MASS SPECTRAL AND CHROMATOGRAPHIC STUDIES ON A SERIES OF REGIOISOMERS AND ISOBARIC DERIVATIVES RELATED TO METHYLENEDIOXYMETHAMPHETAMINES

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Tamer A. Awad

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Signature of Author

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Date of Graduation

DISSERTATION ABSTRACT

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The popular drug of abuse 3,4-methylenedioxymethamphetamine (MDMA) has regioisomeric and isobaric substances of mass equivalence, which have similar analytical properties and thus the potential for misidentification. Direct regioisomers of MDMA include ring and side chain methylenedioxy substitution patterns and the indirect regioisomers include methoxymethcathinones. The methoxy methyl ring substituted methamphetamines constitute the major category of isobaric substances evaluated in this study.

The direct and indirect regioisomers of MDMA and also isobaric substances related to MDMA were synthesized and compared to MDMA by using gas chromatographic and spectrometric techniques. The spectrometric studies of the direct regioisomers and

isobaric substances of MDMA indicated that they can not be easily differentiated by mass spectrometry or ultraviolet (UV) spectrophotometry. The synthesized compounds were converted to their perfluoroacyl derivatives, pentafluropropyl amides (PFPA) and heptaflurobutryl amides (HFBA), in an effort to individualize their mass spectra and to improve chromatographic resolution. Derivatized MDMA was easily distingushed from its derivatized direct and indirect regioisomers using mass spectrometry. Unique fragment ions were observed for the various direct regioisomeric side chains and the methoxymethcathinones. However, it was hard to characterize perfluroacyl derivatives of MDMA from most of the derivatized methoxy methyl ring substituted methamphetamines (isobaric substances) of the same side chain substitution pattern. Gas chromatographic studies indicated that the optimum separation of direct and indirect regioisomers of MDMA was obtained when a 100% dimethyl polysiloxane column was used at gradient temperature program rates. Isobaric substances related to MDMA were divided into subset groups based on the methoxy group position on the aromatic ring and were found to have different elution properties than MDMA and therefore misidentification due to co-elution can be eliminated using 100% dimethyl polysiloxane and trifluoropropyl methyl polysiloxane columns. The mass spectral and chromatographic properties of methoxy methyl ring substituted phenyl acetone, as key intermediates in synthesizing methoxymethyl methamphetamines, were also evaluated and the ten ketones were separated from each other as well as from methylenedioxy-2-propanone on a permethylated beta cyclodextrin column using gradient temperature program rates.

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LIST OF ABBREVIATION

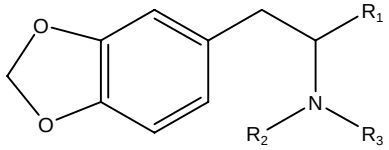
µl	Micro liter
µm	Micrometer
^{o}C	Degree centigrade
2,3-MDMA	2,3-Methylenedioxymethamphetamine
3,4-MDMA	3,4-Methylenedioxymethamphetamine
BDB	3,4-Methylenedioxyphenylpropane-2-amine
CNS	Central nervous system
EI	Electron impact
ev	Electron volt
f.d.	Film Depth
GC	Gas chromatography
GC-MS	Gas chromatography- mass spectroscopy
HFBA	Heptafluorobutryl amide
HIAA	5-hydroxyindoleacetic acid
HMAA	4-Hydroxy-3-methoxy-methamphetamine
HMPA	Hexamethyl phosphoramide
HPLC	High performance liquid chromatograpy
I.C.	Inhibetrory concentration
i.d.	Internal diameter
IVC	Intracerebroventricular
LAH	Lithium aluminium hydride
LC	Liquid chromatography
LDA	Lithium di-isopropyl amine
m	Meter
MAO	Monamine oxidase

MBDB	*N*-Methyl-3,4-methylenedioxy-phenylbutanamine
MDA	3,4-Methylenedioxyamphetamine
MDEA	3,4-Methylenedioxyethyl-amphetamine
MDP-2-P	1-(Methylenedioxyphenyl)-2-propanone
MeI	Methyl iodide
min	Minute(s)
ml	milliliter
mm	Millimeter
MS	Mass spectroscopy
MW	Molecular weight
NBS	N-Bromosuccinimide
NIR	Near infrared
nm	nanometer
NMR	Nuclear magnetic resonance
PCC	Pyridinium chlorochromate
PFPA	Pentafluoroperopyl amide
PMK	1-(Methylenedioxyphenyl)-2-propanone
RedAl	Sodium bis(2-methoxyethoxy) aluminum hydride
RT	Room temperature
SAR	Structure activity relationship
THA	2,4,5-trihydroxyamphetamine
THF	Tetrahydrofuran
THM	2,4,5-trihydroxymethamphetamine
TPH	Tryptophan hydroxylase
TPH	Tryptophan
UV	Ultra violet
λ_{max}	Maximum absorbtion at wave length λ

1 LITERATURE REVIEW

1.1 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 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 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.

1.2 Pharmacology of 3,4-MDMA

1.2.1 History

The synthesis of MDA was first reported in 1910 by the German chemists Mannich and Jacobsohn [Thiessen and Cook, 1973]. Animal studies of the pharmacological properties of MDA were carried out in 1939 and included central nervous system stimulatory activity, sympathomimetic effects, and convulsions at high doses [Gunn *et al.,* 1939]. MDA was patented as an ataractic, an appetite suppressant and an antitussive [Shulgin and Nichols, 1978]. Other studies recommended the use of MDA in psychotherapy for its ability to enhance emotions and empathy without producing sensory disruption or hallucination [Naranjo *et al.,* 1967].

Merck was the first company to synthesize and patent MDMA as a potential appetite suppressant in 1914 but due to the lack of commercial interest, the patent expired and MDMA did not become available on the public market. In the 1970s some behavioral effects of MDMA were examined and its lethal dose in several animal species was determined [Hardman *et al.,* 1973]. The first comprehensive report of the pharmacological actions of MDMA in humans appeared in 1978 [Shulgin and Nichols 1978] and it reported that MDMA produced "an easily controlled altered state of consciousness with emotional and sensual overtones". In addition, it was suggested that MDMA might be useful as an adjunct in psychotherapy. In 1985, intense scientific and social interest in MDMA was generated by a decision on the part of the Drug

Enforcement Administration (DEA) in the United States to severely restrict MDMA use by placing it on schedule I of controlled substances [Lawn, 1986].

Since 1986, large numbers of MDMA and MDA derivatives were synthesized in clandestine laboratories as part of custom drugs design. The motivation for custom designing was to avoid police detection and prosecution since it often takes years for the government to detect, classify, and pass legislation against a dangerous designer drug. For example MDEA became popular in the USA only after the placement of MDMA on Schedule I and enjoyed a brief expansion in use until the "designer drug" legislation of 1986, which outlawed the sale of analogs of controlled substances [Beck, 1990]. Another motive was to improve an existing drug and the designer drug. A good example of this effect is 3-methyl fentanyl, an illegal analog of the basic synthetic narcotic drug fentanyl, which is 500 times more potent than pure natural heroin. A market demanding motive for designer drugs was to remove unwanted side effects from an existing drug and the best example was when illicit chemists produced the designer drug Ecstasy which gives the pleasurable effects of the methamphetamines without the unwanted stimulant side effects.

1.2.2 Behavioral effects

Despite differences in potency, time of onset and duration of action, MDA, MDMA and MDEA have all been reported to produce very similar central and peripheral effects in humans. The central effects are described as an easily controlled altered state of

consciousness, with heightened sense of well being, increased tactile sensations, increased perception of an inwardly focused experience and a strong desire to be with and converse with people, without significant perceptual distortion or hallucinations [Hegadoren *et al*., 1999].

3,4-Methylenedioxymethamphetamine was also found to produce hyperthermia and the "serotonin syndrome" in laboratory animals [Green *et al.,* 1995]. 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 convulsions and death. The syndrome results from processes that increase the function of 5-HT in the brain.

The peripheral effects of MDMA are mainly sympathomimetic in nature, mediated by the release of the neurotransmitter norepinephrine. Clinical trials on humans showed that these effects include increased heart rate, elevated systolic and diastolic blood pressure and mydriasis [Harris et al. 2002; Tancer and Johanson 2001]. Most of these symptoms appear within two hours of drug administration [Tancer and Johnson 2003]. Other common effects include tremor, palpitations, diaphoresis, increased salivation, grinding of teeth and tightened jaw muscles [Hegadoren *et al.,* 1999].

The most common reported side / adverse effects of MDMA are drowsiness, muscle aches and general fatigue, depression lasting 1-2 days, difficulty in concentrating, paranoia and short-lived anxiety and irritability [Hegadoren *et al.,* 1999]. Other newly reported side effects are dry mouth or throat [Hernandez-Lopez et al. 2002]. The side

effects increase with successive doses; while the positive subjective effects diminish [Green et al., 1996] with successive doses.

It has been suggested that 3,4-methylenedioxyphenylalkylamines may represent a novel class of pharmacological agents, labeled entactogens [Nichols *et al*., 1986]. The term entactogen is derived from the Greek roots "en" for within or inside and "gen" meaning to produce or originate and the Latin root "tactus" for touch. Hence, the connotation of entactogen is that of producing a "touching within", in reference to the drugs' ability to promote inward reflection and positive self-assessment. These compounds which include MDA, MDMA, MDEA and *N*-methyl-3,4-methylenedioxy-phenylbutanamine (MBDB) do not fit the pharmacological profile of either phenethylamine hallucinogens such as mescaline or psychomotor stimulants such as amphetamine. However, MDMA and related compounds do have stimulant simillarities to both mescaline and amphetamine

1.2.3 Neurochemical effects

Despite MDMA's structural similarity to amphetamine, investigations in animals have indicated that it is not simply an amphetamine-like compound. Although, the neurochemical actions of amphetamine are mediated primarily through dopamine (DA) release, MDMA and its analogues are more potent serotonin (5-HT) releasers than DA *in vitro* [McKenna *et al.,* 1991]. Schmidt *et al.* reported that MDMA released 5-HT from striatal slices at concentrations about 10-fold lower than those that were required to

stimulate DA-release [Schmidt *et al.,* 1987]. Rudnick and Wall demonstrated that MDMA directly and indirectly stimulates 5-HT efflux through the plasma membrane and vesicular membrane transport systems responsible for 5-HT re-uptake and storage in nerve terminals [Rudnick and Wall, 1992]. In plasma membranes MDMA inhibits 5-HT transport and imipramine (inhibitor of the 5-HT-transporter) binding by direct interaction with the Na^+-dependent 5-HT transporter. In membrane vesicles, which contain the vesicular biogenic amine transporter, MDMA inhibits ATP-dependent 5-HT accumulation and stimulates efflux of previously accumulated 5-HT. Stimulation of vesicular 5-HT efflux is due to dissipation of the transmembrane pH difference generated by ATP hydrolysis and to direct interaction with the vesicular amine transporter. It has been suggested that MDMA induces monoamine release by interacting with the monoamine carriers to reverse the direction of neurotransmitter flow [Hekmatpanah and Peroutka, 1990]. In addition, MDMA can also increase extracellular levels of 5-HT, DA and norepinephrine (NE) by inhibiting re-uptake and by delaying metabolism through inhibition of monoamine oxidase (MAO). Thus, MDMA might increase the synaptic monoamine level by at least three different mechanisms: increased release, inhibited uptake and by MAO-inhibition. A major mechanism by which MDMA may acutely affect neuronal excitability in the brain is by increasing extracellular levels of serotonin and catecholamines and thereby indirectly activating 5-HT-, DA- and NE-receptors. However, changes in neuropeptide neurotransmission also may mediate some of the effects of MDMA [White *et al.,* 1996]. Although a study reporting dopamine toxicity in non-human primates raised concerns that MDMA might possess dopamine toxicity

(Ricaurte et al. 2002). Other studies have not found detectable dopamine toxicity in human ecstasy users (Mithoefer et al. 2003)

Other neurochemical effects reported were elevated levels of plasma cortisol and prolactin, in rats, associated with high doses of MDMA [Pacifici et al., 2001 and 2004].

Furthermore, it has been reported that acetylcholine release from the prefrontal cortex and striatum is increased by intravenous administration of MDMA and these increases are paralleled by a dose-dependent activation of spontaneous behavior. Unfortunately the mechanism of the stimulation of acetylcholine release by MDMA is unclear [Acquas *et al.,* 2001].

1.2.4 Neurotoxicity

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 many functions where Seratonin is believed to play an important role. Indeed, functional abnormalities seen in MDMA users that may be related to seratonin injury include cognitive deficits, altered sleep architecture, altered neuroendrocrine fuction, altered behavioral responses to seratonin selective drugs, and increased impulsivity [McCann *et al.,* 2000].

There are several theories of neurotoxicity under investigation, including the possibility that metabolites formed from demethylation of MDMA might be toxic

[Tucker *et al.,* 1994]. The oxidation of metabolites produces free radicals, which in turn induce oxidative stress and membrane damage. The free radical theory of MDMA-induced neurodegeneration has been supported by *in vitro* and *in vivo* works [Green *et al.,* 1996; Colado *et al.,* 1997].

The report of Ricaurte *et al*. in 1985 showed that a single large dose (10 mg/kg) or repetitive low dose (1.25 mg/kg twice daily x 4 days) of MDA produced lasting decreases in biochemical markers for serotonergic function in rat forebrain [Ricaurte *et al*., 1985]. Similar findings were subsequently obtained with MDMA, which produced similar reductions but was less potent than MDA [Scmidt, 1987; Stone *et al*., 1986]. The loss of seratonin content appears to occur in two phases. The first phase is an initial release of seratonin followed by a recovery within 24 hours. The second phase, the long term decrease in seratonin, occurs within approximately 3 days and persists for up to a year [Schmidt, 1987]. Specifically, markers reduced by MDMA treatment are seratonin, its metabolite 5-hydroxyindoleacetic acid (5-HIAA), and the number of 5-HT uptake sites [Schmidt, 1987; Stone *et al*., 1986]. Tryptophan hydroxylase (TPH) activity is also reduced [Scmidt and Taylor, 1988; Stone *et al*., 1986]. Since this enzyme is required for the synthesis of seratonin from the amino acid tryptophan (TRP), the net effect is decreased seratonin levels in the central nervous system (Figure 2).

However, it is probably not the MDMA molecule that causes the irreversible inhibition of TPH, since it has no effect on hydroxylase activity *in vitro* [Schmidt and Taylor, 1987]. Three potential mechanisms for the inhibition of TPH by MDMA have been proposed; (1) some form of oxidative stress within the neuron may oxidize

functional thiol groups within the TPH molecule, (2) a toxic metabolite of MDMA may be responsible for TPH inhibition, or (3) activation of the autoreceptor by released 5-HT may decrease TPH activity [Sprague *et al.,* 1998].

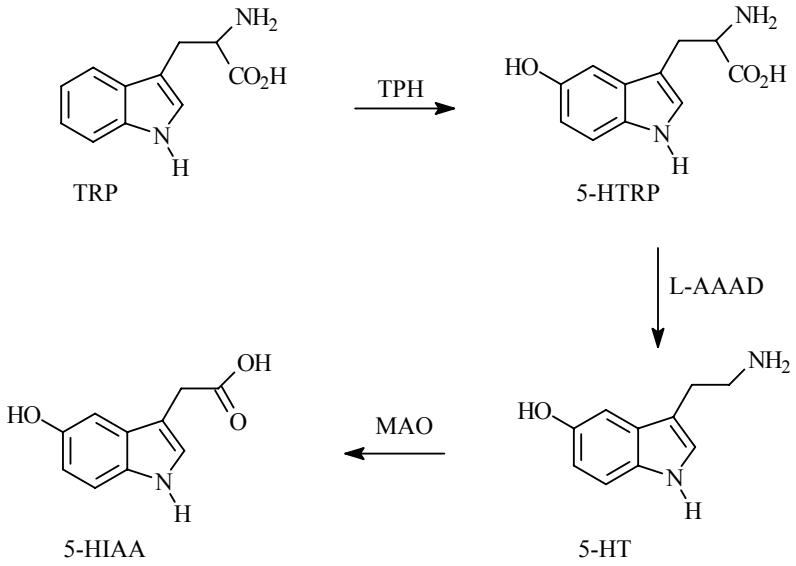


Figure 2: The synthesis and metabolism of serotonin (5-HT).

In addition, serotonin depletion and dopamine receptor activity have been suggested to be key elements in serotonergic neurotoxicity. Sprague *et al.* has proposed an integrated hypothesis for MDMA induced selective serotonergic neurotoxicity. The schematic representation of the hypothesis is shown in Figure 3. MDMA induces an acute release of seratonin and DA. This effect has been shown to be independent of calcium and is thought to be due to seratonin-MDMA exchange. This acute release leads to depletion of intraneuronal seratonin stores. Seratonin released also activates post-synaptic 5-$HT_{2A/2C}$ receptors located on GABA interneurons. 5-$HT_{2A/2C}$ receptor

activation then results in a decrease in inhibitory GABAergic transmission leading to an increase in DA release and synthesis. The excessive DA released is then transported into depleted seratonin terminal. Once concentrated within the seratonin terminal, the DA is deaminated by MAO-B located within the terminal. One of the products of this deamination process is hydrogen peroxide, which may lead to lipid peroxidation and the selective destruction of the seratonin terminal [Sprague *et al.,* 1998]. Some studies showed that the prenatal MDMA exposure induces long-term alterations in the dopaminergic and serotonergic functions in the rat [Galineau L et al 2005]**.** Studies indicated that the serotonin depletion was evident as short as 1 hour(in the hippocampus) and 24 hours (in the striatum) after the first MDMA dose (10 mg/kg) and remained reduced 78 hours later [Williams MT et al 2005]. The pharmacokinetic profile of MDMA in squirrel monkeys after different routes of administration was detemined and found that plasma levels were highly correlated with regional brain serotonin deficits observed 2 weeks later [Mechan A. et al 2006].

An important factor that has been shown to enhance the neurotoxic capacity of MDMA in animal models is the ambient temperature [Malberg et al. 1996; Malberg and Seiden 1998; Malpass et al. 1999], which could have direct implications for dance clubs where MDMA is typically used. Generally, higher doses of MDMA and higher ambient temperature produce hyperthermia; lower doses and lower ambient temperature produce hypothermia, while intermediate levels of dose and ambient temperature may produce biphasic response patterns of hypothermia followed by hyperthermia. For example, a single administration of MDMA, which produces a hyperthermic response (2°C rise in

rectal temperature) at an ambient temperature of 24°C, produces a hypothermic response 1.5°C at an ambient temperature of 11°C [Dafters, 1994]. Malberg and Seiden reported that small (2°C) changes in ambient temperature have a large affect on core body temperature neurotoxicity in MDMA-treated animals. At low ambient temperatures hypothermia and protection against neurotoxicity were obserced, and at high ambient temperatures they reported hyperthermia that correlated with increased neurotoxicity [Malberg and Seiden, 1998]. *In vivo* studies carried on rats discovered a link between skeletal muscle uncoupling proteins in MDMA-mediated hyperthermia. However the mechanisms by which MDMA interacts with skeletal muscle mitochondria is still unknown since these studies found no evidence of uncoupling of oxidative phosphorylation [Rusyniak DE et al 2005].

Research about hypothermia/hyperthermia associated with the administration of MDMA has been extended to higher species animals in an attempt to understand the human model. While some limited prior evidence suggested racimic (+/-) MDMA does not produce hyperthermia in chair-restrained monkeys [Bowyer, J.Fet al 2003]. Another study indicated that hyperthermia is induced by MDMA in unrestrained rhesus monkeys [Tafee MA. et al 2006]. Recent studies indicated that caffeine (10 mg/kg) enhanced the acute toxicity of MDMA (15 mg/kg) and MDA (7.5 mg/kg) in group housed rats through promoting hyperthermia and serotonergic loss [McNamara R. et al 2006].

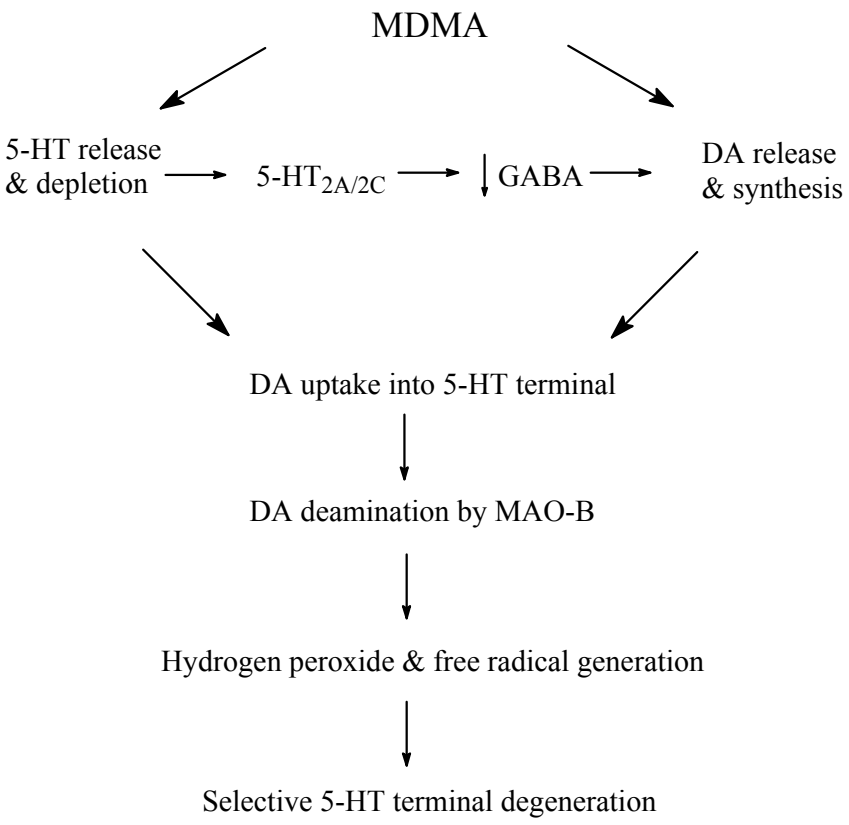


Figure 3: An integrated hypothesis for the development of selective 5-HT terminal degeneration following MDMA.

1.2.5 Metabolism

The methylenedioxyphenylalkylamines undergo two overlapping metabolic pathways: *O*-demethylenation of the methylenedioxy group into dihydroxy derivatives followed by methylation of one of the hydroxy groups and successive degradation of the side chain to *N*-dealkyl and deamino-oxo metabolites [Ensslin *et al*., 1996; Maurer, 1996]. The propanamines MDA, MDMA and MDEA are additionally metabolized to

glycine conjugates of the corresponding 3,4-disubstituted benzoic acids (hippuric acids), unlike the butanamines BDB and MBDB. All hydroxy metabolites are excreted as glucuronic acid and/or sulfate conjugates [Maurer *et al*., 2000]. The proposed scheme for the metabolism is shown in Figure 4.

Demethylenation of methylenedioxyphenylalkylamines to the toxic catechols is mainly catalyzed in humans and rats by CYP2D6.1 and CYP3A4.2 isoenzymes. In humans, MDMA and MBDB could also be demethylenated by CYP1A2. *N*-demethylation of MDMA and MBDB is predominantly catalyzed in rats and in humans by CYP1A2, and *N*-deethylation of MDEA by CYP3A2/4 [Maurer *et al*., 2000].

One of the main metabolites of MDMA is 4-hydroxy-3-methoxy-methamphetamine (HMMA) and it can be detected in plasma and in urine [Helmlin HJ et al 1996]. Plasma concentrations observed for HMAA are quite similar to those corresponding to MDMA. Urinary recovery of HMMA is about 13-14% of 100 mg MDMA dose in 24 h, while MDMA recovery is 24% for the same dose. MDA, formed by *N*-demethylation of MDMA, appears to be a minor metabolite, representing 8-9% of the concentrations of MDMA. This finding is further supported by the fact that MDA urinary recovery is about 1% of the dose administered [De la Torre *et al*., 2000]. Alpha-methyldopamine is a major metabolite of MDA and is readily oxidized to the o-quinone, followed by conjugation with glutathione (GSH). Because the conjugation of quinones with GSH frequently results in preservation or enhancement of biological (re)activity, the metabolites was tested for its effect on depletion of 5-HT and found to cause long term depletion[Miller RT et al 1997; Bai f. et al 1999]. Some studies indicated that these

metabolites, under hyperthermic conditions are more neurotoxic than the parent drugs [Capela JP et al 2006].

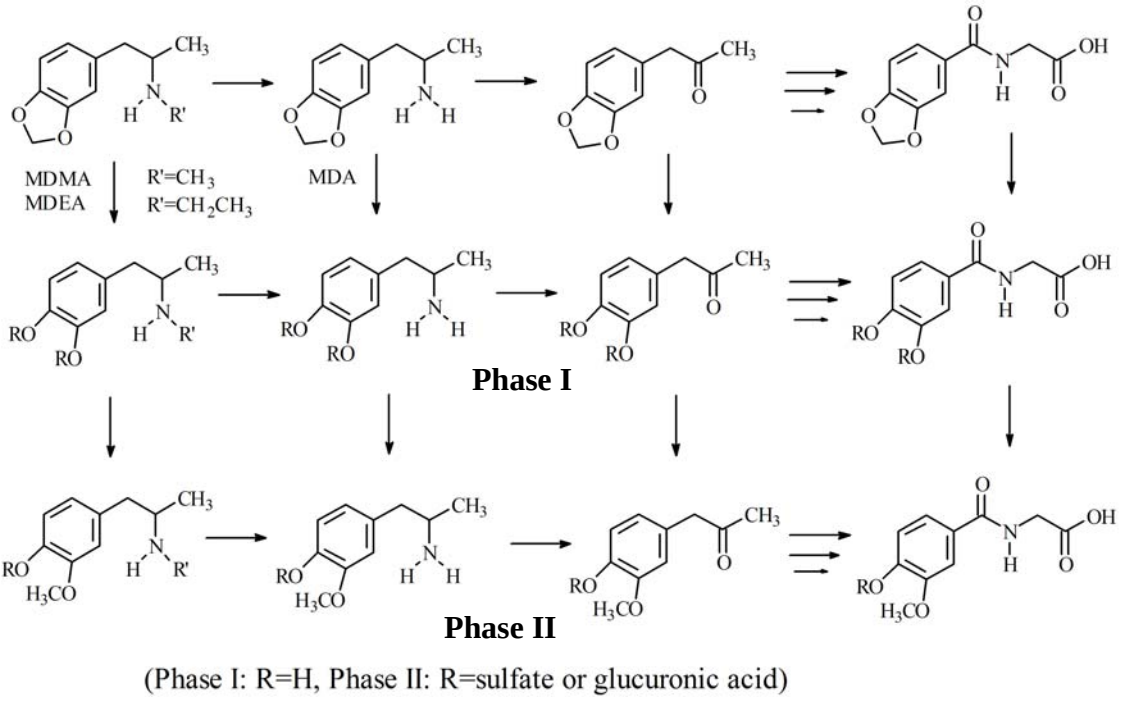


Figure 4: The proposed scheme for the metabolism of the methylenedioxyphenyl-propanamines MDA, MDMA, and MDEA.

Although studies indicate that HMAA induces hyperthermia, however HMAA by itself is not believed to be responsible for altering the striatal dopamine concentration, after i.p. administration, indicates that HHMA is metabolized to other compounds which are responsible for changes [Escobedo I. et al 2004].

Other metabolites of MDMA formed by aromatic hydroxylation have been detected [Lim and Foltz, 1991]. Hydroxylation takes place at positions 2, 5 and 6 of the 3,4-methylenedioxyphenyl ring, however the 6 position hydroxylation is the most common. 2,4,5-Trihydroxymethamphetamine (THM), a potent neurotoxin which is analogous to 6-hydroxydopamine, can be obtained from further metabolism of 6-hydroxy-MDMA (Figure5) [Chu *et al*., 1996]. Respectively, the aromatic hydroxylation of MDA followed by demethylenation yields 2,4,5-trihydroxyamphetamine (THA). Both THA and THM decrease tryptophan hydroxylase (TPH) activity shortly after i.c.v. administration [Elayan *et al*., 1993].

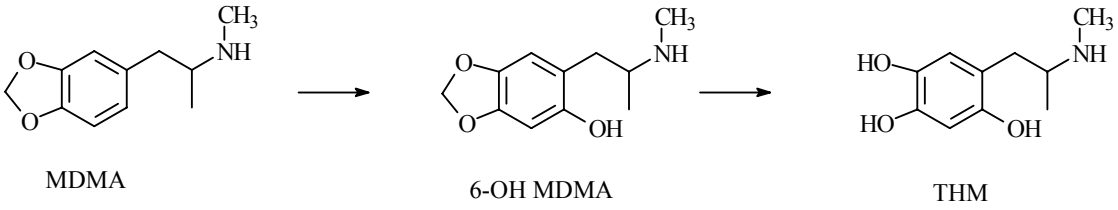


Figure 5: Aromatic hydroxylation of MDMA.

Among MDMA enantiomers, the S-(+)-MDMA was found to be more rapidly [Cho *et al*., 1990] and extensively [Fitzgerald *et al.,* 1989] metabolized than R-(-)-MDMA. The estimated half life for the individual enantiomers were 73.8 and 100.7 min for S-(+) and R-(-)-MDMA, respectively [Cho *et al*., 1990].

1.2.6 Structure-activity relationships of MDMA-like substances

The central nervous system (CNS) activity produced by substituted phenylethylamines ranges from pure stimulant activity to absolute hallucinogenic activity. Modification of the general phenethylamine structure, such as addition of a substituent or alteration of substitution pattern, can significally change or abolish the CNS action of the parent compound. In structure-activity relationship (SAR) studies of MDMA and related substances, there are at least four areas for structural modification (Figure 6).

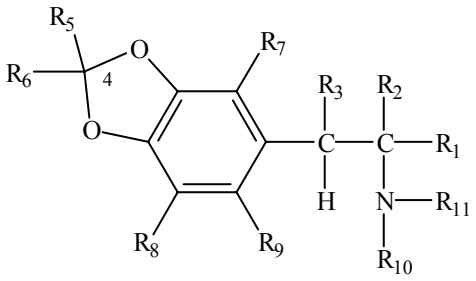


Figure 6: Structural modifications of methylenedioxyphenylethylamines.

First, the variable nature of the amine substituents (R_{10} and R_{11}) including the incorporation of the nitrogen atom in a ring system. A second area is the side chain on the alpha carbon (R_1 and R_2) and a third area is the substitutions on the beta carbon (R_3). Finally, the nature and the location of the ring substituents (R_5 - R_9) can be varied.

1.2.6.1 Amine Substituents

The *N*-substituted MDA derivatives studied for analgesic action and CNS activity showed that only the *N*-methyl (R_{10}=CH_3, R_{11}=H), *N*-ethyl (R_{10}=CH_2CH_3, R_{11}=H), and *N*-hydroxy (R_{10}=OH, R_{11}=H) compounds were active. Structure-activity studies for MDA in several animal assays and in humans indicated that an increase in N-substituent bulk is accompanied by a decrease in central activity. [Braun *et al*., 1980]. The onset of effects ranges from 30 to 60 minutes with MDA to within 30 minutes with MDMA and MDEA [Hegadoren *et al.*, 1999]. Furthermore, duration of action is about 8 hours for MDA and it is about 6 hours for MDMA while MDEA shows a shorter duration of action of 3 to 4 hours.

1.2.6.2 Side chain length and branching

The branched MDA-type molecules having a three carbon side chain (R_1=CH_3, R_2=H) are the most active compounds [Braun *et al*., 1980]. Decreasing the side chain by one carbon (R_1=H, R_2=H) produces unbranched phenethyl derivatives which exhibit reduced stimulant or hallucinogenic activity or both [Glennon *et al*., 1982]. Increasing the side chain by one carbon (R_1=CH_2CH_3, R_2=H) gives butane analogs. The primary amine (R_1=CH_2CH_3, R_2=H, R_{10}=R_{11}=H), 3,4-methylenedioxy-phenylbutanamine (BDB), has both hallucinogenic and stimulant effects [Bronson *et al*., 1995]. The monomethylated derivative of BDB, MBDB, is a less potent stimulant than BDB and *N*,*N*-dimethylated

derivative (MMBDB) is behaviorally inactive [Bronson *et al*., 1995]. However, MBDB is reported to have novel CNS effects with neither stimulant nor hallucinogenic properties [Nichols *et al*., 1986]. In that study MBDB and MDMA were found to be generally similar in effect, with two exceptions. First, the onset of action of MBDB was slower than for MDMA. Secondly, MBDB seemed to produce less euphoria and less stimulant properties than MDMA.

In the case of amphetamine, additional branching of the alkyl side chain produces phentermine (R_1=CH_3, R_2=CH_3) and a decrease in stimulant activity [Biel and Bopp, 1978]. CNS stimulation is deficient in the case of the ethyl homolog (R_1=CH_3, R_2=CH_2CH_3). Phentermine analogs (R_1=CH_3, R_2=CH_3) of MDA and MDMA have been studied and the latter proved to lack MDMA-like activity [Shulgin and Shulgin, 1991]. This indicates that the trend of decreased stimulant activity with increased branching may also hold true for MDMA-like compounds.

1.2.6.3 Beta substitution

By analogy with the amphetamine/phenylpropanolamine and methamphetamine/ ephedrine pairs, hydroxy substitution (R_3=OH) of MDA leads to a decrease in CNS stimulant activity [Biel and Bopp, 1978]. Oxidation of the hydroxy group produces aminoketones (R_3=O) cathinone and methcathinone, respectively. Examination of cathinones indicates a stimulant effect similar to that of their nonoxygenated counterparts [Biel and Bopp, 1978]. β-Keto MDMA (Methylone) was reported to have similar effect

but less potent than MDMA, and therefore it would seem that the effect of carbonyl-oxygen introduction is to decrease potency [Dal Cason et al., 1997]. Recent studies showed that methylone was threefold less potent than the nonketo drugs at inhibiting platelet serotonin accumulation. It was similar in potency to methamphetamine and MDMA on the catecholamine transporters. IC_{50}'s for serotonin accumulation were10-fold higher than the respective values for methamphetamine and MDMA [Cozzi NV et al, 1999].

1.2.6.4 Ring substitution

Addition of one methoxy group (R_7=OCH_3, R_8=R_9=H) in either *ortho* ring position (2- or 6- methoxy) greatly increases the hallucinogenic activity of MDA. On the other hand, *meta* substition (5-methoxy) produces a less potent derivative [Biel and Bopp, 1978]. Dimethoxylation of 3,4-MDA at positions R_7 and R_8 or R_8 and R_9, results in greater hallucinogenic activity than the parent compound [Shulgin, 1978].

Addition of alkyl substituents (steric bulk) to the dioxole ring reduces CNS activity. For example ethylidenedioxyamphetamine (R_5=CH_3, R_6=H) has stimulant activity somewhat less than that of MDA, while isopropylidenedioxyamphetamine (R_5=R_6=CH_3) appears inert until convulsant doses are reached [Nichols *et al.*, 1989].

1.3 Synthesis of MDMA

Different methods have been reported for the synthesis of MDMA, but in clandestine laboratories the commercial availability of precursor chemicals and the ease of synthetic procedures with high yields govern the choice of the synthetic route. The four common MDMA synthesis procedures are illustrated in this chapter and include the reductive amination route, the Leuckart reaction, the nitropropene route and the bromopropane route. Reductive amination is the most direct approach, which involves treatment of the commercially available ketone 1-(3,4-methylenedioxyphenyl)-2-propanone (piperonyl-methylketone, PMK or MDP-2-P) with an amine under reducing conditions (Figure 7). Based on this synthetic route, the sale of the key precursor, MDP-2-P, was regulated in the United States under the Chemical Diversion and Trafficking Act (CDTA) as of March 1989.

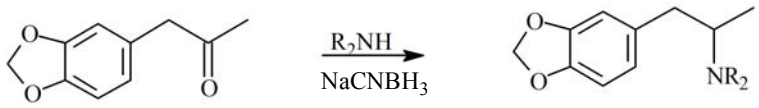


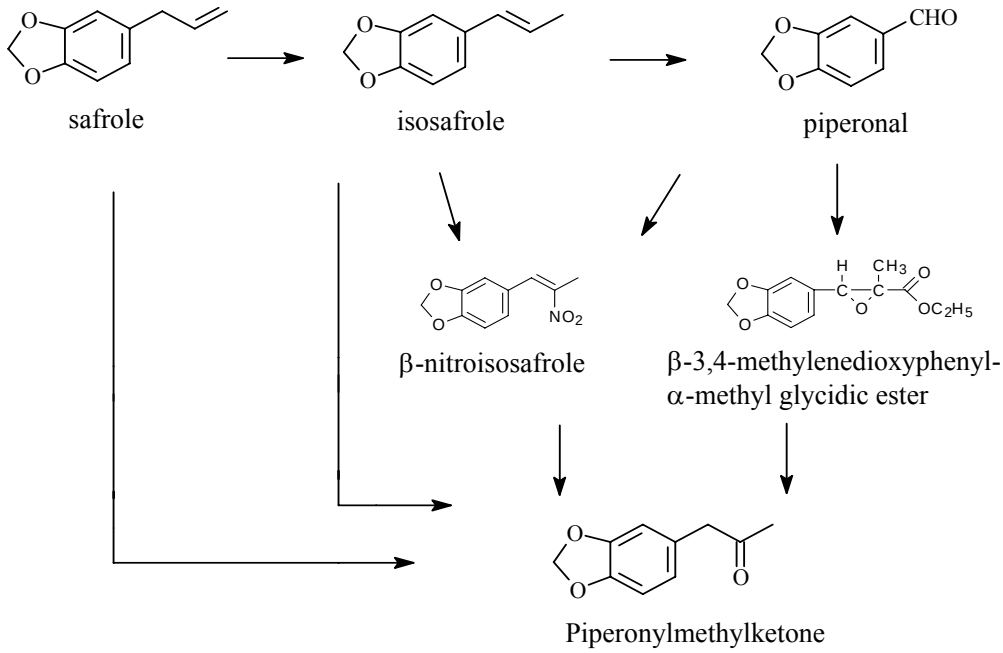
Figure 7: Preparation of 3,4-methylenedioxyphenyl-2-propanamines from PMK by reductive amination.

Regulation of precursor chemicals has provoked clandestine chemists to seek alternative methods for MDMA synthesis. The most frequently used precursors are

piperonal, safrole, and isosafrole, which can be converted to PMK or used directly for the synthesis of MDMA. In this chapter the synthesis of precursors and methylenedioxyphenyl-2-propanamines is discussed. The wide variety of methods used to prepare amphetamines has been adopted for the synthesis of MDMA and related compounds and some of those methods are included in this discussion.

1.3.1 Synthesis of precursors

The most often used precursors are safrole, isosafrole, piperonal and piperonylmethylketone (PMK) and the relationship between these compounds is illustrated in Scheme 1. All four compounds are now commercially available. Safrole and isosafrole are usually encountered as the starting materials in preparation of PMK, whereas piperonal generally serves as the primary precursor for β-nitroisosafrole or glycidic acid esters.



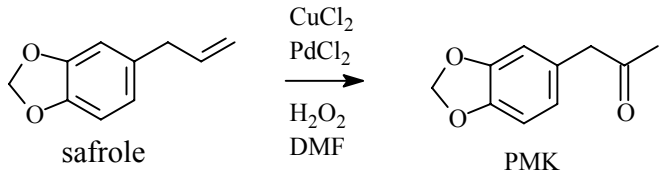
Scheme 1: Relationships of the precursors and MDP-2-P (PMK) synthesis.

1.3.1.1 Safrole as starting material

Safrole is a naturally occurring compound and is a major component of the essential oil from the sassafras tree which is native to North America [Guenther, 1949]. The main volatile oil can be found in the leaves 80-90%, Furthermore, safrole is a minor component of mace and nutmeg, both of which contain 5-methoxysafrole (myristicin) as a major aromatic ingredient [Leung and Foster, 1996].

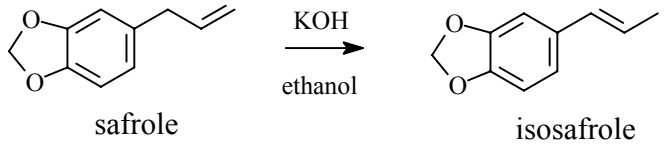
Direct synthesis of PMK can be carried out by oxidation of safrole (Scheme 2). This procedure is called the Wacker-type process which utilizes 30% hydrogen peroxide solution as the oxidizing agent in the presence of palladium chloride as a catalyst and

cuprous chloride as a co-oxidant. The latter function is to reoxidize the Pd to Pd (II) [Dal Cason *et al.,* 1984].



Scheme 2: Oxidation of safrole to PMK.

Safrole can be easily isomerized to the conjugated alkene, isosafrole, by refluxing with ethanol/potassium hydroxide solution (Scheme 3) and distillation can be used to separate the isosafrole from the unreacted safrole [Lukaszewski, 1978]. The product contains both *cis* and *trans*-isomers of isosafrole. The *trans*-isosafrole is thermodynamically more stable than the *cis*-isomer, so with longer reaction times, the amount of the *trans*-isomer is increased in respect to the *cis* isomer.

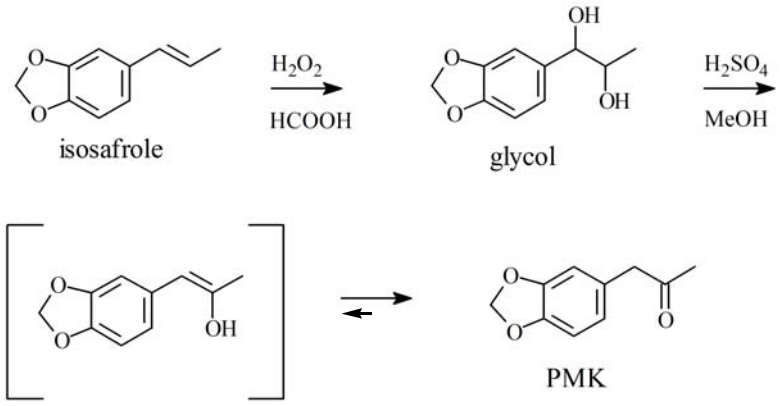


Scheme 3: Isomerization of safrole to isosafrole.

1.3.1.2 Isosafrole as starting material

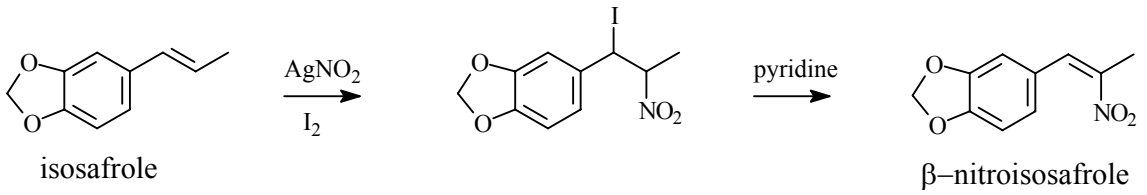
Isosafrole can be converted to PMK through the reaction with formic acid and hydrogen peroxide followed by treatment with sulfuric acid. [Lukaszewski, 1978].

Oxidation of an olefinic compound with formic acid and hydrogen peroxide results in the formation of the *trans* glycol (Scheme 4). Treatment of the glycol with sulfuric acid will result in dehydration which upon tautomerization yields PMK.



Scheme 4: Oxidation of isosafrole to PMK.

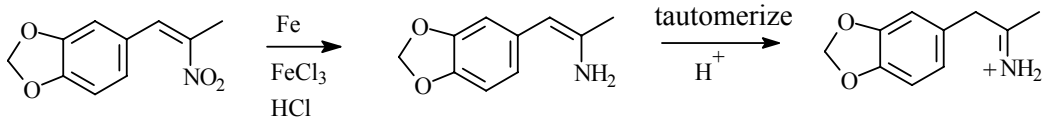
β-Nitroisosafrole is another common intermediate that can be converted to PMK or reduced directly to MDA. β-Nitroisosafrole can be synthesized either from isosafrole or piperonal. Addition of the nitro group to isosafrole can be carried out by nitryl iodide [Hassner *et al*., 1969] or tetranitromethane [Shulgin, 1964]. Nitryl iodide is generated in situ by the reaction of silver nitrite with iodine. Nitryl iodide undergoes regioselective addition to styrene to form the 1-iodo-2-nitrostyrene which upon treatment with base generated the beta-nitrostyrene (Scheme 5).



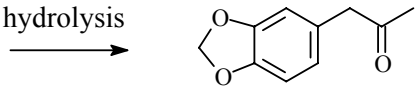
isosafrole β–nitroisosafrole

Scheme 5: Addition of nitro-group to isosafrole

The conversion of β-nitroisosafrole to PMK may be achieved by reacting β-nitroisosafrole with iron and hydrochloric acid in the presence of a catalytic amount of ferric chloride using a two phase solvent system, toluene and water [Heinzelman, 1963]. The nitro group is first reduced to form the enamine, 1-(3,4-methylenedioxyphenyl)-2-aminopropene, which tautomerizes to the imine and undergoes hydrolysis to PMK (Scheme 6).



β–nitroisosafrole



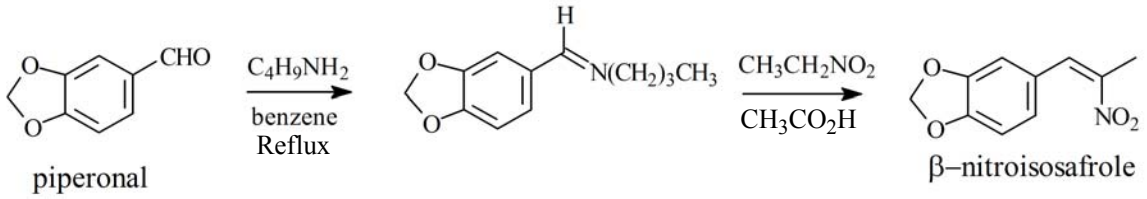
PMK

Scheme 6: Synthesis of PMK from β-nitroisosafrole

1.3.1.3 Piperonal as starting material

Piperonal is a common precursor used for PMK synthesis through the β-nitroisosafrole route. It can be converted to β-nitroisosafrole by reaction with nitroethane

in the presence of a suitable catalyst in a two step process [Hass *et al*., 1950]. The first step is the formation of an imine from refluxing piperonal and n-butylamine using a water separator followed by heating the imine with nitroethane to yield β-nitroisosafrole. (Scheme 7). β-Nitroisosafrole may then be converted to PMK as described in Scheme 6 earlier in chapter 1.3.1.2.

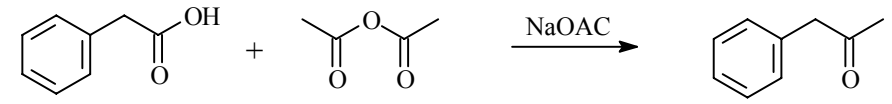


Scheme 7: Synthesis of β-nitroisosafrole from piperonal, nitroethane and butylamine.

1.3.1.4 3,4-Methylenedioxyphenylacetic acid as starting material

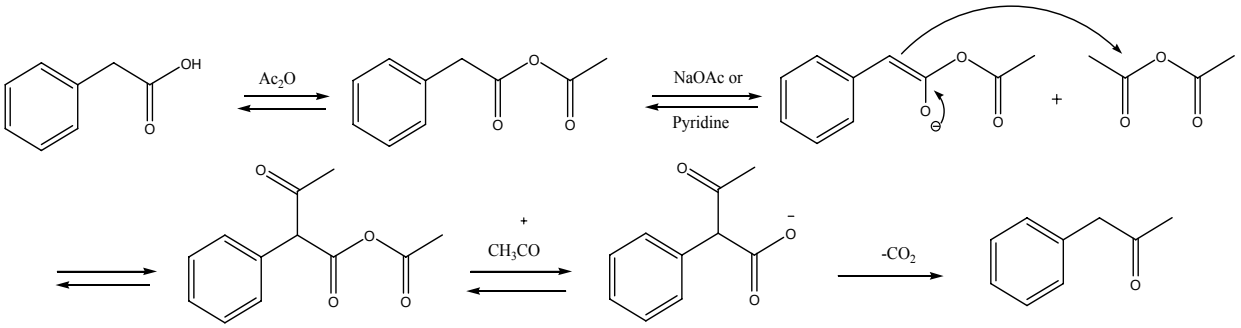
An alternative starting material for PMK is the commercially available 3,4-methylenedioxyphenylacetic acid. Since phenyl acetic acid is one of the commonly used methods to synthesize phenyl-2-propanone (benzyl methyl ketone, BMK), the precursor of amphetamines, it was expected that the same method could be applied in the synthesis of PMK. One of the BMK methods is the base catalyzed condensation of phenylacetic acid and acetic anhydride with sodium acetate in so-called Dakin-West reaction (Scheme 8). Other bases such as sodium bicarbonate, sodium hydroxide, potassium hydroxide and pyridine have been employed with varying degrees of success [Hanel, 1992]. The

possible mechanism of this reaction was described by Buchanan [Buchanan, 1988]. The mechanism may be conceptually broken down into the sequence shown in Scheme 9.



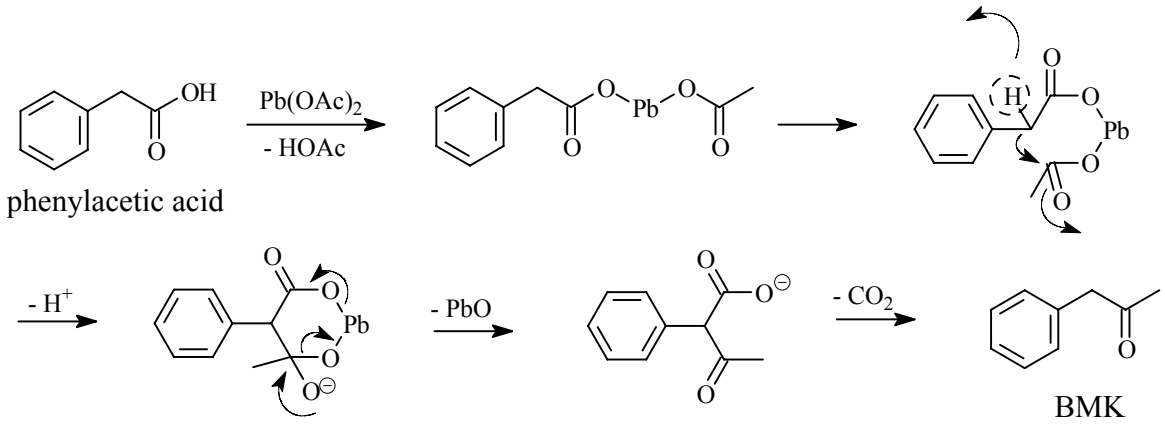
phenylacetic acid BMK

Scheme 8: Synthesis of BMK by base catalyzed condensation of phenylacetic acid and acetic anhydride.



Scheme 9: The Dakin-West reaction of phenylacetic acid and acetic anhydride producing BMK.

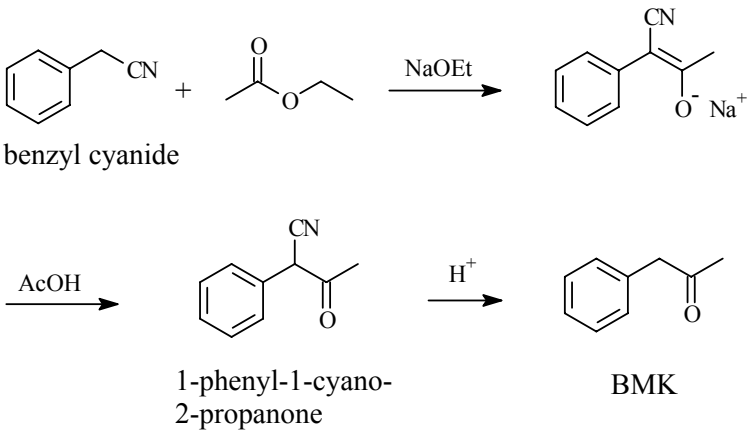
Another method for the manufacture of BMK is the dry distillation of phenylacetic acid with lead (II) acetate in benzene [Allen *et al.,* 1992]. The mechanism for the reaction has been proposed to proceed through the six-membered ring intermediate (Scheme 10).



Scheme 10: The proposed reaction mechanism of phenylacetic acid and lead (II) acetate to form BMK.

1.3.1.5 3,4-Methylenedioxyphenylacetonitrile as starting material

An alternative precursor to PMK may be 3,4-methylenedioxyphenylacetonitrile, based on an analagous reaction starting with phenylacetonitrile (benzyl cyanide) to prepare phenyl acetone [Anon., 1994 and Julian and Oliver, 1984]. In this reaction a solution of benzyl cyanide and ethyl acetate is added to a solution of sodium ethoxide in ethanol and heated at reflux for several hours. Addition of acetic acid will yield the intermediate, 1-phenyl-1-cyano-2-propane, which upon hydrolysis yeilded BMK (Scheme11).

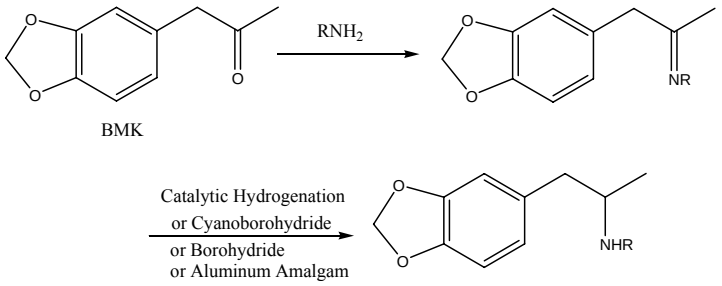


Scheme 11: Synthesis of BMK using benzyl cyanide precursor.

1.3.2 Synthesis of 3,4-methylenedioxyamphetamines

1.3.2.1 The reductive amination route

The most frequently used method to prepare MDMA and other *N*-alkylated MDA derivatives is the reductive amination of PMK. This general method utilizes PMK and an amine (RNH_2) as the starting materials. These two compounds react in an equilibrium to form an imine. The imine is then reduced to the corresponding *N*-alkyl-MDA. The reduction can be carried out by various reducing agents such as hydrogen and a catalyst (catalytic hydrogenation), aluminum amalgam, sodium cyanoborohydride or sodium borohydride (Scheme 12).



Scheme 12: Reductive amination of BMK.

1.3.2.1.1 Catalytic hydrogenation

A significant number of clandestine laboratories in Europe have successfully used catalytic hydrogenation for MDMA manufacture. Reduction of the imine is accomplished on Parr apparatus by treating with hydrogen gas and a suitable hydrogenation catalyst (Raney nickel, palladium or platinum).

1.3.2.1.2 Sodium cyanoborohydride

A mixture of PMK, amine and sodium cyanoborohydride ($NaCNBH_3$) in methanol may be stirred at room temperature for several days. The pH of the mixture is maintained at neutrality by dropwise addition of concentrated hydrochloric acid [Noggle *et al.,* 1987]. Ammonium acetate is used as the nitrogen source in the synthesis of primary amines (MDA), while alkylamines are used in the synthesis of secondary and tertiary amines. The advantage of this procedure lies in the selectivity of the reducing agent. The

$NaCNBH_3$ reduction of aldehydes, ketones and imines is pH dependent and proper control of the pH allows for selectivity of competing reactions [Borch *et al.*, 1971].

1.3.2.1.3 Sodium borohydride

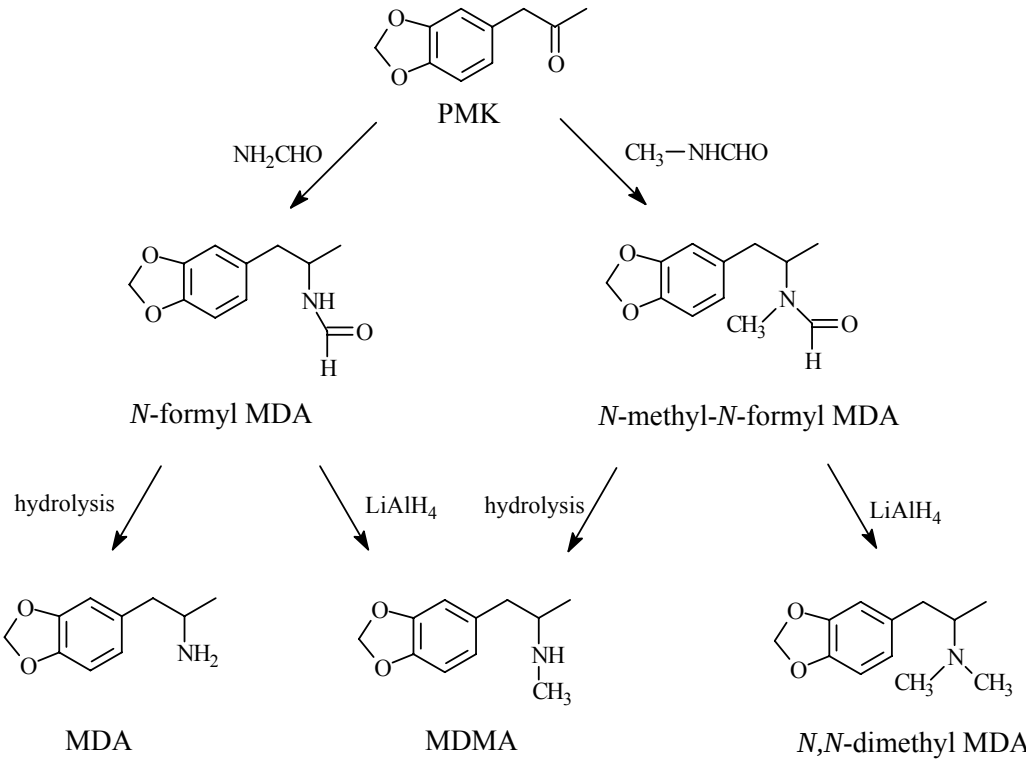
It has been reported that sodium borohydride ($NaBH_4$) as a reducing agent can be used in the synthesis of MDEA [Noggle *et al.,* 1987]. A solution of PMK and aqueous ethylamine is refluxed with sodium borohydride. The reaction mixture was then acidified acidified to pH 1 with concentrated hydrochloric acid.

1.3.2.1.4 Aluminum amalgam

The aluminum foil method is a one step reaction used to prepare methamphetamine and MDMA in clandestine laboratories using dissolving metal reduction, in particular aluminum. In this method, a mixture of PMK and amine (methylamine in the synthesis of MDMA) in alcohol is added slowly and under temperature control to aluminum foil freshly treated with a catalytic amount of mercuric chloride in ethanol. The temperature of the reaction mixture is raised and kept at the boiling temperature of alcohol for several hours [Verweij, 1990].

1.3.2.2 The Leuckart reaction

The Leuckart reaction is one of the oldest and most frequently used methods for the illicit production of amphetamine and methamphetamine. This method was seldom used for the synthesis of the substituted amphetamines [Verweij, 1992]. Reflux of PMK with either formamide, *N*-methylformamide or formic acid results in the formation of the intermediate *N*-formyl or *N*-methyl- *N*-formyl derivative which upon hydrolysis by heating with hydrochloric acid yields MDA or MDMA.

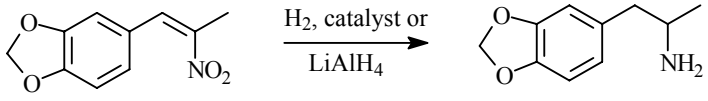


Scheme 13: The Leuckart method in the synthesis of primary, secondary and tertiary 3,4-methylenedioxyamphetamines.

Reduction of these intermediates using lithium aluminum hydride yields the corresponding *N*-methyl-product [Lukaszewski, 1978] (Scheme 13). N-methyl-N-formyl MDA has been observed as a minor component in MDMA dosage forms and likely indicated that the MDMA was synthesized usig the Leuckart method.

1.3.2.3 The nitropropene route

Catalytic metal hydride reduction converts β-nitroisosafrole directly to MDA (Scheme 14). One approach involves hydrogenation of a solution of β-nitroisosafrole in ethanol containing concentrated hydrochloric acid and 5% palladium on carbon as a catalyst[Noggle *et al*., 1991a].

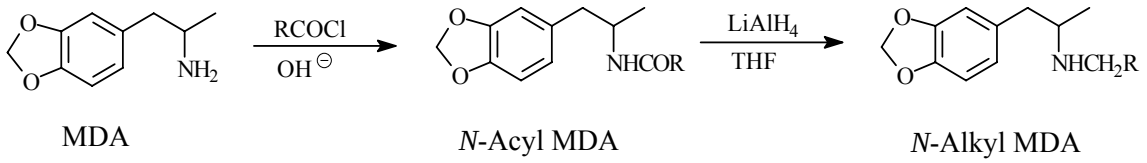


β-nitroisosafrole MDA

Scheme 14: Reduction of β-nitroisosafrole to MDA.

Another approach involves metal hydride reduction of a solution of β-nitroisosafrole in dry tetrahydrofuran (THF) with lithium aluminum hydride [Anon., 1988].

N-alkyl analogs of MDA may be synthesized in a two step sequence involving first acylation of MDA followed by reduction of the resultant amides (Scheme 15). Treatment of MDA with an acid chloride and a base in an organic solvent will yield the corresponding amide [Anon., 1988]. Reduction of the amide to the *N*-alkyl MDA may be carried out by lithium aluminum hydride as described above.

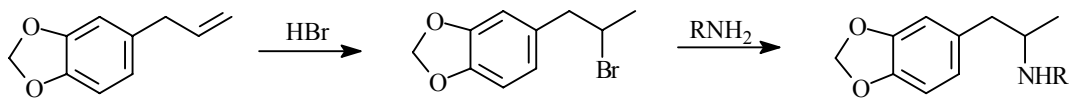


MDA *N*-Acyl MDA *N*-Alkyl MDA

Scheme 15: Acylation of MDA followed by reduction to the *N*-alkyl MDA.

1.3.2.4 The bromopropane route

Long term stirring (7 days) of safrole with 48 % hydrobromic acid yeilds 2-bromo-safrole. Amination, with the corresponding amine in alcohol at room temperature for several days, converts 2-bromo-safrole into MDA or *N*-alkyl MDA [Noggle *et al.,* 1991b] (Scheme 16). It has also been reported that the amination time may be reduced to 7 hours if 2-bromosafrole in alcoholic ammonia or amine solution is heated at 160 °C in the presence of cuprous oxide [Anon., 1988].

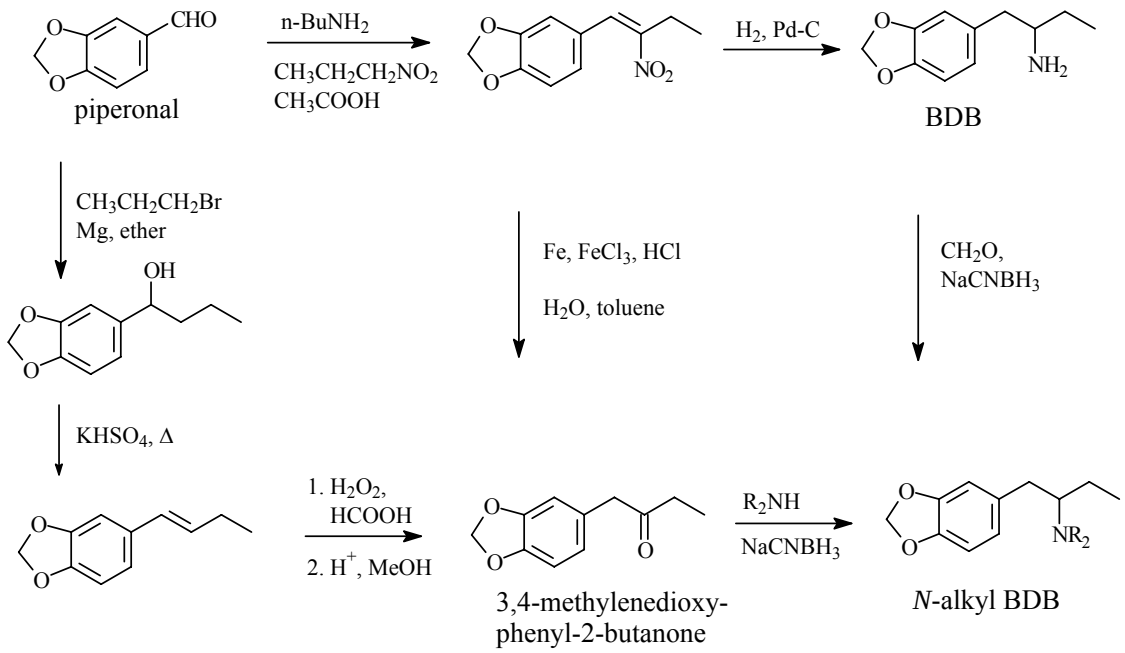


safrole 2-bromosafrole MDA or *N*-alkyl MDA

Scheme 16: Amine displacement reaction of 2-bromosafrole.

1.3.3 Synthesis of 3,4-methylenedioxyphenylbutanamines

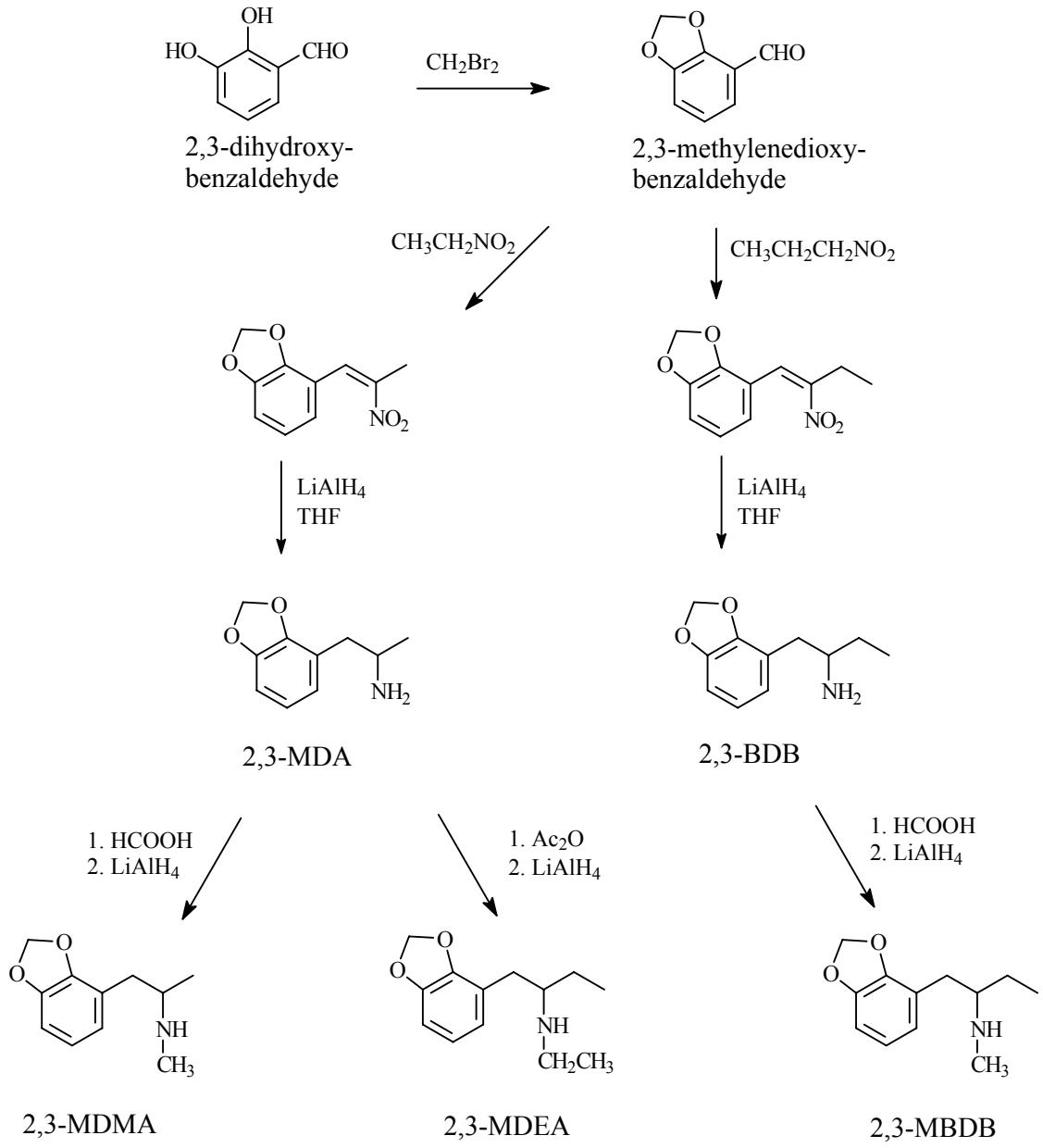
The synthesis of 3,4-methylenedioxyphenylbutanamines were first reported in 1986 [Nichols *et al.,* 1986]. BDB and MBDB were prepared by reductive amination of 3,4-methylenedioxyphenyl-2-butanone. The ketone was prepared from piperonal by treating with propylmagnesium bromide. Dehydration of the resulting alcohol yielded 3,4-methylenedioxyphenyl-2-butene, which was oxidized to the desired ketone (Scheme 17). An alternative way to prepare 3,4-methylenedioxyphenylbutanamines is the condensation between piperonal and nitropropane [Clark *et al.,* 1995a]. 3,4-Methylenedioxy-phenyl-2-nitrobutene can be reduced directly to BDB by catalytic hydrogenation or converted to the ketone by treatment with iron, ferric chloride and hydrochloric acid. Reaction of the ketone with ammonium acetate or alkylamine affords BDB or *N*-alkyl BDBs. addtionally, when BDB is treated with an excess of formaldehyde in the presence of sodium cyanoborohydride, *N*,*N*-dimethyl BDB was obtained.



Scheme 17: Synthesis of 3,4-methylenedioxyphenylbutanamines.

1.3.4 Synthesis of 2,3-methylenedioxyphenalkylamines

The 3,4-methylenedioxyphenalkylamines may be prepared via a large number of synthetic routes due to the wide availability of various precursors. However, synthetic routes to ring regioisomers of the 2,3-methylenedioxyphenalkylamines are limited because of the availability of fewer commercial precursor compounds. Only one precursor, 2,3-methylenedioxy-benzaldehyde is commercially available and can be prepared from 2,3-dihydroxybenzaldehyde by treating with dibromomethane [Soine *et al.,* 1983].



Scheme 18: Synthesis of the 2,3-methylenedioxyphenalkylamines.

The general procedure for synthesis of 2,3-methylenedioxybenzaldehyde and the corresponding MDAs and BDBs is outlined in Scheme 18. Condensation of 2,3-methylenedioxybenzaldehyde with nitroalkane under basic conditions yields the 2-nitroalkenes, which upon reduction with lithium aluminium hydride yields the primary amines, 2,3-MDA and 2,3-BDB. The *N*-methyl and *N*-ethyl-analogs may be prepared by acylation of primary amines followed by lithium aluminium hydride reduction [DeRuiter *et al.,* 1998]. On the other hand, other synthetic routes to the 2,3-methylenedioxyphenalkylamines might be applied by first converting 2-nitroalkene to the corresponding ketone (2,3-PMK or 2,3-methylenedioxyphenyl-2-butanone), which was described in chapter 1.3.1.2.

1.4 Analytical Methods Used to Identify and Separate 3,4-MDMA

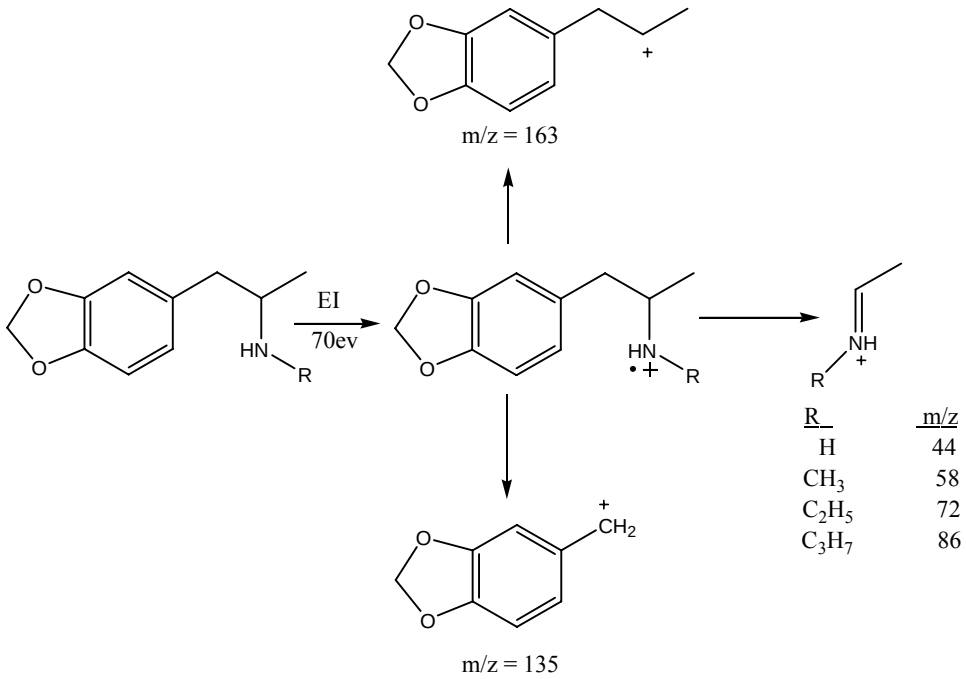
1.4.1 Spectroscopy

1.4.1.1 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 19. 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].



Scheme 19: Mass fragments (EI) of 3,4-methylenedioxyamphetamines.

1.4.1.2 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.

1.4.1.3 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].

1.4.1.4 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].

1.4.1.5 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].

1.4.2 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.

1.4.2.1 High-performance liquid chromatography (HPLC)

1.4.2.1.1 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 1.

Table 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	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 humor, urine	Hypersil BDS C_{18}	FL	Clauwaert *et al*., 2000
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

1.4.2.1.2 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 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 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 3 [Clark *et al.,* 1996].

Table 3: Reversed 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

1.4.2.2 Gas chromatography (GC)

1.4.2.2.1 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 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 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,	urine	DB5-MS	MS (EI, PCI)	Boer *et al.,* 1997

HMMA, HMA)				
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

1.4.2.2.2 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 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.

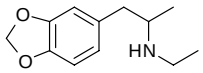
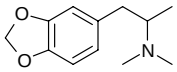
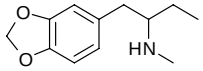
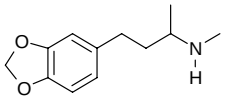
Table 5: Retention times (min) of HFBA derivatives of amphetamines on packed and capillary columns [Lillsunde and Korte, 1991].

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 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 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

1.4.3 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.

1.4.3.1 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.

L.Aalberg reported the 10 direct side chain and ring regioisimers of MDMA including N-ethyl-3,4-methylenedioyphenylethanamine, N,N-dimethyl-3,4-methylenedioxyphenethanamine 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 [L. 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-methylmethamphetamine, 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-propanamine, *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 [L. Aalberg et al. 2003].

L.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 180^0C 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 [L. 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 [L. Aalberg et al., 2004].

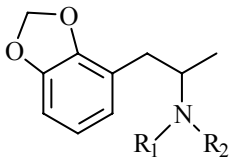
1.4.3.2 Differentiation of regioisomeric 2,3- and 3,4-methylenedioxyphen-alkylamines and isobaric substances by mass spectroscopic methods.

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.

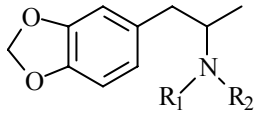
In their study Borth *et al*. were able to differentiate 18 regioisomeric methylene-dioxyphenyl-2-propanamines and methylenedioxyphenyl-2-butanamines (Figure 8). The mixture of compounds was analyzed by GC on a methylsilicone stationary phase and an insufficient separation was obtained. Several compounds (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].



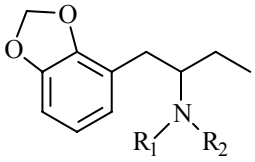
a: $R_1 = H$; $R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = H$; $R_2 = C_2H_5$
d: $R_1 = CH_3$; $R_2 = CH_3$

1



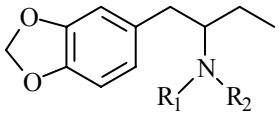
a: $R_1 = H$; $R_2 = H$
b: $R_1 = H$; $R_2 = CH_3$
c: $R_1 = H$; $R_2 = C_2H_5$
d: $R_1 = CH_3$; $R_2 = CH_3$

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

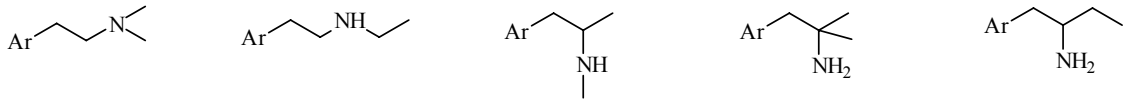
The CI-CID spectra of all ring substituted regioisomers generate a very intense or base peak ion at m/z 135 via cleavage of the benzyl bond by the charge of ammonium cation [Borth *et al.,* 2000b]. The 2,3-methylenedioxy isomeric compounds formed a significant $[C_7H_7O_2]^+$ ion at m/z 123 with the 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.

1.5 Statement of Research Objectives

The broad objective of this research is to investigate the analytical chemistry of the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA). The research will place special emphasis on the specificity of the methods used to identify MDMA and studies will be designed to test and challenge the methods used for the identification of this drug of abuse. These studies will focus on the design and evaluation of compounds capable of producing similar analytical properties to MDMA. The ability to distinguish between these compounds directly enhances the specificity of the analysis for the target molecule.

The initial goal in this project is to prepare samples of the nine direct regioisomeric substances related to MDMA. All ten compounds have the same molecular weight and should yield major fragments in their mass spectra of equivalent mass. The mass spectrum is often the confirmatory piece of evidence in the identification of drugs of abuse and other legally controlled substances. While the mass spectrum is often considered a specific "fingerprint" for an individual compound, other substances can produce very similar or almost identical mass spectra. As demonstrated in previous studies for methamphetamine, the methamphetamine side chain can exist in five regioisomeric forms each yielding equivalent fragments in their mass spectra [Clark *et al.,* 1995b]. 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 (Figure 9). Previous reports indicated that these ten could be separated

chromatographically, however mass spectral discrimination among some of these compounds was based only on relative abundances of some common fragment ions [Aalberg et al.]. Fragmentation occurs in these molecules primarily by an alpha-clevage reaction to yield the benzylic fragment ($ArCH_2^+$) and the substituted imine ($C_3H_8N^+$), thus while other regioisomeric forms exist only these ten can yield fragment ions of the same mass as MDMA. In this study we will use perfluroacyl derivatization in order to improve chromatographic separation and/or to individualize mass spectra of these compounds in an effort to specifically discriminate these unique regioisomers from the drug of abuse, MDMA.



N,*N*-dimethyl-methylenedioxyphenyl-2-ethanamine

N-ethyl-methylene-dioxyphenyl-2-ethanamine

N-methyl-methylene-dioxyphenyl-2-propanamine

α-methyl-methylene-dioxyphenyl-2-propanamine

methylenedioxy-phenyl-2-butanamine

Ar = 2,3 or 3,4-methylenedioxyphenyl

Figure 9: The side chain regioisomeric 2,3- and 3,4-methylenedioxyphenethyl-amines of MW 193 yielding major fragment ions at 58 and 135/136.

In addition, previous studies by Aalberg et al evaluated the analytical properties of another set of ten regioisomeric compounds of mass spectral equivalence of MDMA. Para-methoxymethcathinone was among the compounds prepared and evaluated. The study did not involve the possibility of MDMA co-elution with other methoxymethcathinones substitution patterns (ortho and meta-methoxymethcathiones).

The three methoxymethcathinones are to be prepared and evaluated. (Figure 10) This would be the first reported study to evaluate all three of these compounds.

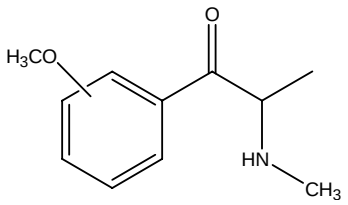


Figure 10: Structure of indirect regioiosmer of MDMA, methoxymethcathinones, of MW 193 yielding major fragment ions at 58 and 135.

The third goal of this work is to evaluate other substances which may yield mass spectra equivalent to that of MDMA. Isobaric substances are defined as compounds of the same nominal mass but of different elemental composition. Thus, for example the methoxy-methyl disubstitution pattern (mass 46, C_2H_6O) is isobaric with methylenedioxy (mass 46, CH_2O_2) disubstitution on the aromatic ring. Therefore, other ring substitution patterns have the potential to produce mass spectra with fragments of equivalent mass to those of 3,4-MDMA. Methoxymethylmethamphetamines share the same side chain substitution of MDMA (Figure 15). Furthermore there is a possibility of co-elution of any or some of these compounds with MDMA in some chromatographic systems. The substitution pattern of both methoxy and methyl groups on the aromatic ring gives a possibility of 10 total compounds. All methoxymethyl methamphitamines were to be prepared and evaluated.

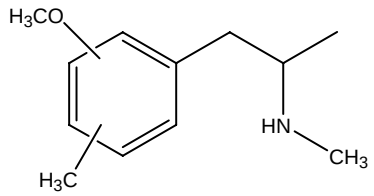


Figure11: Structure of isobaric substances related to MDMA, methoxy methyl methamphetamines, of MW 193 yielding major fragment ions at 58 and 135.

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 "counterfeit substances" from the actual drug of abuse. Those substances which co-elute with the drug of abuse will clearly be misidentified. The ultimate concern then is "if the analyst has never analyzed the counterfeit substances, how can she/he be sure that these compounds would not co-elute with the drug of abuse?" The significance of this question is related to many factors, chief among these is the efficiency of the chromatographic system and the number of possible counterfeit substances.

Following the initial evaluation of the thirteen regioisomeric and isobaric substances to determine their potential to yield a mass spectrum similar to MDMA, the second phase of this work will be a study of the chromatographic properties of these compounds using gas chromatography. The goal of such studies is to compare the structure retention relationships for these compounds and to determine methods to

maximize the chromatographic resolution of these compounds and to determine those conditions which provide maximum resolution between the drug of abuse, MDMA, from the nondrug substances.

2 SYNTHESIS OF THE REGIOISOMERIC AND ISOBARICS AMINES RELATED TO MDMA

Regioisomeric and isobaric substances are considered a significant challenge for the analytical techniques used to identify specific molecules. This is considered extremely important when some of these molecules are legally controlled drugs of abuse and others may be uncontrolled, non-drug species. Methylenedioxyphenethylamines have direct and indirect side-chain and ring substituent regioisomers as well as isobaric substances of equal molecular weight and fragmentation products of identical mass. The direct regioisomers are those substances containing the methylenedioxyphenyl ring system which yields the methylenedioxy benzyl carbocation fragment ($C_8H_7O_2^+$, m/z 135) in the mass spectrum. The indirect regioismers of MDMA consist of those substances which do not contain the methylenedioxyphenyl system yet their empirical formula $C_8H_7O_2^+$ yielding m/z 135, the methoxymethcathinones are perhaps the most likely possibility. The ten methoxy-methyl substituened benzyl carbocation ($C_9H_{11}O^+$, m/z 135) are the isobaric substances evaluated in this study. In this chapter the synthesis of these direct and indirect regioisomeric and isobaric substances related to MDMA are described while their analytical properties including chromatographic separations are discussed in chapter 3.

2.1 Synthesis of the Direct Regioisomers of MDMA.

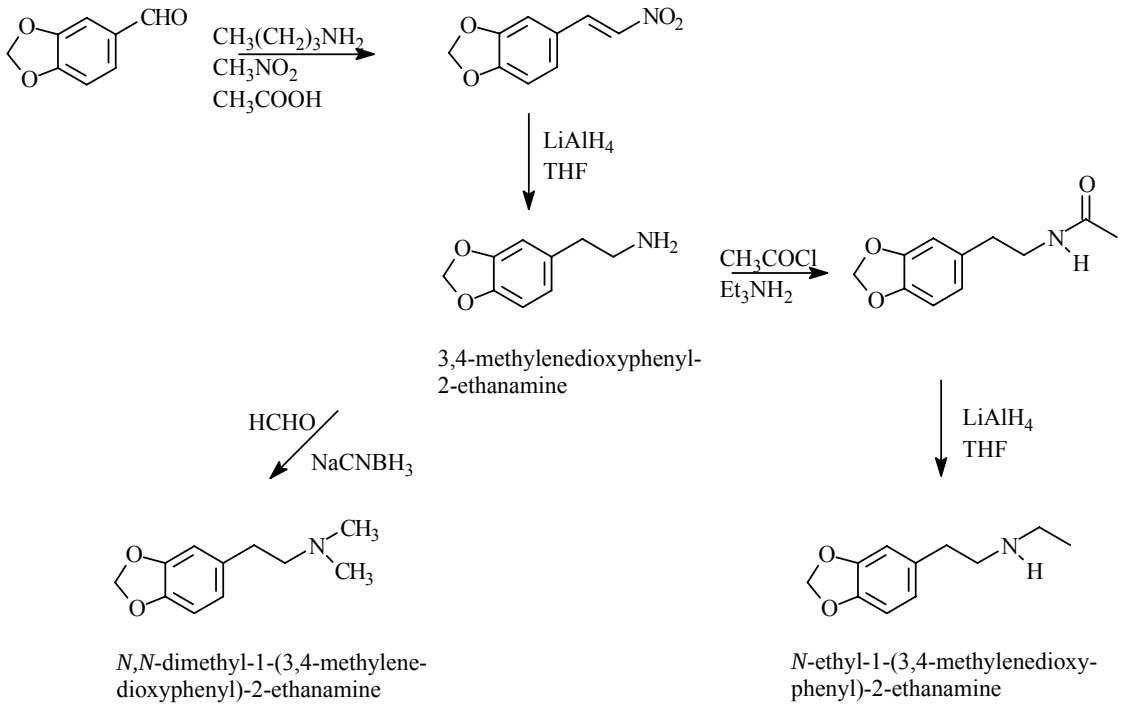
The fusion of the methylenedioxy group in the 2,3- or 3,4- position of the aromatic ring, yields regioisomerism of the aromatic portion of the phenethylamine type molecule. In addition, the alkyl side chain can be modified in various ways to yield regioisomers producing the same mass spectral fragmentation patterns. These modifications result in addition to *N*-methyl-methylenedioxyphenyl-2-propanamine (MDMA) a unique set of a total of ten side-chain regioisomeric 2,3- and 3,4-methylenedioxy-phenethylamines having a molecular weight of 193 yielding major regioisomeric mass spectral fragments at m/z 58 and 135/136. The structures of the ten direct regioisomers are shown in Figure 15 (chapter3).

2.1.1 Synthesis of methylenedioxyphenyl-2-ethanamines.

Synthesis of methylenedioxyphenyl-2-ethanamines was accomplished by synthesizing a primary amine intermediate, 3,4-methylene-dioxyphenyl-2-ethanamine, followed by introduction of an ethyl group or two methyl groups on nitrogen. The synthetic procedure is outlined in Scheme 20. The first step in the procedure was to synthesize the intermediate product, 3,4-methylene-dioxyphenyl-2-ethanamine, via the reduction of 1-(3,4-methylenedioxyphenyl)-2-nitroethene. The synthesis of 1-(3,4-methylenedioxyphenyl)-2-nitroethene was carried out using piperonal as a starting material. A solution of piperonal and *n*-butylamine in benzene was heated at reflux to

form the *n*-butylimine. The imine was dissolved in glacial acetic acid and allowed to react with nitromethane to form the desired 1-(3,4-methylenedioxyphenyl)-2-nitroethene.

The direct reduction of 1-(3,4-methylenedioxyphenyl)-2-nitroethene to 1-(3,4-methylenedioxy-phenyl)-2-ethanamine was accomplished by lithium aluminum hydride. The reduction was done by adding a solution of 1-(3,4-methylenedioxyphenyl)-2-nitroethene in dry tetrahydrofuran (THF) dropwise to a cool stirred suspension of lithium aluminum hydride in dry THF.



Scheme 20: Synthesis of *N*,*N*-dimethyl-1-(3,4-methylenedioxy-phenyl)-2-ethanamine and *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine.

1-(3,4-Methylenedioxyphenyl)-2-ethanamine was then used as a precursor for the synthesis of *N*,*N*-dimethyl and *N*-ethyl 1-(3,4-methylenedioxyphenyl)-2-ethanamine. The synthesis of *N*,*N*-dimethyl-derivative was a one step reaction, which was carried out by stirring a solution of 1-(3,4-methylenedioxyphenyl)-2-ethanamine, 37% formaldehyde and sodium cyanoborohydride in methanol at room temperature for 3 days. The reaction mixture was monitored periodically, and concentrated hydrochloric acid was added to maintain the pH at neutrality.

The *N*-ethyl-derivative was prepared by reduction of the acetamide of 1-(3,4-methylenedioxy)-2-ethanamine. The first step in the reaction sequence was the preparation of the amide using acetyl chloride in THF in the presence of triethylamine. The resulting amide was reduced to the corresponding amine using lithium aluminum hydride. The 2,3-methylenedioxyphenylethanamines were prepared by the same methods using 2,3-methylenedioxybenzaldehyde as starting the material.

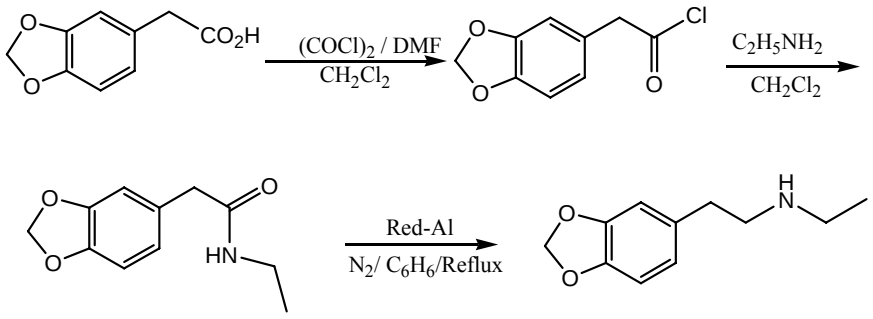
2.1.2 Alternative methods to synthesize N-ethyl-1-(3,4 methylenedioxyphenyl)-2-ethanamine.

Alternative synthetic methods were used to synthesize N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine using different starting materials. These methods involve synthesis of N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine either from 3,4-methylenedioxyphenylacetic acid or from 3,4-methylenedioxyphenyl acetaldehyde.

2.1.2.1 Synthesis of N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine from 3,4-methylenedioxyphenyl acetic acid.

3,4-Methylenedioxyphenylacetic acid was used as a precursor for the synthesis of *N*-ethyl 1-(3,4-methylenedioxyphenyl)-2-ethanamine. The synthetic procedure is outlined in Scheme 21. 3,4-Methylenedioxyphenylacetic acid was converted to 3,4-methylenedioxyphenyl acetyl chloride by refluxing with oxalyl chloride and a catalytic amount of dimethyl formamide in methylene chloride. Dropwise addition of ethylamine to a cooled solution of the crude 3,4 methylenedioxyphenyl acetyl chloride in methylene chloride followed by stirring overnight at room temperature afforded N-ethyl-1-(3,4 methylenedioxyphenyl) acetamide which was then recrystallized from benzene/ hexane.

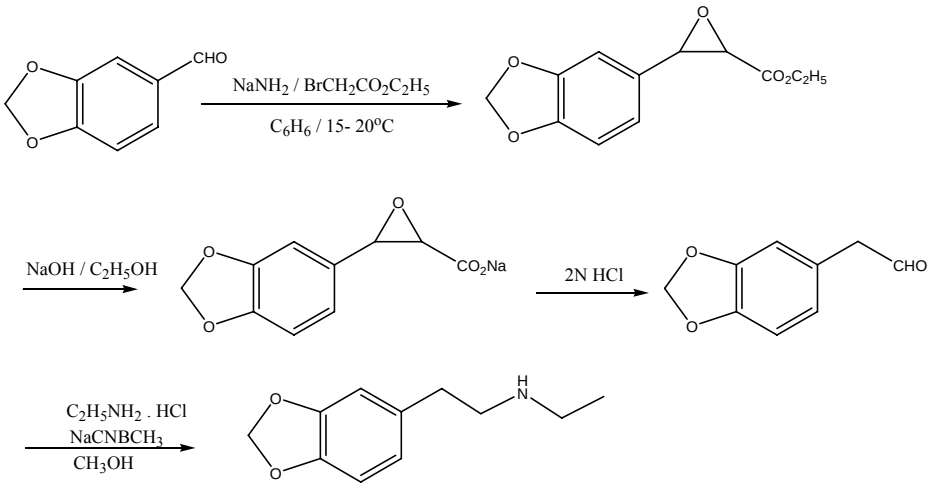
N-ethyl-1(3,4 methylenedioxyphenyl) acetamide in dry benzene was reduced by overnight refluxing with Red al under an atmosphere of nitrogen to yield *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine.



Scheme 21: Synthesis of *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine from 3,4-methylenedioxyphenyl acetic acid.

2.1.2.2 Synthesis of N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine from 3,4-methyelnedioxyphenyl acetaldehyde.

3,4-Methylenedioxyphenyl acetaldehyde was used as a precursor for the synthesis of N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine. The synthetic procedure is outlined in Scheme 22. Sodium amide was added to a cooled mixture of piperonal and ethyl bromoacetate in dry benzene to afford 3,4-methylenedioxyphenethyl glycidic acid ethyl ester.



Scheme 22: Synthesis of *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine from 3,4-methylenedioxyphenylacetaldehyde.

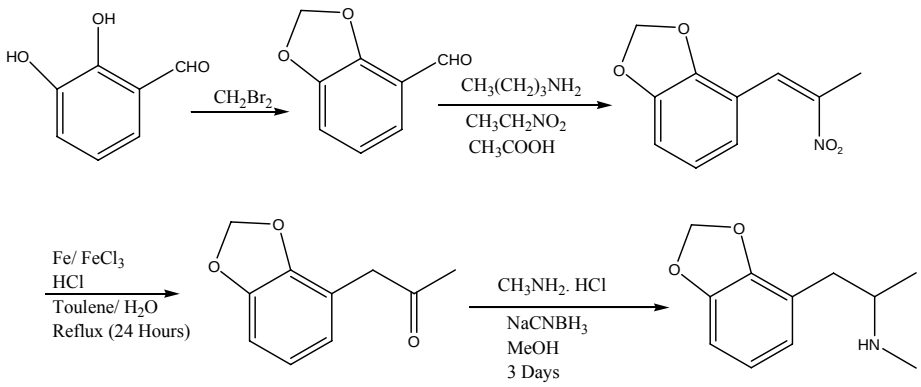
Ester hydrolysis was accomplished by stirring with sodium hydroxide in ethanol to yield 3,4-methylenedioxyphenethyl glycidic acid sodium salt. Warming 3,4-

methylenedioxyphenethyl glycidic acid sodium salt with hydrochloric acid yielded 3,4-methylenedioxyphenethyl glycidic acid which spontaneously decarboxylated to give 3,4-methylenedioxyphenyl acetaldehyde. Reductive amination of 3,4-methylenedioxyphenyl acetaldehyde with ethylamine hydrochloride and sodium cyanoborohydride in methanol at room temperature for 3 days gave the desired amine. The reaction mixture was monitored periodically, and concentrated hydrochloric acid was added to maintain the pH at neutrality

2.1.3 Synthesis of 2,3-MDMA.

All the required 2,3-methylenedioxyphenyl regioisomers can be prepared following the same synthetic procedures used in the synthesis of the 3,4-regioisomers by using the corresponding 2,3-methylenedioxyphenyl starting materials. The required 2,3-methylenedioxybenzaldehyde is not commercially available, yet can be easily prepared from 2,3-dihydroxybenzaldehyde [Soine *et al.,* 1983]. The additional step in the synthesis of 2,3-MDMA followed those already described and are outlined in Scheme 23.

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



Scheme 23: Synthesis of 2,3-MDMA.

The second step in the reaction sequence was the reaction between 2,3-piperonal and nitroethane to yield 2,3-methylenedioxynitropropene. The *n*-butylimine derivative was first formed from 2,3-piperonal and *n*-butylamine followed by the introduction of nitroethane. The reaction mixture was heated at reflux for an hour and 2,3-methylenedioxynitropropene was obtained after purification.

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

The last step in the synthesis of 2,3-MDMA was the reductive amination of 2,3-methylenedioxyphenylacetone with methylamine hydrochloride and sodium cyanoborohydride in methanol. The reaction mixture was stirred at room temperature for three days and the pH maintained at 7 by adding concentrated hydrochloric acid.

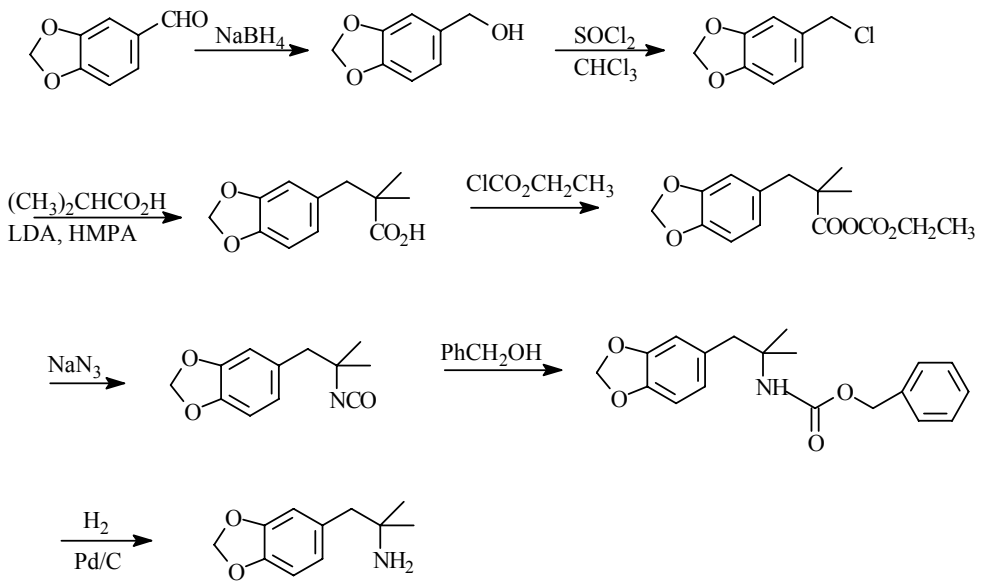
2.1.4 Synthesis of methylenedioxyphentermines.

3,4- and 2,3-methylenedioxyphentermine were synthesized from 3,4- and 2,3-methylenedioxybenzaldehyde, respectively. Since the same method was used to prepare the 2,3- and 3,4-regioisomers, only the synthesis of 3,4-methylenedioxyphetermine is shown in Scheme 24.

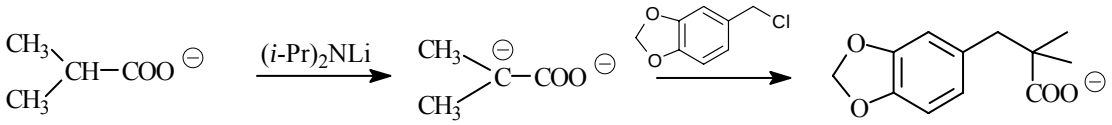
Piperonal was reduced to the benzyl alcohol using excess sodium borohydride. The alcohol was then converted to the benzyl chloride using thionyl chloride in refluxing chloroform. Methylenedioxybenzyl chloride was isolated by Kugelrohr distillation and used in the alpha-alkylation of isobutyric acid (Scheme 25).

The α-position of carboxylic acids can be alkylated by the conversion of their salts to dianions through treatment with a strong base such as lithium diisopropylamine (LDA). The use of Li^+ as the counterion is important, because it increases the solubility of the dianionic salt in the reaction medium [Smith and March, 2001]. LDA was prepared *in situ* from diisopropylamine (in THF) and *n*-butyllithium (in hexane). Isobutyric acid and hexamethylphosphoramide were added dropwise to the resulting mixture. After the mixture was warmed to room temperature, methylenedioxybenzyl chloride was added.

The 2,2-dimethyl-1-(3,4-methylenedioxyphenyl)-propionic acid was isolated as a yellow crystalline solid.



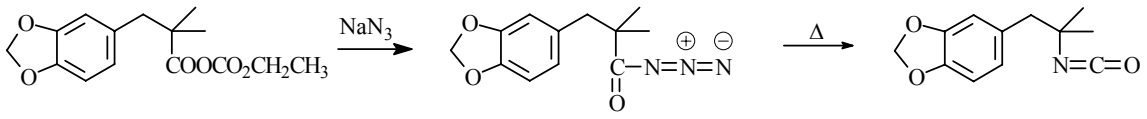
Scheme 24: Synthesis of 3,4-methylenedioxyphentermine.



Scheme 25: Alkylation of isobutyric acid salt.

The product acid was treated with ethyl chloroformate in acetone followed by addition of sodium azide in water. The acyl azide formed was converted to the

corresponding isocyanate by heating a toluene solution of the crude material at 100°C until nitrogen evolution ceased (Scheme 26).



Scheme 26: Preparation of α,α-dimethyl-3,4-methylenedioxyphenylethanisocyanate by the Curtius rearrangement.

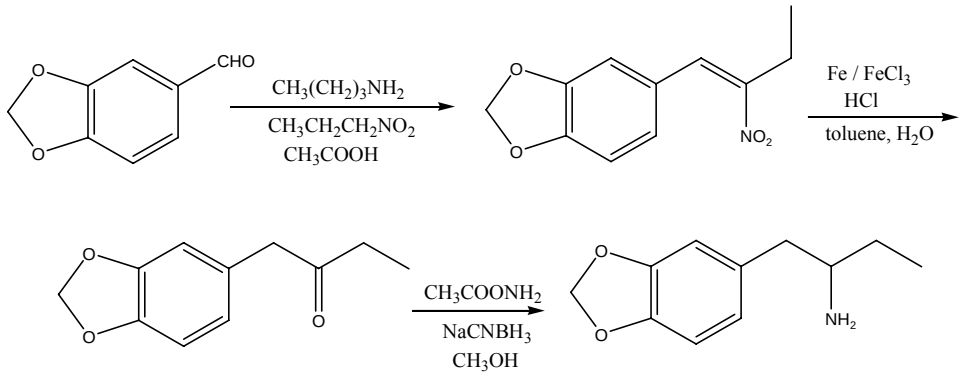
The isocyanate derivative was dissolved in benzyl alcohol and heated to yield 1-[N-(benzyloxycarbonyl) amino]-1,1-dimethyl-2-(3,4-methylenedioxyphenyl)ethane as an amber colored oil. Hydrogenation of the carbamate, 1-[*N*-(benzyloxycarbonyl)amino]-1,1-dimethyl-2-methylenedioxyphenylethane over 10% palladium on carbon yielded 3,4-methylenedioxyphentermine (Scheme 24).

2.1.5 Synthesis of methylenedioxyphenyl -2-butanamine (BDB).

Synthesis of 1-(3,4-methylenedioxyphenyl)-2-butanamine was accomplished by synthesizing a ketone intermediate, 1(3,4-methylenedioxyphenyl)-2-butanone, followed by reductive amination. The synthetic procedure is outlined in Scheme 27. The first step in the procedure was to synthesize the intermediate product, 1-(3,4-methylene-dioxyphenyl)-2-butanone, via reduction followed by hydrolysis of 1-(3,4-

methylenedioxyphenyl)-2-nitrobutene. The synthesis of 1-(3,4-methylenedioxyphenyl)-2-nitrobutene was carried out using piperonal as a starting material. A solution of piperonal and *n*-butylamine in benzene was heated at reflux to form the *n*-butylimine. The imine was dissolved in glacial acetic acid and allowed to react with nitropropane to form the desired 1-(3,4-methylenedioxyphenyl)-2-nitrobutene.

The conversion of 1-(3,4-methylenedioxyphenyl)-2-nitrobutene to 1-(3,4-methylene-dioxyphenyl)-2-butanone was accomplished by reacting the nitrobutene with iron and hydrochloric acid in the presence of a catalytic amount of ferric chloride in two phase solvent system, toluene and water. The reaction mixture was stirred vigorously at reflux for 24 hours. During the reaction the nitro group was first reduced to form 1-(3,4-methylenedioxyphenyl)-2-aminobutene, which tautomerized to the imine and then hydrolyzed to the ketone.



Scheme 27: Synthesis of 1-(3,4-methylenedioxyphenyl)-2-butanamine.

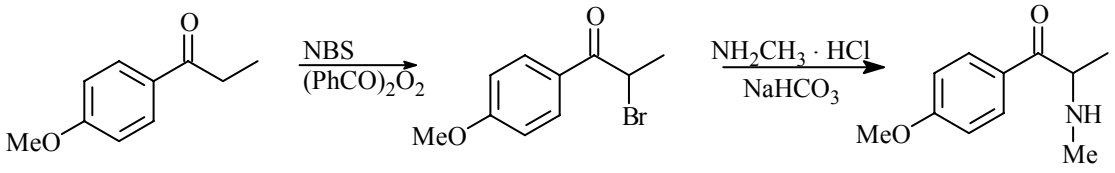
The last step in the synthesis of 1-(3,4- methylenedioxyphenyl)-2-butanamine was the reductive amination of 1-(3,4-methylenedioxyphenyl)-2-butanone. The ketone was dissolved in methanol, and then ammonium acetate and sodium cyanoborohydride were added. The reaction mixture was stirred at room temperature for three days and pH maintained at 7 by adding concentrated hydrochloric acid (Scheme 27).

2.2 Synthesis of the Indirect Regioisomers of MDMA, the Methoxymethcathinones.

There are other regioisomers, which do not contain the methylenedioxy substitution pattern in the aromatic ring, yet they can yield the same mass spectrum as MDMA. For example, a combination of aromatic ring and side chain modifications yields regioisomeric methoxymethcathinones which are considered indirectly regioisomeric with the controlled drug substance 3, 4-methylenedioxymethamphetamine, 3, 4-MDMA. The various isomeric forms of the methoxymethcathinones have mass spectra essentially equivalent to 3,4-MDMA, all have molecular weights of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. While not a direct regioisomer of MDMA, the methoxy benzoyl $(C_8H_7O_2)^+$ fragments have the same mass and empirical formula as the methylenedioxybenzyl $(C_8H_7O_2)^+$ cation occurring at m/z 135. The analytical properties of these compounds are discussed in chapter 3.

2.2.1 Synthesis of *p*-methoxymethcathinone.

The synthesis of *p*-methoxymethcathinone was carried out using *p*-methoxypropiophenone as a starting material. Bromination of the α-position followed by bromine displacement with methylamine gave *p*-methoxymethcathinone (Scheme 28).



Scheme 28: Synthesis of *p*-methoxymethcathinone.

The bromination was achieved by dissolving the starting material in carbontetrachloride followed by addition of *N*-bromosuccinimide (NBS), and a catalytic amount of benzoyl peroxide. NBS is considered a highly regioselective brominating agent at position α to a carbonyl group and benzoyl peroxide served as an initiator in this free radical substitution reaction [Smith and March, 2001]. The reaction mixture was heated at reflux for 21 hours then filtered, and the solvent was then evaporated under reduced pressure to yield *p*-methoxy-α-bromo-propiophenone that was purified using Kugelrohr distillation.

A solution of the bromo-ketone in acetonitrile was added dropwise to a mixture of methylamine hydrochloride and sodium bicarbonate in acetonitrile. The reaction mixture

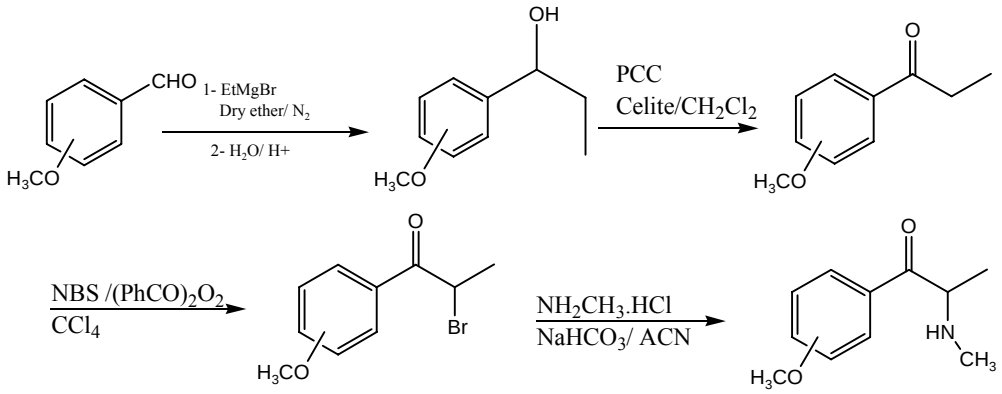
was stirred at room temperature overnight. Evaporation of the solvent followed by isolation of the basic fraction gave light yellow oil, *p*-methoxymethcathinone, which was converted to the corresponding hydrochloride salt using gaseous Hydrochloric acid.

2.2.2 Synthesis of *o*-and *m*-methoxymethcathinones.

The method utilizes the appropriately substituted benzaldehyde as starting material. The synthetic steps used to prepare all three methoxymethcatinones are shown in Scheme 29. The first step in the reaction sequence involved converting the appropriately substituted methoxybenzaldehyde to the substituted methoxyphenyl-propan-1-ol.

A solution of the appropriately substituted methoxybenzaldehyde in dry diethylether was maintained under an atmosphere of dry nitrogen. Ethyl magnesium bromide solution in diethylether was then added dropwise and the reaction mixture was stirred at -20 ^{o}C for two hours. The resulting substituted methoxyphenyl-propan-1-ol in methylene chloride was stirred over night at room temperature with PCC and celite to yield the appropriate substituted methoxypropiophenones which were purified using Kugelrohr distillation.

Methoxypropiophenones were converted to the corresponding methoxymethcathinones via the bromination of the α-position followed by bromine displacement with methylamine to yeild the correspondin*g* methoxymethcathinones using the same procedures stated in section 2.2.1.



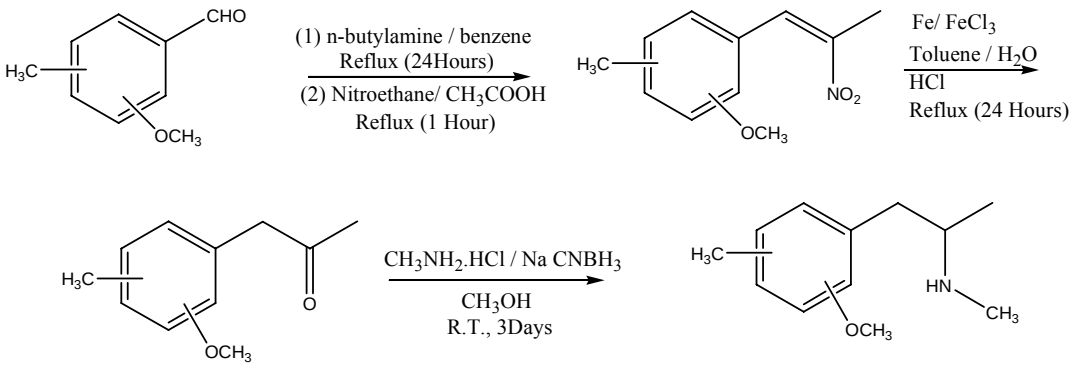
Scheme 29: Synthesis of o-,m- and p-methoxymethcathinones.

2.3. Synthesis of Isobaric Substances Related to MDMA, the Methoxy Methyl Methamphetamines.

There are other isobaric substances, which do not contain the methylenedioxy substitution pattern in the aromatic ring, yet they are able to yield the same mass spectrum as the controlled drug substance MDMA. Among these substances is the methoxy methyl substituted methamphetamines. The various methoxy methyl substitution patterns of this group 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. The 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 The analytical properties of these compounds are discussed in chapter 3.

Ten uniquely substituted methoxy methyl methamphetamines can be synthesized from the appropriately methoxy methyl substituted benzaldehydes by converting them to the corresponding methoxy methyl phenyl-2-propanones followed by reductive amination.

The synthesis of the methoxy methyl methamphetamines was accomplished by synthesizing ketone intermediates, appropriately substituted methoxy methyl phenyl -2-propanones, followed by reductive amination. The synthetic procedure is outlined in Scheme 30. The first step in the procedure was to synthesize the intermediate products, substituted (methoxy-methyl-phenyl)-2-propanones, via condensation followed by hydrolysis of the substituted (methoxy-methyl-phenyl)-2-nitropropene.



Scheme 30: Synthesis of ring substituted methoxy methyl methamphetamines from the corresponding methoxy methyl benzaldehydes.

The syntheses of (methoxymethylphenyl)-2-nitropropenes were carried out using the corresponding aldehydes as a starting material. A solution of appropriately substituted

methoxy methyl benzaldehyde and *n*-butylamine in benzene was heated at reflux to form the *n*-butylimine. The imine was dissolved in glacial acetic acid and allowed to react with nitroethane to form the desired (methoxymethylphenyl)-2-nitropropenes.

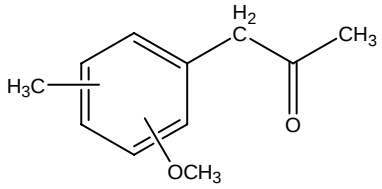
The conversion (methoxy-methyl-phenyl)-2-nitropropenes to the corresponding ketones was accomplished by treating the nitropropenes with iron and hydrochloric acid in the presence of a catalytic amount of ferric chloride in a two phase solvent system of toluene and water.

The reaction mixture was stirred vigorously at reflux for 24 hours. During the reaction the nitro group was first reduced to form (methoxy-methyly-phenyl)-2-aminopropenes, which tautomerized to the imine and then hydrolyzed to give the corresponding (methoxy- methyl-phenyl)-2-propanones.

The ten regioisomeric methoxy-methyl-phenyl-2-propanones are considered the key intermediate to synthesize the methoxy methyl methamphetamine. All the (methoxy-methyl-phenyl)-2-propanones synthesized were identified by H^1NMR spectroscopy (Table7).

The last step in the synthesis of ten isobaric compounds was the reductive amination of appropriately substituted (methoxy methyl phenyl)-2-propanones. The ketones were dissolved in methanol, and then methylamine hydrochloride and sodium cyanoborohydride were added. The reaction mixtures were stirred at room temperature for three days and the pH was maintained at 7 using concentrated hydrochloric acid.

Table 7: H^1NMR data for the 10 regioisomeric (methoxy methylphenyl) -2 propanones.

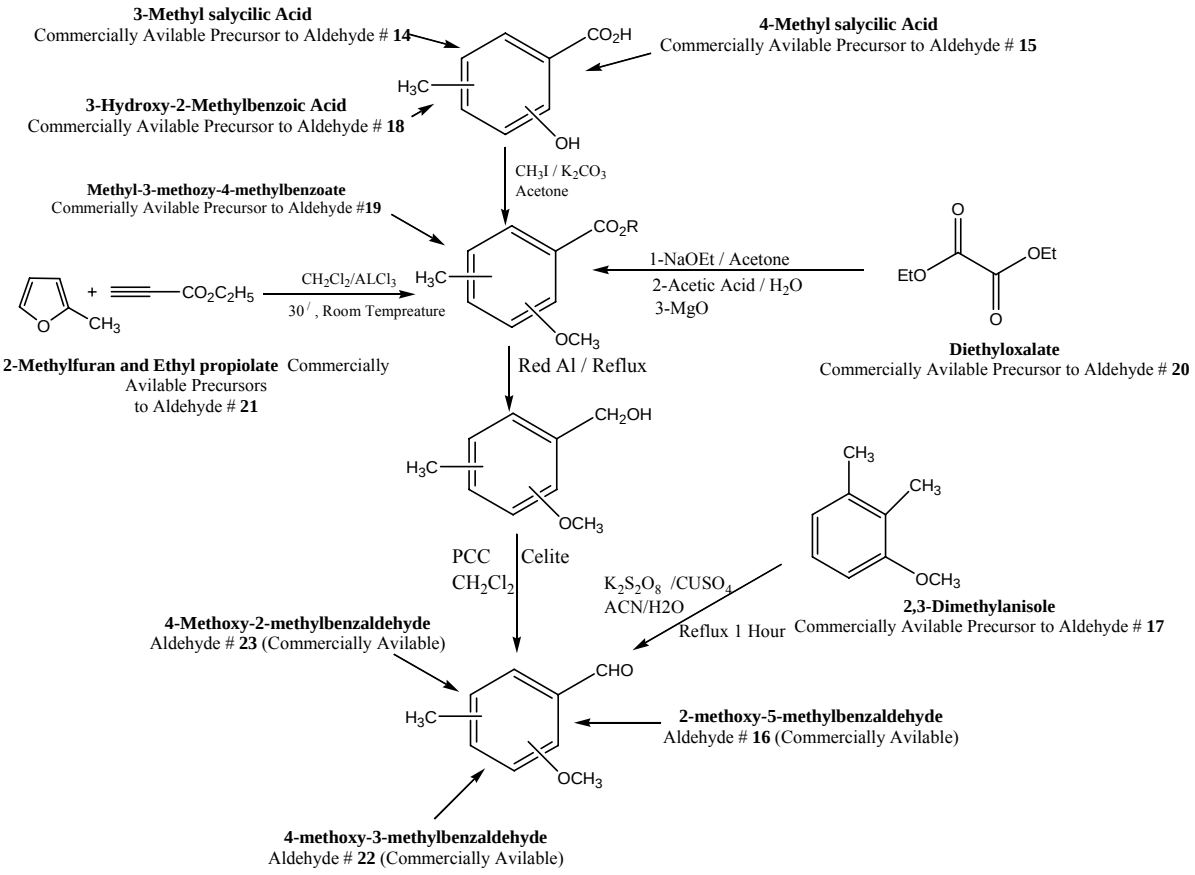


Functional Group	Ar-OCH_3		Ar-CH_3		Ar-CH_2-CO-	-$COCH_3$	Ar-H
	Position	Chemical Shift	Position	Chemical Shift	Chemical Shift	Chemical Shift	Chemical Shift
Chemical Shift (δ ppm)	2	3.763	3	2.297	3.674	2.147	(6.907,2H) (7.042,1H)
	2	3.909	4	3.345	3.790	2.120	6.698-6.818
	2	3.856	5	2.550	3.61	2.114	6.734-6.919
	2	3.663	6	2.269	3.379	2.136	(6.654-6.850,2H) (7.124-7.337,1H)
	3	3.74	2	2.085	3.656	2.075	7.092-6.706,3H
	3	3.818	4	2.091	3.596	2.051	6.62-7.05,3H
	3	3.758	5	2.295	3.386	2.123	6.544-6.610,3H
	5	3.759	2	2.155	3.640	2.119	6.679-7.089, 3H
	4	3.810	3	2.217	3.593	2.135	(7.725,2H) (7.007,1H)
	4	3.819	2	2.191	3.612	2.098	(6.722,2H) (7.00,1H)

2.3.1 Synthesis of substituted methoxy methyl benzaldehydes.

There are only three commercially available methoxy methyl benzaldehydes, 2-methoxy-5-methylbenzaldehyde, 4-methoxy-3-methylbenzaldehyde and 4-methoxy-2-methylbenzaldehde, that were used to synthesize 2-methoxy-5-methyl methamphetamine

(compound 16), 4-methoxy-3-methyl methamphetamine (compound 22) and 4-methoxy-2-methylmethamphetamine (compound 23), respectively.



Scheme 31: Summary of methods used in synthesizing substituted methoxy methyl benzaldehydes.

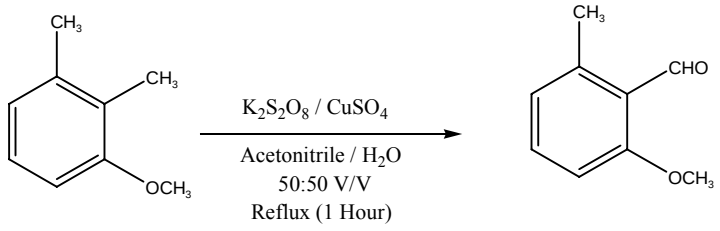
Three other substitution patterns were synthesized from commercially available hydroy-methyl benzoic acids, 3-methyl salicylic acid, 4-methyl salicylic acid and 3-hydroxy-2-methyl benzoic acid. One substitution pattern was prepared from

commercially available methyl ester of 3-methoxy-4-methyl benzoic acid and another from 2,3-dimethylanisole. Thus a total of eight of the required methoxy-methyl-benzaldehydes were available as the appropriately substituted aromatic system. The remaining two aldehydes were prepared by selective synthetic methods which formed the desired substitution patterns; the synthetic routes are summarized in Scheme 31 and described individually in the next sections.

2.3.2 Synthesis of 2-methoxy-6-methylbenzaldehyde.

Hauser and Ellenberger reported that the ortho- methyl group in 2,3-dimethylanisole could be selectively oxidized by refluxing with potassium persulfate and copper sulfate pentahydrate in 50:50 acetonitrile: water (Scheme 32) to yeild 2-methoxy-6-methylbenzaldehyde [Hauser and Ellenberger,1987].

The resulting 2-methoxy-6-methylbenzaldehyde was converted to 2-methoxy-6-methylmethamphetamine (compound 17) using the same procedures illustrated in Scheme 30.

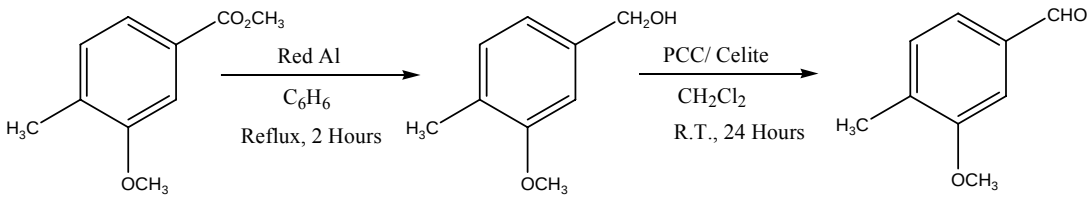


Scheme 32: Synthesis of 2-methoxy-6- methylbenzaldehyde.

2.3.3 Synthesis of 3-methoxy-4-methylbenzaldehyde.

3-methoxy-4-methylbenzaldehyde was synthesized from the commercially available methyl-3-methoxy-4- methyl benzoate. The ester was selectively reduced to the corresponding alcohol, 3-methoxy-4-methyl benzyl alcohol by refluxing with Red Al for two hours. The resulting alchol was selectevly oxidized to 3-methoxy-4-methyl benzaldehyde by overnight stirring with pyridinium chlorochromate and celite in methylene chloride (Scheme 33).

The resulting benzaldehyde was converted to 3-methoxy-4-methyl methamphetamine (compound 19) by following the same procedures outlined in Scheme 30.



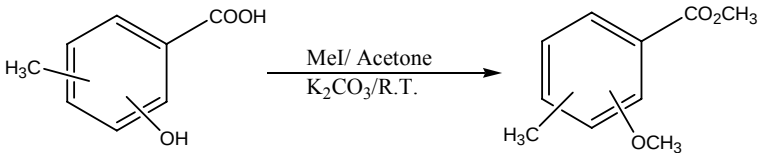
Scheme 33: Synthesis of 3-methoxy-4- methylbenzaldehyde.

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

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

The acids, 3-methyl salicylic acid, 4-methyl salicylic acid and 3-hydroxy-2-methyl benzoic acid were individually stirred with excess methyl iodide in dry acetone and in the presence of potassium carbonate at room temperature for three days yielding methyl- 2-methoxy-3-methylbenzoate, methyl- 2-methoxy-4-methylbenzoate and methyl-3-methoxy-2-methylbenzoate respectively (Scheme 34).

The resulting esters were selectively reduced to the corresponding benzyl alcohols, 2-methoxy-3-methylbenzyl alcohol, 2-methoxy-4-methylbenzeyl alcohol and 3-methoxy-2-methylbenzyl alcohol, followed by selective oxidation to the corresponding benzaldehydes, 2-methoxy-3-methylbenzaldehyde, 2-methoxy-4-methylbenzaldehyde and 3-methoxy-2-methylbenzaldehyde, using the same procedures outlined in Scheme 33.

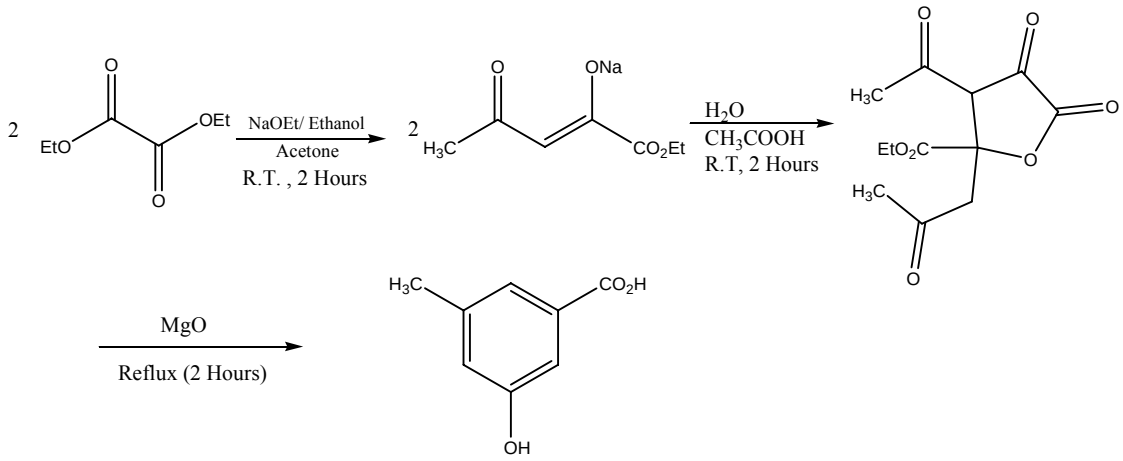


Scheme 34: Synthesis of methyl- ring substituted methoxy methyl -benzoate from the corresponding hydroxyl methyl benzoic acids.

The resulting benzaldehydes were converted to the corresponding methoxymethyl methamphetamines, 2-methoxy-3-methyl methamphetamine (compound 14), 2-methoxy-4-methylmethamphetamine (compound 15) and 3-methoxy-2-methylmethamphetamine (compound 18) using the same procedures outlined in Scheme 30.

2.3.5 Synthesis of 3-methoxy-4-methylbenzaldehyde.

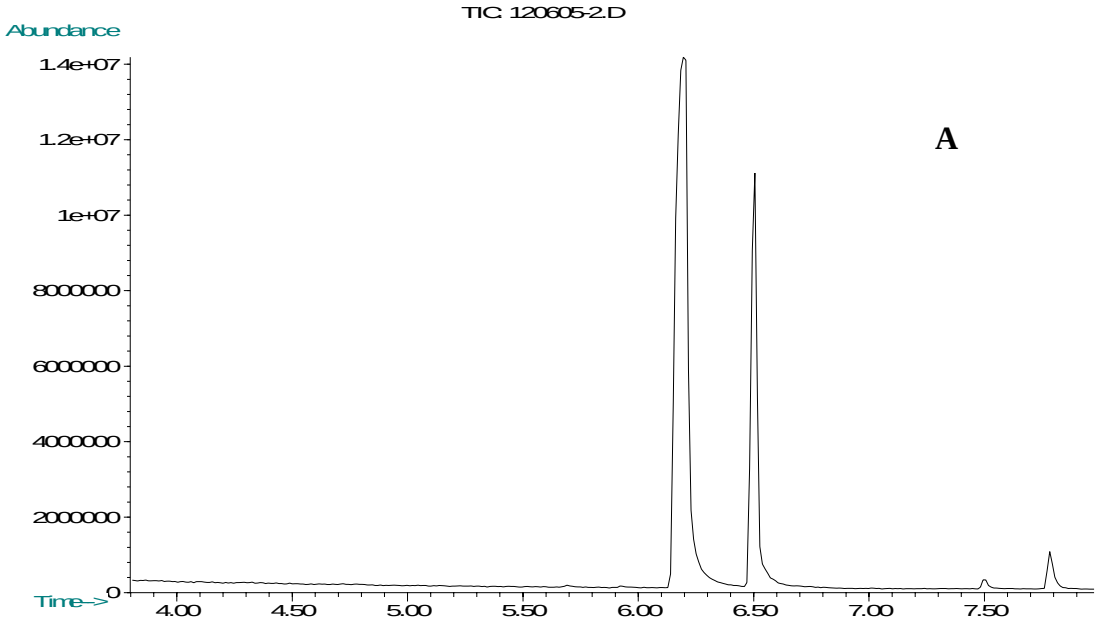
Overnight condensation of acetone and diethyl oxalate in presence of sodium ethoxide under nitrogen atmosphere yielded ethyl sodium acetopyrovate which was converted to 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester by stirring in a mixture of water and glacial acetic acid (1:1 v/v) for two hours at room temperature. 3-Hydroxy-5-methyl benzoic acid was obtained from 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester by refluxing with magnesium oxide in water for two hours [Turner and Gearien, 1952](Scheme 35).



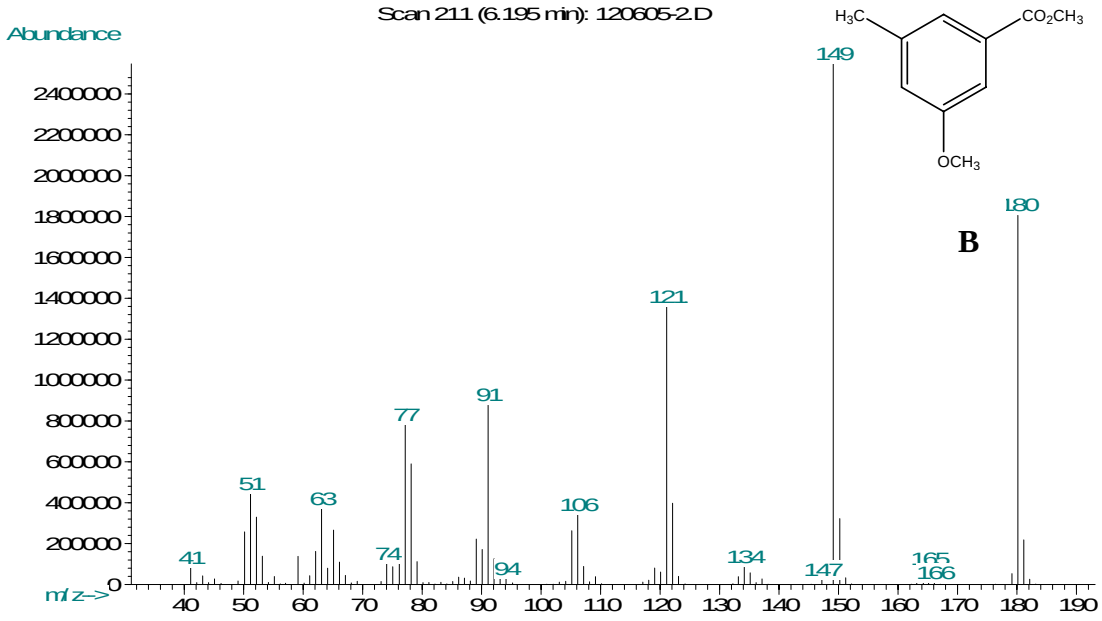
Scheme 35: Synthesis of 3-hydroxy-5-methyl benzoic acid.

3-hydroxy-5-methyl benzoic acid was converted to methyl-3-methoxy-5-methyl benzoate using the same procedures outlined in Scheme 34, however GC-MS analysis of the reaction mixture showed two peaks of m/z 180/149 and 194/149 which indicated a mixture of methyl-3-methoxy-5-methyl benzoate and ethyl -3-methoxy-5-methyl benzoate respectively (Figure 12). These data suggest that during the reaction of 3-acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester with magnesium oxide, a mixture of both 5-hydroxy-3-methlybenzoic acid and ethyl-5-hydroxy-3-methyl benzoate was formed. No attempt at separation of the two products was carried out since the following step includes selective reduction of the ester functional groups following the procedures outlined in Scheme 33 and both compounds yielded one alcohol, 3-methoxy-5-methyl benzyl alcohol (Figure13).

Figure 12: GC (A) and mass spectra of methyl-3-methoxy-5-methyl benzoate (B) and ethyl -3-methoxy-5-methyl benzoate (C).



Time (minutes)



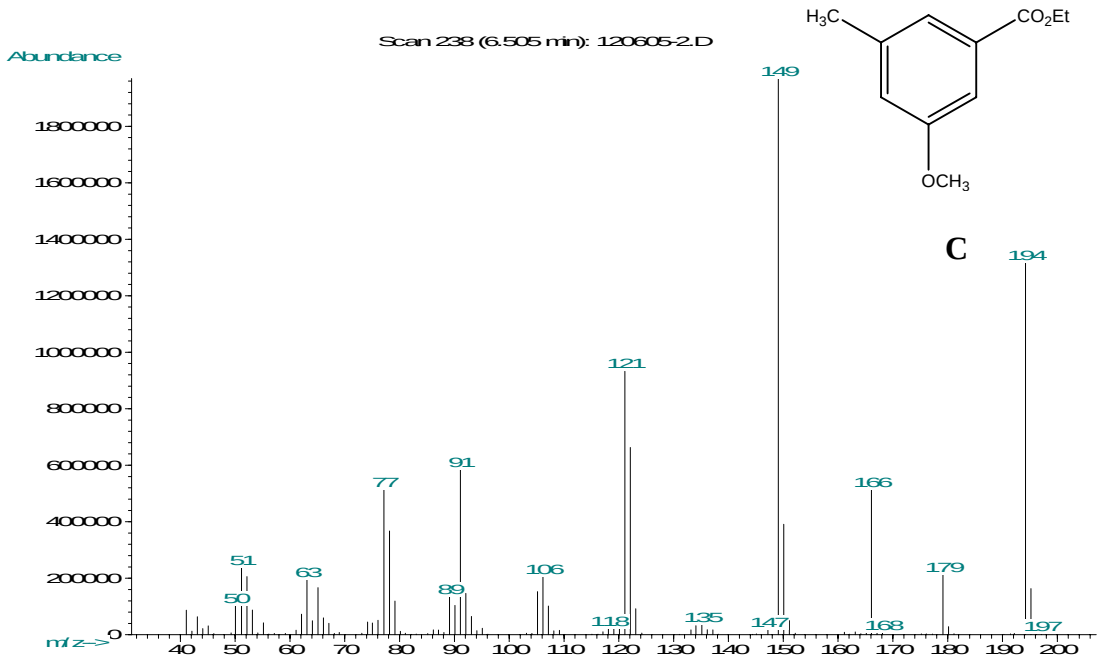
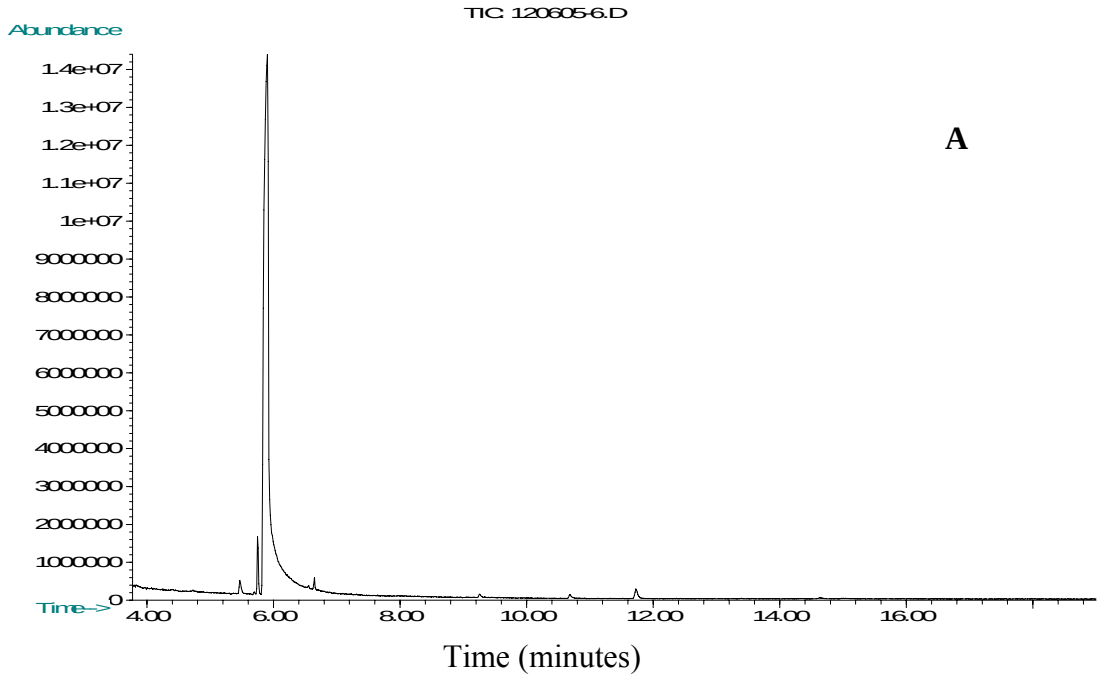
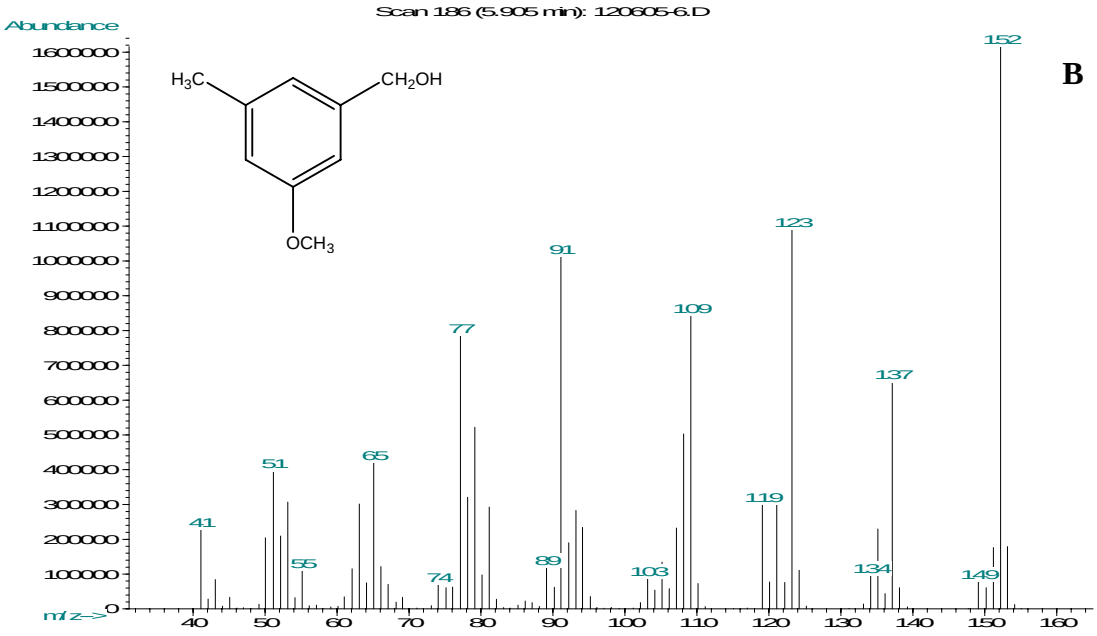


Figure 13: GC (A) and mass spectrum (B) of 3-methoxy-5-methyl benzyl alcohol.



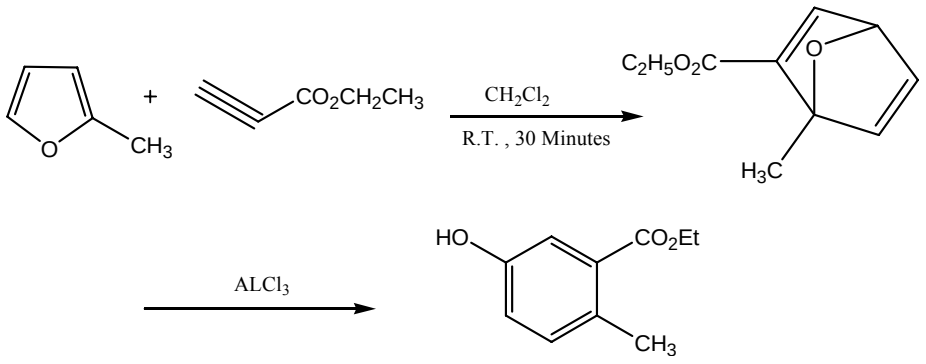


3-Methoxy-5-methyl benzyl alcohol was selectively oxidized to 3-methoxy-5-methylbenzaldehyde using the same procedures outlined in Scheme 13. The resulting benzaldehyde was converted to 3-methoxy-5-methyl methamphetamine (compound 20) using procedures outlined in Scheme 11.

2.3.6 Synthesis of 5-methoxy-2-methylbenzaldehyde.

The Diels Alder type condensation between 2-methyl furan and ethyl propiolate in the presence of anhydrous aluminum chloride yielded ethyl-5-hydroxy-2-methyl benzoate [Randad and Erickson, 2000]. The reaction procedure is outlined in Scheme 36. A solution of 2-methylfuran in methylene chloride was added dropwise to a solution of ethyl propiolate and anhydrous aluminum chloride in methylene chloride and the

resulting reaction mixture was stirred at room temperature for 30 minutes. The reaction was terminated by addition of water and ethyl-5-methoxy-2-methyl benzoate was synthesized by refluxing 5-hydroxy-2-methyl benzoic acid ethyl ester with methyl iodide and potassium carbonate in dry acetone over night. The procedures are outlined in Scheme 34.



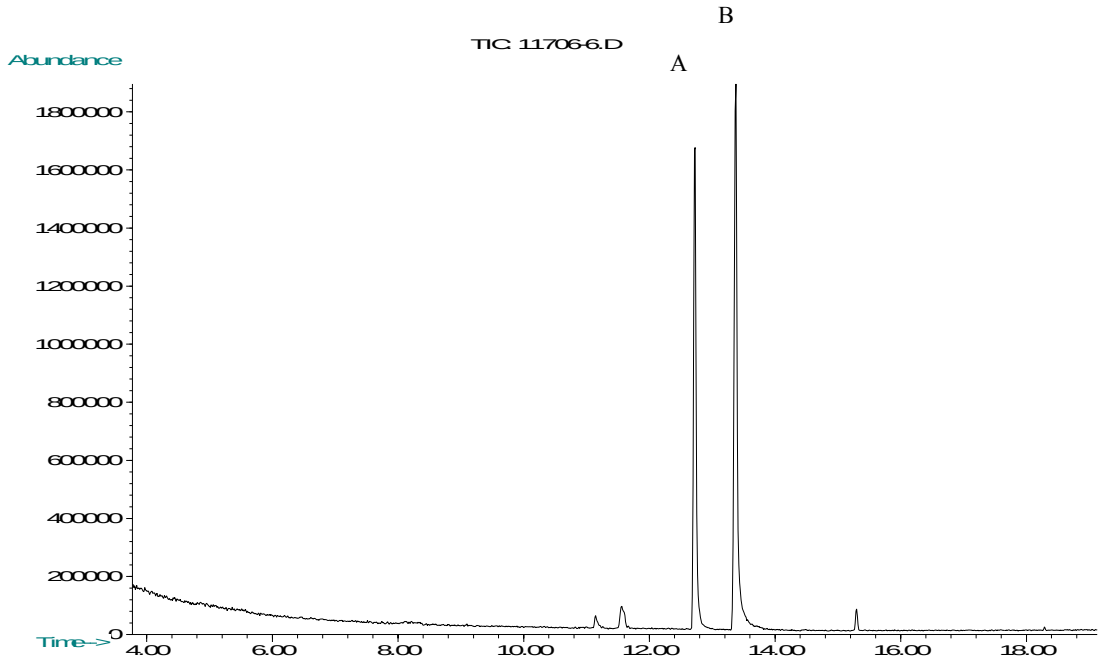
Scheme 36: Synthesis of ethyl-5-hydroxy-2-methylbenzoate.

Synthesis of 5-methoxy-2-methyl benzaldehyde was carried out using the same procedures outlines in Scheme 33, where selective reduction of methyl-5-methoxy-2-methyl benzoate using Red-Al yielded 5-methoxy-2-methyl benzyl alchol which was then selectively oxidized using pyridinium chlorochromate and celite in methylene chloride to yield the desired methoxy methyl substituted benzaldehyde. The initial cycloaddtion reaction is reported to be regiospecific yielding only 1-methyl-2-carbethoxy-7-oxobicyclo [2.2.1] heptadiene intermediate shown in Scheme 36. If the other intermediate had formed (i.e.1-methyl-3-carbethoxy-7-oxobicyclo [2.2.1] heptadiene) the resulting

aldehyde would be 2-methoxy-5-methyl benzaldehyde. These corresponding ketones were separated using GC (Figure14) and allowed successful monitoring of the above reaction sequance. Furthermore mass spectra show m/z 105 characteristic of 2-methoxy-5-methylphenyl acetone and all o-methoxy substituted methylphenyl acetones (chapter 3).

5-Methoxy-2-methyl benzaldehyde was converted to 5-methoxy-2-methyl methamphetamine (compound 21) using procedures outlined in Scheme 30.

Figure 14: Capillary gas chromatographic separation of 2-methoxy-5-methylphenyl acetone (A) and 5-methoxy-2-methylphenyl acetone (B).



Time (minutes)

3 ANALYTICAL STUDIES OF REGIOISOMERIC AND ISOBARIC SUBSTANCES RELATED TO MDMA

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

substances. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of abuse.

All the regioisomers, direct and indirect as well as isobaric compounds have a strong possibility to be identified as 3,4-MDMA, by some analytical methods especially mass spectrometry. In this chapter, all direct ring and side chain regioisomers, and some indirect regioisomers as well as a group of isobaric compounds of 3,4-MDMA are compared by chromatographic and spectroscopic techniques, and methods for their differentiation are explored. The regioisomers and isobars numbered in this chapter follow the same numbering system through out this document.

3.1 Mass Spectrometry and Gas Chromatographic Studies of 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**.** Their mass spectral properties are discussed in chapter 3.1.1 and 3.1.2. While the chromatographic separation of the derivatized primary and secondary amines will be discussed in chapter 3.1.3.

3.1.1. 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.

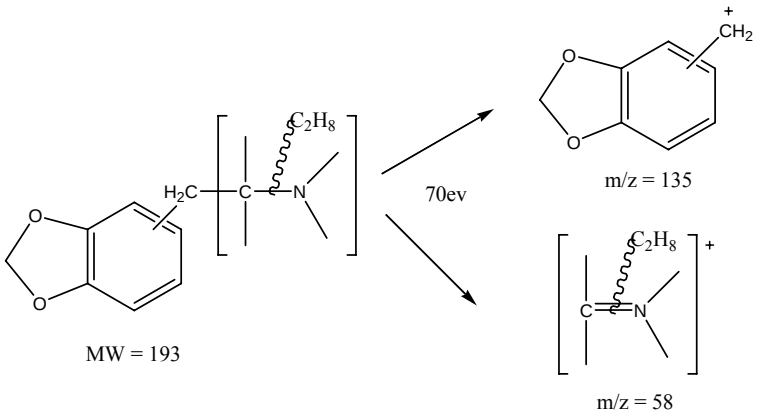
There are nine other methylenedioxy substituted regioisomers (a total of ten compounds) of the 3,4-MDMA molecule (MW=193) which yield α-cleavage fragments at m/z 58 and 135/136 during analysis by mass spectrometry (Scheme37). The mass spectra in Figure 15 are for the ten possible direct regioisomers 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

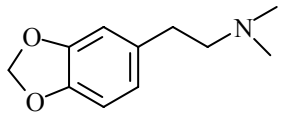
possibility. Furthermore, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest



Scheme 37: General mass spectral fragmentation for the methylenedioxy regioisomers of MDMA (compounds 1-10).

Figure 15 : Structures and mass spectra of methylenedioxy substituted side-chain regioisomers.

Structure of regioisomers **Mass spectrum**



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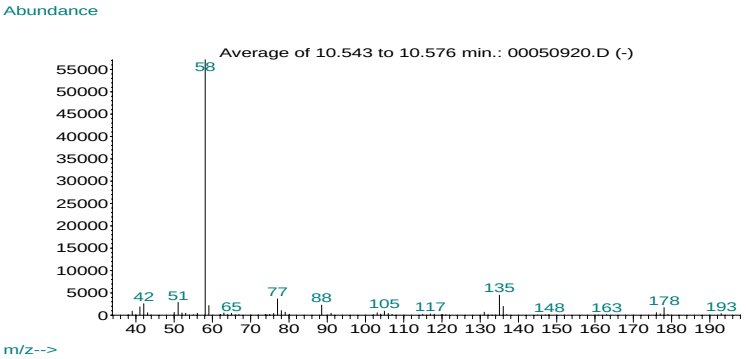

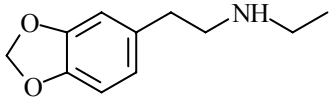

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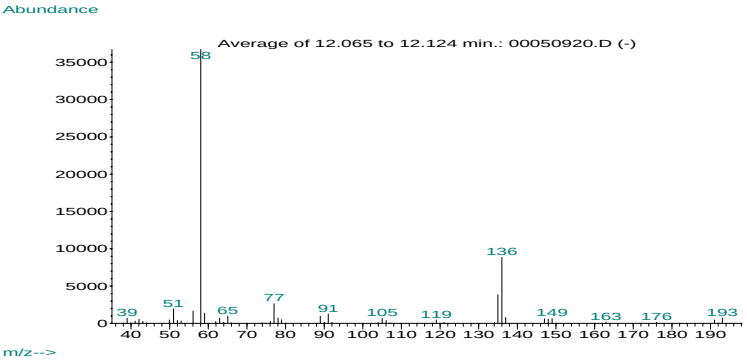

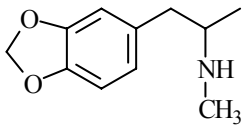

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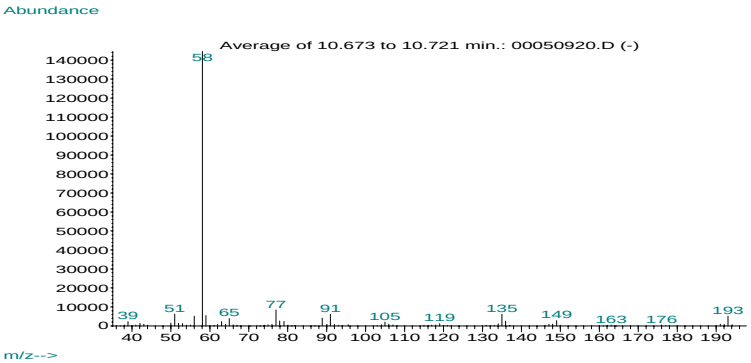

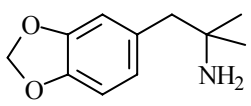

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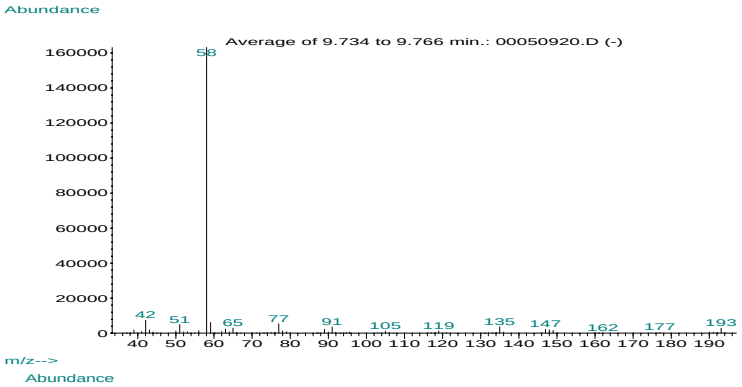
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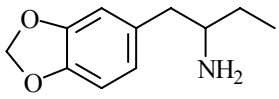
m/z-->



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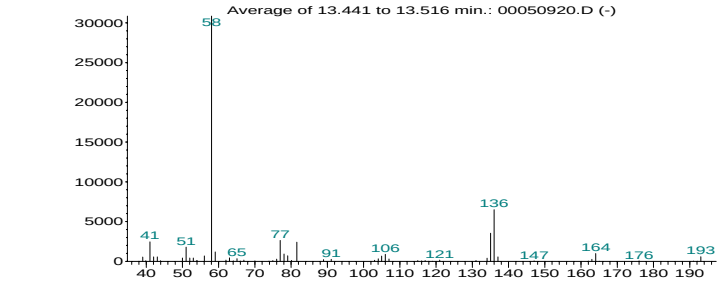
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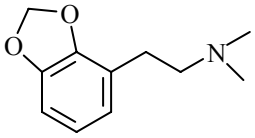
m/z-->



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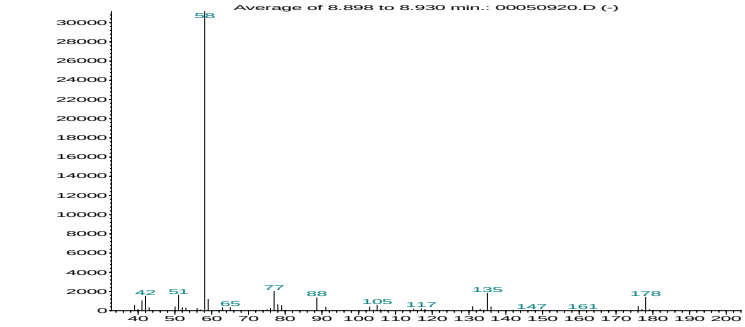
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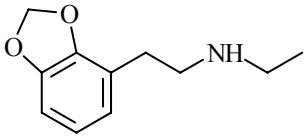
m/z-->



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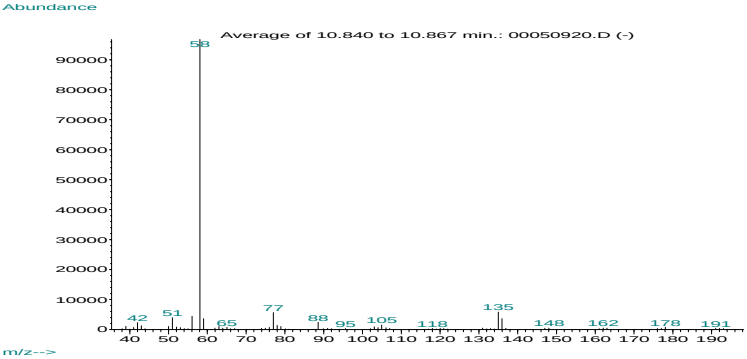
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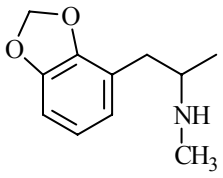
m/z-->



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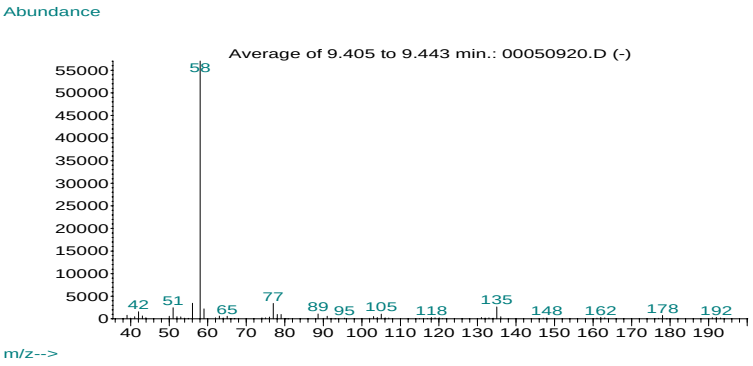

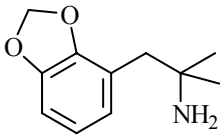

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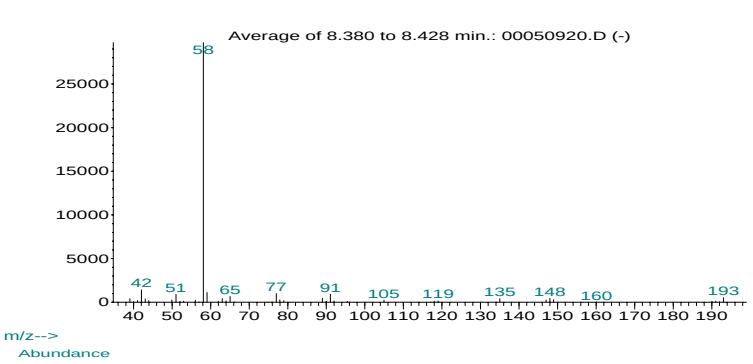

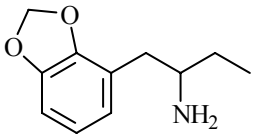

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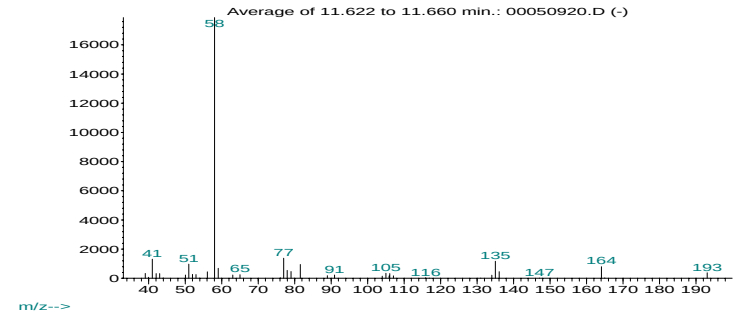


3.1.2. Mass spectral studies of perfluroacyl derivatives of 3,4- MDMA and its direct regioisomers

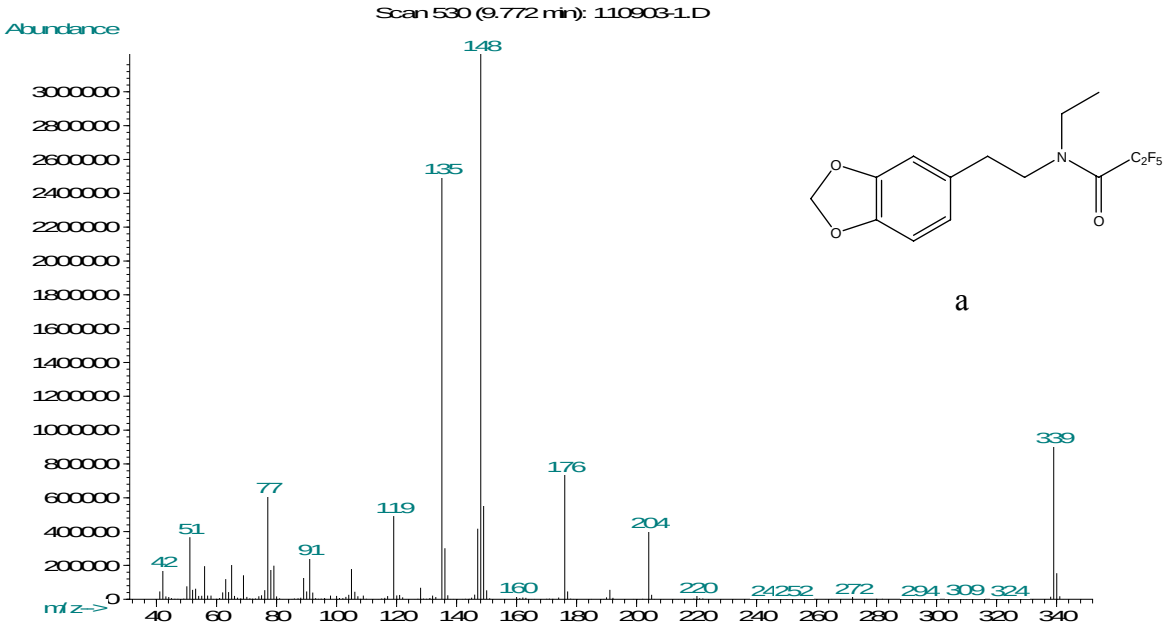
The perfluoroacylated derivatives of the eight primary and secondary amines (Compounds 2-5 and 7-10) were prepared (Chapter 4) and evaluated for their ability to individualize the GC-MS properties of the compounds in this uniquely regioisomeric

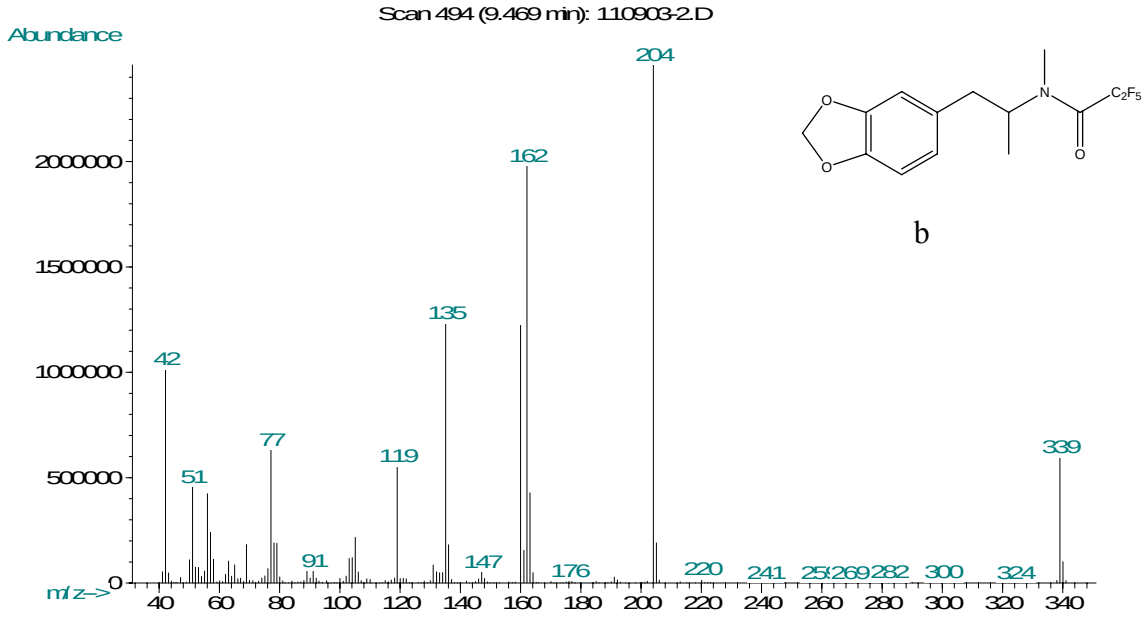
series and to maintain or improve chromatographic resolution. Of course, the two tertiary amines, compounds 1 and 6, would not form stable amide derivative.

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 16 and 17, 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 16: Mass Spectra of the PFPA derivatives of compounds (2-5) and (7-10).

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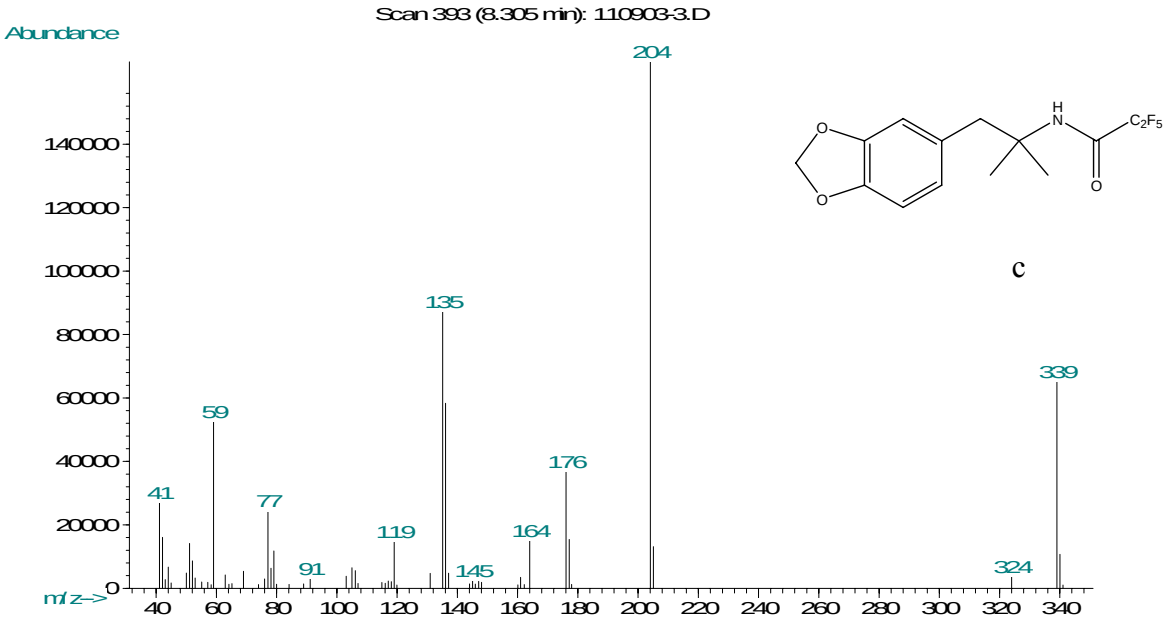
Abundance
m/z-->
204
162
135
42
77
119
339
51
91
147
176
220
241
25
269
282
300
324
O
N
C2F5

b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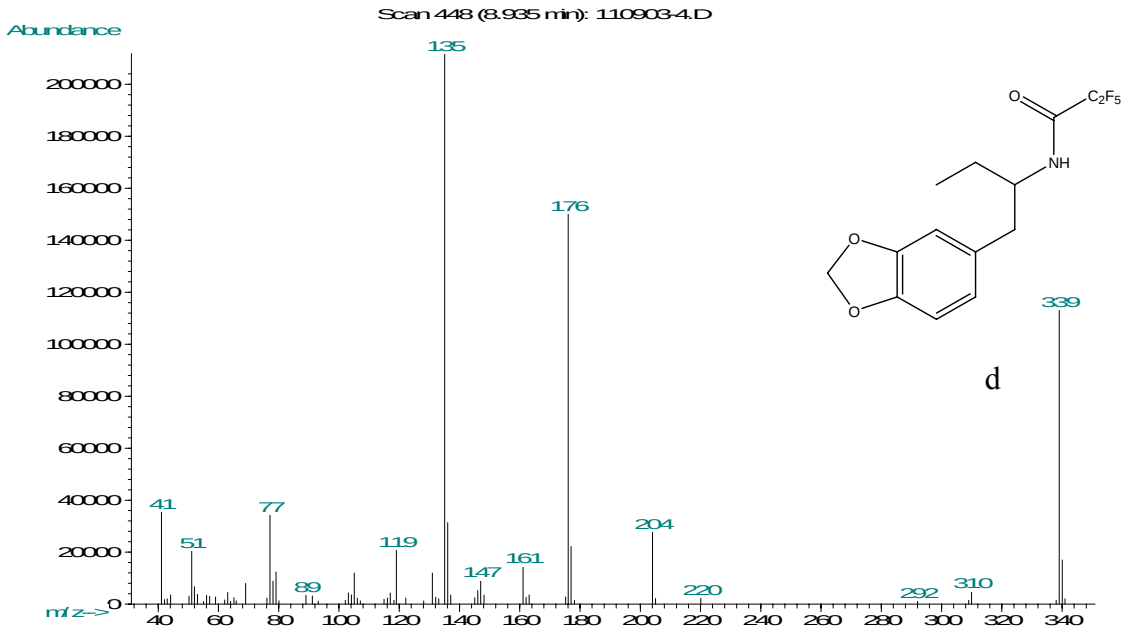
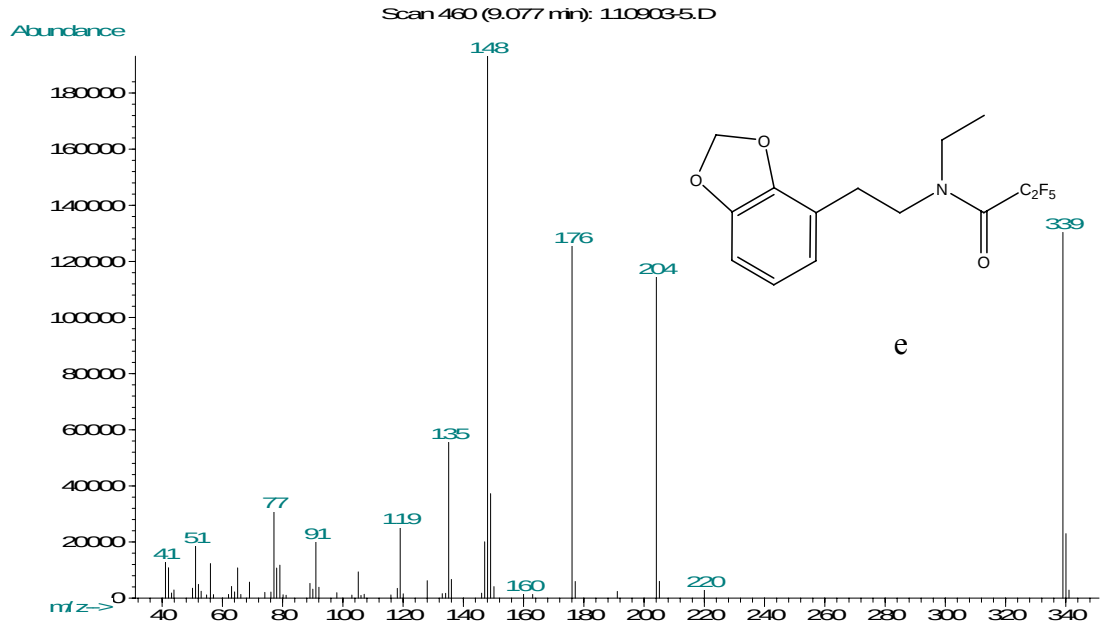
Abundance
m/z-->
204
135
339
59
176
41
77
119
164
91
145
324
H
N
C2F5

c


Scan 448 (8.935 min): 110903-4.D
Abundance
m/z-->
135
176
339
41
51
77
89
119
147
161
204
220
292
310
O
C_2F_5
NH
O
O
d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Abundance
m/z-->
148
176
204
339
135
77
91
119
41
51
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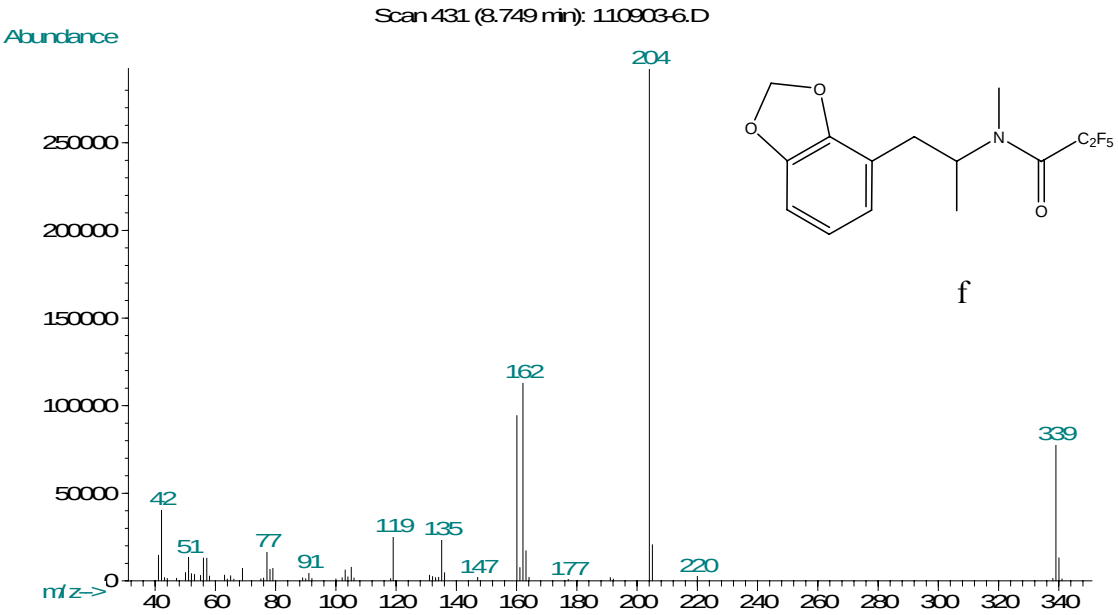
O
N
C_2F_5
O
e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Abundance
m/z-->
204
162
339
42
51
77
91
119
135
147
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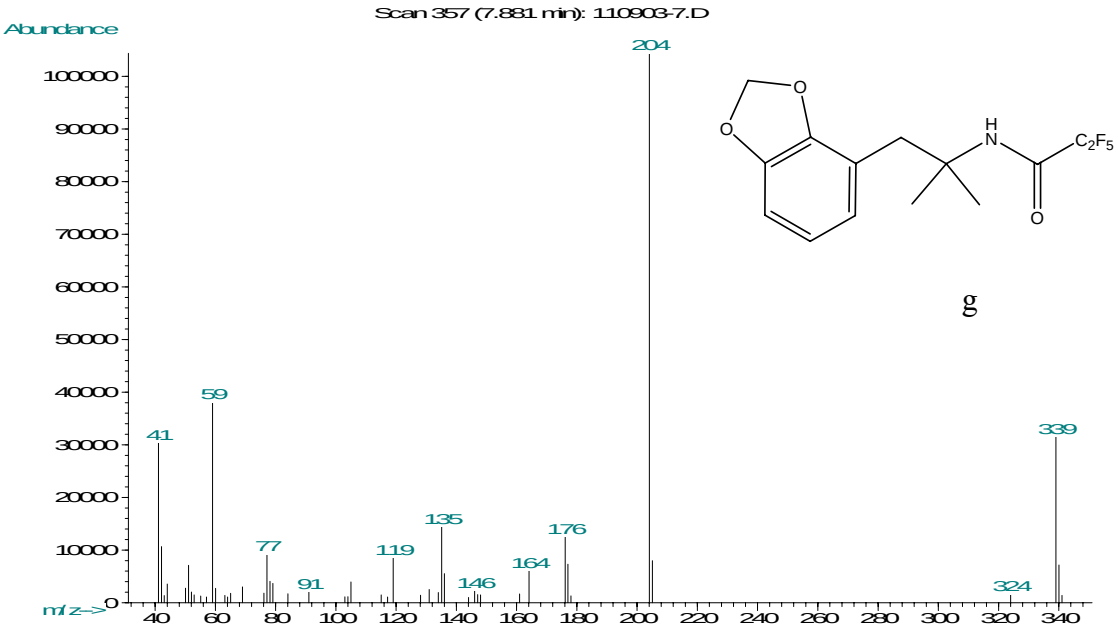
O
O
N
C_2F_5
O

f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Abundance
m/z-->
204
59
41
339
77
91
119
135
146
164
176
324
O
O
H
N
C_2F_5
O

g

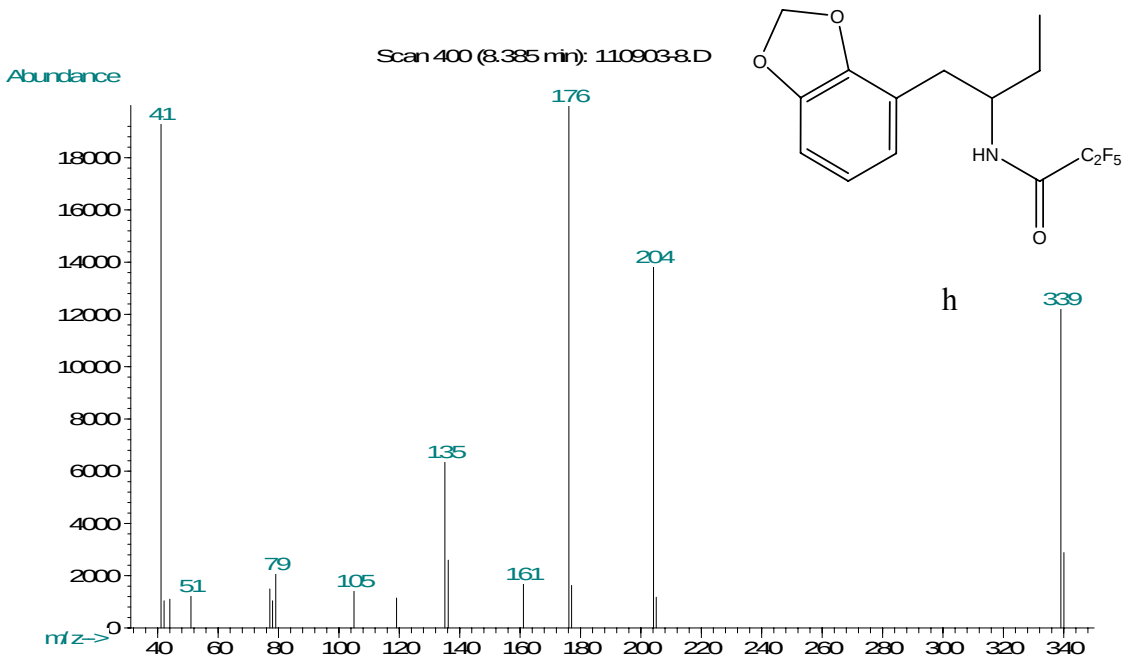
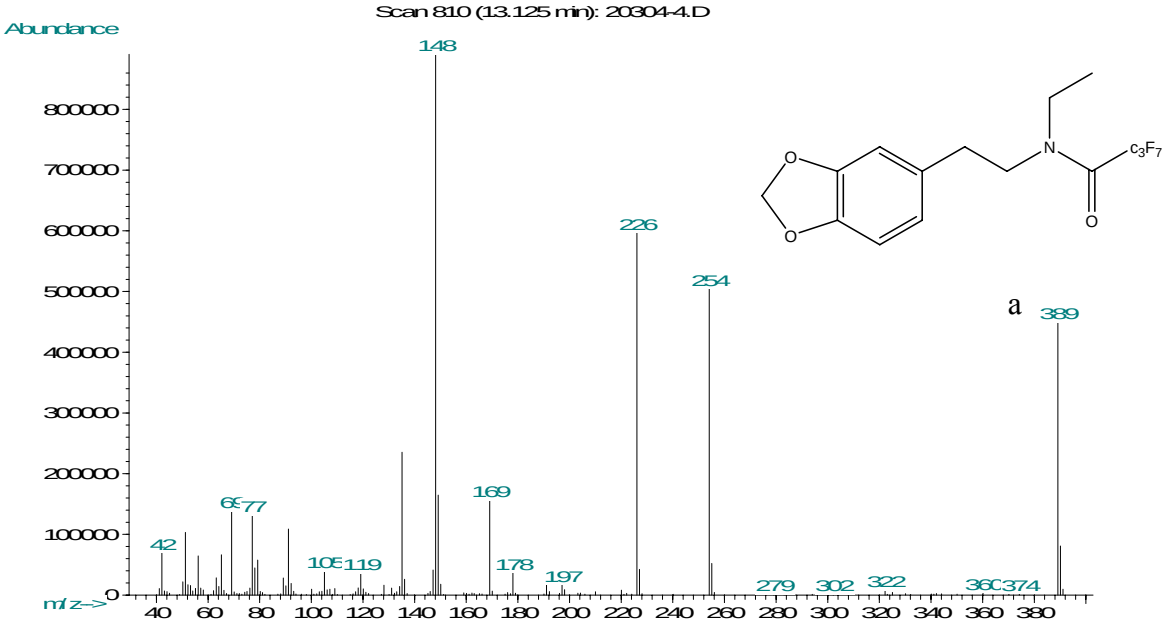
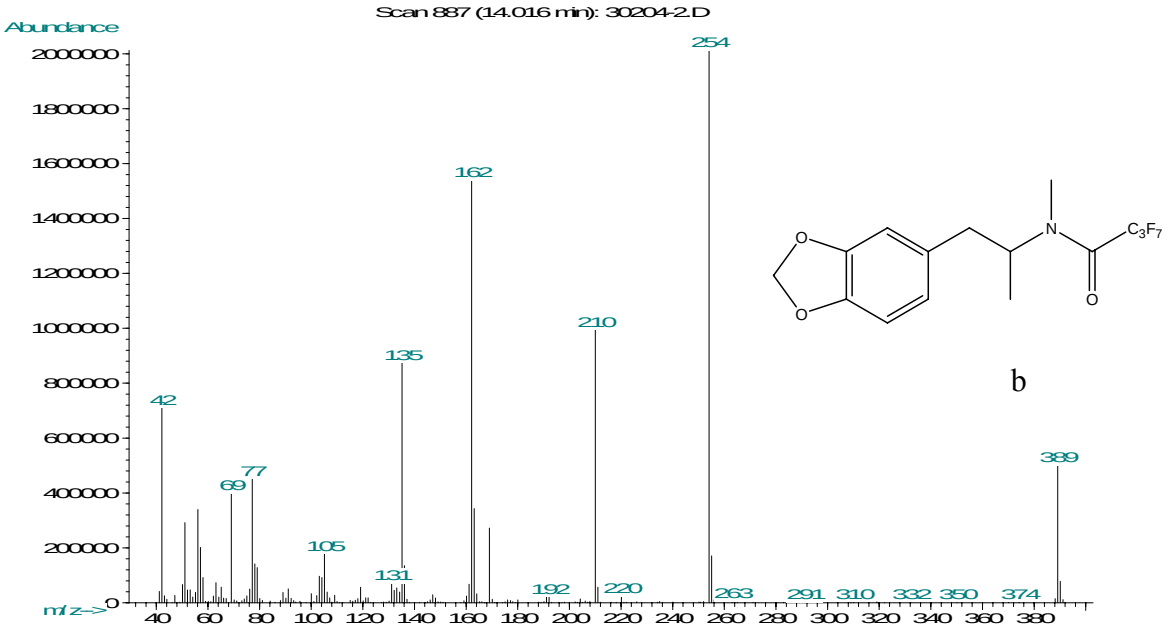
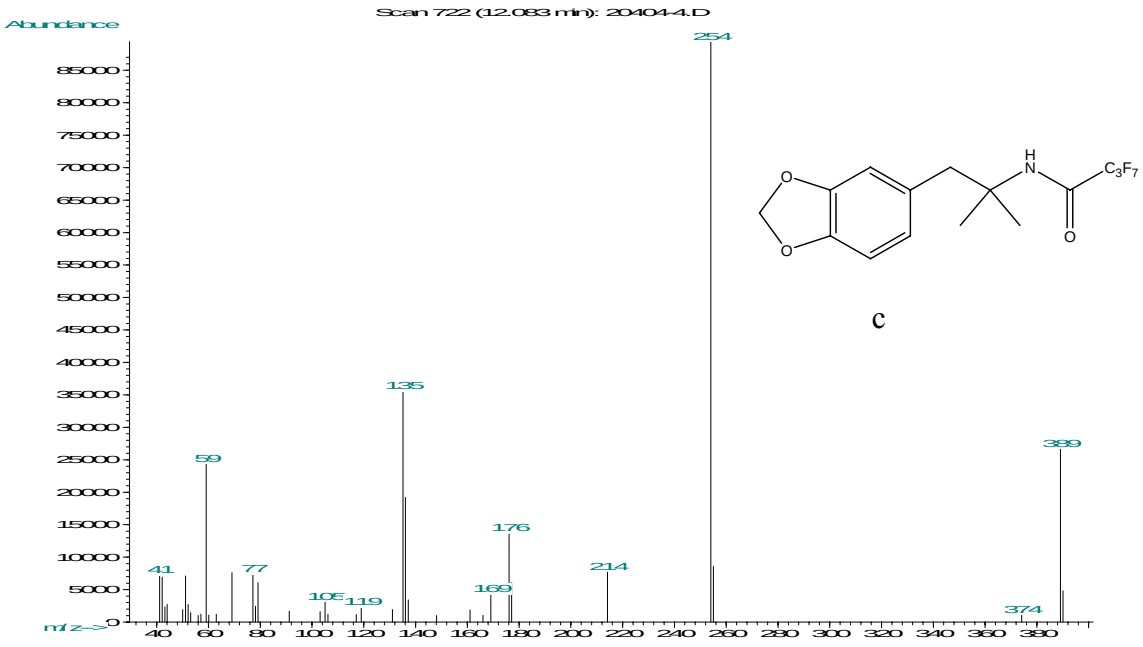


Figure 17: Mass Spectra for the HFBA derivatives of compounds (2-5) and (7-10).

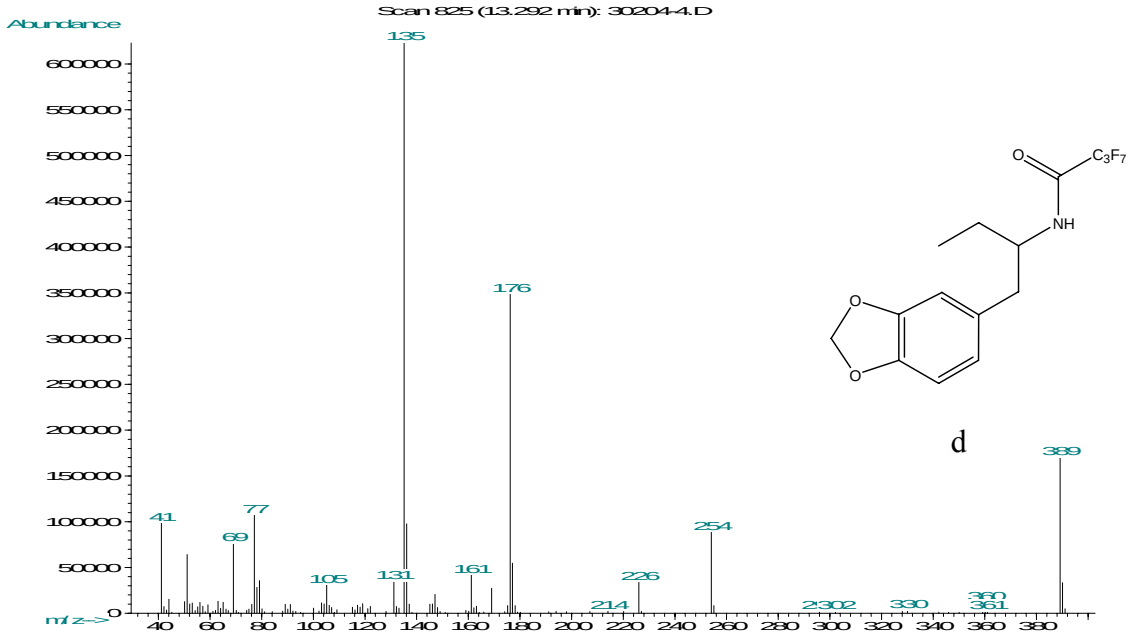


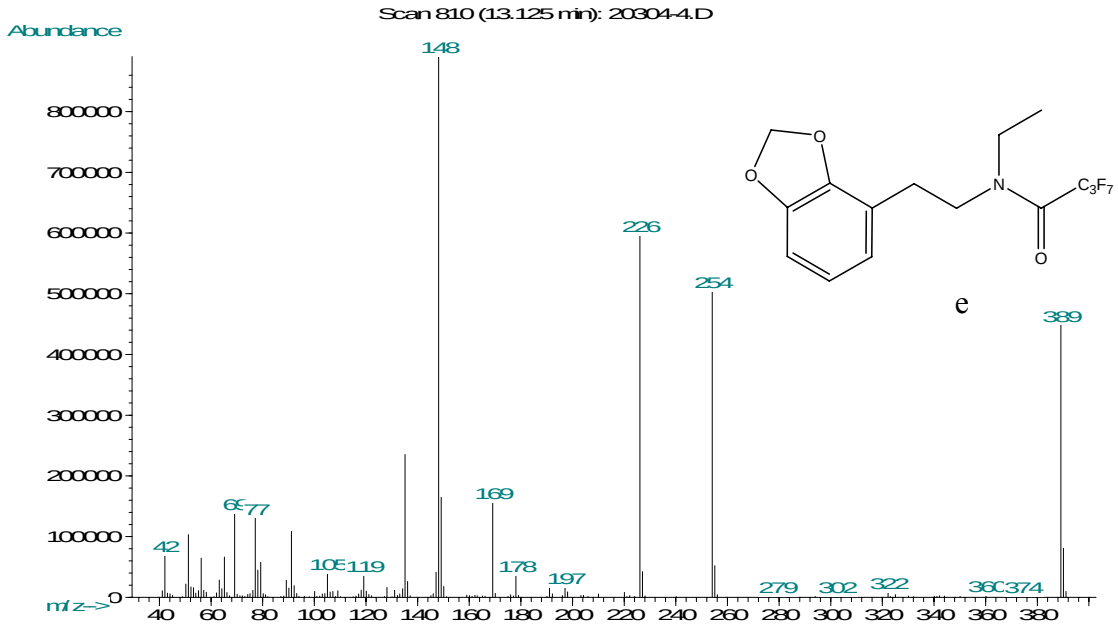

Scan 887 (14.016 min): 30204-2.D
Abundance
m/z-->
254
162
210
135
42
77
69
105
131
192
220
263
291
310
332
350
374
389
O
O
N
O
C3F7

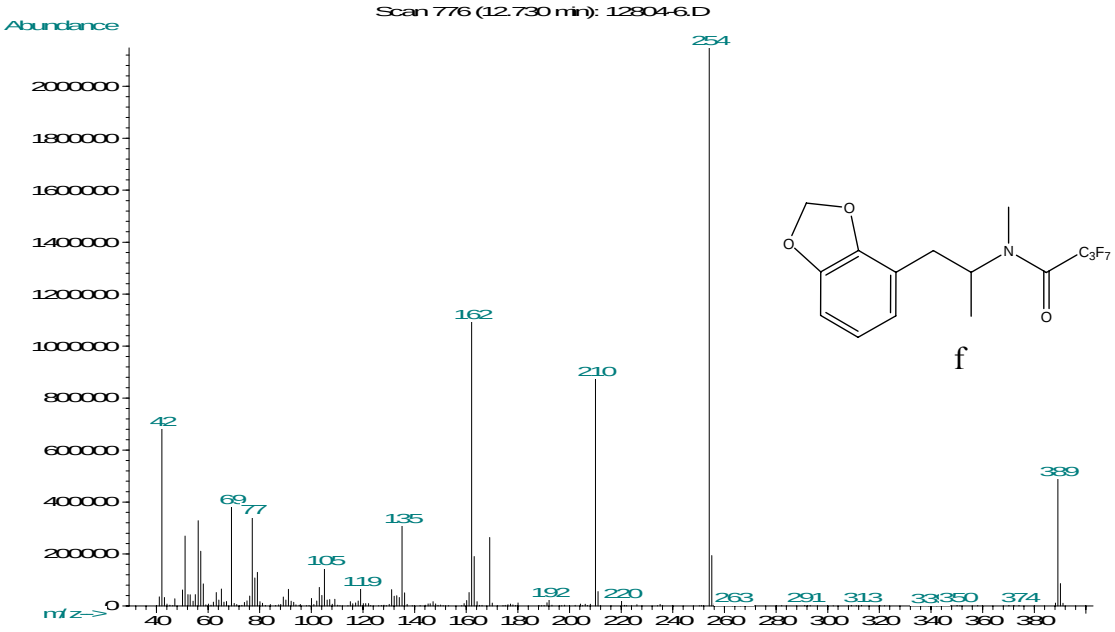
b


Scan 722 (12.083 min): 20404-4.D
Abundance
m/z-->
254
135
59
389
176
214
41
77
105
119
169
374
H
N
O
O
O
C3F7

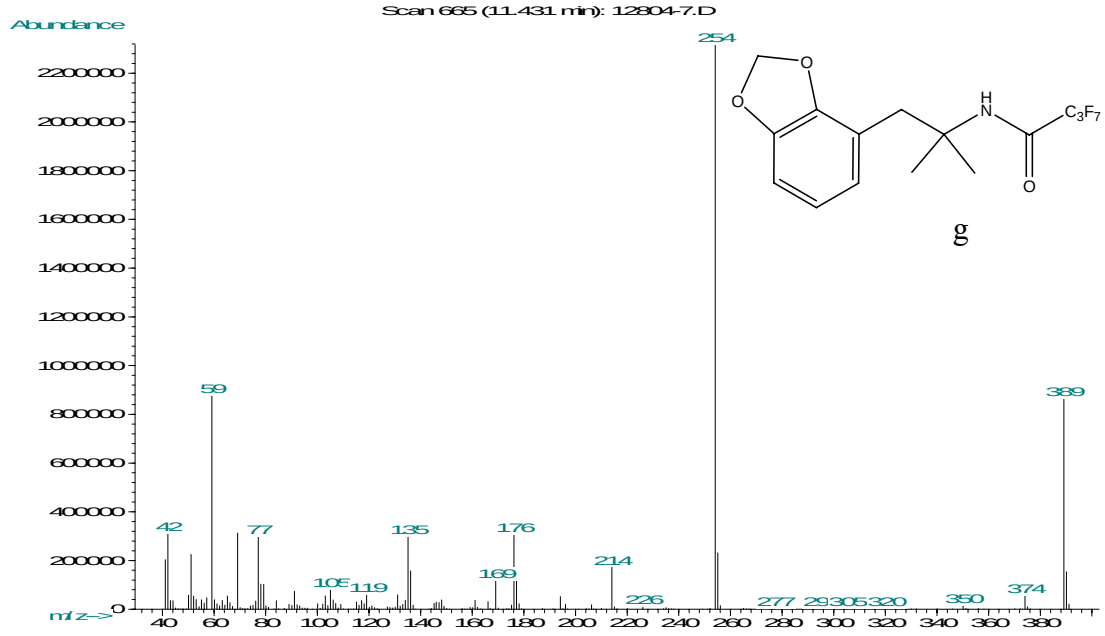
c


Scan 825 (13.292 min): 30204-4.D
Abundance
m/z-->
135
176
41
77
69
105
131
161
214
226
254
302
330
360
361
389
O
C3F7
NH
O
O
d

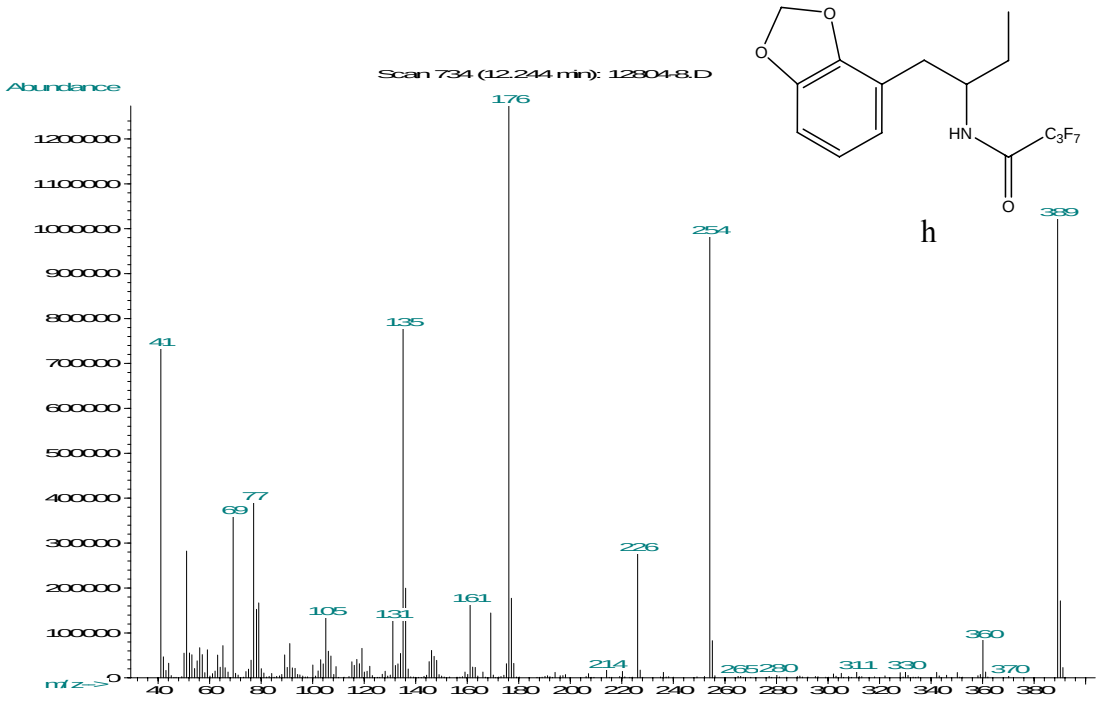

Scan 810 (13.125 min): 20304-4.D
Abundance
m/z-->
148
226
254
389
169
77
42
119
178
197
279
302
322
374
O
O
N
C3F7
O
e


Scan 776 (12.730 min): 12804-6.D
Abundance
m/z-->
254
162
210
42
69
77
135
105
119
192
220
263
291
313
333
350
374
389
O
O
N
C3F7
O

f

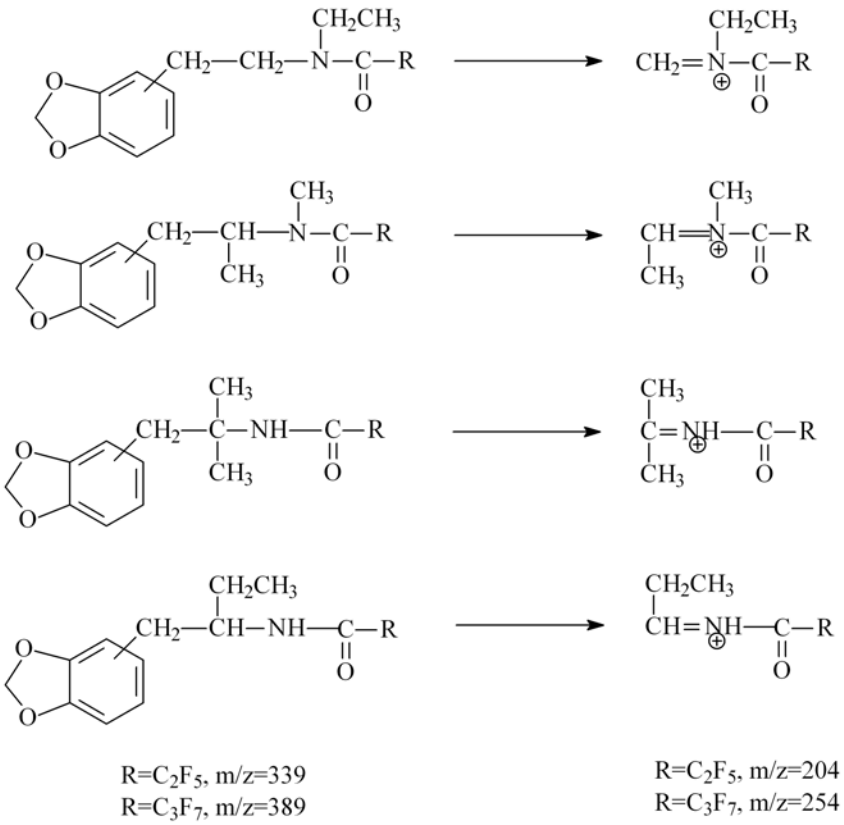

Scan 665 (11.431 min): 12804-7.D
Abundance
m/z-->
254
59
42
77
135
176
214
169
105
119
226
277
293
305
320
350
374
389
O
O
H
N
C3F7
O

g



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 38. The methylenedioxybenzyl cation at m/z 135 is a fragment common to all the spectra in Figures 16 and17. 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

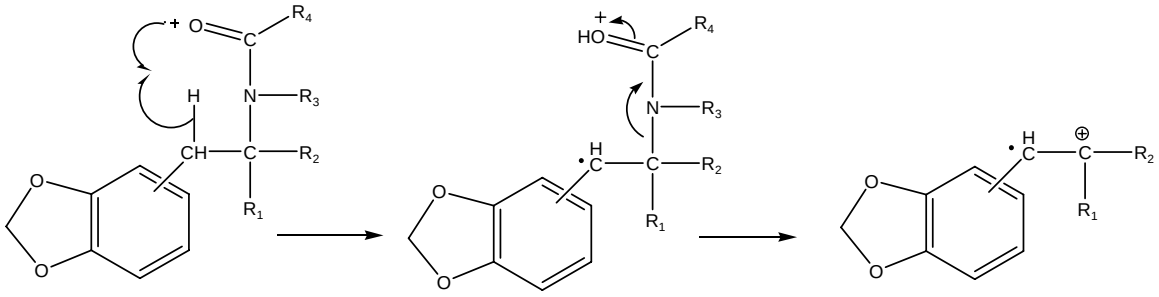
mass significantly individualize the mass spectra and provide specific structure information.



Scheme 38: Formation of the $(M\text{-}135)^+$ ions in the perfluoroacyl-derivatives of the regioisomeric amines.

The mass spectra in Figures 16 and 17 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.

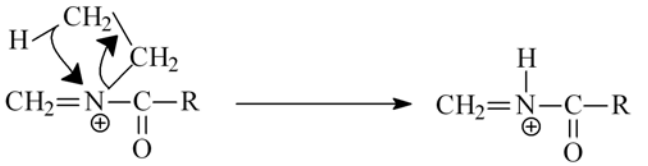
The spectra for the N-ethyl derivatives in Figures 16a, 16e and 17a, 17e) show a base peak at m/z 148 corresponding to the alkane radical cation which occurs from hydrogen rearrangement and subsequent fragmentation of the alkyl carbon to nitrogen bond of the phenethylamine side chain (Scheme 39).



Scheme 39: Mechanism for the formation of the alkane radical cation in the perfluoroacyl-derivatives of the regioisomeric amines.

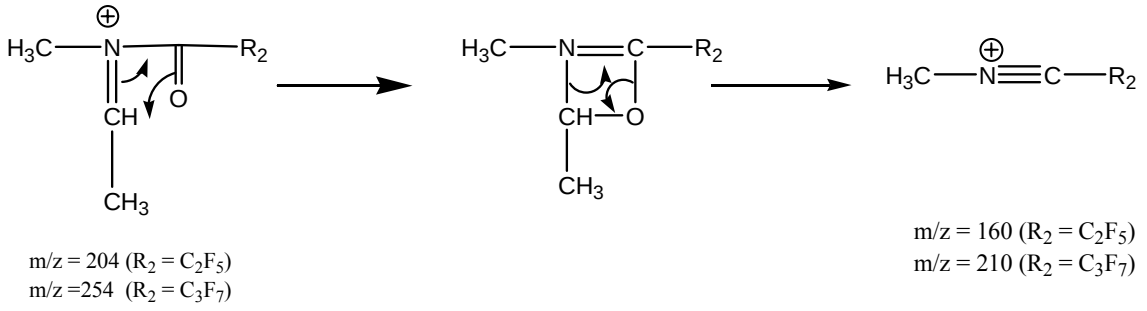
This ion at m/z 148 would only occur for the N-ethyl regioisomer. The spectra in Figures 16b, 16f and 17b, 17f show the 2,3- and 3,4-methylenedioxyphenylpropane hydrocarbon ion at m/z 162, identifying this molecules as the PFPA and HFBA derivatives of 2,3- and 3,4-methyelenedioxymethamphetamines, respectively. The proposed mechanism for the formation of the hydrocarbon fragment is illustrated in Scheme 39. 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 16a and 16e) 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 16 c, d, g, h and 17 c, d, g, h). The m/z 176 ion in the spectra for the PFPA derivatives of the N-ethyl regioisomers (Figures 16a and 16e) is a rearrangement of the m/z 204 ion resulting in the loss of mass 28 (the N-ethyl group) via hydrogen transfer (Scheme 40). 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 17a and 17e. 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.

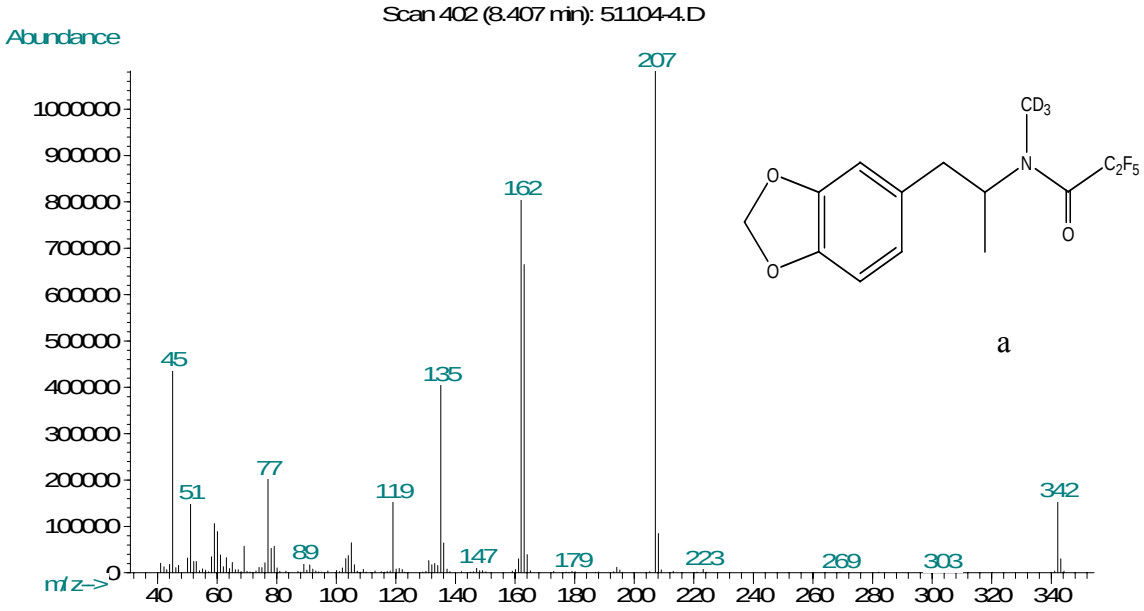


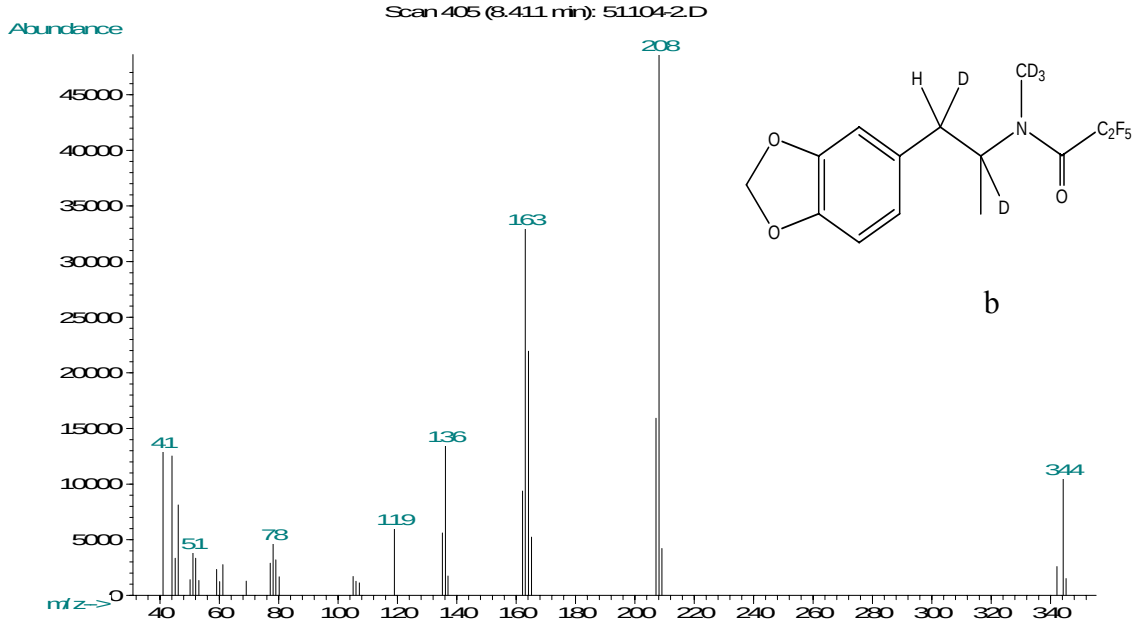
Scheme 40: Mechanism for the loss of mass 28 from the perfluoroacyl-N-ethyl-imine cation.

A comparison of the PFPA derivatives for 3,4- and 2,3-methylenedioxymethamphetamine (Figures 16b and 16f) with the HFBA derivatives (Figures 17b and 17f) 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 41. 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 18. 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 41. An equivalent fragmentation pathway has been reported [C.R. Clark et al, 1995] for methamphetamine.



Scheme 41: Formation of m/z 160 and 210 from the PHPA and HFBA derivatives of 2,3- and 3,4-MDMA


Scan 402 (8.407 min): 51104-4.D
Abundance
m/z-->
207
162
45
135
77
51
119
89
147
179
223
269
303
342
CD3
N
C2F5
O
O
O
a


Scan 405 (8.411 min): 51104-2.D
Abundance
m/z-->
208
163
41
136
51
78
119
344
H
D
CD3
N
C2F5
O
O
O
D
b

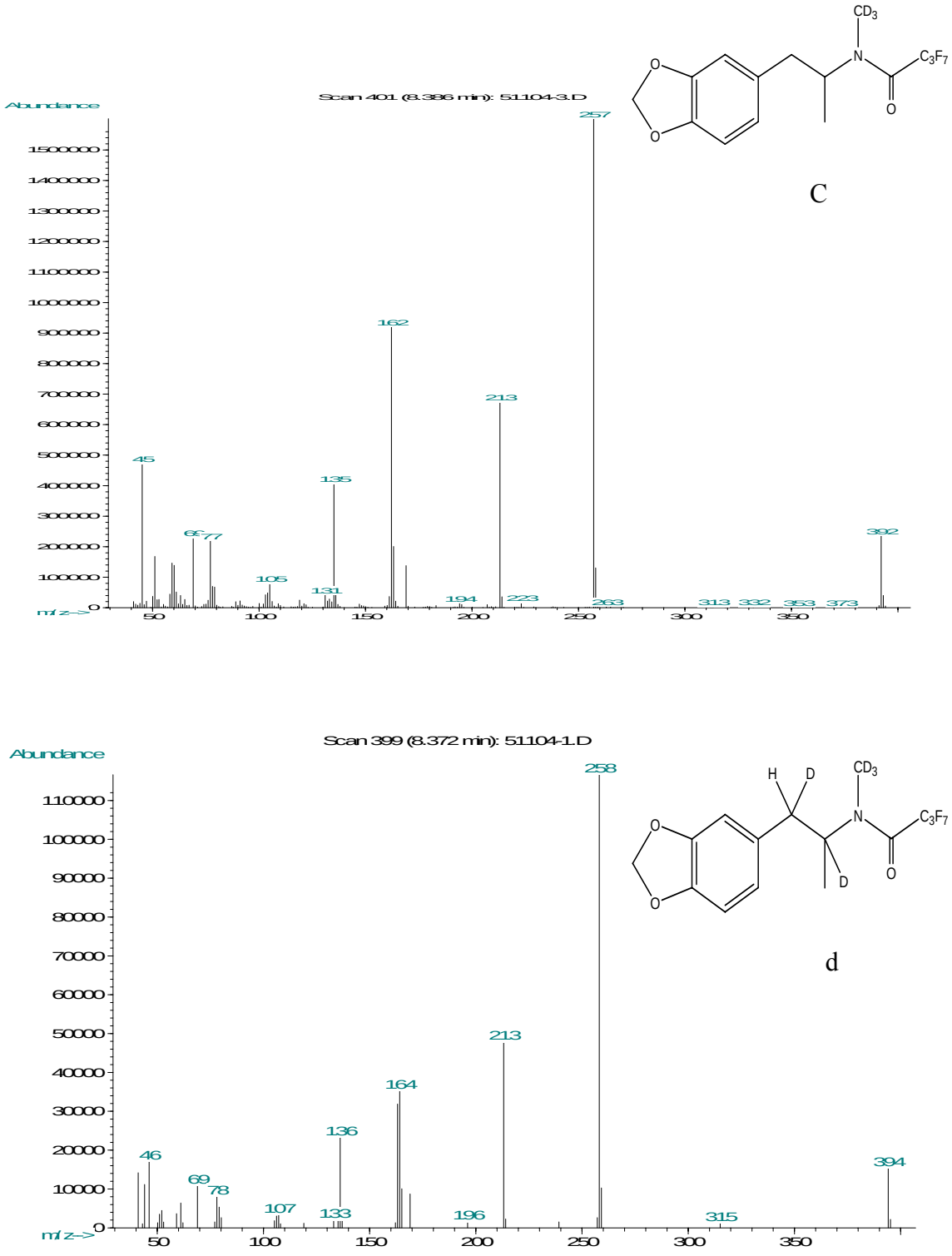
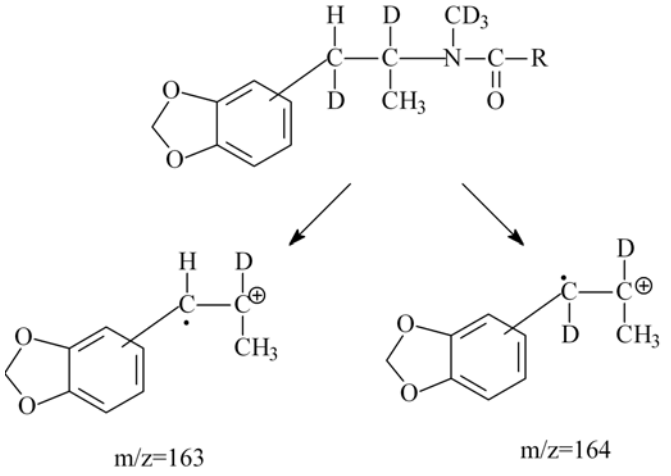


Figure 18: Mass spectra of the PFPA and HFBA derivatives for the d_3 and d_5- MDMA.

The spectra for the derivatives of d_3- and d_5-MDMA in Figure 18 also lend support to the proposed mechanism for the formation of the alkene fragment at m/z 162 illustrated in Scheme 39. The exact structure for the d_5-MDMA is shown in Scheme 42, 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 42: Formation of m/z 163 and 164 in the perfluoroacyl-derivatives of d_5-MDMA.

3.1.3. 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. 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.

Table 8 shows the relative retention of these compounds compared to N-methyl-3, 4-methyelenedioxyphenyl-2-propanamine (MDMA) under identical chromatographic conditions. 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). 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 19 and 20

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. This program was used to collect retention times of compounds 2-5 and 7-10 in their underivatized, HFBA and PFPA forms on Rtx-5. The same program was also used to collect retention data of the PFPA derivatives of the same compounds on Rtx-1. The second temperature program used to collect the retention data of the underivatized and 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.

The resulting elution order and resolution are quite similar. In fact the elution order is the same for all the chromatograms in Figures 19 and 20. 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.

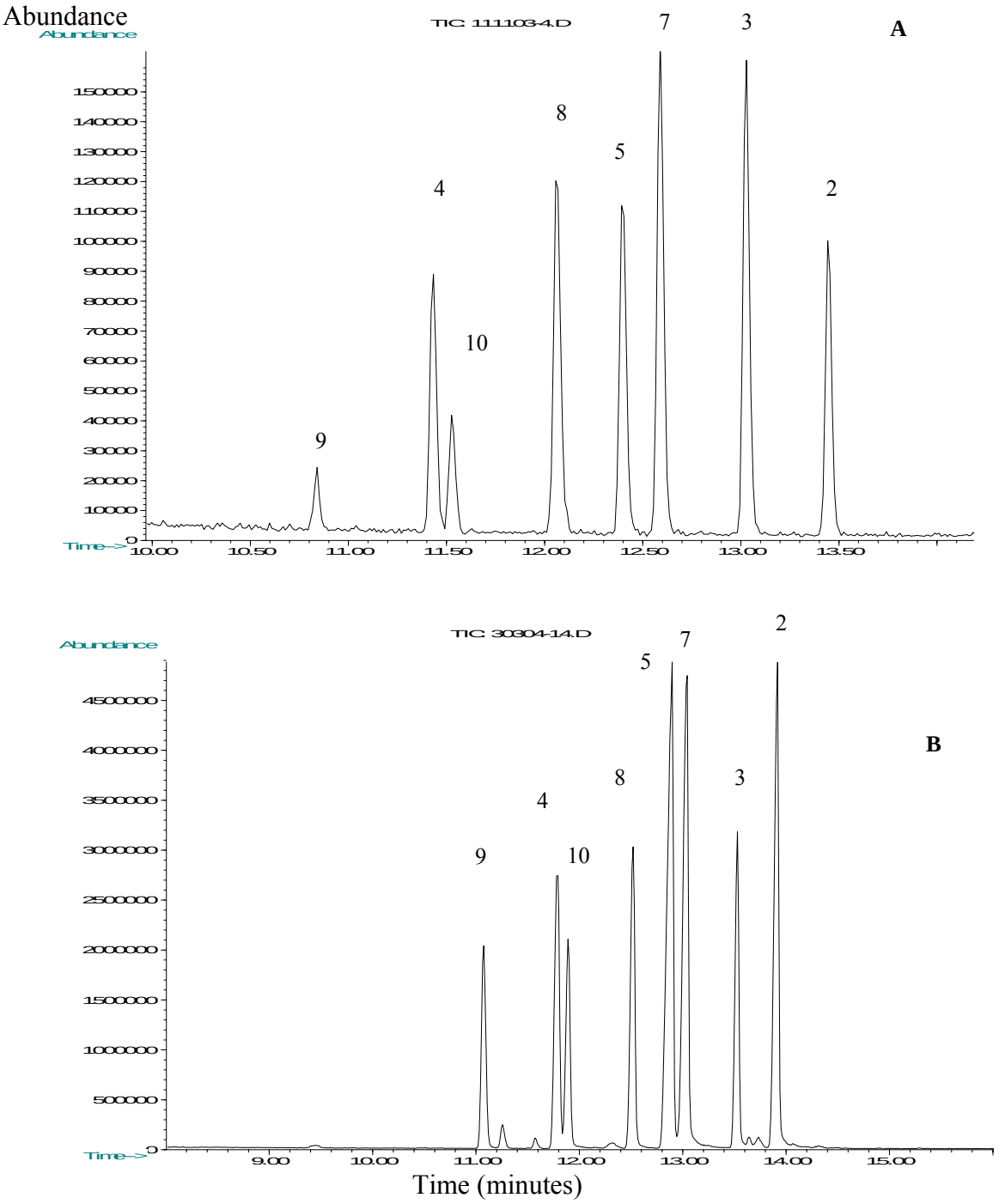


Figure 19: Capillary gas chromatographic separation of the PFPA derivatives of compounds (2-5) and (7-10). Columns used: A Rtx-1; B Rtx-5.

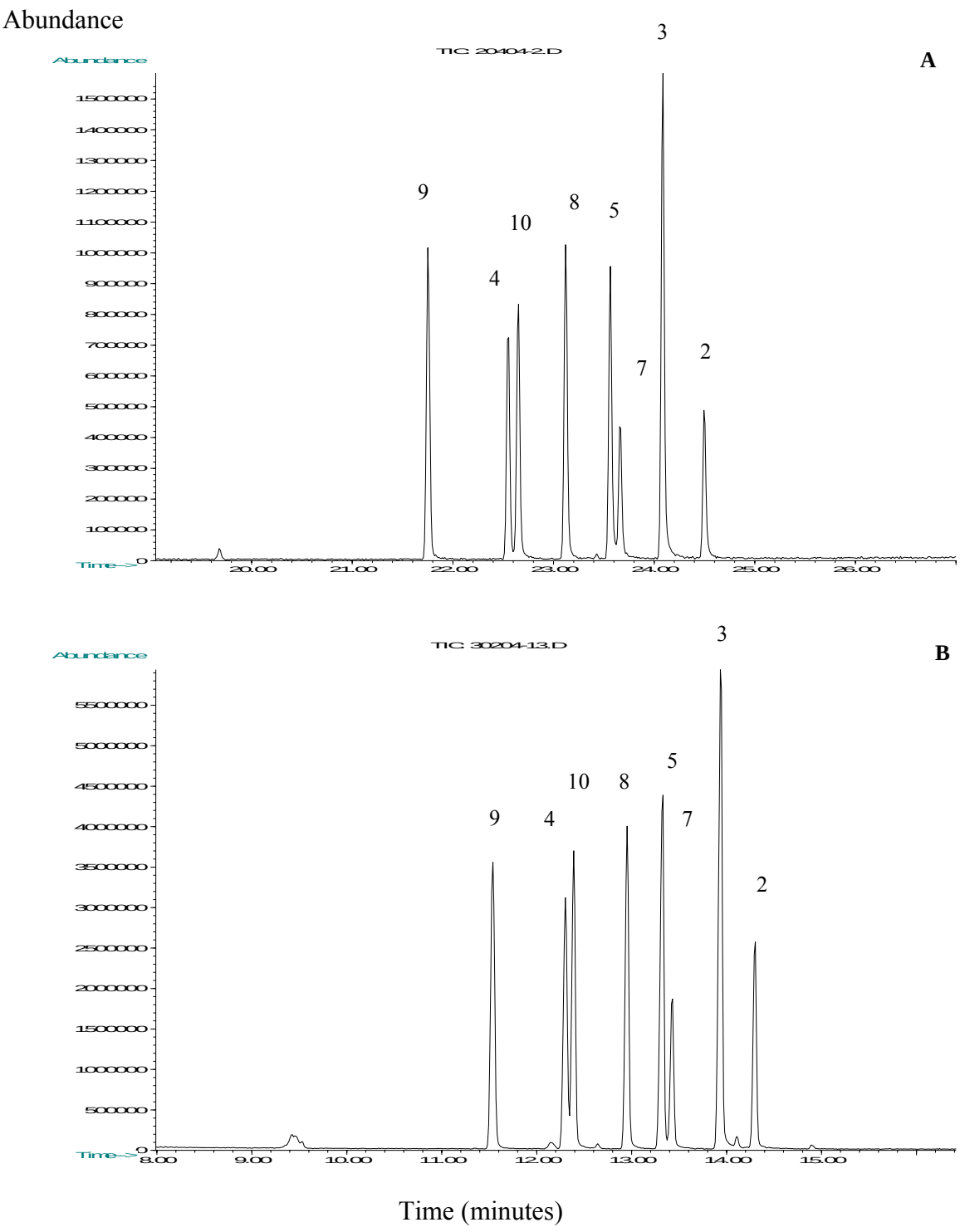


Figure 20: Capillary gas chromatographic Separation of HFBA derivatives of compounds (2-5) and (7-10). Columns used: A Rtx-1; B Rtx-5.

Table 8: Retention data of compounds 2-5 and 7-10 underivatized, HFBA and PFPA derivatives on Rtx-1 and Rtx-5.

Compound Number	Rtx-1◊			Rtx-5◊◊		
		Derivatives▲			Derivatives▲	
	Underivatized**	HFBA derivatives**	PFPA derivatives*	Underivatized*	HFBA derivatives*	PFPA derivatives*
2	1.021	1.017	1.033	1.044	1.023	1.021
3	1.0 (19.731 min.)	1.0 (24.087 min)	1.0 (13.029min)	1.0 (10.171 min.)	1.0 (14.01 min)	1.0 (13.596 min.)
4	0.923♦	0.936	0.877	0.984♦	0.887	0.872
5	0.975■	0.978	0.951	1.083■	0.954	0.958
7	0.972■	0.983	0.967	1.079■	0.959	0.966
8	0.942	0.960	0.923	0.955	0.934	0.926
9	0.885	0.903	0.833	0.918	0.828	0.861
10	0.926♦	0.940	0.884	0.982♦	0.895	0.879

◊ Rtx-1 is a 30m x 0.25mm-I.d. column coated with o.25 µm 100% dimethyl polysiloxane
◊◊Rtx-5 is a 30 m x 0.25mm-I.d. column coated with o.25 µm 95% dimethyl-5%diphenyl polysiloxane
* Temperature program used TP-1
** Temperature program used TP-2
▲Abbreviations: PFPA, pentafluoropropionamide; HFBA, heptafluorobutyrylamide.
♦ and ■ compounds that share the same sign co-elute on the same separation column.
-Results are the average of three experiments

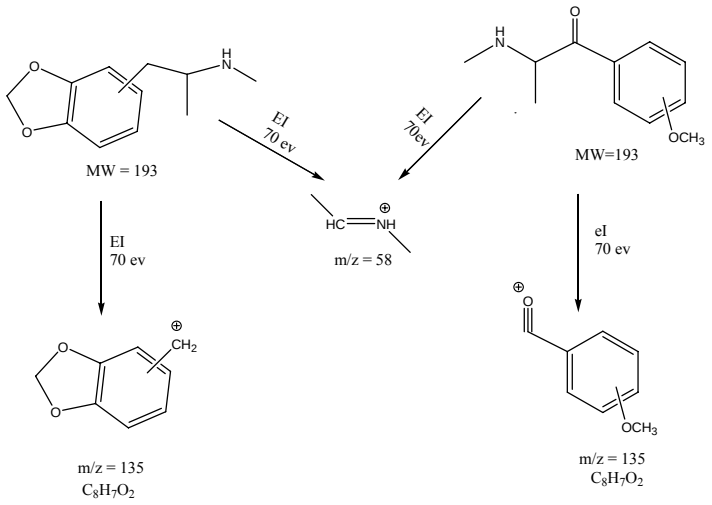
3.2 Mass Spectrometry and Gas Chromatographic Studies of Indirect Regioisomers Related to 3,4-MDMA, the Methoxymethcathinones.

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. In chapter 3.2.1 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 in chapter 3.2.2.

3.2.1 Mass spectral studies of the undreivatized and the perfluroacyl 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 21) show a base peak at m/z 58 as seen for 2,3- and 3,4-MDMA (Figure 15). The major fragmentation pattern for the methoxymethcathinones is shown in Scheme 43. 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.

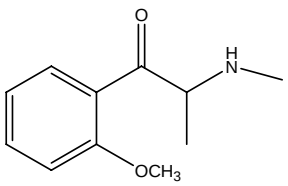


Scheme 43: EI fragmentation pattern of the underivatized 2,3- ,3,4-MDMAs and the methoxymethcathinones

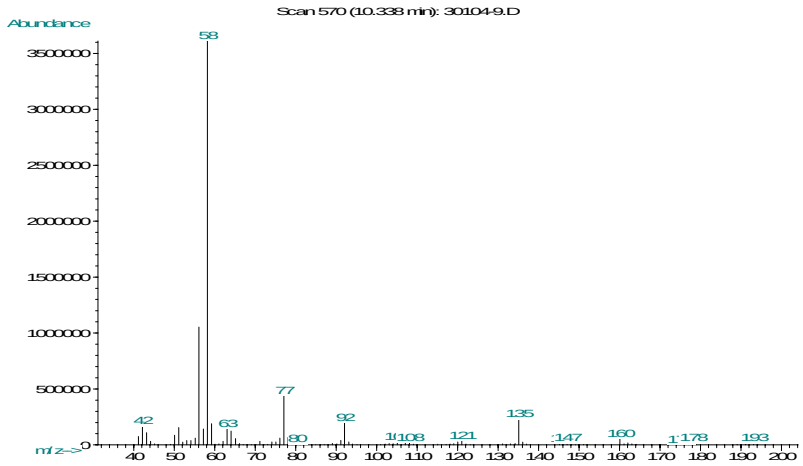
Figure 21: Mass spectra of the underivatized methoxymethcathinones.

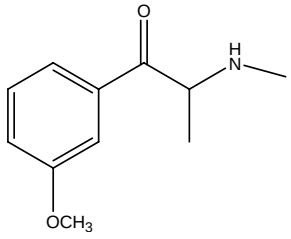
Structure of regioisomers

Mass spectrum

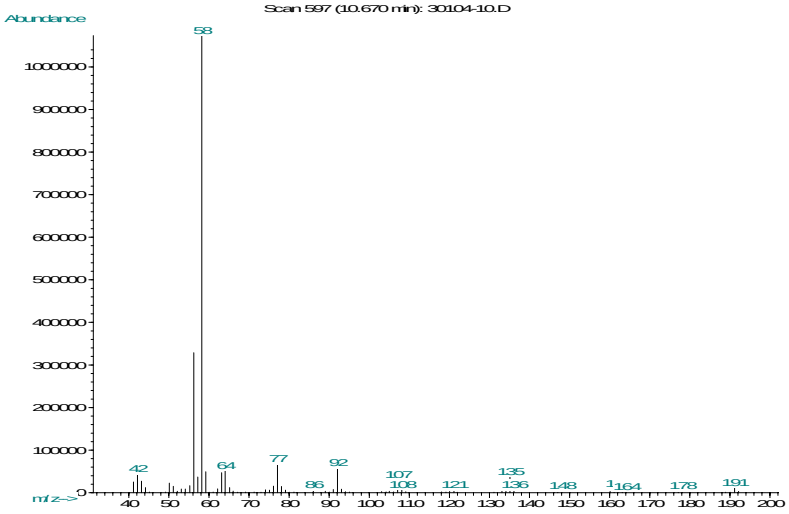


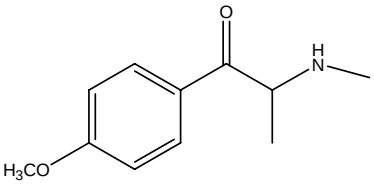
11



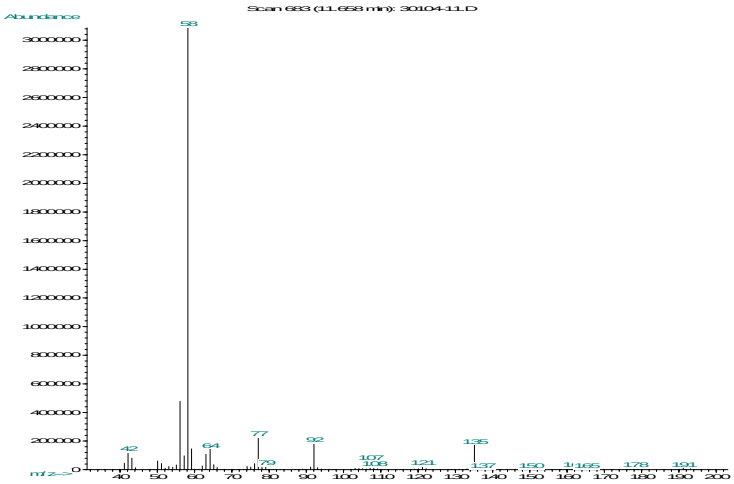


12

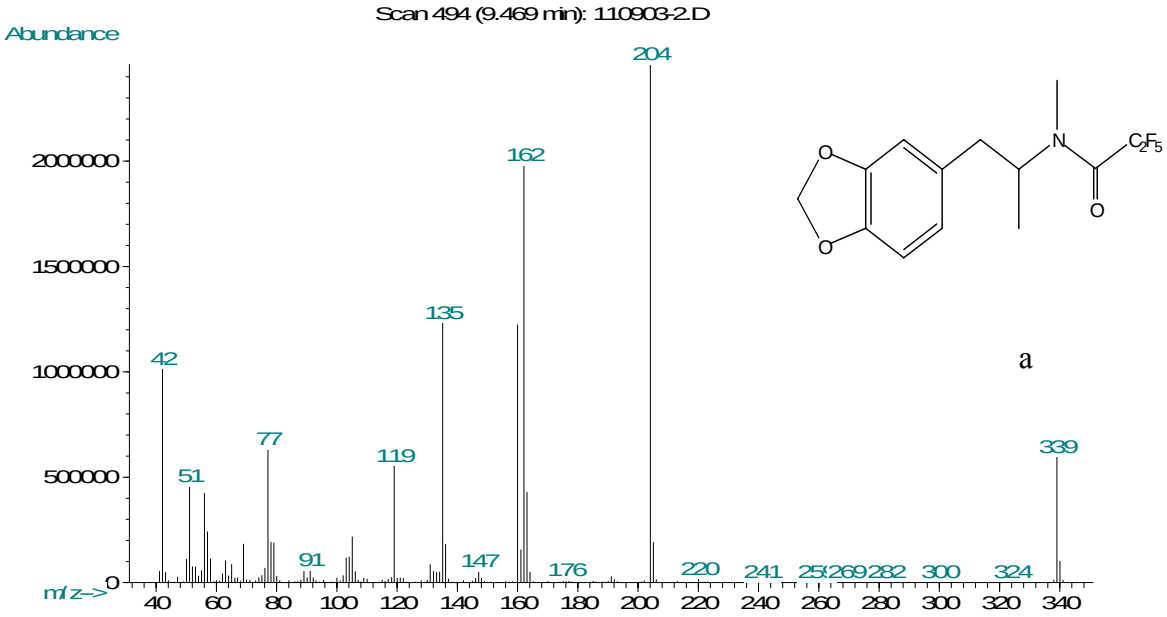


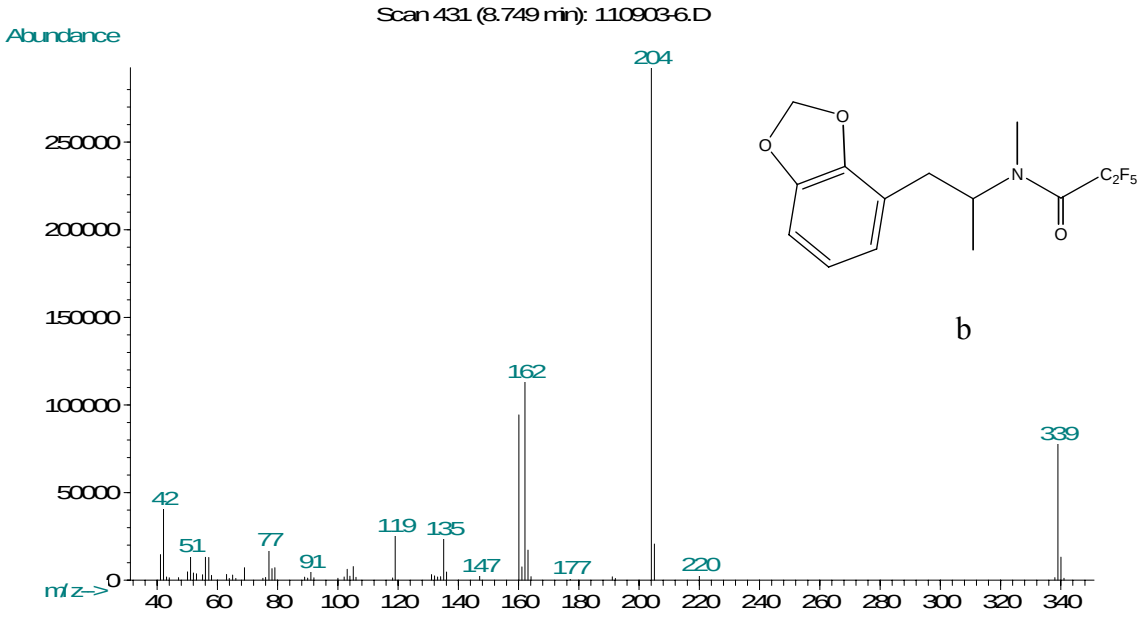


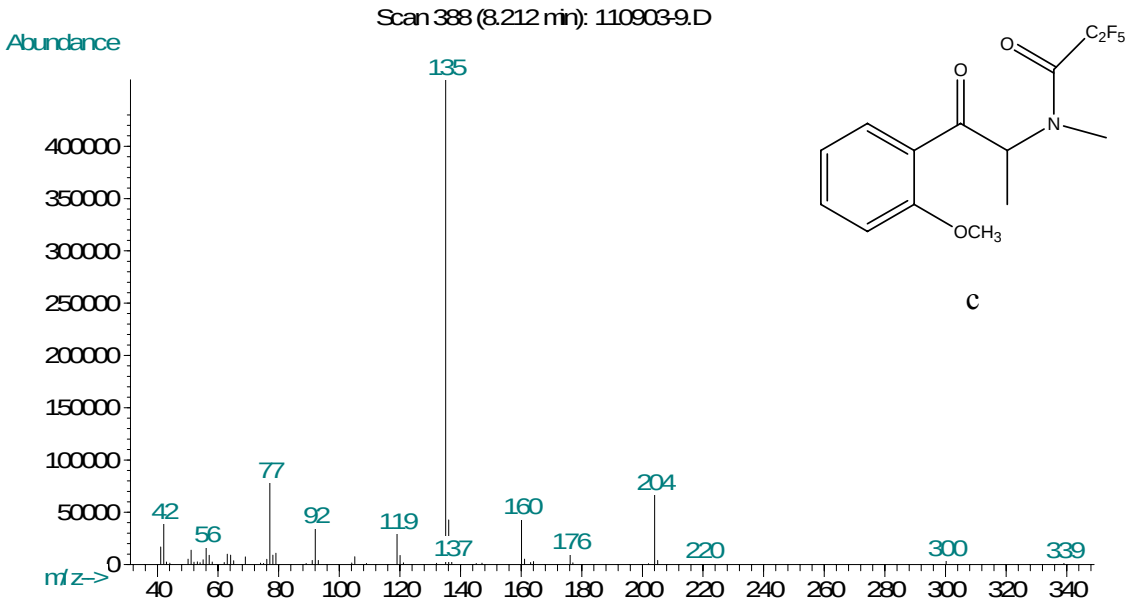
13



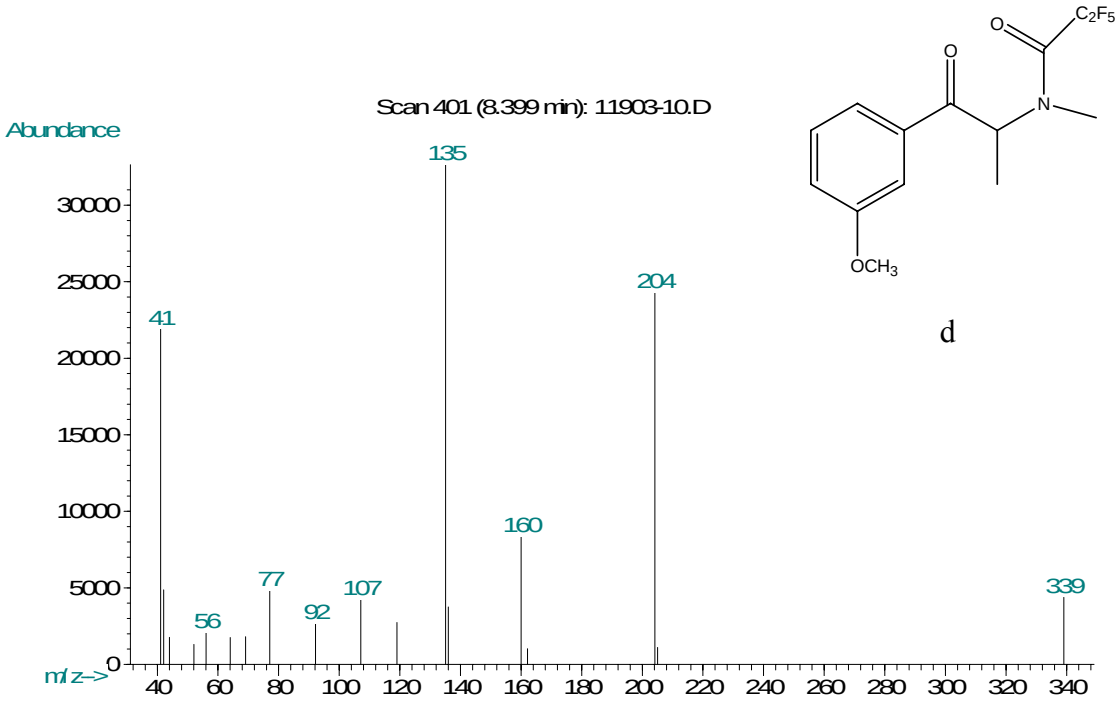
Since the mass spectra of the methoxymethcathinones is almost identical to the drug of abuse 3,4-MDMA, perfluoroacylated derivatives of 3,4 and 2,3-methylenedioxymethamphetamines and their regioisomeric secondary amines, ortho, meta and para-methoxymethcathinones, were prepared and evaluated in an effort 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 22 and 23, respectively.


Scan 494 (9.469 min): 110903-2.D
Abundance
204
162
135
42
77
119
51
91
147
176
220
241
25
269
282
300
324
339
m/z-->
a


Scan 431 (8.749 min): 110903-6.D
Abundance
204
162
339
42
51
77
91
119
135
147
177
220
m/z-->
b


Scan 388 (8.212 min): 110903-9.D
Abundance
m/z-->
135
77
42
56
92
119
137
160
176
204
220
300
339
O
O
N
C_2F_5
OCH_3

c


Scan 401 (8.399 min): 11903-10.D
Abundance
m/z-->
135
204
41
160
77
107
92
56
339
O
O
N
C_2F_5
OCH_3

d

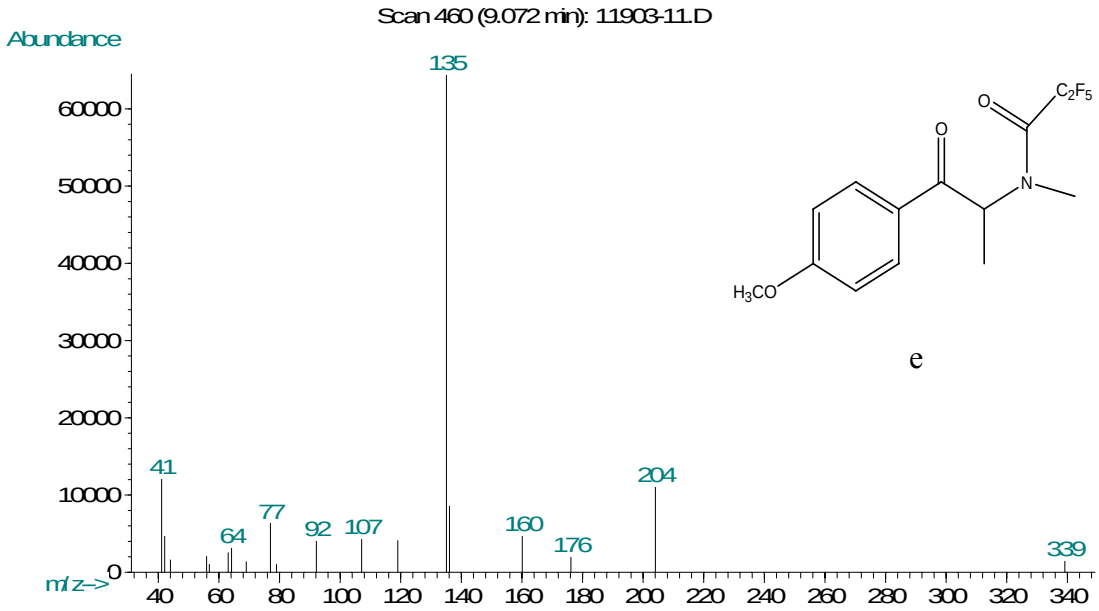
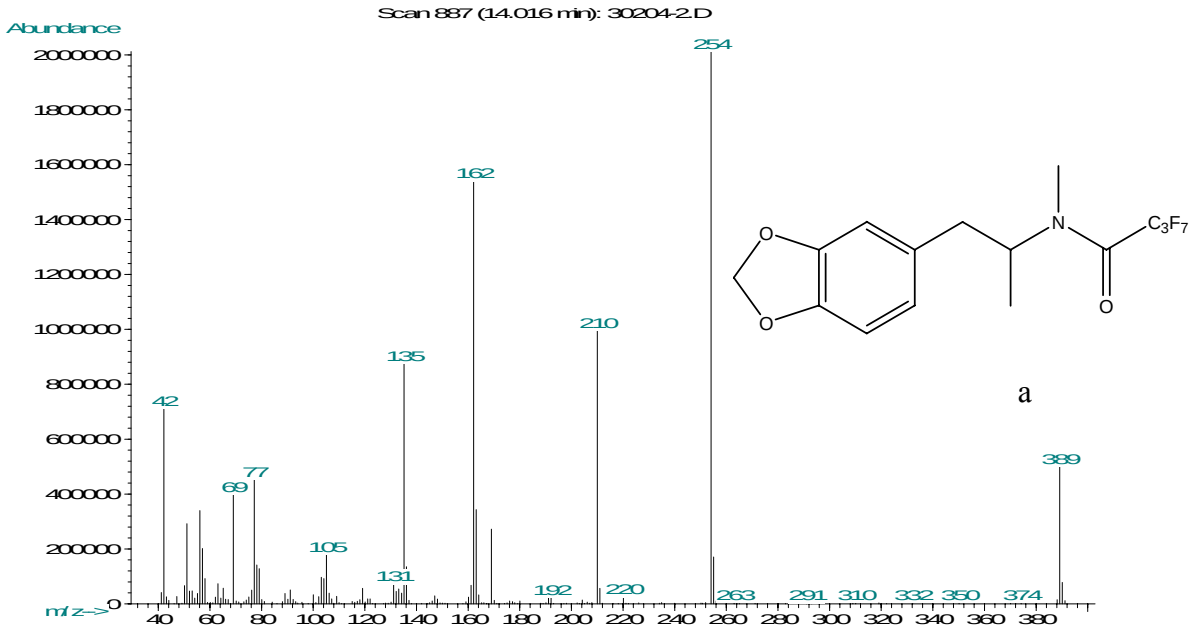
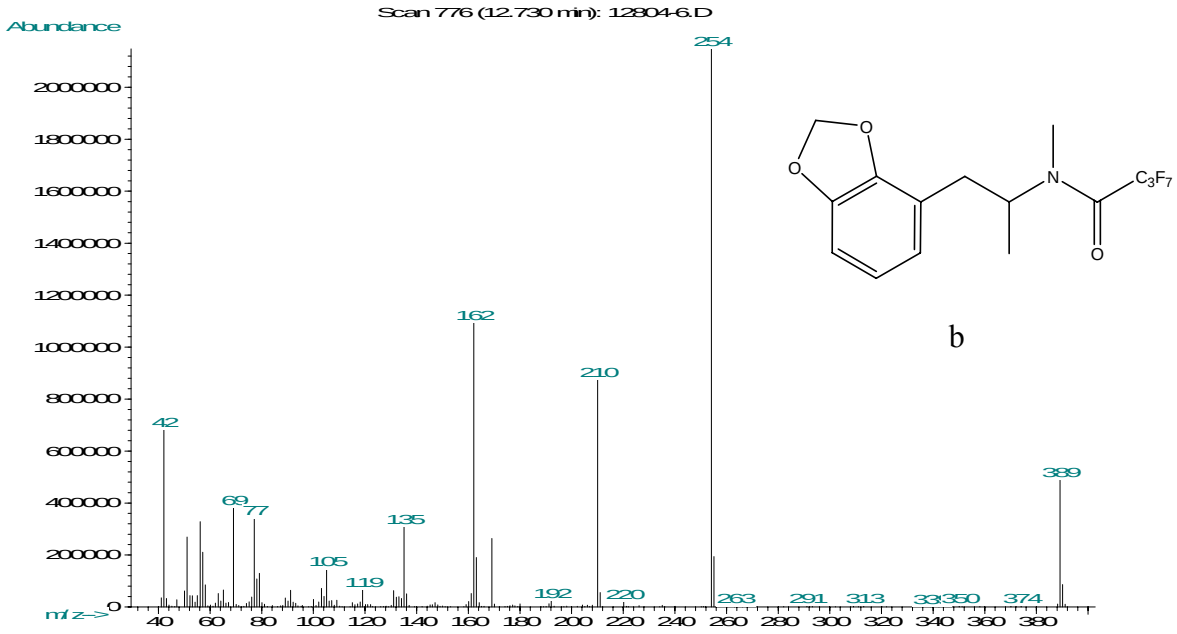


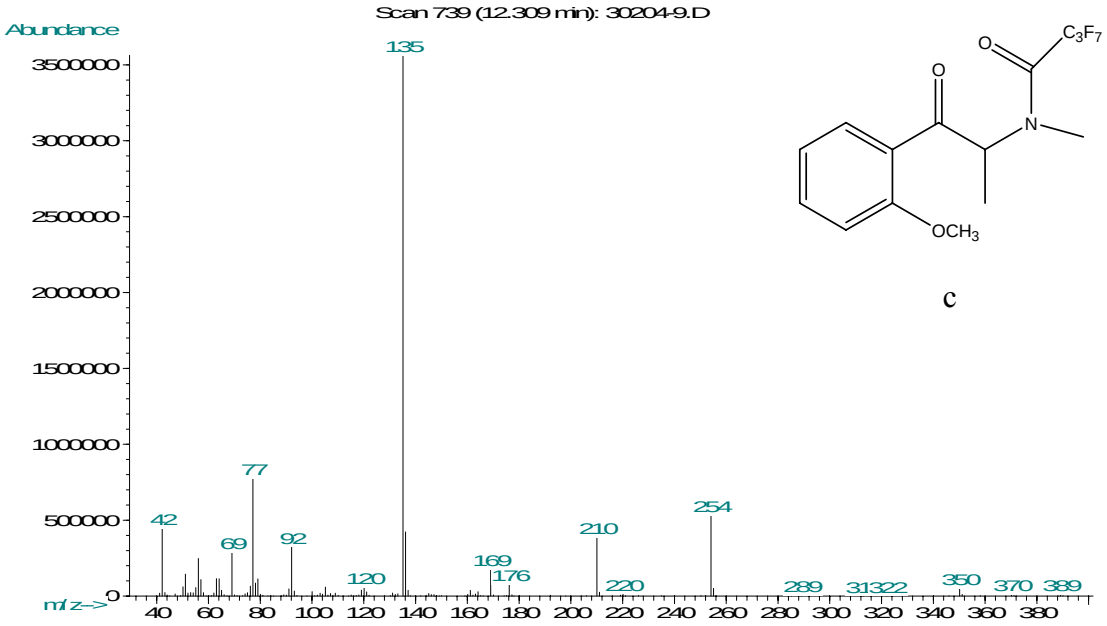
Figure 22: Mass spectra of the PFPA derivatives of 3,4-MDMA (a); 2,3-MDMA (b) ;ortho (c); meta (d) and para (e)-methoxymethcathinones.


Scan 887 (14.016 min): 30204-2.D
Abundance
m/z-->
254
162
210
135
42
77
69
105
131
192
220
263
291
310
332
350
374
389
O
O
N
C_3F_7
O

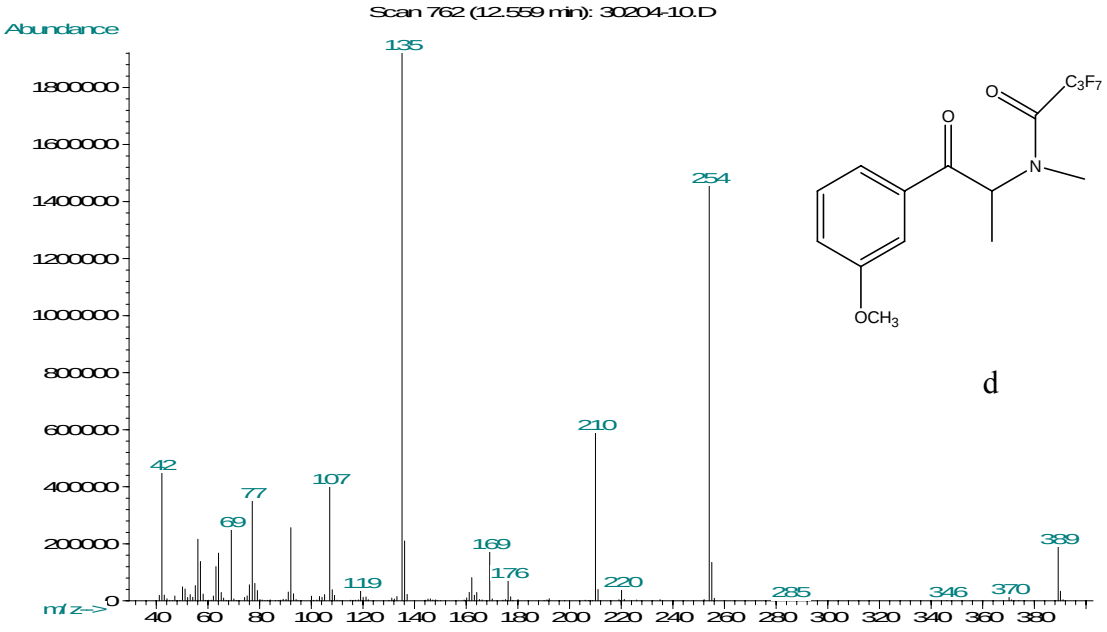
a


Scan 776 (12.730 min): 12804-6.D
Abundance
m/z-->
254
162
210
42
69
77
135
105
119
192
220
263
291
313
333
350
374
389
O
O
N
C_3F_7
O

b


Scan 739 (12.309 min): 30204-9.D
Abundance
m/z-->
135
77
42
69
92
120
169
176
210
220
254
289
313
22
350
370
389
O
O
N
C3F7
OCH3

c


Scan 762 (12.559 min): 30204-10.D
Abundance
m/z-->
135
254
210
42
77
107
69
169
176
119
220
285
346
370
389
O
O
N
C3F7
OCH3

d

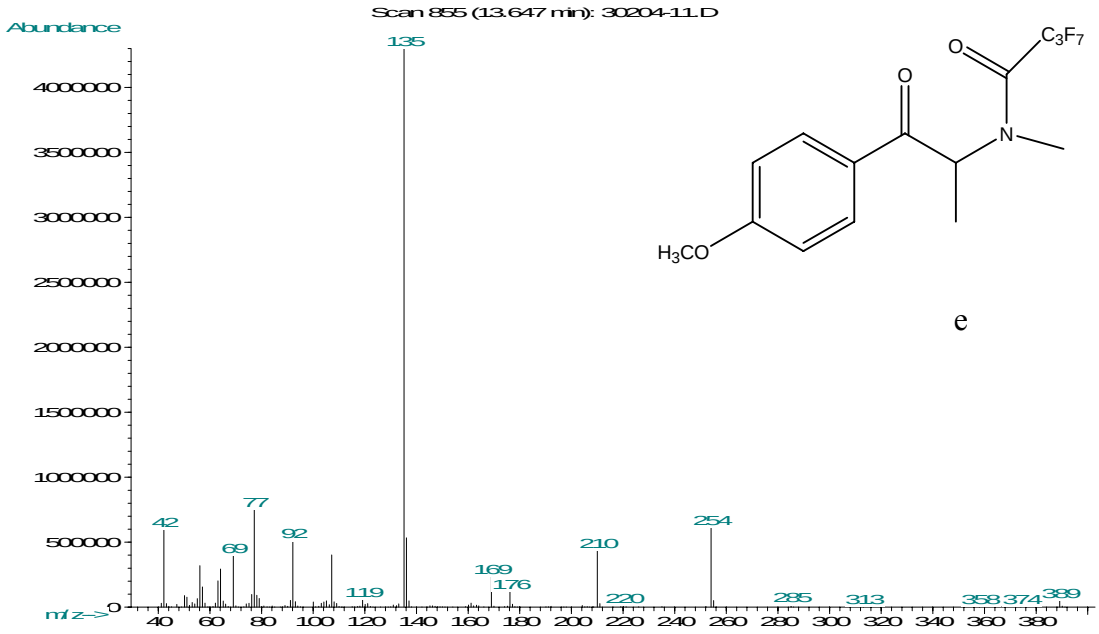
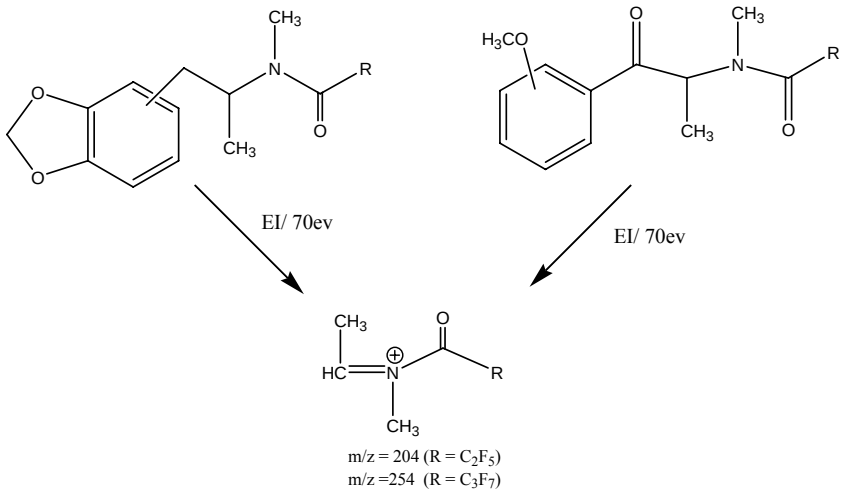


Figure 23: Mass Spectra for the HFBA derivatives of 3,4-MDMA (a); 2,3-MDMA (b); ortho (c); meta (d) and para (e)-methoxymethcathinones.

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 44

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 22 and 23). 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.



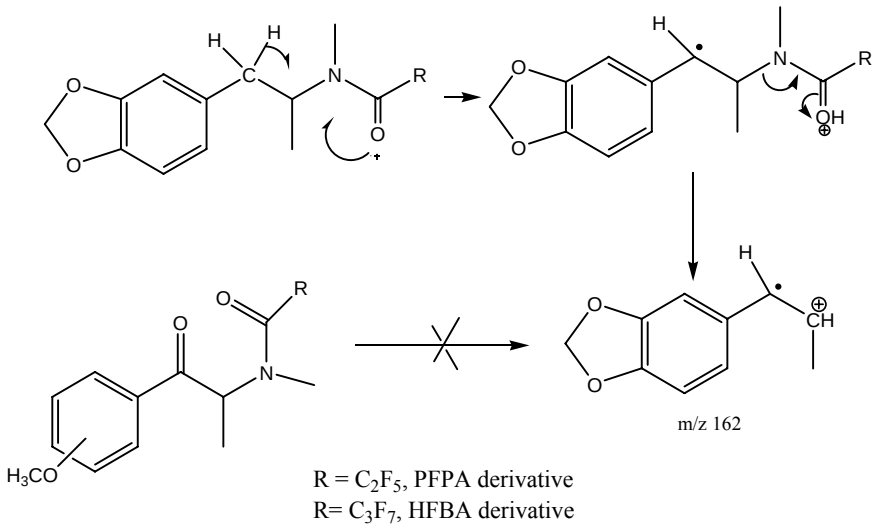
Scheme 44: Formation of m/z 204 and m/z 254 for the PFPA and HFBA derivatives of 2,3-, 3,4-MDMAs and methoxymethcathinones

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 22 and 23 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 22a, 22b, 23a and 23b 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 3.1.2 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 45). 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 22a and 22b) with their HFBA derivatives (Figures 23a and 23b) 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 3.1.2 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 41). 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 45: Benzylic hydrogen transefer to form m/z 162 occur only in MDMA perfluroaceyl derivatives but not for methoxymethcathinones.

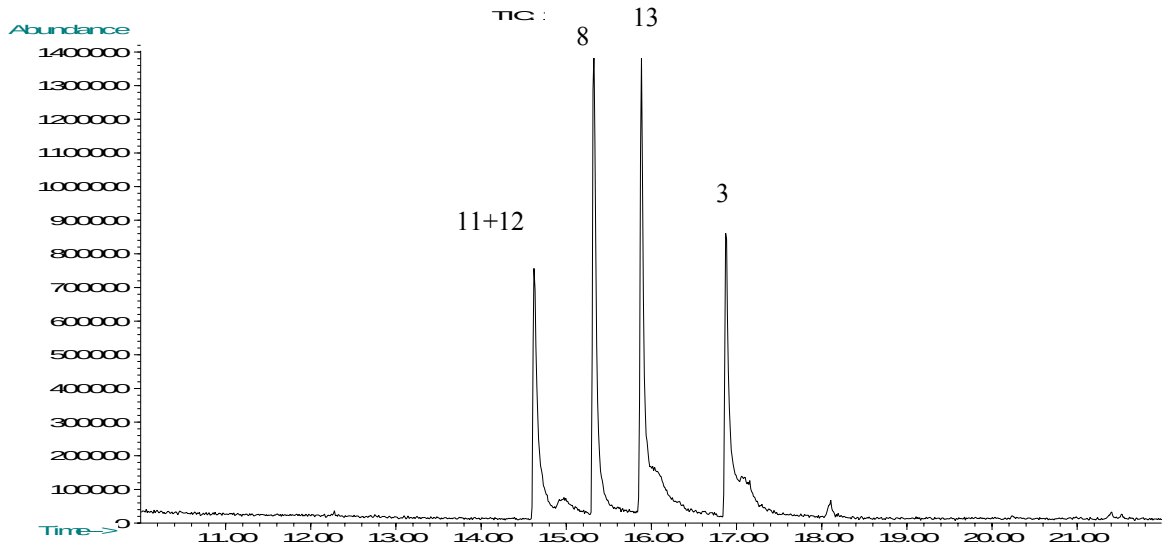
3.2.2 Gas chromatographic separation of 2,3-MDMA and 3,4 MDMA from the methoxymethcathinones

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 24).

The PFPA and HFBA derivatives showed improved resolution when compared to the underivatized amines. Table 9 shows the relative retention of these compounds compared to 3,4-MDMA under identical chromatographic conditions. Several temperature programs were evaluated and the best compromises between resolution and analysis time were used to generate the data in Table 9 and the chromatograms in Figures 25 and 26.

The two chromatograms for the PFPA derivatives (Figure 25) 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 25 and 26. In each case the derivatized 2-methoxymethcathinone (compound 11) eluted first followed closely by the 3-methoxymethcathinone derivative (compound 12). The third compound to elute is the 2,3-MDMA derivative (compound 8) and the derivatized 4-methoxymethcathinone (compound 13) is the forth peak in each chromatogram. The derivatized form of 3,4-MDMA (compound 3) showed the greatest retention in each chromatogram.



Time (minutes)

Figure 24: Capillary gas chromatogram for a physical mixture of underivatized 3,4 MDMA, 2,3-MDMA and methoxymethcathinones**.**

Table 9: Retention data of the underivatized, HFBA and PFPA derivatives of 3,4-MDMA, 2,3-MDMA and the methoxymethcathinones collected using Rtx-1 and Rtx-5 stationary phases

Compounds' Number	Rtx-1◊			Rtx-5◊◊		
		Derivatives▲			Derivatives▲	
	Underivatized**	HFBA derivatives*	PFPA derivatives**	Underivatized*	HFBA derivatives*	PFPA derivatives*
3	1.0 (11.108 min.)	1.0 (13.556 min.)	1.0 (9.390 min.)	1.0 (11.679min.)	1.0 (13.932 min.)	1.0 (13.546 min.)
8	0.889	0.927	0.932	0.878	0.929	0.926
11	0.848♦	0.873	0.875	0.833♦	0.978	0.870
12	0.848♦	0.894	0.892	0.833♦	0.898	0.886
13	0.925	0.967	0.964	0.920	0.970	0.967

◊ Rtx-1 is a 30m x 0.25mm-I.d. column coated with o.25 µm 100% dimethyl polysiloxane
◊◊Rtx-5 is a 30 m x 0.25mm-I.d. column coated with o.25 µm 95% dimethyl-5%diphenyl polysiloxane
* Temperature program used was TP-1
** Temperature program used was TP-6
▲Abbreviations: PFPA, pentafluoropropionamide; HFBA, heptafluorobutyrylamide.
♦ Compounds co-elute with each other
-Results are the average of three experiments

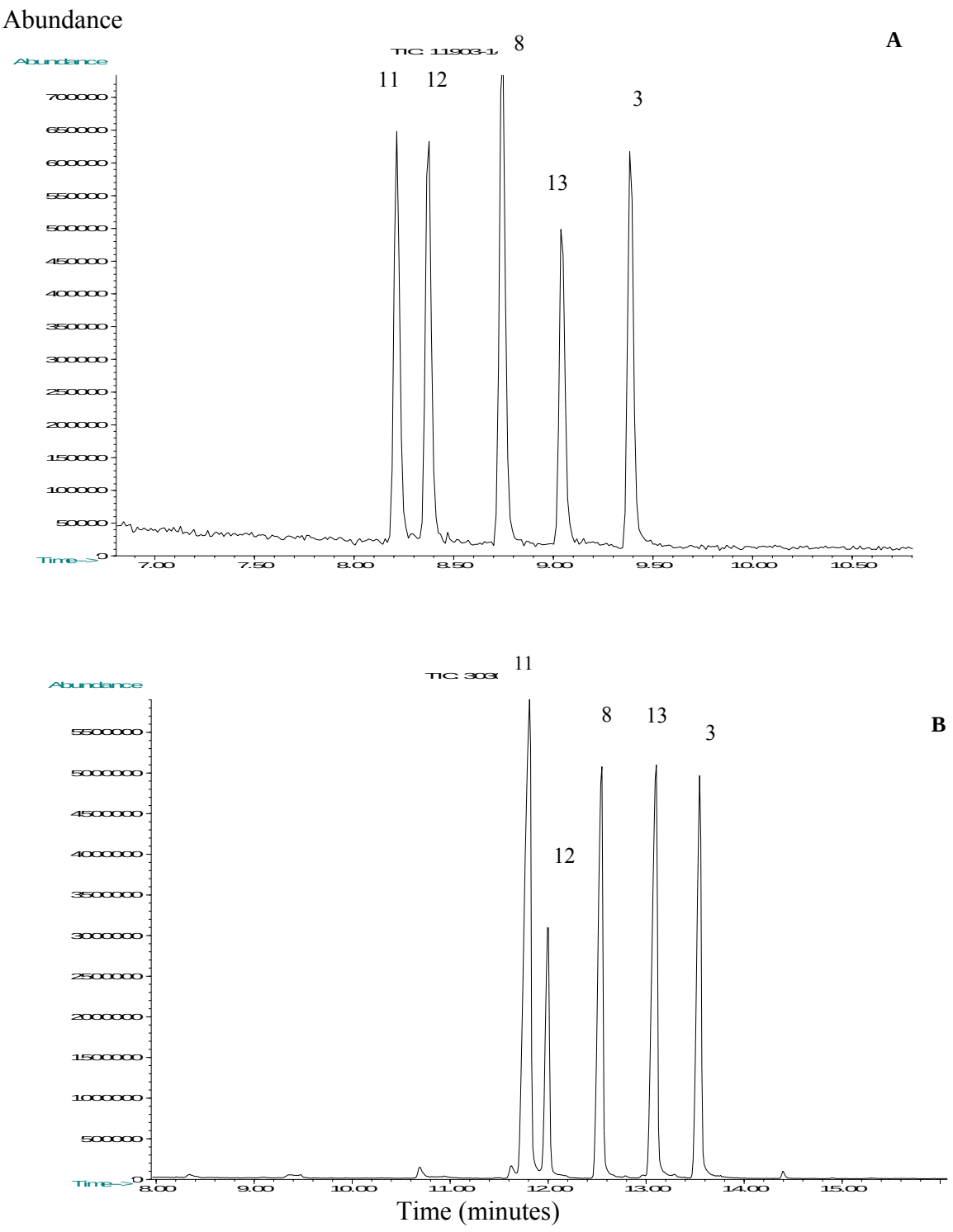


Figure 25: Capillary gas chromatographic separation of PFPA derivatives of compounds 3, 8, 11, 12 and 13. Columns used: A Rtx-1; B Rtx-5.

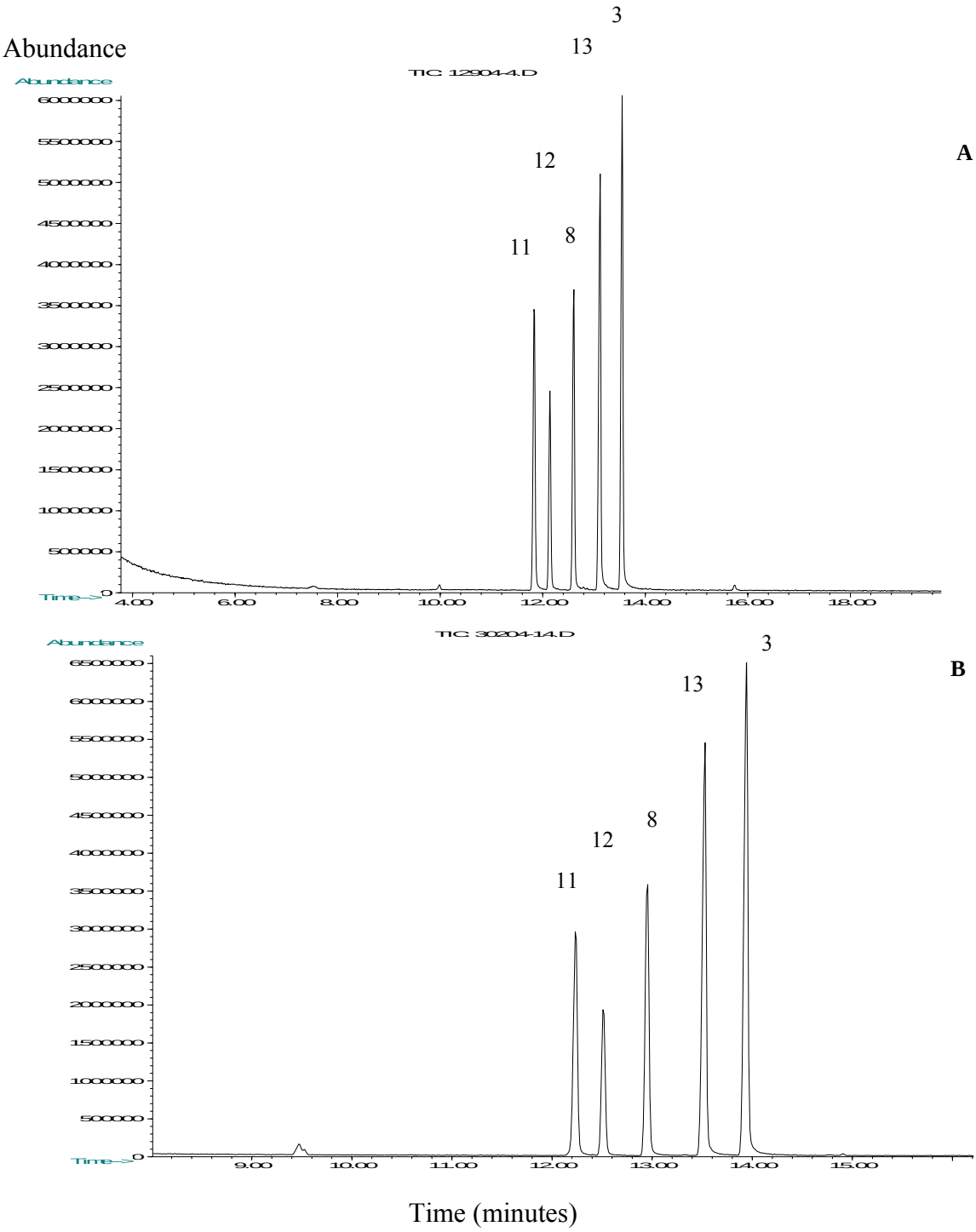


Figure 26: Capillary gas chromatographic separation of HFBA derivatives of compounds 3, 8, 11, 12 and 13. Columns used; A: Rtx-1; B: Rtx-5.

3.3 Ultraviolet, Mass Spectral and Gas Chromatographic Studies of Isobaric Substances Related 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 is the methoxy methyl substituted methamphetamines. The ultraviolet properties of these substaces will be discussed in chapter 3.3.1 while their mass spectral properties will be sudied in chapter 3.3.2 and 3.3.3. The chromatographic separation of the underivatized and derivatized amines will be discussed in chapter 3.3.4.

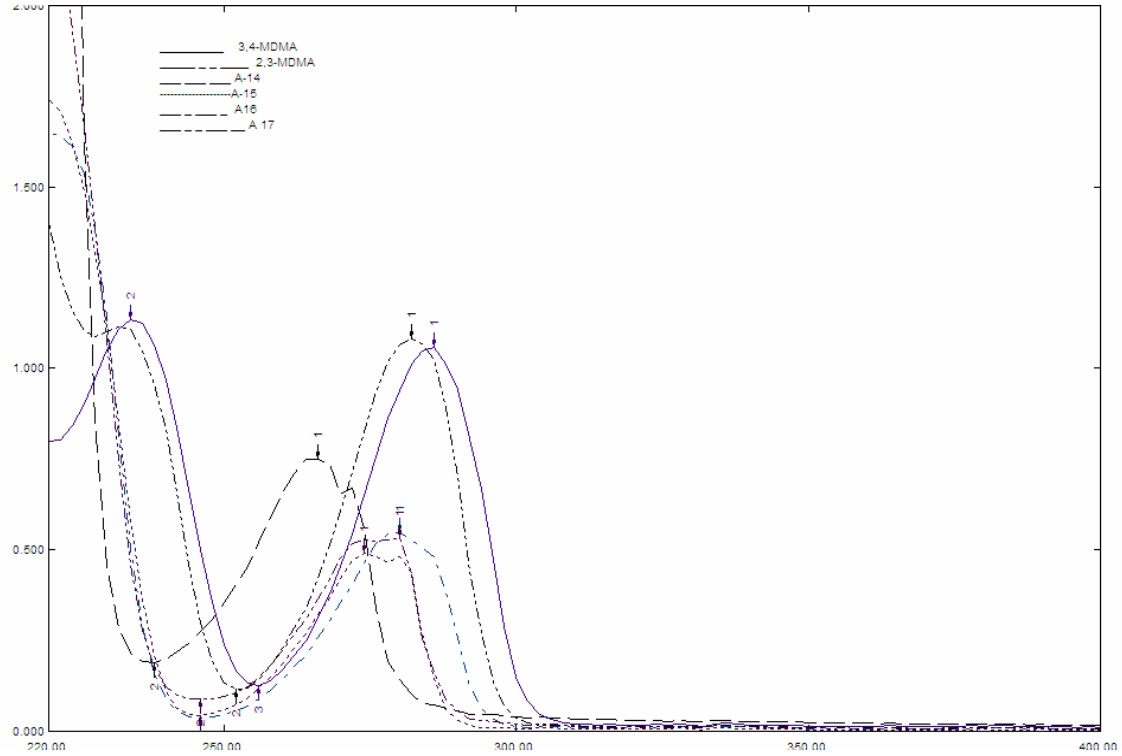
3.3.1. Ultraviolet spectrophotometric studies of methoxy methyl methamphetamines compared to 2,3- and 3,4-MDMA.

The UV spectra were obtained from aqueous solutions of the analytes dissolved in 1N hydrochloric acid at a wavelength range between 220 and 400 nm.

It is already known that the methylenedioxyphenyl group in 3,4-MDMA is a strong chromophore in the UV range with two major absorption bands in the 285 nm and 235 nm range with the absorptivity slightly higher at 285 nm [Noggle *et al.*, 1987].

The 2,3-methylenedioxyphenyl regioisomer shows quite similar absorption properties except the absorption at 235 nm is weaker and appears only as a shoulder (Figure 27). The methoxy methyl methamphetamines with the methoxygroup in the ortho postion (compounds 14-17) show almost identical UV spectra with both 3,4-MDMA and 2,3-MDMA as illustrated in Figure 27, only 2-methoxy-3-methylmethamphetamine (compound 14) shows a different absorption maximum occuring at 266nm.

Absorbance



Wave lenghth (nm)

Figure 27: UV-spectra of compounds 14-17, 2,3-MDMA and 3,4-MDMA

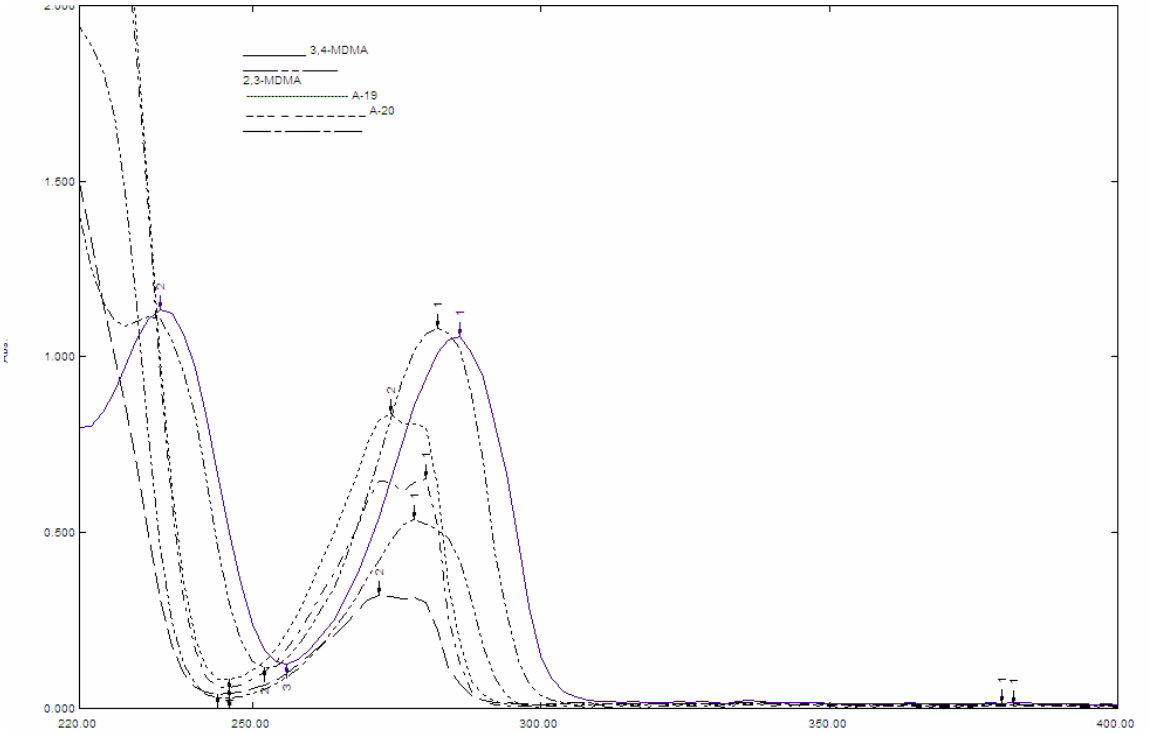


Figure 28: UV-spectra of compounds 18-21, 2,3-MDMA and 3,4-MDMA

The isobaric methoxy methyl methamphetamines with the methoxy group in the meta postion show λ_{max} at a range of 272-280 nm which is slighltly lower than those observed for both 3,4- and 2,3- MDMA (Figure 28). However the maximum peaks of these compounds intersects with peak shoulders of both 3,4- and 2,3- MDMA's indicating that discrimination between these compounds and the target drug of abuse can not be achived using direct spectrophotometry.

The ring substituted methoxy methyl methamphetamines with the methoxy group in the para postion show a different λ_{max} at 274 nm (Figure 29). Since the λ_{max} of both compounds intersects with the shoulders of the peak maxima of both 3,4- and 2,3-

MDMA's, it will be very hard to determine metha methoxy methyl methamphetamines when exist in a physical mixture with either or both 2,3- and 3,4-MDMA

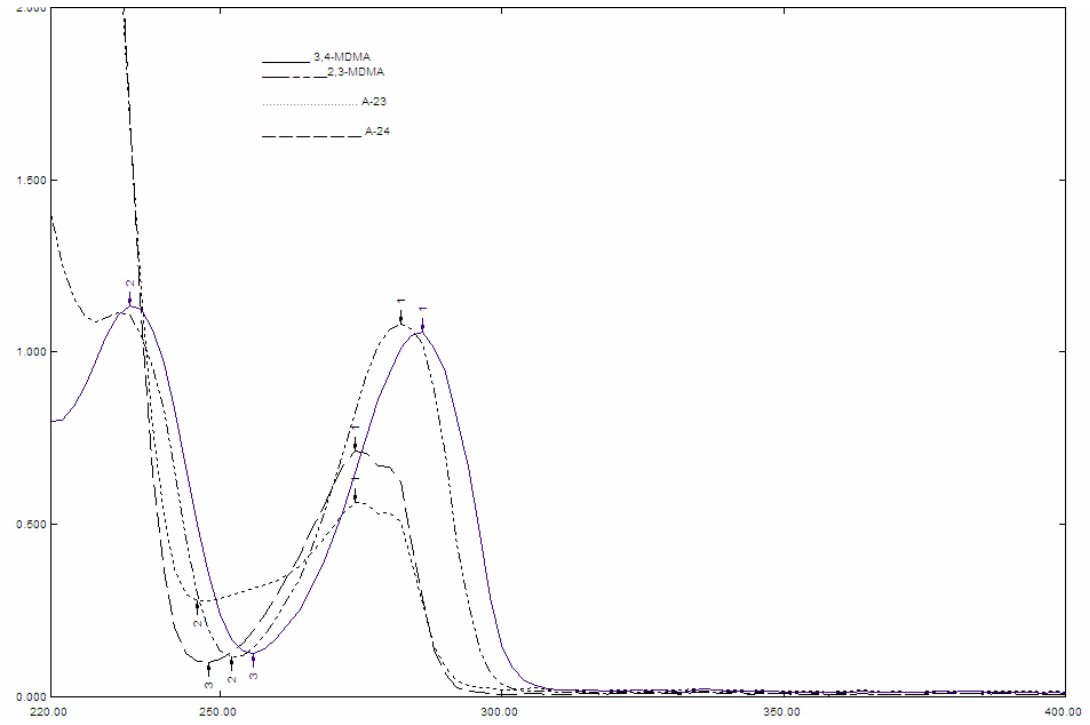


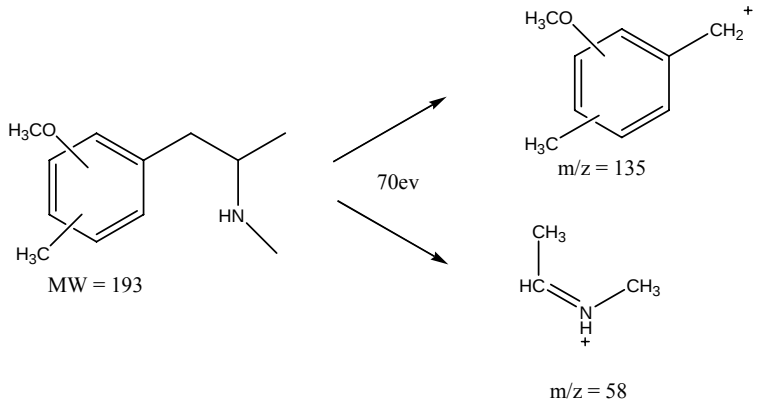
Figure 29: UV-spectra of compounds 22, 23, 2,3-MDMA and 3,4-MDMA

3.3.2. Mass spectral studies of isobarics related 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.

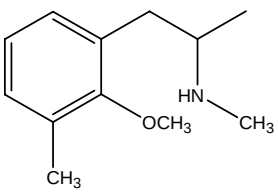
The various methoxy methyl ring substitution patterns of methamphetamine have the potential to yield mass spectra essentially equivalent to 3,4-MDMA and 2,3-MDMA, all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136 (Figure 30). The 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 46).



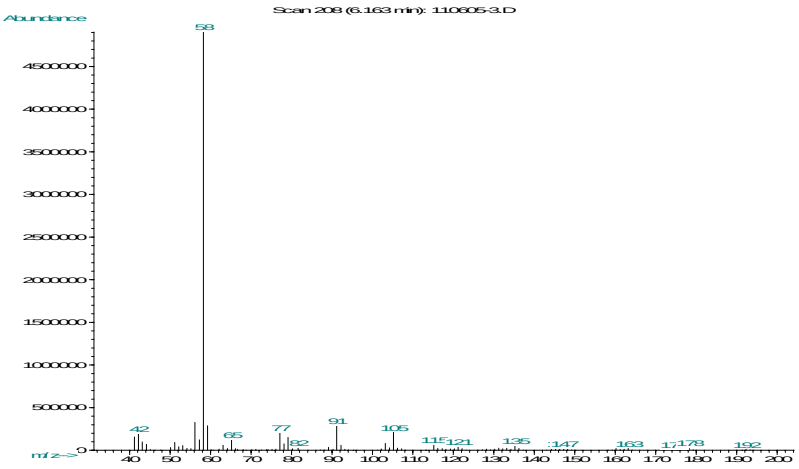
Scheme 46: General mass spectral fragmentation for the ring substituted methoxy methyl methamphetamines (compounds 14-23).

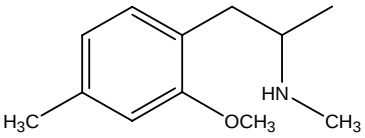
Figure 30: Structures and mass spectra of ring substituted methoxy methyl methamphetamines

Structure of Isobarices | **Mass spectrum**



14





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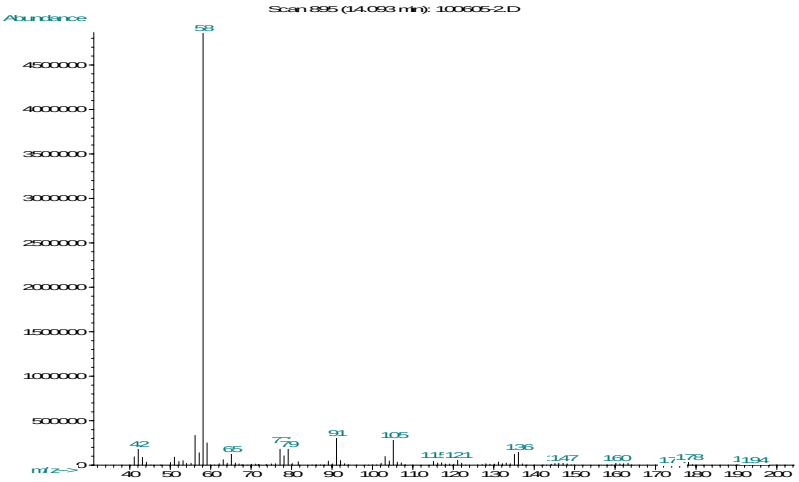
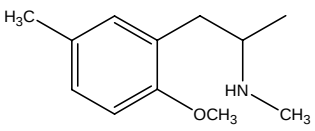
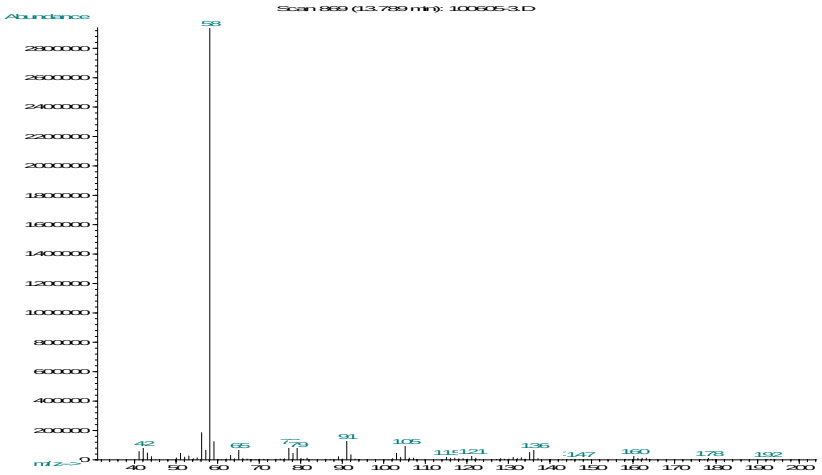
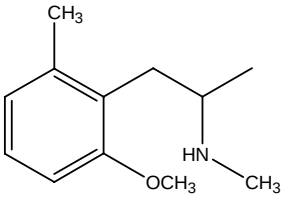


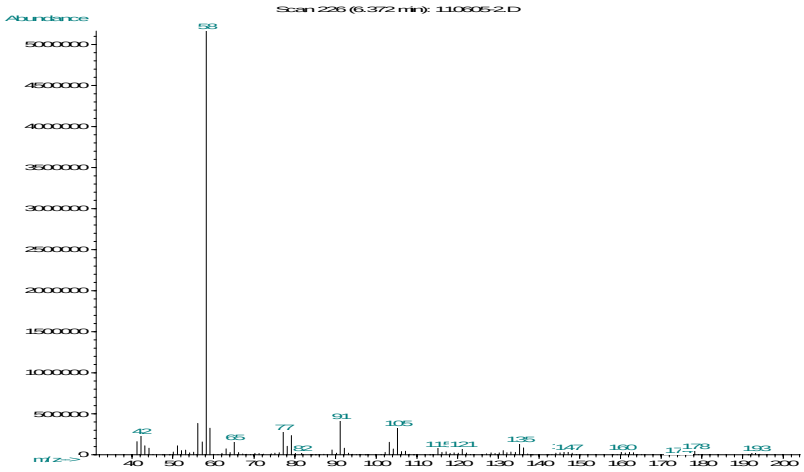

H3C
HN
OCH3
CH3

16


Scan 869 (13.789 min): 100605-3.D
Abundance
m/z-->


CH3
HN
OCH3
CH3

17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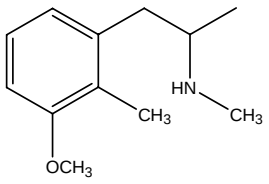
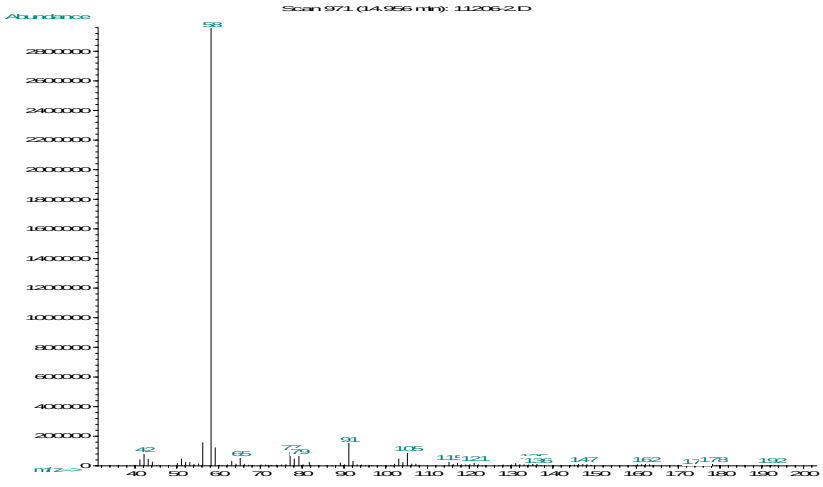
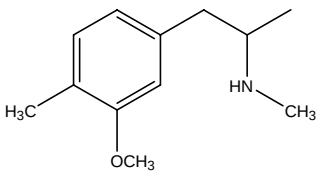
Abundance
m/z-->

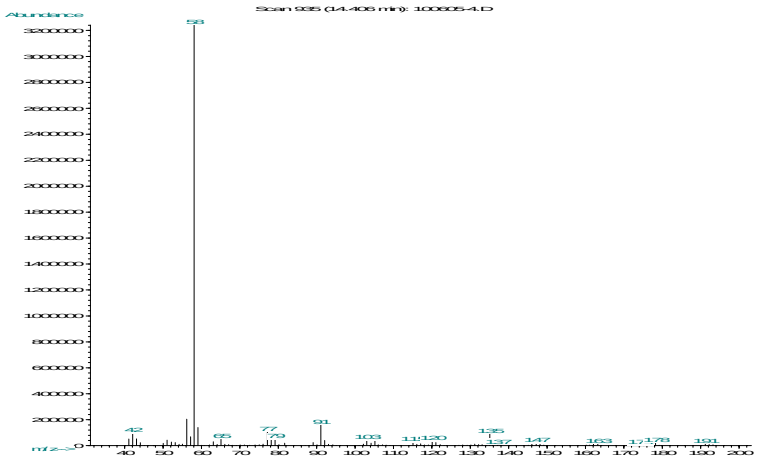

HN
CH3
CH3
OCH3

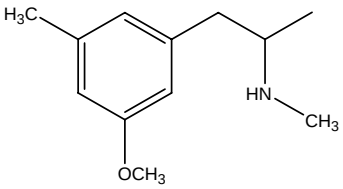
18


Scan 971 (14.956 min): 111206-2.D
Abundance
m/z-->

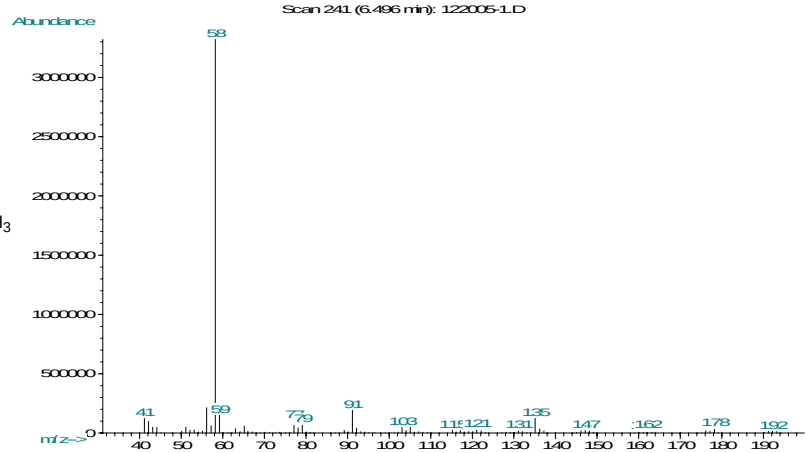

H3C
HN
CH3
OCH3

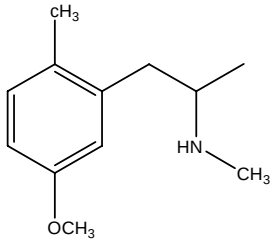
19


Abundance
Scan 935 (14.406 min): 100605-1.D
58
42
65
77
79
91
103
111120
135
137
147
163
178
181
m/z-->

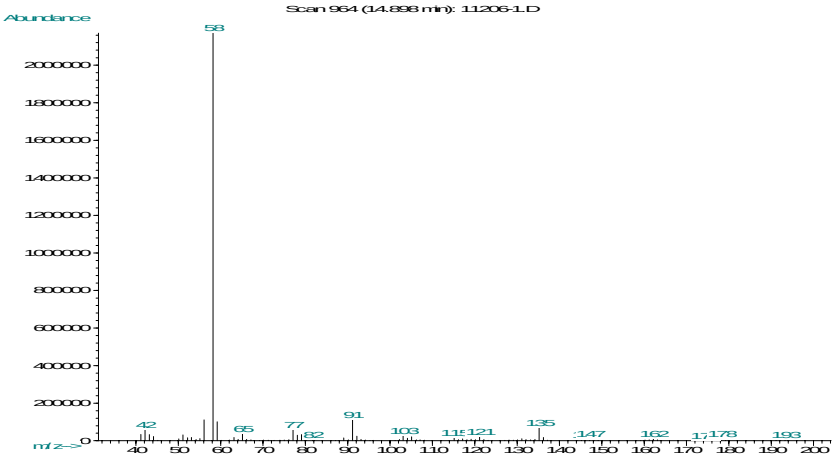

H3C
HN
CH3
OCH3

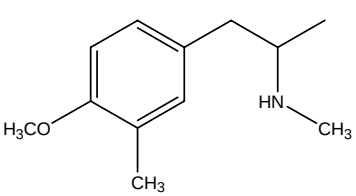
20


Abundance
Scan 241 (6.496 min): 122005-1.D
58
41
59
77
79
91
103
111121
131
135
147
162
178
192
m/z-->

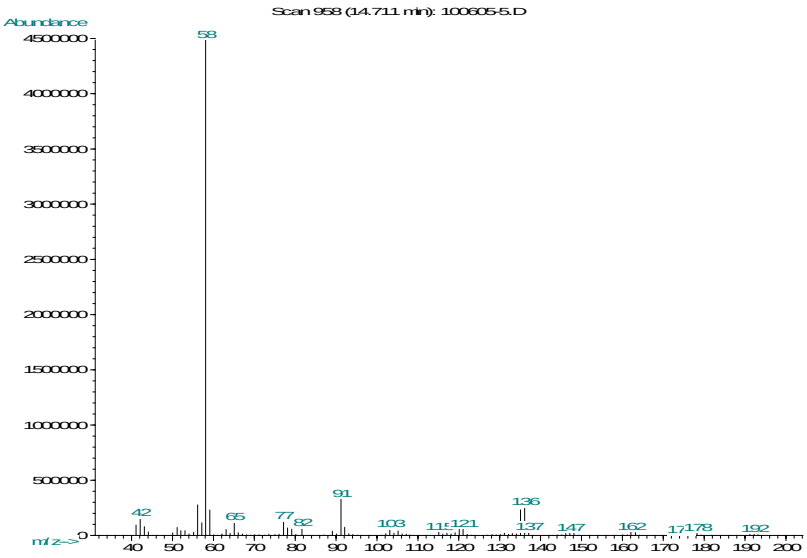

CH3
HN
CH3
OCH3

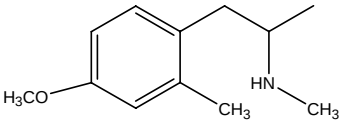
21


Abundance
Scan 964 (14.898 min): 111206-1.D
58
42
65
77
82
91
103
111121
135
147
162
178
193
m/z-->

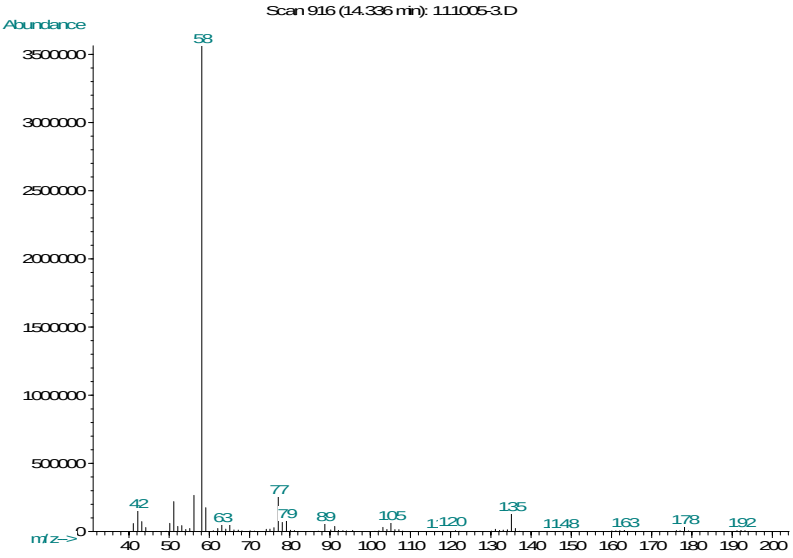


22





23



The mass spectra for the ten ring substituted methoxy methyl methamphetamines in Figure 30 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

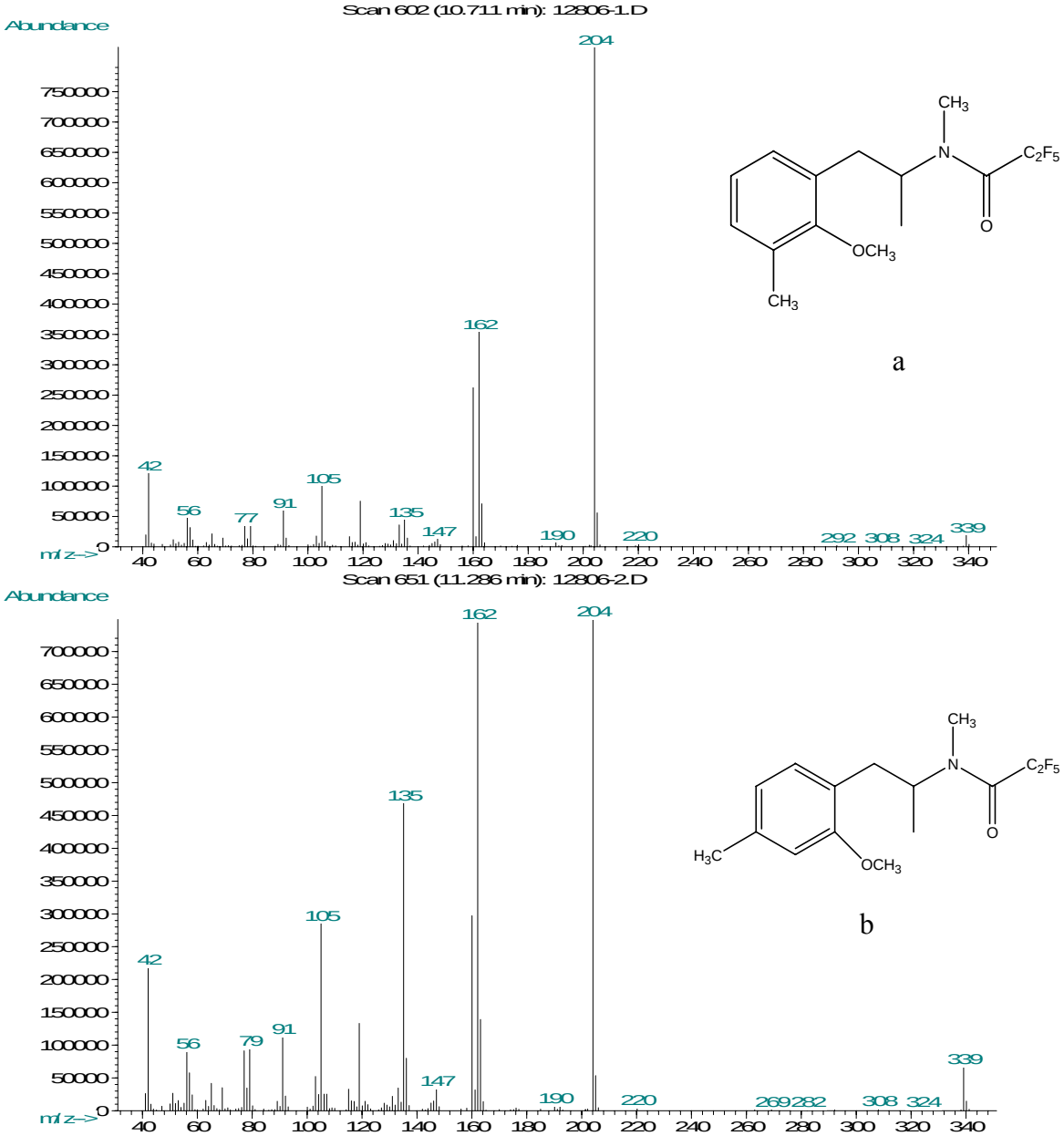
individual identification of any one of these substances. This constitutes a significant analytical challenge, where the use of gas chromatography-mass spectrometry (GC-MS) must be based primarily upon the ability of the chromatographic system to separate the "counterfeit substance" from the actual drug of interest. Addtionally, the ability to distinguish between these regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

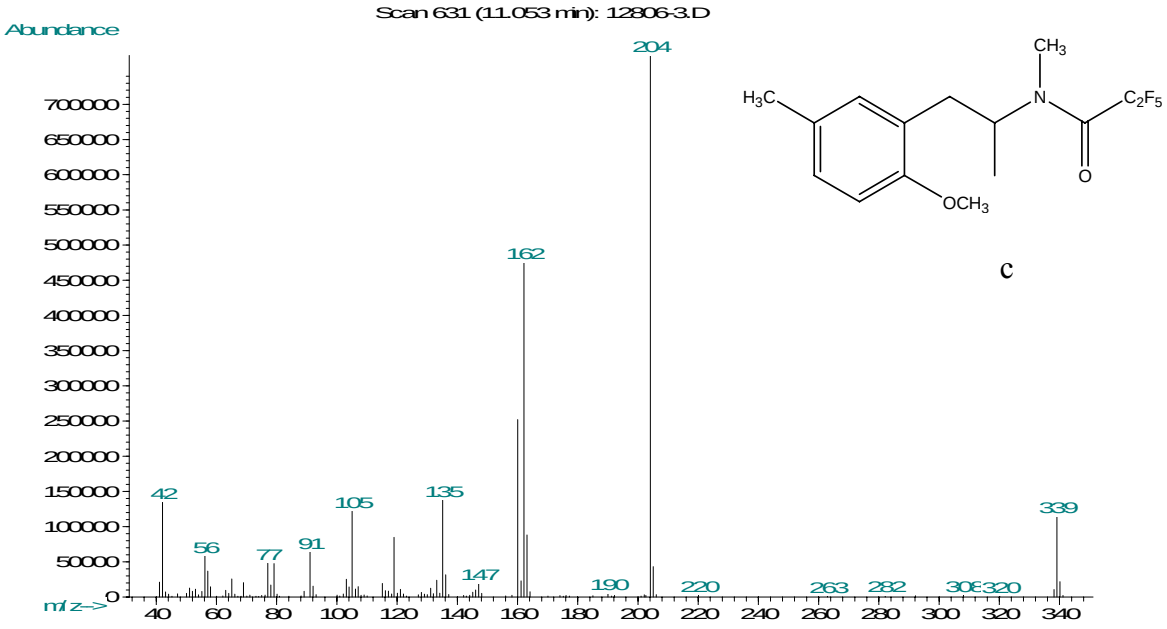
3.3.3. Mass spectral studies of perfluroacyl derivatives of isobaric substances (methoxymethyl methamphetamines) compared to 2,3- and 3,4-MDMA

The perfluoroacylated derivatives of the ten methoxy methyl methamphetamines were prepared (Chapter 4) 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 [F. W. McLafferty et al, 1993]. The PFPA and HFBA derivatives of the side chain regioisomers of 2,3- and 3,4-MDMA were described previously in 3.1.2. 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,

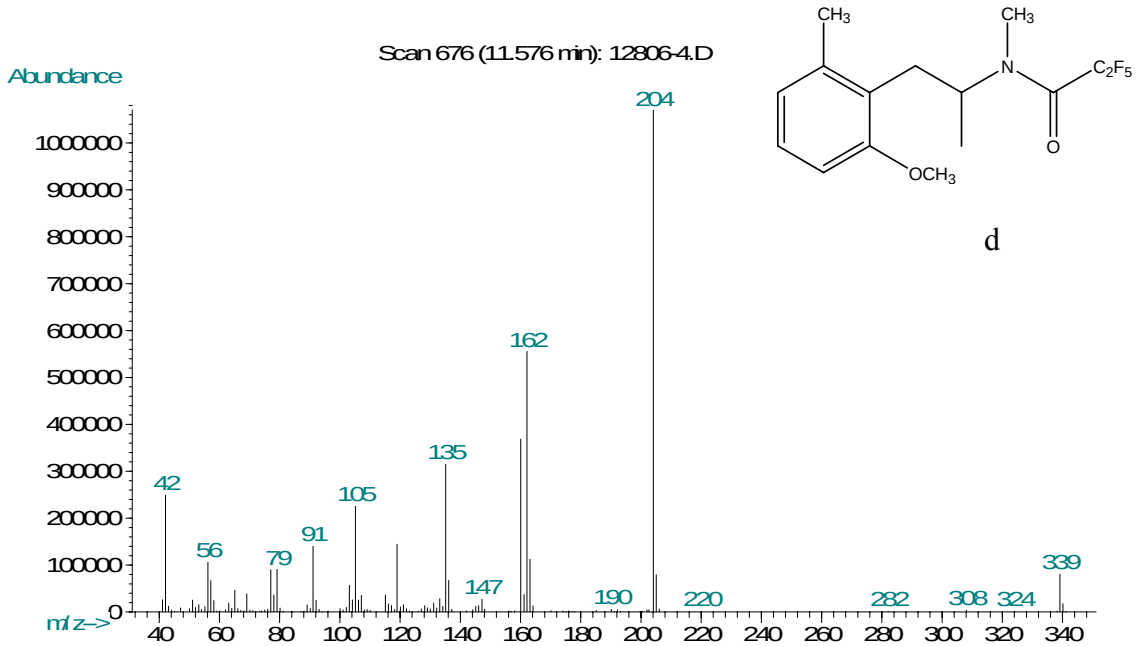
perfluroacylation may not allow for complete compound individualization based only on the observed mass spectrum. The mass spectra for the ten pentafluoropropionyl (PFPA) and heptflourobutryl (HFBA) amides are shown in Figures 31 and 32, while those of 3,4 and 2,3-MDMA are shown in Figures 16b, 16f, 17b and 17f, respectively

Figure 31: Mass spectra of the PFPA derivatives of compounds 14-23

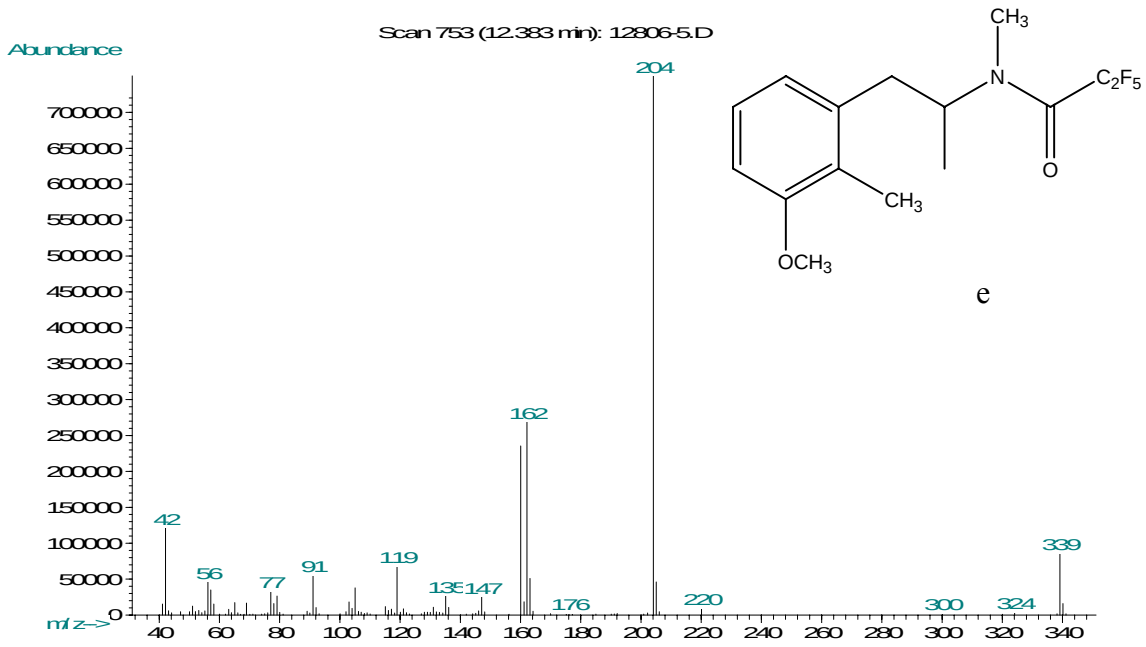



Scan 631 (11.053 min): 12806-3.D
Abundance
m/z-->
204
162
42
56
77
91
105
135
147
190
220
263
282
308
320
339
CH_3
H_3C
N
C_2F_5
O
OCH_3

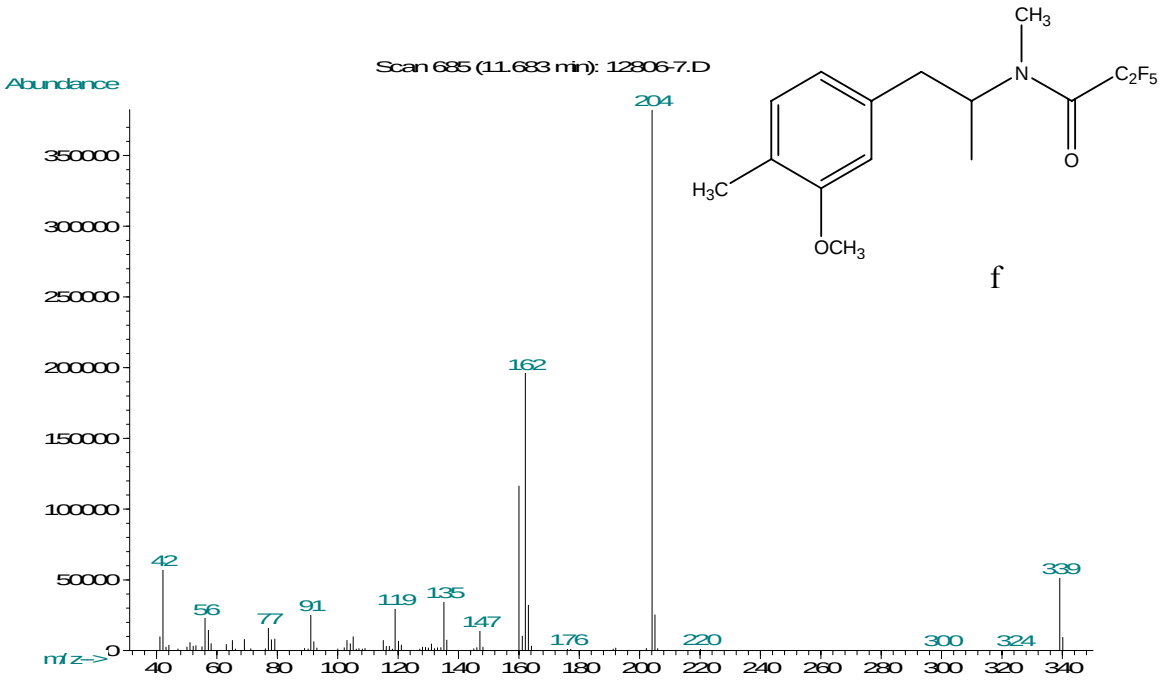
c


Scan 676 (11.576 min): 12806-4.D
Abundance
m/z-->
204
162
42
56
79
91
105
135
147
190
220
282
308
324
339
CH_3
CH_3
N
C_2F_5
O
OCH_3

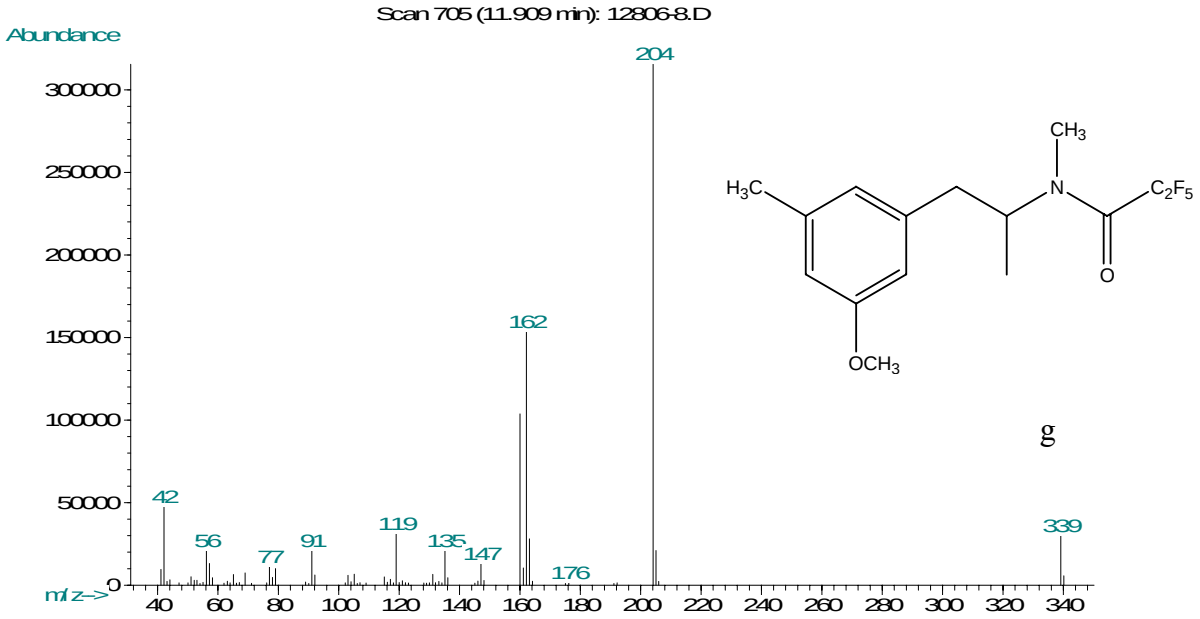
d


Scan 753 (12.383 min): 12806-5.D
Abundance
m/z-->
204
162
42
91
119
56
77
135
147
176
220
300
324
339
CH3
N
C2F5
O
CH3
OCH3

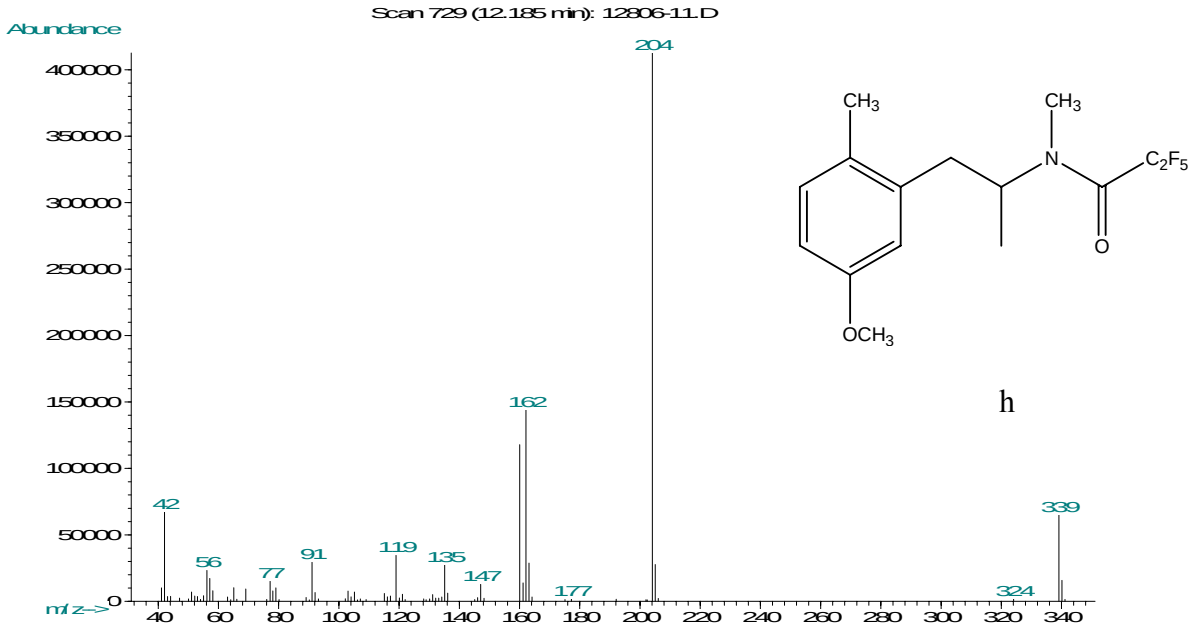
e


Scan 685 (11.683 min): 12806-7.D
Abundance
m/z-->
204
162
42
91
119
135
56
77
147
176
220
300
324
339
CH3
N
C2F5
O
H3C
OCH3

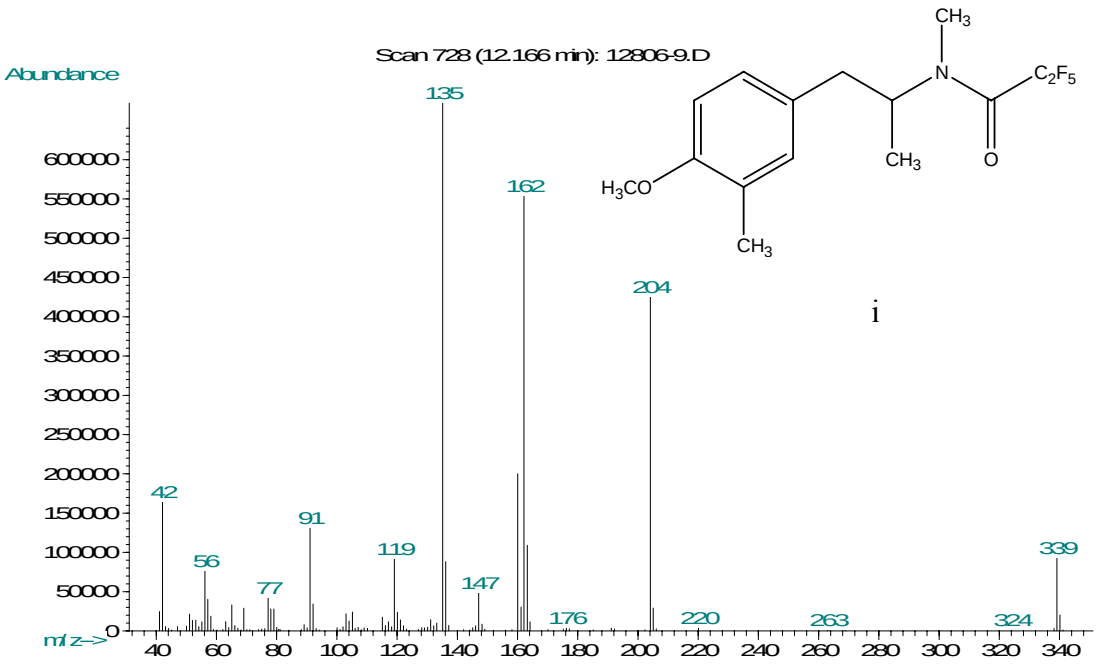
f


Scan 705 (11.909 min): 12806-8.D
Abundance
m/z-->
204
162
42
56
77
91
119
135
147
176
339
CH3
H3C
N
C2F5
O
OCH3

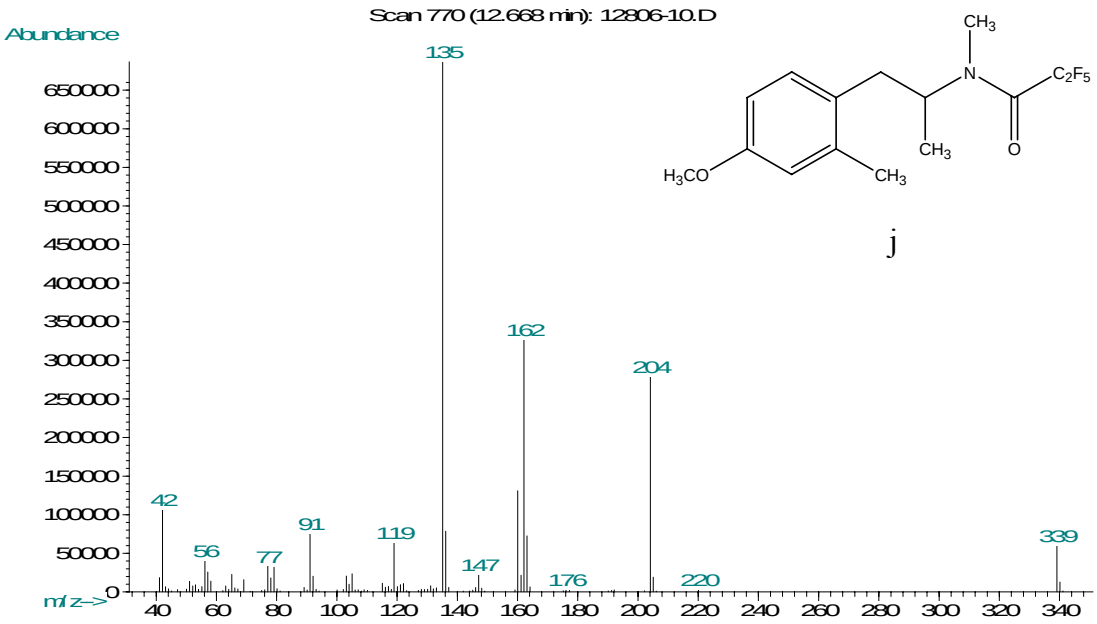
g


Scan 729 (12.185 min): 12806-11.D
Abundance
m/z-->
204
162
42
56
77
91
119
135
147
177
324
339
CH3
CH3
N
C2F5
O
OCH3

h

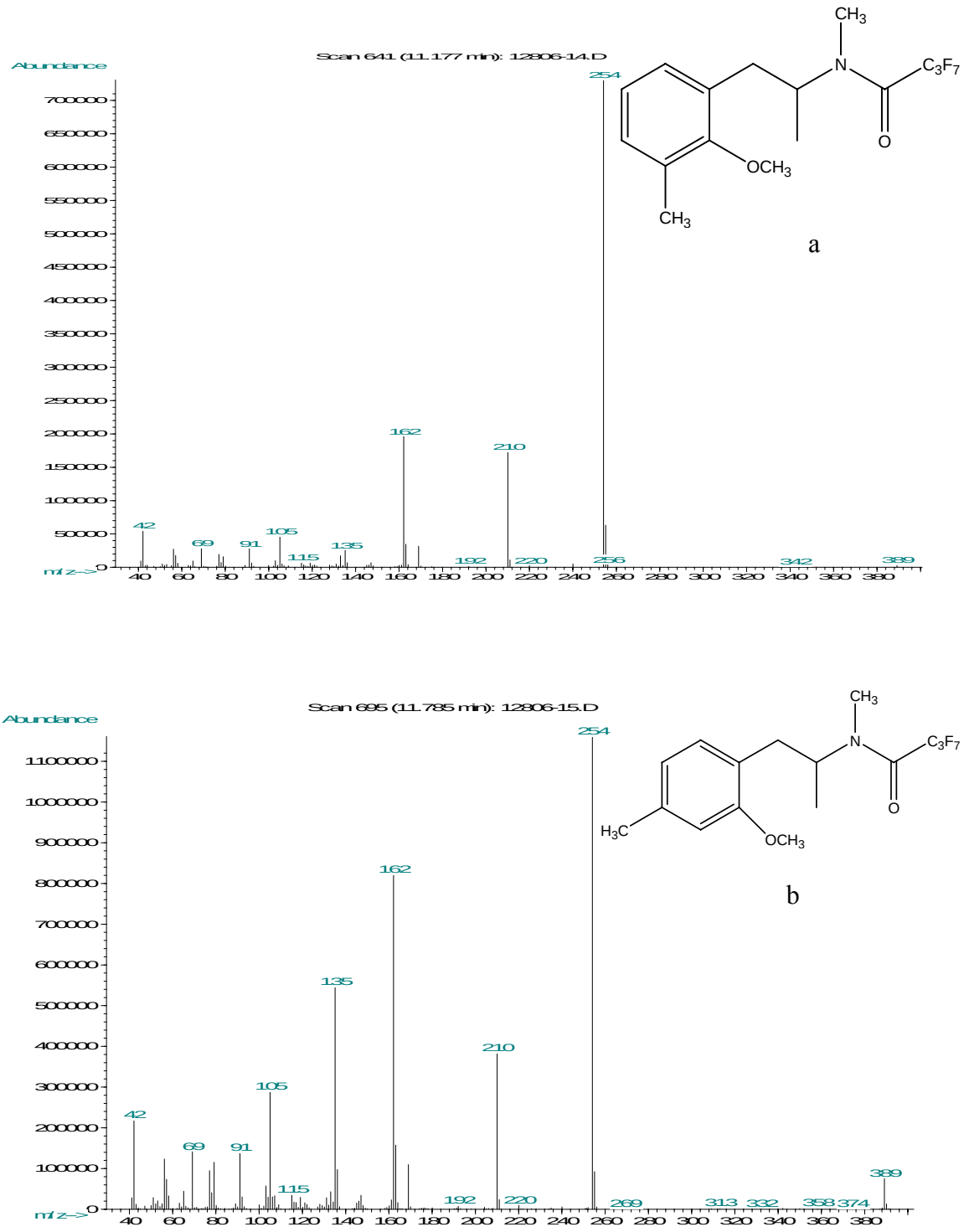

Scan 728 (12.166 min): 12806-9.D
Abundance
m/z-->
135
162
204
42
91
119
56
77
147
176
220
263
324
339
CH3
N
C2F5
O
H3CO
CH3
CH3

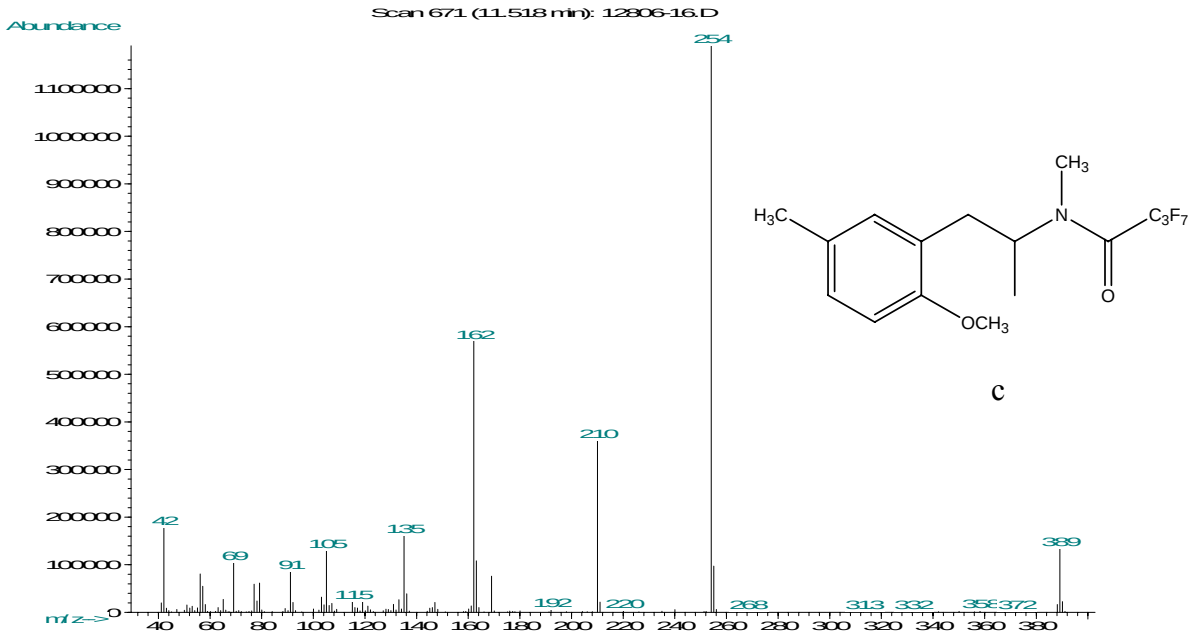
i


Scan 770 (12.668 min): 12806-10.D
Abundance
m/z-->
135
162
204
42
91
119
56
77
147
176
220
339
CH3
N
C2F5
O
H3CO
CH3
CH3

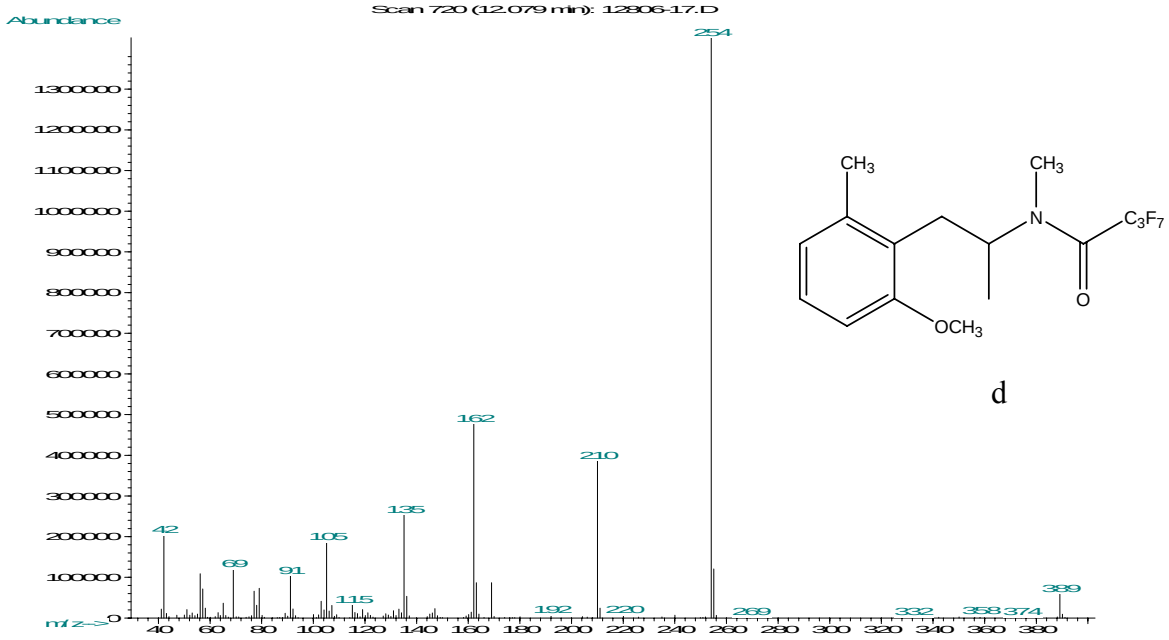
j

Figure 32: Mass spectra of the HFBA derivatives of compounds 14-23

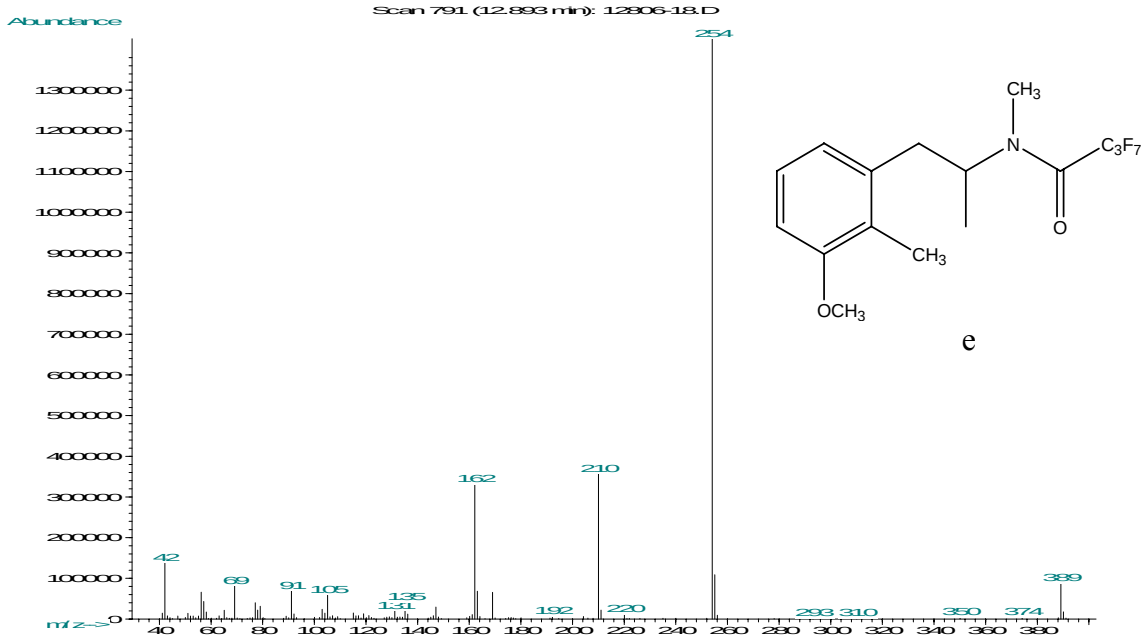



Scan 671 (11.518 min): 12806-16.D
Abundance
m/z-->
254
162
210
42
69
91
105
115
135
192
220
268
313
332
389
CH3
H3C
N
C3F7
O
OCH3

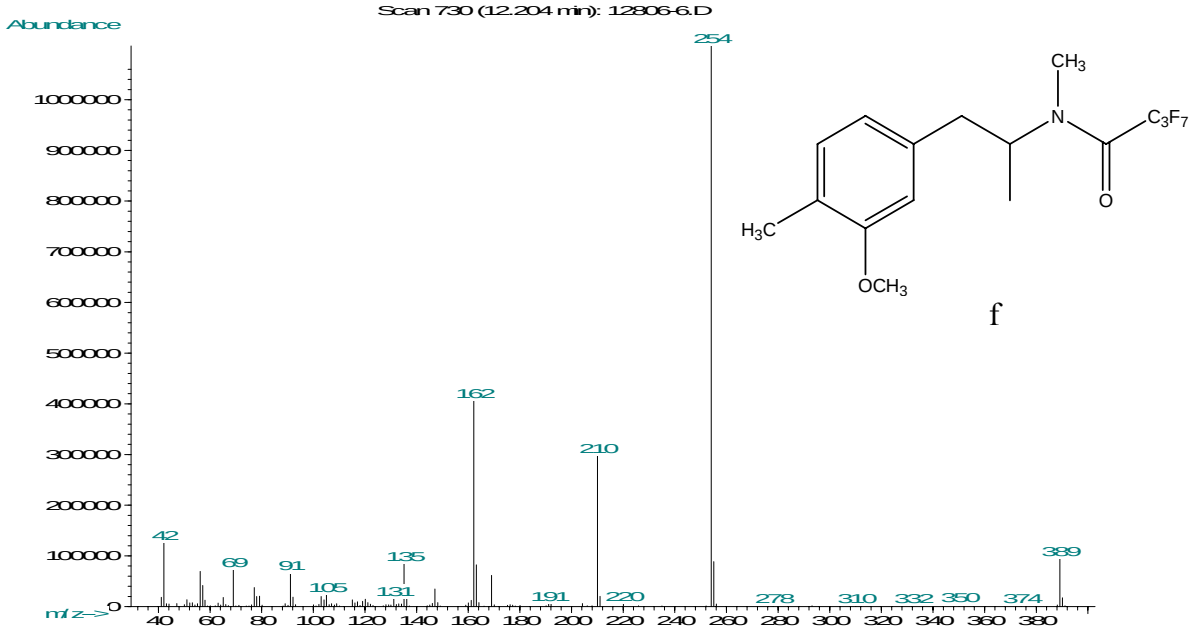
c


Scan 720 (12.079 min): 12806-17.D
Abundance
m/z-->
254
162
210
42
69
91
105
115
135
192
220
269
332
358
374
389
CH3
CH3
N
C3F7
O
OCH3

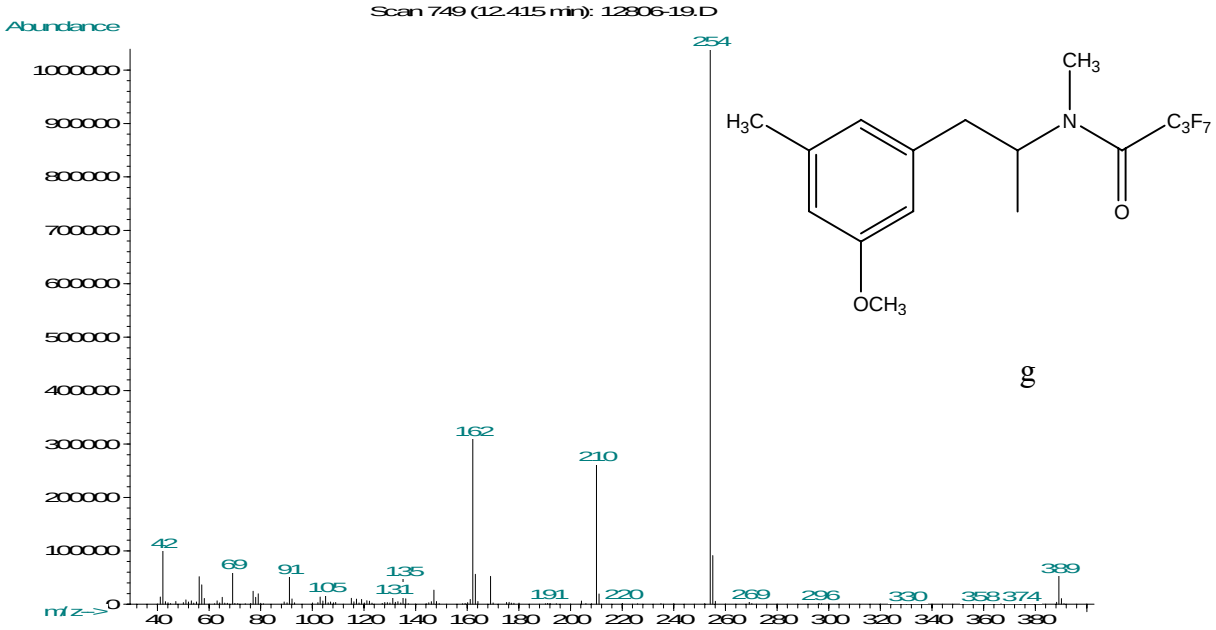
d


Scan 791 (12.893 min): 12806-18.D
Abundance
m/z-->
254
210
162
42
69
91
105
135
131
192
220
293
310
350
374
389
CH3
N
C3F7
O
CH3
OCH3

e


Scan 730 (12.204 min): 12806-6.D
Abundance
m/z-->
254
162
210
42
69
91
135
105
131
191
220
278
310
332
350
374
389
CH3
N
C3F7
O
H3C
OCH3

f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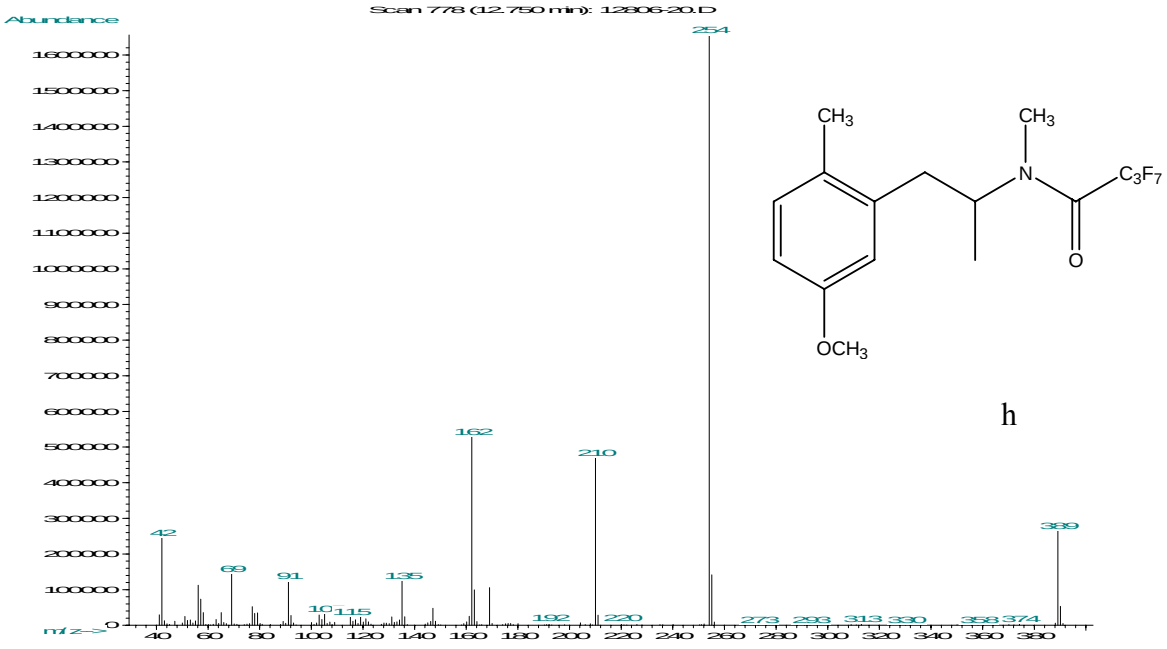
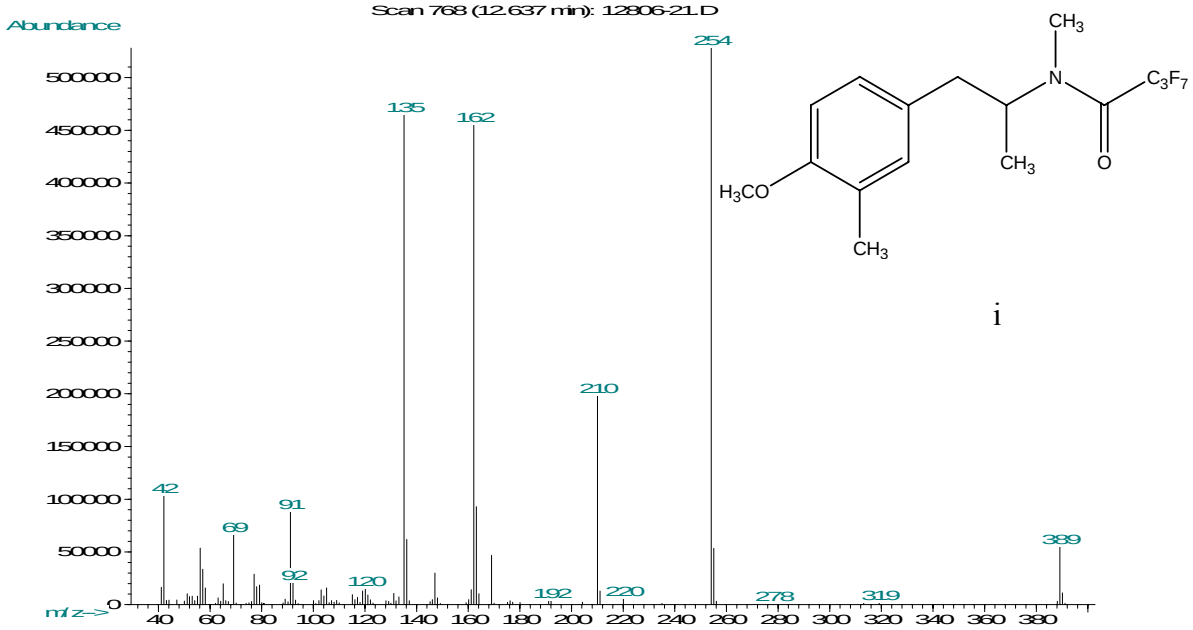
Abundance
m/z-->
254
162
210
42
69
91
105
131
135
191
220
269
296
330
358
374
389
CH3
H3C
N
C3F7
O
OCH3

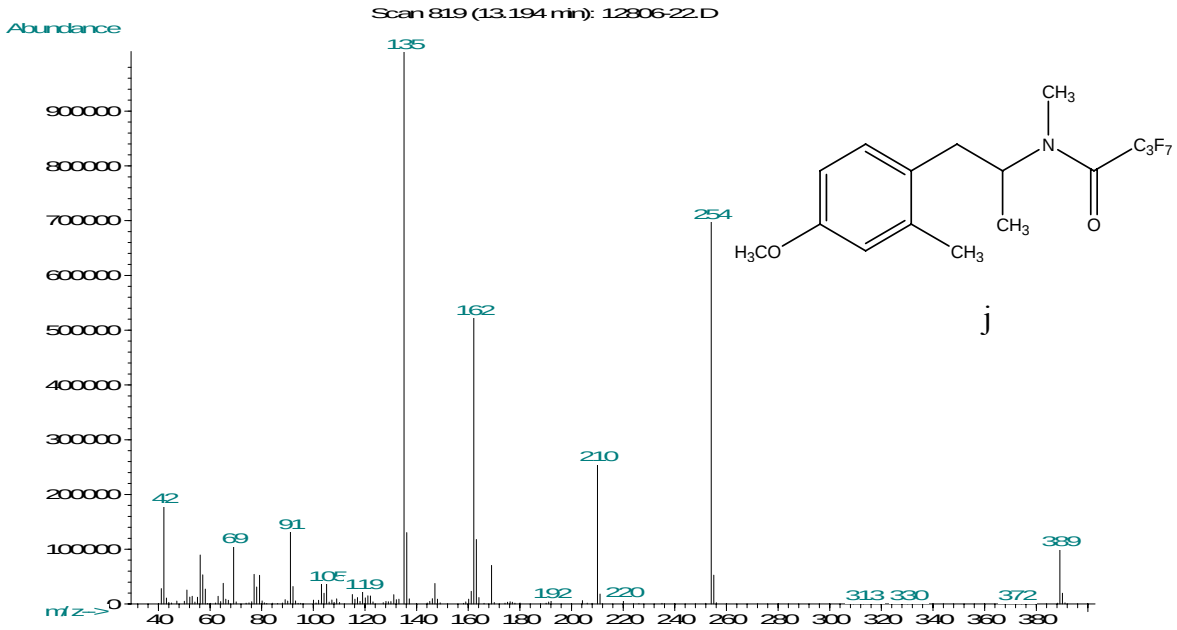
g


Scan 778 (12.750 min): 12806-20.D
Abundance
m/z-->
254
162
210
42
69
91
135
192
220
273
296
313
330
358
374
389
CH3
CH3
N
C3F7
O
OCH3

h

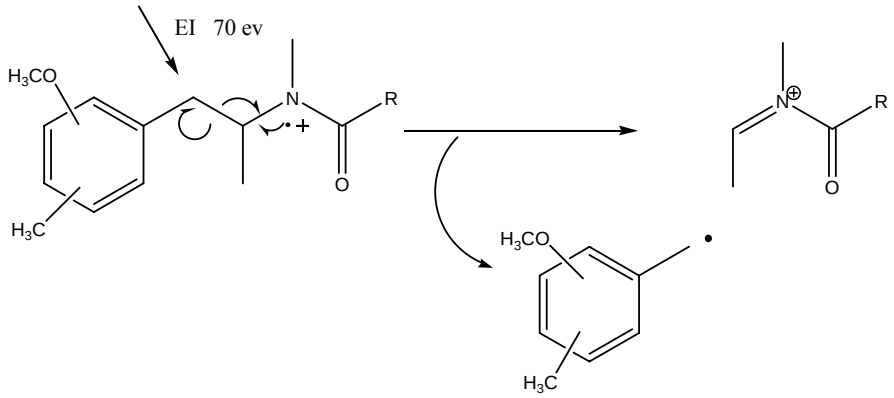

Scan 768 (12.637 min): 12806-21.D
Abundance
m/z-->
254
135
162
210
42
91
69
92
120
192
220
278
319
389
CH3
N
C3F7
O
CH3
H3CO
CH3

i


Scan 819 (13.194 min): 12806-22.D
Abundance
m/z-->
135
254
162
210
42
91
69
105
119
192
220
313
330
372
389
CH3
N
C3F7
O
CH3
H3CO
CH3

j

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 $(M-135)^+$ species. The general fragmentation pattern and structures for the m/z 204 and 254 ions are shown in Scheme 47. The methoxymethylbenzyl cation and radical cation at m/z 135/136 is also a common fragment in most of the spectra in Figures 31 and 32 (see Scheme 46). 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.



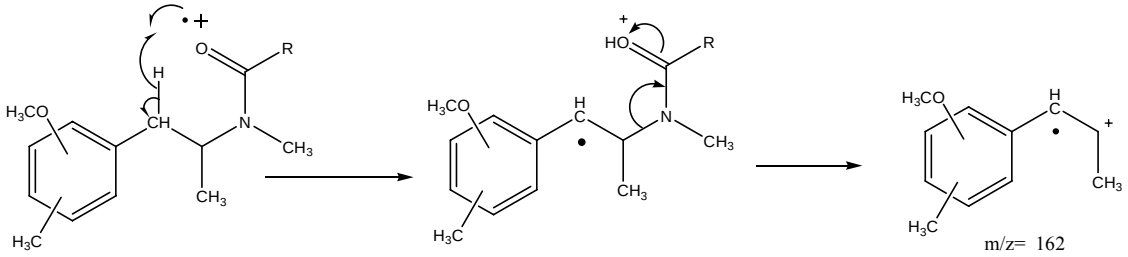
Scheme 47: Formation of m/z 204 (R=C_2F_5) and m/z 254 (R= C_3F_7) from perfluoroaceyl derivatives of methoxy methyl methamphetamines.

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 31 and 32 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 31 and 32) 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 48). 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 31) and at m/z 210 in the HFBA derivatives (Figues 32)

Additionally the PFPA and HFBA derivatives of d_3- and d_5-MDMA, in Figure 18 and discussed earlier in this chapter, also lend support to the proposed strucure for the formation of the alkene fragment at m/z 162 illustrated in Schemes 39 and 42. A comparison of the PFPA derivatives (Figure31) with the HFBA derivatives (Figure 32) 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

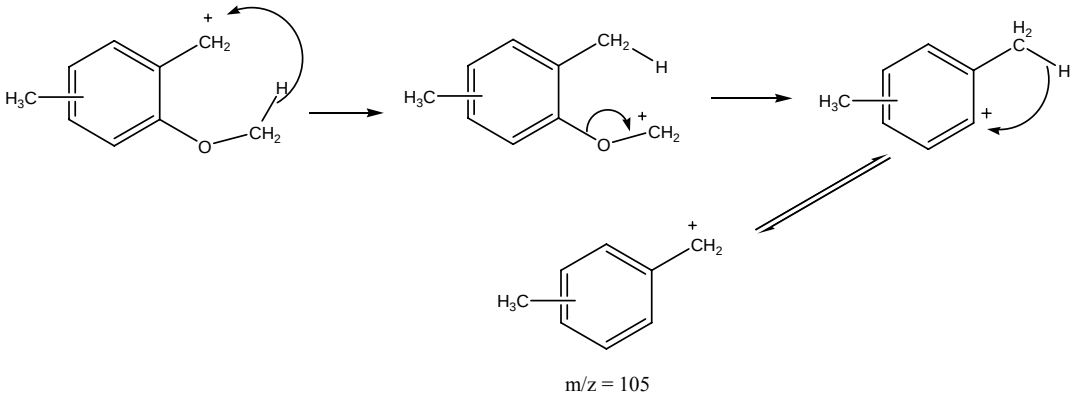
respectively. The PFPA and HFBA derivatives of d_3- and d_5-MDMA, (Figure 18) showed that the m/z 160 and m/z 210 ions contain the N-methyl group and supports the proposed structure of the characteristic nitrile fragment.The suggested mechanism of forming these masses was illustrated previously in Scheme 41.



R = C_2F_5 PFPA derivatives
R= C_3F_7 HFBA Derivatives

Scheme 48: 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 31a, b, c, and d for the PFPA derivatives and Figure 32a, 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 49).



Scheme 49: 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 14-17) all show m/z 105 ion formed through the mechanism described in Scheme 49. 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 14-17.

The PFPA and HFBA derivatives of compounds 18, 19, 20 and 21 (methoxy group in the meta postion of the aromatic ring) can be differentiated from the PFPA and HFBA derivatives of compounds 14, 15, 16 and 17 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 22 and 23, 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.

3.3.4. 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 collect the retention data in Tables 10 and 11 and to generate the twelve chromatograms for the PFPA and HFBA derivatives in Figures 34-39, 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 34-39.

Tables 10 and 11 show the relative retention of these compounds compared to N-methyl-3,4-methyelenedioxyphenyl-2-propanamine (3,4-MDMA) under identical chromatographic conditions. The stationary phases compared were the relatively nonpolar phases, 100% dimethyl polysiloxane (Rtx-1), 95% dimethyl-5% diphenyl polysiloxane (Rtx-5), 65%. dimethyl -35% diphenyl polysiloxane (Rtx-35) and the more polar trifluoropropyl methyl polysiloxane (Rtx-200).

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 15 co-elutes with compound 17 while underivatized compound 19 co-elutes with underivatized compounds 20 and 22 on Rtx-1 stationary phase. Each of the PFPA and HFBA derivatives of compounds 21 and 22 co-elute, also the HFBA derivatives of compounds 17 and 19 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 19, compound 15 co-elutes with compound 17, compound 18 co-elutes with compound 21 and finally compound 20 co-elutes with compound 22. 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 8 co-elutes with compound 20 and compound 21 co-elutes with compound 22. Three sets of the HFBA derivatives were found to co-elute, compound 8 co-elutes with compound 20, compound 14 co-elutes with compound 16 and finally compound 18 co-elutes with compound 21on the same column.

Three sets of underivatized compounds were found to co-elute on Rtx-35, compound 17 co-elutes with compound 19, compound 18 co-elutes with compound 21 and finally compound 20 co-elutes with compound 22. 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 18 co-elutes with compound 22 and compound 17 co-elutes with compound 19. The HFBA derivatives of compounds 15, 21 and 22 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 20 and 22 co-elute (Figure 33). The PFPA derivatives allow more distinctive differentiation between compounds 20 and 22 based on mass spectrometry. Compound 22 has a base peak at m/z 135 compared to m/z 204 base peak for compound 20. Thus a sample containing compound 20 or 22 could be identified based on mass spectrometry.

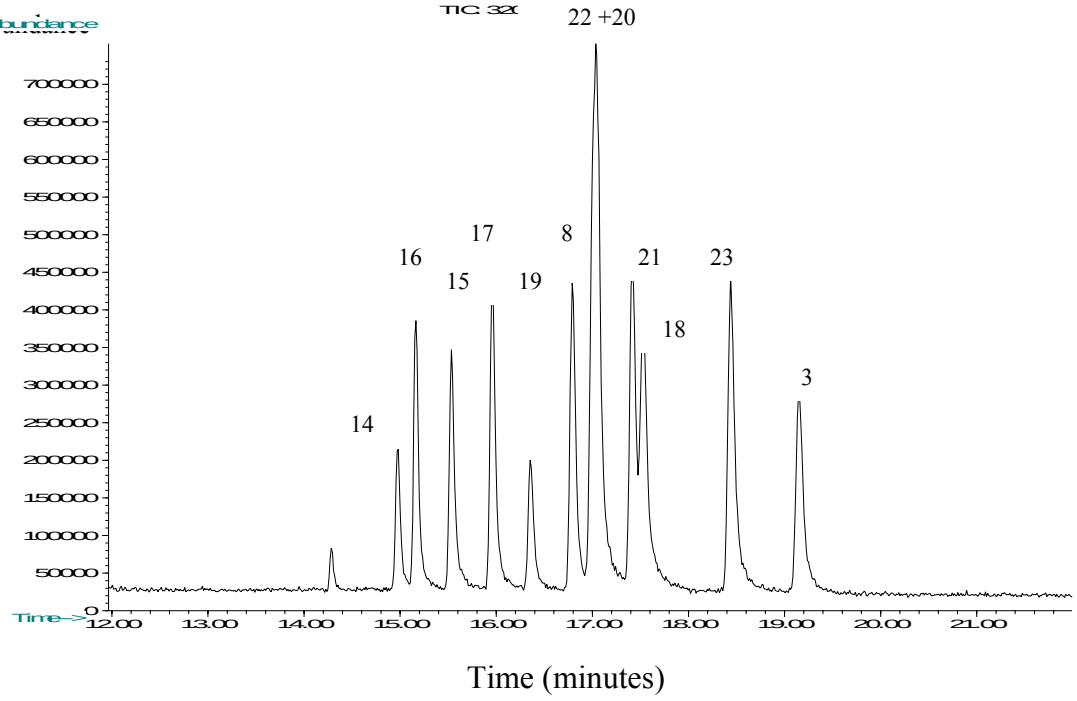


Figure 33: Capillary gas chromatograph of a physical mixture of the PFPA derivatives of compounds 14-23, 2,3-MDMA and 3,4-MDMA. Column used: RTX-200.

Table 10: Retention data of the underivatized, HFBA and PFPA derivatives of compounds 2,3-, 3,4-MDMA and ring substituted methoxy methyl methamphetamines collected on Rtx-1 and Rtx-35

Compound Number	Rtx-1[◊]			Rtx-35[◊◊]		
		Derivatives[▲]			Derivatives[▲]	
	Underivatized	HFBA	PFPA	Underivatized	HFBA	PFPA
3	1[◊] (14.909 min)	1 (18.931 min)	1 (18.497 min)	1 (18.333 min)	1 (20.893 min)	1 (20.909 min)
8	0.995	0.955	0.951	0.996	0.962	0.952[⌂]
14	0.864	0.898	0.891	0.867	0.880	0.878
15	0.932[■]	0.926	0.924	0.922	0.957[♦]	0.908
16	0.911	0.916	0.912	0.908	0.897	0.897
17	0.933[■]	0.941[■]	0.938	0.933[■]	0.920	0.925[■]
18	0.992	0.982	0.980	0.973[♦]	0.966	0.966[♦]
19	0.972[♦]	0.949[■]	0.946	0.934[■]	0.930	0.928[■]
20	0.976[♦]	0.960	0.958	0.953[⌂]	0.943	0.944
21	0.996	0.973[♦]	0.968[♦]	0.970[♦]	0.957[♦]	0.957[⌂]
22	0.997[♦]	0.970[♦]	0.957[♦]	0.954[⌂]	0.957[♦]	0.961[♦]
23	1.013[◊]	0.993	0.993	0.984	0.978	0.977

[◊] Rtx-1 is a 30m x 0.25mm-I.d. column coated with o.25 µm 100% dimethyl polysiloxane
[◊◊]Rtx-35 is a 30 m x 0.25mm-I.d. column coated with o.25 µm 65% dimethyl-35%diphenyl polysiloxane
[▲]Abbreviations: PFPA, pentafluoropropionamide; HFBA, heptafluorobutyrylamide.
[♦], [⌂] and [■] compounds that share the same sign co-elute on the same column.
Results are the average of three experiments.

Table 11: Retention data of the underivatized, HFBA and PFPA derivatives of compounds 2,3-, 34-MDMA and ring substituted methoxy methyl methamphetamines collected on Rtx-5 and Rtx-200.

Compound Number	Rtx-5◊			Rtx-200◊◊		
		Derivatives▲			Derivatives▲	
	Underivatized	HFBA	PFPA	Underivatized	HFBA	PFPA
3	1◊ (15.682 min)	1◊ (19.387 min)	1◊ (19.022 min)	1◊ (17.298 min)	1 (19.854 min)	1 (19.139 min)
8	0.955•	0.956•	0.953•	0.946•	0.873	0.876
14	0.859	0.916■	0.889	0.849	0.777	0.781
15	0.930■	0.923	0.921	0.904	0.803	0.811
16	0.910	0.912■	0.909	0.891	0.784	0.792
17	0.937■	0.938	0.935	0.918	0.827	0.832
18	0.985♦	0.979	0.977	0.959•	0.915	0.914
19	0.953•	0.947	0.944	0.933⌂	0.851	0.853
20	0.969⌂	0.957•	0.956•	0.973	0.887⌂	0.890⌂
21	0.984♦	0.970	0.968■	0.993■	0.908	0.908
22	0.969⌂	0.968	0.967■	0.939⌂	0.882⌂	0.889⌂
23	1.003◊	0.992◊	0.991◊	0.994◊	0.961	0.962

◊ Rtx-5 is a 30m x 0.25mm-I.d. column coated with o.25 µm 95% dimethyl-5% diphenyl polysiloxane
◊◊Rtx-200 is a 30 m x 0.25mm-I.d. column coated with o.25 µm trifluoropropyl methyl polysiloxane
▲Abbreviations: PFPA, pentafluoropropionamide; HFBA, heptafluorobutyrylamide.
♦, ⌂, •, ◊ and ■ compounds that share the same sign co-elute on the same separation column.
Results are the average of three expirments

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 14-23) 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.

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 14-17, along with 2,3-MDMA and 3,4-MDMA, the elution order of the perfluroacyl derivatives of these compounds was compound 14 followed by 16, 15, 17, 2,3-MDMA- and finally 3,4-MDMA (compound 3). The elution order, on Rtx-1 and Rtx-35, was the same for this subset of compounds. (Figures 34 and 35). 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 18-21, along with 2,3-MDMA and 3,4-MDMA, the PFPA derivative of compound 19 elutes first followed by 2,3-MDMA-PFPA, 20-PFPA, 21-PFPA, 18-PFPA and finally

3,4-MDMA-PFPA(compound 3); (Figure 36A). The elution order is the same for the HFBA derivatives of the same compounds on Rtx-1 (Figures 37A). This elution order has changed on Rtx-35, where compound 20 elutes before 2,3-MDMA, and most significantly 2,3-MDMA (compound 8) and 21 co-elute. This change in elution order takes place for the PFPA and HFBA of these compounds (Figures 36b and 37b) 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 22, 23, 2,3-MDMA and 3,4-MDMA, the elution order of the perfluroacyl derivatives was 2,3-MDMA followed by 22, 23 and finally 3,4-MDMA. The elution order was the same on both Rtx-1 and Rtx-35 (Figures 38 and 39). 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.

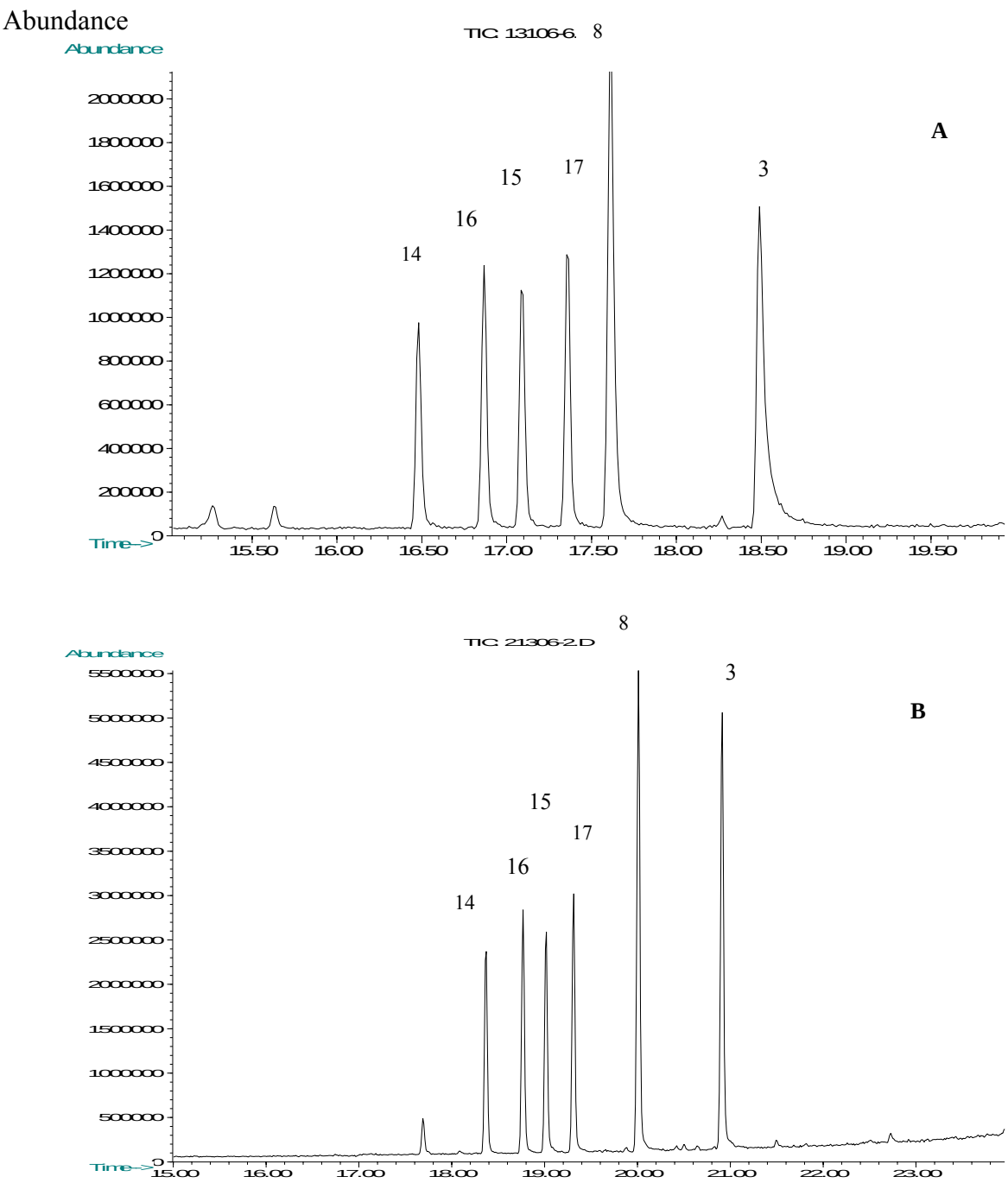


Figure 34: Capillary gas chromatographic separation of PFPA derivatives of compounds 14-17, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

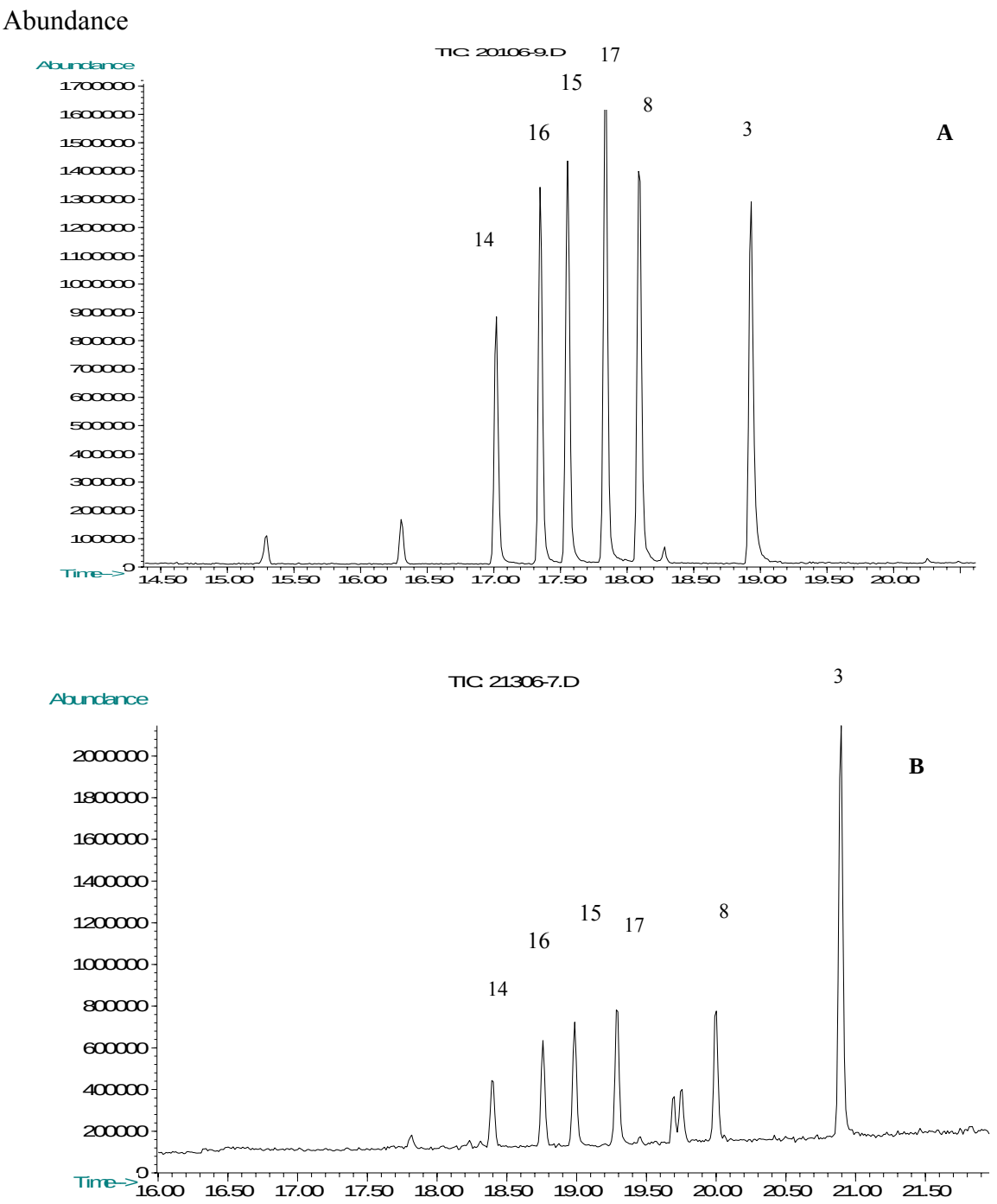


Figure 35: Capillary gas chromatographic separation of HFBA derivatives of compounds 14-17, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

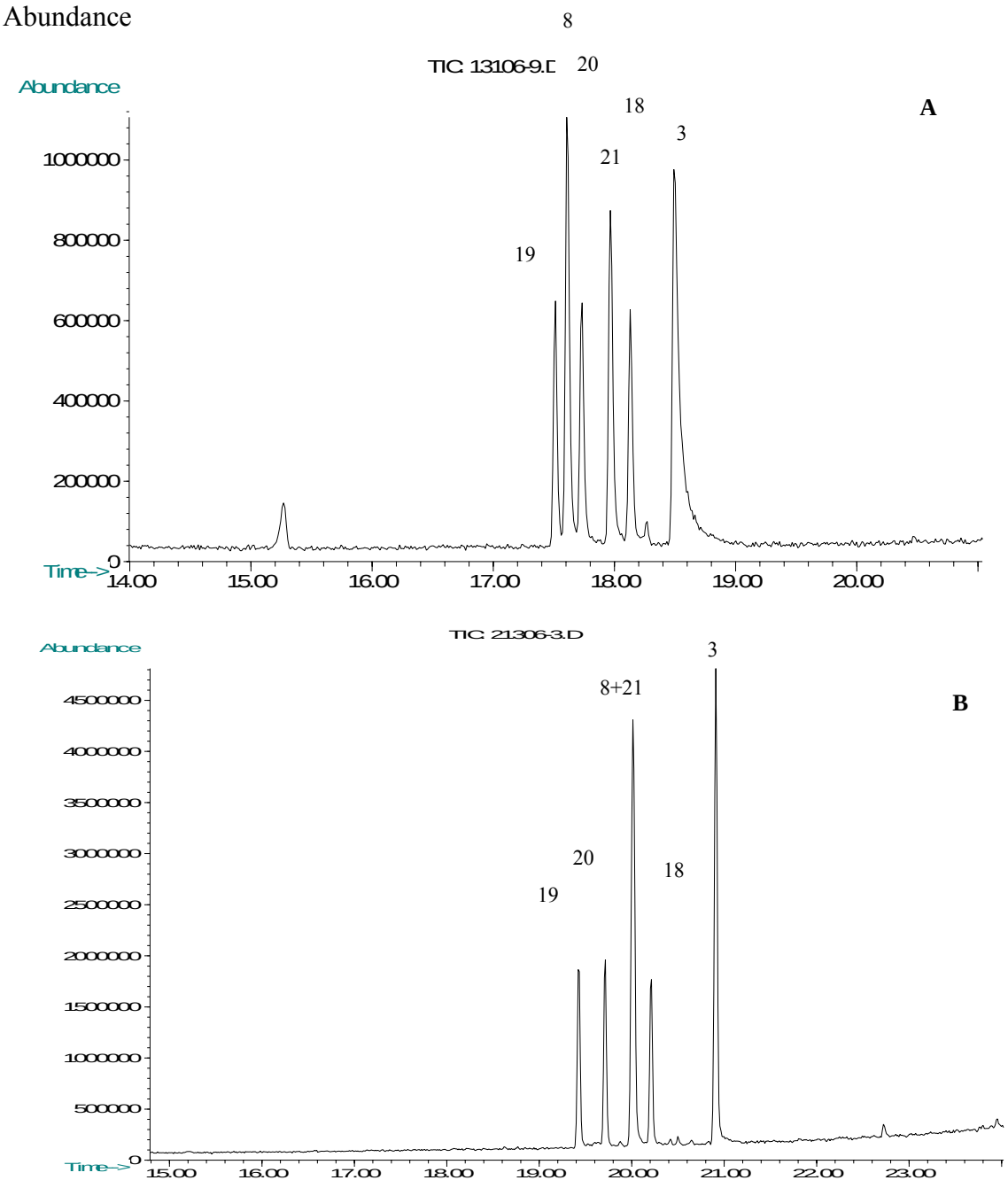


Figure 36: Capillary gas chromatographic separation of PFPA derivatives of compounds 18-21, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

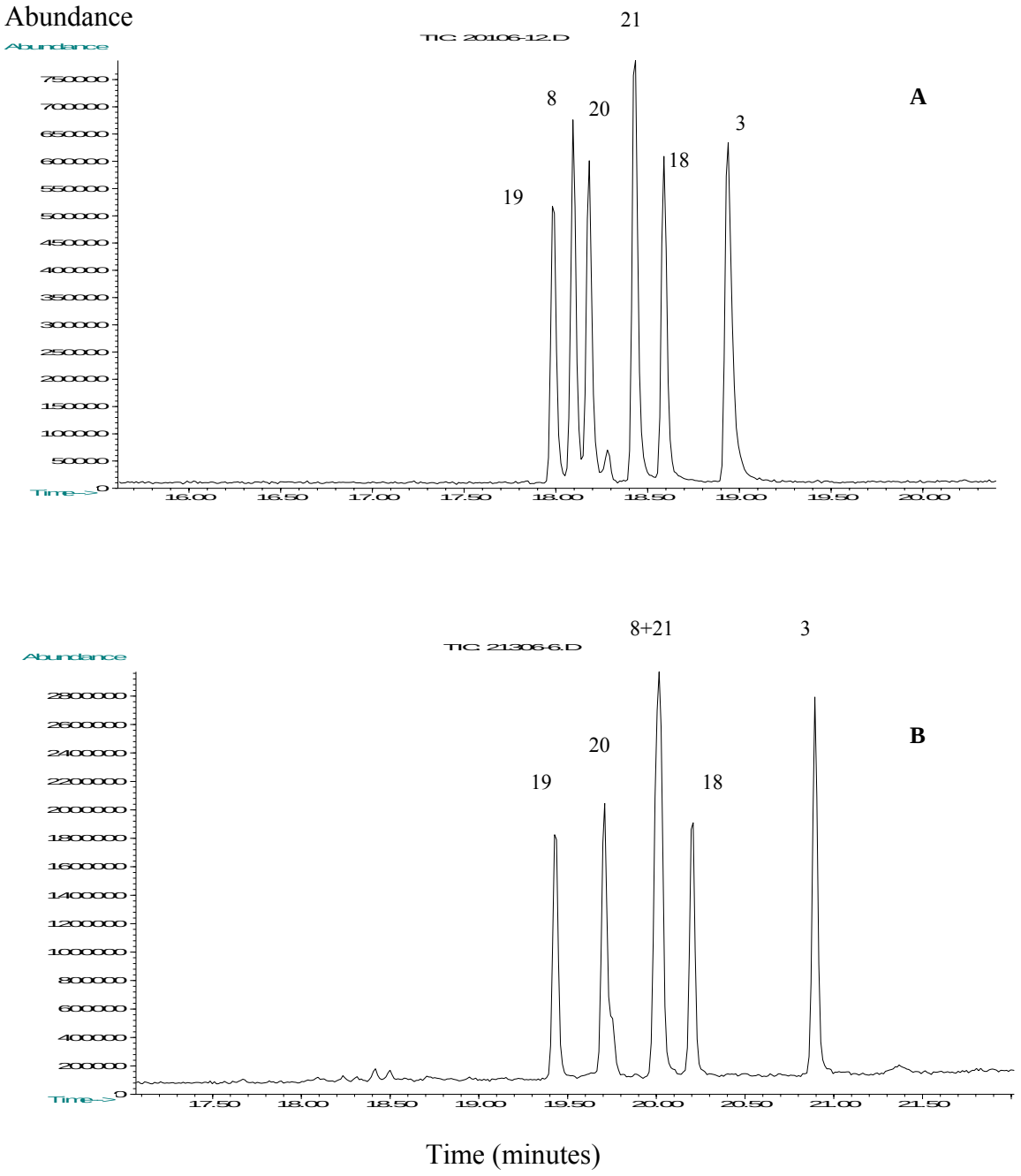


Figure 37: Capillary gas chromatographic separation of HFBA derivatives of compounds 18-21, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35**.**

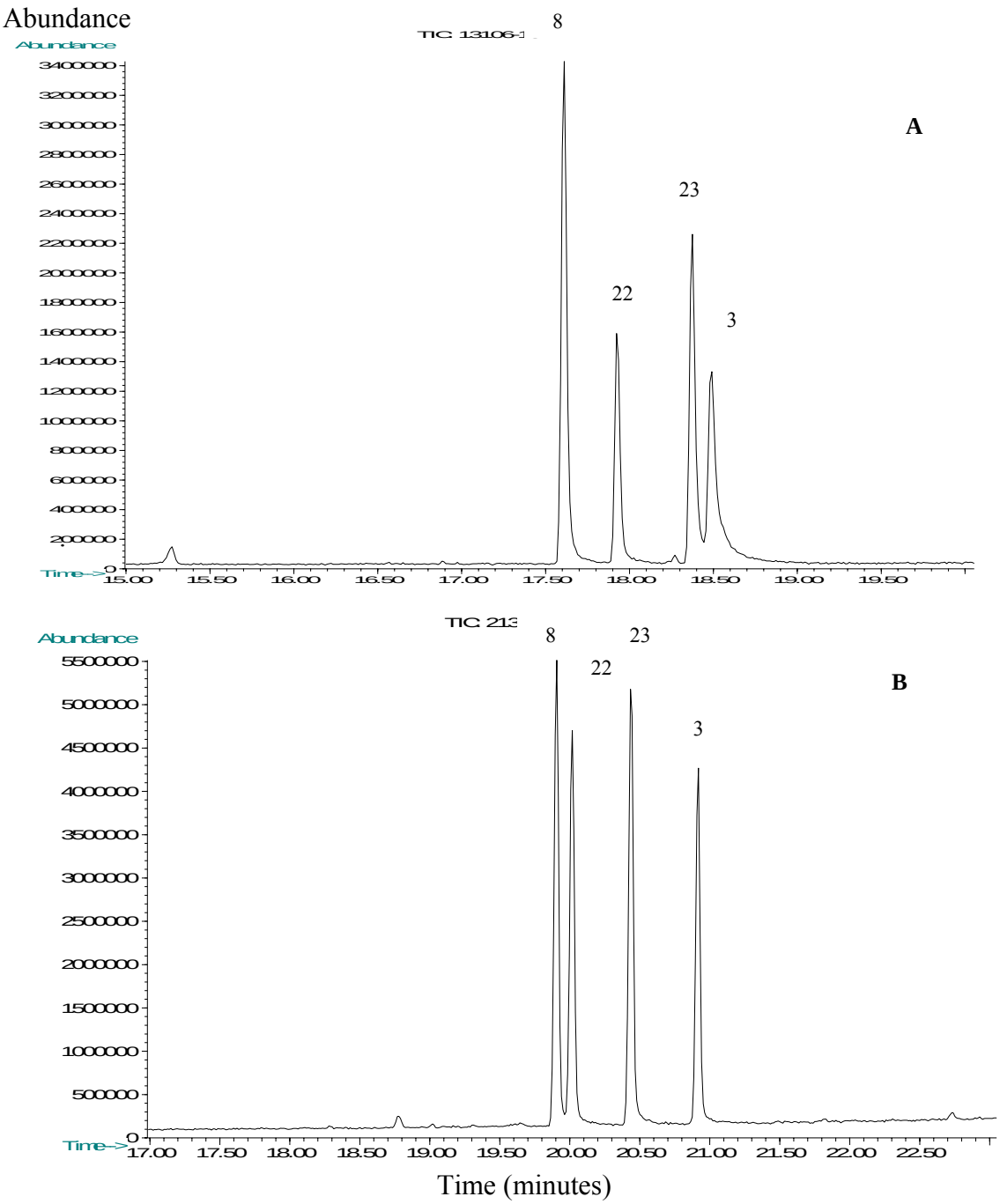


Figure 38: Capillary gas chromatographic separation of PFPA derivatives of compounds 22, 23, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

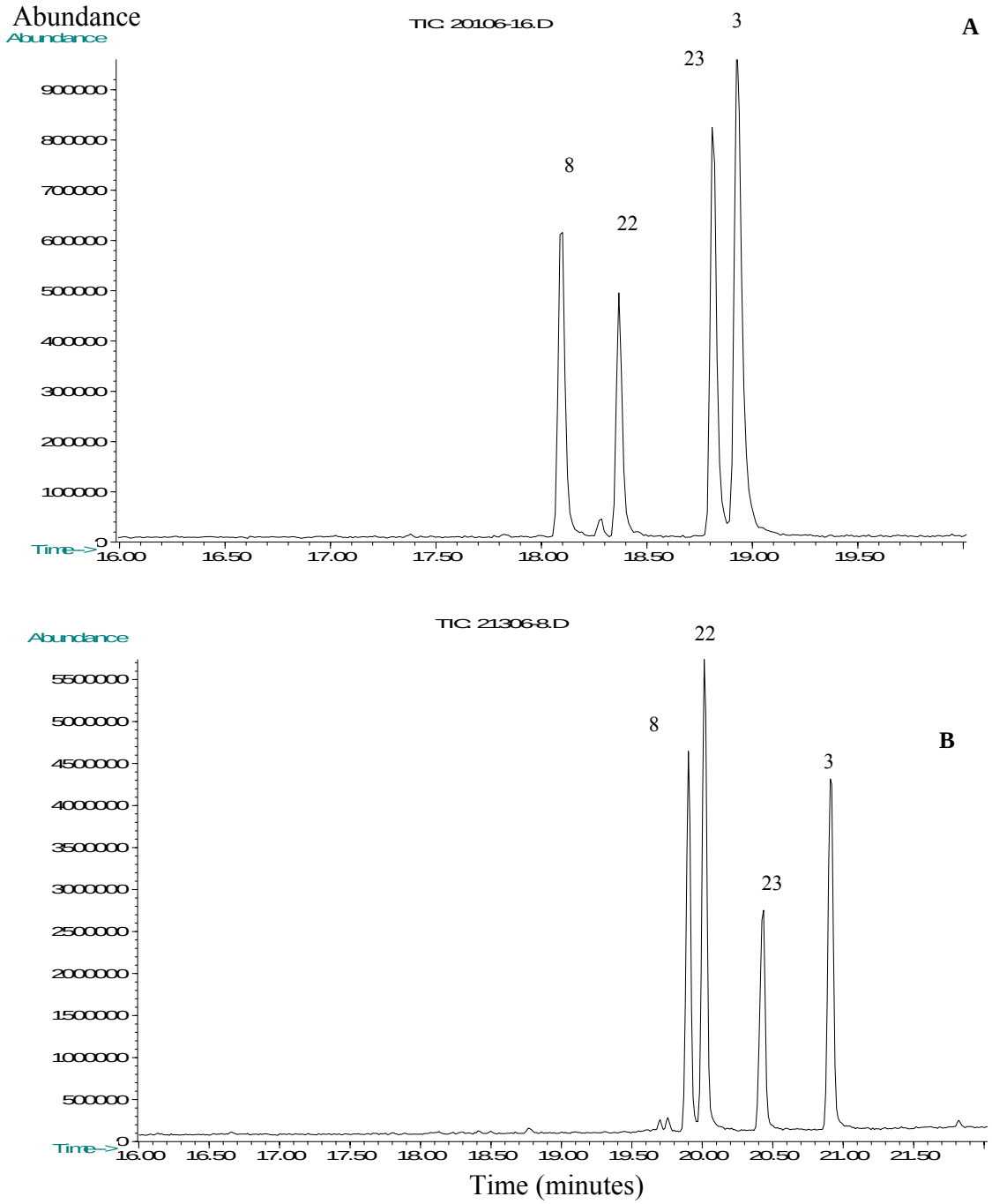


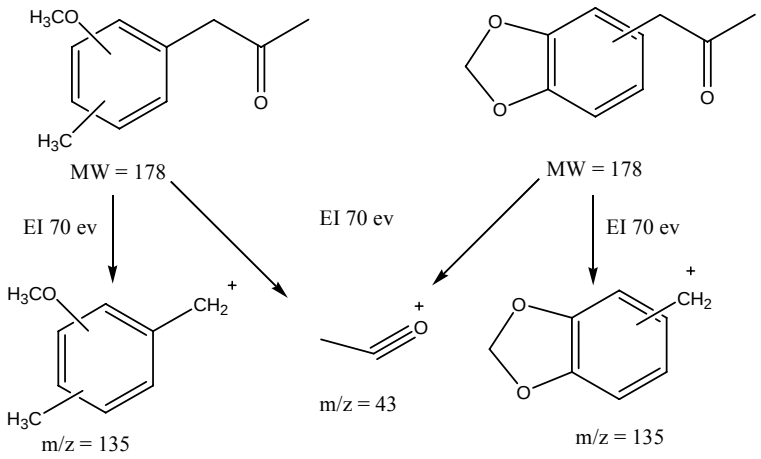
Figure 39: Capillary gas chromatographic separation of HFBA derivatives of compounds 22, 23, 2,3-MDMA and 3,4-MDMA. Columns used: A RTX-1; B RTX-35.

3.4 Mass Spectral and Gas Chromatographic Studies of Ring Substituted Methoxy Methyl Phenyl -2-Propanones

The methoy methyl phenyl-2-propanones are the key intermediate in the synthesis of methoxy methyl amphetamines. These compounds are isobaric ketones related to 3,4 methylenedioxyphenyl-2-propanone, a controlled precursor for the drug of abuse 3,4-MDMA. The synthetic procedures to prepare this set of ketones were discussed in chapter 2, while their mass spectral properties will be discussed in chapter 3.4.1 and the chromatographic properties of these compounds will be discussed in chapter 3.4.2

3.4.1 Mass spectral studies of methoxy meythyl phenyl-2-propanones.

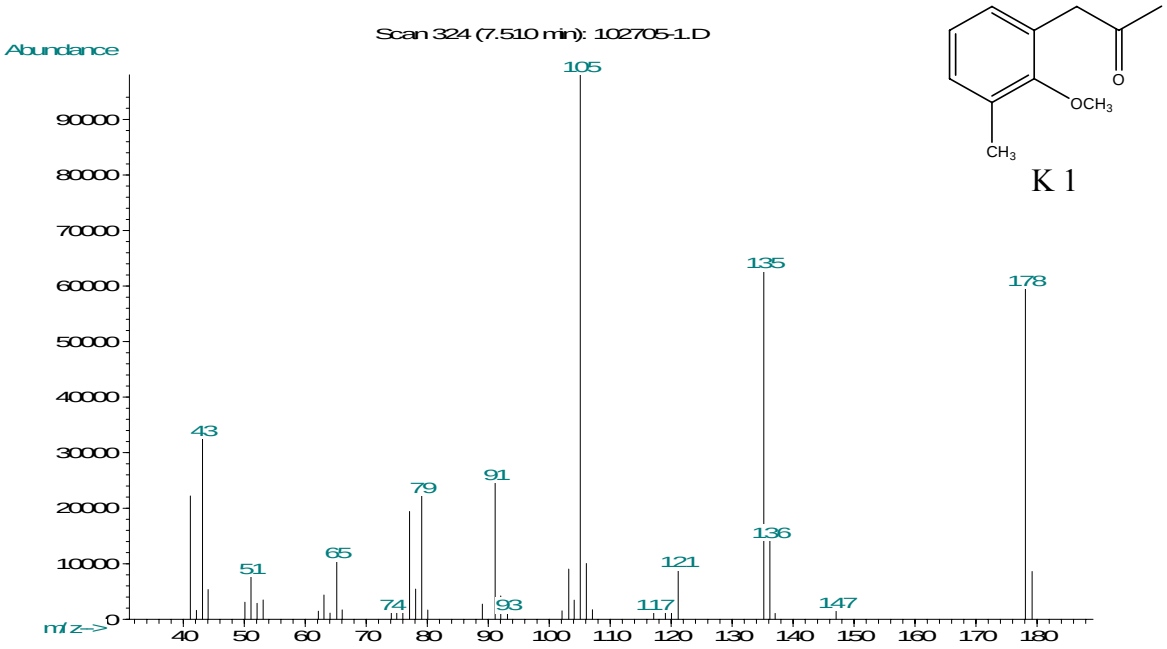
The mass spectrum of 3,4-methylenedioxyphenyl-2-propanone shows a molecular ion at m/z 178 and major fragment ions at m/z 135/136 and at m/z 43 for the acetyl $(CH_3CO)^+$ fragment. The various isomeric forms of the methoxy methyl phenyl-2-propanones have the potential to produce mass spectra essentially equivalent to 3, 4-methylenedioxyphenyl-2-propanone, all have molecular weight of 178 and can yield major fragment ion in their electron ionization mass spectra at m/z 135/136 and m/z 43. For these individual regioisomers, the 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 m/z 43 is the acetyl cation produced from the alpha cleavage between the carbonyl carbon and benzylic carbon (Scheme 50).

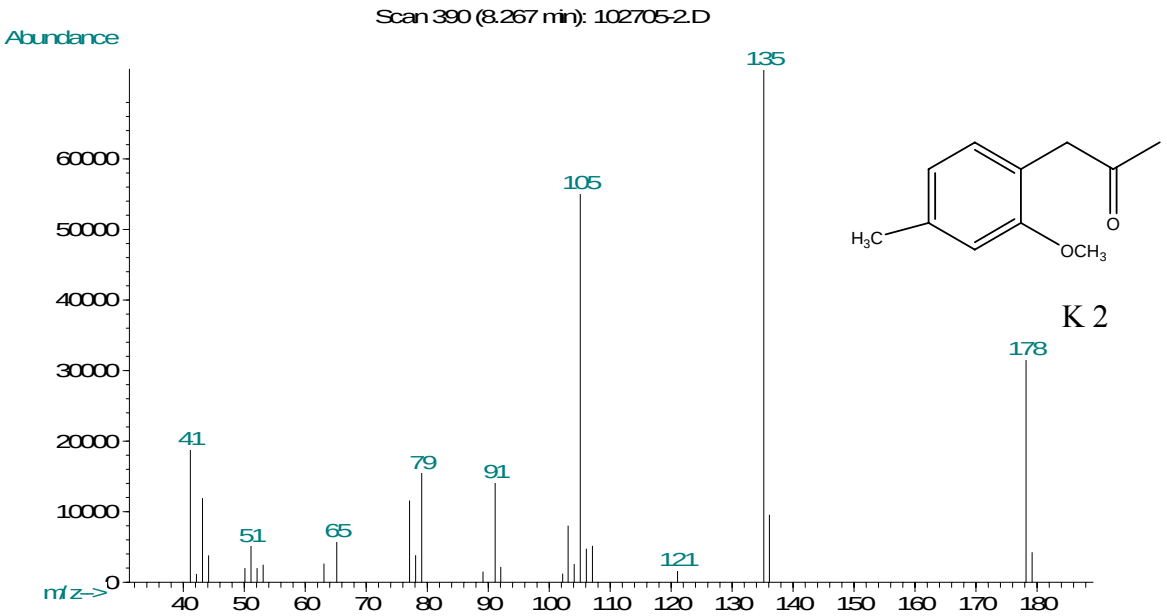


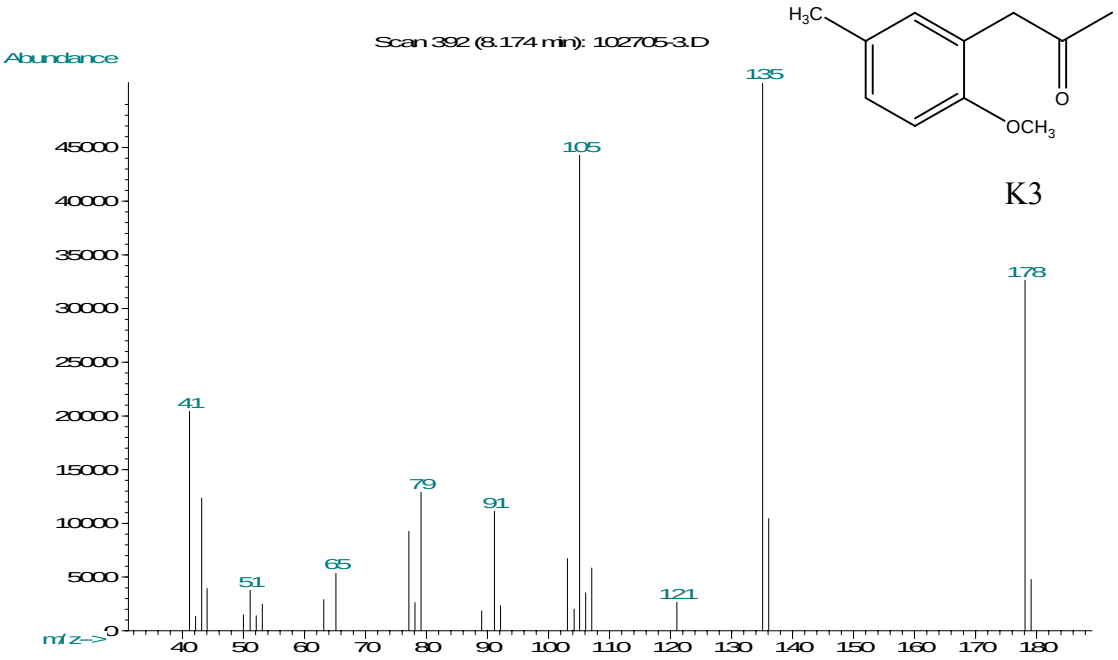
Scheme 50: General mass spectral fragmentation for the ring substituted methoxy methyl phenyl acetones.

The mass spectra of the 10 substituted methoxy methyl phenyl acetones are shown in Figure 40. The methoxy methyl ring substituted phenyl acetones with the methoxy group in the ortho position are characterized by m/z 105; in fact m/z 105 is the base peak in the mass spectra of 2-methoxy-3-methyl phenyl acetone (Figure 40-K1). This ion likely arrises from loss of mass 30 (CH_2O) from the initial benzylic cation at m/z 135. The m/z 105 ion is a significant fragment only when the methoxy-group in the ketone is ortho to the acetone side-chain and the site of initial benzylic cation formation. This m/z 105 ion can be formed by 1,6-hydride shift from the hydrogen atom of the methoxy group to the benzyl cation followed by loss of formaldehyde (Scheme 51). This ion at m/z 105 also appears in the mass spectra of 3-methoxy-2-methyl phenyl acetone, and may be attributed to rearrangement of the initial benzylic cation to the adjacent methyl group which is then ortho to the methoxy group.

Figure 40: Mass spectra of ring substituted methoxy methyl phenyl acetones.



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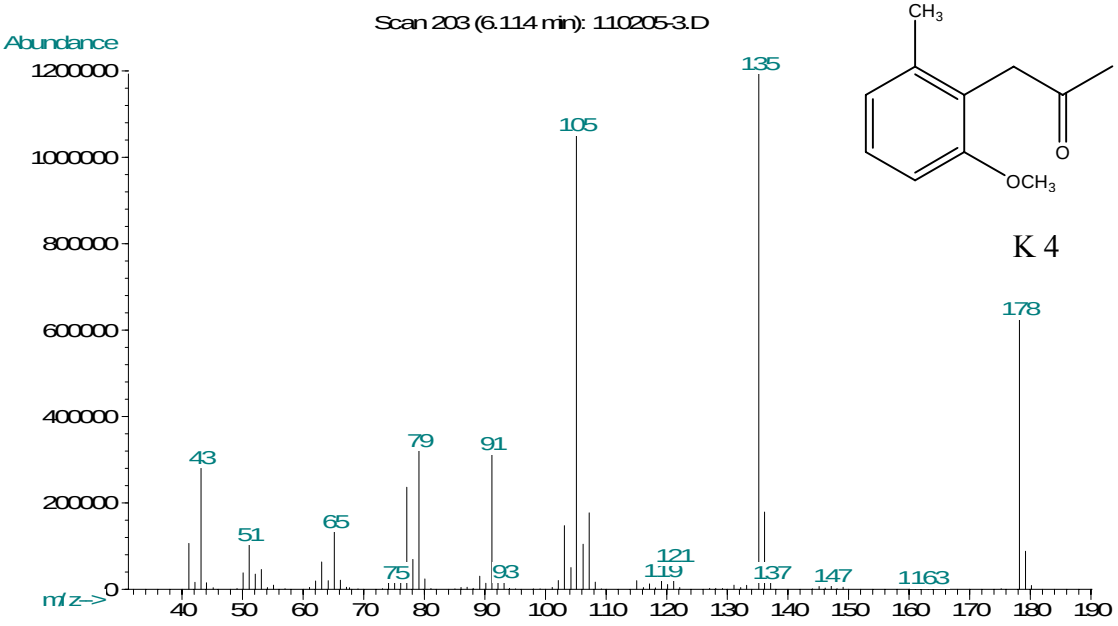
Abundance
m/z-->
H3C
O
OCH3
K3
135
105
178
41
79
91
65
51
121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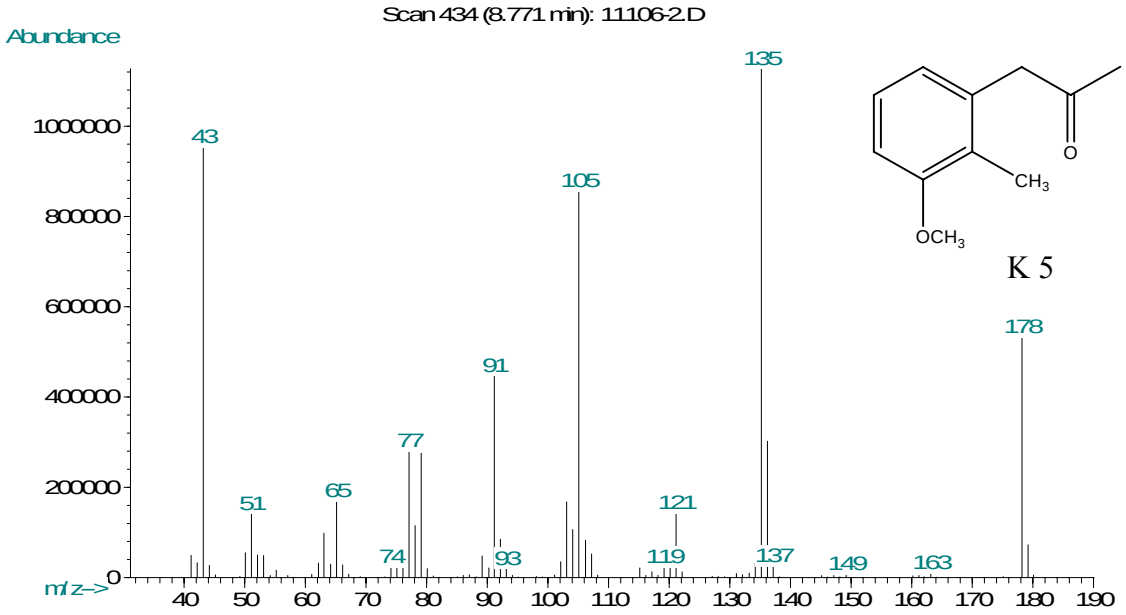
Abundance
m/z-->
CH3
O
OCH3
K 4
135
105
178
43
79
91
51
65
75
93
121
119
137
147
1163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m/z-->
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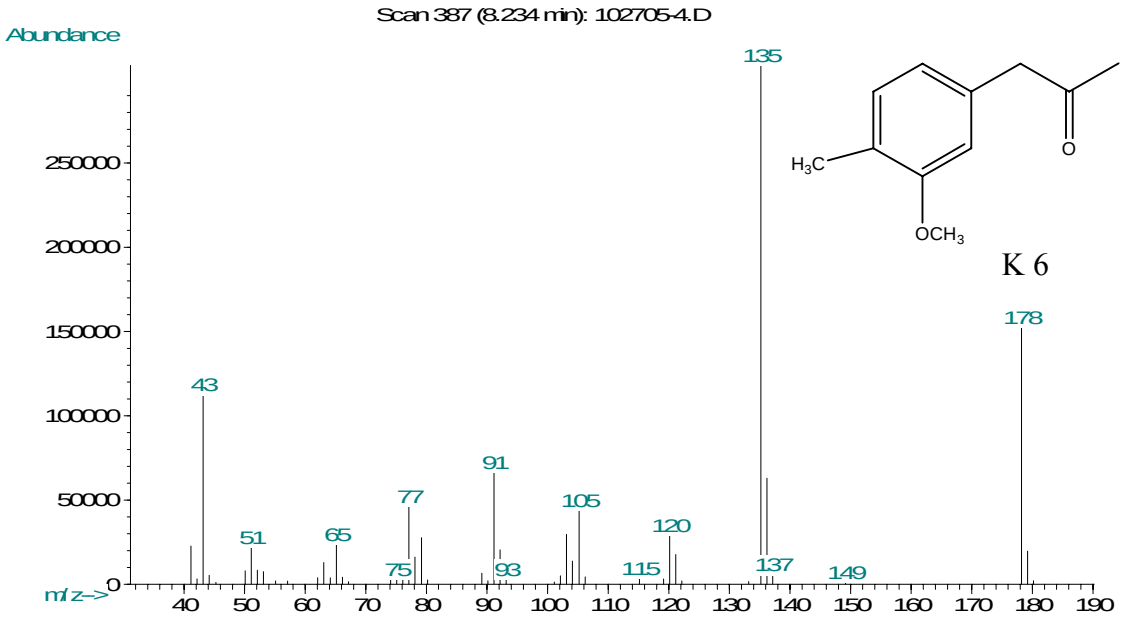
800000
600000
400000
200000
0
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
43
51
65
74
77
91
93
105
119
121
135
137
149
163
178
O
CH3
OCH3

K 5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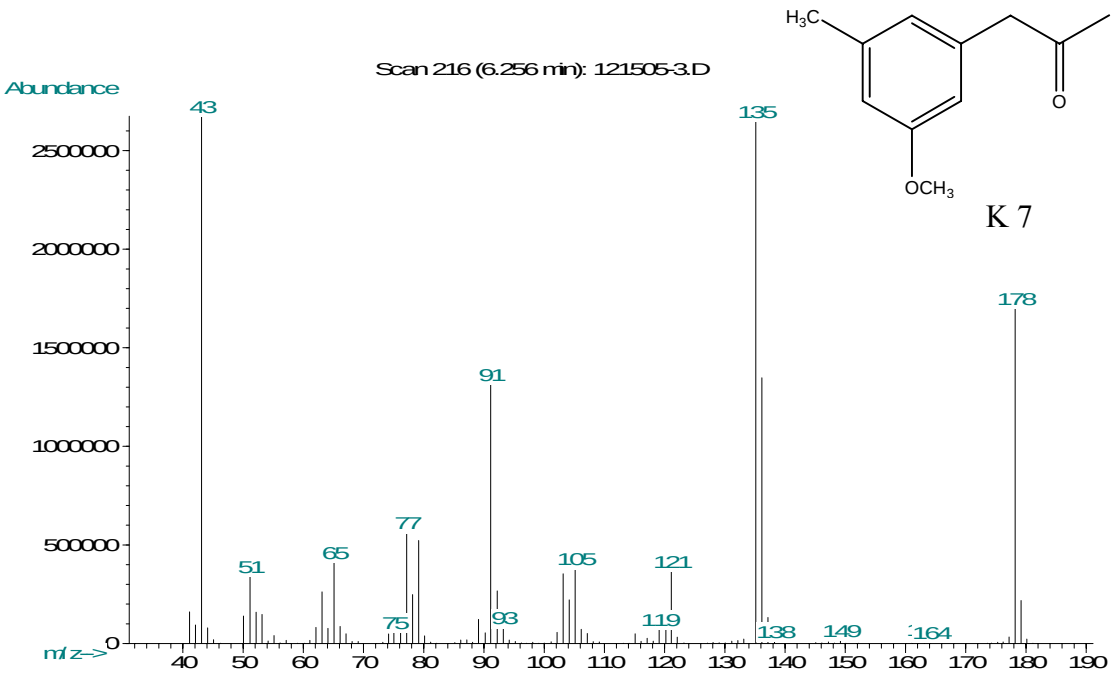
Abundance
m/z-->
250000
200000
150000
100000
50000
0
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
43
51
65
75
77
91
93
105
115
120
135
137
149
178
H3C
O
OCH3

K 6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Abundance
m/z-->
H3C
O
OCH3
K 7
43
135
178
91
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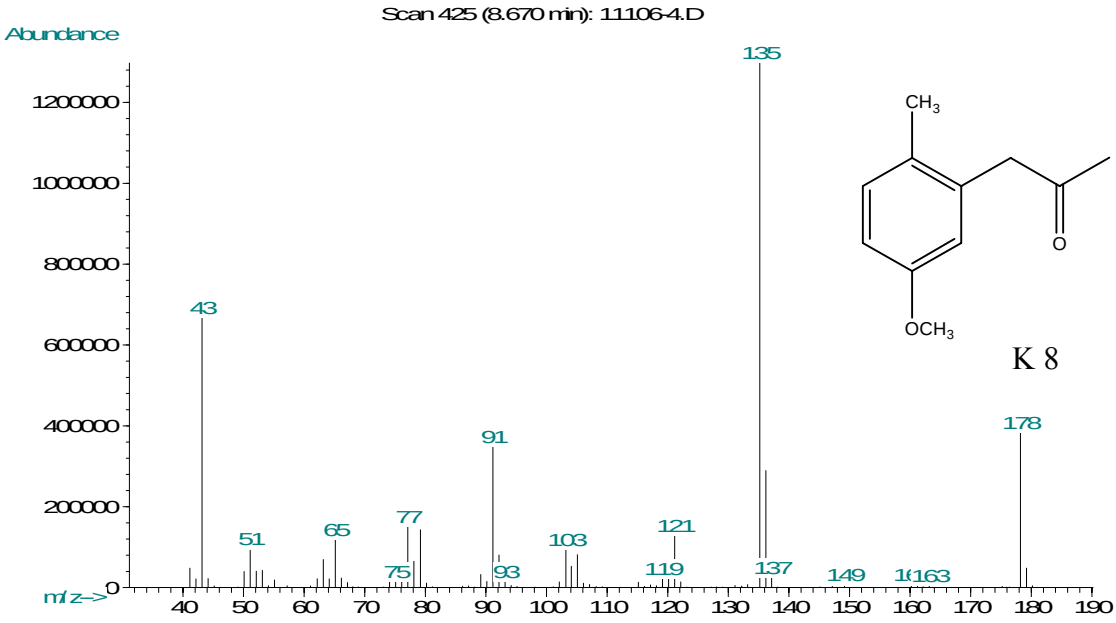
51
65
105
121
75
93
119
138
149
164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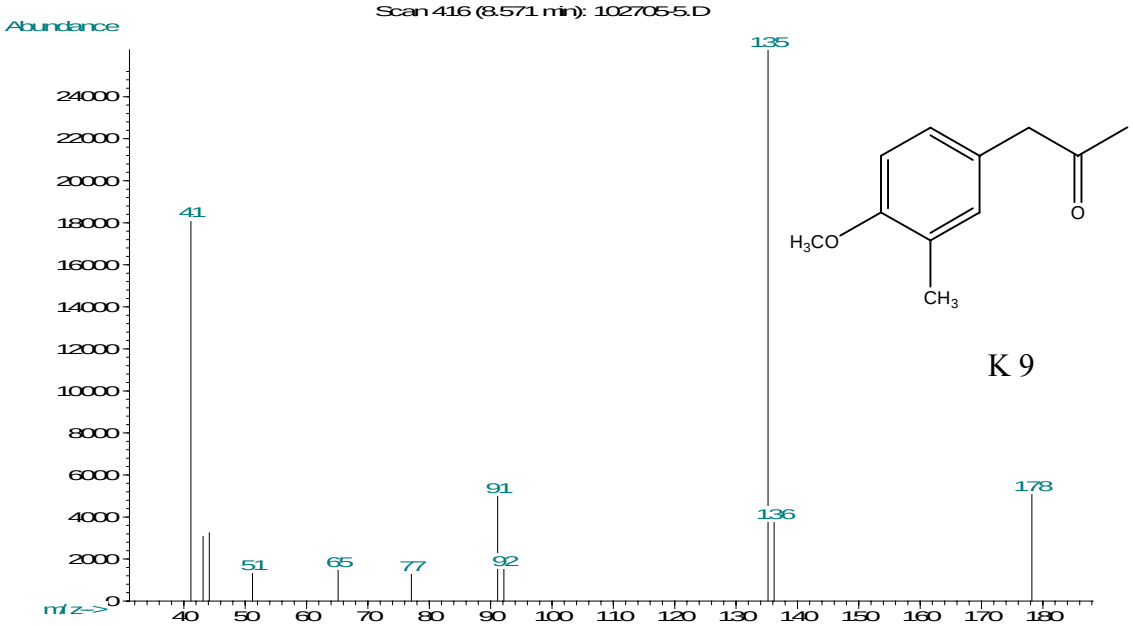
Abundance
m/z-->
CH3
O
OCH3
K 8
135
43
178
91
77
51
65
103
121
75
93
119
137
149
163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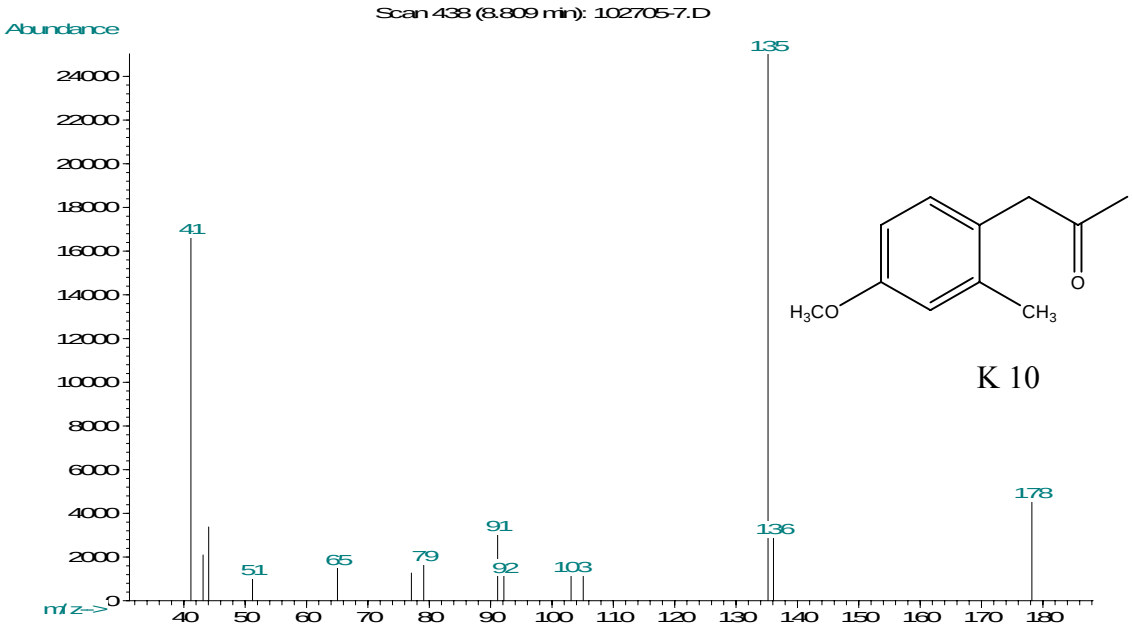
Abundance
m/z-->
135
41
91
136
178
51
65
77
92
H3CO
CH3
O

K 9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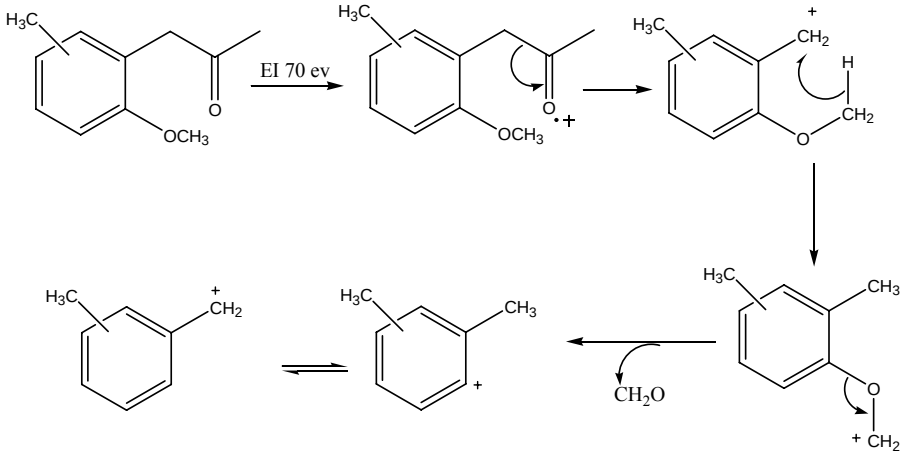
Abundance
m/z-->
135
41
91
136
178
51
65
79
92
103
H3CO
CH3
O

K 10



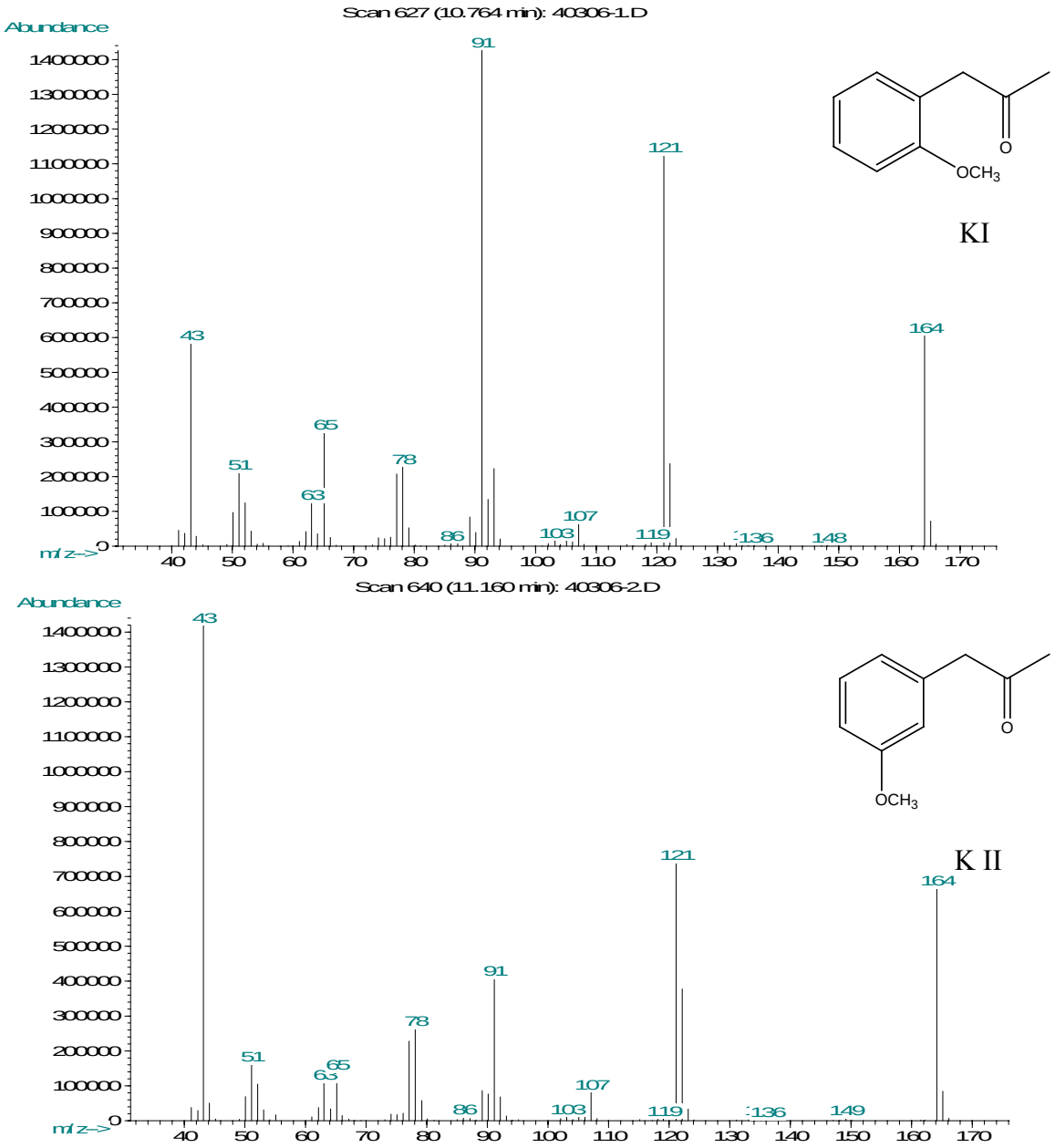
Scheme 51: Formation of m/z 105 from ortho methoxy ring substituted benzyl carbocation.

The suggested mechanism was supported by screening the mass spectra of the commercially available 2-, 3-, and 4-methoxy phenyl acetones (KI-KIII). The mass spectra of all three methoxy ring substituted phenyl acetones are shown in Figure 41. All mass spectra (KI-KIII) show m/z 121 which is analogus to m/z 135 for K1-10 and m/z 91 which is analogus to m/z 105. The m/z 91 ion is the base peak for 2-methoxy phenyl acetone only and this ion is less significant in the 3- and 4-methoxy isomers. The m/z 91 ion would also be the loss of mass 30 from the initial benzylic cation at m/z 121 for this model system. The 3-methoxy-phenylacetone shows m/z 43 as the base peak and the 4-methoxy-isomer shows m/z 121 as the base peak.

The gas chromatographic separations of the 3 isomers of methoxy-phenylacetone are shown in Figure 42. The compounds were separated on an Rtx-200 Stationary phase using a temperature program that holds column temperature at 100 ^{o}C for 1 minute then

the temperature was ramped up to 180 ^{o}C at a rate of 9 ^{o}C/ minute. Column temperature was held at 180 ^{o}C for 2 minutes then was ramped up to 200 ^{o}C at a rate of 9 ^{o}C/ minute and set at 200 ^{o}C for 5 minutes (TP-1).

Figure 41: Mass spectra of 2-, 3- and 4-methoxy phenyl acetones



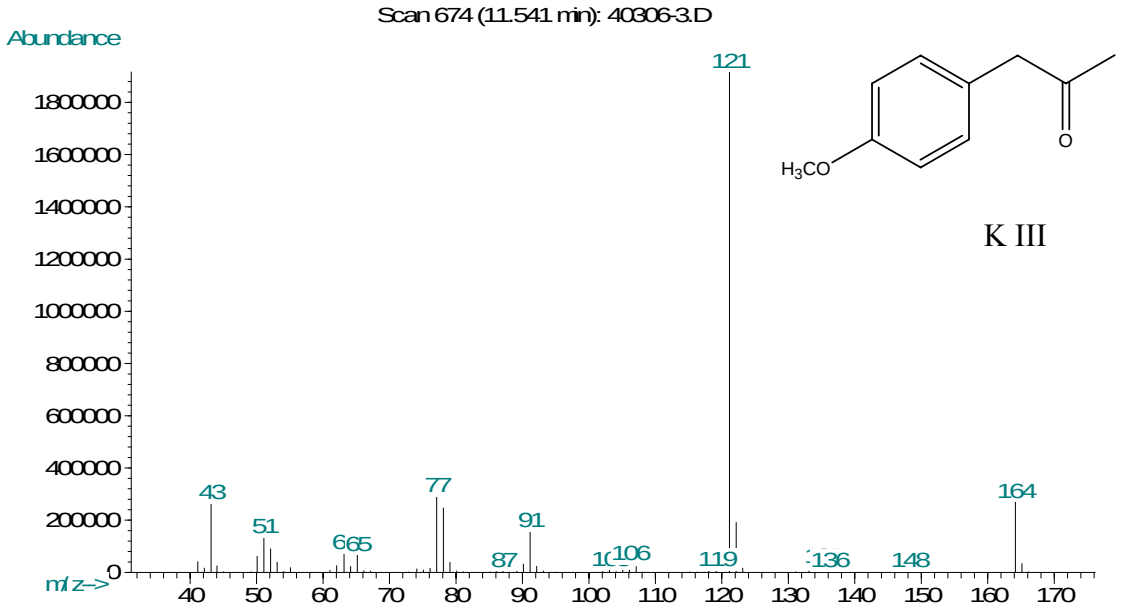
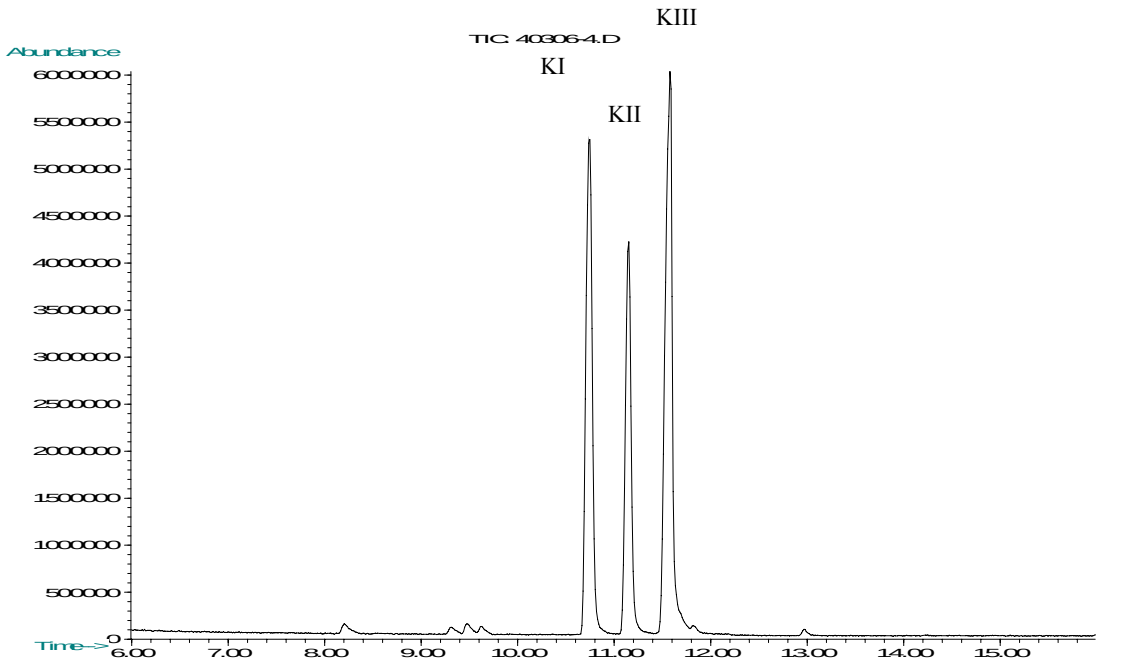


Figure 42: Capillary gas chromatographic separation of 2-(KI), 3-(kII) and 4-(kIII) methoxyphenyl acetones on Rtx-200



3.4.2 Gas chromatographic separation of ring substituted methoxy methyl phenyl acetones

Methoxy methyl ring substituted phenyl acetones were compared on three stationary phases using capillary columns of the same dimensions, 30m x 0.25mm and 0.25um depth of film. Several temperature programs were evaluated and used to collect retention data in Table 12 however only one column (Rt-βDEXcst-TM) and only one temperature program (TP-5) were able to separate the 10 ketones (Figure 43). The program was to hold the column temperature at 70 ^{o}C for 2 minutes and the temperature was ramped up to 150 ^{o}C at a rate of 2.5 ^{o}C/minute. The column temperature was held on 150 ^{o}C for three minutes and finally was ramped up to 200 ^{o}C at a rate of 15 ^{o}C/minute and held the temperature at 200 ^{o}C for 5 minutes.

From the retention data collected, a direct comparison between Rtx-1 and Rtx-200 pases showed increasing retention on the more polar Rtx-200 phase and fewer coeluting compounds using identical temperature programs. The Rtx-1 pase gave very similar retention properties for K2, K4 and K6 with the most retained compound K 10 eluting in 8.857 minutes. The Rtx-200 column using the same temperature program produced ention time of 13.221 minutes for K10 and showed K5 and K7 to have similar retention properties. The elution order of the 10 ketones on the beta cyclodextrin column (Rt-βDEXcst-TM) was found to be different from the one on both Rtx-1 and Rtx-200 using the same temperature program (Figure 44).

Table 12: Retention data of ring substituted methoxymethyl phenyl acetones collected on Rtx-1, Rtx-200 and Rt-βDEXCst-TM.

Compound Number	Rtx-1[◊]	Rtx-200[◊◊]	Rt-βDEXcst-TM [◊◊◊]		
	TP-1	TP-1	TP-3	TP-4	TP-5
K1	0.845	0.837	0.8194	0.8826	0.872
K2	0.932[♦]	0.924	0.9024	0.9313	0.9502
K3	0.92	0.903	0.8746	0.9179	0.9251
K4	0.937[♦]	0.92	0.8854	0.9269	0.9406
K5	0.99	0.959[⌂]	0.9513[⌂]	0.967[⌂]	0.9768
K6	0.93[♦]	0.897	0.9211	0.9497	0.9655
K7	0.96	0.949[⌂]	0.9553[⌂]	0.9706[⌂]	0.9821
K8	0.985	0.978	0.9692	0.9797	0.9868
K9	0.976	0.942	0.972	0.9821	0.9915
K10	1 (8.857 minutes)	1 (13.221 minutes)	1 (13.705minutes)	1 (19.348 minutes)	1 (39.497 minutes)

[◊] Rtx-1 is a 30m x 0.25mm-I.d. column coated with o.25 µm 100% dimethyl polysiloxane
[◊◊]Rtx-200 is a 30 m x 0.25mm-I.d. column coated with o.25 µm trifluoropropyl methyl polysiloxane
[◊◊◊] Rt-βDEXcst-TM is a 30 m x 0.25mm-I.d. column coated with o.25 µm 14% cycloporopyl phenyl-86% dimethyl polysiloxane
TP- Temperature programs used
[♦], and [⌂], compounds that share the same sign co-elute on the same separation column
-Results are the average of three experiments.

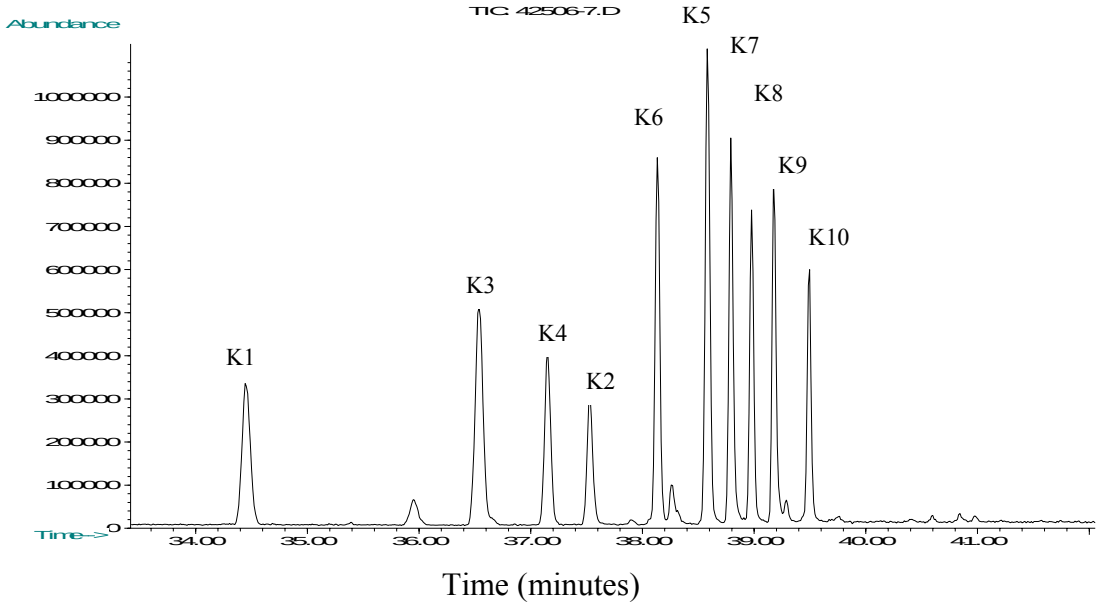


Figure 43: Capillary gas chromatographic separation of methoxy methyl phenyl acetones (K1-K10) on β cyclodextrin column (Rt-βDEXcst-TM).

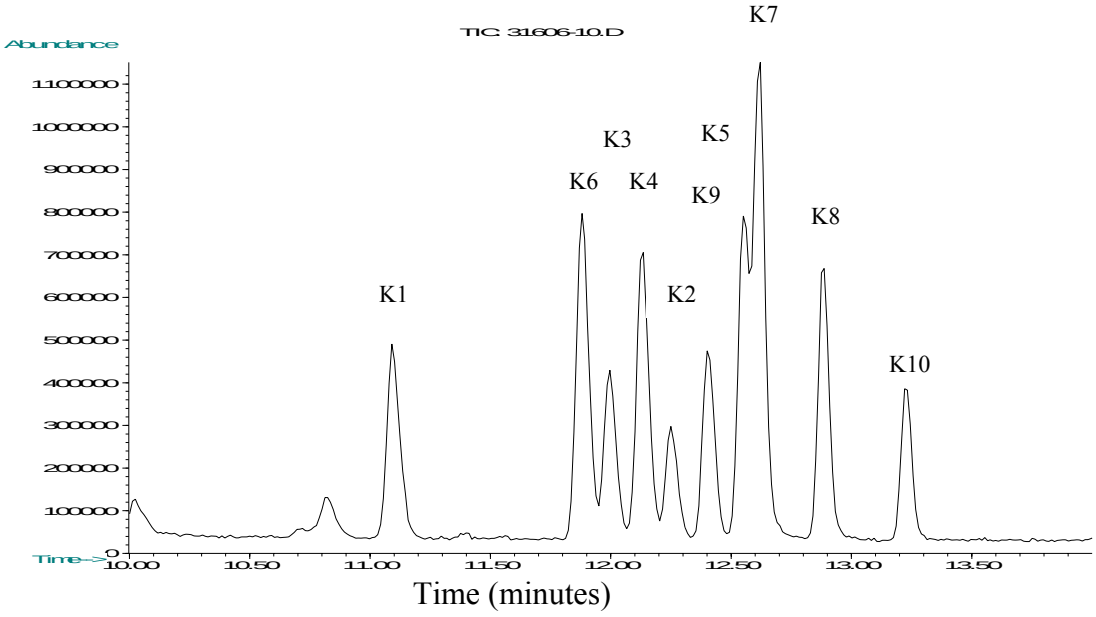


Figure 44: Capillary gas chromatographic separation of methoxy methyl phenyl acetones (K1-K10) on Rtx-200.

All ten methoxy methyl substituted phenyl acetones were seprated from methylenedioxy-2-propanone, the precursor ketone of 3,4MDMA, on the Rt-βDEXcst-TM column. MDP-2-P was eluted last at 40.893 minutes. Figure 45

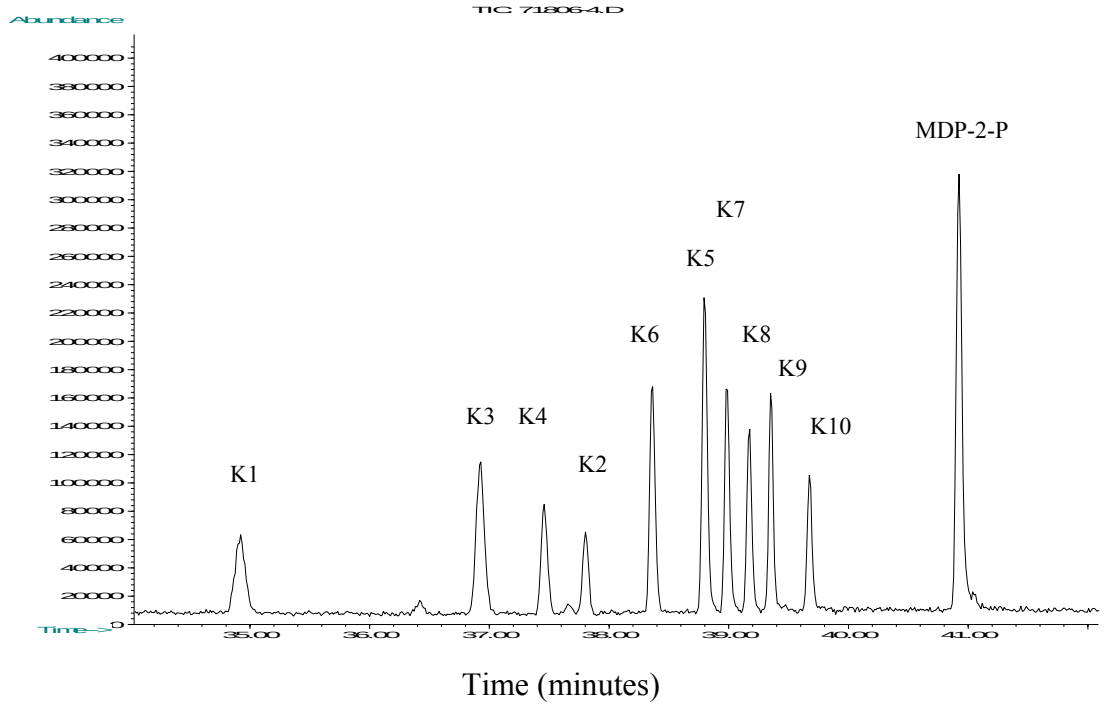


Figure 45: Capillary gas chromatographic separation of methoxy methyl phenyl acetones (K1-K10) and MDP-2-P on β cyclodextrin column (Rt-βDEXcst-TM).

Cyclodextrins have been used extenseivly in separation science because they have shown to discriminate between postional isomers, functional groups, homologues and enantiomers.The unusual properties of cyclodextrins originate in their unique structure. In general cyclodextrins are water soluble oligosaccharides with a hydrophilic surface and a hydrophobic interior cavity. They are capable of forming inclusion compounds with a wide range of hydrophobic molecules including organic moites,

inorganic ions and metallo-organic species. Entrapment inclusion occurs without the formation of formal chemical bonds [Braga et al, 2006].

Formation of inclusion complexes between the ligand and cyclodextrin was thought to be a result of hydrophobic interactions between the ligand and the relatively hydrophobic cavity of the cyclodextrin coupled with polar interactions between appropriate substituents on the ligand and the polar rim of cyclodextrin. The unmodified cyclodextrin rim are lined with primary hydroxyl groups on one side and secondary hydroxyls on the other side of the cavity. Both hydroxyl groups may be functionalized with hydrophobic or hydrophilic groups to enhance complex forming ability and selectivity towards certain analytes [Szejtli and Osa, 1996].

Three major typs of cyclodextrins are known α-, β-and γ-CD. The α-CD contains six, β-CD seven and γ-CD eight glucose units. The utility of underivatized cyclodextrins in GC applications was limited because of their high crystallinity while their insolubility in most organic solvents made them difficult to formulate into GC stationary phases. However some functionalized cyclodextrins form viscous oils suitable for GC stationary phase coatings and have been used either neat or diluted in polysiloxane polymer as chiral stationary phases for gas chromatographic applications [Schneiderman and Stalcup, 2000].

The permethylated derivative of betacyclodextrin in cyanopropyl-dimethyl polysiloxane is one of these derivatives that have been developed in the past few years for stereochemical separation purposes. The column used during this project, Rt- βDEXcst, showed that the ring substituted methoxy methyl phenyl acetones with the methoxy group in the ortho postion (K1-K4) elute first followed by phenyl acetones where the methoxy groups are in the meta postion (K5-K8) and the last eluting ketones were those who have

the methoxy groups in the para postion (K9 and K 10). Figure 44 showes all ketones well resolved, however the elution of all ten regioisomeric ketones required an analysis time of almost 40 minutes.

3.5 Conclusions

The milestones for this dissertation have been divided into three main stations that include synthesis and chromatographic studies of a) ring and side chain regioisomers related to MDMA, b) methoxymethcathinones as indirect regioisomers of the drug of abuse Ecstacy, and c) ring substituted methoxymethyl methamphetamine as isobaric substances related to 3,4 MDMA.

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, methoxybenzoyl or methoxymethyl benzyl groups. Thus the traditional electron impact mass spectrum provides little structural information for differentiating among these 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 any of the other isomers. This elimination process may be accomplished on the basis of chromatography alone but ultimately would require the analyst to use reference samples of all amines. The reference samples would be necessary to determine if any of the isomeric methylenedioxyphenethylamines co-eluted with MDMA.

The first part of the project involved the synthesis of nine ring and side chain substances that constitute direct regioisomers of MDMA. These compounds were synthesized from the corresponding commercially available 3,4-methylenedioxy benzaldehyde and 2,3-dihydroxybenzaldehydes. Derivatization of the eight primary and secondary amines with various acylating agents yielded amides with similar resolution pattern of the underivatized amines by capillary gas chromatography on Rtx-1 and Rtx-5 stationary phases. However the perfluoroacyl derivatives significantly individualized the

mass spectra for these amides and allowed 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 were essentially equivalent for chromatographic purposes however; the HFBA derivatives offered more unique fragment ions for additional discrimination among these regioisomeric substances and MDMA

The second milestone in this project was to synthesize the methoxymethcathinones as indirect regioisomers to MDMA. All three methoxymethcathinones were synthesythed from the corresponding commercially available anisaldehydes. The synthesized compounds were evaluated for their chromatographic and mass spectral properties compared with 3,4- and 2,3-MDMA. Under regular chromatographic condtions used to determine MDMA, there was co-elution between ortho- and meta-methoxy methcathinones. Derivatization of these amines with various perfluro acylating agents yielded amides with improved resolution compared to the underivatized amines by capillary gas chromatography on Rtx-1 and Rtx-5 stationary phases. These perfluoroacyl derivatives significantly individualized 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 charactrestic to MDMA amides. The amides of the methoxy methcathinones do not yield the m/z 162 ion since no benzylic hydrogen is available for rearrangments. that were not formed for the methoxymethcathinones. The PFPA and HFBA derivatives are essentially equivalent for chromatographic purposes.

The third phase of this work involved the preparation of ring substituted methoxy methyl methamphetamines as isobaric substaces related to MDMA. These compounds are characterized by having the same side chain as MDMA. All synthesis procedures led to intermediate ketones, methoxymethyl phenyl acetones, which upon reductive amination gave the corresponding methamphetamine. Unlike the first two phases, some of the starting materials are commercially available while others are not. The commercially available starting materials were 2-methoxy-5-methylbenzaldehyde, 4-methoxy-3-methylbenzaldehyde and 4-methoxy-2-methylbenzaldehde. Other benzaldehydes were synthesized from the corresponding commercially avilable hydroxyl methyl benzoic acids, 3-methyl salyclic acid, 4-methyl salyclic acid and 3-hydroxy-2-methyl benzoic acid. One substitution pattern was prepared from the commercially available methyl ester of 3-methoxy-4-methyl benzoic acid and another from 2,3-dimethylanisole. Thus a total of eight of the required methoxy-methyl-benzaldehydes were available as the appropriately substituted aromatic system. The remaining two aldehydes were prepared by selective synthetic methods which formed the desired substitution pattern. These synthetic routes utilized commercially available acetone, diethyl oxalate, 2-methyl furan and ethyl propiolate as starting materials.

The prepared ring substituted methoxy methyl methamphetamines were evaluated for their chromatographic and mass spectral properties compared to 3,4- and 2,3-MDMA. The mass spectra studies showed almost idental mass spectra of all ten methoxy methyl methamphetamines and 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. The 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. The PFPA derivatives allow more distinctive differentiation between the two para methoxy methyl methamphetamines based on mass spectrometry.

Different stationary phases and temperature programs were used in an effort to separate these compounds from 2,3- and 3,4-MDMA. The best resolution, among the evaluated columns, was achieved on Rtx-200 where in a physical mixture of the 12 compounds 11 peaks were observed, only the perfluroacyl derivatives of two para methoxy methyl methamphetamines co-elute. The perfluroacyl derivatives of these compounds were divided into three subsets based on the ring substitution pattern of the methoxy group. All perflourocayl derivatives of methoxy methyl methamphetamines in subsets were resolved in a physical mixture with 2,3- and 3,4- MDMA on Rtx-1 and Rtx-5.

Since the methoxymethyl phenyl acetones were key intermediates in synthesizing ring substituted methoxy methyl methamphetamines, their mass spectral and chromatographic properties were also evaluated during the course of this work. The mass spectral studies were able to distinguish the methoxy methyl phenyl acetones with the methoxy group in the ortho postion from other substitution patterns based on the significant peak at m/z 105. The best chromatographic separation was achived on a permethylated betacyclodextrin column where the methoxy methyl phenyl acetones with the methoxy in the ortho postion elutes first followd by regioisomers with the methoxy

group in the meta postions while regioisomers with the methoxy group in the para postion elute last. All ten methoxymethyl phenyl acetones were separated from methylenedioxy-2-propanone, the precursor of 3,4-MDMA, whick eluted last on the same column.

4 EXPERIMENTAL

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

4.1.1 Materials

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

3,4-Methylenedioxyphenylacetone, piperonal, 2,3-dihydroxybenzaldehyde, *o*-anisaldehyde, *m*- anisaldehyde, *p*-anisaldehyde, *p*-methoxypropiophenone, 2,3-dimethyl anisole, 3-methyl salicylic acid, 4-methyl salicylic acid, 2-methoxy-phenylacetone,4-methoxy-2-methyl benzaldehyde, 4-methoxy-3-methylbenzaldehyde, methylamine hydrochloride, ethylamine hydrochloride, *N*-methyl formamide, benzaldehyde, isobutyric acid, 2-methylbutyric acid, valeric acid, 1-nitropropane, *N*-bromosuccinimide, methylene bromide, methyl iodide, hexamethylphosphoramide, diisopropylamine, ethyl chloroformate, sodium azide, acetyl chloride, thionylchloride, benzoyl peroxide, copper(II)oxide, 60% sodium hydride, 10% palladium on carbon, lithium aluminum hydride, sodium cyanoborohydride, nitroethane, ethylchloroacetate, sodium amide, ethyl-3-bromopropionate, ethyl-2-bromobutrate, propylbromoacetate, methyl-2-bromoproprionate,4-methoxy-3-methylbenzaldehyde, methylphenylketone, 3M methyl magnesium bromide in ether, 3 M ethyl magnesium bromide in ether, potassium persulfate, cupper sulfate, *n*-butylamine and sodium borohydride, Sodium bis(2-

methoxyethoxy) aluminum hydride (Red-Al) in toluene, 2-methyl furan, 2.5 M *n*-butyllithium in hexane, powdered iron were purchased from Sigma (St. Louis, MO, USA). MDMA-d_5 was purchased from Cerilliant Corporation (Round Rock, Texas, USA). Pentafluropropionic anhydride and heptaflurobutric anhydride were purchased from UCT (Bristol, PA, USA). 2-methoxy-5-methyl benzaldehyde was purchased Trans World Chemicals (Rockville, MD, USA). 3-methoxy-4-methyl benzoic acid methyl ester was purchased from TCI America (Portland, OR, USA). Methyl idodide was purchased from Acros Organics (Morris Plains, NJ, USA).

HPLC grade acetonitrile, methylenechloride, methanol, toluene, tetrahydrofuran and ferric chloride were purchased from Fisher Scientific, (Atlanta, GA, USA). Diethyl ether, 2-propanol, methylene chloride, carbon tetrachloride, benzene, tetrahydrofuran (THF) and chloroform were purchased from Fisher Scientific (Fair Lawn, N.J., USA).

4.1.2 Instruments

GC-MS analysis was performed with 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. 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).

H^1NMR data were collected for samples disoved in deuterated chloroform using a Brucker Avance 250 (250MHz) instrument with the chemical shifts being reported as δ ppm downfield from tetramethylsilane

Ultra violet spectra were collected on Shimadzu UV-3600 Series in the absorbance measuring mode with 1.0 nm slit width and time constant of 0.1 sec.

4.1.3 GC- Columns

Different capillary GC columns were evaluated throughout the course of this work, however only columns showed best compromises between resolution and analysis time are illustrated in Table 13. 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 13: 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
Rtx-200	trifluoropropyl methyl polysiloxane
Rt-βDEXcst-TM	14% cyanopropyl phenyl – 86 % dimethylpolysiloxane

4.1.4 Temperature Programs

Different temperature programs were evaluated throughout the course of this work, however only programs showing the best compromises between resolution and analysis time are illustrated in table14.

Table 14: List of temperature programs used.

Temperature program Name	Injector Temperature ^{0}C	Detector Temperature ^{0}C	Program setup
TP-1	250	280	Hold column temperature at 100^{o}C for 1 minute then the temperature was ramped up to 180^{o}C at a rate of 9^{o}C/ minute. Column temperature was held at 180^{o}C for 2 minutes then was ramped up to 200^{o}C at a rate of 9^{o}C/ minute and set at 200^{o}C for 5 minutes
TP-2	250	280	Hold column temperature at 70^{o}C for 1 minute then the temperature was ramped up to 150^{0}C at a rate of 7.5^{o}C/ minute. Column temperature was held at 150^{o}C for 2 minutes then was ramped up to 250^{o}C at a rate of 10^{o}C/ minute and set at 250^{o}C for 5 minutes
TP-3	200	200	Hold column temperature at 100^{o}C for 1 minute then the temperature was ramped up to 180^{o}C at a rate of 9^{o}C/ minute. Column temperature was held at 180^{o}C for 2 minutes then was ramped up to 200^{o}C at a rate of 9^{o}C/ minute and set at 200^{o}C for 10 minutes

TP-4	200	200	Hold column temperature at 70°C for 1 minute then the temperature was ramped up to 150°C at a rate of 7.5°C/ minute. Column temperature was held at 150°C for 2 minutes then was ramped up to 200°C at a rate of 10°C/ minute and set at 200°C for 10 minutes
TP-5	200	200	Hold column temperature at 70°C for 2 minute then the temperature was ramped up to 150°C at a rate of 2.5°C/ minute. Column temperature was held at 150°C for 3 minutes then was ramped up to 200°C at a rate of 15°C/ minute and set at 200°C for 5 minutes
TP-6	250	280	Hold column temperature at 100°C for 1 minute then the temperature was ramped up to 180°C at a rate of 20°C/ minute. Column temperature was held at 180°C for 2 minutes then was ramped up to 250°C at a rate of 10°C/ minute and set at 200°C for 5 minutes

4.2 Synthesis of Regioisomeric and Isobaric Substances Related to MDMA

4.2.1 Synthesis of direct regioisomers related to MDMA

4.2.1.1 Preparation of *N,N*-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (1)

A mixture of piperonal (20.0 g, 0.133mol) and *n*-butylamine (76 ml, 0.768mol) in benzene (500 ml) was refluxed overnight using a Dean Stark trap to remove

water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (40 ml), and nitromethane (8.11 g, 0.133mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, yellow crystals of 1-(3,4-methylenedioxyphenyl)-2-nitroethene were formed in the reaction mixture and additional acetic acid (50 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. Crude yellow crystals of 1-(3,4-methylenedioxyphenyl)-2-nitroethene were isolated by filtration, washed with water, air dried and then recrystallized from 2-propanol (50 ml) to give 69% (17.6 g, 0.091 mol) yield.

Dry THF (20 ml) was added dropwise to lithium aluminum hydride (2.0 g, 0.053 mol) under nitrogen followed by the dropwise addition of 1-(3,4-methylenedioxyphenyl)-2-nitroethene (2.0 g, 0.010 mol) in dry THF (30 ml). The mixture was refluxed for one hour and then stirred at room temperature overnight. The reaction was quenched by adding a mixture of water (2 ml) and THF (25 ml), 2N sodium hydroxide (2 ml) in THF (10 ml), and water (4 ml) in THF (10 ml). The mixture was filtered and the solvent of the filtrate was evaporated under reduced pressure. The residue was suspended in water (30 ml) and acidified to pH 1 by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3 x 25 ml) then was alknized by the addition of sodium hydroxide pellets. The basic aqueous suspension was extracted with methylene chloride (3 x 30 ml) and the organic combined extract was dried over anhydrous sodium sulfate. The solvent was filtered and evaporated to yield 1-(3,4-methylenedioxyphenyl)-2-ethanamine (0.53 g, 0.0032 mol, 32%) as a yellow oil.

A mixture of 1-(3,4-methylenedioxyphenyl)-2-ethanamine (0.70 g, 0.0042 mol), 37% formaldehyde (2.3 g, 0.025 mol) and sodium cyanoborohydride (1.06 g, 0.0168 mol) in methanol (50 ml) was stirred at room temperature for three days. The solvent was evaporated under reduced pressure and the obtained yellow oil was stirred in acidic water overnight and the resulting aqueous acidic solution was washed with methylene chloride (3 x 25 ml). The aqueous layer was alkalinized by the addition of sodium hydroxide pellets and extracted with methylene chloride (3 x 30 ml). The combined organic extract was dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to yield a yellow oil, *N*,*N*-dimethyl-1-(3,4-methylenedioxy-phenyl)-2-ethanamine. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the *N*,*N*-dimethyl-1-(3,4-methylenedioxy-phenyl)-2-ethanamine hydrochloride salt (0.30 g, 0.0016 mol, 37%). MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.2 Preparation of *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (2) from 3,4- Methylenedioxyphenyl ethanamine

3,4-Methylenedioxyphenyl ethanamine (0.53 g, 0.0032 mol) and triethylamine (0.65 g, 0.0064 mol) were dissolved in THF (25 ml). Acetyl chloride (0.50 g, 0.0064 mol) in THF (10 ml) was added dropwise to the reaction mixture and then it was refluxed overnight. The reaction mixture was allowed to cool at room temperature and the crystals of triethylamine hydrochloride were filtered off. The filtrate was evaporated under reduced pressure and the residue was dissolved in methylene chloride. The organic layer

was washed with acidic water, brine and saturated sodium bicarbonate solution then dried over anhydrous sodium sulfate. Filtration followed by evaporation of the organic solvent gave N-acetyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (0.43 g, 0.0021 mol, 66%) as a brown oil.

Dry THF (10 ml) was added dropwise to lithium aluminum hydride (0.43 g, 0.011 mol) under nitrogen atmosphere followed by dropwise addition of N-acetyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (0.43 g, 0.0021 mol) in dry THF (10 ml). The reaction mixture was allowed to reflux overnight. The reaction was quenched by adding a mixture of water (1 ml) and THF (5 ml) followed by 2N sodium hydroxide (1 ml) in THF (5 ml), and water (2 ml) in THF (5 ml). The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was suspended in water (30 ml) and acidified to pH 1 using concentrated hydrochloric acid. The aqueous acid layer was washed with methylene chloride (2 x 25 ml). The acidic aqueous layer was alkalinized by the addition of sodium hydroxide pellets. The basic aqueous suspension was extracted with methylene chloride (3 x 30 ml) and the combined organic extract was dried over anhydrous sodium sulfate. The solvent was then filtered evaporated under reduced pressure to give *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine as a light yellow oil. *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine was converted to the hydrochloride salt by dissolving in dry diethyl ether followed by the passing of hydrochloric acid gas in the solution. Light buff crystals of *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine hydrochloride (0.010 g, 0.00004 mol, 2%) were formed and separated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed

4.2.1.3 Preparation of *N*-ethyl-1-(3, 4-methylenedioxyphenyl)-2-ethanamine (2) from 3,4- methyelnedioxyphenyl acetic acid

3,4- Methylenedioxyphenyl acetic acid (5.0g, 27mmol) was dissolved in methylene chloride (75 ml) and the solution was kept cold using ice. A solution of oxalyl chloride (6.85 g, 54mmol) in methylene chloride (25ml) was then added dropwise followed by the addition of 8-10 drops of dimethylformamide. The reaction mixture was refluxed for 3 hours and the organic layer was evaporated under reduced pressure to give crude 3,4-methylenedioxyphenylacetyl chloride.

Ethylamine (10 ml 0.015mol) in methylene chloride (15 ml) was cooled using acetone/ice and then a solution of 3,4-methylenedioxyphenylacetyl chloride (2.61g, 0.0015 mol) in methylene chloride (15 ml) was added dropwise. The mixture was stirred overnight at room temperature. The organic layer was evaporated under reduced pressure and the residue was dissolved in methylene chloride (60 ml). The organic layer was washed sequentially with 2N hydrochloric acid solution (2 x 15 ml), 2N sodium hydroxide solution (2 x 15 ml) and water (3x 20 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give N-ethyl-1(3,4 methylenedioxyphenyl) acetamide which was crystallized from benzene/ hexane to give a yield of 74%.

N-Ethyl-1(3,4 methylenedioxyphenyl) acetamide (1.95g, 0.009 mol) in dry benzene (20 ml) was added dropwise to RedAl (1.91g, 0.009mol) under atmosphere of nitrogen and the reaction mixture was refluxed overnight. The reaction was terminated by the addition of ethanol and water (50 ml each) and the organic layer was separated and

evaporated under reduced pressure. The residue was suspended in water (30 ml) and acidified to pH 1 using concentrated hydrochloric acid. The aqueous acid layer was washed with methylene chloride (2 x 25 ml). The acidic aqueous layer was alkalinized by the addition of sodium hydroxide pellets. The basic aqueous suspension was extracted with methylene chloride (3 x 30 ml) and the combined organic extract was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine as a light yellow oil. *N*-Ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine was converted to the hydrochloride salt by dissolving in dry diethyl ether followed by the passing of hydrochloric acid gas in the solution. Light buff crystals of *N*-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine hydrochloride (1.62 g, 0.0084 mol, 83%) were formed and separated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.4 Preparation of *N*-ethyl-1-(3, 4-methylenedioxyphenyl)-2-ethanamine (2) from 3,4 methyelnedioxyphenylacetaldehyde

Sodium amide (1.72g, 0.04 mol) was added dropwise to an ice cooled mixture of piperonal (5.0g, 0.033mol) and ethyl bromoacetate (5.66g, 0.033 mol) in dry benzene (50 ml). The mixture was allowed to stir for two hours at 15-20 ^{o}C. The reddish colored reaction mixture was then poured over crushed ice and the organic layer was separated. The aqueous layer was washed with benzene (3 x 20ml). The combined organic extract was washed with distilled water (3 x 30ml) then dried over anhydrous

sodium sulfate. Benzene was filtered and evaporated under reduced pressure to yield crude 3,4-methylenedioxyphenethylglycidate.

Crude 3,4-methylenedioxyphenethylglycidate (7.86g, 0.033mol) was dissolved in ethanol (30 ml) and sodium hydroxide (1.33 g, 0.033mol) was added slowly followed by the addition of distilled water (5.0 ml). The mixture was stirred overnight at room temperature to give white crystals of 3,4-methylenedioxyphenylglycidic acid sodium salt that were collected by filtration under reduced pressure. Crystals were washed with ether and methanol (50 ml each) and air dried.

3, 4- Methylenedioxyphenylglycidic acid sodium salt was added to 50 ml 2N hydrochloric acid solution and the mixture was warmed gently for 1.5 hour. An oily layer of 3,4-methylenedioxyphenylacetaldehde was formed. The desired aldehyde was extracted by benzene (70 ml) and the benzene layer was washed once with water and dried over anhydrous sodium sulfate. The benzene was filtered and evaporated under reduced pressure to give a yellow oil of 3,4-methylenedioxyphenylacetaldehde which was purified by kugelrohr distillation.

3,4-Methylenedioxyphenylacetaldehde (1.0g, 0.006mol) along with ethylamine hydrochloride (2.74g, 0.061 mol) and sodium cyanoborohydride (3.78g, 0.061 mol) were dissolved in methanol (50 ml) and the reaction mixture was stirred at room temperature for 3 days. Methanol was evaporated under reduced pressure and the resulting residue was stirred in acidic water (50 ml) overnight at room temperature. The aqueous layer was washed with methylene chloride (3 x 20 ml) and was alkalinized using sodium hydroxide pellets. The aqueous layer was then extracted with methylene chloride (3 x 30 ml) and the organic layer was dried over anhydrous sodium sulfate. Methylene

chloride was filtered and evaporated under reduced pressure to give a yellow oil of N ethyl-1-(3, 4-methylenedioxyphenyl)-2-ethanamine. The oil was dissolved in dry ether and gaseous hydrochloric acid was passed to give white crystals of N-ethyl-1-(3, 4-methylenedioxyphenyl)-2-ethanamine hydrochloride (0.6 g, 0.031 mol, 60 %) were separated by filtration and air dried. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.5 Preparation of α,α-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine (4)

Diisopropylamine (5.6 g, 0.055mol) was added to 75 ml of dry THF, under an atmosphere of nitrogen and the solution was cooled with external dry ice/isopropanol. A solution of 2.5 M *n*-butyllithium in hexane (24 ml, 0.06mol) was then added dropwise and the mixture was allowed to warm to room temperature and stirred for 5 minutes. The reaction mixture was then cooled again in the dry ice bath and isobutyric acid (2.2 g, 0.025mol) was added dropwise followed by the addition of 5 ml of hexamethylphosphoramide. The reaction mixture was allowed to warm to room temperature and stirred for 30 minutes. 3,4-Methylenedioxybenzyl chloride (4.25 g, 0.025 mol) was then added dropwise and the mixture was stirred overnight at room temperature.

The reaction mixture was poured into 50 ml of 10% hydrochloric acid and THF was evaporated under reduced pressure. The acidic aqueous residue was extracted with diethyl ether (3 x 30 ml). The combined ether extracts were washed with 10%

hydrochloric acid, and then extracted with saturated sodium carbonate solution. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with diethyl ether (3 x 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and the organic solvent was evaporated under reduced pressure. The obtained 2,2-dimethyl-(3,4-methylenedioxy-phenyl)propionic acid (3.0 g, 0.013mol, 53%) crystallized spontaneously.

2,2-Dimethyl-(3,4-methylenedioxyphenyl)propionic acid (2.7 g, 0.012mol) and triethylamine (1.23 g, 0.012mol) were dissolved in water (2 ml) and diluted with acetone, sufficient to maintain a clear solution at ice-bath temperature. A solution of ethyl chloroformate (1.52 g, 0.014mol) in 10 ml of acetone was added dropwise to the 0°C solution, followed by the addition of a solution of sodium azide (0.98 g, 0.015mol) in water (8 ml) and the reaction mixture was stirred for 45 minutes at room temperature. The aqueous phase was extracted with toluene (3 x 30 ml) and the combined toluene extracts was washed with water and dried over anhydrous sodium sulfate. This organic layer was filtered and heated at 100°C until nitrogen evolution has ceased, which required about 30 minutes. The solvent was evaporated under reduced pressure and the residue was dissolved in 10 ml of benzyl alcohol and heated overnight at 100°C. Excess benzyl alcohol was removed by Kugelrohr distillation to yield 1-(*N*-(benzyloxycarbonyl)amino)-1,1-dimethyl-2-(3,4-methylenedioxyphenyl)-ethane as an amber oil residue.

The oily residue of 1-(*N*-(benzyloxycarbonyl)amino)-1,1-dimethyl-2-(3,4-methylenedioxyphenyl)-ethane was dissolved in ethanol (50 ml) and 10% palladium on carbon (0.5 g) was added. The reaction mixture was hydrogenated for 24 hours under 50 psi. The carbon was removed by filtration over celite and the organic solvent was

evaporated under reduced pressure. The residue was dissolved in acidic water, washed with methylene chloride (3x 20 ml). The aqueous layer was alkalinized with sodium hydroxide and extracted with methylene chloride (3 x 30 ml) and the organic layer was dried using anhydrous sodium sulfate overnight. Sodium sulfate was removed by filtration and the solvent was evaporated under reduced pressure to give α,α-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine as a residue. The residue was dissolved in diethyl ether. Hydrochloric acid gas was introduced until white crystals of α,α-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine hydrochloride were formed (0.62 g, 0.0027 mol, 23%) and isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.6 Preparation of 1-(3,4-methylenedioxyphenyl)-2-butanamine (5)

A mixture of piperonal (20.0 g, 0.133mol) and *n*-butylamine (76 ml, 0.768 mol) in benzene (500 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as an oil. The imine was dissolved in glacial acetic acid (40 ml), and nitropropane (11.83 g, 0.133mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, yellow crystals of 1-(3,4-methylenedioxyphenyl)-2-nitrobutene were formed in the reaction mixture and additional acetic acid (50 ml) was added. The mixture was cooled at room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. Yellow crude crystals of 1-(3,4-methylenedioxyphenyl)-2-nitrobutene

were isolated by filtration, washed with water, air dried and then recrystallized from 2-propanol (50 ml) to give 70 % (17.85 g, 0.092 mol) yield.

1-(3,4-Methylenedioxyphenyl)-2-nitrobutene (3.3 g, 0.015mmol) was dissolved in toluene (15 ml) and 15 ml of water. The resulting solution was mixed with powdered iron (4.49 g, 0.088mol), ferric chloride (0.90 g, 0.006mol) and concentrated hydrochloride acid (6 ml). The mixture was stirred vigorously and refluxed over a day. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5 N hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 1-(3,4-methylenedioxyphenyl)-2-butanone (1.25 g, 0.0065 mol, 46.9%) as a yellow oil.

1-(3,4-Methylenedioxyphenyl)-2-butanone (1.25 g, 0.0065mol) was dissolved in methanol (50 ml) followed by the addition of ammonium acetate (5.01g, 0.065mol) and sodium cyanoborohydride (10.7g, 0.163mol). The reaction mixture was stirred at room temperature for 4 days and the acidity was maintained during that time using glacial acetic acid. Methanol was evaporated under reduced pressure and the resulting residue was stirred in acidic water (50 ml) overnight at room temperature. The aqueous layer was washed with methylene chloride (3 x 20 ml) and was alkalinized using sodium hydroxide pellets. The aqueous layer was then extracted with methylene chloride (3 x 30 ml) and the organic layer was dried over anhydrous sodium sulfate. Methylene chloride was filtered and evaporated under reduced pressure to give a yellow oil of N -

ethyl-1-(3, 4-methylenedioxyphenyl)-2-ethanamine. The oil was dissolved in dry ether and gaseous hydrochloric acid was passed to give white crystals of 1-(3,4-methylenedioxyphenyl)-2-butanamine hydrochloride (1.0 g, 0.005mol, 80 %) which was filtered and air dried. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.7 Preparation of *N,N*-dimethyl-1-(2,3-methylenedioxy-phenyl)-2-ethanamine (6)

2,3-Dihydroxybenzaldehyde (5.0 g, 0.036mol) and potassium carbonate (18.75 g, 0.136mol) were dissolved in 50 ml of DMF. Methylene bromide (18.9 g, 7.6 ml, 0.10mol) was added dropwise at room temperature, followed by addition of copper (II) oxide (0.010 g). The reaction mixture was refluxed for 2 hours and additional methylene bromide (18.9 g, 7.6 ml, 0.10 mol) was added. The mixture was allowed to reflux overnight. The mixture was first vacuum filtered and then DMF was removed by Kugelrohr distillation. The brown oil obtained was suspended with water and extracted with methylene chloride (3x 25 ml). The combined organic extract was washed with 5% potassium hydroxide solution, brine and 2N hydrochloric acid. The methylene chloride was evaporated and the obtained oil was distilled by Kugelrohr apparatus (100°C/ 3 mmHg), which gave 2,3-methylenedioxybenzaldehyde (3.2 g, 0.021 mol, 59%) as a light yellow oil.

A mixture of 2,3-methylenedioxybenzaldehyde (3.2 g, 0.021 mol) and *n*-butylamine (12.16 ml, 0.123 mol) in benzene (100 ml) was refluxed overnight using a

Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as an oil. The imine was dissolved in glacial acetic acid (20 ml), and nitromethane (1.3 g, 0.02 mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, yellow crystals of 1-(2,3-methylenedioxyphenyl)-2-nitroethene were formed in the reaction mixture and additional acetic acid (20 ml) was added. The mixture was cooled at room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. Crude yellow crystals of 1-(2,3-methylenedioxyphenyl)-2-nitroethene were isolated by filtration, washed with water, air dried and then recrystallized from 2-pronanol (50 ml) to give 62.5% (2 g, 0.010 mol) yield.

Dry THF (20 ml) was added dropwise to lithium aluminum hydride (2.0 g, 0.053mol) under nitrogen followed by the addition of 1-(2.3-methylenedioxyphenyl)-2-nitroethene (2.0 g, 0.010 mol) in dry THF (30 ml) dropwise. The mixture was refluxed for one hour and then stirred at room temperature overnight. The reaction was quenched by adding the mixture of water (2 ml) and THF (25 ml), 2N sodium hydroxide (2 ml) in THF (10 ml), and water (4 ml) in THF (10 ml). The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was suspended in water (30 ml) and acidified to pH 1 by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3 x 25 ml) then was alknized by the addition of sodium hydroxide pellets. The basic aqueous suspension was extracted with methylene chloride (3 x 30 ml) and the organic layer was dried with anhydrous sodium sulfate. The solvent was evaporated to yield 1-(2,3-methylenedioxyphenyl)-2-ethanamine (0.60 g, 0.0036 mol, 36%) as a yellow oil.

A mixture of 1-(3,4-methylenedioxyphenyl)-2-ethanamine (0.70 g, 0.0042 mol), 37% formaldehyde (2.3 g, 0.025 mol) and sodium cyanoborohydride (1.06 g, 0.0168 mol) in methanol (50 ml) was stirred at room temperature for three days. The solvent was evaporated under reduced pressure and the obtained yellow oil was stirred in acidic water (25ml) overnight. The aqueous acidic solution was washed with methylene chloride (3 x 15 ml). The aqueous layer was alkalinized by the addition of sodium hydroxide pellets and extracted with methylene chloride (3 x 25 ml). The combined organic extracts were dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to yield a yellow oil, *N*,*N*-dimethyl-1-(2,3-methylenedioxy-phenyl)-2-ethanamine. The oil was dissolved in dry diethyl ether, and hydrochloric acid gas was added to form the *N*,*N*-dimethyl-1-(2.3-methylenedioxy-phenyl)-2-ethanamine hydrochloride salt (0.50 g, 0.0026 mol, 61.6%). MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.8 Preparation of *N*-ethyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine (7)

2,3-Methylenedioxyphenylethanamine (0.53 g, 0.0032 mol) and triethylamine (0.65 g, 0.0064 mol) were dissolved in THF (25 ml). Acetyl chloride (0.50 g, 0.0064 mol) in THF (10 ml) was added dropwise to the reaction mixture which was then allowed to reflux overnight. The reaction mixture was cooled to room temperature and the crystals of triethylamine hydrochloride were removed by filteration. The filtrate was evaporated under reduced pressure and the residue was dissolved in methylene chloride. The organic layer was washed with acidic water, brine and saturated sodium bicarbonate solution then

dried over anhydrous sodium sulfate. Filtration followed by evaporation of the organic solvent gave N-acetyl-1-(2,3-methylenedioxy)-2-ethanamine (0.40 g, 0.0019 mol, 61.4%) as a brown oil.

Dry THF (10 ml) was added dropwise to lithium aluminum hydride (0.43 g, 0.011 mol) under nitrogen atmosphere followed by dropwise addition of N-acetyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine (0.43 g, 0.0021 mol) in dry THF (10 ml). The reaction mixture was allowed to reflux overnight. The reaction was quenched by adding the mixture of water (1 ml) and THF (5 ml) follwed by 2N sodium hydroxide (1 ml) in THF (5 ml), and water (2 ml) in THF (5 ml). The mixture was filtered and the solvent of the filtrate was evaporated under reduced pressure. The residue was suspended in water (30 ml) and acidified to pH 1 using concentrated hydrochloric acid. The aqueous acid layer was washed with methylene chloride (3 x 15 ml). The acidic aqueous layer was alkalinized by the addition of sodium hydroxide pellets. The basic aqueous suspension was extracted with methylene chloride (3 x 25 ml) and the organic layer was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give *N*-ethyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine as a light yellow oil. *N*-ethyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine was converted to the hydrochloride salt by dissolving in dry diethyl ether followed by the addition of hydrochloric acid gas in the solution. Light buff crystals of *N*-ethyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine hydrochloride (0.2 g, 0.00008 mol, 40%) were formed and isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.9 Preparation of 2,3-methylenedioxymethamphetamine (8)

2,3-Dihydroxybenzaldehyde (5.0 g, 0.03 mol) and potassium carbonate (18.75 g, 0.136mol) were dissolved in 50 ml of DMF. Methylene bromide (18.9 g, 7.6 ml, 0.10mol) was added dropwise at room temperature, followed by addition of copper (II) oxide (0.010 g). The reaction mixture was refluxed for 2 hours and additional methylene bromide (18.9 g, 7.6 ml, 0.10mol) was added. The mixture was allowed to reflux overnight. The mixture was first vacuum filtered and then DMF was removed by Kugelrohr distillation. The obtained brown oil was suspended with water and extracted with methylene chloride (3x 30 ml). The combined organic extract was washed with 5% potassium hydroxide solution, brine and 2N hydrochloric acid. The methylene chloride was evaporated and the obtained oil was distilled by Kugelrohr apparatus (100°C/ 3 mmHg), which gave 2,3-methylenedioxybenzaldehyde (3.2 g, 0.021mol, 59%) as light yellow oil.

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

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

2,3-Methylenedioxyphenyl-2-propanone (1.12 g, 0.0063mol) was dissolved in methanol (40 ml) followed by addition of methylamine hydrochloride (4.25 g, 0.063mol) and sodium cyanoborohydride (0.60 g, 0.0095 mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (50 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 20 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 30 ml). The combined organic extract was dried with magnesium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 2,3-methylenedioxymethamphetamine (0.80 g, 0.0041mol, 66%)

were obtained by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.10 Preparation of α,α-dimethyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine (9)

Sodium borohydride (1.62 g, 0.042mol) was added to 2,3-methylenedioxy-benzaldehyde (3.2 g, 0.021 mol) in 2-propanol (50 ml). The reaction mixture was stirred at room temperature overnight. Additional sodium borohydride (0.8 g, 0.02mol) was added and the reaction mixture was stirred at room temperature over three days. The reaction was quenched by addition of ice and the mixture was allowed to stir overnight. The 2-propanol was evaporated under reduced pressure and the water layer was extracted with methylene chloride and evaporated to give 2,3-methylenedioxy-benzylalcohol as white crystals (3.0 g, 0.0197 mol, 94%).

Thionyl chloride (2.58 g, 0.0217 mol) was added dropwise to 2,3-methylenedioxy-benzylalcohol (3.0 g, 0.0197 mol) in 20 ml of chloroform. The mixture was refluxed over three hours. Chloroform was evaporated under reduced pressure and benzene was added to the residue twice and evaporated under reduced pressure. The residue obtained was distilled by Kugelrohr apparatus, which gave 2,3-methylenedioxybenzyl chloride (2.1 g, 0.012 mol, 63%) as a colorless liquid.

To a 30 ml portion of dry THF, under an atmosphere of nitrogen, there was added diisopropylamine (2.8 g, 0.0275 mol), and the solution was cooled with external dry ice/isopropanol. Then a solution of 2.5 M *n*-butyllithium in hexane (12 ml, 0.03mol) was

added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 5 minutes, then cooled again in the dry ice bath. Isobutyric acid (1.1 g, 0.0125mol) was added dropwise, followed by the addition of 2.6 ml of hexamethylphosphoramide and the mixture was allowed to warm to room temperature and stirred for 30 minutes. A portion of 2,3-methylenedioxybenzyl chloride (2.1 g, 0.012 mol) was added dropwise and the mixture was stirred overnight at room temperature.

The reaction mixture was poured into 30 ml of 10% hydrochloric acid and the THF was evaporated under reduced pressure and the acidic aqueous residue was extracted several times with diethyl ether. The pooled ether extracts were washed with 10% hydrochloric acid, and then extracted with saturated sodium carbonate solution. This was acidified with concentrated hydrochloric acid and extracted with diethyl ether. The diethyl ether was dried with anhydrous magnesium sulfate, and evaporated under reduced pressure. The obtained light yellow oil of 2,2-dimethyl-(2,3-methylenedioxyphenyl)-propionic acid (1.95 g, 0.0088 mol) crystallized spontaneously.

2,2-Dimethyl-(2,3-methylenedioxyphenyl)propionic acid (1.95 g, 0.0088mol) and triethylamine (0.89 g, 0.0088mol) were dissolved in water (2 ml) and diluted with sufficient acetone to maintain a clear solution at ice-bath temperature. A solution of ethyl chloroformate (1.11 g, 0.010mol) in 7 ml of acetone was added dropwise to the 0°C solution, followed by the addition of a solution of sodium azide (0.71 g, 0.011mol) in water (6 ml). Stirring was continued for 45 minutes at room temperature. The aqueous phase was extracted with toluene, which was washed with water and dried with magnesium sulfate. This organic solution was heated (100°C) until nitrogen evolution has ceased, which required about 30 minutes. The solvent was removed under

vacuum and the residue was dissolved in 10 ml of benzyl alcohol. This solution was heated (100°C) overnight. Removal of the excess benzyl alcohol by Kugelrohr distillation left a residue of 1-[N-(benzyloxycarbonyl) amino]-1,1-dimethyl-2-(2,3-methylenedioxyphenyl)-ethane as an amber oil. The oily residue was dissolved in ethanol (30 ml) and 10% palladium on carbon (0.25 g) was added. The reaction mixture was hydrogenated for 1.5 hours (50 psi). The carbon was removed by filtration through Celite. The solvent was removed under reduced pressure and the residue was dissolved in acidic water, washed with methylene chloride, the water layer was alkalinized with sodium hydroxide and extracted with methylene chloride. The solvent was evaporated under reduced pressure and dissolved in diethyl ether. Hydrochloric acid gas was introduced until white crystals were formed. Filtration of the crystals gav eα,α-dimethyl-1-(2,3-methylenedioxyphenyl)-2-ethanamine (0.57g, 0.0030mol, 34%) which were recrystallized from diethyl ether and 2-propanol. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.1.11 Preparation of 1-(2,3-methylenedioxyphenyl)-2-butanamine (10)

A mixture of 2,3-methylenedioxybenzaldehyde (5.0 g, 0.033mol) and *n*-butylamine (19 ml, 0.192mol) in benzene (125 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as greenish oil. The imine was dissolved in glacial acetic acid (10 ml), and nitropropane (2.98 g, 0.034mol) was added dropwise and the reaction mixture was refluxed for one

hour. During reflux, yellow crystals of 1-(2,3-methylenedioxyphenyl)-2-nitrobutene were formed in the reaction mixture and additional glacial acetic acid (15 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. Crude yellow crystals of 1-(2,3-methylenedioxyphenyl)-2-nitrobutene were isolated by filtration, washed with water, air dried and then recrystallized from 2-pronanol (15 ml) to give a 68 % (3.4 g, 0.017mol) yield.

1-(2,3-Methylenedioxyphenyl)-2-nitrobutene (3.3 g, 0.015mmol) was dissolved in toluene (15 ml) and 15 ml of water. The resulting solution was mixed with powdered iron (4.49 g, 0.088mol), ferric chloride (0.90g, 0.006mol) and concentrated hydrochloride acid (6 ml). The mixture was stirred vigorously and refluxed over a day. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5 N hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 1-(2,3-methylenedioxyphenyl)-2-butanone (1.5 g, 0.0078 mol, 55.2%) as a yellow oil.

1-(2,3-Methylenedioxyphenyl)-2-butanone (1.25 g, 0.0065 mol) was dissolved in methanol (50 ml) followed by the addition of ammonium acetate (5.01g, 0.065mol) and sodium cyanoborohydride (10.7g, 0.163 mol). The reaction mixture was stirred at room temperature for 4 days and the acidity was maintained during that time using glacial acetic acid. Methanol was evaporated under reduced pressure and the resulting residue was stirred in acidic water (50 ml) overnight at room temperature. The

aqueous layer was washed with methylene chloride (3 x 20 ml) and was alkalinized using sodium hydroxide pellets. The basic aqueous layer was then extracted with methylene chloride (3 x 30 ml) and the organic layer was dried over anhydrous sodium sulfate. Methylene chloride was filtered and evaporated under reduced pressure to give a yellow oil of 1-(2,3-methylenedioxyphenyl)-2-butanamine. The oil was dissolved in dry ether and gaseous hydrochloric acid was passed to give white crystals of 1-(2,3-methylenedioxyphenyl)-2-butanamine hydrochloride. The crystals were filtered and air dried to give 0.8 g yield (0.004mol, 64 %). MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.2 Synthesis of indirect regioisomers related to MDMA, the methoxymethcathinones.

4.2.2.1 Preparation of o-methoxymethcathinone (11)

A solution of o-methoxybenzaldehyde (2.72g, 0.020mol) in 50 ml of dry diethylether was added to a flask and maintained under an atmosphere of dry nitrogen. Ethyl magnesium bromide solution in diethyl ether (8ml, 0.024mol) was then added dropwise and the reaction mixture was stirred at -20 ^{o}C for two hours. The reaction was quenched using 1N hydrochloric acid (25 ml) and the ether layer was separated, washed with water and dried over anhydrous sodium sulfate over night. The ether layer was then filtered and evaporated under reduced pressure to yield the 1-(2-methoxy-phenyl)-propan-1-ol (2.49 g, 15 mmol, 91.5 %).

1-(2-Methoxy-phenyl)-propan-1-ol (2.49 g, 0.015mol) in methylene chloride (60 ml) were stirred over night at room temperature with PCC (6.5 g, 0.030 mol) and Celite (6.5 g). The reaction mixture was then diluted with diethylether (150 ml) and stirred for 30 minutes, and then vacuum filtered through a pad of silica gel (200-400 mesh) under vacuum. The organic layer was evaporated under reduced pressure to yield 1-(2-methoxy-phenyl)-propan-1-one which was purified using Kugelrohr distillation.

1-(2-Methoxy-phenyl)-propan-1-one (5.0g, 0.03mol) was dissolved in carbon tetrachloride (200ml) along with n-bromosuccinimide (5.49 g, 0.03mol) and a catalytic amount of benzoyl peroxide (10 mg). The mixture was refluxed over night, filtered and the solvent evaporated under reduced pressure yielding 2-bromo-1-(2-methoxy-phenyl)-propan-1-one which were purified using Kugelrohr distillation.

2-Bromo-1-(2-methoxy-phenyl)-propan-1-one (5.41 g, 0.0223 mol) in acetonitrile (50 ml) was added dropwise to a mixture of methylamine hydrochloride (15.0 g, 0.223 mol) and sodium bicarbonate (18.7 g, 2.23 mol) in acetonitrile (50 ml). The mixture was stirred at room temperature for 3 days, filtered and the solvent was evaporated under reduced pressure. Water (50 ml) was added to the residue and acidified with concentrated hydrochloric acid and the aqueous acidic solution was washed with methylene chloride (3 x 25 ml). The aqueous layer was alkalinized with sodium hydroxide pellets and extracted with methylene chloride (3 x 50 ml). The methylene chloride layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give o-methoxymethcathinone as light yellow oil. o-Methoxymethcathinone was dissolved in dry ether and converted to the hydrochloride salt using gaseous hydrochloric acid. The yield was (1.0g, 0.0043 mol, 19.2 %). MS,

molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.2.2 Preparation of m-methoxymethcathinone (12)

A solution of 3-methoxybenzaldehyde (2.72g, 0.020mol) in 50 ml of dry diethylether was added to a flask and maintained under an atmosphere of dry nitrogen. Ethyl magnesium bromide solution in diethylether (8ml, 0.024mol) was then added dropwise and the reaction mixture was stirred at -20 ^{o}C for two hours. The reaction was then quenched using 1N hydrochloric acid (25 ml) and the ether layer was separated, washed with water and dried over anhydrous sodium sulfate over night. The ether layer was filtered and evaporated under reduced pressure to yield the 1-(3-methoxy-phenyl)-propan-1-ol (2.5 g, 0.015 mol, 91.9 %).

1-(3-Methoxy-phenyl)-propan-1-ol (2.49g, 0.015mol) in methylene chloride (60 ml) were stirred over night at room temperature with PCC (6.5 g, 0.030mol) and Celite (6.5 g). The reaction mixture was then diluted with diethylether (150 ml) and stirred for 30 minutes and then filtered on a pad of silica gel (200-400 mesh) under vacuum. The organic layer was evaporated under reduced pressure to yield 1-(2-methoxy-phenyl)-propan-1-one which was purified using Kugelrohr distillation.

1-(3-Methoxy-phenyl)-propan-1-one (5.0g, 0.030mol) was dissolved in carbon tetrachloride (200ml) along with N-bromosuccinimide (5.49 g, 0.030 mol) and a catalytic amount of benzoyl peroxide (10mg). The mixture was refluxed over night,

filtered and the solvent evaporated under reduced pressure yielding 2-bromo-1-(3-methoxy-phenyl)-propan-1-one which were purified using Kugelrohr distillation.

2-Bromo-1-(3-methoxy-phenyl)-propan-1-one (5.41g, 0.0223 mol) in acetonitrile (50 ml) was added dropwise to a mixture of methylamine hydrochloride (15.0 g, 0.223 mol) and sodium bicarbonate (18.7g, 2.23 mol) in acetonitrile (50ml). The mixture was stirred at room temperature for 3 days, filtered and the solvent was evaporated under reduced pressure. Water (50ml) was added to the residue and acidified with concentrated hydrochloric acid and the aqueous acidic solution was washed with methylene chloride (3 x 25ml). The aqueous layer was alkalinized with sodium hydroxide pellets and extracted with methylene chloride (3 x 50ml). The methylene chloride layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give 3-methoxymethcathinone as light yellow oil. 3-methoxymethcathinone was dissolved in dry ether and converted to the hydrochloride salt gaseous hydrochloric acid (1.5g, 0.0065mol, 28.8 %). MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.2.3 Preparation of p-methoxymethcathinone (13)

1-(4-Methoxyphenyl)-propan-1-one (5.0g, 0.0305mol) was dissolved in 200 ml of carbontetrachloride and *N*-bromosuccinimide (5.49g, 0.0305mol) and catalytic amount of benzoyl peroxide (0.01g) were added. The mixture was refluxed for 21 hours. The reaction mixture was filtered and the solvent was evaporated under reduced pressure to yield p-methoxy-2-bromo-propiophenone (6.5g, 0.0267mol, 88%).

p-Methoxy-2-bromo-propiophenone (5.41g, 0.0223mol) in acetonitrile (20 ml) was added dropwise to the mixture of methylamine hydrochloride (15.0g, 0.223mol) and sodium bicarbonate (18.7g, 0.223mol) in acetonitrile (80ml). The reaction mixture was stirred at room temperature overnight. The mixture was filtered and the solvent was evaporated under reduced pressure. Water (50ml) was added to the residue and acidified with concentrated hydrochloric acid and washed with methylene chloride (3 x 20ml). The aqueous layer was alkalinized with sodium hydroxide pellets and extracted with methylene chloride (3 x 30ml). The methylene chloride was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to give light yellow oil, which was dissolved in a mixture of anhydrous diethyl ether and 2-propanol. Hydrochloric acid gas was introduced and white crystals of *p*-methoxymethcathinone hydrochloride (1.3g, 0.0056mol, 25%). MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3 Synthesis of Isobaric substances related to MDMA, the methoxy-methyl-methamphetamines.

4.2.3.1 Preparation of 2-Methoxy-3-Methyl-methamphetamine (14)

Dimethyl sulfate (47.6ml, 0.503mol) and potassium carbonate (60g, 0.434mol) were added to a solution of 3-methyl salicylic acid (25.5g, 0.168mol) in dry acetone (300 ml) and the reaction mixture was stirred at room temperature for one week. The mixture was gravity filtered and the residue was washed with acetone (3 x 30 ml) and the

combined filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30ml), followed by the addition of triethylamine (70ml) and the solution was stirred at room temperature for 30 minutes. The reaction mixture was then transferred to a separatory funnel and washed with 6N hydrochloric acid solution (2x25 ml), water (4x50ml) and brine solution (2x50ml) respectively. The organic layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure. GC-MS analysis of the product showed that methylation of the hydroxyl group did not go to completion and 2 major peaks were found representing 3-methyl salicylic acid methyl ester and 2-methoxy -3-methylbenzoic acid methyl ester in the ratio of 3:1. This residue was then dissolved in dry acetone (300 ml) followed by the addition of methyl iodide (20g) and potassium carbonate (15.5g) and the reaction mixture was stirred for five days at room temperature. Solids were removed by filtration and the organic layer was evaporated under reduced pressure to yield 2-methoxy-3-methyl benzoic acid methyl ester.

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

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

A mixture of 2-methoxy-3-methylbenzaldehyde (10.0g, 0.0666mol) and *n*-butylamine (38ml, 0.384mol) in benzene (250ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as an oil. The imine was dissolved in glacial acetic acid (60ml), and nitroethane (5.0g, 0.0666 mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(2-methoxy-3-methyl phenyl)-2-nitropropene was separated as dark oil which was extracted by methylene chloride (4 x 30ml). The combined organic extract was washed with water (4 x 15ml) and was dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to give 1-(2-methoxy-3-methyl phenyl)-2-nitroepropene (6.3g, 0.030 mol, 59.3%).

Crude 1-(2-methoxy-3-methylphenyl)-2-nitropropene (6.2g, 0.030mol) was dissolved in toluene (30ml) and water (30ml). The resulting solution was mixed with powdered iron (8.98g, 0.176mol), ferric chloride (1.80g, 0.012mol) and concentrated

hydrochloride acid (12ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30ml) and water (30ml) were added and the mixture was gravity filtered. The residue was washed with additional toluene and water. The organic layer was then separated, and washed with 5N hydrochloric acid solution, water and saturated sodium bicarbonate solution (3 x 15ml each). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. Kugelrohr distillation of the crude product afforded 1-(2-methoxy-3-methylphenyl)-2-propanone (3.55 g, 0.0198 mol, 66.6%) as a yellow oil.

1-(2-Methoxy-3-methylphenyl)-2-propanone (1.0g, 0.006mol) was dissolved in methanol (30ml) followed by addition of methylamine hydrochloride (3.75 g, 0.056mol) and sodium cyanoborohydride (0.53g, 0.0084mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (30ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 15ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20 ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 2-methoxy-3-methylmethamphetamine hydrochloride (0.7g, 0.0036mol) were isolated by filtration.

MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.2 Preparation of 2-methoxy-4-methylmethamphetamine (15)

Dimethyl sulfate (47.6ml, 0.503mol) and potassium carbonate (60g, 0.434mol) were added to a solution of 4-methyl salicylic acid (25.5g, 0.168mol) in dry acetone (300 ml) and the reaction mixture was stirred at room temperature for one week. The mixture was gravity filtered and the residue was washed with acetone (3x 30ml) and the combined filtrate was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (30ml), followed by the addition of triethylamine (70ml) and the solution was stirred at room temperature for 30 minutes. The reaction mixture was then transferred to a separatory funnel and washed with 6 N hydrochloric acid solution (2x25 ml), water (4x50ml) and brine solution (2x50ml) respectively. The organic layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure. GC-MS analysis of the product showed that methylation of the hydroxyl group did not go to completion. 2 major peaks were found, one representing 4-methyl salicylic acid methyl ester and 2-methoxy-4-methylbenzoic acid methyl ester in the ratio of 3:1. Residue was then dissolved in dry acetone (300ml) followed by the addition of methyl iodide (20g) and potassium carbonate (15.5g) and the reaction mixture was stirred for five days at room temperature. Solids were removed by filtration and the organic layer was evaporated under reduced pressure to yield 2-methoxy-4-methyl benzoic acid methyl ester.

Red Al (83ml) was added to a solution of 2-methoxy-4-methyl benzoic acid methyl ester (24.0g, 0.145mol) in benzene (200ml) under nitrogen. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (60ml) and water (60ml), then the organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100ml) and the organic layer was washed with water (3x30ml). Methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 2-methoxy-4-methyl benzyl alcohol.

Pyridinium chlorochromate (28.1g, 0.130mol) and celite (28.1g) were added to a solution of 2-methoxy-4-methylbenzyl alcohol (10g, 0.085mol) in methylene chloride (250ml) and the reaction mixture was stirred at room temperature overnight. Reaction mixture was diluted with ether (100ml) and stirred for 30 minutes then filtered over a pad of silica gel (200-400 mesh) and the residue was washed with ether (3x30ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 2-methoxy-4-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 2-methoxy-4-methylbenzaldehyde (10.0g, 0.0666mol) and *n*-butylamine (38ml, 0.384mol) in benzene (250ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (60ml), and nitroethane (5.0g, 0.0666mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(2-

methoxy-4-methyl phenyl)-2-nitropropene was separated as dark oil which was extracted by methylene chloride (4 x 30ml). The combined organic extract was washed with water (4 x 15ml) and was dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to give 1-(2-methoxy-4-methyl phenyl)-2-nitroepropene (5.0 g, 0.024 mol, 47.1%).

Crude 1-(2-methoxy-4-methylphenyl)-2-nitropropene (5.0g, 0.024mol) was dissolved in toluene (30ml) and water (30ml). The resulting solution was mixed with powdered iron (7.24g, 0.139mol), ferric chloride (1.45g, 0.010mol) and concentrated hydrochloride acid (9. ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The residue was washed with additional toluene and water. The organic layer was then separated, and washed with 5N hydrochloric acid solution, water and saturated sodium bicarbonate solution (3x 15ml). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. Kugelrohr distillation of the crude product afforded 1-(2-methoxy-4-methylphenyl)-2-propanone (2.0 g, 0.0111 mol, 56.3%) as a yellow oil.

1-(2-Methoxy-4-methylphenyl)-2-propanone (1.0g, 0.006mol) was dissolved in methanol (30ml) followed by addition of methylamine hydrochloride (3.75 g, 0.056mol) and sodium cyanoborohydride (0.53g, 0.0084mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (30ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was

washed with methylene chloride (3x 15ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 2-methoxy-4-methylmethamphetamine (0.5g, 0.0026mol) were isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.3 Preparation of 2-methoxy-5-methylmethamphetamine (16)

A mixture of 2-methoxy-5-methyl benzaldehyde (10.0 g, 0.0666mol) and *n*-butylamine (38 ml, 0.384mol) in benzene (250 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (60 ml), and nitroethane (5.0g, 0.0666mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(2-methoxy-5-methylphenyl)-2-nitropropene was separated as dark green oil which was extracted by methylene chloride (4 x 30 ml). The combined organic extract was washed with water (4 x 15 ml) and was dried over anhydrous sodium sulfate. The organic layer

was filtered and evaporated under reduced pressure to yield 1-(2-methoxy-5-methyl phenyl)-2-nitroepropene (6.9g, 65%).

Crude 1-(2-methoxy-5-methylphenyl)-2-nitropropene (6.2g, 0.030mmol) was dissolved in toluene (30 ml) and 30 ml of water. The resulting solution was mixed with powdered iron (8.98g, 0.176mol), ferric chloride (1.80g, 0.012mol) and concentrated hydrochloride acid (12 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5 N hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 1-(2-methoxy-5-methylphenyl)-2-propanone (3.25g, 0.0182mol, 61%) as yellow oil.

1-(2-Methoxy-5-methylphenyl)-2-propanone (3.0g, 0.0168mol) was dissolved in methanol (40 ml) followed by addition of methylamine hydrochloride (11.25g, 0.168mol) and sodium cyanoborohydride (1.59g, 0.0252mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (50 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 20 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 30 ml). The combined organic

extract was dried over anhydrous sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 2-methoxy-5-methylmethamphetamine hydrochloride (1.5g, 0.0077mol) were obtained by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.4 Preparation of 2-methoxy-6-methylmethamphetamine (17)

A solution of copper sulfate pentahydrate (18.04g, 0.0723mol) and potassium persulfate (60.55g, 0.22mol) in water (250 ml) was added dropwise to a solution of 2,3-dimethyl anisole (9.82, 0.073mol) in acetonitrile (250ml) and the reaction mixture was refluxed for 45 minutes. The reaction mixture was cooled to room temperature, solvent volume was reduced under reduced pressure and 2-methoxy-6-methylbenzaldehyde was extracted using methylene chloride (4x 35 ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford crude 2-methoxy-6-methylbenzaldehyde.

A mixture of 2-methoxy-6-methylbenzaldehyde (10.0g, 0.066mol) and *n*-butylamine (38 ml, 0.384mol) in benzene (250 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (60 ml), and nitroethane (5.0g, 0.066mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50 ml) was added. The mixture was cooled to room temperature,

poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(2-methoxy-6-methylphenyl)-2-nitropropene was separated as dark green oil which was extracted by methylene chloride (4 x 30 ml). the combined organic extract was washed with water (4 x 15 ml) and was dried over anhydrous sodium sulfate.the organic layer was filtered and evaporated under reduced pressure to yield of 1-(2-methoxy-6-methylphenyl)-2-nitroepropene (7.2 g, 67.8%).

Crude 1-(2-methoxy-6-methylphenyl)-2-nitropropene (6.2g, 0.030mol) was dissolved in toluene (30 ml) and 30 ml of water. The resulting solution was mixed with powdered iron (8.98g, 0.176mol), ferric chloride (1.80g, 0.012mol) and concentrated hydrochloride acid (12 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5 N hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 1-(2-methoxy-6-methylphenyl)-2-propanone (2.96g, 0.0166mol, 55.6%) as yellow oil.

1-(2-Methoxy-6-methylphenyl)-2-propanone (1.5g, 0.0084mol) was dissolved in methanol (40 ml) followed by addition of methylamine hydrochloride (6.67g, 0.084mol) and sodium cyanoborohydride (0.75g, 0.0126mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (50 ml),

and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 15 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20 ml). The combined organic extract was dried over anhydrous sodiumsulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 2-methoxy-6-methylmethamphetamine hydrochloride (1.0g, 0.0051mol) were obtained by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.5 Preparation of 3-methoxy-2-methylmethamphetamine (18)

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

Red Al (26.5 ml) was added to a solution of 3-methoxy-2-methyl benzoic acid methyl ester (7.86g, 0..0433mol) in benzene (100ml) under an atmosphere of nitrogen. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml), then the organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (75

ml) and the organic layer was washed with water (3x30 ml). The methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 3-methoxy-2-methyl benzyl alcohol (5.85g, 85.4 %)

Pyridinium chlorochromate (15.1g, 0.076mol) and celite (15.1g) were added to a solution of 3-methoxy-2-methylbenzyl alcohol (5.85g, 0.038mol) in methylene chloride (150ml) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with ether (100 ml) and stirred for 30 minutes then it was filtered over a pad of silica gel (200-400 mesh) and the residue was washed with ether (3x30 ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 3-methoxy-2-methylbenzaldehyde which was purified by Kugelrohr distillation (4.71g, 82%).

A mixture of 3-methoxy-2-methylbenzaldehyde (4.71g, 0.0312mol) and *n*-butylamine (17.89ml, 0.181mol) in benzene (100 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (60 ml), and nitroethane (2.34g, 0.0312mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(3-Methoxy-2-methylphenyl)-2-nitropropene was separated as dark oil which was extracted by methylene chloride (4 x 30 ml). The combined organic extract was washed with water (4 x 15 ml) and was dried over anhydrous sodium sulfate. The

organic layer was filtered and evaporated under reduced pressure to yield 1-(3-methoxy-2-methylphenyl)-2-nitroepropene (6.4 g, 99.3%).

Crude 1-(3-methoxy-2-methylphenyl)-2-nitropropene (6.2g, 0.030mol) was dissolved in toluene (30 ml) and water (30 ml). The resulting solution was mixed with powdered iron (8.98g, 0.176mol), ferric chloride (1.80g, 0.012mol) and concentrated hydrochloride acid (12 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The residue was washed with additional toluene and water. The organic layer was then separated, and washed with 5N hydrochloric acid solution, water and saturated sodium bicarbonate solution (3 x 15 ml each). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product afforded 1-(3-methoxy-2-methylphenyl)-2-propanone (4.01g, 0.0225mol, 75.1%) as yellow oil.

1-(3-Methoxy-2-methylphenyl)-2-propanone (2.0g,0.012mol) was dissolved in methanol (60 ml) followed by addition of methylamine hydrochloride (7.5g, 0.112mol) and sodium cyanoborohydride (1.06g, 0.0168mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (30 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 15 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20 ml). The combined organic extract was dried over anhydrous

sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 3-methoxy-2-methylmethamphetamine hydrochloride (1.3. g, 0.006mol) were isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.6 Preparation of 3-methoxy-4-methylmethamphetamine (19)

Red-Al (11.2g, 0.55mol) was added to a solution of 3-methoxy-4-methyl benzoic acid methyl ester (5.0g, 0.028mol) in benzene (100ml) under nitrogen. The reaction mixture was refluxed for 2 hours and the reaction was terminated by the addition of ethanol (50ml) and water (50ml), then the organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100 ml) and the organic layer was washed with water (3x30 ml). Methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 3-methoxy-4-methylbenzyl alcohol.

Pyridinium chlorochromate (18.2g, 0.084mol) and celite (18.1g) were added to a solution of 3-methoxy-4-methylbenzyl alcohol (6.5g, 0.043mol) in methylene chloride (200ml) and the reaction mixture was stirred at room temperature overnight. Reaction mixture was diluted with ether (100 ml) and stirred for 30 minutes then it was filtered over a pad of silica gel (200-400 mesh) and the residue was washed with ether (3x30 ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 3-methoxy-4-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 3-methoxy-4-methylbenzaldehyde (3.8g, 0.025mol) and *n*-butylamine (14.44ml, 0.146mol) in benzene (150 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (30 ml), and nitroethane (1.9g, 0.025mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (30 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(3-Methoxy-4-methylphenyl)-2-nitropropene was separated as dark oil which was extracted by methylene chloride (3 x 15 ml). The combined organic extract was washed with water (3 x 15 ml) and was dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to yield 1-(2-methoxy-4-methylphenyl)-2-nitroepropene (2.5g, 27.1%).

Crude 1-(3-methoxy-4-methylphenyl)-2-nitropropene (5.0g, 0.024mol) was dissolved in toluene (30 ml) and water (30 ml). The resulting solution was mixed with powdered iron (7.24g, 0.139mol), ferric chloride (1.45g, 0.010mol) and concentrated hydrochloride acid (9.6 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The residue was washed with additional toluene and water. The organic layer was then separated, and washed with 5N hydrochloric acid solution, water and saturated sodium bicarbonate solution (3x 15 ml each). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr

distillation of the crude product afforded 1-(3-methoxy-4-methylphenyl)-2-propanone (3.6g, 0.020mol,) as yellow oil.

1-(3-Methoxy-4-methylphenyl)-2-propanone (1.34g, 0.0075mol) was dissolved in methanol (30 ml) followed by addition of methylamine hydrochloride (5.1g, 0.075mol) and sodium cyanoborohydride (0.73g, 0.012mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (30 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 15 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20 ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 3-methoxy-4-methyl methamphetamine hydrochloride (1.0g, 0.0052mol) were isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.7 Preparation of 3-methoxy-5-methylmethamphetamine (20)

Sodium metal (25.5g, 1.1mol) was added in 250 mg portions over 2.5 hours to absolute ethanol (500ml) in an ice cooled dry three neck flask under nitrogen and the mixture was stirred overnight. Acetone (58.93g, 1.0mol) and diethyl oxalate (146.27g)

were then added dropwise over 3 hours and the resulting thick yellow mixture was stirred for additional 2 hours. The resulting ethyl sodium acetopyrovate was collected by vacuum filtration was dried over night.

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

Magnesium oxide (45.3g, 1.12 mol) was added in three portion to a suspension of 3-Acetyl-4,5-dioxo-2-(2-oxo-propyl)-tetrahydro-furan-2-carboxylic acid ethyl ester (85.5g, 0.277 mol) in water (1540 ml) and the reaction mixture was refluxed for 2 hours. The reaction mixture was then filtered under vacuum and the residue was washed with hot water. The filtrate volume was reduced under reduced pressure and it was then cooled to room temperature. Hydrochloric acid gas was then bubbled to afford 3-hydroxy-5-methyl benzoic acid that was isolated by gravity filtration and dried overnight.

Methyl iodide (33.53g, 0.236mol) and potassium carbonate (32.71g, mol) were added to a solution of 3-hydroxy-5-methylbenzoic acid (12g, 0.0667mol) in dry acetone and the reaction mixture was refluxed for 24 hours. The reaction mixture was cooled to room temperature and solids were removed by filtration and the filtrate was evaporated under reduced pressure. GC-MS monitoring of the reaction showed 4 peaks of m/z 180/149, 166/149, 194/135 and 180/135 that represents ethyl-3-methoxy 5-methyl

benzoate, methyl-3-hydroxy-5-methyl benzoate and ethyl-3-hydroxy-5-methyl benzoate, respectively. Excess methyl iodide was added and the reaction was refluxed for another 24 hours. GC-MS analysis of the reaction mixture showed only two peaks of m/z 180/149 and 194/149 indicating the formation if methyl-3-methoxy-5-methyl benzoate and ethyl -3-methoxy-5-methyl benzoate, respectively. The reaction mixture was cooled to room temperature and solids were removed by gravity filtration. The residue was washed with acetone and the combined organic extract was evaporated under reduced pressure to afford a mixture of methyl-3-methoxy-5-methyl benzoate and ethyl-3-methoxy-5-methyl benzoate.

Red-Al (60 ml) was added to a solution of the crude methyl-3-methoxy-5-methyl benzoate and ethyl-3-methoxy-5-methyl benzoate (23g) in benzene (200 ml) and the reaction mixture was refluxed for 2 hours. The reaction was terminated by the addition of ethanol (50ml) and water (50ml) and then the organic layer was separated and evaporated under reduced pressure. The residue was dissolved in methylene chloride (100 ml) and the organic layer was washed with water (3x30 ml). Methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 3-methoxy-5-methylbenzyl alcohol.

Pyridinium chlorochromate (28.0 g, 0.130mol) and celite (28g) were added to a solution of 3-methoxy-5-methylbenzyl alcohol (10.0g, 0.066mol) in methylene chloride (200ml) and the reaction mixture was stirred at room temperature overnight. Reaction mixture was diluted with ether (100 ml) and stirred for 30 minutes then it was filtered over a pad of silica gel (200-400 mesh) and the residue was washed with ether (3x30 ml).

The combined organic filtrate was evaporated under reduced pressure to afford crude 3-methoxy-5-methylbenzaldehyde which was purified by Kugelrohr distillation.

A mixture of 3-methoxy-5-methylbenzaldehyde (5.0g, 0.032mol) and *n*-butylamine (19.0ml, 0.193mol) in benzene (150 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (30 ml), and nitroethane (2.5g, 0.032mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (30 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(3-methoxy-5-methylphenyl)-2-nitropropene was separated as dark oil which was extracted by methylene chloride (3 x 15 ml). The combined organic extract was washed with water (3 x 15 ml) and was dried over anhydrous sodium sulfate. The organic layer was filtered and evaporated under reduced pressure to yield 1-(2-methoxy-5-methylphenyl)-2-nitroepropene (5.o g, 0.024 mol).

Crude 1-(3-methoxy-5-methylphenyl)-2-nitropropene (5.0g, 0.024mol) was dissolved in toluene (30 ml) and water (30 ml). The resulting solution was mixed with powdered iron (7.24g, 0.139mol), ferric chloride (1.45g, 0.010mol) and concentrated hydrochloride acid (9.6 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The residue was washed with additional toluene and water. The organic layer was then separated, and washed with 5 N hydrochloric acid solution, water and saturated sodium bicarbonate solution (3x 15 ml each). The organic layer was

dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product afforded 1-(3-methoxy-5-methylphenyl)-2-propanone (2.5 g, 0.014mol) as a yellow oil.

1-(3-Methoxy-5-methylphenyl)-2-propanone (1.51g, 0.0085mol) was dissolved in methanol (30 ml) followed by addition of methylamine hydrochloride (5.73g, 0.085mol) and sodium cyanoborohydride (0.83g, 0.014mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (30 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 15 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20 ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 3-methoxy-5-methyl methamphetamine hydrochloride (1.0 g, 0.0052mol) were isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.8 Preparation of 5-methoxy-2-methylmethamphetamine (21)

A solution of 2-methylfuran (6.56g, 0.08mol) in methylene chloride (30.0 ml) was added dropwise to a solution of ethyl propiolate (7.84g, 0.08mol) and anhydrous aluminum chloride (10.64g, 0.0596mol) in methylene chloride (120ml) and the resulting reaction mixture was stirred at room temperature for 30 minutes .The reaction mixture was then shacken vigorously with water and the organic layer was separated. The organic layer was extracted with 5% sodium hydroxide solution (3x 30 ml) and the combined aqueous basic layer was acidified with concentrated hydrochloric acid. The acidified aqueous layer was extracted with ethyl acetate (4 x30 ml) and the combined organic layer was dried over anhydrous sodium sulfate. The organic layer was then filtered and evaporated under reduced pressure to afford ethyl 5-hydroxy-2-methylbenzoate.

Methyl iodide (33.53g, 0.236mol) and potassium carbonate (32.71g, 0.237mol) were added to a solution of ethyl 5-hydroxy-2-methylbenzoate (12g, 0.0667mol) in dry acetone and the reaction mixture was refluxed for 24 hours. The reaction mixture was cooled to room temperature and solids were removed by filtration and the filtrate was evaporated under reduced pressure. GC-MS monitoring of the reaction showed 2 peaks of m/z 180/149 and 194/149 methyl 5-methoxy 2-methyl benzoate and ethyl 5-methoxy-2-methyl benzoate, respectively.

Red-Al (60 ml) was added to a solution of the crude methyl-5-methoxy-2-methyl benzoate and ethyl -5-methoxy-2-methyl benzoate (10.32g) in benzene (200 ml) and the reaction mixture was refluxed for 2 hours. The reaction was terminated by the addition of ethanol (50ml) and water (50ml), and then the organic layer was separated and

evaporated under reduced pressure. The residue was dissolved in methylene chloride (100 ml) and the organic layer was washed with water (3x30 ml). Methylene chloride layer was dried over anhydrous sodium sulfate for 5 hours then filtered and evaporated under reduced pressure to yield crude 5-methoxy-2-methylbenzyl alcohol (6.7g, 0.44mol).

Pyridinium chlorochromate (17.29 g, 0.088mol) and celite (17.29g) were added to a solution of 5-methoxy-2-methylbenzyl alcohol (6.7g, 0.044mol) in methylene chloride (150ml) and the reaction mixture was stirred at room temperature overnight. Reaction mixture was diluted with ether (100 ml) and stirred for 30 minutes then it was filtered over a pad of silica gel (200-400 mesh) and the residue was washed with ether (3x30 ml). The combined organic filtrate was evaporated under reduced pressure to afford crude 5-methoxy-2-methylbenzaldehyde which was purified by Kugelrohr distillation (5.80g, 87.3%).

A mixture of 5-methoxy-2-methylbenzaldehyde (5.8g, 0.038mol) and *n*-butylamine (22.0 ml, 0.223mol) in benzene (100 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as oil. The imine was dissolved in glacial acetic acid (60 ml), and nitroethane (2.88g, 0.0384mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(5-Methoxy-2-methylphenyl)-2-nitropropene was separated as dark oil which was extracted by methylene chloride (4 x 30 ml). The combined organic extract was washed with water (4 x 15 ml) and was dried over anhydrous sodium sulfate. The

organic layer was filtered and evaporated under reduced pressure to yield 1-(5-methoxy-2-methylphenyl)-2-nitroepropene (6.2 g, 96.2%).

Crude 1-(5-methoxy-2-methylphenyl)-2-nitropropene (6.2g, 0.030mol) was dissolved in toluene (30 ml) and water (30 ml). The resulting solution was mixed with powdered iron (8.98g, 0.176mol), ferric chloride (1.80g, 0.012mol) and concentrated hydrochloride acid (12 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The residue was washed with additional toluene and water. The organic layer was then separated, and washed with 5N hydrochloric acid solution, water and saturated sodium bicarbonate solution (3 x 15 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product afforded 1-(5-methoxy-2-methylphenyl)-2-propanone (4.41 g, 0.0248mol, 82.6%) as yellow oil.

1-(5-Methoxy-2-methylphenyl)-2-propanone (2.0g,0.012mol) was dissolved in methanol (60 ml) followed by addition of methylamine hydrochloride (7.5g, 0.112mol) and sodium cyanoborohydride (1.06g, 0.0168mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (30 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 15 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 20 ml). The combined organic extract was dried over anhydrous

sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 5-methoxy-2-methylmethamphetamine hydrochloride (1.30. g, 0.007 mol) were isolated by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.9 Preparation of 4-methoxy-3-methylmethamphetamine (22)

A mixture of 4-methoxy-3-methylbenzaldehyde (5.0g, 0.0333mol) and *n*-butylamine (19ml, 0.192mol) in benzene (125 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as an oil. The imine was dissolved in glacial acetic acid (40 ml), and nitroethane (2.50g, 0.0333mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(4-methoxy-3-methylphenyl)-2-nitropropene was separated as olive green oil which was extracted by methylene chloride (3x30ml). the combined organic extract was washed with water (3x15ml) and was dried over anhydrous sodium sulfate.the organic layer was filtered and evaporated under reduced pressure to yield 1-(4-methoxy-3-methylphenyl)-2-nitroepropene (3.35 g, 65%).

Crude 1-(4-methoxy-3-methylphenyl)-2-nitropropene (3.1g, 0.015mol) was dissolved in toluene (15 ml) and 15 ml of water. The resulting solution was mixed with

powdered iron (4.49g, 0.088mol), ferric chloride (0.90g, 0.006mol) and concentrated hydrochloride acid (6 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5N hydrochloric acid, water and saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 1-(4-methoxy-3-methylphenyl)-2-propanone (1.50 g, 0.0084mol, 56.25%) as yellow oil.

1-(4-Methoxy-3-methylphenyl)-2-propanone (1.0g, 0.0056mol) was dissolved in methanol (40 ml) followed by addition of methylamine hydrochloride (3.75g, 0.056mol) and sodium cyanoborohydride (0.53g, 0.0084mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (50 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 20 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 30 ml). The combined organic extract was dried over anhydrous sodium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was bubbled into the solution to form the hydrochloride salt. White crystals of 4-methoxy-3-methyl methamphetamine hydrochloride (0.60g, 0.0031mol) were obtained

by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.2.3.10 Preparation of 4-methoxy-2-methylmethamphetamine (23)

A mixture of 4-methoxy-2-methylbenzaldehyde (5.0g, 0.0333mol) and *n*-butylamine (19ml, 0.192mol) in benzene (125 ml) was refluxed overnight using a Dean Stark trap to remove water. The reaction mixture was then cooled to room temperature and the solvent was evaporated under reduced pressure to yield the crude imine as an oil. The imine was dissolved in glacial acetic acid (40 ml), and nitroethane (2.50g, 0.0333mol) was added dropwise and the reaction mixture was refluxed for one hour. During reflux, additional acetic acid (50 ml) was added. The mixture was cooled to room temperature, poured over crushed ice and acidified to pH 1 using concentrated hydrochloric acid. 1-(4-methoxy-2-methylphenyl)-2-nitropropene was separated as olive green oil which was extracted by methylene chloride (3 x 30 ml). the combined organic extract was washed with water (3 x 15 ml) and was dried over anhydrous sodium sulfate.the organic layer was filtered and evaporated under reduced pressure to yield 1-(4-methoxy-2-methyl phenyl)-2-nitroepropene (3.5 g, 65%).

Crude 1-(4-methoxy-2-methylphenyl)-2-nitropropene (3.1g, 0.015mmol) was dissolved in toluene (15 ml) and 15 ml of water. The resulting solution was mixed with powdered iron (4.49g, 0.08mol), ferric chloride (0.90g, 0.006mol) and concentrated hydrochloride acid (6 ml). The mixture was stirred vigorously and refluxed overnight. After cooling to room temperature, toluene (30 ml) and water (30 ml) were added and the

mixture was gravity filtered. The precipitate was washed with additional toluene and water. The toluene layer was separated, and washed with 5N hydrochloric acid solution, water and saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was evaporated. Kugelrohr distillation of the crude product gave 1-(4-methoxy-2-methylphenyl)-2-propanone (2.25g, 0.0126mol, 84.4%) as yellow oil.

1-(4-Methoxy-2-methylphenyl)-2-propanone (1.0g, 0.0056mol) was dissolved in methanol (40 ml) followed by addition of methylamine hydrochloride (3.75g, 0.056mol) and sodium cyanoborohydride (0.53g, 0.0084mol). The reaction mixture was stirred at room temperature for three days and the pH was maintained at 7 by adding concentrated hydrochloric acid. The reaction mixture was evaporated under reduced pressure to yield a white solid. The solid was suspended in cold water (50 ml), and slowly acidified by the addition of concentrated hydrochloric acid. The acidic aqueous layer was washed with methylene chloride (3x 20 ml). The aqueous layer was made alkaline by the addition of sodium hydroxide pellets and the resulting basic suspension was extracted with methylene chloride (3x 30 ml). The combined organic extract was dried with magnesium sulfate, filtered and evaporated to yield colorless oil. The oil was dissolved in anhydrous diethyl ether, and hydrochloric acid gas was added to form the hydrochloride salt. White crystals of 4-methoxy-2-methyl methamphetamine hydrochloride (0.63 g, 0.0033mol,) were obtained by filtration. MS, molecular weight 193, m/z 58 [100%], no other fragment ions of 10% or greater were observed.

4.3 Preparation of the Perfluoroacyl Derivatives

Each perfluoroamide was prepared individually from the hydrochloride salts of the regioisomers by dissolving approximately 0.3 mg (1.33×10^{-5} mol) of each amine in 50µl of ethyl acetate followed by addition of large excess (250 µl) of the appropriate derivatizing agent (pentafluropropionic anhydride or heptaflurobutric anhydride) and the derivatization reaction mixtures were incubated in capped tubes at 70 ^{o}C for 20 minutes. Following incubation each sample was evaporated to dryness under a stream of air at 55^{o}C and reconstituted with 200µl of ethyl acetate and 50µl of pyridine.

4.4 Preparation of the Pyridinium chlorochromate

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

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