

## REVIEW ARTICLE

# Abuse of Xylazine by Human and its Emerging Problems: A Review from Forensic Perspective

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## ABSTRACT

Xylazine is a sedative, analgesic and muscle relaxant widely applied in the veterinary field. However, owing to its depressant effect, xylazine has become a substance of abuse by humans. Misuse of xylazine not only triggers unwanted consequences (death), but also linked with various crimes. Google Scholar, PubMed and SciFinder were used to retrieve articles and case reports in relation to the misuses of xylazine and established analytical methods for forensic investigation until November 2021. Literatures reported the accidental and intended poisoning of xylazine, recreational use of xylazine and as an adulterant in recreational drugs. In addition to being a facilitator of crime and sexual assault, it is administered illegally to food producing animals as a sedative and to sports animals as a doping agent. Problems associated with the abuse of xylazine were highlighted in this review, covering the unknown prevalence of xylazine abuse and the need to revise the regulatory status of xylazine. In addition, limited screening and confirmatory methods that can be readily utilised to detect xylazine either alone or simultaneously with other substances of abuse, particularly useful for forensic toxicology and narcotic section were available in the literature. As a conventionally used veterinary drug, xylazine is undoubtedly a potentially hazardous drug, and the investigations on its potential abuse would enhance routine forensic examination to keep pace with the status of illicit drugs. *Malaysian Journal of Medicine and Health Sciences* (2022) 18(4):190-201. doi:10.47836/mjmhs18.4.26

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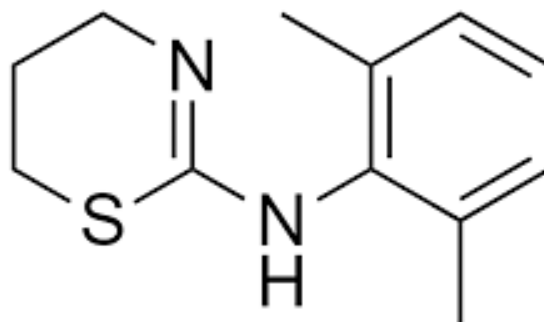
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## INTRODUCTION

Drug abuse continues to be a challenging issue globally that affects the well-being of the global population (1). Although prescription medications have been actively reported to be misused by humans (2), a veterinary drug, xylazine is currently being misused by humans (3). Xylazine, also known as N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine, is an  $\alpha_2$ -agonist sedative widely used in the veterinary field (4). Xylazine (chemical formula of C<sub>12</sub>H<sub>16</sub>N<sub>2</sub>S (Fig. 1) and molecular mass of 220.34 g/mol) was first developed by Farbenfabriken Bayer in the Federal Republic of Germany as a medicinal drug for the treatment of hypertension (5). Xylazine belongs to the thiazine group and serves as a central nerve system depressant (6). However, it was reported to cause bradycardia and hypotension. Xylazine is approved by the Food and Drug Administration (FDA) only for veterinary use in water injectable solution or crystalline powder marketed in various brands, such as Anased®, Rompun® and Sedazine® (7,8), and it is no longer used clinically by humans (7–9). Nevertheless, it has become a substance of abuse by humans.



**Figure 1: Chemical structure of xylazine**

To date, the prevalence of xylazine misuse remains unclear. However, xylazine is reported to be misused recreationally in several cities and countries across United States and Canada (10–15). In South-East Asia, including Thailand and Singapore, xylazine is used as a drugging agent (16,17). Based on the documented effects of xylazine and its status, it was revealed a high potential for xylazine to emerge as another “ketamine scenario”, threatening the well-being of society. Historically, ketamine was synthesized primarily for the use as anesthetic for Vietnam soldiers (18). Similar to the xylazine, ketamine was also a widely used in veterinary

field to initiate anesthesia (19). Later, ketamine was misused recreationally especially in “rave” scene and in drink spiking (20,21).

This review seeks to discuss xylazine and its exploitation from a forensic perspective and the potential problems that may arise owing to its misuse. As the misuse of xylazine could result in another “ketamine scenario”, readily validated analytical methods should be made available to detect this drug. Herein, supportive literatures were presented to pave the way for the establishment of appropriate and effective screening methods as well as confirmation methods that can be used by the forensic community, especially from forensic toxicology and narcotic section to detect xylazine alone or simultaneously with other drugs.

## SEARCH STRATEGY

Google Scholar, PubMed and SciFinder were used to retrieve the relevant articles using keywords: “xylazine and intoxication”, “xylazine and human”, “xylazine and abuse”, “xylazine and prevalence”, and “xylazine and analytical”. Relevant articles cited in the publications recovered from search engines were also collected. Google (website, blog, newsletter) was also used to identify recent cases on the misuse of xylazine with keywords, “xylazine and crimes” and “xylazine and spiked”. Literature reports published in English language until November 2021 were included in this review.

## XYLAZINE AS A VETERINARY MEDICATION

In veterinary field, xylazine is primarily used to induce a state of sedation with a shorter interval of analgesic, preanesthetic prior to general or local anesthesia, immobilisation agent for large wild animals as well as emetic agent for cats (22,23). As an  $\alpha_2$ -agonist, xylazine provides effects by stimulating the central  $\alpha_2$ -receptors and inhibit the release of peripheral norepinephrine, causing bradycardia, hypotension, and reduced cardiac output (24). Nevertheless, xylazine might also bind to cholinergic, serotonergic, dopaminergic,  $\alpha_1$ -adrenergic, histaminergic, and opiate receptors with an initial paradoxical hypertension upon administration (9).

Xylazine can be administered intravenously, intramuscularly, intraperitoneal, subcutaneously and orally for its sedative and muscle relaxant effect (22,25). It is available in the market in liquid solution at either 20, 100, or 300 mg/mL. This veterinary drug is dose-dependent, requires a small amount to initiate effects (4). Dosages of xylazine to induce effects were varied depending on the species and mode of administration. A 0.55 to 8.82 mg/lb of xylazine was suggested as administering dosage for dogs, cats, horses, elk and deer (26). Meanwhile, a lower xylazine dosage (i.e. 0.044 to 0.33 mg/kg) can initiate effects in certain species such as cattle, sheep and goat as they are sensitive to the effects

of xylazine (22).

Instead of single administration, xylazine can be co-administered with other drugs to achieve the profound effects. For instance, xylazine had been used together with lidocaine to achieve more prolonged analgesic effects in animals (27–29). When combined with ketamine, xylazine exhibits short term anesthetic and stable cardiopulmonary function during surgical procedure in horse (30). Addition of acepromazine to the xylazine-ketamine formulation was reported to have further prolonged the anesthetic period (31).

Nonetheless, care must be exercised with xylazine due to its reported drawbacks. It produces milder effects than other  $\alpha_2$ -adrenergic agonists (clonidine, detomidine, and medetomidine) (4), while it could potentially induce certain undesired effects, such as transient dose-dependent hyperglycemia, bradycardia, or even death when administered alone (32). Attention is also required when xylazine is to use with other drugs such as acepromazine and other central nervous system (CNS) depressants which could induce additive CNS depression (22).

## ABUSE POTENTIAL OF XYLAZINE

Although xylazine is used legitimately as tranquilizer in veterinary science and research, there are still reservations regarding its usage in specific industries, particularly the drug industry for humans, animal production industry, and animal doping industry. Currently, xylazine is not listed as a controlled substance by the Drug Enforcement Administration (DEA) in the United States (33). In Australia (34) and Canada (35), xylazine is also not enlisted as a controlled substance. Therefore, pharmacist could dispense this sedative if a prescription is provided. In South-East Asia, xylazine is categorized as a controlled substance in Thailand (36). It is scheduled as a poison in Malaysia (37) and Singapore (38) where this sedative can only be dispensed by a licensed pharmacist in both countries.

At the early stage of xylazine development, it was tested in different species, including humans, as a potential sedative-hypnotic, analgesic, and anesthetic drug. However, due to its central nervous system depressant effects, the FDA banned its use by humans (7). The uncontrolled intake of xylazine could cause hazardous side effects and even death. Several reports associated with xylazine misuse by humans were reviewed by Fyffe (39) and Ruiz-Colyn et al. (3). The unconventional use of xylazine has also been reported in food-producing animals (40) as well as sport animals (41).

## Xylazine in Accidental and Intended Poisoning

Of forensic importance, accidental and intended poisoning cases owing to xylazine and its related drugs have been reported (3,39). Fyffe (39) described

ten cases of xylazine intoxication that occurred from 1979 to 1988. Seven cases were related to intended xylazine intoxication, one was related to accidental intoxication, and two were anecdotal intoxication cases. Subsequently, Ruiz-Colyn et al (3) assessed 43 xylazine intoxication cases reported over 35 years up to 2013. Approximately two-thirds of the cases reviewed were either accidental intoxication or suicidal cases (3). In recent years, two intended xylazine poisoning cases (7,42) and an accidental xylazine intoxication case (43) were reported in the literature.

### Recreational drug

Xylazine, though not as popular as other drugs employed recreationally, has been reported in the literature. Xylazine is either inhaled or snorted (44) and is used in combination with other medications (3) to give the extended effects desired by users, which comes at the risk of multiple concomitant adverse effects (45).

Xylazine was first documented in 2000s as a recreational drug or adulterant to other drugs in Puerto Rico, commonly addressed by local as “Anestesia de Caballo” (46). In a survey conducted between 2007 and 2013 in Puerto Rico (San Juan, Rio Piedras, Ponce), 73.4% out of the 451 drugs users was found to use xylazine together with heroin (47). Rodríguez et al. (48) detected xylazine in approximately 40% of syringes collected from drug users from Puerto Rico (San Juan, Mayaguez, and Aguadilla), with a high prevalence rate of xylazine abuse especially in areas involving cattle farming due to ease of accessibility. In another demographic study occurred at Puerto Rico, more than 80% of 89 recruited drug users claimed to use xylazine recreationally via injection (80.7%), sniffing (14.2%), and smoking (1.4%) (49).

Xylazine was reported with its misuses in Texas, Philadelphia, New York, Washington, Kentucky, and Vermont of the United States (10,13–15,50). A total of 76 xylazine exposure cases were reported to Texas Poison Center between 2000 and 2014 (10). Polydrug samples seized in Philadelphia, Pennsylvania showed that xylazine was detected, increasing from 5% in 2015 to 25% in 2019 (13). In New York, 2.2% of the 357 syringes collected in conjunction to syringe exchange program between March and June 2017 were detected with xylazine (15). Meanwhile, residual xylazine was detected in 6.6% from a total of 1187 syringes collected from a needle exchange program in Washington, District of Columbia from September 2020 to May 2021 (50). 1.5% of the total 515 seized drugs from Kentucky and Vermont was also tested with the presence of xylazine (14).

Apart from the United States, xylazine was also detected in drug checking service of Canada (11,12). For instances, four of the 1714 samples submitted to drug checking center at Vancouver and Surrey were

detected with xylazine (June to November 2018) (11). In Toronto’s Drug Checking Service, xylazine was first detected in 7.2% of 1073 samples from September 2020 to February 2021 (12).

As xylazine was not permitted for human use, the effects and health consequences of xylazine administration could not be fully understood. Xylazine users were prone to skin ulcers and human immunodeficiency virus (HIV) behaviors (47,49). The development of skin ulcers could be due to skin oxygenation delayed wound healing and thus, an increased risk of infection (49). Furthermore, approximately one-fifth of xylazine users overdosed while approximately one-third reported signs of growing injection frequency (49). One significant concern was the relatively low health state, including increased fatality in the users as compared to those that took other drugs without consuming them with xylazine (48). An increase of overdose deaths with xylazine detected was evident where the number of overdose deaths with xylazine detected had been increased from 40 cases (2010 to 2015) to 262 cases (2019) in Philadelphia (13). In Connecticut, the number of xylazine-involved deaths was reported at 71 cases (2019) and 141 cases (2020) (51). Up to February 2021, 35 xylazine-involved deaths have been identified in Connecticut (51). Therefore, the recreational use of xylazine could seriously deteriorate the well-being of its users, and thus deserves the attention of the health and law enforcement authorities.

### Adulterant to Other Drugs

Various adulterants and diluents are found in street drugs; they are either used in economical-motivated adulteration to increase profit or more commonly in product-property adulteration to enhance the desire effects or reduce the unwanted effects (52). Adulterants are often active pharmacological substances which may mimic the effects of the base drug (53). In most instances, they are usually costly and cannot be easily acquired on the market. On the other hand, diluents are mixed to bulk the base drug and are often cheaper and readily available (54). A distinctive characteristic of adulterant and diluent is that the likelihood of adulterants to impose a toxic effect on drug users are higher than that of diluents (14). Table I summarizes drugs that have been abused, adulterated, used concomitantly, or detected concurrently with xylazine in toxicological analyses.

Heroin and fentanyl are the most reported illicit drugs adulterated with xylazine. “Tranq dope” is referred by Philadelphian as heroin or fentanyl that adulterated with xylazine (13). According to the literature, heroin might be consumed with xylazine because of their synergistic pharmacological effects (3). A semi-structured interview reported that drug addicts who took xylazine and heroin together experienced a “high” sensation that could not result from consuming either drug alone (46). However, this combination was also reported to be potentially life-

**Table I: Reported drugs that co-present with xylazine**

Classification	Drug of abuse	Legal Status	Reference
Analgesic	Heroin	Schedule I (33)	(12–15,46,48,49,55,56)
	Fentanyl	Schedule I (33)	(11–13,55–59)
	Carfentanil	Schedule II (33)	(11,12)
	Codeine	Schedule II (33)	(55)
	Morphine	Schedule II (33)	(55)
	Butorphanol	Schedule IV(33)	(10)
	Methadone	Schedule II (33)	(13,55)
	Phenacetin	Non-medical use	(11,12)
Stimulant	Cocaine	Schedule II (33)	(10,13,46,48,49,55–58)
	Methamphetamine	Schedule II (33)	(11,12,56)
	Methylphenidate	Schedule II (33)	(42)
	Caffeine	Psychoactive	(11)
Anaesthetic	Ketamine	Schedule III (33)	(60,61)
	Combination of Tiletamine & Zolazepam	Schedule III (33)	(10)
	Detomidine	Veterinary Usage	(10)
	Lidocaine	Prescription	(55)
	Procaine	Prescription	(55)
	Phencyclidine	Schedule II (33)	(55)
Barbiturate	Pentobarbital	Schedule II (33)	(10,60)
	Phenytoin	Prescription	(10)
Antipsychotic	Haloperidol	Prescription	(16)
Antimalarial	Quinine	Prescription	(55)
Benzodiazepine	Alprazolam	Schedule (33)	(55)
	Etizolam	Schedule IV (62)	(12)
	Clorazepate	Schedule IV (33)	(10)
Antihistamine	Diphenhydramine	Over-the-counter (OTC)	(55)
	Hydroxyzine	Prescription	(55)
Selective serotonin reuptake inhibitor (SSRI)	Citalopram	Prescription	(55)
Tetracyclic antidepressant	Mirtazapine	Prescription	(55)
Calcium channel blocker	Diltiazem	Prescription	(55)
Fentanyl precursor	4-anilino-N-phenethyl-4-piperidine (4-ANPP)	Schedule II (63)	(12,55)
Non-steroidal anti-inflammatory drug (NSAID)	Ibuprofen	OTC	(55)
	Naproxen	OTC	(55)

threatening (13,55–57).

Stimulants, such as cocaine, were found to be adulterated with xylazine. However, the adulteration of xylazine with other stimulants, such as methamphetamine, is rare. Cocaine is frequently added to a mixture of heroin and xylazine as a speedball (a mix of heroin and cocaine) (48). This combination is believed to be able to neutralize the depressant effects generated by heroin and xylazine (46). Xylazine was also reported to be used together with ketamine, tiletamine and zolazepam, butorphanol, detomidine, pentobarbital, as well as phenytoin (10). Thus, the prevalence of xylazine as a concomitant drug or as an adulterant to other drugs should be studied. Local clandestine laboratories in different regions might have different adulteration practices regarding xylazine. Such information would assist in profiling illicit drugs for forensic intelligence, both domestically and internationally.

### Drug Facilitated Crime and Drug Facilitated Sexual Assault

Drug facilitated crimes (DFCs) involve the incapacitation of victims using drugs (64). One frequently reported DFC is drug-facilitated sexual assault (DFSA) (64) where the victim's drink is spiked. Gamma hydroxybutyrate

(GHB), ketamine, diazepam, and benzodiazepine (65) are common substances associated with DFC; however, xylazine was recently identified (16,17,66,67).

Our literature search revealed that none of the DFC cases involving xylazine was fatal. The perpetrators were only fined for committing a felony crime. A teen had been fined for “placing foreign objects in edibles” and “second degree recklessly endangering safety” in a case where he spiked his stepfather's drink with cow tranquilizer (xylazine) (67). A man was found guilty of spiking the victim's drink with “love potion” containing xylazine together with haloperidol (16), the latter of which is used to treat schizophrenia in humans (68). Drinks spiked with xylazine have been used to render elderly victims' unconscious before a robbery (17). An attempted sexual abuse case of a four-year-old boy, who involved the use of xylazine, was also reported, exemplifying the need for precautionary actions by the law enforcement authorities (66).

### Prohibited Substance in Animal Production Industry

With the higher demand for protein-based products (69), for instance, an estimated 40 kg per person in the United States and Australia in 2018 (70), there is increased motivation to produce high-quality presentable meat.

To avoid pale soft exudative meats produced by animals that are stressed because of transportation to the abattoir, tranquilizers are administered before slaughtering (40). Caution must however be exercised in administering veterinary drugs to food-producing animals as excessive residues could be harmful to human health. The potential effects due to misuse of xylazine in such animals included the development of antimicrobial drug resistance, hypersensitivity reaction, carcinogenicity, mutagenicity, teratogenicity, and disruption of intestinal flora (71).

Xylazine is commonly administered to dog, cat, deer, sheep, goats, and elk (22); however, its use in livestock for human or animal consumption is prohibited in the United States (71,72). Nonetheless, xylazine is allowed for use in livestock under two circumstances, namely for emergency use only and when it carries a withdrawal interval (WDI) twice beyond the requirement by FDA before the products can be consumed by human. They shall comply to both the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) and 21 CFR part 530 (Extralabel drug use in animals) under the FDA regulations. WDI referred to minimum period between the last dose of medical administration and the production of animal-derived products for food (73). The Food Animal Residue Avoidance Databank (FARAD) had suggested a WDI of slaughter and milk at 10 days and 24 hours, respectively following IM administration of xylazine to cattle (74). Meanwhile, the Title 7 of the Code of Federal 89 Regulations (CFR) Section 205.603 recommended that the slaughter and milk shall fulfill WDI of at least 8 days and 4 days, respectively when xylazine is administered to food-producing animals (75).

Xylazine was reported to be administered to food-producing animals (40), and was detected as residues, though not frequently, in the monitoring program in Europe (76,77). Consumption of xylazine-contaminated meats by human might cause unwanted consequences as described by Haight (78). In the case report, two people had developed unpleasant behavior (one felt "high" for a few hours and the other drove over 100 km on the next day and fell asleep during a job interview) after consuming elk sedated with xylazine (78). Such cases highlighted the need to monitor xylazine abuse in meat production.

From human food safety perspective, xylazine is not approved for consumption by food-producing animals (72). First, xylazine could be a potential carcinogen; however, its carcinogenic potential is yet to be assessed completely (72). Second, the metabolites of xylazine, 2,6-dimethylaniline, was reported as carcinogen in an animal study (79). Based on the Federal Law, the use of carcinogenic-potent substance in livestock is prohibited unless it can be proved that the meat of livestock is free from any residual contamination via method of detection approved by FDA. More toxicity studies on

xylazine, covering the carcinogenic, reproductive, and developmental impacts shall be explored to ensure the human food safety.

### **Doping Agent in Animal Sports**

Since ancient times, animals have been exploited by humans as a tool of amusement and a form of entertainment. One of these amusements includes horse racing. Animal racing has been exploited for monetary gain. As a result, doping practices (either doping to win, doping to lose, or doping to mask the effects of other drugs) are sometimes adopted (80). Xylazine is a widely used as pain relief and sedative in horse racing (41). However, it has also been reported as a doping agent used in horses (primarily doping to lose) (80). Positive regulatory results were reported in United States (81), Australia (82) and France (83).

Xylazine is regulated by the Association of Racing Commissioners International (ARCI) as a class 3 foreign substance (84). To ensure fairness of the game and welfare of the competed horse, the horses are not allowed to race under the influence of xylazine (85). Therefore, ARCI has set a withdrawal time of 48 hours with an interim threshold of 0.01 ng/mL in plasma via IV at a dosage of 1.1 mg/kg (81,85,86). Compared to other abusive drugs, limited studies were found regarding the illegitimate application of xylazine for doping practice.

### **ABUSE OF XYLAZINE: A PROBLEM OF CONCERN**

As with many other drugs, the misuse of xylazine is not a rare occurrence. Such misuse has occurred over the past years, mainly with its use as a suicidal agent, and recently, as a recreational drug and an adulterant for drugs and component used in DFC. In this section, several issues regarding the abusive potential of xylazine, especially as an emerging recreational drug, are highlighted.

#### **Unknown Prevalence of Xylazine Misuse as a Recreational Drug and Adulterant**

Although some studies have reported the use of xylazine as a recreational drug; in general, the available information is not extensive and is limited to certain regions or specific incidents. As a result, the prevalence of its abuse in other areas remains unambiguous. The presence of xylazine, especially in seized drugs, was seldom reported. Laboratories often only report the controlled substance regulated by law (14), especially when their standard operating procedure is designed to detect only the analytes of interest. Therefore, the prevalence of this drug remains unknown.

Investigating the prevalence of xylazine abuse is crucial as it could help to generate accurate data for devising more efficient drug policies, such as a stricter control scheme. Although xylazine is regulated as a controlled substance (Thai law) or a poison (Malaysia Poison Act), enforcement on its possession and use still



requires improvement. A devised drug policy based on local need is required to combat various crimes of drug-related smuggling, trafficking, DFC, DFSA, as well as street crimes such as petty theft and robbery. Global commitment and mutual agreement to collect information on the prevalence of this drug should be initiated for forensic intelligence as this can increase the awareness of law enforcement authorities and governmental agencies, in this case, on the potential abuse of xylazine.

### **Regulatory Status of Xylazine**

Owing to the unknown prevalence of xylazine worldwide, a more systematic data collection of its use or abuse in various scenarios should be initiated before revising its regulatory status. Generally, xylazine is approved for veterinary use, specifically for non-food producing animals and is available in pharmacies through prescription by licensed physician in most countries. Accessibility to xylazine is one of the risk factors contributing to the increase in its abuse (48). As xylazine can cause another “ketamine scenario”, policy makers should consider listing xylazine as a controlled substance in their respective country. Prevalence studies revealing the severity of its abuse should also be emphasized.

### **Availability of a Screening Assay to Detect Xylazine and Other Drugs**

The ability to perform a quick screening for xylazine is essential when a prompt clinical decision needs to be made on the type of emergency treatments to be administered for the suspected intoxication. Such screening narrows down the possible identity of drugs in toxicological cases and assists a forensic chemist in the narcotics department to identify the potential drugs in the seized evidence. Routinely, immunoassay and color tests are widely employed as the screening tests. In an emergency setting, the chromatographic technique is rarely used as it is time-consuming (87). The immunoassay is thus preferred as it enables rapid analysis in intoxication cases involving drugs from biological samples. The color test is commonly used as a presumptive test for seized drugs and is based on color change.

To date, no immunoassays and color tests have been discovered to concurrently screen for the presence of xylazine and other recreational drugs in the forensic settings (40). However, a radioreceptor assay was developed to simultaneously detect xylazine and detomidine (88). Unlike the immunoassay, the radioreceptor assay involves a rivalry between analytes and labelled ligands to bind to receptors. Extracted xylazine or detomidine must compete with tritiated clonidine to bind to  $\alpha_2$ -adrenoceptors in rat brain homogenates. Based on this assay, the detection limit of xylazine could be up to 2.5 ng/mL; this assay was found to be useful in pharmacokinetic studies of  $\alpha_2$ -agonists in

various animals (88).

Radioreceptor assay would be useful; however, such instrument is not available in most medical and forensic laboratories. As the number of substance abuse differs from time to time, and the number may be prone to increase, it is crucial to develop a rapid screening test applicable for xylazine and a wide range of drugs. Recently, Fiorentin et al. (89) developed and validated a screening method using FLIR Griffin™ G510 portable gas chromatograph mass spectrometer (GC-MS), targeting 24 drugs of abuse, including xylazine. Based on their findings, the limit of detection for xylazine was 40  $\mu\text{g/mL}$  (89). To date, there is still limited screening tests available for detecting xylazine alone or with other drugs of abuse. Thus, discovering an appropriate strategy that not only focuses on the development of immunoassay and color test, but also portable and mini size detection kits for field settings, is warranted.

### **Availability of a Validated Analytical Method for the Detection of Xylazine and Other Drugs**

For forensic purposes, a two-tier analysis is preferable as a second analysis can complement the first screening analysis for quick identification. A confirmatory assay should be well-equipped with high sensitivity and high specificity as an analytical technique. High sensitivity is important whenever trace analysis is required, mostly in caseworks related to DFC and DFSA. Meanwhile, specificity is required when a chemist is needed to identify a drug in a severely contaminated matrix, such as blood or a spiked drink. Nonetheless, both immunoassay and the color test might lack both qualities.

Several validated methods have been reported for the detection of xylazine, mainly in veterinary toxicology, emphasizing veterinary residue monitoring in meat products to ensure consumer safety (90–92). However, validated methods that can be readily utilized in forensic narcotics and forensic toxicology to combat crimes are less likely to be reported. Table II describes the analytical techniques successfully applied in forensic caseworks for the detection of xylazine alone or with other drugs, as well as the xylazine metabolite, 2,6-dimethylaniline (DMA).

Barroso et al. (93) performed a toxicology analysis using solid-phase microextraction (SPE) coupled with GC-MS to detect xylazine that peaked at 5.91 min in human blood. Meanwhile, Ruiz-Colyn et al. (57) developed an ultra-high-performance liquid chromatography (UPLC-MS-MS) method to simultaneously detect xylazine, free morphine, codeine, 6-acetylmorphine, cocaine, and benzoylecgonine in human blood; this method was characterized by minimal sample preparation, small sample volume (0.25 mL of blood sample), and short separation time (2.5 min). Although the method had advantages for detecting xylazine, its advanced and sophisticated instrumentation would not always be

**Table II: Analytical methods applied in forensic samples to detect xylazine, its metabolites and/or other drugs**

Analytical Techniques	Analytes of Interest	Sample	Sample Preparation	LOD of xylazine	Recovery of xylazine	Working range	Reference
GC-MS	Xylazine	SPE	Human blood	2 ng/mL	83.8 ± 9.14% to 94 ± 5.4%	0.01 - 3.5 µg/mL	(93)
UPLC-MS/MS	Xylazine, free morphine, Codeine, 6-AM, cocaine and benzoylecgonine	Protein precipitation	Human blood	1 ng/mL	110.1% (Low concentration) and 102.5% (High concentration)	10 - 1000 ng/mL	(57)
UHPLC-QTOF/MS	Xylazine and DMA	SPE	Blood	0.2 ng/mL	80.1% to 83.3%	2 - 1000 ng/mL	(94)
LC-MS/MS	Xylazine and DMA	Protein and phospholipid removal, SPE and supported liquid extraction (SLE)	Blood	0.2 ng/mL	101.2 ± 6.5% to 112.5 ± 4.7%	2 - 1000 ng/mL	(95)
			Urine	0.4 ng/mL	96.9 ± 6.2% to 117.4 ± 4.3%		
Voltammetry (DPV technique)	Xylazine	Protein precipitation	Human serum	42 nM	98.92% to 100.57%	13.3 - 320.0 ng/mL	(96)
			Pharmaceutical xylazine formulation	42 nM	100.08 ± 0.74% to 100.16 ± 0.52%		
Voltammetry (DPV technique)	Xylazine	No sample treatment	Synthetic urine	27 µg/L	96 ± 4%	0.5 - 256 µmol/L	(97)
Voltammetry (AdSV technique)	Xylazine	No sample treatment	Spiked beverages	0.1 mg/L <sup>-1</sup>	80.08 ± 0.2% to 108.1 ± 0.3%	0.4 - 6.0 mg/L <sup>-1</sup> and 6.0 - 80.0 mg/L <sup>-1</sup>	(98)

available in forensic laboratories worldwide.

Besides the chromatography technique, an electroanalytical method has also been discovered (96–98). Recently, electroanalytical methods have gained the attention of the forensic community as they can be miniaturized, and involve minimal contact with toxic solvents, such as acetonitrile and dichloromethane. El-Shal and Hendawy (97) established an electroanalytical method using a multiwalled carbon nanotube (MWCNT), 1-n-butyl-3-methylpyridinium hexafluorophosphate ion crystal (BMH), sodium dodecyl sulphate (SDS), and the MWCNT-BMH-SDS electrode in a buffer of pH 7 with the differential pulse voltammetry (DPV) technique to quantify levels of xylazine. Mendes et al. (96) developed an electroanalytical method using the DPV technique and a pH of 7 but used a glassy carbon electrode to detect xylazine in synthetic urine. Based on adsorptive stripping voltammetry (AdSV) mean, a portable electrochemical device with a graphene nanoplatelets-modified screen-printed carbon electrode (GNPs/SPCE) as the electrode was also developed and validated to detect xylazine in spiked beverages (98). Notably, spiked beverage is valuable evidence that can be recovered from crime scenes following DFCs (17,99,100). Good sensitivities and recoveries were reported with the methods established using the electrochemical approach (96–98).

As xylazine has a high metabolic rate, some studies proposed the detection of xylazine as well as one of its primary metabolites, DMA. Two methods that have been applied successfully in real cases for the concurrent detection of xylazine and DMA were established by Gao et al. (94,95) Ultra-high-performance liquid chromatography coupled with quadrupole-time of flight mass spectrometry (UHPLC-QTOF) (94) and liquid chromatography-tandem mass spectrometry (LC-MS/

MS) (95) were used to detect xylazine after extraction from blood and urine using different procedures, namely SPE and the protein and phospholipid removal cartridge extraction method, respectively. The latter extraction method was suggested to have greater extraction efficiency, producing better experimental outcomes. The presence of xylazine in animal tissues was also analyzed using LC-MS/MS with a reported LOD at 0.06 µg/kg in animal-derived food products (101).

As highlighted by Solimini et al. (52), a validated analytical method should be established to detect xylazine, which has been proven to cause hazardous effects on humans either when consumed alone or with other illicit drugs, especially heroin and speedball that will increase the fatality rate. More importantly, an analytical method that can simultaneously detect xylazine and one or more of its metabolites in addition to DMA should be established. The method should be applied in forensic toxicology and should be able to recognize the rapid metabolism of xylazine following administration and for delayed sampling after the occurrence of DFC and DFSA (102). Examples of metabolites include N-(2,6-dimethylphenyl) hydroxy-oxo-5,6-dihydro-4H-1,3-thiazin-2-amine and N-(4-hydroxy-2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine. DMA was recognized to not be a well-suited target analyte in xylazine intoxication cases as it could also originate from lidocaine (102).

Our review suggests that the responsible parties within the forensic community should take appropriate steps to establish a more effective analytical method to be utilized by forensic organizations. Many of the developed methods, including those published in the literature, should be tested in real case scenarios to verify their appropriateness and effectiveness. Active research in forensic drug laboratory should involve the

exploration of drug detection, rather than being confined within the standard methods that only analyze the drugs of interest. This is particularly important whenever the identity of seized illicit drug is unknown or is being adulterated with many different substances, as well as in wiped samples collected from the containers of spiked drinks. Dissemination of information and sharing of technology would help to resolve forensic problems in a more effective manner.

## CONCLUSION

This review highlights that a veterinary drug, xylazine, has been misused in several forms: as a recreational drug; as an adulterant to other recreational drugs; as a drug in DFC and DFSA; and as a source of accidental and intended poisoning. As a conventionally used veterinary drug, xylazine is undoubtedly a potentially hazardous drug as its consumption could lead to fatalities. This drug can also be abused. More prevalence studies on xylazine and other common illicit drugs should be carried out using accurate and validated analytical techniques. Such studies would enable the establishment of a relevant and useful drug policy for crime prevention and control. Additionally, there is a need to derive an analytical method as well as protocols for extraction that can be readily utilized by the forensic science community to detect xylazine. Research has been conducted as a preparation step for another “ketamine scenario”, which could be caused by xylazine. As drug-related crimes are always the primary concern affecting societal well-being worldwide, the authors believe that investigations on the potential abuse of xylazine would enhance routine forensic examination to keep pace with the status of illicit drugs.

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