GC-MS AND GC-IRD STUDIES ON ETHOXYPHENETHYLAMINES RELATED TO MDEA, MDMMA AND MBDB

by

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Abstract

Three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equal mass have been reported as drugs of abuse in recent years. These compounds are 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxy-N,N-dimethylamphetamine (MDMMA), and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). Ring substituted ethoxyphenethylamine regioisomers were synthesized. These regioisomers are compounds with an isobaric relationship to the controlled drug substances MDEA, MDMMA and MBDB, all have molecular weights of 207 and major fragment ions in their electron ionization mass spectra at *m/z* 72 and 135/136. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary amines were evaluated in GC–MS studies. The mass spectra for these derivatives were significantly individualized and the resulting unique fragment ions allowed for specific identification. These perfluoroacyl derivatives showed reasonable gas chromatographic resolution on the non-polar stationary phase Rtx-1. GC-IRD studies provided structure-IR spectral relationships used for the discrimination of the three target drugs (MDEA, MDMMA and MBDB) from the other nine ring substituted ethoxyphenethylamine regioisomers. In addition GC-MS and IRD studies were done on the ketone precursors of the

ethoxyphenethylamines and ketone precursors of the drug of abuse MDEA, MDMMA and MBDB. The ketones have shown reasonable gas chromatographic resolution on the non-polar stationary phase Rtx-1. GC-IRD studies provided structure-IR spectral relationships used for the discrimination of the ketone precursors of the drugs (MDEA, MDMMA and MBDB) from the other six ring substituted ethoxyphenethyl ketones regioisomers.

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1. LITERATURE REVIEW

1.1. Introduction:

There is a worldwide concern over the increase in the usage of so called "designer drugs" among teens and young adults in many countries. Among the different classes of designer drugs are ring-substituted amphetamines like 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxyphenyl-2-butanamine (BDB), N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) and 3,4-methylenedioxy-N,N-dimethylamphetamine (MDMMA). These ring-substituted amphetamines became more popular in recent years mainly within the 'rave' dance music culture; most users consider these agents to cause little, if any, adverse effects. However frequent arrests, serious undesired reactions and late night E.R. visits associated with their use have been commonly reported. Also, fatalities due to the use of these drugs, mainly MDMA "ecstasy" either directly (toxicity) or indirectly (e.g. motor vehicle accident) have been reported over the past decades. Data collected by Yacoubian (2003)

explored ecstasy usage trends in the United States during the 1990s, confirming that there is a increase in the usage of ecstasy; in the United Kingdom, a community survey of the illegal use of controlled drugs in that country showed that roughly 4% of 16-59 years olds have taken ecstasy [Cole et al., 2003]. Ecstasy and other ring-substituted amphetamines can be found in a variety of dosage forms and can be administrated by different routes. Oral administration in the form of tablets or capsules, with dosages ranging from 120-130 mg for MDMA and 100-200 mg for MDEA is the most common route. Other less common routes can be inhalation as a powder or use as a suppository. Theses ring-substituted amphetamines can also be administered parentally, because of the high solubility of the amine salts in water [Freudenmann et al., 2004]. In humans, the duration of action after administering MDMA is between 4-6 hours, 8-12 hours for MDA and a shorter duration of 2-3 hours was reported for MDEA [Freudenmann et al., 2004].

The term "ecstasy" was originally used as a street name for only MDMA; but currently it is used for the whole group of the methylenedioxyphenylalkylamine analogs such as MDA, MDEA and MBDB. One reason is that all these substances (MDMA, MDEA, MDA and MBDB) have very similar pharmacological profiles. In addition, there has been an increasing trend of selling ecstasy tablets and capsules that contain not only MDMA, but a mixture of other drugs of abuse and impurities [Ecstasy Data, 1996-2007]. It is also common that ecstasy is mixed with a combination of adulterants such as other amphetamines, caffeine, ephedrine, pseudoephedrine [Smith et al., 2002] and acetaminophen [Kalasinsky et al., 2004].

Table 1: Street names of some of the popular drugs of abuse.

Agent	Street Names
DOB	Bromo-DMA, Golden Eagle, Bromo-STP, Tile,100X
DOM	Pink Wedge, STP(Serenity, Tranquility, and Peace)
MDA	Harmony, Love, Love Drug, Speed for lovers.
MDEA	Eve.
MDMA	Adam, California Sunrise, Dove, Ecstasy, XTC, E.
MBDB	Eden, Methyl-J
BDB	J

These designer drugs are produced in clandestine laboratories and the major region of manufacturing these drugs are countries in Western Europe [Freudenmann, 2004]. Clandestine manufacturing laboratory goals are often to prepare substances that have pharmacological profiles that are sought after by the user population, in addition to creating substances that circumvent existing local and international laws. In some European countries, new drug substances cannot be immediately considered as illicit drugs as a result of the substance-by-substance scheduling approach. Such scheduling approach contributes to clandestine experimentation into individual substances within a class of drugs with similar pharmacological profiles or perhaps yielding substances of increased potency. In the USA, continued designer exploration has resulted in Controlled Substances Analog Act legislation to upgrade the penalties associated with clandestine use of all compounds of a structural series. Thus, identification of new ring-substituted amphetamines and other designer drugs is essential and a significant task for forensic laboratories.

1.2. Pharmacology:

1.2.1. History:

In 1910, two German chemists, G. Mannish and W. Jacobson, first synthesized MDA [Freudenmann et al., 2006]. In the late 1930s animal studies of the pharmacological properties of MDA showed that this compound had CNS stimulatory activity, sympathomimetic effects, and convulsions at high doses [Gunn et al., 1939]. MDA has been patented as an appetite suppressant, antitussive and as an ataractic [Shulgin & Nichols, 1978].

Two years later (1912) MDMA was first synthesized, and was patented as a potential appetite suppressant by Merck pharmaceuticals. However the patent expired years later, without MDMA being commercially available. In the 1970s the behavioral effects and lethal dose of MDMA were determined on several animal species [Hardman et al., 1973] and before the end of the decade the first comprehensive report of the pharmacological actions of MDMA in humans was reported [Shulgin & Nichols, 1978]. These reports showed that MDMA produced "an easily controlled altered state of consciousness with emotional and sensual overtones" and was suggested that MDMA might be useful as an adjunct in psychotherapy. In the mid 1980s the Drug Enforcement Administration (D.E.A.) in the United States placed MDMA on schedule I of controlled substances and thereby, restricted its use. This resulted partly from the controversy surrounding its' use, abuse potential and potential for toxicity that was raised by the US media [Lawn, 1986; Hegadoren et al., 1999].

In addition to MDMA, MDA was also added to the schedule I of controlled substances. Since that time there was an increase in custom drug design and synthesis of a number of derivatives of MDMA and MDA in clandestine laboratories; without expensive or bulky equipment, mainly based in some Europe countries like Belgium and Holland [Freudenmann et al., 2004]. The motivation for custom designing was to avoid different law enforcement agency detection and prosecution since it often takes years for the government to detect, classify, and pass laws against a designer drug of abuse. One example, in the United States, the popularity of MDEA (the N-ethyl analog of the controlled substances MDA) started to rise only after the placement of MDMA on Schedule I. MDEA popularity continued to grow until the Control Substance Analog Act legislation in 1986, which outlawed the sale of analogs of controlled substances [Beck, 1990]. Consequently many isomers of illicit drugs based on their structural similarity were controlled rather than scheduling each drug individually. However in Germany MDEA remained legal until 1991 and until 1993 in Holland [Freudnmann et al., 2004].

Another focus of drugs designed by clandestine laboratories was to improve an existing designer drug by ether increasing the drugs potency or by removing unwanted adverse effects.

Today MDEA, MDMA, MBDB and MDMMA are listed along with other ring-substituted amphetamines on Schedule I according to the Control Substance Act in the USA, Schedule III Control Drugs and Substance Act in Canada, and class A according to the Misuse of Drugs Act in the UK, and on similar lists in other countries all over the world.

Table 2: Milestones, from the history of MDMA, MDA, MDEA and MBDB. [Freudenmann et al., 2004].

Date	Event
1909-1910	German chemists C. Mannich and W. Jacobsohn first synthesized MDA.
1912-1914	MDMA first synthesized in 1912 at pharmaceutical company Merck, as an intermediate byproduct in a chemical pathway for a styptic agent; patent assigned to Merck April 27th 1914.
1953-1954	First formal animal study in five species using MDMA and seven other psychotropic drugs (University of Michigan); secret, US army-sponsored study, unpublished until 1973
1960	First regular scientific paper on MDMA (in Polish) describing an MDMA synthesis.
1978-1980	MDEA first mentioned in scientific publications by A. Shulgin and co-workers.
1984	MDMA's street name 'ecstasy' was coined in California
1985-1988	MDMA listed as a schedule I controlled substance in the US; MDEA surfaced as a legal substitute ("designer drug"), but became schedule I on august 13th 1987 as well in the US.
1986	Mass production of ecstasy in Belgium, Holland, Germany and Poland.
1987	First report of fatalities after polydrug intoxication including MDEA [Dowling et al.]
1996	First report of fatal MDEA mono-intoxication [Iwersen & Schmoldt]
2001	The FDA approved controlled clinical trials of MDMA in psychotherapy of Posttraumatic Stress Disorder (PTSD).
2003	MDA, MDMA, MDEA, MBDB and MDMMA were listed on the most restrictive category of abused substances in the USA (schedule I), UK (Class A) and banned in most others countries.

1.2.2. Pharmacodynamics:

It has been reported that MDA, MDMA and MDEA produce very similar central and peripheral effects in humans, with differences in potency, time of onset and duration of action. The central effects are described as an easily controlled altered state of consciousness, with heightened sense of well being, increased tactile sensations, 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]. The pharmacologic profile of the 3,4-methylenedioxyphenylalkylamines are significantly different from other drugs of abuse thus 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 and "gen" meaning to produce and the Latin root "*tactus*" for touch. Hence, the connotation of entactogen is that of producing a "touching within", an indication of the drug's ability to promote inward reflection and positive self-assessment. The 3,4-methylenedioxyphenylalkylamine compounds that include MDA, MDMA, MDEA and MBDB do not fit the pharmacological profile of either psychomotor stimulants such as amphetamine or phenethylamine hallucinogens such as mescaline. On the other hand, MDMA and MDEA and related compounds do have stimulant similarities to both amphetamine and mescaline.

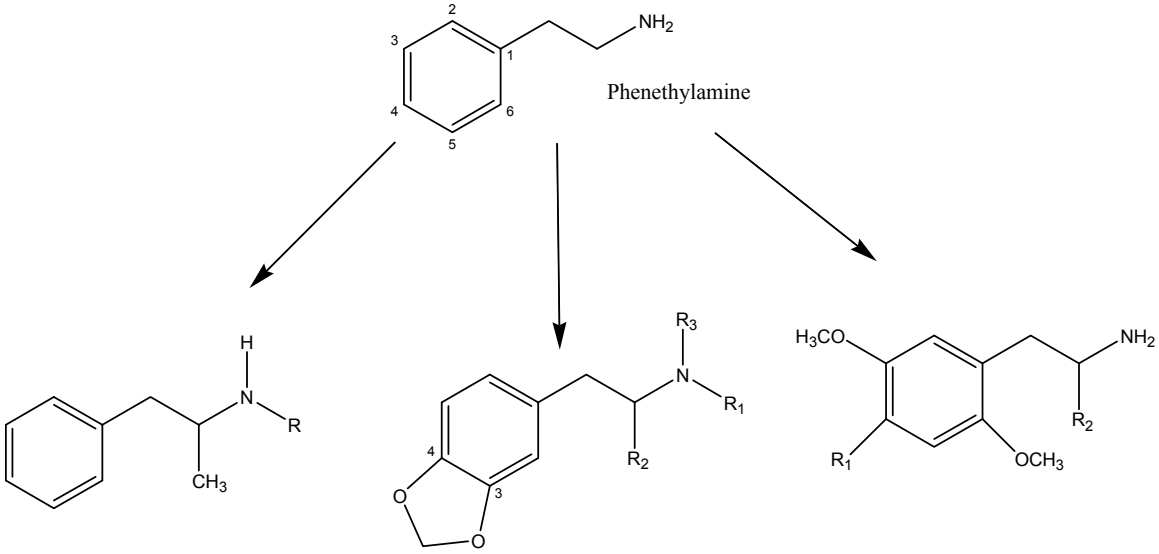
The peripheral effects of MDA, MDMA MDEA are for the most part sympathomimetic in nature and is mediated by the release of catecholamine

neurotransmitters. Studies shown these drugs cause a number of effects on the human body, among them are an increased heart rate, elevated systolic and diastolic blood pressure, mydriasis, tremor palpitation [Tancer et al., 2001; Harris et al., 2002], diaphoresis, increased salivation, tightened jaw muscles and grinding of teeth [Hegadoren et al., 1999] and cause a number of adverse effect. Reports of drowsiness, muscle aches, general fatigue and depression, difficulty in concentrating, paranoia and short-lived anxiety and irritability have been seen [Hegadoren et al., 1999], in addition to dryness of the mouth and/or throat [Hernandez-Lopez et al., 2002].

Chemically, these and all other ring-substituted amphetamines, amphetamine and some hallucinogens consist of an aromatic ring and an aliphatic side chain, with a chiral carbon in the alpha position to a nitrogen atom (Figure 1). Also ring-substitute phenethylamine are chemically similar to some neurotransmitters mainly noradrenaline (NE, norepinephrine), dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT). These phenethylamines interact with the associated transmitter system in the central nervous system (CNS). Amphetamines show a psychomotor stimulation by a net release of dopamine due to a reverse transport in the plasma membrane of the dopamine transporter [Freudnmann et al., 2004].

MDEA, MDMA and MDA evoke their effect by several different mechanisms; they act mainly by indirect serotonergic mechanism in the central nervous system [Nichols, 1986; Kalant, 2001; Crespi et al., 1997; Freudenmann et al., 2004]. These substances also release noradrenaline and dopamine from intracellular stores [Rothman, 2001]. Studies have also shown that ring-substitute phenethylamines trigger a net release of 5-hydroxytryptamine and inhibit its re-uptake in the serotonin system [Kalant, 2001;

Freudenmann et al., 2004]. MBDB also has been found to increase the concentration of dopamine, serotonin, and noradrenaline but to a smaller degree than both MDMA and MDEA [Meyer et al., 2009].



Amphetamines (stimulants)	Entactogens	Phenethylamine hallucinogens
Amphetamine (R=H)	MDA (R_1= R_3=H, R_2=CH_3)	DOM (R_1= R_2=CH_3)
Methamphetamine (R=CH_3)	MDMA (R_1= R_2=CH_3, R_3=H)	DOE (R_1= C_2H_5, R_2=CH_3)
	MDEA (R_1= C_2H_5, R_2=CH_3, R_3=H)	DOB (R_1= Br, R_2=CH_3)
	MBDB (R_1= CH_3, R_2=C_2H_5, R_3=H)	DOI (R_1= I, R_2=CH_3)
	MDMMA (R_1= R_2=R_3=CH_3)	

Figure 1: Structural similarity of phenethylamine and its derivatives [Freudenmann et al., 2004].

At the molecular level, MDEA, MDMA and MDA's primary site of action is a serotonin transporter (SERT), a membrane-bound protein in the 5-hydroxytryptamine

vesicles and presynpatic plasma membrane, acting as a substrate-type 5-hydroxytryptamine releaser [Rothman et al., 2002]. SERT is also the site of action of selective serotonin reuptake inhibitors (SSRIs) antidepressants. Causing major drug-drug interaction when ring-substituted phenethylamines are co-administered with SSRI antidepressants. For example when MDEA is given with fluoxetine, it may lead to serotonin syndrome and/or arterial hypertension [Freudenmann et al., 2004; Maurer, 2000]. Among other effects on the biological system, ring-substituted phenethylamines have been shown to inhibit monoamine oxidases [Leonardi et al., 1994]; they also have weak intrinsic activity, acting as a direct agonist of 5-hydroxytryptamine, alpha, beta, and dopaminergic receptors subtypes [Freudenmann et al., 2004]. A mild hallucinogenic effect of ring-substitute phenethylamines can be due to their affinity to the postsynaptic 5-hydroxytryptamine subtype-2 receptors.

1.2.3. Metabolism and Elimination:

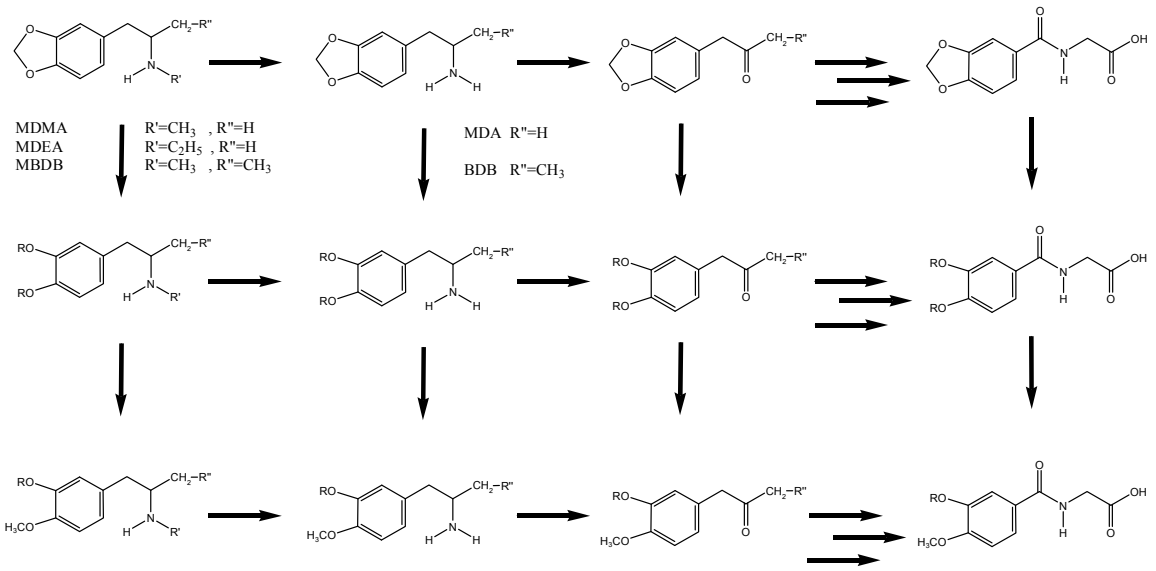
The metabolic pathways that methylenedioxyphenylalkylamines include O-demethylation of the methylenedioxy group yielding a dihydroxy derivative followed by methylation of one of the hydroxy groups and successive degradation of the side chain to N-dealkyl and deamino-oxo metabolites [Ensslin et al., 1996; Maurer, 1996]. The propanamines MDA, MDMA and MDEA also are metabolized to glycine conjugates of the corresponding 3,4-disubstituted benzoic acids (hippuric acids), unlike the butanamine MBDB. The hydroxy metabolites are excreted as glucuronic acid and/or sulfate

conjugates [Maurer et al., 2000]. Demethylation of methylenedioxyphenylalkylamines to the toxic catechols is mainly catalyzed in humans and rats by CYP2D6 isoenzymes.

In humans, MDMA can be demethylated by the isoenzymes CYP1A2. N-demethylation of MDMA is predominantly catalyzed in rats and in humans by the isoenzyme CYP1A2; however the N-deethylation of MDEA is by the isoenzymes CYP3A2/4 [Maurer et al., 2000]. A major metabolite of MDMA is 4-hydroxy-3-methoxymethamphetamine (HMMA). The HMMA metabolite can be detected in both plasma and in urine samples [Helmlin et al., 1996].

MDA, formed by N-demethylation of MDMA, appears to be a minor metabolite, representing less than 10% 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]. A major metabolite of MDA is α-methyldopamine which is readily oxidized to the ortho-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 were tested for effects on depletion of 5-HT and found to cause long term depletion[Miller et al., 1997; Bai et al., 1999].

The metabolism of MBDB is comparable to MDMA/MDEA. In phase I, it is N-dealkylated to its active metabolite 3,4-methylenedioxybutanamine (BDB) by the isoenzymes CYP1A2. MBDB can also undergo demethylenated to the corresponding dihydroxy compound 1,2-dihydroxy-4-[2-(methylamino)butyl]benzene (DHMBB) followed by conjugation in phase II [Maurer,1996; Meyer 2009; Kronstrand,1996; Kintz 1997; Maurer et al., 2000].



(Phase I: R=H, Phase II: R= Sulfate or Glucuronic acid)

Scheme 1: General scheme of the proposed metabolism of the MDA, MDMA, MBDB and MDEA in humans and rats.

1.3. Toxicology:

1.3.1 Acute Effects:

Deaths linked to MDMA and MDEA misuse were first reported in the United States in the 1980s [Carter et al., 2000]. The use of such substances as mood enhancers has steadily increased, due to the erroneous belief that MDMA, MDEA and other ring-substituted amphetamines are relatively safe drugs. Most cases of MDMA and MDEA toxicity result in mild symptoms that can include: agitation, hypertension, tachycardia, mydriasis, trismus, and diaphoresis. Fatigue and difficulty concentrating are also commonly reported symptoms. Occasionally, intense dysphoria (severe depression,

anxiety), confusion or delirium can occur. These effects have been widely reported for MDMA and to a lesser extent for MDEA; data currently available for MBDB concerning toxicity are very limited [Meyer et al., 2009]. This may be attributed to the increased popularity of MDMA and users do not usually consume MBDB alone but more often ecstasy pills contain a mixture of MBDB and other ring-substituted phenethylamines.

Severe overdoses due to ecstasy appear to follow a clear pattern of toxicity characterized by hyperthermia, hyperkalemia, acidosis, dysrhythmias, disseminated intravascular coagulation, rhabdomyolysis, seizures, and acute renal failure. Ecstasy-associated morbidity and mortality have been related to hyponatremia, dehydration, hyperthermia, hypertensive crisis, and cardiac dysrhythmias [Micromedex® Healthcare Series].

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

A wide range of possible acute complications are associated with use of ring-substituted amphetamines ranging from psychiatric disorders to life threatening medical conditions. One complication of MDEA is it can trigger acute psychotic disorders leading to anxiety and panic attacks [Iwersen et al., 1996; Gouzoulis et al., 1993; Hermle et al., 1993].

The use of MDEA and MDMA may also cause a condition called the “serotonin syndrome” [Sternbach, 1991] a serious medical conditions that require rapid treatment.

This syndrome includes an elevated body temperature (hyperthermia), intense physical activity and a hot environment contribute to the life threatening increase of body temperature [Freudenmann et al., 2004]. Body temperatures in excess of 43.8ºC have been reported due to the toxic action of MDMA [Green et al., 2004]. MDMA and MDEA also involve hyperrflexia and tremors (neuromuscular), in addition to hyperactivity, agitation and confusion (psychopathologic) and often nausea and vomiting (gastrointestinal). Severe cases of the syndrome can lead to rhabdomyolysis, disseminated intravascular coagulation and renal failure [Freudenmann et al., 2004].

Cardiovascular complications of MDEA use include arterial hypertension, tachycardia, arrhythmia and acute heart failure [Freudenmann et al., 2004]. One study found a noticeable increase in systolic blood pressure and heart rate after administration of MDMA [Mas et al., 1999]. MDEA produced a similar response for systolic blood pressure and heart rate, however with a lower increase in diastolic blood pressure [Gouzoulis et al., 1993]. Other reported complications included respiratory failure, cerebral seizures, convulsions and hepatitis [Freudenmann et al., 2004].

Since clandestine laboratories are the major source of different ring-substituted amphetamines, users may concomitantly consume substances even more toxic than the supposed drug or may ingest doses greatly higher than "labeled."

1.3.2. Chronic Effects:

There are no definitive data describing the chronic effects of ring-substituted amphetamines, due to the absence of follow-up data from survivors of acute toxicity with

MDEA and MBDB among other ring-substituted amphetamines [Hegadoren et al., 1999]. One reason is many ring-substituted amphetamines (MDMA, MDEA, MBDB and MDMMA) are not usually found in a pure form, but as mixtures of different amphetamine analogs. Although some studies have predicted the possibility of neurotoxic effects in humans, due to long term effects of MDMA [McCann et al., 1994], a study by Morland (2000) concluded that teenagers do seem to have a higher risk to neurotoxicity than adult users of ecstasy. In addition Morland maintains that it is still not clear yet if the long term effect of ecstasy use are either revisable or dose-depended. Thus the potential long-term effects in the CNS, renal, liver function or other systems in the body remain unknown.

Concern has been expressed regarding the use of ring- substituted amphetamines during pregnancy; there are only a few studies on the effect of ecstasy on human pregnancy. Its use may lead to teratogenic effects including congenital heart disease and malformation [Freudenmann et al., 2004]. A clinical study conducted by the UK National Teratology and In-formation Service (NTIS) collected prospective follow-up data between the years 1989 to 1998 on 136 pregnancies following primarily 1st trimester exposure to ecstasy. Of the remaining 78 live born infants – 35% of these women had elective terminations and 10% had miscarriages - over 15% had congenital malformations, which is 5 -7 fold higher than the expected incidence of 2 -3% [Craig, 2001]. There have been equally few animal studies published on the teratogenic potential of ecstasy and these results have been mixed [Craig, 2001]. No specific information on the relative teratognicity of MDMA, MDA, MDEA, MBDB and MDMMA are available.

Substance dependence of MDMA and MDEA are considered to be low, this was based on the observation that discontinuation of the substance showed a lack of tolerance and withdrawal effects. This was also supported by the consumption patterns (often used on weekends only and self-limiting) [Freudenmann, 2004]. Animal studies show that the dependence potential of MBDB appears to be small, probably even smaller than that of MDMA [EMCDDA, 1998].

2. OBJECTIVES AND RATIONALE

Methylenedioxyphenethylamines such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) are structurally similar to amphetamines and mescaline (a psychedelic phenethylamine). All three compounds are known to produce very similar peripheral and psychoactive central effects in humans with slight differences in time of onset, potency and duration of action [Braun et al., 1980 and Hegadoren et al., 1999]. The homologous primary amine, 3,4-methylenedioxyphenylbutanamine (BDB), has both hallucinogenic and stimulant effects [Bronson et al., 1995]. N-methyl-BDB (MBDB) has been reported to have novel central nervous system (CNS) effects with neither stimulant nor hallucinogenic properties [Nichols et al., 1986]. MBDB and MDMA are reported to be generally similar in effect with slight differences in potency [Nichols et al., 1986]. It has been suggested that the 3,4-methylenedioxyphenyl-alkylamines represent a novel class of pharmacological agents, labeled entactogens, a term used in reference to the drugs' ability to promote inward reflection and positive self-assessment [Kalant, 2001]. This set of compounds, which includes MDA, MDMA, 3,4-methylenedioxy-N,N-dimethylamphetamine (MDMMA), MDEA, and N-methyl-3,4-methylenedioxy-

phenylbutanamine (MBDB), do not fit the pharmacological profile of either phenethylamine hallucinogens or psychomotor stimulants. Indeed, MDEA, MDMMA and MBDB have already appeared as components of street drug samples.

Mass spectrometry is usually the confirmatory piece of evidence for the identification of drugs in forensic and other regulatory laboratories. However, there are many isomeric compounds that produce essentially equal mass spectra making the differentiation of target drug from imposter molecule(s) a challenge in many analytical situations. These compounds are usually either positional isomers "regioisomers" in the alkyl side chain or in the aromatic ring substitution pattern or compounds of isobaric relation "isobaric compounds" (same nominal mass with different elemental composition).

In forensic drug chemistry the need for the identification of the different isomeric compounds and regioisomeric differentiation has been addressed in a number of drug categories [Awad et al., 2005; 2006; 2007; 2008]. Ring and side chain regioisomers of ethoxyphenethylamines are considered isobaric to MDEA, MDMMA, and MBDB. Combination of the three possible positions of the ethoxy group on the aromatic ring along with the three regioisomeric side chains yielded all nine structural possibilities related to MDEA, MDMMA and MBDB (Figure 2). The compounds in this set of phenethylamines all have molecular weight of 207 with the potential to produce a mass spectrum with a major fragment ions at *m/z* 72 for the side chain imine fragment $(C_4H_{10}N)^+$. The 3,4-methylenedioxybenzyl fragment $(C_8H_7O_2)^+$ is isobaric to the ring substituted ethoxybenzyl fragment $(C_9H_{11}O)^+$, both yielding *m/z* 135. When other compounds exist with the ability to produce nearly identical mass spectra as the drug of

interest, the identification by gas chromatography–mass spectrometry (GC–MS) must focus on the ability of the chromatographic system to separate the imposter molecules from the drug of interest. While nuclear magnetic resonance (NMR) can be a useful method for differentiation of these regioisomers, it is not a technique with direct application for all areas of regulatory analysis. Most forensic drug samples are not of sufficient purity for direct NMR analysis and NMR is not usually applicable to the analysis of drugs in biological samples. Thus, the analysis of these drugs must depend heavily on chromatographic methods. Mass spectral similarities along with possible co-elution, under identical chromatographic conditions, make the differentiation of the controlled substance from imposter molecules a significant challenge to forensic chemists. The lack of reference materials further complicates the problem. Also, the retention properties and resolution of analyzed compounds depend mainly on the column efficiency, composition, dimensions, temperature programming, etc.

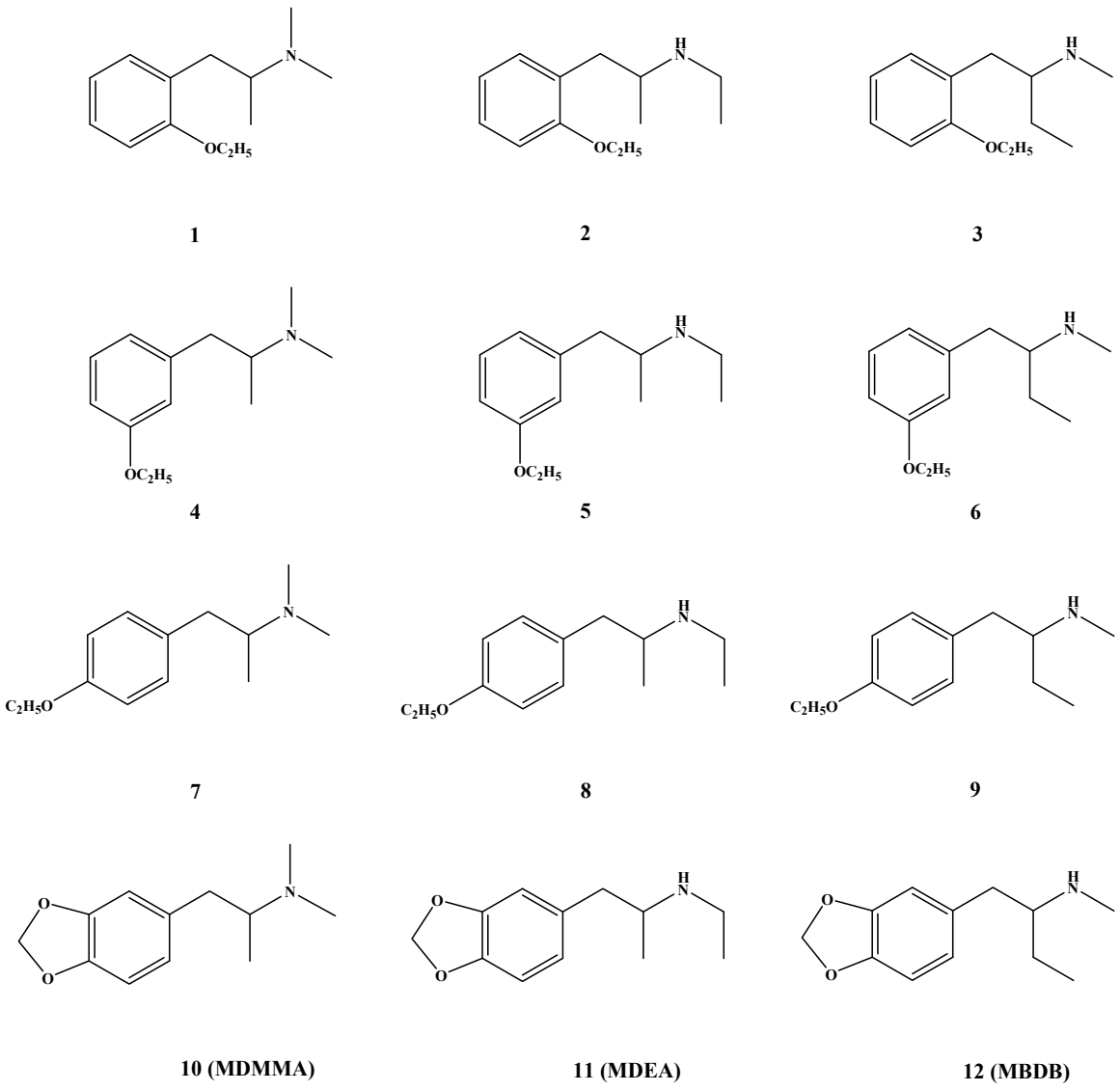


Figure 2: Structures of the regioisomeric and isobaric compounds in the study.

Previous reports [Aalberg et al., 2003] described the synthesis and mass spectral evaluation of methylenedioxy ring and side chain regioisomeric compounds related to MDEA, MDMMA, and MBDB. It was concluded that the mass spectra of the underivatized compounds provided very little structural information for the specific differentiation among these regioisomers [Aalberg et al., 2003]. Derivatization by

perfluoroacylation individualized the mass spectrum for each compound and allowed, along with chromatographic separation, a specific identification of the target compounds from the regioisomeric and isobaric imposter molecules [Awad et al., 2005].

Infrared spectroscopy is considered a confirmation method for the identification of organic compounds due to the uniqueness of infrared spectra for very similar molecules. GC-FTIR spectroscopy is characterized by scanning quickly enough to obtain IR spectra of peaks eluting from the capillary columns. Thus this technique combines the separation power of GC with the identification power of IR. The GC-IR has been successfully used in the identification of amphetamine isomers [Duncan et al., 1988] as well as methamphetamine and phentermine [Kempfert, 1998]. Recently, GC-IRD has been successfully applied for the differentiation of 10 different compounds of ring and side chain substituted regioisomers and isobaric compounds related to the controlled substance 3,4-MDMA [Belal et al., 2009].

The goal of this project is to develop analytical methods for the differentiation of the nine ethoxyphenethylamines from the isobaric methylenedioxyphenethylamines related to MDMMA, MDEA and MBDB. The synthesis of the nine ring and side chain regioisomers of ethoxyphenethylamines is the initial goal of this project. Gas chromatographic studies will attempt to resolve these regioisomeric and isobaric substances in underivatized and derivatized forms. The secondary amines will be derivatized in an effort to individualize their mass spectra and obtain more specific ions that would help discriminate among these regioisomeric compounds. Additionally GC-IR studies will be evaluated in an effort to establish vapor phase absorption and

regioisomeric structural relationships. GC-MS and IRD studies were done on the ketone precursors of the ethoxyphenethylamines and ketone precursors of the drugs of abuse MDEA, MDMMA and MBDB in an effort to develop additional methods of compound identification.

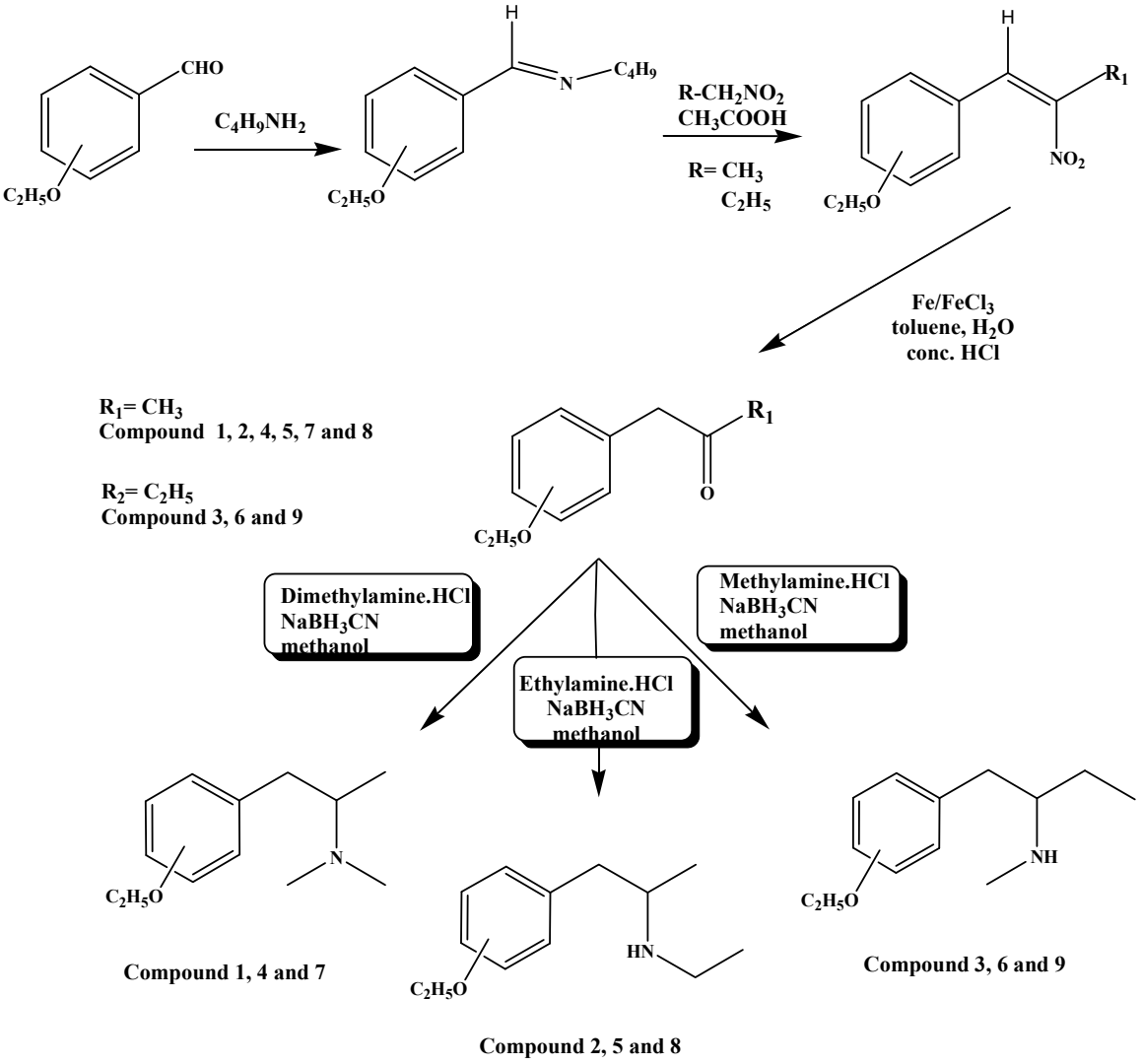
3. RESULTS AND DISCUSSION

3.1. Analytical Studies of Regioisomeric and Isobaric Substances Related to MDMMA, MDEA and MBDB:

3.1.1 Preparation of the Regioisomers:

The structures of all twelve compounds prepared and evaluated in this study are shown in Figure 2. The general method for the preparation of the twelve regioisomeric amines began with the condensation of appropriately substituted aldehydes with n-butylamine followed by reaction with the appropriate nitroalkane (nitroethane or 1-nitropropane), resulting in formation of the corresponding 2-nitroalkenes. Treatment of the 2-nitroalkenes with $Fe/FeCl_3$ in a mixed solvent system of toluene and aqueous acid resulting in the formation of the ketone intermediates. In this reaction the nitro group is first reduced to yield the corresponding 2-aminoalkene, which tautomerizes to the imine which is hydrolyzed to yield the corresponding ketone. The ketones were purified by Kugelrohr distillation. Then reductive amination was carried out by adding sodium cyanoborohydride with the appropriate amine hydrochloride (dimethyl, ethyl or methyl) in methanol, yielding the desired amines which were converted into the hydrochloride

salts using gaseous hydrochloric acid. Scheme 2 shows the general synthesis pathway of ring and side chain regioisomers of ethoxyphenethylamine.



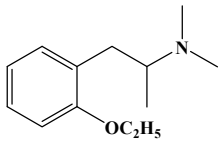
Scheme 2: General synthesis of the ring and side chain regioisomers of the ethoxyphenethylamines.

3.1.2. Mass Spectral Studies:

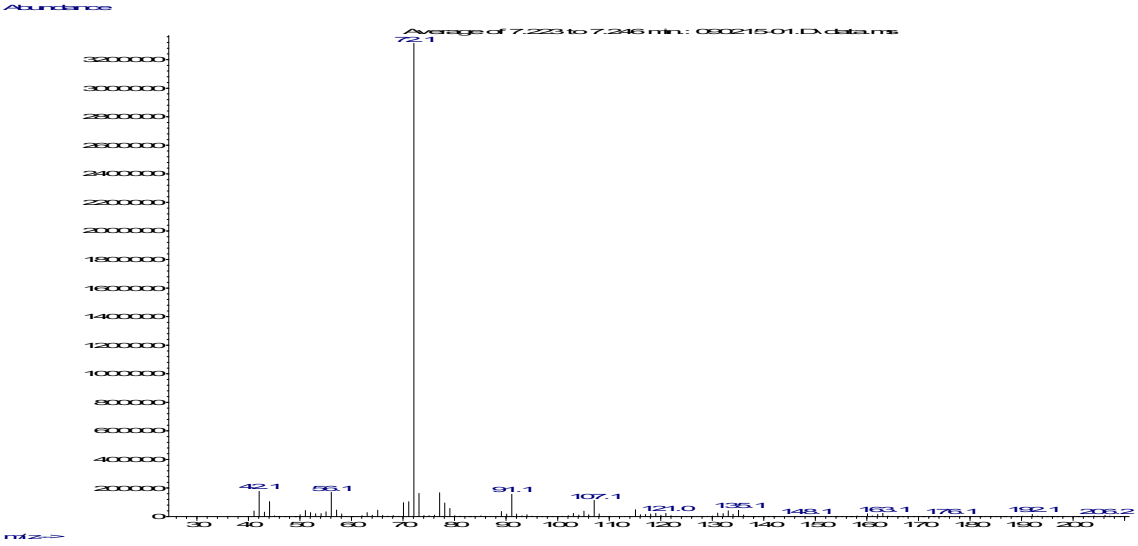
3.1.2.1 Mass Spectral Studies of Isobaric and Regioisomers of MDMMA, MDEA and MBDB:

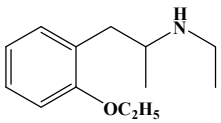
Mass spectrometry is the primary method for confirming the identity of drugs in forensic samples. Figure 3 shows the electron impact mass spectra of all twelve compounds including the three drugs of abuse; MDMMA, MDEA and MBDB. The mass spectra of all regioisomeric compounds (M.W.=207) are characterized by a base peak at *m/z* 72 formed by an α-cleavage reaction involving the carbon–carbon bond of the ethyl linkage between the aromatic ring and amine nitrogen. Other less abundant peaks were observed at *m/z* 135/136 that would represent the ethoxybenzyl and 3,4-methylenedioxybenzyl cation and radical cation fragments respectively as well as other ions of low relative abundance. The general fragmentation pathway for these compounds is shown in Scheme 3. The ultimate identification of any one of these amines with the exclusion of the other eleven regioisomeric substances cannot be based on their mass spectra alone. This lack of mass spectral specificity in addition to the possibility of chromatographic co-elution with any of the reference drugs in this study (MDMMA, MDEA and MBDB) could result in misidentification of the target drug. Furthermore, the lack of available reference samples for the nine regioisomeric ring substituted ethoxyphenethylamines compounds complicates the individual identification of any one of these substances to the exclusion of others. This constitutes a significant analytical challenge, where the specific identification by GC–MS must be based primarily upon the ability of the chromatographic system to separate these regioisomeric imposter molecules from the actual drug of interest. Additionally, the ability to distinguish between these

regioisomers directly enhances the specificity of the analysis for the target drugs of interest.

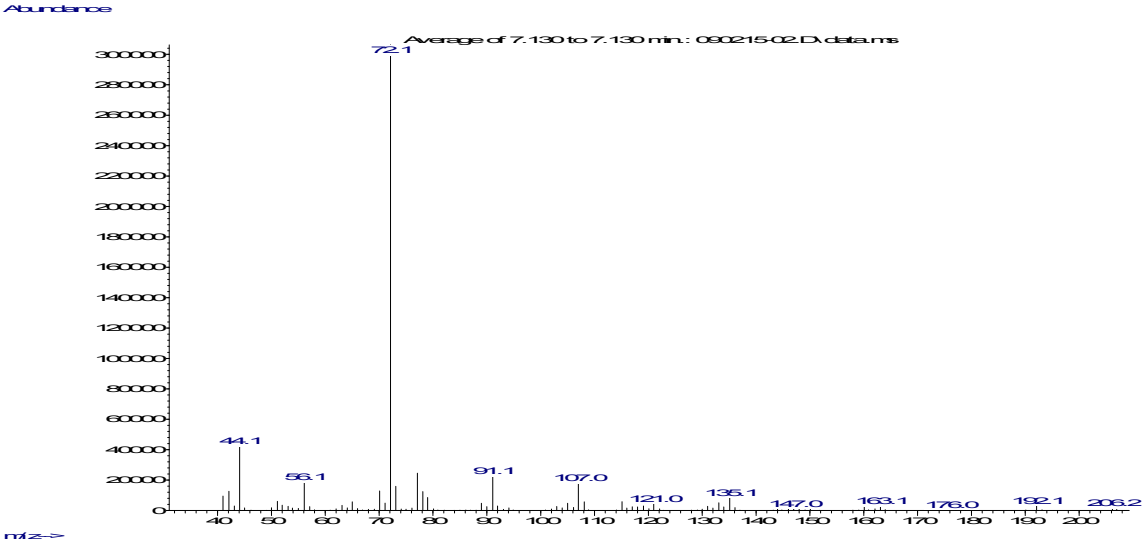


1




H
N
OC2H5

2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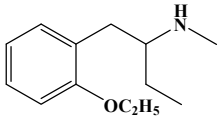
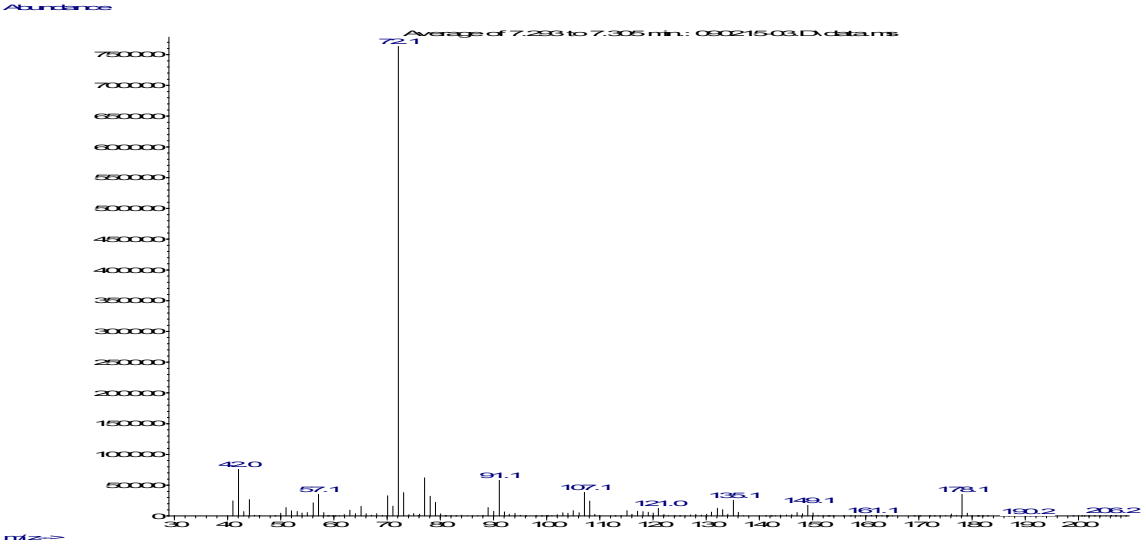
163.1
176.0
192.1
206.2
m/z-->


H
N
OC2H5

3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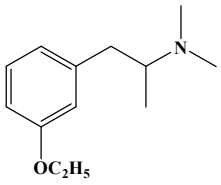
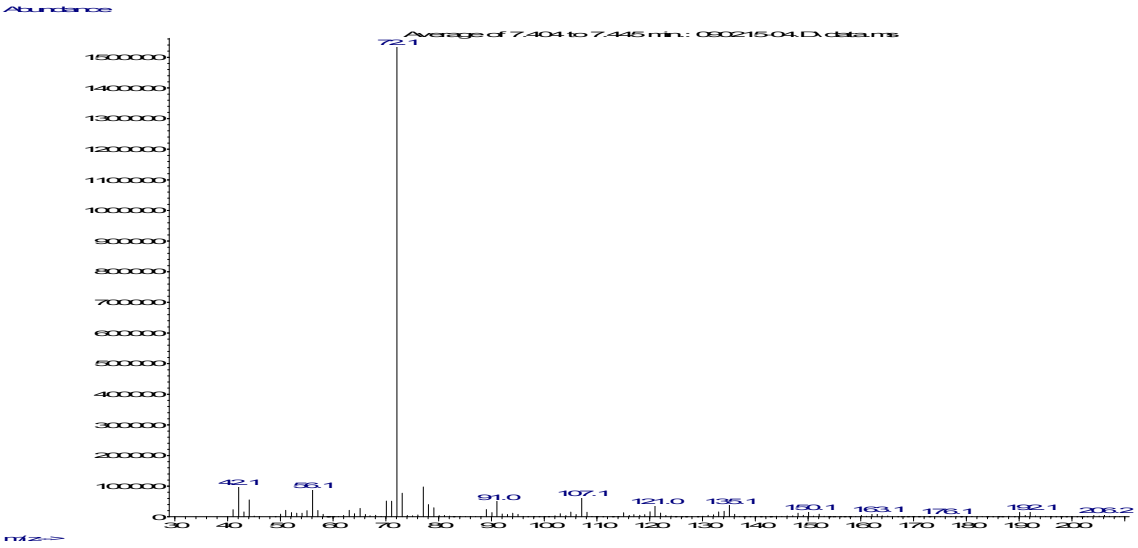
161.1
178.1
190.2
206.2
m/z-->


N
OC2H5

4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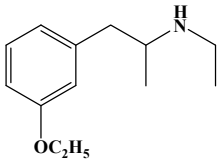
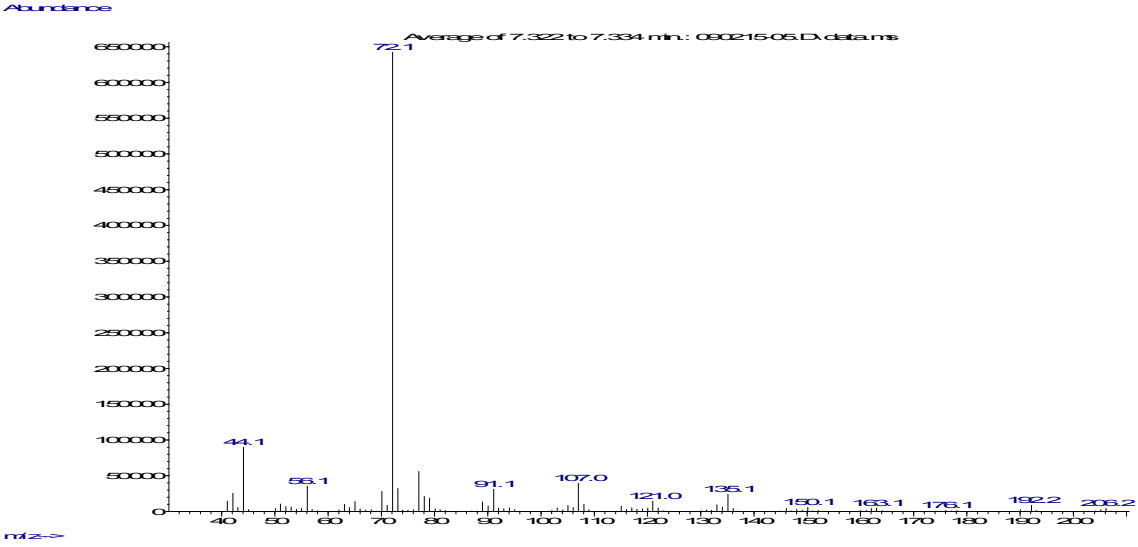
56.1
91.0
107.1
121.0
135.1
150.1
163.1
178.1
192.1
206.2
m/z-->


H
N
OC2H5

5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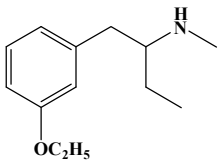
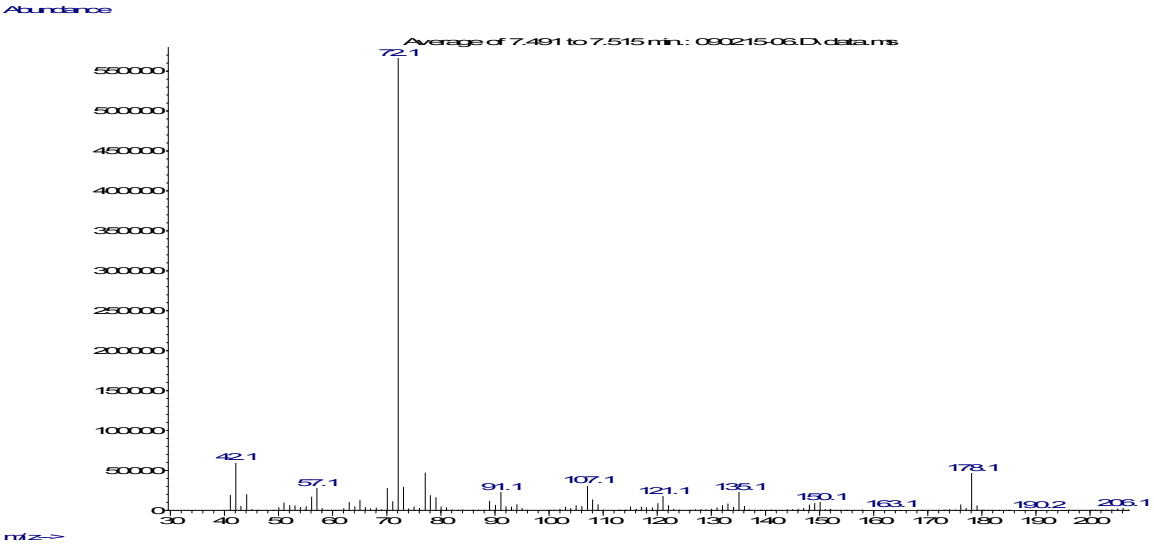
150.1
163.1
178.1
192.2
206.2
m/z-->


H
N
OC2H5

6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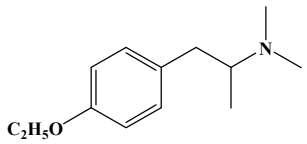
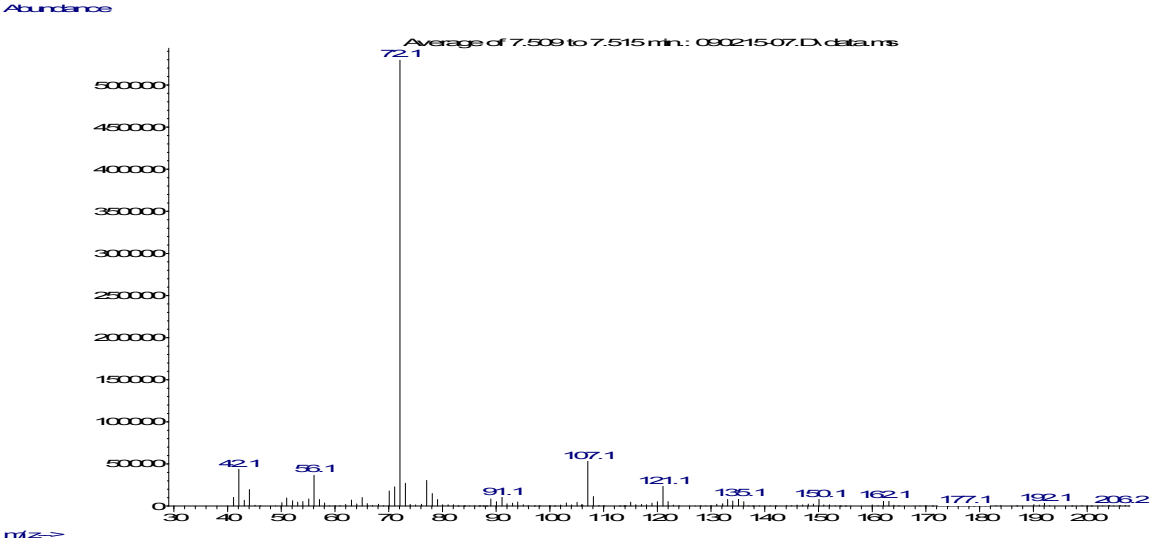
57.1
91.1
107.1
121.1
135.1
150.1
163.1
178.1
190.2
206.1
m/z-->


N
C2H5O

7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91.1
107.1
121.1
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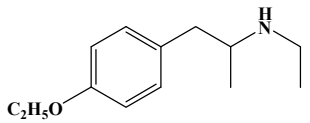
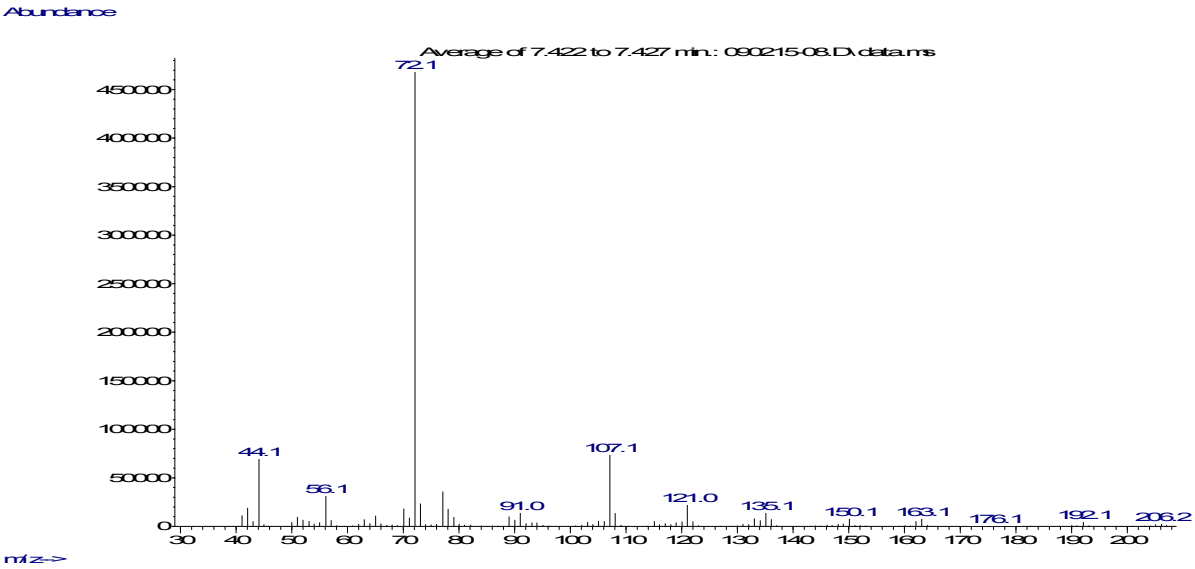
162.1
177.1
192.1
206.2
m/z-->


H
N
C_2H_5O

8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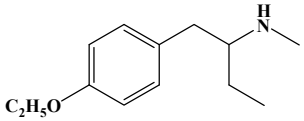
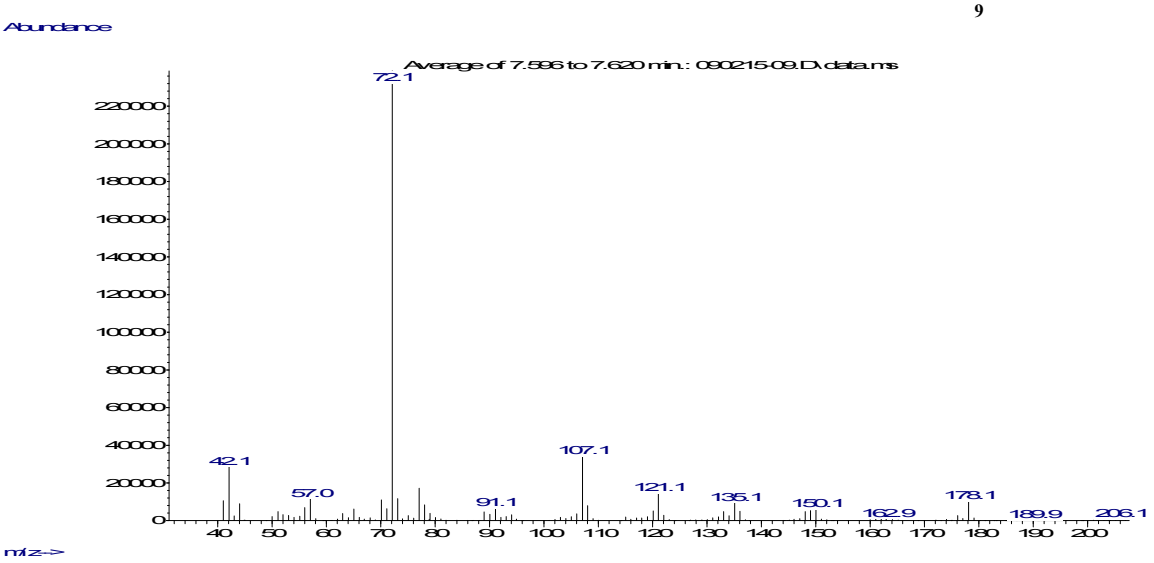
121.0
135.1
150.1
163.1
176.1
192.1
206.2
m/z-->


H
N
C_2H_5O

9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57.0
91.1
107.1
121.1
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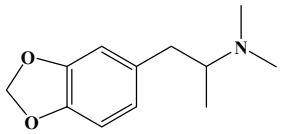
162.9
178.1
189.9
206.1
m/z-->



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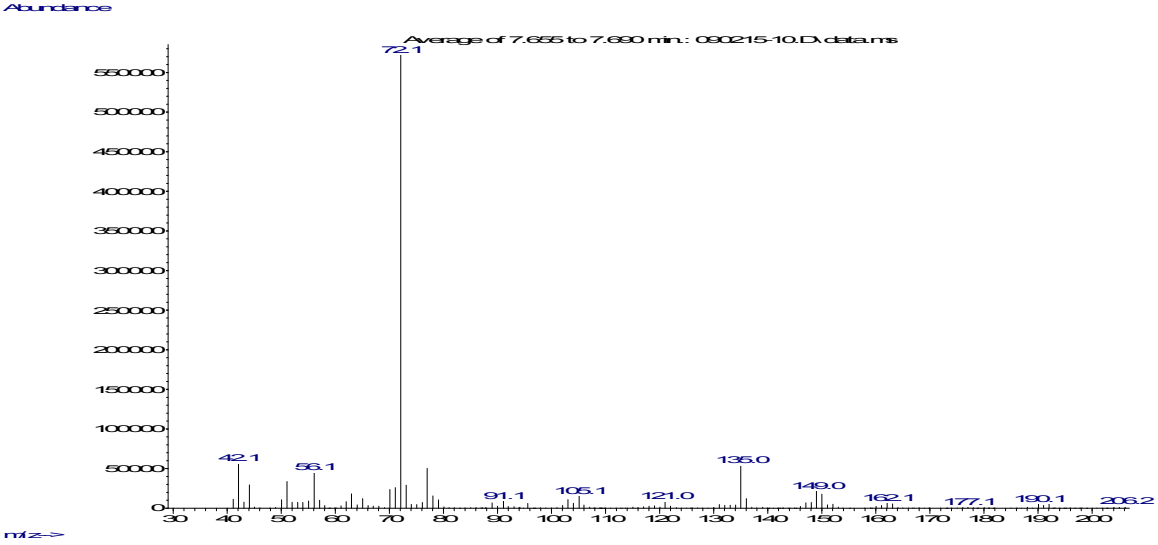
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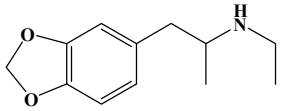
56.1
91.1
105.1
121.0
135.0
149.0
162.1
177.1
190.1
206.2
m/z-->



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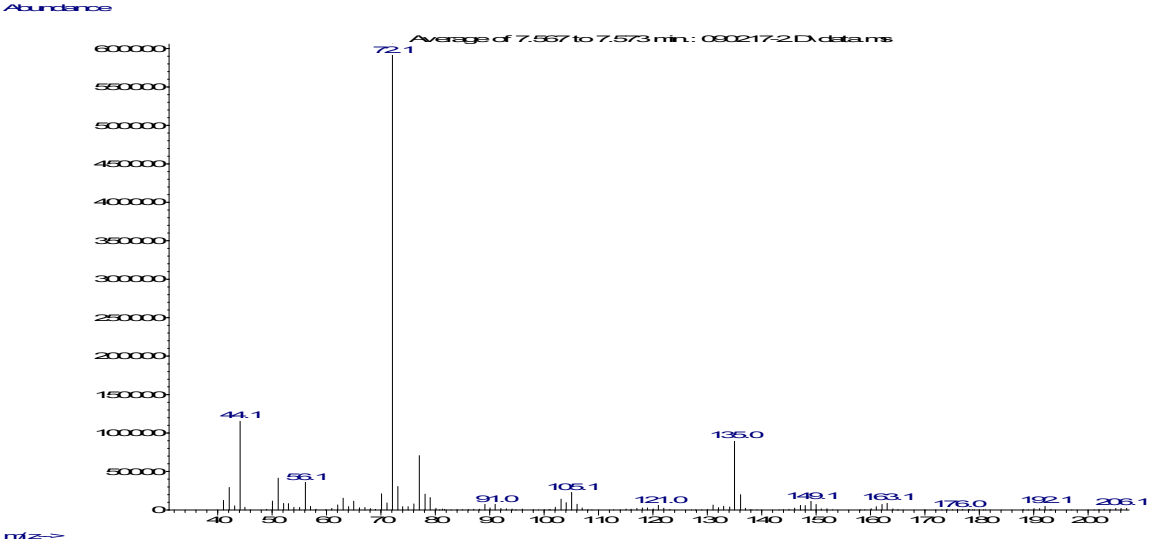
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91.0
105.1
121.0
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163.1
178.0
192.1
206.1
m/z-->

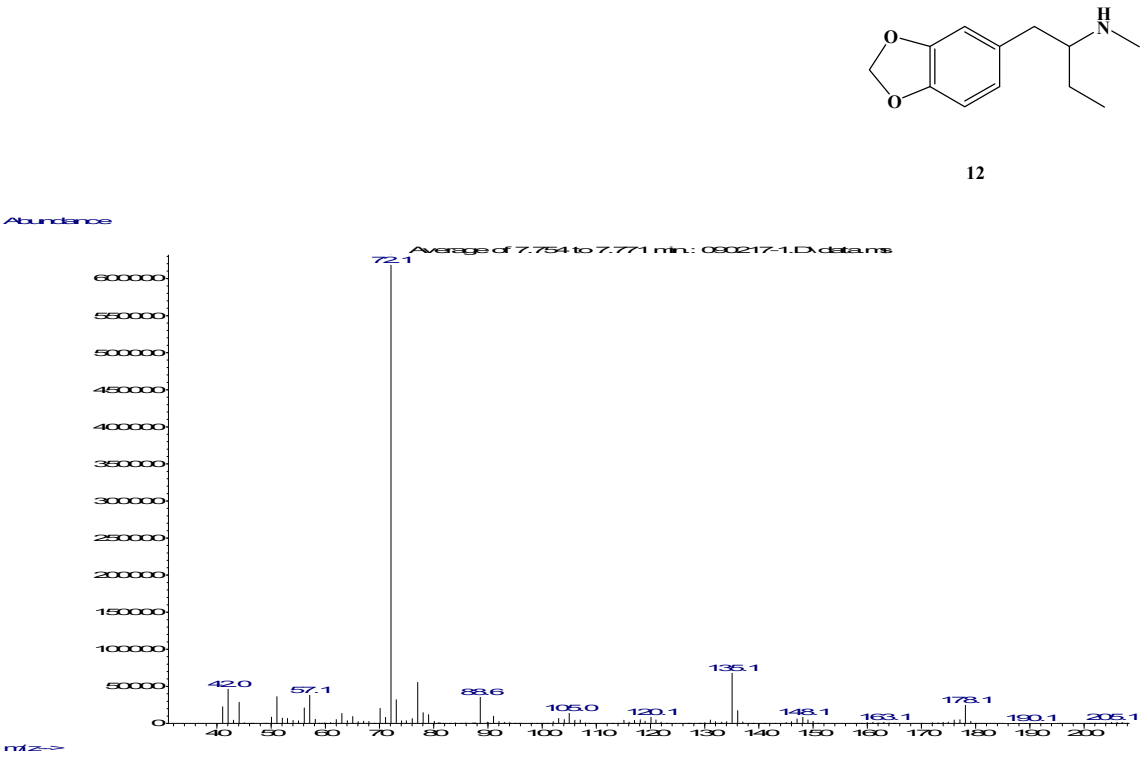
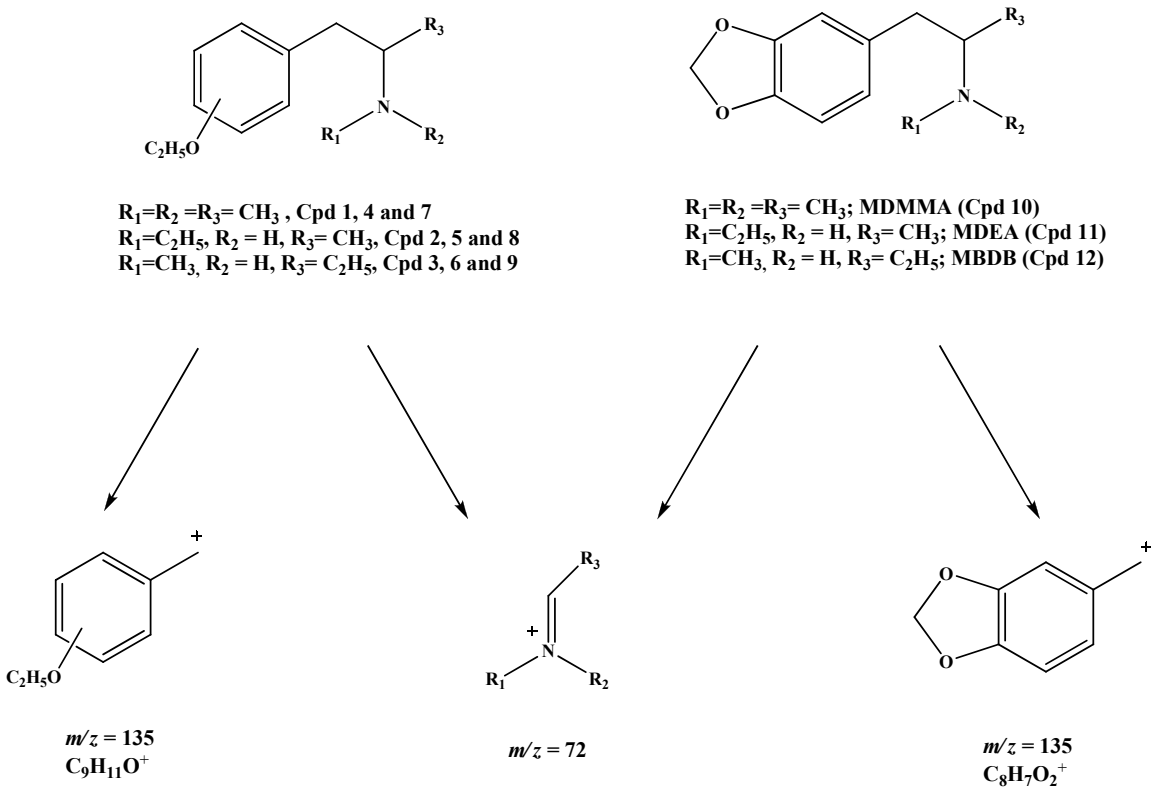


Figure 3: Mass spectra of compounds 1-12.

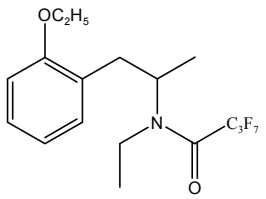


Scheme 3: EI fragmentation pattern for compounds 1-12.

3.1.2.2. Mass Spectral Studies of the Perfluoroacyl Derivatives of the Isobaric and Regioisomeric of MDMMA, MDEA and MBDB:

Various perfluoroacyl derivatives of the regioisomeric secondary amines were prepared (the derivatization procedure is mentioned in section 4.3.) and evaluated in an effort to individualize their mass spectra and to maintain or improve chromatographic resolution. The acylation of the amines significantly lowers the basicity of nitrogen and allows fragmentation pathways to play a more prominent role in the mass spectrum [Awad et al., 2005; 2006; 2007; 2008]. The heptafluorobutryl, pentafluoropropionyl and trifluoroacetyl derivatives of compounds 2, 3, 5, 6, 8, 9, 11 and 12 were evaluated for

their ability to individualize the mass spectra through the formation of unique and specific marker ion fragments. Compounds 1, 4, 7 and 10 are tertiary amines and do not form a stable amide derivative.



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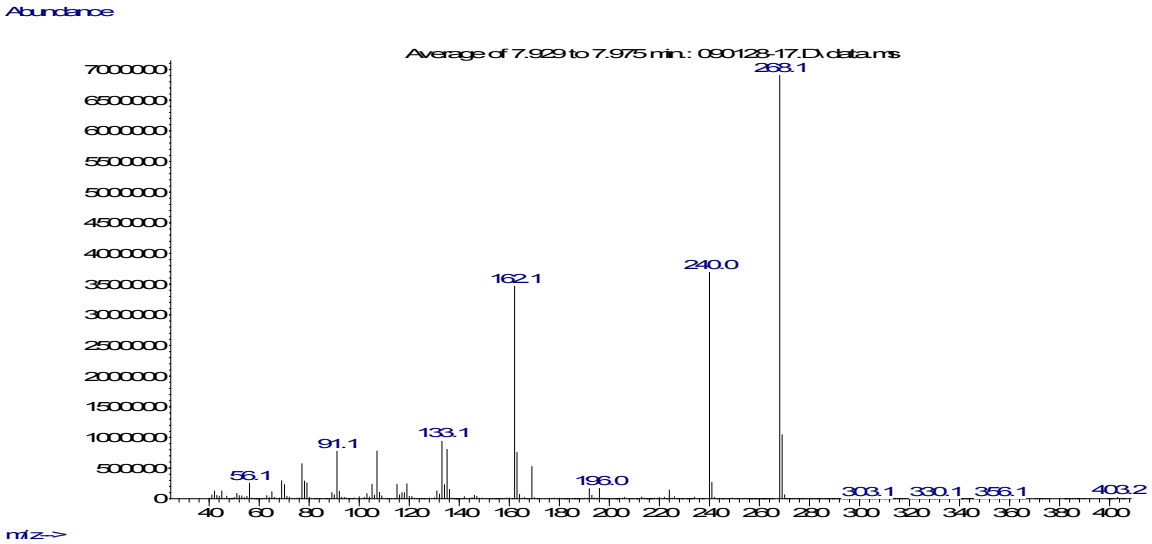
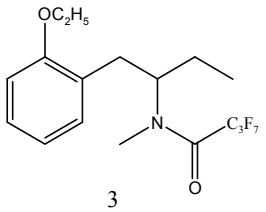
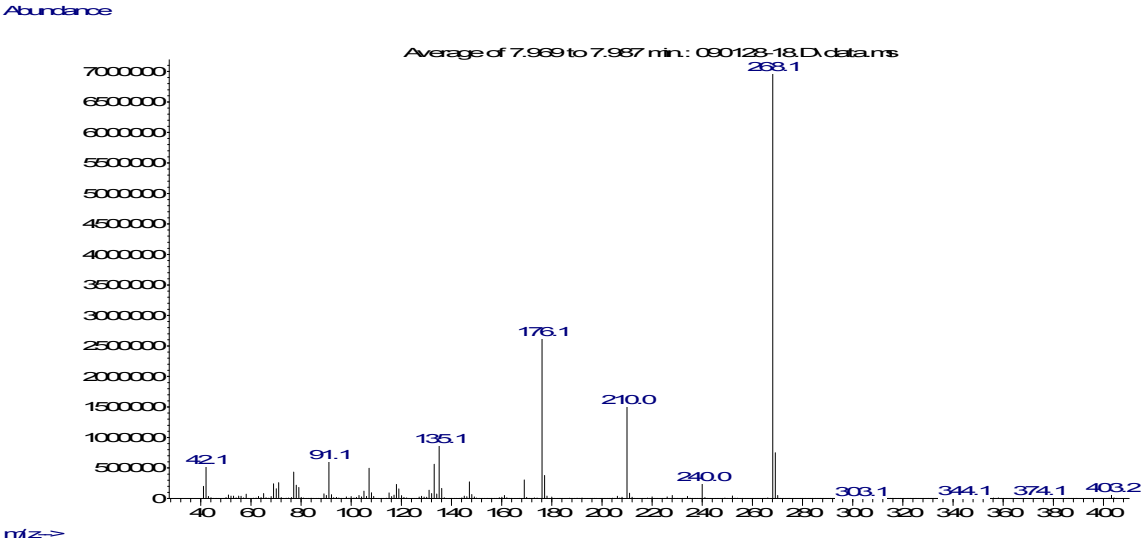



OC2H5
N
C3F7
O

3


Abundance
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210.0
135.1
91.1
42.1
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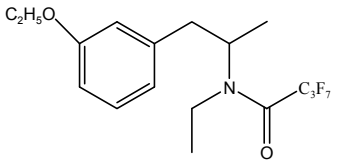
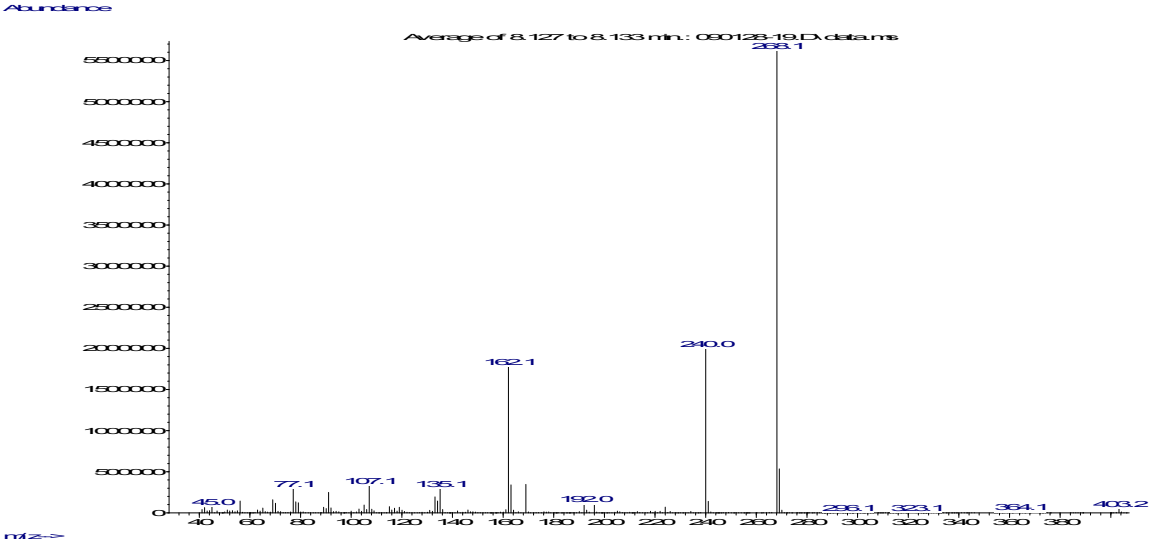
303.1
344.1
374.1
403.2
m/z-->


C2H5O
N
C3F7
O

5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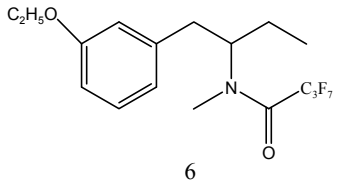
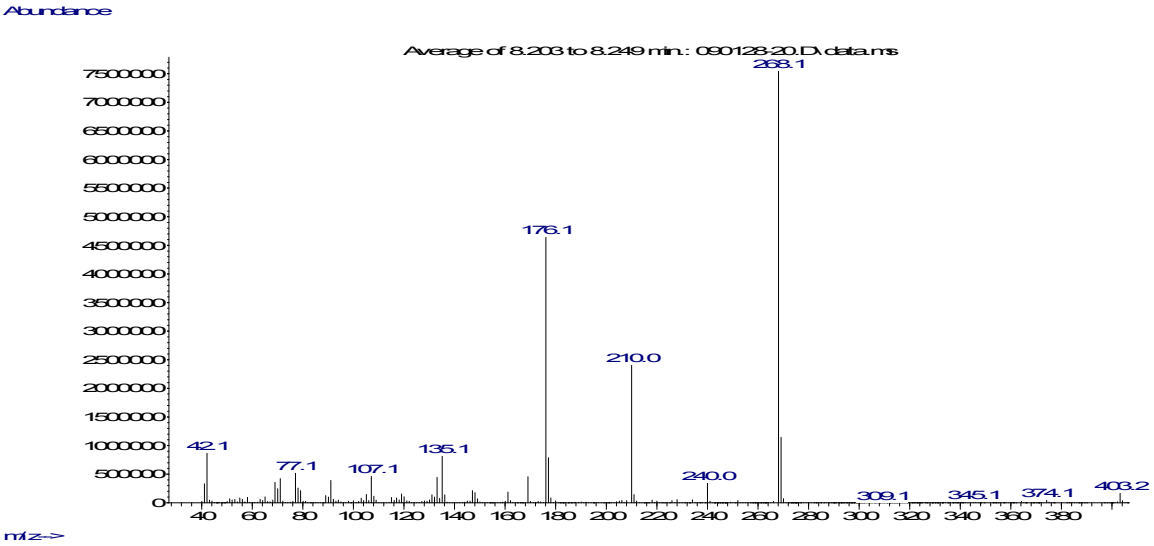
192.0
296.1
323.1
364.1
403.2
m/z-->


C_2H_5O
N
C_3F_7
O

6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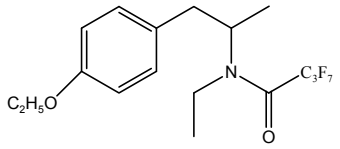
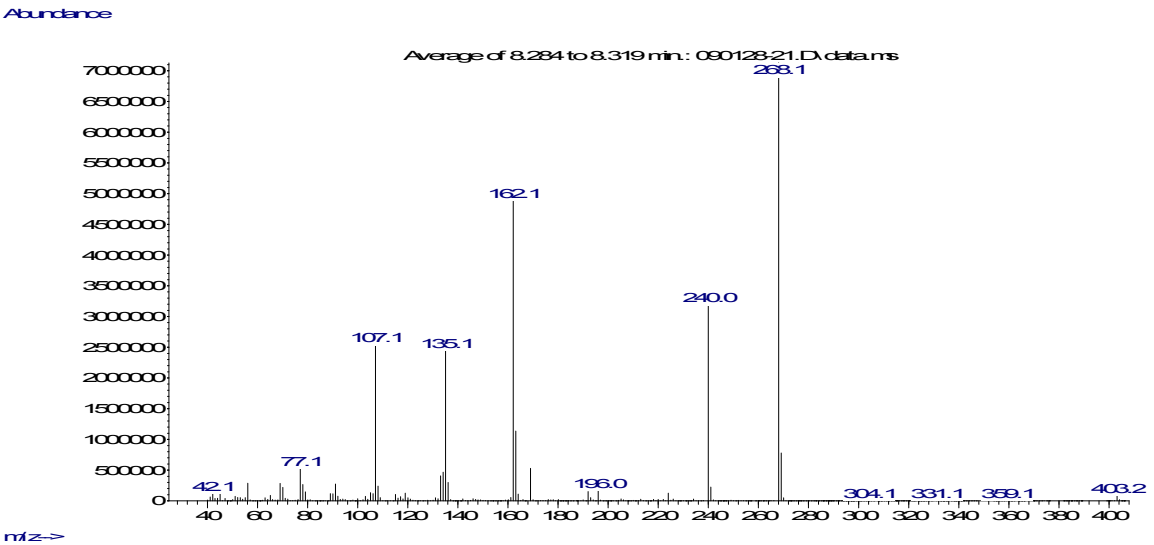
309.1
345.1
374.1
403.2
m/z-->


C_2H_5O
N
C_3F_7
O

8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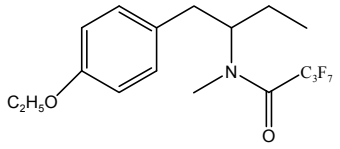
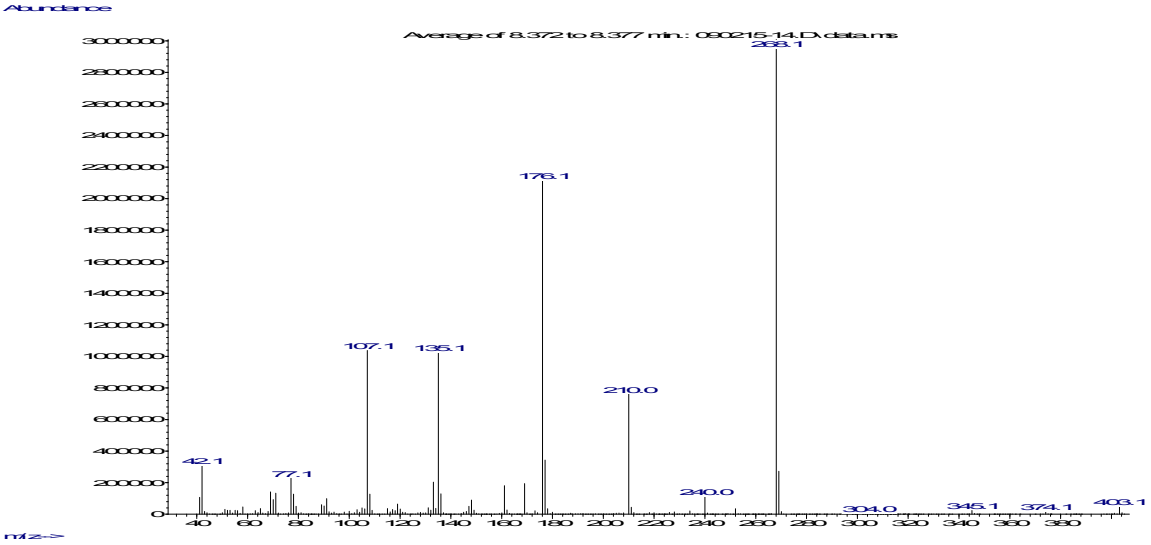
304.1
331.1
359.1
403.2
m/z-->


C_2H_5O
N
C_3F_7
O

9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240.0
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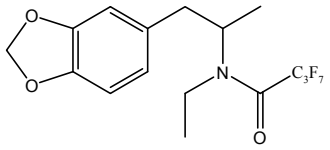
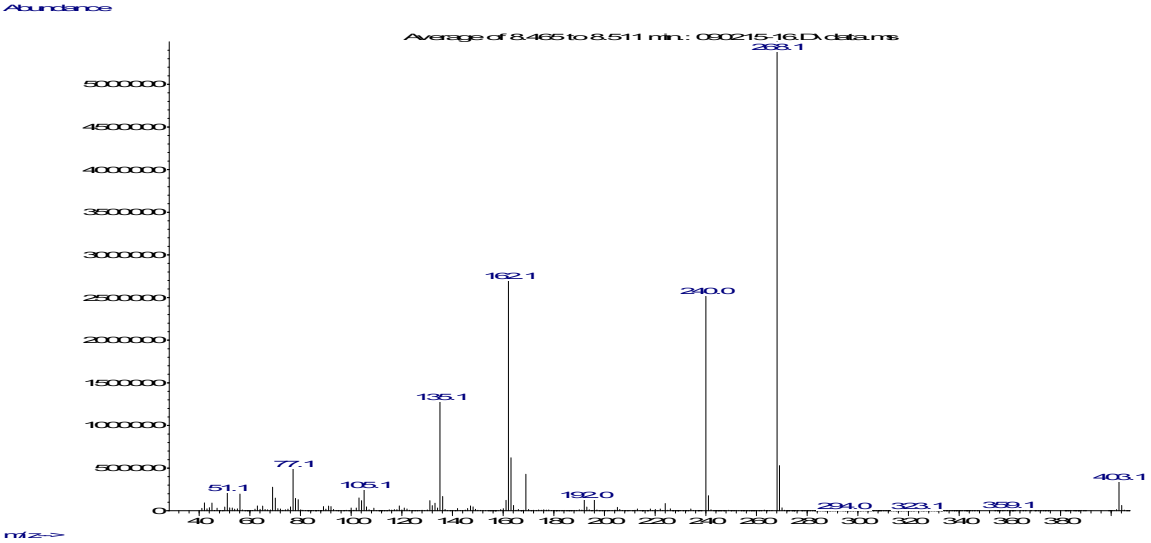
345.1
374.1
403.1
m/z-->


O
O
N
C_3F_7
O

11


Abundance
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268.1
162.1
240.0
135.1
77.1
51.1
105.1
192.0
294.0
323.1
359.1
403.1
m/z-->

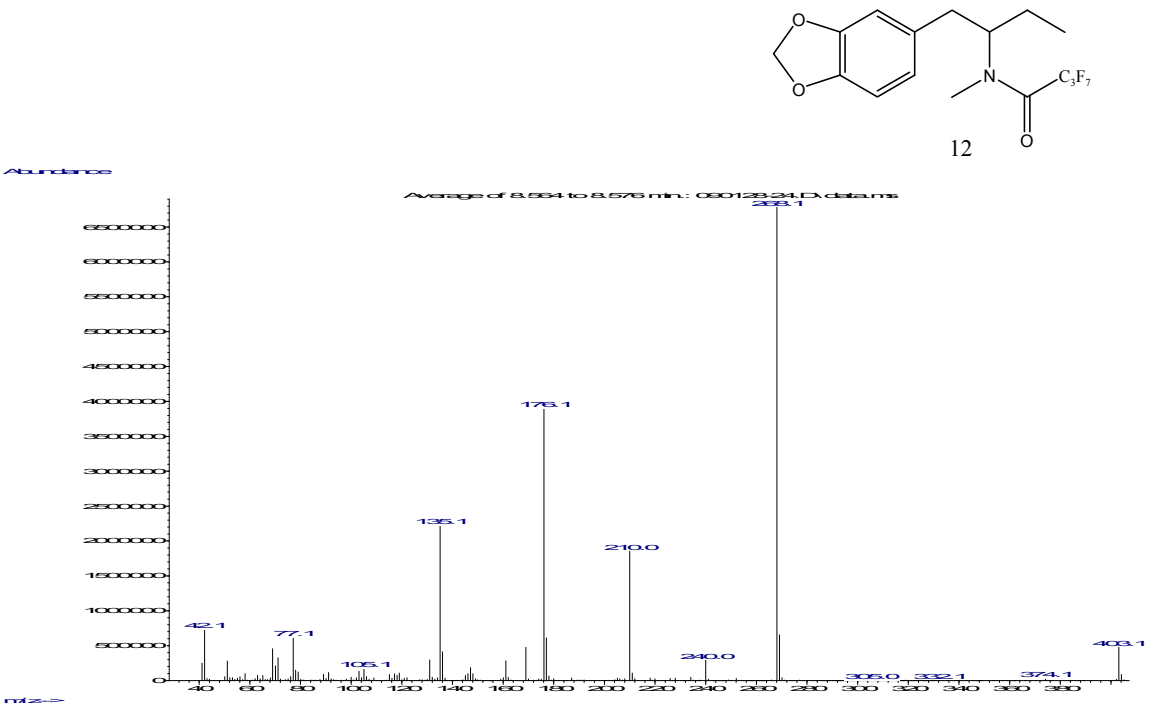
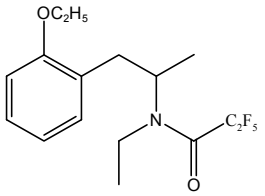
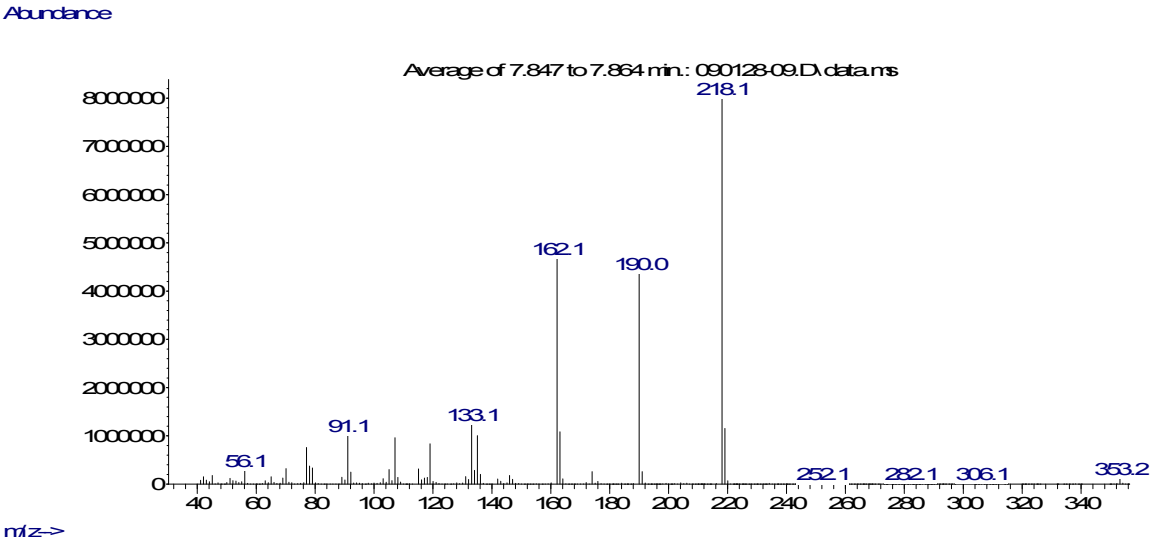


Figure 4: Mass spectra of the HFBA derivatives of compound 2, 3,5,6,8,9,11 and 12


OC2H5
N
C2F5
O

2


Abundance
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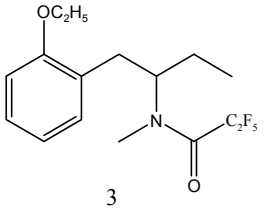
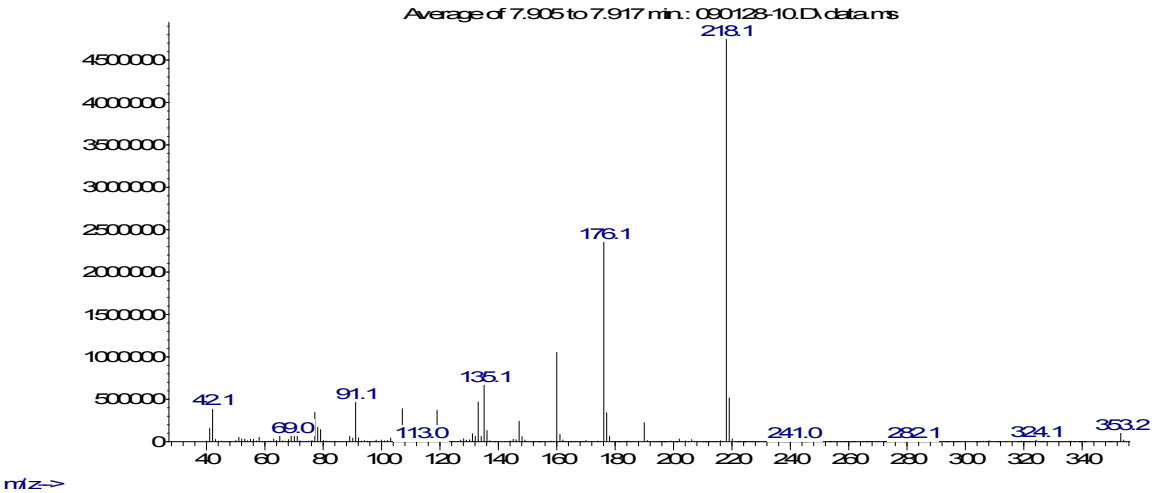
218.1
162.1
190.0
91.1
133.1
56.1
252.1
282.1
306.1
353.2
m/z-->


OC2H5
N
C2F5
O

3


Abundance
Average of 7.905 to 7.917 min.: 090128-10.D\data.ms
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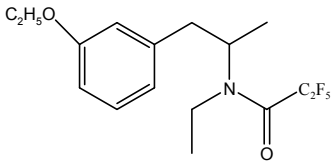
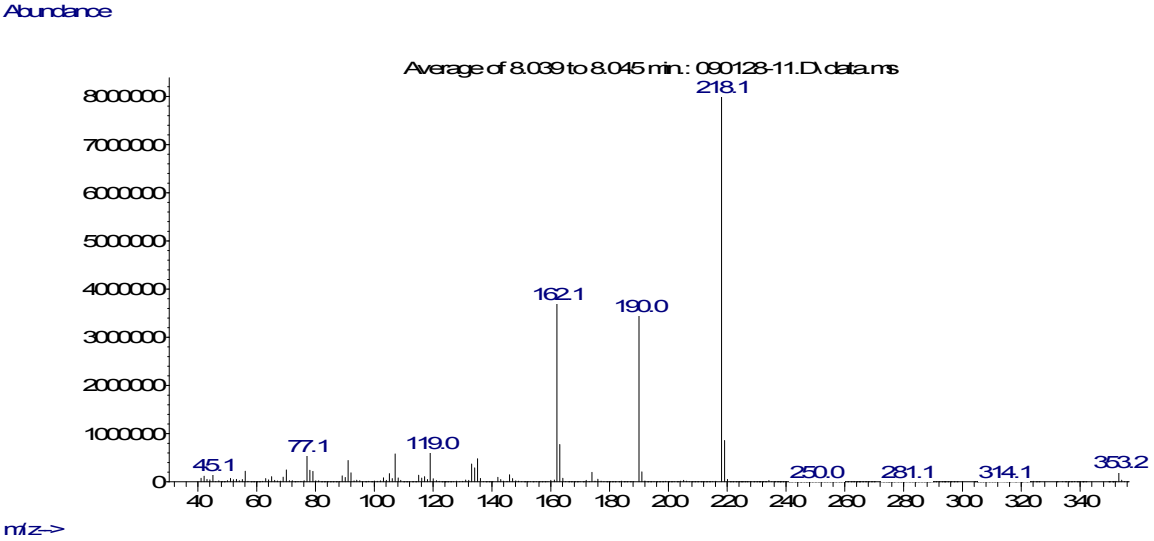
176.1
135.1
91.1
42.1
69.0
113.0
241.0
282.1
324.1
353.2
m/z-->


C2H5O
N
C2F5
O

5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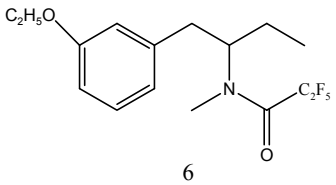
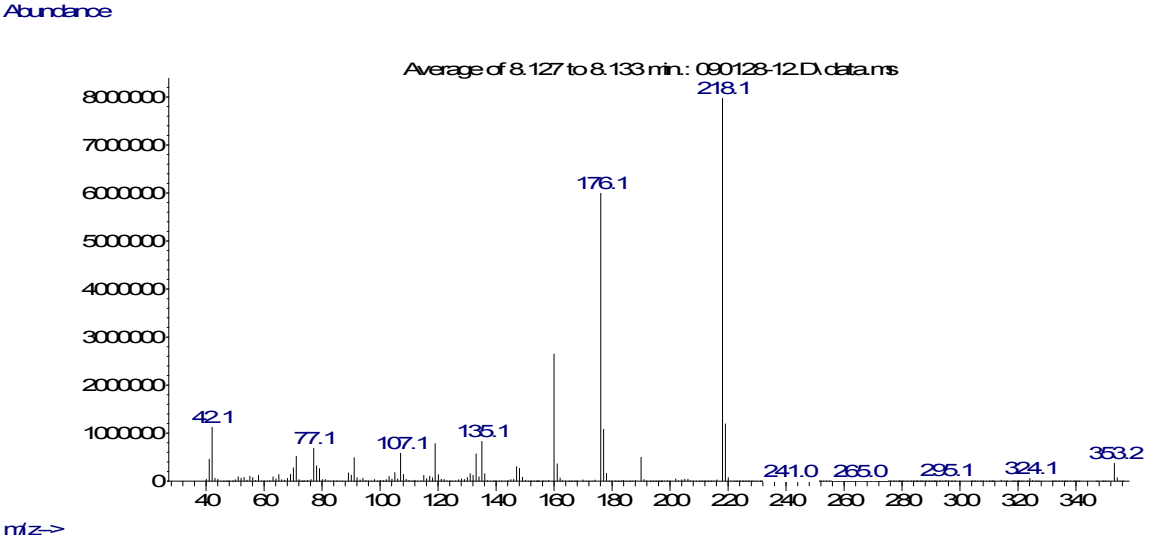
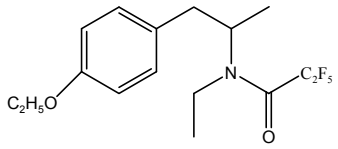
162.1
190.0
77.1
119.0
45.1
250.0
281.1
314.1
363.2
m/z-->

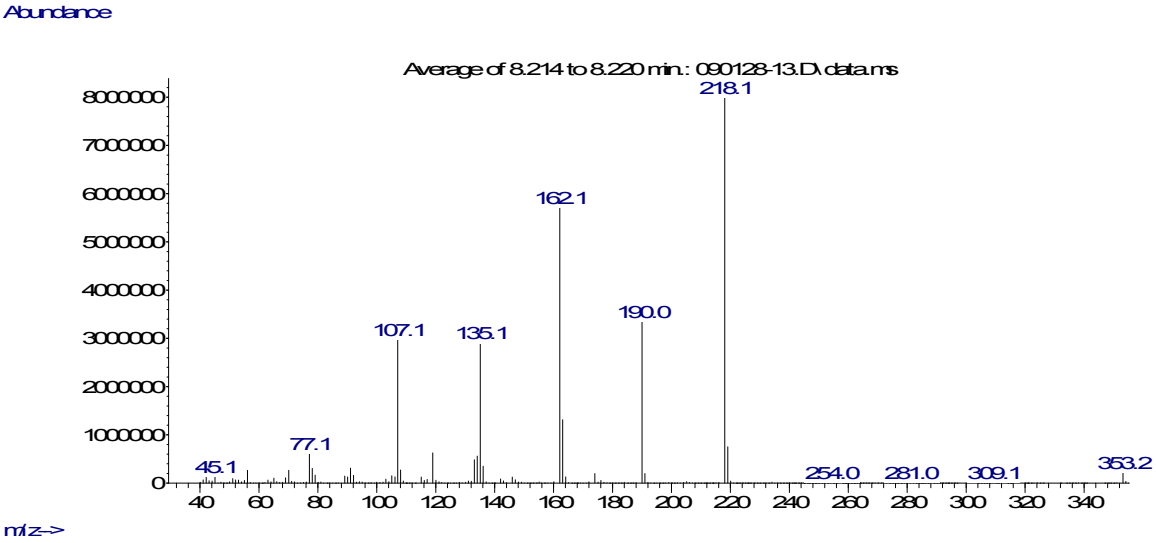

C2H5O
N
C2F5
O

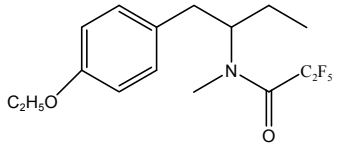
6


Abundance
Average of 8.127 to 8.133 min.: 090128-12.D\data.ms
218.1
176.1
42.1
77.1
107.1
135.1
241.0
265.0
295.1
324.1
353.2
m/z-->

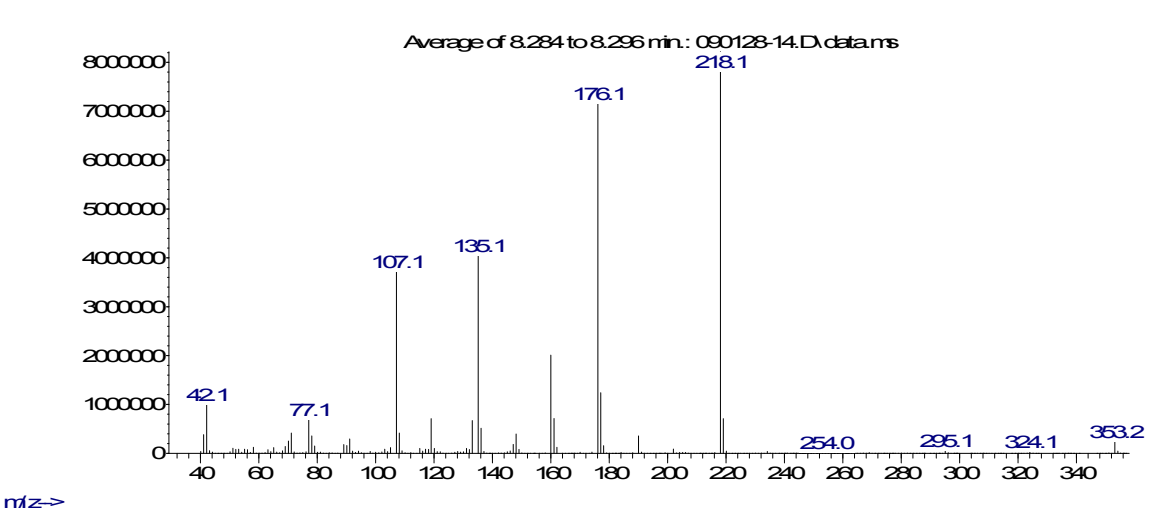

C_2H_5O
N
C_2F_5
O

8


Abundance
Average of 8.214 to 8.220 min.: 090128-13.D\data.ms
218.1
162.1
190.0
107.1
135.1
77.1
45.1
254.0
281.0
309.1
353.2
m/z-->


C_2H_5O
N
C_2F_5
O

9


Abundance
Average of 8.284 to 8.296 min.: 090128-14.D\data.ms
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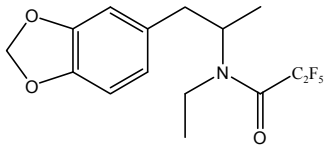
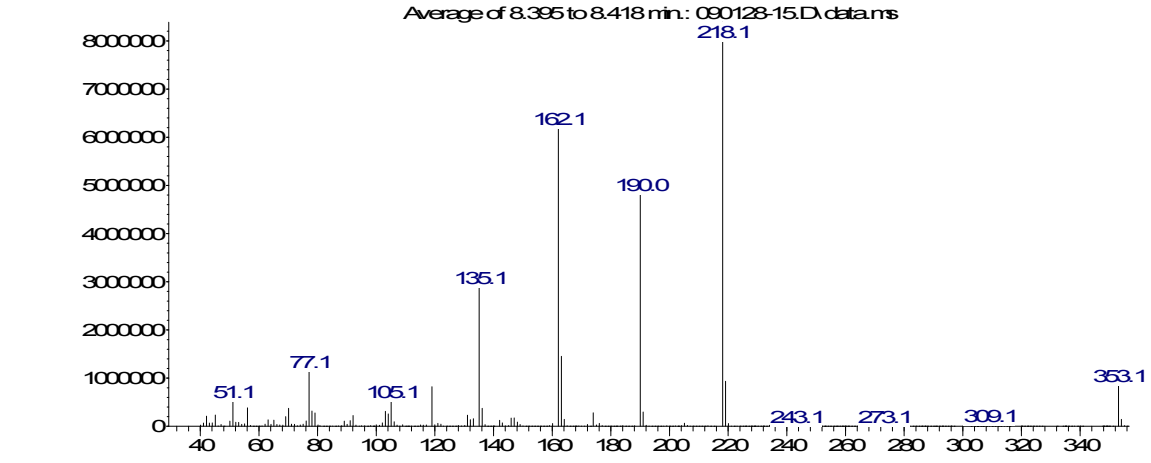
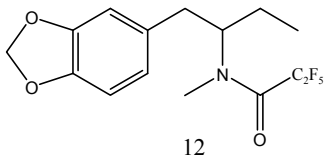
176.1
135.1
107.1
42.1
77.1
254.0
295.1
324.1
353.2
m/z-->



11





12

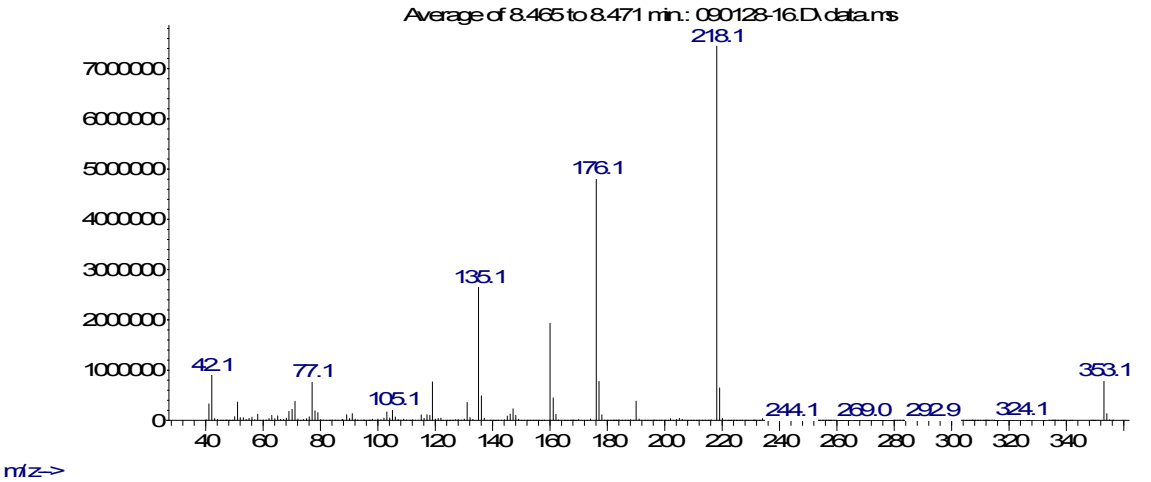
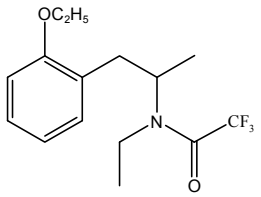
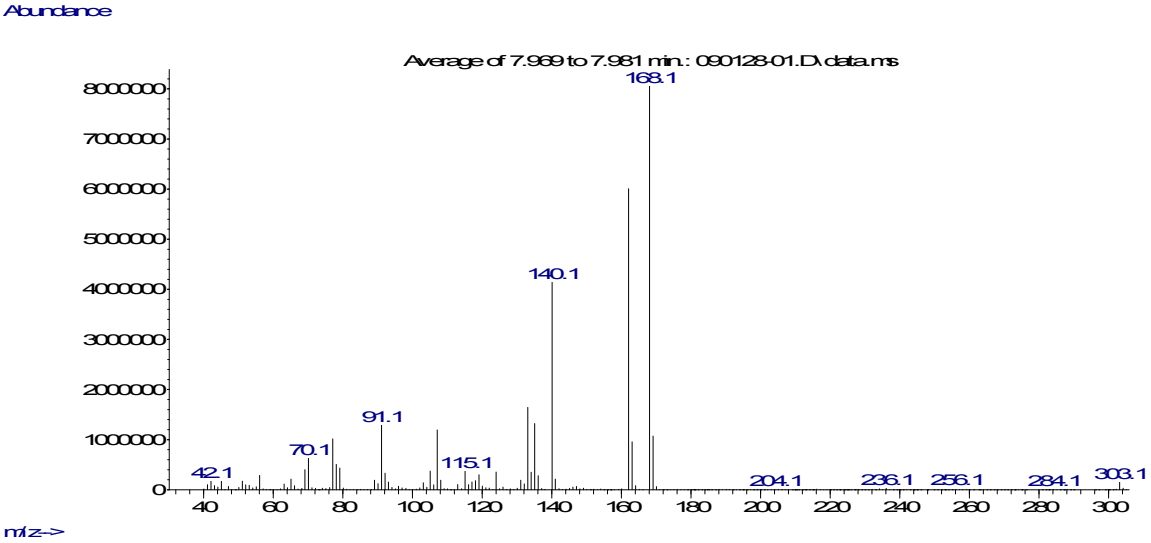
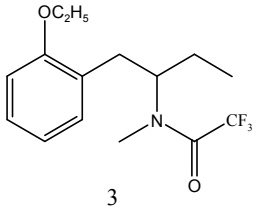


Figure 5: Mass spectrum of the PFPA derivatives of compound 2, 3, 5, 6, 8, 9, 11 and 12.

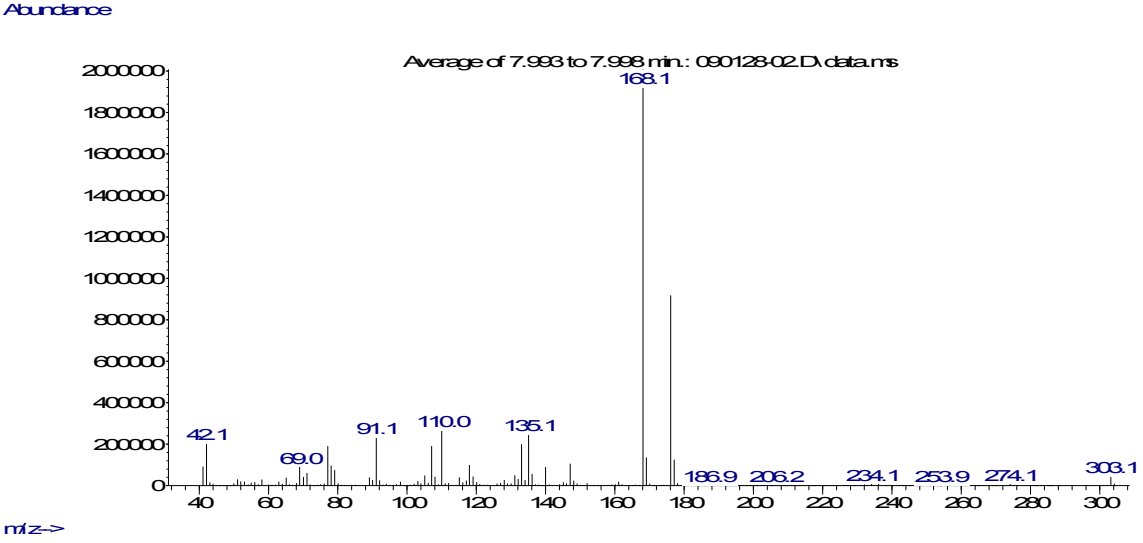

OC2H5
N
CF3
O

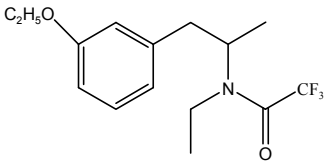
2


Abundance
Average of 7.969 to 7.981 min.: 090128-01.D\data.ms
168.1
140.1
91.1
70.1
115.1
42.1
204.1
236.1
256.1
284.1
303.1
m/z-->

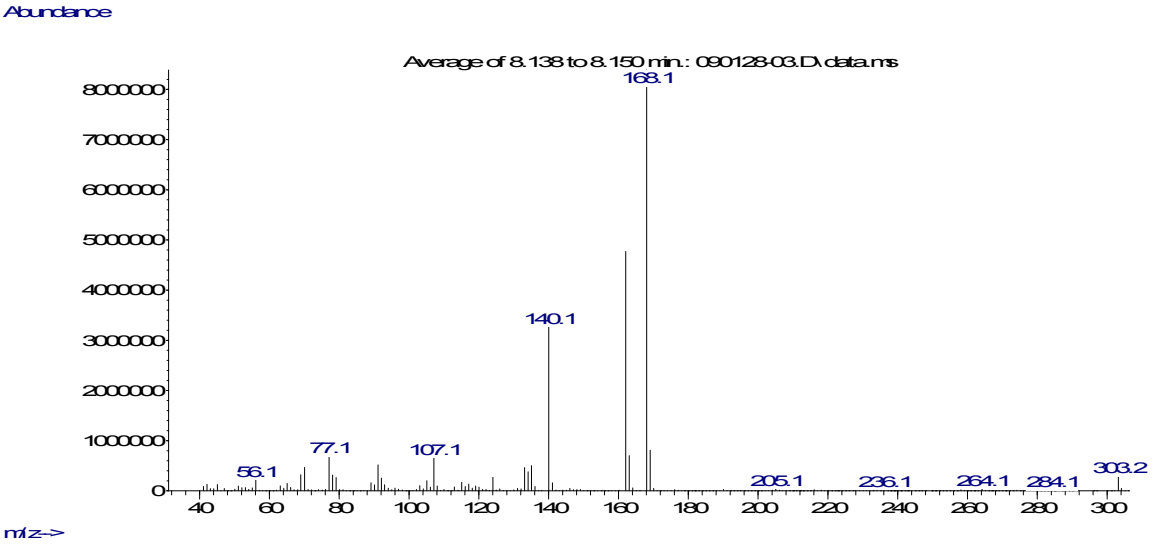

OC2H5
N
CF3
O

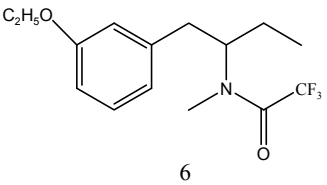
3


Abundance
Average of 7.993 to 7.998 min.: 090128-02.D\data.ms
168.1
42.1
91.1
110.0
135.1
69.0
186.9
206.2
234.1
253.9
274.1
303.1
m/z-->

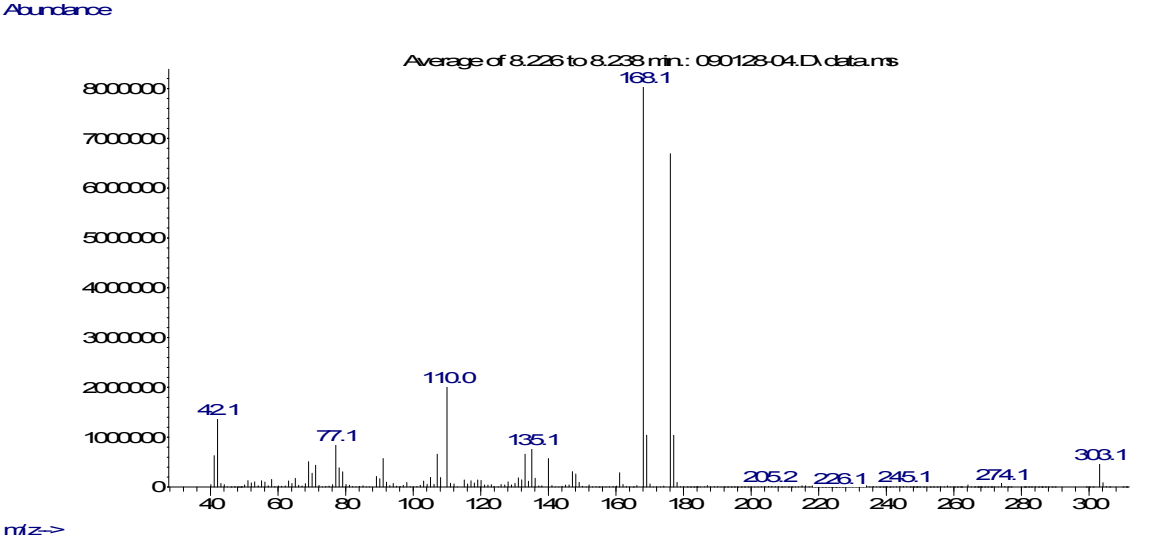

C2H5O
N
CF3
O

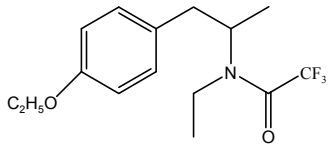
5


Abundance
Average of 8.138 to 8.150 min.: 090128-03.D\data.ms
168.1
140.1
77.1
107.1
56.1
205.1
236.1
264.1
284.1
303.2
m/z-->

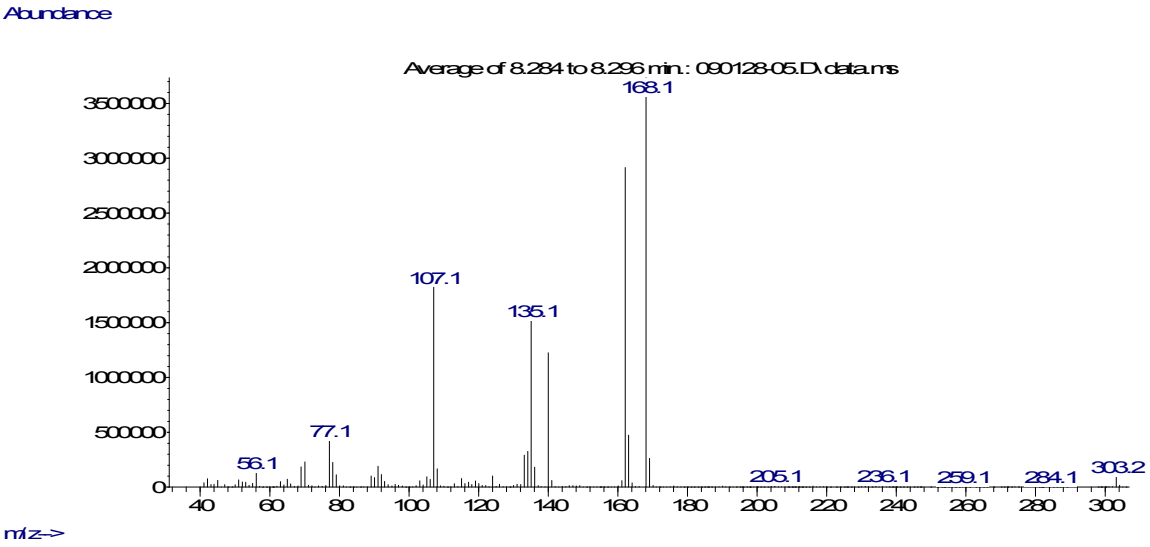

C2H5O
N
CF3
O

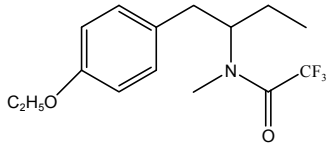
6


Abundance
Average of 8.226 to 8.238 min.: 090128-04.D\data.ms
168.1
110.0
42.1
77.1
135.1
205.2
226.1
245.1
274.1
303.1
m/z-->

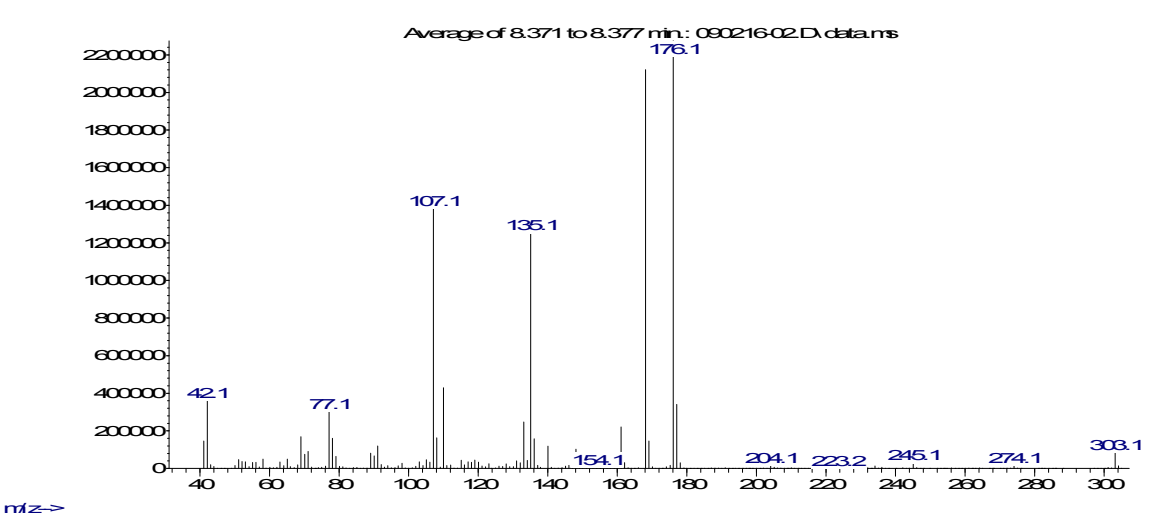

C_2H_5O
N
CF_3
O

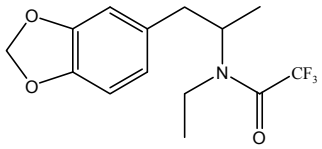
8


Abundance
Average of 8.284 to 8.296 min.: 090128-05.D\data.ms
168.1
107.1
135.1
77.1
56.1
205.1
236.1
259.1
284.1
303.2
m/z-->

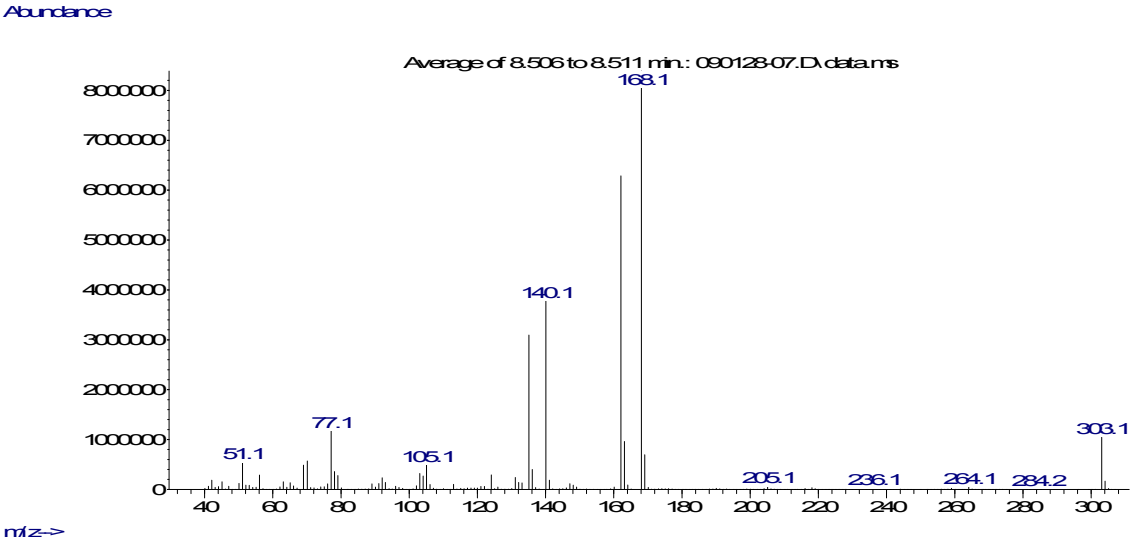

C_2H_5O
N
CF_3
O

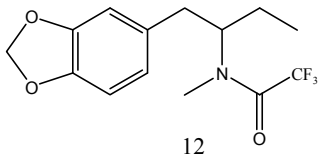
9


Abundance
Average of 8.371 to 8.377 min.: 090216-02.D\data.ms
176.1
107.1
135.1
42.1
77.1
154.1
204.1
223.2
245.1
274.1
303.1
m/z-->



11





12

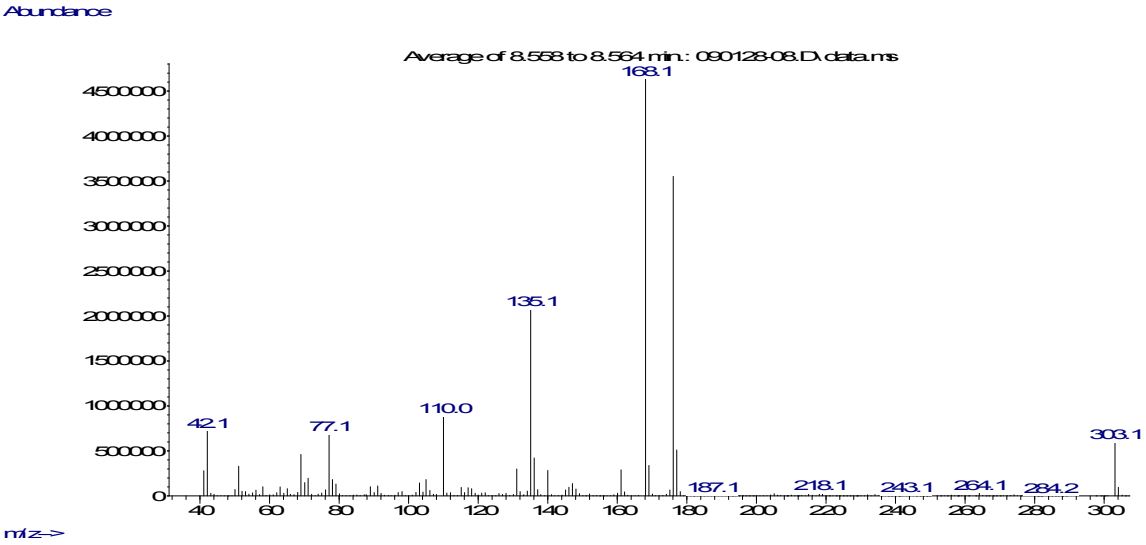
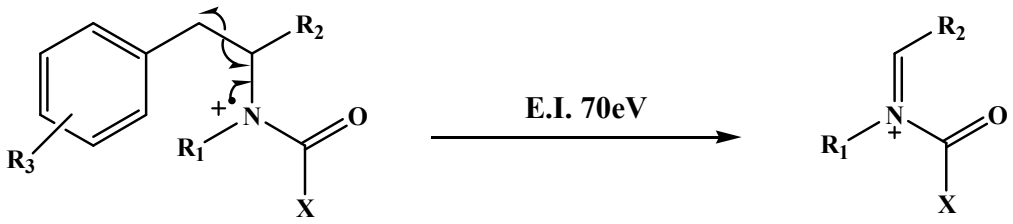


Figure 6: Mass spectra of the TFA derivatives of compound 2, 3, 5, 6, 8, 9, 11 and 12

The mass spectra for the eight heptaflourobutryl (HFBA), pentafluoropropionyl (PFPA) and trifluoroacetyl amides (TFA) are shown in Figure 4, 5 and 6, respectively. From these spectra, a base peak occurs at *m/z* 268, 218 and 168 which corresponds to the loss of 135 mass units from the molecular ion at 403, 353 and 303 for HFBA, PFPA and TFA amides, respectively. These ions at *m/z* 268, 218 and 168 are the HFBA, PFPA and TFA imine species, likely formed from the α-cleavage of the amide nitrogen to eliminate the corresponding ethoxybenzyl or 3,4-methylenedioxybenzyl fragments. Thus these ions are analogous to *m/z* 72 in the underivatized species because all these ions represent the $(M–135)^+$ species (Scheme 4). Also these spectra show *m/z* 135 that corresponds to the ethoxybenzyl and 3,4-methylenedioxybenzyl cations formed through the ionization of the aromatic ring in a similar manner to the underivatized species.

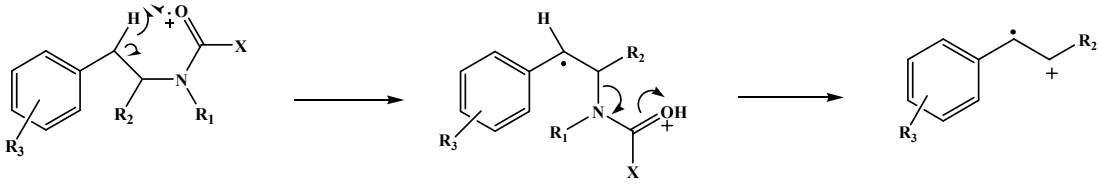


R_1 = C_2H_5, R_2= CH_3, R_3 = OC_2H_5 ,Cpd 2, 5, 8
R_1 = CH_3 , R_2=C_2H_5, R_3 = OC_2H_5 ,Cpd 3, 6, 9
R_1 = C_2H_5, R_2= CH_3, R_3 = OCH_2O ,Cpd 11
R_1 = CH_3 , R_2=C_2H_5, R_3 = OCH_2O ,Cpd 12
X = CF_3 , M.W. = 303
X = C_2F_5, M.W.= 353
X = C_3F_7, M.W.= 403

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

Scheme 4: Formation of the acylimine fragment species via α-cleavage fragmentation pathway.

The decreased role for the α-cleavage reaction in the fragmentation of these amides allows the formation of more diagnostic ions for each individual isomer. Acylation, in particular the perflouroacylation, weakens the bond between nitrogen and the α-carbon of the substituted phenethyl group, allowing the formation of charged hydrocarbon species of increased relative abundance. These alkene radical cations of varying mass are formed due to the transfer of a benzylic hydrogen to the ionized carbonyl oxygen followed by the loss of a neutral amide species (Scheme 5). The resulting alkene radical cations significantly individualize the mass spectra and provide specific information concerning the side chain structure. The mass spectra in Figures 4, 5 and 6 illustrate the role of the alkene fragments at *m/z* 162 and 176 in side chain identification. These ions identify the number of carbons in the hydrocarbon chain attached directly to the aromatic ring in an uninterrupted manner.



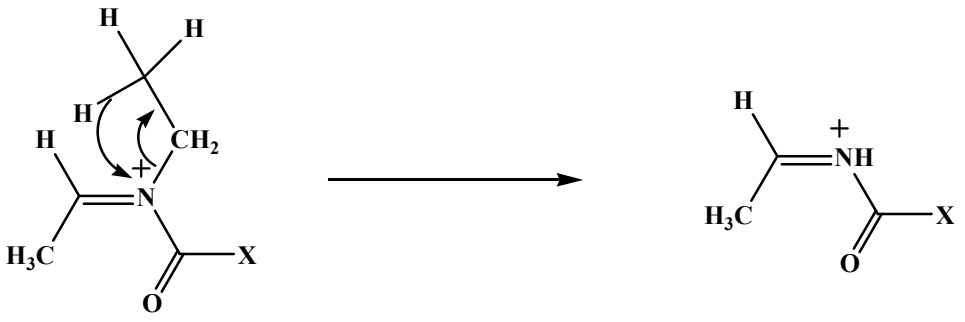
R_1 = C_2H_5, R_2= CH_3, R_3 = OC_2H_5 ,Cpd 2, 5, 8
R_1 = CH_3 , R_2=C_2H_5, R_3 = OC_2H_5 ,Cpd 3, 6, 9
R_1 = C_2H_5, R_2= CH_3, R_3 = OCH_2O ,Cpd 11
R_1 = CH_3 , R_2=C_2H_5, R_3 = OCH_2O ,Cpd 12
X = CF_3, M.W. = 303
X = C_2F_5, M.W.= 353
X = C_3F_7, M.W.= 403

m/z = 162 , R_2=CH_3, R_3 = OC_2H_5; Cpd 2,5,8
m/z = 176 , R_2=C_2H_5, R_3 = OC_2H_5; Cpd 3,6,9
m/z = 162 , R_2=CH_3, R_3 = OCH_2O; Cpd 11
m/z = 176 , R_2=C_2H_5, R_3 = OCH_2O; Cpd 12

Scheme 5: Formation of alkene radical cation.

In Figures 4, 5 and 6, the *m/z* 162 is characteristic for compounds 2, 5, 8 and 11 (group 1) while the corresponding alkene fragment at m/z 176 is specific for compounds 3, 6, 9 and 12 (group 2). Thus the alkene radical cations divide these regioisomers into two main groups based on number of carbons attached directly to the aromatic ring. Additionally the mass spectra of the HFBA derivatives for compounds 2, 5, 8 and 11 are characterized by the presence of a unique ion at *m/z* 240. The equivalent ion for the PFPA and TFA derivatives of these compounds occur at *m/z* 190, *m/z* 140. The ion at *m/z* 240 shown for the HFBA derivatives of the group 1 compounds occurs from the imine fragment (*m/z* 268) of the N-ethyl species via hydrogen rearrangement followed by the loss of the ethylene group to give the corresponding characteristic ion at *m/z* 240. The equivalent fragmentation pathway occurs with the loss of ethylene from the imine fragments at *m/z* 218 and 168 of the PFPA and TFA derivatives respectively (Scheme 6). Differences in the relative abundances of *m/z* 162 and 240 (in the case of HFBA derivatives) compared to the base peak at *m/z* 268 provides some degree of discrimination among the compound in group 1. Conversely, the mass spectra of group 2 (Compounds 3, 6, 9 and 12) of the HFPA derivatives are characterized by *m/z* 210 (160 and 110 for the, PFPA and TFA derivatives, respectively), a characteristic fragment for the N-methylamine substitution pattern. This ion is formed by rearrangement of the imine cation followed by the loss of propanaldehyde to give the *m/z* 210 ion in the HFBA derivatives and in an analogous manner m/z 160 and 110 for PFPA and TFA, respectively. An analysis of the masses of the components, which make up the fragment at *m/z* 210 (in the case of HFBA) include for example C_3F_7 (169 mass units) and CH_3 (15 mass units), leaving only a mass of 26 available for the total of 210. The mass 26 would

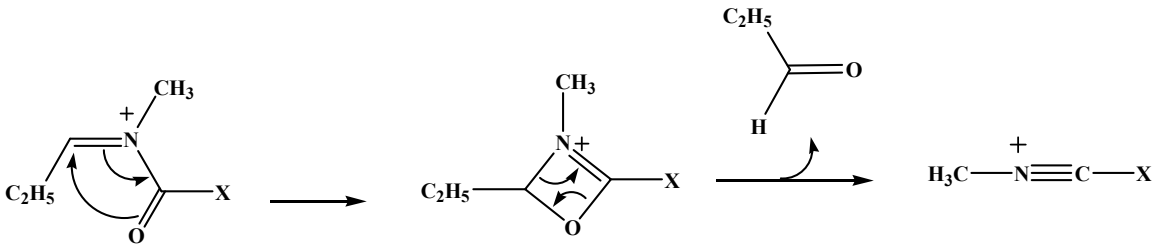
correspond to CN and the proposed mechanism for the formation of $(C_3F_7CNCH_3)^+$ is shown in Scheme 7. An equivalent fragmentation pathway has been previously reported [Awad et al., 2005]. Analogous ions formed through the same fragmentation pathways are seen for the PFPA at m/z 160 and TFA at *m/z* 110 due to the loss of propanaldehyde from the base peak for all the compounds of group 2.



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

X = CF_3, *m/z* = 140
X = C_2F_5, *m/z* = 190
X = C_3F_7, *m/z* = 240

Scheme 6: Formation of *m/z* 140, 190 and 240 for the HFBA, PFPA and TFA derivatives of compounds 2, 5, 8 and 11 (group1).

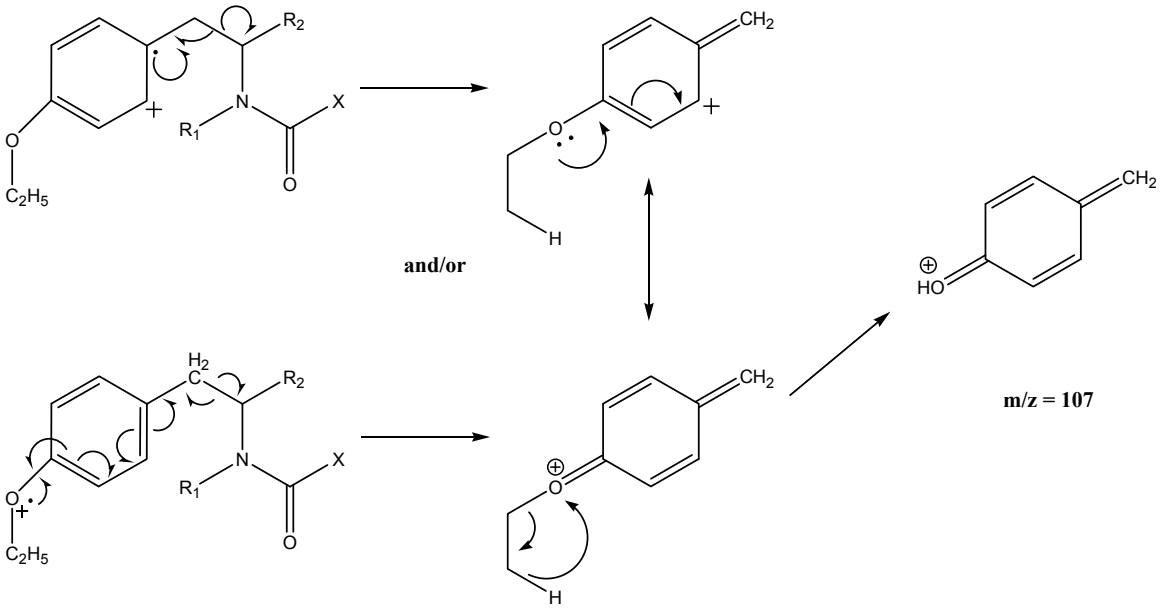


X = CF_3, *m/z* = 168
X = C_2F_5, *m/z* = 218
X = C_3F_7, *m/z* = 268

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

Scheme 7: Formation of m/z 110, 160 and 210 for the HFBA, PFPA and TFA derivatives of compounds 3, 6, 9 and 12 (group 2).

Mass spectra of perfluoroacyl derivatives of the ring substituted ethoxyphenethylamines showed a unique ion fragment at *m/z* 107. This ion at *m/z* 107 represents the loss of 28 mass units (ethylene, C_2H_4) from the ethoxybenzyl cation at *m/z* 135 (Scheme 8). Although the relative abundance varies significantly, the *m/z* 107 ion is present in all mass spectra of the perfluoroacyl derivatives of these compounds (2, 3, 5, 6, 8 and 9) and offers a unique fragment ion to discriminate the ethoxy ring substitution pattern from the methylenedioxy ring substitution pattern. The *m/z* 107 ion is also present in relatively low abundance in the mass spectra of the underivatized ethoxyphenethylamines shown in Figure 3.



$R_1 = C_2H_5$, $R_2 = CH_3$; Cpd 2, 5, 8
$R_1 = CH_3$, $R_2 = C_2H_5$; Cpd 3, 6, 9
X = CF_3, M.W. = 303
X = C_2F_5, M.W. = 353
X = C_3F_7, M.W. = 403

Scheme 8: Formation of *m/z* 107 for compounds 2, 3, 5, 6, 8 and 9.

3.1.3. Vapor-phase Infra-Red Spectrophotometry:

Infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas-chromatography with infrared detection (GC-IRD) was evaluated for differentiation among the 12 regioisomeric amines in this study. Infrared detection should provide compound specificity without the need for chemical derivatization of the parent drug molecule. The use of infra-red spectrophotometry as a supportive method in the identification of drugs in forensic laboratories has been widely documented [Belal et al., 2009; Duncan, 1988]. The vapor-phase infrared spectra for compounds 1-12 are shown in Figure 7. The spectra were generated in the vapor-phase following sample injection into the gas chromatograph. Each compound shows a vapor-phase IR spectrum with absorption bands in the regions 650 – 1700 cm^{-1} and 2700 – 3100 cm^{-1}. Both regions are useful in the identification and differentiation among this set of compounds.

Examination of the vapor-phase infra-red spectra these compounds show characteristic peaks in the range of 2700 cm^{-1} and 3100 cm^{-1}, based principally on the nitrogen substitution pattern. Hence the compounds can be classified into 3 main groups based on substitution on the nitrogen atom, regardless the ring substitution pattern. The first group contains compounds 1, 4, 7 and 10 which are N,N-dimethyl-tertiary amines and their IR spectra show characteristic peaks of medium intensity around 2782, 2827 cm^{-1} and also peaks of higher intensity near 2940 and 2975 cm^{-1}. The second group is the secondary amines (compounds 2, 5, 8 and 11) with an ethyl group as the second group on the nitrogen atom. Their IR spectra show almost identical absorption spectrum at the 2700-

3100 cm^{-1} region, with a medium absorption peak near 2933 cm^{-1} and a high intensity peak at 2971 cm^{-1}. The last group, also secondary amines are compounds 3, 6, 9 and 12. These compounds all have the nitrogen atom connected to a methyl group, as well as longest uninterrupted alkyl side chain attached to the aromatic ring. Their infra-red spectra show weak absorption peaks near 2801 cm^{-1} and 2886 cm^{-1} and stronger absorption doublet peak near 2940 and 2970 cm^{-1}.

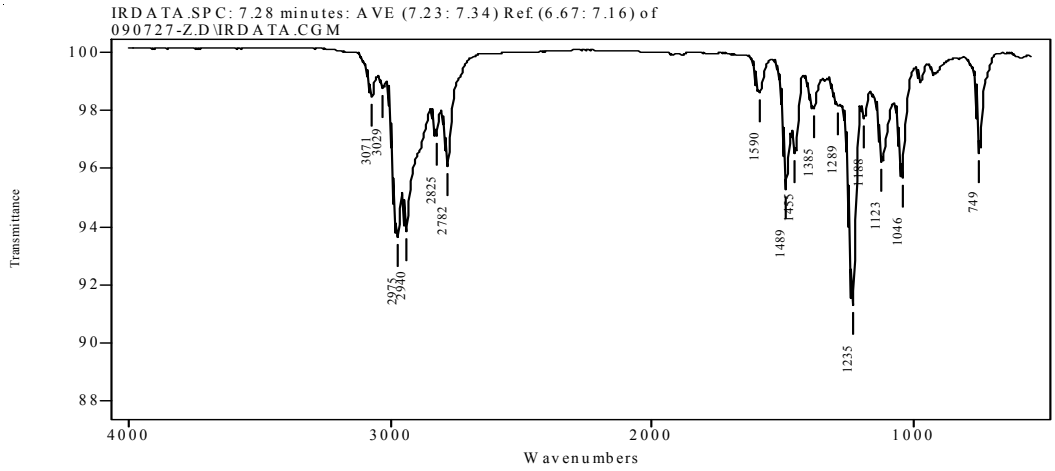
Additionally, it was observed that the IR spectra in the region of 650 – 1700 cm^{-1} can be useful in the differentiation of ring substitution patterns when the side chain is held constant. Hence it can be used to differentiate compounds within each of the three N-substitution patterns described above. In the first group (N,N-dimethyl substitution pattern) compound 1 is characteristic by a medium intensity absorption peak at 749 cm^{-1}, which is absent in the IR spectra of other compounds within the same group. In addition, compound 1 shows a strong absorption band at 1235 cm^{-1} which is shifted to 1254, 1239 and 1246 cm^{-1} in the IR spectra of compounds 4, 7 and 10, respectively. Another aspect that is characteristic for compound 1 is the doublet peak at 1455 cm^{-1} and 1489 cm^{-1}, which is shifted to 1447 cm^{-1} and 1486 $cm^{-1}$for compound 4. The doublet peak of greater intensity occurs at 1443 cm^{-1} and 1489 cm^{-1} for compound 10, while compound 7 shows a high intensity singlet peak at 1509 cm^{-1}.

The IR spectrum of compound 4 shows a characteristic singlet absorption peak at 1601 cm^{-1} which does not exist in the IR spectra of the other compounds in the group but instead, peaks of different absorption intensities were observed at 1590, 1609 and 1608 cm^{-1} for compounds 1, 7 and 10, respectively. Compound 7 could be identified by the

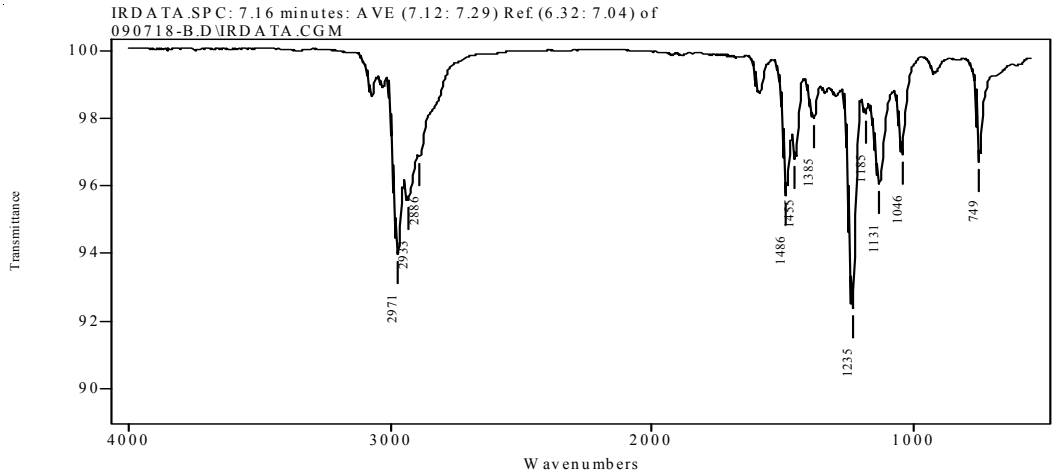
strong peak at 1509 cm^{-1} and finally compound 10 shows a characteristic strong peak at 1489 cm^{-1}. Differentiation between ring substitution patterns within the other two groups of nitrogen substitution pattern could be carried out in an analogous manner.

The IR spectrum of the three 3,4-methylenedioxyamptamines (MDEA, MDMMA and MBDB) all show characteristic strong peak at 1489 cm^{-1} and 1246 cm^{-1} and a medium intensity absorption peak at 1443 cm^{-1} and 1050 cm_{-1} , these peaks can be used to differentiate between the three 3,4-methylenedioxyamptamines from the other nine ethoxyphenethylamine regioisomers.

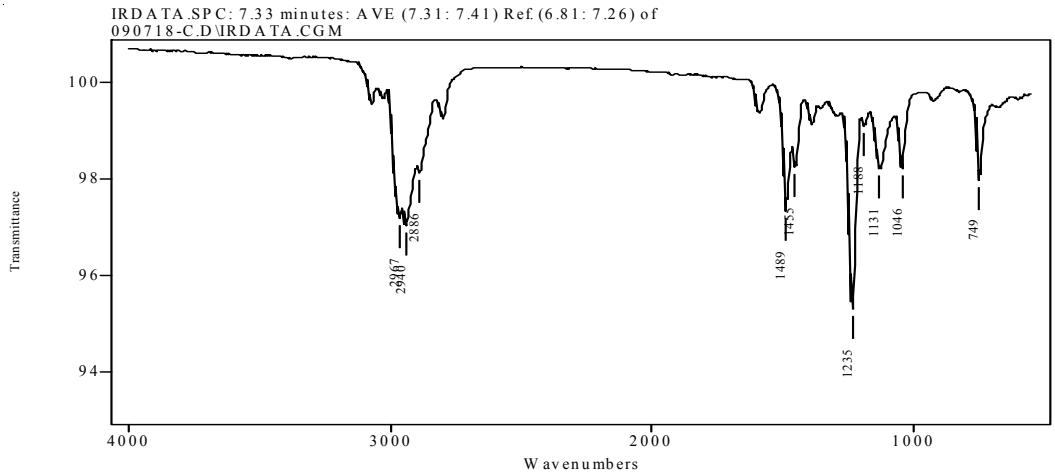
Vapor Phase IR Spectrum of compound 1



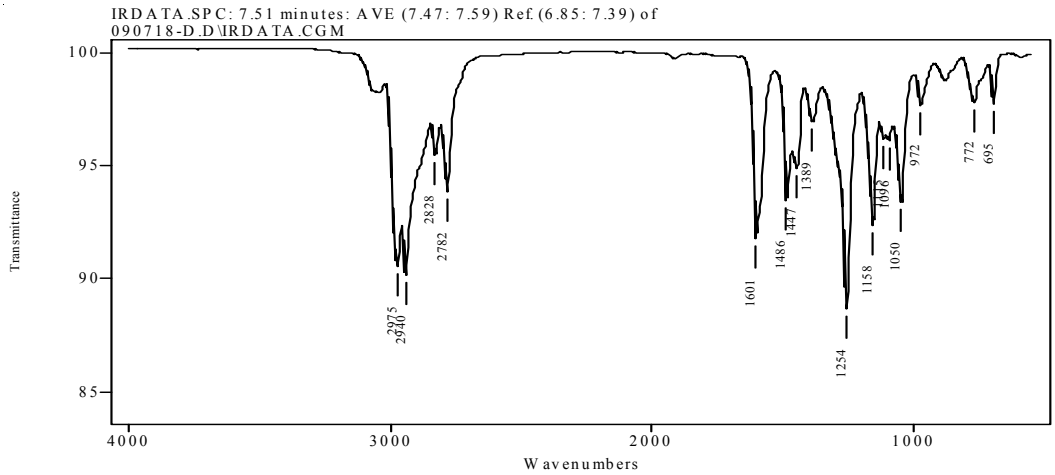
Vapor Phase IR Spectrum of Compound 2



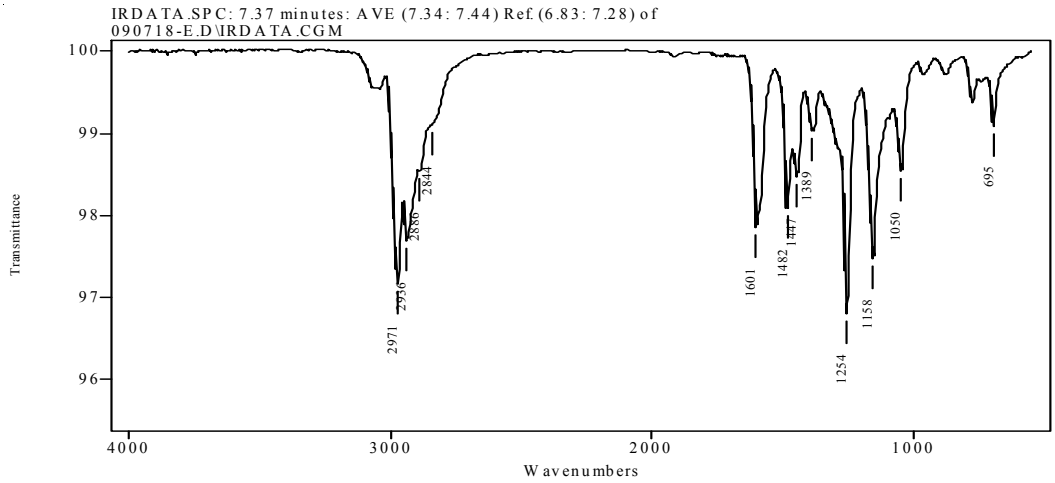
Vapor Phase IR Spectrum of Compound 3



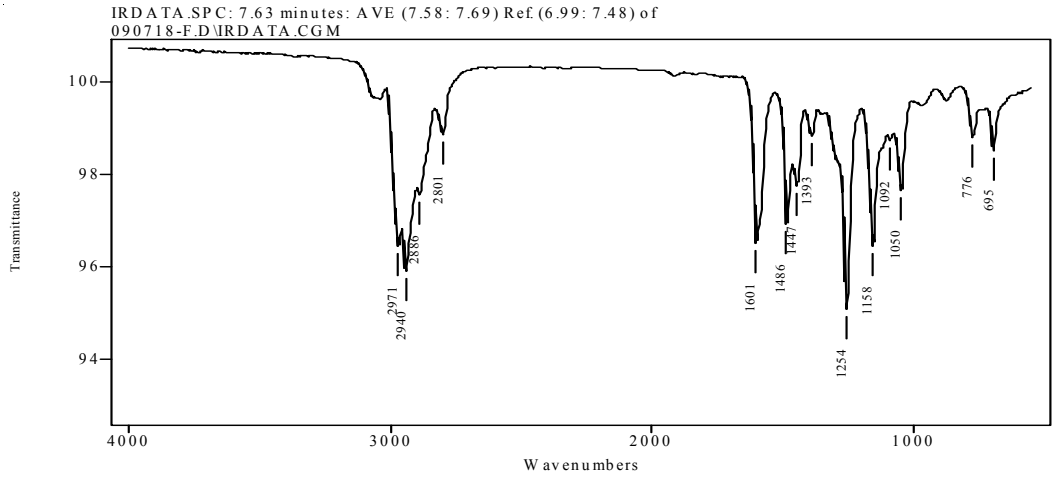
Vapor Phase IR Spectrum of Compound 4



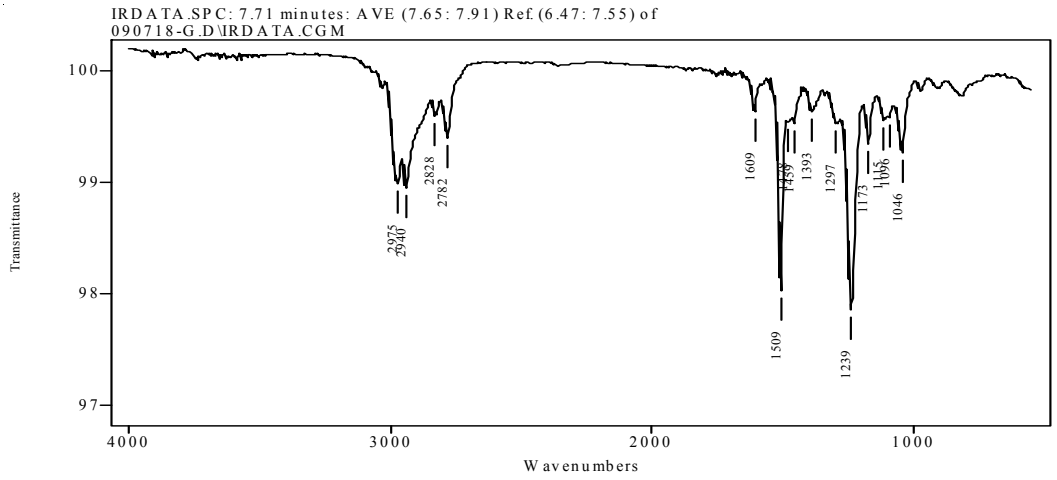
Vapor Phase IR Spectrum of Compound 5



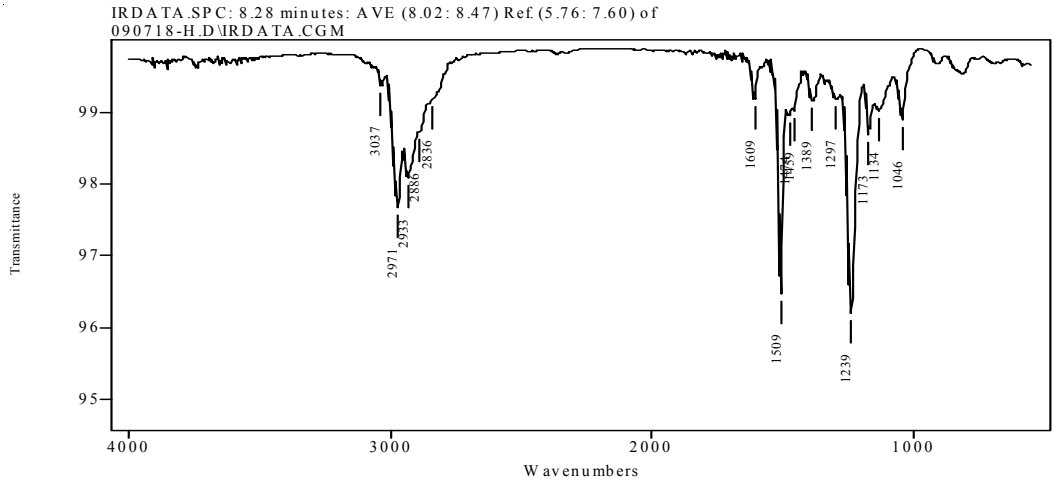
Vapor Phase IR Spectrum of Compound 6



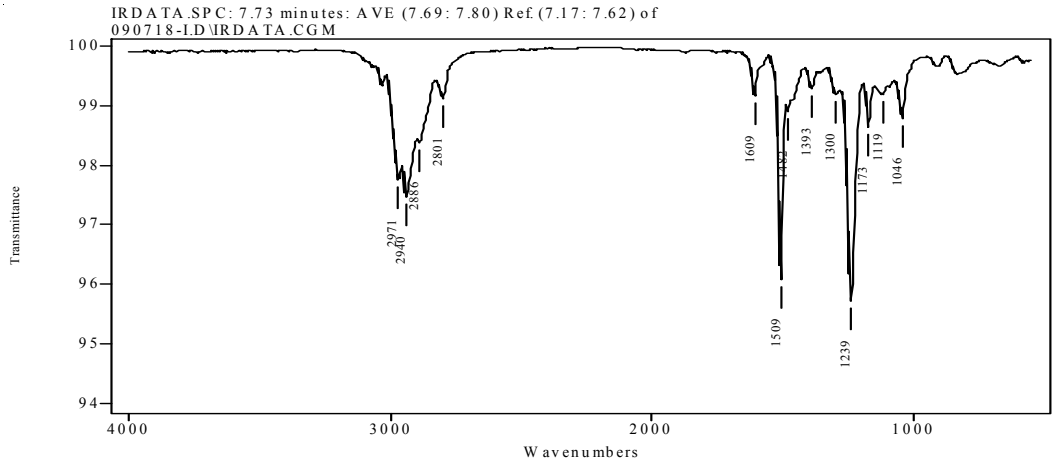
Vapor Phase IR Spectrum of Compound 7



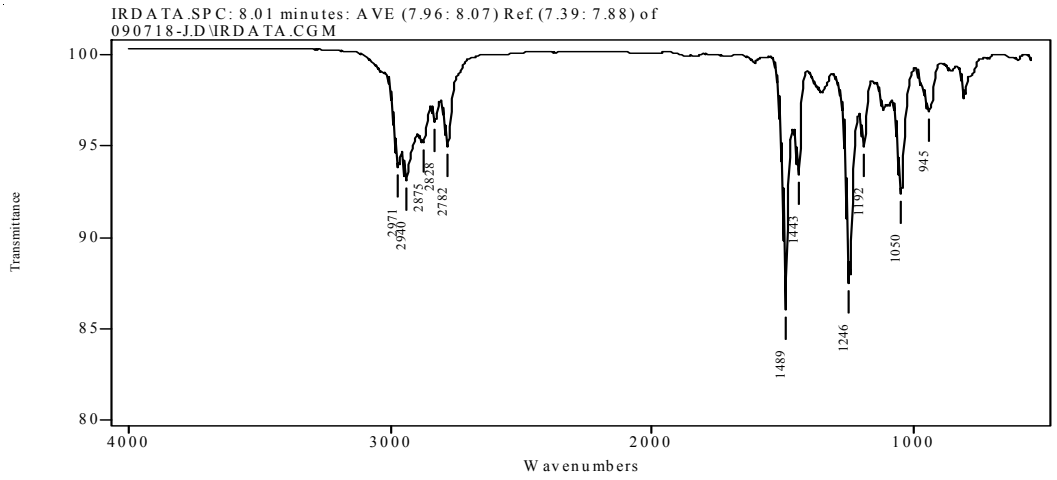
Vapor Phase IR Spectrum of Compound 8



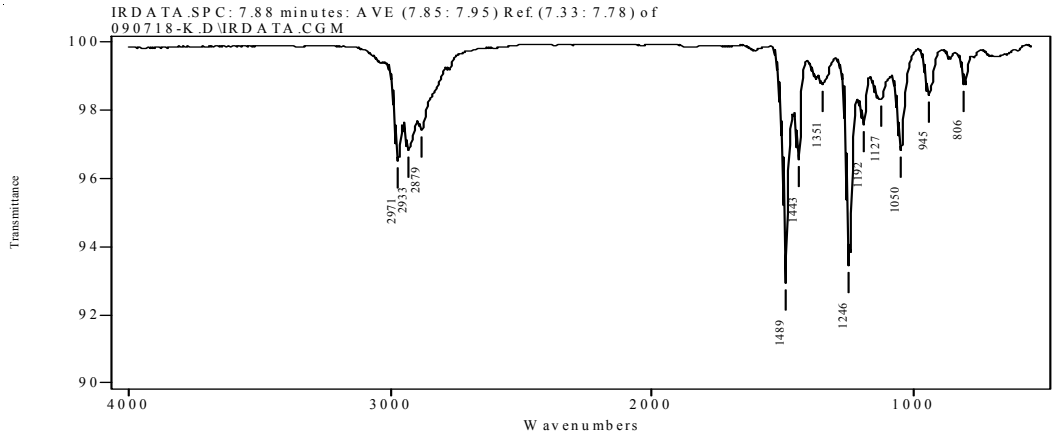
Vapor Phase IR Spectrum of Compound 9



Vapor Phase IR spectrum of Compound 10



Vapor Phase IR spectrum of Compound 11



Vapor Phase IR Spectrum of Compound 12

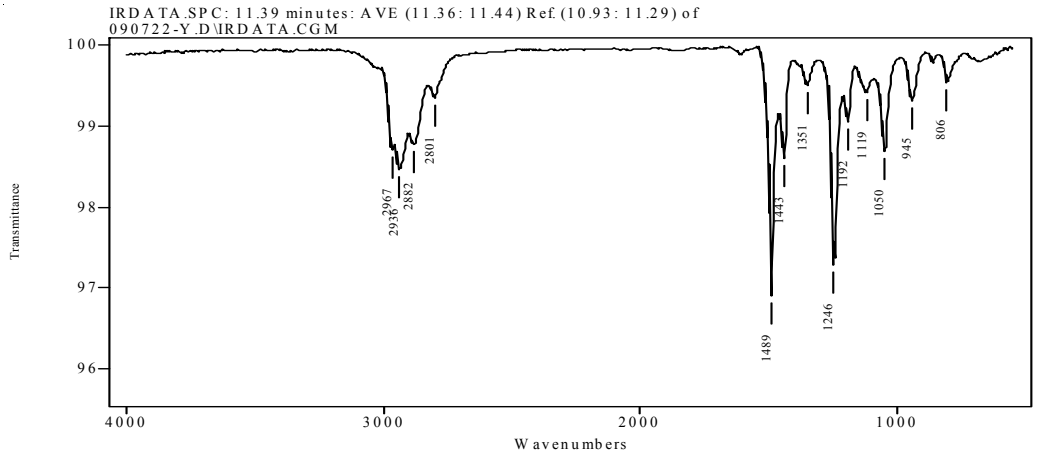


Figure 7: Vapor Phase IR spectra of compounds 1-12.

3.1.4. Gas Chromatography:

3.1.4.1. Gas Chromatography of Underivatized Amines:

Several stationary phases and different temperature programs were evaluated in an effort to resolve the underivatized 12 regioisomeric and isobaric amines involved in this study. The retention properties of the amines were compared on three different columns Rtx-1, Rxi-50 and on a more polar Rtx-200. Table 3 shows the observed retention times of all twelve amines using the same temperature program. Due to their structural similarity some of these amines have a gas chromatographic behavior and mass spectra that differ only slightly.

Separation of the underivatized amines was achieved on a 50% phenyl – 50% methyl polysiloxane column (Rxi-50), The temperature program started with a initial temperature of 70ºC for 1 minute, then the temperature was ramped up to 150ºC at a rate of 5ºC/min held at150ºC for 2 minutes, then increased to 250ºC at a rate of 10ºC/min and held for 15 minutes (TP-2). The chromatogram (Figure 8) shows almost co-elution between compounds 6, 7 and 9, 10 which supports the need of specific methods beyond chromatographic elution properties to identify these compounds. Derivatization eliminates such co-elution simply because compound 7 and 10 will not form stable acylated derivatives; hence they will have different retention properties from the acylated derivative of both compounds 6 and 9 in this limited series of compounds. Different mixtures of the underivatized amines showed that, when the ring substitution pattern is held constant, the N-ethyl side chain isomer elutes before the N,N-dimethyl isomer and

the N-methyl (the longest un-interrupted alkyl side chain attached to the aromatic ring) elutes last. (Figures 9, 10 and 11). On the other hand, when the side chain is held constant, the ring substituted ethoxy isomer in the ortho position elutes before the meta then the para and finally the 3,4-methylenedioxy substitution pattern elutes last (Figure 12, 13 and 14).

Table 3: Retention time (min.) of the underivatized amines of compounds 1-12.*

Column Used	Compound Number												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Rtx-1	7.235	7.165	7.311	7.421	7.33	7.503	7.515	7.427	7.608	7.66	7.573	7.765	
Rxi-50	6.256	6.191	6.35	6.539	6.386	6.566	6.546	6.478	6.657	6.716	6.654	6.843	**Retention Time(min.)**
Rtx-200	9.493	9.388	9.627	9.796	9.668	9.936	9.919	9.802	10.076	10.228	10.111	10.397	

* Columns Rtx-1, Rxi-50 and RTX-200. Temperature Program: Injection temperature 250°C, detector temperature 280°C , initial temperature 70°C hold for 1 min, then ramped up to 250°C at a rate of 30°C /min (TP-1).

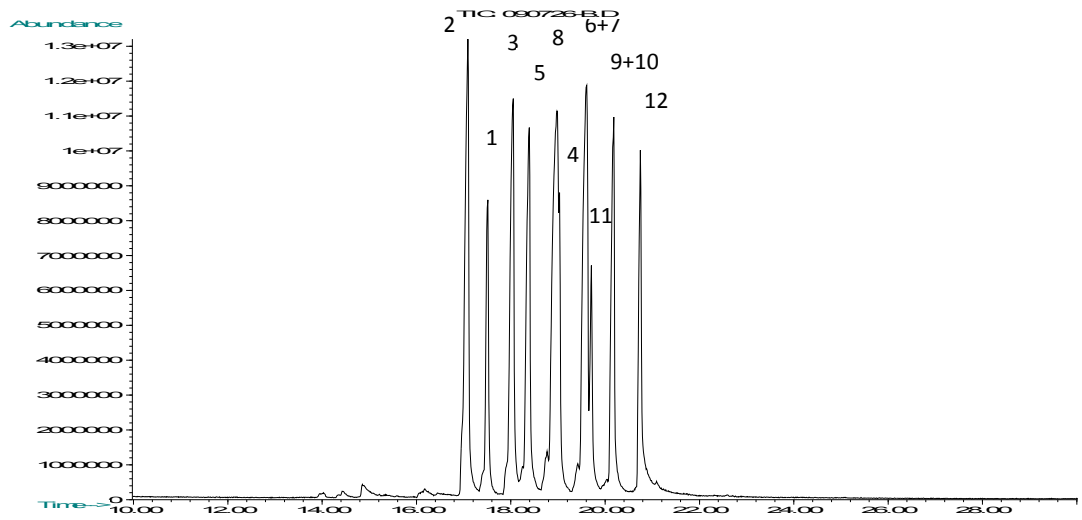


Figure 8: Capillary gas chromatograph of physical mixture of compounds 1 - 12 [underivatized], Column used Rxi-50, temperature program (TP-2).

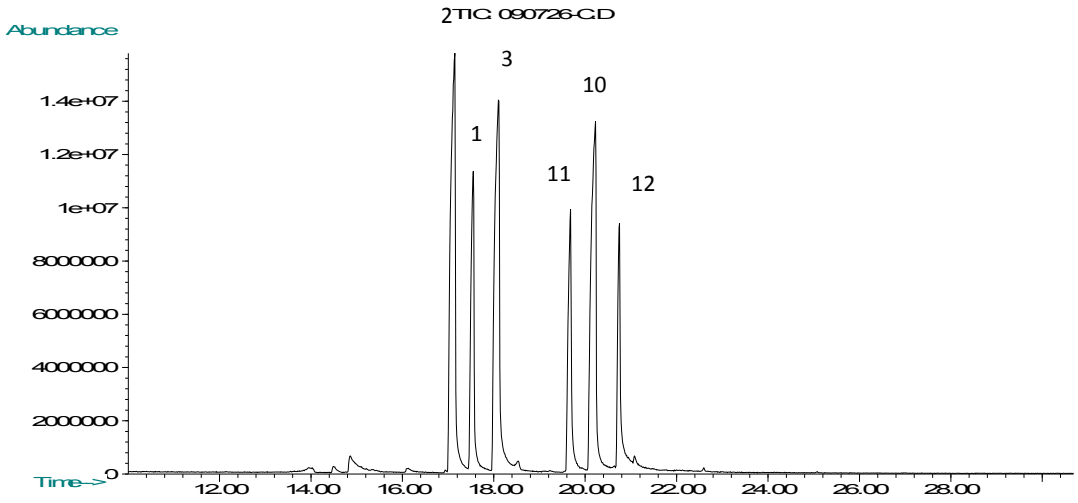


Figure 9: Capillary gas chromatograph of physical mixture of compounds 1,2,3,10,11 and 12 [underivatized], Column used Rxi-50, temperature program (TP-2).

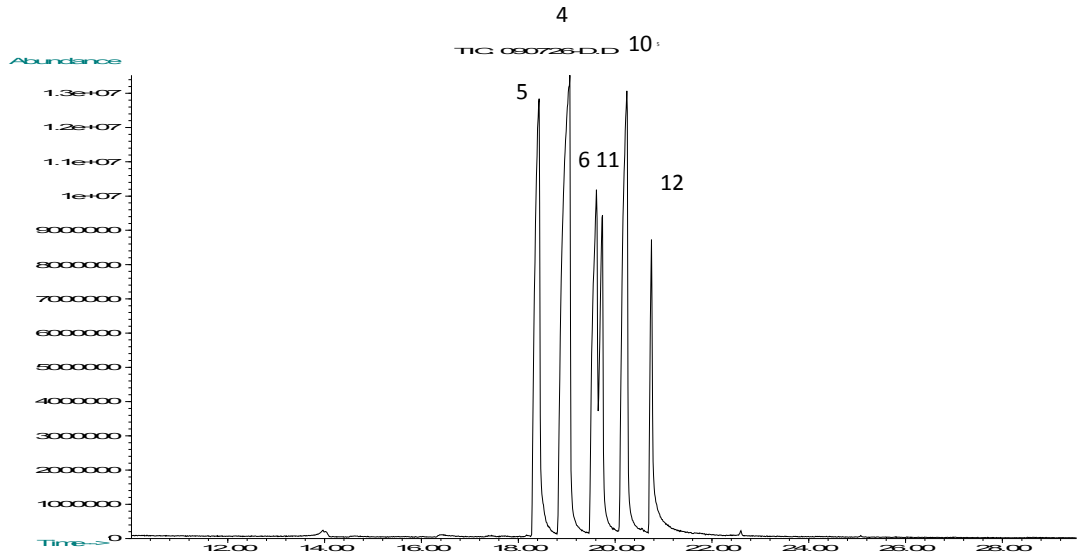


Figure 10: Capillary gas chromatograph of physical mixture of compounds 4,5,6,10,11 and 12 [underivatized], Column used Rxi-50, Temperature program (TP-2).

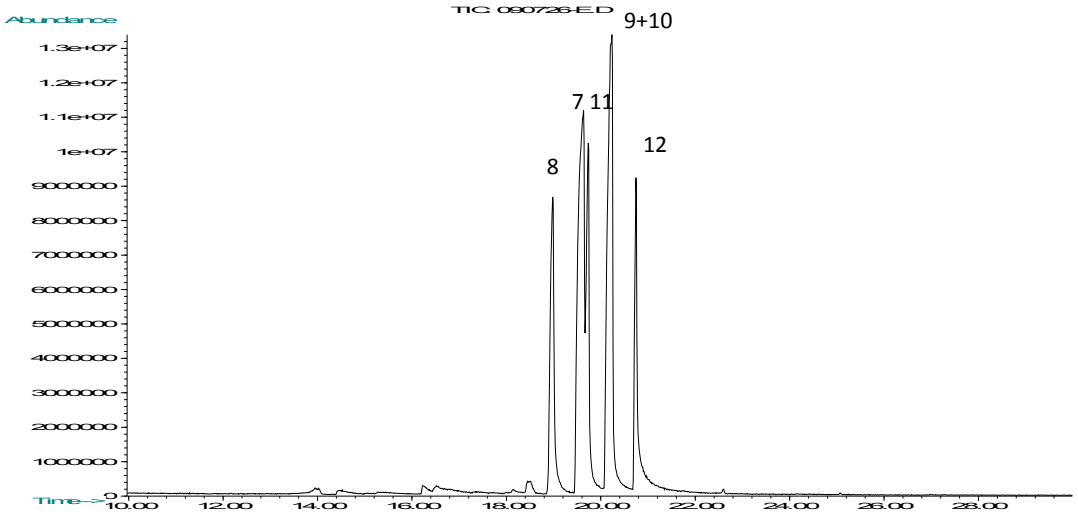


Figure 11: Capillary gas chromatograph of physical mixture of compounds 7,8,9,10,11 and 12 [underivatized], Column used Rxi-50, Temperature program (TP-2).

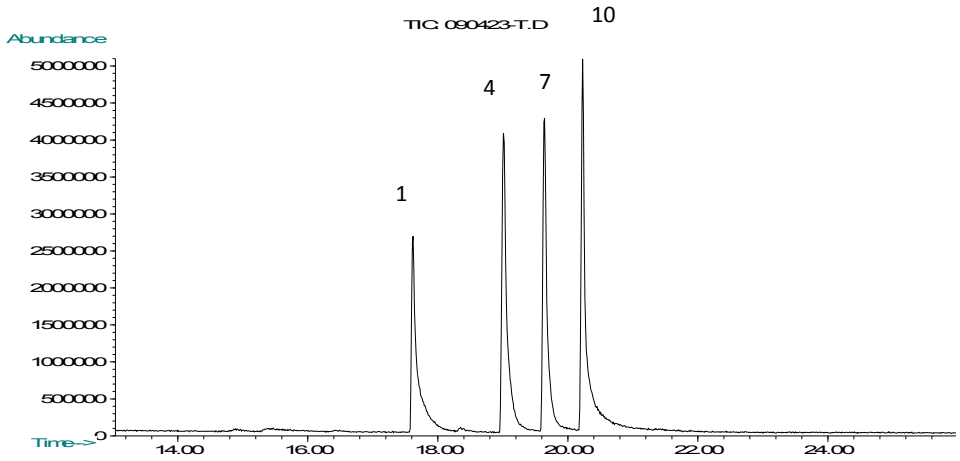


Figure 12: Capillary gas chromatograph of physical mixture of compounds 1, 4, 7 and 10 [underivatized], Column used Rxi-50, Temperature program (TP-2).

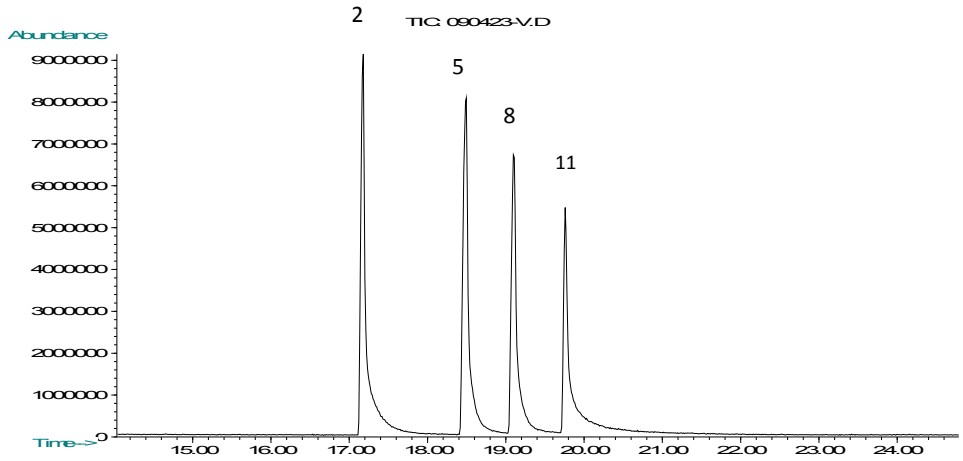


Figure 13: Capillary gas chromatograph of physical mixture of compounds 2, 5, 8 and 11[underivatized], Column used Rxi-50, Temperature program (TP-2).

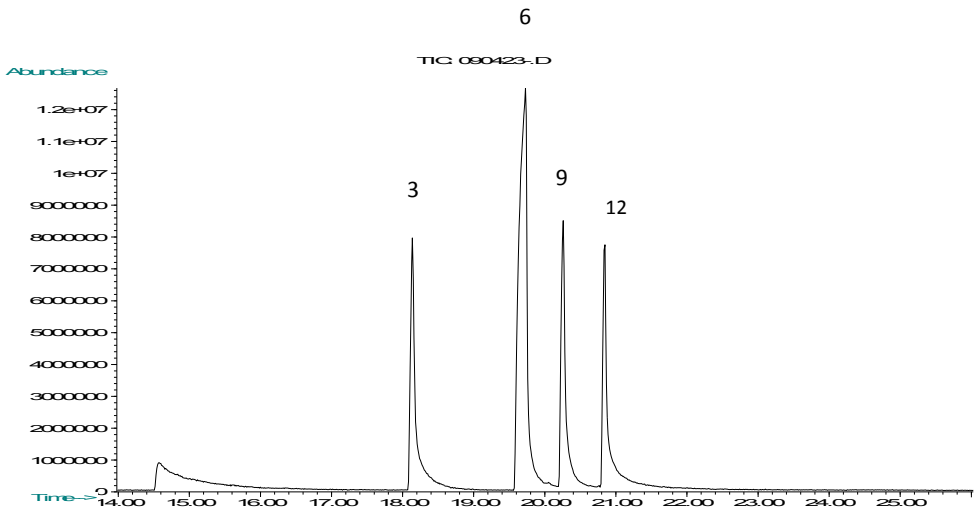


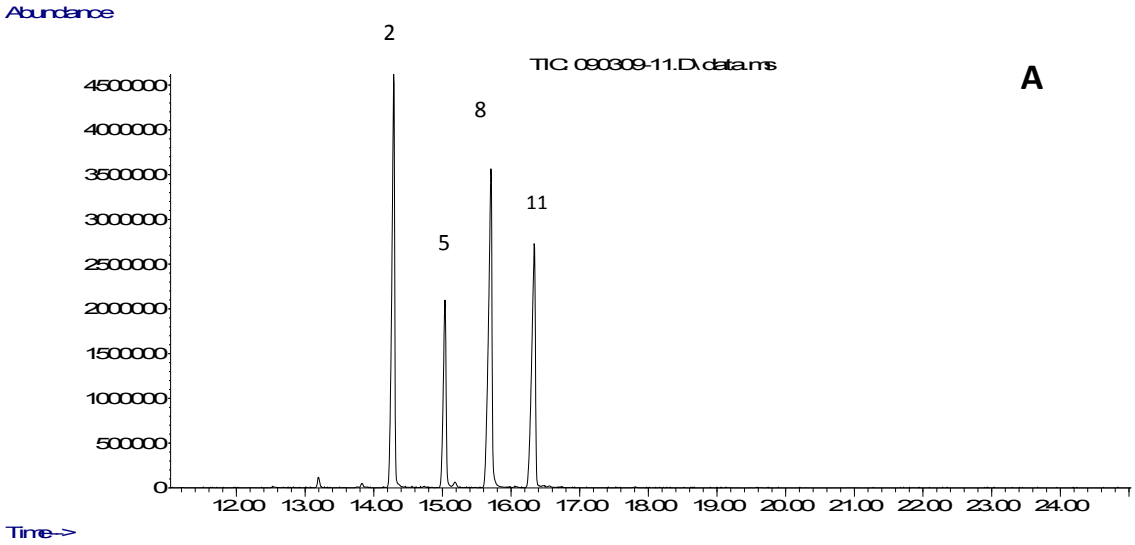
Figure 14: Capillary gas chromatograph of physical mixture of compounds 3, 6, 9 and 12 [underivatized], Column used Rxi-50, temperature program (TP-2).

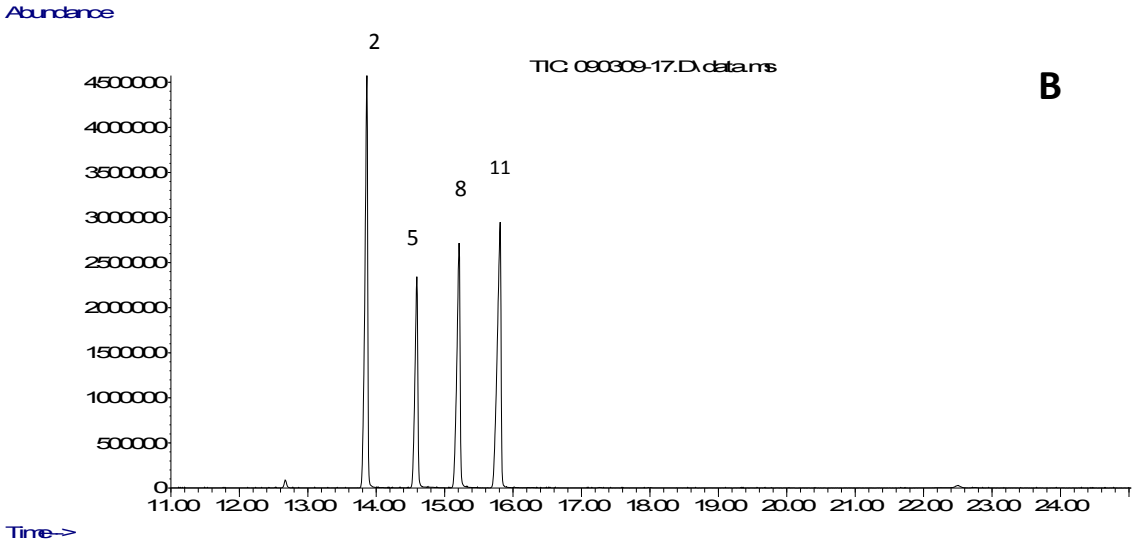
3.1.4.2. Gas Chromatography of Perfluoroacyl Derivatives:

A relatively nonpolar stationary phase of 100% dimethyl polysiloxane (Rtx-1) showed good resolution and adequate peak symmetry for the perfluoroacyl derivatives of the secondary amines, the derivatization process is mention section 4.3. The temperature program started at 100ºC for 1 minute, then ramped up to 180ºC at a rate of 9ºC per minute held for 2 minutes and increased to 200ºC at a rate of 10ºC/min and held for the remainder of the run (TP-4).

The chromatographic separation studies began by dividing the perfluoroacyl derivatives (HFBA, PFPA and TFA) into subset groups based on both the similarities of their ring and side chain substitutions. The first group consists of the HFBA, PFPA and TFA derivatives of the N-ethyl same side chain, regardless of differences in the ring

substations (Figure 15 A-C). The three perfluoroacyl derivatives of the N-methyl side chain group are shown in Figure 16 A-C. In all three subsets the ring substituted ethoxy isomer in the ortho position elutes before the meta then the para and finally the 3,4-methylenedioxy substitution pattern elutes last.





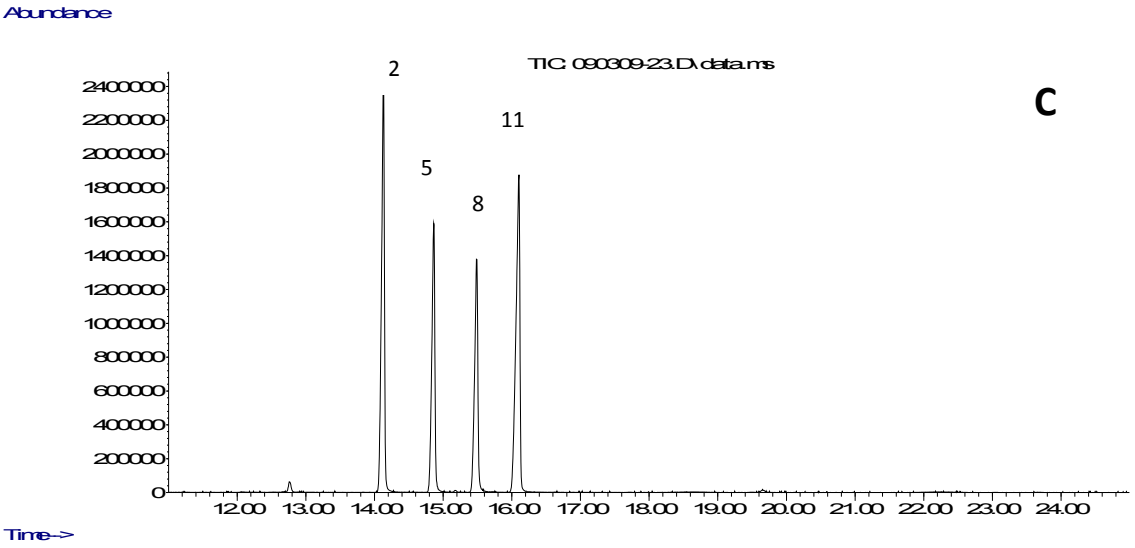
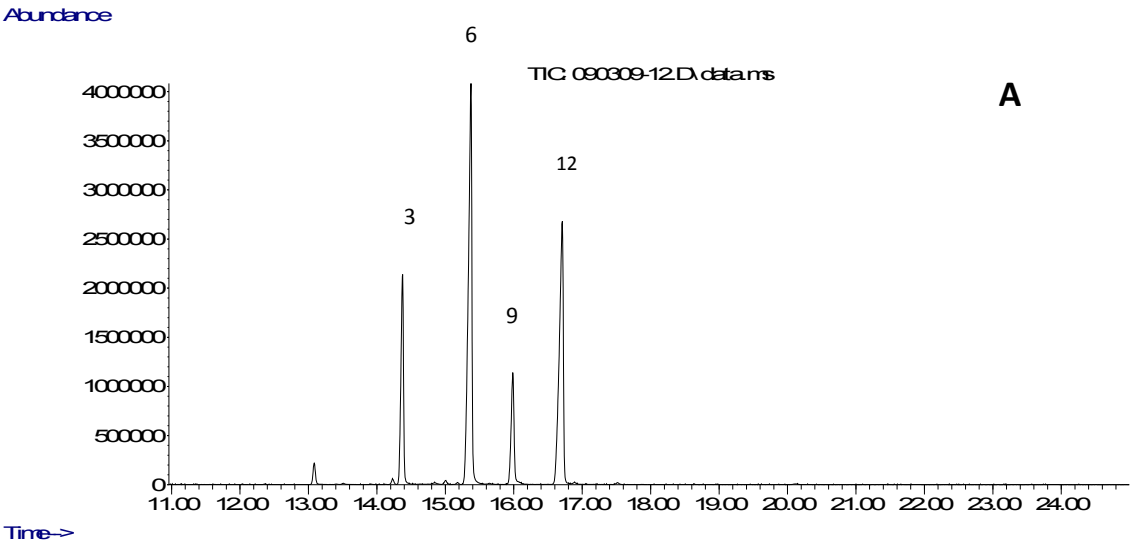


Figure 15: Capillary gas chromatograph of the perfluoroacyl derivatives of compounds 2, 5, 8 and 11. (A) HFBA, (B) PFPA and (C) TFA. Column Rxi-1, temperature program (TP-4).



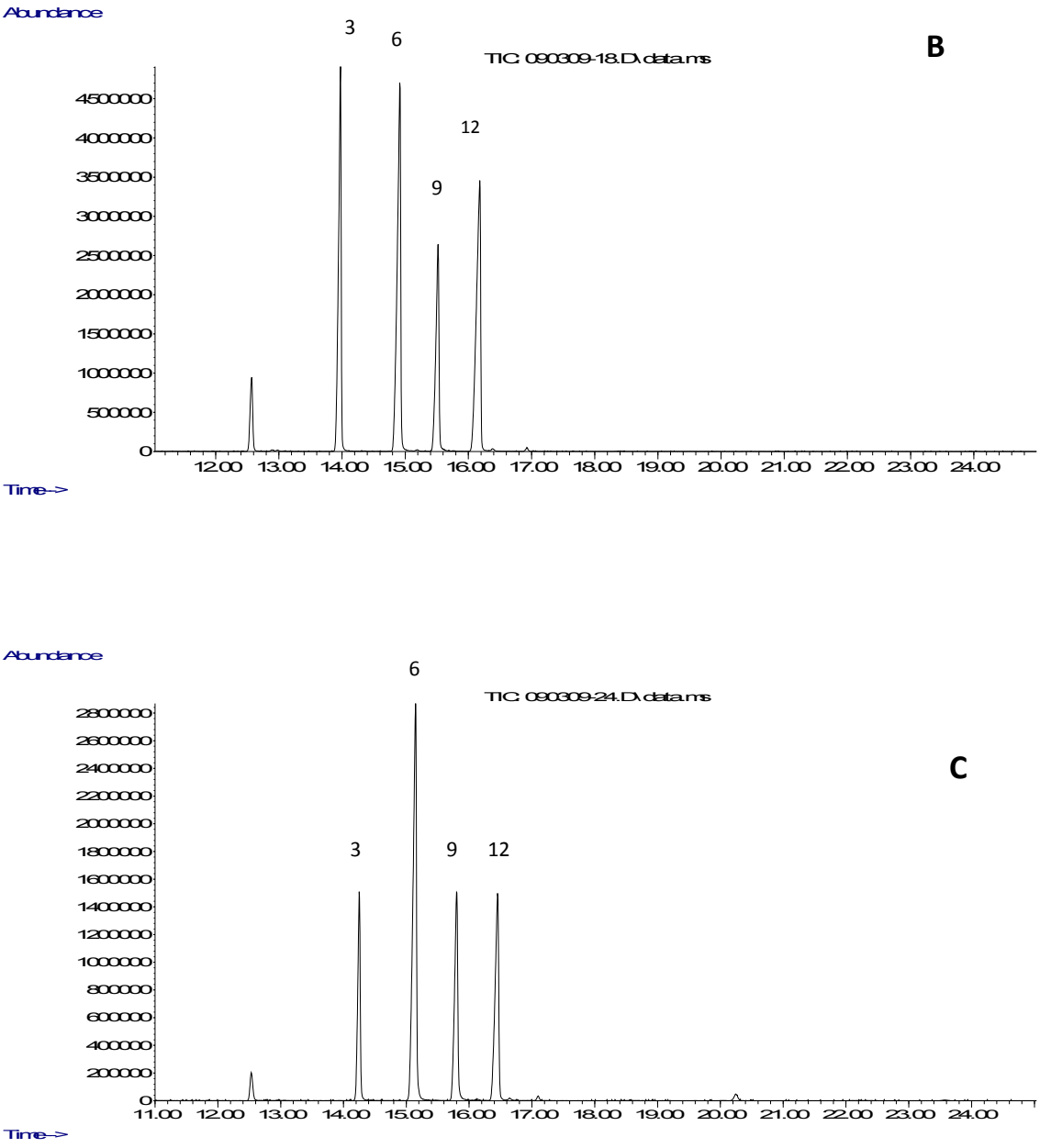
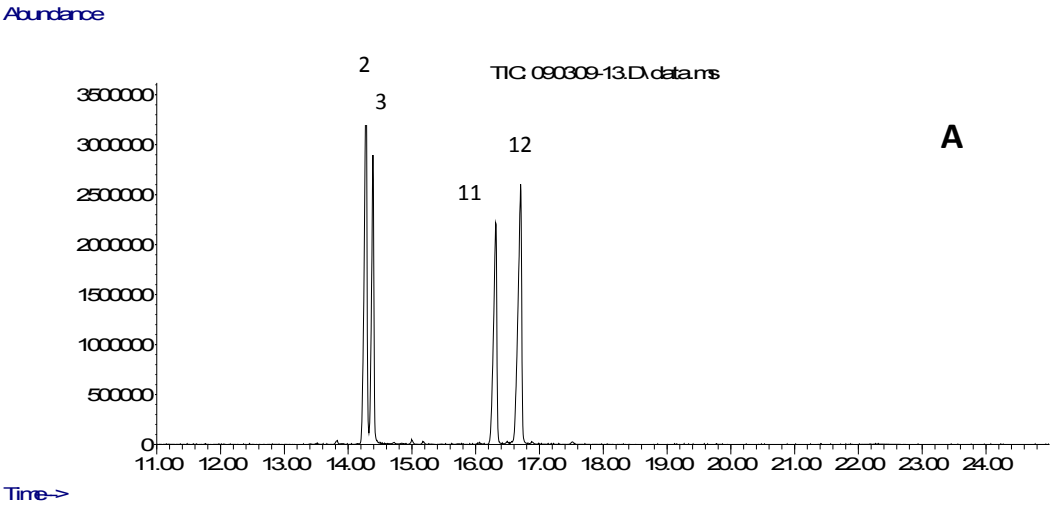
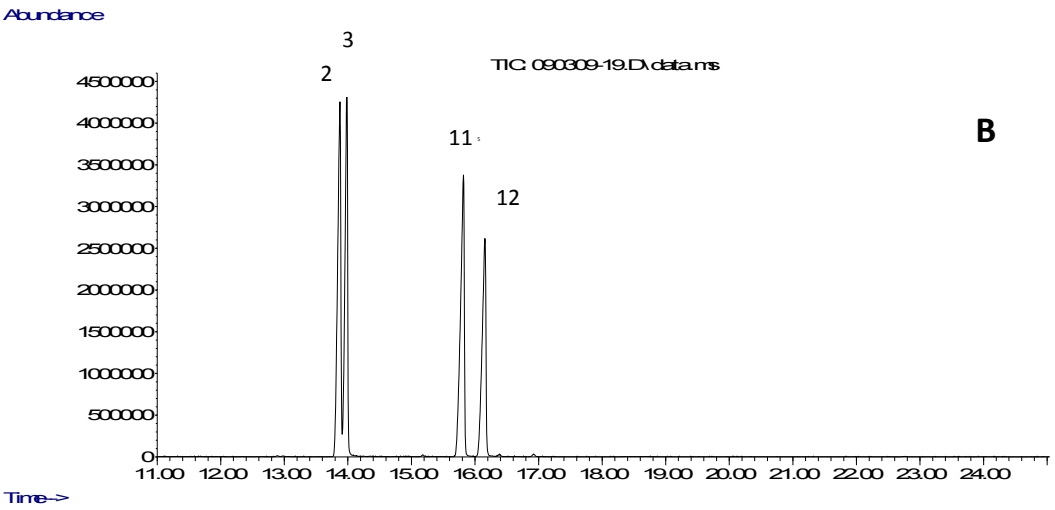


Figure 16: Capillary gas chromatograph of the perfluoroacyl derivatives of compounds 3, 6, 9 and 12. (A) HFBA, (B) PFPA and (C) TFA. Column Rxi-1, temperature program (TP-4).

The next group of chromatographic separation was based on preparing a number of physical mixtures were the perfluoroacyl derivatives with the same ring substitution

pattern were mixed with the perfluoroacyl derivatives of compounds 11 and 12. In all cases, the shorter un-interrupted alkane side chain amides elutes before the longer one when the ring substitution pattern is held constant within the limited series of compounds in the study. In other words, the N-ethyl isomer elutes before the N-methyl isomer of the same ring position. Also, the derivatized drugs of abuse (compounds 11 and 12) elute after the ethoxy ring substituent's (Figure 17, 18 and 19).





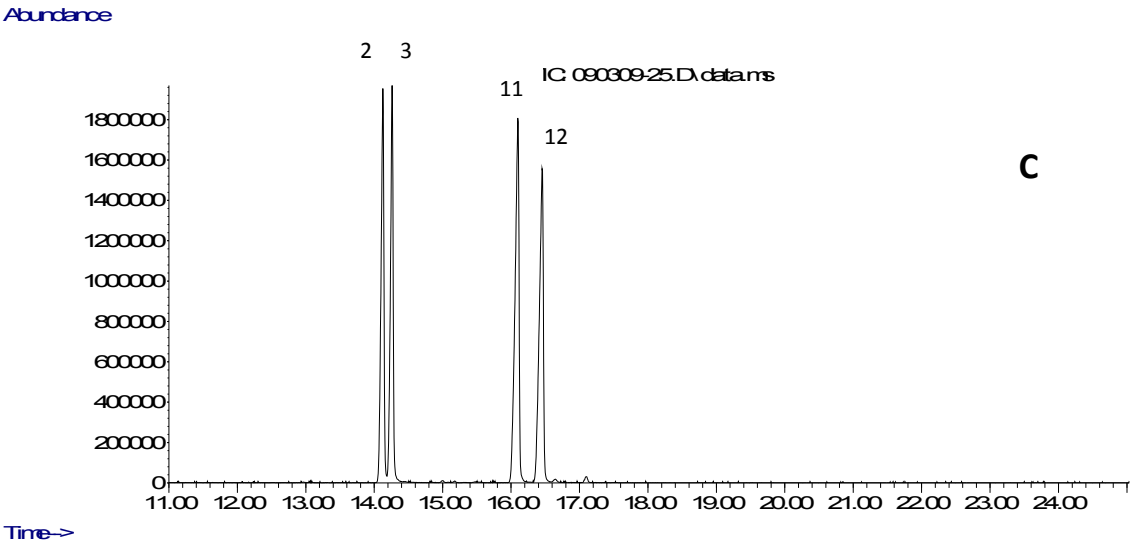
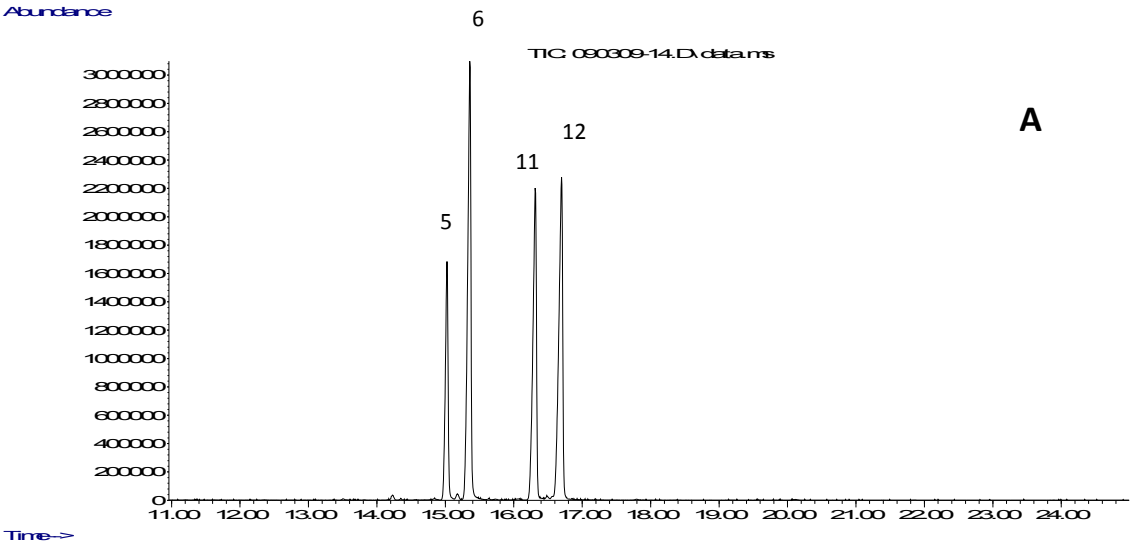


Figure 17: Capillary gas chromatograph of the perfluoroacyl derivatives of compounds 2,3,11 and 12. (A) HFBA, (B) PFPA and (C) TFA. Column Rxi-1, temperature program (TP4).



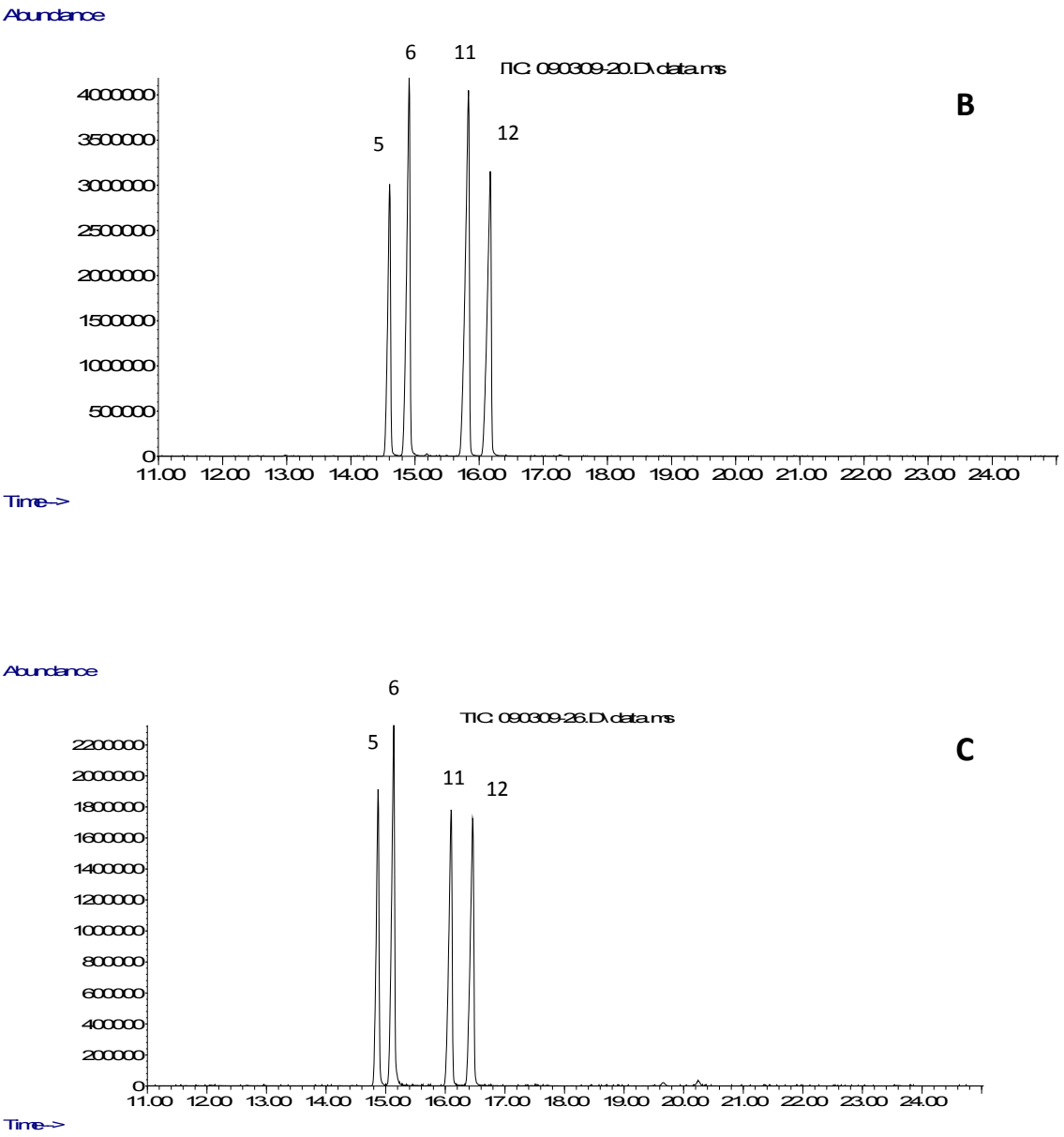
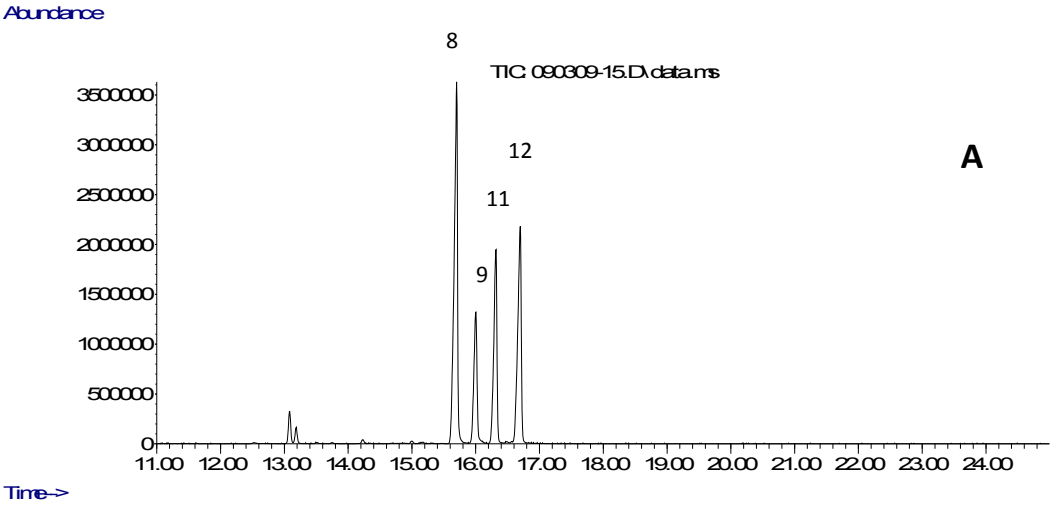


Figure 18: Capillary gas chromatograph of the perfluoroacyl derivatives of compounds 5, 6, 11 and 12. (A) HFBA, (B) PFPA and (C) TFA. Column Rxi-1, temperature program (TP-4).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A
11
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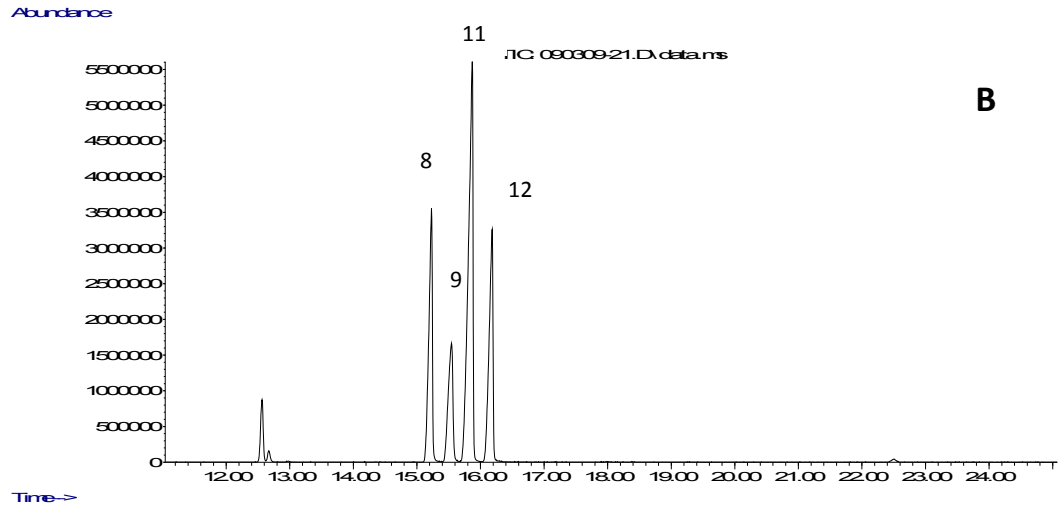
Time-->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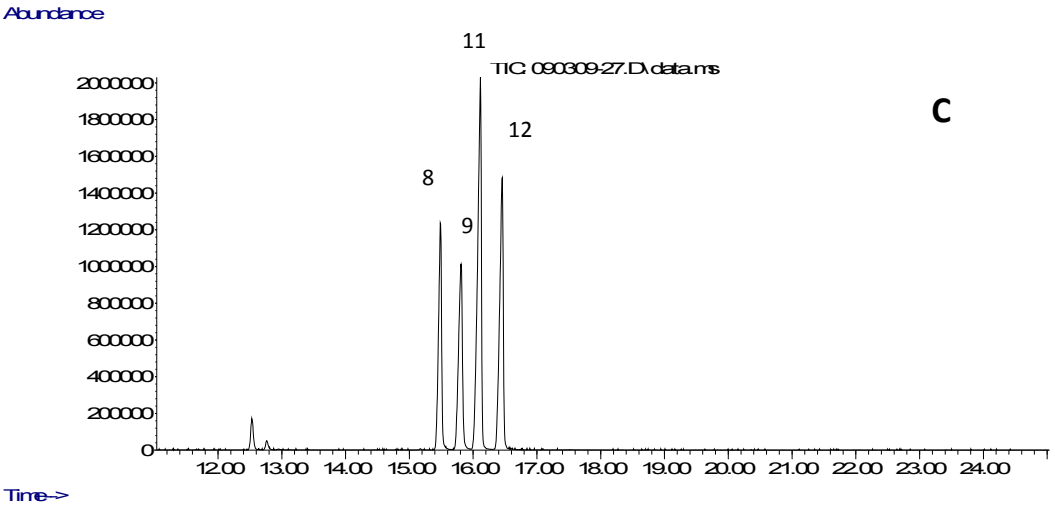
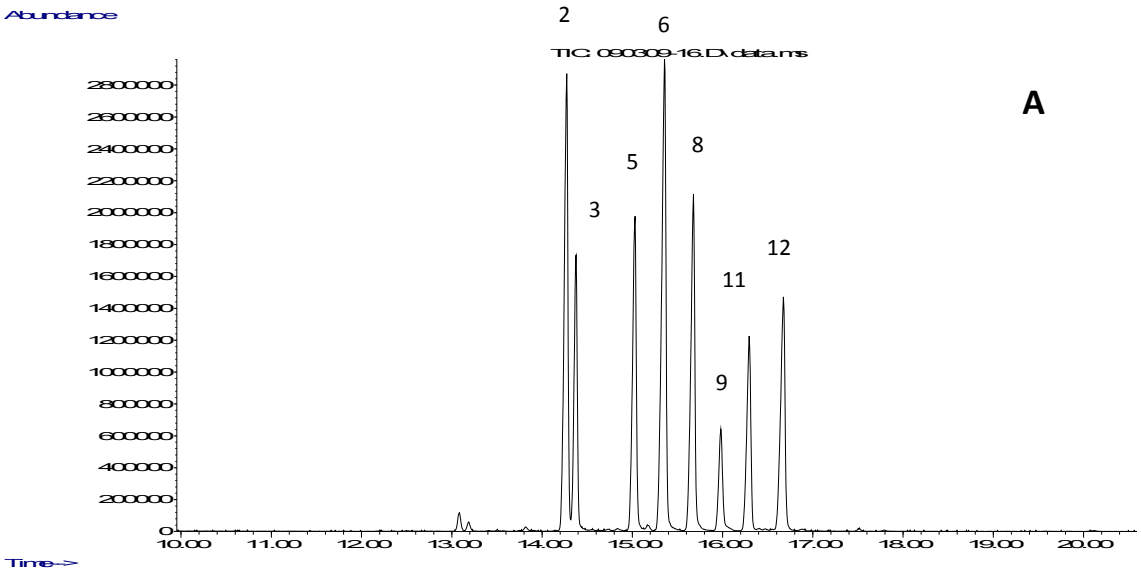
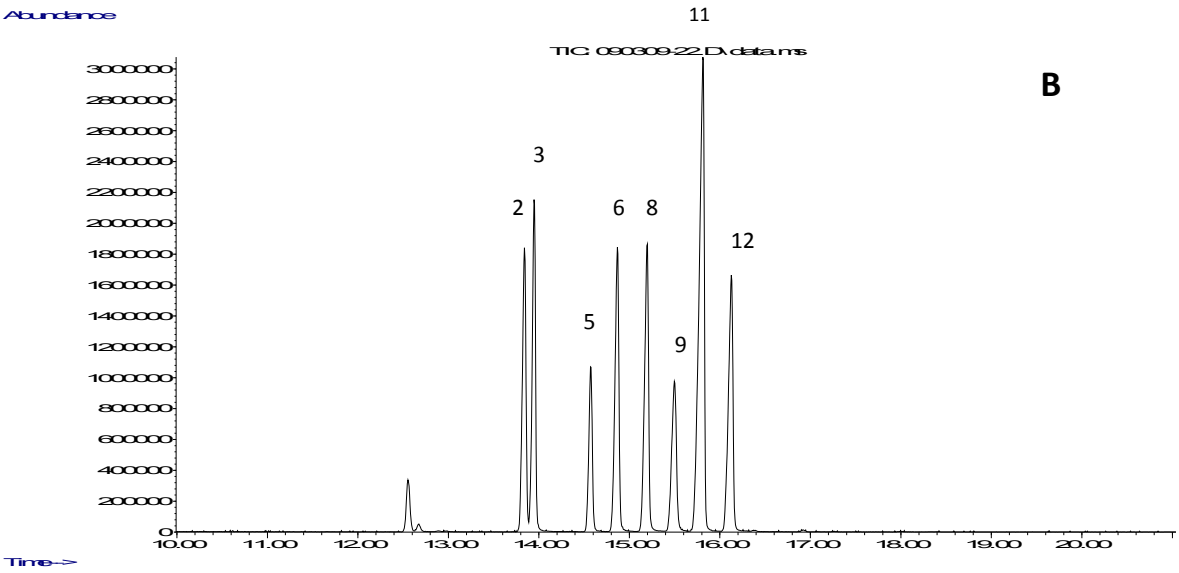


Figure 19: Capillary gas chromatograph of the perfluoroacyl derivatives of compounds 8,9,11 and 12. (A) HFBA, (B) PFPA and (C) TFA. Column Rxi-1, temperature program (TP-4).

The separations of the all eight HFPA, PFPA and TFA derivatives are shown in figure 20 A, B and C, respectively. The chromatogram showed the elution order followed the same rationale as before ware the ring substituted ethoxy isomer in the ortho position elutes before the meta then the para, in addition the N-ethyl isomer elutes before the N-methyl isomer of the same ring position and finally the derivatized drugs of abuse (compounds 11 and 12) elute last. Thus, GC coupled with MS offers significant discrimination among this set of compounds and GC-IRD provides confirmatory structure – IR spectral details among the studied compounds.


Abundance
TIC: 090309-16.D\data.ms
2
6
5
8
3
12
11
9
A
Time-->
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00


Abundance
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11
3
2
6
8
12
5
9
B
Time-->
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12.00
13.00
14.00
15.00
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17.00
18.00
19.00
20.00

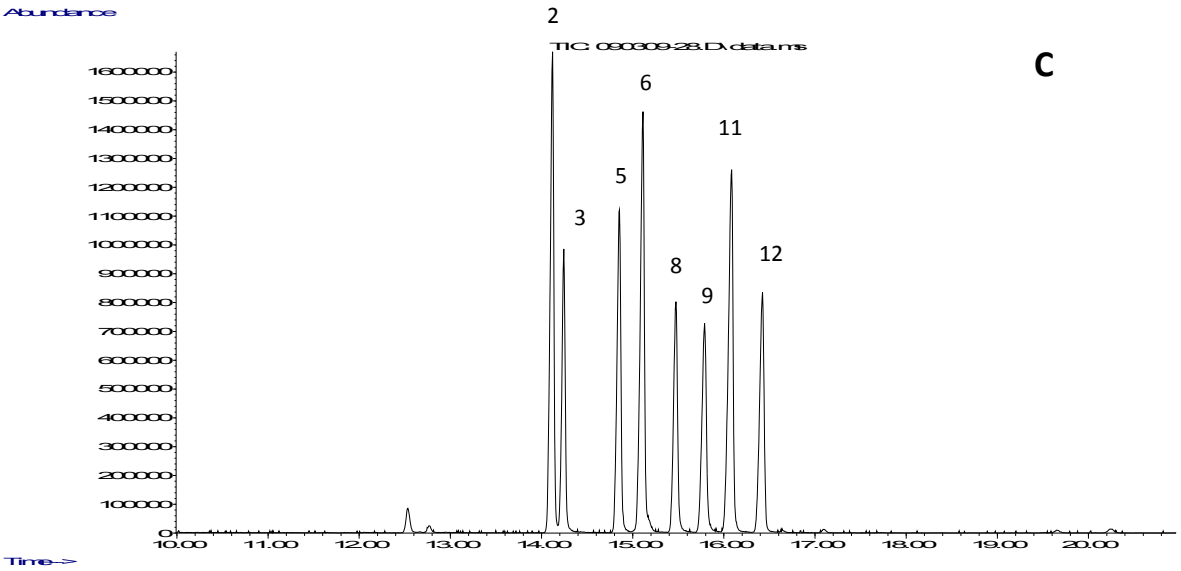


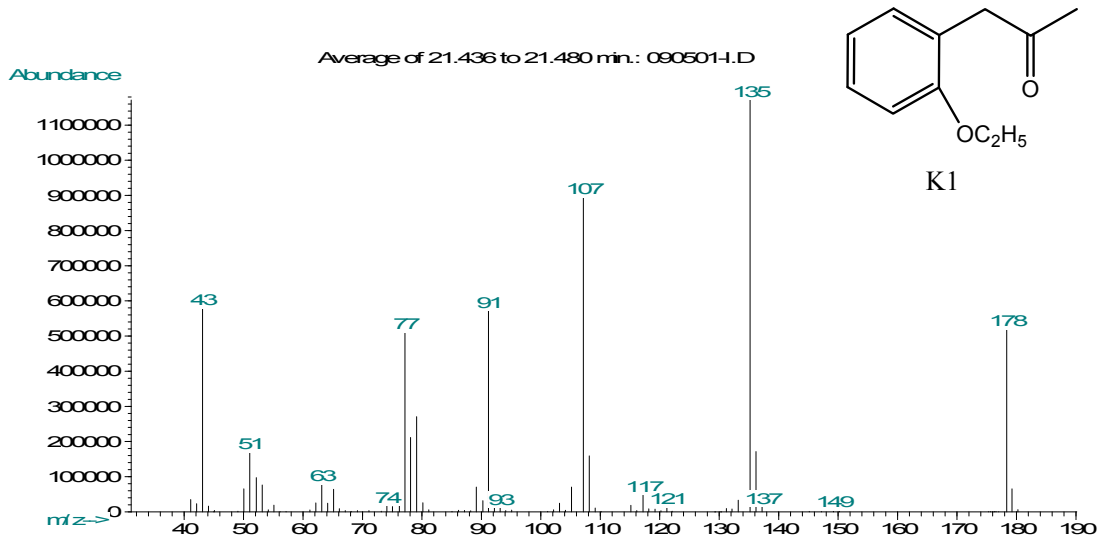
Figure 20: GC separation of the perfluoroacyl derivatives of compounds 2, 3, 5, 6, 8, 9, 11 and 12. (A) HFBA, (B) PFPA and (C) TFA. Column Rxi-1, temperature program (TP4).

3.2. Analytical Studies of Regioisomeric Ring Substituted Ethoxyphenyl-propan-2-one and Ethoxyphenylbutan-2-one Related to Ketone Precursors of MDEA, MDMMA or MBDB:

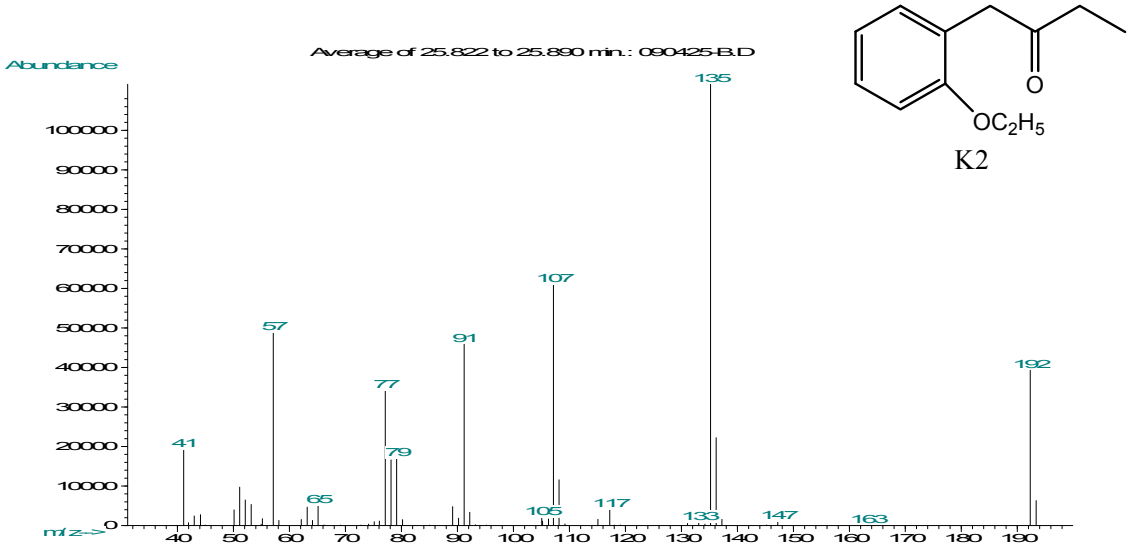
3.2.1. Mass Spectral Studies of Isobaric and Regioisomers of Ketone Precursors of MDMMA, MDEA or MBDB:

Figure 21 shows the EI mass spectra of all eight ketones used for the synthesis of the amines (see section 3.1.1). Compound K7 is the precursor ketone to the controlled substance MDMMA and MDEA. Compounds K1, K3 and K5 are the isobaric ketones to compound K7 that would produce the ring substituted ethoxy analog all have same molecule weight at 178 (group1). Compound K8 is the precursor ketone to the controlled substance MBDB. Compounds K2, K4 and K6 are the isobaric ketones to compound K8 that would produce the ring substituted ethoxy analog all have same molecule weight at 192 (group2).

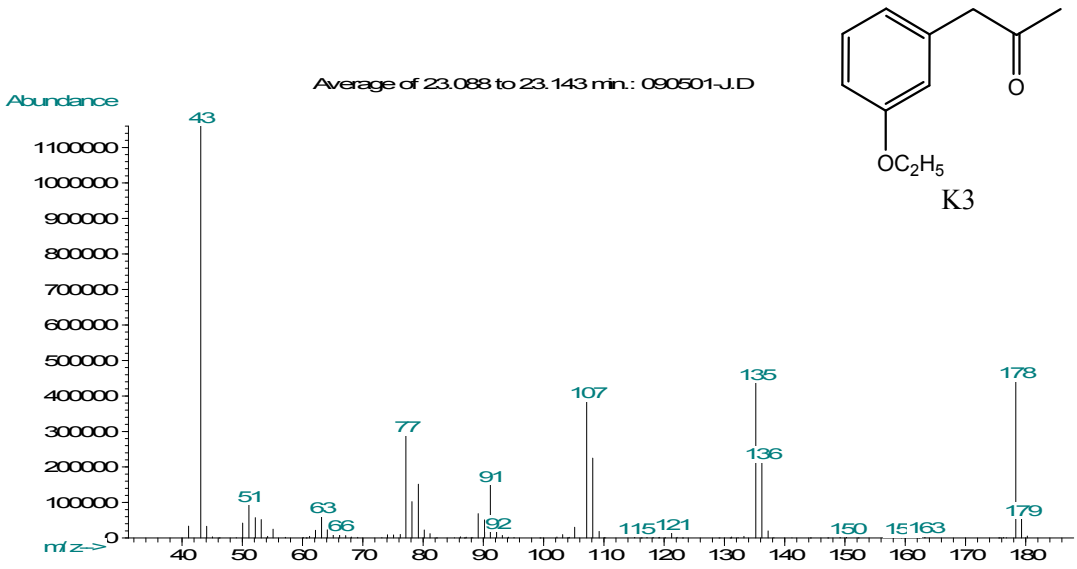
The mass spectra of the ketones in group 1 all yield a molecular ion at *m/z* 178, in addition to other fragment at *m/z* = 136, 135, 91, 77 and 43, however they show differences in there abundance intensity depending on the position of the ring substitution to the acetone group. The mass spectra of the ketones in group 2 all yield a molecular ion at *m/z* 192, in addition to other fragments at *m/z* = 136, 135, 91, 77 and 57. These fragments show differences in their relative abundance depending on the position of the ring substituent relative to the side chain group (Figure 21).


Average of 21.436 to 21.480 min.: 090501-I.D
Abundance
m/z-->
135
107
43
91
77
178
51
63
74
93
117
121
137
149
O
OC2H5

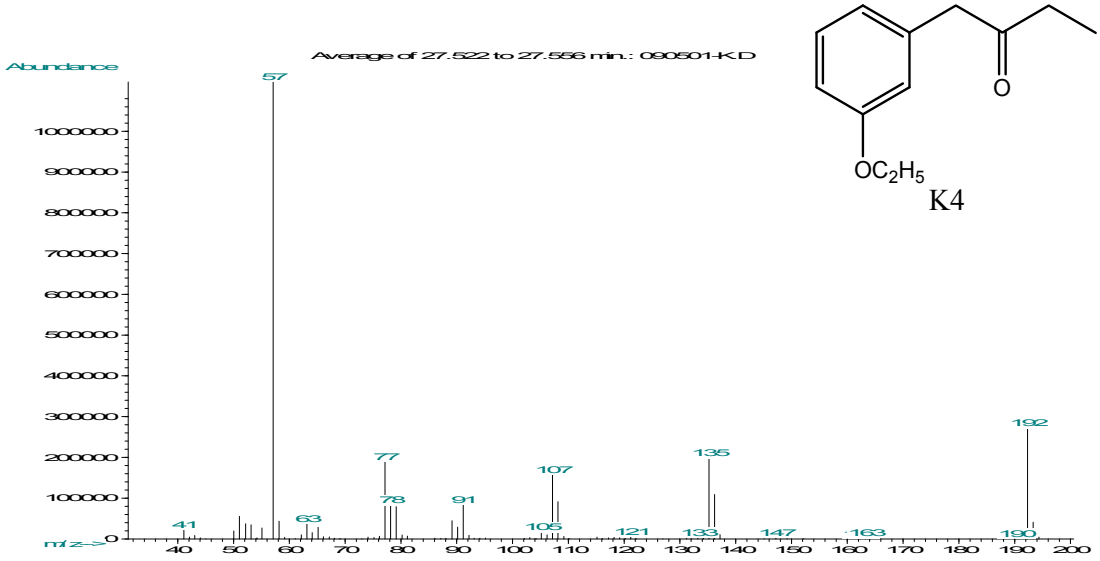
K1


Average of 25.822 to 25.890 min.: 090425-B.D
Abundance
m/z-->
135
107
57
91
77
192
41
79
65
105
117
133
147
163
O
OC2H5

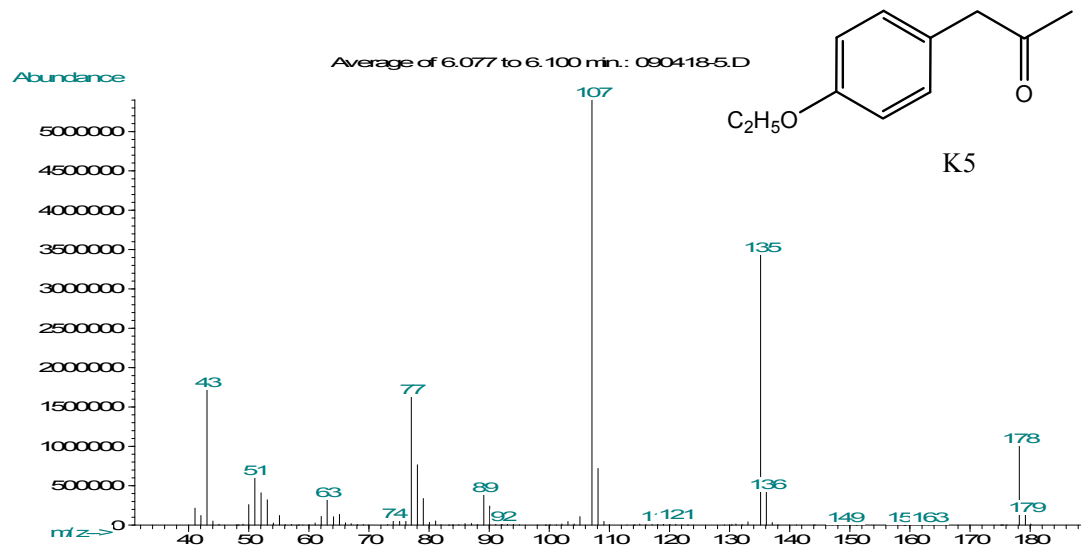
K2


Average of 23.088 to 23.143 min.: 090501-J.D
Abundance
m/z-->
43
51
63
66
77
91
92
107
115
121
135
136
150
163
178
179
O
OC2H5

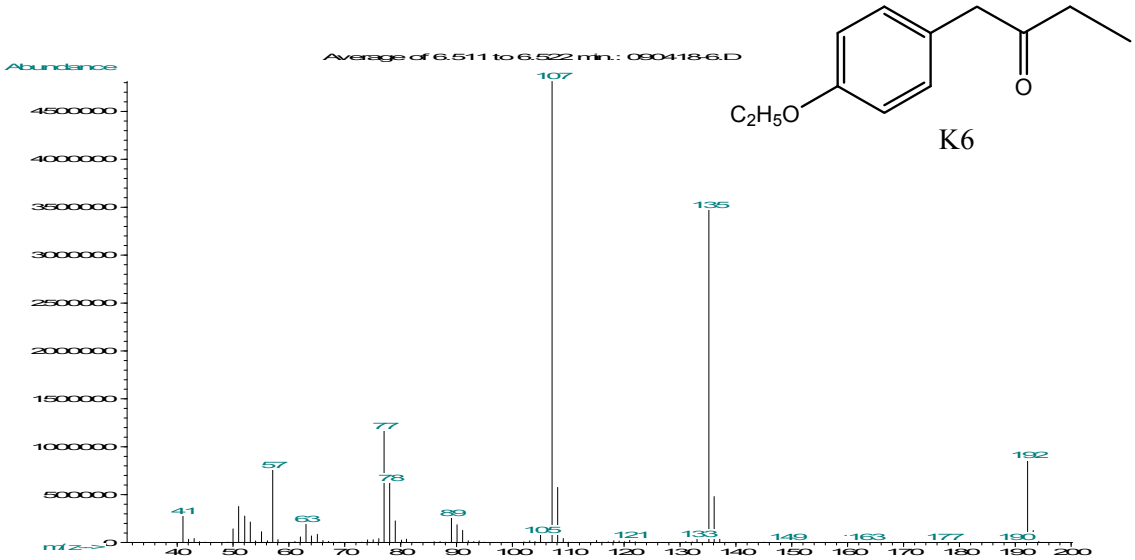
K3


Average of 27.522 to 27.556 min.: 090501-K.D
Abundance
m/z-->
57
41
63
77
78
91
105
107
121
133
135
147
163
190
192
O
OC2H5

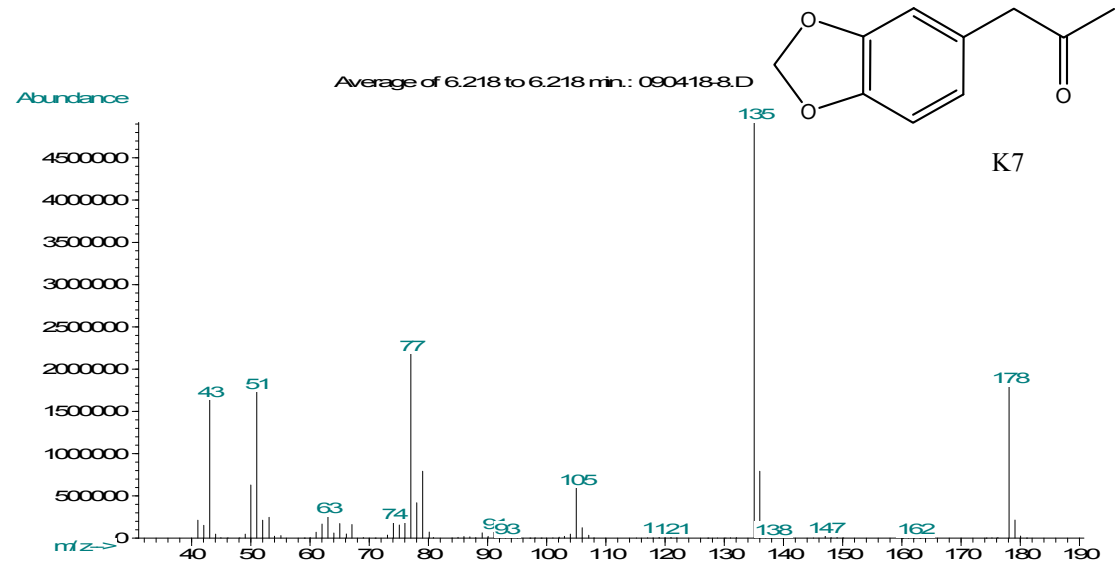
K4


Average of 6.077 to 6.100 min.: 090418-5.D
Abundance
m/z-->
107
135
43
77
178
51
63
89
136
74
92
121
149
163
179
C_2H_5O
O

K5


Average of 6.511 to 6.522 min.: 090418-6.D
Abundance
m/z-->
107
135
77
78
57
192
41
63
89
105
121
133
149
163
177
190
C_2H_5O
O

K6



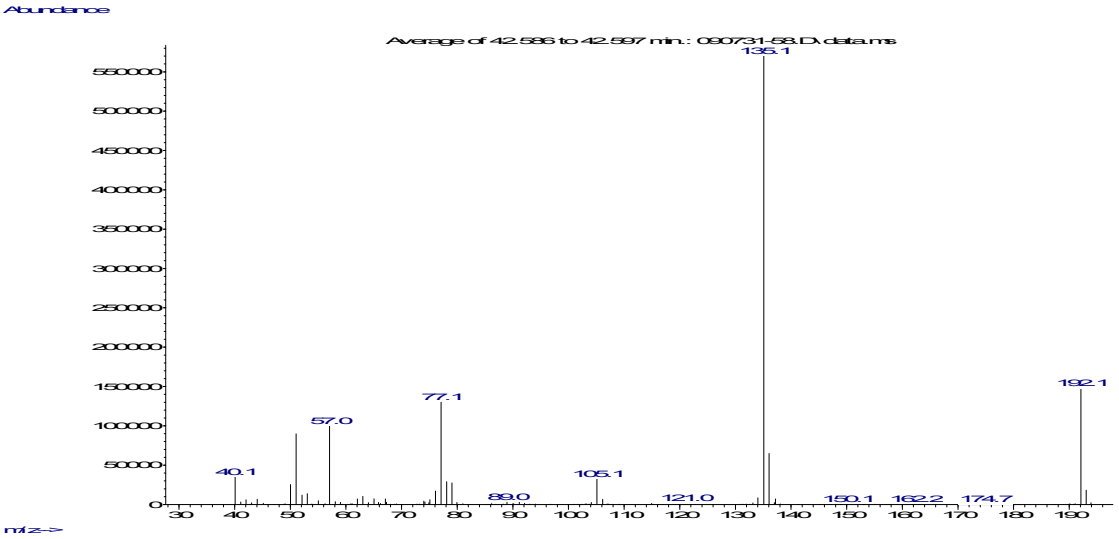
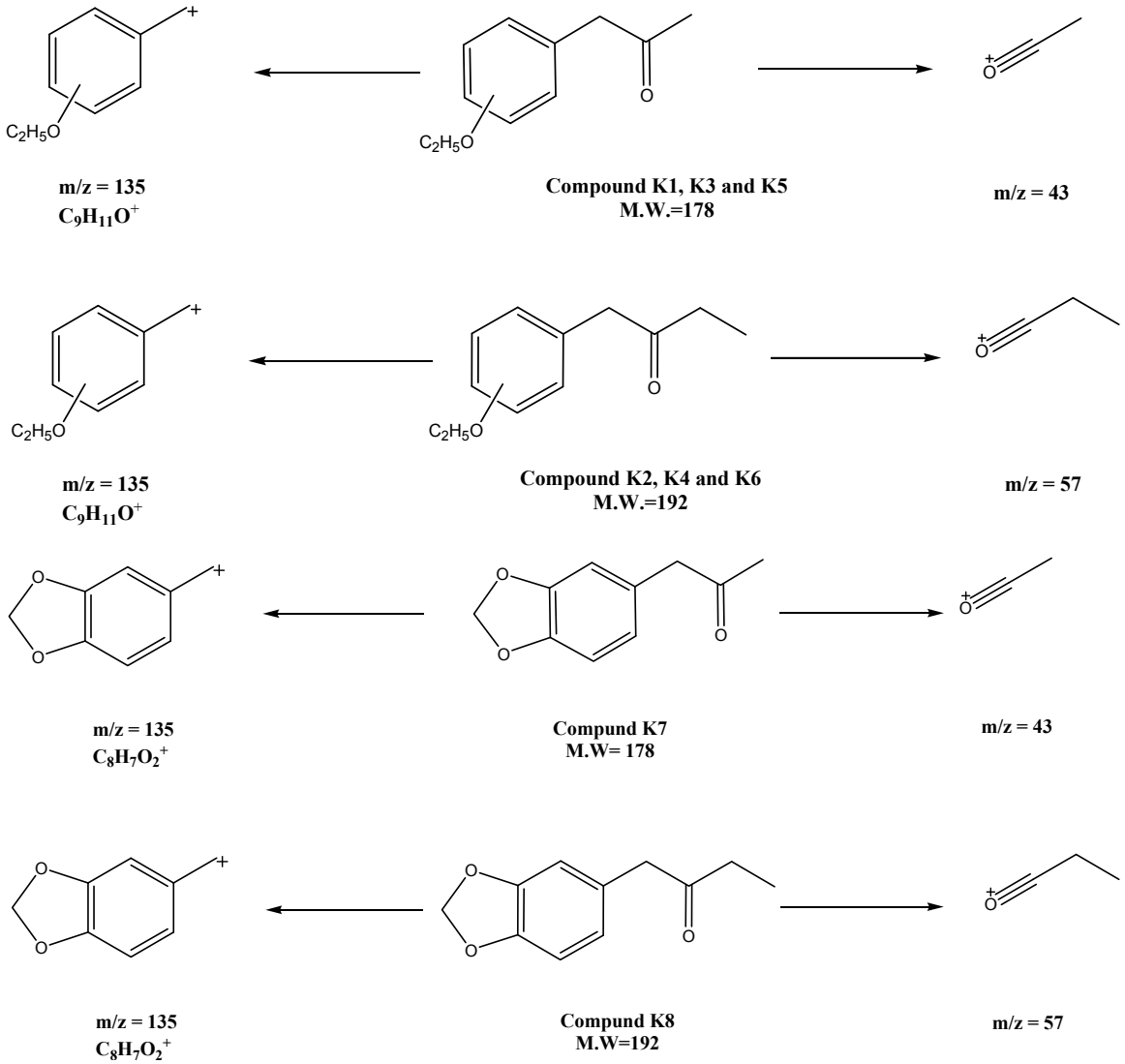
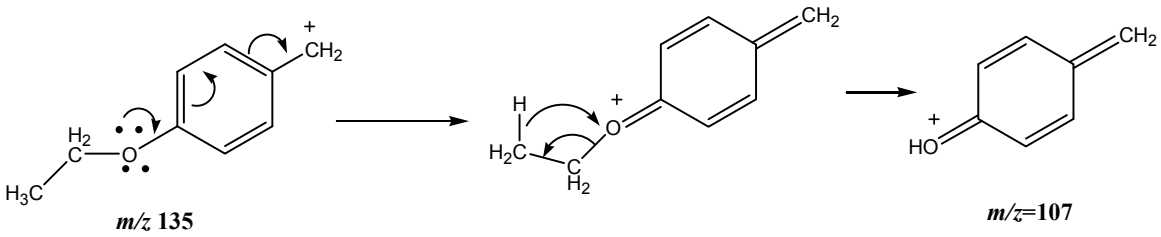


Figure 21: Mass spectra of compounds K1-K8.

Fragmentations at *m/z* 135/136 were observed in the mass spectra of both groups. This ion represents the ethoxybenzyl and 3,4-methylenedioxybenzyl cation and radical, respectively, formed through the ionization of the aromatic ring. In fact, the ethoxybenzyl cation is the base peak of the ketone having the ethoxy-ring substituted at the ortho position (K1 and K2) and as well as the ring substituted 3,4-methylenedioxy ketones (K7 and K8). Another major fragment ions are at *m/z* 43 and 57 for group 1 ketones (K1, K3, K5 and K7) and group2 (K2, K4, K6 and K8), respectively. These ions formed by an α-cleavage reaction involving the carbon–carbon ring and carbonyl oxygen (Scheme 9). These fragments at *m/z* 43 and 57 are the base peak for the ketones where the ethoxy group is in the meta position and consequently can be used as characteristic fragments to discriminate these two ketones from the other ketones involved in this study. A fragment at *m/z* 107 was observed in the mass spectra of the ring substituted ethoxyphenyl-2-propanones and ethoxyphenyl-2-butanones regioisomers (K1–K6) of different relative abundance representing the loss of 28 mass units (ethylene, C_2H_4). This fragment is formed through the loss of 28 mass unit (C_2H_4) from the ethoxybenzyl cation at *m/z* 135(Figure 21) This ion is absent in the mass spectra of the 3,4-methylenedioxyl ketones and a discriminatory fragment between the ethoxy ring substitution pattern from the methylenedioxy ring substitution pattern which lacks this fragment in there mass spectra. The *m/z* 107 is the base peak in K5 and K6 where the ethoxy ring substitution at the para position The general fragmentation pathways for these compounds are shown in (Scheme 9 and 10).



Scheme 9: EI fragmentation pattern for compounds K1-K8.



Scheme 10: Formation of *m/z* 107 in the mass spectra of ring substituted ethoxyphenyl-propan-2-ones and ethoxyphenylbutan-2-ones.

3.2.2. Vapor Phase Infra-red Spectrophotometer:

As described earlier, infrared spectrometry is often used as a confirmatory method for drug identification in forensic drug analysis. Gas chromatography with infrared detection (GC–IRD) was evaluated for differentiation among the eight regioisomeric ketones in this study. The use of infra-red spectrophotometry as a supportive method in the identification of drugs in forensic laboratories has been widely documented [Belal, 2009; Awad, 2009; Duncan, 1988].

The IR-spectra of compounds K1-K8 were generated in the vapor-phase following sample injection into the gas chromatograph and spectra are shown in figure 22. Each compound shows a vapor-phase IR spectrum with absorption bands in the regions 650–1700 cm^{-1} and 2900–3100 cm^{-1}. The regions between 650–1700 cm^{-1} is most useful in the identification and differentiation among this set of compounds, however the regions of 2900–3100 cm^{-1} did not provide unique bands. In general, variations in the side chain composition results in small variations in the IR spectrum of the carbonyl

stretching band near 1725-1730 cm^{-1}, even when the ring substitution pattern was kept constant. On the other hand, if the side chain composition is kept constant and only the ring substitution is changed, this results in a noticeable differences of their IR absorption bands in the region 650 –1650 cm^{-1}.

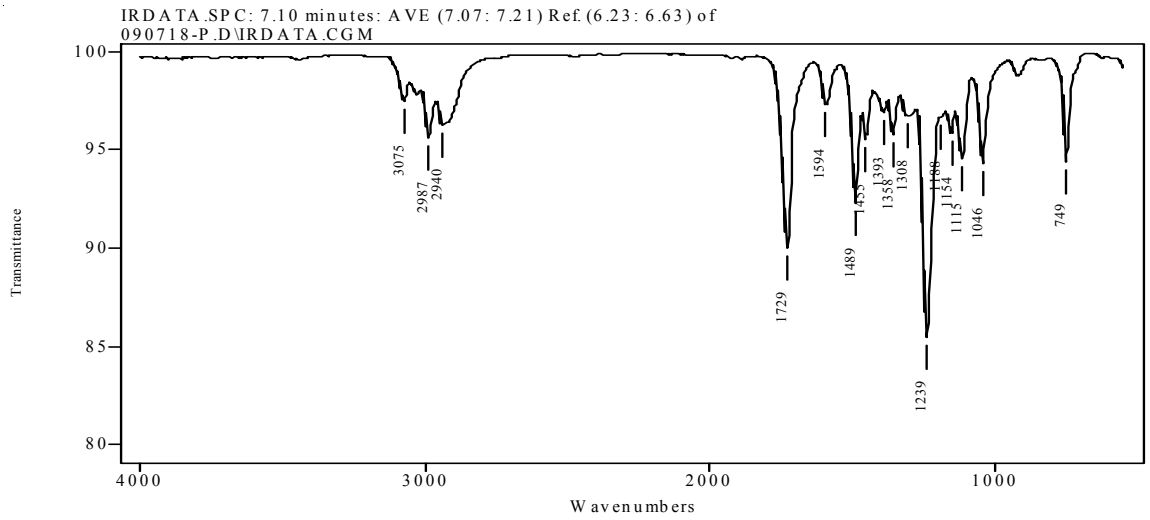
These compounds show that when the ring substitution is kept constant the infra-red spectrum 650 –1700 cm^{-1} are almost identical. These ketones show a strong absorption peak from carbonyl group near 1729 cm^{-1}. The eight ketones were divided to two groups based on the number of carbons of the carbonyl side chain attached to the ring (group1: K1, K3, K5 and K7) and (group2: K2, K4, K6 and K8).

In the first group, compound K1 is characteristic by a medium intensity absorption peak at 749 cm^{-1}, which is absent in the IR spectra of all other compounds within the same group. Additionally, compound K1 shows a strong absorption band at 1239 cm^{-1} which is shifted to 1254, 1242 and 1246 cm^{-1} in the IR spectra of compounds K3, K5 and K7, respectively. Another characteristic peak for compound K1 is the medium intensity singlet peak at 1489 cm^{-1}, which is shifted to 1486 cm^{-1} for K3. A singlet peak of high intensity is shown at 1509 cm^{-1} for K5 and 1489 cm^{-1} for K7.

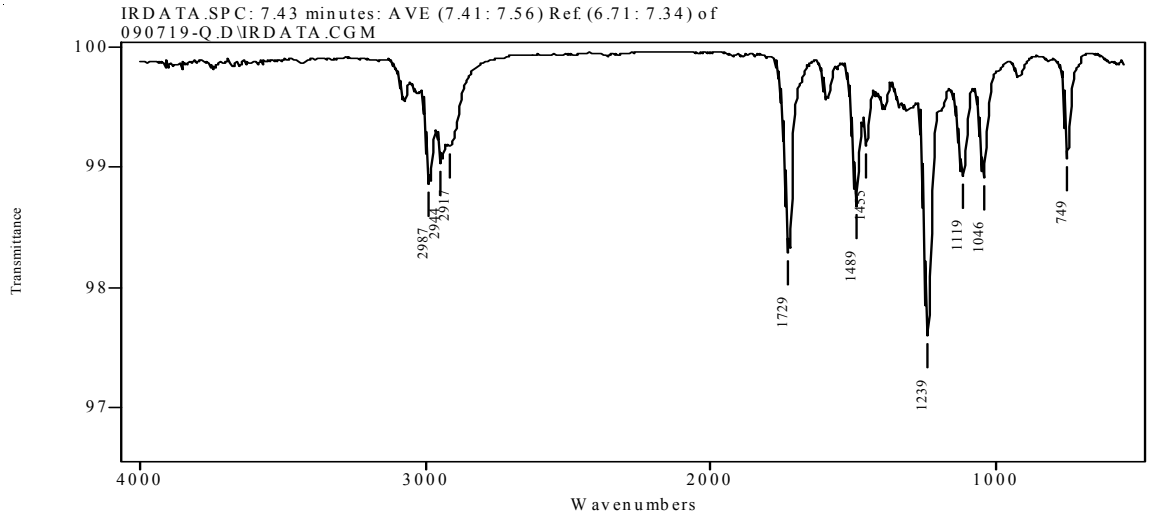
The IR spectrum of compound K3 shows a characteristic singlet absorption peak near 1601cm^{-1} which does not exist in the IR spectra of the other compounds in the group but instead, peaks of different absorption intensities were observed at 1594, 1613 and 1611 cm^{-1} for K1, K5 and K7, respectively. Compound K5 could be identified by the strong peak at 1509 cm^{-1} and finally K7 show a characteristic strong peak at 1489 cm^{-1}.

Differentiation between ring substitution patterns within the other groups of side chain substitution pattern could be carried out in an analogous manner.

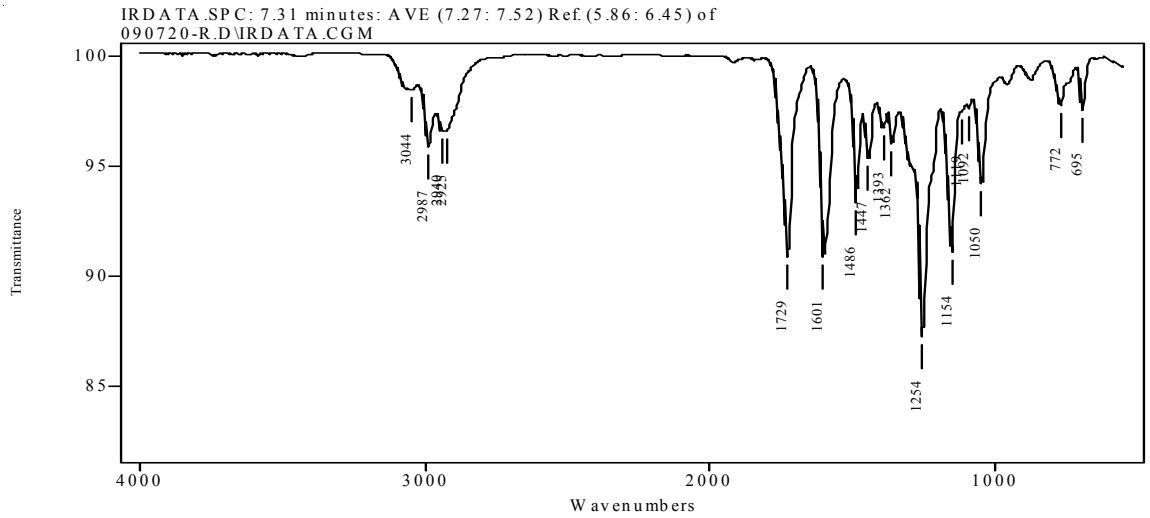
Vapor phase IR spectra of 1-(2-ethoxyphenyl)-propan-2-one (K1)



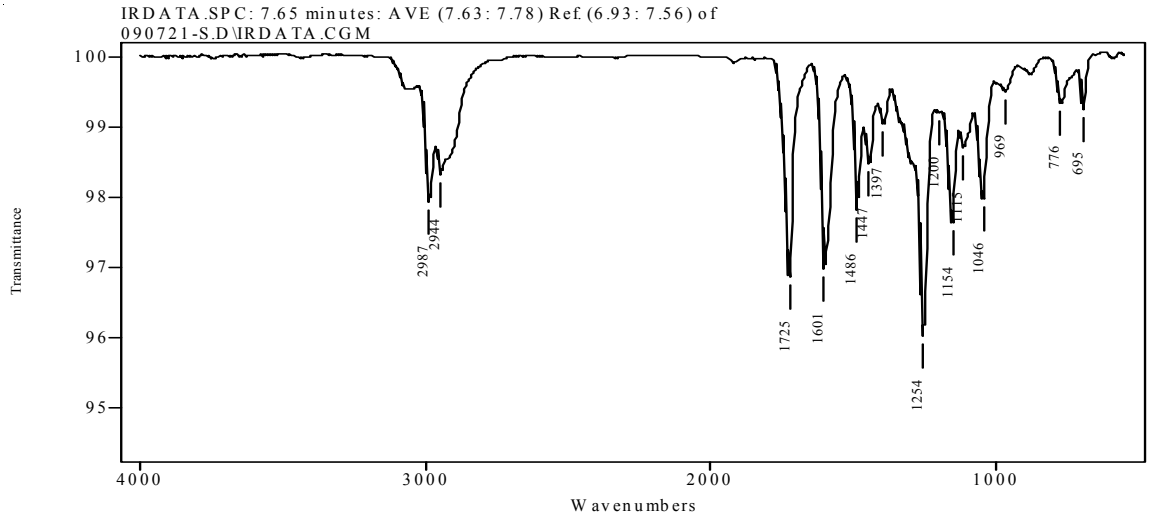
Vapor phase IR spectra of 1-(2-ethoxyphenyl)-butan-2-one (K2)



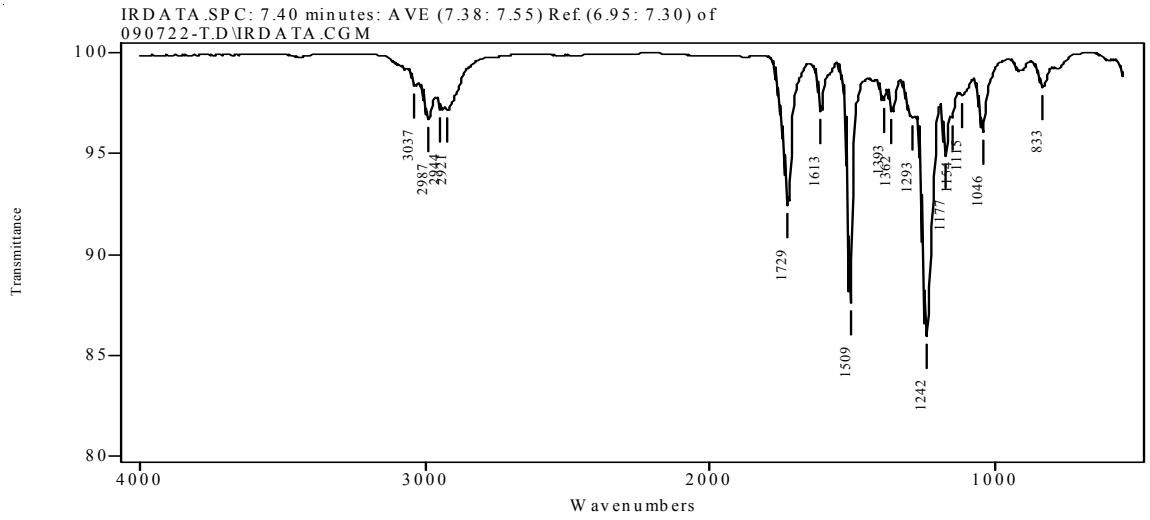
Vapor phase IR spectra of 1-(3-ethoxyphenyl)-propan-2-one (K3)



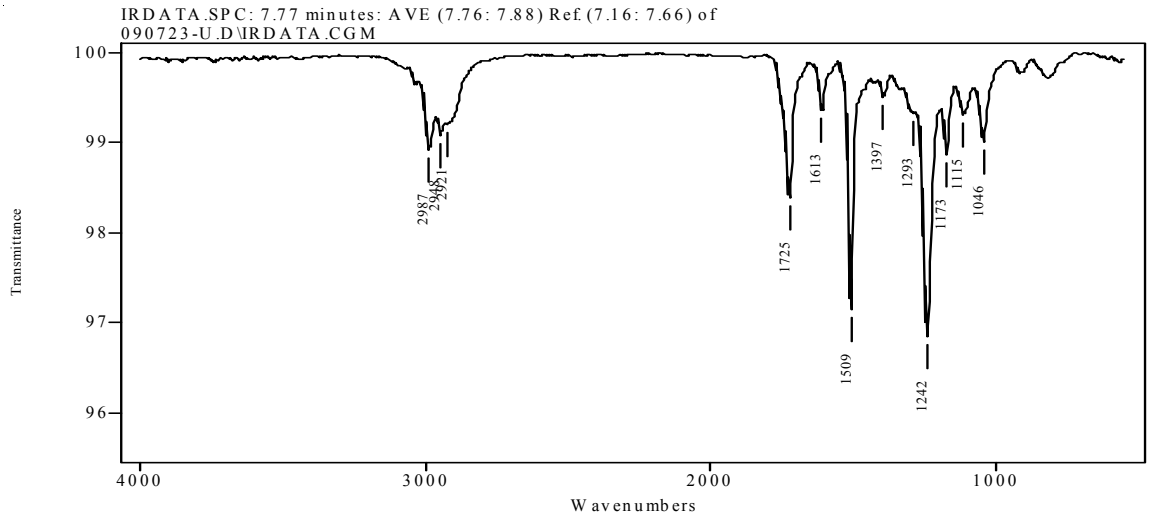
Vapor phase IR spectra of 1-(3-ethoxyphenyl)-butan-2-one (K4)



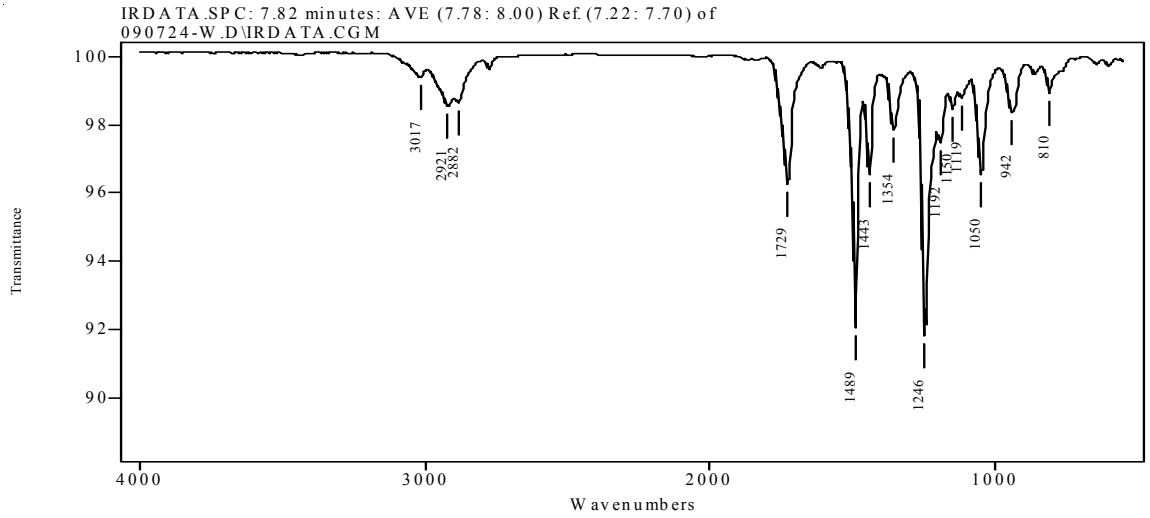
Vapor phase IR spectra of 1-(4-ethoxyphenyl)-propan-2-one (K5).



Vapor phase IR spectra of 1-(4-ethoxyphenyl)-butan-2-one (K6).



Vapor phase IR spectra of 1-(3,4-methylenedioxyphenyl)-propan-2-one (K7)



Vapor phase IR spectra of 1-(3,4-methylenedioxyphenyl)-butan-2-one (K8)

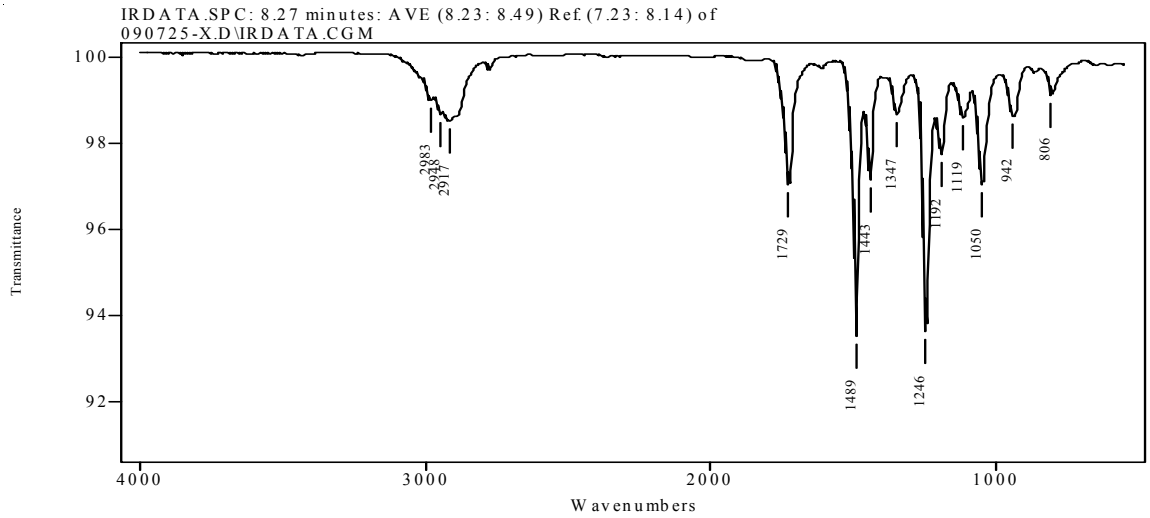


Figure 22: Vapor phase IR spectra of compounds K1-K8.

3.2.3. Gas Chromatography:

GC-MS analysis was carried on two GC-MS interments; one was a 7890A gas chromatograph, 7683B auto injector and a 5975C VL mass selective detector (Agilent Technologies, Santa Clara, CA). The mass spectral scan rate was 2.86 scans per second. The GC was operated in splitless mode with a carrier gas (helium grade 5) and flow rate of 0.7 ml/min .The mass spectrometer was operated on electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230ºC. Samples were diluted in HPLC grade acetonitrile as both an individual solutions and as physical mixtures and then introduced directly into the GC via the auto injector using an injection volume of 1 µl. The columns used were 30 m × 0.25 mm i.d. coated with 0.25 µm 100% dimethyl polysiloxane (Rtx- 1) and 30 m × 0.25 mm i.d. coated with 0.25 µm trifluoropropylmethylpolysiloxane (Rtx- 200). The second GC-MS instrument was HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). The MS was operated in the electron impact (EI) mode using ionization voltage of 70 eV and a source temperature of 230ºC. Samples were dissolved in high-performance liquid chromatography-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and manually introduced (1µL), individually, using a 10-µL Hamilton syringe (Hamilton Co., Reno, NV). The column used was 30 m × 0.25 mm i.d. coated with 0.5 µm 50% phenyl – 50% methyl polysiloxane (Rxi-50).

The temperature program used was to hold oven temperature at 70^{o}C for 1 minute, ramped up to 150^{o}C at a rate of 2.5^{o}C per minute, held at 150^{o}C for 3 minutes

then ramped up to 250^{o}C at a rate of 15^{o}C per minute and maintained at 250^{o}C for the reminder of the run.

For each of the eight compounds in the study, there chromatographic properties were evaluated as individual compounds and in physical mixtures. Ketones were divided into two subsets based on their molecular weight. The first subset consists of the four compounds of regioisomeric ring substituted ethoxyphenyl-2-propanone (K1, K3 and K5) and 3,4- methylenedioxyphenyl-2-propanone (K7). The second subset contain the other ketones of the regioisomeric ring substituted ethoxyphenyl-2-butanone (K2, K4 and K6) and 3,4-methylenedioxyphenyl-2-butanone (K8). Several stationary phases and different temperature programs were evaluated in an attempt to achieve the best compromise between resolution and retention time.

Figure 23 and 24 shows the chromatographic separation of the compounds in subset 1 and subset 2 respectively on the relatively nonpolar 100% dimethyl polysiloxane capillary column (Rtx-1). The elution order in both chromatograms is the same rational with the compounds having the ethoxy ring substitution at the ortho position elutes first followed by the meta then para position and finally 3,4-methylenedioxy ring substituted ketones. Figure 25 shows chromatographic separation of all 8 compounds in the study. The elution order of within both subset remain unchanged, however the shorter un-interrupted carbon side chain of the ketones (K1, K3, K5 and K7) elutes before the longer one (K2, K4, K6 and K8).

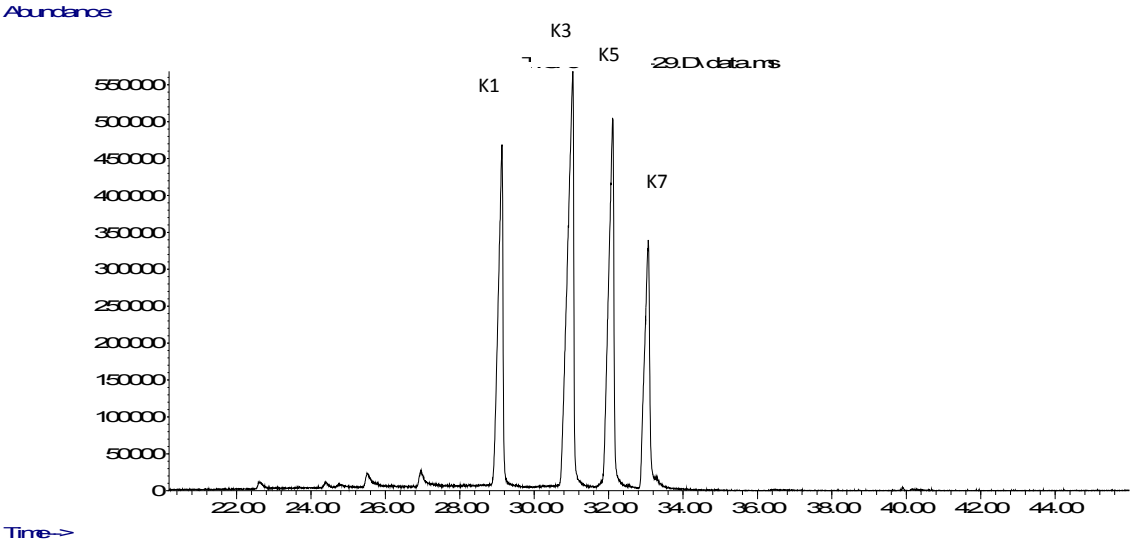


Figure 23: Gas chromatographic separation of compounds K1, K3, K5 and K7.Column used Rtx-1, temperature program (TP-5).

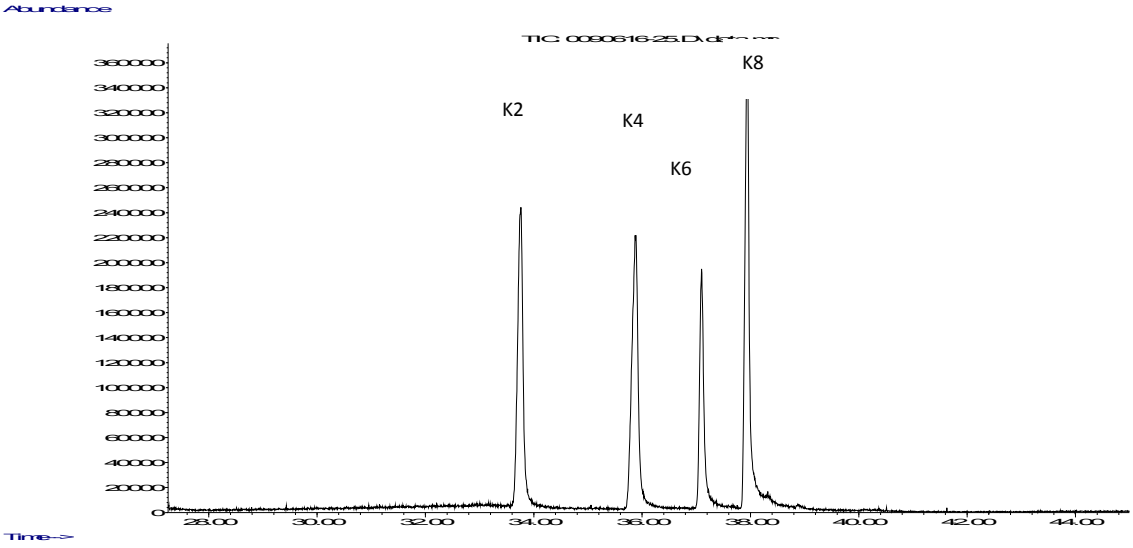


Figure 24: Gas chromatographic separation of compounds K2, K4, K6 and K8. Column used Rtx-1, temperature program (TP-5).

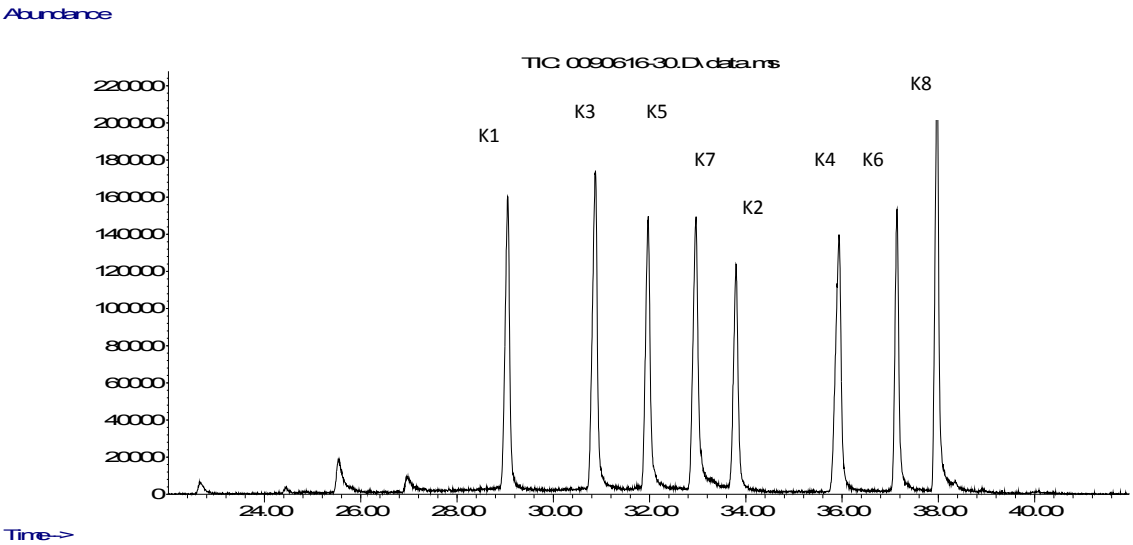


Figure 25: Chromatographic separation of compounds K1- K8. Column used Rtx-1, temperature program (TP-5).

Retention times of the studied compounds differ acceding to differences in column polarity. Ketones are found to be less retained in polar columns such as Rxi-50 compared to the nonpolar column Rtx-1 (table 4).

Table 4: Retention times (min) of compounds K1-K8*.

Column Used	Compound K1	K2	K3	K4	K5	K6	K7	K8	
Rtx-1	**28.824**	**33.575**	**30.631**	**35.632**	**31.744**	**36.955**	**32.700**	**37.806**	**Retention Time(min.)**
Rxi-50	**21.647**	**25.699**	**23.168**	**27.543**	**24.185**	**29.035**	**24.887**	**29.559**	
Rtx-200	39.100	39.957	40.546	40.668	41.268	41.356	41.910	42.697	

* Columns Rtx-1, Rxi-50 and RTX-200. Temperature Program (TP5).

Thus, GC coupled with MS offers significant discrimination among this set of compounds, and GC-IRD provides additional confirmatory structure – IR spectral details among the studied compounds.

4. EXPERIMENTAL

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

4.1.1 Materials:

The starting materials used for the synthesis of the regioisomers and isoboric compounds involved in this study were piperonal, 2-ethoxybenzaldehyde, 3-hydroxybenzaldehyde, 4-ethoxybenzaldehyde, n-butylamine, nitroethane, 1-nitropropane, iron powder, methylamine hydrochloride, dimethylamine hydrochloride, ethylamine hydrochloride, potassium carbonate and sodium cyanoborohydrde. Chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Benzene, toluene, hydrochloric acid, glacial acetic acid, methylene chloride, methanol, ferric chloride, acetone, 2-propanol, anhydrous sodium sulfate and HPLC grade acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

4.1.2 Instruments:

GC-MS analyses were carried on two GC-MS interments. Instrument 1 was a Agilent 7890A gas chromatograph, 7683B auto injector and a 5975C VL mass selective

detector (Agilent Technologies, Santa Clara, CA). Samples were introduced via the auto injector using an injection volume of 1 μL.

The second GC-MS instrument was a HP-5890 GC coupled with a HP-5970 mass selective detector (Hewlett Packard, Palo Alto, CA). Samples were dissolved in HPLC-grade acetonitrile (Fisher Scientific, Fair Lawn, NJ) and manually introduced (1μL), individually, using a 10-μL Hamilton syringe (Hamilton Co., Reno, NV).

The mass spectral scan rate was 2.86 scans per second. The GC was operated in splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 ml/min .The mass spectrometer was operated in electron impact (EI) mode using an ionization voltage of 70 eV and a source temperature of 230ºC.

GC-IRD studies were carried out on a Hewlett-Packard 5890 Series II gas chromatograph and a Hewlett-Packard 7673 auto-injector coupled with a Analytical Solutions and Providers Infrared detector II(IRD II), obtained from Analytical Solutions and Providers (Covington, KY). The vapor phase infrared detector (IRD) spectra were recorded in the range of 4000 – 650 cm^{-1} with a resolution of 8 cm^{-1} and a scan rate 1.5 scans per second. The IRD flow cell and the transfer line temperatures were held at 280ºC and the GC was operated in the splitless mode with a carrier gas (helium grade 5) flow rate of 0.7 mL/min and a column head pressure of 10 psi. Samples were dissolved and diluted in HPLC-grade acetonitrile and introduced via the auto injector using an injection volume of 1 μL.

4.1.3. GC-Columns:

A number of capillary GC columns were evaluated throughout the course of this study, however only columns showing the best compromises between resolution and analysis time are illustrated in Table 5. All columns used were purchased from Restek Corporation (Bellefonte, PA). Inlet pressure was converted according to the constant flow mode and the total flow was 60 ml/min.

Table 5: List of columns used and their composition.

Column Name	Column Composition	Column Dimensions
Rtx-1	100% dimethyl polysiloxane	30m X 0.25mm-I.d. X 0.25 μm (fd)
Rtx-200	trifluoropropyl methyl polysiloxane	30m X 0.25mm-I.d. X 0.25 μm (fd)
Rxi-50	50% dimethyl-50%diphenyl polysiloxane	30m X 0.25mm-I.d. X 0.5 μm (fd)

4.1.4 Temperature Programs:

Different temperature programs were tried to improve resolution. The following temperature programs were evaluated:

- Program 1 (TP1): Injection temperature 250ºC, detector temperature 280ºC , initial temperature 70ºC hold for 1 min , then ramped up to 250ºC at a rate of 30ºC/min.
- Program 2 (TP2): Injection temperature 250C, detector temperature 250C , initial temperature 70ºC hold for 1 minute, then ramped up to 150ºC at a rate

of 5ºC/min hold for 2 minutes, and increased to 250ºC at a rate of 10ºC/min hold for 15 minutes.

- Program 3(TP3) : Injection temperature 250C, detector temperature 250C , initial temperature 70ºC hold for 1 minute , then ramped up to 150ºC at a rate of 7.5ºC/min hold for 2 minutes, and increased to 250ºC at a rate of 10ºC/min hold for 15 minutes.
- Program 4(TP4) : Injection temperature 250C, detector temperature 280C , initial temperature 100ºC hold for 1 minute, then ramped up to 180ºC at a rate of 9ºC/min hold for 2 minutes, and increased to 200ºC at a rate of 10ºC/min hold for 5 minutes.
- Program 5(TP5): Injection temperature 250C, detector temperature 280C , initial temperature 70ºC hold for 1 minute, then ramped up to 150ºC at a rate of 2.5ºC/min hold for 3 minutes, and increased to 250ºC at a rate of 15ºC/min hold for 10 minutes.

4.2. Synthesis of Regioisomers of Ethoxyphenethylamines:

4.2.1. Synthesis of N,N- dimethyl-1-(2-ethoxyphenyl)-2-propanamine (1):

N-butylamine (76 ml, 0.766 mol) was added to a solution of 2-ethoxybenzaldehyde (20.0 g, 0.133 mol) in benzene (200 ml) and the resulting mixture was refluxed overnight using a Dean-Stark trap. The solvent was then evaporated under reduced pressure then mixed with nitroethane (10.3 g, 0.137 mol) and glacial acetic acid (100 ml). The mixture was refluxed for 2.5 hours then quenched using a mixture of water and ice, and the pH

was adjusted to about 1 using concentrated hydrochloric acid. The resulting solution of 1-(2-ethoxyphenyl)-2-nitropropene was washed with water and extracted with methylene chloride (3 X 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure, then purified using Kugelrohr distillation to yield 14.5 g, (72.5%) of the formed nitropropene. The 1-(2-ethoxyphenyl)-2-nitropropene (14.5 g,0.07 mol) was dissolved in toluene (70.5 ml) and water (70.5 ml) followed by the addition of concentrated hydrochloric acid (30 ml). Then ferric chloride (4.2 g, 0.026 mol) and iron (20.9 g, 0.375 mol) were added and the mixture was shaken vigorously and refluxed over 24 hours. Following reflux the mixture was cooled to room temperature then filtered. The organic layer was isolated and washed with 6 N hydrochloric acid solution (3 X 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting 2-ethoxyphenylacetone (3.83 g, 26.4%) was purified using Kugelrohr distillation. Reductive amination was carried out by adding dimethylamine hydrochloride (3.78 g, 0.0842 mol) and sodium cyanoborohydrde (0.8 g, 0.0126 mole) to a solution of 2-ethoxyphenylacetone (1.5 g, 0.0084 mol) in methanol. The reaction was stirred at room temperature for 3 days, then quenched using water and ice. Methanol was evaporated under reduced pressure, and additional amount of water (30 ml) was added. N,N-dimethyl-1-(2-ethoxyphenyl)-2-propanamine was extracted with methylene chloride (3 X 30 ml), and the organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting amine was converted to the hydrochloride salt using gaseous hydrochloric acid to obtain white crystals of N,N-

dimethyl-1-(2-ethoxyphenyl)-2-propanamine hydrochloride (0.65 g, 0.0031 mol, 43.33%).

4.2.2. Synthesis of N-ethyl-1-(2-ethyoxyphenyl)-2-propanamine (2):

N-butylamine (76 ml, 0.766 mol) was added to a solution of 2-ethoxybenzaldehyde (20.0 g, 0.133 mol) in benzene (200 ml) and the resulting mixture was refluxed overnight using a Dean-Stark trap. The solvent was then evaporated under reduced pressure then mixed with nitroethane (10.3 g, 0.137 mol) and glacial acetic acid (100 ml). The mixture was refluxed for 2.5 hours then quenched using a mixture of water and ice, and the pH was adjusted to about 1 using concentrated hydrochloric acid. The resulting solution of 1-(2-ethoxyphenyl)-2-nitropropene was washed with water and extracted with methylene chloride (3 X 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure, then purified using Kugelrohr distillation to yield 14.5 g, (72.5%) of the formed nitropropene. The 1-(2-ethoxyphenyl)-2-nitropropene (14.5 g,0.07 mol) was dissolved in toluene (70.5 ml) and water (70.5 ml) followed by the addition of concentrated hydrochloric acid (30 ml). Then ferric chloride (4.2 g, 0.026 mol) and iron (20.9 g, 0.375 mol) were added and the mixture was shaken vigorously and refluxed over 24 hours. Following reflux the mixture was cooled to room temperature then filtered. The organic layer was isolated and washed with 6 N hydrochloric acid solution (3 X 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting 2-ethoxyphenylacetone (3.83 g, 26.4%) was purified using Kugelrohr distillation.

Reductive amination was carried out by adding ethylamine hydrochloride (3.78 g, 0.0842 mol) and sodium cyanoborohydrde (0.8 g, 0.0126 mole) to a solution of 2-ethoxyphenylacetone (1.5 g, 0.0084 mol) in methanol. The reaction was stirred at room temperature for 3 days, then quenched using water and ice. Methanol was evaporated under reduced pressure, and additional amount of water (30 ml) was added. N- ethyl-1-(2-ethyoxyphenyl)-2-propanamine was extracted with methylene chloride (3 X 30 ml), and the organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting amine was converted to the hydrochloride salts using gaseous hydrochloric acid to obtain white crystals of N-ethyl-1-(2-ethyoxyphenyl)-2-propanamine hydrochloride(0.72 g, 0.0035 mol, 48.0%).

4.2.3 Synthesis of N-methyl-1-(2-ethoxyphenyl)-2-butanamine (3):

N-butylamine (76 ml, 0.766 mol) was added to a solution of 2-ethoxybenzaldehyde (20 g, 0.133 mol) in benzene (200 ml) and the resulting mixture was refluxed overnight using a Dean-Stark trap. The solvent was then evaporated under reduced pressure then mixed with 1-nitropropane (15.4 g, 0.173 mol) and glacial acetic acid (100 ml). The mixture was refluxed for 2.5 hours then quenched using a mixture of water and ice, and the pH was adjusted to about 1 using concentrated hydrochloric acid. The resulting solution of 1-(2-ethoxyphenyl)-2-nitrobutene was washed with water and extracted with methylene chloride. The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure, then purified using Kugelrohr distillation to yield 13.8 g (69.0 %) of the formed nitrobutene. The 1-(2-ethoxyphenyl)-2-

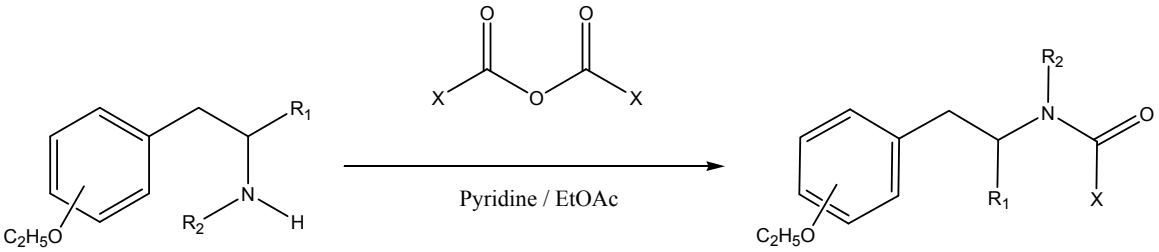
nitrobutene (13.8 g,0.062 mol) was dissolved in toluene (67 ml) and water (67 ml) followed by the addition of concentrated hydrochloric acid (28 ml). Then ferric chloride (4.01 g, 0.025mol) and iron (19.96 g, 0.358 mol) were added and the mixture was shaken vigorously and refluxed over 24 hours. Following reflux the mixture was cooled to room temperature then filtered. The organic layer was isolated and washed with 6 N hydrochloric acid solution (3 X 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting 1-(2-ethoxyphenyl)-buta-2-one (2.7 g, 19.5%) was purified using Kugelrohr distillation. Reductive amination was carried out by adding methylamine hydrochloride (2.42 g, 0.078 mol) and sodium cyanoborohydrde (0.74 g, 0.0117 mole) to a solution of 1-(2-ethoxyphenyl)-buta-2-one (1.5 g, 0.0078 mol) in methanol. The reaction was stirred at room temperature for 3 days, then quenched using water and ice. Methanol was evaporated under reduced pressure, and additional amount of water (30 ml) was added. N- methyl-1-(2-ethoxyphenyl)-2-butanamine was extracted with methylene chloride (3 X 30 ml), and the organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting amine was converted to the hydrochloride salt using gaseous hydrochloric acid to obtain white crystals of N-methyl-1-(2-ethoxyphenyl)-2-butanamine hydrochloride(0.62 g, 0.003 mol, 41.3%).

The synthesis of the remaining isobaric and regioisomers were done in the same manner, but with the use of appropriately substituted benzaldehyde ; the synthesis of 3-ethoxybenzaldehyde involves an extra step in the beginning of the synthesis by starting with 20.0 g (0.16 mol) of 3-hydroxybenzaldehyde in a solution of potassium carbonate

(66.3 g , 0.48 mol) in 150 mL of anhydrous acetone. Then iodoethane (74.88 g, 0.48 mol) was added dropwise, the mixture was refluxed over 3 hours the resulting solution was filter and then evaporated under reduced pressure to obtain the 3-ethoxybenzaldehyde (18.5g, 92.5%) .

4.3. Derivatization procedure:

Each perfluoroamide was prepared individually from each of the secondary amine regioisomers by dissolving approximately 0.3 mg (1.45×10^{-6} mol) of each amine in 50 µL of ethylacetate, followed by addition of an excess (250 µL) of the appropriate derivatizing agent (TFAA, PFPA or HFBA), and the resulting mixtures were incubated in capped tubes at 70°C for 20 min. Following incubation, each sample was evaporated to dryness under a stream of air at 55°C and reconstituted with 200 µL of ethylacetate and 50 µL of pyridine. A portion of each final solution (50 µL) was diluted with HPLC grade acetonitrile (200 µL) to give the working solutions.



R_1= CH_3, R_2=C_2H_5; Cpd 2, 5 and 8
R_1= C_2H_5, R_2=CH_3; Cpd 3, 6 and 9

X=CF_3
C_2F_5
C_3F_7

Scheme 11: General scheme showing the derivatization of the secondary amines using HFBA, PFPA and TFA.

5. SUMMARY AND CONCLUSIONS

Three regioisomeric methylenedioxyphenethylamines (MDEA, MDMMA and MBDB), having the same molecular weight and major mass spectral fragments of equivalent mass, have been reported as drugs of abuse. Ring substituted ethoxy phenethylamines have an isobaric relationship with the controlled substances. The synthesis of theses ethoxyphenethylamines began with the appropriate substituted benzaldehyde to yield the appropriate substituted phenyl ketones. The ketones were converted to the desired amines by reductive amination. Mass spectrometry studies were done on the underivatized amines, showing almost identical mass spectra with major fragment ions at *m/z* = 72 and 135. Chromatographic resolution of the underivatized compounds was attempted on an Rxi-50 column and yielded some co-eltions. Derivatization of the secondary amines with various perfluoroacylation agents yields amides that significantly individualized their mass spectra and allowed for specific side chain identification. The individualization is the result of formation of unique marker ions at m/z 107, 176 and 162 or as a result of differences in the relative abundances of some common ions. The GC-IRD yield structure IR absorption relationships for the 12 amines in the study offering additional structural confirmation without the need for

chemical derivatization of the compound. Chromatographic resolution of the perfluoroacyl amides was achieved on a relatively non polar Rtx-1 stationary phase.

In addition GC-MS and IRD studies were done on the ketone precursors of the ethoxyphenethylamines and ketone precursors of the drug of abuse MDEA, MDMMA and MBDB.

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