DETERMINATION OF DELTA ^{13}C VALUES OF AMPHETAMINE AND METHAMPHETAMINE USING GAS CHROMATOGRAPHY/ COMBUSTION/ ISOTOPE RATIO MASS SPECTROMETRY (GC/C/IRMS)

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by

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ SYMBOLS

AP	Amphetamine
µL	Microliter
‰	Per mil
CF	Continuous Flow
CNS	Central Nervous System
CV	Coefficient of variation
DCC	Doping Control Centre
DI	Dual Inlet
EA	Element Analyzer
EI	Electron ionization
EP	Ephedrine
GC/C/IRMS	Gas Chromatography/Combustion/ Isotope Ratio Mass Spectrometry
GC/MS	Gas Chromatography /Mass Spectrometry
HCl	Hydrochloric acid
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High performance liquid chromatography
IRMS	Isotope Ratio Mass Spectrometry
KOH	Potassium hydroxide
LLE	Liquid Liquid Extraction
m/z	mass-to-charge ratio
MA	Methamphetamine
MDMA	3,4 methylenedioxymethamphetamine
MSD	Mass Selective Detector
mg	Milligram
mL	Milliliter
NADA	National Anti Drug Agency
*p*EP	Pseudoephedrine
RSD	Relative standard deviation
SD	Standard Deviation
SIM	Selective ion Monitoring

SPE	Solid-phase extraction
TC	Temperature Conversion
Temp	Temperature
VPDB	Vienna-Pee-Dee Belemnite
WADA	World Anti Doping Agency
δ	Delta value
μg/mL	Microgram per milliliter

PENENTUAN NILAI DELTA ^{13}C AMFETAMINA DAN METAMFETAMINA MENGGUNAKAN KROMATOGRAFI GAS/ PEMBAKARAN/ NISBAH ISOTOP SPEKTROMETRI JISIM (GC/C/IRMS)

ABSTRAK

Penyalahgunaan dadah perangsang jenis amfetamina (ATS) telah dianggap sebagai masalah yang paling meluas di seluruh dunia dan juga dianggap sebagai ancaman kepada keselamatan negara. Kajian ini memberi tumpuan kepada analisis isotop stabil karbon menggunakan nisbah isotop spektrometri jisim (IRMS) dengan membangun dan mengesahkan kaedah pengesanan amfetamina dan metamfetamina. Kemampuan untuk mengukur taburan isotop semulajadi dengan ketepatan dan kejituan yang tinggi telah meningkatkan penggunaan Gas Kromatografi/ Pembakaran/ Nisbah Isotop Spektrometri Jisim (GC/C/IRMS) untuk pelbagai aplikasi dalam tahun kebelakangan ini. Urin yang ditambahkan dengan piawai amfetamina dan metamfetamina telah diekstrak menggunakan kaedah pengekstrakan cecair dan kemudiannya dilarutkan dengan fasa bergerak untuk pembersihan lanjut dan pemisahan menggunakan kromatografi cecair berprestasi tinggi (HPLC). Fraksi ini telah dikumpul dan disuntik ke dalam GC/C/IRMS. GC/C/IRMS menunjukkan nilai δ^{13}C amfetamina adalah -24.72 ±0.6‰, manakala metamfetamina adalah -28.90 ±0.15‰. Sampel piawa pepejal amfetamina dan metamfetamina yang sama juga telah dianalis untuk menentukan nilai δ^{13}C setiap piawai dengan menggunakan Penganalis unsur isotop nisbah spektrometri jisim jenis FLASH (EA/IRMS); nilai ini kemudiannya dibandingkan dengan keputusan yang diperoleh dari GC/C/IRMS. EA/IRMS menunjukkan keputusan yang setanding dengan keputusan yang diperoleh menggunakan GC/C/IRMS; amfetamina menunjukkan nilai δ^{13}C sebanyak -24.90

±0.03‰, manakala metamfetamina menunjukkan $\delta^{13}C$ sebagai -28.99 ±0.03‰. Sampel menggunakan kaedah pembersihan HPLC telah dianalisa dan dibandingkan dengan kaedah yang tidak menjalani pembersihan oleh HPLC. Sampel urin yang telah melalui pembersihan menggunakan HPLC, menunjukkan hasil yang lebih baik dan jelas untuk amfetamina dan metamfetamina kerana pendekatan pembersihan ini telah meminimakan latar belakang analisis di dalam sampel urin. Pengesahan kaedah telah dilakukan untuk menunjukkan kekhususan, kebolehulangan, ketepatan dan kejituan serta pemulihan menggunakan pendekatan ini. Kaedah pengesahan telah menunjukkan peratus pemulihan adalah melebihi 80%. Kejituan intra-hari dan inter-hari adalah kurang daripada 10% di dalam sampel urin kawal kualiti. Kesimpulanya, protokol yang dibangunkan menggunakan GC/C/IRMS didapati sesuai bagi penentuan $\delta^{13}C$ untuk amfetamina dan metamfetamina di dalam sampel urin.

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ABSTRACT

Drug abuse of amphetamine-type stimulants (ATS) has been considered as the most widespread problem around the world and also regarded as a threat to national security. This study is focused on stable carbon isotope analysis by isotope ratio mass spectrometry (IRMS) to develop and validate a method for amphetamine and methamphetamine analysis. The ability to measure isotope distribution in natural abundance with high accuracy and precision has increased the application of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) for various applications in recent years. Urine Samples spiked with amphetamine and methamphetamine standards were extracted using liquid–liquid extraction and subsequently, reconstituted with the mobile phase for further purification and separation using High Performance Liquid Chromatography (HPLC). These fractions were collected and analysed using the GC/C/IRMS. GC/C/IRMS showed δ^{13}C value of -24.72 ±0.6‰ and -28.90 ±0.15‰ for amphetamine methamphetamine, respectively. The solid samples of the same amphetamine and methamphetamine standards were also analysed to determine the δ^{13}C of each standard by using the FLASH elemental analyzer isotope ratio mass spectrometry (EA/IRMS); these were then compared with the results obtained from the GC/C/IRMS. EA/IRMS showed comparable results with those obtained with GC/C/IRMS; amphetamine showed the δ^{13}C value of -24.90 ±0.03‰, while methamphetamine showed δ^{13}C result of -28.99 ±0.03‰. Samples were also analysed following HPLC clean-up and compared to those that did not undergo

HPLC clean-up. The urine samples that were subjected to HPLC clean-up, showed better and clear results for amphetamine and methamphetamine as this approach has minimal background interference. Method validation was performed to show specificity, repeatability, accuracy and precision as well as recovery using this approach. The validated method showed that the recoveries were greater than 80%, while the intra and inter day precision was less than 10% in urine. As a conclusion, the developed protocol using GC/C/IRMS was found to be appropriate for the determination of $\delta^{13}C$ for amphetamine and methamphetamine in spiked urine samples.

CHAPTER 1
INTRODUCTION

1.1 Introduction

Drug abuse of amphetamine-type stimulants (ATS) has been considered as the most widespread problem around the world (Kurashima et al., 2009). Malaysia is one of the golden triangle countries, which has resulted with the highest level of heroin, ATS and other illicit substance use, and this maybe contributed by foreign immigration from other countries (Tanguay, 2011). ATS is easily synthesized in clandestine laboratories from several chemical precursors (Kurashima, et al., 2009).

Amphetamines are stimulant drugs that increase the activity of central nervous system and nowadays, they are mostly abused by drug addicts and athletes in sport (George, 2000). East Asian countries have shown a marked increase in the use of amphetamine-type-stimulants (ATS) (Hatim, 2008). Amphetamines are drugs that include amphetamine and methamphetamine; these have contributed to the drug abuse problems globally. In Japan, methamphetamine is the most highly abused drugs It was also found that more than 80% of violation cases were related to drug addiction (Inoue, Iwata, & Kuwayama, 2008). The most famous street names for methamphetamine are ice, speed and shabu. In 2008 the National Anti Drug Agency (NADA) of Malaysia identified 8,870 addicts of which 1,126 of them were ATS users and dependents (Hatim, 2008). From January to November of 2011, it was found that the drugs used by addicts were mostly heroin (38.83%), morphine (31.11%) and methamphetamine (14.50%), and

about 15.10 % was detected using ATS; these include ecstasy, syabu and amphetamine pills (AADK, 2011).

Synthetic methamphetamine has a very high rates of illicit abuse in many countries, and in order to track and limit the production of these drugs, many methods have been developed (David, Hibbert, Frew, & Hayman, 2010). In forensic science, the question concerning the analytical approach to determine the origin of drugs that are found at different places and at different times is often raised (Mas, Beemsterboer, Veltkamp, & Verweij, 1995). A drug profiling technique is often performed to provide a set of specific selected characteristics of the drugs. Organic impurity profiling is one of the drug profiling techniques used for the identification of the precursor, the synthetic pathway and the intrinsic characteristic of the seized drugs (Iwata et al., 2008). Recent studies have focused on isotope ratio mass spectrometry (IRMS) as the most recent profiling technique (Collins et al., 2010) and very useful for the analysis of amphetamines, the precursors and their metabolites in order to trace the seized methamphetamine. IRMS is a technique that can determine the origin of the compounds (Kurashima, et al., 2009). Presently gas chromatography/combustion/isotope ratio mass spectrometry(GC/C/IRMS) is considered as one of the powerful instrument and tools for source identification of abused drugs (Hernández, 2008). Isotopes are considered as the fingerprint of the compounds (Benson, Lennard, Maynard, & Roux, 2006). The natural abundance isotope consist of light and heavy isotopes which are ^{12}C, ^{13}C, ^{14}N, ^{15}N, ^{16}O, ^{17}O,^{18}O, ^{1}H, ^{2}H, ^{32}S, ^{33}S and ^{34}S .

The IRMS is measured by an equation called delta value δ (Benson, et al., 2006). These isotopes are expressed as δ, relative to Vienna Pee Dee Belemnite (VPDB) standard for carbon or nitrogen (Iwata, et al., 2008).

$$\delta\,[‰] = \frac{(R\ \text{Sample} - R\ \text{Standard})}{R\ \text{Standard}} \times 1000 \quad \text{.....(1.1)}$$

Many studies have indicated that IRMS technique saves time; it has a short analysis time in forensic analysis work (Kurashima, et al., 2009). There are several researchers that have studied the detection of amphetamines using IRMS, but most have dealt with tablets only. The compound 3,4-methylenedioxymethamphetamine (MDMA) is the most widely used illicit drugs that has been analyzed and reported by IRMS (Matsumoto et al., 2008).

Amphetamine type stimulants (ATS) in recent years have become a great concern as a public health issue especially in East Asia and the consumption of these drugs has rapidly increased among the population. ATS has shown high rates of illicit abuse, because it is easily synthesized in clandestine laboratories. Gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS), is a powerful instrument used to determine and trace the origins of seized drugs. This thesis will present a studyon the method of determining Delta carbon 13 $\delta^{13}C$ of amphetamine and methamphetamine as an approach in determining the source of drug origin in positive urine samples using GC/C/IRMS and to achieve this goal, the present study is focused on the following objectives:

1.2 Research Objectives

1. Purification of amphetamine and methamphetamine in urine samples using HPLC clean up after pretreatment of the samples using liquid-liquid extraction.
2. Determination of the Delta values based on carbon 13 ($\delta^{13}C$) for the positive urine samples containing amphetamine and methamphetamines using GC/C/IRMS.
3. Develop and validate a GC/C/IRMS method for amphetamine and methamphetamine analysis.

1.3 Thesis outline

The main body of this thesis consists of a general introduction and background, literature reviews, material and methods, results and discussion, conclusions and recommendations for future study.

CHAPTER ONE is a general introduction on the wide spread abuse of drugs and the recent drugs profiling techniques.

CHAPTER TWO covers the general information and definition about stimulants. It provides a brief background about amphetamines and methamphetamines as well as an explanation of the physical and chemical properties of each drug. It Include the effects of amphetamines and the metabolism of these drugs. The definition of isotope ratio and the way it is measured will also be described. This chapter provides the principle of some instruments that will be used to analyze amphetamines and methamphetamine in this thesis like HPLC, GC/C/IRMS and GC/MS.

CHAPTER THREE describes the approach and the methodologies for the extraction of compounds from urine, separation by HPLC and determination of $\delta\ ^{13}C$ values on the IRMS with discussion of each instrument that has been employed during the study.

In CHAPTER FOUR, experimental results and the discussions are presented from studies using IRMS.

CHAPTER FIVE which is the last chapter includes the conclusion of the whole study in the thesis and recommendation for future work on urine analysis for drugs of abuse using IRMS.

CHAPTER 2
LITERATURE REVIEWS

This chapter is an introductory chapter that presents (1) a general background of the central nervous system stimulant, mainly amphetamine and methamphetamine, (2) followed by an overview of the principles and kinetics of absorption, distribution, metabolism and excretion of each stimulant, (3) an overview of Isotope Ratio Mass Spectrometry, and (4) finally this chapter will review analytical techniques that were used for urine analysis.

2.1 Stimulants Drugs

Stimulants are drugs which affect the central nervous system (CNS) of the brain. They are psychoactive drugs that excite the body functions and cause several physiological effects. These include for example enhanced alertness, wakefulness, elevated mood and many other symptoms. Stimulants are widely used throughout the whole world as medicine and as illicit substances by abusers and drug addicts (NDRI, 2007). Drug abuse is considered as the most serious social problems throughout the world. Some drugs are produced naturally by plants and others have resulted from chemical synthesis (Inoue, Iwata, & Kuwayama, 2008). There are general terms called amphetamines–type stimulants which are synthetic drugs, chemically related to the parent compound amphetamine. They can be distinguished from other psychoactive drugs like heroin, cocaine and cannabis, which are plant derivatives (NDRI, 2007). Amphetamine and its derivatives are considered as orchids of the psychoactive drugs

(Sulzer et al., 2005). These stimulant drugs include amphetamine (1-phenyl-2-aminopropane), methamphetamine (1-phenyl-2-methylaminopropane) and their salts (Takayama & Hayakawa, 2005). Due to the extensive use of amphetamines, drug testing of amphetamines is performed as a routine test in forensic toxicology laboratories (Grefslie, Krogh, & Rasmussen, 1999).

2.2 History of Amphetamine and Methamphetamine

Amphetamines play a major role among the class of stimulant drugs which have the greatest effect on the central nervous system (CNS) and considered as the most highly potent of stimulant drugs. These stimulants generally include three closely related drugs: amphetamine, dextroamphetamine and methamphetamine (King & Ellinwood, 1997). Amphetamine (AP) was first synthesized in 1887 in Germany by a Romanian chemist called Lazãr Edeleanu, who named the compound as phenylisopropylamine. From 1933 until 1934 it was used by medical doctors as an inhaler drug for decongestant under the name of Benzedrine (Rasmussen, 2006). Amphetamine was used as drugs to increase the alertness in soldiers and to decrease fatigue during World War II. For many years amphetamine has been used to treat many kinds of diseases like asthma, obesity (as diet pills), depression and narcolepsy.\

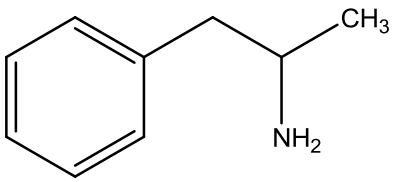
A related compound called methamphetamine which is an amphetamine derivative, was first synthesized in Japan in 1920 by a Japanese pharmacologist known as Akira Ogata (Rasmussen, 2008). The name methamphetamine (MA) was derived

from elements of the chemical structure of the compound which is methyl alpha-methylphenylamine. In 1943 in the United State, methamphetamine was approved to treat many disorders such as narcolepsy, mild depression, post encephalitic Parkinsonism, chronic alcoholism and arteriosclerosis (Miller, Hajdukovic, & Erman, 1993).

Amphetamine and methamphetamine are the most powerful synthetic stimulants that are widely abused. Amphetamine and methamphetamine are chiral compounds which exist as a pair of enantiomers. The(S) or (+) or *d*- form called dextro-AP or MA, while (R) or (-) or *l*-form refers to levo-AP or MA. The *d*- form of AP and MA is the most potent isomers and widely abused, while the *l*-form is used in Vicks vapor inhaler to relieve nasal congestion. Amphetamines can be found as the racemic compound that includes a mixture of both levo- and dextro- amphetamine (Phinney & Sander, 2004).

2.3 Amphetamine

Amphetamine (IUPAC name: 1-phenypropane-2-amine) is one of the metabolites of methamphetamine (Phinney & Sander, 2004). Amphetamine is considered as one of the most widely abused stimulants in sport (J.George, 2000). The chemical structural formula of amphetamine is shown in Figure 2.1 .



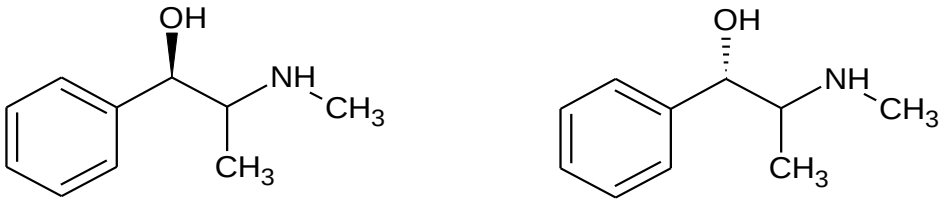
Chemical Formula: $C_9H_{13}N$
Molecular Weight: 135.21

Figure 2.1 Chemical structure of amphetamine (adapted from Golub et al., 2005)

2.3.1 Amphetamine Synthesis

Amphetamine can be extracted from the natural plant known as ephedra. The genus *Ephedra* and the tree *cathedulis* in Arabic is known as *khat* and as *myrrha* in most of East Africa. *Ephedra sinica* in China is known as *Ma huang*, which means looking for trouble. The herb has been used as herbal medicine for many centuries for treating asthma and as a decongestant. This herb has been abused over many years as stimulants, from which ephedrine is considered as the major active component.

In the present days ephedrine (EP) and pseudoephedrine (*p*EP) are the most common precursor for some laboratories that produces methamphetamines for the illicit markets (Sulzer, et al., 2005). The chemical structure of both EP and *p*EP is shown in Figure 2.2. Amphetamine synthesis can be performed by serial alkylation of methyl acetoacetate with dimethyl sulfate and benzyl chloride, and then followed by hydrolysis and deacetylation to produce 2-phenylpropionic acid. This is followed by a reaction with thionyl chloride and ammonia to form 2-phenylpropionamide. Upon treatment with sodium hypochlorite it will form a racemic mixture of amphetamine (phenyl 1-2 propane) (Golub et al., 2005).



Ephedrine Pseudoephedrine

Figure 2.2 Chemical structural formula of ephedrine and pseudoephedrine (adapted from Sulzer, et al., 2005)

2.3.2 Physical and Chemical Properties of Amphetamine

The chemical formula of AP is $C_9H_{13}N$ and the molecular weight is 135.21. By weight, amphetamine is about 79.95% of carbon, 9.69% hydrogen and about 10.36% of nitrogen (Budavari, 2001). Amphetamine is an acrid and colorless volatile liquid with strong odor. The boiling point is about 200- 203°C. Amphetamines are slightly soluble in water, ethers and alcohol. Amphetamine sulfate is insoluble in ether (Golub, et al., 2005). Abused amphetamine commonly comes under different names all around the world with street names such as Amp, Bennies, Black Beauties, Brown, Hearts, Speed, Uppers, Cranks, Fives, Whiz, and Louee (Greene, Kerr, & Braitberg, 2008). The famous trade names for amphetamine is Benzedrine (mix of *d*,*l*-AP) and Dexedrine (*d*-AP); both are marketed for medical purposes. The optical isomers of AP show different pharmacological effects. The *d*-isomers is more potent than the *l*-isomers in affecting the CNS, while in cardiovascular system the *l*-isomers is more potent than the *d*-isomers (Flotz, Fentiman, & Flotz, 1980).

2.3.3 Physical effects of Amphetamine

Amphetamine is considered as one of the three major types of CNS stimulants that are currently abused in sport. Amphetamines produce a variety of clinical effects in the body system; in the cardiovascular system, it will cause tachycardia, hypertension, arrhythmias, vasospasm, acute coronary syndrome, acute cardiomyopathy and hypotension. The CNS will be affected with symptoms like agitation, paranoia,

hallucination, hyperreflexia, euphoria, anorexia, hyperthermia and coma. Amphetamine also affects the gastrointestinal system by causing hepatitis, nausea, vomiting, diarrhea and gastrointestinal ischaemia. In cases of amphetamine overdoses and chronic toxicity, it can cause behavioral illness, vasculitis, cardiac valve disease, cardiomyopathy and pulmonary hypertension. The other effects are muscle rigidity, tremor and tachypnoea (Greene, et al., 2008).

2.3.4 Metabolism and excretion of Amphetamine

Exposing amphetamine drugs to a biological system may lead to the conversion to various metabolites as a result of enzymatic reaction in blood, kidney, intestinal mucosa and liver (Sukkwan, 2006).The basic Amphetamine metabolites are p-hydroxylamphetamine and p-hydroxynorephedrine. Both of these metabolites have the same effects as the parent compound amphetamine (George, 2000). They occur in significant levels in the urine. Amphetamine is deaminated by cytochrome P_{450} to p-hydroxyamphetamine and phenylacetone, which is oxidized into benzoic acid and excreted as the glycine conjugate into the urine. A minor component of amphetamine will be oxidized and converted into norephedrine (Sukkwan, 2006). The metabolic pathways of amphetamine are illustrated in Figure 2.3. The excretion of the unchanged drug into the urine ranged between 10 to 60 percent depending on the urinary pH (Flotz, et al., 1980).

According to some studies, following ingestion of 10 mg of d-amphetamine sulfate, the peak blood concentration of approximately 35 ng/mL was reached in 2 hours. This is followed by a huge decline of plasma level of the drug with an elimination half-life of 11-13 hours over the next 48 hours. About 45% of the ingested dose are excreted into the urine as unchanged drug (Flotz, et al., 1980). Another study showed that, following oral doses of 2.5 to 15 mg of AP, peak plasma concentration of 30 to 170 μg/mL was reached in 2 hours and the elimination half-life was 8-12 hours. Amphetamine starts to appear in the urine within 20 minutes of the administration of the drug (Sukkwan, 2006).

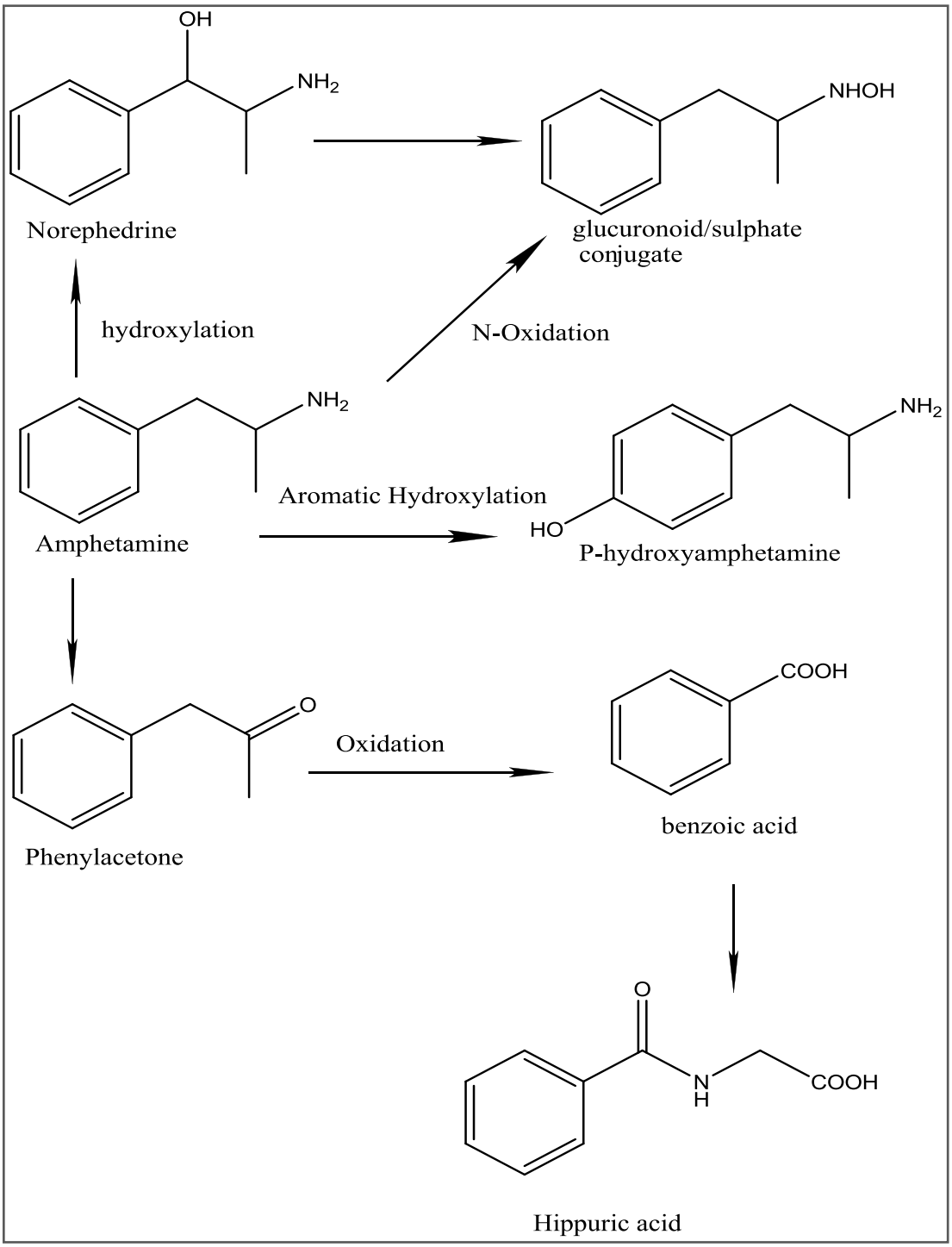
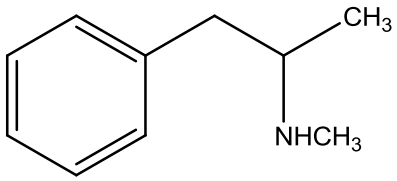


Figure 2.3 Metabolic pathway of amphetamine (adapted from Sukkwan, 2006)

2.4 Methamphetamine

Methamphetamine (MA) (IUPAC name: N-methyl-1-phenylpropane-2-amine) is one of the most illicit derivative which belongs to the class of amphetamines (Logan, 2002). In Japan MA was considered as the most common drug abused ,and over 80% of violation cases of drug control laws are related to methamphetamine (Inoue, et al., 2008). The structural chemical formula of methamphetamine is shown in Figure 2.4.



Chemical Formula: $C_{10}H_{15}N$
Molecular Weight: 149.23

Figure 2.4 Chemical structure of methamphetamine (adapted from Golub, et al., 2005)

2.4.1 Methamphetamine Synthesis

Methamphetamine drug is a synthetic derivative of ephedrine, which is contained in a natural plant known as Ephedra (Jirovský *et al.*, 1998). MA could be manufactured by several routes, starting with 1-phenyl-2-propane by reductive amination with methyl amine. The product of the reaction is a racemic mixture of MA. Another approach in the synthesis of MA involves the reduction of *l*-ephedrine (*l*-EP) or *d*-pseudoephedrine (*d*-PEP) to produce *d*-MA (Inoue, et al., 2008). These reductions have utilized several materials including red phosphorus with hydroiodic acid or another reduction using sodium or lithium in condensed liquid ammonia (Logan, 2002).

2.4.2 Physical and Chemical Properties of Methamphetamine

The chemical formula of MA is $C_{10}H_{15}N$ and the molecular weight is 149.23. By weight MA is about 80.48% of carbon, 10.13% hydrogen and about 9.39% of nitrogen (Budavari, 2001). Methamphetamine is colorless, odorless and a crystal-like powder. The pure methamphetamine has an oil-like texture (Freye & Levy, 2009). The methamphetamine hydrochloride is a white powder with a melting point of 170-175°C (Logan, 2002). It is soluble in ethanol and diethyl ether. The MA-hydrochloride is readily soluble in water (Golub, et al., 2005). The appearance of methamphetamine hydrochloride is shown in Figure 2.5.

Figure 2.5 Picture of methamphetamine hydrochloride as a powder (left) and Crystal-Meth (right); due to its higher purity, the latter has a crystal-like appearance named ice (adapted from Freye & Levy, 2009)

Methamphetamine is similar to amphetamine in that it exists in two isomeric forms which are (R) or (-) and (S) or (+) form. The name *d*- and *l*- form refers to the dextrorotatory or levorotatory properties which relates to the optical activity towards plane-polarized light (Logan, 2002). Determination of enantiomers ratio can help in determining whether these drugs have originated from licit sources or illicit synthetic sources (Logan, 2002). The most commonly abused names are: Solid, Meth, Speed, whiz, Fast, Base, Pure, Rabbit, Tail, Wax, liquid, Red speed, liquid red, Ox blood, Crystal-ice, d-meth, glass, Batu and Shabu (Greene, et al., 2008).

2.4.3 Physical effects of methamphetamine

Methamphetamine produces a number of symptoms which is dependent on the dosage and they include euphoria, restlessness, impression of internal power and self-believe, fatigue suppression and loss of appetite, high self-confidence, increased energy, hyperthermia, aggression, cardiac arrhythmia, hallucination and coma (Jirovský, et al., 1998). The effects from smoking and intravenous administration of methamphetamine can include increase in the heart rate, stroke volume and cardiac output for the first 30 minutes of taking the dose. High doses of methamphetamine administration can cause intense exhilaration, extreme wakefulness, rapid flow of ideas, increased physical and mental capacity, talkativeness, rapid speech and intensive sexual arousal (Logan, 2002).

2.4.4 Metabolism and excretion of Methamphetamine

Metabolism of methamphetamine consists of two major pathways: N-demethylation of methamphetamine to form amphetamines which subsequently follow several metabolic pathways. Aromatic hydroxylation of methamphetamine produce 4-hydroxyamphetamine followed by the subsequent formation of 4-hydroxynorephedrine (Golub, et al., 2005). In some studies it was found that 43% of methamphetamine is excreted unchanged within 24 hours. About 15% was found as para-hydroxyamphetamine and 4-7% as amphetamine. Norephedrine and the partly conjugated p-hydroxy metabolites, phenylacetone and benzoic acid can also be found in

urine (Jirovský, et al., 1998). The metabolic pathway of methamphetamine was summarized in Figure 2.6.

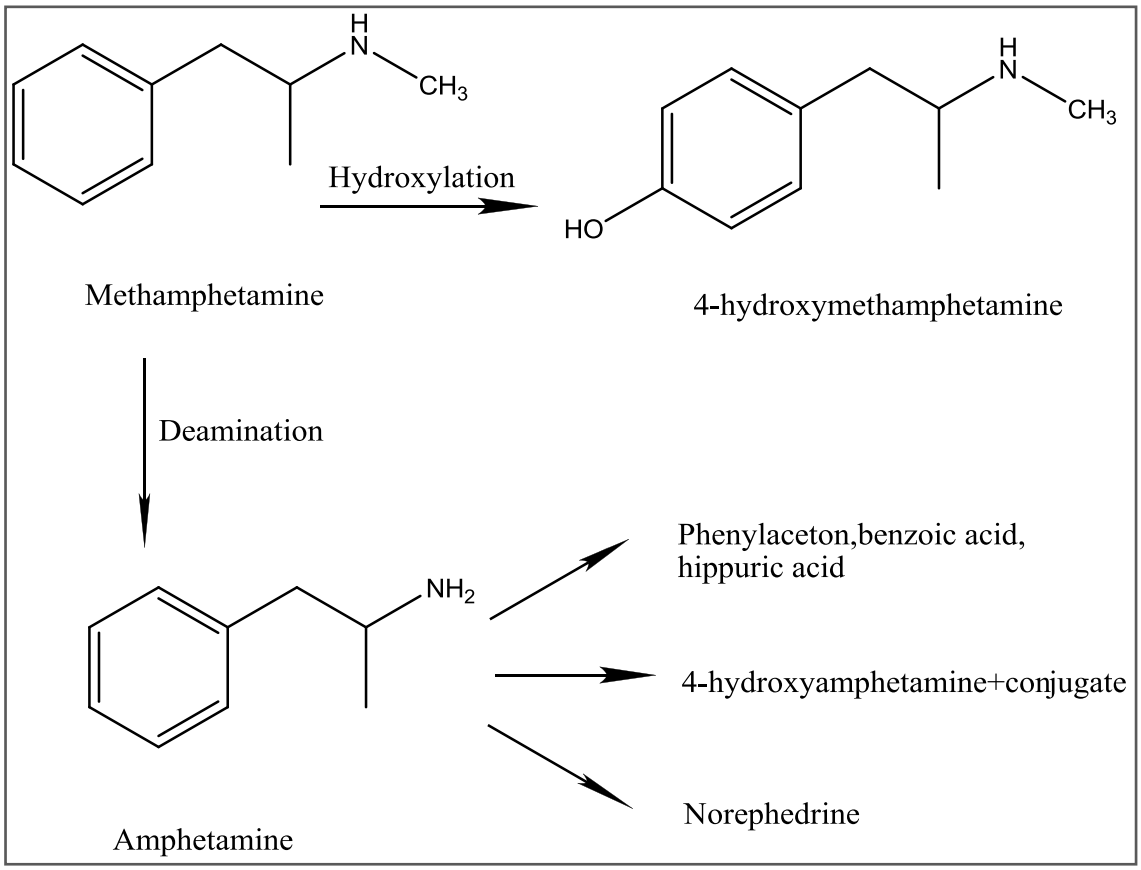


Figure 2.6 Metabolic pathways of methamphetamine adapted from (Jirovský, et al., 1998)

The half life of methamphetamine has been reported as 12 hours in human. Methamphetamine and its metabolites are excreted into the urine in the free form. It was found in some studies that 37-45% of MA doses are eliminated as unchanged drug and about 7 % eliminated as amphetamine following 22 mg intake by inhalation or 15.5 mg by intravenous exposure. The percentage of the parent compound excreted differs and varies according to the urine pH (Golub, et al., 2005).

2.5 Gas Chromatography/ Mass Spectrometry GC/MS

GC/MS is a combination of two powerful techniques; the GC separates the components of the mixture and MS provides data and information to help identify the components. In GC, the sample are vaporized and carried out by a carrier gas such as helium or hydrogen from the injector moving throughout the column ending into the detector (Kitson, Larsen, & McEwen, 1996). MS is a graphic representation of the ions which is separated according to their mass-to-charge ratio *m/z*. The result output is shown in the form of (*x, y)* plot, the *x*-axis refers to mass-to-charge ratio scale and the *y*-axis refers to the intensity scale (Kitson, et al., 1996). Presently, GC/MS technique plays an important role in the analysis and separation of volatile compounds (McNair, Miller, & MyiLibrary, 2008).

2.6 Confirmation of Amphetamines Using GC/MS

GC/MS is the most widely used method for the confirmation of positive screening tests for amphetamine and methamphetamine, because of its high specificity and sensitivity (Hsu, Chen, & Liu, 2009). GC/MS is also considered as the gold standard in toxicological analysis (Kraemer, 1998). There are many publication about stimulants analysis using GC/MS. Cody discussed the determination of methamphetamine enantiomers ratios in urine by GC/MS (Cody, 1992). Goldberger and Cone (1994) reviewed confirmatory tests for drugs in the workplace using gas chromatography-mass spectrometry (Goldberger & Cone, 1994). Most of the GC/MS procedures for the determination of amphetamine and methamphetamine in urine follow the same principles, which include extraction, derivatization, separation and finally detection. Extraction was performed using liquid– liquid extraction (LLE) or solid-phase extraction (SPE). Amphetamines have been determined using different detection mode; such as full scan, selective ion- monitoring (SIM) and electron ionization (EI) (Hsu, et al., 2009).

2.7 Isotope Ratio Mass Spectrometry (IRMS)

2.7.1 Principle of Isotope Ratio

Isotopes are atoms for one element which have the same number of protons and electrons but differing number of neutrons (Sulzman, 2007). Isotopes are divided into two categories which is stable and unstable isotopes (Hoefs, 2009). There are about 300 different stable isotopes and over 1200 unstable isotopes (Hoefs, 2009). Stable isotopes are those with stable energy and do not decay. Isotopes become stable when the neutrons and the protons become quite similar in numbers (Sulzman, 2007). Isotope is derived from the Greek word which means (an equal place) (Hoefs, 2009). Isotopes comes in the form of ${}^{m}_{n}\mathrm{E}$, where *m* refers to the mass number which is the sum of protons and neutrons in the nucleus. The subscript *n* refers to the atomic number of an element E (Hoefs, 2009). Therefore ${}^{\mathbf{12}}_{\mathbf{6}}\mathrm{C}$ is an example of the carbon isotope which has six protons and six neutrons (Hoefs, 2009). Every element has a light isotope and one or two heavy isotopes. Examples of light isotopes are: carbon (^{12}C), nitrogen (^{14}N), oxygen (^{16}O), hydrogen (^{1}H) and sulfur (^{32}S). Examples of the corresponding heavy isotopes are ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{2}H, ^{33}S and ^{34}S (Benson et al., 2006). It is a fact that every biochemical process involves substances that contain one of these elements C, N, H, O and S. We found them in the air we breathe, in the food we eat, in the drinks we consume and in the surrounding environment that we live in (Hernández, 2008). According to some studies, Meier-Augenstein and Liu (2004) stated that "the ability to measure the isotope ratios of more than one element in a single molecule allows stable isotope fingerprinting of

molecules. The more isotopes that can be measured, generally the better the chances are of individualizing substances". Isotopes fingerprinting can be used to trace the drugs showing the common source of the drug, whether it is naturally found or manufactured (Hernández, 2008). Stable isotope ratio measurement becomes one of the most important technique and increasingly used in forensic science to detect the illicit and abused drugs to support drug crime investigations (Collins et al., 2010).

An organization of World Anti Doping Agency (WADA), intended to fight doping in athletics. The $\delta^{13}C$ helped to distinguish between exogenous and endogenous steroids. It was found that, the ratio of testosterone to epitestosterone in urine can indicate synthetic steroids use (Ehleringer, Cerling, & West, 2007). The use of stable IRMS has shown that synthetic testosterone has lower $\delta^{13}C$ value than the endogenous hormone (Hernández, 2008).

2.7.1.1 Measuring Isotope Ratios

The natural abundance isotope ratio data are generally reported as delta values (**δ**) which are expressed in units per mil (mil=thousand) and written as ‰ (Benson, et al., 2006). Delta value can be calculated and measured according to the following formula:

$$\delta = \frac{(R\ \text{Sample} - R\ \text{Standard})}{R\ \text{Standard}} \times 1000 \quad \ldots\ldots (2.1)$$