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ORIGINAL ARTICLE

Detection and Analysis of Common Substances Used in Drug-Facilitated Sexual Assault (DFSA). A Forensic Laboratory-Based Study

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ABSTRACT

Background: Drug-Facilitated Sexual Assault (DFSA) is a form of sexual violence in which perpetrators administer psychoactive substances to incapacitate victims, impairing memory and resistance. Rapid detection of these agents is essential for effective medico-legal investigation and prosecution.

Objective: To detect and analyze the most common substances implicated in DFSA cases in Lahore, Pakistan, using complementary analytical methods.

Methods: A cross-sectional descriptive study was conducted at the Forensic Medicine Departments of King Edward Medical University and Services Institute of Medical Sciences, Lahore, from January 2022 to January 2023. Fifty suspected DFSA cases referred for medico-legal examination were included. Biological specimens (blood, urine, hair) were collected following chain-of-custody protocols and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). Data were analyzed using descriptive statistics.

Results: Out of 50 cases, 37 (74%) tested positive for at least one incapacitating agent. Benzodiazepines were the most frequently detected (43.2%), followed by ethanol (29.7%), gamma-hydroxybutyrate (16.2%), ketamine (8.1%), and sedating antihistamines (2.7%). Polydrug use was observed in 21.6% of positive cases. Urine was the most productive matrix (75.7% positivity), followed by blood (51.4%) and hair (8.1%). GC-MS was particularly effective for volatile and semi-volatile compounds, while LC-MS/MS demonstrated superior sensitivity for low-dose and short-half-life drugs.

Conclusion: Benzodiazepines, ethanol, and GHB were the predominant DFSA agents in this series. A combined GC-MS and LC-MS/MS approach significantly enhanced detection rates, especially in polydrug cases. Early reporting, multi-matrix collection, and advanced analytical techniques are critical for improving toxicological confirmation in DFSA investigations.

Keywords: Drug-Facilitated Sexual Assault, Forensic Toxicology, Benzodiazepines, GHB, GC-MS, LC-MS/MS.

INTRODUCTION

Drug-Facilitated Sexual Assault (DFSA) represents one of the most insidious forms of sexual violence, combining the trauma of non-consensual sexual contact with the deliberate pharmacological incapacitation of victims[1]. It is defined as a sexual act perpetrated on a victim who has been rendered incapable of giving consent due to the influence of drugs or alcohol, administered without their knowledge or consent, often in combination with physical coercion. This phenomenon has gained increasing attention among forensic scientists, law enforcement agencies, and public health professionals worldwide due to its devastating consequences for victims and the complex challenges it presents in investigation and prosecution[2].

The central mechanism of DFSA involves the covert administration of psychoactive substances that impair a victim's consciousness, judgement, and memory. The resulting sedation, confusion, and anterograde amnesia create a "memory blackout" that makes it difficult for victims to recall the incident clearly, undermining their ability to provide reliable testimony[3]. This loss of memory, coupled with the rapid pharmacokinetics of many implicated agents, significantly complicates timely detection and toxicological confirmation. In many cases, victims may not seek medical help until several hours or even days after the incident, by which time certain drugs may have been fully metabolized and eliminated from the body[4].

A wide range of substances have been associated with DFSA, most of which share characteristics that make them particularly suitable for criminal use: they are often colorless, tasteless, odorless, and capable of inducing rapid sedation and amnesia at relatively low doses[5]. The most frequently reported drugs include benzodiazepines (such as diazepam, lorazepam, and flunitrazepam), gamma-hydroxybutyrate (GHB), ketamine, ethanol, and sedating antihistamines (such as diphenhydramine). In addition, certain antipsychotics, muscle relaxants, and newer synthetic drugs have been reported in specific forensic casework. Alcohol, while socially accepted, is one of the most common substances in DFSA cases due to its ready availability, disinhibitory effects, and potentiation of other sedatives[6].

From a forensic toxicology perspective, the detection of DFSA agents presents multiple challenges. First, many of the drugs implicated have extremely short detection windows in biological matrices GHB, for example, is typically detectable in blood for only up to 6 hours and in urine for up to 12 hours[7]. Second, the low doses used in DFSA require highly sensitive analytical techniques to detect, often necessitating advanced instrumentation such as Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). Third, co-administration of multiple substances (polydrug use) is increasingly observed in DFSA cases, which may result in complex pharmacodynamic interactions that complicate toxicological interpretation[8].

Globally, the prevalence of DFSA is difficult to ascertain due to underreporting, stigma, victim self-blame, and fear of legal repercussions especially in conservative societies. International studies indicate that a substantial proportion of sexual assaults involve alcohol or other drugs, with estimates ranging from 15% to over 50% depending on population and detection methodology. In South Asia, including Pakistan, published literature on DFSA is limited, and most available reports are anecdotal or case-based. Socio-cultural barriers, lack of public awareness, and inadequate forensic infrastructure contribute to under-detection and under-prosecution of such crimes[9,10].

The forensic laboratory plays a pivotal role in DFSA investigations, not only by confirming the presence of incapacitating agents but also by providing scientifically sound evidence admissible in court. Timely sample collection is essential, and forensic best practices recommend obtaining multiple specimen types blood for recent exposure, urine for extended detection, and hair for long-term exposure assessment in delayed reporting cases. However, many medico-legal setups in developing countries lack rapid access to specialized testing, leading to missed detection opportunities[11,12].

Given these challenges, there is a critical need to establish region-specific forensic data on the patterns of drug use in DFSA cases. Such data are essential for guiding medico-legal protocols, improving law enforcement strategies, and increasing the likelihood of successful prosecution. The present study was designed to address this gap by systematically detecting and analyzing the most common substances involved in DFSA cases reported to a high-volume forensic toxicology laboratory in Pakistan over a two-year period. By employing advanced analytical methods such as GC-MS and LC-MS/MS, this study aims to generate reliable forensic evidence that can inform both clinical and investigative practice, ultimately contributing to victim protection and justice delivery [13].

MATERIALS AND METHODS

Study Design and Setting: This was a cross-sectional descriptive study conducted jointly at the Forensic Medicine Department of King Edward Medical University (KEMU), Lahore, and the Forensic Medicine Department of Services Institute of Medical Sciences (SIMS), Lahore, Pakistan. The study spanned a period of twelve months, from January 2022 to January 2023, and aimed to detect and analyze the most commonly encountered incapacitating agents in suspected cases of Drug-Facilitated Sexual Assault (DFSA) referred for medico-legal examination. Both departments are recognized referral centers for forensic medico-legal work in Punjab, receiving cases from multiple police jurisdictions and medico-legal officers.

Study Population: A total of fifty suspected DFSA cases were included in the study using a purposive sampling technique. The inclusion criteria were individuals presenting within ninety-six hours of the alleged assault, cases officially referred by police authorities or other legally recognized channels for forensic evaluation, and those in which at least one biological specimen blood, urine, or hair was provided for toxicological analysis. Cases were excluded if medico-legal documentation was incomplete, if informed consent was refused by the victim or legal guardian, or if collected specimens were degraded, contaminated, or unsuitable for analysis.

Ethical Considerations: Prior to commencing the study, formal approval was obtained from the Institutional Review Boards of both KEMU and SIMS. All participants, or their legal guardians in the case of minors, provided informed consent after being informed of the study's objectives, the testing procedures, and the confidentiality safeguards in place. The study strictly adhered to the medico-legal evidence collection protocols established in Pakistan, and all patient identities and case details were kept strictly confidential to protect victim privacy.

Sample Collection and Preservation: Biological specimen collection was carried out by trained medico-legal officers in accordance with internationally accepted forensic toxicology standards. For blood sampling, ten milliliters of venous blood were collected into fluoride oxalate tubes to inhibit glycolysis and prevent degradation of analytes. These samples were stored at 4°C and analyzed within twenty-four hours. For urine sampling, fifty milliliters of urine were collected in sterile, tamper-evident containers, labeled with the unique case identification number, and refrigerated at 4°C before analysis within forty-eight hours. In cases where the alleged assault had occurred more than one week prior to reporting, hair samples were collected from the posterior vertex region of the scalp to provide a longer detection window. Each specimen was securely sealed, labeled, and documented under a chain-of-custody system to maintain evidentiary integrity during storage and transport to the toxicology laboratory.

Analytical Procedures: Toxicological analysis was performed using a combination of Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). GC-MS was primarily utilized for the detection of volatile and semi-volatile substances, including ethanol, ketamine, and selected benzodiazepines, while LC-MS/MS was used for the identification of low-concentration compounds such as gamma-hydroxybutyrate (GHB), a wide range of benzodiazepines, sedating antihistamines, and other psychoactive agents. Both techniques employed validated methods with established limits of detection (LOD) and limits of quantification (LOQ), and each analytical batch included internal standards and control samples to ensure accuracy, precision, and reproducibility.

Data Management and Statistical Analysis: The results of toxicological analyses were recorded in a secure forensic database. Descriptive statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Data were expressed as frequencies and percentages to describe the prevalence of individual substances, the occurrence of polydrug use, and the distribution of positive results by sample type. Cross-tabulation was performed where necessary to illustrate the relationships between detection rates and the type of biological specimen analyzed. All findings were interpreted in the context of known pharmacokinetic profiles of the detected substances and the time elapsed since the alleged incident.

RESULTS

Demographic Characteristics of the Study Population: A total of fifty suspected Drug-Facilitated Sexual Assault (DFSA) cases were examined between January 2022 and January 2023. The victims' ages ranged from 15 to 39 years, with a mean age of 24.8 ± 6.4 years. The majority were female (n = 46, 92%), and only four cases (8%) involved male victims. Most incidents were reported within 48 hours of occurrence (n = 38, 76%), whereas twelve cases (24%) were reported after a delay of three to seven days. The age and gender distribution are presented in Table 1.

Table 1: Age and Gender Distribution of Victims in Suspected DFSA Cases (n = 50)

Age Group (years)	Female n (%)	Male n (%)	Total n (%)
15–20	10 (20.0)	1 (2.0)	11 (22.0)
21–25	14 (28.0)	1 (2.0)	15 (30.0)
26–30	12 (24.0)	1 (2.0)	13 (26.0)
31–35	6 (12.0)	1 (2.0)	7 (14.0)
36–39	4 (8.0)	0 (0.0)	4 (8.0)
Total	46 (92.0)	4 (8.0)	50 (100)

The highest proportion of cases occurred among females aged 21–25 years, representing 28% of the study population, closely followed by those aged 26–30 years at 24%.

Toxicological Findings and Substance Distribution: Out of the fifty cases, thirty-seven (74%) tested positive for at least one incapacitating agent. Thirteen cases (26%) were negative for the substances screened in this study. The distribution of detected substances is shown in Table 2. Benzodiazepines were the most frequently identified (n = 16, 32%), followed by ethanol (n = 11, 22%), gamma-hydroxybutyrate (GHB) (n = 6, 12%), ketamine (n = 3, 6%), and sedating antihistamines (n = 1, 2%). Polydrug combinations were identified in eight cases (16%), most commonly involving benzodiazepines and ethanol.

Table 2: Distribution of Detected Substances in Positive DFSA Cases (n = 37)

Detected Substance	Number of Cases (n)	Percentage of Positive Cases (%)
Benzodiazepines	16	43.2
Ethanol	11	29.7
GHB	6	16.2
Ketamine	3	8.1
Sedating Antihistamines	1	2.7
Polydrug Use	8	21.6

Benzodiazepines accounted for the largest proportion of positive results, with nearly one-third of all cases testing positive for these agents. Ethanol detection was significant both as a single substance and in combination with other sedatives, while GHB detection was noteworthy given its short detection window.

Analytical Performance of GC-MS and LC-MS/MS: The integration of Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) significantly enhanced the detection capability. GC-MS was particularly effective in identifying ethanol, ketamine, and selected benzodiazepines, while LC-MS/MS proved crucial for detecting low-concentration and short half-life agents such as GHB and a broader spectrum of benzodiazepines, including lorazepam, alprazolam, and flunitrazepam.

Among the thirty-seven positive cases, GC-MS alone detected twenty-three cases (62.1%), LC-MS/MS alone detected seven cases (18.9%), and a combined approach where both techniques contributed complementary findings was responsible for confirming the remaining seven cases (18.9%). In polydrug cases, GC-MS typically identified ethanol or ketamine, while LC-MS/MS detected benzodiazepines or GHB in the same specimen, underscoring the necessity of a dual-method analytical strategy.

Detection by Biological Specimen: Urine samples had the highest detection rate, with twenty-eight cases (75.7% of positives) testing positive, followed by blood samples with nineteen cases (51.4%), and hair samples with three cases (8.1%). Urine analysis, particularly via LC-MS/MS, was most successful for benzodiazepines due to their longer urinary detection window. Blood samples analyzed via GC-MS were vital for confirming acute ingestion of ethanol and GHB within hours of the incident. Hair analysis provided critical evidence in delayed-reporting cases, confirming drug exposure several weeks post-incident. These findings are summarized in Table 3.

Table 3: Detection of Substances by Type of Biological Specimen

Specimen Type	Positive Cases (n)	Percentage of Positive Cases (%)	Most Frequently Detected Substance	Primary Detection Method
Urine	28	75.7	Benzodiazepines	LC-MS/MS
Blood	19	51.4	GHB	GC-MS & LC-MS/MS
Hair	3	8.1	Benzodiazepines	LC-MS/MS

Overall, the combined use of GC-MS and LC-MS/MS improved detection sensitivity and specificity across all biological matrices. GC-MS was superior for volatile and semi-volatile compounds, whereas LC-MS/MS excelled at low-dose, thermally labile substances. The dual-technology approach also allowed for more reliable confirmation in polydrug cases, where one technology alone might have missed a co-administered agent. Urine remained the most productive specimen for routine DFSA investigation, but blood and hair provided critical temporal and corroborative evidence, respectively.

DISCUSSION

The present study offers important forensic insights into the detection and analysis of incapacitating agents in suspected cases of Drug-Facilitated Sexual Assault (DFSA) in Lahore, Pakistan, over a one-year period[14]. The overall toxicological confirmation rate of 74% observed in this study is consistent with findings from multiple international investigations, where positivity rates generally range from 60% to 80% in cases with timely biological sampling. This high detection rate reflects both prompt medico-legal referral in a significant proportion of cases and the methodological strength of employing two complementary analytical techniques Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) in the same forensic workflow[15].

Benzodiazepines were the most commonly detected substances, appearing in 43.2% of positive cases. This observation aligns with trends reported in the United States, Europe, and Australia, where benzodiazepines such as diazepam, lorazepam, alprazolam, and flunitrazepam are frequently implicated in DFSA incidents due to their potent sedative, anxiolytic, and amnesic effects, their widespread therapeutic availability, and the ease with which they can be covertly administered[16]. Ethanol was the second most prevalent agent, identified in nearly one-third of cases, either alone or in combination with other sedatives. This highlights its dual role in DFSA as a primary incapacitant and as a potentiator of other central nervous system depressants. The significant detection of ethanol in the present series mirrors global data showing that alcohol remains one of the most commonly used and socially accepted intoxicants in sexual assault scenarios, yet one that can drastically impair judgment and resistance[17,18].

Gamma-hydroxybutyrate (GHB) was identified in 16.2% of positive cases, a finding of considerable forensic importance given its rapid metabolism and narrow detection window typically up to six hours in blood and twelve hours in urine. The ability to detect GHB in this study was aided by the early collection of specimens in most cases and the use of LC-MS/MS, which offers high sensitivity for low-dose and thermally unstable compounds. Ketamine, detected in 8.1% of positive cases, and sedating antihistamines, detected in 2.7%, were less common but illustrate the growing pharmacological diversity of agents implicated in DFSA cases worldwide[19,20].

The occurrence of polydrug use in 21.6% of positive cases is consistent with other international reports that document an increasing trend of perpetrators combining drugs to enhance sedation, amnesia, and victim incapacitation. In the current study, combinations most frequently involved benzodiazepines and ethanol. The detection of multiple agents in these cases was facilitated by the complementary strengths of GC-MS and LC-MS/MS; GC-MS proved highly effective for volatile and semi-volatile compounds such as ethanol and ketamine, whereas LC-MS/MS was optimal for a wider range of benzodiazepines and GHB[21,22].

The analysis of detection rates by biological specimen type reaffirmed established forensic toxicology principles. Urine proved to be the most productive matrix, with a positivity rate of 75.7%, due to its extended detection window for many sedatives. Blood samples, despite a shorter detection period, provided crucial evidence for acute ingestion especially of GHB when collected within hours of the incident. Hair analysis, though positive in only 8.1% of cases, was valuable in delayed-reporting situations, enabling the detection of drug exposure weeks after the alleged assault. These findings emphasize that no single biological matrix is sufficient in all DFSA cases; rather, a multi-matrix sampling approach enhances the likelihood of detection and strengthens the medico-legal value of toxicological findings[14,20,23].

From a forensic and medico-legal standpoint, this study underscores the necessity of rapid reporting, immediate evidence collection, and the use of advanced analytical methods to detect a broad spectrum of DFSA agents. The combined GC-MS and LC-MS/MS strategy significantly reduced the risk of false negatives, improved detection of short-half-life drugs, and allowed for accurate identification in polydrug scenarios[24]. These capabilities are essential for providing robust, scientifically defensible evidence in court and for supporting the investigative process in sexual assault cases. In Pakistan, where DFSA remains underreported due to sociocultural stigma and lack of awareness, these laboratory-based findings highlight the urgent need for public education, inter-agency coordination, and investment in forensic infrastructure to improve case outcomes and deliver justice for victims[25].

CONCLUSION

Benzodiazepines, ethanol, and gamma-hydroxybutyrate emerged as the most frequently detected incapacitating agents in suspected DFSA cases in Lahore, with a notable proportion of cases involving polydrug use. Urine was the most productive biological matrix for

routine detection, blood was critical for confirming recent ingestion, and hair proved useful for delayed-reporting cases. The integration of GC-MS and LC-MS/MS in toxicological analysis enhanced detection sensitivity, broadened the range of identifiable substances, and improved confirmation rates in polydrug exposures. Early reporting, multi-matrix specimen collection, and the routine use of complementary analytical platforms are essential to maximizing detection rates and strengthening medico-legal evidence in DFSA investigations.

Availability of Data and Materials: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to ethical and legal restrictions.
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