DETERMINATION OF XYLAZINE IN DRUG-SPIKED DRINKS USING GAS CHROMATOGRAPHY FOR FORENSIC INVESTIGATION

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by

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LIST OF SYMBOLS

±	More or less
pA	Peak area
R^2	Linear regression
v/v	Volume per volume
w/v	Weight per volume

LIST OF UNITS

%	Percent
µL	Microlitre
eV	electron volt
g	Gramme
kg	Kilogramme
mg	Milligramme
min	Minute
mL	Millilitre
ng	Nanogramme
ºC	degree Celsius
pg	Picogramme
V	Voltage

LIST OF ABBREVIATIONS

CD	Codeine
CF	Caffeine
CI	Chemical ionisation
CNS	Central nervous system
CRM	Certified reference material
D_C	Distribution coefficient
DCM	Dichloromethane
DDA	Dangerous Drug Act
DFC	Drug facilitated crime
DFSA	Drug facilitated sexual assault
DLLME	Dispersive liquid-liquid microextraction
D_M	Partition coefficient
e.g.	*Exempli grati*- for example
ECD	Electron capture detector
ECG	Electrocardiogram
ED	Emergency department
EI	Electron impact ionisation
et al.	*et alia*- and others
FDA	Food and Drug Administration
FID	Flame ionisation detector
GABA	Gamma-aminobutyric acid
GC	Gas Chromatography
GHB	Gamma hydroxybutyrate
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
HR	Heroin
i.e.	*id est*- that is
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IS	Internal standard
KET	Ketamine
LC	Liquid chromatography

LC-HR-MS^n	Liquid chromatography coupled with linear ion trap high resolution multistage mass spectrometry
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantitation
LSD	Lysergic Acid Diethylamide
MDMA	3,4-methylenedioxymethamphetamine
MEPS	Microextraction by packed sorbent
MM	6-Monoacetylmorphine
MP	Morphine
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NADA	National Anti-Drug Agency
NaOH	Sodium hydroxide
NPD	Nitrogen phosphorous detector
NSAID	Non-steroidal anti-inflammatory drugs
OJP	Office of Justice Programme
PCM	Paracetamol
QC	Quality control
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
RSD	Relative standard deviation
SD	Standard deviation
SPE	Solid phase extraction
SPME	Solid phase microextraction
STD	Standard
TLC	Thin layer chromatography
UHPLC	Ultra-high-pressure liquid chromatography
UNODC	United Nations Office on Drugs and Crime
USA	United States of America
UV	Ultraviolet
UV-Vis	Ultraviolet – visible
XYZ	Xylazine

LIST OF APPENDICES

PENENTUAN XILAZIN DALAM MINUMAN YANG DIMASUKKAN DADAH DENGAN MENGGUNAKAN KROMATOGRAFI GAS UNTUK PENYIASATAN FORENSIK

ABSTRAK

Xilazin, sejenis ubat haiwan yang dikenali dengan kesan sedatif, anestesia dan analgesia telah dilaporkan penggunaannya dalam aktiviti jenayah disebabkan dadah (DFC) kebelakangan ini. Mangsa menjadi tidak berupaya selepas pengambilan minuman yang ditambah dadah dan seterusnya terdedah kepada risiko rompakan dan gangguan seksual. Kajian ini bertujuan untuk membangunkan suatu kaedah kromatografi gas berasaskan makmal untuk mengesan dan menentukan kuantiti xilazin dalam sampel minuman yang ditambah dadah yang wujud dalam bentuk cecair, titisan dan kering. Dalam kebanyakan situasi, sampel minuman yang disyaki dimasukkan dadah terdapat di tempat kejadian tetapi tahap kepekatan xilazin berkemungkinan amat rendah atau dibatasi oleh jumlah yang boleh diperolehi semula untuk pengujian forensik. Dalam kajian ini, suatu kaedah kromatografi gas-spektrometri jisim telah dibangunkan untuk mengesan kehadiran xilazin dan satu kromatografi gas-pengesanan pengionan nyalaan telah ditentu-sahkan untuk penentuan kuantiti sebatian tersebut. Seterusnya, xilazin daripada empat sampel minuman berlainan dalam tiga bentuk fizikal, yang berkemungkinan menyerupai tetapan forensik, telah diperolehi dan ditentukan. Satu kaedah yang sah untuk penentuan identiti dan kuantiti xilazin dalam 15 minit telah dibangunkan. Had pengesanan dan had pengkuantitian bagi kaedah tersebut telah ditentukan masing-masing pada 0.08 μg/mL dan 0.26 μg/mL. Suatu kelinearan yang baik telah dicapai dengan pekali korelasi sama dengan 0.997. Kejituan intra-hari dan antara hari untuk kaedah tersebut masing-masing adalah dalam julat 0.5

– 0.7% dan 1.6 – 3.9%. Ketepatan yang baik juga dilaporkan antara 97.3% dan 100.7%. Bagi perolehan xilazin daripada empat minuman berbeza yang diuji dalam kajian ini, jumlah xilazin yang terkandung dalam sampel minuman bentuk cecair boleh dikuantitikan dengan peratusan perolehan lebih daripada 77%. Kajian ini menunjukkan isipadu sampel minuman tertinggal yang terhad dan juga sampel kering tidak menghalang perolehan semula dadah yang disasarkan. Xilazin surih yang wujud dalam bentuk sisa titisan atau kering dalam suatu bekas boleh dikesan pada peratusan perolehan semula sekurang-kurangnya 40%. Kesimpulannya, suatu kaedah kromatografi gas telah berjaya dibangunkan untuk penentuan sisa-sisa xilazin dalam minuman yang ditambah dadah. Hal ini dapat menyediakan teknik penyiasatan berguna kepada juruanalisis makmal dan pegawai tempat kejadian dalam penyiasatan DFC.

DETERMINATION OF XYLAZINE IN DRUG-SPIKED DRINKS USING GAS CHROMATOGRAPHY FOR FORENSIC INVESTIGATION

ABSTRACT

Xylazine, a veterinary drug known for its sedative, anaesthesia, and analgesia effects, had recently reported its utilisation in drug facilitated crimes (DFC). Victims are incapacitated after consumption of drug-spiked drinks and subsequently exposed to the risk of robbery and sexual assault. This study aimed to establish a laboratory-based gas chromatography method to detect and quantify xylazine in drug-spiked drink samples appearing in liquid, droplet, and dry forms. In many instances, the suspected drug-spiked drink samples are available at the scene but the concentration level of xylazine could be very low or restricted by its recoverable amount for forensic testing. In this study, a gas chromatography-mass spectroscopy method was developed to detect the presence of xylazine, and a gas chromatography-flame ionisation detector method was validated for quantification of the substance. Subsequently, xylazine from four different drink samples present in three different physical forms, simulating the possible forensic settings; were recovered and determined. A validated method for the determination and quantification of xylazine within 15 minutes was reported. The limit of detection and limit of quantification of the method were determined at 0.08 μg/mL and 0.26 μg/mL, respectively. A good linearity was achieved with correlation coefficient of 0.997. Intra-day and inter-day precisions for the method were ranged at 0.5 - 0.7% and 1.6 – 3.9%, respectively. Good accuracy was also reported, ranging between 97.3% and 100.7%. For the recoveries of xylazine from four different drinks tested in this study, the amount of xylazine contained in a liquid drink sample could be quantified with recoveries greater than 77%. This study evidenced that limited

volume of leftover spiked drink samples, and even the dried samples; did not hinder the recovery of the targeted drug. Trace xylazine that appeared in residual droplets or dried form within a container can be detected at a recovery percentage at least 40%. To conclude, a gas chromatographic method was successfully established for the determination of residual xylazine in drug-spiked drinks that can then provide valuable investigative technique to laboratory analysts and crime scene officers in DFC investigations.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

1.1.1 Misuse of Drugs

According to the World Health Organisation (WHO), drug is chemical substance that alters the normal function when it is administered into the body of a living organism. It can be used in the treatment, prevention, cure or diagnosis of disease, or to enhance the physical or mental well-beings (WHO, 2021). Drugs can be classified based on their therapeutic uses, sites of action, impacts towards body system, and their respective molecular structures. Globally, drug misuse is considered as one of the greatest threats to the society. Such act involves the consumption of drugs which harms the physical or mental health of an individual and the society (Oppenheimer, 1991). Generally, drugs had been used in both the medical treatment and for non-medical or recreational purposes. The latter is frequently associated with various crimes, especially involving the plant cultivation, clandestine manufacturing, trafficking, smuggling, packaging, distribution, and sale of the illicit drugs. These activities would subsequently lead to the risks of crimes, including homicide, drug-facilitated sexual assault (DFSA), robbery and many others (OJP, 1998; Johnson, 2003).

Under the legislation of Dangerous Drug Act (DDA) 1952, the trafficking, cultivation, manufacturing, dealing and possession of drugs are considered as crimes. DDA 1952 is the key regulation against the drug-related offences in Malaysia, and it describes the punishment under the Act. Meanwhile, Poison Act 1952 (revised 1989) regulate the import, possession, manufacture, compounding, storage, transportation,

sale and use of poisons. Accomplices in case related to illicit drugs, an individual charged under the Act is also punishable with imprisonment, whipping or a death sentence.

Figure 1.1 shows the statistics of drug addicts and substance abuse in Malaysia between 2018 and 2020 as recorded by the National Anti-Drug Agency (NADA). It was noted the number of drug addicts and substance abuse reported per year has reached a peak in 2019 about 142,199 individuals; however, the number has drop in following year (NADA, 2020). Moreover, the statistics also included those drug addicts who were detected and recorded by the law enforcement authorities, where a huge proportion might not be recorded. The high number had indirectly suggested the demand of drug addicts for the illicit drug, requiring attention by Malaysian government. Whenever there is a demand, the supply would increase simultaneously either through domestic manufacturing or trafficking from other countries into Malaysia.

Illicit drug problems are frequently highlighted in the mass media but their interlink to other criminal acts, including assault, gambling, robbery *etc.* are often overlooked. In additional of misuse of illicit drugs, dismantling of clandestine drug laboratories and tackling of drug trafficking activities could also be linked with other severe crimes, especially when they involve the use of firearms and ammunition. The issue related to illicit drug shall not be treated as a single case but could be associated with other criminal activities, indicating the importance of forensic drug analysis.

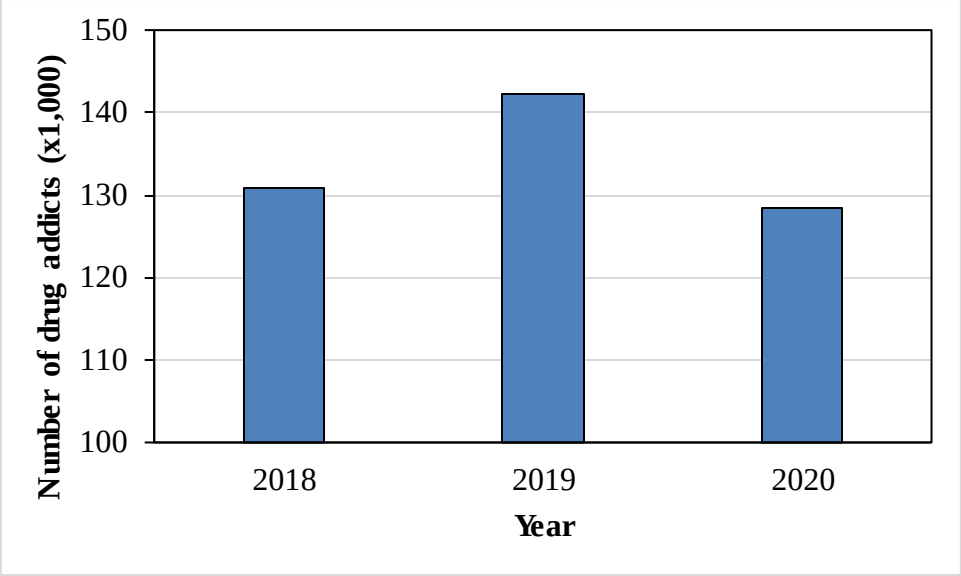

Figure 1.1: Number of drugs addicts and substance abuse in Malaysia from year 2018 to 2020 (NADA, 2020).

NADA (2020) also reported the types of drugs used by the drug addicts in Malaysia, as depicted in Figure 1.2, covering only those drug addicts detected by the law enforcement authorities. Based on the Figure 1.2, opiate-based drugs (red colour bar), including both heroin and morphine, had dominated the Malaysian's drugs market in 2016. However, it was found that methamphetamine (white and grey colour bars) in crystalline and tablet forms had overtaken the top rank starting from the year of 2017. This could be due to the increased number of clandestine laboratories dismantled in the country, where majority of them involved in producing illicit methamphetamine (UNODC, 2020). Additionally, illicit drugs including marijuana, psychoactive pills, and others (kratom leaves, depressant, dissociative, hallucinogens, inhalant, and sedatives) were also encountered by law enforcement authorities but in relatively lower frequencies. Among the detected substances, veterinary drug was also misused by the drug addicts to gain the desired effects. The most common drug under such category is the ketamine.

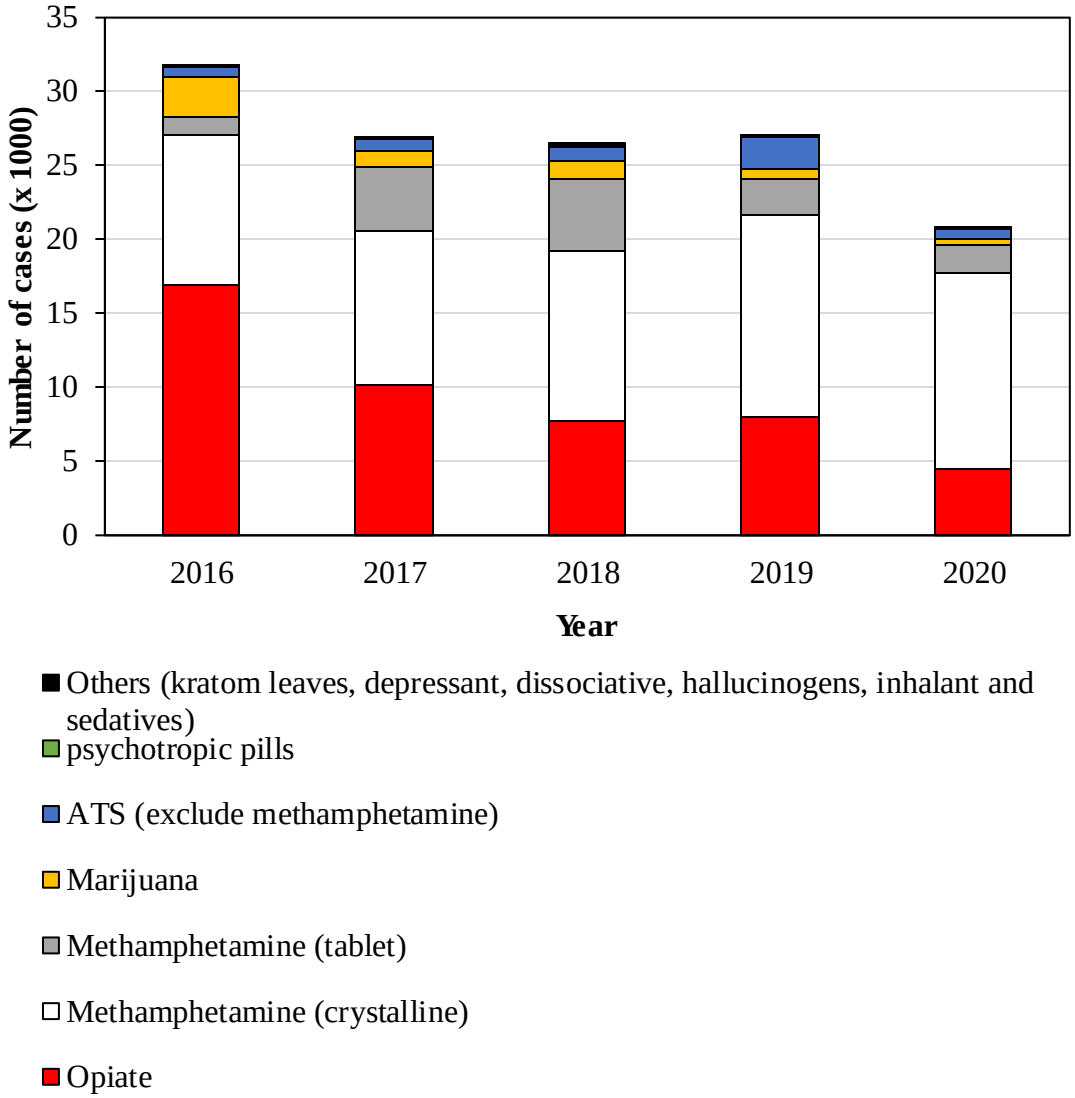

Figure 1.2: Type of drugs use among the drugs addicts in the Malaysia from year 2016 to 2019 (NADA, 2020).

1.1.2 Misuse of Veterinary Drugs by Human

Misuse of drug is one of the major problems faced by many countries (Oppenheimer, 1991; Erramouspe *et al.*, 2002; Johnson, 2003). According to Food and Drug Administration (FDA), a consumption of more than the prescribed dose for the medicinal effect, or wrongly prescribed to someone are classified as drug misuse (Food and Drug Administration, 2017). In fact, the intentional administration of veterinary

medications into human is a form of substance misuse (Erramouspe *et al.*, 2002; Perrin, 2015). Analgesic, anti-inflammatory medications, anti-arthritis medications, systemic antibiotics, topical anti-infectives, and topical corticosteroids are among the reported veterinary medications misused by human. These drugs were used for self-medication among the licensed veterinary practitioners in the United States (Erramouspe *et al.*, 2002). Specifically, non-steroidal anti-inflammatory drugs were the most frequently drug misused by nearly half of the respondents (47.6% out of 319 respondents) for muscular aches and pains, sprains and neuralgia purposes in a report (Erramouspe *et al.*, 2002). More recent cross-sectional studies have suggested that the diversion of opioid analgesic prescription and unlawful channelling of drug from veterinary sources to the illicit marketplace and to people who were not prescribed for had appeared to be increasing (Wood, 2015; Russell *et al.*, 2018).

In the United States, people involved with rodeo, horse racing, health care, rural area residents, and those who are lacking health insurance were perceived to be the groups most likely to misuse veterinary drugs. Having an independent self-sufficient attitude, the easily accessible to veterinary medications at lower cost, and a belief that veterinary medications acquired stronger effects than comparable human medications are among the reasons of veterinary drugs being misused (Erramouspe *et al.*, 2002). Therefore, issues related to misuses of veterinary drugs can be dangerous and therefore shall be revised and planned for immediate attention by the governmental agencies to minimise the potential risks in human health.

Veterinary medications can be lethal to human if they are consumed extra-label, which means they are used in a way that is not listed on the drug's label. Note that the

target substance in this study, xylazine, is also a veterinary drug. Upon human consumption, xylazine can act as a poison, causing the central nervous system (CNS) depression, bradycardia, hypotension, and even death (Stillwell, 2003; Elejalde *et al.*, 2003; Bayramoglu *et al.*, 2016; Krongvorakul *et al.*, 2018; Nunez *et al.*, 2021). In 2007, a profile on the poisoning admissions in Malaysia revealed that 17% of the poisoning cases was caused by non-opioid analgesics, anti-pyrectics and anti-rheumatic, as shown in Table 1.1 (Rajasuriar *et al.*, 2007). In more recent years (2010 to 2015), analgesic poisoning was reported at approximately 14.7% among the group of pharmaceutical agents meanwhile veterinary poisoning was reported about 0.2%. The related statistical data is shown in Table 1.2 (Alwan *et al.*, 2020). Although the actual substances that involved in the poisoning cases were not retrievable; nonetheless, the misuse of xylazine, which is frequently coupled with ketamine, should also be a concern.

Table 1.1: Poisoning admissions by substances (Rajasuriar *et al.*, 2007)

Poisoning substance	Admissions	
	No. of cases	Percentage (%)
Pharmaceutical substances	11916	54.88
• (Non-opioid analgesics, anti-pyrectics and anti-rheumatics)	3738	17.21
Chemical substances	9798	45.12
Total	**21714**	**100**

Table 1.2: Poisoning cases according to the groups of pharmaceutical agents between year 2010 to year 2015 (Alwan *et al.*, 2020)

Pharmaceutical agent	Admissions	
	No. of cases	Percentage (%)
Analgesic	1616	14.7
Veterinary	22	0.2
Other pharmaceutical	9360	85.1
Total	**10998**	**100**

*The term (other pharmaceutical) refers to drug listed in the article other than analgesic and veterinary.

1.1.3 Veterinary Analgesics

The US Federal Food, Drug, and Cosmetic Act defines animal drug, also known as veterinary drug, as any drug intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease, and to affect the structure or any bodily function in animals (FDA, 2020). Veterinary drugs are composed of a broad groups of pharmaceuticals exhibiting many different chemical and therapeutic properties (Oh *et al.*, 2009; Marazuela, 2017).

Analgesic animal drug can be classified into two major classes, namely the opioids and non-steroidal anti-inflammatory drugs (NSAIDs) (Yamaoka and Auckburally, 2013). Opioids are drugs that naturally produced from opium or made partially or fully synthetic such as oxycodone and fentanyl, while NSAIDs cover those non-opioid substances such as aspirin and ibuprofen. These drugs are useful adjuncts in the treatment of postsurgical pain (Brownstein, 1993; Prescriber, 1996; White, 2005). The structure of opioids resembles opium in terms of physiological effects or addictive properties, and they are likely to bind to the opioid receptor that coupled with G-proteins

in the brain. They are alkaloids derived from plant materials *i.e.* morphine and heroin and also subset of opioids (Prescriber, 1996; Lindesmith, 2008). Additionally, there are several minor classes, including analgesic adjuvants such as lidocaine, ketamine and alpha-2 adrenergic agonists (Yamaoka and Auckburally, 2013). They are mainly administered into animals through feed, drinking water or by injection, depending on the intended purposes (Reig and Toldrá, 2008; Marni *et al.*, 2017). The use of analgesics drug in veterinary medicine is very common, but the reports in research and from the perspective of economy and health issues were still lacking. This study focuses on analysing a veterinary drug, xylazine.

1.1.4 Xylazine and Its Abuse

Xylazine is also known as *N*-(2,6-dimethylphenyl)-5,6-dihydro-4*H*-1,3-thiazin-2-amine ($C_{12}H_{16}N_2S$). The chemical structure of xylazine is shown in Figure 1.3. It was the first non-opiate α_2-agonist sedative that had been widely used in veterinary medicine (Torruella, 2011; Clarke and Trim, 2013). This substance was developed by Farbenfabrikin Bayer AG in the Federal Republic of Germany in 1962. As a thiazole drug, it has a molecular mass of 220.34 g/mol. A secondary amine is bonded to a benzene with two methyl groups and a thiazine ring.

At room temperature, xylazine exists in solid form as pure crystalline powder. Xylazine hydrochloride exhibited polymorphism as it has four crystalline structures. Each polymorph structures showed different intermolecular interactions, affecting its physical properties and relative stability (Zvirgzdins *et al.*, 2014). Polymorphic structure with higher density has proved to possess higher stability (Bērziņš *et al.*, 2010; Zvirgzdins *et al.*, 2014).

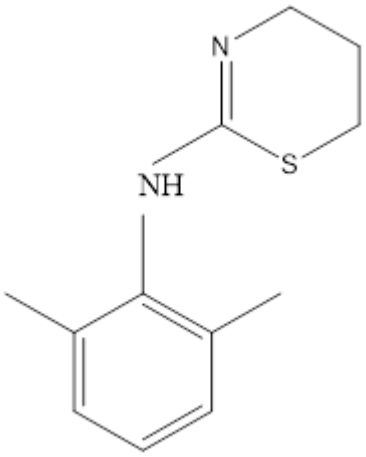

Figure 1.3: Chemical structure of xylazine.

Apart from their similar structures, xylazine possesses similar properties to clonidine carrying high lipid solubility and high volume distribution (Spoerke *et al.*, 1986). A highly lipid-soluble drug generally has rapid metabolism and good cell penetration, resulting in high volume distribution. A drug with high volume distribution can be extensively distributed into tissues and cells (Li *et al.*, 2019). Note that the molecular weight and melting point of xylazine are 220.34 g/mol and 140ºC, respectively (PubChem, 2021), and upon examination on xylazine properties in PubChem database, xylazine has generally fulfilled the *Lipinski's rule of 5* where a molecule should have molecular weight of less than 500, a *log P* of less than 5, hydrogen bond donor of less than 5 and hydrogen bond acceptor of less than 10, and polar surface of 140 $Å^2$, to have good permeation and absorption (Lipinski *et al.*, 1997; Anban *et al.*, 2020; PubChem, 2021). These physiochemical parameters could affect the interaction between the drug molecule and the lipophilic cell membrane on the blood-brain barrier *e.g.*, high-lipid soluble drug. In other words, drug with low number of hydrogen bonding and polar surface area is readily crosses the hydrophobic phospholipid bilayer to diffuse into the brain (Li *et al.*, 2019).

Although xylazine is specifically for veterinary application; yet misuse of xylazine by human was reported in several states and countries, including the Philadelphia (Wong *et al.*, 2008), Puerto Rico (Torruella, 2011; Reyes *et al.*, 2012), Canada (Tobias *et al.*, 2020), and Texas (Forrester, 2016). More recently, xylazine was found to be spiked into drinking water in Thailand (Krongvorakul *et al.*, 2018) and Singapore (Elena, 2016) with criminal intentions. Despite the misuse of xylazine which had been reported in neighbouring countries (Thailand and Singapore), the prevalence of xylazine in Malaysia, nonetheless, still remains unclear. This could probably be due to the unavailability of an established and validated method for the detection of xylazine, and the acquisition for the determination of this drug is not initiated by the investigating parties.

1.1.5 Legislation of Xylazine

In the United States, xylazine is not scheduled as a controlled substance (DEA, 2020). Nevertheless, it is listed as controlled substance and could be accessed under the prescription by licensed pharmacists in Canada and Australia under the Controlled Drugs and Substances Act 1996 and Poison Standard February 2018, respectively. In Thailand, xylazine is enlisted as controlled substance due to the increasing drugging incidents involving xylazine (National News Bureau of Thailand, 2013). According to FDA (2017), xylazine is approved only for veterinary use, but its use in "*food-producing animals*" is specifically unapproved (Chamberlain and Brynes, 1998). Under Code of Federal Regulations (2017), xylazine can be used in horses, wild deer, elks, dogs, and cats to produce sedation effect, as an analgesic, and a preanesthetic to local anaesthesia (FDA, 2017). However, it was also noted that the drug is approved for use in food

animals in Canada, United Kingdom, France, Germany, and Switzerland (Chamberlain and Brynes, 1998).

In Malaysia, xylazine is not listed as controlled drug in Dangerous Drug Act 1952, but it is regulated by Poison Act 1952 (revised 1989) and is only accessible to veterinary personnel or licensed parties. Though it is widely marketed as veterinary sedative, analgesic, and muscle relaxant, xylazine is classified as analgesic under Group B Poison Category of Poison Act 1952. Note that the Poisons Act (1952) deals with the regulation of poisons or drugs which are not scheduled under the Dangerous Drug Act 1952, whereas the Dangerous Drug Act (1952) specifically deals with the regulations of opium, dangerous drugs, and related materials. Under the Poison Act 1952, Group B poison *i.e.* xylazine shall not be sold or supplied by retail to any person except licensed practitioners. Those who sold or supplied xylazine without prescription, upon conviction, could be sentence with a fine of a maximum of RM 5,000.00 or imprisonment for up to two (2) years or both (Poison Act 1952).

1.2 Problem Statement

Misuse of drug is a global problem that has haunted the modern society. Recreational drug use might seem to be harmless but prolonged use of such drug might cause addiction, and potentially lead to drug facilitated crime. Drug-facilitated crime occurred when a victim was subjected to criminal act under the incapacitation of alcohol or drug (Shbair and Lhermitte, 2010). In Malaysia, there were cases where drug syndicate targeted teenagers through selling drugs by hiding them inside common products or by spiking into their drinks, to get them addicted. By this way, they managed to retain more customers for the illicit drugs, considering as their marketing strategy

(Kumar, 2018; Basyir, 2020). It was also reported that drinks spiked with drugs are often consumed by teenagers, especially at nightclubs and wild parties (Kumar, 2018).

Besides recreational purpose, drinks spiked with drugs are frequently colourless and odourless, and they can provide sedative effect upon their consumption. As a result, these drug-spiked drinks are often associated with crime such as date-rape and theft. Several drugging incidents using xylazine were reported in Thailand for robbery and alleged sexual assault purposes (Krongvorakul *et al.*, 2018; The Star, 2020). Xylazine was also reported to be misused by humans, especially as an adulterant to other drugs to enhance drug effect (Ruiz-Colón *et al.*, 2014). However, the presence of xylazine is not tested during the forensic routine analyses in Malaysia, and therefore whether it has been used in drug facilitated crimes (DFC) or adulteration in Malaysia remains unexplored. The prevalence of xylazine in the country is also unknown. Therefore, a method allowing the detection of xylazine should be established.

Historically, ketamine was documented to be illegally use and abuse due to its anaesthetic effect. It was also reported as an emerging date-rape drug used for DFSA purposes that commonly spiked into the drinks of victims (Li *et al.*, 2011; Khey *et al.*, 2013; Mohamed *et al.*, 2019). Similar to xylazine, ketamine is a commonly used drug in veterinary medicine for induction of anaesthesia, especially in small animals (Hartsfield, 1992). Additionally, anaesthetic ketamine and tranquiliser xylazine are often used in combination to produce effective analgesia in animals (Niedorf *et al.*, 2003; Shbair *et al.*, 2010a; Meyer *et al.*, 2013; Giroux *et al.*, 2015). Based on the status of xylazine and its documented effects, it demonstrates a high potential for xylazine to emerge as another "ketamine scenario" which might cause severe threat to the society.

To deter such recurrence, an immediate step forward action on the lengthy method development and validation from the forensic science communities is requested, and therefore this project was proposed.

1.3 Objectives

1.3.1 General Objective

The general objective of the study was to detect and quantify xylazine through a laboratory-based gas chromatography (GC) technique for forensic investigative purposes.

1.3.2 Specific Objectives

To achieve the main objective, specific objectives are set as follows:

i To develop and validate a GC technique in analysing xylazine.

ii To establish a liquid-liquid extraction protocol to extract xylazine from various drinks.

iii To investigate the recovery percentages of xylazine from various spiked drink samples, simulating the real case scenarios.

1.4 Significance of Study

Due to the worldwide drug misuses phenomenon that threaten the society, identifying an unknown substance as a drug or poison is a routine and crucial in forensic analysis. Foreseeing the potential of xylazine as another ketamine that had greatly affected our society and country, a proactive action shall aid in minimising the impact. An established analytical method for detection and quantitation of xylazine shall allow the addressing on the identity of unknown substance as xylazine if it was misused. The method would enable the investigation on the prevalence of xylazine which remains

unclear to date, either as recreational drugs, as adulterant, or as an ingredient that had been spiked into drinks.

The establishment of a suitable sample processing protocol to detect drugs residue in spiked drink samples would aid in the forensic investigation in cases involving DFC. As the drink spiking was frequently used to drug victims for committing crimes, this study would be beneficial, allowing the recovery of drugs residue from different drinks, in this case, xylazine. Quantifying the drugs in spiked drinks could provide details on the usage of drugs during forensic investigation, particularly the amount of xylazine used in those incidents.

CHAPTER 2

LITERATURE REVIEW

2.1 Drug Facilitated Crimes

Drug Facilitated Crimes (DFCs) are phenomena involving the administration of psychoactive substances without the victim's knowledge, and subsequently inducing incapacitation and facilitating criminal acts such as theft, robbery, and sexual assault (Rocca *et al.*, 2016; Wang *et al.*, 2018). Most DFCs involved the use of strong CNS depressant drugs to control and prevent the victims from fighting off their attackers. More specifically, crimes involving non-consensual sexual acts under the influence of drug is called DFSA. In other words, DFSA occurred without victim's valid consent because of drugs, including alcohol. Since 1980s, DFSA cases is often classified as "date-rape", describing rape perpetrated by victim's social escort or someone known to victim as like they were in a date together (Hall and Moore, 2008; Shbair and Lhermitte, 2010). Victims of DFC are usually under the amnesic effects of the administered drug; therefore in most cases, the victims might not be clearly remembered the pre-events leading to the assault (LeBeau and Montgomery, 2010).

Most drugs involved in DFCs are believed to provide amnesic, sedative-hypnotic, anxiolytic, narcotic, and muscle relaxant or the disinhibitory effects (Rocca *et al.*, 2016). There are more than 50 drugs that have been reported to be associated with DFCs. Amongst, ethanol was the most common type, followed by cannabinoids, cocaine, benzodiazepines, amphetamines, and gamma hydroxybutyrate (GHB) (Elsohly and Salamone, 1999; Hall and Moore, 2008). These drugs were found to produce rapid onset effects, muscle relaxation and anterograde amnesia (Milhorn, 2018). The ideal

features of these intoxicating substance such as odourless, colourless and tasteless, making them easier to be spiked into a food or drink and consumed by the victim unconsciously (Gharedaghi *et al.*, 2018). According to a clinical-epidemiological study carried out among commuters, about 45% of the poisoning cases involved the use of drug and happened during transportation, dominantly in public buses. Victims also reported that some offenders tricked them by eating the same source of food or drink to gain their trust while offering them the drugged food or drink (Uddin *et al.*, 2018).

In a study involving the analysis of thousands of urine samples from individuals who claimed to have been sexually assaulted under drug facilitation, approximately 67% of the urine samples were detected with alcohol either alone or in combination with other drugs. Cannabis was reported to be the second most prevalent drug after alcohol which could be primarily due to recreational uses trailed by medical purposes (Hindmarch *et al.*, 2001; National Academies of Sciences and Medicine, 2017).

In Malaysia, the common date rape drugs are Rohypnol® (flunitrazepam), GHB and ketamine which could induce powerful incapacitating effects from five to thirty minutes upon administration. These drugs were chosen by the rapist could most probably due to their amnesia, muscle relaxant and profound sedative effects (Hindmarch and Brinkmann, 1999; Fernandez and Mohamad Nor, 2019).

2.1.1 Forensic Analysis in DFCs

When DFCs happened, blood and urine of the victims are routinely collected for toxicological analyses. Additionally, alternative samples such as hair can also be collected as drugs were proven to be detected in hair for months and years depending

on the length of the hair sample (LeBeau, 2009; Gautam *et al.*, 2014). In certain instances, some drugs can only be detected in blood for the first to second hour after administration, while in urine, it could be up to 4 to 5 days depending on the metabolism of that drug (Rocca *et al.*, 2016). Drugs and metabolites are generally concentrated in urine; therefore, they can be appeared over longer periods of time to be detected as compared to blood specimens (Humbert *et al.*, 2014). Investigation on several patients presenting at emergency department (ED) who claimed that their drink had been spiked with unknown substances revealed that drugs with short detection window could be still detected in patients (urine and blood) within a time period of 18 hours after being drugged as compared to those patients who appeared later than 18 hours (Hughes *et al.*, 2007). In fact, if a blood specimen can be collected within short period after drug ingestion, a combination of blood and urine specimens could provide clear picture of administration of the drug (Humbert *et al.*, 2014), allowing the possibility for positive determination.

When dealing with biological samples, false negative outcome could be resulted due to the delayed sample collection and detection despite being drugged. Therefore, in certain cases, victim claimed that his drink has been spiked with drug but tested negative (Rocca *et al.*, 2016). In addition, victim under incapacitation of drugs often experienced amnesic effect, causing him/her unable to clearly remember the sequence of events before assault. When the victims realised on the incidents, the substance can no longer be detected in the urine or blood (LeBeau and Montgomery, 2010; Rocca *et al.*, 2016).

2.1.2 Drugs involved in DFCs

Benzodiazepines, non-benzodiazepine hypnotics (zolpidem and zopiclone), anti-histaminic and neuroleptic drugs, tricyclic antidepressants, GHB, cannabinoids, amphetamines and opiates are some of the drugs reported used for DFCs (Rocca *et al.*, 2016). Most DFC-related drugs carry similar properties including fast-acting, strong CNS depressants and their effects can be enhanced when used in combination with alcohol (LeBeau, 2009). The chance to detect the presence of these drugs are varied based on their retention in the biological samples; therefore, the missing well-timed blood and urine sample often resulted in missing the administered drug (Rocca *et al.*, 2016).

Since most of the drugs used in DFCs come in solid and as tablet formulation, it frequently requires no preparation before spiking into someone's drinks. Tablets with insoluble cellulose-based fillers might need to be pre-dissolved into small volume of ethanol-based solution, and further filtered prior to spiking (LeBeau, 2009). The following sections briefly described the illicit drugs encountered in DFCs and their behaviours.

GHB is used in DFCs due to its strong sedative, amnesiac effects as well as its rapid elimination from body upon administration. The use of GHB is quite difficult to be proven as GHB appears as metabolite of gamma-aminobutyric acid (GABA) in human body (Lemaire-Hurtel and Alvarez, 2014). Note that GABA serves as the main inhibitory neurotransmitter in the brain that reduces the activity of the neurons to which it binds. Upon consumption, it produces deep unconsciousness within 10 to 15 minutes

that could last up to four hours. Commonly, the drug is ingested orally and rapidly absorbed into the body (Wells, 2001).

Other than GHB as the most common type, the group of benzodiazepines is a large class of drug which is generally prescribed as anxiolytics, anticonvulsants, anaesthetic adjuncts, hypnotics, muscle relaxants, and they are commonly used to treat obsessive–compulsive disorders (Madea and Mubhoff, 2009). Additionally, it is recreationally abused in combination with other illicit drugs such as opiates and cocaine. The drug can be consumed orally, intravenously, and intramuscularly. Through oral ingestion, it can be absorbed into bloodstream at 100% absorption due to its high lipid solubility (LeBeau, 2009).

Generally, benzodiazepines could reach the peak blood concentration from 30 minutes to six hours in post-administration. The drug can be classified based on their elimination half-lives *i.e.* short-acting such as midazolam and triazolam and long-acting such as diazepam and clonazepam which could cause severe "hangover" side effect (Lemaire-Hurtel and Alvarez, 2014).

Amphetamine can be administered through oral ingestion, nasal inhalation, injection or smoking. Amphetamine can cause physical and mental stimulation; and thus, euphoria can be observed after administration. Euphoria produced through nasal inhalation and oral ingestion are less intensive and took time to develop as compared to other administration routes of amphetamine *i.e.* smoking and injection (Shbair *et al.*, 2010b). Amphetamines produce norepinephrine and dopamine pre-synaptically that responsible for stimulant and euphoria effects, respectively. The psychotic effects upon

its consumption include severe anxiety, unpleasant hallucinations, as well as panic attacks, and they can last up to four hours.

Despite being CNS stimulant, amphetamine is occasionally encountered in DFSA either in opportunistic DFSA or predatory DFSA, as it lower inhibitions and boost sex drive. Opportunistic DFSA is where the intoxication with drug have been self-administered by the victim meanwhile in predatory DFSA, the drug was covertly administered into victim by the perpetrator (Grela *et al.*, 2018).

Ketamine, a popular club drug, is a dissociative anaesthetic which is particularly common in animal related applications. There were reports regarding theft from veterinarian clinics due to the availability of the drug (LeBeau, 2009). Ketamine can be injected intravenously, intramuscularly, subcutaneously, through nasal insufflation, smoking inhalation as well as oral ingestion (Beynon *et al.*, 2008). Most drug users used ketamine due to its hallucinogenic properties. Victims may appear awaken but they are actually in a trancelike state when ketamine was administered (LeBeau, 2009).

Cocaine is clinically known as anaesthetic agent; however, it is illegally consumed due to its psychotropic effects. Cocaine can be administered through inhalation, smoking, injection or oral ingestion. The psychotropic effects of cocaine begin within seconds when it is smoked or injected unlike oral administration or nasal insufflation, where the peak effects could take longer time to develop up to 15 to 60 minutes (Shbair *et al.*, 2010b).

Cocaine usually come in white powdery form and sometimes, in form of white lumps, also known as crack (Grela *et al.*, 2018). Cocaine, as a strong CNS stimulant, increases the dopamine and further causing euphoria (Negrusz *et al.*, 2005; Shbair *et al.*, 2010b). Therefore, it is often associated with opportunistic DFSA (Grela *et al.*, 2018). Besides, it can increase the norepinephrine that eventually raises the blood pressure and heart rate (Shbair *et al.*, 2010b). Its effect on serotonin could increase body temperature and decrease one's appetite. However, the presence of cocaine might be correlative to the abuse of other drugs which might suggest risky behaviour of the victim (Negrusz *et al.*, 2005).

MDMA, also known as ecstasy, is a chemical derivative of amphetamine (Shbair *et al.*, 2010b). Unlike other drugs used to commit DFC, MDMA has both hallucinogenic and stimulant properties. With unwilling administration of MDMA to an individual, it could enhance the individual's sense, including desirable feeling associated with touch which likely cause sexual contact (LeBeau, 2009). This drug is typically ingested orally and rapidly metabolised into its metabolites *i.e.* methylenedioxyamphetamine (MDA) (Krasowski, 2019). Upon administration, MDMA can be detected in urine up to four days, given that highly sensitive instrumentation was utilised (LeBeau, 2009; Harper *et al.*, 2017).

Opioid is a class of substances that includes compounds extracted from the poppy seed, as well as semisynthetic and synthetic compounds with similar properties that interact with opioid receptors in the brain. Opioids possess strong CNS depressant and analgesic properties. In DFSA cases, it was reported that victims could sense and awake due to sudden infliction of pains caused by penetration or body movement

(LeBeau, 2009). The use of opioids could minimise the likelihood of awakening during sexual assault as opioids can produce sedation and muscle relaxation (Shbair *et al.*, 2010b). Such drugs have also been reported with good absorption through parenteral administration as compared to gastrointestinal ingestion (LeBeau, 2009).

In recent years, there was an increase in abuse of non-benzodiazepine hypnotics such as zolpidem and zopiclone. Note that these drugs are generally used in short-term treatment of insomnia (Krasowski, 2019). Despite having different structural properties, these drugs mimic benzodiazepines by binding to GABA receptor, and thus inducing rapid and strong CNS depressant effects (Madea and Mubhoff, 2009). There are also other drugs such as barbiturates, antipsychotics that have been reported to be used in DFCs. They mainly possess similar properties as in other sedative drugs, causing incapacitation to victims.

As stated above, these drugs generally reported to be associated DFCs and had been the focus of many forensic investigations conducted by law enforcement agencies. Many literatures had reported analytical developments in the toxicological investigation of drugs involved in DFCs and their significances towards the investigation (Humbert *et al.*, 2014; Negrusz and Gaensslen, 2016; Krasowski, 2019).

2.2 Xylazine as α_2-adrenergic Agonists

For several decades, α_2-adrenergic agonists have been used in the veterinary medicine to obtain sedation, analgesia, and muscle relaxation in a great variety of domestic and exotic species. α_2-adrenergic agonists refer to drugs that bind to α_2-receptors in the brain stem and in the periphery that inhibit sympathetic activities. The

first drug belonged this group that had been used in veterinary medicine was the xylazine (Giovannitti Jr *et al.*, 2015).

Xylazine, as an α_2 agonists, causes both sedative and analgesic effects in animals. At low doses, both effects are dose-dependent, while higher dose acts to lengthen the duration of sedation and increase the risk of adverse effects (Sinclair, 2003). Xylazine is used to induce sedation for minor diagnostic and surgical procedures either alone or in combination with other drugs *i.e.* benzodiazepines and opioids, as well as in complement with injectable *i.e.* ketamine, thiopental, propofol and inhalational anaesthetics *i.e.* halothane, isoflurane (Lemke, 2004). Besides, xylazine in combination with other sedatives, tranquilisers, and analgesics also have suggested better epidural analgesia with faster onset, prolonged duration, as well as increased sedation (Greene and Thurmon, 1988; Dart, 1999; Hubbell, 2009).

Xylazine was also reported to act like oxytocin; in pregnant animals, it increases the contraction of uterus, and eventually increases the intrauterine pressure (Fish *et al.*, 2011). Besides, xylazine can prolong the duration of increased intrauterine pressure in cases of delayed uterine clearance (De Lille *et al.*, 2000). In small animals *i.e.* cats, α_2-agonists cause stimulation of the chemoreceptor trigger zone to induce emesis (Hubbell, 2009). The effects of xylazine can be reversed by administration of selective antagonists, such as yohimbine and atipamezole (Sinclair, 2003).

2.3 Effects of Xylazine

Xylazine can cause several biological effects *i.e.* bradycardia, hypotension, and respiratory depression upon consumption either by human or animals (Ruiz-Colón *et*

al., 2014; Gomaa *et al.*, 2015). As a strong α_2-adrenergic agonist that produces sedation and muscle relaxation effects, it stimulates the central α_2-receptors and decreases the release of norepinephrine and dopamine in the CNS (Shi *et al.*, 2016). Stimulation of vascular α-adrenoceptors by direct injection of clonidine-like drug *i.e.* xylazine causes vasoconstriction which could further lead to a transient increase in blood pressure followed by a decrease afterwards. In worst-case scenario, the occurrence of bradycardia and dramatic could reduce cardiac output resulted from α_2-agonists effects on preganglionic fibres in the thoracic spinal cord (Hurley *et al.*, 2013).

α_2-agonists bind with receptors and intrinsically change the membranes of α_2-adrenoceptors, diminishing release of norepinephrine. Norepinephrine is responsible in producing arousal, and if it is blocked, sedation is resulted (Hurley *et al.*, 2013). However, optimum sedation could be failed to achieve if the endogenous catecholamine levels increased as it could interfere with α_2-agonist-induced reductions in excitatory neurotransmitter release (Sinclair, 2003). Xylazine could also cause hypotension by increasing systemic blood pressure (Hsu *et al.*, 1989). The central and peripheral adrenoceptors are stimulated, and they further affect the cardiovascular function. Since xylazine reduces the norepinephrine outflow within the CNS, the sympathetic tone gets dampened and further reduced the heart rate, leading to bradycardia (Sinclair, 2003).

It is important to point out that α_2-agonists were reported to induce hyperglycaemia, an increase in blood glucose level by suppressing insulin release and stimulating glucagon release, or both from pancreatic islets (Greene and Thurmon, 1988). Hyperglycaemia associated with xylazine is contributed by the actions at both α_2- and α_1-adrenoreceptors. α_2-agonists can cause respiratory depression which