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BAKALÁŘSKÁ PRÁCE

2020 Mutale Jane Mulenga

UNIVERZITA PARDUBICE

FAKULTA CHEMICKO-TECHNOLOGICKÁ

IDENTIFICATION AND DETERMINATION OF NARCOTIC AND PSYCHOTROPIC SUBSTANCES IN HAIR AND URINE

Mutale Jane Mulenga

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Zásady pro vypracování:

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ANOTACE

Tato bakalářská práce se zabývá klasifikací, extrakcí a stanovením nelegálních drog. V první kapitole je bakalářská práce zaměřena na chemickou klasifikaci nelegálních drog, jejich využití a účinky. Ve druhé části se věnuje pozornost extrakci a analýze nelegálních drog v biologických materiálech (tj. moči, krvi a vlasech). A v poslední části se pojednává o stanovení v těchto jednotlivých biologických materiálech.

KLÍČOVÁ SLOVA

drogy, moč, krev, vlasy, extrakce, stanovení, kvantitativní analýza

TITLE

Classification, extraction and determination of illicit drugs

ANNOTATION

This bachelor thesis deals with the classification, extraction and determination of illicit drugs. In the first chapter, the author focuses on the chemical classification of illicit drugs, their uses and effects. In the second part, she pays attention to the extraction and analysis of illicit drugs in biological materials (i.e. urine, blood and hair). And lastly, the determination in these individual biological materials.

KEYWORDS

Illicit drugs, urine, blood, hair, extraction, determination, quantitative analyses, liquid-liquid extraction, solid-phase extraction.

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Abbreviations

6-MAM	6-monoacetylmorphine
ADHD	Attention Deficit Hyperactivity Disorder
CBD	Cannabidiol
CE	Capillary Electrophoresis
CFME	Continuous Flow Microextraction
CNS	Central Nervous System
CSA	Controlled Substances Act
CZE	Capillary Zone Electrophoresis
DLLME	Dispersive Liquid-Liquid Microextraction
DUI	Driving Under the Influence
DVB	Divinyl benzene
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GC	Gas Chromatography
HF-LPME	Hollow Fibre Liquid-Phase Microextraction
HPLC	High Performance Liquid Chromatography
HS-SDME	Headspace Single Drop Microextraction
HS-SPME	Headspace Solid Phase Microextraction
IPA	Iso-propyl alcohol
LC	Liquid Chromatography
LLE	Liquid-Liquid Extraction
LLLME	Liquid-Liquid-Liquid Microextraction
LSD	Lysergic acid diethylamide
MDA	Methylenedioxyamphetamine

MDEA	Methylenedioxy-N-ethyl amphetamine
MDMA	Methylenedioxymethamphetamine
MS	Mass Spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular Weight
OF	Oral Fluid
PCP	Phencyclidine
PDMS	Poly(dimethyl)siloxane
PFAA	Pentafluoro propionic anhydride
PFP-OH	Pentafluoro propanol
RSD	Relative Standard Deviations
SDME	Single-Drop Microextraction
SFC	Supercritical Fluid Chromatography
SLE	Supported Liquid Extraction
SPE	Solid-Phase Extraction
SPME	Solid-Phase Microextraction
THC	Tetrahydrocannabinol
THC-COOH	Carboxy tetrahydrocannabinol
UHPLC	Ultra-High Performance Liquid Chromatography

Introduction

A drug is any chemical substance that alters normal bodily function when absorbed into the body of a living organism. It can either originate from a natural source such as a plant or fungi or can be synthesised from a chemical [1, 2].

This first part of this thesis will focus primarily on the classification of illicit or recreational drugs. These are basically psychoactive drugs or drugs that affect the mind, mood or other mental processes. They are usually taken for any other reason than medical benefits such as for enjoyment or entertainment purposes. Illicit drugs cause a chemical reaction in the brain which affects feelings, thoughts and behaviour. This can be extremely harmful and can lead to addiction, self-damage and even death [1].

The second part is about the analysis of illicit drugs me in biological materials such as urine, blood and hair and how these samples are prepared for analysis using the different types of extraction methods.

The third part describes how the determination of these drugs is carried out using different quantitative techniques like gas and high performance liquid chromatography, which are used most frequently. At the end is a case report, which was done at the Norwegian institute of public health. At the end are tables summarizing all the information regarding the analysis of different drugs and their metabolites in whole blood, oral fluid, urine and hair.

The manufacture, importation, possession, use and distribution of drugs, controlled substances and chemicals used to produce them is regulated by the federal U.S. drug policy known as The Controlled Substances Act (CSA) Title II of the Comprehensive Drug Abuse Prevention and Control Act of 1970. The CSA places regulated chemicals into five (5) categories called schedules, depending on the material's pharmaceutical use, potential harmfulness, and how likely it is to cause addiction [3]. Below is a summary of the schedules:

- **Schedule 1:** Highly addictive substances with little or no medical use. These include *marijuana (cannabis), heroin, 3,4-methylenedioxymethamphetamine (MDMA or ecstasy)* and *Lysergic acid diethylamide (LSD)* [2].

- **Schedule 2:** Highly addictive substances with a severely restricted medical use. These include *cocaine, phencyclidine (PCP),* and many *amphetamine and barbiturate formulations* [2].

- **Schedule 3:** Substances with less potential for abuse than schedule 1 and 2 drugs and have a recognised medical use. These are also prescription drugs. Schedule 3 drugs include *codeine, ketamine,* and *anabolic steroids* [2].

- **Schedule 4:** Substances with lower potential for abuse than schedule 3 drugs and that possess a medical use. They are also prescription drugs and treated similarly to schedule 3 substances. Schedule 4 drugs include *chlordiazepoxide (Librium), dexfenfluramine (Redux), ethchlorvynol (Placidyl), zolpidem (Ambien),* and *many tranquilizers* and *weight control agents* [2].

- **Schedule 5:** Substances with less abuse potential than schedule 4 drugs and that have medical use. Many of these preparations are available without a prescription. Schedule 5 drugs include *codeine and opiate preparations used in cough syrups* [2].

However, the term "illegal drugs" is not used because the defination of what is legal differs from country to country and because some drugs that are abused or overdosed, or even prescribed medically or sold over the counter lead to addiction and are legal drugs [1].

1 CHEMICAL CLASSIFICATION OF ILLICIT DRUGS

The categorization of drugs can be done in several ways. In the medical and pharmacological industries, a drug can be categorized by its chemical activity or by the way in which it treats. For example, medications used to prevent seizures are called anticonvulsants, while drugs that break down mucus and relieve congestion are called mucolytic drugs. With reference to addiction treatment and rehabilitation, the classification of drugs frequently used are the following five (5) classes regulated by the controlled substances Act [4]:

- Cannabis,
- Depressants,
- Narcotics,
- Stimulants,
- Hallucinogens.

1.1 Cannabis (marijuana)

Cannabis also known as marijuana, is a mind-altering drug derived from the cannabis plant (*cannabis sativa* or *cannabis indica*). It is either used for medical or recreational purposes [5]. The main psychoactive part of the cannabis plant is tetrahydrocannabinol (**THC, figure 1).** Cannabis can be smoked, vaporized, ingested through food or as an extract [6].

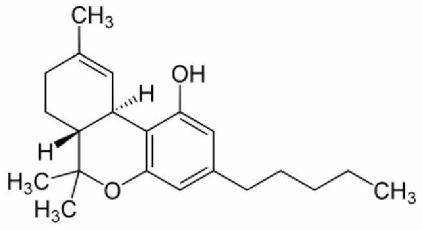

Figure 1: Molecular structure of Tetrahydrocannabinol (THC) [93]

1.1.1 Uses of marijuana

Marijuana is also comprised of another chemical called Cannabidiol (**CBD, figure 2**). This is the substance usually linked with creating medical benefits. Unlike THC, CBD does not cause someone to be high. However, the medical benefits as well as the breeding methods of cannabis plants with a high level of CBD and low level of THC for medical use are still being studied [7].

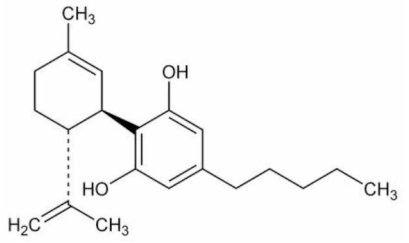

Figure 2: Molecular structure of Cannabidiol (CBD) [94]

There is not much evidence proving that cannabis can be used to reduce nausea and vomiting in patients undergoing chemotherapy, to help improve appetite in people with HIV/AIDS and to treat chronic pain and muscle spasms. However, an artificial type of THC known as Drabinol, approved by the Food and Drug Administration (FDA) is used as an appetite stimulant for people with AIDS and as an antiemetic or drug that prevents vomiting for people receiving chemotherapy. The use of marijuana for other medical purposes is not enough in terms of safety or efficiency [8-11].

Cannabis can cause both psychoactive and physiological effects when used. The direct effects of consuming cannabis are relaxation as well as euphoria (the "high" or "stoned" feeling), a general change of awareness sensations, high sex drive and misrepresentations in the perception of time and space [12].

Other effects when misused include change in physical appearance, auditory and/or visual illusions or hallucinations and ataxia or lack of control of bodily movements

due to the selective impairment of polysynaptic reflexes. In some cases, cannabis can lead to detachment states such as detachment from oneself as well as reality [13].

1.1.2 Pharmacokinetics and Pharmacodynamics of Marijuana

When a person smokes marijuana, THC is quickly transferred from the lungs into the bloodstream. The blood then transports the chemical to the brain and other organs throughout the body. When ingested, THC absorbs slower in the body, hence feeling the effects after about half an hour to an hour. THC acts on precise brain cell receptors that typically react to natural THC-like chemicals which help in normal brain development and function. Marijuana over-activates the parts of the brain that have the highest amount of these receptors hence causing the "high" that people feel [14]. When a person is new to marijuana use, the exposure to THC levels is higher and they stand a greater chance of a harmful reaction [15].

1.1.3 Physical Effects

- **Breathing problems:** People who smoke marijuana can usually have similar breathing problems such as daily cough and phlegm, frequent lung illness and infections as those who smoke tobacco as the marijuana smoke irritates the lungs. So far, researchers have not fully determined the higher risk of lung cancer in marijuana smokers [16].
- **Increased heart rate:** Marijuana increases heart rate about 3 hours after smoking. This might the possibility of heart attack. Therefore, vulnerable people like children, people with asthma or older people and those with heart problems may be at higher risk [16] [25].
- **Problems with child development during and after pregnancy:** A study suggested that during a drug test, women are twice as likely to test positive for marijuana use than they say in self-reported procedures [17]. This is a huge concern for medical professionals as the use of marijuana during pregnancy may cause lower birth weight and a higher risk of both brain and behavioural problems in babies [18-20]. Studies also suggest that relative amounts of THC are excreted into mothers' breast milk [21].

- **Intense Nausea and Vomiting:** Consistent, prolonged use of marijuana can lead to Cannabinoid Hyperemesis Syndrome. This causes users to experience a series of severe nausea, vomiting, and dehydration [22].

Although it is possible to fail a drug test after inhalation of second-hand marijuana smoke, it's unlikely because a very minimal amount of THC is released in the air when exhaled. Even though THC was found in blood, there wouldn't be enough to fail a blood test [23, 24].

Studies on animals suggest that exposure to addictive substances like THC could change the way in which the brain responds to other drugs. For instance, the repeated exposure of young rodents to THC later showed an enhanced response to other addictive substances like morphine and nicotine in some parts of the brain, hence showed addiction-like behaviours [26-29].

The extensive use of marijuana can cause addiction, causing health and social problems in a person's life. People who start using marijuana before the age of 18 are 4 to 7 times more likely to develop a marijuana addiction. However, people addicted to marijuana report mild withdrawal symptoms that make it hard for them to quit such as insomnia, loss of appetite, anxiety, grumpiness as well as cravings [30, 31].

1.2 DEPRESSANTS

Depressants are mind altering or psychoactive drugs that can either be in form of pharmaceutical drugs or illicit drugs that slow down the activity of the central nervous system (CNS). This causes a person to become less cautious and slows down functions like breathing and heart rate. Drugs classified as depressants include [32]:

- **Alcohol,**
- **Barbiturates,**
- **Benzodiazepines.**

1.2.1 Alcohol

Alcohol, also chemically known as ethanol, is a psychoactive drug found in alcoholic beverages such as beer, wine and distilled spirits or hard liquor as the active ingredient. Alcohol is one of the oldest and most commonly used recreational substances causing alcohol intoxication or drunkenness. Alcohol consumption can cause both short and long-term effects depending on the length of consumption [33, 34].

Short-term effects include:

- Sedation,
- Increased sociability,
- Decreased anxiety,
- Cognitive, memory, motor and sensory impairment.

Long term effects include:

- Significant permanent damage to the brain,
- Liver disease,
- Birth defects,
- Cancer,
- Death.

Ethanol consumption can lead to death when blood alcohol levels reach 0.5 % or more while levels less than 0.1 % can cause intoxication and unconsciousness often happening at 0.3-0.4 % [35].

1.2.2 Barbiturates

Barbiturates (**figure 3**) are central nervous depressants that cause muscle relaxation by reducing nerve activity. They also decrease heart rate, breathing and blood pressure. All barbiturates affect gamma-aminobutyric acid (GABA), a neurotransmitter

(chemical) which enables nerves to communicate with each another. Barbiturates are all chemical derivatives of barbituric acid, hence the name barbiturate [36].

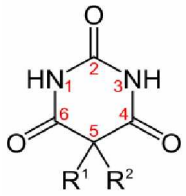

Figure 3: Basic structure of a barbiturate including numbering scheme [37]

Table 1: Some examples of barbiturates and their IUPAC names [37]

Short Name	**R^1**	**R^2**	**IUPAC Name**
Allobarbital	CH_2CHCH_2	CH_2CHCH_2	5,5-diallylbarbiturate
Amobarbital	CH_2CH_3	$(CH_2)_2CH(CH_3)_2$	5-ethyl-5-isopentyl-barbiturate
Aprobarbital	CH_2CHCH_2	$CH(CH_3)_2$	5-allyl-5-isopropyl-barbiturate
Alphenal	CH_2CHCH_2	C_6H_5	5-allyl-5-phenyl-barbiturate
Barbital	CH_2CH_3	CH_2CH_3	5,5-diethylbarbiturate
Brallobarbital	CH_2CHCH_2	CH_2CBrCH_2	5-allyl-5-(2-bromo-allyl)-barbiturate
Pentobarbital	CH_2CH_3	$CHCH_3(CH_2)_2CH_3$	5-ethyl-5-(1-methylbutyl)-barbiturate
Phenobarbital	CH_2CH_3	C_6H_5	5-ethyl-5-phenylbarbiturate
Secobarbital	CH_2CHCH_2	$CHCH_3(CH_2)_2CH_3$	5-[(2*R*)-pentan-2-yl]-5-prop-2-enyl-barbiturate; 5-allyl-5-[(2R)-pentan-2-yl]-barbiturate

Uses

Barbiturates can create a number of effects ranging from mild sedation to total anaesthesia. They are used as anxiolytics, hypnotics and anticonvulsants. Barbiturates can cause addiction both physical and psychological hence, have rapidly been replaced by benzodiazepines in medical routines such as anxiety and insomnia treatments and because benzodiazepines have a lower risk of overdose. This is because an antidote for

barbiturate overdose is also scarce. Despite this, barbiturates are still used for different purposes such as general anaesthesia, epilepsy, migraine treatment, euthanasia and capital punishment [37].

The most commonly abused barbiturates include amobarbital (Amytal), pentobarbital (Nembutal), and secobarbital (Seconal). This is because these barbiturates cause effects within a very short time after being taken. A combined use of amobarbital and secobarbital known as Tuinal is also widely use. Short-acting barbiturates are frequently prescribed as sedatives and sleeping pills. These pills begin to cause effects after about 15 to 40 minutes after being taken and their effects last from 5 to 6 hours [37].

Effects

As people get older, the body becomes less capable of getting rid of barbiturates. Therefore, people above the age of 65 are at higher risk of suffering the harmful barbiturate effects such as drug dependence and accidental drug overdose. However, pregnant women and babies are also at a higher risk. When taken during pregnancy, barbiturates pass through the placenta to the foetus and when the baby is born may experience withdrawal symptoms as well as trouble breathing. Furthermore, when breastfeeding mothers take barbiturates, the drug may be transmitted to their babies through breast milk [38, 39].

1.2.3 Benzodiazepines

Benzodiazepines (**BZD, BZs, figure 4**) also known as "benzos", are a group of psychoactive drugs which heighten the effect of the neurotransmitter GABA at the $GABA_A$ receptor, causing sedation, hypnosis, muscle relaxation as well as anxiolytic and anticonvulsant effects. When overdosed, short-acting benzodiazepines may cause memory loss and detachment. However, benzodiazepines are used in treating anxiety, muscle spasms, insomnia, seizures, alcohol withdrawal, agitation and as a premedication for medical or dental procedures [40, 41].

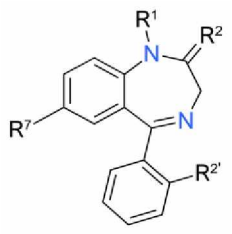

Figure 4: Basic structure of benzodiazepines [95]

List of examples of generic and brand names for benzodiazepines [42]:

- Alprazolam (Xanax),
- Clobazam (Onfi),
- Clonazepam (Klonopin),
- Clorazepate (Tranxene),
- Chlordiazepoxide (Librium),
- Diazepam (Valium, Diastat, Acudial, Diastat),
- Estazolam (Prosom),
- Lorazepam (Ativan),
- Oxazepam (Serax),
- Temazepam (Restoril),
- Triazolam (Halcion).

Even when prescribed by a doctor, benzodiazepines can cause serious addiction especially in people with a drug or alcohol abuse history. However, when used for a very long period, people are more likely to develop a tolerance for them hence needing higher doses during the treatment of medical conditions. Benzodiazepines are frequently abused by young adults by crushing them up and sniffing and sometimes taken whole as a pill. These drugs however have adverse effects such as; nightmares, agitation, aggression and amnesia [42].

Signs and symptoms of addiction include:

- Problems sleeping,
- Diarrhoea,
- Vomiting,

- Nausea,
- Goose bumps,
- Uncontrollable leg movements,
- Bone and muscle pain.

Benzodiazepines can change the chemistry of the brain, hence making the recovery from addiction difficult [42].

1.3 NARCOTIC ANALGESICS

Narcotics analgesics also known as narcotics, opiates, opioid analgesics or simply opioids are a category of drugs used as a relief from relative to severe acute or chronic pain. Analgesic is another term used for pain relief medication. Narcotic analgesics are amongst the most commonly used analgesics for pain relief hence being amongst the most abused drugs resulting in millions of people having a substance abuse disorder involving prescribed analgesics [43].

Narcotic analgesics bind to opioid receptors which are part of the opioid system that is responsible for pain, pleasure and addiction behaviour. These receptors are also found elsewhere in the body such as the lungs and stomach but exist more abundantly in the brain and spine (CNS) [43].

1.3.1 Opium

Opium (*Lachryma papaveris)* or "poppy tears" is obtained from the dried latex of opium poppy (*Papaver somniferum*). Opium latex comprises of approximately 12 % of the analgesic alkaloid morphine, other closely related opiates such as codeine and thebaine and non-analgesic alkaloids such as papaverine and noscapine. Morphine is used to produce heroin and other synthetic opioids for either medicinal or illegal drugs trade purposes by chemically processing it [44].

With the advancement of prescription pain medication over the past years, it is important to know the difference between the terms opiate, opioid and narcotic and how they work.

1.3.2 Opiates

Opiates are the natural substances extracted from opium while opium can be extracted from opium poppy. Opium contains opiates such as morphine (**figure 5**) and codeine (**figure 6**) [45].

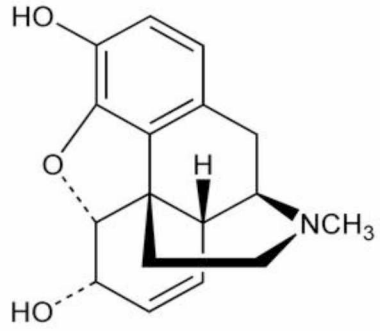

Figure 5: Molecular structure of Morphine [96]

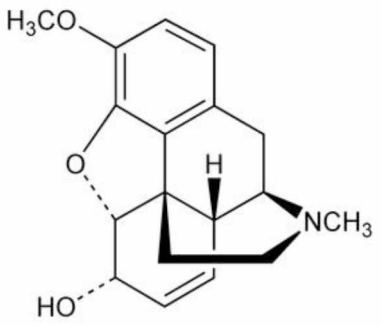

Figure 6: Molecular structure of Codeine [97]

1.3.3 Opioids

Like opiates, opioids also bind to opioid receptor system (mu, kappa, delta) but do not occur naturally. Therefore, opioids can either be synthetic for example fentanyl (**figure 7**) and methadone (**figure 8**) or semi-synthetic for example oxycodone (**figure 9**) and hydrocodone (**figure 10**). Synthetic opioids are chemically manufactured, while semi-synthetic opioids are a result of chemical modifications to natural opiates [45].

Although opioids are prescribed pain relief medications, they can have adverse effects such as drowsiness and can cause addiction. For this reason, the use of prescribed opioids is regulated by the Controlled Substances Act in the United States. Nonetheless, not all opioids are prescribed for pain relief. Heroin (**figure 11**) is an example of an illegal and non-prescribed opioid derived from morphine and is usually abused by injection [45].

Examples of synthetic opioids

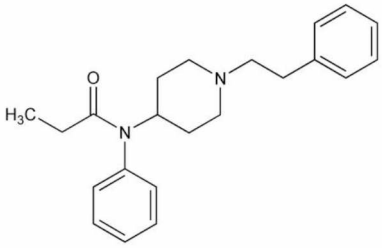

Figure 7: Molecular structure of Fentanyl [98]

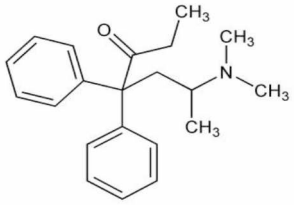

Figure 8: Molecular structure of Methadone [99]

Examples of semi-synthetic opioids

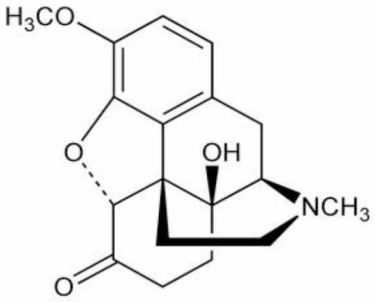

Figure 9: Molecular structure of Oxycodone [100]

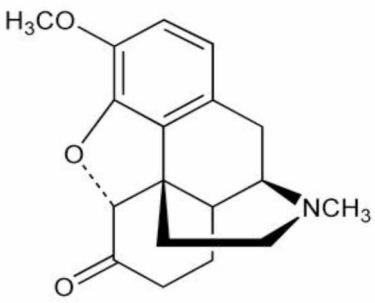

Figure 10: Molecular structure of Hydrocodone [101]

Example of a non-prescription opioid

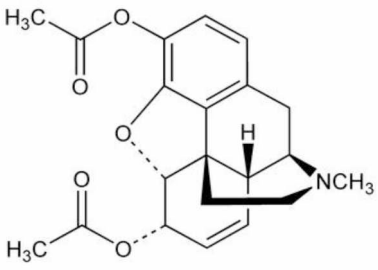

Figure 11: Molecular structure of Heroin [102]

1.3.4 Narcotics

Opioids are generally classified under narcotics. But because the term narcotics is usually associated with illegal drugs, its use in the medical industry has fallen out. However, the term narcotic refers to a substance that causes insensibility or narcosis [45].

1.4 STIMULANTS

As the name suggests, stimulants are drugs that stimulate or enhance alertness, hence leading to increased attention, energy and high blood pressure. These drugs have generally been used throughout medical history to treat asthma, obesity, different neurological and respiratory problems. Previously, stimulants were regarded as safe. But as use increased, the dangers became more obvious and have since been less prescribed and accepted unless in the most severe cases [46].

As of today, stimulants are only used to treat very few disorders such as Attention Deficit Hyperactivity Disorder (ADHD) and narcolepsy. Even when prescribed for such cases, these drugs are used as a last resort only when other forms of treatment have failed [46].

Drugs that can be classified as stimulants include:

- Caffeine,
- Nicotine,
- Cocaine,
- Amphetamine,
- Methamphetamine,
- MDMA(MethylenDioxyMethAmphetamine).

1.4.1 Caffeine

Caffeine (**figure 12**) is a compound which belongs to the class of chemicals called xanthine which are naturally found in coffee, tea and in smaller amounts cocoa or chocolate, soft drinks as well as energy drinks in larger amounts. Caffeine is the world's

most common and frequently used stimulant. It is also used in some medications to help increase or reduce the effects of the primary ingredient fatigue and drowsiness [47].

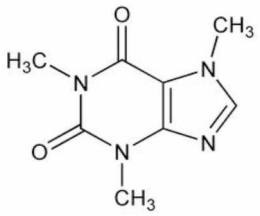

Figure 12: Molecular structure of Caffeine [103]

Caffeine has inconsistent effects on learning and memory but improves reaction time, concentration and motor coordination. However, depending on the size and tolerance of a person, the amount of caffeine needed to produce these effects differs from person to person with effects arising about an hour after consumption and subsiding after about 3 to 4 hours [48, 49].

1.4.2 Nicotine

Nicotine (**figure 13**) is the active chemical ingredient found in tobacco and can exist in many forms such as, cigars, cigarettes and smoking cessation aids like nicotine patches and electronic cigarettes. Nicotine usually used worldwide due to its stimulating and relaxing effects. This happens because of glucose being released from the liver and adrenaline from the adrenal medulla. Users report feelings of relaxation, alertness and calmness [50, 52, 53].

Nicotine binds with the nicotinic acetylcholine receptor, causing several effects such as an increase in the activity of dopaminergic neurons in the midbrain reward system and a decrease in the expression of monoamine oxidase in the brain. The most common source of nicotine, which is tobacco can be very addictive ranking 6th amongst the most harmful of 20 drugs assessed by a multi-criteria decision analysis. It was also determined at 3 % below cocaine and 13 % above amphetamines [50, 51].

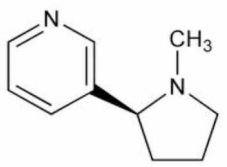

Figure 13: Molecular structure of Nicotine [104]

1.4.3 Cocaine

Cocaine (**figure 14**) is a very strong addictive stimulant extracted from coca plant leaves. Despite its use in the medical industry for some medical purposes such as anaesthesia during surgery, cocaine is an illegal drug. As a street drug, it appears as a fine, white, crystal powder often mixed with corn starch, talcum powder or flower as well as other drugs such as amphetamine to increase profits [54]

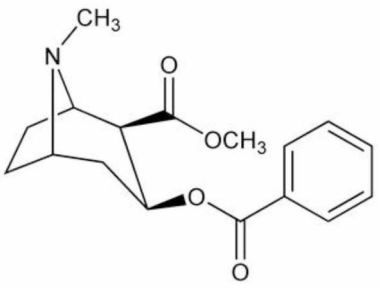

Figure 14: Molecular structure of Cocaine [105]

Cocaine users normally sniff it through the nose, rub it into their gums or inject it into the bloodstream by either dissolving the powder alone in water or as a combination with heroin called a "speedball". Some users smoke processed cocaine in the form of a crystal rock called "freebase cocaine", which is heated to produce vapours which are then inhaled into the lungs [54].

Cocaine elevates the amount of the natural chemical messenger in the brain called dopamine which controls pleasure and movement. Usually, the brain releases dopamine in response to potential rewards such as the smell of good food. It is then recycled back into the cell from which it was released, cutting off the signal between nerve cells.

However, cocaine prevents the recycling of dopamine, hence causing extreme amounts to build up between nerve cells. This accumulation of dopamine eventually disturbs normal brain communication and causes cocaine's "high". Cocaine users take the drug repeatedly within a short time at increasingly higher doses to keep their high [54].

1.4.4 Amphetamine

Amphetamine (**figure 15**), which is extracted from alpha-methylphenethylamine is a powerful CNS stimulant used in the treatment of ADHD, narcolepsy, obesity, nasal congestion and depression. Amphetamine has been known to be used as a cognitive enhancer and to enhance athletic performance and as a recreational drug, is often used as an aphrodisiac and a euphoriant. Despite being a prescription drug, the unauthorized possession and distribution of amphetamine is highly restricted due to the major health risks in relation to its recreational use [55-57].

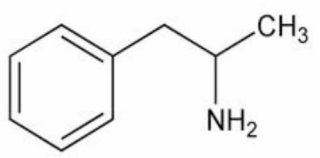

Figure 15: Molecular structure of Amphetamine [106]

1.4.5 Methamphetamine

Methamphetamine (**figure 16**), usually known as "meth" is a crystalline powder with chemical properties like those of amphetamine. Methamphetamine is like cocaine in the way it stimulates the CNS but has more intense and long-lasting effects than those of cocaine. Methamphetamine is a powerful CNS stimulant usually used recreationally and less commonly as an alternative medication for ADHD and obesity [58, 59].

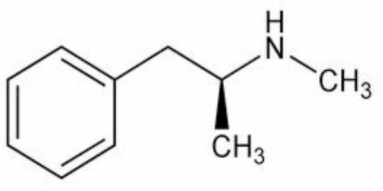

Figure 16: Molecular structure of Methamphetamine [107]

When taken in low doses, methamphetamine can increase concentration and energy and cause mood elevation in people with fatigue and can also cause loss of appetite and promote weight loss. However, at a higher dose can induce psychosis, seizures, breakdown of skeletal muscle and bleeding in the brain. When used over a long period of time in higher doses, it can be very addictive and can cause unpredictable, rapid mood swings, violent behaviour as well as psychotic behaviour such as paranoia, hallucinations and delusions. Methamphetamine is known recreationally for its ability to increase energy and sexual desire to a point where users are continuously able to engage in sexual activity for several days[60].

1.4.6 3,4-MethyleneDioxyMethamphetamine

Commonly known as ecstasy (E), 3,4-Methylenedioxymethamphetamine (**MDMA, figure 17**) is a psychoactive drug belonging to the substituted methylenedioxyphenethylamine and amphetamine classes of drugs and is mainly used recreationally. MDMA can cause increased empathy, euphoria and enhanced sensations. Effects of MDMA begin after about 30-45 minutes when taken orally and last for about

3-6 hours. As of 2017, there has not been approval for any use of MDMA in the medical industry [61-63].

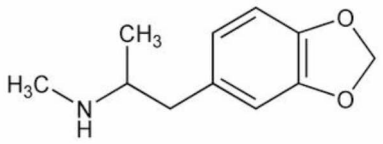

Figure 17: Molecular structure of 3,4-Methylenedioxymethamphetamine [108]

MDMA can cause addiction and has negative effects such as memory loss, insomnia, paranoia, grinding of teeth, impaired vision, sweating, rapid heartbeat, depression and fatigue. There have also been reported cases of death due to an increase in body temperature and dehydration. MDMA increases the release and slows the re-uptake of the neurotransmitters serotonin, dopamine and norepinephrine in parts of the brain and has psychedelic effects [61, 64, 65].

1.5 HALLUCINOGENS

Hallucinogens are drugs that cause major distortions of reality by changing the user's thinking processes and perception. They are known to change a person's perception far differently than most drugs. The effects of these drugs can cause one to experience new and prolonged consciousness as well as synaesthesia (mixed sensory experiences, such as seeing sounds or hearing colours). Hallucinogens also cause other common negative effects such as; hallucinations, misconception of time, detachment experiences like not feeling connected to reality or one's body [66].

Some of the more common hallucinogens include:

- LSD,
- Psilocybin (magic mushrooms),
- peyote (mescaline),
- Ketamine (Special K),
- PCP (phencyclidine).

1.5.1 Lysergic Acid Diethylamide

Lysergic acid diethylamide (**LSD, figure 18**) is a mind-altering drug famous for its psychological effects such as delusions, anxiety and paranoia. LSD is addictive and is mostly used recreationally and is often sold on a cube of sugar, gelatine or blotter paper. The drug is usually swallowed, kept under the tongue or injected into the bloodstream. A dose as low as 20–30 micrograms can produce an effect. When in pure form, LSD is a clear or white crystalline, odourless, compound. It is sensitive to ultraviolet light, oxygen and chlorine and can last for a long period of time when kept away from light and moisture at a low temperature [66-68].

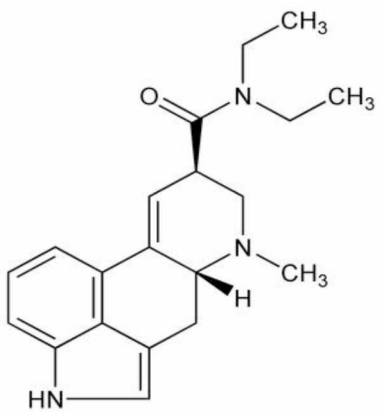

Figure 18: Molecular structure of Lysergic Acid Diethylamide [109]

1.5.2 Psilocybin (Magic Mushrooms)

Psilocybin (4-phosphoryloxy-N, N-dimethyltryptamine, **figure 19**) also known as "magic mushroom" like any other naturally occurring hallucinogens is found in several types of mushrooms. When taken, the body changes it to psilocin which produces psychedelic effects like those of LSD but only last for a shorter period. Even though the recommended dose may be in the range of milligrams, unknown amounts are often taken as they are taken through the consumption of mushrooms or tea [69].

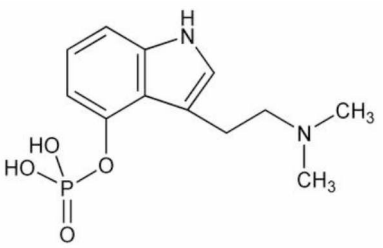

Figure 19: Molecular structure of Psilocybin [110]

1.5.3 Peyote (Mescaline)

Peyote (mescaline, **figure 20**) are the buttons found on many species of cactus indigenous to Mexico. Even though the drug contains several phenethylamine alkaloids, its primary mind-altering ingredient is mescaline and may be produced synthetically [69].

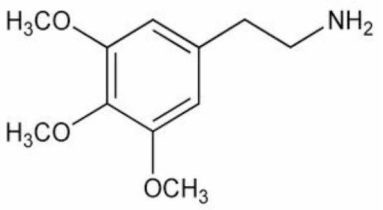

Figure 20: Molecular structure of Mescaline [111]

1.5.4 Ketamine

Ketamine (**figure 21**) is a drug used as an anaesthetic during human and animal surgery. Most of the ketamine sold on the streets for recreational purposes comes from veterinary offices. Most manufacturers sell the drug as powder or as pills even though it is available as an injectable liquid [66].

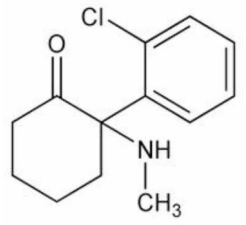

Figure 21: Molecular structure of Ketamine [112]

Apart from providing pain relief, sedation and memory loss, ketamine also causes a trance-like state. However, breathing and heart function usually remain functional during its effects. Effects usually start about 5 mins after being injected and last up to 25 minutes. The drug is also used for chronic pain and as a sedative in intensive care [70, 71].

1.5.5 Phencyclidine

Phencyclidine (**PCP, figure 22**), also known as "angel dust" among other names, is a psychoactive drug that was initially made as an anaesthetic, but patients treated with it experienced several adverse effects while recovering from it. It may be in form of tablet, liquid, crystal, capsule or powder. It can either be sniffed, ingested or smoked. Sometimes, cigarettes are dipped into a PCP solution before being sold. PCP can cause visual hallucinations, paranoia and a sense of invulnerability [66, 72, 73].

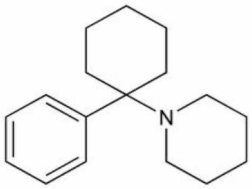

Figure 22: Molecular structure of Phencyclidine [113]

2 EXTRACTION OF ILLICIT DRUGS

Extraction is the process of separating one or more compounds of interest (analytes) from another compound (usually referred to as the sample or matrix) for further processing and analysis to be done usually using a fluid (an extracting solvent). Although extractions into the gas phase and on to solid sorbents are also common [78].

2.1 Sample Preparation

The preparation of the sample that is being analysed is usually the most crucial and difficult in-terms of both time and effort taken in the separation of the analyte from the matrix as each matrix has its own exceptional challenges. For instance, urine contains a high concentration of salt, plasma contains a high number of phospholipids, whole blood contains red blood cells which frequently must be lysed and so on. The type of extraction technique to be used depends on the different characteristics of the analyte and the matrix [83].

For a good bioanalysis to be achieved, biological sample collection procedures must be properly carried out. Hence, the state of the samples must be preserved from the time they are collected to the time they are analysed. Plasma is the most common matrix but according to the characteristics of the drug and the way in which the drug is metabolised, blood or serum may be more suitable for analysis [83].

Sometimes to help further understand the performance of the drug being analysed, it is also convenient to measure the drug concentration in urine, especially if a high amount of the drug is excreted through this specific path. Therefore, the most important factor during the collection of biological samples is to quickly collect them and making sure they are stored at the desired temperature, making an unstable drug in the matrix stable and making sure that the samples are correctly labelled [83].

2.1.1 Blood Samples

Whole blood is an important tool for quick and short-term investigation of illicit drugs in forensic toxicology especially in situations where no other sample is available. The detection of illicit drugs can be difficult due to the difference of functional groups associated with different analyte classes. It is therefore difficult to separate all analytes using a single procedure without compromising extract cleanliness [86].

Amongst other extraction techniques like solid-phase extraction (SPE), solid-phase microextraction (SPME), one of the easy and preferred methods for separation of drugs from any biological fluids such as blood or urine is liquid-liquid extraction (LLE). This is because it is less costly as it does not require chemicals of high purity and expertise while handling and can be done with basic laboratory setup. An example of a basic laboratory setup for the extraction of acidic, basic and neutral drugs from blood can be done as shown in the summary below (**figure 23**). [87].

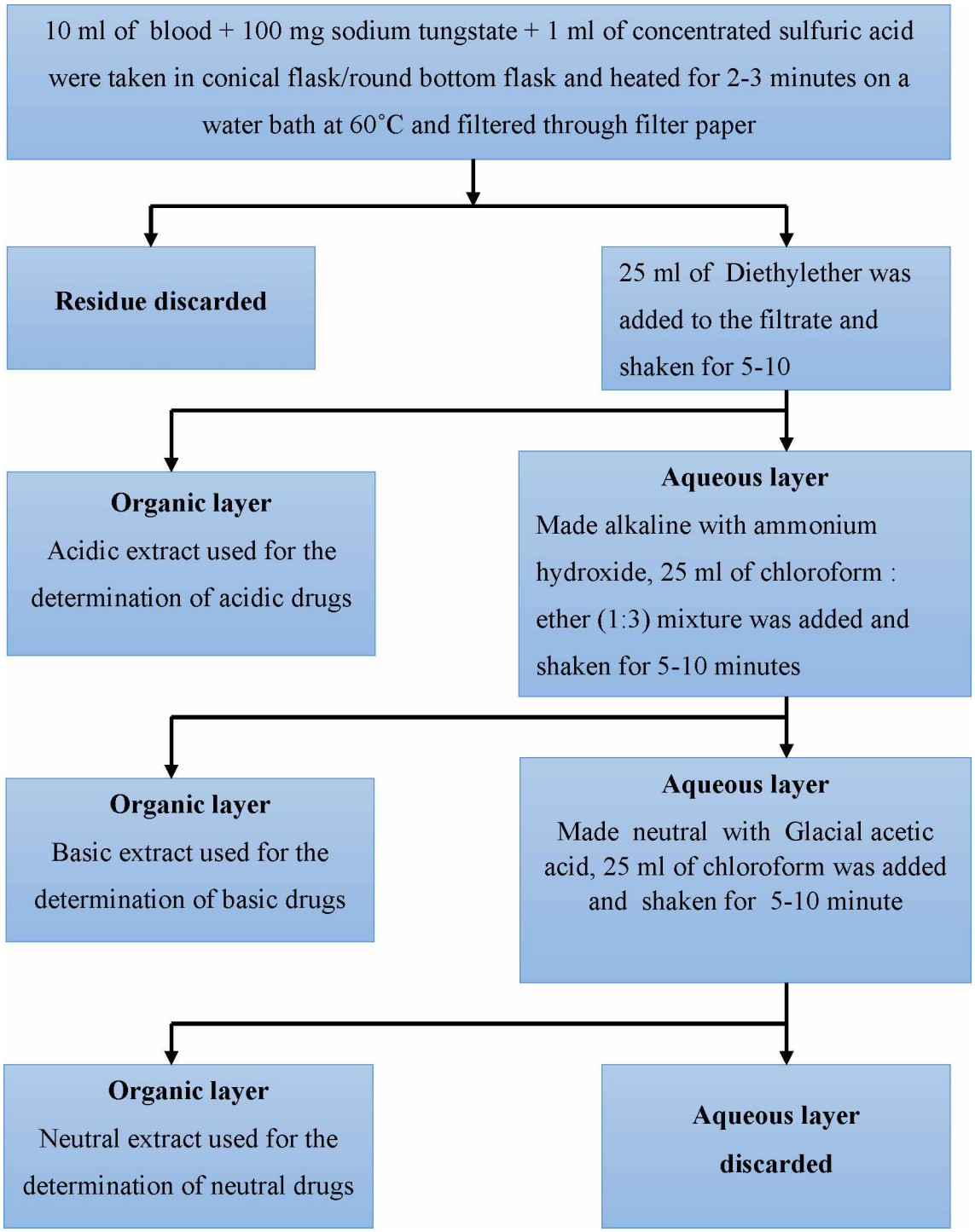

Figure 23: Basic laboratory setup for the extraction process of drugs from blood [87]

2.1.2 Urine Samples

Urine is a convenient sample for toxicological analysis because a reasonably high concentration of drugs and their metabolites accumulate in urine and the window period for a drug to be detected in urine is usually longer than that in blood, hence allowing any possible potential drugs or poisons to be detected [82].

Samples of urine are usually diluted with water or buffer at a suitable pH. Sometimes, hydrolysis using strong acids, bases or enzymes is carried out to change the conjugates into compounds that are easier to extract. Using strong acids or bases can cause the analyte to degrade, and this is why the use of enzymes such as beta-glucuronidase are suitable. However, the use of enzymes can be time consuming. Therefore, the most frequently used techniques are LLE or SPE [84].

The application of a new chip based miniature LLE system with divided flow for the preparation of samples in gas chromatography (GC) to detect amphetamine in urine samples has been reported. The microextraction system produced good results in terms of being reliable, effecient and flexible, hence allowing its automated operation and/or use in the field [84]. Like blood, extraction of acidic, basic and neutral drugs from urine can also be done using a basic laboratory setup as shown in the summary below (**figure 24**)[87].

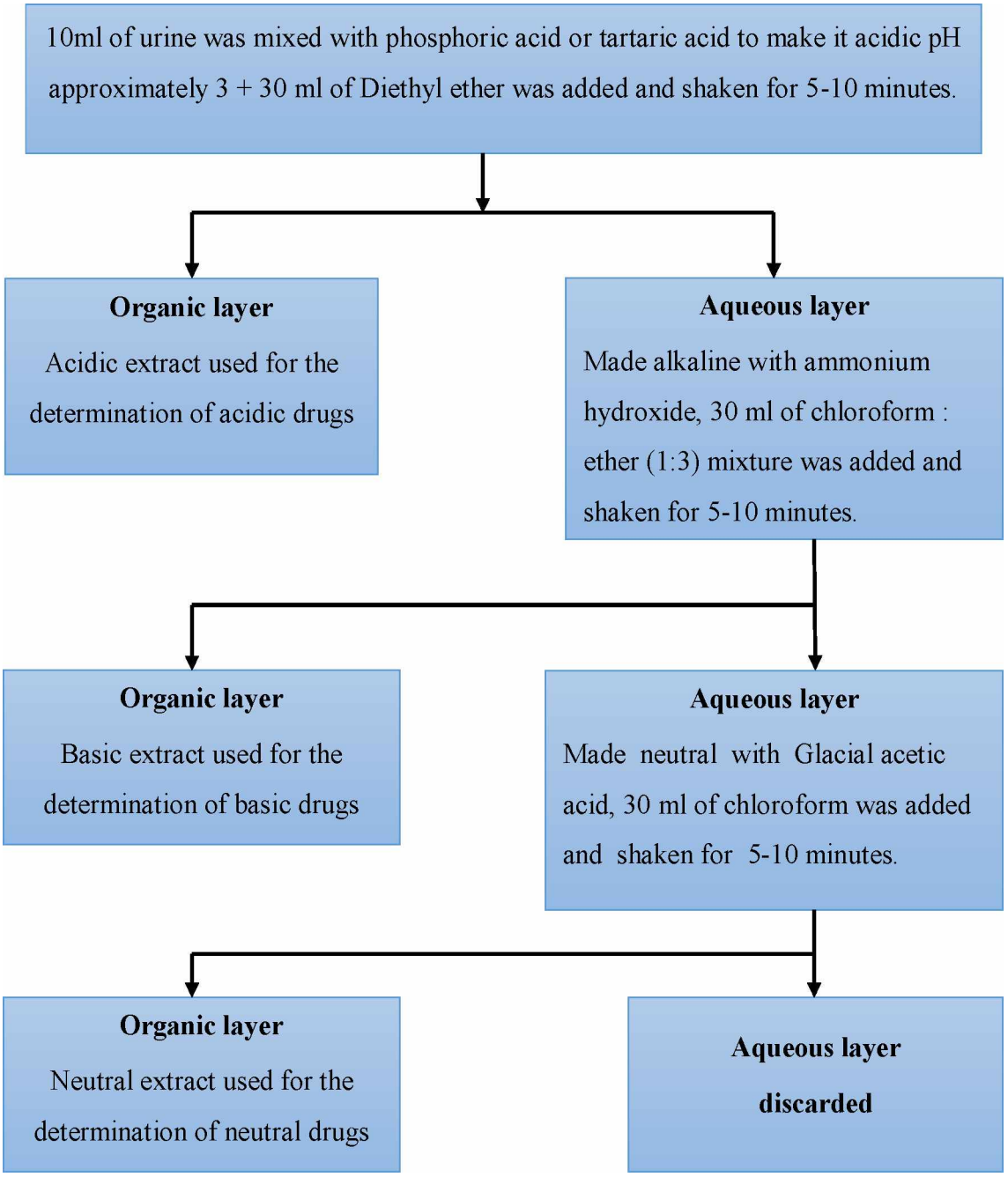

Figure 24: Basic laboratory setup process for the extraction process of drugs from urine [87]

2.1.3 Hair samples

Hair analysis has become of increasing importance in the detection of substances of abuse, both in clinical and forensic toxicology investigations. Hair analysis has certain advantages over other biological materials (blood and urine), as it has a longer window of detection which means that drugs can still be detected even after a long period of time. It is also easier to collect and is more stable as a sample as it can easily be stored at room temperature and does not really need to be analysed immediately after collection [77, 84].

However, hair samples can still be exposed to exterior contamination and still must undergo decontamination procedures to remove impurities from the environment such as cosmetics, sweat or any other surface material due to environmental exposure to prevent getting false positives. Therefore, the hair samples are usually washed using detergents, organic solvents and aqueous solvents and then later dried and cut into smaller pieces [84].

Extraction techniques, which are the most important steps in hair analysis are used to recover the toxins from the hair matrix. Some of the methods used include alkaline, acidic or enzymatic hydrolysis and microwave extraction. LLE or SPE are used as clean up procedures. The use of microextraction methods in the extraction of drugs from hair is gaining more importance as in the case of blood or urine [84,85].

2.2 Liquid-Liquid Extraction

Liquid-Liquid Extraction (LLE) is a method based on the selective transfer of an analyte from one solution (water, aqueous solution) to another (a water-immiscible organic solvent) [79].

The main disadvantages of this type of extraction are the formation of emulsions, the use of large sample volumes and toxic organic solvents. For these reasons, LLE is said to be a time-consuming method. New so-called miniaturized extraction techniques have emerged over the years, with the main drawbacks of LLE being suppressed as they require small amounts of the sample as well as the organic solvent to be used [79, 84]. These techniques include single-drop microextraction (SDME), hollow fibre liquid phase

microextraction (HF-LPME), and dispersive liquid-liquid dispersion micro-extraction (DLLME) [79].

Liquid–liquid extraction is normally considered to be an indirect method of separation because it involves the introduction of new material for a separation to be carried out, whereas direct methods, such as distillation, do not require any new material. However, there are many situations during separation where introducing a new element is the only practical option, such as: [80]

- Separation of liquids with close or similar boiling points,
- Separation of liquids that do not easily change from solid or liquid to a vapor,
- Recovery of materials with high boiling points,
- Reduction in the cost of evaporation,
- Replacement of fractional crystallisation,
- Separation of materials that are sensitive to heat,
- Separation of mixtures of liquids whose boiling points and composition remains constant throughout distillation (azeotrope),
- Separation according to the properties of the chemical.

Sample Preparation

Most of the drugs are easily extracted from alkaline plasma into relatively non-polar solvents, and *n*-heptane, *n*-hexane and *n*-pentane are mostly used, usually with a small amount of iso-amyl (iso-pentyl) or iso-propyl alcohol (IPA) to reduce adsorptive losses. The benzamides, being more polar, require a more polar extraction solvent such as chloroform. More polar solvents may be required if metabolites are to be quantified, and diethyl ether, dichloromethane and ethyl acetate can be used [81].

2.3 Single-Drop Microextraction

Single-Drop Microextraction (SDME) is a very fast and inexpensive extraction technique. It is practically free of solvents, due to the large reduction in the volume fraction of the sample. Only small portion of the analyte is extracted (concentrated) for analysis. Four types of SDME can be used for the extraction of inorganic analytes. These include [79]:

- Direct single-drop microextraction (Direct-SDME)-**figure 25 A**),
- Headspace single-drop microextraction (HS-SDME)-**figure 25 B**),
- Liquid–liquid–liquid microextraction (LLLME)-**figure 25 C**),
- Continuous flow microextraction (CFME).

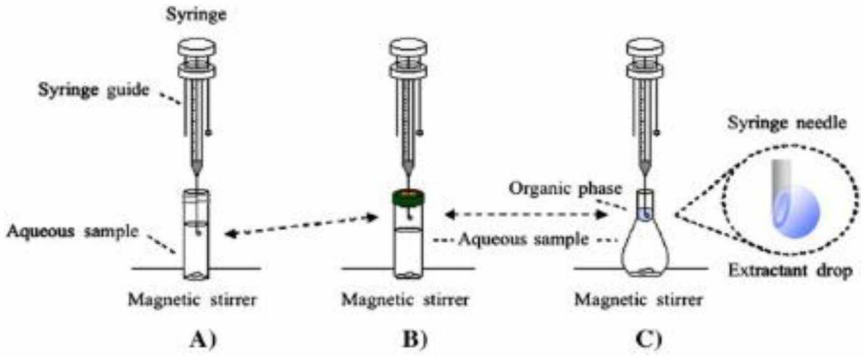

Figure 25: Illustration of (A) Direct-SDME, (B) HS-SDME and (C) LLLME [79]

2.3.1 Direct Single-Drop Microextraction

In direct single-drop microextraction (Direct-SDME), a micro-drop of the extractant phase from the micro-syringe needle is exposed to a stirred aqueous sample for a certain period. The drop is then taken back into the micro-syringe needle and injected into the detector which then gives out the analytical signal. The transfer of the analytes from the sample to the extractant goes on until a thermodynamic equilibrium is reached or the extraction is stopped. In direct-SDME, the extractant phase should be water-immiscible and the analytes should be more soluble in the extractant phase than in the sample solution [79].

The main disadvantage of using direct-SDME is that the drop is stirred at a high rate or high temperature causing the drop to become unstable especially when the sample is not cleaned properly. Solvents which are highly soluble in water and have a low boiling point are not suitable to be used for direct-SDME because they evaporate and dissolve at a high rate [79].

2.3.2 Headspace Single-Drop Microextraction

HS-SDME is a technique which helps prepare the sample for extraction and preconcentration of volatile or semi-volatile compounds. This is done by exposing the micro-drop to the headspace on top of the sample. A hanging drop containing the derivatizing agent is exposed to the gaseous phase for microextraction. The transfer in the headspace happens at a fast rate due to the massive diffusion coefficients in the gaseous phase [79].

For the use of HS-SDME, organic solvents and aqueous drops which have a high boiling point can be used as extractant phases for inorganic analytes. HS-SDME is used for clean and compatible matrices. For HS-SDME to be successful, volatile analytes that produce volatile species are needed. In the organic field, HS-SDME is often used in combination with GC [79].

2.3.3 Liquid–Liquid–Liquid Microextraction

LLLME is a type of microextraction used for ionizable analytes. In LLLME, analytes are extracted from the aqueous stirred sample into an organic layer that has a lower density than water. Stirring the aqueous sample can lead to indirectly induced convection in the organic layer and the aqueous micro-drop due to the momentum transfer between both interfaces. By adjusting the pH of the aqueous solution and micro-drop, the neutral form of the analyte extracted by the organic solvent can be obtained, ionized and extracted into the drop [79].

In comparison to other SDME methods, LLLME is more difficult to accomplish. As a result, HPLC or CE can be used after preconcentration with LLME as long as the extract is in aqueous form [79].

2.3.4 Continuous-Flow Microextraction

Continuous-flow microextraction (**CFME – figure 26 A**) is another type of SDME in which extraction is done in a glass chamber instead of a vial. Instead of stirring, the sample is continuously pumped at a constant flow rate until the glass chamber is full. A drop is then formed at the tip of a micro-syringe needle which then comes in contact with a fresh and flowing solution of the sample [79].

The rate at which the sample flows must ensure an effective microextraction of analytes that do not have bubbles or dislodgement. For the drop at the tip of the needle to be stable, samples must be thoroughly clean. Another version of this microextraction called cycle-flow microextraction (**figure 26 B**) can also be used by introducing it into the waste outlet tube from the sample reservoir [79].

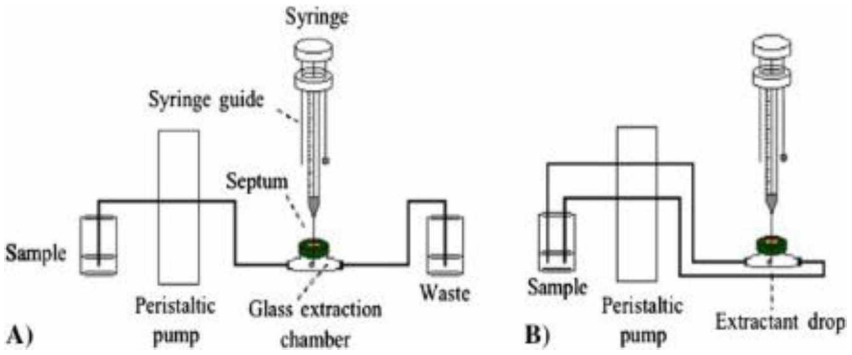

Figure 26: Illustration of (A) CFME and (B) Cycle-Flow Microextraction [79]

2.4 Hollow Fibre Liquid Phase Microextraction

Hollow Fibre Liquid Phase Microextraction (HF-LPME) is a technique that allows the extraction and concentration of an analyte from complex samples in a simple and cost-effective manner. In the two-phase sampling mode (HF-LPME), the analyte is

separated from the aqueous sample to a water-immiscible extracting agent deposited in the pores of the hollow fibre made of polypropylene supported by a micro-syringe [79].

2.5 Dispersive Liquid-Liquid Microextraction

Dispersive liquid–liquid microextraction (DLLME) is an easy and quick microextraction technique based on the use of a suitable extractant, that is a few microlitres of an organic solvent that has a high density such as tetrachlorometane, chloroform, carbon disulphide, nitrobenzene, bromobenzene, chlorobenzene or 1,2-dichlorobenzene, and a disperser solvent with high miscibility in both extractant and aqueous phase such as methanol, ethanol, acetonitrile or acetone [79]. Below is a summary of the DLLME process (**figure 27**).

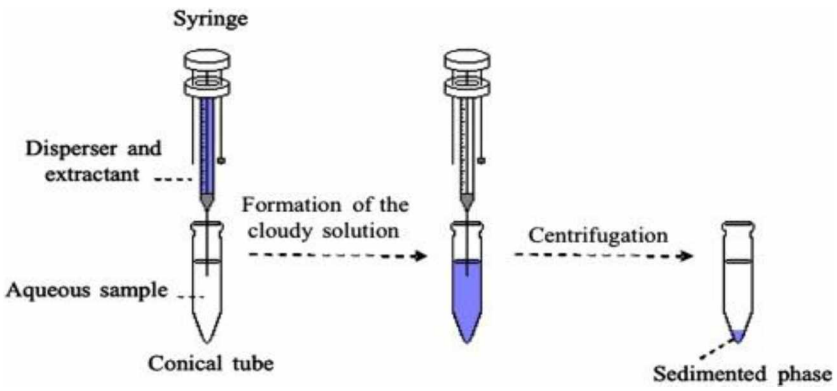

Figure 27: Illustration of the DLLME procedure [79]

2.6 Solid-Phase Extraction

Solid-Phase Extraction (SPE) is an extraction technique that requires the use of solid particles and a chromatographic packing material to separate the different elements of a sample chemically. SPE was originally thought of as a replacement for LLE due to its suitable properties for sampling as it does not require the transportation and storage of bulk samples for processing by the receiving laboratory [83, 88].

Several factors such as selectivity, density, toxicity, volatility, reactivity and miscibility with an aqueous media must be taken into consideration when choosing a solvent to extract a drug from the matrix. The process of choosing a suitable SPE extraction sorbent is dependent on understanding the type of interaction between the analyte and the sorbent. Therefore, knowing the hydrophobic, polar and ionogenic properties of both the solute and the sorbent can help understand this interaction between the analyte and the sorbent. The most common types of interaction mechanisms in SPE are van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions) and cation-anion interactions (ionic interactions) [83].

Extraction steps in SPE

Conditioning: The solvent is passed through the SPE material using methanol to wet the bonded functional groups [83].

Equilibration: The sorbent is treated with a solution that is similar in polarity, pH, etc. to the sample matrix to maximise retention. The same aqueous solution that the sample is prepared in must be used [83].

Sample Load: The sample is then introduced so that analytes are extracted onto the sorbent. An aqueous solvent must be used [83].

Washing: This is done using the strongest aqueous solution that will not elute the target compounds. Increasing the organic percentage, increasing or decreasing the pH, changing the ionic strength for thorough clean-up. Dry the cartridge to remove all water [83].

Elution: This is done using the weakest organic solvent that will remove all the target analytes. Elution of polar target compounds occurs best in polar solvents then change the pH and increase the ionic strength [83].

2.6.1 Example of hair analysis using SPE

A report describing a simple and fast method used to extract amphetamines from urine. Two techniques were used and compared. One of the two techniques used was (SupelMIP™) which uses a molecularly imprinted SPE and is designed specifically for

the extraction of amphetamines. The other technique was a recently released extraction technique which uses a conventional hydrophilic polymer SPE phase. A group of amphetamines were extracted from urine samples using the two techniques using the procedures described in the table below [116].

Table 2: Comparison of SupelMIP SPE and Conventional Hydrophilic Polymer SPE Method [116]

SupelMIP SPE	**Conventional Hydrophilic Polymer SPE**
Sample Pre-Treatment	
1. Samples of human urine were spiked with amphetamines and diluted with ammonium acetate buffer with a pH of 8 in the ratio 1:1 (v/v). The pH was adjusted using NH3 or CH3COOH.	1. Samples of human urine were spiked with amphetamines. Spiked and blank urine were acidified using 100 µl of 5M HCl per 10 ml urine.
SPE Procedure	
Amphetamines- 25 mg/3ml 2. The molecularly imprinted phase was conditioned and equilibrated with 1 ml of CH_3OH and 1 ml of 10 mM ammonium acetate buffer (pH 8).	Amphetamines- 30 mg/1ml 2. The SPE phase was conditioned and equilibrated with 1 ml of CH_3OH and 1 ml of deionized H_2O.
3. 1 ml of the pre-treated sample was then loaded onto the cartridge.	3. 1 ml of the pre-heated sample was then loaded onto the cartridge.
4. The sample was then washed using the scheme; • 2x 1ml of deionized H_2O without letting the column dry!	4. The sample was washed with 1 ml 5% CH_3OH containing 2% NH_4OH and with 1 ml 20% CH_3OH containing 2% NH_4OH.

• 1ml 60/40 CH_3CN/deionized H_2O followed by 5-10 minute vacuum (-1 bar, 20 in Hg or -70 kPa) to dry the column. 1 ml 1% CH_3COOH in CH_3CN • 1 ml 1% CH_3COOH in CH_3CN	
5. The amphetamines were then eluted with 2x 1 ml 1% HCOOH in CH_3OH and applied -0.4 bar (-12 in Hg) between each fraction.	5. The amphetamines were then eluted with 0.5 ml 20% CH_3OH with CH_3COOH.
6. Evaporation was done under N_2 until it was dry and reconstituted with 150 µl LC mobile phase before LC-MS-MS analysis.	6. Evaporation was done under N_2 and reconstituted with 150 µl LC mobile phase before LC-MS-MS analysis.

The lower limit of quantification (LLOQ) for each analyte was estimated for both techniques. The SupelMIP SPE achieved a high selectivity and for further demonstration of this, a urine sample was spiked with 15 pg/ml of amphetamine and extracted and analysed using LC-MS-MS. In comparison with both techniques, an amphetamine peak was detected using the SupelMIP SPE method while the conventional hydrophilic polymer SPE method had no response to the amphetamine [116].

Without the use of internal standard corrections, the calibration curves of the two techniques were compared to the matrix-matched samples for both methods by summarising the percentage of ion suppression at 10 and 100 ng/ml. It was observed that the ion suppression for the SupelMIP SPE was significantly less and achieved lower LOQs/LODs ten times less than those of the conventional hydrophilic polymer SPE method [116].

However, due to the high selectivity of the SupelMIP SPE method, it offered an increased recovery and reliability for better sensitivity, precision and accuracy [116].

2.7 Solid-Phase Microextraction

Solid-phase microextraction (SPME) is a technique used for the quick pre-treatment of laboratory samples. This technique has many advantages as it does not alter the chemical properties and the concentrations of the analytes. This is because only a very small amount of the analyte is taken from the samples, hence resulting in more representative information and more accurate characterization of the analytical process [89].

To date, a number of SPME configurations have been developed such as fiber, stirrer, vessel walls, suspended particles, tube and membrane. Fiber SPME being the most widely used technique. Selecting a suitable SPME fiber is the basis of achieving good analysis results. There are four main conditions usually used in the selection of a suitable fiber coating for a specific application and are as follows [89]:

1. The molecular weight (MW) and size of the analytes,
2. The polarity of the analytes,
3. The concentration levels and type of analytes,
4. The complexity of the sample.

Below is a list of the general rule for the selection of SPME commercial fiber towards different analytes depending on their molecular weight, volatility and polarity [89].

Table 3: Selection of SPME commercial fibre [89]

Analyte type	**Recommended SPME fibre**
Gas and low MW compounds (MW 30–225)	75 µm/85 µm carboxen/PDMS
Nonpolar and volatile compounds (MW 60–275)	100 µm PDMS
Volatile, amino, and nitro aromatic compounds (MW 50–300)	65 µm PDMS/DVB
Polar and semi-volatile compounds (MW 80–300)	85 µm PA
Nonpolar and semi-volatile compounds (MW 80–500)	30 µm PDMS

Nonpolar and high MW compounds (MW 125–600)	7 µm PDMS
Alcohols and polar compounds (MW 40–275)	60 µm PEG
Aromatic compounds (volatile and semi-volatile C_3–C_{20}) (MW 40–275)	50/30 µm DVB/carboxen/PDMS

Normally, SPME can be paired with differnt types of seperation and quantification systems such as GC, High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE), or Supercritical Fluid Chromatography (SFC) combined with conventional detectors. The type of analytical system is chosen according to the properties of the analyte especially its volatility and distribution behavior [89].

For drug purposes, SPME was first used for the analysis of forensic drugs due to their non-polarity and volatility, which was advantageous for the SPME-GC application paired with poly(dimethyl)siloxane (PDMS) fiber. But, with the development of solid sorbent coatings, especially the divinyl benzene (DVB)-related products, a wider range of drugs can be successfully analyzed. This created a way for the application to to semi-volatile drugs like cocaine and benzodiazepines [89].

2.7.1 Example of hair analysis using SPME

The analysis of hair is mostly used for the long-term monitoring of drug and alcohol users. Headspace-Solid Phase Microextraction (HS-SPME) is one of the methods used during hair analysis as it produces highly pure extracts with no interferences. It also only requires a single step to measure some drugs such as ketamine, nicotine, amphetamines, phencyclidine and antidepressants. Below is an illustration of the HS-SPME equipment (**figure 28**) used for the sampling of hair [115].

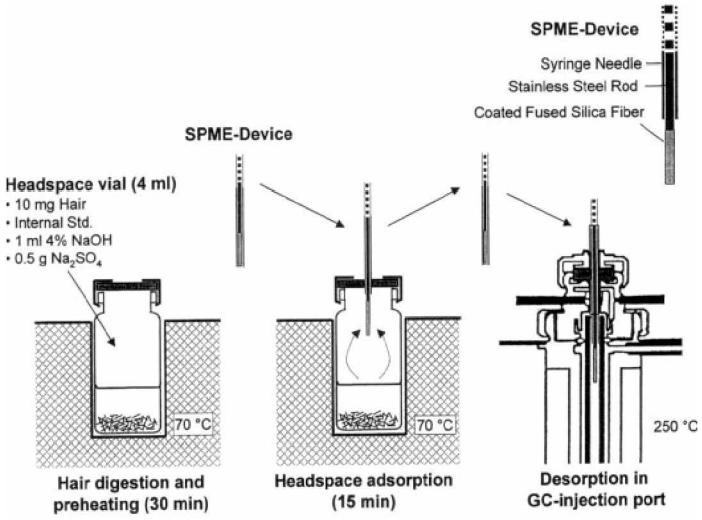

Figure 28: Illustration of HS-SPME equipment [115]

A HS-SPME technique was developed by Gentili and others to detect amphetamines with the use of 100 µm Polydimethylsiloxane (PDMS) fibre for extraction at a temperature of 71°C using MS detection. The confirmation of positive samples was done using Tandem mass spectrometry (MS/MS) as well as positive chemical ionisation techniques. The sensitivity of the technique depends on the analyte and ranges between 0.7-1.9 µg/g. The technique is appropriate for clinical, forensic, epidemiological uses [115].

3 DETERMINATION OF ILLICIT DRUGS

The extraction methods mentioned above are followed by a quantitative analysis to determine the how much of the substance is present in the sample. This is done with the use of certain chromatographic methods such as liquid and/or gas chromatography. These are the most commonly used for quantitative analysis.

3.1 High-Performance Liquid Chromatography

High pressure liquid chromatography, usually called high performance liquid chromatography (HPLC or LC) is a seperation technique frequently used in the pharmaceutical industry for analysis of pharmaceuticals, biomolecules, polymers and other organic compounds. HPLC is carried out in the liquid phase in which the analytes are seperated from their samples through the distribution between a mobile phase (the flowing liquid) and a stationary phase (sorbents inside the column). The concentration of each separated component in the column discharge is monitored by an online detector which then creates a chromatogram [90].

HPLC usually requires the use of a combination of water and either methanol or acetonitrile containing a non-volatile buffer such as phosphate buffer and other inorganic additives as a mobile phase. However, for Liquid Chromatography - Mass Spectrometry and Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS), these non-volatile additives can cause possible MS contamination as well as strong ion suppression effect and can therefore not be recommended. Instead, volatile additives such as formic or acetic acid (0.1% or lower) or ammonium acetate (2-10 mM) as salts are used [91].

For the maintenance of constant chromatographic conditions, the pH of the mobile phase should be two units above or below pKa. Octadecyl (C18) is the most commonly used and for polar metabolites, short-chained bonded phases like Octane (C8), phenyl or cyano are more suitable [91].

To solve the issue of retention, ion-paring reagent is added to the mobile phase. The neutral ion pars that are formed increase retention and the shape of the peak. During LC-MS/MS analyses, the use of ion-paring reagents like trifluoroacetic acid and other

peerfluorated acids are suitable for basic analytes and nucleoside phosphates for acidic analytes. However, the use of these additives, especially trifluoroacetic acid, should be used at low concentrations as they cause ion suppression [91].

3.2 Ultra-High Performance Liquid Chromatography

Ultra-High Performance Liquid Chromatography (UHPLC) requires the use of a reduced particle diameter from 5.0 µm to 1.7 µm which would result in an increase of efficiency, resolution, sensitivity and speed. For quick analyses, sub-2µm particle column dimensions are usually 50x2 mm. One additional benefit of UHPLC is the low consumption of mobile phase as it saves at least 80 % in comparison to HPLC [91].

An additional benefit of UHPLC is the low consumption of mobile phase, where it saves at least 80 % compared to HPLC. The high pressure due to decreased particle size require a chromatographic system that can withstand such high pressure (instruments nowadays up to 1200 bars). To avoid clogging, the filtration of both samples and solvents through 0.2 µm filters is recommended. The combination of UHPLC with MS/MS provides an even more powerful support in pharmacokinetic studies as it has advantages such as short analysis time, enhanced separation efficiency and high detection sensitivity. [91].

3.3 Gas Chromatography

Gas chromatography with mass spectrometry (GC-MS) is usually used to analyse small amounts of organically extractable, non-polar, volatile compounds and highly volatile compounds that may undergo chromatographic analysis. The analysis of polar compounds such as metabolites from biological matrices using GC-MS involves the extraction of analytes into volatile organic solvents either directly or by derivatization which increases the volatility of previously non-volatile organic compounds [91].

Most analytes require a long time-consuming sample preparation as well as derivatization to become stable, volatile and open to the ionization technique. This disadvantage called for the direction of GC-MS to LC-MS. LC-MS is advantageous in

the study of drug metabolism of low dosed and large drugs. Nevertheless, GC-MS may also be beneficial usually in clinical and forensic toxicology or doping control. GC-MS is receiving wider recognition in different classes of antidepressant agents, representing 6% of general analytical methods for determination of antidepressants and their metabolites [91].

4 Case study report

Norwegian Institute of Public Health

The purpose of this study examine if drugs identified in blood were found in oral fluid and if analysis of opiates found in oral fluid is as conclusive as in urine. Samples of blood, urine and oral fluid were collected from 100 drivers suspected of driving under the influence of drugs. Oral fluid and blood were analysed using LC-MS/MS methods and urine by immunological methods. In blood and urine, positive results were obtainted using chromatographicmethods. The analytical method comprised of 25 the most frequently abused drugs and some metabolites in Norway [92].

4.1 Urine

Samples of urine were tested for amphetamines, barbiturates, cannbis, cocaine, methadone opiates, LSD, buprenorphine and benzodiazepines by immunological methods using Hitachi 917. Additionally, pH and creatinine were measured. Validation analyses were carried out using LC-MS/MS for benzodiazepines and UHPLC-MS/MS for opiates and cocaine. The analysis of amphetamines was done using GC-MS technique revised from a whole blood technique which involves basic LLE with cyclohexane followed by derivatisation by pentadecafluorooctanoyl chloride (PFOC) and GC–MS analysis [92].

For the analysis of THC-COOH (a metabolite of THC formed in the human body after the use of cannabis), the urine samples were hydrolysed at a temperature of 60 °C in a basic solution followed by LLE with hexane:ethylacetate (7:1, v:v), derivatisation by

pentafluoropropionic anhydride (PFAA) and pentafluoropropanol (PFP-OH) and GC–MS analysis. Both GC–MS analyses were performed on Varian capillary columns with an internal diameter of 0.25 mm, 0.4 μm film thickness, VF-1MS columns, with a length of 12.5 and 15 m, respectively [92].

4.2 Blood

Blood samples are generally analysed for the detection of drugs that can cause impairment. Whole blood samples were analysed using UPLC-MS/MS. Opiods and cocaine/benzoylecgonine validation samples were prepared and confirmed with a UPLC–MS/MS method as that of urine. THC and amphetamines were confirmed with GC-MS, while benzodiazepines were confirmed with UPLC–MS/MS and methadone and zopiclone were confirmed with LC–MS. However, the institute does not coduct the analysis of THC-COOH in blood, because this metabolite is not active and is present in very low concentrations. The oral fluid samples were kept at a temperature of −20 °C. After defrosting, extraction of drugs was done using LLE followed by UPLC-MS/MS [92].

The table below (Table 3) indicates the analytical cut-off values for whole blood, urine and oral fluid samples. Drugs found in urine were reported as “detected” if the determined value was higher than the analytical cut-off. Creatinine correction was not made [92].

Table 4: Cut off levels for analysis in whole blood, oral fluid and urine [92]

	Whole blood (ng/mL)	**Oral fluid (ng/mL)**	**Drug cut-off level in oral fluid**	**Urine quantification (ng/mL)**	**Urine screening (ng/mL)**
6-MAM	9.8	1	0.8	33	20
7-Aminoflunitrazepam	NA	0.2	0.1	28	NA
7-Aminoclonazepam	NA	0.6	0.7	29	NA
7-Aminonitrazepam	NA	0.5	0.6	25	NA
Alprazolam	9.3	0.5	0.5	31	NA
Amphetamine	41	5	24	135	300
Benzodiazepines	NA	NA	NA	NA	200
Benzoylecgonine	58	10	7	58	30
Buprenorphine	0.9	2.3	NA	193	5
Cannabis	NA	NA	NA	NA	20
Clonazepam	9.5	0.6	0.5	NA	NA
Codeine	9.0	2	8	60	NA
Cocaine	61	1	1.8	61	NA
Diazepam	57	0.4	0.4	NA	NA
Flunitrazepam	1.6	0.2	0.3	NA	NA
Lorazepam	9.6	0.6	NA	32	NA
MDA	54	6	NA	1434	NA
MDEA	104	6	NA	207	NA
MDMA	58	26	NA	77	NA

Methadone	62	10	7.7	62	300
EDDP	NA	NA	NA	111	NA
Methamphetamine	45	5	15	149	NA
Morphine	8.6	4	7	29	NA
N-Desmethyldiazepam	54	0.4	0.7	135	NA
Nitrazepam	14	0.6	0.4	NA	NA
Opiates	NA	NA	NA	NA	300
Oxazepam	287	0.6	5	143	NA
THC/THC-COOH[a]	0.63	0.6	0.6	10	NA
Zolpidem[b]	15	1	NA	6	NA
Zopiclone[b]	19	1.3	1	4	NA

NA: not analysed.

THC was analysed in oral fluid and blood while THC-COOH in urine. Buprenorphine was analysed in oral fluid and whole blood while buprenorphine glucuronide in urine.

a. Analysis only in urine if identified in oral fluid. Cut off levels in oral fluid were reported in oral fluid without a buffer for example, corrected with a factor of 2 compared to measured values.

b. Analysed only in urine if detected in oral fluid. Cut off levels in oral fluid is reported in oral fluid without buffer, for examples, it is corrected with a factor of 2 in comparison to measured values.

4.3 Results

Blood and oral fluid were collected in all the 100 cases. In 7 cases, very little or no urine was recieved, hence these cases were excluded. In 5 of the cases in which amphetamine and/or methamphetamine were detected, very little urine was received for analysis. However, drugs of abuse were detected in all cases. The comparison of results in oral fluid and urine are shown in the table below (Table 4). MDMA, MDA, MDEA, zolpidem, and lorazepam were not detected in any of the samples. However, this might have been the first study analysing a large number of abused drugs taken from one person at the same time using chromatographic quantification in blood, urine and oral fluid [92].

Table 5: The number of drugs detected in the different matrixes [92]

	Detected in OF or blood or urine	**Detected in OF**	**Detected in blood**	**Detected in urine**	**Detected in blood and OF**	**Detected in urine and OF**	**Detected in blood and urine and OF**
Benzodiazepines and *z*-hypnotic							
7-Amino-clonazepam/clonazepam[a]	32	31	25	29	25	28	22
7-Amino-flunitrazepam/flunitrazepam[a]	3	3	1	3	1	3	1
7-Amino-nitrazepam/nitrazepam[a]	10	9	5	10	5	9	5
Alprazolam	20	18	13	17	13	15	13
N-desmethyldiazepam	31	27	22	27	22	23	20
Oxazepam	28	13	2	28	2	13	2
Zopiclone	6	6	0	5	0	5	0
Cannabis (THC/THC-COOH)[b]	46	35	25	45	23	34	23
Opioids							
6-MAM	26	26	0	19	0	19	0
Codeine	20	20	5	18	5	18	5
Methadone	8	8	6	7	6	7	6

Buprenorphine/buprenorphine glucuronide[c]	6	2	1	6	1	2	1
Morphine	22	22	15	20	15	19	15
Central stimulants and metabolites							
Amphetamine	39	39	23	39	23	39	23
Benzoylecgonine	18	16	12	18	12	16	12
Cocaine	21	20	5	16	5	15	5
Methamphetamine	41	41	31	41	31	40	31

OF: Oral Fluid

a. The parent drugs clonazepam, flunitrazepam and nitrazepam for the 7-amino benzodiazepines were analysed in blood and compared to the results of the metabolites in oral fluid and urine.

b. For cannabis, THC was discovered in blood and oral fluid and THC-COOH is discovered in urine.

c. Buprenorphine is analysed as buprenorphine glucuronide in urine.

4.3.1 Urine, blood and Oral Fluid Summary

The analysis showed a good link between the urine results and oral fluid for amphetamines, cocaine/benzoylecgonine, methadone, opiates, zopiclone and benzodiazepines as well as the 7-amino-benzodiazepines. Cocaine and the heroin marker 6-monoacetylmorphine (6-MAM) were usually detected in oral fluid and urine. Concentrations higher than the cut-off values in both oral fluid and urine were discovered in 15 of the 22 cases positive for morphine, in 18 of the 20 cases positive for codeine and in 19 of the 26 cases positive for 6-MAM [92].

Cannabis use was established through the detection of THC in oral fluid and THC-COOH in urine. Cannabis was also established in both oral fluid and urine in 34 of the 46 cases. In 11 cases, cannabis use was confirmed by a positive result only in urine and in 1 case only in oral fluid. However, all the drug groups found in blood were also found in oral fluid [92].

Because all the necessary drugs found in blood were found in oral fluid and the analysis of the opiate results in oral fluid was more accurate than in urine, oral fluid might be a replacement for urine in driving under the influence (DUI) cases. The sampling saves time and is also less meddlesome for drivers [92].

4.4 Hair

In this study, a LC-MS/MS technique for drug testing in hair was created and authorised. 0.45 mL of acetonitrile/25 mM formic acid (5:95 v/v) and 50 µl of deuterated internal standards were added to 20 mg of hair. The sample was then incubated for 18 hours in a water bath at a temperature of 37 °C. With the use of a Zorbax SB-Phenyl column (2.1 x 100 mm, 3.5-µm particle), separation by LC was done followed by an execution of mass detection by positive ion mode electrospray LC-MS/MS. This mass detection was performed on the following drugs: 7-aminoclonazepam, 7-aminoflunitrazepam, oxazepam, diazepam, alprazolam, zopiclone, zolpidem, carisoprodol, meprobamate, buprenorphine, and methadone, nicotine, cotinine, morphine, 6-monoacetylmorphine, codeine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, cocaine, benzoylecgonine and 7-aminonitrazepam [114].

Table 6: Calibration range, LOQ, within-assay precision, between-assay precision, accuracy and recovery results [114]

	Analyte	**Calibration range (ng/mg)**	**LOQ (ng/mg)**	**Theoretical concentration (ng/mg)**	**Within -assay RSD (n=10) (%)**	**Between -assay RSD (n=10) (%)**	**Accuracy (n=10) (%)**
1	Nicotine	0.25 - 2.5	0.75	0.39 1.93	6.7	13 12	-4.3 -18
2	Cotinine	0.25 - 2.5	0.0125	0.38 1.91	3.2	2.7 4.1	-6.6 -7.6
3	Morphine	0.25 - 2.5	0.005	0.39 1.94	5.1	4.2 5.1	-3.1 -2.6
4	Amphetamine	0.25 - 2.5	0.025	0.36 1.82	2.0	5.7 4.5	-3.3 -5.6
5	Methamphetamine	0.25 - 2.5	0.0125	0.38 1.89	2.6	4.0 5.5	1.9 -1.8
6	Codeine	0.25 - 2.5	0.0125	0.37 1.86	2.7	3.8 4.7	-18 -18
7	7-Aminonitrazepam	0.05 – 2.5	0.005	0.077 1.88	3.9	5.4 4.3	6.1 -0.4
8	MDMA	0.25 - 2.5	0.005	0.38 1.89	4.4	3.4 4.8	-7.7 -7.4
9	6-MAM	0.25 - 2.5	0.0125	0.38 1.91	3.7	4.8 4.9	-24 -21
10	Benzoylecgonine	0.05 – 2.5	0.0025	0.077 1.89	3.4	5.4 5.4	0.3 -7.3
11	Zopiclone	0.05 – 2.5	0.005	0.084 1.86	9.2	9.1 12	-13 -13
12	7-Aminoclonazepam	0.05 – 2.5	0.0125	0.078 1.90	11	6.6 5.6	-2.6 -10
13	Cocaine	0.25 - 2.5	0.0125	0.38 1.92	7.2	6.6 5.9	-18 -17
14	Meprobamate	0.25 - 2.5	0.025	0.39 1.95	8.7	9.5 6.0	-19 -17
15	Zolpidem	0.05 – 2.5	0.01	0.083 1.84	9.6	7.6 5.9	-9.5 -14
16	7-Aminoflunitrazepam	0.05 – 2.5	0.01	0.079 1.94	6.8	5.3 3.9	-1.1 -13
17	Buprenorphine	0.05 – 2.5	0.05	0.077 1.88	11	15 6.9	7.2 -6.5
18	Oxazepam	0.05 – 2.5	0.05	0.078 1.91	11	11 8.6	16 -6.4
19	Carisoprodol	0.25 - 2.5	0.0125	0.37 1.86	9.7	8.6 9.6	-16 -21
20	Methadone	0.25 - 2.5	0.0125	0.42 2.12	12	9.4 7.5	-13 -12
21	Diazepam	0.05 – 2.5	0.025	0.079 1.94	11	8.2 9.4	16 -11
22	Alprazolam	0.05 – 2.5	0.01	0.078 1.92	8.8	9.3 8.2	9.1 -8.5

4.4.1 Hair Summary

Within-assay relative standard deviations (RSD) ranged from 2.0 % to 12 % and between-assays from 2.7 % to 15 %. Accuracies ranged from 24% to 16% and recoveries from 25% to 100%. Therefore, the LC-MS/MS was proven to be efficient for the drug quantification in hair and has since been used at the Norwegian institute of public [114].

5 Conclusion

Illicit drugs are substances that alter a person's normal bodily function by stimulating the central nervous system causing psychedelic effects such as addiction, hallucinations, paranoia, delusions, euphoria, loss of time and perception to reality or even death. Due to their adverse effects, most of these drugs are strictly prohibited.

The first part focused on the the classification of these drugs into five classes, there physical and psychological long and short-term effects, their medical and recreational uses and the methods in which they are taken. These classes included; cannabis, depressants, narcotics, stimulants and hallucinogens.

The second part focused on the sampling and some common extraction methods such as LLE and SPE. These extraction techniques are important and therefore need to be carried out before quantification because they help purify the analyte from the matrix or sample hence increasing the concentration of the analyte. The samples that were used for the extration and determination of the drugs and their metabolites were urine, blood and hair.

The last part focused on the different quantitative analyses such as LC and GC combined with a mass detector and a case study which was carried out at the norweigian institute of public health using hair, blood and urine samples to determine if drugs detected in blood could be found in oral fluid and if analysis of opiates identified in oral fluid is as conclusive as in urine. All drugs detected in blood were also found in oral fluid, and ingestion of heroin, by the specific marker 6-MAM, was more frequently confirmed by analysing oral fluid than urine. For the basic drugs (opiates, opioids, cocaine, amphetamines) the concentrations were, as expected, higher in oral fluid than in whole blood. This was also the case for THC, benzoylecgonine and zopiclone, while for the benzodiazepines the concentrations were lower and for the hair analysis proved to be efficient and favourable over urine analysis for both research and rehabilitation purposes [92].

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