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Drug-facilitated sexual assaults (DFSA): a serious underestimated issue

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Abstract. – OBJECTIVE: Drug-facilitated sexual assault (DFSA) is a nonconsensual sexual act in which the victim is incapacitated or unconscious due to the effects of alcohol, a drug and/or other intoxicating substances. Dozens of drugs (including ethanol) can potentially be used to commit sexual assaults, but γ-hydroxybutyric acid (GHB) and flunitrazepam are the most common "date rape drugs".

MATERIALS AND METHODS: Multidisciplinary databases were browsed using the following search terms: "drug-facilitated sexual assault", "chemical submission", "date rape", "rape drugs", and "drink-spiking". Moreover, a search for reports was conducted on Institutional websites to identify documentation published by international agencies or institutions. Articles and reports were independently evaluated by each author.

RESULTS: There are no accurate estimates of the number of DFSA occurring each year, although assaults are increasingly reported. Many DFSA, however, are still not reported. Victims are reluctant to report incidents because of embarrassment, guilt or perceived responsibility, or because they do not clearly remember the assault. Moreover, most of the drugs typically used in sexual assaults are rapidly metabolized, making them undetectable in routine drug screenings.

CONCLUSIONS: Most of the substances involved in DFSA, with the exception of alcohol, are under international control and scheduled under the United Nations Single Convention on Narcotic Drugs of 1961 and the Convention on Psychotropic Substances of 1971. However, several psychotropic substances and antihistamines used in sexual assaults are still not under international control, allowing for trafficking, often *via* the Internet and courier. The absence of international control makes it difficult to obtain accurate data on the nature and the extent of the problem.

Key Words:
Drug-facilitated sexual assault, Urine and hair analysis, Epidemiology.

Introduction

Drug-facilitated sexual assault (DFSA) is a form of sexual violence against an individual incapacitated by a mind-altering substance, such as alcohol or "date rape drugs". It is estimated that 75% of all acquaintance rapes involve alcohol and/or drugs. Drugs, when used with alcohol, can result in a loss of consciousness and a loss of the ability to consent to sexual intercourse[1]. Most DFSA victims are women and perpetrators are men, although men can also be the victims. DFSA occur in three specific circumstances: (1) when the victim involuntarily ingests an intoxicating substance, (2) when the victim ingests both voluntarily and involuntarily an intoxicating substance, and (3) when the victim voluntarily ingests an intoxicating substance[2]. The psychoactive substance most commonly associated with DFSA is alcohol, but levels of recreational drug use are rising and drug markets are expanding, providing faster and cheaper ways to incapacitate a victim. Using drugs for nonconsensual sexual activity has led to the introduction of terms, such as "date rape drugs" and "drink spiking". Accurate data on the incidence of sexual assaults is not available, and reports to police are likely to significantly understate the actual numbers. The low reporting rate may be due to many factors, including psychological barriers (shame

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and embarrassment), cultural beliefs, fear of stigmatization, and lack of confidence in the criminal justice system.

The drugs the most commonly involved in sexual assaults are central nervous system (CNS) depressants. These substances can alter a victim's behaviour, ranging from loss of inhibition to loss of consciousness, and are often associated with anterograde amnesia. Dozens of drugs (including ethanol) can be used in DFSA. γ-hydroxybutyric acid (GHB) and flunitrazepam (Rohypnol®) are the most common "date rape drugs". However, scientific reports demonstrated that numerous CNS depressants and over-the-counter, prescription, and illegal drugs are also involved. Typical drugs used in DFSA include:

- Benzodiazepines (i.e., Valium®, Xanax® or Rohypnol®);
- Antidepressants (i.e., Elavil® or Zoloft®);
- Muscle relaxants (i.e., Soma® or Flexeril®);
- Antihistamines (i.e., Benadryl®);
- Over-the-counter sleep aids (i.e., Unisom®);
- Hallucinogens (i.e., ecstasy, marijuana, or ketamine);
- Opioids (i.e., Vicodin® or Oxycontin®).

DFSA drugs are typically found at raves, dance clubs, and bars, but they are also sold in schools, on college campuses and at private parties. Many drugs can also be purchased *via* Internet while others, such as prescription benzodiazepines, are often available at home. The most commonly used substance is ethanol, as it is legal, inexpensive, and there is usually no need to force consumption.

- Ethanol is the most common CNS depressant in DFSA. It can impair judgement and reduce inhibition and, in larger quantities, can cause loss of physical control and consciousness. The effects of most drugs with anxiolytic, sedative or hypnotic properties may be significantly increased when taken with alcohol.
- Benzodiazepines also are CNS depressants, although most are controlled under drug or medicine legislation. Flunitrazepam (widely known as "roofies") is the most common benzodiazepine in DFSA. Flunitrazepam is marketed under the trade name of Rohypnol® in many European countries as a powerful sedative/hypnotic prescription drug. It is tasteless, odourless, and dissolves in liquid. When flunitrazepam began being involved in sexual assault cases through "drink spiking", the manufacturer (Roche Pharmaceuticals, Basel, Switzerland) modified the product formulation and added a blue dye fizzing in liquids. The colorant, however, is not present in flunitrazepam from illicit sources. In France, additional manufacturing restrictions have been implemented. Benzodiazepines can induce confusion, impaired thinking, and memory loss, drowsiness, sleepiness and fatigue, impaired coordination and dizziness[3]. Benzodiazepines are found in forms of tablets, capsules, and injectables[4]. They are lipophilic and therefore less soluble in polar solvents, such as water and ethanol. However, a higher lipophilicity ensures a faster onset of action.
- GHB is a prescription CNS depressant in parts of Europe and in the United States, where it is marketed as an anaesthetic and a treatment for alcohol withdrawal symptoms. In 2005, the European Medicines Agency (EMA) also authorized GHB use as a medicine to treat narcolepsy with cataplexy in adults. In March 2001, GHB was listed as a Schedule IV drug in the 1971 United Nations (UN) Convention on Psychotropic Substances, thereby compelling European Union (EU) Member States to control the substance under their own national legislation. GHB induces sedation and anaesthesia and has a generally steep dose-response curve with a high interindividual variability. A small dose increase can cause loss of physical control and consciousness. It also increases sex drive, lowers inhibitions, and induces memory lapses, drowsiness, and dizziness. Degenhardt et al[5] highlighted the potential risk of GHB, reporting that over 50% of individuals who had voluntarily used the drug for recreational purposes had experienced unconsciousness following use. GHB is used in amnesia treatment with a 50 mg/kg dose, and as an analgesic at 10-20 mg/kg. Intoxication can occur above 15 mg/kg and doses higher than 50 mg/kg are toxic, while a 4-g dose is lethal[6]. GHB is quickly metabolized with a half-life varying between 20 min and 1 h[6].
- GHB is easily manufactured from its precursors γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), which are widely used solvents in industry. GBL and 1,4-BD are rapidly converted to GHB upon ingestion. GBL and 1,4-BD are not controlled in most countries, but Sweden, Italy, and Latvia enforced controls similar to those for GHB. In the United Kingdom, restrictive measures are being currently examined.

GHB and GBL onset of action is 20-60 min and 10-30 min, respectively[7].
- Ketamine, a Schedule III short-acting anesthetic for humans and animals, is another common drug in DFSA. It is a general anesthetic inducing a feeling of depersonalization and derealization. The victim can be aware of the assault, but unable to move or fight back. Ketamine can also cause amnesia.

DFSA drugs are typically odorless, colorless, and tasteless when mixed in a drink. GBL is an exception due to its bitter taste, although it is easily concealed with flavorsome drinks. Within 30 minutes of ingestion, the victim may struggle to talk or move and may lose consciousness. Because of the drug, the victim may have little to no recollection of the events, and many assaults are not reported.

Not everyone is similarly affected by drugs and it is difficult to accurately predict the effects of any drug on a particular individual. The effects may vary depending on the drug, the dose ingested, and whether the drug is mixed with alcohol or other drugs. Other influencing factors are the victim's weight, gender and metabolism, and technical considerations, such as how rapidly medical assistance is provided. Depending on the substance, the initial effects of a drug can be unnoticed or become quickly apparent:

- Nausea;
- Loss of bowel or bladder control;
- Breathing difficulties;
- Lightheadedness with little to no alcohol consumption;
- Sudden increase in dizziness, disorientation or blurred vision;
- Sudden body temperature change that can be signaled by sweating or chattering teeth;
- Waking up with little to no memory of recent events.

Many of these drugs are quickly eliminated from the body, from 12 to 72 hours[8]. Rohypnol® is eliminated within 36-72 hours and GHB within 10-12 hours. GBL leaves the urinary system within 6 hours and the bloodstream within 24 hours[9].

The aim of this review is to provide an updated outline of drugs involved in sexual assaults. The authors also provide epidemiological data of the phenomenon and discuss the analytical strategies and methods currently available to identify these substances.

Materials and Methods

Reports from 256 female patients who were admitted (voluntarily or accompanied by their parents if under 18 years of age) to the Sexual Assaults Centre of Careggi University Hospital in Florence, Italy, between January 2010 and July 2018 were examined. The centre received victims from the "Area Vasta Firenze", including 4 cities and more than 1,500,000 inhabitants. The procedure for identification and data and sample collection was described in an agreement protocol between the Sexual Assault Centre and the Unit of Forensic Toxicology of the University of Florence, approved by the Medical Ethical Committee[10].

Other epidemiological data were retrieved from PubMed, PsycINFO, and Scopus databases using the search terms "drug-facilitated sexual assault", "chemical submission", "date rape", "rape drugs", and "drink-spiking" to identify relevant studies to include in the review. Moreover, reports were retrieved from the websites of international agencies or institutions, including the United Nations Office on Drugs and Crime (UNODC), the World Health Organization (WHO), and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Articles and reports were independently evaluated by each investigator involved in the review. This study focused on victims of alleged DFSA aged 16 years and over, when toxicology results were reported.

Results

Most studies were published in the United States, followed by the United Kingdom and Europe with only one study in Australia and Africa. Alcohol was the most commonly detected substance and co-exposure to other drugs was common. Cannabinoids and benzodiazepines were frequently detected.

Discussion

Epidemiology

It is difficult to estimate the number of DFSA occurring every year considering the low reporting rates[11], although assaults are increasingly reported. Victims are often reluctant to report incidents because of embarrassment, guilt or perceived responsibility, or because they do not

clearly remember the assault. Moreover, most of the drugs used during sexual assaults are rapidly absorbed by the body and metabolized, making difficult the detection of these drugs in routine urine and blood drug screenings.

Over the past ten years, the number of DFSA reports increased in the United States[12], the United Kingdom[13], France[14], the Netherlands[15], and Australia[16], where serious concerns were expressed about the incidence of alcohol and drug use in nonconsensual sexual activity.

Italy/scientific literature: Only few cases were reported in the literature[17,18]. Barbiturates, morphine, and GHB were involved in isolated cases[19].

Italy/Careggi admissions: A total of 95 patients (37.1% of n = 256) were tested positive for at least one substance. Alcohol was the most detected substance (n = 57), followed by cannabis (n = 19), cocaine (n = 15), and opiates/methadone (heroin: n = 5, morphine: n = 1, methadone: n = 6); benzodiazepines and amphetamine were found in 13 and 2 cases, respectively. Only one case of GHB exposure was observed, while new psychoactive substances were not detected. Sedative drugs were found in patients suspecting a DFSA attempt[10].

France: Biological samples from 35 DFSA victims (19 males and 16 females) were collected over an 8-month period in 1995. Initial immunoassay screening detected benzodiazepines in 48.5% urine samples, followed by cannabis (detection of tetrahydrocannabinol) (17.1%), alcohol (11.0%), phenothiazines (8.6%), opiates (8.6%), tricyclic antidepressants (5.7%), and barbiturates (2.9%)[20].

France: Over a 9-year period from 2003 to the end of 2011, 473 cases of probable chemical submission or DFSA were identified. The study differenciated chemical submission, characterized by a premeditated choice of substance by the offender, and forced ingestion, when the offender acted alone or as part of a group. Forced ingestion represented 8% of cases (29 women and 11 men). Victims were predominantly female (n = 295, 62%) with a 0.6 sex ratio, and were aged between 2 months and 90 years. Mean age was 29.8 years for the 452 victims over age 1, when age was reported. Sex and age distribution showed that 15 to 39-year-old females were more frequently victims of drug-facilitated crimes (DFC), with a 36% peak for 20 to 29-year-old females. Male victims were predominantly aged between 20 and 39 with two peaks of 22%, although a 17% peak was observed for males under 15. Voluntary alcohol ingestion was reported in 46% subjects over 18 or old enough to use another non-therapeutic substance (435 cases over the age of 12 years), while this information was unavailable for 132 cases (30%). Cannabis use was reported by 16% victims[21].

UK: Over a 3-year period from 2000-2002, the Forensic Science Service (FSS) studied 1014 cases of alleged DFSA[22]. Exposure to alcohol, common drugs, and usual drugs of abuse was analytically documented. Most of blood and/or urine samples (81%) contained alcohol. Nearly 50% cases were tested positive for alcohol and/or other incapacitating drugs. Illicit drugs were detected in 35% cases, cannabis being the most commonly detected substance (26%) followed by cocaine (11%). In only 2% of cases (n = 521), a sedative or a disinhibiting drug was detected, although its presence could not be explained by the victim. These drugs were: benzodiazepines (n = 512), MDMA (n = 53), antihistamines (n = 52), GHB (n = 52), zopiclone (n = 51), and mirtazapine (n = 51). Similar results were reported by Beynon et al[23] a few years later. Papers from the UK after 2006 all cite Operation Matisse[24], a 12-month study on DFSA from 2004-2005 and including 120 victims of alleged DFSA. In this study, sexual assaults were reported within 72 hours. Controlled or prescription drugs were detected in 48% of cases, cannabis (20%), and cocaine (17%) being the most common substances. GHB was detected in two cases, but flunitrazepam was not identified.

Northern Ireland: Data from the Forensic Science Northern Ireland (FSNI) database from 1999 to 2005 were examined. Blood and/or urine were analyzed, but the methods were not detailed. In 2005, alcohol was involved in 65% cases, and contained alcohol, drugs or both were involved in 78% cases. Drugs were identified in 20 of 51 cases (39%). Analgesic drugs were the common substances (n = 511). Recreational drugs (cannabis, MDMA) were found in eight samples. Six samples were tested positive for benzodiazepines. More than one drug was involved in almost half of the cases[25].

Netherlands: Data from the Netherlands Forensic Institute (NFI) were published by Bosman et al[26] in 2011. Forensic cases were reported over a 3-year period (January 2004 to December 2006), including sexual assault cases with

or without blood and/or urine collection. A total of 134 cases of alleged DFSA were screened for drugs of abuse, prescription drugs, and GHB, and 108 were screened for alcohol. Most victims were women (94%). Twenty-seven percent of the cases were negative. Alcohol alone or together with other drugs was the most common finding (51 of 108 cases). A wide range of drugs was detected: cocaine (14%), benzodiazepines (10%), MDMA, and 3,4-methylene dioxyamphetamine (MDA) (10%), cannabis (10%), amphetamine (4%), GHB (detected in only two cases), and ketamine (detected in one case). Sedative therapeutic drugs were also found in single cases: amitriptyline, codeine, methadone, and zolpidem. A combination of drugs was often found.

Germany: From 1995 to 1998, the Munich Department of Forensic Medicine (Bavaria) registered 92 DFC cases. From 1997 to 2006, the Bonn Department of Forensic Medicine (North Rhine-Westphalia) reported a 10-fold increase in the number of investigations on alleged DFSA, currently reaching 40 to 50 cases per year. Madea and Musshoff[27], the authors reported that benzodiazepines were the most commonly used substances, followed by other hypnotic agents (zopiclone, GHB >10 mg/mL in urine), antihistamines (diphenhydramine), sedating antidepressants, and other illegal drugs (MDMA).

Denmark: A total of 167 blood samples from sexual assault victims in the Aarhus area were studied over a 2.5-year period. Twenty (12%) victims suspected an exposure to a "date rape drug". Seventeen of the 20 victims reported alcohol consumption before the assault, but only four (20%) were tested positive for alcohol. Benzodiazepines were identified in 25% cases, either alone or in combination with other drugs. Meprobamate, phenobarbital, oxycodone, methylphenidate, and amphetamine were each detected once. Tetrahydrocannabinol and/or 11-nor-9-carboxy-tetrahydrocannabinol were detected in three cases. The authors noted that a high amount of alcohol had been ingested in several cases in which no sedative drugs had been detected. As observed in other studies, alcohol was the most prominent substance, and therefore is a high-risk factor of DFSA[27,28].

Norway: In Norway, 730 female patients were admitted to the Sexual Assault Center in Trondheim from July 2003 to December 2010. Blood and/or urine specimens were obtained from 264 of 730 patients for toxicological analyses. Fifty-seven (22%) of the 264 victims suspected a proactive DFSA. Twenty-two of the 57 patients (38%) were positive for alcohol only; 13 (22%) were positive for at least one drug other than alcohol. Five patients were positive for benzodiazepines (diazepam and/or oxazepam, clonazepam), one was positive for morphine and oxycodone, 2 were positive for cannabis and 4 were positive for amphetamines; several patients tested positive for more than one drug[29].

Poland: A 15-fold increase in DFSA cases was reported from 2000-2002 to 2003-2004. The most common substances detected in blood and/or urine were amphetamine and cannabis, while alcohol, MDMA, benzodiazepines, propranolol, and lidocaine were detected in few cases only. No precise data were reported[30].

Belgium: No comprehensive data on DFSA were currently published in Belgium[19].

Spain: The National Institute of Toxicology and Forensic Sciences of Madrid conducted a descriptive and retrospective study on alleged DFSA cases reported from 2010-2013. A total of 152 of 445 sexual assault cases were examined. Biological specimens collected for toxicological analysis included blood (28.9%), urine (15.8%) or both (53.9%) and were mostly collected between 6 and 12 h after the incidents (40.33% of documented cases). Toxicological analyses were positive in 85.5% cases, with ethanol (76.9%), pharmaceuticals (36.1%, mainly benzodiazepines), and illicit drugs (29.2%, mainly cocaine), either alone or in combination[31].

United States: From 1996 to 2000, specimens from 3303 victims of suspected DFSA were collected[32]. A total of 61% victims tested positive for at least one substance and 39% samples were negative. A single drug was detected in 64% positive specimens; two drugs were detected in 22% specimens; and three or more were detected in 14% specimens. Alcohol, either alone (44%) or in combination with other substances (23%), was the most common substance, present in 67% positive samples. Cannabis was the second most prevalent drug as cannabinoids were detected in 19% of all samples and 30% of positive samples. Benzodiazepines accounted for 15% of all positive samples. Cocaine was detected in 14%, amphetamines in 11%, GHB in 5%, and opiates (morphine, codeine and heroin, and their me-

tabolites) in 4% of positive cases. Propoxyphene and barbiturates were detected in 2% of positive cases.

United States: Alleged DFSA cases were reported from four states (Texas, California, Minnesota, and Washington) between January 2002 and March 2004[33]. Biological specimens from 144 cases (16.8%) were tested. All victims were female and over 18; 60.4% were 25 or younger. A total of 45.8% victims self-reported alcohol use, but only 9.7% samples were positive for alcohol. Alcohol was followed by cannabis (9.0% of self-report, confirmed by the detection of tetrahydrocannabinol and/or 11-nor-9-carboxy-delta-9-tetrahydrocannabinol in 32.6% samples), cocaine (5.6% of self-report, confirmed in 18.1% samples), and amphetamines (3.5% of self-report, confirmed in 6.9% of samples). All victims denied voluntary use of opiates and benzodiazepines, but these substances were detected in 6.9% and 3.5% cases, respectively.

North America: Among 45 alleged DFSA cases in 2015, 58% tested positive for cannabis, whilst 43% were positive for alcohol, 26% for cocaine, 13% for amphetamines, 11% for benzodiazepines and opiates, 5% for methamphetamines, and 1% for methadone. "Other" or undetermined drugs were identified in 33% of cases[34].

United States: One thousand cases from 38 American states and territories, from March 2015 until June 2016, were examined. When gender was indicated (n = 613), most of the victims (91.7%) were females, and the mean age was 26.8 years. Blood and/or urine samples were tested. A total of 21.6% cases were negative for intoxicating substances (n = 216). A hundred and one different substances were detected. Ethanol was the most prevalent substance, detected in 30.9% cases (n = 309), followed by cannabinoids (tetrahydrocannabinol, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, and/or 11-hydroxy-tetrahydrocannabinol) in 28.8% cases (n = 288), amphetamine/methamphetamine (16.5%, n = 165), cocaine/metabolites (10.4%, n = 104), and clonazepam/metabolite (7.6%, n = 76). Mean, median and range concentrations of ethanol in blood (n = 309) were 98.6 mg/dL, 82.0 mg/dL, and 9.2-366 mg/dL, respectively. Ethanol and cannabinoids were the most frequent combination. California was the state with the highest number of cases (n = 260), followed by Maryland (n = 79), Massachusetts (n = 66), and Mississippi (n = 54). The top five compounds detected in urine were cannabinoids, ethanol, amphetamine/methamphetamine, cocaine/metabolites, and clonazepam, while the top five compounds detected in blood were ethanol, cannabinoids, amphetamine/methamphetamine, clonazepam, and alprazolam. Regarding polysubstance use, 276 cases were positive for one substance, while 220 cases were positive for two substances, 109 cases for three substances, 63 cases for four substances, 37 cases for five substances and 79 cases for six or more substances[35].

Canada: Demographic data were retrospectively studied over an extended period (1993-2002) in British Columbia, although no data were provided regarding alcohol and drug use in DFC/DFSA. The authors showed that the incidence of hospital reported DFSA has steadily increased since 1999 and that young women in their teens are particularly vulnerable to this form of sexual assault. Du Mont et al[36] also conducted a prospective cohort study in seven hospital-based sexual assault treatment centers in Ontario (2005-2007). In 178 cases of suspected DFSA, reported between June 2005 and March 2007, 96.2% victims were female, and the mean age was 25.8 years[36]. A total of 85% victims reported alcohol use before the alleged assault; 25.6% reported use of over-the-counter medications; 29.4% reported use of prescription medications; and 25.5% reported illicit drug use. Alcohol (30.9% of urine samples) was the most commonly detected substance, followed by cannabinoids (33.7%), cocaine (21.4%), antidepressants (16.4%; citalopram was the most commonly detected [6.7%]), benzodiazepines (11.3%; lorazepam was the most commonly detected [6.2%]), amphetamines (7.3%), MDMA (7.3%), codeine (4.5%), morphine (3.9%), antipsychotics (3.4%), methadone (1.1), GHB (1.1%), and ketamine (1.1%). Flunitrazepam (Rohypnol®) was not detected.

South Africa: A number of 107 victims of suspected DFSA from the Victoria Hospital Clinical Forensic Unit in Cape Town were examined between October 2013 and 30 June 2016. Biological specimens were screened for drugs of abuse. Most of the patients were female (n = 104, 97%), aged 18 to 25 (n = 54, 50%), and were admitted to the Clinical Forensic Unit within 24 h of the assault (n = 78, 73%). Altogether, 30 patients (28%) reported a history of mental health issues, drugs and/or alcohol use

or before sexual abuse. Samples included blood (n = 40, 37%), urine (n = 96, 90%) and/or hair (n = 2), as well as breath for ethanol measurements (58 prospective cases, 54%). Specimens were positive for drugs and/or ethanol in 72 patients (67%), with drugs other than ethanol being detected in 60 patients (56%). Samples were positive for drugs and/or ethanol in 72 patients (67%), with multiple drugs detected in 35 cases and a single drug detected in 37 cases. The most common substances detected were stimulants (methamphetamine, n = 30; cocaine, n = 10) and sedative hypnotics (methaqualone, n = 15; doxylamine or diphenhydramine, n = 13). Combinations of methaqualone, diphenhydramine, and methamphetamine were identified in 15 cases. Benzodiazepines were detected in 4 patients, although benzodiazepine use was not self-reported. Among the 32 negative cases, 22 patients reported ethanol use (69%), and 3 reported use of recreational drugs (9%)[37].

Australia: A total of 76 alleged DFSA cases among 434 cases of sexual assault (17.5%) were identified over a one-year period (2002-2003)[38]. Most victims were females, and the average age was 25.6 years. The median delay from alleged incident to time of examination was 20 h. Alcohol consumption was reported in 77% cases prior to the assault. Alcohol was detected in 37% cases, with an average blood alcohol concentration of 0.11% at the time of examination. Forty-nine percent reported using prescription medications and 26% reported using recreational drugs. Non-self-reported drugs were detected in 15 cases. The drugs detected included cannabis (n = 4), antidepressants[39], amphetamines[40], benzodiazepines[39], and opiates[40].

Analytical Strategies to Detect Drugs Involved in DFSA

Matrices

When a DFSA is reported, the choice of an appropriate specimen is crucial for toxicological investigations. Depending on the time that has passed since the sexual assault was reported, there are different approaches on how to choose the biological matrix to be analyzed.

When different samples of high volume and allowing longer windows of detection are available, the matrix of choice is urine (up to five days). Parent compounds and metabolites can be detected in blood for a shorter time (up to two days). If urine and blood specimens are not available, hair should be tested within four weeks of the reported assault. Urine is preferred to blood since it allows for a longer window of detection of drugs and metabolites and the sampling is easier and less invasive. Many drugs are quickly absorbed by the body and metabolized; the sooner the urine sample is collected the higher the chance of detection[41]. A positive urine sample usually is a sufficient proof of exposure. On the contrary, a negative result often requires further investigation. Nevertheless, the period of detection depends on the pharmacological profile of the specific substances tested[41]. Positive blood samples can document drug exposure within a shorter period of time when compared to urine. Blood concentrations give information on drug pharmacological effects and pharmacokinetics and may corroborate the victim's symptoms. In case of whole blood analysis, the parent drugs are targeted, and the detection of metabolites is possible in only few cases.

Interestingly, several "date rape drugs", such as GHB are also endogenous substances produced by the human body. In this concern, the analysis of multiple matrices can be performed to obtain complementary information to differentiate endogenous production from exogenous administration[41,42]. Since hair can retain xenobiotics for a large period, it is the matrix of choice in case of late DFSA reporting. Segmental analysis of hair provides chronological information about the suspected drug consumption, differentiating occasional, and chronic consumption. Hair analysis allows to identify endogenous GHB concentrations (under 12 ng/mg)[42]. Exogenous GHB can be identified in hair samples 7 days after exposure[43]. Nevertheless, GHB can be detected as a metabolite of the prodrug GBL, which can be administered instead of GHB since it is cheaper and easier to purchase[44].

In more complex cases in which blood and urine tests are not enough to prove exposure, the investigation of alternative matrices can be considered. For instance, oral fluid (OF) and nails have been recently evaluated as potential biological specimens to document drug exposure in DFSA. OF consists of saliva and other debris and food products in the oral cavity. Hydrophilic xenobiotics can enter OF from the bloodstream by passive diffusion and drugs can be detected as glucuronidated metabolites. Several factors influence the elimination of drugs in OF, such as the pKa of molecules, the pH of oral cavity and systemic diseases. Although OF drug concentrations

are not clearly correlated to blood concentrations and there is currently no proper biomarker to normalize OF drug concentrations, OF testing can provide qualitative information on drug exposure. Nails is a keratinized matrix accumulating drugs over time. Drugs and metabolites are incorporated during the bidirectional growth of nails, but they are also incorporated through occupational exposure. Like OF, the correlation between blood and nail concentrations is not clearly understood, but nail analysis can provide useful information in cases of late DFSA reporting.

Sampling

The systematic collection of biological specimens is the first phase of DFSA investigations. Incorrect sampling procedure can affect the results of the analysis due to the loss of target compounds. According to the UNODC guideline[41], biological evidence should be collected as soon as possible, ideally before any medication is administered to the subject. Custody chain is highly recommended. Timing of sampling is crucial, specifically when faced to substances undergoing fast metabolism, such as GHB. In case of suspected GHB administration, whole blood samples should be collected within eight hours and urine samples should be collected in less than twelve hours after exposure. Endogenous levels of GHB can be detected in urine (less than 1 mg/dL) and blood (less than 4 mg/mL) after twelve hours following intake[43]. Each matrix investigated in DFSA cases requires particular care on sampling and storage:

Urine: Urine should be collected as soon as possible after the suspected DFSA. Although international guidelines suggest collecting urine specimens within 5 days after drug administration, the pharmacokinetic of the substances should be considered for the interpretation of analytical results. Since urine is the matrix of choice for drug screening, a minimum volume of 50 mL should be collected and splitted into two sterile plastic containers with screw cap. Preservatives are not required, but they may be useful in case of *Candida albicans* contamination. The first sample should be used for screening and confirmatory tests, and the second sample should be stored at -18°C if further testing is needed. If the analysis cannot be conducted within 24 h after collection, all samples should be stored at -18°C[41].

Blood: According to international guidelines, whole blood should be collected as a complementary matrix to urine, preferably within 48 h of the alleged DFSA. Collection of blood samples should be perfomed by trained specialized personnel using appropriate disposable syringes and sterile tubes. A gas syringe is recommended for the analysis of volatile xenobiotics, such as alcohol or chloroform. A preservative, such as 2.5 g/L sodium fluoride or 2 g/L potassium oxalate, should be added to the test tubes to prevent degradation. Skin disinfection with ethanol or other volatile solvents should be avoided to not interfere with ethanol quantification in blood. Like urine, at least two 5-mL samples should be collected and immediately stored at 2-8°C, or at -18°C if the analysis cannot be conducted within 24 h[41].

Hair: Hair specimens should be collected at least 4 weeks after the reported assault. Pubic, axillary, torso or leg hair may be collected instead of head hair when the subject presents alopecia, is shaved or applied massive cosmetic treatment that can invalidate analytical results. International guidelines recommend collecting at least 100-150 mg hair, cutting the samples as close as possible to the scalp due to the kinetics of incorporation in hair. It is important to tightly attach hair strands to not lose their orientation for segmental analysis. Due to the stability of the matrix, samples can be stored at room temperature in antistatic envelopes[41].

Oral fluid and nails: Since OF and nails are considered as alternative matrices, there are currently no guidelines on sampling. However, several recommendations can be found in the literature. OF can be collected by passive drooling, expectoration, and salivary stimulation or *via* a collection device. Different types of devices are commercially available and are generally preferred to other sampling techniques. Several sampling kits do not require particular care for the storage of the samples[45]. Nails can be collected with disposable sterile nailclippers to avoid sample contamination and cut as close as possible to the nail bed. Due to the stability of the matrix, nail samples can be stored in sealed bags at room temperature[46].

Analytical Strategies

Many drugs used in DFSA may induce similar clinical symptoms. It is not possible to conclude that symptoms are due to drug exposure without clear analytical evidence. Moreover, a negative toxicological result does not exclude drug exposure, as the drug and its metabolites may have

been fully metabolized and eliminated at the time of sample collection. In addition, due to drug adulteration, several drug may be administered simultaneously[47], acting with a synergistic effect[48]. The final result also depends on the screening and confirmation methods. As such, they should be sensitive and reliable enough to guarantee the viability of those results[41].

Concerning urine and blood analysis, several techniques are recommended. Volatile compounds testing can be performed using head-space-gas chromatography (HS-GC) with mass spectrometry (HS-GC-MS)[48] or flame ionization (HS-GC-FID) detectors. As an alternative to HS, solid-phase microextraction (SPME) can be used[41]. For drug screening, metabolites, and non-volatile compounds, instruments should operate in full-scan acquisition in MS and spectrophotometry UV-Visible, and spectra should be further compared to references to confirm drug detection[41]. When available, urine samples should be screened for a predefined selection of substances likely to be used in DFSA, such as ethanol, drugs of abuse, and pharmaceuticals, including GHB, flunitrazepam, and ketamine[49]. Gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) or diode array detector (LC-DAD) are the most recommended technologies for DFSA, with optimized analytical methods to specifically target "date rape drugs" and their metabolites to detect low concentrations[50-52]. In general screening procedures, GC-MS tests are performed after sample derivatization. Unfortunately, in routine practice, most samples are delivered to testing laboratories a long time after collection, jeopardizing the detection of low drug concentrations. To improve detection, it is recommended to use MS/MS detection, such as LC-MS/MS or GC-MS/MS, due to high sensitivity and selectivity[41]. For ethanol detection, gas chromatography coupled to a flame ionization detector with (GC-HS-FID) or withouth (GC-FID) head-space sampler is recommended. Metabolites biomarkers of ethanol, such as ethylglucuronide (EtG) and ethyl sulfate (EtS) are often targeted as they are detectable in GC-MS or LC-MS/MS.

In hair, confirmatory methodologies can be used for identifying GHB and cannabinoids, with GC-MS/MS or LC-MS/MS detection[41]. Illicit and prescribed drugs can be detected by GC-MS, GC-MS/MS, and LC-MS/MS[41,53], and benzodiazepines and hypnotics can be detected by LC-MS/MS[41,52-55].

Conclusions

DFSA is an underestimated issue. Many drugs are involved and many of them are easily obtainable. To combat this phenomenon, two different strategies must be developed and implemented. The first priority is to increase public awareness, to help victims recognize the effects of "date rape drugs" and to urge them to reach emergency services for a correct diagnosis and better care. The second priority is to inform toxicologists on the best analytical strategies and most informative biological matrices to document DFSA.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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