Investigating the role of routine drug analyses in survivors of sexual offences admitted to the Clinical Forensic Unit at Victoria Hospital

By

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DECLARATION

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degrees Celsius
µL	Microliter
AAFS	American Academy of Forensic Sciences
ACE	Acetaminophen
ACN	Acetonitrile
ADHD	Attention-deficit/hyperactivity disorder
ALP	Alprazolam
AMP	Amphetamine
AMI	Amitriptyline
ASB	Academy standards Board
BEN	Benzoylecgonine
CAL	Calibrator
CBD	Cannabidiol
CE	Capillary electrophoresis
CET	Cocaethylene
CFU	Clinical Forensic Unit
CNS	Central nervous system
COC	Cocaine
COD	Codeine
CRM	Certified reference material
CV	Coefficient of variation
DT	Detection time
DFC	Drug facilitated crime
DFSA	Drug Facilitated sexual assault
DFSO	Drug Facilitated sexual offence
DIP	Diphenhydramine
DOA	Drugs of abuse
FPS	Forensic Pathology Services
FTU	Forensic Toxicology Unit
HQC	High quality control
HPLC	High-performance liquid chromatography
HCD	Hydrocodone
ISTD	Internal standard

L	Liter
LC	Liquid chromatography
LOD	Limit of detection
LQC	Low quality control
MA	Massachusetts
MET	Methamphetamine
MEQ	Methaqualone
mg	Milligram
MQC	Middle quality control
MRM	Multiple reaction monitoring
MS	Mass spectrometry
n	Number of participants
NC	North Carolina
ND	None detected
NH4OH	ammonium hydroxide
NH4CH3CO2	Ammonium acetate
PA	Pennsylvania
PAOH	Pan American Organization of Health
PQC	Positive quality control
PTSD	Post-traumatic stress disorder
RT	Retention time
Rpm	Revolutions per minute
SACENDU	South African Community Epidemiology Network on Drug Use
SANSU	South African National Accreditation System
THC	Delta-9-tetrahydrocannabinol
THC-COOH	11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol
TX	Texas
$t_{1/2}$	Half-life
UK	United Kingdom
USA	United States of America
UNODC	United Nations Office on Drugs and Crime
UPLC-MS/MS	Ultra-performance liquid chromatography-tandem mass spectrometry
WHO	World Health Organization

ABSTRACT

Introduction: Toxicological analysis is an important component of drug-facilitated sexual assault (DFSA) investigations, as it allows for identification and interpretation of substances involved. Currently, forensic toxicological analyses are not routinely provided to DFSA survivors in South Africa. The aim of this study was to investigate the utilisation and applicability of a targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for common drugs of abuse in cases of suspected drug facilitated sexual offences.

Methods: Blood and urine samples from 17 consenting adult survivors, who reported to the Clinical Forensic Unit at Victoria Hospital (August 2022 – December 2022) in Cape Town, South Africa, were analysed using a validated LC-MS/MS method targeting 31 common drugs of abuse. Samples were prepared for analysis using a Waters Ostro® pass-through plate extraction for blood and simple dilution extraction for urine. Case histories were obtained from participants by the attending medical practitioner using a standardised data collection sheet.

Results: Majority of the participants reported to the clinic within 24 hours after the alleged offence (64.7%). Several participants reported consuming alcohol (64.7%), medicinal drugs (23.5%), or recreational drugs (35.3%) prior to, or at the time of the offence. A psychoactive drug was detected in 58.8% of cases. Methamphetamine (and its metabolite amphetamine) were the most frequently detected analytes (41.2% and 35.3% of cases, respectively).

Conclusion: Accurate and reliable toxicological analysis is vital in processing DFSO cases. This study determined that the analytical method is useful in DFSO cases as over half of the participants tested positive for at least one drug, and most self-reported recreational drugs were included in the panel. Recommendations include expanding the panel to include additional pharmaceutical drugs and incorporating ethanol analysis into the routine workflow to provide comprehensive testing to survivors of DFSO and support the criminal justice system in South Africa.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Sexual violence is a global issue that approximately one-third of all women have experienced at some point in their lives, and is not restricted by sex, age, ethnicity, or social class (Costa, Lavorato & Baldin, 2020; Hiddink-Til, Teunissen & Lagro-Janssen, 2021). Due to sexual assault being a human rights violation and the negative psychological and social impact on the survivor, sexual violence was defined as a global health problem in 1993 by the Pan American Organization of Health (PAOH) and World Health Organization (WHO) (Costa, Lavorato & Baldin, 2020). Sexual assault is defined in international legislation, and typically includes both penetrative (rape) and non-penetrative assault (indecent assault), with the focus being on the lack of consent from the victim (Hurley, Parker & Wells, 2006; Juhascik et al., 2007; Tiemensma & Davies, 2018). In South African legislation, as defined in the Criminal Law (Sexual Offences and Related Matters) Amendment Act 32 of 2007, the term sexual offence is used, and it includes any type of sexual act (penetrative or non-penetrative) performed on someone who cannot or did not provide consent. For example, taking advantage of individuals that cannot provide consent, such as individuals with intellectual disabilities or individuals who are intoxicated (South African Government, 2007).

The consequences of sexual violence are felt by the victim in both the physical and mental aspects of their lives (Costa, Lavorato & Baldin, 2020). Short-term consequences that the victim could potentially also have to cope with include pregnancy, sexually transmitted infections, reproductive tract infections and other potential injuries that occurred during the assault (Costa, Lavorato & Baldin, 2020). The long-term consequences may include sexual and gynecological disorders, as well as mental health issues such as depression and post-traumatic stress disorder (PTSD) (Anderson et al., 2019; Costa, Lavorato & Baldin, 2020; Hiddink-Til, Teunissen & Lagro-Janssen, 2021). Children and young adults who experience sexual abuse are more likely to have physical and mental health issues that could lead to suicidal ideation (Lopez-Castroman et al., 2013) or engagement in riskier behaviour such as excessive drinking and drug consumption to cope with the trauma or remaining in the same dangerous environment (Steele et al., 2019).

Multiple studies have been conducted globally to determine the prevalence of sexual assault. A survey was conducted in 2015 on 1000 students at universities in the United Kingdom. It was reported that 33.3% of female and 12.5% of male students had been sexually assaulted

(Goldhill, 2015). In 2009, a national study comprising of 5001 women was conducted in the United States which revealed that 18% of all women have been raped in their lifetime, however only one out of six of these victims reported the assault to the police (Kilpatrick et al., 2007; Wille et al., 2021).

Sexual offences are also a concern on the African continent. According to Beyene et al. (2019), the lifetime prevalence of sexual offences among female students attending academic institutions in sub-Saharan Africa ranged from 21% to 29%. Beyene et al. (2019) also investigated 23 studies that included 40,409 participants with 11,511 cases of lifetime sexual violence based in Sub-Saharan Africa and determined that the pooled estimate of the lifetime prevalence of sexual violence was 26.2%. A study was conducted, investigating the worldwide prevalence of non-partner sexual violence. Central sub-Saharan Africa had the highest estimated frequency of 21% and southern sub-Saharan Africa was the second highest with an estimated frequency of 17.4% (Abrahams et al., 2014). In South Africa, it was found that 24.9% of women living in Rustenburg, North West Province, had been sexually violated, and 9.0% of these women experienced sexual offences by the age of 15 years (Steele et al., 2019). Meinck et al. (2016) also found that children aged 10 to 17 years in the South African provinces Mpumalanga and the Western Cape had a 9% prevalence for experiencing sexual abuse. Orkin, Hirschowitz & Worku (2000) studied national and provincial South African surveys from 1998, which indicated that survivors of rape were usually between 16 and 25 years old and had been raped by men they had known with and 34.6% reporting they had been raped by relatives or partners (Orkin, Hirschowitz & Worku, 2000). The Rape Surveillance Project of the University of South Africa reported that rapes reported to the police were mainly those committed by strangers (55.3%) (Orkin, Hirschowitz & Worku, 2000). This could be an indication that individuals are more likely to report sexual assaults if the perpetrator is unknown to them. The lifetime prevalence for sexual offences at a university campus in the Eastern Cape for both male and female students was 37.9% (Ajayi, Mudefi & Owolabi, 2021).

A popular term in the present social climate is “rape culture", which refers to a prevailing acceptance or normalisation of sexual abuse by certain societies (Ajayi, Mudefi & Owolabi, 2021). To understand the motivation and determine the prevalence of rape (penetrative sexual offence) in South Africa, Jewkes et al. (2010) conducted an anonymous survey in the Eastern Cape and KwaZulu-Natal. Of the 1,686 men who participated in the in the study, 27.6% had forced a woman to have sex without consent and 3% had forced a man to have sex without consent. Most of the men had raped more than once and 7.7% had raped more than 10 times

(Jewkes et al., 2010). Based on the results of the study, the main motivation for rape was due to a sense of entitlement and the use of sexual violence on individuals as a form of punishment was observed in half of the men who raped. The men would often rape a woman, or her child in some instances, to punish the woman (Jewkes et al., 2010). Of the men who had raped, 5% had raped a child under the age of 15 years, the motivation behind these rapes were mainly boredom and opportunity as the men believed the young child would not tell anyone (Jewkes et al., 2010).

Despite a large body of research, it is difficult to estimate the exact number of sexual assaults that occur each year in various countries due to underreporting (Juhascik et al., 2007; Grela, Gautam & Cole, 2018; Costa, Lavorato & Baldin, 2020; Wille et al., 2021; Skov et al., 2022). The main reasons for underreporting are largely attributed to the survivor fearing backlash from the perpetrator, feeling ashamed and at fault for the sexual assault, not trusting the judicial system, or fear of how people would perceive them if they spoke up (Anderson et al., 2019; Costa, Lavorato & Baldin, 2020; Grela, Gautam & Cole, 2018; Hiddink-Til, Teunissen & Lagro-Janssen, 2021; Juhascik et al., 2007; Skov et al., 2022; Tiemensma & Davies, 2018; Wille et al., 2021).

1.2 Drug-facilitated sexual assault

The United Nations Office on Drugs and Crime (UNODC) defines drug facilitated crimes (DFCs) as criminal acts that are carried out by administering a substance to an individual with the intention of impairing their behaviour, perceptions, or decision-making ability; or by taking advantage of an impaired person after they voluntarily consume an incapacitating substance (UNODC, 2011; Wille et al., 2021). Any form of sexual assault that occurs as a DFC is referred to as a drug-facilitated sexual assault (DFSA) (Wille et al., 2021).

DFSA is therefore defined as assaults in which victims are subjected to non-consensual sexual acts, while they are incapacitated or unconscious because of the effects of alcohol and/or drugs and are therefore prevented from resisting, or unable to consent (Anderson, Flynn & Pilgrim, 2017; Anderson et al., 2019; Costa, Lavorato & Baldin, 2020; Juhascik et al., 2007; Poulsen et al., 2021; Skov et al., 2022; Tiemensma & Davies, 2018; Wille et al., 2021). As mentioned previously, South Africa's legislation classifies both penetrative and non-penetrative sexual assaults as sexual offences, therefore in South Africa the term drug-facilitated sexual offence (DFSO) may be used.

DFSOs can be divided into two categories, proactive DFSO and opportunistic DFSO. Proactive DFSO is an active or forcible administration of a psychoactive or incapacitating substance with the intent to render an individual intoxicated or unconscious to facilitate non-consensual sexual activity. Whereas, in opportunistic DFSO, the perpetrator is not directly involved with the intoxication of the individual, however, the perpetrator takes advantage of an individual's intoxicated state to engage in non-consensual sexual activity (Anderson, Flynn & Pilgrim, 2017; Anderson et al., 2019; Costa, Lavorato & Baldin, 2020; Juhascik et al., 2007; Poulsen et al., 2021; Skov et al., 2022; Tiemensma & Davies, 2018; Wille et al., 2021). Although the mainstream narrative portrays proactive DFSOs as the main form of DFSO, research indicates that opportunistic DFSOs are more frequent (Grela, Gautam & Cole, 2018; Hiddink-Til, Teunissen & Lagro-Janssen, 2021).

Determining the true prevalence of DFSO is challenging as survivors of sexual assaults might not wish to disclose their use of drugs or alcohol at the time of the assault, or due to alcohol and drugs being metabolised and excreted from the body prior to sample collection (Grela, Gautam & Cole, 2018; Juhascik et al., 2007; Skov et al., 2022). Another concern is that survivors may be afraid to self-report voluntary drug and alcohol usage, in fear that it could impact their treatment or future legal proceedings.

To determine the proportion of DFSO cases which may be proactive, multiple studies have been conducted by testing blood and urine samples taken from consenting survivors of suspected DFSO cases along with a self-report of their alcohol and drug use prior to the sexual assault (Hurley, Parker & Wells, 2006; Juhascik et al., 2007; Tiemensma & Davies, 2018; Busardo et al., 2019; Skov et al., 2022). These studies compare the toxicological analysis results to that of the self-reported drug and alcohol usage of the survivor. Unexpected toxicological results occurred when a drug was positive in a sample, but the individual stated they had not voluntarily consumed that drug (Hurley, Parker & Wells, 2006; Juhascik et al., 2007; Skov et al., 2022). Results from a study conducted by Hurley, Parker and Wells (2006), found that 20% of suspected DFSO cases had unexpected toxicological results, indicating possible proactive DFSOs. Skov et al. (2022) identified five different studies conducted in the United Kingdom, Australia, Denmark, Norway, and Spain that attempted to estimate the prevalence of proactive DFSA (Scott-Ham & Burton, 2005; Hurley, Parker & Wells, 2006; Juhascik et al., 2007; Birkler et al., 2012; Hagemann et al., 2013; Caballero, Quintela & Landeira, 2017). Results indicated that the prevalence of suspected proactive DFSO was between 2 and 22% of total DFSA cases (Skov et al., 2022). Another study based in the United

Kingdom identified only 2.1% of DFSO cases to likely be due to pro-active DFSO (Scott-Ham & Burton, 2005). In this study, temazepam (which is also an active metabolite of diazepam) was the most commonly seen unsuspected drug in these cases (Scott-Ham & Burton, 2005). Grela, Gautam and Cole (2018) further identified a study in Australia where antidepressants were the most common seen drug followed by cannabis, benzodiazepines, amphetamines, and opioids in suspected proactive DFSA cases (Hurley, Parker & Wells, 2006).

Studies have shown that the main drugs that are frequently detected in toxicological analysis are drugs that are commonly abused in the population of question and this is because most reported cases are opportunistic (Poulsen et al., 2021). Therefore, the drugs that hindered the individual's ability to react or provide consent may be those that they obtain themselves and take willingly. As each population is unique in terms of drug consumption, knowledge on the drug use patterns in a specific population is important to ensure testing methods are targeted appropriately to increase the probability of successfully detecting a substance. The prevalence of opportunistic DFSO can be determined based on the self-reported case history of the survivor and the toxicological results. If the history and toxicological results correspond, the case may be considered an opportunistic DFSO case.

Delays between the time of the suspected DFSO and sample collection, decreases the probability that an analyte will be detected due to the physiological elimination of drugs from the body. Delayed collection of samples is detrimental to toxicological investigations as test results could be negative even if the survivor did indeed administer a drug (Tiemensma & Davies, 2018). This may result in fewer positive results for drugs in suspected DFSO cases, which could underestimate the true prevalence of DFSO. The type of sample being tested is also relevant. For example, drugs are typically detected for longer periods in urine as they are metabolised and removed from the blood circulation to be excreted. In a local study conducted by Tiemensma and Davies (2018) only 32.5% of blood samples that had been collected less than 24 hours after the suspected DFSO were positive for drugs, whereas 58.3% of urine samples that were collected less than 48 hours after the DFSO were positive for drugs. Studies have reported that survivors who declared alcohol consumption but had extended sampling time from the assault, often produced negative results for alcohol (Anderson, Flynn & Pilgrim, 2017; Tiemensma & Davies, 2018). Alcohol is widely reported as the most common substance involved in DFSO cases (Bertol et al., 2018; Skov et al., 2022), which highlights the need for survivors to report to a forensic clinic as soon as possible to receive care and collect evidential samples.

The medical practitioners and trained rape counsellors at the clinical forensic units (CFU) offer the patients support through counselling, medico-legal examination and evidence collection, treatment of injuries, testing for pregnancy or sexually transmitted diseases, and provide post-exposure prophylaxis against HIV. The CFU is open 24 hours every day of the year and a rape crisis counselor and enrolled nurse assistant is always available to assist someone entering the unit (Friends of Victoria, 2024). The CFU treat patients that have experienced rape, intimate partner violence, gender-based violence and child abuse free of charge (Friends of Victoria, 2024). The CFU is one of 51 Thuthuzela Care Centres in South Africa, the centres assist with opening of police cases, arranging counselling and court preparation for individuals that wish to report the offence (South Africa Government, 2024).

1.3 Common drugs in DFSO cases

Findings from multiple DFSO studies have shown that the main substance involved in DFSO is alcohol, followed by illicit impairing drugs such as: narcotic analgesics, central nervous system depressants, stimulants, cannabinoids, and hallucinogens (Costa, Lavorato & Baldin, 2020; Grela, Gautam & Cole, 2018; Juhascik et al., 2007; Tiemensma & Davies, 2018). The use of these drugs changes the perception of the user and reduces their information processing ability, which would decrease their ability to provide consent or even be aware of what is occurring if highly intoxicated.

1.3.1 Analgesics

Narcotic analgesics cause pain relief, sedation, and a feeling of euphoria. In high doses the effects of narcotic analgesics include stupor, coma, and respiratory depression, these effects increase and individuals' susceptibility to DFSO as a perpetrator could take advantage of them in these states. Narcotic analgesics are commonly referred to as opioids and includes opium, opium derivatives, and their semi-synthetic and synthetic substitutes (UNODC, 2011; WHO, 2023). Opiates are natural alkaloid constituents of the milky substance in the opium poppy plant and this includes codeine and morphine (UNODC, 2021; World Health, 2023). Opioids that are synthesised from natural opiates are called semi-synthetic opioids, such as: buprenorphine, heroin, hydrocodone, hydromorphone, and oxycodone. Fully synthetic opioids are made in a laboratory, without using natural opiates. Fentanyl, methadone, and tramadol are examples of fully synthetic opioids (UNODC, 2021).

Analgesics are the one of the most detected drug groups in DFSO cases (Skov et al., 2022). For example, Skov's et al., (2022) found that codeine and morphine were the most commonly detected drugs in Northern Ireland DFSO cases (Busardo et al., 2019).

1.3.2 Central nervous system depressants

Central nervous system (CNS) depressant drugs temporarily diminish the normal functioning of the brain and CNS causing sedation. Side effects of this drug class include slurred speech, loss of motor coordination, weakness, and blurred vision. Alcohol is the most misused CNS depressant, and other examples include barbiturates, benzodiazepines, and methaqualone.

Benzodiazepines are often misused for their sedative or calming effects (Sonone, Jadhav & Singh Sankhla, 2021). Self-treatment or coping motives for sleep, tension and negative emotions are the most common reasons for misuse of benzodiazepines (Kapil et al., 2014; Votaw et al., 2019). However, previous studies indicate that they are also misused out of curiosity and for recreational purposes (Chen et al., 2011; Kapil et al., 2014). Benzodiazepines are often associated with DFSOs due to their ability to cause sedation. The side effects of benzodiazepines include drowsiness, memory loss, impaired coordination, and confusion. Administration of benzodiazepine drugs can therefore cause an individual to have diminished awareness of their surroundings and increased drowsiness, making them vulnerable to acts of non-consensual sexual activity (Grela, Gautam & Cole, 2018).

The most commonly detected benzodiazepines in DFSO studies across the world were diazepam, alprazolam, oxazepam, clonazepam, temazepam and zolpidem (Skov et al., 2022). France had the highest prevalence (82%) of positive results for benzodiazepines, while a study in Cape Town, South Africa reported very low rates of detected benzodiazepines (3.7%) (Djezzar et al., 2009; Tiemensma & Davies, 2018).

1.3.3 CNS stimulants

CNS stimulants are psychoactive drugs that increase alertness, attention and concentration and elevate an individuals' moods by exciting the cortical areas, brain stem and the spinal cord (Romach, Schoedel & Sellers, 2014; Couch, White & De Gray, 2020).

Examples of stimulants are methamphetamine, amphetamine, and 3,4-methylenedioxymethamphetamine (MDMA). Amphetamines result in more dopamine and norepinephrine to be in the individual's system increasing the sense of euphoria and excitement (Reyes-Parada, Iturriaga-Vasquez & Cassels, 2020). Amphetamines are encountered in DFSO

cases as they lower an individuals' inhibitions, increase euphoria and susceptibility to suggestions, thereby potentially causing an individual to engage in more risky behaviour (Grela, Gautam & Cole, 2018). Amphetamines were detected or self-reported (2-13%) in multiple DFSO studies across USA, UK and Canada (Anderson, Flynn & Pilgrim, 2017). South Africa had the highest prevalence of methamphetamine between the studies, it was detected in 28% of the positive samples (Tiemensma & Davies, 2018; Skov et al., 2022).

1.3.4 Cannabinoids

Cannabis is a psychoactive drug that is produced from the *cannabis sativa* plant. The main psychoactive component of cannabis that affects the brain is delta-9-tetrahydrocannabinol (Δ-9-THC) (Atakan, 2012; Grotenhermen, 2003). THC and other cannabinoid compounds interact with G-protein-coupled cannabinoid receptors in the brain and peripheral tissues which results in a change in the activity of endogenous chemical mediators (Grotenhermen, 2003; Atakan, 2012). This change in activity results in a change in the individual's perception and mood. The desired effects of cannabis usage are an increased sense of euphoria and relaxation. Some effects of cannabis that could increase the individual's risk of being taken advantage of are impairment to short term and working memory, psychomotor coordination, and concentration, as well as potential increased arousal. Cannabinoids were detected in between 0.6% and 33.7% of samples across 19 studies (Skov et al., 2022). In nine of the studies cannabinoids were the most prevalent drug (Skov et al., 2022). Canada, USA, New Zealand, and the United Kingdom (18.6% - 33.7%) had the highest rate of detection of cannabinoids (Skov et al., 2022). The lowest rate of cannabinoid detection was seen in Taiwan which had no positive samples for cannabinoids, followed by France, Norway, Sweden, Northern Ireland, Italy, and Spain which ranged from 0.6% to 11.4% (Skov et al., 2022). A study that included 107 participants in South Africa similarly reported a low rate of detection for cannabis in suspected DFSO cases (2%), however the method used for the analysis of cannabinoids was not optimised, likely resulting in an underestimation (Tiemensma & Davies, 2018).

1.3.5 Drug-drug interactions

Co-ingestion of more than one drug is common and can cause an increase in impairment, which may result in a lower dosage of a certain drug producing an intoxicating effect (Schepis et al., 2016; Huang et al., 2018). Alcohol is often consumed in conjunction with other drugs such as cannabis or other CNS depressants or stimulants (Nattala et al., 2012). Schepis et al. (2016) identified that 72.6% of non-medical drug users commonly practice co-ingestion. Betrol et al.

(2018) found that alcohol is most commonly co-ingested with cannabis and benzodiazepines. The combination of these substances and alcohol may cause the individual to have decreased reaction capability, memory loss, dizziness and lack of concentration (Betrol et al., 2018), thereby increasing the individual's vulnerability to a perpetrator.

Further studies have identified that benzodiazepines are commonly co-ingested with alcohol (Jones, 2019; Pergolizzi et al., 2022). When a benzodiazepine drug, such as alprazolam, is administered with alcohol the metabolic rate of alprazolam decreases, leading to an increased blood concentration of alprazolam. This would result in more of the drug crossing the blood-brain-barrier, thereby enhancing the toxicity of alprazolam, even if a relatively small quantity of the drug was consumed (Huang et al., 2018). Both alcohol and benzodiazepines are CNS depressants, which leads to a greater decrease in CNS functioning. It has also been reported that benzodiazepines can cause amnesia when consumed with alcohol or opioids (Madea & Mußhoff, 2009).

McCabe et al. (2015) reported that prescription stimulant medications are commonly co-ingested with alcohol and cannabis, especially among high school students and young adults (McCabe et al., 2015). The authors speculated that this could be due to the high availability of alcohol and cannabis to adolescents. Adolescents and young adults might underestimate the risks of the use consuming another drug while on prescription medication or they might underestimate the risk of consuming prescription medication for recreational purposes (McCabe et al., 2015).

There are multiple reasons for co-ingestion of drugs, however the most common are to obtain a sense of euphoria and relief from anxiety (Nattala et al., 2012). Co-ingestion of drugs can increase the desired effects or prevent unwanted effects of certain drugs. An example is cocaine, which is often consumed with alcohol. In this instance, the alcohol reduces some of cocaine's negative effects such as anxiety, tension and clenching (Nattala et al., 2012).

1.4 Challenges in interpretation

Interpretation of toxicology results in DFSO cases can be challenging due to multiple aspects. Firstly, time delays between the incident and reporting to the clinic can decrease the likelihood of a substance being detected in the biological sample(s) (Wille et al., 2021). Some drugs have a faster elimination time frame than others which shortens the detection window between the administration of the drug and sample collection. Urine has a longer detection window;

however, it is not used to quantify the drug and only as an indication if a drug was detected. A negative result for a drug therefore does not indicate that the drug was not taken at the time of the assault, it indicates that no drug was present at (above the detection limit) at the time of sample collection (Wille et al., 2021). Interpretation without sufficient case information is challenging, as multiple factors such as age, body-mass index (BMI), drug usage (naïve versus chronic users), multiple drugs consumed will impact the elimination rate of the drug (Skov et al., 2022). On the other hand, a positive result should be assessed with all relevant case history, as it is possible that the individual consumed the drug after the alleged assault (Wille et al., 2021).

Therefore, to assist with interpretation of toxicological results from individuals with multiple drugs detected, a self-reported history of the drugs consumed prior to a DFSO is very important. Knowing if the individual is a chronic drug user is also vital as this might affect their tolerance to certain substances which needs to be considered when interpreting drug concentrations.

There is currently no legislation requirements in South Africa for the evaluation and detection limits of drugs in DFSO crimes. Various methods are used across South Africa for the detection of drugs in DFSO crimes depending on the laboratory the samples were sent to, this can cause confusion in the courts when they are shown various methods with different detection limits. Creating a standardised testing method for drugs involved in DFSO crimes in South Africa could greatly benefit the toxicology laboratories and the legal teams at court.

1.5 Knowledge gap and motivation for study.

There is a paucity of research in South Africa relating to DFSO. As highlighted by Tiemensma and Davies (2018), there is a need for further research to better understand DFSO and how to aid the survivors and legal system with reliable toxicological results. Currently, forensic toxicological analyses are not routinely offered to DFSO survivors. Due to the criminal nature of these cases, a reliable and efficient toxicology service is required to provide evidence that can withstand scrutiny in court. This research will also provide further insight into the prevalence of DFSO, and the types of impairing substances commonly involved to improve public health policies. The implementation of a reliable toxicology service can provide valuable information to individuals involved and support the legal and health care systems, by guiding intervention and prevention efforts.

The Forensic Toxicology Unit (FTU) was established within the Forensic Pathology Services (FPS), Western Cape Department of Health with the aim of strengthening toxicological

services in South Africa. The FTU recently developed and validated a LC-MS/MS method for the detection and quantification of 31 common drugs of abuse (DOA) in ante-mortem and post-mortem specimens. The LC-MS/MS method was developed to provide a single analysis that targets the most prevalent drugs detected in the local post-mortem casework. The FTU selected the analytes based on a review of historical toxicology results in the Western Cape and selected the most prevalent drugs and their metabolites. The FTU also included analytes which are recommended in the ASB Standard 119 for the Analytical Scope and Sensitivity of Forensic Toxicology in Medicolegal Death investigations (ASB, 2021). To strengthen DFSO toxicological testing in South Africa, this study aimed to investigate the applicability of this analytical method in cases of suspected DFSO.

1.6 Aims and Objectives

The aim of this study was to investigate the utilisation and applicability of a novel multi-drug analytical method in routine DFSO cases involving survivors who presented to the Clinical Forensic Unit at Victoria Hospital in Cape Town, South Africa. This was achieved through the following objectives:

i. Collection of biological specimens (blood and urine), from consenting adults (>18 years) who presented for suspected DFSOs at Victoria Hospital.

ii. Preparation of samples and conducting a targeted analysis for 31 common drugs of abuse using LC-MS/MS.

iii. Investigation of the prevalence and type of drugs detected to provide a descriptive analysis on the demographic and circumstantial profile of survivors with respect to findings.

CHAPTER 2: MATERIALS AND METHODS

2.1 Sample collection and setting

The study population included consenting suspected DFSO survivors, 18 years and older, who presented for treatment to the Clinic Forensic Unit (CFU) at Victoria Hospital. Cases were included as a suspected DFSO based on either self-reporting by the survivor, if the attending doctor had reason to believe (during the examination of the patient) that an intoxicating substance may have been involved during the offence, or if a DFSO could not be excluded. Survivors of sexual offences from a specific geographical area that covers 22 police stations in Cape Town, are referred to and treated at the CFU (Tiemensma & Davies, 2018). The CFU treats approximately 35 cases (adult and paediatric) of alleged sexual offences per month.

The study was approved, prior to commencement, by the University of Cape Town Human Research Ethics Committee (HREC REF:231/2022) and the Western Cape Government Ethics committee (Ref: WC-202206-28) (Appendix D).

Blood and urine samples were collected from adult survivors (≥ 18 years), who reported to the CFU within two weeks of the suspected DFSO incident. A two-week cut-off period was implemented due to the elimination of drugs from the body and increased likelihood that drugs would not be detected in blood or urine after two weeks, while considering that individuals may delay presenting to the CFU following the alleged incident. The samples were collected over a five-month period, from August 2022 to December 2022. Informed consent was obtained by the attending doctor from all survivors prior to the collection of samples. Basic demographic information and relevant case history was also collected by the treating medical practitioner using the template provided in Appendix A.

Blood samples were collected in a 4 mL vacutainer containing sodium fluoride and potassium oxalate to preserve the specimen. The urine samples were collected in a sterile urine collection cup with no preservatives. Both specimens were stored at 4°C within the facility prior to being transported to the FTU laboratory. Once received at the FTU laboratory, the samples were stored in a designated fridge at 4°C before analysis and the sample information was entered into an internal research database as per standard laboratory procedures. All samples were analysed within six months from the date of sample collection.

2.2 Chemicals and reagents

Zinc sulphate ($ZnSO_4$) and ammonium acetate ($NH_4CH_3CO_2$) used to prepare the lysis buffer were obtained from Alfa Aesar (Haverhill, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Formic acid, acetonitrile, and methanol (HLPC grade) were obtained from Honeywell (Charlotte, NC, USA). A 25% ammonium hydroxide solution obtained from Alfa Aesar (Haverhill, MA, USA) was used to prepare the reconstitution solution. Ultra-pure water was obtained in-house using a Millipore Direct Q3 UV water purification system (Merck, Darmstadt, Germany). Certified reference material for analytes and deuterated internal standards were obtained from Restek Corporation (Bellefonte, PA, United States), LGC standards (Teddington, UK) and Cerilliant corporation (Round Rock, TX, USA) as per Appendix B.

2.3 Sample preparation

2.3.1 Blood

Blood samples were extracted using Ostro™ Pass-Through Sample Preparation plates obtained from Waters Corporation (Milford, MA, USA). A volume of 150 µL of lysis buffer (0.1 M $ZnSO_4$/0.1 M $NH_4CH_3CO_2$) was added to each well of the plate to disrupt the cell membranes. This was followed by adding 50 µL of blood, which was mixed by aspirating up-and-down.

To remove the proteins, 600 µL of acetonitrile (ACN) containing 0.1% formic acid and internal standards were added to each well. The internal standards (ISTDs) listed in Appendix B.2 were added to compensate for analytical variability and the final (post-extraction) concentration of each ISTD was 60 ng/mL. The Ostro plate was then vortexed at 500 rpm for 2 minutes using a microplate shaker (Capp, Nordhausen, Germany).

A positive pressure manifold (Waters, Milford, MA, USA) was used to elute the sample into a collection plate at 5 psi. The eluents were then transferred into 1.5 mL microcentrifuge tubes and evaporated to dryness in a MiVac Quattro Evaporator (Genevac, Ipswich, England) at 45°C. The sample was then reconstituted in 50 µL of diluent [containing equal parts of 2% ACN and 1% formic acid (component A) and 5% ammonium hydroxide (NH_4OH) in ACN:methanol (1:1) (component B)] and vortexed thoroughly. The reconstituted samples were further centrifuged (Labnet International, Edison, NJ, USA) at 8 000 rpm for 5 minutes and the supernatant transferred to an HPLC vial which was loaded onto the autosampler for injection.

2.3.2 Urine

A simple ‘dilute-and-shoot’ procedure was used to prepare urine samples for analysis. A volume of 150 µL acetonitrile containing 0.1% formic acid and internal standards (Appendix B.2) was aliquoted into microcentrifuge tubes to which 150 µL of urine was added. The final (post-extraction) concentration of each ISTD was 30 ng/mL. The microcentrifuge tubes were vortex mixed (Benchmark Scientific, Sayreville, NJ, USA) at 2000 rpm for 2 minutes, and subsequently centrifuged for 5 minutes at 13000 rpm. A volume of 100 µL supernatant was then transferred to a HPLC vial and diluted with 150 µL 2% acetonitrile and 1% formic acid (component A) and 5% ammonium hydroxide (NH_4OH) in ACN:methanol (1:1) (component B). After a brief vortex to ensure the vial components are adequately mixed, the vial was loaded onto the autosampler for injection.

2.4 Instrumental analysis

All samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) a on a Waters ACQUITY I-Class UPLC coupled to a XEVO TQD mass spectrometer (UPLC-MS/MS) (Milford, MA, USA), for the detection and quantification of multiple common drugs of abuse (DOA) listed in Table 2.2. Liquid chromatography-tandem mass spectrometry, or ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), combines the separation of analytes using liquid chromatography with the detection of the analytes based on their charge-to-mass ratio using mass spectrometry (Stefani *et al.*, 2022). It is a highly sensitive and specific technique and is therefore particularly suited for targeted quantitative methods in forensic toxicology. The method was previously validated by laboratory staff in accordance with the recommendations of the American Academy of Forensic Sciences (AAFS) Standards Board (ANSI/ASB) Standard 036 (AAFS, 2019) and South African National Accreditation System (SANAS) TG 41-03. Blood samples were analysed quantitatively and urine samples qualitatively.

A 5 µL aliquot of the extract was injected onto an ACQUITY BEH C18 column (130 Å, 1.7 µm particle size, 100 mm x 2.1 mm I.D.) fitted with an ACQUITY UPLC BEH C18 VanGuard pre-column (130 Å, 1.7 µm particle size., 5 mm x 2.1 mm I.D.) (Waters, Milford, MA, USA). The column temperature was maintained at 40°C and sample temperature at 10°C. The conditions of the liquid chromatograph and mass spectrometer are provided in Table 2.1. The multiple reaction monitoring conditions and retention times for each analyte are provided in Table C.1 and C.2. Analysis of the instrumental data was carried out using MassLynx software

v4.2. The instrumental data was processed using defined parameters set for qualitative and quantitative criteria with regards to peak integration, retention time, ion rations, calibration standards and quality controls. The chromatographic data was automatically evaluated using peak quality criteria and further confirmed by visual inspection.

Table 2.1: LC and MS conditions

LC conditions		**MS conditions**	
Column temperature:	40°C	**Ionization mode:**	ESI + (positive)
Sample temperature:	10°C	**Acquisition mode:**	Multiple reaction monitoring (MRM)
Mobile phase A:	0.1% formic acid in MilliQ water	**Capillary voltage:**	2.5 kV
Mobile Phase B:	0.1% formic acid in acetonitrile	**Desolvation temp:**	400°C
Seal wash & purge solvent:	9:1 water: acetonitrile	**Desolvation gas flow:**	850 L/Hr
Wash solvent:	1:9 water:acetonitrile	**Source temp:**	150°C
Injection Volume:	5 mL	**Cone gas flow:**	5 L/Hr
LC gradient program: 7-minute run time			
Time (min)	**Flow (mL/min)**	**% Mobile phase A**	**% Mobile phase B**
0.00 (Initial)	0.6	98	2
3.33	0.6	33	67
4.00	0.6	10	90
5.50	0.6	10	90
6.00	0.6	98	2
7.00	0.6	98	2

2.6 Calibrators and quality control

Calibrators and quality control samples were prepared in-house using certified reference material. The calibrator and control concentrations used for the analysis of blood and urine samples are provided in Table 2.2.

Table 2.2: Calibrator and quality control concentrations for each analyte.

#	Analyte	Blood calibrators (mg/L)							Blood quality controls (mg/L)				Urine quality controls (mg/L)	
		1	2	3	4	5	6	7	LOD	LQC	MQC	HQC	LOD	PQC
1	6-AM	0.010	0.025	0.050	0.125	0.250	0.500	-	0.005	0.030	0.200	0.400	0.010	0.020
2	Acetaminophen	0.100	0.250	0.500	1.250	2.500	5.000	-	0.050	0.300	2.000	4.000	0.100	0.200
3	Alprazolam	0.005	0.010	0.025	0.050	0.125	0.250	0.500	0.0025	0.015	0.200	0.400	0.005	0.010
4	Amitriptyline	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100
5	Amphetamine	0.020	0.050	0.100	0.250	0.500	1.000	-	0.010	0.060	0.400	0.800	0.050	0.100
6	Benzoylecgonine	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100
7	Buprenorphine	0.004	0.010	0.020	0.050	0.100	0.200	-	0.002	0.012	0.080	0.160	0.010	0.020
8	CBD	-	-	-	-	-	-	-	-	-	-	-	0.020	0.040
9	Clobazam	0.010	0.020	0.050	0.100	0.250	0.500	1.000	0.005	0.030	0.400	0.800	0.010	0.020
10	Cocaethylene	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100
11	Cocaine	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100
12	Codeine	0.010	0.025	0.050	0.125	0.250	0.500	-	0.010	0.030	0.200	0.400	0.020	0.040
13	Diazepam	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.010	0.020
14	Diphenhydramine	0.040	0.080	0.200	0.400	1.000	2.000	4.000	0.010	0.120	1.600	3.200	0.050	0.100
15	Fentanyl	0.001	0.002	0.005	0.010	0.025	0.050	0.100	0.500*	0.003	0.040	0.080	0.001	0.002
16	Hydrocodone	0.010	0.025	0.050	0.125	0.250	0.500	-	0.005	0.030	0.200	0.400	0.020	0.040
17	Hydromorphone	0.010	0.025	0.050	0.125	0.250	0.500	-	0.005	0.030	0.200	0.400	0.020	0.040
18	Ketamine	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100
19	MDA	0.010	0.020	0.050	0.100	0.250	0.500	1.000	0.005	0.030	0.400	0.800	0.025	0.050
20	MDMA	0.010	0.020	0.050	0.100	0.250	0.500	1.000	0.005	0.030	0.400	0.800	0.025	0.050
21	Methadone	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100
22	Methamphetamine	0.020	0.040	0.100	0.200	0.500	1,000	2.000	0.010	0.060	0.800	1.600	0.025	0.050
23	Methaqualone	0.050	0.100	0.250	0.500	1.250	2,500	5.000	0.025	0.150	2.000	4.000	0.050	0.100
24	Methcathinone	0.020	0.050	0.100	0.250	0.500	1,000	-	0.010	0.060	0.400	0.800	0.020	0.040
25	Morphine	0.010	0.025	0.050	0.125	0.250	0.500	-	0.005	0.030	0.200	0.400	0.020	0.040
26	O-DSMT	0.010	0.020	0.050	0.100	0.250	0.500	1.000	0.005	0.030	0.400	0.800	0.100	0.200
27	Oxycodone	0.010	0.025	0.050	0.125	0.250	0.500	-	0.010	0.030	0.200	0.400	0.050	0.100
28	Oxymorphone	0.010	0.025	0.050	0.125	0.250	0.500	-	0.005	0.030	0.200	0.400	0.020	0.040
29	THC	-	-	-	-	-	-	-	-	-	-	-	0.020	0.040
30	THC-COOH	0.010	0.025	0.050	0.125	0.250	0.500	-	0.005	0.030	0.200	0.400	0.020	0.040
31	Tramadol	0.020	0.040	0.100	0.200	0.500	1.000	2.000	0.010	0.060	0.800	1.600	0.050	0.100

*In µg/L

2.7 Data handling and analysis

All samples and case information collected were anonymised using unique laboratory and study numbers. Upon receipt at the FTU, specimen details were accessioned into a restricted designated sample repository research database. Instrumental raw data from the LC-MS/MS analysis was processed using MassLynx software v4.2 (Waters, Massachusetts, USA). All results remain anonymous and personal information was not included in the study. Case history information and analytical results were documented in a Microsoft Excel database accessible only to the student and supervisors. Basic descriptive statistics with simple summaries were performed on the generated data.

CHAPTER 3: RESULTS

3.1 Case history

A total of 17 individuals were included in the study, of which case information sheets were received for 15 participants (Table 3.1). The majority (n=11, 73.3%) of participants reported to the CFU within 24 hours (Table 3.1). All participants were female (n=15, 100%), between the ages of 18-29 years (n=9, 60%) with clear histories or vague recollections of the offence (n=11, 73.3%), most of the offences were reported to have occurred in a private residence (n=11, 73.3%), one of which occurred in the survivor's own residence. Most of the perpetrators were unknown (n=8, 53.3%) to the participants. The majority (n=13, 86.7%) of participants reported the offence to the South African Police Service (SAPS).

Table 3.1: Case history of 15 survivors of suspected DFSO that presented to the Victoria Hospital's Clinical Forensic Unit.

Patient characteristics	Number of cases (% of total participants)
Sex	
Female	15 (100)
Male	0 (0)
Patient age (years)	
18-19	3 (20)
20-29	6 (40)
30-39	5 (33.3)
40-49	1 (6.7)
Was the perpetrator known?	
Yes	7 (46.7)
No	8 (53.3)
Details of alleged offence	
Clear history	5 (33.3)
Vague recollection	6 (40)
No recollection	4 (26.7)
Type of sexual offence	
Vaginal	9 (60)
Oral	1 (6.7)
Combination	3 (20)
Unknown	2 (13.3)
Place of alleged offence	
Own residence	1 (6.7)
Other residence	10 (66.7)
Public	3 (20)
Other (car)	1 (6.7)
Associated physical violence or injuries	
Yes	6 (40)
No	9 (60)
Reported to South African Police (SAPS)	
Yes	13 (86.7)
No	2 (13.3)
Patient suspected they are a victim of DFSO	
Yes	8 (53.3)
No	5 (33.3)
Uncertain	2 (13.3)
Time between alleged offence and medical exam	
< 12 hours	3 (20)
< 24 hours	8 (53.3)
48 -72 hours	1 (6.7)
> 72 hours	3 (20)

All participants were asked about their alcohol, medicinal, and recreational drug use history and grouped based on their self-reported usage as shown in Figure 3.1. Eleven participants reported consuming alcohol (73.3%), of which five (33.3%) reported consuming alcohol alone.

Most participants reported drinking prior to the offence, and one drank at the time of the offence. Wine was the most common drink consumed, and the majority (n=9 out of 12 that

reported drinking, 75%) of the participants reported drinking more than two units of alcohol (wine, beer, or spirits). Four (26.7%) of the participants reported medicinal drug usage prior to the offence, the drugs mentioned included Xanax (alprazolam), antidepressants, ADHD medication, Dopaquel (quetiapine) and antiretrovirals. Six (40%) participants reported recreational drug usage, including, dagga (cannabis), tik (methamphetamine), magic mushrooms (psilocybin) and buttons (methaqualone). All were administered prior to the offence, one was taken orally (magic mushrooms), and the remaining substances were smoked/inhaled. In two cases, the substance was administered more than 72 hours before the incident and was thus unlikely to have played a role in the offence. Alcohol was consumed in combination with recreational drugs in 3 cases (20%), this included the use of cannabis (n=1) and methamphetamine (n=2) while or on the same day they were consuming alcohol.

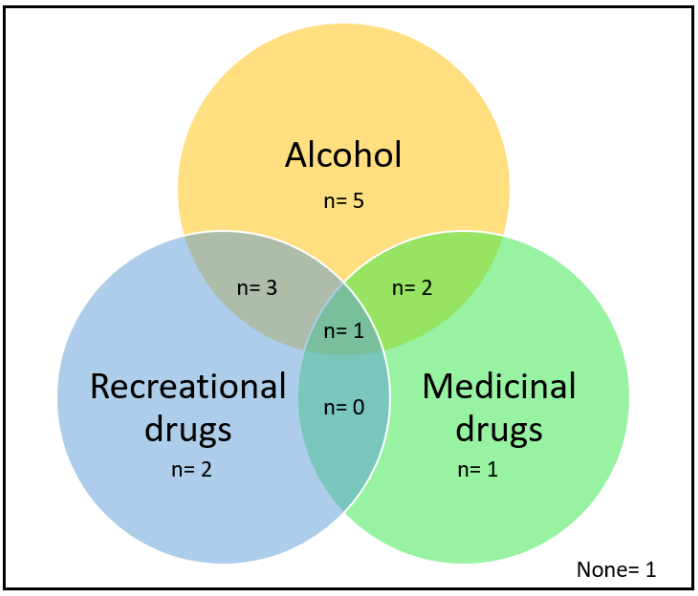

Figure 3.1: Venn diagram of self-reported drug and alcohol use.

3.2 Toxicological analysis

Blood and urine samples were collected from all the participants, the results of which are presented in Table 3.2.

Table 3.2: Results obtained from the analysis of blood and urine samples for multiple drugs of abuse using LC-MS/MS

Case	Time between alleged offence and sample collection	Blood	Urine**
01	28 – 32 hours	THC-COOH (0.048 mg/L)	THC-COOH
02	3 days	Acetaminophen (0.21 mg/L)	ND
03	13.5 hours	Methamphetamine (0.026 mg/L)	Amphetamine, methamphetamine
04	< 24 hours	ND	Methamphetamine
05	18.5 hours	Acetaminophen (7.7 mg/L)	ND
06	5 hours	ND	ND
07	8.5 hours	Acetaminophen (0.20 mg/L)	ND
08	Unknown	Acetaminophen (0.14 mg/L)	ND
09	5.5 hours	Methamphetamine (0.12 mg/L), methaqualone (0.99 mg/L)	Amphetamine, methamphetamine, methaqualone
10	Unknown	Methamphetamine (<0.020 mg/L)*, methaqualone (0.15 mg/L)	Amitriptyline, amphetamine, methamphetamine
11	13 hours	THC-COOH (0.031 mg/L)	THC-COOH
12	13.5 hours	Methamphetamine (0.079 mg/L), methaqualone (0.99 mg/L), THC-COOH (0.022 mg/L)	Amphetamine, diphenhydramine, methamphetamine, methaqualone
13	23.75 hours	ND	ND
14	15.80 hours	ND	Amphetamine, methamphetamine
15	4 – 6 days	ND	Amphetamine, methamphetamine
16	3 days	ND	ND
17	18 hours	Acetaminophen (0.84 mg/L), benzoylecgonine (0.12 mg/L), codeine (0.010 mg/L)	Benzoylecgonine, cocaethylene, cocaine, codeine

*Below the limit of quantitation.
**Acetaminophen not analysed in urine.
ND = none detected

Drugs were detected in blood in 11 cases (64.7%), and in urine in 10 cases (58.8%). In blood, acetaminophen was most frequently detected (n=5, 29.4%), followed by methamphetamine (n=4, 23.5%) and THC-COOH (n=3, 17.6%). Methamphetamine was the most frequently detected drug in urine samples (n=7, 41.2%) followed by its metabolite, amphetamine (n=6, 35.3%). Excluding acetaminophen, drugs (or metabolites of drugs) that may cause CNS impairment were detected in ten cases (58.8%). The majority (n=11, 73.2%) of the patients (for which case information was available) reported to the clinic for sample collection within 24 hours, the longest delay in sample collection to the alleged assault was 4-6 days.

The self-reported drug and alcohol usage was compared to the drugs that were detected in the blood and urine samples for each of the individuals (Table 3.3). Two participants (08 and 10) did not have completed data information sheets; therefore, no self-reported drug and alcohol usage history was available. The majority (n=10, 66.7%) of participants self-reported drug use did not match the findings in their biological samples. Most (n=8, 53.3%) of the participants suspected that they were involved in a DFSO. Participants that did not believe (n=5, 33.3%) or were uncertain (n=2, 13.3%) if they were involved in a DFSO, reported alcohol or the use of a psychoactive drug at the time of the incident. All participants, besides case 02, 12 and 14, that reported recreational drug use reported usage a few hours (<12 hours) prior to the incident. Case 02 reported smoking cannabis approximately 12 hours prior to the incident. Case 12 reported that they had been forced to smoke methamphetamine 13 hours prior to the incident. Case 14 noted that they had consumed recreational mushrooms a month prior to the incident.

Table 3.3: Self-reported drug and alcohol use compared to drug findings.

Case	Self-reported alcohol use	Self-reported medicinal drug use	Self-reported recreational drug use (time in hours between last administration and medical examination)	Patient suspects they were involved in a DFSO (suspected substance)	Detected drugs
01	No	No	Cannabis (< 24)	No	THC-COOH
02	Yes	No	Cannabis (> 72)	No	Acetaminophen
03	Yes	Antidepressants, ADHD treatment, Dopaquel (antipsychotic)	Methamphetamine (< 24)	Methamphetamine	Amphetamine, methamphetamine
04	Yes	No	No	Uncertain	Methamphetamine
05	Yes	No	No	Yes†	Acetaminophen
06	Yes	Antiretrovirals*	No	No	ND
07	Yes	Antiretrovirals*	No	No	Acetaminophen
08‡	Unspecified	Unspecified	Unspecified	Unspecified	Acetaminophen
09	No	No	Methaqualone (< 24)	No	Amphetamine, methamphetamine, methaqualone
10‡.	Unspecified	Unspecified	Unspecified	Unspecified	Amitriptyline, Amphetamine, methamphetamine, methaqualone
11	Yes	No	No	Yes†	THC-COOH
12	Yes	No	Tik (< 24)	Alcohol	Amphetamine, diphenhydramine, methamphetamine, methaqualone, THC-COOH
13	Yes	Antidepressants	No	Yes†	ND
14	Yes	No	Mushrooms (> 72)	Yes†	Amphetamine, methamphetamine
15	No	No	No	Methamphetamine	Amphetamine, methamphetamine
16	Yes	No	No	Yes†	ND
17	Yes	No	No	Uncertain	Acetaminophen, benzoylecgonine, cocaethylene, cocaine. codeine

*Tenofovir, lamivudine, dolutegravir; †Unknown substance; ‡Incomplete data sheet; ND = None detected

CHAPTER 4: DISCUSSION AND CONCLUSION

Drug-facilitated sexual offences (DFSOs) are criminal offences in South Africa; however, toxicological analysis is not a public service that is routinely offered to survivors of sexual offences. With limited availability of toxicological testing for these cases, interpretation of the survivor's intoxication and impaired ability to provide consent may often be limited to the individual's self-reported alcohol and/or drug usage (Tiemensma & Davies, 2018). In a previous study conducted by Tiemensma and Davies (2018); urine, blood and/or hair were screened for drugs of abuse. The authors reported that 67% of the cohort of survivors of suspected DFSO that reported to Victoria Hospital CFU tested positive for drugs and/or alcohol, indicating that impairing substances are frequently involved in sexual offences and highlighting the need for routine toxicological testing.

Valid and reliable toxicological analysis is essential in DFSO cases for multiple reasons. Firstly, findings that are produced from validated methods can be used in court to support a testimony and aid in a successful prosecution or conviction. Secondly, the results could potentially help survivors of assaults to understand and begin processing the traumatic event and hopefully assist in their healing process. Thirdly, the results from the analysis can provide the attending doctor with vital information required for the treatment of their patients. Lastly, the results can create awareness to the public of how drugs are involved in sexual offences and help to create a baseline for possible intervention strategies, as well as contribute towards the knowledge of the drug usage in the general population or community.

4.1 Case histories

To shed light on some of the circumstances surrounding each of the alleged DFSOs that were included in this study, data information sheets were created and completed during the examination by the attending medical practitioner. The location of the assault largely occurred in a place of residence, which agreed with previous studies. Tiemensma & Davies (2018) reported that 57% of sexual offences occurred in someone's home. Similarly, in a study conducted by Hagemann et al. (2013), 61% of the offences were experienced in a private space. This is most likely because in private spaces there is more opportunity to commit an offence without witnesses or someone else's knowledge.

Most perpetrators were unknown to the survivors (n= 8 out of 15, 53.3%). The relation between the perpetrator and the survivor, whether they were known or unknown to them, in this study contradicted findings from previous studies who found that approximately 30% or less of the offences were conducted by unknown perpetrators (Tiemensma & Davies, 2018; Hagemann et al., 2013; Hiddink-Til, Teunissen & Lagro-Janssen, 2021; Wille et al., 2021). The difference seen could be due to the smaller sample size that was used in this study as all previous studies had a sample size above 70. However, it could also indicate that more opportunistic offences are occurring from unknown individuals that gain access to the same residence as the survivor.

A high police reporting rate was observed during the study. The number of offences reported to the police was similar to what was reported by Tiemensma & Davies, (2018), where 91% of individuals reported the offence to the police. A study by Hagemann et al., (2013) also had a high rate of reporting (67.5%). Two cohorts of survivors of sexual violence that reported to a clinic in the Netherlands, consisted of individuals that presented between 2013 and 2016 (n=83) and the second group between 2017 and 2020 (n=270). Both groups had a lower rate of reporting of 44% and 63%, respectively (Hiddink-Til, Teunissen & Lagro-Janssen, 2021). However, the above studies have higher reporting rates than other literature, which estimated only one in six survivors of sexual assault will report the offence to the police (Kilpatrick et al., 2007; Wille et al., 2021) and that majority of rape survivors would only report if they were in a life threating state or if the perpetrator was unknown to them (Chen & Ullman, 2010; Spohn, 2020). This is most likely since individuals who are comfortable with providing consent to being a part of a study or report to a forensic clinic are more likely to feel comfortable with reporting the offence to law enforcement, perhaps due to having support from friends, family or a medical practitioner. The services offered at the clinic could also impact the decision of the patient to report the crime to police, due to the support provided by specialist counselors (Friends of Victoria, 2024; South Africa Government, 2024).

A large portion (n=6, 40%) of the patients presented with indications of experiencing physical violence or injuries that were associated with the offence. This could have increased the need for them to report to the clinic. Mechanisms that could increase the reporting rate to the police include having more dedicated clinical units available, reducing the stigma surrounding sexual offences, improved awareness on what to do if you have been a victim of a sexual offence, as well as awareness surrounding the characteristics of sexual offences as some individuals might not register that they have been a victim of a sexual offence, especially if it involves a partner

(Kilpatrick et al., 2007; Steele et al., 2019). The process of reporting should be as simple as possible to prevent any additional stress or trauma to the survivor. A study conducted by Walker & Louw (2005) investigated how survivors of sexual offences found their interaction with the Court for Sexual Offences in Bloemfontein, Free State, South Africa. The study included 49 survivors, of which 75.6% found the police were prepared to assist them and 42.9% reported that they felt the police emotionally supported them during the initial stages following the offence. However, survivors stated that they found the attending nurses to not show sympathy to their situation, only 34.5% of the survivors experienced the medical staff as emotionally supportive. While the feedback regarding the police officer's preparedness seemed to mainly be positive, the survivor's experience in feeling emotionally supported by medical practitioners was lacking. It is thus important that law enforcement and medical personnel are suitably trained to manage DFSO cases and should be encouraged to show compassion towards survivors to decrease additional stress following a traumatic event.

Participants reported on whether they suspected they experienced a DFSO. Five (33.3%) reported that they did not suspect they were a victim of DFSO and two (13.3%) reported that they were uncertain. However, all five participants who reported that they did not suspect they were a victim of DFSO had consumed either alcohol and/or drugs prior to the sexual offence that took place. Both participants that were uncertain if they were victims of DFSO had reported drinking prior to the offence. The United Nations Office on Drugs and Crime defines DFSO's as; any form of sexual assault that occurs while an individual is incapacitated or unconscious because of the effects of alcohol and/or drugs and is therefore prevented from resisting or unable to consent (UNODC, 2011). This can either be due to purposefully administering the drug to the victim or by taking advantage of an individual that has voluntarily consumed any incapacitating substance (Juhascik et al., 2007; Anderson, Flynn & Pilgrim, 2017; Tiemensma & Davies, 2018; Anderson et al., 2019; Costa, Lavorato & Baldin, 2020; Wille et al., 2021; Mognetti et al., 2022; Skov et al., 2022).

Public perception mainly focuses on proactive DFSO's, when the perpetrator administers a drug to the individual without their knowledge or consent. This focus has potentially caused multiple individuals to believe that if they willingly consumed alcohol or drugs and then were sexually assaulted that it no longer classifies as a DFSO or that they could be partly responsible for the action of the perpetrator. This is highlighted in this study as seven individuals reported

they were uncertain or did not believe they experienced a DFSO and shows the need for educational campaigns to inform the public about what constitutes a DFSO.

4.2 Toxicology results

While the blood concentration for the drugs was reported on, it remains challenging to interpret as these concentrations do not reflect the concentrations or level of intoxication at the time of the offence. Multiple factors will affect the level of impairment, for example, the individual's metabolism, tolerance, and possible drug-drug or drug/alcohol interactions due to polydrug use. Tolerance to certain drugs is developed when an individual repeatedly uses a drug and requires a higher dose to produce the same effect. The level of impairment will also depend on the route of administration for certain drugs. When a drug is taken orally instead of smoked, less of the drug will reach the circulatory system, as the drug will have to go through first-pass metabolism resulting in some of the drug being metabolised before it enters the circulatory system. Drugs such as cannabis, when administered orally, have limited solubility in the gastrointestinal tract causing less of the drug to be absorbed and available to cause an effect (Johnson, Miskelly & Rindelaub, 2022).

Urine is an excretory substance and drugs in the body have thus been metabolised by the time they are detected. Results from urine analyses are usually only used as an indication of whether the drug was present in the individual's system and cannot be used to comment on the level of intoxication. Urine samples are thus seldom used for quantitative analysis due to the difficulty in interpretation of results. However, a large majority of drugs have a longer detection window in urine compared to blood (Yang & Lewandrowski, 2001). The longer detection periods in urine make it a valuable sample to use for the detection of drugs. In the clinical setting, an advantage of urine sampling is also that it is less invasive (Yang & Lewandrowski, 2001).

Although alcohol analysis was outside the scope of this study, a high prevalence (n=14, 82.3%) of one or more drugs in the study population was observed when compared to other studies. A study done by Hagemann et al. (2013) detected alcohol (ethanol) and/or drugs in 59% of their study population. However, when looking at drugs other than alcohol, only 14% of their population tested positive. The study performed by Hagemann et al., (2013) had similar results to that of Hiddink-Til, Teunissen & Lagro-Janssen, (2021) which detected psychotropic substances in 24% and 11% of cases in their two cohorts. In this study 58.8% (n=10) of cases tested positive for a psychotropic substance, which is substantially higher than the previously mentioned studies. Tiemensma & Davies (2018) reported that 56% of their population had used

drugs other than alcohol, which is similar to results from our study. The difference between Hagemann et al., (2013) and Hiddink-Til, Teunissen & Lagro-Janssen, (2021) studies to this study and Tiemensma & Davies (2018) could be due to the different locations of the populations that were being used in the studies. As this study and Tiemensma & Davies (2018) was conducted at the same facility, substance use patterns between the studies may bear resemblance.

The majority (n=11, 64.7%) of the blood samples were positive for at least one drug. However, four of these samples only tested positive for acetaminophen, an over-the-counter analgesic. Seven (41.2%) blood samples were positive for at least one other drug. This is similar to the observation made by Tiemensma and Davies (2018), who found that 32.5% of the blood samples were positive for drugs, whereas 61.4% of the urine samples tested positive for drugs.

Methamphetamine was detected in seven (41.2%) urine samples. All except one of the samples that tested positive for methamphetamine also tested positive for the metabolite amphetamine. Crystal methamphetamine, colloquially known as 'tik', is an illicit stimulant recreational drug which is very popular in the Western Cape (Orwa & Nyabadza, 2019; Okafor et al., 2020). Amphetamine-type stimulants are commonly found in DFSO cases as they lower an individuals' inhibitions, increase euphoria and susceptibility to suggestions (Grela, Gautam & Cole, 2018).

Only four of the seven urine samples that had a positive result for methamphetamine had a blood concentration above the limit of detection. Each of the participants who had a positive detection for methamphetamine in urine presented to the clinic within one day of the alleged assault except for two, one had an incomplete data form and the other presented after four to six days after the alleged assault. Urine has a longer detection window than blood for methamphetamine, as the half-life of methamphetamine is 9-24 hours in urine and can usually be detected up to 72 hours after the final dose, with around 70% of methamphetamine excreted from the urine within 24 hours (Cruickshank & Dyer, 2009; Hardey & Kelley, 2022). Whereas the plasma half-life of methamphetamine is approximately ten hours (Cruickshank & Dyer, 2009). The difference in detection window is the most likely reason for the difference in positive and negative results for the blood samples, as individuals who reported to the clinic within approximately 13 hours tested positive for methamphetamine in the blood as well as the urine, whereas those that reported after 15 hours did not have detectable concentrations of methamphetamine in the blood. Two participants who tested positive for methamphetamine

reported being forced to smoke tik, therefore these findings are in keeping with the case history. The difference in results between the blood and urine samples for these cases highlight the importance of early sampling to obtain reliable toxicological evidence.

Two samples that were positive for methamphetamine were also positive for methaqualone, one of which self-reported the use of tik and the other the use of mandrax (methaqualone). Methaqualone (mandrax or "buttons") is also widely used in South Africa, often in combination with crystal methamphetamine. Methaqualone has sedative effects that reduce an individual's ability to consent to sexual activity or to react to a dangerous situation (Bonner et al., 2022). The sedative effect of methaqualone could last up to six hours depending on the individual (McCarthy, Myers & Siegfried, 2005). These sedative effects increase an individual's vulnerability to DFSO. Methaqualone was detected in three cases, one of which the case history was not available for. For two cases it was reported that the drugs, one reported methaqualone usage and one Tik, were administered less than 24 hours before the medical examination and just prior to the alleged assault. The half-life of the elimination phase for methaqualone in blood is between 19.6 and 41.5 hours (Inger et al., 2022), therefore this finding supports the case history that the individual could have been under the influence of the drug at the time of the assault. Diphenhydramine is an antihistamine that can be used to treat allergies and coughs (Sicari & Zabbo, 2023). It is also a component of mandrax, which was first produced in 1965 by combining methaqualone and diphenhydramine (Kelly & Psych, 1973).

Mandrax is widely used in South Africa, often in combination with crystal methamphetamine. Mandrax was the third most reported substance in the Western Cape by individuals who seek substance abuse treatment, following alcohol and cannabis in 2015 (Dada et al., 2015; Bonner et al., 2022). The August 2023 SACENDU report determined mandrax as the fifth most observed substance at specialist substance use treatment centers (SACENDU, 2023). Methaqualone was detected in 17.6% (n=3) of the cases, this is substantially higher than SACENDU (2023) 0.3% of individuals that had methaqualone detected, however this difference is most likely due to the difference in sample size our study consisted of 17 participants whereas the SACENDU report for the Western Cape included 1953 participants. A study performed by Rabie et al. (2020) that consisted of 729 men from two peri-urban settlements outside of Cape Town found that 57% of the participants self-reported the use of mandrax and 18% reported the use of tik. Diphenhydramine was detected in a case which also had methamphetamine, methaqualone and amphetamine detected in the urine. The blood sample of this case was positive for methamphetamine, methaqualone and THC-COOH. The

detection of methaqualone and diphenhydramine together is therefore likely an indication of mandrax usage. The fact that methaqualone and methamphetamine were detected in samples from participants that stated the use of either tik or mandrax is interesting and may suggest that the individual was unaware of possible poly-drug use at the time of the incident.

The inactive metabolite of cannabis, 11-Nor-9-carboxy-THC (THC-COOH), was detected in both urine and blood samples of three cases. THC and its metabolites (tetrahydrocannabinol carboxylic acid (9-carboxy-THC), 11-carboxy-THC and 11-hydroxy-THC) can be found almost immediately after smoking (Grotenhermen, 2003; Huestis, 2007; Lucas, Galettis & Schneider, 2018). The effects will generally start to decrease after two to three hours (Grotenhermen, 2003; Lucas, Galettis & Schneider, 2018). Smoking cannabis produces psychoactive effects faster than ingestion of cannabis as ingested cannabis has to first go through first-pass metabolism and enter the circulatory system before the desired effect will be produced (Lucas, Galettis & Schneider, 2018). THC-COOH is the primary form of cannabis that is detected in the urine with a urinary excretion half-life of 30 - 60 hours (Sharma, Murthy & Bharath, 2012). THC-COOH can be detected three to seven days after exposure to cannabis (Musshoff & Madea, 2006), which causes interpretation of the drugs effect during the offence to be difficult. In addition, THC-COOH is an inactive metabolite and cannot be used to interpret impairment. Cannabinoids are highly lipophilic and easily move into well-vascularised organs such as the lung, heart, brain, and liver (Lucas, Galettis & Schneider, 2018). If an individual chronically smokes or ingests cannabis, cannabinoids may accumulate in adipose tissues and liver (Lucas, Galettis & Schneider, 2018). This can make toxicological testing for cannabinoids difficult to interpret because if cannabis is detected in a sample it will be difficult to comment on if the individual could have been under the influence at the time of the assault, therefore an accurate drug usage history is required. It would take longer to fully remove the drug from the body of a chronic user and cause small quantities of the drug to be present in the bloodstream for extended periods. Two of the cases where THC-COOH was detected, reported that they regularly used recreational drugs, however, it is unknown whether they were chronic users of cannabis and if this finding was incidental from previous use prior to the offence.

Cocaine and the metabolites, benzoylecgonine and cocaethylene, were detected in one (5.9%) case. Benzoylecgonine is the major metabolite of cocaine, it is formed due to the hydrolysis of cocaine in the liver and excreted in the urine. Cocaethylene is formed in the liver when ethanol is present in the liver with cocaine (Blaho et al., 2000; Jones, 2019; Pergolizzi et al., 2022), therefore it is produced when an individual consumes both alcohol and cocaine. Cocaine is

frequently taken in combination with alcohol, and cocaethylene ($t_{1/2}$ = 2 hours) has a longer half-life than cocaine ($t_{1/2}$ = 1 hour) and may cause individuals to experience more intense and longer lasting psychoactive effect (Jones, 2019; Pergolizzi et al., 2022). The detection of cocaethylene in the urine provides confirmation that the participant had consumed cocaine and alcohol together. This is in keeping with the case history as the participant did report consuming alcohol.

Three participants had no drugs detected in their urine or blood samples. These individuals did not report the use of any drugs; however, they did report consuming alcohol. According to the case histories, two of these individuals consumed a large amount of alcohol, one of which reported consuming a liter of wine and the other 10 beers. The third reported drinking one unit of wine. They all stated that they do not suspect that they experienced a DFSO, as discussed previously this highlights the need for awareness campaigns about DFSOs as often the public seems to be under the impression that an offence which occurs following the voluntary use of alcohol does not constitute a DFSO. Individuals need to be made aware that consuming any substance which causes impairment and reduced ability to consent can be deemed a DFSO. In these cases, an impairing substance, other than alcohol, may not have been involved, however it is possible that they had consumed a drug that is not included in the drug panel.

4.3 Opportunistic and Proactive DFSO

The drugs detected in the blood and urine samples were compared to the drugs which the participants self-reported (Table 3.3). A third of the participants (n=5, 33.3%) had unexpected positive toxicology results for drugs that could cause CNS impairment. It is possible that this could indicate the occurrence of proactive DFSO, however it is also possible that the individuals did not accurately report their drug usage. Without being certain of the accuracy of the self-reported drug usage, and given the limited sample size, it remains challenging to make a reasonable conclusion on this finding.

The presence of these substances indicates that there is a possibility the participants were under the influence of an impairing substance at the time of the alleged assault. The individuals who willingly administered a substance all reported doing so a few hours prior to the assault, except for case 14 who consumed mushrooms the previous month. However, the drugs detected in their sample were amphetamine and methamphetamine, indicating more recent methamphetamine use.

Majority (n=10, 58.8%) of the participants had consumed illicit or psychotropic drugs at some point. This is higher than the Hiddink-Til et al. (2021) study on sexual assault with 11% and 24% of the cases, in the two previously discussed cohorts, to have psychotropic substances present. This study included all survivors of sexual offences who reported to a sexual assault center within eight days after the assault. The lower percentage of cases positive for drugs is most likely because all sexual offences that occurred were included, whereas a large portion (41.7%) of the participants in this study suspected that they were a victim of DFSO. Hagemann et al. (2013) found that 19% of their study population were positive for drugs other than alcohol, and likewise to the previous study, Hagemann et al. (2013) included all patients who presented to the clinic. Similar results were reported by Tiemensma & Davies (2018) who included all patients who presented to the clinic and had biological samples collected and determined that 56% of the cases were positive for drugs other than alcohol. The higher percentage of cases that are positive for drugs is likely due to the study having a higher percentage of participants who suspected they had experienced a DFSO, or it can be due to the high prevalence of substance abuse in the local population.

4.4 Applicability of FTU's novel multi-drug analytical method in routine DFSO

The novel multi-drug analytical method that was developed by the FTU was used during this study to determine if the method could be utilised for routine DFSO casework.

Different populations will have varied drug use patterns and thus understanding the drug trends in the country or city is crucial to developing efficient methods in forensic toxicology. The FTU's drug of abuse panel includes all the drugs mentioned previously and when compared to the self-reporting recreational drug use from the participants the most reported drugs were cannabis, methamphetamine, and methaqualone, all of which are included in the drug panel. This highlights the effectiveness of the method and suggests that it would be beneficial to incorporate this method in routine drug testing of DFSO cases in the Western Cape. The FTU's method has been validated according to local and international guidelines (SANAS and ASB) which allows the results to withstand scrutiny in court during medico-legal investigations.

Auckloo & Davies (2019) conducted a post-mortem study on violent fatalities and found that 98% of these cases tested positive for alcohol. In this current study 73.3% of the participants (who had their data information sheet completed) reported that they had consumed alcohol prior to or during the alleged assault. Alcohol is commonly abused in South Africa and can lead to individuals being in vulnerable positions as seen in this study and the post-mortem study

(Auckloo & Davies, 2019). Therefore, it is imperative that accurate and reliable alcohol analyses are added to the scope of testing available to DFSO survivors.

4.5 Limitations of the study

Although this was a proof-of-concept study and the number of participants was sufficient, including a larger sample size could provide a more accurate representation of the prevalence of DFSO in sexual offence cases and the type of drugs commonly seen in these types of cases. To limit the stress that participating in the study could add to the survivor, attending medical practitioners were tasked with completing the data information sheets. This resulted in incomplete or missing information in certain cases, which could not be recovered. Standardised procedures for the collection of information and samples are recommended for future studies. As is often the case with DFSOs, the toxicology findings are compromised by delays between the alleged incident and sample collection. From the cases included in this study, the time from the alleged incident to sample collection varied up to six days which should be considered when interpreting findings. Lastly, as described, the LC-MS/MS method utilised is a targeted analysis which means that any substance which is not on the panel will not be detected in this analysis. An expanded panel and/or comprehensive screen with targeted confirmation should thus be considered for future research.

4.6 Conclusion

The aim of this study was to determine if the novel multi-drug analytical method developed by the Forensic Toxicology Unit is applicable to DFSO cases. Sexual offences are a major problem in South Africa and a large portion of the population use recreational drugs or regularly consume alcohol. The use of alcohol and recreational drugs increases an individual's vulnerability to DFSO. Amongst the study participants 64.7% of the survivors reported consuming alcohol prior to or at the time of the offence, 23.5% reported the use of medicinal drugs and 35.3% reported the use of recreational drugs. The toxicological analysis detected one or more psychoactive drugs in 58.8% of the cases. In 47.1% of cases the substance(s) detected did not match what was self-reported or knowingly consumed, indicating possible proactive DFSO. In conclusion, the method that was developed by the Forensic Toxicology Unit is applicable to DFSO cases as it includes the commonly abused drugs of the Western Cape population. Possible future studies could include developing and validating a method for determining blood alcohol concentration and enhancing the drug panels to support drug facilitated crime case work in South Africa.

REFERENCES

American Academy of Forensic Sciences. 2019. *Standard Practices for Method Validation in Forensic Toxicology*. 1st ed. V. ANSI/ASB Standard 036. Colorado Springs: AAFS Standards Board. Available: www.asbstandardsboard.org.

American Academy of Forensic Sciences. 2021. *Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations.* 1st ed. ANSI/ASB Standard 119. Colorado Springs: AAFS Standards Board. Available: www.asbstandardsboard.org.

Abrahams, N., Devries, K., Watts, C., Pallitto, C., Petzold, M., Shamu, S. & García-Moreno, C. 2014. Worldwide prevalence of non-partner sexual violence: A systematic review. *The Lancet*. 383(9929):1648–1654. DOI: 10.1016/S0140-6736(13)62243-6.

Ajayi, A.I., Mudefi, E. & Owolabi, E.O. 2021. Prevalence and correlates of sexual violence among adolescent girls and young women: findings from a cross-sectional study in a South African university. *BMC Women's Health*. 21:1-8. DOI: 10.1186/s12905-021-01445-8.

Anderson, L.J., Flynn, A. & Pilgrim, J.L. 2017. A global epidemiological perspective on the toxicology of drug-facilitated sexual assault: A systematic review. *Journal of Forensic and Legal Medicine*. 47:46–54. DOI: 10.1016/j.jflm.2017.02.005.

Anderson, L.J., Flynn, A., Drummer, O., Gerostamoulos, D. & Schumann, J.L. 2019. The role of voluntary and involuntary drug and alcohol consumption and premorbid mental health factors in drug-facilitated sexual assault. *Forensic Science, Medicine, and Pathology*. 15(3):382–391. DOI: 10.1007/s12024-019-00124-3.

Atakan, Z. 2012. Cannabis, a complex plant: Different compounds and different effects on individuals. *Therapeutic Advances in Psychopharmacology*. 2(6):241–254. DOI: 10.1177/2045125312457586.

Bertol, E., Di Milia, M.G., Fioravanti, A., Mari, F., Palumbo, D., Pascali, J.P. & Vaiano, F. 2018. Proactive drugs in DFSA cases: Toxicological findings in an eight-years study. *Forensic Science International*. 291:207–215. DOI: 10.1016/j.forsciint.2018.08.032.

Beyene, A.S., Chojenta, C., Roba, H.S., Melka, A.S. & Loxton, D. 2019. Gender-based violence among female youths in educational institutions of Sub-Saharan Africa: A systematic review and meta-analysis. *Systematic Reviews*. 8(1). DOI: 10.1186/s13643-019-0969-9.

Birkler, R.I.D., Telving, R., Ingemann-Hansen, O., Charles, A.V., Johannsen, M. & Andreasen, M.F. 2012. Screening analysis for medicinal drugs and drugs of abuse in whole blood using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOF-MS)--toxicological findings in cases of alleged sexual assault. *Forensic Science International*. 10(222(1–3)):154–61. DOI: 10.1016/j.forsciint.2012.05.019. Epub 2012 Jul 6.

Blaho, K., Logan, B., Winbery, S., Park, L. & Schwilke, E. 2000. Blood cocaine and metabolite concentrations, clinical findings, and outcome of patients presenting to an ED. *American Journal of Emergency Medicine*. 18(5):593–598. DOI: 10.1053/ajem.2000.9282.

Bonner, C.P., Browne, F.A., Carney, T., Shangase, N., Ndirangu, J.W., Myers, B. & Wechsberg, W.M. 2022. Mandrax use, sexual risk, and opportunities for pre-exposure prophylaxis among out-of-school adolescent girls and young women in Cape Town, South Africa. *South African Medical Journal*. 112(5):341–346. DOI: 10.7196/SAMJ.2022.v112i5.15971.

Busardo, F.P., Vari, M.R., Trana, D., Malaca, S., Carlier, J. & Luca, N.M. 2019. Drug-facilitated sexual assaults (DFSA): a serious underestimated issue. *European Review for Medical and Pharmacological Sciences*. 23:10577–10587. DOI: 10.26355/eurrev_201912_19753.

Caballero, C., Quintela, O. & Landeira, A. 2017. Alleged drug-facilitated sexual assault in a Spanish population sample. *Forensic Chemistry*. 4:61–66. DOI: 10.1016/j.forc.2017.02.009.

Chen, Y. & Ullman, S.E. 2010. Women's reporting of sexual and physical assaults to police in the national violence against women survey. *Violence Against Women*. 16(3):262–279. DOI: 10.1177/1077801209360861.

Chen, K.W., Berger, C.C., Forde, D.P., D'adamo, C., Weintraub, E. & Gandhi, D. 2011. Benzodiazepine Use and Misuse Among Patients in a Methadone Program. *BMC Psychiatry*. 11:90. DOI: 10.1186/1471-244X-11-90.

Costa, Y.R. de S., Lavorato, S.N. & Baldin, J.J.C.M. de C. 2020. Violence against women and drug-facilitated sexual assault (DFSA): A review of the main drugs. *Journal of Forensic and Legal Medicine*. 74:1-8. DOI: 10.1016/j.jflm.2020.102020.

Couch, G.A., White, M.P. & De Gray, L.E. 2020. Central nervous system stimulants: basic pharmacology and relevance to anaesthesia and critical care. *Anaesthesia and intensive care medicine*. 21(10):503–511. DOI: 10.1383/anes.2006.7.2.60.

Cruickshank, C.C. & Dyer, K.R. 2009. A review of the clinical pharmacology of methamphetamine. *Addiction*. 104(7):1085–1099. DOI: 10.1111/j.1360-0443.2009.02564.x.

Dada, S., Erasmus, J., Burnhams, N.H., Parr, C., Bhana, A., Timol, F., Fourie, D., Kitshoff, D., et al. 2015. Monitoring alcohol, tobacco and other drug abuse trends in South Africa (phase 38). *SACENDU*. 18(2):1–22. Available: https://www.researchgate.net/publication/284046057_Monitoring_alcohol_and_drug_abuse_trends_in_South_Africa_Phase_27 [2023, November 25].

Djezzar, S., Questel, F., Burin, E., Dally, S. & the French Network of centers for evalution and information on pharmacodependence. 2009. Chemical submission: results of 4-year French inquiry. *International journal of legal medicine* . 123:213–219. DOI: 10.1007/s00414-008-0291-x.

Friends of Victoria. 2024. *Clinical Forensic Medicine Department / Victoria Hospital Thuthuzela Care Centre*. Available: https://www.friendsofvictoriahospital.org/forensics/ [2024, February 27].

Goldhill, O. 2015. *I was sexually assaulted by a fellow student – but my university did nothing*. Available: https://www.telegraph.co.uk/women/womens-life/11340661/I-was-sexually-assaulted-by-a-fellow-student-but-my-university-did-nothing.html [2023, November 04].

Grela, A., Gautam, L. & Cole, M.D. 2018. A multifactorial critical appraisal of substances found in drug facilitated sexual assault cases. *Forensic Science International*. 292:50–60. DOI: 10.1016/j.forsciint.2018.08.034.

Grotenhermen, F. 2003. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clinical Pharmacokinetics*. 42:327–360. DOI: https://doi.org/10.2165/00003088-200342040-00003.

Hagemann, C.T., Helland, A., Spigset, O., Espnes, K.A., Ormstad, K. & Schei, B. 2013. Ethanol and drug findings in women consulting a Sexual Assault Center - Associations with clinical characteristics and suspicions of drug-facilitated sexual assault. *Journal of Forensic and Legal Medicine*. 20(6):777–784. DOI: 10.1016/j.jflm.2013.05.005.

Hardey, S. & Kelley, R. 2022. *How long does meth stay in your system?* Available: https://americanaddictioncenters.org/meth-treatment/how-long-in-system [2023, November 22].

Hiddink-Til, A., Teunissen, T.A.M. & Lagro-Janssen, A.L.M. 2021. Trends in victim, incident and care characteristics of a Dutch sexual assault center. *Journal of Forensic and Legal Medicine*. 81:102183. DOI: 10.1016/j.jflm.2021.102183.

Huang, Z., Xu, Z., Wang, H., Zhao, Z.Q. & Rao, Y. 2018. Influence of ethanol on the metabolism of alprazolam. *Expert Opinion on Drug Metabolism & Toxicology*. 14(6):551–559. DOI: 10.1080/17425255.2018.1483338.

Huestis, M.A. 2007. Human cannabinoid pharmacokinetics. *Chem Biodivers.* 4(8):1770-1804. DOI: 10.1002/cbdv.200790152.

Hurley, M., Parker, H. & Wells, D.L. 2006. The epidemiology of drug facilitated sexual assault. *Journal of Clinical Forensic Medicine*. 13(4):181–185. DOI: 10.1016/j.jcfm.2006.02.005.

Inger, J.A., Mihan, E.R., Kolli, J.U., Lindsley, C.W. & Bender, A.M. 2022. Dark Classics in chemical neuroscience: methaqualone. *ACS Chem. Neurosci.* 2023. 14(3):340-350. DOI: 10.1021/acschemneuro.2c00697.

Jewkes, R., Sikweyiya, Y., Morrell, R. & Dunkle, K. 2010. Why, when and how men rape. *SA Crime Quarterly*. 34:23–31. DOI: https://doi.org/10.17159/2413-3108/2010/i34a874.

Johnson, O.E., Miskelly, G.M. & Rindelaub, J.D. 2022. Testing for cannabis intoxication: Current issues and latest advancements. *WIREs Forensic Science*. 4(4):e1450. DOI: 10.1002/wfs2.1450.

Jones, A.W. 2019. Forensic Drug Profile: Cocaethylene. *Journal of Analytical Toxicology*. 43(3):155–160. DOI: 10.1093/jat/bkz007.

Juhascik, M.P., Negrusz, A., Faugno, D., Ledray, L., Greene, P., Lindner, A., Haner, B. & Gaensslen, R.E. 2007. An estimate of the proportion of drug-facilitation of sexual assault in four U.S. localities. *Journal of Forensic Sciences*. V. 52. 1396–1400. DOI: 10.1111/j.1556-4029.2007.00583.x.

Kapil, V., Green, J.L., Le Lait, C., Wood, D.M. & Dargan, P.I. 2014. Misuse of benzodiazepines and Z-drugs in the UK. *British Journal of Psychiatry*. 205(5):407–408. DOI: 10.1192/bjp.bp.114.149252.

Kelly, M.G. & Psych, M.R.C. 1973. A drug of abuse-Mandrax. *Journal of the Irish Medical Association*. 66(16):456–457. Available: https://www.drugsandalcohol.ie/6575/1/611-0566.pdf [2023, November 25].

Kilpatrick, D.G., Resnick, H.S., Ruggiero, K.J., Conoscenti, L.M. & Mccauley, J. 2007. *Drug-facilitated, Incapacitated, and Forcible Rape: A National Study*. Charleston. DOI: http://dx.doi.org/10.3886/ICPSR20626.

Lopez-Castroman, J., Melhem, N., Birmaher, B., Greenhill, L., Kolko, D., Stanley, B., Zelazny, J., Brodsky, B., et al. 2013. Early childhood sexual abuse increases suicidal intent. *World Psychiatry*. 12(2):149–154. DOI: 10.1002/wps.20039.

Lucas, C.J., Galettis, P. & Schneider, J. 2018. The pharmacokinetics and the pharmacodynamics of cannabinoids. *British Journal of Clinical Pharmacology*. 84(11):2477–2482. DOI: 10.1111/bcp.13710.

Madea, B. & Mußhoff, F. 2009. K.-o.-mittel: Häufigkeit, wirkungsweise, beweismittelsicherung. *Deutsches Arzteblatt*. 106(20):341–347. DOI: 10.3238/arztebl.2009.0341.

McCabe, S.E., West, B.T., Schepis, T.S. & Teter, C.J. 2015. Simultaneous co-ingestion of prescription stimulants, alcohol and other drugs: A multi-cohort national study of US adolescents. *Human Psychopharmacology*. 30(1):42–51. DOI: 10.1002/hup.2449.

McCarthy, G., Myers, B. & Siegfried, N. 2005. Treatment for Methaqualone dependence in adults. *Cochrane Database of Systematic Reviews*. (April, 2005). DOI: 10.1002/14651858.cd004146.pub2.

Meinck, F., Cluver, L.D., Boyes, M.E. & Loening-Voysey, H. 2016. Physical, emotional and sexual adolescent abuse victimisation in South Africa: prevalence, incidence, perpetrators and locations. *J Epidemiol Community Health*. 70(9):910–916. DOI: 10.1136/jech.

Mognetti, B., Bo, M., Berta, G.N., Canavese, A., Castagna, P., Collini, F., Santa, V., Salomone, A., et al. 2022. Sexual Harassments Related to Alcohol and Drugs Intake: The Experience of the Rape Centre of Turin. *International Journal of Environmental Research and Public Health*. 19(22). DOI: 10.3390/ijerph192215090.

Musshoff, F. & Madea, B. 2006. Review of biologic matrices (urine, blood, hair) as indicators of recent or ongoing cannabis use. *Ther Drug Monit*. 28(2):155–63. Available: http://journals.lww.com/drug-monitoring.

Nattala, P., Leung, K.S., Abdallah, A. Ben, Murthy, P. & Cottler, L.B. 2012. Motives and simultaneous sedative-alcohol use among past 12-month alcohol and nonmedical sedative users. *Am J Drug Alcohol Abuse*. 38(4):359–364. DOI: 10.3109/00952990.2011.643987.

Okafor, C.N., Stein, D.J., Dannatt, L., Ipser, J., van Nunen, L.J., Lake, M.T., Krishnamurti, T., London, E.D., et al. 2020. Contingency management treatment for methamphetamine use disorder in South Africa. *Drug and Alcohol Review*. 39(3):216–222. DOI: 10.1111/dar.13019.

Orkin, M., Hirschowitz, R. & Worku, S. 2000. *Quantitative research findings on rape in South Africa*. Pretoria: Statistics South Africa. Available: https://www.statssa.gov.za/publications/Rape/Rape.pdf [2023, November 25].

Orwa, T.O. & Nyabadza, F. 2019. Mathematical modelling and analysis of alcohol-methamphetamine co-abuse in the Western Cape Province of South Africa. *Cogent Mathematics & Statistics*. 6(1):1641175. DOI: 10.1080/25742558.2019.1641175.

Pergolizzi, J., Breve, F., Magnusson, P., LeQuang, J.A.K. & Varrassi, G. 2022. Cocaethylene: When Cocaine and Alcohol Are Taken Together. *Cureus*. 14(2):e22498. DOI: 10.7759/cureus.22498.

Poulsen, H., McCarthy, M.J., Baker, J., Verma, A., Moir, H.J., Brodie, T., Thatti, B., Trotter, G., et al. 2021. Toxicological Assessment of the Role of Alcohol and Drugs in Drug-Facilitated Sexual Assault Cases in New Zealand. *Journal of Analytical Toxicology*. 45(1):44–52. DOI: 10.1093/jat/bkz110.

Rabie, S., Bantjes, J., Gordon, S., Almirol, E., Stewart, J., Tomlinson, M. & Rotheram-Borus, M.J. 2020. Who can we reach and who can we keep? Predictors of intervention engagement and adherence in a cluster randomized controlled trial in South Africa. *BMC Public Health*. 20(1):275. DOI: 10.1186/s12889-020-8357-x.

Reyes-Parada, M., Iturriaga-Vasquez, P. & Cassels, B.K. 2020. Amphetamine derivatives as monoamine oxidase inhibitors. *Frontiers in Pharmacology*. 10:1590. DOI: 10.3389/fphar.2019.01590.

Romach, M.K., Schoedel, K.A. & Sellers, E.M. 2014. Human abuse liability evaluation of CNS stimulant drugs. *Neuropharmacology*. 87:81–90. DOI: 10.1016/j.neuropharm.2014.04.014.

South African Community Epidemiology Network on Drug Use. 2023. *Monitoring alcohol, tobacco and other drug trends (South Africa): July - December 2022*.

South African National Accreditation System. 2020. *Recommended guidelines for the verification and validation of methods in forensic chemistry*. Available: https://www.sanas.co.za/Publications%20and%20Manuals%20Files/TG%2041-03.pdf [2024, February 20].

Schepis, T.S., West, B.T., Teter, C.J. & McCabe, S.E. 2016. Prevalence and correlates of co-ingestion of prescription tranquilizers and other psychoactive substances by U.S. high school

seniors: Results from a national survey. *Addictive Behaviors*. 52:8–12. DOI: 10.1016/j.addbeh.2015.08.002.

Scott-Ham, M. & Burton, F.C. 2005. Toxicological findings in cases of alleged drug-facilitated sexual assault in the United Kingdom over a 3-year period. *Journal of Clinical Forensic Medicine*. 12(4):175–186. DOI: 10.1016/j.jcfm.2005.03.009.

Sharma, P., Murthy, P. & Bharath, M.M.S. 2012. Chemistry, metabolism, and toxicology of cannabis: clinical implications. *Iranian J Psychiatry*. 7(4):149–156. PMID: 23408483. PMCID: PMC3570572.

Sicari, V. & Zabbo, C.P. 2023. *Diphenhydramine*. V. StatPearls [Internet]. Treasure Island: StatPearls Publishing. Available: https://www.ncbi.nlm.nih.gov/books/NBK526010/ [2024, February 26].

Skov, K., Johansen, S.S., Linnet, K. & Nielsen, M.K.K. 2022. A review on the forensic toxicology of global drug-facilitated sexual assaults. *European Review for Medical and Pharmacological Sciences*. 26:183–197. DOI: 10.26355/eurrev_202201_27767.

Sonone, S., Jadhav, S. & Singh Sankhla, M. 2021. A Forensic Aspect on Drug Facilitated Sexual Assault. (February). DOI: 10.15406/frcij.2021.09.00341.

South Africa Government. 2024. *Thuthuzela care centres*. Available: https://www.gov.za/TCC [2024, February 27].

South African government. 2007. *Criminal law (sexual offences and related matters) amendment act 32 of 2007*.

Spohn, C. 2020. Sexual assault case processing: The more things change, the more they stay the same. *International Journal for Crime, Justice and Social Democracy*. 9(1):86–94. DOI: 10.5204/ijcjsd.v9i1.1454.

Steele, S.J., Abrahams, N., Duncan, K., Woollett, N., Hwang, B., O'Connell, L., Van Cutsem, G. & Shroufi, A. 2019. The epidemiology of rape and sexual violence in the platinum mining district of Rustenburg, South Africa: Prevalence, and factors associated with sexual violence. *PLoS ONE*. 14(7):e0216449. DOI: 10.1371/journal.pone.0216449.

Stefani, N.T., French, D., Jannetto, P.J., Rappold, B.A. & Clarke, W.A. 2022. Liquid chromatography–tandem mass spectrometry for clinical diagnostics. *Nature reviews.* 2(1): 96-99. DOI: 10.1038/s43586-022-00175-x.

Tiemensma, M. & Davies, B. 2018. Investigating drug-facilitated sexual assault at a dedicated forensic centre in Cape Town, South Africa. *Forensic Science International*. 288:115–122. DOI: 10.1016/j.forsciint.2018.04.028.

United Nations Office on Drugs and Crime. 2011. *Guidelines for the forensic analysis of drugs facilitating sexual assault and other criminal acts*. Available: www.unodc.org.

Votaw, V.R., Geyer, R., Rieselbach, M.M. & McHugh, R.K. 2019. The epidemiology of benzodiazepine misuse: A systematic review. *Drug and Alcohol Dependence*. 200:95–114. DOI: 10.1016/j.drugalcdep.2019.02.033.

Walker, S.P. & Louw, D. 2005. The Court for Sexual Offences: Perceptions of the victims of sexual offences. *International Journal of Law and Psychiatry*. 28(3):231–245. DOI: 10.1016/j.ijlp.2005.04.001.

World health organisation. 2023. *Opioid overdose*. Available: https://www.who.int/news-room/fact-sheets/detail/opioid-overdose [2024, February 26].

Wille, S.M.R., Van Dijck, K., Van Assche, A., Di Fazio, V., Ramiréz-Fernandéz, M.D.M., Vanvooren, V. & Samyn, N. 2021. The interest of a systematic toxicological analysis combined with forensic advice to improve the judicial investigation and final judgment in drug facilitated sexual assault cases. *Pharmaceuticals*. 14(5). DOI: 10.3390/ph14050432.

Yang, J.M. & Lewandrowski, K.B. 2001. Urine drugs of abuse testing at the point-of-care: clinical interpretation and programmatic considerations with specific ž / reference to the Syva Rapid Test SRT. *Clinica Chimica Acta*. 307:27–32. Available: www.elsevier.comrlocaterclinchim.

APPENDIX A: INFORMED CONSENT AND CASE INFORMATION SHEET

A.1 Case information sheet

Investigating the role of routine drug analyses in survivors of sexual offences admitted to the Clinical Forensic Unit at Victoria Hospital

Clinical Forensic Unit, Victoria Hospital, in collaboration with Division of Forensic Medicine, University of Cape Town

1. Basic information

Study number	**CP-2022-XX**
Date of birth *(dd/mm/yyyy)*	
Sex	☐ Male ☐ Female
Date and time of examination	**Date:** / / **Time:** h

2. Case history

Approximate date and time of alleged incident		**Date:** / /			**Time:** h
Did the patient report to the clinic ≤ 2 weeks after the assault? *If yes, collect blood and urine samples.*				□ Yes	□ No
Details of incident:					
□ Clear history		□ Vague recollection		□ No recollection	
Place of the alleged incident:					
□ Own residence	□ Other residence	□ Workplace	□ Bar/ club/ restaurant	□ Public space	□ Other _____
Was the perpetrator known to the participant?				□ Yes	□ No
Type of sexual assault:					
Penetrative:	□ Oral	□ Vaginal	□ Anal	□ Non-penetrative	
Associated physical violence or injuries present?				□ Yes	□ No
Has the incident been reported to SAPS?				□ Yes	□ No
Alcohol history					
Self-reported alcohol use?				□ Yes	□ No
If yes, when was alcohol consumed:					
□ **Prior** to the incident		□ **At** the time of the incident		□ **After** the incident	
Type of alcohol:					

□ Beer	□ Wine	□ Spirits	□ Other _____
Number of units/drinks consumed?			
Approximate date and time of first drink? / /			h
Approximate date and time of last drink? / /			h
Medicinal drug history			
Self-reported medicinal or pharmaceutical drug use?		□ Yes	□ No
If yes, when was medication(s) consumed:			
□ **Prior** to the incident	□ **At** the time of the incident	□ **After** the incident	
Type of medication:			
□ Prescription	□ Non-prescription		
Name or kind of medication and dosage:			
Approximate date and time of last dose? / /			h
Recreational drug history			
Self-reported recreational drug use?		□ Yes	□ No
If yes, when was drug(s) consumed:			
□ **Prior** to the incident	□ **At** the time of the incident	□ **After** the incident	
Name or type of drugs used:			
Route of administration:			
□ Oral	□ Intravenous	□ Snorted	□ Inhaled/smoked
Approximate date and time of last administration? / /			h
Regular recreational drug user?		□ Yes	□ No
Medical examination			
Were any of the following symptoms reported/observed? *(please tick all that apply)*			

□ Drowsiness / sedation	□ Confusion	□ Loss of consciousness	□ Amnesia	□ Nausea	□ Dizziness
□ Slurred speech	□ Weakness	□ Seizures	□ Pupil size reaction	□ Impaired vision	□ Paralysis
□ Loss of inhibitions	□ Aggression	□ Hallucinations / Dissociations	□ Vomiting	□ Diarrhea	□ Impaired coordination

Does the participant suspect they are a victim of drug-facilitated sexual assault?	□ Yes	□ No	□ Uncertain
If yes, is there a specific substance known or suspected?		□ Yes	□ No
Please specify:			
Is there any evidence / suspicion that the patient is under the influence of drugs or alcohol?		□ Yes	□ No
If yes, please describe:			
Additional medical or case history: *(Relevant history, especially if any conditions present which may influence alcohol and/or drug metabolism and elimination, such as liver and GIT conditions)*			

3. Sampling information

Please collect the following samples, and label specimens with the study number, type of specimen, and collection date (dd/mm/yyyy): (Eg. CP_XX_Urine_21032022).

- Approximately 4 mL of blood in a grey top tube
- 60 to 100 mL of urine in a sterile urine collection cup

Place labelled blood and urine in the designated refrigerator in the Clinical Forensic Unit.

Type of specimen(s)	**Sample collection date** (dd/mm/yyyy)	**Sample collection time** (hh:mm)	**Collected by**
Blood			
Urine			

_____

Name of Attending Medical Practitioner

_____ _____

Signature **Date** (*dd/mm/yyyy*)

For any queries please contact: Jade Mader, Tel: 021 650 5492 / 083 377 5354

A.2 Informed consent form

Investigating the role of routine drug analyses in survivors of sexual offences admitted to the Clinical Forensic Unit at Victoria Hospital
Study number: CP-XX-2022

Researchers: Casey Peters, Jade Mader and Bronwen Davies

Why is this study being done?
A drug-facilitated sexual offence (DFSO) is a criminal offence in South Africa; however, routine testing for drugs in these cases is not always done. This results in the loss of valuable evidence in DFSO cases. Whether you chose to use drugs or alcohol or not, this may affect your ability to consent (say "yes" or "no") to a sexual activity. This study hopes to get more information on drug use in sexual offence cases to be able to improve the testing available to survivors of sexual assault. If you are willing to allow your doctor to collect blood and/or urine samples for this study, please read the rest of this form.

Who can take part in the study?
Adult (18 years and older) survivors of a sexual offence who report to the Clinical Forensic Unit within the first two weeks after the incident.

What will happen to your samples?
If you decide to take part in the study, ± 4 mL of blood (1 tube) and 60-100 mL urine (1 small cup) will be collected (once-off). The doctor will draw blood from your arm during their examination and you will be asked to collect your own urine sample in a collection cup (in private). Your samples will then be tested for 31 common drugs that are used in Cape Town.
A unique study and laboratory number will be given to your samples to ensure your personal details remain anonymous.

Your personal information (name, surname or ID number) will not be used or mentioned anywhere in the study. If drugs are found in your samples, this will have no negative consequences to you. Your genetic material or DNA **will not** be studied; this study only looks at drug use. The anonymous information collected from you will be safely stored and only the researchers mentioned above will have access to it.
There will be no negative consequences if you choose not to take part or wish to withdraw at any stage of the study. If you decide that you do not want to be part of this study (after signing this form), your samples will be disposed of and not tested, and your information will be removed from the study.

Storage of samples
The samples collected at the Clinical Forensic Unit for this study will either be discarded (thrown away) upon completion of the study, or, if you allow, will be stored for up to five years for future DFSO research. If any other research is done using these samples the research will first undergo a complete review by the University of Cape Town's Human Research Ethics Committee. No personal information (name, surname or ID number) will be used for any future studies. If you do not want your samples to be stored for any future research, all samples will be discarded after this study is complete (less than one year). Please indicate your choice by selecting **yes** or **no** below.

Benefits of this study
This study will help to better understand drug use in sexual offence cases. It will allow laboratories to develop new methods for drug testing that could help survivors of sexual assault.

Will the results of the research be shared with you?
The test results will be shared in confidence with your doctor at the Clinical Forensic Unit. You may ask your doctor for the results, however, because this is a research study the results may not be used as evidence for any legal proceedings.

Contact information
If you have any questions about the study or would like more information, please feel free to contact Jade Mader on 021 650 5492 or 083 377 5354.

This study has been approved by the University of Cape Town Human Research Ethics Committee (Reference number: 231/2022).
Contact number: 021 406 6492

If you understand the study and still wish to participate, please complete the consent form by circling **yes** or **no** and sign below.
I confirm that:

I have read and understood the contents of this form and give permission to be included in this study.	Yes	No
I have been informed about the purpose, sample collection procedures, potential benefits and risks of this study.	Yes	No
I understand that blood and urine samples will be collected by my doctor, if I present within two weeks of the assault.	Yes	No
I understand that these samples will be used for research, if I give permission.	Yes	No
I understand that I can withdraw my permission from the study, with no negative consequences, at any time, whether prior to the study or during the study.	Yes	No
If I do not give permission, I understand that this will not affect my treatment or have any negative effects to me.	Yes	No
Storage of samples		
I give permission to store my samples for future DFSO research (up to 5 years) that has been approved by an appropriate research ethics committee.	Yes	No

_____ _____
Patient Signature Date (*dd/mm/yyyy*)

_____ _____
Attending Medical Practitioner Signature Date (*dd/mm/yyyy*)

APPENDIX B: PRODUCT INFORMATION FOR CERTIFIED REFERENCE MATERIALS

Table B.1: Bulk stock concentration and manufacturers of certified reference materials used for preparation of calibrators and controls.

#	Standard	Concentration	Manufacturer
1	6-Acetylmorphine	0.1 mg/mL	LGC Standards
2	Acetaminophen	1.0 mg/mL	LGC Standards
3	Alprazolam	1.0 mg/mL	Restek Corporation
4	Amitriptyline	1.0 mg/mL	LGC Standards
5	Amphetamine	1.0 mg/mL	LGC Standards
6	Benzoylecgonine	1.0 mg/mL	LGC Standards
7	Buprenorphine	1.0 mg/mL	LGC Standards
8	Cannabidiol (CBD)	1.0 mg/mL	Restek Corporation
	Delta-9-Tetrahydrocannabinol (THC)		
9	Clobazam	1.0 mg/mL	Restek Corporation
10	Cocaethylene	1.0 mg/mL	LGC Standards
11	Cocaine	1.0 mg/mL	LGC Standards
12	Codeine	1.0 mg/mL	LGC Standards
13	Diazepam	1.0 mg/mL	Restek Corporation
14	Diphenhydramine	1.0 mg/mL	LGC Standards
15	Fentanyl	1.0 mg/mL	LGC Standards
16	Hydrocodone	1.0 mg/mL	LGC Standards
17	Hydromorphone	1.0 mg/mL	LGC Standards
18	Ketamine	1.0 mg/mL	LGC Standards
19	3,4-Methylenedioxyamphetamine (MDA)	1.0 mg/mL	LGC Standards
20	3,4-Methylenedioxymethamphetamine (MDMA)	1.0 mg/mL	LGC Standards
21	Methadone	1.0 mg/mL	LGC Standards
22	Methamphetamine	1.0 mg/mL	LGC Standards
23	Methaqualone	1.0 mg/mL	LGC Standards
24	Methcathinone	1.0 mg/mL	LGC Standards
25	Morphine	1.0 mg/mL	LGC Standards
26	O-Desmethyltramadol (O-DSMT)	1.0 mg/mL	LGC Standards
27	Oxycodone	1.0 mg/mL	LGC Standards
28	Oxymorphone	1.0 mg/mL	LGC Standards
29	11-Nor-9-Carboxy-Delta-9-THC (THC-COOH)	0.1 mg/mL	Restek Corporation
30	Tramadol	1.0 mg/mL	LGC Standards

Table B.2: Bulk stock concentration and manufacturers of internal standards for each drug anaylsed in the DOA panel I method for blood and urine

Internal standards used for quantitative analysis in whole blood			
#	**Internal standard**	**Concentration**	**Manufacturer**
1	6-Acetylmorphine D3	1.0 mg/mL	LGC Standards
2	Acetaminophen D4	1.0 mg/mL	LGC Standards
3	Alprazolam D5	0.1 mg/mL	LGC Standards
4	Amitriptyline D3	0.1 mg/mL	LGC Standards
5	Amphetamine D6	1.0 mg/mL	LGC Standards
6	Benzoylecgonine D3	0.1 mg/mL	LGC Standards
7	Buprenorphine D4	1.0 mg/mL	Cerilliant Corporation
8	Cocaethylene D3	0.1 mg/mL	LGC Standards
9	Cocaine D3	1.0 mg/mL	LGC Standards
10	Codeine D3	0.1 mg/mL	LGC Standards
11	Diazepam D5	0.1 mg/mL	Restek Corporation
12	Diphenhydramine D3	0.1 mg/mL	LGC Standards
13	Fentanyl D5	0.1 mg/mL	LGC Standards
14	Hydrocodone D3	0.1 mg/mL	LGC Standards
15	Hydromorphone D3	0.1 mg/mL	LGC Standards
16	Ketamine D4	0.1 mg/mL	LGC Standards
17	MDA D5	1.0 mg/mL	LGC Standards
18	MDMA D5	0.1 mg/mL	LGC Standards
19	Methadone D3	1.0 mg/mL	LGC Standards
20	Methamphetamine D5	1.0 mg/mL	LGC Standards
21	Morphine D3	1.0 mg/mL	LGC Standards
22	Methaqualone D7	0.1 mg/mL	LGC Standards
23	O-desmethyl-cis-tramadol D6	0.1 mg/mL	Cerilliant Corporation
24	Oxycodone D3	1.0 mg/mL	LGC Standards
25	Oxymorphone D3	1.0 mg/mL	LGC Standards
26	THC-COOH D3	0.1 mg/mL	LGC Standards
27	Tramadol-13C D3	0.1 mg/mL	LGC Standards
Internal standards used for qualitative analysis in urine			
1	Doxepin-D3	10 µg/mL	Restek Corporation
	Diazepam-D5		

APPENDIX C: MULTIPLE REACTION MONITORING PARAMETERS

Table C.1: Multiple reaction monitoring parameters (whole blood)

#	Analyte	Precursor ion (m/z)	Product ions (m/z)		DT (secs)	CV (V)	CE (eV)		RT (min)
1	6-acetylmorphine	328.2	165.1	211.1	0.006	52	35	25	1.31
2	6-acetylmorphine-d3	331.1	165.0	-	0.006	45	50	-	1.30
3	Acetaminophen	152.0	110.1	93.2	0.007	40	22	20	1.08
4	Acetaminophen-d4	156.2	114.1	-	0.007	30	15	-	1.08
5	Alprazolam	309.0	205.0	281.1	0.024	50	43	30	2.75
6	Alprazolam-d5	314.1	210.1	-	0.024	60	42	-	2.74
7	Amitriptyline	278.2	91.1	105.1	0.024	44	20	24	2.56
8	Amitriptyline-d3	281.3	91.1	-	0.024	38	30	-	2.57
9	Amphetamine	136.2	119.1	91.1	0.006	25	10	20	1.31
10	Amphetamine-d6	142.0	93.1	-	0.006	36	26	-	1.30
11	Benzoylecgonine	290.1	168.1	105.0	0.006	36	18	36	1.55
12	Benzoylecgonine-d3	293.1	171.1	-	0.006	78	22	-	1.55
13	Buprenorphine	468.2	101.0	396.2	0.024	60	42	38	2.30
14	Buprenorphine-d4	472.3	59.1	-	0.024	96	48	-	2.30
15	Clobazam	301.0	259.0	224.0	0.031	40	18	32	2.99
16	Cocaethylene	318.2	196.2	82.1	0.024	42	20	30	2.04
17	Cocaethylene-d3	321.2	85.2	-	0.024	80	34	-	2.04
18	Cocaine	304.1	182.1	82.1	0.034	40	18	28	1.84
19	Cocaine-d3	307.2	185.2	-	0.034	42	20	-	1.84
20	Codeine	300.1	215.2	165.1	0.006	54	26	38	1.20
21	Codeine-d3	303.0	215.0	-	0.006	50	26	-	1.20
22	Diazepam	285.1	154.0	193.1	0.031	50	26	30	3.13
23	Diazepam-d5	290.0	154.1	-	0.031	56	28	-	3.11
24	Diphenhydramine	256.2	167.1	152.0	0.024	22	16	50	2.24
25	Diphenhydramine -d3	259.2	167.1	-	0.024	22	14	-	2.24
26	Fentanyl	337.2	105.1	188.2	0.024	48	38	22	2.18
27	Fentanyl-d5	342.2	105.1	-	0.024	86	48	-	2.17
28	Hydrocodone	300.1	199.1	171.0	0.006	60	30	44	1.37
29	Hydrocodone-d3	303.2	199.1	-	0.006	94	34	-	1.37
30	Hydromorphone	286.1	185.1	157.1	0.008	66	32	42	1.00
31	Hydromorphone-d3	289.0	184.9	-	0.008	50	30	-	0.99

#	Analyte	Precursor ion (m/z)	Product ions (m/z)		DT (secs)	CV (V)	CE (eV)		RT (min)
32	Ketamine	238.1	125.0	207.0	0.006	28	24	12	1.55
33	Ketamine-d4	242.1	129.0	-	0.006	62	26	-	1.55
34	MDA	180.2	163.2	133.1	0.006	18	8	16	1.33
35	MDA-d5	185.1	110.1	-	0.006	36	22	-	1.33
36	MDMA	194.1	163.1	105.0	0.006	24	11	24	1.40
37	MDMA-d5	199.1	165.1	-	0.006	46	14	-	1.40
38	Methadone	310.2	265.2	105.0	0.024	34	16	28	2.61
39	Methadone-d3	313.2	105.0	-	0.024	64	26	-	2.61
40	Methamphetamine	150.1	91.0	119.0	0.006	25	16	12	1.39
41	Methamphetamine-d5	155.1	91.9	-	0.006	52	18	-	1.39
42	Methaqualone	251.1	91.1	132.0	0.024	98	40	26	2.76
43	Methaqualone-d7	258.2	98.0	-	0.024	52	44	-	2.75
44	Methcathinone	164.0	131.0	105.0	0.006	24	18	26	1.20
45	Morphine	286.1	201.1	165.1	0.016	54	28	34	0.88
46	Morphine-d3	288.9	200.9	-	0.016	50	26	-	0.88
47	O-DSMT	250.1	58.0	-	0.006	54	16	-	1.35
48	O-DSMT-d6	256.2	64.1	-	0.006	54	16	-	1.35
49	Oxycodone	316.1	256.2	241.1	0.006	44	26	26	1.30
50	Oxycodone-d3	319.2	244.0	-	0.006	40	32	-	1.30
51	Oxymorphone	302.1	227.0	242.1	0.011	44	28	24	0.93
52	Oxymorphone-d3	305.1	230.0	-	0.011	36	30	-	0.93
53	THC-COOH	345.1	327.3	299.3	0.063	40	20	25	4.09
54	THC-COOH-d3	348.2	196.1	-	0.063	42	32	-	4.09
55	Tramadol	264.2	58.0	-	0.038	24	16	-	1.71
56	Tramadol-13C-d3	268.2	58.0	-	0.038	32	18	-	1.71

Table C.2: Multiple reaction monitoring parameters (urine)

#	Analyte	Precursor ion (m/z)	Product ions (m/z)		DT (secs)	CV (V)	CE (eV)		RT (mins)
1	6-acetylmorphine	328.2	165.1	211.1	0.011	52	35	25	1.31
2	Alprazolam	309.0	205.0	281.1	0.034	50	43	30	2.75
3	Amitriptyline	278.2	91.1	105.1	0.034	44	20	24	2.56
4	Amphetamine	136.2	119.1	91.1	0.011	25	10	20	1.31
5	Benzoylecgonine	290.1	168.1	105.0	0.011	36	18	36	1.55
6	Buprenorphine	468.2	101.0	396.2	0.034	60	42	38	2.30
7	CBD	315.2	193.1	123.0	0.080	40	22	32	4.32
8	Clobazam	301.0	259.0	224.0	0.034	40	18	32	2.99
9	Cocaethylene	318.2	196.2	82.1	0.034	42	20	30	2.04
10	Cocaine	304.1	182.1	82.1	0.052	40	18	28	1.84
11	Codeine	300.1	215.2	165.1	0.011	54	26	38	1.20
12	Diazepam	285.1	154.0	193.1	0.034	50	26	30	3.13
13	Diazepam-d5	290.0	154.1	-	0.034	56	28	-	3.11
14	Diphenhydramine	256.2	167.1	152.0	0.034	22	16	50	2.24
15	Doxepin-d3	283.1	106.9	-	0.034	48	26	-	2.31
16	Fentanyl	337.2	105.1	188.2	0.034	48	38	22	2.18
17	Hydrocodone	300.1	199.1	171.0	0.011	60	30	44	1.37
18	Hydromorphone	286.1	185.1	157.1	0.014	66	32	42	1.00
19	Ketamine	238.1	125.0	207.0	0.011	28	24	12	1.55
20	MDA	180.2	163.2	133.1	0.011	18	8	16	1.33
21	MDMA	194.1	163.1	105.0	0.011	24	11	24	1.40
22	Methadone	310.2	265.2	105.0	0.034	34	16	28	2.61
23	Methamphetamine	150.1	91.0	119.0	0.011	25	16	12	1.39
24	Methaqualone	251.1	91.1	132.0	0.034	98	40	26	2.76
25	Methcathinone	164.0	131.0	105.0	0.011	24	18	26	1.20
26	Morphine	286.1	201.1	165.1	0.024	54	28	34	0.88
27	O-DSMT	250.1	58.0	-	0.011	54	16	-	1.35
28	Oxycodone	316.1	256.2	241.1	0.011	44	26	26	1.30
29	Oxymorphone	302.1	227.0	242.1	0.017	44	28	24	0.93
30	THC	315.2	193.1	123.0	0.080	40	22	32	4.64
31	THC-COOH	345.1	327.3	299.3	0.080	40	20	25	4.09
32	Tramadol	264.2	58.0	-	0.063	24	16	-	1.71

APPENDIX D: EHTICAL APPROVAL FORMS

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 0866: fax: +27 21 483 6058
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: WC_202206_028
ENQUIRIES: Dr Sabela Petros

University of Cape Town
Anzio Road
Observatory
Cape Town
7925

For attention: Mrs Jade Mader, Ms Casey Peters, Ms Bronwen Davies

Re: Investigating the role of routine drug analyses in survivors of sexual offences admitted to the Clinical Forensic Unit at Victoria Hospital

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries in accessing the following sites:

Victoria Hospital	**Dr Graeme Dunbar**	**021 799 1211**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted and the constraints caused by the Covid-19 epidemic above are respected and adhered to.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final feedback (**Annexure 9**) within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. In the event where the research project goes beyond the *estimated completion* date which was submitted, researchers are expected to complete and submit a progress report (**Annexure 8**) and an updated ethics clearance letter to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
4. The reference number above should be quoted in all future correspondence.

Yours sincerely

PROF. V ZWEIGENTHAL
DIRECTORATE: HEALTH INTELLIGENCE
DATE: 29 July 2022

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

07 June 2022

HREC REF: 231/2022

Ms J Mader
Division of Forensic Medicine & Toxicology
Level 2 Falmouth Building- FHs
Email: jade.mader@uct.ac.za
Student: PTRCAS006@myuct.ac.za

Dear Ms Mader

PROJECT TITLE : INVESTIGATING THE ROLE OF ROUTINE DRUG ANALYSES IN SURVIVORS OF SEXUAL OFFENSES ADMITTED TO THE CLINICAL FORENSIC UNIT AT VICTORIA HOSPITAL-
(MASTERS MISS CASEY PETERS)

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19. Please refer to guidance letter dated 02 February 2022 on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Approval is granted for one year until the 30 June 2023.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Miss Casey Peters will also be involved in this study

Please quote the HREC REF 231/2022 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/6/2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	3/6/2023

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

HUMAN RESEARCH ETHICS COMMITTEE
- 2 JUN 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Please clarify your plan for research-related activities during COVID-19 lockdown

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	2023/06/02		
HREC REF Number	231/2022	Current Ethics Approval was granted until	30 June 2023
Protocol title	Investigating the role of routine drug analyses in survivors of sexual offences admitted to the Clinical Forensic Unit at Victoria hospital		
Principal Investigator	Jade Mader		
Department / Office Internal Mail Address	Division of Forensic Medicine and Toxicology, Level 1, Entrance 3, Falmouth Building, Anzio Road, Cape Town.		
1.1 Does this protocol receive US Federal funding?		☐ Yes	✓ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
✓	Data collection is complete, data analysis only
Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository.	

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		37
Total number of records or specimens collected, reviewed or stored since last progress report		n/a
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	☐ Yes	✓ No

4. Signature

Signature of PI		Date	2023/05/31