Review Article

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Analytical Advances in Date Rape Drug Detection: A Comparative Study of Metabolite Analysis in Blood and Urine Samples

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Abstract

Date rape drugs (DRDs) are particularly toxic for the reason of their quick metabolism and covert administration. The detection and elimination of date rape drugs (DRDs) is crucial for preventing drug-facilitated sexual assault (DFSA). This study compares and contrasts the most current developments in identifying DRD metabolites.The present research offers an in-depth examination of analytical advancements for the identification of DRD metabolites in urine and blood samples.

With an emphasis on medications such as ketamine, flunitrazepam (Rohypnol), and gamma-hydroxybutyric acid (GHB), we looked into the metabolic pathways and detection windows of these chemicals. The urine samples may not provide the same level of immediateness as blood samples when it comes to acute forensic examination, even though they offer longer detection periods because of slower metabolite excretion. On the other hand, blood samples are more accurate just after drug administration, which makes them essential for quick forensic analysis.

This review research expands knowledge of DRD detection by offering a thorough evaluation of the accuracy of different analytical approaches in distinct biological matrices. It promotes the use of a dual-sample strategy to enhance forensic investigations and aid in the prosecution of DFSA cases by utilizing the advantages of both blood and urine examinations.

Keywords: Drug facilitated sexual assault, Date rape drug, Metabolite

INTRODUCTION

Date rape refers to a condition in which two persons in anintimate relationship engage in non-consensual sexual activity. It is also defined as a specific kind of sexual assault in which there is, or has been, a personal social relationship of some sort between the suspect and the victim, ranging from a first date to a long-term partnership. The scientific term for 'date rape' is 'Drug facilitated sexual assault'. Anyone using a medication that impairs judgment or behaviour runs the danger of engaging in unsafe or undesired sexual conduct. The most frequent "drug" connected to sexual assaults is alcohol.[1]

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These medicines have become quite popular among abusers as they not only physically weaken or affect the victim, but also slow down the victim's mental ability to the point where the victim is unable to think and recall anything. These medications have no taste, smell, or colour, they can be incorporated into the victim's food, drinks or beverages easily, giving the perpetrators a chance to use them as a convincing tool. Detecting the presence of these drugs through the victim's blood, hair or urine sample is difficult as they have a special feature or property called 'half-life' which makes it tough to predict their existence. The half-life is the time the body needs to partially metabolize the medication that has been consumed, resulting in the drug's volume in the body being reduced to half of what it was initially.[2]

To prevent date rape and date rape drug use, the first measure that can be initiated is education. This initiative can be started by educating society about date rapes, the drugs that are used and the way that drugs are used. Understanding the basic human right, that they have the right to say 'no' when they are not comfortable is important. Knowing the drugs, how to protect a drink, and different signs and symptoms to look for can help in preventing date rapes.[3]

Types of Date Rape Drugs

- **Alcohol:** It is well recognized that alcohol is at the top list among the drugs that increase the risk of sexual assault. It is the drug that is mostly chosen or consumed by people at clubs, parties or dates. Studies have proven that consumption of alcohol either by the perpetrator or the victim or by both was found in about half of all sexual assault cases. The use of alcohol leads to physical as well as cognitive impairment.[3]
- **Gamma-Hydroxybutyric Acid (GHB):** GHB also known as G, soap or liquid ecstasy. This substance occurs naturally in the central nervous system (CNS), Small citrus fruits and in almost every animal in a minimal quantity. It is a salty powdery substance that can also be prepared using industrial chemicals. The drug has a soapy and salty taste.[3]

  Metabolic pathway of GHB: When ingested, rapid absorption of the drug takes place in the gastrointestinal tract. The molecules of GHB are distributed through the bloodstream to the different body organs and tissues. It is capable of crossing the blood-brain barrier, resulting to its psychoactive effects. Firstly, the GHB molecules get metabolized in liver with the help of the enzyme called GHB dehydrogenase (GHBDH) to succinic semialdehyde (SSA). The succinic semialdehyde is then further metabolized by another enzyme called succinic semialdehyde dehydrogenase (SSADH) to succinic acid. This succinic acid then enters into the Krebs cycle, where it breakdowns into water and carbon-dioxide. A very small amount of unchanged GHB is then gets eliminated in the urine.[4] Only about 1% of the ingested GHB is eliminated in urine due to the extensive metabolism. Overdose of the drug can lead to stroke, coma or even death.[5]
- **Benzodiazepines:** This drug is basically used to treat insomnia, anxiety, and various other health issues. Commonly used benzodiazepines drugs that are used to commit date rapes are Flunitrazepam, temazepam, and midazolam. These drugs have hypnotic effects. Flunitrazepam and temazepam are generally chosen by perpetrators because they are soluble in ethanol.[3]
  a. **Flunitrazepam:**It is a derivative of benzodiazepine that is water-soluble and chemically it is represented as 5-(o-fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2H-1,4-benzodiazepin-2-one. Flunitrazepam are marketed as "Roofies" and "Rohypnol"commonly.[6] It acts on the GABA neurotransmitter in brain with the help of GABA-A receptors. Flunitrazepam gets metabolized in the liver to 7-aminoflunitazepam as major metabolite and also to desmethyl-flunitrazepam and 3-hydroxyflunitrazepam as minor metabolites. Its effect lasts up to 12 hours.[7]
  b. **Temazepam:** It is commercially known as "Restoril" and chemically represented as 7-chloro-1, 3-dihydro-3-hydroxy-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one. Temazepam undergoes metabolism primarily in the liver through conjugation. Its quantity in blood is found to be about 36% while only 2% in urine.[8]
  c. **Midazolam:** It has a fast and short-acting metabolism. It can be administrated intravenously, intramuscularly, orally, intranasally, etc. Generally it has an elimination half-life of 1.5-4 hours. In the body,Midazolam gets metabolized by hydroxylation and conjugation. It exhibits about 94% plasma protein binding

and approximately 0.5% is excreted unchanged.[9]

d. **Diazepam:** Diazepam is marketed under the brand name "Valium"[10]. It is a sedative drug and acts as a depressant in the central nervous system when ingested. It is mixed with alcohol or opioids to get more stronger effects.[11] It is known for its hypnotic, antispasmodic, antiepileptic and anxiolytic properties.[12]

e. **Clonazepam**: It is also known as Klonopin.[13] It possesses an intermediate onset of action.[14] It is known for its long half-time and strong potency. It functions as an agonist of GABA-A receptors. Clonazepam exhibits a wide spectrum of effects against various seizure diseases.[15]

f. **Alprazolam**: It has a rapid onset of action and its duration of action is generally short.[13] Often it is prescribed to manage anxiety and panic disorders. In healthy adults, its mean plasma half-life is nearly 11.2 hours.[16]

g. **Flurazepam**: It has a fast action and long duration mechanism.[13] The blood concentration of flurazepam following administration is incredibly low and only becomes noticeable a few hours following a single dosage.[17] It acts as a sleeping aid in the treatment of insomnia. However, an overdose of flurazepam may lead to dizziness, lethargy and daytime drowsiness.[18]

h. **Triazolam**: It has a rapid action and short duration mechanism.[13] It is triazolobenzodiazepine with hypnotic properties. Compared to medications with longer half-lives like flurazepam, nitrazepam, or flunitrazepam, it would seem more appropriate as a hypnotic.[19]

Metabolic pathway of benzodiazepines

After the consumption of the drug, its molecules are well absorbed by the gastrointestinal tract and are distributed throughout the brain and central nervous system. The metabolites of benzodiazepines are highly protein bound and accumulate easily in lipid-rich areas. The molecules then undergo extensive metabolism through two main phases in the liver:

Phase I: Most of the benzodiazepines are metabolized via cytochrome P450 (CYP450) enzymes. The phase includes oxidation reactions like hydroxylation, nitroreduction and N-dealkylation.

Phase II: This phase deals with the conjugation of the molecules with glucuronide leading to an increase in water solubility of the metabolites, which further facilitates their excretion through urine or feces.[20]

Benzodiazepines can be classified based on their elimination half-life:

i. **Short-acting**: The composition that has an elimination half-life of less than 5 hours. They are known for their hypnotic effects and also a few residual effects. Examples: Midazolam and triazolam.

ii. **Intermediate-acting**: Their elimination half-life is around 5-24 hours. They are generally used for anxiety purposes. Examples: Alprazolam, Flunitrazepam and Temazepam.

iii. **Long-acting**: They have an elimination half-life of more than 24 hours that allows them to remain active in the body for a long period of time. Example: Diazepam, Clorazepate.[21]

- **Ketamine:** This drug is generally used for its hallucinogenic effects. In pharmacies, it is sold in liquid form which can be injected or directly ingested. In clubs, it is sold as dried powder form and is generally smoked with the mixture of tobacco or marijuana.[3]

Metabolic pathway of ketamine

After ingestion, ketamine undergoes a series of steps to get metabolised in the body. N-demethylation of ketamine takes place with the help of cytochrome P450 enzyme resulting in the formation of norketamine and dehydronorketamine. They further undergo hydroxylation. Both ketamine and its metabolites get metabolized through glucuronidation which increases the water solubility in the metabolites facilitating excretion. About 2% unchanged, 2% in norketamine form, 16% in dehydronorketamine and 80% in hydroxylated form of ketamine and its metabolites are excreted through urine.[22]

Analytical Techniques for Detection of Drugs

Most date rape medicines are quickly metabolized by the body and are thereforehard to identify. Biological samples are often obtained when the drug's effects have already worn off and just trace levels are still present in the victim's bodily fluids. Some of the methods are mentioned below for the detection of drugs from blood and urine samples: -

For Blood

- In laboratories, liquid chromatography with the combination of tandem mass

spectrometry has become more and more common. Compared to immunoassay, this method has a greater specificity, can identify several compounds from just one sample, measures analytes in diverse matrices, and significantly reduces analytical interference.[24]

- Dried blood spot is another technique that is capable of detecting drug abuse in adults and it can measure the level of medication. It can also predict a newborn's prenatal drug exposure.[25,26,27]
- An alternative method that is used to detect drugs is capillary electrophoresis with advantages like great selectivity, efficiency of separation is high, short duration of time, automatability, etc. It is also used by collaborating mass spectrometry for much better results.[28]

For Urine

- At present, immunoassay is the most common technique used for the analysis of urine samples for drug testing. It provides qualitative results. In some cases, for better results, a 2-step approach is carried out i.e. the screening of immunoassay is followed by GC-MS (gas chromatography-mass spectrometry) as a confirmatory process.[29,30]
- Urine specimen validity test (SVT) is a set of tests performed on a sample of urine to decide whether the sample is authentic, unharmed and suitable for drug detection. The key components of SVT are creatinine levels, specific gravity, pH levels, oxidising adulterants, temperature, nitrites and glutaraldehyde.[30]
- The Urine drug test (UDS) refers to a diagnosis method for the detection of a specific drug or its metabolites in a urine sample. This process generally screens for amphetamine, methamphetamine, benzodiazepines, barbiturates, marijuana, cocaine, PCP, methadone, opioids and alcohol.[31]

Challenges in The Field

- Urine samples must be taken within 72 hours of flunitrazepam exposure for any detection to occur. Sensitive analytical testing must then be performed on the samples.[23]
- In contrast to Rohypnol, which can be traced for up to 72 hours, GHB can become undetectable in just 12 hours.[23]
- An investigation's toxicological and prosecutorial outcomes may be significantly impacted by a delay in specimen collection.
- Due to various factors, including a lack of qualified laboratories, the prosecution's weak case in court, lack of universal scientific criteria for determining the validity of certain forensic science branches, and delays in the rape survivor's medical examination, the judgement rate in rape cases is very low.[32]
- Because there are so many different and unique molecules in the therapeutic classes of medications that might be utilized, their analysis is also rather difficult.[33]
- The GHB molecule having tiny size and nature of polarity make the chromatographic analysis difficult which has been proven in many cases.[33]

Future Direction of Analytical Methods

- Various new methods have been introduced for the examination of date-rape-drugs, out of which the maximum concentrates on using GC/MS (gas chromatography-mass spectroscopy), LC/MS (liquid chromatography-mass spectroscopy), or CE/MS (capillary electrophoresis combined with mass spectroscopy) for determining their presence in biological samples or serum.[33,35]
- An experiment has been successfully employed for detecting the presence of ketamine in a sample using the process of X-ray diffraction and MDI jade 9 software.[34]
- In high-volume testing scenarios, the screening-confirmatory test approach of ELISA screening and GC/MS analysis appears to be effective in KET research.[36]

Ethical and Privacy Issues

- The investigator must treat every person as an independent agent with the power to set personal objectives and make informed choices following the moral standard of respect for persons.[37]
- Proper documentation of the impacts that have been created on the victim's health is done by the health professionals.[38]
- It is important to keep the victim's identity and personal information confidential to prevent them from possible reprisals and social disgrace.
- It is necessary to protect the victim's medical records privacy. These documents should not be accessed or disclosed without authorization, to avoid breaching of the

victim's privacy and cause more harm.

CONCLUSION

This study examined the developments in analytical methods for identifying date rape drugs, with a particularly highlighting the comparison between the metabolic analyses of urine and blood samples. It demonstrates how important it is to use contemporary analytical methods to increase the sensitivity, specificity, and general dependability of these illegal chemical detections. Analyzing blood and urine samples side by side reveals unique benefits and drawbacks specific to each biological matrix. Blood samples give more quick and accurate picture of the drug's presence, even though its detection window is shorter. In contrast, urine samples broaden the window of detection, making it possible to identify drug usage long after the drug has been metabolized. This distinction is important because sample collection timing can have a big impact on the analysis's result in forensic investigations.

In conclusion, the advancements in analytical techniques for date rape drug detection have improved our forensic capabilities. By continuing to refine these methods and addressing existing challenges, we can further enhance the accuracy and reliability of drug detection.

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