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REVIEW ARTICLE

Benzodiazepines in Drug-Facilitated Sexual Assault: Pharmacology, Forensic Detection, and Emerging Challenges

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ABSTRACT

However, benzodiazepines are one of the most widespread drugs used in DFSA, second only to alcohol across all the cases. In fact, such drugs are ideal candidates for drink-spiking due to their rapid effect on the brain, significant anterograde amnesia, and inability to alter the taste, color, or smell of a drink. Furthermore, since benzodiazepines are prescribed by physicians on an extremely wide scale for anxiety or insomnia treatment, these drugs are easily available to criminals. This paper will review the mechanisms of action of normal and 'designer' benzodiazepines, vulnerable population groups, as well as the samples used in laboratory examinations of suspected DFSA. The paper will also analyze techniques used in testing for presence of benzodiazepines, including screening and mass spectrometry as well as newly developed lateral flow immunoassay strips. Finally, it will highlight several important problems of current testing, such as rapid excretion of the drug, inadequate standardization of laboratory techniques for identification of 'designer' drugs, as well as legal issues faced by victims.

KEYWORDS

• Benzodiazepines • Drug-Facilitated Sexual Assault (DFSA) • Forensic Toxicology • Designer Benzodiazepines • Date Rape Drugs • LC-MS/MS • Biological Matrices • Drug Screening

Key Messages: Benzodiazepines are widely used in drug-facilitated sexual assault (DFSA) due to rapid sedation and amnesia effects. This review covers their pharmacology, detection methods like LC-MS/MS and emerging point-of-care sensors, and current forensic and legal challenges.

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INTRODUCTION

The drug facilitated sexual assault (DFSA) occurs in a situation where an individual gets sexually assaulted due to excessive intoxication or drugging such that the individual cannot provide consent.[1-4] Researchers usually categorize the DFSA into three categories including the administration of drugs without the knowledge of the victim, a mixture of both drugs and the unknown one and the voluntary consumption of the drugs by the victim. Although the fact that the victim had voluntarily taken drugs would not reduce the severity of the crime according to the courts.

Date-rape drugs usually possess similar characteristics: they are highly potent even in minute quantities, they are excreted rapidly by the body, and induce drowsiness and amnesia.[2,3,6] Clinicians usually recognize several specific symptoms in patients who become victims: the person appears much more intoxicated than he or she should be, considering the amount of alcohol intake, the patient suffers from significant memory gaps, confusion, and bruising without explanation.[5,7] Unfortunately, exact numbers of the problem are not available due to the reluctance of victims to report such cases. Estimates say that up to 86.8% of DFSA cases remain unreported.[8] However, the available statistics are quite alarming. Thus, in Denmark, nearly half of 12,000 sexual assaults reported each year include drugs and alcohol use.[9] In Australia, the incidence of DFSA amounted to 60.45 per 100,000 individuals in 2021.[10] In the US, according to surveys, over 11 million women were raped during their lives while being under the influence of drugs or alcohol.[11]

Why are there so many users of benzodiazepines in criminal populations? One major factor is because they are widely available. They are regularly prescribed for anxiety, insomnia, spasms, and convulsions by doctors. Due to their widespread availability, they are easily available on the black market for abusers to access.[12,13] All research studies show that benzodiazepines are one of the five most commonly abused substances in DFSA tests, just behind alcohol.[9,10,14] For instance, in one Danish lab, they were found in 9.8% of patients, being the most frequently encountered prescribed substance.[9]

Unfortunately, "designer" benzodiazepines have exacerbated the issue recently. The fact of the matter is that the illicit modification of the drug's molecular structure allows for its being legal according to the drug laws while retaining or even amplifying the effects of the drug in making people unconscious and forgetting everything that happened.[15,16] Such cases have been increasing ever since 2014. The fact of the matter is that such substances are either sold through the dark web or masquerade as prescription pills. It is exceptionally difficult to detect them in labs. In reality, routine drug screenings are unable to detect them, reference standards cannot be obtained easily, and the impact of certain "designer" benzodiazepines remains unknown.[15,1,8]

This review includes such issues as follows. Firstly, the mechanism of benzodiazepine efficacy in the use for spiking drinks is considered. Secondly, prevalence of their presence in the samples is described. Thirdly, the types of samples that should be investigated and timing issues are discussed. Fourthly, laboratory methods of detection of benzodiazepines are presented. Fifthly, frequent errors in interpretation of the laboratory results and trends in development of the analytical technology are revealed.

PHARMACOLOGY OF BENZODIAZEPINES AND THEIR RELEVANCE TO DFSA

1. Mechanism of Action

The danger of these drugs lies in their influence on the brain. The benzodiazepine acts through increasing the brain's GABA neurotransmitter, which leads to reduced brain activity. The drug binds to the GABA-A receptors and increases the frequency of openings of the chloride channel. The result is decreased activity of the brain cells and sedation of the nervous system as a whole.[13,1] Clinically, the benefits include decreased anxiety, muscle relaxation, anti-epileptic action and inducing sleep. However, for sexual crimes, the desired effect is anterograde amnesia (inability to form memories).[13,2,1] The GABA-A receptor has several functions depending on its part. Alpha-1 is responsible for inducing sleep and amnesia, alpha-2 and alpha-3 – for relaxing muscles and decreasing anxiety.[13,20]

This difference in the mode of binding is crucial in practical application. For instance, fast-acting drugs such as midazolam and

triazolam have an immediate strong binding with the alpha-1 subunit, rendering the victim unconscious and causing amnesia.[13,21] Moreover, flunitrazepam (also known as Rohypnol) is commonly used in date-rape incidents because of its high potency, being seven to ten times more potent than diazepam. Even a tiny amount of the drug, which is non-lethal, will leave the person unconscious and helpless, making it impossible for the victim to recall what happened afterwards.[22-24] Finally, these drugs are readily soluble in drinks without affecting their color, smell, and taste.[10,24,25]

2. Pharmacokinetics and the Forensic Detection Window

The major issue with this test lies in the short half-life of these drugs, since many victims are not examined on time and the drug is no longer present in their system.[22,6] This particular type of drug is metabolized by the liver with the help of certain enzymes such as CYP3A4 and CYP2C19 and it gets converted into metabolites. Then it gets eliminated through the kidneys in form of urine.[20,22] But each person is unique and some people have certain genetic factors which cause them to metabolize drugs at a very slow pace and therefore, they will show a positive result for a couple of days.

There are a few issues associated with the short time window for blood tests. Typically, the parent compound can be detected in blood for less than 24 hours. For instance, flunitrazepam does not stay in blood for more than 60 to 72 hours, although the victim is either unconscious or experiences anterograde amnesia for 8 to 12 hours. This fact implies that the drug may have left blood before a victim wakes up and seeks help.[22,24] Urinary analysis is preferred due to longer detection periods from 1 to 5 days. In order to detect the drug presence in the specimen, laboratories use enzymatic hydrolysis.[5,25,26] As far as the long-acting drugs such as diazepam, which remain in the organism for 20 to 80 hours, there is the presence of nordiazepam compound which is detectable during 7 days. The interpretation of such results can be challenging. Indeed, since diazepam decomposes into several types of benzodiazepines such as temazepam and oxazepam, the detection of three compounds may mean that the victim took one dose of diazepam.[10,26]

3. Designer Benzodiazepines

Structure of Benzodiazepines

Benzodiazepines have a benzene ring fused to a seven-membered diazepine ring, with yet another ring fused to a precise position (R5). The molecule is highly modifiable. Unethical chemists exploit this to synthesize designer drugs. Incorporation of atoms such as fluoride, chlorine, or bromine, or the introduction of an additional ring, increases the binding affinity of the drug to the GABA-A receptor. Therefore, designer drugs produced in this manner are highly potent hypnotic and amnestic agents compared to their legitimate counterparts.[15,16,25]

An excellent example is flualprazolam, which is almost exactly the same drug as Xanax (alprazolam) except for an additional fluorine atom. However, this single difference makes it three to four times more potent with 1 mg resulting in complete memory loss.[16] Flualprazolam was discovered in robbery cases in China in 2017, and two years later, it became known in more than 1,600 cases in the United States.[16,1,7] As a result, the drug was banned worldwide in 2020.[16,1,7] Flubromazolam and clonazolam are among other designer drugs that are extremely potent with only 0.5 mg causing total memory erasure.[1,7,21] According to one laboratory report, the number of cases involving designer drugs increased ten times from 2020 to 2023. Bromazolam, etizolam, and clonazolam were identified as the most common of them. Since they are sold as counterfeit prescriptions, their victims do not even know what they are ingesting.[15,16,1,7]

EPIDEMIOLOGY OF DFSA AND THE ROLE OF BENZODIAZEPINES

1. Prevalence and Victim Profiles

The prevalence of DFSA is challenging to estimate due to the fact that only a few cases get reported since the substances quickly leave the body. Nevertheless, from the statistics available, young females appear to be the primary victims. According to a study conducted in Denmark, the peak rate of DFSA occurs in girls between 15 to 19 years, with a marked decline in women above 40 years (OR=0.38).[9] The risk of becoming a victim of DFSA is higher on weekends and evenings as well as in places such as bars and clubs.

Additionally, incidents of DFSA increase when the encounter takes place on the same day as the attack. Furthermore, there is a higher occurrence of DFSA by several perpetrators as compared to sexual assault without drugs.

In Australia, research shows that around half of all sexual assaults include the use of some sort of drug or alcohol, which equals 365,700 cases over a decade. Still, out of all of them, only 43,900 are ever reported to the police.[8,10] Memory impairment is a major component of these offenses—with 25% to 50% of victims unable to recall any of what happened, which makes investigation and prosecution of offenders difficult.[9,20] Also, it usually takes victims quite some time before they come forward—the same Danish study showed that only 55.4% of the victims had consulted a doctor or gone to the police within 24 hours of being sexually assaulted.[9] When forensic experts manage to examine the victim, benzodiazepines often show up in tests of the victims.[9,10,14] However, it should be remembered that this type of drugs is highly popular and used widely as a prescription medicine. In Australia, there are 24,000 benzodiazepines written for women per 100,000 people annually. As a result, testing of the victim for this drug is not proof of spiking.[10]

2. Polydrug Use and Synergistic Effects

The occurrence of a single benzodiazepine in a drug test is rare. Most of the time, the victim uses several drugs, the most frequent being alcohol. Moreover, cannabis, cocaine, opioids, antidepressants, allergy medication, or even various kinds of benzodiazepines can be found in a drug test.[9,14,26] For instance, a research conducted in Denmark among suspected cases showed that 37% of positive drug tests were mixed drug use cases including three or more medications at once.[14] The combination of benzodiazepines and alcohol is especially dangerous as both drugs affect the same receptors in the brain. Together, they result in extreme drowsiness, discoordination, and breathing difficulties which are much stronger than effects caused by the consumption of any of these drugs individually. The main indicator of a spike of a victim's drink is a state of impairment which is much higher than blood alcohol levels allow.[13,22,24] This becomes especially critical for people who regularly use prescribed drugs such as antidepressants or antipsychotics since the addition of a benzodiazepine to such a cocktail results in a lethal shutdown of the nervous system.[10]

BIOLOGICAL MATRICES FOR FORENSIC DETECTION

1. Blood and Urine

The analysis of blood collected immediately following the crime scene would give the most accurate picture of the level of substance in the victim's system, and it is almost impossible to falsify.[25-27] Nevertheless, due to rapid clearance from blood plasma (up to several hours), the collection of blood is not possible. This explains why laboratories heavily rely on urine. The advantage of collecting urine is that there is no need for any needles, and metabolites can be detected within 1 to 5 days – this kind of sample is considered the gold standard for drug identification.[22,6,5,25] Currently, in laboratories, the enzyme beta-glucuronidase is used to hydrolyze urine samples. Modern genetically modified beta-glucuronidase enzymes make the process highly efficient, allowing to isolate 97% of drugs.[28]

2. Alternative Matrices

This is due to the large detection window of hair, making it possible to detect the presence of drug use over months or even years after ingestion. Once the drug is ingested into the blood system, it gets absorbed by the hair follicles as hair is growing. With the help of hair segments, researchers are able to roughly determine the time when the victim consumed the drug.[27,29] However, hair tests have serious limitations. The darker color of hair means the higher content of melanin, which increases its ability to bind to these drugs and creates biased testing results. Furthermore, the chemical processing of hair (bleaching and coloring) destroys the traces of the drug in hair. Besides, the drug appears in the grown part of hair after about 7 to 10 days after ingestion.[27,29]

Oral fluid (saliva) can be used as another good source of recent drug consumption because its concentration correlates with the concentration of free drug in the bloodstream, and it comprises mainly the drug itself rather than its metabolites.[27,29] However, while oral fluid is very easy to collect non-invasively using an absorbent device, the main problem lies in collecting a very small volume of fluid (typically less than 1 ml), requiring special

sensitivity of lab analysis devices, as well as the dependence of the results on individual characteristics (natural production of saliva).

The vitreous humor (eye fluid) is also quite useful in case of an autopsy because it is protected from environmental contaminants and bacterial contamination, as well as any post-mortem alterations by being enclosed within the eye.[29,30] However, due to its high protein binding (80%-99%), benzodiazepines do not occur in eye fluid, and their concentration is typically lower in eye fluid compared to blood plasma; it is lower even tenfold, leading to its under detection by conventional screening methods.[29,30]

The testing of dried bloodstains on clothes represents a new technique that facilitates the collection of evidence if the assault is reported late. It was demonstrated that drugs such as alprazolam, clonazepam, diazepam, and lorazepam can be detected and quantitated in cotton dried stains for up to 96 hours after collection at room temperature.[31] The level of the substance remains unchanged after the sample is dried since all metabolic processes cease after that point. Besides, the stained material can be used to test DNA and blood and thus allows getting different types of evidence from a single sample.[31] There are also developments of sweat patches, such as the PharmChek patch, that allow following drug use for up to two weeks and bridging short windows in urine and long windows in hair, but we still lack studies regarding detection of benzodiazepines in sweat.[27,29]

ANALYTICAL METHODS FOR BENZODIAZEPINE DETECTION IN DFSA

1. Screening Methods

Lab testing is typically done in two phases. In the first phase, they conduct a screening test (immunoassay) to determine which general family of drugs is involved. Then, in the second phase, they conduct an extensive confirmatory test which gives information about the particular drug present and its concentration.[25,26,32] Typically, standard immunoassays such as ELISA, CEDIA, FPIA, and KIMS tests are conducted first since they are relatively cheap and quicker. However, there are major problems with using such screening techniques in date rape cases because they lack sensitivity and specificity. They are prone to producing false positives and negatives and do not help in identifying particular drugs within the family.[25,26]

Such tests have known limitations. For example, the FPIA test is not sensitive enough to detect flunitrazepam or triazolam if their level is close to toxic because such small amounts cannot be detected by the test.[25,26] The CEDIA and KIMS tests are also unable to detect low amounts of these substances.[25,26] Detection of designer benzodiazepines via such tests is a lottery since a drug may simply go unnoticed. For example, the presence of loprazolam and meclonazepam is undetectable by the test at low concentrations (above 300 micrograms per liter).[15] In order to increase sensitivity, researchers recommend adjusting cut-off points (for example, increasing ELISA cut-off from 30% to 70%) which allows detecting positive cases that cannot be identified through regular testing.[15] However, no matter how good a test is, one cannot trust the results for court use since one has to corroborate positive findings through sophisticated apparatus.[25,26,32]

2. Confirmatory and Quantitative Methods

The procedure used in the determination and quantification of the substance under investigation includes liquid chromatography tandem mass spectrometry (LC-MS/MS) in a mode known as MRM mode.[26,32] Modern LC-MS/MS techniques allow for detection of over 40 substances including old school benzodiazepines, newer designed ones as well as their metabolites. The sensitivity of the method allows for detection of substances as low as sub-nanogram levels compared to older technologies, which was not possible before. Special calibration procedures, such as 1/x and 1/x^2 weighting, are required for obtaining accurate results.[15]

Another technique is the use of liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS), where specialized Q-TOF or Orbitrap instruments are employed for wide screening of drugs. With this technique, labs can test for several thousand drugs at once, including newly designed ones, which may lack detection formula templates yet. Results are then compared to libraries that hold over 5,000 reference substances and thousands of other suspicious substances.[32] Nonetheless, one problem with LC-HRMS is that it typically uses positive charge ionization (ESI+). Substances, which can be analyzed

only in negative charge ionization (ESI-), may be entirely overlooked unless a dual run is performed.[32] Gas chromatography-mass spectrometry (GC-MS) technique is also applied due to the presence of large reference libraries. Yet, benzodiazepines need to be derivatized before testing in order to withstand heat inside the instrument, which may cause mistakes at times.[33,34]

For that reason, it is necessary for a comprehensive study to employ a number of machines. For instance, the lab may make use of LC-HRMS to detect any substance which is not known, LC-MS/MS to determine the exact amount of the drug, GC-FID to test the blood alcohol content, and GC-MS to analyze the other date rape drugs such as GHB.

3. Emerging Detection Technologies

Since lab tests take time, researchers are building quick-test devices that can be used on the spot. These tools are meant to check drinks in real time, preventing assaults before they happen and saving the drink as evidence.[1,8,35] One cool method uses electrical signals to detect designer benzodiazepines directly in drinks. Scientists tested four designer drugs—clonazolam, flualprazolam, flubromazolam, and diclazepam—spiked into soda, vodka, and wine. The test found 92% to 103% of the drug in under five minutes using a small, battery-powered sensor. More importantly, this sensor could tell the difference between very similar drugs (like flualprazolam and clonazolam) in the same drink, which normal screen tests cannot do.[1,8]

For an even simpler test, scientists have created paper test strips using cobalt chemistry. Dipping the paper strip into a drink for 2 to 3 seconds changes its color if midazolam or diazepam is present. A smartphone app can then scan the color within ten minutes, correcting for the color of the drink (like dark soda or red wine) to give an accurate reading.[35] Similarly, combining thin-layer chromatography with smartphone scanning offers a cheap, green way to test samples in poor areas.[36] Other techniques, like capillary electrophoresis (MEKC), can match GC-MS sensitivity for some drugs, and researchers are working on 'lab-on-a-chip' chips to make portable testing even easier.[37]

Table 1: Summary of Analytical Methods for Benzodiazepine Detection in DFSA

Method/ Technique	Category	Sample Matrix	Capabilities / Sensitivity	Limitations	Ref.
Immunoassays (ELISA, CEDIA, FPIA, KIMS)	Screening (Tier 1)	Blood, urine	Rapid, low-cost, first-line class-level screen; sensitivity improved by raising cut-offs (e.g., ELISA 30%–70%)	Low sensitivity/specificity; false positives/negatives; FPIA misses near-toxic flunitrazepam/triazolam levels; CEDIA/KIMS miss low concentrations; misses designer benzodiazepines (e.g., loprazolam, meclonazepam <300 μg/L); not court-admissible alone	15,25, 26,32
Liquid chromatography–tandem mass spectrometry (LC-MS/MS, MRM mode)	Confirmatory /Quantitative (Tier 2)	Blood, urine	Detects 40+ substances incl. metabolites; sub-nanogram sensitivity; requires 1/x and $1/x^2$ calibration weighting for accuracy	Requires known reference standards for each target compound; specialized, costly instrumentation	15,26, 32
Liquid chromatography–high resolution mass spectrometry (LC-HRMS; Q-TOF/ Orbitrap)	Confirmatory/ Wide untargeted screening	Blood, urine	Simultaneous screening for thousands of drugs, incl. newly designed ones; matched against libraries of 5,000+ reference substances	Typically uses positive ionization (ESI+); substances detectable only in negative mode (ESI-) may be missed unless a dual run is performed	32
Gas chromatography–mass spectrometry (GC-MS)	Confirmatory	Blood, urine, beverages	Large, well-established reference spectral libraries	Benzodiazepines must be derivatized to withstand heat, introducing potential error	33,34
Gas chromatography with flame ionization detection (GC-FID)	Confirmatory (adjunct)	Blood	Used alongside LC-HRMS/ LC-MS/MS/GC-MS in a multi-instrument workflow to quantify blood alcohol content	Not specific to benzodiazepines; measures alcohol only	32

table Cont....

Method / Technique	Category	Sample Matrix	Capabilities / Sensitivity	Limitations	Ref.
Electrochemical sensor (electrified liquid–liquid interface)	Emerging point-of-care	Beverages	Detects 92–103% of spiked drug within <5 minutes using a small battery-powered device; discriminates structurally similar designer drugs (e.g., flualprazolam vs. clonazolam)	Validated on a limited panel (4 designer benzodiazepines: clonazolam, flualprazolam, flubromazolam, diclazepam)	1,8
Colorimetric paper strips (cobalt chemistry) + smartphone app	Emerging point-of-care	Beverages	Color change after 2–3 second dip; smartphone app reads result within 10 minutes, correcting for beverage color	Validated for midazolam and diazepam only; qualitative/semi-quantitative	35
Thin-layer chromatography (TLC) / HPTLC + smartphone imaging	Emerging, low-resource testing	Beverages, biological samples	Low-cost, "green" alternative suited to resource-limited settings; dual-detection (imaging + densitometry) variants available	Lower sensitivity and resolution than LC-MS/MS	36
Capillary electrophoresis (micellar electrokinetic chromatography, MEKC)	Emerging	Biological samples, beverages	Can match GC-MS sensitivity for some drugs	Not yet validated across the full range of benzodiazepines and designer analogues	37
"Lab-on-a-chip" microfluidic devices	Emerging point-of-care (in development)	Beverages	Aim to make portable, real-time testing more accessible	Still under development; not yet validated for routine forensic use	37

Abbreviations: ELISA: Enzyme-Linked Immunosorbent Assay; **CEDIA:** Cloned Enzyme Donor Immunoassay; **FPIA:** Fluorescence Polarization Immunoassay; **KIMS:** Kinetic Interaction of Microparticles in Solution; **LC-MS/MS:** Liquid Chromatography-Tandem Mass Spectrometry; **MRM:** Multiple Reaction Monitoring; **LC-HRMS:** Liquid Chromatography-High Resolution Mass Spectrometry; **Q-TOF:** Quadrupole Time-of-Flight; **ESI+:** Positive Electrospray Ionization; **ESI-:** Negative Electrospray Ionization; **GC-MS:** Gas Chromatography-Mass Spectrometry; **GC-FID:** Gas Chromatography with Flame Ionization Detection; **GHB:** Gamma-Hydroxybutyrate; **MEKC:** Micellar Electrokinetic Chromatography; **TLC:** Thin-Layer Chromatography; **HPTLC:** High-Performance Thin-Layer Chromatography; **DFSA:** Drug-Facilitated Sexual Assault; **Ref.:** Reference.

CHALLENGES IN FORENSIC DETECTION AND INTERPRETATION

1. The Time Window Problem

The primary forensic problem associated with benzodiazepines is the disparity between the length of time in which the effects of the substance linger on the victim compared with its duration in the victim's body. This is exploited by the attackers since the substances clear out of the body very quickly - even faster than the victim recovers from memory loss and recognizes the assault, reaching a clinic for testing.[29,10] Research has proven that victims of DFSA seek treatment at hospitals much later than other assault victims, with the possibility of detecting the substance in their body fading extremely rapidly after 24 hours have passed.[9,14]

Another bit of evidence that is overlooked is the spiked drink. Benzodiazepines tend to be relatively stable in drinks. For instance, diazepam remains relatively stable in just about all kinds of beverages, but temazepam deteriorates quite fast when present in hot beer.[33,38] It was possible to detect flunitrazepam and diazepam using a technique involving microwave extraction of dried drink droplets and subsequent detection by LC-MS at concentrations of 25 ng/mL in beverages using small amounts of fluids.[38] Essentially, the glass retains the drug evidence for longer periods than the victim's body does.

2. Designer Benzodiazepines and Analytical Gaps

New designer benzodiazepines create a huge black spot in testing, not sensitivity problem. In case the laboratory does not possess the standards of the drug in question and does not have a predetermined testing method, the machine would simply miss the drug altogether, no matter how advanced it is.[15,1,7,32] Screening methods are highly unreliable when testing these drugs. Designer drugs may give a positive reaction in the initial screening process, however, during the subsequent confirmation step, fail it due to the fact that the machine was not adjusted to search for that particular chemical substance.

Another designer drug may not be detectable through the screening process at all and will be missed from the very beginning.[15,1,7] Finally, distinguishing similar isomers, such as methylclonazepam and meclonazepam is highly complicated due to the fact that both substances have the same molecular weight. Distinguishing one from another is possible only with chemical standards.

The metabolic pathways of these substances create additional problems. Designer benzodiazepines share the same liver pathway as any regular pharmaceutical substance, so they usually result in the very same metabolites being found. For instance, the designer substance diclazepam metabolizes into lorazepam. When lorazepam is detected in the urine sample, there might be no way to determine whether the person ingested regular prescription lorazepam or was drugged with illegal diclazepam. The importance of the issue is obvious.[1,7,21] In order to solve the problem, labs need to keep track of updating their reference database and the latest information on newly emerging drugs provided by international drug agencies (EMCDDA, UNODC), as well as forums for users.[32]

3. Postmortem Considerations

Fatal intoxications become even more complex due to the phenomenon known as postmortem redistribution. It involves a leakage of molecules of psychoactive substances from such organs as brain, liver, heart, lungs, and kidneys into the peripheral blood vessels once the bloodstream stops functioning in a deceased patient.[29,30] Thus, it is better for a toxicologist to conduct tests using peripheral blood drawn from the leg (femoral or iliac veins). It is extremely important since benzodiazepines and soporifics are detected in combination in 80%-90% of drug fatalities in some regions.[30]

If no leg blood samples can be taken, it is possible to use samples of heart blood or thoracic fluid, since concentrations of substances in them are almost the same for most benzodiazepines. Muscle tissue can also be used; however, certain psychoactive drugs accumulate there in much higher concentrations than in blood (e.g. clonazepam, nitrazepam, flunitrazepam).[30] Another significant problem is the continuous formation of 7-amino metabolites of nitro-type benzodiazepines during postmortem storage of a body. In this way, the ratio of parent compound to metabolite could be higher than 30:1, which could lead toxicologists to the mistake in estimating the dose.[30]

DISCUSSION

Use of benzodiazepines in the context of drug-facilitated crimes has existed since the 1990s and remains one of the most frequently reported types of psychoactive substances involved in such cases around the globe.[11,22,23,39] There is a range of factors that makes them so popular among attackers: high availability of this group of substances, deep sedative and amnestic effect, ability to dissolve almost undetectable in drinks and rapid elimination from the organism before the victim will manage to reach the hospital.

Designer benzodiazepines are another whole story. The list of prescription drugs that existed once had turned into the large number of different synthetic substances which can be obtained illegally through special networks designed especially to evade any control in the sphere of drugs.[15,16,1,7,21] The case of flualprazolam clearly demonstrates how fast the process goes: this drug appeared on Chinese market in 2017 and in just two years was detected in more than 1,600 cases in the USA. The example proves how quickly new psychoactive substances are able to overcome the legal and analytical limitations.[16,1,7]

The current screening methods (immunoassays) are definitely inadequate in resolving this issue, despite the fact that most laboratories start with them. For example, routine screens such as FPIA fail to detect therapeutic levels of flunitrazepam and triazolam, whereas CEDIA and KIMS fail to detect trace amounts of DFSA.[25,26] In addition, current screening methods are inadequate in detecting new designer drugs. This is why laboratories should develop comprehensive screening procedures focused on designer drugs, instead of using those which produce false negatives.

This issue also has significant social and legal dimensions. In cases when the presence of a benzodiazepine in the victim's body is detected, law enforcement officers, doctors, and even jurors tend to be very suspicious and usually believe that the drug was willingly taken by the victim for recreational purposes, not spiked.[9,10]

Such a presumption is particularly unfair for those who really need such drugs due to anxiety or sleeping problems. The offender can simply use the victim's prescription as the reason for his/her behavior claiming that the victim has overdosed on her own drugs. It implies that individuals with psychological problems are exposed to a two-fold threat.

Fortunately, advances in technology have provided solutions to some of these limitations on testing. Quick test devices such as the hand-held beverage screening device and the paper strip tests for drugs target the prevention of crime through the testing of beverages prior to consumption.[1,8,35] In contrast, the testing of dried blood samples and chemical stains on clothing can identify the presence of a drug several days later, well after the drug has exited the body.[31,36] Although these new advances cannot address the issue of victim non-reporting, they provide valuable tools for law enforcement and forensic toxicologists.

CONCLUSION

Benzodiazepines continue to be one of the most hazardous types of drugs used in sexual assaults involving drugging. Their popularity stems from their availability, quick action, ability to cause amnesia, and ability to dissolve unnoticed in liquids. This is even more of an issue with the increasing prevalence of designer and potent types of the drug.

In order to address this issue, it is necessary to take action from different directions. Firstly, hospitals must systematically obtain blood samples along with urine samples of the victim as well as hair testing in case the report is made weeks after the incident.[27,29] Secondly, laboratories must stop using basic drug screens and adopt advanced drug screens such as high resolution screening (LC-HRMS) and quantitation (LC-MS/MS).[15,32] Lastly, a standardized protocol must be established for obtaining empty cups, beverages, and clothes from crime scene investigations.[31,33,38]

Last but not least, legal definitions for drink spiking and drug-facilitated sexual assault, as well as severe punishments for those who commit such offenses, are necessary. At the same time, international laws must ban the use of new designer drugs more quickly, since it takes years for authorities to classify these substances.[16,1,7,21,23] Further investigation is required into designer benzodiazepines' pharmacokinetics, development of alternative specimen sampling methods, and assessment of effectiveness of new legislation and prevention programs.

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