TITLE

RECOVERY STUDY OF DANGEROUS DRUGS IN SOFT DRINKS USING LIQUID-LIQUID EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR (GC-FID)

by

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	Percent
<	Less Than
°C	Degree Celsius
°C/min	Degree Celsius per Minute
ATS	Amphetamine-Type Stimulants
cm/sec	Centimetre per Second
CoSCN	Cobalt Thiocyanate
DEA	Drug Enforcement Administration
DOC	Department of Chemistry
ELISA	Enzyme-Linked Immunosorbent Assay
ESI	Electrospray Ionisation
FD	Fluorescence Detector
FDA	Food and Drugs Administration
g	Gram
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionisation Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS-EI	Electron Impact Ionisation Gas Chromatography-Mass Spectrometry
GHB	Gamma-hydroxybutyrate
HCl	Hydrogen Chloride
HPLC	High Performance Liquid Chromatography
HPLC-UV	High Performance Liquid Chromatography-Ultraviolet
IMS	Ion Mobility Sspectrometry

kg	Kilogram
kPa	Kilopascal
kPa/min	Kilopascal per Minute
L	Litre
LC	Liquid Chromatography
LC-HRMS	Liquid Chromatography-High Resolution Mass Spectrometry
LC-MS	Liquid Chromatography-Mass spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LLE	Liquid-Liquid Extraction
LSD	D-lysergic Acid Diethylamide
M	Molarity
m	Metre
m/z	Mass per Charge Ratio
MDA	3,4-methylenedioxyamphetamine
MDE	N-ethyl-3,4-methylendioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
mg	Milligram
mg/mL	Milligram per Mililitre
min	Minute
MIP	Molecularly Imprinted Polymer
mL	Millilitre
mL/min	Millilitre per Minute
mm	Millimetre
NaOH	Sodium Hydroxide
NCI	Negative-Ion Chemical Ionisation

NIDA	National Institute of Drug Abuse
nm	Nanometre
N-OH MDA	N-hydroxy-3,4-methylenedioxyamphetamine
PCI	Positive-Ion Chemical Ionisation
PDMS	Polydimethyl Siloxane
PFBC	Pentafluorobenzyl Chloride
PSPME	Planar Solid Phase Microextraction
RM	Ringgit Malaysia
RSD	Relative Standard Deviation
SD	Standard Deviation
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SPME-IMS	Solid Phase Microextraction-Ion Mobility Spectrometry
TIS	TurboIonSpray
UK	United Kingdom
UNODC	United Nation Office of Drugs and Crime
UPLC	Ultra-Performance Liquid Chromatography
UPLC-ESI-MS	Ultra-Performance Liquid Chromatography-Electrospray Ionisation-Mass Spectrometry
USA	United States of America
UV	Ultraviolet
μL	Microliter
μm	Micrometre

KAJIAN PEMULIHAN DADAH BERBAHAYA DALAM MINUMAN RINGAN DENGAN KAEDAH PENGEKSTRAKAN CECAIR-CECAIR DIIKUTI KROMATOGRAFI GAS-PENGESAN PENGIONAN API (GC-FID)

ABSTRAK

Parti, 'rave' dan kelab malam sering dikait rapat dengan dadah rekreasi, yang juga dikenali sebagai 'dadah kelab'. Melarutkan dadah haram, seperti ketamine dan 3,4-methylenedioxymethamphetamine (MDMA), ke dalam bentuk cecair untuk disamarkan sebagai mimuman ringan dalam botol, merupakan salah satu kaedah penyamaran dadah yang terkini. Oleh itu, satu kajian pemulihan telah dijalankan untuk menghasilkan kaedah pengesanan dan pengukuran kuantiti dadah tertentu yang terkandung dalam minuman ringan. Kajian ini bertujuan untuk mengkaji keberkesanan kaedah pengekstrakan cecair-cecair (LLE) yang digunakan untuk mengekstrak dadah daripada minuman ringan. LLE dengan kloroform membolehkan pengekstrakan ketamine dan MDMA daripada sampel minuman ringan. Analisis kromatografi gas-spektrometri jisim (GC-MS) telah digunakan untuk mengesahkan kehadiran dadah yang dikehendaki dalam ekstrak, diikuti analisis kromatografi gas-pengesan pengionan api (GC-FID) untuk mengukur kuantiti dadah yang dikehendaki yang diekstrak daripada minuman ringan. Bagi analisis GC-MS, kualiti kesepadanan spectrum jisim untuk MDMA (78% – 87%) lebih rendah daripada ketamine (98%) dan standard internal (91%). Dalam kajian pemulihan dengan pengekstrakan berulang-ulang atas sampel yang sama, kuantiti dadah yang telan diekstrak adalah konsisten bagi jus blackcurrant (RSD = 6.77%) dan teh limau ais (RSD = 5.38%), tetapi tidak konsisten bagi teh hijau (RSD = 15.52%) dan jus oren (RSD = 29.43%). Dalam kajian pemulihan dengan menggunakan sampel kes yang dibubuhi amaun ketamine tetap, purata peratusan pemulihan ketamine daripada jus blackcurrant ialah 87.27 ± 5.72 %.

Keluk regresi untuk pemulihan ketamine yang didapati linear (R^2 = 0.997), menunjukkan kejituan kaedah tersebut. Kesimpulannya, prosedur analitikal yang merangkumi LLE, GC-MS dan GC-FID dalam kajian pemulihan didapati berkesan untuk mengekstrak, mengesan dan mengukur kuantiti ketamine and MDMA dalam minuman ringan berlarut dadah.

RECOVERY STUDY OF DANGEROUS DRUGS IN SOFT DRINKS USING LIQUID-LIQUID EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR (GC-FID)

ABSTRACT

Raves and night clubs are always associated with the recreational drugs, which are also referred as 'club drugs'. Dissolving illegal drugs, such as ketamine and 3,4-methylenedioxy-methamphetamine (MDMA), into liquid forms to be disguised as bottled soft drinks is one of the current drug concealment methods. Hence, a recovery study to develop suitable method for detection and quantification of the amount of specific drugs contained in the soft drinks is proposed. The objective of this study is to study the recovery efficiency of liquid-liquid extraction (LLE) method used to extract drugs from the soft drinks. LLE with chloroform allowed the extraction of ketamine and MDMA from the sample soft drinks. Gas Chromatography–Mass Spectrometry (GC-MS) analysis was used to confirm the presence of desired drugs in the extracts, followed by Gas Chromatography–Flame Ionisation Detector (GC-FID) analysis to quantify the amount of desired drugs extracted from the soft drinks. In GC-MS analysis, it was found that the quality of mass spectral match for MDMA (78% – 87%) was lower compared to ketamine (98%) and internal standard (91%). In recovery study of repeated extraction on same sample, it was found that the amount of drugs recovered were consistent for blackcurrant juice (RSD = 6.77%) and ice lemon tea (RSD = 5.38%), but inconsistent for green tea (RSD = 15.52%) and orange juice (RSD = 29.43%). In recovery study using spiked samples, the average recovery percentage of ketamine from blackcurrant juice was 87.27 ± 5.72 %. The regression curve for recovery of ketamine was found to be sufficiently linear (R^2 = 0.997), which indicated that the method was accurate. In brief, the analytical

procedures of LLE, coupled with GC-MS and GC-FID in recovery study were found reliable for the extraction, detection and quantitation of ketamine and MDMA in the drug-laced soft drinks.

CHAPTER 1

INTRODUCTION

The fastest growing classes of recreational drugs, which was referred as 'club drugs', have been associated with the all-night parties, including raves and night clubs (Couper and Logan, 2000). Substances referred to in the classification of club drugs include 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), gamma-hydroxybutyrate (GHB), ketamine, Rohypnol®, D-lysergic acid diethylamide (LSD) and methamphetamine (National Institute of Drug Abuse (NIDA), 2016). These drugs have been used for personal excitement or being spiked in drinks. Among the drugs, ketamine and MDMA (Figure 1.1) are particularly common in night parties, and therefore have been identified for this study.

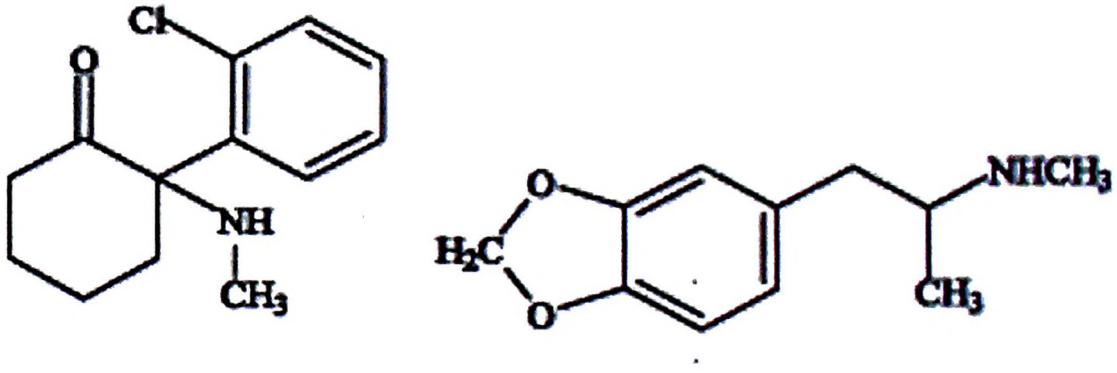

Figure 1.1: Chemical Structure of Ketamine (Left) and MDMA (right) (Wu et al., 2008).

1.1 Background of the Study

Drugs remain as the global threat to health and development, including Malaysia. According to the United Nation Office of Drugs and Crime, UNODC, the number of illicit drug users was estimated to be 246 million people at about 5.2% among the world population in 2013 (UNODC, 2015b). It was observed that the prevalence of drugs users remained stable for past three years (UNODC, 2015b). However, the increase of world population reflected the continuous increasing trend of the number of drug users (UNODC, 2015b). The increased demand of illegal narcotic drugs has triggered the drug trafficking and smuggling business to meet continuous demand as evident by frequent news reported on drug trafficking and smuggling activities (Ab Hamid *et al.*, 2012). This indicates the number drug trafficking and smuggling that went through the security system could be high.

1.2 Emerging Trends and Concerns in Malaysia

In Malaysia, the trafficking of amphetamine-type stimulants (ATS) into the country for both domestic use and as transit point for international business remains a problem. The clandestine laboratories manufactured significant quantities of crystalline methamphetamine and 'ecstacy' using the precursors as well as chemicals that had been smuggled into Malaysia or diverted from the licit market. Furthermore, the manufacturing of illicit nimetazepam (also known as Erimin 5) and ketamine, the cultivation of the local plant kratom (also known as '*ketum*'), as well as the most widely used opiates, which include heroine and illicit morphine, delineate the current trends in Malaysia (UNODC, 2013).

Table 1.1: Rank of use of selected drugs in Malaysia, 2010-2014 (National Anti-drugs Agency (AADK), 2016).

Drug type	2010	2011	2012	2013	2014
Opiates*	1	1	1	1	1
Opium	7	7	7	•	•
Methamphetamine	3	2	2	2	2
Cannabis	4	3	3	3	3
ATS pills**	2	4	4	4	4
Psychotropic pills	6	6	6	5	6
Others***	5	5	5	6	5

*Referred to heroine and morphine
**Referred to ecstasy and amphetamine
***Including cocaine, ketamine, and codeine
• = not reported

In 2014, 7.94% of all drug users in Malaysia used ATS, including amphetamine and MDMA. The prevalence of cocaine, ketamine and codeine has increased in recent years. Opiates, particularly heroine and morphine remains the primary drug used in Malaysia. Approximately 65% of all drug users used heroine and illicit morphine respectively (AADK, 2016).

Table 1.2: Trend in use of selected drugs in Malaysia, 2010-2014 (AADK, 2016).

Drug type	2010	2011	2012	2013	2014
Opiates*	•	↔	↑	↑	↓
Opium	•	↓	↔	•	•
Methamphetamine	•	↑	↓	↓	↑
Cannabis	•	↓	↓	↓	↓
ATS pills**	•	↓	↓	↑	↑
Psychotropic pills	•	↓	↑	↓	↓
Others***	•	↓	↓	↓	↑

↑ = increasing, ↓ = decreasing, ↔ = stable, • = not reported
*Referred to heroine and morphine
**Referred to ecstasy and amphetamine
***Including cocaine, ketamine, and codeine

There has been a reported increase of ecstasy seizures in Malaysia in 2012. However, ecstasy was known to be the least used ATS in 2010 and 2012. But, in other point of view, Malaysia has been identified as a transit country for ecstasy seized by several countries in the region in recent years. In Singapore, almost all of the seizures in 2012 and 2013, and almost two-thirds of the seizures in 2011 had been transited via Malaysia. Additionally, all seizures in Brunei Darussalam in 2011 and 2013, and a quarter of seizures in New Zealand in 2011 had reportedly also been transited through Malaysia. Therefore, the increase of seizures in Malaysia might point to growing transit trafficking of ecstasy (UNODC, 2015a).

The most commonly used new psychoactive substances (NPS) in Malaysia were ketamine. Most ketamine found in Malaysia was smuggled from southern India. However, is was observed that ketamine seizures and ketamine-related arrests in Malaysia had decreased significantly over the last few years. Ketamine seizures in Malaysia have been comparatively lower compared to other countries in East and South-East Asia region. There are also signs that a large share of the amount of ketamine seized in the region was intended for the domestic market (UNODC, 2015a).

1.3 Concealment of Club Drugs

Drugs are often transported into the targeted area or country through shipment hidden in food cans, exported coffee bags, prepared food such as pickles, fruits, and frozen food such as fish or shrimp (Ismail and Jaafar, 2015). These food items are used as medium to conceal drugs to avoid detection. For club drugs, dissolving drugs into liquid forms in bottled alcohol or soft drinks are the upcoming trend. The chieftain was hardly detected due to indirect involvement of many people and stages in smuggling. Very often, the drug products were

seized by authorities but the responsible people for the crime often escape except the packers (Ismail and Jaafar, 2015). The following were the recent cases of drug smuggling using food and drinking products reported in Malaysia.

1.3.1 Liquid Cocaine in Cans

In November 2012, a ship from South America heading to Mozambique which made a stop at Port Klang in Malaysia was suspected to have loaded with drug shipment. Using the portable drug testing kits, Police and Custom officers have found a tin to have tested positive for controlled drug substances among over 100 cans of processed coconut milk and pineapples. The forensic scientists from the Department of Chemistry (DOC) Malaysia were called upon to examine seizure containing approximately 2300 cans. The physically identical cans have made the inspection of all cans, that weighed about 400 g each, difficult to be tested unless opened. Those tested positive cans contained about half of its weight with liquidised cocaine (Ismail and Jaafar, 2015).

1.3.2 Drugs in Mixed-pickles

In July 2013, 40 boxes containing mixed pickles in a factory storing food items such as vermicelli, tomato sauce, imported fruit pickles and salts in Nilai Industrial Park, Selangor were seized and examined by the forensic scientists from DOC Malaysia. Packets of suspected controlled substances were believed to have been supplied from the neighbouring country. A total of 100 kg of high grade heroine was recovered from 195 packets of mixed pickles (Ismail and Jaafar, 2015).

1.3.3 Drugs-in-drinks Syndicates

In August 2015, a drug syndicate which disguised RM38,340 worth of liquid methamphetamine in flavoured water bottles attempted to distribute the goods to the local market. Seven people were arrested and 246 bottles containing drugs were seized during the raids conducted at Shamelin Perkasa in Cheras and Pandan Perdana in Ampang, Kuala Lumpur. Ketamine, combined with ecstasy were believed to be processed into various flavoured water bottles such as ice lemon tea, Ribena, apple and orange juice. The drugs were distributed for entertainment and private parties, that cost from RM70 to RM150 per bottle (Malaysiandigest.com, 2015).

In October 2015, police have crippled a syndicate selling soft drinks laced with ecstasy and ketamine at entertainment outlets in Kuala Lumpur. The activity was uncovered after five people were arrested in multiple raids by police and Special Tactical Intelligence Narcotic Group of Royal Malaysian Police. Interrogation shows that the customers were aware of the drugs in the drinks, pricing at RM350 for 500 mL. A total of 1,225 bottles containing drug-spiked drinks, 10,015 ecstasy and 39 erimin pills, and 109 g of ketamine worth RM 1.14 million were seized during the raid (Todayonline.com, 2015).

In January 2016, police have crippled another syndicate selling bottled drinks laced with drugs with the arrest of a suspect in a workshop at Salak Selatan, Selangor. One hundred and twenty bottles of 355 mL orange juice drinks worth RM30,000 containing ketamine, Erimin-5 and Ecstasy (each bottle was sold for RM200 to RM300) were seized by police. A transparent plastic packet containing 266 g of Syabu were also seized in this case (Ashwin, 2016).

1.4 Objectives

The main objective of this study is to study the recovery efficiency of the liquid-liquid extraction (LLE) of ketamine and MDMA from soft drinks. The specific objectives are listed as following:

- To extract the ketamine and MDMA from respective soft drinks using LLE method with chloroform.
- To detect and confirm the presence of ketamine and MDMA in the extracts using gas chromatography-mass spectrometry (GC-MS).
- To quantify the amount of ketamine and MDMA extracted from the soft drinks using gas chromatography-flame ionisation detector (GC-FID).

1.5 Significance of the Study

It was observed that the bottled soft drinks laced with drugs such as ketamine and ecstasy had become the current trend of drug smuggling in Malaysia. There was no recovery study of dangerous drugs on the soft drinks as sample matrices being done in DOC. Hence a recovery study need to be done to develop a suitable method in order to detect and quantify the amount of specific drugs contained in the soft drinks at the DOC to prepare for the increase of such samples submitted for analysis. The result of this study may provide useful information on the recovery efficiency of the method used in the Narcotic casework for improved quality assurance of such analyses.

CHAPTER 2

LITERATURE REVIEW

2.1 Brief History

2.1.1 Ketamine

Ketamine is a type of dissociative anaesthetic with effect of hallucination. It was developed in 1960s, derived from phencyclidine by Dr Calvin Lee Stevens of Wayne State University for a pharmaceutical company named Parke-Davis. In 1964, the first experiment was conducted by injecting ketamine into human subjects to study its safety and general anaesthetic properties. However, several side effects were observed on the human subjects, including the feelings of floating and numbness of their limb. To reduce the adverse effects of ketamine, sedatives or tranquilizers, such as diazepam, midazolam and propofol were found to be more useful in order to reduce the emergence delirium of ketamine (Domino, 2010).

In 1970, the United States Food and Drug Administration (FDA) approved ketamine for utilisation as short-acting anaesthetics in human and animals. It was then given to injured American soldiers during the Vietnam War due to its large margin of safety. Due to the extensive abuse of ketamine, the Drug Enforcement Administration (DEA) was established in 1973. It was followed by the formation of the drug scheduling system (Domino, 2010).

Ketamine was distributed under the trade names Ketalar®, Ketaset®, and Vetamine®. Approximately 16,000 dispensed ketamine prescription were widely used in medical field (Domino, 2010). In clinical settings, it was widely used in many area of medicine, including

paediatrics, emergency and pain management (White *et al.*, 1982). Ketamine is also widely used as veterinary medicine. However, the sterile ketamine vials, intended for veterinary use were diverted for recreational use (Domino, 2010). Recreational use of ketamine was first reported in West Coast of the United States in 1970s (Siegel, 1978).

Ketamine was known to be a drug with LSD-like hallucination properties, but was easier to measure and adjust the balance of drug dosage (McCambridge *et al.*, 2006). It has a shorter half-life and better dose response compared to LSD. Ketamine became popular as recreational drug in 1990s (Hillhouse and Porter, 2015), and it appeared as complementary of ecstasy pills on the UK dance scene (Jansen, 1993). Ketamine was sold using street names such as Bump, Cat Tranquilizer, Cat Valium, Jet, Jet K, K, Ket, Kit Kat, Kizzo, Monkey Business, Monkey Mix, Purple, Special K, Special La Coke, Super Acid, Super K, Super Acid, and Vitamin K (Domino, 2010; DEA, 2015a). In 1999, ketamine, including its salts, isomers, and salts of isomers, were listed as a Schedule III non-narcotic substance under the Federal Controlled Substances Act in USA (DEA, 2015a). It has currently acceptable medical use but some potential of abuse (DEA, 2015a).

2.1.2 Methylenedioxymethamphetamine (MDMA)

In order to search for haemostatics, MDMA was first synthesised from hydrastinine at the Germany pharmaceutical company Merck, in 1912 and it was patented in 1914 (Freudenmann *et al.*, 2006). Its first pre-clinical pharmacological test was conducted since 1927, while searching for the adrenaline like substances (Freudenmann *et al.*, 2006). Toxicological tests were conducted by Merck chemist in 1952 (Freudenmann *et al.*, 2006). The toxic effects of MDMA were studied using five different species of laboratory animals at the University of

Michigan from 1953 and 1954 (Hardman *et al.*, 1973; Shulgin, 1986). However, the evaluated effects of MDMA in human were still remained unclear until 1970s (Freudenmann *et al.*, 2006; Sessa, 2007).

The use of MDMA as an agent for psychotherapy was pioneered by Dr Leo Zeff from California (Holland, 2001). As a psychedelic psychotherapist, Zeff substituted LSD (in 1960s) with MDMA along with his studies on the psychoactive effects of MDMA (Holland, 2001). While the administration of MDMA in psychedelic treatment was still legal, he had introduced MDMA to over 4000 patients (Holland, 2001). The patients who had undergone this treatment went on to become 'self-therapists' (Holland, 2001). This cause the growth of clinicians using MDMA in and around California (Sessa, 2007). Although some research trials exist without double-blind placebo control, there are still case reports and informal therapy outcome studies that were considered successful (Stolaroff, 2004).

Greer and Tolbert (1986) described the therapeutic methods and the subjective reports of 29 patients under MDMA-assisted therapy in early 1980s. They found that there were no significant complication, physically or mentally, observed upon taking the drug (Greer and Tolbert, 1986). In contrast, most of them reported positive individual effects, improved behaviours, and the ease of relationship problems after their therapies (Greer and Tolbert, 1986). Continuously, in the later review among 80 patients between 1980 to 1985, Greer (1998) listed the careful protocols and experimental techniques, in order to enhance the drug-assisted therapeutic session. However, MDMA clinical research were prohibited since 1985.

Same as LSD, MDMA was attempted to be shifted from legitimate medical use to its administration by a wider population in public since 1980s (Sessa, 2007). However, MDMA had been leaked from the medical community and became drug of recreational use among youngsters in the night club and party scenes (Sessa, 2007). The common street names of MDMA include Adam, Beans, Clarity, Disco Biscuit, E, Ecstasy, Eve, Go, Hug Drug, Lover's Speed, MDMA, Peace, STP, X, and XTC (DEA, 2015b). Hence, the number of negative reports regarding the uncontrolled usage of MDMA out of medical context increased significantly (Sessa, 2007). Therefore, using emergency measures, DEA had made the drug a Schedule One controlled substance in USA in 1985 (Sessa, 2007).

2.2 Abuse of Ketamine and MDMA

Ketamine, along with the other club drugs, has become popular among adolescents and young adults at all-night party scenes, such as dance clubs and raves. For medical purposes, ketamine is manufactured as an injectable prescription liquid formulation (Figure 2.1), as well as in powder or tablet formulation (DEA, 2015a).

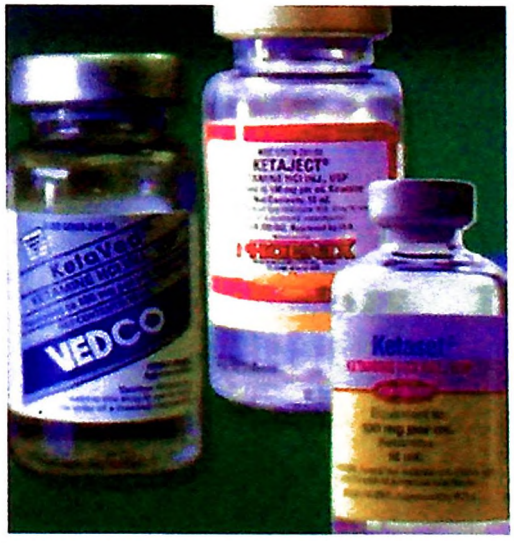

Figure 2.1: Vials containing liquid ketamine (DEA, 2015a).

For illicit ketamine (Figure 2.2), powdered ketamine can be synthesised from pharmaceutical ketamine by evaporating the liquid to form crystals, which are then ground into powder. Powdered ketamine is cut into lines, also known as bumps, followed by snorting or smoking, typically in marihuana or tobacco in cigarettes. Liquid ketamine is injected, intravenously or intramuscularly, which possess higher bioavailbility. Liquid ketamine can be also mixed into alcohol or soft drinks due to its tasteless, odourless, and colourless in nature. In the street use, ketamine is found by itself or often in combination with MDMA, amphetamine, methamphetamine, or cocaine (DEA, 2015a; Smith *et al.*, 2002a).

Figure 2.2: Illicit ketamine in various forms (DEA, 2015a).

The recreational use of MDMA mainly involves swallowing tablets (Figure 2.3), which are sometime crushed and snorted to achieve more rapid onset of effects. Occasionally, it was smoked but rarely injected. Many other compounds may also be found as adulterants in tablets, including aspirin, caffeine, dextromethorphan, pseudoephedrine, ketamine, LSD, and paramethoxyamphetamine. Street MDMA is also available in powdered form. MDMA abusers usually take MDMA by 'stacking', three or more tablets at once, or by 'piggy-backing', taking a series of tablets over short period of time (DEA, 2015b). 'Candy flipping', which is co-intake of MDMA and LSD, is a trend among young adults (DEA, 2015b). The MDMA use is often

accompanied by characteristic paraphernalia, including pacifiers and candy suckers, to avoid the bruxism upon intake of the drug. Since MDMA is usually abused with other drugs, such as marijuana, as well as alcohol or soft drinks, it is considered as a dangerous party drug that can cause death (DEA, 2015b; Smith *et al.*, 2002a).

Figure 2.3: MDMA tablets, also known as ecstasy pills (DEA, 2015b).

2.3 Preliminary Tests for Ketamine and MDMA

Several detection techniques for ketamine and MDMA were developed in the laboratory. The most common preliminary tests are the colour tests (Sarwar, 2006; UNODC, 2009). The next is the screening test, particularly the enzyme-linked immunosorbent assay, ELISA (Cheng *et al.*, 2007; Laloup *et al.*, 2005). These preliminary tests were rapid and suitable for initial testing, before proceeding to analytical instrumentations, and suitable in routine forensic caseworks.

2.3.1 Colour Tests

Colour test for the identification of ketamine in forensic laboratories are important for preliminary screening. In 2006, only one colour test, the Janovshy test, was available for screening purposes. The Janovsky reagent is alkaline meta-dinitrobenzene, which react with ketamine to form purple-coloured ring as positive result (Rees and Wasem, 2000; Shadan and Abd. Rahim, 2005). In early 2007, Morris (2007) reported a modified cobalt thiocyanate, CoSCN colour test for ketamine that is highly specific for ketamine. In powdered form only, ketamine HCl turns blue to dark blue with neutral CoSCN test; while for acidified ketamine, in both powdered and liquid form, it resulted in a pink solution with the typical CoSCN test (Morris, 2007). However, these two tests were rather insensitive, with detection limit of about 1.25 mg (Sarwar, 2006). Subsequently, Sarwar (2006) developed a new reagent, which is the mixture of gold bromide solution and sodium hydroxide solution. Upon adding ketamine, the reagent changed from brownish yellow colour into deep purple colour within approximately one minute, that turns to a dark, blackish-purple colour within approximately two minutes (Sarwar, 2006). The test is simple, easy to perform, nearly twice as sensitive as the Janovsky and Morris tests, and highly specific (Sarwar, 2006).

To distinguish MDMA from other ATS, a series of colour tests can be utilised. The first method is the concentrated sulphuric acid, which reacts with methylendioxyamphetamine type compounds, including N-ethyl-3,4-methylendioxyamphetamine (MDE), N-hydroxy-3,4-methylendioxyamphetamine (N-OH MDA), 3,4-methylendioxymethamphetamine (MDMA) and 3,4-methylendioxyamphetamine (MDA), to form a violet colour. These compounds can be further separated by using Simon reagent (sodium nitroprusside and acetaldehyde, followed by sodium carbonate), which only MDE and MDMA give positive results. Upon reaction, the

brilliant deep purple blue colour develops at once. To further differentiate MDE and MDMA, two reagents can be used. First, MDMA reacts with Karo reagent (potassium persulfate in concentrated sulphuric acid) to form a yellowish-green colour, which then develops into deep back colour. Second, a few drops of ammonium dichromate and concentrated sulphuric acid is added to MDMA, deep olive green colour develops at once, which green colour develops later.

2.3.2 Enzyme-linked Immunosorbent Assay (ELISA)

More recently, ELISA has become increasingly popular among the forensic toxicology community due to its relative ease to use, growing potential for automation, and suitability for use with biological samples without the need of sample preparations, as well as applicability to low sample volumes (Laloup *et al.*, 2005). For ketamine and its metabolites, ELISA is usually used for detection in urine samples, and shown to have high efficiency, high sensitivity, and high specificity, low false positive or negative rate (Cheng *et al.*, 2007). However, the samples that have only its metabolites, such as norketamine and dehydronorketamine, without ketamine itself, may be missed by this screening methods (Cheng *et al.*, 2007). On the other hands, MDMA is detected from various type of biological samples, such as oral fluid and plasma (Laloup *et al.*, 2005). ELISA serves a fast and accurate screening technique for the detection of plasma samples and oral fluid specimens, which are MDMA/MDA positive (Laloup *et al.*, 2005).

2.4 Extraction Methods for Ketamine and MDMA

The main purpose of the extraction steps is to isolate the drugs of interest from sample matrices. The common sample matrix that involved in researches on ketamine and MDMA are oral fluid, sweat, urine, hair and human plasma. There were also researches that extract and

detect the common volatile components from the MDMA drug samples by air sampling in the large open or closed environment such as cargo container (Gura *et al.*, 2009; Lai *et al.*, 2008).

In the recent studies, the most common method for extraction of ketamine and MDMA is the solid phase extraction (SPE) (Aboul-Enein and Hefnawy, 2005; Cheng and Mok, 2004; Cheng *et al.*, 2007; De Martinis *et al.*, 2007; Harun *et al.*, 2010; Jenkins *et al.*, 2004; Kim *et al.*, 2008; Nema *et al.*, 2011; Peters *et al.*, 2005; Phonchai *et al.*, 2012; Pirnay *et al.*, 2006; Scheidweiler and Huestis, 2006; Wang *et al.*, 2005). Other extraction methods are solid phase microextraction (SPME) of MDMA (Gura *et al.*, 2009; Lai *et al.*, 2008), micropulverise extraction of ketamine (Favretto *et al.*, 2013; Inagaki *et al.*, 2009), and liquid-liquid extraction (LLE) of ketamine and MDMA (Chèze *et al.*, 2007; Chou *et al.*, 2004; Da Costa and Chasin, 2004).

2.4.1 Solid Phase Extraction (SPE)

For SPE methods on ketamine and MDMA, various brands of cartridges were utilised by the researchers. Examples are Altech C18 (Cheng and Mok, 2004), Clean Screen ZSDAU020 (Pirnay *et al.*, 2006), Drug Clean™ C (Kim *et al.*, 2008), Oasis HLB (Cheng *et al.*, 2007; Phonchai *et al.*, 2012), Oasis MCX (Jenkins *et al.*, 2004), Sep-Pak C18 (Aboul-Enein and Hefnawy, 2005), SPEC C18AR/MP1 columns (Scheidweiler and Huestis, 2006), SPEC MP1 disk (De Martinis *et al.*, 2007), SPEC®-PLUS-3ML-DAU Column (Wang *et al.*, 2005) and Toxitube A® (Chèze *et al.*, 2007). SPE gave high recoveries (Aboul-Enein and Hefnawy, 2005), where sample loss from SPE was negligible compared to evaporation method, and can be compensated by an equivalent loss of the internal standard (Jenkins *et al.*, 2004). SPE also

removes the endogenous interferences and hence little or no matrix suppression or enhancement can be observed (Aboul-Enein and Hefnawy, 2005; Jenkins *et al.*, 2004).

Modifications on SPE cartridge, in term of sorbent materials were done by researchers, in order to enhance the extraction of ketamine from biological samples. An anti-ketamine molecularly imprinted polymer (MIP) was synthesised by Harun *et al.* (2010) and used as the sorbent in SPE protocol to isolate ketamine and its metabolite from human hair extracts. As the imprinting effect, higher percentage of ketamine bound to the MIP than the non-imprinted polymer as SPE sorbent. The effect could be enhanced by non-polar solvent, such as chloroform. With that, the matrix effects caused by the complex matrix in hair samples could be minimised. The analytes with different shapes and functional groups from those of the template ketamine had low affinity to MIP, this indicated the shape selectivity of the ketamine MIP. The authors reported that MIP columns were reusable, robust and able to withstand a range of different pH and solvent conditions (Harun *et al.*, 2010). Hence it is suitable in extracting ketamine in hair samples from chronic users as well as those obtained after single exposure, which was in trace amount (Harun *et al.*, 2010).

The monolith with reactive hydroxyl group on its surface enables it to function as a miniature SPE cartridge that prepared by Nema *et al.* (2011). The SPE cartridge was then used to extract ketamine from urine to study its extraction efficiency. The prepared cartridge was found robust in extracting ketamine efficiently and repeatedly without any significant deterioration in its performance (Nema *et al.*, 2011). The variations caused by ion suppression of the extract and recoveries of sample urine were reported to be small. This suggest that the properties of monolith were consistent to be used as a miniature SPE cartridge for extracting ketamine from urine (Nema *et al.*, 2011).

For estimating the amounts of MDMA, MDA and methamphetamine in urine, a simple SPE method was developed by Phonchai *et al.* (2012). The pH of urine sample was adjusted to 12 prior to perform SPE using Oasis HLB. The MDMA, MDA, methamphetamine, as well as the internal standard, diphenylamine (DPA) are weak base and in unionised forms and interact well with the SPE sorbent in alkaline condition. A 2-dimensional-SPE with one-wash step was chosen since it was simple and provided a high efficiency of extraction for MDMA, MDA, and methamphetamine from urine samples. The authors used one-wash step to prevent the effective elution of interfering compounds in urine samples. the SPE method developed was proven to be highly effective for successful determination of MDMA, MDA and methamphetamine in the urine drug abuse suspects.

2.4.2 Solid Phase Microextraction (SPME)

Detection of vapours emanating from hidden illicit drugs in large open or closed spaces has been a challenging task. The detection of trace levels of MDMA in a complex and high throughput environment requires a fast sampling device. Instead, it can be achieved by sampling the volatile compound of MDMA, piperonal. Conventional methods of direct air sampling of drugs is considered ineffective, due to the nature of the drugs, which is low volatility, making them impossible to be sampled using headspace technique. SPME was reported to be an effective and rapid air sampling technique for the extracting the volatile analytes in a confined space, such as cargo container, accumulating in sufficient concentration. Additionally, no potential compounds that commonly found in cargo container can interfere in detecting piperonal (Lai *et al.*, 2008).

Gura *et al.* (2009) presented a novel SPME device for enhanced air sampling and to target volatile markers and odour signatures of MDMA. The sampling method was basically on unique static samplers, planar substrates coated with sol-gel polydimethyl siloxane (PDMS) nanoparticles, also known as planar solid phase microextraction (PSPME). The results showed that the novel device exhibit significant improvement over the fibre SPME in extraction of piperonal vapours using standards as well as case samples, which present in traced amount (Gura *et al.*, 2009). The sampling using PSPME can be done within few seconds to achieve positive identification. The PSPME has an operational advantage over the fibre SPME because the PSPME can be inserted directly into the desorber for analysis without the need for an interface or modification to the front end of the analyser (Gura *et al.*, 2009). This makes PSPME applicable to a number of instrumental analyses already deployed in the fields as well as in forensic laboratories.

2.4.3 Micropulverised Extraction

For extracting ketamine from hair samples, a trend in reduction of sample pre-treatment and isolation has recently emerged in the forensic hair analysis community, including simultaneous pulverisation/extraction procedure. Considering the basic nature of ketamine, the micropulverised method can efficiently extract the drug and its metabolites from hair. Briefly, the washed whole hair was put in a vial with two stainless steel beads, acetonitrile as organic solvents, trifluoroacetic acid as internal standard, working solution, and water, followed by shaking using a pulveriser (Favretto *et al.*, 2013; Inagaki *et al.*, 2009).

Inagaki *et al.* (2009) compared the micropulverised extraction method with conventional acidic methanol extraction, which extract ketamine by directly immersing the hair sample into organic solvent, methanoic trifluoroacetic acid, together with 2-aminobiphenyl as internal standard, followed by sonication and allowing to stand overnight at room temperature. The precisions obtained in the case of micropulverised extraction were slightly better than those obtained in the case of acidic methanol extraction (Inagaki *et al.*, 2009). This could be explained as due to its simpler pretreatment method and bypassing complicated processes such as evaporation, derivatisation or SPE. Hence it is more reliable, rapid, and can be completed within a short time period (Inagaki *et al.*, 2009). Therefore, the micropulverised extraction method is considered to be highly useful for the analysis of ketamine in hair samples (Inagaki *et al.*, 2009).

2.5 Liquid-liquid Extraction (LLE)

Liquid-liquid extraction (LLE) is one of the most commonly used sample-preparation techniques (Chiu *et al.*, 2010). This method separates compounds based on differing solubilities in two contacting liquid phases, which are insoluble or partially soluble to each other (Chiu *et al.*, 2010). Commonly, the two liquid phases consist of an aqueous and a non-polar organic phase (Chiu *et al.*, 2010). LLE is widely used for sample preparations that involve analytes having lower molecular weight, such as drugs (Buhrman *et al.*, 1996; Chen *et al.*, 2008; Ji *et al.*, 2008; Lee *et al.*, 2008; Nirogi *et al.*, 2006).

Various types of organic solvents were used as the organic phase in the LLE methods. For examples, hexane was used in simple LLE while ethyl acetate was used in back-extraction of drug SR 27417 from human plasma (Buhrman *et al.*, 1996). Ethyl acetate was used in LLE

to isolate triptolide from the biological matrix, human whole blood (Chen *et al.*, 2008). Ethyl acetate was also chosen as an extraction solvent in the extraction of doxazosin from human plasma, in order to obtain the selectivity and good recovery (Ji *et al.*, 2008). Butyl acetate was used in a one-step LLE to extract etodolac from human plasma, where the recovery results indicate no significant ion suppression by co-eluting endogenous substances in this extraction method (Lee *et al.*, 2008).

For LLE of ketamine from urine, triethylamine/cyclohexane was used as the organic solvent (Chou *et al.*, 2004). Pentafluorobenzyl chloride (PFBC) was then added to chemically derivatise ketamine and its metabolite, norketamine, in order to enhance their instrumental responses and mass spectrum uniqueness, to afford more proper and easier selection of quantifier and qualifier ions, as well as to achieve more evidential identification and quantitation (Chou *et al.*, 2004). For LLE of MDMA from urine and hair, hexane/ethylacetate mixture was chosen as the organic solvent (Chèze *et al.*, 2007; Da Costa and Chasin, 2004). It require less amount of solvents, simple, fast, has presented precise results and high recovery percentage, and is acceptable by forensic laboratory studies (Da Costa and Chasin, 2004).

An analytical technique, which included LLE method, was developed by Nirogi *et al.* (2006) and validated for the quantification of olanzapine in human plasma. Prior for minimising ion suppression and matrix effects in the study, six organic solvents, diethyl ether, ethyl acetate, hexane, dichloromethane, chloroform and butyl tert-methyl ether, and the mixtures in various proportions and combinations were evaluated by the authors (Nirogi *et al.*, 2006). They found that a mixture of diethyl ether and dichloromethane, with in volume ratio of 7:3, performed an optimal extraction yield (Nirogi *et al.*, 2006). A clean chromatogram for

blank plasma sample and yield the highest recovery for the analyte from the human plasma was reported using this mixture (Nirogi *et al.*, 2006).

In the study by Buhrman *et al.* (1996), a comparison of two LLE methods in extracting the drug SR 27417 from human plasma were carried out. They were hexane LLE versus liquid-liquid back extraction, which ethyl acetate was used twice in the extraction method. Although the extraction efficiencies of the two LLE methods were similar, the ethyl acetate back extraction method possessed nearly zero ion suppression. The alteration of pH value of the samples made the drug of interest dissolve in desired phase without other matrix components. This enhance the process efficiency of the back-extraction. With that, compared to hexane LLE, the back-extraction has better precision and accuracy, fewer coeluting matrix components, less variability, and lower limit of quantification (Buhrman *et al.*, 1996).

Usually, LLE yields rather clean extract with high reproducibility. However, there were some drawbacks which are inevitable. First, the process often requires preconcentration before analysis (Chiu *et al.*, 2010). It needs large amounts of costly toxic solvents, and can be time consuming (Chiu *et al.*, 2010). LLE is also difficult to be automated. There were efforts to make LLE automated by automating the mixing and phase separation steps by the researchers. For examples, LLE methods in the 96-well-plate format (Wang *et al.*, 2006), and turbulent flow (Xu *et al.*, 2005), were implemented in the quantitation studies. However, these automated techniques hindered LLE method to become popular. There are other methods that showed to give out better yields than LLE methods. Nonetheless, LLE is still routinely used by the forensic laboratories, with good recoveries.

2.6 Analytical Instrumentations for Ketamine and MDMA

In recent studies, liquid chromatography (LC) and gas chromatography (GC) analyses are utilised by researchers for ketamine and MDMA analysis. LC was the most frequently used technique in analysis of ketamine from various types of biological sample such as hairs, urine, and human plasma, with the aids of extraction methods reviewed in the previous section. For examples, liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Cheng and Mok, 2004; Harun *et al.*, 2010; Nema *et al.*, 2011; Wang *et al.*, 2005), liquid chromatography-mass spectrometry (LC-MS) (Favretto *et al.*, 2013; Inagaki *et al.*, 2009), and high performance liquid chromatography (HPLC) (Aboul-Enein and Hefnawy, 2005).

There were numerous studies that utilised GC as analytical instrument in analysis of MDMA in liquid biological samples such as urine, oral fluid, sweat and blood with aids of mainly SPE methods. For examples, gas chromatography-mass spectrometry (GC-MS) (De Martinis *et al.*, 2007; Peters *et al.*, 2005; Pirnay *et al.*, 2006; Scheidweiler and Huestis, 2006), and gas chromatography-flame ionisation detector (GC-FID) (Phonchai *et al.*, 2012). Ion mobility spectrometry (IMS) was also used in detecting the volatile compound of MDMA, particularly piperonal, in order to detect the presence of MDMA in an large open or closed place such as cargo container (Gura *et al.*, 2009; Lai *et al.*, 2008).

2.6.1 Liquid Chromatography (LC) Techniques

2.6.1.1 Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

LC-MS/MS can be used in the analysis of ketamine and MDMA as it provides both screening and confirmation capabilities. Sample preparation time can be reduced and sample integrity can be improved. With the aid of highly specific extraction methods, such as SPE, this method can be sufficiently sensitive in order to detect specific drugs that well-separated from their metabolites (Cheng and Mok, 2004). This technique is suitable for drug monitoring programme or routine screening in high throughput forensic laboratories. Specific LC programme need to be set in order to effectively separate the possible interfering compounds from the target compound, as well as to minimise the matrix effects (Cheng and Mok, 2004; Harun *et al.*, 2010).

The MS/MS was also coupled with ionisation devices, such as TurboIonSpray (TIS) and electronspray ionisation (ESI), to enhance the separation of the fragmented ions. TIS is similar to ESI, that is most ionisation process in liquid phase before being detected by mass spectrometer, but the ionisation occur in the gas phase. It is the transfer process that ions present in the sample liquid phase into the gas phase. With that, the parameters of sensitivity, corresponding to the collective efficiencies in both liquid and gas phases are considered during the analysis process. The method was sensitive, specific, and accurate, and provided easy operation to detect and quantitate ketamine and MDMA as well as their metabolites up to eight days in urine after single intake. It was a powerful technique for the determination of low levels of drugs in hair after a single dose like drug-facilitated crimes (Chèze *et al.*, 2007). Segmented