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Violence against women and drug-facilitated sexual assault (DFSA): A review of the main drugs

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ABSTRACT

Sexual violence is a universal phenomenon without restriction to sex, age, ethnicity or social class that causes devastating effects in the physical and mental health spheres, in the short-term and long-term, such as pregnancy, sexually transmitted infections (STI) and greater susceptibility to psychiatric symptoms, especially depression. Some cases of sexual assault and rape are based on the use of so-called drug-facilitated sexual assault (DFSA), which cause victims' loss of consciousness and inability to defend, making them vulnerable to violence. Thus, this article aimed to review the literature on gender violence and the drugs used to facilitate sexual assault, addressing their mechanism of action and pharmacokinetics, as well as drug detection times in human body and types of forensic identification. It is understood that the knowledge of these drugs and their pharmacological and diagnostic mechanisms should be widely disseminated, especially about sensitivity tests and the time the drug remains in the body, which would validate the promotion of evidence to prove abuse, and, thus, being able to give a promising outcome to cases of aggression, which is extremely beneficial for women.

1. Introduction

It is still difficult to measure the actual number of sexual violence cases, as well as the type and prevalence of the drug used, as there are several factors involved, such as the victim's hesitation in reporting abuse, whether due to embarrassment, lack of support, as well as discouragement, because the blame for the violence is usually attributed to the woman; the time elapsed between the fact and the complaint; the time taken to collect biological material for analysis; and the lack of adequate instrumentation for detection, since the most used drugs for this purpose, according to the literature, have a short half-life, being quickly metabolized and eliminated from the victim's body, without leaving a trace.[1]

Violence against women is a highly complex social phenomenon and serious paradoxes, considering their ethical, cultural, political, religious aspects, as well as in attempts to explain it through the most varied currents and trends. Considered a serious public health problem, recognized by the World Health Organization (WHO), violence against women cannot go unnoticed by any health professional who has contact with victims in their services.[2,3]

Among the various types of violence to which women are exposed just because they are women, sexual violence is the one that most reveals the manifestation of power that marks the hierarchical social relations between the genders.[4] There are several types of sexual violence: rape, attempted rape, indecent assault, obscene acts and harassment, which can occur concomitantly even with other types of physical violence (bodily injury, attempted femicide, mistreatment, and threats).[5]

In this context, sexual violence is a universal phenomenon, in which there is no restriction of sex, age, ethnicity or social class, which has been and still is present in different contexts throughout history. Although it also affects men, women are the main victims, at any stage of their lives. However, women who are still children and adolescents are at greater risk of suffering this type of aggression.

This violence causes devastating effects in the physical and mental spheres, which can be observed in the short-term and long-term. Short-term physical consequences include pregnancy, reproductive tract infections and sexually transmitted infections (STIs). In the long-term, these women may develop gynecological and sexual disorders.

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Besides, women with a history of sexual violence are also at greater risk of developing psychiatric disorders, especially depression, panic, suicide attempts, abuse and drug addiction.[6,7]

Because of the seriousness of this issue, both in the violation of human rights and the physical terms as well as in the psychological and social impact that it causes to victims, sexual violence was, in 1993, defined as a global public health problem recognized by the Pan American Organization of Health (PAHO) and WHO.[2,3]

Regarding the phenomenon of sexual violence against women, several forms of executing it have been used throughout history and are currently gaining ground in this scenario. Among them, there is the use of substances that modify the behavior to facilitate this act, as well as other crimes, such as thefts, homicides, and lightning kidnappings.

Date rape drugs is the most common term used to define these drugs and they are becoming more common, since they are substances that, because their discrete organoleptic characteristics, can be easily placed in drinks or administered directly in the victim's mouth. For this reason, in recent years, drug-facilitated sexual assault (DFSA) has become a growing concern in the health community, although the incidence is difficult to quantify, since victims often take time to report and not all of them receive timely medical care or undergo toxicology tests. Besides, date rape drugs are usually taken with ethanol and can exhibit similar toxicodynamic effects, leading to a misdiagnosis of classic ethanol intoxication.[8–10] In time, in this case, the crime occurs when a person is subjected to non-consensual sexual acts while incapacitated or unconscious due to the effect of ethanol, of medication and/or other intoxicating substance and is therefore prevented from resisting and/or unable to give consent.

Thus, in this article, a literature review was carried out addressing the main date rape drugs and also describing its pharmacological features, such as pharmacokinetics, mechanisms of action, time of detection in the human body and forms of forensic diagnosis.

2. Date rape drugs

Date rape drugs consist of a range of chemical substances that are used to facilitate crimes, such as rape.[8,11,12] In Brazil, this coup is known as "Good night, Cinderella", and it can also be considered as a public health problem, since it causes physical and psychological damage to its victims, in addition to affecting women from all social segments.

According to Society of Forensic Toxicologists guides,[13] the main drugs involved in DFSA are: analgesics (fentanyl, codeine, tramadol); antidepressants (citalopram, fluoxetine, amitriptyline); antihistamines (diphenhydramine and hydroxyzine); some antipsychotics such as clozapine and barbiturates such as phenobarbital. However, the most common drugs are ethanol, benzodiazepines, gamma-hydroxybutyrate (GHB) and ketamine (KET).

The toxicological analysis for the determination of these drugs can be done in several biological matrices, such as urine, plasma, whole blood, oral fluid and hair. In general, in forensic toxicological analyzes, blood is considered the gold standard sample for monitoring drug use. However, it allows the detection of drugs and/or their metabolites for only 24–48 h after the use and the sample collection outside the hospital environment is difficult to perform.[14]

The detection of date rape drugs is complex because these active ingredients are potent, and, therefore, administered in small doses, and they are also easy to be eliminated by the body, which results in low concentrations in biological fluids. Since the victims usually take a long time to make the complaint, when it occurs, the drugs would have already been eliminated from the blood and urine, which are the main analysis matrices.[15] Because of this, proving the use of date rape drugs still represents a major challenge for Forensic Toxicology.

Therefore, the use of oral fluid as an alternative matrix to blood has been growing over the last decades, mainly because it is an easily obtained sample, has a non-invasive and low-cost collection, in addition to having difficulty tampering with the collected material. However, this matrix needs to be collected as soon as possible after the occurrence of abuse facilitated by the drug.[16]

The hair sample can also be considered an important alternative matrix in cases of drug detection, due to the possibility of detection for a long time after use. This biological matrix has been decisive in cases where the victim reports what happened and takes the exam weeks or months after the aggression.[8]

It is understood that the methods used to determine date rape drugs in different biological matrices must have a high identification capacity.[17] The more sensitive the analytical method, the larger the detection window and the greater the probability of positive results. Some methodologies used to confirm the samples are gas chromatography-mass spectrometry (GC/MS); gas chromatography-tandem mass spectrometry (GC-MS/MS); liquid chromatography coupled to mass spectrometry (LC/MS); and liquid chromatography-tandem mass spectrometry (LC-MS/MS).[14]

2.1. Ethanol

Ethanol or ethyl alcohol ($H_3C–CH_2–OH$), popularly known as "alcohol", is one of the most widely used drugs and produces countless effects directly on neurochemical systems. It is a colorless, volatile, flammable and water-soluble liquid. This substance has been consumed by human since the beginning of history and is responsible for causing greater morbidity and mortality, in addition to generating more costs with public health assistance than all other illicit drugs. Besides, it induces amnesia, loss of motor coordination and mental confusion,[18] effects that facilitate the action of sexual predators, making this compound a date rape drug.

The effects of ethanol by a neurochemical view is still poorly understood. This is due to the lack of pharmacological specificity of ethanol and the lack of pharmacological tools for studying this drug. However, the effects of ethanol on the nervous system have been characterized by other ways, using biochemical, behavioral and electrophysiological approaches.[19]

Symptoms of intoxication range from expansive affection to uncontrolled mood swings, emotional outbursts evolving into dizziness, lack of motor coordination and mental disorientation. In more severe cases of intoxication, the functions of the central nervous system (CNS) can be quite depressed, causing a condition similar to general anesthesia and progressing to alcoholic coma.[20]

The main enzymatic systems responsible for the oxidation of ethanol are dehydrogenases, namely alcohol dehydrogenase and aldehyde dehydrogenase and, to a lesser extent, the cytochrome P450-dependent monooxygenases. The half-life of alcohol is approximately 4 h and the elimination rates are between 0.1 and 0.25 g/L/h. Some factors influence the rate of alcohol elimination, such as the tolerance rate (among people who drink socially and chronically) and differences in alcohol dehydrogenase levels. In this case, women are more sensitive to alcohol, since they have lower levels of this enzyme, which is responsible for gastric metabolism of ethanol.

The use of alcohol is the easiest way to get the victim into a state of chemical submission. A breathalyzer is a device used to estimate blood alcohol levels from the exhaled air.[21] For toxicological analysis to detection of blood alcohol, Guidelines for the Forensic analysis of drugs facilitating sexual assault and other criminal acts[8] recommends the use of gas chromatography with flame ionization detection by direct injection (GC-FID) or headspace (HS-GC-FID). This method is considered precise in the quantification of the volatile compound ethanol in body fluids, besides its accuracy, sensitivity and the possibility of automation.[22,23] Poulsen et al.[24] describes that ethanol can be detected by HS-GC-FID at levels down to 1 mg/100 mL. The analysis of ethanol in samples collected a long time after the incident could generate false negative results. In these cases, the analysis of its metabolites ethylglucuronide and ethyl sulfate are by GC/MS or LC-MS/MS is recommended.[8,25]

2.2. Ketamine (KET)

Ketamine (KET) is a compound marketed as a racemic mixture of its two optically active enantiomers, S-(+) and R-(−). The chemical structure of KET is shown in Fig. 1. KET, despite presenting some restrictions of use, is still a very important drug in anesthesiology, as it is a safe alternative for anesthesia in patients with some respiratory and cardiovascular limitations.[26] It could be found as a white crystalline powder or dissolved in a liquid and can be administered via intramuscular, rectal, nasal and oral routes. However, the intravenous and muscular routes are the most used in clinical practice, as they guarantee a greater drug bioavailability.

KET is a fast-acting anesthetic that produces hallucinogenic effects, such as auditory and visual distortions. Its use is allowed only when administered by a physician or a veterinarian, being used for starting and maintaining anesthesia. This compound induces a different type of anesthesia called dissociative, which is characterized by a cataleptic behavior, resulting from a functional and electrophysiological dissociation between the thalamus-neocortical and limbic systems, causing amnesia and paralysis. However, the patient does not completely lose the consciousness, keeping alert with eyes open, in a trance state.[27]

In Brazil, ketamine is a controlled drug listed in C1 list of substances and drugs subject to special control according Ordinance SVS/MS n. 344/1998, the Brazilian Controlled Drugs and Substances Act.[28] In this country, this substance is registered as an injectable solution, whose main indication is as an anesthetic in surgical interventions that do not require muscle relaxation or as an anesthetic adjunct to complement other low-power drugs in anesthesia.[29]

Hallucinations caused by the use of this drug are directly related to the dose and are potentiated when administered with alcoholic beverages. Even so, it is still used in hemodynamic patients, for inducing anesthesia in patients with asthma and for sedating agitated children in intensive care units.[30]

Currently, this substance has been used both as a recreational drug, due to its hallucinogenic effects, as well as a date rape drug, to induce amnesia and to facilitate the sexual abuse.[31] When used as date rape drug, KET can be inhaled, smoked[32] or even diluted in drinks, in which the drug becomes imperceptible to the victims, due to its discrete organoleptic characteristics.[33]

KET, unlike other hypnotic anesthetics, such as propofol, has little action on gamma-aminobutyric acid (GABA) receptors. Both KET and its metabolites are N-methyl D-Aspartate (NMDA) receptor antagonists. However, KET's pharmacological targets are not limited to this receptor. It was observed that this drug interacts, even with less specificity, with several other receptors and ion channels, including dopaminergic, serotonergic, sigma, opioids, and cholinergic receptors, besides acting on hyperpolarization-activated cyclic nucleotide-gated channels.[34]

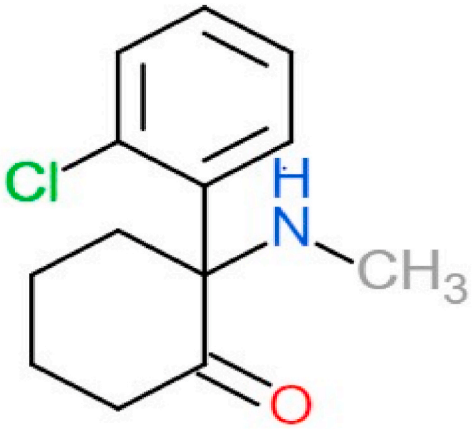

Fig. 1. Chemical structure of KET.

The extensive hepatic metabolism of KET begins with a N-demethylation reaction, giving the metabolite norketamine (NKET). This reaction is catalyzed by cytochrome P450 isoforms CYP2B6 and CYP3A4. The isoform CYP3A4 catalyze the demethylation of (S)-ketamine more quickly than (R)-ketamine, while the isoform CYP2B6 demethylates both KET enantiomers with similar efficiency. After the biotransformation of KET into NKET, this metabolite is oxidized to hydroxynorketamine (HNK) (Fig. 2).[35]

The administration of KET by intramuscular route ensures a bioavailability around 93%, reaching peak plasma concentrations in 5–30 min. However, the oral bioavailability of KET is low, ranging from 16% to 29%, with peak concentrations achieved within 20–120 min. This low value occurs due to the extensive first pass hepatic metabolism, which KET goes through when administered orally.[36]

On the other hand, intranasal administration is considered a good alternative. In addition to being less invasive, it results in rapid systemic absorption and is not subject to first pass effect when compared to other administration routes. KET is very fat-soluble, so it is widely distributed to tissues. Approximately 90% of KET dose is excreted in urine in 72 h. About 2% of the KET is eliminated in its unchanged form, another 2% is excreted as NKET and 16% as HNK.[37]

NKET is still the main product for toxicological analysis and can be detected in a urine sample by GC/MS. NKET is a less active metabolite and is found in lesser quantities in biological matrices, but has a longer half-life, which justifies its identification in forensic analyzes in cases of date rape drugs.[38] Gas chromatography is usually used to analyze KET and several drugs of forensic field because of its low cost, high selectivity and sensitivity and easy operation.[39] Efficient extraction methods based on automatic solid phase extraction (SPE) apparatus were described by Cheng et al.[40] and Kim et al.,[41] which allow a safe and fast protocol, with lower solvent use than other extraction methods, like liquid-liquid extraction. Cheng et al.[40] adopted GC/MS methodology to analyze KET and NKET in urine, achieving good accuracy and precision results, with a limit of detection (LOD) to KET and NKET of 15 and 5 ng/mL and a limit of quantification (LOQ) of 15 and 20 ng/mL, respectively. A positive ion chemical ionization – gas chromatography – mass spectrometry (PCI–GC–MS) was adopted by Kim et al.,[41] allowing LOD and LOQ for both analytes of 25 and 50 ng/mL, respectively. Xu and Liu[42] also used GC-MS to determine KET in urine, but they adopted a dispersive liquid-liquid microextraction (DLLME), which is simple, rapid, allowing a high recovery and enrichment factors compared to the previous cited extraction method. The LOD and LOQ values of KET were 0.91 and 3.03 ng/mL, respectively, indicating good chromatographic results.

KET is a non-volatile compound and can be a hard derivatizing one, which can compromise, in several cases, its analysis with its metabolites and other compounds by GC.[39] Fernandez et al.[43] developed a method based on LC-MS/MS to analyze simultaneously multiple drug classes in urine, including ketamine. They validated a highly sensitive method, which includes an SPE step, presents high recovery. The LOD and LOQ of KET were 0.125 and 0.5 ng/mL, indicating better performance than GC methods previously described. Lin et al.[44] have also validated a method based on LC-MS/MS to analyze ketamine in the presence of multiple drugs, comparing its precision, accuracy and specificity with GC/MS methods and, thus, recommending the liquid chromatography for use as a confirmation assay and quantification method.

The detection of KET in oral fluid deduces that there is a recent use of this substance, whereas the detection of this drug and/or its biotransformation products in urine does not necessarily indicate this recent use.[33] In urine samples, there is the possibility of detecting KET and/or its metabolites for 72 h, which may vary according to individual characteristics of the victims, administration routes and doses. After about 2 h of the crime, KET's biotransformation products can now be identified in urine by immunoassays,[38] despite being a rarely used method by laboratories.[45] Cheng et al.[46] proposed a two-step testing strategy, based on the use of Neogen ELISA as a preliminary test and GC/MS as a

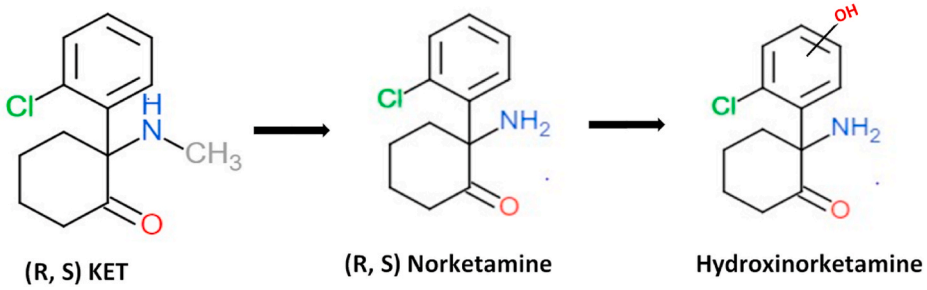

Fig. 2. Chemical structure of KET and its main metabolites.

confirmatory test to determine KET in urine. Besides the validation of a sensitive, simple and rapid chromatographic method and the verification of sensitivity, specificity and low rates of false positive and negative results with ELISA method, the study indicates that ELISA screening followed by GC/MS analysis seems to be an efficient strategy of screening-confirmation test in high-volume testing situations to analyze KET.

In whole blood samples, a positive result indicates that the victim was exposed to KET less than 48 h, whereas, in hair sample analysis, it is possible to obtain a larger analytical window, which may indicate the use of the drug weeks or even months ago, depending on the hair collected for the analysis, considering that the hair grows 1 cm per month.[8,14] For determination of KET and its metabolites in blood or hair, liquid chromatography coupled to mass spectrometry is commonly used. Ultrahigh Performance Liquid Chromatography - tandem Mass Spectrometry (UHPLC-MS/MS) combined with the extraction method DLLME was successfully used to determine ketamine and other drugs in whole blood samples, with LOD and LOQ of 0.5 and 2 ng/mL for KET, respectively.[47] Strano-Rossi et al.[48] applied UHPLC–MS/MS in multiple reaction monitoring (MRM) mode to develop a simple high-throughput screening method for the determination psychoactive compounds, including KET, in hair matrix, with LOD and LOQ of 2 and 10 pg/mg for KET, respectively. The use of liquid chromatography combined with tandem mass spectrometry allows analysis with high selectivity and an efficient analytes separation, as well as fewer sample preparation steps, simultaneously determination and quantification of analytes with different properties, leading to shorter analysis time.[49]

2.3. Benzodiazepines

Benzodiazepines form a drug class widely used worldwide in the treatment of seizures, sleep disorders and anxiety. These drugs are classified in two different ways to guide their use in clinical practice, and they are: (1) according to the affinity for the GABA binding site - an ionotropic receptor; and, (2) according to the pharmacokinetics, and this way concerns the metabolism of these drugs, which determines their half-life.[50]

Benzodiazepines bind to the alpha subunit of GABA type A receptors ($GABA_A$), enhancing the action of GABA, thus inhibiting neuronal excitability and increasing the conductance of the chlorine ion (Cl^-) channel. The higher concentration of Cl^- ions, the more the target cell is hyperpolarized, and as a result, the neuron firing rate decreases. This decrease causes all the pharmacological effects of benzodiazepines, which are mainly sedation and muscle relaxation.[51]

The main clinical uses of benzodiazepines are as sedative-hypnotic, anticonvulsant and anxiolytic. In Brazil, Clonazepam, Bromazepam and Alprazolam are among top-selling drugs and are controlled drugs listed in B1 list of psychotropic substances subject to "B" recipe notification according to Ordinance SVS/MS n. 344/1998.[28]

All drugs of this class have high partition coefficients. However, lipophilicity can vary up to 50 times depending on the lipophilic contribution and electronegativity of substituent groups present in its structure. Based on their half-life, benzodiazepines can be classified into three categories: ultrashort-acting benzodiazepines, which have a half-life shorter than 6 h, such as triazolam; intermediate-acting benzodiazepines, with a half-life of 6–24 h, like estazolam; and long-acting benzodiazepines, with a half-life longer than 24 h, such as diazepam and flurazepam.[52] Flurazepam itself has a short half-life, which ranges from 2 to 3 h, but its main active metabolite, *N*-desalkylflurazepam, has a long half-life, ranging from 47 to 100 h.[33] The chemical structures of main benzodiazepines used in clinical practice are shown in Fig. 3.

Benzodiazepines are associated with a significant number of DFSA. The most cited drug in this class in DFSA cases is flunitrazepam (Rohypnol®), but there is a wide variety of benzodiazepines, also used for this purpose, such as bromazepam, alprazolam, midazolam, oxazepam, diazepam, lorazepam and clonazepam.[15]

To analyze the cases of DFSA, it must be considered that the analyte in biological samples can be at very low concentrations, due to two main reasons: the high potency of these drugs, that is, they can achieve the desired effects with the administration of low doses; and/or the short half-lives of these drugs associated with the delay of hours or days between the crime and the analysis. Moreover, the drug identification in sample is more important than its quantification. However, due to the delay in reporting DFSA cases and the short detection time of most of these drugs in blood and urine, these sample analyses is complicated.[53]

Flunitrazepam is the well-known benzodiazepine used as a rape facilitator and the most mentioned associated with these crimes. However, diazepam and temazepam, the active metabolite of diazepam, generated after hepatic metabolism (Fig. 4), are the drugs most found in sample analyses of DFSA cases. The use of flunitrazepam as a date rape drug has spread in such proportions that F. Hoffmann-La Roche AG (Roche), manufacturer of Rohypnol®, reformulated the pill, making its dissolution more difficult and generating a bright blue color when in contact with a solvent. However, there are still other formulations available on the market that contain flunitrazepam, such as Hipnodorm®, sold in Australia and produced by Alphapharm Pty Ltd., and Rohydorm®, sold in Brazil and produced by EMS Sigma Pharma Ltda.[54]

Flunitrazepam is also one of the most prescribed short-term benzodiazepines, due to its sedative and hypnotic potential. In clinical practice, it is used to treat insomnia, tension, anxiety (treatment and prevention) and seizures. Its main side effects are the poor-quality sleep, speech and balance impairments and psychological and physical dependence. The use of this drug as a rape facilitator is attractive mainly because it causes memory blackouts.[55] The chemical structures of flunitrazepam and its metabolites are shown in Fig. 5. These compounds are extremely important in forensic context, since their detection in biological samples indicates the recent use of this drug.

The use of LC-MS/MS for analysis of benzodiazepines in hair is recommended by UNODC.[8] Rust et al.[56] validated a method to quantify 21 benzodiazepines and 3 "z-drugs" based on LC-MS/MS and sample extraction with methanol and a methanolic/aqueous solution, with selectivity and lower limits of quantification between 0.6 and 16 pg/mg. Although methanol is a solvent that allows an efficient extraction when a lot of substances must to be simultaneously analyzed and a limit

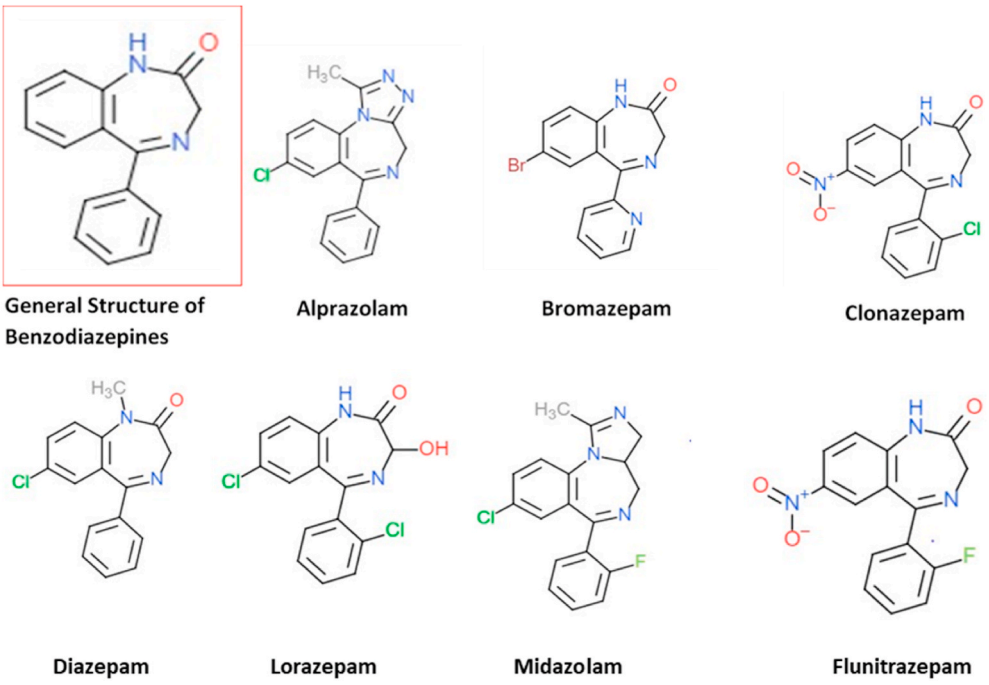

Fig. 3. Chemical structure of the main benzodiazepines.

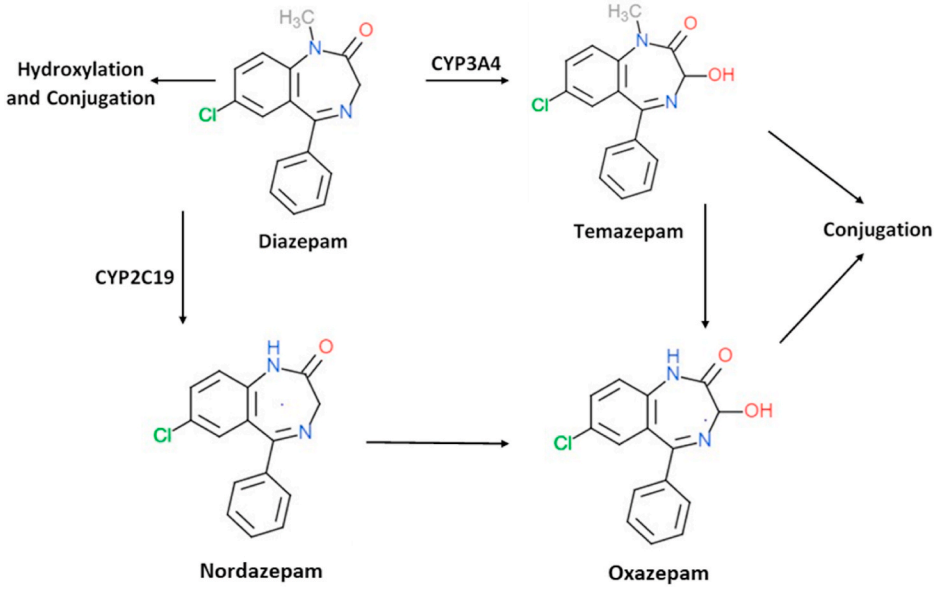

Fig. 4. Diazepam metabolism.

quantity of hair is available, Morini et al.[57] observed a better sensitivity of analysis of benzodiazepines using LC-MS/MS with a phosphate extraction than with methanolic extraction. While the methanolic extraction allows a LOQ between 1 and 20 pg/mg of hair, with the phosphate extraction, a LOQ between 0.1 and 5 pg/mg is reached, that could be important in cases of DFSA in which a single dose of the drug is used.

UNODC[8] also recommend the analytical laboratories efforts in detection of benzodiazepines in urine, considering that the common use of this drug class in DFSA cases. Several methods using LC-MS/MS have already been validated, differing as to the sample extraction procedure and the limits of detection. Quintela et al.[58] adopted a solid-phase extraction with a mixed-mode phase, reaching LODs lower than 0.05 μg/L, while Salomone et al.[59] obtained LODs ranged from 0.5 to 30 μg/L, using an enzymatic hydrolysis and liquid-liquid extraction in sample preparation. Magalhães et al.[60] adopted a method of extraction based on liquid-liquid extraction with low-temperature partitioning and analysis using liquid chromatography combined with high-resolution mass spectrometry, obtaining LODs for benzodiazepines in urine lower than 5 μg/L.

The use of immunoassay methods for screening benzodiazepines in biological matrices of DFSA victims can be adopted as long as the cut-offs informed by the manufacturer are higher than those recommended in DFSA investigations or with the revalidation of the method for lower cut-offs.[8]

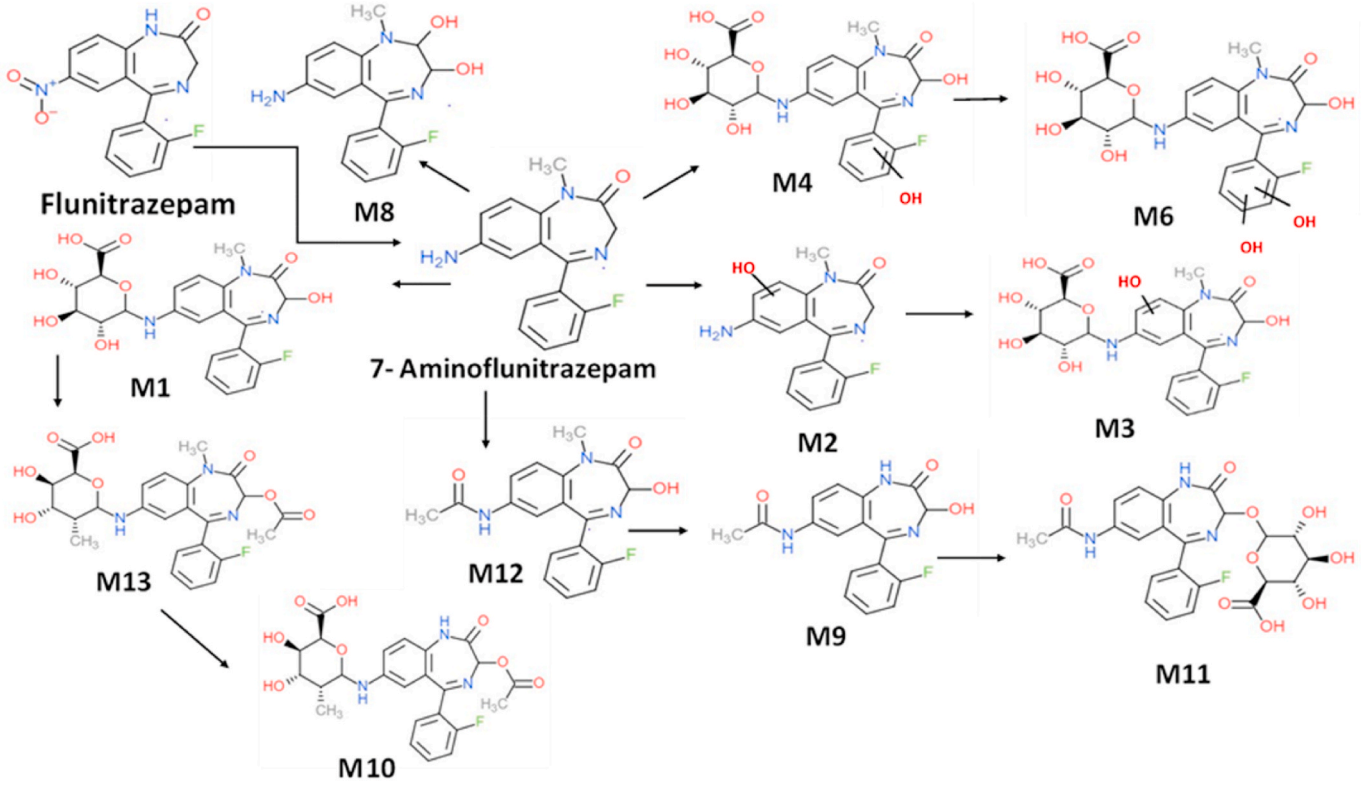

Fig. 5. Chemical structures of flunitrazepam and its metabolites.

2.4. Gamma-hydroxybutyrate (GHB)

Known as gamma-hydroxybutyric acid or gamma-hydroxybutyrate, GHB is an isostere of the neurotransmitter GABA and the similarities between their chemical structures is shown in Fig. 6. This substance was discovered in the 1960s, by the French researcher Henri Laborit, whose intention was to discover a compound that could be a GABA analog, and, thus, use it in the treatment of seizures.[1] Later, it was discovered that GHB is an endogenous compound present in the CNS, specifically in the brain, and a precursor to the inhibitory neurotransmitter GABA. Throughout the studies, some pharmacological properties have been discovered that have made GHB potentially useful as an intravenous anesthetic. However, its clinical use was discontinued due to its inability to induce analgesia, its unpredictable anesthetic effect and the difficulty in adjusting therapeutic doses.[61] Moreover, it induces several side effects such as delusions, muscle contraction, seizures and coma, and so it was no longer used for therapeutic purposes. However, at the end of the 20th century, GHB resurfaced as a drug of abuse, becoming known as the "rape drug" and as the "liquid ecstasy".[62]

GHB can stimulate the release of growth hormone (GH) and, for this reason, it has been widely used by bodybuilders over the last decades. Its use as date rape drug is due to its euphoric and CNS depressant effects.[63] The use of GHB or its analogs, γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), which are both rapidly metabolized to GHB, is associated with cases of intoxication and death from respiratory depression.[64]

Even though GHB is an illicit drug, its analogs GBL and 1,4-BD are freely marketed, as they are chemical components used in the manufacture of cleaning products, pesticides and plastics. Thus, these products are easily accessible, and when ingested, they are rapidly metabolized to GHB, exerting the same clinical effects as this compound. They can also be used as synthetic precursors to produce GHB.[65]

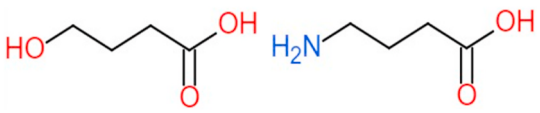

Fig. 6. Chemical structure of GHB (left) and GABA (right).

GHB is widely used in liquid form, which can be administered orally, but it is also found in powder or tablet form. Both GHB and its precursors have discrete organoleptic characteristics, which facilitates the adulteration of beverages.[1] In Ordinance SVS/MS n. 344, 12/1998, GHB is listed in the B1 List of psychotropic substances.[28]

A study indicates a new trend of use the legal analog GBL instead of the illegal GHB and the risk of this new context. GBL is also orally administered as a liquid, but it has a higher bioavailability than that of GHB due to its higher lipophilicity and faster effects are observed.[66]

The exact mechanism of action of GHB is still poorly understood. However, it is known that they act on the GABA type B receptor ($GABA_B$) as a neuromodulator. This drug still has clinical relevance as it is useful in the treatment of narcolepsy symptoms, including R.E.M. (Rapid Eye Movement) sleep disorders, cataplexy, hallucinations and sleep paralysis.[67]

The onset of GHB effects, such as a date rape drug or a recreational drug, varies according to the administered dose. When ingested, GHB is absorbed quickly, reaching a peak plasma concentration in 30–90 min. While the half-life of GHB is short at low doses, high doses result in slow and prolonged absorption.[68]

It is important to note that GHB has a sharp dose-response curve and has a small therapeutic index, that is, the dose to obtain therapeutic effects is close to the dose necessary to cause intoxication, and GHB intoxication results in a similar sedative state to catalepsy or seizure.[67]

The most of the GHB dose undergoes first-pass metabolism by cytochrome P450 and the main biotransformation pathway is an oxidation catalyzed by GHB dehydrogenase, a cytosolic $NADP^+$-dependent enzyme. This reaction gives succinic semialdehyde as product, which is an important compound in forensic analysis (Fig. 7).[63] GHB is predominantly eliminated in the urine after undergoing biotransformation and is practically undetectable in this matrix 12 h after its administration. GHB metabolites (Fig. 7) are important in forensic analyzes for the

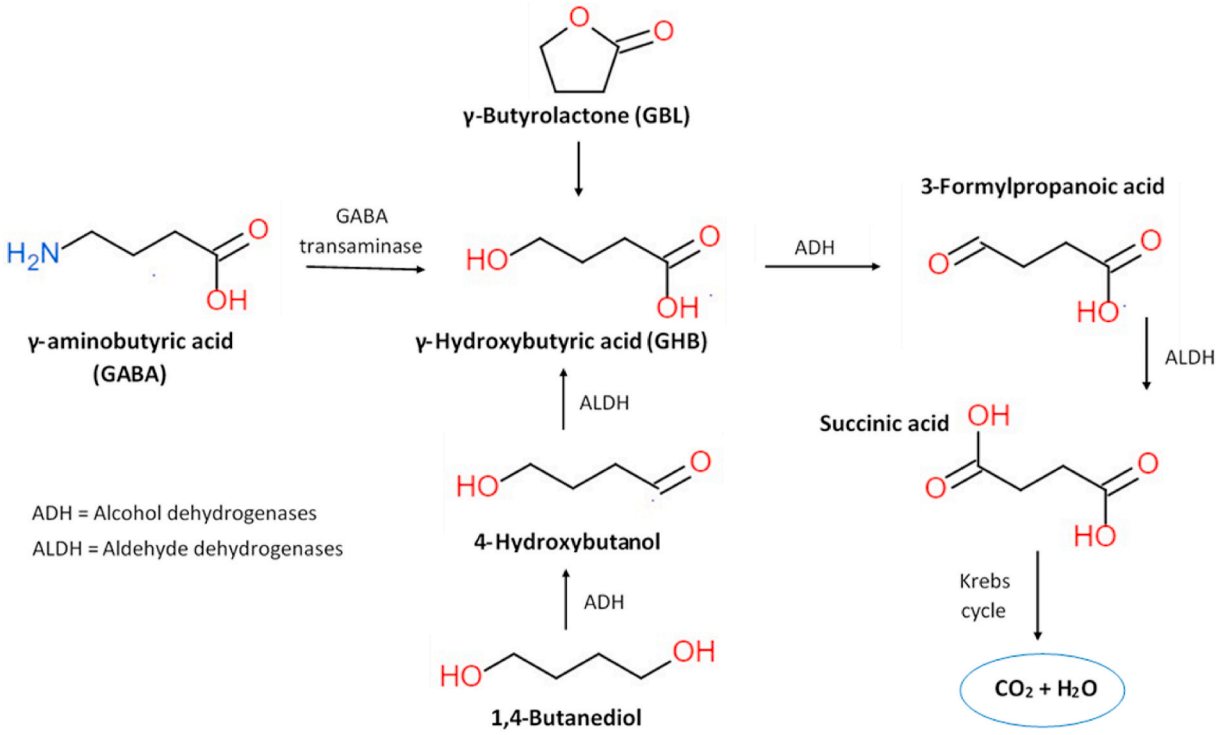

Fig. 7. GHB metabolism and its main metabolites.

detection of GHB in biological samples during the investigative process of cases involving the use of date rape drugs. However, in standard samples (urine and blood), detection is only possible when the victim's contact with the substance has been recent.

Due to the endogenous nature of GHB, the interpretation of the analytical results must be done carefully, with the quantification of the analyte being an important step. UNODC[8] (2011) recommends a cut-off concentration for GHB in urine of 10 mg/L and in blood of higher than 2 mg/L.

Several efforts to develop a practical protocol to be applied in all suspected cases of GHB-facilitated sexual assault have been done. Based on a study case of hair GHB decay after a single dose administration in DFSA case[69] and a literature review of hair testing of endogenous GHB and concentrations determined also after a single GHB administration in DFSA cases,[70] the authors suggest some analytical steps. Considering the high variability of endogenous GHB concentrations detected in hair of drug-free individuals, which complicates the definition of a reliable cut-off, in cases of suspect of GHB-facilitated sexual assault, the authors suggest the victim's sample collection in two moments: one hair sample as soon as possible after the GHB, cut into 3 or 5 mm segments, to determine the endogenous GHB level, and a second hair sample, one month after the exposure, proceeding with the same treatment and analysis as done for the first sample and comparing the concentration of GHB in each hair segment. The first segment is discharged because of the risk of sweat contamination. Bertol et al.[71] determined that when the ratio between the target segment and the mean values of all the others is between 3.52 and 5.63, there is a high probability of GHB consumption a month ago. If the victim has not washed his/her hair, Busardo et al.[70] also indicate the analytical methodology described by Kintz[72] which suggests the comparative analysis of the proximal hair segment with the other hair segments without decontamination to detect the GHB excreted in sweat and determine the consumption shortly after the exposure.

The use of gas and liquid chromatography are both described to analyze GHB in biological matrices. Bertol et al.[73] described the similar performance of GC-MS and LC-MS/MS for quantifying endogenous and exogenous GHB hair concentrations. For the two methods, a liquid-liquid extraction in ethyl acetate is adopted after hair digestion in NaOH, including a trimethylsylil derivatization step for GC/MS. GHB-d6 was used as internal standard. The LODs for GC/MS and LC-MS/MS were 0.4 and 0.5 ng/mg, respectively, and a LOQ of 0.6 ng/mg for both methods. GHB have a low molecular weight, besides being high polarity and thermal instability, which implies the need for its derivatization for GC analysis.[74]

Villain et al.[75] validated a method based on GC/MS to analyze GHB in blood and urine. The extraction method was the same for both matrices, which involved a neutral extraction with acetonitrile followed by a derivatization step with N,O-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA + TMCS). The LODs found for blood and urine were 0.1 and 0.2 mg/L respectively and the LOQ was 1 mg/L for both matrices.

Considering the expansion of use of GHB analogs as an alternative to GHB use,[66] the detection of these compounds in biological matrices is also important in a forensic context. A simultaneously analysis of GHB, GBL and 1,4-BD in urine using LC-MS/MS was validated by Wood et al.[74] For sample preparation, the urine was just diluted in an aqueous solution of the internal standard. The LOQs for all analytes were 1 mg/L. Johansen and Windberg[76] also used LC-MS/MS to identify and quantify in whole blood these three compounds plus gama-valerolactone (GVL), a compound that is in vivo converted to gama-hidroxyvalerate, that has similar effect to GHB. The samples were prepared using acidic methanol and the detection was in MRM mode. The method was specific and sensitive, with a LOD and LOQ of 0.5 and 1.0 mg/kg, respectively.

3. Drug combination

As reviewed by Anderson and colleagues,[77] the detection of more than one drug in drug positive samples of sexual assault victims is considerable common. The use of more than one drug that can affect CNS function can expose the victims to a situation of greater vulnerability, by intensifying their loss of perception and reaction, besides it can increase the risk of intoxication. Fiorentin and Logan[33] analyzed 1000

cases of suspected DFSA in the United States and observed that just one drug was used in 27.6% of cases, while two or more drugs were detected in 50.8% of samples. Jones et al.[78] found a drug combination in more than 20% of positive cases. Du Mont et al.[79] detected both alcohol and other drug in 18.0% of samples, while Scott-Ham and Burton[80] detected both alcohol and illicit drugs in 15.0% of analyzed cases of DFSA. The poly-drug use (the use of three or more drugs) is described by Scott-Ham and Burton[80] and ElSohly and Salamone.[81] The use of both alcohol and two illicit drugs or three illicit drugs was observed by Scott-Ham and Burton[80] in 3.1% and 0.9% of cases, respectively, while the use of three illicit drugs was identified in 1.3% of cases. The use of four illicit drugs was detected in only one case of 1014 samples. While ElSohly and Salamone[81] detected only one drug class in 39.0% samples, positive samples for two drug classes were 21.3% of all cases. 13.0% of cases were positive for three drug classes, while 8.3% for four or more drug classes.

Besides being the main date rape drug used alone, Ethanol seems to be also the most prevalent drug in drug combinations. The most frequent drug combination found by Fiorentin and Logan,[33] ElSohly and Salamone[81] and Scott-Ham and Burton[80] was ethanol and cannabinoids, corresponding to between 6 and 8% of the cases. Other common combination found by Fiorentin and Logan[33] and ElSohly and Salamone[81] were ethanol associated with benzodiazepines. Hagemann et al.[82] identified this last drug combination as the most frequent (3.4% of total cases) in samples of female patients assisted at Sexual Assault Center at St. Olavs University Hospital, Trondheim, Norway, between 2003 and 2010. Jones et al.[78] also identified the use of benzodiazepines in combination with ethanol as one of the most frequent drug combinations in samples of female victims of alleged sexual assault in Sweden, corresponding to 4.0% of positive cases. Other illicit drugs, like amphetamines and cocaine, are also common when drug combination is detected, as described by Fiorentin and Logan,[33] Jones et al.,[78] Scott-Ham and Burton.[80] Although drugs that affect CNS have properties to facilitate the sexual assault, Scott-Ham and Burton[80] and Jones et al.[78] observed that several positive cases for prescription drugs, like antidepressants, benzodiazepines and analgesics, confirmed the use of the medication detected, which can suggest that this drug may not be associated with the sexual incident. However, the use of these drugs or the concomitant use with ethanol or other drugs may have been a factor in facilitating the rapist's action. Moreover, in most studies, the authors focus on the analysis of substances commonly associated with DFSA cases, which can compromise the complete crime understanding when the rape is facilitated by over-the-counter drugs with sedative or other psychoactive effects or even prescription drugs non-screened in routine.[83]

4. Discussion

Sexual violence is a major public health concern and the statistics regarding this phenomenon are still divergent and do not represent the reality, since a significant number of victims do not report the incidence to the police because of several factors, such as the attacker's threat and the shame to have being submitted to this act. The detection of these drugs by conventional analytical methods, which use standard samples (blood and urine), often becomes impracticable due to the victim's delay in reporting, which in itself is due to fear, shame, unawareness of the correct procedures and/or the slowness of the system itself, being a challenge to forensic toxicology, considering the complexity of this issue. This is because most of the drugs used in these crimes have a short half-life and considering toxicological analyzes with the standard samples mentioned above, 45 days is a very long period and makes the detection of drugs unfeasible. This is because 45 days are, in many cases, a short period, for psychologically fragile women to understand, accept and denounce the violence suffered. There are indeed toxicological analyzes for the detection of drugs in the long term, but these demand high-cost chromatographic methods that require careful sample pretreatment procedures.[9,84,85]

It is, therefore, important to elaborate more accurate and punctual measures, for example, the increase of drug control by regulatory authorities in order to suppress sexual violence facilitated by the drugs described in this review. Thus, there is a need to focus on health actions for female public with the aim to instruct about how to prevent this con and, mainly, the ways to look for effective assistance when a DFC happens.

5. Conclusion

This work also highlights the difficulties in criminal cases involving the use of date rape drugs, since, to guarantee more reliable results, toxicologists should have access to sensitive and specific methods able to detect traces of compounds in the blood, urine or hair. For this, they need to have validated methods to detect potential drugs associated with DFC, the knowledge about the best matrices to analysis according to the delay of sample collection after crime and all equipment to proceed with the detection. Finally, knowing all the limitations of the methods and considering the factors related to the pharmacokinetics and toxicokinetics of drugs, toxicologists must interpret the analytical data with caution and inform the judicial authorities their perceptions.

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