Final Technical Report

Synthesis and Analytical Profiles for Regioisomeric and Isobaric Amines Related to MDMA, MDEA and MBDB: Differentiation of Drug and non-Drug Substances of Mass Spectral Equivalence

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Abstract: This project has focused on issues of resolution and discriminatory capabilities in controlled substance analysis providing additional reliability for forensic evidence and analytical data on regioisomeric and isobaric phenethylamines of forensic interest. The broad objective of this research is improved specificity in the analytical methods used to identify MDMA, MBDB, MDEA, and related phenethylamine controlled substances. This improved specificity comes from methods which allow the forensic analyst to eliminate non-drug imposter substances as the source of analytical data matching that of the controlled drug substance. This project has developed methods to discriminate between the methylenedioxyphen-ethylamine drugs and those regioisomeric and isobaric molecules having the same molecular weight and major fragments of equivalent mass (i.e. identical mass spectra). The work has emphasized those analytical methods commonly employed in forensic laboratories for confirmation of drug identity: gas chromatography, mass spectrometry, infrared spectrometry. When compounds exist which produce the same mass spectrum (same

MW and fragments of equivalent mass) as the drug of interest, the identification by GC-MS must be based entirely upon the ability of the chromatographic system to resolve these substances.

The initial phase of this work involved the organic synthesis of the direct regioisomeric substances related to MDMA, MBDB, and MDEA. We also prepared representative samples of the indirect regioisomeric molecules (such as the 2, 3, and 4-methoxymethcathinones) and the isobaric substances, compounds of the same nominal mass but different elemental composition (for example 2, 3, and 4-ethoxyphenethylamines and the 10 different ring substitution patterns for the methoxy-methyl-phenethylamines). Additional compounds were prepared in related categories to further refine the developed analytical methods and confirm/challenge experimental observations. A number of deuterium labeled analogs has been prepared to establish mass spectral fragmentation patterns. Other isomeric series were prepared such as the methoxymethylene-substitution phenyl ring pattern for the MDMA and the MBDB side chain series and the 2-, 3-, and 4-ethoxyphenyl compounds for the MBDB series. Overall, in this project more than 90 phenethylamines having a regioisomeric or isobaric relationship to MDMA or MBDB have been synthesized and evaluated.

The chromatographic retention properties for each series of isomers have been evaluated by gas chromatographic techniques on a variety of stationary phases. These studies

established structure-retention relationships (elution orders) for the side chain regioisomers and the isobaric amines on a number of stationary phases.

Mass spectrometry studies have focused on the underivatized free amines and have clearly established the significant similarity in the mass spectrum for the side chain and ring regioisomers. Derivatization studies have established methods for differentiation of side chain substitution patterns yielding unique fragment ions of significant abundance in the EI mass spectrum for these compounds. The perfluoroacyl derivatives provide maximum mass spectral information for compound individualization and in a number of regioisomeric subsets the HFBA derivatives provide more data for molecular individualization than PFPA or TFA derivatives. A detailed background study established the advantages of perfluoroacyl derivatives over other alkyl or aryl amide derivatives. A second detailed background study provided the scientific evidence for the mechanism of fragmentation producing the unique m/z 110, 160 and 210 ions for the methamphetamine side chain in the TFA, PFPA and HFBA derivatives respectively. The mass spectral studies clearly show that the individual side chain isomers of m/z58 and m/z72 can be identified by specific fragment ions. Additionally unique ions help to point the direction of investigation for ring substitution pattern. For example the unique fragment ions at m/z107 in the spectrum of ethoxy-substituted phenethylamines allows its' differentiation from methylenedioxyphenethylamines. Vapor phase infrared spectrometry following separation by gas chromatography (GC-IRD) provided confirmation of the nature and position of aromatic ring substituent.

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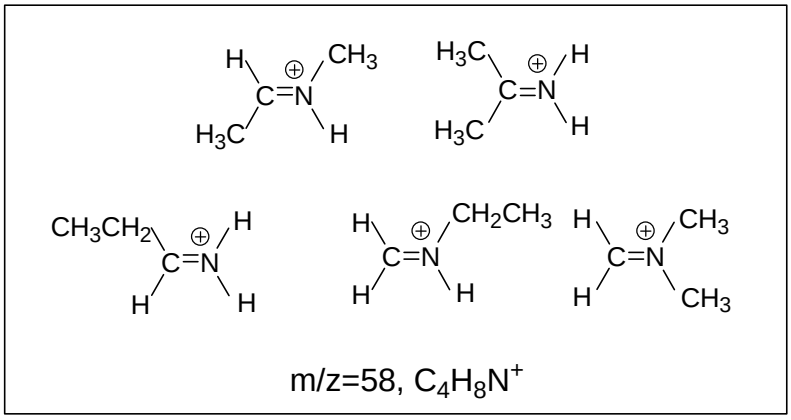
Executive Summary: In this project, we have synthesized over 90 regioisomeric and isobaric phenethylamines of mass spectral equivalence and evaluated the analytical profiles of these amines. The work has emphasized those analytical methods commonly employed in forensic laboratories for confirmation of drug identity: gas chromatography, mass spectrometry, infrared spectrometry. The synthetic efforts focused on those regioisomeric and isobaric substances most likely to appear in future forensic samples. In some cases compounds were prepared to complete a set of all the possibilities in a series when some of the regioisomers have already appeared in clandestine samples. In other cases the compounds prepared were available directly or indirectly from commercially available precursor substances. We have collected, cataloged and categorized the mass spectra for the free amines and several derivatives of each of the 90 amines; studied the gas chromatographic retention properties of various subsets of the amines and their derivatives; collected and evaluated vapor phase infrared spectra for these compounds. As of the date of this writing (January, 2011) twenty scientific publications resulting from this area of research are in print or accepted for publication in Forensic Science International and Journal of Chromatographic Science. A few other manuscripts related to this project are yet to be written. Additionally 12 presentations have been made at national meetings during this project period.

The overall goal of this project was a comprehensive study of those regioisomeric and isobaric amines capable of producing mass spectra equivalent to those of MDMA, MBDB and MDEA. The availability of all the necessary compounds to establish and

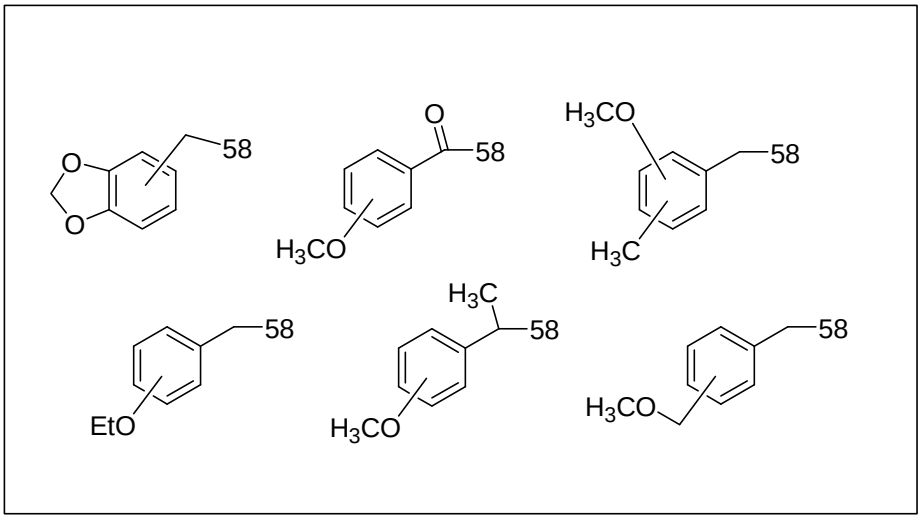
prove the structure-retention, structure-fragmentation and other structure-property analytical experiments was the starting point for this research. The mass spectra of phenethylamines are characterized by a base peak formed from an amine initiated alpha-cleavage reaction of the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine group. In methamphetamine and ring substituted methamphetamines (for example 3,4-methylenedioxymethamphetamine), the alpha-cleavage reaction yields the substituted imine fragment at m/z 58 and the benzyl fragment or substituted benzyl fragment at the appropriate mass.



Regioisomeric Forms of m/z 58

In this project the substituted benzyl fragment for 3,4-MDMA and its regioisomeric and isobaric equivalents occurs at m/z 135. There are four side chain regioisomers of methamphetamine with the potential to yield mass spectra essentially equivalent to methamphetamine yielding regioisomeric imine fragment ions in their electron ionization mass spectra at m/z 58. Thus, for every individual ring substitution

pattern there are five side chains yielding equivalent m/z 58 fragment ions (the methamphetamine side chain and four regioisomeric equivalents). The ring substitution pattern in methylenedioxymethamphetamine (MDMA) doubles the number of isomers to ten, the five side chain isomers in which the methylenedioxy-ring is substituted in a 3,4-manner and the five in which the methylenedioxy-ring is substituted in a 2,3-pattern. Thus, there are nine other amines with the potential to produce a mass spectrum essentially the same as that of 3,4-MDMA. Most of these potential designer analogues have the strong possibility to be misidentified as 3,4-MDMA based on mass spectrometry. In addition to the ten amines containing the methylenedioxy-group, other substitution patterns have the potential to produce mass spectra with fragments of equivalent mass to those of 3,4-MDMA.



Example of Regioisomeric and Isobaric Compounds in this Study

For example, the reported stimulant methoxy-methcathinones are uniquely isomeric with the MDMAs having the same MW, elemental composition and expected major fragment ions of equal mass to those of the MDMAs. Additionally isobaric substitution patterns especially those likely to undergo few if any unique/characteristic fragmentations such as methoxy-methyl disubstitution of the methamphetamine aromatic ring, ethoxy-monosubstitution as well as methoxymethylene- monosubstitution are compounds with the potential to produce El mass spectra essentially equivalent to that of 3,4-MDMA. Each methoxy-methyl, methoxymethylene- or ethoxy-substitution pattern would produce 5-side chain isomers analogous to methamphetamine with the potential to yield mass spectra equivalent to 3,4-MDMA. The o, m, and p-ethoxy substituents would yield a total of 15-isomers, 15 for the methoxymethylenes and the methoxy-methyl disubstituted aromatic ring system (10 unique disubstitution patterns) can result in over 50 isomers.

Representative samples have been prepared in this study for each aromatic ring substitution pattern and each side chain regioisomer. A complete set of all side chain regioisomers was prepared in enough categories to confirm mass spectral methods for specific side chain identification. The number of side chain regioisomers for the MBDB series doubles to 20 for each ring substitution pattern and the base peak for these compounds is m/z72. In this project efforts were concentrated on preparing compounds necessary to confirm methods developed for the MDMA isomers (m/z58). When the number of mass spectral equivalent isomeric substances is relatively small, chromatographic separation and reference standard

availability is not a major concern. However, the continued designer exploration of some drug categories will likely produce even greater numbers of regioisomeric and isobaric substances especially among the phenethylamines. As the number of compounds having mass spectral equivalence increases so does the challenge of specific identification via chromatographic resolution and other analytical methods. In this project we have systematically examined the analytical profiles of compounds from each structural category. The results of the work will allow the forensic analyst to differentiate among most of the regioisomeric and isobaric equivalents of MDMA without the need for reference standards of all these compounds.

The mass spectra in almost all the compounds prepared in this study showed the expected m/z 58 and 135 ions with no features for specific differentiation. Upon derivatization with perfluoroacyl reagents such as trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl characteristic and distinguishing ions were obtained in many cases. These are specific ions which occur for differentiation and not just changes in the relative abundance of common ions. In every case specific ions to identify the side chains were obtained. The individualization is the result of fragmentation of the alkyl carbon-nitrogen bond yielding hydrocarbon fragments at m/z 148, 162 and 176 as well as other unique fragments from these regioisomeric amides. The N-ethylamides show a specific ion to identify the ethyl group by loss of mass 28 and the methamphetamine side chain yields a unique ion at m/z110, 160 and 210 for the TFA, PFPA and HFBA derivatives

respectively. Thus, following chemical derivatization the variations in regioisomeric m/z58 side chains can be specifically identified. This is possible due to specific ions identifying the number of carbons attached directly to the aromatic ring in an uninterrupted manner and the nature of the substituent attached to nitrogen. During the course of this work the mass spectral fragmentation mechanisms and proof of structure for these diagnostic ions have been confirmed by homologation experiments and deuterium labeling studies. These additional compounds were synthesized in order to confirm the details of the observed mass spectral fragmentation pathways.

In an additional fundamental study, a series of acylation reagents were evaluated to find the reagent yielding maximum specific fragmentation information as well as appropriate chromatographic properties. The perfluoroacyl derivatives of MDMA show excellent chromatographic properties and unique mass spectral fragment ions. The perfluoroalkyl amides of TFAA, PFPA and HFBA yield a unique series of mass spectral fragments at m/z 110, 160 and 210 respectively. Additionally, the base peaks in these mass spectra occur at relatively higher masses at m/z 154, 204 and 254 respectively. These ions are the perfluoroacylimines resulting from loss of 135 mass units (3,4-methylenedioxybenzyl) from the molecular ion. This $(M-135)^+$ species also occurs for the acetyl, propionyl and butyryl amides however these ions rearrange through loss of the acyl group to yield a common ion at m/z 58. Thus, the base peak for the non-fluorinated hydrocarbon derivatives is the same as that observed for the underivatized MDMA, m/z 58. The

formyl, d_3-acetyl, d_5-benzoyl and pentafluorobenzoyl derivatives provided chromatographic and confirmatory mass spectral evidence for fragmentation pathways.

In many cases the ring substituent can be identified following perfluoroacylation as well. In some cases the position of ring substitution is not available from the mass spectral data. In all cases compounds having identical mass spectra in the derivatized and underivatized form, can be differentiated by vapor phase infrared spectrometry. GC-IRD studies yield individual spectra to differentiate primarily between ring substituents. Thus these techniques in concert provide molecular individualization for most of these compounds. The differentiation by IRD of the methcathinones is straight forward due to the carbonyl absorption in this set of compounds.

Similar studies for the precursor substances synthesized in this study have helped to provide analytical details for the substituted phenylacetones and 2-butanones and related ketones. Additionally the tertiary amines cannot yield stable amide derivatives for side chain or ring substituent identification. These compounds can only be differentiated by underivatized mass spectra, vapor phase infrared spectrometry and other techniques.

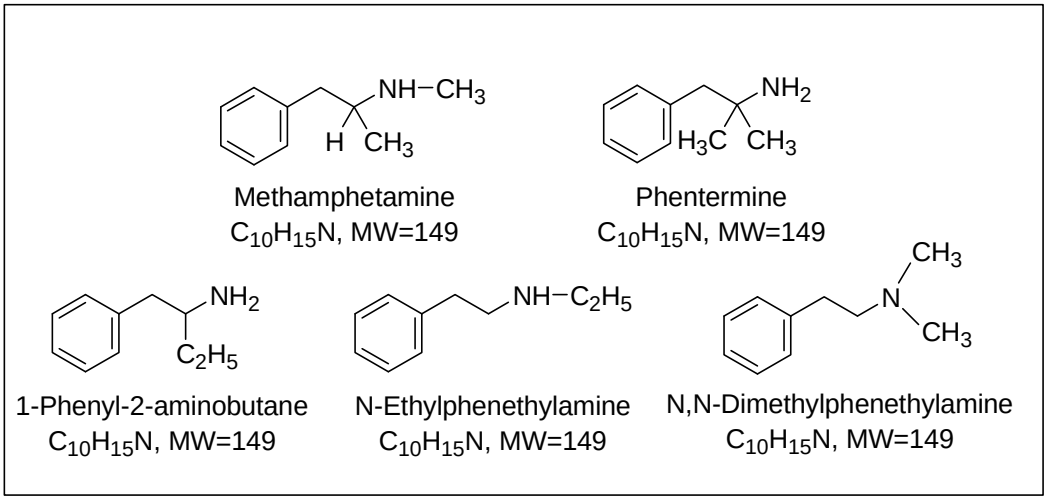
Main Body of the Final Technical Report

I. Introduction

I.1 Statement of the Problem

3,4-Methylenedioxymethamphetamine, MDMA, is a Schedule I drug under the Controlled Substances Act (CSA). Primarily illicitly manufactured in and trafficked from Europe, MDMA is the most popular of the club drugs. DEA reporting indicates widespread abuse of this drug within virtually every city in the United States. The mass spectrum is often the confirmatory piece of evidence in the identification of drugs in the forensic laboratory. While the mass spectrum is often considered a specific "fingerprint" for an individual compound, there are other substances which produce very similar or almost identical mass spectra. Many of these compounds which yield the same mass spectrum are positional isomers of side-chain or aromatic ring substituents. Such compounds having MS-equivalency and similar elution properties, perhaps coelution, represent a serious analytical challenge for the forensic chemist. The ability to distinguish between these isomers directly enhances the specificity of the analysis for the controlled substance. If other compounds exist which have the potential to produce the same mass spectrum as the drug of interest then the identification by GC-MS must be based entirely upon the ability of the chromatographic system to separate the isomeric substance from the actual drug. Those substances which coelute with the drug of abuse will be misidentified. The ultimate concern then is "if the laboratory has never analyzed the counterfeit substances, how can the analyst be sure that these compounds would not coelute

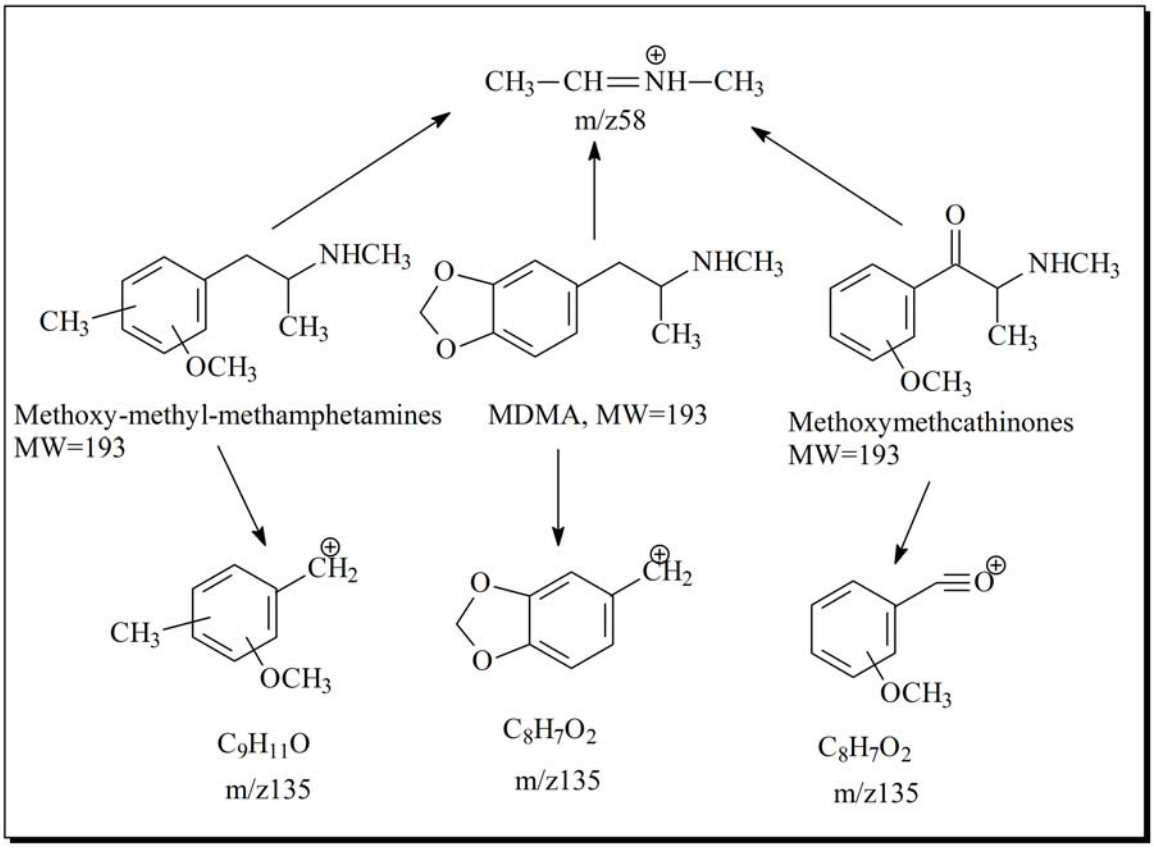
with the controlled drug?" The courts expect forensic drug chemistry to identify a substance as an individual compound, not report it as an unknown member of a large group of regioisomeric/isobaric substances. Methamphetamine is an excellent example which illustrates the possibility of counterfeit amines based on side chain isomerism. The El mass spectrum for methamphetamine is dominated by the m/z 58 fragment and the m/z 91 fragment with very little molecular ion at m/z 149. There are four other side chain isomers of methamphetamine having a molecular weight of 149 which yield an imine fragment of m/z58 and an unsubstituted benzyl fragment of m/z 91.



Methamphetamine and Regioisomeric Side Chains Yielding m/z 58.

However, the high resolution capabilities of capillary GC coupled with the limited number of compounds (a total of five for the methamphetamines having an unsubstituted aromatic ring) can solve the issue in this case. Aromatic ring substitution makes this issue much more

challenging in forensic drug analysis. When the number of mass spectral equivalent isomeric substances is relatively small, chromatographic separation and reference standard availability is not a major concern. However, the continued designer exploration of some drug categories will likely produce even greater numbers of regioisomeric and isobaric substances especially among the phenethylamines. As the number of compounds having mass spectral equivalence increases so does the challenge of specific identification via chromatographic resolution and other analytical methods.



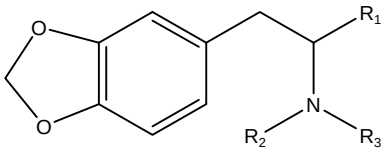
Major Fragment Ions for Substituted Methamphetamines

The results of this project allow the forensic analyst to differentiate among most of the regioisomeric and isobaric equivalents of MDMA and minimizes the need for a large number of reference standards of all these compounds.

I.2 Literature Citations and Review

I.2.1 Introduction

Designer drug exploration of the methylenedioxyphenethylamines has produced several drugs of abuse in recent years. These derivatives include 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethyl-amphetamine (MDEA), 3,4-methylenedioxyphenyl-2-butanamine (BDB) and 2-methylamino-1-(3,4-methylenedioxyphenyl)butane (MBDB). The methylenedioxy-derivatives of amphetamine and methamphetamine represent the largest group of designer drugs (Figure I.2-1).



MDA: $R_1 = CH_3$, $R_2 = R_3 = H$

MDMA: $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = H$

MDEA: $R_1 = CH_3$, $R_2 = C_2H_5$, $R_3 = H$

BDB: $R_1 = C_2H_5$, $R_2 = R_3 = H$

MBDB: $R_1 = C_2H_5$, $R_2 = CH_3$, $R_3 = H$

Figure I.2-1: Chemical structures of methylenedioxyphenalkyl amine derivatives.

MDMA, also known as eccstcy, with its stimulant and hallucinogenic effects in humans is the most commonly used derivative of this series and has become a major drug of abuse in recent years. In 2003, the estimated annual production of ecstacy worldwide was 100-125 tons and the estimated retail price for that drug alone was 63.74 billion US dollars for 1.4 billion tablets. In the same year eight million people were reported to abuse Ecstacy alone [UNODCCP, 2003]. In 2005, the reported consumption of ecstacy among US students indicated that 1.7 % of the eight grade students in the US had consumed the drug and this increased to 2.6% among tenth grade students and as high as 3.0 % among students in grade twelve [MTF, 2005]. About 0.1 % of the global population (age 15 and above) consume ecstasy and significantly higher ratios, 0.5 – 2.4%, have been reported from countries in the Oceania region, Western Europe and North America. West Europe and North America together account for almost 85% of global consumption. Use of ecstasy, however, is increasingly spreading to developing countries as well.

Tablets or capsules are the most common way to administer MDMA, however the powder also can be snorted, smoked or—in rare cases—injected (TCADA 2002). Drug effects may last up to six hours and are known to include the production of profoundly positive feelings, empathy for others, elimination of anxiety, and extreme relaxation. MDMA is also said to suppress the need to eat, drink, or sleep, enabling users to endure two- to three-day parties. For a while, it has been clear that many tablets sold as ecstasy do not always contain MDMA as an active substance and in many cases these tablets are notoriously impure [Ecstasy Data, 1996-2006]. Ecstasy tablets are prepared in clandestine laboratories and, during most of the last decade; Western Europe has been the world's

major manufacturing region. The most frequently mentioned country of origin was Belgium followed by Germany, the UK, Spain and the USA. The most frequently mentioned source countries located in Eastern Europe were the Baltic countries, Poland and Belarus. China, Indonesia and Thailand were the most frequently reported source countries located in Asia. In Africa, the Republic of South Africa, and in South America, Colombia, were identified as source countries for ecstasy [UN ODCCP, 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 immediatley considered 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 MDA derivatives and other designer drugs is essential and a significant task for forensic laboratories.

I.2.2 Analytical Methods Used to Identify and Separate 3,4-MDMA

I.2.2.1 Spectroscopy

I.2.2.1.1 Mass spectrometry

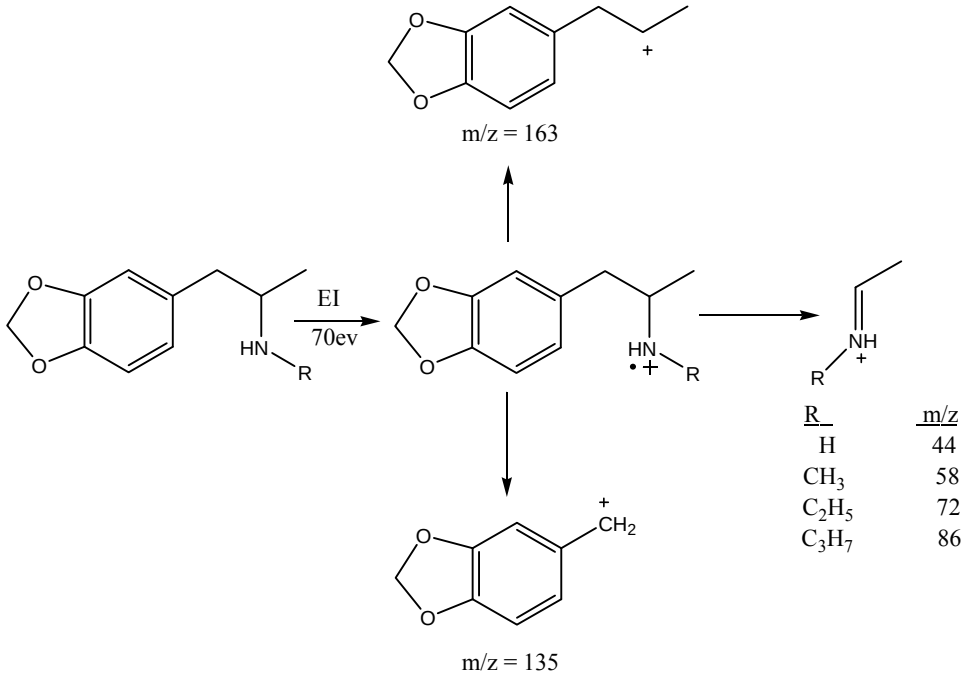
Gas chromatography-mass spectrometry (GC-MS) is the main tool used for the detection and identification of unknown drugs in forensic and other drug screening laboratories. The mass spectra of 3,4-methylenedioxyamphetamines are characteristic and

can be used for differentiation from other phenethylamines [Noggle *et al.,* 1988]. The electron impact (EI) mass spectra for 3,4-methylenedioxyamphetamines exhibit weak to extremely weak molecular ions which, in some cases, may not be evident without computer enhancement. The base peaks result from the alpha cleavage involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine nitrogen producing the products shown in Scheme I.2.2-1. Furthermore a peak at m/z 135, which corresponds to methylenedioxybenzyl cation, is characterstic for these compounds.

Unfortunately, the EI technique is often insufficient, alone, to discriminate between structurally similar phenethylamines. The amine dominated fragmentation reactions in these compounds often yields low mass fragments of similar mass and little (if any) molecular ion species. The differentiation of these compounds is only achieved by means of derivatization and chromatographic methods. Borth *et al.,* reported regioisomeric differentiation of 2,3- and 3,4-methylenedioxyphenalkylamines by using collision-induced dissociation (CID) mass spectrometry under EI and chemical ionization (CI) [Borth *et al.,* 2000].

In a recent study, [Sachs and Woo, 2007] described the use of the low mass region between mass 39 and 58 for differentiation between methamphetamine and its four possible regioisomers. All five regioisomeric phenethylamines yield m/z 58 as the imine base peak therefore the additional two carbons of the side chain are bonded to the fragment imine species. The reported method [Sachs and Woo, 2007] for differentiation between the compounds uses m/z 39 as a reference ion and compares its relative abundance to the ions at m/z 41, m/z 42 and m/z 56. The relative abundance of each of

the three ions (m/z 41, 42 and 56) was characteristic for each of the five side chain regioisomers. The use of the m/z 39 as a pseudo-internal standard was platform independent and reported to be consistent among numerous published reports on similar compounds [Sachs and Woo, 2007].



Scheme I.2.2-1: Mass fragments (EI) of 3,4-methylenedioxyamphetamines.

Methamphetmaine was the only one of the five regioisomeric amines which showed both m/z 41 and m/z 56 in greater abundance than m/z 39. The mechanistic fragmentation process for the formation of each of these diagnostic low mass ions was described in detail. However, the disadvantage of the method is the relatively low intensity of these low mass ions and the lack of a unique and characteristic ion for each of the regioisomeric side chains (i.e. all five compounds show fragment ions at m/z 39, 41, 42 and 56 and differentiation is based on the relative abundance of these ions).

I.2.2.1.2 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. The chemical shift data with proton assignments, appropriate proton-proton J-coupling as well as ^{13}C-NMR shift assignments for MDMA as the free base and hydrochloride salt in deuterochloroform has been reported [Dal Cason *et al.,* 1997].

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. Lee et al. reported the differences in chemical shifts of the solid state NMR and solution NMR for MDMA [Lee *et al.,* 2000]. This study described the differences between chemical shifts of MDMA·Hydrochloric acid in ecstasy tablets and pure crystals.

I.2.2.1.3 Infrared (IR) spectroscopy

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. Common absorptions for 3,4-methylenedioxyamphetamines were reported [Young, 2000]. Because of the polymorphic crystalline structure of the hydrochloride salt compared to the base form, different infrared spectra were determined for MDMA hydrochloride alone [Shulgin, 1986]. Near infrared (NIR) spectroscopy (1100-2500 nm) offers a possibility for fast and nondestructive screening of ecstasy tablets. Modern NIR equipment is portable and easy to handle so that confiscated samples can be measured on the spot and in real time. It has been shown that differentiation of placebo, amphetamine and ecstasy samples is possible [Sonderman and Kovar, 1999].

Infra red detector coupled with GC also had significant application in the identification of controlled substances in presence of their isomers. Generally isomers other than optical isomers yield different IR spectra. Diastereomers resulting from two or more chiral centers also exhibit different infrared spectra [Lanig et al, 2003]. Polymorphism in the solid phase can yield variations in the infrared spectrum of an individual compound. Analytes in the solid phase can crystallize in different ways, allowing for different crystal forms and different intermolecular interactions resulting in significant variability in the IR spectrum of an individual compound [Lanig et al, 2003]. GC-FTIR spectroscopy is characterized by scanning quickly enough to obtain IR spectra of peaks eluting from the capillary columns. Thus, this technique combines the separation power of GC with the identification power of IR and is not affected by polymorphism since the IR spectra are obtained in the vapor phase. GC-IR and MS have been successfully used for the identification of some amphetamine isomers [Duncan and

Soine, 1988]. The GC-IR Spectra of methamphetamine and phentermine have been previously reported [Kempfert, 1988] along with other non-phenethylamine forensic compounds.

I.2.2.1.4 Raman spectroscopy

Raman spectroscopy also has been used to study ecstasy tablets [Bell *et al.,* 2000a]. The use of far-red (785 nm) excitation reduces interfering background luminescence and therefore the level of fluorescence background, even in untreated samples, is sufficiently low which makes it possible to obtain good quality data in reasonable times. It was shown that Raman methods can be used to distinguish between ecstasy analogs and the compounds can be identified even in a mixture with bulking agents in ecstasy tablets. The spectra can also be used to identify bulking agents, the relative concentration of drug to bulking agent and the degree of hydration of the active compounds. Bell *et al*. found that composition profiling by Raman methods is a fast and effective method of discriminating between ecstasy tablets manufactured in different ways and with different drug feed stocks [Bell *et al.,* 2000b]. Recently a surface-enhanced Raman scattering (SERS) spectroscopy method was developed for a rapid determination of MDMA in illicit samples [Sagmuller B. et al., 2001].

I.2.2.1.5 Ultra violet (UV) spectroscopy

The 3,4-methylenedioxyphenyl group is a strong chromophore in the UV range with two major absorption bands at 285 nm and 235 nm range with the absorptivity slightly higher at 285 nm [Noggle *et al.,* 1987]. Because of their common chromophore, a

variety of 3,4-methylenedioxyamphetamines and 3,4-methylenedioxyphenyl-2-butanamines show similar UV absorption properties and therefore are not discriminated by UV-spectroscopy [Clark *et al.,* 1995].

I.2.2.2 Chromatography

Chromatography has played a key role in forensic drug separation and identification over the past 35 years. Gas chromatography (GC) coupled with several detection methods such as flame ionization (FID), nitrogen phosphorus (NPD), electron capture (EC), and mass spectrometry (MS) has been used for the analysis of different *N*-substituted analogues of 3,4-methylenedioxyamphetamines. The determination of MDMA, MDA and MDEA in biological samples has mainly been carried out using GS-MS [Clauwaert *et al.,* 2000; Peters FT et al., 2005]. Actually, GC-MS is considered the method of choice in forensic laboratories. High-performance liquid chromatography (HPLC) with different detectors is another often applied technique in the determination of *N*-substituted analogs of 3,4-methylenedioxyamphetamines. Most of the literatures describe the identification and quantitative determination of MDA, MDMA, and MDEA, and their metabolites in biological samples. Derivatization is mainly required in the analysis of biological samples to improved sensitivity and selectivity, while such a technique is not usually applied in the analysis of non-biological samples such as drug forms.

I.2.2.2.1 High-performance liquid chromatography (HPLC)

I.2.2.2.1.1 Analysis of biological samples.

The determination of MDA, MDMA and MDEA in biological samples has been done by HPLC with various detectors, such as ultraviolet (UV), diode array detection (DAD), fluorescence (FL), electrochemical (ED), mass spectrometry (MS), and mass spectrometry –mass spectrometry (MS-MS) and these studies are reviewed in Table I.2-1.

Table I.2-1: HPLC methods to separate 3,4-methylenedioxyamphetamines from biological matrices.

Separated compounds	Matrix	Column	Detector	Reference
MDA, MDEA, MDMA, MBDB	Oral fluids	Kromasil 100 C8	FL	Concheiro et al. 2005
MDA, MDEA, MDMA	urine	octadecyl C_{18}	FL	De Costa JL et al., 2004
R-MDA, S-MDA, R-HME, S-HME, R-MDE, S-MDE	Plasma urine	chiral-CBH	FL	Buechler J et al. 2003
MDA, MDMA, MDEA, amphetamine, methamphetamine, ephedrine	plasma oral fluids	Hypersil BDS C_{18}	MS-MS	Wood M. et al. 2003
MDMA, MDA, amphetamine	Blood, urine, postmortem tissue	Hypersil BDS phenyl	MS	Mortier KA et al., 2002
R-MDA, S-MDA, R-MDMA, S-MDMA, R-MDEA, S-MDEA	plasma	ChiralDex, LiCrospher 60 RP-select B	FL	Brunnenberg and Kovar, 2001
MDA, MDMA, MDEA	blood, vitreous	Hypersil BDS C_{18}	FL	Clauwaert *et al*., 2000

	humor, urine			
MDA, MDEA, MDMA, MBDB, 2-CB and some phenethylamines	serum	Superspher 100 RP 18	APCI-MS	Bogusz *et al.*, 2000
MDA, MDMA, MDEA	hair	PLRP-S	FL	Tagliaro *et al*., 1999
amphetamine, methamphetamine, MDA, MDMA, MDEA	urine	Silica column (APEX)	UV-visible	Talwar *et al*., 1999
MDA, MDMA, MDEA, MBDB	urine, serum, saliva	LiChrocart-Li-Crospher 100 RP-18	FL	Mancinelli *et al.*, 1999
amphetamine, methamphetamine, MDA, MDMA, MDEA	serum	Superspher Select B a ECOcart	APCI-MS, DAD/UV	Bogusz *et al.*, 1997
MDA, MDMA	plasma, urine	Spherisorb ODS-1	DAD	Helmlin *et al*., 1996
MDA, MDMA, MDEA	whole blood	Whatman silica Partisphere	ED	Michel *et al*., 1993

I.2.2.2.1.2 Analysis of non-biological samples.

Reversed phase LC is the main technique used for separation and quantitation of the active substances, usually 3,4-methylenedioxy-amphetamines, in ecstasy tablets. Usually the column of the choice is conventional C_{18} or a base-deactivated C_{18} stationary phase (Table I.2-2). Mancinelli *et al.* have developed a HPLC-fluorimetric procedure suitable for different matrices, dosage forms and biological samples, to determine MDA, MDMA, MDEA, and MBDB [Mancinelli *et al.,* 1999]. This procedure was carried out in basic

isocratic conditions and because of the high pH (11.4) the analytes are non-ionized. The analysis time is less than 10 min and the first eluting compound is MDA (4.94 min) followed by MDEA (6.77 min), MBDB (7.53 min) and last eluting one is MDMA (9.50 min).

Sadeghipour and Veuthey also reported the use of fluorimetric detection in determination of MDA, MDMA, MDEA, and MBDB [Sadeghipour and Veuthey, 1997]. The selectivity of the method was verified not only with common substances, which can appear in seized tablets, but also with some drugs of abuse such as cocaine, morphine, amphetamine, methamphetamine, etc. The selectivity of the method is based on that only methylenedioxylated amphetamines are natively fluorescent and therefore detectable. The optimal mobile phase condition was determined as 20 mM NaH_2PO_4 solution (adjusted to pH 3.8)-acetonitrile (85:15, v/v). Under these conditions, the analytes were ionized and analysis time was less than 6 min. The elution order was MDA, MDMA, MDEA and the last eluting compound was MBDB.

In addition, Sadeghipour *et al*. have developed a reversed-phase LC method with UV detection for separation and quantitation of five amphetamines (amphetamine, methamphetamine, MDA, MDMA, and MDEA) and ephedrine in the presence of adulterants in illicit drugs [Sadeghipour *et al.*, 1997]. The comparison of a regular reversed phase column (RP18 Nucleosil 100) and a base-deactivated column (RP18-AB Nucleosil 100) was demonstrated and as a result, the base-deactivated phase gave higher efficiency and lower peak asymmetry and capacity factors which allowed a rapid separation of amphetamines with good resolution. The mobile phase composition was optimized by studying the influence of pH, buffer composition and the organic solvent

type. The best results were obtained with acetonitrile concentrations between 7 and 10% and with phosphate buffer (pH adjusted to between 3.4 and 3.8).

The reversed phase separation of *N*-alkyl MDAs has been achieved on a C_{18} stationary phase and a ternary mobile phase [Noggle *et al.*, 1987 and 1988]. The ternary mobile phase consisted of pH 3 phosphate buffer, acetonitrile, and methanol containing triethylamine. The use of triethylamine as a competing base (silanophile) was necessary on μBondapak C_{18} stationary phase to prevent peak tailing.

Table I.2-2: HPLC methods to separate 3,4-methylenedioxyamphetamines from non-biological matrices.

Separated compounds	Column	Detector	Reference
MDA, MDMA, MDEA, MBDB	LiChrocart-LiCrospher 100 RP-18	FL	Mancinelli et al., 1999
MDA, MDMA; MDEA; MBDB		FL	Sadeghipour and Veuthey, 1997
MDA, MDMA, MDEA, and other phenethylamines	RP18 Nucleosil 100, RP18-AB Nucleosil 100	FL	Sadeghipour et al., 1997
MDEA, MDMMA, MBDB, MDP-3-MB	Bondclone C_{18}	UV	Clark et al., 1996
MDA, MDMA, MDEA, MDMMA	Bondclone C_{18}	UV	Clark et al., 1995a
BDB, MBDB, MDP-2-EB, MDP-2-MMB, MDP-2-OHB	Bondclone C_{18}	UV	Clark et al., 1995a
MDP-3-B, MDP-3-MB MDP-3-EB, MDP-3-MMB, MDP-3-OHB	Bondclone C_{18}	UV	Clark et al., 1995a
MDMA, BDB, MDP-3-B	Bondclone C_{18}	UV	Clark et al., 1995a

MDA, MDMA, NOHMDA	Nucleosil C_{18}	UV	Valaer et al., 1990
MDA, MDMA, NOHMDA	Deltabond C_8	UV	Valaer et al., 1990
MDA, MDMA, MDMMA, MDEA, N-isopropyl MDA, N-n-propyl MDA, NOHMDA	μBondapak C_{18}	UV	Noggle et al., 1988
MDA, MDMA, MDEA, N-isopropyl MDA, N-n-propyl MDA, N-isobutyl MDA, N-n-butyl MDA	μBondapak C_{18}	UV	Noggle et al., 1987

The elution order of the *N*-alkyl MDAs is based on the relative lipophilicity of the derivatives and thus retention increases with the size of the alkyl chain on the nitrogen. The *N,N*-dimethyl MDA (MDMMA) elutes before *N*-ethyl MDA (MDEA), and the branched isopropyl derivative elutes before *N*-n-propyl MDA [Noggle *et al.*, 1988]. Under these conditions the *N*-hydroxy MDA (NOHMDA) has the highest retention (25 min) which has been explained by higher polarity and lower basicity of the compound compared to the other *N*-alkyl MDAs. The pK_a value for NOHMDA has been determined by titration to be 6.22, which is much lower than the pK_a values of other *N*-alkyl MDAs (cirka 10) [Valaer *et al.,* 1990]. The separation of NOHMDA from MDA and MDMA was improved by using a Deltabond C_8 stationary phase which enabled the separation without the need for competing base and in a shorter analysis time (10 min) [Valaer *et al.,* 1990].

Clark *et al.* reported the separation studies of *N*-substituted MDAs, *N*-substituted-2-butanamines and *N*-substituted-3-butanamines [Clark *et al.,* 1995a]. The separation of the compounds was accomplished using a C_{18} stationary phase (Bondclone C_{18}) with an

acidic mobile phase. The elution order under these conditions was according to the size of the *N*-substituent. The *N*-substituted MDAs eluted in the same order as previously; MDA (5.08 min), MDMA (5.87 min) and MDEA (6.68 min). The elution order of *N*-substituted-2-butanamines and *N*-substituted-3-butanamines was similar. The three-carbon side chain propanamines (MDAs) had lower capacity factors than the 2-and 3-butanamines when comparing compounds with identical *N*-substituents. In addition, 3-butanamines display higher capacity factors than the 2-butanamines of the same *N*-substituent in every case. When compounds with the same molecular weight as MDMA were compared, it was found that MDMA eluted first, followed by BDB and the last compound to elute was MDP-3-B [Clark *et al.,* 1995a]. The separation of compounds with the same molecular weight as MDEA was also studied under the same conditions and the results are shown in Table I.2-3 [Clark *et al.,* 1996].

Table I.2-3: Reverse**d phased LC separation of MDEA and its regioisomers.**

Compound	**R_t (min)**
MDMMA	7.93
MDEA	8.77
MBDB	10.91
MDP-3-MB	12.60

I.2.2.2.2 Gas chromatography (GC)

I.2.2.2.2.1 Analysis of biological samples.

Publications on GC procedures with different detectors used for the determination of 3,4-methylenedioxyamphetamines from biological samples are reviewed in Table I.2-4. A mass spectrometer is the most specific detector for drug testing. Several manuscripts describe drug testing using GC with less specific detectors. For example Drummer *et al*. identified amphetamine, methamphetamine, MDMA, MDA, and other drugs of forensic interest in blood using GC-NPD [Drummer *et al*., 1994]. In addition, the analysis has been performed by a dual channel GC combined with a NP and an EC detector [Lillsunde *et al.,* 1996]. Ortuño *et al.* stated that when analyzing plasma samples with GC-NPD, good chromatographic separation and adequate sensitivity of underivatized compounds can be achieved but the same approach is not applicable for urine [Ortuño *et al.*, 1999]. When GC-MS is used for screening blood samples, the selected ion monitoring (SIM) mode is often applied [Marquet *et al.,* 1997].

Table I.2-4: GC procedures for the identification and/or quantification of 3,4-methylene-dioxy amphetamines from biological samples.

Separated compounds	Matrix	Column	Detector	Reference
Enantiomers of MDEA, MDMA, MDA	blood	HP5-MS	MS(EI)	Peters FT et al., 2005
Enantiomers ofMDA, MDMA, MDEA, amphetamine, methamphetamine	urine		MS(EI)	Paul BD et al., 2004
MDMA, MDEA	hair	DB5-MS	MS (SIM)	Girord and Staub, 2000
MDA, MDMA, MDEA, MBDB	serum	DB5-MS	MS (SIM)	Weinman *et al.,* 2000

MDMA and its metabolites (MDA, HMMA, HMA)	plasma, urine	Ultra-2	NPD	Ortuño *et al.,* 1999
R and S-MDMA and its chiral metabolites (MDA, HMMA, HMA)	urine	DB5-MS	MS (EI, PCI)	deBoer *et al.,* 1997
MDA, MDMA, MDEA, amphetamine, methamphetamine	blood	HP5-MS	MS (EI, SIM)	Marquet *et al.,* 1997
MDA, MDMA, MDEA, amphetamine, methamphetamine	urine	SPB-5	MS (EI, CI, SIM)	Dallakian *et al*., 1996
MDA, MDMA, MDEA, MBDB	urine	FSC HP-5	MS (EI, SIM)	Kronstrand, 1996
MDA, MDMA, amphetamine, methamphetamine, etc.	blood	FSC HP-5	NPD, ECD	Lillsunde *et al.,* 1996
MDA, MDMA, MDEA, BDB, MBDB	urine	HP1	MS (SIM)	Maurer, 1996
MDA, MDMA, amphetamine, methamphetamine	blood	FSC BP-5	NPD	Drummer *et al*., 1994
MDA, MDMA, MDEA	urine	FSC DP-5	MS (CI, SIM)	Lim *et al*., 1992

I.2.2.2.2.2 Analysis of non-biological samples.

O'Connell and Heffron established a GC-MS procedure to determine the principal amphetamines, MDMA, MDEA, MDA, cocaine and pharmacologically active impurities in ecstasy tablets [O'Connell and Heffron, 2000]. The tablets were ground into powders and dissolved in ethanol. The column used was a HP-1 fused-silica capillary column column (60 m x 0.25 mm id) with a 1 μm film thickness of methylsilicone.

Lillsunde and Korte have reported a method for analyzing 12 ring and *N*-substituted amphetamine-derivatives in body fluids or seized materials by GC combined either with

MS, EC or NP detector [Lillsunde and Korte, 1991]. GC-MS was used for identification with a packed column, 2% SP-2110 / 1% SP-2510. GC-ECD and GC-NPD was used for quantitation with a fused silica capillary column, SE-54. Derivatization was done with heptafluorobutyric anhydride and then most of the 12 amphetamines examined were separated and their retention times are listed in Table I.2-5. Only MDEA and 2,5-dimethoxy-4-ethylamphetamine as well as 3,4,5-trimethoxyamphetamine and 3-methoxy-4,5-methylenedioxyamphetamine co-eluted. On the other hand, the mass spectra are characteristic and can be used to distinguish these compounds from each other.

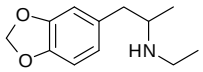
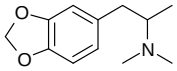
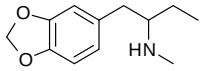
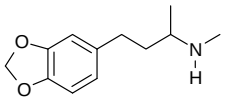
Table I.2-5: Retention times (min) of HFBA derivatives of amphetamines on packed and capillary columns [Lillsunde and Korte, 1991].

Compound (HFBA-derivative)	R_t (packed column)	R_t (capillary column)
amphetamine	3.3	2.74
methamphetamine	3.8	3.61
N-ethylamphetamine	4.1	3.95
4-methoxyamphetamine	5.8	4.77
MDA	7.5	5.77
2,5-dimethoxyamphetamine	6.9	6.24
MDMA	7.5	6.87
2,5-dimethoxy-4-ethylamphetamine	7.8	7.23
MDEA	7.8	7.25
3,4,5-trimethoxyamphetamine	8.8	7.76
3-methoxy-4,5-methylenedioxyamphetamine	8.8	7.77
4-bromo-2,5-dimethoxyamphetamine	10.2	8.70

Gas chromatographic separation of MBDB, MDEA, *N,N*-dimethyl-3,4-methylenedioxy-amphetamine (MDMMA) and *N*-methyl-1-(3,4-methylenedioxyphenyl)-3-butanamine (HMDMA) has been achieved on a 12 m x 0.20 mm id methylsilicone column (HP-1) [Noggle *et al.,* 1995]. These compounds have the same molecular weight and similar retention properties (Table I.2-6). HMDMA has the highest retention time and the other three compounds elute over approximately 0.25 minutes with MDEA and MDMMA eluting before MBDB. In this system compounds having the C_3 carbon side chain attached to the aromatic ring elute before the two aryl-C_4 butanamines.

The use of mass spectrometry as a detector does not provide significant data for differentiation among MDEA, MBDB and MDMMA since these regioisomeric compounds also yield regioisomeric fragment ions of equal mass. HMDMA can be easily distinguished from the other three compounds because the difference in mass spectrum produced by substitution of the methylamino group at the 3-position of the butanamine side chain. Pentafluoropropionylamide (PFPA) derivativatization of MDEA, HMDMA, and MBDB, was used to improve mass spectrometric differentiation [Clark *et al.,* 1996]. The GC analysis of the three PFPA derivatives showed slightly higher retention for derivatized compounds and they eluted in the same order as underivatized compounds. Tertiary amines such as MDMMA do not form stable PFPA-derivatives and therefore it can be easily identified since the mass spectrum remains unchanged after acylation with PFPA.

Table I.2-6: GC separation of MDEA, MDMMA, MBDB and HMDMA.

Compound	Structure	R_t
MDEA		7.23
MDMMA		7.35
MBDB		7.50
HMDMA		7.68

I.2.3 General Problems for Identification and Separation.

Regioisomerism at the aromatic ring and the alkyl side-chain of the methylenedioxyalkylamines produces a variety of compounds that have very similar analytical properties. The methylenedioxy ring can be fused to the aromatic in a 2,3- or 3,4-pattern, yielding regiosiomerism of the aromatic portion of the molecule. Identification of 2,3-methylenedioxy-phenalkylamines is of importance to forensic chemists, although those compounds are not as likely to appear on the clandestine market. Alkyl side-chain regioisomerism is most significant when imine fragments of equivalent mass and similar abundance appear in the mass spectra of these compounds. Some of the alkyl side-chain regioisomers can be differentiated by derivatization [Clark *et al.,* 1995b], but for example 2,3- and 3,4-methylenedioxyphenalkyl-amines cannot be differentiated by derivatization [DeRuiter *et al.,* 1998].

Further more there are other compounds that do not necessarily contain the methylenedioxy ring, however they constitute indirect regioisomeric and isobaric substances related to the drug of abuse 3,4-MDMA. These substances have the same molecular weight and are capable of producing mass spectra similar to 3,4-MDMA. The co-elution of one or more of these substances with MDMA remains a possibility. Additionally the lack of reference materials for these substances complicates the identification procedure.

I.2.3.1 Differentiation of regioisomeric 2,3- and 3,4-methylenedioxy phenalkyl amines and isobaric substances related to MDMA and MDEA by chromatographic methods.

The analytical properties of the 2,3-MDAs, such as 2,3-MDA, 2,3-MDMA, 2,3-MDEA, and 2,3-MDMMA, has been compared to the corresponding 3,4-MDAs [Casale *et al.,* 1995]. The EI mass spectra of 2,3- and 3,4-MDAs showed fragments of the same mass with only slight differences in relative intensity. The distinguishable difference is the relative abundance of ions at m/z 135 and m/z 136. Such differences cannot be considered significant from the analytical point of view, in particular when the analytes must be detected in a chromatographic run and the mass spectra of the compounds of interest show interferences from co-chromatographing substances. Gas chromatographic studies of the regioisomers on the methylsilicone stationary phase showed that all four 2,3-MDAs were easily resolved from its respective 3,4-regiosiomers and their retention times were significantly less than the corresponding 3,4-substituted MDA. Naturally, the regioisomeric MDAs could each be differentiated by proton NMR via variances in both chemical shifts and overall peak patterns. However, NMR is not a technique, which is

commonly used in the forensic laboratories and therefore the differentiation has usually depended on chromatographic and mass spectrometric methods.

DeRuiter *et al.* studied liquid chromatographic and mass spectral methods to differentiate 2,3- and 3,4-methylenedioxyphenyl ring substitution regioisomers of MDMA, BDB, MDEA, and MBDB [DeRuiter *et al.*, 1998]. The derivatization of the side-chain regioisomers to pentafluoropropionylamide (PFPA) derivatives enabled differentiation by mass spectroscopy. The fragmentation of the bond between the alkyl side-chain and the nitrogen in the PFPA-derivatized amines yielded prominent ions that identified the nature of the hydrocarbon chain attached directly to the aromatic ring. The reversed-phase LC system consisting of a C_{18} stationary phase (Hypersil Elite C_{18}) and a mobile phase of 30% methanol in pH 3 phosphate buffer gave an excellent separation of regioisomeric MDMA and BDBs. The first eluting compound was 3,4-MDMA followed by 2,3-MDMA and then 3,4-BDB and last eluting compound was 2,3-BDB. A similar elution order was obtained when regioisomeric MDEA and MBDBs were separated on the same stationary phase but by using a different mobile phase composition. The optimum isocratic separation was achieved when the mobile phase was 10% acetonitrile in pH 3 phosphate buffer. The two compounds with the C_3 alkyl chain attached to the aromatic ring eluted first; the 3,4-MDEA eluted before the 2,3-MDEA, and the two compounds with C_4 alkyl group showed greater retention. The observed elution order was the same; first are eluting compounds with shorter alkyl chains attached to the aromatic ring and secondly, the 3,4-regioisomers elute before the 2,3-regioisomers, for several C_{18} stationary phases in both methanol- and acetonitrile-modified acidic aqueous systems. In addition, four regioisomers of 3,4-MDMA and 3,4-MDEA were analyzed by GC on a

methylsilicone stationary phase under temperature-programmed conditions. 3,4-MDMA and its regioisomers eluted over a 0.6 min time window from 6.7 to 7.3 min and 3,4-MDEA and its regioisomers produced an elution range of 0.5min (7.0 to 7.5 min). The elution order of the compounds was not mentioned.

Aalberg et al. reported the use of Dry Lab software for the separation of direct and indirect regioisomers related to the drug of abuse 3,4-MDMA and 3,4 MDEA using LC. The Dry lab software uses initial four runs under identical conditions using two different gradient times and two different temperatures. By entering the collected retention data along with column dimensions, mobile phase composition, flow rate and column efficiency, the software will generate three dimensional resolution map and suggest the best conditions for separating the desired compounds in a physical mixture.

Aalberg reported the 10 direct side chain and ring regioisimers of MDMA including N-ethyl-3,4-methylenedioyphenylethanamine, N,N-dimethyl-3,4-methylene-dioxyphenethanamine, 3,4-methylenedioxyphetramine, 3,4-methylenedioxyBDB and their 2,3- ring regioisomers. The HPLC study showed the rentention time increase with side chain length of the uninterrupted hydrocarbon attached to the aromatic ring. Base line separation of these compounds was carried out on an XTerra column at 40°C with tG of 90 min (initial 3% methanol, final 30% methanol in pH 3 phosphate buffer). On Supelcosil ABZ^{+}Plus column a better sepration was obtained at 41°C with a tG of 230 min (initial 3% methanol, final 40% methanol in pH 3 phosphate buffer). Dry lab helped in determining the optimum isocratic separation of these compounds on a Supelcosil ABZ^{+}Plus with 2% methanol and pH 3 phosphate buffer at 45°C [Aalberg et al. 2003].

The indirect regioisomers of 3,4-MDMA included in the Aalberg study were p-ethoxymethamphetamine, 1-*p*-ethoxyphenyl)-2-butanamine, 4-methoxy-3-methylmeth-amphetamine, 1-(3-methyl-4-methoxyphenyl)-2-butanamine, α,α-dimethyl-1-(3-methyl-4-methoxyphenyl)-2-ethanamine, *N*-methyl-1-(2-methoxyphenyl)-1-methyl-2-propan-amine, *N*-methyl-1-(3-methoxyphenyl)-1-methyl-2-propanamine, *N*-methyl-1-(4-methoxyphenyl)-1-methyl-2-propanamine and p-methoxymethcathinone. The sepration of these substances was only achieved on Supelcosil ABZ^{+}Plus and XTerra columns, when acetonitrile was used as an organic modifier in the mobile phase. Separations conditions were gradient mobile phase with a gradient time of 20.90 min at 40°C, starting from 14% and ending at 26% acetonitrile in pH 3 phosphate buffer. It was very hard to get a base line separation for both *N*-methyl-1-(3-methoxyphenyl)-1-methyl-2-propanamine and *N*-methyl-1-(4-methoxyphenyl)-1-methyl-2-propanamine because of the similarity of their retention properties. The Supelcosil ABZ^{+}Plus offered the best resolution of all the 19 compounds with the gradient mobile phase (initial mobile phase consisted of 3% acetonitrile and pH 3 phosphate buffer, and the final composition, 9.6% acetonitrile, was reached in 35 min) at room temperature [Aalberg et al. 2003].

Aalberg et al. also reported gas chromatographic separations for the above described substances. For the 2,3- and 3,4- regioisomers directly related to the drug of abuse MDMA, optimum separation was obtained using a 35% phenylmethylsilicone phase (DB35MS) and temperature programming of 180^{0}C as intial temperature and an increase in rate of 0.3 ^{0}C/ minute. These conditions were determined by the retention modeling software, Dry Lab [Aalberg et al., 2004].

Other indirect ring and side chain regioisomers and isobaric substances related to MDMA were separated on non polar Ultra 2 column (25 m, 0.2 mm, 0.33μm) with a temperature program rate of 7.3°C/min. The analysis time was decreased on a narrow pore column (Hp-5) by optimizing the temperature program through segmented temperature ramp using Dry Lab software. A base line separation of all the 19 direct, indirect and isobaric substances related to MDMA was not obtained in the study [Aalberg et al., 2004].

I.2.3.2 Differentiation of regioisomeric 2,3- and 3,4-methylenedioxyphen-alkylamines and isobaric substances by mass spectroscopic methods.

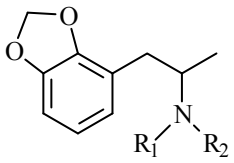
The underivatized methylenedioxyphenalkylamines give virtually identical EI mass spectra containing mainly intense immonium ions as mentioned earlier. The differentiation of some methylenedioxyphenalkylamines can be achieved by means of derivatization using various chromatographic methods. The use of tandem mass spectrometry (MS-MS) is reported to give additional information contained in the collision induced dissociation (CID) mass spectra of molecular ions using EI and especially methane CI [Borth *et al.*, 2000a]. CID mass spectra are obtained by parent ions colliding with an inert gas during the passage through the reaction chamber of a tandem mass spectrometer.

In their study Borth *et al*. were able to differentiate 18 regioisomeric methylene-dioxyphenyl-2-propanamines and methylenedioxyphenyl-2-butanamines (Figure I.2-2). The mixture of compounds was analyzed by GC on a methylsilicone stationary phase and

an insufficient separation was obtained. Several compounds (3a and 1c or 4a, 2c and 3b or 4b, 3c and 3d or 4c and 4d) co-eluted.

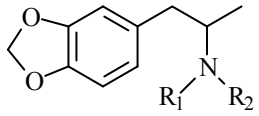
In general, the molecular ion CID mass spectra using EI were distinct, except in the case of compounds 4e and 4f the molecular ions did not have sufficient intensity for recording daughter ion mass spectra. The EI-CID mass spectra of all 2,3-methylenedioxyphenethylamines derivatives (1a-d and 3a-d) are dominated by immonium base peak ions with the general formula $[C_nH_{2n+n}N]^+$ (m/z 44, 58, 72, etc.) resulting from an α-cleavage reaction. In contrast the EI-CID mass spectra of 3,4-methylenedioxy isomeric compounds show significant or base peaks ions at m/z 136. This signal was explained to be due to a rearrangement of nitrogen H atoms to the aromatic *ortho* position eliminating an imine or by a specific six-center H-rearrangement of a γ-H-atom of the alkyl side chain to the aromatic ring eliminating a neutral enamine [Borth *et al.,* 2000a].

The CI-CID spectra of all ring substituted regioisomers generate a very intense or base peak ion at m/z 135 via cleavage of the benzyl bond by the charge of ammonium cation [Borth *et al.,* 2000b]. The 2,3-methylenedioxy isomeric compounds formed a significant $[C_7H_7O_2]^+$ ion at m/z 123 with the mass of a protonated methylenedioxybenzene. The 3,4-methylenedioxy ring-substituted compounds do not show this ion to a significant extent, but they show a significant homologous $[C_8H_9O_2]^+$ ion at m/z 137 by a formally benzylic bond cleavage. Therefore, the study of Borth et al using CI-CID mass spectrometry suggests that, the ion at m/z 123 indicates the 2,3-ring-substituted phenethylamine isomers and the ion at m/z 137 the 3,4-ring-substituted isomers.



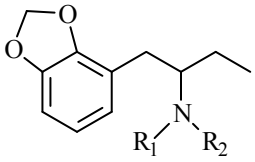
a: $R_1 = H$; $R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = H$; $R_2 = C_2H_5$
d: $R_1 = CH_3$; $R_2 = CH_3$

1



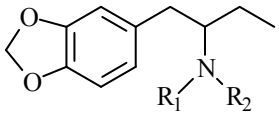
a: $R_1 = H$; $R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = H$; $R_2 = C_2H_5$
d: $R_1 = CH_3$; $R_2 = CH_3$

2



a: $R_1 = H$; $R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = H$; $R_2 = C_2H_5$
d: $R_1 = CH_3$; $R_2 = CH_3$

3



a: $R_1 = H$; $R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = H$; $R_2 = C_2H_5$
d: $R_1 = CH_3$; $R_2 = CH_3$
e: $R_1 = H$; $R_2 = n\text{-}C_3H_7$
f: $R_1 = H$; $R_2 = i\text{-}C_3H_7$

4

Figure I.2-2: Chemical structures of 18 regioisomeric methylenedioxyphenyl-2-propanamines and methylenedioxyphenyl-2-butanamines [Borth *et al.*, 2000a].

I.3 Statement of Hypothesis or Rationale for the Research

Analytical specificity in forensic drug analysis requires knowledge of all possible isomers in order to identify an individual compound and eliminate all other possibilities. Knowledge of the properties of all the possible regioisomeric/isobaric substances is essential in order to specifically identify any one of these compounds. The precursor chemicals are available to synthesize any of these compounds and several have already appeared in clandestine drug samples. It is perhaps a matter of time until one appears which has GC retention properties and MS equivalence to MDMA or MBDB. The goal of this work is to have the data and methods for differentiation available to prevent misidentification at that future time.

II. Methods (Narrative Descriptions of the Experimental Design)

II.1 Synthesis Design

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

Synthetic Methods Not Available

II.2 Methods (Synthetic materials):

Synthetic Methods Not Available

II.3 Analytical Methods

II.3.1 Instruments

GC-MS analysis was performed on two diggerent GC-MS. the first GC-MS is an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto , CA) using Helium (grade 5.0) as carrier gas. The mass spectrometer was operated on the electron impact (EI) mode using ionization voltage of 70 ev and a source temperature of 230 ^{o}C [Instrument 1]. Samples were dissolved in HPLC grade acetonitrile (Fisher Scientific NJ, USA) and manually introduced (1 µL), individually and in a physical mixture using a 10µL Hamilton syringe (Hamilton Co., Reno Nevada, USA).

The Second GC-MS is GC–MS analysis was performed using an Agilent Technologies (Santa Clara, CA) 7890A gas chromatograph and an Agilent 7683B auto injector coupled with a 5975C VL Agilent mass selective detector. The mass spectral scan rate was 2.86 scans/s. The GC was operated in splitless mode with a helium (grade 5) flow rate of 0.7 mL/min and the column head pressure was 10 psi (68.946 kPa). The

MS was operated in the electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230 8C. The GC injector was maintained at 250 8C and the transfer line at 280 ^{0}C [Instrument 2]

GC–IRD studies were carried out on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 auto injector coupled with a Hewlett-Packard 5965B infrared detector obtained from Analytical Solutions and Providers, Covington, Kentucky. The vapor phase infrared detector (IRD) spectra were recorded in the range of 4000–550 cm_1 with a resolution of 8 cm_1 and a scan rate 1.5 scans/s. The IRD flow cell temperature as well as the transfer line was 280 8C and the GC was operated in the splitless mode with a carrier gas (helium grade 5) flow rate of0.7 mL/min and a column head pressure of 10 psi[Instrument 3].

H^{1}NMR for the identification of raction intermediates were collected for samples dissolved in deuterated chloroform using a Brucker Avance 250 (250MHz) instrument with the chemical shifts being reported as δ ppm downfield from tetramethylsilane [Instrument 4]

II.3.2 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 II.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 (fd) with o.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 except for Rt-βDEXcst-TM, the injector temperature was adjusted to 200°C as recommended by the manufacturer.

Table II.1: List of columns used and their compostion.

Column Name	Column Composition
Rtx-1	100% Dimethyl polysiloxane
Rtx-5	95% dimethyl-5%diphenyl polysiloxane
Rtx-35	65% dimethyl-35%diphenyl polysiloxane
Rxi-50	50% phenyl to 50% methyl polysiloxane
Rtx-200	trifluoropropyl methyl polysiloxane
Rt-βDEXcst-TM	14% cyanopropyl phenyl – 86 % dimethylpolysiloxane

III. Results

During the course of this project we have prepared a large number of ring and side-chain substituted phenethylamines and compared and evaluated the analytical properties of these compounds. This work has included the 5 side chain isomers of 3,4-methylenedioxy ring system yielding the m/z 58 base peak in the EI-mass spectrum (3,4-MDMA and the 4 side chain isomers as well as the equivalent 2,3-methylenedioxy series of compounds. Additional studies have characterized the 2-, 3-, and 4-methoxymeth-cathinones which can be considered indirect isomers since these compounds have the same elemental composition as the MDMA series.

A number of individual synthetic and analytical studies have evaluated the 2-, 3-, and 4-ethoxyphenethylamines as well as the 10 different ring substitution patterns for the methoxy-methyl-phenethylamines. Furthermore the 2-, 3-, and 4-methoxy-alpha-methyl-methamphetamines were prepared and evaluated as well.

The methamphetamine side-chain isomer of every ring substitution pattern was prepared and evaluated in this study.

A series of all ten of the MBDB (m/z 72) side chain isomers was prepared and evaluated as well as of the more common side chains for several other ring substitution patterns.

Well over 90 compounds were evaluated in this project which allowed us to confirm specific structure property relationships for differentiation among most of these isomers.

The next chapters provide the details of specific studies as an example of each category of compounds evaluated in this project.

Chapter 1
Mass Spectrometry and Gas Chromatographic Studies on Direct Regioisomers to 3,4-MDMA.

There are nine other methylenedioxy-substituted phenethylamines with the potential to produce a mass spectrum essentially the same as 3,4-MDMA, five of them being 2,3-methylenedioxy ring substituted regioisomers**.**

Mass spectral studies of direct regioisomers of 3,4-MDMA (methylenedioxyphenyl substituted side-chain regioisomers).

The mass spectra of the phenethylamine drugs of abuse including 3,4-MDMA are characterized by a base peak formed by an α-cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-MDMA (MW=193) the α-cleavage reaction yields the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively) and the substituted imine fragment at m/z 58. Thus, the mass spectrum for 3,4-MDMA contains major ions at m/z 58 and 135/136 as well as other ions of low relative abundance.

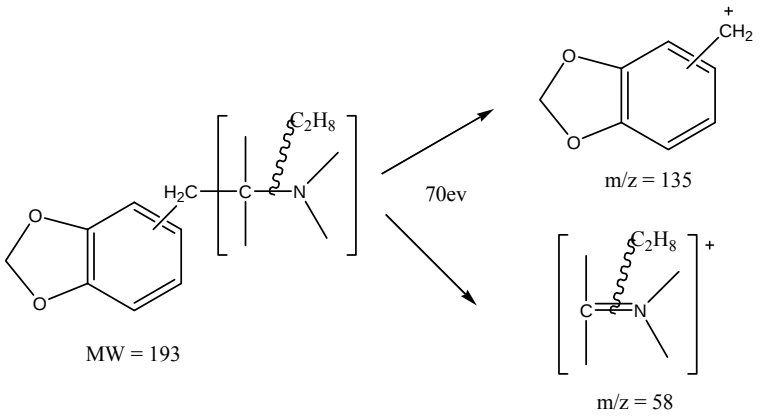
There are nine other methylenedioxy substituted regioisomers (a total of ten compounds) of the 3,4-MDMA molecule (MW=193) which yield α-cleavage fragments at m/z 58 and 135/136 during analysis by mass spectrometry (Scheme 1.1). The mass spectra in Figure 1.1 are for the ten possible direct regioisomers of the MDMA molecule. The first five side chain regioisomers show the methylenedioxy-group fused to the aromatic ring in a 3,4-manner while in compounds 6 through10 the substitution is in the 2,3-manner. All compounds show the expected fragments (m/z 58 and 135), and in

addition, most of the compounds show the molecular ion at m/z 193. In a direct comparison of the 3,4-regioisomers versus the 2,3-regioisomers with the identical side chain, the major difference is the greater relative abundance of the radical cation at m/z 136 for the 3,4-substitution pattern in most cases.

Clark et al [1996, 1998] and Aalberg et al. [2000, 2003] in previous work have described the analytical properties of these unique direct regioisomeric equivalences to the drug of abuse 3,4-methylenedioxymethamphetamine. These results illustrated that the mass spectrum alone cannot be used to identify an individual compound within this group to the exclusion of all others. Their observations [Aalberg et al., 2000 and 2004] illustrated that 3,4-MDMA (3) and *N*-ethyl-2,3-methylenedioxyphenyl-2-ethanamine (7) co-eluted using some common gas chromatographic stationary phases and conditions. However, additional studies [Aalberg et al., 2004] have identified capillary gas chromatographic phases and conditions for the complete resolution of compounds 1-10. Optimum separation was obtained using a 35% phenylmethylsilicone phase (DB35MS) and temperature programming conditions determined by retention modeling software (Dry Lab).

While these studies have shown that all ten compounds can be resolved, the lack of mass spectral specificity makes the specific identification of MDMA (with the exclusion of all other regioisomers) a significant challenge. The lack of available reference samples for all ten of these regioisomeric molecules further complicates the individual identification of any one of these substances. When other compounds exist with the potential to produce the same or nearly identical mass spectrum as the drug of interest, the identification by gas chromatography-mass spectrometry (GC-MS) must be

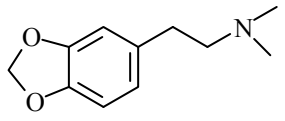
based primarily upon the ability of the chromatographic system to separate the "counterfeit substance" from the actual drug of interest. If not, those substances co-eluting with the target drug in chromatographic systems could be misidentified as the target drug. Without the appropriate standards a thorough method validation is not possible, and thus co-elution of drug and non-drug combinations would remain a possibility. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest



Scheme 1.1: General mass spectral fragmentation for the methylenedioxy regioisomers of MDMA (compounds 1-10).

Figure 1.1: Structures and mass spectra of methylenedioxy substituted side-chain regioisomers.

Structure of regioisomers **Mass spectrum**



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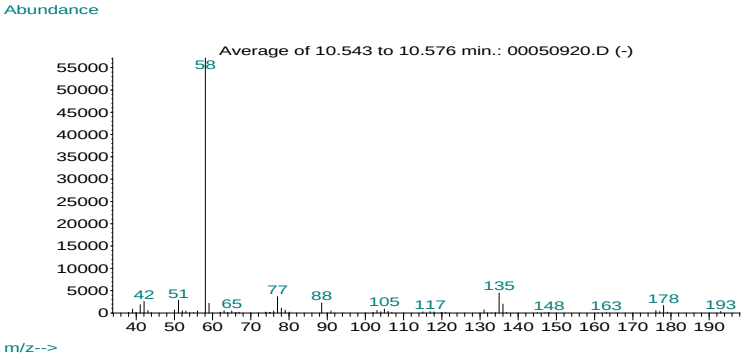

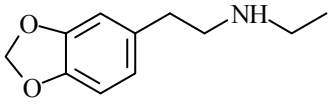

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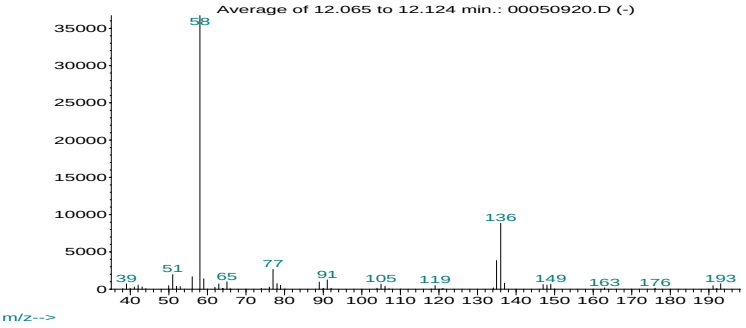

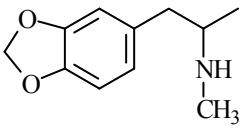

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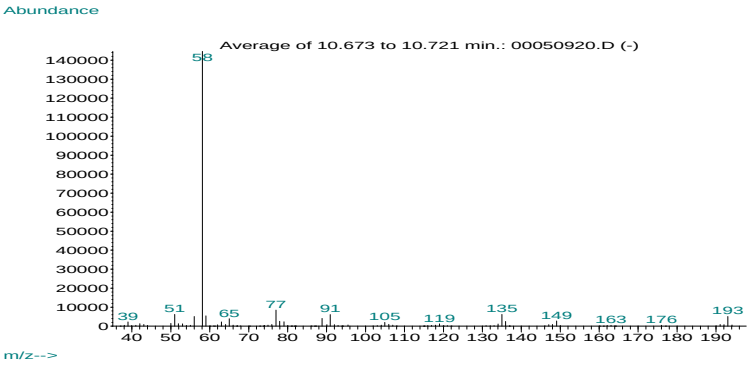

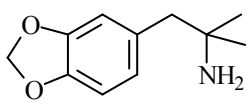

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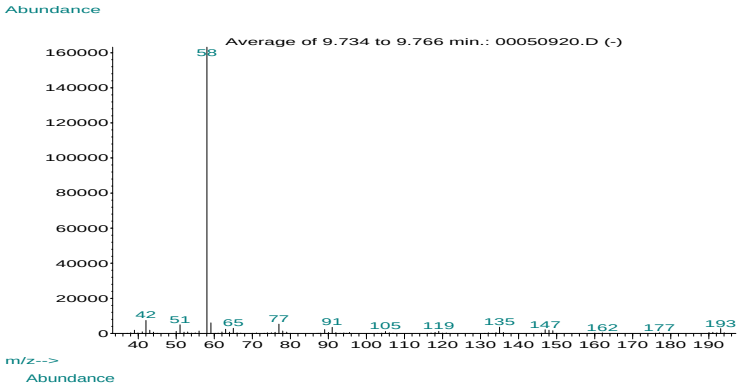

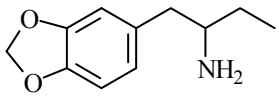

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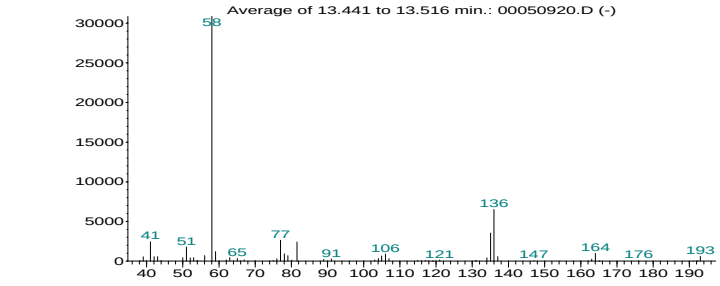

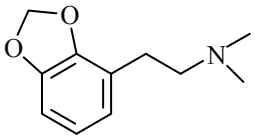

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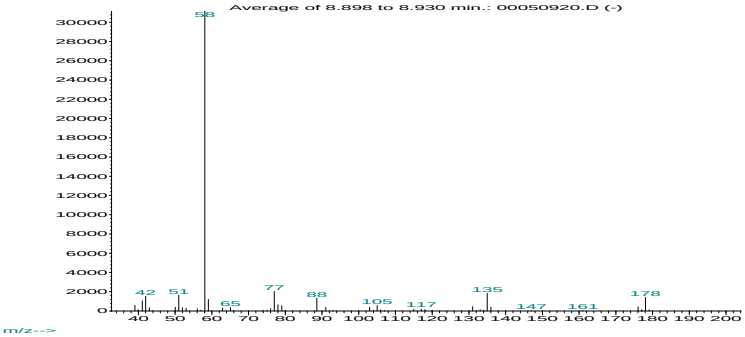

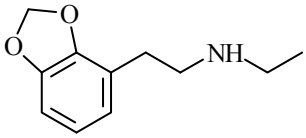

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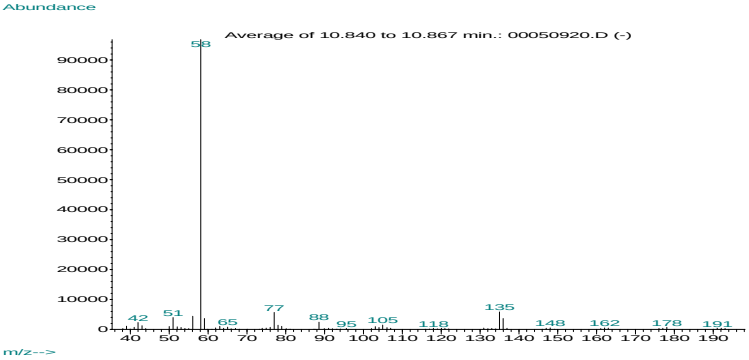

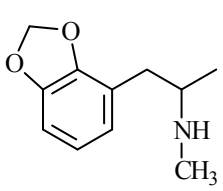

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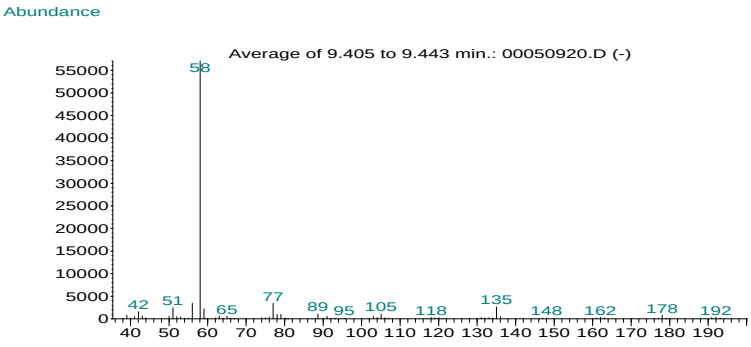

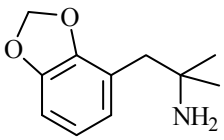

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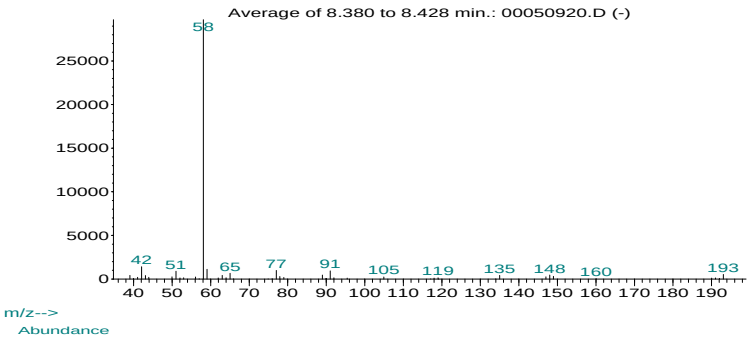

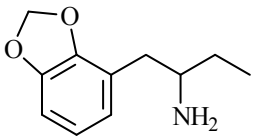

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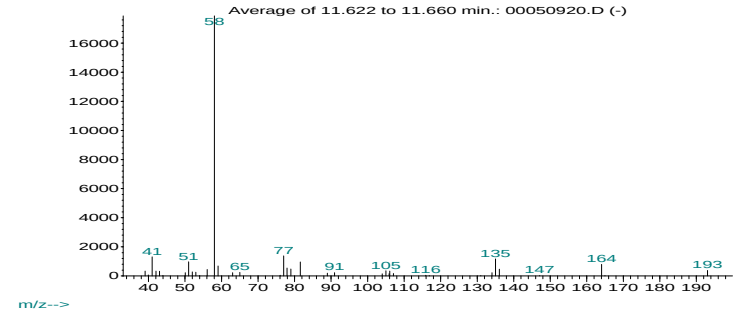


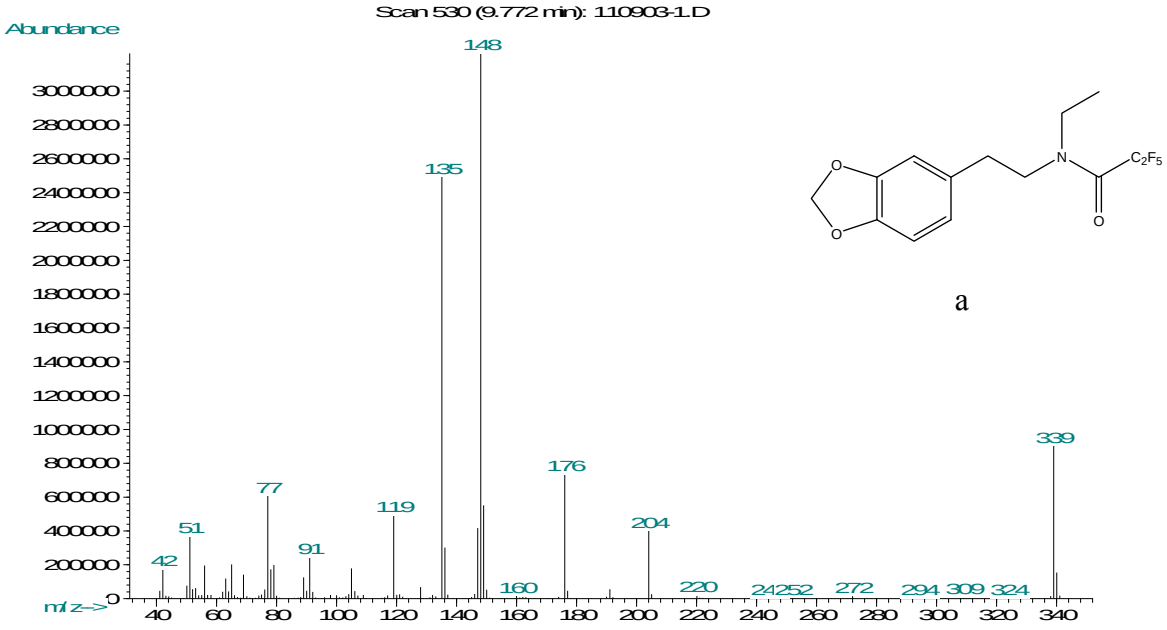
Mass spectral studies of perfluroacyl derivatives of 3,4- MDMA and its direct regioisomers

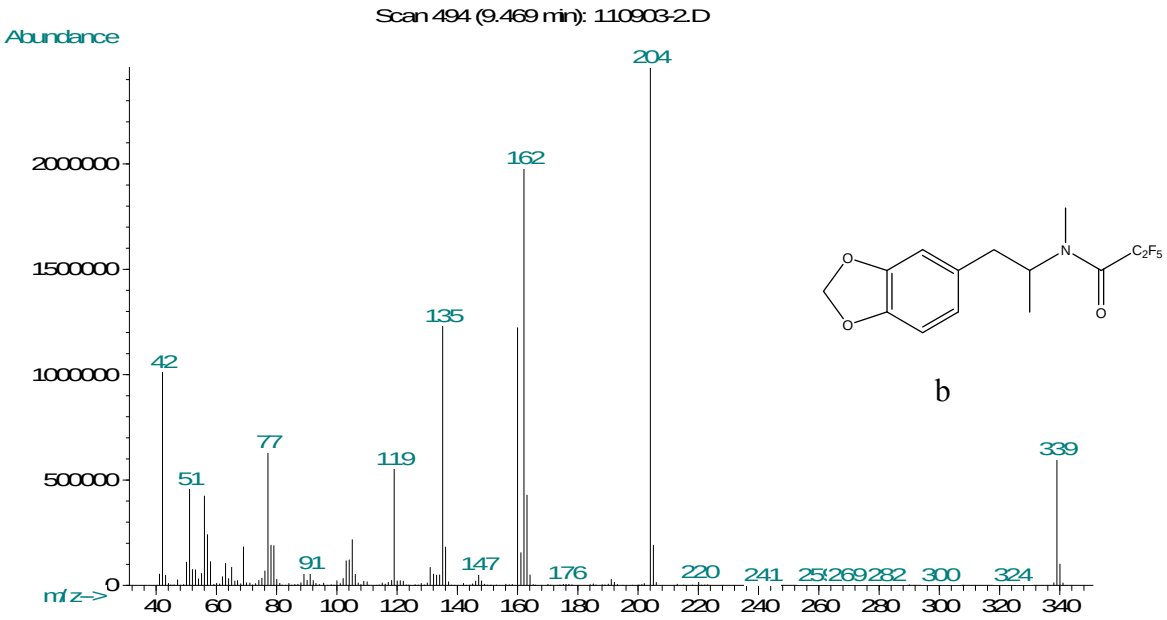
The perfluoroacylated derivatives of the eight primary and secondary amines (Compounds 2-5 and 7-10) were prepared and evaluated for their ability to individualize the GC-MS properties of the compounds in this uniquely regioisomeric series and to maintain or improve chromatographic resolution. Of course, the two tertiary amines, compounds 1 and 6, would not form stable amide derivative.

Acylation of the amines significantly lowers the basisty of nitrogen and can allow other fragmentation pathways to play a more prominent role in the mass spectrum [F. W. McLafferty et al, 1993]. The mass spectra for the eight pentaflouropropionyl and heptflourobutryl amides are shown in Figures 1.2 and 1.3, respectively

From these spectra a common peak occurs at m/z 204 and 254 which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for PFPA and HFBA amides, respectively.

Figure 1.2: Mass Spectra of the PFPA derivatives of compounds (2-5) and (7-10).





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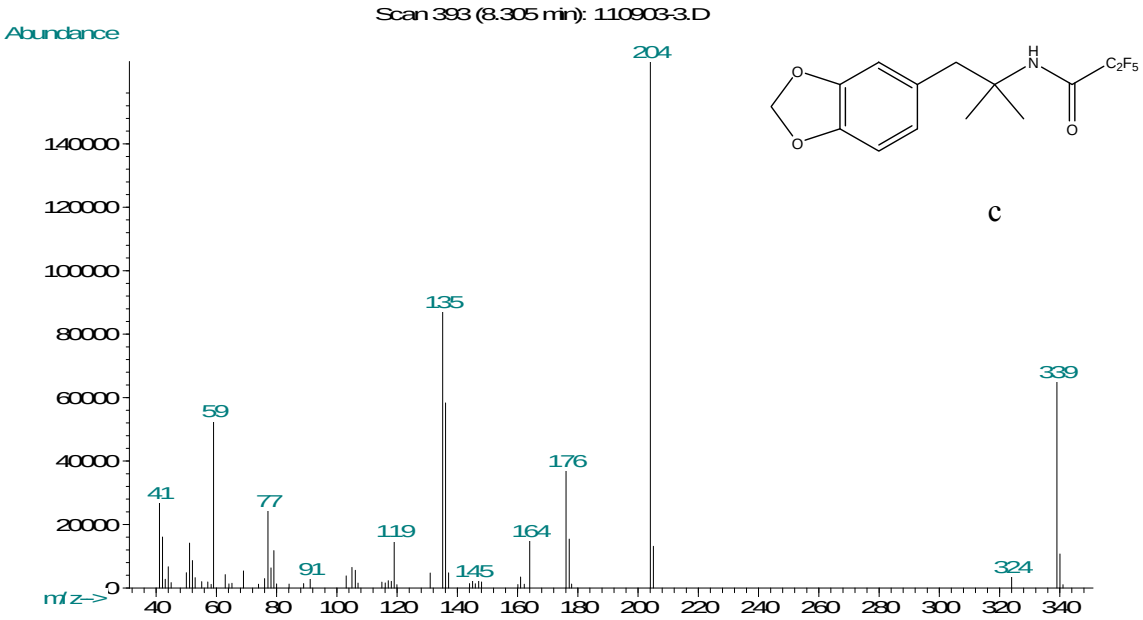

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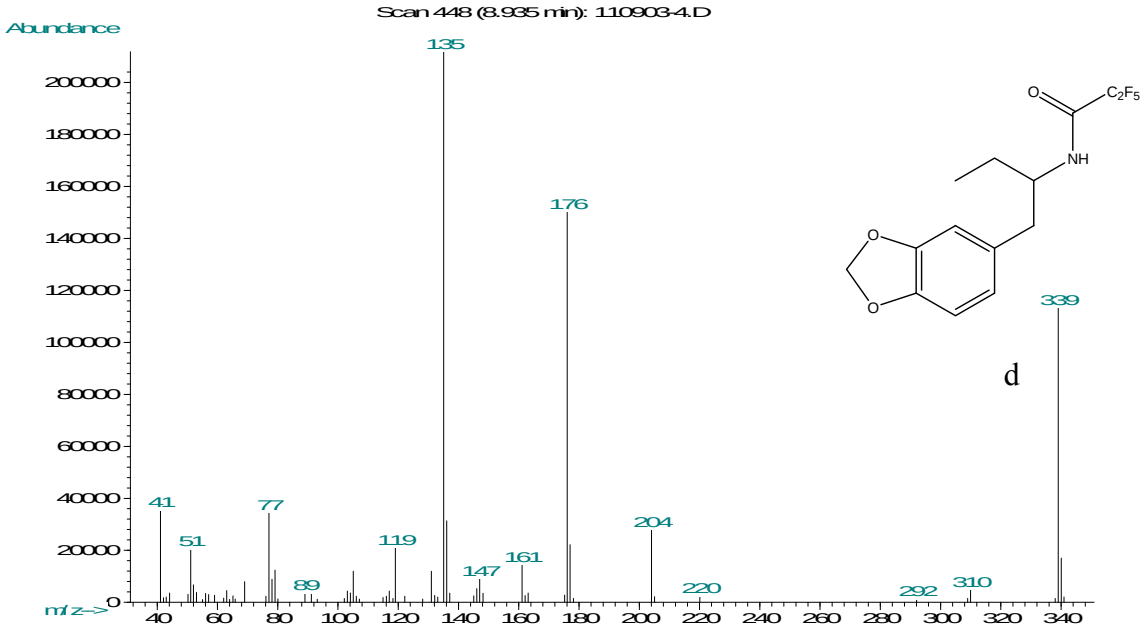

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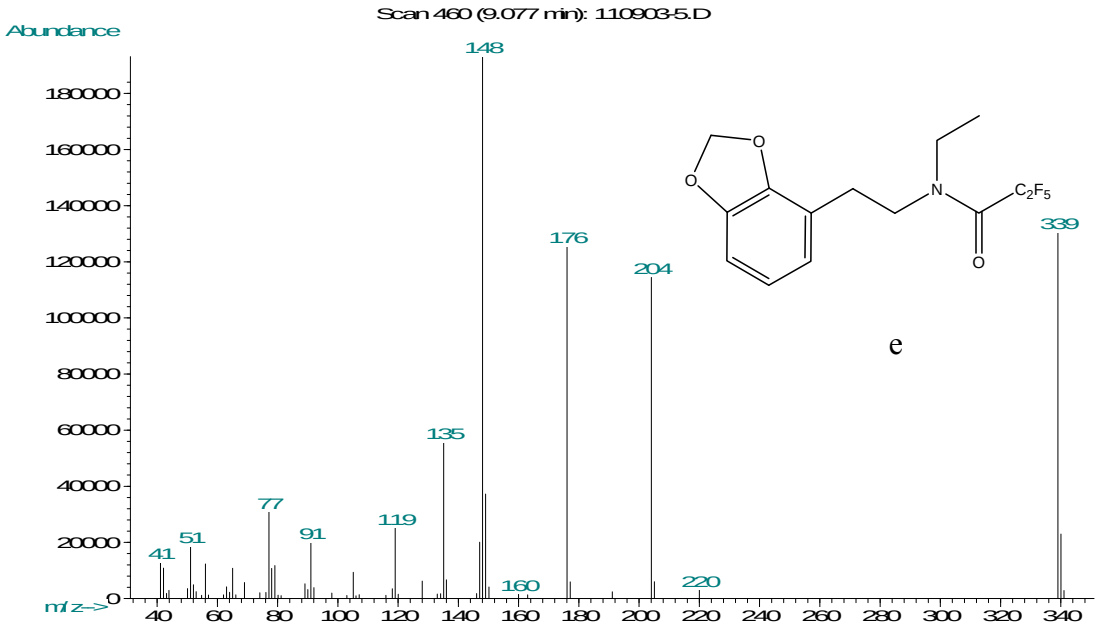

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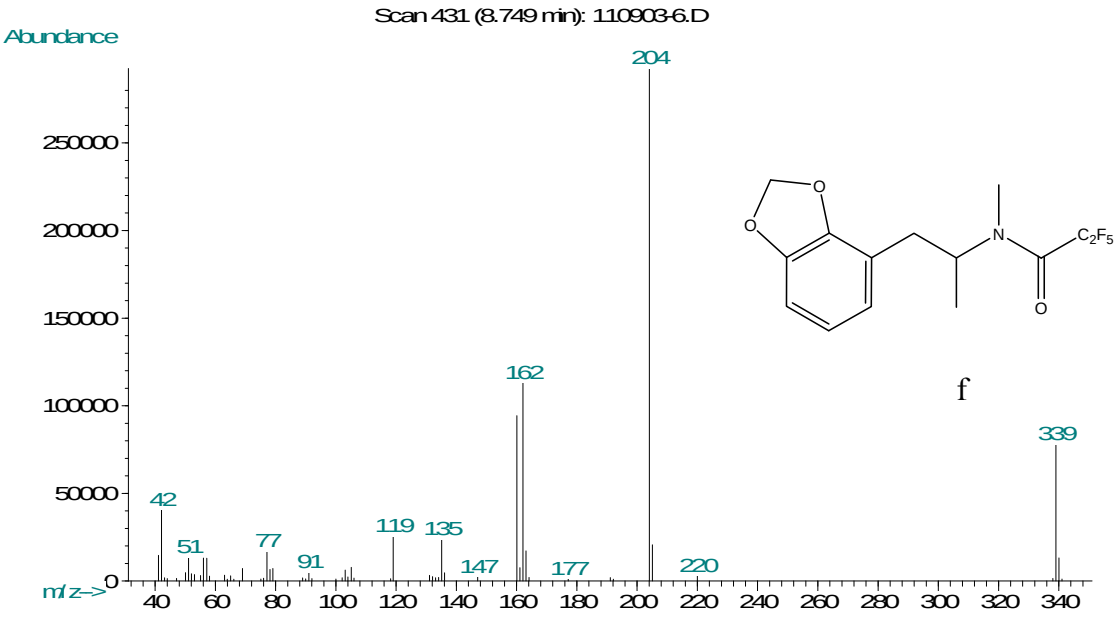

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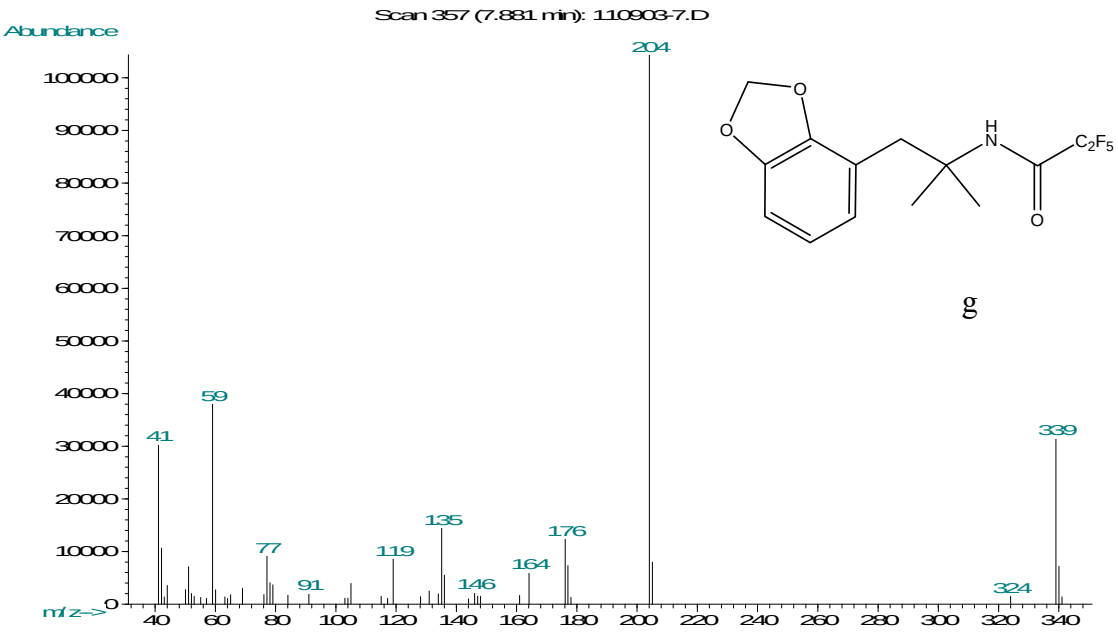

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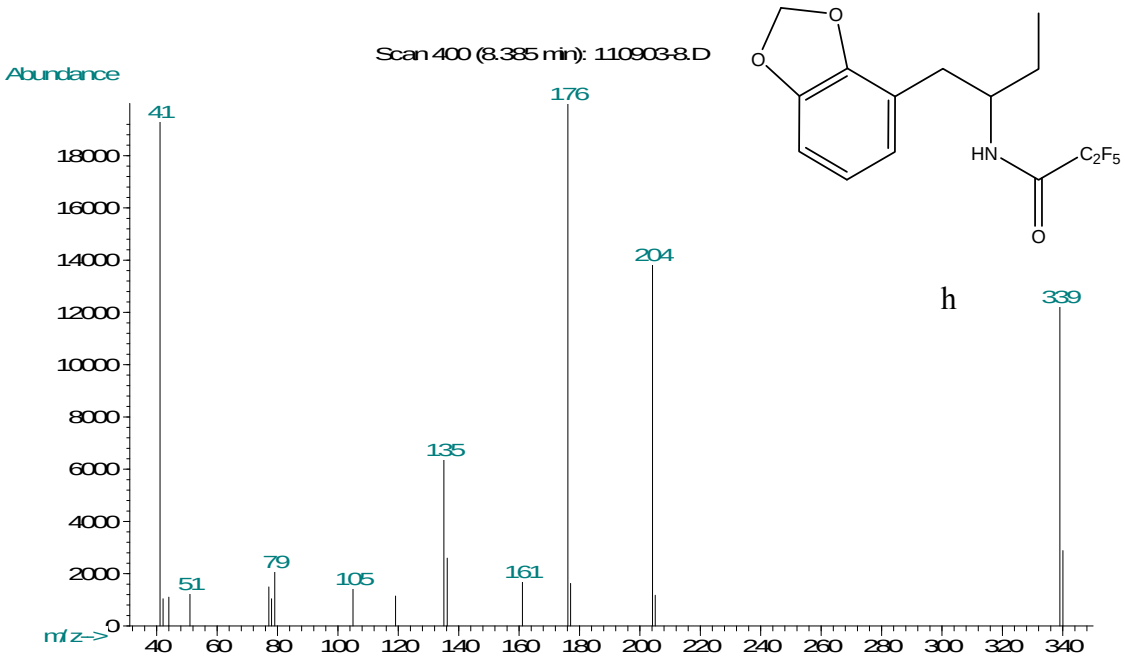
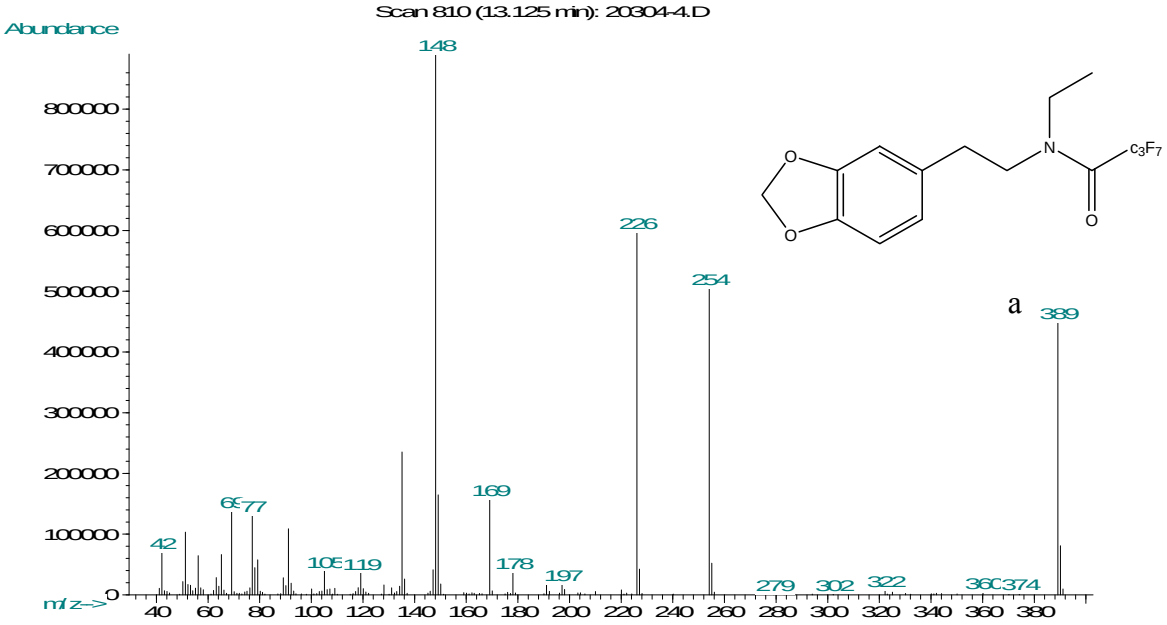
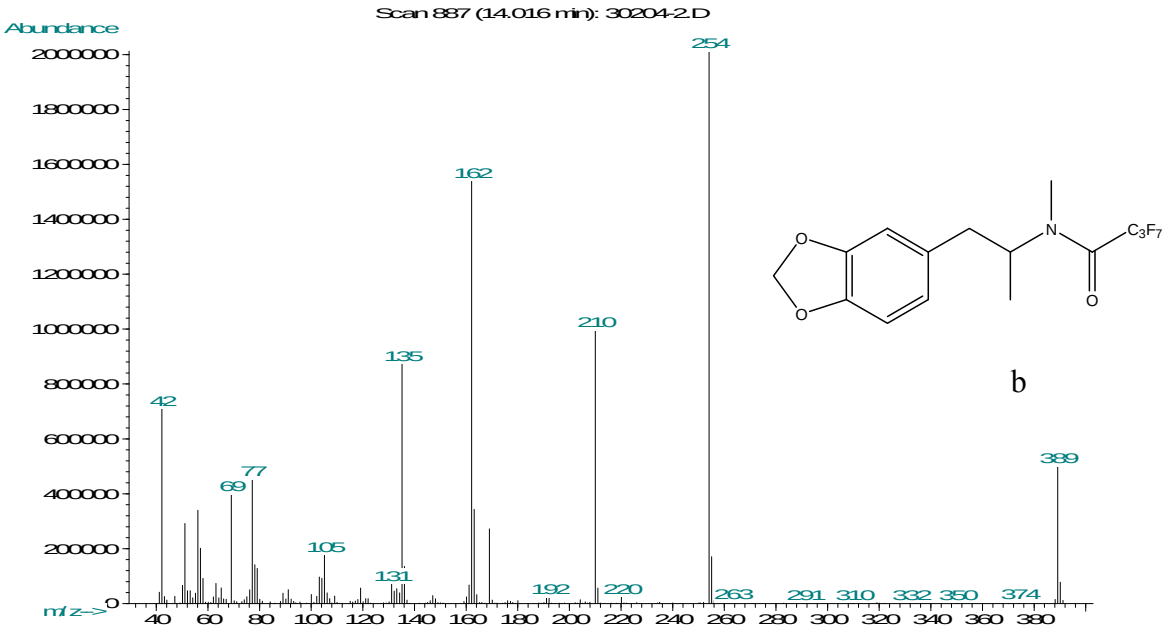


Figure 1.3: Mass Spectra for the HFBA derivatives of compounds (2-5) and (7-10).





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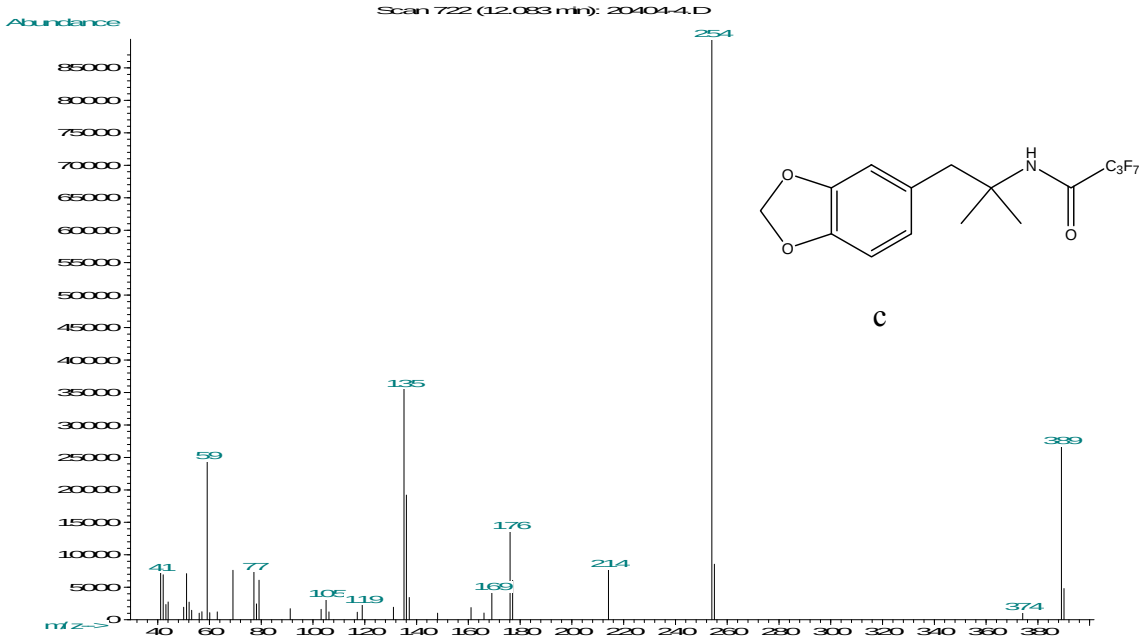

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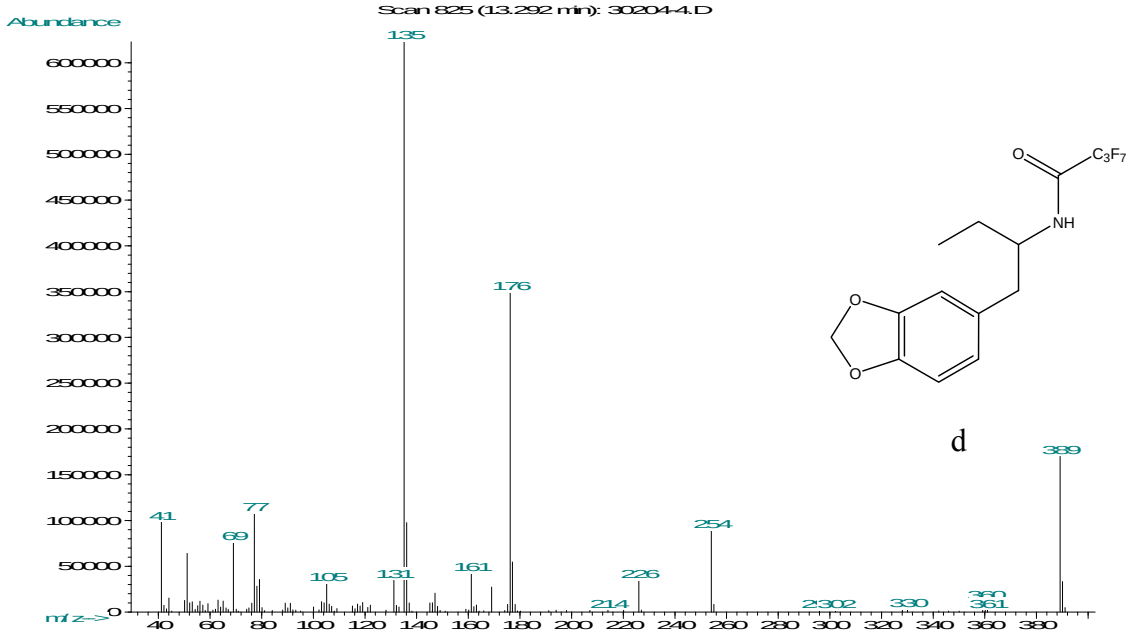

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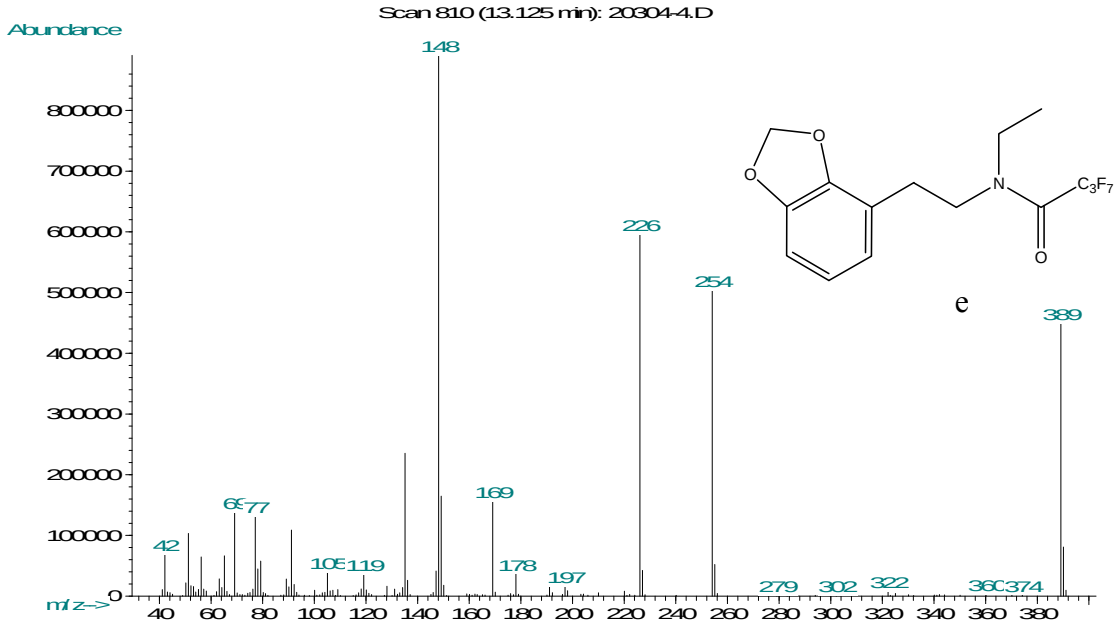

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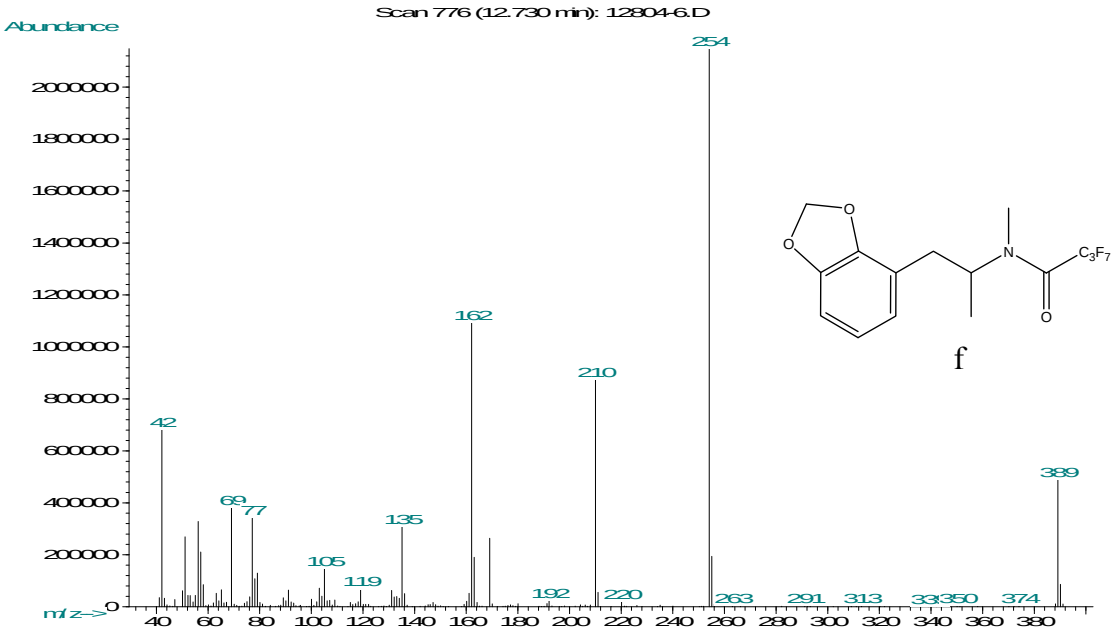

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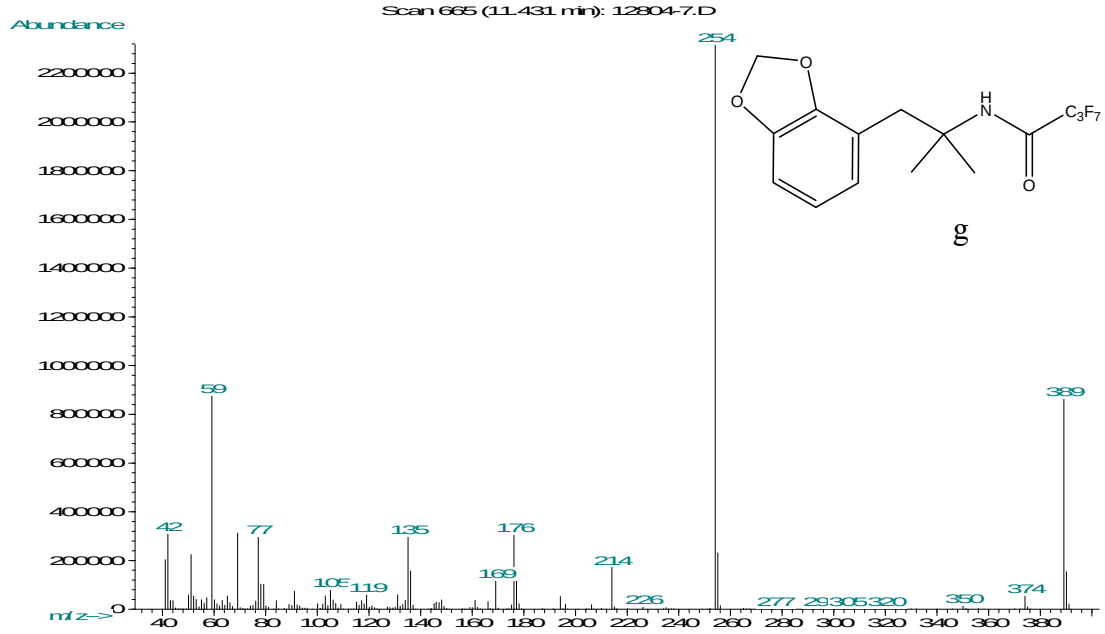

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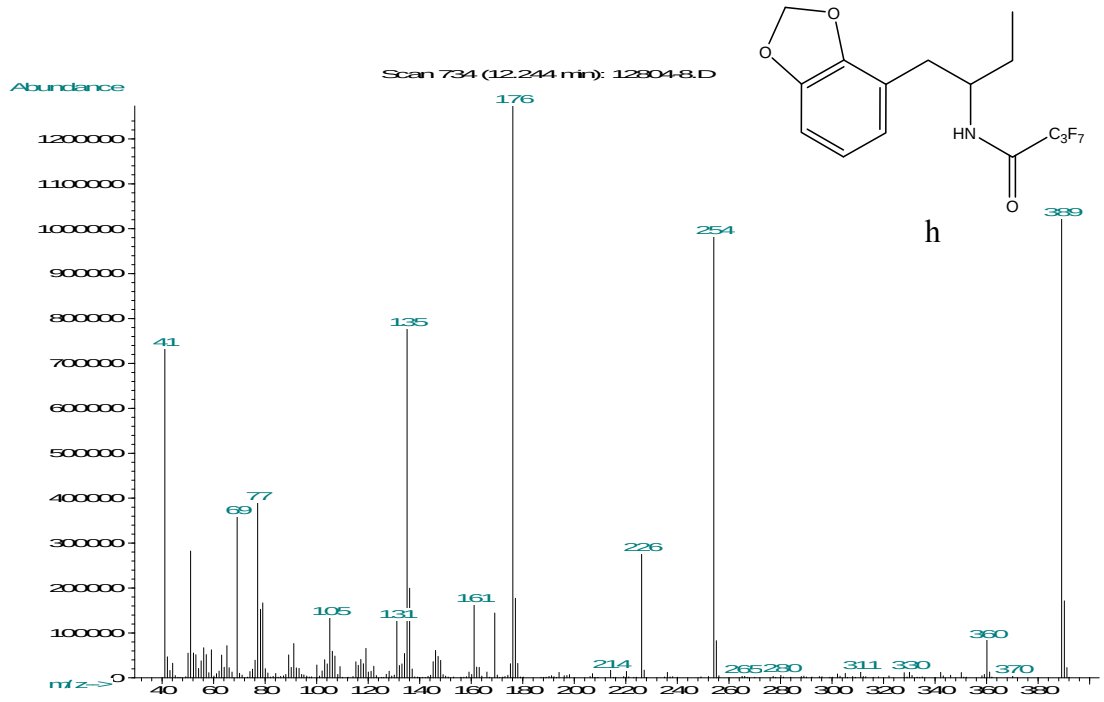
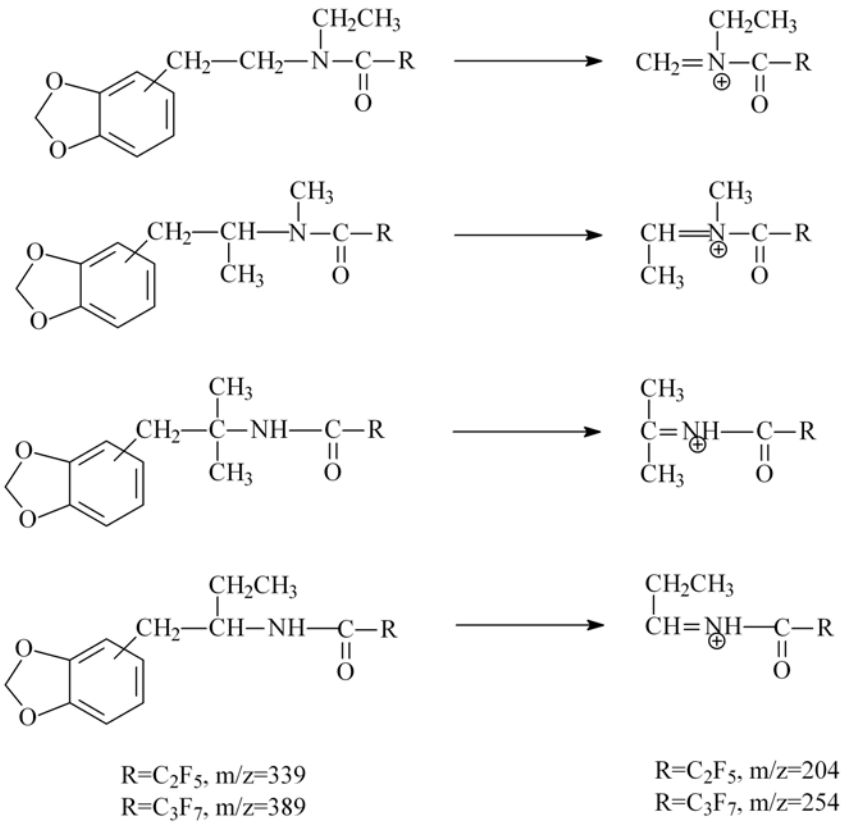


These ions at m/z 204 and 254 is the PFPA and HFBA imine species likely formed from the alpha cleavage of the amide nitrogen to eliminate the 2, 3- and 3, 4-methylenedioxybenzyl radical, thus the m/z 204 and 254 ions in PFPA and HFBA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M\text{-}135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Scheme 1.2. The methylenedioxybenzyl cation at m/z 135 is a fragment common to all the spectra in Figures 1.2 and 1.3. The decreased role for alpha cleavage reaction in the fragmentation of these amides allows the formation of ions more diagnostic of each individual side chain isomer. Acylation, and in particular the perflouroacylation, weakens the bond between nitrogen and the alpha-carbon of the substituted methylenedioxyphenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying

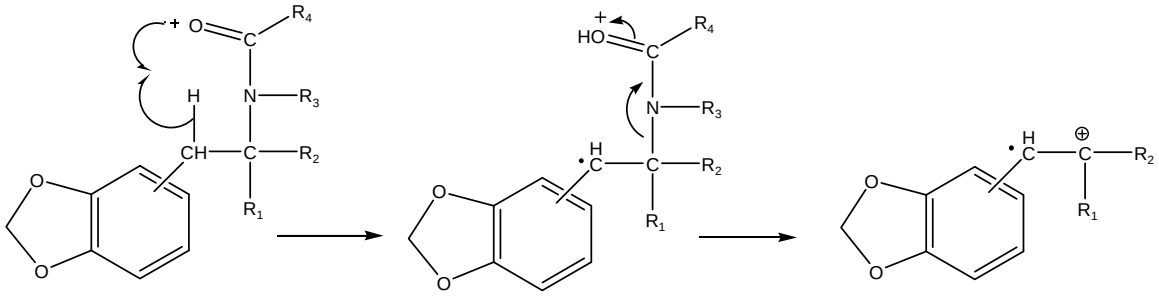
mass significantly individualize the mass spectra and provide specific structure information.



Scheme 1.2: Formation of the (M-135)$^+$ ions in the perfluoroacyl-derivatives of the regioisomeric amines.

The mass spectra in Figures 1.2 and 1.3 illustrate the role of hydrocarbon fragments at m/z 148, 162 and 176 in the electron impact mass spectral differentiation among these side chain regioisomers.

The spectra for the N-ethyl derivatives in Figures 1.2a, 1.2e and 1.3a, 1.3e) show a base peak at m/z 148 corresponding to the alkane radical cation which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (Scheme 1.3).

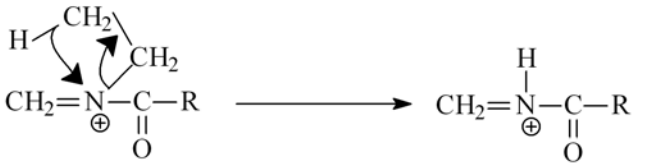


R_1=R_2=H, m/z=148
R_1=H, R_2=CH_3, m/z = 162
R_1=R_2=CH_3, m/z = 176
R_1=H, R_2=C_2H_5, m/z = 176

Scheme 1.3: Mechanism for the formation of the alkane radical cation in the perfluoroacyl-derivatives of the regioisomeric amines.

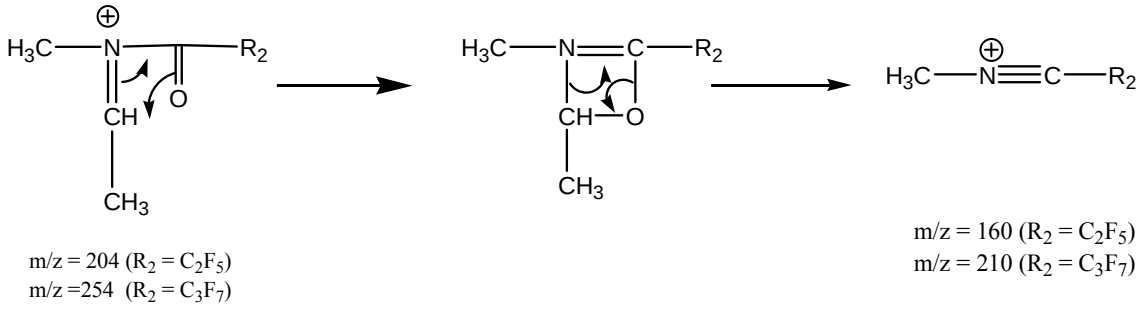
This ion at m/z 148 would only occur for the N-ethyl regioisomer. The spectra in Figures 1.2b, 1.2f and 1.3b, 1.3f show the 2,3- and 3,4-methylenedioxyphenylpropane hydrocarbon ion at m/z 162, identifying this molecules as the PFPA and HFBA derivatives of 2,3- and 3,4-methyelenedioxymethamphetamines, respectively. The proposed mechanism for the formation of the hydrocarbon fragment is illustrated in Scheme 2.3. The spectra for the PFPA and HFBA derivatives of the primary amines 4, 5, 9 and 10 show ions at m/z 176 from the corresponding 2,3- or 3,4-methylenedioxyphenyl alkyl radical cation. This m/z 176 results from hydrogen rearrangement and subsequent fragmentation of alkyl carbon to nitrogen bond. The lower abundance of m/z 176 for the 2, 3- and 3, 4-methylenedioxyphentramines (compounds 4 and 9) may be attributed to steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

While the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the N-ethylphenethylamines (Figures 1.2a and 1.2e) is the appearance of an ion at m/z 176 in addition to the base peak at m/z 148. The 176 ion suggests a four carbon chain directly attached to the aromatic ring as occurs for the alpha-ethyl- and alpha, alpha-dimethyl-phenethylamines (Figures 1.2 c, d, g, h and 1.3 c, d, g, h). The m/z 176 ion in the spectra for the PFPA derivatives of the N-ethyl regioisomers (Figures 1.2a and 1.2e) is a rearrangement of the m/z 204 ion resulting in the loss of mass 28 (the N-ethyl group) via hydrogen transfer (Scheme 1.4). This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the N-ethyl-phenethylamines shown in Figure 1.3a and 1.3e. The loss of 28 mass units from the acylimine fragment at m/z 254 yields the equivalent fragment ion at m/z 226. Thus, the HFBA derivatives may offer more unique characteristic ions for individualization of these regioisomeric substances.



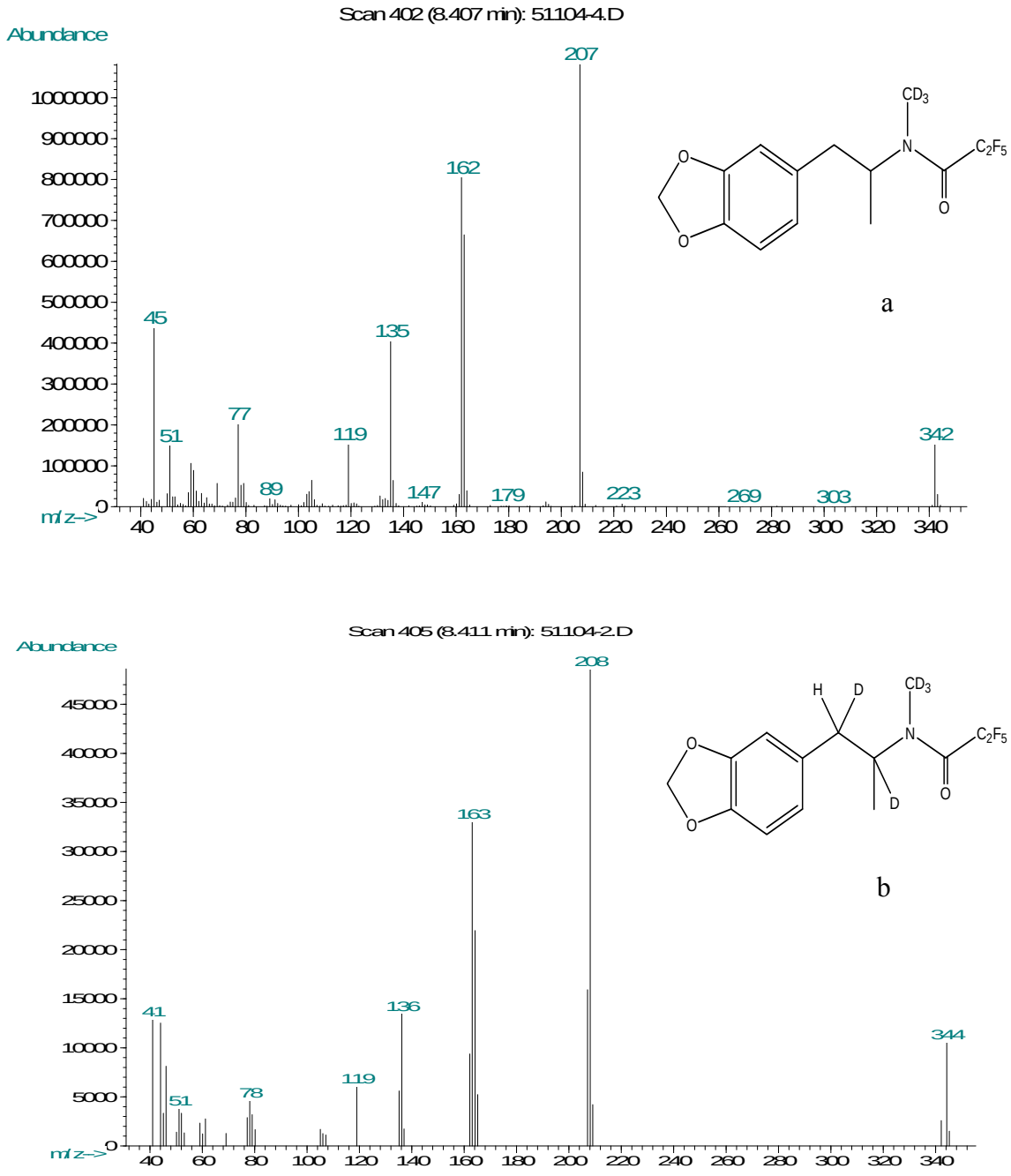
Scheme 1.4: Mechanism for the loss of mass 28 from the perfluoroacyl-N-ethyl-imine cation.

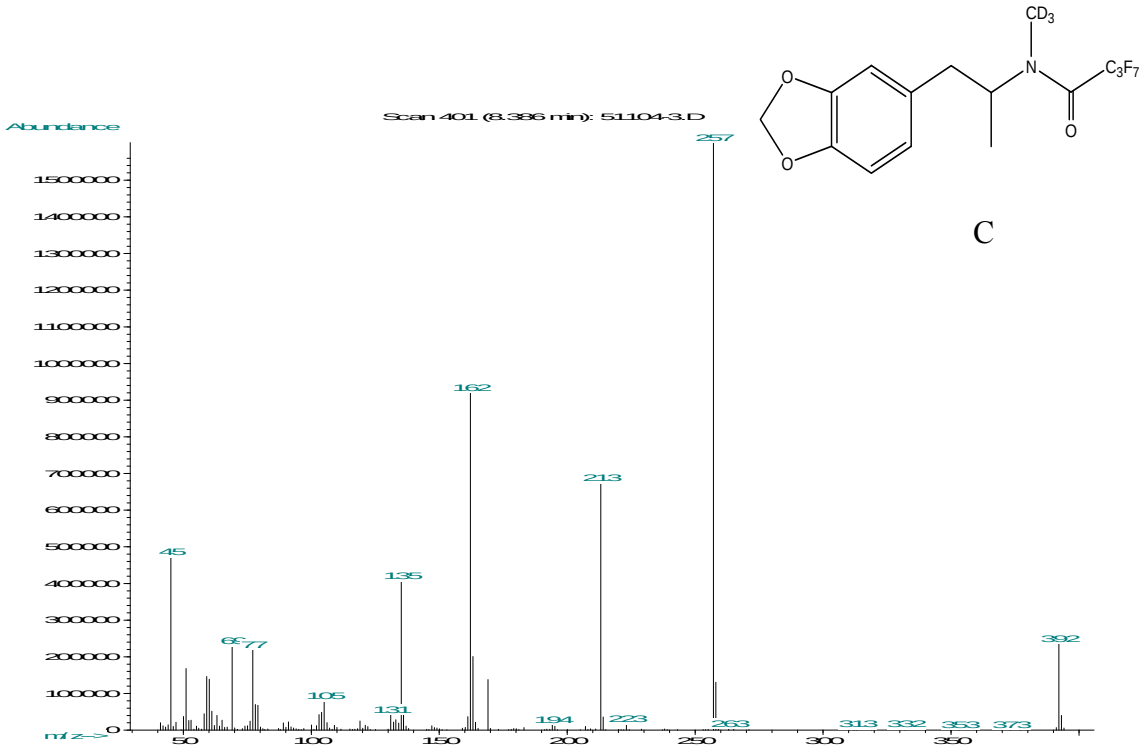
A comparison of the PFPA derivatives for 3,4- and 2,3-methylenedioxymethamphetamine (Figures 2.2b and 2.2f) with the HFBA derivatives (Figures 1.3b and 1.3f) indicated unique ions at m/z 160 and m/z 210. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7, respectively the suggested mechanism of forming these masses can be illustrated in Scheme 1.5. Additional information about these ions can be obtained by a comparison of the mass spectra for the PFPA and HFBA derivatives of 3,4-MDMA and NCD_3-3,4-MDMA (MDMA-d_3) in Figure 1.4. The corresponding ions in these spectra occur at m/z 163 and 213 and the equivalent ions for the derivatives of d_5-MDMA also occur at m/z 163 and 213. Thus, an analysis of the masses of the components which make up the fragment at m/z 160 for example include C_2F_5 (119 mass units) and CH_3 (15 mass units) leaving only a mass of 26 available for the total of 160. The mass 26 would correspond to CN and the proposed mechanism for the formation of $(C_2F_5CNCH_3)^+$ is shown in Scheme 1.5. An equivalent fragmentation pathway has been reported [C.R. Clark et al, 1995] for methamphetamine.

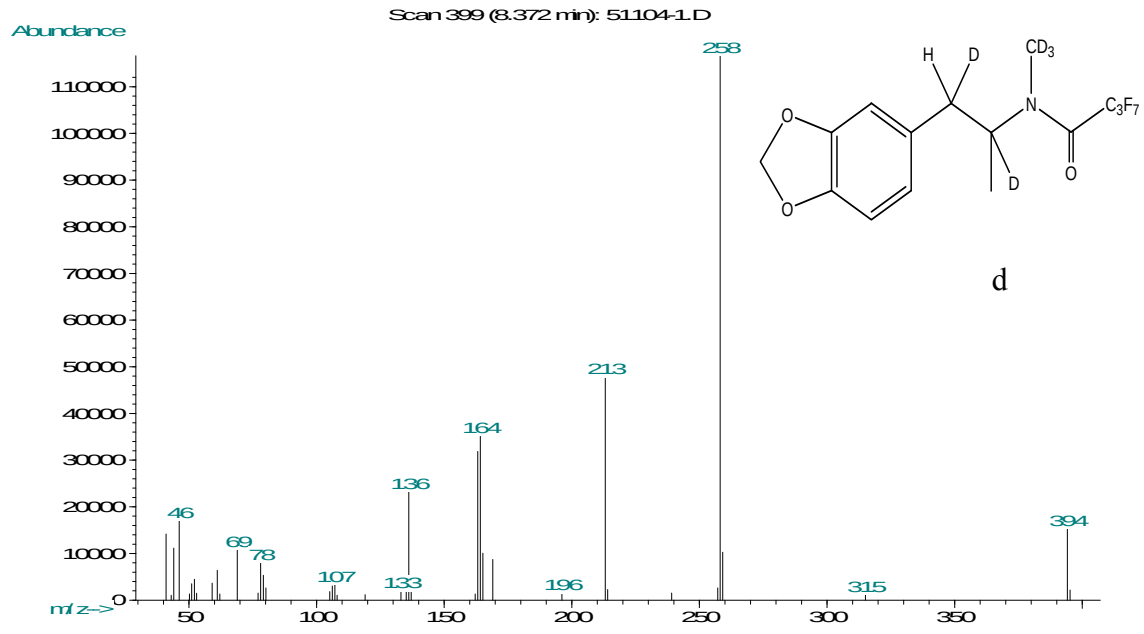


Scheme 1.5: Formation of m/z 160 and 210 from the PHPA and HFBA derivatives of 2,3- and 3,4-MDMA

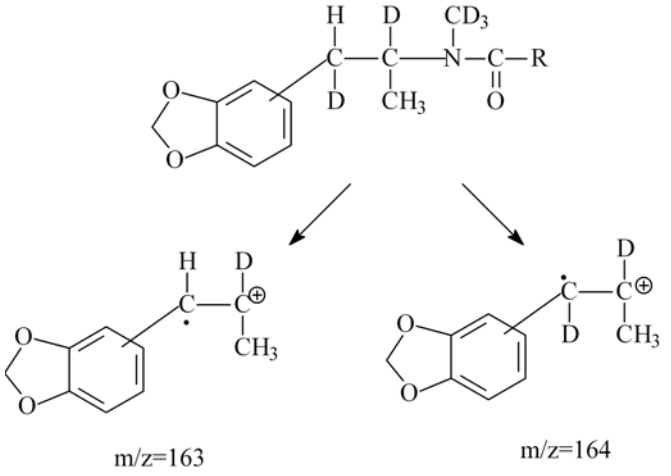
Figure 1.4: Mass spectra of the PFPA and HFBA derivatives for the d_3 and d_5-MDMA.







The spectra for the derivatives of d_3- and d_5-MDMA in Figure 2.4 also lend support to the proposed mechanism for the formation of the alkene fragment at m/z 162 illustrated in Scheme 1.3. The exact structure for the d_5-MDMA is shown in Scheme 1.6, the benzylic position contains one hydrogen and one deuterium and the transfer fragmentation can occur to remove either species to form the alkene radical cation. Thus, the resulting alkene can yield ions at m/z 163 or 164 depending on the probability of transfer.



Scheme 1.6: Formation of m/z 163 and 164 in the perfluoroacyl-derivatives of d_5-MDMA.

Gas chromatographic separation of perfluroacyl derivatives of 3,4-MDMA and their direct regioisomers

The PFPA and HFBA derivatives of the eight primary and secondary amines were compared on two stationary phases using capillary columns of the same dimensions, 30m x 0.25mm and 0.25um depth of film using instrument 1. Previous studies on the

chromatographic properties of the underivatized compounds [Aalberg et al., 2000 and 2004] have shown that other compounds in this series co-eluted with MDMA using some common gas chromatographic stationary phases and conditions.

The stationary phases compared were the relatively nonpolar phases, 100% dimethyl polysiloxane (Rtx-1) and 95% dimethyl-5% diphenyl polysiloxane (Rtx-5). Several temperature programs were evaluated and the best compromises between resolution and analysis time were used to generate the data in Table 8 and the chromatograms in Figures 1.5 and 1.6

The two chromatograms for the PFPA derivatives in Figure 19 were generated using two temperature programs. The first program used was to hold the column temperature at 100 ^{o}C for 1 minute, ramped to 180 ^{o}C at 9 ^{o}C/minute, hold at 180 ^{o}C for 2 minutes ramp to 200 ^{o}C at 10 ^{o}C/minute [TP-1]. This program was used to separate compounds 2-5 and 7-10 in their HFBA and PFPA forms on Rtx-5. The same program was also used to separate the PFPA derivatives of the same compounds on Rtx-1. The second temperature program used to resolve the HFBA derivatives of compounds 2-5 and 7-10 on Rtx-1. The program was set up to hold the column temperature at 70 ^{o}C for 1 minute, ramped to 150 ^{o}C at 7.5 ^{o}C/minute, hold at 150 ^{o}C for 2 minutes ramp to 250 ^{o}C at 10 ^{o} /minute[TP-2].

The resulting elution order and resolution are quite similar. In fact the elution order is the same for all the chromatograms in Figures 1.5 and 1.6. The chromatograms show that the 2,3-isomer elutes before the corresponding 3,4-isomer for all the side chain regioisomers. For example, 2,3-MDMA elutes before 3,4-MDMA, and this pattern holds for all side chain regioisomers. When the ring substitution pattern is held constant (ie 2,3-

or 3,4-) and the side chain elution order is evaluated the two secondary amides elute before the two tertiary amides. Additionally, in every case in this limited set of compounds the branched side chain elutes before the straight chain isomer when the ring substitution pattern and the degree of amide substitution are constant. Therefore, the 2,3-phentermine-PFPA elutes first followed by 2,3-BDB-PFPA (both secondary amides), then 2,3- MDMA-PFPA, and N-ethyl 2,3-methylenedioxyphenethylamine-PFPA the two tertiary amides. Perhaps the most useful information in these chromatograms is the relative elution of the derivatized controlled substance MDMA and its closest eluting regioisomeric equivalents. Both the PFPA and HFBA derivatives of MDMA elute between the N-ethyl-2,3- and 3,4-methylenedioxyphenethylamine PFPAs and HFBAs, both the N-ethyl regioisomers show very distinct mass spectra with several characteristic ions to differentiate these compounds from the drug of abuse MDMA. Thus, derivatization methods coupled with both chromatographic and mass spectral procedures can allow for the complete characterization of the side chain substitution pattern for these ten uniquely isomeric substances, however, the HFBA derivatives offer more unique fragment ions for additional discrimination among these regioisomeric substances.

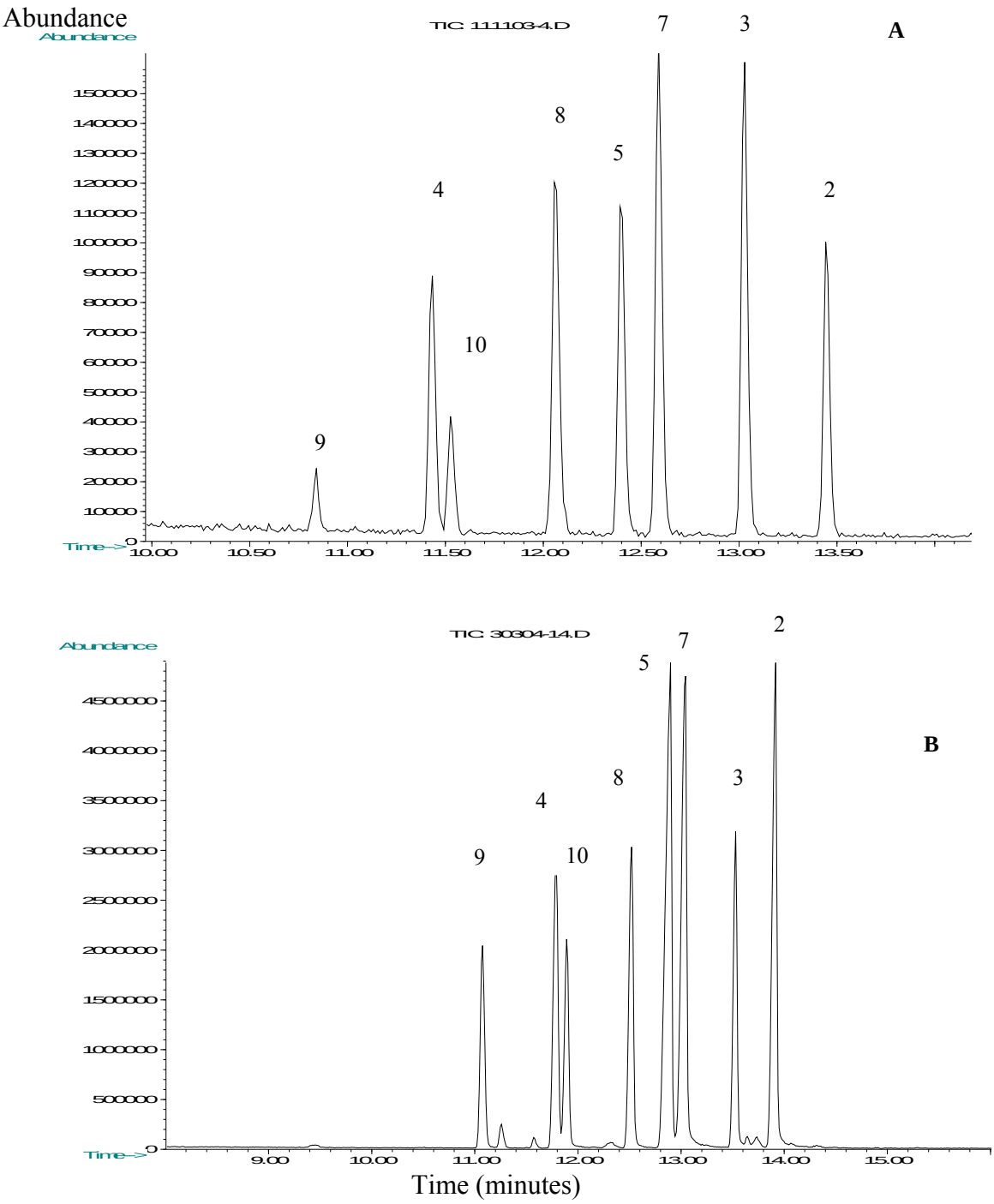


Figure 1.5: Capillary gas chromatographic separation of the PFPA derivatives of compounds (2-5) and (7-10). Columns used: A Rtx-1; B Rtx-5.

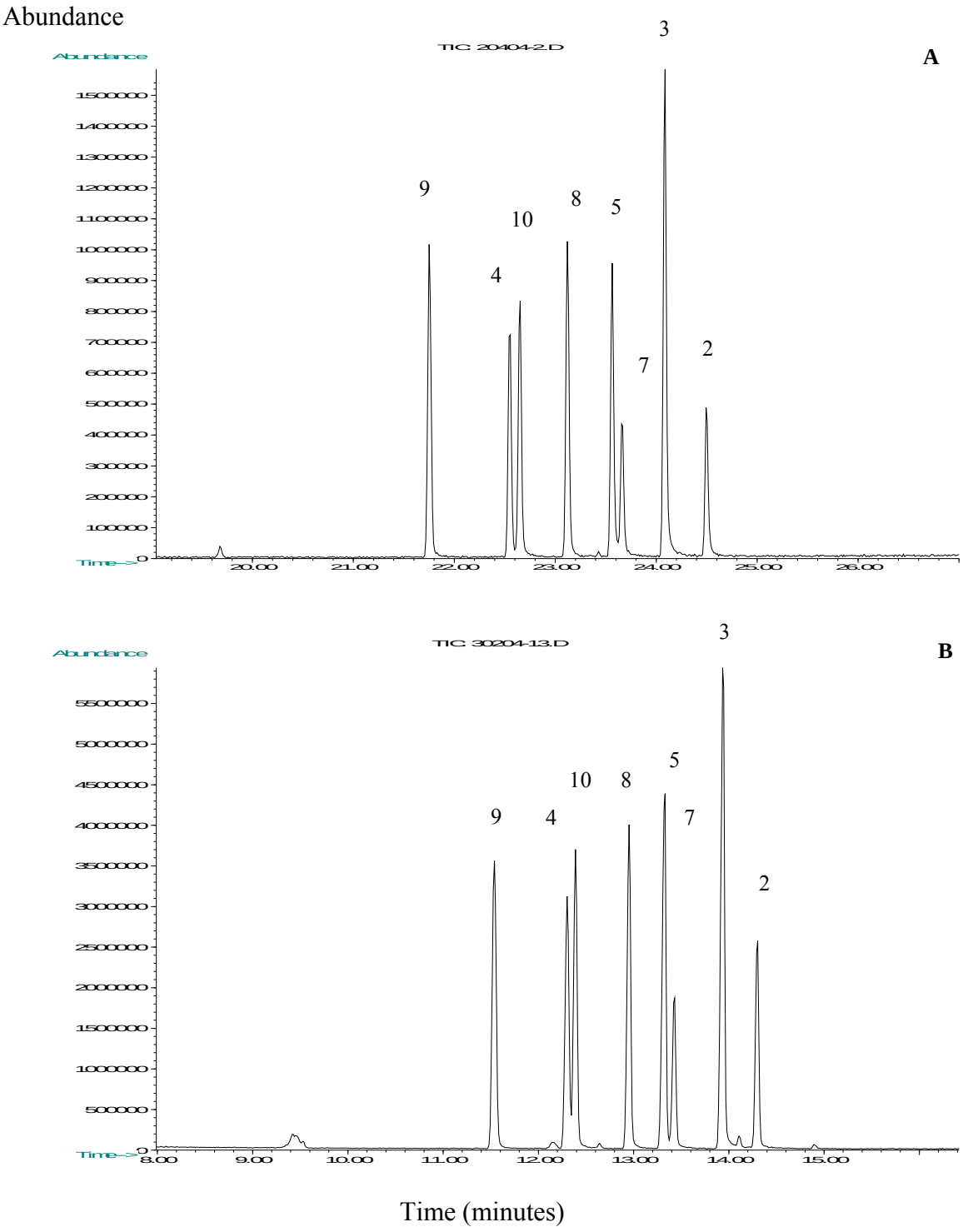


Figure 1.6: Capillary gas chromatographic Separation of HFBA derivatives of compounds (2-5) and (7-10). Columns used: A Rtx-1; B Rtx-5

Conclusions of Chapter 1

3,4-MDMA and nine other methylenedioxyphenethylamines are a unique subset of regioisomeric molecules; each compound has a molecular weight of 193 and yields a base peak at *m/ z* 58 in the MS from the loss of the corresponding methylenedioxybenzyl group. Thus, the traditional EI MS provides little stru ctural information for diff e rentiating among these 10 compounds. Because of the unique similarity of these compounds by MS, the specific identification of a compound such as 3,4-MDMA requires methods to eliminate any of the other nine isomers.

This elimination process may be accomplished on the basis of chromatography alone but would ultimately require the analyst to use reference samples of each of the 10 amines. The reference samples would be necessary to determine if any of the isomeric methylenedioxyphenethylamines coeluted with 3,4-MDMA. Derivatization of the eight primary and secondary amines with various acylating agents yields amides with similar resolution to the underivatized amines by capillary GC on RTX-1 and RTX- 5 stationary phases. However the perfluoroacyl derivatives significantly individualize the mass spectra for these amides and allow for specific identification. The individualization is the result of fragmentation of the alkyl carbon–nitrogen bond yielding hydrocarbon fragments at *m/ z* 148, 162, and 176, as well as other unique fragments from these regioisomeric amides. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes. However, the HFBA derivatives offer more unique fragment ions for additional discrimination among these regioisomeric substances.

Chapter 2

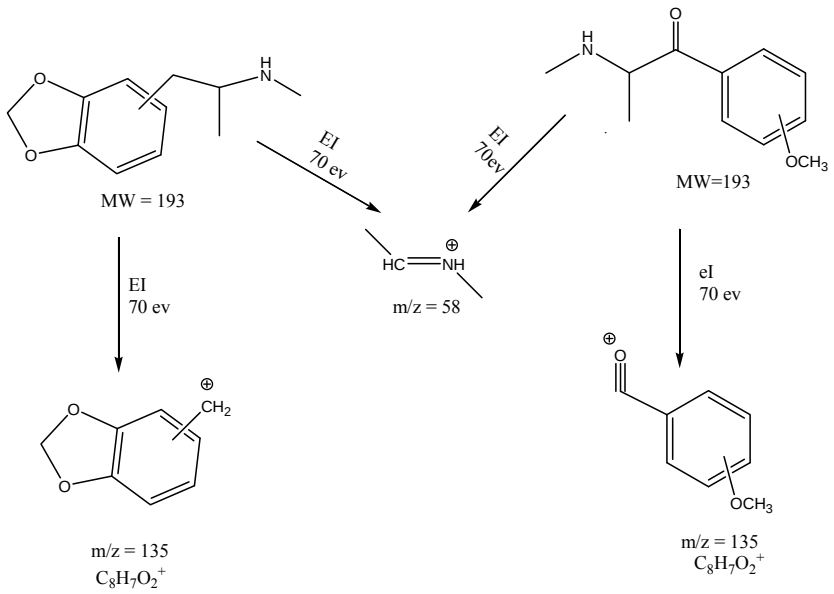
Mass Spectrometry and Gas Chromatographic Studies of Indirect Regioisomers Related to 3,4-MDMA, the Methoxymethcathinones.

The methoxymethcathinones constitutes a set of indirect regioisomers of the controlled drug substance 3,4-methylenedioxymethamphetamine. The various isomeric forms of the methoxymethcathinones have mass spectra essentially equivalent to 3, 4-MDMA, all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. For these individual regioisomers the m/z 135/136 ion is the methoxybenzoyl carbocation $(C_8H_7O_2)^+$ not the methylenedioxybenzyl carbocation $(C_8H_7O_2)^+$. The specific identification and differentiation between these compounds and the drug of abuse 3,4-MDMA must be based on a combination of mass spectral data as well as chromatographic resolution of these regioisomeric substances. The mass spectra of the underivatized methoxymethcathinones as well as their perfluroacetyl derivatives will be compared with 3,4- and 2,3- MDMA. The chromatographic separation of the underivatized and derivatized methoxymethcathinones from 2,3- and 3,4-methylenedioxymethamphetamines will be discussed.

Mass spectral studies of the underivatized and the perfluoroacyl derivatives of the methoxymethcathinones

Methoxymethcathinones represent a potentially significant challenge for analytical drug chemistry. All the regioisomeric methoxymethcathinones have the same molecular weight (193) and the same side chain as the drug of abuse 3,4-MDMA.

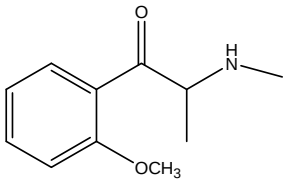
The mass spectra for the three methoxymethcathinone regioisomers (Figure 2.1) show a base peak at m/z 58 as seen for 2,3- and 3,4-MDMA (Figure 2.1). The major fragmentation pattern for the methoxymethcathinones is shown in Scheme 2.1. The methoxybenzoyl $(C_8H_7O_2)^+$ fragment has the same mass and empirical formula as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. Furthermore the m/z 58 ion in the methoxymethcathinones is the same imine structure as that obtained in the mass spectra of both 3,4- and 2,3-MDMA.



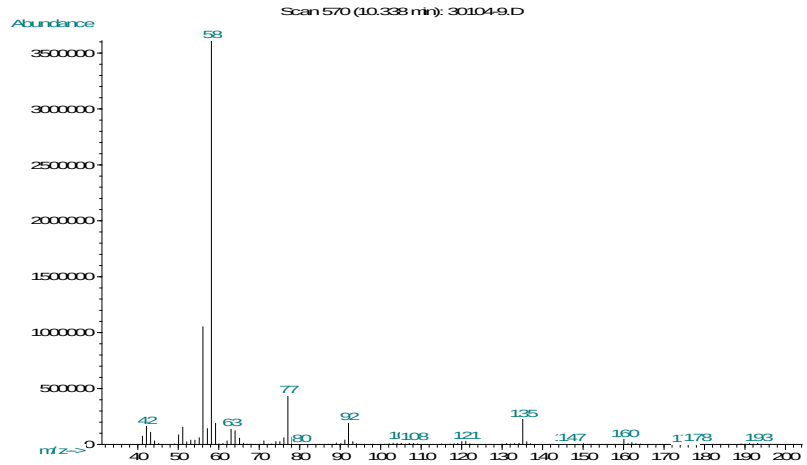
Scheme 2.1: EI fragmentation pattern of the underivatized 2,3- ,3,4-MDMAs and the methoxymethcathinones

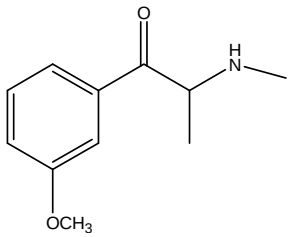
Figure 2.1: Mass spectra of the underivatized methoxymethcathinones.

Structure of regioisomers **Mass spectrum**

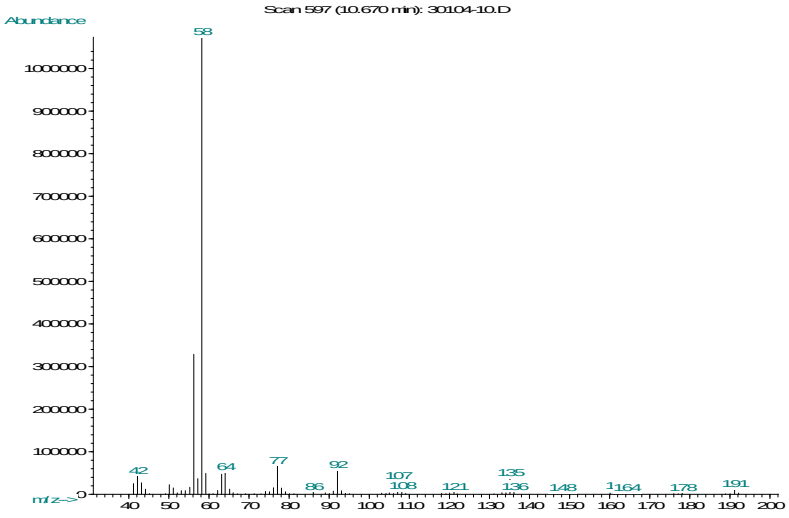


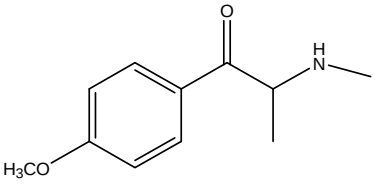
1



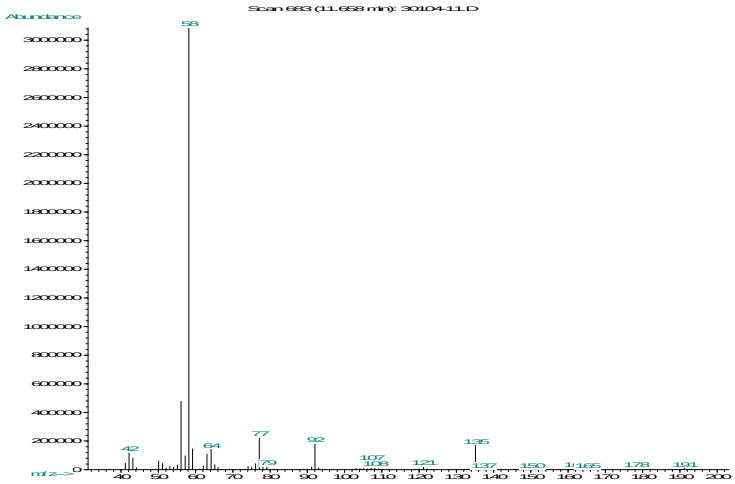


2



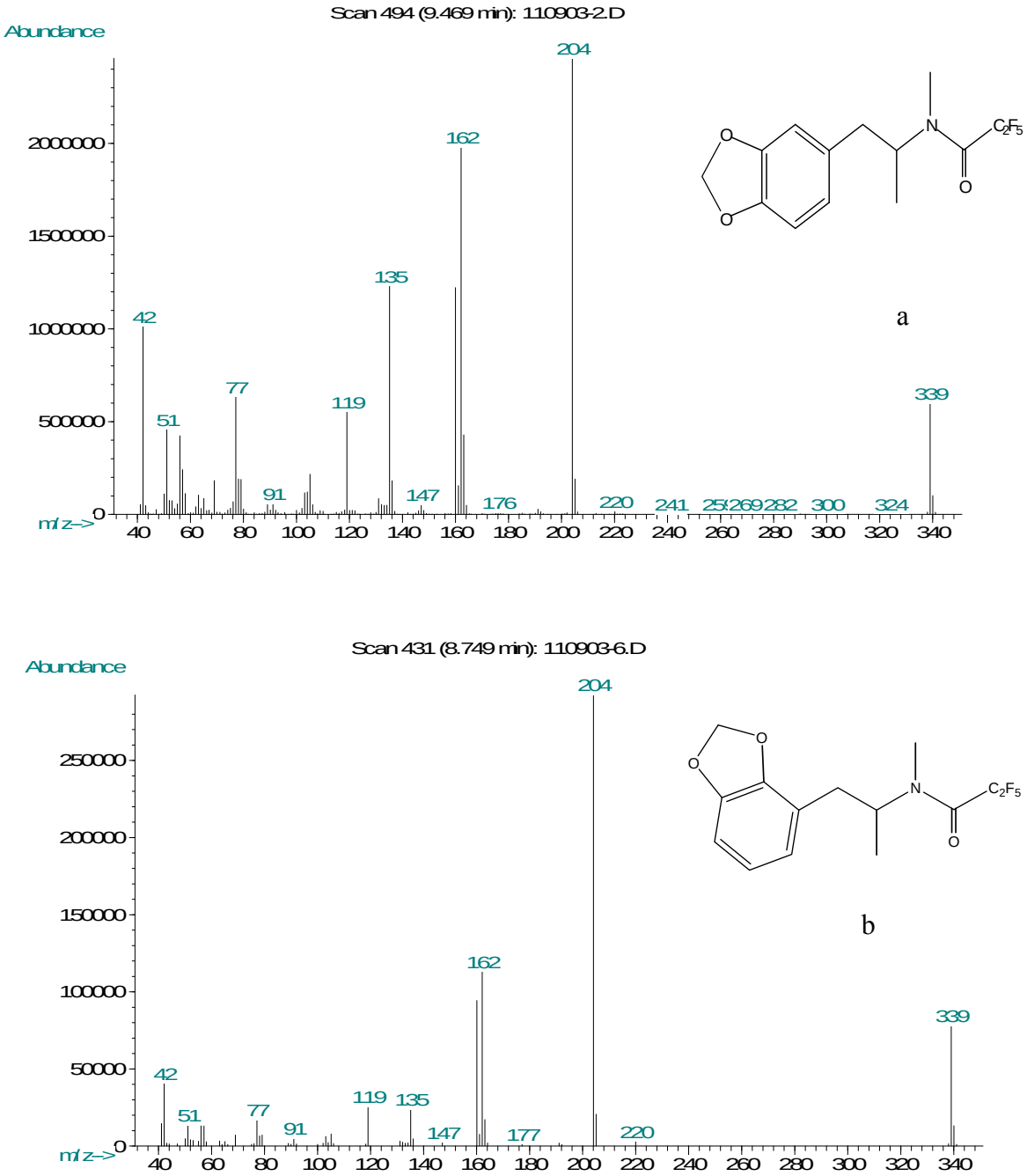


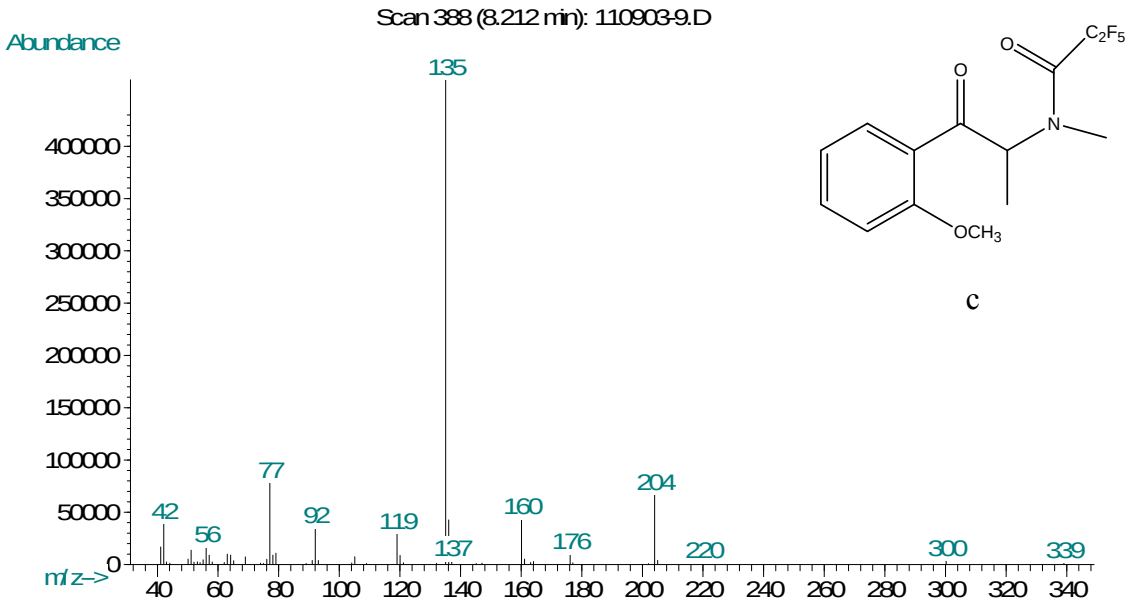
3



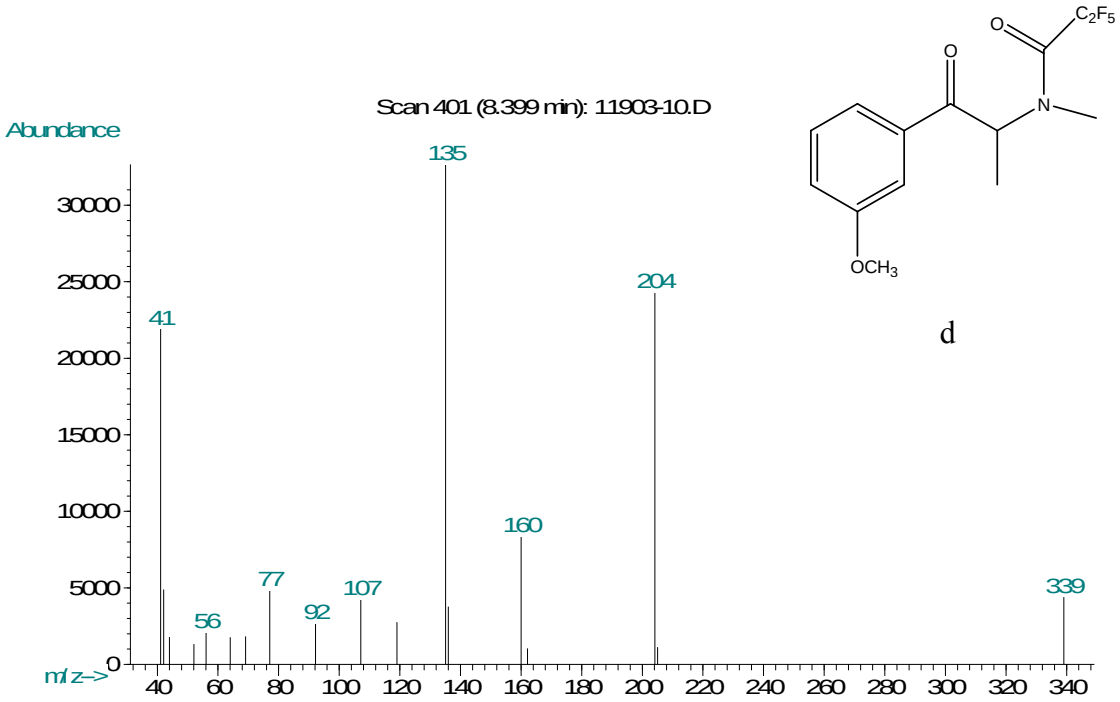
Since the mass spectra of the methoxymethcathinones is almost identical to the drug of abuse 3,4-MDMA, perfluoroacylated derivatives of 3,4- and 2,3-methylenedioxymethamphetamines and their regioisomeric secondary amines, ortho, meta and para-methoxymethcathinones, were prepared and evaluated in an effort to individualize their mass spectra and to improve chromatographic resolution. Acylation of the amines significantly lowered the basicity of nitrogen and allowed other fragmentation pathways to play a more prominent role in the mass spectrum. The mass spectra for the five pentaflouropropionyl and heptflourobutryl amides are shown in Figures 2.2 and 2.3, respectively.

Figure 2.2: Mass spectra of the PFPA derivatives of 3,4-MDMA (a); 2,3-MDMA (b) ;ortho (c); meta (d) and para (e)-methoxymethcathinones.





c



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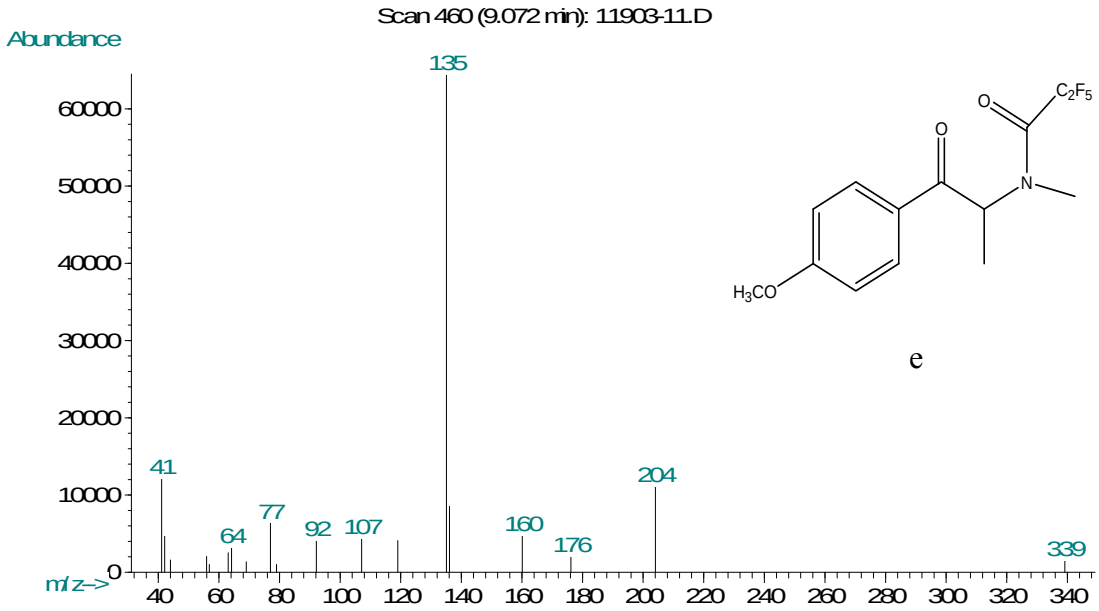
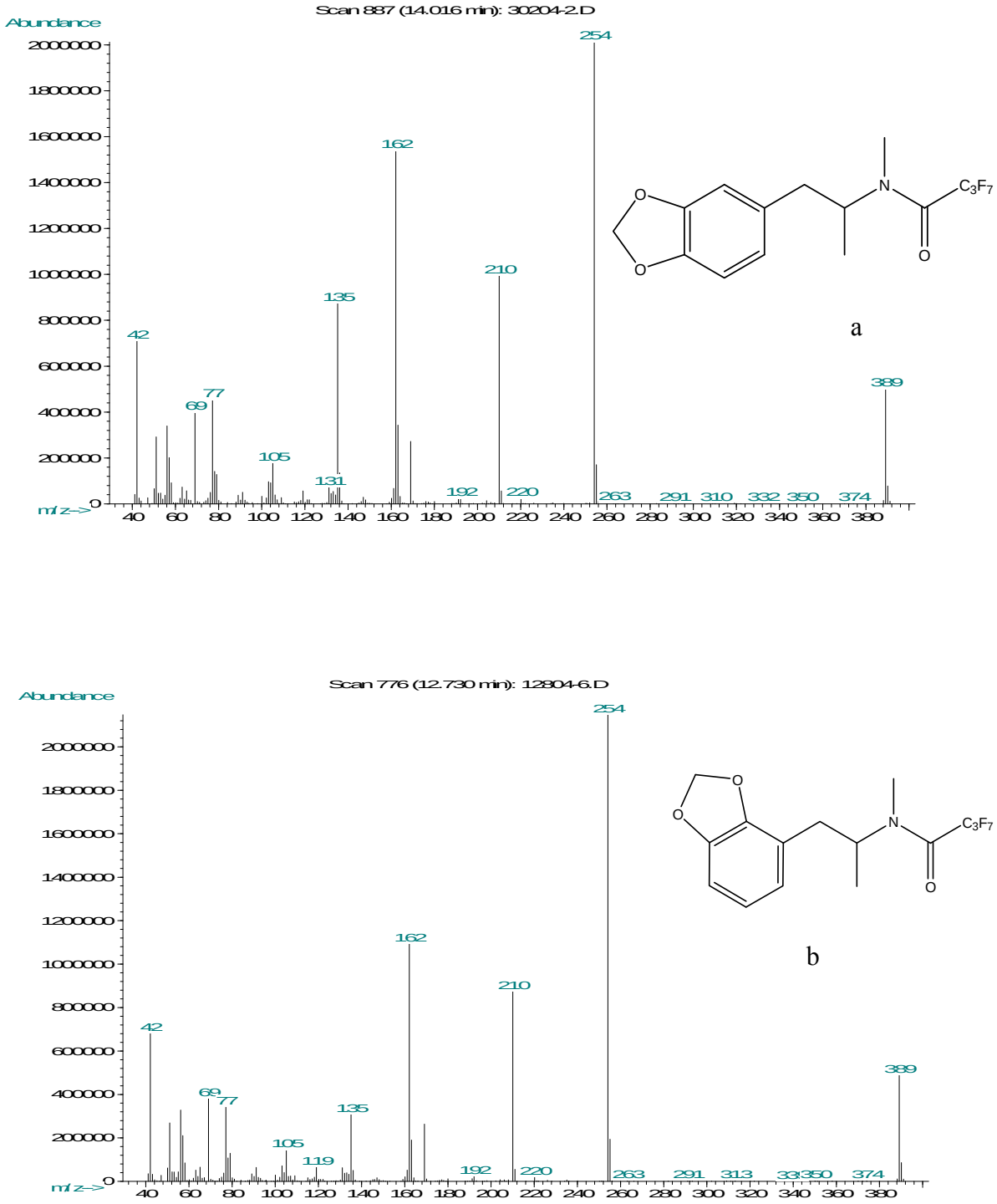
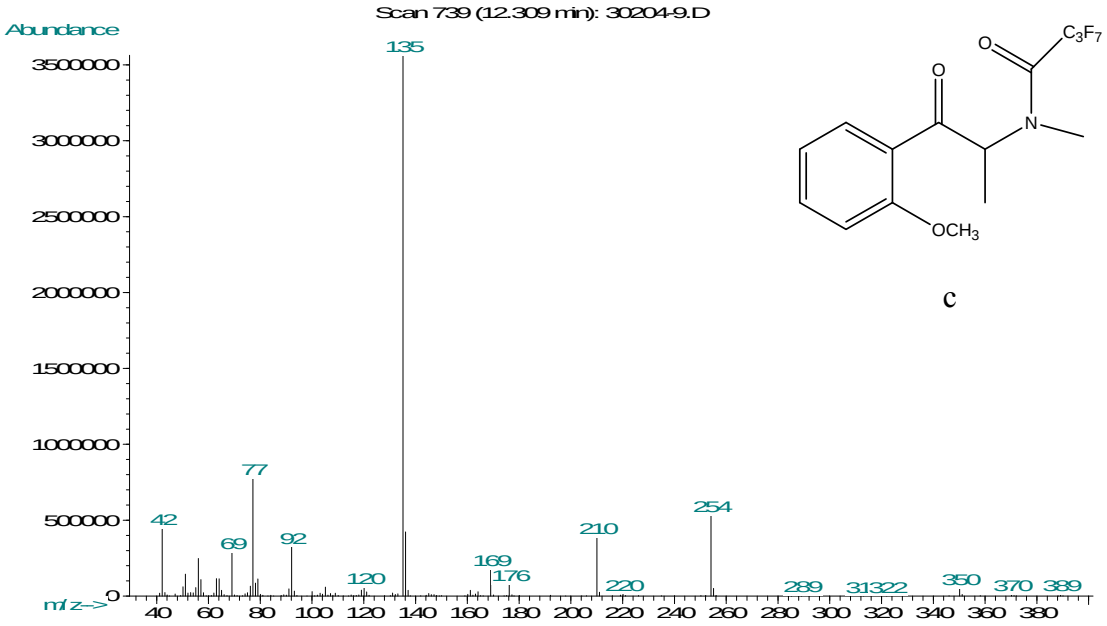


e

Figure 2.3: Mass Spectra for the HFBA derivatives of 3,4-MDMA (a); 2,3-MDMA (b); ortho (c); meta (d) and para (e)-methoxymethcathinones.





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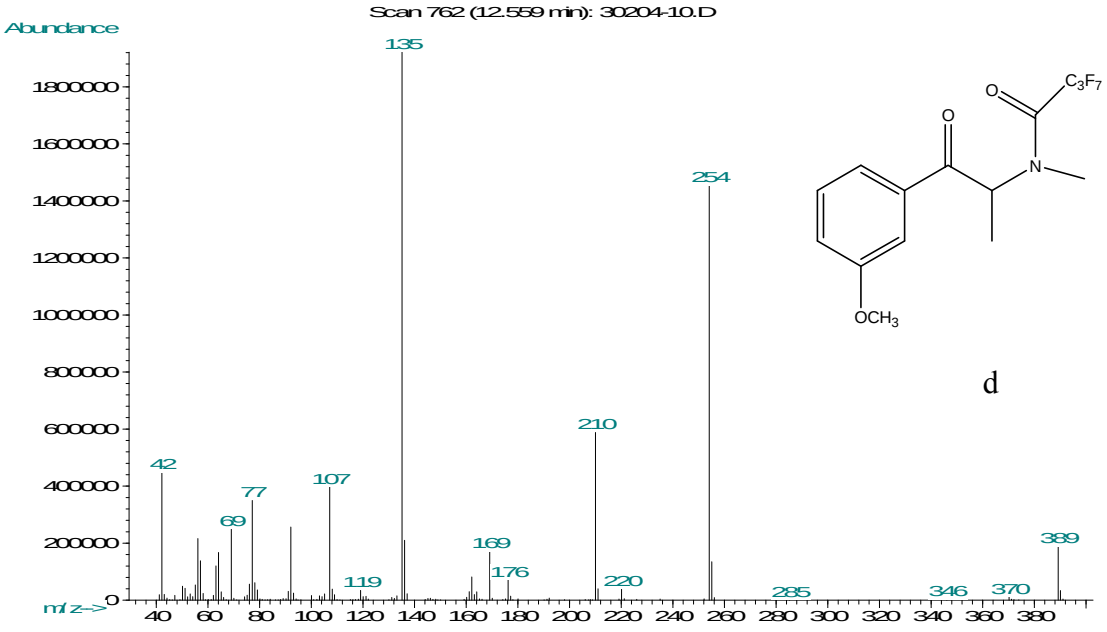

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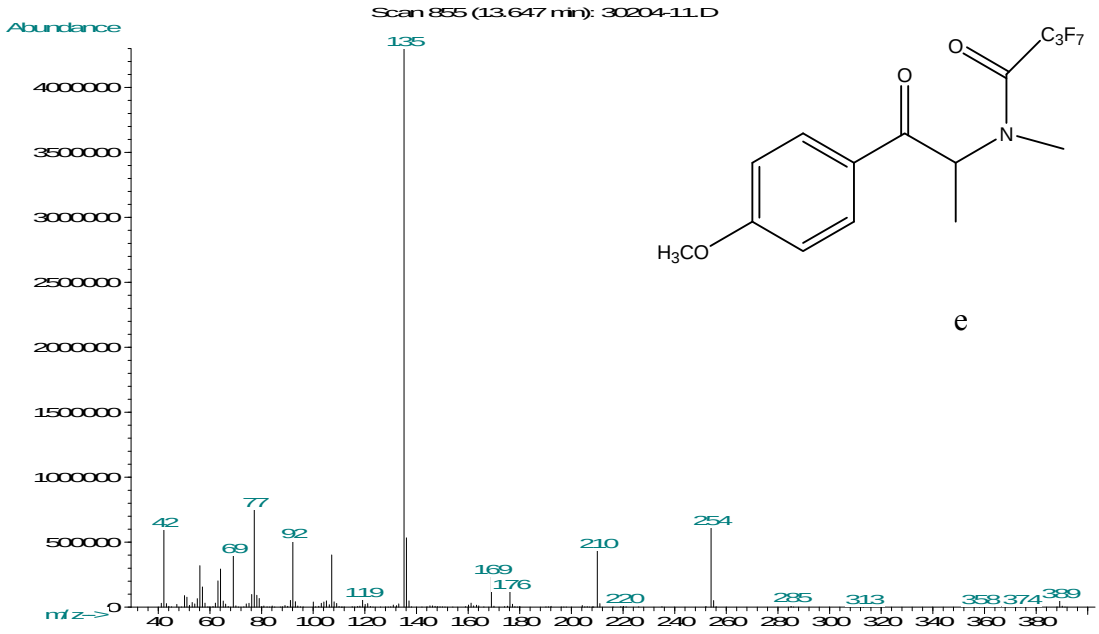
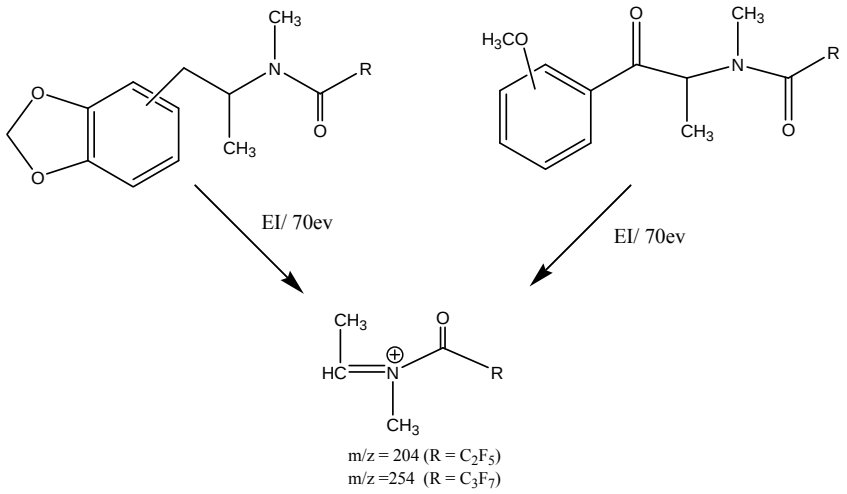


e

From these spectra a common peak occurs at m/z 204 and 254 which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for PFPA and HFPA amides. This ion at m/z 204 and 254 is the PFPA and HFPA imine species likely formed from the alpha cleavage of the amide nitrogen to eliminate the 2, 3- and 3, 4-methylenedioxybenzyl and methoxybenzoyl radicals. Thus the m/z 204 and 254 in PFPA and HFPA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M-135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Scheme 2.2

The relative abundances for the m/z 204 and 254 ions are always higher in the 3,4- and 2,3-methylenedioxymethamphetamines than in the methoxymethcathinones The methylenedioxybenzyl and the methoxybenzoyl cations at m/z 135 are fragments common to all the spectra (Figures 3.2 and 3.3). The relative abundance of m/z 135 in perfluoroacyl derivatives of methoxymethcathinones is higher than that observed for 3,4- and 2,3-methylenedioxymethamphetamines. The m/z 135 ion is the base peak in the mass spectra for the derivatives of the methoxymethcathinones likely due to the additional carbonyl site for initial radical cation formation in these compounds.



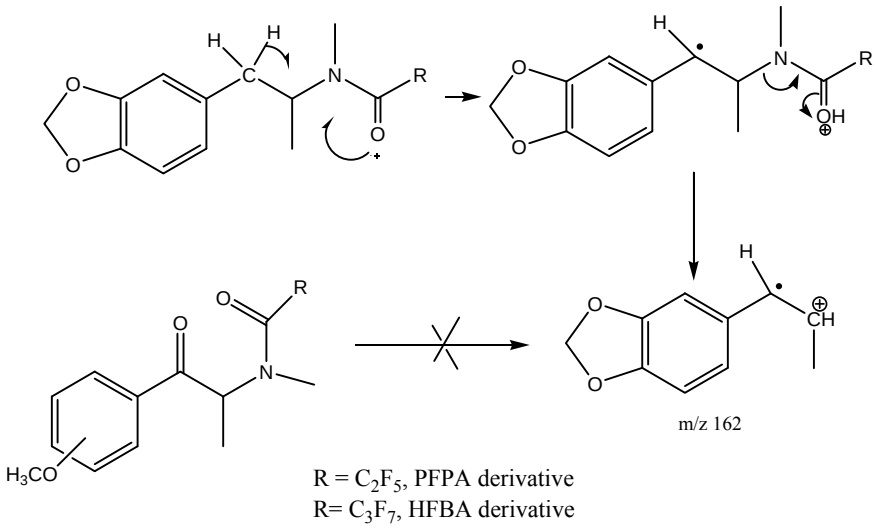
Scheme 2.2: Formation of m/z 204 and m/z 254 for the PFPA and HFBA derivatives of 2,3-, 3,4-MDMAs and methoxymethcathinones

The decreased role for alpha cleavage reaction in the fragmentation of these amides as a result of perflouroacylation which weakens the bond between nitrogen and the alpha-carbon of the substituted methylenedioxyphenethyl group, allowing the

formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass significantly individualize the mass spectra and provide specific structural information. The mass spectra in Figures 2.2 and 2.3 illustrate the role of the hydrocarbon fragment at m/z 162 in the electron impact mass spectral differentiation among these regioisomeric compounds. The spectra in Figures 2.2a, 2.2b, 2.3a and 2.3b show the 2,3- and 3,4-methyelenedioxyphenylpropene radical cation at m/z 162, identifying these molecules as the PFPA and HFBA derivatives of 2,3- and 3,4-methyelenedioxy methamphetamines, respectively. The formation of the m/z 162 ion has been described chapter 1 and requires the transfer of hydrogen from the benzylic carbon. This fragmentation mechanism does not take place in the PFPA and HFBA derivatives of the methoxymethcathinones due to the absence of benzylic hydrogen (Scheme 2.3). One can conclude that the presence of alkene ions at m/z 162, can be used to identify the side chain of 3,4- and 2,3-methyelenedioxymethamphetamines and exclude the regioisomeric methoxymethcathinones. Conversely, the base peak at m/z 135 as well as the absence of the m/z 162 ion would identify one of these substances as a methoxymethcathinone regioisomer.

A comparison of the PFPA derivatives between the 3,4- and 2,3-methylenedioxymethamphetamine (Figures 2.2a and 2.2b) with their HFBA derivatives (Figures 3.3a and 3.3b) indicates unique ions at m/z 160 and m/z 210. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7, respectively. These unique ions have been fully characterized in chapter 1 using deuterated analogs of 3,4-MDMA and methamphetamine. These ions at m/z 160 and 210 are the result of a rearrangement decomposition of ions 204 and 254

respectively (Scheme 1.2). The m/z 204 and 254 ions have the same structure whether generated from derivatives of the MDMAs or the methoxymethcathinones. Therefore these unique ions do not provide any information to differentiate between the two groups of substances.



Scheme 2.3: Benzylic hydrogen transefer to form m/z 162 occur only in MDMA perfluroaceyl derivatives but not for methoxymethcathinones.

Gas chromatographic separation of 2,3-MDMA and 3,4 MDMA from the methoxymethcathinones

Gas chromatographic properties of the PFPA and HFBA derivatives of the 2,3- and 3,4-methylenedioxymethamphetamines and 2-, 3-, and 4-methoxymethcathinones were compared on two stationary phases using capillary columns of the same dimensions, 30m x 0.25mm and 0.25um depth of film. The stationary phases compared in this study

were the relatively nonpolar phases, 100% dimethyl polysiloxane (Rtx-1) and 95% dimethyl-5% diphenyl polysiloxane (Rtx-5). The underivatized compounds were not completely resolved with 2-methoxymethcathinone co-eluting with 3-methoxymethcathinone using these common gas chromatographic stationary phases and some common temperature programming conditions (Figure 2.4).

The PFPA and HFBA derivatives showed improved resolution when compared to the underivatized amines. Several temperature programs were evaluated and the best compromise between resolution and analysis time were used to generate the chromatograms in Figures 2.5 and 2.6.

The two chromatograms for the PFPA derivatives (Figure 2.5) were generated using two different temperature programs; the resulting elution order and resolution are quite similar. In fact the elution order is the same for all the chromatograms shown in Figures 2.5 and 2.6. In each case the derivatized 2-methoxymethcathinone (compound 1) eluted first followed closely by the 3-methoxymethcathinone derivative (compound 2). The third compound to elute is the 2,3-MDMA derivative (compound 4) and the derivatized 4-methoxymethcathinone (compound 3) is the forth peak in each chromatogram. The derivatized form of 3,4-MDMA (compound 5) showed the greatest retention in each chromatogram.

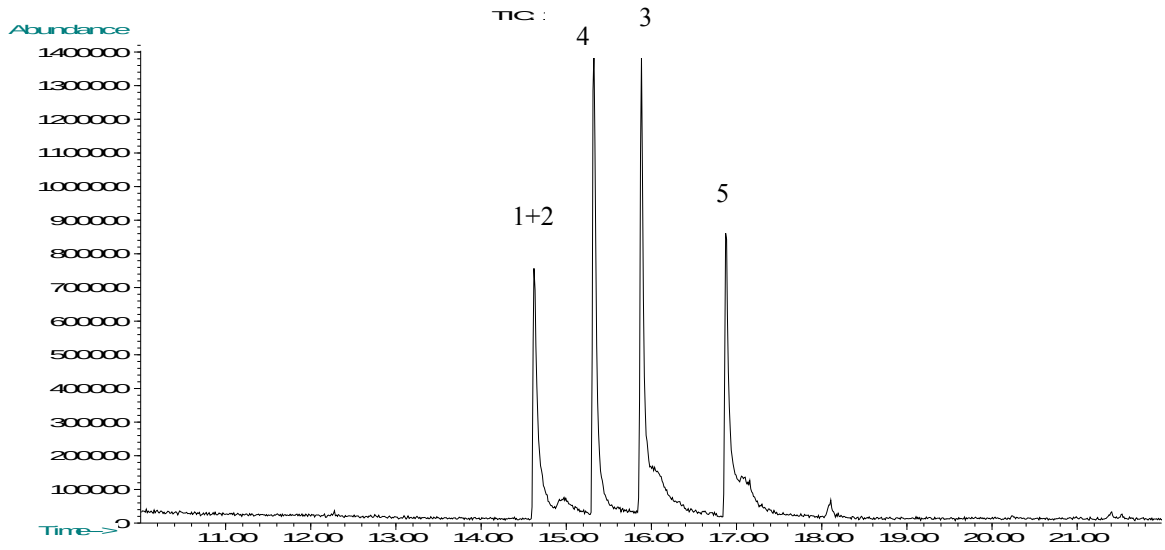


Figure 2.4: Capillary gas chromatogram for a physical mixture of underivatized 3,4 MDMA, 2,3-MDMA and methoxymethcathinones.

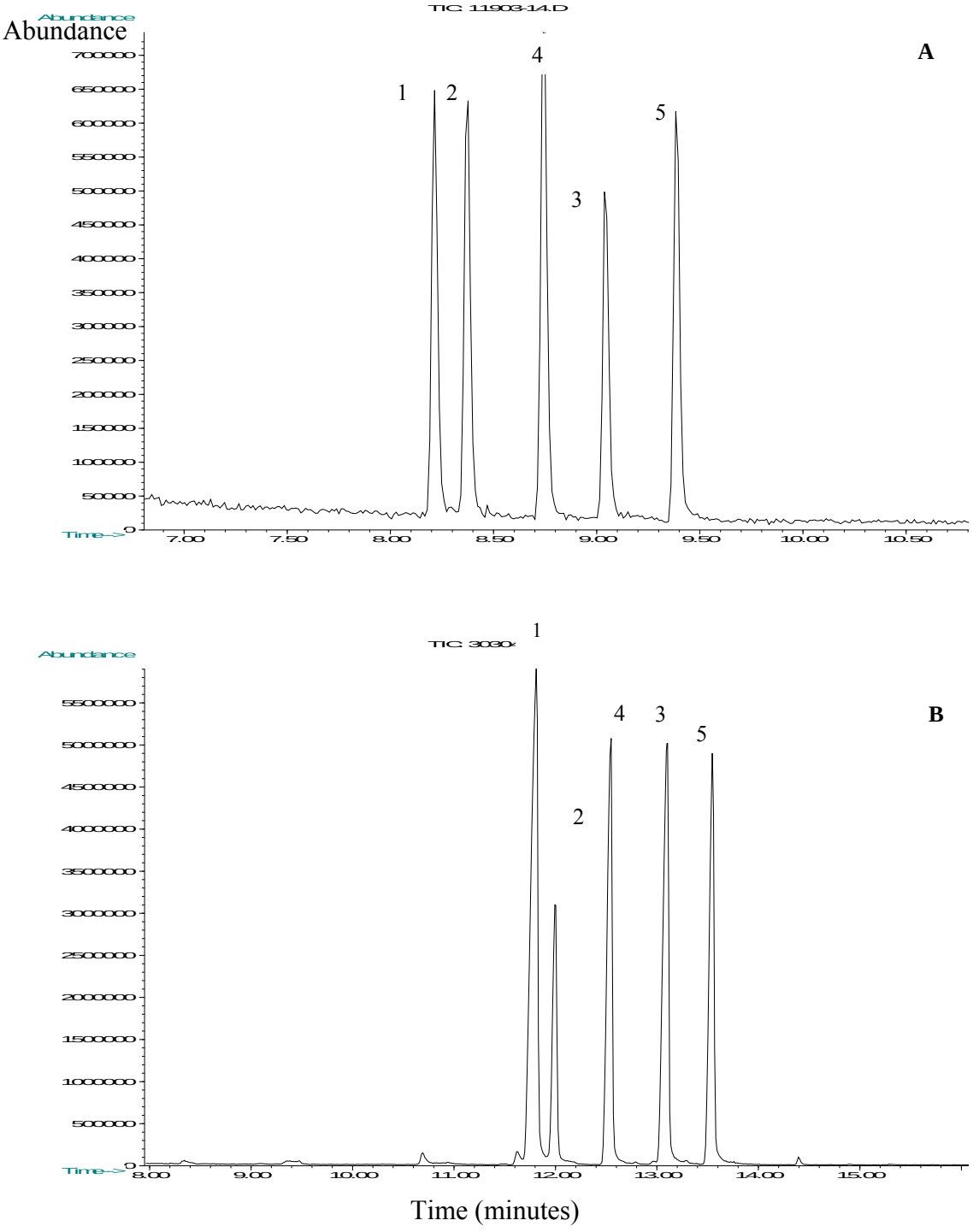


Figure 2.5: Capillary gas chromatographic separation of PFPA derivatives of compounds 1-5. Columns used: A Rtx-1; B Rtx-5.

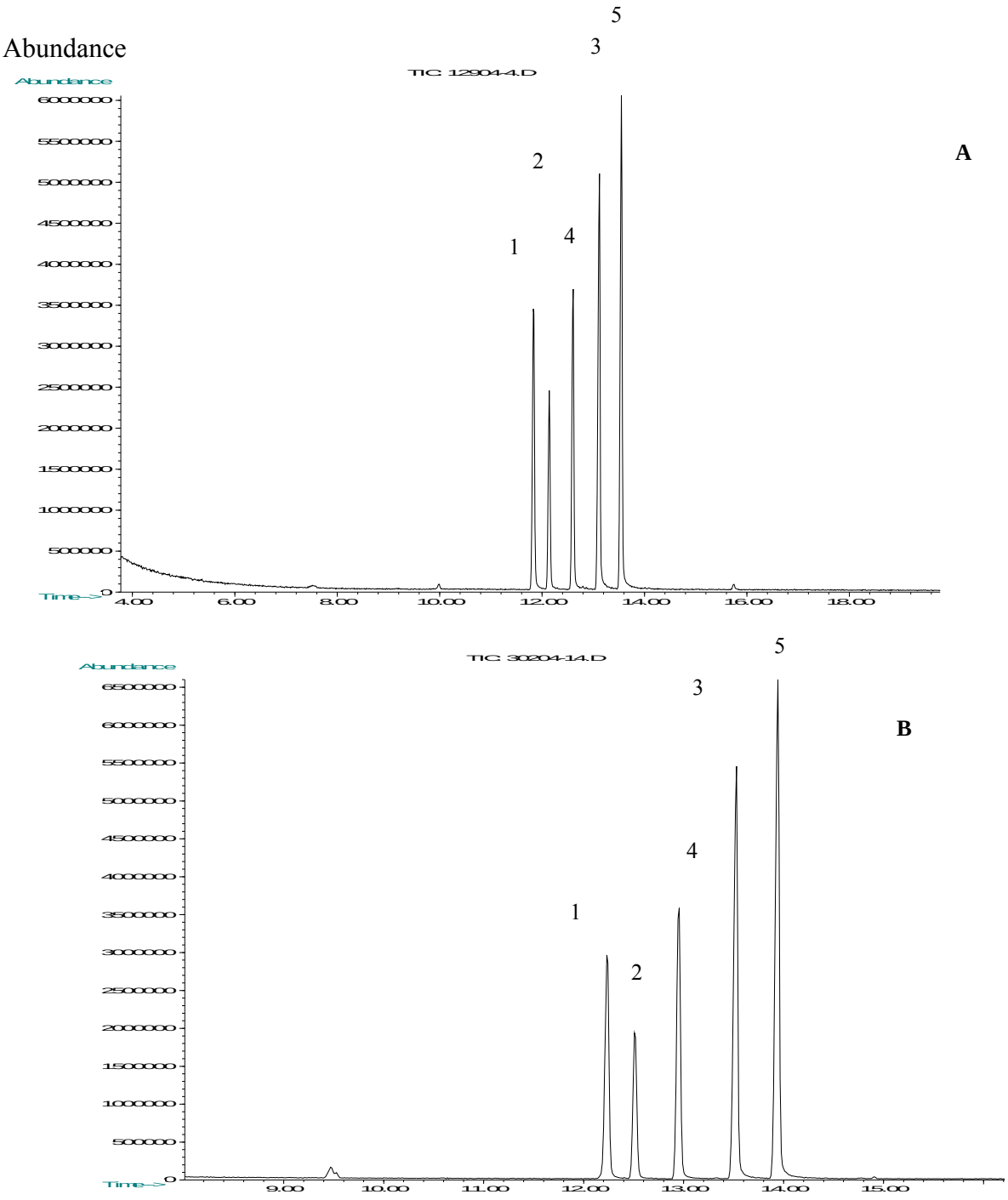


Figure 2.6: Capillary gas chromatographic separation of HFBA derivatives of compounds 1-5. Columns used; A: Rtx-1; B: Rtx-5.

Conclusion of Chapter 2

3,4- and 2,3-MDMA and 2-, 3-, and 4-methoxymethcathinone are a unique subset of regioisomeric molecules; each compound has a Mw of 193 and yields a base peak for the N-methyl imine of acetaldehyde at m/z 58 in the mass spectrum from the loss of the corresponding $C_8H_7O_2$ methylenedioxybenzyl and methoxybenzoyl groups, respectively. Thus, the traditional EI-MS provides little structural information for differentiating among these five compounds. Because of the unique similarity of these compounds by MS, the specific identification of a compound such as 3,4-MDMA requires methods to eliminate the other four isomers. This elimination process may be accomplished on the basis of chromatography alone but would ultimately require the analyst to use reference samples of each of the five amines. Derivatization of these amines with various acylating agents yields amides with improved resolution compared with the underivatized amines by capillary GC on Rtx-1 and Rtx-5 stationary phases. These perfluoroacyl derivatives significantly individualize the mass spectra for these amides. The individualization is the result of fragmentation of the alkyl carbon–nitrogen bond, yielding hydrocarbon fragments at m/z 162 as well as other unique fragments from the MDMA amides that were not formed for the methoxymethcathinones. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes; however, the HFBA derivatives offer more unique fragment ions for additional mass spectral discrimination among these regioisomeric substances.

Chapter 3

Mass Spectrometry and Gas Chromatographic Studies on Acylated Derivatives of a Series of Side Chain Regioisomers of 2-Methoxy-4-Methyl Phenethylamines

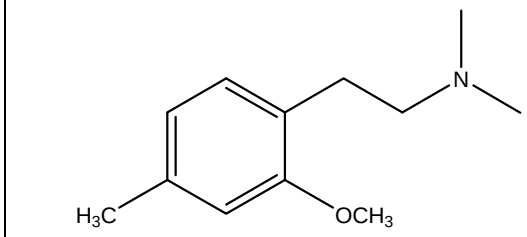
In this chapter GC-MS studies are described for a series of side chain isomers of 2-methoxy-4-methylphenethylamines. These isomers have an isobaric relationship (equal mass, different elemental composition) to the controlled substance 3,4-MDMA.

Mass Spectral Studies on Chain Regioisomers of 2-Methoxy-4-Methyl Phenethylamines

Mass spectrometry is the primary method for confirming the identity of drugs and other substances of abuse in forensic samples. The five side chain regioisomers of 2-methoxy-4-methylphenethylamine (Figure 3.1, Compounds 1-5) have the potential to yield mass spectra essentially equivalent to 3,4-MDMA (and 2,3-MDMA). All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136 (Figure 3.2). The m/z 58 ion in the methoxy methyl phenethylamine is regioisomeric with that obtained in the mass spectra of both 2,3 and 3,4-MDMA. Additionally, the isobaric methoxy methyl benzyl $(C_9H_{11}O)^+$ fragments have the same nominal mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135 (Scheme 3.1). The individual mass spectra for 2,3- and 3,4-MDMA are also

presented in Figure 3.2 (Compounds 6 and 7). The data in Figure 3.2 show that the mass spectra do not provide any major fragment ions to differentiate among these compounds.

Figure 3.1: Structures of the side chain regioisomers of 2-methoxy-4-methylphenethylamines, and 2,3- and 3,4-MDMA.



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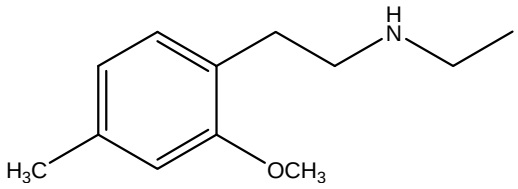

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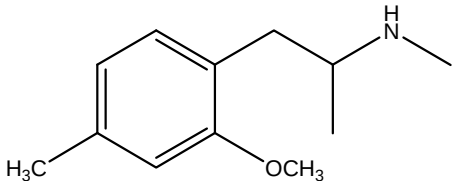

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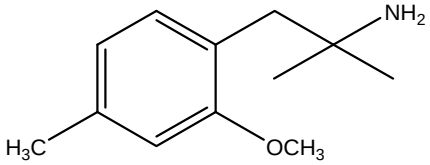

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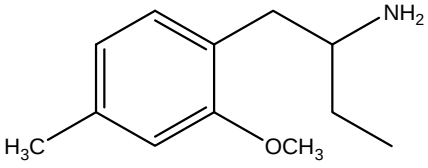

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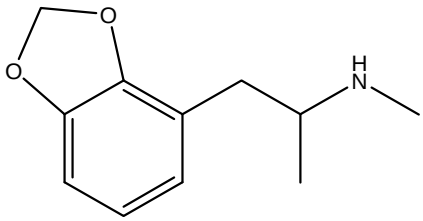

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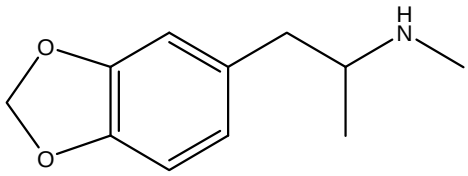
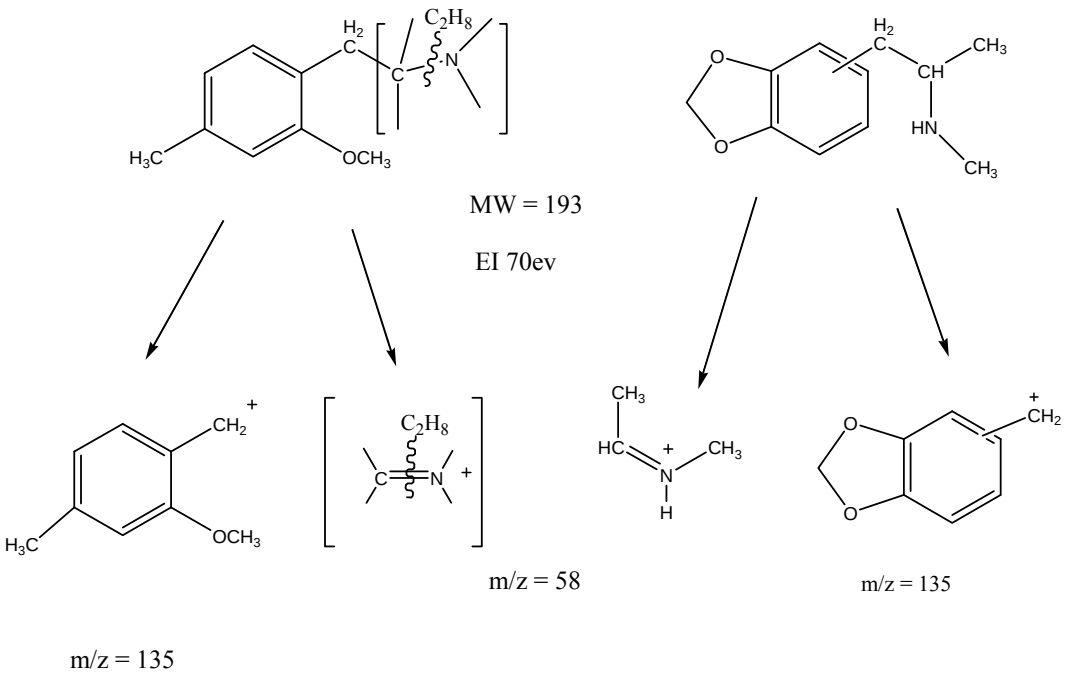
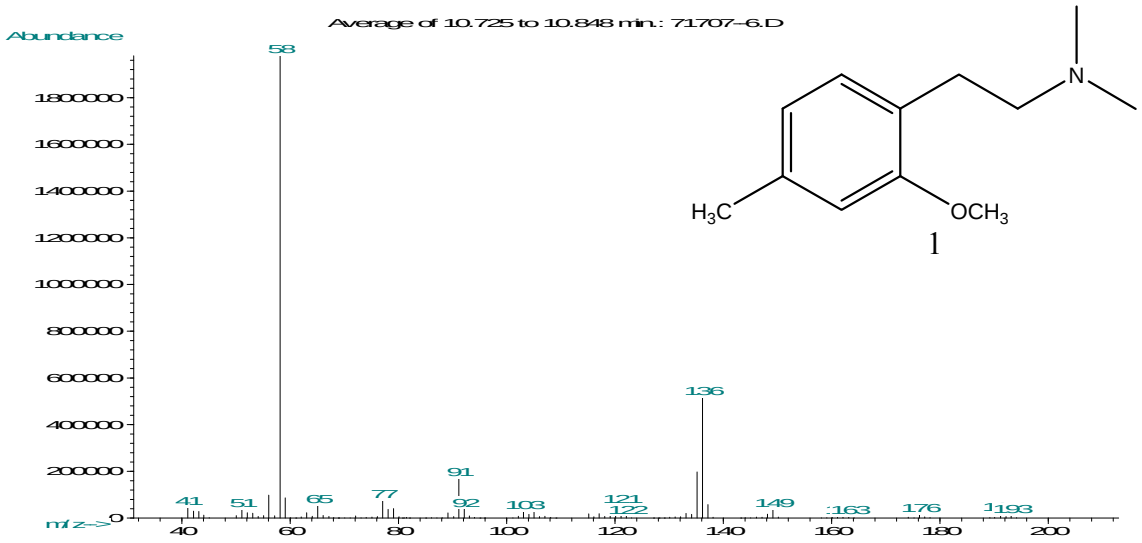


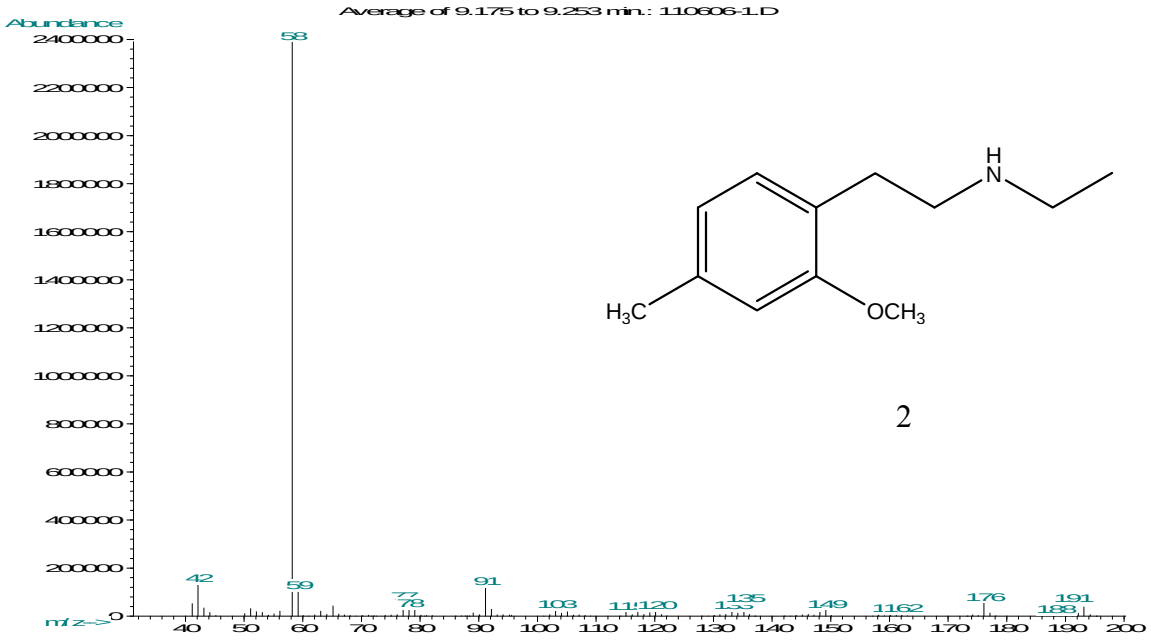
7 (3,4-MDMA)



Scheme 3.1: EI fragmentation pattern for compounds 1-7.

Figure 3.2: Mass Spectra of the underivatized amines 1-7.





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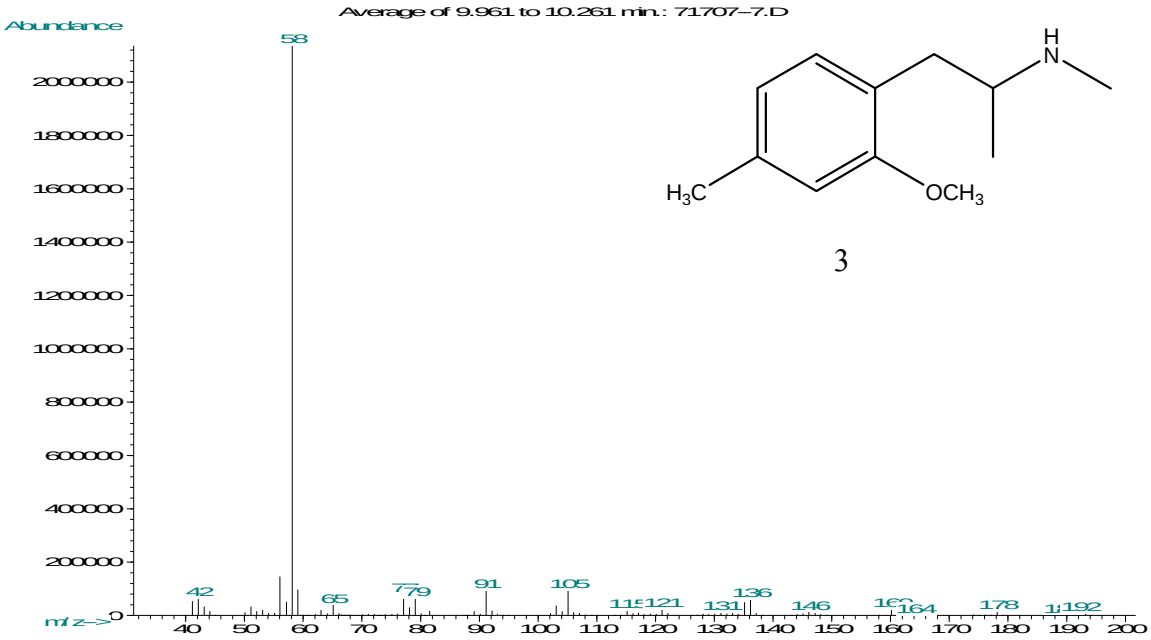

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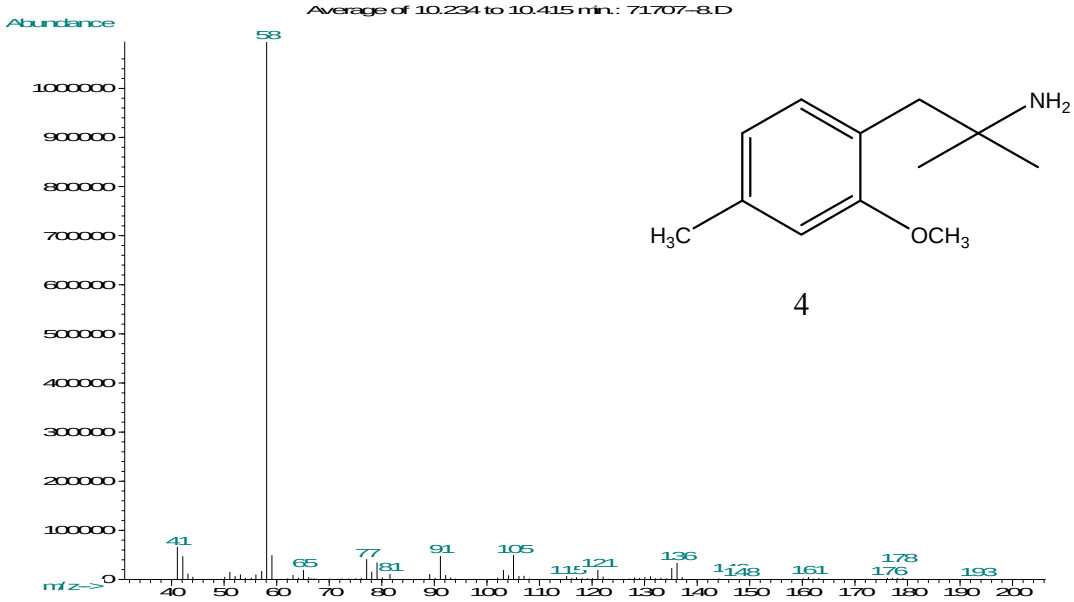

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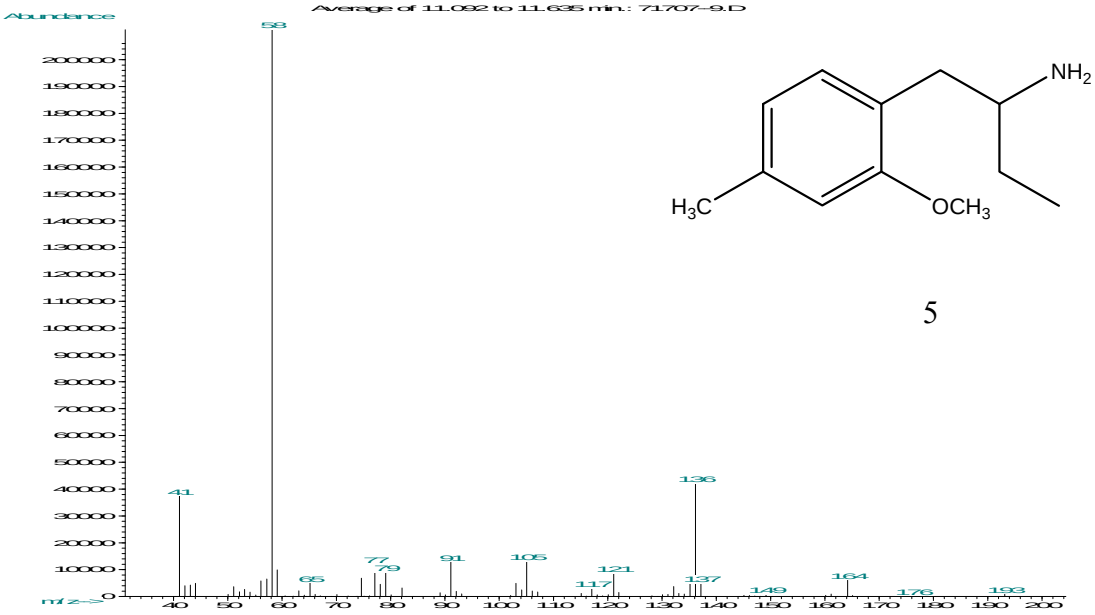

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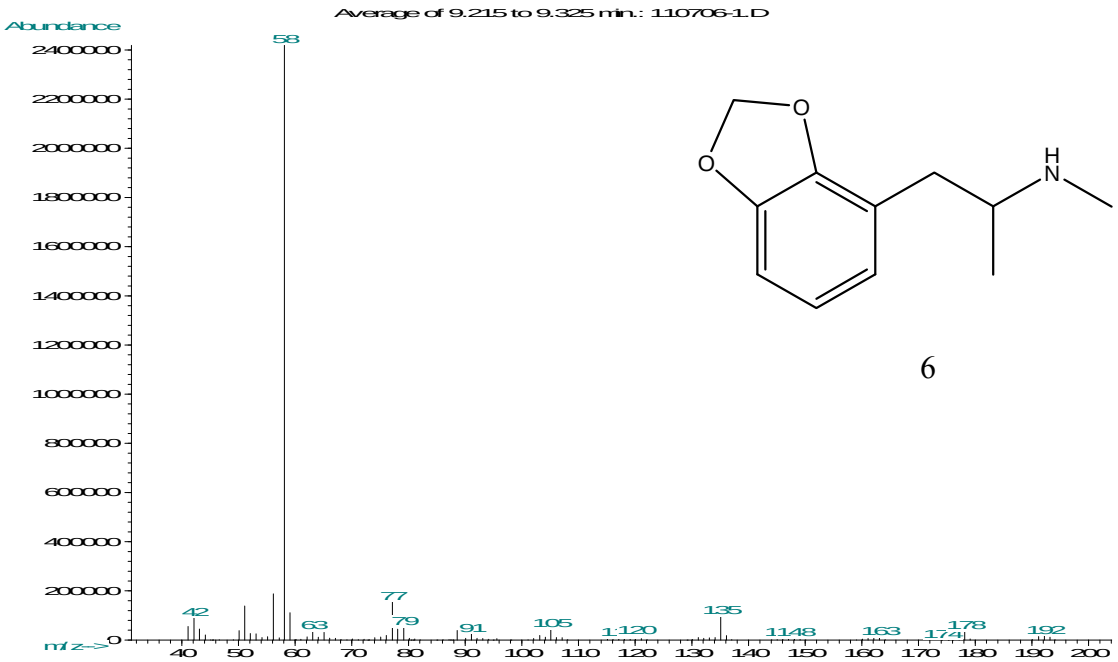

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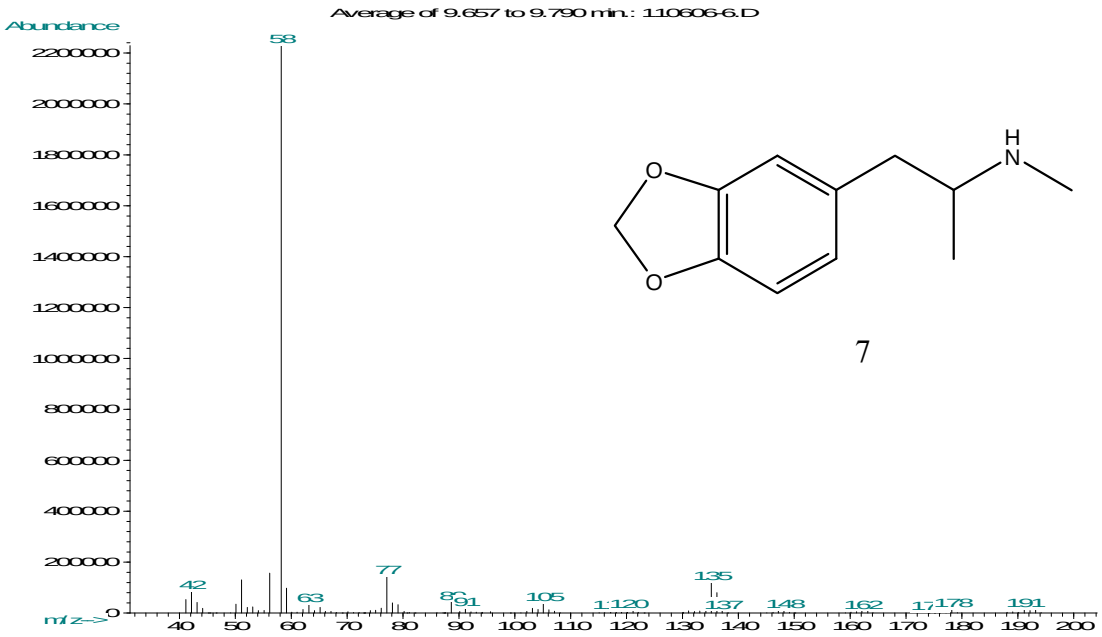

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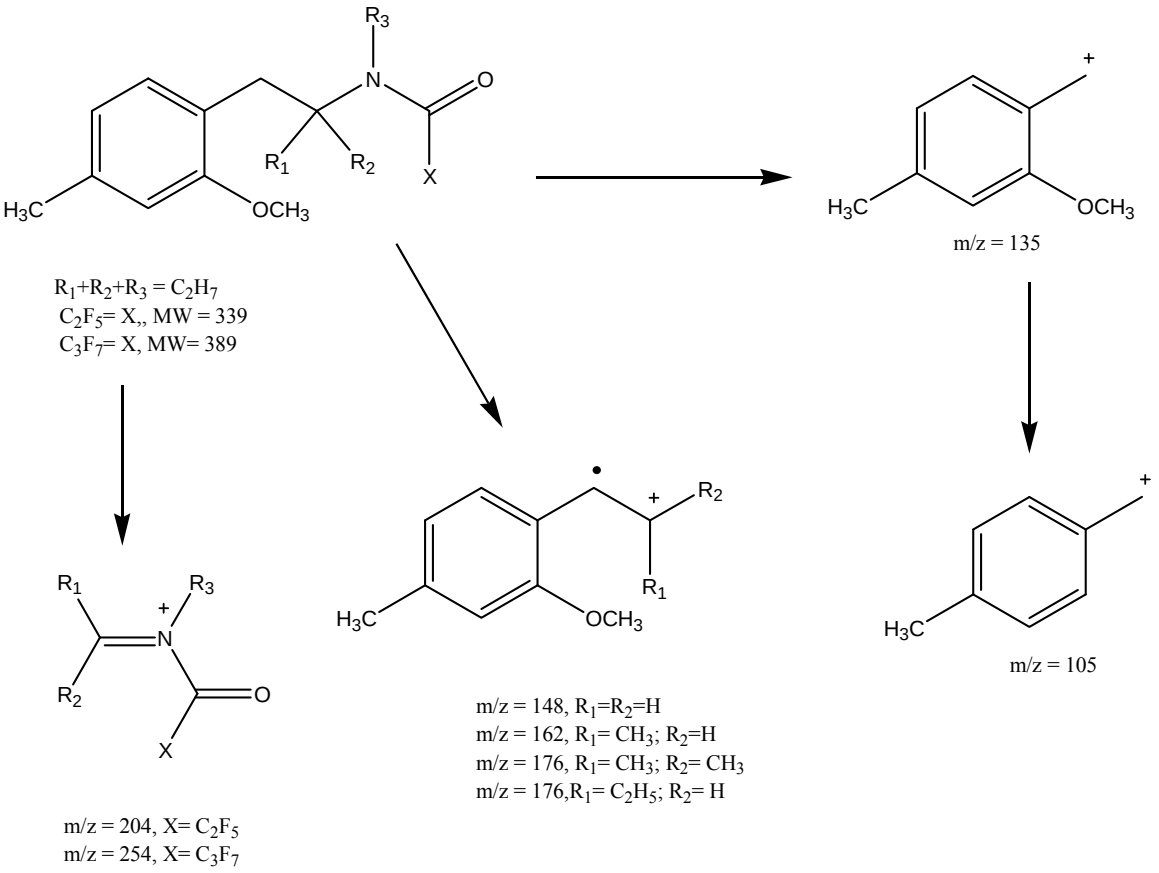


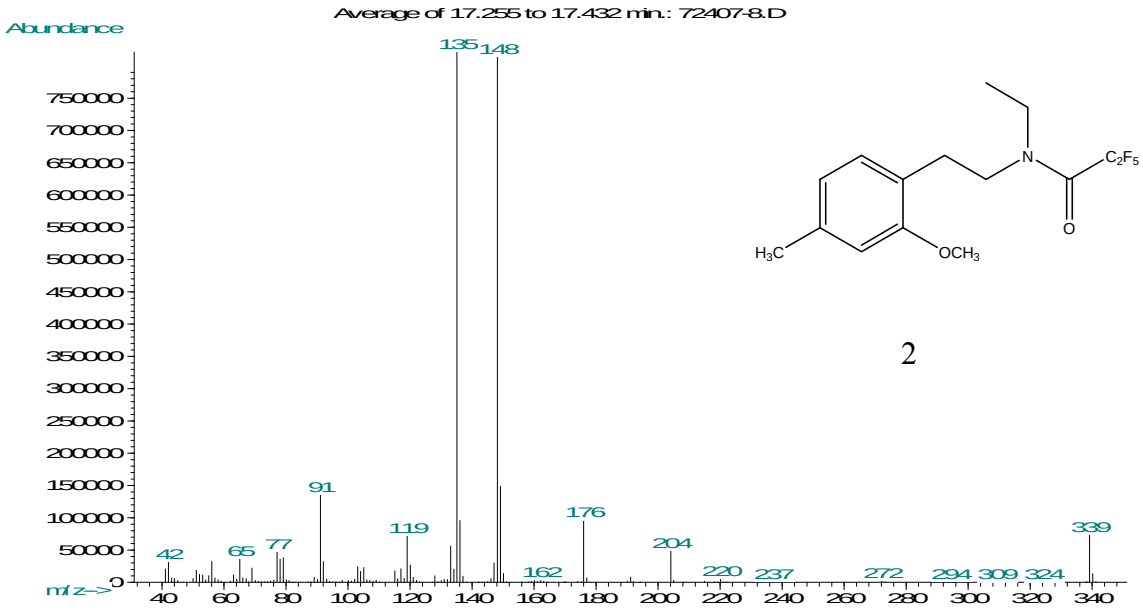
Scheme 3.2: Mass spectral fragmentation products for the acylated 2-methoxy-4-methyl phenethylamines.

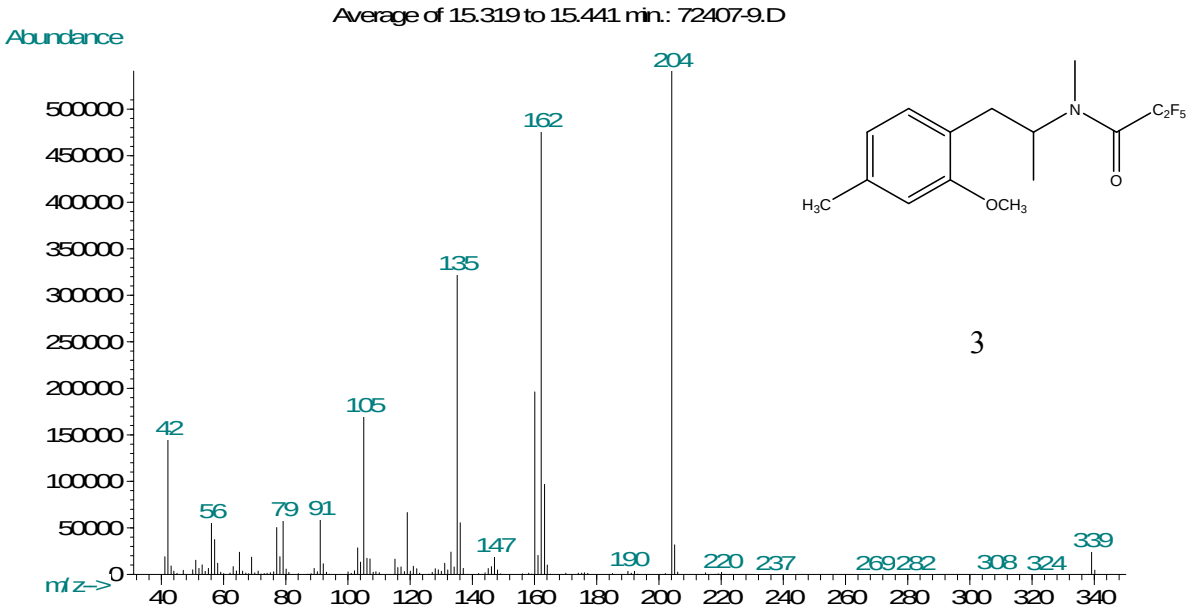
In the next phase of this study various perfluoroacylated derivatives of the regioisomeric primary and secondary amines were prepared and evaluated in an effort to individualize their mass spectra and provide unique marker ions for specific identification. Generally, acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum.

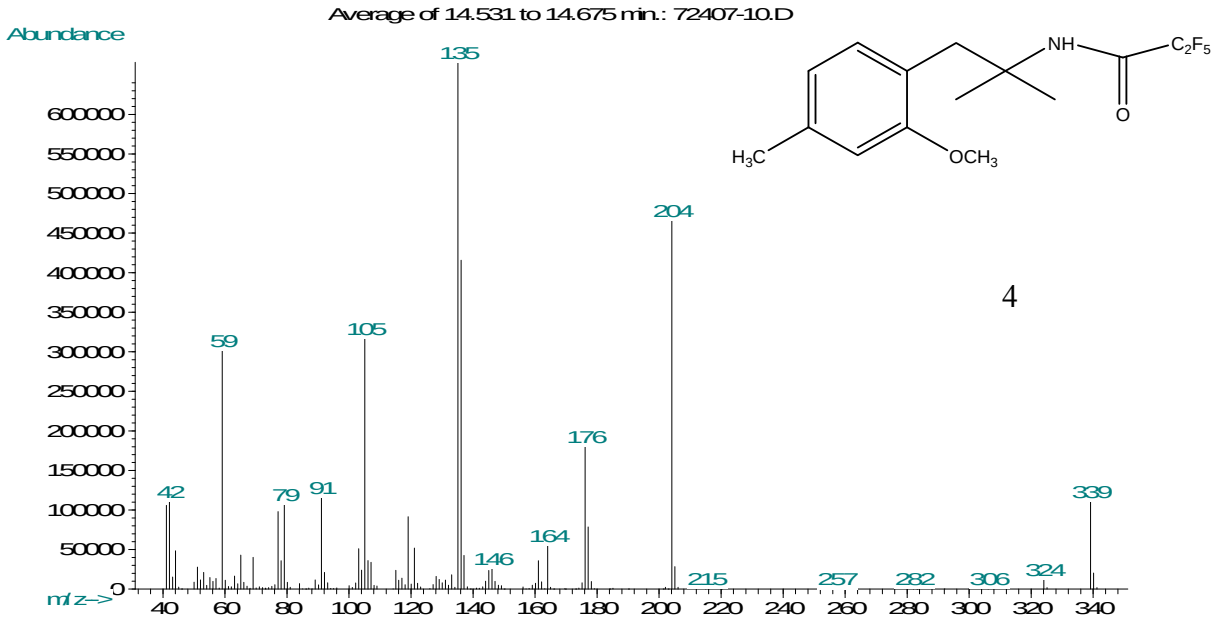
The mass spectra for the pentafluoropropionyl and heptfluorobutyryl amides are shown in Figures 3.3 and 3.4, respectively. From these spectra a common peak occurs at m/z 204 and 254 which corresponds to the loss of 135 mass units from the molecular ions

at 339 and 389 for PFPA and HFBA amides. The m/z 204 and 254 ions in the PFPA and HFBA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M-135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Scheme 3.2. The 2-methoxy-4-methylbenzyl cation (m/z 135) and the methylenedioxybenzyl cation (m/z 135) are fragments common to all spectra in Figures 3.3 and 3.4. However, the 2-methoxy-4-methylbenzyl cation at m/z 135 in the perfluoroacyl derivatives shows a very high relative abundance. Indeed the m/z 135 ion is the base peak in all the PFPA derivatives of compounds 2,4,5 and in the HFBA derivatives of compounds 4 and 5. The remaining two HFBA derivatives of compounds 2 and 3 and the PFPA derivative of compound 3 show the m/z 135 ions as a major fragment of at least 90% relative abundance. This would suggest that the perfluoroacyl derivatives of compounds 2, 3, 4 and 5 offer a distinct discrimination between the methylenedioxy and the 2-methoxy-4-methyl substitution patterns based on the difference in relative abundances of the substituted benzyl cation at m/z 135.

Figure 3.3: Mass Spectra of the PFPA derivatives of compounds 2-7.







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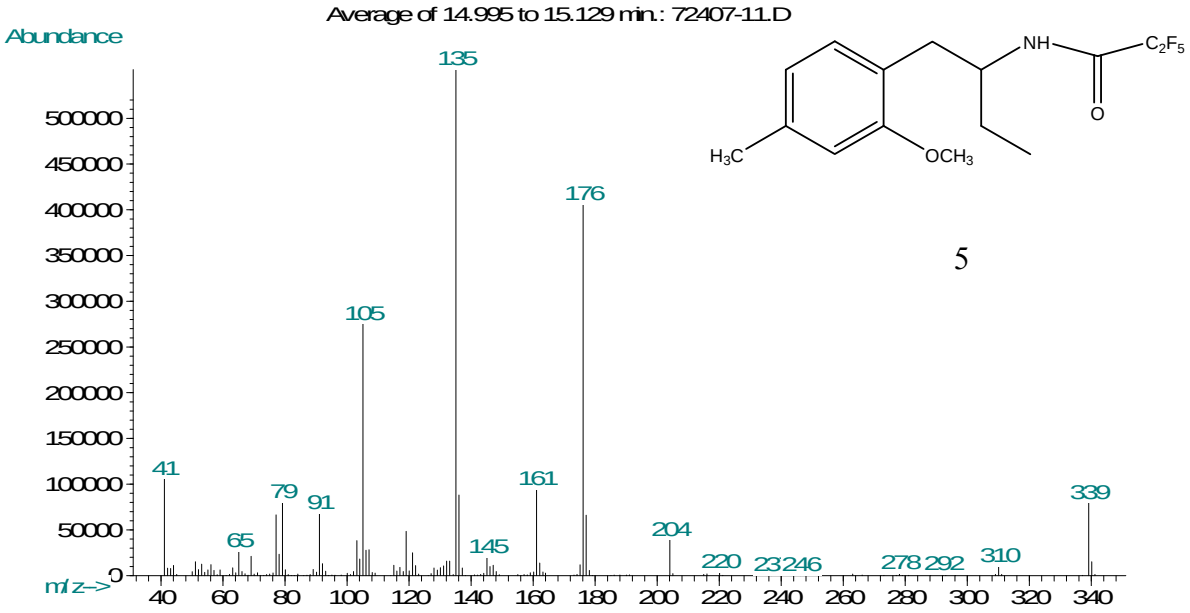

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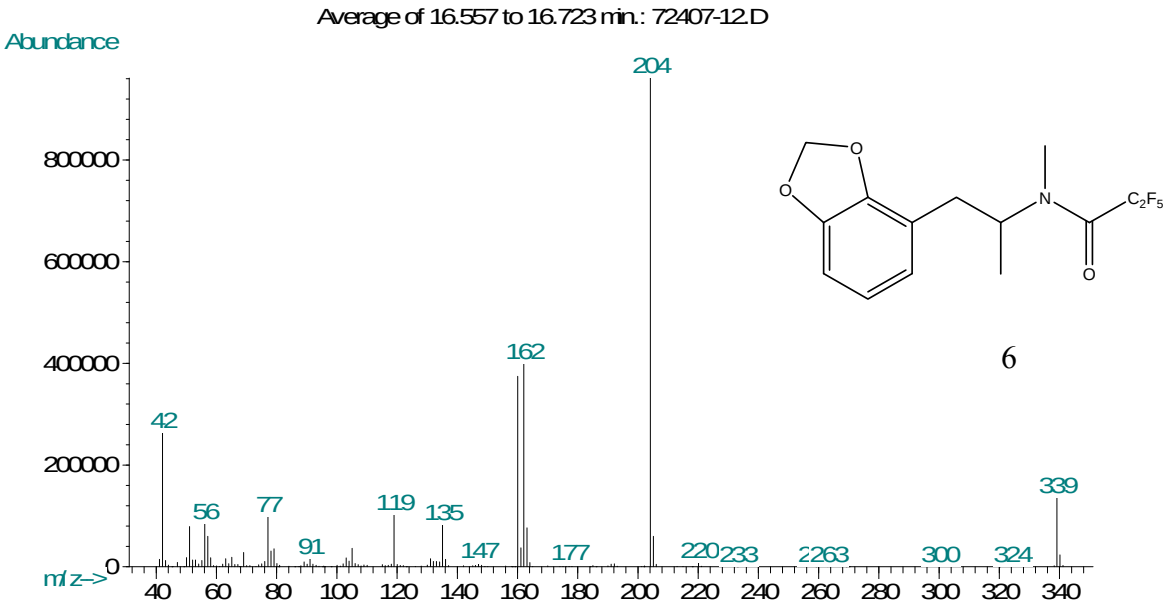

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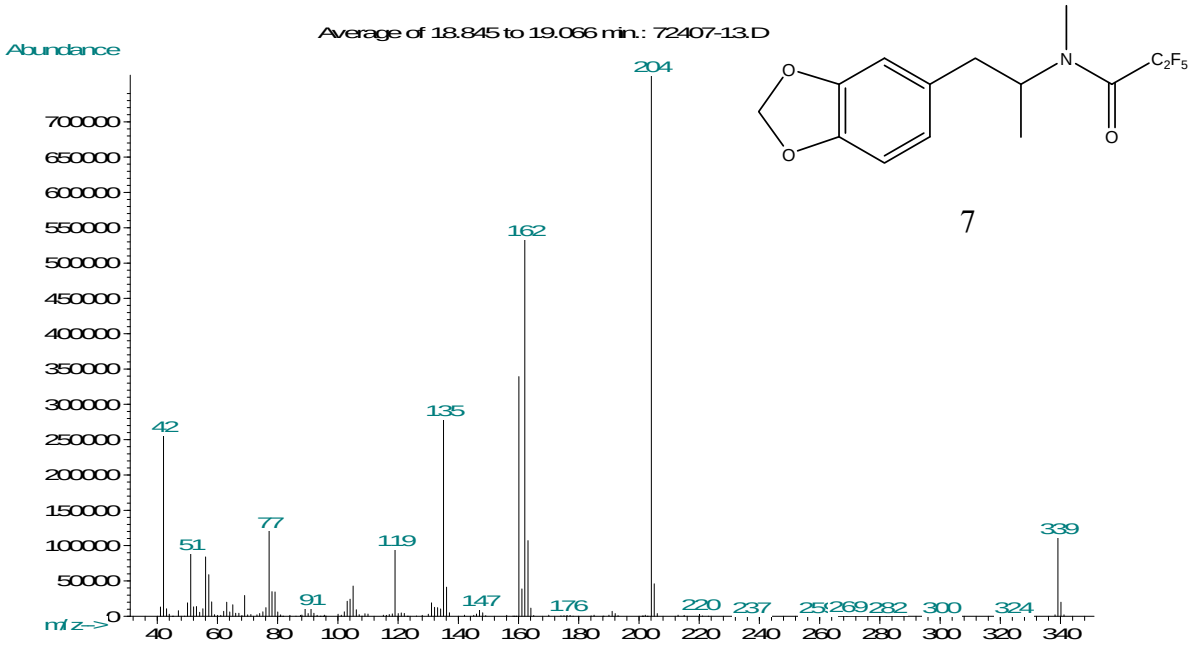

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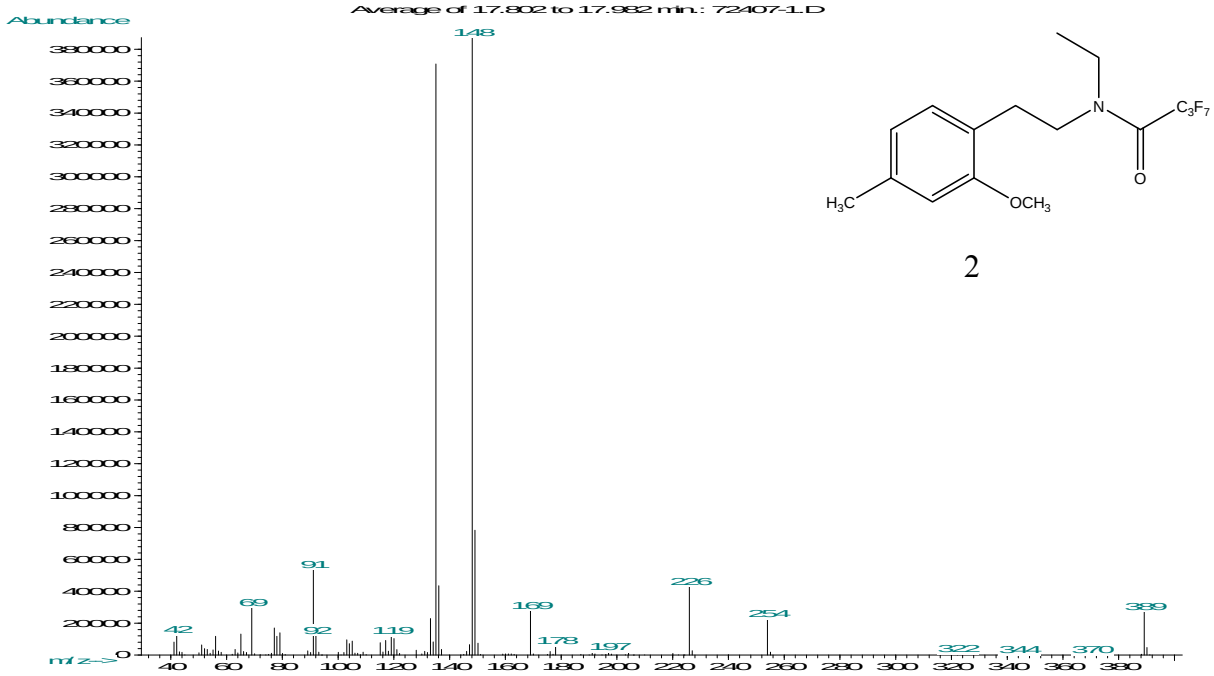
Figure 3.4: Mass spectra of the HFBA derivatives of compounds 2-7.



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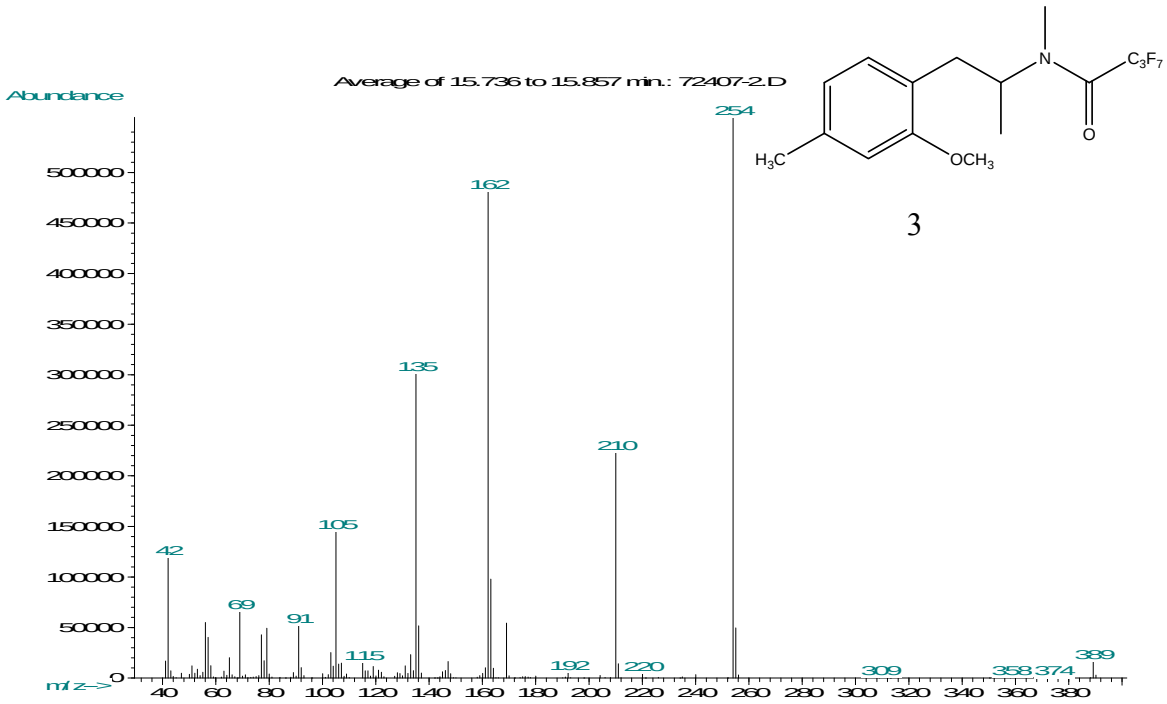

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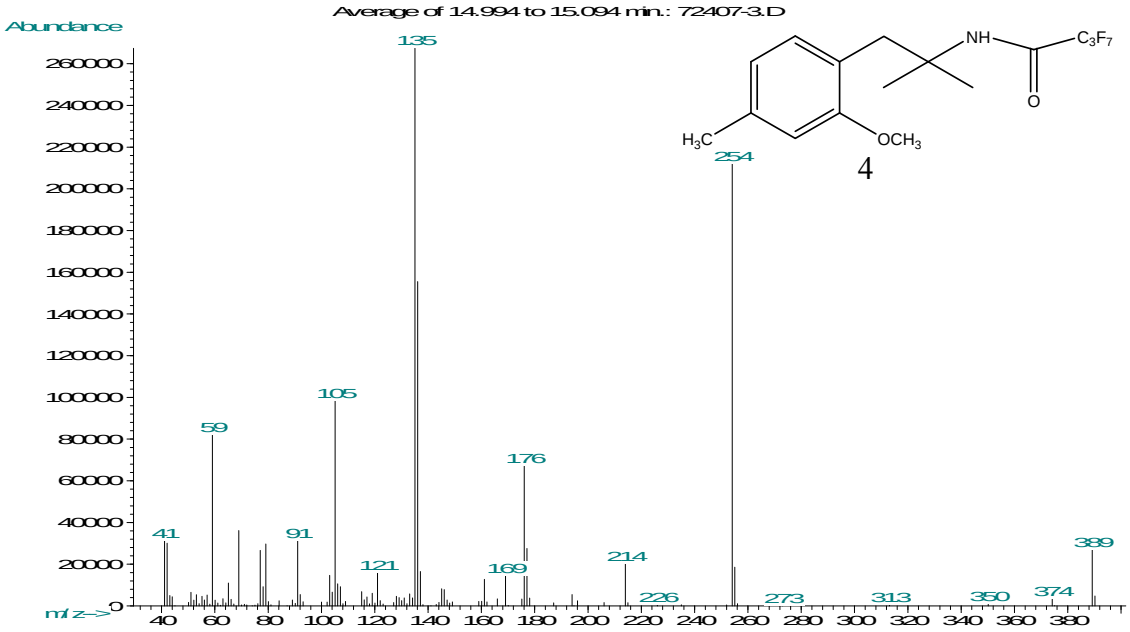

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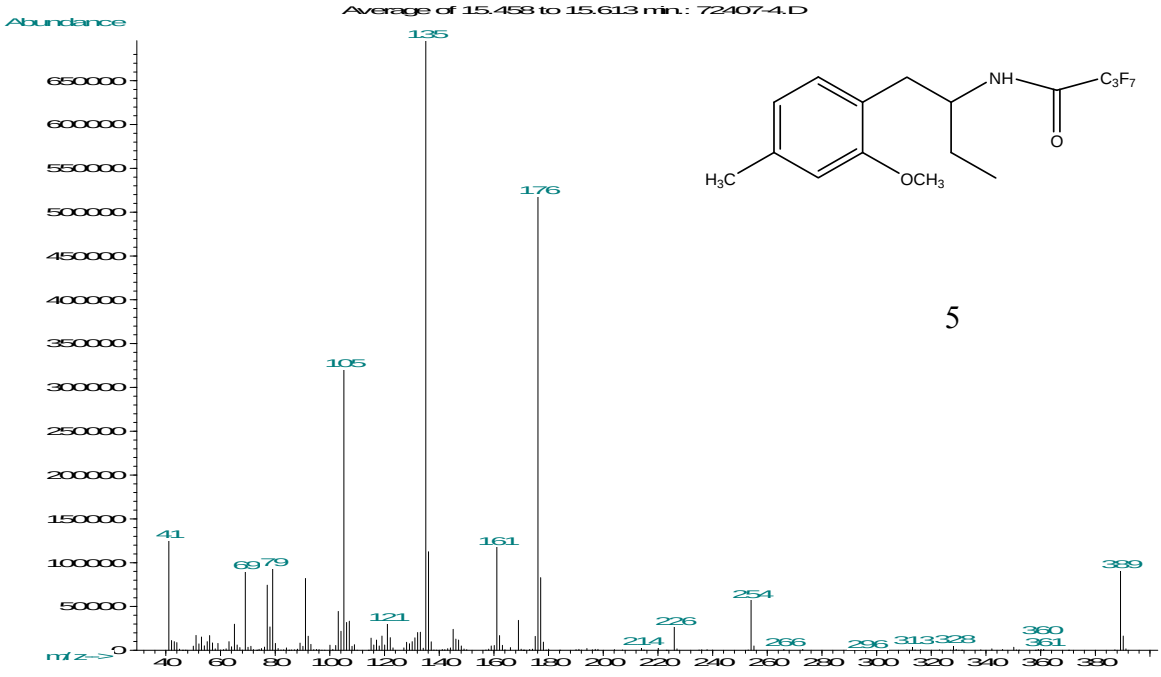

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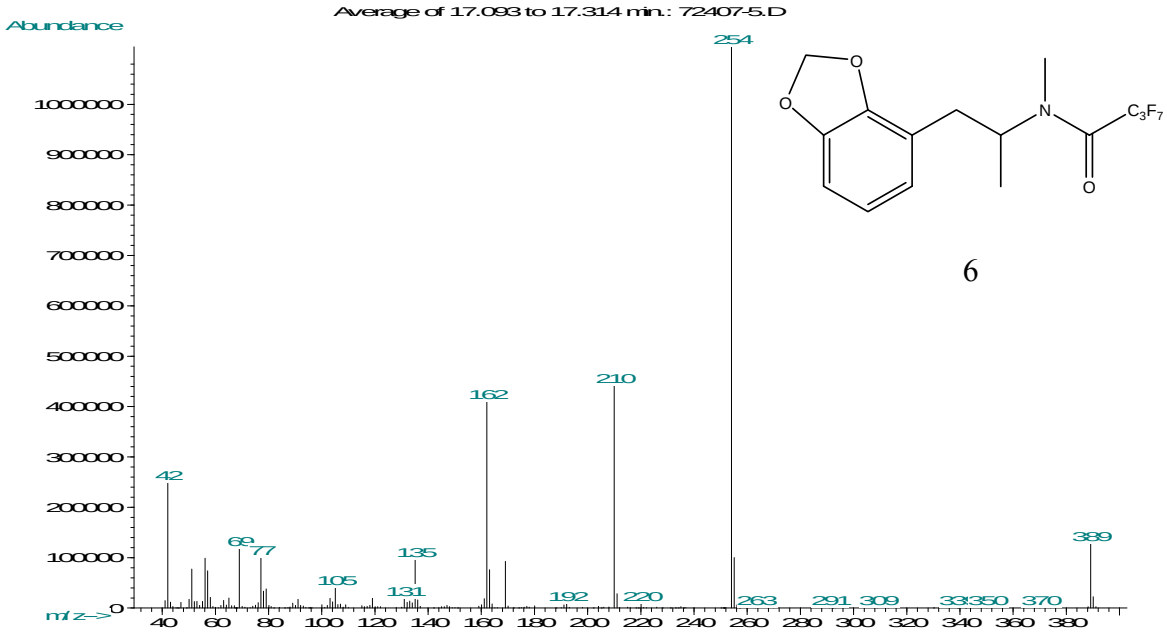

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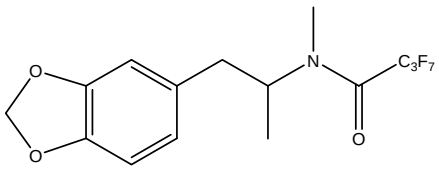

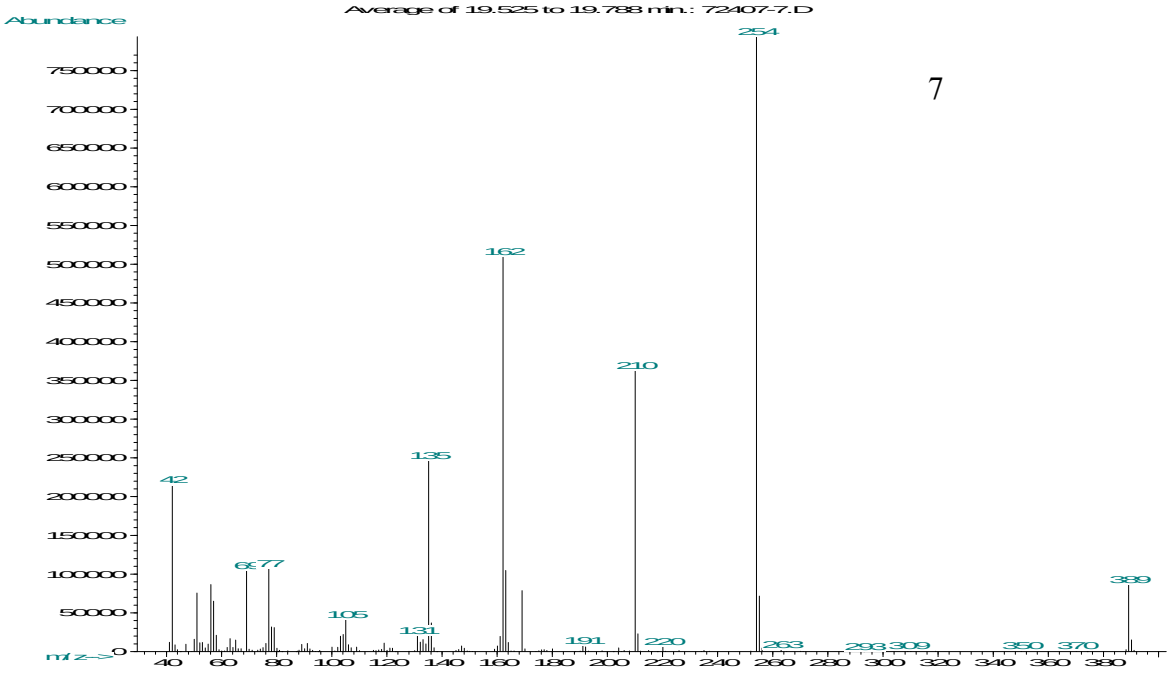

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The decreased role for the alpha cleavage reaction in the fragmentation of these amides allows the formation of more diagnostic ions of each individual isomer. Acylation weakens the bond between nitrogen and the alkyl carbon of the phenethyl side chain, allowing the formation of charged side chain specific hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass significantly individualize the mass spectra and provide specific structure information. The spectra for the N-ethyl isomer (compound 2) in Figures 3.3-2 and 3.4-2 show a base peak at m/z 148 corresponding to the alkene radical cation which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (see Scheme 3.2). This ion at m/z 148 would only occur for the N-ethyl regioisomer. The spectra in Figures 3.3-3, 3.3-6, 3.3-7, 3.4-3, 3.4-6 and 3.4-7 show the substituted phenylpropene hydrocarbon radical cation at m/z 162, identifying these molecules as the PFPA and HFBA derivatives of 2-methoxy-4-methylmethamphetamine and the 2,3-, 3,4-methylenedioxymethamphetamines respectively. The spectra for the PFPA and HFBA derivatives of the primary amines (compounds 4 and 5) show ions at m/z 176 from the corresponding substituted phenylbutene radical cation. The lower abundance of m/z 176 for the 4-methoxy-3-methylphentramine (compound 4) may be the result of steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

While the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the N-ethylphenethylamines (Figure 3.3-2) is the appearance of an ion at m/z 176 in addition to the base peak at m/z 148. The 176 ion suggests a four carbon chain directly attached to

the aromatic ring as occurs for the alpha-ethyl- (compound 5) and alpha, alpha-dimethyl- (compound 4) phenethylamines (Figures 3.3-4, 3.3-5 and 3.4-4, 3.4-5). The m/z 176 ion in the spectra for the PFPA derivatives of the N-ethyl regioisomers (Figure 3.3-2) is a rearrangement of the m/z 204 ion resulting in the loss of mass 28 (the N-ethyl group) via hydrogen transfer This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the N-ethyl-phenethylamines shown in Figure 3.4-2. The loss of 28 mass units from the acylimine fragment at m/z 254 yields the equivalent fragment ion at m/z 226. Thus, the HFBA derivatives may offer more characteristic ions for individualization of these regioisomeric substances.

A comparison of the mass spectra for the PFPA and HFBA derivatives of all three ring substituted methamphetamines (Compounds 3, 6 and 7) indicates unique ions at m/z 160 and m/z 210 (see Figures 3.3-3, 3.3-6, 3.3-7; 3.4-3, 3.4-6, and 3.4-7). This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7 respectively. This ion has been described in previous chapters as the cationic methylated nitrile $C_2F_5CNCH_3$ or $C_3F_7CNCH_3$. The equivalent fragmentation further supported by the analysis of the mass spectra of the PFPA and HFBA derivatives of d_3- and d_5-MDMA in previous studies [Chapter 1]. This fragmentation pathway appears unique to the acylated N-methyl C-methyl pattern (methamphetamine side chain).

The presence of relatively high abundances of the m/z 105 ion in the spectra of the PFPA and HFBA derivatives of compound 3 allows for significant discrimination of this 2-methoxy-4-methylmethamphetamine from the two isobaric methylenedioxy-

methamphetamines isomers (compounds 6 and 7). This ion at m/z 105 represents the loss of 30 mass units (formaldehyde, CH_2O) from the methoxymethylbenzyl cation at m/z 135. Previous reports suggested that the m/z 105 ion is characteristic of all o-methoxy substitution patterns regardless the position of the methyl group on the aromatic ring [Chapter 6]. However, the absence of this fragment from the spectra of the derivatives of compound 2 suggests the necessity of further structure fragmentation studies for these compounds.

Gas Chromatographyic separation of the perflouroacyl derivatives of 2- methoxy-4-methylphenethylamines from 2,3- and 3,4-MDMA

The PFPA and HFBA derivatives of the six primary and secondary amines were compared on two stationary phases using two GC-MS systems, The relatively nonpolar 100% dimethyl polysiloxane (Rtx-1) and the more polar trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and one program showing the best compromise between resolution and analysis time was used to generate the chromatograms in Figures 3.5 and 3.66. The elution order was the same for both stationary phases and all temperature programs evaluated in this study. The chromatograms of the perfluoroacyl compounds show that the 2,3-MDMA elutes before the corresponding 3,4-isomer, which elutes last. When the ring substitution pattern is held constant (2-methoxy-4-methyl), the side chain elution order is secondary amides before the tertiary amides and in this limited set of examples branched isomers elute before the more linear ones. Therefore, the amides of compound 4 elute first followed by the amide

of compound 5 (both secondary amides), then the amides of compound 3 (the methamphetamine side chain) and finally the amides of compound 2 (the more linear of these two tertiary amides). The amides of 2,3-MDMA elute between the secondary and tertiary amides in this group of compounds.

Perhaps the most useful information in these chromatograms is the relative elution of the derivatized controlled substance 3,4-MDMA and its closest eluting regioisomeric and isobaric equivalents. Both the PFPA and HFBA derivatives of 3,4-MDMA elute last and the N-ethyl-2-methoxy-4-methylphenethylamine PFPAs and HFBAs are the closest eluted compounds in the 2-methoxy-4-methyl phenethylamine series. The isobaric acylated N-ethyl amides show very distinct mass spectra with several characteristic ions to differentiate it from the corresponding amides of the drug of abuse 3,4-MDMA. Thus, derivatization methods coupled with both chromatographic and mass spectral procedures can allow for the complete differentiation of the side chain substitution pattern of the 2-methoxy-4-methylphenethylamines from 3,4-MDMA and its regioisomer, 2,3-MDMA. Additionally in this limited study, the Rtx-200 stationary phase provided the greatest resolution of the amides of compound 7 (3,4-MDMA) from all the other isomers.

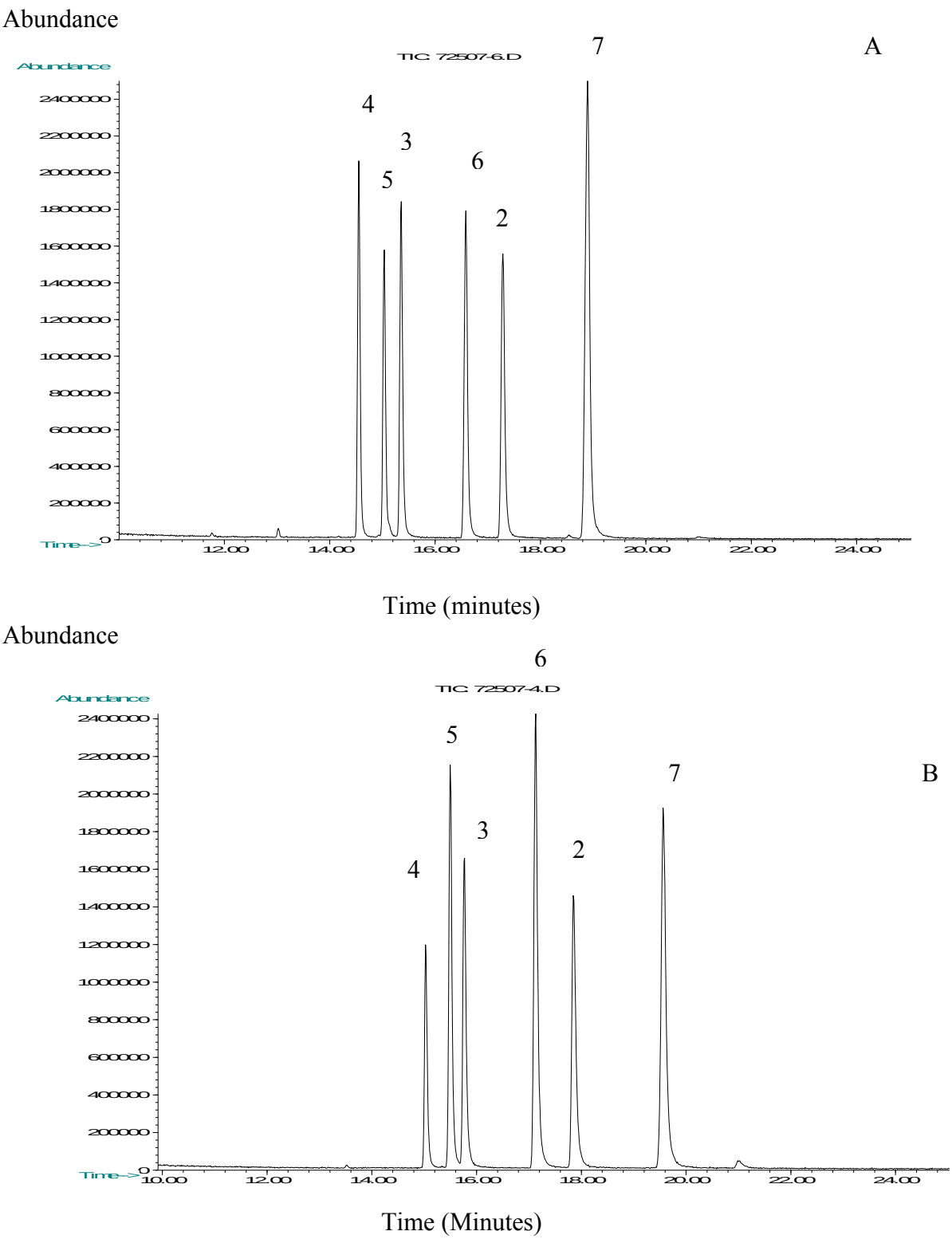


Figure 3.5: Chromatograms of the PFPA (A) and HFBA (B) derivatives of compounds 2-7. GC-MS system 1, Rtx-200 column.

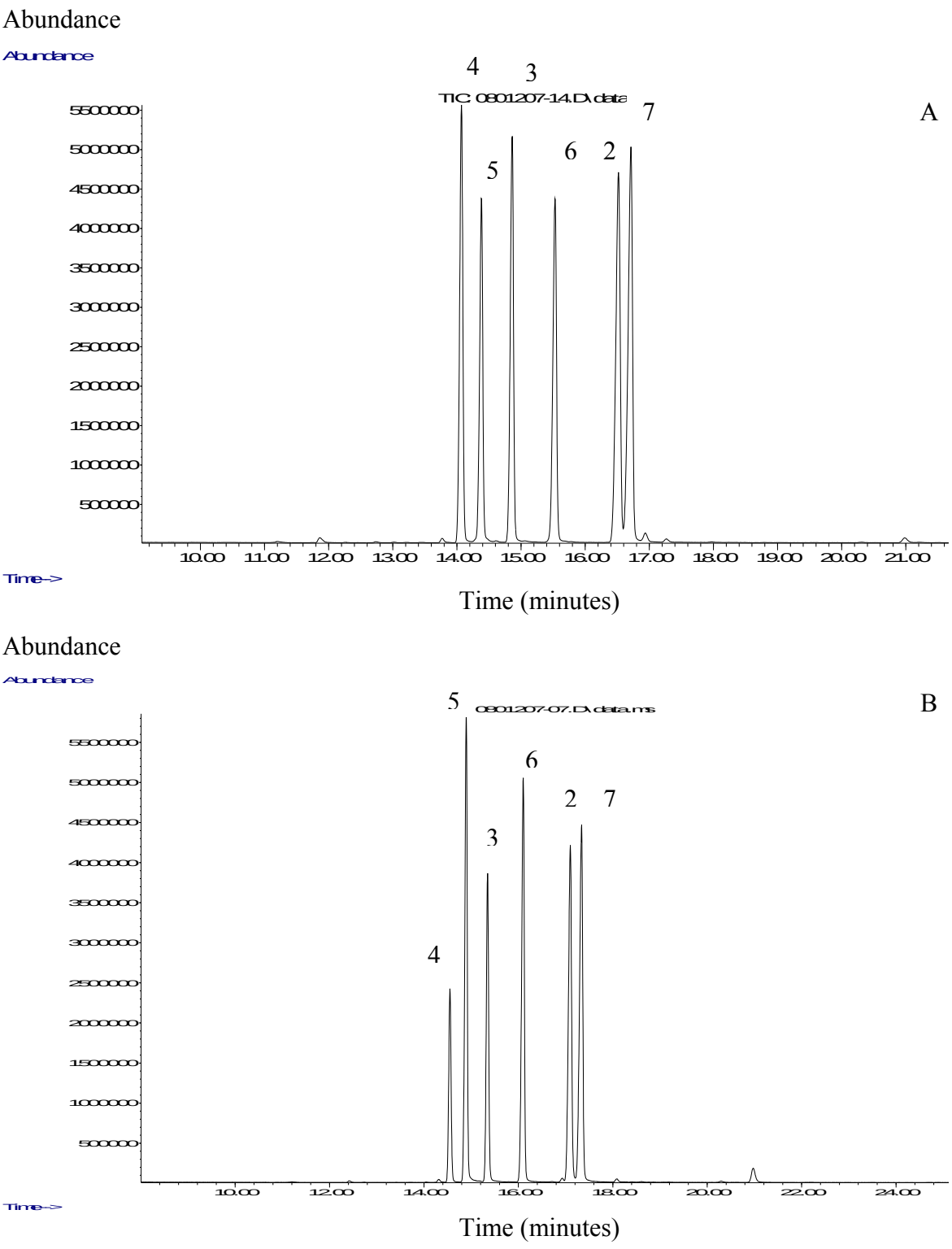


Figure 3.6: Chromatograms of the PFPA (A) and HFBA (B) derivatives of compounds 2-7. GC-MS system2, Rtx-1 column.

Conclusion of Chapter 3

Differentiation of the side chain substitution pattern of the 2-methoxy-4-methylphenethylamines from 3,4-MDMA and its regioisomer, 2,3-MDMA was accomplished using a combination of gas chromatography and mass spectrometry. Derivatization of the primary and secondary amines with various acylating agents yields amides with improved resolution compared to the underivatized amines by capillary gas chromatography on Rtx-1 and Rtx-200 stationary phases.

Additionally, the perfluoroacyl derivatives significantly individualize the mass spectra for these amides and allow for unambiguous identification. The individualization results from fragmentation of the alkyl carbon-nitrogen bond yielding characteristic hydrocarbon fragments at ***m/z*** 105, 148, 162, and 176 as well as other unique fragments.

Chapter 4

GC-MS Analysis of Acylated Derivatives of the Side Chain Regioisomers of 4-Methoxy-3-Methyl Phenethylamines Related to Methylenedioxymethamphetamine

In this chapter all the side chain regioisomeric possibilities of 4-methoxy-3-methyl phenethylamines, as a representative of the m/z58 side chain regioisomers of the methoxy group in the 4 position, will be compared from the mass spectral and chromatographic aspects to 2,3- and 3,4-MDMA.

Mass Spectral studies of 4-methoxy-3-methyl phenethylamines along with 2,3- and 3,4- MDMA

Mass spectrometry is the primary method for confirming the identity of drugs and other substances of abuse in forensic samples. The mass spectra of phenethylamines are characterized by a base peak formed from an amine initiated alpha-cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-methylenedioxymethamphetamine (MW=193) the alpha-cleavage reaction yields the substituted imine fragment at m/z 58 and the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-methylenedioxymethamphetamine contains major ions at m/z 58 and 135/136 as well as other ions of low relative abundance. There are a total of five regioisomeric forms of the m/z 58 imine species.

The five side chain regioisomers of 4-methoxy-3-methylphenethylamine (Compounds 1-5) have the potential to yield mass spectra essentially equivalent to 3,4-MDMA and 2,3-MDMA (structures shown in Figure 4.1). All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136 (Figure 4.2). The isobaric methoxy methyl benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. Furthermore the m/z 58 ion in the methoxy methyl phenethylamine is regioisomeric with that obtained in the mass spectra of both 2,3 and 3,4-MDMA (Scheme 4.1). The individual mass spectra for 2,3- and 3,4-MDMA are also presented in Figure 4.2 (Compounds 6 and 7).

Figure 4.1: Structures of side chain regioisomers of methamphetamine

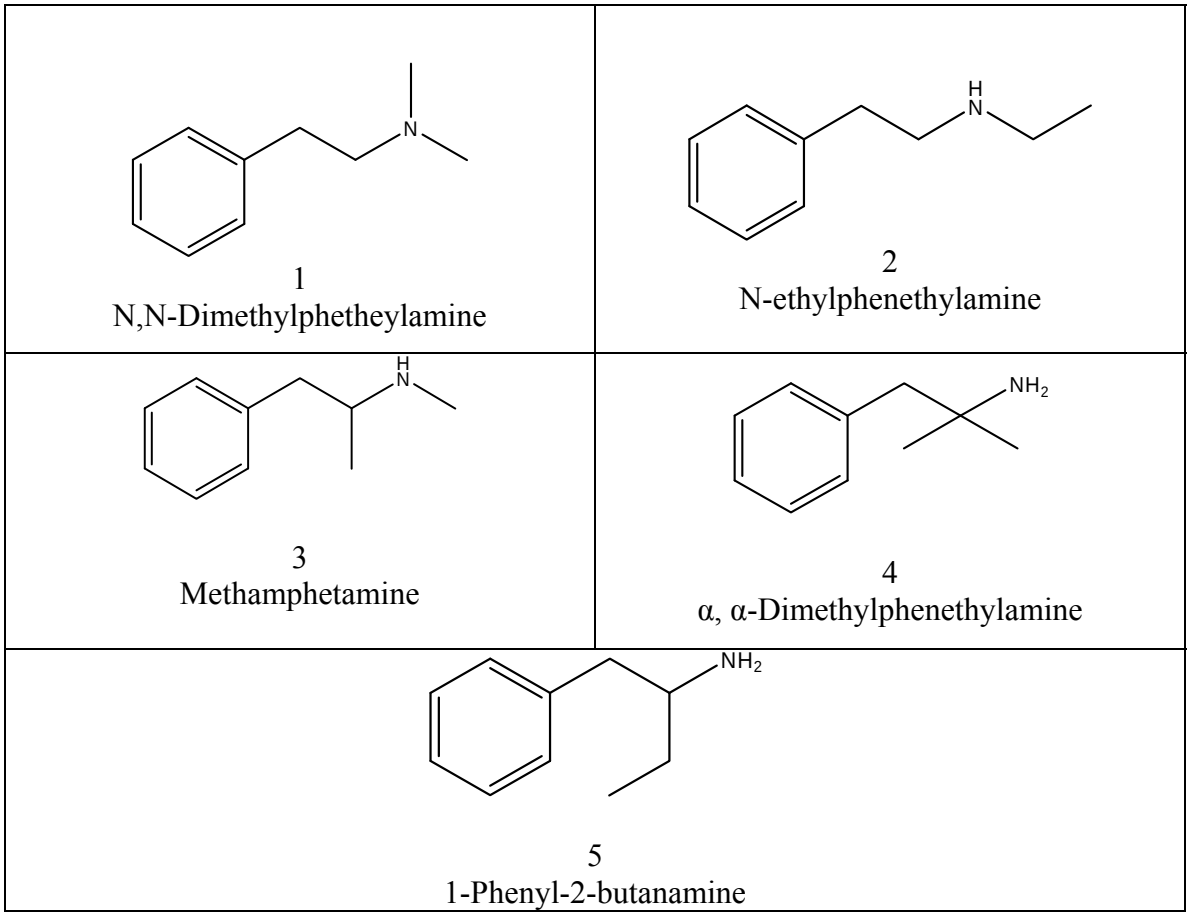
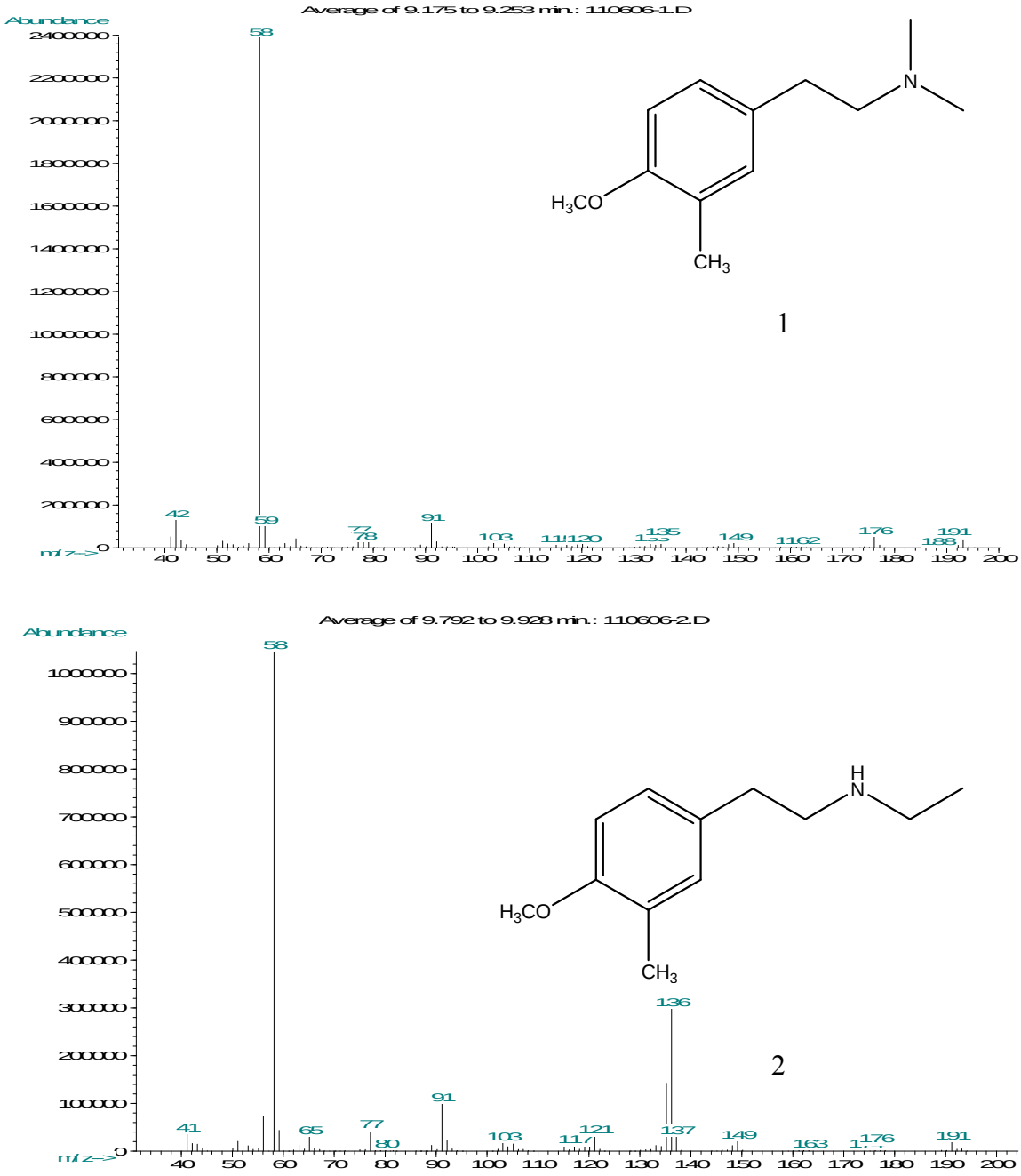
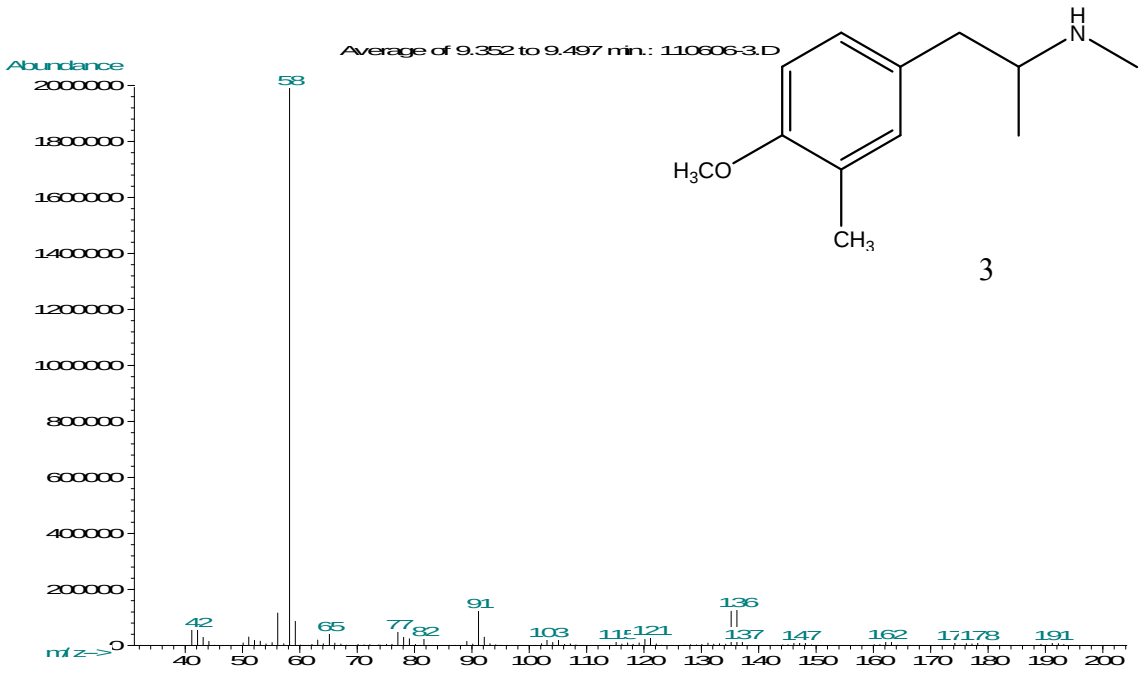


Figure 4.2: Mass Spectra of the undreivatized amines:





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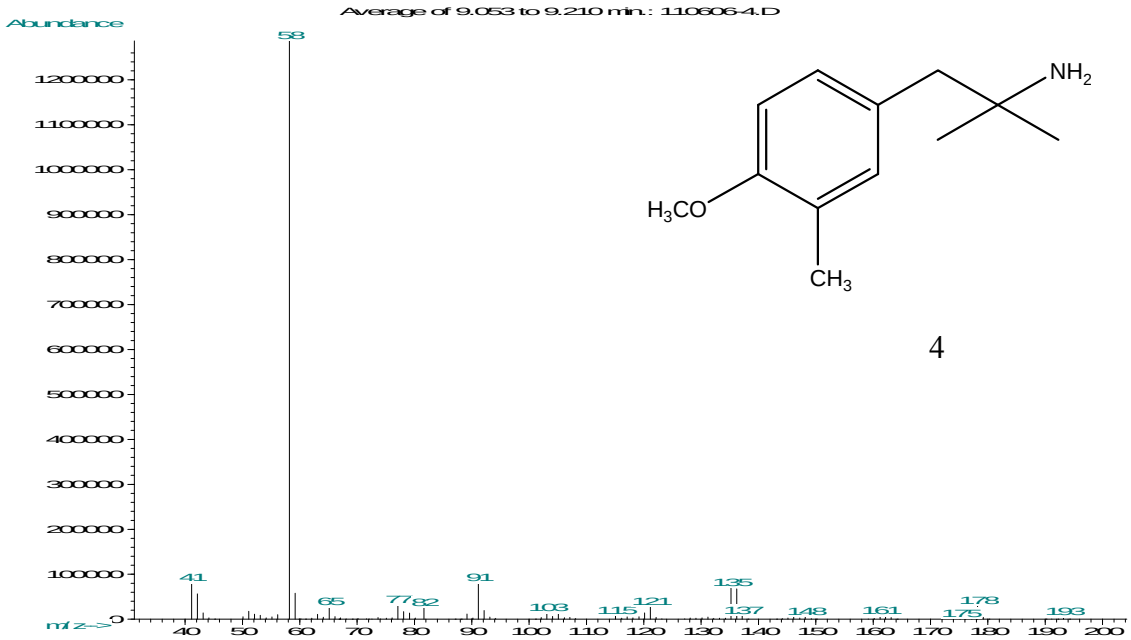

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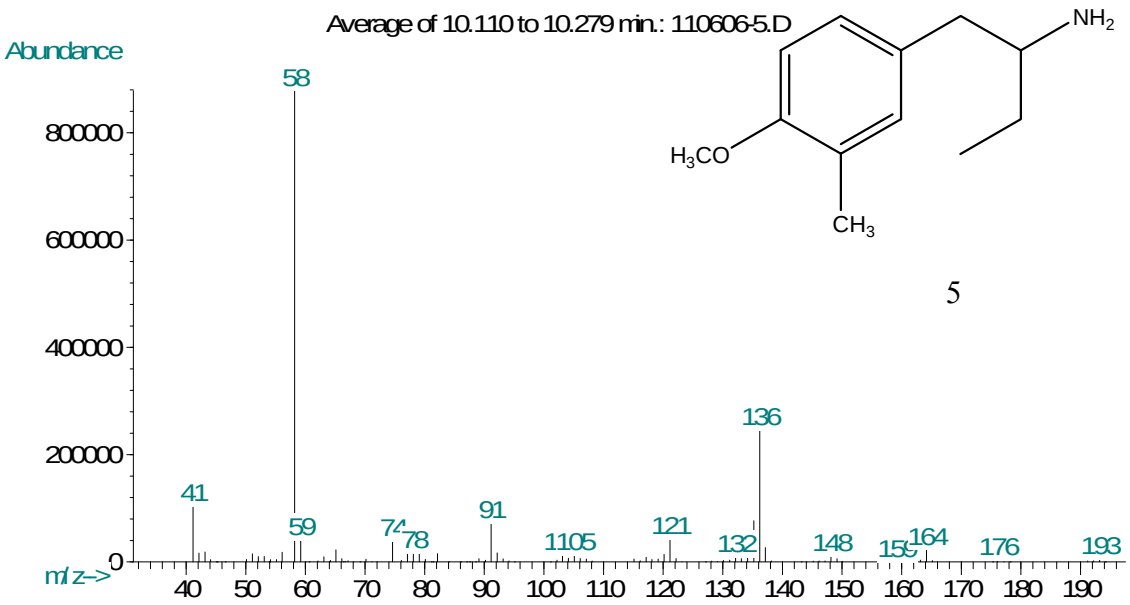

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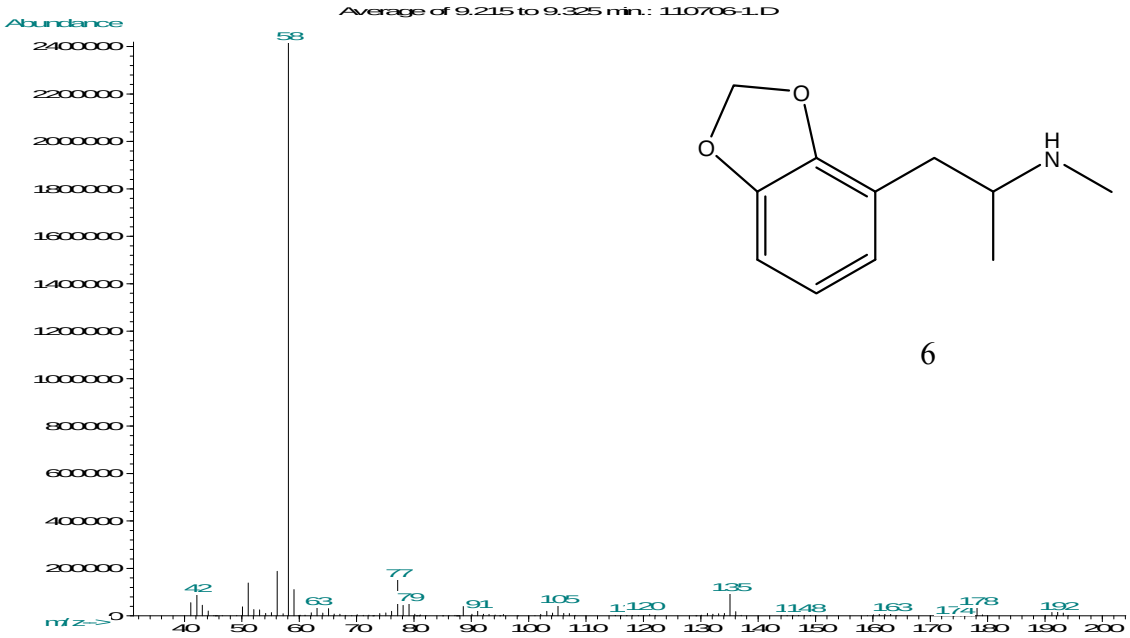

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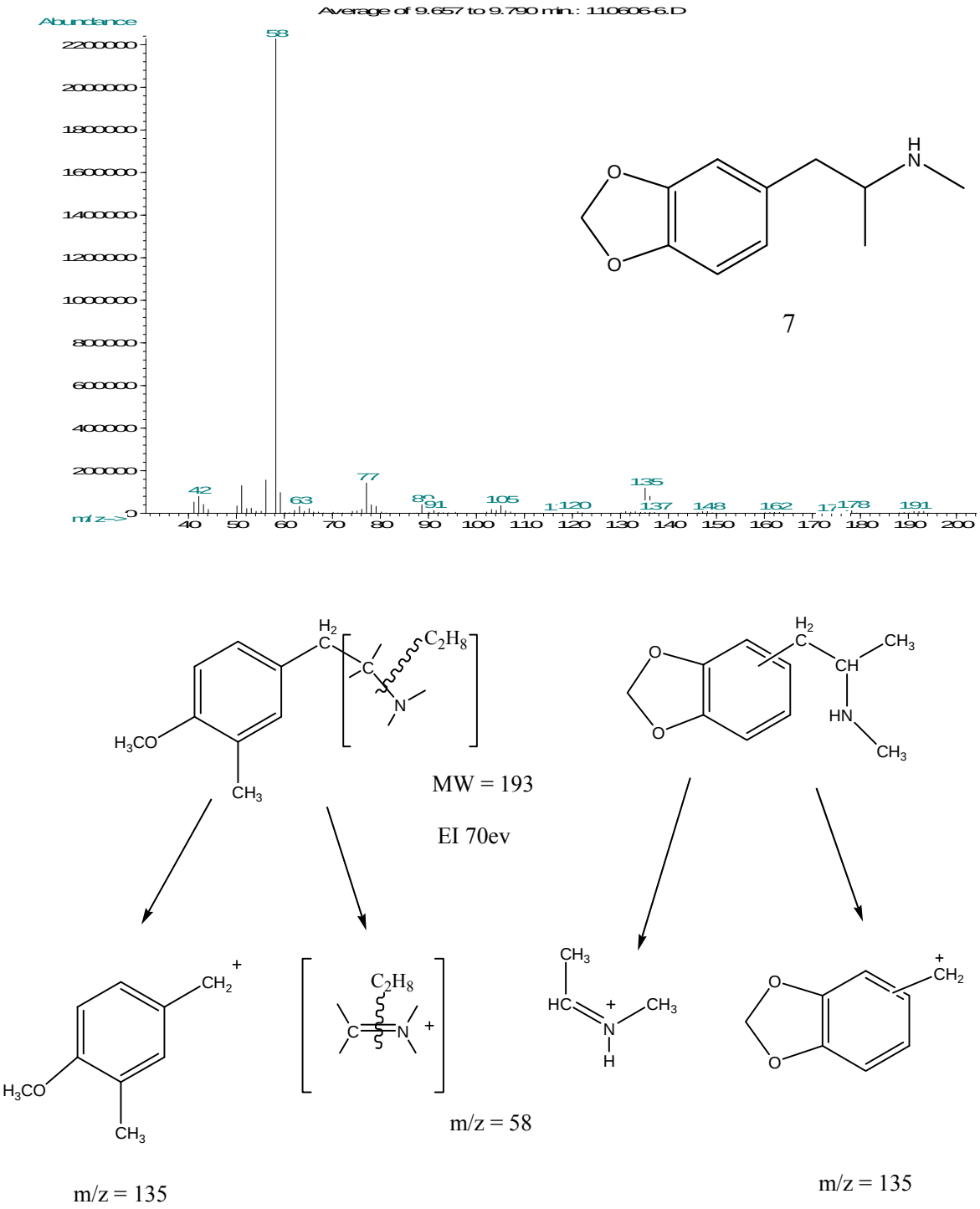


Scheme 4.1: EI fragmentation pattern of the underivatized compounds 1-7

This lack of mass spectral specificity in addition to the possibility of chromatographic co-elution, with 3,4-MDMA, could result in misidentification of the

target drug. Furthermore, the lack of available reference samples could complicate the individual identification of any one of these substances. This constitutes a significant analytical challenge where the specific identification by gas chromatography-mass spectrometry (GC-MS) must be based primarily upon the ability of the chromatographic system to separate the regioisomeric/isobaric non-drug substance from the actual drug of interest. Additionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

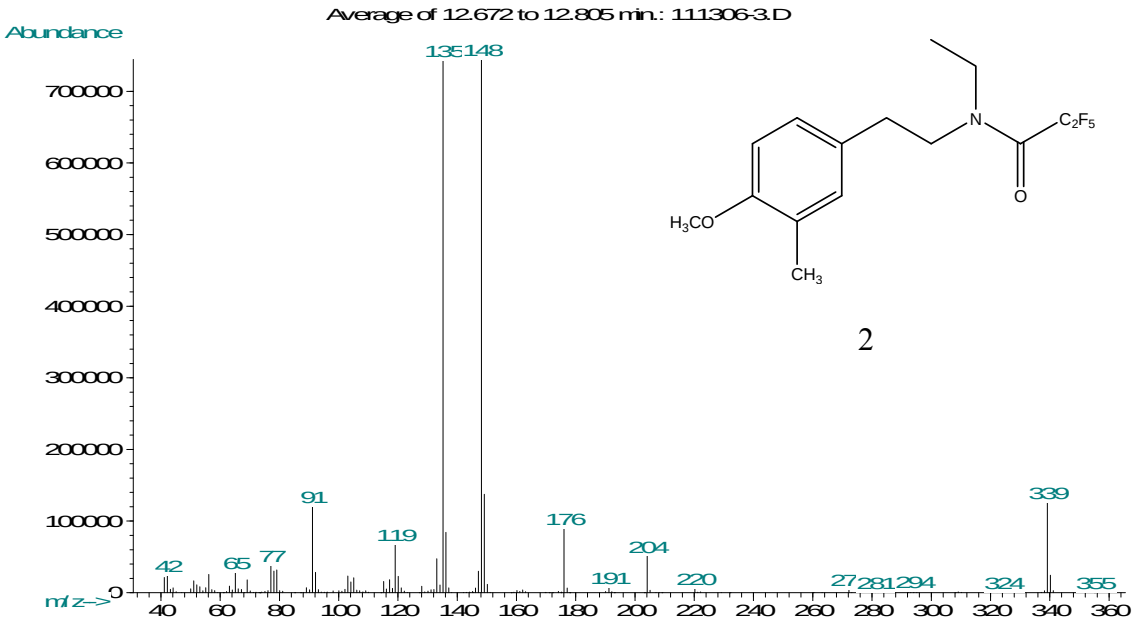
In the next phase of this study various perfluoroacylated derivatives of the regioisomeric primary and secondary amines were prepared and evaluated in an effort to individualize their mass spectra and provide unique marker ions for specific identification. Acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum.

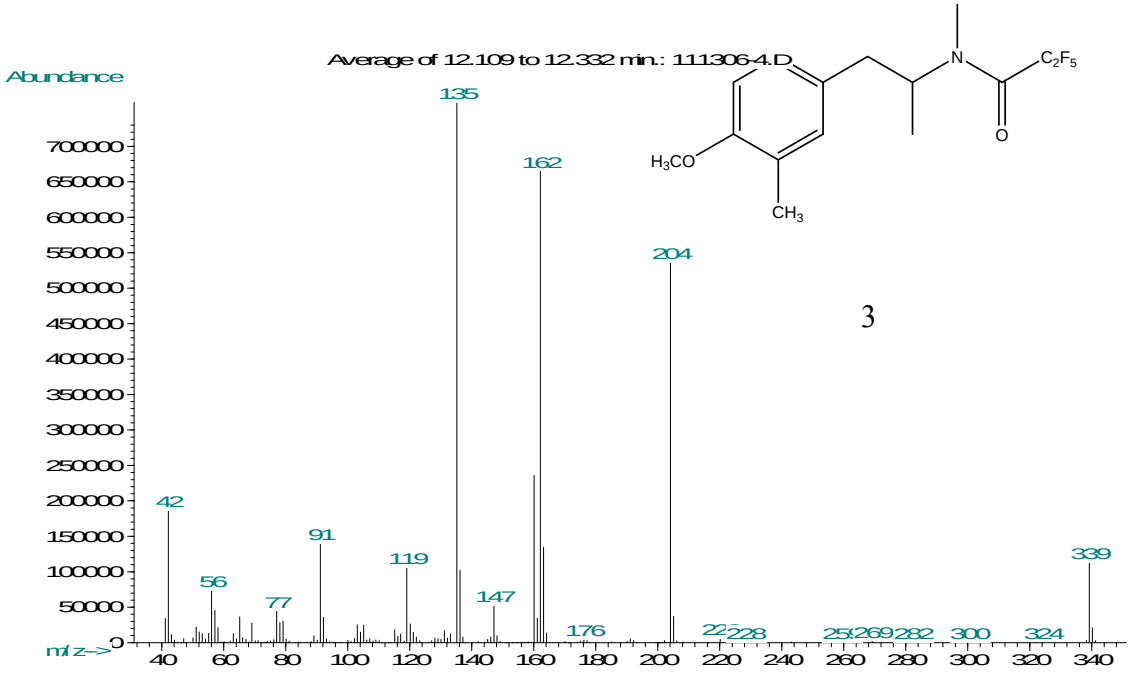
The mass spectra for the twelve pentafluoropropionyl and heptfluorobutryl amides are shown in Figures 4.3 and 4.4, respectively. From these spectra a common peak occurs at m/z 204 and 254 which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for PFPA and HFPA amides. This ion at m/z 204 and 254 is the PFPA and HFPA imine species likely formed from the alpha cleavage of the amide nitrogen to eliminate the 4-methoxy-3-methylbenzyl radical as well as the 2, 3- and 3, 4-methylenedioxybenzyl radical. Thus the m/z 204 and 254 ions in the PFPA and HFPA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M-135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Scheme 4.2. The 4-methoxy-3-methylbenzyl cation

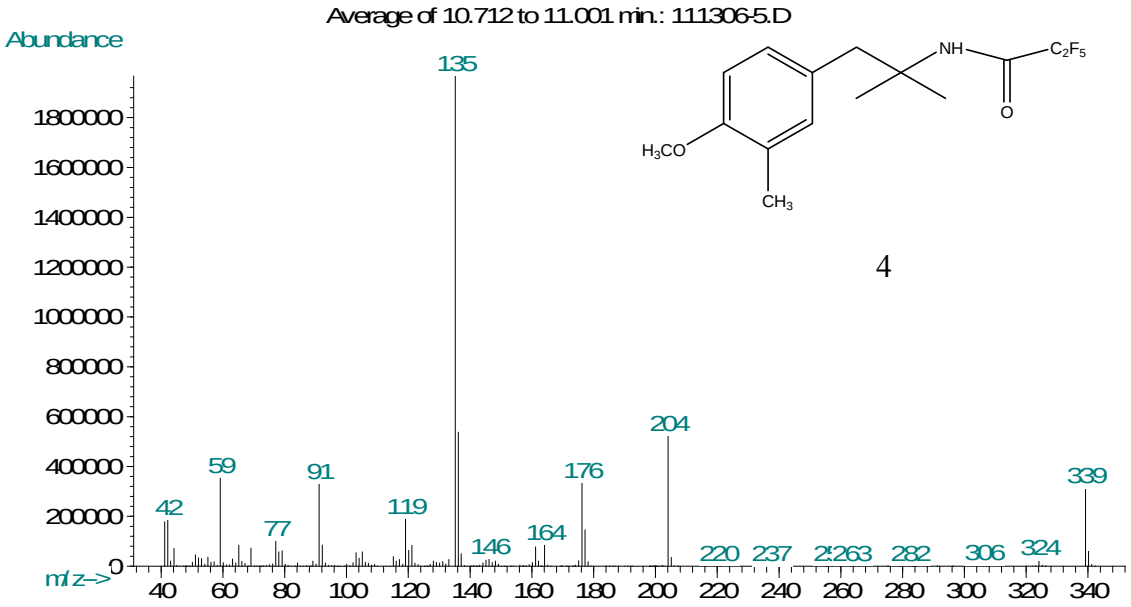
and the methylenedioxybenzyl cation (m/z 135) are fragments common to all spectra in Figures 4.3 and 4.4. However, the 4-methoxy-3-methylbenzyl cation at m/z 135 in the perfluoroacyl derivatives shows a very high relative abundance. Indeed the m/z 135 ion is the base peak in all the PFPA derivatives of compounds 2-5 and in the HFBA derivatives of compounds 4 and 5. The remaining two HFBA derivatives of compounds 2 and 3 show the m/z 135 ions as a major fragment of at least 90% relative abundance. This would suggest that the pentafluoroacyl derivatives offer a distinct discrimination between the methylenedioxy and the 4-methoxy-3-methyl substitution patterns based on the difference in relative abundances of the substituted benzyl cation at m/z 135.

The decreased role for the alpha cleavage reaction in the fragmentation of these amides allows the formation of ions more diagnostic of each individual isomer. Acylation weakens the bond between nitrogen and the alkyl carbon of the phenethyl side chain, allowing the formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass significantly individualize the mass spectra and provide specific structure information. The mass spectra in Figures 4.3 and 4.4 illustrate the role of hydrocarbon fragments at m/z 148, 162 and 176 in the electron impact mass spectral differentiation among these regioisomers.

Figure 4.3: Mass Spectra of the PFPA derivatives of compounds 2-7.







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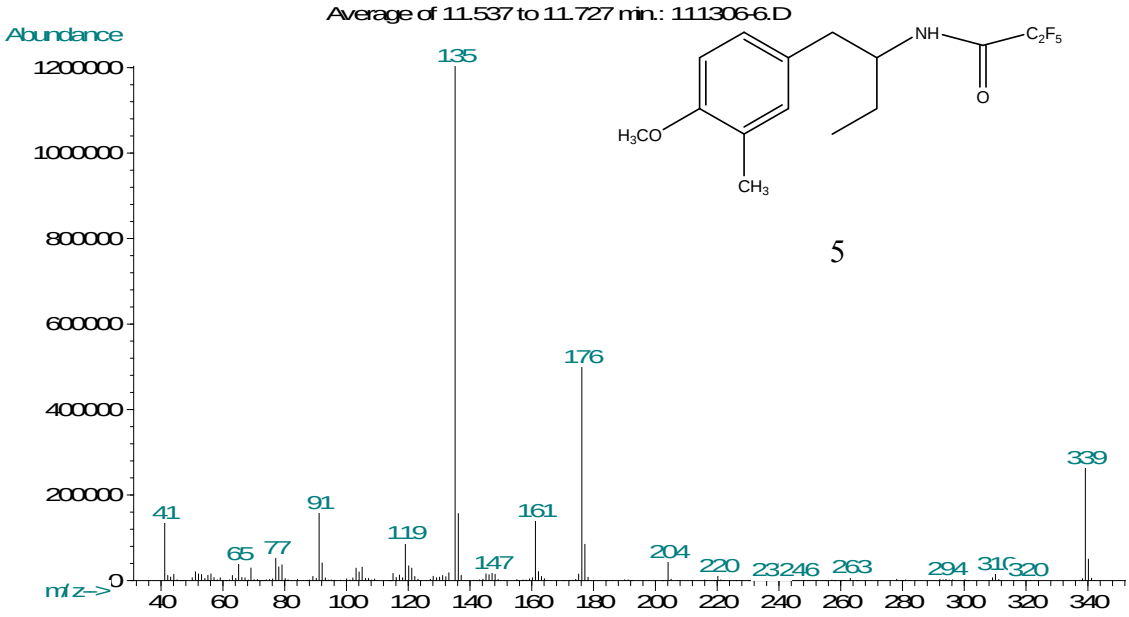

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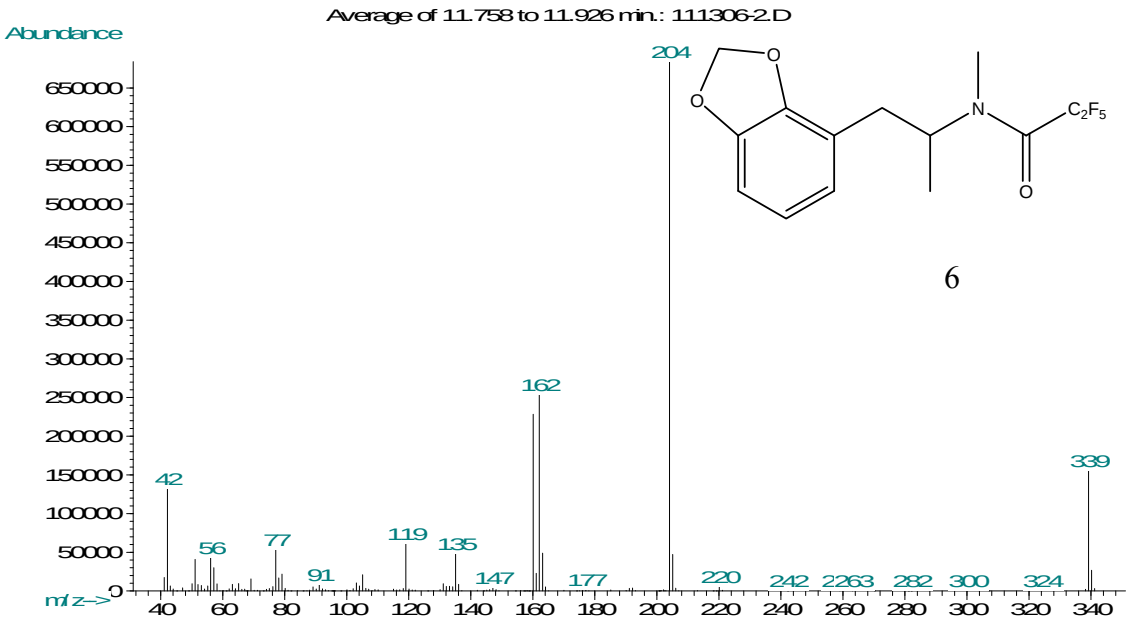

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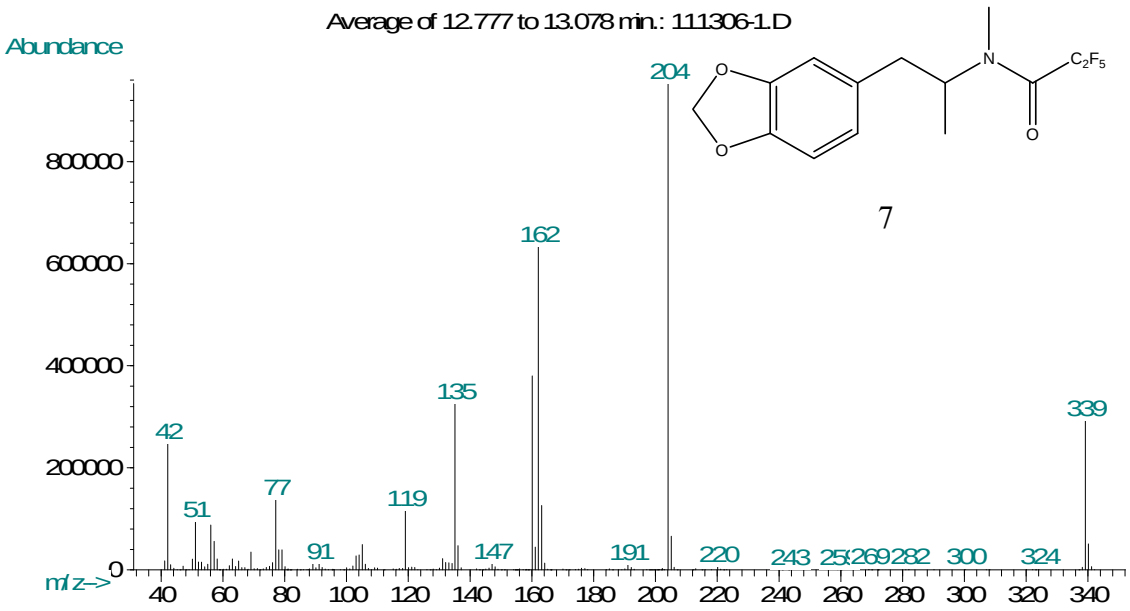

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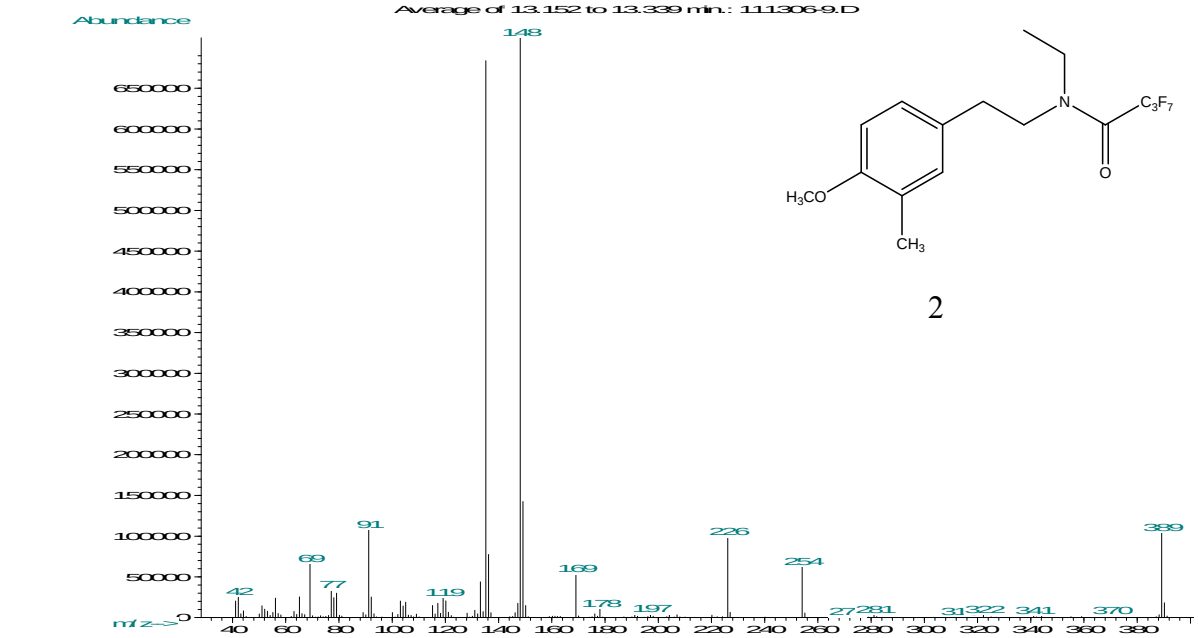
Figure 4.4: Mass spectra of the HFBA derivatives of compounds 2-7



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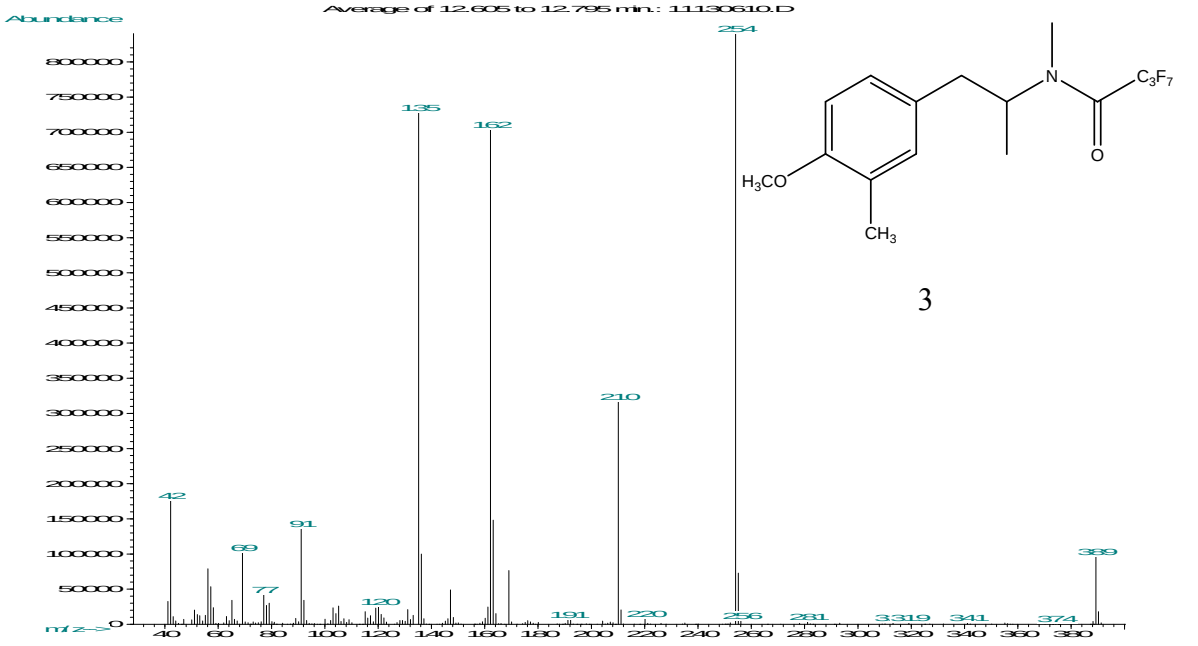

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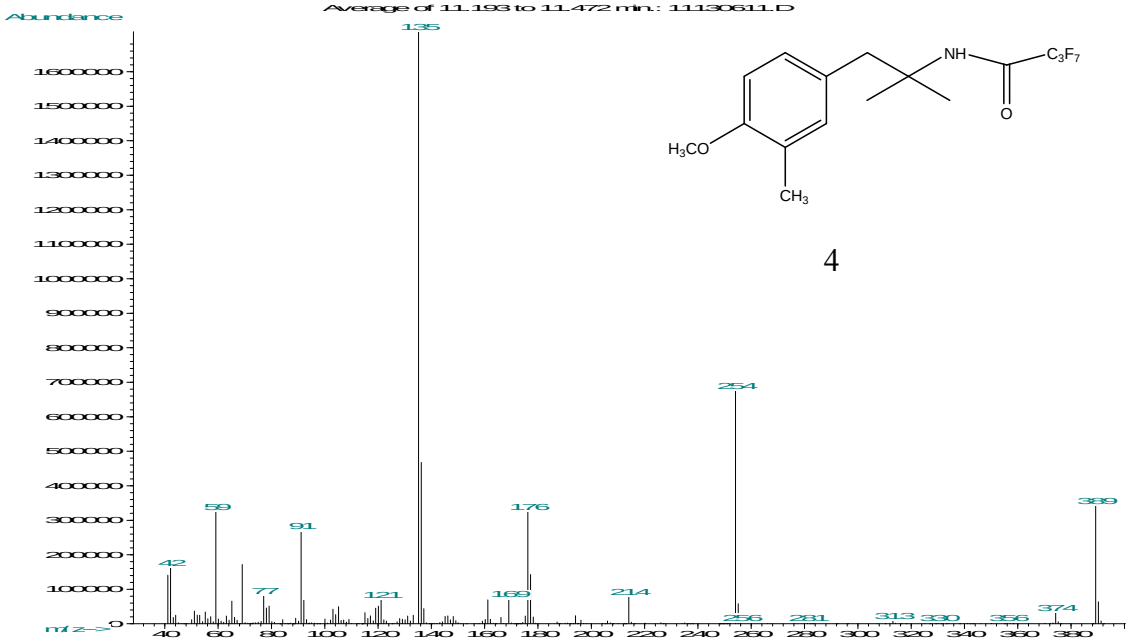

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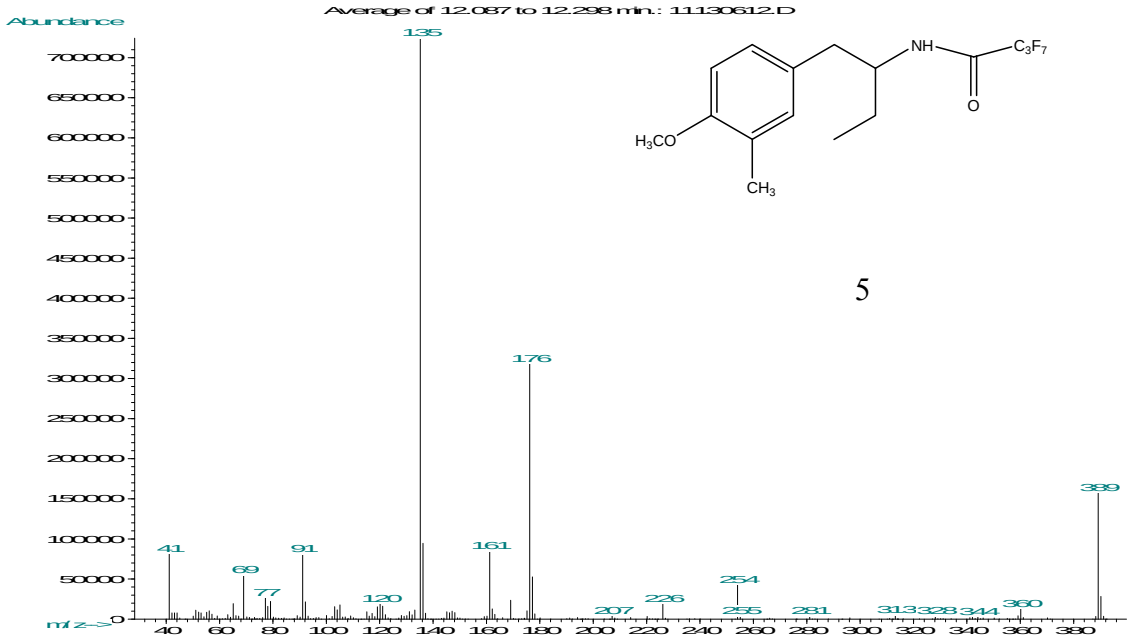

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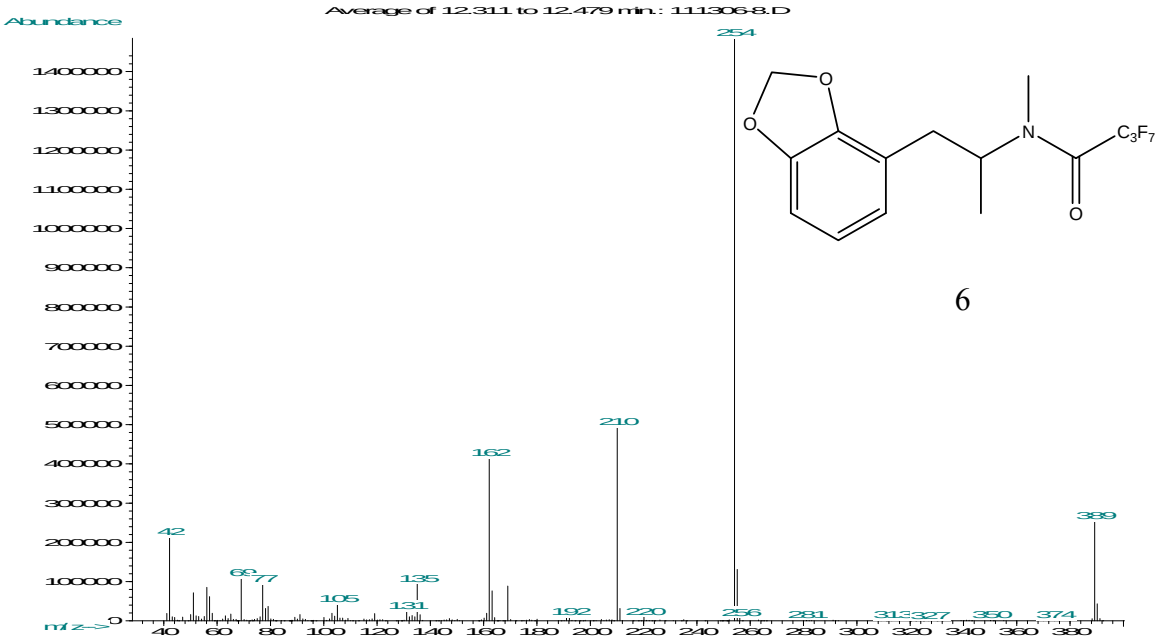

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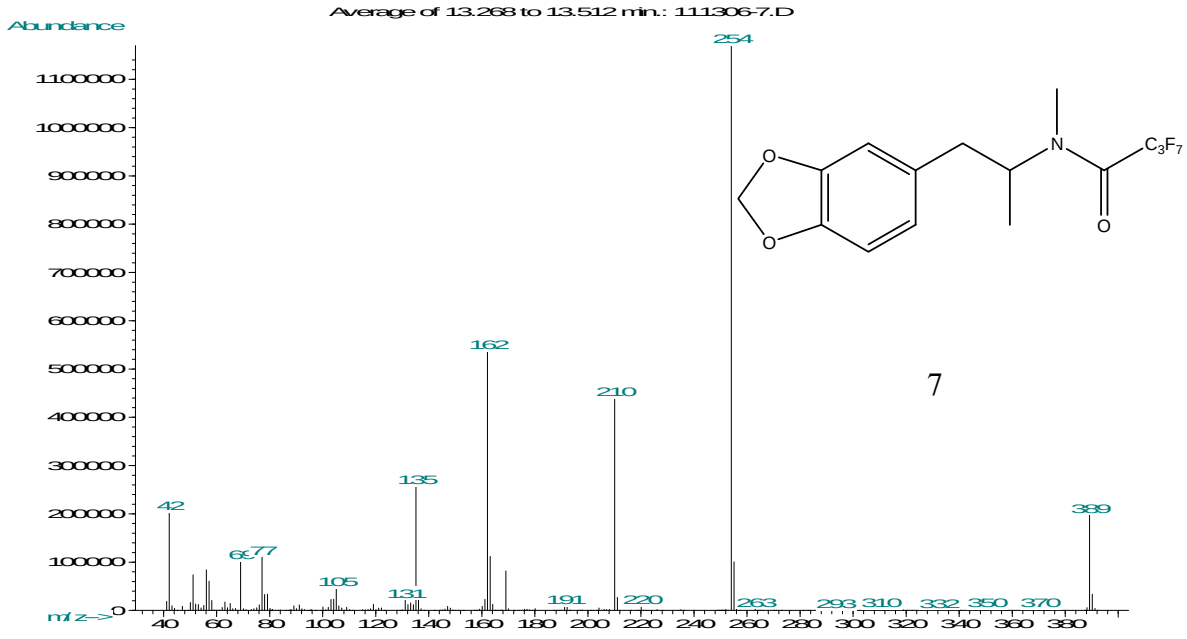

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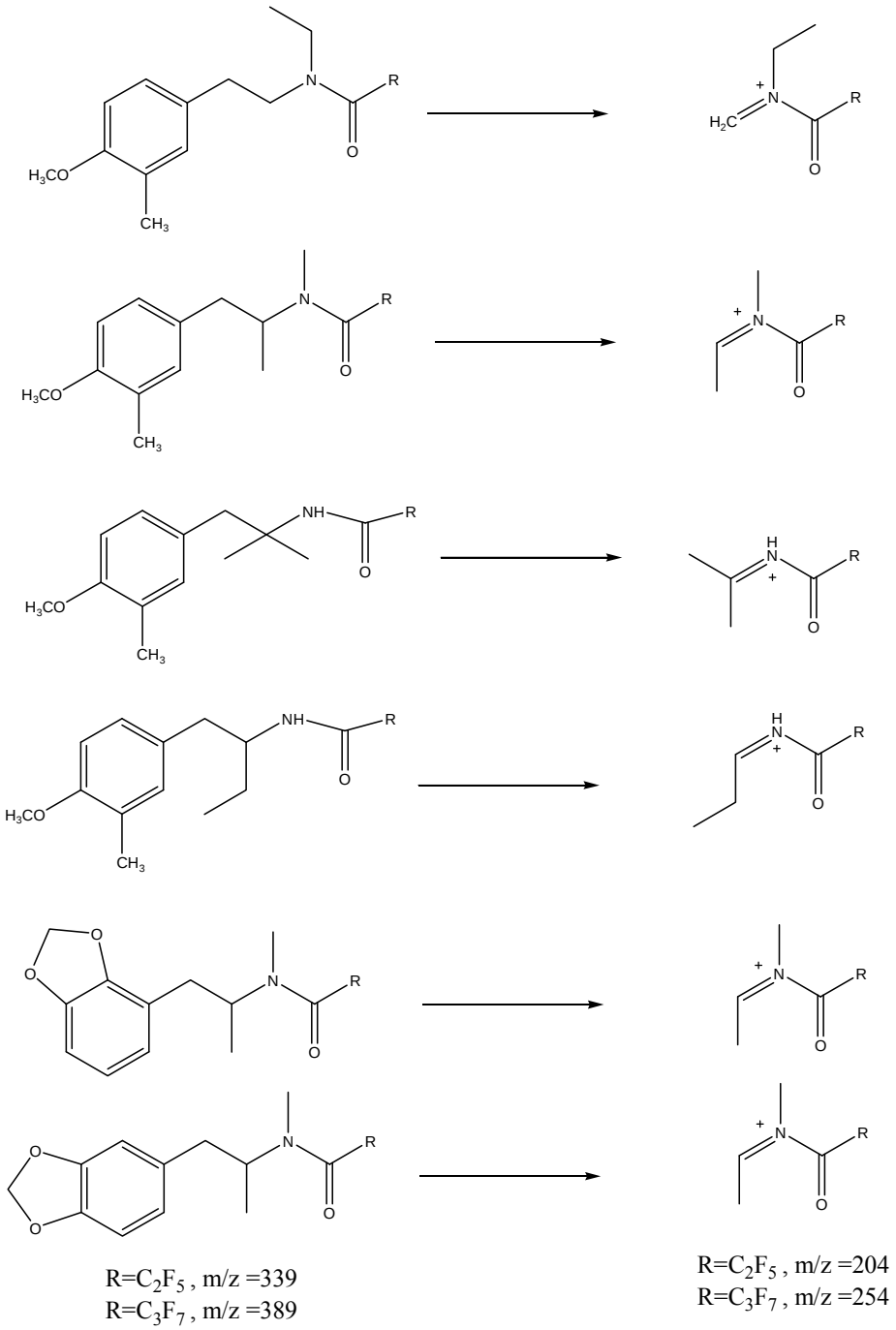
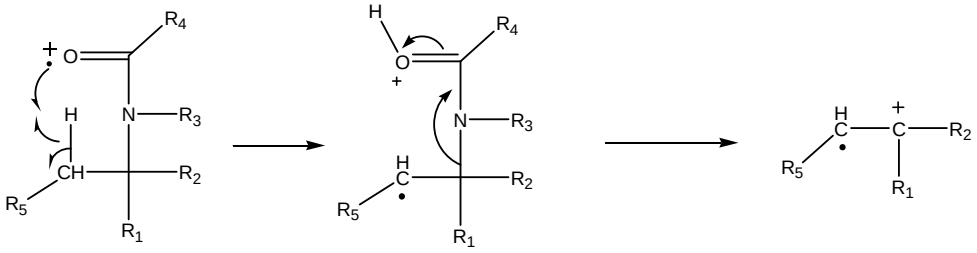


Scheme 4.2: Formation of the $(M-135)^+$ ions in the perfluoroacyl derivatives of the regioisomeric 4-methoxy-3-methylphenethyl amines, 2,3- and 3,4-MDMA.

The spectra for the N-ethyl isomer (compound 2) in Figures 4.3-2 and 4.4-2 show a base peak at m/z 148 corresponding to the alkene radical cation which occurs from

hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (see Scheme 4.3). This ion at m/z 148 would only occur for the N-ethyl regioisomer. The spectra in Figures 4.3-3, 4.3-6, 4.3-7, 4.4-3, 4.4-6 and 4.4-7 show the substituted phenylpropane hydrocarbon ion at m/z 162, identifying these molecules as the PFPA and HFBA derivatives of 4-methoxy-3-methylmethamphetamine and the 2,3-, 3,4-methylenedioxymethamphetamines respectively. The proposed mechanism for the formation of the hydrocarbon fragment is illustrated in Scheme 4.3.



R_3 = C_2H_5 (Compound 2)
CH_3 (Compounds 3,6,7)
H_2 (Compound 4,5)
R_4 = C_2F_5, PFPA Derivatives
C_3F_7, HFBA Derivatives
R_5 = 4-Methoxy-3-methyphenyl (Compounds 2-5)
= 2,3-Methylenedioxyphenyl (Compound 6)
=3,4-Methylenedioxyphenyl (compound 7)

R_1=R_2 = H, m/z= 148 (Compound 2)
R_1= H, R_2= CH_3, m/z =162 (Compounds 3,6,7)
R_1=R_2 = CH_3, m/z =176 (Compound 4)
R_1=H, R_2= C_2H_5, m/z=176 (Compound 5)

Scheme 4.3: Mechanism for the formation of the alkene radical cation in the perfluroacyl derivatives ofthe regioisomeric 4-methoxy-3-methylphenethyl amines, 2,3- and 3,4-MDMA.

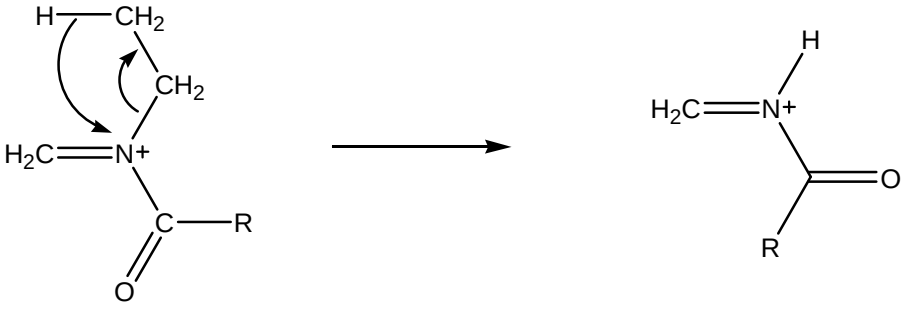
However the base peak of m/z 135 in the spectra of the PFPA derivative of compound 3 offer a significant discrimination of 4-methoxy-3-methylmethamphetamines over its two isobaric methylenedioxymethamphetamines isomers (compounds 6 and 7) . The spectra for the PFPA and HFBA derivatives of the primary amines (compounds 4

and 5) show ions at m/z 176 from the corresponding substituted phenylbutene radical cation. This m/z 176 results from hydrogen rearrangement and subsequent fragmentation of alkyl carbon to nitrogen bond. The lower abundance of m/z 176 for the 4-methoxy-3-methylphentramine (compound 4) may be attributed to steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

While the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the N-ethylphenethylamines (Figure 4.3-2) is the appearance of an ion at m/z 176 in addition to the base peak at m/z 148. The 176 ion suggests a four carbon chain directly attached to the aromatic ring as occurs for the alpha-ethyl- (compound 5) and alpha, alpha-dimethyl- (compound 4) phenethylamines (Figures 4.3-4, 4.3-5 and 4.4-4, 4.4-5). The m/z 176 ion in the spectra for the PFPA derivatives of the N-ethyl regioisomers (Figure 4.3-2) is a rearrangement of the m/z 204 ion resulting in the loss of mass 28 (the N-ethyl group) via hydrogen transfer (see Scheme 4.4). This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the N-ethyl-phenethylamines shown in Figure 4.4-2. The loss of 28 mass units from the acylimine fragment at m/z 254 yields the equivalent fragment ion at m/z 226. Thus, the HFBA derivatives may offer more characteristic ions for individualization of these regioisomeric substances.

A comparison of the mass spectra for the PFPA and HFBA derivatives of all three ring substituted methamphetamines (Compounds 3, 6 and 7) indicates unique ions at m/z 160 and m/z 210 (see Figures 4.3-3, 4.3-6, 4.3-7; 4.4-3, 4.4-6, and 4.4-7). This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each

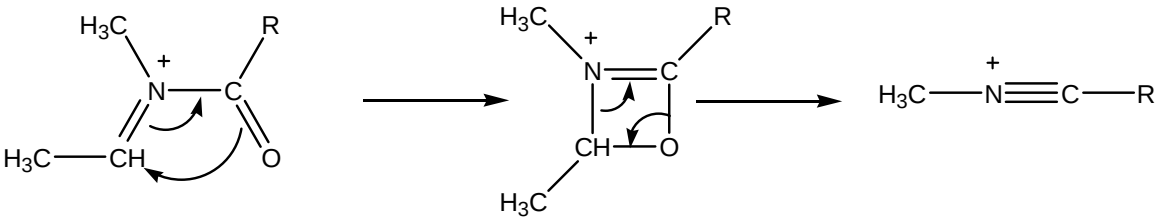
derivative, C_2F_5 and C_3F_7 respectively. An analysis of the masses of the components which make up the fragment at m/z 160 for example include C_2F_5 (119 mass units) and CH_3 (15 mass units) leaving only a mass of 26 available for the total of 160. The mass 26 would correspond to CN and the proposed mechanism for the formation of $(C_2F_5CNCH_3)^+$ is shown in Scheme 4.5. An equivalent fragmentation pathway has been further supported by the analysis of the mass spectra of the PFPA and HFBA derivatives of d_3- and d_5-MDMA in chapter 1.



R=C_2F_5, m/z = 204
R=C_3F_7, m/z = 254

R=C_2F_5, m/z = 176
R=C_3F_7, m/z = 226

Scheme 4.4: Mechanism for the loss of mass 28 from the perfluoroacyl-N-ethyl-imine cation.



Scheme 4.5: Mechanism for the formation of the m/z 160 and m/z 210 ions in the mass spectra of the perfluoroacyl derivatives of the ring substituted methamphetamines (Compounds 3, 6 and 7).

Gas Chromatography of the regioisomeric 4-methoxy-3-methylphenethyl amines, 2,3- and 3,4-MDMA.

The PFPA and HFBA derivatives of the six primary and secondary amines were compared on two stationary phases of the same dimensions, 30 m x 0.25 mm and 0.25 μm depth of film. The stationary phases compared in this study were the relatively nonpolar phase 100% dimethyl polysiloxane (RTX-1) and the more polar trifluoropropyl methyl polysiloxane (Rtx-200). Several temperature programs were evaluated and one program showing the best compromise between resolution and analysis time was used to generate the chromatograms in Figure 4.5. The chromatograms of the perfluoroacyl compounds show that the 2,3-MDMA elutes before the corresponding 3,4-isomer, which elutes last. When the ring substitution pattern is held constant (4-methoxy-3-methyl), the side chain elution order is secondary amides before the tertiary amides and in this limited set of examples branched isomers elute before the more linear ones. Therefore, the

amides of compound 4 elute first followed by the amide of compound 5 (both secondary amides), then the amides of compound 3 (the methamphetamine side chain) and finally the amides of compound 2 (the more linear of the tertiary amides). The amides of 2,3-MDMA elute between the secondary and tertiary amides in this group of compounds. Perhaps the most useful information in these chromatograms is the relative elution of the derivatized controlled substance 3,4-MDMA and its closest eluting regioisomeric and isobaric equivalents. Both the PFPA and HFBA derivatives of 3,4- MDMA elute last and the N-ethyl-4-methoxy-3-methylphenethylamine PFPAs and HFBAs are the closest eluted compounds in the 4-methoxy-3-methyl phenethylamine series. The isobaric N-ethyl amides show very distinct mass spectra with several characteristic ions to differentiate it from the corresponding amides of the drug of abuse 3,4-MDMA. Thus, derivatization methods coupled with both chromatographic and mass spectral procedures can allow for the complete differentiation of the side chain substitution pattern of the 4-methoxy-3-methylphenethylamines from 3,4-MDMA and its regioisomer, 2,3-MDMA.

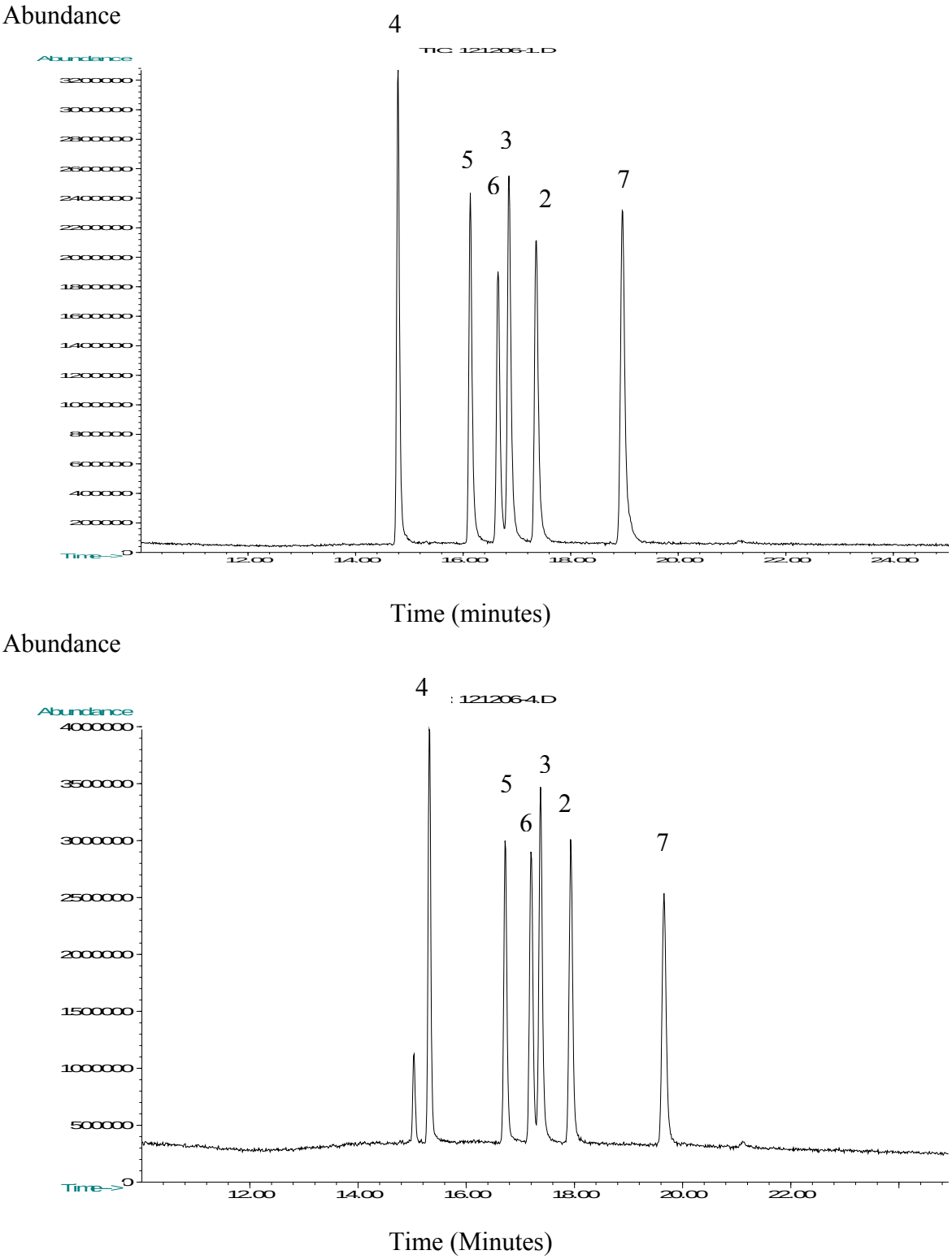


Figure 4.5: GC Chromatograms of the PFPA (A) and HFBA (B) derivatives of compounds 2-7. Rtx-200 column.

Conclusions of Chapter 4

3,4-MDMA , 2,3-MDMA and the five side chain regioisomers of 4-methoxy-3-methyl phenethylamines are a unique subset of regioisomeric and isobarc molecules; each compound has a molecular weight of 193 and yields a base peak at m/z 58 in the mass spectrum from the loss of the corresponding methylenedioxybenzyl and 4-methoxy-3-methylbenzyl groups respectively. Thus the traditional electron impact mass spectrum provides little structural information for differentiating among these seven compounds. Because of the unique similarity of these compounds by mass spectrometry, the specific identification of a compound such as 3,4-MDMA requires methods to eliminate other regioisomeric and isobaric substances.

This elimination process could be accomplished on the basis of chromatography alone but ultimately would require the analyst to use reference samples of the other substances. Derivatization of the primary and secondary amines with various acylating agents yields amides with improved resolution compared to the underivatized amines by capillary gas chromatography on TRx-1 and TRX-200 stationary phases. Additionally, the perfluoroacyl derivatives significantly individualize the mass spectra for these amides and allow for specific identification. The individualization is the result of fragmentation of the alkyl carbon-nitrogen bond yielding characteristic hydrocarbon fragments at m/z 148, 162 and 176 as well as other unique fragments.

Chapter 5

Gas Chromatographic and Mass Spectral Studies on Acylated Side Chain Regioisomers of 3-Methoxy-4-methyl-phenethylamine and 4-Methoxy-3-methyl-phenethylamine

This chapter describes the MS and GC behavior of a set of selected compounds having nonequivalent ring substituents at the 3- and 4-position of the aromatic ring. Thus, these ten compounds represent all the possible regioisomeric methoxy methyl phenethylamines having the same 3,4 substitution pattern as 3,4-MDMA.

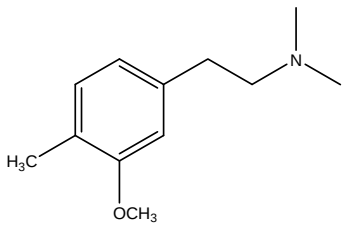
Mass Spectral Studies on Side Chain Regioisomers of 3-Methoxy-4-methyl-phenethylamine and 4-Methoxy-3-methyl-phenethylamine

Mass spectrometry is the primary method for confirming the identity of drugs and related substances in forensic samples. The mass spectra of phenethylamines are characterized by a base peak formed from an amine initiated alpha-cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-methylenedioxymethamphetamine (MW=193) the alpha-cleavage reaction yields the substituted imine fragment at m/z 58 and the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-methylenedioxymethamphetamine contains major ions at m/z 58 and 135/136 as well as other ions of low relative abundance [Chapter 1].

The side chain regioisomers of 3-methoxy-4-methyl phenethylamine and 4-methoxy-3-methyl phenethylamine (Compounds 1-10, Figure 5.1) have the potential to

yield a mass spectrum essentially equivalent to 3,4-MDMA. All have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136 (Figure 5.2). The individual mass spectra for 2,3- and 3,4-MDMA are also presented in Figure 5.2 (Compounds 11 and 12). The isobaric methoxy-methyl-benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. Furthermore the m/z 58 ion in the ring substituted methoxy-methyl phenethylamine is regioisomeric with that obtained in the mass spectra of both 2,3 and 3,4-MDMA (Scheme5.1). This lack of mass spectral specificity for the isomers shown in Figure 5.2, in addition to the possibility of chromatographic co-elution with 3,4-MDMA, could result in misidentification in this series of drugs and drug-like substances

Figure 5.1: Structures of Side Chain Regioisomers of 3-Methoxy-4-methyl-phenethylamine, 4-Methoxy-3-methyl-phenethylamine, 2,3- and 3,4-MDMA



N,N-Dimethyl-3-methoxy-4-methyl phenethylamine

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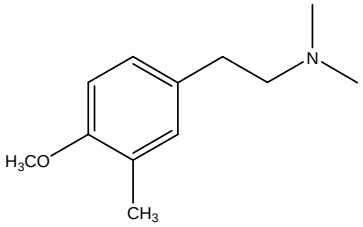

N,N-Dimethyl-4-methoxy-3-methyl phenethylamine

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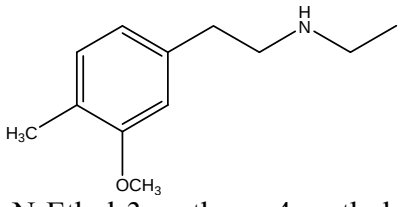
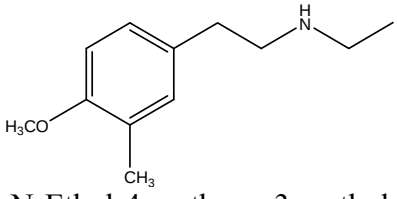


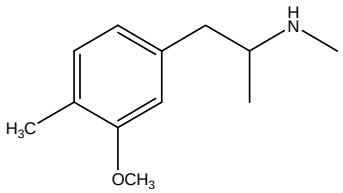
N-Ethyl-3-methoxy-4-methyl phenethylamine

2



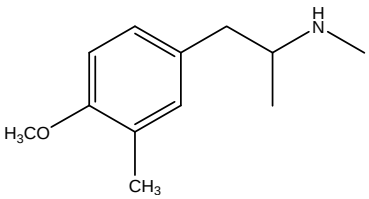
N-Ethyl-4-methoxy-3-methyl phenethylamine

7



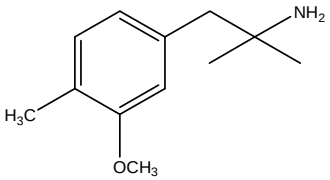
3-Methoxy-4-methyl methamphetamine

3



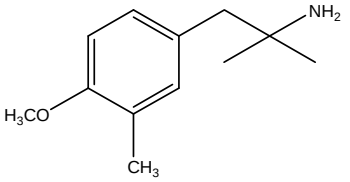
4-Methoxy-3-methyl methamphetamine

8



3-Methoxy-4-methyl phentramine

4



3-Methoxy-4-methyl phentramine

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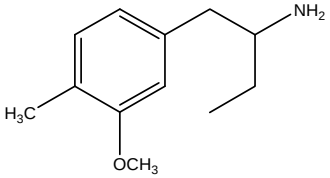

3-Methoxy-4-Methyl BDB

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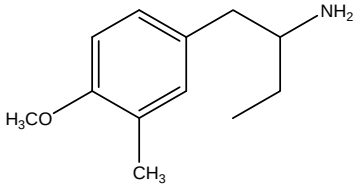

4-Methoxy-3-Methyl BDB

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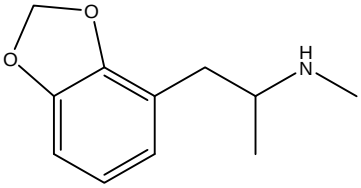
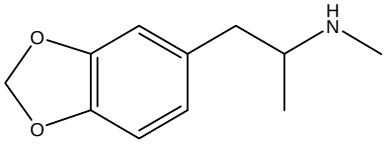


11

(2,3-MDMA)



12

(3,4-MDMA)

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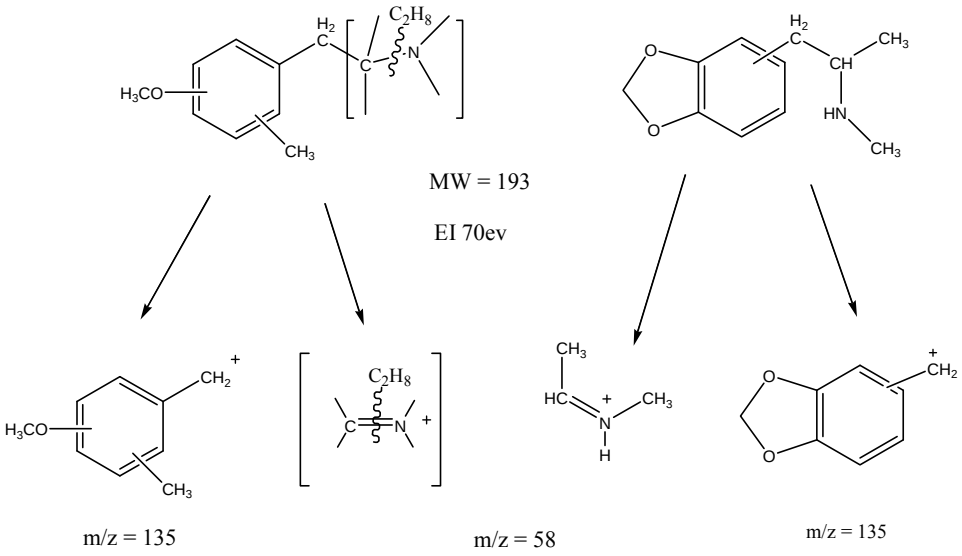
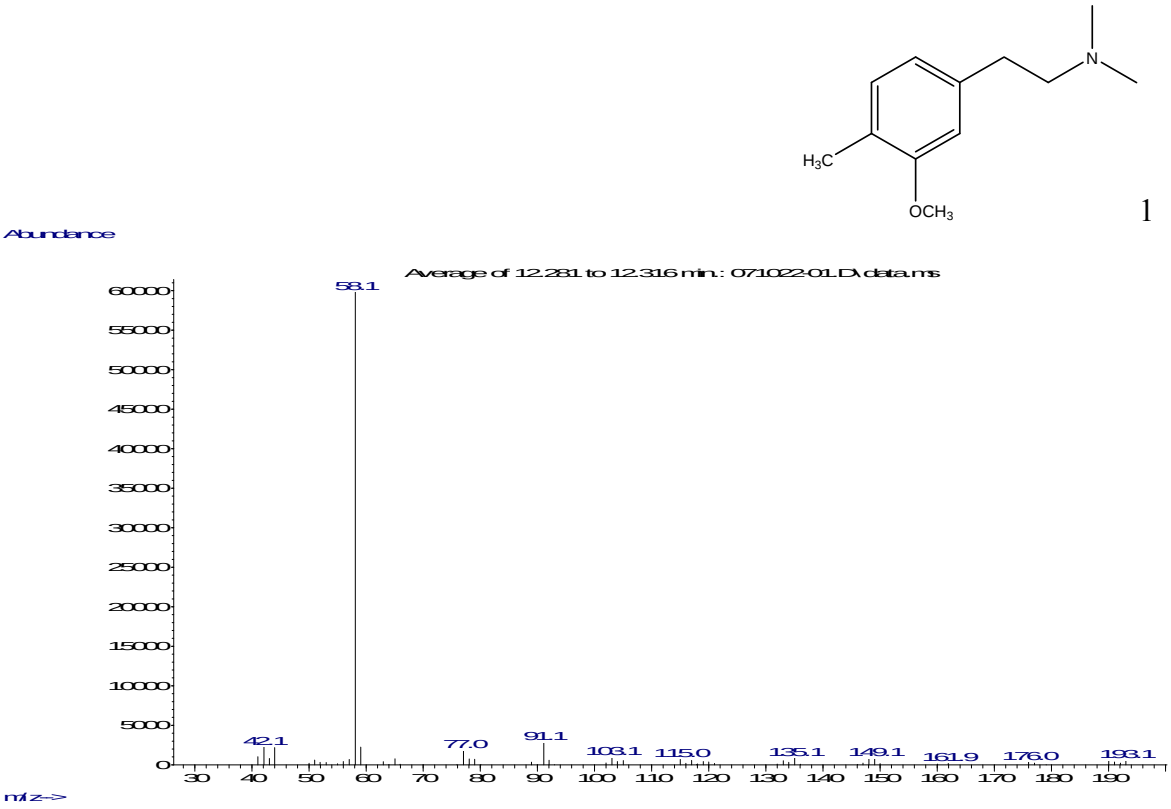
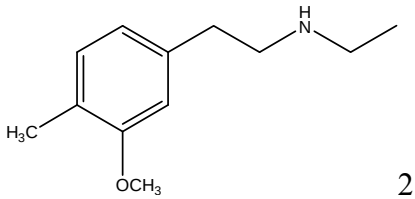


Scheme 5.1: Structures of the major EI fragments for 3-Methoxy-4-methyl-phenethylamine, 4-Methoxy-3-methyl-phenethylamine, 2,3- and 3,4-MDMA

Figure 5.2: Mass Spectra of 3-Methoxy-4-methyl-phenethylamine, 4-Methoxy-3-methyl-phenethylamine, 2,3- and 3,4-MDMA.





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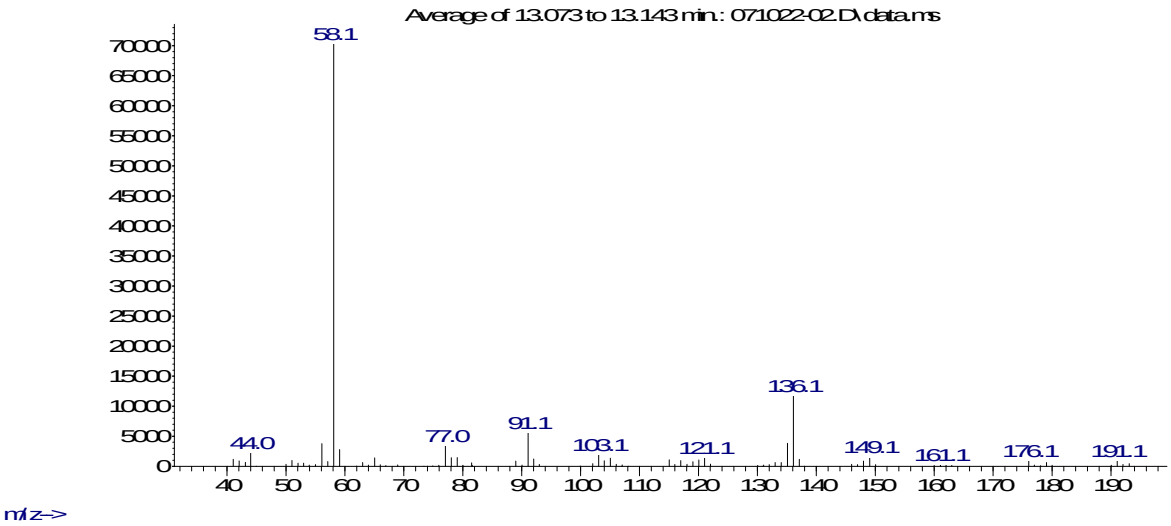

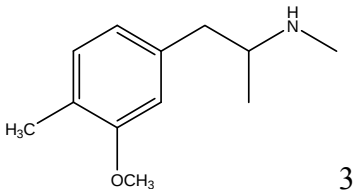

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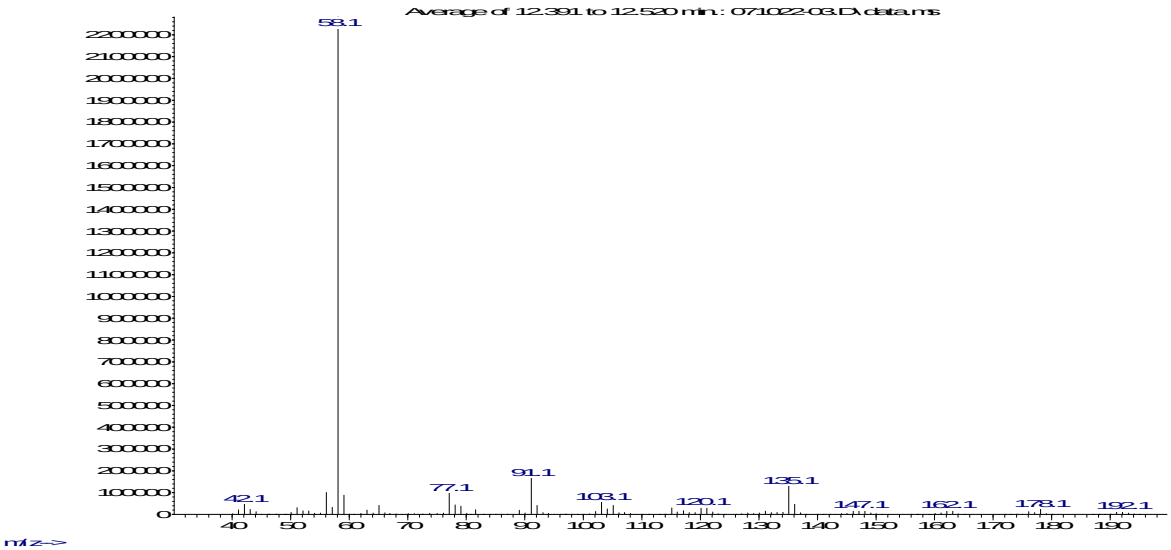

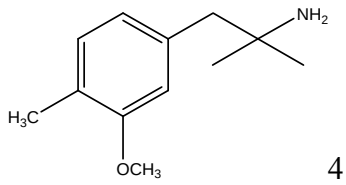

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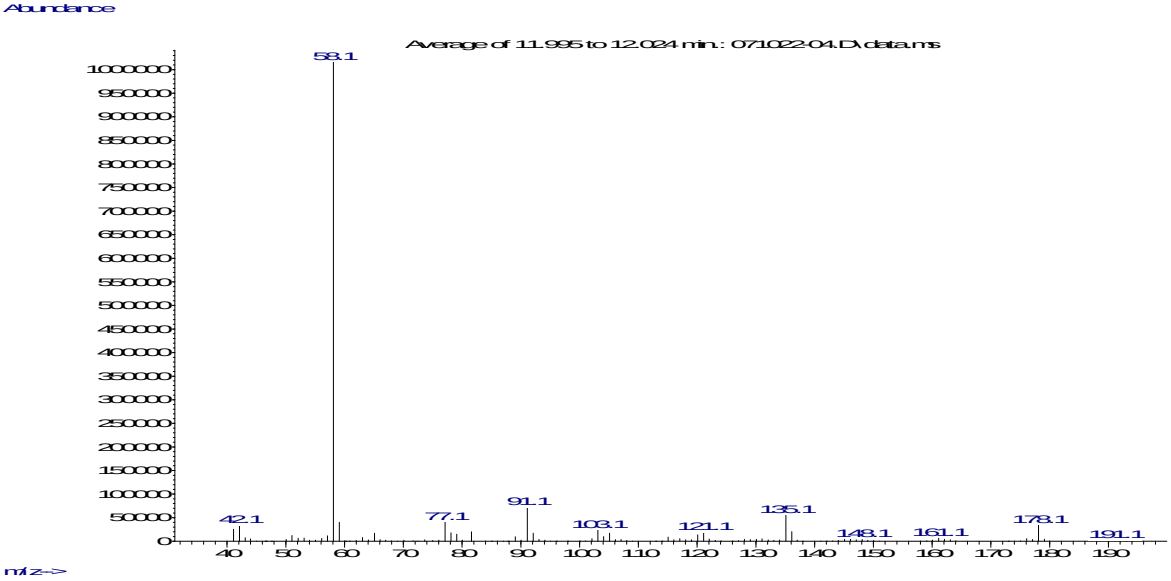

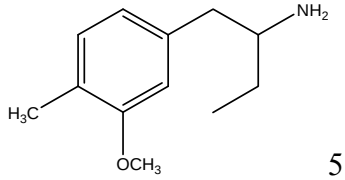

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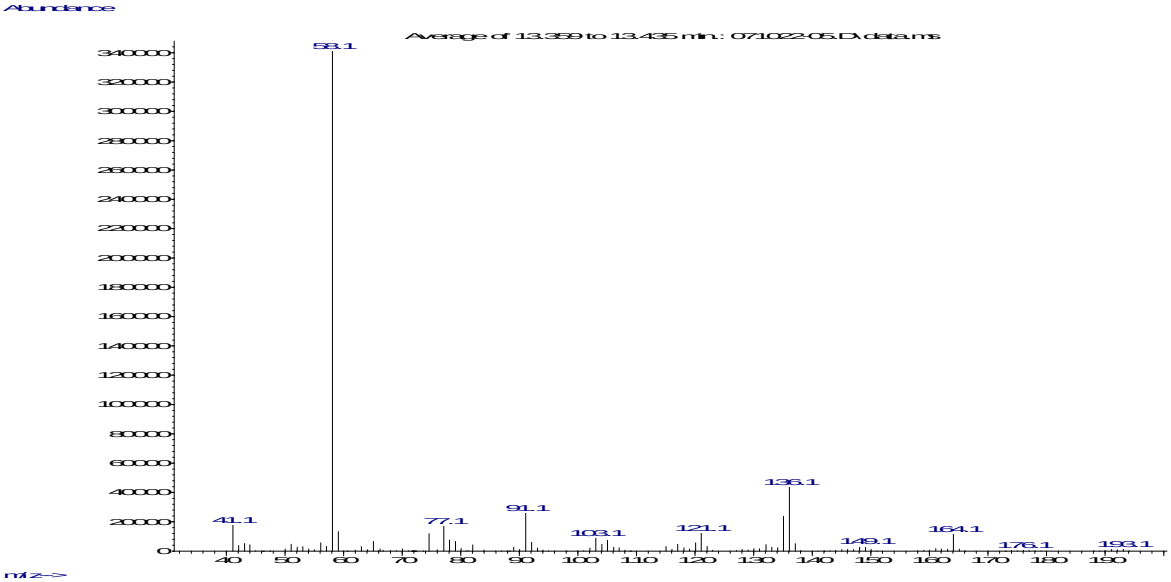

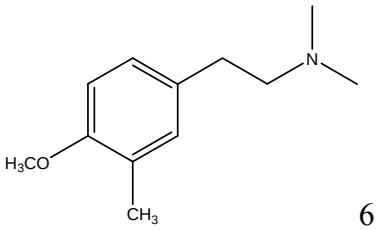

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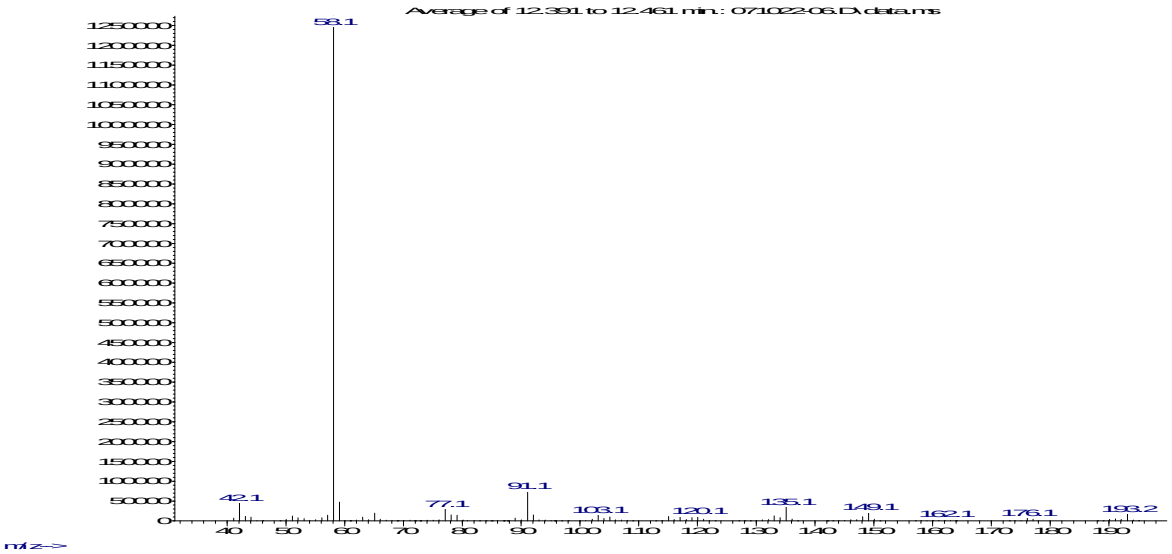

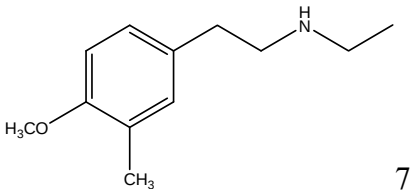

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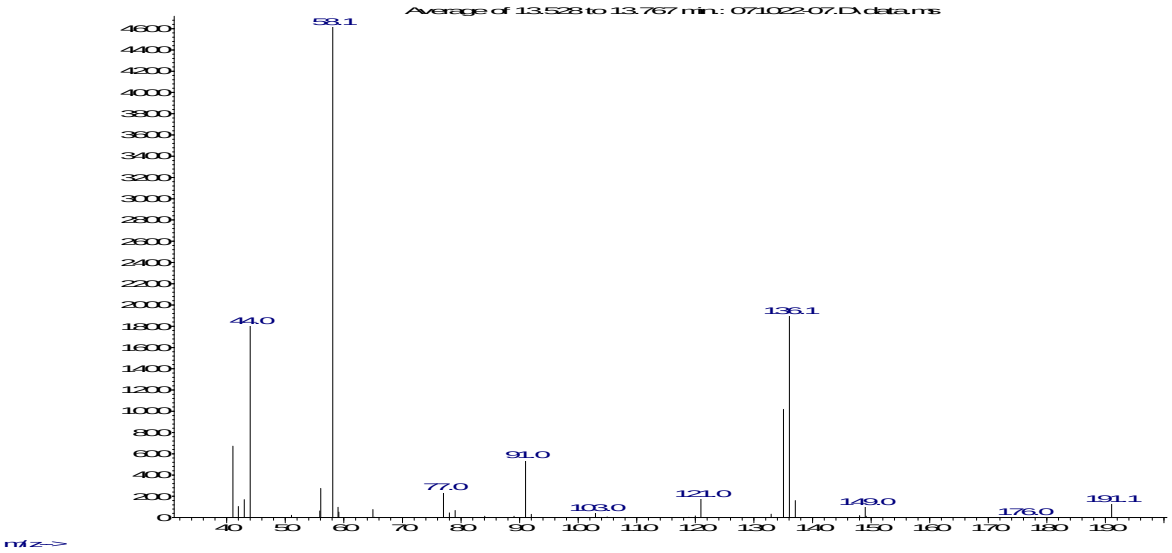

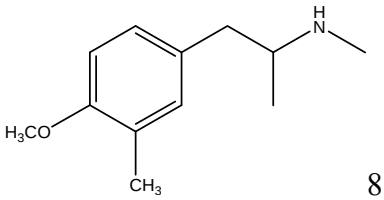

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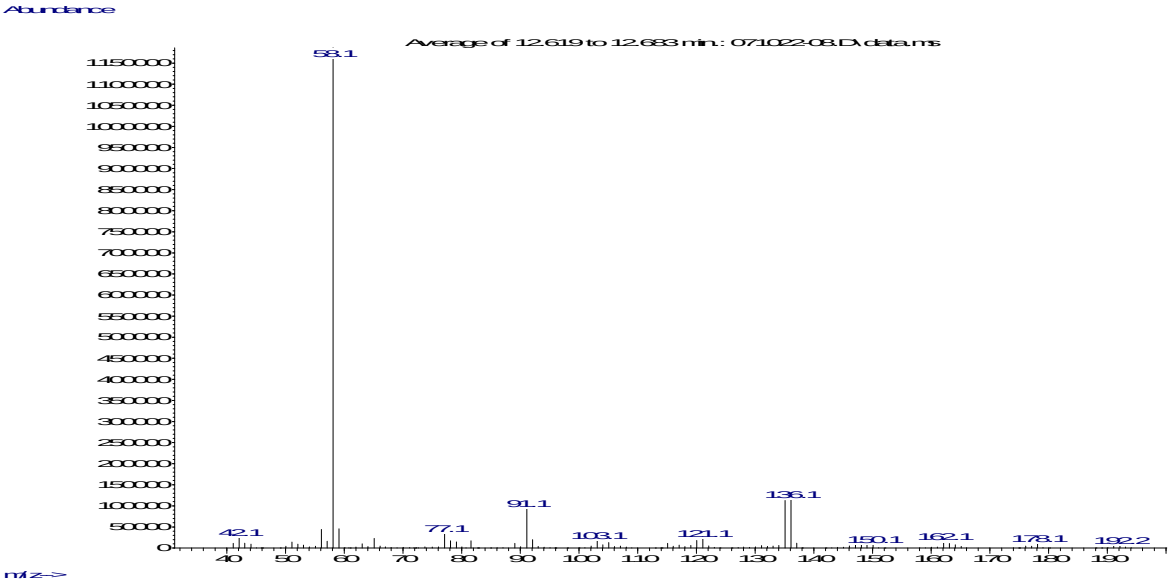

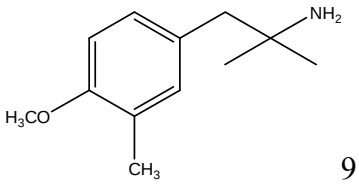

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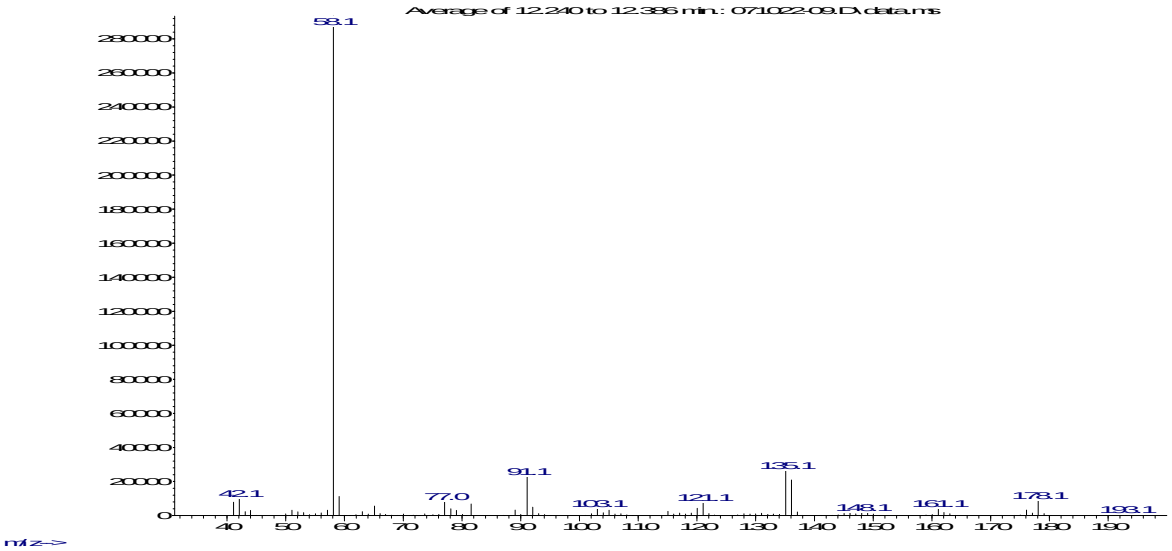

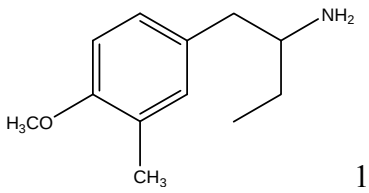

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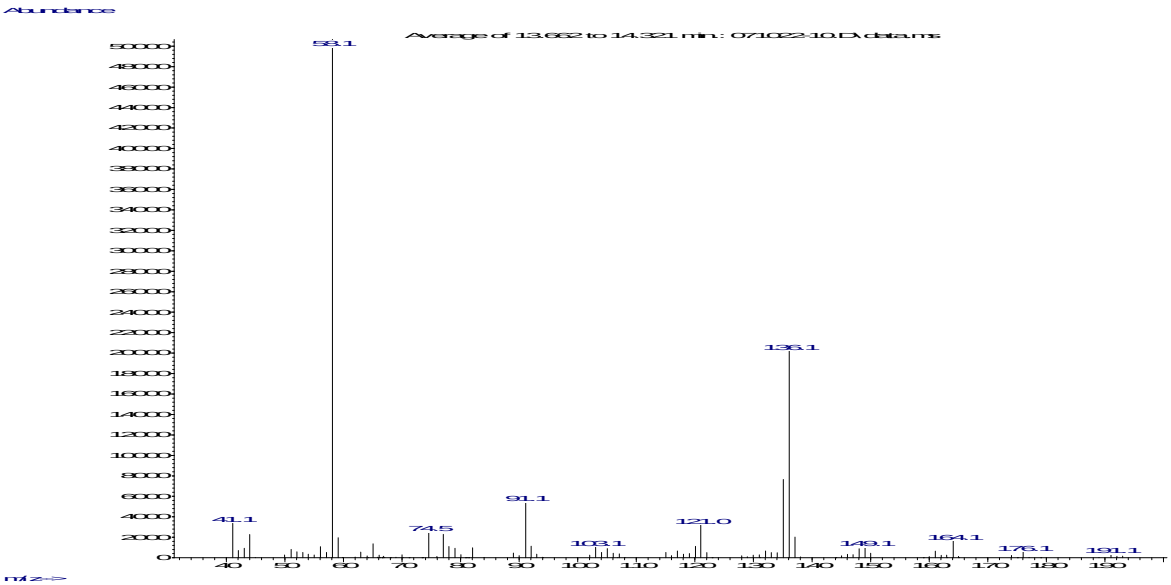

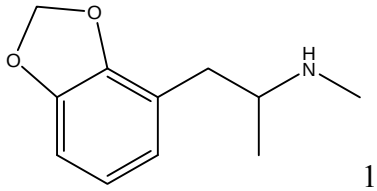

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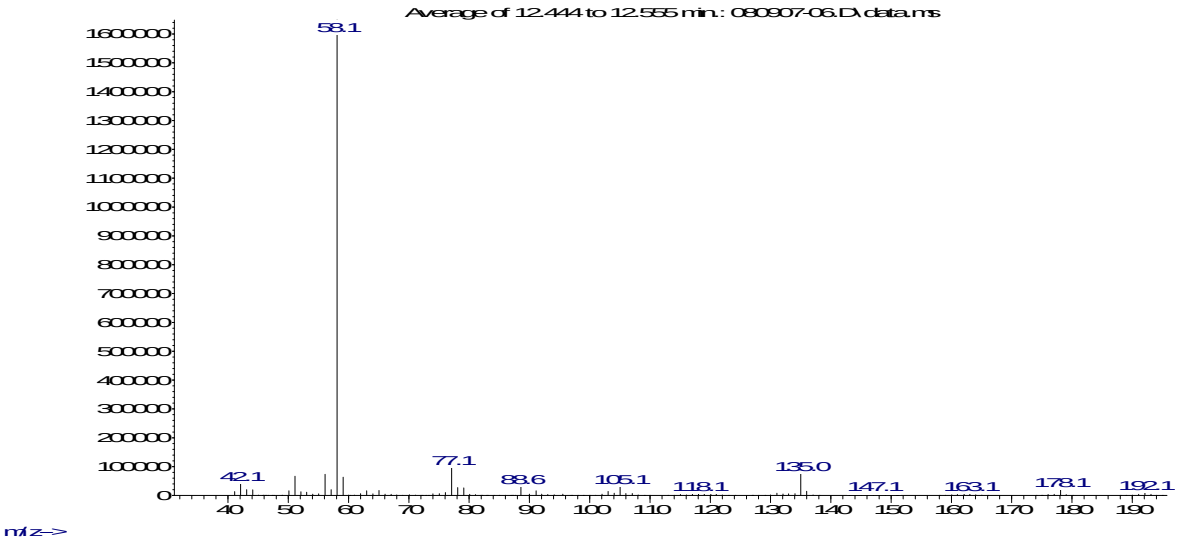

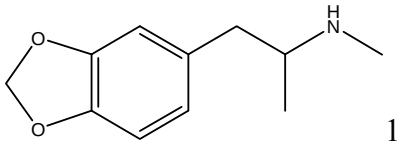

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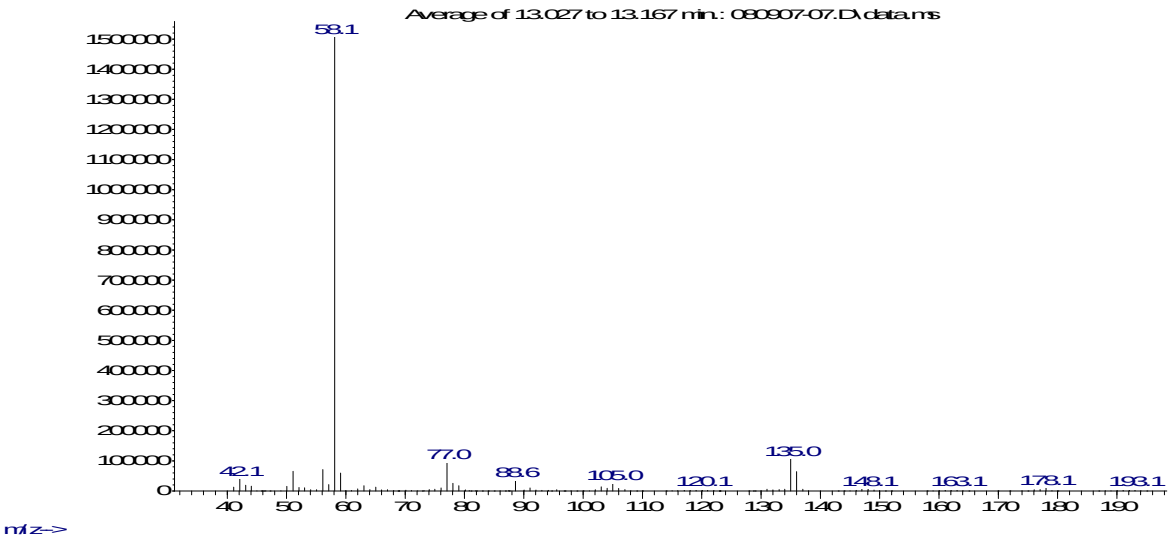
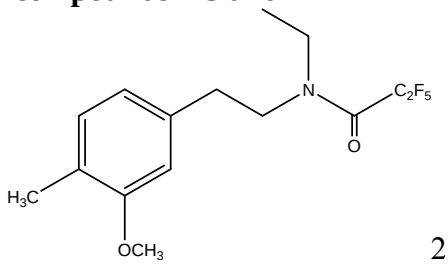


The second phase of this study involved the preparation and evaluation of various perfluoroacylated derivatives of the regioisomeric primary and secondary amines, in an effort to individualize their mass spectra via formation of unique marker ions and improved chromatographic resolution. Acylation of the amines generally lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum. Of course the tertiary amine (compounds 1 and 6) do not form a stable amide derivative.

The mass spectra for the twenty pentafluoropropionyl and heptfluorobutryl amides are shown in Figures 5.3 and 5.4, respectively. From these spectra a common peak occurs at m/z 204 and 254 which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for PFPA and HFPA amides. This ion at m/z 204 and 254 is the PFPA and HFPA imine species likely formed from the alpha cleavage of the amide

nitrogen to eliminate the methoxy-methyl-benzyl or methylenedioxybenzyl radical. Thus the m/z 204 and 254 in PFPA and HFPA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M-135)^+$ species (Scheme 5.2). The 3-methoxy-4-methylbenzyl cation, 4-methoxy-3-methylbenzyl cation and the methylenedioxybenzyl cation (m/z 135) are fragments common to all spectra in Figures 5.3 and 5.4.

Figure 5.3: Mass spectra of the PFPA derivatives of compounds 2-5 and 7-12



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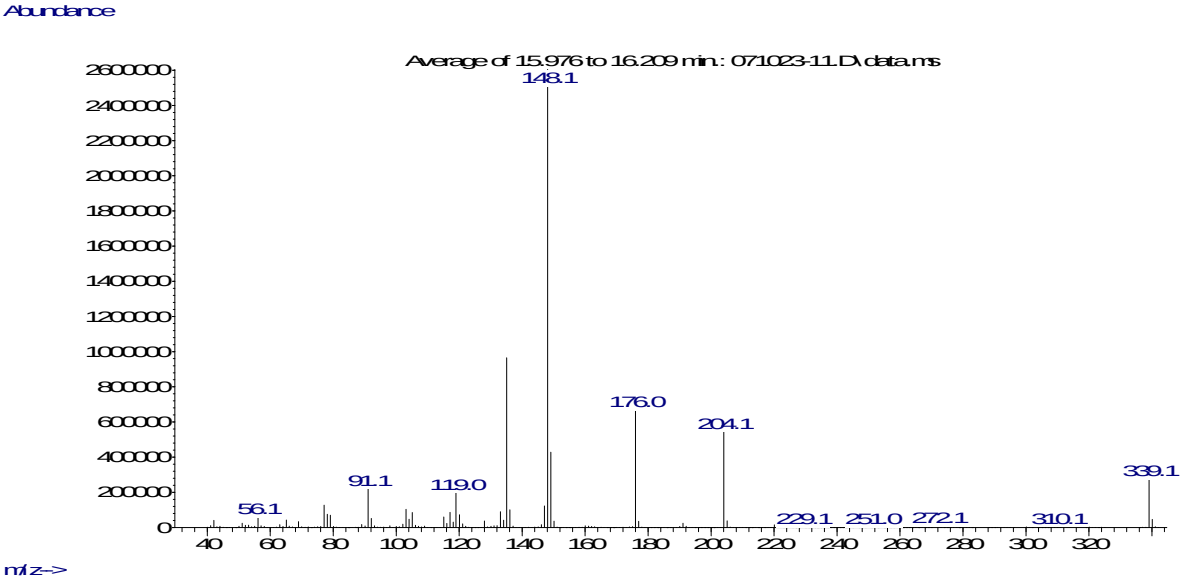

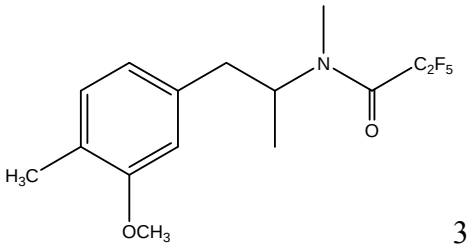

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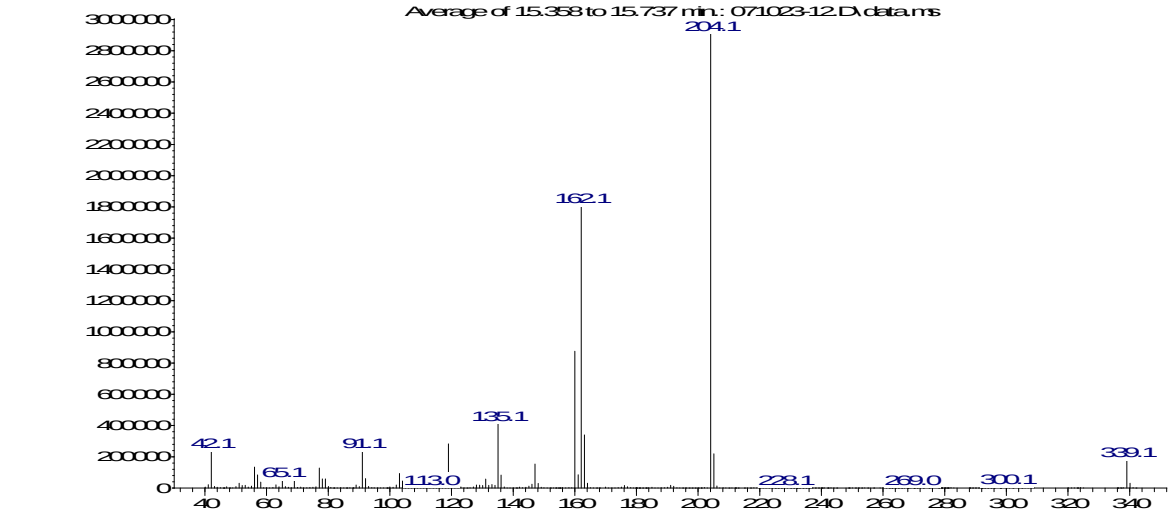

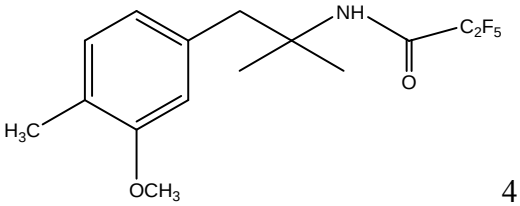

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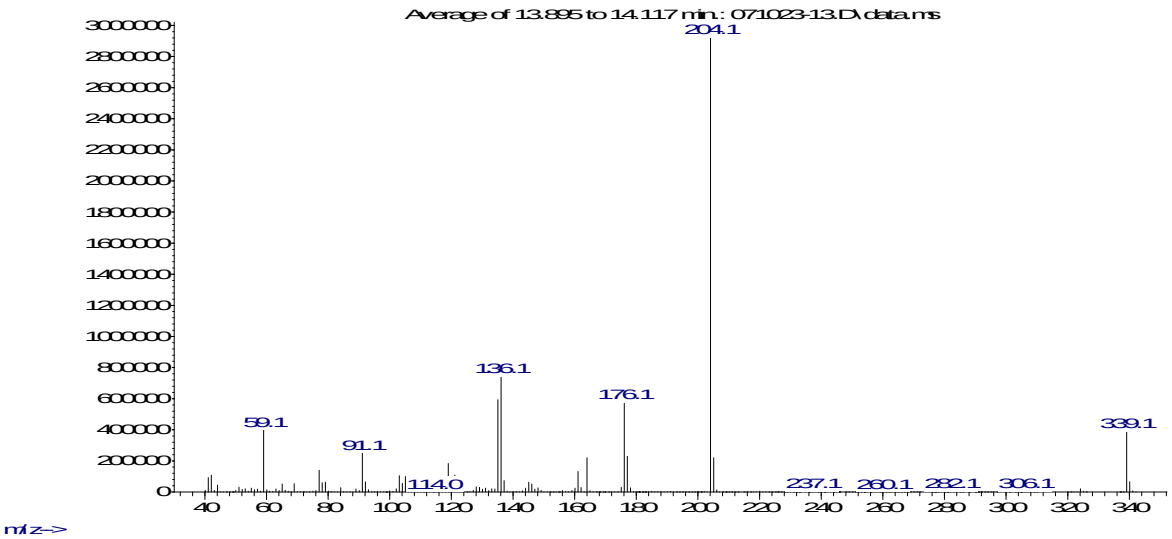

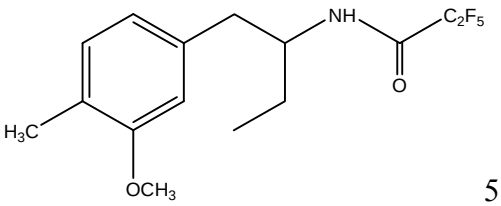

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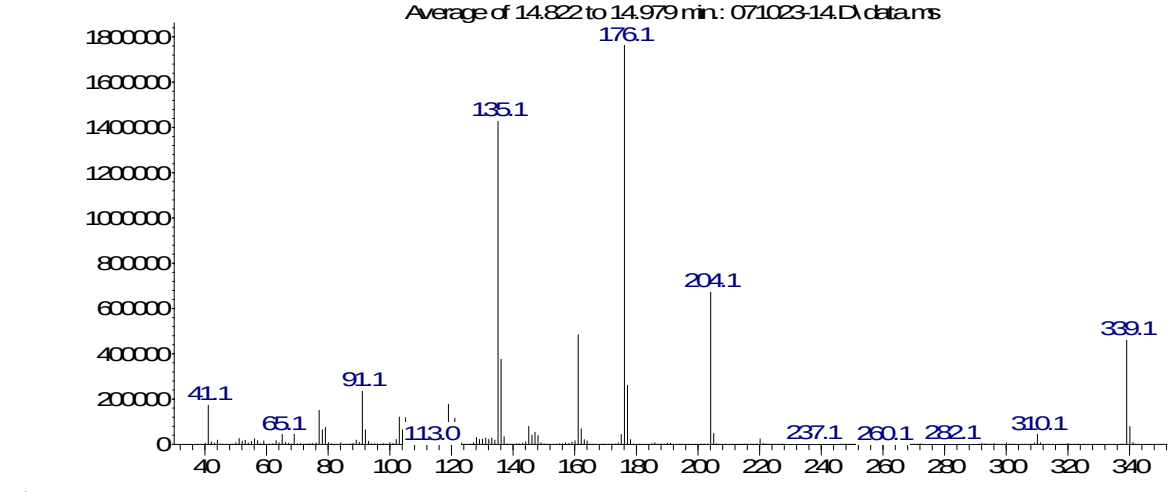

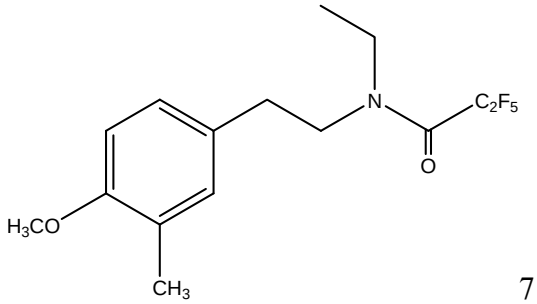

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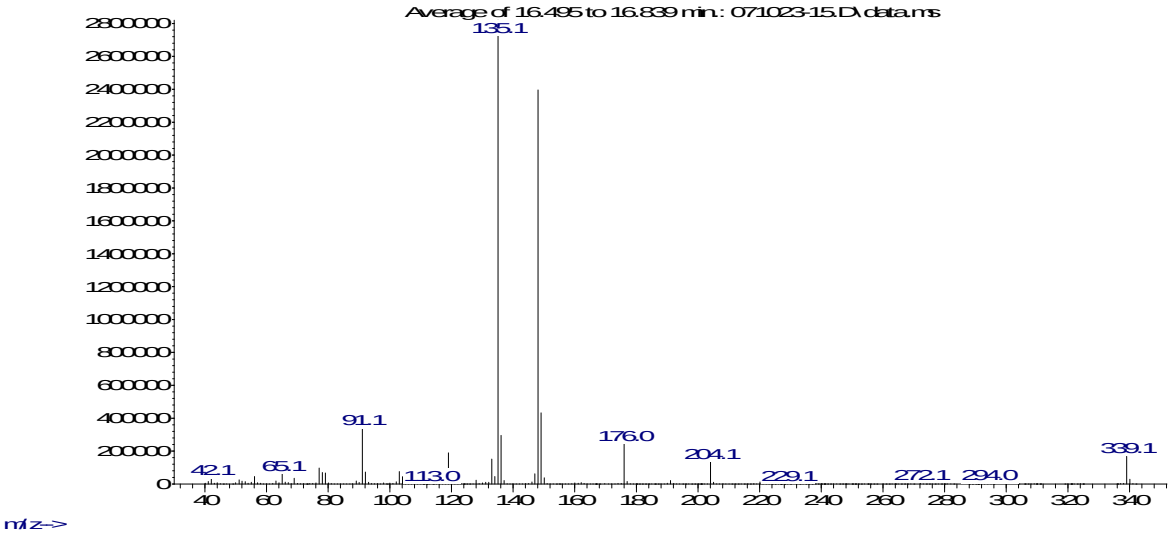

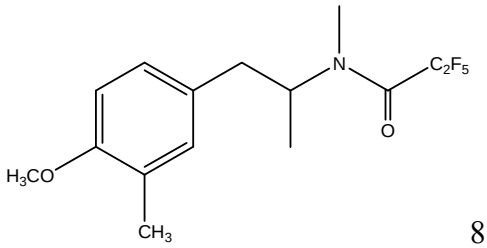

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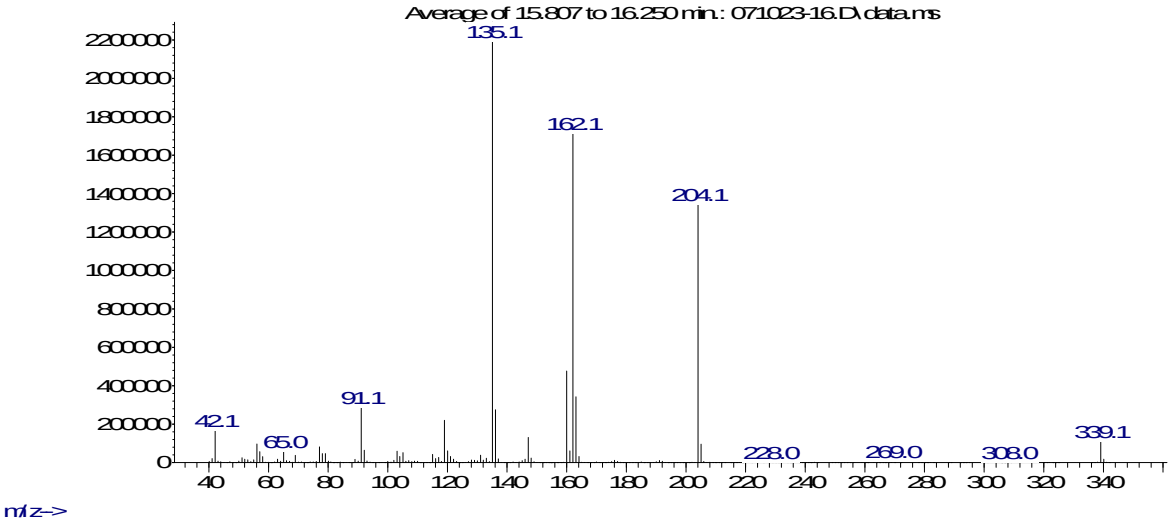

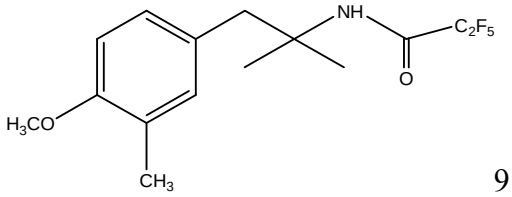

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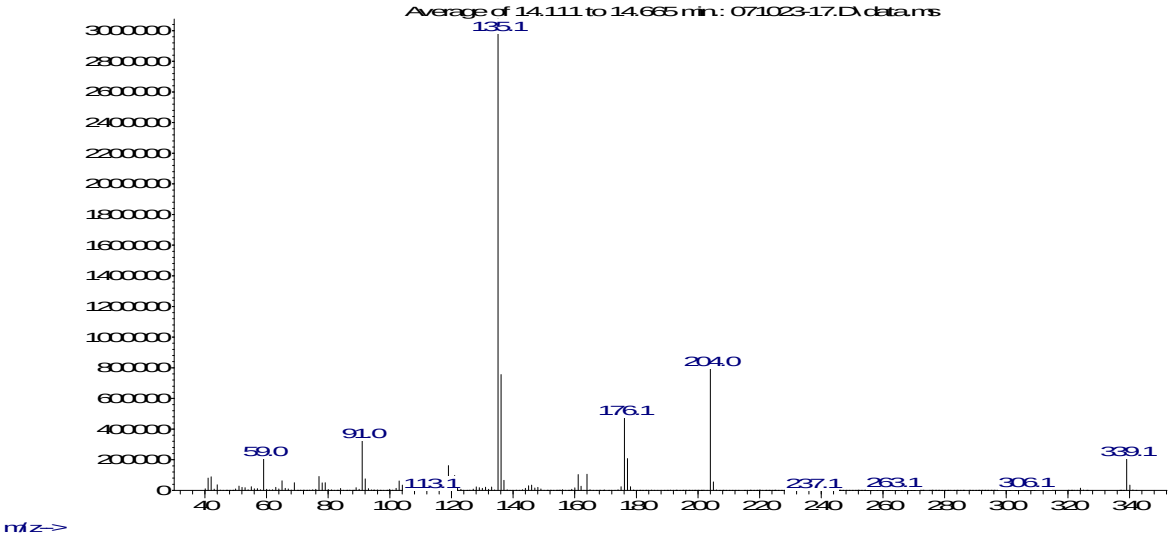

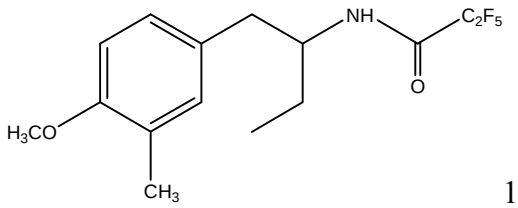

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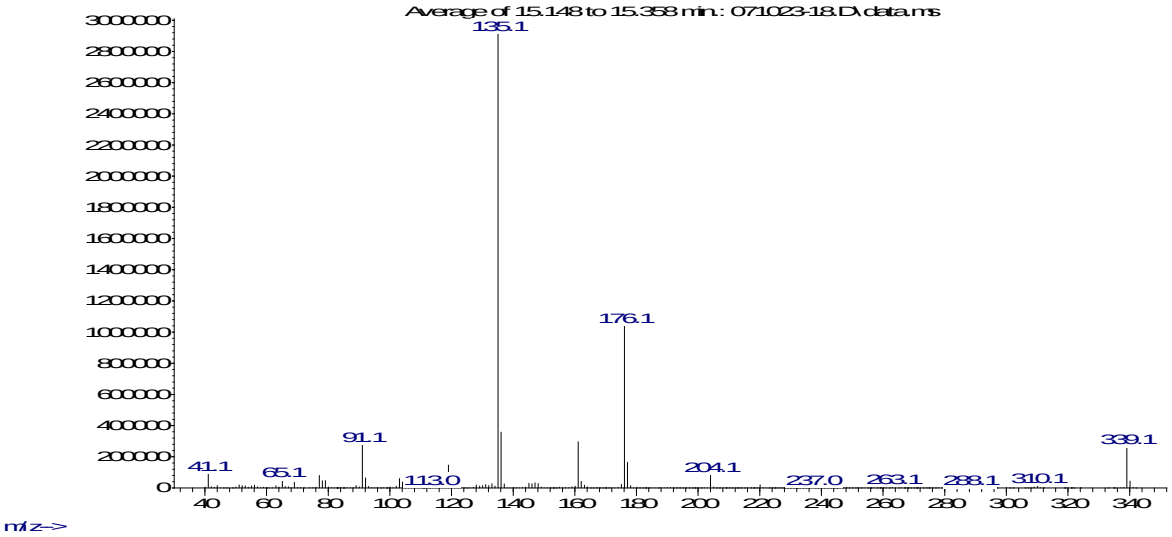

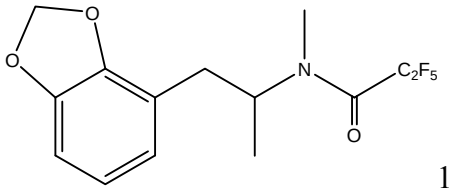

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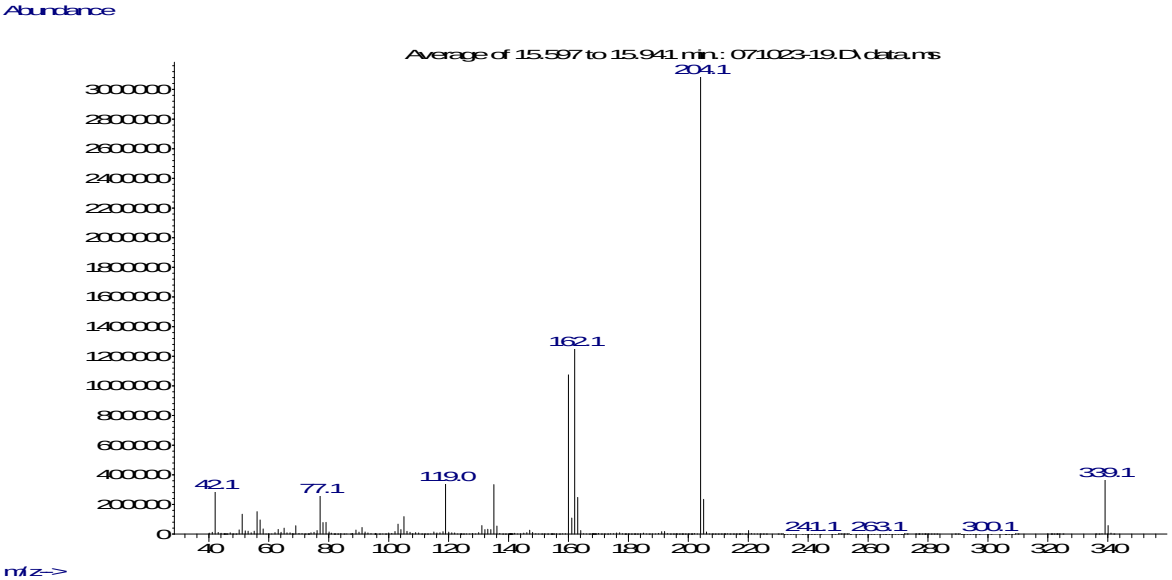

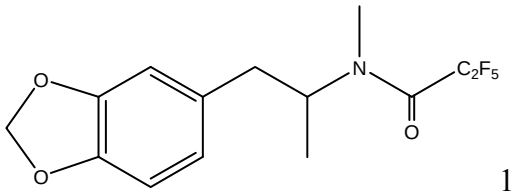

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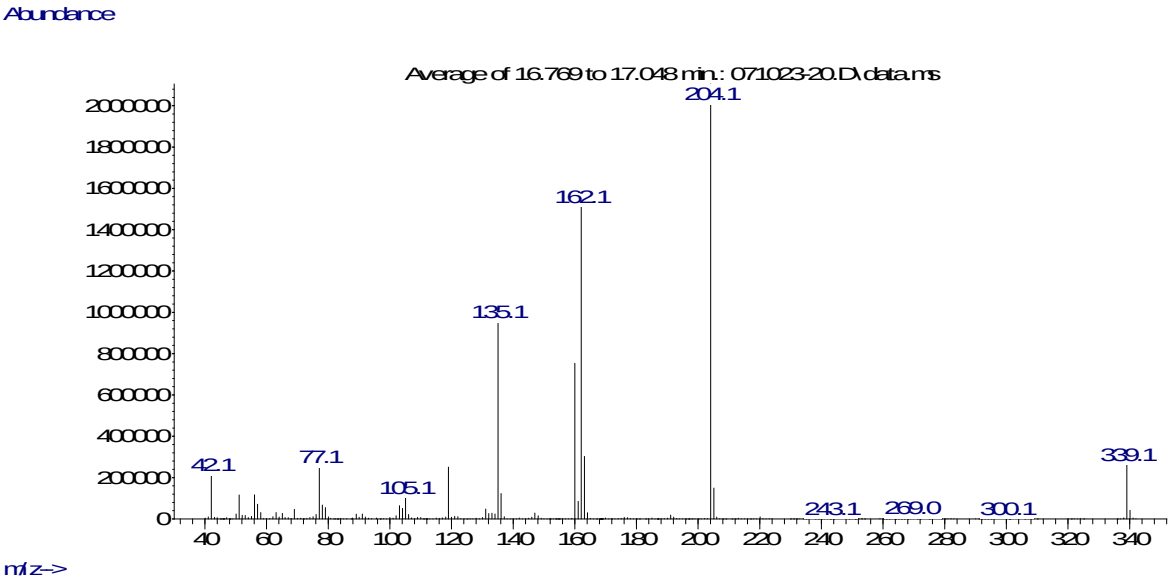
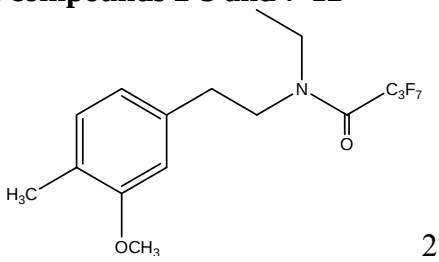


Figure 5.4: Mass Spectra of the HFBA derivatives of compounds 2-5 and 7-12



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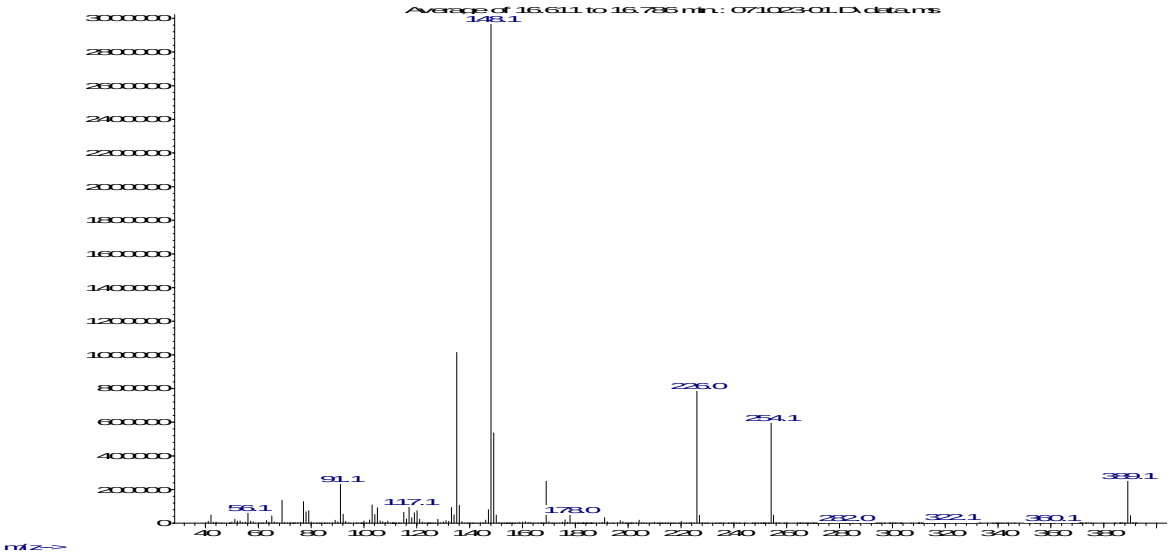

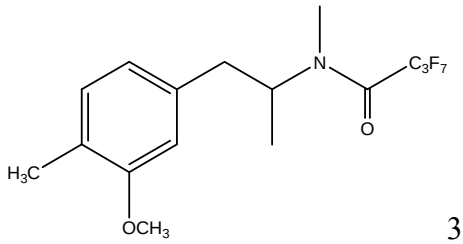

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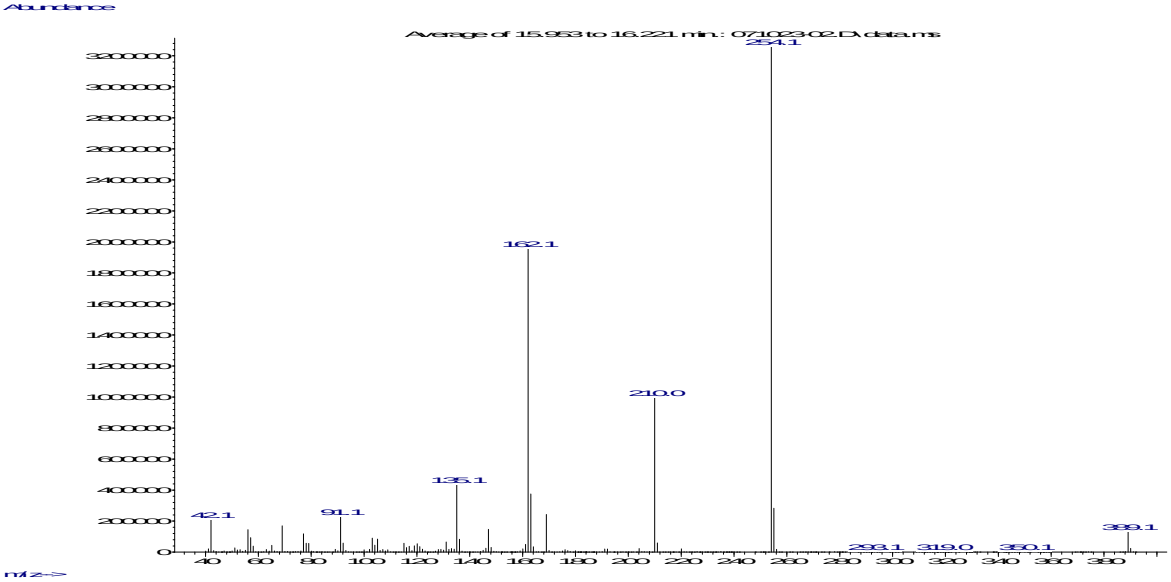

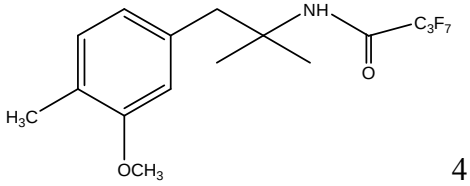

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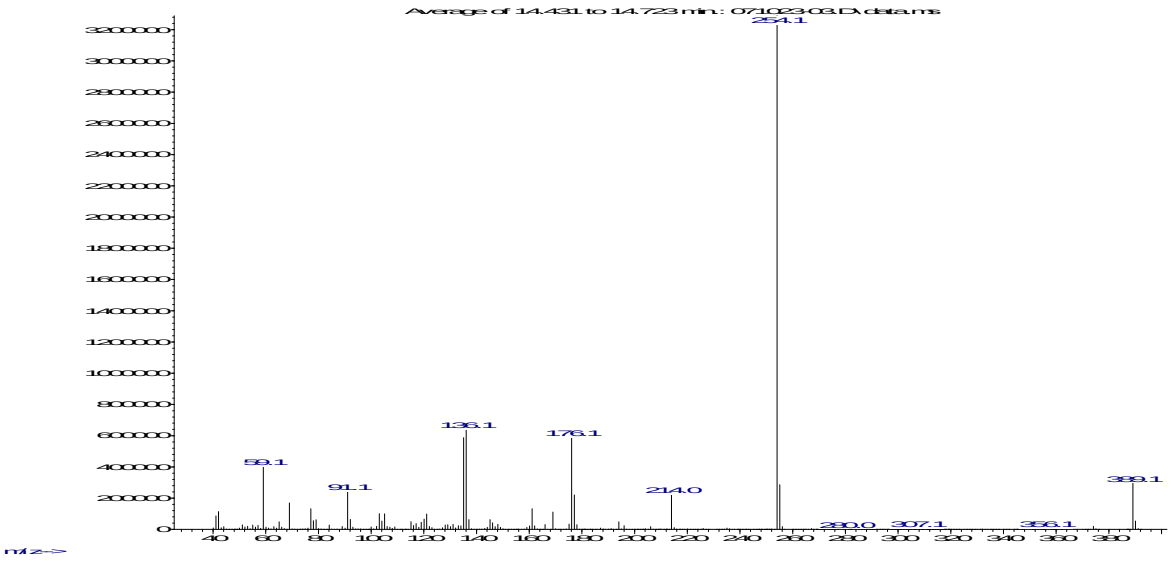

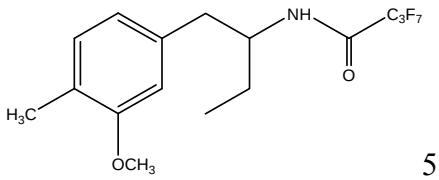

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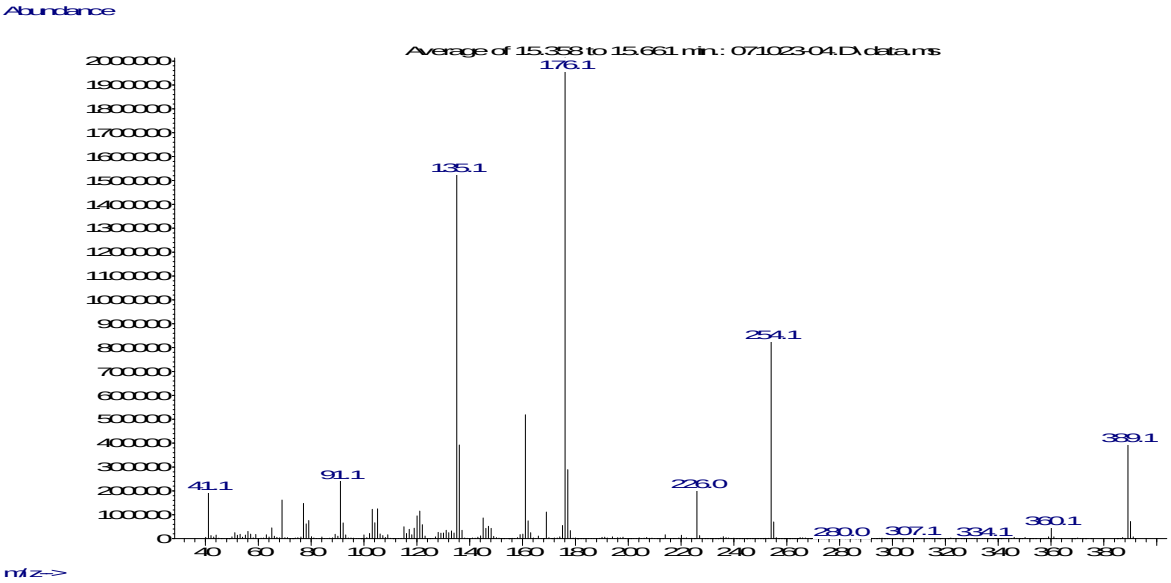

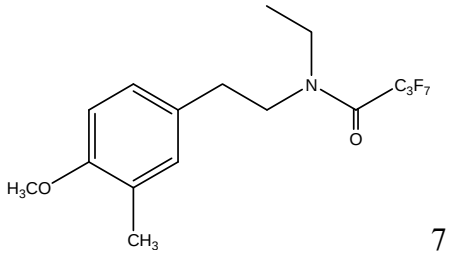

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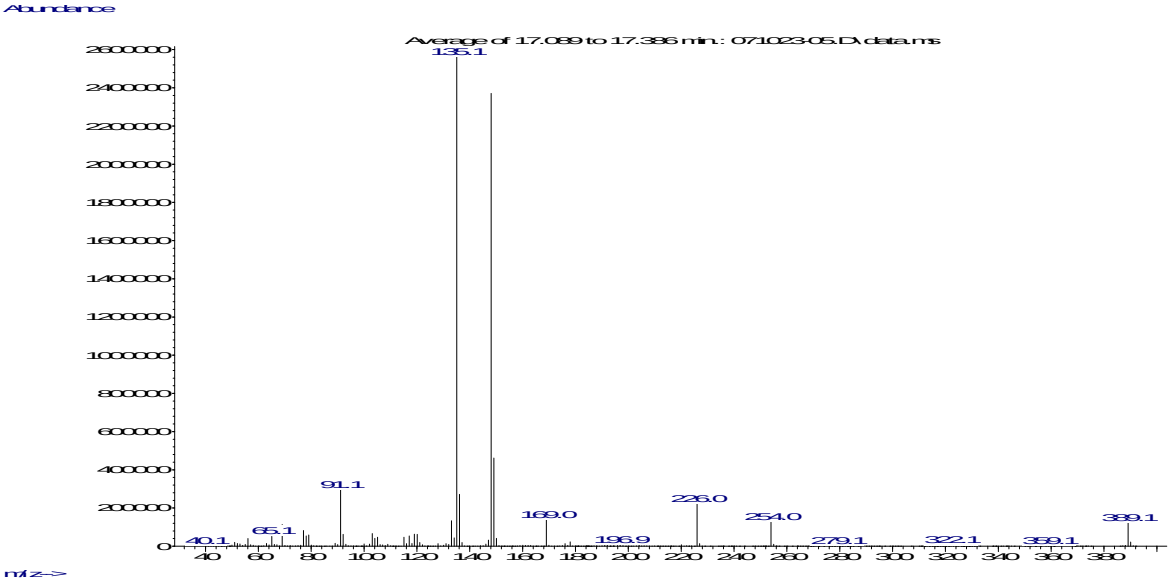

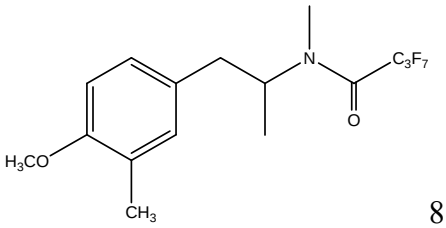

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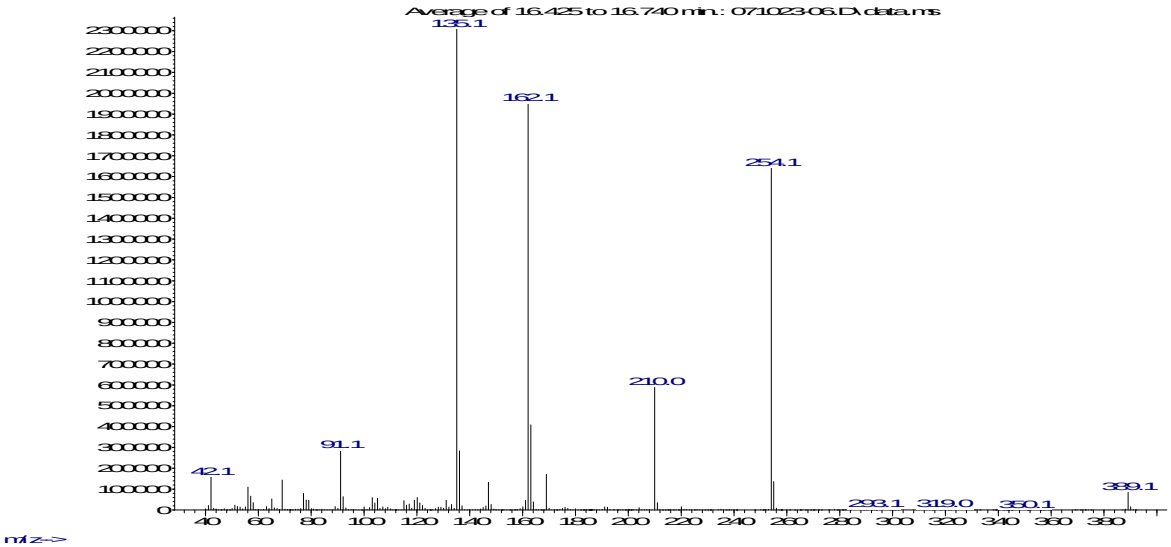

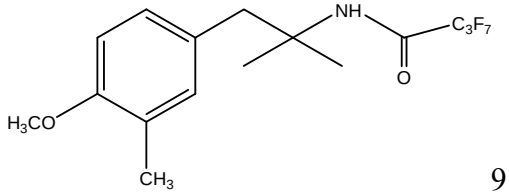

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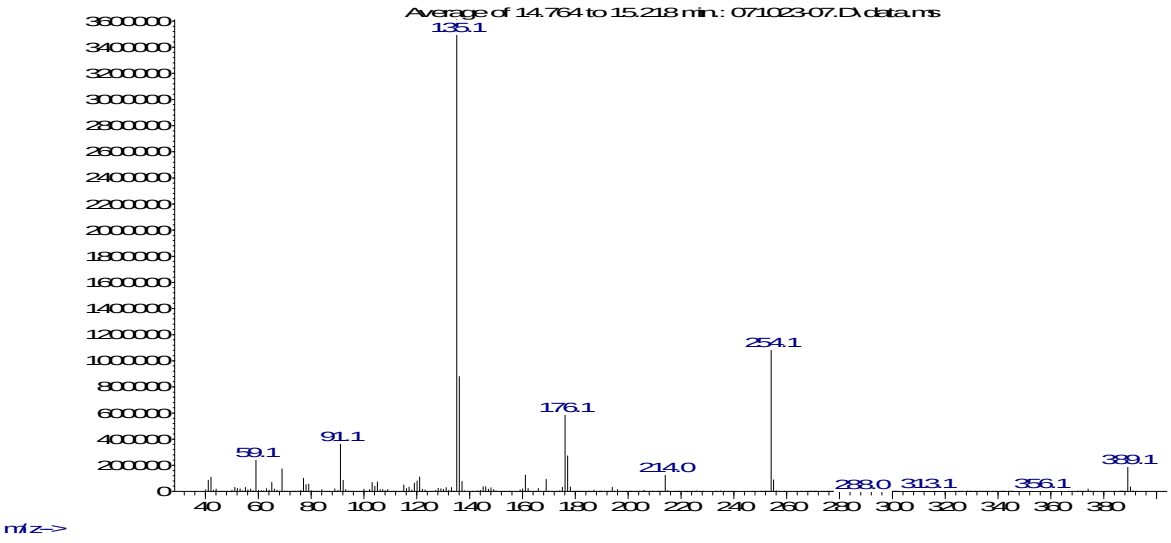

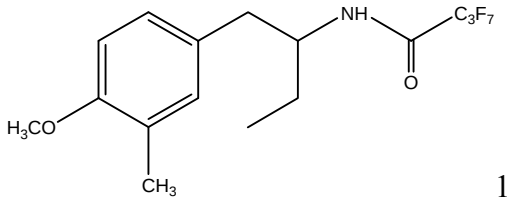

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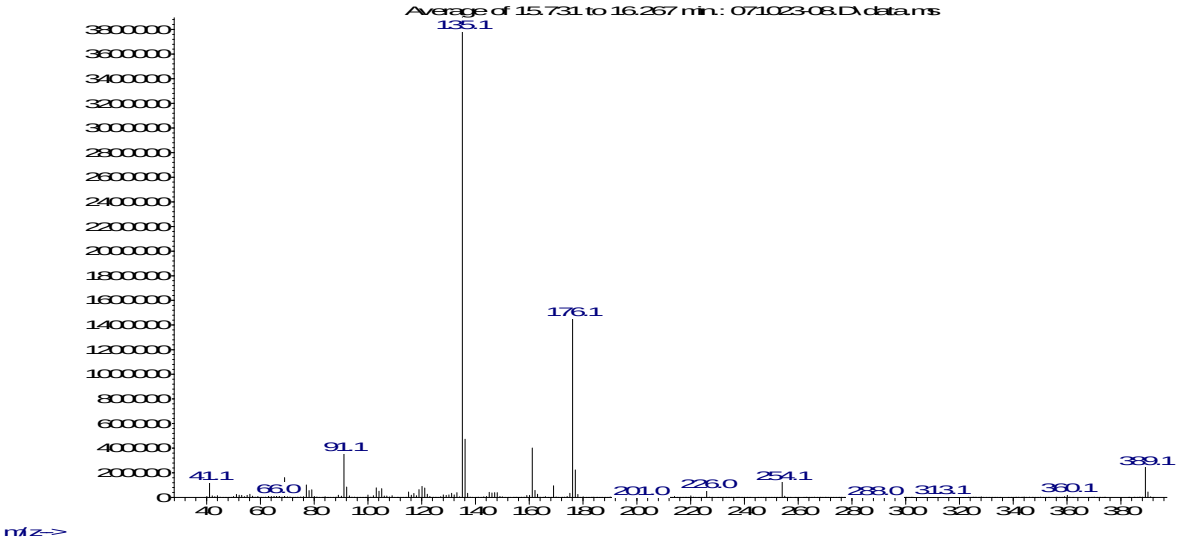

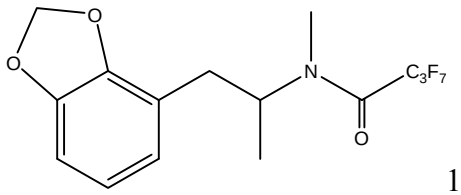

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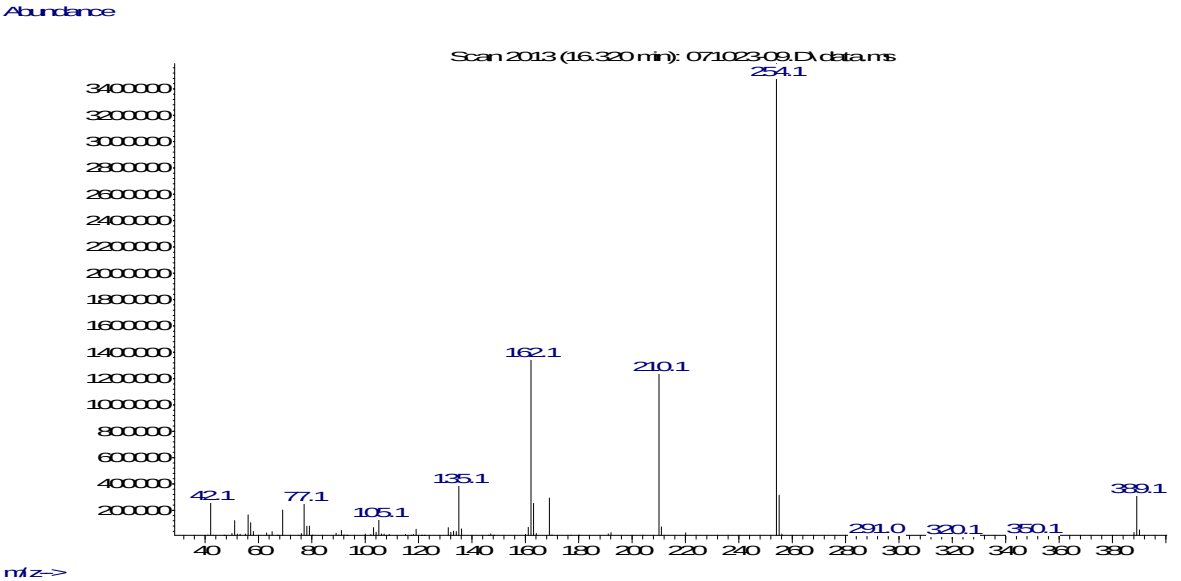
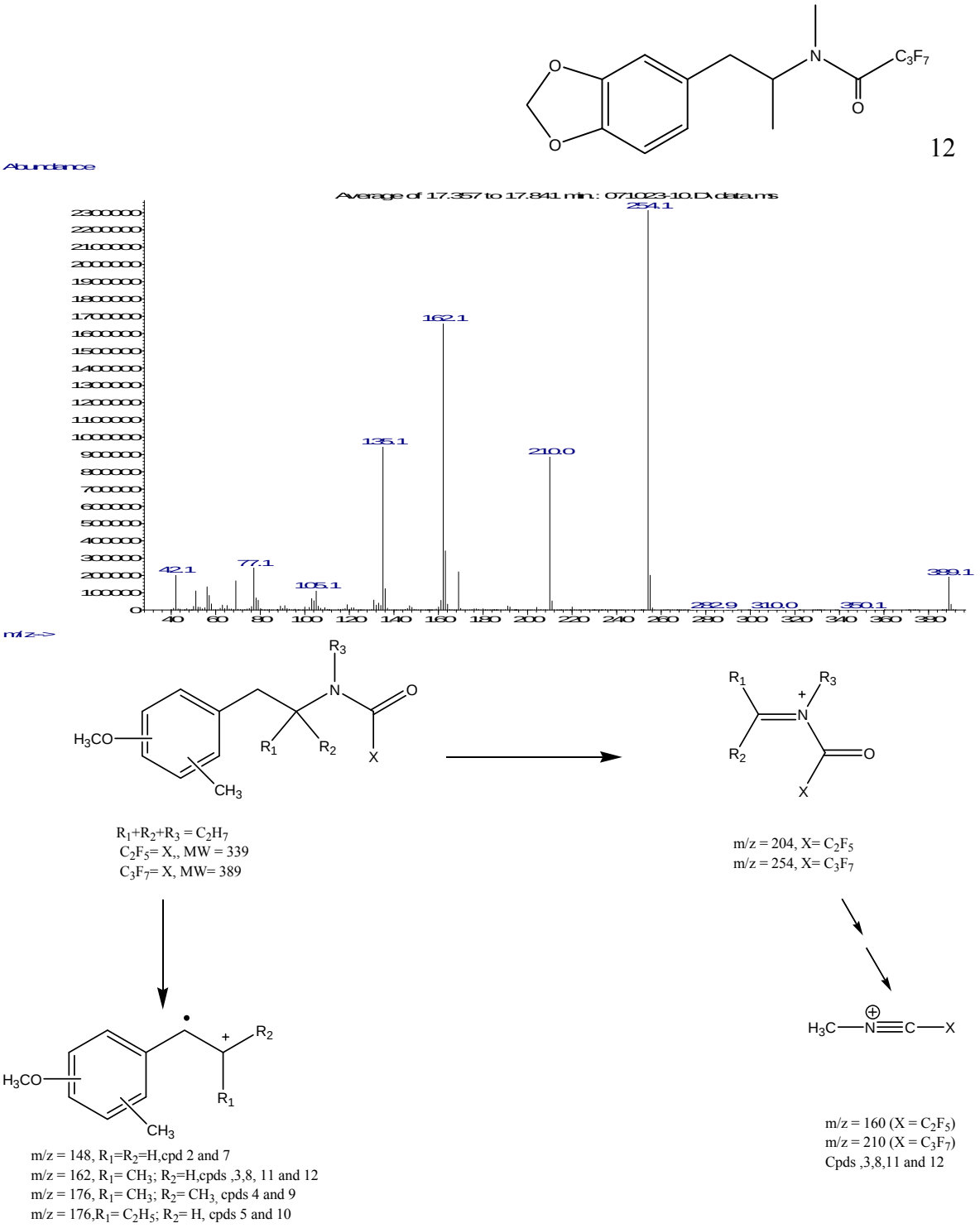




Scheme 5.2: Structures of the major EI fragments for the perfluoroacylated 3-Methoxy-4-methyl-phenethylamines and 4-Methoxy-3-methyl-phenethylamines

Indeed the m/z 135 ion is the base peak in all the PFPA and HFBA derivatives of compounds 7-10 and this increased relative intensity may serve as an indicator ion for discrimination of the 4-methoxy-3-methyl ring substitution pattern from other ring substitution patterns in this study. The decreased role for alpha cleavage reaction in the fragmentation of these amides allows the formation of ions more diagnostic of each individual isomer. Acylation weakens the bond between nitrogen and the alpha-carbon allowing the formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass identify the number of carbons attached directly to the aromatic ring. The mass spectra in Figures 5.3 and 5.4 show hydrocarbon fragments at m/z 148, 162 and 176 for a two-carbon, three-carbon and four-carbon chain attached directly to the aromatic ring.

The spectra for the N-ethyl derivatives in Figures 5.3-2, 5.-7, 5.4-2 and 5.4-7 show a base peak at m/z 148 corresponding to the two-carbon alkene radical cation which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain. This ion at m/z 148 would only occur for the N-ethyl regioisomer. The relative abundance of both m/z 148 and 135 offer a clear discrimination of compound 2 from its direct regioisomer (compound 7) as well as from the other isomers. The spectra in Figures 5.3-3, 5.3-8, 5.3-11, 5.3-12, 5.4-3, 5.4-8, 5.4-11 and 5.4-12 show the ring substituted methoxy-methyl or the methylenedioxyphenylpropene hydrocarbon ion at m/z 162 (the three-carbon alkene radical cation), identifying this molecules as the PFPA and HFBA derivatives containing the methamphetamine side chain, compounds 3, 8, 11 and 12 respectively. The spectra for the PFPA and HFBA derivatives of the primary amines 4, 5, 9 and 10 show ions at

m/z 176 from the four-carbon alkene radical cation of the 3-methoxy-4-methyl- and 4-methoxy-3-methyl-phenethylamines. This m/z 176 results from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond yielding the methoxymethyl-phenylbutene radical cation. The lower abundance of m/z 176 for compounds 4 and 9 may be attributed to steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

While the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the N-ethylphenethylamines (Figures 5.3-2 and 5.3-7) is the appearance of an ion at m/z 176 in addition to the base peak at m/z 148. Based on the previous discussion, the m/z 176 ion suggests a four carbon chain directly attached to the aromatic ring as occurs for the alpha-ethyl- and alpha, alpha-dimethyl-phenethylamines (Figures 5.3-4, 53-5, 5.3-9, 5.3-10 and 5.4-4, 5.4-5, 5.4-9, 5.4-10). The m/z 176 ion in the spectra for the PFPA derivatives of the N-ethyl regioisomers (Figures 5.3-2 and 5.3-7) is a rearrangement of the m/z 204 ion resulting in the loss of mass 28 (the N-ethyl group) via hydrogen transfer. This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the N-ethyl-phenethylamines shown in Figures 5.4-2 and 5.4-7. The loss of 28 mass units from the acylimine fragment at m/z 254 yields the equivalent fragment ion at m/z 226. Thus, the HFBA derivatives may offer more characteristic ions for individualization of these regioisomeric substances compared to the PFPA derivatives.

A comparison of the PFPA derivatives for compounds 3, 8, 11 and 12 (Figures 5.3-3, 5.3-8, 5.3-11 and 5.3-12) with their HFBA derivatives (Figures 5.4-3, 5.4-8, 5.4-

11 and 5.4-12) indicates unique ions at m/z 160 and m/z 210. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7 respectively. Additional information about these ions were obtained in previous deuterium labeling studies which confirmed that methyl group on nitrogen is a part of this resulting fragment. The remaining mass 26 would correspond to CN and the proposed structures of m/z 160 and 210 are shown in Scheme 5.2.

Gas Chromatography of 3-Methoxy-4-methyl-phenethylamine, 4-Methoxy-3-methyl-phenethylamine, 2,3- and 3,4-MDMA

The PFPA and HFBA derivatives of the primary and secondary amine side chain regioisomers of the ring substituted methoxy methyl phenethyl amines, 2,3-MDMA and 3,4-MDMA were compared on a 100% dimethyl polysiloxane (Rtx-1) stationary phase. Several temperature programs were evaluated and one program showing the best compromise between resolution and analysis time was used to generate the chromatograms in Figure 5.5. The chromatograms show that when the ring substitution pattern is held constant (i.e. 3-methoxy-4-methyl- or 4-methoxy-3-methyl-) the two secondary amides elute before the two tertiary amides. Additionally, in every case in this limited set of compounds the branched side chain elutes before the straight chain isomer when the ring substitution pattern and the degree of amide substitution are constant, regardless of the derivatizing agent. Therefore, the alpha, alpha-dimethyl isomer elutes first followed by the alpha-ethyl isomer (both secondary amides), then the methamphetamine, and N-ethyl phenethylamines (the two tertiary amides).

When the side chain is held constant, the 3-methoxy-4-methyl ring substitution pattern elutes before the 4-methoxy-3-methyl ring substitution pattern and this elution order is the same for both the PFPA and the HFBA derivatives. Perhaps the most useful information in these chromatograms is the relative elution of the derivatized controlled substance 3,4-MDMA and its closest eluting regioisomeric equivalents. Both the PFPA and HFBA derivatives of 3,4-MDMA elute after the N-ethyl-3-methoxy-4-methyl phenethylamine and 4-methoxy-3-methylphenethylamine PFPAs and HFBAs, both the N-ethyl regioisomers show very distinct mass spectra with several characteristic ions to differentiate these compounds from the drug of abuse 3,4-MDMA. Thus, derivatization methods coupled with chromatographic and mass spectral procedures can allow for the characterization and differentiation of these ten uniquely isomeric substances. The individualization is possible without the need for reference samples of all these uniquely similar substances.

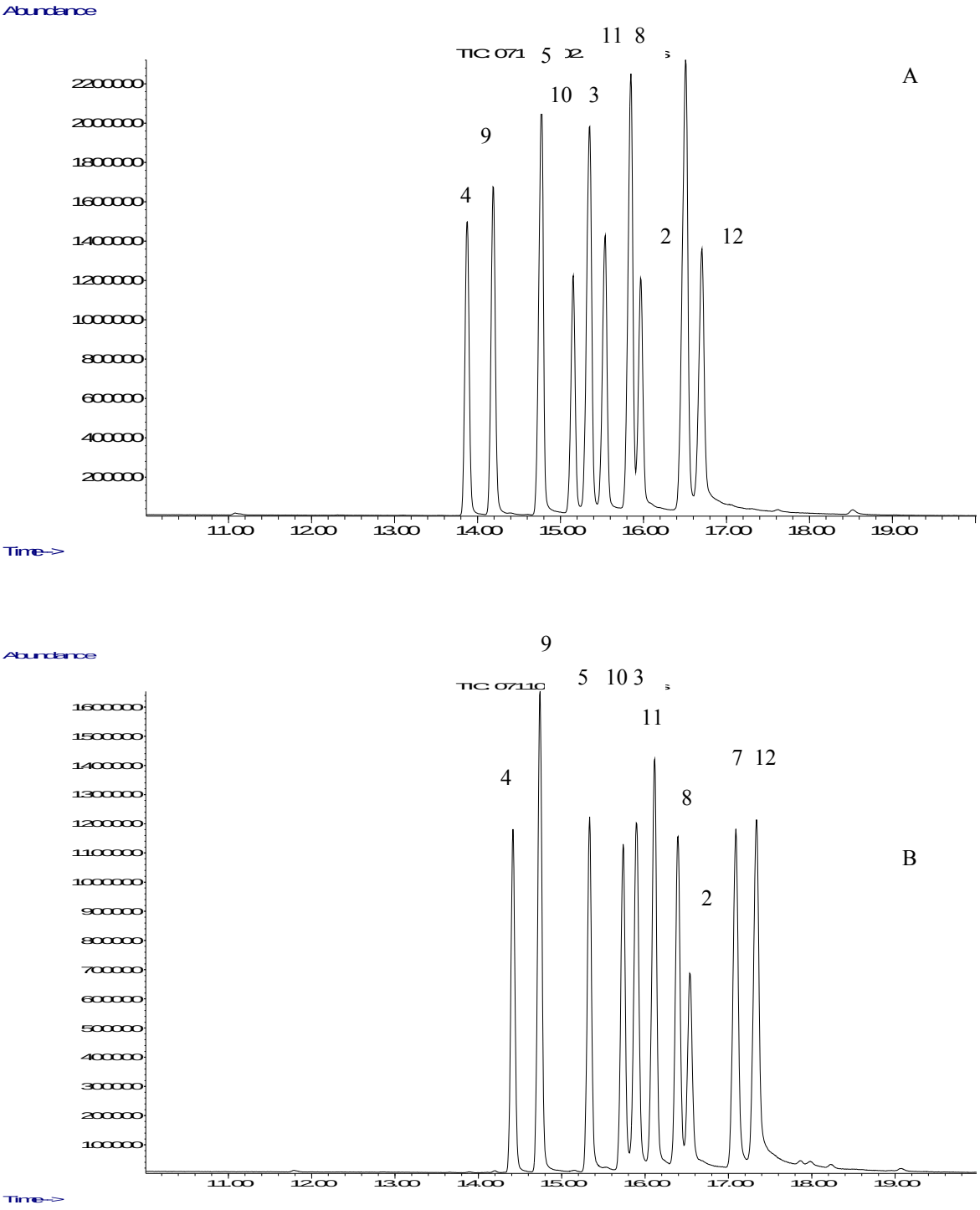


Figure 5.5: GC Separation of the PFPA (A) and HFBA (B) derivatives of compounds 2-5 and 7-12; Rtx-1 column.

Conclusions of Chapter 5

3,4-MDMA , 2,3-MDMA and ten side chain regioisomers of 3-methoxy-4-methyl-phenethylamine and 4-methoxy-3-methyl-phenethylamine are a unique subset of regioisomeric and isobaric molecules; each compound has a molecular weight of 193 and yields a base peak at m/z 58 in the mass spectrum from the loss of the corresponding methylenedioxybenzyl or the mass equivalent isobaric ring substituted methoxy methyl benzyl groups. Thus the traditional electron impact mass spectrum provides little structural information for differentiating among these ten compounds.

Derivatization of the eight primary and secondary amines with various acylating agents yields amides which significantly individualize the mass spectra for these amides and allow for specific identification. The individualization is the result of fragmentation of the alkyl carbon-nitrogen bond yielding hydrocarbon fragments at m/z 148, 162 and 176 as well as other unique fragments from these regioisomeric amides. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes however; the HFBA derivatives offer more unique fragment ions for additional discrimination among these regioisomeric substances. Chromatographic resolution of the acylated amines was achieved on relatively non polar stationary phase Rtx-1 (100% dimethyl polysiloxane).

Chapter 6

Mass Spectral and Gas Chromatographic Studies of Methoxy Methyl Methamphetamines Isobaric to 3, 4-MDMA.

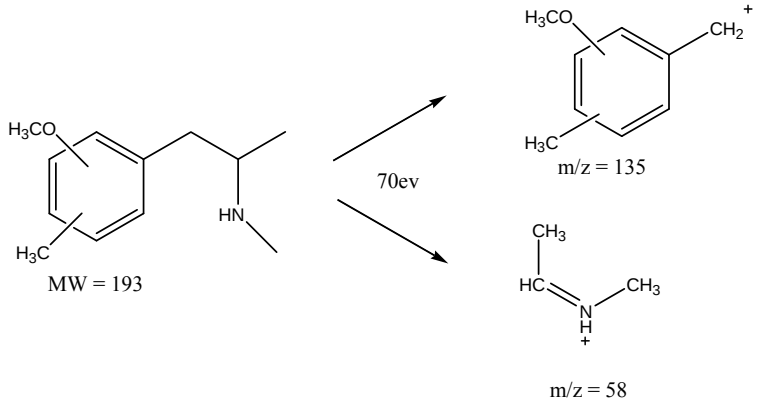
Isobaric substances are compounds of the same nominal mass but with different elemental composition. There are isobaric substances, which do not contain the methylenedioxy substitution pattern in the aromatic ring, yet they are able to yield the same major fragments in their mass spectra as the controlled drug substance MDMA. Among these MDMA isobaric substances are the ring substituted methoxy methyl methamphetamines.

Mass Spectral Studies of Methoxy Methyl methamphetamines Isobaric to 3,4-MDMA

The mass spectrum of 3,4-MDMA is characterized by a base peak formed by an α-cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-MDMA (MW=193) and its direct regioisomer, 2,3-MDMA, the α-cleavage reaction yields the 3,4-methylenedioxybenzyl and 2,3-methylenedioxybenzyl fragments at mass 135/136 (for the cation and the radical cation, respectively) and the substituted imine fragment at m/z 58. Thus, the mass spectrum for 3,4-MDMA contains major ions at m/z 58 and 135/136 as well as other ions of low relative abundance.

The various methoxy methyl ring substitution patterns of methamphetamine have the potential to yield mass spectra essentially equivalent to 3,4-MDMA and 2,3-MDMA,

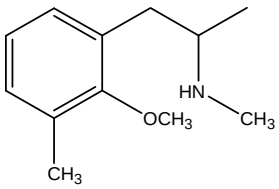
all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136 (Figure 6.1). The isobaric methoxy methyl benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. Furthermore the m/z 58 ion in the methoxymethamphetamine is the same imine structure as that obtained in the mass spectra of both 3,4 and 2,3-MDMA (Scheme 6.1).



Scheme 6.1: **General mass spectral fragmentation for the ring substituted methoxy methyl methamphetamines.**

Figure 6.1: Structures and mass spectra of ring substituted methoxy methyl methamphetamines

Structures **Mass spectra**



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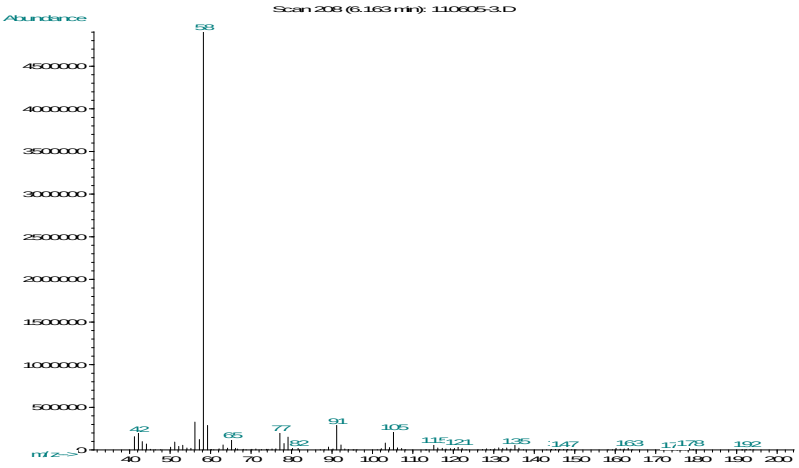

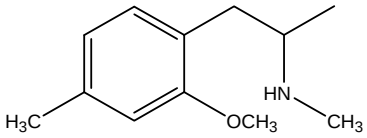

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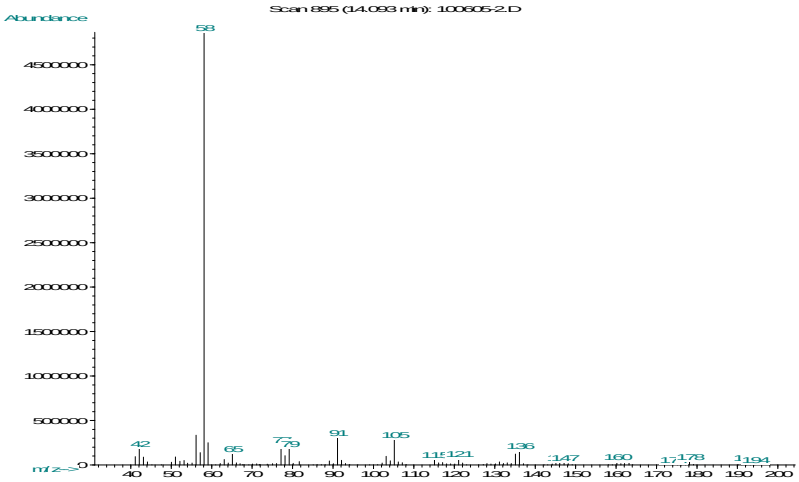

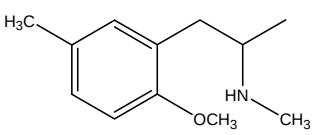

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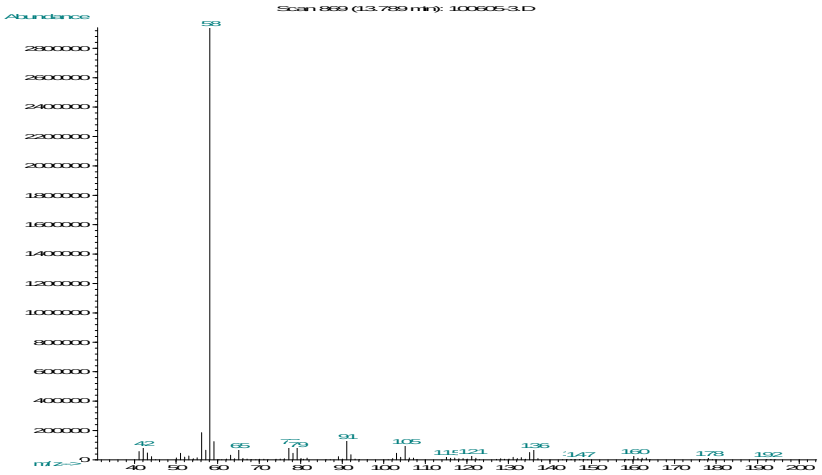

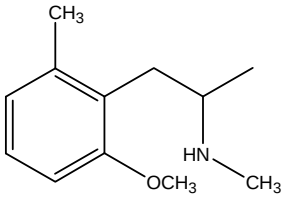

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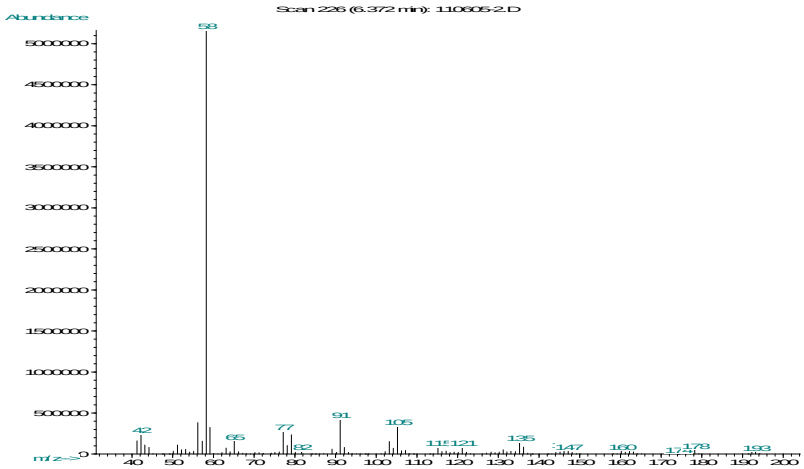

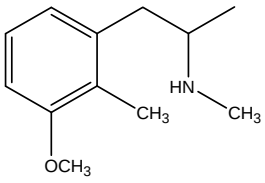

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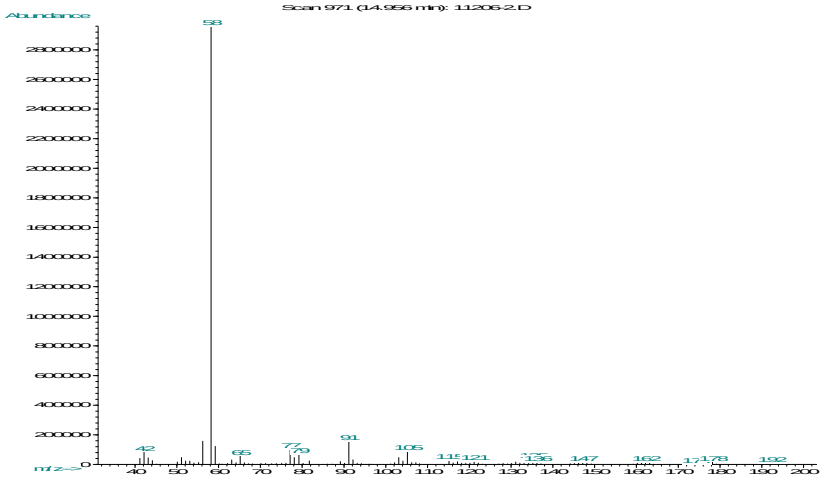

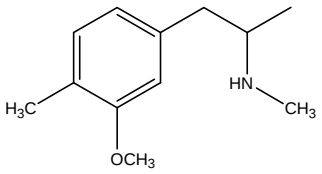

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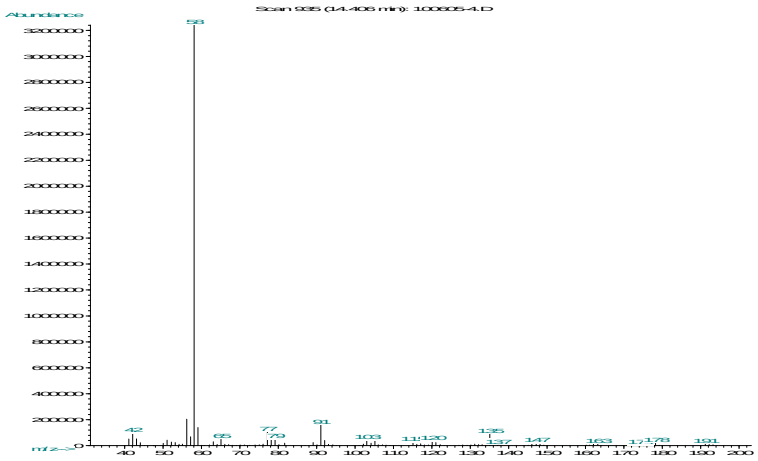

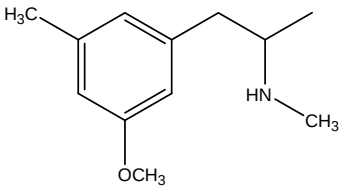

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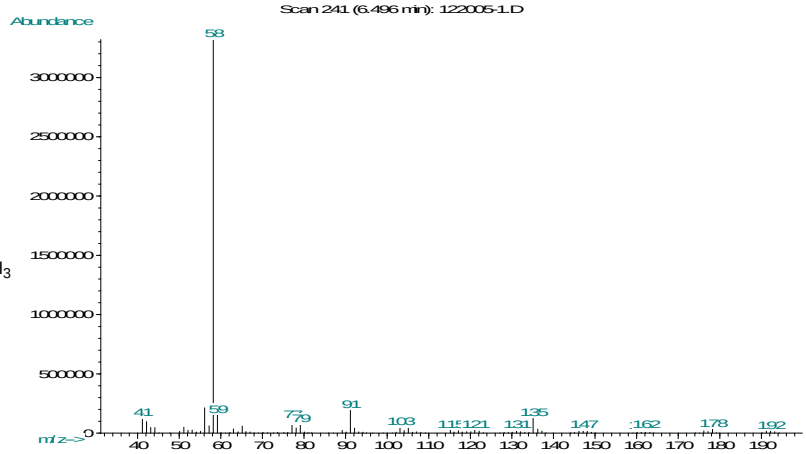

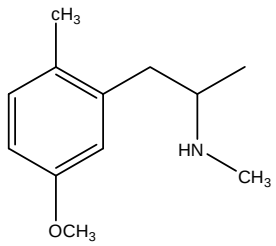

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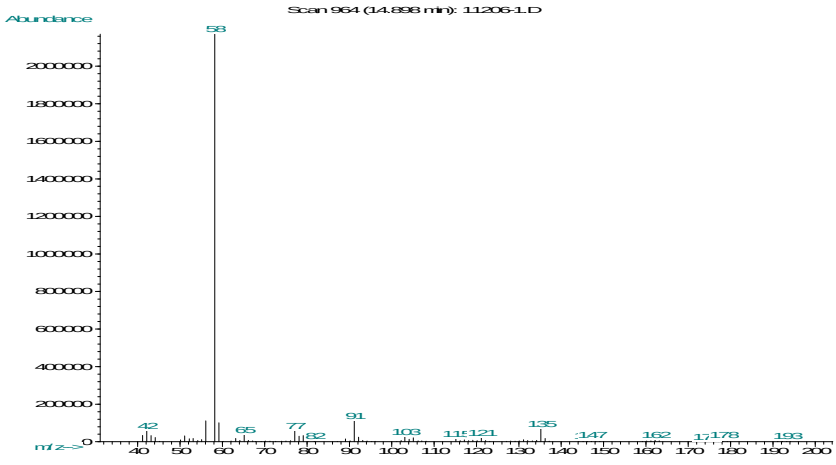

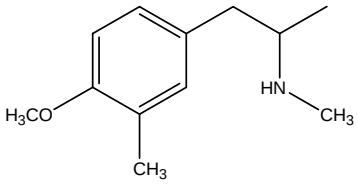

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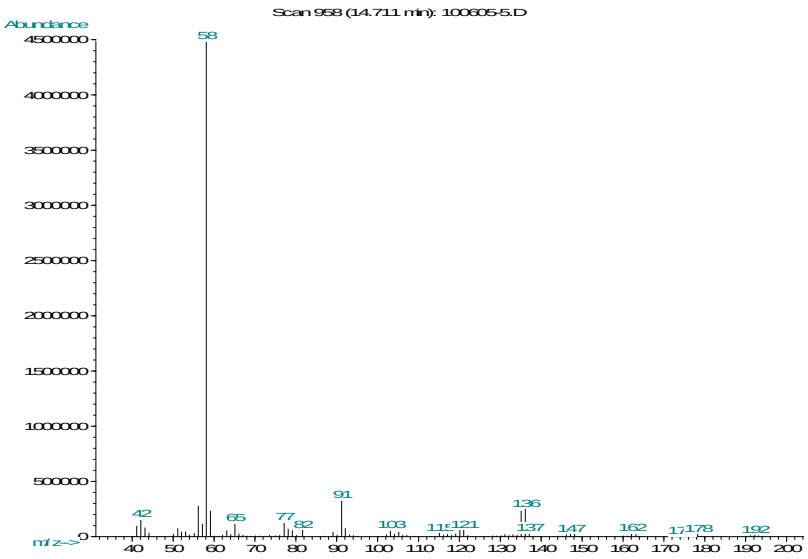

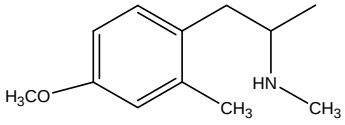

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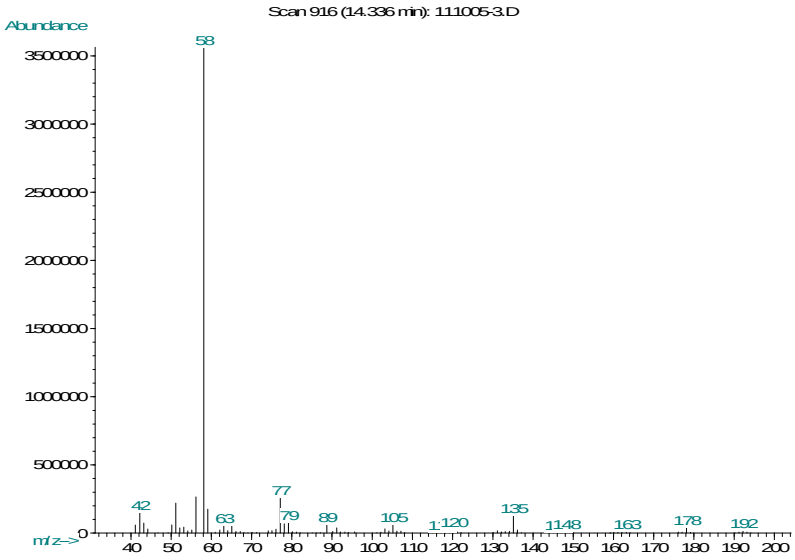


The mass spectra for the ten ring substituted methoxy methyl methamphetamines in Figure 6.1 show only the major fragment ion at equivalent masses. This lack of mass

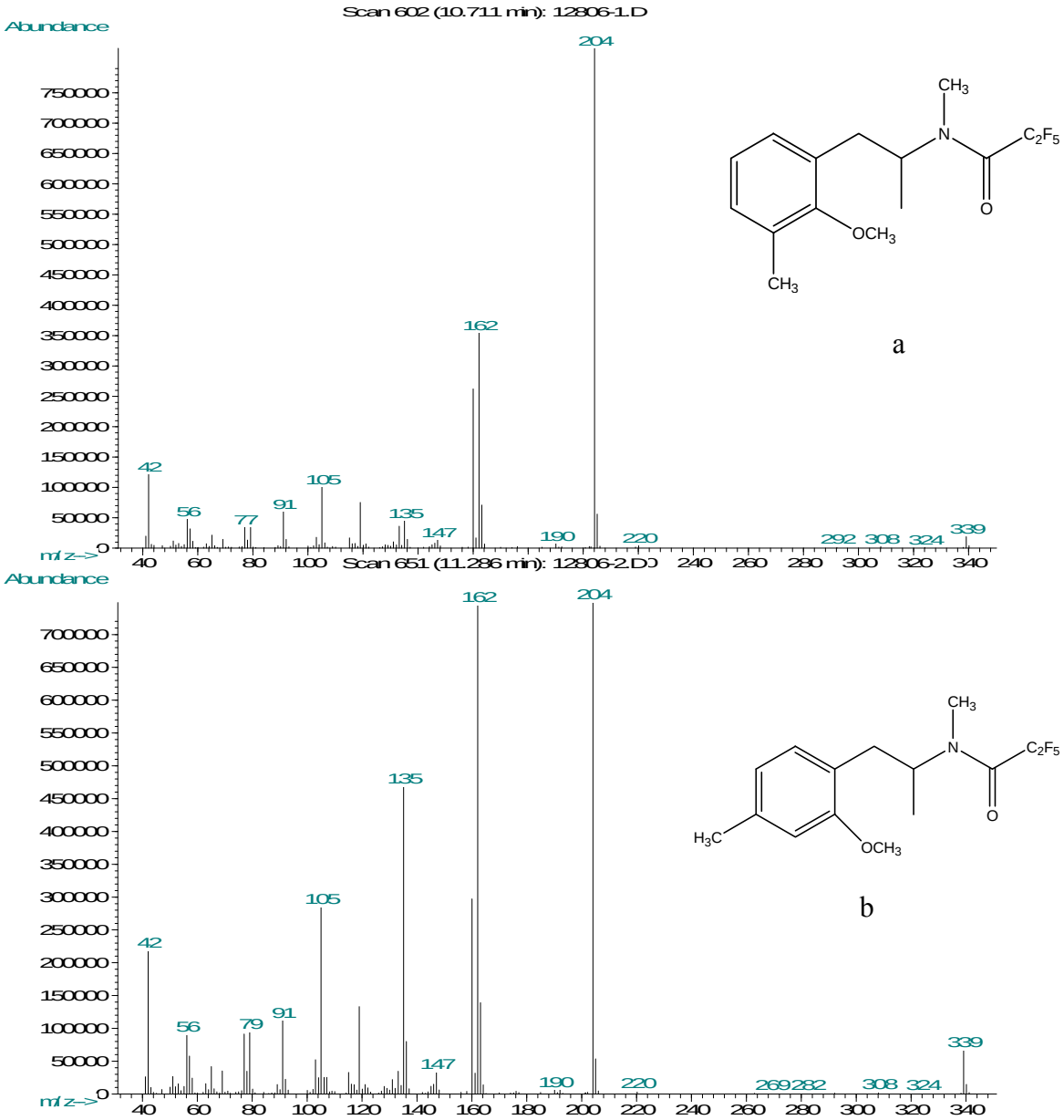
spectral specificity in addition to the possibility of chromatographic co-elution, with 3,4-MDMA, could result in misidentification of the target drug. Furthermore, the lack of available reference samples for all ten of these isobaric molecules complicates the individual identification of any one of these substances. This constitutes a significant analytical challenge, where the use of gas chromatography-mass spectrometry (GC-MS) must be based primarily upon the ability of the chromatographic system to separate the "counterfeit substance" from the actual drug of interest. Additionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

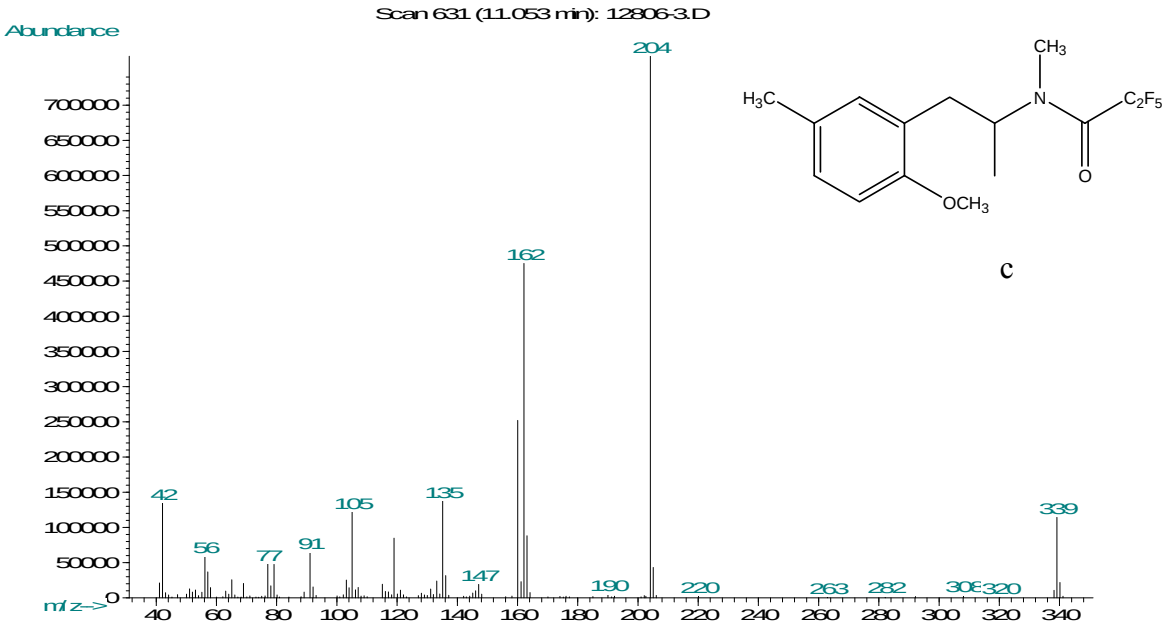
Mass Spectral Studies of Perfluroacyl Derivatives Methoxymethyl Methamphetamines compared to 2,3- and 3,4-MDMA

The perfluoroacylated derivatives of the ten methoxy methyl methamphetamines were prepared and evaluated for their ability to individualize the mass spectral properties of these compounds and to maintain or improve chromatographic resolution. Acylation of the amines significantly lowers the basicisty of nitrogen and can allow other fragmentation pathways to play a more prominent role in the mass spectrum. These perfluoroacyl derivatives allowed the unique fragmentation reactions which characterized and served to individualize the side chain regioisomers. However, in these substances the perfluoroacyl derivatives were less successful at differentiating the 2,3- from the 3,4- ring substitution pattern of the identical side chain. Since all ten of the methoxy methyl methamphetamines have the same side chain and have ten different substitution patterens, perfluroacylation may not allow for complete compound individualization based only on

the observed mass spectrum. The mass spectra for the ten pentafluoropropionyl (PFPA) and heptflourobutryl (HFBA) amides are shown in Figures 6.2 and 6.3. For comparative purposes in this chapter 2,3- and 3,4-MDMA will be given the compounds number 11 and 12.

Figure 6.2: Mass spectra of the PFPA derivatives of methoxy methyl methamphetamines, 2,3- and 3,4-MDMA





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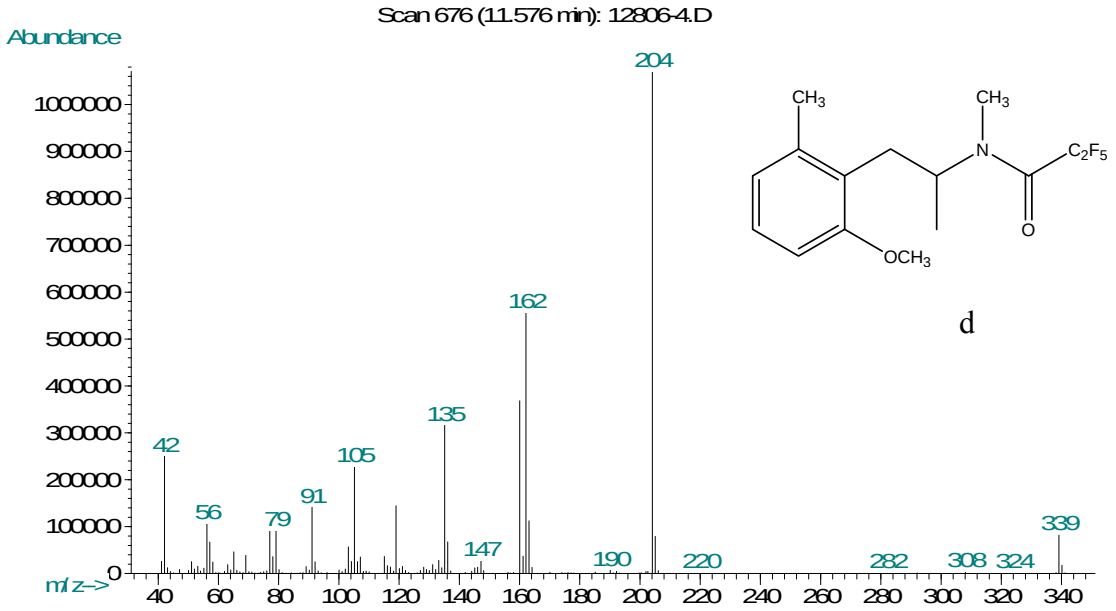

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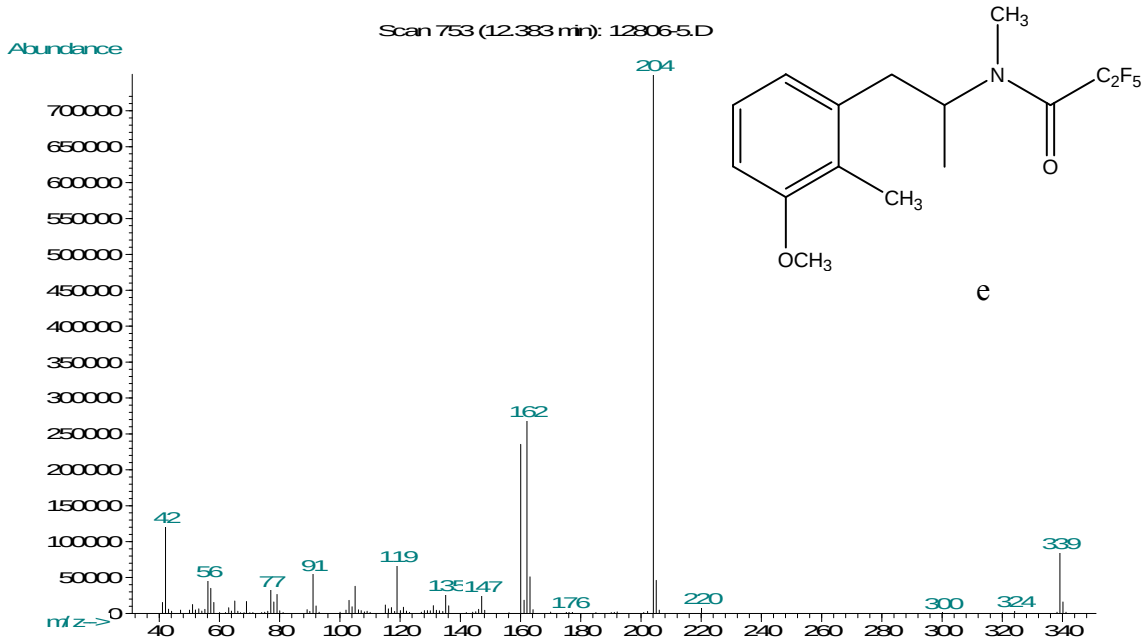

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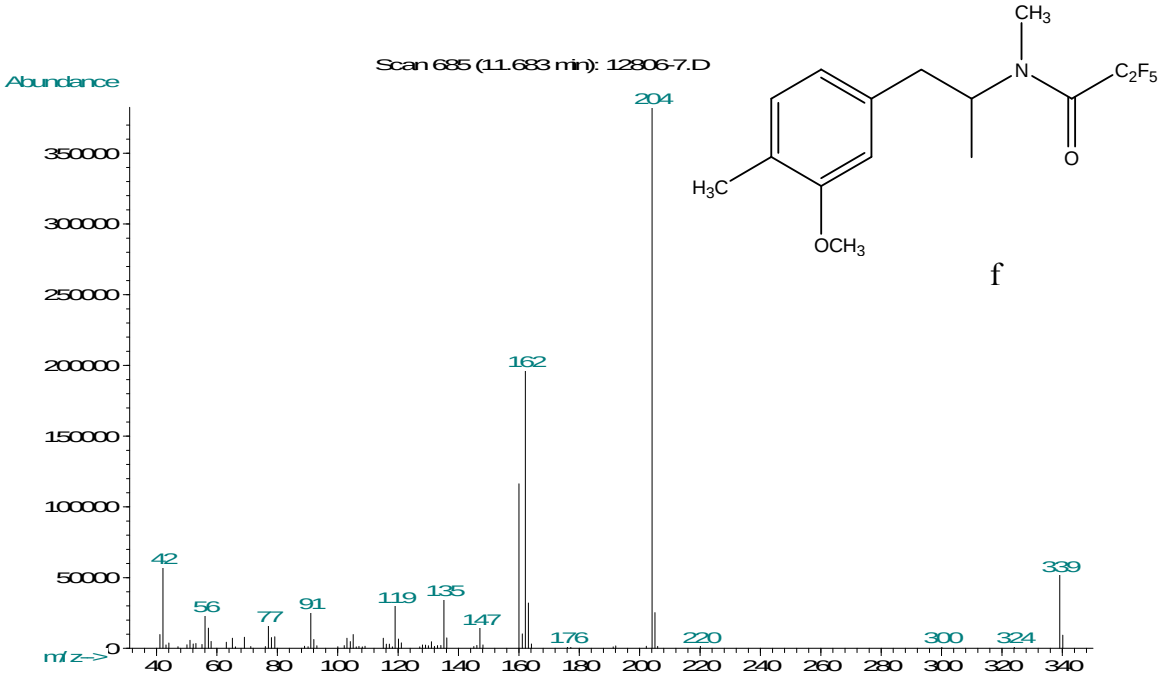

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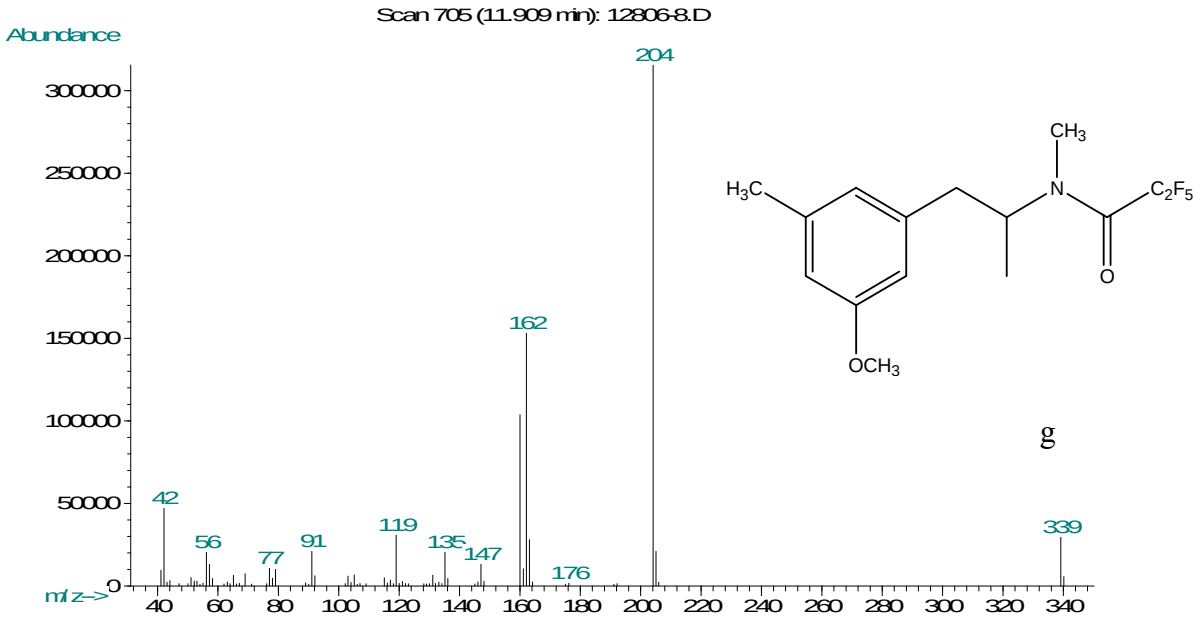

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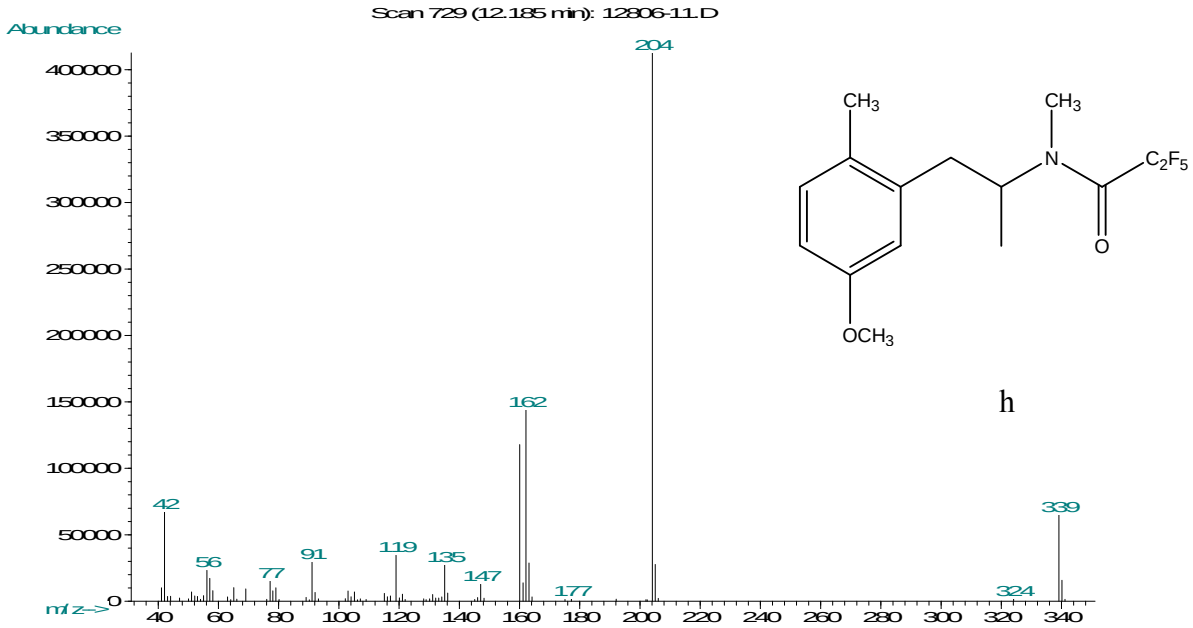

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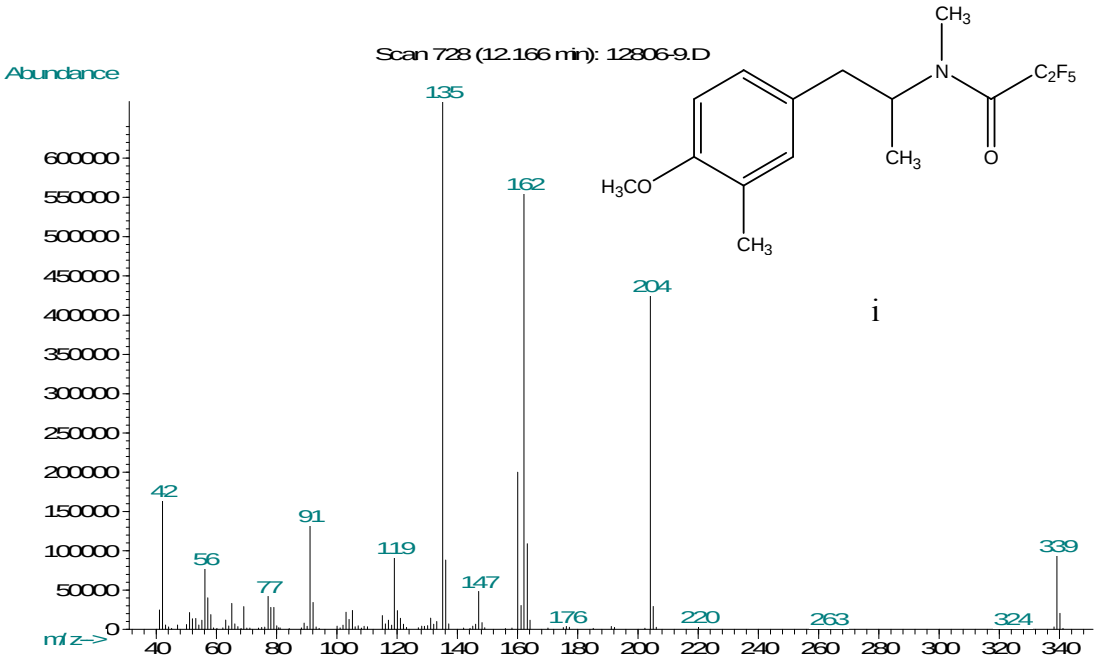

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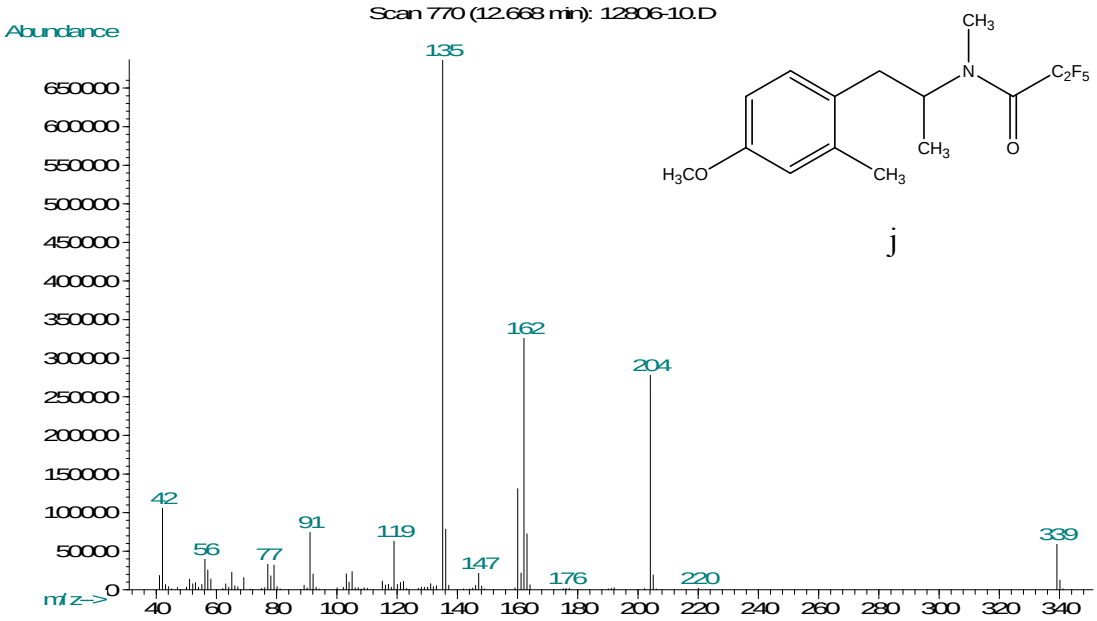

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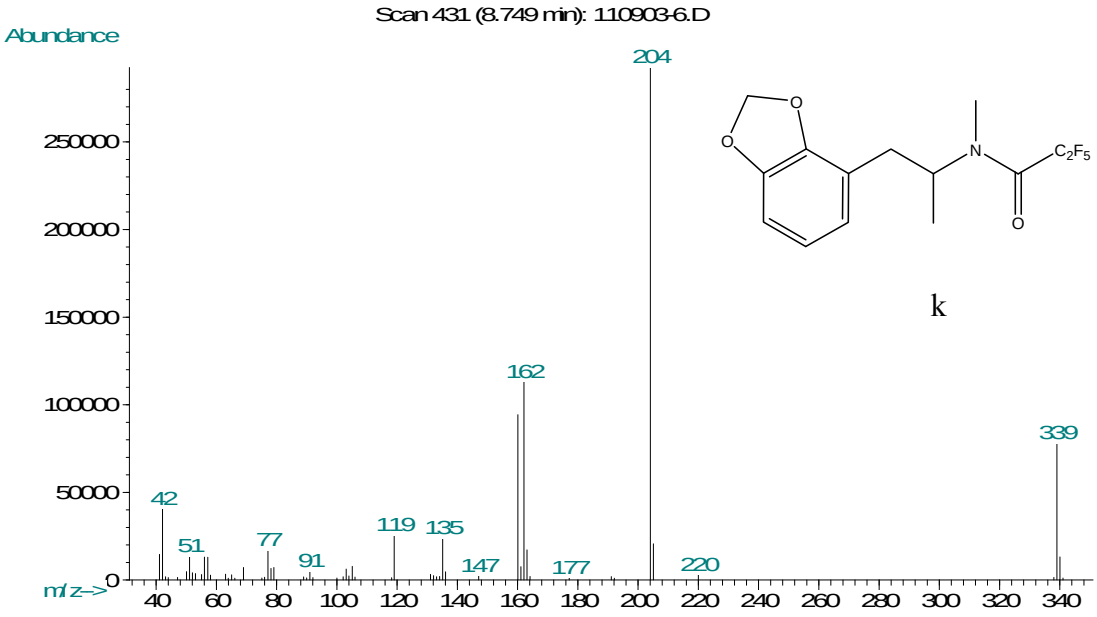

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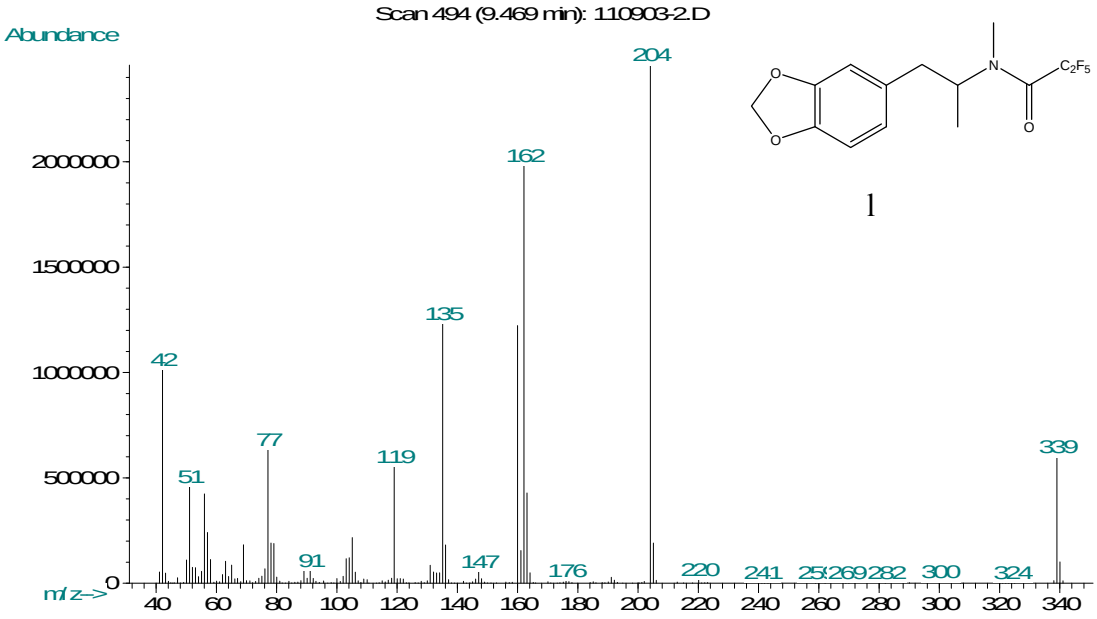

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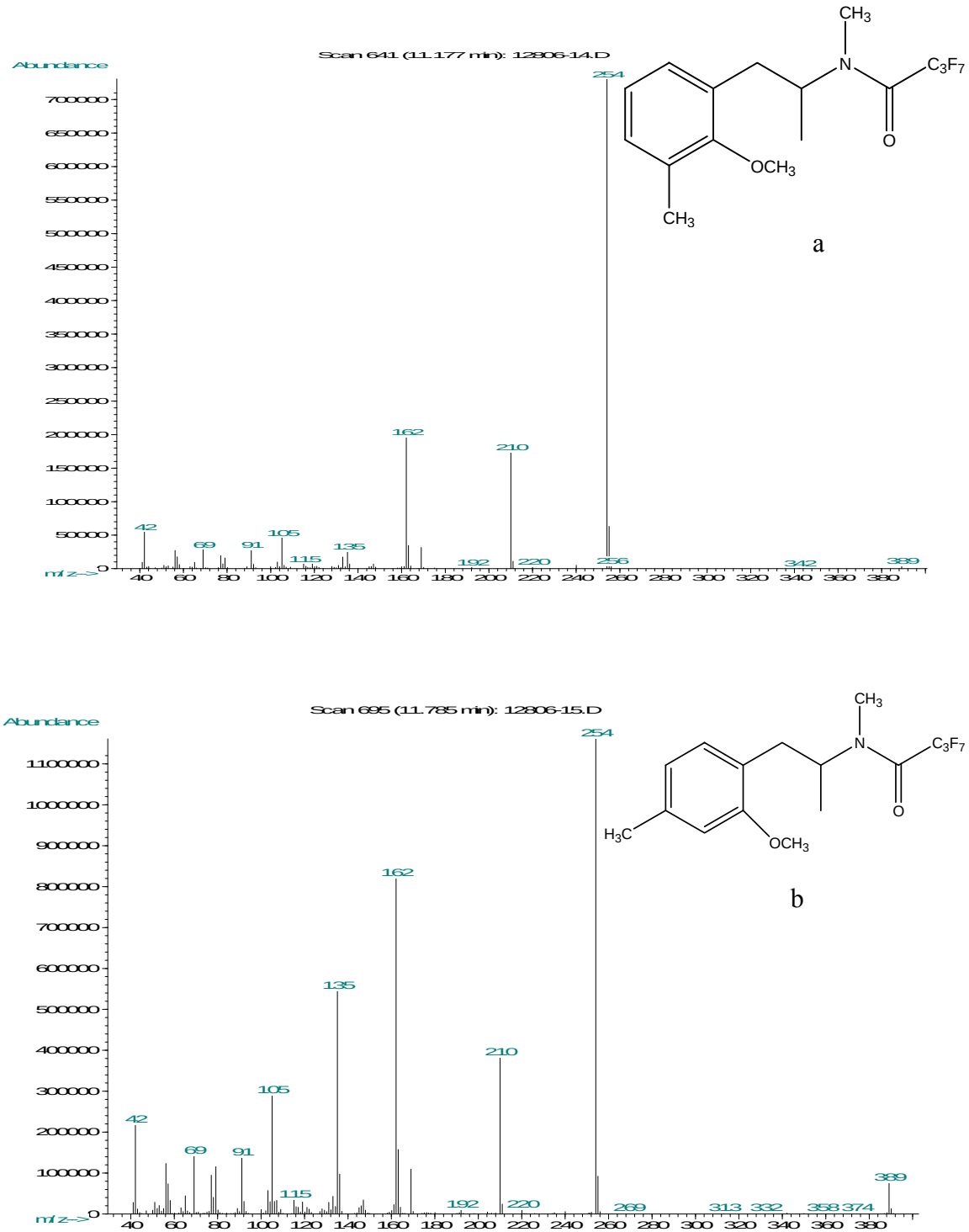
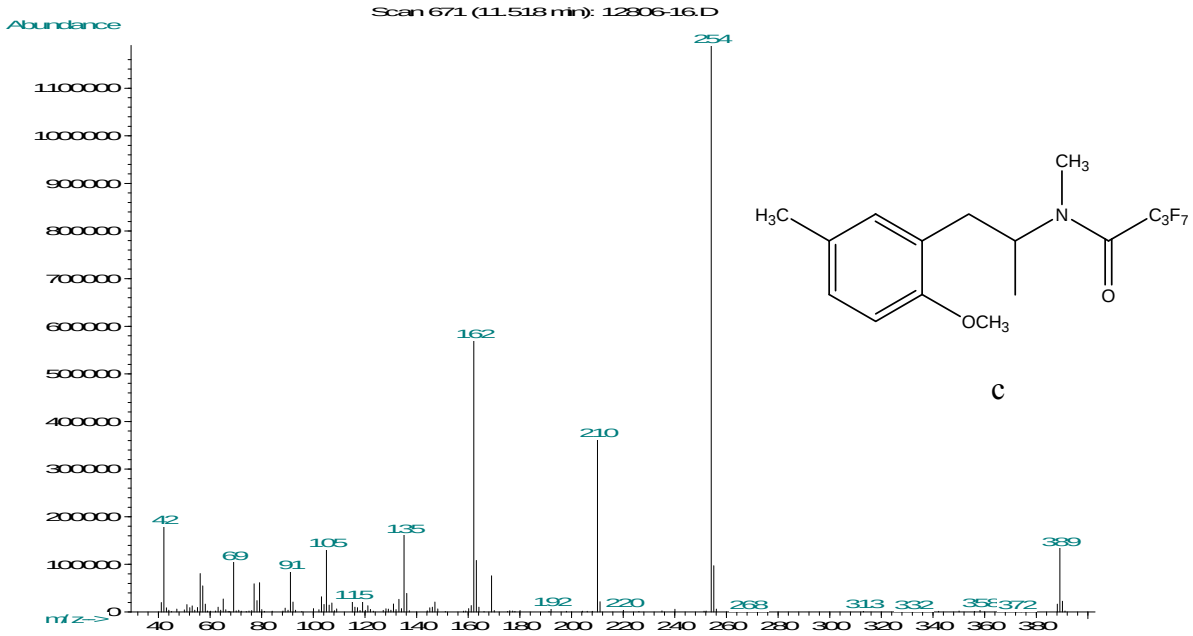
Figure 6.3: Mass spectra of the HFBA derivatives of methoxy methyl methamphetamines, 2,3- and 3,4-MDMA





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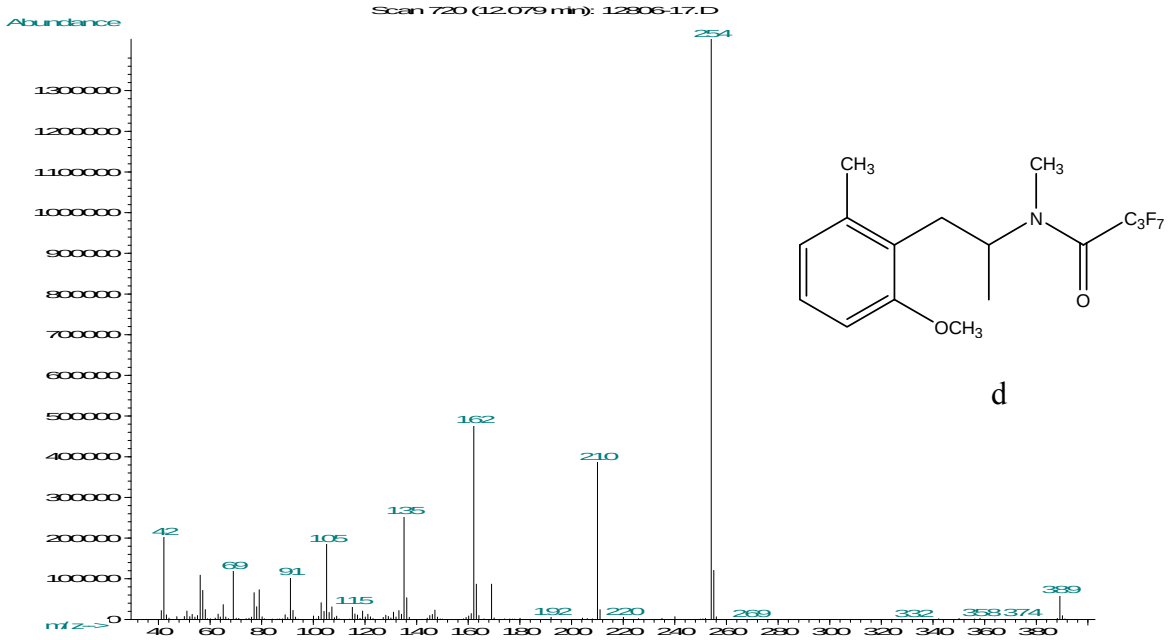

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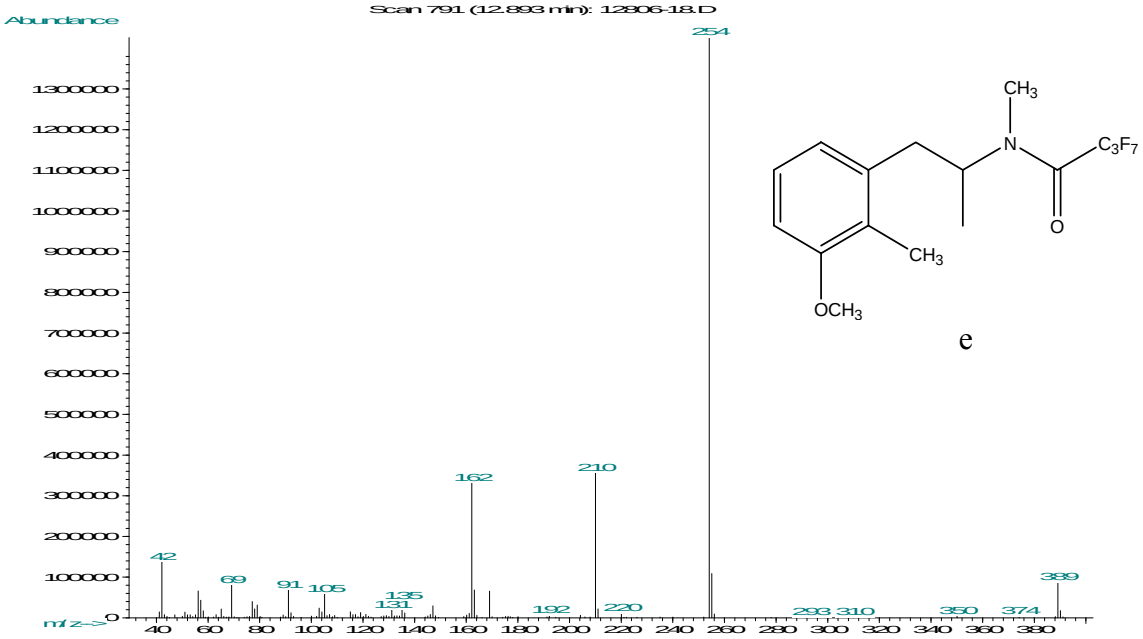

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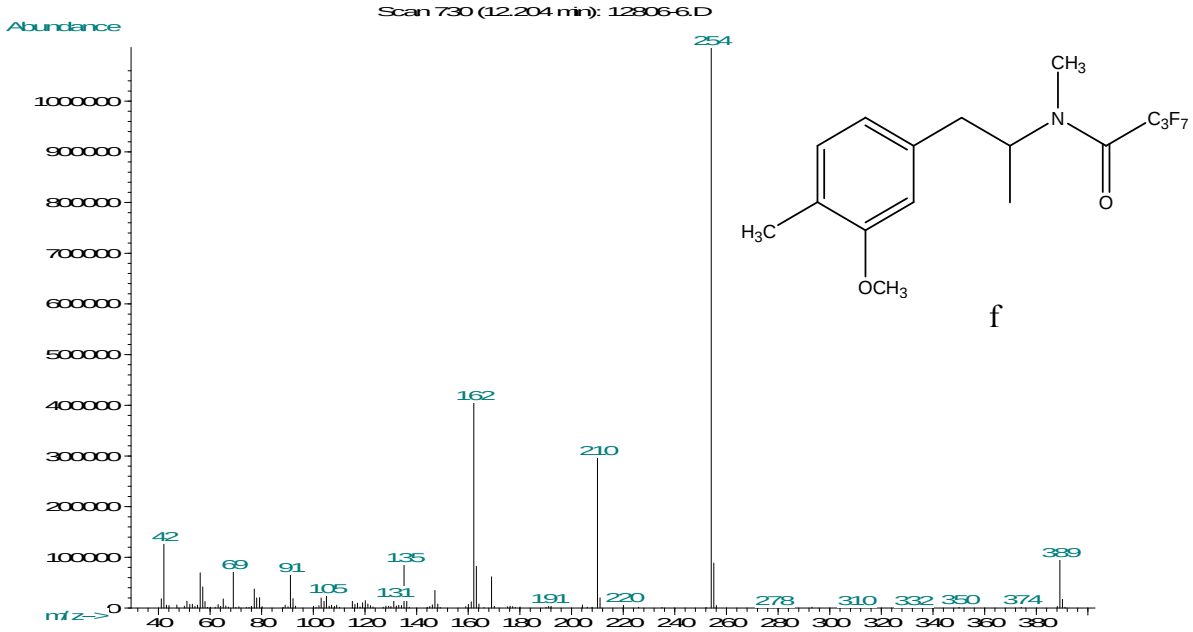

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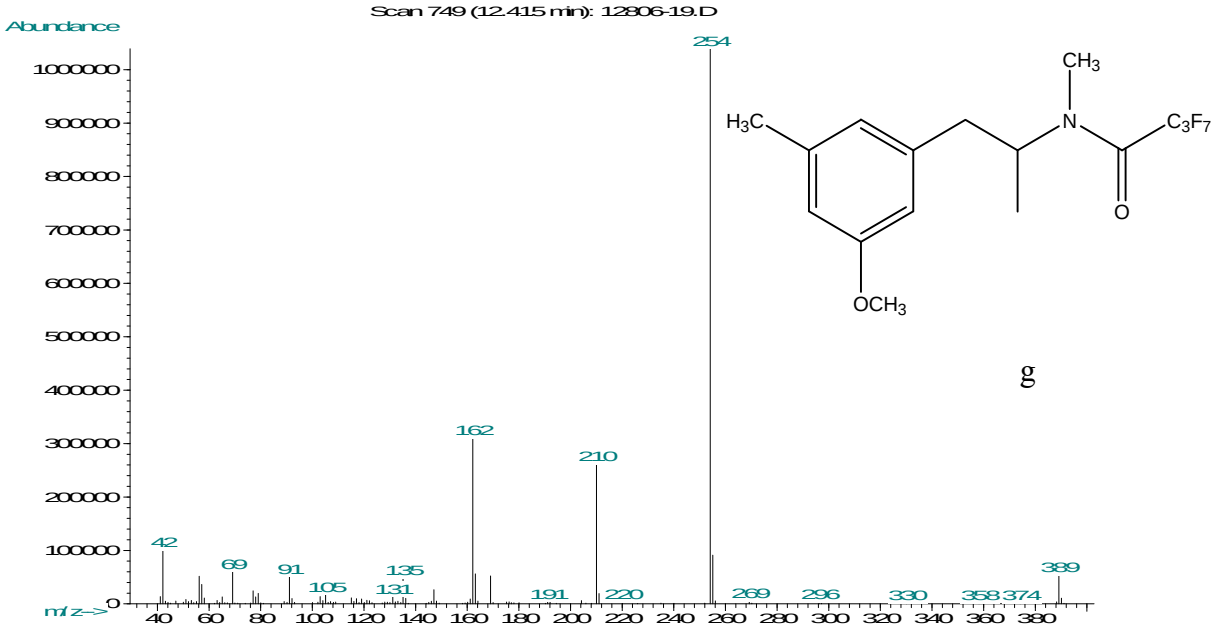

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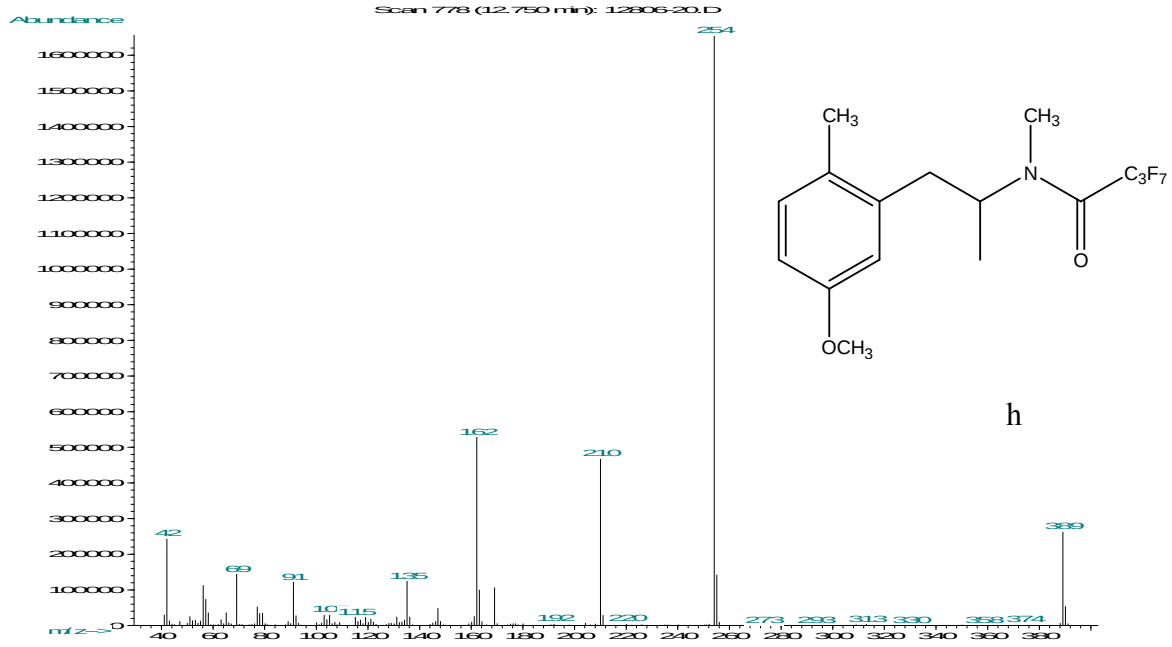

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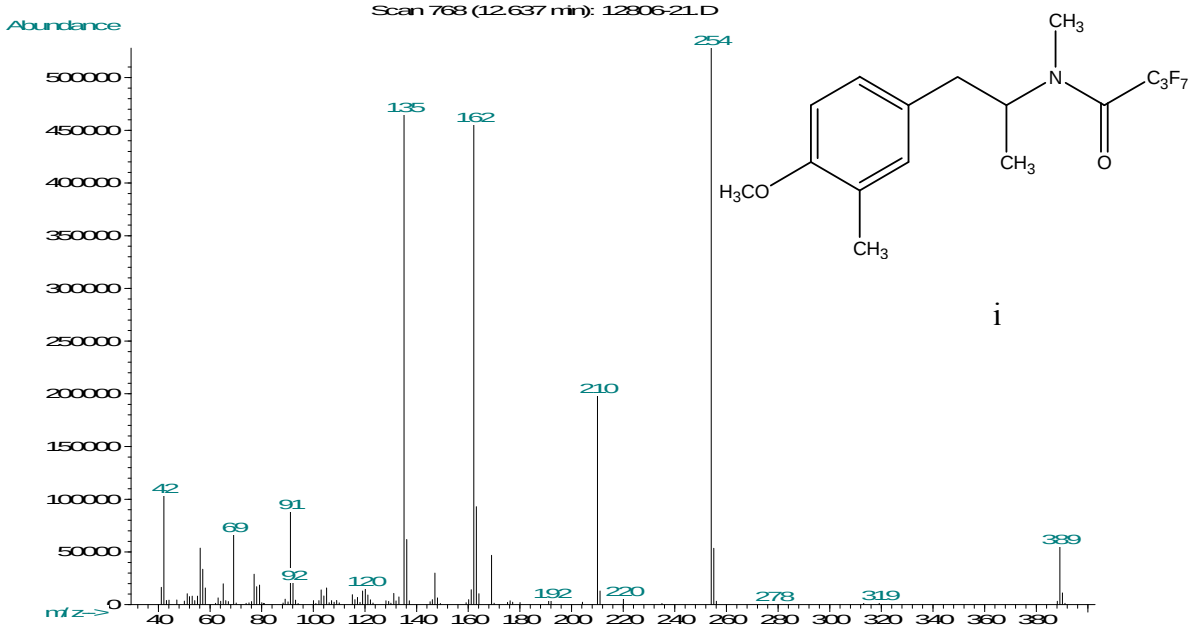

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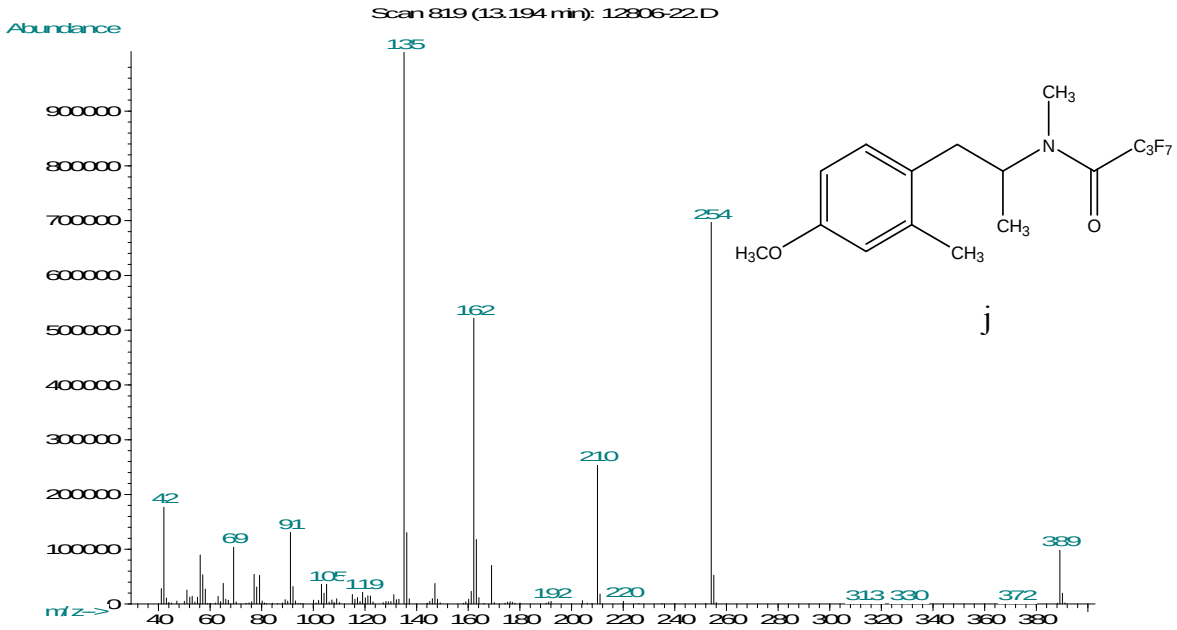

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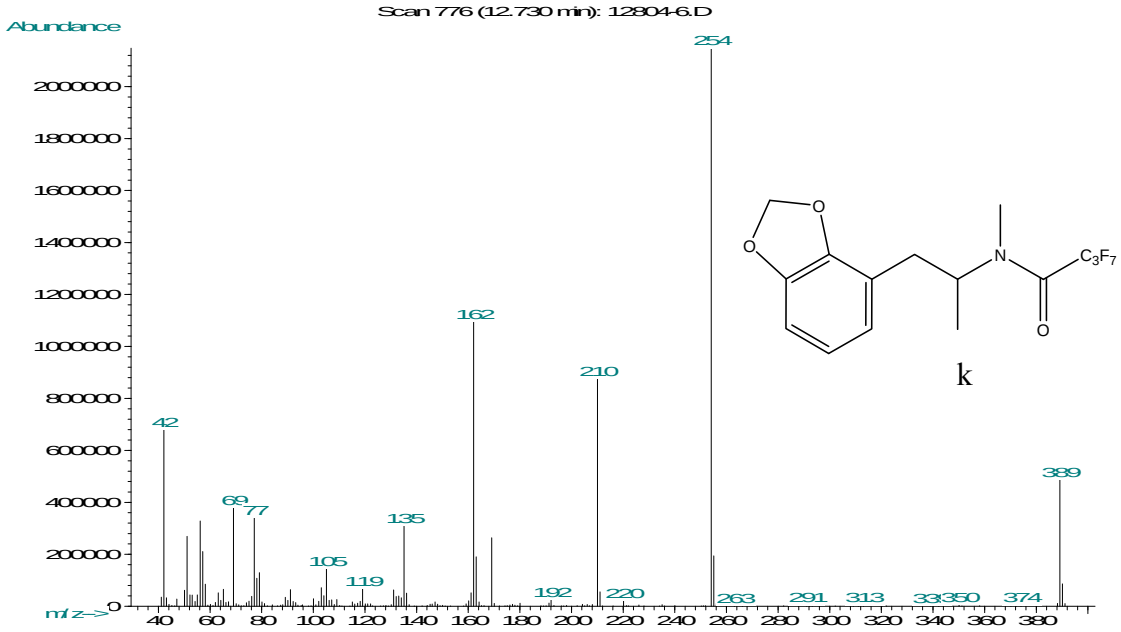

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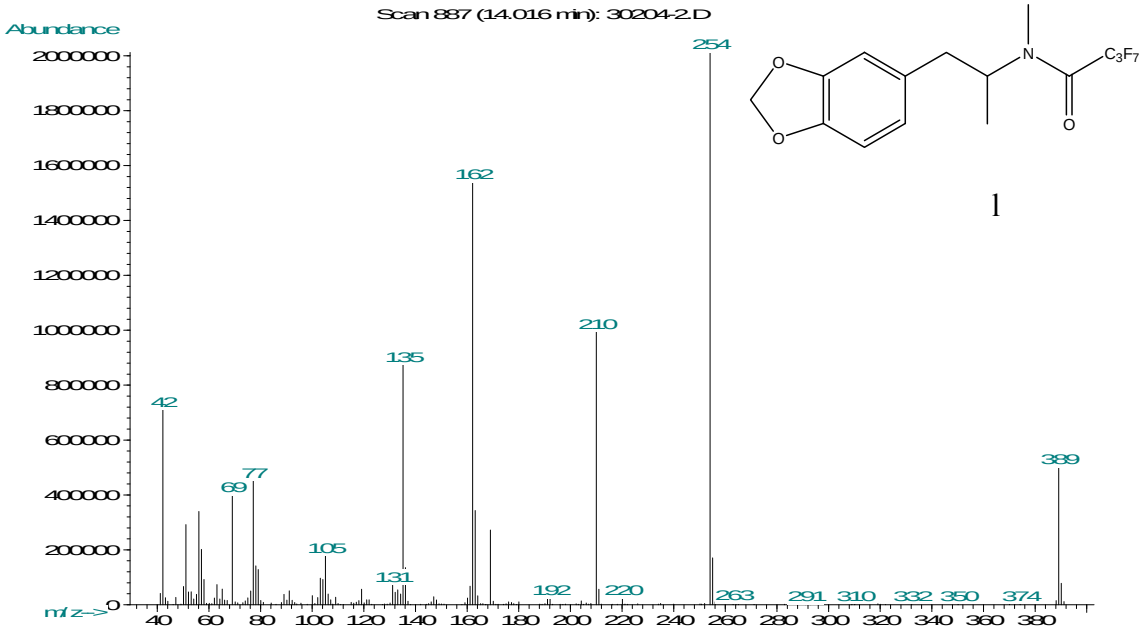
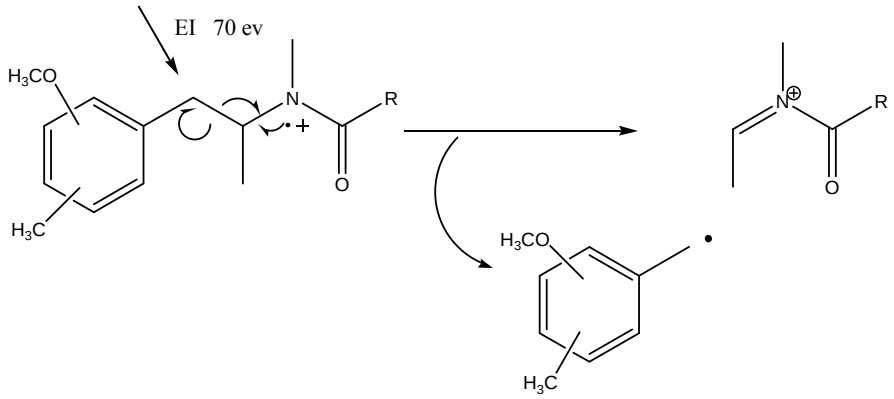


l

All these spectra show major high mass fragment ion at m/z 204 or 254 corresponding to the loss of 135 mass units from the molecular ion 339 and 389 from the PFPA and HFBA amides, respectively. The ions at m/z 204 and 254 are the PFPA and

HFBA imine species likely formed from the alpha cleavage of the amide nitrogen to eliminate the methoxymethylbenzyl radical. Thus the m/z 204 and 254 ions in PFPA and HFBA amides are analogous to m/z 58 in the underivatized species because all these ions represent the $(M\text{-}135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Scheme 6.2. The methoxymethylbenzyl cation and radical cation at m/z 135/136 is also a common fragment in most of the spectra in Figures 6.2 and 6.3. The identical fragmentation pathways for the PFPA and HFBA derivatives of 2,3- and 3,4-MDMA produced ions of the same structure at m/z 204 and 254 and isobaric ions at equivalent masses for the benzylic species at m/z135/136.



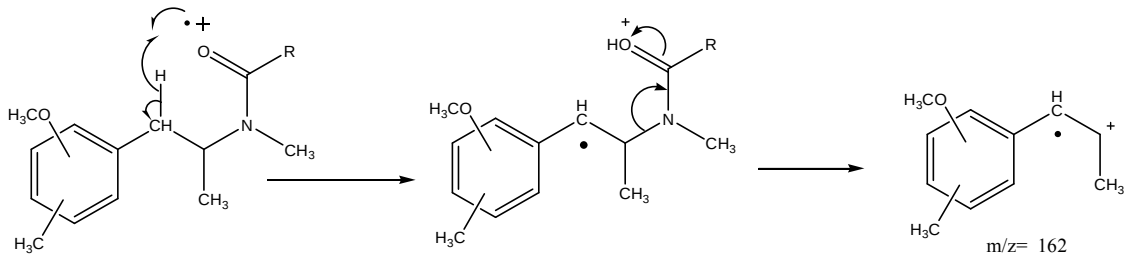
Scheme 6.2: Formation of m/z 204 (R=C_2F_5) and m/z 254 (R= C_3F_7) from perfluoroaceyl derivatives of methoxy methyl methamphetamines.

Acylation, and in particular the perflouroacylation, weakens the bond between nitrogen and the alpha-carbon of the substituted methoxy methyl phenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. The mass spectra in Figures 6.2 and 6.3 illustrate the role of hydrocarbon fragments at

m/z 162, 105 and 210 in the electron impact mass spectral differentiation among these isobaric compounds.

The mass spectra for all derivatives (Figures 6.2 and 6.3) show a common peak at m/z 162 corresponding to the alkene radical cation which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (Scheme 6.3). The isobaric methylenedioxyphenylpropene radical cation is observed in the mass spectrum of the PFPA and HFBA derivatives of 2,3- and 3,4-MDMA.The presence of the m/z 162 ion (as described previously) indicates that a 3-carbon chain is attached directly to the aromatic ring in an uninterrupted manner. The companion ion identifying the substituent on nitrogen as the N-methyl group occurs at m/z160 in the PFPA derivatives (Figure 6.2) and at m/z 210 in the HFBA derivatives (Figure 6.3)

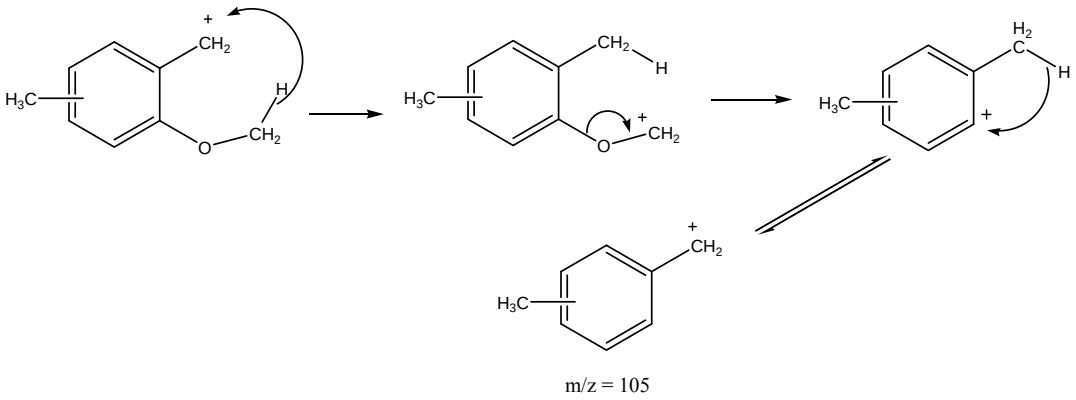
Additionally the PFPA and HFBA derivatives of d_3- and d_5-MDMA, in Figure 1.4 and discussed earlier in chapter 1, also lend support to the proposed structure for the formation of the alkene fragment at m/z 162 illustrated in Schemes 1.5 and 1.6 in chapter 1. A comparison of the PFPA derivatives (Figure 6.2) with the HFBA derivatives (Figure 6.3) indicated unique ions at m/z 160 and m/z 210. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7 respectively. The PFPA and HFBA derivatives of d_3- and d_5-MDMA, (Figure 1.4; chapter 1) showed that the m/z 160 and m/z 210 ions contain the N-methyl group and supports the proposed structure of the characteristic nitrile fragment.The suggested mechanism of forming these masses was illustrated previously in Scheme 1.5; chapter 1.



R = C_2F_5 PFPA derivatives
R= C_3F_7 HFBA Derivatives

Scheme 6.3: Formation of m/z 162 from perfluoacyl derivatives of methoxy methyl methamphetamines.

The mass spectra of the 2-methoxy-substituted methyl methamphetamines derivatives shown in Figure 6.2 a, b, c, and d for the PFPA derivatives and Figure 6.3 a, b, c and d for the HFBA derivatives show a more prominent m/z 105 ion than the other substitution patterns. This ion at m/z 105 represents the loss of 30 mass units (formaldehyde, CH_2O) from the methoxymethylbenzyl cation at m/z 135. The further loss of formaldehyde (CH_2O) from those benzylic cations having an ortho-methoxy group can be attributed to a 1,6-hydride shift from the carbon of the methoxy group to the methylene of the methoxymethyl benzyl cation followed by another hydride rearrangement and loss of formaldehyde to give the methyl benzyl cation (Scheme 6.4).



Scheme 6.4: Formation of m/z 105 form methoxymethylbenzyl radical.

As expected in this study, acylation of the side chain nitrogen in these isobaric methamphetamines did not individualize the resulting mass spectra. Thus, differentiation among these compounds and differentiation from 3,4-MDMA remains a significant challenge for chromatographic studies. However, the mass spectra obtained for the PFPA and HFBA derivatives do provide information which could allow these ten methoxymethylmethamphetamines to be divided into three subsets based on the position of the methoxy-group ring substitution.

The mass spectra of the PFPA and HFBA derivatives of the ortho-methoxy subset (compounds 1-4) all show m/z 105 ion formed through the mechanism described in Scheme 6.4. This ion does not occur to any significant extent in the derivatives of the meta-methoxy of para-methoxy subsets. The m/z 105 ion is not observed in the mass spectrum of the PFPA and HFBA derivatives of 2,3- and 3,4-MDMA. Thus the m/z 105 ion may distinguish this ortho-methoxy subset from MDMA but no specific ions were observed to distinguish among the members of this subset, compounds 1-4.

The PFPA and HFBA derivatives of compounds 5-8 (methoxy group in the meta postion of the aromatic ring) can be differentiated from the PFPA and HFBA derivatives

of compounds 1-4 by the absence of m/z 105. Perfluroacylation did not offer an advantage in distinguishing these compounds from each other and from the MDMAs.

The para-methoxy subset, the PFPA and HFBA derivatives of compounds 9 and 10, shows a very different distribution of ions than that observed for either of the other two subsets. The low mass ions at m/z 135 and m/z 162 show a very high relative abundance and actually appear as the base peak in several spectra. These are the only derivatives not showing the perfluoroacylimine at m/z 204 or m/z 254 as the base peak.

In summary, perfluoroacylation did not allow mass spectrometry to individualize these compounds. The presence of the m/z 105 ion suggests the methoxy group is in the ortho-position of the aromatic ring while the significant abundance of the low mass ions at m/z 135 and m/z 162 indicates the methoxy group is substituted at the para-position of the aromatic ring. The meta-substituted aromatic ring methoxy group isomers did not show any unique fragments to distinguish them from 2,3- and 3,4-MDMA.

Gas chromatographic separation of the perfluroacyl derivatives of ring substituted methoxy methyl methamphetamines, 2,3- and 3,4- MDMA.

The underivatized and PFPA and HFBA derivatives of 2,3- and 3,4-MDMA and their isobaric substituted methoxy methyl methamphetamines were compared on four stationary phases using capillary columns of the same dimensions, 30m x 0.25mm and 0.25um depth of film.

Several temperature programs were evaluated, however only one program yielding the best compromises between resolution and analysis time was used to generate the twelve chromatograms for the PFPA and HFBA derivatives in Figures 6.5-6.10,

respectively. The program (TP-2) was set up to hold the column temperature at 70 ^{o}C for 1 minute, ramped to 150 ^{o}C at 7.5 ^{o}C/minute, hold at 150 ^{o}C for 2 minutes and finally ramped to 250 ^{o}C at 10 ^{o}C/minute. Only chromatograms generated on Rtx-1 and Rtx-35 are shown in Figures 6.5-6.10.

The co-elution of some underivatized, PFPA and HFBA derivatives of 2,3-, 3,4-MDMA and their isobaric substances varied from one stationary phase to another. Underivatized compound 2 co-elutes with compound 4 while underivatized compound 6 co-elutes with underivatized compounds 7 and 9 on Rtx-1 stationary phase. Each of the PFPA and HFBA derivatives of compounds 8 and 9 co-elute, also the HFBA derivatives of compounds 4 and 6 co-elute on the Rtx-1 stationary phase.

Four sets of underivatized compounds were found to co-elute on Rtx-5, 2,3-MDMA co-elutes with compound 6, compound 2 co-elutes with compound 4, compound 5 co-elutes with compound 8 and finally compound 7 co-elutes with compound 9. In case of the PFPA derivatives of the 2,3- , 3,4-MDMA and their isobaric substituted methoxy methyl methamphetamine, there are two sets of compounds co-elute namely compound 11 co-elutes with compound 7 and compound 8 co-elutes with compound 9. Three sets of the HFBA derivatives were found to co-elute, compound 11 co-elutes with compound 7, compound 1 co-elutes with compound 3 and finally compound 5 co-elutes with compound 8on the same column.

Three sets of underivatized compounds were found to co-elute on Rtx-35, compound 4 co-elutes with compound 6, compound 5 co-elutes with compound 8 and finally compound 7 co-elutes with compound 9. In case of the PFPA derivatives of the 2,3-, 3,4-MDMA and their isobaric substituted methoxy methyl methamphetamine, there

are two sets of compounds which co-elute where compound 5 co-elutes with compound 9 and compound 4 co-elutes with compound 6. The HFBA derivatives of compounds 2, 8 and 9 co-elute on the same column.

The best resolution, among the evaluated columns, was achieved on Rtx-200 where in a physical mixture of the 12 PFPA derivatized compounds yielded 11 peaks with compounds 7 and 9 co-elute (Figure 6.4). The PFPA derivatives allow more distinctive differentiation between compounds 7 and 9 based on mass spectrometry. Compound 9 has a base peak at m/z 135 compared to m/z 204 base peak for compound 7. Thus a sample containing compound 7 or 9 could be identified based on mass spectrometry. The similarity in chromatographic properties among these regioisomeric and isobaric molecules in the derivatized and underivatized form provides for a significant chromatographic challenge. However, all four of the stationary liquid phases evaluated in this study successfully resolved 3,4-MDMA from the other isomers. The variation in chromatographic selectivity among the phases resulted in various coelutions within the isobaric methoxy methyl methamphetamines. Since mass spectrometry of the perfluoroacyl derivatives of the isobaric methoxymethyl methamphetamines (compounds 1-10) successfully divided these compounds into subsets based on the ring position of the methoxy group, the chromatographic properties were evaluated using the same subsets. The chromatographic properties of each subset of compounds was compared to 2,3- and 3,4-MDMA.

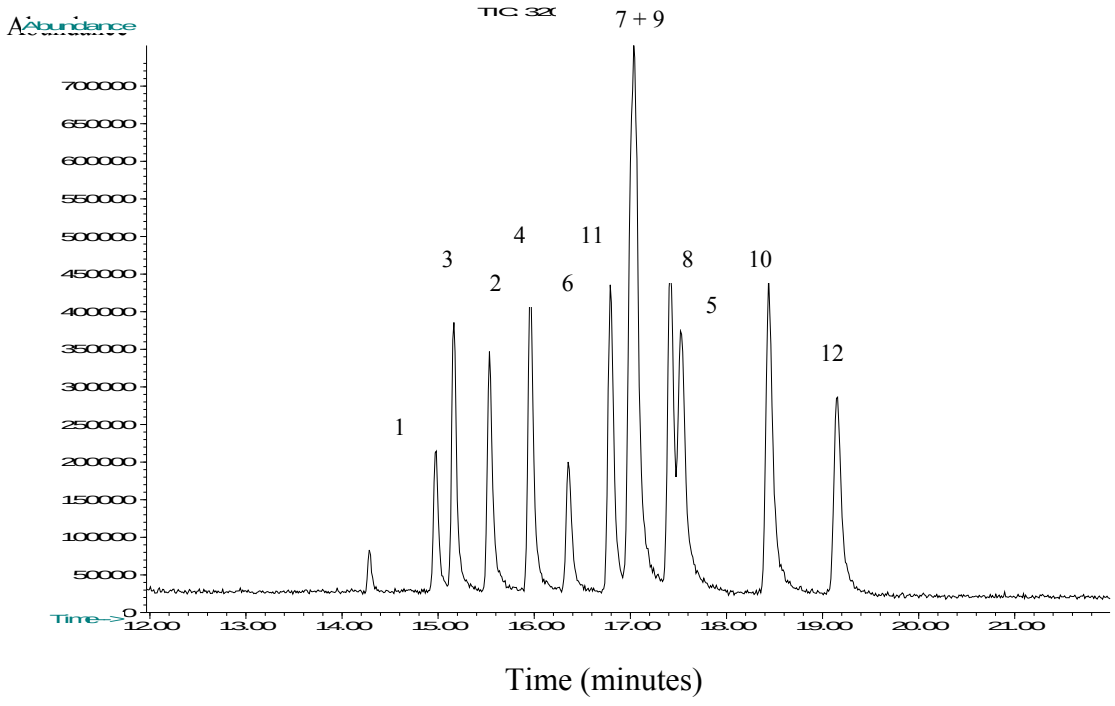


Figure 6.4: Capillary gas chromatograph of a physical mixture of the PFPA derivatives of methoxy methyl methamphetamines (Compounds 1-10), 2,3- (11) and 3,4-MDMA (12) . Column used: RTX-200.

In all the chromatographic studies 3,4-MDMA elutes last in every subset and in every form (derivatized and underivatized). In the first subset (the ortho-methoxy substituted aromatic ring), compounds 1-4, along with 2,3-MDMA and 3,4-MDMA, the elution order of the perfluroacyl derivatives of these compounds was compound 1 followed by 3, 2, 4, 2,3-MDMA- and finally 3,4-MDMA (compound 12). The elution order, on Rtx-1 and Rtx-35, was the same for this subset of compounds. (Figures 6.5 and 6.6). This subset of isobaric amines was identified as having a significant m/z 105 ion in their mass spectra.

In the second subset (the meta-methoxy substituted aromatic ring), compounds 5-8 along with 2,3-MDMA and 3,4-MDMA, the PFPA derivative of compound 6 elutes

first followed by 2,3-MDMA-PFPA, 7-PFPA, 8-PFPA, 5-PFPA and finally 3,4-MDMA-PFPA(compound 12); (Figure 6.7A). The elution order is the same for the HFBA derivatives of the same compounds on Rtx-1 (Figures 6.8A). This elution order has changed on Rtx-35, where compound 7 elutes before 2,3-MDMA, and most significantly 2,3-MDMA (compound 11) and 8 co-elute. This change in elution order takes place for the PFPA and HFBA of these compounds (Figures 6.7b and 6.8b) which suggests that Rtx-35 is not the best column for resolving this subset of compounds. It is this subset of isobaric amines that showed no distinguishing characteristics (neither unique fragment ions nor unique relative abundance of fragment ions) in their mass spectra.

In the third subset (the para-methoxy substituted aromatic ring), compounds 9, 10, 2,3-MDMA and 3,4-MDMA, the elution order of the perfluroacyl derivatives was 2,3-MDMA followed by 9, 10 and finally 3,4-MDMA. The elution order was the same on both Rtx-1 and Rtx-35 (Figures 6.9 and 6.10). It is this subset of compounds which showed mass spectra most easily distinguished from the other subsets and from 2,3- and 3,4-MDMA.

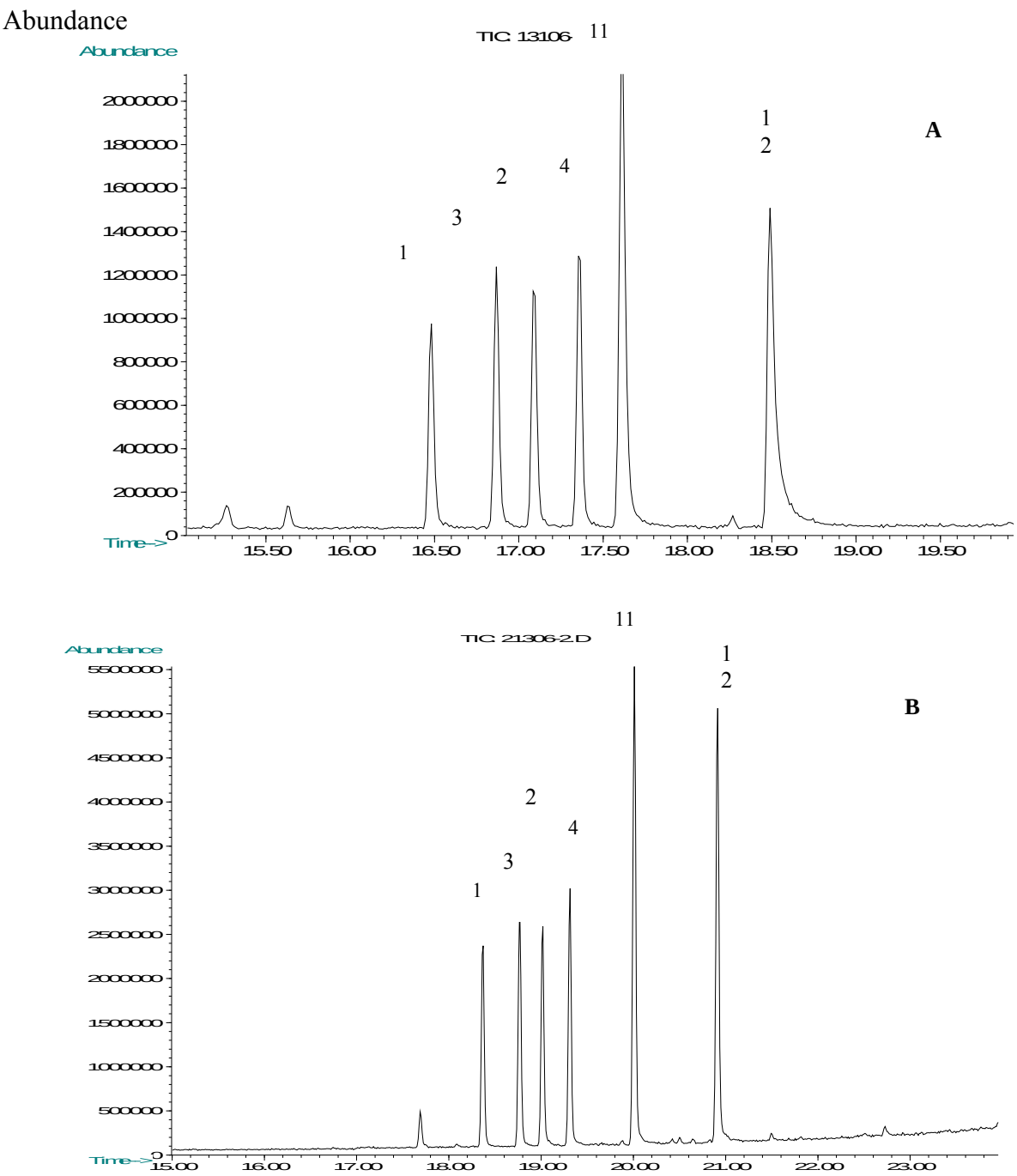


Figure 6.5: Capillary gas chromatographic separation of PFPA derivatives of compounds 1-4, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

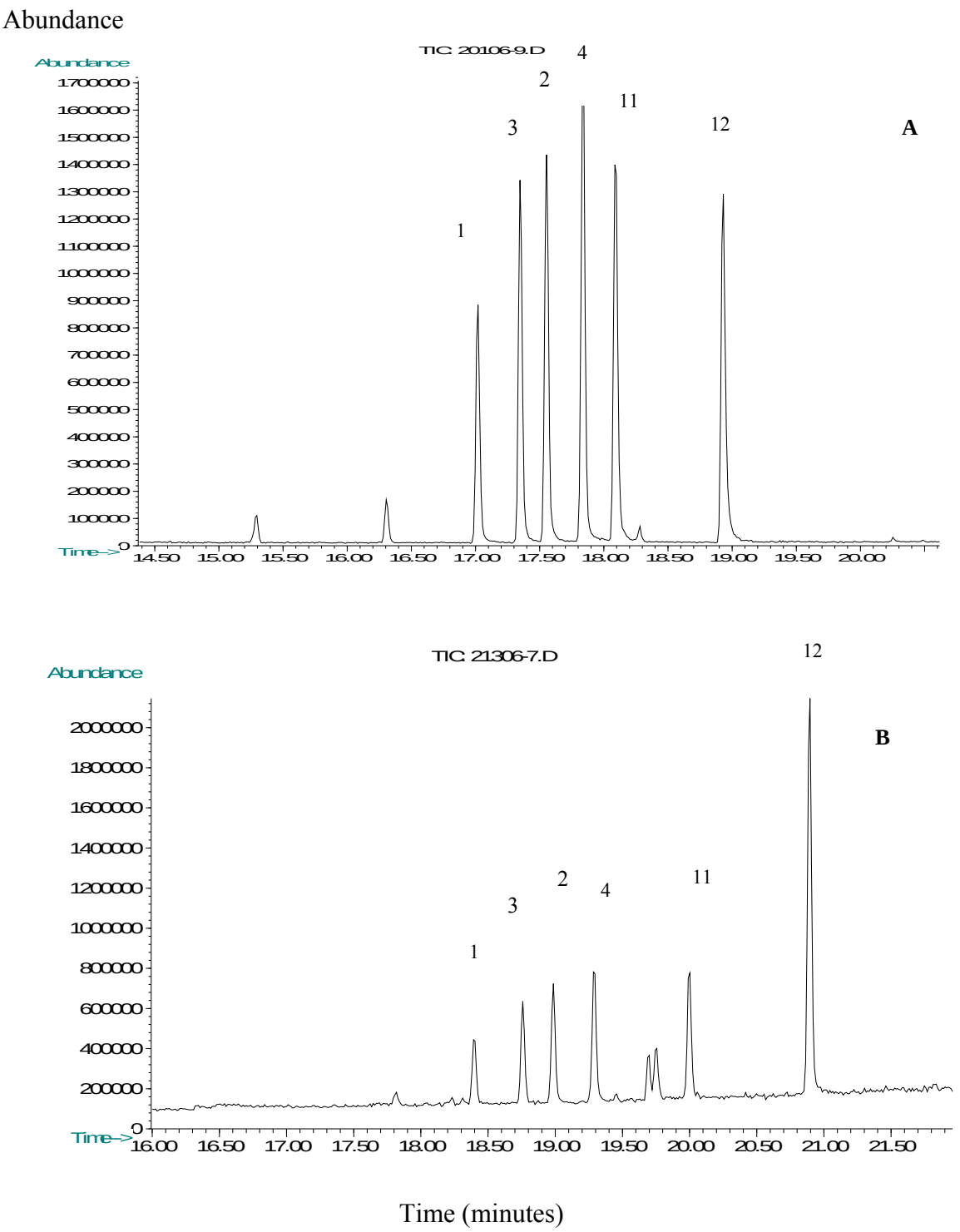


Figure 6.6: **Capillary gas chromatographic separation of HFBA derivatives of compounds 1-4, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.**

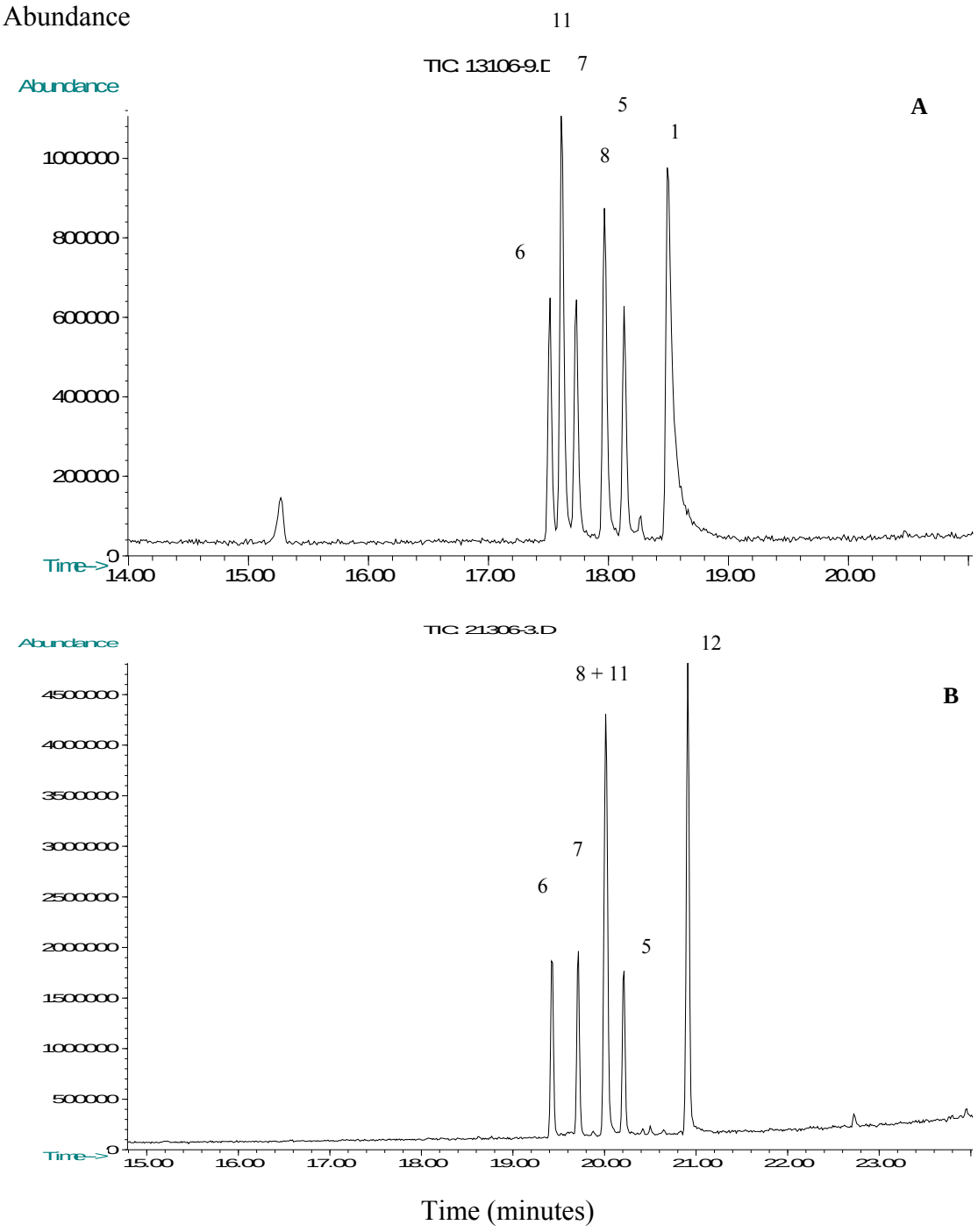


Figure 6.7: Capillary gas chromatographic separation of PFPA derivatives of compounds 5-8, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

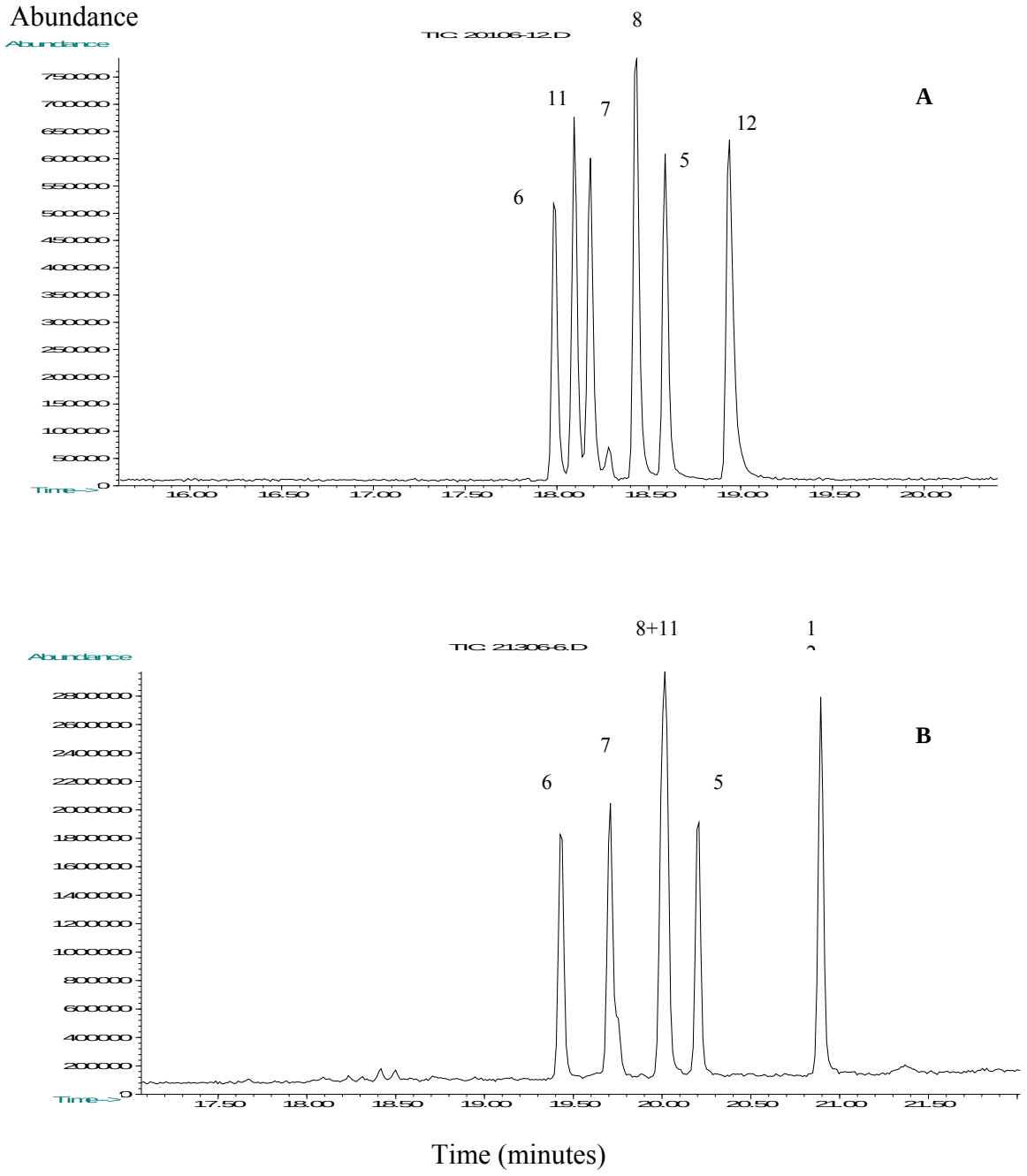


Figure 6.8: Capillary gas chromatographic separation of HFBA derivatives of compounds 5-8, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

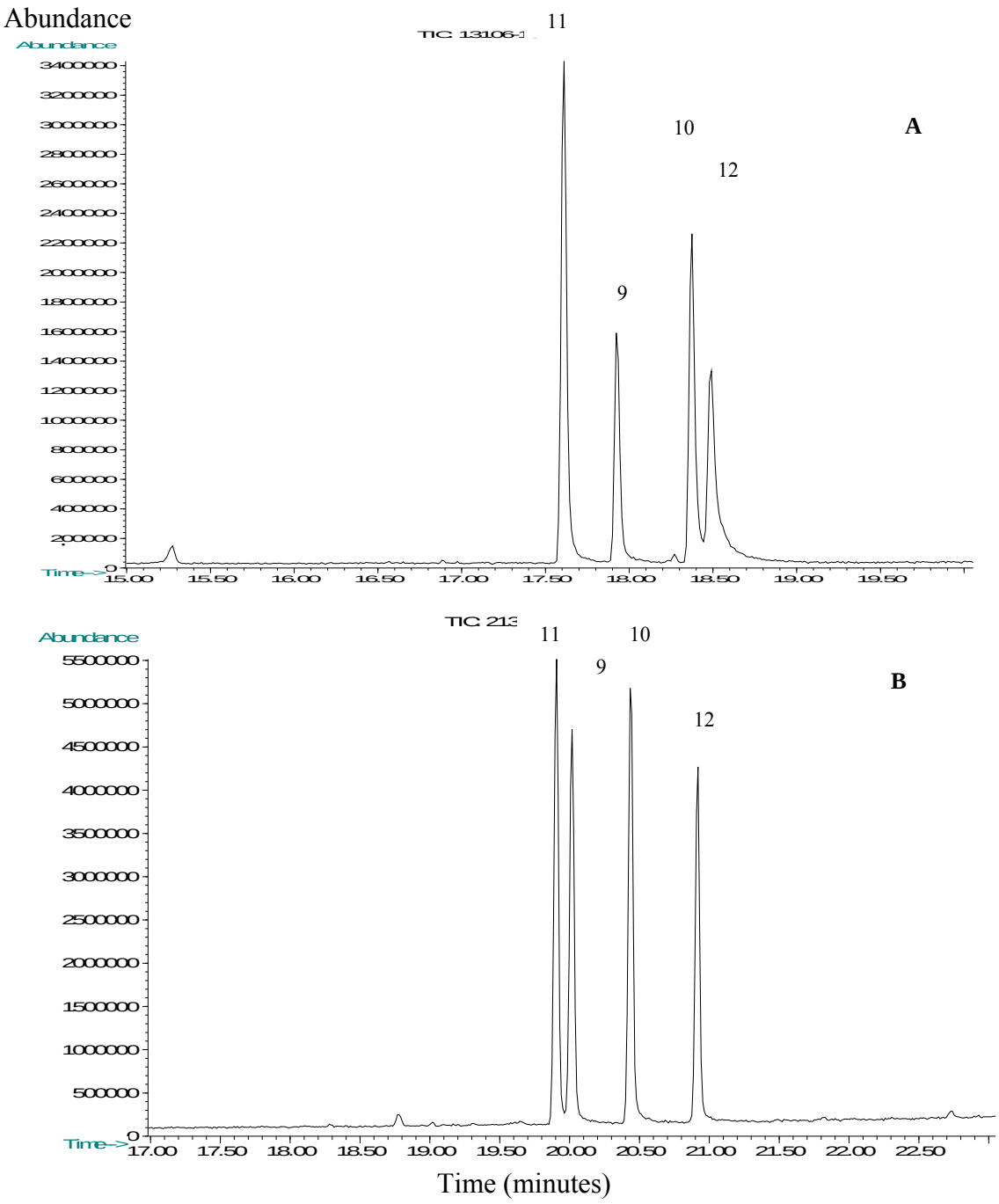


Figure 6.9: Capillary gas chromatographic separation of PFPA derivatives of compounds 9-10, 2,3-MDMA (11) and 3,4-MDMA(12) . Columns used: A RTX-1; B RTX-35.

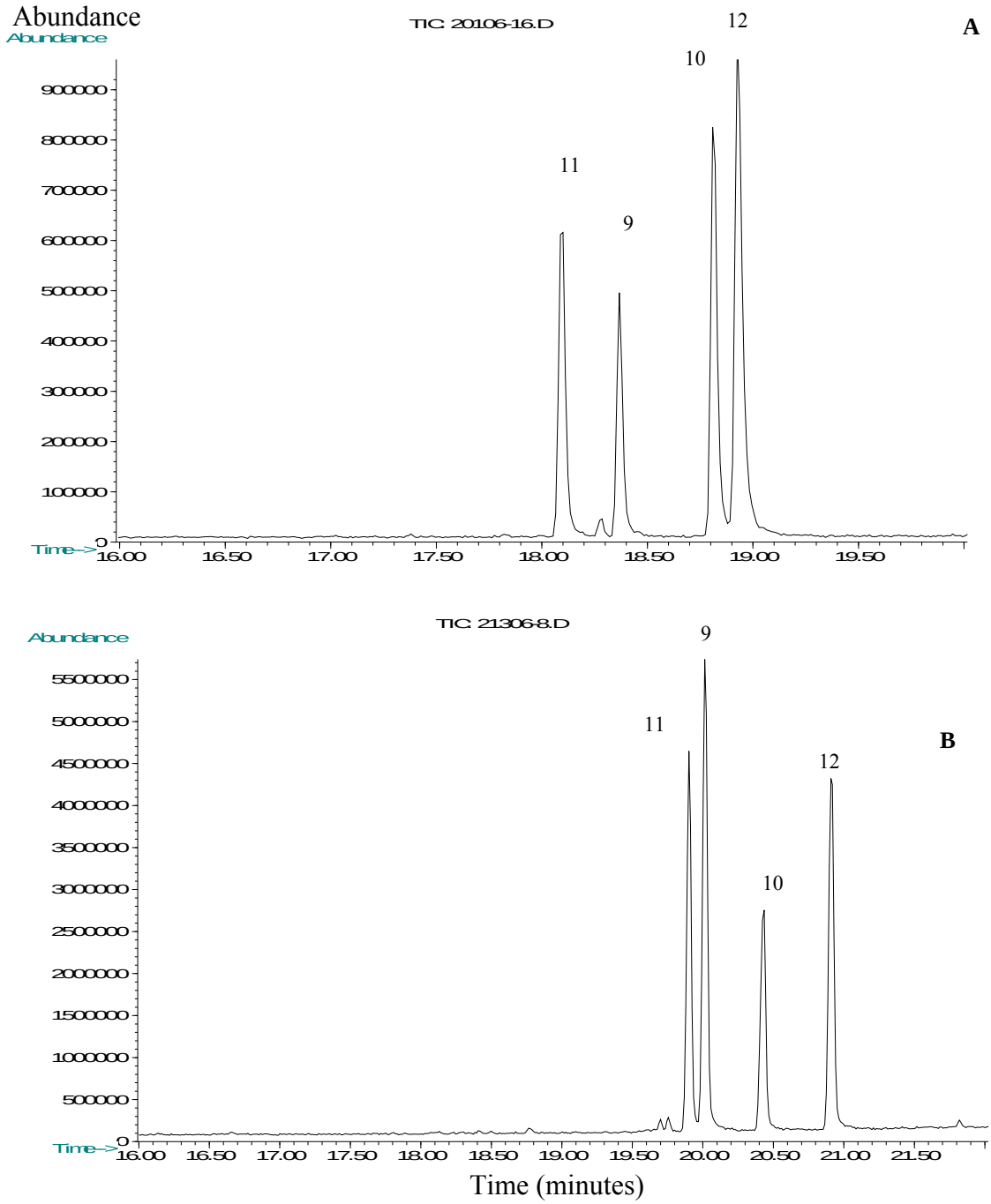


Figure 6.10: Capillary gas chromatographic separation of HFBA derivatives of compounds 9-10, 2,3-MDMA(11) and 3,4-MDMA(12). Columns used: A RTX-1; B RTX-35.

Conclusions of Chapter 6

The methoxy methyl methamphetamines have an isobaric relationship to the 2,3- and 3,4-methylenedioxymethamphetamines and represent a unique analytical challenge in forensic drug chemistry. Each of these compounds has a molecular weight of 193 and yields a base peak for the N-methyl imine of acetaldehyde at m/z 58 in the mass spectrum. This ion represents the loss of 135 mass units from the molecular ion corresponding to the substituted benzyl species, $C_8H_7O_2$ (methylenedioxybenzyl) and $C_9H_{11}O$ (methoxy methyl benzyl). Thus, the traditional electron impact mass spectrum provides little structural information for differentiating among these 12 compounds. Because of the unique similarity of these compounds by MS, the specific identification of a compound such as 3,4-MDMA requires methods to eliminate the other isomers. The ring-substituted methoxy methyl methamphetamines were evaluated for their chromatographic and mass spectral properties compared to 2,3- and 3,4-MDMA. The mass spectra studies showed almost identical mass spectra for all 10 methoxy methyl methamphetamines and 2,3- and 3,4-MDMA.

The perfluoroacylated derivatives of the 10 methoxy methyl methamphetamines were prepared and evaluated for their ability to individualize the mass spectra of these compounds and to maintain or improve chromatographic resolution. The perfluoroacylation process did not produce unique mass spectra characteristic of each compound. However, derivatization did subdivide the methoxy methyl methamphetamines into subgroups based on the position of the methoxy group on the aromatic ring relative to the alkylamine side-chain. The presence of the m/z 105 ion suggests the methoxy group is in the 2-position relative to the alkylamine side-chain, and

the significant abundance of the low mass ions at m/z 135 and m/z 162 indicates the methoxy group is substituted at the 4-position of the aromatic ring. The 3-substituted aromatic ring methoxy group isomers did not show any unique fragments to distinguish them from 2,3- and 3,4-MDMA as the perfluoroacylated derivatives.

Different stationary phases and temperature programs were used in an effort to separate the methoxy methyl methamphetamines from 2,3- and 3,4-MDMA. The best resolution among the evaluated columns was achieved on Rtx-200, where a mixture of the 12 compounds gave 11 peaks and only the PFPA derivatives of methoxy methyl methamphetamines 7 and 9 coelute. The perfluoroacyl derivatives of the methoxy methyl methamphetamines were divided into three subsets based on the ring substitution pattern of the methoxy group. The derivatives of the individual subsets were each resolved on Rtx-1.

Chapter 7

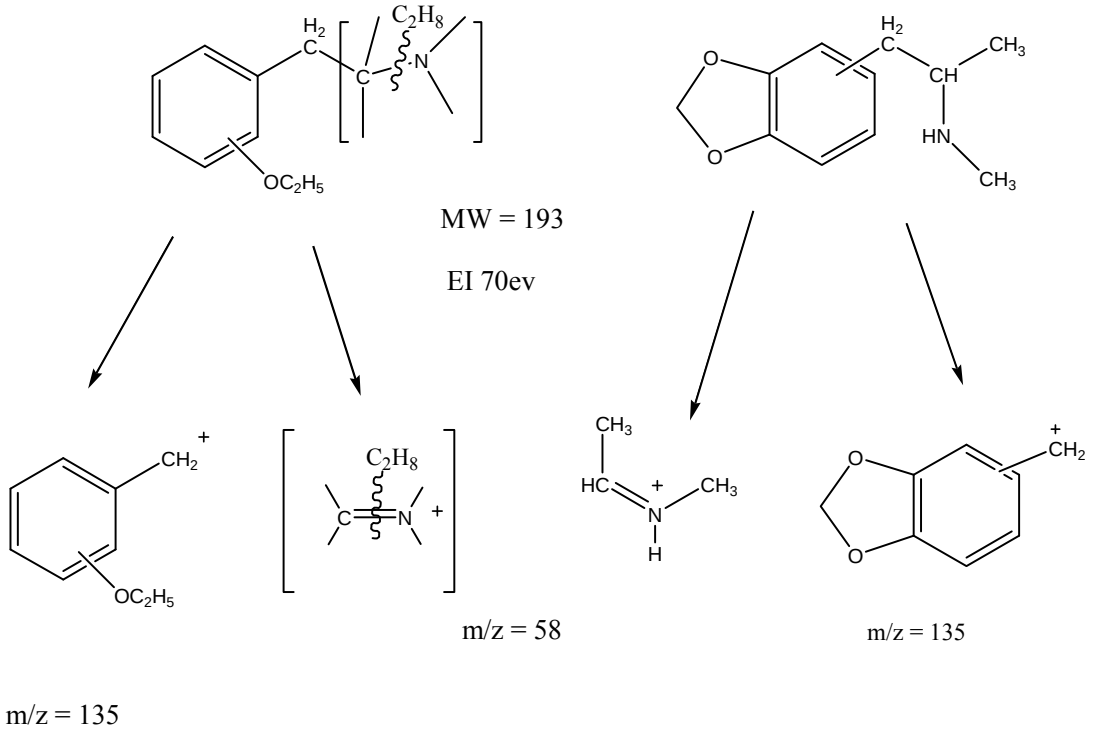
GC–MS Analysis of Ring and Side Chain Regioisomers of Ethoxyphenethylamines

The target compounds of this chapter are a series of ring substituted ethoxy phenethylamines with molecular weight 193 and the potential to produce mass spectra with major fragment ions at ***m/z*** 58 for the imine and ***m/z*** 135/136 for the substituted benzyl fragment. The major fragment ions observed in the EI mass spectrum for 3,4-MDMA (MW = 193) occur at equivalent masses.

Mass spectrometry of Ring and Side Chain Regioisomers of Ethoxyphenethylamines

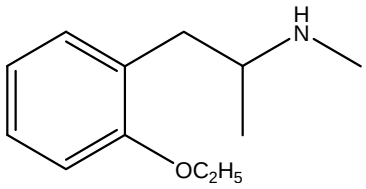
MS is the primary method for confirming the identity of drugs and related substances in forensic samples. The mass spectra of phenethylamines are characterized by a base peak formed from an amine initiated alpha-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and the amine. In 3,4-methylenedioxymethamphetamine (MW = 193) the alpha-cleavage reaction yields the substituted imine fragment at ***m/z*** 58 and the 3,4-methylenedioxybenzyl fragment at mass 135/136 (for the cation and the radical cation, respectively). Thus, the mass spectrum for 3,4-methylenedioxymethamphetamine contains major ions at ***m/z*** 58 and 135/136 as well as other ions of low relative abundance. The ring substituted ethoxy methamphetamines (Compounds 1–3, Figure 7.1) have the potential to yield a mass spectrum essentially equivalent to 2,3- and 3,4-MDMA. There are five possible side chain regioisomers for each one of these ring substitution patterns.

The five side chain regioisomers of 2-ethoxyphenethylamine included in this study are shown in Figure 7.1 (Compounds 1, 4–7). All five side chain regioisomers have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at ***m/z*** 58 and 135/136 (Scheme 7.1). The individual mass spectra for 2,3- and 3,4-MDMA are also presented in Scheme 7.1 (Compounds 8 and 9). The isobaric ethoxy benzyl $(C_9H_{11}O)^+$ fragments have the same mass as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at ***m/z*** 135. Furthermore the ***m/z*** 58 ion in the 2-ethoxyphenethylamines is regioisomeric with that obtained in the mass spectra of both 2,3 and 3,4-MDMA (Figure 7.2).



Scheme 7.1: EI fragmentation pattern for ring substituted ethoxy phenethylamines.

Figure 7.1: Structures of the ring substituted ethoxymethamphetamines, side chain regioisomerics of 2-ethoxyphenethylmethamphetamines, and 2,3- and 3,4-MDMA.



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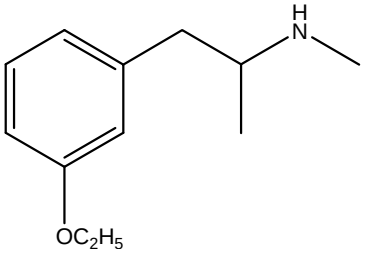

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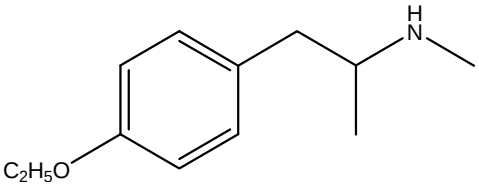

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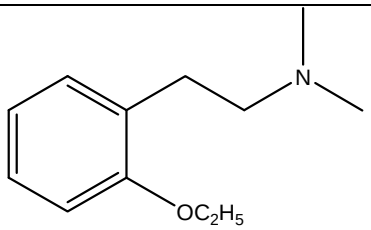

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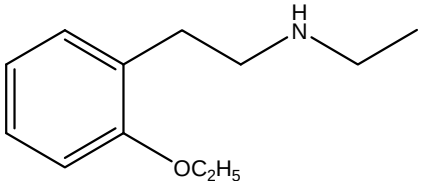

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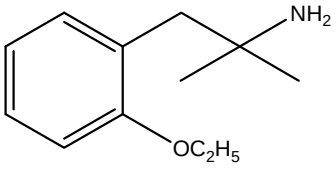

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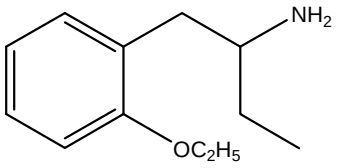

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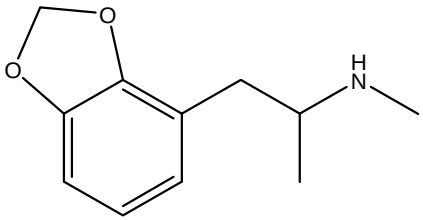

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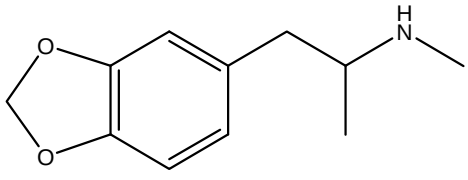
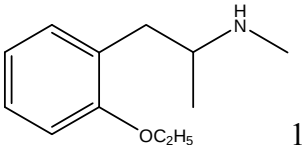


7 (3,4-MDMA)

This lack of mass spectral specificity for the isomers shown in Figure 7.2, in addition to the possibility of chromatographic co-elution with 3,4-MDMA, could result in misidentification in this series of drugs and drug-like substances.

Figure 7.2: Mass Spectra of compounds ring substituted ethoxy Phenethylamines, 2,3- and 3,4-MDMA.



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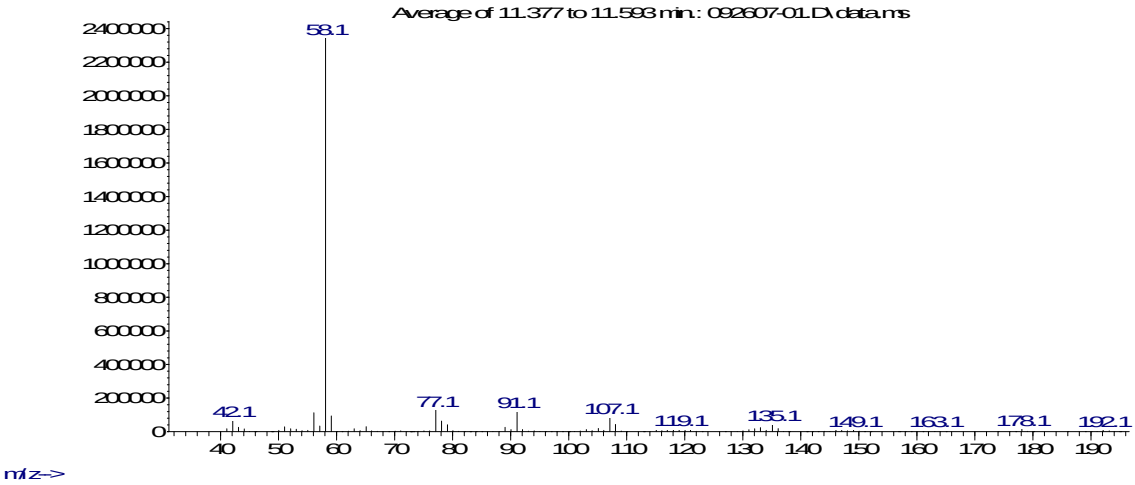

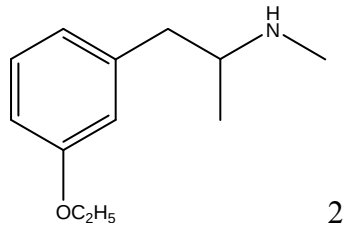

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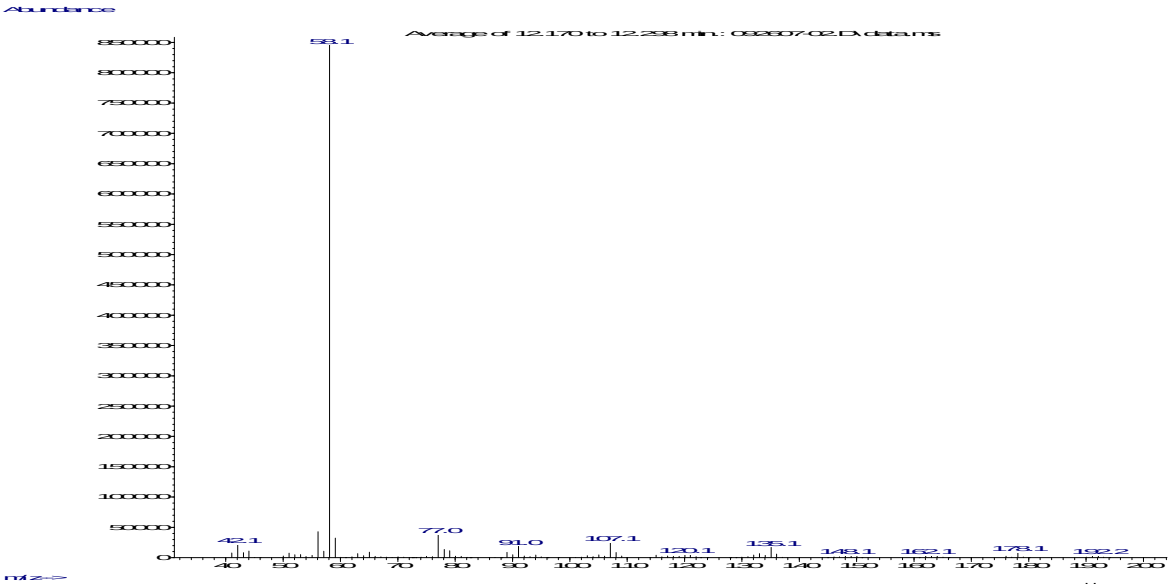

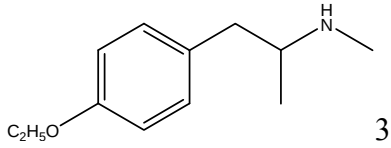

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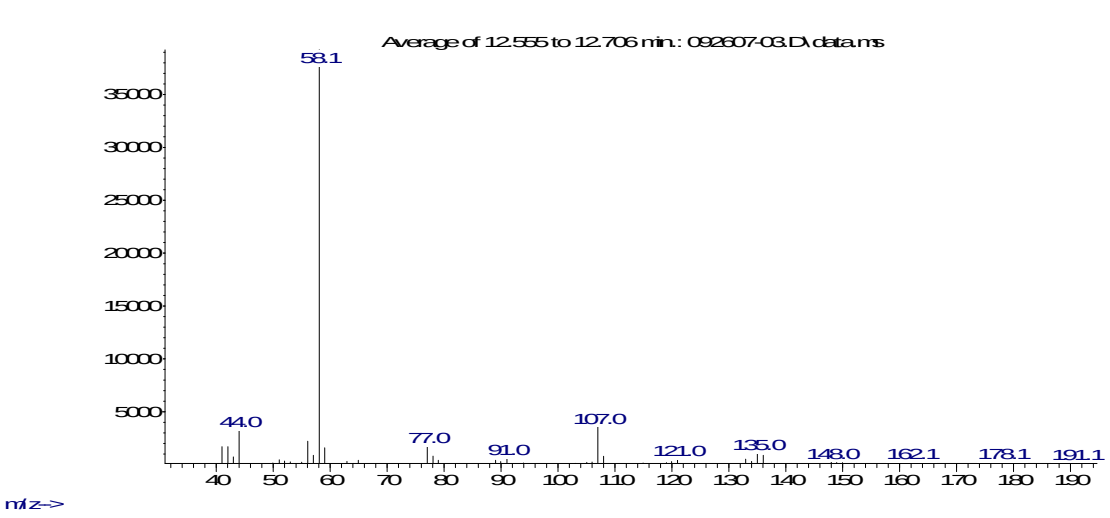

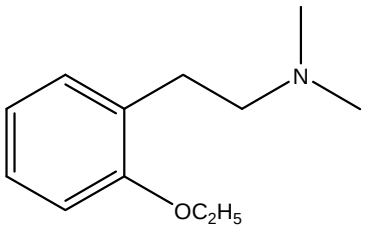

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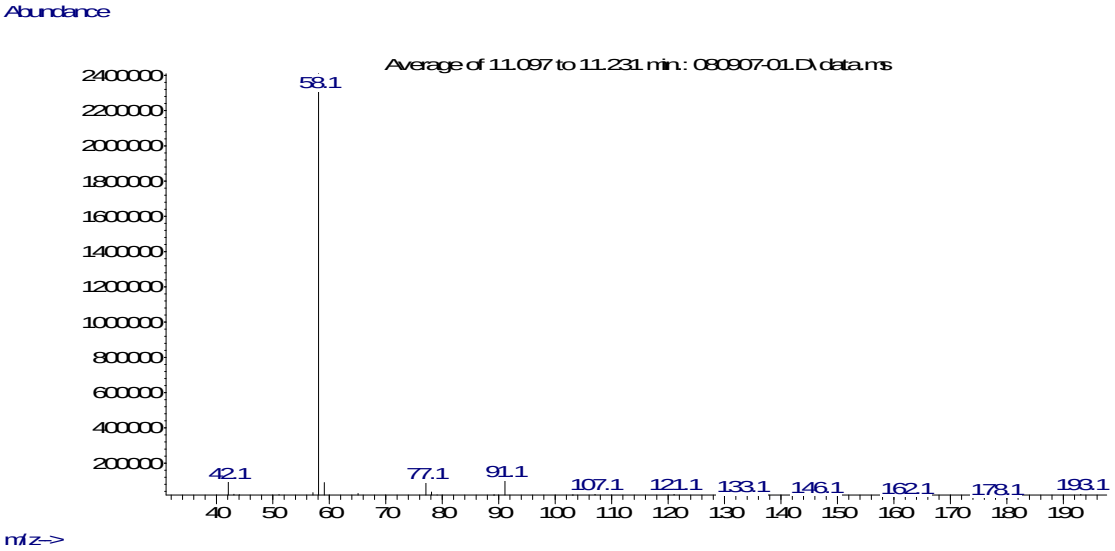

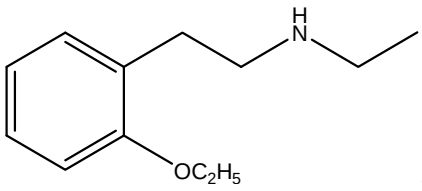

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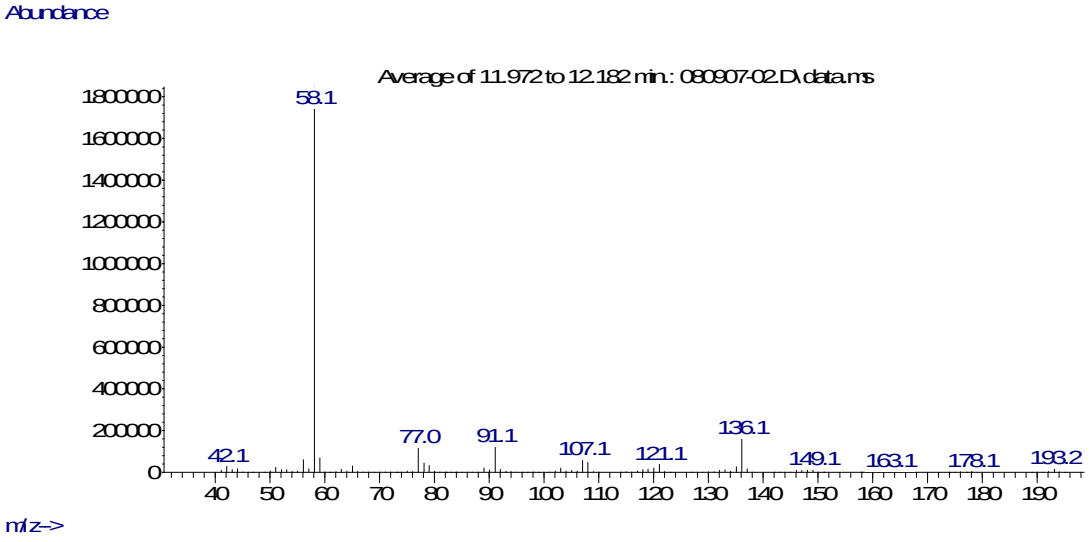

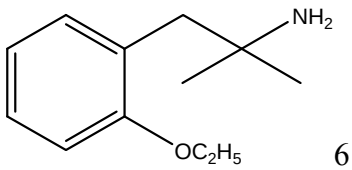

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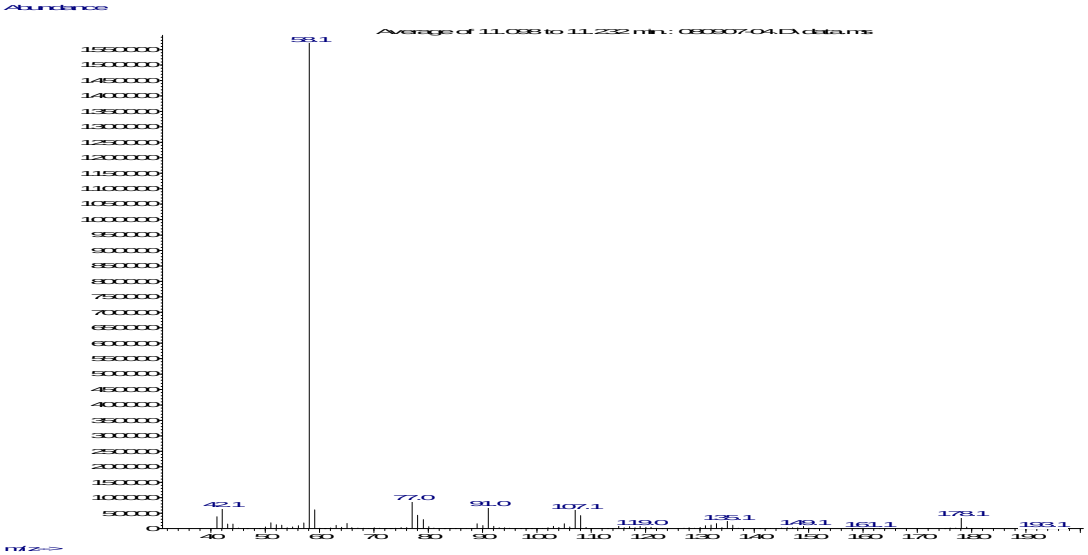

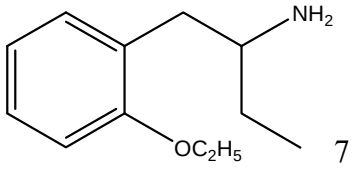

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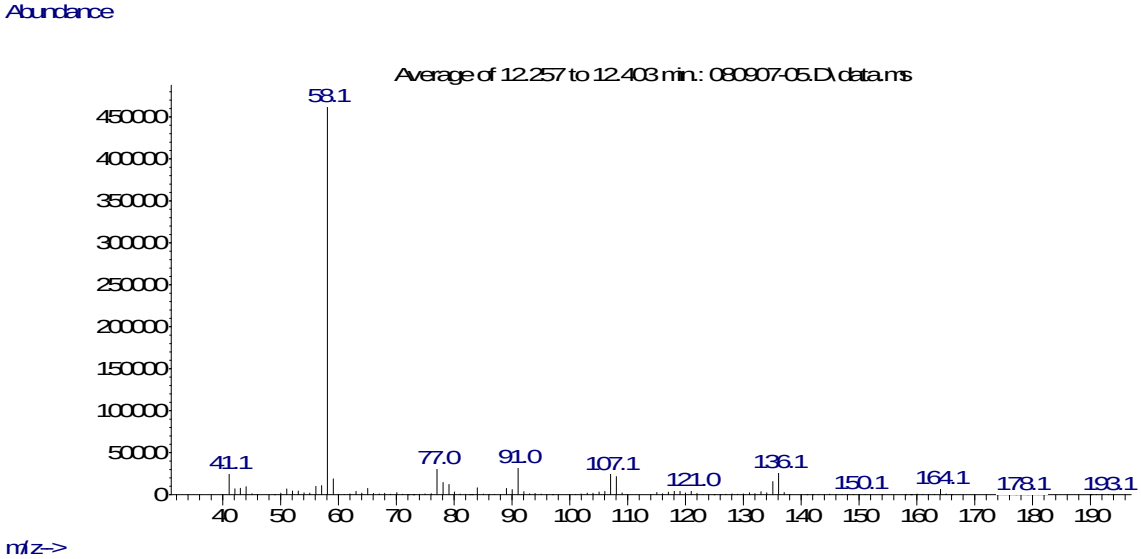

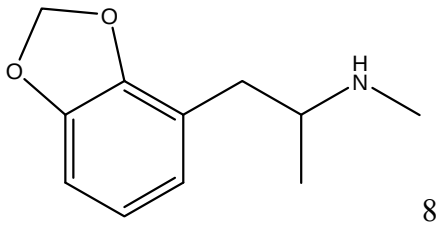

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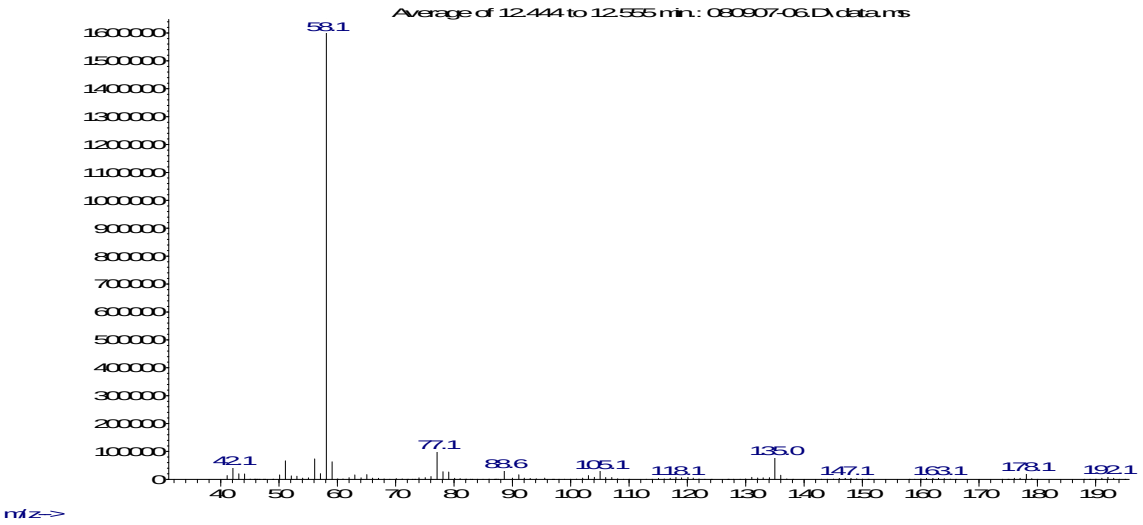

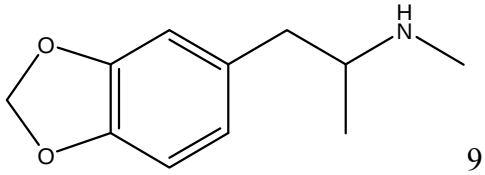

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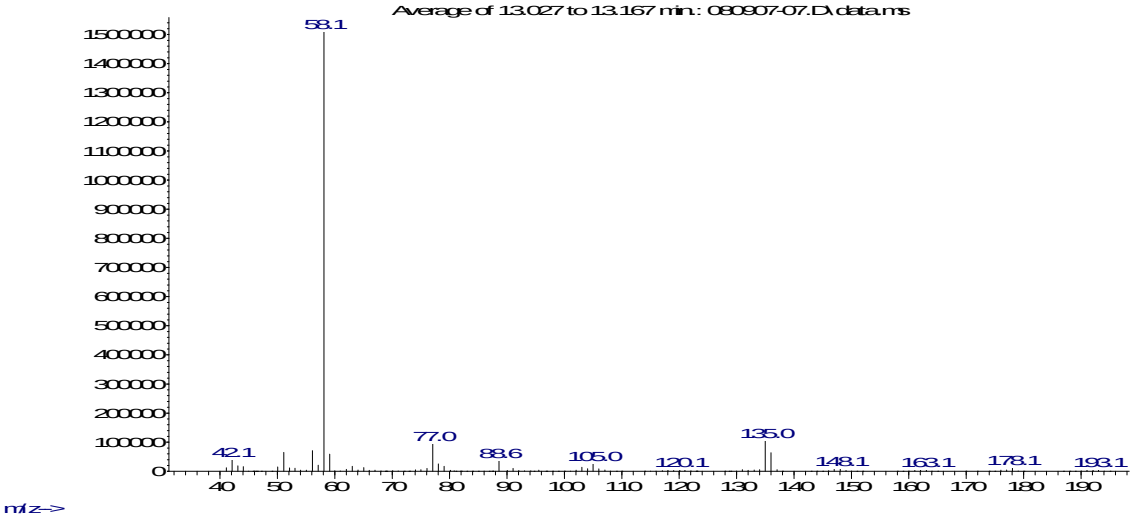
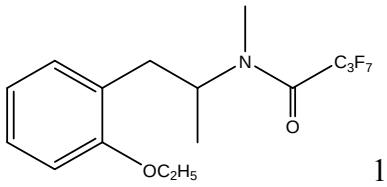


In the next phase of this study, various Acylated derivatives of the ring substituted ethoxy methamphetamines and the primary and secondary amines of the side chain regioisomers of 2-ethoxyphenethylamine were prepared and evaluated in an effort to individualize their mass spectra and provide unique marker ions for specific identification. Acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the resulting mass spectrum. Of course the tertiary amine (compound 4) does not form a stable amide derivative. The mass spectra for the pentafluoropropionyl and heptafluorobutyryl amides are shown in Figures 7.3 and 7.4, respectively. Perfluroacyl derivatives of the ring substituted ethoxy methamphetamines (Compounds 1–3) have almost identical mass spectra with the derivatized 2,3- and 3,4-MDMA (Compounds 8 and 9) except for a unique ion at ***m/z*** 107. This ion at ***m/z*** 107 represents the loss of 28 mass units (ethylene, C2H4) fromthe ethoxybenzyl cation at ***m/z*** 135 (Scheme 7.2). Although the relative abundance varies significantly, the***m/z*** 107 ion is present in all mass spectra of the PFPA and HFPA of compounds (1–3 and 5–7) and offers a unique fragment ion to discriminate these compounds from 3,4- and 2,3-MDMA. The ***m/z*** 107 ion is also present in relatively low abundance in the mass spectra of the underivatized ethoxy amines shown in Figure 7.2.

Figure 7.3: Mass Spectra of the HFBA derivatives of ring substituted ethoxy Phenethylamines, 2,3- and 3,4-MDMA.



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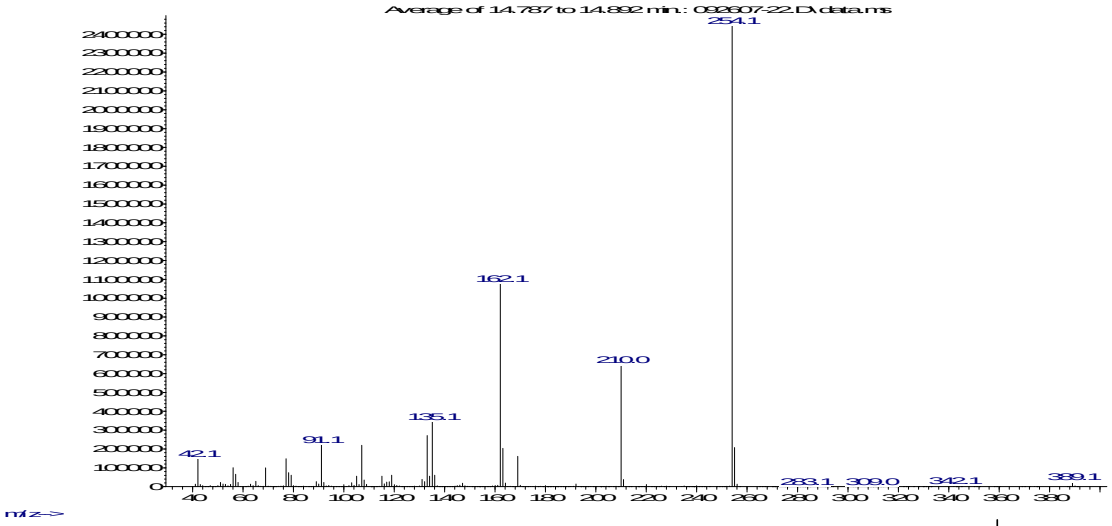

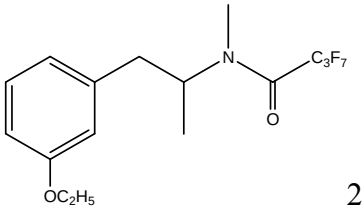

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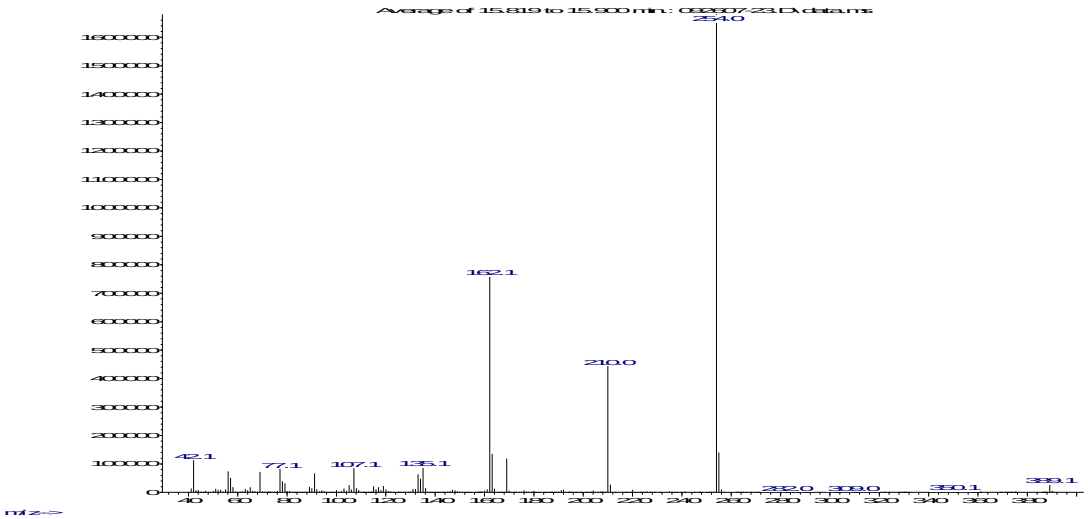

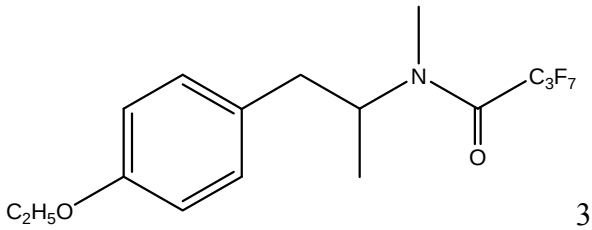

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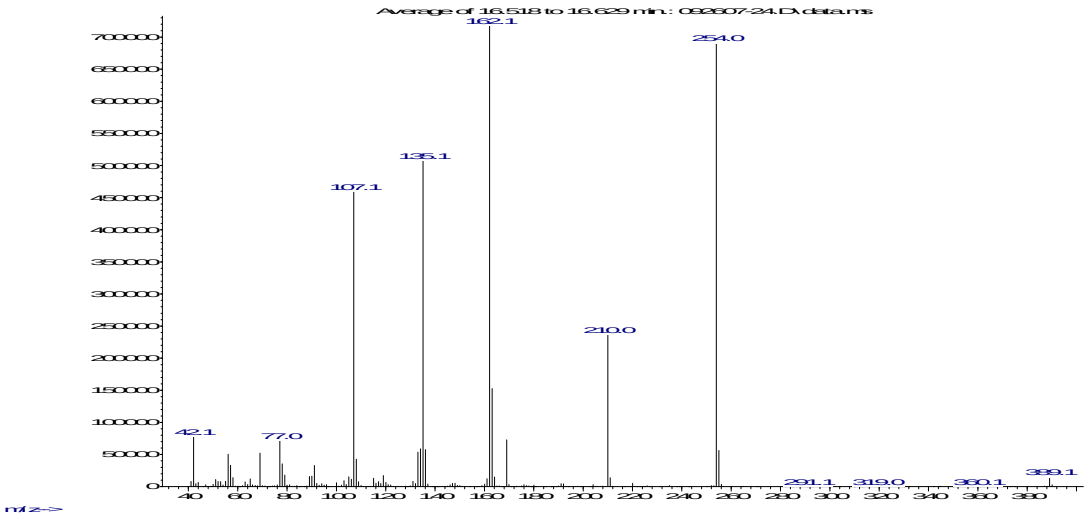

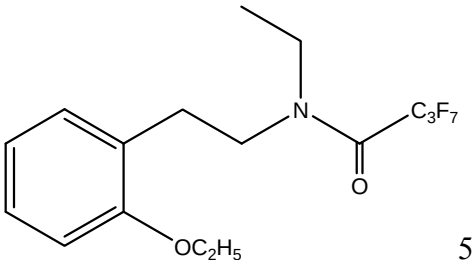

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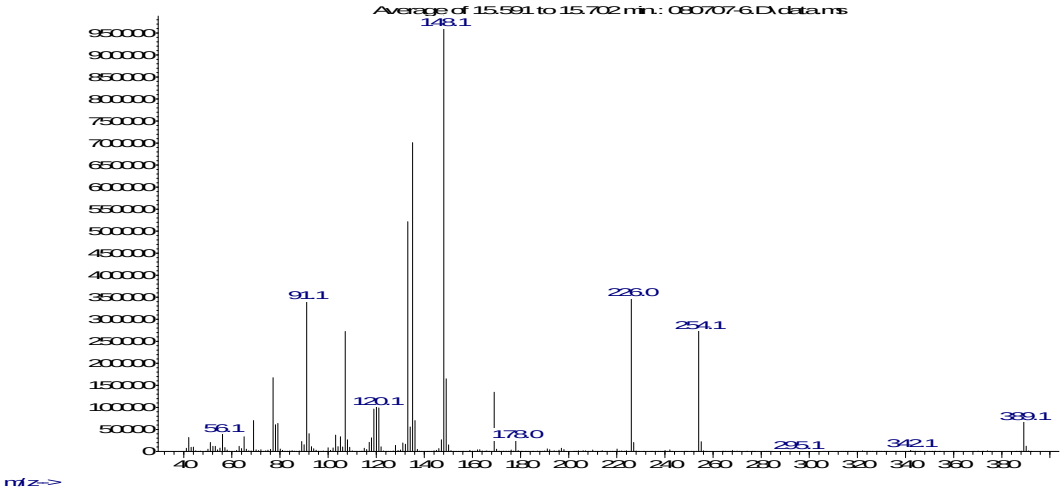

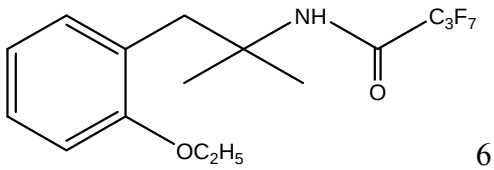

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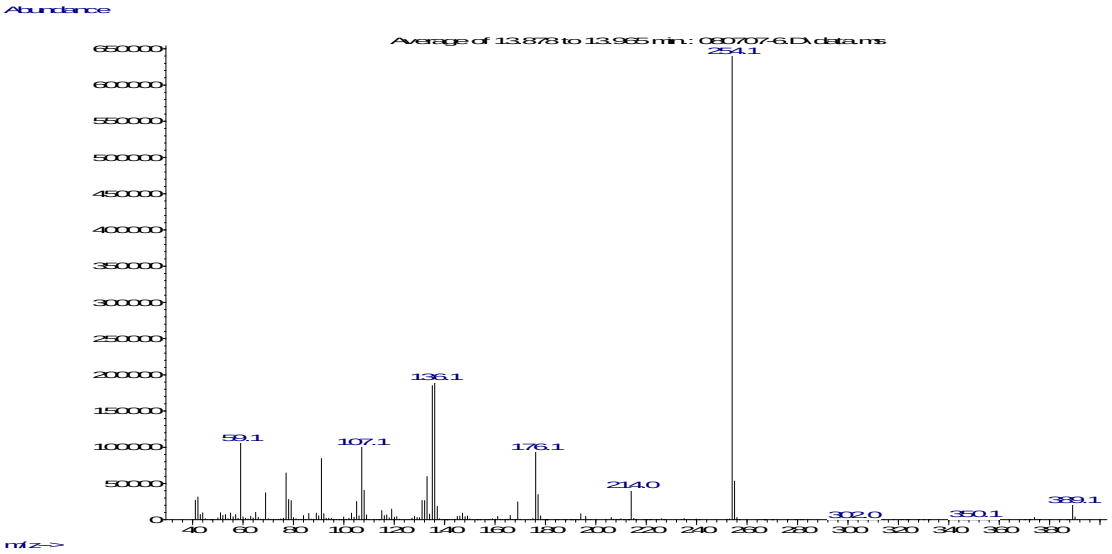

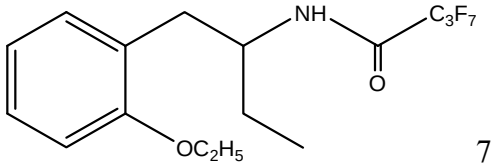

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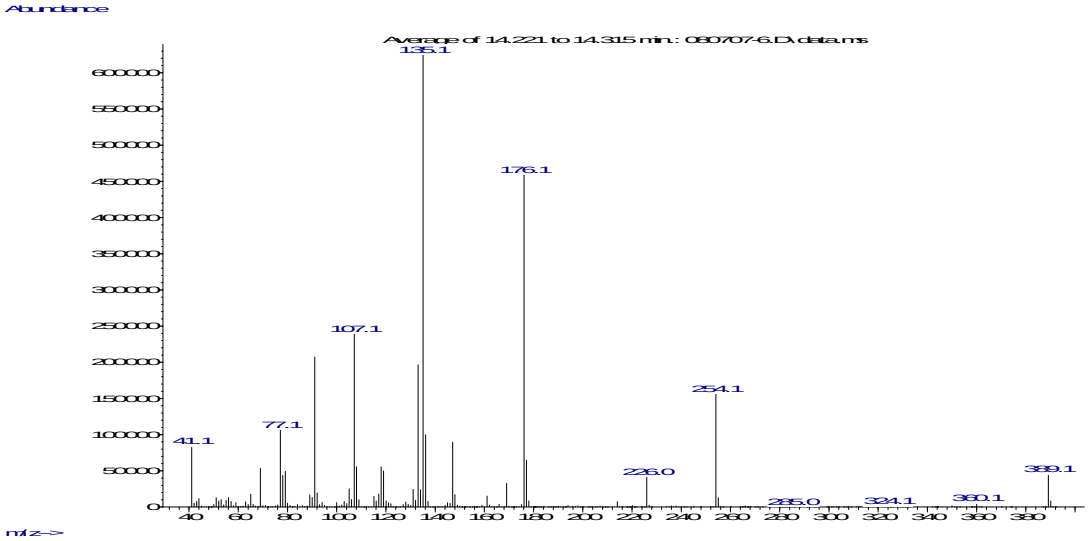

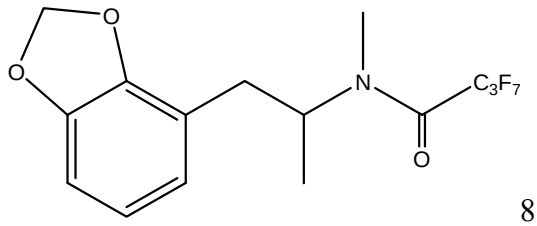

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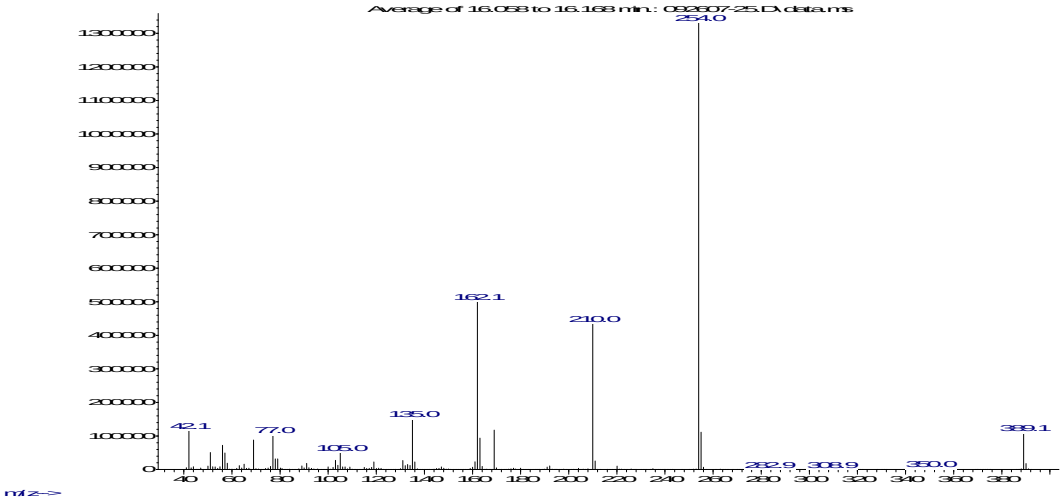

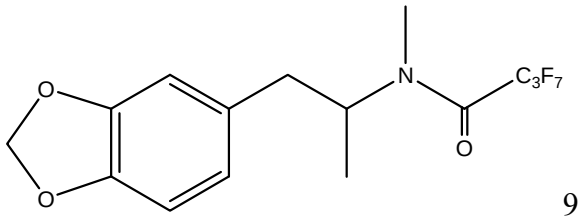

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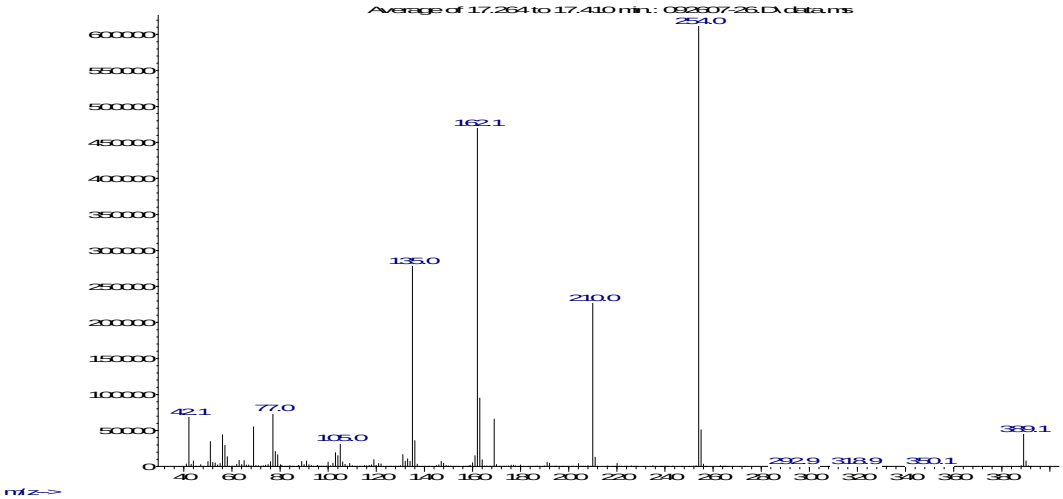
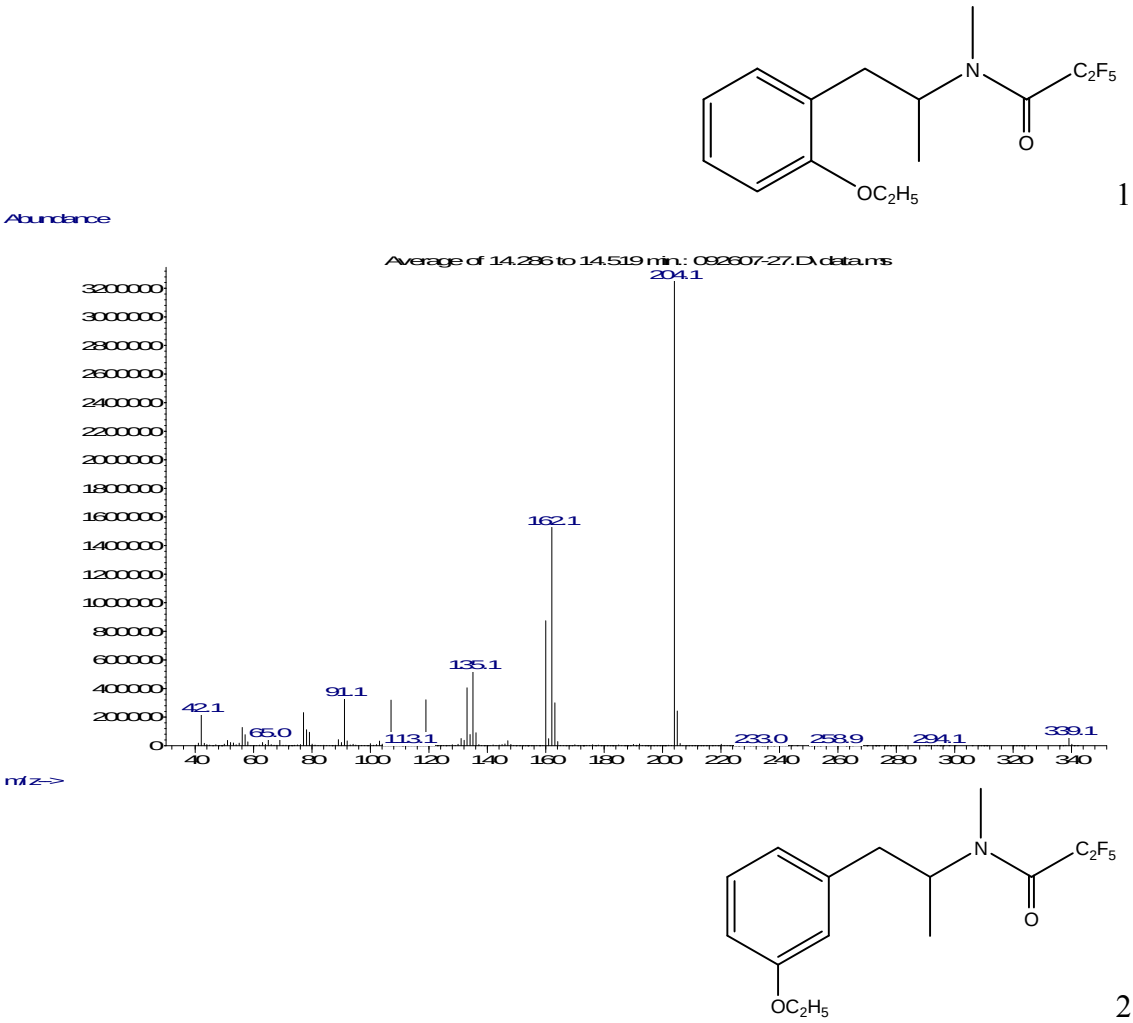
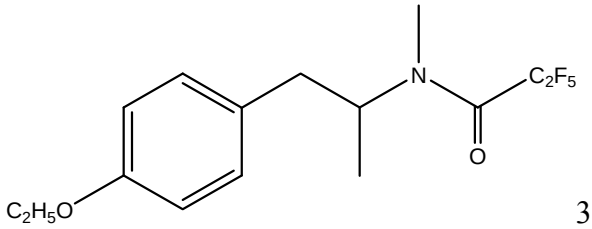


Figure 7.4: Mass Spectra of the PFPA derivatives of the ring substituted ethoxy Phenethylamines, 2,3- and 3,4-MDMA.





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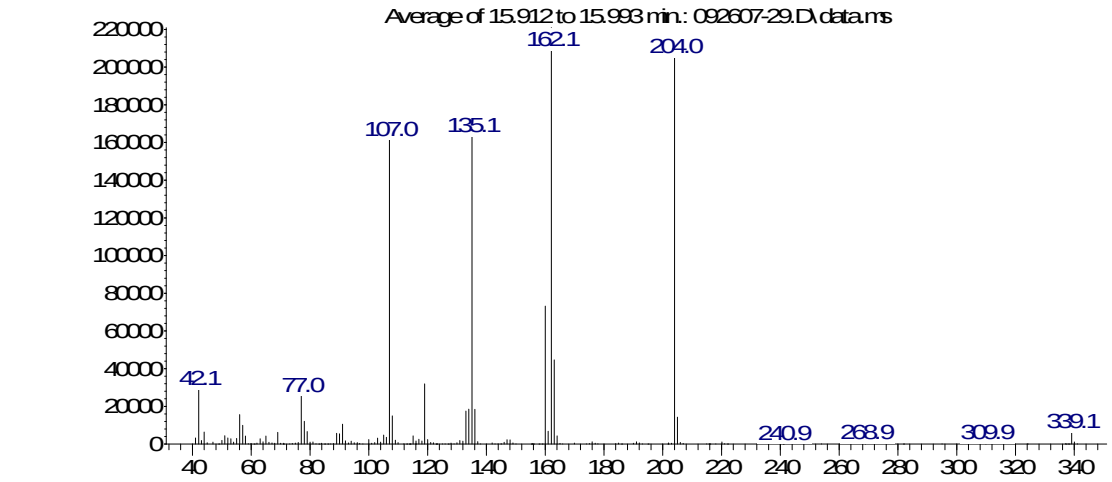

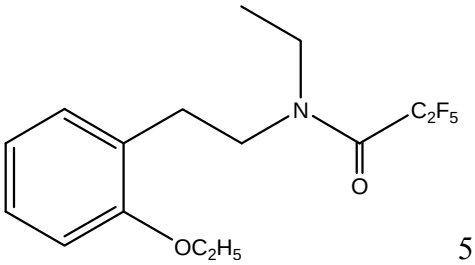

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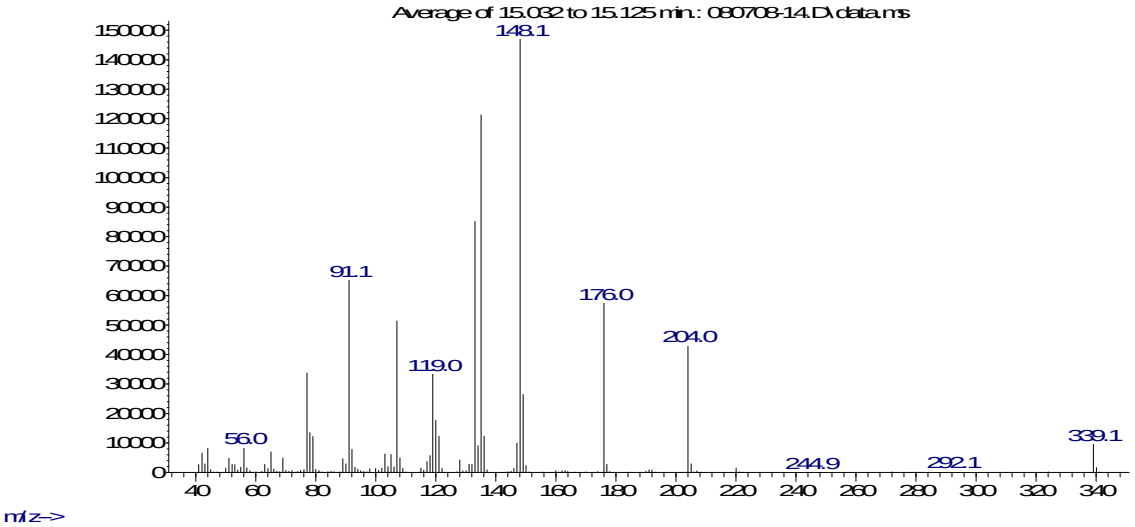

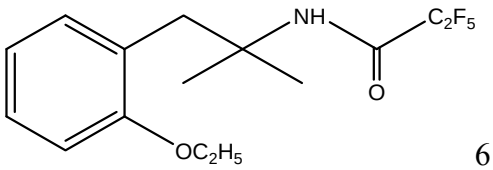

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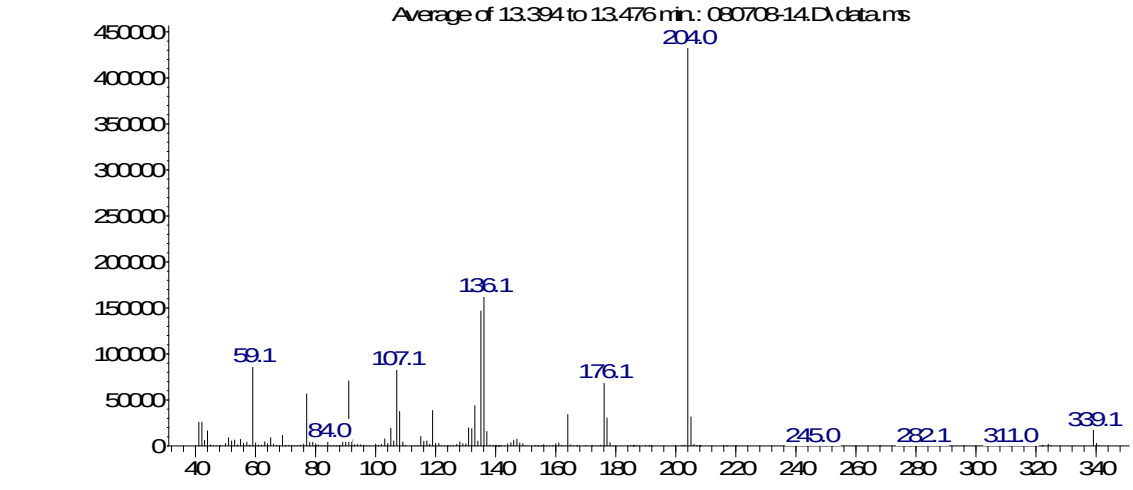

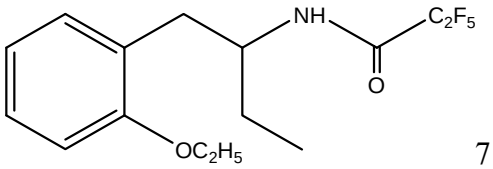

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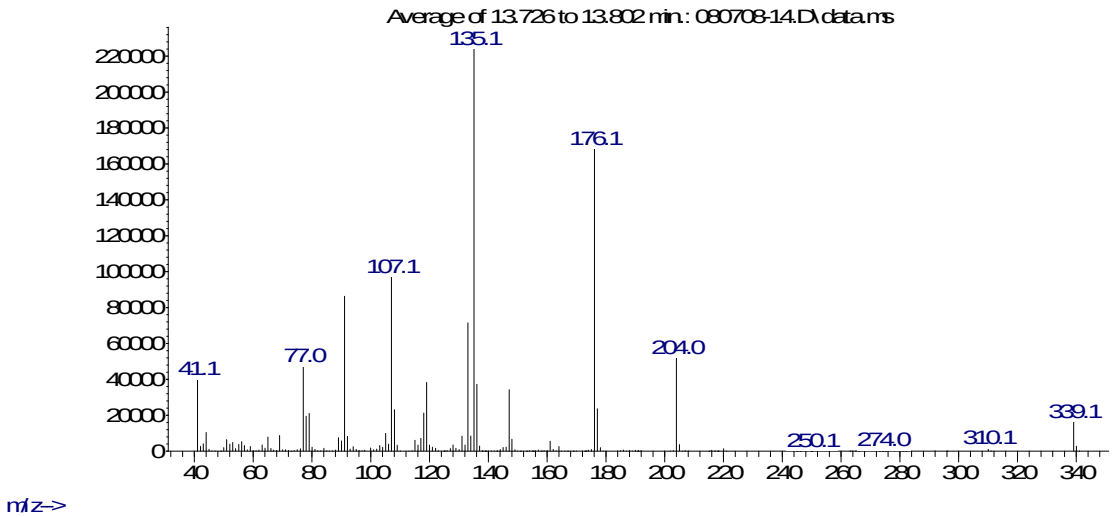

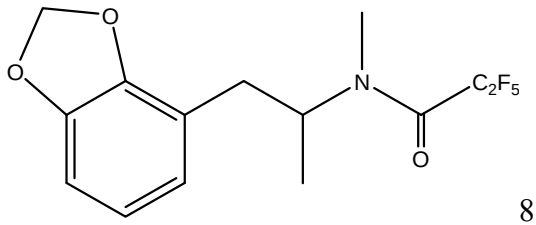

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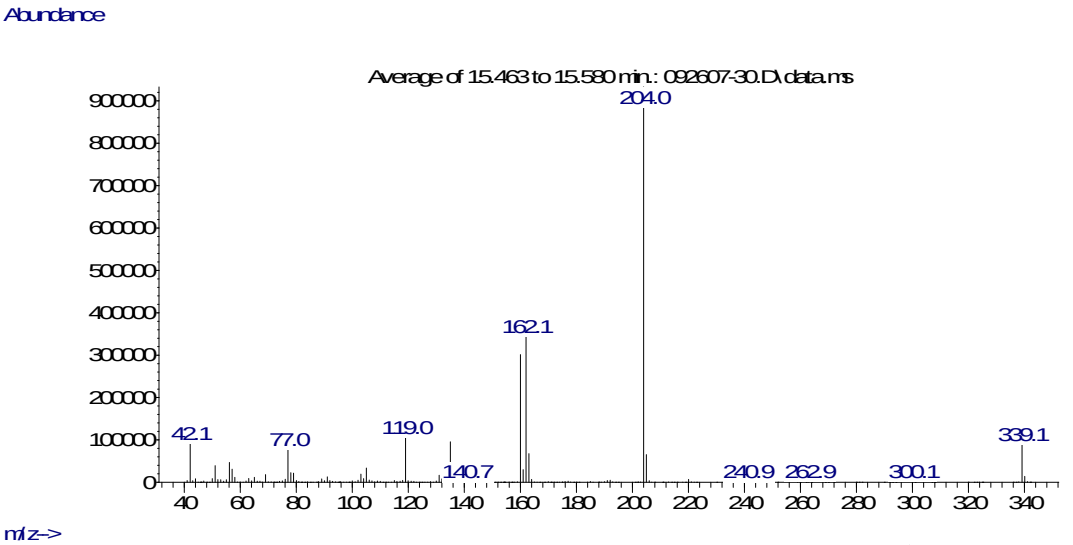

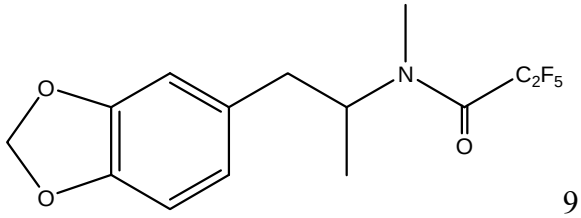

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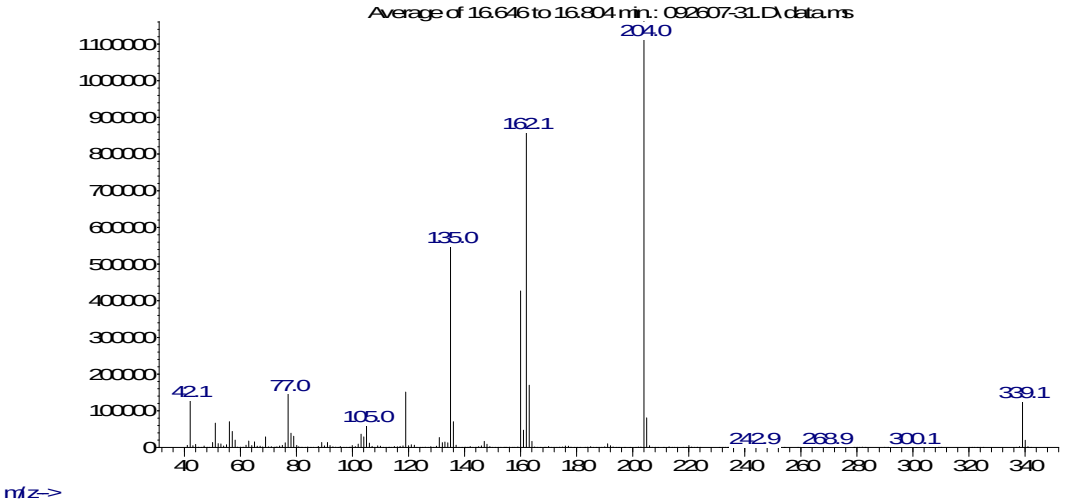
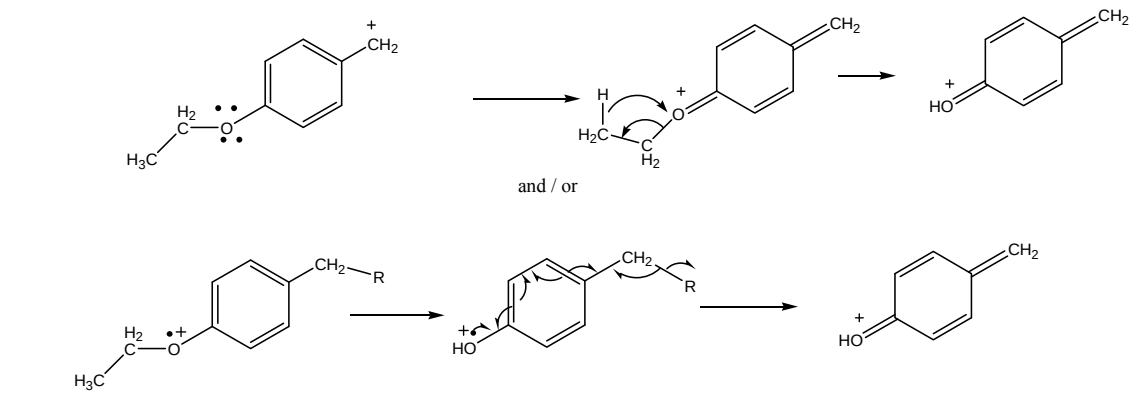


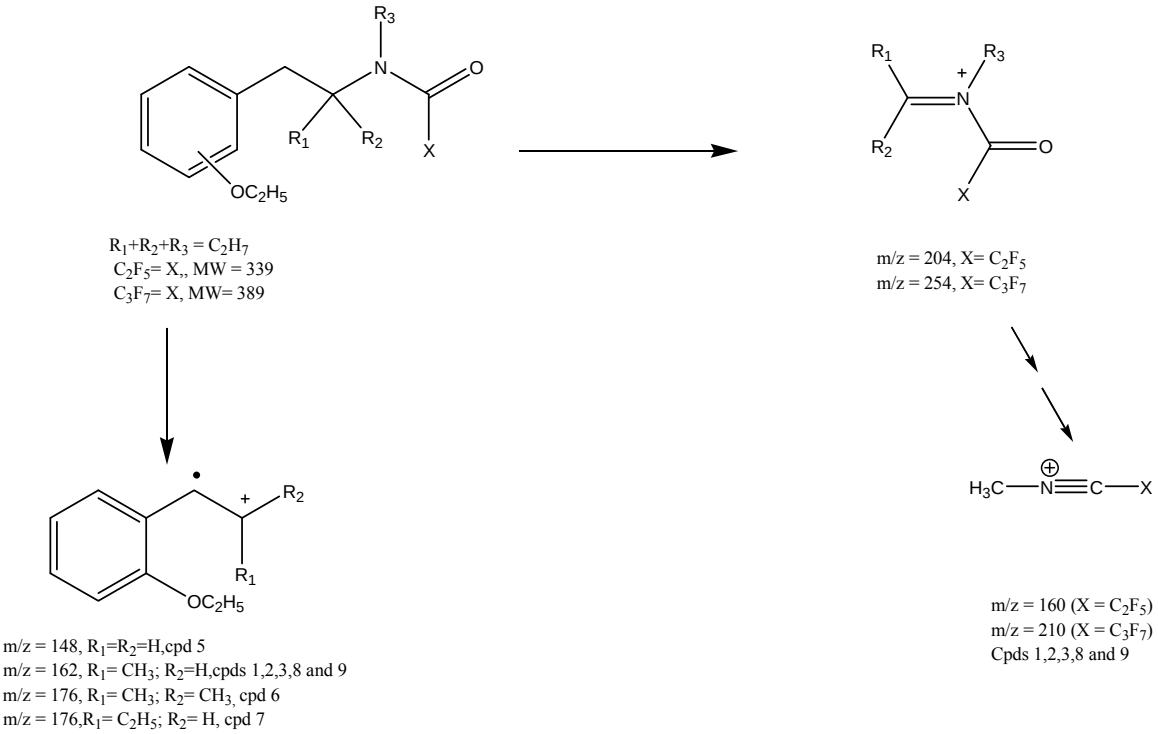


Scheme 7.2: mass spectral fragmentation of ethoxyphenethylamines yielding cation at m/z 107

These spectra in Figures 7.3 and 7.4 show a common peak at ***m/z*** 204 and 254, which corresponds to the loss of 135 mass units from the molecular ions at 339 and 389 for the PFPA and HFBA amides. This ion at ***m/z*** 204 and 254 is the PFPA and HFBA imine species formed from the alpha cleavage of the amide nitrogen to eliminate the ethoxy benzyl radical or themethylenedioxybenzyl radical. Thus the ***m/z*** 204 and 254 ions in the PFPA and HFBA amides are analogous to ***m/z*** 58 in the underivatized species because all these ions represent the (M-135)+ species. The general fragmentation pattern and structures for the ***m/z*** 204 and 254 ions are shown in Scheme7.3. The ethoxy benzyl cation (***m/z*** 135) and the methylenedioxybenzyl cation (***m/z*** 135) are fragments common to all spectra in Figures 7.3 and 7.4. However, the ethoxy benzyl cation in the perfluoroacyl derivatives shows a very high relative abundance in some of these isobaric compounds.

Indeed the***m/z*** 135 ion is the base peak in the PFPA andHFBA derivatives of Compound 7. This would suggest that the perfluoroacyl derivatives offer some level of

discrimination between the methylenedioxy and ethoxy ring substitution patterns based on the difference in relative abundances of the substituted benzyl cation at ***m/z*** 135.



Scheme7.3: Mass spectral fragmentation products for the acylated ethoxy phenethylamines.

The decreased role for the alpha cleavage reaction in the fragmentation of these amides allows the formation of ions more diagnostic of individual side chain isomers. Acylation weakens the bond between nitrogen and the alkyl carbon of the phenethyl side chain, allowing the formation of charged hydrocarbon species of increased relative abundance. These hydrocarbons of varying mass significantly individualize the mass spectra and provide specific structural information. The mass spectra in Figures 7.3 and 7.4 illustrate the role of hydrocarbon fragments at ***m/z*** 148, 162, and 176 in the electron impact mass spectral discrimination among the side chain regioisomers. The spectra for

the ***N***-ethyl isomer (compound 5) in Figures 7.3-5 and 7.4-5 show a base peak at ***m/z*** 148 corresponding to the alkene radical cation which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (see Scheme 7.3). This ion at ***m/z*** 148 would only occur for the ***N***-ethyl regioisomer. The spectra in Figures 7.3-1, 7.3-2, 7.3-3, 7.3-4,7.3-9, 7.4-1, 7.4-2, 7.4-3, 7.4-4 and 7.4-9 show the substituted phenylpropane hydrocarbon ion at ***m/z*** 162, identifying these molecules as the

PFPA and HFBA derivatives of 2-, 3-, 4-ethoxymethamphetamines and the 2,3-, 3,4-methylenedioxymethamphetamines, respectively. However, the base peak of ***m/z*** 162 and relatively high abundance ***m/z*** 107 in the spectra of the perfluroacyl derivatives of compound 3 offer a significant discrimination of 4-ethoxymethamphetamines over the 2-, 3-ethoxymethamphetamines and the two isobaric methylenedioxymethamphetamines isomers (Compounds 1, 2, 8, and 9 in Figures 7.3 and 7.4). The spectra for the PFPA and HFBA derivatives of the primary amines (compounds 6 and 7 in Figure 7.3 and 7.4) show ions at ***m/z*** 176 from the corresponding substituted phenylbutene radical cation. The lower abundance of ***m/z*** 176 for the 2-ethoxy phentermine (Compound 6) may be attributed to steric inhibition of hydrogen transfer in the alpha, alpha-dimethyl substitution pattern.

While the alkene ions at 148, 162, and 176 help to identify the side chain regioisomers, one complicating factor in the PFPA derivatives for the ***N***-ethylphenethylamines [Figure 7.4-5] is the appearance of an ion at ***m/z*** 176 in addition to the base peak at ***m/z*** 148. The 176 ion might suggest a four carbon chain directly attached to the aromatic ring as occurs for the alpha-ethyl- (Compound 7) and alpha, alpha-

dimethyl- (Compound 6) phenethylamines [Figures 7.3-6, 7.3-7, and 7.4-6, 7.4-7]. The ***m/z*** 176 ion in the spectra for the PFPA derivatives of the ***N***-ethyl regioisomers [Figure 7.4-5] is a rearrangement of the ***m/z*** 204 ion resulting in the loss of mass 28 (the ***N***-ethyl group) via hydrogen transfer. This coincidental common mass from two different fragmentation pathways is confirmed by examining the mass spectra for the HFBA derivatives of the ***N***-ethyl-phenethylamines shown in Figure 7.3-5. The loss of 28 mass units from the acylimine fragment at ***m/z*** 254 yields the equivalent fragment ion at***m/z*** 226. Thus, the HFBA derivatives may offer more characteristic ions for individualization of these regioisomeric substances.

A comparison of the mass spectra for the PFPA and HFBA derivatives of all ring substituted methamphetamines (Compounds 1, 2, 3, 8, and 9) indicates unique ions at ***m/z*** 160 and ***m/z*** 210 [see Figures 7.3-1, 7.3-2, 7.3-3, 7.3-8, 7.3-9, 7.4-1, 7.4-2, 7.4-3, 7.4-8 and 7.4-9]. This mass difference of 50 (CF_2) suggests these ions contain the perfluoroalkyl group for each derivative, C_2F_5 and C_3F_7, respectively. An evaluation of the masses of the components which make up the fragment at ***m/z*** 160 for example include C_2F_5 (119 mass units) and CH_3 (15 mass units).

Previous deuterium labeling studies have confirmed that methyl group on nitrogen is a part of this resulting fragment (Chapter 1). The remaining mass 26 would correspond to CN and the proposed structures of ***m/z*** 160 and 210 are shown in Scheme 7.3. An equivalent fragmentation pathway has been reported for methamphetamine and further supported by the analysis of the mass spectra of the PFPA and HFBA derivatives of d_3- and d_5-MDMA in a previous study (Chapter1).

Gas Chromatography of Ring substituted Ethoxy Phenethylamines, 2,3- and 3,4-MDMA.

The PFPA and HFBA derivatives of the ring substituted ethoxy methamphetamines and the primary and secondary side chain regioisomers of the 2-ethoxyphenethylamine were compared on the relatively non polar 100% dimethyl polysiloxane (Rtx-1).

Four physical mixtures were prepared, two containing the HFBA and the PFPA derivatives of 2-, 3-, and 4-ethoxymethamphetamine along with 2,3- and 3,4 MDMA, while the other two contained the side chain regioisomers of 2-ethoxyphenethylamine (Compounds 1, 5, 6 and 7) with the isobaric 2,3- and 3,4-MDMAs. Several temperature programs were evaluated and one program showing the best compromise between resolution and analysis time was used to generate the retention data in Table I and the chromatograms in Figures 7.5 and 7.6. Chromatograms shows that 3,4- MDMA has the highest affinity for the stationary phase among this set of compounds both in the free amine form and the two amide forms investigated in this study.

The chromatograms in Figure 7.5 show the separation of the PFPA (Figure 7.5A) and HFBA (Figure 7.5B) derivatives of the five compounds having the methamphetamine side chain. This common side chain allows for a comparison of relative stationary phase affinity as a function of ring substituents in this set of compounds. The 2-ethoxy substitution pattern shows the least retention and compound 1 has the lowest retention time. The 3-ethoxymethamphetamine (Compound 2) elutes second followed by 2,3-MDMA (Compound 8) then the 4-ethoxy substituent compound 3. The compound showing the highest retention time and eluting last is 3,4-MDMA (Compound 9). Among

the ethoxy substituents the 2-isomer shows the least retention followed by the 3-isomer, and the 4-ethoxy isomer has the greatest retention on the Rtx-1 phase. The elution order is the same for both the PFPA and HFBA derivatives with the HFBA derivatives having slightly greater retention under identical chromatographic conditions.

The chromatograms in Figure 7.6 show the separation of the side chain regioisomers as the ring substitution pattern is held constant (2-ethoxy). The side chain elution order is secondary amides (Compounds 6 and 7) before the tertiary amides (Compounds 1 and 5) and in this limited set of examples branched isomers elute before the more linear ones. Therefore, the amides of compound 6 elute first followed by the amide of compound 7 (both secondary amides), then the amides of compound 1 (the methamphetamine side chain) and finally the amides of compound 5 (the more linear of the tertiary amides).

The methylenedioxy isomers (Compounds 8 and 9) elute later than any of the side chain isomers of the 2-ethoxy ring substitution pattern. Thus, these chromatographic results coupled with the mass spectral data allow for the individualization of each member of this series. The derivatized side chain isomers separated in Figure 7.6 can be differentiated by unique fragment ions in their mass spectra in addition to the well resolved chromatographic results. The compounds having a common side chain are well resolved in Figure 7.5 and the ***m/z*** 107 fragment ion allows for the identification of those compounds having the ethoxy ring substituent.

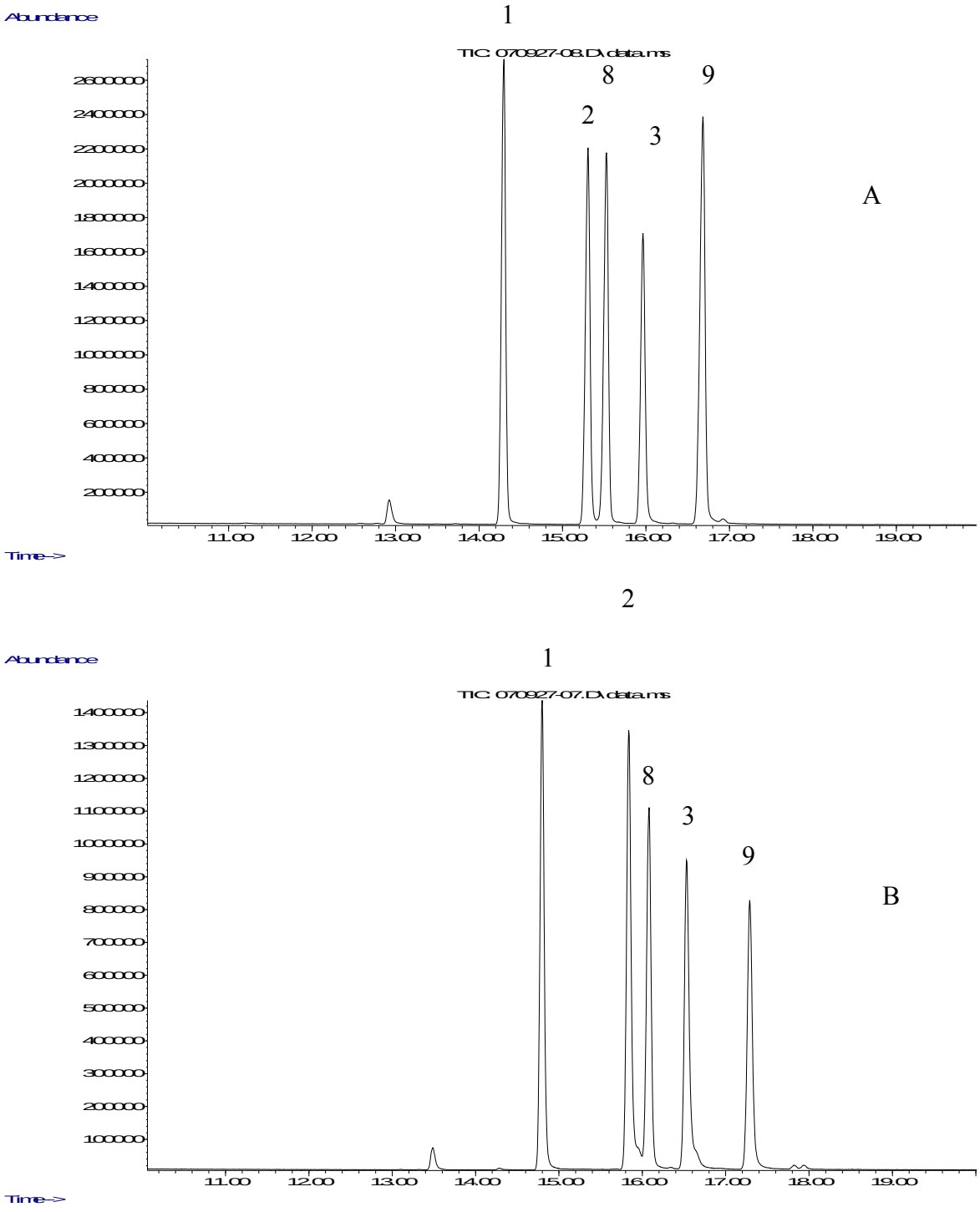


Figure 7.5: GC Separation of the PFPA (A) and HFBA (B) derivatives of compounds 1-3,8 and 9; Rtx-1 column

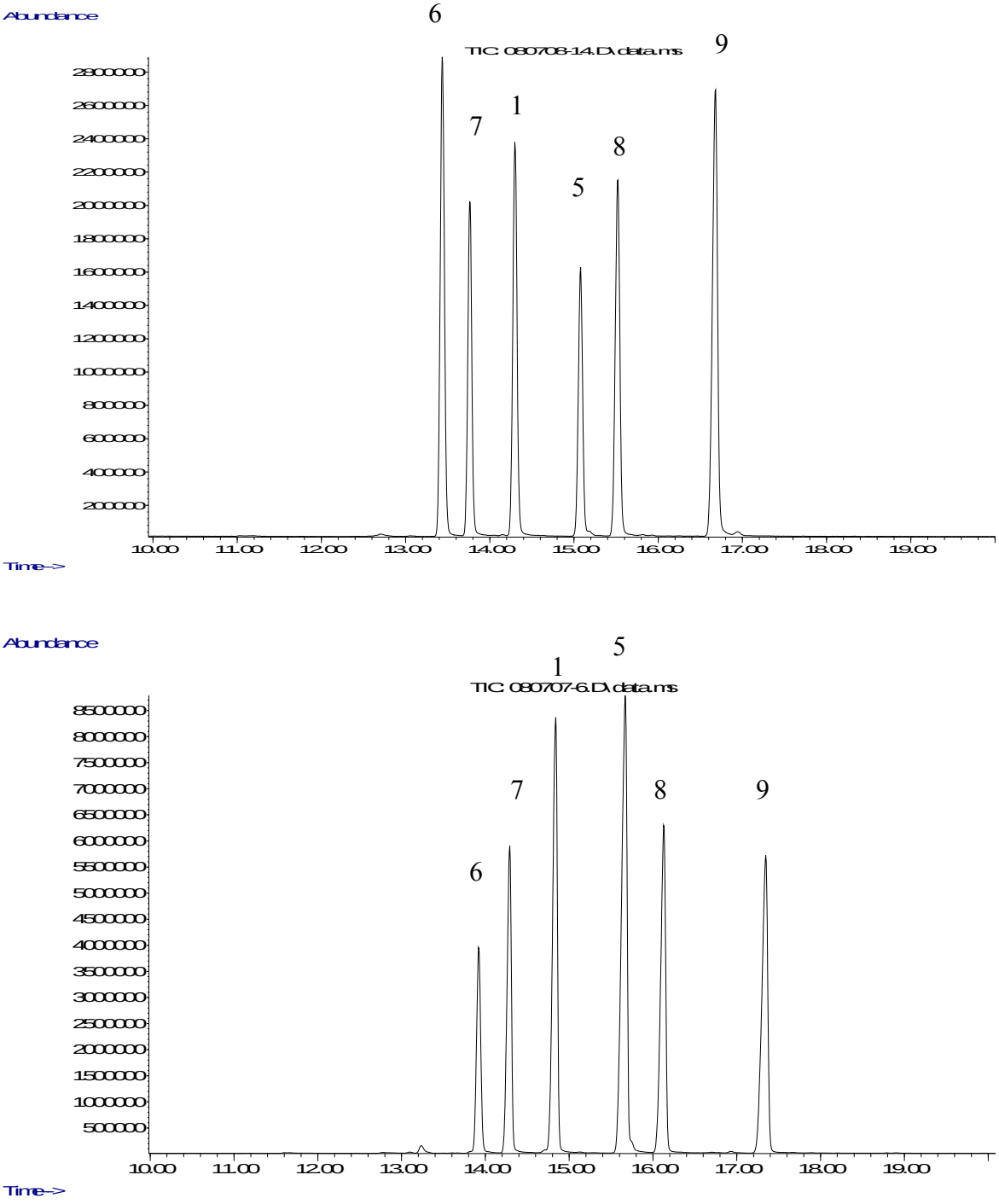


Figure 7.6: GC Separation of the PFPA (A) and HFBA (B) derivatives of compounds 1, 5,6,7,8 and 9; Rtx-1 column

Conclusions of Chapter 7

3,4-MDMA, 2,3-MDMA, and the three ring substituted ethoxy methamphetamines are a unique subset of regioisomeric and isobaric molecules; each compound has a molecular weight of 193 and yields a base peak at ***m/z*** 58 in the mass spectrum from the loss of the corresponding methylenedioxybenzyl and ring substituted ethoxybenzyl groups, respectively. The traditional electron impact mass spectrum provides little structural information for differentiating among these seven compounds. Derivatization with acylating agents yields amides with improved resolution compared to the underivatized amines by capillary gas chromatography on the Rtx-1 stationary phase.

Additionally, the perfluoroacyl derivatives significantly individualize the mass spectra for these amides and allow for specific identification. The ring substituted ethoxy methamphetamines are characterized from 2,3- and 3,4-MDMA by the presence of ***m/z*** 107. Side chain regioisomers yield unique hydrocarbon fragment ions at ***m/z*** 148, 162 and 176.

Chapter 8

GC-MS Studies on Direct Side Chain Regioisomers Related to Substituted Methylenedioxyphenethylamines; MDEA, MDMMA and MBDB

There are an additional seven direct side chain regioisomeric phenethylamines related to the controlled substances MDEA, MDMMA, and MBDB. The structures of this set of compounds are shown in Figure 8.1.

A previous report from our research group included the synthesis and mass spectral evaluation of these ten regioisomeric compounds. The mass spectra of the underivatized compounds provided very little structural information for the specific differentiation among these regioisomers even though these regioisomers were separated by capillary gas chromatography.

In this chapter, derivatization of the primary and seconday amines was carried out in an effort to obtain more specific ions that would help discriminating among the members of this set of compounds.

Mass Spectral Studies

Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 8.2 shows representative electron impact mass spectra of the three drugs of abuse; MDEA, MBDB and MDMMA, involved in the study (Compounds 6, 7, 9). The MS spectra of all ten underivatized regioisomers were previously reported. The mass spectra of the ten regioisomeric compounds (MW=207) are characterized by a

base peak at *m/z* 72 formed by α-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and amine nitrogen.

Figure 8.1: Structures of the side chain regioisomeric phenethylamines related to MDEA, MDMMA, and MBDB.

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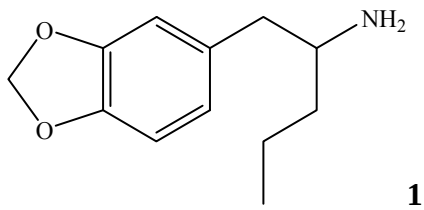
<td> 
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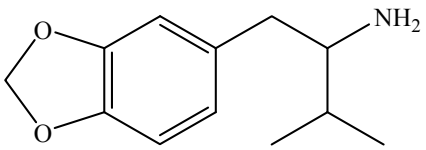
<td> 
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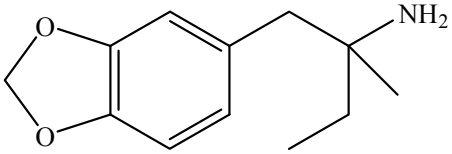
<td> 
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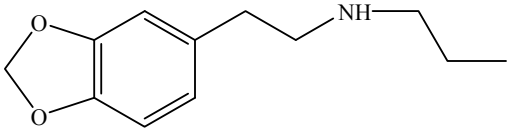
<td> 
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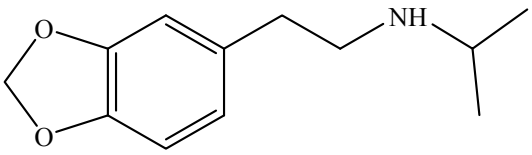
<td> 
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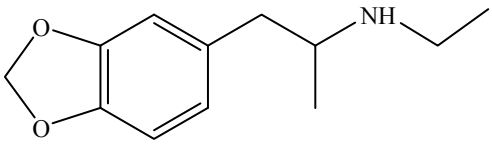
<td> 
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MDEA
3,4-Methylenedioxy-N-ethylamphetamine</td>
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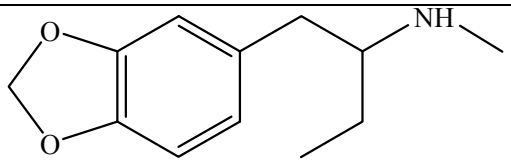
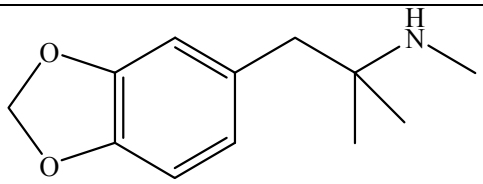


7

MBDB
N-methyl-1-(3,4-
Methylenedioxyphenyl)-2-butanamine



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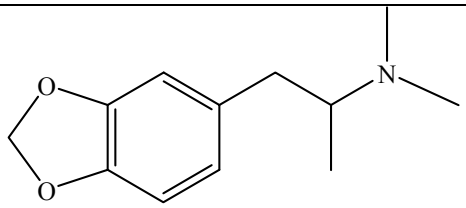
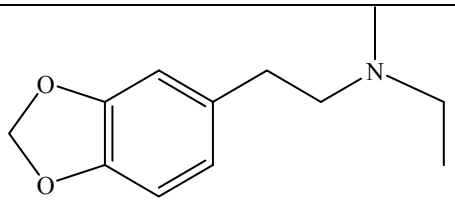
N-methyl-3,4-
methylenedioxyphentermine



9
MDMMA
3,4-Methylenedioxy-N,N-
dimethylamphetamine



10

N-ethyl-N-methyl-3,4-

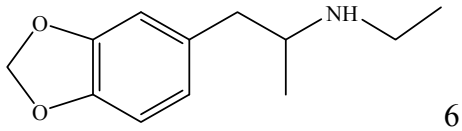
methylenedioxyphenyl)-2-ethanamine

Other less abundant peaks were observed at *m/z* 135/136 from the 3,4-methylenedioxybenzyl cation and radical cation fragments, respectively as well as other ions of low relative abundance. The EI mass spectra of these regioisomers show some variation in the relative intensity of the major ions with only one or two minor ions that might be considered side-chain specific fragments. Thus, the ultimate identification of any one of these amines with the elimination of the other nine regioisomeric substances can not depend on their mass spectra alone.

This lack of mass spectral specificity in addition to the possibility of chromatographic co-elution with any of the drugs of abuse; MDEA, MBDB and MDMMA could result in misidentification of the target drug. Furthermore, the lack of available reference samples for the seven regioisomeric 3,4-methylenedioxy-

phenethylamines compounds complicates the individual identification of any one of these substances. This constitutes a significant analytical challenge, where the specific identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate the regioisomeric substance from the actual drug of interest. Additionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

Figure 8.2: EI Mass Spectra of MDEA (compound 6) MBDB (compound 7) and MDMMA (compound 9).



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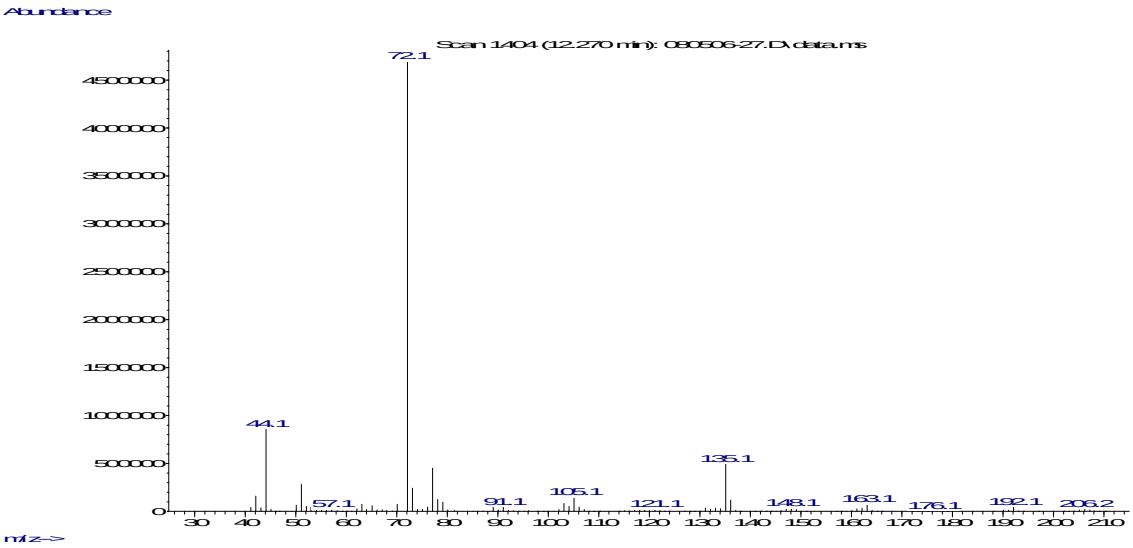

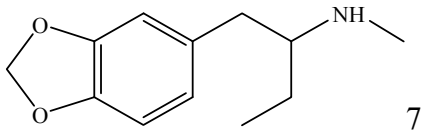

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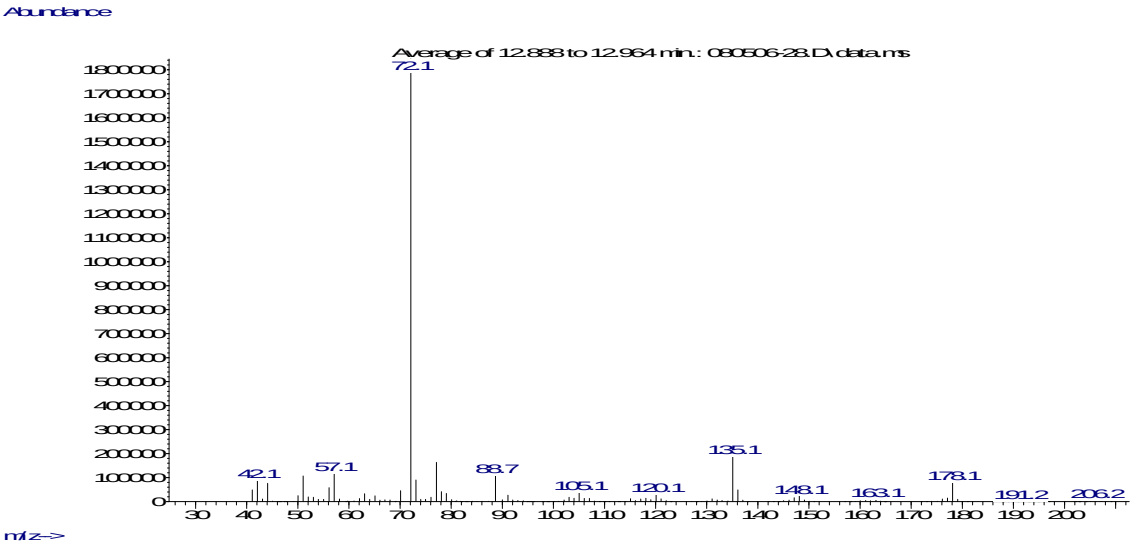

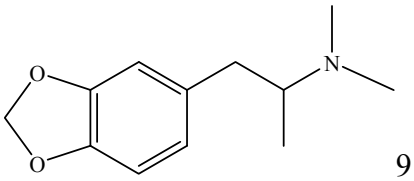

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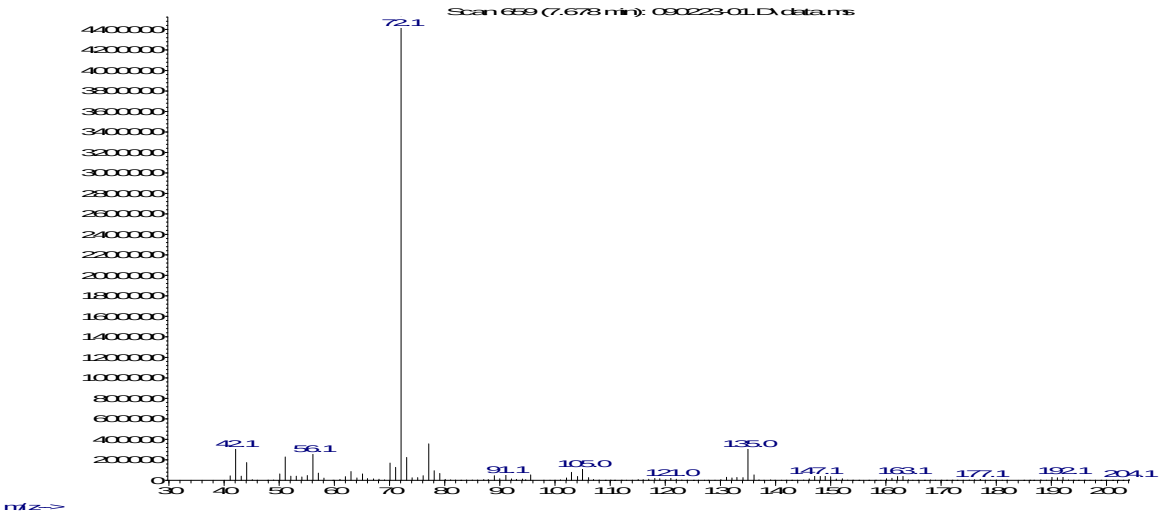


Various perfluoroacyl derivatives of the regioisomeric primary and secondary amines (Compounds 1-8) were prepared and evaluated in an effort to individualize their

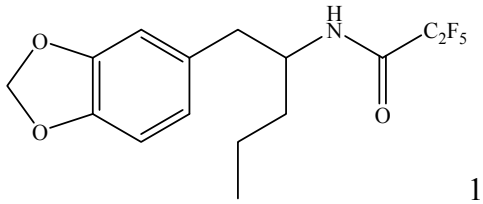
mass spectra and maintain or improve chromatographic resolution. Acylation of the amines significantly lowers the basicity of nitrogen and can allow other fragmentation pathways to play a more prominent role in the mass spectrum. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of compounds 1-8 were evaluated for their ability to individualize the mass spectra through the formation of specific fragments. Compounds 9 and 10 are tertiary amines and do not form a stable amide derivative.

The mass spectra for the eight pentaflouropropionyl (PFPA) amides are shown in Figure 8.3. The spectra for the PFPA derivatives are representative of all the perfluoroacyl amides in this study. From these spectra, a common peak occurs at *m/z* 218 which corresponds to the loss of 135 mass units from the molecular ion at 353. This ion is the PFPA imine species, likely formed from the α-cleavage of the amide nitrogen to eliminate the 3,4-methylenedioxybenzyl fragment at *m/z* 135. Thus this ion is analogous to *m/z* 72 in the underivatized species because it represents the $(M–135)^+$ species.

The decreased role for the α-cleavage fragmentation of these amides allows the formation of more diagnostic ions for each individual isomer. Acylation, and in particular the perflouroacylation, weakens the bond between nitrogen and the α-carbon of the substituted phenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. These alkenes (radical cations) of varying mass are formed due to the transfer of a benzylic hydrogen to the ionized carbonyl oxygen followed by the loss of a neutral amide species. The resulting alkene radical cations (3,4-methylenedioxyphenylalkenes) significantly individualize the mass spectra and provide specific structural information. The mass spectra in Scheme 8.1 illustrate the role of the

alkene fragments at *m/z* 148, 162, 176 and 190 in identification of these regioisomers. These ions identify the number of carbons in the hydrocarbon chain attached directly to the aromatic ring in an uninterrupted manner.

Figure 8.3: Mass spectra of the PFPA derivatives of compounds 1-8.



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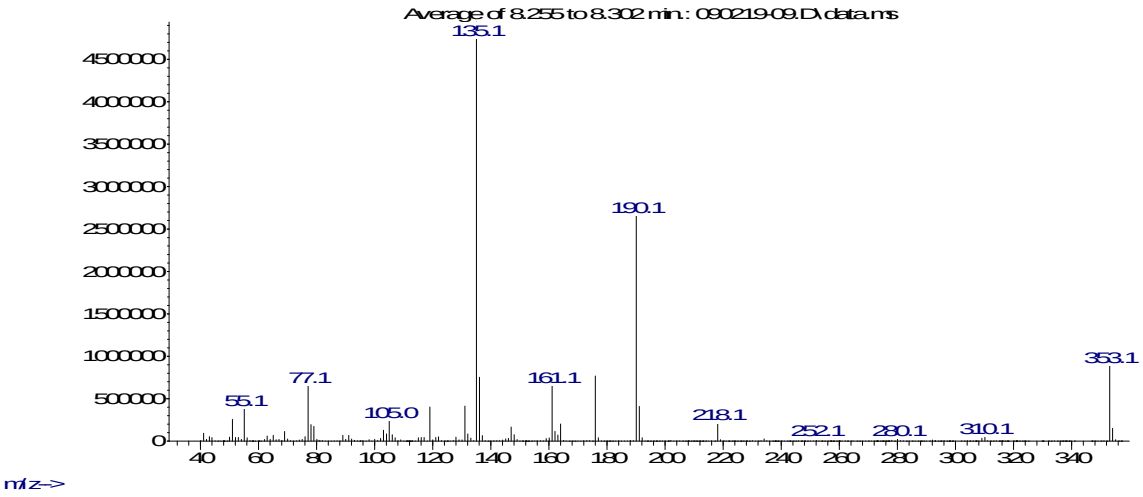

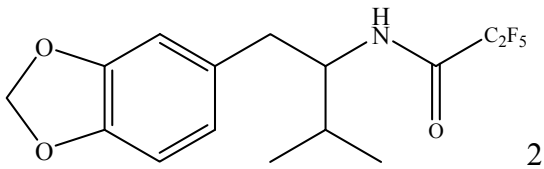

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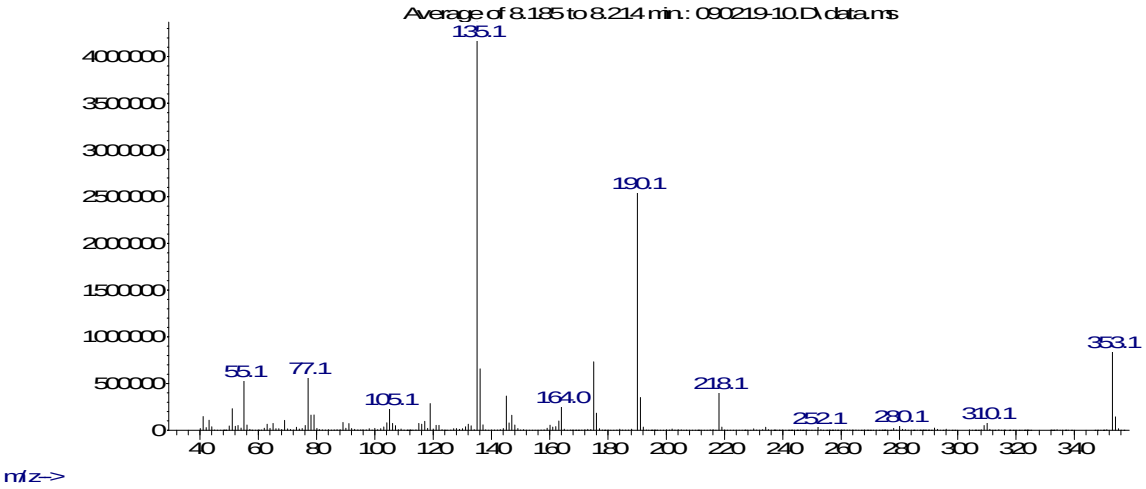

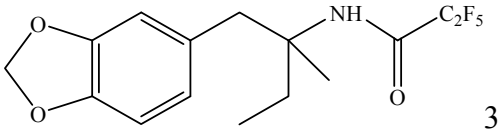

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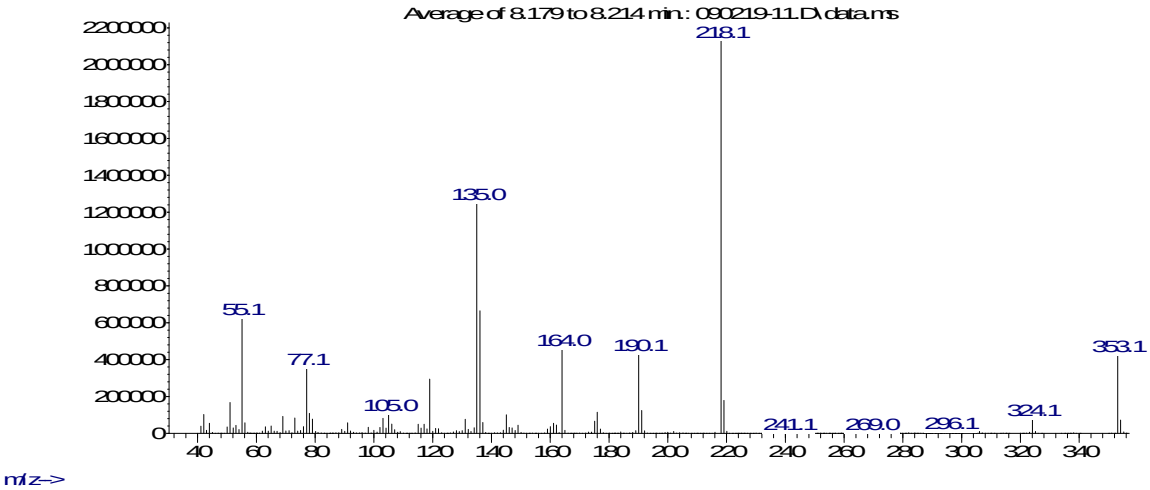

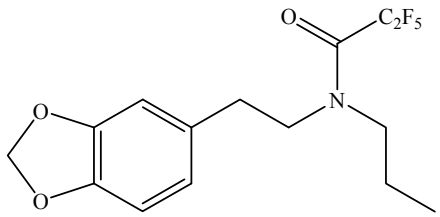

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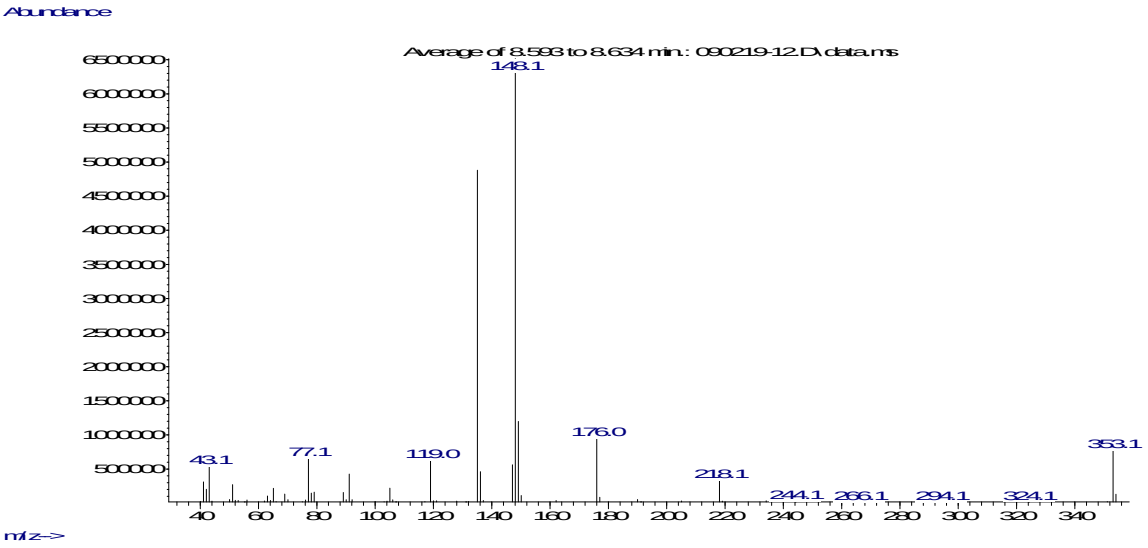

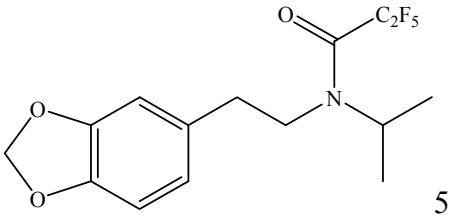

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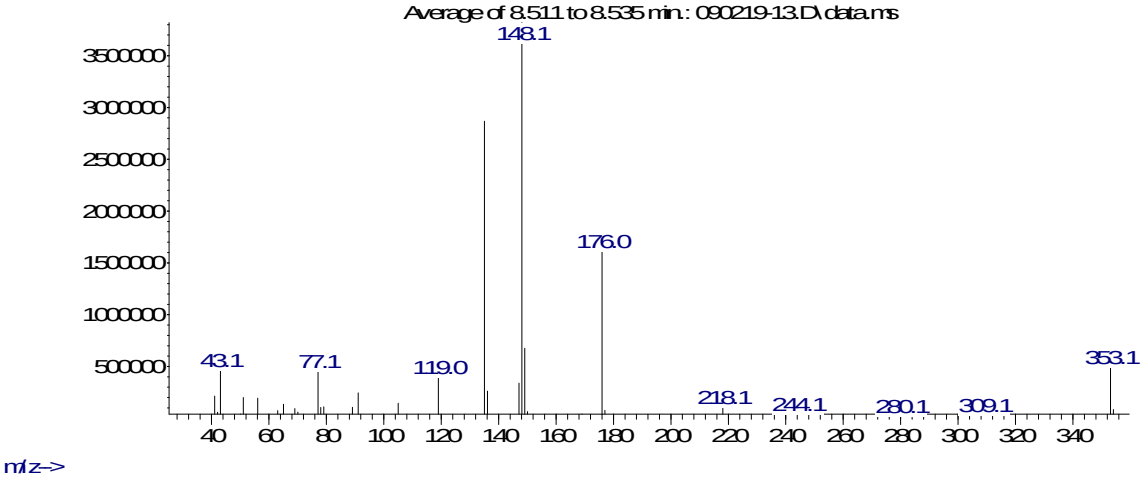

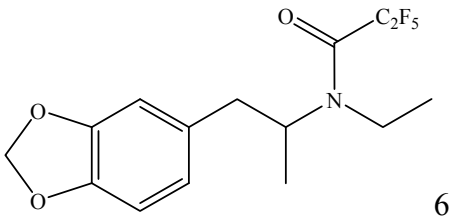

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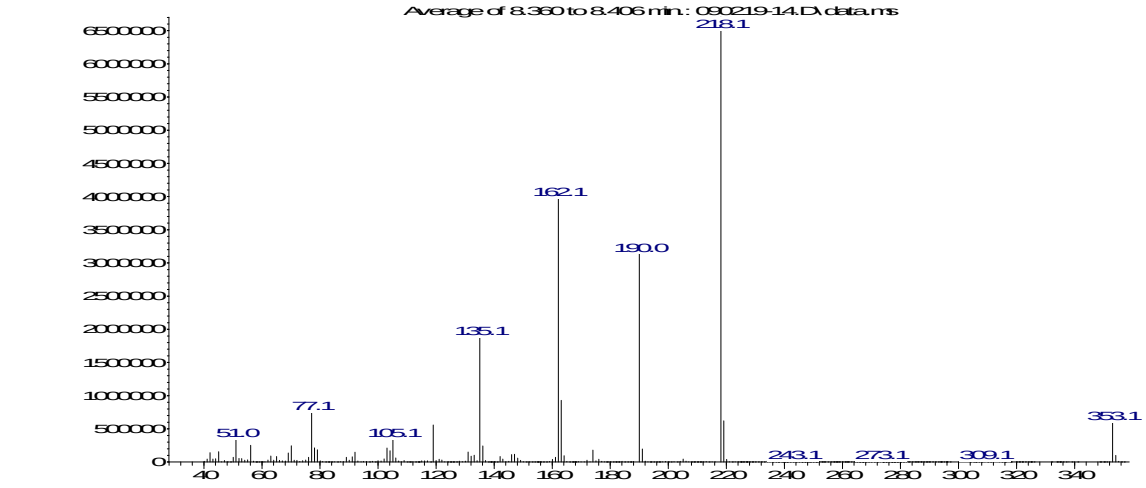

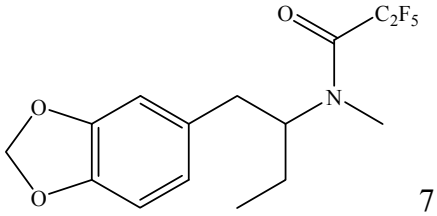

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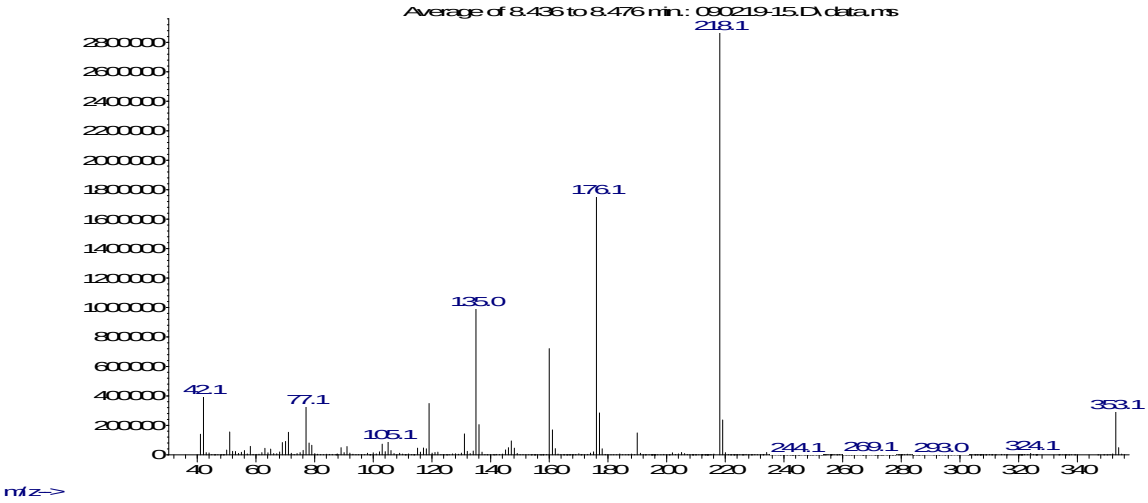

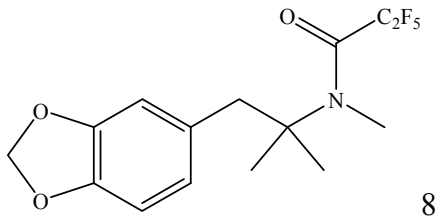

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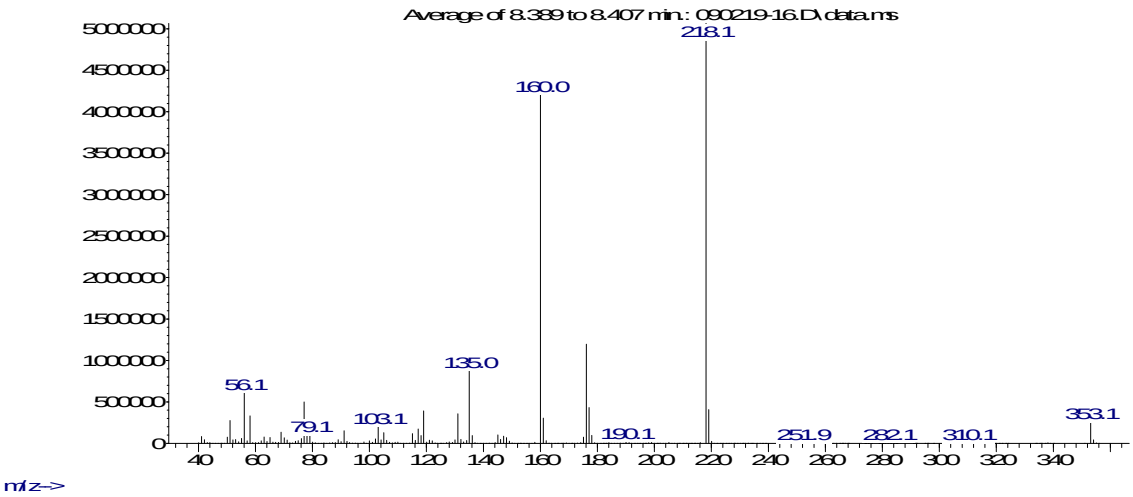
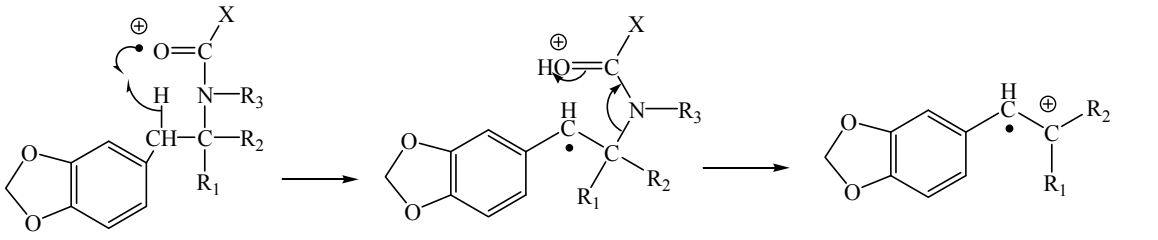


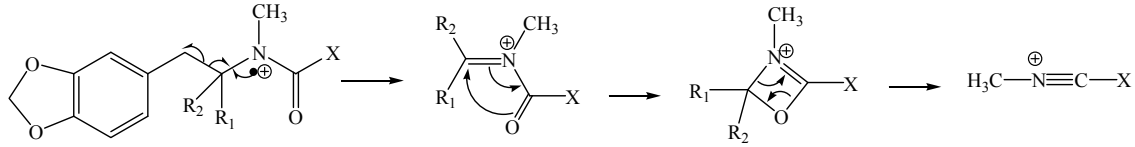
Further examination of the mass spectra of the PFPA derivatives for the eight regioisomers (Figure 8.3) indicates unique ions at *m/z* 160 for both compounds 7 and 8. An analysis of the masses of the components, which make up the fragment at *m/ z* 160 include for example C_2F_5 (119 mass units) and CH_3 (15 mass units), leaving only a mass of 26 available for the total of 160. The mass 26 would correspond to CN and the proposed mechanism for the formation of $(C_2F_5CNCH_3)^+$ is shown in Scheme 8.2. The analogous ion occurs at *m/z* 110 and *m/z* 210 for the TFA and HFBA derivatives, respectively. An equivalent fragmentation pathway has been previously reported and this fragment is characteristic of N-methyl substituted phenethylamine such as compound 7 and 8.



X= CF_3, TFA
C_2F_5, PFPA
C_3F_7, HFBA

R_1=H, R_2= C_3H_7, *m/z*=190, cpd 1
R_1=H, R_2= $CH(CH_3)_2$, *m/z*=190, cpd 2
R_1=CH_3, R_2= C_2H_5, *m/z*= 190, cpd 3
R_1=H, R_2=H, *m/z*=148, cpds 4 and 5
R_1=H, R_2= CH_3, *m/z*=162, cpd 6
R_1=H, R_2= C_2H_5, *m/z*= 176, cpd 7
R_1=CH_3, R_2= CH_3, *m/z*= 176, cpd 8

Scheme 8.1: Mechanism of the formation of the alkene radical cation in the perfluoroacyl derivatives of the primary and secondary regioisomeric amines



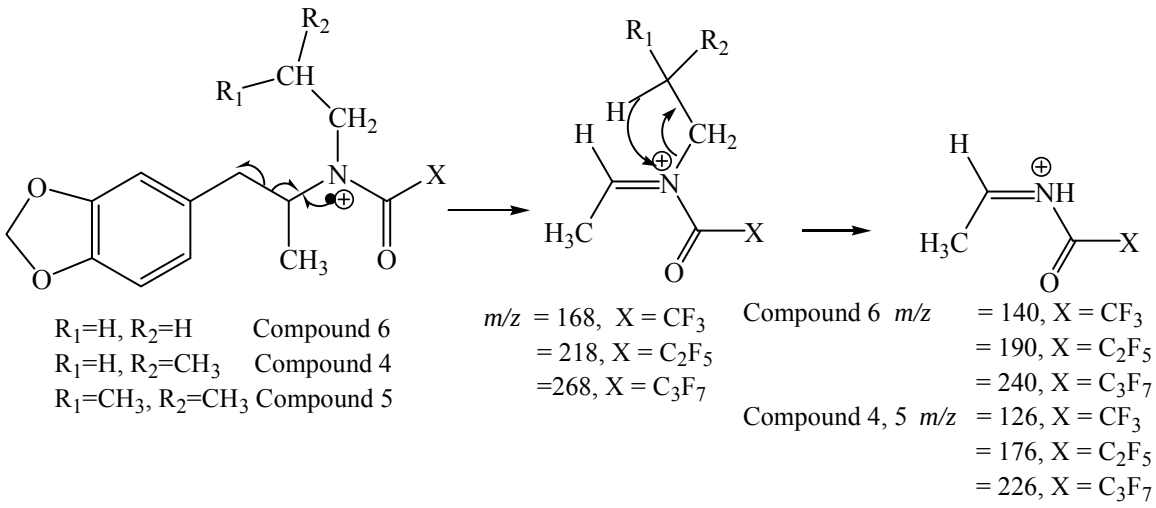
R_1 = H, R_2 = C_2H_5, cpd 7
R_1 = CH_3, R_2 = CH_3, cpd 8
X = CF_3, TFA
C_2F_5, PFPA
C_3F_7, HFBA

m/z = 168, X = CF_3
= 218, X = C_2F_5
=268, X = C_3F_7

m/z = 110, X = CF_3
= 160, X = C_2F_5
= 210, X = C_3F_7

Scheme 8.2: Formation of *m/z* 110, 160, 210 for compounds 7 and 8

An important fragmentation pathway characteristic to the PFPA derivative of compound 6 (the only N-ethyl compound in this limited series) and compounds 4, 5, which are N-propyl and isopropyl amines is illustrated in Scheme 8.3. This results in the formation of fragment ions at *m/z* 190 (Compound 6) or *m/z* 176 (Compounds 4, 5) for their PFPA amides. These ions came from the imine fragment at *m/z* 218 through hydrogen rearrangement and subsequent cleavage of the N-alkyl group on the nitrogen. This occurs only with the N-alkyl group of ethyl or larger.



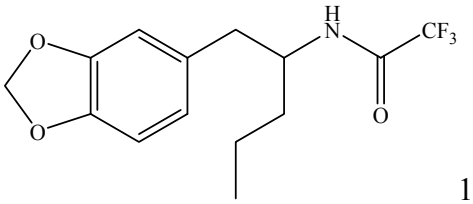
Scheme 8.3 Formation of *m/z* 140, 190 and 240 for compound 6 and *m/z* 126, 176 and 226 for compounds 4 and 5.

Derivatization categorized this limited set of compounds into three subset groups depending on the mass recorded for base peak. The first group includes compounds 1 and 2 having *m/z* 135 as the base peak in their PFPA derivatives. The second group comprises compounds 4 and 5 having a base peak at *m/z* 148. Finally, the third group includes compounds 3, 6, 7 and 8 which are characterized by having the fragment ions at *m/z* 218 as the base peak.

Moreover, derivatization also enables the discrimination among some members within the same group. For the second group, compounds 4 and 5 could be differentiated by the difference in the relative abundance of the fragment ions at *m/z* 176. Among the members of the third group, compounds 7 and 8, which are the only two N-methyl compounds in this series, could be characterized by the fragment ions at *m/z* 160. In addition, they can also be differentiated by inverse intensities of the relative abundances of fragment ions at *m/z* 160 and 176.

The fragment ion *m/z* 190 in the mass spectra of the PFPA derivative of compound 6 (Scheme 8.3) can be confused with the same mass fragment ion found in the mass spectra of the PFPA derivatives of compounds 1-3 formed by another fragmentation pathway (Scheme 8.1). The TFA and HFBA derivatives eliminated such confusion through the observance of analogous ions at *m/z* 140 and 240 for both derivatives respectively formed through the same fragmentation pathway shown in Scheme 11.3. Figure 11.4 shows representative mass spectra of the TFA derivatives of compounds 1-3 and 6.

Figure 8.4: Mass spectra of the TFA derivatives of compounds 1-3 and 6.



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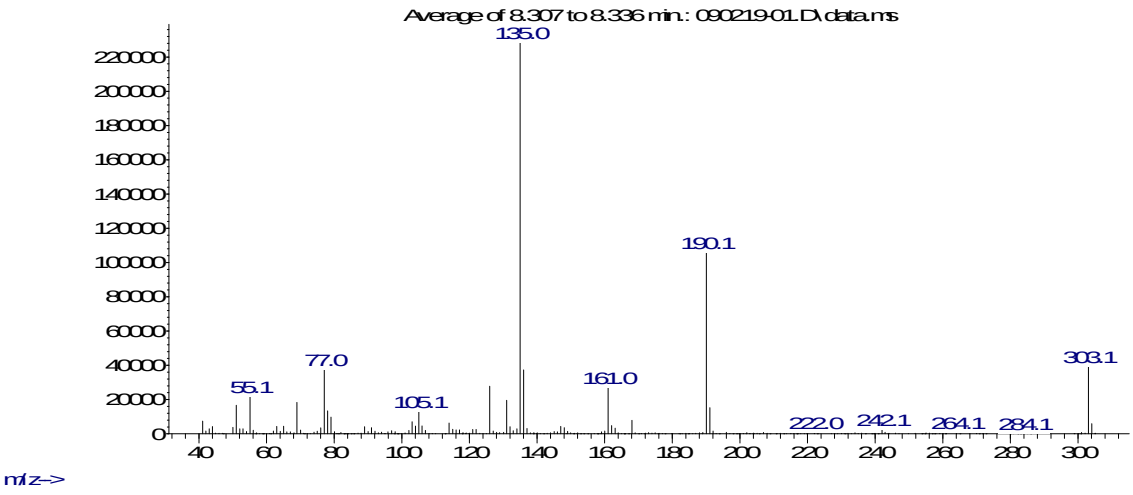

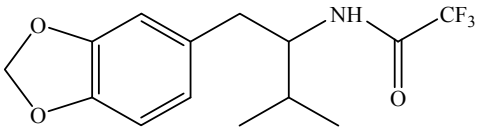

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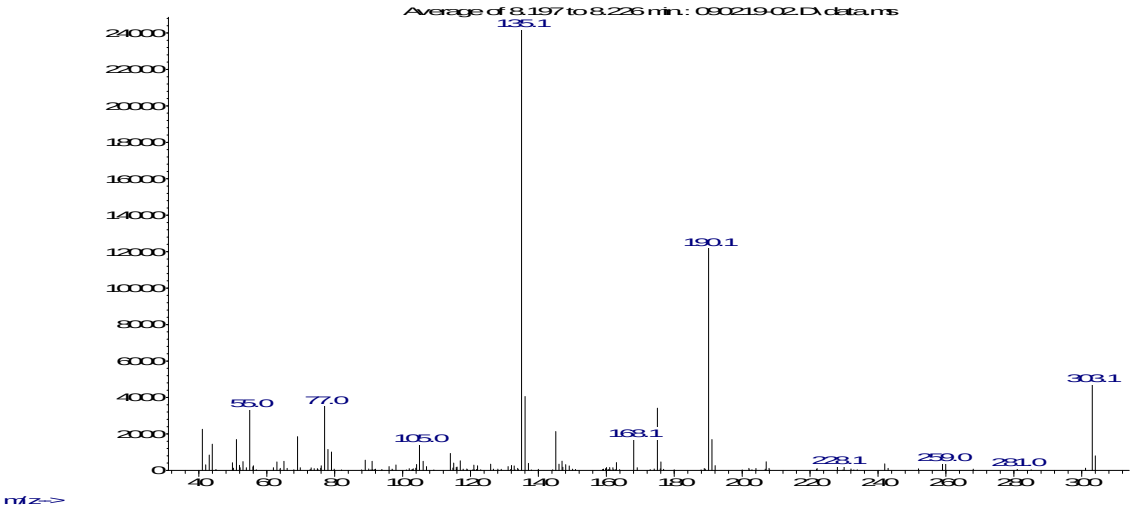

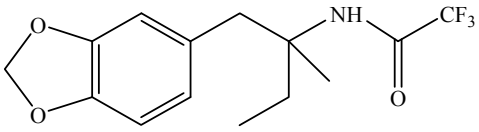

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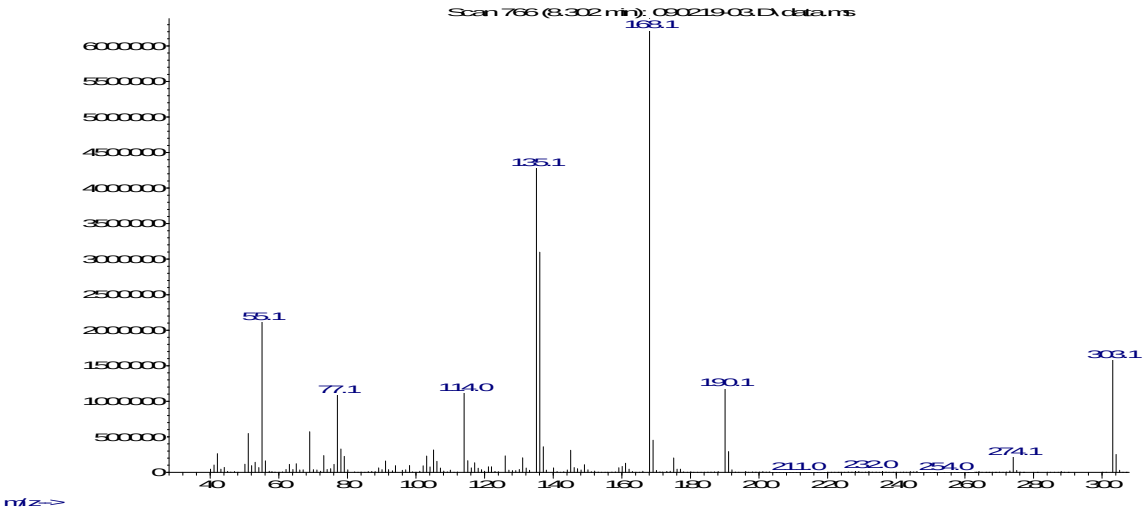

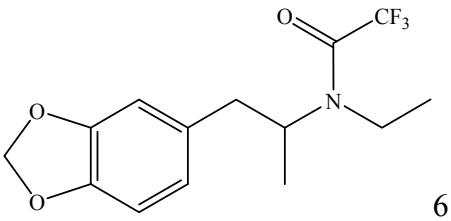

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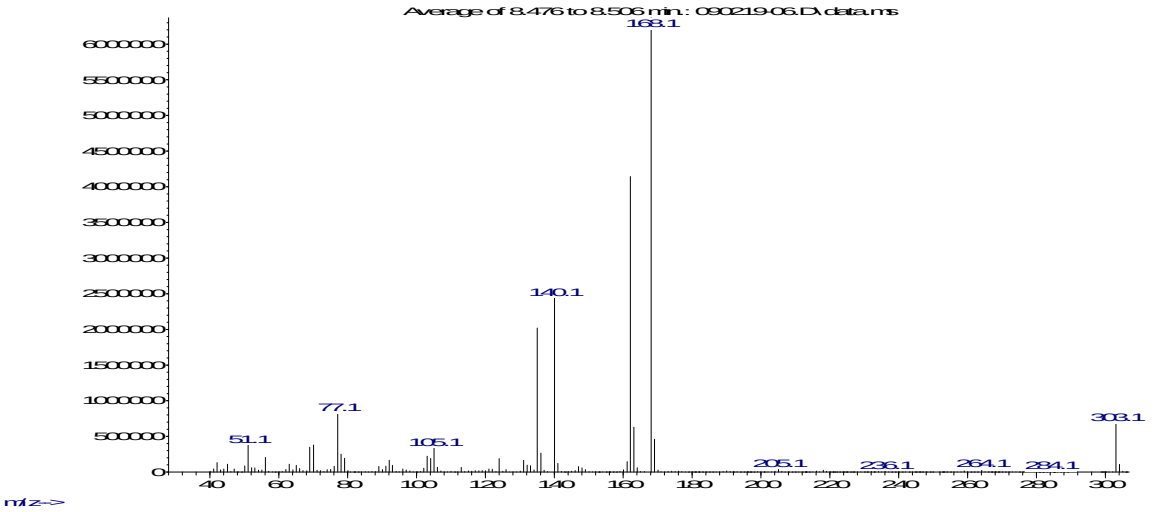
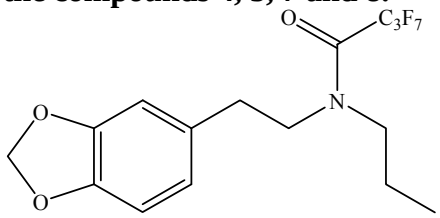


The ion at *m/z* 176 can be formed through two different fragmentation pathways (Scheme 8.1 and 8.3) for the PFPA derivatives of compounds 4, 5, 7 and 8. The use of TFA and HFBA as derivatizing agents resolved this problem by providing characteristic peaks at *m/z* 126 and 226 for the TFA and HFBA derivatives of compounds 4 and 5, respectively. Figure 8.5 shows representative mass spectra of the HFBA of these four compounds. Hence, TFA and HFBA derivatives provide more structure specific fragment ions than the PFPA analog.

Figure 8.5: Mass spectra of the HFBA derivatives of the compounds 4, 5, 7 and 8.



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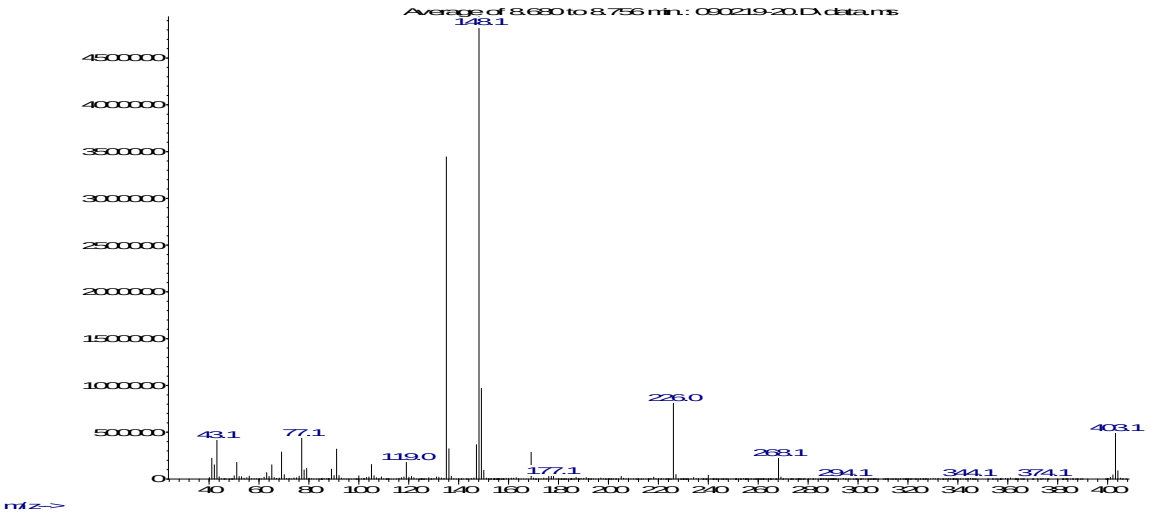

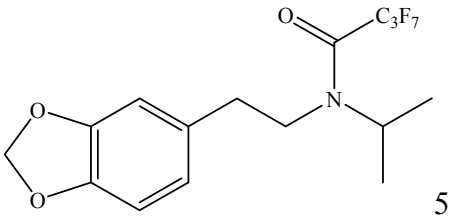

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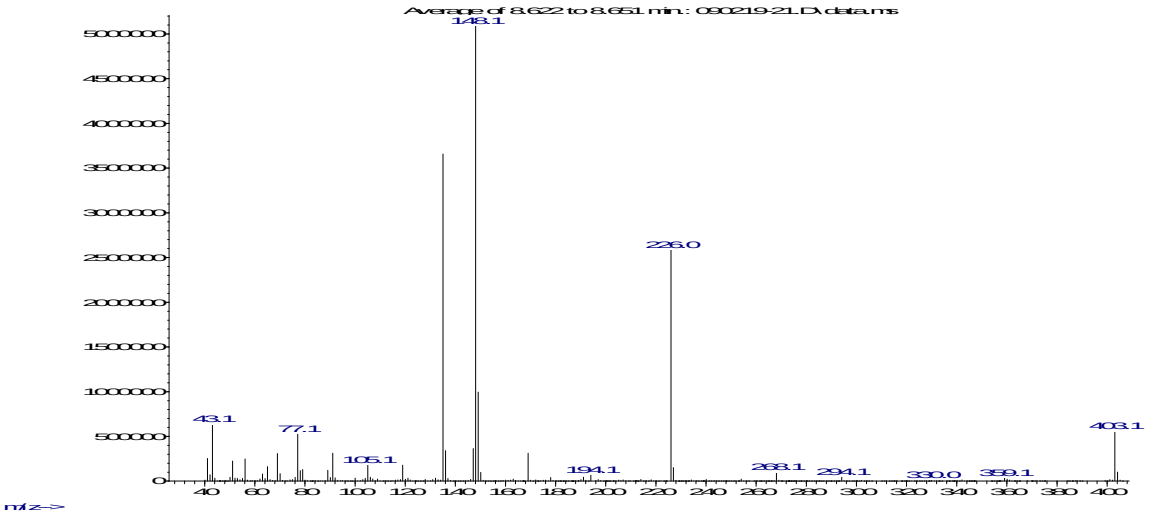

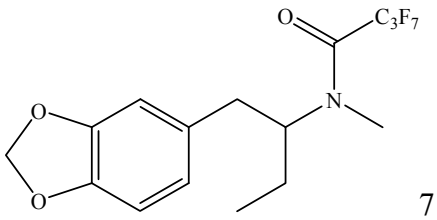

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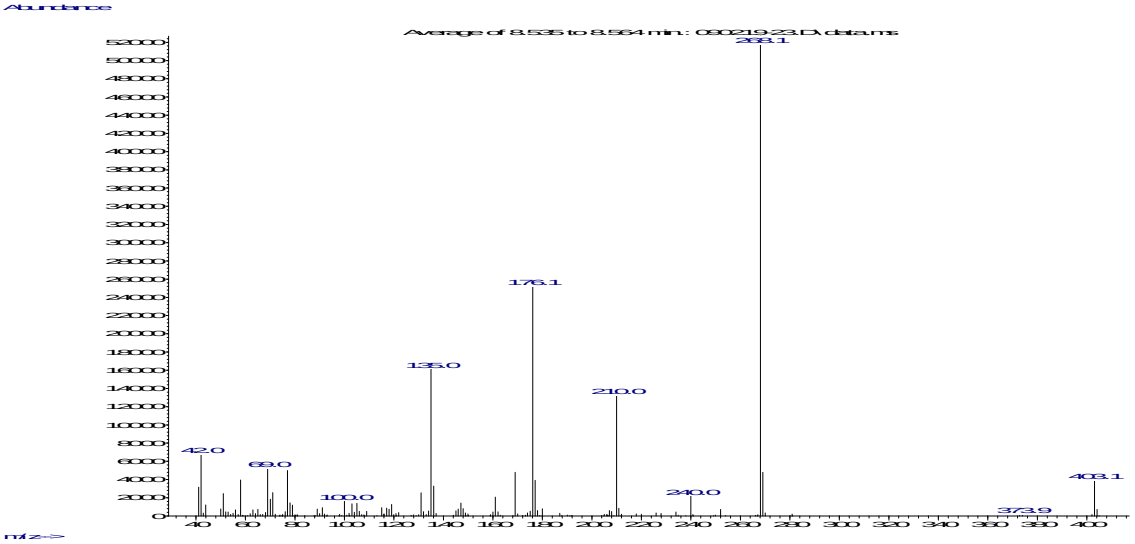

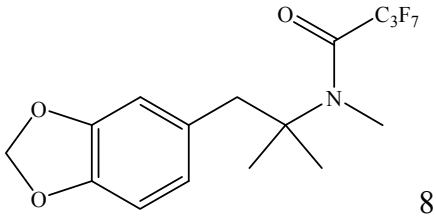

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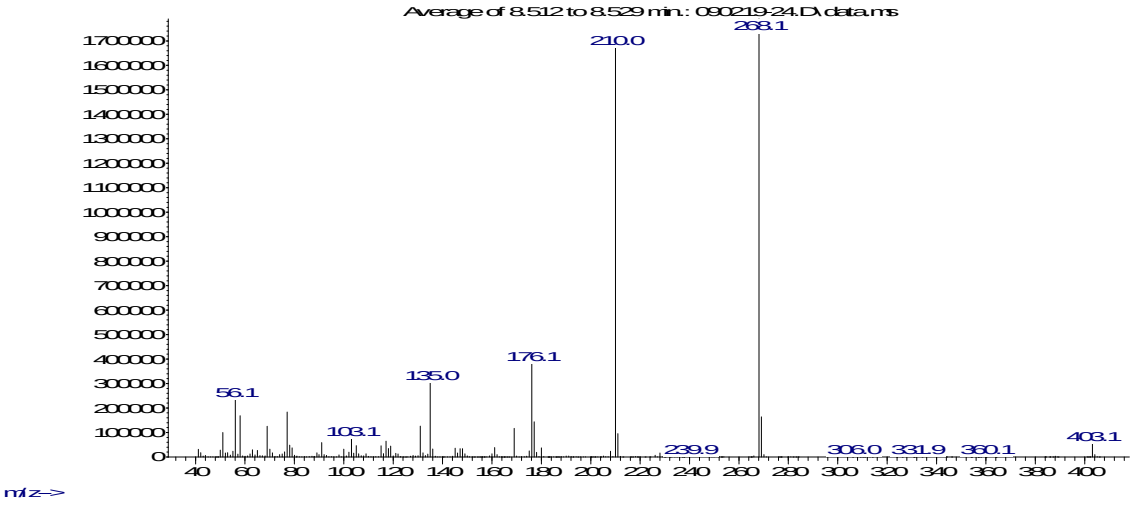


Gas Chromatography of Side Chain Regioisomeric Phenethylamines Related to the Controlled Substances MDEA, MDMMA, and MBDB.

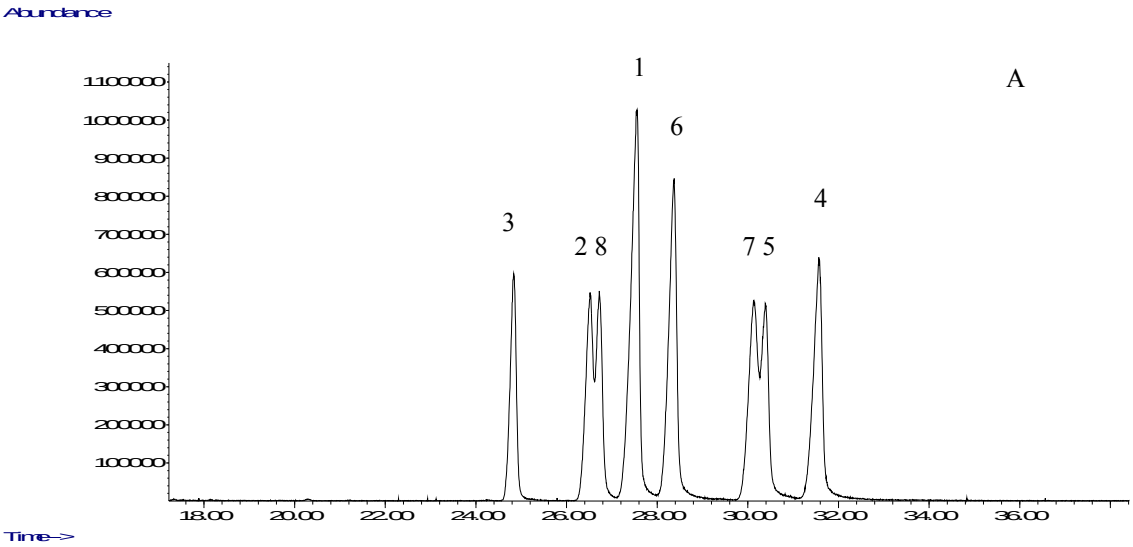
GC-MS is a powerful tool that combines the identification power of mass spectrometry with the separation power of gas chromatography. The separation of a physical mixture of the compounds 1-10 in the underivatized form was the subject of a previous report from our laboratory.

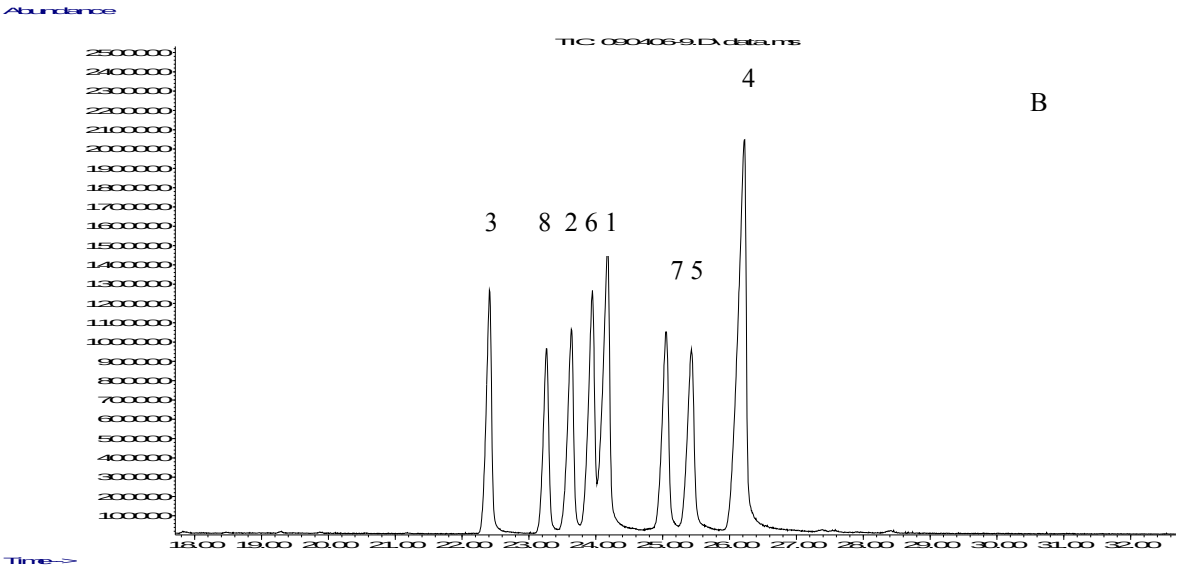
Several stationary phases and different temperature programs were evaluated in an effort to resolve the TFA, PFPA and HFBA derivatives in this study. The Rtx-200 phase provided adequate resolution for these compounds of closely related retention properties. However, different temperature programs were necessary for resolving each set of perfluoroacyl derivatives.

The separation was performed using three temperature programs for each derivative studied. Program one was used to separate the trifluoroacetyl (TFA) derivatives and consisted of an initial hold at 100°C for 1.0 min, ramped up to 180°C at a rate of 12°C min^{-1}, held at 180°C for 2.0 min then ramped to 200°C at a rate of 10°C min^{-1} and held at 200°C for rest of the run duration. The second program was used to resolve the pentafluoropropionic anhydride (PFPA) derivatives. It started at 100°C for 1.0 min, ramped up to 180°C at a rate of 7.5°C min^{-1}, held at 180°C for 2.0 min then ramped to 200°C at a rate of 10°C min^{-1} and held at 200°C. The third program utilized to separate the heptafluorobutyric anhydride (HFBA) derivatives had an initial oven temperature at 100°C for 1.0 min, followed by temperature increase at a rate of 9°C min^{-1} to reach

180°C, held at 180°C for 2.0 min then ramped to 200°C at a rate of 10°C min^{-1} and held at 200°C for the remaining of the run.

Figure 8.6 shows the chromatographic separation of all derivatized forms (TFA, PFPA and HFBA) of compounds 1-8. The chromatogram shows that the derivatives of the primary amines (compounds 1-3), the most branched side chain (compound 3) elutes first and the least branched (compound 1) elutes last. For the secondary amines (compounds 4-8), the perfluroamides elute in a similar pattern. Thus the compound of greatest side chain branching (compound 8) elutes first followed by compound 6, 7, 5 and the most linear tertiary amide (compound 4) elutes last. The resolution of the PFPA and HFBA derivatives was better than that of the TFA derivatives. However both TFA and HFBA derivatives provide more structurally specific ions in the mass spectrum to help in discriminating among these compounds. Hence, the HFBA derivatives are considered the best choice to discriminate by mass spectrometry and resolve by gas chromatography this set of regioisomeric phenethylamine derivatives.





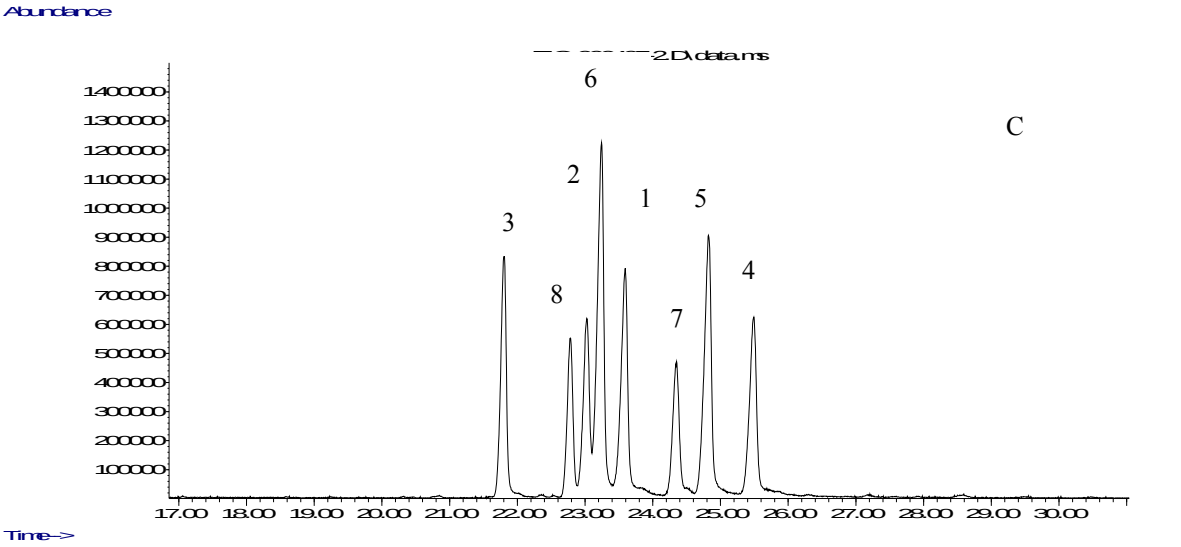


Figure 8.6 Gas chromatographic separation of A: TFA; B: PFPA and C: HFBA derivatives of compounds (1-8), Column used; Rtx-200.

Conclusion of Chapter 8

Three regioisomeric methylenedioxyphenethylamines (MDEA, MDMMA, and MBDB), having the same molecular weight and major mass spectral fragments of equivalent mass, have been reported as drugs of abuse. Seven additional phenethylamines have a side chain regioisomeric relationship with the controlled substances. Derivatization of the eight primary and secondary amines with various perfluroacylating agents yields amides which significantly individualized their mass spectra and allowed for specific side chain identification. The individualization is the result of formation of unique marker ions or as a result of differences in the relative abundances of some common ions. The trifluoroacetyl and heptafluorobutryl derivatives offer more unique fragment ions over pentafluoropropionyl derivatives. Chromatographic resolution of the perfluroacyl amides was achieved on a relatively polar stationary phase Rtx-200.

Chapter 9

GC-MS Evaluation of a Series of Acylated Derivatives of 3,4-Methylenedioxymethamphetamine

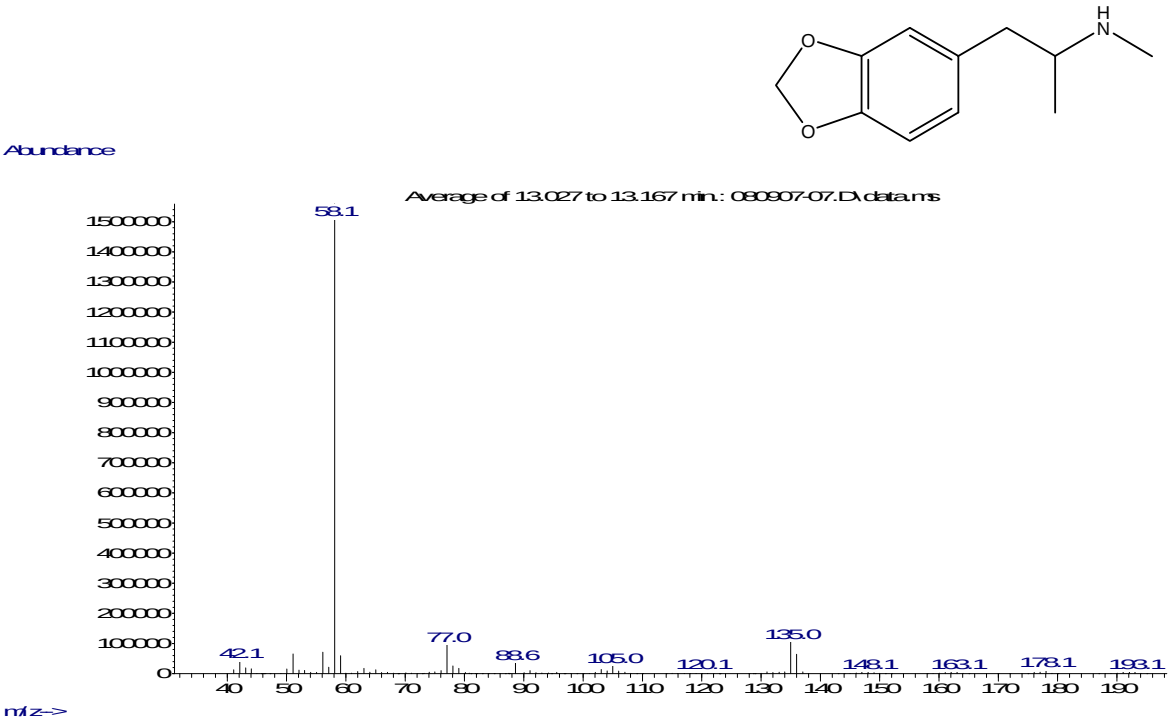
The aim of this chapter is to study and compare the mass spectral properties of MDMA after derivatization with different reagents commonly used for amine acylation. The second task is to evaluate the retention properties of each acyl derivative under chromatographic conditions regularly used by forensic chemists. The derivatizing agents

used in this study included trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), heptafluorobutric anhydride (HFBA), pentafluorobenzoyl chloride, acetic anhydride, propionic anhydride , butyric anhydride and benzoyl chloride. Additionally, the formyl, d_3-acetyl and d_5-benzoyl derivatives were prepared to confirm mass spectral fragmentation pathways.

Mass Spectrometry of Acylated Derivatives of 3,4-Methylenedioxymethamphetamine

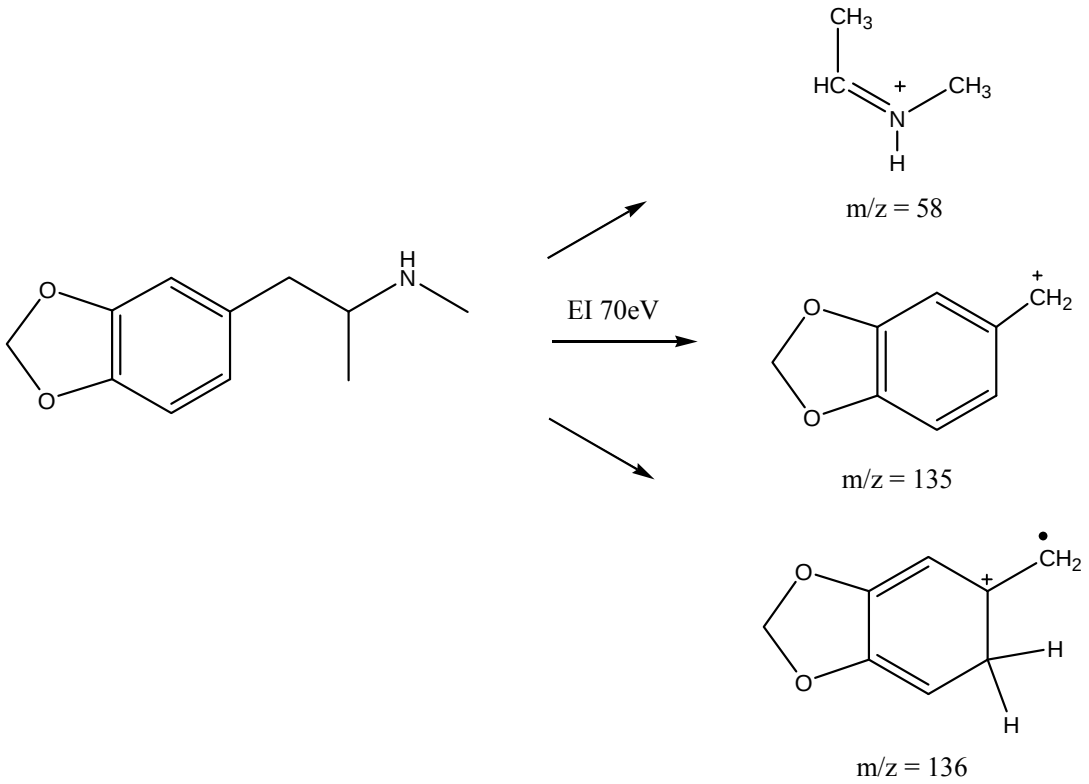
Mass spectrometry is the principal method used for confirming the identity of drugs and related substances in forensic samples. Figure 9.1 shows the structure of 3,4-MDMA and the mass spectrum for the underivatized amine. The mass spectrum for 3,4-MDMA is presented here primarily for comparison with the various acylated derivatives reported in this study. The fragmentation of phenethylamines is generally characterized by a base peak from an amine initiated alpha-cleavage reaction breaking the carbon-carbon bond of the ethyl linkage between the aromatic ring and the nitrogen atom of the amine group. With regards to 3,4-MDMA, initial ionization of nitrogen followed by cleavage of the alpha bond yields the m/z 58 imine cation, the base peak in the MDMA mass spectrum.

Figure 9.1: Mass Spectrum of the underivatized MDMA



Formation of the molecular ion via ionization of the aromatic pi-electrons followed directly by alpha-cleavage yields the benzylic cation at m/z 135 and hydrogen rearrangement from the equivalent molecular ion followed by alpha-cleavage initiated at the distant radical site yields the radical cation at m/z 136 in Figure 9.1. The structures for these fragment ions are shown in Scheme 9.1

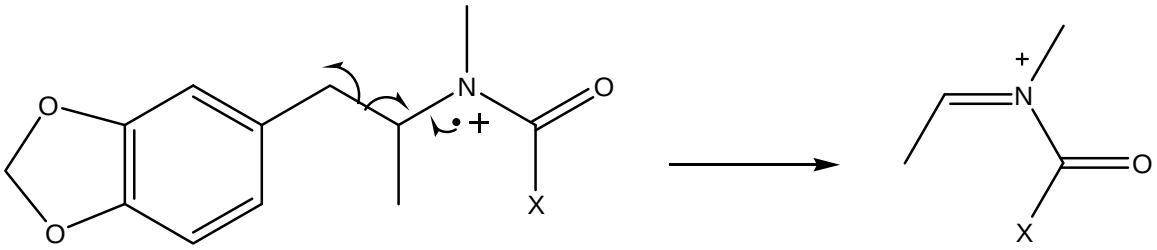
The initial group of derivatives evaluated in this study were the simple alkyl amides of 3,4-MDMA obtained by derivatization with acetic, propionic and butyric anhydrides. Acylation of the amine results in significantly lower nitrogen basicity and this often results in the formation of unique and characteristic fragment ions.



.

Scheme 9.1. EI fragmentation pattern for the underivatized MDMA.

Figure 9.2 (A, B and C) shows the mass spectra for the homologous acetyl, propionyl and butyryl amides. These three spectra show molecular ions of low relative abundance at m/z 235, 249 and 263 respectively. Additionally, a second homologous series of fragment ions $(M-135)^+$ occurs at m/z 100, 114 and 128 suggestive of the acylated imine fragment for each of the amides. The mass spectrum in Figure 9.2D provides evidence to support these structural assignments and shows the equivalent ion at m/z 103 for the d_3-acetamide. The structures for the acylated imine fragments included in this study are shown in scheme 9.2



X = CF_3, 289
= C_2F_5, 339
= C_3F_7, 389
= C_6F_5, 387
= H, 221
= CH_3, 235
= C_2H_5, 249
= C_3H_7, 263
= C_6H_5, 297
= CD_3, 238
= C_6D_5, 302

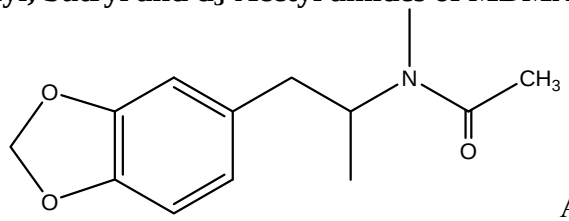
m/z = 154, X = CF_3
= 204, X = C_2F_5
= 254, X = C_3F_7
= 252, X = C_6F_5
= 86, X = H
= 100, X = CH_3
= 114, X = C_2H_5
= 128, X = C_3H_7
= 162, X = C_6H_5
= 103, X = CD_3
= 167, X = C_6D_5

Scheme 9.2: Imine fragmentation pattern of acylated derivatives of MDMA.

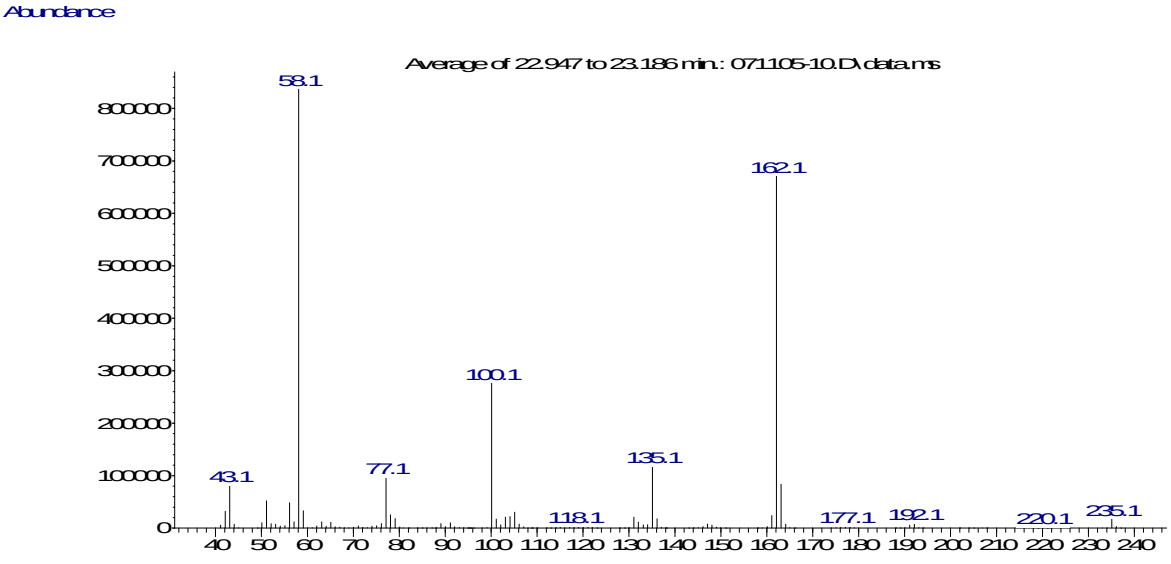
The spectra in Figure 9.2A, B and C show two prominent ions occurring at the same mass for all three derivatives. The base peak for these derivatives is the m/z 58 ion and this ion is equivalent to the base peak in the underivatized amine whose mass spectrum is shown in Figure 9.1.

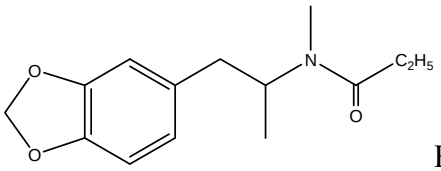
The m/z 58 ion in the spectrum of these derivatives is likely the result of hydrogen rearrangement from the alkyl group of the acyl imine fragments at m/z 100, 114 and 128 to yield the common imine fragment at m/z 58. This fragmentation pathway is illustrated in Scheme 9.3. Furthermore, the mass spectrum in Figure 9.2D for the d_3-acetamide shows the deutrated imine species at m/z 59 providing support for the structural assignment and fragmentation pathway

Figure 9.2. Mass spectra of acetyl, propanyl, butryl and d_3-Acetyl amides of MDMA

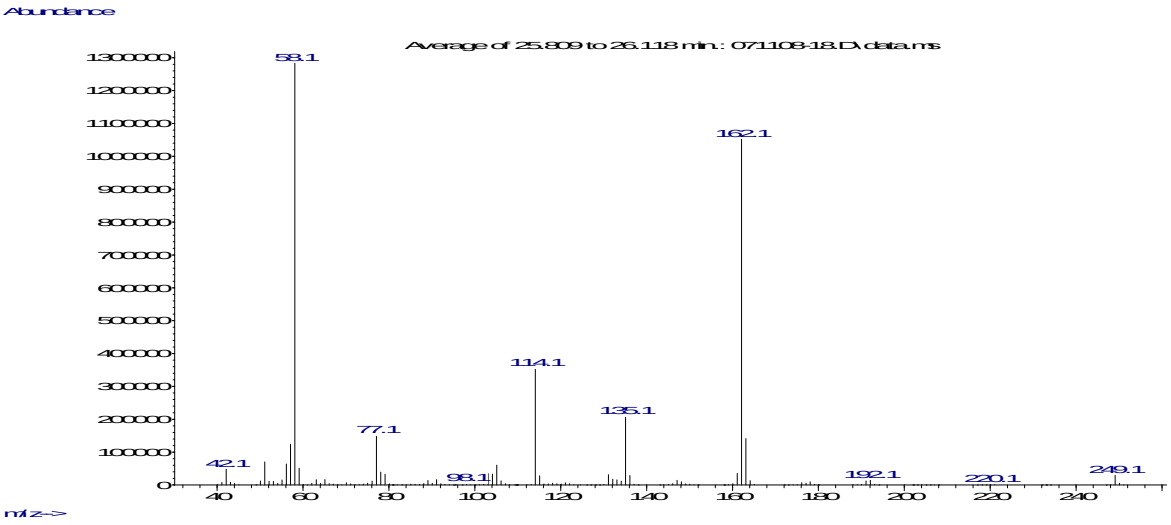


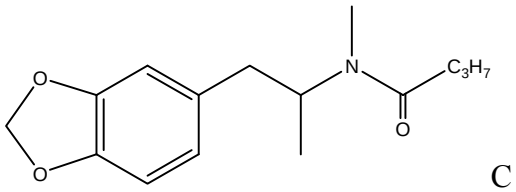
A



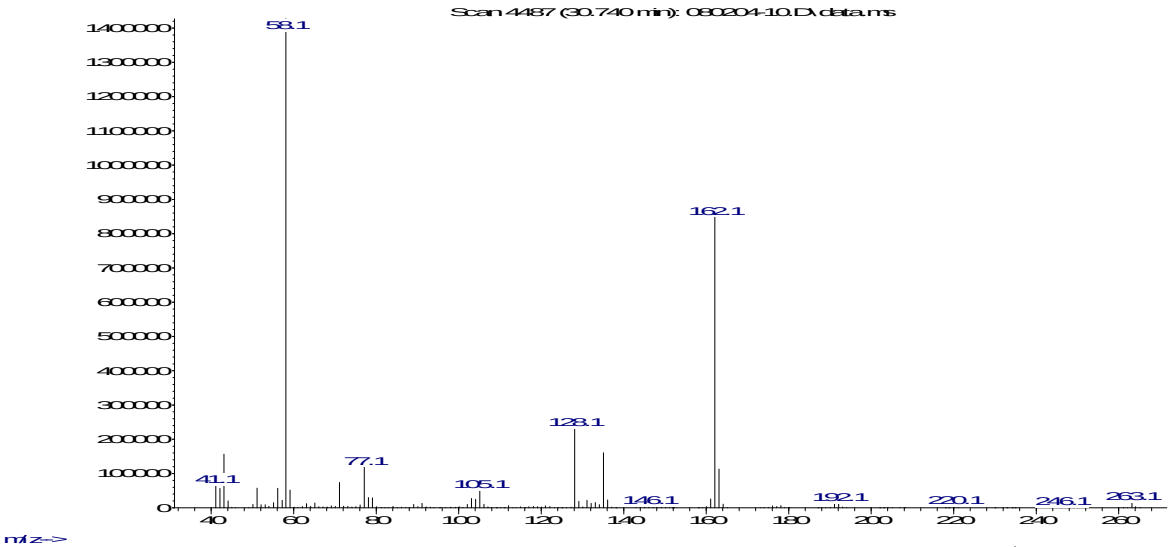


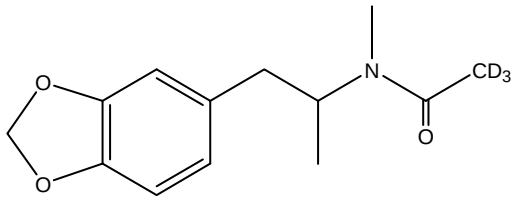
B





C





D

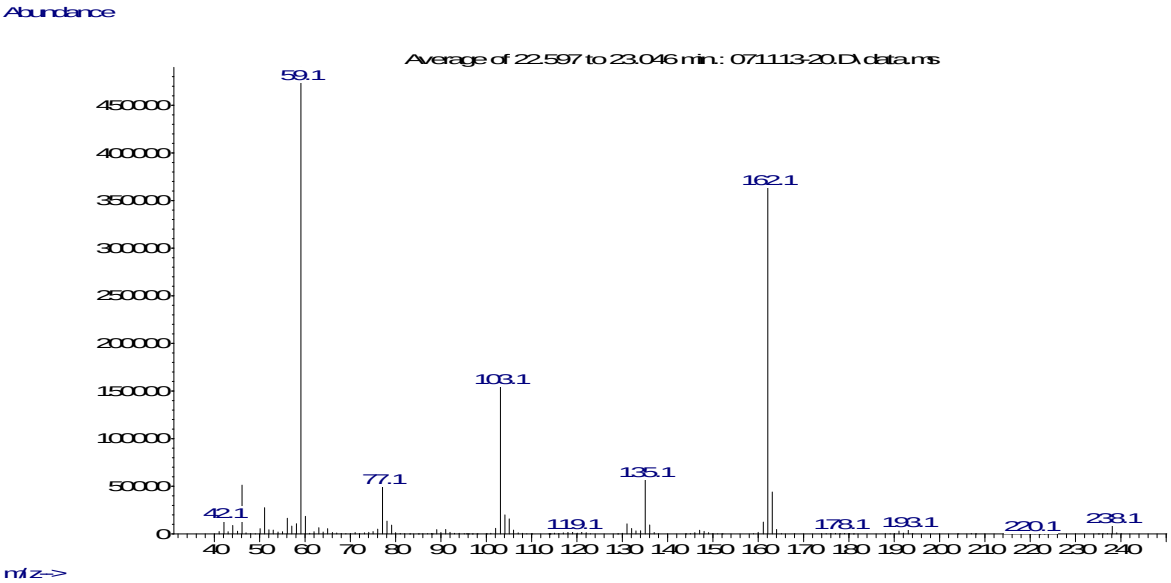
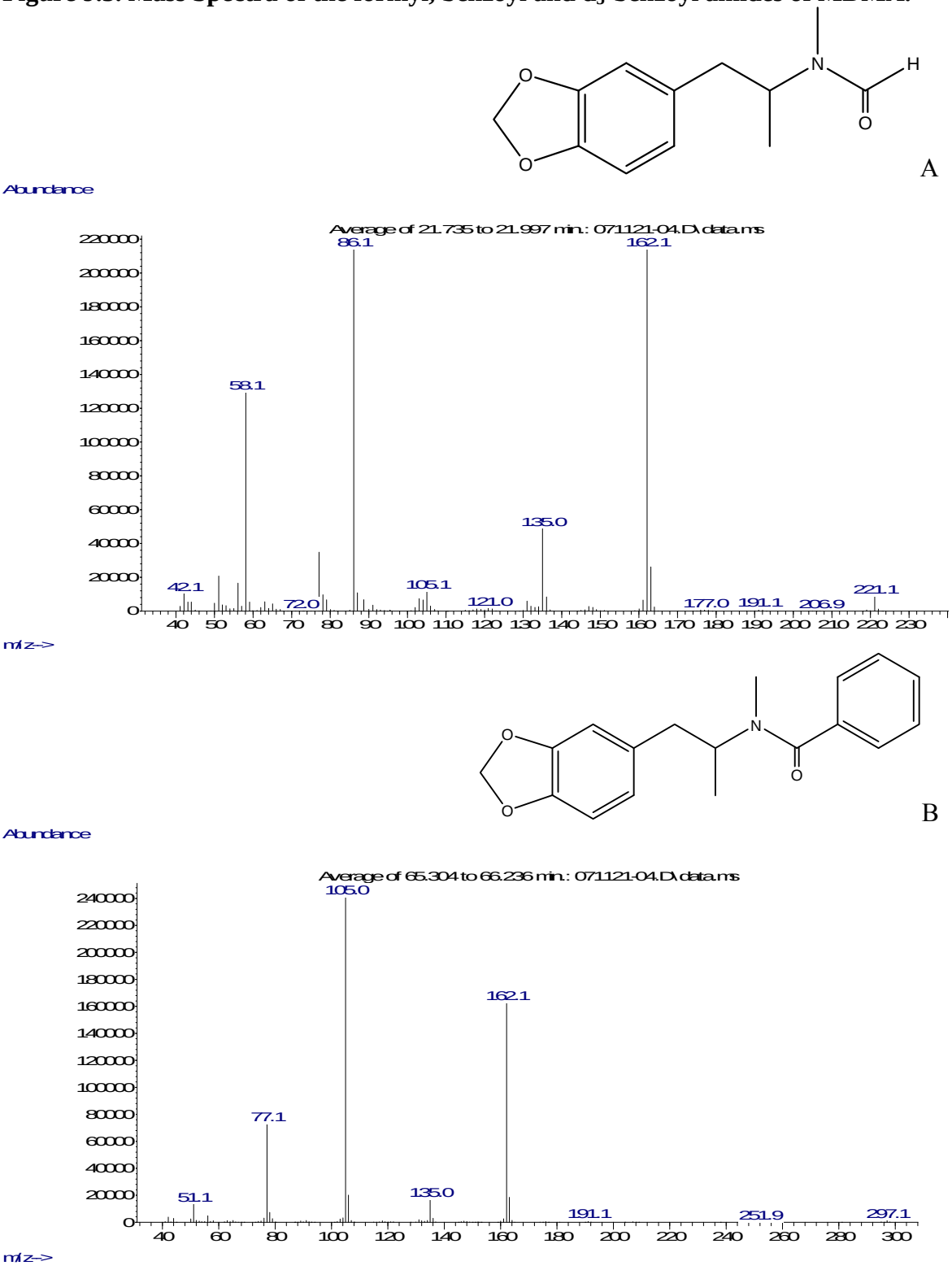
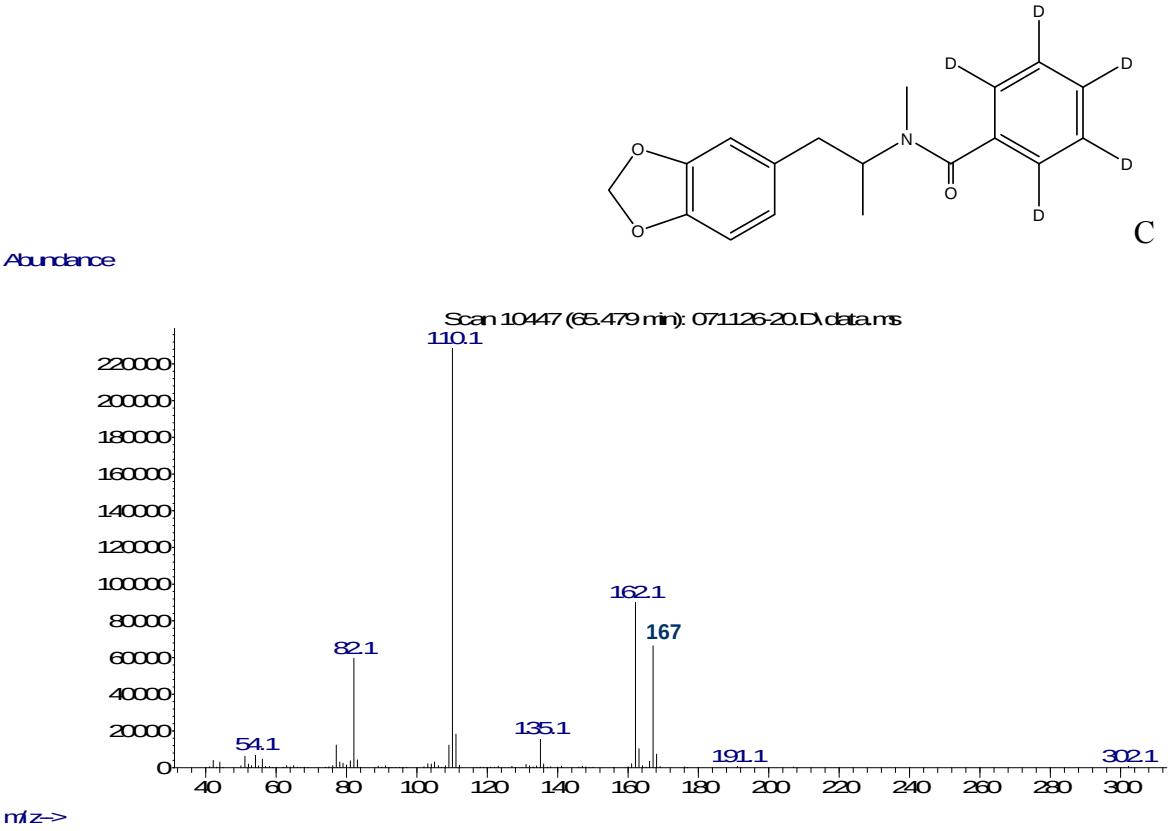
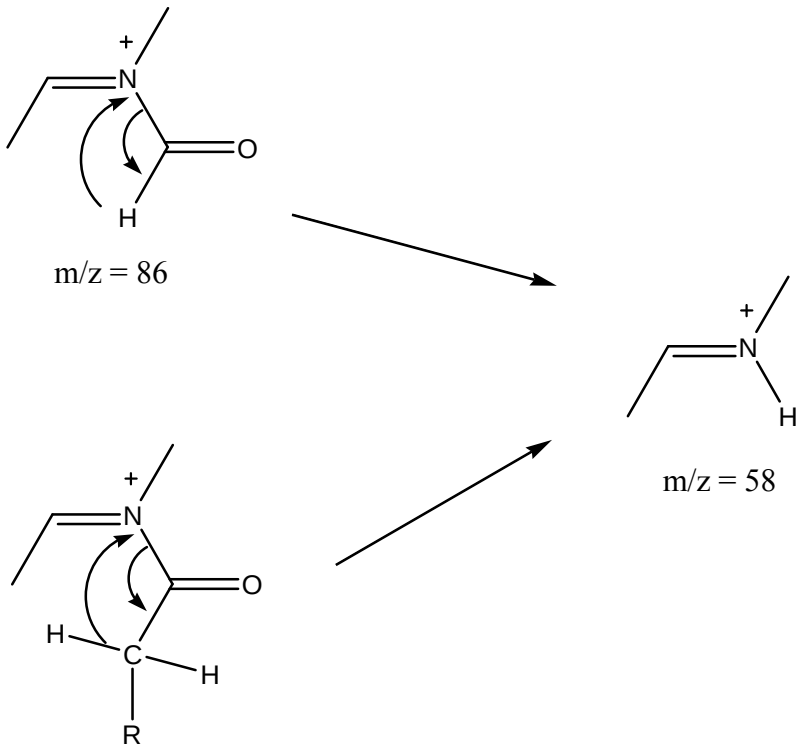


Figure 9.3. Mass Spectra of the formyl, benzoyl and d_5-benzoyl amides of MDMA.

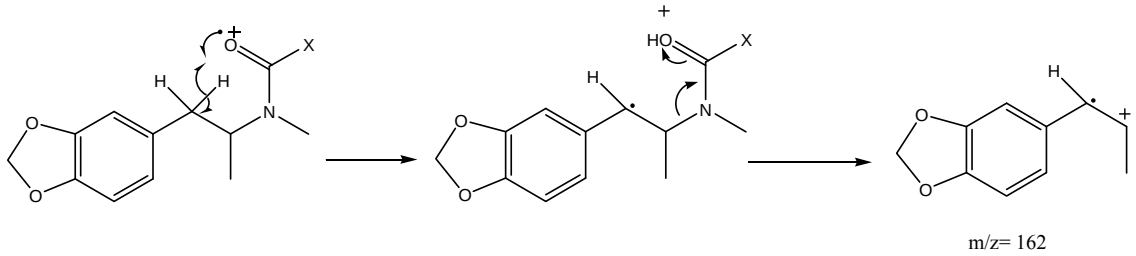




The formamide (N-formyl) and benzamide (N-benzoyl) derivatives were prepared and evaluated to determine the scope of the hydrogen rearrangement in the further decomposition of the acyl imine species. The mass spectra of these compounds are shown in Figure 9.3A-B. As shown in Scheme 9.3, the hydrogen of the formyl group does transfer in significant abundance; however the resulting m/z 58 fragment is not the base peak for this compound. The decreased likelihood of hydrogen transfer through the 3-membered ring transition leaves the formyl imine species (m/z 86) as the more prominent ion in its mass spectrum. The benzamide in Figure 9.3B does not show any indication of a m/z 58 ion demonstrating that the aromatic hydrogens do not migrate in the same manner as the hydrogen of the alkyl groups in the previously described spectra.



Scheme 9.3 Formation of m/z 58 from the N-formyl, acetyl propionyl and butyryl imine fragments



Scheme 9.4 Formation of m/z 162 in all amide derivatives of MDMA

The other common fragment seen in all the mass spectra in Figure 9.2 and those in Figure 9.3A and B is the m/z 162 ion. This fragment ion is the 3,4-methylenedioxyphenylpropene radical cation occurring through hydrogen transfer from

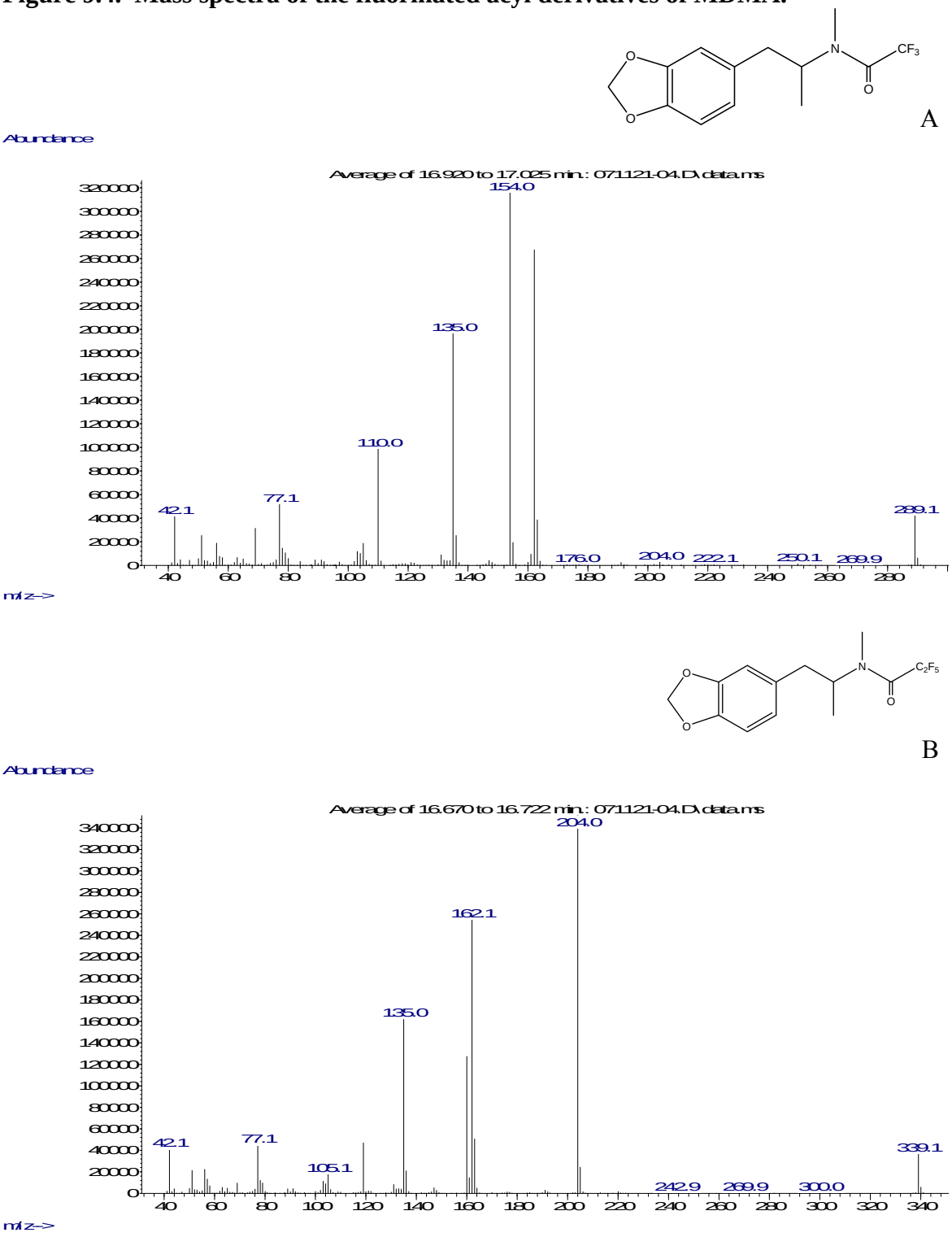
the propyl group to the carbonyl oxygen followed by loss of the neutral amide species. This mechanism for formation of the m/z 162 radical cation is shown in Scheme 9.4. An interesting uniqueness was observed in the mass spectrum of the benzamide derivative in Figure 9.3B. Two separate fragmentation pathways can yield ions at m/z 162, the 3,4-methylenedioxyphenylpropene radical cation and the benzoylimine cation. Figure 9.3C shows confirmation of this point with the mass spectrum for the d_5-benzamide. The d_5-benzoylimine is shifted to m/z 167 as expected while the 3,4-methylenedioxyphenylpropene fragment remains at m/z 162. Additionally, the benzoyl fragment ($C_6H_5CO^+$) at m/z 105 in Figure 9.3B is shifted to m/z 110 for the d_5-benzoyl derivative.

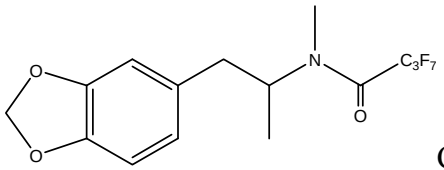
The mass spectra for all the derivatives described to this point have some disadvantages for the identification of amines such as 3,4-methylenedioxymethamphetamine. The derivatives formed from alkanoic carboxylic acid anhydrides (acetic, propionic and butyric) fragment to yield base peaks at m/z 58 equivalent to the underivatized amines. Thus without the aid of chromatographic separation, the completeness of the derivatization process itself could be questioned. The loss of significant ion current as m/z 58 is a drawback to the use of these derivatives for forensic analysis and the only unique major ion for identification of 3,4-MDMA is the m/z 162 ion and this ion appears regardless of the acylating species. The benzamide derivative yields significant ion current at unique masses such as m/z 105 and 162. However the benzamide derivative of 3,4-MDMA was observed to have quite high GC retention properties as will be described later in this study. These relatively high retention properties may limit this derivative utility in some applications.

The mass spectra for a series of perfluoroacyl derivatives of 3,4-MDMA are shown in Figure 9.4. One of the major features of these spectra is the absence of m/z 58 ion. This ion does not occur since no hydrogen is available for migration from the acyl portion of these derivatives. The spectra in Figure 9.4A, B, C and D further show common ions at m/z 135 and m/z 162. These ions are the 3,4-methylenedioxybenzyl cation and the 3,4-methylenedioxyphenylpropene radical cation respectively. The formation of these ions occurs in an analogous manner to that described earlier in this report. The three perfluoroalkyl derivatives, trifluoroacetyl, pentafluoropropionyl and heptafluorobutyryl amides show several ions in a homologous series separated by 50 mass units (CF_2). The m/z 154, m/z 204 and m/z 254 ions in these three spectra represent the perfluoroacylimine species (see Scheme 9.2). These ions are the base peaks in the three spectra and without hydrogen atom to migrate from the acyl group, no m/z 58 decomposition ion is formed. Additionally, these spectra do not show any evidence of analogous fluorine transfer decomposition products.

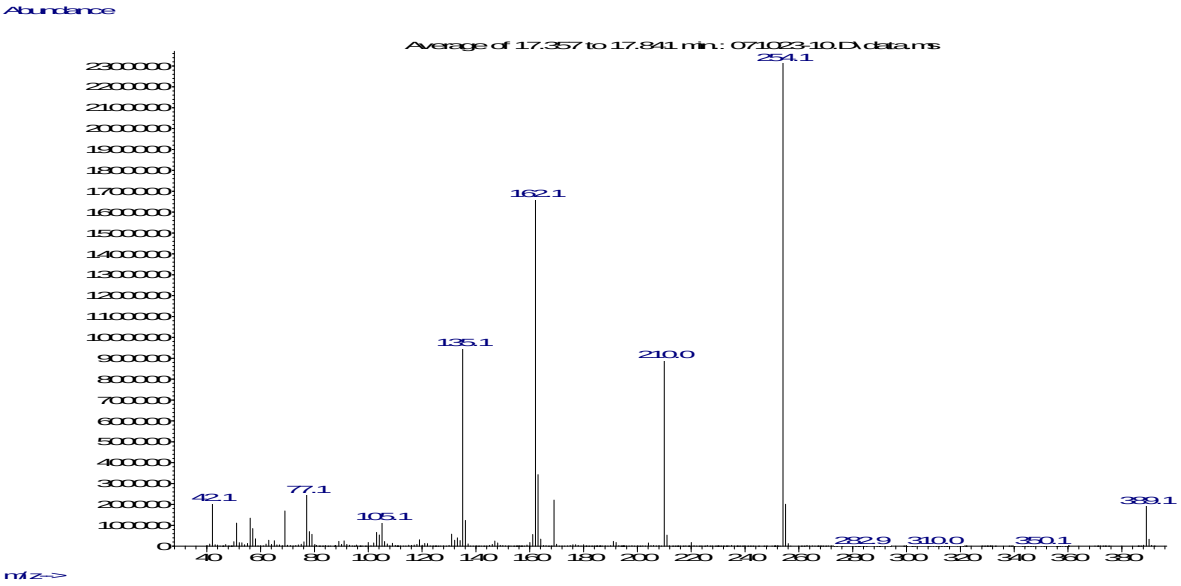
The second homologous series of ions in Figure 9.4A, B and C occur at m/z 110, 160 and 210 respectively. This series of ions differing by 50 mass ions (CF_2) indicates that the perfluoro fragment is a component of these ions. Previous studies in chapter 1 using d_3-3,4-MDMA in which the three deuterium atoms are bonded to the N-methyl group (N-CD_3) have shown that all three deuterium labels are contained in the m/z 160 ion for the PFPA derivative. This leaves only 26 mass units (CN) to account for all the mass of the m/z 160 ion. The mechanism and account for these ions is shown in Scheme 17.5.

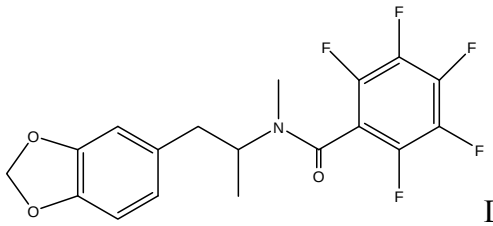
Figure 9.4. Mass spectra of the fluorinated acyl derivatives of MDMA.



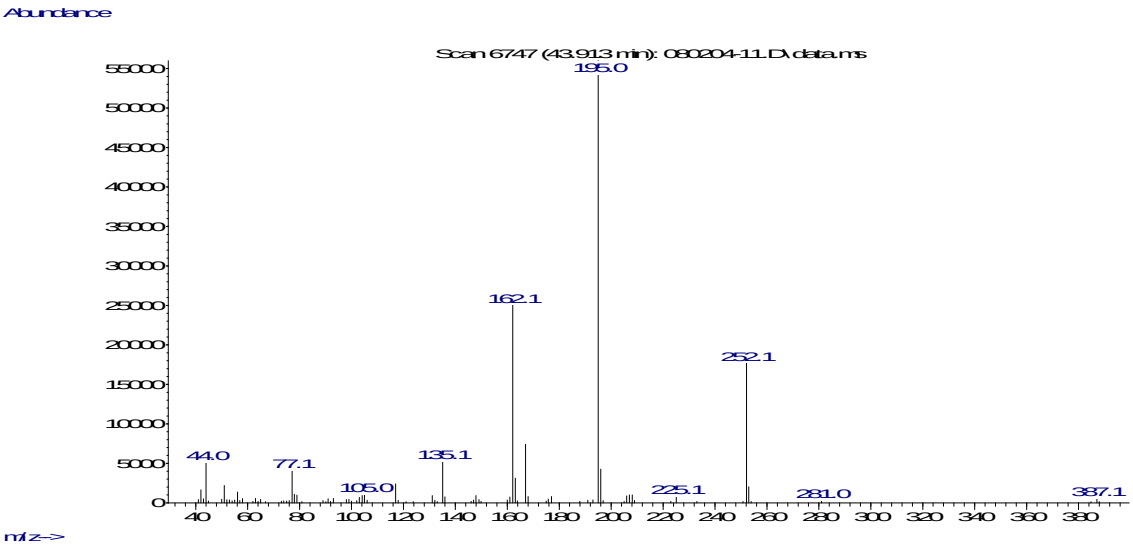


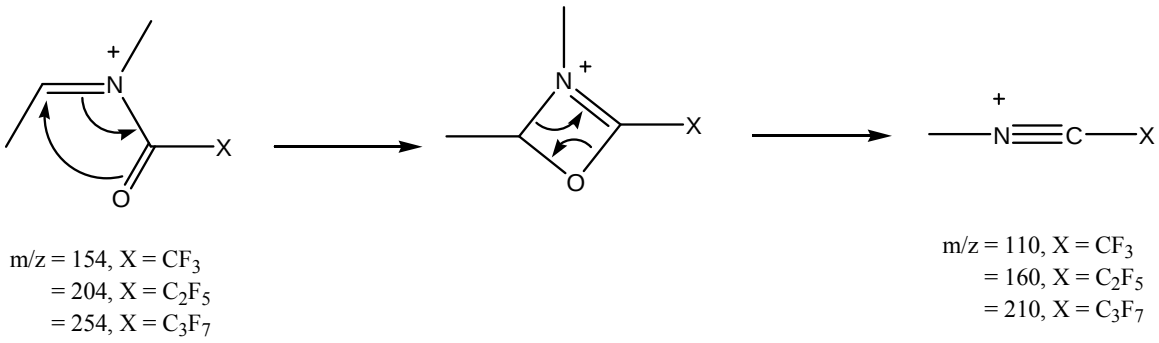
C





D



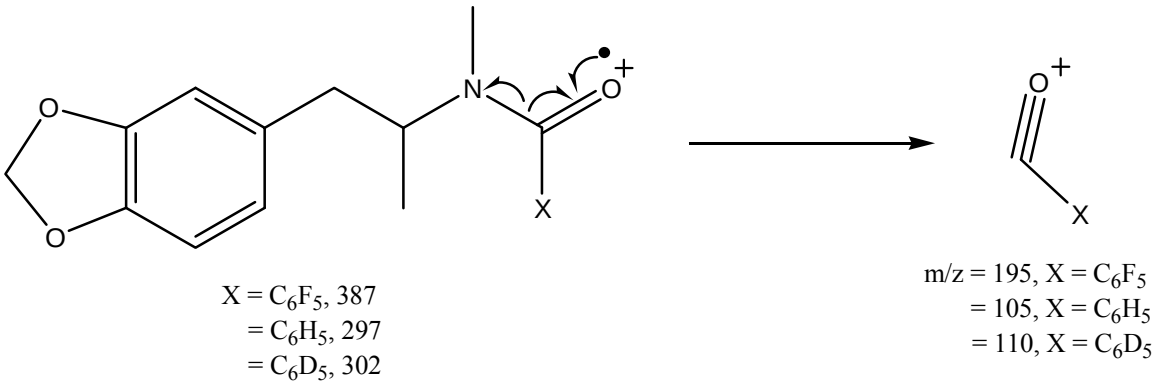


Scheme 9.5: Formation of m/z 110, 160 and 210 in the spectra of the TFAA, PFPA and HFBA derivatives of MDMA.

Based on our studies of numerous phenethylamines, this mechanism appears to be specific for the perfluoroacyl derivatives of N-methyl substituted compounds. Next chapter will address the scope of this mechanism.

While this project did not examine any perfluoroacyl derivatives in this homologous series beyond the heptafluorobutyramide, the perfluorooctanoyl (PFO) derivatives of 3,4-MDMA and methamphetamine have been reported. These derivatives show a major fragment ion at m/z 410 which extends this series of rearrangement ions (see Scheme 9.5) to higher chain homologues. The additional four CF_2 groups in the PFO derivative beyond the HFBA derivative account for the 200 additional mass units. The work of Westphal et al further described the PFO derivative of d_5-MDMA and clearly showed that only three deuterium labels were incorporated into the analogous fragment ion at m/z 413. The base in the mass spectrum of the PFO derivative of MDMA occurs at m/z 454 which is the perfluoroacylimine species and analogous to the base peaks in Figure 9.4A, B and C. Thus these data are consistent with the experimental observations reported in this study.

The remaining spectrum in Figure 9.4D is for the pentafluorobenzamide and the base peak at m/z 195 is the pentafluorobenzoyl cation. This ion is analogous to the m/z 105 and m/z 110 ions for the benzoyl and d_5-benzoyl cations respectively (Scheme 9.6). These ions would be major fragments in the benzamide of most amines and thus do not provide ions characteristic of 3,4-MDMA.



Scheme 9.6. Formation of m/z 195, 105 and 110 from the pentafluorobenzoyl, benzoyl and d_5-benzoyl derivatives of MDMA.

Gas Chromatography

The chromatogram in Figure 9.5 provides a comparison of the acyl group structure and relative GC retention properties on a nonpolar stationary phase, 100% dimethylpolysiloxane, Rtx-1. Several temperature programs were evaluated in this project and one program showing the best compromise between resolution and analysis time was used to generate the chromatogram in Figure 9.5.

The perfluoroalkylamides are the first group of derivatives to elute on the dimethylpolysiloxane stationary phase. These amides elute much earlier than their corresponding non-fluorinated hydrocarbon counterparts. Both the fluorinated and non-fluorinated alkyl derivatives elute before the aromatic amides. In every case of direct comparison, the perfluoro species eluted much earlier than its non-fluorinated

hydrocarbon counterpart. The most obvious example of this effect can be seen for the benzamides in the last two peaks in the chromatogram (Figure 9.5). The high relative retention for the benzamides coupled with the lack of major fragment ions could limit their value in MDMA analysis and identification. The alkyl hydrocarbon derivatives provide significantly increased chromatographic retention yet show the m/z 58 ion as the base peak as does the derivatized amine, MDMA. Thus, the perfluoroalkyl derivatives offer excellent chromatographic properties as well as a significant number of characteristic fragment ions for MDMA identification.

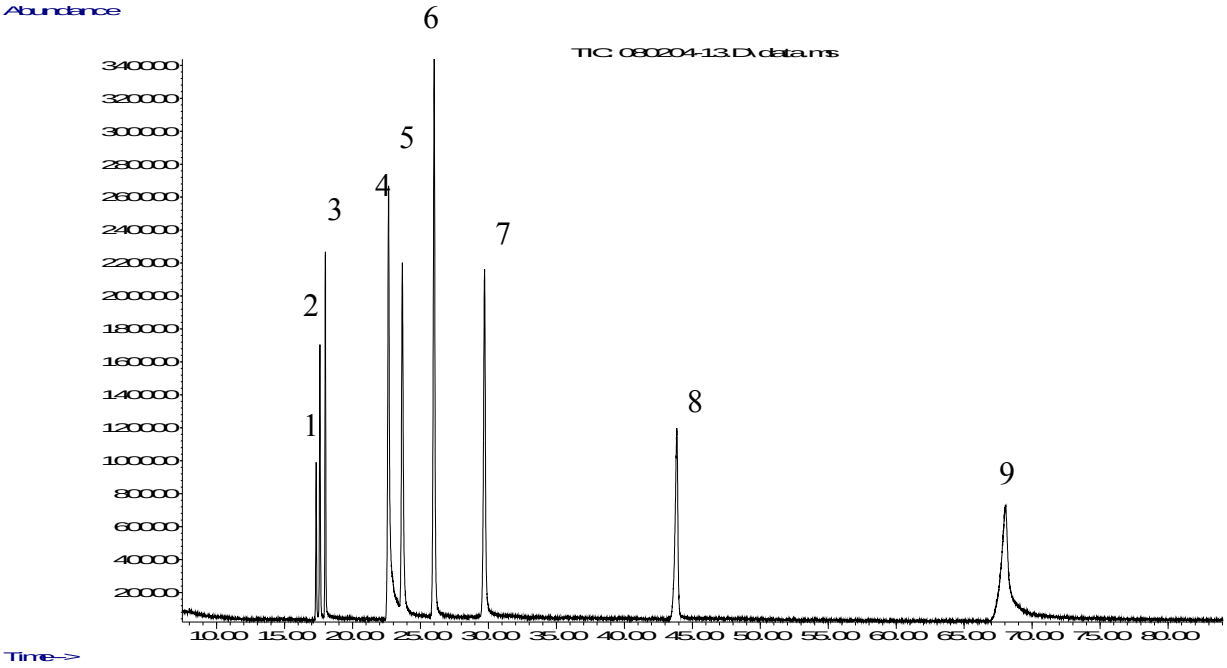


Figure 9.5. Gas chromatographic separation of MDMA derivatives. Peaks: 1, PFPA; 2, TFAA; 3, HFBA; 4, N-formyl; 5, acetyl; 6, propoinyl; 7, butyrl; 8, pentaflurobenzoyl and 9, benzoyl amides.

Conclusions of Chapter 9

The perfluoroacyl derivatives of MDMA show excellent chromatographic properties and unique mass spectral fragment ions. The perfluoroalkyl amides of TFAA, PFPA and HFBA yield a unique series of mass spectral fragments at m/z 110, 160 and 210 respectively. Additionally, the base peaks in these mass spectra occur at relatively higher masses at m/z 154, 204 and 254 respectively. These ions are the perfluoroacylimines resulting from loss of 135 mass units (3,4-methylenedioxybenzyl) from the molecular ion. This $(M-135)^+$ species also occurs for the acetyl, propionyl and butyryl amides however these ions rearrange through loss of the acyl group to yield a common ion at m/z 58. Thus, the base peak for the non-fluorinated hydrocarbon derivatives is the same as that observed for the underivatized MDMA, m/z 58. The formyl, d_3-acetyl, d_5-benzoyl and pentafluorobenzoyl derivatives provided chromatographic and confirmatory mass spectral evidence for fragmentation pathways.

IV. Conclusions

IV.1 Discussion of Findings.

The compounds investigated in this project are a series of closely related regioisomeric and isobaric amines having mass spectra essentially equivalent to that of MDMA, MDEA and MBDB. Mass spectral equivalence in the underivatized amines shows that MS methods do not provide confirmation of drug identity and molecular individualization. Additional scientific data for molecular individualization can be obtained following simple chemical derivatization with perfluoroacylating reagents. Perfluoroacylamines provide specific and unique mass spectral fragments for side chain and some ring substituent identification. Vapor phase infrared spectrometry (GC-IRD) provides additional confirmation for other ring substituents and for those compounds not forming stable acylation products. Thus the results of this research have provided improved specificity in the analytical methods used to identify these phenethylamine controlled substances. This improved specificity comes from methods which allow the forensic analyst to eliminate non-drug imposter substances as the source of analytical data matching that of the controlled drug substance.

IV.2 Implications for policy and practice:

The results of this project allow the forensic analyst to differentiate among most of the regioisomeric and isobaric equivalents of MDMA. This can be accomplished without having access to a large number of reference standards of these compounds.

IV.3 Implications for further research:

Perhaps the first implication from the results of this work is an educational program to efficiently and effectively transfer the findings to practicing forensic analysts. While we have published extensively on this subject in the scientific literature, a one or two day short course would allow us to put these data in the hands of forensic drug chemists in an organized and effective manner. The course would include background educational material on mass spectra and infrared interpretation as well as chromatographic theory. The course could be offered in conjunction with regional meetings of forensic scientists over a period of a year to 18 months and reach numerous individual forensic scientists.

Retention index studies for all the ring substituted amines of the same side-chain, especially the methamphetamine side-chain compounds. Since the methamphetamine side chain is perhaps the most likely to appear in new designer drugs, retention indexing studies on standardized GC columns and stationary phases could further decrease the need for reference standards in identification of these regioisomeric and isobaric amines. Such studies should be done in cooperation with collaborators in forensic science laboratories.

Liquid Chromatographic studies on unique stationary phases to provide retention/separation/resolution data on as large a subset of these compounds as possible. Again concentrating on those compounds having the same side chain structure. These are

the compounds for which mass spectrometry provides the least information for molecular individualization.

In concert with LC studies MS-MS techniques can be explored for evidence of specific fragment ions identifying aromatic ring substitution patterns. The initial phase of such a study would be to compare the fragment patterns for all the possible regioisomeric substitution patterns in each series. Since we have all the compounds available already, our group is well positioned to begin this study immediately.

V. References

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VI. Dissemination of Research Findings

VI.1 Scientific Publications Related to this Project:

Ashley L Thigpen, Jack DeRuiter and C Randall Clark, "GC-MS Studies on the Regioisomeric 2,3- and 3,4-Methylenedioxyphenethylamines Related to MDEA, MDMMA and MBDB," J. Chromatogr. Sci., 45, 229 (2007).

Tamer Awad, Jack DeRuiter and C. Randall Clark, "Chromatographic and Mass Spectral Studies on Methoxy Methyl Methamphetamines Related to 3,4-Methylenedioxy-methamphetamine," J. Chromatogr. Sci., 45, 466 (2007).

Tamer Awad, Jack DeRuiter and C. Randall Clark, "GC-MS Analysis of Regioisomeric Ring Substituted Methoxy Methyl Phenylacetones," J. Chromatogr. Sci., 45, 458 (2007).

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C. Randall Clark, Tamer Awad and Jack DeRuiter, "GC-IRD Analysis of Regioisomeric Substituted Phenethylamines of Mass Spectral Equivalence," Chromatography Today, 1 #4, 27-30 (2008).

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Tamer Awad, Tarek Belal, Hadir M. Maher, C. Randall Clark and Jack DeRuiter. "GC-MS Studies on Side Chain Regioisomers Related to Substituted Methylenedioxy-phenethylamines; MDEA, MDMMA and MBDB" J. Chromatogr. Sci., 48, 726-732 (2010).

VI.2 Scientific Presentations Related to this Project:

Synthesis and Analytical Profiles for Regioisomeric and Isobaric Amines Related to MDMA, MDEA and MBDB: Differentiation of Drug and non-Drug Substances of Mass Spectral Equivalence—Progress Presentation

C. Randall Clark, 2007 General Forensics R&D Grantees Meeting, , San Antonio, Texas, February **2007**.

Synthesis and Analytical Profiles for Regioisomeric and Isobaric Amines Related to MDMA, MDEA and MBDB: Differentiation of Drug and non-Drug Substances of Mass Spectral Equivalence—Progress Presentation

C. Randall Clark, 2007 General Forensics R&D Advisory Group, Scottsdale, Arizona, October **2007**.

GC-MS Analysis of the Ring and Side Chain Regioisomers of Ethoxyphenethylamines

C. Randall Clark, Tamer Awad and Jack DeRuiter. American Chemical Society National Meeting, New Orleans, Louisiana, USA; April 10, **2008**.

Synthesis and Analytical Profiles for Regioisomeric and Isobaric Amines Related to MDMA, MDEA and MBDB: Differentiation of Drug and non-Drug Substances of Mass Spectral Equivalence—Progress

C. Randall Clark, 2009 General Forensics R&D Grantees Meeting, Denver, Colorado. February **2009.**

GC-IRD Identification of Isomeric Phenethylamines of Mass Spectral Equivalence C. Randall Clark, Tamer Awad, Tarek Belal and Jack DeRuiter, Pittsburgh Conference, Chicago, Illinois, March 8, **2009**.

GC-MS Studies on Side Chain Regioisomers Related to the Substituted Methylenedioxyphenethylamines MDEA, MDMMA AND MBDB, Tamer Awad, Jack DeRuiter and C. Randall Clark, Pittsburgh Conference, Chicago, Illinois, March 8, **2009**.

GC-MS and GC-IRD Studies on the Ring Isomers of N-Methyl-1-Methoxyphenyl-1-Methyl-2-Propanamines Related to 3,4-MDMA, Tamer Awad, Hadir M. Maher, Jack DeRuiter and C. Randall Clark, American Chemical Society Meeting, Salt Lake City, Utah, April, **2009**.

Abdullah M. Al-Hossaini, Tamer Awad, Jack DeRuiter and C. Randall Clark,
"GC-MS and GC-IRD Studies on Ethoxy- and Methoxymethyl-phenethylamines: Isobaric Substances Related to the Methylenedioxyphenethylamine Drugs,"
Pittsburg Conference on Analytical Chemistry (Pittcon), March 2, 2010, Orlando, FL.

Tamer Awad, Karim M.Abdel-Hay, Jack DeRuiter and C. Randall Clark
"Comparison of GC-IRD and GC-MS Methods for the Identification of Isomeric Drug Substances: Piperazines and Phenethylamines," Pittsburg Conference on Analytical Chemistry (Pittcon), March 4, 2010, Orlando, FL.

C. Randall Clark, "Comparison of GC-MS and GC-IRD Methods for the Identification of Isomeric Substituted Phenethylamine and Related Drug Substances," Southwestern Association of Forensic Scientists (SWAFS), Grapevine TX, Sept 23, 2010.