

Pharmacology and toxicology of xylazine: quid novum?

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Abstract. – The current opioid overdose crisis is characterized by the presence of unknown psychoactive adulterants. Xylazine is an alpha-2 receptor agonist that is not approved for human use but is commonly used in veterinary medicine due to its sedative and muscle-relaxant properties. Cases of human intoxication due to accidental or voluntary use have been reported since the 1980s. However, reports of adulteration of illicit opioids (heroin and illicit fentanyl) with xylazine have been increasing all over Western countries. In humans, xylazine causes respiratory depression, bradycardia, and hypotension-posing individuals, using xylazine-adulterated opioids. We present a narrative review of the latest intoxication cases related to xylazine, to bring awareness to readers and also to help pathologists to detect and deal with xylazine cases.

Key Words:

Xylazine, Cut agent, Adulterant, Analytical methods.

Introduction

Xylazine (5,6-dihydro-2-(2,6-xylidino)-4H-1,3-thiazine) was first synthesized in Germany in 1962 as an antihypertensive drug¹. It is also known as Rompun[®], Anased[®], Sedazine[®], and Chanazin^{®2}. It shares some pharmacological properties with a number of structurally related drugs, including phenothiazines, tricyclic antidepressants, and clonidine³. Xylazine acts by stimulating alpha-2 receptors in the central and peripheral nervous systems⁴. As a result, the brain's stern vasomotor center is inhibited, resulting in bradycardia and transient hypertension, followed by sustained hypotension⁵. This compound can be administered in a variety of ways, including intravenously, intramuscularly, subcutaneously, and orally⁶. Xylazine is rapidly absorbed, metabolized into various intermediates, and broken down into final organic sulfate products in the mammalian

body. The main xylazine biotransformation pathway is most likely thiazine ring breakdown, with 2, 6-dimethylaniline (DMA) as the main product^{7,8}. It is rapidly distributed after intravenous administration, primarily to the central nervous system and kidneys⁹, and is then metabolized in the liver, with up to 70% of the dose excreted in the urine in dogs¹⁰. Xylazine is a commonly used veterinary tranquilizer employed to sedate large animals for antipsychotic purposes, particularly before surgical procedures⁶. Currently, xylazine is a non-narcotic drug used in veterinary medicine as an analgesic, sedative, and muscle relaxant for large animals such as horses, ruminants, and deer. In humans, xylazine was studied as a sedative-hypnotic, analgesic, and anesthetic drug, but it was rejected due to its frequent association with severe hypotension⁵. Xylazine also shows peripheral autonomic effects, reducing centrally mediated sympathetic tone via interneural norepinephrine release, resulting in bradycardia, hypotension, and decreased cardiac output¹¹. This compound has recently been discovered to have an affinity for cholinergic, serotonergic, dopaminergic, alpha-1 adrenergic, H2-histaminergic, and conceivably opiate receptors¹¹.

Several antagonists have been used to treat xylazine intoxication. Tolazoline can be used to treat unresponsive bradycardia and hypotension, but it has also been linked to hypertension, arrhythmias, and tachycardia¹¹. Yohimbine, another alpha-2 antagonist, has been shown in humans to counteract the sedative effects of xylazine¹². Atropine has been used to treat bradycardia and hypotension in humans⁹ and naloxone was administered with no effect¹³. Further testing of the drug revealed central nervous system (CNS) effects which halted its further investigation in humans¹⁴. Xylazine is not currently approved by the US Food and Drug Administration (FDA) for use in humans but is approved for use in a variety of animals (dogs, cats, horses, deer, and elk)¹.

Illicit drugs such as cocaine and heroin are often adulterated with other agents to mimic or enhance the drugs effects¹⁵. Because of the similar pharmacologic effects, synergistic effects can occur when xylazine is used with heroin resulting in an increase in fatal cases. According to the literature, the dosages of xylazine known to produce toxicity and fatality in humans vary from 40 to 2,400 mg¹², with postmortem blood concentrations of xylazine ranging from trace to 16 mg/L and non-fatal blood or plasma concentrations ranging from 0.54 to 4.6 mg/L². Thangada et al¹⁶ and Nunez et al¹⁷ described an outbreak of overdose deaths involving xylazine, usually mixed with fentanyl. When used as an adulterant, it causes bradycardia, central nervous system depression, respiratory depression, and severe hypotension in humans, and chronic use has been linked to physical deterioration. Furthermore, literature reports that for many fatalities caused by overdose, adulterants played a determinant role in the cause of death¹⁸. In Puerto Rico, xylazine has been found as an adulterant of heroin in street drugs since 2000¹⁹. It is also reported that xylazine is used as a drug of abuse itself or a drug administered for criminal intent such as sexual assault, robbery, or homicidal intent.

A review² was previously published regarding xylazine effects after its administration. In that study, Ball et al² presented the most common types of xylazine exposures, reporting that the majority were unintentional and intentional misuse or abuse. The authors also discussed common symptoms after xylazine consumption. We aim to present a comprehensive narrative review of the literature regarding fatal and non-fatal cases after xylazine consumption. Literature that identified and quantified xylazine in conventional and non-conventional biological matrices is also presented in light of the most recent xylazine intoxication cases and new analytical methods.

Methods

A literature search was performed on PubMed and the following keywords were included: “Xylazine” (3,547), “Xylazine and Adulterant” (14), “Xylazine and Cut Agent” (1), “Xylazine and Cut” (12), “Xylazine and Human” (361). Further research manuscripts were retrieved through the reference lists of selected articles and from reports and websites of international drug agencies. Six of the co-authors screened all articles

independently to determine their relevance to the present review and were included if at least two co-authors selected the article. Only papers published in the English language were reviewed.

Reported Cases Related to Xylazine Consumption

A total of 48 fatal cases related to xylazine consumption have been reported over the past years^{1,3,5,7,9,11,13,10-38}. **Supplementary Table I** outlines the gender, age, route of administration, symptoms, case description, and concentration found for each xylazine-related case report.

Of all the reported cases (48), twenty-five^{3,5,7,9,11,13,21-23,25-28,30,31,33,35,39} were non-fatal scenarios that required medical support, and twenty-three^{1,20,24,29,34,36,38} resulted in fatalities (Figure 1).

Of the 23 deceased, two²⁰ were homicides, five^{9,11,13,24,38} were suicides (1 by hanging and 4 by xenobiotic intoxication), and nineteen^{1,5,7,13,21-23,25-31,33-36,39} were recreational drug overdoses. In seventeen^{1,3,29,30,33-36,38,39} of 19 cases, xylazine was used as an adulterant in association with fentanyl (7 cases), morphine (13 cases), cocaine (7 cases), benzoylcegonine (9 cases), methadone (1 case), alprazolam (1 case), codeine (4 cases), 6-acetylmorphine (6 cases). Figure 2 shows the total number of cases where xylazine was consumed with the different drugs of abuse mentioned above.

Parent drugs and respective metabolites are described separately since the information available is related to biological samples. It is the case of cocaine and morphine and their main metabolite: benzoylcegonine and 6-acetyl morphine, respectively.

The non-fatal cases resulted in intoxication with the following symptoms: bradycardia, hypertension, hypotension, central nervous system depression, drowsiness, loss of consciousness, disorientation, ataxia, dysarthria, respiratory depression, miosis, and hyperglycemia. A case of ulcers in the lower limbs and a case of withdrawal symptoms due to chronic use of xylazine are reported. The intoxications occurred accidentally in 8 cases, in 3 cases of suicidal attempt, in 3 cases of self-medication, in 3 cases of administration with criminal intent, and in 8 cases during an assumption with recreational purpose. Among the cases of intoxication, when xylazine was taken for recreational purposes, only one case reported a co-assumption of opioids. In one case, it was administered with paroxetine,

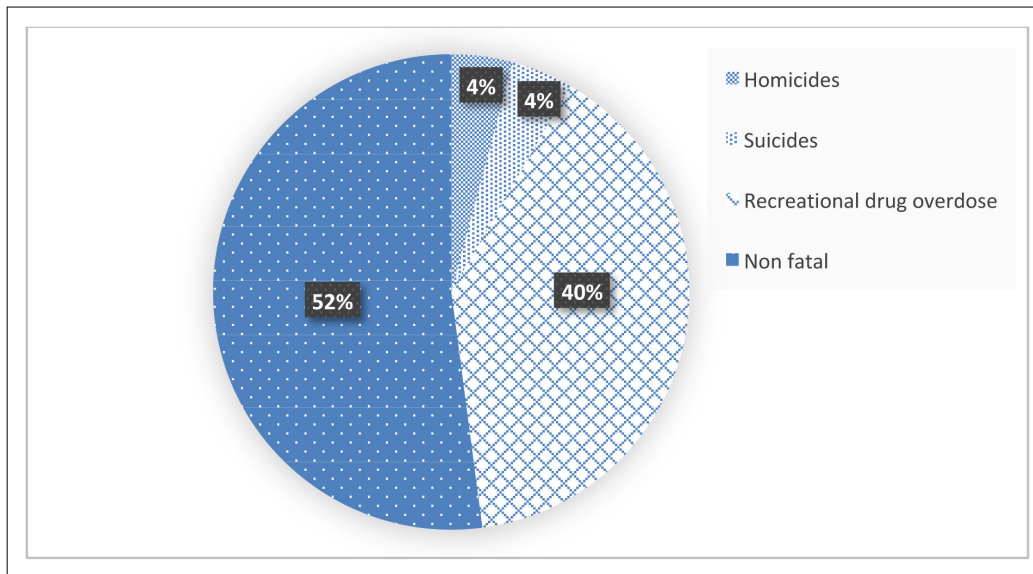


Figure 1. Percentages of fatal and non-fatal cases related to xylazine consumption assessed in biological fluids (years 1981-2023, n=48 total cases).

in one case with ketamine, and in one case with ketamine and phenobarbital, but also consumption of xylazine alone was reported. Even if the reported cases of intoxication have always required medical intervention, it is evident that the greatest danger of xylazine is when this sub-

stance is associated with opioids and fentanyl. According to the literature, the trend of recent years highlights that while the older articles report accidental or recreational intoxications that occurred only with the use of xylazine, the latest articles report intoxications that occurred with

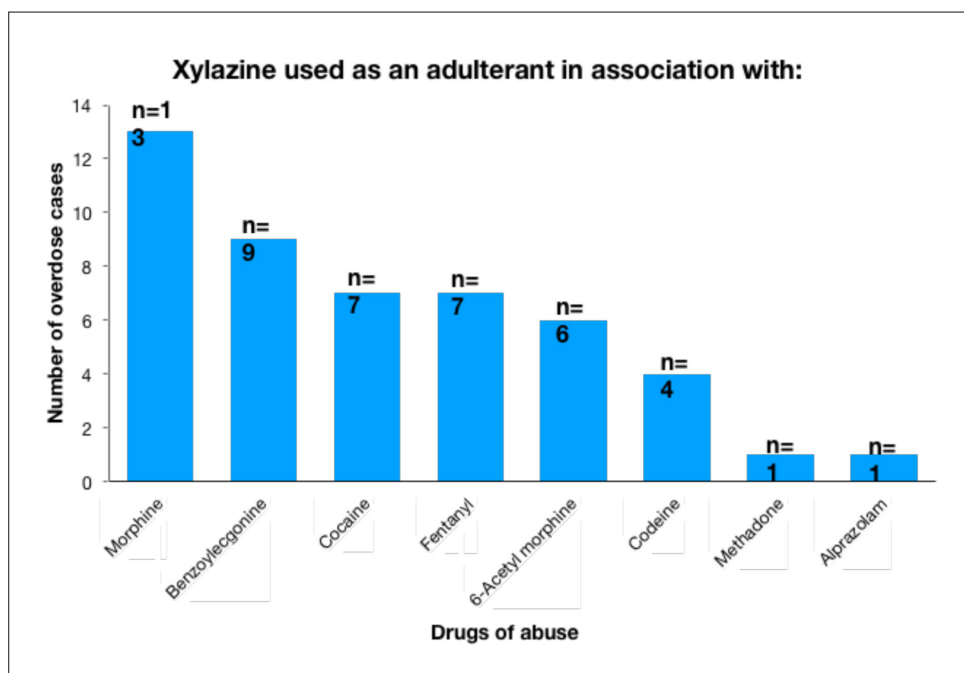


Figure 2. Number of overdose cases where xylazine was found as cutting agent for different drugs of abuse (years 1981- 2023).

xylazine used as an adulterant, in association with opioids and cocaine. This trend also emerges from several published aggregated data³⁹ concerning the xylazine issue in Canada, Porto Rico, and the United States. Between January and December 2019, among the 45,676 overdose deaths reported to the “State Unintentional Drug Overdose Reporting System” (SUDORS), 826 (1.8%) xylazine-positive, and 531 (1.2%) xylazine-involved deaths were identified in 25 and 23 states, respectively. Xylazine was listed as a cause of death in 64.3% of deaths in which it was detected, and, among all xylazine-involved deaths, one or more other drugs were also listed as a cause of death, and 98.7% of xylazine-positive deaths and 99.1% of xylazine-involved deaths had fentanyl (including analogs) listed as a cause of death. Cocaine and heroin were listed as a cause of death in 32.1% and 26.0% of xylazine-positive deaths, respectively, and in 29.6% and 28.4% of xylazine-involved deaths, respectively. Moreover, in 2019, a total of 1,200 deaths from unintentional drug overdoses were reported¹⁶ in Connecticut, with 70 (5.8%) decedents positive for xylazine. During the period January-July 2020, 666 deaths from drug overdoses were reported¹⁶ in Connecticut, with 76 (11.4%) positive for xylazine. Among 146 xylazine-positive deaths during 2019 and 2020, test results for all but one (99.3%) were positive for fentanyl. Toxicology analysis revealed that 85.6% of xylazine-fentanyl deaths included other substances: cocaine, heroin, benzodiazepines, ethanol, and gabapentin. In Cook County, the Cook County Medical Examiner’s Office and Cook County Health analyzed⁴⁰ suspected substance-related deaths from January 2017 to October 2021 for the presence of xylazine and co-occurring substances. Xylazine testing became standard in Cook County for suspected drug overdose deaths. A total of 236 xylazine-associated deaths were reported⁴⁰ during the study period and xylazine-associated deaths increased throughout the study period. Fentanyl or fentanyl analogs were detected on forensic testing in most xylazine-involved deaths (99.2%). Other common co-occurring substances included diphenhydramine, cocaine, quinine, and naloxone.

This review, in agreement with what has been found in the literature, highlights that most recent fatalities due to xylazine are associated with illicit drugs, particularly fentanyl and its analogs. The danger of xylazine results in the synergistic effect it exerts in association with opioids since both substances result in an inhibition of the

central nervous system. Pursuing new research and raising awareness of xylazine as a cutting substance can be helpful in guiding overdose prevention and helping clinicians in the treatment of overdoses involving xylazine. Currently, a specific antidote is not available for xylazine, but naloxone is still indicated in suspected cases of potentially fatal overdose because most cases co-occur with opioids. Cardiovascular and respiratory support is fundamental to the management of serious xylazine toxicity. Healthcare providers should know that cases of suspected overdose involving opioids, that are refractory to naloxone administration, could involve xylazine toxicity.

Estimates of xylazine detection in overdose deaths might be underestimated. More articles regarding cases of poisoning related to xylazine should be published, and is necessary to expand postmortem testing for xylazine and co-occurring substances (as in Cook County) to better define the role of xylazine in opioid-related deaths.

Analytical Methods with Biological Matrices

Over the years, not a lot of analytical method-regarding xylazine detection have been developed. Perhaps because xylazine’s use has recently raised interest in adulteration purposes. This could imply that there is a greater interest in detecting this substance now that new intoxication cases are being reported. This review includes analytical methods developed in the last two decades (see Table I). Studies without information regarding analytical data were not considered nor included in this table, since the aim was to analyze and discuss different analytical parameters from different authors’ approaches. Further information regarding method validation was not included because it was only available from a few studies. Nevertheless, validation parameters such as recovery, accuracy, precision, limits of detection (LOD), and limit of quantification (LOQ) were described when available. From a total of 19 cases (see Table I), seven used urine as a matrix of choice^{6,11,25,33,41,42,43}, followed by blood^{1,3,5}, serum^{25,42}, and gastric fluid²⁵. All analytical methods included chromatographic separations and mass spectrometry detection. Gas chromatography coupled to mass spectrometry (GC-MS)^{3,5,11,25,33,34,41,42}, liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS)^{11,25}, and ultra-high performance liquid chromatography coupled to Quadrupole Time of Flight mass spectrometer (UHPLC-QTOF)⁶ analytical methods are most common for forensic

Table I. Analytical methods to determine xylazine in conventional and non-conventional biological matrices.

Sample	Sample preparation	Method	Mobile phase	Linear range	LOD (ng/mL) ($\mu\text{g/mL}$)	LOQ (ng/mL)	Concentration ($\mu\text{g/mL}$)	References
U P	SPE, Evaporate, Resuspend LLE; Centrifuge; Evaporate; Resuspend Resuspend	GC-MS LC-MS/MS	Helium A: Triethylammonium phosphate 0.02 M pH3; B: Acetonitrile	NA 25-800	NA 5	NA 25	20 $\mu\text{g/L}$ NA	Olmos-Carmona et al ⁴¹ Hoffmann et al ¹¹
U GF	Dilute Dilute	GC-MS GC-MS	Helium Helium	NA NA	NA NA	NA NA	NA NA	Hoffmann et al ¹¹ Hoffmann et al ¹¹
B	Centrifuge; LLE; Evaporate; Resuspend	GC-MS	Helium	0.250-1	NA	NA	570	Stillwel ¹³
B	Dilute; Centrifuge; SPE; Evaporate; Resuspend	GC-MSD (mass selective detector)	Helium	0.010-3.5	2	10	1,500	Barroso et al ⁵
U B	SPE NA	EI GC-MS EI GC-MS	Helium Helium	0.050-5 NA	NA NA	NA NA	NA NA	Wong et al ³⁴ Wong et al ³⁴
B	PP; SPE; Evaporate; Resuspend	UHPLC-MS/MS	A: Water with 0.1% FA; B: Acetonitrile with 0.1% formic acid	10-1,000	1	10	461	Ruiz-Colón et al ¹
P	Hydrolysis; LLE; Microwave- assisted acetylation	GC-MS	Helium	0.100-1	NA	NA	300	Meyer et al ³³
U	Hydrolysis; LLE; Microwave- assisted acetylation	GC-MS	Helium	0.100-1	NA	NA	300	Meyer et al ³³
U	PP; LLE; Microwave-assisted acetylation	LC-MS coupled to TF LXQ	A: 50 mM ammonium B: Acetonitrile with 1 mL/L formic acid formate pH3;	0.100-1	NA	NA	300	Meyer et al ³³
B	SPE; Evaporation; Resuspend	UHPLC-QTOF	A: Water: 0.1% FA B: Acetonitrile	0.002-1	0.2	0.6	NA	Gao et al ⁶
U	SPE; Evaporation; Resuspend	UHPLC-QTOF	A: Water: 0.1% formic acid B: Acetonitrile	0.002-1	0.4	1	95	Gao et al ⁶
S	LLE	GC-MS	Helium	50-1,000	0.006	0.017	53	Andresen-Streichert et al ⁴²
U	LLE	GC-MS	Helium	NA	NA	NA	630	Andresen-Streichert et al ⁴²
S	SPE; Evaporate; Resuspend	LC-MS/MS	A: 5 mM ammonium acetate with 0.1% FA; B: Acetonitrile with 0.1% formic acid	0.010-0.750	1	10	57	Ramon et al ²⁵
U	SPE; Evaporate; Resuspend	LC-MS/MS	A: 5 mM ammonium FA acetate with 0.1% B: Acetonitrile with 0.1% FA;	0.010-0.751	NA	NA	294	Ramon et al ²⁵
GF	SPE; Evaporate; Resuspend	GC-MS	Helium	NA	NA	NA	NA	Ramon et al ²⁵

U, urine; B, blood; P, plasma, S, serum, GF, gastric fluid, NA, not available; SPE, solid phase extraction; LLE, liquid-liquid extraction; PP, protein precipitation; GC-MS, gas chromatography coupled to mass spectrometry; LC-MS/MS, liquid chromatography coupled to mass spectrometry in tandem; GC-MSD gas chromatography coupled to mass selective detection; EI GC-MS, gas chromatography with cold electron ionization; UHPLC-MS/MS, ultra-high performance liquid chromatography coupled to mass spectrometry in tandem; LC-MS TF LXQ, liquid chromatography and mass spectrometry with linear ion trap; UHPLC-QTOF, ultra-high performance liquid chromatography coupled to Quadrupole Time of Flight mass spectrometer.

toxicology purposes. When dealing with urine samples, various approaches can be taken. GC-MS was used by both Meyer et al³³ and Andersen-Streichert et al⁴² to detect xylazine. The first author³³ used a sample volume of 500 μL and liquid-liquid extraction (LLE) as an extraction technique, whereas the second author⁴² used a sample volume ten times larger and an additional hydrolysis step during sample preparation. There is no data on LOD or LOQ, but a concentration of 0.630 $\mu\text{g}/\text{mL}$ xylazine was discovered. Meyer et al³³ also used LC-MS/MS to investigate protein precipitation (PP) before LLE with only 100 μL urine. Again, no data on the LOD or LOQ achieved is available, but the authors were able to detect xylazine at a concentration of 0.3 $\mu\text{g}/\text{mL}$. The authors^{25,43} also used LC-MS/MS as a chromatographic instrument of choice. Korn et al⁴³ used a simple sample preparation using 100 μL urine, reaching a LOD and LOQ of 10 ng/mL and detecting a concentration range between samples of 0.010-12 $\mu\text{g}/\text{mL}$ xylazine. The same method presented a linear range of 50 to 500 $\mu\text{g}/\text{mL}$, values of 27% recovery, 6% accuracy, 3.5%, and 5.2% for intra- and inter-day precision and quantifying xylazine with a concentration of 0.010 $\mu\text{g}/\text{mL}$. Krongvorakul et al²⁵ used a small amount of 10 μL sample and extracted it using solid phase extraction (SPE), choosing a calibration range from 0.01 to 0.751 $\mu\text{g}/\text{mL}$, reaching a sample concentration of 0.294 $\mu\text{g}/\text{mL}$ xylazine. Concerning blood samples, Stillwell³ used a 5 mL sample and an LLE technique that allowed quantification of 0.57 $\mu\text{g}/\text{mL}$ xylazine. As for Barroso et al⁵, with 10 times lower sample volume, and SPE reached LOD and LOQ of 2 and 10 ng/mL , respectively. The same authors used a calibration curve ranging from 0.01 to 3.50 $\mu\text{g}/\text{mL}$ with a concentration found for xylazine of 1.5 $\mu\text{g}/\text{mL}$. Both authors^{3,5} used gas chromatography coupled to mass spectrometry in tandem (GC-MS/MS) as chromatographic separation. Ruiz-Colón et al¹ used 250 μL blood and extracted it with PP and SPE reaching a LOD of 1 ng/mL and a LOQ of 10 ng/mL . This last analysis¹ was performed in ultra-high performance liquid chromatography coupled to mass spectrometry in tandem (UHPLC-MS/MS), quantifying 0.461 $\mu\text{g}/\text{mL}$ xylazine. Serum samples were also tested for the presence of xylazine. For example, Andresen-Streichert et al⁴² used a 500 μL sample and LLE with further GC-MS analysis, detecting 0.053 $\mu\text{g}/\text{mL}$ xylazine. Krongvorakul et al²⁵ used a small amount of 10 μL and SPE, reaching a LOD of 0.001 $\mu\text{g}/$

mL and LOQ of 0.010 $\mu\text{g}/\text{mL}$ using LC-MS/MS being able to detect 0.057 $\mu\text{g}/\text{mL}$ xylazine in a real sample. Only one case was reported using plasma samples¹¹, where authors used 1 mL sample and LLE as extraction technique, reaching a LOD and LOQ of 5 and 25 ng/mL , respectively. Furthermore, just one case with gastric fluid, where Krongvorakul et al²⁵ use only 2 μL sample and extraction was performed with an SPE technique and analyzed with an LC-MS/MS. No information regarding LOD or LOQ was found.

Overall, urine was the most used sample to determine xylazine. In fact, this matrix is a great biological specimen for determining a different class of drugs, and it is a well-known and widely used matrix in toxicological analysis, workplace drug testing, and road safety. It is usually abundant, simple to obtain, and allows for more easily performed drug screening (when compared to blood testing). SPE and LLE are the most chosen techniques to analyze xylazine^{1,3,5,6,11,25,33,34,41,42}. More rigorous sample preparation like these ones can reduce background noise and improve detectability. Authors use PP as an additional preparation step when dealing with blood and urine samples³³. In fact, PP is a simple sample preparation method that provides adequate extraction efficiency while simplifying extraction and preparation time. Other authors³³ used hydrolysis as sample preparation for urine and plasma, where they applied a technique from a previous study concerning new cathinone-derived designer drugs' metabolism. When using liquid chromatography, choosing a buffer as a component of the mobile phases can help to maintain optimal conditions to elute the compounds properly and it was used by some of the authors^{11,25,33}. Low LOD is key for identifying this compound. LOD values, when described, range from 0.001 to 10 ng/mL , whereas LOQ values go from 0.010 to 25 ng/mL . Overall, only 9 presented the concentration found for xylazine from the total number of cases. When present, case reports show concentrations for xylazine ranging from 0.053 to 1.5 $\mu\text{g}/\text{mL}$. Another factor to consider is when these methods detected other drugs alongside xylazine, indicating the possibility of xylazine and other compounds' policonsumption, such as paroxetine³, ketamine³³, fentanyl³⁴, heroin³⁴, morphine, 6-acetyl morphine, codeine, cocaine, benzodiazepine¹, and others⁴⁴. According to the literature, xylazine dosages known to cause toxicity and death in humans range from 40 to 2,400 mg ²⁰, with postmortem blood concentrations ranging from

trace to 16 µg/mL, and non-fatal blood or plasma concentrations ranging from 0.54 to 4.6 µg/mL¹. As a result, there is a postmortem concentration overlap between fatal and non-fatal cases, implying that there is no defined safe, toxic, or fatal concentration. Furthermore, interpreting results becomes even more difficult when xylazine is present in combination with other abused drugs. More research is needed to determine the relationship between xylazine blood concentrations and the presence of other drugs. These measurements will be critical in determining xylazine's role in the cause of death.

Ultimately, synthetic compound polysubstance use has been linked to severe exacerbations of the US overdose crisis⁴⁵. Xylazine is a veterinary tranquilizer that is becoming more common in overdose deaths due to the proliferation of illicitly manufactured fentanyl. Nonetheless, xylazine is not routinely tested in many jurisdictions and is not thoroughly tracked on a national scale⁴⁶. The appearance of xylazine in the illicit drug supply introduces a slew of unknowns and potential hazards for drug users. The public health system must respond by increasing testing to determine the prevalence of xylazine, identifying its potential toxicity at different levels of exposure, and implementing protective measures to avoid harm. Authors⁴⁶ have discussed the growing threat posed by new psychoactive substances, as well as the dynamics and developments that have led to their spread. This includes the risk of new adulteration practices, such as fentanyl and its analogs, posing a serious health risk due to increased toxicity. Other authors^{47,48} provided a broader context for screening and detection techniques, as well as perspectives and potential proposals to improve their efficacy. Certainly, there is currently little testing capable of detecting xylazine in drug supplies, limiting public health intervention, harm reduction strategies, or the development of novel treatment strategies.

Conclusions

Xylazine is a non-approved alpha-2 receptor agonist used in veterinary medicine for its sedative and muscle-relaxant properties. Reports of xylazine adulteration of illicit opioids (heroin and fentanyl) have been increasing, putting them at risk of fatal overdose. Because of limited clinical experience with xylazine, it becomes crucial to identify the most common signs and symptoms of xylazine exposure and treat-

ments used in human cases. Analytical methods able to detect and quantify xylazine in different biological matrices have also been reported in the literature and were described point by point. To help clinicians to better understand how xylazine consumption over the years, this review presents the latest case reports, where symptoms, and concentrations.

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Authors' Contributions

SM, FP, and FPB conceived the design of the manuscript. SM, FP, MP, AK, and PB performed the literature search, and FPB revised it. All the authors have been involved in drafting the manuscript and revising it critically for important intellectual content, and all of them have given final approval for the version to be published.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

Data Availability

The data supporting this article are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

Not applicable.

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