CHROMATOGRAPHIC AND MASS SPECTRAL STUDIES ON MASS

EQUIVALENT SUBSTITUTED PHENETHYLAMINES RELATED

TO MDEA, MDMMA AND MBDB

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CHROMATOGRAPHIC AND MASS SPECTRAL STUDIES ON MASS EQUIVALENT SUBSTITUTED PHENETHYLAMINES RELATED TO MDEA, MDMMA AND MBDB

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THESIS ABSTRACT

CHROMATOGRAPHIC AND MASS SPECTRAL STUDIES ON MASS EQUIVALENT SUBSTITUTED PHENETHYLAMINES RELATED TO MDEA, MDMMA AND MBDB

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The goal of this project is to evaluate the GC-MS properties of a series of twelve regioisomeric and isobaric ring substituted phenethylamines with identical molecular weight and major fragmentation ions. Three regioisomeric 3,4-methylenedioxyphenethyl -amines have been reported as components of clandestine drug samples in recent years. These drugs of abuse are a subset of a total of six methylenedioxyphenethylamines of identical molecular weight yielding regioisomeric fragment ions of equal mass in the electron impact mass spectra.

This project also evaluated six isobaric methoxy-methylphenethylamines of the same molecular weight and expected mass spectral fragmentation products of equal mass.

The procedures described in this study will provide for the specific identification of any one of these compounds to the exclusion of the other possible regioisomeric/isobaric amines.

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1. LITERATURE REVIEW

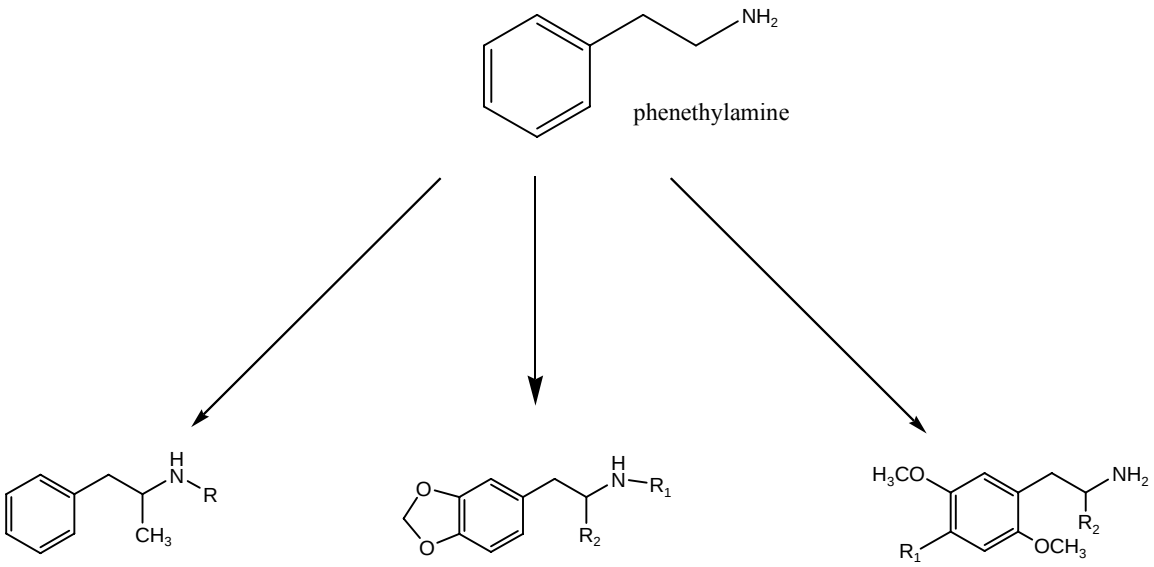
1.1 Introduction

In the second part of the 1990s, a trend of escalating ecstasy use was observed all over the world. Although mostly identified with 3,4-methylenedioxymethamphetamine (MDMA), tablets containing 3,4-methylenedioxyamphetamine (MDA), 3,4-methylendioxyethylamphetamine (MDEA) and 2-methylamino-1-(3,4-methylenedioxyphenyl)-butane (MBDB) have been frequently referred to as "ecstasy" as well. The methylenedioxyamphetamines, (MDMA, MDA and MDEA) are all novel psychoactive compounds with structural similarities to amphetamine and the psychedelic phenethylamine, mescaline. The methylenedioxy derivatives of amphetamine and methamphetamine represent the largest group of "designer drugs".

Methamphetamine was developed in the early 20th century from its parent drug, amphetamine and was used originally in nasal decongestants and bronchial inhalers. Like amphetamine, it causes decreased appetite, increased energy and a general sense of well being (Hegadoren, 1999). The term "ecstasy" is no longer used just for MDMA, but for the whole group of ring-substituted amines (MDA, MDMA, MDEA and MBDB) because they are chemically and pharmacologically nearly identical. In some cases the clandestine drug samples referred to as "ecstasy" contain mixtures of the ring substituted amines (Freudenmann, 2004).

Entactogens is another name commonly used for these drugs. Although it was originally coined for MDMA and MBDB only; the name is based on the belief that the substances help individuals experience empathy enhancement or a "touching-within" (Freudenmann, 2004). MDMA is the most commonly used derivative of this series. It has both stimulant and hallucinogenic effects in humans. The most common way to administer MDMA is orally, usually in tablet form or capsule form and its effects last approximately 4-6 hours (Hegadoren, 1999).

Ecstasy tablets are prepared in clandestine laboratories and during most of the last decade; Western Europe has been the world's major manufacturing region (Freudenmann, 2004). The goal of clandestine manufacturers is to prepare substances with pharmacological profiles that are sought after by the using population. These manufacturers are also driven by the desire to create substances that fall outside national and/or international control regimes in order to bypass existing laws and to avoid prosecution. This offers room for clandestine experimentation into individual substances within a class of drugs with similar pharmacological profiles. This approach is not only used to bypass the legal system but also to produce even more potent substances from non-controlled starting materials (Aalberg, 2002). Thus, identification of all potential MDA derivatives in clandestine drug products has become essential and highly challenging for forensic chemists.



Amphetamines (stimulants)	Entactogens	Hallucinogens
Amphetamines (R=H) Methamphetamine ($R=CH_3$)	MDA ($R_1=H$, $R_2=CH_3$) MDMA ($R_1=CH_3$, $R_2=CH_3$) MDEA ($R_1=C_2H_5$, $R_2=CH_3$) MBDB ($R_1=CH_3$, $R=C_2H_5$)	DOM ($R_1=CH_3$, $R_2=CH_3$) DOE ($R_1=C_2H_5$, $R_2=CH_3$) DOB ($R_1=Br$, $R_2=CH_3$) DOI ($R_1=I$, $R_2=CH_3$)
Street names: Amphetamine: speed, uppers Methamphetamine: meth, chalk, tweak, crank, ice, crystal, glass	MDA: love drug MDMA: ecstasy, XTC, E, adam, M&M, California sunrise, hug drug, clarity, essence, lover's speed, stacy MDEA: eve, intellect MBDB: methyl-J, eden	DOM: "STP" (serenity, tranquility and peace)

Figure 1: Phenethylamine and its derivatives. Three important classes of substances of abuse are derived from phenethylamine : 1) amphetamines (stimulants) lack of any ring-substitution, 2) ring-substituted amphetamines (RSAs) with an aromatic substitution in the ring position 3 and 4 (methylenedioxy group), 3) phenethylamine hallucinogens with a 3-fold ring-substitution.

1.2 History

The first reported synthesis of a methylendioxyamphetamine (MDA) was in 1910 by the German chemists Mannich and Jacobsohn. Its pharmacological properties were reported in animals in 1939 and included marked sympathomimetic effects (CNS stimulatory activity and convulsions) at high doses. MDA in turn has been coined an antitussive, an ataractic and an appetite suppressant. Its current popularity as a recreational drug and its name, the "love" drug, arise from its emotion-enhancing or entactogenic qualities (Hegadoren, 1999).

The first public recording of the preparation and properties of MDMA was in 1914 when it was patented by Merck as a potential appetite suppressant. However, the patent expired without being commercially available on the public market. MDMA was largely ignored by the scientific community until the 1970s when, as part of a larger study of mescaline analogues, some of MDMAs behavioral effects were examined (Hardman et al., 1973). The first comprehensive report of pharmacological actions of MDMA in humans appeared in 1978 (Shulgin, 1978) and it reported that MDMA produced "an easily controlled altered state of consciousness with emotional and sensual overtones." Prior to this, the drug had been used in clinical practice as an adjunct to psychotherapy for its ability to encourage openness of emotional expression and to facilitate interpersonal communication and intimacy (Shulgin, 1990). In 1985, intense scientific and social interest in MDMA was generated by a decision on the part of the Drug Enforcement Agency (DEA) in the United States to severely restrict MDMA

use by placing it on Schedule I of controlled substances (Lawn, 1986). Controversy over its use, its abuse potential and its potential for toxicity spilled over into the media and was largely responsible for this placement (Hegadoren, 1999).

The ethyl derivative (MDEA) became popular in the USA only after the placement of MDMA on Schedule I and expanded in use briefly until the "designer drug" legislation of 1986 which outlawed the sale of analogues of controlled substances (Hegadoren, 1999).

Table 1: Short history of MDA, MDMA, MDEA and MBDB (Freudenmann, 2004):

Date	**Event**
1909-1910	German chemists C. Mannich and W. Jacobsohn first synthesized MDA
1912-1914	MDMA (not under this name) first synthesized in 1912 at pharmaceutical company E. Merck, Darmstadt, Germany, as an intermediate byproduct in a chemical pathway for a styptic agent; patent assigned to Merck April 27th 1914
1978-1980	MDEA first mentioned in scientific publications by A. Shulgin and co-workers
1985-1988	MDMA listed as a schedule I controlled substance in the US and it was also federally banned in other countries; MDEA surfaced as a legal substitute ("designer drug"), but became schedule I on August 13th 1987 as well.
1985	Techno-music and ecstasy use become a mass phenomenon in Europe in the 1990s; mass production of ecstasy in Belgium, The Netherlands, Germany and Poland

1986	First report of fatalities after polydrug intoxication including MDEA Dowling et al.].
Jan 28th 1991	MDEA federally controlled in Germany
1996	First report of fatal MDEA mono-intoxication [Iwersen & Schmoldt]
2003	Currently MDA, MDMA, MDEA and MBDB are listed in the most restrictive category of abused substances in the USA (schedule I), UK (Class A), and Germany (appendix 1 of the BTM, 16th edition from November 28th, 2001) as well as in other countries.

1.3 Pharmacology

1.2.1 Behavioral Effects

MDA MDMA and MDEA have all been reported to produce very similar central and peripheral effects in humans. However, there are differences in potency, time of onset and duration of action. The central effects are described as an easily controlled altered state of consciousness with heightened sense of well being, increased tactile sensations, increase perception of inwardly focused experience, and a strong desire to be with people, without significant perceptual distortion or hallucination. Although MDA has been known to produce hallucinations at higher than typical doses, this effect appears to be either abolished or diminished to visual distortions rather than well formed hallucinations by N-alkylations (as in the cases of MDMA and MDEA) (Hegadoren, 1999).

MDA is more potent as a hallucinogen than MDMA and MDEA with a typical dose range of 60-120mg, while single doses of 100-200mg. of MDMA and MDEA are common. The onset of effects range form 30-60 minutes with MDA to within 30 minutes with MDMA and MDEA. Duration of action is longer for MDA (~ 8 hours) than MDMA (~ 6 hours) and MDEA (~ 3-4 hours) (Hegadoren, 1999). MDMA has been shown to produce hyperthermia and the "serotonin syndrome" in laboratory animals. This syndrome consists of a complex series of behaviors including enhanced locomotor activity, reciprocal forepaw treading, head weaving, piloerection, hind limb abduction, proptosis, ataxia, unawareness, leading finally to convulstions and death (Green et al., 1995). The most common reported side effects in humans are drowsiness, muscle aches and general fatigue, depression lasting 1-2 days, difficulty sleeping and concentrating, paranoia and short lived anxiety and irritability (Hegadoren, 1999). The side effects increase with successive doses, while the positive subjective effects diminish (Green and Goodwin, 1996). It was originally considered that the after effects limit the frequency of use and tend to encourage variety in the choices of recreational drug ingested. This apparent sensitization seen in the unwanted after effects and the tolerance that develops to the positive effects have been reinterupted as supportive evidence of neurodegeneration (Hegadoren, 1999).

The peripheral effects of MDA, MDMA and MDEA are largely sympathomimetic in nature, mediated by the release of norepinephrine. These effects include tachycardia, elevated blood pressure, mydriasis, tremor, palpitations and diapnoresis. Other common effects include increase salivation, bruxism (grinding of

the teeth) and trismus (tightening of the jaw muscles) (Hegadoren, 1999).

It has been suggested that 3, 4-methylenedioxyphenylalkylamines may represent a novel class of pharmacological agents, labeled entactogens. These compounds which include MDA, MDMA, MDEA and MBDB do not fit the pharmacological profile of either phenethylamine hallucinogens or psychomotor stimulants. The prototypic entactogen was the α-ethyl derivative of MDMA, MBDB. MBDB was proposed as an entactogen devoid of hallucinogenic properties and which supposedly had a less complex array of behavioral effects in comparison with MDA. Subjects given MBDB supported its entactogenic properties, reporting a pleasant state of introspection, enhanced communication and a pronounced sense of empathy, similar to MDMA except the onset of action was slower and gentler with less euphoria (Nichols et al., 1986).

1.2.1 Cognitive Effects

There has been considerable interest in acute and chronic cognitive effects on the methylenedioxyamphetamine analogues, especially with respect to memory and learning. Experimental data for animals has shown that dose, dosing regimen and species are all important factors in the ability of these drugs to produce any cognitive effects. Non-human primates appear to be more sensitive than rats to large changes in cognitive testing. Acute administration of low doses of MDMA produced deficits in tests involving time estimation, motivation and learning, but had no effect on attention or short term memory. However, chronic treatment with escalating doses of MDMA produced

behavioral tolerance in the same operant tasks. The tolerance could still be demonstrated for some of the tasks when the animals were administered a challenge dose of MDMA 6-18 months after the completion of the chronic MDMA treatment (Frederick, 1995 and Frederick, 1997).

Recreational users have not reported specific changes in cognitive performances related to learning and memory. Varying degrees of "mental confusion" after acute MDMA ingestion have been reported, but no objective testing was included. A single dose of MDEA (140mg) given to volunteers failed to adversely affect the performance on several numerical tests. However, MDMA users have been shown to possess deficiencies on tests involving immediate and delayed word recall (Hegadoren, 1999).

1.3.3 Metabolism

The methylenedioxyphenylalkylamines undergo two overlapping metabolic pathways: O-demethylenation (to split the ring) of the methylenedioxy group to dihydroxy-derivatives followed by methylation of one of the hydroxy groups and successive degradation of the side chain the N-dealkyl and deamino-oxo metabolites. In contrast to the butanamines, BDB and MBDB, the propanamines, MDA, MDMA and MDEA are additionally metabolized to glycine conjugates of the corresponding 3, 4-disubstituted benzoic acids (hippuric acids). The hydroxy metabolites of all the drugs are excreted as glucuronic acid and/or sulfate conjugates (Maurer et al., 2000).

Quantitatively, the second metabolic pathway that breaks down the side chain of

the molecule by N-dealkylation is less important. In urine, (after 140mg of MDEA), unchanged MDEA and 3, 4-dihydroxyethylamphetamine (DHEA) can be detected for 33-62 hours after ingestion and MDA for 32-36 hours after ingestion. Initially detectable are also traces of 3, 4-dihydroxyamphetamine (DHA), 4-hydroxy-3-methoxyamphetamine (HMA), piperonyl acetone, 3, 4-dihydroxyphenyl acetone and 4-hydroxy-3-methoxyphenyl acetone (Freudenmann, 2004).

1.4 Toxicology

1.4.1. Acute Effects

The subjective experience under ecstasy varies greatly and depends on several factors such as the dose, presence of additional substances in the drug product, frequency of previous exposure to ecstasy, baseline mood, concomitant use of other drugs, etc. In general, the experience consists of "amphetamine-like" stimulation, mild "DOM-like" hallucinations and "entactogenic" effects. The effects of ring substituted amines seem to lie in between classic amphetamines and phenethylamine hallucinogens which are derived from the same parent compound (Freudenmann, 2004).

In the early 1970s reports of fatalities associated with the ingestion of MDA began appearing. A patient treated in a hospital emergency room after ingestion of MDA presented as unresponsive, with increased heart rate, hyperthermia, generalized rigidity, dilated pupils, and hyperreflexia. After 24 hours there was a deepened

unresponsiveness, increased pulmonary distress, decreased blood pressure, and continued hyperthermia resistant to any cooling techniques applied. The patient died without his/her conditions ever stabilizing (Hegadoren, 1999).

There are many case reports that describe post mortem and non-fatal clinical courses related to either MDMA or MDEA use. The clinical characteristics of mental status changes, restlessness, hyperthermia, hyperreflexia, and myoclonus are reminiscent of serotonin syndrome defined as a toxic hyperserotonergic state (Hegadoren, 1999).

The recommended treatment for toxic reactions related to the ingestion of MDA, MDMA and MDEA are generally supportive with ventilation assistance, cooling measures, anticonvulsants and fluid replacements. Rehydration is recommended but the rate of correction must take into account the degree of existing hyponatremia (Hegadoren, 1999).

Table 2: Psychotropic effects of MDEA in healthy humans (Freudenmann, 2004)

Symptoms	Pooled Data (%)	Study 1 N=8(males) day time, personal contact to scientist , systemic ratings	Study 2 N=6(females) night time,no personal; contact to scientist no systemic ratings
Hypervigilance, increased drive.	100	8/8	6/6
Euphoria, relaxation, peaceful satisfaction.	50	4/8	3/6
Dysphoria, irritation.	7	1/8	0/6

Feeling of happiness.	21	2/8	1/6
"Entactogenic effects": introspection, loss of anxiety, happy self acceptance, controlled communicative openness.	21	3/8	0/6
Altered perception of time	21	2/8	1/6
Altered visual, tactile, acoustic perception: colors more intense, blurred contours, things bigger/ smaller, sounds louder, haptic impressions "as if through cotton wool".	50	4/8	3/6
Depersonalization, derealization.	7	0/8	1/6
Religious-mystique experiences.	21	1/8	2/6
Psychotic state: acoustic/visual hallucinations, delusions, loss of control, anxiety.	7	0/8	1/6
No loss of control.	93	8/8	5/6
Disturbed concentration (subjective).	43	4/8	2/6
Severe cognitive dysfunction.	N/A	0/0	N/A

1.4.2. Chronic Effects

Unfortunately there are no follow-up data available from the survivors of acute toxicity related to ingestion of any of the methylenedioxyamphetamine analogues. Thus, potential long term or permanent disturbances in liver functions, thermoregulation or other systems affected by acute ingestion cannot be ruled out (Hegadoren, 1999).

What can be discussed is the issue of substance dependence. In general, the addictive potential for ecstasy is considered to be low.

This conclusion is based on the lack of tolerance and withdrawal effects after discontinuation, as well as on it s consumption pattern (use on weekends only, often self-limiting, strong influence on sociocultural factors). Criteria for substance dependence according to the current edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (Jansen, 1999) are not met in many ecstasy users. However, some recent case reports indicate that ecstasy does indeed have the potential to evoke substance dependence (Freudenmann, 2004).

1.5 Psychopathology

In addition to the psychological risks inherent in the use of recreational drugs in general, the methylenedioxyamphetamine analogues have been associated with a number of psychiatric symptoms. Numerous case reports document diverse symptoms arising from a variety of use patterns from a single dose to chronic use of MDMA. The symptoms include paranoid psychosis of varying duration, persistent anxiety, depression and panic disorder (Freudenmann, 2004). More recently, the risk of suicide in two individuals was highlighted (Hegadoren, 1999). These individuals presented with very different histories, symptoms and outcomes. The first individual had a six year history of MDMA use and complained of persistent depressive episodes with suicidal thoughts and significant anxiety. The second individual was believed to be a first time user, who became very depressed over a recent breakup with a girlfriend with in three hours of ingestion and two days later died from a self-inflicted gunshot wound to the head.

MDMAs ability to influence 5-HT systems might be acting as a catalyst to create the abnormal pattern(s) of 5-HT neurotransmission believed to underlie many psychopathological disorders, particularly mood disorders (Hegadoren, 1999).

There is substantial evidence that many former ecstasy users seek professional help because of various neuropsychiatric symptoms. They suffer from cognitive problems that effect verbal and visual memory, decision making and problem solving as well as affective (atypical depression or anxiety) and psychotic symptoms (paranoid ideas, altered visual or acoustic perception, depersonalization and flashbacks) (Freudenmann, 2004).

1.6 Neurotoxicity

It is the area of potential neurotoxicity in humans that is most controversial. There is a large body of literature from animal studies that demonstrates significant long term neurochemical and morphological changes in 5-HT neurons in response to administration of the methylenedioxy analogues (Hegadoren, 1999). Presently there is no direct evidence that MDMA is neurotoxic to humans, although indirect evidence does suggest the possibility of neurotoxicity. If serotonergic neurotoxicity does occur in MDMA users, one might predict changes in some of the functions where 5-HT is believed to play a major role. Indeed functional abnormalities seen in MDMA user that may be related to 5-HT injury include cognitive deficits, altered sleep patters, altered neuroendocrine

function, altered behavioral responses to 5-HT selective drugs and increased impulsivity (McCann et al., 2000).

1.6.1 Criteria for Neurotoxicity

Kleven and Seiden summarized four criteria that have been most widely used to establish neurotoxicity of amphetamine analogues: (a) long lasting depletions of 5-HT or dopamine (b) a decrease in high affinity uptake sites for 5-HT or dopamine (c) decreased affinity of synthetic enzymes for 5-HT and dopamine and (d) alterations in neuronal morphology. All four of these criteria have been studied in relation to the methylenedioxy analogues in animals (Kleven, 1992).

A single dose of MDMA (10mg/kg) in rats produced a biphasic effect, with acute depletion of 5-HT, most prominent at 3-6 hours after administration and recovery seen by 24 hours, followed by another phase of 5-HT that was seen one week after the drug treatment. The second phase of 5-HT depletion was followed by a decreased number of 5-HT uptake sites. The hippocampus and the frontal cortex were the most sensitive regions to the depleting effects of MDMA (Hegadoren, 1999). Dose-dependent decreases in tryptophan hydroxylase activity (the enzyme that is involved in the synthesis of 5-HT) have also been recorded after the administration of MDA or MDMA. Using a number of different experimental techniques, all three methylenedioxy analogues of amphetamine have been proven to be capable of producing morphological changes to the 5-HT neurons (Hegadoren, 1999). Although most researchers have not been able to

demonstrate that 5-HT cell bodies are adversely affected by these drugs, recent studies using a human serotonergic cell line showed that MDMA produced apoptotic response in the cells by inducing DNA fragmentation (Simantov, 1997).

That the amphetamine analogues affect the chemistry of neural processes comes as no surprise when one considers how closely their structures resemble those of the neurotransmitters (Freeman, 2002). Figure 2 shows the structural similarity of these compounds.

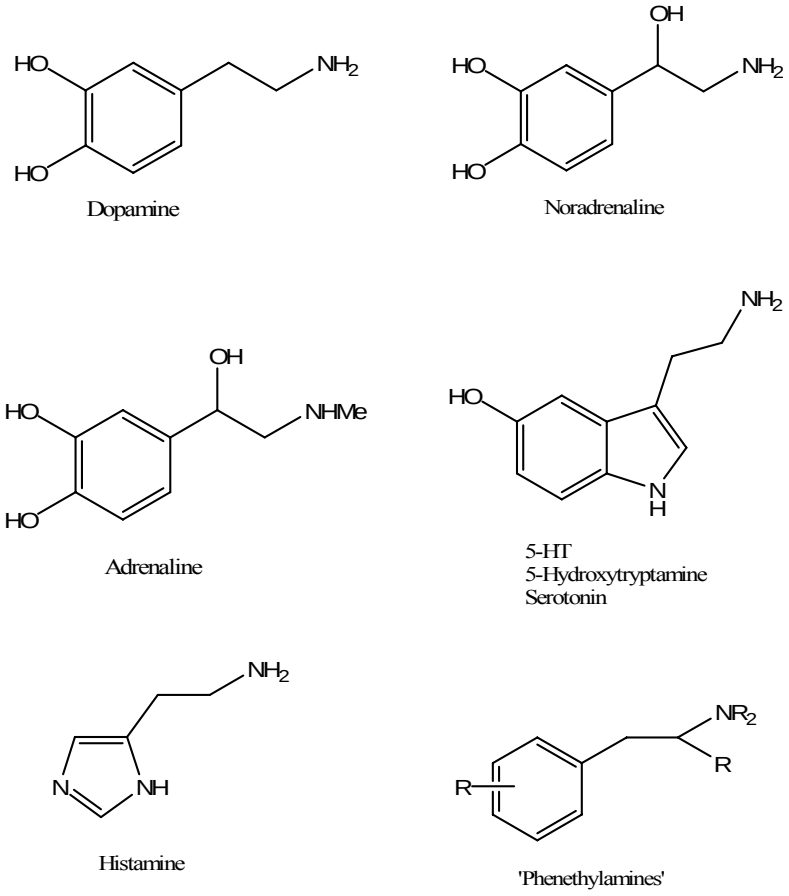


Figure 2: The structural similarities between phenethylamines and neurotransmitters

1.6.2. Recovery and Reinnervation

The degree of recovery and the time required for recovery to take place after administration of these drugs also appear to be dependant on dose, dosing regiment, specific biological markers examined, the brain region studied, the species and the specific methylenedioxy analogue (Hegadoren, 1999). For example, Battaglia found full recovery of the number of cortical 5-HT uptake sites in rats by 12 months, following an initial 90% loss of the sites after MDMA (20mg/kg twice a day for four days) (Battaglia, 1988). Ricuarte reported partial recovery of the levels of 5-HT in rats 5 months after a drug treatment regime of 20mg/kg two times a day for four days, then repeated one week later (Ricuarte, 1992). In contrast, monkeys examined 14 weeks and 18 months after multiple doses of MDMA continued to exhibit profound loss of 5-HT uptake sites and decreased levels of 5-HT in specific brain regions (Insel, 1989).

1.6.3 Implications for Humans

Despite the need to use high doses of MDMA or MDA to demonstrate significant and long lasting neurochemical and morphological changes, it would be foolish to dismiss the animal literature and conclude that these drugs have little neurotoxic risks to its users. It is well known that the rate and degree of metabolism of many drugs varies considerably among various species. In addition, many factors argue against a simple dose response relationship in the neurotoxic potential in humans. The lack of a dose

response relationship in the emergence of acute toxic reactions in humans suggests that other factors mediate individual responses to these drugs. An important factor that has been shown to enhance the neurotoxic capacity of MDMA in animal models is the ambient temperature which could have direct implications for dance clubs where most of the use of these drugs is reported. Core temperature is also important in terms of neurotoxic potential, as demonstrated by animal experiments where deficits in 5-HT parameters were blocked by drugs that prevented MDMA-induced hyperthermia (Hegadoren, 1999).

1.7 Abuse Potential

There is mounting epidemiological data that the methylenedioxy analogues of amphetamine are being heavily used, which has prompted some researchers to investigate whether the analogues should be considered drugs of abuse. The relative or absolute abuse potential of MDMA, MDA and MDEA has not been assessed in humans, but there are a number of studies in animals that would suggest that these drugs have at least moderate abuse potential in humans. Although there is controversy over the definition of "drugs of abuse" and members of this grouping can have widely differing mechanisms of action, the ability to act as a powerful operant reinforcer and to produce psychomotor stimulation are considered fundamental properties. Common behavioral paradigms used to study drugs of abuse in animals include exploratory locomotor activity, conditioned place preference, drug self-administration, intracranial self-stimulation and drug

discrimination (Hegadoren, 1999).

Some new epidemiological trends include 1) increased drug trafficking, more professional production and distribution that lead to: a higher availability and greater use of ecstasy worldwide, 2) change in consumption patterns: growing street market for home use, no longer a mere party drug for weekends, simultaneous use of other substances in order to modulate effects and 3) change in user profile: ecstasy consumers tend to be older, more "multi-drug" users. So called "safe use recommendations" (see www.dancesafe.org) contributed to the use of refreshing drinks and regular "chill outs" during rave weekends. This positive development is, however, counteracted by a growing number of substance mixtures in ecstasy pills and multi-drug use, which raises clinical concern due to possible drug interactions (Freudenmann, 2004).

1.8 Drug Discrimination Studies

In the drug discrimination model, an animal (usually a rat) is trained to perform one function (i.e. Pressing the left lever rather than the right one) when a drug is administered, and a different function if given a placebo. Drug-appropriate response occurs either after administration of the training dose of the original drug of when a new drug is perceived by the animal to be similar (Oberlender and Nichols, 1989) to the original drug.

Phenethylamines represent a very broad and interesting series of psychoactive substances that produce one or more of several different discriminative stimulus

effects in animals depending on the substitution pattern present in the molecule (Glennon, 1989). Psychoactive phenethylamines with demonstrated abuse potential can be divided into three categories: (a) hallucinogens (b) central stimulants and (c) a third type typified by MDMA based on different discrimination training profiles. Several examples of hallucinogens have been widely used as training drugs in drug discrimination studies. These include 5-methoxy-N, N-dimethyltryptamine (5-OMe DMT), (+) lysergic acid diethylamide (LSD), mescaline and 1-(2, 5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Glennon, 1991). Stimulus generalization occurs between these agents regardless of which are used as the training group. In the drug discrimination studies of phenethylamines, the most commonly used hallucinogenic training drug is DOM which has a phenethylamine structure (Fig1). DOM stimulus generalization is highly dependent upon location and number of methoxy groups present in the phenethylamine (Aalberg, 2002).

Amphetamine probably represents the prototypical central stimulant and most related stimulants possess a phenethylamine moiety (Fig 1). Although both optical isomers of amphetamine have been employed as training drugs in drug discrimination studies, (+) amphetamine is the more prevalent and the more potent of the two (Young and Glennon, 1986).

A third type of stimulus effect is that produced by MDMA. However, MDMA possesses some amphetamine like stimulant characteristics and therefore N-methyl-1-(4-methoxyphenyl)-2-aminopropane (PMMA) (Fig 1) has been proposed to be a “cleaner” MDMA-like agent than MDMA itself (Glennon et al., 1997). An MDMA

stimulus has been demonstrated to generalize to PMMA and it was found to be three times more potent than MDMA. PMMA differs somewhat from MDMA in that it is not recognized by its (+) amphetamine trained animals. Although the stimulus affects of PMMA are not well understood, it is obvious that they are different from those of either DOM or amphetamines (Glennon and Higgs, 1992). Also, although MDMA is not usually considered to be hallucinogenic, it partially substitutes for a DOM-stimulus and there is at least one documented report where high doses of MDMA have produced visual hallucinations (McCann and Ricuarte, 1991). Both optical isomers are able to produce MDMA-like effects and S (+) MDMA is more potent than R (-) MDMA (Nichols and Oberlender, 1989).

Neither optical isomer of MDEA resulted in amphetamine or DOM-like stimulus generalization. Racemic MDEA is capable of producing MDMA-like stimulus effects in MDMA-trained animals (Glennon and Misenheimer, 1989).

MBDB can also generalize to MDMA and/or PMMA. Stimulus generalization occurs to S (+) MBDB and R (-) MBDB. These compounds failed to substitute for (+) amphetamine in tests of stimulus generalization using (+) amphetamine trained animals. This suggests that MBDB, although capable of producing MDMA-like stimulus effects, are probably best represented as PMMA-like (Rangisetty et al., 2001).

The compounds of interest in this research topic are 12 phenethylamines, three 3,4-methylenedioxyphenethylamines, three 2,3-methylenedioxyphenethylamines, three 4-methoxy,2-methyl-phenethylamines, and three 4-methoxy, 3-methyl-phenethylamines. The last six compounds are PMMAs which is also an interesting point to make since

MDMA, MDEA and MBDB all generalize to PMMAs which are “cleaner” drugs.

1.9 Current Recreation Use

Data on the current prevalence of use of these drugs is sparse, although there are survey results that suggest that they continue to experience increasingly wider popularity. Although the most common drug was being made during this period (1978-1981) was methamphetamine (378 out of 751 labs), 16 illegal labs were found to be specifically making MDA (Frank, 1983). Kirsch reported that approximately 10,000 doses of MDMA were being distributed on a monthly basis by a single lab in California in 1976 and that number increased to 30,000 by 1984 and to 500,000 just prior to MDA and MDMA being placed on Schedule I of the Controlled Substances Act in 1985 (Kirsch, 1986). In 1987, out of 369 college undergraduates interviewed at a major American university, 39% of the students admitted to having used MDMA at least once during the preceding year (Hegadoren, 1999). A random 1990 survey of illicit drug use by undergraduates at a British university revealed that 24% of those surveyed had used MDMA. An opinion poll conducted in Britain reported that 31% of people between the ages of 16 and 25 had taken MDMA, mostly at dance clubs (Harris Research Center, 1992). Over 90% of participants (n=135) in a Glasgow dance scene had tried MDMA at least once (Forsyth, 1996). Other epidemiological studies involving 15 and 16 year old students have reported that 5-9% of them had used MDMA (Texas School Survey, 1997).

The production of MDMA continues to be concentrated in Western Europe,

particularly in the Netherlands and Belgium with an increase in production observed in Eastern Europe (Interpol, 2005). MDMA has been seized mainly in Western and Central Europe (54%) and Oceania (26%). The majority of precursors required to manufacture MDMA originate in China. Globally the volume of MDMA seizures has declined to the point where there are now 37% less than in the peak year in 2002. This may reflect declining production rates in European centers. In the United States there are also indications of declining rates of use of MDMA (NDIC, 2005), contrasting with the strong rise in seizures in Oceania and East and South East Asia.

A national study of MDMA users shows that the availability of MDMA remains stable. Sixty- one percent of these surveyed considered MDMA to be 'very easy' to obtain and 35% considered it to be 'easy'. Over 2/3 (68%) of the national sample reported that they typically used more than one tablet (Dunn, 2005). The majority of users were also likely to use other drugs with MDMA.

2. OBJECTIVES AND RATIONALE

The primary objective of this project is to evaluate the GC-MS properties of a series of twelve regioisomeric and isobaric ring substituted phenethylamines of molecular weight 207 and expected major fragment ions of m/z 72. Three regioisomeric 3,4-methylenedioxyphenethylamines having equal molecular weight and major mass spectral fragments of equivalent mass have been reported as components of clandestine drug samples in recent years. These drugs of abuse (MDEA, MDMMA and MBDB) are a subset of a total of six methylenedioxyphenethylamines of molecular weight 207 yielding regioisomeric fragment ions of equal mass (m/z 72 and 135/136) in the electron impact mass spectra. Some of these compounds may be pharmacologically inactive and others' pharmacological properties are unknown, but all have the strong possibility to be identified as MDEA, MDMMA and/or MBDB by mass spectrometry. Additionally this project will evaluate six isobaric methoxy-methylphenethylamines of the same molecular weight and expected mass spectral fragmentation products of equal mass. The methoxy-methylphenethylamines selected for evaluation in this project are 4-methoxy-3-methyl and 4-methoxy-2-methyl-substituted phenyl rings since these precursor aldehydes are commercially available and are the more likely candidates for designer drug exploration. The structures for all twelve compounds included in this project are in Figure 3.

Regioisomer differentiation is a significant issue in forensic drug chemistry and has been addressed in a number of drug categories (Clark et al., 1995, Clark et al., 1996, Aalberg et al., 2000). The ability to distinguish between these regioisomeric substances directly increases the specificity of the analysis for the target drug of abuse. The mass spectrum is usually the confirmatory piece of evidence for the identification of drugs of abuse in forensic laboratories. There can be other compounds with essentially equal mass spectra as the drugs of abuse. For major drugs of abuse such as the amphetamines and MDMAs, there are many positional isomers (regioisomers) in the alkyl side chain or in the aromatic ring substitution pattern which can yield nearly an identical mass spectrum. While nuclear magnetic resonance (NMR) can be a useful method for differentiation of these regioisomers, it is not a technique with direct application for all areas of forensic drug chemistry (including biological samples) and not always readily available in most laboratories. Thus, the analysis of these "street drug" samples must depend heavily on chromatographic methods as well as mass spectrometry.

When other compounds exist with the ability to produce nearly identical mass spectra as the drug of interest, the identification by gas chromatography-mass spectrometry (GC-MS) must focus on the ability of the chromatographic system to separate the "non-drug" regioisomers from the drug of interest. The regioisomers that co-elute with the drugs of interest in chromatographic separations could be mistaken for the drug of abuse itself. Without the appropriate standards, thorough method validation is not possible, and thus co-elution of the regioisomer (the non-drug) with the drug would remain a possibility.

The targets of this study were all six methylenedioxyphenethylamines (see Figure 3) with molecular weight 207 and the potential to produce mass spectrum with major fragment ions at m/z 72 for the imine and m/z 135/136 for the benzyl fragment. Additionally this project will evaluate six isobaric methoxy-methylphenethylamines of the same molecular weight and expected mass spectral fragmentation products of equal mass. Therefore, analysis of the underivatized regioisomers by electron ionization mass spectrometry alone may not provide significant data for the specific differentiation of one of these regioisomers to the exclusion of all others. The specific identification must be based on a combination of mass spectral data as well as chromatographic resolution of the regioisomeric and isobaric substances.

Mass spectrometry was used to evaluate the compounds for confirmation of their similar fragmentation ions. The derivatization procedures were used to attempt to individualize the spectra of the compounds. The gas chromatogram was used to attempt to separate the parent amines and the derivatives using various stationary phases and temperature programs.

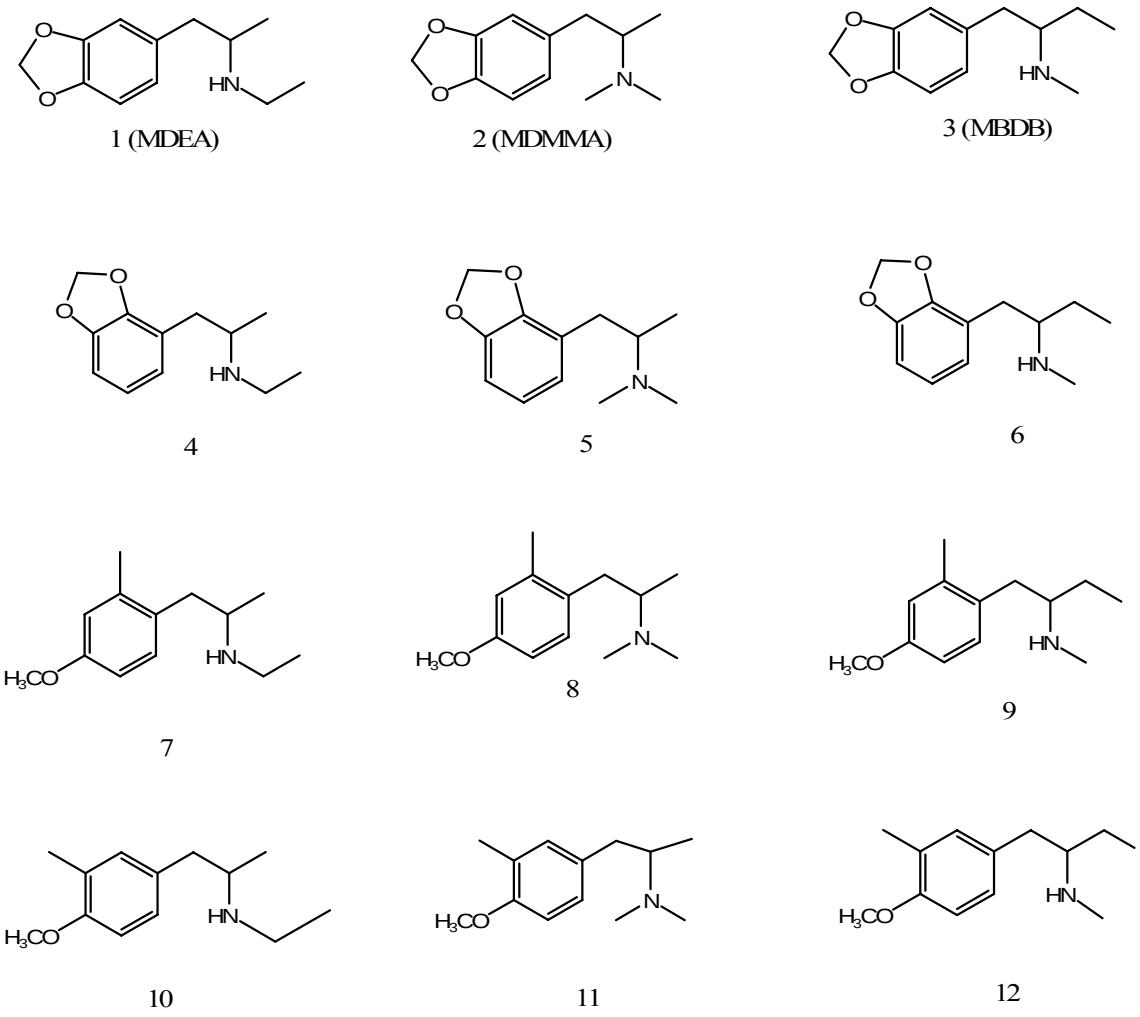


Figure 3: Structures of all 12 amines included in this study.

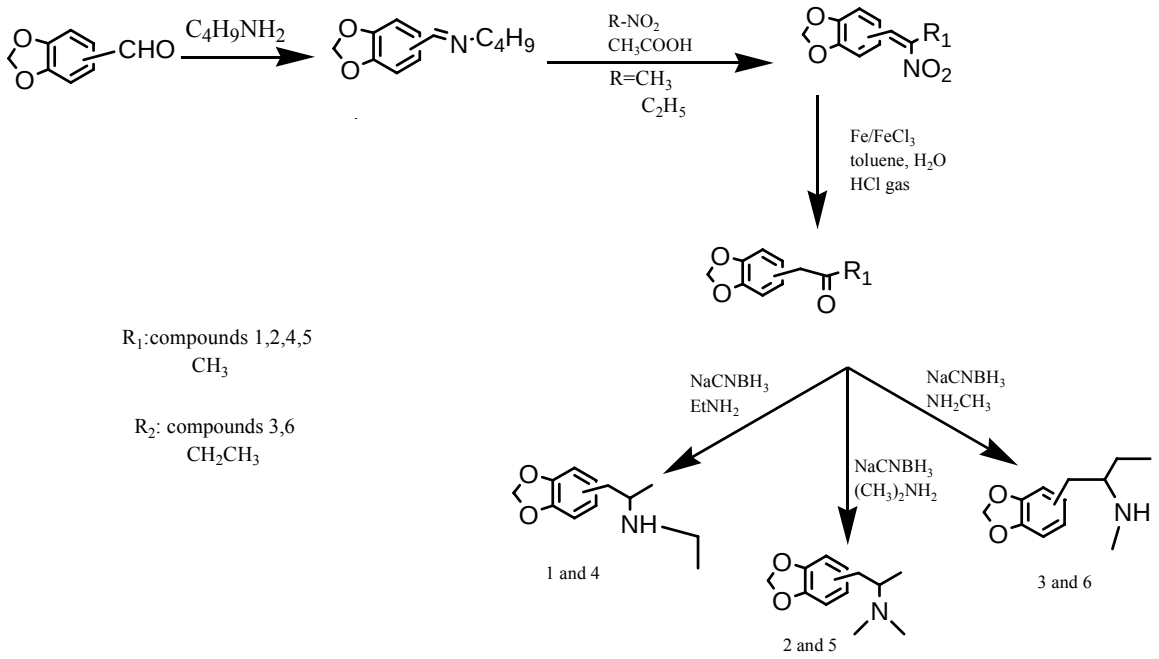
3. RESULTS AND DISCUSSION

3.1 Preparation of the regioisomers

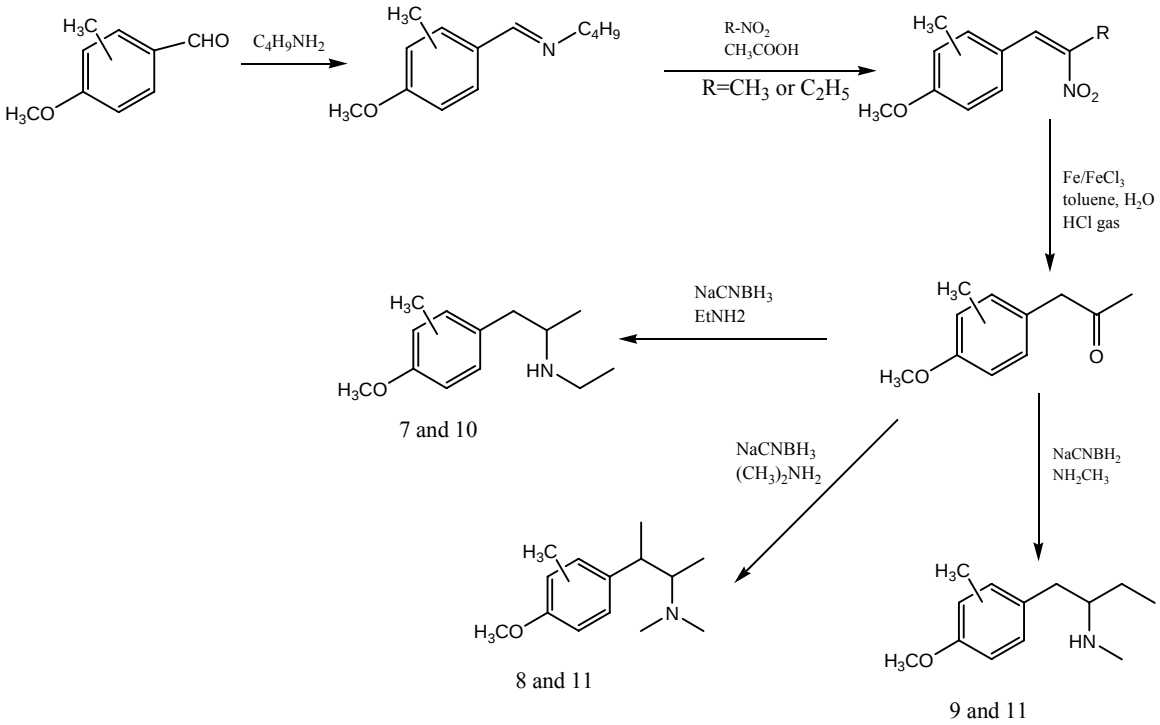
The general methods for the preparation of the twelve regioisomeric (2, 3- and 3, 4-methylenedioxy- and 4-methoxy, 3-methyl and 4-methoxy, 2-methyl-) phenethylamines are outlined in Scheme 1 and Scheme 2 and the structures of all twelve compounds prepared and evaluated in this study are shown in Figure 3. The synthesis for these compounds begins with 2, 3- and 3, 4-methylenedioxybenzaldehyde (piperonal) and 4-methoxy, 3-methyl and 4-methoxy, 2-methyl benzaldehyde as starting materials. The method of preparation for 2, 3-methylenedioxybenzaldehyde has been described previously from our laboratory and piperonal and the methoxt-methylbenzaldehydes are (Aalberg, 2000) commercially available materials.

Synthesis begins by combining the appropriate benzaldehyde with n-butyl amine and benzene and allowing the mixture to reflux overnight with removal of water via a Dean-Stark trap. The resulting n-butylimine is allowed to react with the appropriate nitroalkane (nitropropane or nitroethane). Reduction of the resulting 2-nitroalkene is accomplished with iron and ferric chloride in refluxing toluene. Initial reduction occurs at the nitro group to yield the enamine which upon hydrolysis yields the desired ketone.

The ketones were purified by Kugelrohr distillation then reacted with the appropriate amine hydrochloride (dimethyl, methyl or ethyl) and sodium cyanoborohydride in methanol. This reductive amination yields the desired amines which were converted into the hydrochloride salts using gaseous hydrochloric acid. Combination of the appropriate aldehyde, nitroalkane and alkyl amine allowed for the synthesis of the twelve amines shown in Figure 3.



Scheme 1: General synthesis of compounds 1-6.



Scheme 2: General synthesis of compounds 7-12.

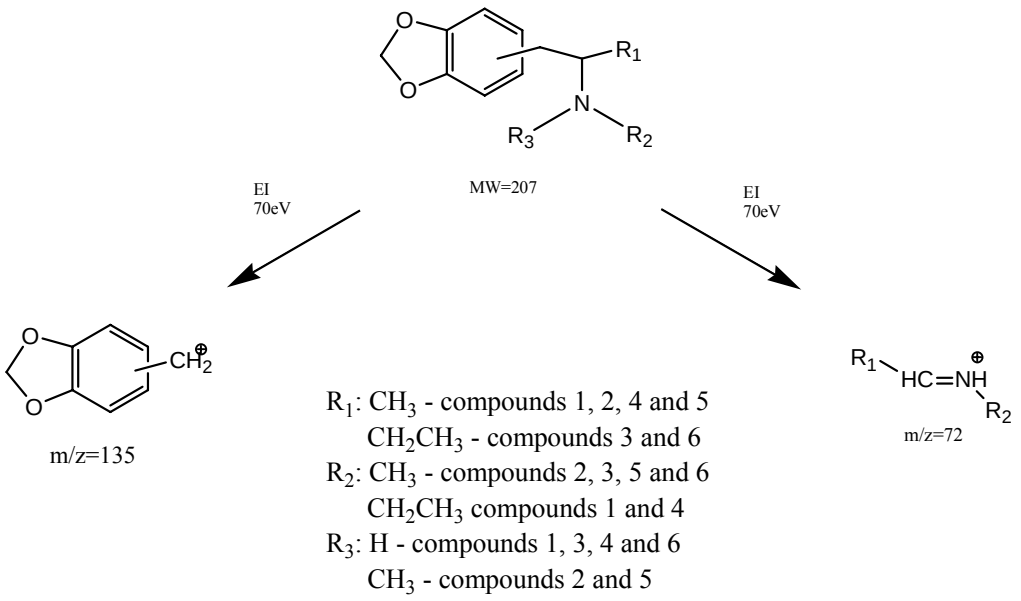
3.2 Mass Spectral Studies of the Regioisomers

3.2.1 Mass spectra of the amines

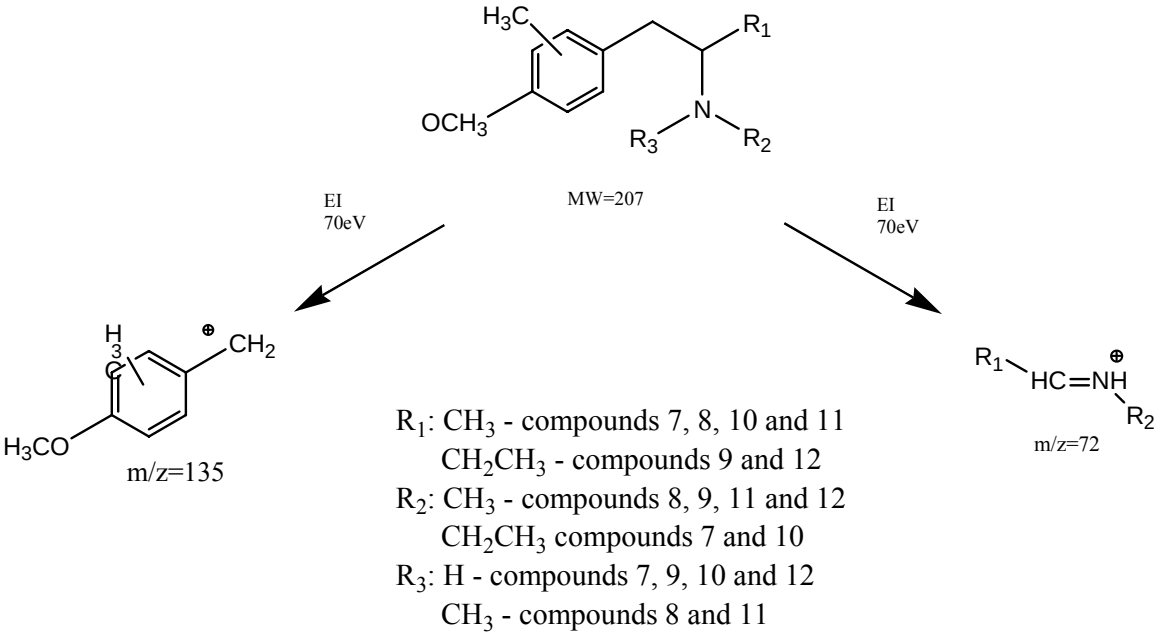
Figure 4 shows the EI mass spectra for the twelve regioisomeric phenethylamines included in this study. Mass spectrometry is the primary method for confirming the identity of drugs of abuse in forensic samples. These spectra indicate that very little structural information is available for differentiation among these regioisomers because the major fragment ions occur at equal masses. The mass spectra are characterized by a base peak formed by an alpha cleavage reaction involving the carbon-carbon

bond of the ethyl linkage between the aromatic ring and the amine. The alpha cleavage reaction yields the regioisomeric butylimine fragments at m/z 72 and the 2, 3- , 3, 4-methylenedioxybenzyl and the 4-methoxy, 3-methyl and 4-methoxy, 2-methyl benzyl cation fragment at m/z135 and the radical cation at m/z 136. The fragmentation patterns and the structures of the fragment ions are shown in Scheme 3. These regioisomers all have essentially the same mass spectra and their specific identification by mass spectrometry represents a significant challenge for analytical drug chemistry. This is especially significant in this series of amines since compounds 1, 2 and 3 have all been reported in recent years as components of clandestine drug samples (Hegadoren et al., 1999 and Aalberg et al., 2003).

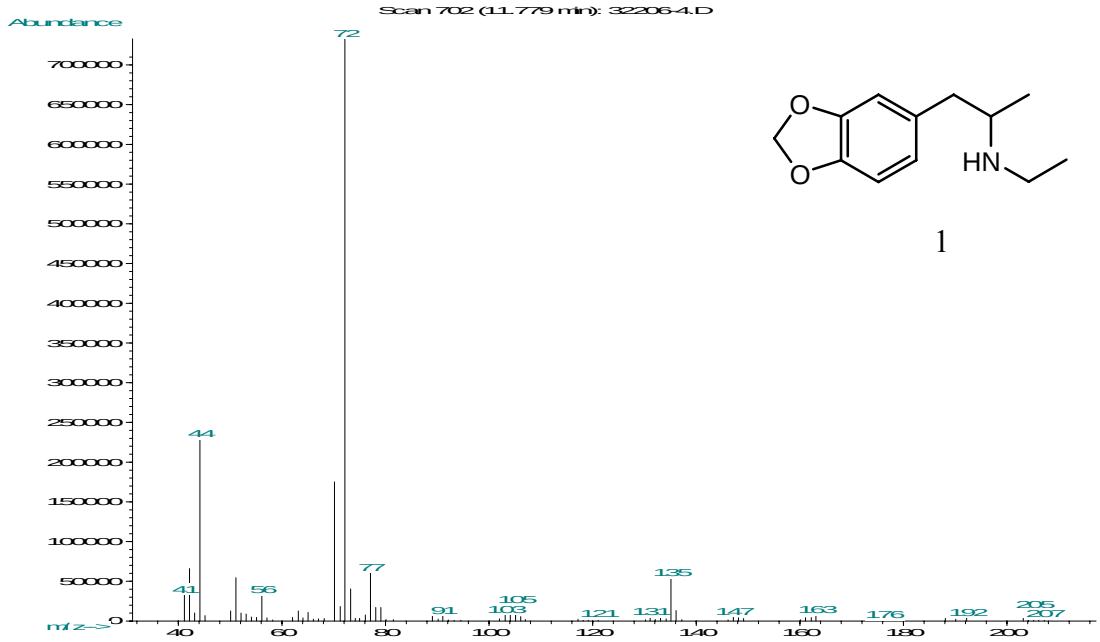
The m/z 44 ion in the spectra for compounds 1, 4, 7 and 10 occurs from the loss of ethylene from the N-ethyl group of the base peak (m/z 72). The m/z 44 ion is a more prominent peak in the methylenedioxy-substituted amines (compounds 1 and 4) than in the methoxy-methyl substituted amines (compounds 7 and 10) However this low mass ion is present in the mass spectra for many substituted phenethylamines as well as other compounds and does not provide enough diagnostic information to individualize these mass spectra. These spectra also contain ions of much lower relative abundances which do not provide sufficient information for differentiation among theses regioisomers.



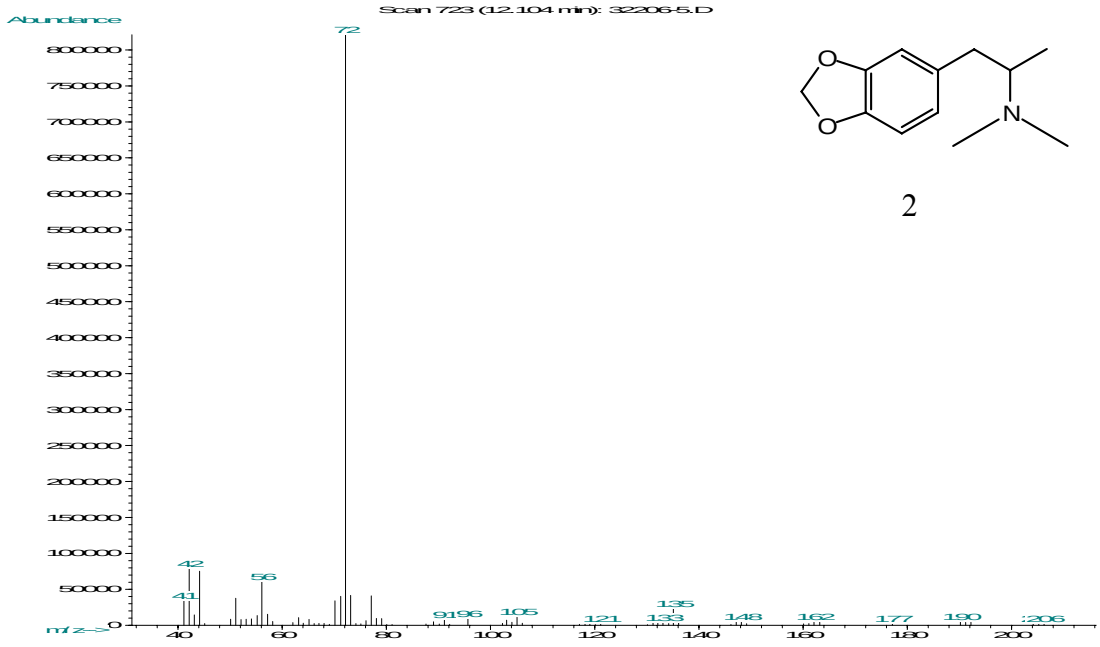
Scheme 3: General mass spectral fragmentation for the underivatized amines (1-6) included in this study.



Scheme 4: General mass spectral fragmentation for the underivatized amines (7-12) included in this study.


Scan 702 (11.779 min): 32206-4.D
Abundance
m/z-->
72
44
77
41
56
135
91
105
103
121
131
147
163
176
192
205
207
O
O
HN

1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Abundance
m/z-->
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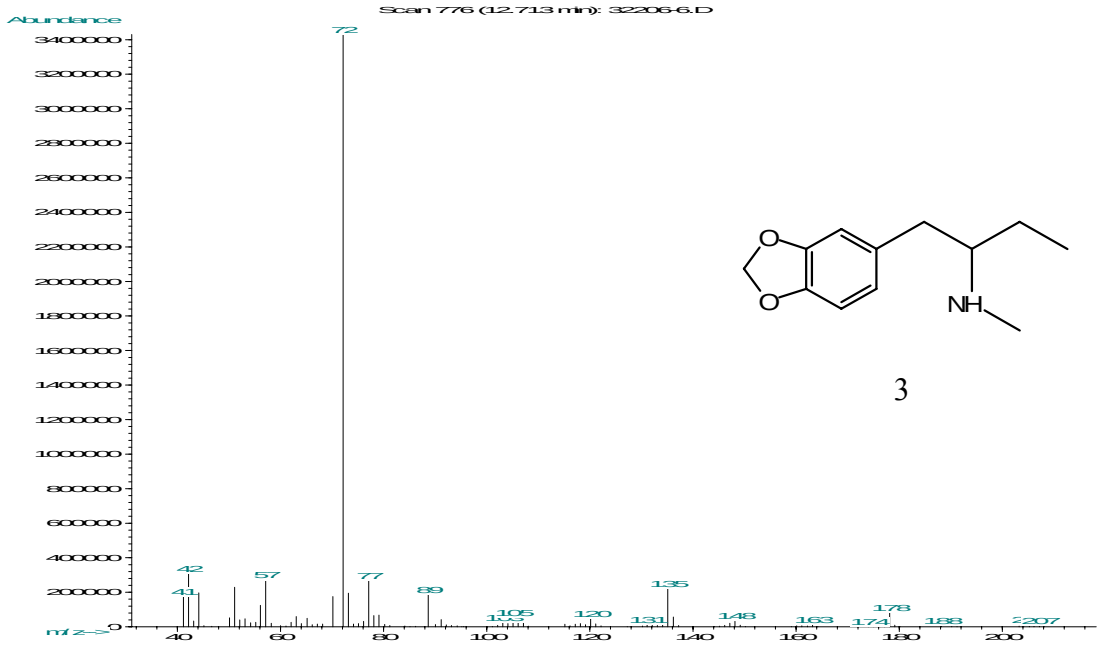
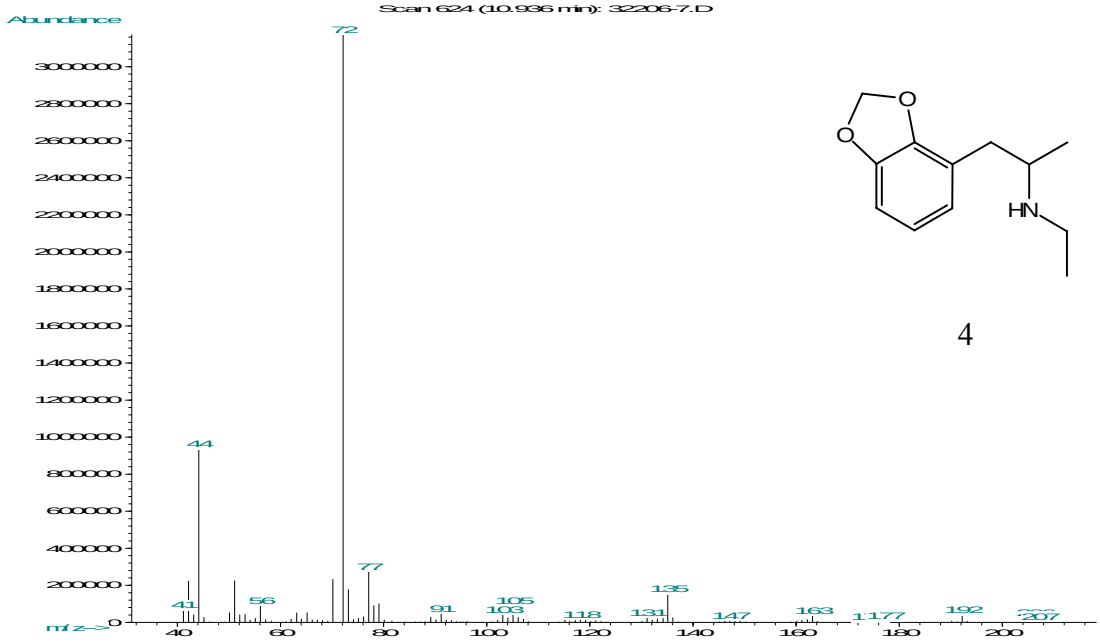
42
41
56
91
96
105
121
135
133
148
162
177
190
206
O
O
N

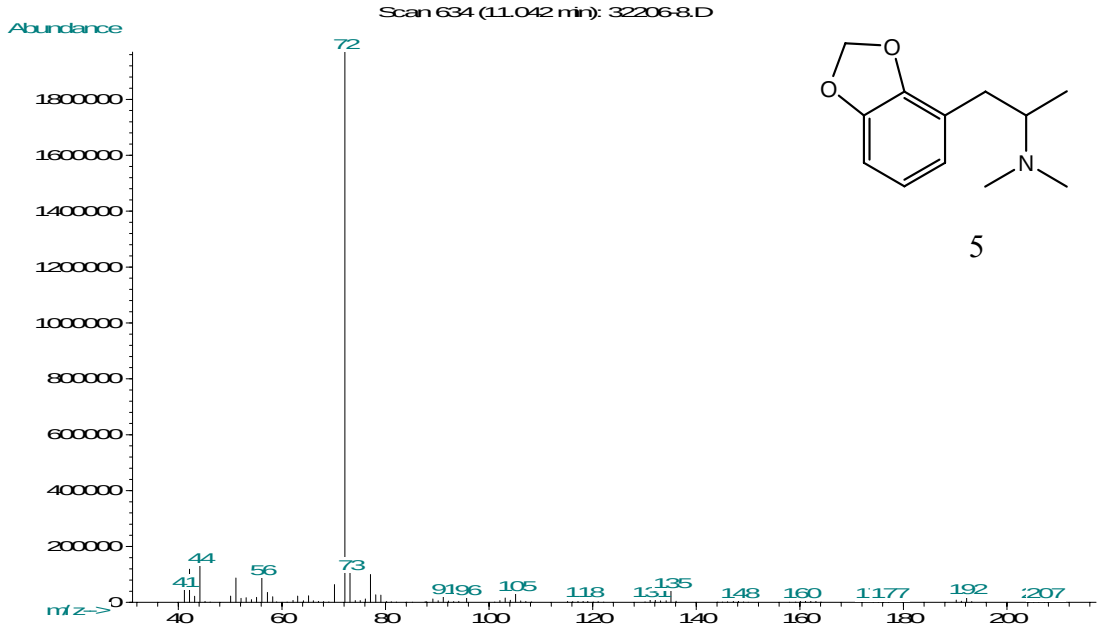
2


Scan 776 (12.713 min): 32206-6.D
Abundance
m/z-->
72
42
41
57
77
89
105
135
148
163
178
207
O
O
NH

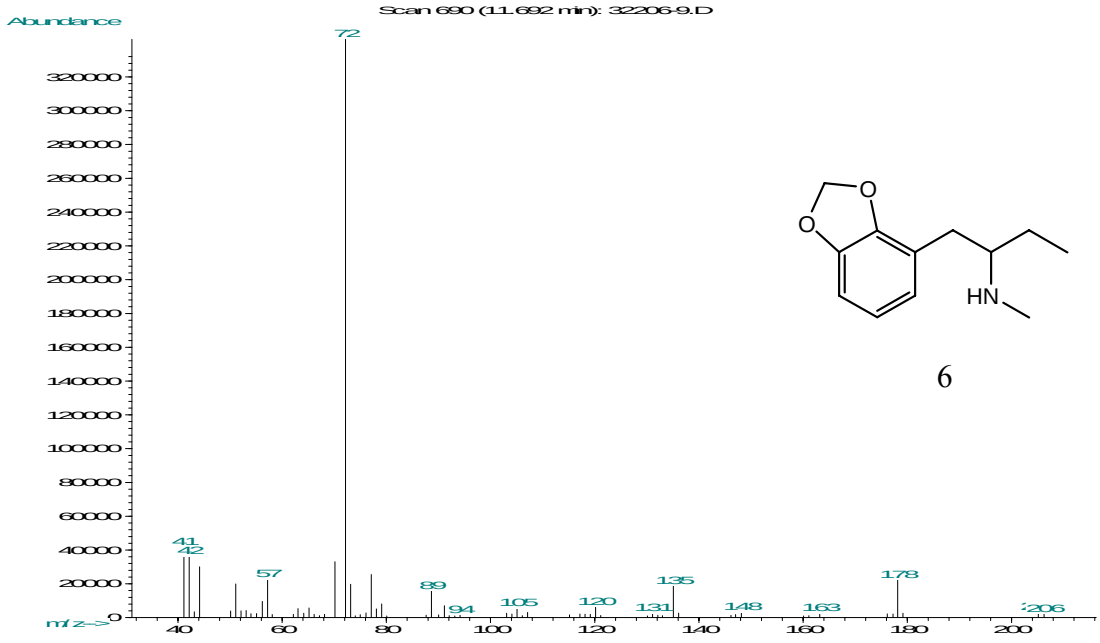
3


Scan 624 (10.936 min): 32206-7.D
Abundance
m/z-->
72
44
41
56
77
91
105
103
118
131
135
147
163
177
192
207
O
O
HN

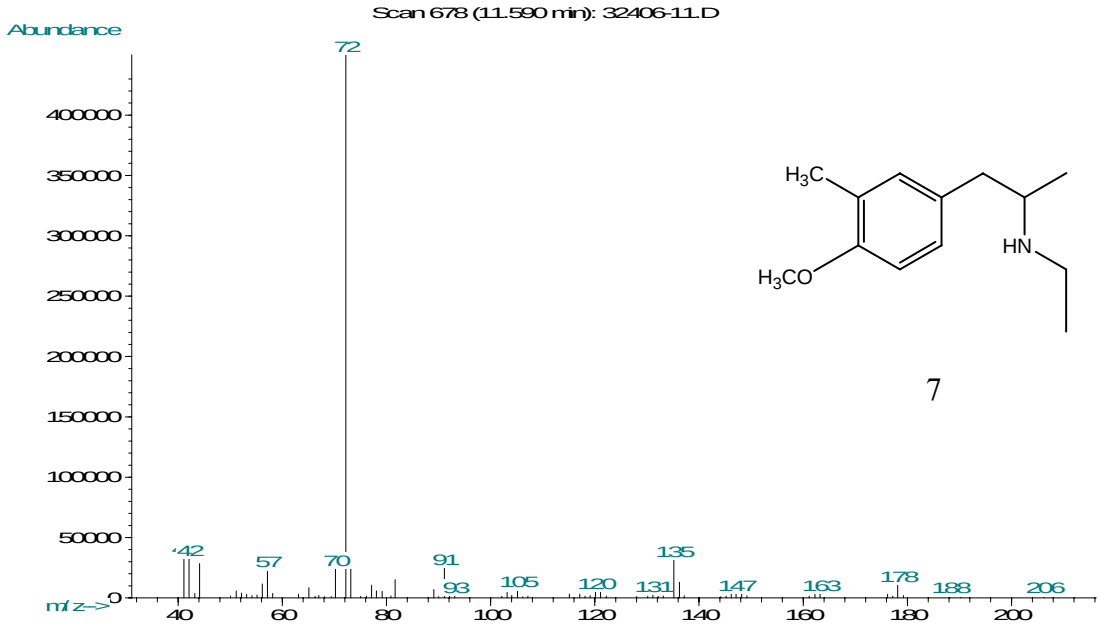
4


Scan 634 (11.042 min): 32206-8.D
Abundance
m/z-->
72
44
41
56
73
91
96
105
118
131
135
148
160
177
192
207
O
O
N

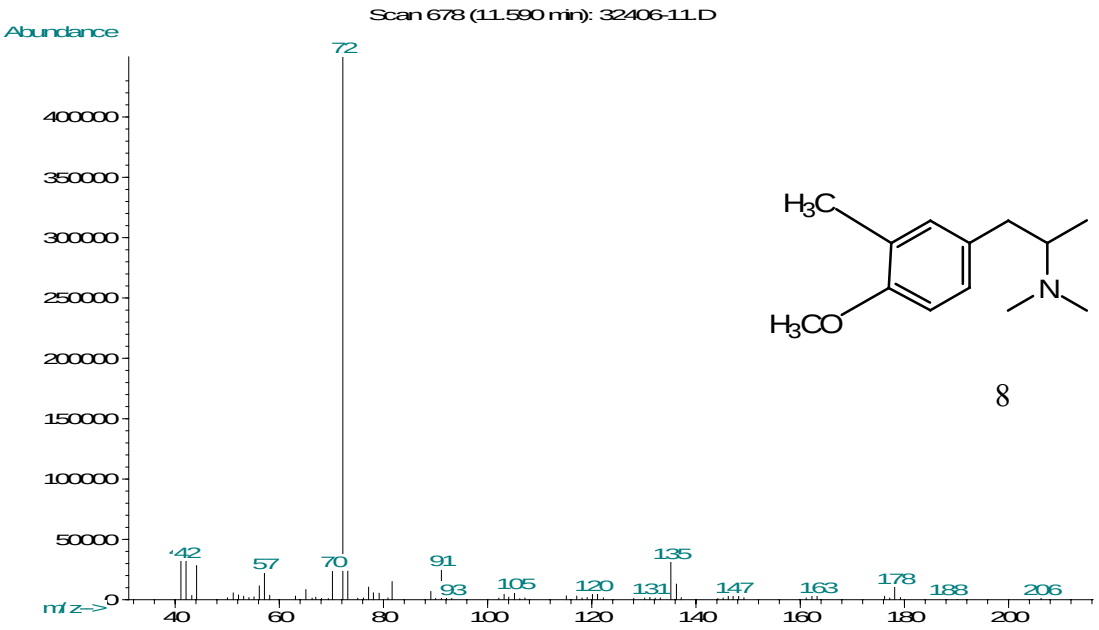
5


Scan 690 (11.692 min): 32206-9.D
Abundance
m/z-->
72
41
42
57
89
94
105
120
131
135
148
163
178
206
O
O
HN

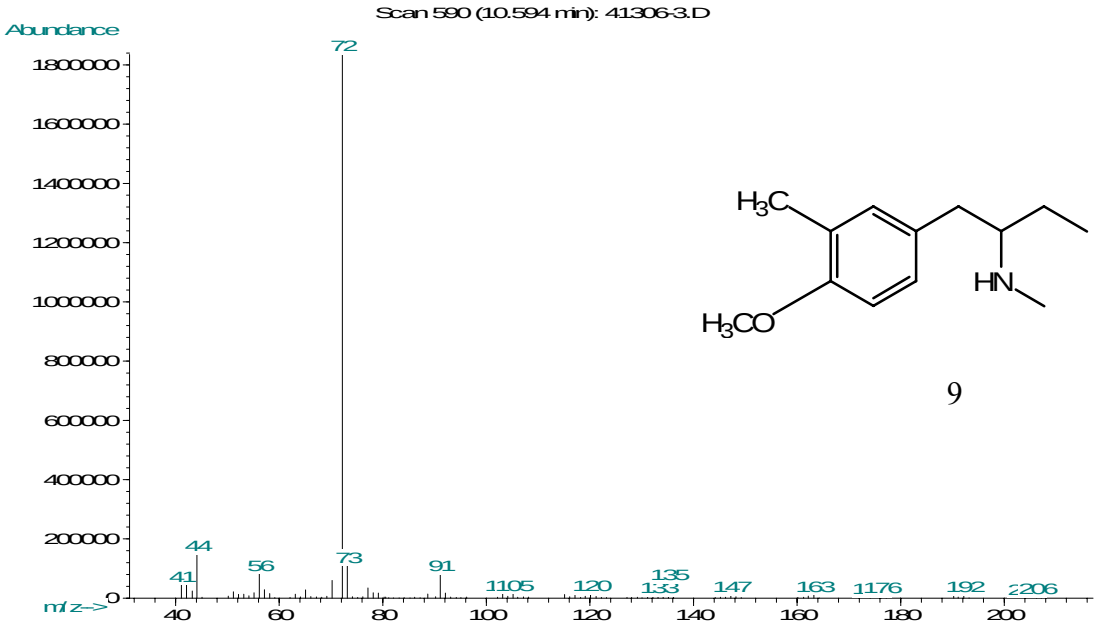
6


Scan 678 (11.590 min): 32406-11.D
Abundance
72
400000
350000
300000
250000
200000
150000
100000
50000
0
42
57
70
91
93
105
120
131
135
147
163
178
188
206
m/z-->
40
60
80
100
120
140
160
180
200
H3C
H3CO
HN

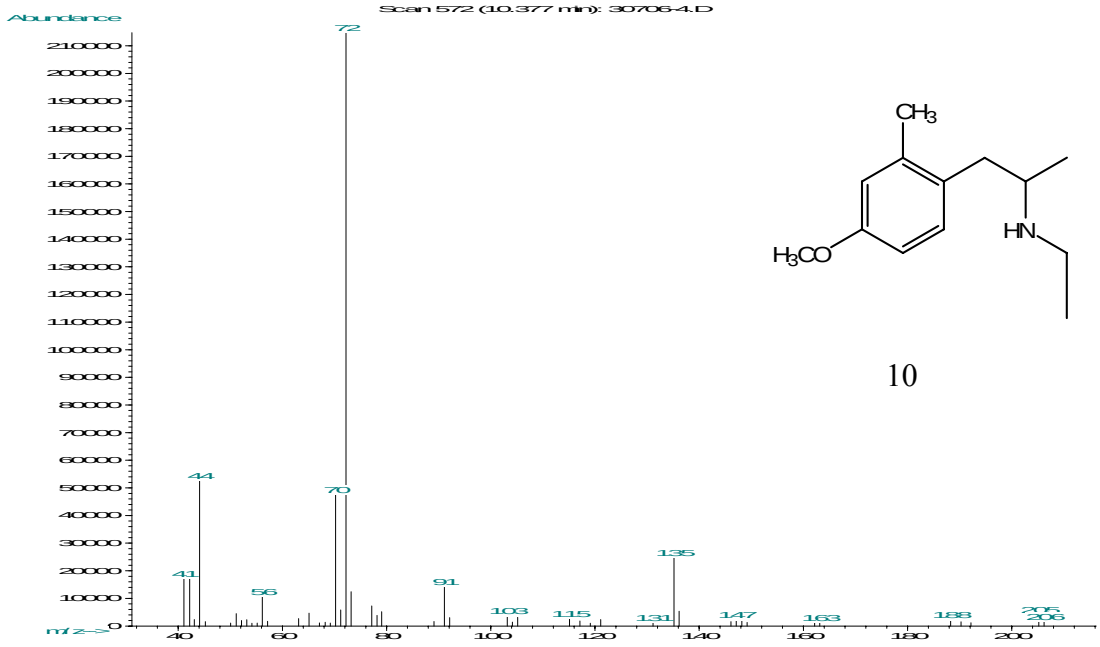
7


Scan 678 (11.590 min): 32406-11.D
Abundance
72
400000
350000
300000
250000
200000
150000
100000
50000
0
42
57
70
91
93
105
120
131
135
147
163
178
188
206
m/z-->
40
60
80
100
120
140
160
180
200
H3C
H3CO
N

8


Scan 590 (10.594 min): 41306-3.D
Abundance
m/z-->
72
44
56
73
91
41
1105
120
135
133
147
163
1176
192
2206
H3C
HN
H3CO

9


Scan 572 (10.377 min): 30706-4.D
Abundance
m/z-->
72
44
70
41
56
91
103
115
131
135
147
163
188
205
206
CH3
HN
H3CO

10

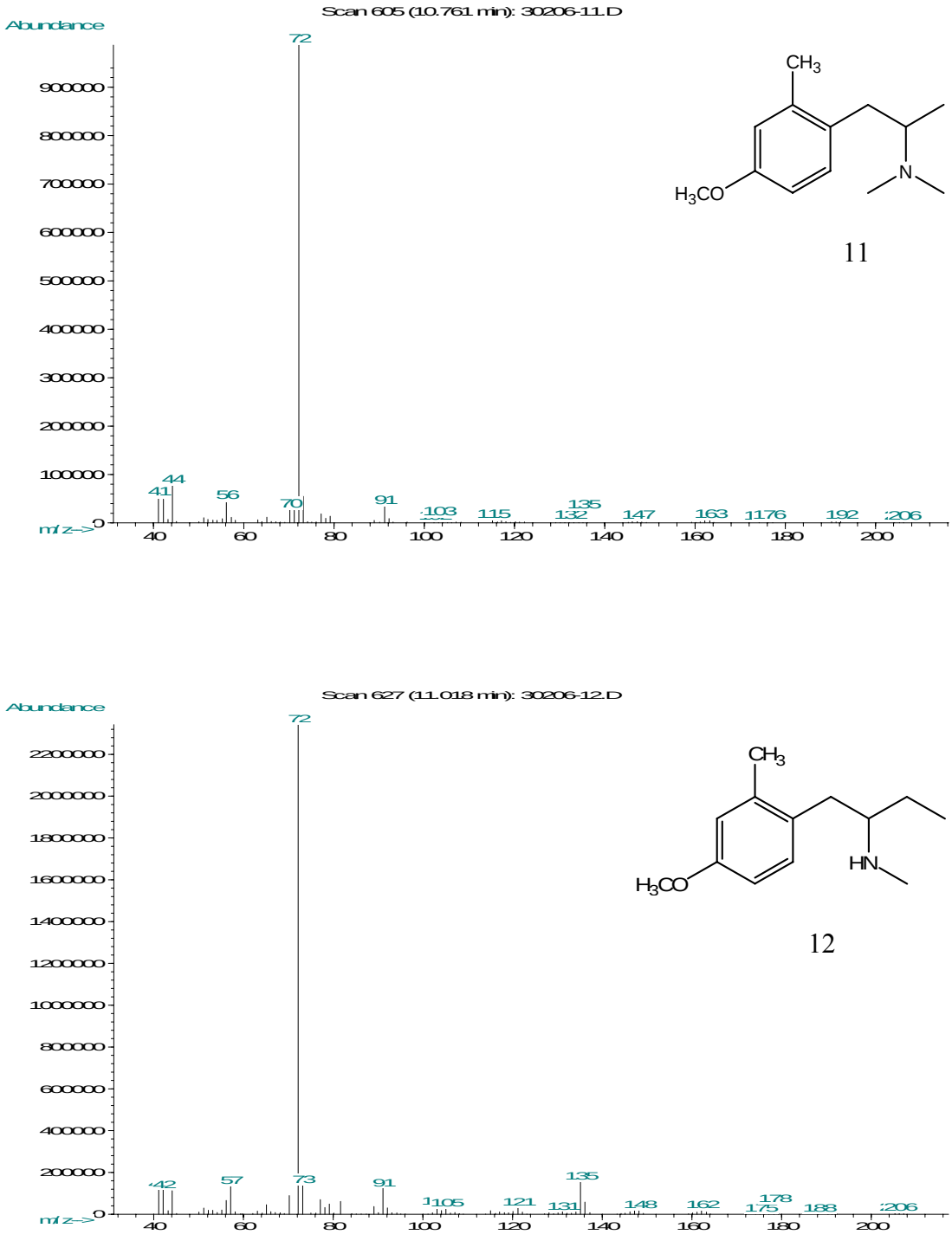


Figure 4 : Mass spectra of the 12 underivatized amines included in this study

3.2.2 Mass Spectra of the Perfluoroacyl- derivatives

In the next part of this study, various perfluoroacylated derivatives of the phenethylamines were prepared and evaluated in an effort to individualize the mass spectra as well as maintain or improve chromatographic resolution. Acylation of the amines greatly lowers the basicity of the nitrogen and this often allows other fragmentation pathways to play a more prominent role in the mass spectrum (Awad et al., 2005). The pentafluoroproprionyl (PFPA) and heptafluorobutryl (HFBA) derivatives of the twelve amines were evaluated for their ability to individualize the mass spectra and provide unique ions for compound identification and differentiation. The mass spectra for the eight pentafluoroproprionyl and the eight heptafluorobutryl amides of the secondary amines (the tertiary amines do not form stable acylation products) are shown in Figures 5 and 6. For the PFPA and HFBA derivatives the spectra show a common base peak at 218 and 268 which corresponds to the loss of 135 mass units from the molecular ions at 353 and 403. The ions at m/z 218 and 268 are the PFPA and HFBA imine species likely formed form the alpha cleavage reaction of the amide nitrogen. Thus the m/z 218 and 268 ions in the mass spectrum of the PFPA and HFBA amides are analogous to the m/z 72 in the underivatized species. The fragmentation pattern and structures of the fragment ions for the derivatized compounds are shown in Scheme 5 and Scheme 6.

There are two major diagnostic pathways in the mass spectrum of the PFPA and HFBA derivatives which allows differentiation of the side chain regioisomers. The first of these is the alkene fragment observed at m/z 162 and m/z 176; these ions occur in

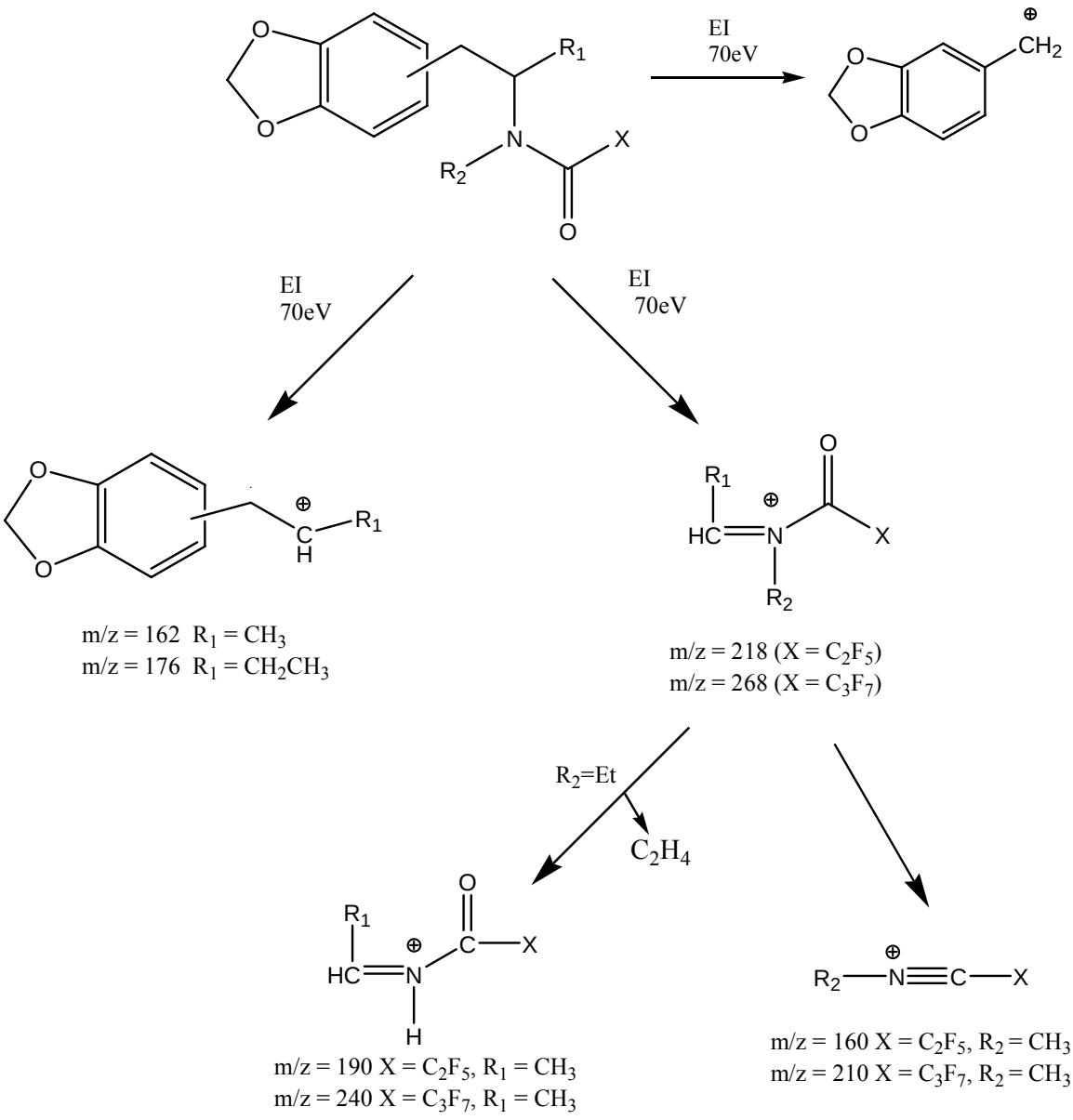
the spectrafor both the PFPA and HFBA derivatives indicating the perfluoroacyl moiety is not a component of these ions. The m/z 162 ion occurs in the mass spectrum for the PFPA and HFBA derivatives of compounds 1, 4, 7 and 10, the N-ethyl regioisomers. This alkene fragment is the radical cation (see Scheme 3) resulting from cleavage of the bond between nitrogen and the alkyl carbon of the hydrocarbon side chain. This bond cleavage occurs following an initial hydrogen rearrangement yielding the radical cation species at m/z 162. Thus, the m/z 162 ion is indicative of the C3 alkene chain attached to the aromatic ring. The analogous fragmentation pathway for the PFPA and HFBA derivatives of compounds 3, 6, 9 and 12 yields a fragment ion at m/z 176 indicating a C4 side chain attached directly to the aromatic ring.

The second diagnostic fragmentation pathway does contain the perfluoracyl group or a portion of the perfluoroacyl groups and therefore appears at different masses for the PFPA and HFBA derivatives. The loss of mass 28 (the N-ethyl group lost as ethylene) from the base peak in the mass spectrum of compounds 1, 4, 7 and 10 appears at m/z 190 and m/z 240 for the PFPA and HFBA derivatives, respectively. While these ions can occur in the C-ethyl regioisomers (compounds 3, 6, 9 and 12) the loss of ethylene from these derivatives is a much less prominent ion. When the side chain consists of an N-methyl group (compounds 3, 6, 9 and 12) the base peak undergoes a rearrangement fragmentation reaction to yield the m/z 160 and m/z 240 for the PFPA and HFBA derivatives respectively (see Scheme 3). The structure of this ion has been confirmed by deuterium labeling experiments in other series of N-methyl-phenethylamines (Clark et al., 1995 and Awad et al., 2005). Thus, the mass spectra of these perfluoroacyl

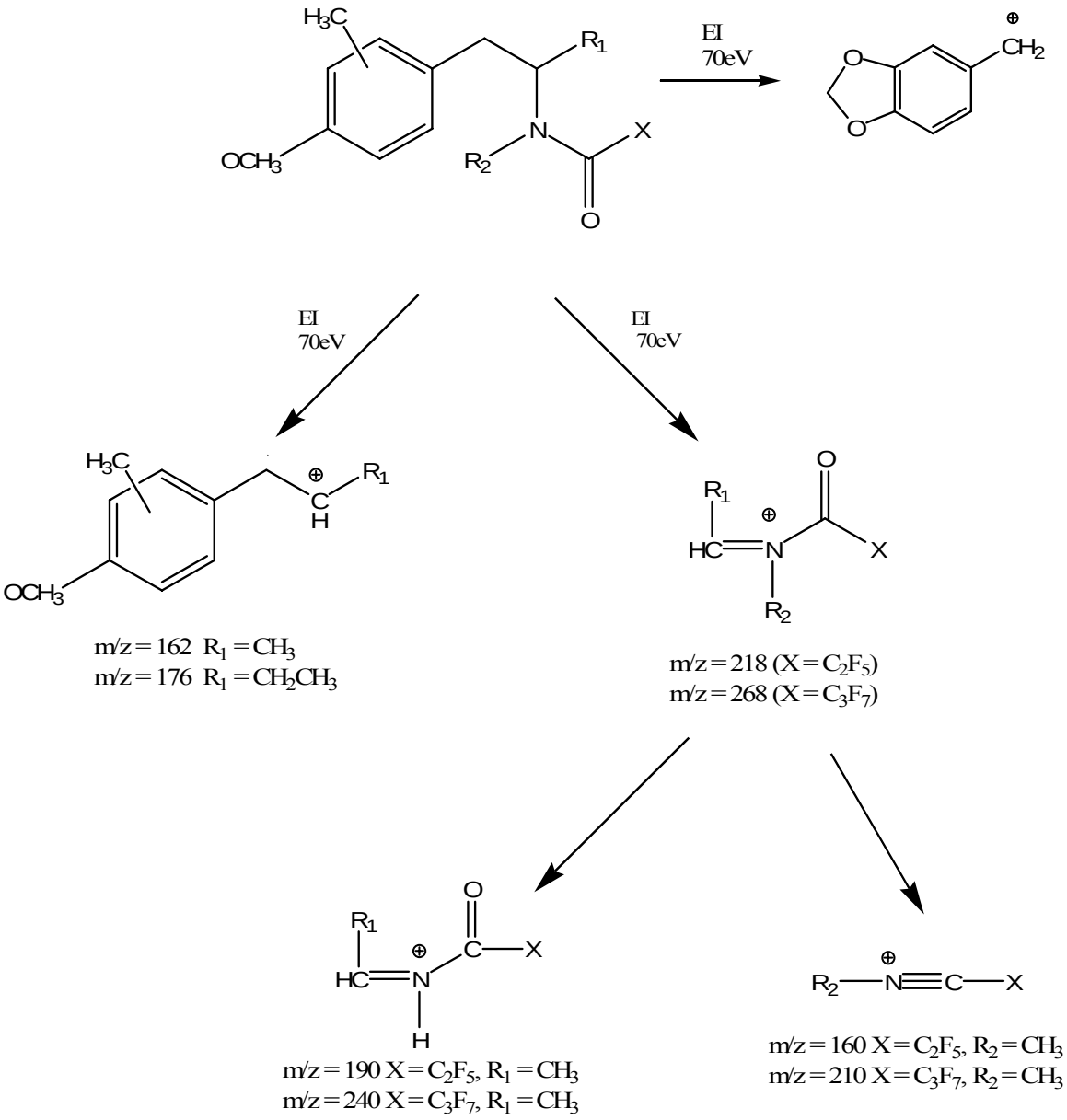
derivatives allow identification of the carbon side chain attached directly to the aromatic ring and identification of the alkyl group bonded to nitrogen.

A comparison of the PFPA derivatives of MDEA and MBDB in Figure 5 illustrates the use of these diagnostic ions to distinguish between these compounds. The mass spectrum for the PFPA derivatives of MDEA shows a significant fragment and m/z 162 identifying the C3 carbon chain attatched directly to the aromatic ring and the m/z 190 ion representing the loss of 28 from the base peak and identifying the alkyl group on nitrogen as the ethyl moiety. The spectrum for the PFPA of MBDB shows ions at m/z 176 for the C4 carbon chain and the m/z 160 ion identifying the N-alkyl group as methyl. These same ions occur in the PFPA derivatives of other amines in Figure 5 allowing clear differentiation between these regioisomeric amines. These amines produced essentially identical mass spectra in the underivatized form. Analogous ions are present in the spectra of the HFBA derivatives in Figure 6.

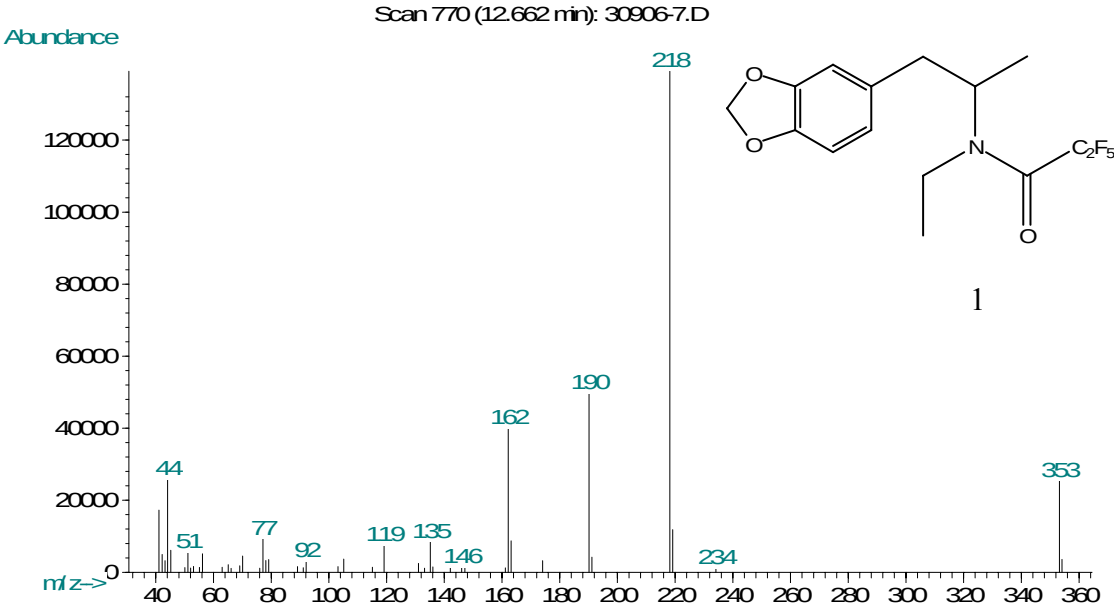
A comparison of the methylenedioxy-substituted compounds 1, 3, 4 and 6 with the methoxy-methyl amines 7, 9, 10 and 12 shows a significant ion at m/z 135 for the methoxy-methyl compounds. The more abundant m/z 135 ion appears to allow differentiation between the two sets of ring substituents, 2, 3- and 3, 4-methylenedioxy verses 4-methoxy-3-methyl / 4-methoxy-2-methyl. The two benzylic cations have an isobaric relationship, methylenedioxybenzyl and methoxy-methylbenzyl and each occur at m/z 135.



Scheme 5: General fragmentation of the PFPA and HFBA derivatives of compounds of the methylenedioxy substituted phenethylamines (compounds 1-6).



Scheme 6: General fragmentation pattern for the PFPA and HFBA derivatives of the methoxy-methyl substituted phenethylamines (compounds 7-12).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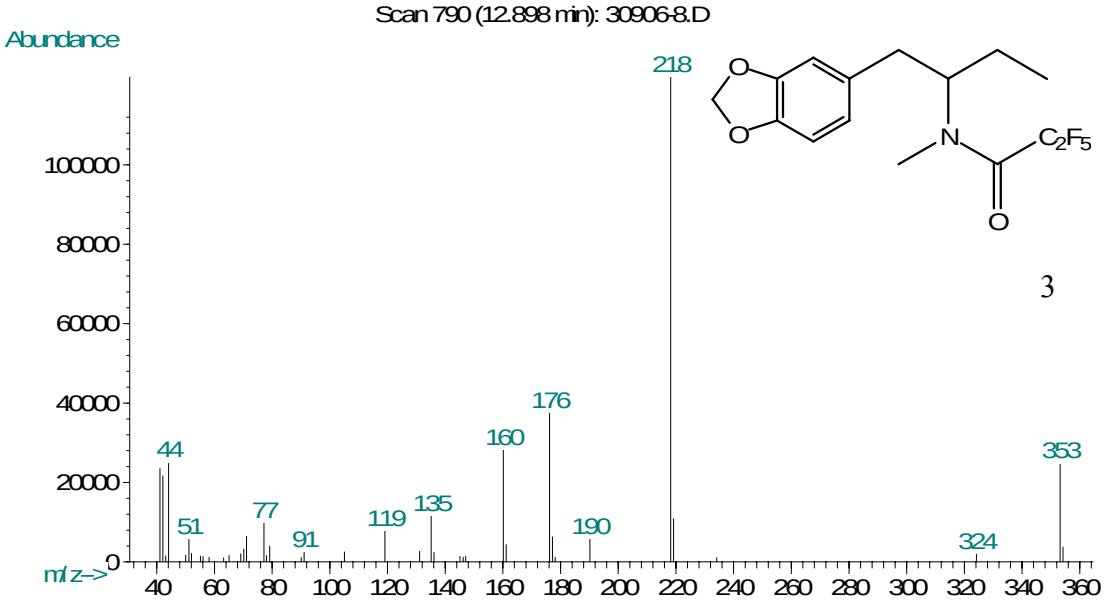
Abundance
m/z-->
218
190
162
44
353
77
51
92
119
135
146
234
O
O
N
C2F5
O

1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Abundance
m/z-->
218
176
160
44
353
77
51
91
119
135
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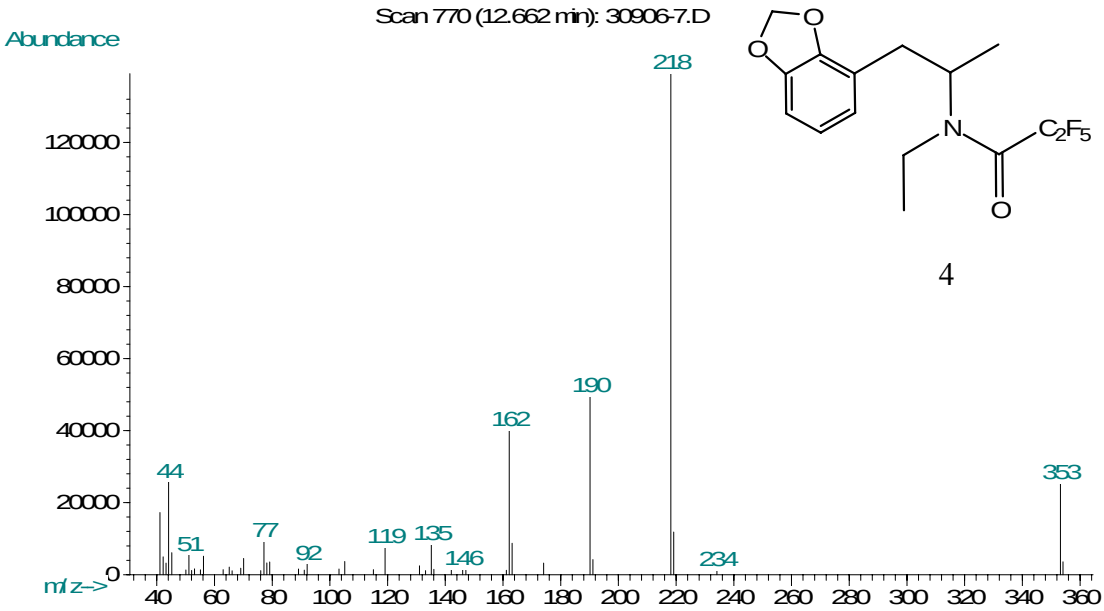
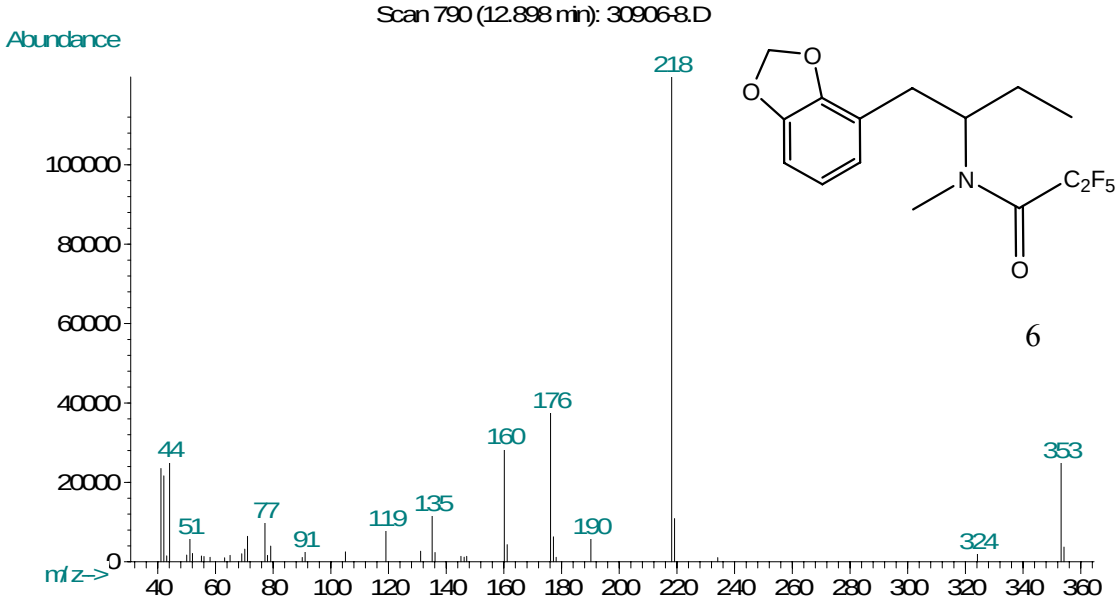
O
N
C2F5
O

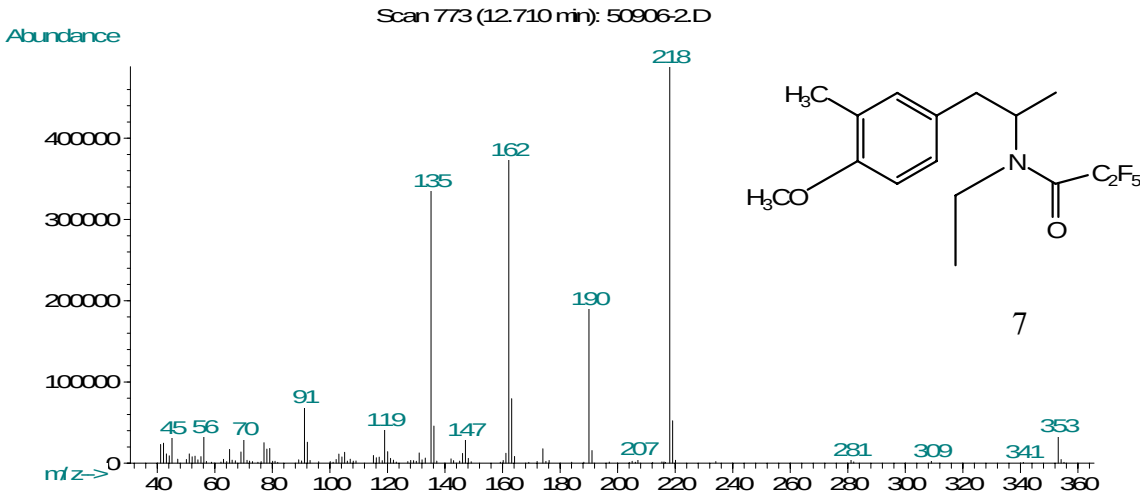
3


Scan 770 (12.662 min): 30906-7.D
Abundance
m/z-->
218
190
162
44
353
77
51
92
119
135
146
234
O
O
N
C_2F_5
O

4


Scan 790 (12.898 min): 30906-8.D
Abundance
m/z-->
218
176
160
44
353
77
51
91
119
135
190
324
O
O
N
C_2F_5
O

6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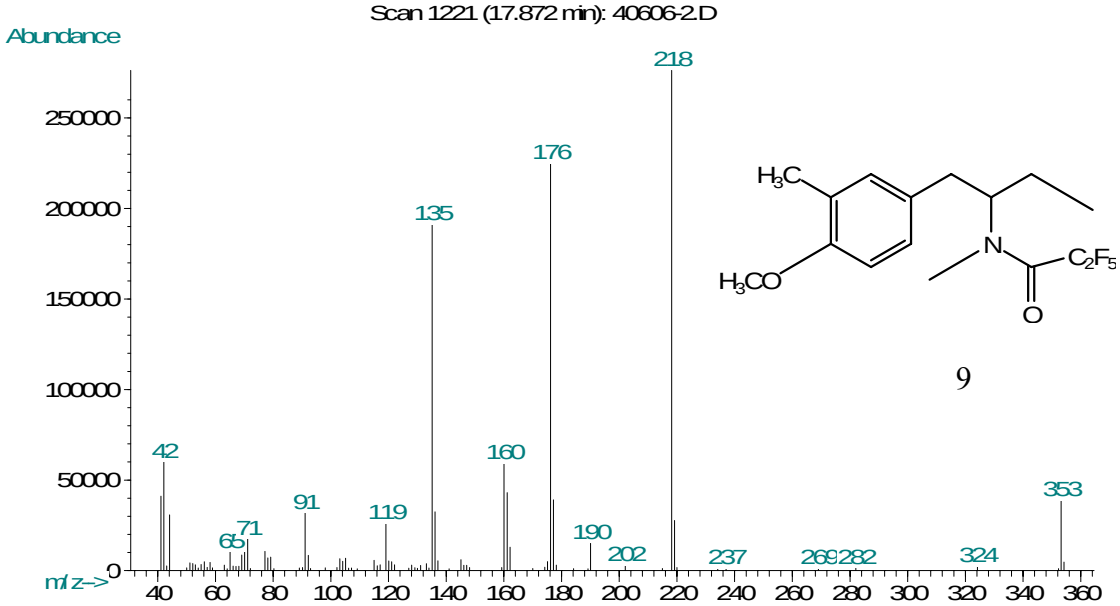
Abundance
m/z-->
218
162
135
190
91
119
147
45
56
70
207
281
309
341
353
H3C
H3CO
N
C2F5
O

7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Abundance
m/z-->
218
176
135
160
42
91
119
71
65
190
202
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269
282
324
353
H3C
H3CO
N
C2F5
O

9

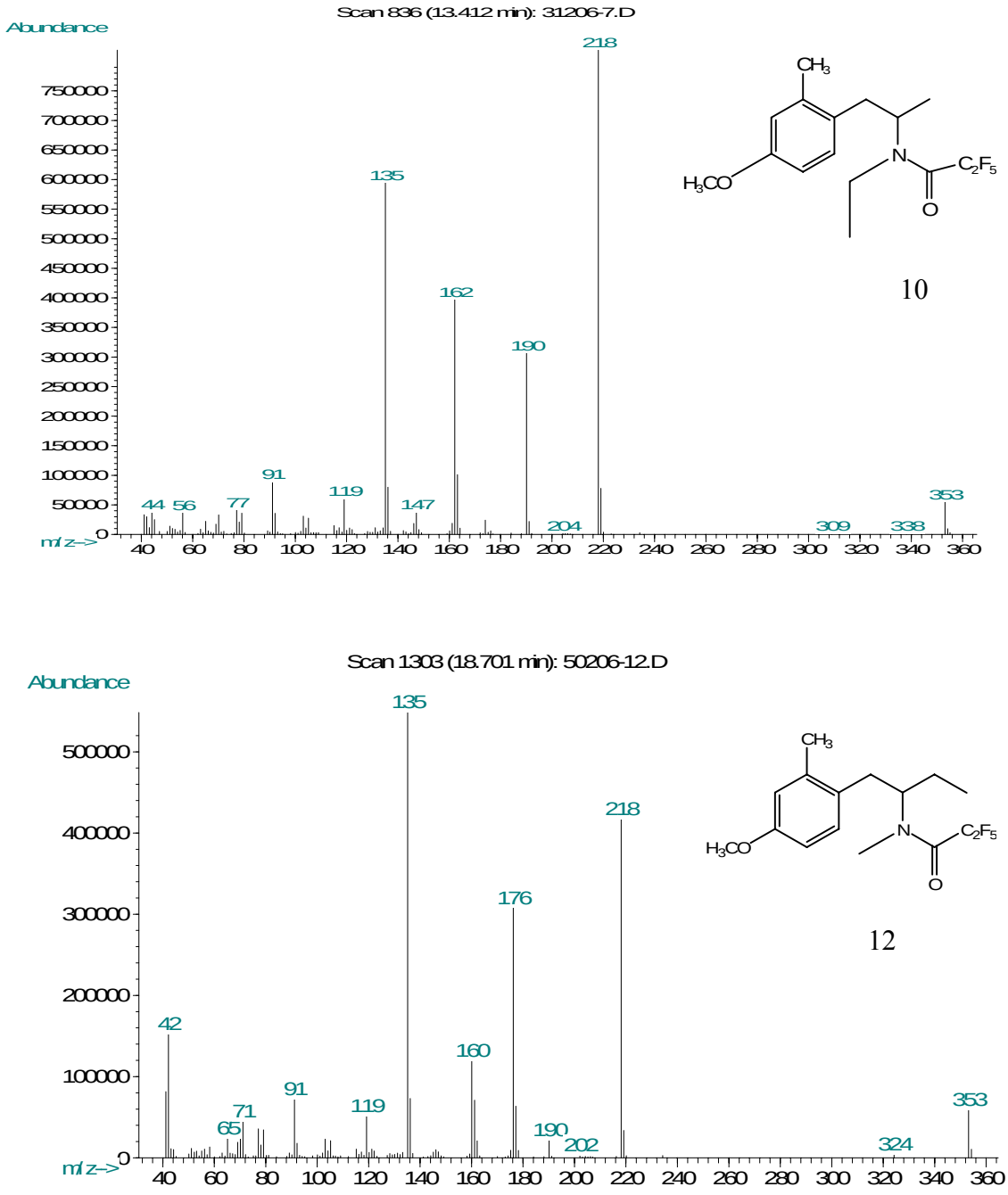
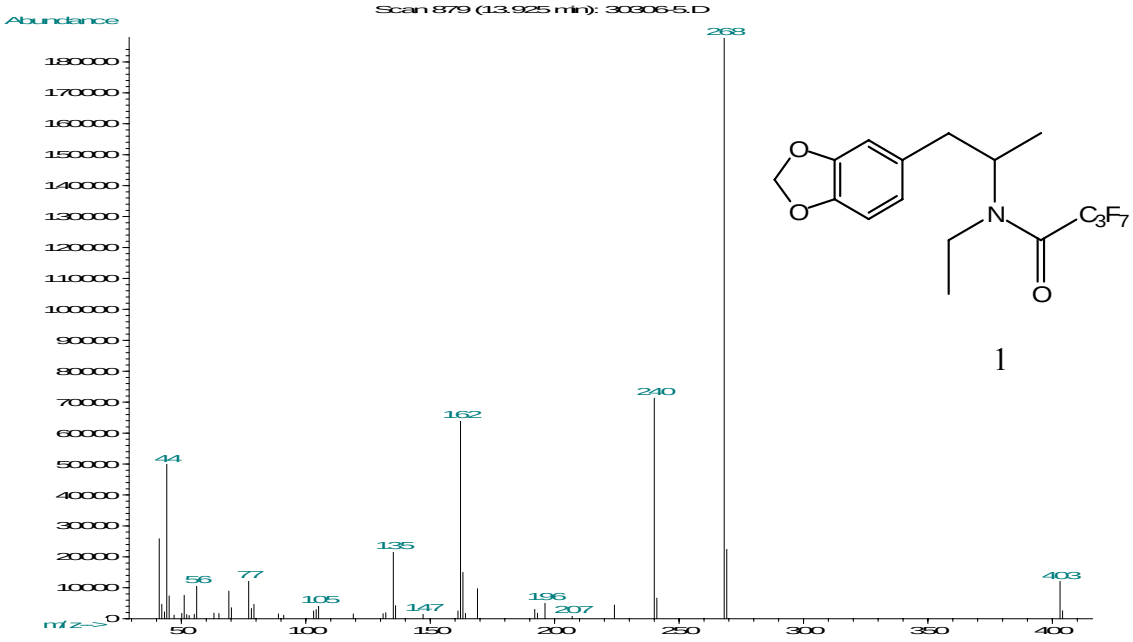


Figure 5: Mass spectra of the PFPA derivatives included in this study.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Abundance
m/z-->
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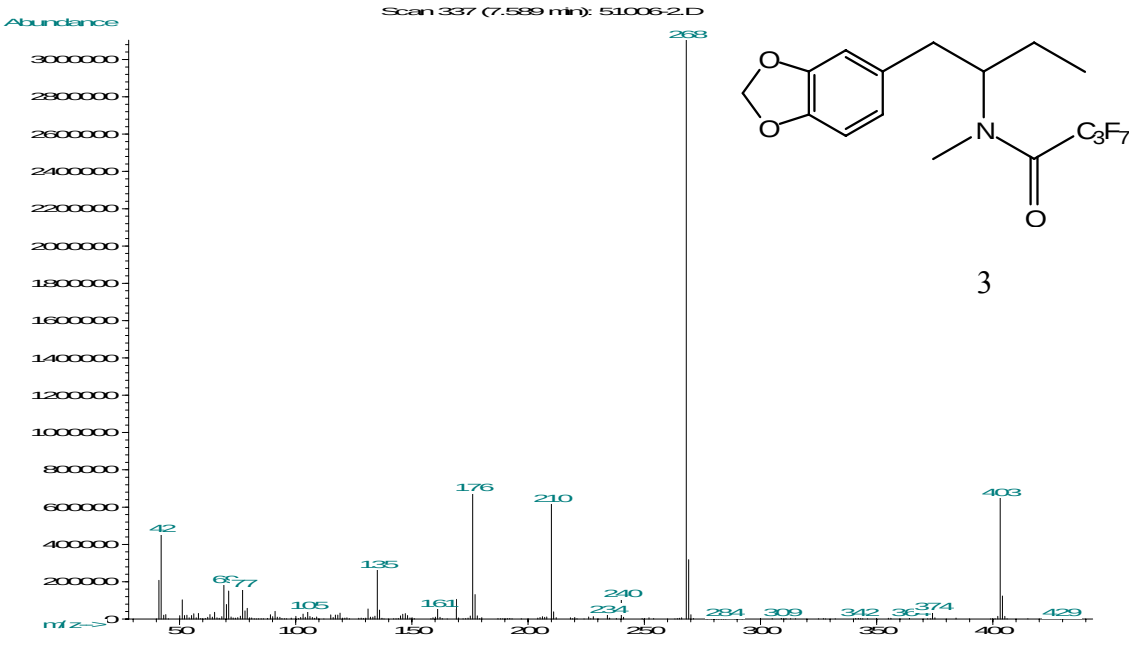
240
162
44
135
56
77
105
147
196
207
403
O
O
N
O
C3F7

1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Abundance
m/z-->
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176
210
403
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66
77
105
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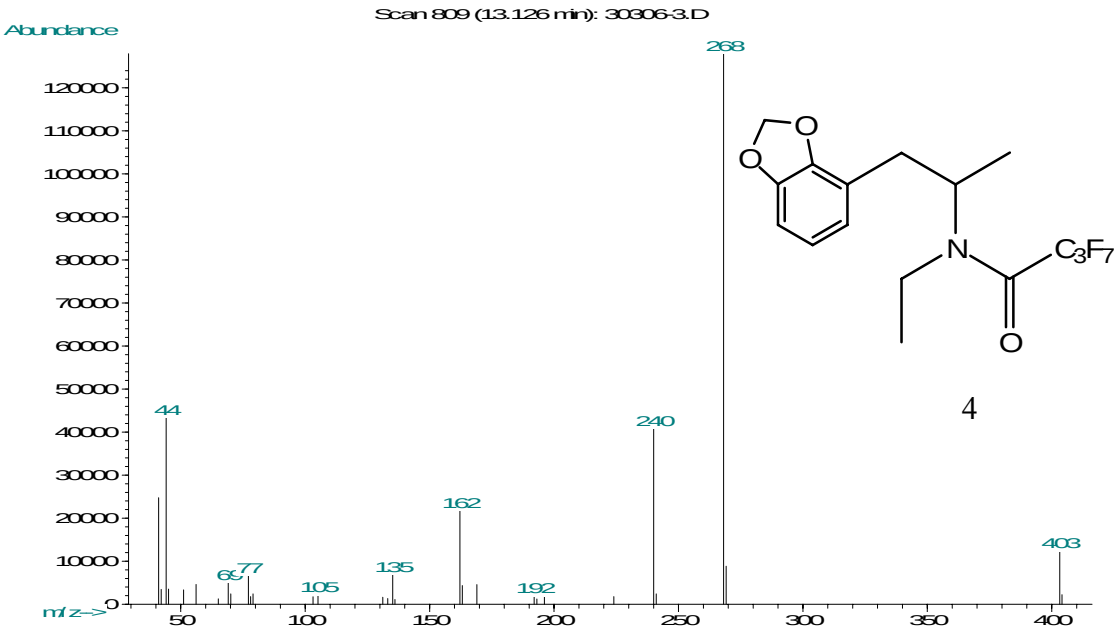
342
374
429
O
O
N
O
C3F7

3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Abundance
m/z-->
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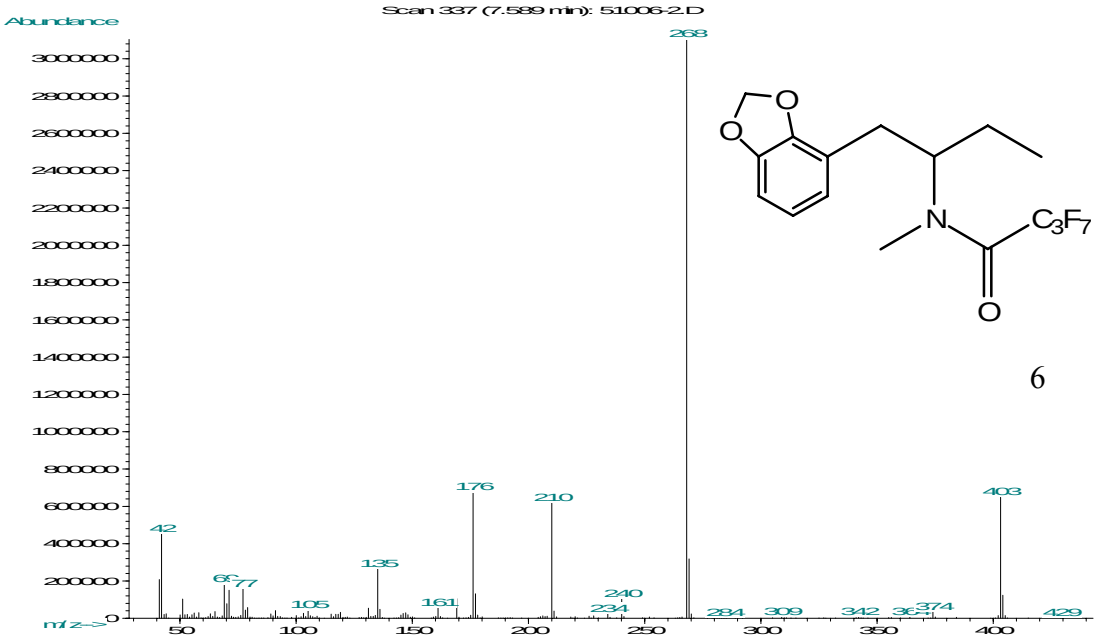
44
240
162
403
77
105
135
192
O
O
N
C3F7
O

4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Abundance
m/z-->
268
176
210
403
42
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77
105
161
240
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342
374
429
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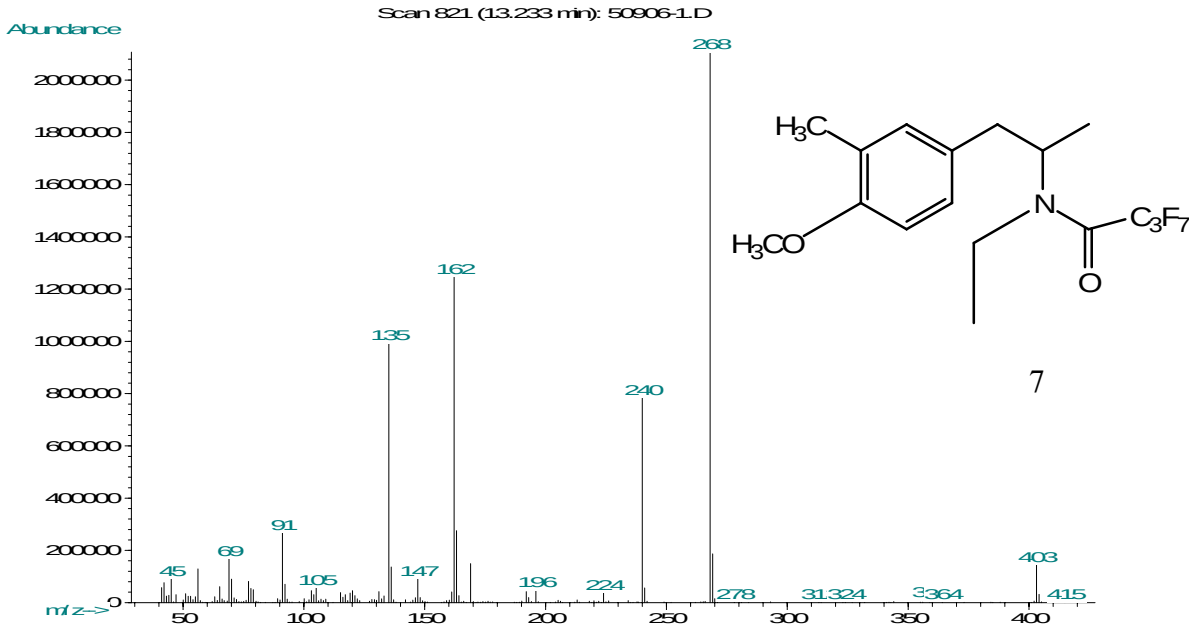
O
N
C3F7
O

6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Abundance
m/z-->
268
162
135
240
91
69
45
105
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196
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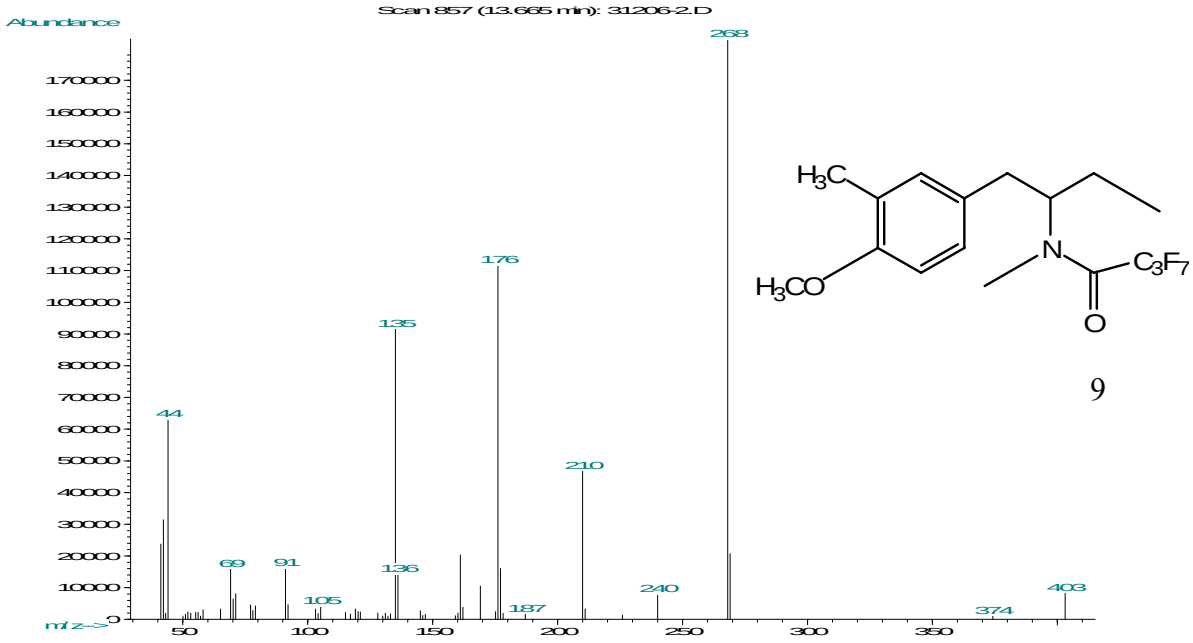
415
H3C
H3CO
N
C3F7
O

7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Abundance
m/z-->
268
176
135
44
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374
403
H3C
H3CO
N
C3F7
O

9

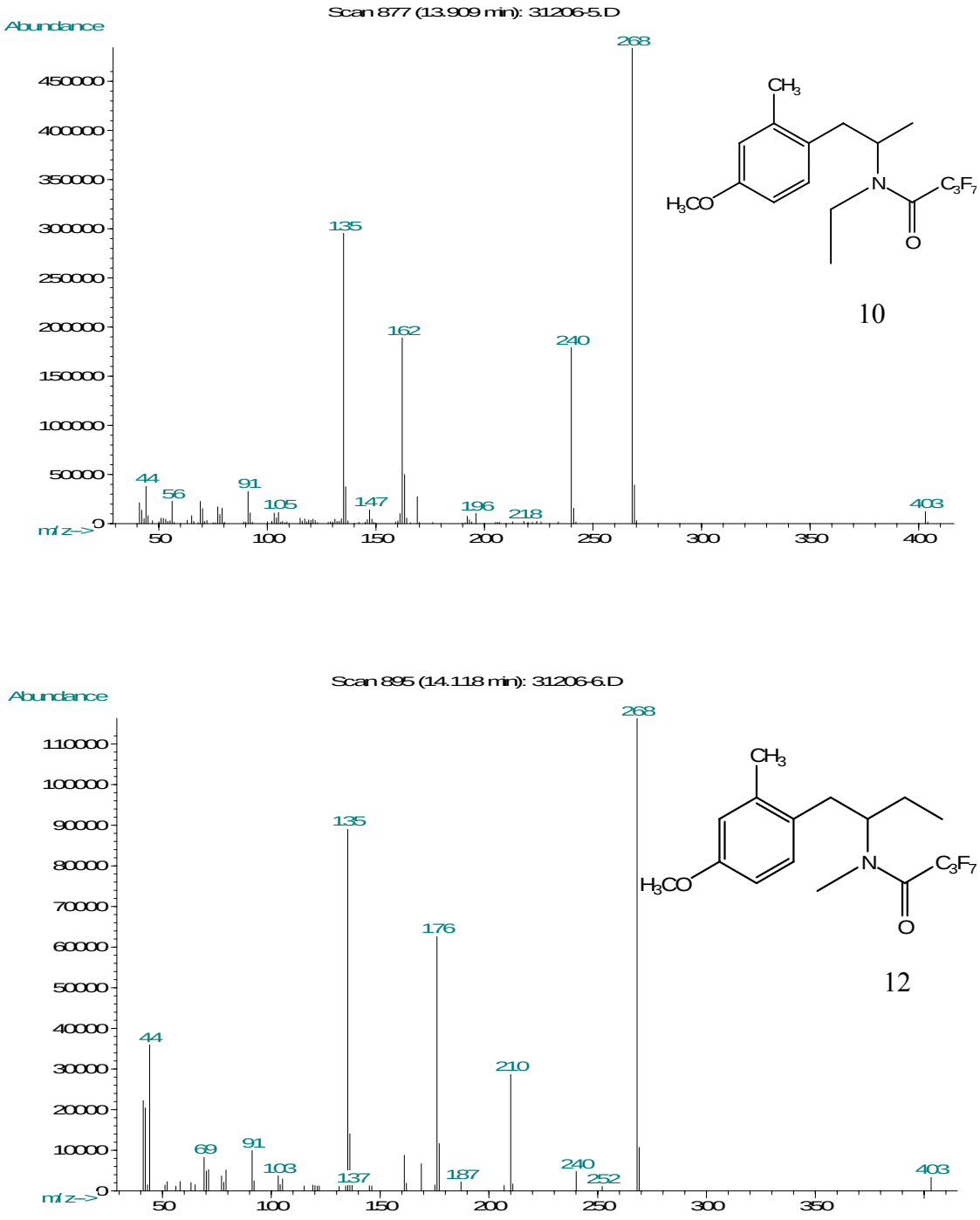


Figure 6: Mass spectra of HFBA derivatives included in this study.

3.3 Gas Chromatography

3.3.1 Gas Chromatography of the Amines

When other compounds exist that have the potential to produce nearly identical mass spectra as the drug of interest, the separation of the "nondrug regioisomers" from the actual drug is a critical issue. Mass spectrometry alone does not provide enough information to distinguish between the 12 regioisomers in this study as the underivatized species. Therefore, the identification by GC-MS must depend heavily on the ability of the chromatographic system to separate the drug molecules from the non-drug regioisomers.

The gas chromatographic separation of amines 1-6 is shown in Figure 7. This separation was obtained using a 30m x 0.25mm id column with a 0.25μm film of trifluoropropylmethyl polysiloxane, Rtx-200 (Figure 7A), and a permethylated beta cyclodextran, Rtx- beta cyclodextran (Figure 7B), column of the same dimensions. The retention times are greater on the Rtx-βDEX stationary phase and the resolution and peak shape are also better on the Rtx-βDEX stationary phase. The chromatograms in Figure 7 shows that the three 2, 3-methylenedioxyphenethylamines, (compounds 4, 5 and 6), elute before any of the three 3, 4-methylenedioxyphenethylamines, (compounds 1, 2 and 3). The order of side chain elution within the individual ring substitution patterns is N-ethyl followed by N, N-dimethyl in the arylpropylamine series. The N-methyl derivative of the arylbutanamine elutes third within each ring substitution series. Thus, the longest continuous hydrocarbon side chain (C4) shows the greatest retention on the Rtx-

200 and Rtx-beta cyclodextran phases. The retention properties of these amines were also compared on a less polar (100%) dimethyl polysiloxane column (Rtx-1) and 95% dimethyl-5% diphenyl polysiloxane (Rtx-5) of the same column dimensions and film thickness as described above. The observed elution order of the side chain regioisomers and ring regioisomers was the same as that obtained for the more polar phase, Rtx-200. However, in our experiments using the same temperature programs as used in Figure 7, complete resolution of all six compounds was not obtained on these columns. Compounds 1 and 6 co-elute under these conditions.

For the second subset of compounds included in this study (compounds 7-12), the 4-methoxy-3-methyl and the 4-methoxy-2-methyl substituded phenethylamines, separation was also carried out on the same four columns described above and complete resolution was only achieved on the Rtx-200 column (Figure 8). The elution order was 7 (first), 8, 9, 10, 11 and 12 (last). The observed elution order for these compounds again showed the two arylpropylamines eluting before the arylbutamine isomer. Since both series have a 4-methoxy group substituted on the aromatic ring, the position of the ring methyl group determines the elution order for the compounds with identical side chains. In all cases the 3-methyl series (compounds 7-9) elute before the 2-methyl series (compounds 10-12) of the same side chain. In fact, all three of the 3-methyl isomers elute before any of the 2-methyl isomers on the Rtx-200 column. The other three stationary phases investigated in this study did not provide complete resolution for compounds 7-12. On the Rtx-1 column, compounds 8 and 10 co-eluted, on the Rtx-5 column, compounds 8 and 9 co-eluted and on the Rtx-βDEX column, compounds 9 and 10

co-eluted. The two chromatograms obtained on the Rtx-200 column (Figures 9A and 10A) clearly point out the reason for subdividing the compounds in this study into two groups, the methylenedioxy substituted regioisomers (compounds 1-6) and the methoxymethyl regioisomers (compounds 7-12). The two groups (all twelve compounds) would yield a significant amount of co-elution if analyzed in one sample. The similar elution properties for there underivatized amines as well as their very similar mass spectra demonstrates the value of the previously described derivatization methods to aid in the differentiation among these isomeric substances. Furthermore, the mass spectral differences between the derivatives of the methylenedioxy compounds (1-6) and the methoxy-methyl amines (7-12) provided the basis for dividing the chromatographic studies into these two subsets.

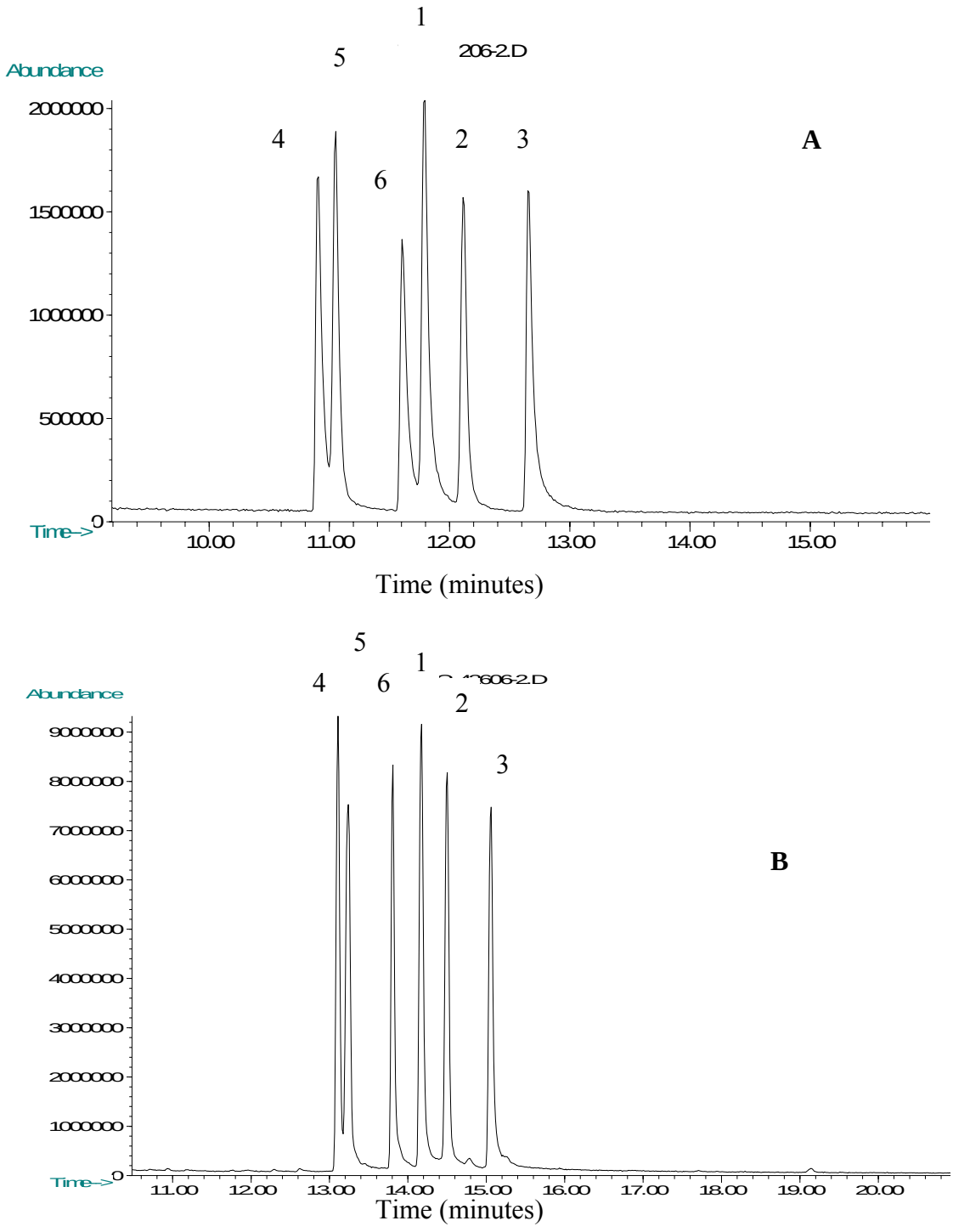


Figure 7: Capillary gas chromatographic separation of the underivatized

compounds 1-6. Columns used: Rtx-200 (A) and Rtx-beta dextran (B).

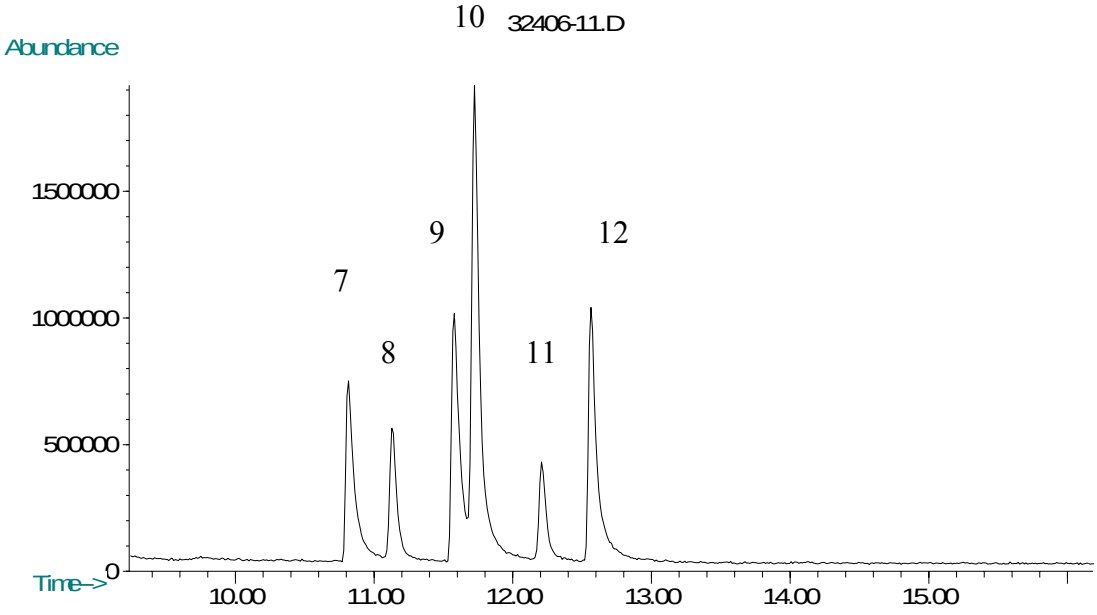


Figure 8: Capilaary gas chromatographic separation of the underivatized compounds 7-12, column used: Rtx-200.

3.3.2 Gas chromatography of the derivatives

The gas chromatographic properties of the PFPA and HFBA derivatives of the eight secondary amine regioisomers were compared on four columns: a dimethyl polysiloxane (Rtx-1), a dimethyl-diphenyl polysiloxane (Rtx-5), a trifluoropropylmethyl polysiloxane (Rtx-200) and a cyclodextran (Rtx-βDEX). The PFPA and HFBA derivatives showed improved resolution57when compared to the underivatized

amines. Several temperature programs were evaluated and the best compromises between resolution and analysis time were used to generate the data in the chromatograms in Figures 9-14. The complete separation of all 8 derivatives was unsuccessful. The chromatographic separation of the PFPA and HFBA derivatives of the methylenedioxy substituted amines (compounds 1, 3, 4 and 6) are shown in Figures 9 and 10. Figure 9 shows the separation of the PFPA derivatives of amines 1, 3, 4 and 6 on the Rtx-200 and the beta-cyclodextrin phases. These two phases showed the best resolution for all the derivatized amines. The elution order observed is the same on both stationary phases and the same as that observed for the underivatized forms of these amines. The 2,3-methylenedioxy amines (compounds 4 and 6) elute before either of the 3,4-methylenedioxy amines (compounds 1 and 3). Within each ring substitution (2,3- or 3,4-) the C3 side chain isomer (compound 1 or 4) elutes before the C4 side chain isomer (compounds 3 or 6).

The HFBA derivatives in Figure 10 show the same elution order and very similar retention times on the same columns using an identical temperature program. In general, the HFBA derivatives show slightly greater retention than the PFPA derivatives for compounds 1, 3, 4 and 6 when comparing the same stationary phase, the Rtx-200 or Rtx-βDEX. Resolution of the derivatives was excellent on both phases.

The separation obtained for the PFPA and HFBA derivatives of the methoxy-methyl substituted isomers (7, 9, 10 and 12) are shown in Figures 11 and 12. The two best stationary phses for maximum resolution of the derivatives are Rtx-200 and the Rtx-beta dextran. The resolution of these derivatized amines is far superior on the

beta dextran phase than the Rtx-200 stationary phase. These derivatives (both PFPA and HFBA) show significant peak tailing on the Rtx-200 phase (Figures 11A and 12A). The peak shapes on the beta dextran column are significantly improved over the Rtx-200 column. The side chain elution order observed for these methoxy-methyl phenethylamine derivatives is the same as that observed for the methylenedioxy series, the C3 side chains (compounds 7 and 10) eluting before the C4 side chain (compounds 9 and 12). The elution order of the ring substitution pattern is the same as that observed for the underivatized amines. The 4-methoxy-3-methyl isomers elute before the 4-methoxy-2-methyl isomers.

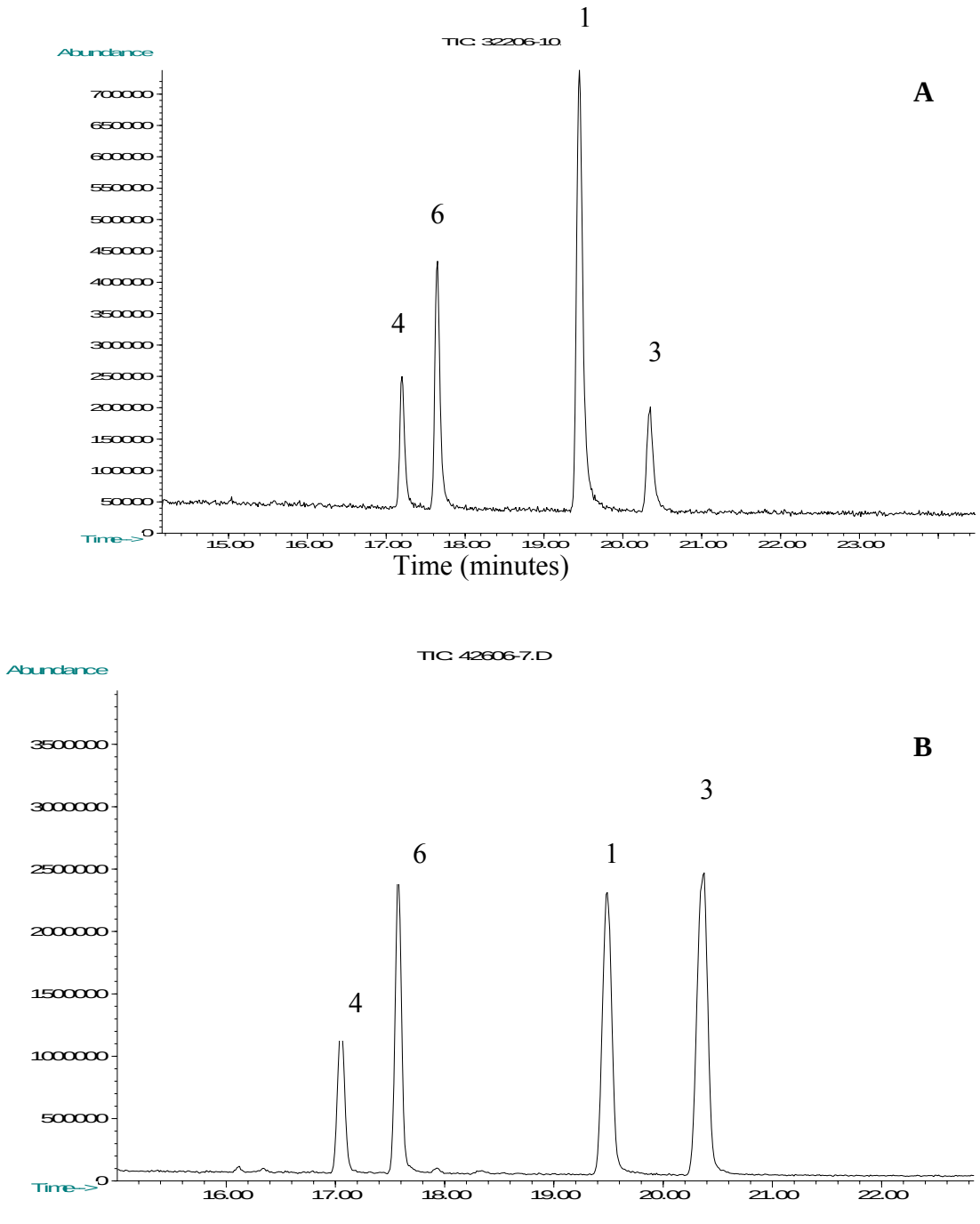


Figure 9: Capillary gas chromatographic separation of PFPAs of compounds 1, 3, 4 and 6. Column used: Rtx-200 (A) and Rtx-beta dextran (B)

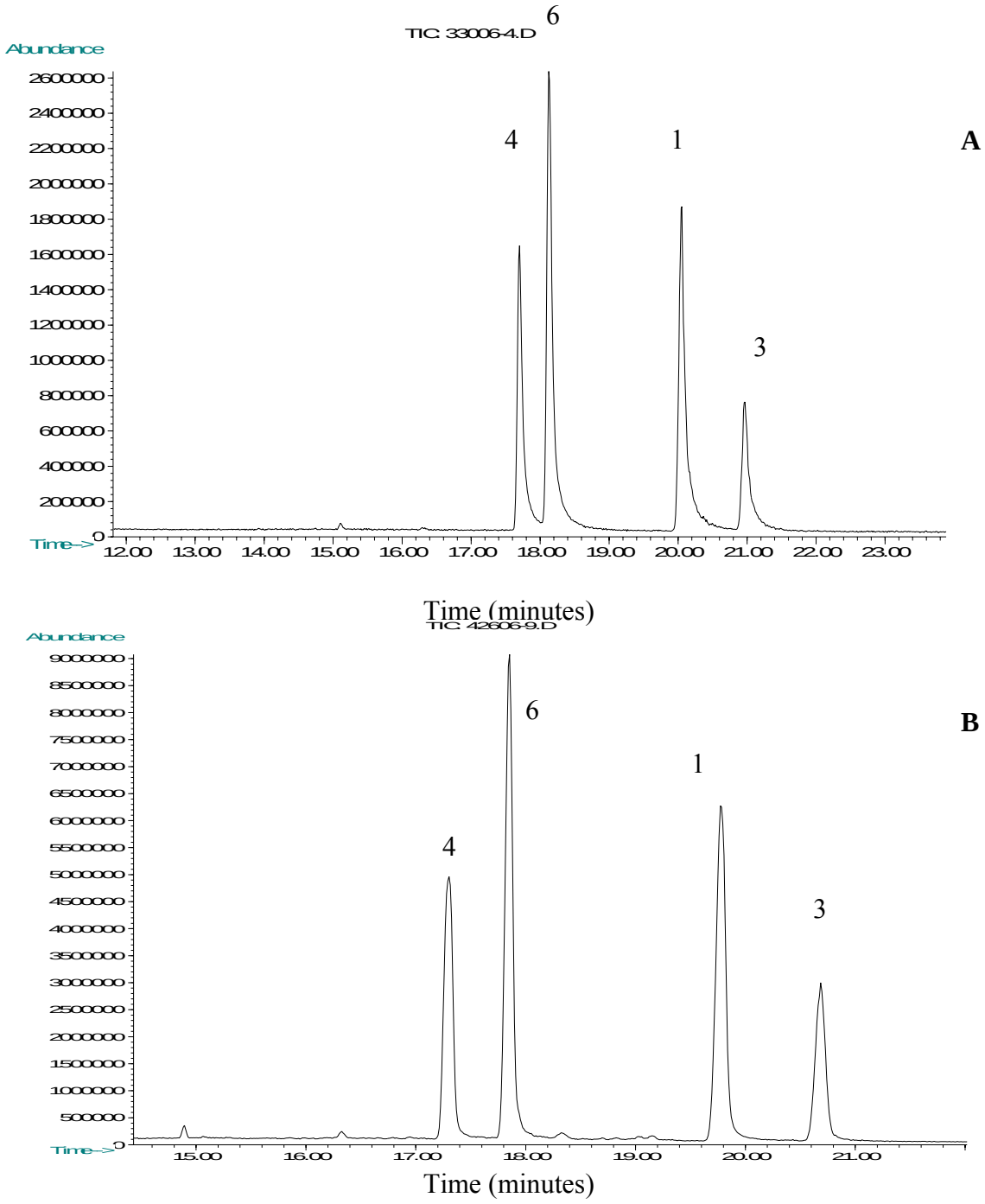


Figure 10: Capillary gas chromatographic separation of HFBAs of compounds1, 3, 4, and 6. Column used: Rtx-200 (A) and Rtx-β dextran (B)

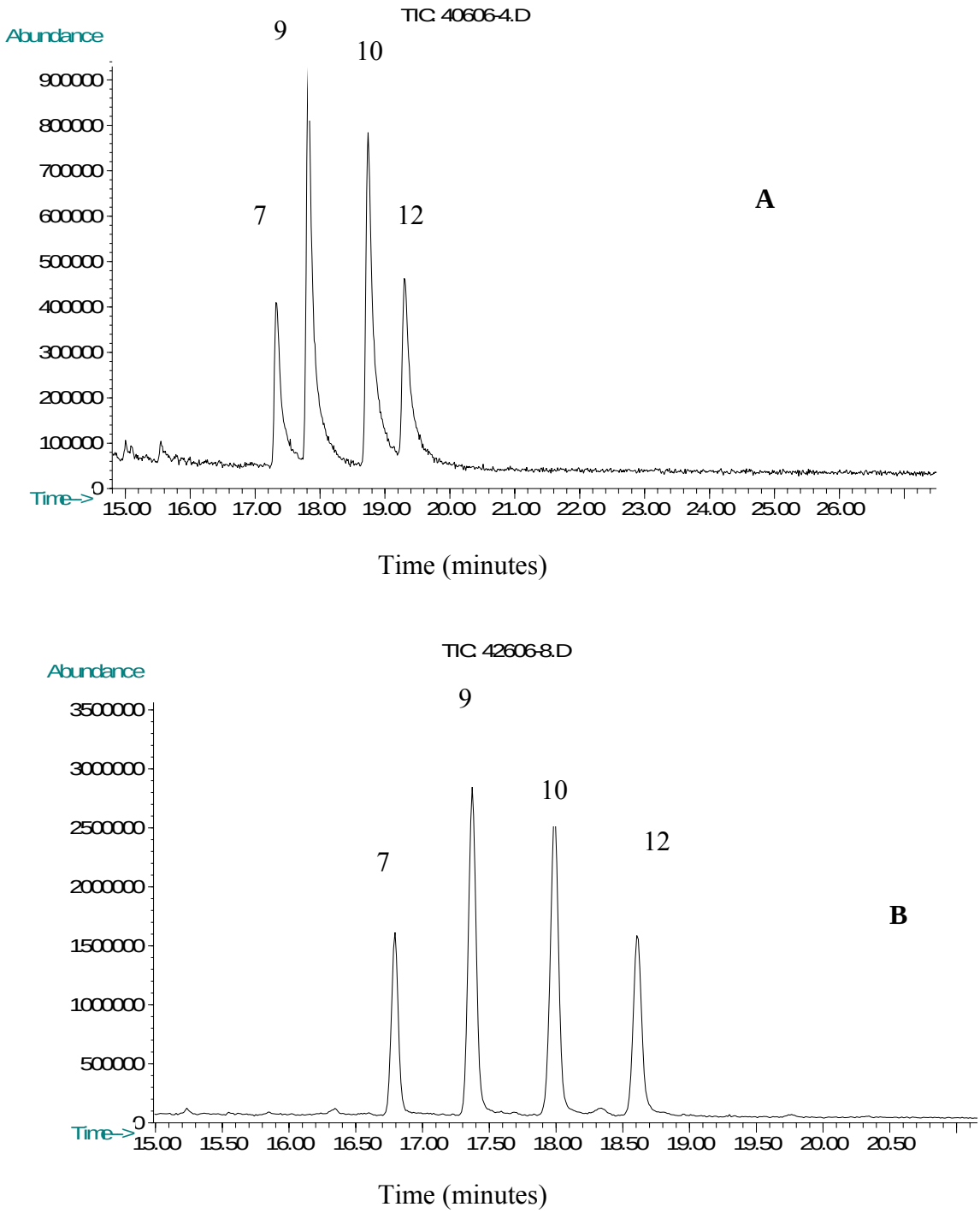
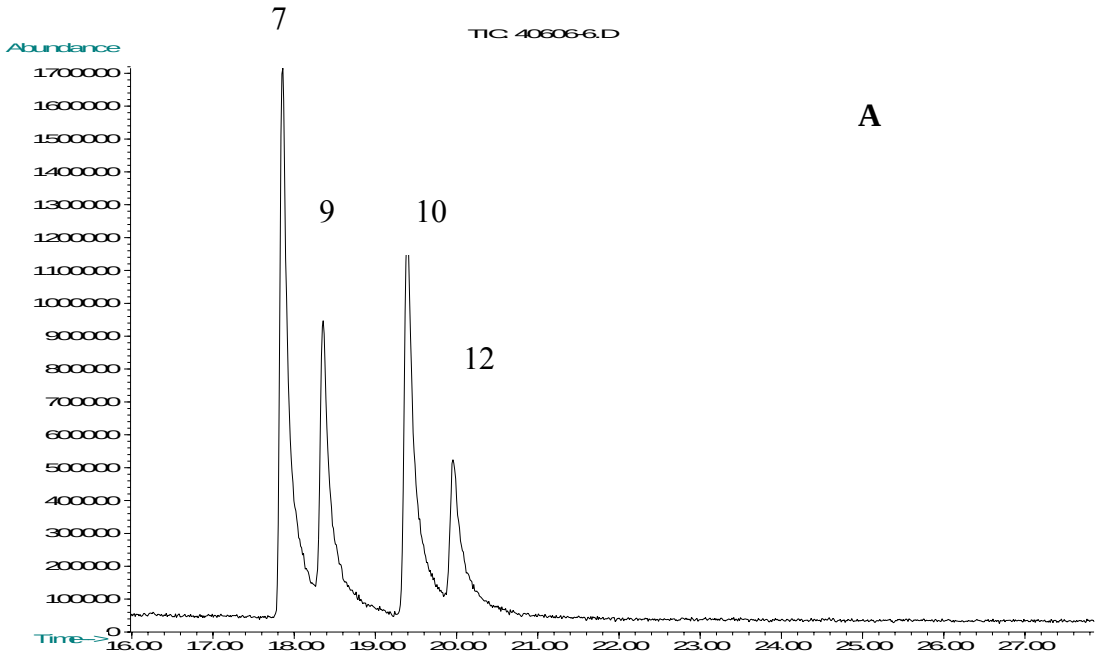


Figure 11: Capillary gas chromatographic separation of PFPA derivatives of comounds7-12 columns used (A) Rtx-200 (B) Rtx-βDEX .



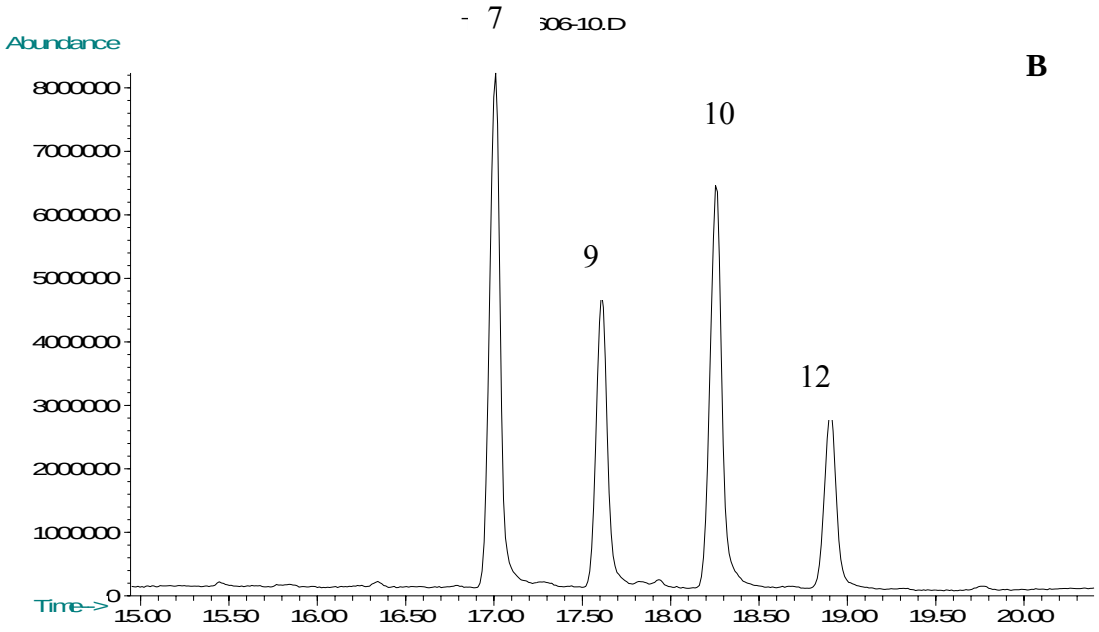
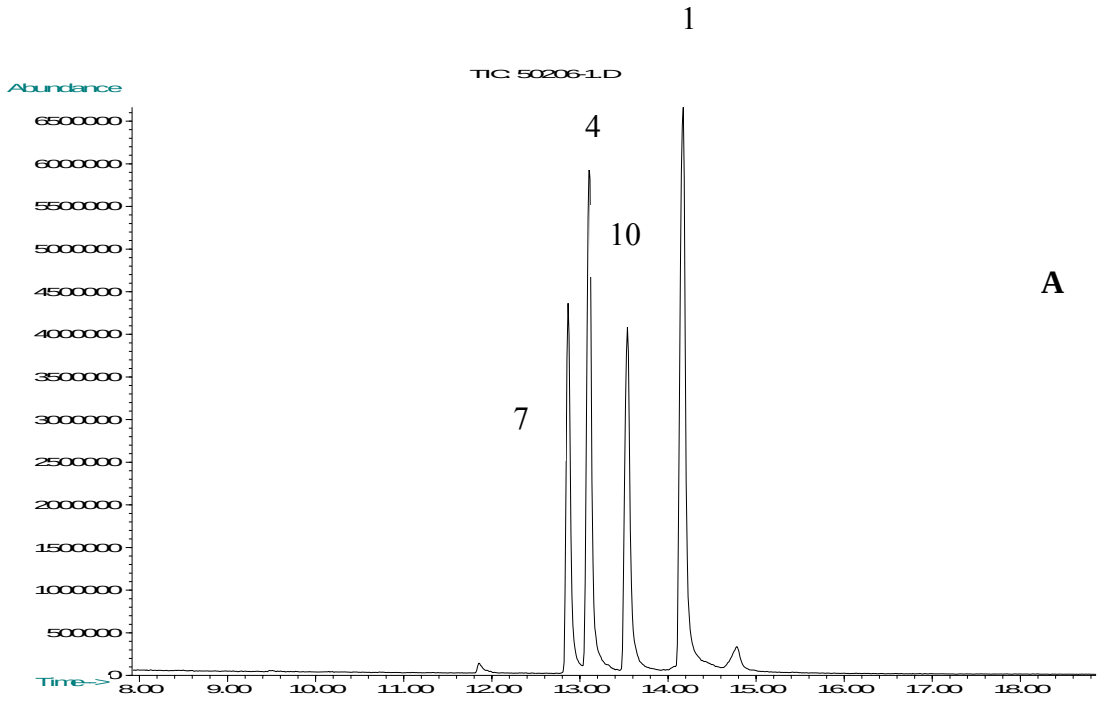


Figure 12: Capillary gas chromatographic separation of HFBA derivatives of compounds 7-12 columns used: (A) Rtx- 200 (B) Rtx-βDEX

3.3.3 Gas chromatography of the regioisomeric and isobaric ring substituents

The third chromatographic study in this project was to evaluate the comounds having identical side chains. These comparisons were made for both the underivatized amine and the PFPA and HFBA derivatives. The mass spectrometry studies on the PFPA and HFBA derivatives had clearly established that specific fragment ions were available to distinguish among the different side chains. Furthermore the nature of the ring substituted methylenedioxy or methoxy-methyl compounds showed significant and characteristic differences in fragment ion abundance. Thus the chromatographic properties of the same side chain subsets is perhaps the most significant for the ability to specifically identify one of these substances to the exclusion of all the other amines in this study. The separation was carried out on the Rtx-βDEX and Rtx-200 stationary phases with great resolution (Figures 13 and 14). In the case of the N-ethyl side chains the elution order was the same for both the underivatized and the derivatized phases. The first compound to elute was compound 7 (4-methoxy-3-methyl-ethylamphetamine) followed by compound 4 (2, 3-methylenedioxyethylamphetamine). The third compound to elute was compound 10 (4-methoxy-2-methyl-ethylamphetamine) followed by compound 1 (3, 4-MDEA). In all phases, compound 1 showed the greatest retention. Compounds that possessed the N, N-dimethyl side chain (cannot be derivatized) could not be resolved with compounds 2 and 5 co-eluting. Compounds with the N-methyl, C-ethyl side chain were also resolved in both the underivatized and the derivatized

forms. The first to elute was compound 9 (4-methoxy-3-methyl-phenethylamine) followed by compound 6 (2, 3-MBDB). The third compound to elute was compound 12 (4-methoxy- 2-methyl-phenethylamine) followed by compound 3 (3, 4-MBDB). Compound 3 showed the greatest retention in both the underivatized and the derivatized phases.



Time (minutes)

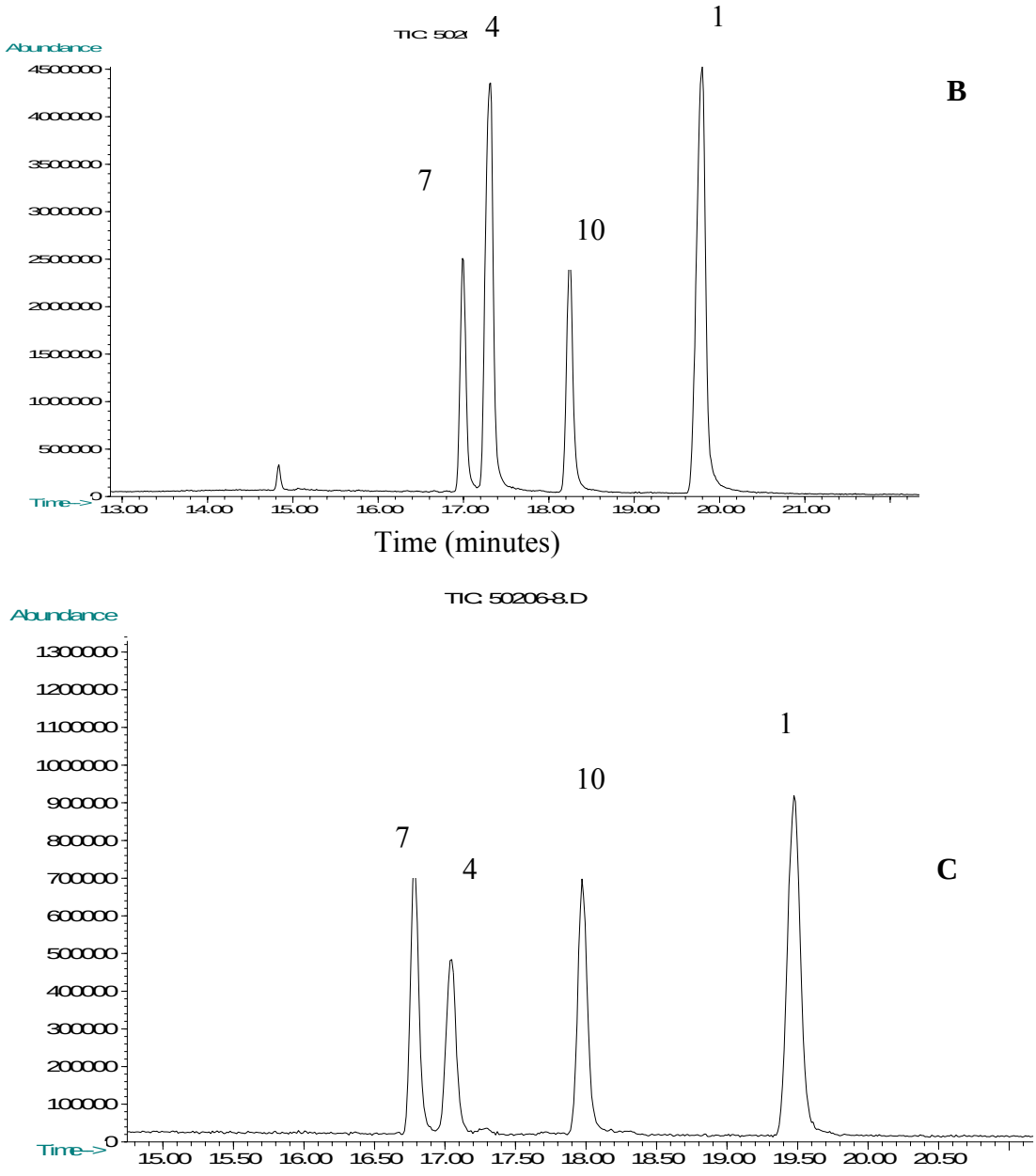
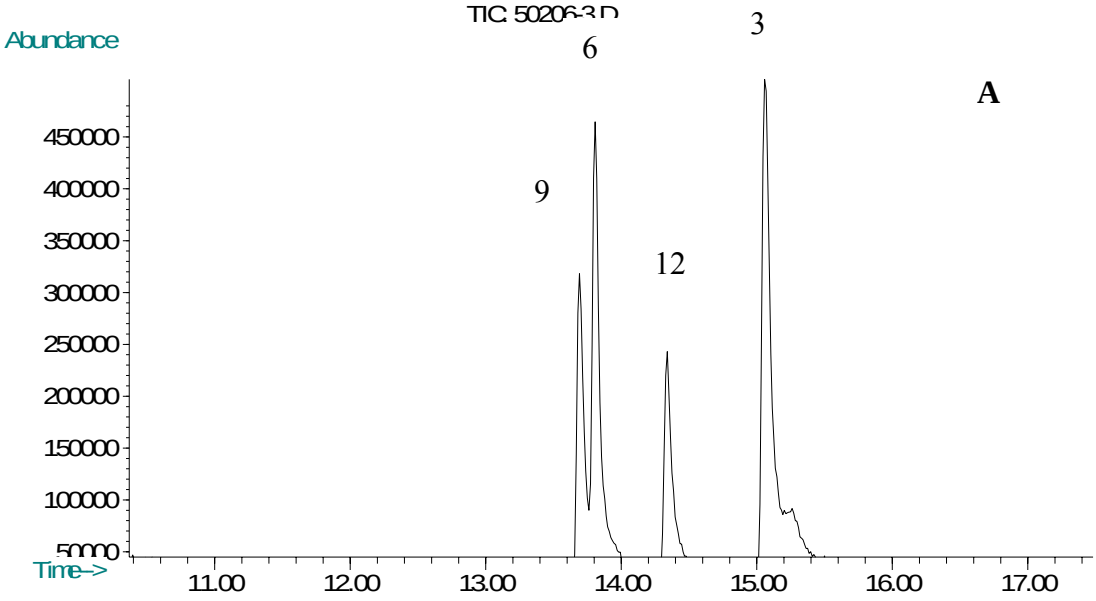
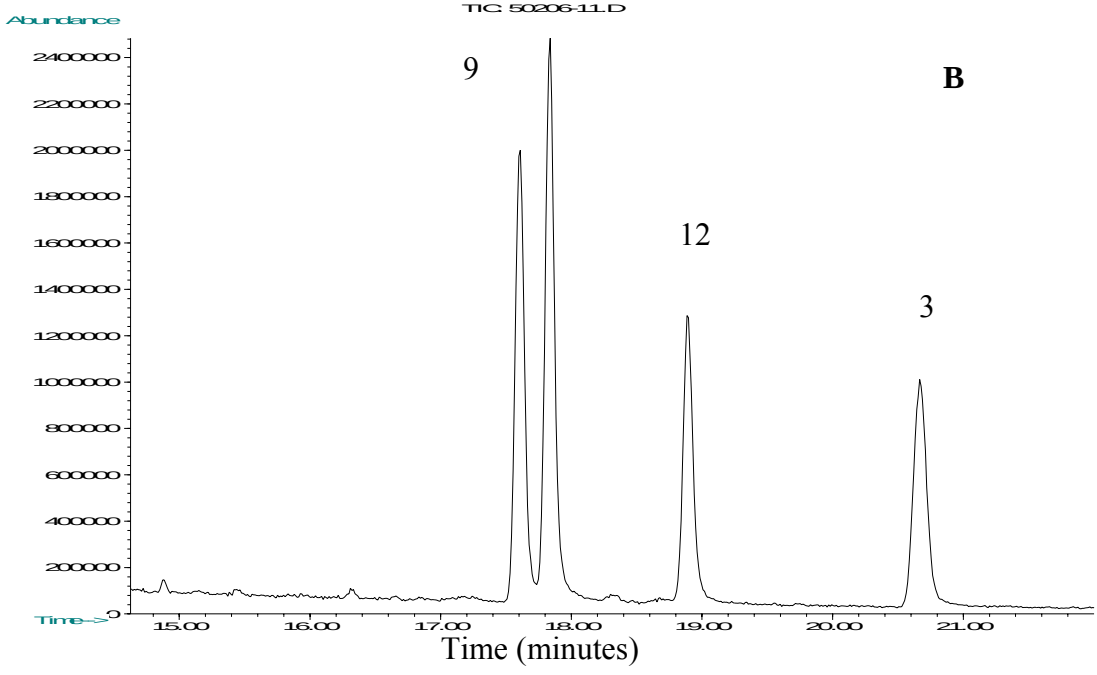


Figure 13: Capillary gas chromatographic separation of compounds containing the N-ethyl side chain; column used: Rtx-βDEX (A) underivatized (B) PFPA (C) HFBA.



Time (minutes)

6



Time (minutes)

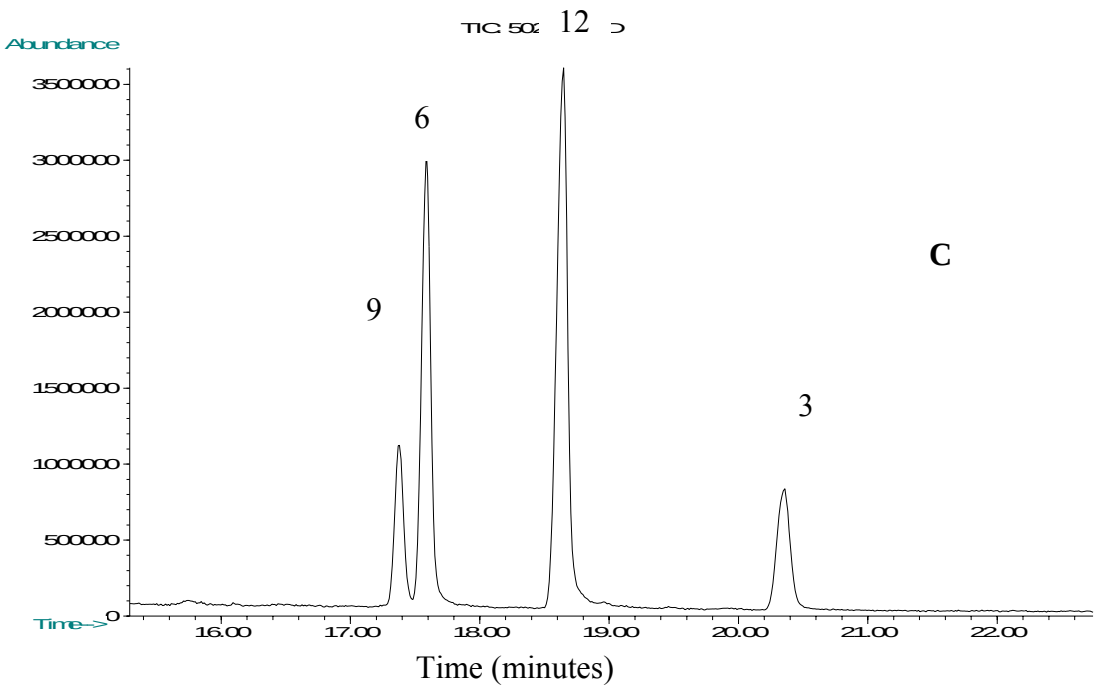


Figure 14: Capillary gas chromatographic separation of compounds containing the N-methyl side chain; column used: Rtx-βDEX (A) underivatized (B) PFPA (C) HFBA

4. EXPERIMENTAL

GC/MS analysis was performed with an HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). The MS was operated in the electron impact (EI) mode using ionization voltage of 70 eV and a source temperature of 230°C. Samples were dissolved in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and manually introduced (1µL), individually and in a physical mixture, using a 10-µL Hamilton syringe (Hamilton Co., Reno, NV).

The separation was carried out on a 30-m × 0.25-mm i.d. column coated with 0.25-µm 100% dimethyl polysiloxane (Rtx-1), a 30-m × 0.25-mm i.d. column coated with 0.25-µm 95% dimethyl-5% diphenyl polysiloxane (Rtx-5), a 30-m × 0.25-mm i.d column coated with 0.25-µm trifluoropropylmethyl polysiloxane (Rtx-200) and a 30-m × 0.25-mm i.d column coated with 0.25-µm 86% dimethyl polysiloxane and 14% cyanopropylphenyl (Rtx-βDEXcst), all obtained from Restek corporation (Bellefonte, PA).

The retention data was generated using two temperature programs. Program 1 consisted of an initial hold at 100°C for 1.00 min., ramped up to 180°C at a rate of 9°C/min and held at 180°C for 2.00 min., then ramped to 200°C at a rate of

10°C/min and held at 200°C for 5.00 min. Program 2 consisted of the exact same temperature

conditions with the only difference being between the temperatures of the injector and the detector. For program 1 the injector temperature was 250°C and the detector temperature was 280°C. For program 2 the injector temperature and the detector temperatures were the same, both being 200°C.

4.1 Synthesis of regioisomers of MDEA, MDMMA and MBDB

The synthesis of the regioisomers in this study begins with 5 grams of the appropriately substituted benzaldehyde (2, 3-methylenedioxybenzaldehyde, 3, 4-methylenedioxybenzaldehyde (piperonal), 4-methoxy-2-methyl or 4-methoxy- 3-methyl). Piperonal was obtained from Aldrich Chemical Company (Milwaukee, Wisconsin) and 2, 3-methylenedioxybenzaldehyde was made from 2, 3-dihydroxybenzaldehyde (Aldrich Chemical Company) according to previously reported methods [8]. A sample of 2, 3-dihydroxybenzaldehyde was added to a potassium carbonate solution of DMF (dimethylformamide) followed by methylene bromide and copper oxide. The solution was refluxed and 75 mL of water were added and the mixture extracted with methylene chloride, dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure.

4.2 Synthesis of 2, 3-methylenedioxybenzaldehyde

The synthesis of 2, 3-methylenedioxybenzaldehyde involves an extra step in the beginning of the synthesis to make 2, 3-MDEA, MDMMA and MBDB. Begin with 5 grams of 2,3-dihydroxybenzaldehyde was added to 18.75 grams (0.126 moles) of a potassium carbonate solution in 50 mL of DMF (dimethylformamide), followed by the addition of 7.6 mL (18.9 grams, 0.1 moles) of methylene bromide added drop wise along with 0.1 grams of copper oxide. The solution was refluxed overnight and 75 mL of water were added to the mixture before extraction with methylene chloride which was then dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure. The resulting 2, 3 methylenedioxybenzaldehyde was purified using Kugelrohr distillation. The remaining steps are the same for the preparation of all the regioisomers.

4.3 Synthesis of the phenethylamine regioisomers

5 grams of the appropriately substituted methylenedioxybenzaldehyde was added to 20 mL n-butylamine in 62.5 mL of benzene. The mixture was refluxed overnight using a Dean-Stark trap and the solvent evaporated to dryness under reduced pressure. The appropriate nitroalkane (nitroethane or nitropropane) (2.6mL) was added and the mixture refluxed for one hour followed by addition of 12.5 mL of glacial acetic acid, 12.5 mL of water and ice. The resulting mixture was acidified with concentrated hydrochloric

acid and extracted into methylene chloride, and the organic phase washed with water and dried over sodium sulfate. The solution was filtered and evaporated to dryness under reduced pressure and 5 grams of the resulting nitrostyrene was added to a mixture of 25 mL of toluene, 22.5 mL of water and 9 mL of concentrated hydrochloric acid containing 13.5 grams of ferric chloride and 6.7 grams of iron. The mixture was refluxed and filtered and the toluene layer was isolated and extracted into concentrated hydrochloric acid, washed with water then extracted into saturated sodium bicarbonate and washed with water again. The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting ketone was purified using Kugelrohr distillation.

Five grams of the appropriate ketone was added to 2.5 grams of the appropriately substituted amine hydrochloride, 0.8 grams of sodium cyanoborohydride and 40 mL of methanol and the mixture stirred over the weekend. Upon completion the methanol was evaporated to dryness under reduced pressure. The residue was suspended in water and extracted with methylene chloride, and the organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting basic fraction gave light yellow oils which were converted to the hydrochloride salts by adding 30 mL of dry ether and bubbling through gaseous hydrochloric acid.

4.4 Derivatization Procedure

Each perfluoroamide was prepared individually from the hydrochloride salts by dissolving approximately 0.3 mg. (1.5 x 10^-6 moles) of each amine in 50 µL of ethyl acetate followed by the addition of a large excess (250 µL) of the appropriate derivatizing agent (pentafluoroproprionic anhydride or heptafluorobutric anhydride). The derivatization reaction mixtures were incubated in capped vials at 70 °C for 20 minutes. Following incubation, each sample was evaporated to dryness under a stream of air at 55 °C and reconstituted with 200 µL of ethyl acetate and 50 µL of pyridine. A portion of this solution was further diluted with HPLC grade acetonitrile and volumes of 1µL of the resulting solutions were injected into the GC-MS for analysis

5. SUMMARY AND CONCLUSIONS

The goal of this project was to evaluate the GC-MS properties of a series of twelve regioisomeric and isobaric ring substituted phenethylamines of molecular weight 207 and expected major fragment ions of m/z 72. The three regioisomeric 3, 4-methylenedioxyphenethylamines (MDEA, MDMMA and MBDB) have appeared as components of clandestine drug samples in recent years. The twelve compounds (see Figure 3) were synthesized using the appropriate aldehyde and a combination of the appropriate nitroalkane and alkyl amines. Mass spectral studies of the underivatized amines showed very little specific structural information. The mass spectra are characterized by a base peak formed by an alpha cleavage reaction involving the carbon-carbon bond of the ethyl linkage between the aromatic ring and the amine. The alpha cleavage reaction yields the regioisomeric butylamine fragments at m/z 72 and the 2,3- and 3,4- methylenedioxybenzyl and the 4-methoxy-3-methyl and 4-methoxy-2-methyl benzyl cation fragment at m/z 135 and the radical cation at m/z 136. The various perfluoroacyl derivatives of the phenethylamines were prepared and evaluated in an effort to individualize the mass spectra. There are two major diagnostic pathways in the mass spectrum of the PFPA and HFBA derivatives which allows differentiation of the side chain regioisomers.

The first of these is the alkene fragment observed at m/z 162 and m/z 176; these ions occur in the spectra for both the PFPA and HFBA derivatives.

The second diagnostic pathway involves loss of mass 28 from the base peak for the N-methyl derivatives and a characteristic rearrangement product ion for the N-methyl derivatives. The rearrangement ion occurs at m/z 160 and m/z 240 for the PFPA and HFBA derivatives, respectively. Thus, derivatization allowed for the specific identification of the side chains in these regioisomeric and isobaric amines

GC-MS analysis was carried out on several temperature programs and also several different columns with the Rtx-βDEX giving the best resolution in most cases. The methylenedioxy subset (compounds 1-6) were well resolved on the beta dextran column with the 2, 3 regioisomers eluting before the 3, 4 regioisomers and the side chain elution order was constant in all experiments. In the methoxy-methyl subset (compounds 7-12), the 3-methyl regioisomers eluted before the 2-methyl regioisomers and the same side chain elution order was maintained. All the derivatized amines (PFPA and HFBA) showed excellent resolution on the beta dextran column and good resolution on the Rtx-200 stationary phase. The observed elution orders were the same as in the underivatized amines. The mass spectrometry studies on the PFPA and HFBA derivatives had clearly established that specific fragment ions were available to distinguish among the different side chains. A comparative study of the underivatized and derivatized amines of the same side chain showed the same elution order on the beta dextran stationary phase. The observed elution order was the 4-methoxy-3-methylphenethylamines followed by the 2, 3-methylenedioxyphenethylamines, then the 4-methoxy-2-methylphenethylamines and

finally 3, 4-methylenedioxyphenethylamines. In all our chromatographic studies the 3, 4-methylenedioxy regioisomer showed the highest retention. The procedures described in this study will provide for the specific identification of any one of these compounds to the exclusion of any of the other possible regioisomeric/isobaric amines.

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