COMPARATIVE STUDY OF THE UTILITY OF ELECTRON IONIZATION AND CHEMICAL IONIZATION MASS SPECTROMETRY FOR CONFIRMATION AND QUANTIFICATION OF STIMULANTS IN URINE

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LIST OF ABBREVIATIONS/ SYMBOLS

6-MAM	6-monoacetylmorphine
AP	Amphetamine
BSTFA	N, O-bis-trimethylsilyl-trifluoroacetamide
BTFA	Bis(trifluoroacetamide)
CI	Chemical Ionization
CNS	Central nervous system
DCC	Doping Control Centre
EI	Electron impact
GC	Gas Chromatograph
GC-MS	Gas Chromatography Mass Spectrometry
HCL	Hydrochloric acid
HFBA	Heptafluorobutyric anhydride
HFBCL	Heptafluorobutyric chloride
HFPA	Heptafluorobutyryl
HS-SPME	Headspace solid-phase microextraction
ITQ	Ion Trap Quadrupole
KOH	Potassium hydroxide
LC/MS	Liquid Chromatography Mass Spectrometry
LLE	Liquid-liquid Extraction
LOD	Limit of detection
LOQ	Limit of quantification
M	Molar
m/z	mass-to-charge ratio
$M^{+\bullet}$	Positive radical ion
MA	Methamphetamine
MBDB	Methylenedioxyphenyl-N-methyl-2-butanamine
MBTFA	N-methylbis(trifluoroacetamide)
MCF	Methyl chloroformate
MDA	3,4 methylenedioxyamphetamine
MDE	3,4-methylenedioxyethylamphetamine
MDMA	3,4 methylenedioxymethamphetamine
mg	Milligram
mL	Millilitre
MSTFA	*N*-methyl-*N*-trimethylsilyl-trifluoroacetamide
MSTFAACTIVATEDI	N-Methyl-N-trimethylsilyltrifluoroacetamide activated I
MTBSTFA	N-methyl-n-(tert- butyldimethylsilyl) trifluoroacetamide)

NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCI	Negative chemical ionization
NH_4OH	Ammonium hydroxid
NICI	Negative ion chemical ionization
PCI	Positive chemical ionization
PFA	Pentafluoropropionyl
PFPA	Pentafluoropropionic anhydride
TBDMS	*Tert*-Butyldimethylsilyl
TFA	Ttrifluoroacetyl
TFAA	Trifluoroacetic anhydride
TFAI	1-(Trifluoroacetyl)imidazole
TMA	Trimethylamine
TMCS	Trimethylchlorosilane
TMSI	Trimethylsilylimidazole
WADA	World Anti Doping Agency

KAJIAN PERBANDINGAN MENGGUNAKAN KAEDAH PENGIONAN ELEKTRON DAN PENGIONAN KIMIA SPEKTROMETRI JISIM UNTUK PENGESAHAN DAN KUANTIFIKASI DADAH PERANGSANG DALAM AIR KENCING

ABSTRAK

Prosedur analisis untuk mengesan dadah perangsang dalam air kencing menggunakan gas kromatografi-spektrometri jisim (GC-MS) merupakan satu cabaran yang perlu dihadapi oleh makmal anti-doping. Penggunaan reagen terbitan untuk kompoun sasaran akan memberikan bentuk kromatogram yang lebih baik, mengurangkan polariti kompoun dan meningkatkan ciri-ciri kromatografi gas, menyediakan pemisahan yang baik dan menghasilkan spektrum jisim yang berintensiti tinggi untuk kedua-dua pengenalpastian dan analisis kuantitatif, sekali gus meningkatkan pengesanan sesuatu kompoun. Tindak balas sililasi, asetilasi dan alkilasi adalah kaedah terbitan yang paling berkesan dan biasa digunakan untuk GC-MS. Kajian ini memberi tumpuan kepada lima dadah perangsang terlarang daripada senarai dadah terlarang WADA iaitu Amfetamina, Metamfetamina, Efedrina, Metilefedrina dan Katina. Apabila kompoun ini diterbitkan dengan pelbagai jenis reagen terbitan, had pengesanan boleh diturunkan dengan ketara. GC-MS secara tradisionalnya menggunakan kaedah pengionan elektron yang menghasilkan produk ion serpihan jisim yang rendah; ini membuatkan pengesahan kompoun menjadi sukar. Apabila kompoun terbitan dianalisa menggunakan pengionan kimia (CI) dalam GC-MS, ion berproton [M + H]+ serta [M +29]+ dan [M+41]+ akan diperolehi dan boleh digunakan sebagai ion pengesahan. Kajian ini menunjukkan bahawa penggunaan TMSTFA sebagai reagen terbitan dan analisis menggunakan

kaedah pengionan kimia (CI) boleh memenuhi kriteria pengenalan kualitatif selaras dengan kriteria WADA. Dalam kajian ini, had pengesanan bahan-bahan ambang iaitu efedrina, metilefedrina dan katina, didapati 50% lebih rendah daripada paras ambang yang ditentukan oleh WADA; had pengesanan efedrina dan metilefedrina adalah masing-masing 39.0 ng/mL dan 1250 ng/mL manakala bagi katina, had pengesanan adalah 39.0 ng/mL. Nilai ambang yang ditetapkan oleh WADA sebaliknya adalah lebih tinggi, 10 µg/mL bagi efedrina dan metilefedrina, dan 5 µg/mL bagi katina.

COMPARATIVE STUDY OF THE UTILITY OF ELECTRON IONIZATION AND CHEMICAL IONIZATION MASS SPECTROMETRY FOR CONFIRMATION AND QUANTIFICATION OF STIMULANTS IN URINE

ABSTRACT

An analytical procedure for the detection of stimulants in urine using gas chromatography-mass spectrometry (GC-MS) is one of the analytical challenges facing anti-doping laboratories. The use of derivatising reagents for the target compounds will provide better peak shapes, reduce the polarity and enhance the gas chromatographic properties, providing a good separation and mass spectra with high abundance for both identification and quantitative analysis, thus enhancing the detectability of a compound. Silylation, acylation and alkylation reactions are the most effective and common derivatization methods that are used for the GC. This research was focused on five banned stimulants from the WADA prohibited list which were amphetamine, methamphetamine, ephedrine, methylephedrine and cathine. When these compounds were derivatised with various derivatising reagents the limits of detection can be lowered considerably. Traditional GC/MS using electron impact ionisation suffers from product of low mass fragment ions making confirmation of these analytes difficult. When these derivatised compounds were analysed using chemical ionisation (CI) in the GC-MS, the protonated ion $[M+H]^+$ as well as the $[M+29]^+$ and $[M+41]^+$ ions were obtained to be used as confirmatory ions. This research showed that using two steps derivatization by MSTFA followed by MBTFA and analysis using chemical ionisation (CI) can fulfil the identification criteria for qualitative identification of compounds in line with the WADA criteria. In this research the LOD of the threshold substances; ephedrine, methylephedrine,

cathine, were lower than 50% of the WADA threshold concentration; LOD of ephedrine and methylephedrine were 39.0 ng/ml and 1250 ng/ml and for cathine the LOD was 39.0 ng/ml. The WADA threshold on the other hand were much higher, 10µg/ml for ephedrine and methylephedrine and 5µg/ml for cathine.

CHAPTER 1
INTRODUCTION

1.1 Background

In the field of sport, the analytical challenge facing anti-doping laboratories is to detect the presence of forbidden substances through the analysis of body fluids. One of the analytical techniques that has been successfully shown to provide evidence of abuse is gas chromatography coupled to mass spectrometry (GC/MS) (Hemmersbach & de la Torre, 1996).

GC/MS is useful tool for chemical analysis, especially when used together with MS/MS have more recently been introduced. MS/MS has the advantage of identifying compounds by first; selecting an ion of single mass to charge (m/z) value in the first MS, then subjecting it to fragmentation using specific collision energy following which the m/z ions in the second MS were detected (Huestis & Smith, 2006).

Some compounds cannot be analyzed by a particular method in GC/MS because of the following: they are not amenable to the analytical technique as they cannot be volatilized or they are not stable under these technical conditions. The chemical structure of these compounds has to be modified to enable analysis by the desired technique. The interaction between the compounds themselves or between the compounds and the column in some analysis can lead to poor peak resolution or unsymmetrical peaks which makes the identification of the peak difficult. Sometimes a very small amount of compound in a complex matrix need to be analysed, so it is

important to extend the range of detectability to very low levels either by improved detector design or increasing the detectability of the compound itself. Some of these problems may be solved through the use of derivatization techniqes.

Derivatization plays an important role in gas chromatography (GC) through the following ways: it increases the analyte volatility and improves the chromatographic characteristics by decreasing the polarity in addition to increasing the detector sensitivity of the analyte (Robert J, 1999). At the same time derivatization will not change the arrangement of the structure during derivative formation; in addition there will be no sample losses during the reaction and it will not interact with the GC column. Most derivatives help to replace active hydrogen atom attached to the compound with the one which is more stable and less polar. The derivative of the original analyte will show more volatility and more symmetrical peaks by reducing the peaks tailling. Different types of derivatizing reagent can be used in GC/MS but some are preferred for certain types of detectors; for example trimethylsilyl and *tert*-butyldimethylsilyl derivatives are preferred for GC/MS with positive ion electron impact (EI) detection as it increases the detectability of the compounds and it is easily formed. Fluorinated derivatives are formed in cases where negative ion chemical ionization mass spectrometry (NICI/MS) with electron capture detector (Robert J, 1999).

This research will focus on five banned stimulant from the World Anti Doping Agency WADA prohibited list which are: amphetamine , methamphetamine , ephedrine , methyl ephedrine and cathine derivatized with five different derivatising reagents. These are: N-Methyl-N-trimethylsilyltrifluoroacetamide activated I

(MSTFA ACTIVATED I), *N*-Methyl-bis(trifluoroacetamide) (MBTFA),1 (trifluoroacetyl)imidazole (TFAI), pentafluoropropionic anhydride (PFPA), heptafluorobutyric anhydride (HFBA) and two steps derivatization by MSTFA followed by MBTFA.

1.2 Research Objectives

The measurable objectives of this study are listed as follows:

1. The study will investigate various derivatization reagents that can be used for functional groups on the molecular structures of the stimulants (Amphetamine, Methamphetamine, Ephedrine, Methyl ephedrine and cathine) to achieve the limits of detection in the range of 50-100 times better than traditional GC/MS analysis.

2. Conduct analysis using the various derivatives in both Chemical ionization (CI) and Electron ionization (EI) in GC-MS to determine which of these techniques will achieve the best sensitivity with the different types of the derivatising reagents.

CHAPTER 2
Literature review

2.1 Stimulants

Stimulants refer to a class of drugs that include psychomotor stimulants, central nervous system stimulants and sympathomimetic amines. These compounds activate the body mentally and physically by increasing the heart and breathing rates (Colmain & Council, 2010) (Lu *et al.*, 2010). As a result they help in reducing tiredness and muscle fatigue and increase stamina, especially in training and competition among athletes.

On the other hand, stimulants have adverse effects of which addiction is the most serious but it also causes hypertension, anxiety, irregular heartbeat, and stroke which can lead to death (Lu *et al.*, 2010). There are exceptions in that some stimulants are included in the 2012 monitoring program of WADA prohibited list because they are used as a medication. An example is adrenaline which is used in the preparation of local anesthetic substances such as xylocaine. There are many other types of stimulants which are not prohibited, if their concentration in the urine does not exceed a certain specified limit. For example , cathine, ephedrine, methyl ephedrine and pseudoephedrine are nonprohibited stimulants when their concentrations are less than 5, 10, 10 and150 µg/mL respectively (Colmain & Council, 2010).

2.2 Amphetamine

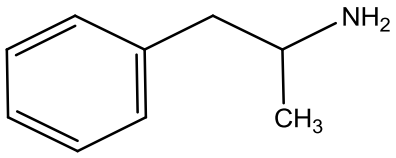
Amphetamine is one of the major types of central nervous system (CNS) stimulants (George, 2000) which was first synthesized in Germany in 1887. It has been used during the World War II as a stimulant. Amphetamine is also considered as a medication in different clinical cases such as shock, encephalitis, postural hypotension and obesity (Greene *et al.*, 2008).

2.2.1 Chemical and physical structure of amphetamine

The chemical name for amphetamine is α-Methylphenethylamine with the formula ($C_2H_{13}N$) (Foltz *et al.*, 1980). The chemical structure is shown in Figure 2.1.

It is one of the derivatives of phenylethylamines which are similar to adrenaline in composition. If the ethyl chain is modified by replacement of various hydrogen atoms which can occur either at the alkyl carbon atoms, aromatic ring or terminal amino nitrogen group, the resulting compound can give different clinical effects (Greene *et al.*, 2008).

Amphetamine physically is a colorless liquid with a boiling point of 200ºC to 203ºC (Clarke, 1986); it is soluble in acid, alcohol and ether but only slightly soluble in water (Foltz *et al.*, 1980).



AMPHETAMINE

Figure 2.1 Chemical structure of Amphetamine (Sukkwan, 2006)

2.2.2 Amphetamine metabolism

Amphetamine is metabolised to several metabolites through the following pathways: deamination to produce phenylacetone which is further oxidized to benzoic acid. It also undergoes β-hydroxylation to produce norephedrine and hydroxylation to produce o-hydroxynorephedrine. In Figure 2.2, the metabolic pathway of amphetamine is illustrated (Sukkwan, 2006).

Amphetamine is excreted in the urine within 20 minutes of administration (Sukkwan, 2006) as the following; 30% as unchanged drug, 3% as p-hydroxyamphetamine, 3% as phenylacetone, 20% as benzoic acid and 5% as p-hydroxynorephedrine (Foltz *et al.,* 1980) . Excretion of the unchanged drug depends on the urinary pH. In alkaline urine, 45% of the dose is excreted within 24hr and 2% of it as unchanged drug. On the other hand in acidic urine, 78% of the dose is excreted within 24hr and 68% as the unchanged drug (Sukkwan, 2006) .

2.2.3 Amphetamine properties and effects

Amphetamine exerts the following effects: increased stamina, excitement, concentration and mental aptitude, increase in physical energy and decreased appetite (George, 2000; Greene *et al.*, 2008). Because of these properties, most of the amphetamine abusers are students undergoing examination, drivers who travel long distances and athletes that require enhanced performance and endurance (Kintz *et al.*, 1989).

The major side effects that appear in the amphetamine abusers are confusion, delirium, palpitation, sweating, hypertension, tachycardia and joint and muscle pain but there are some effects that depends on the drug dosage; these include anxiety, irresponsible behavior, restlessness and depression (George, 2000).

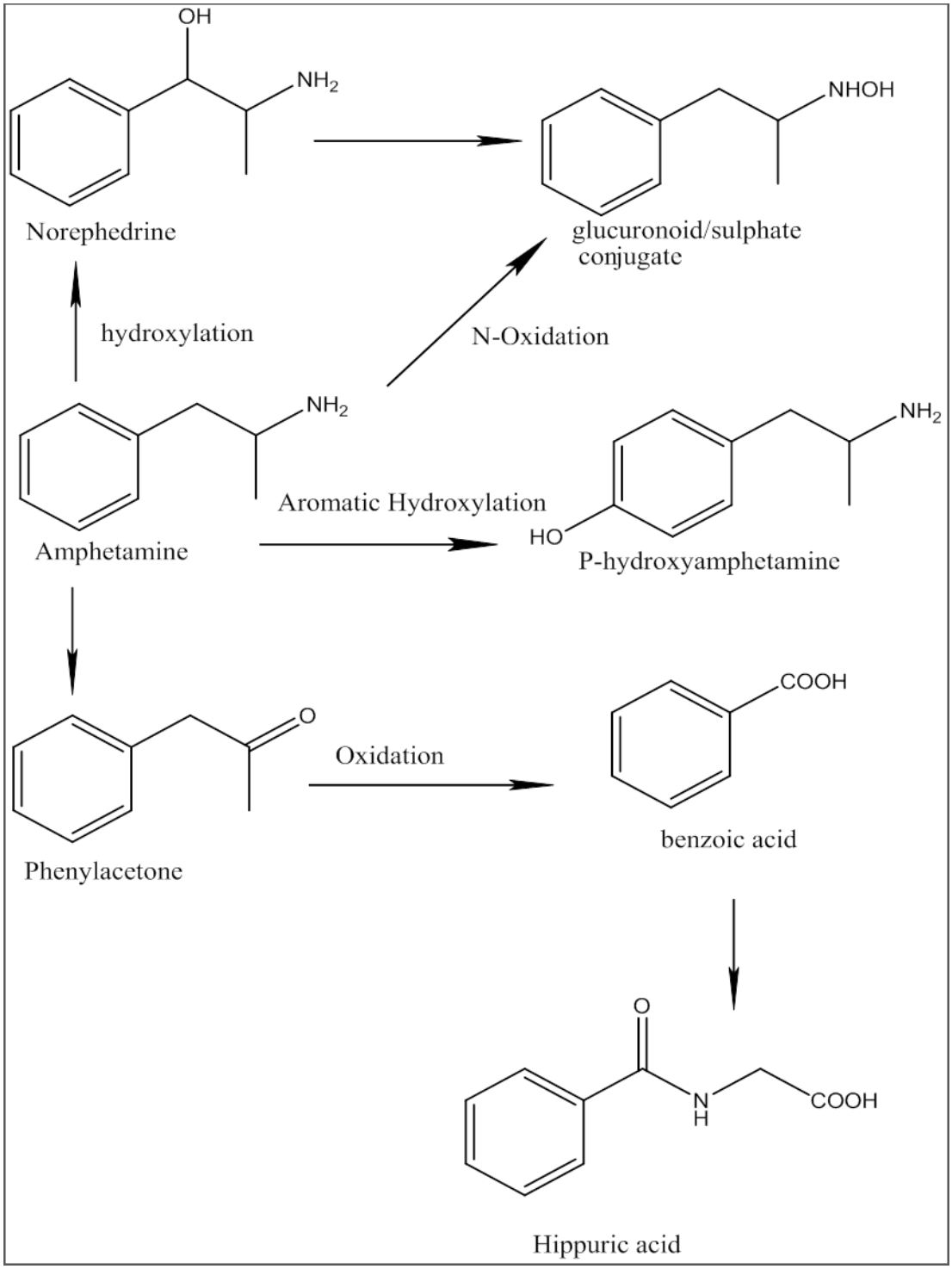


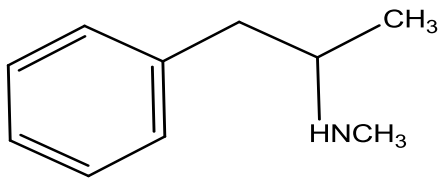
Figure 2.2 Metabolic pathway of amphetamine, adapted from (Sukkwan, 2006)

2.3 Methamphetamine

Methamphetamine is one of the more popular central nervous system (CNS) stimulants; it is a derivative from the class of amphetamines and its first large scale production took place in 1919 in Japan, it is popularly known as crystal meth, ice, speed and crank (Logan, 2002).

2.3.1 Chemical and physical structure of methamphetamine

Methamphetamine is the common name for N-methyl-1-phenylpropan-2-amine with chemical formula of $C_{10}H_{15}N$ (Logan, 2002). The chemical structure is illustrated in Figure 2.3.

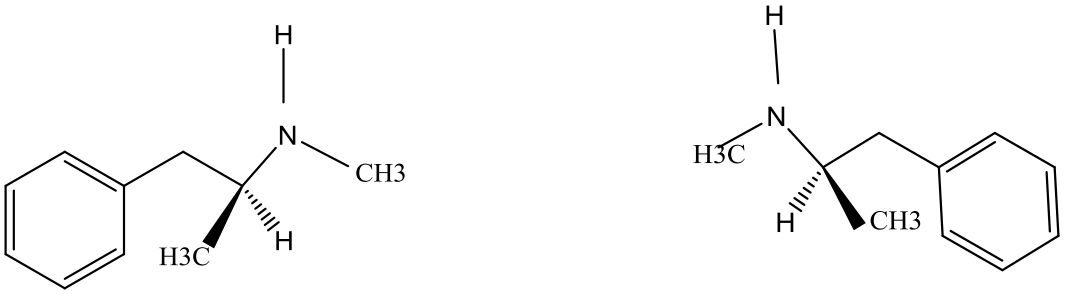


METHAMPHETAMINE

Figure 2.3 Chemical structure of methamphetamine (Foltz *et al.*, 1980)

Methamphetamine is produced via chemical synthesis to produce either the powder or crystal form of the drug. This will also contain small amounts of

intermediate chemical compounds such as ephedrine and pseudoephedrine. These compounds like amphetamine, possess a chiral centre (Inoue et al., 2008) . Therefore methamphetamine can exist in the form of two optical isomers which are; D-Methamphetamine and L-Methamphetamine (Figure 2.4) These isomers are similar in chemical reactions, melting points and solubility but they differ in smell and their pharmacological effects in the body because they fit into the receptors in different ways (Freye & Levy, 2009).



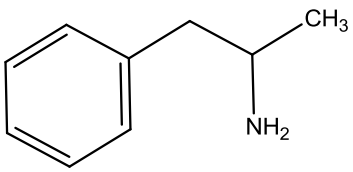
D-methamphetamine L-methamphetamine

Figure 2.4 Molecular structures of both optical isomers of methamphetamine differing only in their ability to rotate plane polarized light; adapted from (Homer *et al.*, 2008).

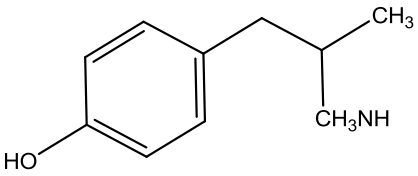
physically Methamphetamine is colorless and odorless, with a boiling point of 212ºC (Foltz et al., 1980). Pure methamphetamine has an oily texture which makes it insoluble in water but with good solubility in ether and ethanol. On the other hand, methamphetamine hydrochloride which is the salt form of methamphetamine, is soluble in water, ethanol and chloroform but insoluble in ether; the melting point of the salt is 173ºC (Freye & Levy, 2009).

2.3.2 Methamphetamine Metabolism

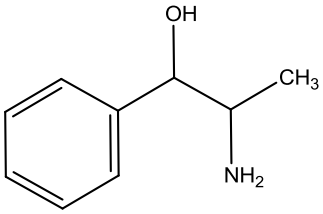
Methamphetamine is metabolized in the liver by three mechanisms: (1) N-demethylation to produce amphetamine, catalyzed by cytochrome P4502D6 (Figure 2.5.a), (2) aromatic hydroxylation via cytochrome P4502D6 to produce primarily 4-hydroxymethamphetamine (Figure 2.5.b.) and (3) β-hydroxylation to produce norephedrine (Figure 2.5.c) (Cruickshank & Dyer, 2009). It must be considered at this point that there are many drugs that are metabolized to both amphetamine. For example ; benzphetamine (didrex), an anti-obesity drug (Cawley & Rizzo, 2005), is metabolized to desmethylbenzphetamine but also to D-methamphetamine and amphetamine; Deprenyl, a drug used in the Parkinson's disease, is unusual in that it is metabolized to L-amphetamine and L-methamphetamine; Famprofazone (Gewodin), used as a painkiller and an antipyretic drug, is metabolized to D- and L-methamphetamine and amphetamine. Hence, analysis of the enantiomers together with testing of the ingested drug metabolites, taking into account the amphetamine and methamphetamine concentrations, will help to verify the source of the measured drug (Logan, 2002).



AMPHETAMINE
(a)



4--HYDROXYMETHAMPHETAMINE
(b)



NOREPHEDRINE
(c)

Figure 2.5 Chemical structures of amphetamine metabolites produced in the liver via: (a) N-demethylation, (c) aromatic hydroxylation and (b) β-hydroxylation (Foltz *et al.*, 1980)

Methamphetamine is excreted in urine depending on the pH; in normal urine pH (6-8), 37-54% of the dose of methamphetamine is excreted as the parent drug with 4-7% as D-amphetamine (Beckett & Rowland, 1965)

2.3.3 Clinical effects of Methamphetamine

General immediate effects of the methamphetamine include euphoria, decreased appetite, increased energy and the effect may remain for 4 to 12 hours depending on the dose (Wilkins, 2002).

Methamphetamine can be used in different ways either by snorting, injecting, smoking or by oral administration (Wilkins, 2002). Some symptoms are associated with the route of administration. For example, taking methamphetamine intravenously may lead to antisocial behavior and speeds up symptoms of

schizophrenia. The “rush” or “flash” which takes a few minutes is described as extremely pleasurable; the latter can be due to smoking of amphetamine while the oral consumption or snorting of amphetamine produce only euphoria but not a rush. Other symptoms that appear in case of addiction of methamphetamine are fatal lung and kidney disorder, decreased immune system, brain and liver damage and stroke (Freye & Levy, 2009)

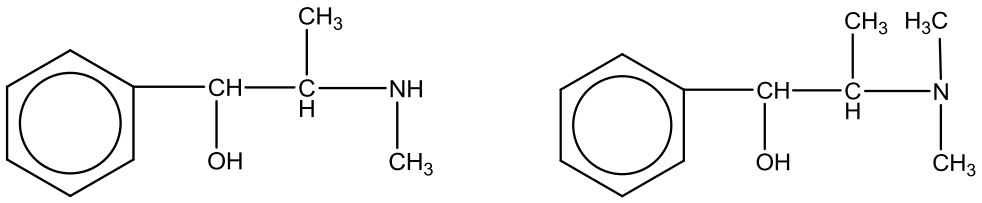
2.4 Ephedrine and Methyl ephedrine

Ephedrine is one of the natural alkaloid extracted from plant of the genus *Ephedr*a (Amare *et al.*, 2011) which is one of the traditional herbs used by the Chinese for medication purposes for more than 5000 years. Although *Ephedra* has six optically active alkaloid which are: (+)-pseudoephedrine, (-)-ephedrine, (-)-N-methyl ephedrine, (+)-N-methyl pseudoephedrine, (-)-norephedrine, and (+)-nor pseudoephedrine, the major alkaloid (-)-ephedrine comprises 30-90% of the total amount and is considered one of the first alkaloid that was extracted from *Ephedra* (Abourashed *et al.*, 2003).

2.4.1 Chemical and physical properties of ephedrine and methyl ephedrine

Ephedrine and methyl ephedrine molecular weights are 165 and 179 respectively with chemical formula $C_{10}H_{15}NO$ and $C_{11}H_{17}NO$ respectively Figure 2.6 shows the chemical structure of ephedrine and methylephedrine.

Ephedrine is characterized physically as having a waxy solid texture which comes as colorless crystals, granules and decomposed gradually when exposed to the light. It has a melting point of 40°C to 43°C and it is soluble in water, chloroform and ether but less soluble in ethanol while methylephedrine is soluble in chloroform and ether (Tilstone & Stead, 1986).



Ephedrine Methyl-ephedrine

Figure 2.6 Chemical structures of Ephedrine and Methyl-ephedrine; adapted from (Spyridaki *et al.*, 2001).

2.4.2 Ephedrine sources and synthesis

Ephedrine can be obtained via three methods as shown in Figure 2.7; the first method is through the production of natural ephedrine from *Ephedra* plants. In this method, the composite output is of natural origin and this is what is applied in the ephedrine factories in china. The second method is a semi-synthetic process which is through the fermentation of sugar followed by amination and this is commonly found in India, in this method the nitrogen source is methylamine (CH_3NH_2), which is added in step two of the manufacturing process.. The third method is chemical synthesis by bromination of propiophenone followed by amination (Makino *et al.*, 2007).

2.4.3 Chemical and pharmacological effects of ephedrine

Ephedrine and pseudoephedrine are both classified under sympathomimetic alkaloids because of their direct effects on the sympathetic nervous system; this is because it acts on the α and β-sympathetic receptors on the cell membranes found on most body organs such as heart, lungs and surrounding blood vessels.

These receptors are sensitive to ephedrine and norepinephrine (Powers, 2001) resulting in the increase in heart rate, peripheral vasoconstriction, bronchodilation and central nervous system stimulation. For this reason, ephedrine has many therapeutic uses such as for weight loss (Abourashed *et al.*, 2003), treatment of bronchial asthma and as spinal anesthesia (Amare *et al.*, 2011).

Pharmacological effects that can be associated with ephedrine and considered to be minor side effects are palpitation, restlessness, headache, insomnia and anxiety. On the other hand, there are serious side effects which include severe hypertension, irregular heartbeat, mental disorder, hepatitis, stroke and intracranial hemorrhage (Powers, 2001)

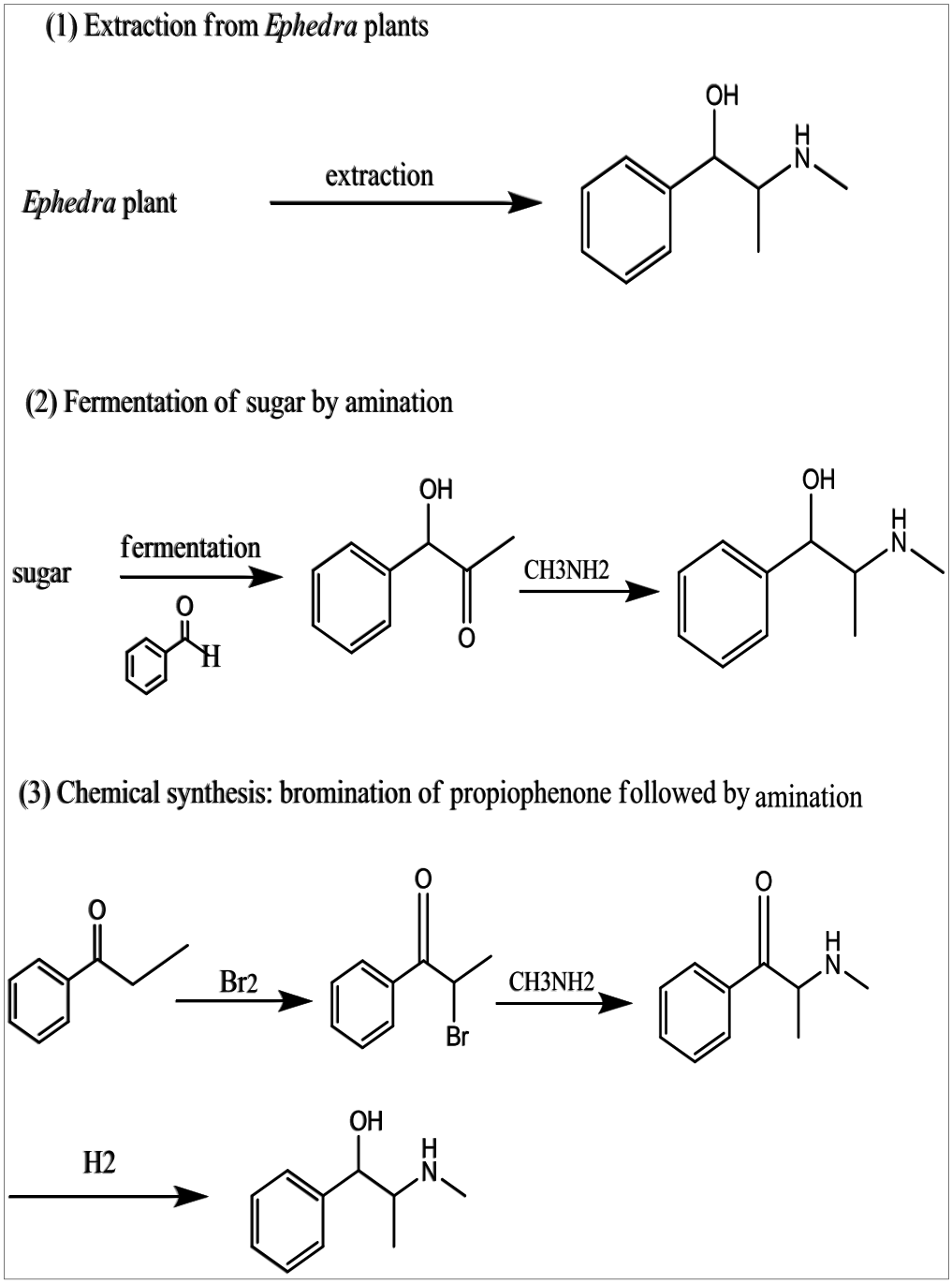


Figure (2.7) Production schemes for ephedrine adapted from Makino *et al.*, 2007.

2.5 Cathine

Cathine or D-norpseudoephedrine, Figure (2.8), is one of the active alkaloids of Khat (Feyissa & Kelly, 2008), a plant used in eastern Africa and southern Arabia; the fresh leaves is chewed and the dried form is consumed as tea (Zelger *et al.*, 1980). Cathine is produced as a result of enzymatic conversion of cathinone which is one of the other active alkaloid of Khat. During khat maturation it is also formed by the reduction of cathinone during drying and storage of Khat.

2.5.1 Chemical and physical structure of cathine

Cathine is the popular name for (+) norpseudoephedrine with chemical formula of ($C_9H_{13}NO$); the molecular weight is 151.2 and the melting point of the crystal form of cathine is 77 - 78ºC and it is soluble in ethanol, chloroform and ether (Clarke, 1986).

2.5.2 Clinical effects of cathine

A study (Mwenda et al; 2004) has shown that cathine may affect the reproductive system in reducing the utero placental blood flow, maternal weight gain and maternal myoendometrial blood flow. On the other hand cathine may increase placental vascular resistance and maternal blood pressure (Mwenda *et al.*, 2004).

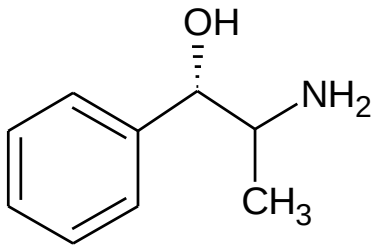


Figure 2.8 Chemical structure of norpseudoephedrine adapted from (Marais & Laurens, 2009)

2.6 Derivatization

Derivatising reagents are chemical compounds that are used to replace the unstable hydrogen atom attached to the heteroatom (Robert J, 1999) in functional groups such as hydroxyl, carboxylic acid and amines (Segura *et al.*, 1998) with less polar and more stable group. This will increase the volatility of the analyte which leads to improved chromatographic characteristic that can occur either by decreasing the analyte polarity or increasing the detector sensitivity (Robert J, 1999) .

2.6.1 Application of the derivatising reagents in Gas Chromatography Mass Spectrometry (GC-MS)

When target compounds are injected into the Gas chromatograph, the results of the analysis must be based on the mass spectra criterion which sometimes requires improvement through derivatization reagents. One of these criteria is better peak shapes without what is known as ghost peaks which can be formed as a result of column adsorption especially for thermo labile and polar compounds. Using the derivatising reagents will reduce the polarity and enhance the gas chromatographic

properties. Another criterion that is taken into account is the separation of closely related compounds which cannot be separated in the underivatized form; derivatising reagents can help in providing a good separation and mass spectra with high abundance for both identification and quantitative analysis which are usually based on monitoring of at least three ions. Derivatising agents also enhances the detectability of a compound. Using derivatising reagents can provide high electron affinity groups such as halogen atoms which increase the ionization efficiency in negative chemical ionization (NCI) (Segura *et al.*, 1998), thereby reducing the limit of detection of the particular compound .

2.6.2 Disadvantages of derivatising reagents in Gas chromatography mass spectrometry (GC-MS)

Formation of multiple derivatives is an indicator of unsuccessful derivatization and this can occur because of incomplete reaction of derivatization of polyfunctional compounds. Other obstacles that may be faced during derivatization is the side products of the derivatization reaction and its effect on the stability of the derivatives formed. For example the halogen acids that may be formed through acylation with anhydrides and acyl halides can produce dehydration or enolization which are the side reaction. In this case, neutralization is required (Segura *et al.*, 1998).

2.6.3 Types of derivatives useful in GC

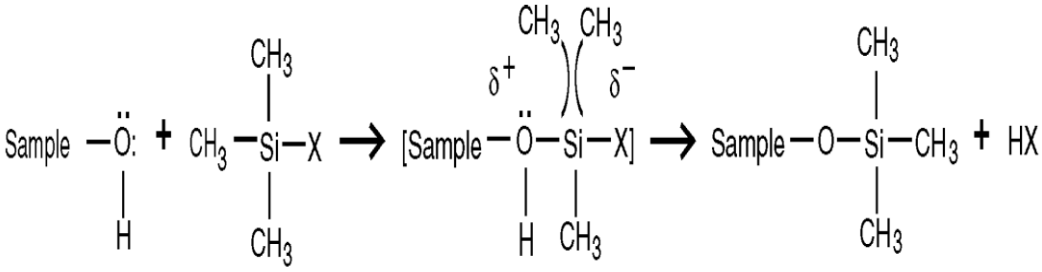
Prior to analysis of the compounds with functional groups that contain active hydrogens (OH, COOH, SH and NH), they should be treated with an appropriate derivatising reagent to avoid reduction of volatility and thermal instability which may lead to the interaction with fused silica or the stationary phase resulting in a broad peak (Danielson *et al.*, 2000). Silylation, acylation and alkylation reactions are the most effective and common derivatization methods that are used for GC (Danielson *et al.*, 2000).

2.6.3.1 Silylation

Silylation is one of the most commonly used derivatization procedure for GC-MS analyses and it is formed by the displacement of active proton in OH, NH or SH groups by an alkylsilyl group. The functional groups that are present in an organic compound can be converted to silyl ester or ether but the ease of formation of silyl derivatives differ from the most to the least efficient according to the following: alcohol > phenols > carboxylic acids > amines > amides.

The most common silylation derivatising reagent which is used in GC-MS analyses are the TMS-amides; these include *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) (Figure.2.9) and N-O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) (Figure.2.9). Because of their high volatility and silylating power, these derivatising reagents has been widely used for analytical purposes. Trimethylsilylimidazole (TMSI) (Figure.2.9) also has strong silylation affinity for carboxyl and hydroxyl groups but not for amino groups. *Tert*-

Butyldimethylsilyl (TBDMS) derivatives are also used to increase the stability and to give good mass spectrometric fragmentation but the disadvantage of TBDMS is the difficulty they present for the derivatising of sterically hindered groups (Segura *et al.*, 1998). The general reaction for the formation of trialkylsilyl derivatives is shown in scheme 2.1



Scheme 2.1: General reaction mechanism for the formation of trialkylsilyl derivatives for trimethylchlorosilane, X = Cl, adapted from (Orata)

A study published in 2009 (Bertrand R.Brunet *et al.*, 2008) reported the use of BSTFA with trimethylchlorosilane (TMCS) as a derivatising reagent to prove that the method permits the simultaneous quantification of methadone, and heroin, cocaine and metabolites in human sweat (Brunet *et al.*, 2008). BSTFA and N-methyl-n-(tert-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) are required as derivatising reagents to convert ethanolamines which result from degredation of nitrogen mustard to (TMS) and (TBDMS) esters. (Kenar & Alp, 2011).

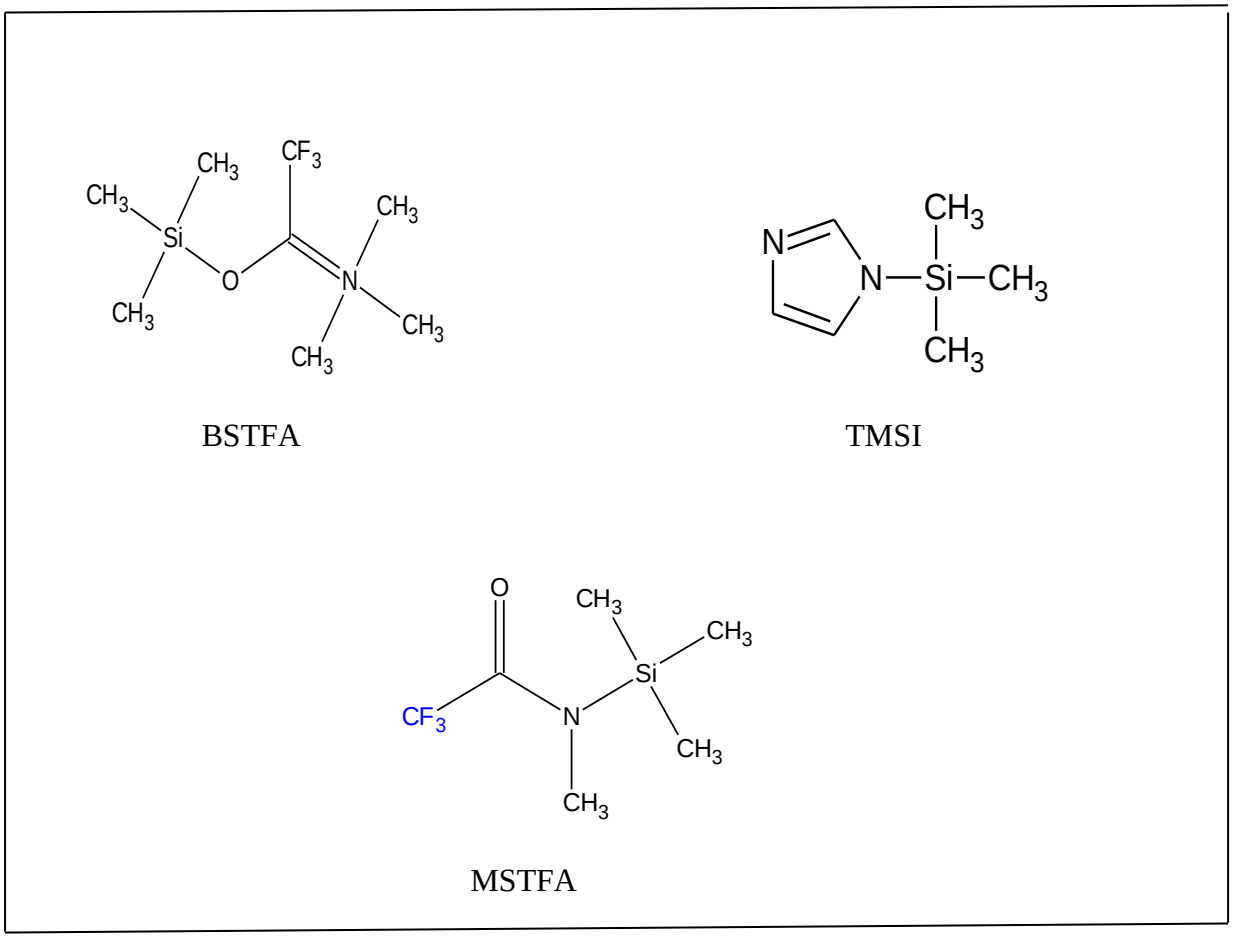


Figure 2.9 Chemical structure of BSTFA, MSTFA and TMSI (Danielson *et al.*, 2000)

2.6.3.2 Acylation

Acylation is another derivatization method that is used to convert compounds with active hydrogens such as (OH, NH, SH) into ester, amide, and thioester respectively by using a carboxylic acid or its derivative (Danielson *et al.*, 2000). Acylation derivatives can be obtained as a result of the reaction between different functional groups such as amine, alcohol, thiols, amides, enols, phenols, sulfonamides, unsaturated compounds and aromatic rings with acylation reagents. There are three main types of reagents: acyl halides, acid anhydrides and reactive acyl derivatives. The most popular acyl derivatives are haloalkylacyl because of its role in increasing the electron affinity of compounds and create highly sensitive

analysis when NCI-MS is used. Other acyl derivatives which are the perfluoroacyl derivatives are widely used because of their advantage in having a high m/z value spectra; these derivatives are: trifluoroacetyl (TFA), heptafluorobutyryl (HFB) and pentafluoropropionyl (PFP). Acylation reactions using HFBA, PFPA and TFAA derivatization reagents are shown in schemes 2.2, 2.3 and 2.4 respectively.

$$C_3F_7OCOCOC_3F_7 + H\text{-}Y\text{-}R \rightarrow C_3F_7OC\text{-}Y\text{-}R + C_3F_7OC\text{-}OH \quad (2.2)$$

$$C_2F_5OCOCOC_2F_5 + H\text{-}Y\text{-}R \rightarrow C_2F_5OC\text{-}Y\text{-}R + C_2F_5OC\text{-}OH \quad (2.3)$$

$$CF_3OCOCOCF_3 + H\text{-}Y\text{-}R \rightarrow CF_3OC\text{-}Y\text{-}R + CF_3OC\text{-}OH \quad (2.4)$$

Schemes 2.2, 2.3, 2.4: Derivatization reactions using HFBA, PFPA and TFAA respectively: Y = O, NH, NR´, R, R´ = Alk, Ar, adapted from (Mohd, 2012)

In addition to these acyl derivatives, there is what is known as trifluoroacetylation which can be applied to amine, hydroxyl, and thiol groups by using N-methylbis(trifluoroacetamide) (MBTFA) or bis(trifluoroacetamide (BTFA) which is characterized by high volatility and do not interfere in the GC analysis . There are many articles that have presented results of the use of perfluoroacyl derivatives in the determination of amphetamine and related drugs in urine. In 1996, Jon Jonsson *et al* published their articles that mentioned the use of trifluoroacetic anhydride (TFA) as a derivatising agent for the determination of amphetamine in authentic samples by (GC-MS) and compared the results to those that used another derivatising agent, the chloroformate derivatives by using Nitrogen Phosphorus Detector Gass Chromatograph (GC-NPD) (Jonsson et al., 1996). Another article

published in 2009 by Tamer Awad *et al* on the use of perfluoroacyl derivative (heptafluorobutyrlamides and pentafluoropropionylamides) to distinguish between primary and secondary regioisomeric amines (3-methoxy-4-methylphenethylamines and 4-methoxy-3-methyl-phenethylamines); the study showed that both PFPA and HFBA derivatives are suitable for chromatographic separation purposes, However, the HFBA derivative offered more unique fragment ions which provide additional distinction among the regioisomeric substances (Awad *et al.,* 2009). In an article published in 2002, Huang et al proved that the combination of heptafluorobutyric chloride (HFBCl) and HFBA as a derivatizing reagents by using headspace solid-phase microextraction (HS-SPME) method achieved high sensitivity for amphetamine and methamphetamine analysis; the use of this new acylation technique which is based on water hydrolysis of derivatizing reagents is because the amphetamines cannot be acylated effectively in heated aqueous solution (Huang et al., 2002). In a research conducted in 2001, presented the determination of the ring substituted amphetamines that were abused by a group of recovering opiate abusers participating in a methadone maintenance programme in a Dublin Drug Rehabilitation Centre; in this analysis the use of MBTFA as a derivatizing reagents for the determination of the amphetamine type stimulants (Kavanagh *et al.*, 2001). N-TFA-O-TMS is a derivative formation that is produced from trimethylsilylation reagent which is MSTFA followed by MBTFA which is a trifluoroacylating reagent. This derivative is used for amino acids, phenolalkylamines, and hydroxyamines because of its stability in solution and provides excellent chromatographic properties.