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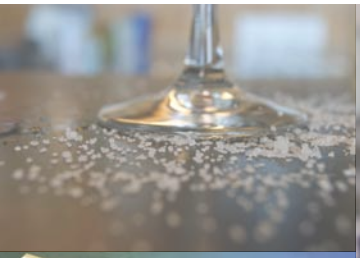

Guidelines for the

Forensic analysis of drugs facilitating sexual assault and other criminal acts

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Guidelines for the forensic analysis of drugs facilitating sexual assault and other criminal acts

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Contents

List of abbreviations

APCI	atmospheric pressure chemical ionization
BSTFA	N,O-bis trimethylsilylfluoroacetamide
CI	chemical ionization
DFC	drug-facilitated crime
DFSA	drug-facilitated sexual assault
CID	collisonally induced dissociation
CNS	central nervous system
CRM	certified reference material
DMSO	dimethyl sulfoxide
DMT	dimethyl tryptamine
EI	electron impact ionization
EQC	external quality control
ESI	electrospay ionization
GC	gas chromatography
GC-FID	gas chromatography with flame ionization detection
GC-MS	gas chromatography with mass spectrometry detection
GC-MS-MS	gas chromatography with tandem mass spectrometry detection
GHB	gamma-hydroxybutyric acid, gamma-hydroxybutyrate
GBL	gamma-butyrolactone
HR-MS	high resolution mass spectrometry detection
HS-GC-FID	headspace gas chromatography with flame ionization detection
HS-GC-MS	headspace gas chromatography with mass spectrometry detection
IQC	internal quality control
LLoD	lower limit of detection
LLoQ	lower limit of quantification
LC-DAD	liquid chromatography with diode array detection
LC-MS	liquid chromatography with mass spectrometry detection
LC-MS-MS	liquid chromatography with tandem mass spectrometry detection
LLE	liquid-liquid extraction
LSD	lysergic acid diethylamide
MAM	6-monoacetyl morphine
MDA	3,4-methylenedioxyamphetamine

MDMA	3,4-methylenedioxymethamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
MBDB	methylbenzodioxolylbutanamine
MRPL	minimum required performance limit
MS-MS	tandem mass spectrometry detection
MSTFA	N-methyl-N-trimethylsilylfluoroacetamide
NaF	sodium fluoride
NCI	negative chemical ionization
PMA	paramethoxyamphetamine
SPE	solid phase extraction
SPME	solid phase micro extraction
SRM	selective reaction monitoring
STA	systematical toxicological analysis
THC	Δ-9-tetrahydrocannabinol
UHPLC-MS-MS	ultra high pressure liquid chromatography with tandem mass spectrometry detection
UHPLC-DAD	ultra high pressure liquid chromatography with diode array detection
Z-drugs	zolpidem, zopiclone and zaleplon

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1. Introduction

1.1. Background

Drug-facilitated crime (DFC) is a general term that includes rape or other sexual assault, robbery, money extortion, as well as the deliberate maltreatment of the elderly or children under the influence of psychotropic substances. DFCs are criminal acts carried out by means of administering a substance to a person with the intention of impairing behaviour, perceptions or decision-making capacity. It also extends to taking advantage of an impaired person, without their consent, after their voluntary intake of an incapacitating substance. While the covert use of drugs to facilitate crime has occurred over the centuries, it has recently been highlighted by a significant increase in reports of DFC worldwide.

Psychoactive substances used in DFCs may alter the victim's degree of consciousness, state of awareness, judgement and memory. Such substances can make the victim vulnerable and unable to fight off their attacker. In addition, they can be used to sedate the victim in order to facilitate easier transport by the perpetrator.

The perpetrator of a DFC can either be a stranger or an acquaintance. Most substances used in DFCs are potent fast-acting central nervous system (CNS) depressants with effects that mimic severe alcohol intoxication or general anesthesia. The resulting pharmacological effects may include relaxation, euphoria, lack of inhibition, amnesia, impaired perception, difficulty in maintaining balance, impaired speech, drowsiness, loss of motor function, vomiting, incontinence, unconsciousness and possibly death. This may lead police to assume that the victim was drunk rather than drugged and thus impact the investigation. In many instances, the perpetrator is well aware of the effects of the administered drug.

Drug-facilitated sexual assault (DFSA), which is a subset of DFC, occurs when a person (male or female) is subjected to sexual act(s) while they are incapacitated or unconscious due to the effect(s) of ethanol, a drug and/or other intoxicating substance, and as a result unable to resist or consent to such acts. Substances may be administered covertly to an intended victim or victims, or a perpetrator may take advantage of a victim after voluntary ingestion of the substance.

The use of the term "date rape" by the media in cases of sexual assault, to describe DFSA, may be misleading. The media has focused on only a few of the drugs such as Rohypnol®, GHB and ketamine that can be used in DFSA. However, there are many other substances that can be used to facilitate such crimes including alcohol, over-the-counter medicines, other psychoactive prescription drugs and illicit

substances. Many substances give additive depressant effects when combined with alcohol and may be considerably easier to obtain than those given prominence in the media; for example, perpetrators have been known to use their own prescription medicines to incapacitate others.

The true prevalence of DFCs is unknown. Many studies suggest that fewer than 20 per cent of sexual assaults are reported to law enforcement agencies. In DFSA cases the impact that central nervous system depressant drugs have on memory and consciousness result in even fewer DFSAs being reported as compared to sexual assault not involving drugs.

Factors which complicate investigations in DFC include:

- Lack of experience among investigators, medical personnel, laboratories and prosecutors in handling in DFC cases,
- Lack of recognition of the crime by law enforcement agencies,
- Delays in reporting the incident,
- A broad range of substances that may be used.

Currently, no international standards are in place to facilitate the detection and identification of the substances that may be used in DFC. In addition there is no uniform system for defining and collecting statistical data on DFC.

Several countries have reported an increase in the non-medical use of psychotropic substances and expressed concern about the abuse of these substances. The United Nations Commission on Narcotic Drugs adopted a resolution 53/7 (53rd session, 2010) on "International cooperation in countering the covert administration of psychoactive substances related to sexual assault and other criminal acts" which inter alia requested UNODC to analyse the phenomenon of drug-facilitated sexual assault or other criminal acts and develop guidelines for forensic analysis to identify the presence of psychoactive substances. As a follow up to this resolution, UNODC organized a meeting of international subject-matter experts on 23-25 March 2011 at the UNODC Headquarters in Vienna to develop these guidelines.

1.2. Purpose and scope of the manual

This manual is one of a series of UNODC LSS publications on guidelines, best practices and recommended methods of analysis of drugs under international control and related substances. Developed as a practical guide of best practices and logical procedures, this manual will assist in the investigation, analytical detection and prosecution in DFC cases. It is intended for worldwide use with the aim of improving investigational and analytical capabilities. Specifically, it provides guidance to:

- Investigators and medical professionals as to requirements for successful evidence collection including sample collection and storage.
- Analytical toxicologists to carry out analysis of these substances and interpret results in cases of DFCs.

The manual outlines the investigative and analytical challenges related to DFC and emphasizes the importance of evidence collection as a basis for further investigation. In this regard, it also provides recommendations on practical tools for the collection of evidence. Further, it addresses the limitations of the analytical toxicological investigation and other issues that may impact the interpretation of results. Detailed consideration is given to all analytical aspects important in the detection and identification of substances and interpretation of results in the context of DFSA cases. References to validated methods for analysis of blood, urine and hair samples are given in bibliography. The manual emphasizes the importance of collaboration of all those involved in the investigation and the importance of collecting consistent data.

While the emphasis of this document is on DFSA, similar considerations apply in the investigation of other drug-facilitated crimes (DFC), such as robbery, money extortion, human trafficking and the abuse of the elderly, children and mental health patients.

2. Investigative and analytical challenges

There are a number of investigative and analytical challenges that may occur in any case in which drugs were used to facilitate the crime. An awareness and appreciation of these challenges is important for a successful investigation.

When the victim of a DFC is unclear about the events leading up to the assault because of the amnesiac effects of the drug(s) administered, it may lead to a delay in reporting the incident, if it is even reported at all. A considerable amount of time may be spent trying to fill in memory gaps by speaking with friends who were with the victim or even with the assailant, if the person is known to the victim. The possibility that the victim may have been completely unconscious when the crime occurred and, thus has no idea that he or she was assaulted, complicates reporting even further. It is unlikely in these cases, for the victim ever to report the crime, unless something arouses his or her suspicion. As an investigator, it is important to recognize and be sensitive to these reasons for the delay in reporting of DFCs.

Once individuals report that they feel they may have been a victim of DFSA, it is critical that appropriate biological specimens, for example urine (see section 3), are quickly collected. Some of the drugs used are detectable in urine only for short periods of time post-administration, some for less than one day post-exposure, while others may be detectable in urine for four or more days after the alleged offence, depending of the screening and confirmation methods. A delay of just one or two hours in the collection of a blood specimen for toxicological testing may lead to an administered substance being missed.

The investigator must obtain complete information about any substances voluntarily ingested by the complainant. This includes an estimate of the amount of alcohol consumed in the period leading up to the alleged assault, any recreational drugs taken, as well as prescription or over-the-counter medications that may have been recently used. It is vital that the victim tells the truth about this information if a prosecution is to succeed. Assertions about substances used may be verified by analysing for metabolites, specific markers, or segmental analysis of hair collected at least one month after the alleged incident.

Many investigations have failed to unveil complete information on ingested drug(s) due to the apprehension of some victims that admission to the voluntary use of a drug, such as cannabis, could prejudice the outcome of the investigation or court proceedings. Investigators must assure the victim that such information is needed to help explain the incapacitation that was experienced. Investigators must also remember that even if the victim voluntarily consumed the drugs that in the event

incapacitated him or her, it should not be misconstrued that the person did so with the intent of becoming a victim of crime.

In addition to the collection of biological evidence from the complainant, evidence should also be collected from the crime scene(s). All evidence should be collected using proper chain-of-custody procedures in order to ensure their authenticity, integrity and traceability.

The CNS depressants that may be used in DFSA present many analytical challenges. Many are highly potent and hence are given in very low doses. The drugs that may be encountered are not limited to illicit drugs, but include prescription and over-the-counter medications that may be readily available to most perpetrators. The low doses that may be used as well as the different physicochemical properties of many of these compounds often make it difficult for laboratories to detect their presence using routine analytical methodologies, hence more sensitive methodology and instrumentation has to be applied. Furthermore, investigators, health-care workers and laboratory personnel may not be aware of the range of drugs that may be used to facilitate crimes. This may result in the analysis focusing on only a few suspected drugs and missing the drug that was actually used. There are over 50 drugs known to have been used in DFSA, and each year new drugs are introduced that may be encountered in such cases. The large number of compounds that may be encountered presents a serious challenge to the toxicology laboratory charged with performing sensitive, comprehensive screening tests for all of these drugs. A thorough investigation of the circumstances of each case can provide the laboratory with information as to the drugs to focus on for a better probability of success.

It is important to remember that many of the drugs that feature in DFSA, including alcohol, may give rise to similar clinical symptoms in a complainant. Therefore, it is not possible to conclude that incapacitation reported by a complainant is due to a particular substance without evidence that the substance (or a specific marker/metabolite) is present in a sample from the complainant. Additionally, since most drugs are metabolized and eliminated from the body at different rates, it should never be assumed that a negative toxicological finding is proof that an incapacitating drug was not present at the time of the alleged assault.

The analysis of samples collected during investigation of DFSA must be performed by well-trained staff in an appropriate forensic toxicology laboratory. Such analyses are not routine in most forensic toxicology laboratories and generally require advanced instrumentation that may not be available in all laboratories. It is important that selective and appropriately validated analytical procedures are employed that are capable of detecting these drugs and their metabolites with the highest possible sensitivity. It is therefore advisable that properly collected and stored evidential specimens are sent to an adequately equipped analytical toxicology laboratory instead of immediate and partial analysis carried out in a laboratory that does not have the adequate analytical capacity for this purpose.

Different analytical strategies may be required for different specimens (urine, blood, saliva, scene residues, vomit, stained clothing, and hair) submitted for analysis. For example, urine samples may require hydrolysis in order to facilitate detection of metabolites excreted as conjugates, while analysis of blood and hair may focus more on the parent drug.

Finally, the interpretation of the toxicological findings can be challenging. Identification of any drug or metabolite in a biological specimen is generally proof of exposure, but simply detecting a compound at best may substantiate other evidence as to possible incapacitation at the time of the alleged offense. Further, given the individual variations in the rate at which drugs are metabolized, it is usually difficult to assess the dose or the exact time of exposure. Information from other sources such as scene residues can provide strong corroborative evidence. On the other hand, failure to detect a drug or metabolite, i.e. a negative result, does not always mean that the drug was not ingested. Some compounds (for example GHB, ethanol) occur naturally in the human body, so quantitative information is vital in the interpretation of results.

3. Evidence collection

The initial interview of the alleged victim, subsequent examination by a health-care professional and systematic collection of biological specimens are important stages in the first phase of a DFSA investigation (see annex 4, "Example of information collection worksheet for DFSA cases"). While the alleged victim's care is of prime importance; focus should also be on preserving evidence of the crime. Evidence of sexual assault (vaginal and anal swabs for spermatozoa and eventual DNA testing, description and photos of haematoma, evidence of other injury) must be carefully collected and documented by the healthcare professional. It is important that the health-care professional has the proper forensic training and is qualified to collect evidence that will be used in criminal cases.

Biological evidence should be collected as soon as possible using an adequate DFSA evidence collection kit, and should be accompanied by proper chain of custody documentation. Biological samples must be collected ideally before any medication is administered to the victim, but if this is not possible, all such medications must be documented. Specimens should be properly labelled with the date and time of collection and the collector's initials. Collected specimens should be immediately sealed and stored securely. A major advancement in investigating DFSA is to allow that a urine sample is taken from the complainant as soon as the incident is reported: this could be done by trained police officers.

While each case has its own history and peculiarities that may warrant the use of one specimen over another, urine is usually the specimen of choice for a toxicological investigation of suspected DFSAs. Compared to blood, urine samples allow for a longer window of detection of drugs and metabolites. These specimens should be collected and refrigerated as quickly as possible. The sooner a urine specimen is obtained after the alleged event, the greater the chance of detecting drugs that are quickly eliminated from the body.

3.1. Evidence collection kits

While DFCs that do not involve a sexual assault may require collection of only a urine and blood sample, sexual assault cases may require the collection of more evidence. It is imperative that medical facilities that are responsible for initiating the collection of biological samples from a possible victim of crime have appropriate evidence collection kits available, including appropriate urine and blood tubes for specimen collection for toxicological analysis.

Sexual assault evidence kits should contain:

- Evidence collection instructions and directives
- Unique identification information for each kit and for each item in the kit
- Self-sealing bags for each exhibit
- Evidence seal
- Paper bags for clothing and objects
- Paper (covering floor surface) to collect evidence while complainant undresses
- 5 ml blood tubes with sodium fluoride/potassium oxalate preservatives (recommended concentrations for NaF 2.5 g/l and potassium oxalate 2 g/l) for toxicological analysis (blood tubes should be completely filled)
- Buccal swabs or 5 ml blood tube with potassium-EDTA for genetic analysis
- Two sterile plastic 30 ml urine containers without preservatives
- Sterile DNA free swabs for body cavities and surfaces (for example to sample for traces of sperm, blood and saliva)
- Physiological saline solution for vaginal or anal rinsing and/or wet sterile swabs, if needed
- Wooden applicators to collect evidence from under nails
- Gloves, hair net and mask for collector
- Chain of custody form, with medical report and standard questionnaire for the health-care professional (complainant's full name, date and time of sampling, date and time of alleged assault, use of drugs and medication a week prior to the assault, date and time of last consented sexual relations, time of previous urination, etc.)

The chain of custody of evidence must be initiated and specimens should be submitted to a forensic toxicology laboratory that is capable of screening for a wide range of compounds at high sensitivity. If local regulations require the hospital's own laboratory to conduct some of the analyses, duplicate samples of the hospital-tested samples should be collected, if possible, for submission to the forensic laboratory. However, these preliminary/screening assays that may be performed at the hospital should be as wide and inclusive as possible with due consideration of the specificity and the detection limits of the screening technique.

3.2. Sample transfer and storage

Evidence collected from the victim of a suspected DFC must be properly sealed and secured. Biological specimens should be stored at 2-8 °C to help prevent

degradation. Specimens should be transported refrigerated to the laboratory as quickly as possible; in any event minimizing the time they are kept at temperatures above 25 °C.

3.3 Biological samples and sampling

Urine

Urine should be collected in any case in which the complainant reports within the first 120 hours (5 days) after the alleged assault. While in reality many of the drugs listed in annex 1 may have been eliminated from urine in less than 120 hours, a few may remain at low concentration.

A minimum of 50 ml of urine should be collected in at least two sterile containers (no preservative needed) and stored at 2-8 °C. If it is not possible to analyse samples within 24 hours, it is advisable to store the samples in a freezer (–18 °C). Unused samples should be stored in a freezer in case further analysis is requested for at last 12 months.

Whole blood

Blood should be collected in addition to urine, preferably within 48 hours of the alleged incident. Blood sampling should be performed with disposable syringes; the use of ethanol or other solvents with volatile fractions should be avoided in skin disinfection. At least two 5 ml samples should be collected in blood tubes containing compounds such as NaF and potassium oxalate (recommended concentrations for NaF 2.5 g/l and potassium oxalate 2 g/l) to prevent degradation and clotting with one sample used for analyses and the other retained in case of a need for a defence analysis. The blood samples should be refrigerated (at 2-8°C) as soon as possible. If it is not possible to conduct analysis within 24 hours, it is advisable to preserve the sample by storage in a freezer (after separating the plasma). Furthermore, it is advisable that sample residues are stored in a freezer (–18 °C) in case further analyses are requested at a later date. In cases where blood plasma may need to be separated by centrifugation from blood cells prior to analysis, the separation should be done before the freezing of whole blood.

It should be noted that the timeframes provided for detection of drugs in urine and blood are general guidelines and that many drugs will no longer be detectable in conventional samples such as urine, four or five days after ingestion.

Head hair

In cases of late reporting of the alleged assault or if chronic exposure to a drug must be assessed, head hair should be collected at least four weeks after the alleged assault. At least two hair samples (thickness of a pencil) should be cut as close to the scalp as possible (see the figure 1 in annex 5). It is very important that hair is sampled in a strict manner by properly trained personnel. A sampling protocol is given in annex 5. In cases of a shaven head, pubic, axillary, torso or leg hair may also be collected for analysis, although the interpretation of the quantitative results in these cases remains very difficult.

When segmental analysis is not possible (if only axillary, torso or leg hair are available), analysis could be eventually limited to a qualitative analysis, because the growth rate is not well established, as happens in head hair. Consequently, a positive result in these type of hair samples indicates that the alleged victim consumed the compound at any time, but not necessarily at the time of the assault.

Hair samples should be stored at room temperature, in a dry environment protected from light.

Other biological samples

In some cases, vomit from the scene of the alleged assault or from the clothes of the complainant may be a useful specimen. If a drug is not fully absorbed before vomiting occurs, the drug may be detected at relatively high amounts in a vomit stain. If collected, vomit or a vomit stain should be stored preferably frozen.

3.4. Other samples

If the scene of the alleged assault is searched, cups, drinking glasses, bottles, containers and liquids that may contain residues of drugs should be collected and submitted for analysis. Other items of evidence that could prove useful to the investigation include plates, foods, pharmaceutical products and prescriptions for medicines. Photographic/video (cameras, video recorders) and electronic evidence from computers may also prove useful to the case as there have been several instances when the perpetrator(s) have recorded the assault. For trace evidence, clothing, sheets and bedding, sexual devices, condoms, etc. should be collected with the classical precautionary measures for DNA analysis.

Police and crime scene investigators are normally trained to collect such evidence. Care should be taken that scene residues are packed individually in order to avoid cross-contamination of biological samples, especially if volatile compounds are implicated in the alleged assault.

4. Analytical considerations

Detection of DFSA and other DFC related substances may be a very demanding task requiring highly sensitive and selective analytical techniques in an appropriately equipped and staffed laboratory. Practical issues which need to be considered in establishing an analytical screening procedure for biological specimens (blood, urine and hair) for a wide range of substances related to these cases include sample size, speed of analysis, sensitivity and specificity of the methods.

The outcome or results of analysis of substances in urine may depend on the analytical method used. For example, many immunoassays will not detect all the known benzodiazepines. In addition, exposure to some benzodiazepines may not be detected after 2-3 days due to the typical high detection limits of immunoassays. In contrast, the use of some liquid chromatography-mass spectrometry/mass spectrometry (LC-MS-MS) methods may allow for the detection of benzodiazepines for four days or longer after ingestion of a dose likely to cause incapacitation.

False negative results due to the use of insufficiently sensitive methods may dissuade further investigation of the allegations and might discourage the victim from taking the matter further. Subsequently, immunoassays and enzymatic techniques, which have high detection limits, should be avoided. In case sufficient sample is available to permit further analyses, these techniques can be employed for preliminary screening, but very cautiously. It must also be recognized that a negative result may be due to insufficient sensitivity and that a positive result requires confirmation with a more selective technique. It must be ensured, when employing a preliminary screening technique, that a sufficient sample amount is available for further and confirmatory analyses.

In case of late sampling or use of techniques with low sensitivity or poor specificity/selectivity, the collection of larger sample amounts, preferably combined with a more efficient extraction of the analyte, or with concentration of an extract before chromatographic analysis, should be considered.

4.1. Substances encountered in DFSA and other DFC cases

The drugs used in DFSA and other DFC have one or more of the following properties: they can cause sedation, anterograde amnesia, are odourless and tasteless, dissolve readily in alcoholic or other beverages, are fast-acting (within 30 minutes or

so of administration), have a generally short plasma half-life (a few hours) and generally require a low dose to be effective (exceptions include ethanol, GHB, and GBL and related compounds).

It appears that almost any drug with mildly sedative properties may be used by a perpetrator. The availability of the drug to the perpetrator is a very important criteria to select a drug to commit drug facilitated crime. For example, over the counter drugs can be purchased by anyone. Prescription drugs may be available via a legitimate prescription, through medical services, or they might be bought on the Internet or on the street**.**

Substances which have been encountered in DFSA and other DFC cases are listed in annex 1.

4.2. Procedures and analytical strategy

As in all other forensic settings, bias towards any of the parties (complainant, suspected perpetrator, or others) must be avoided. Therefore, the reliability of qualitative and quantitative analytical findings is an essential requisite for correct toxicological interpretation. To this end, methods adopted in DFSA investigations should be properly validated for at least the following parameters; selectivity, calibration model (linearity), accuracy and precision, lower limit of detection (LLoD), lower limit of quantification (LLoQ), and analyte stability. Further parameters that may require validation are recovery, matrix effects (particularly important for LC-MS techniques), and ruggedness (robustness).

Only validated procedures based on adequately sensitive and selective analytical techniques, by hyphenated chromatographic and spectroscopic techniques such as LC-DAD, LC-MS, LC-MS-MS, GC-MS and GC-MS-MS, should be employed whenever available. However, LC-MS, LC-MS-MS and GC-MS-MS methods are highly recommended. If this is not possible, samples should be properly stored in a freezer (–18 °C) and referred for analyses to a specialized laboratory.

The confirmation of a compound's identity is an essential requisite in the forensic setting. In this regard, it must be recognized that hyphenated chromatographic and mass spectroscopic techniques provide, by definition, two sets of analytical data on the compound (retention behaviour and mass spectral data) that are sufficient for a proper confirmation for forensic purposes.

Quantification in blood, and for some substances also in urine (for example alcohol, GHB), should be performed whenever possible in order to make inference on the time of administration, the magnitude of the dose, and on the likely incapacitating effect of the drug. However, it must be stressed that these aspects of interpretation should be approached with extreme caution owing to the many factors involved. In

suspected DFSA a broad spectrum screen should be applied even if a single compound is strongly suspected or detected. Any compounds identified in urine should be looked for and measured in blood, if an appropriate sample is available.

The analytical strategy to be adopted depends on the time of sampling in relation to the alleged incident and on the specimen(s) available. Higher concentrations and/or longer detection times (typically up to 120 hours) usually apply to urine, although metabolites also need to be detected in this matrix. Parent compounds can be detected in blood for a short period of time (usually not longer than two days). Whenever urine and blood are not available or are sampled too late, consideration should be given to the detection of the parent compound in a hair sample collected at least four weeks after the event. It should be borne in mind that the detection of metabolites in hair may be useful for differentiation between external contamination and use.

Annex 2 lists the substances that should be first targeted in the analysis of urine, with minimum required performance limits (MRPL). Parent drugs and target analytes (metabolites) are included.

4.3. Analytical methodology

Urine and blood analysis

The following techniques are recommended for urine and blood analysis:

- *Volatile substances*—the analysis can be achieved by head space gas chromatography with either flame ionization detection (HS-GC-FID) or with mass spectrometry detection (HS-GC-MS);

  When headspace GC is used as the identification and detection method, special attention should be paid to the choice of conditions for sample preparation (sample pH, ionic strength, phase ratio, HS sample volume, incubation time and temperature), the GC oven-temperature programme and column specifications (polarity, film thickness) in order to optimize sensitivity and selectivity.

  When classical headspace equipment is not available, solid-phase micro extraction (SPME) is an alternative. Different SPME fibre types allow the adsorption of volatile and semi-volatile compounds onto the fibre, from which they are thermally desorbed in the GC injector. However, this technique requires a practical experience, particularly in DFSA and other DFC cases.

- *Non-volatile organic compounds*—Screening for drugs, metabolites and other non-volatile organic compounds should be performed using techniques able to acquire the full-scan spectrometric (MS) and spectroscopic (UV-Vis) data of the chromatographic eluate with a sufficient scan speed. Further,

comparisons should be made between unknown spectra and spectra obtained from authentic reference standards. The use of high resolution mass-spectrometry (HR-MS) for the identification of unknowns by accurate measurement of their mass-to-charge ratio and isotope pattern, if any, is also a viable alternative. Whatever the analytical technique adopted, its limitations (for example poor performance of polar and high molecular weight compounds for GC, or on thermolabile compounds) must be taken into the consideration.

Target analysis for medicinal drugs and drugs of abuse is recommended by GC-MS and LC-DAD or, if available, LC-MS-MS. In such cases, the use of methods optimized for the target analyte is very useful. However, a general screening method by GC-MS, combining derivatization and comparison with a recent spectra library may help to identify low levels of specific metabolites. But as samples are most often collected late, very low concentrations of the substances are expected: hence it is highly recommended to use LC-MS-MS or GC-MS-MS due their higher sensitivity and selectivity.

- *Ethanol* should be analysed by gas chromatography with flame ionization detection by direct injection (GC-FID) or head space (HS-GC-FID).

  The detection of ethanol conjugated metabolites (ethylglucuronide, ethyl sulfate) by LC-MS/MS or, after derivatization, by GC-MS may be considered in order to confirm or exclude the ingestion of alcoholic beverages when alcohol is not detected in blood or urine.

Hair analysis

The following techniques are recommended for hair analysis:

- GC-MS, GC-MS-MS and LC-MS-MS for illicit and prescription drugs
- LC-MS-MS for hypnotics, benzodiazepines and benzodiazepine-like drugs
- GC-MS-MS (or LC-MS-MS) for GHB and cannabinoids

If hair analysis has to be performed, preliminary appropriate washing of the sample is mandatory in order to minimize the risk of surface contamination. The washings should also be analysed. Hair segmentation is highly recommended to differentiate between only one and chronic consumption.

Recommendations for sample preparation

Sample preparation is an essential step of any analytical procedure, especially when high sensitivity is required. Adequate sample preparation results in an increase in method sensitivity and selectivity and can reduce matrix effects. Even when highly

selective detectors such as MS-MS or HR-MS are adopted, the beneficial impact of sample preparation should not be disregarded. On the other hand, artifact formation, loss of analyte or extract contamination should always be considered during method development.

Hydrolysis

Glucuronidation, a conjugation reaction involving the human UDP-glucuronosyltransferase (UGT) family of enzymes, plays an important role in the metabolic fate of many drugs. The enzymatic hydrolysis of urine may be required to ensure the detection of compounds and/or metabolites excreted as conjugates (unless reference standards of the conjugates are available).

Although enzymatic hydrolysis may be time-consuming, it has advantages of producing cleaner samples for analysis. The milder conditions result in better analyte stability during the hydrolysis process and thus reduce artifact formation. Different types of enzymes are commercially available, but the most frequently used are β-glucuronidase from *E. coli* or *Helix pomatia*, combined with arylsulfatase.

Sample procedure for the enzymatic hydrolysis of glucuronides

To 1 ml urine, add a suitable internal standard (as a glucuronide) in 1-2 ml of a suitable buffer (pH 5.2). Add β–glucuronidase (approx. between 1 000 and 20 000 units per ml urine) and arylsulfatase, if required. Incubate at 37°C overnight (approx. 16 h) or at least 90 minutes at 50°C. After incubation, adjust the pH of the solution appropriately for liquid-liquid or solid-phase extraction.

Chemical (for example with strong acid) hydrolysis may also be used, but it results in a loss of selectivity owing to degradation of the compounds of interest (for example for benzodiazepines). However, it can be considered as a viable, cheap and rapid alternative for specific analytes when their stability under hydrolysis conditions has been assessed.

Extraction

The extraction of analytes from a sample is of analytical importance and usually results in increased sensitivity and selectivity/specificity. It can be performed by liquid-liquid extraction (LLE) or solid phase extraction (SPE).

LLE exploits the relative affinity for, or partitioning of the analyte between two immiscible liquid systems, usually an organic solvent and an aqueous buffered solution. The process is based on well-defined thermodynamic relationships and has a

wide dynamic range. LLE has advantages of being fast, inexpensive and efficient, and works especially well with urine. However, LLE may involve high solvent consumption and care must be taken to avoid the formation of emulsions during extraction.

Extraction from aqueous samples (for example urine, blood) should be performed at an appropriate pH value with reference to the pKa of the target analytes. For screening purposes, extraction should be performed at various pH-values (for example pH 2-3 and pH 8-9). Saturation with neutral salts (for example NaCl) is recommended. A phase ratio (organic/aqueous) of 1:2 should be the aim in order to avoid co-extraction of a large amount of interferences.

SPE may be used as an alternative to liquid-liquid extraction. When appropriate sample dilution has been used, it will allow continuous flow of the specimens through the SPE cartridges and avoid clogging. SPE, which is suitable for both large and small sample volumes, typically requires use of less solvent than LLE and results in high extraction efficiency. The exploitation of the relative affinities of drug substances for the wide range of available solid phase chemistries and mechanisms (for example hydrophobic/hydrophilic interactions, ion-exchange, immuno-affinity) and appropriate selection of sample loading, washing or eluting buffers/solvents by the analyst results in cleaner and highly concentrated samples for analysis. This results in increased sensitivity and selectivity.

The availability of highly selective analytical techniques, such as those obtained for hyphenation of chromatographic and spectroscopic methods for example LC-MS, has led to the development of methods for "direct analysis" for certain analytes by means of the so-called "dilute and shoot" technique. Although this practice brings many advantages in terms of avoiding the possible drawbacks of sample preparation and of increasing the sample throughput, its application should be preceded by a thorough method validation, especially with reference to matrix effects.

Derivatization

Gas chromatography (GC) is a technique used in the detection and identification of organic compounds that are volatile and stable up to 350°C or so. The volatility of the desired analyte may be inherent (for example ethanol) or improved with derivatization. The process of derivatization increases the spectrum of substances that can be analysed by GC and can be performed prior to, or during the extraction. An important prerequisite for this approach is the availability of reference data (for example retention times, mass spectra) for the corresponding derivatives of toxicologically relevant compounds.

Silylating agents, for example TMCS (trimethylchlorosilane), but also BSTFA or MSTFA, are able to react with a wide spectrum of functional groups as hydroxyl-, carboxyl- , and amino-groups to produce volatile products suitable for GC analysis. This makes the process particularly suitable for systematic toxicological analyses (STA) and the most used in GC analysis. Silylating reagents have an added advantage

of not requiring removal of excess reactant prior to GC analysis. However, silyl derivatives are very sensitive to moisture so the reaction should occur under strict anhydrous conditions. Moreover, deposition of silica at the detector may be a problem.

Alternative derivatization procedures involve acetylation of compounds with amine and hydroxyl functional groups using acetic anhydride, and methylation of acidic groups using iodomethane. Derivatization procedures with mixtures of perfluorinated alcohols and anhydrides are widely applied, as well.

Example procedure for derivatization by silylation

To the dry residue after solvent extraction, add 20 µl of BSTFA containing 1% TMCS (this reagent is ready-to-use commercially available, and the manufacturers provide information of its potential use). Mix by vortex. Incubate at 80°C for 15 minutes.

Example procedure for derivatization by methylation

Prepare a solution of TMAH at 0,5g/ml in water (may be kept for six months at 20°C). Extemporaneously, add 100 µl TMAH to 2.0 ml DMSO. Add 200 µl of this reagent to the dry residue after solvent extraction. Mix by vortex. Leave for 2 minutes at laboratory temperature. Add 50µl iodomethane (work under extractor hood), vortex, and incubate at room temperature for 15 minutes. Stop reaction with 200 µl 0.1N HCl.

Best practice recommendations for laboratory analysis

Fully validated procedures according to internationally accepted standards should be available and employed for qualitative and quantitative analysis. Appropriate internal standards should be adopted. The use of stable isotope labeled internal standards (deuterium/carbon 13) is encouraged for mass spectrometric techniques.

The following recommendations should be considered by the laboratory when analysing specimens from suspected DFSA cases:

- Measurement of blood and urine concentrations must be done from the first available biological specimen; back calculation may be of some help if any alcohol is found. If ethanol intake is suspected or should be excluded, but is not detected in the blood and urine samples due to late sampling, the determination of conjugated ethanol metabolites may be performed.

- Enzymatic hydrolysis rather than acidic hydrolysis should be used for general purpose screening. It also allows lower detection limits in the benzodiazepine assays.
- Particular effort should be devoted to the detection of benzodiazepines and benzodiazepine-like drugs (Z-drugs) in urine, because of their frequent involvement in these cases. Different methods have been used successfully (for example GC-MS after hydrolysis, solvent extraction and derivatization; NCI-GC-MS after hydrolysis and SPE extraction; NCI-GC-MS-MS).

  In hair analysis, deuterated analogs of the compounds under study have to be added at a low enough concentration to avoid the isotopic contribution.
- When using an immunoassay for screening, the limits of sensitivity of this analytical technique should be recognized. When the immunoassay is devoted to a group of compounds (for example benzodiazepines), the limits of detection for the most common compounds and/or metabolites should be specifically assessed, noting that the cut-offs proposed by the manufacturer may be too high for their application in DFSA investigations. However, lower cut–off levels may be applied when proper revalidation of the method is performed.
- In general, the use of immunoassays in DFSA cases is discouraged. If immunoassays are used in a laboratory, sensitive chromatographic assays are absolutely mandatory for screening and confirming drug classes.

Example of an analytical strategy

Alcohol determination in blood and urine

Quantitative value of ethanol should be determined in blood and urine samples. If the analysis for ethanol is negative, particularly in cases of late sampling, the determination of ethylglucuronide and sulphate may be considered.

Hydrolysis of urine

Urine aliquot should be submitted to enzymatic hydrolysis before extraction for non-volatile organic compounds.

Extractions

Different solvents may be employed and each laboratory has to improved the best suitable after testing in the usual conditions of the laboratory.

1. *Extraction from blood and urine:*
   (a) GHB: neutral extraction
   (b) Cannabinoids: acidic extraction

(c) Other psychoactive substances: LLE at different pH values of the sample for acidic/neutral and basic analytes

2. *Extraction from hair:*

(a) GHB: after digestion in NaOH

(b) Barbiturates: acidic extraction

(c) Other psychoactive substances: LLE after incubation in Sörensen buffer

(d) Cannabinoids and amphetamines: LLE after digestion with NaOH and derivatization

Instrumental analysis

1. *GC-MS*

(a) Columns: for a general screening, classical non-polar 5% phenyl 95% methylpolysiloxane capillary columns are a good compromise.

(b) Detectors: when electron impact ionization (EI) is used, a derivatization step may be required. Negative chemical ionization (NCI) or positive chemical ionization (PCI) increases sensitivity and specificity.

2. *LC-MS-MS*

(a) Columns: most of the screening methods are based on reversed and normal phase columns. With the large number of different columns on the market, it is important for a laboratory to evaluate the column of interest under their own unique conditions.

(b) Detectors:

- Atmospheric pressure ion sources, either electrospray (ESI) or atmospheric pressure chemical ionization (APCI) may be used. The former is preferred for polar analytes, the latter for thermally stable and less polar analytes.
- If MS-MS is not available, fragmentation can be obtained before the MS analyser by collisionally induced dissociation (CID). However, spectra heavily contaminated with chemical noise are typically obtained.
- MS-MS detection should be adopted whenever available owing to its better selectivity. Triple quadrupole instruments offer more versatility and better quantification performance than ion-trap instruments, although at higher cost.

3. *Examples of analytical conditions (refer to the Bibliography for fully detailed methods)*

(a) GHB in blood and urine:

- Extraction is performed in acidic conditions with ethyl acetate, after adding GHB-D6 as an internal standard;

- Detection is performed by GC-MS, after derivatization with BSTFA.

(b) GHB in hair:

- Extraction is performed with ethyl acetate after incubation in NaOH at 80°C;
- Detection is performed by GC-MS-MS, after derivatization with BSTFA.

(c). Cannabis in blood:

- Extraction is performed with hexane/ethylacetate (2/1: vol/vol);
- Detection is performed by GC-MS-MS, after derivatization with BSTFA.

(d) Cannabis in hair:

- Extraction is performed with hexane/ethylacetate (2/1: vol/vol) on hair after incubation in NaOH;
- Detection is performed by GC-MS-MS, after derivatization with BSTFA.

(e) Other psychoactive substances and street drugs, in blood, urine and hair:

- Extraction is performed with liquid-liquid extraction (LLE) for blood and urine, and by hexane/ethylacetate (2/1: vol/vol) in hair, after digestion with NaOH at 80°C. Extraction by sonication/ incubation with methanol is a better choice for labile compounds as opiates and cocaine. Filtration is needed.
- Instrumental analysis is performed by LC-ESI-MS-MS with a C18 column, using SRM mode and PCI (except for barbiturates: NCI).

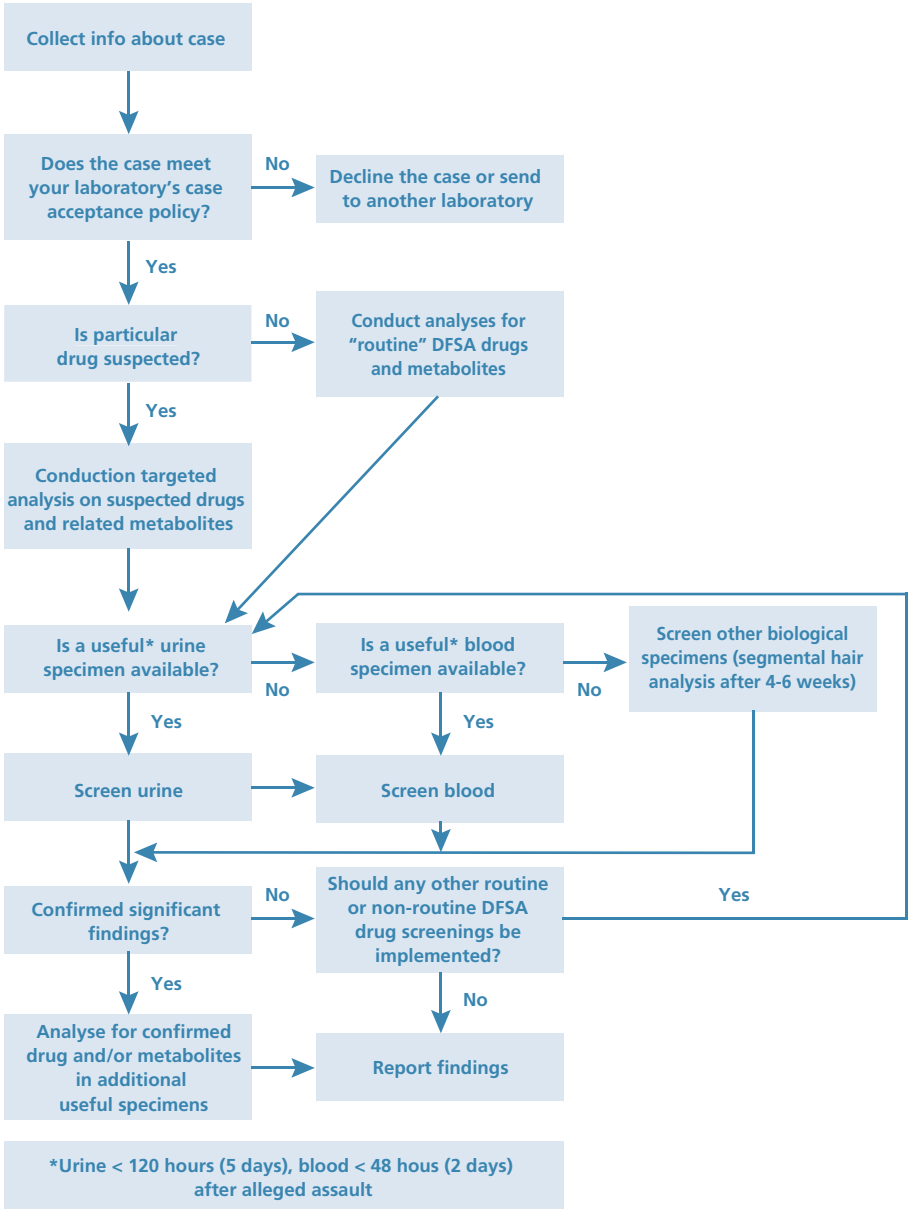
A number of source parameters have to be specifically adjusted and optimized for each analyte on a LC-MS instrument in use in the laboratory, as they might vary significantly between different manufacturers (ion source temperature, gas flows, fragmentor voltage, collision energy). Scan speed should be adjusted to obtain a minimum of 10 scans across the analyte peak to obtain an adequate quantification performance. Identification should be performed by monitoring at least two specific reactions (involving the pseudomolecular ion or a high mass fragment as precursor and avoiding poorly specific product ions, such as fragments resulting from the loss of water).

The linear dynamic range should be assessed. These may be relatively narrow, typically 10-500 µg/l in blood or urine and 100-500 pg/mg in hair. Matrix effect and ion suppression must be tested.

Due to the frequent upgrades of the LC-MS-MS (or GC-MS-MS) instruments by the suppliers, each laboratory has to optimize its own methodology: for example, one can have one method for neuroleptics and antidepressants and another for benzodiazepines. The aim is to obtain the best sensitivity and to achieve the analysis (and interpretation) as quickly as possible: solving DFSA and DFC cases can be considered as an emergency, in a judicial point of view.

Example of a flow chart for the toxicological analysis

The laboratory should have documented procedures for the overall process of handling DFSA cases. Below is an example of a strategy on how to handle toxicological analysis in DFSA cases.



Reference: LeBeau, M. A.: Laboratory management of drug-facilitated sexual assault cases; *Forensic Science Review,* 22:113;2010.

4.4. Reference compounds

The availability of reference standards for parent and/or metabolites is a prerequisite for qualitative and quantitative analysis. Certified reference materials (CRMs) can be used if available. CRMs have one or more property values are certified by a procedure, which establishes their traceability to an accurate realization of the unit in which the property values are expressed. Each certified value is accompanied by an uncertainty at a stated level of confidence.

In the absence of CRMs, commercial reference standards should be used. These reference standards/materials should be sufficiently pure to make them suitable for calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. These do not provide formal traceability but are useful and less expensive alternatives for initial method development

The exchange of analytical standards (and of analytical data as well) between laboratories should be encouraged, in accordance with national and international legislation.

4.5. Minimum required performance limits (MRPL)

In order to ensure that all laboratories report the presence of DFSA related substances in a uniform way, a minimum routine detection capability for testing methods should established. While it is inevitable that some laboratories will be able to identify a wider range or lower concentrations of substances than other laboratories, it is also recognized that minimum required performance levels (MRPL) must be met by all laboratories dealing with these cases.

MRPLs are technical performance parameters with which all laboratories should comply when testing for the presence of a DFSA related substance. They neither represent a threshold, nor a limit of detection (LLoD) or of quantification (LLoQ). Positive results may still be obtained with procedures with LLoDs below the established MRPL values. On the other hand, false negatives may result from the adoption of analytical methods with LLoDs above the proposed MRPLs.

Substances which should be first targeted in DFSA analysis with minimum required performance limits are listed in annex 2.

4.6. Factors out of the control of the forensic toxicologist

The validity of laboratory analysis or data is inextricably linked to the quality control measures adopted from the collection of evidence, through documentation,

transportation and storage up to the receipt of samples by the forensic toxicologist. Some of these factors, which are out of the control of the forensic toxicologist, include the clinical examination, collection and storage of evidence by the law enforcement/health professionals, and preliminary screening where applicable.

One of the most common shortfalls in clinical examination at the emergency unit/forensic emergency unit is that the clinician may not be aware of the possibility that a DFSA may have occurred. This could result from the fact that the victim may not be aware that a drug has been placed surreptitiously in his/her drink, or that the victim does not want to disclose his/her voluntary consumption of an illicit drug prior to the suspected incident. Blood and urine should be collected immediately by the clinician who should note the date and time of the collection. It is also the responsibility of the clinician to collect a sufficient volume of blood and urine. Finger-prick blood specimens and buccal swabs are of poor evidentiary value and similarly the collection of hair on the day of the suspected event.

Sample storage conditions at the hospital are also not under the control of the forensic toxicologist. A specific person should be tasked with the responsibility of storing the samples in a secured environment, under temperature-controlled conditions.

The importance of ongoing communication between the toxicologist and the investigating police team should be emphasized. This enables the results to be discussed, provides an opportunity to request further information (if needed) and to establish whether there is a legitimate reason for the presence of any drugs detected in case samples.

4.7. Staff skill requirements and equipment considerations

The complex nature of DFSA cases and the concomitant analytical challenges necessitates well-trained scientists and sufficiently equipped laboratories.

Staff should have skills in the fundamental aspects of analytical chemistry, forensic- and clinical toxicology as well as in pharmacology, including a sound background in drug metabolism and pharmacokinetics. The analytical chemistry training should include knowledge and use of hyphenated techniques such as chromatography-mass spectrometry (GC-MS, LC-MS and Tandem MS technologies), sample preparation and extraction procedures applicable to trace analysis, method validation, data handling and reporting of results. The respect for human dignity and confidentiality of personal information are essential attributes and should be part of their daily operation. Staff should have the ability to interact with investigating officers and with the criminal courts.

In view of the financial outlay required to fully equip a forensic toxicology laboratory in order to fulfil requirements mentioned above, it is recommended that analysis be directed to designated laboratories, which have the analytical capacity to reach the minimum required performance limits (MRPL). Adequate sampling, evidence collection and storage should still be performed at a local level to ensure that the results produced by the regional laboratories are scientifically accurate and valid.

5. Interpretation of results

As stated earlier, a negative toxicological finding does not exclude the use of a drug in a possible DFSA case. Negative findings may be due to:

- Late sample collection resulting in drug and/or metabolite concentrations below the MRPL of the laboratory. Note that the table in annex 3 shows the half lives ($T_{1/2}$) for some drugs. This may be useful in evaluating the time that a drug would be expected to remain in the blood or urine after ingestion and may assist in the estimation and verification of the time at which the complainant claims to have lost his (her) consciousness.
- Use of a substance that is unknown to the laboratory and/or which is beyond its analytical capability (for example new "designer drugs" or substances showing high-potency at low concentrations).
- Breaking down on storage for some drugs (for example zopiclone and new drugs such as methcathinones). If it is not possible to analyse samples within 24 hours, freezing samples (–18 °C) as soon as possible is the best option to avoid this degradation.

Any medication or procedures used in treatment of the complainant should be considered in interpretation. Concomitant administration of a diuretic drug for instance, may result in urine dilution causing the concentration of the suspected compound to be below the MRPL sooner than would be the case if a single compound were administered. Co-administration of alcohol may have a profound effect on the pharmacokinetics and pharmacodynamics of the administered drug. Therefore, results should be reviewed in context of the entire investigation and case history.

5.1. Urine

A positive identification in urine is normally sufficient proof that the victim was exposed to a drug within a period of one to five days before the sample was collected. It should be noted that the period of detection depends on the substance, as well as the dose that was administered. The practice of correlating the urinary concentration at the time of sampling with the dose and effect of the drug at the time of exposure is discouraged.

Due to the endogenous nature of GHB, caution must be taken in the interpretation of positive findings. GHB concentrations have been shown to increase *in vitro* in urine samples during the storage. Therefore, the actual recommended cut-off concentration for endogenous GHB in urine is 10 mg/l to help to distinguish endogenous from exogenous GHB.

5.2. Blood

A positive finding in blood may be proof of an exposure to a drug within a shorter time period compared to urine (usually less than 48 hours). Blood concentration may provide information on the possible pharmacological effect at the time of the alleged incident. A concentration of a drug in blood, along with pharmacokinetic information may be used to predict and correlate the symptoms described by the victim. Anterograde amnesia and/or unconsciousness may result in difficulty to accurately estimate the time of the suspected incident.

Finding GHB in the blood may help for the interpretation of the results obtained in the urine. Like in urine, GHB concentrations have been shown to increase *in vitro* in blood samples during the storage. Therefore, researchers have suggested that an adequate cutoff for GHB as high as 2 mg/l if blood is collected aseptically and stored at +4°C. GHB aciduria is a rare genetic disorder (succinic semialdehyde dehydrogenase deficiency) in which the endogenous GHB levels in blood and urine of its patients are increased.

One has to differentiate endogenous production from exogenous administration and the strongest interpretation of any GHB findings can be made when you have complimentary findings in urine and blood.

5.3. Hair

A positive finding in hair may provide proof of an exposure during the growth period analysed. Segmental analysis is important to provide information on the appropriate time period during which the suspected crime occurred. Segmental hair analysis can provide information as to whether the substance was taken regularly before the alleged incident or if the substance had been ingested only in a short timeframe that corresponded to the moment of the incident. It is important that segmental hair analysis results be considered in the context of other evidence to support the case. The mean growth speed of head hair 1.0 +/- 0.2 cm per month is commonly used. However, the growth speed for some hair could be as low as 0.6 cm per month or up to 2 cm per month.

Special attention has to be given to the hair analysis of GHB. As GHB is an endogenous compound, normal levels of endogenous GHB in each individual vary. The strand of hair has to be cut in 5 to 10 small segments (0.3 to 0.5 cm long) and each segment analysed for GHB in order to identify if one segment has GHB concentration 10 times higher than the others, suggesting possible administration of exogenous GHB.

6. Data collection

Information available on DFSA and other DFC is primarily based on anecdotes and limited data are available on its frequency and current trends. Effective policies to address the increase in DFSA and other DFC require the availability of accurate and reliable data and information, including the types of substances used and prevalence, in other to define national and regional trends. The required data needs to have a high degree of certainty and must be the result of cooperation between all the agencies involved: the police, the medical personnel, the forensic toxicologist and the judicial authorities. The data collection procedures used in some countries, for example surveys, responses to charity helplines, governmental statistics, data published by scientists in meetings, need to be standardized to facilitate comparability of data.

Based on limited available data, it would seem that most of the substances implicated in DFSA and other DFC cases (except alcohol) are under international control and scheduled under the United Nations Single Convention on Narcotic Drugs, 1961 and the Convention on Psychotropic Substances, 1971. However, psychotropic substances such as GBL and some antihistamines used in sexual assault cases, remain outside international controls, although controls exist at national level in some countries. Such disparities in national and regional legislations allow trafficking of psychoactive substances through different countries, often via the Internet and courier, and make it difficult to obtain accurate data on the nature and the extent of the problem.

While expert perception points to an increase in the number of DFSA and other DFC cases, constraints exist with regard to the availability and collection of data. Victims may be unwilling to go to the police and/or the hospital to be examined. Police records in some countries can, in principle, provide some information. But they cannot comprehensively describe the phenomena since DFSA are often not reported to the authorities and, when they are, they may be classified under more generic offenses (such as rape). The actual prevalence of sexual assaults, particularly DFSA cases, is underreported and only very little information and statistics are available. Forensic laboratories may not always report their data on DFSA and governmental health organizations do not collect such data in all countries. In recognition of the difficulties in obtaining accurate data on the subject, the Commission on Narcotic Drugs (CND) in its resolution 53/7 (2010) invited member states and regional organizations to promote research into the administration of psychoactive substances for sexual assault or other criminal purposes with a view to measuring the extent of the phenomenon, ascertaining the modi operandi of assailants and identifying the psychoactive substances used, whether under

international control or not. The Commission further noted that it is essential to highlight the problem and that an improvement in national capacity to collect data is needed.

In order to support countries trying to enhance the quality and availability of data on DFSA and other DFC, there is the need to develop standards on how to collect the data through population-based surveys and administrative recording systems on crime and criminal justice. Victim surveys are potentially a good tool to collect data on DFSA and other DFC as they reach out to the potential victims. Standard guidelines should be developed to improve the current recording system for crimes in order to ensure that DFSA and other DFCs are properly recorded and the data analysed.

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Annexes

Annex 1. Actual substances encountered in DFSA and other DFC cases

GHB

GHB, GBL, 1,4-BD, valerolactone

Benzodiazepines

Alprazolam
Bromazepam
Chlordiazepoxide
Clobazam
Clonazepam
Clorazepate
Clotiazepam
Cloxazolam
Diazepam
Estazolam
Flunitrazepam
Loprazolam
Lorazepam
Lormetazepam
Medazepam
Midazolam
Nitrazepam
Nordazepam (=nordiazepam)
Oxazepam
Phenazepam
Prazepam
Temazepam
Tetrazepam
Triazolam

Z-drugs (hypnotics)

Zaleplon
Zolpidem
Zopiclone

Antihistamines and others

Antihistamines

- Brompheniramine
- Cetirizine
- Chlorpheniramine
- Cyclobenzaprine
- Diphenhydramine
- Doxylamine
- Hydroxyzine
- Niaprazine

Others

- Aceprometazine
- Alimemazine
- Amitriptyline
- Chloral hydrate
- Clonidine
- Clozapine
- Cyamemazine
- Dextromethorphan
- Haloperidol
- Meprobamate
- New antidepressants
- Oxomemazine
- Valproic acid

Barbiturates

- Amobarbital
- Barbital
- Pentobarbital
- Phenobarbital
- Secobarbital

Opiates and opioids (licit narcotic analgesics)

- Codeine
- Dihydrocodeine
- Hydromorphone
- Methadone
- Morphine
- Oxycodone

Street drugs and traditional drugs of abuse

Cannabinoids

Natural (THC)
Synthetic cannabinomimetics (Spice, etc.)

Opiates

Heroin
Morphine

Cocaine

Cocaine and crack cocaine

Amphetamines

Amphetamine
PMA
MBDB
MDA
MDEA
MDMA
Methamphetamine

Others

Atropine
Ayahuasca
Cathinone and cathinone derivatives
Hallucinogenic mushrooms
Kawa-kawa
Ketamine
LSD
Mescaline
Phencyclidine
Piperazine group
Poppers
Salvinorine A
Scopolamine

Ethanol (alcohol)

Annex 2. Substances that should be targeted for in the analysis of urine, with minimum required performance limits (MRPLs), including parent drugs and metabolites

This list is a comprehensive list and each laboratory needs to select the substances which are most commonly used in their region and/or country.

> Reference: Recommended Maximum Detection Limits for common DFSA drugs and metabolites in urine samples, Drug-Facilitated Sexual Assault Committee, Society of Forensic Toxicologists (SOFT).

Laboratories are encouraged to screen at the proposed detection limits or lower according to their current capacity.

GHB

Gamma-hydroxy butyrate	10 mg/l

Benzodiazepines

Alprazolam and alpha-OH-alprazolam	10 µg/l
Bromazepam and OH-bromazepam	10 µg/l
Chlordiazepoxide	10 µg/l
Clobazam	10 µg/l
Clonazepam and 7-aminoclonazepam	5 µg/l
Clotiazepam	10 µg/l
Diazepam	10 µg/l
Estazolam	10 µg/l
Flunitrazepam and 7-aminoflunitrazepam	5 µg/l
Loprazolam	10 µg/l
Lorazepam	10 µg/l
Lormetazepam	10 µg/l
Midazolam	10 µg/l
Nitrazepam and 7-aminonitrazepam	5 µg/l
Nordiazepam	10 µg/l
Oxazepam	10 µg/l
Phenazepam	5 µg/l
Prazepam	10 µg/l
Temazepam	10 µg/l
Tetrazepam	10 µg/l
Triazolam and 4-OH-triazolam	5 µg/l

Z-drugs (hypnotics)

Zaleplon	10 µg/l
Zolpidem and metabolites	10 µg/l
Zopiclone and metabolites	10 µg/l

Antihistamines and others

Aceprometazine	10 µg/l
Alimemazine	10 µg/l
Amitriptyline and nortriptyline	10 µg/l
Brompheniramine and desmethyl-B	10 µg/l
Carisoprodol and meprobamate	50 µg/l
Cetirizine	10 µg/l
Chlorpheniramine and desmethyl-C	10 µg/l
Citalopram and desmethylcitalopram	10 µg/l
Clonidine	10 µg/l
Cyamemazine	10 µg/l
Cyclobenzaprine	10 µg/l
Desipramine	10 µg/l
Diphenhydramine	10 µg/l
Dextromethorphan	10 µg/l
Doxepin and desmethyldoxepin	10 µg/l
Doxylamine and desmethyldoxylamine	10 µg/l
Fluoxetine and norfluoxetine	10 µg/l
Haloperidol	10 µg/l
Hydroxyzine	10 µg/l
Imipramine	10 µg/l
Niaprazine	10 µg/l
Oxomemazine	20 µg/l
Paroxetine	10 µg/l
Sertraline and norsertraline	10 µg/l
Valproic acid	50 µg/l

Barbiturates

Amobarbital	25 µg/l
Butalbital	25 µg/l
Pentobarbital	25 µg/l
Phenobarbital	25 µg/l
Secobarbital	25 µg/l

Narcotics and non-narcotics analgesics

Codeine	10 µg/l
Dextromethorphan	10 µg/l
Dihydrocodeine	10 µg/l
Fentanyl	10 µg/l
Hydrocodone	10 µg/l
Hydromorphone	10 µg/l
Meperidine (pethidine)	10 µg/l
Methadone	10 µg/l
Morphine	10 µg/l
Oxycodone	10 µg/l
Pethidine	10 µg/l
Propoxyphene and norpropoxyphene	10 µg/l

Street drugs and miscellaneous drugs

Cannabinoids

THC-COOH	10 µg/l

Opiates

6-mono-acetyl-morphine (MAM)	10 µg/l
Morphine	10 µg/l

Cocaine

Benzoylecgonine	50 ng/ml
Cocaine	50 µg/l
Cocaethylene	50 µg/l
Methylecgonine	50 µg/l

Amphetamines

Amphetamine	10 µg/l
Methamphetamine	10 µg/l
MBDB	10 µg/l
MDA	10 µg/l
MDEA	10 µg/l
MDMA	10 µg/l

Ketamine and norketamine	1 µg/l
Lysergic acid (LSD)	1 µg/l
Phencyclidine	10 µg/l
Piperazine group	10 µg/l
Scopolamine	10 µg/l

Ethanol	0.1 g/l
Ethyl glucuronide	100 µg/l

Annex 3. Half-lives ($T_{1/2}$), therapeutic and toxic concentrations for select CNS depressants

$\mathbf{T_{1/2}}$ may be useful in evaluating the time that a drug would be expected to remain in the blood or urine after ingestion and may assist in the estimation and verification of the time at which the complainant claims to have lost his(her) consciousness.

References: Baselt R., 2011; Drummer O. H., 2001, Moffat A. C. *et al,* 2011.

Molecule	*Therapeutic concentrations in the blood (µg/l)*	*Toxic concentrations in the blood (µg/l)*	*$T_{1/2}$ (hours)*
Alprazolam	5-50	75	12-15
Alimemazine	50-400	>500	6-18
Bromazepam	80-200	300-500	8-19
Cetirizine	250-450	NA	6.5-10
Chlordiazepoxide	400-2 000	5 000	20-40
Clobazam	100-600	NA	10-20 (metab: 50)
Clonazepam	10-80	100-120	19-40
Clotiazepam	10-700	1 000-5 000	4
Diazepam	250-1 500	5 000	20-30
Cyamemazine	50-400	NA	10
Estazolam	55-100	1 000	10-24
Doxylamine	50-400	NA	10
Flunitrazepam	1-15	50	20
Haloperidol	5-40	>500	10-40
Hydroxyzine	50-90	>100	13-27
Loprazolam	5-10	NA	6-23
Lorazepam	20-250	300	12
Lormetazepam	1-25	NA	10
Meprobamate	5 000-20 000	>50 000	6-17
Midazolam	40-100	1 000-1 500	2-3
Nitrazepam	10-180	200-500	20-25
Nordazepam	200-2 000	2 000	65
Oxazepam	200-2 000	3 000	8
Prazepam	10-200	1 000-5 000	metab: 65
Temazepam	20-900	1 000	5-8
Tetrazepam	50-600	6 000	10-26
Triazolam	2-20	200	1.5-3 (metab: 4)
Zolpidem	30-300	500	1.5-4.5
Zopiclone	10-50	150	3.5-6.5

NA: data non available
metab: metabolite

Annex 4. Example of information collection worksheet for DFSA cases

Reference: LeBeau M. A.: Laboratory management of drug-facilitated sexual assault cases; *Forensic Science Review,* 22:113:2010.

Drug-facilitated sexual assault information collection worksheet

Agency: _____ City: _____

Contact Person: _____ Phone: _____

Name of Victim: _____ Name of Suspect (s): _____

Case Number(s): _____ Date and Time(s) of Assault: _____

Date of Contact: _____ Examiner Collecting Information: _____

_____

1. Were any specimens collected and what were they? _____
2. When were the specimens collected (date and times)? _____
3. What symptoms did the victim describe? _____

_____

4. Were there any witnesses? If so, how did they describe the victim? _____

_____

5. How long did the victim have amnesia or was the victim unconscious? _____

_____

6. Did the victim consume any alcohol? If so, how much (types of alcohol, size of drinks, over how many hours, etc.)? _____
7. Did the victim voluntarily take any drugs (recreational, prescription or over-the-counter)? If so, which ones, how much and when? _____
8. Did the victim urinate prior to providing any specimens? If so, approximately how many times? Please indicate the time of the previous urination. _____
9. What is known about the suspect in regard to occupation, hobbies, drug history and medical history? _____
10. What recreational and prescription drugs does the suspect have ready access to?

_____

11. Additional notes of interest: _____

_____

Annex 5. Example of hair collection checklist

Reference: FBI Laboratory, Chemistry Unit, United States.

Collection steps for hair specimens for analysis of exposure to drugs

Hair has been used as a specimen to evaluate people's exposure to certain drugs and poisons for many years. While this specimen does not allow for as comprehensive a drug screen as more common specimens (for example blood and urine), it does allow for assessment of exposure over longer periods of time (i.e. months for hair as opposed to hours or days for blood and urine). For this reason, hair becomes a particularly useful specimen when there is a significant delay between the suspected last time that exposure to the drug took place and when collection actually occurs. Typically, head hair is utilized.

It is advised to wait at least four weeks after suspected drug exposure before collecting the hair samples. Cutting the hair is not allowed during this period of time. The following steps, which vary greatly from those required to take hair samples for trace evidence examination, should be followed for the collection of hair submitted for drug testing (two hair clippings should be collected and packaged separately):

Step one: Assemble all collection materials to include:

- Chain-of-custody and consent forms (if applicable)
- White envelope (letter size)
- Evidence tape or evidence bag
- Aluminum foil (optional)
- Scissors
- Twist tie

Step two: Label two white envelopes to include:

- Name of the person from whom the hair is collected
- Location that hair is collected from
- Collection date
- Name of the person collecting the hair

Step three: Using a twist tie, secure a twist of hair (about the diameter of a pencil) from the crown of the head (figure 1).

Step four: Cut the hair as close to the scalp as possible (figure 1).

Step five: Ensure the cut hair is tightly secured with the twist tie and place into the white envelope. Samples may be secured with aluminum foil before mailing in order to retain orientation. Seal envelope. Secure with evidence tape or place into evidence bag.

Step six: *Repeat* steps 3 through 5 in order to collect a second sample.

Figure 1. Cut hair next to scalp.

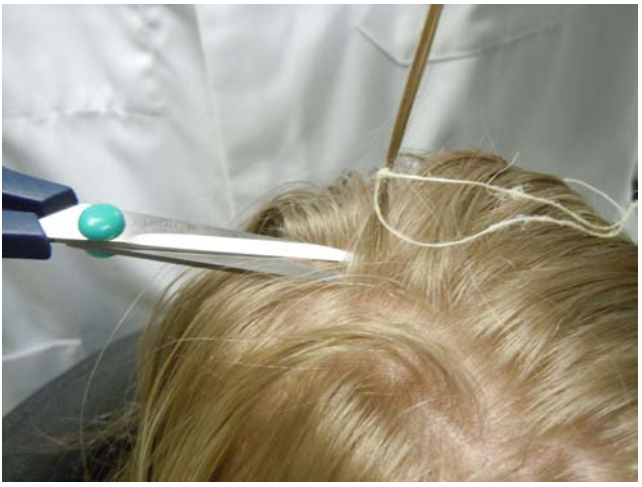

Photo credit: Laboratoire Toxlab.

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