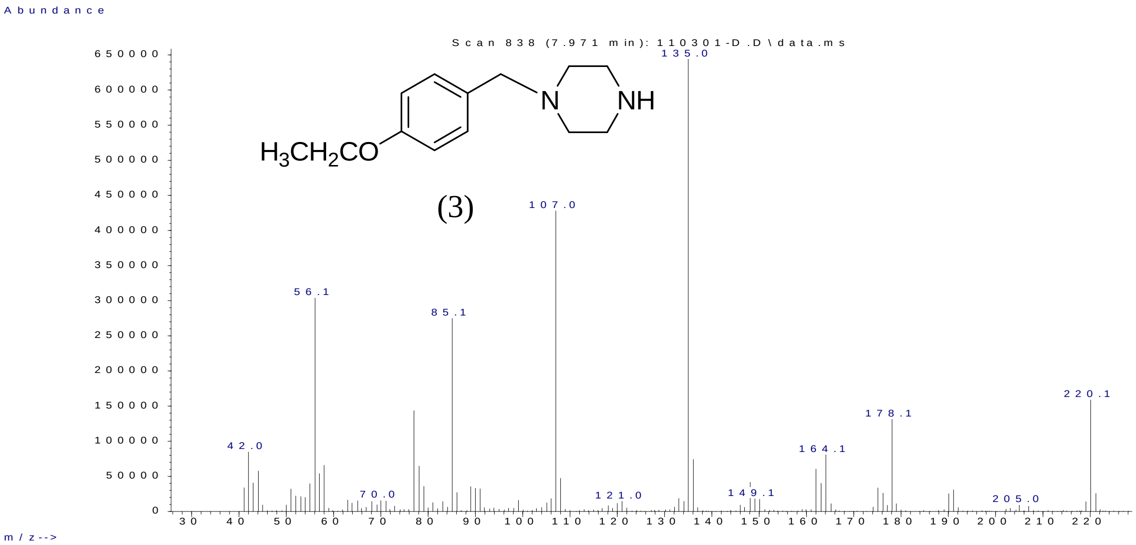
164.1
149.1
70.0
121.1
205.1
m/z-->


Abundance
Scan 838 (7.971 min): 110301-D.D\data.ms
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N
NH
(3)
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42.0
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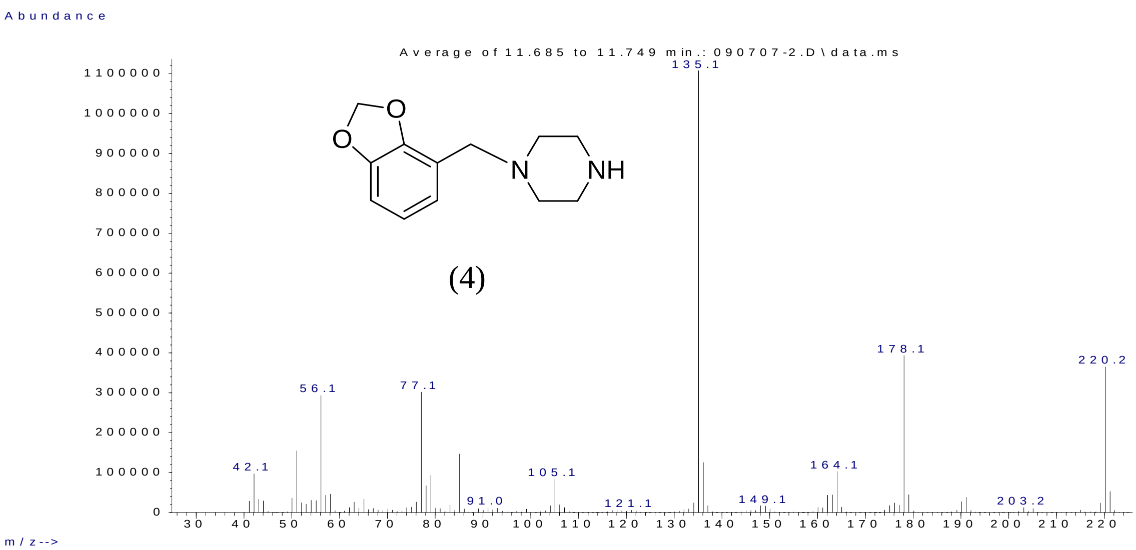
121.0
149.1
164.1
178.1
205.0
220.1
m/z-->


Abundance
Average of 11.685 to 11.749 min.: 090707-2.D\data.ms
O
O
N
NH
(4)
135.1
178.1
220.2
56.1
77.1
42.1
91.0
105.1
121.1
149.1
164.1
203.2
m/z-->

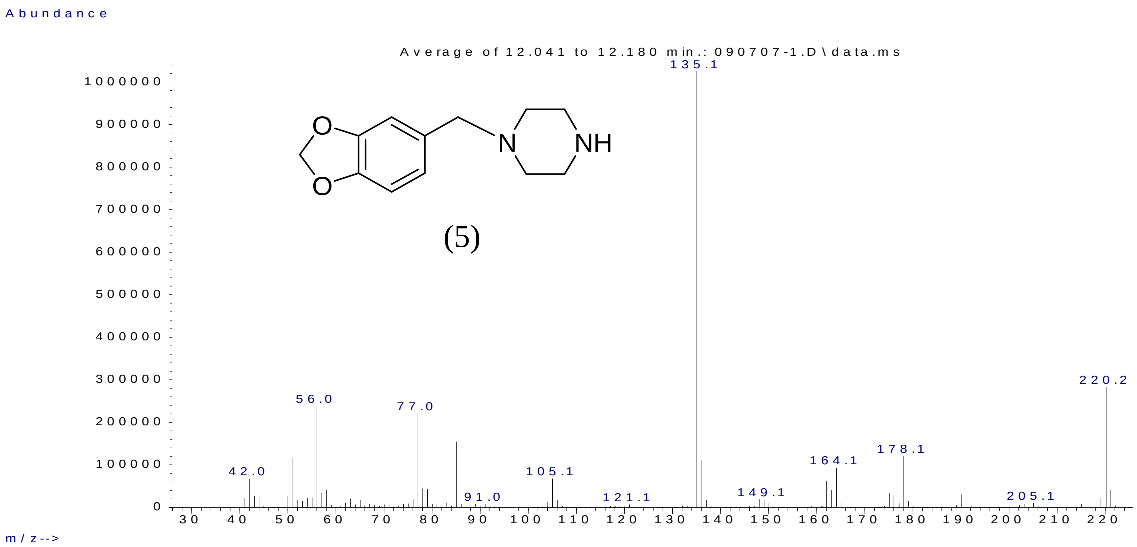
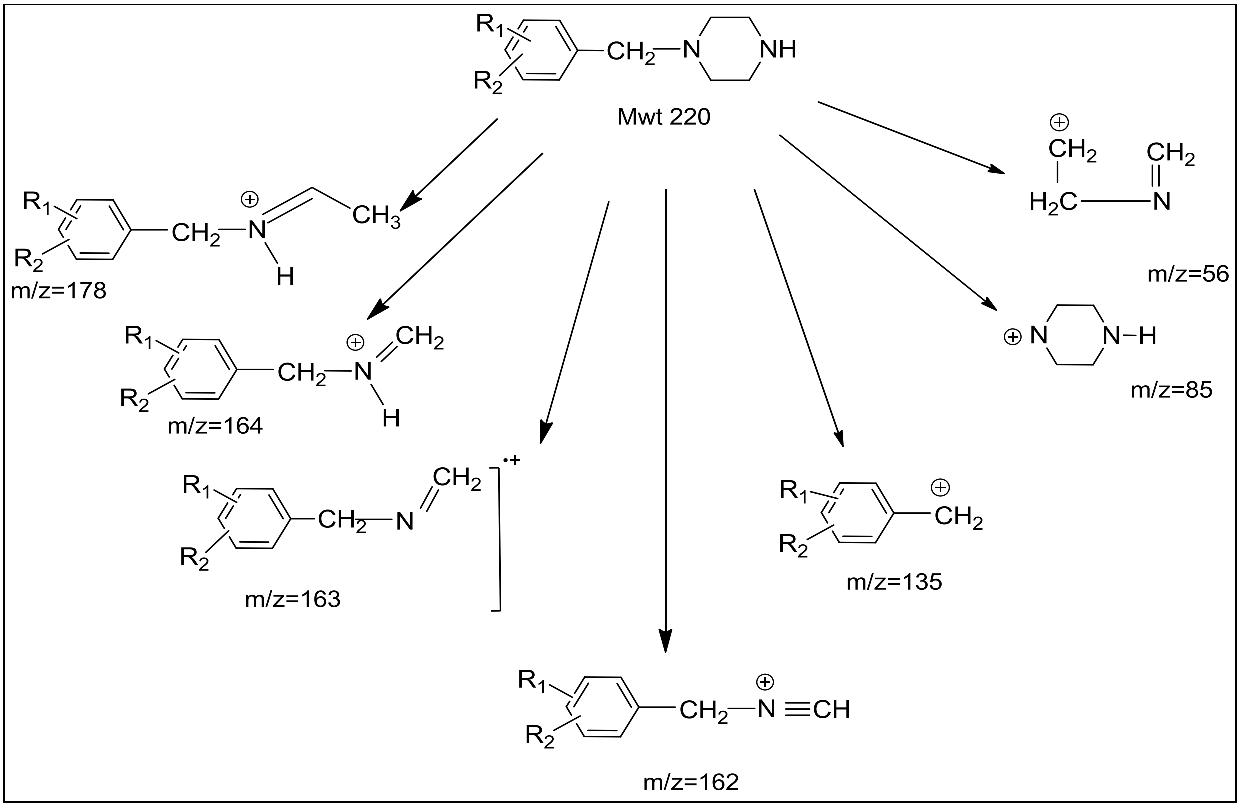


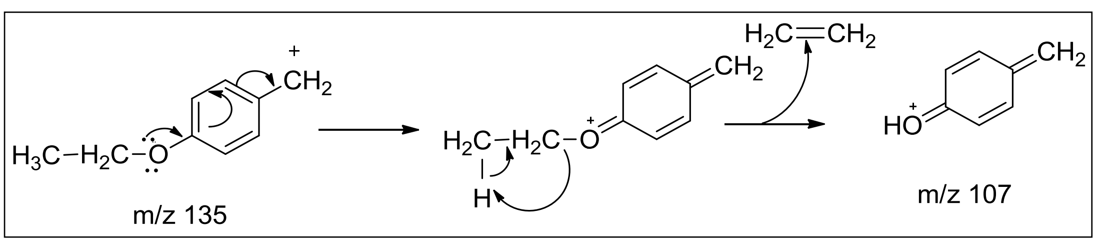
Fig. 8. EI mass spectra of the methylenedioxy and ethoxybenzylpiperazines in this study.



$R_1 = OCH_2CH_3$, R_2 = H in case of EBP

R_1, R_2 = dioxymethylene in case of MDBP

Scheme 19. Mass spectral fragmentation pattern of the underivatized methylenedioxy and ethoxybenzylpiperazines under EI of 70eV.



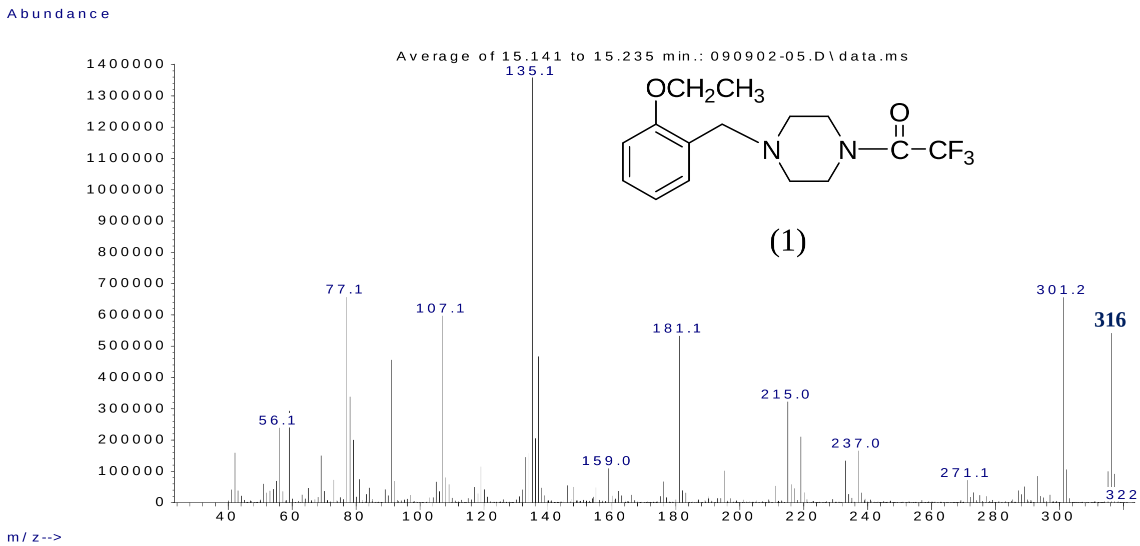
Scheme 20. Mass spectral fragmentation of the ethoxybenzylpiperazines yielding the fragment cation at m/z 107.

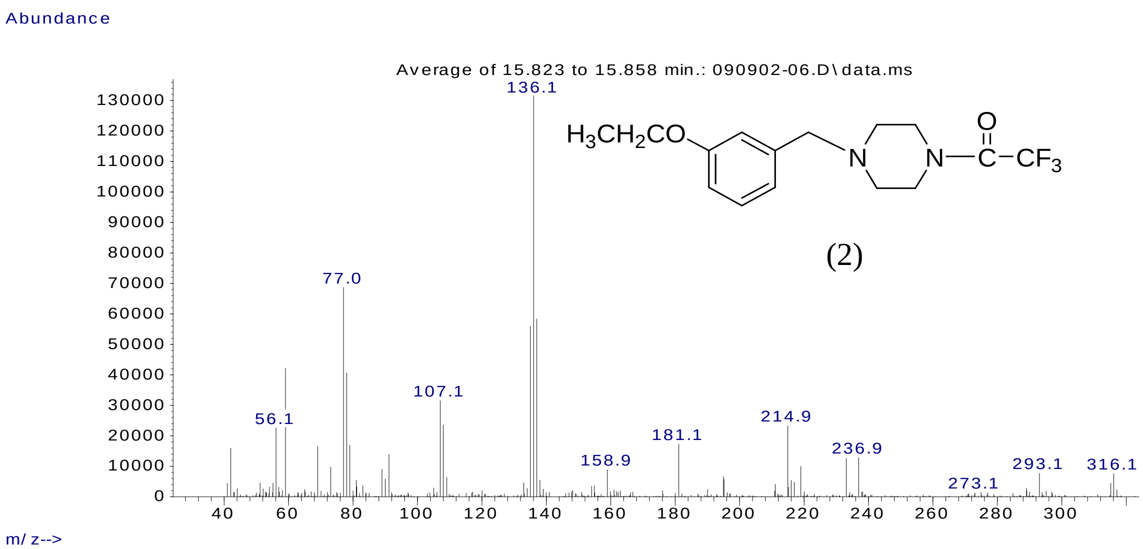
of the ethoxybenzylpiperazines from their isobaric methylenedioxy compounds.

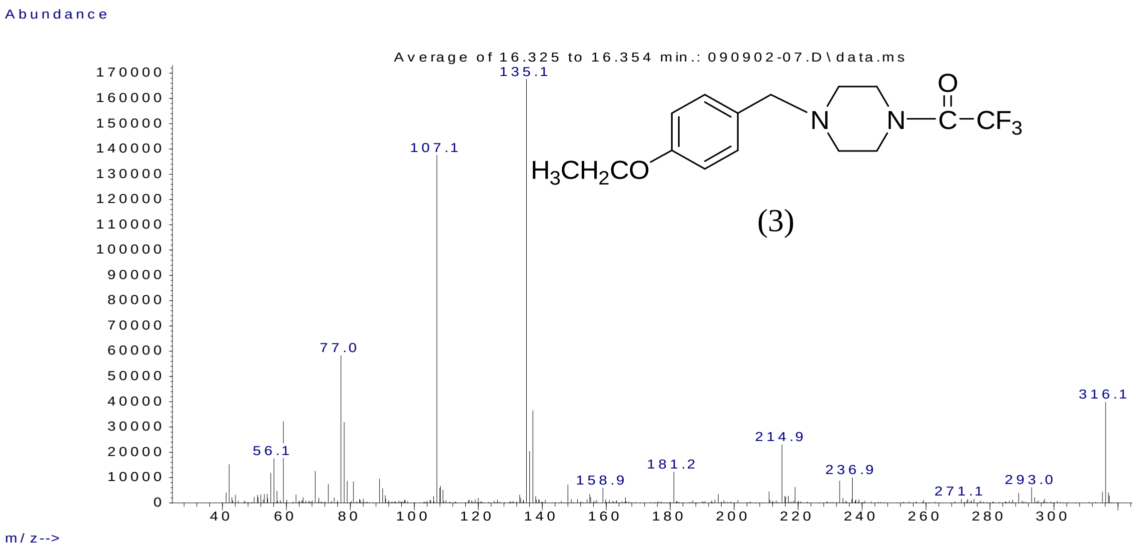
The second phase of this study involved the preparation and evaluation of perfluoroacyl derivatives of the isomeric benzylpiperazines, in an effort to individualize their mass spectra and identify additional unique marker ions for these five compounds.

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 9 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 $(M\text{-}135)^+$ ion for each amide. The ion at *m/z* 219 was observed in the spectra of all derivatives and is likely formed by the elimination of the acyl moiety. Those ions occurring at *m/z* 69, 119 and 169 are the perfluoroalkyl cations trifluoromethyl, pentafluoroethyl or heptafluoropropyl from the appropriate amides. 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 $[M\text{-}15]^+$ 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.

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

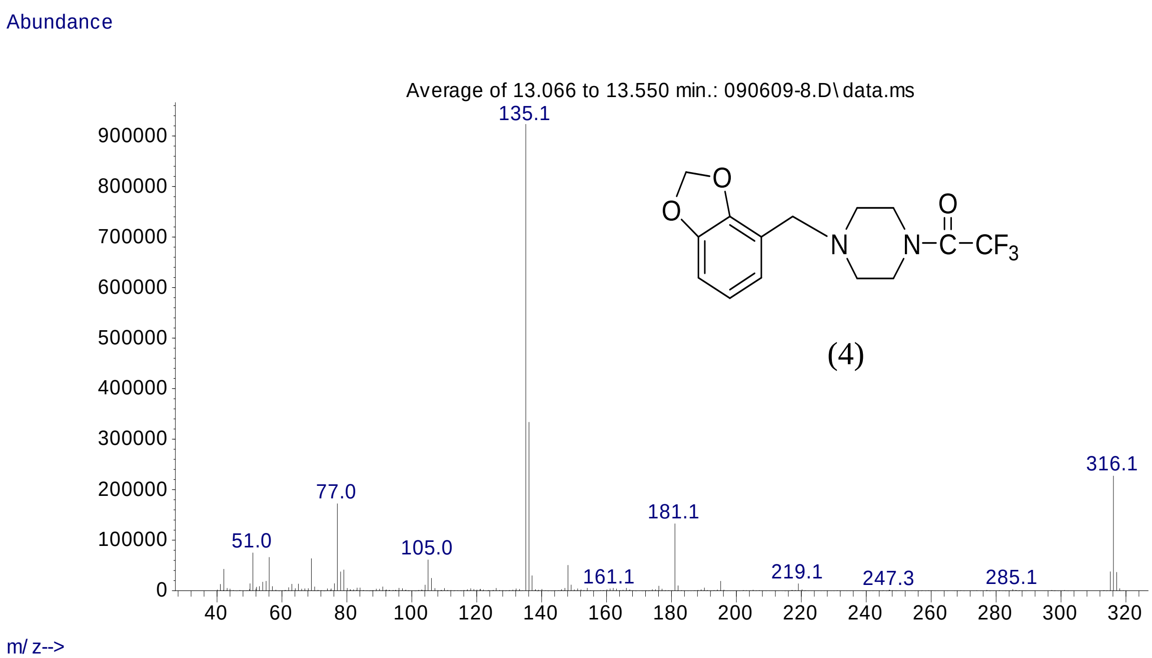






Abundance
Average of 16.325 to 16.354 min.: 090902-07.D\data.ms
135.1
107.1
77.0
56.1
158.9
181.2
214.9
236.9
271.1
293.0
316.1
m/z-->
H_3CH_2CO
N
N
O
C
CF_3

(3)



Abundance
Average of 13.066 to 13.550 min.: 090609-8.D\data.ms
135.1
77.0
51.0
105.0
161.1
181.1
219.1
247.3
285.1
316.1
m/z-->
O
O
N
N
O
C
CF_3

(4)

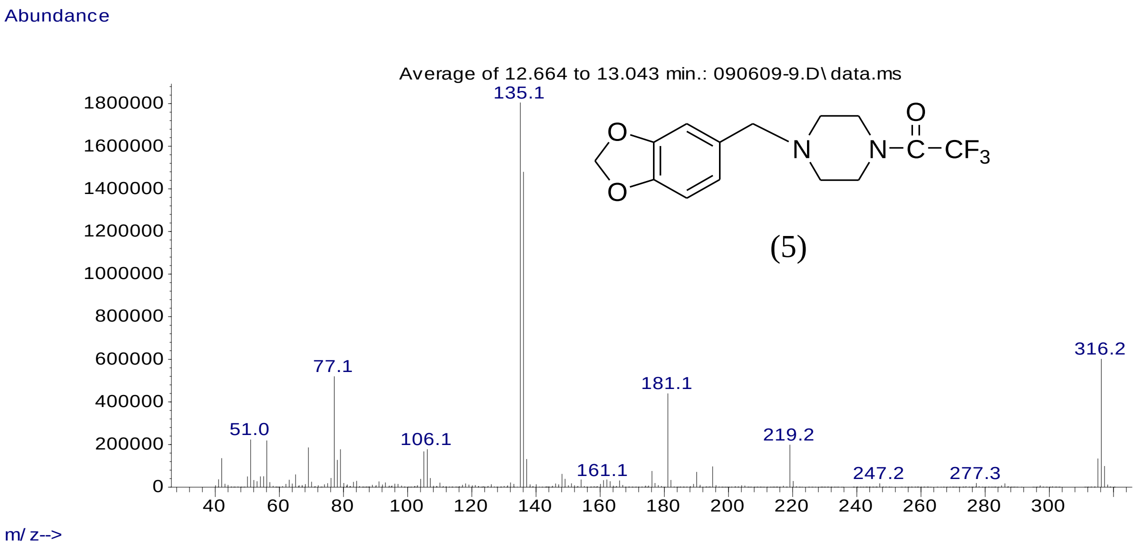


Fig. 9. Mass spectra of the trifluoroacetyl derivatives of the five piperazine compounds in this study.

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 10 shows the GC-TOF-MS exact mass analysis of the 3,4-methylenedioxybenzyl cation (m/z=135) for compound 5. The upper panel (10A) shows the expected/calculated mass for the $C_8H_7O_2$ elemental composition. The lower panel (10B) 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 11A and 11B 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 11 confirm the elemental composition C_7H_7O for the unique rearrangement ion at m/z 107 seen in the ethoxybenzylpiperazine compounds.

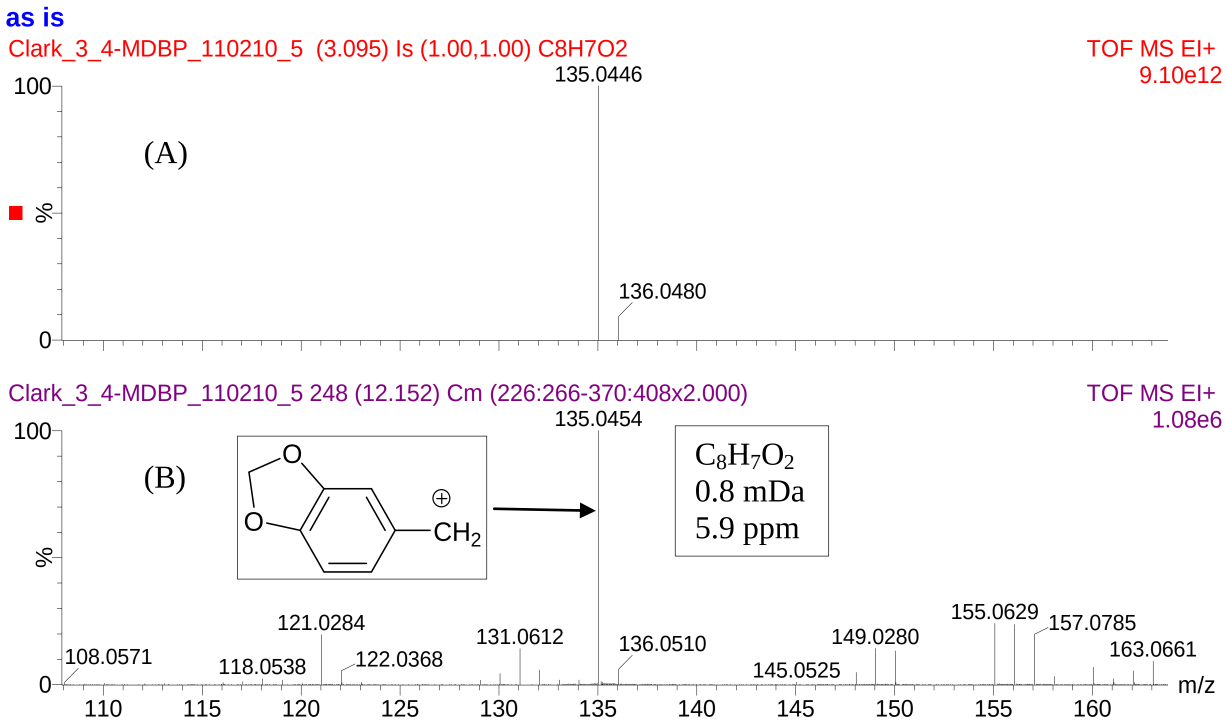


Fig. 10. GC-TOF mass spectral analysis of the m/z 135 ion for 3,4-methylenedioxybenzylpiperazine. 10A= calculated mass for $C_8H_7O_2$; 10B= experimental results.

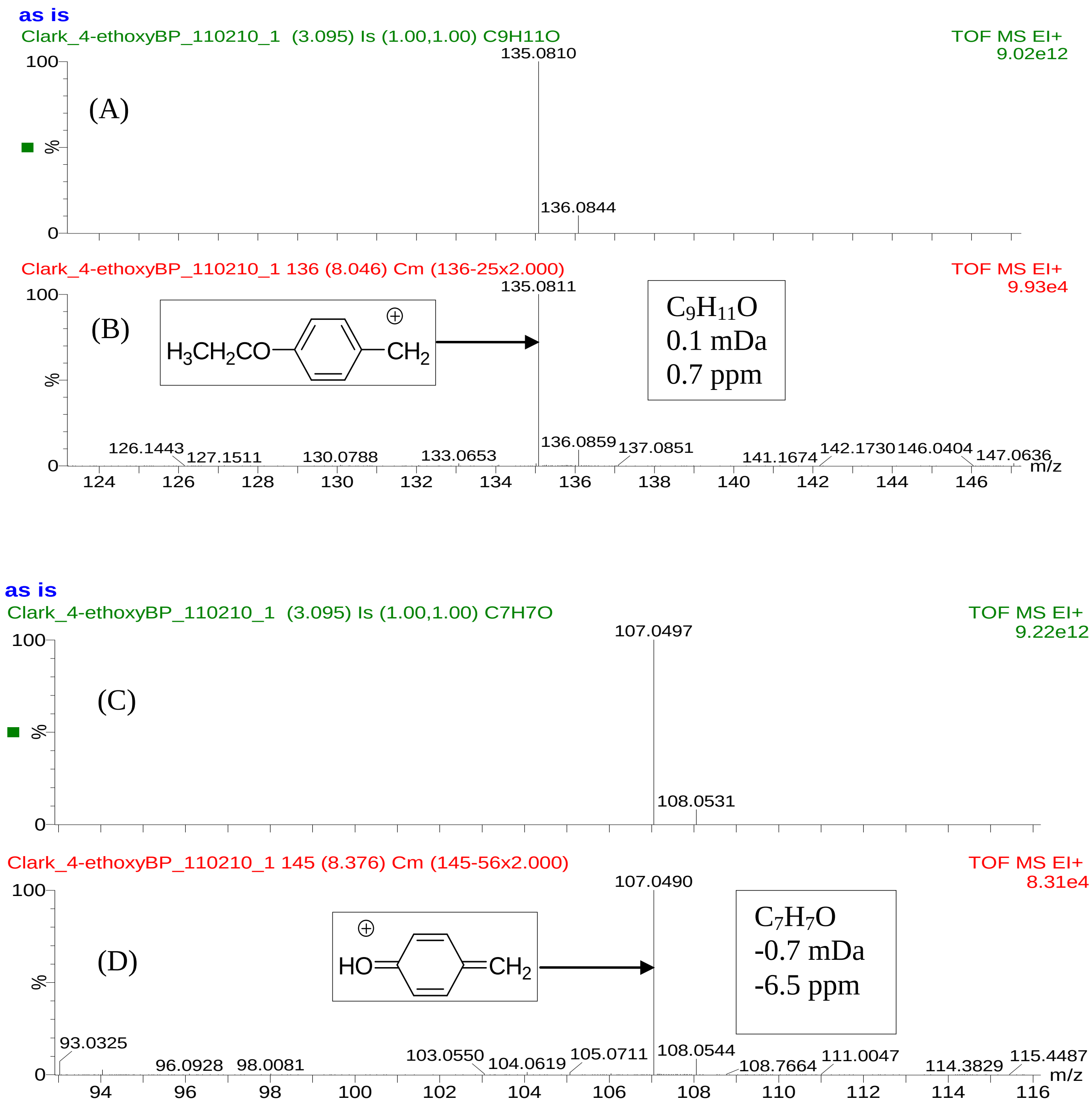
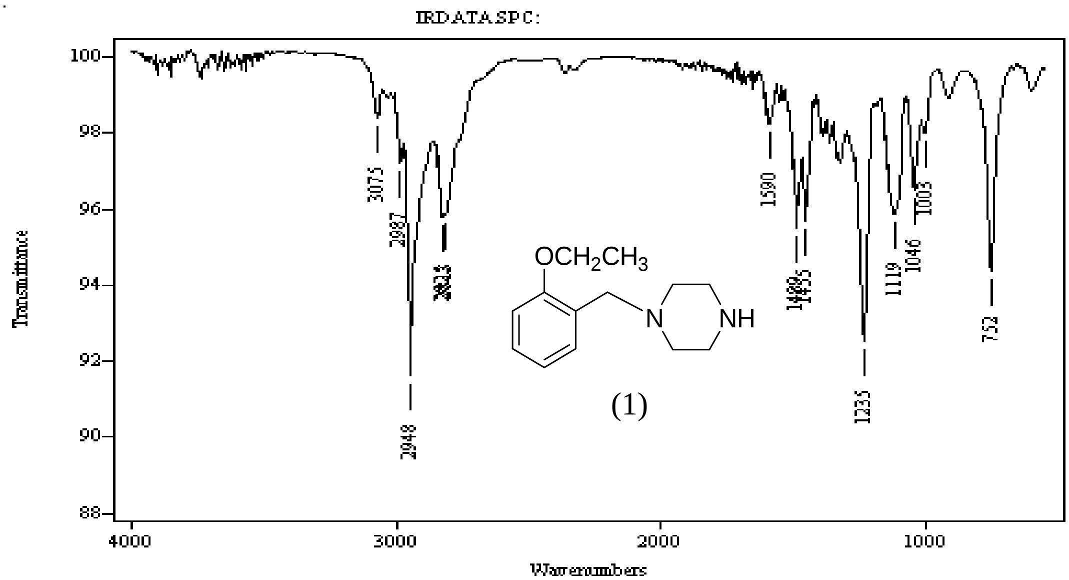


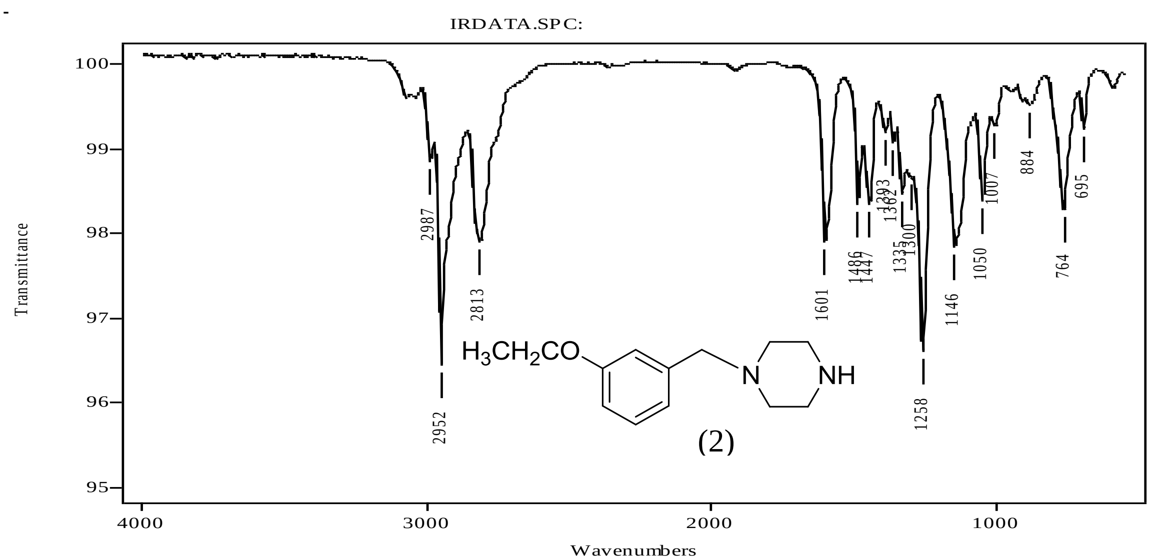
Fig. 11: GC-TOF mass spectral analysis of the m/z 135 and m/z 107 ions for 4-ethoxybenzylpiperazine. 11A= calculated mass for $C_9H_{11}O$; 11B= experimental results. 11C= calculated mass for C_7H_7O; 11D= experimental results.

3.2.2. Vapor-phase Infra-Red Spectrophotometry

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 12. 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 section 3.1.2 (p.g. 53).


IRDATA.SPC:
Transmittance
Wavenumbers
OCH2CH3
N
NH
(1)
3075
2987
2948
2823
1590
1480
1455
1235
1119
1046
1003
752


IRDATA.SPC:
Transmittance
Wavenumbers
H3CH2CO
N
NH
(2)
2987
2952
2813
1601
1486
1447
1393
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1335
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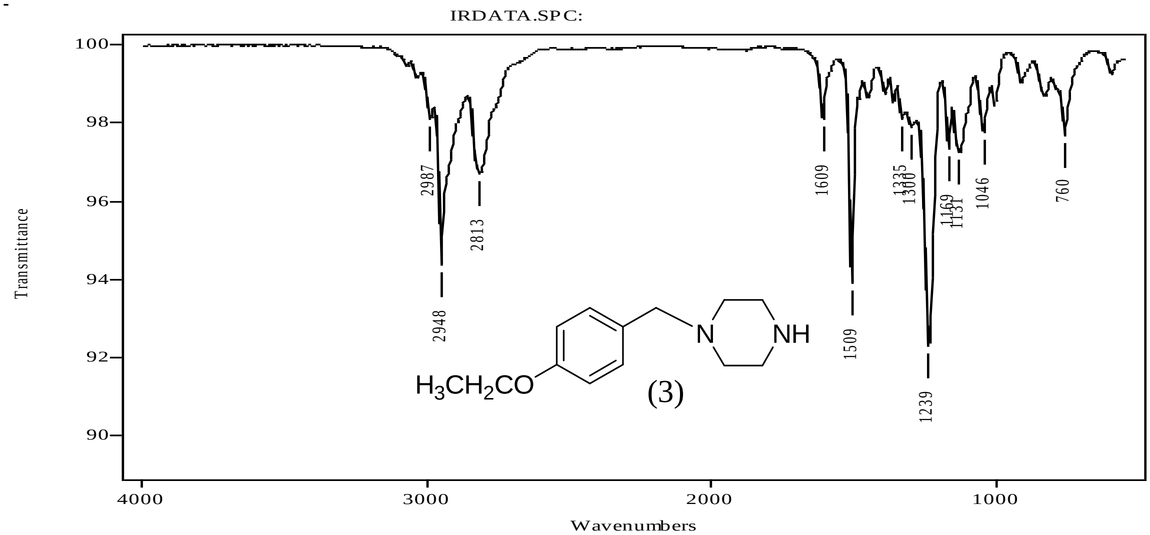
1050
1007
884
764
695


IRDATA.SPC:
Transmittance
Wavenumbers
100
98
96
94
92
90
4000
3000
2000
1000
2987
2948
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1609
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1335
1300
1239
1169
1131
1046
760
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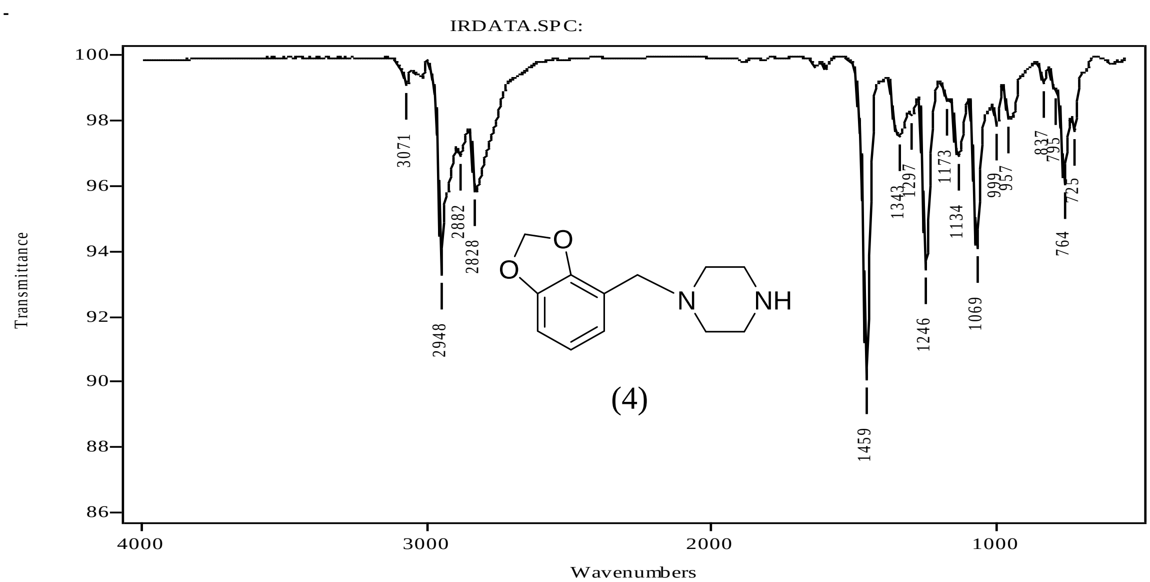
N
NH
(3)


IRDATA.SPC:
Transmittance
Wavenumbers
100
98
96
94
92
90
88
86
4000
3000
2000
1000
3071
2948
2882
2828
1459
1343
1297
1246
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1134
1069
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837
795
764
725
O
O
N
NH
(4)

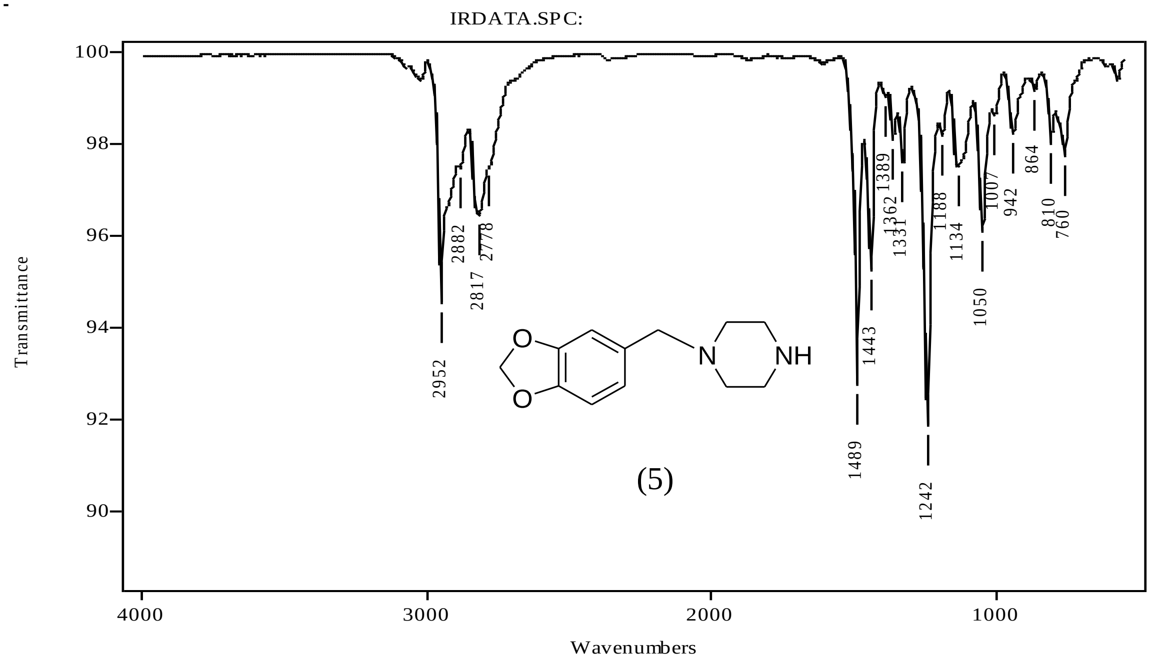


Fig. 12: Vapor phase IR spectra of the five methylenedioxy and ethoxybenzylpiperazines.

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.

3.2.3. Gas Chromatographic Separation

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 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.

Several temperature programs were evaluated and the chromatogram in Figure 13 is a representative of the results obtained for all samples on this stationary phase. In Figure 13 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.

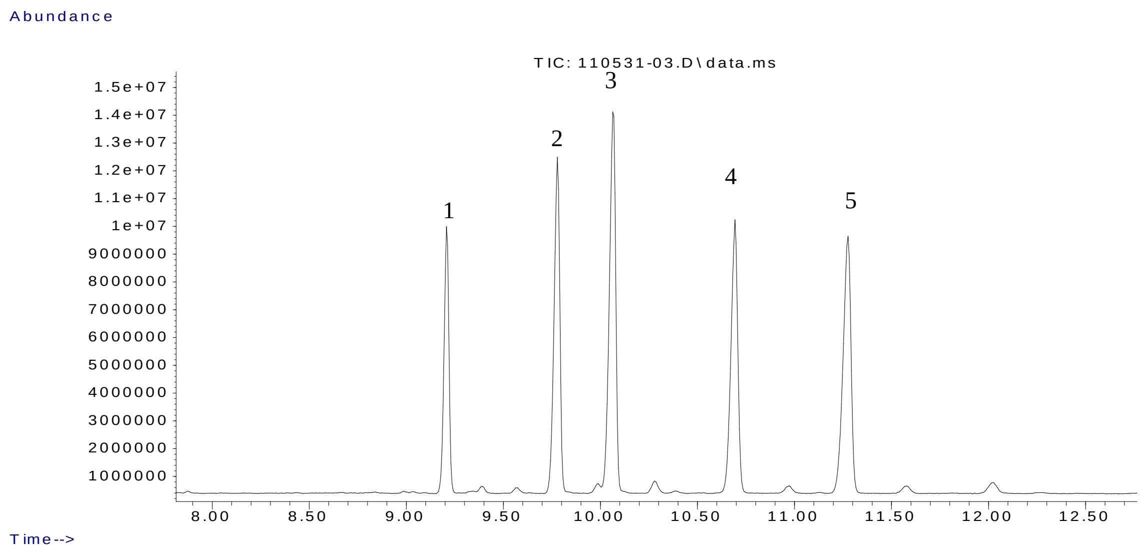


Fig. 13. Gas chromatographic separation of the heptafluorobutyryl derivatives of the five piperazine isomers using Rxi-50 column. The number over the peak corresponds to the compound number.

3.2.4. 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.

3.3. 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.

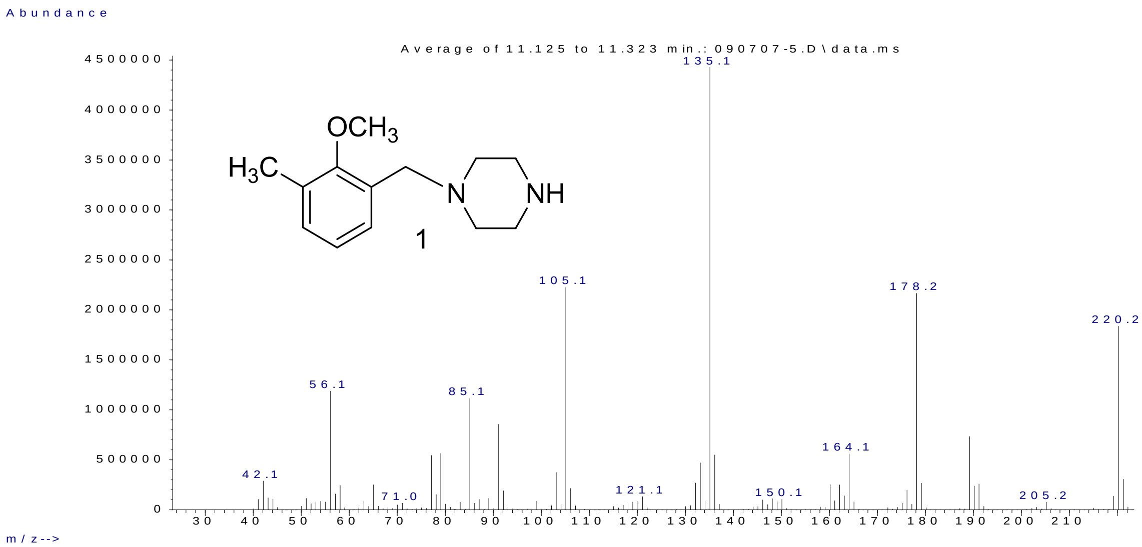
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the six isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized 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.

3.3.1. Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 14 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 Scheme 21 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 Scheme 22. Those methoxymethylbenzylpiperazines with the methoxy group in the ortho position relative to the side chain are characterized by a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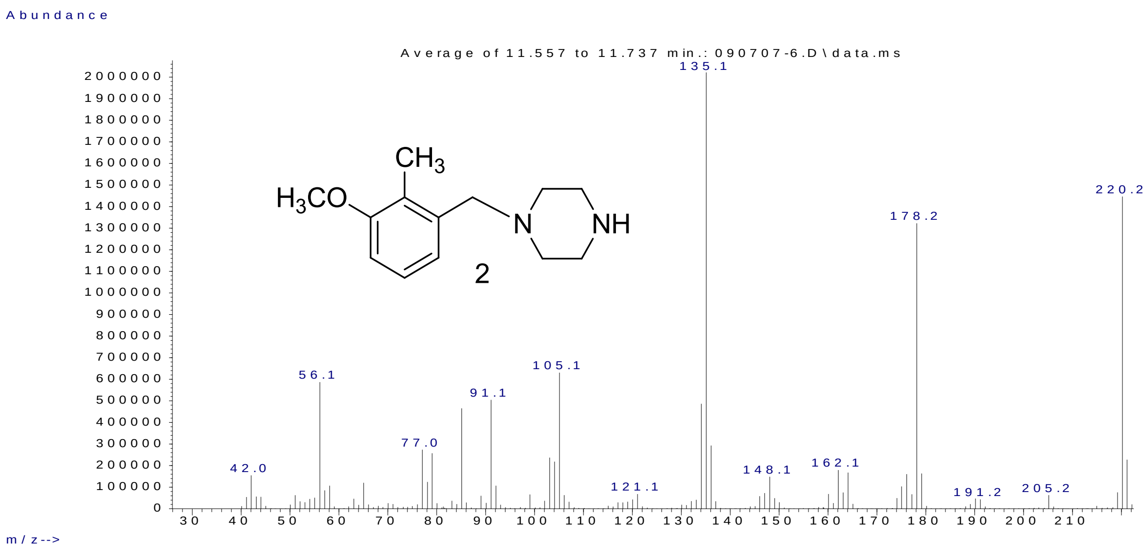
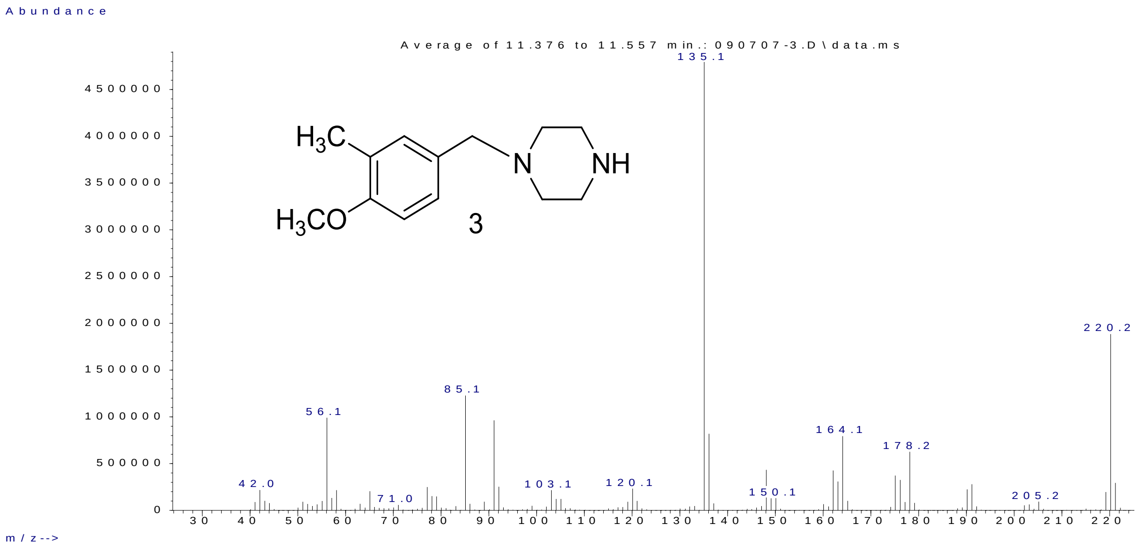
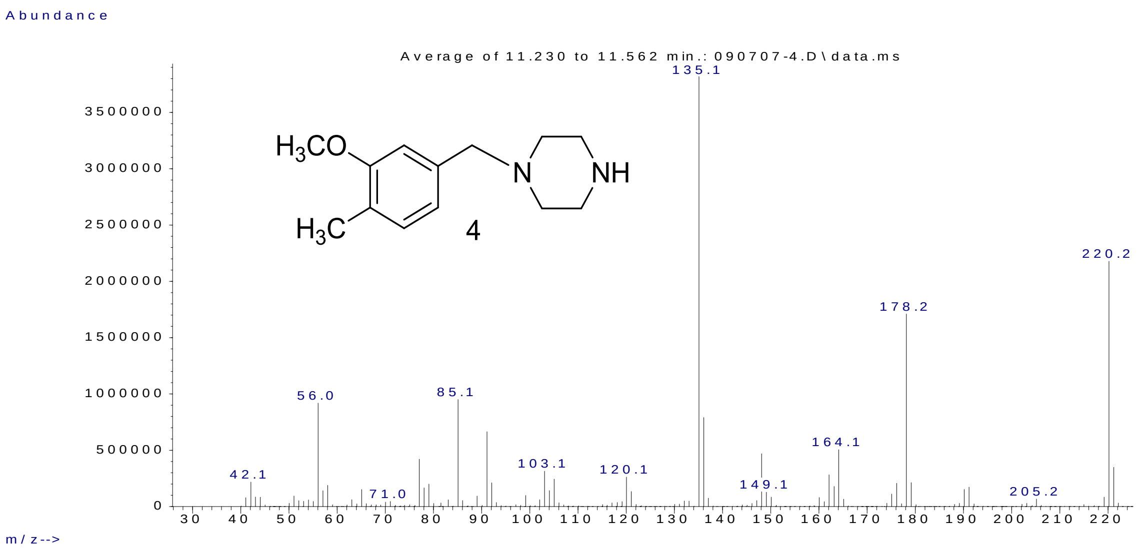
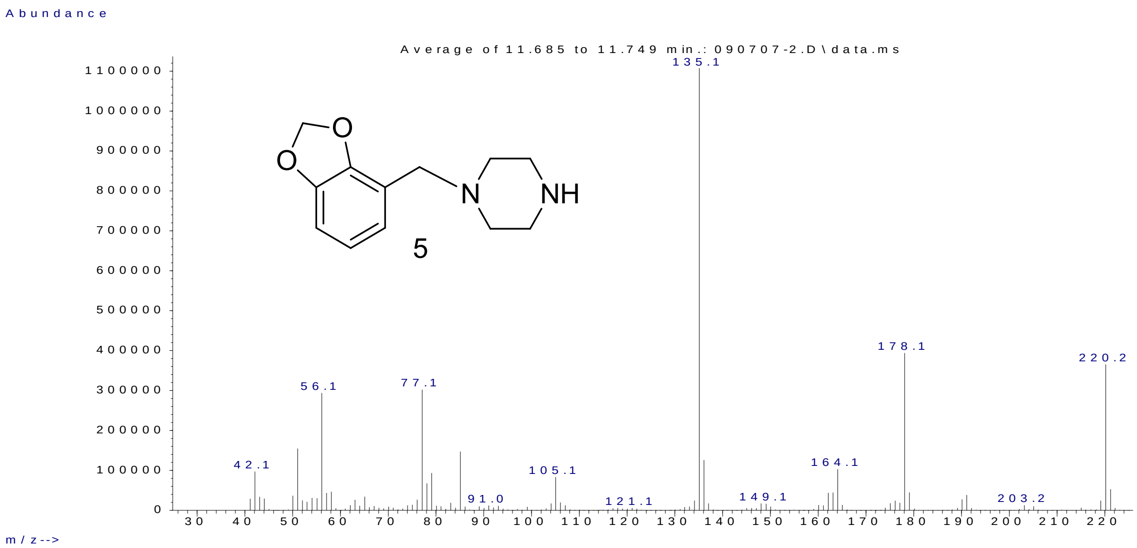
OCH3
H3C
N
NH
1
135.1
105.1
178.2
220.2
56.1
85.1
164.1
42.1
71.0
121.1
150.1
205.2
m/z-->


Abundance
Average of 11.557 to 11.737 min.: 090707-6.D\data.ms
CH3
H3CO
N
NH
2
135.1
220.2
178.2
56.1
105.1
91.1
77.0
42.0
148.1
162.1
121.1
191.2
205.2
m/z-->


Abundance
Average of 11.376 to 11.557 min.: 090707-3.D\data.ms
H_3C
H_3CO
N
NH
3
135.1
220.2
85.1
56.1
164.1
178.2
42.0
103.1
120.1
71.0
150.1
205.2
m/z-->


Abundance
Average of 11.230 to 11.562 min.: 090707-4.D\data.ms
H_3CO
H_3C
N
NH
4
135.1
220.2
178.2
56.0
85.1
164.1
42.1
103.1
120.1
149.1
71.0
205.2
m/z-->



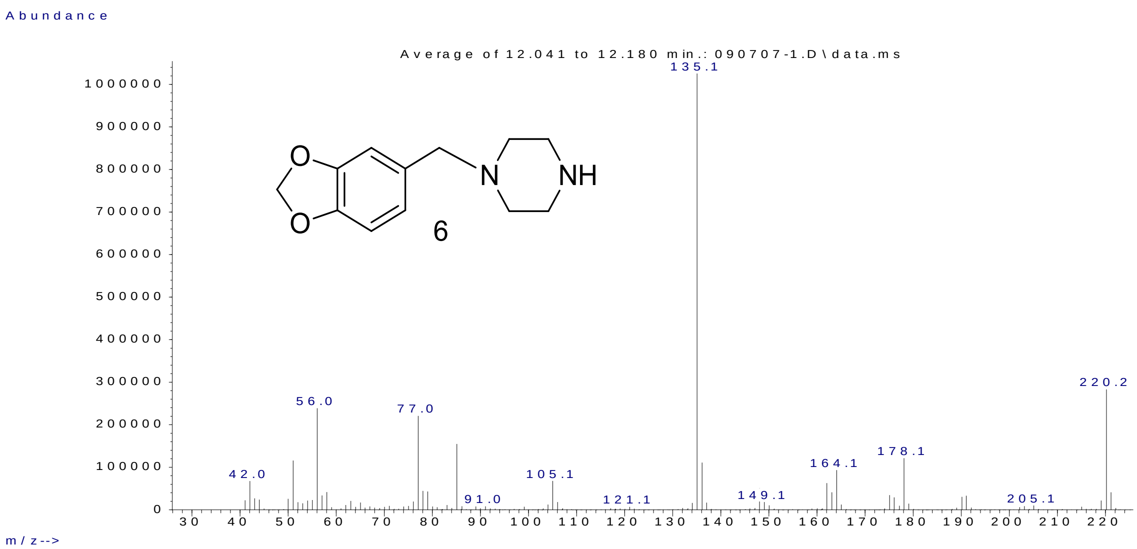
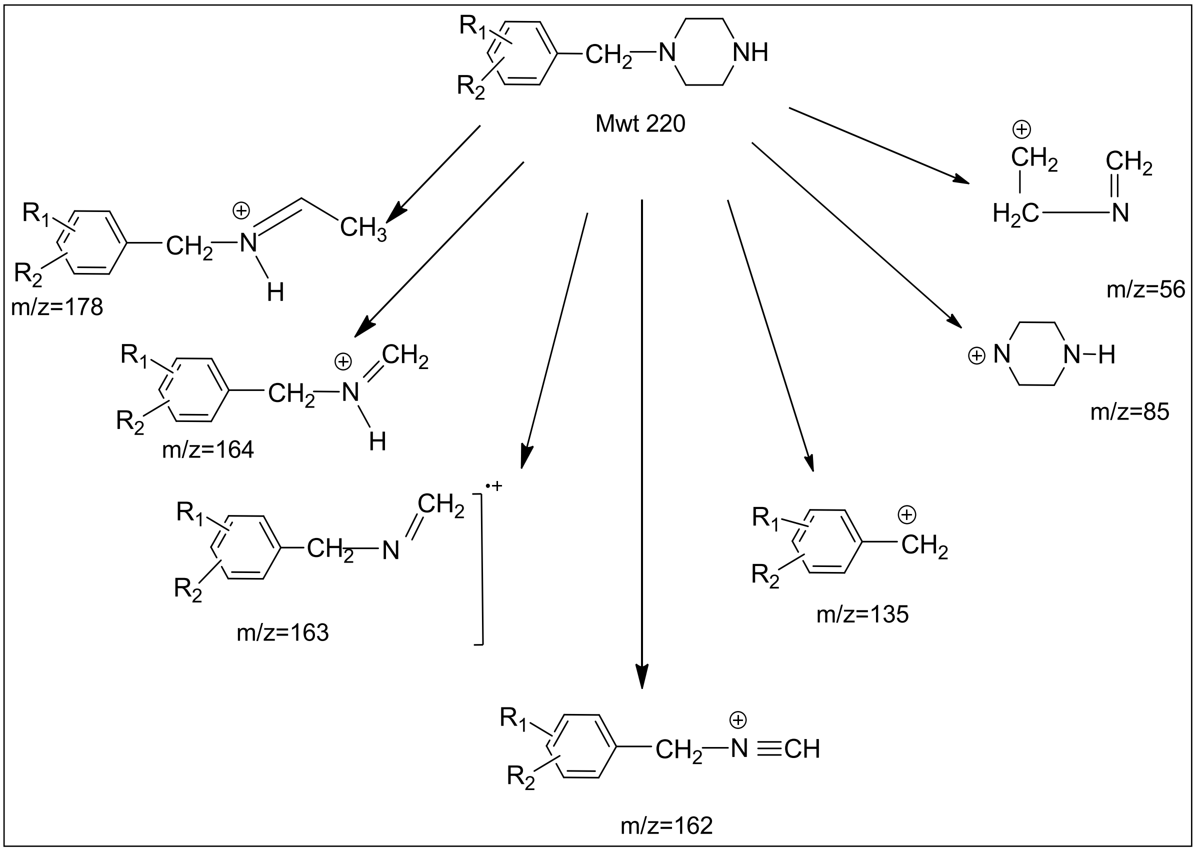
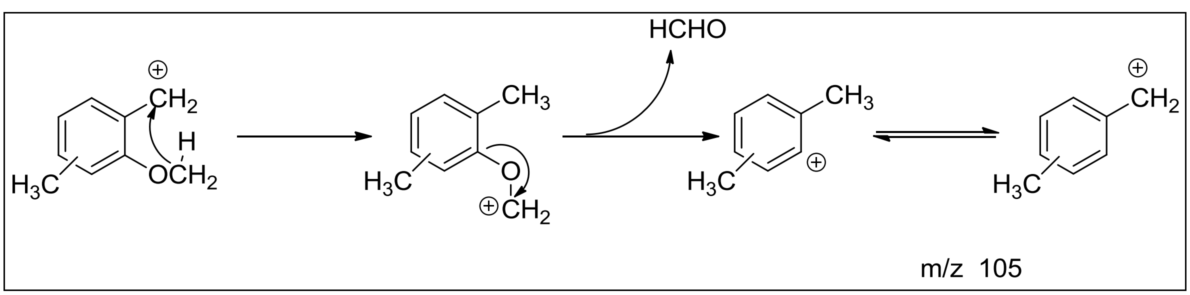


Fig. 14. EI mass spectra of the six methylenedioxy and methoxymethylbenzylpiperazines.



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

Scheme 21. EI mass spectral fragmentation pattern of the methylenedioxy and methoxymethylbenzylpiperazines.

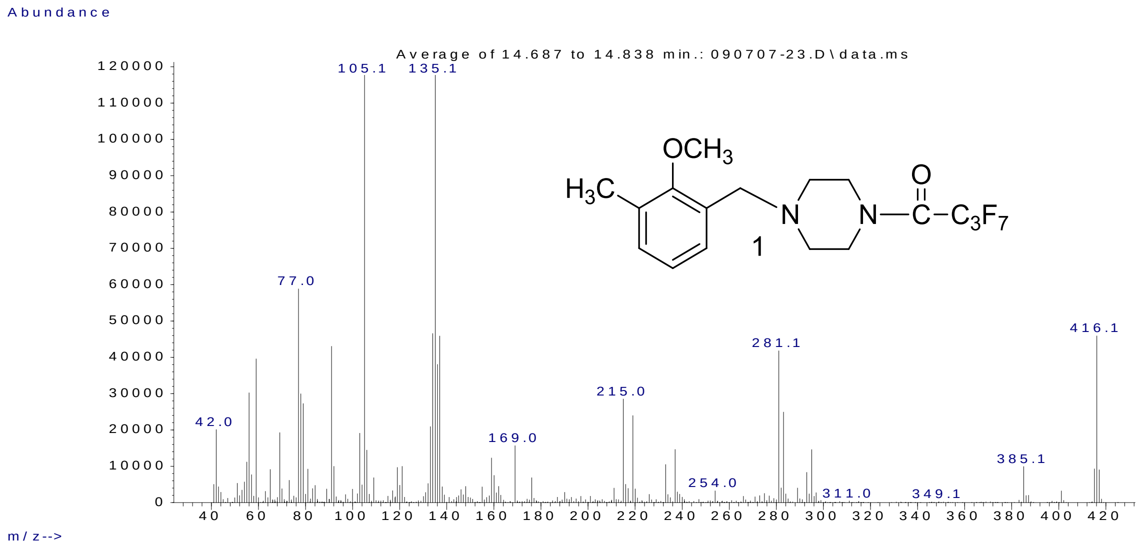


Scheme 22. Mechanism for the formation of the m/z 105 ion in the mass spectra of the underivatized and derivatized 2-methoxy regioisomers of the methoxymethylbenzylpiperazines.

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 Scheme 22. 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 and Maher *et al*, 2009].

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 15 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 substituted benzyl cation. Furthermore, an additional fragment ion series occurring at *m/z* 181, 231 and 281 for the TFA, PFPA and HFBA amides.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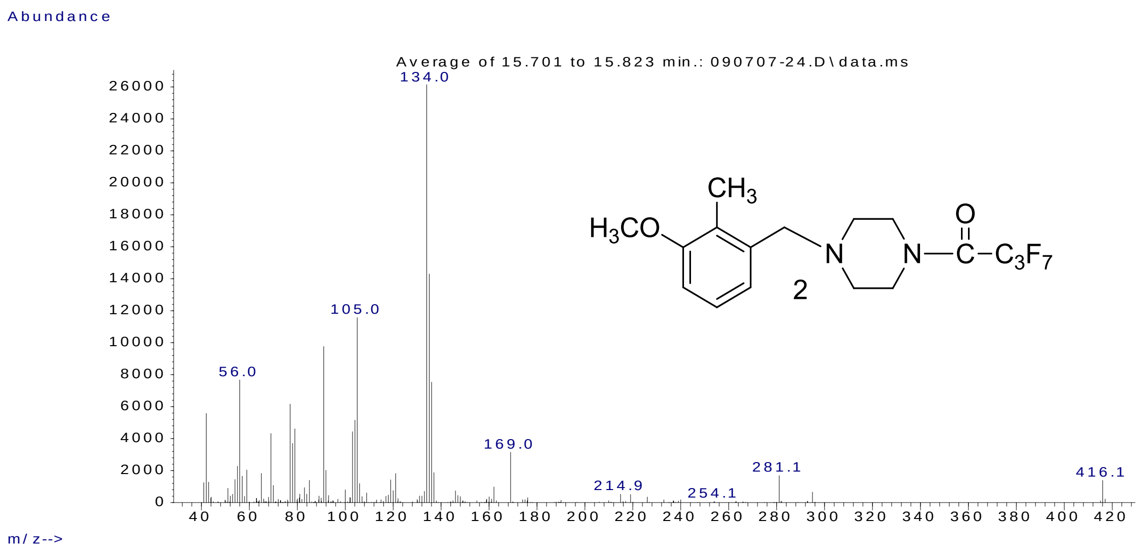
105.1
135.1
77.0
42.0
169.0
215.0
254.0
281.1
311.0
349.1
385.1
416.1
m/z-->
1


Abundance
Average of 15.701 to 15.823 min.: 090707-24.D\data.ms
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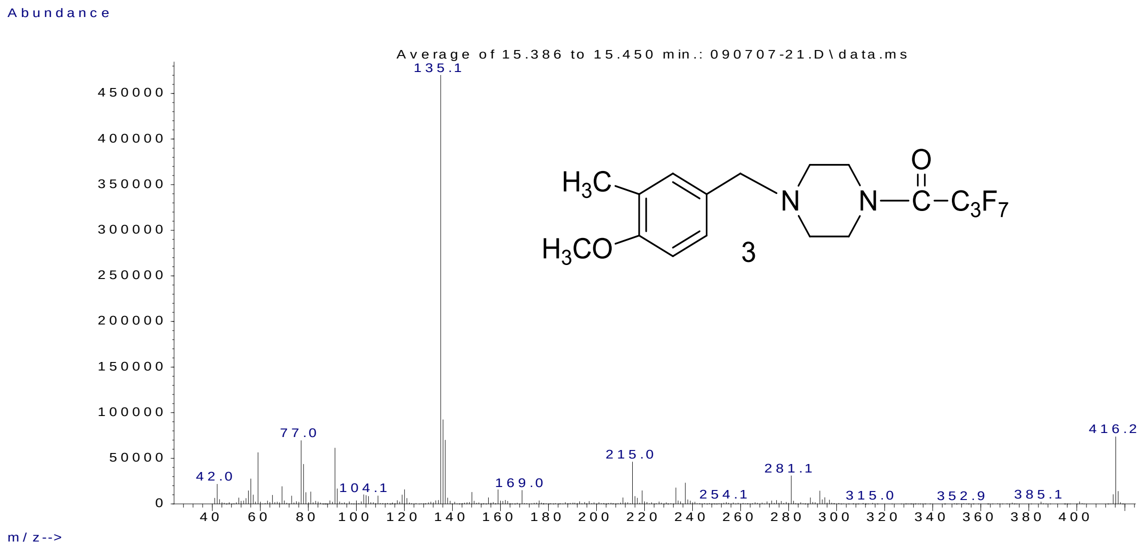
105.0
56.0
169.0
214.9
254.1
281.1
416.1
m/z-->
2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77.0
42.0
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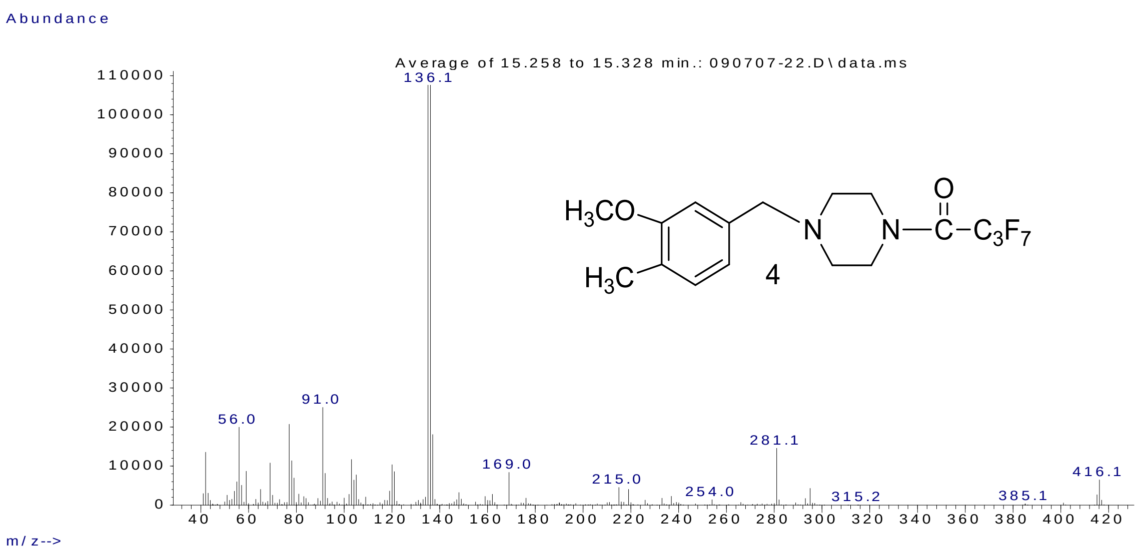
169.0
215.0
254.1
281.1
315.0
352.9
385.1
416.2
3
m/z-->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169.0
215.0
254.0
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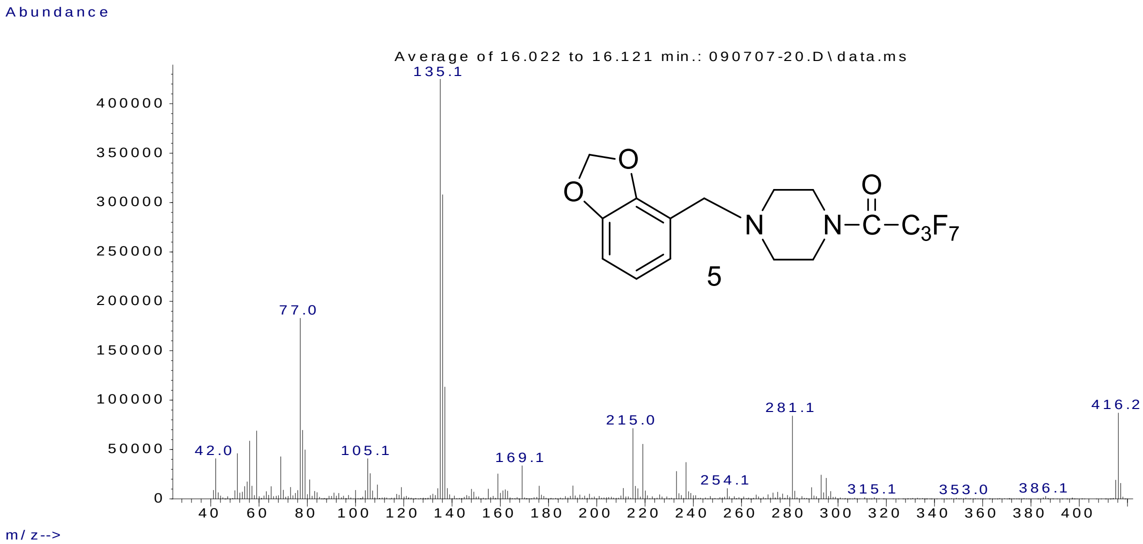
416.1
4
m/z-->



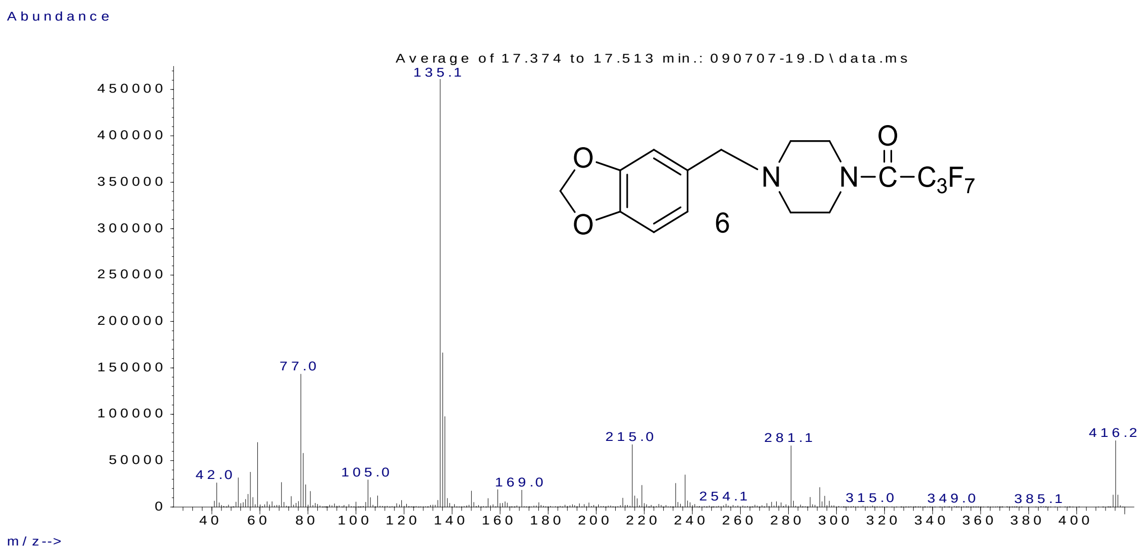


Fig. 15. EI mass spectra of the heptafluorobutyrylamides for the six substituted benzylpiperazines in this study.

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 16 shows the GC-TOF-MS exact mass analysis of the 3,4-methylenedioxybenzyl cation (m/z=135) for compound 6. The upper panel (16A) shows the expected/ calculated mass for the $C_8H_7O_2$ elemental composition. The lower panel (16B) 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 17A and 17B 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.

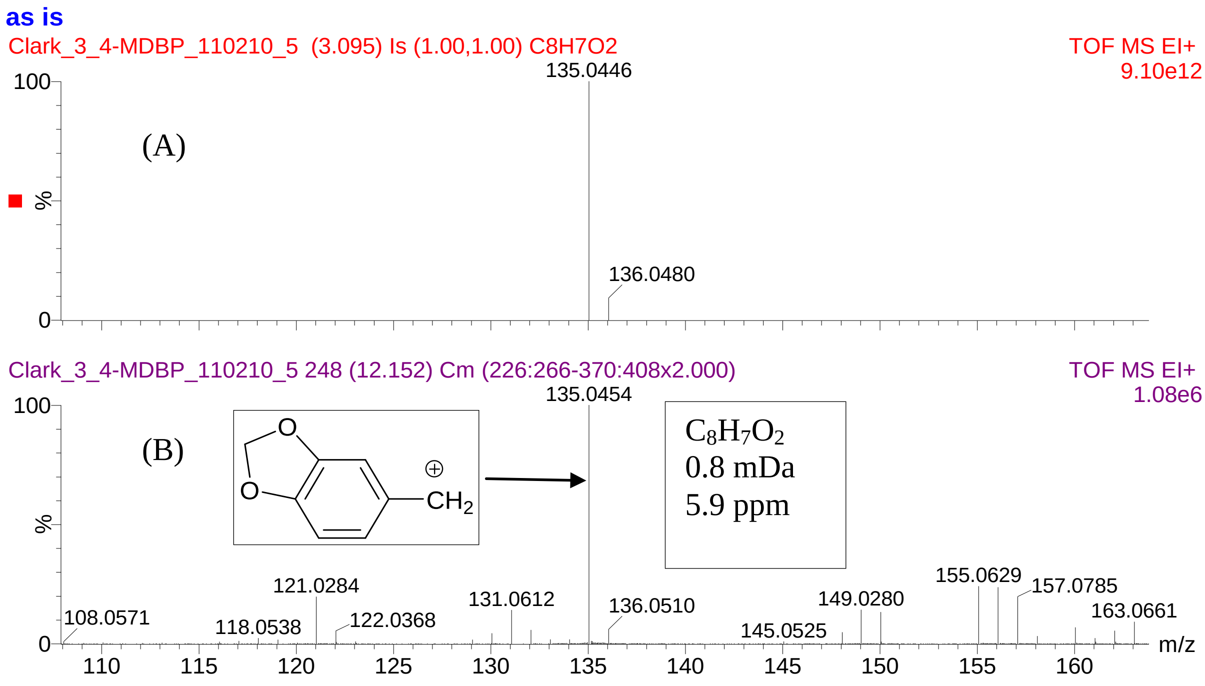


Fig. 16. GC-TOF mass spectral analysis of the m/z 135 ion for 3,4-methylenedioxybenzylpiperazine. 16A= calculated mass for $C_8H_7O_2$; 16B= experimental results.

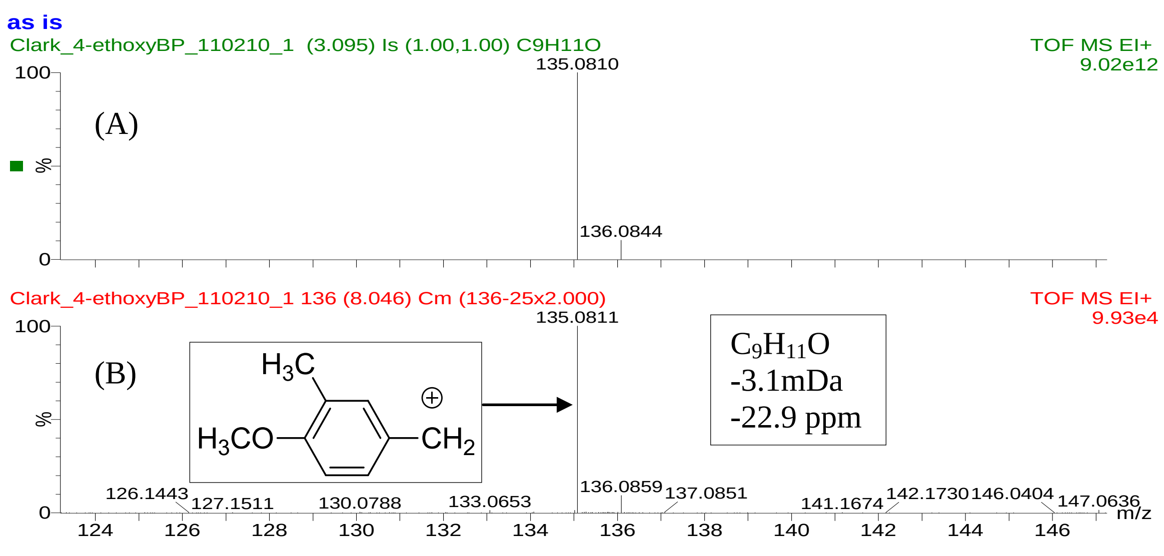


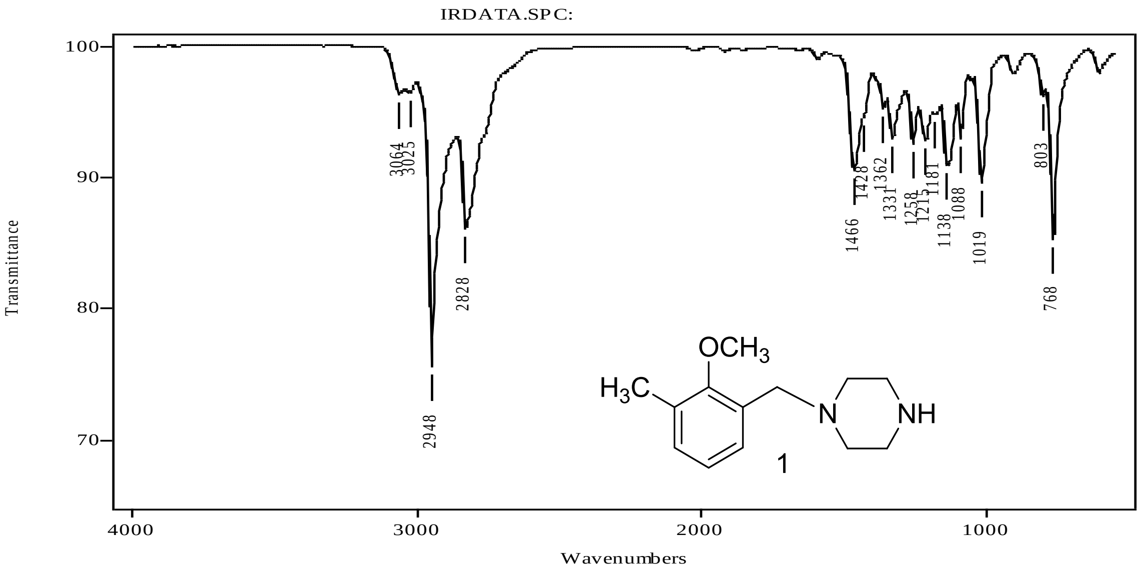
Fig. 17: GC-TOF mass spectral analysis of the m/z 135 ion for 4-methoxy3-methylbenzylpiperazine. 17A= calculated mass for $C_9H_{11}O$; 17B= experimental results.

3.3.2. Vapor-phase Infra-Red Spectroscopy

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 18. 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 section 3.1.2 (p.g. 53).

-



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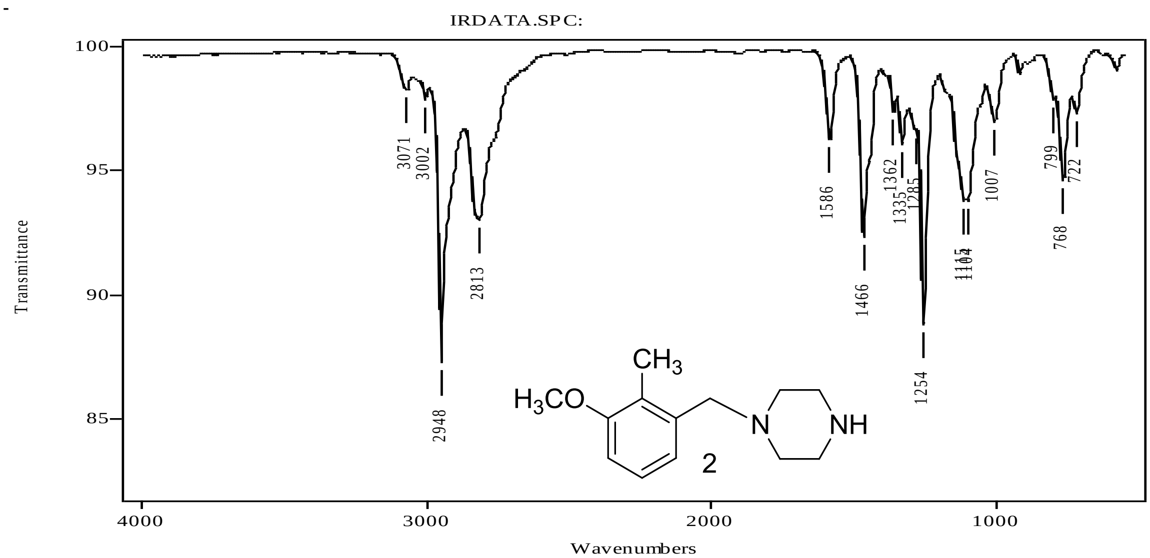
Transmittance
Wavenumbers
100
90
80
70
4000
3000
2000
1000
3064
3025
2948
2828
1466
1428
1362
1331
1258
1215
1181
1138
1088
1019
803
768
OCH3
H3C
N
NH
1

-



IRDATA.SPC:
Transmittance
Wavenumbers
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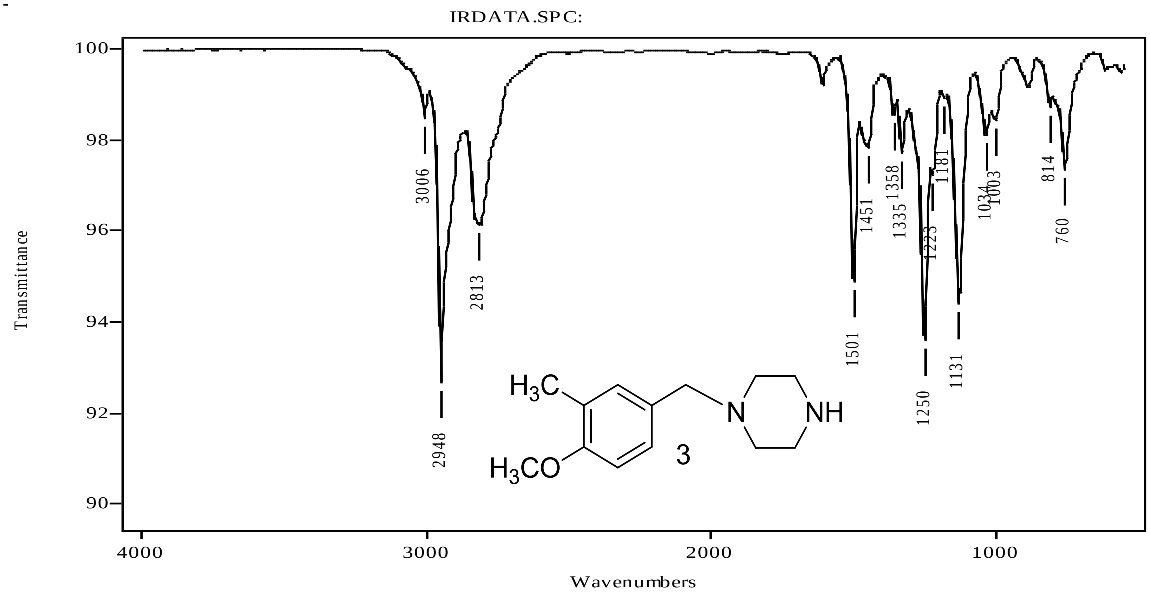
95
90
85
4000
3000
2000
1000
3071
3002
2948
2813
1586
1466
1362
1335
1285
1254
1115
1104
1007
799
768
722
CH3
H3CO
N
NH
2



IRDATA.SPC:
Transmittance
Wavenumbers
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98
96
94
92
90
4000
3000
2000
1000
3006
2948
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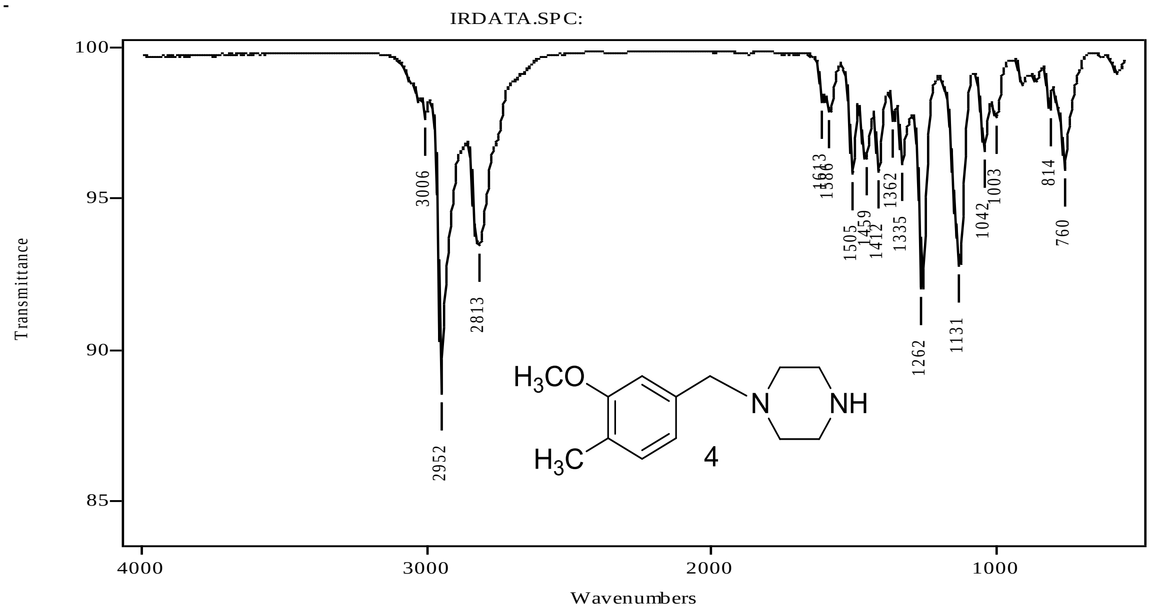
H_3CO
N
NH
3



IRDATA.SPC:
Transmittance
Wavenumbers
100
95
90
85
4000
3000
2000
1000
3006
2952
2813
1613
1586
1505
1459
1412
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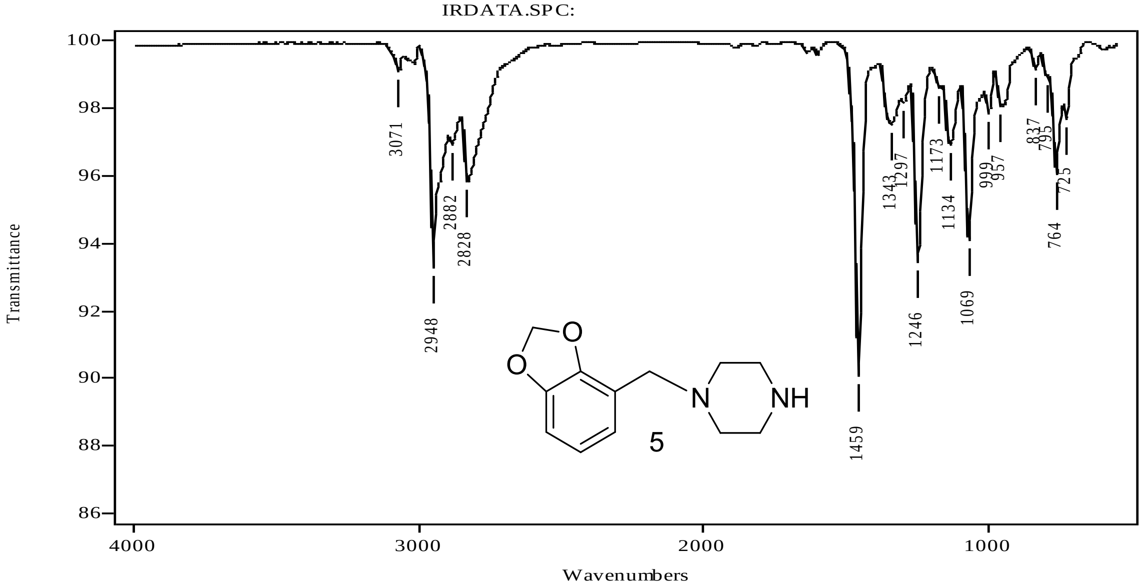
1335
1262
1131
1042
1003
814
760
H_3CO
H_3C
N
NH
4

-



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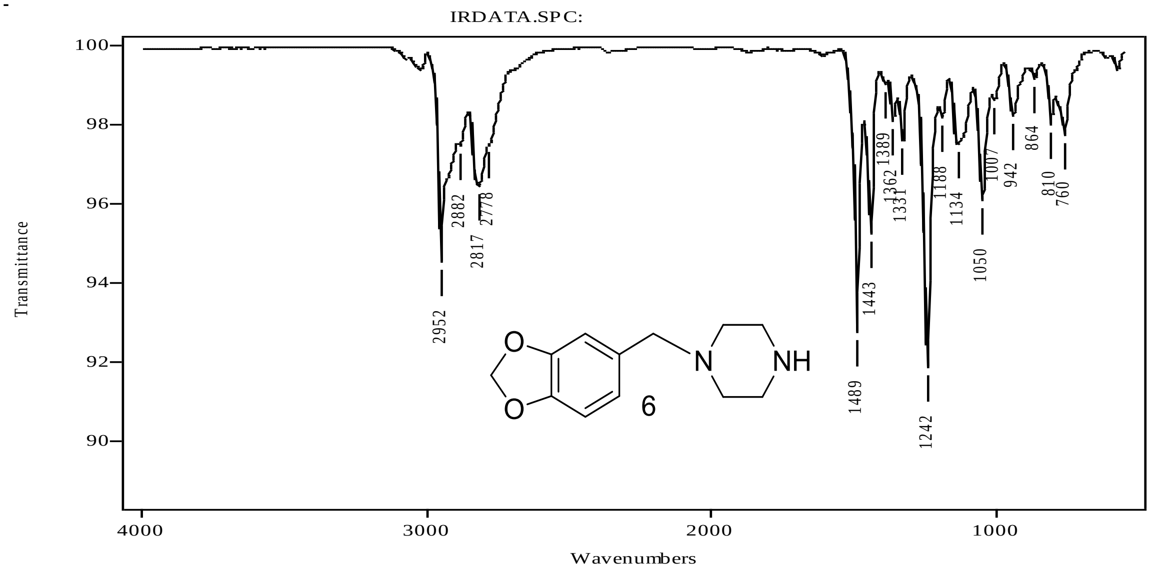


Fig. 18: Vapor phase IR spectra of the six underivatized methylenedioxy and methoxymethylbenzylpiperazines.

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 19 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.

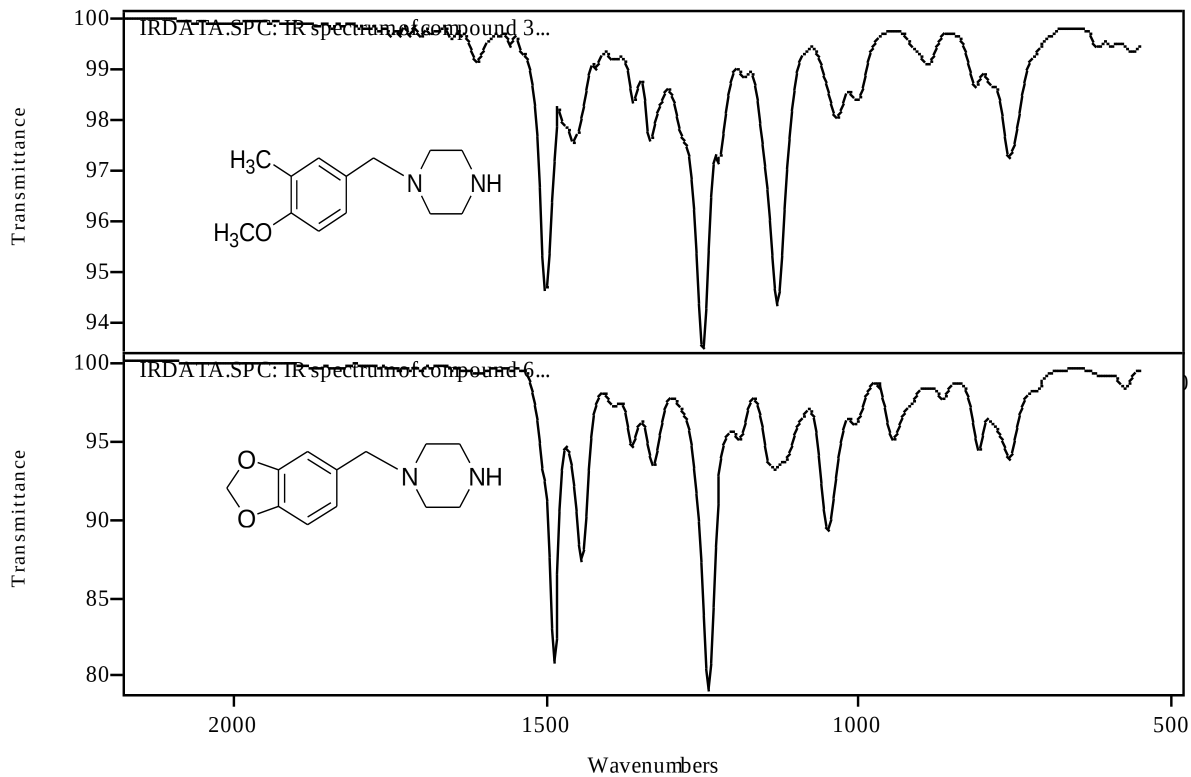


Fig. 19: Vapor phase IR spectra of compounds 3 and 6 in the region between 650 – 1800 cm^{-1}.

3.3.3. Gas Chromatographic Separation

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^{o}C for 1 minute, ramped up to 180^{o}C at a rate of 12^{o}C per minute followed by a hold at 180^{o}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 20 is a representative of the results obtained for all samples on this stationary phase.

In Figure 20 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.

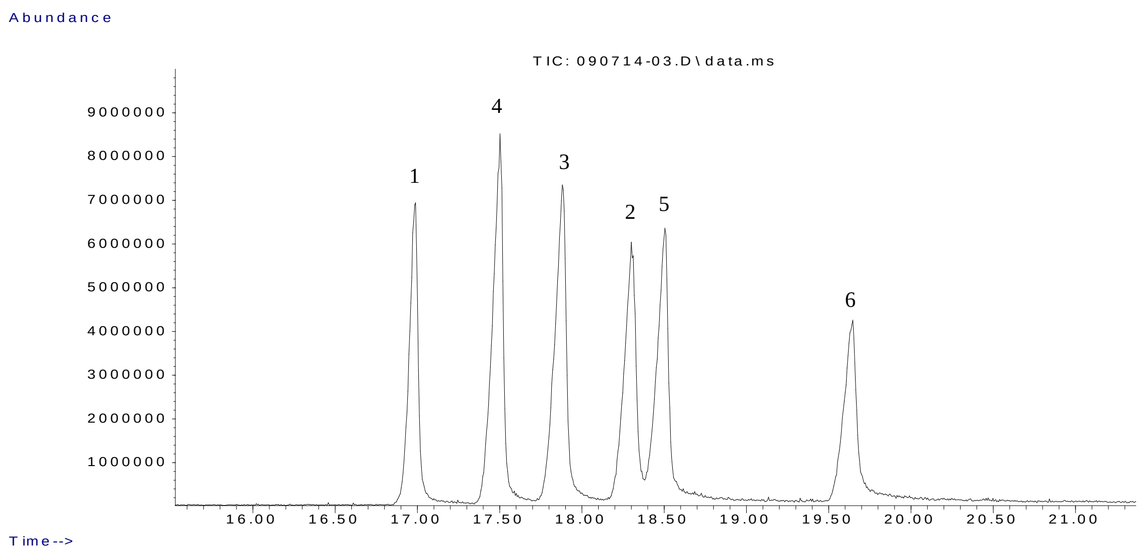


Fig. 20. Gas chromatographic separation of the six underivatized benzylpiperazines on Rtx-200 column. The numbers over the peaks correspond to the compound numbers.

3.3.4. 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.

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

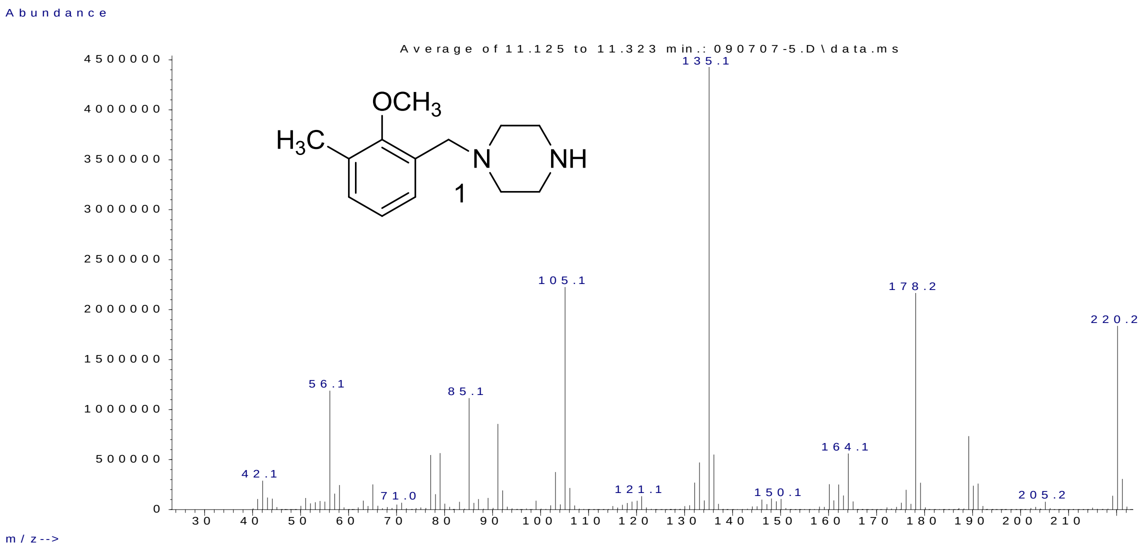
The substituted benzylpiperazines, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP), its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) and all ten possible isobaric ring substituted methoxymethylbenzylpiperazines (MMBPs) have almost identical mass spectra. Perfluoroacylation of the secondary 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.

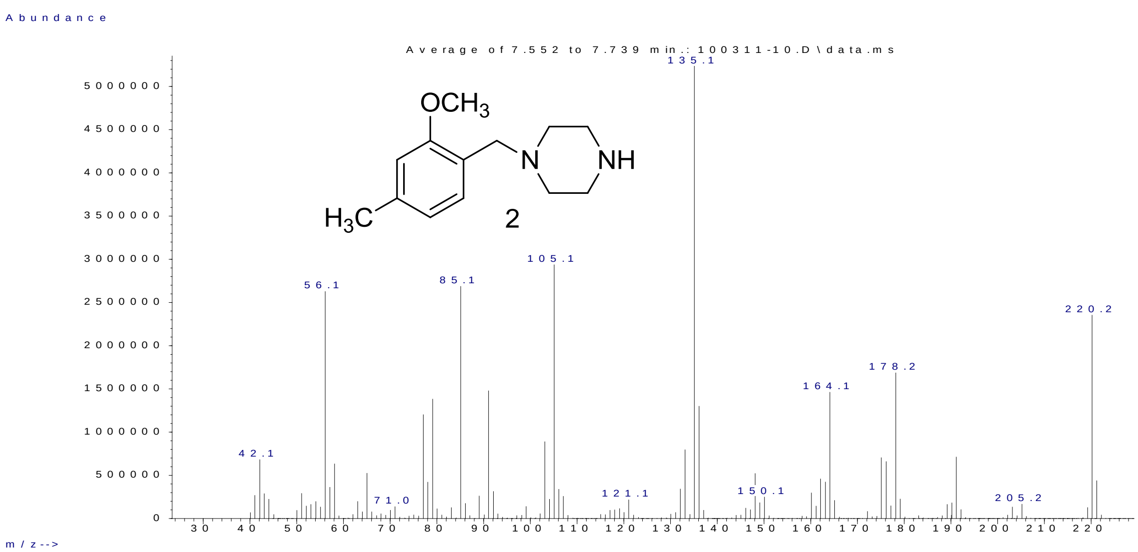
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the twelve isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The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

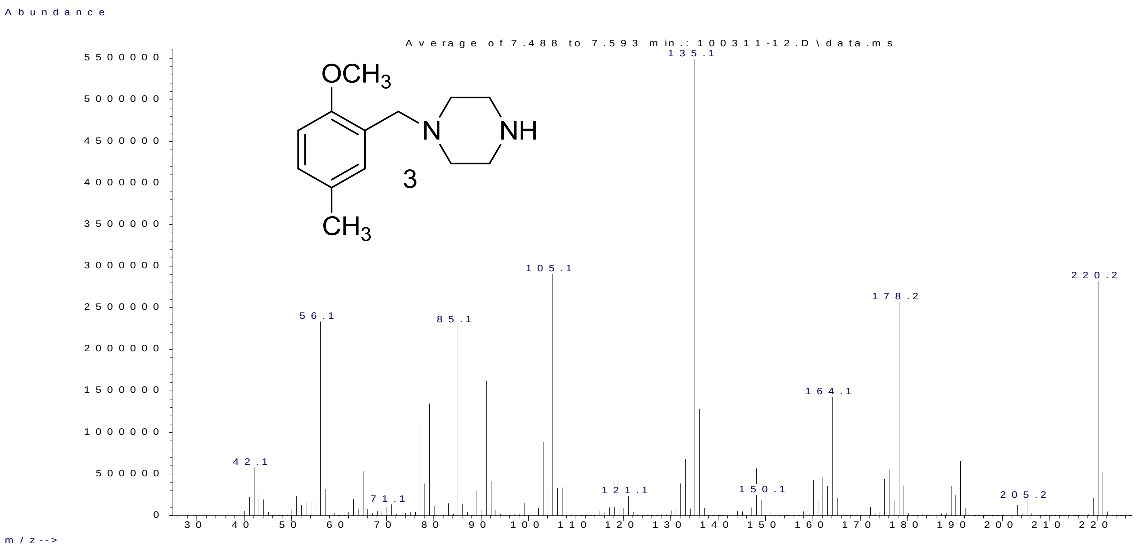
3.4.1. Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 21 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 section (3.3.1). 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 Scheme 21 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 section 3.3.1 which is characteristic for all the ortho-methoxy ring substituted compounds and was described in Scheme 22 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 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 section 3.3.1.



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NH
3
CH3
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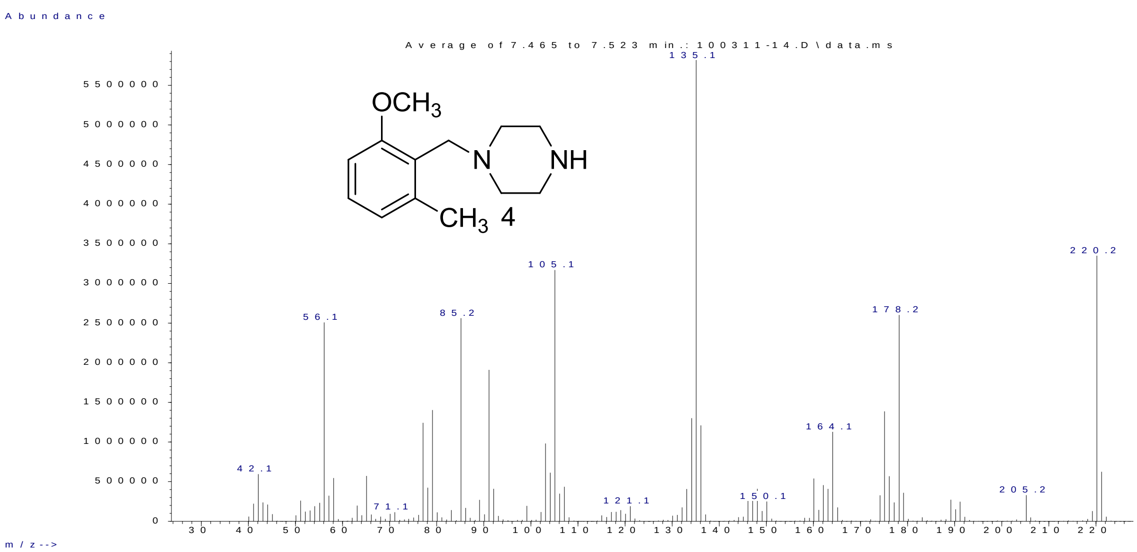
178.2
56.1
85.1
164.1
42.1
121.1
150.1
205.2
71.1
m/z-->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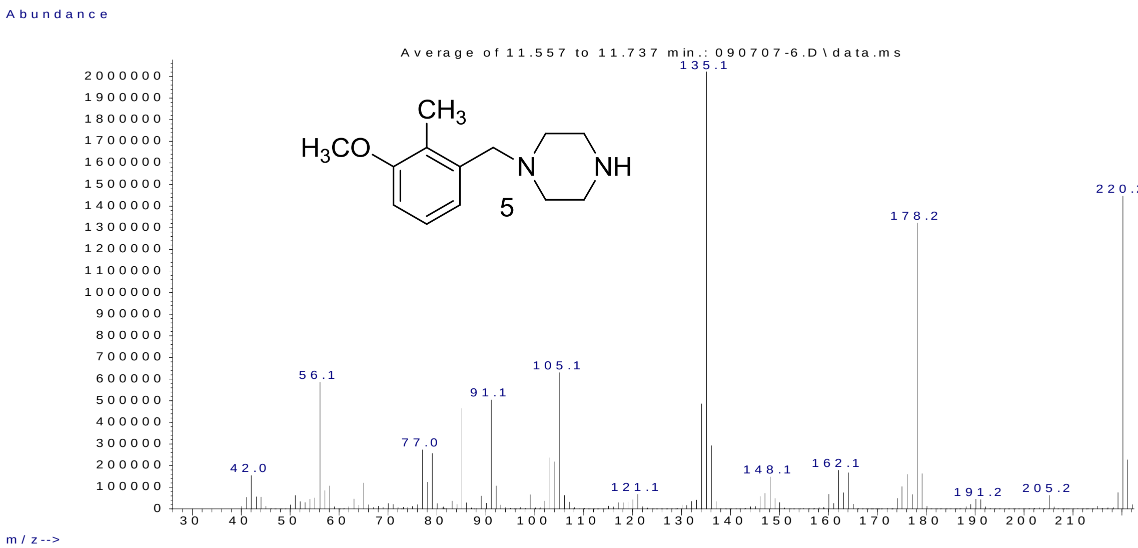
N
NH
CH3 4
135.1
220.2
105.1
56.1
85.2
178.2
164.1
42.1
121.1
150.1
205.2
71.1
m/z-->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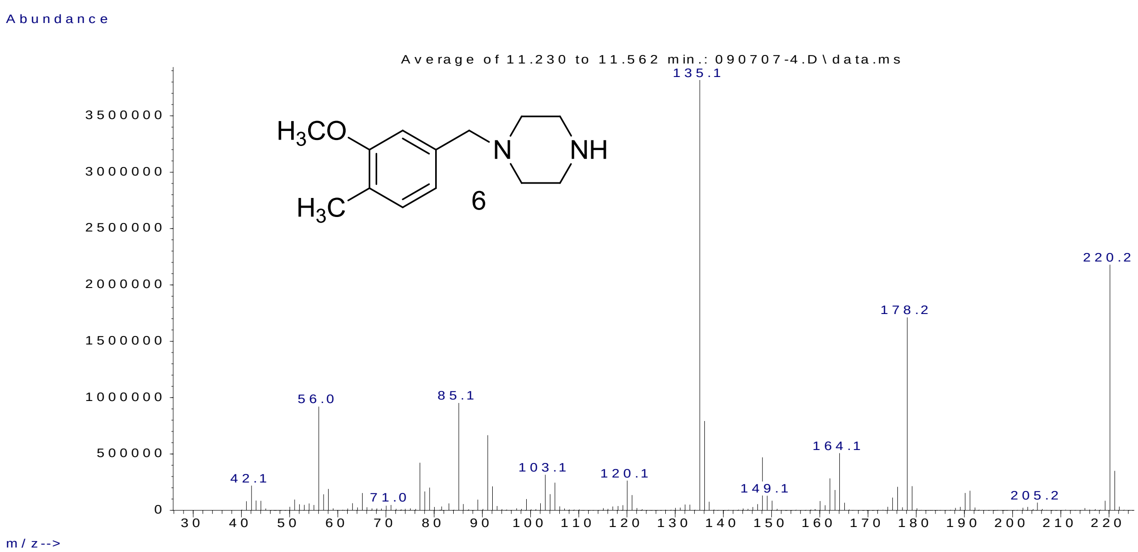
CH_3
N
NH
5
135.1
220.2
178.2
105.1
56.1
91.1
77.0
42.0
121.1
148.1
162.1
191.2
205.2
m/z-->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H_3C
N
NH
6
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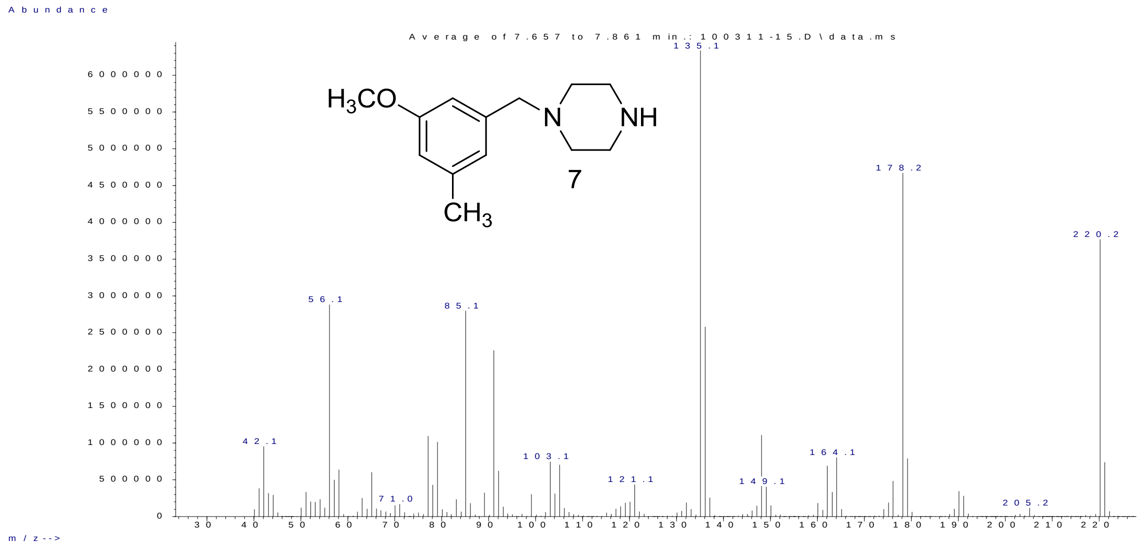
178.2
85.1
56.0
164.1
103.1
120.1
42.1
149.1
71.0
205.2
m/z-->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NH
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CH_3
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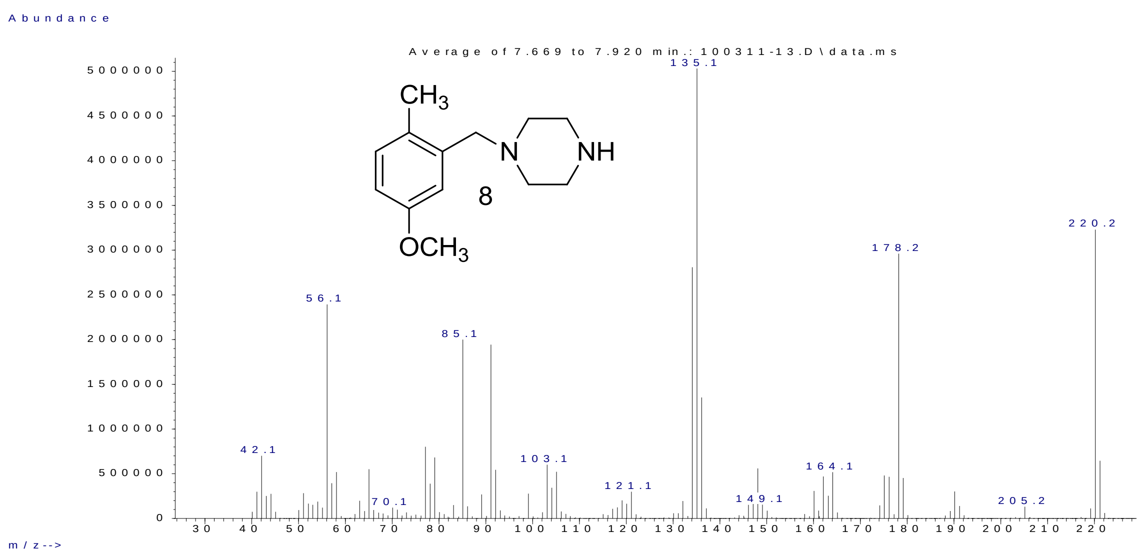
220.2
56.1
85.1
42.1
103.1
164.1
121.1
149.1
71.0
205.2
m/z-->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NH
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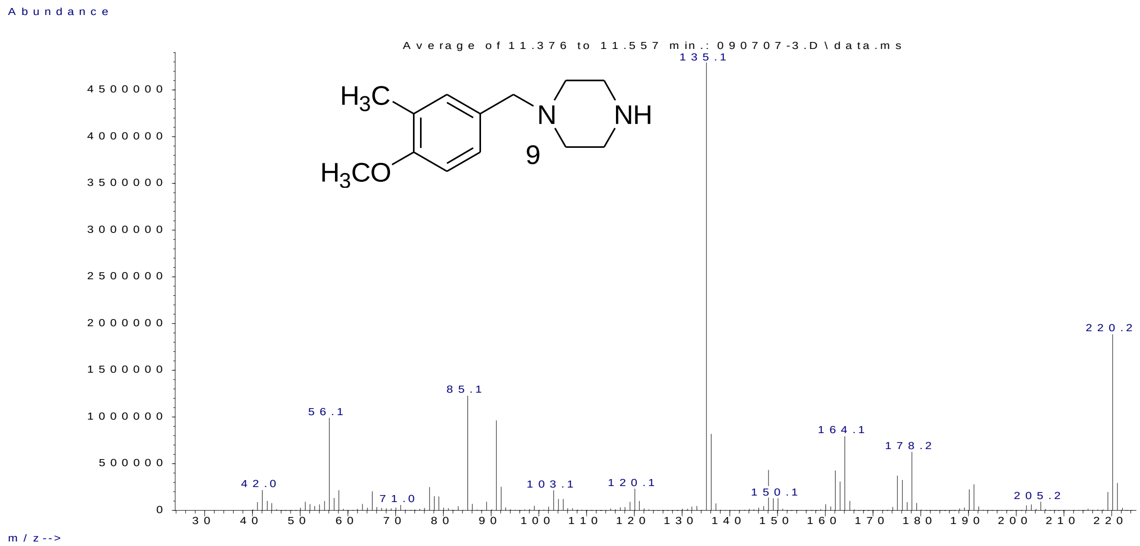
103.1
164.1
121.1
149.1
70.1
205.2
m/z-->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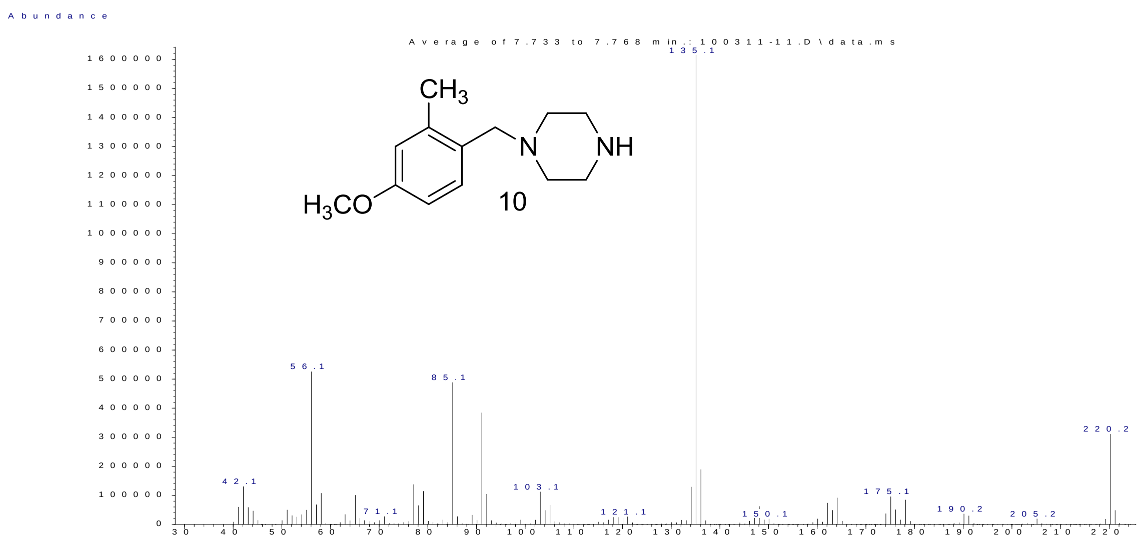
120.1
150.1
71.0
205.2
m/z-->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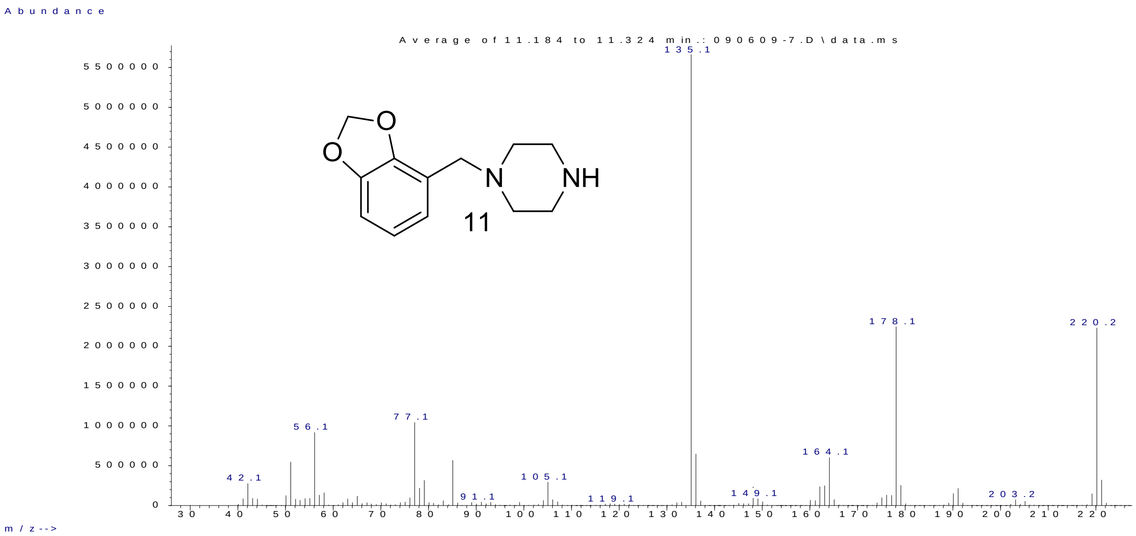
175.1
71.1
121.1
150.1
190.2
205.2
m/z-->



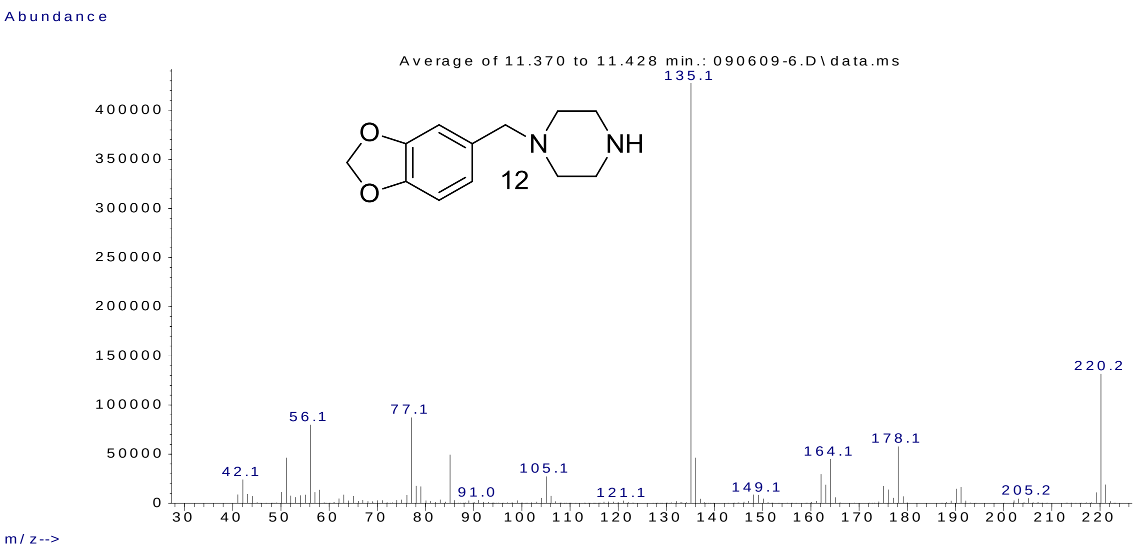
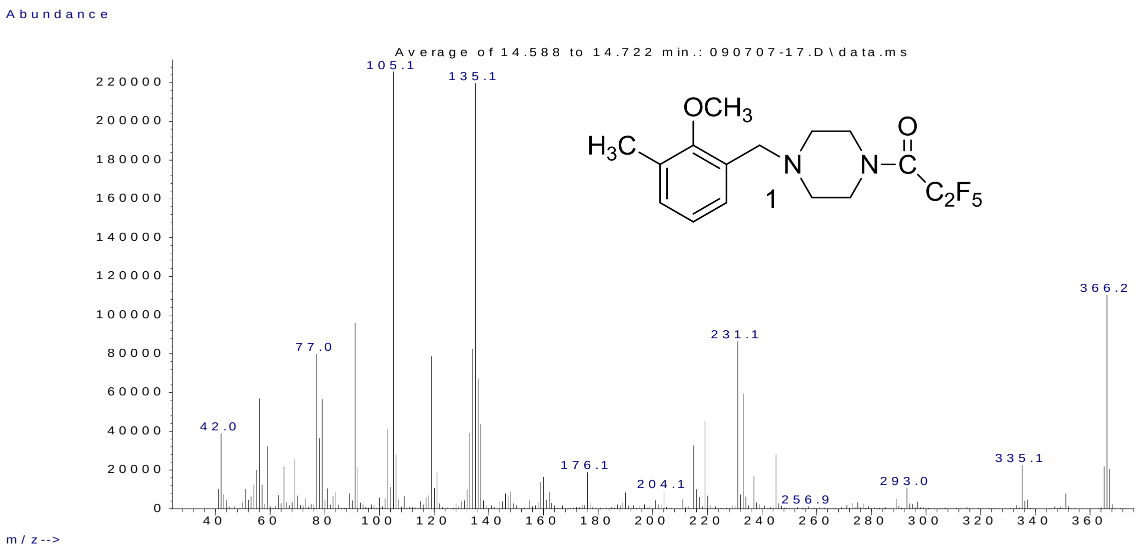


Fig. 21. EI mass spectra of the 12 benzylpiperazines in this study.

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 22 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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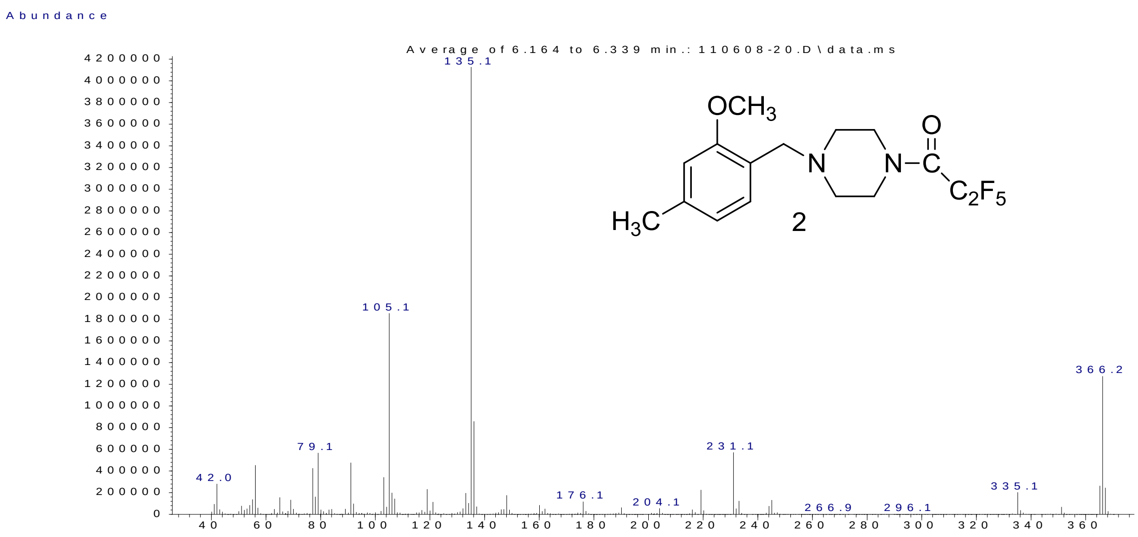
C2F5
1
m/z-->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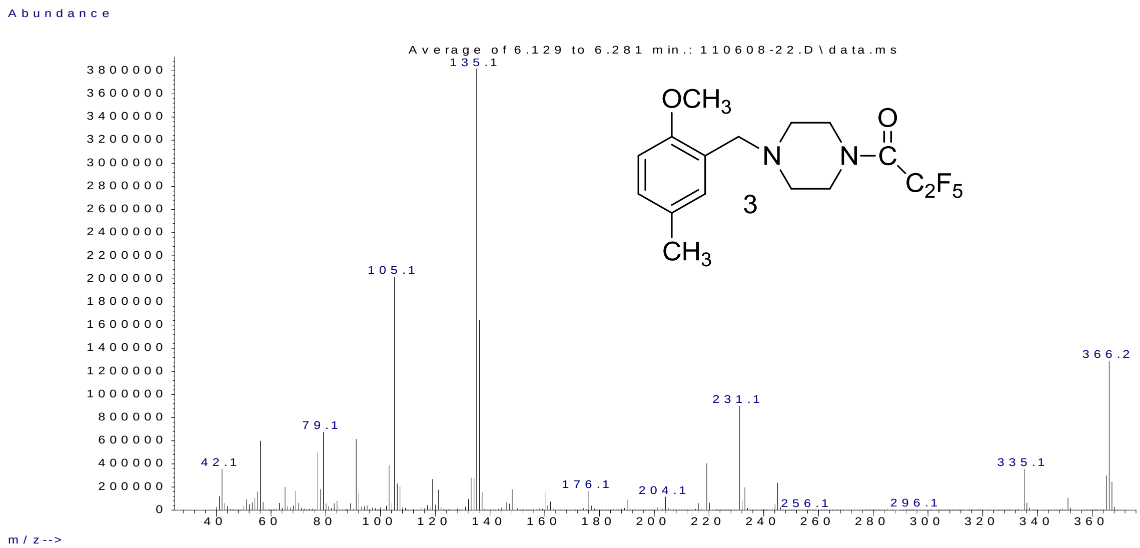
C2F5
2
m/z-->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335.1
366.2
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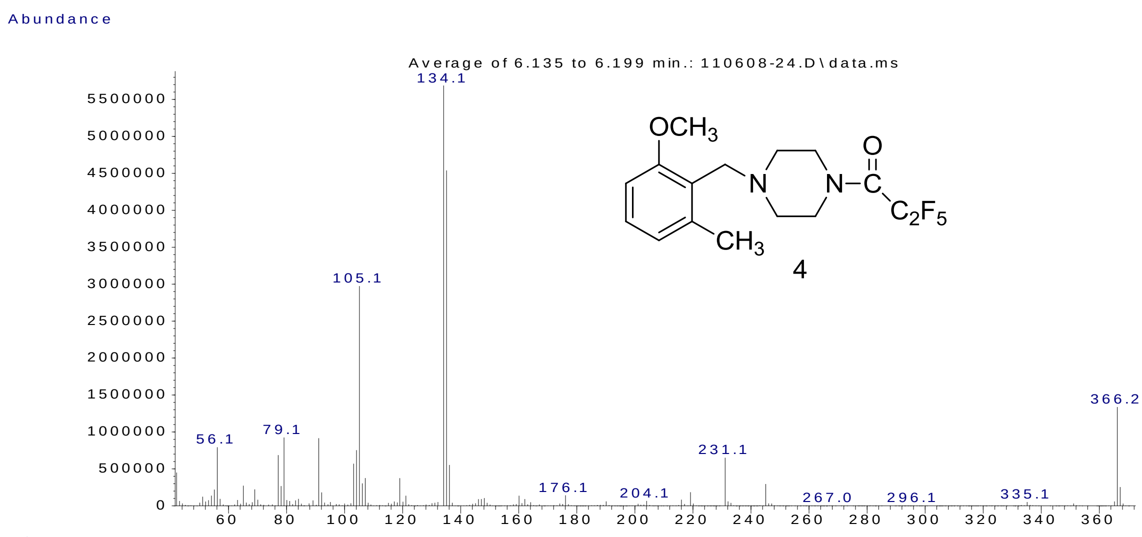
N
N
O
C
C2F5
CH3
3
m/z-->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O
C
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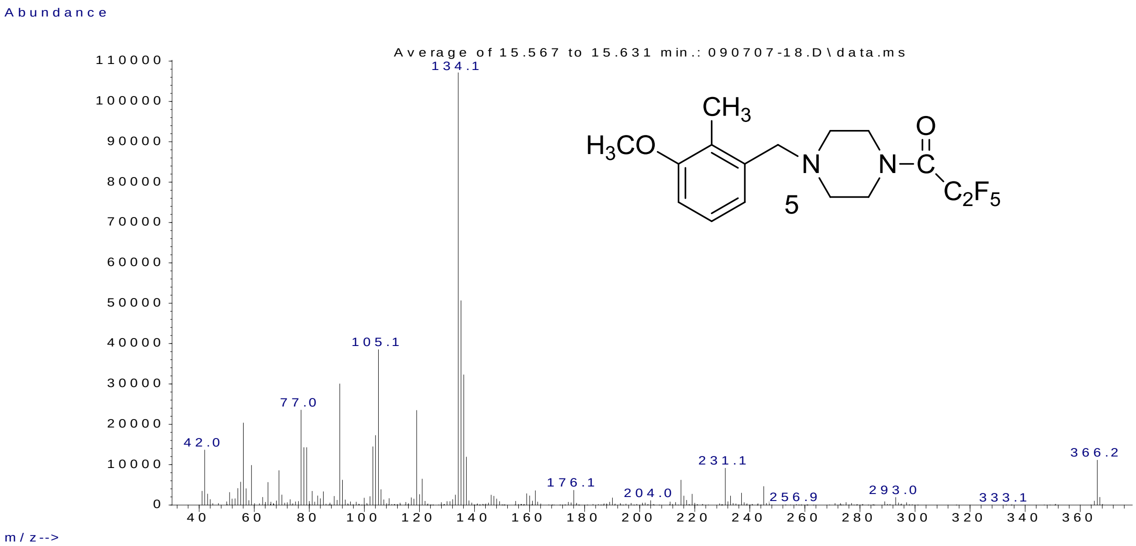
CH3
4
m/z-->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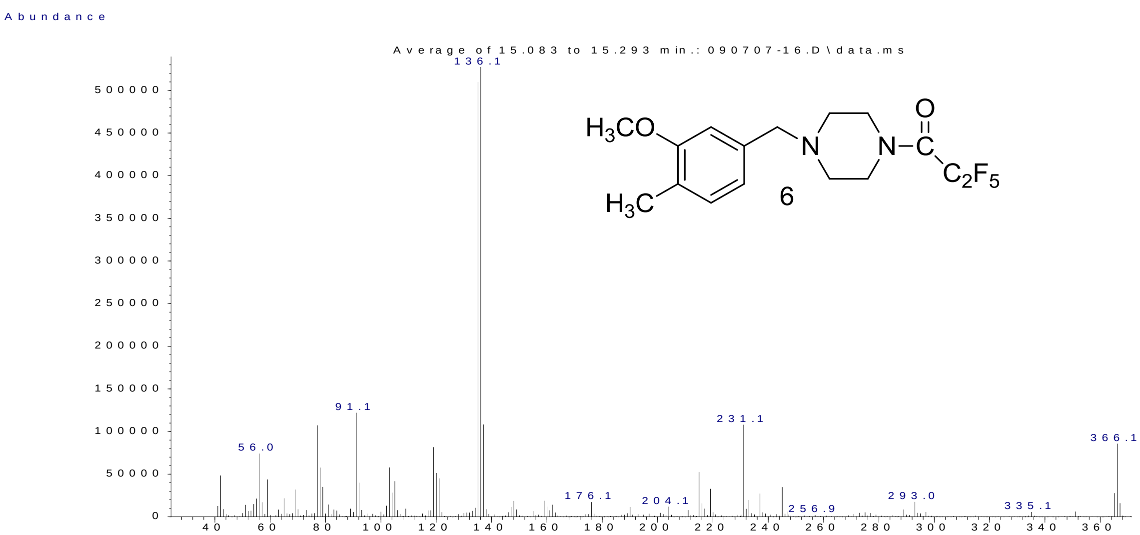
C_2F_5
5
m/z-->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C
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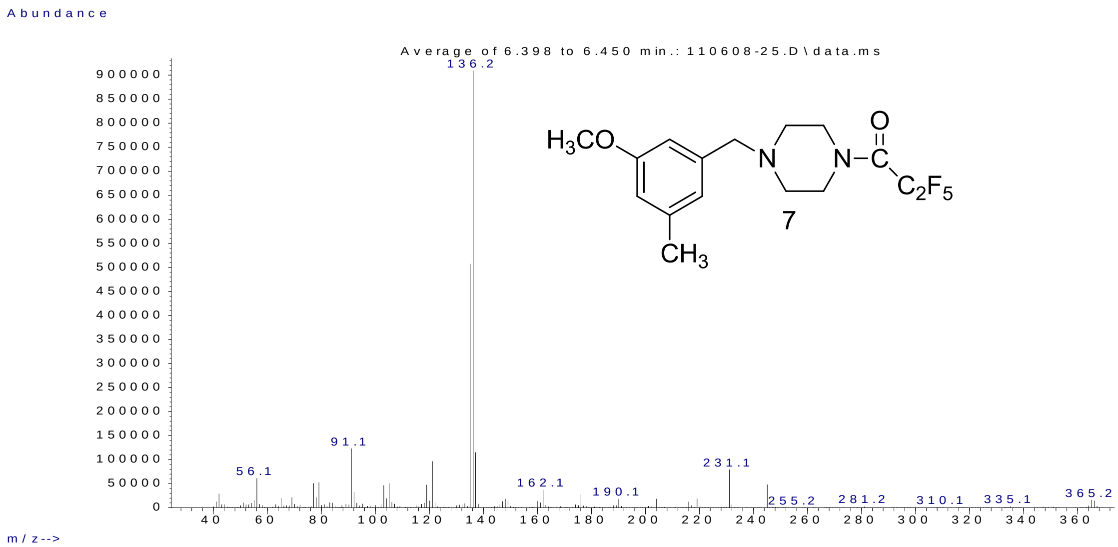
6
m/z-->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91.1
56.1
162.1
190.1
231.1
255.2
281.2
310.1
335.1
365.2
m/z-->
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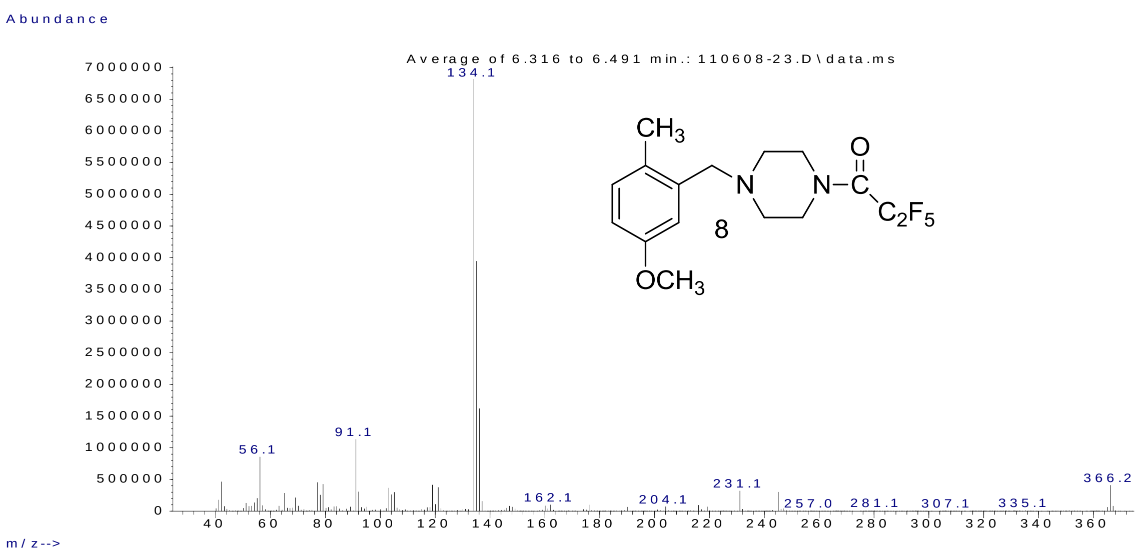
CH3
N
N
O
C
C2F5
7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91.1
56.1
162.1
204.1
231.1
257.0
281.1
307.1
335.1
366.2
m/z-->
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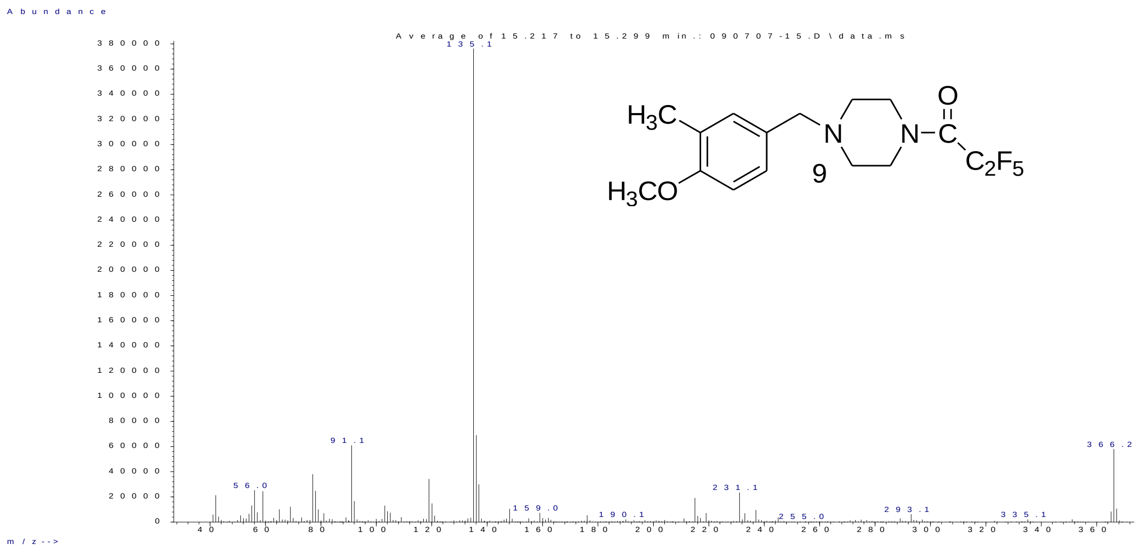
N
N
O
C
C2F5
OCH3
8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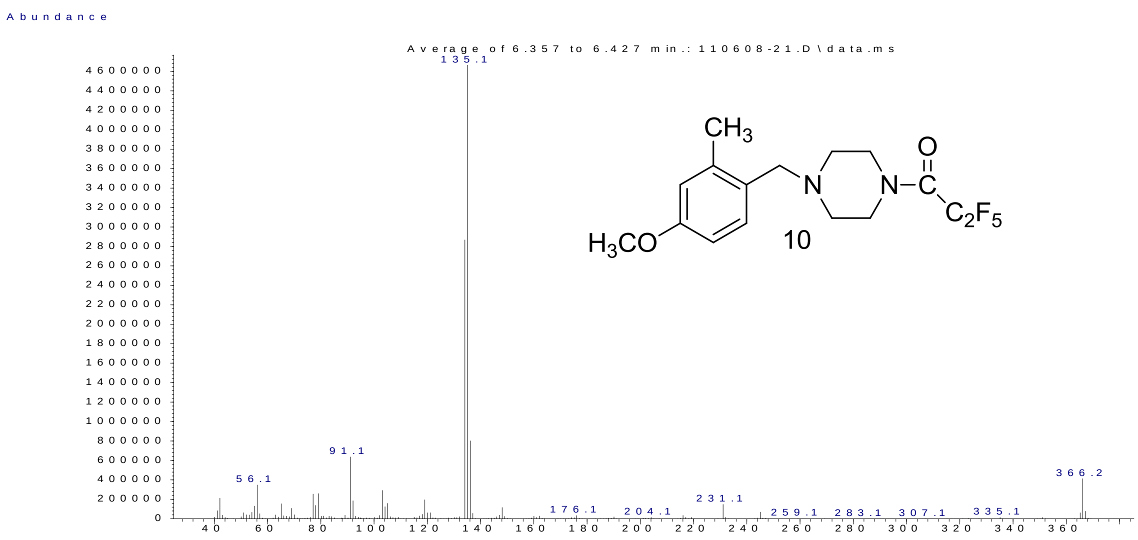
91.1
56.0
159.0
190.1
231.1
255.0
293.1
335.1
366.2
m/z-->
9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56.1
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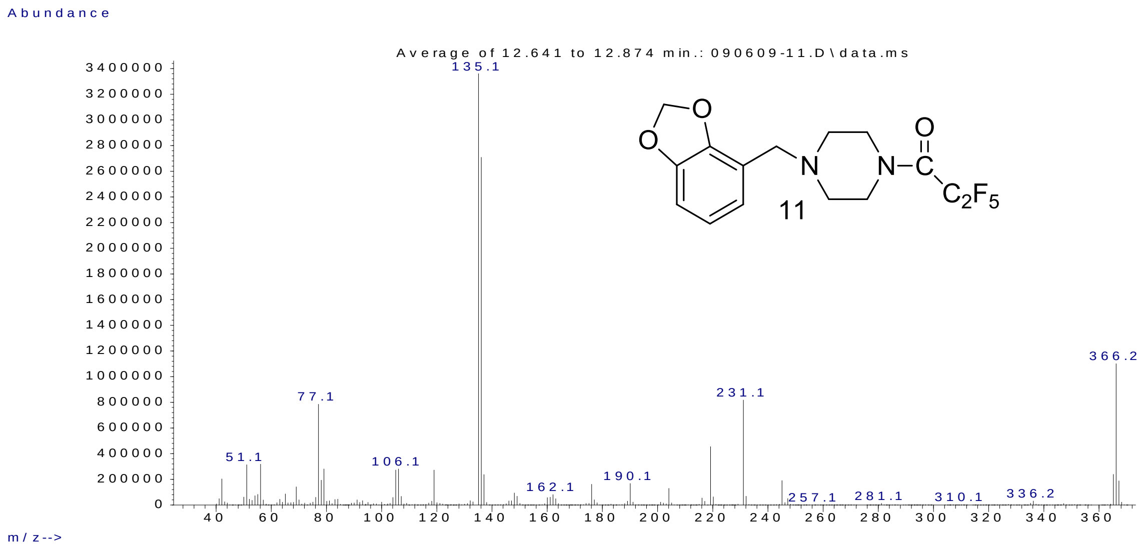
204.1
231.1
259.1
283.1
307.1
335.1
366.2
m/z-->
10



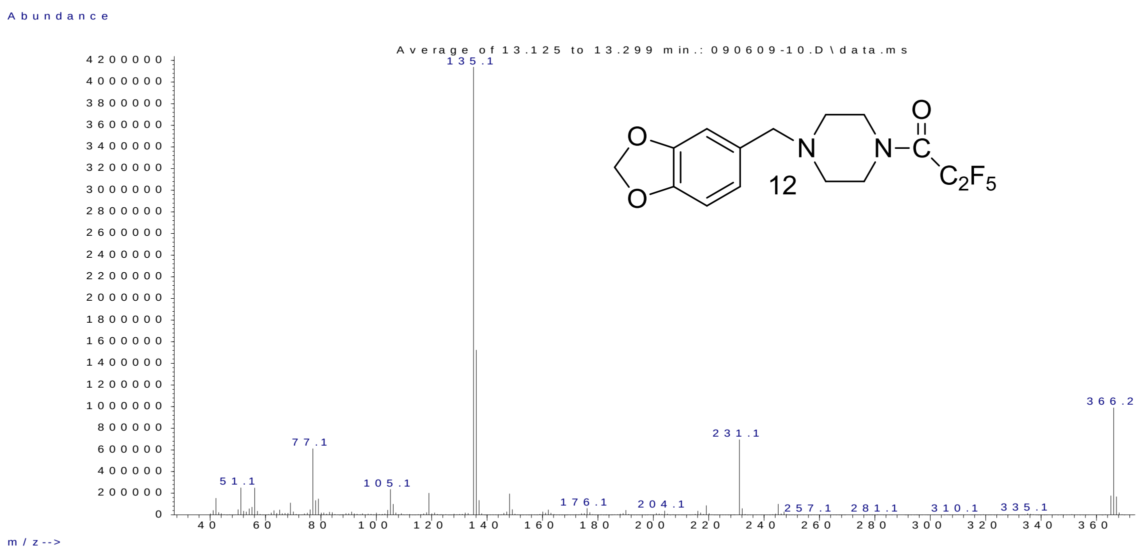


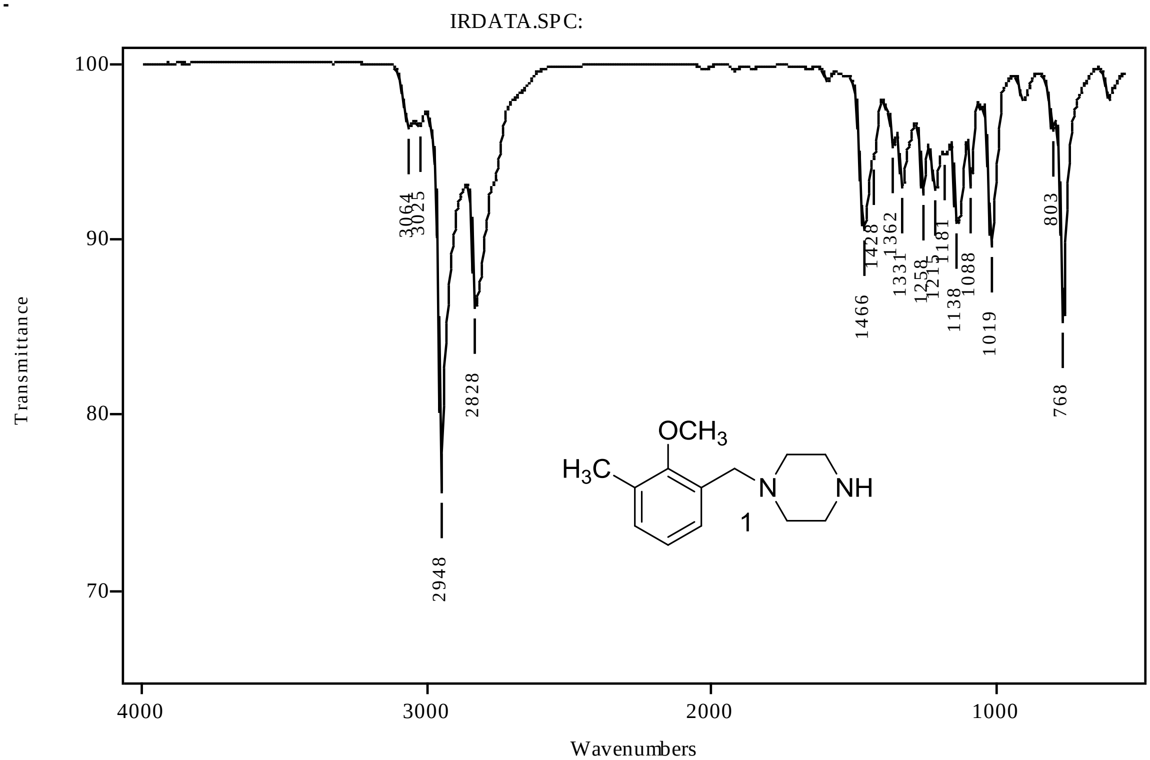
Fig. 22. EI mass spectra of the pentafluoropropionylderivatives of the 12 benzylpiperazines in this study.

3.4.2. Vapor-phase Infra-Red Spectroscopy

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 23. 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}. In the preceding section 3.3.2, 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 compounds11 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-MDBP. The 2,3-MDBP regioisomer has a relatively strong IR band at 1069 cm^{-1} which is shifted to a medium intensity peak at 1050 cm^{-1} in the 3,4-regioisomer. The vapor phase IR spectrum of the 3,4-MDBP regioisomer can be distinguished from that of the 2,3-regioisomers by at least three IR bands of

-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Transmittance
Wavenumbers
3064
3025
2948
2828
1466
1428
1362
1331
1258
1215
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1019
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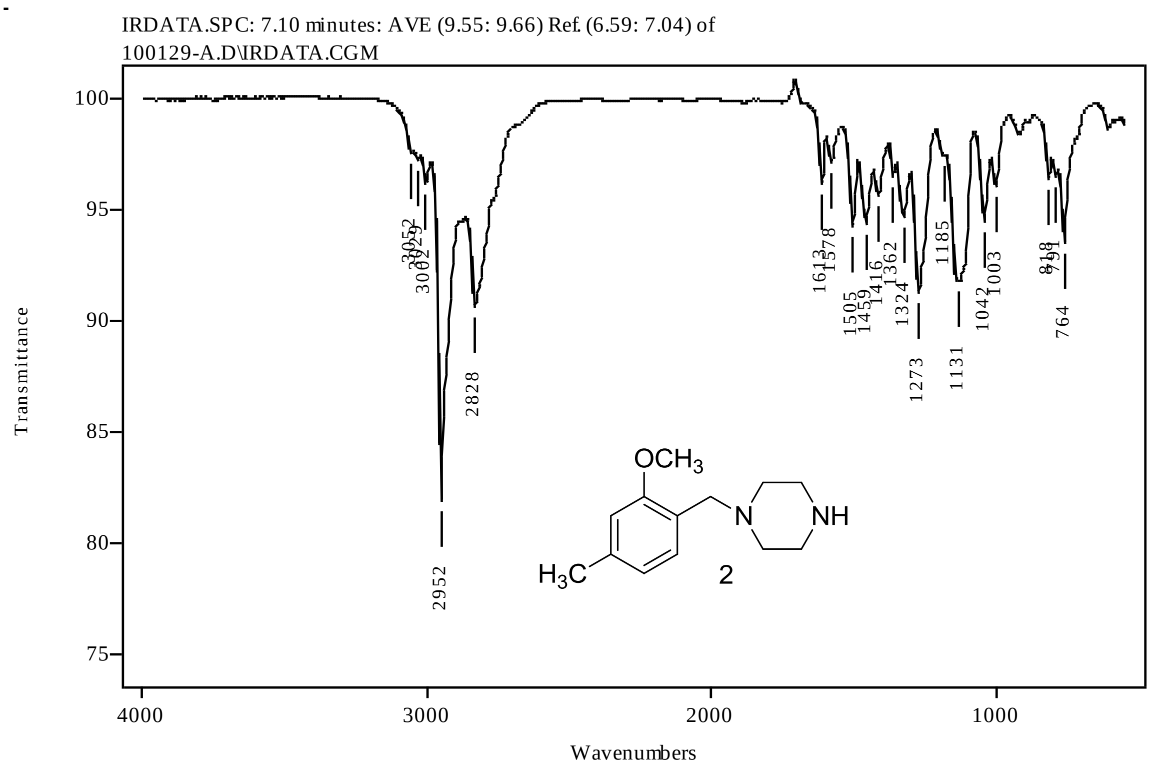
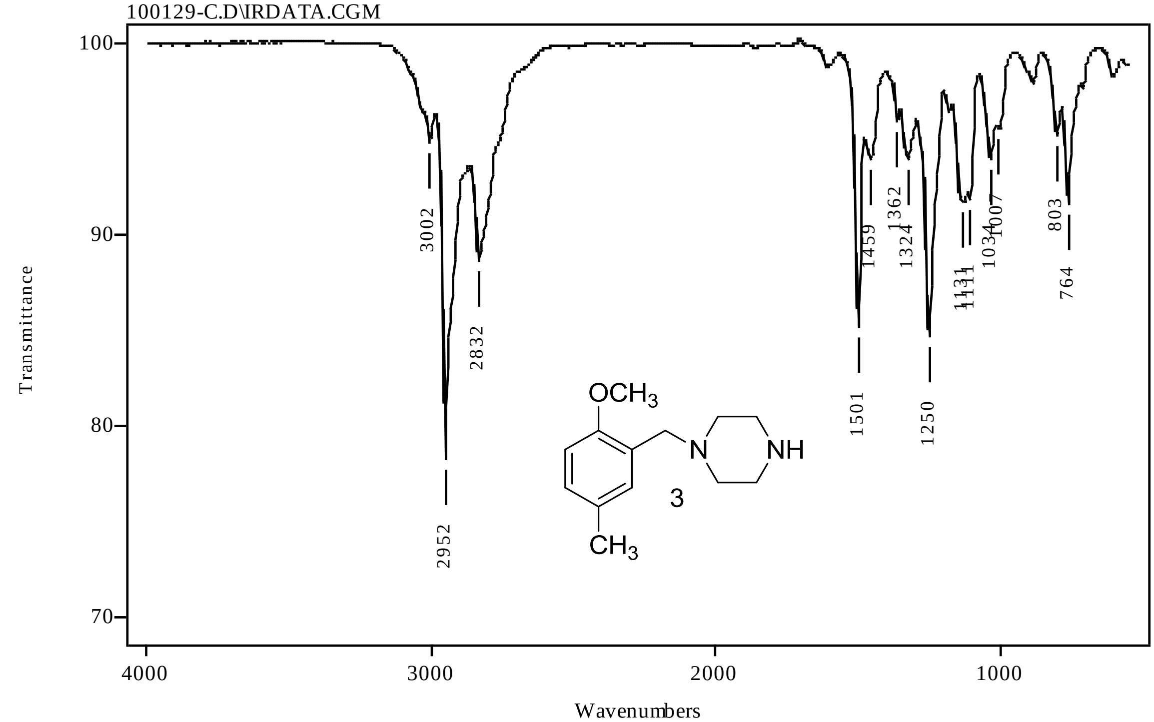
768
OCH3
H3C
N
NH
1

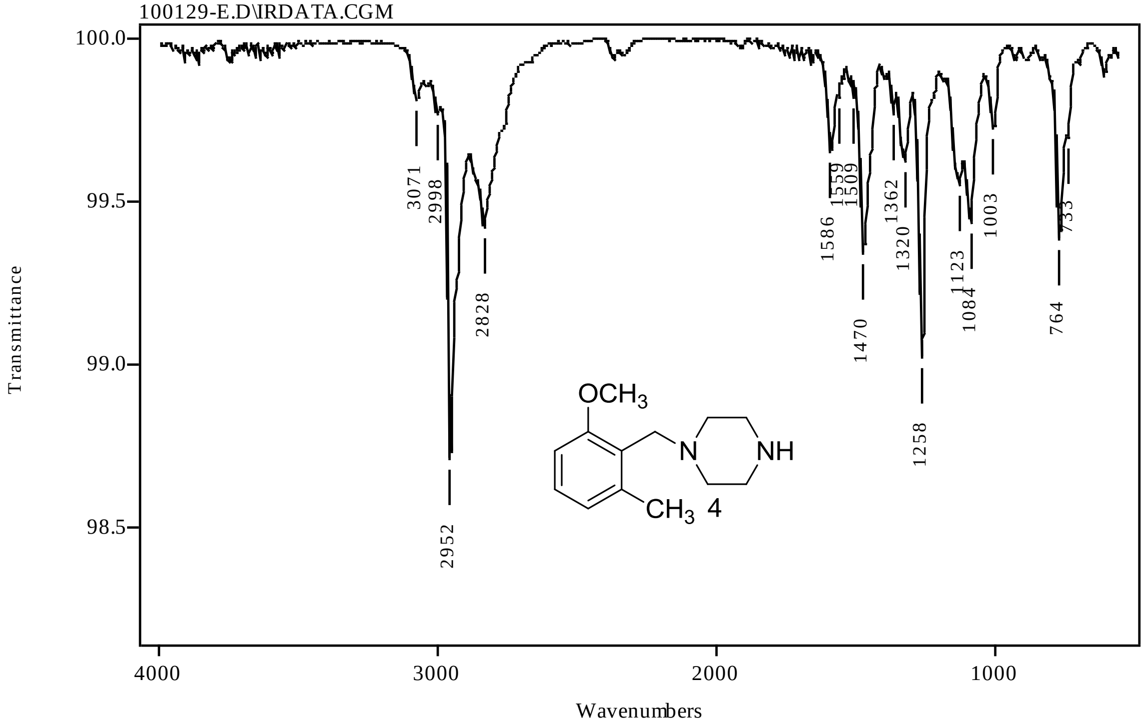
-


IRDATA.SPC: 7.10 minutes: AVE (9.55: 9.66) Ref. (6.59: 7.04) of
100129-A.D\IRDATA.CGM
Transmittance
Wavenumbers
3052
3029
3002
2952
2828
1613
1578
1505
1459
1416
1362
1324
1273
1185
1131
1042
1003
818
791
764
OCH3
H3C
N
NH
2

IRDATA.SPC: 6.84 minutes: AVE (9.40: 9.48) Ref. (6.19: 7.02) of
100129-C.D\IRDATA.CGM



IRDATA.SPC: 9.32 minutes: AVE (9.30: 9.78) Ref. (7.33: 9.26) of
100129-E.D\IRDATA.CGM



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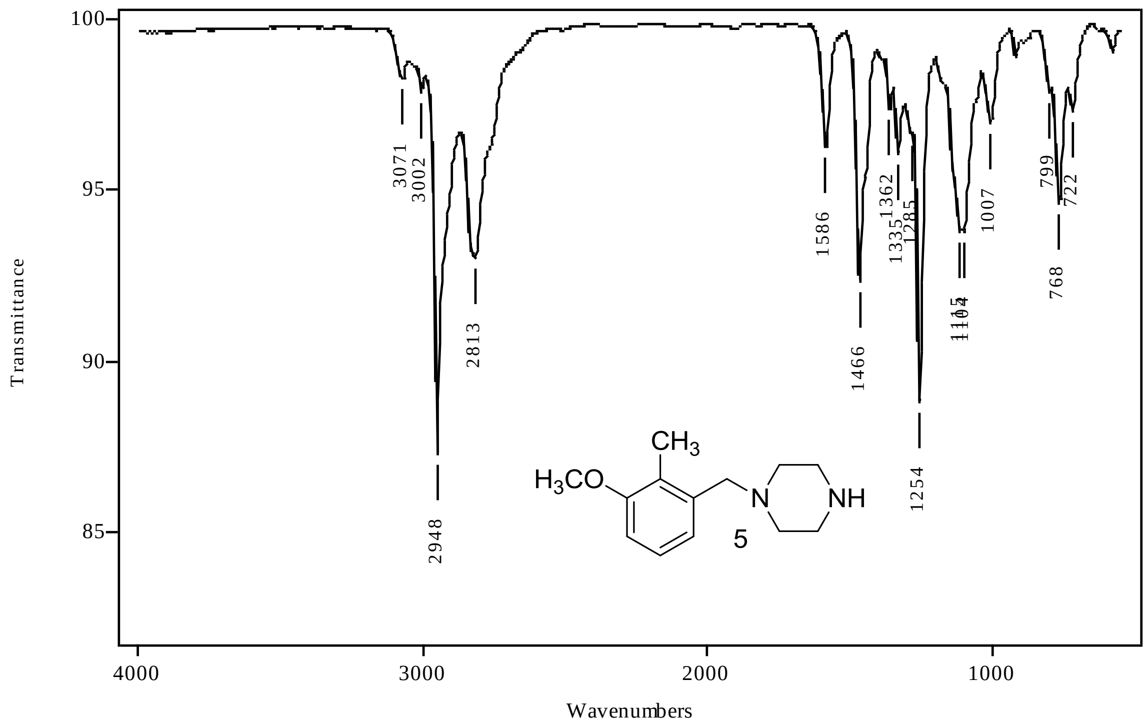
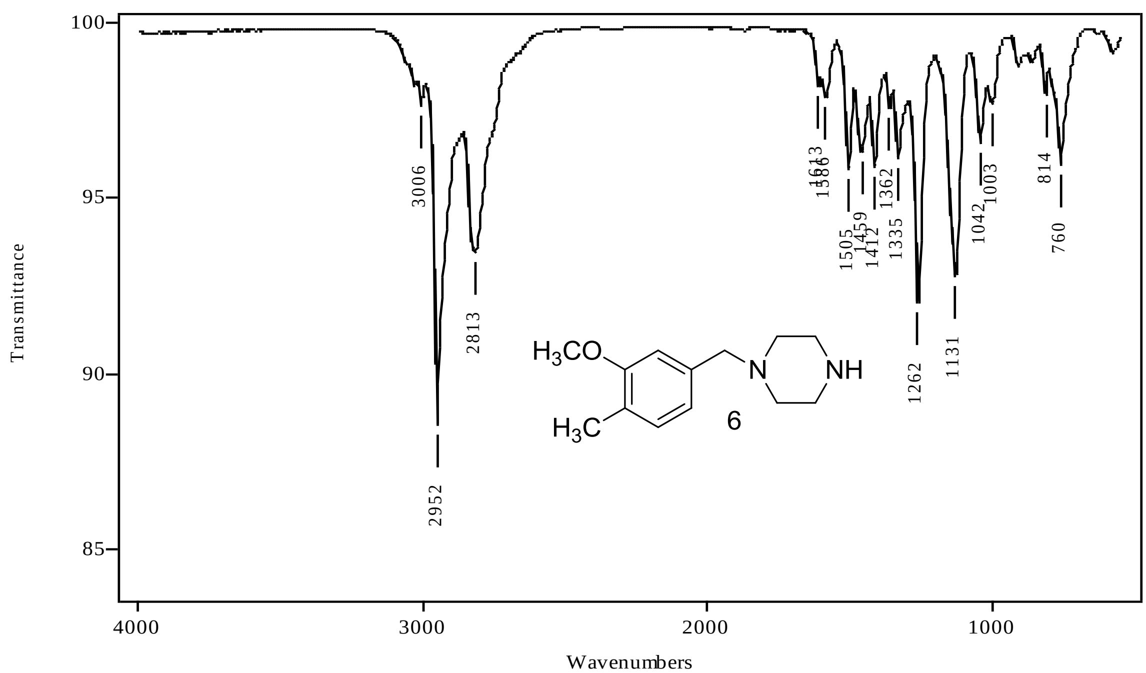


IRDATA.SPC:



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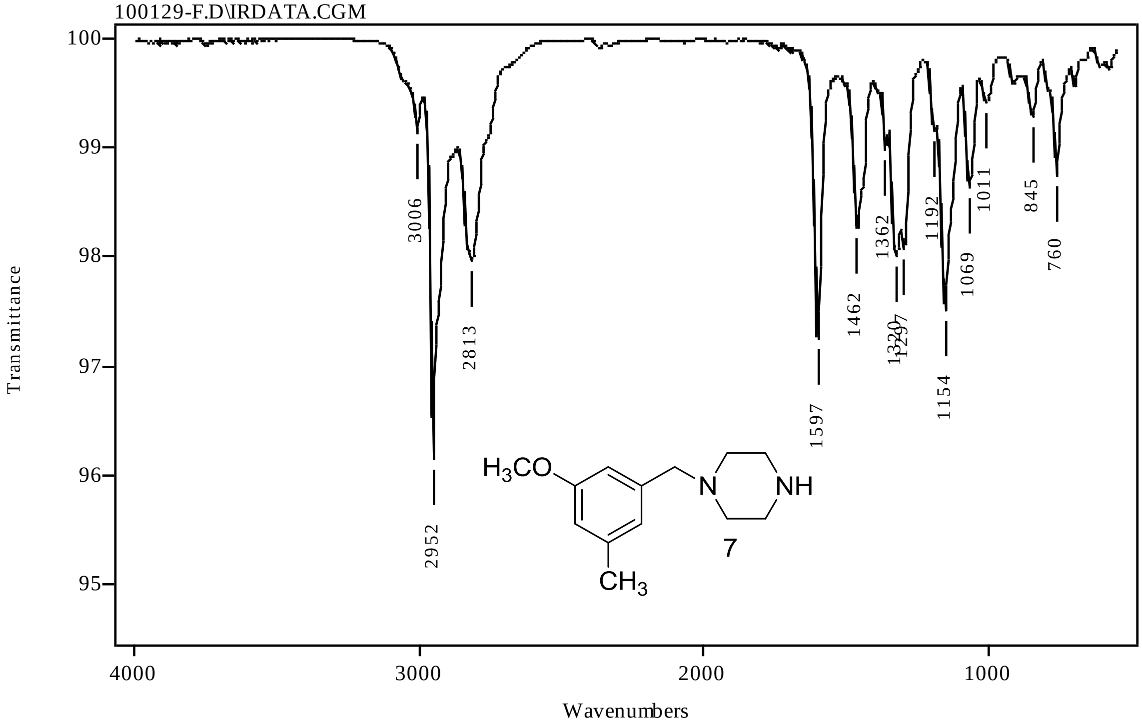
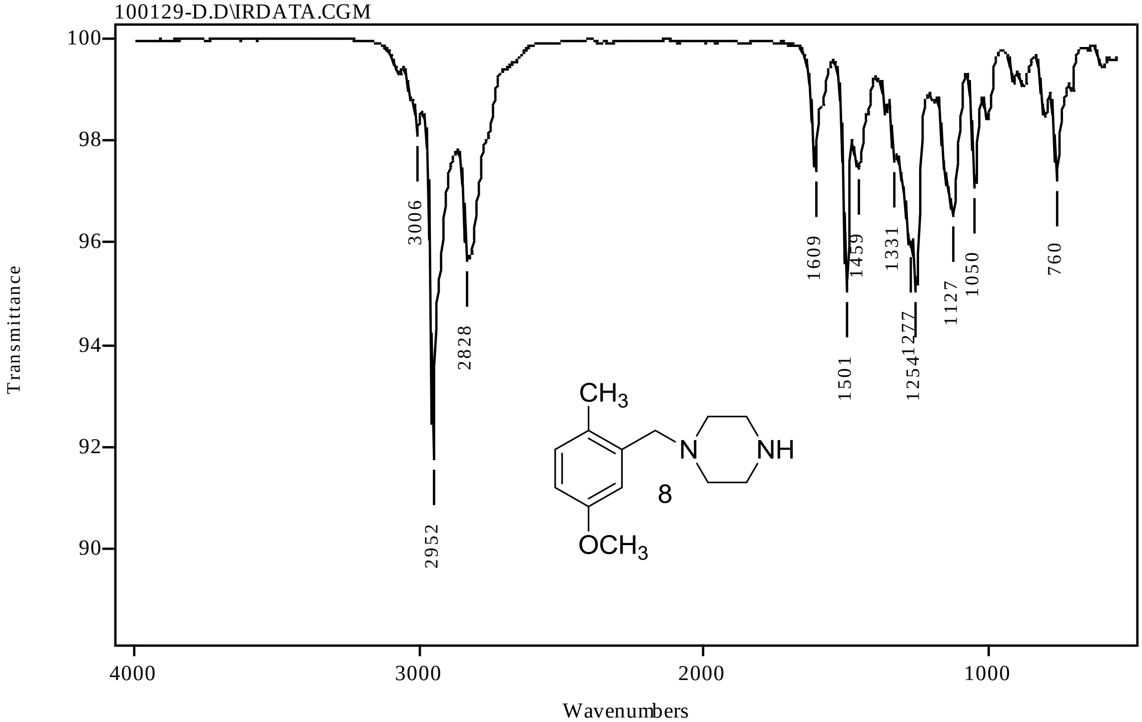
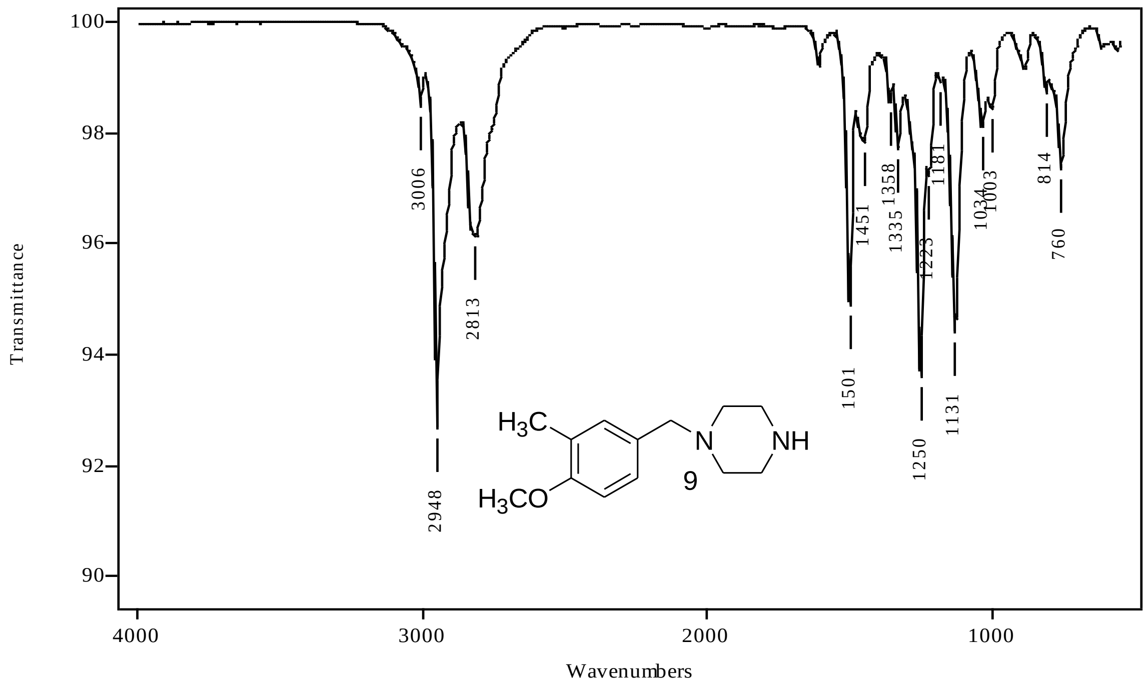


IRDATA.SPC: 9.86 minutes: AVE (9.79: 10.24) Ref. (7.91: 9.70) of
100129-D.D\IRDATA.CGM



IRDATA.SPC:



IRDATA.SPC: 3.94 minutes: AVE (9.98: 10.04) Ref. (6.81: 6.97) of
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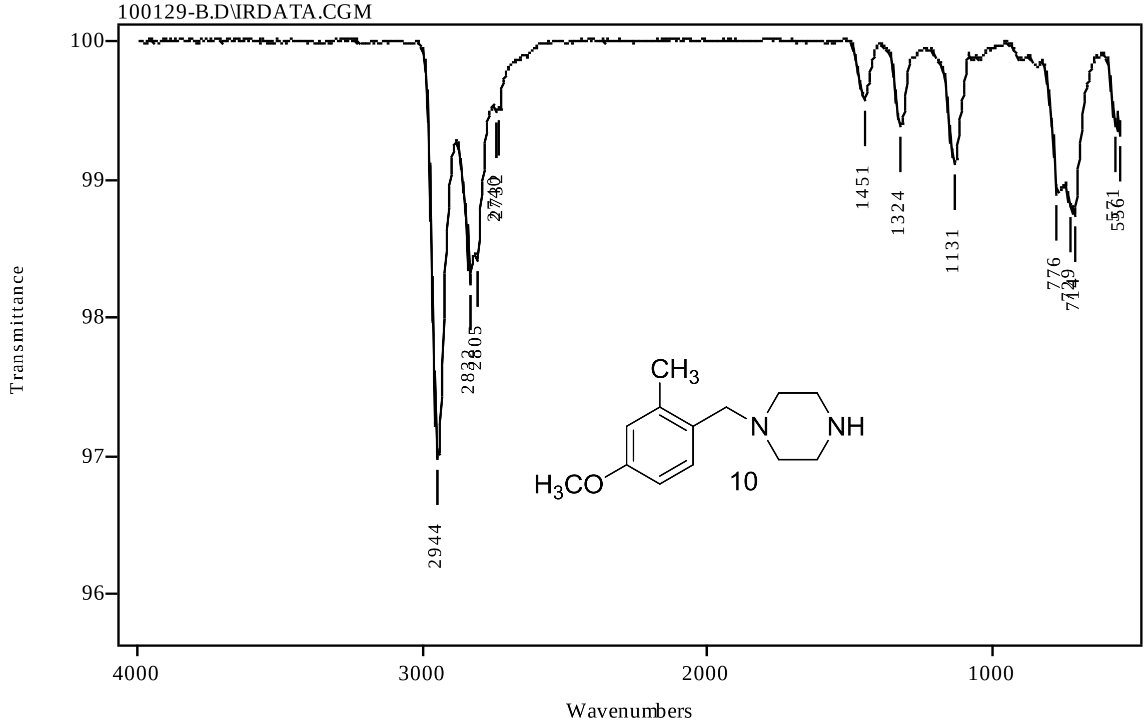
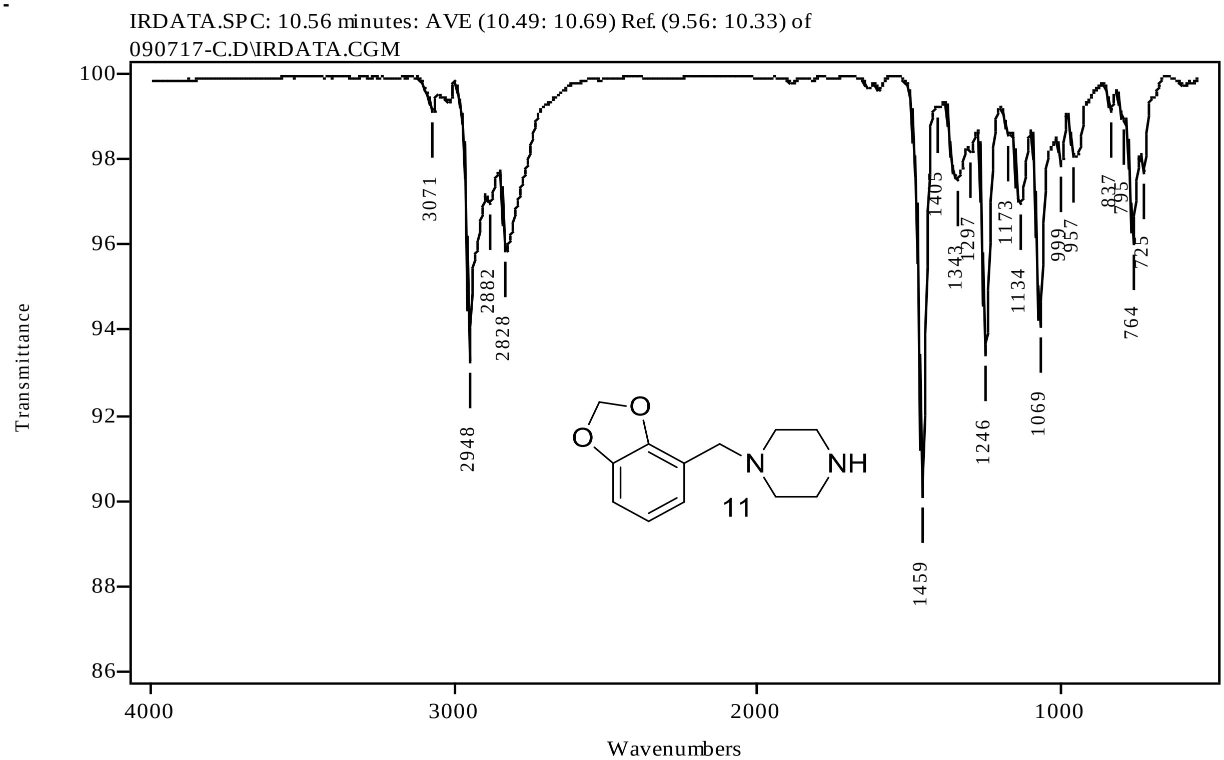




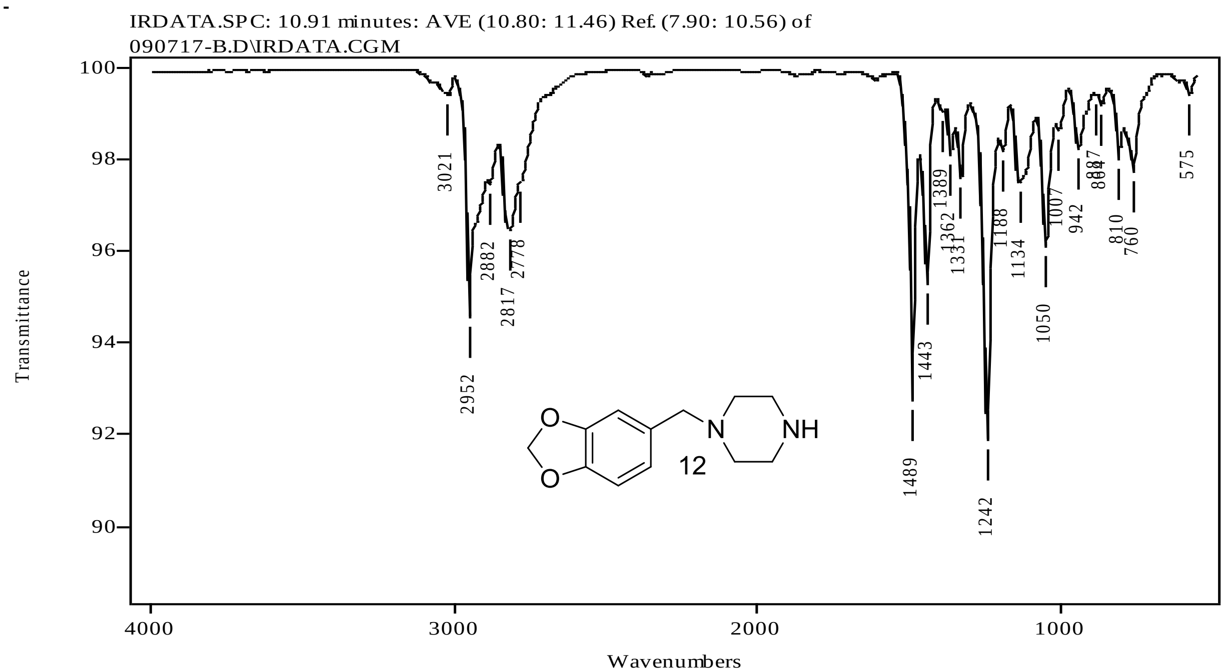


Fig. 23: Vapor phase IR spectra of the 12 underivatized methylenedioxy and methoxymethylbenzylpiperazines.

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.

3.4.3. Gas Chromatographic Separation

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 70^{o}C for 1 minute, ramped up to 150^{o}C at a rate of 7.5^{o}C per minute followed by a hold at 150^{o}C 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 24-27 are representatives of the results obtained for all samples on this stationary phase.

In Figure 24 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 24.

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 25. The second subgroup includes those compounds with the meta methoxy group (compounds 5-8) in addition to compounds 11 and 12 and their chromatographic separation is in Figure 26. 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 27.

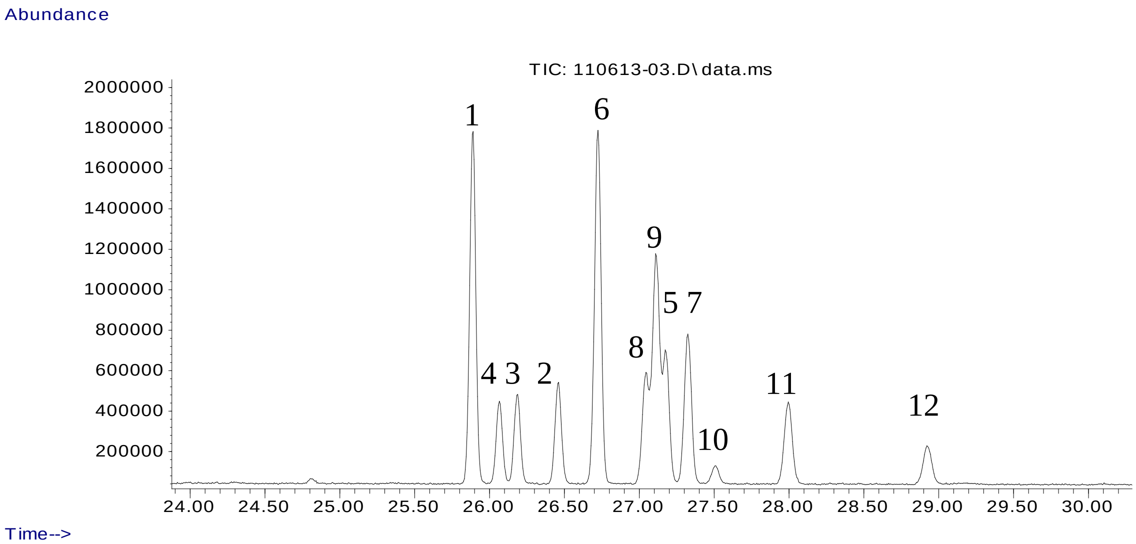


Fig. 24. Gas chromatographic separation of the pentafluoropropionyl derivatives of the 12 piperazine isomers using Rtx-35 column. The number over the peak corresponds to the compound number.

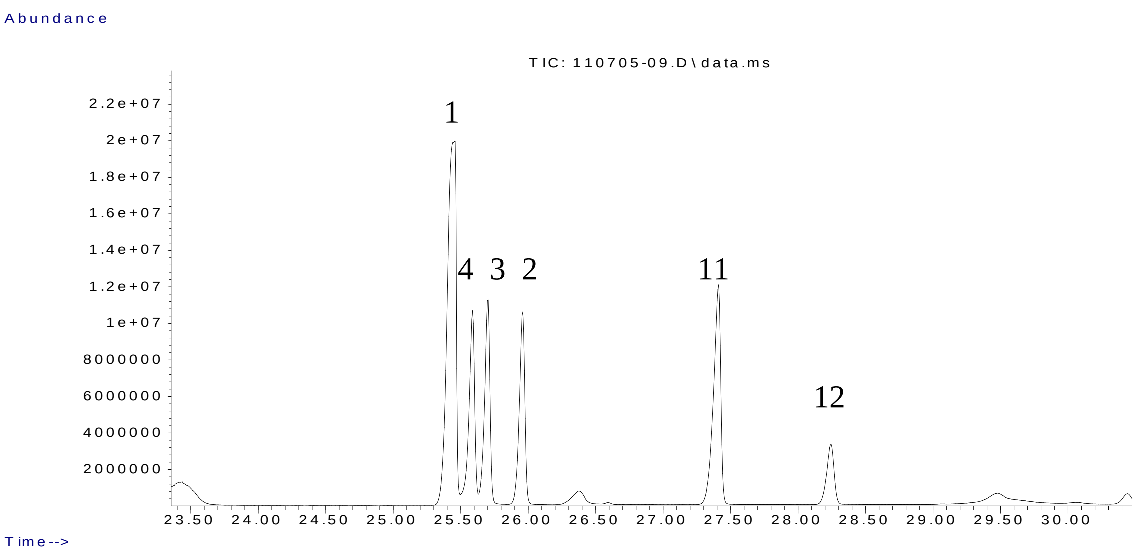


Fig. 25. Gas chromatographic separation of the pentafluoropropionyl derivatives of the MMBPs with o-methoxy group and methylenedioxypiperazines using Rtx-35 column. The number over the peak corresponds to the compound number.

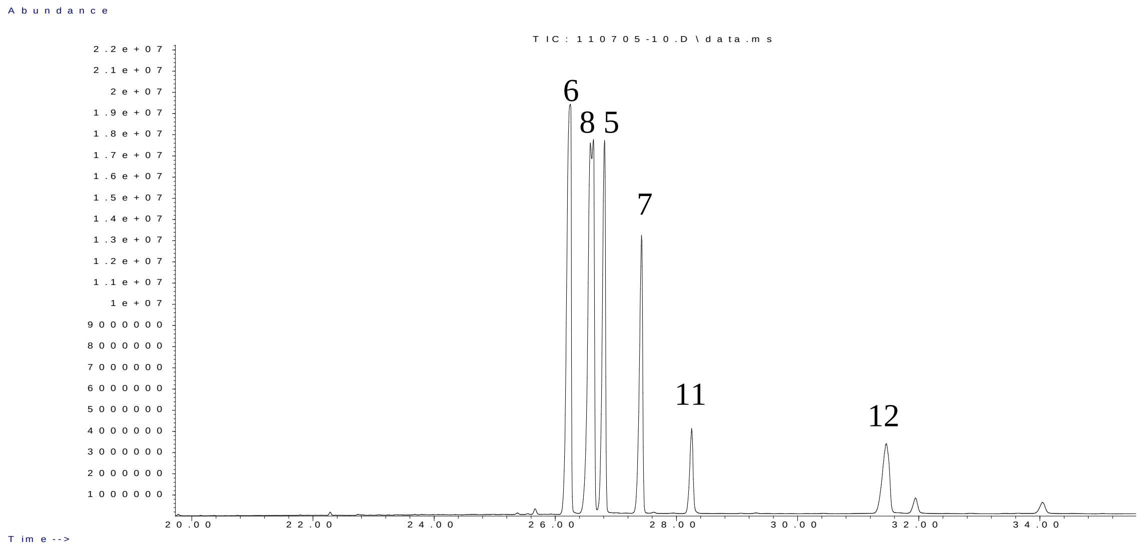


Fig. 26. Gas chromatographic separation of the pentafluoropropionyl derivatives of the MMBPs with m-methoxy group and methylenedioxypiperazines using Rtx-35 column. The number over the peak corresponds to the compound number.

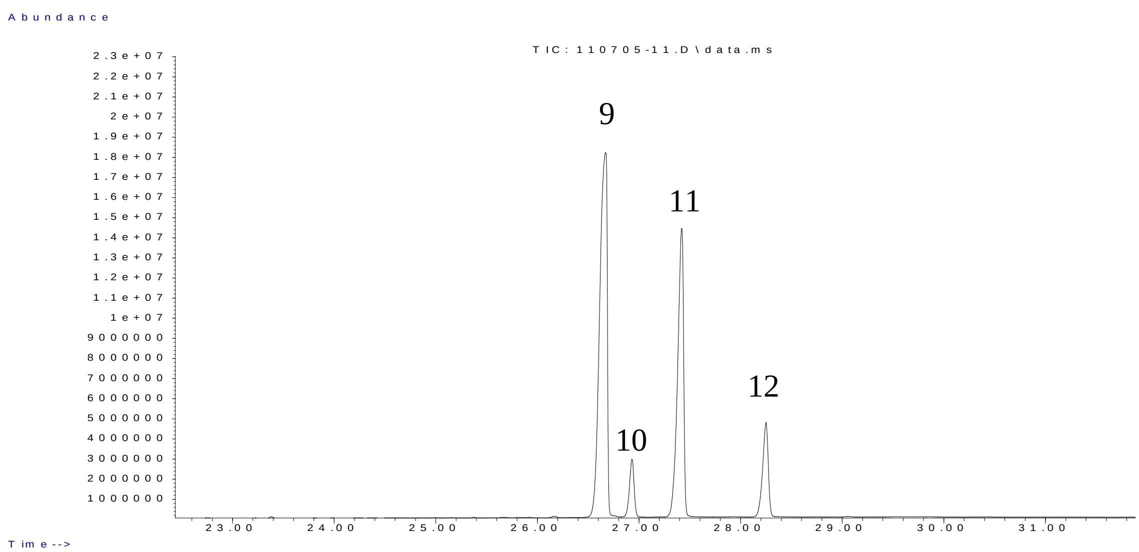


Fig. 27. Gas chromatographic separation of the pentafluoropropionyl derivatives of the MMBPs with p-methoxy group and methylenedioxypiperazines using Rtx-35 column. The number over the peak corresponds to the compound number.

3.4.4. 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.

3.5. 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.

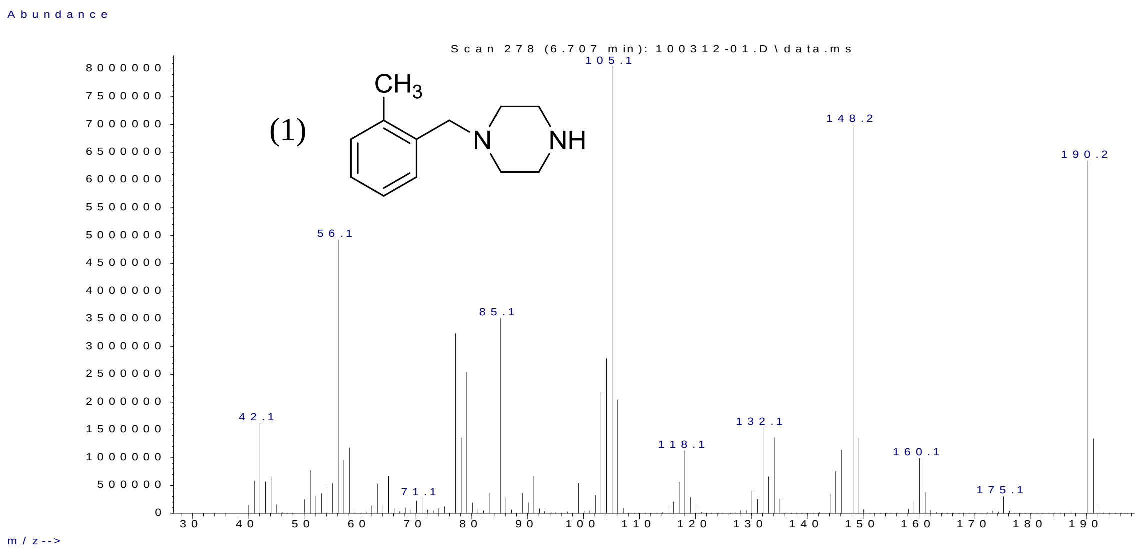
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the four isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized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

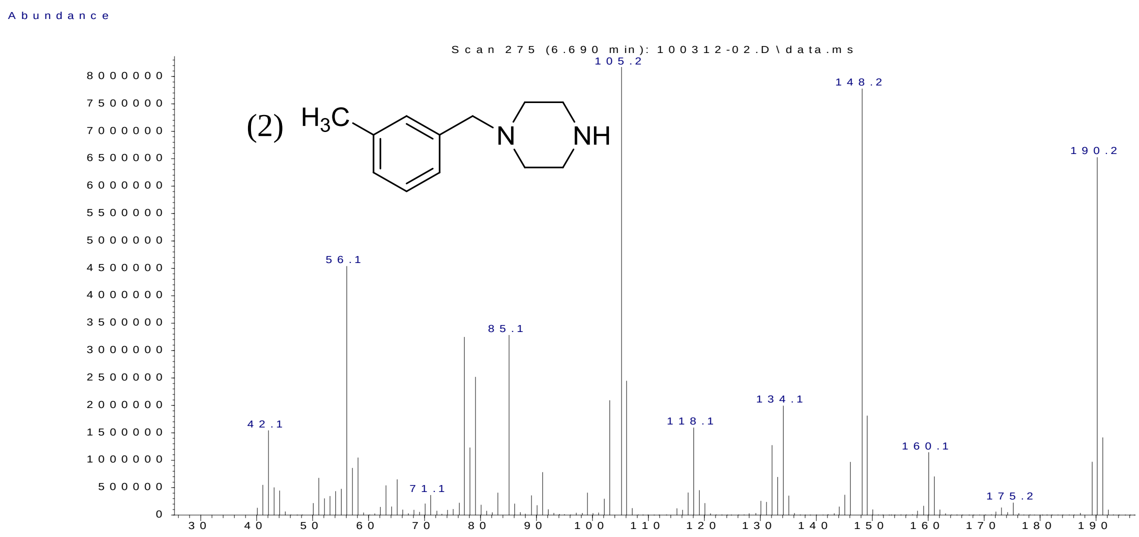
(Rtx-200). Gas chromatography coupled with time-of-flight mass spectrometry provides an additional means of differentiating between the isobaric methylbenzylpiperazine and benzoylpiperazine which have equivalent nominal masses but are different in their elemental composition and exact masses.

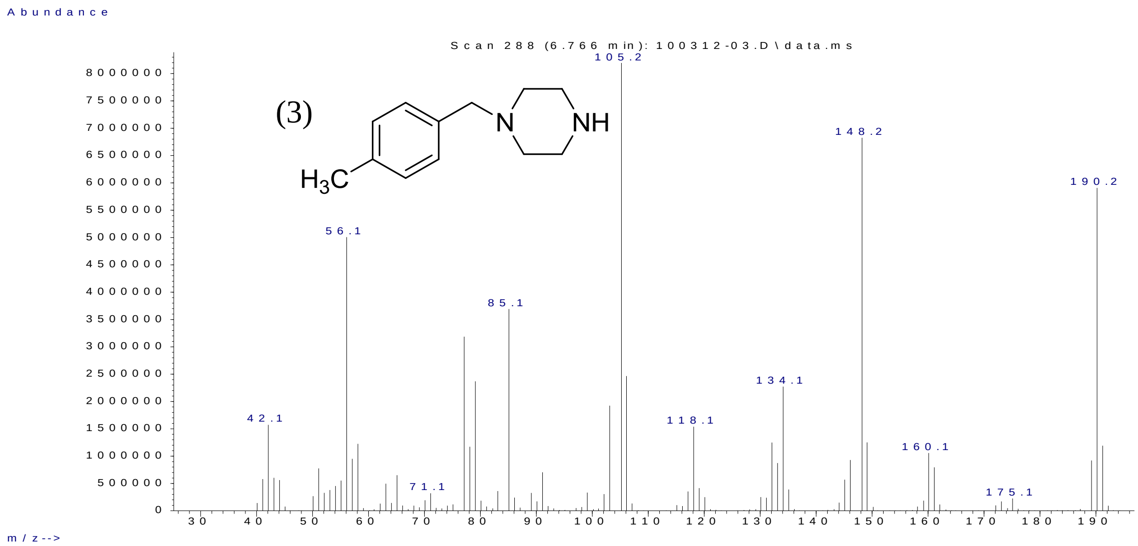
3.5.1. Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 28 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 Schemes 23 and 24 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.

The proposed structure for the m/z 122 $(C_7H_8NO)^+$ ion is shown in the fragmentation scheme in Scheme 24 and the equivalent ion has been confirmed by exact mass GC-TOF-MS analysis for the ring substituted methoxybenzoylpiperazines and will be discussed later in section 3.9.1. 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







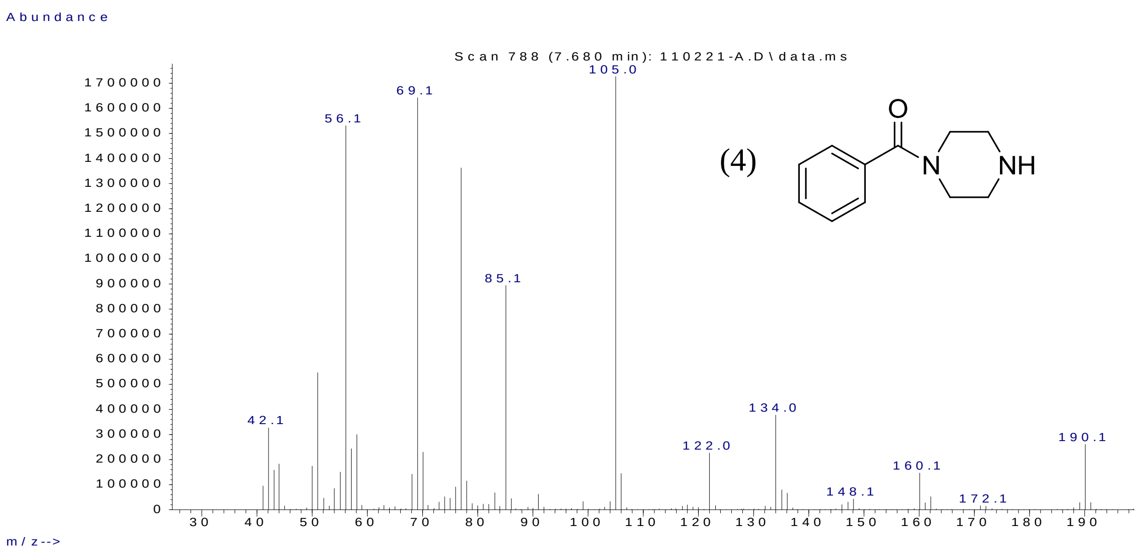
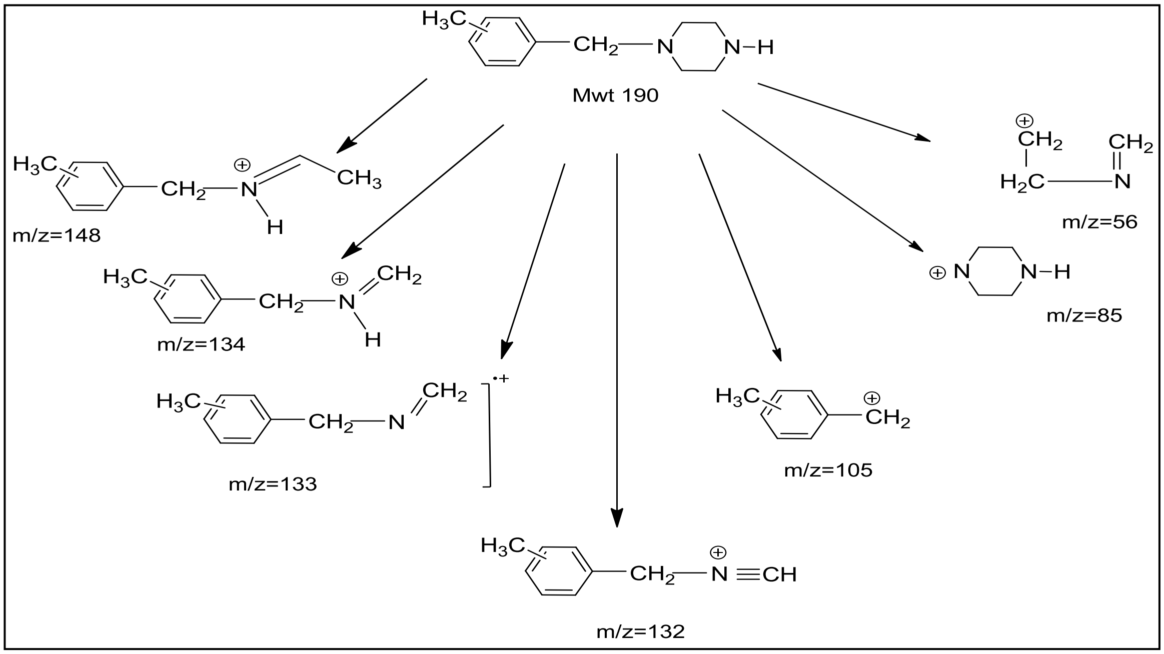
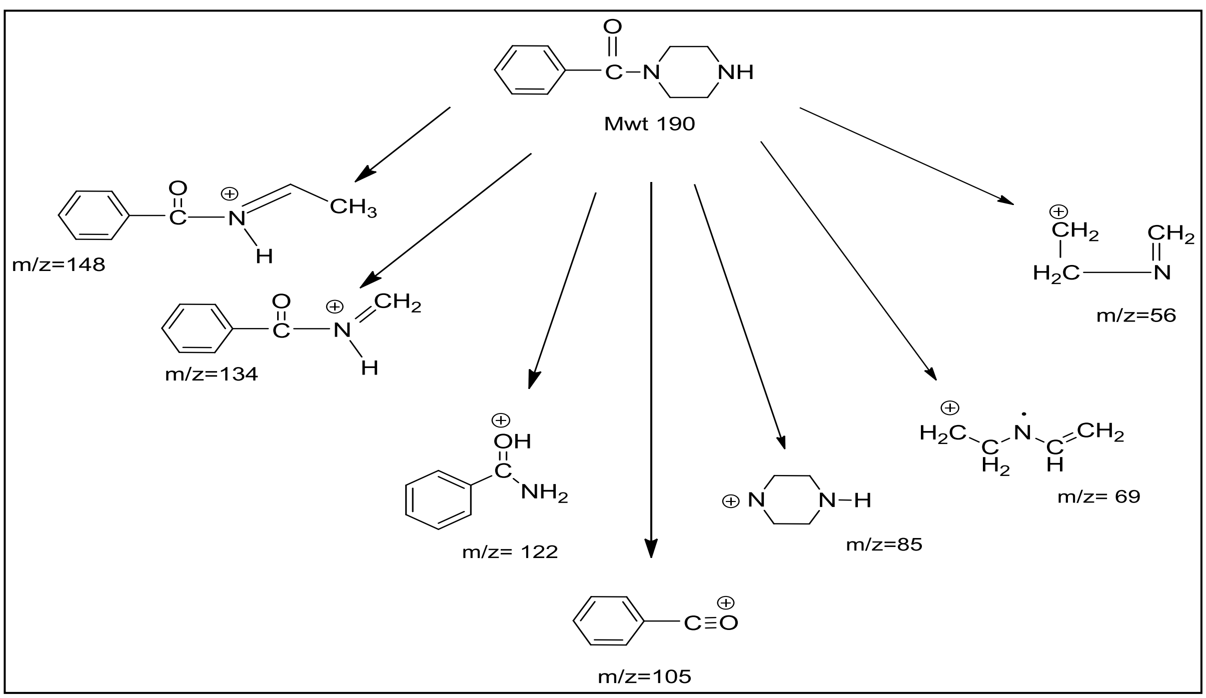


Fig. 28: Mass spectra of the four underivatized piperazines in this study.



Scheme 23. Mass spectral fragmentation pattern of the underivatized methylbenzylpiperazines under EI (70eV) conditions.



Scheme 24. Mass spectral fragmentation pattern of the underivatized benzoylpiperazine under EI (70eV) conditions.

the deuterium labeled form of Compound 4 is shown in Figure 29. The mass spectrum in Figure 29 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 30.

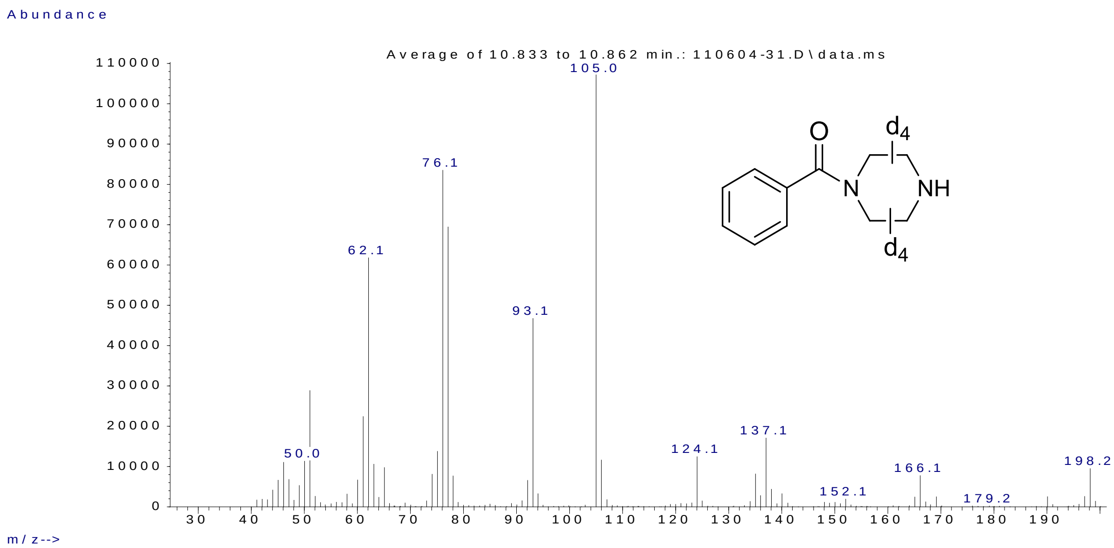


Fig. 29: Mass spectrum of the benzoyl-d_8-piperazine.

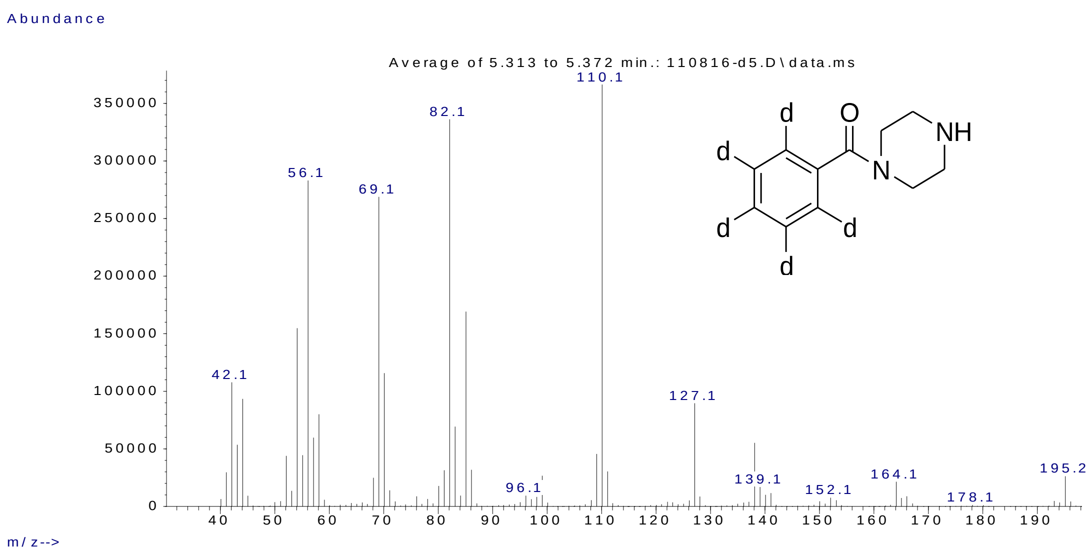


Fig. 30: Mass spectrum of the d5-benzoylpiperazine.

The mechanism of formation of the characteristic ions at m/z 122 and m/z 69 in the benzoylpiperazine is illustrated in Scheme 25. 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 Scheme 25 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 31). This compound is prepared using the same procedure outlined in Scheme 14 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 29 as the corresponding fragment is shifted 7 Da higher to become m/z 76.

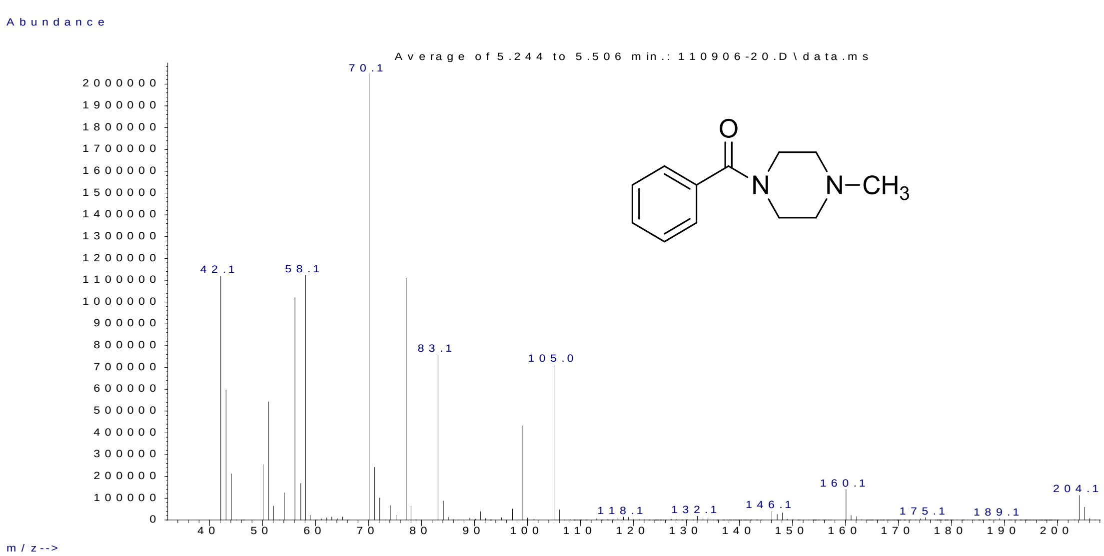
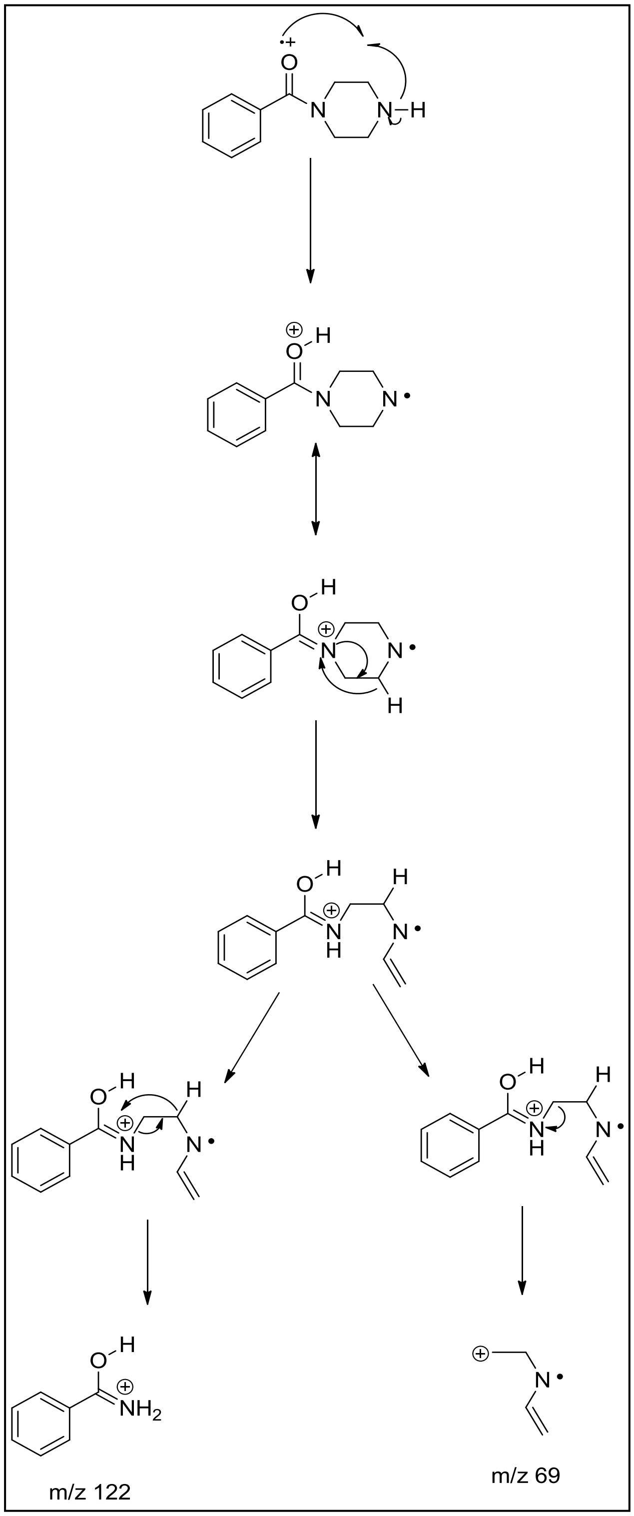
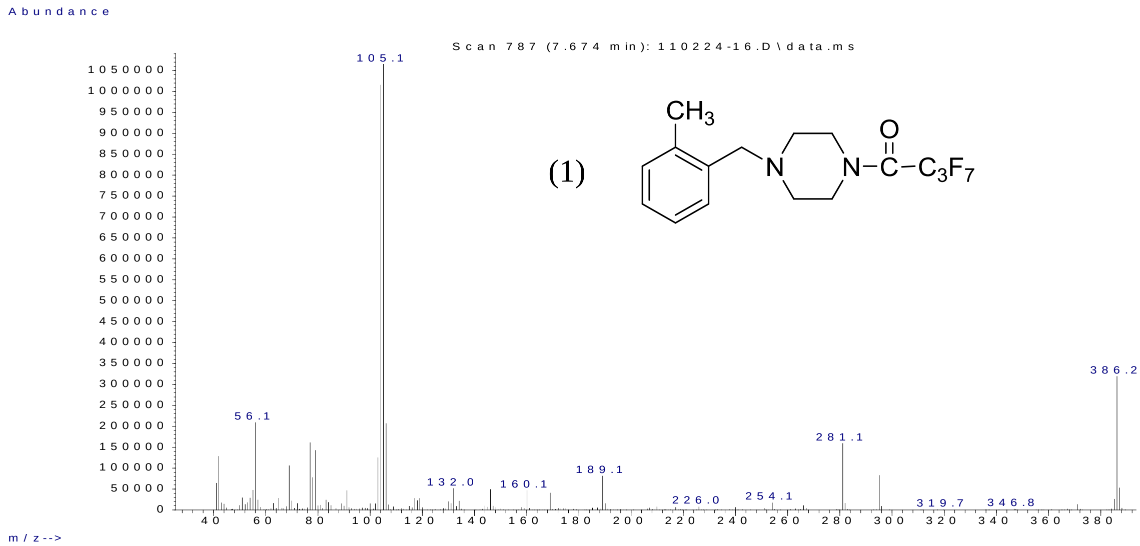


Fig. 31. Mass spectrum of the N-methylbenzoylpiperazine.



Scheme 25. Formation of the m/z 122 and m/z 69 ions in the benzoylpiperazine (BNZP).

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 32 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 $(M\text{-}105)^+$ 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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O
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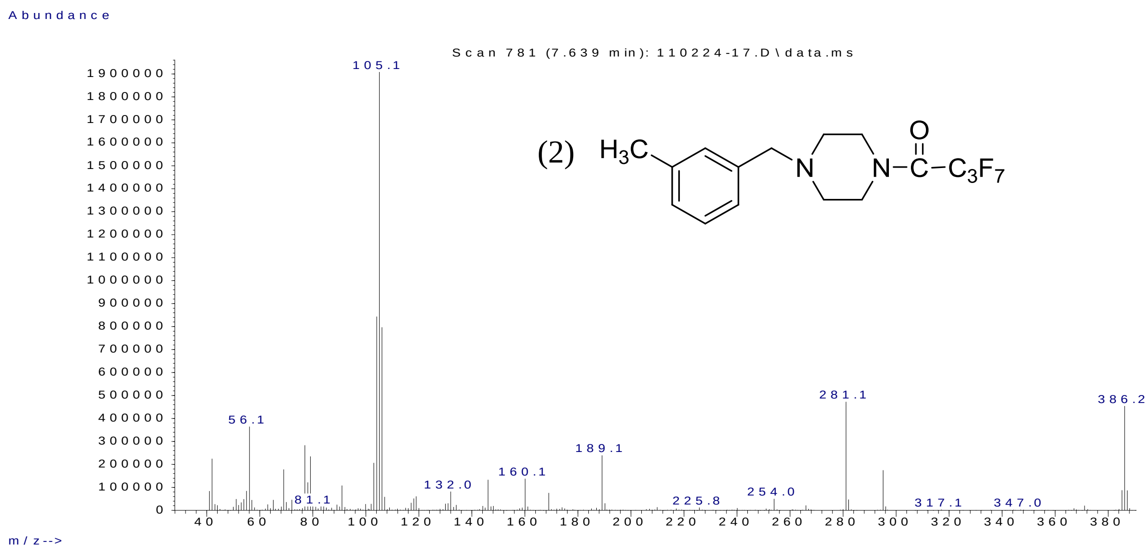
281.1
319.7
346.8
386.2
m/z-->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(2)
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N
O
C
C_3F_7
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225.8
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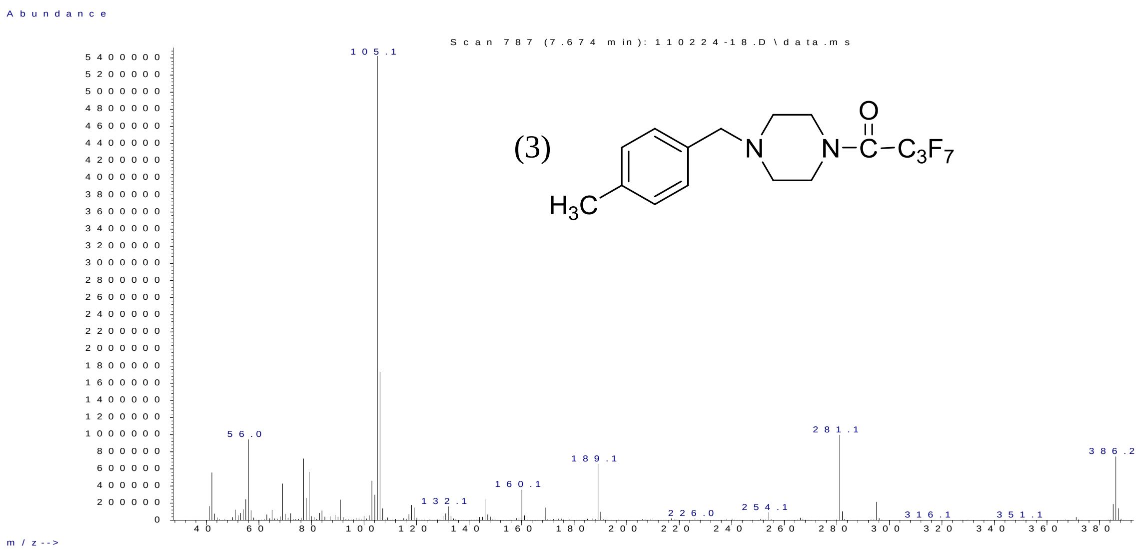
281.1
317.1
347.0
386.2
m/z-->



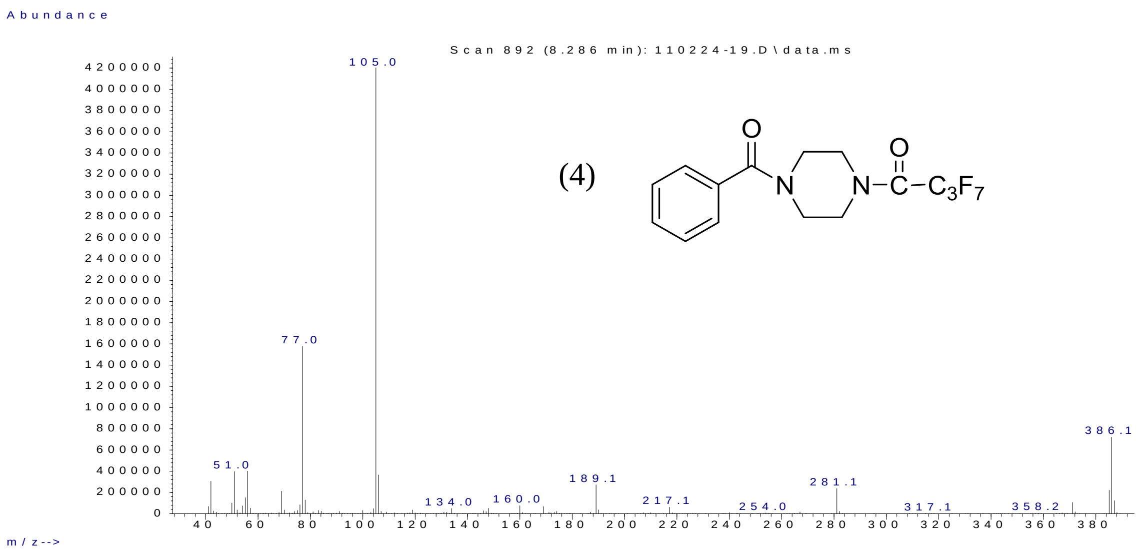


Fig. 32. Mass spectra of heptafluorobutyryl derivatives of the four piperazine compounds in this study.

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.

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 33 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 (33A) shows the expected/calculated mass for the C_8H_9 elemental composition. The lower panel (33B) 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. Figures 33C and 33D 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.

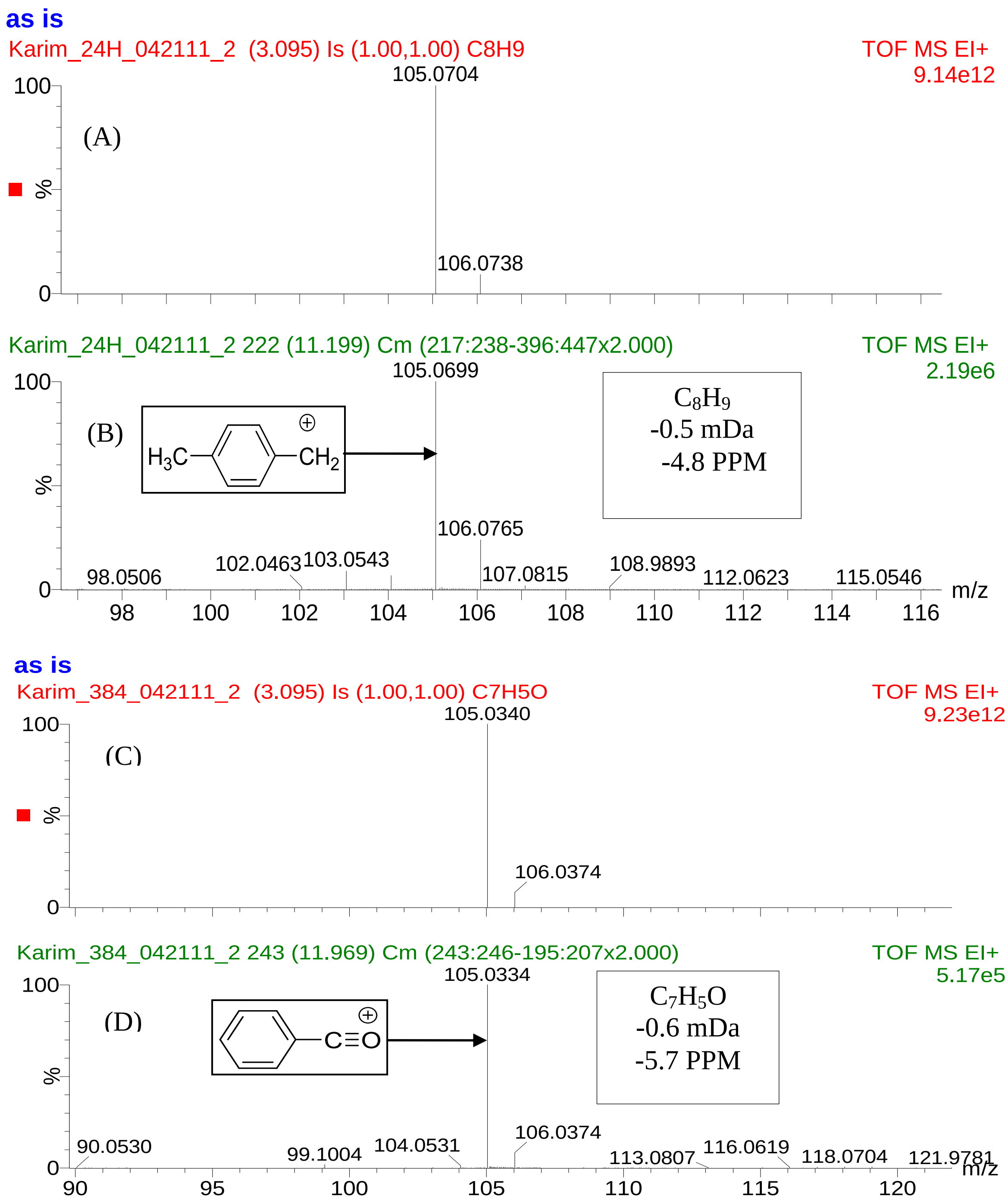


Fig. 33. GC-TOF mass spectral analysis of the m/z 105 ion for 4-methylbenzylpiperazine and for benzoylpiperazine. 33A= calculated mass for C_8H_9; 33B= experimental results. 33C= calculated mass for C_7H_5O; 33D= experimental results.

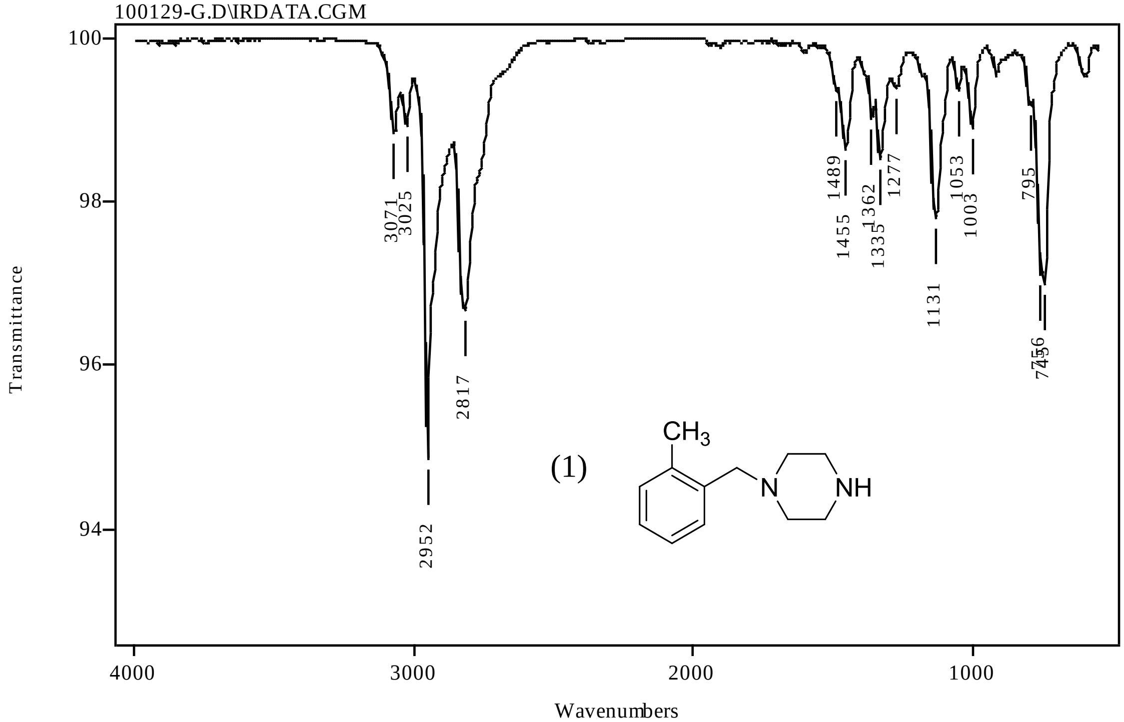
3.5.2. Vapor-phase Infra-Red Spectrophotometry

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the 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 34. 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} [Kempfert, 1988]. 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}.

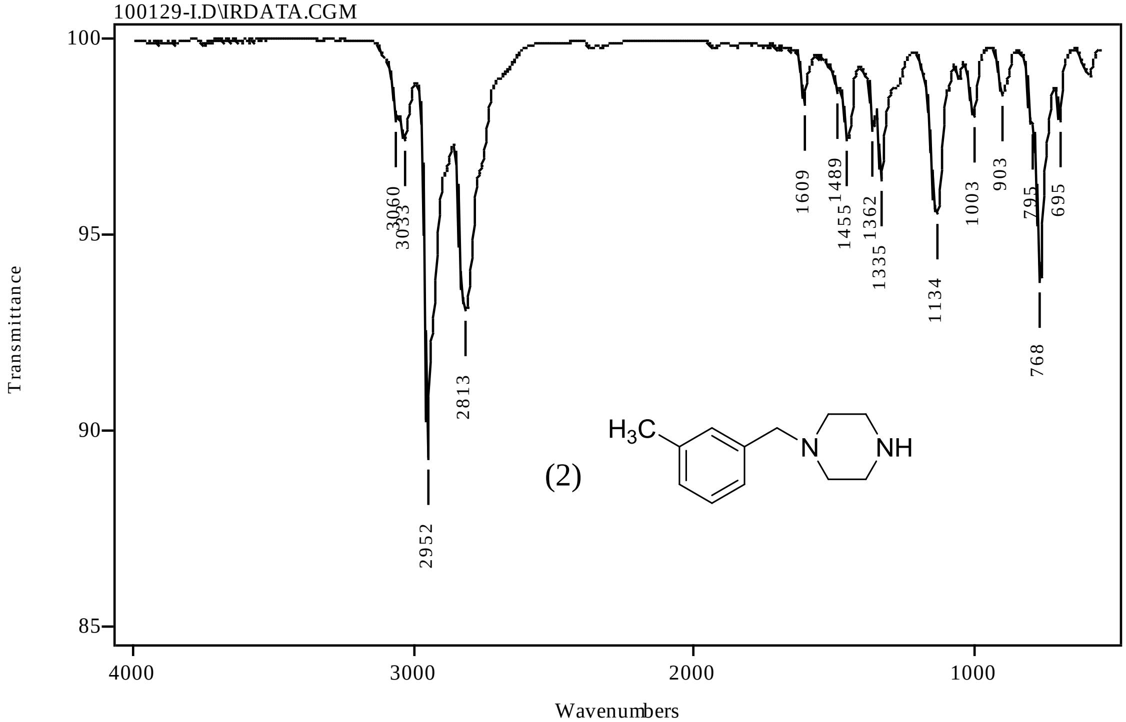
The benzoylpiperazine shows a characteristic strong singlet IR band at 1671 cm^{-1} corresponding to the carbonyl group stretching which can distinguish this benzoylpiperazine from the three ring substituted methylbenzylpiperazines. In addition, this compound shows other strong characteristic singlets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

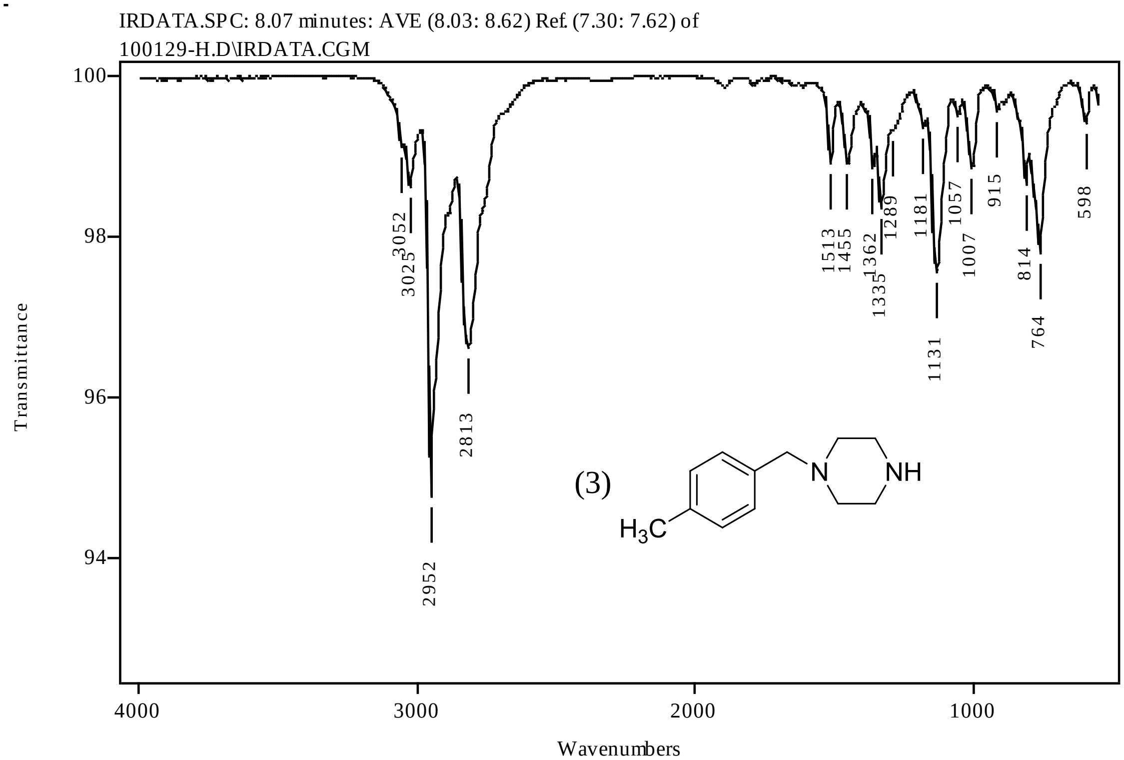
The three ring substituted methylbenzylpiperazines share almost the same IR features in the region of 2700 – 3100 cm^{-1}. However, they can be differentiated by the positions and intensities of several IR peaks in the region of 650 – 1700 cm^{-1}. Compound 3 shows a medium intensity doublet at 1513 cm^{-1}, 1455 cm^{-1} which is shifted to a weak intensity doublet at 1489 cm^{-1}, 1455

IRDATA.SPC: 8.00 minutes: AVE (7.97: 8.55) Ref. (7.27: 7.54) of
100129-G.D\IRDATA.CGM



IRDATA.SPC: 8.03 minutes: AVE (7.99: 8.35) Ref. (6.43: 7.91) of
100129-I.D\IRDATA.CGM





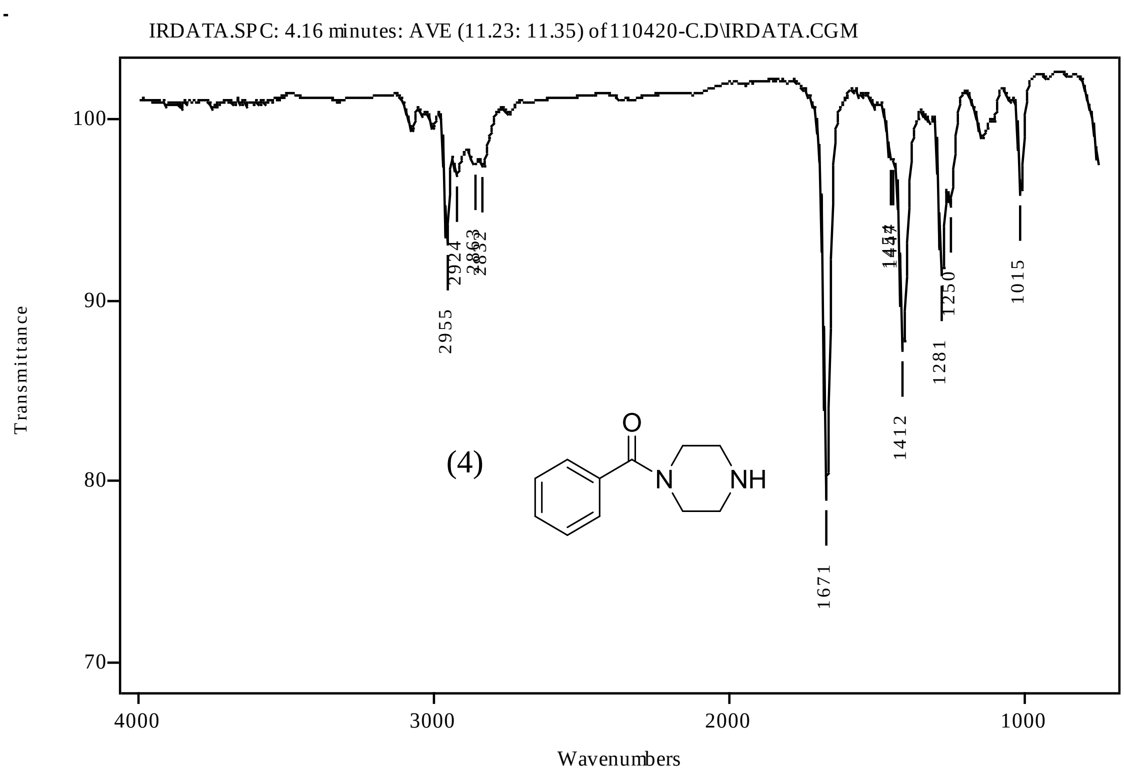


Fig. 34. Vapor phase IR spectra of the four piperazine compounds in this study.

cm^{-1} in compounds 1 and 2. Compound 2 shows a medium peak at 1134 cm^{-1} which is shifted to a peak at 1131 cm^{-1} in both compounds 1 and 3. Compound 2 also has a medium intensity peak at 1609 cm^{-1} which is absent in compounds 1 and 3. These results 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.

3.5.3. Gas Chromatographic Separation

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 35 is representative of the results obtained for all samples on this stationary phase.

In Figure 35 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.

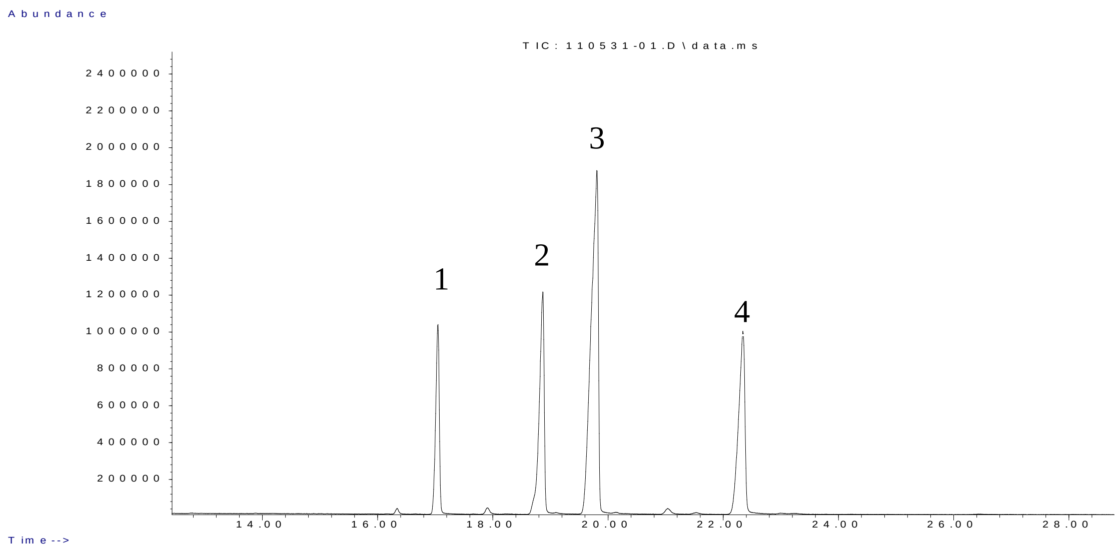


Fig. 35. Gas chromatographic separation of the four pentafluoropropionyl derivatives using Rtx-200 column. The number over the peak corresponds to the compound number.

3.5.4. 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.

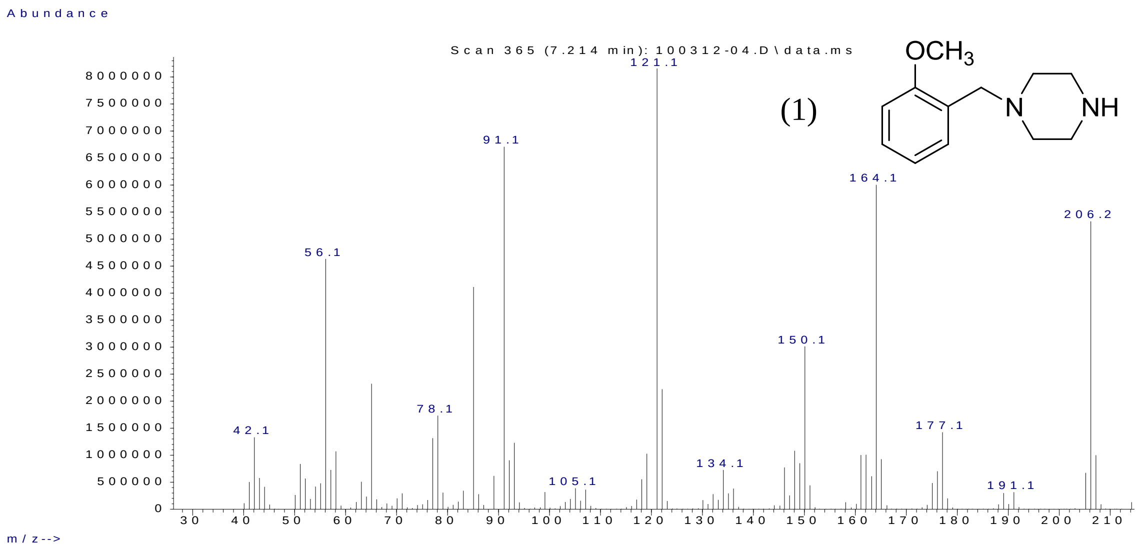
3.6. Differentiation of the monomethoxybenzylpiperazines (OMeBPs) by GC-IRD and GC-MS

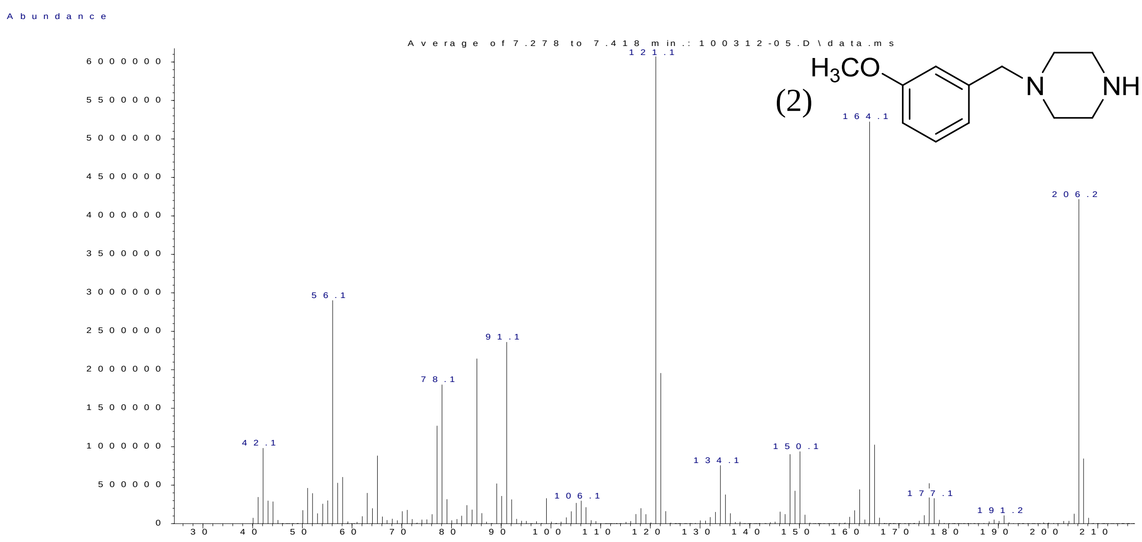
Three ring substituted methoxybenzylpiperazines (OMeBPs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not 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).

3.6.1. Mass spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 36 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 Scheme 26 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)


Abundance
Scan 365 (7.214 min): 100312-04.D\data.ms
OCH3
N
NH
(1)
121.1
91.1
164.1
206.2
56.1
150.1
78.1
42.1
177.1
134.1
105.1
191.1
m/z-->


Abundance
Average of 7.278 to 7.418 min.: 100312-05.D\data.ms
H3CO
N
NH
(2)
121.1
164.1
206.2
56.1
91.1
78.1
42.1
150.1
134.1
106.1
177.1
191.2
m/z-->

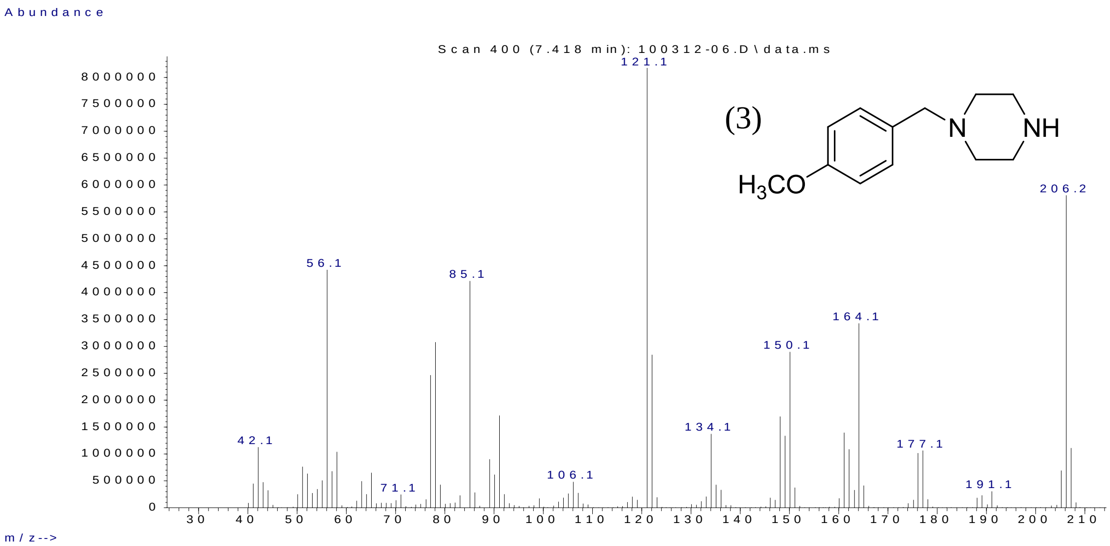
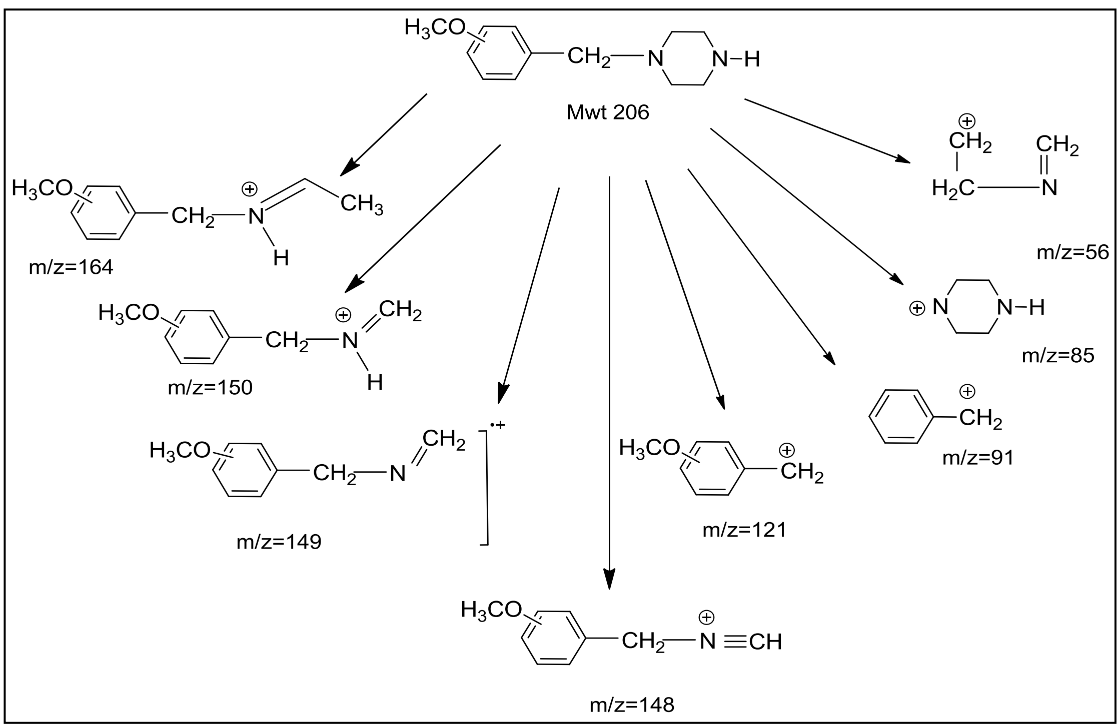
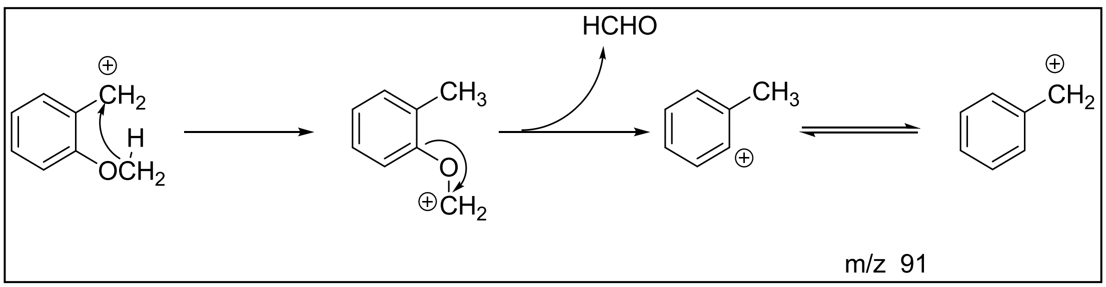


Fig. 36. EI mass spectra of the three methoxybenzylpiperazines.



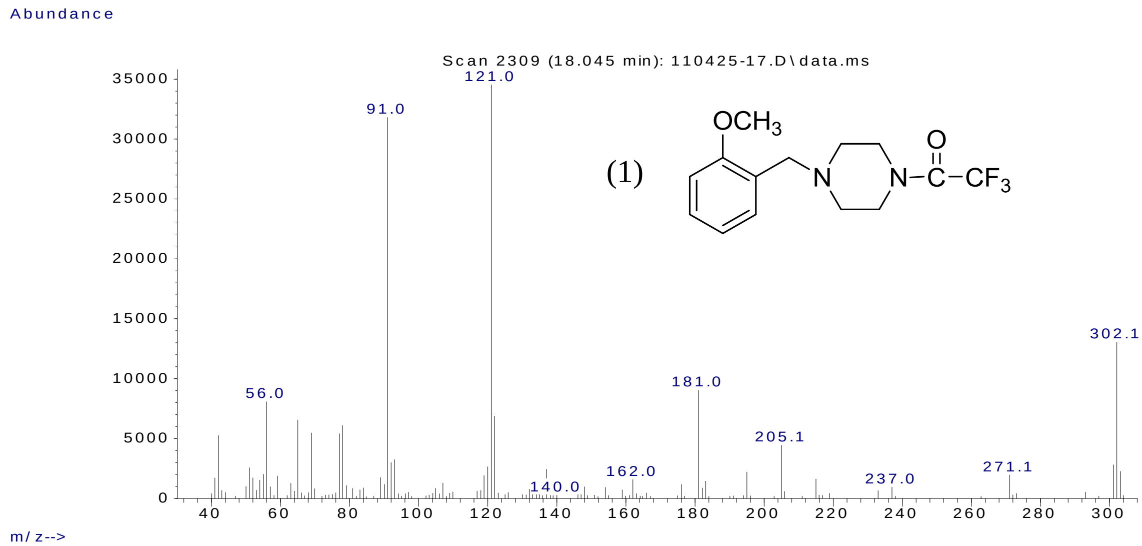
Scheme 26. EI mass spectral fragmentation pattern of the underivatized methoxybenzylpiperazines.

have almost identical mass spectra to each other. An additional fragmentation pathway similar to that described before in section 3.3.1 which is characteristic for the ortho-methoxy ring substituted compound (compound 1) is described in Scheme 27. 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 Scheme 27.

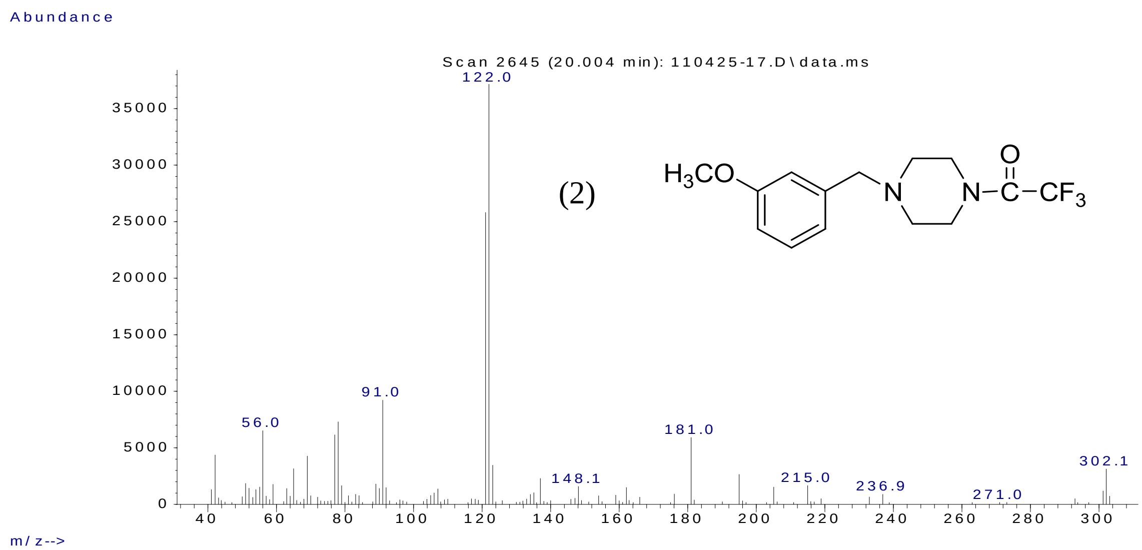


Scheme 27. Mechanism for the formation of the m/z 91 ion in the mass spectra of the regioisomers of the methoxybenzylpiperazines.

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 37 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 $(M-121)^+$ 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.



Abundance
Scan 2309 (18.045 min): 110425-17.D\data.ms
121.0
91.0
56.0
140.0
162.0
181.0
205.1
237.0
271.1
302.1
(1)
OCH3
N
N
O
C
CF3
m/z-->



Abundance
Scan 2645 (20.004 min): 110425-17.D\data.ms
122.0
91.0
56.0
148.1
181.0
215.0
236.9
271.0
302.1
(2)
H3CO
N
N
O
C
CF3
m/z-->

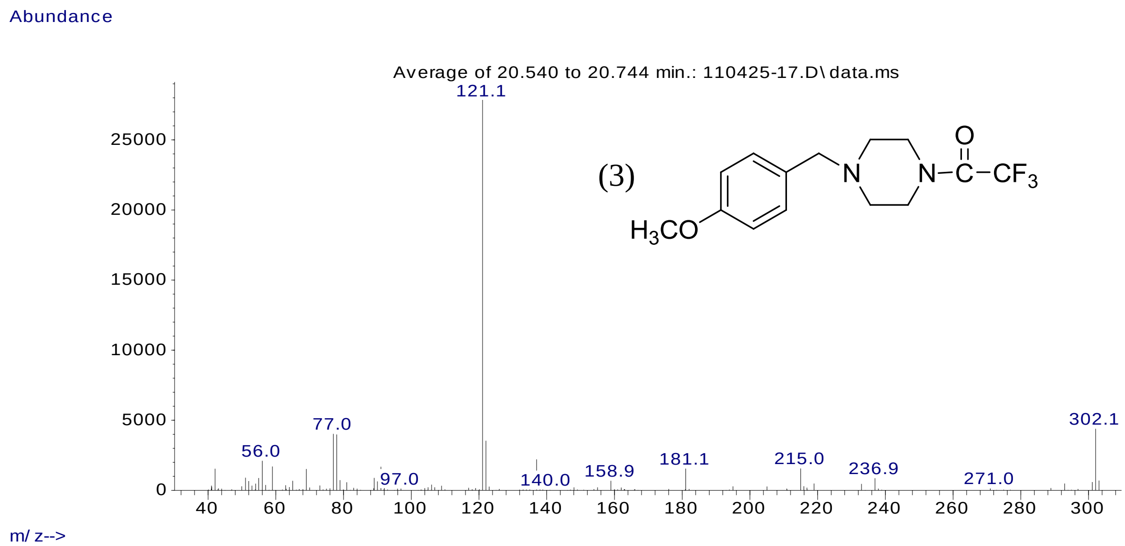
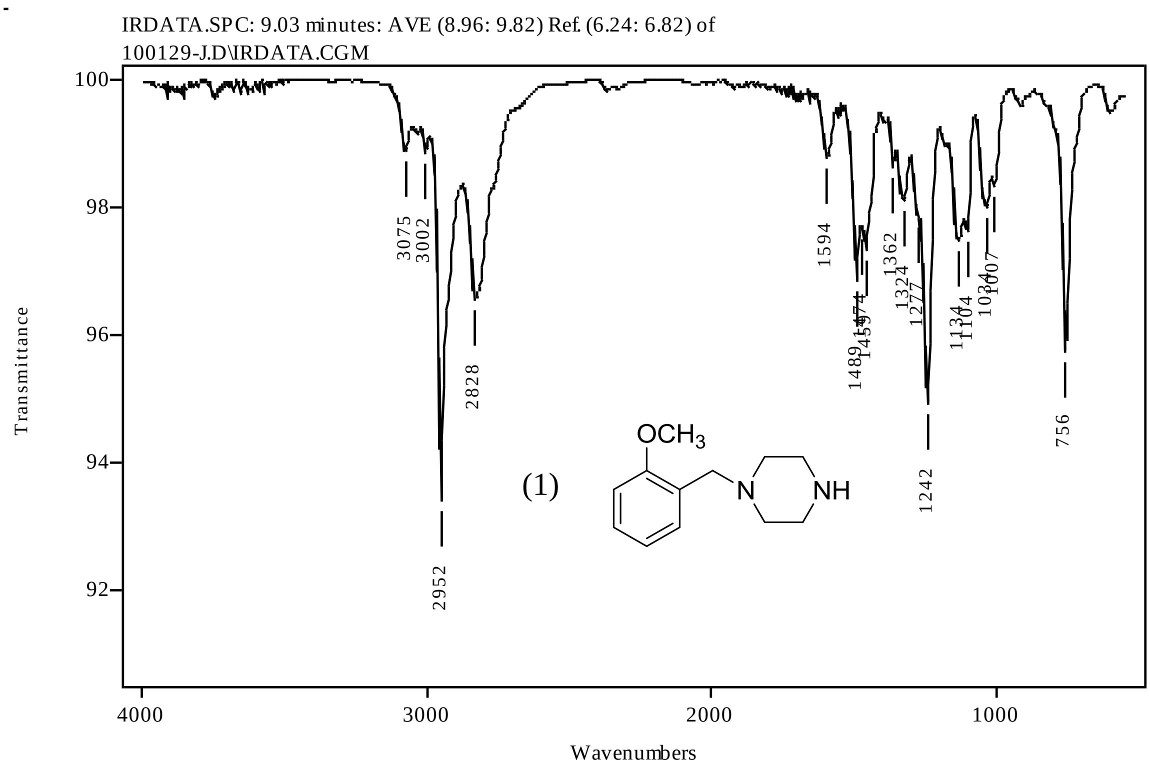


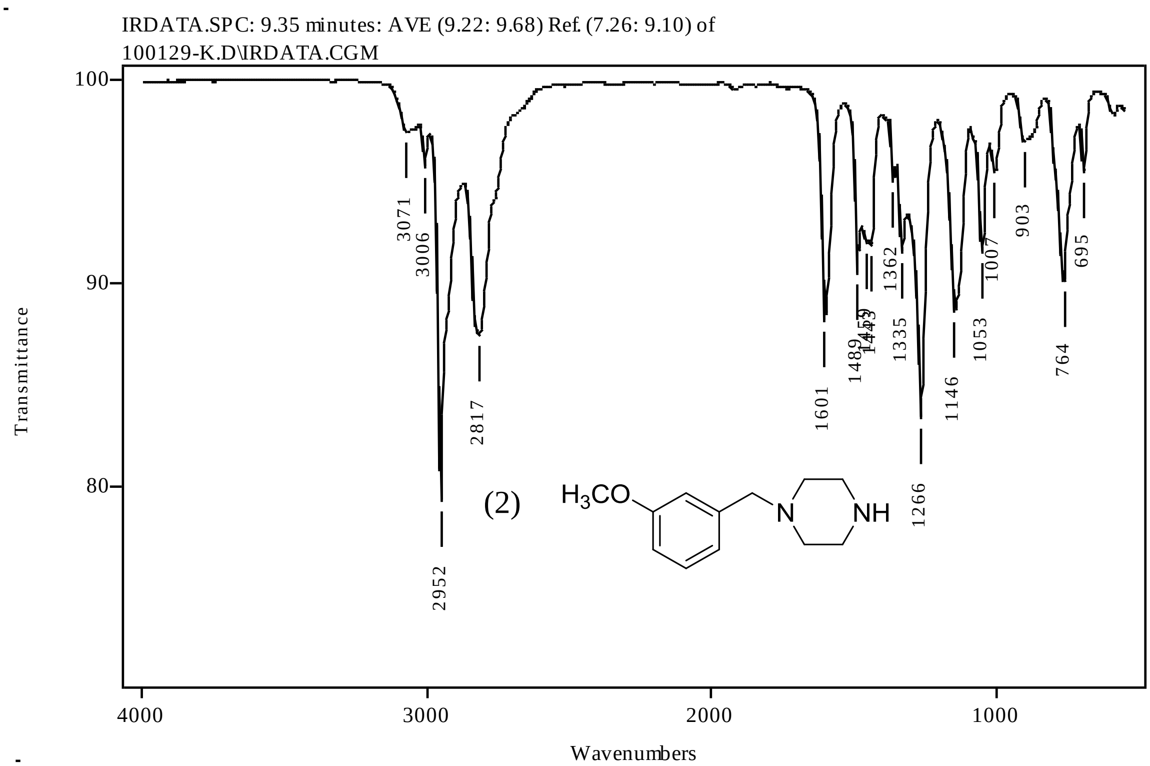
Fig. 37. Mass spectra of trifluoroacetyl derivatives of the three piperazine compounds.

3.6.2. Vapor-phase Infra-Red Spectrophotometry

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the 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 38. 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.





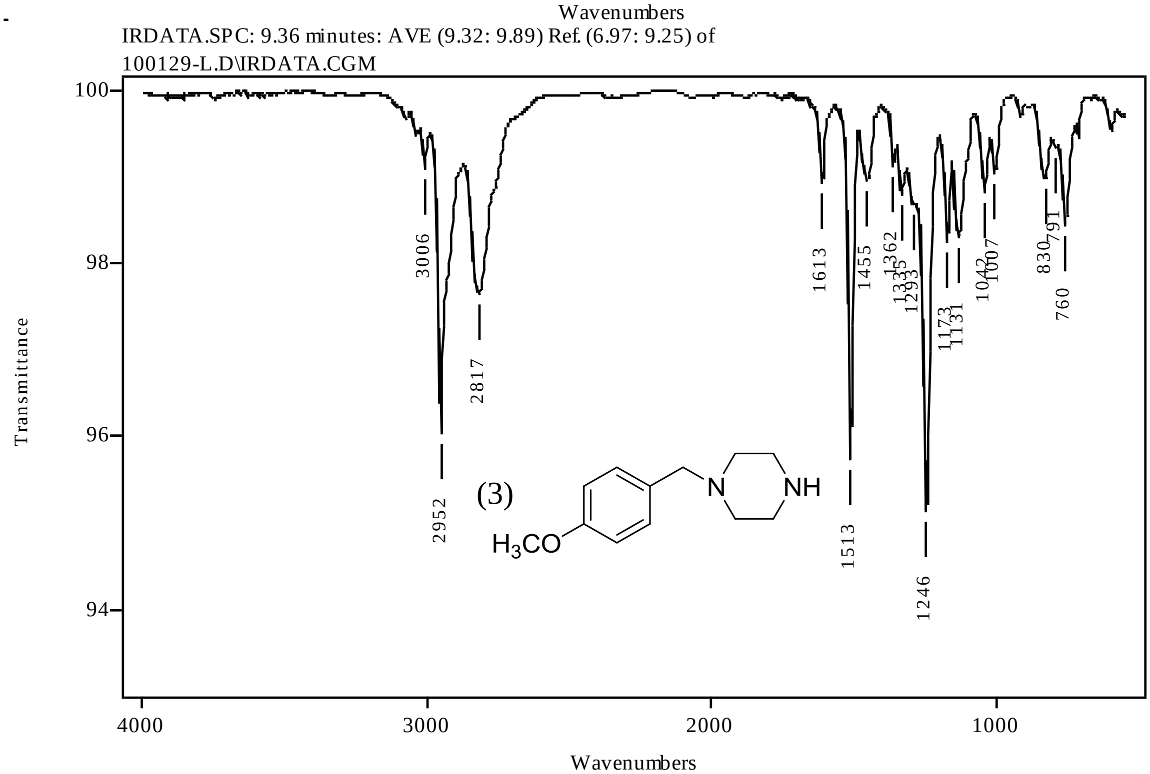


Fig. 38. Vapor phase IR spectra of the three methoxybenzylpiperazines.

3.6.3. Gas Chromatographic Separation

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 39 and 40 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.

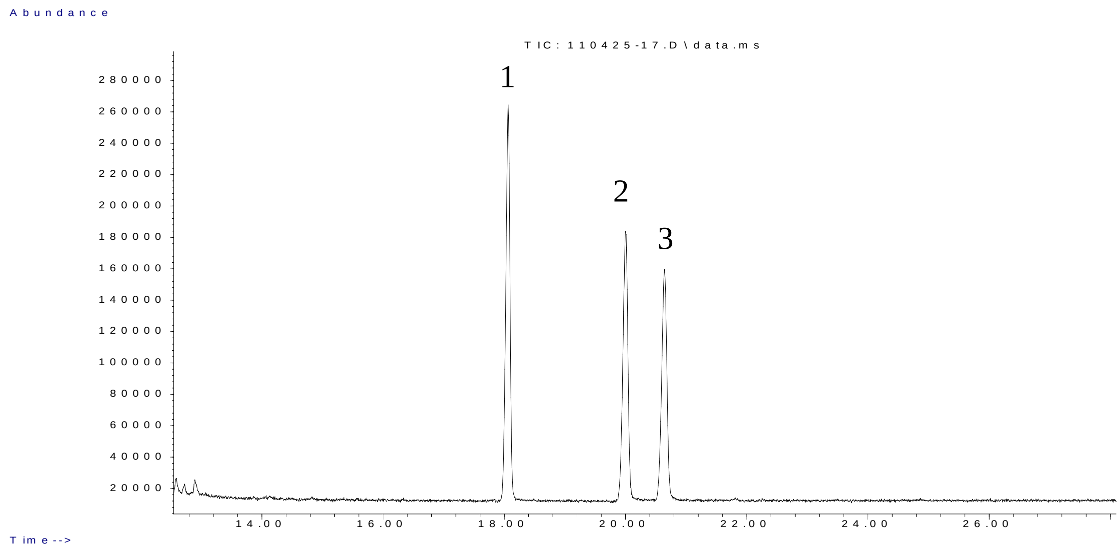


Fig. 39. Gas chromatographic separation of the trifluoroacetyl derivatives of the OMeBPs using Rtx-200 column. The number over the peak corresponds to the compound number.

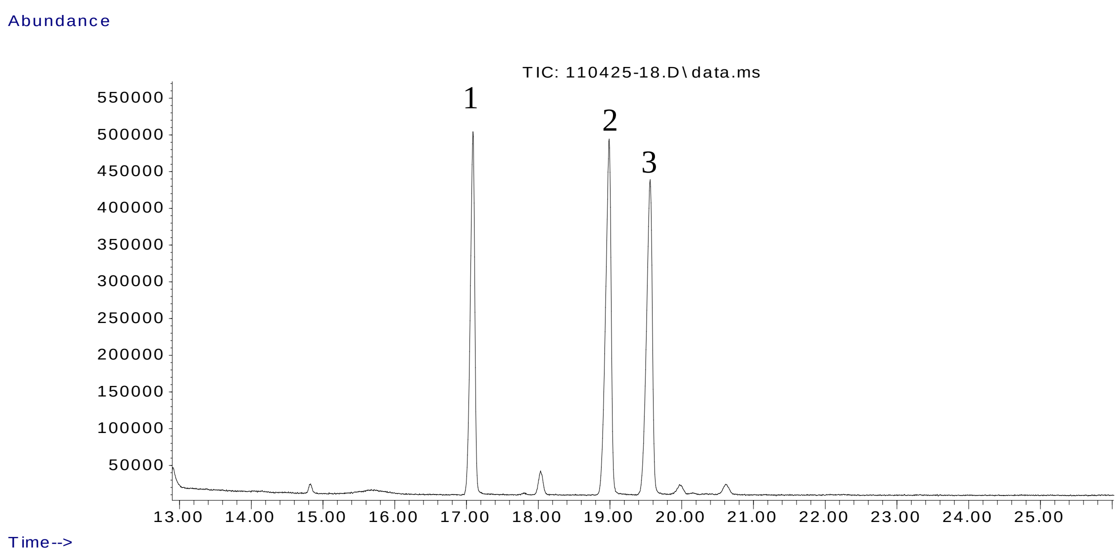


Fig. 40. Gas chromatographic separation of the pentafluoropropionyl derivatives of the OMeBPs using Rtx-200 column. The number over the peak corresponds to the compound number.

3.6.4. Conclusion

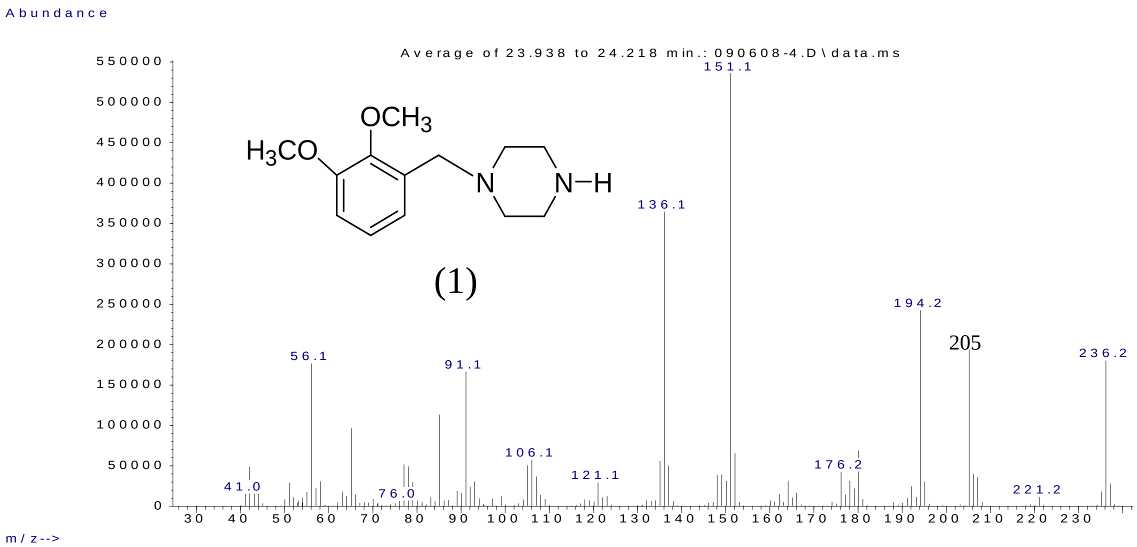
The three regioisomeric methoxybenzylpiperazines have a regioisomeric relationship to each other. These three piperazines yield very similar fragment ions in their mass spectra Chemical derivatization (perfluoroacylation) did not offer any additional unique marker ions to allow identification of one 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.

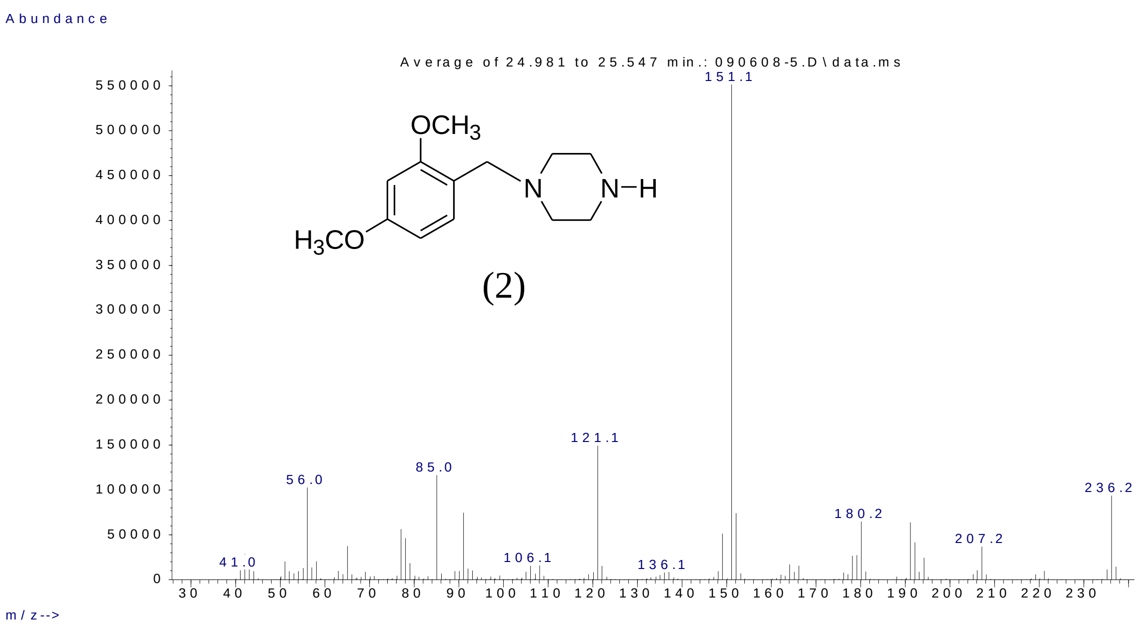
3.7. GC-MS and GC-IRD Studies on the Six Ring Regioisomeric Dimethoxybenzylpiperazines (DMBPs)

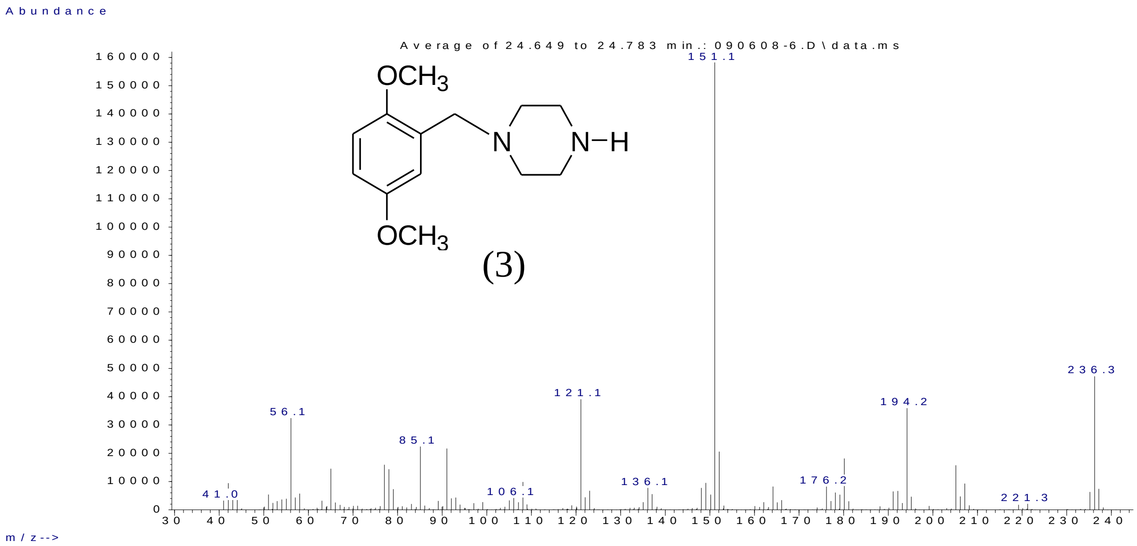
Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the differentiation between the six regioisomeric dimethoxybenzylpiperazines. These six regioisomeric substances are resolved by GC and the vapor phase infrared spectra clearly differentiate among the six dimethoxybenzyl 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.

3.7.1. Mass Spectral Studies

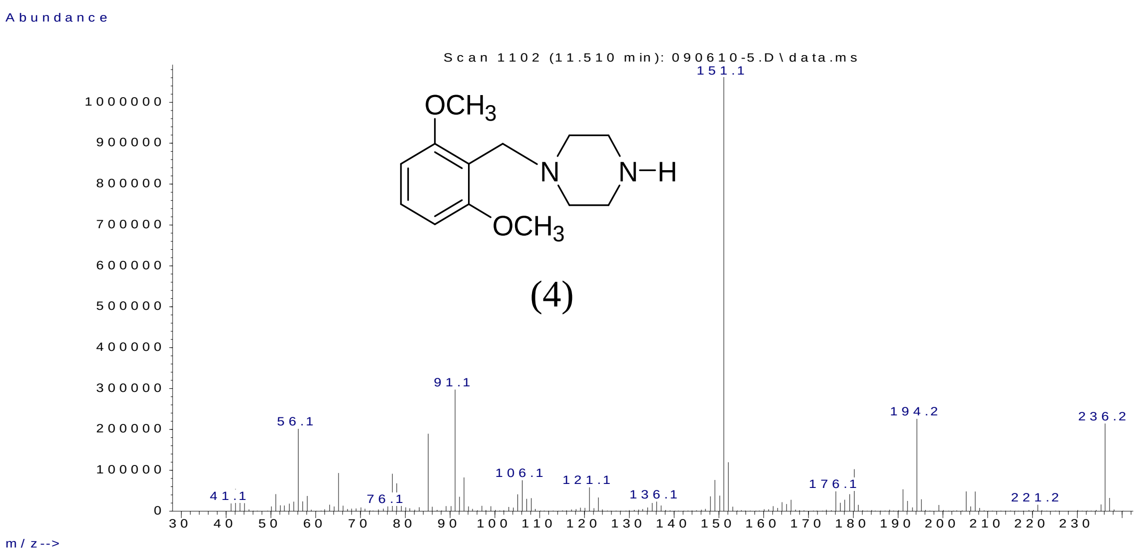
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 41 shows the EI mass spectra of the six regioisomeric dimethoxybenzylpiperazines (Compounds 1-6). The mass spectra in Figure 41 indicate that very little structural information is available for differentiation 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 Scheme 28. 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.


Abundance
Average of 23.938 to 24.218 min.: 090608-4.D\data.ms
H3CO
OCH3
N
N-H
(1)
151.1
136.1
194.2
205
236.2
56.1
91.1
106.1
121.1
176.2
41.0
76.0
221.2
m/z-->

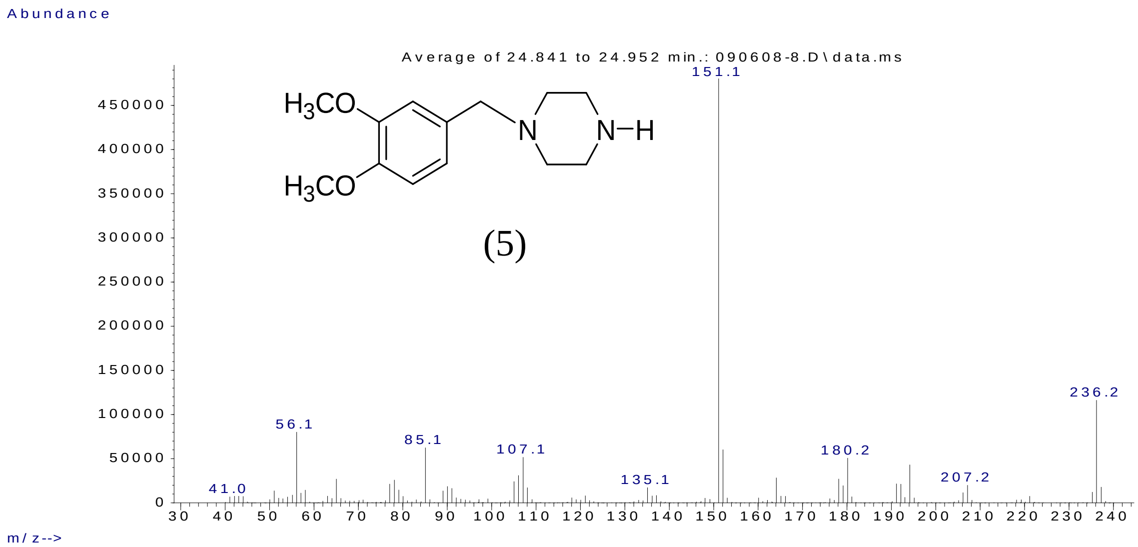

Abundance
Average of 24.981 to 25.547 min.: 090608-5.D\data.ms
OCH3
H3CO
N
N-H
(2)
151.1
121.1
85.0
56.0
236.2
180.2
207.2
106.1
136.1
41.0
m/z-->


Abundance
Average of 24.649 to 24.783 min.: 090608-6.D\data.ms
OCH3
N
N−H
OCH3
151.1
236.3
121.1
194.2
56.1
85.1
136.1
176.2
106.1
41.0
221.3
m/z-->

(3)


Abundance
Scan 1102 (11.510 min): 090610-5.D\data.ms
OCH3
N
N−H
OCH3
151.1
91.1
194.2
236.2
56.1
106.1
121.1
136.1
176.1
41.1
76.1
221.2
m/z-->

(4)



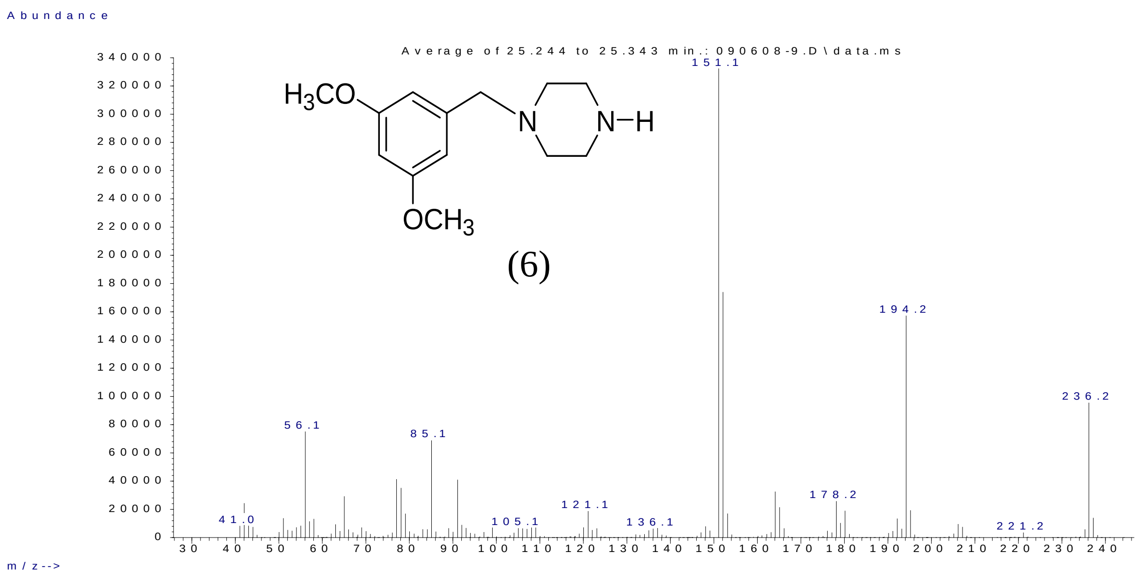
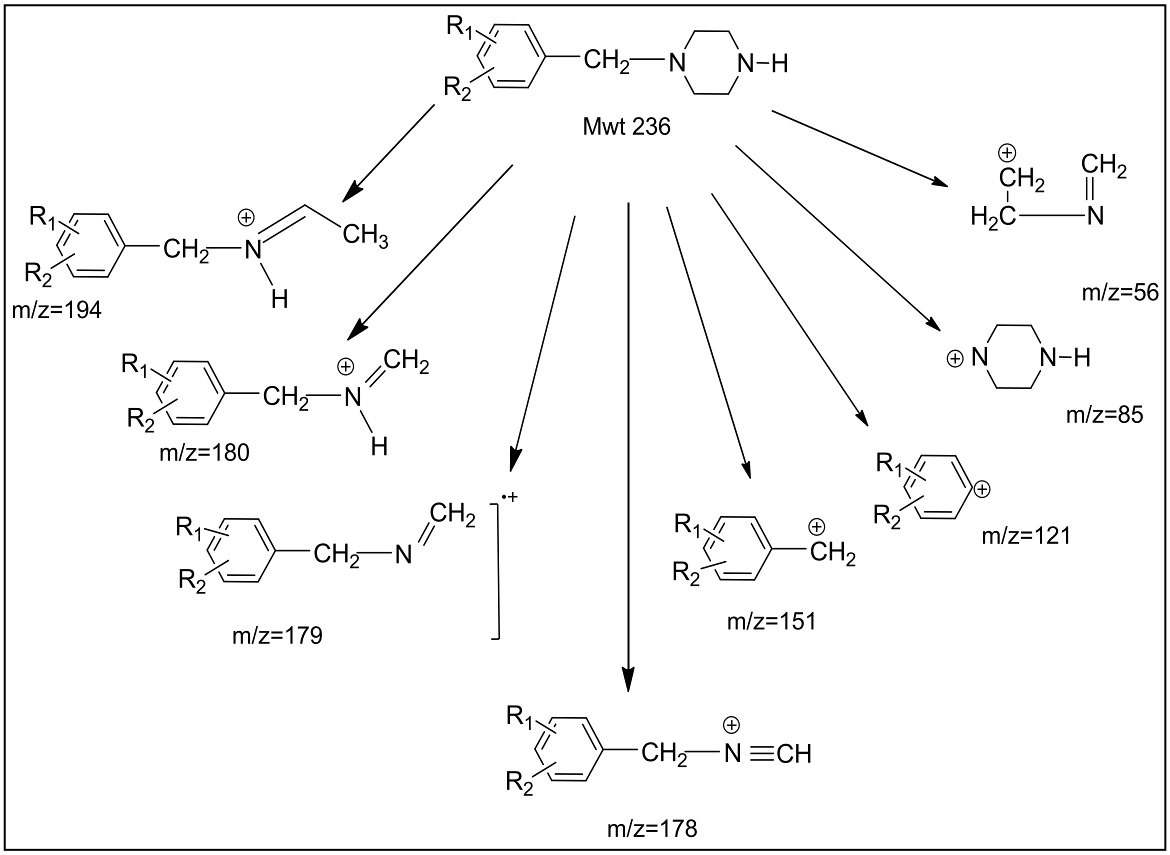


Fig. 41. EI mass spectra of the six dimethoxybenzylpiperazines.

Exact mass analysis using GC-TOF-MS confirmed the m/z 136 ion as the elemental composition $C_8H_8O_2$. Figure 42 shows the exact mass measurement results for the m/z 136 ion in the 2,3-isomer. The upper panel (42A) shows the expected/calculated mass for the $C_8H_8O_2$ elemental composition and the lower panel (42B) 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 Scheme 29. 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.



$R_1 = R_2 = OCH_3$

Scheme 28. EI mass spectral fragmentation pattern of the underivatized dimethoxybenzylpiperazines.

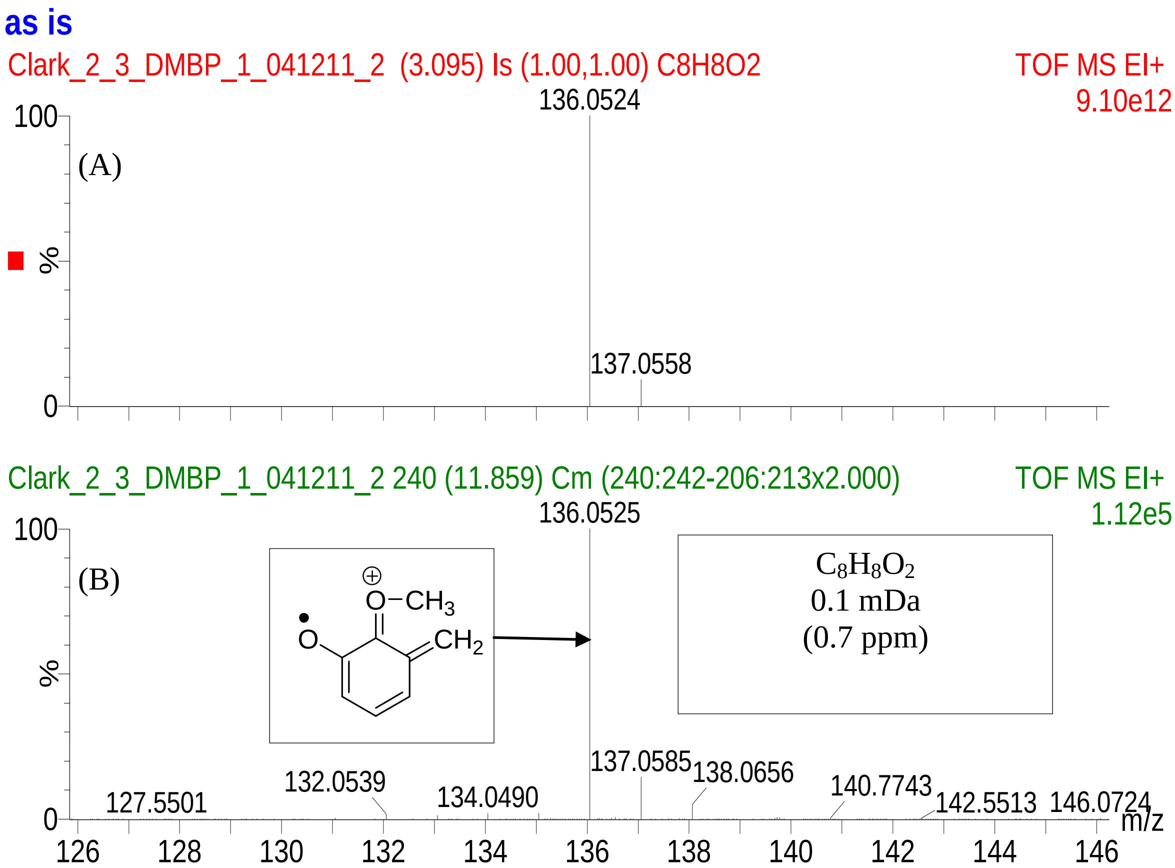
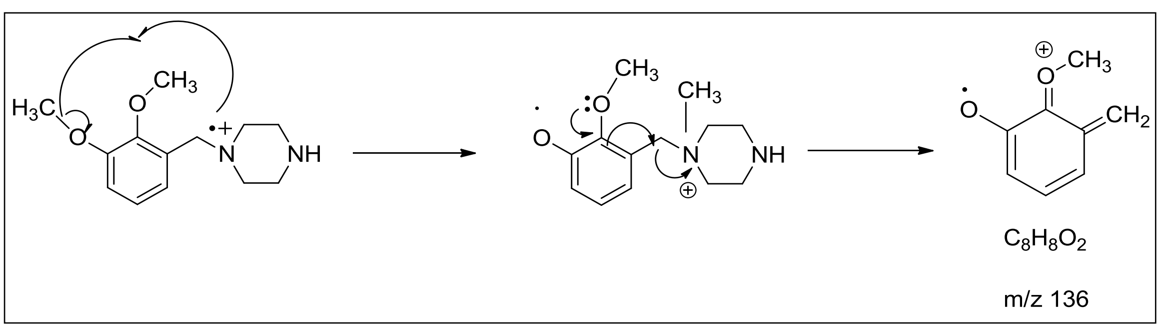


Fig. 42. GC-TOF mass spectral analysis of the m/z 136 ion for 2,3-dimethoxybenzylpiperazine.

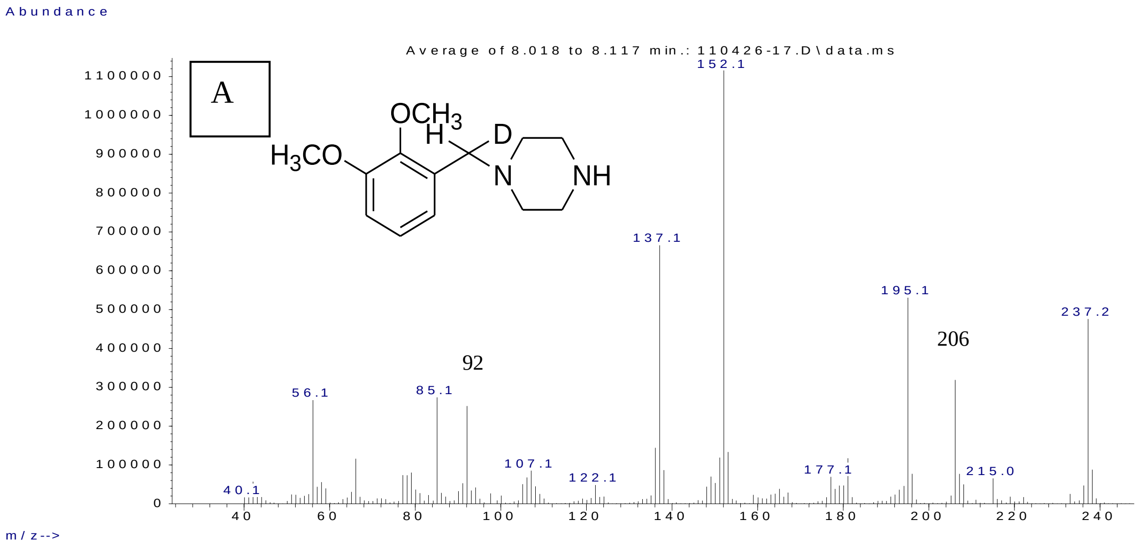


Scheme 29. Proposed mechanism for the formation of the m/z 136 ion in the mass spectrum of 2,3- dimethoxybenzylpiperazine.

The mass spectra for the three deuterium labeled forms of Compound 1 are shown in Figure 43. The spectrum in Figure 43A 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 43B). The methyl groups of both methoxy substituents are labeled with deuterium (di-OCD_3) in this form of Compound 1. The spectrum in Figure 43B 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 43C 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 Scheme 29. The mass spectrum in Figure 43C 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 Scheme 29 shows the migration of the methyl group from the 3-methoxy substituent to the piperazine nitrogen to yield a quaternary 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


Abundance
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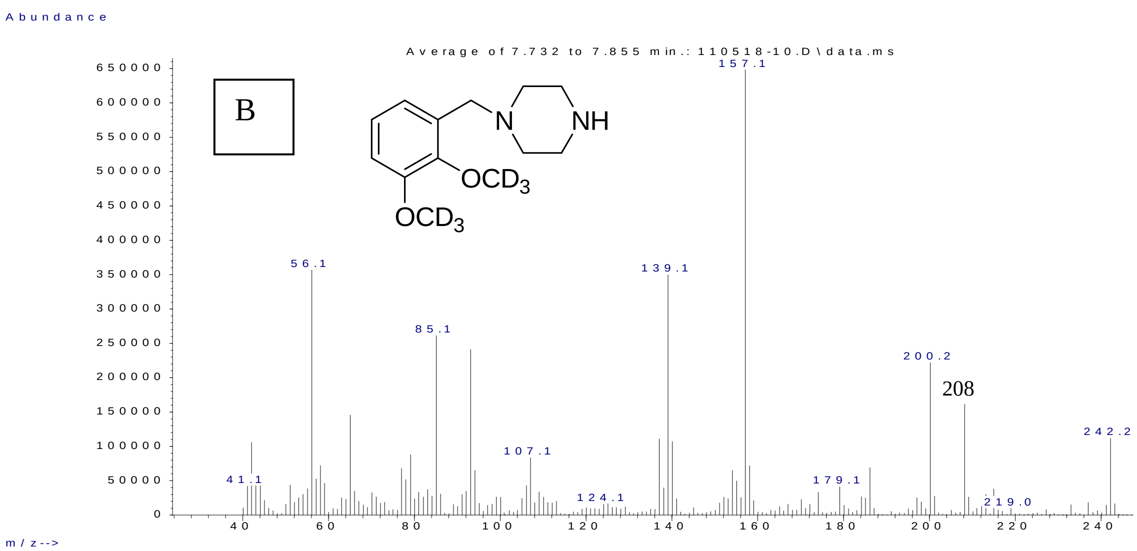
A
OCH3
H3CO
H
D
N
NH
152.1
137.1
195.1
237.2
206
92
56.1
85.1
107.1
122.1
177.1
215.0
40.1
m/z-->


Abundance
Average of 7.732 to 7.855 min.: 110518-10.D\data.ms
B
N
NH
OCD3
OCD3
157.1
56.1
139.1
85.1
200.2
208
242.2
107.1
41.1
179.1
124.1
219.0
m/z-->

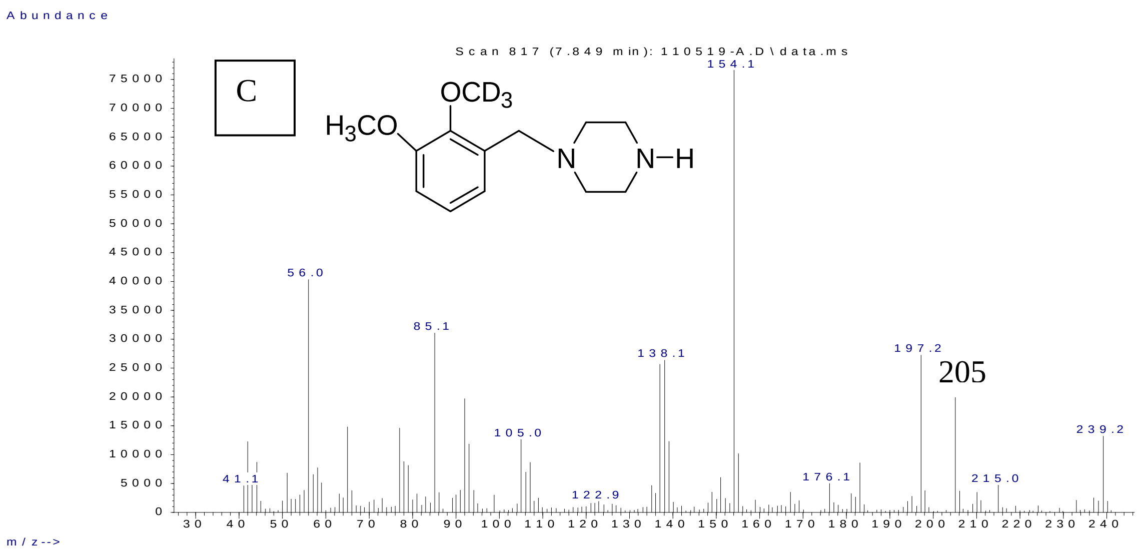


Fig. 43. Mass spectra for d_1, d_6, and d_3-2,3-dimethoxybenzylpiperazine.

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 44 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 Scheme 29. The mass spectrum in Figure 44 is for

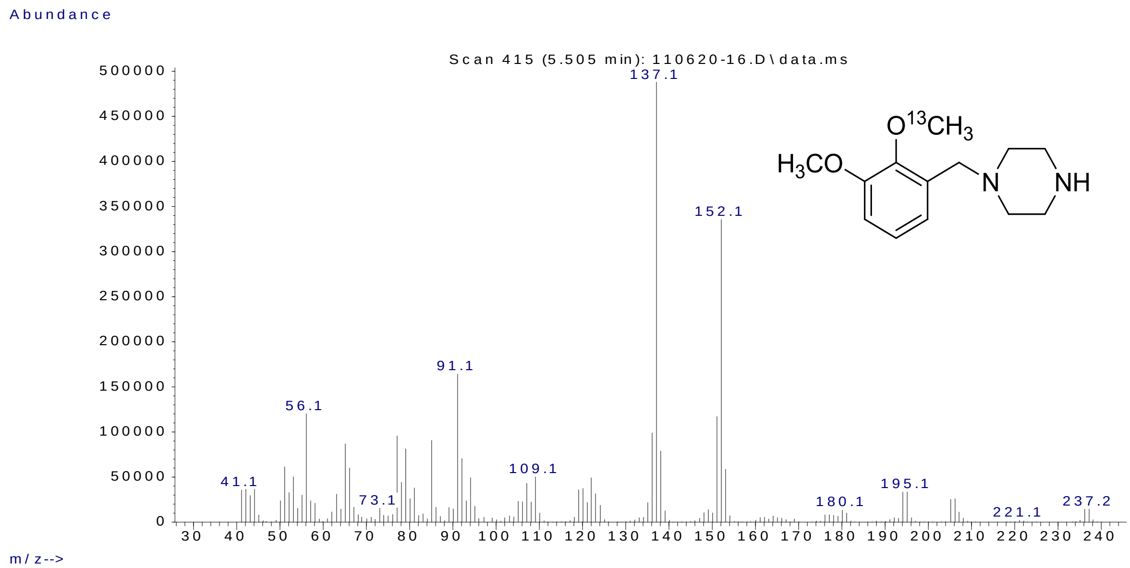
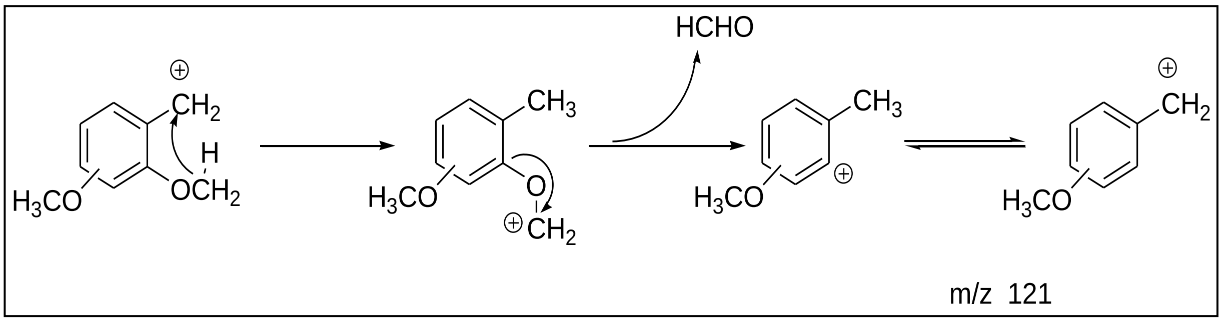


Fig. 44. Mass spectrum for the 2-^{13}Cmethoxy-3-methoxybenzylpiperazine.

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.

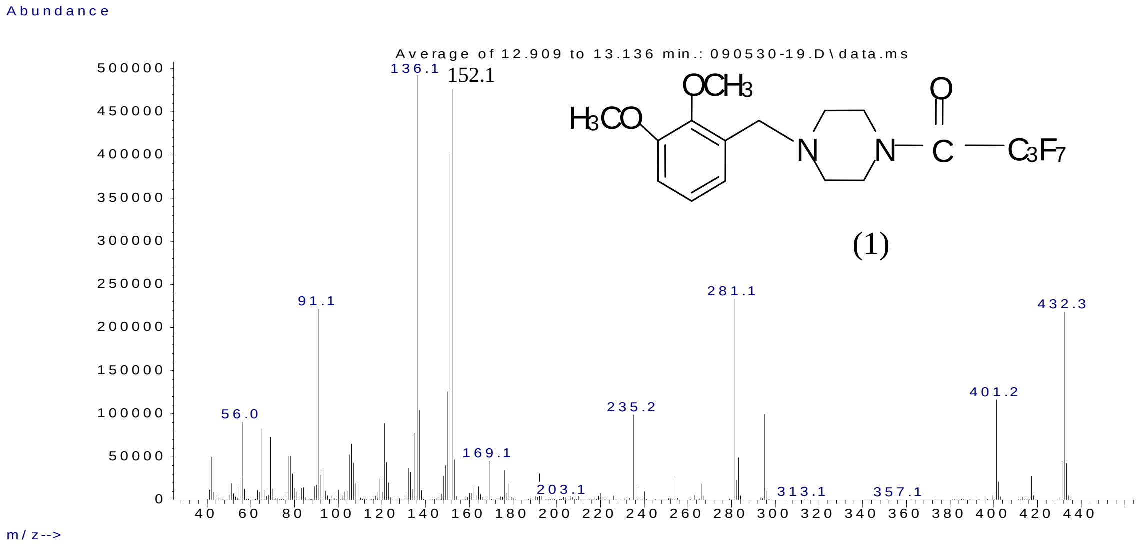
The 2,3-dimethoxybenzylpiperazine (Figure 41) shows a second unique ion at m/z 205 $(M-31)^+$ likely from the direct loss of a methoxy group from the molecular ion. The mass spectra in Figures 43B and 43C for the deuterated forms of Compound 1 confirm that the m/z 205 specifically results from loss of 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 Scheme 30. 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. 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 Scheme 30. 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]



Scheme 30. 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.

The mass spectra for the six heptafluorobutyryl amides are shown in Figure 45 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.


Abundance
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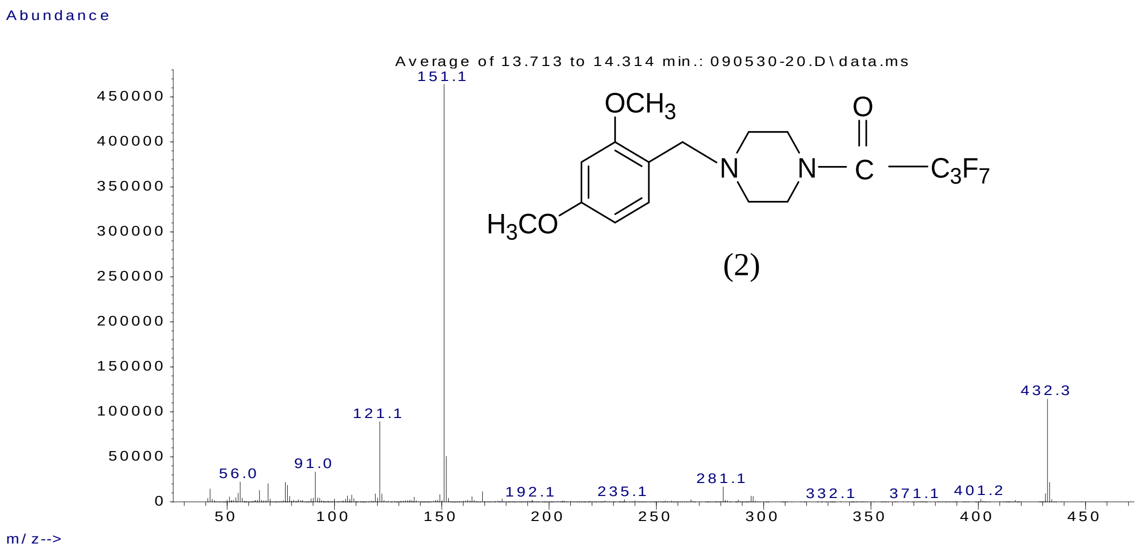
136.1
152.1
91.1
56.0
169.1
203.1
235.2
281.1
313.1
357.1
401.2
432.3
OCH3
H3CO
N
N
C
O
C3F7
(1)
m/z-->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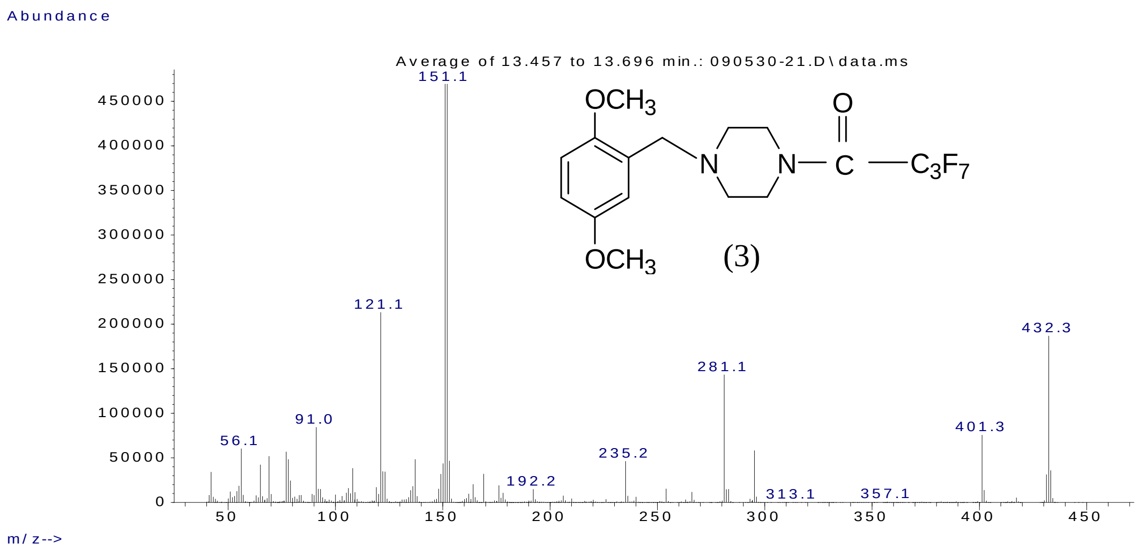
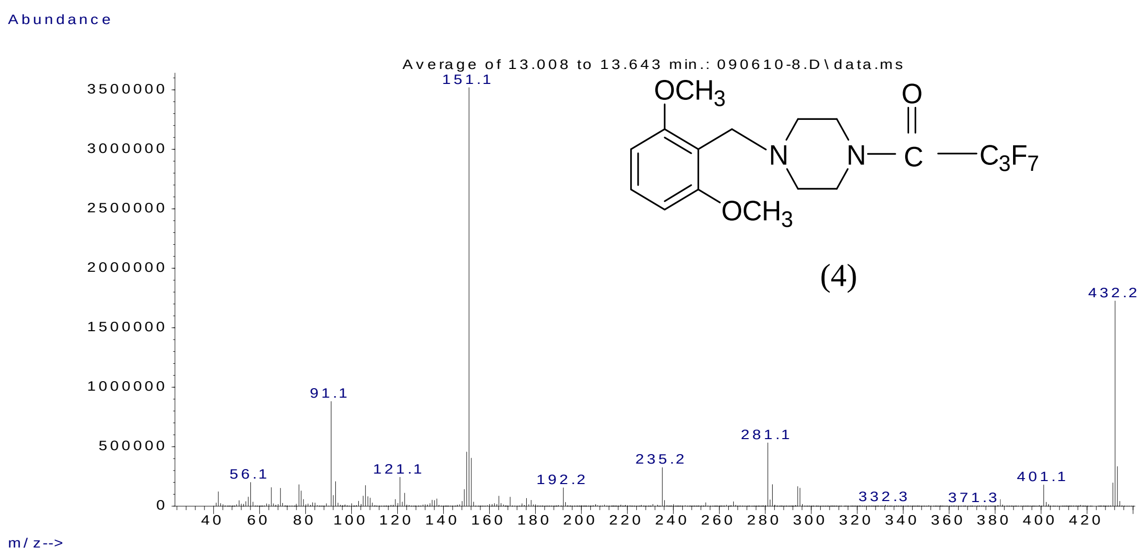
151.1
121.1
56.0
91.0
192.1
235.1
281.1
332.1
371.1
401.2
432.3
OCH3
H3CO
N
N
C
O
C3F7
(2)
m/z-->


Abundance
Average of 13.457 to 13.696 min.: 090530-21.D\data.ms
OCH3
O
N
N
C
C3F7
OCH3
(3)
151.1
121.1
432.3
281.1
91.0
56.1
401.3
235.2
192.2
313.1
357.1
m/z-->


Abundance
Average of 13.008 to 13.643 min.: 090610-8.D\data.ms
151.1
OCH3
O
N
N
C
C3F7
OCH3
(4)
432.2
91.1
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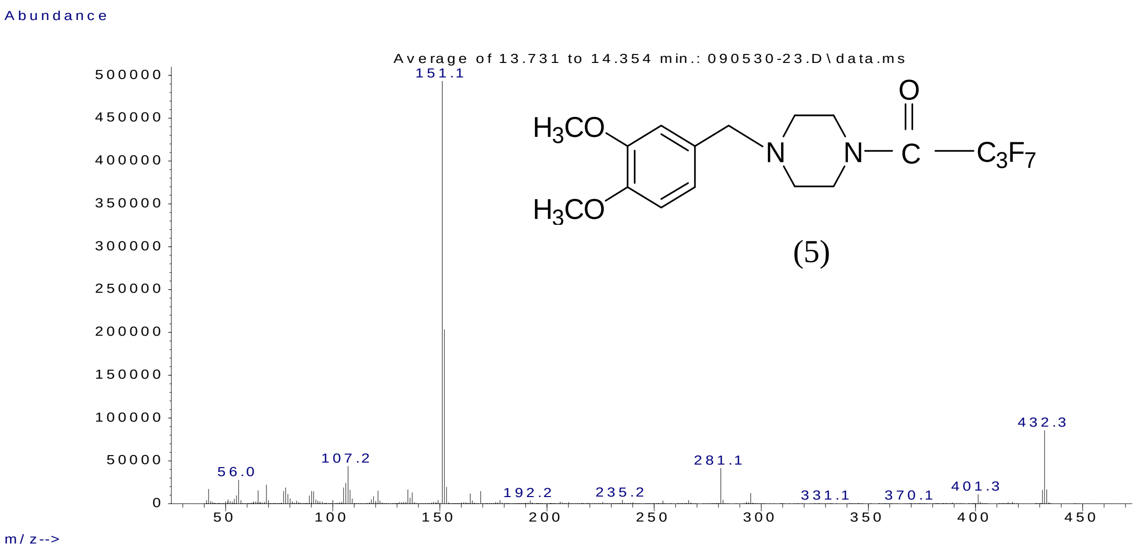
121.1
192.2
401.1
332.3
371.3
m/z-->



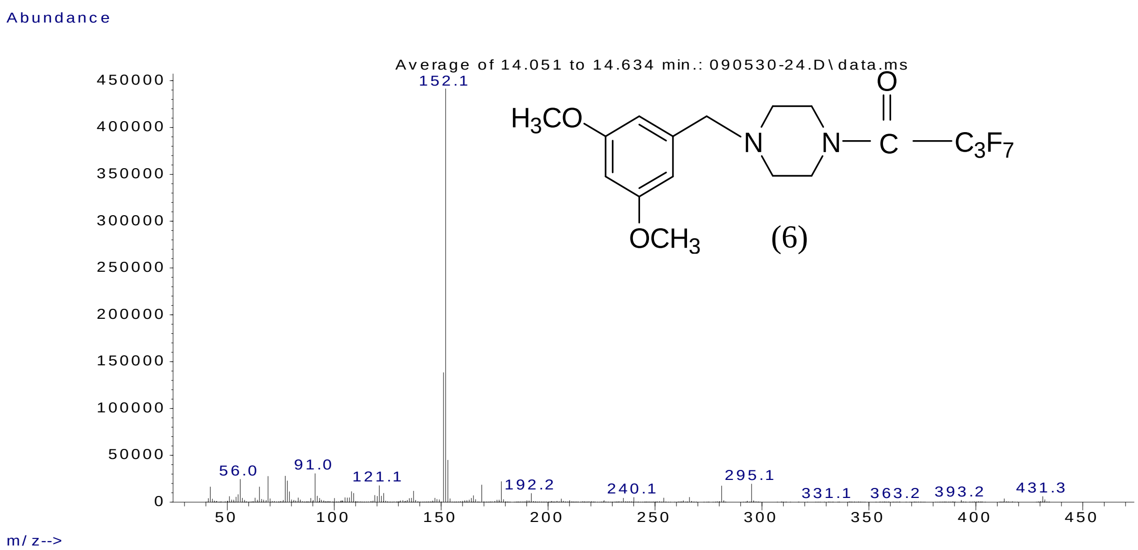
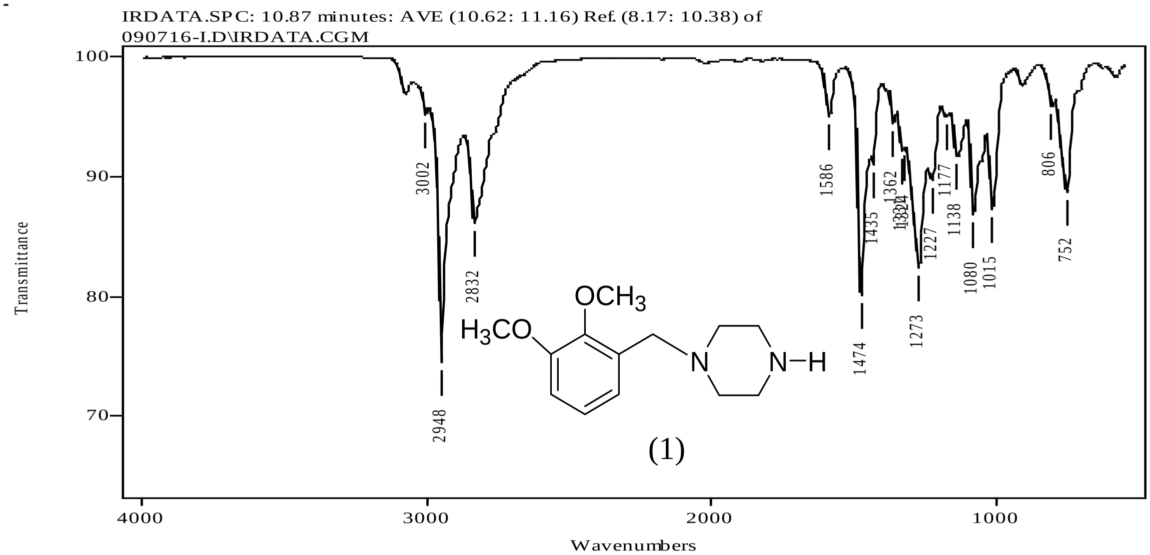


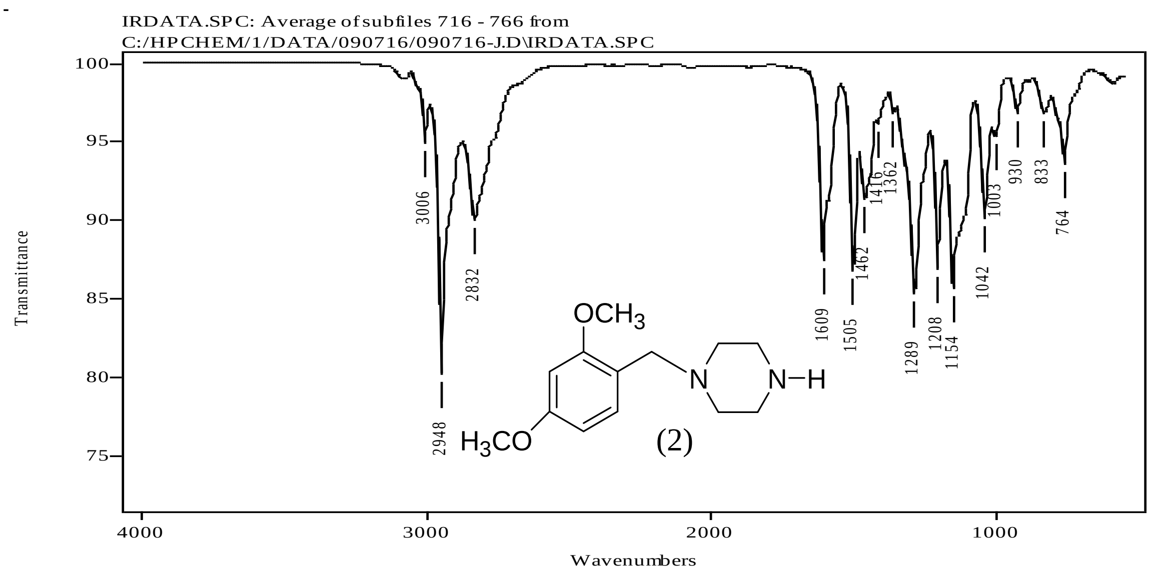
Fig. 45. MS spectra of heptafluorobutyryl derivatives of the six dimethoxybenzylpiperazine compounds.

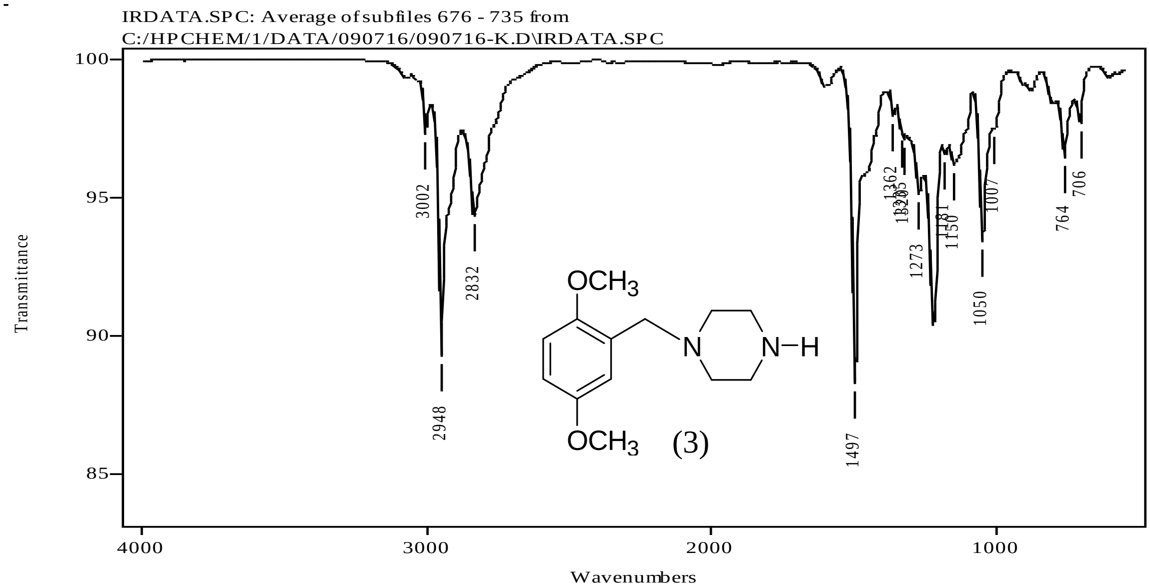
3.7.2. Vapor-phase Infra-Red Spectrophotometry

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the six regioisomeric DMBPs. Infrared detection could 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 46. 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


IRDATA.SPC: 10.87 minutes: AVE (10.62: 11.16) Ref. (8.17: 10.38) of
090716-I.D\IRDATA.CGM
Transmittance
Wavenumbers
3002
2948
2832
1586
1474
1435
1362
1273
1227
1177
1138
1080
1015
806
752
OCH_3
H_3CO
N
N–H
(1)


IRDATA.SPC: Average of subfiles 716 - 766 from
C:/HPCHEM/1/DATA/090716/090716-J.D\IRDATA.SPC
Transmittance
Wavenumbers
3006
2948
2832
1609
1505
1462
1416
1362
1289
1208
1154
1042
1003
930
833
764
OCH_3
H_3CO
N
N–H
(2)


IRDATA.SPC: Average of subfiles 676 - 735 from
C:/HPCHEM/1/DATA/090716/090716-K.D\IRDATA.SPC
Transmittance
Wavenumbers
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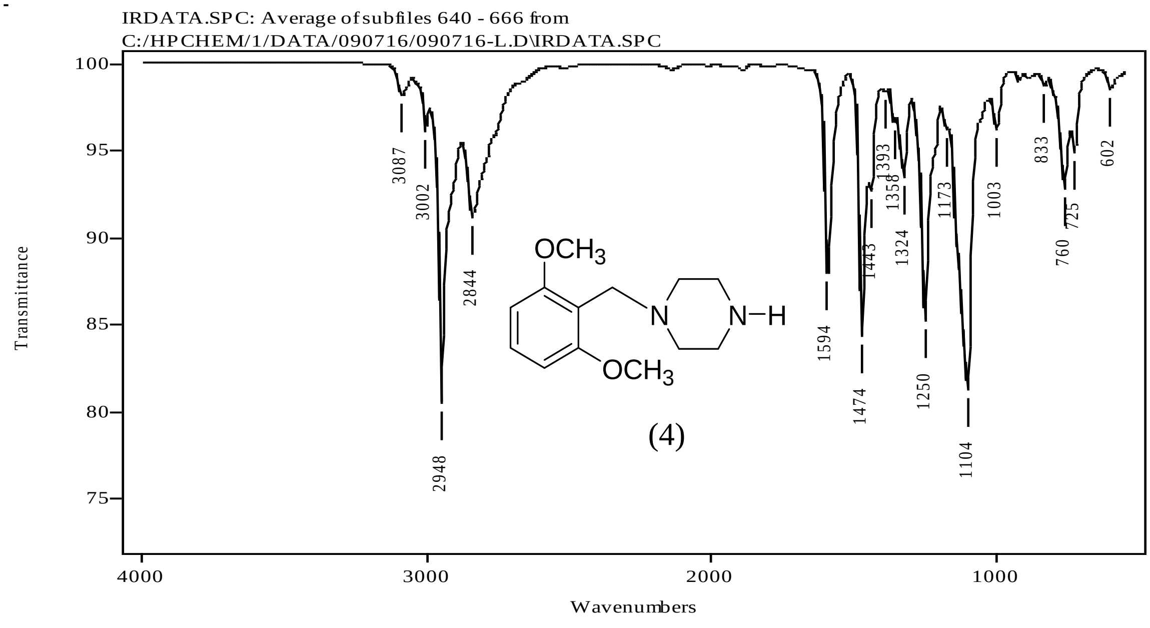
3000
2000
1000
100
95
90
85
3002
2948
2832
1497
1362
1273
1181
1150
1050
1007
764
706
OCH3
OCH3
N
N–H
(3)


IRDATA.SPC: Average of subfiles 640 - 666 from
C:/HPCHEM/1/DATA/090716/090716-L.D\IRDATA.SPC
Transmittance
Wavenumbers
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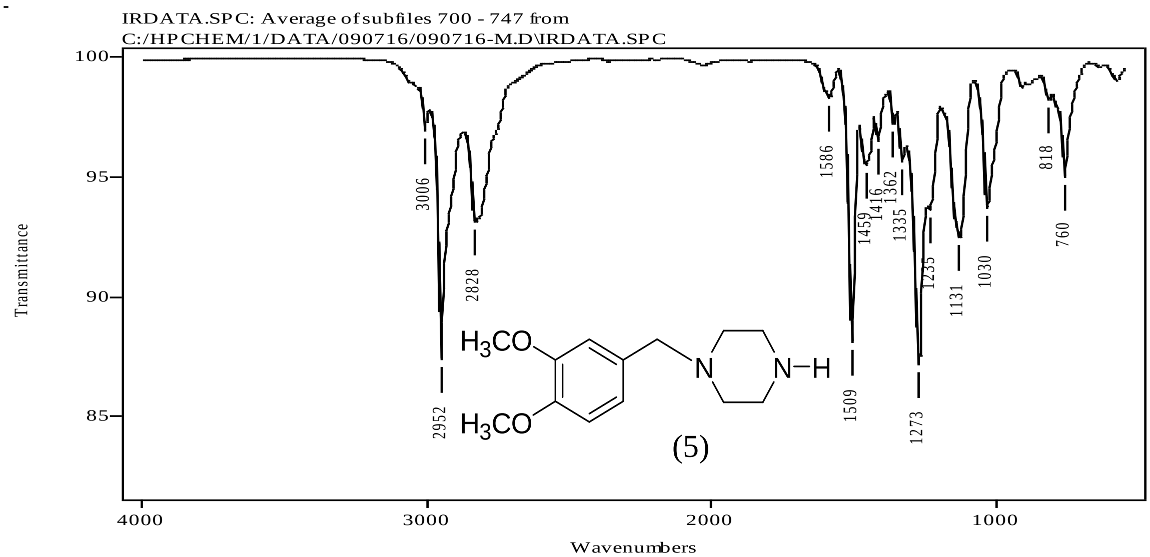
3000
2000
1000
100
95
90
85
80
75
3087
3002
2948
2844
1594
1474
1443
1393
1358
1324
1250
1173
1104
1003
833
760
725
602
OCH3
OCH3
N
N–H
(4)



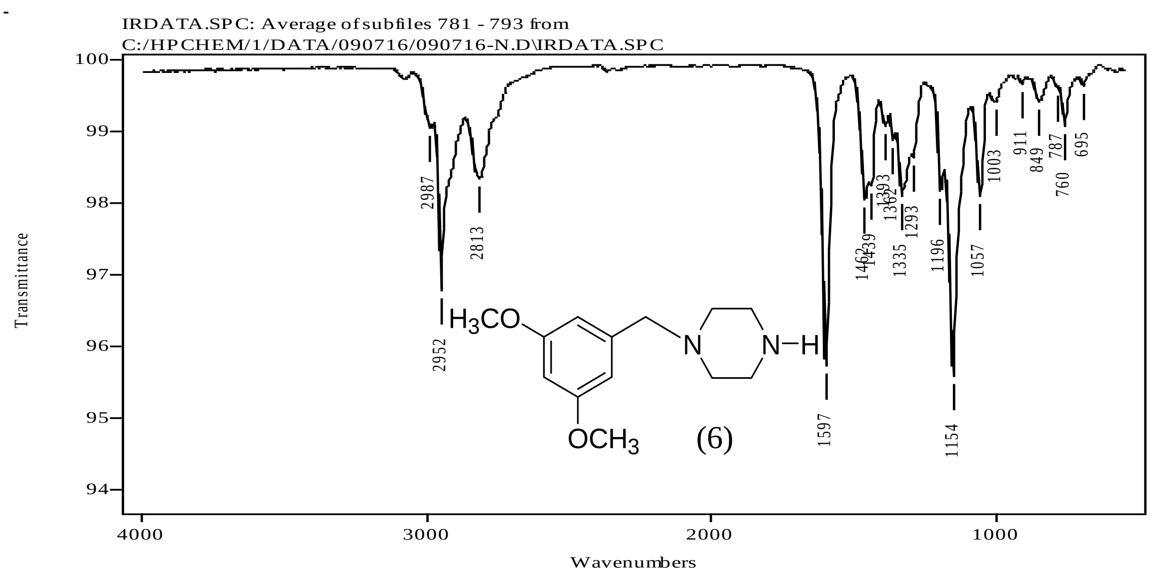


Fig. 46. Vapor phase IR spectra of the six dimethoxybenzylpiperazines.

strong intensity appearing at 1273 cm^{-1} compared to a peak of strong intensity at 1154 cm^{-1} in the 3,5-isomer, a 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.

3.7.3. Gas Chromatographic separation

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 47 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.

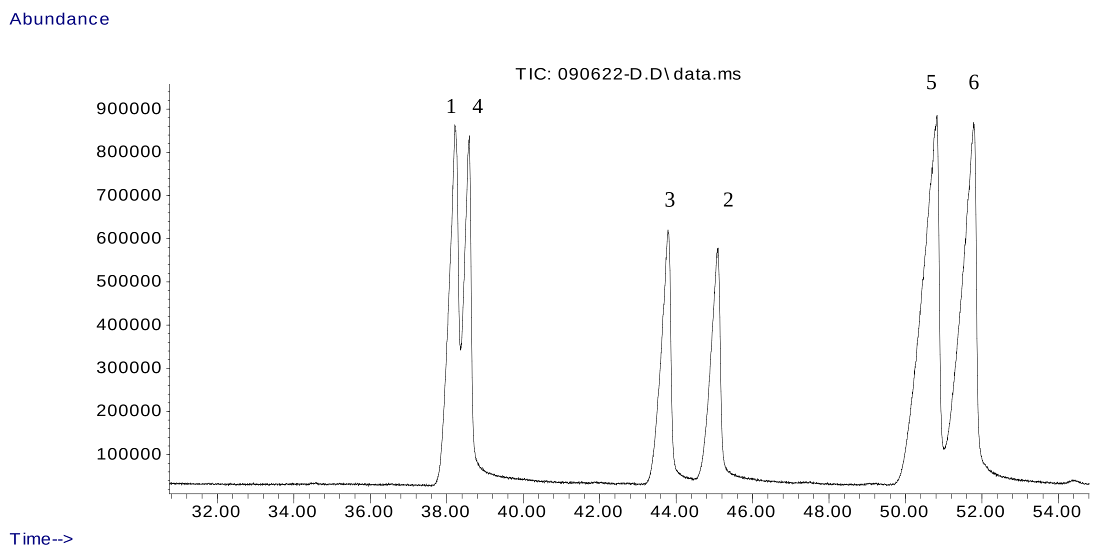


Fig. 47. Gas chromatographic separation of the pentafluoropropionyl derivatives of the DMBPs using Rtx-200 column. The number over the peak corresponds to the compound number.

3.7.4. 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.

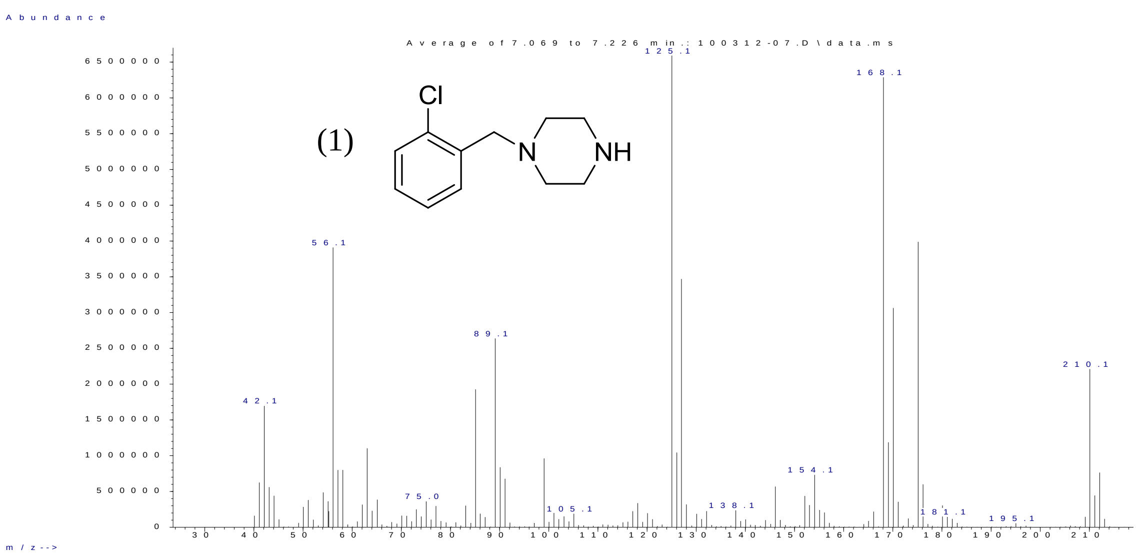
3.8. Differentiation of the Chlorobenzylpiperazines (ClBPs) by GC-IRD and GC-MS

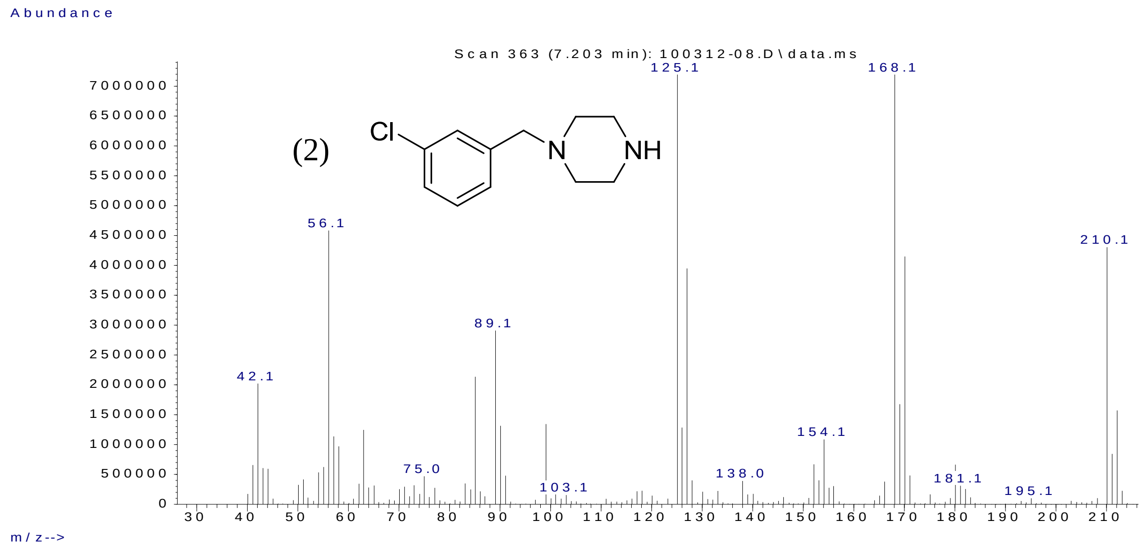
Three ring substituted chlorobenzylpiperazines (ClBPs) have equal mass and many common mass spectral fragment ions. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter 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).

3.8.1. Mass Spectral Studies

Figure 48 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 Scheme 31 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)





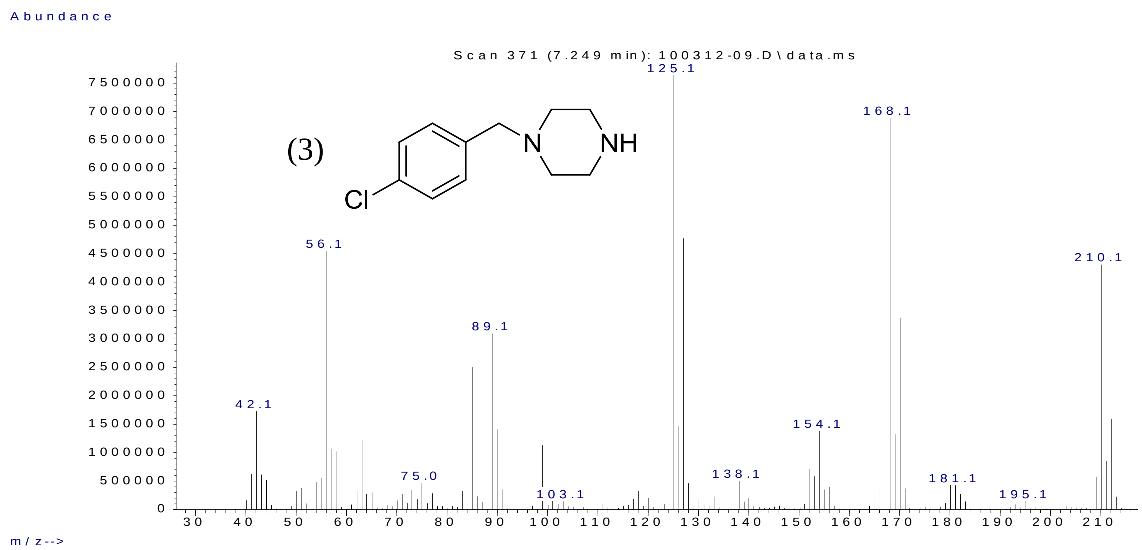
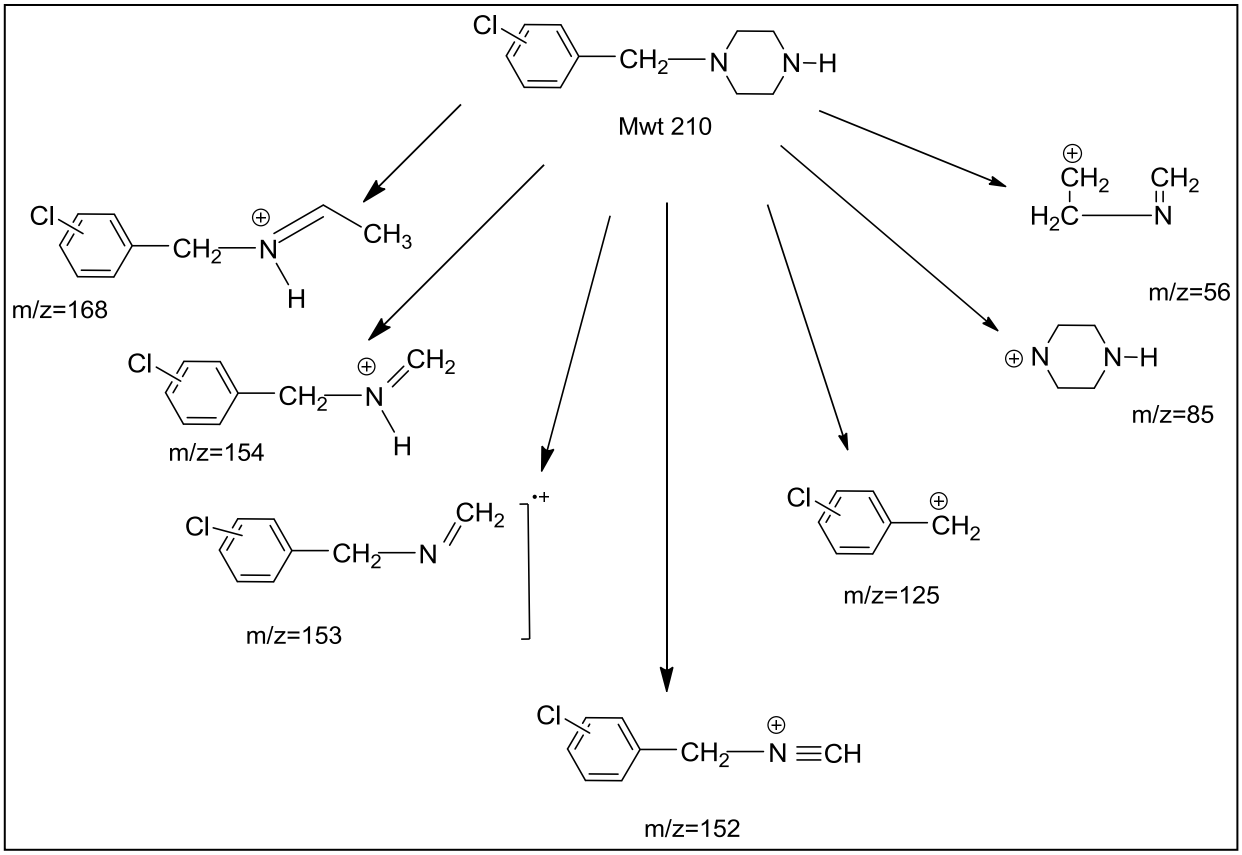
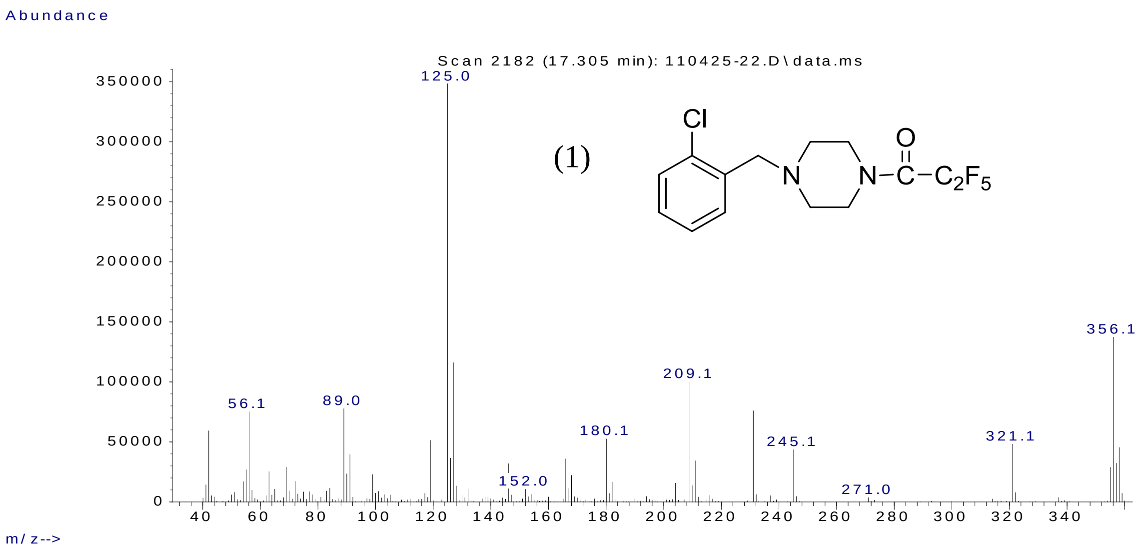


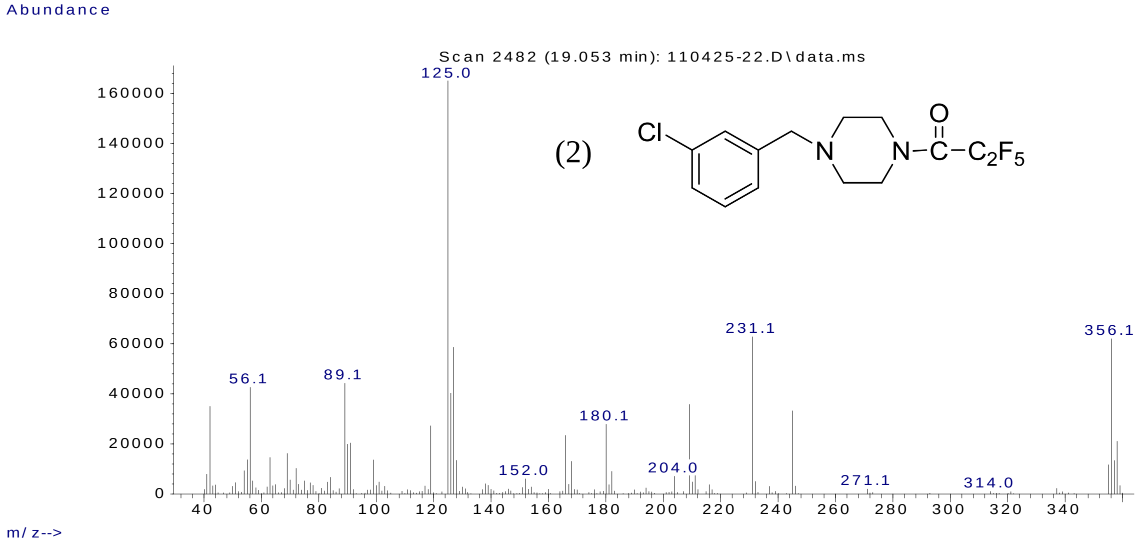
Fig. 48. EI mass spectra of the three chlorobenzylpiperazines.



Scheme 31. 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 49 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 $(M\text{-}125)^+$ 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.





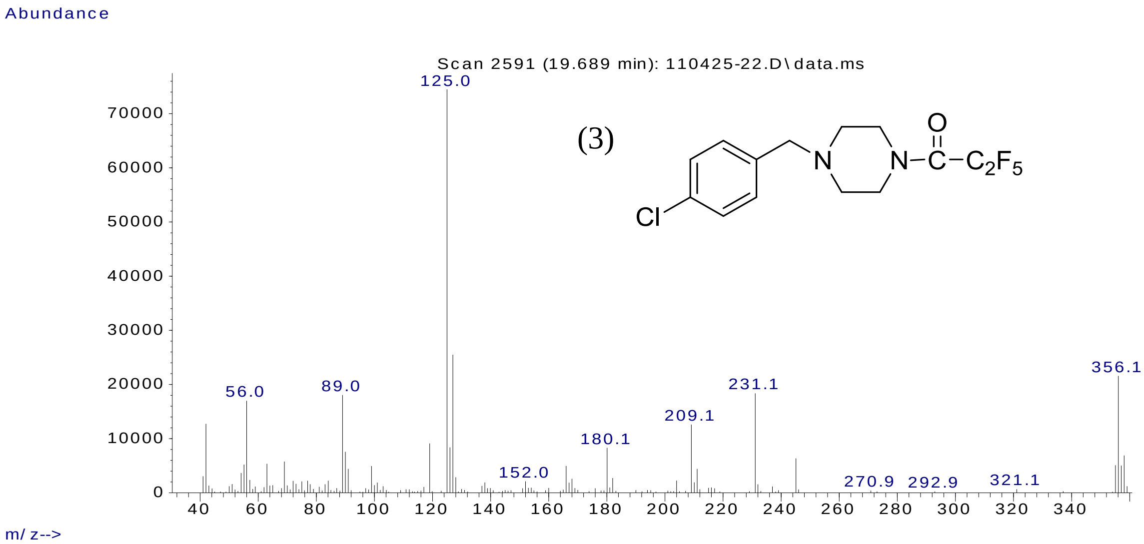
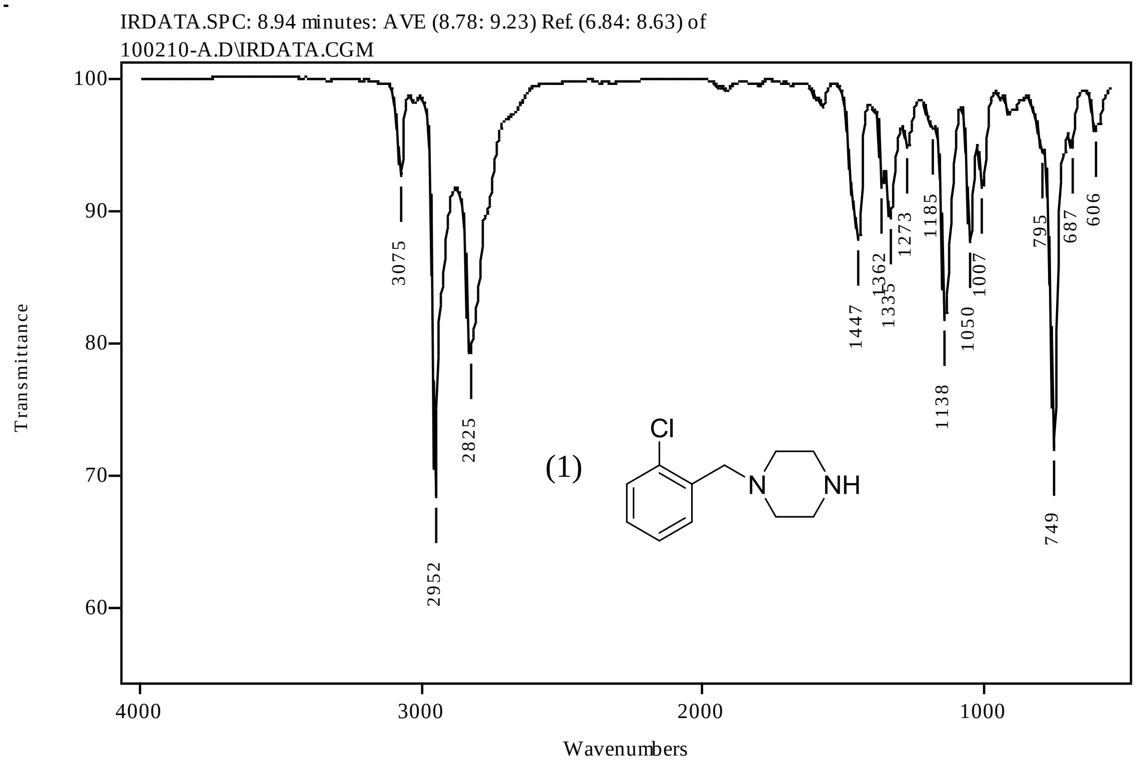


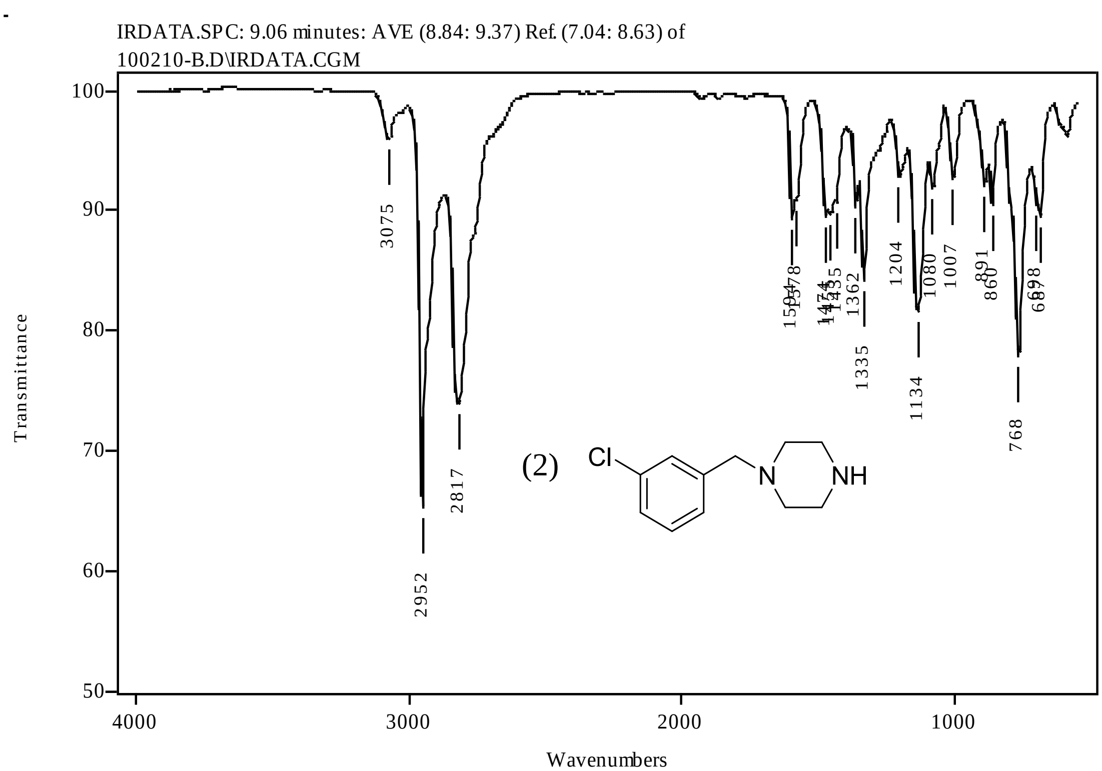
Fig. 49. Mass spectra of pentafluoropropionyl derivatives of the three chlorobenzylpiperazine compounds.

3.8.2. Vapor-phase Infra-Red Spectrophotometry

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the 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 50. 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.


IRDATA.SPC: 8.94 minutes: AVE (8.78: 9.23) Ref. (6.84: 8.63) of
100210-A.D\IRDATA.CGM
Transmittance
Wavenumbers
(1)
Cl
N
NH
3075
2952
2825
1447
1362
1335
1273
1185
1138
1050
1007
795
749
687
606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100210-B.D\IRDATA.CGM
Transmittance
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NH
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1204
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1007
891
860
768

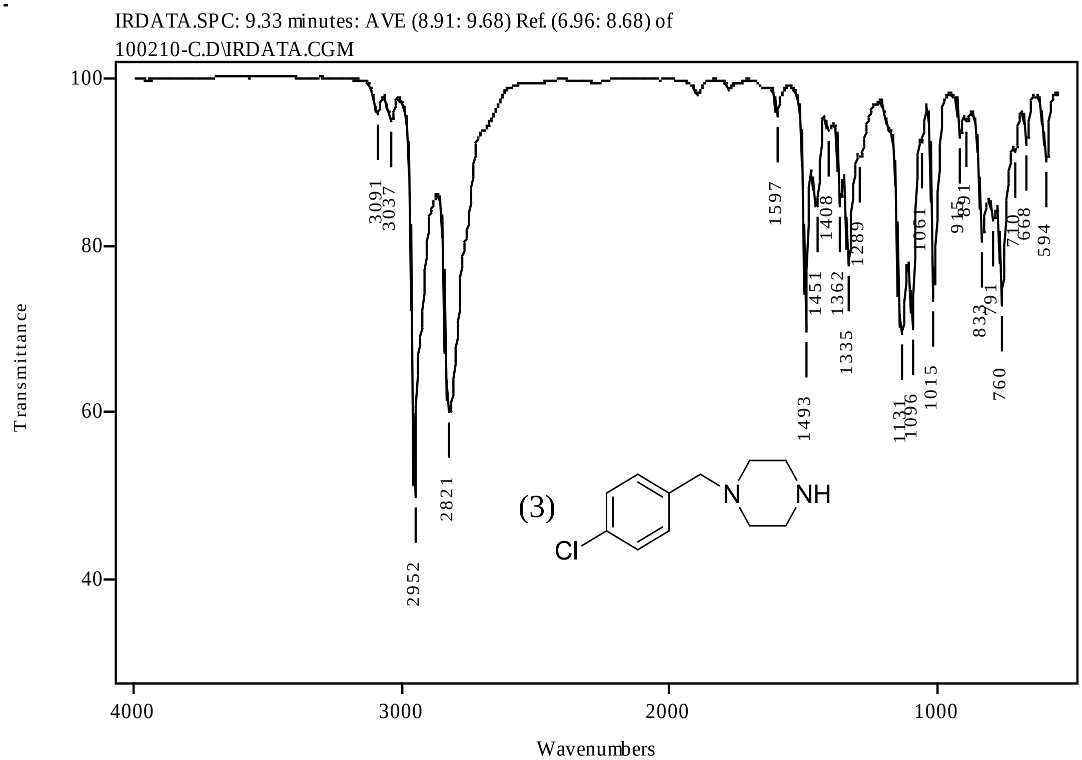


Fig. 50. Vapor phase IR spectra of the three chlorobenzyl piperazines.

3.8.3. Gas Chromatographic Separation

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 51 and 52 are representatives of the results obtained for all samples on this stationary phase.

In Figures 51 and 52 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.

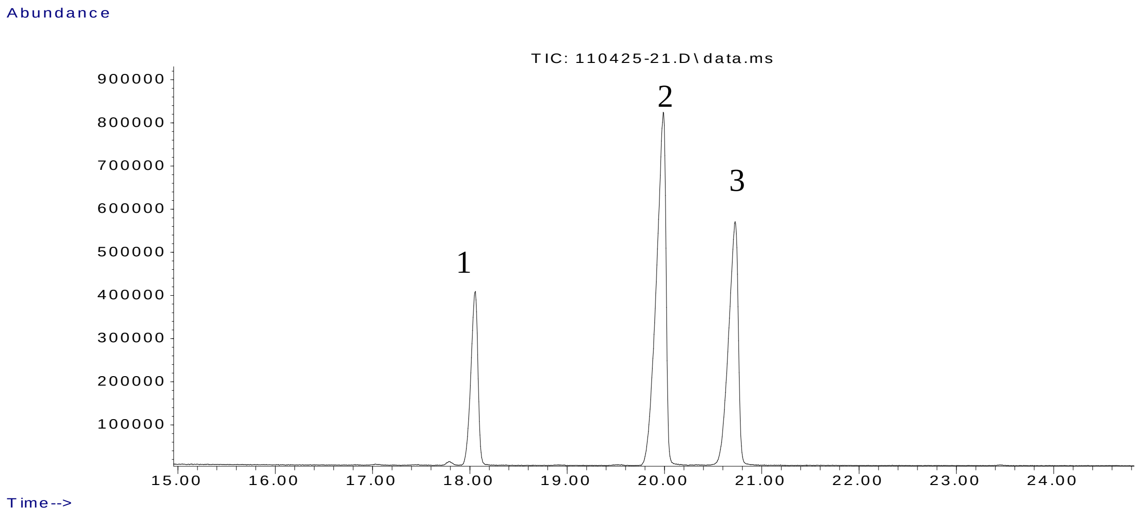


Fig. 51. Gas chromatographic separation of the trifluoroacetyl derivatives of ClBPs using Rtx-200 column. The number over the peak represents the compound number.

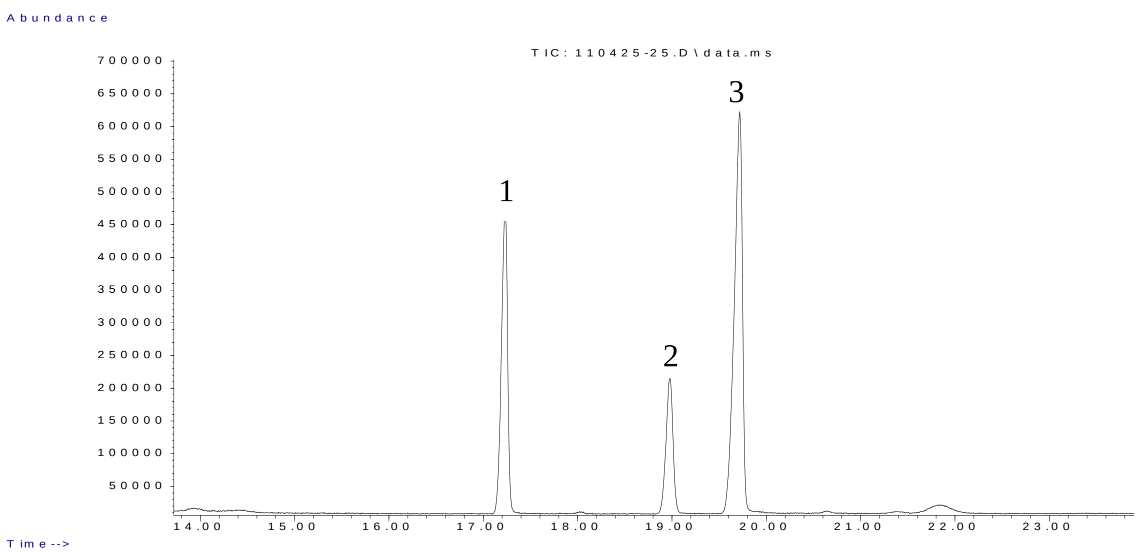


Fig. 52. Gas chromatographic separation of the pentafluoropropionyl derivatives of ClBPs using Rtx-200 column. The number over the peak represents the compound number.

3.8.4. 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.

3.9. Differentiation of Methylenedioxybenzylpiperazines (MDBPs) and Methoxybenzoylpiperazines (OMeBzPs) 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.

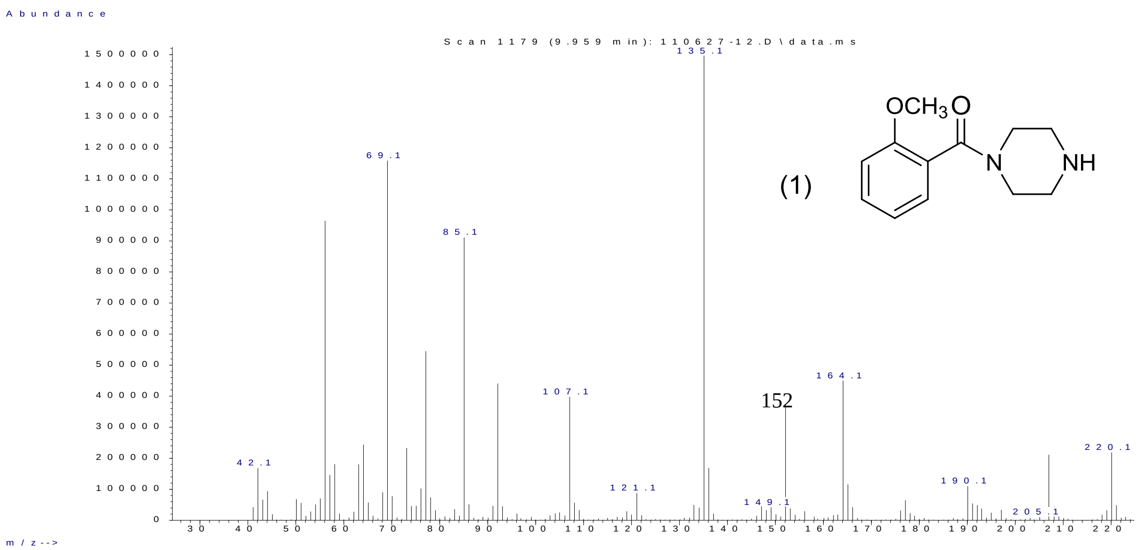
Gas chromatography coupled to infrared detection (GC-IRD) provides direct confirmatory data for the identification of the carbonyl containing compounds and the differentiation of the psychoactive designer drug 3,4-MDBP from its direct (2,3-MDBP) and indirect (OMeBzPs) regioisomers. The mass spectra in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

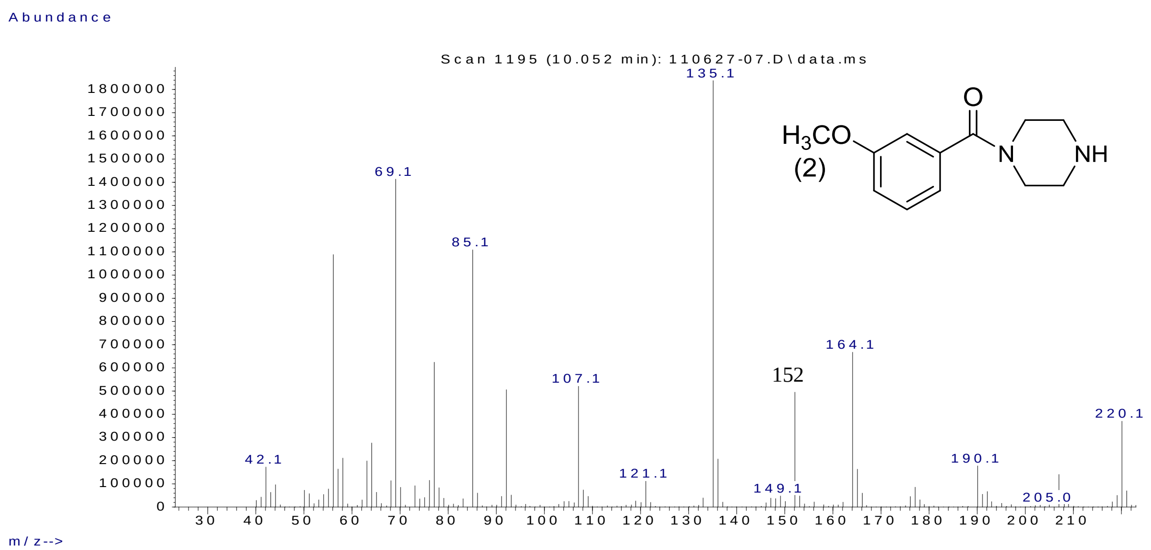
3.9.1. Mass spectral studies

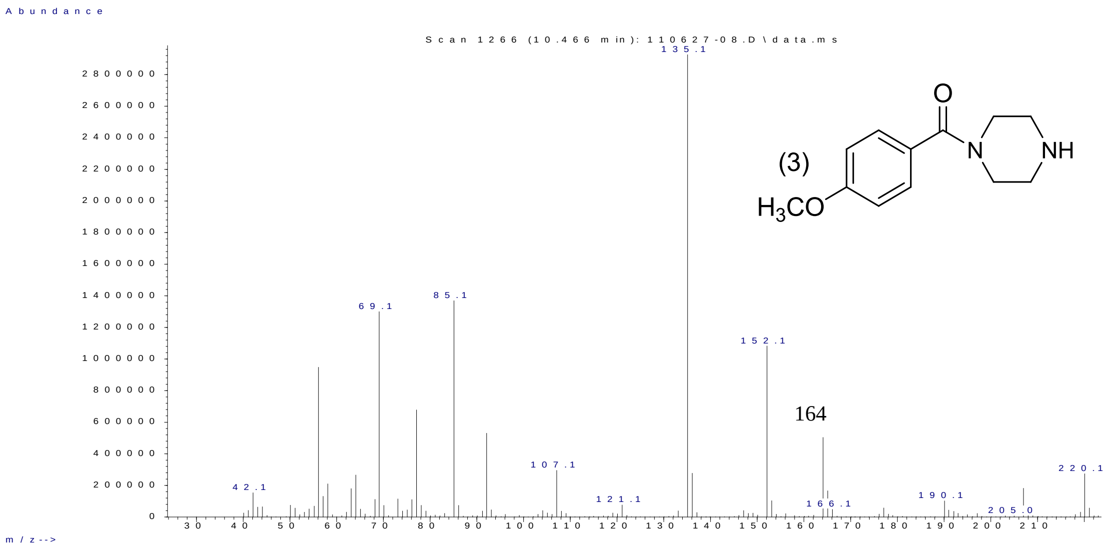
Figure 53 shows the EI mass spectra of all five isomeric piperazines (Compounds 1-5). The ions of significant relative abundance common to the five isomers likely arise from fragmentation of the piperazine ring. The mass spectra of the five piperazines show the fragment ions at m/z 178, 164, 135, 85 and 56 as well as other ions of low relative abundance. The proposed structures of these fragment ions are shown in Schemes 18 and 32 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 54 shows the exact mass measurement results for the m/z 152 ion in the 4-methoxybenzoylpiperazine. The upper panel (54A) shows the expected/calculated mass for the

$C_8H_{10}NO_2$ elemental composition and the lower panel (54B) shows the experimental results along with the degree of agreement (0.0 mDa, 0.0 ppm) between the calculated and experimental



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69.1
85.1
152.1
164
107.1
42.1
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220.1
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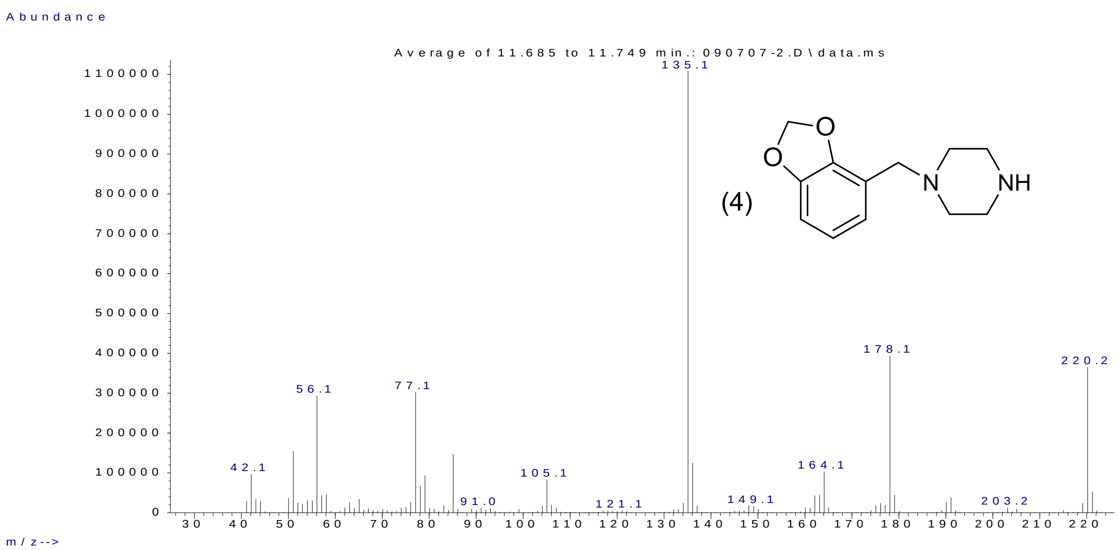
N
NH
H3CO
m/z-->


Abundance
Average of 11.685 to 11.749 min.: 090707-2.D\data.ms
135.1
178.1
220.2
56.1
77.1
42.1
105.1
164.1
91.0
121.1
149.1
203.2
(4)
O
O
N
NH
m/z-->

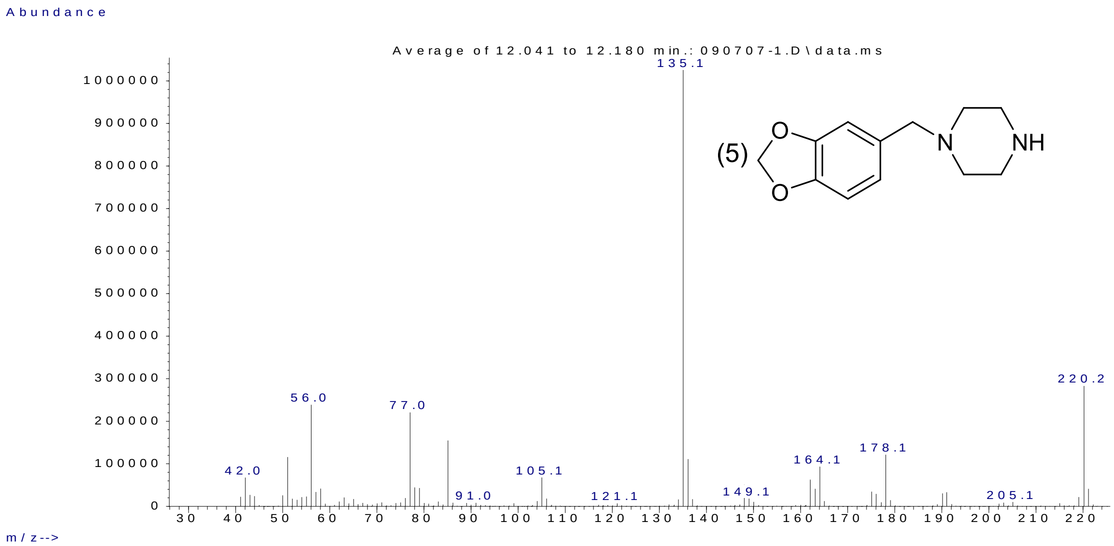
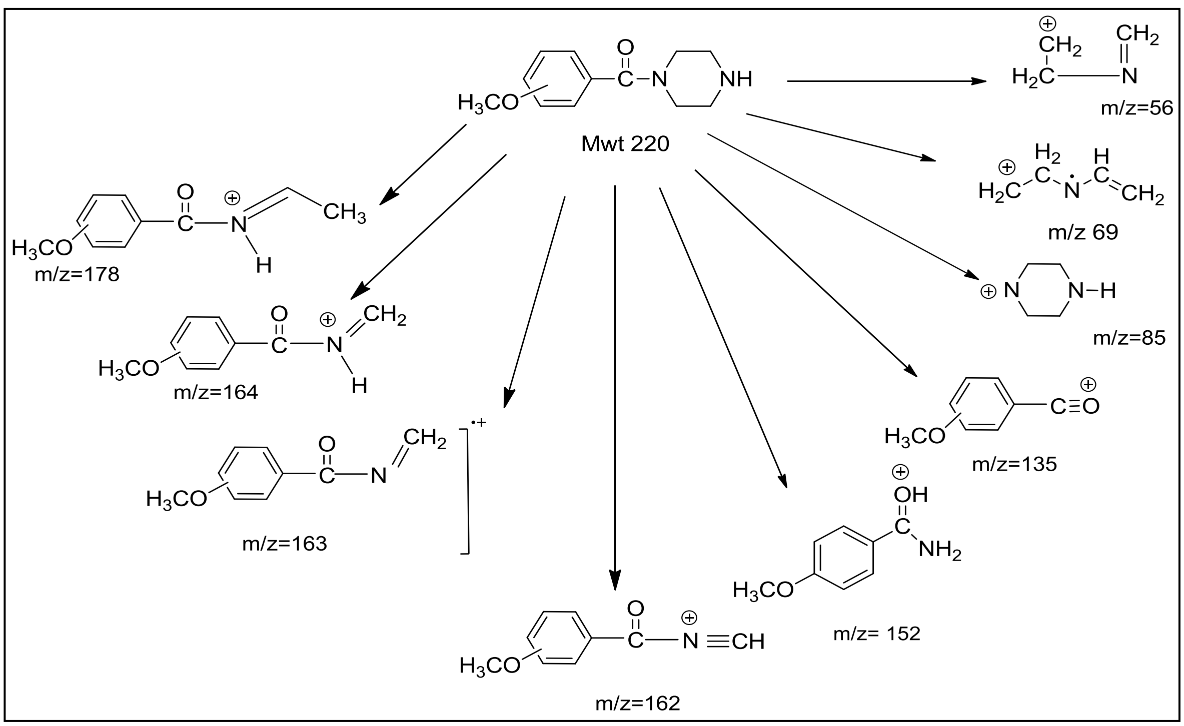


Fig.53. Mass spectra of the underivatized methylenedioxybenzylpiperazines and methoxybenzoylpiperazines in this study.



Scheme 32. Mass spectral fragmentation pattern of the underivatized methoxybenzoylpiperazines under EI (70eV) conditions.

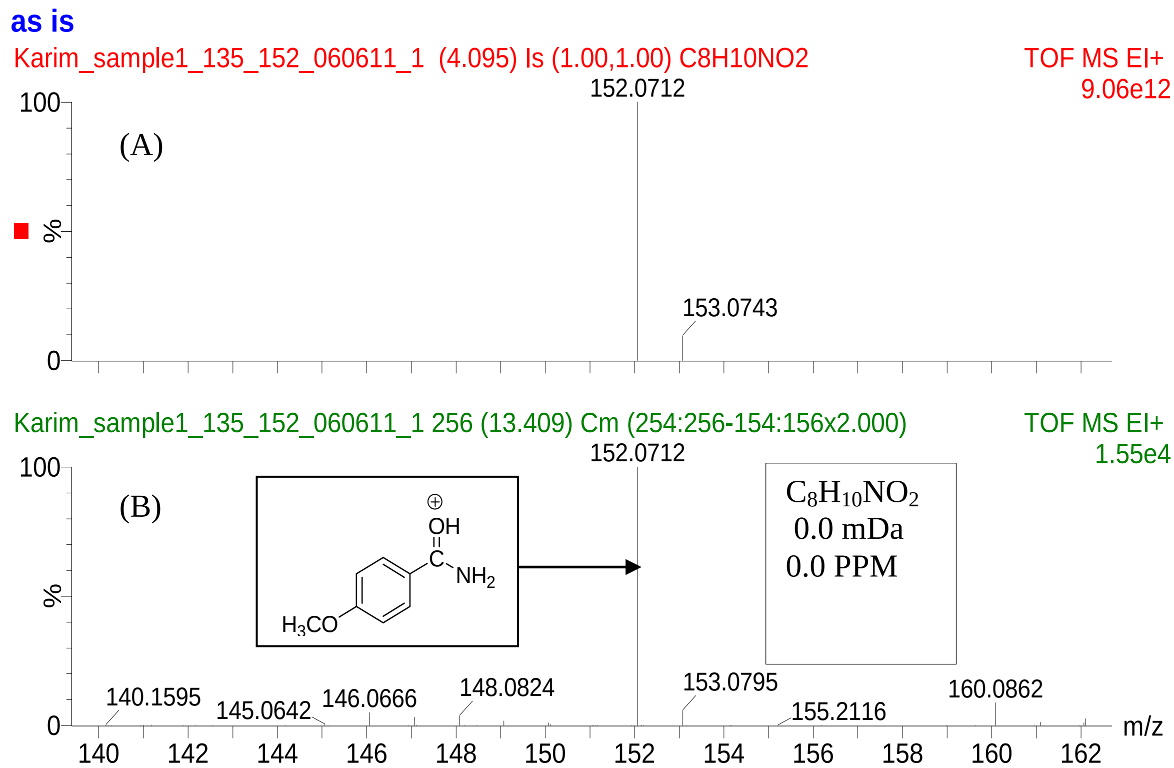


Fig. 54. GC-TOF mass spectral analysis of the m/z 152 ion for 4-methoxybenzoylpiperazine. 54A= calculated mass for C8H10NO2; 54B= experimental results.

results.

The proposed structure for the m/z 152 $C_8H_{10}NO_2$ ion is shown in Figure 54. 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 55. The mass spectrum in Figure 55 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 Scheme 25. 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 55 as the corresponding fragment is shifted 7 Da higher to become m/z 76.

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 56 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

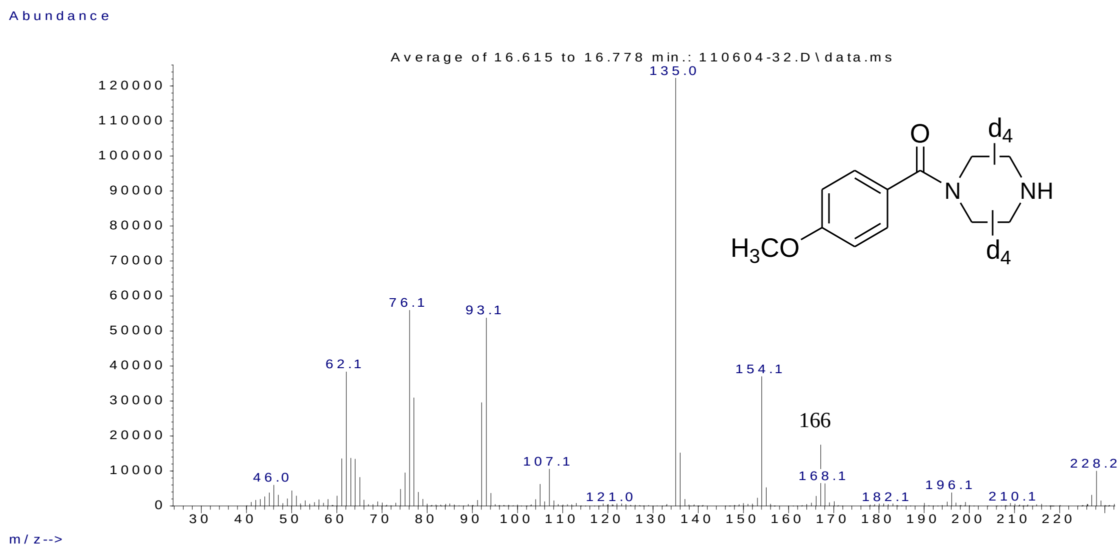
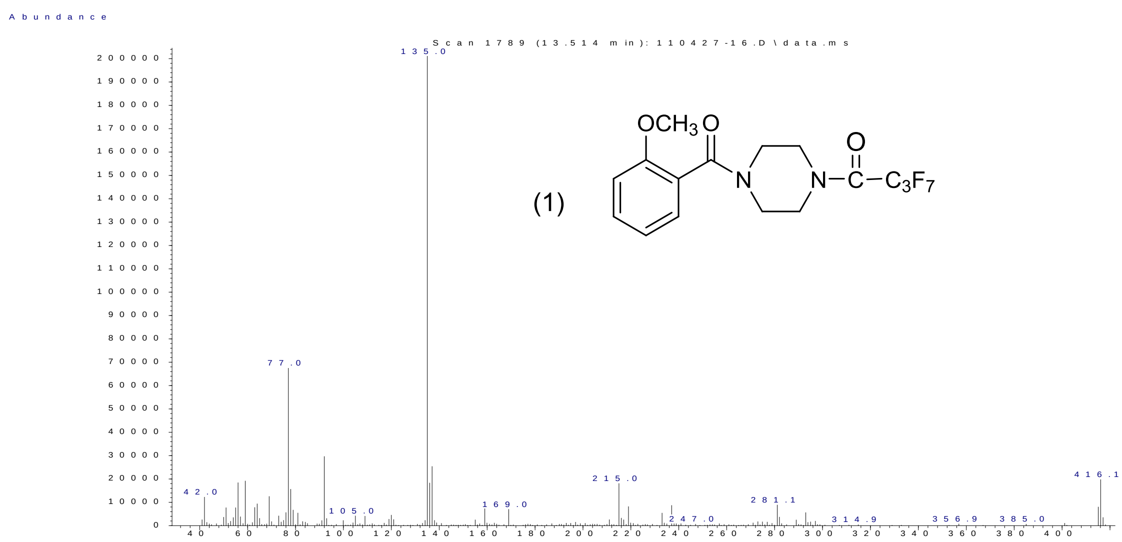
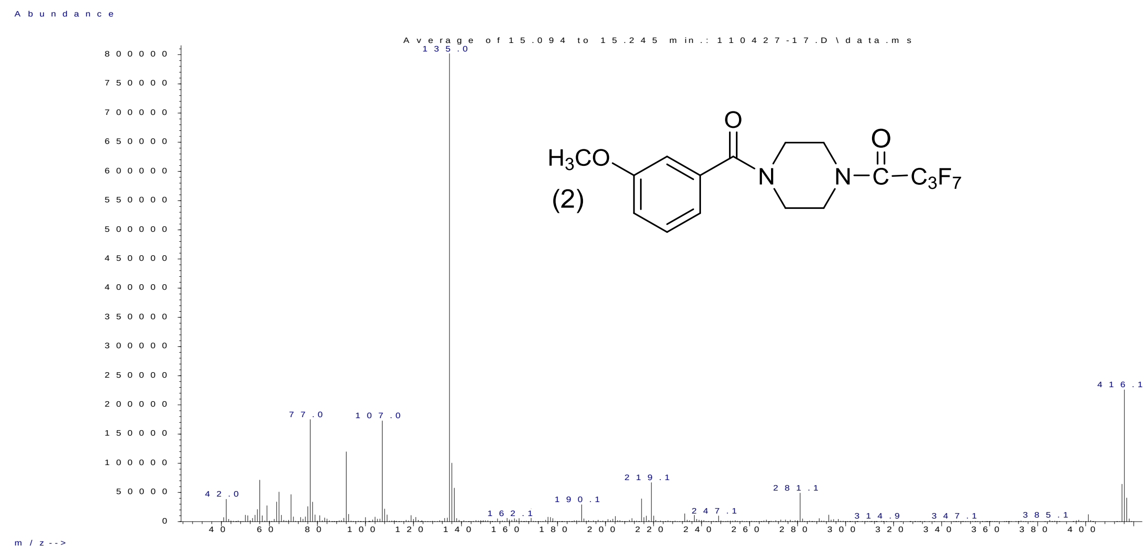


Fig. 55. Mass spectrum of the 4-methoxybenzoyl-d_8-piperazine.

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77.0
107.0
42.0
162.1
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219.1
247.1
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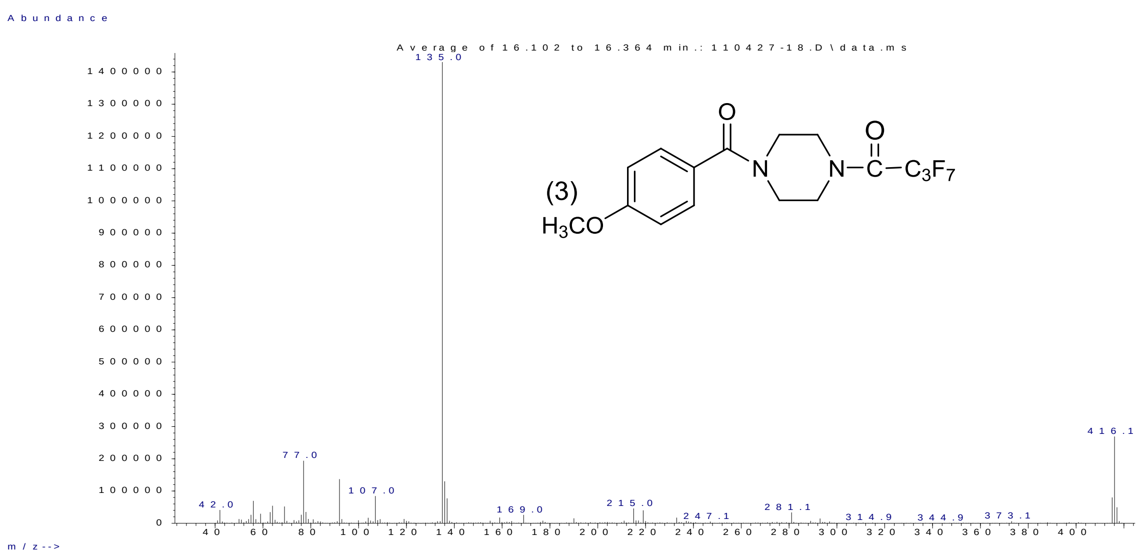
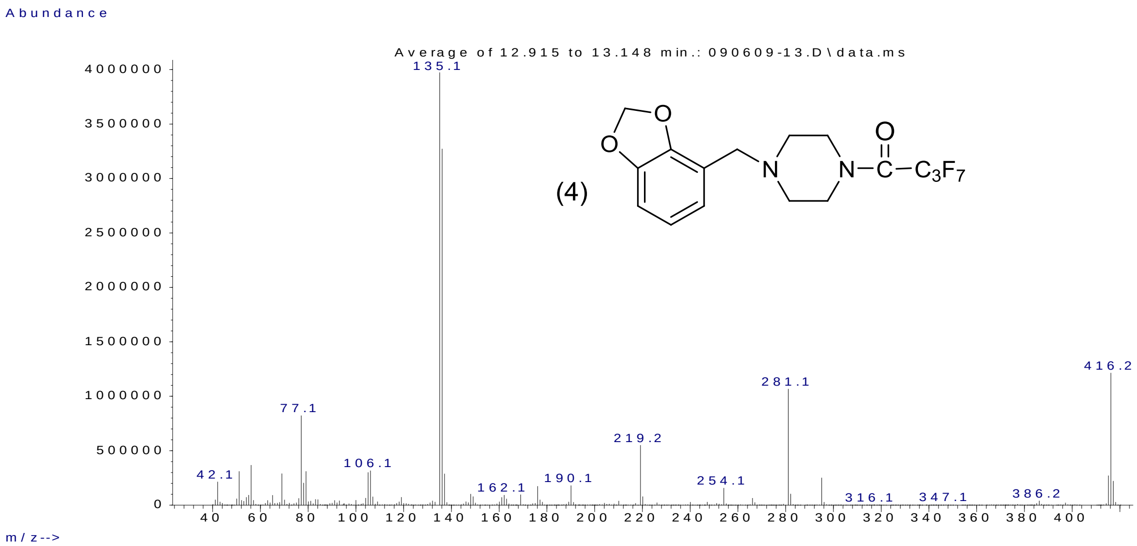
N
O
O
C
C_3F_7


Abundance
Average of 16.102 to 16.364 min.: 110427-18.D\data.ms
135.0
416.1
77.0
107.0
42.0
169.0
215.0
247.1
281.1
314.9
344.9
373.1
m/z-->
(3)
H_3CO
N
N
O
O
C
C_3F_7



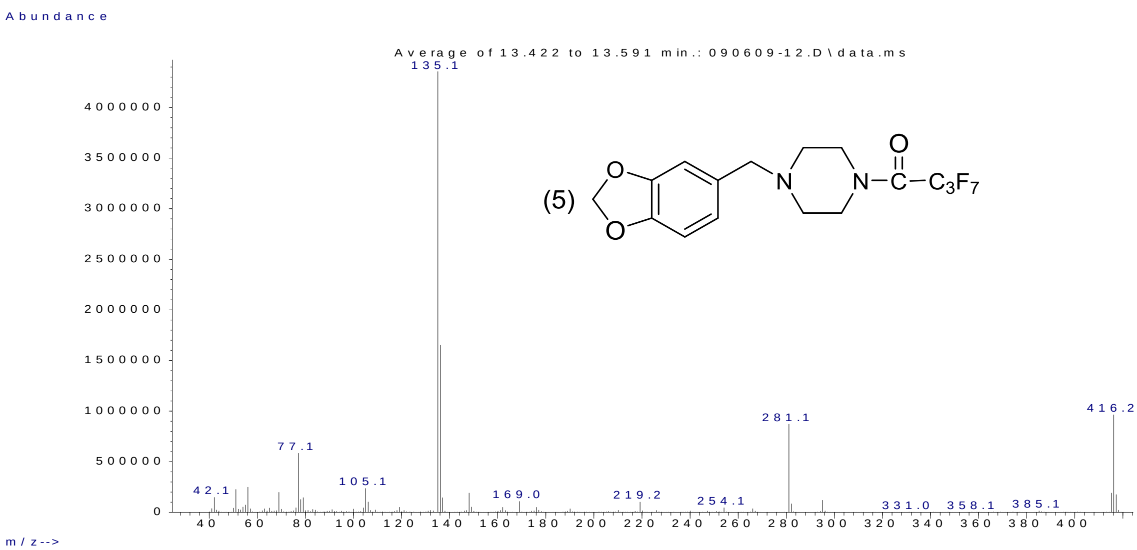


Fig. 56. Mass spectra of the heptafluorobutyryl derivatives of the five piperazine compounds in this study.

respectively corresponds to the $(M-135)^+$ 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.

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 57 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 (57A) shows the expected/ calculated mass for the $C_8H_7O_2$ elemental composition. The lower panel (57B) 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 57C and 57D 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.

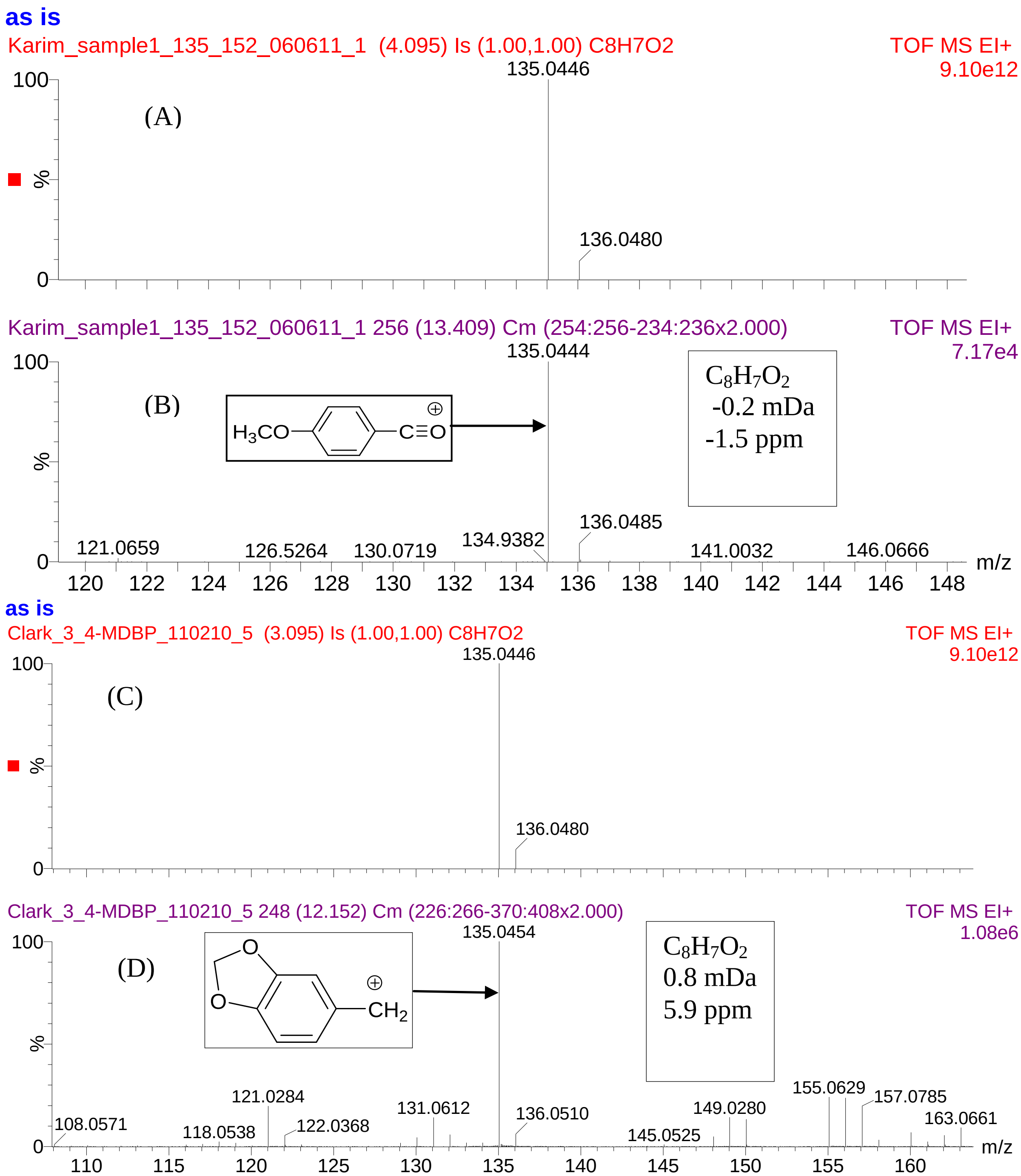


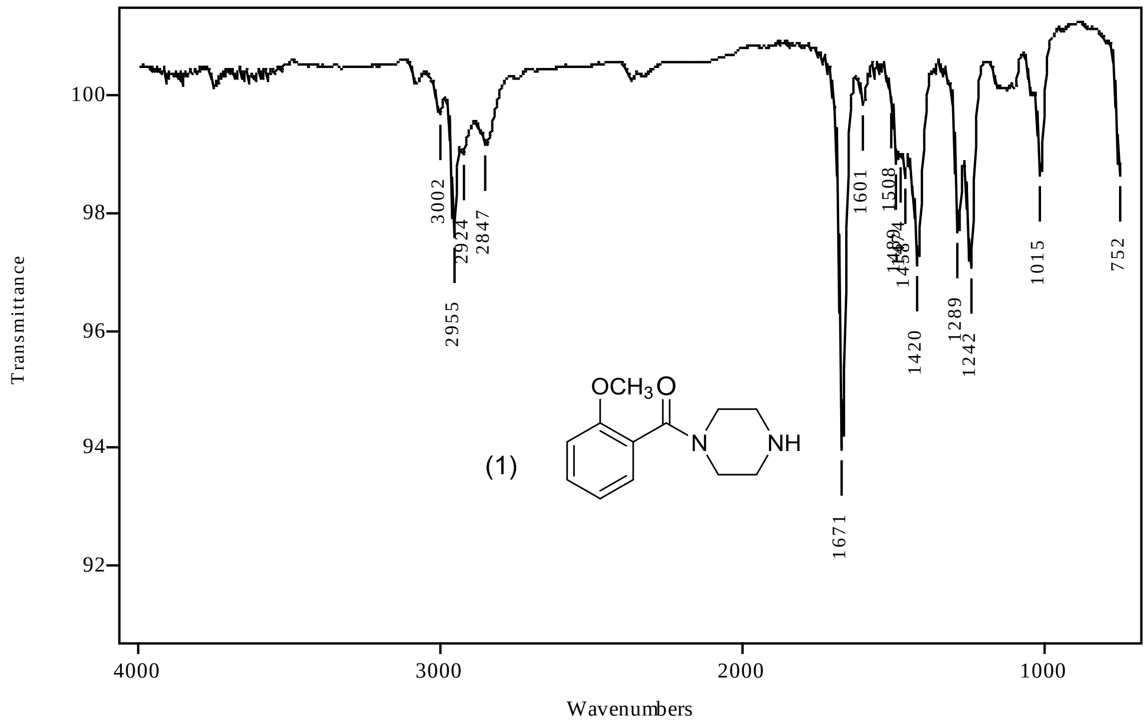
Fig. 57. GC-TOF mass spectral analysis of the m/z 135 ion for 4-methoxybenzoylpiperazine and 3,4-methylenedioxybenzylpiperazine. 57A= calculated mass for C8H7O2; 57B= experimental results. 57C= calculated mass for C8H7O2; 57D= experimental results.

3.9.2. Vapor-phase Infra-Red Spectroscopy

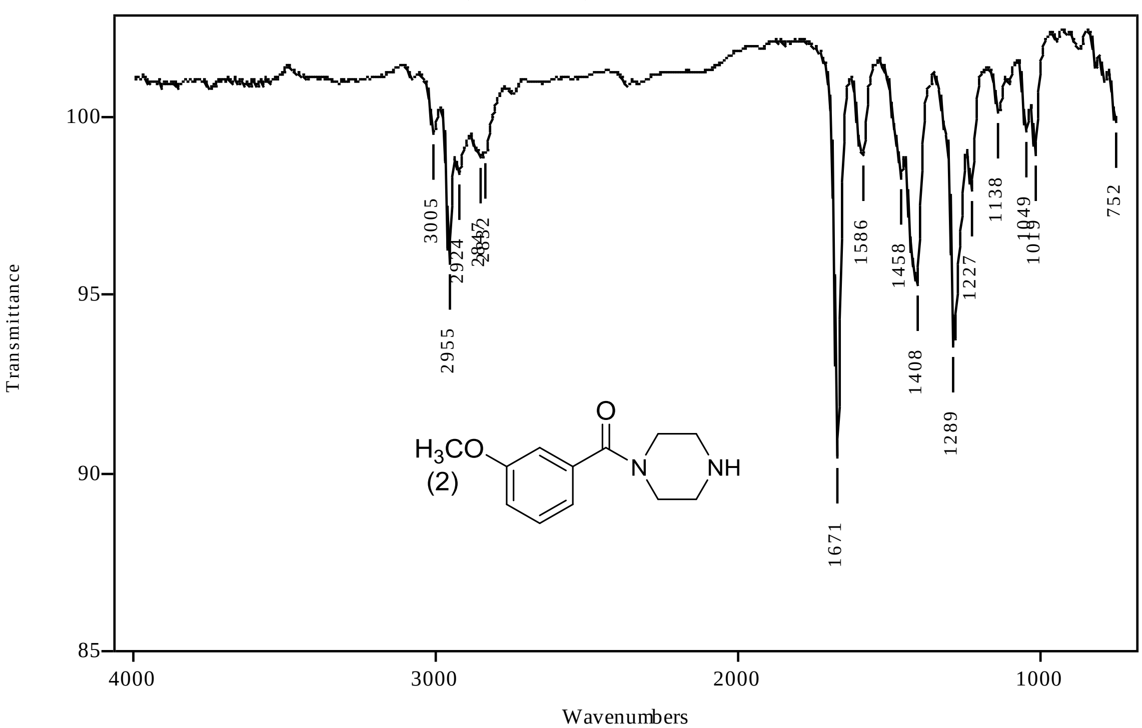
Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the five isomeric piperazines. The vapor phase infrared spectra for the five piperazines are shown in Figure 58. 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

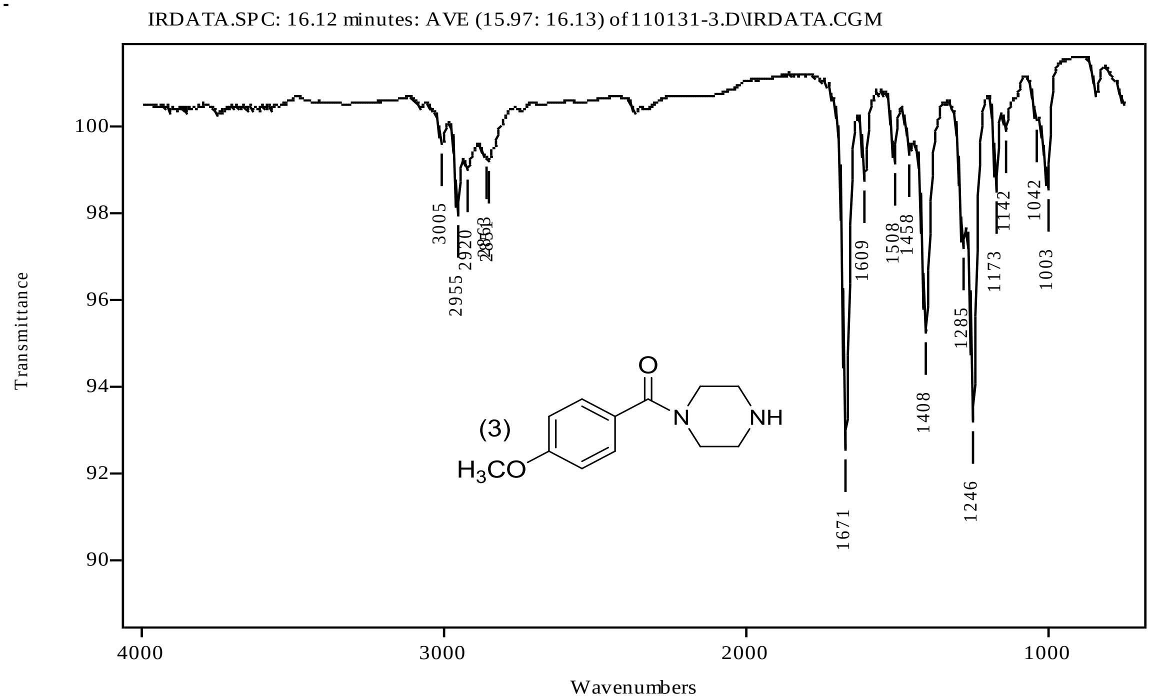
IRDATA.SPC: 4.78 minutes: AVE (13.06: 13.19) of 110131-1.D\IRDATA.CGM



IRDATA.SPC: 4.15 minutes: AVE (16.32: 16.32) of 110131-2.D\IRDATA.CGM



-


IRDATA.SPC: 16.12 minutes: AVE (15.97: 16.13) of 110131-3.D\IRDATA.CGM
Transmittance
Wavenumbers
3005
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2920
2863
2831
1671
1609
1508
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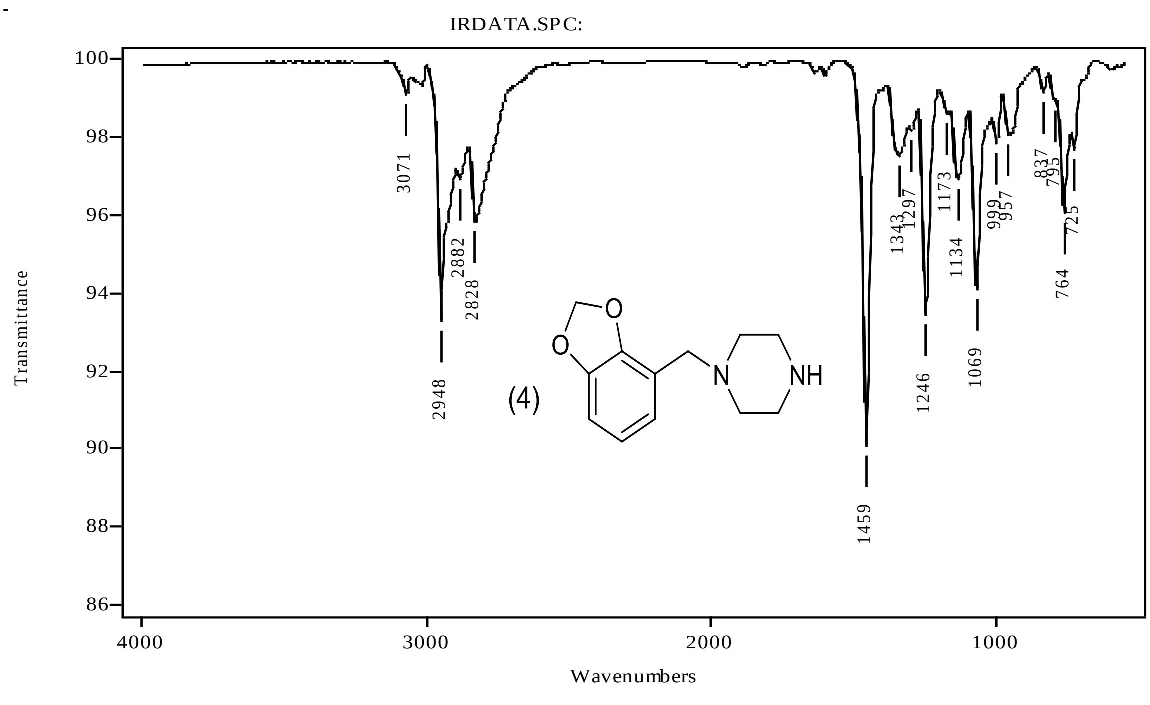
1408
1285
1246
1173
1142
1042
1003
(3)
O
N
NH
H3CO

-


IRDATA.SPC:
Transmittance
Wavenumbers
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2882
2828
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(4)
O
O
N
NH

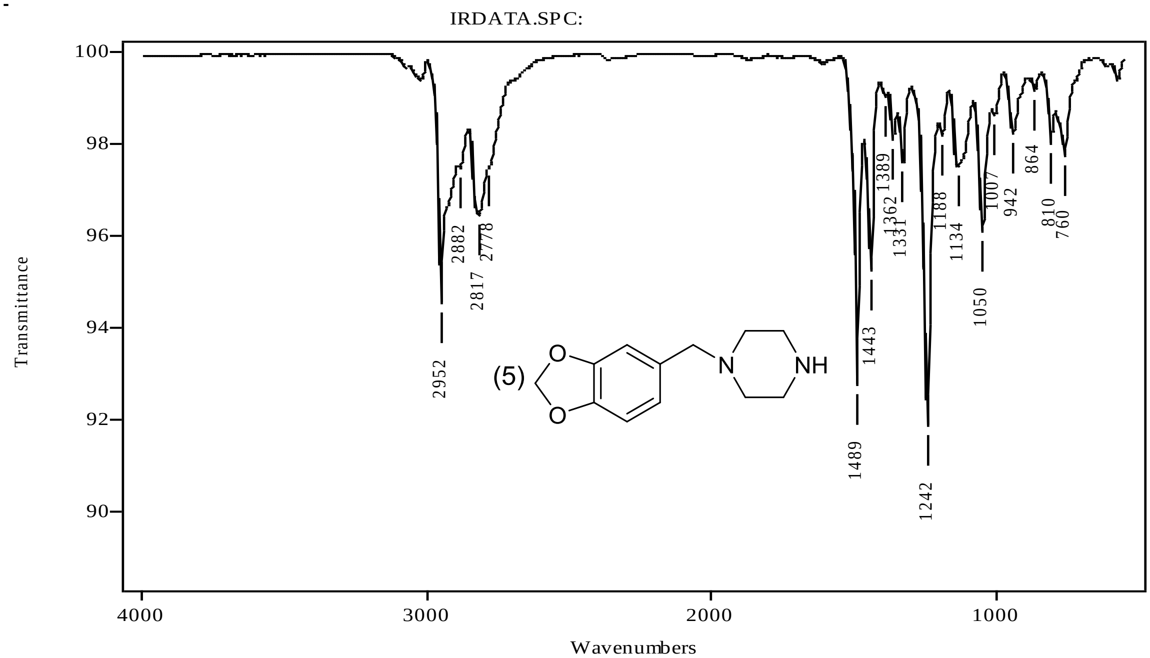


Fig. 58. Vapor phase IR spectra of the five piperazines involved in this study.

compound 2.

The infrared spectra and results for the two MDBPs have been previously discussed in details in section 3.1.2 (p.g. 53).

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.

3.9.3. Gas Chromatographic Separation

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^{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.

The chromatograms in Figure 59 are representatives of the results obtained for all samples on this stationary phase. In Figure 59A and 59B 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.

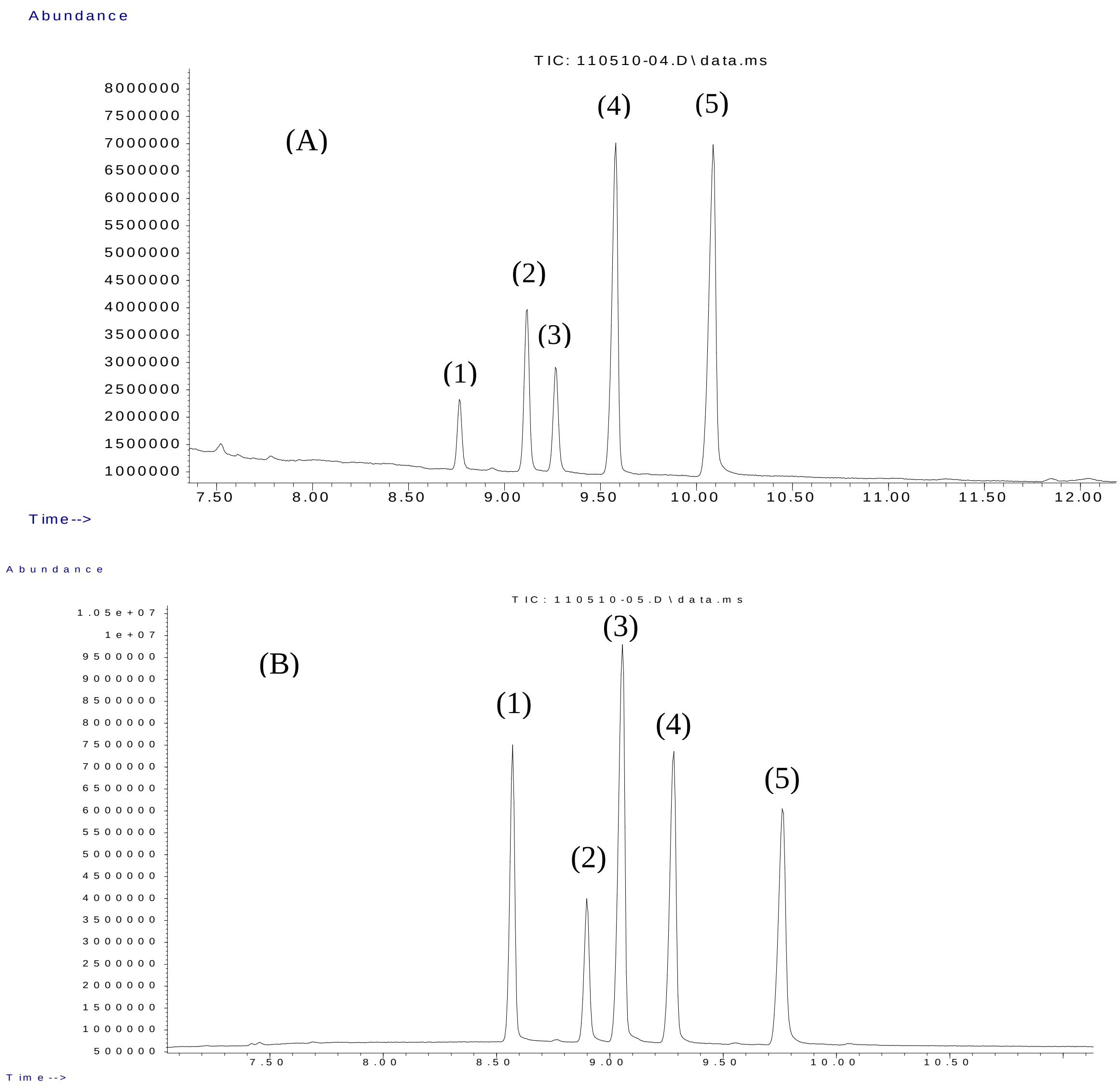


Fig. 59. Gas chromatographic separation of the (A) pentafluoropropionyl derivatives and (B) heptafluorobutyryl derivatives of the five piperazines using Rtx-200 column. The number over the peak represents the compound number.

3.9.4. Conclusion

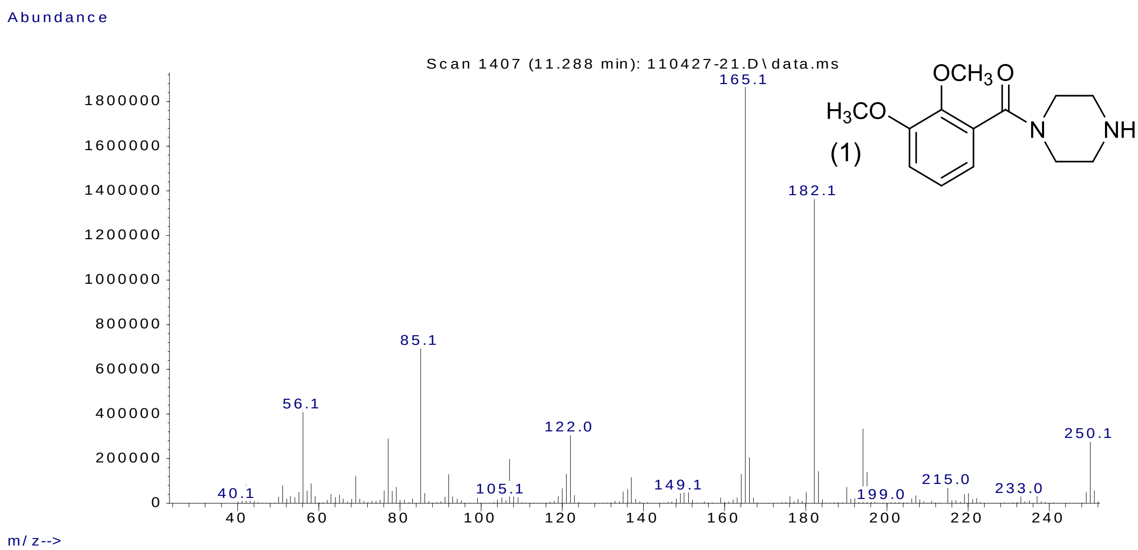
The three methoxybenzoylpiperazines have an indirect regioisomeric relationship to the controlled substance 3,4-MDBP and its regioisomer 2,3-MDBP. The five regioisomeric piperazines yield very similar fragment ions in their mass spectra with only the three methoxybenzoylpiperazine regioisomers showing 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.

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

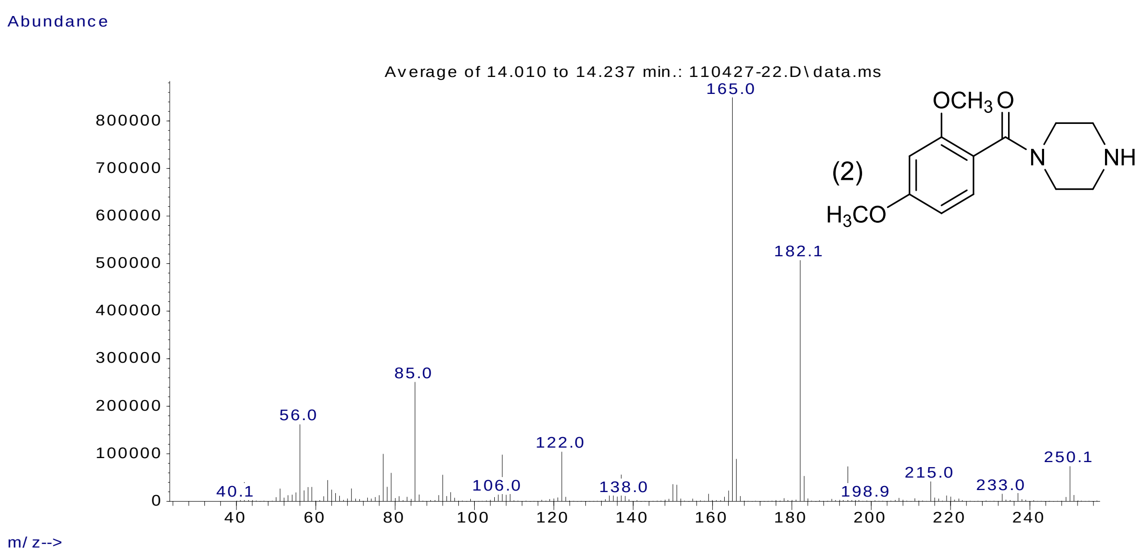
Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the differentiation between the six regioisomeric dimethoxybenzoylpiperazines. These six regioisomeric substances are well resolved by GC and the vapor phase infrared spectra clearly differentiate among the six dimethoxybenzoyl substitution patterns. However, the mass spectra for the six 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 six isomers to the exclusion of the other compounds. Perfluoroacylation of the secondary amine nitrogen for each of the six regioisomers was conducted 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 of structure.

3.10.1. Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 60 shows the EI mass spectra of the six regioisomeric dimethoxybenzoylpiperazines (Compounds 1-6). The mass spectra in Figure 60 indicate that very little structural information is available for differentiation among these isomers since all the major fragment ions occur at equal masses.



Abundance
Scan 1407 (11.288 min): 110427-21.D\data.ms
165.1
182.1
85.1
56.1
122.0
250.1
40.1
105.1
149.1
199.0
215.0
233.0
m/z-->
OCH3 O
H3CO
N
NH
(1)



Abundance
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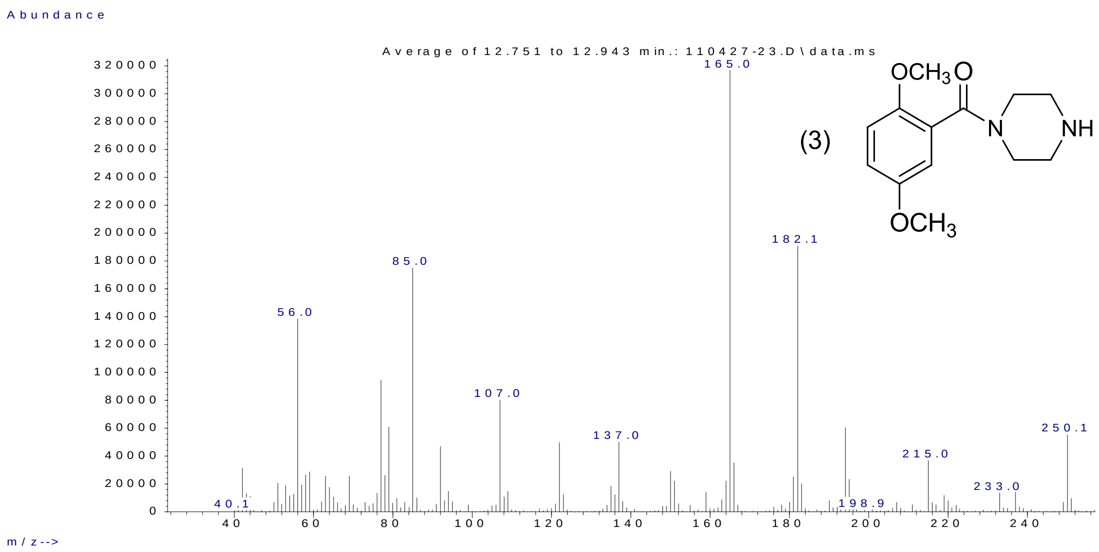
165.0
182.1
85.0
56.0
122.0
250.1
40.1
106.0
138.0
198.9
215.0
233.0
m/z-->
OCH3 O
N
NH
(2)
H3CO


Abundance
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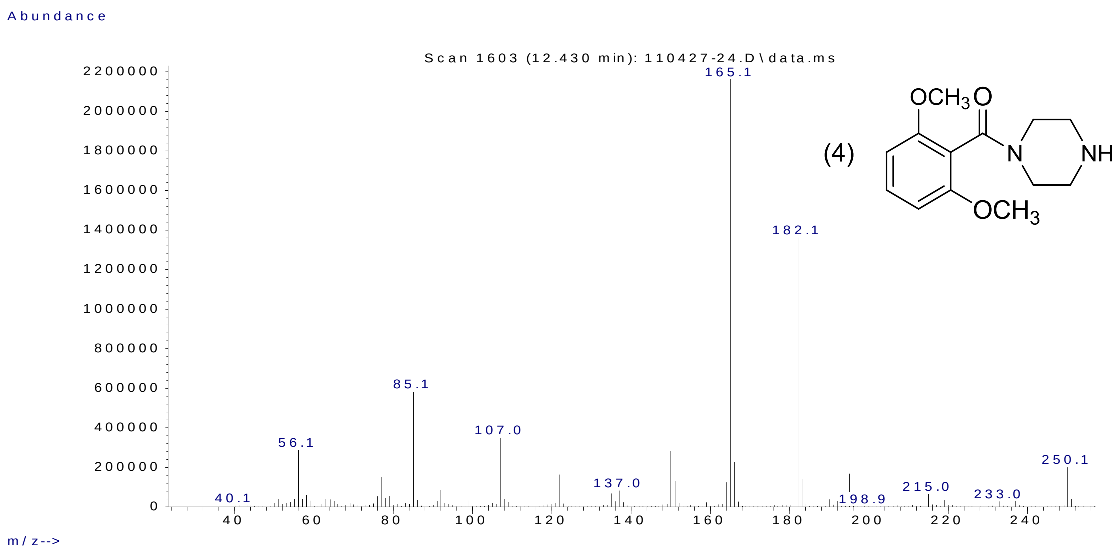
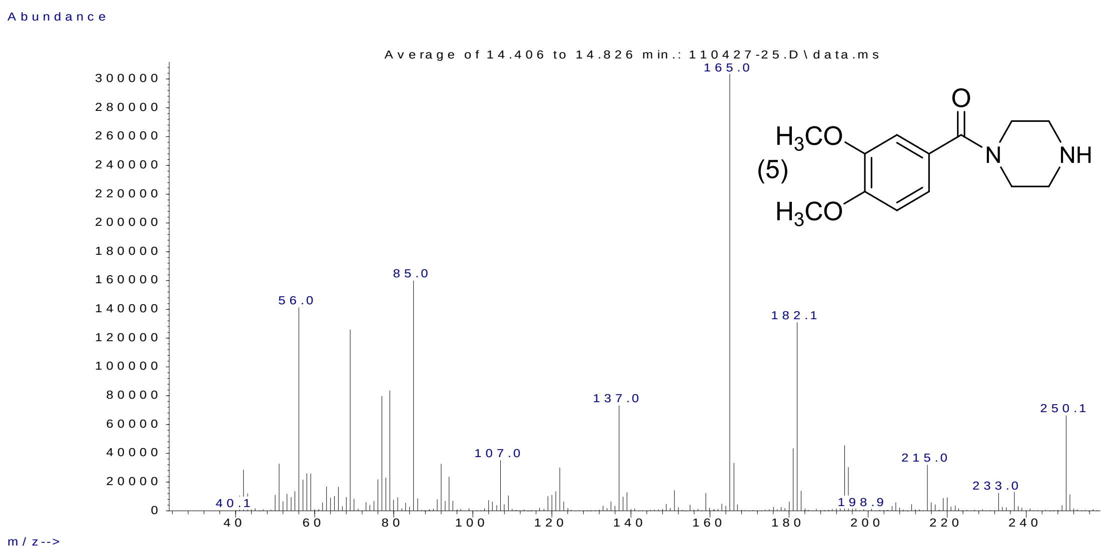
165.0
182.1
85.0
56.0
107.0
137.0
250.1
215.0
233.0
198.9
40.1
(3)
m/z-->


Abundance
Scan 1603 (12.430 min): 110427-24.D\data.ms
165.1
182.1
85.1
56.1
107.0
137.0
250.1
215.0
233.0
198.9
40.1
(4)
m/z-->



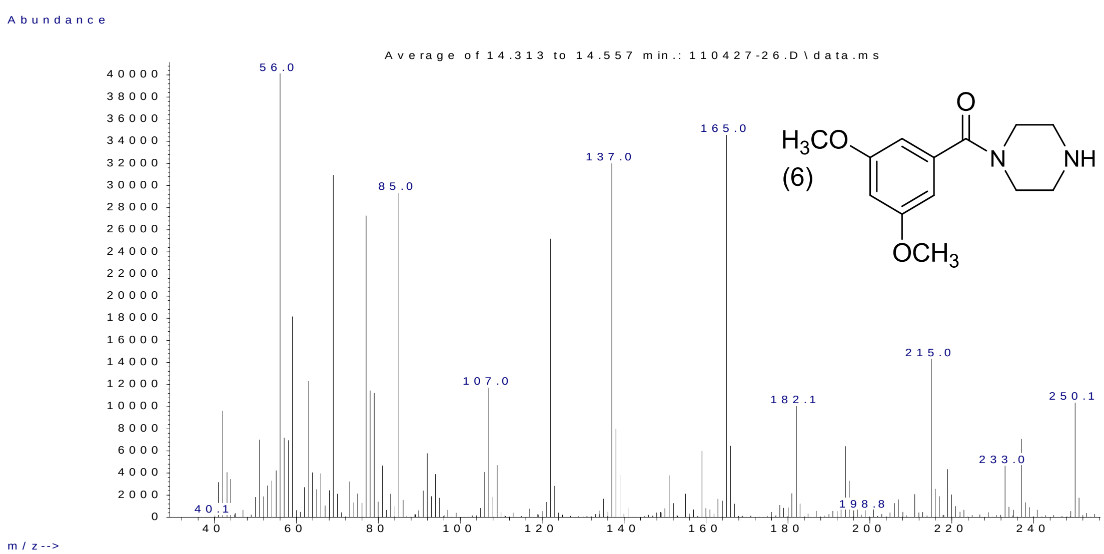
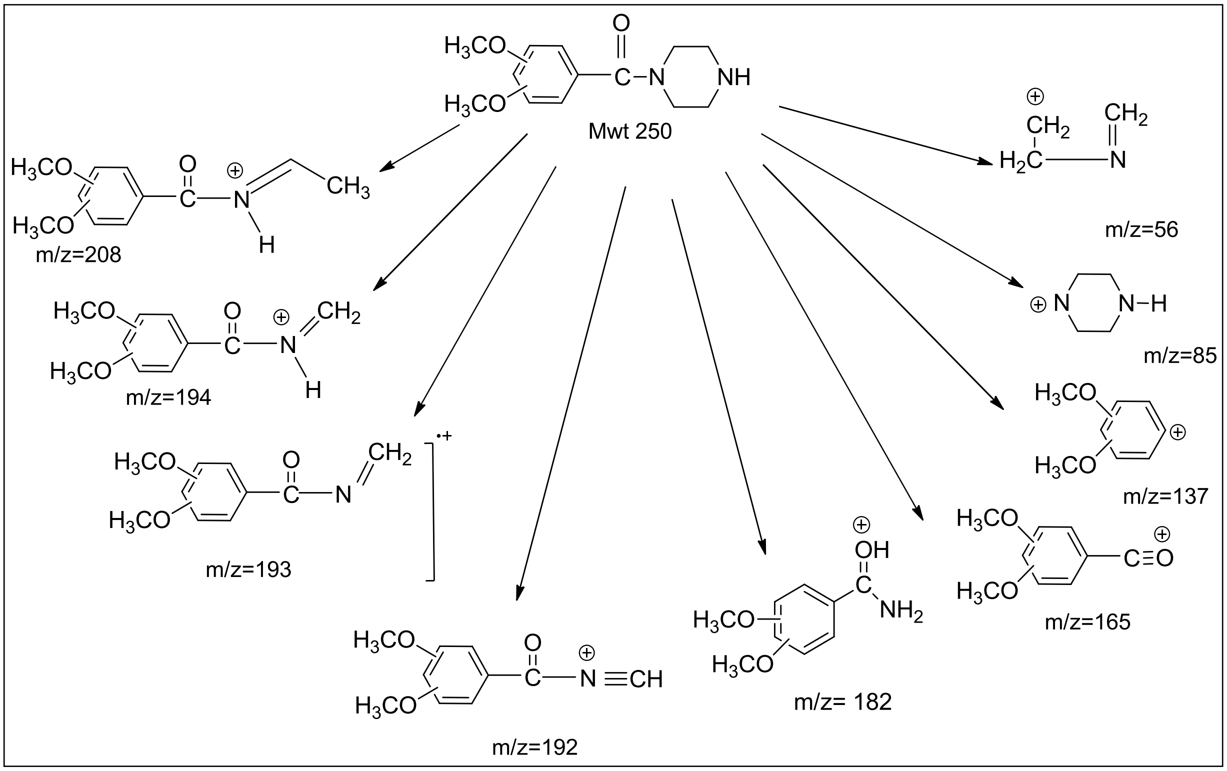


Fig. 60. Mass spectra of the underivatized six dimethoxybenzoylpiperazines.

The fragmentation pathways for dimethoxybenzoylpiperazine DMBP yield the fragment ions at m/z 208, 194, 193, 192, 165, 137, 85 and 56 as shown in Scheme 33. The structures for the fragmentation in the the six DMBzP regioisomers are likely equivalent. The relative abundances are slightly different for the six. Thus, mass spectrometry does not provide confirmation of identity for an individual DMBzP regioisomer.

Another common fragment ion occurs at m/z 182 in the mass spectra of the six regioisomers. The proposed structure for the formation of the m/z 182 $C_9H_{12}NO_3$ ion is shown in Scheme 33 and has also been described for the methoxybenzoylpiperazines in section 3.9.1. The suggested structure for this fragment involves the formation of the protonated 3,4-dimethoxybenzamide. The proposed structure for the m/z 182 ion is supported by the mass spectrum of the 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 in an ice-bath. The mass spectrum for the deuterium labeled form of Compound 5 is shown in Figure 61. The mass spectrum in Figure 61 shows that two deuterium atoms remain as a part of the ion in question since the mass increased by 2 Da to m/z 184 in this case.



Scheme 33. Mass spectral fragmentation pattern of the underivatized dimethoxybenzoylpiperazines under EI (70eV) conditions.

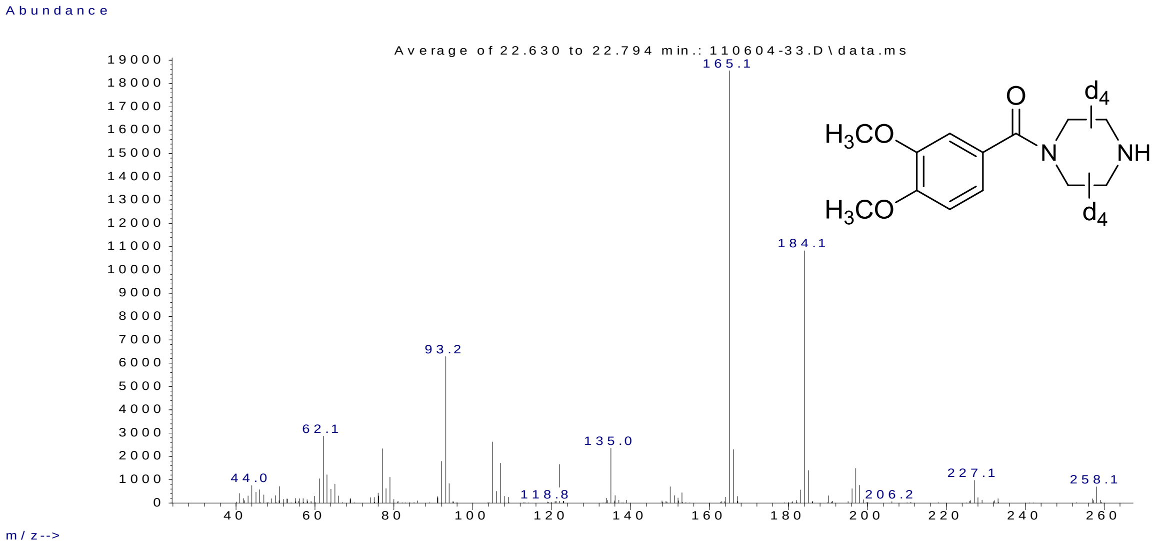
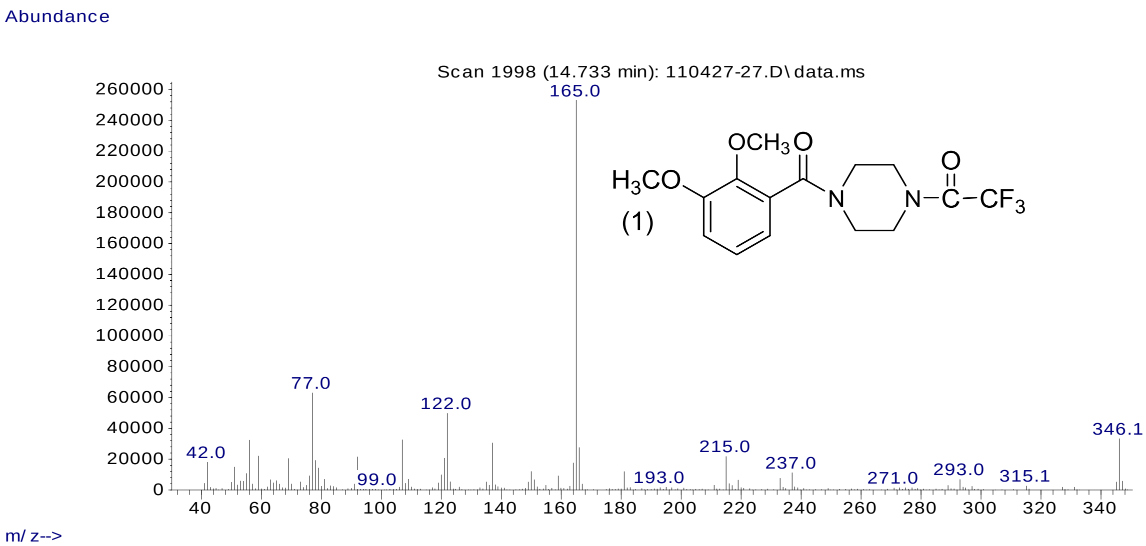


Fig. 61. Mass spectrum of the 3,4-dimethoxybenzoyl-d_8-piperazine.

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 compounds. Acylation lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum.

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 62 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 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 is no significant difference between the MS spectra of the six compounds. Thus, acylation of the six piperazines does not give characteristic fragments that help to discriminate among the six regioisomers.


Abundance
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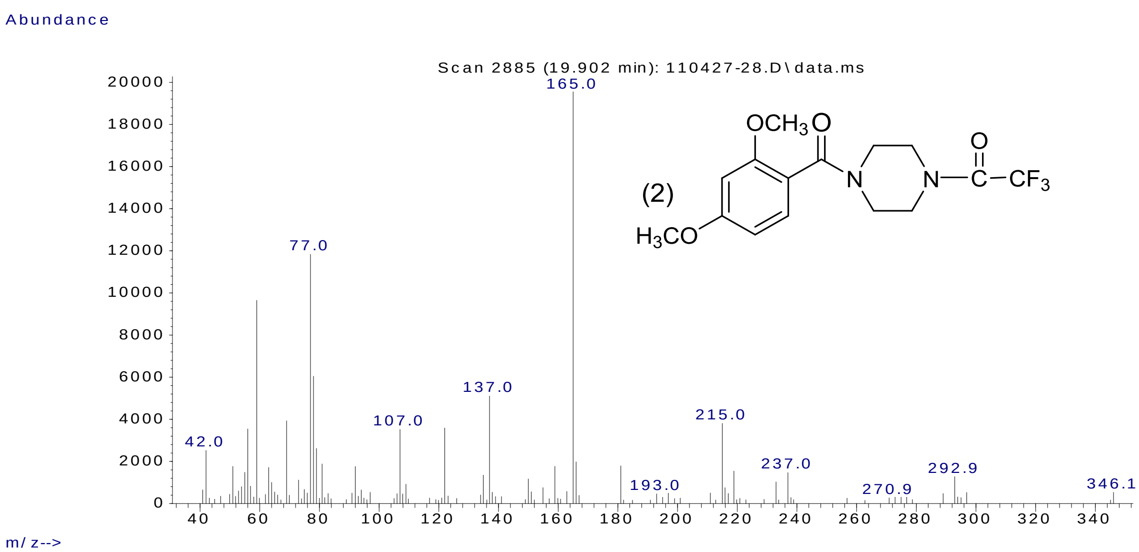
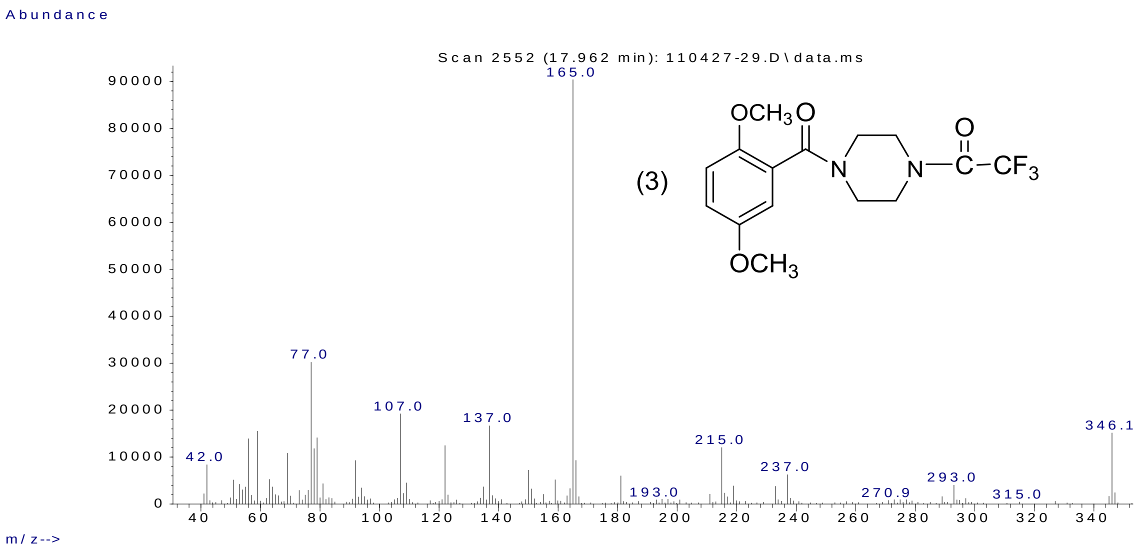
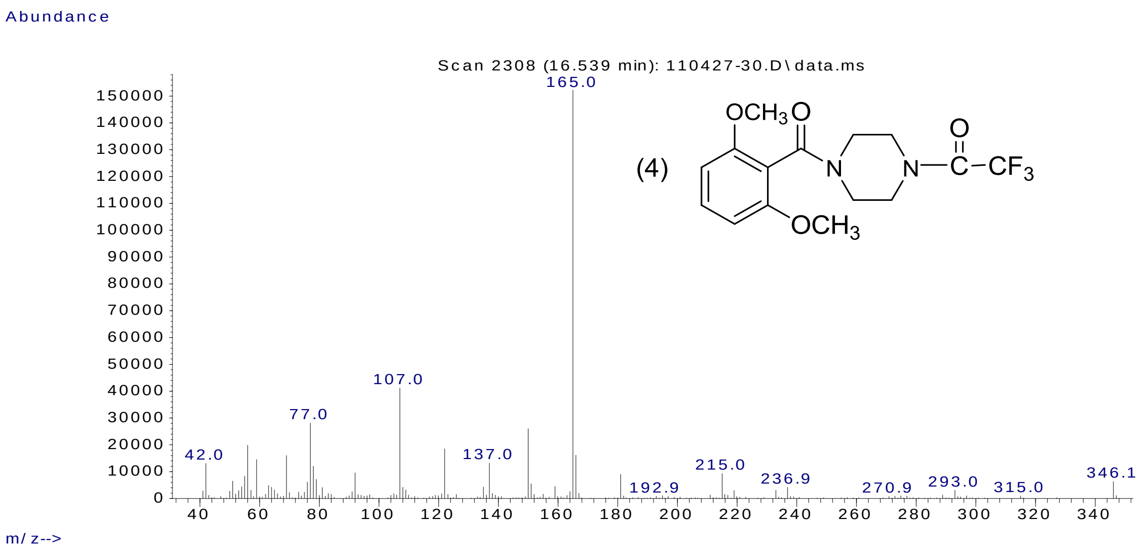
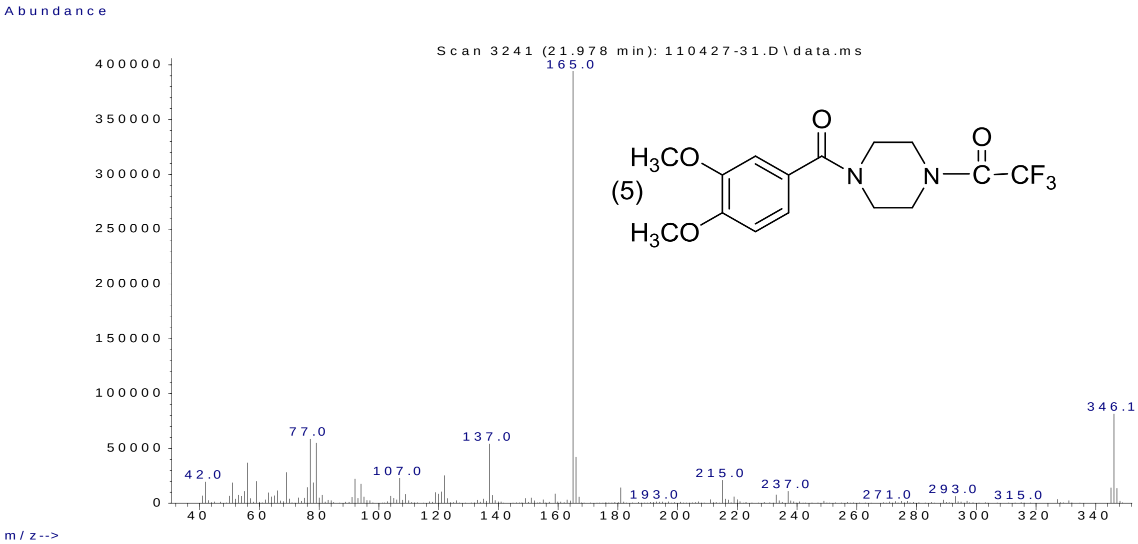
77.0
122.0
42.0
99.0
215.0
237.0
193.0
271.0
293.0
315.1
346.1
OCH_3 O
H_3CO
(1)
N
N
O
C
CF_3
m/ z-->


Abundance
Scan 2885 (19.902 min): 110427-28.D\ data.ms
165.0
77.0
137.0
107.0
42.0
215.0
237.0
193.0
270.9
292.9
346.1
OCH_3 O
(2)
H_3CO
N
N
O
C
CF_3
m/ z-->


Abundance
Scan 2552 (17.962 min): 110427-29.D\data.ms
(3)
m/z-->


Abundance
Scan 2308 (16.539 min): 110427-30.D\data.ms
(4)
m/z-->



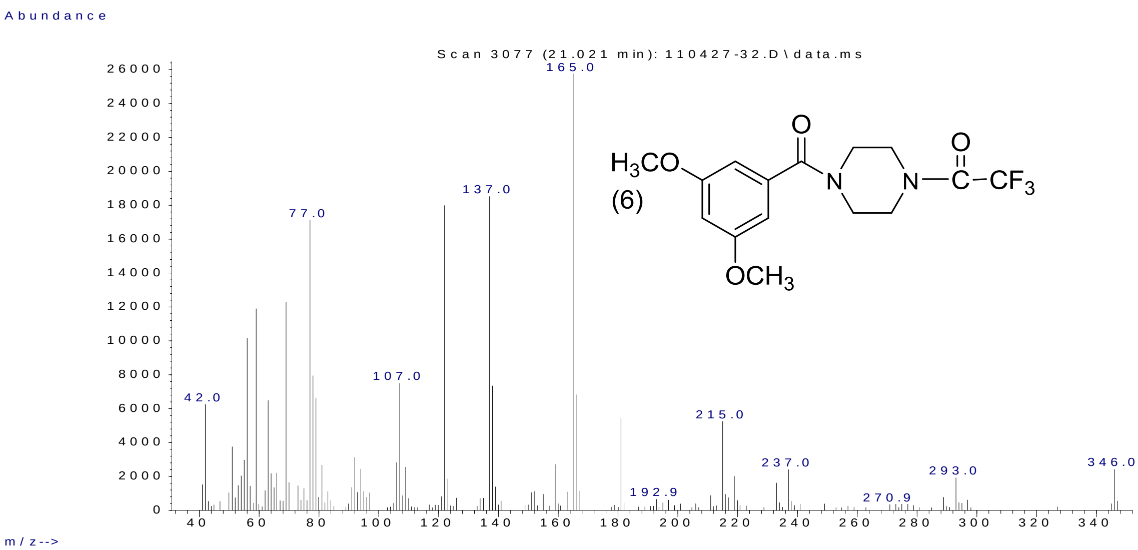
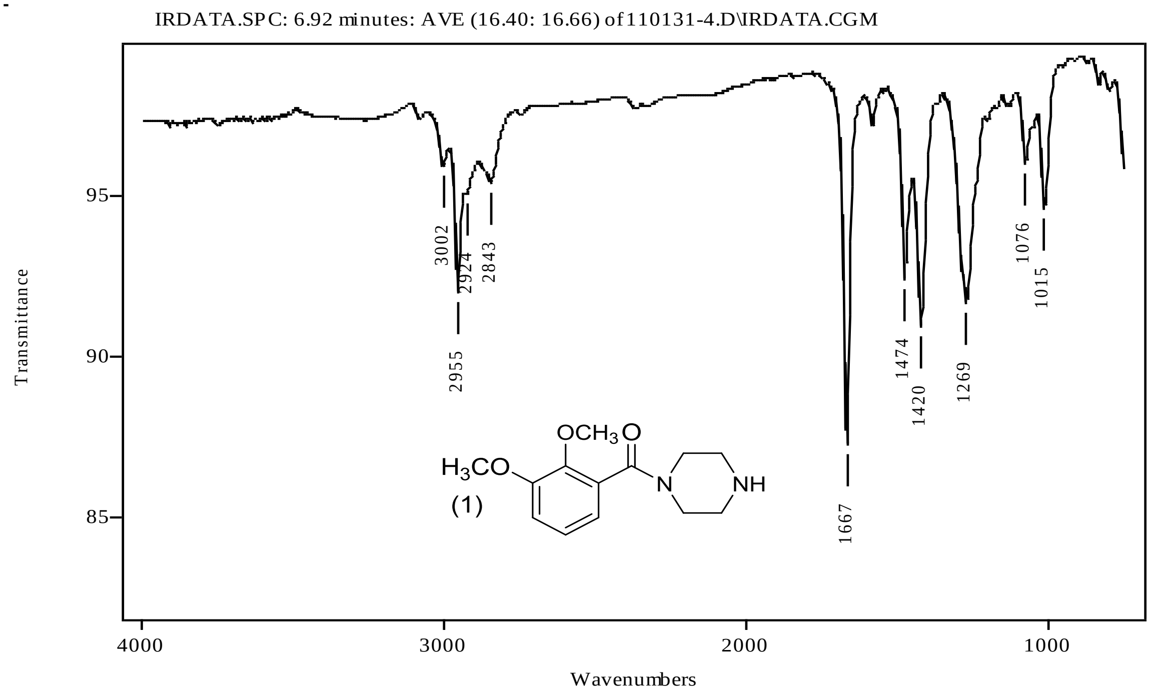


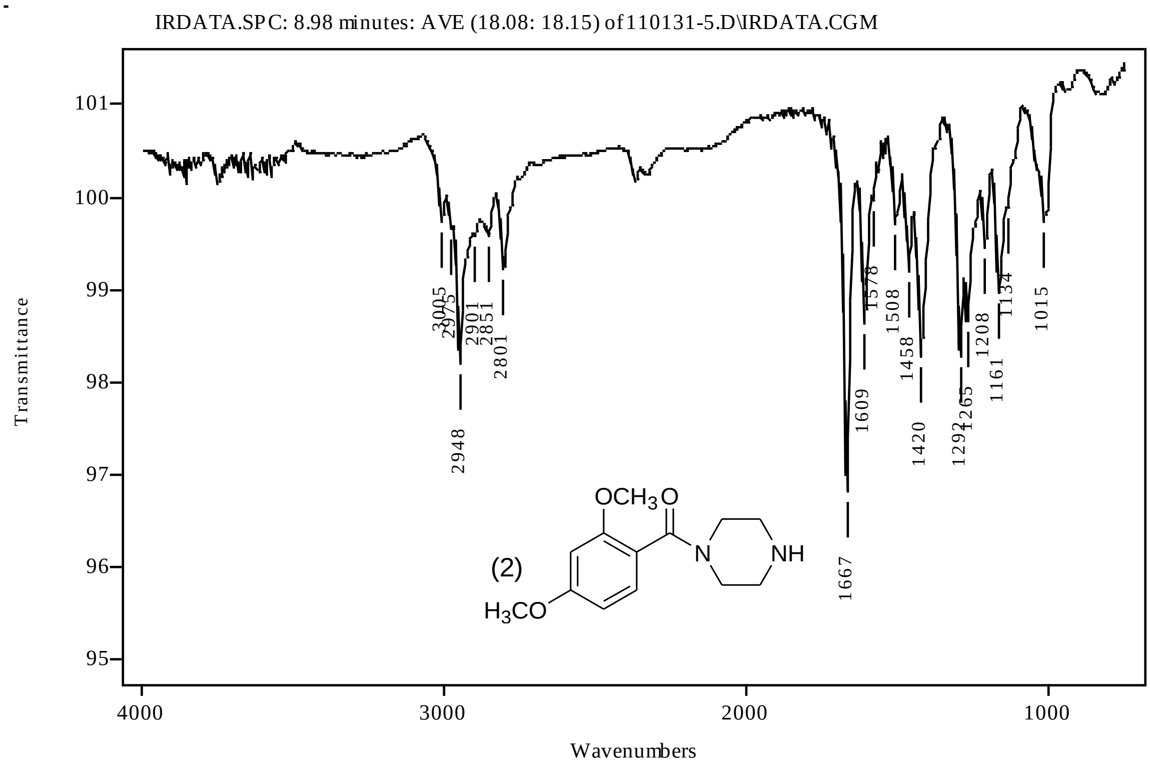
Fig. 62. Mass spectra of the trifluoroacetyl derivatives of the six dimethoxybenzoylpiperazine compounds.

3.10.2. Vapor-phase Infra-Red Spectrophotometry

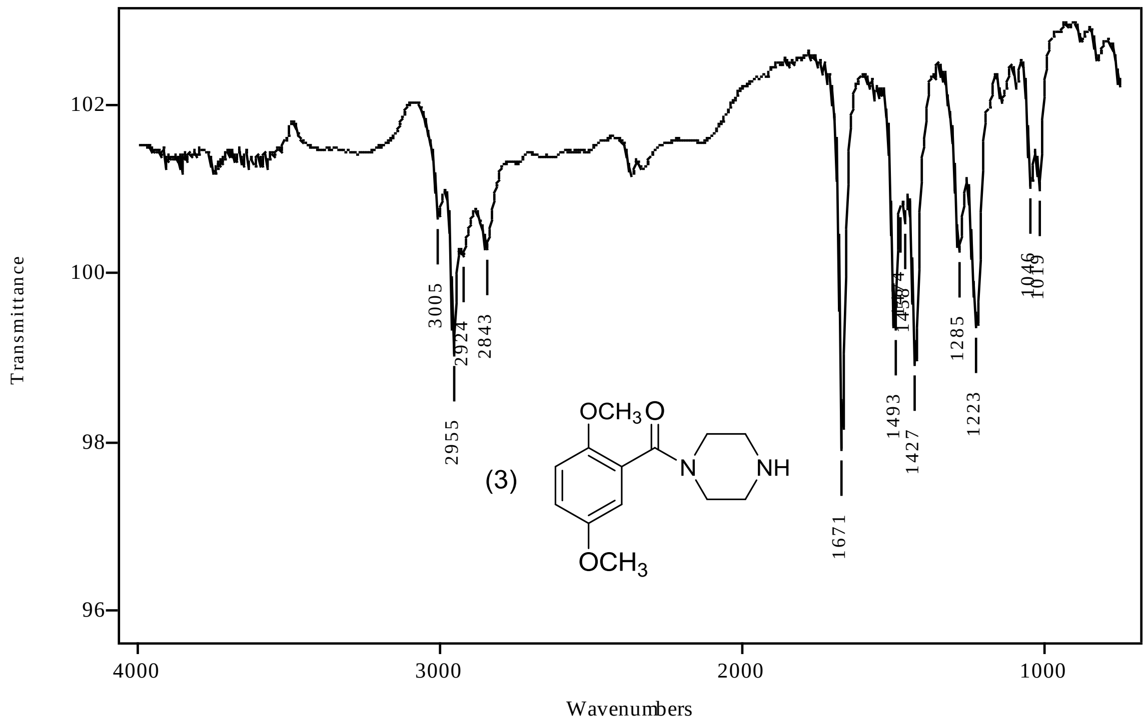
Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the six regioisomeric DMBzPs. Infrared detection should provide compound specificity without the need for chemical modification of the drug molecule. The vapor-phase infrared spectra for the six underivatized piperazines are shown in Figure 63. 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 IR spectra of the six regioisomers share a common IR absorption band at 1667 cm^{-1} corresponding to the carbonyl group stretching. 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-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-

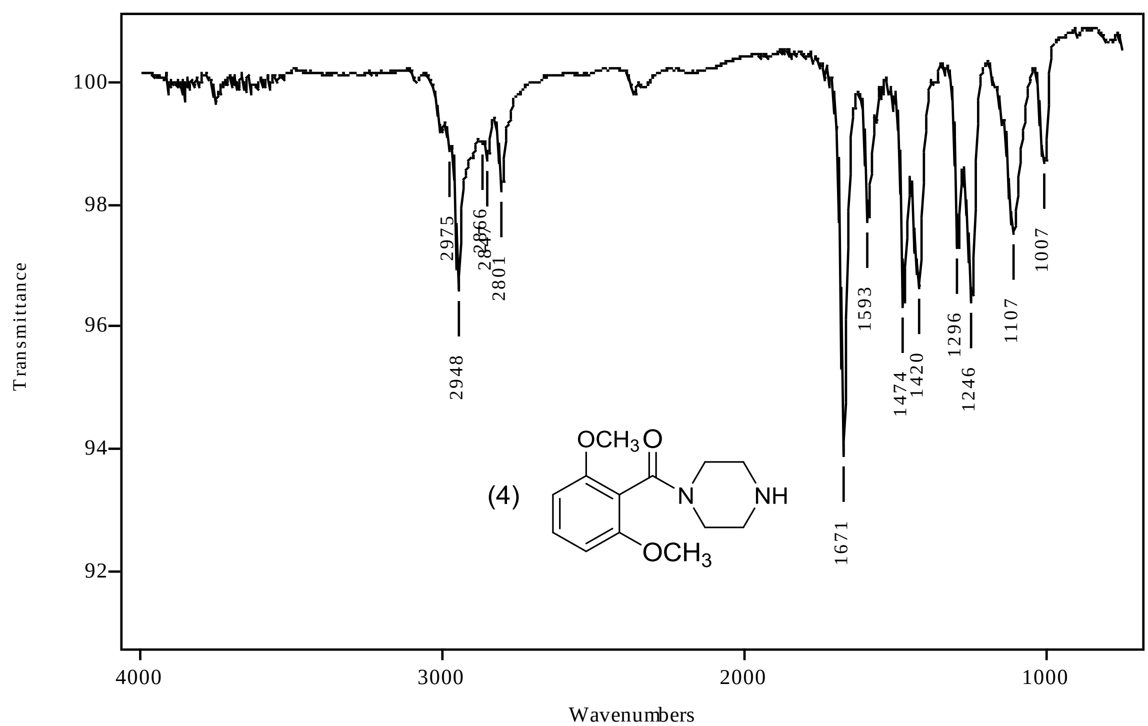

IRDATA.SPC: 6.92 minutes: AVE (16.40: 16.66) of 110131-4.D\IRDATA.CGM
Transmittance
Wavenumbers
4000
3000
2000
1000
95
90
85
3002
2924
2843
2955
1667
1474
1420
1269
1076
1015
OCH3
O
H3CO
N
NH
(1)

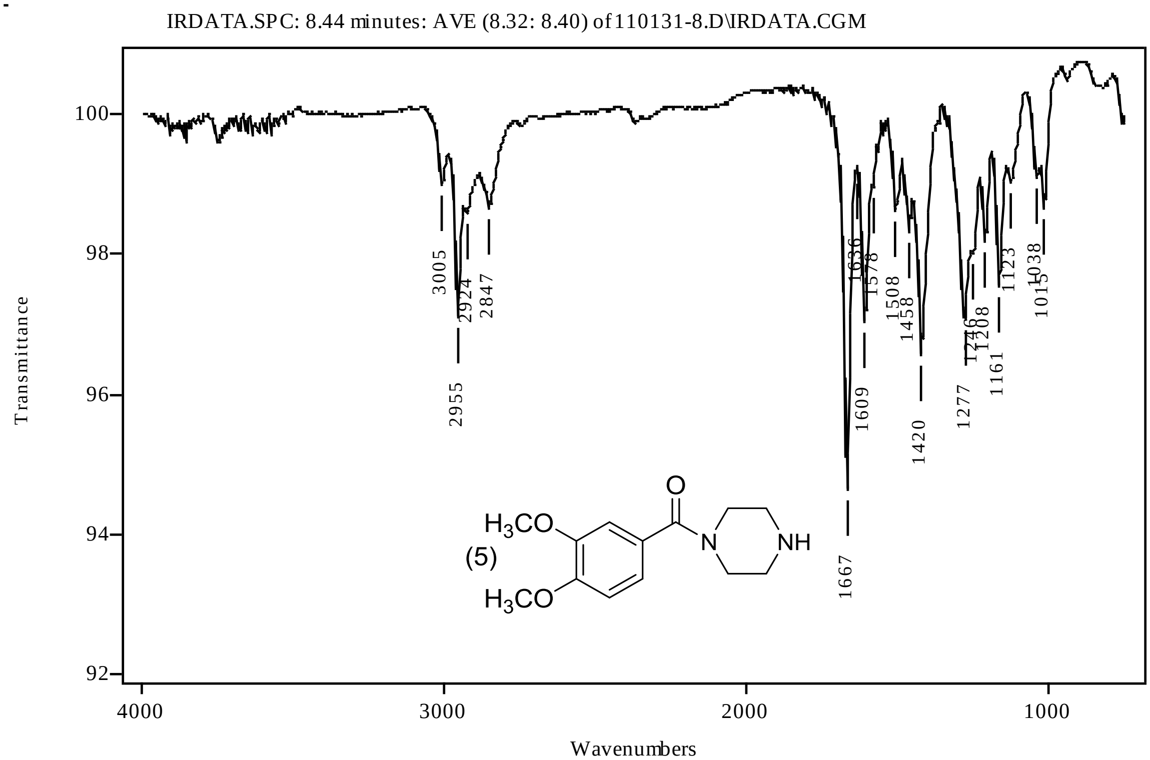

IRDATA.SPC: 8.98 minutes: AVE (18.08: 18.15) of 110131-5.D\IRDATA.CGM
Transmittance
Wavenumbers
4000
3000
2000
1000
101
100
99
98
97
96
95
3005
2975
2901
2851
2801
2948
1667
1609
1578
1508
1458
1420
1292
1265
1208
1161
1134
1015
OCH3
O
N
NH
(2)
H3CO

IRDATA.SPC: 4.18 minutes: AVE (20.83: 21.05) of 110131-6.D\IRDATA.CGM



IRDATA.SPC: 15.60 minutes: AVE (15.55: 15.60) of 110131-7.D\IRDATA.CGM





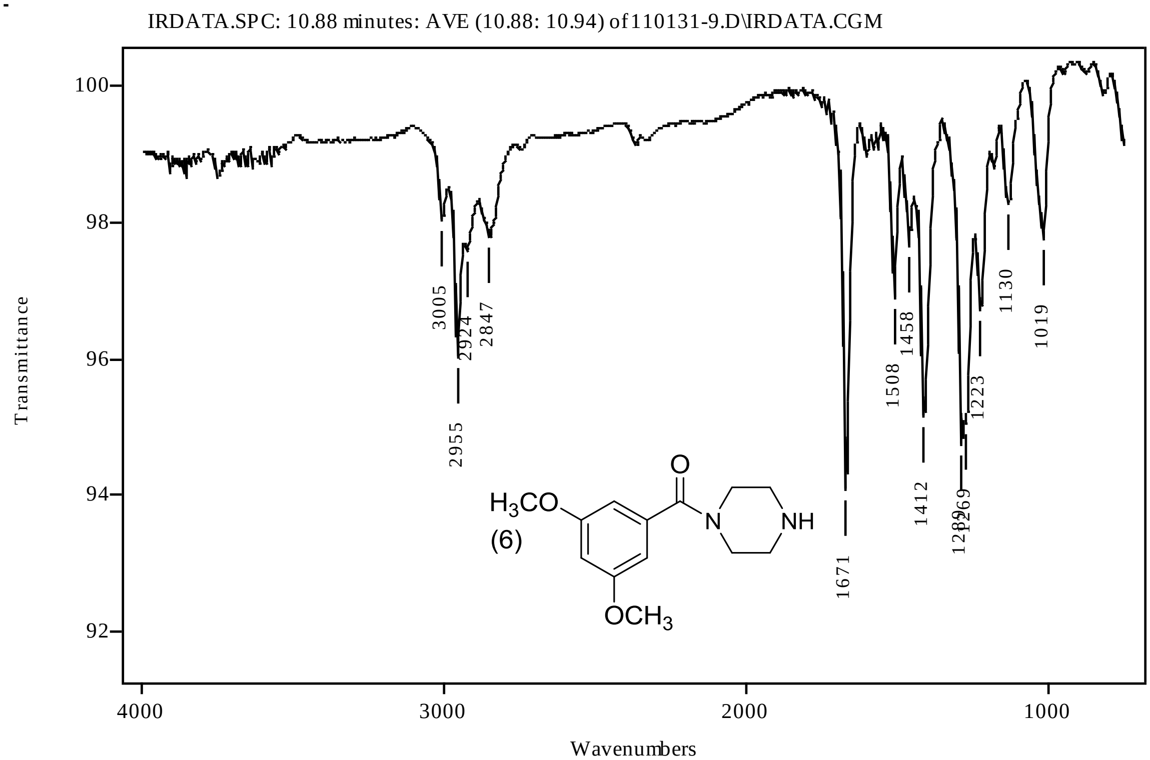


Fig. 63. Vapor phase IR spectra of the six dimethoxybenzoyl piperazines.

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.

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 set of regioisomeric dimethoxybenzoylpiperazine compounds.

3.10.3. Gas Chromatographic Separation

Gas chromatographic separation of the derivatized piperazines was accomplished on an Rtx-200 (100% trifluoropropyl methyl polysiloxane) stationary phase using a capillary column (30m × 0.25mm, 0.5-μm film thickness). 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, held at 250°C for 20 min. The representative chromatogram in Figure 64 shows the separation of the PFPA derivatives of the dimethoxybenzoylpiperazines. 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.

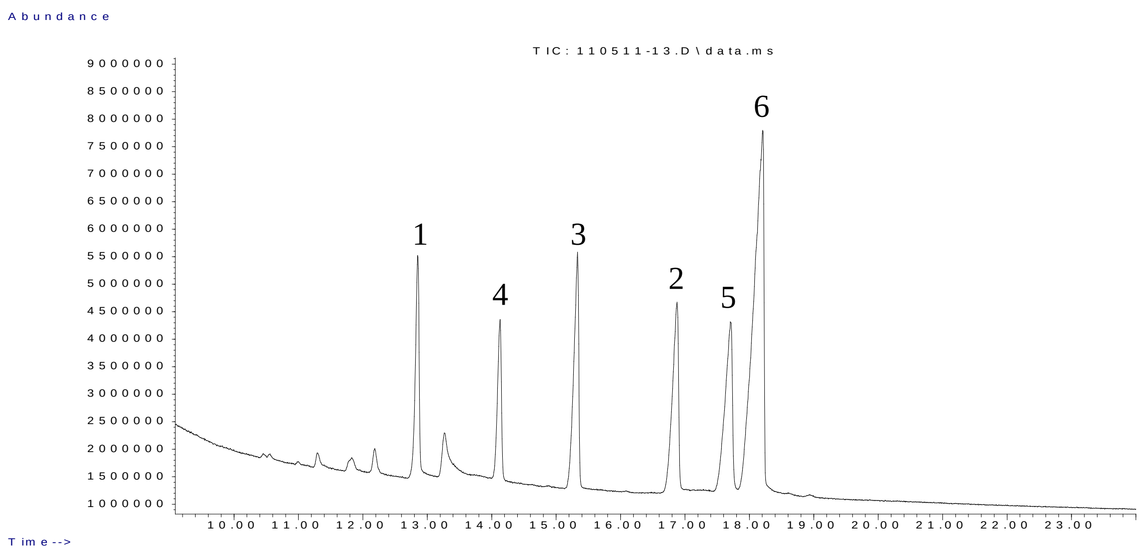


Fig. 64. Gas chromatographic separation of the pentafluoropropionyl derivatives of the dimethoxybenzoylpiperazines using Rtx-200 column. The number over the peak corresponds to the compound number.

3.10.4. 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.

3.11. 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.

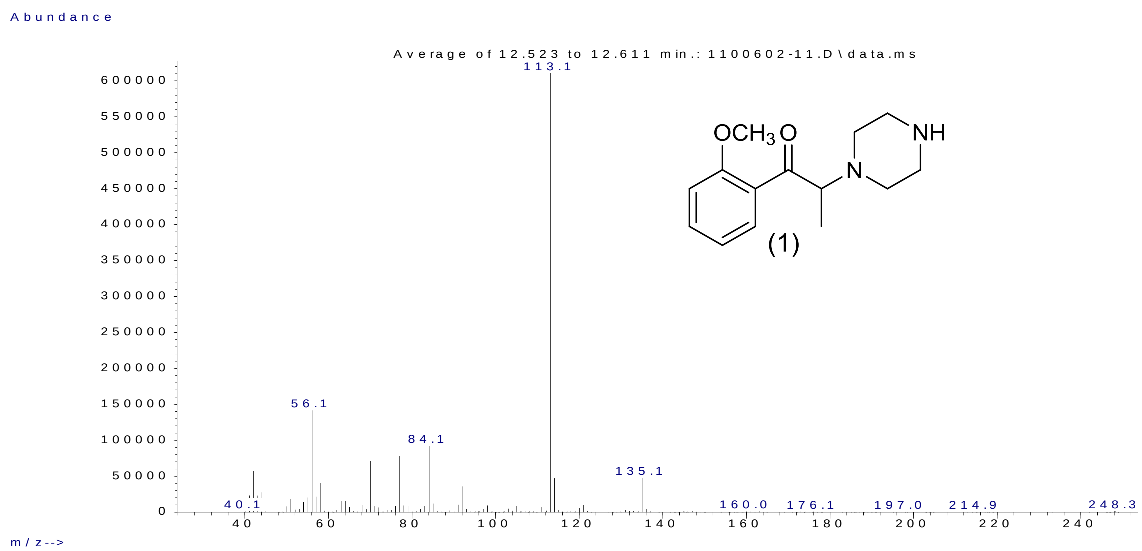
Gas chromatography coupled to infrared detection (GC-IRD) provides direct confirmatory data for the identification of the carbonyl containing compounds and the differentiation of the 3,4-MDPPP from its direct (2,3-MDPPP) and indirect (OMePPPOs) regioisomers. The vapor phase infrared spectra provide for 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 is composed of 5% diphenyl and 95% dimethyl polysiloxane (Rtx-5).

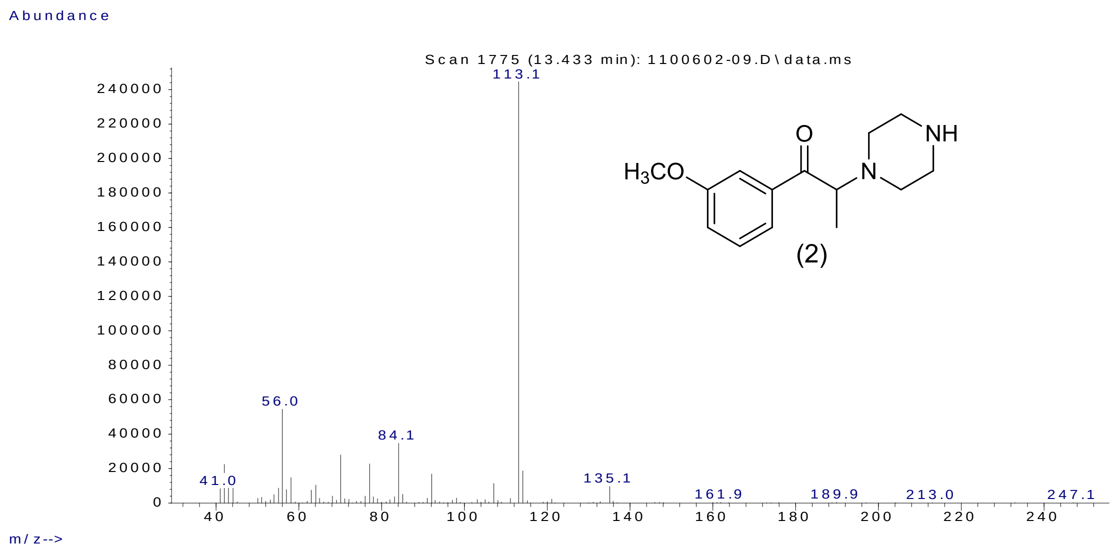
3.11.1. Mass Spectral Studies

Figure 65 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 in addition to the alpha cleavage (α-cleavage) products. The mass spectra of the five piperazines show the 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 Schemes 34 and 35. 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 occurring at m/z 135. The mass spectra for the ring substituted OMePPPOs (Compounds 1-3) have almost identical mass spectra to each other and to the MDPPPs isomers (Compounds 4 and 5).

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 66 shows the mass spectra of the heptafluorobutryl amides of the five studied compounds as representatives of all the perfluoroacylated piperazines. The molecular ion peaks for the five 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 perfluoroacyl 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* 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 other in this set of compounds.




Abundance
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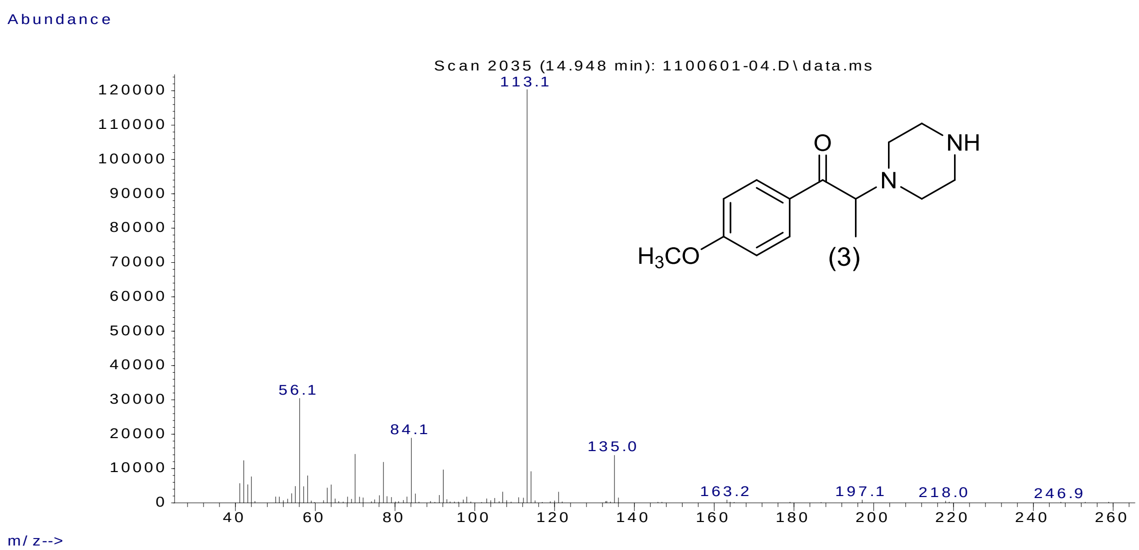
113.1
56.0
84.1
41.0
135.1
161.9
189.9
213.0
247.1
m/z-->
H3CO
O
NH
N
(2)


Abundance
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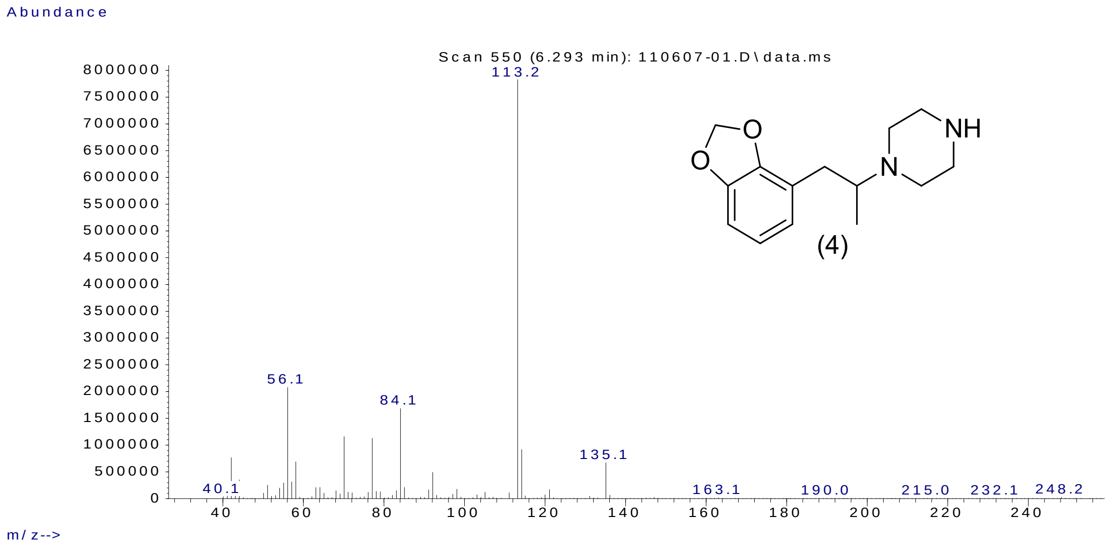
84.1
135.0
163.2
197.1
218.0
246.9
m/z-->
O
NH
N
H3CO
(3)



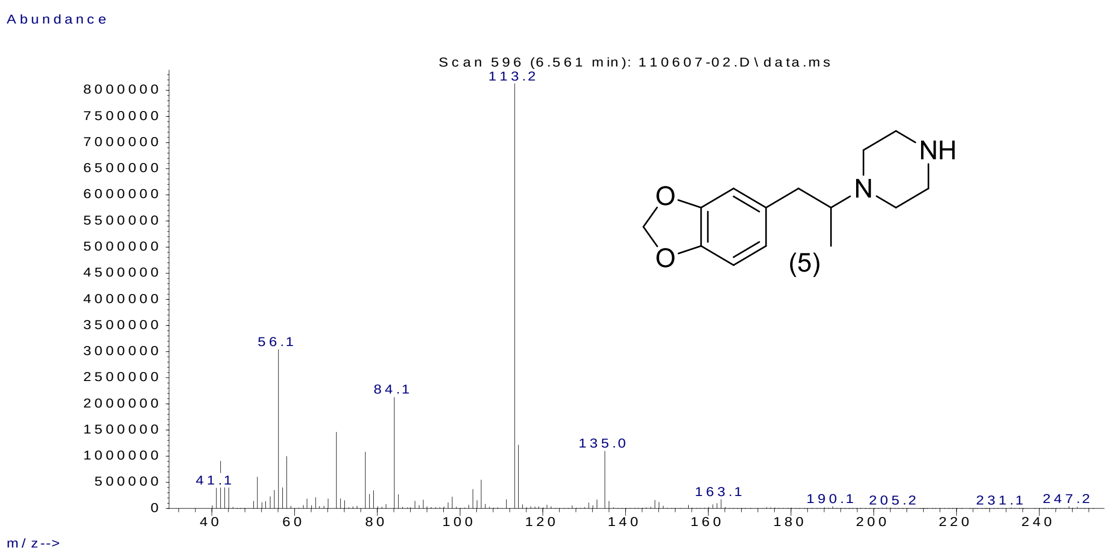
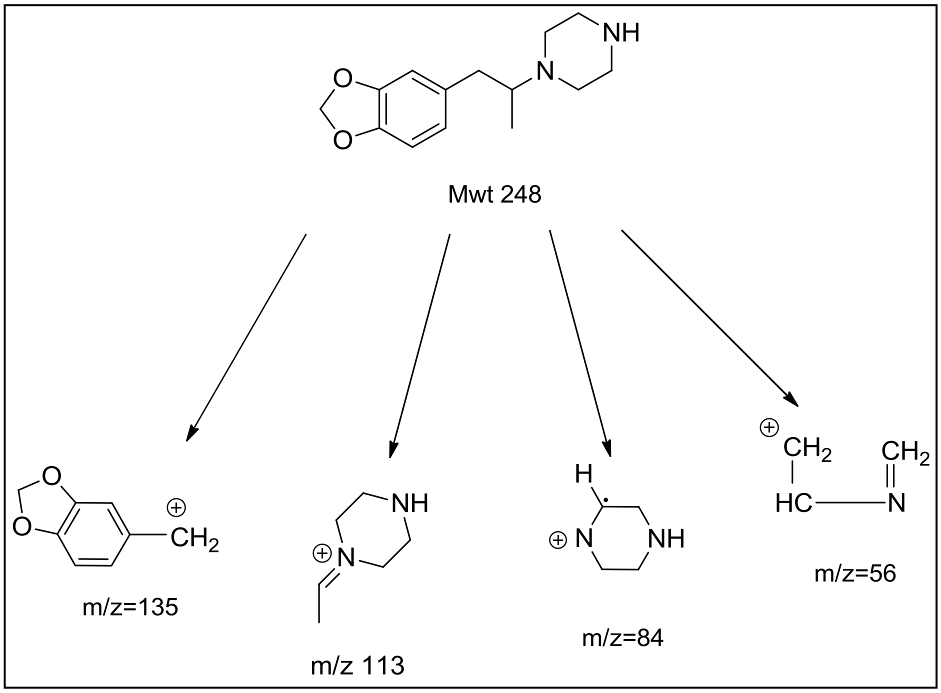
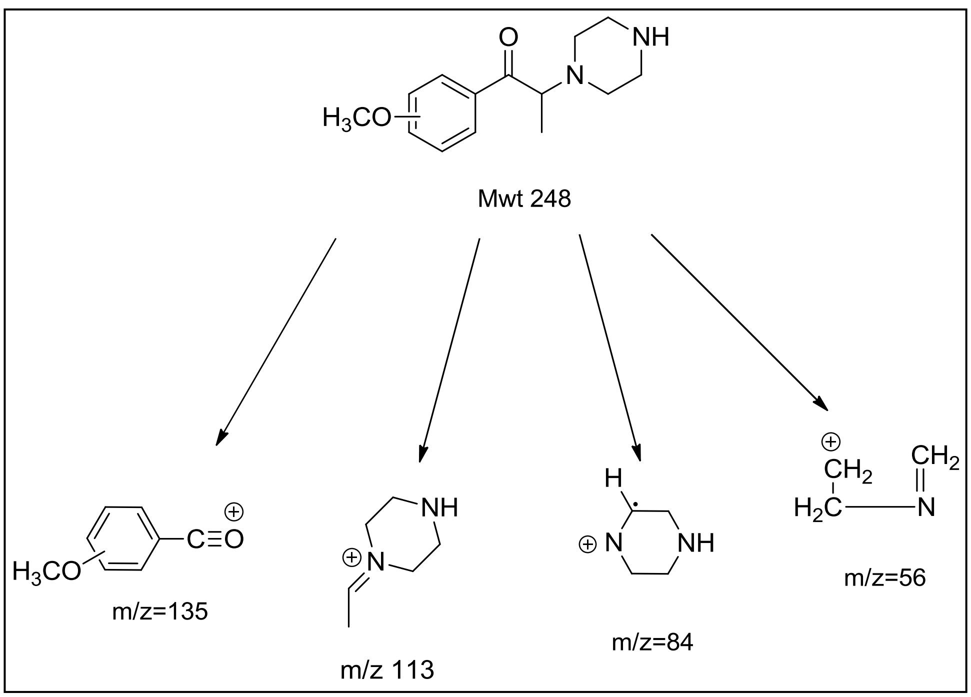


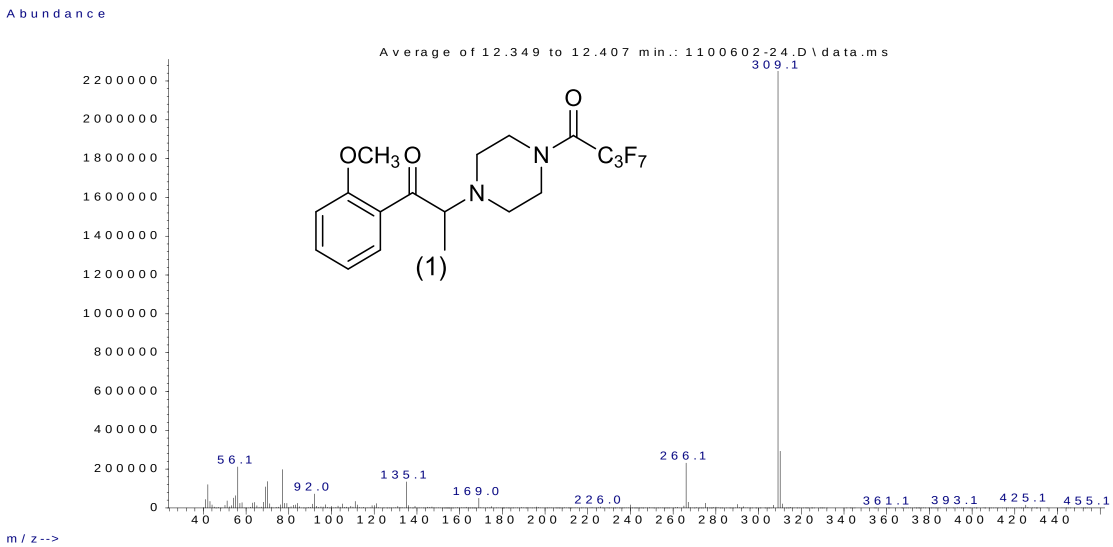
Fig. 65. Mass spectra of the five underivatized piperazines in this study.



Scheme 34. Mass spectral fragmentation pattern of the underivatized 1-(3,4-methylenedioxyphenyl)-2-piperazinopropane (3,4-MDPPP) under EI (70eV) conditions.



Scheme 35. Mass spectral fragmentation pattern of the underivatized 1-(methoxyphenyl)-2-piperazinopropanones (OMePPPOs) under EI (70eV) conditions.


Abundance
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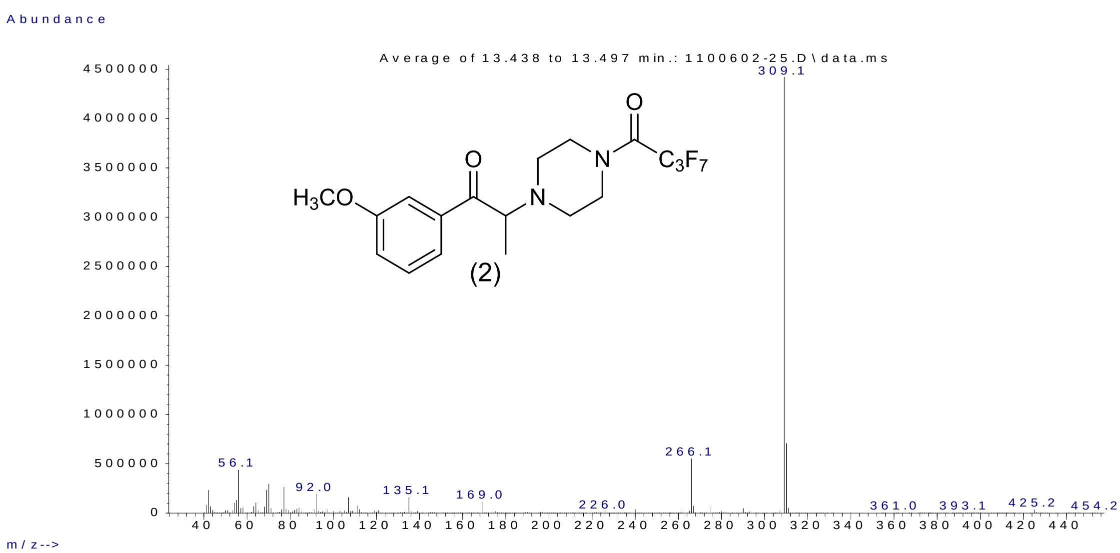
309.1
266.1
56.1
92.0
135.1
169.0
226.0
361.1
393.1
425.1
455.1
OCH3
O
N
N
O
C3F7
(1)
m/z-->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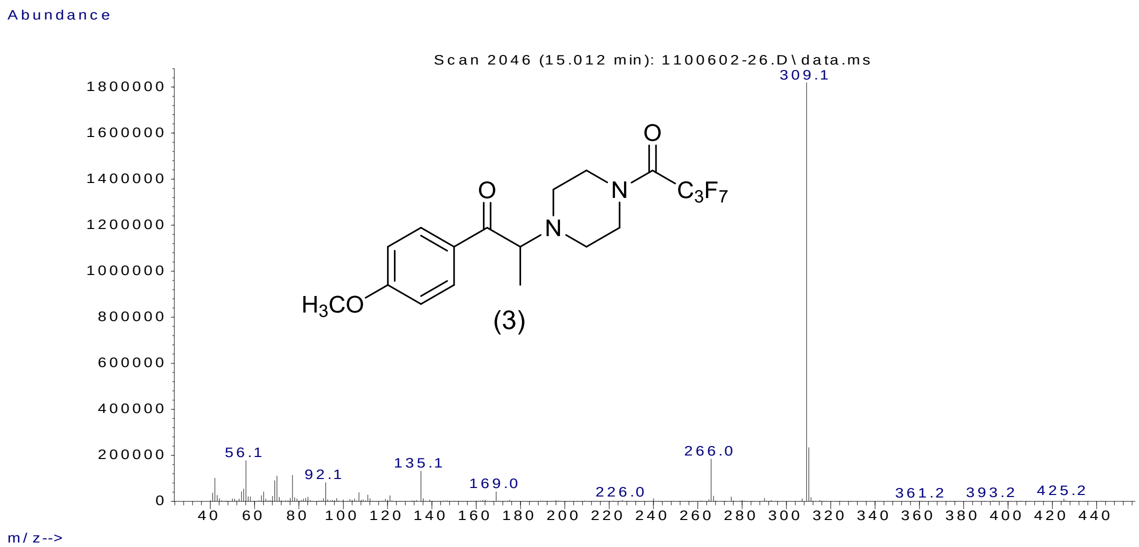
266.1
56.1
92.0
135.1
169.0
226.0
361.0
393.1
425.2
454.2
H3CO
O
N
N
O
C3F7
(2)
m/z-->


Abundance
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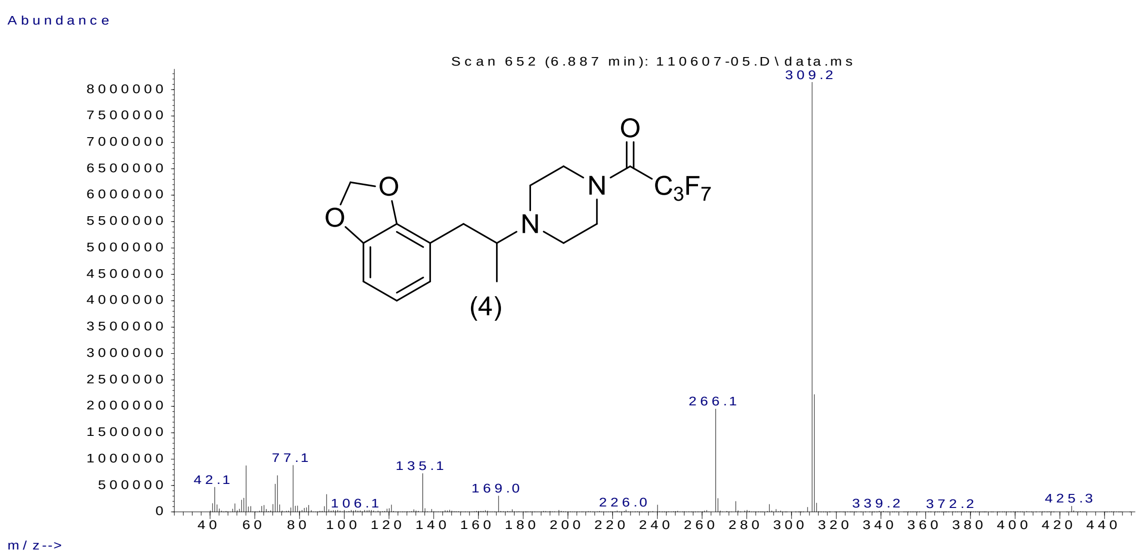
309.1
266.0
56.1
92.1
135.1
169.0
226.0
361.2
393.2
425.2
O
N
C3F7
H3CO
(3)
m/z-->


Abundance
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309.2
266.1
42.1
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226.0
339.2
372.2
425.3
O
N
C3F7
(4)
m/z-->

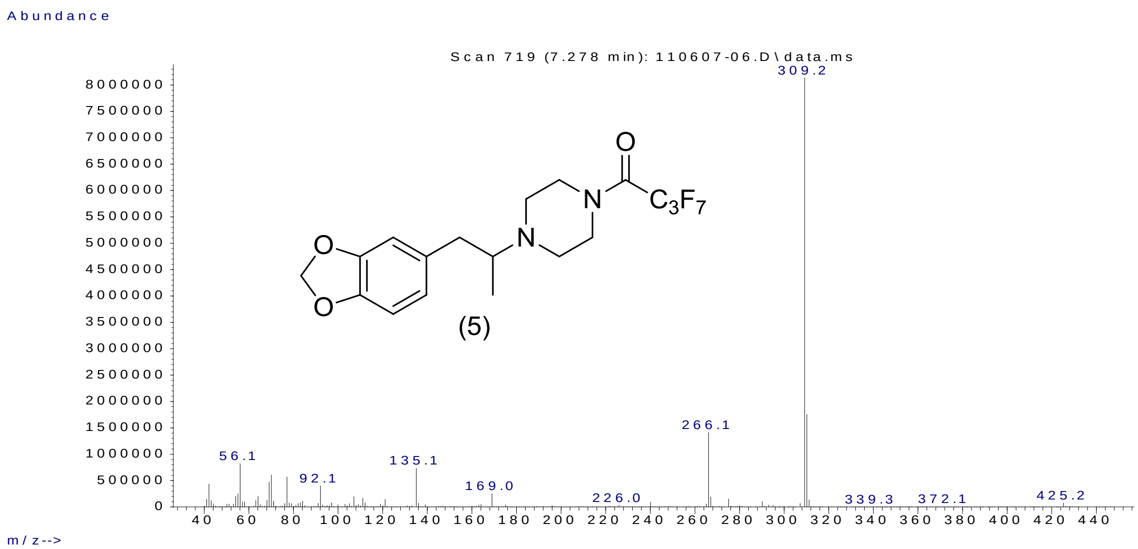


Fig. 66. Mass spectra of the heptafluorobutyryl derivatives of the five piperazines in this study

3.11.2. Vapor-phase Infra-Red Spectroscopy

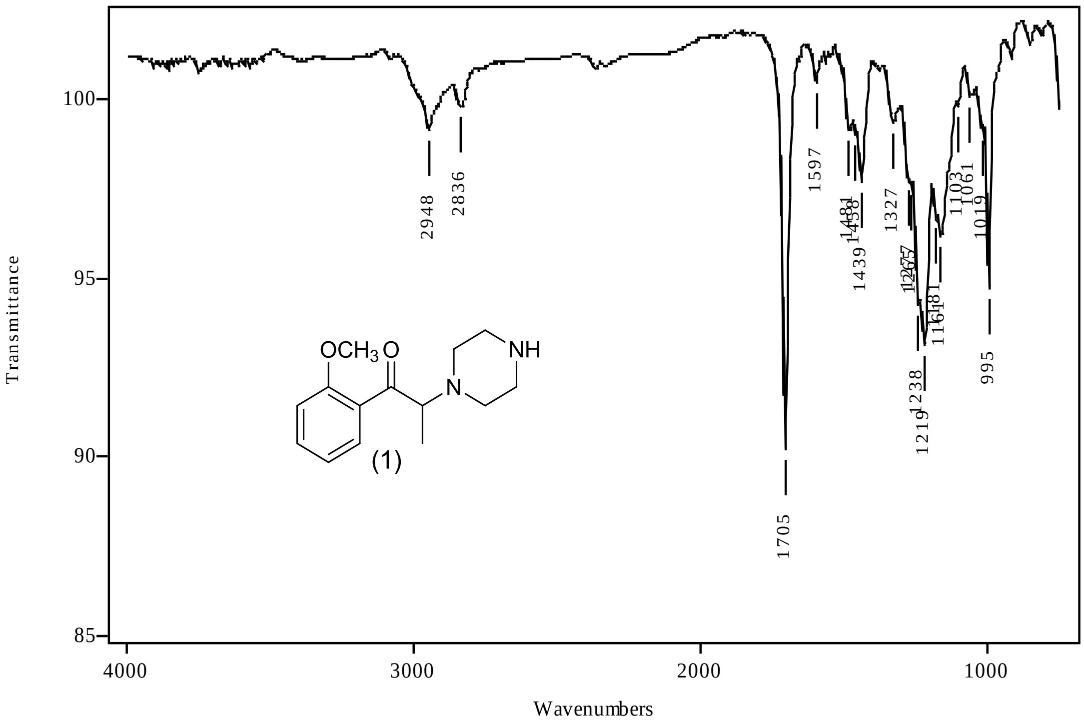
Infrared spectroscopy is often used as a confirmatory method for compound identification in forensic drug analysis. Gas chromatography coupled with infrared detection (GC-IRD) was evaluated for differentiation among the five isomeric piperazines. The vapor phase infrared spectra for the five piperazines are shown in Figure 67. 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 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 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 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.

IRDATA.SPC: 6.15 minutes: AVE (13.23: 13.28) of 110602-A.D\IRDATA.CGM



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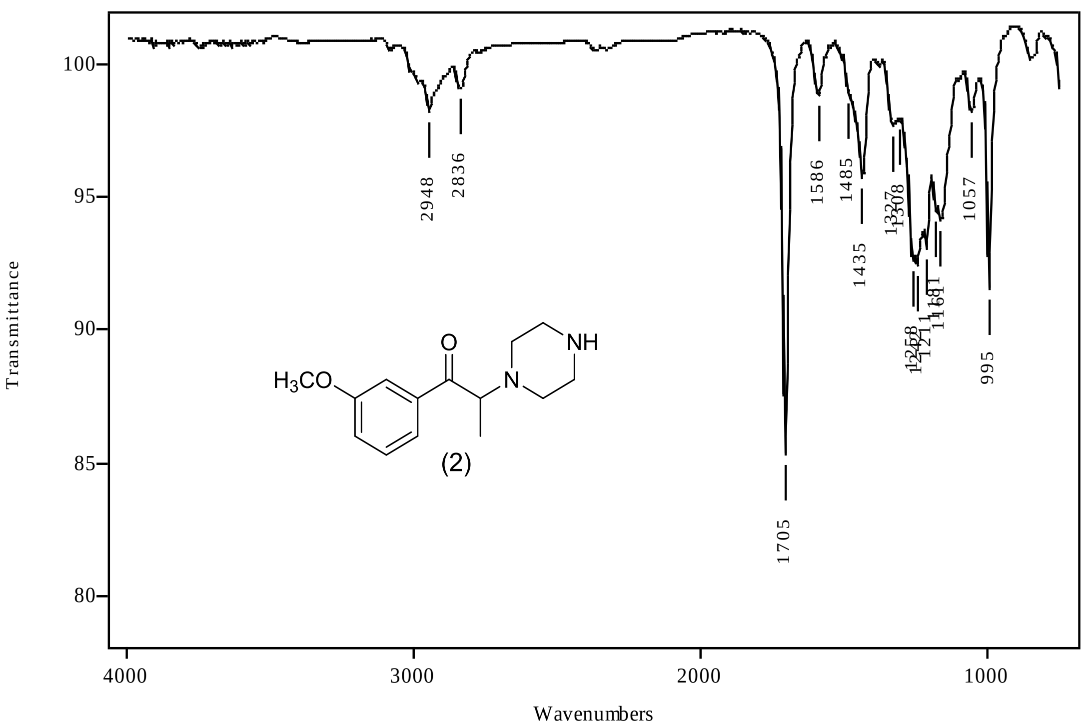

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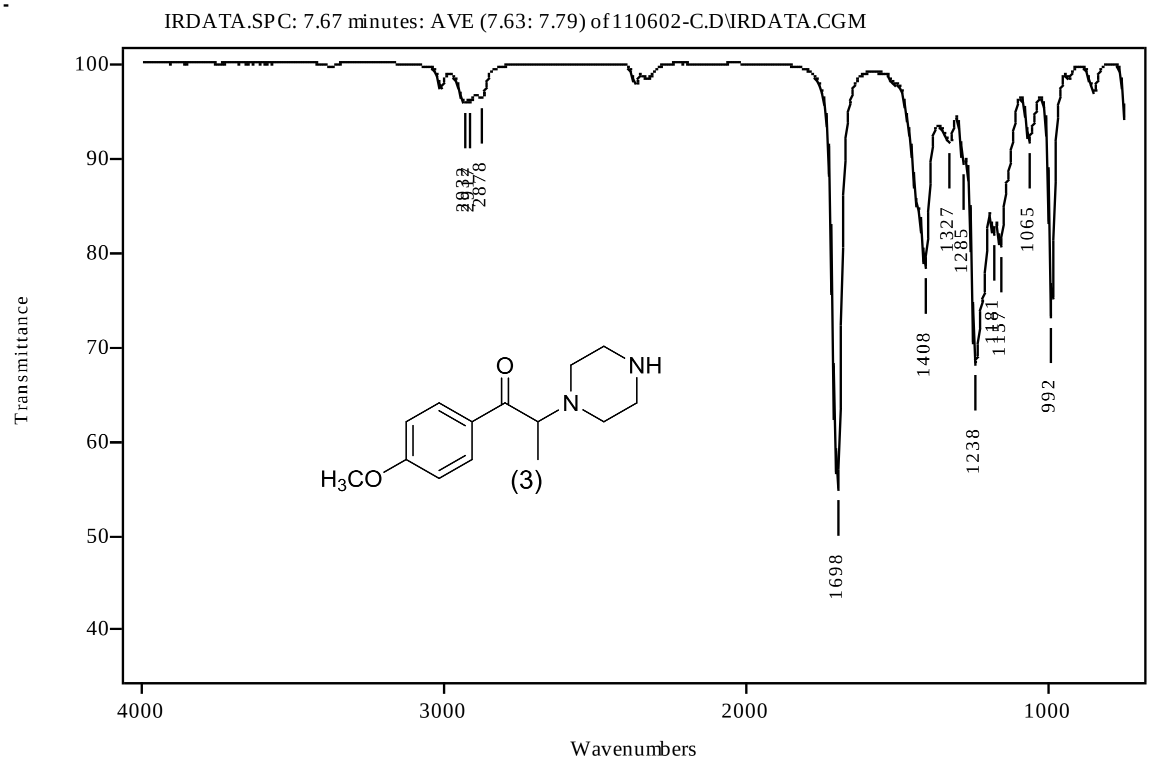
IRDATA.SPC: 7.67 minutes: AVE (7.63: 7.79) of 110602-C.D\IRDATA.CGM
Transmittance
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1408
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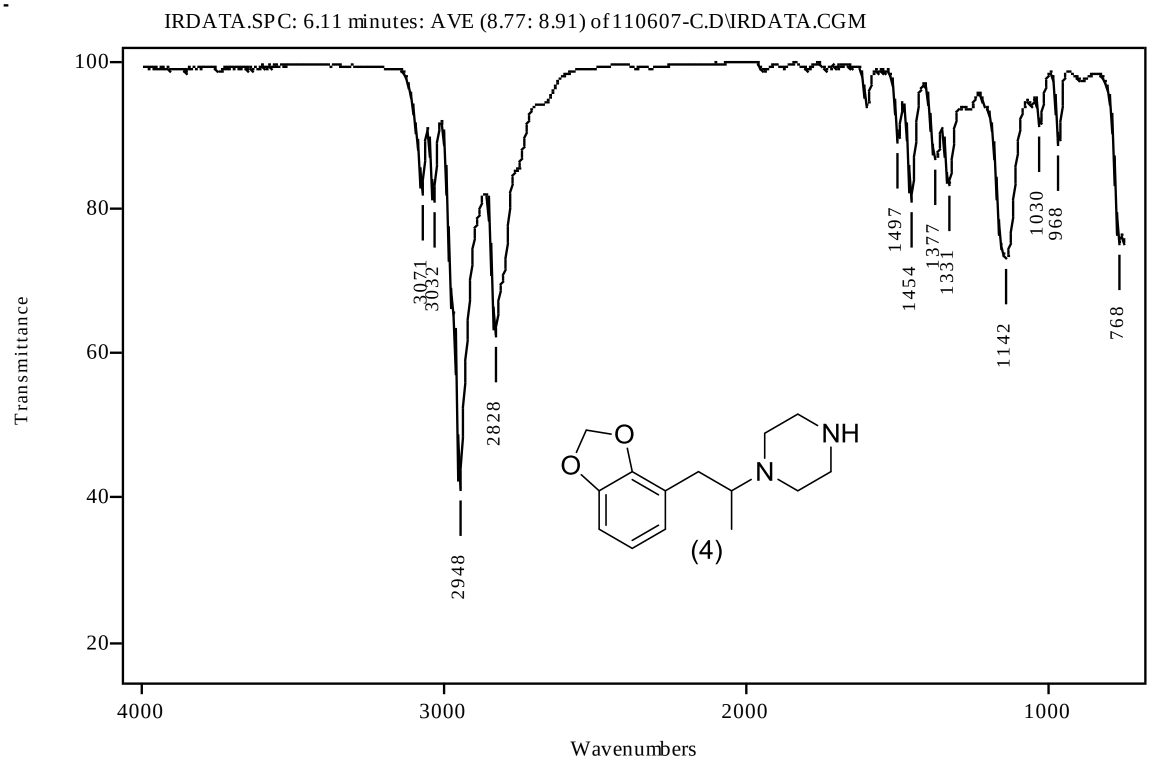
1285
1238
1181
1157
1065
992
O
NH
N
H_3CO
(3)

-


IRDATA.SPC: 6.11 minutes: AVE (8.77: 8.91) of 110607-C.D\IRDATA.CGM
Transmittance
Wavenumbers
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O
O
NH
N
(4)

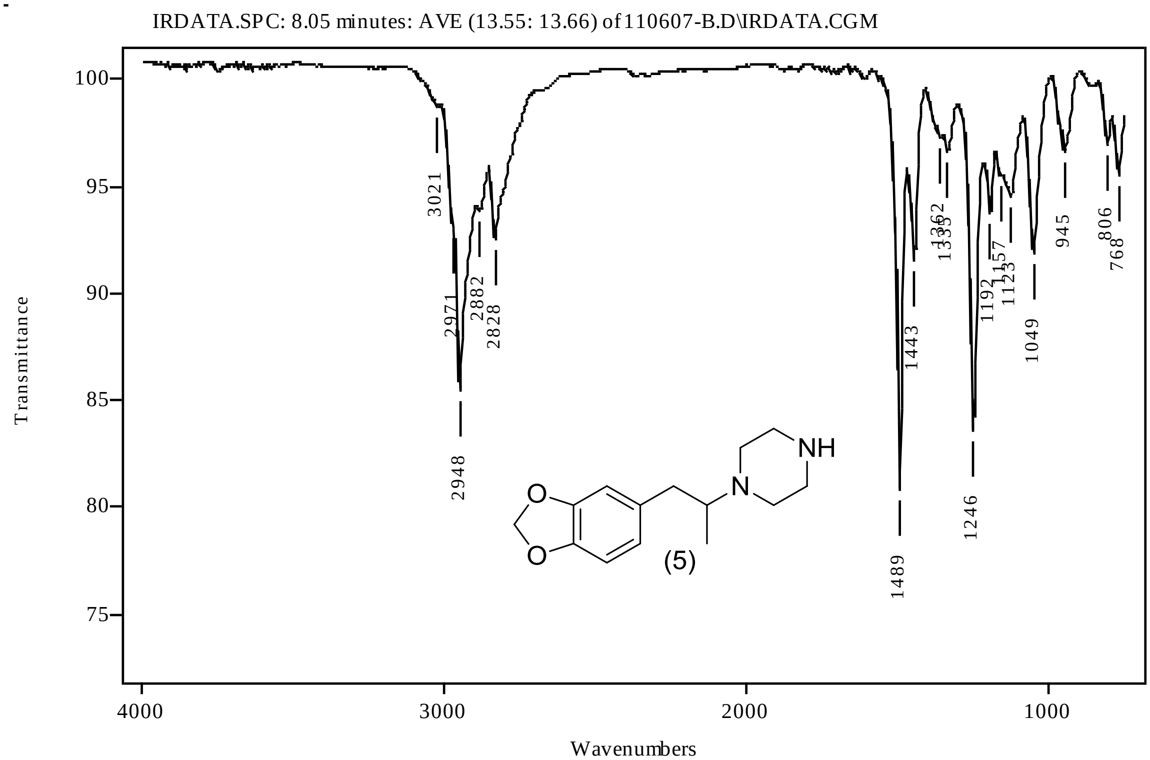


Fig. 67. Vapor phase IR spectra of the five piperazines involved in this study

3.11.3. Gas Chromatographic Separation

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). 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.

The chromatograms in Figure 68 are representatives of the results obtained for all samples on these two stationary phases. In Figure 68A and 68B 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.

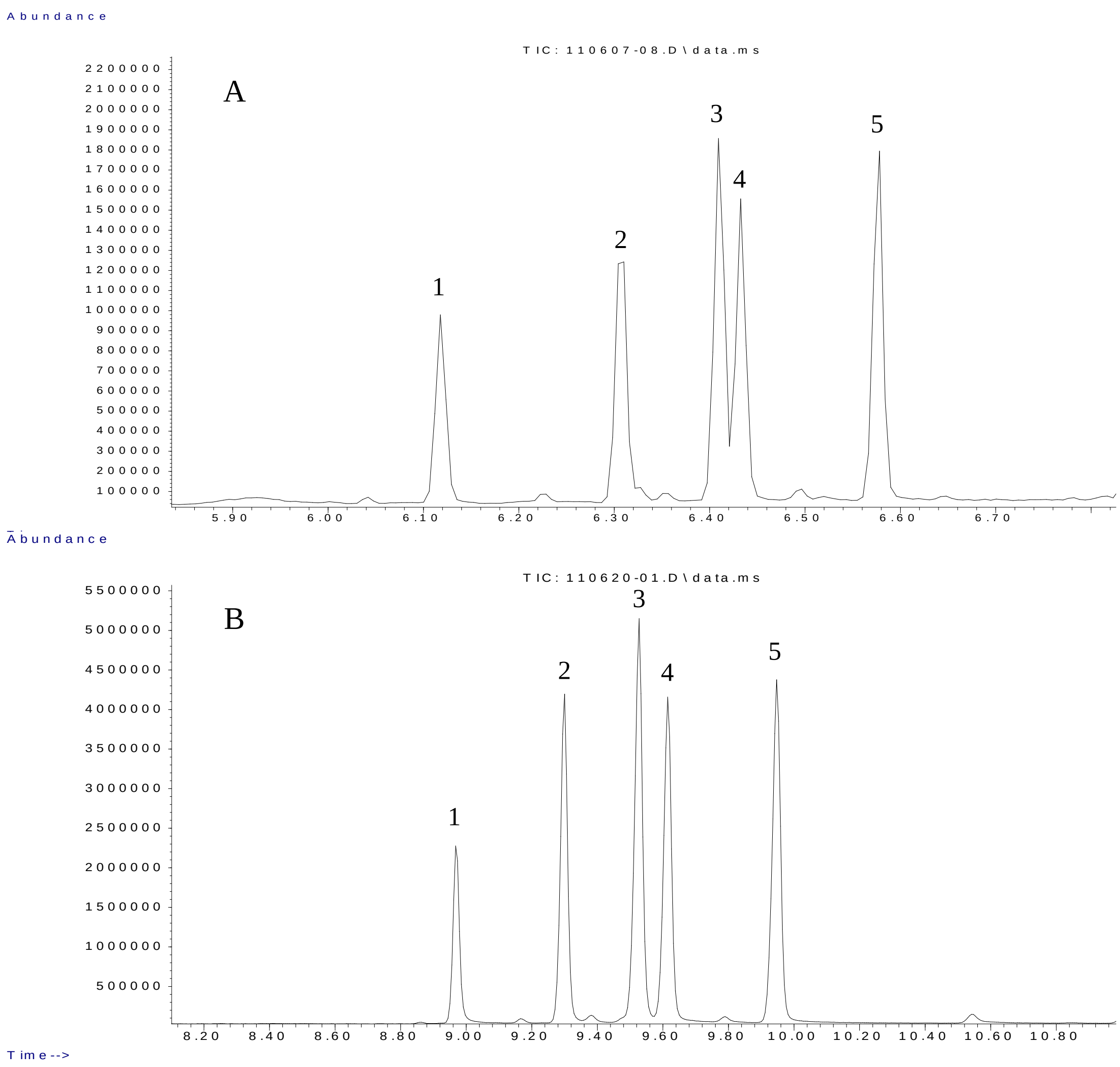


Fig. 68. Gas chromatographic separation of the heptafluorobutyryl derivatives using (A) Rtx-1 and (B) Rtx-5 column. The number over the peak corresponds to the compound number.

3.11.4. 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 the 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.

3.12. 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.

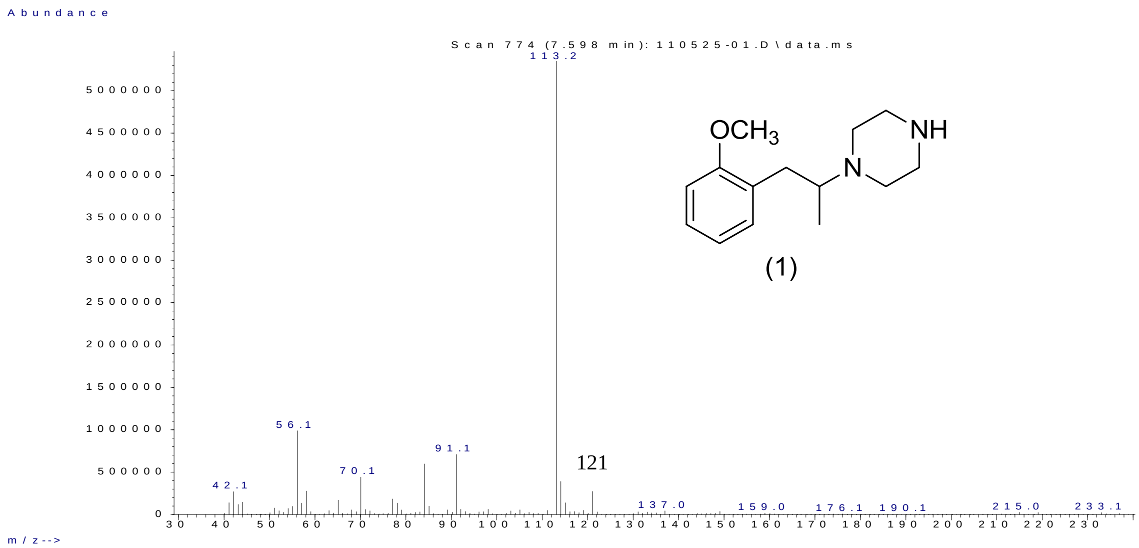
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the three isomers. The mass spectra in combination with the vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The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

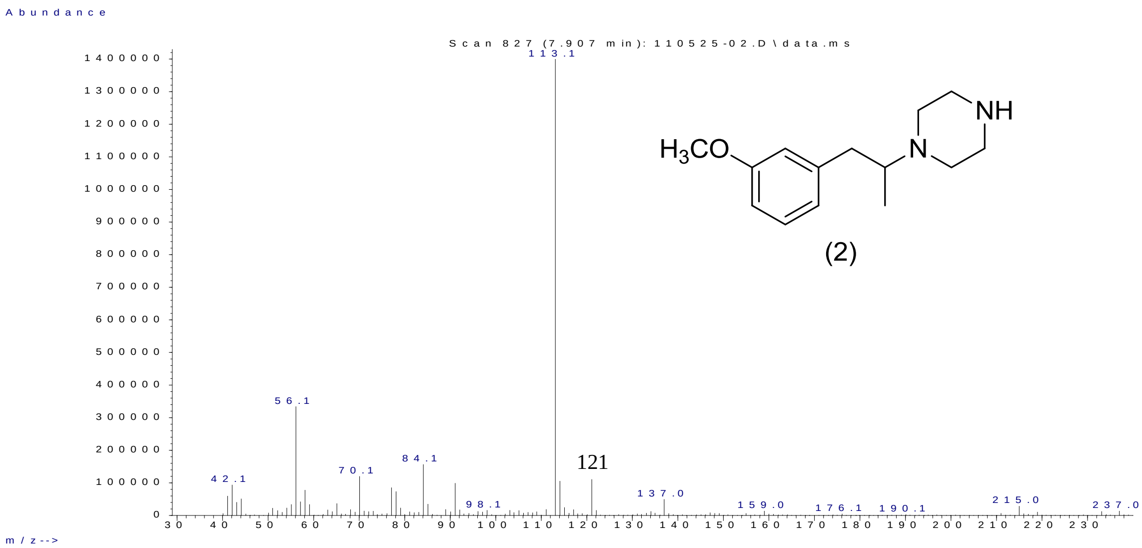
3.12.1. Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 69 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 Scheme 36.

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 70 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 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.





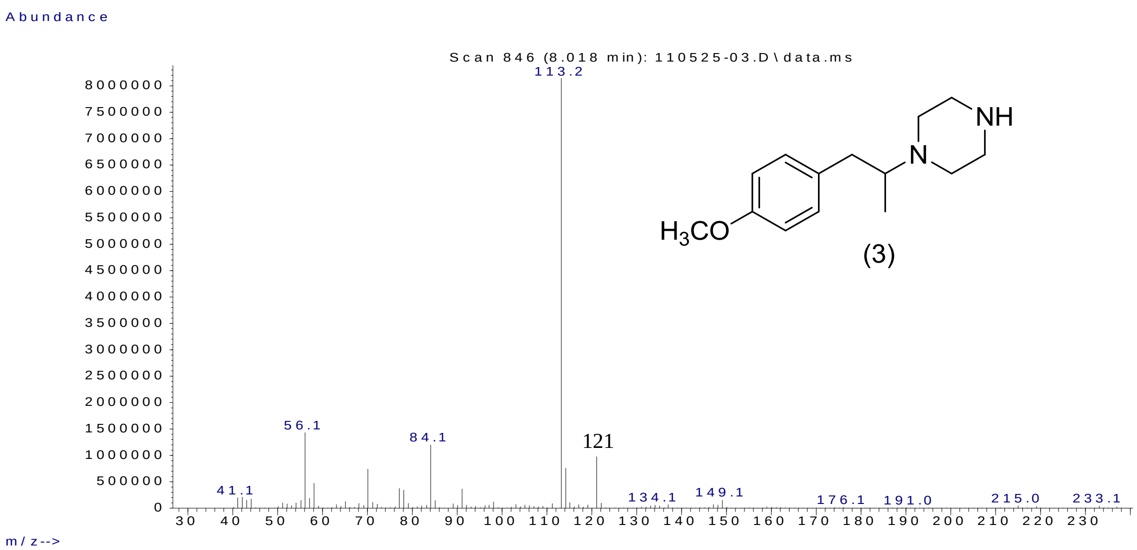
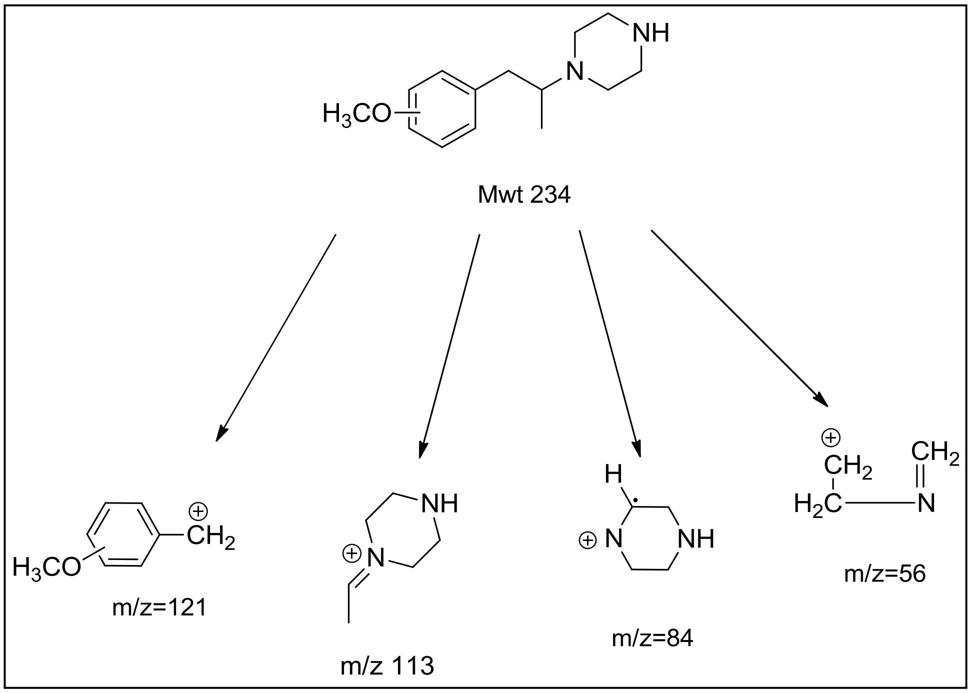
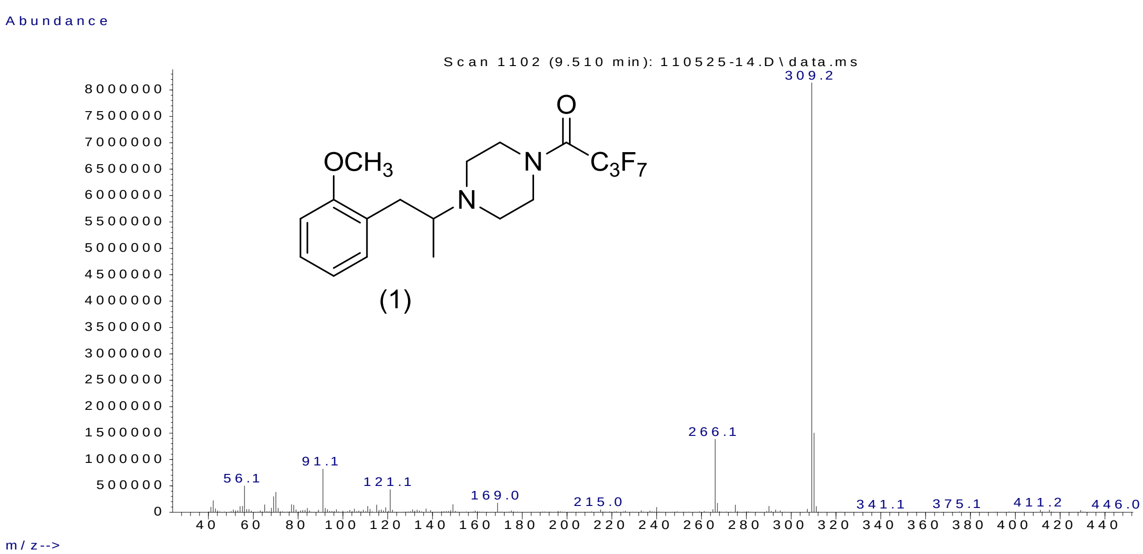
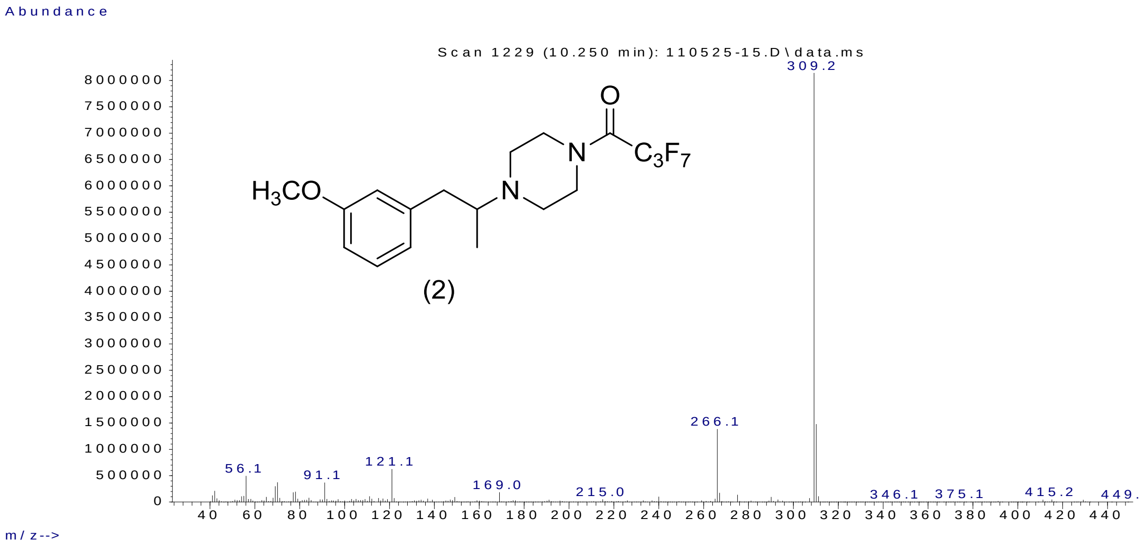


Fig. 69. Mass spectra of the underivatized piperazines in this study.



Scheme 36. Mass spectral fragmentation pattern of the underivatized 1-(methoxyphenyl)-2-piperazinopropanes (OMePPPs) under EI (70eV) conditions.





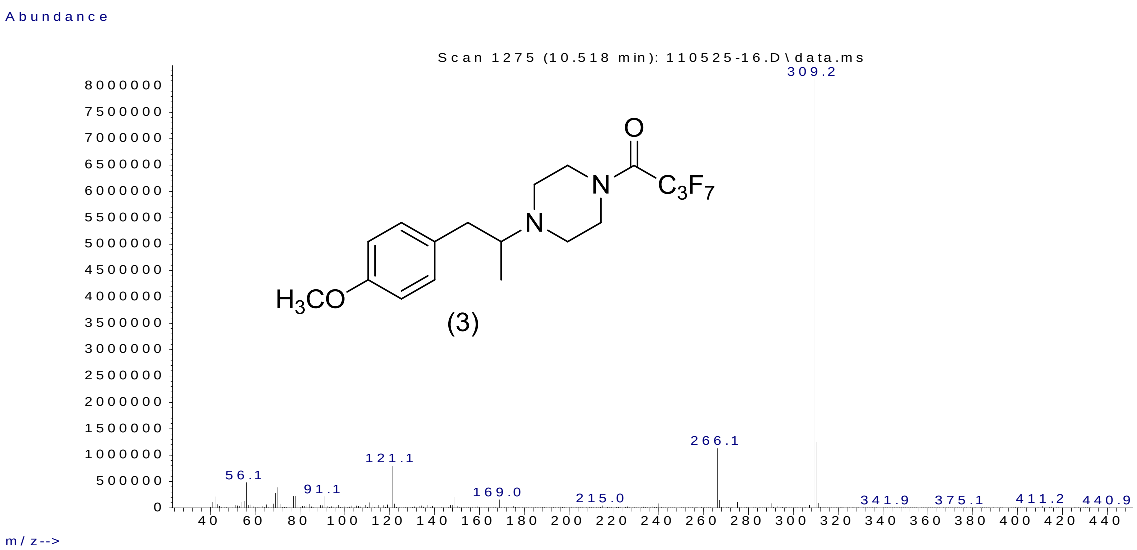
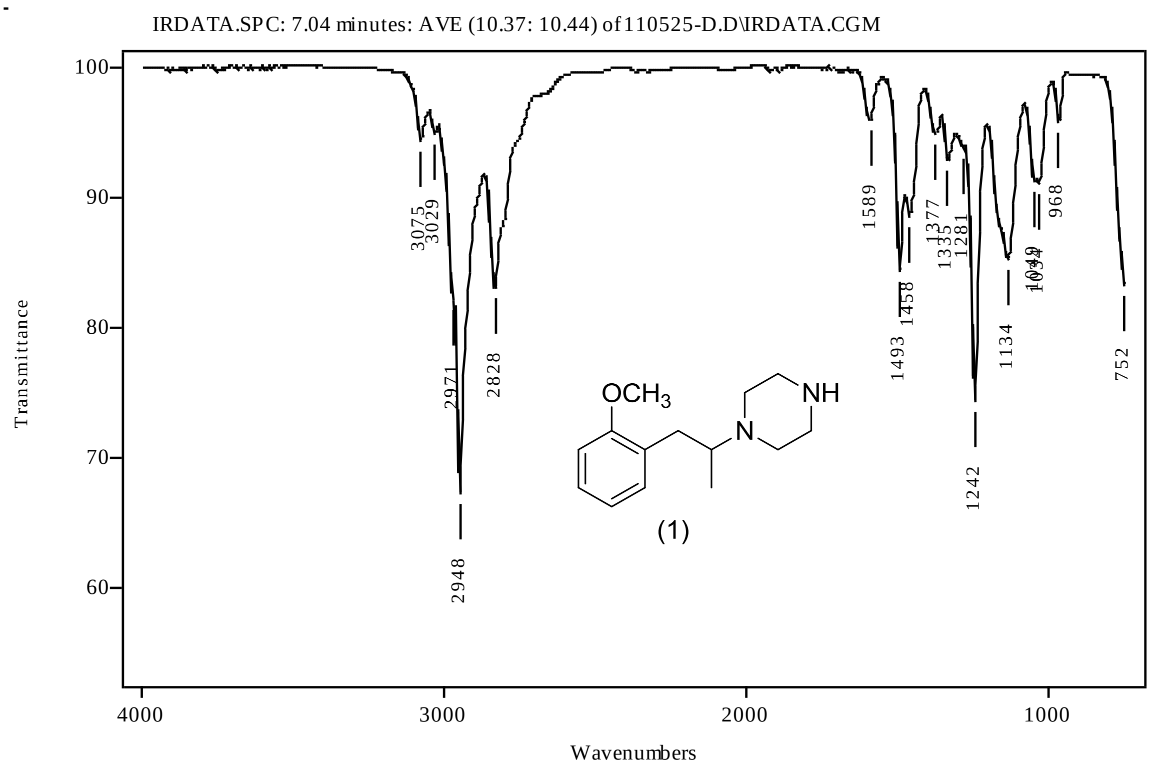


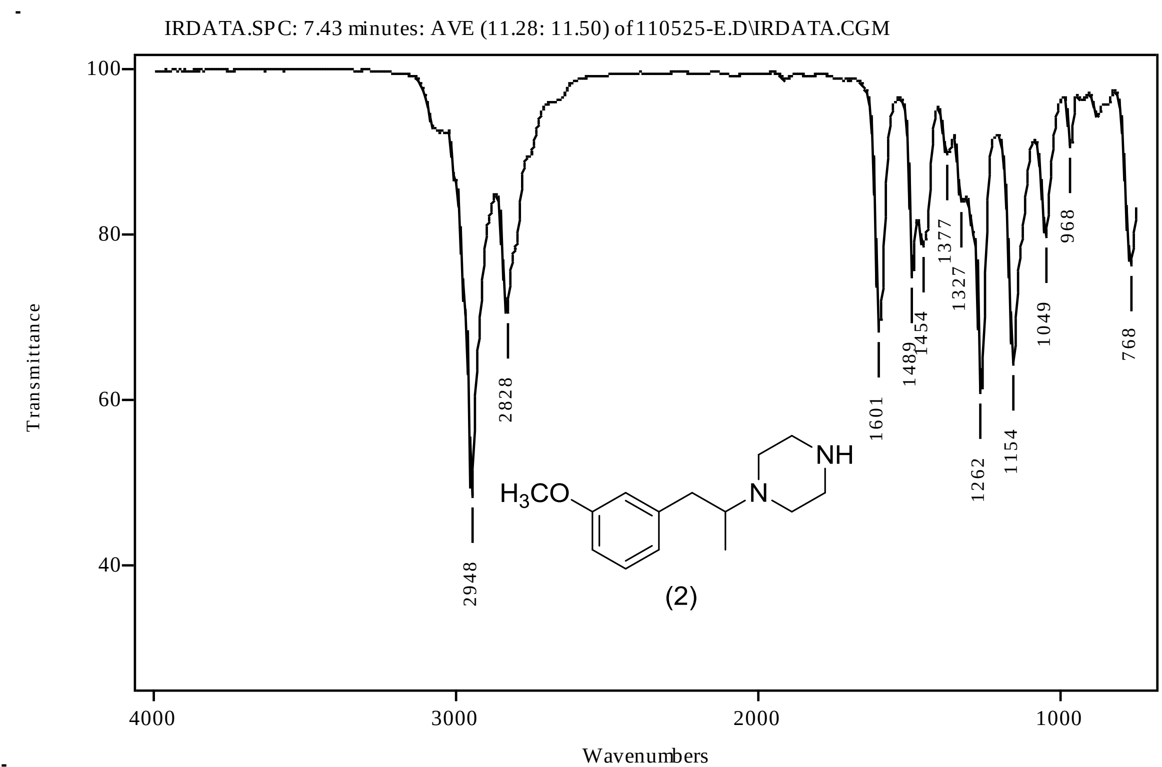
Fig. 70. Mass spectra of the heptafluorobutyryl derivatives of the piperazine compounds in this study.

3.12.2. Vapor-phase Infra-Red Spectroscopy

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 71. 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.





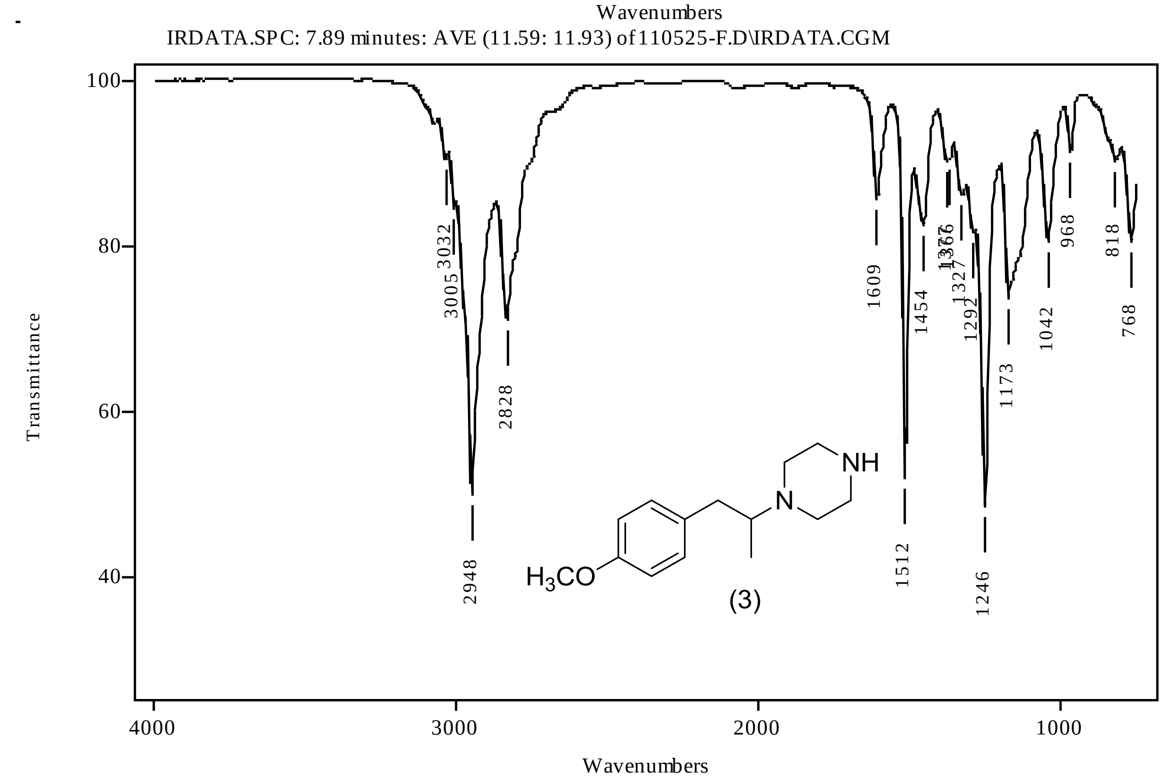


Fig. 71. Vapor phase IR spectra of the piperazines involved in this study.

3.12.3. Gas Chromatographic Separation

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 72 is a representative of the results obtained for all samples on this stationary phase.

In Figure 72 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.

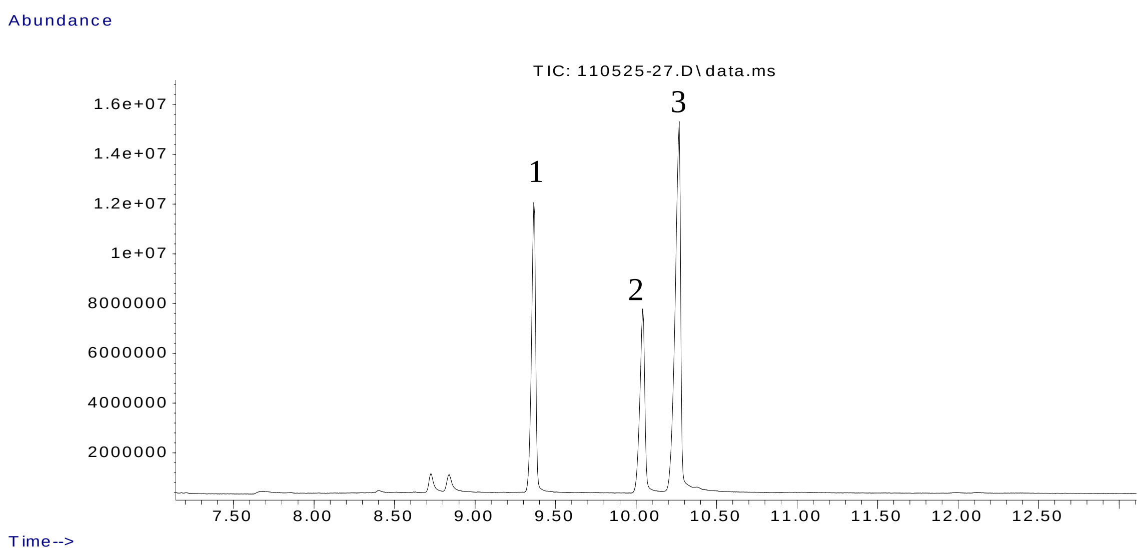


Fig. 72. Gas chromatographic separation of the pentafluoropropionyl derivatives using Rtx-200 column. The number over the peak represents the compound number.

3.12.4. 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.

4. Experimental

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

4.1.1. Materials

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

Piperazine, 3,4-Methylenedioxyphenylacetone, piperonal, 2,3dihydroxybenzaldehyde, 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, d5-benzoyl chloride, d_8-piperazine, 2-ethoxybenzaldehyde, 3-ethoxybenzaldehyde, 4-ethoxybenzaldehyde, 2-chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2,3-dimethoxybenzaldehyde, 2,4-dimethoxybenzaldehyde, 2,5-dimethoxybenzaldehyde, 2,6-dimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 3,5-dimethoxybenzaldehyde, benzoyl chloride, 2,3-dimethoxybenzoylchloride, 2,3-dimethoxybenzoylchloride, 2,4-dimethoxybenzoyl chloride, 2,5-dimethoxybenzoylchloride, 2,6-dimethoxybenzoylchloride, 3,4-dimethoxybenzoylchloride, 3,5-dimethoxybenzoic acid, 2-methoxybenzoylchloride, 3-methoxybenzoylchloride, 4-methoxybenzoylchloride, 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).

4.1.2. 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.

4.1.3. GC-Columns

Different capillary GC columns were evaluated throughout the course of this work, however only columns showed best 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. Column coated (f.d) with 0.25 µm. 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

4.1.4. 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 used

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

4.2. Synthesis of the Regioisomeric and Isobaric Piperazines

4.2.1. Synthesis of the ring substituted benzylpiperazines

4.2.1.1. 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%].

4.2.1.2. Synthesis of the methoxymethylbenzylpiperazines (MMBPs)

4.2.1.2.1. 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%].

4.2.1.2.2. 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%].

4.2.1.2.3. 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%].

4.2.1.2.4. 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%].

4.2.1.2.5. 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%].

4.2.1.2.6. 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%].

4.2.1.2.7. 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%].

4.2.1.3. Synthesis of the ethoxybenzylpiperazines (MMBPs)

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%].

4.2.1.3. Synthesis of the methylbenzylpiperazines (MBPs)

A mixture of either 2-methylbenzaldehyde (o-toluealdehyde) or 3-methylbenzaldehyde (m-toluealdehyde) or 4-methylbenzaldehyde (p-toluealdehyde) (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%].

4.2.1.4. 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%].

4.2.1.5. 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%].

4.2.1.6. 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%].

4.2.2. Synthesis of the ring substituted benzoylpiperazines

4.2.2.1. 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%].

4.2.2.2. 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%].

4.2.2.3. 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.

4.2.2.4. 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 220, m/z 135 [100%].

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

4.2.3.1. 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%].

4.2.3.2. 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%].

4.2.3.3. 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%].

4.3. 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.

4.4. 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].

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