



Faculty of Sciences
Department of Chemical and Forensic Sciences

**DRUG FACILITATED SEXUAL ASSAULT: SURVEY AND METHOD
DEVELOPMENT FOR DETECTION AND STABILITY STUDIES OF
DRUGS COMMONLY ENCOUNTERED IN SEXUAL ASSAULT CASES**

By;
MOLATELO MODIRI D. THUTWA

Student ID number: 15001448
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Supervisors: Prof. Ellen Mwenesongole¹, Prof. Lata Gautam² and Dr Ofentse Mazimba³

¹J. Frank Barefield, Jr Department of Criminal Justice, College of Arts and Sciences, University of Alabama at Birmingham.

²Forensic and Investigative Sciences, School of Life Sciences, Faculty of Science and Engineering, Anglia Ruskin University.

³Department of Chemical and Forensic Sciences, Faculty of Science, BIUST.

October 2023

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Candidate: MOLATELO MODIRI D. THUTWA

Student ID: 15001448

Thesis Titled: DRUG FACILITATED SEXUAL ASSAULT: SURVEY AND METHOD DEVELOPMENT FOR DETECTION AND STABILITY STUDIES OF DRUGS COMMONLY ENCOUNTERED IN SEXUAL ASSAULT CASES

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DEDICATION

This thesis is dedicated to my family especially my sisters and aunts, who have always been my best cheerleaders. I will always appreciate their confidence and trust in me to aim and achieve higher. This achievement means so much to them as it does to me.

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LIST OF ABBREVIATIONS

AET- Alcohol Education Trust

BIUST- Botswana International University of Science and Technology

BPS- Botswana Police Service

BSA- N,O-Bis(trimethylsilyl)acetamide

BSTFA- N,O-Bis(trimethylsilyl)trifluoroacetamide

CNS- Central nervous system

DDT- DDT College of Medicine

DFSA- Drug Facilitated Sexual Assault

DLLME- Dispersive liquid-liquid micro-extraction

GABA- Gamma-aminobutyric acid

GC-MS-Gas chromatography-mass spectrometry

GHB- Gamma hydroxybutyrate

GUC- Gaborone University College of Law and Applied sciences

HFLPME- Hollow fibre liquid phase microextraction

HPLC- High performance liquid chromatography

IDM- Institute of Development Management

LLE- Liquid liquid extraction

LLE- Liquid-liquid extraction

LPME- Liquid phase microextraction

MBTFA- N-methyl-bistrifluoroacetamide

MS/MS-Mass spectrometry/mass spectrometry

MSTFA- N-methyl-N-trimethylsilyl-trifluoroacetamide

NIST- National Institute of Standards and Technology

NMR- Nuclear magnetic resonance

PAR- Peak area ratio

PfBBR- Pentafluorobenzyl bromide

RRT- Relative retention time

RSD- Relative standard deviation

RT- Retention time

SDLPME- Solid drop based liquid-phase microextraction

SOFT- Society of Forensic Toxicologists

SPE- Solid phase extraction

SPME- Solid phase microextraction

SWGTOX- Scientific Working Group for Forensic Toxicology

TMCS- Trimethylsilyl chloride

UB- University of Botswana

UK-United Kingdom

USA- United States of America

WADA- World Anti-Doping Agency

WAR- Women Against Rape

ABSTRACT

Sexual assault is a criminal, social and public health problem that affects both men and women across the world, and Botswana is not immune to this scourge. Drug facilitated sexual assault (DFSA) is a subcategory of sexual assault in which a victim is assaulted while incapacitated and unable to consent due to intoxication by alcohol and/or drugs. It is an increasingly reported crime which has presented various challenges to law enforcement and the society. Research shows that alcohol is the most encountered drug in DFSA, followed by benzodiazepines and anti-depressants. These drugs are rapidly metabolized and since victims delay reporting the assaults due to the physiological effects of the drugs, they are rarely detected in biological samples collected from the victims. In this regard, DFSA cases are not easy to prosecute. Research has also shown that university students may be at an increased risk of sexual assault, particularly DFSA. This study was therefore aimed at assessing the knowledge, prevalence and perception of DFSA among tertiary students in Botswana along with the analysis of drug detectability and stability in common alcoholic beverages using the gas chromatography-mass spectrometry (GC-MS). To address this aim, an extensive literature review was carried out, focusing mainly on grey literature on DFSA in Botswana, and a cross-sectional study was conducted through the use of an online survey which was shared via email and social media platforms. Research on the commonly dispensed pharmaceutical drugs (benzodiazepines, anti-depressants, anti-histamines) associated with DFSA was carried out in pharmacies around Palapye and Gaborone. The common benzodiazepines, antidepressants and antihistamines (diazepam, bromazepam, amitriptyline, chlorphenamine and antazoline), included in this study were spiked in four common alcoholic beverages (chibuku, wine, vodka and savanna) to develop a method to test their detection and stability in the alcoholic beverages using the GC-MS. The review of grey literature indicated that DFSA is very prevalent in Botswana, as the reports indicate that the police record a high number of DFSA cases. The data from pharmacies yielded diazepam and bromazepam as the common benzodiazepines, fluoxetine and amitriptyline as the common anti-depressants, chlorphenamine and loratadine as the common anti-histamines, all of which are controlled pharmaceuticals. The majority of the respondents of the survey (75.2%, n=117) knew what DFSA was and 25.7% indicated that they were well informed on the topic. The study revealed that 48.7% of the respondents had experienced unwanted sexual advances and 12.8% were using alcohol/drugs at the time. Majority of those who experienced unwanted sexual advances were females (66.7%) and 85.7% of the 12.8% who were using

drugs/alcohol at the time of the assault, were females. The study population (51.3%) largely believed that there was lack of support for DFSA victims. Of the five target drugs under investigation, four were detected and confirmed using the GC-MS, with the exception of bromazepam. A GC-MS method was developed and optimised for the simultaneous detection and analysis of antazoline, diazepam, chlorphenamine and amitriptyline in alcoholic beverages. The four drugs were also detectable over a 28 day period after storage at room temperature and at -20 °C, but were not always stable in the beverages when derivatised. This study showed that sexual assault was prevalent among tertiary students and there were cases of DFSA, which mostly affected female students according to the survey results. This requires interventions by law enforcement, non-governmental organizations (NGOs) and the society to facilitate the reporting of sexual assault cases, social and psychological support for victims. In addition, the spiked beverages may provide alternative evidence in the investigation of DFSA cases in Botswana, and so there should be changes in policies on investigations to include suspected spiked drinks where possible.

CHAPTER 1

INTRODUCTION

This chapter introduces the research topic of this study, drug facilitated sexual assault (DFSA), an increasingly growing crime, the challenges with DFSA cases in general and the current gap in research on the matter. The incidence of DFSA cases and common drugs encountered in such cases both globally and locally, is also briefly presented, with the current resolutions including instrumental analysis of samples and proposed approaches.

1.1 Drug Facilitated Sexual Assault

Sexual assault refers to sexual contact or behavior that occurs without the consent of the victim and it includes both penetrative (rape) and non-penetrative (indecent assault) activities [1]. The use of drugs in sexual assault is now being reported in journals and the media, causing a concern about DFSA [2], [3], [4]. This refers to a sexual activity in which the victim lacks capacity to give consent to the act due to intoxication by drugs of abuse, pharmaceutical compounds and/or alcohol [3], [5], [6]. DFSA may be proactive, whereby a drug is directly/forcefully added to the victim's drink by the perpetrator for purposes of sexual assault or it may be opportunistic, whereby the victim of a sexual assault is/was intoxicated with drugs by their own actions [1], [6], [7]. Research shows that sexual assault is a serious problem, especially among women, estimated to affect 64 per 100,000 females every year in the United States of America (USA) [2]. The first cases of DFSA date as far back as the late 1980's in the Western society and it has been increasing in prevalence [2], [8]. DFSA has reportedly increased in incidence across the world as more cases are being reported, even in the remotest regions of small countries such as Botswana and Zimbabwe [9], [10].

As DFSA is becoming more widespread, Botswana is not an exception. These rapidly increasing cases of DFSA in Botswana have gotten the attention of the Botswana Police Service (BPS) and Women against Rape (WAR) association as depicted by the media [9], [10], [11]. According to psychosocial-support stakeholders, DFSA is prevalent in Botswana but remains underpublicized due to different psychological and social factors including guilt, embarrassment and lack of trust in the justice system [12], [13]. As reported in local newspapers, a police spokesperson expressed concern about the increasing number of drug-facilitated rape cases in the country [13], [14], [15]. Many cases of date rape have been reported in Botswana which indicates the existence of a problem with regard

to DFSA. According to the rape statistics produced by a survey conducted by World Population Review in 2021, Botswana was ranked top on the rape statistics in the world at 92.93% per 100,000 people [16]. In addition, the report also states that the number does not include the cases that go unreported to the police [16].

Although there are no published reports, as far as the author is aware, various local news reports indicate that the problem is prevalent in the country as cited by the Botswana Police Service (BPS) statistics i.e., DFSA cases are many but remain underreported [13]. In Botswana, this act is termed rape under section 141 of the Penal Code (Chapter 08:01) of the Laws of Botswana, commencing 10 June 1964, Amendment Act No. 5 of 1998. The Act states that; *“Any person who has unlawful carnal knowledge of another person, or who causes the penetration of a sexual organ or instrument, of whatever nature, into the person of another for the purposes of sexual gratification, or who causes the penetration of another person's sexual organ into his or her person, without the consent of such other person, or with such person's consent if the consent is obtained by force or means of threats or intimidation of any kind, by fear of bodily harm, or by means of false pretences as to the nature of the act, or, in the case of a married person, by personating that person's spouse, is guilty of the offence termed rape”* [17]. In addition, according to the laws of Botswana, sexual harassment is also a violation of the Penal Code as per section 146 of indecent assaults, which states that *“Whoever, intending to insult the modesty of any woman, utters any word, makes any sound or gesture or exhibits any object intending that such word or sound shall be heard, or that such gesture or object shall be seen, by such woman or intrudes upon the privacy of such woman, is guilty of an offence and is liable to imprisonment for a term not exceeding one year”* [17]. Statistics show that BPS recorded 2,047 rape related cases countrywide in 2017 alone, an increase of 31 cases from 2016. They also recorded 1600 rape cases in the first quarter of 2019, noting that most of these cases were date and drug facilitated rape [13], [14]. The BPS also revealed that they notably record a high number of rape cases during the festive season, a period characterized by high alcohol/drug consumption and regularly visiting places of entertainment [9], [11]. This shows the need to improve on the recording of rape or sexual assault cases with the police, to separate DFSA cases from rape cases as well as the formulation of distinct laws and policies for DFSA.

Though sexual assault is not limited to college or university campuses, it is prevalent on campuses or among college and university students [18]. Majority of sexual assault cases occur around the age of 24, at which most people are college or university students, this marks college or university campuses

as high-risk for sexual assault [19], [20]. Studies also suggest that female college and university students are at a higher risk than females of the same age in the general population [3], [21]. Therefore, this research is focused on conducting both survey research among tertiary students in Botswana, and laboratory based studies, to address these challenges.

1.2 Common Drugs Associated with DFSA

The most common psychoactive substance associated with DFSA, especially in Botswana, is alcohol but the expansion of both pharmaceutical and illicit drug markets has provided faster and cheaper ways of incapacitating victims [7], [9], [22], [23]. As a result, there is a risk of proactive DFSA, which commonly occurs in social gatherings such as parties, clubs and bars, as people secretly slip psychoactive drugs into beverages to incapacitate victims [4], [15], [24]. Perpetrators have also resorted to giving victims stronger alcoholic drinks or adding them to their ordinary drinks without the victim's knowledge. Although anyone may fall victim to DFSA, the youth between the 18-25 age range are more exposed to DFSA as they usually regularly visit places of entertainment [24], [25], [26]. The most common drugs reported to be used in these cases (often called date-rape, predatory or sexual assault facilitating drugs) include flunitrazepam (rohypnol), diazepam, gamma hydroxybutyrate (GHB), ketamine, ketorolac (antihistamine in eye drops), ecstasy and benadryl (an antihistamine) [24], [25], [27], [28]. They may be in tablet, powder or liquid form [7], [24], [25]. These drugs are usually sedatives/depressants that slow down activities of the central nervous system thereby causing drowsiness, mild amnesia, slurred speech and muscle incoordination, making the victim more vulnerable to sexual assault [9], [25], [29], [30], [31].

A number of forensic analytical techniques have been developed overtime to detect these date rape or sexual assault facilitating drugs (the terms are used interchangeably in this research), in biological samples such as urine, blood, saliva and hair [30], [31], [32]. Extraction techniques including solid-phase (SPE) and liquid- liquid extraction (LLE) are employed followed by analysis using gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) [30], [31], [32], [33], [34]. LLE has presented various techniques including dispersive liquid-liquid micro extraction (DLLME), which involves the dispersion of small amounts of extraction solvent in an aqueous sample [30], [35]. DLLME leads to rapid partitioning of analytes into the extraction solvent, high enrichment factors and very low solvent use, as compared to other liquid or solid phase extraction methods [34], [35], [36]. Extraction solvents such as diethyl ether, ethyl acetate, chloroform, isopropanol and dichloromethane are

commonly used [32], [37].

According to the BPS, the most common drug encountered locally is alcohol but it is also common for perpetrators to spike victims' beverages with illicit and pharmaceutical drugs at social gatherings, for purposes of sexual assault [13], [15]. Victims of DFSA often report the assault (or incidence) at a later time due to various reasons, such as not recalling the events of the assault, and feelings of guilt and embarrassment [2]. This renders it difficult to trace the drugs in the body as they are rapidly metabolized in the body, so there is hardly concrete evidence with DFSA cases [7], [14], [28], [38]. These cases are therefore rarely prosecuted and so victims of DFSA feel that they do not have enough support [38], [39]. Consequently, most victims allegedly resort to not reporting sexual assault, so there is currently little to no DFSA statistics in Botswana [39]. Adding on to that, in Botswana sexual assault cases are not separated according to the circumstances surrounding them i.e. whether it was drug facilitated or not, except for defilement cases, which are defined under section 147 of the Penal Code (Chapter 08:01) of the Laws of Botswana, commencing 10 June 1964, **Amendment Act No. 21 of 2018**. The Act states that; *any person who unlawfully and carnally knows any person under the age of 18 years is guilty of an offence and on conviction shall be sentenced to a minimum term of 10 years, imprisonment or to a maximum term of life imprisonment*. This suggests that there are no separate statistics on DFSA which could be available but are probably 'hidden' within the rape statistics. There could be a higher number of DFSA victims in the society than thought and so the true incidence of DFSA needs to be investigated. In light of this, DFSA cases present complex challenges and research gaps which are also noted in the experiences of sexual assault expressed by the survey respondents. So, different research angles are required to tackle them so as to improve the criminal justice system of Botswana.

Evidently, many DFSA offences go unreported or are reported too late to collect reliable forensic evidence, which leads to less evidence and witnesses to support the victims' statement. Moreover, most of the drugs are rapidly metabolized making them untraceable in routine drug testing of biological fluids such as urine or blood. It is therefore difficult to investigate DFSA cases and to quantify the degree to which drugs are used to facilitate sexual assault. Studies have also shown that storage conditions of samples for analysis have the potential to alter drug concentrations [37]. The initial concentration of the drug that incapacitated the victim, its stability in different matrices, and the difference between the detected concentration and the initial concentration, is vital in toxicological forensic analysis [33], [37]. So, a gap in research relating to the optimisation and

validation of methods to detect and determine the stability of date-rape drugs in different drinks and storage conditions over time was noted, and is being addressed in this research [33], [37], [40]. In addition, published studies used pure drug standards during method development [37]. To the author's knowledge, pharmaceutical formulations of drugs associated with DFSA, which is what perpetrators would use in real life incidents, have not been used in such studies. A popular locally brewed alcoholic beverage was also included in the research, in addition to globally available alcoholic beverages.

There is evidently a gap in the study and understanding of DFSA in Botswana in terms of how it originates, escalates and its impact in the society, despite it increasing at an alarming rate. Therefore, an extensive study of victimization by either opportunistic or proactive DFSA and the challenges faced by victims is a critical need [29]. Effective measures of studying and investigating such cases through cultivating a better understanding of DFSA, implicated date-rape drugs and other factors surrounding it are also necessary.

This research therefore intends to understand the true scale of DFSA in Botswana through survey based research, to explore the socio-demographics of the affected groups as well as their knowledge and experiences (direct and indirect) on DFSA. So, the main aims are to review literature on DFSA in Botswana so as to evaluate the evolution and state of DFSA and to study the prevalence and knowledge of DFSA in Botswana through a survey. This along with developing, optimising and validating a method aimed at simultaneously determining the detectability and stability of pharmaceutical formulations of common date-rape drugs in spiked alcoholic drinks, as alternative sample types to biological fluids from which date rape drugs may not be detected due to rapid metabolism. This should aid in investigation and prosecution of DFSA cases as the outcomes of this research should inform the revising of policies for DFSA cases to be separated from sexual assault or rape cases. This should also make way for distinct lawmaking and prosecution of DFSA cases, as well as assist forensic analytical laboratories in developing strategies for the storage and analysis of these DFSA samples.

1.3 Problem Statement

Although there are no journal publications on DFSA in Botswana, as far as the author is aware, several newspaper reports have been written citing the high rate of DFSA in Botswana. According to the BPS, the most common drug encountered locally is alcohol but it is also common that perpetrators spike victims' beverages with drugs at social gatherings, for purposes of sexual assault [6], [8].

Victims often report sexual assault at a later time due to various reasons, rendering it difficult to trace the drugs in the body as they are rapidly metabolized in the body, so there is hardly evidence with DFSA cases [16]. Consequently, victims have now resorted to not reporting the assaults as there are less chances of prosecution or conviction because of lack of evidence. The BPS also acknowledges failing to secure convictions due to lack of evidence or witnesses and this shows a gap in research with regards to DFSA in Botswana [7]. Also, not much research has been done with respect to the detection and stability of benzodiazepines, as the most implicated in DFSA cases, in beverages under different storage conditions [17].

Extensive academic and non-academic research carried out over the years has shown that sexual assault including DFSA is a worldwide problem, mostly affecting females [5]. However, to the knowledge of the author, there is currently no study on the prevalence and knowledge of DFSA, in particular, among a given population in Botswana. Studies on the prevalence or knowledge of sexual assault have been reported sparsely in many parts of the world, including South Africa, but none has been reported in Botswana [19], [41]. The true prevalence of DFSA remains a complexity in research because victims may not know with certainty that they were given drugs without their knowledge or whether they were even sexually assaulted [18].

Due to the challenges in detection and investigation because of these drugs, DFSA crimes are rarely prosecuted and conviction rates are significantly lower [4]. This therefore calls for an improved response system to tackling the state of DFSA so as to address shortcomings of research on solving such cases. Hence why the purpose of this study is to review literature on DFSA in Botswana, carry out a survey and evaluate the detectability and stability of date rape or sexual assault facilitating drugs in beverages so as to establish effective measures of resolving DFSA cases in Botswana.

1.4 Research Objectives

1.4.1 Main Objective

To undertake a survey on the prevalence of DFSA in Botswana, and to develop and optimise a simple and sensitive method to analytically evaluate the detectability and stability of common date rape drugs in alcoholic beverages using GC-MS.

1.4.2 Specific Objectives

- 1) To find out the knowledge, experiences and perceptions on DFSA in Botswana through literature review and a survey.
- 2) To identify the common pharmaceutical drugs that are likely to be associated with DFSA.
- 3) To develop and optimise a GC-MS method to detect the selected drugs
- 4) To determine the stability of drugs in alcoholic beverages.

1.5 Research Questions

This research aims at answering the following questions;

- 1) How prevalent is DFSA in Botswana?
- 2) Are people, especially young females, aware of DFSA in Botswana and to what extent?
- 3) How many people, if any, have encountered DFSA in Botswana?
- 4) Which settings pose a high risk of DFSA in Botswana?
- 5) Are the sexual assault facilitating drugs stable in alcoholic beverages and detectable using GC-MS?

1.6 Significance of the Study

This research is critical for all stakeholders such as law enforcement officials, non-governmental organizations (NGOs), academics, the general public as a whole, and will pave way for advanced research on DFSA. Through this study, those who work in this field may gain a better understanding of the complexities involved in DFSA cases thus restoring the integrity of the criminal justice system. This would also aid in developing more knowledge and understanding on DFSA, common sexual assault facilitating drugs and possibly devise strategies of increasing the rate of prosecution of DFSA cases. On assumption that the outcomes of the study are positive relative to the objective, more awareness on DFSA would be created among Botswana, especially young ladies and possible measures to curb and better investigate DFSA may be put in place. This study may also help in presenting to law enforcement, alternative samples of DFSA to biological samples, in which the drug may not be detected. Through the success of this study, interventions or policies can be put in place and recommendations can be made in order to make people aware of DFSA so as to possibly prevent and reduce the crime.

CHAPTER 2

LITERATURE REVIEW

The chapter presents an overview of previously published studies on drug facilitated sexual assault in various parts of the world. Extensive background information on the subject is discussed, covering the incidence of DFSA globally and locally, the common date-rape drugs, sample preparation, and the analytical techniques used. The contents of this chapter essentially inform the choice of the study design as well as instrumental analysis followed for this particular research.

2.1 Prevalence and Incidence of DFSA

Previous studies show that to date, precise data on the incidence of drug facilitated sexual assault has not been published globally. Police reports are likely to significantly make little of the actual numbers of sexual assaults as most victims do not report DFSA, this is alluded to in section 1.2 [26], [42].

A telephone based study was carried out at the Ireland Rape Crisis Network to determine the prevalence of DFSA by studying the reports made by victims [43]. According to the study, the results were misleading as they were low despite the high reports on the helpline, indicating that DFSA cases are underreported. The 2007 statistics indicate that 2.3% of the cases recorded in Ireland were of drug facilitated sexual assault [43]. This gives the impression that the number of DFSAs is small however, that is not the reality. Also, in a recent Swedish laboratory based study, 55% of sexual assault victims had ethanol present in their system [43]. In Ireland, alcohol is involved in about half of all adult sexual assaults [43]. These statistics indicate that the offences of DFSA are very prevalent in Ireland [13], [43].

Though research shows that victims fall victim to both opportunistic and proactive DFSA, drink spiking cases are common and are especially reported in newspaper reports [9], [10], [44], [45]. The UK, USA and Australia are some of the countries in which these cases are rapidly reported and prevalent [44] [45], [46]. In a survey study conducted by the Alcohol Education Trust (AET) in Cambridge, UK, 747 respondents participated and 94 (12.6%) indicated that they have had their drinks spiked before, with a frequency of 15% and 7% among females and males, respectively [44]. As is characteristic of victims of DFSA, only 8% of the victims of drink spiking reported the matter to the police or sought medical assistance.

Another survey conducted separately among 23,000 students from 19 universities around the UK, showed that 11% of the respondents had experienced drink spiking before. In this study, 35% of these incidents took place at private parties and the cases usually occur amongst groups of friends [44].

A clinical based study carried out in 2005 to estimate the incidence of DFSA in USA also showed that DFSA may be underreported in USA. In this study, 4.2% of the subjects tested were estimated to have been victims of DFSA. In the case of opportunistic DFSA, 35.4% of the subjects were estimated to have been victims of DFSA. This study included toxicological analysis coupled with the subject's statements to determine DFSA and so renders the results more reliable [2].

A study, which is the first in Africa to the author's knowledge, was carried out in Cape Town, South Africa, to investigate 107 cases of suspected DFSA victims, depicting the prevalence of DFSA [47]. In this hospital-based study, most of the patients were females between the ages of 18 and 25 years. The results indicated that 58% of the incidents occurred in the late evening/early morning at the home of the perpetrator(s)/a friend and most of the perpetrators were known to the victims. Drugs and/or alcohol were detected in 72 of the patients (67%), with drugs other than alcohol being detected in 60 of the patients (56%), indicating that alcohol/drugs was used to facilitate sexual assault [47]. Most of the cases were suggesting that opportunistic DFSA is more common than proactive, as most victims reported the voluntary use of alcohol or drugs around the time of the assault, or leading to the sexual assault [47].

A cross-sectional study carried out at a South African university to examine the prevalence of sexual violence among adolescent girls and young women revealed that a higher prevalence of sexual assault was reported by victims who heavily consumed alcohol periodically, compared to non-consumers of alcohol [19]. This research also showed that victims who reported heavy periodical consumption of alcohol had a higher likelihood of reporting lifetime prevalence of sexual assault [19]. This essentially proves that the use of drugs, particularly alcohol perpetuates sexual assault especially among women.

In Botswana, although there are no published reports, as far as the author is aware, various news reports indicate that the problem is prevalent in the country as cited by the BPS statistics i.e., date rape cases are many but remain underreported [13]. Statistics show that BPS recorded 2 047 rape related cases countrywide in 2017 alone, an increase of 31 cases from 2016, noting that most cases

were date and drug facilitated rape. The BPS also revealed that in a space of 17 days i.e. from 20th December 2019 to January 5 2020, they recorded 14 rape cases and 13 were date rape cases [11], [14].

In Mogoditshane, a developed village located just by the capital Gaborone in Botswana, DFSA cases are seemingly high and associated with places of entertainment [11], [48]. For instance, 150 rape cases were recorded in 2016 and the number of cases increased significantly to 159 cases in 2017 [48]. The police investigations reportedly categorize most of the cases as date rape and the perpetrators are often friends or acquaintances of the victims. The reports show that most women were victims of sexual assault after their drinks were spiked at places of entertainment [23], [48]. The Officer Commanding Senior Superintendent reported and alerted women about drink spiking drugs which they have discovered to be in high use at most entertainment places in the Gaborone region. According to the police, most of the sexual assaults take place at people's residences and intoxication with alcohol influences these incidents[11], [23], [48].

Furthermore, 109 rape cases were recorded from December 2017- January 2018 and 110 from December 2018-January 2019 in Botswana [10], indicating that sexual assault cases are common during the festive seasons. A part of the statistics i.e. the number of rape cases recorded during the festive seasons of 2017-2020 are shown in Figure 1.

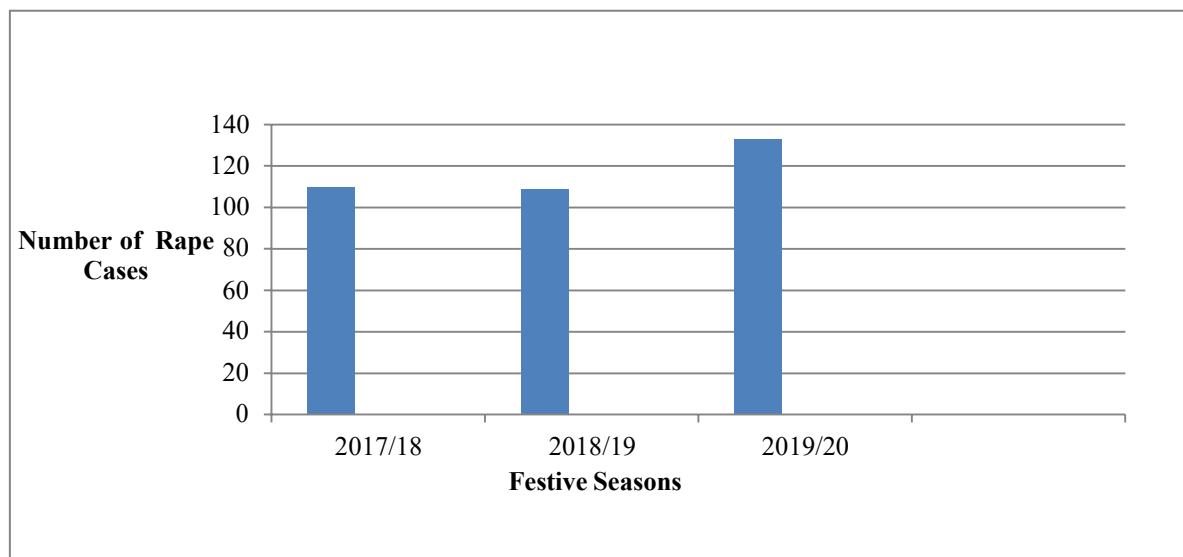


Figure 1: Graph of the number of rape cases recorded by the BPS during the festive seasons of 2017/18-2019/20[11], [13], [49].

2.2 Drugs Commonly Encountered in DFSA

Studies report the use of drugs to incapacitate victims for purposes of sexual assault [24], [25]. This section aims to discuss the background and link to sexual assault of these drugs.

According to the Society of Forensic Toxicologists (SOFT), the key drugs involved in DFSA cases include antidepressants such as fluoxetine and amitriptyline, antihistamines such as diphenhydramine and chlorphenamine [40]. Eye drops are also reportedly encountered in some DFSA cases as they contain active ingredients such as tetrahydrozoline and antazoline, which are anti-histamines [50]. However, the most common drugs encountered during investigations of DFSA are alcohol (ethanol), benzodiazepines, gamma-hydroxybutyrate (GHB) and ketamine [2], [4], [51], [52].

Most of these drugs are under international control, according to the 1961 Single Convention on Narcotic Drugs and the 1971 Convention on Psychotropic Drugs [7], [53], [54]. Consequently, these drugs are also controlled in many countries around the world, including Botswana [53]. However, some of the drugs used for sexual assault are not yet under international control as they possess some pharmaceutical benefits [33]. According to the United Nations Office on Drugs and Crime (UNODC), and the United States Drug Enforcement Agency (DEA), benzodiazepines, including diazepam and bromazepam are controlled drugs placed on schedule IV of controlled substances [38], [40], [53], [55]. Therefore, their sale or use is regulated through medical prescriptions as they have low potential for abuse or dependence [53]. The 1971 United Nations Convention on Psychotropic Substances currently controls 38 benzodiazepines including alprazolam, bromazepam, brotizolam, camazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, cloxazolam, delorazepam, diazepam, estazolam, ethyl loflazepate, etizolam (since 2020), flualprazolam (since 2020), fludiazepam, flurazepam, flunitrazepam, halazepam, haloxazolam, ketazolam, loprazolam, lorazepam, lormetazepam, medazepam, midazolam, nimetazepam, nitrazepam, nordazepam, oxazepam, oxazolam, phenazepam (since 2016), pinazepam, prazepam, temazepam, tetrazepam and triazolam [56].

2.2.1 Alcohol (Ethanol)

Studies show that alcohol is the main drug for increasing victims' risk of being sexually assaulted, mainly perpetuating opportunistic DFSA. It leads to intoxication characterised by symptoms such as sudden affection, mood swings, dizziness, lack of motor coordination and mental disorientation [22], [25], [33]. Also, it may lead to the inhibition of the functions of the central nervous system (CNS), leading to an anaesthesia-like state which advances into alcoholic coma, rendering the victim helpless

[22]. In addition, alcohol has a widespread social use, especially in colleges or universities and this increases the risk of sexual assault by its consumers.

According to a number of studies, alcohol is a commonly known incapacitating drug found in sexual assault cases, and so it is the most studied. Studies show that there is a relationship between alcohol intoxication and the risk of being sexually assaulted [5], [57].

2.2.2 Benzodiazepines

Benzodiazepines are also more commonly used in DFSA as a means of incapacitating victims. Their availability as pharmaceutical drugs and their synergistic effects with various drinks including alcohol, make them more convenient for facilitating sexual assault [24], [51], [58]. They are known to induce conditions such as hypnosis, loss of memory and muscle relaxation in victims. Benzodiazepines are pharmaceutically used worldwide in the treatment of seizures, sleep disorders and anxiety [7], [51]. Due to these properties, benzodiazepines have been used to spike alcoholic and non-alcoholic drinks in drug assisted crimes such as sexual assault, and robbery [33], [38].

Benzodiazepines are associated with a significant number of DFSA cases in many countries [37], [57]. The most cited drug in this class in DFSA cases is flunitrazepam (Rohypnol), but there is a wide variety of benzodiazepines, also used for this purpose, such as bromazepam, diazepam, alprazolam and oxazepam, which are controlled and only distributed by prescription in Botswana [4], [59].

2.2.3 Gamma-hydroxybutyrate (GHB)

Also known as gamma-hydroxybutyric acid, GHB is similar to the neurotransmitter GABA. This drug was discovered as a compound that could be a GABA analog, thus used in the treatment of seizures [51], [52]. The pharmacological properties of GHB discovered during its study proved it potentially useful as an anaesthetic [51], [60]. However, its medical use was discontinued due to its inability to induce analgesia, and side effects such as sedation, drowsiness, muscle contractions and unconsciousness. GHB then emerged as a drug of abuse, being known as a date rape drug [4], [33], [52]. The effects of low doses of the drug can be stimulating and have aphrodisiac effects. It can be found in powder, tablet and liquid forms, which can be administered orally, with a distinctive bitter and/or salty taste, facilitating the adulteration of alcoholic and non-alcoholic beverages [57], [60].

2.2.4 General Use of Controlled Pharmaceutical Drugs

Although these drugs have the ability to facilitate sexual assault as they affect the CNS, it has been reported that positive cases in DFSA investigations may be confirmation of medical use of these

prescription drugs, which suggests that they may not always be associated with the assaults [6], [30], [31]. However, the use of these drugs with ethanol or other drugs may have been a factor in facilitating the act due to the resulting effects in the victims' body [57]. Their use usually correlates to availability and ease of use by both the perpetrators and victims. Since most of these drugs are also pharmaceutical drugs, they tend to be encountered most as they are readily available [33]. Due to the aforementioned factors, benzodiazepines, antihistamines and antidepressants are usually more challenging to determine whether they were used for therapeutic or illicit purposes.

2.3 Instrumental Analysis of Samples

Instrumental analysis of the drugs requires extraction of the target analytes from the relevant aqueous samples. Commonly applied extraction techniques in investigations of DFSA mainly utilize LLE and SPE at times. The factors influencing the choice of carrying out SPE or LLE include the chemical properties of the analytes, the complexity of the sample matrix and the analytical technique to be used for the sample [31], [36], [58], [61]. Different analytical techniques have also been developed over the years, to determine the presence of these drugs in biological specimen such as urine and blood, coupled with extraction techniques to maximise the concentration of the analyte.

2.3.1 Solid-Phase Extraction

Solid-phase extraction is based on the distribution of compounds between the liquid phase (sample) and the stationary phase (adsorbent). It is usually in four stages and involves stationary phase conditioning, which is then followed by deposition of the sample [36], [62].

Solid-phase microextraction (SPME), a type of solid-phase extraction, is widely used in different laboratories [52], [62], [63]. It is more environmentally friendly than LLE and SPE because it does not require solvents, but it still has significant drawbacks, such as the limited lifespan of SPME fibers, the costs, and sample carryover [36], [64].

2.3.2 Liquid-Liquid Extraction (LLE)

The liquid-liquid extraction (LLE) technique is based on the principle that the analyte in one liquid phase is transferred to another immiscible liquid phase. It is commonly used in toxicological analysis to purify and concentrate samples prior to analysis, usually chromatography techniques [30], [52], [58], [64], [65]. The specific properties of the solvent, such as miscibility with water, polarity, density, and toxicity allows for selection of the solvent or combination of solvents for a particular extraction [36]. Similarly, analyte properties such as the structure and lipophilicity allows for

optimisation of extraction and efficiency, based on determination of the extraction yield [65], [66]. The choice of solvents for particular extractions depends on the interaction of physicochemical properties and their capability to dissolve compounds because they possess complementary intermolecular interactions to the target compounds [64].

Liquid phase microextraction (LPME), a type of LLE, provides an alternative to SPME. LPME includes Single Droplet Liquid Phase Micro Extraction (SDLPME), Hollow Thread Liquid Phase Micro Extraction (HFLPME) and Dispersion Liquid Phase Micro Extraction (DLLME) [36], [66]. All three are environmentally friendly, as the amount of organic solvent used is usually in the range of microliters [34], [37]. Also, these methods do not have the high cost and sample carryover issues associated with SPME [37].

2.3.2.1 DLLME

DLLME is an increasingly used technique in chemistry due to its advantages and efficiency [30], [63], [66]. It involves rapid injection of a solvent mixture in an aqueous sample, resulting in a solution consisting of micro droplets of the extract solvent, distributed in an aqueous sample. The mixture allows the analyte to be immediately transferred from the aqueous sample into the organic phase i.e. the solvent [34], [63], [67]. This is achieved due to the large surface area in DLLME compared to the ordinary LLE technique, created by a large number of micro-droplets, thus maximizing extraction and leading to a high enrichment factor [63]. The extraction solvent must be immiscible with water, miscible with the dispersion solvent, and have a high affinity for the target analyte to ensure extraction efficiency [35], [66].

2.3.3 High Performance-Liquid Chromatography (HPLC)

Research shows that HPLC is one of the best methods for quantifying drugs, especially benzodiazepines [30], [34], [58], [59]. However, there is a need for more sensitive analytical methods for determination of drugs in spiked drinks, especially because analyte concentration is usually minute and minimal sample preparation is required [59]. Compared to the GC method, HPLC does not pose the risk of thermal degradation to the target analyte, so the derivatisation reaction is not necessary. A simple sample preparation step including liquid-liquid extraction and solid-phase extraction is usually sufficient [31], [36], [63], [66]. Although many detectors may be used in conjunction with the HPLC, the analysis of drugs is usually best in combination with mass spectrometry (HPLC-MS or MS/MS) [36], [52].

2.3.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is one of the most sensitive and versatile tools for thorough analysis of the structure and interactions of drugs. This technique has also proven useful for qualitative and quantitative measurements of absorbed analytes [4], [33], [36]. The resulting NMR spectrum has detailed information that allows for rapid detection and identification of analytes present in various samples including blood and urine [36]. Another advantageous feature of NMR spectroscopy is its non-destructive nature, which allows subsequent re-analysis of the sample using other methods [36], [68]. The application of nuclear magnetic resonance (NMR) spectroscopy as a method of drug analysis has the advantage of reducing sample preparation time and allowing simultaneous detection and quantification of drugs [68].

2.3.5 Gas Chromatography-Mass Spectrometry

Gas chromatography (GC) combined with mass spectrometry is widely utilised as the analytical technique for the detection and quantification of various drugs, due to its high sensitivity and specificity [4], [30], [31], [52], [59], [69]. Identification of drugs is based on the specific detection of large amounts of ions in the analyte under analysis. The search for the identity of the particular ion obtained is performed by comparison with a particular reference library such as the National Institute of Standards and Technology (NIST) library [34], [36], [66]. GC-MS quantifies and identifies drugs, which often have low detection and quantitation limits. In addition, the use of GC requires that analytes be thermally stable as they may degrade without prior derivatisation [58], [59], [69].

2.3.5.1 Derivatisation

Analysis with GC-MS requires derivatisation of analytes that undergo thermal degradation, as well as to enhance chromatographic separations and detectability [69], [70]. The choice to derivatise depends on the chemical structure of the analyte, the sample matrix as well as the desired changes in the analyte properties. Derivatisation is an important step in drug analysis as most drugs are polar in nature, due to possession of functional groups such as alcohols, phenols, carboxylic acids, amines and amides [71]. The functional groups requiring and involved in derivatisation are the amine (NH and NH₂) and hydroxyl (OH) groups [52].

Five drugs of interest are included in this study, but only two of the drugs require derivatisation as they both possess the amine group (NH) with a labile hydrogen. Figure 2 shows the structures of antazoline and bromazepam, with an indication of the amine derivatising polar groups.

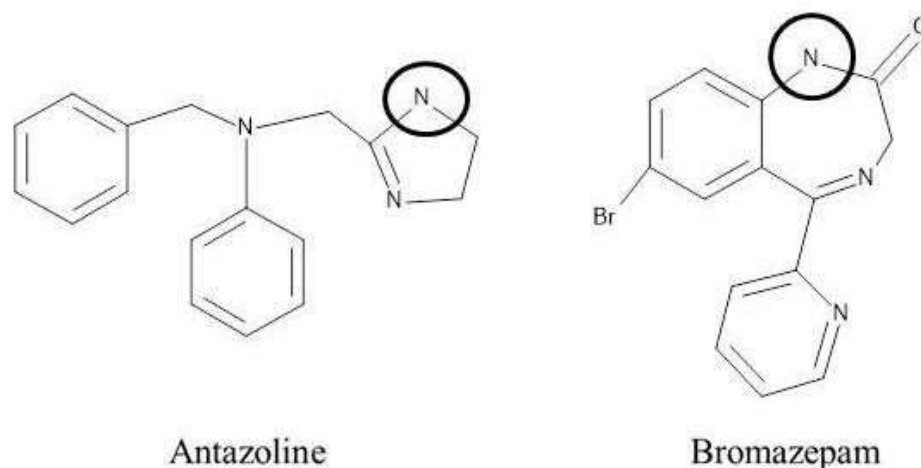


Figure 2: Chemical structures of derivatisable drugs of interest

Derivatisation renders highly polar materials volatile, so that they can be eluted at suitable temperatures eliminating thermal decomposition or molecular re-arrangement [71], [72]. This enables the analysis of compounds that otherwise cannot be readily monitored by GC and also reduces analyte adsorption in the GC system while improving detector response, peak separations and peak symmetry. Common derivatising agents include alkylation agents such as pentafluorobenzyl bromide (PFBBBr), silylating agents such as bistrimethylsilyltrifluoroacetamide (BSTFA), N-methyltrimethylsilyltrifluoroacetamide (MSTFA), bistrimethylsilylacetamide (BSA), trimethylchlorosilane (TMCS), and acylation agents such as N-Methyl-bis (trifluoroacetamide) (MBTFA), pentafluoropropionic acid anhydride (PFAA) and trifluoroacetic acid anhydride (TFAA), which chemically alter compounds through alkylation, silylation or acylation reactions [71], [73], [74].

Also, for reliable and quality analysis, the derivatisation reaction ought to be optimised. Optimisation of the derivatisation reaction involves determining whether the reaction is complete, usually through a set of experiments monitoring the effect of one variable, the derivatising reagent volume or reaction temperature, on the results [74], [75]. Efficiency of the reaction may be determined by measuring the increase in drug peak area against the reaction temperature and time. The presence of one derivative peak and the most intense instrumental response indicates maximum conversion, and hence the optimal derivatisation conditions for that analyte [75].

2.3.5.2 Method Validation

Validation is the process of performing a set of experiments that reliably estimates the reliability of an analytical method. The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for the intended purpose [76], [77]. Various guidelines are available to assist laboratories in designing their own method validation strategies while maintaining the integrity and reliability of the method, such as the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines and Eurachem guidelines [77], [78].

To be fit for the intended purpose, the method must meet certain validation characteristics which include selectivity (specificity), linearity, range, accuracy, precision, limit of detection and quantitation [76], [77]. The key performance tests selected for this research are precision studies and auto-sampler stability. Precision studies are carried out to determine the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the set conditions. Precision may be considered at two levels: intra-assay precision and intermediate precision [76], [78], [79], [80]. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Auto-sampler stability is established to prove the stability of the processed sample in auto sampler compared with processed samples [77], [80]. Table 1 details the significance of these validation parameters as well as the protocol for each parameter.

Table 1: Validation parameters assessed in the study

Validation Parameter	Significance	Protocol used in this study
Auto-sampler stability	Auto-sampler stability study is carried out to determine the stability of the analytes during instrumental analysis, under conditions of analysis i.e. autosampler conditions for the expected maximum time of an analytical run.	Mixed drug standards in different vials, from the same homogenous sample are placed on the auto-sampler and analysed over a period of 24 hours using the GC-MS.
Precision	Precision is carried out to	Mixed drug standards at three

	determine the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample. It is usually measured in terms of imprecision, expressed as relative standard deviation (R.S.D.). Repeatability/ intra-assay precision expresses the precision of measurements under the same operating conditions over a short period of time. Intermediate/ inter-assay precision expresses the precision of measurements over different days, under varied conditions.	different concentrations are analysed and intra-assay precision of the instrument is determined under the same instrumental conditions. Inter-assay precision of the instrument is also determined at the three concentrations over three separate days.
Recovery (%)	Recovery experiments are performed to determine the percentage of the analyte response after sample preparation compared to that of a standard containing the analyte at a concentration corresponding to 100% recovery.	Two different LLE systems are investigated, i.e. chloroform: isopropanol (2:1, v/v) and diethyl ether. To calculate the %recovery, a similar unextracted mixed drug standard is also analysed.

CHAPTER 3

METHODS

This chapter details the study designs utilised for the research, the materials and experimental procedures used for the assessment of the prevalence of DFSA in Botswana, as well as the detection and stability of drugs that are commonly used in facilitating sexual assault. The chapter further highlights the various preliminary studies conducted such as pilot study, signal testing, optimisation of derivatisation method, method validation, and sample extraction.

3.1 The Survey

3.1.1 Study Design

This was a cross-sectional study conducted across tertiary institutions in Botswana with a total of about 40, 534 students [81]. The survey was also open to graduates of the various institutions thus increasing the total target population to over 150,000. Botswana has over 20 tertiary institutions and this includes universities, colleges and brigades. Only 10 were chosen for this study based on high student enrolment. The chosen institutions were Botswana International University of Science and Technology (BIUST), University of Botswana (UB), Botho University (BU), Botswana University of Agriculture and Natural Resources (BUAN), Ba Isago University, Botswana Accountancy College (BAC), Limkokwing University, Gaborone University College of Law and Professional Studies (GUC), Boitekanelo College and Institute of Development Studies (IDM), all of which are in Gaborone except BIUST, which is in Palapye.

A semi-structured online survey was conducted to determine the incidence of DFSA among the population. Participants were 18 years and above and either currently studying or had previously studied at a tertiary institution locally.

The Cochran formula was used to determine the minimum sample size required in this study. The formula is suitable for large populations and allows for calculation of the best possible sample size, with suitable precision and confidence level. The sample size is calculated based on the proportion of people (50%) who are likely to experience or witness DFSA to those who are not likely to [82], [83].

The minimum sample size required for this study was 96 participants, assuming that 50% of the tertiary/graduate population has experienced/witnessed DFSA as calculated using the Cochran formula as follows:

$$n = (Z^2 \times pq)/d^2 ,$$

Where; n=the required minimum sample size for the study, z=Z-score, p=proportion of people who have experienced/witnessed DFSA in the study area, q=1-p, d=the acceptable error level

The calculation of the sample size was based on acceptable error level of 10% (0.1) and a confidence interval (CI) of 95% corresponding to a Z-score of 1.96. These statistical values are within the recommended values for standard deviation (SD) and error levels in social science research [83], [84]. The proportion of people who have either experienced/witnessed DFSA to those who have not is unknown, therefore a standard deviation of 0.5 was used, p=0.5.

3.1.2 Sampling Method

The study participants were selected using the convenient and purposive sampling technique as the link to the online survey was sent to all. The first stage involved selecting the tertiary institutions in Botswana based on highest enrolment of students. The selection of respondents was random as the survey was made available to all through email, Facebook, WhatsApp and Twitter, and participants answered as they received the survey.

3.1.3 Administration and Measurement of Research Instrument

A questionnaire on DFSA was set up in an Online Survey Site, using Google Forms and shared through emails and social media platforms mentioned in section 3.1.2, targeting tertiary students and graduates at 10 institutions across Botswana.

The questionnaire used in this study was adapted from a previous study on the prevalence of DFSA and it was validated through a pilot study [83]. It was used to obtain both qualitative and quantitative data with open-ended and closed-ended questions, respectively. The closed-ended questions were used to collect the sociodemographic data of the participants which included their age range, gender, level of education and knowledge or understanding of the concept and subject area. The open-ended questions were asked to examine the participant's experiences or witnessing of DFSA, what they think are the most common date rape drugs, the most common perpetrators of DFSA, high risk settings and their opinions on how to best curb DFSA.

3.1.4 Data Analysis

Data analysis for this study was carried out using the Statistical Package for Social Sciences (SPSS) version 18.0. The data was first screened for errors and two incomplete responses were found and removed. Manual representational thematic analysis was used to analyse textual data obtained from open-ended questions and presented quantitatively. In questions where respondents could give multiple answers, for both open-ended and closed-ended questions, each answer was analysed as an individual response. Hence, the total of some responses in a category exceeds the 117 total number of respondents. The sociodemographic factors of the respondents were compared with their knowledge and perceptions or opinions on DFSA using inferential statistics. A Pearson χ^2 test was used to examine the impact of independent variables i.e. age, gender, education level, on various responses such as knowledge of DFSA, experience on DFSA and perceptions in the study and $p \leq 0.05$ at 95% CI was considered to be statistically significant.

3.1.5 Data Coding

The survey comprised closed-ended and open-ended survey questions, which allowed free-text answers, leading to a number of free-text responses as well as multiple answers. Nine open-ended questions allowed for free text responses and four closed-ended questions allowed for multiple answers. Respondents answered all which applied to them. Thematic analysis was carried out to extract themes from the free-text responses. Labels were assigned to the responses, representing important themes in each response. Numbers were used as labels for all the responses, to allow for easy organisation (see Appendix III).

The responses or answers were coded exactly as they were given in the survey for closed-ended questions, while for open-ended questions; themes were formed according to the responses received.

3.1.6 Public Involvement and Ethical Approval

Approval from BIUST and the government was granted through a research permit from the Ministry of Tertiary Education, Research, Science and Technology, to carry out this research study. Current students and graduates of ten institutions in Botswana participated in this study by completing the survey online (Table 3 in section 4.1). The pilot study was carried out at BIUST in the Department of Chemical and Forensic Sciences, to determine its feasibility on a larger scale before rolling it out. It was successful as only positive feedback was received. The participants were notified that the survey is strictly anonymous and they did not provide any identification details.

3.2 Method Development, Optimisation and Validation for Detection and Stability of Drugs

3.2.1 Materials and Chemicals

All chemicals used were analytical reagent grade or better. Chloroform, ethyl acetate, isopropanol and methanol were purchased from Fisher Chemicals; eicosane was purchased from Acros Organics; *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) derivatising agent was purchased from Supelco Analytical. The pharmaceutical drugs; diazepam, amitriptyline, bromazepam and chlorphenamine were donated in kind by various pharmacies in Palapye, Botswana, while antazoline (Gemini eye drops) was purchased from the pharmacies and they are shown in Figure 3. Table 2 shows the chemical information of the pharmaceutical drugs.



Figure 3: The pharmaceutical drugs purchased from various pharmacies around Palapye, Botswana

Table 2: Information on the pharmaceutical drugs included in this research

SN	Brand name	Active drug	Molecular weight (g/mol)	Total mass per tablet (g)	Mass of active drug (g)
1	Allergex	Chlorphenamine	274.7	0.2027	4
2	NA	Amitriptyline	277.4	0.1024	25
3	Remedium	Diazepam	284.7	0.1966	5
4	Sandoz	Bromazepam	316.15	0.2060	3

NA; Information was not available



Figure 4: The common beverages among tertiary students in Botswana, used in this research

The beverages used in this study were Savannah cider, 6% alcohol by volume, 4th Street red wine, 8% alcohol by volume, Russian Bear vodka, 43% alcohol by volume and the Chibuku traditionally brewed beer, 4% alcohol by volume (Figure 4). All these beverages are manufactured through fermentation and/or distillation, but have unique composition, especially chibuku which is brewed from fermented sorghum grains locally. All the beverages have distinct characteristics, chibuku is thicker and grainy in composition, vodka is clear and smooth, while wine and savanna are smooth with flavour. They were all purchased from local liquor stores in Palapye, Botswana during the period March 2021–February 2022. The four drinks tested in this research were selected based on a survey that was carried out among tertiary students, to represent common drink types consumed by tertiary students in Botswana.

3.2.2 Commonly Dispensed Pharmaceutical Drugs Often Associated with DFSA

The selection of the target drugs included in this research was based on the most commonly purchased antidepressants, benzodiazepines and antihistamines (including eye drops) from pharmacies in Botswana. The general availability and ease of use of the drugs by the general public was also studied and considered. The list of these drugs was compiled by enquiring through a letter of request and questionnaire at different pharmacies in Gaborone, the capital city and Palapye, which hosts the second biggest university in Botswana (BIUST).

The survey yielded the drugs shown in Figure 3 as the commonly dispensed pharmaceutical drugs in Botswana, which are also easily available to the general public. These may therefore be the potential drugs used in drink spiking incidents in Botswana. These pharmaceutical formulations or drugs were used as sources of the active drug or ingredient so as to simulate the use of the drugs in actual and practical incidents and are referred to as drug standards in this thesis. While they are all controlled substances, antidepressants and benzodiazepines are obtained through a medical prescription, but some eye drops and antihistamines can be easily obtained over-the-counter without a prescription. However, bromazepam was subsequently removed from the study after signal testing due to detectability issues and unavailability of new batches of the same drug in pharmacies countrywide. Antazoline was also removed from the research as it went out of stock countrywide, shortly after bromazepam, however it was included in all the preliminary studies.

3.2.3 Chemical Structures of the Commonly Dispensed Antidepressants, Benzodiazepines and Antihistamines in Botswana

According to the pharmacy survey the most commonly purchased antidepressants, benzodiazepines and antihistamines across the different pharmacies was found to be chlorphenamine, amitriptyline, diazepam, Gemini eye drops (active drug antazoline) and bromazepam. Figure 5 depicts the chemical structures of the aforementioned drugs, which are the drugs included in this research.

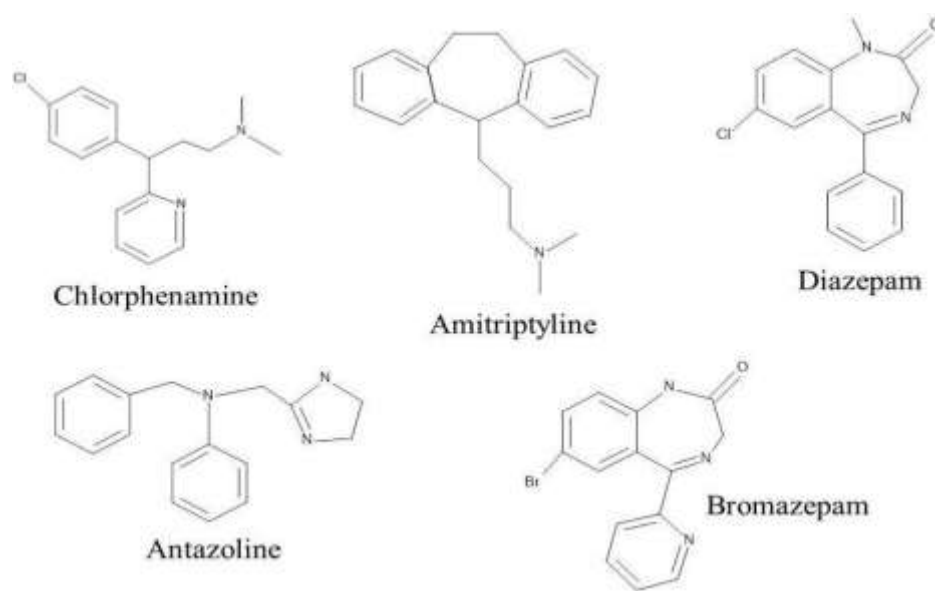


Figure 5: Chemical Structures of the Commonly Dispensed Antidepressants, Benzodiazepines and Antihistamines in Botswana

3.2.4 GC-MS Method

The GC-MS method used was adopted from literature [37]. GC-MS analysis was carried out on an Agilent 5973 mass selective detector coupled to an Agilent 6890N GC system, with a PAL RSI 85 auto sampler. The GC was fitted with an Agilent J&W cross-linked polymethylsiloxane capillary column (HP-5 MS; 30 m; internal diameter 0.320 mm; film thickness 0.25 μ m), utilised with split injection (14:1) at 250 °C. The injection volume was 1 mL. The column flow rate (He carrier gas) was 1.2 mL/min. The oven temperature programme was 150 °C (2 minutes), then increasing at a rate of 20 °C/min to 280 °C and held at 280 °C for 5 minutes. The quadrupole mass analyser was operated in electron impact (EI) mode. The ion source temperature was 230 °C, interface temperature was 300 °C and solvent delay time was 3 minutes. Samples were analysed in full scan. Ethyl acetate blanks were run between all samples, controls and standards to prevent carry over.

3.2.5 Preliminary Tests

3.2.5.1 Preparation of Stock Solutions from Pharmaceutical Formulations

Individual 250 μ g/mL stock solutions of diazepam, bromazepam, amitriptyline, chlorphenamine and antazoline were prepared in methanol. 5 mg of each drug was dissolved in 20 mL of methanol in a 20 mL volumetric flask, to make a final concentration of 250 μ g/mL. All the drugs were in tablet form except the eye drops which were liquid and, an analytical balance was used to weigh the tablets. In preparing the antazoline stock solution, 1 mL of the Gemini eye drops was used as a 400 μ g/mL stock solution of antazoline.

The mass of a tablet of diazepam (5 mg) was found to be 0.1966 g, which was equivalent to 5 mg of diazepam. The tablet was crushed into fine powder using a mortar and pestle and then dissolved in about 5 mL methanol in a beaker. The solution was then transferred quantitatively into a 20 mL volumetric and filled to the mark. After mixing, the solution was allowed to stand before being transferred into a 50 mL storage bottle.

Bromazepam (3 mg) tablet weighed 0.2060 g, equivalent to 3 mg of bromazepam, and 5 mg was calculated to be equivalent to 0.3433 g of the bromazepam tablet. A mass of 0.3433 g of the bromazepam tablets was then weighed. A tablet of chlorphenamine (4 mg) weighed 0.2027 g, equivalent to 4 mg of chlorphenamine, and 5 mg was calculated to be equivalent to 0.2534 g of the chlorphenamine tablet. A mass of 0.2534 g of the chlorphenamine tablets was then weighed. A tablet of amitriptyline (25 mg) weighed 0.1024 g, equivalent to 25 mg of amitriptyline, and 5 mg was

calculated to be equivalent to 0.0205 g of the amitriptyline tablet. 0.0205 g of the amitriptyline tablet was then weighed. The same procedure outline for diazepam above was followed to prepare stock solutions of chlorphenamine, bromazepam and amitriptyline. 1 mL of the Gemini eye drops contains 0.4 mg of antazoline; therefore 1 mL of the eye drops was used as a 400 µg/mL stock solution. The internal standard (eicosane) was also prepared at a concentration of 250 µg/mL in ethyl acetate. A mass of 0.056 g of eicosane was weighed and the same procedure for preparation of stock solutions was followed.

All the stock solutions were stored in dark amber bottles after preparation, closed off with parafilm and stored at 4 °C.

3.2.5.2 Signal Testing/Detection (Derivatised Drugs)

Drug samples of concentrations 25, 50, 75 and 100 µg/mL were prepared in methanol, from the stock solutions, by measuring 10, 20, 30 and 40 µL of each of the stock solutions respectively, to test the detectability and GC-MS signals for each drug. The internal standard was added at 25 µg/mL (10 µL) in each sample. The samples were dried under a gentle stream of nitrogen gas at 30 °C and reconstituted in 100 µL ethyl acetate and analysed with the GC-MS, method presented in section 3.2.4. The above signal testing process was repeated, with reconstituting in 100 µL of the derivatising agent (BSTFA+TMCS, 99:1) and heating to 70 °C for 15 minutes. This was to compare between the derivatised and underivatised analytes during analysis, so as to determine whether derivatisation would significantly improve detection.

3.2.5.3 Mixed Standard Signal Testing

Subsequently, two mixed drug standards were prepared with the drugs at the noted detectable concentrations. After drying under a gentle stream of nitrogen gas at 30 °C, one standard was reconstituted in 100 µL of ethyl acetate and one was reconstituted in 100 µL of BSTFA+TMCS (99:1). The latter was heated to 70 °C for 15 minutes and they were both analysed using the GC-MS.

3.2.5.4 Optimisation of the Derivatisation Method

Four samples of the mixed standards were prepared in vials at concentrations of 50, 75, 100, 100 and 50 µg/mL for diazepam, amitriptyline, antazoline, bromazepam and chlorphenamine, respectively. The four mixed standards were labelled A, B, C and D and they were dried under a gentle stream of nitrogen gas followed by the addition of 100 µL of BSTFA+TMCS (99:1). All the vials were heated to 70 °C with vial A

heated for 15 minutes, vial B for 30 minutes, vial C for 45 minutes and vial D for 60 minutes. They were then analysed using the GC-MS (section 3.2.4).

3.2.5.5 Signal Testing/Detection (Underivatised Drugs)

A mixed drug standard containing chlorphenamine, amitriptyline and diazepam was prepared with the drugs at the noted detectable concentrations. After drying under a gentle stream of nitrogen gas at 30 °C, the standard was reconstituted in 100 µL of ethyl acetate and then analysed using the GC-MS. This was to determine if removing the derivatisation step with the underivatising drugs improved detection.

3.2.6 Method Validation for Qualitative Analysis (Derivatised Drugs)

3.2.6.1 Precision Studies

Precision Studies i.e. intra-assay and inter-assay were carried out over a period of three days. Derivatised mixed drug standards of concentrations 75 µg/mL (chlorphenamine, amitriptyline and diazepam at 75 µg/mL and antazoline at 100 µg/mL) and 100 µg/mL (chlorphenamine, amitriptyline at 100 µg/mL and antazoline at 125 µg/mL) were prepared. The internal standard was also added (10 µL) to a final concentration of 25 µg/mL. The standards were evaporated to dryness under a gentle stream of nitrogen gas and derivatised in 100 µL of BSTFA: TMCS (99:1). The samples were divided into four vials; A, B, C and D, then analysed using the GC-MS, with blanks in between. The four replicates were to allow for statistical variation assessment.

Intra-assay precision of the instrument was determined at the two concentrations (75 µg/mL and 100 µg/mL) over a 5 hour period under the same instrumental conditions.

Inter-assay precision of the instrument was also assessed at the two concentrations (75 µg/mL and 100 µg/mL) over three separate days.

The precision was determined by the relative standard deviation (RSD) of the peak area ratios (PARs) of the samples.

3.2.6.2 Auto-Sampler Stability

Auto-sampler stability study was carried out on derivatised mixed drug standards in order to determine the stability of the derivatised analytes during instrumental analysis.

Derivatised mixed drug standards of concentrations 100 µg/mL (chlorphenamine, amitriptyline at 100 µg/mL and antazoline at 125 µg/mL) were prepared and the internal standard eicosane was added at

25 µg/mL. The standards were evaporated to dryness under a gentle stream of nitrogen gas and derivatised in 100 µL of BSTFA: TMCS (99:1). The mixed drug standard of chlorphenamine, amitriptyline, diazepam and antazoline was divided into 10 vials after preparation. The samples were kept in the auto-sampler and analysed immediately over a period of 25 hours using the GC-MS (section 3.2.4), with at least 9 solvent blanks injected between each sample injection. The analysis time point indicates the time intervals at which the storage stability samples were analysed with. The stability was evaluated by regression analysis on a plot of PAR against the injection time. Instability was indicated by a negative slope, significantly different from zero ($p \leq 0.05$) [85]. Statistical analysis was done with Microsoft Excel 2010.

3.2.7 Method Validation for Qualitative Analysis (Underivatised Drugs)

3.2.7.1 Precision Studies

Precision Studies i.e. intra-assay and inter-assay were carried out over a period of three days. Underivatised mixed drug standards of concentrations 25, 75 and 125 µg/mL (chlorphenamine, amitriptyline and diazepam) were prepared. The internal standard was also added (10 µL) to a final concentration of 25 µg/mL. The standards were evaporated to dryness under a gentle stream of nitrogen gas and reconstituted in 100 µL of ethyl acetate. The samples were vortexed, divided into six vials (A-F) and analysed using the GC-MS (section 3.2.4), with blanks in between each vial. The six replicates were to allow for reduced variation to be detected in this study and to assess statistical variation between more measurements.

Intra-assay precision of the instrument was determined over a 13-hour period under the same instrumental conditions.

Inter-assay precision of the instrument was also assessed at the three concentrations (25, 75 and 125 µg/mL) on three separate days.

The precision was determined by the relative standard deviation (RSD) of the peak area ratios (PARs) of the samples.

3.2.7.2 Auto-Sampler Stability

Auto-sampler stability study was carried out on the underivatised mixed drug standards in order to determine the stability of the analytes during instrumental analysis.

Auto-sampler stability for the underivatised drugs was determined over 24 hours. An underivatised mixed drug standard of 100 µg/mL for chlorphenamine, amitriptyline and diazepam was prepared

from a 250 µg/mL mixed stock solution. The internal standard eicosane was also added (10 µL) at 25 µg/mL. The standards were evaporated to dryness under a gentle stream of nitrogen gas and were reconstituted in 100 µL of ethyl acetate. The sample was then vortexed and divided into 14 vials after preparation. The vials were kept in the auto-sampler and analysed immediately over a period of 24 hours using the GC-MS, with at least 5 solvent blanks injected between each sample injection. The analysis time point indicates the time intervals at which the storage stability samples were analysed with.

The stability was evaluated by regression analysis on a plot of PAR against the injection time. Instability was indicated by a negative slope, significantly different from zero ($p \leq 0.05$) [85]. Statistical analysis was done with Microsoft Excel 2010.

3.2.8 Spiked Beverage Preparation and Analysis

3.2.8.1 Solvent Selection

Two different LLE extraction systems were investigated, i.e. chloroform: isopropyl alcohol (2:1, v/v) and diethyl ether, adopted from literature [37], [63], [86], [87]. A 5 mL aliquot of wine was spiked with a 250 µg/mL mixed drug standard containing 3 drugs (diazepam, amitriptyline and chlorphenamine) to a final concentration of 100 µg/mL, and the internal standard eicosane at a final concentration of 25 µg/mL. Different 0.5 mL aliquots of the spiked mixture were transferred into 6 Eppendorf tubes, labelled A-F. The samples in tubes A-C were extracted with 100 µL x3 of diethyl ether while tubes D-F were extracted using 100 µL x3 of chloroform: isopropanol. The organic layers were transferred into appropriately labelled vials and evaporated to dryness under a gentle stream of nitrogen gas. The samples were then reconstituted in 40 µL of BSTFA+TMCS (99:1) and then heated to 70 °C for 30 minutes then analysed using the GC-MS. In order to enable the calculation of recovery, a similar unextracted sample spiked with the mixed drug standard was prepared, evaporated, derivatised and analysed using the GC-MS (section 3.2.4). The average peak areas ratios of the triplicate analysis were used to determine the %recovery (Equation 1) in each solvent system.

3.2.8.2 Drink Spiking and Storage

The four drinks were degassed for 20 minutes at room temperature using an ultrasonic bath prior to addition of the drugs. The Savanna cider, 4th Street red wine, Russian Bear vodka and Chibuku local beer (20 mL) were each spiked with a mixed drug stock solution to give a final concentration of 100 µg/mL (diazepam, amitriptyline and chlorphenamine). The IS (eicosane) was added to the individual

samples to give a final concentration of 25 µg/mL, immediately prior to extraction. The difference in the spiking concentrations of the drugs is owing to the instrumental response assessed during the signal testing. The spiked beverages were divided into two aliquots and stored in vials. Half of the vials were stored at room temperature, and the rest were stored in the freezer at -18 °C, until liquid-liquid extraction (LLE) and GC-MS analysis was carried out at the appropriate analysis points.

3.2.8.3 Extraction of Drugs from the Spiked Drinks

LLE was performed using chloroform: isopropanol ((2:1, v/v), 100 µL×3) directly after spiking and on unspiked beverages, in triplicate samples. After extraction, the organic phase (bottom) was collected and dried **under a gentle stream of nitrogen gas**. The samples were then reconstituted in 40 µL of BSTFA+TMCS (99:1) and then heated to 70 °C then analysed using the GC-MS. Extraction and analysis of the samples was continued on day 2, day 4, day 6, day 13, day 21 and day 28 to monitor detection and stability of the drugs over a period of 28 days. Stability was evaluated by calculating the %loss of the analyte or drug over the 28 day period, using Equation 2 in section 4.2.11.2.

3.2.9 Statistical Analysis

All experiments were carried out in triplicate, except intra-assay precision studies for the derivatised and underivatised drugs carried out in four and six replicates, respectively. Statistical analysis was carried out using Microsoft Excel across all studies.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter discusses the results obtained from the survey conducted to assess the prevalence, knowledge, perceptions and experiences of DFSA among the youth in Botswana. This chapter also presents the statistical analysis conducted to evaluate the significance and reliability of the data obtained. The data is divided into subsections according to themes developed during data analysis and data coding. The chapter also discusses the results obtained from preliminary and method validation studies undertaken prior to conducting stability studies. The results from the application of the validated and optimised method towards studying the stability of derivatised drugs in alcoholic beverages are also presented.

4.1 The Survey

This survey was aimed at assessing the prevalence of DFSA in Botswana as well as the perception and knowledge of Botswana on the subject, following the method described in section 3.1.

A total of 117 responses were received and Table 3 below shows the number of participants per tertiary institution. This was more than the minimum sample size of 96 required for this study based on the Cochran formula.

Table 3: Number of participants per tertiary institution

Institution Name	Abbreviation	Number of Participants	Percentage of total respondents (%)
Botswana International University of Science and Technology	BIUST	68	58.1
University of Botswana	UB	19	16.2
Botho University	BU	4	3.4
Botswana Accountancy College	BAC	15	12.8
Gaborone Universal College of Law and Professional Studies	GUC	5	4.3
Institute of Development Studies	IDM	2	1.7
DDT College of Medicine	DDT	1	0.9
Botswana University of Agriculture and Natural Resources	BUAN	2	1.7
Gaborone Technical College	GTC	1	0.9

4.1.1 Sociodemographic Factors of the Respondents

The survey targeted tertiary students who were around ages 19-24, which explains why the majority of the respondents were in the 21-24 age groups at 47.9%, followed by the 25-30 age groups at 25.6% (Figure 6).

Females made up 64.1% of the respondents, males made 34.2% and 1.7% preferred not to say their gender. The higher number of respondents being females could be attributed to more females enrolling in higher numbers at tertiary institutions [81].

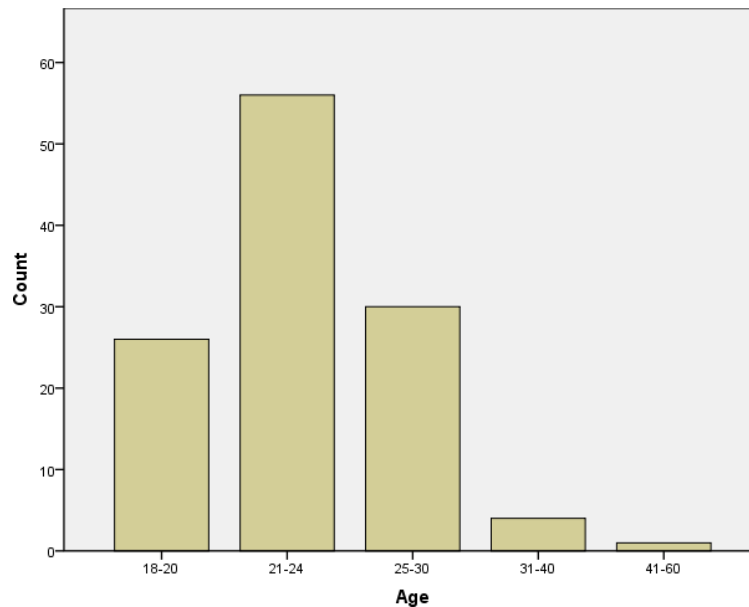


Figure 6: Respondents' Age Groups

Most respondents were scholars at BIUST (57.3%), UB (16.2%) and BAC (12.8 %) (Table 3). In targeting the BIUST students, various platforms including emails, were used as compared to other institutions where only social media was used. DDT and GTC had the least respondents at 0.9% each. BU, IDM and DDT had no male respondents while DDT, BUAN and GTC did not have female respondents (Figure 7).

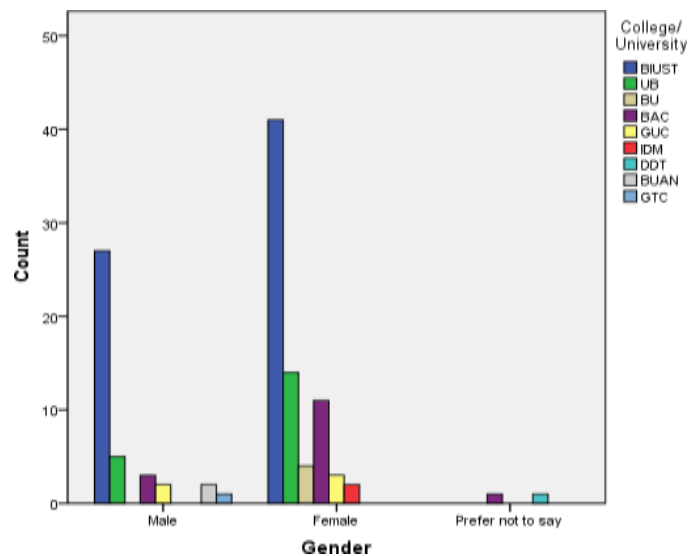


Figure 7: Respondents per Institution of Study by Gender

Respondents with bachelor's degrees/equivalent made up majority of the respondents (57.3%), followed by Master's degree and BGCSE/IGCSE (high school certificates) holders at 17.1% each. There were more female degree holders than males, which could also be owing to females making most of the respondents (Figure 7).

Most respondents, 84.6%, were currently enrolled when responding to the survey while 15.4% were not enrolled. The latter group was most likely graduates from the tertiary institutions, or members of staff.

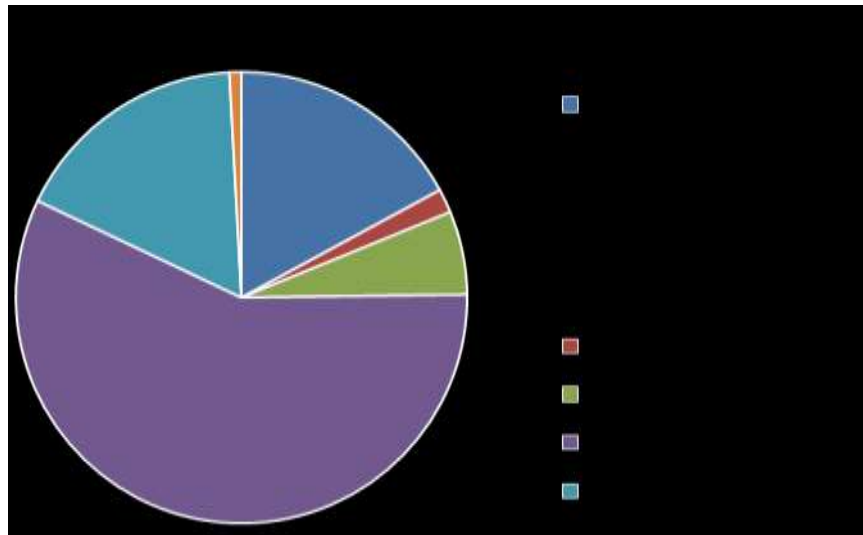


Figure 8: Chart showing the respondents' level of education

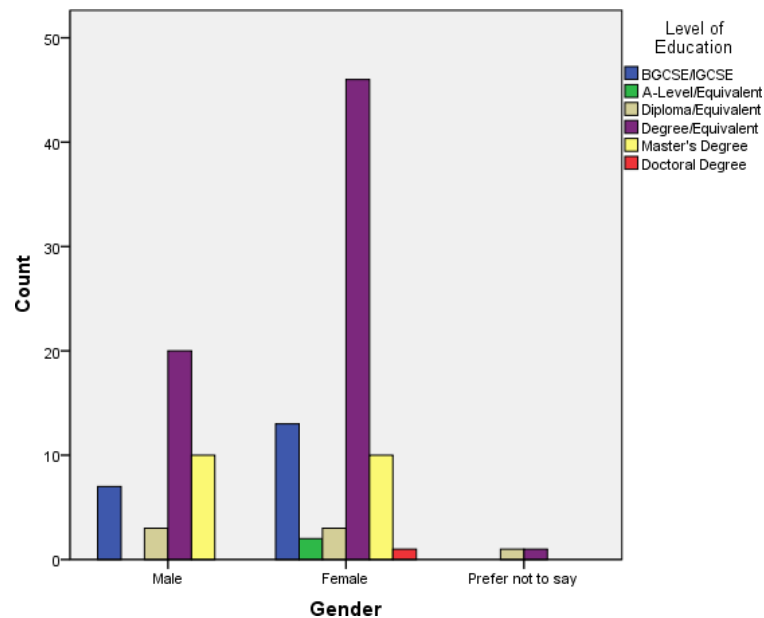


Figure 9: Respondents' Level of Education by Gender

Most of the respondents were degree holders (57.3%), followed by BGCSE/IGCSE and master's degree holders (17.1%) and 74.6% of the degree holders knew what DFSA was while 90% of the Master's degree holders and 70% of the BGCSE/IGCSE holders also knew about the topic (Figures 8, 9 and 10). However the relationship between respondents' level of education and knowledge of DFSA was non-significant ($p=0.430$, $\chi^2=4.887$), see Appendix I.

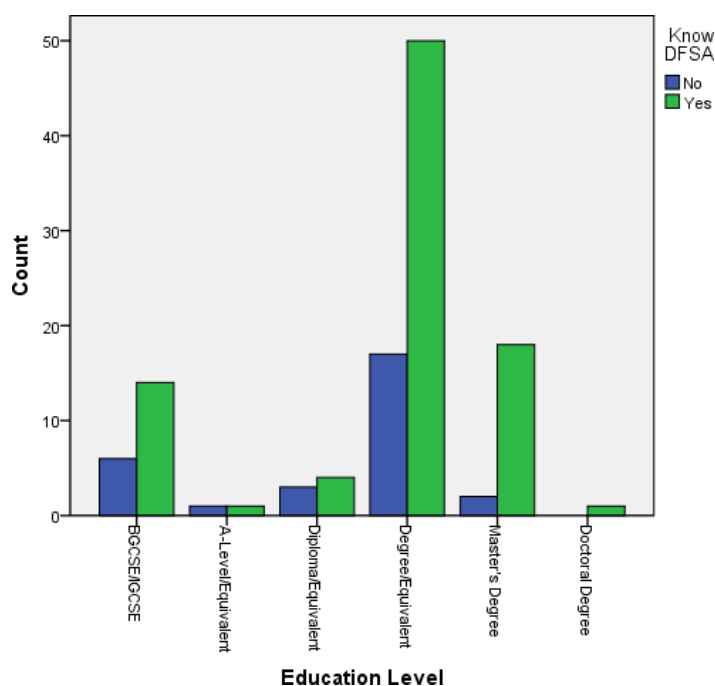


Figure 10:Chart showing the comparison of respondents' level of education and their knowledge of DFSA

Table 4: Respondents' Knowledge of Sexual Assault, DFSA and Information about DFSA

Knowledge of Sexual Assault	Frequency	%	Informed on DFSA	Frequency	%
No	6	5.1	Not well informed	28	24.3
Yes	111	94.9	A bit well informed	62	53.9
Knowledge of DFSA			Very well informed	25	21.7
No	29	24.8	Know About Consent		
Yes	88	75.2	Yes	117	100.0

The data obtained showed that majority of the target population in Botswana has adequate knowledge on the subject as 94.9% of the respondents knew what sexual assault was beforehand; while 75.2% knew what DFSA was (Table 4). In a study carried out at a South African university to find out the prevalence of sexual violence among adolescent girls, majority of the study population had adequate

knowledge of sexual assault and this shows that people usually do have a general knowledge of the broad sexual assault [19]. In addition, 21.7% of the respondents indicated that they were very well informed on DFSA while 52.9% indicated that they are a bit well informed on the topic (Table 4). Of the 64.1% of females, 31% said they were very well informed on DFSA, 49.3% of them said they were a bit well informed and 20.5% were not well informed (Figure 12). Even though a number of females indicate that they are informed on DFSA, a good number (20.5%) was also not well informed. In addition, all respondents indicated they knew that consent is required before a sexual activity.

This study shows that the government's efforts in educating people about sexual assault and drug facilitated sexual assault are effective as 31.3% of the respondents learnt about these at school. However, the majority, 36.8% learnt about sexual assault from the media and 26.4% learnt from the internet (Figure 11).

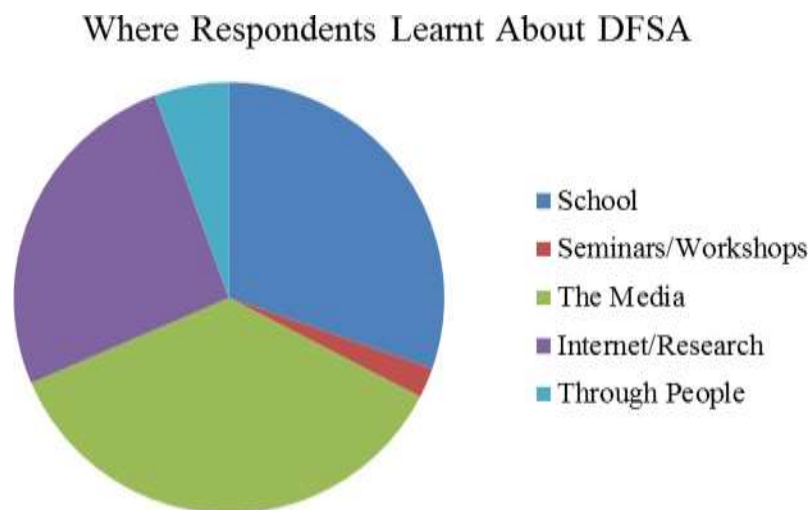


Figure 11: Chart showing where respondents learnt of DFSA

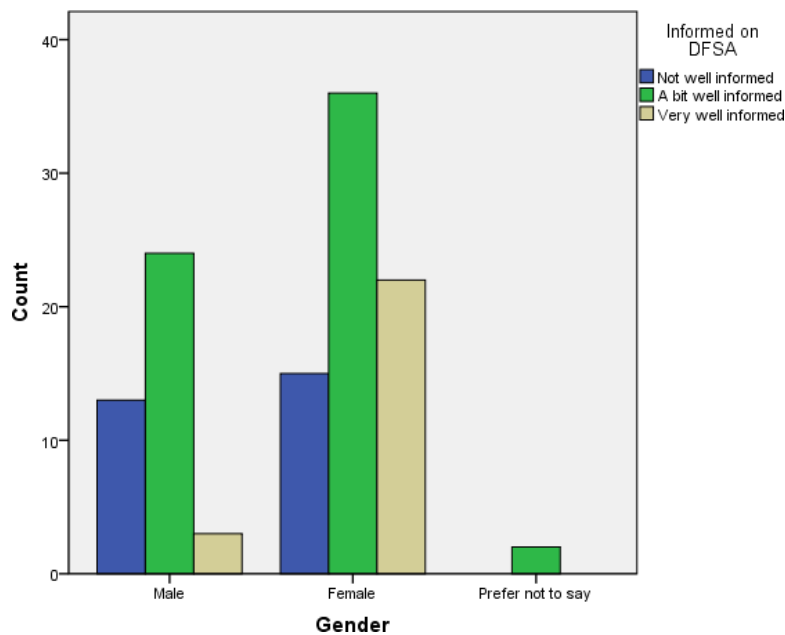


Figure 12: Respondents' level of information on DFSA by gender

4.1.2 The Chances and Risk Factors of Experiencing DFSA in Botswana

According to this study, respondents believed it is unlikely for one to experience drug facilitated sexual assault as 48.7% believe it is not at all likely to experience it, 35.9% believe it is slightly likely, 13.7% believe it is very likely and 1.7% believe it is extremely likely (see appendix II). More females(17.3%) think it is very likely to experience DFSA than males (5%) (Figure 13). This suggests that as females are generally at a higher risk of being sexually assaulted, they may also deem it very highly likely to experience it. There was a significant positive relationship between the gender of the respondents and their opinion on how likely it is to experience DFSA ($p=0.002$, $\chi^2=0.286$).

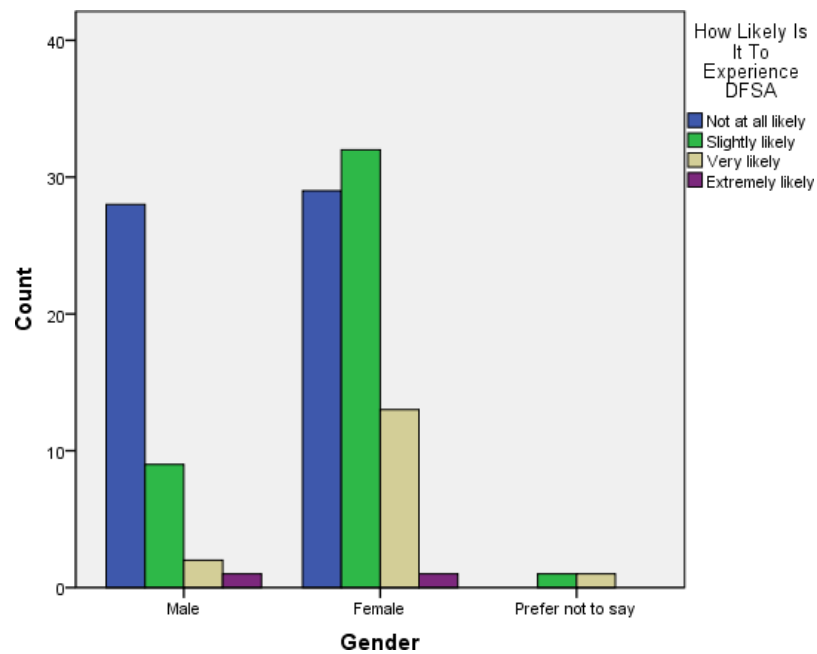


Figure 13: Respondents' opinion on how likely it is to experience DFSA by gender

According to responses, one is more likely to experience DFSA at entertainment events, clubs/bars (37.5%) and entertainment events (37.5%). Likewise, most studies do show that the mentioned are perceived to be high risk areas for DFSA and general sexual victimization [14], [39]. In this study, most cases occurred at clubs/parties (Figure 14), which correlates with many other studies of this nature, where it is common that DFSA usually occurs at clubs and parties or general entertainment settings[1], [5], [37]. One study carried out at a university in the south of USA showed that women who frequently attended group parties were at a higher risk of experiencing incapacitated sexual assault, as the two were positively associated [18].

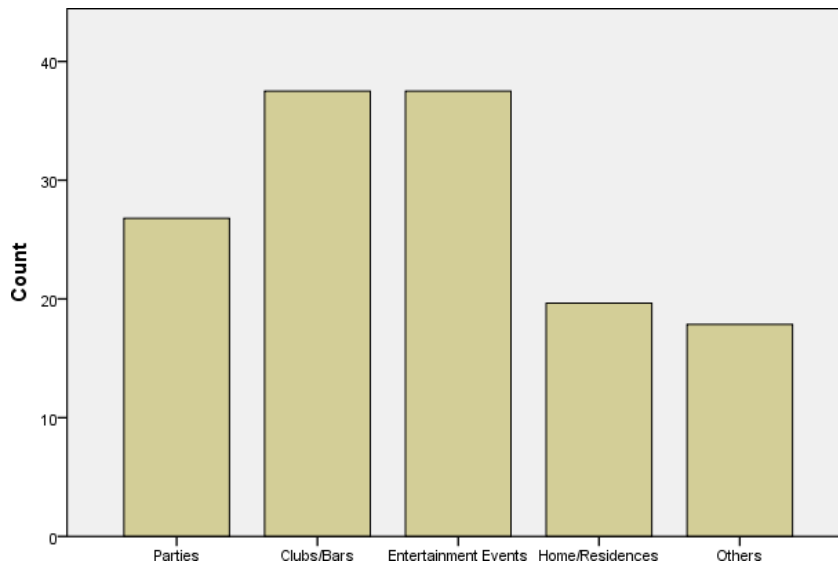


Figure 14: Respondents' opinions on where one is likely to experience DFSA by gender

Most respondents do not often hear of DFSA incidents at their college/university (55.6%), 29.1% never hear of the incidents while 13.7% often hear of the incidents and 1.7% hear of the cases very often (Figure 15). GUC, despite a low number of respondents, has incidences of DFSA as 40% of the respondents who often hear of the incidents at their college or university are from GUC, followed by BAC (20%), [see appendix I](#). Also, BAC and BIUST each had one respondent indicating that they hear of the incidents very often (Figure 15). There was no significant relationship between how often the respondents hear of DFSA incidents and their university or college ($p=0.282$, $\chi^2=27.501$), see Appendix I. In one campus sexual assault study, it was shown that there was usually a lot of sexual assault cases or incidents on campuses as majority of the women indicated that they have experienced sexual assault [18], and this could be the case in Botswana.

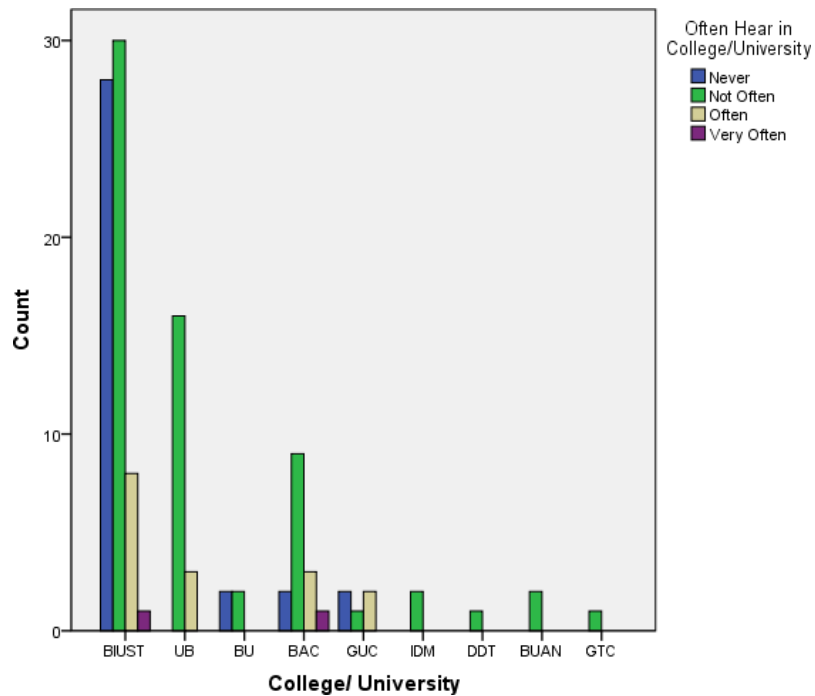


Figure 15: Respondents' institution of study and how often they hear of DFSA cases

Most people believe DFSA occurs at night at clubs/bars (51.3%), parties (41.7%), residences (31.3%), entertainment events (26.1%) and others (11.3%). This corresponds with most studies [23], [41] and the views of respondents as to which are high risk areas for DFSA in Botswana (Table 5).

Table 5: Respondents' Opinion of When and Where DFSA Occurs

When and Where Does DFSA Occur	Frequency	%
Parties	48	41.7
Clubs/Bars	59	51.3
Entertainment Events	30	26.1
Homes/Residences	36	31.3
Others	13	11.3

Most studies on DFSA indicate that perpetrators use sedatives to render the victims vulnerable [24], [25], [29], and this coincides with the knowledge/perceptions of the study population. Of the 117 respondents, 37.3% of the respondents cited alcohol as the number one substance involved in DFSA cases, followed by marijuana at 28.3%, sedatives/date-rape drugs at 26.4%, cocaine at 17.9% and

other substances including viagra and aphrodisiacs at 5.7% (Table 6). This agrees with other studies, [9], [22], [88], as they indicated that alcohol was the most used drug in incapacitating victims. Lastly, 14.2% of the respondents did not know substances that are involved in DFSA cases.

Table 6: Respondents' Opinion of the Substances Involved in DFSA Cases

Substances Involved in DFSA Cases	Frequency	%
Alcohol	40	37.7
Marijuana	30	28.3
Cocaine	19	17.9
Sedatives/Date-rape drugs	28	26.4
Do not know	15	14.2
Others	6	5.7

The main perpetrators of DFSA are believed to be males (70.9%), according to 65.1% of female respondents (Figure 16). There was a significant relationship between respondents' gender and their opinion of the main perpetrators of DFSA ($p=0.056$, $\chi^2=9.205$), see Appendix I. Some said both males and females are the main perpetrators (20.5%), while 8.5% believe females are the main perpetrators. This indicates that more females perceive males as the main perpetrators as most of them are sexually victimized by males [5], [41].

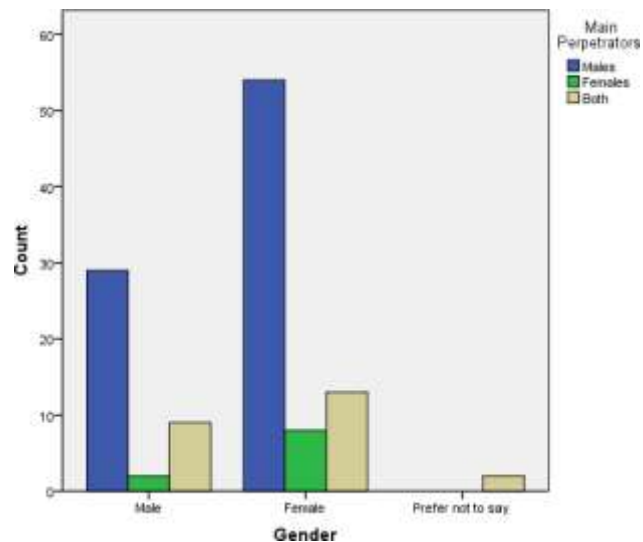


Figure 16: Respondents' opinions of the main perpetrators of DFSA by gender

4.1.3 Experiences on DFSA in Botswana

Of the 48.7% of respondents who have experienced unwanted sexual advances towards them, the majority were females (66.7%). Some respondents (6%), preferred not to say whether they have experienced unwanted sexual advances (Figure 17). As stated in section 1.1, unwanted sexual advances are categorised as indecent assaults in Botswana and are a violation of the laws of Botswana [17]. There was a significant positive relationship between gender and experience on unwanted sexual advances ($p=0.000$, $\chi^2=28.721$).

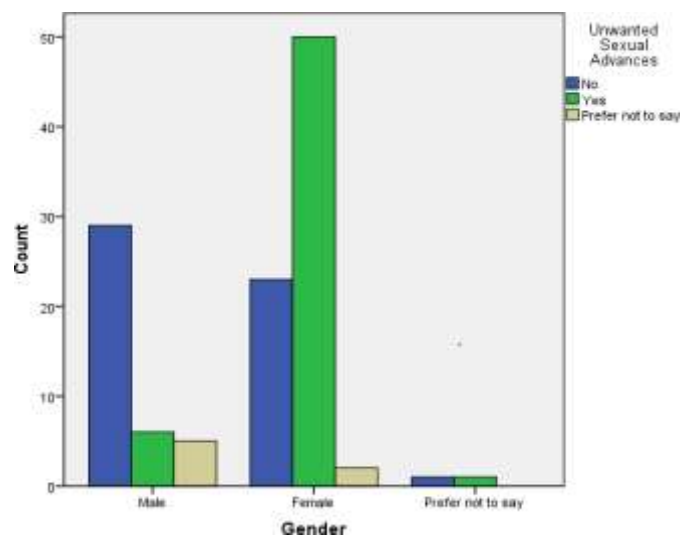


Figure 17: Respondents who have experienced unwanted sexual advances by gender

This study suggests that people are likely to be sexually violated by their friends/acquaintances as the results of this study show that 41.3% of the victims of unwanted sexual advances were violated by their friend/acquaintance, 24.6% by a stranger, and 21.3% by a fellow college/university student. Some of the victims experienced this from their partners, ex-partners and colleagues (Table 7). These results correlate with a number of other studies in showing that a lot of victims of sexual assault were victimized by people they are familiar with. [41], [89].

Table 7: Victims of Unwanted Sexual Advances with the Person/Perpetrator

Relationship With The Person	Frequency	%
Partner	3	4.9
Ex-Partner	6	9.8
Colleague	5	8.2
Fellow College/University student	13	21.3
Friend/Acquaintance	25	41.0
Stranger	15	
Family Member	7	24.6
Others	8	11.5

According to the study, most of the incidents took place at the victim's residence (34.4%), some at the perpetrator's place of residence (27.9%), university residence (24.6%) and some at other places such as cars, parties, bars/nightclubs. This shows that homes or residences are actually also high risk places for sexual assault, unlike the general perception that this can only happen at entertainment settings (Figure 18). The studied population had indicated that it is most likely to experience DFSA at nightclubs and parties, which tallies with other published research [1], [5], however, this particular study indicates that DFSA also has a high chance of occurring at private residences.

Where Did the Incident Take Place

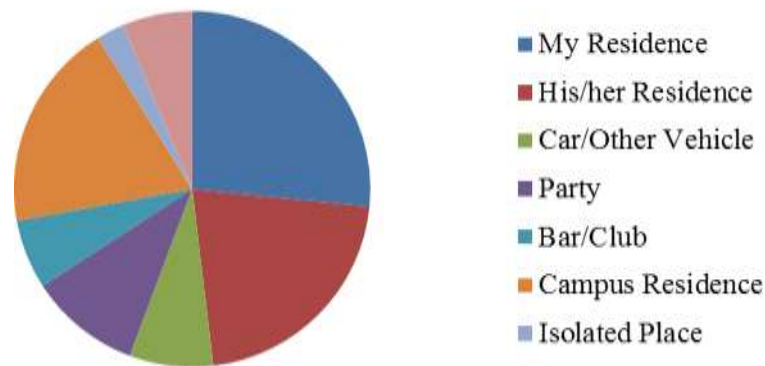


Figure 18: Chart showing places where unwanted sexual advances took place

The respondents who indicated that they were using alcohol/drugs at the time of the incident, 85.7% were females (12) and 14.3% were males (2), see Appendix I. All the respondents who indicated they were drugged beyond the ability to give consent were females. Though a small percentage of the respondents stated that they experienced DFSA (12.8%), this indicates that it indeed exists in Botswana. Also, as majority of those who were using alcohol/drugs are females (85.7%), this indicates that opportunistic DFSA may also be common among women in Botswana (Figures 19-21).

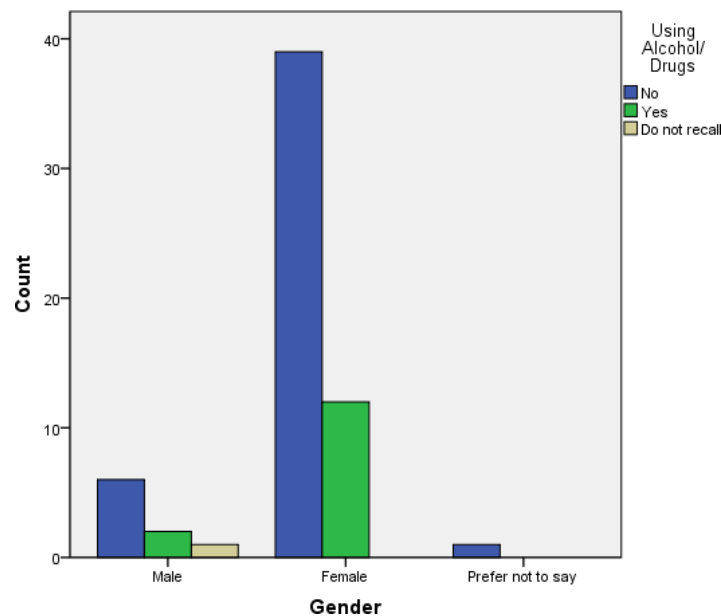


Figure 19: Respondents who were using alcohol/drugs by gender

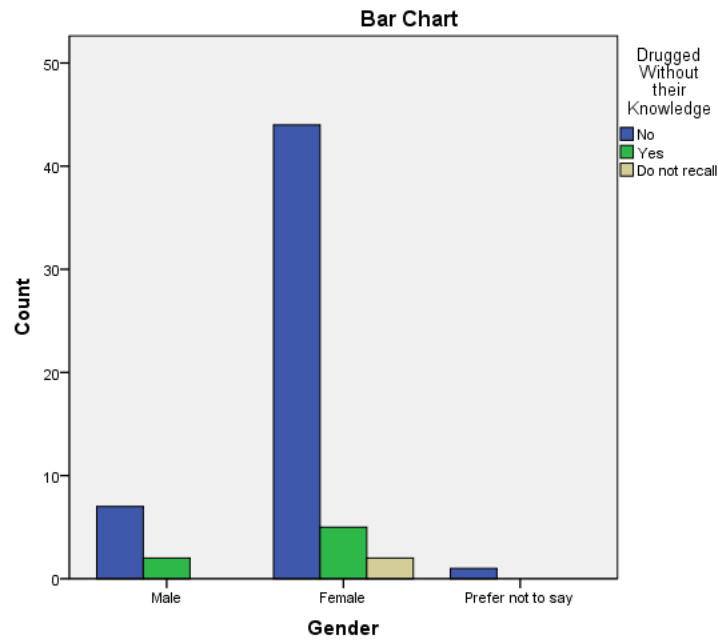


Figure 20: Respondents who believed they were drugged without their knowledge by gender

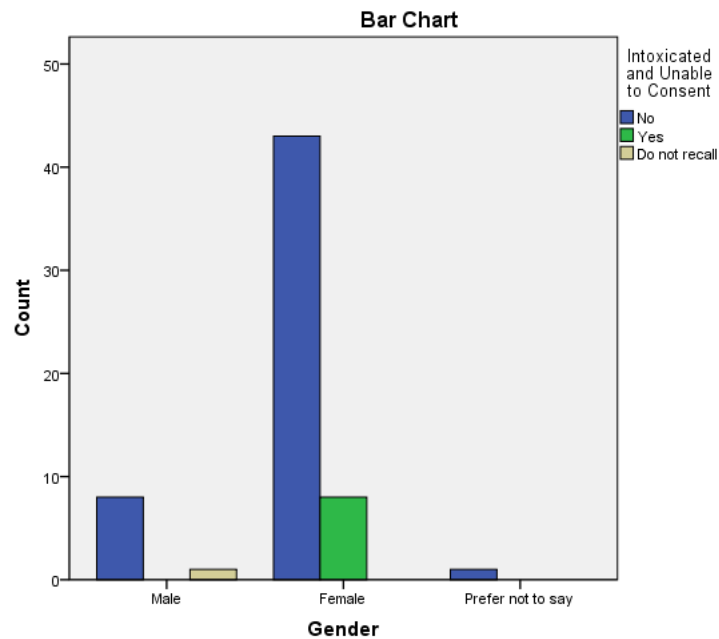


Figure 21: Respondents who were intoxicated and unable to consent by gender

In line with previous studies which indicated that the majority of victims of sexual assault do not report the incidents [19], [47] in this study 40.2% of respondents said they did not report to police or

tell anyone about the incident, 5.1% did not recall whether they did or not, while only 8.5% reported the incidents. From the respondents who told someone (friends/family members), none of them reported to the police (Table 8). In a sexual assault climate survey carried out at the University of New Brunswick, most victims (74%) had told someone about the incident, but typically a close friend, and rarely health care providers, security or law enforcement [41]. The results of this study align with those of the climate survey, and both studies suggest that the current state of law enforcement towards supporting victims of sexual assault may not be satisfactory.

Table 8: Responses on Reporting Unwanted Sexual Advances

Did You Report or Tell Anyone	Frequency	%
No	47	40.2
Yes	10	8.5
Do not recall	6	5.1
Who Did You Report To or Tell		
A friend	5	4.3
Sibling	1	0.9
Parent	1	0.9
Colleague	1	0.9
Other Family Members	1	0.9

Similar to other published studies [1], [20], victims had various reasons for not reporting which included believing nothing happened (12%) therefore there is no need to report, fear/confusion (7.7%), no reason or do not know (9.4%), fear of not being believed (6.8%), feeling ashamed (4.3%) and that reporting is a tedious process (0.9%) (Figure 22).

Reasons for Not Reporting Unwanted Sexual Advances

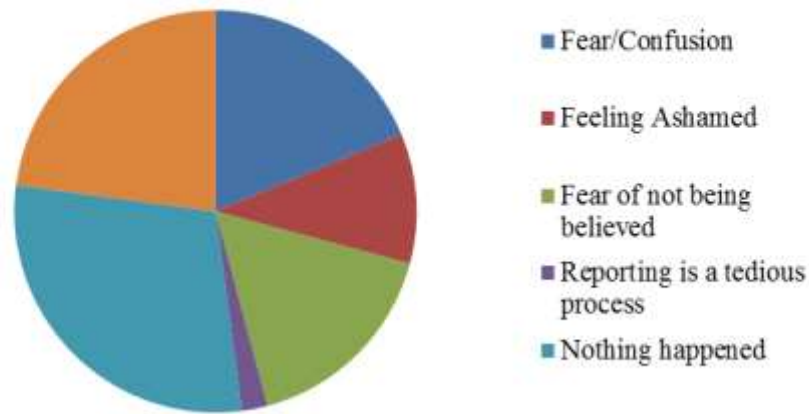


Figure 22: Respondents' Reasons for Not Reporting Unwanted Sexual Advances

Some respondents (19.7%) knew someone who has experienced DFSA, 24.8% of respondents have experienced drink spiking while 2.6% preferred not to say whether they have ever witnessed drink spiking or not. Most people witnessed drink spiking at entertainment events/settings (41.9%), residences (19.4%), clubs/bars (19.4%) and other places such as restaurants as well as hearing from friends (22.6%) (Table 9).

The study population showed adequate knowledge of where to get help or support after sexual assault as 40.2% of the study population was a bit informed on where to get help/support after sexual assault, 24.8% are very informed and 12% are not informed on where to find help/support. This indicates that a good number (approximately 65%) of people are informed on where to get help or support after sexual assault and so awareness and support on sexual assault should be improved. Though some respondents had knowledge of where to get help or support after sexual assault, however some (approximately. 35%), do not know and so awareness and support on sexual assault should be improved.

Table 9: Respondents' Experiences/Witnessing of Drink Spiking

Do You Know Anyone Who Experienced DFSA	Frequency	%
No	53	45.3
Yes	23	19.7
Do not Know	16	13.7
Ever Witnessed Drink Spiking		
No	60	51.3
Yes	29	24.8
Prefer not to say	3	2.6
Where Did You Witness Drink Spiking		
Media	3	9.7
Club/Bar	6	19.4
Entertainment Event	13	41.9
Home/Residence	6	19.4
Others	7	22.6

4.1.4 Perceptions and Social Influences on DFSA

The data suggests that there is problems with victim support as 51.3% of the respondents believe that there is not enough support for sexual assault victims, 16.2% think there is and 10.3% preferred not to say whether there is enough support. As a higher number of the respondents do not believe that there is enough support for victims. This points towards an opportunity with regards to how DFSA cases can be handled in Botswana.

Though some respondents know where to get help or support after sexual assault, they do not believe that there is enough support for victims. Also, most indicated various reasons as to why they did not report their experience with sexual assault or DFSA and they gave suggestions as to how the system can be improved to better support victims and also curb sexual assault. 42.9% of the respondents indicated that there should be a change within law enforcement, there should be stricter laws for perpetrators and the reporting process should be made easier. Of the respondents, 26.5% of the respondents cited that there needs to be more education and awareness among the population, 20.4%

believe that there should be counselling offered to victims, 8.4% believe that there should be special sexual assault referral centers for victims and others said that there should be a 24hr professional helpline (2.0%) that is accessible to all (Table 10).

Table 10: Respondents' Suggestions on What Can be Done to Assist Victims

What Can Be Done To Assist Victims	Frequency	%
Education/Awareness	13	26.5
Counselling	10	20.4
Easy reporting/Stricter Laws	21	42.9
24hr Helpline	1	2.0
Sexual Assault Referral Centers	4	8.2
Others	2	4.1

According to this study, most respondents have lost faith in the systems in place of reporting sexual assault at their institutions of study, as 12.8% say that the victim is blamed when they report sexual assault, 8.5% say that no investigations are conducted and little is done to solve the case, 7.7% say that the case is dismissed due to lack of evidence, 4.3% believe that the cases take too long to be solved and 6.8% indicated that they do not know or have never heard of what happens when a victim reports a case of sexual assault (Table 11). In the UNB sexual assault climate survey [41], most victims indicated that they did not report the sexual assault incidents because they did not think it would help or believe it would be taken seriously [41]. Only 12% of the respondents say that appropriate measures are taken once a case of sexual assault is reported at their institution of study. These results are consistent with the findings of other studies as majority also indicated that authorities do not take sexual assault cases as seriously as they should. Victims of sexual assault generally do not seek medical assistance, counselling or report to the police as shown by this study and others [2], [14], [41], [89]. In another study sexual assault survey carried out in Washington, the results differ with those of this study as a good number of the victims indicated that they sought medical assistance (41%) and counselling (44%) and they found all to be helpful. However, a small percentage of the victims reported to the police (15%) and a small number (10%) of the victims found the services to be unhelpful [89].

Table 11: Respondents' Opinions of What Happens When One Reports Sexual Assault at their Institution of Study

What Happens When One Reports Sexual Assault	Frequency	%
Case is dismissed	9	7.7
Appropriate measures are taken	14	12.0
No investigations/Little is done	10	8.5
Cases take too long	5	4.3
Victim blaming	15	12.8
Do not know/Never heard	8	6.8

The respondents were asked who they think is the most responsible for minimizing DFSA in Botswana; 72.8% selected the society, 66.3% selected him/herself (i.e. individuals), 53.3% selected the police, 48.9% selected family, 47.8% selected the government (Table 12). The respondents selected all the five choices, showing that 'everyone' is responsible. From a review of published studies, education or awareness on sexual assault for both victims and perpetrators, as well as improving the resources and response for victims, are amongst the most important measures to be taken in minimizing DFSA, which should also apply in Botswana [18]. Many other studies on sexual assault also cite that the curbing or reduction of sexual assault requires a collective effort by society [18], [89].

This study has shown the prevalence, experience and knowledge of sexual assault or DFSA within the study population. These results are new findings in Botswana and they will aid in devising interventions or policies and recommendations in order to increase awareness on DFSA thereby reducing the crime.

Table 12: Respondents' Opinions on who is Responsible for Minimizing DFSA and Reasons

Who Is Responsible For Minimizing DFSA	Frequency	%
Himself/ herself	61	66.3
Police	49	53.3
Government	44	47.8
Family	45	48.9
Society	67	72.8
Reasons		
Individual responsibility	19	27.5
DFSA requires unity	31	44.9
Legislation should be toughened	6	8.7
The victim is never wrong	2	2.9
The society can do better	14	20.3

4.1.5 Limitations of the Survey

There are a few study limitations; the data was collected from tertiary students and graduates who may have been more exposed to education and awareness on DFSA than the general population. As such, the findings cannot be generalised across all Batswana. The survey questionnaire was administered online and a lot of people may not have had access to the internet and also, some people may not have been active on social media thereby decreasing the chances of obtaining more responses. This led to a low number of participants from the target population compared with other studies of the same nature. However, the eventual sample size was still representative of the study population and above the required minimum sample size. Also, as is common with online surveys, respondents may have underreported or exaggerated responses. The design or formulation of some questions was imprecise which was not identified during pilot phase of this research. This has led to challenges with the interpretation and presentation of data as some responses were ambiguous. In light of this, there is need for further research on a larger scale, to assess the knowledge and prevalence of DFSA in Botswana.

4.2 Method Development for Detection and Stability Studies of Drugs in Alcoholic Beverages

4.2.1 Commonly Dispensed Pharmaceutical Drugs Associated with DFSA in Botswana

Table 13 shows the commonly purchased and dispensed pharmaceutical drugs, which were likely to be involved in DFSA cases, according to a survey conducted among pharmacies in Botswana, as presented in section 3.2.1.

Table 13: The Commonly Dispensed Pharmaceutical Drugs Associated with DFSA in Pharmacies around Botswana

	Antihistamines	Antidepressants	Benzodiazepines	Eye drops
Toro Pharmacy-Palapye	Chlorphenamine	Quetiapine Olanzapine Amitriptyline Sertraline Fluoxetine	Diazepam Alprazolam	Sufr blue Eye gene
The Medics Centre-Palapye	Chlorphenamine Loradine Cetirizine Promethazine	Amitriptyline Fluoxetine Imipramine	Diazepam Bromazepam Zolpidem	Gemini Eye gene Predmild Spersadex
Pulse Pharmacy-Palapye	Chlorphenamine Loratadine Doxylamine Phenylephrine Diphenhydramine Dextromethorphan	No data	No data	Gemini Eye gene Spersallerg Oxylene Refresh tears Refresh liquid gel
First Care Pharmacy-Palapye	Chlorphenamine Loratadine Cetirizine	Fluoxetine Duloxetine Escitalopram	Loprazolam	Brimonidine Eye gene Spersallerg Lerobunolol Refresh tears Pred-mild Pred-forte

Noble Pharmacy- Gaborone	Cetirizine Loratadine	Olazepine Fluoxetine Paroxetine	Bromazepam Diazepam	Combigan Eye gene Spersallerg Refresh tears
Friends Pharmacy- Gaborone	Cetirizine Allergex Claritine	Amitriptyline	Bromazepam Diazepam	Tamigen Eye gene Spersallerg
Clinicare Pharmacy- Gaborone	Loratadine Chlorphenamine Promethazine Desloratadine	Amitriptyline Fluoxetine Paroxetine Duloxetine	Bromazepam Diazepam Clonazepam Alprazolam	Oxymetazoline Chloramphenicol Prednisolone Sodium Chromochate

4.2.2 Signal Testing

Preliminary studies were conducted on mixed drug standards, so as to develop a method to simultaneously analyse the five drugs investigated in this research. Chromatograms and mass spectra of individual underivatised drugs were initially obtained for comparison with chromatograms and mass spectra of the derivatised individual drugs and mixed drug standards. In addition, mass spectra were compared with published literature or the National Institute of Standards and Technology (NIST) mass spectral search program [Version 2.0(2)].

4.2.3 Detection of the Derivatised Drugs

Underivatised and derivatised mixed drug standards of chlorphenamine, amitriptyline, antazoline, bromazepam and diazepam were analysed and Figure 23 shows the results of analysis of the mixed drug standards, at 100 µg/mL. When analysing the individual drug standards, chlorphenamine, amitriptyline and diazepam were detectable at 50 µg/mL, antazoline at 75 µg/mL and bromazepam was only detectable at 100µg/mL, and therefore the mixed drug standard was prepared at 100µg/mL taking into account the minimum detectable concentration of bromazepam. Based on the chromatogram of the underivatised mixed drug standard in Figure 23A, antazoline was not detected as a peak was not present as depicted in Table 14. This was possibly because it requires derivatisation to be detected using the GC-MS. In that case, the chromatogram of the derivatised mixed drug standard in Figure 23B, depicts peaks for four drugs; chlorphenamine, amitriptyline, antazoline and

diazepam. However, while other drugs were completely derivatised with BSTFA at 70°C for 15 minutes, antazoline was only detected upon heating at 70°C for 30 minutes. Bromazepam was not detected in both the derivatised and underivatised mixed standard, despite being detected individually at the same concentration.

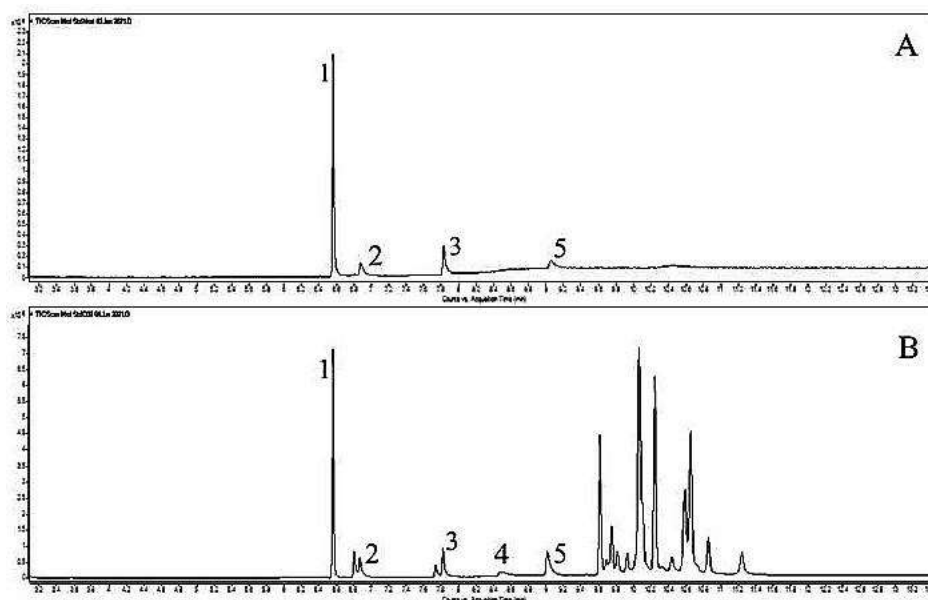


Figure 23: Chromatograms of the mixed drug standards

A: underivatised mixed drug standard and B: derivatised mixed drug standard

1- Eicosane (Internal Standard), 2-Chlorphenamine, 3-Amitriptyline, 4-Antazoline, 5-Diazepam

Table 14: The retention times and relative retention times of the analytes in the underivatised and derivatised mixed drug standards

Analyte	Underivatised mixed standard		Derivatised mixed standard	
	Retention time (minutes)	Relative Retention Time (RRT)	Retention Time (minutes)	Relative Retention Time (RRT)
Internal Standard	6.566	-	6.561	-
Chlorphenamine	6.881	1.05	6.870	1.05
Amitriptyline	7.825	1.19	7.814	1.19
Antazoline	ND	-	8.472	1.29
Diazepam	9.055	1.38	9.032	1.38
Bromazepam	ND	ND	10.331	DI

ND: Not detected, DI: Detected individually but not in the mixed standard, -: Not calculated

Relative Retention Time (RRT) = $RT_{\text{analyte}}/RT_{\text{internal standard}}$; RT: Retention time

In analysing the individual underivatised and derivatised drugs, chlorphenamine was detected at a retention time of 6.8 minutes, amitriptyline at a retention time of 7.8 minutes, antazoline at a retention time of 8.4 minutes, diazepam at a retention time of 9.0 minutes and bromazepam at a retention time of 10.3 minutes. The internal standard, eicosane, was also added prior to derivatising and was detected at a retention time of 6.5 minutes (Table 14). Additionally, the relative retention times (RRTs) for both the derivatised and underivatised drugs (i.e. chlorphenamine, amitriptyline and diazepam) correlated indicating that these drugs are not affected by derivatisation. RRTs in chromatography are usually used because they correct for variables in analytical conditions that may affect retention time, thereby standardizing retention time for accuracy [90]. Furthermore, according to the World Anti-Doping Agency (WADA) guidelines, the RRT of the analyte in the sample should be within $\pm 0.5\%$ from that of the same analyte in a reference used [91]. Therefore, comparison of the RRTs of the derivatised and underivatised drugs correlate and do not differ as shown in Table 14, and therefore meet the acceptance criteria. Bromazepam was however not detected when derivatised in a mixed drug standard, and Figure 23B shows the chromatogram of the derivatised mixed drug standard. Bromazepam may not be detectable in its derivatised form due to background interferences, inability of the compound to derivatise under the derivatisation conditions applied, or thermal

instability of the analyte. However, literature indicates that silylation of bromazepam should render it thermally stable [32]. Also, the derivatised mixed drug standard may have contained other structurally similar compounds or compounds interfering with the detection of bromazepam, however other studies show the detection of bromazepam reliably in mixed drug standards [36], [87]. All the drugs were detected in both the derivatised and underderivatised mixed standards, except bromazepam, despite increasing its concentration to 150 µg/mL and varying the instrument parameters to optimise detection of all the target drugs as part of the preliminary testing. Due to this, bromazepam was removed from the study owing to poor instrumental response and subsequent lack of availability from pharmacies nationwide.

In the chromatogram of the derivatised mixed drug standard there are noticeable interferences and co-elutions in the matrix for chlorphenamine and amitriptyline, while the internal standard, antazoline and diazepam were not co-eluting with other compounds. According to the NIST Library, the compounds co-eluting closely with chlorphenamine and amitriptyline were identified as hexadecanoic acid and octadecanoic acid trimethylsilyl esters. These compounds may be emanating from the derivatising agent, BSTFA+TMCS (99:1), as they were not present in the mixed underderivatised drug standard (Figure 23A) and blank samples. However, all the drugs of interest were distinguishable from other peaks and their retention times as they were consistently distinct. Due to derivatisation, there are multiple artefacts in the sample matrix shown by the peaks eluting from 9.6 minutes (Figure 23B). These peaks as well as the co-eluting peaks may be compounds arising from incomplete derivatisation of some compounds in the sample matrix, column bleed and artefacts or compounds in the derivatising agent. According the NIST Library, these are all trimethylsilyl ethers/esters, possibly arising from the derivatising agent, they include octadecadienoic acid, galactopyranose, 6-0- (trimethylsilyl), cyclic 1,2,3,4 butylboronate, hexadecanoic acid, octadecanoic acid and mannobiose, octakis (trimethylsilyl) ether [71].

4.2.4 Confirmation of the Derivatised Drugs

In addition, the detected drugs in section 4.2.3 were confirmed using the ion fragment patterns observed on their mass spectra. Figures 24-27 compare the mass spectra of the detected derivatised drugs and the spectra from the NIST Library. Diagnostic ions or confirmation ions were selected according to literature i.e. from the NIST website and previously published work as well as in comparison to the NIST Library [92], [93], [94]. According to the criteria for chromatographic-mass spectrometric confirmation of the identity of analytes by the WADA, at least three diagnostic ions are

required for confirmation [91]. The diagnostic ions were selected based on a combination of the most abundant ions and those with significant relative molar mass as per their mass spectral patterns [95]. According to the mass spectra, there is evidence of interference from other matrix components on quantifier and confirmation ions, as some of the fragment ions between the reference and sample spectra did not match. This may have been due to the presence of other matrix components and variance in detector response [96]. However, the quantifier ions were distinguishable from other peaks and confirmation was made by using 3 diagnostic ions i.e. the quantifier and confirmation ions for each analyte or drug. The WADA criterion for chromatographic-mass spectrometric confirmation of identity of analytes and the Standard for Mass Spectral Data Acceptance in Forensic Toxicology, state that the relative abundances of any of the diagnostic ions should be within $\pm 20\%$ of the corresponding relative abundances of the same ions in the reference used [91], [97], [98], and this is also evaluated.

Chlorphenamine

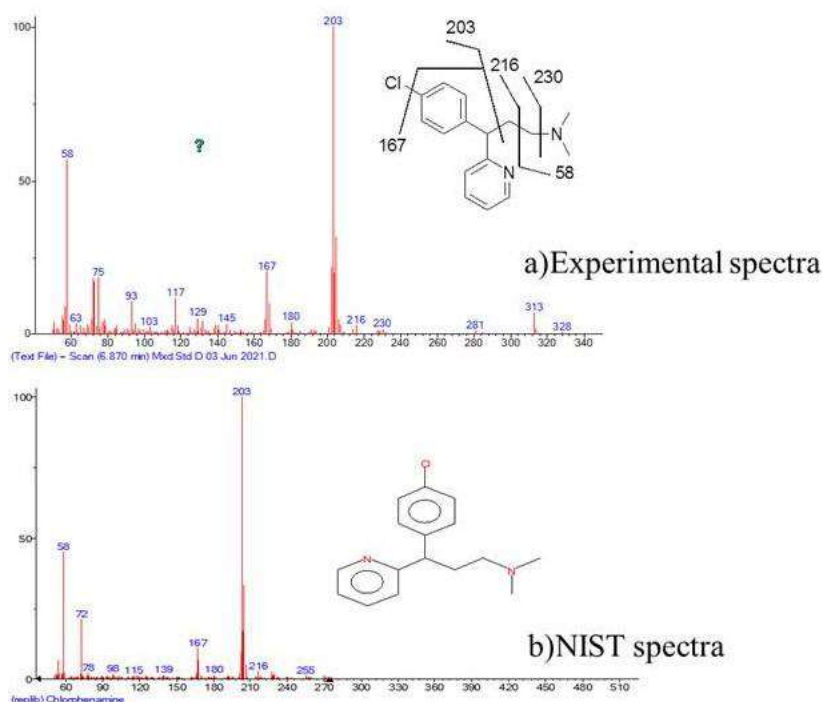


Figure 24: Mass spectra of chlorphenamine in the derivatised mixed standard and the NIST Library spectra

The base peak or quantifier ion; m/z 203 and two qualifier ions; m/z 167, m/z 58 for chlorphenamine were used for confirmation [99]. According to the NIST library, these are ions diagnostic of chlorphenamine in mass spectrometry analysis. These ions were observed in the spectra in Figure 24

which was comparable to the spectra obtained from the NIST Library as the reference spectra in

Figure 24. Table 15 shows the comparison of diagnostic ion intensities in both the mass spectra for comparison. It can be observed that the relative abundances in both spectra were not significantly different and therefore met the confirmation acceptance criteria, except m/z 58, which exceeded the criteria, according to the WADA guidelines [91], [97]. Since different sample preparation and analysis conditions were used between this research and the source of the NIST Library spectra, the results are bound to be slightly different as observed.

Table 15: Comparison of diagnostic ion intensities between chlorphenamine mass spectra

Diagnostic ion	Relative Ion Intensities (%)	
	Experimental spectra	NIST spectra
203	99.9	99.9
167	21.5	26.0
58	79.8	61.9

NIST Library ionisation source: EI

Amitriptyline

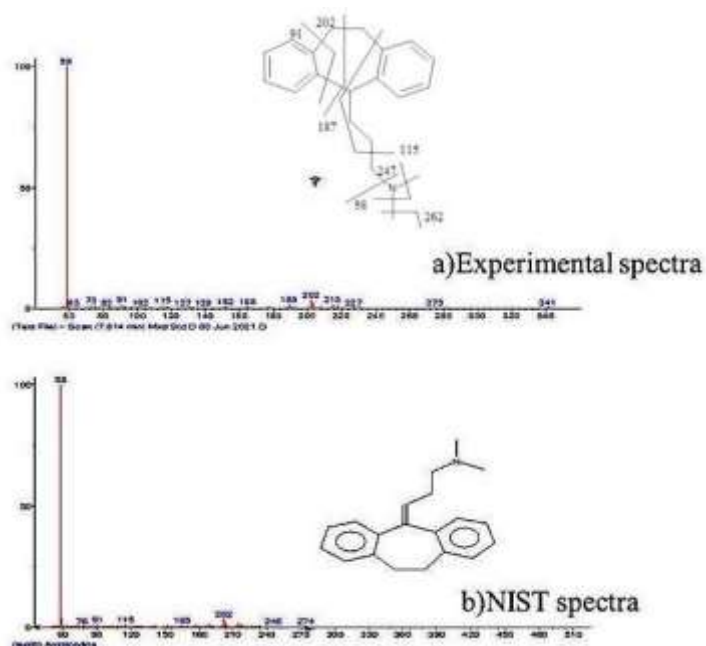


Figure 25: Mass spectra of amitriptyline in the derivatised mixed standard and the NIST Library spectra

The base peak or quantifier ion m/z 58, and two qualifier ions (m/z 202, m/z 91) for amitriptyline [94] were used for confirmation (Figure 25). The intensities of the diagnostic ions were comparable to the NIST Library spectra. Only the base peak had a relative ion intensity greater than 50% while the other ions' intensities were below 5%. All the diagnostic ion intensities met the acceptance criteria, being within $\pm 20\%$ of the relative abundances of diagnostic ions observed in the NIST Library mass spectra. Table 16 shows the comparison of diagnostic ion intensities according to the experimental and library mass spectra.

Table 16: Comparison of diagnostic ion intensities between the amitriptyline mass spectra

Diagnostic ion	Relative Ion Intensities (%)	
	Experimental spectra	NIST spectra
58	99.9	99.9
202	3.5	5.0
91	1.6	1.7

NIST Library ionisation source: EI

Antazoline

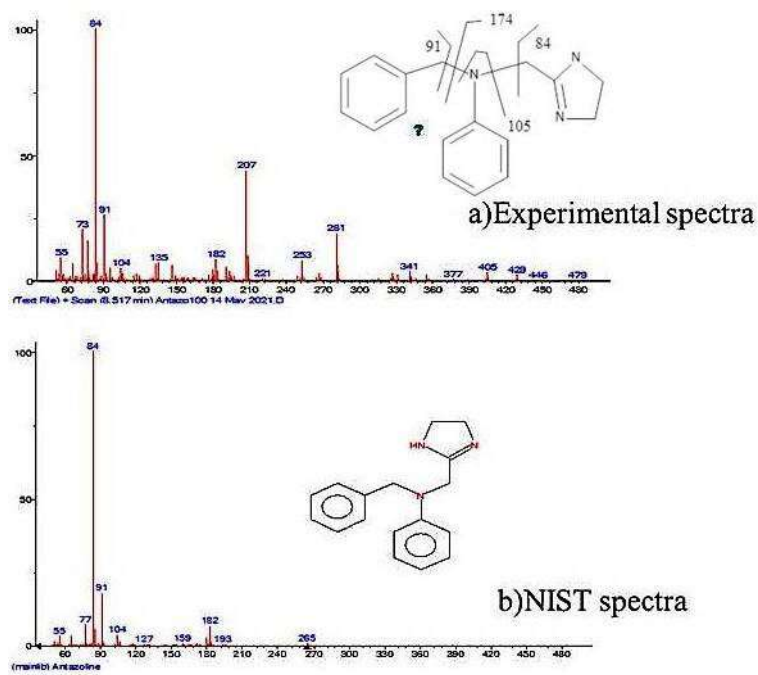


Figure 26: Mass spectra of antazoline in the derivatised mixed standard and the NIST Library spectra

The base peak or quantifier ion m/z 84, and two qualifier ions (m/z 182, m/z 91) for antazoline were used for confirmation. All these ions were observed in Figure 26 which was comparable to the NIST Library spectra as the reference. Likewise, the ion intensities in the mass spectra met the WADA acceptance criteria [91] for reliable confirmation of antazoline except m/z 91, which did not meet the criteria in comparison to the NIST Library spectra. Table 17 shows the comparison of fragment ion intensities according to the antazoline mass spectra.

Table 17: Comparison of diagnostic ion intensities between antazoline mass spectra

Diagnostic ion	Relative Ion Intensities (%)	
	Experimental spectra	NIST spectra
84	99.9	99.9
91	28.1	18.0
182	7.1	6.8

NIST Library ionisation source: EI

Diazepam

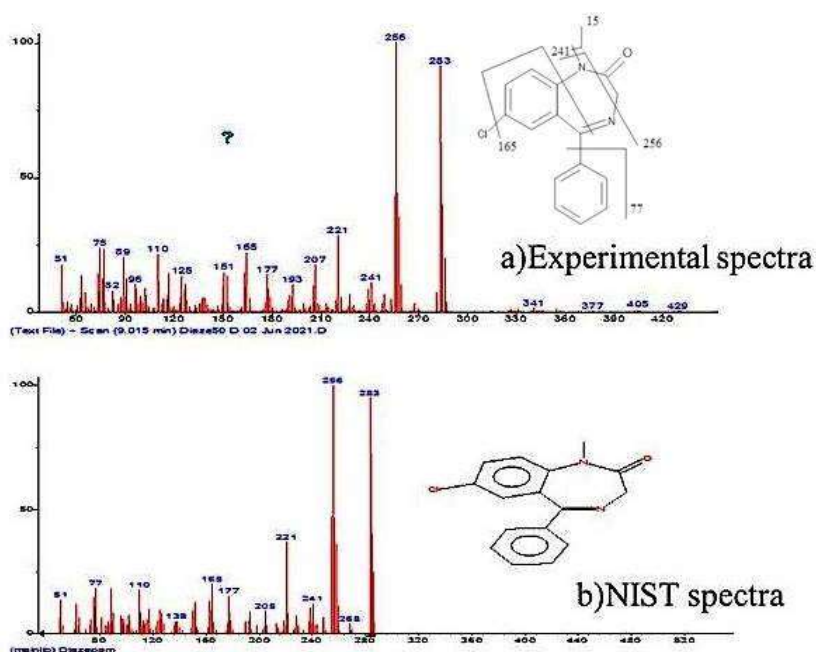


Figure 27: Mass spectra of diazepam in the derivatised mixed standard and the NIST Library spectra

The base peak or quantifier ion (m/z 256), and two qualifier ions (m/z 283, m/z 165) for diazepam [93], [92] were used for confirmation (Figure 27). All the observed diagnostic met the acceptance criteria by not differing by more than $\pm 20\%$ with the relative abundances of the corresponding ions in the NIST Library spectra [91], [97]. Table 18 shows the comparison of diagnostic ion intensities according to both diazepam mass spectra.

Table 18: Comparison of fragment ion intensities between the diazepam mass spectra

Diagnostic ion	Relative Ion Intensities (%)	
	Experimental spectra	NIST spectra
256	99.9	99.9
283	95.6	94.9
165	19.1	20.1

NIST Library ionisation source: EI

The relative abundances reported in tables 15-18 were indicative of the base peak ions having a 99.9% intensity, similar to the reference spectra from the NIST Library. The intensities of the diagnostic ions in the samples were mostly lower than that of the reference spectra, with exceptions of some of the ions, especially with chlorphenamine, however, they successfully confirm the detection of the drugs, within the pharmaceutical drug sample matrix. Most of the diagnostic ions met the acceptance criteria, and the exceptions are due to the difference in analytical conditions between the source of the reference spectra and this study. Also, the molecular ions for chlorphenamine and antazoline were not present in the spectra which was of importance when electron ionisation (EI) was used as the ion source [93]. The absence of molecular ion peak may have been due to interferences from other compounds or interfering background signal [96].

As the derivatised drugs gave better results in terms of detection, peak resolution, shape and intensity, the derivatisation method was optimised to obtain maximum efficiency.

4.2.5 Optimisation of the Derivatisation Method

Optimisation of the derivatisation method was carried out so as to maximise conversion of the analytes (antazoline and bromazepam) to their silylated compounds. The derivatisation condition investigated was reaction time, while reagent volume and the temperature were kept constant at 40

μL and 70°C , respectively. Reaction efficiency was investigated at 15, 30, 45 and 60 minutes. The aim of selecting the high temperature was an attempt to improve the degree of conversion as well as to reduce the derivatising time. The peak area ratios (PARs) of the analytes were used to determine the optimum derivatisation reaction time and they were calculated by dividing the peak area of the drug with the peak area of the internal standard. The PARs of each of the drugs were plotted against the derivatisation reaction time and the results are depicted in Figure 28.

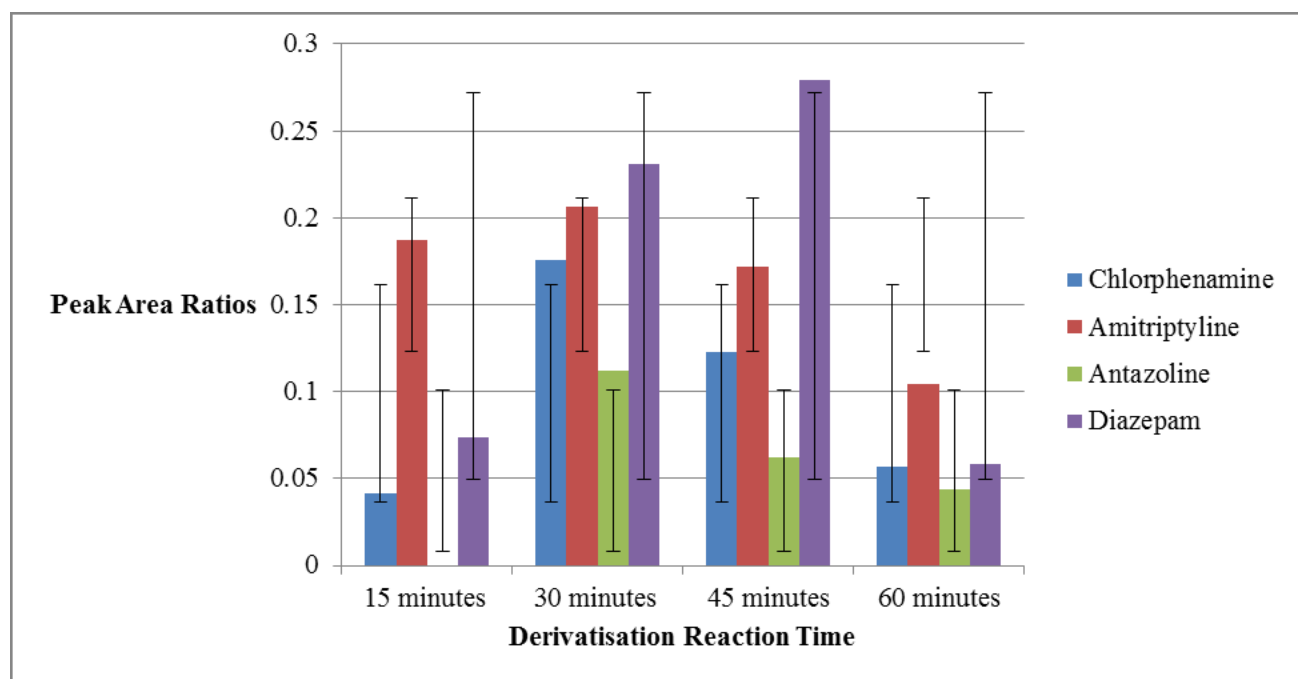


Figure 28: Graph of peak area ratios (PARs) vs derivatisation reaction time

Figure 28 depicts the results of drug derivatisation at 15, 30, 45 and 60 minutes. The derivatisation reaction was regarded as complete when there was no change or increase in peak area with increasing reaction time [75]. In addition, the number of peaks in the chromatogram, their retention time and intensities were used to assess the completion of the derivatisation reaction.

All except one of the drugs i.e. diazepam, exhibited to be fully derivatised after 30 minutes. This was because all the four compounds reach their maximum PAR at 30 minutes after which the PAR decreases (except diazepam), possibly due to degradation. Also, the suitable 30 minutes reaction time indicated improved peak intensity and shape, separation efficiency and chromatographic resolution [72]. Though the peak area and peak area ratio of diazepam increased at 45 minutes reaction time, the peak shapes and resolution were poor compared to the lower reaction time. Based on the results from

The detected drugs, the derivatisation conditions of 70 °C for 30 minutes were selected as the most suitable conditions.

Derivatising the drugs for 15 minutes did not allow for the detection of antazoline, indicating that the analyte was not fully derivatised and therefore undetected, while at increased reaction times the peak intensities of the other analytes decreased indicating low detectability and possible degradation. Derivatising at the increased reaction times also resulted in broader peaks and poor peak shape [100]. A high variability between the data, which is depicted by the large error bars, was also noticed indicating that there may be fluctuations with analyte measurements, possibly introduced by interferences from derivatisation [96]. In one study carried out to optimise the derivatisation reaction for the analysis of different pharmaceutical drugs, including amitriptyline, using BSTFA and 1% TMCS in pyridine and ethyl acetate (2:1:1, v/v/v), heating for 30 minutes at 60 °C was found to be the most suitable derivatisation condition [101].

4.2.6 Detection of the Underivatised Drugs

The purpose of this research was to develop, optimise and validate the analytical method intended to detect and determine the stability of the commonly dispensed antidepressants, benzodiazepines and antihistamines in Botswana. As a derivatisation step was included and optimised due to the derivatising drugs, antazoline and bromazepam, the results showed that derivatisation introduced some extra peaks, a degree of background noise and high variations in the data. Therefore, all the preliminary tests were also carried out without the derivatisation step upon noticing these discrepancies in the data, which may have arisen from derivatisation. This was to evaluate whether extra peaks and noise may be avoided and better quality peaks may be achieved. In this case, antazoline was not included in these preliminary studies as it required derivatisation to be detected using the GC-MS, and it was already out of stock in the country. The detection of the drugs of interest was also studied without the derivatisation step to determine any improvements in detection and quality of the results. The three underivatising drugs chlorphenamine, amitriptyline and diazepam were successfully detected in the underivatised mixed standard at 25 µg/mL. Figure 29 and Table 19 shows the chromatogram of the underivatised mixed standard and the individual analytes with their retention times.

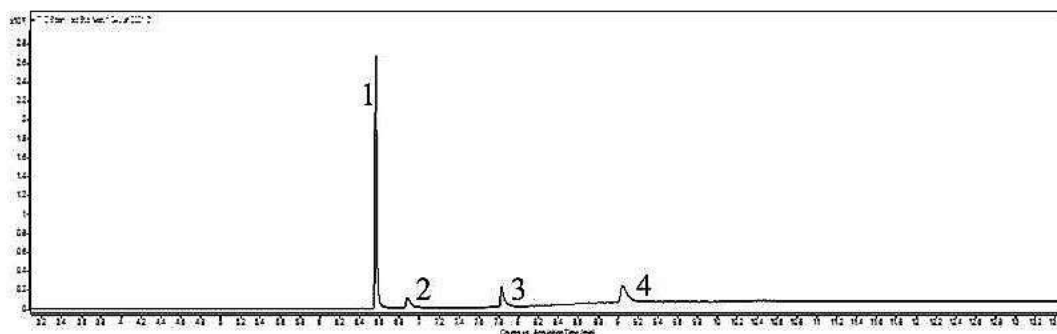


Figure 29: Chromatogram of the underivatised mixed drug standard at 25µg/mL (1-Internal Standard, 2-Chlorphenamine, 3-Amitriptyline, 4-Diazepam)

Table 19: Retention times and relative retention times of the analytes in the underivatised mixed drug standard

Analyte	Retention Time (mins)	Relative Retention Time (RRT)
Internal Standard	6.641	-
Chlorphenamine	6.948	1.05
Amitriptyline	7.904	1.19
Diazepam	9.061	1.36

Analytes detected at 25 ug/mL concentration

Relative Retention Time (RRT) = $RT_{\text{analyte}} / RT_{\text{internal standard}}$; RT: Retention time

As shown in Figure 29, the three drugs were detected underivatised at a concentration of 25 µg/mL, while initially they were only detectable at a concentration of 50 µg/mL. The RRTs remained largely unchanged further confirming identification of the drugs. The observed difference in instrumental sensitivity may have been due to the regular maintenance and replacement of instrument hardware overtime (liners, septum, column). The column for instance was changed (with a similar make-up) and if the GC column is replaced with another column of the same properties, slight variations of the retention times are expected [102]. Though this was a result of instrument maintenance, the outcomes were positive for the research as the minimum detectable concentration of the drugs was lower. Evidently, without derivatisation, detection and confirmation significantly improved as there were no co-elutions or interferences in the sample matrix. This was shown by the lack of extra peaks in the chromatogram in Figure 29 and figure 23A, compared to Figure 23B of the derivatised mixed drug standard. All the analyte peaks were separated and distinguishable from each other. Similarly, the

detected underivatised drugs were then confirmed using the mass spectra and the selected diagnostic ions.

4.2.7 Confirmation of the Underivatised Drugs

The mass spectra of the underivatised drugs and reference spectra corresponded, confirming that the detected drugs are the drugs of interest. Appendix IV shows the mass spectra of chlorphenamine in the underivatised mixed standard, comparing it to the reference spectra, as an example. To further confirm the detected drugs, Table 20 shows the confirmation ions of each of the drugs as well as comparison of the relative ion intensities.

The base peaks and two confirmatory ions for each of the three drugs were used for confirmation. All the quantifier and qualifier ions were also observed in the spectra listed in appendix IV and were comparable to the NIST Library as the reference spectra. Table 20 shows the comparison of fragment ion intensities according to both the mass spectra of the underivatised drugs and the reference from the library. The diagnostic ions met the acceptance criteria, except chlorphenamine (m/z 58), which did not meet the criteria according to WADA guidelines [91], [97]. This is also the case for confirmation of derivatised drugs in section 4.2.4, the m/z 58 of chlorphenamine was out of the acceptance criteria. Amitriptyline (m/z 202) also exceeded the acceptance criteria in the underivatised mixed drug standard.

Table 20: Comparison of diagnostic ion intensities between the analytes' mass spectra

Fragment ion/Peak	Relative Ion Intensities (%)	
	Drug Standard Spectra	Reference spectra
Chlorphenamine		
203	99.9	99.9
167	17.2	20.7
58	26.1	42.7
Amitriptyline		
58	99.9	99.9
202	7.0	5.0
91	1.9	1.7
Diazepam		
256	99.9	99.9
283	93.6	94.9
165	16.5	20.1

The relative abundances of each of the fragment ions in the samples reported on Table 20 correlated with those from the NIST Library, at the same ions abundance. Though some fragment ions had high abundances similar to confirmatory ions of the derivatised drugs, the intensities were quite similar. However, higher relative abundances of the confirmation ions as well as more distinct mass spectral patterns were observed for the derivatising drugs (Tables 15-18). This can therefore lead to more reliable positive identification of analytes in complex matrices for the derivatised drugs.

The data presented shows that chlorphenamine, amitriptyline, and diazepam were all detectable when derivatised and underderivatised at minimum concentrations of 50 µg/mL and 25 µg/mL, respectively. Antazoline, which was not detectable without derivatisation, was detected at a minimum concentration of 75 µg/mL when derivatised. Therefore, mixed drug standards were to be prepared at a concentration of 100 µg/mL, considering the minimum detectable concentration of antazoline as well as to make up for extraction losses during LLE. According to the mass spectra, the three drugs were detected at similar concentrations when derivatised and when not derivatised.

4.2.8 Method Validation for Qualitative Analysis

4.2.8.1 Precision Studies

Precision was assessed for the four drugs using GC-MS. Both intra-assay and inter-assay precision of the derivatised and underivatised drugs (prior to subsequent removal of the derivatising step) were assessed and the results are discussed in the following sections.

4.2.8.2 Intra-assay precision for the Derivatised Drugs

The intra-assay precision of the instrument was evaluated at a lower (75 µg/mL) and higher (100 µg/mL) drug concentration, due to the minimum detectable concentration of antazoline, as described in section 4.2.6. Table 21 shows the RSDs calculated from the mixed drug standards analysis from different vials across the two concentrations, over 6 hours.

Table 21: Intra-assay precision

Drug	Intra-assay precision, n=4			
	75 µg/mL		100 µg/mL	
	Average PAR	RSD (%)	Average PAR	RSD (%)
Chlorphenamine	0.14	21.25	0.25	27.48
Amitriptyline	0.28	7.18	0.46	3.60
Antazoline	0.05	14.99	0.09	7.41
Diazepam	0.28	9.58	0.52	5.87

Diazepam, amitriptyline and antazoline across the two concentrations yielded RSDs well below the ≤ 15% criteria normally set for higher concentrations and the ≤20% criteria normally set for lower concentration ranges, except chlorphenamine at both concentrations. This was indicative of good repeatability of the method for these drugs, according to The Federal Drug Agency (FDA) and the Scientific working group for forensic toxicology (SWGTOX) standard practices for method validation in forensic toxicology [66], [69], [77]. The RSDs of the drugs at 75 µg/mL ranged from 7.18% to 21.25% and at 100 µg/mL, the RSDs ranged from 3.60% to 27.48%. Three of the four drugs showed good repeatability and reproducibility as the RSDs were below the ≤15% criteria normally set for higher concentrations. The RSDs for diazepam at 75 and 100 µg/mL were 9.58% and 5.87%,

respectively. Antazoline showed 14.99% and 7.41% RSDs at 75 and 100 µg/mL, respectively. The RSDs at 75 and 100 µg/mL for amitriptyline were 7.18 and 3.60 %, respectively. Chlorphenamine, which exhibited RSDs that did not meet the criteria for good repeatability according to the Federal Drug Agency, had RSDs of 21.25 and 27.48% at 75 and 100 µg/mL, respectively. The higher RSDs may have been due to higher fluctuations in detector response and degradation reactions [103]. Also, the RSDs are noticeably higher at 75 µg/mL compared with the higher concentration, 100 µg/mL, suggesting that there is good precision at the higher concentration and poor precision at the lower concentration for diazepam, amitriptyline and antazoline. These data correlates with the Horwitz curve theory, which suggests that as the concentration of an analyte decreases, the RSD is likely to increase [103], [104]. This may be because random errors and background noise usually increase with a decrease in concentration [95], [103]. The Horwitz function may however not necessarily cover every possible outcome in analytical science, but it is widely used in research [103].

All the drugs across the two concentrations indicated good precision except chlorphenamine. The high RSDs, especially for chlorphenamine, could be due to the drug undergoing degradation reactions or hydrolysis of the drug. They could also be attributed to errors introduced during the sample preparations as well as interfering co-eluent [35], [96], as it was described in sections 4.2.4 and 4.2.7 in confirming chlorphenamine, that the relative intensity of the diagnostic ion m/z 58, exceeded the acceptance criteria for both the derivatised and underivatised drugs. This could be suggesting that there are interferences with the detection and/or confirmation of chlorphenamine thus affecting the precision.

4.2.8.3 Inter-assay Precision for the Derivatised Drugs

The inter-assay precision of the instrument was also evaluated at low (75 µg/mL) and high (100 µg/mL) drug concentrations. Table 22 shows the RSDs calculated from analysing the mixed drug standards from different vials across the two concentrations, over three days.

Table 22: Inter-assay precision

Drug	Intra-assay precision, n=3			
	75 µg/mL		100 µg/mL	
	Average PAR	RSD (%)	Average PAR	RSD (%)
Chlorphenamine	0.10	28.34	0.22	22.84
Amitriptyline	0.24	17.00	0.39	18.59
Antazoline	0.04	23.46	0.07	20.76
Diazepam	0.20	38.65	0.50	4.83

In assessing the inter-assay precision of the derivatised drugs, somewhat high RSDs for all the drugs were observed, indicating poor inter-assay precision for the drugs. Likewise, the data correlated with the Horwitz curve theory [104] as the RSDs for the higher concentration, 100 µg/mL RSDs were mostly lower than those obtained at 75 µg/mL, except for chlorphenamine. The RSDs for chlorphenamine were 28.34 and 22.84% at 75 and 100 µg/mL respectively, amitriptyline had RSDs of 17.00 and 18.59% at 75 and 100 µg/mL, respectively. The RSDs of antazoline were 23.46 and 20.76% at 75 and 100 µg/mL, respectively. Lastly, diazepam had a very high RSD of 38.65% at 75 µg/mL and a lower RSD of 4.83% at 100 µg/mL. Diazepam showed good precision at the higher concentration and showed poor precision at the lower concentration. On the other hand, amitriptyline showed good precision at the lower concentration (75 µg/mL) but showed poor precision at 100 µg/mL, with an RSD of 18.59%. All the drugs i.e. diazepam, chlorphenamine, amitriptyline and antazoline across the two concentrations yielded RSDs over the $\leq 15\%$ criteria normally set for higher concentrations and the $\leq 20\%$ criteria normally set for lower concentration ranges [95], except diazepam and amitriptyline at 100 µg/mL and 75 µg/mL, respectively. This was indicating poor repeatability of the method for these drugs, according to Scientific working group for forensic toxicology (SWGTOX) standard practices for method validation in forensic toxicology and The Federal Drug Agency (FDA) [66], [69], [77].

Similar studies carried out to determine the precision of drugs report that the methods and analytes exhibit good precision within the acceptable criteria [32], [59], [105], however, that was not the case in this study. The poor inter-assay precision of the drugs especially at 75 µg/mL, may be attributed to minimum detectable concentrations of the drugs being high. The drugs were detected at minimum concentrations of 50 and 75 µg/mL and hence this may have been introducing high fluctuations in

detector response [96], as precision decreases as concentration approached the limit of detection [103]. In addition, there seems to be interferences from co-elutions and high fluctuation with analysis of the derivatised drugs (Figure 23B) [106]. Despite the poor inter-assay precision of the drugs, the precision of the method can be considered consistent, since it was tested on low concentrations of the analytes with regards to instrument sensitivity and based on the minimum detectable concentrations.

The drugs or analytes requiring derivatisation i.e. bromazepam and antazoline, were already removed from the study due to shortage of pharmaceutical stock in the country, and so the derivatisation step was removed prior to proceeding to precision studies and auto-sampler stability with the remaining underivatised drugs in sections 4.2.8.4 and 4.2.9.2. Since bromazepam and antazoline were removed as target analytes, the derivatisation step was removed from the method, which also saves time. This is because diazepam, chlorphenamine and amitriptyline, the available drugs, do not require derivatisation and so could be analysed underivatised.

4.2.8.4 Intra-Assay Precision for the Underivatised Drugs

The validation parameters were also conducted with only diazepam, amitriptyline and chlorphenamine (without derivatisation). Precision was assessed for the three drugs using the GC-MS. Both intra-assay (Table 23) and inter-assay precision (Table 24) were assessed.

The intra-assay precision of the instrument was evaluated at low (25 µg/mL), medium (75 µg/mL) and high (125 µg/mL) drug concentrations, as the minimum detectable concentration of the three drugs improved due to factors described in section 4.2.6. Table 23 shows the RSDs calculated from the analysis of the mixed drug standards from different vials across the three concentrations, on the same day.

Table 23: Intra-assay precision

Drug	Intra-assay precision, n=6					
	25 µg/mL		75 µg/mL		125 µg/mL	
	Average PAR	RSD (%)	Average PAR	RSD (%)	Average PAR	RSD (%)
Chlorphenamine	0.23	13.31	0.29	13.20	0.66	3.18
Amitriptyline	0.31	8.67	0.40	12.93	0.83	5.74
Diazepam	0.41	8.99	0.54	11.92	1.17	4.87

The RSDs for chlorphenamine analysed at 25, 75 and 125 µg/mL were 13.31, 13.20 and 3.18%, respectively. Amitriptyline yielded RSDs of 8.67, 12.93 and 5.74% at 25, 75 and 125 µg/mL, respectively. While diazepam RSDs at 25, 75 and 125 µg/mL were 8.99, 11.92 and 4.87%, respectively.

All the RSDs across the three concentrations were well below the $\leq 15\%$ criteria normally set for higher concentrations and the $\leq 20\%$ criteria set for lower concentrations, indicating good repeatability of the method for the three drugs [95]. Therefore, RSDs for the drugs across the three concentrations indicated good precision.

The data also shows that RSDs at 25 µg/mL, the lowest concentration assessed, were higher than RSDs at 125 µg/mL (Table 23), the highest concentration assessed for all the three drugs. This corresponds with the Horwitz curve theory [104]. The RSDs obtained at 125 µg/mL were all below 6%, while the RSDs obtained at 25 µg/mL ranged from 8.67% to 13.31%. Thus the data suggested that precision slightly declined as the concentration approached the limit of detection [103].

4.2.8.5 Inter-assay precision for the Underivatised Drugs

The inter-assay precision of the instrument was also evaluated at low (25 µg/mL), medium (75 µg/mL) and high (125 µg/mL) drug concentrations. Table 24 shows the RSDs calculated from analysing the mixed drug standards from different vials across the three concentrations, over three different days.

Table 24: Inter-assay precision

Drug	Inter-assay, n=3					
	25 µg/mL		75 µg/mL		125 µg/mL	
	Average PAR	RSD (%)	Average PAR	RSD (%)	Average PAR	RSD (%)
Chlorphenamine	0.24	2.66	0.31	5.65	0.64	2.52
Amitriptyline	0.32	3.33	0.43	5.31	0.81	2.30
Diazepam	0.45	6.60	0.58	6.74	1.11	3.70

All of the RSDs across the three concentrations were well below the $\leq 15\%$ criteria normally set for higher concentrations and the $\leq 20\%$ criteria normally set for lower concentrations [95], [107]. The

RSDs ranged from 2.30-6.74% for all the drugs across the three concentrations. Similarly, lower RSDs were obtained at the higher concentration of 125 µg/mL than at 25 µg/mL, however this trend is not observed at 75 µg/mL as the RSDs were higher than those at 25 µg/mL. This corresponds with the Horwitz theory [103]. The RSDs obtained at 125 µg/mL were all below 4%. The Scientific working group for forensic toxicology (SWGTOX) standard practices for method validation in forensic toxicology also state that the RSDs should be within the $\leq 20\%$ criteria, and the data meets this criteria [77].

In comparison, the drugs show better precision when they were not derivatised than when they were derivatised. This suggested that derivatisation introduces fluctuations in the analysis of the drugs, probably due to the presence of artefacts in the sample matrix, as shown by the chromatogram in Figure 23B. The intra-assay precision of the underivatised drugs (chlorphenamine, amitriptyline and diazepam) ranged from 2.30 to 13.31%, and that of the same drugs derivatised ranged from 4.83 to 38.65%. This shows that higher RSDs were obtained from the same drugs when they were derivatised than when they were not derivatised. Also, for the common mixed drug standard concentration between the two studies, 75 µg/mL, the underivatised drugs yielded lower RSDs in both the inter-assay and intra-assay precision compared to the derivatised drugs which yielded higher RSDs, as shown in Tables 21-24. This correlates with earlier discussions in sections 4.2.4 and 4.2.6, where derivatised samples led to poorer results than underivatised samples since derivatisation introduced coelution, artefacts and background noise. However, in both studies the data correlated with the Horwitz cure theory. Overall, amitriptyline showed better precision and chlorphenamine showed less precision in both studies.

4.2.9 Auto-Sampler Stability

Likewise, the drugs were assessed for their auto-sampler stability under the two modes of reconstitution or preparation (derivatised and underivatised drugs).

4.2.9.1 Auto-Sampler Stability of the Derivatised Drugs

The 24-hour auto-sampler stability of the derivatised drugs was determined for chlorphenamine, amitriptyline, antazoline and diazepam, and Figure 30 shows the results.

All the drugs showed that they were stable on the auto-sampler, except antazoline which was the only derivatisable drug. Though derivatisation should improve stability, derivatised antazoline was not stable on the auto-sampler for 24 hours. While research and data is available on the stability of

antazoline in biological specimen [108], [109], little research has been carried out with regards to the analysis and stability of antazoline in alcoholic beverages, to the knowledge of the author.

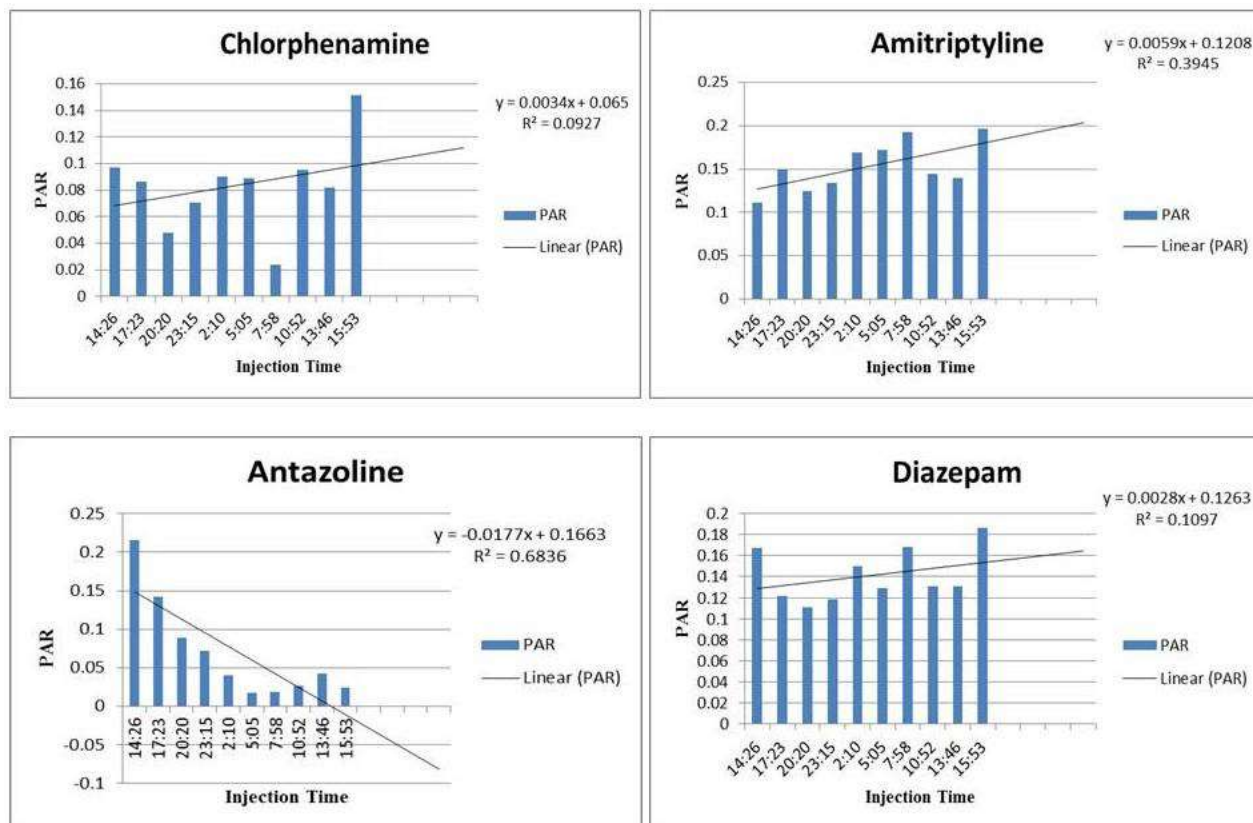


Figure 30: Graphs of auto-sampler stability for the individual derivatised drugs

The PARs of each of the analytes in the analysed mixed drug standards over the 24 hours were used to generate bar graphs as illustrated on Figure 30 above, and the gradients or slopes were extracted from the respective graphs. The change in PAR from time point zero was used to evaluate the stability. Here, instability was indicated by a negative slope, significantly different from zero ($p \leq 0.05$). The slopes from all the drugs, except one, were not significantly different from zero ($p \leq 0.05$) indicating stability during the analysis period. The slopes were 0.0034, 0.0059 and 0.0028 for chlorphenamine, amitriptyline and diazepam, respectively. However, antazoline had a slope significantly different from zero (-0.0177), indicating instability [85]. This was also indicated by the higher correlation coefficient (0.6836) compared to the other three drugs. Figure 30 shows the slope and correlation coefficient (R^2) from the linear regression equations.

Most of the fluctuations for the four drugs were observed between 20:20 and 7:58 (injections after about 6 hours), indicating less stability during this period. Antazoline, which was unstable, possibly undergoes degradation reactions during sample preparation and analysis, due to temperature and moisture changes [108]. In a study carried out to determine the stability of antazoline in human plasma [109], antazoline solutions were found to be stable under laboratory bench conditions (20 °C) and under different storage conditions (4 °C and -20 °C). Antazoline was also stable in the autosampler for at least 24 h, when extracted with ethyl acetate and reconstituted in aqueous methanol. The data obtained was in contrast to the results obtained in this study [109]. As the derivatising drug in the mixed standard, it may be incompletely derivatised or unstable in the conditions under which it is studied. In addition, the results of a study on the analysis of antazoline and tetryzoline hydrochloride (a similar drug in terms of chemical structure) indicated that the drugs are susceptible to degradation, to different extents under the different conditions in aqueous solutions [108].

In conclusion, based on these results, for overnight runs on the GC-MS instrument, sample vials of derivatised drugs should be kept refrigerated and put on the auto-sampler to ensure that they would not stand for more than 20 hours before injection.

4.2.9.2 Auto-Sampler Stability of the Underivatised Drugs

The 24-hour auto-sampler stability was also assessed for the underivatised drugs, chlorphenamine, amitriptyline and diazepam and Figure 31 shows the results.

The slopes generated from regression analysis for all the drugs were not significantly different from zero ($p \leq 0.05$) indicating stability during the analysis period. The slopes were 0.0009, 0.0117 and 0.0003 for chlorphenamine, amitriptyline and diazepam, respectively. High fluctuations were observed between 5:21 and 18:17 (injections after about 11 hours), for chlorphenamine and amitriptyline, indicating less stability during this period. This may have been due to instrumental variation such as detector response variations and temperature changes, leading to high deviations in the peak areas of the analytes [96]. These results correspond to the precision studies results, showing that the underivatised drugs exhibit good precision, are stable, portray minimal fluctuations and less degradation during analysis. In conclusion, based on these results, for overnight runs on the GC-MS instrument, sample vials of underivatised drugs should be kept refrigerated and put on the auto-sampler to ensure that they would not stand for more than 24 hours before injection.

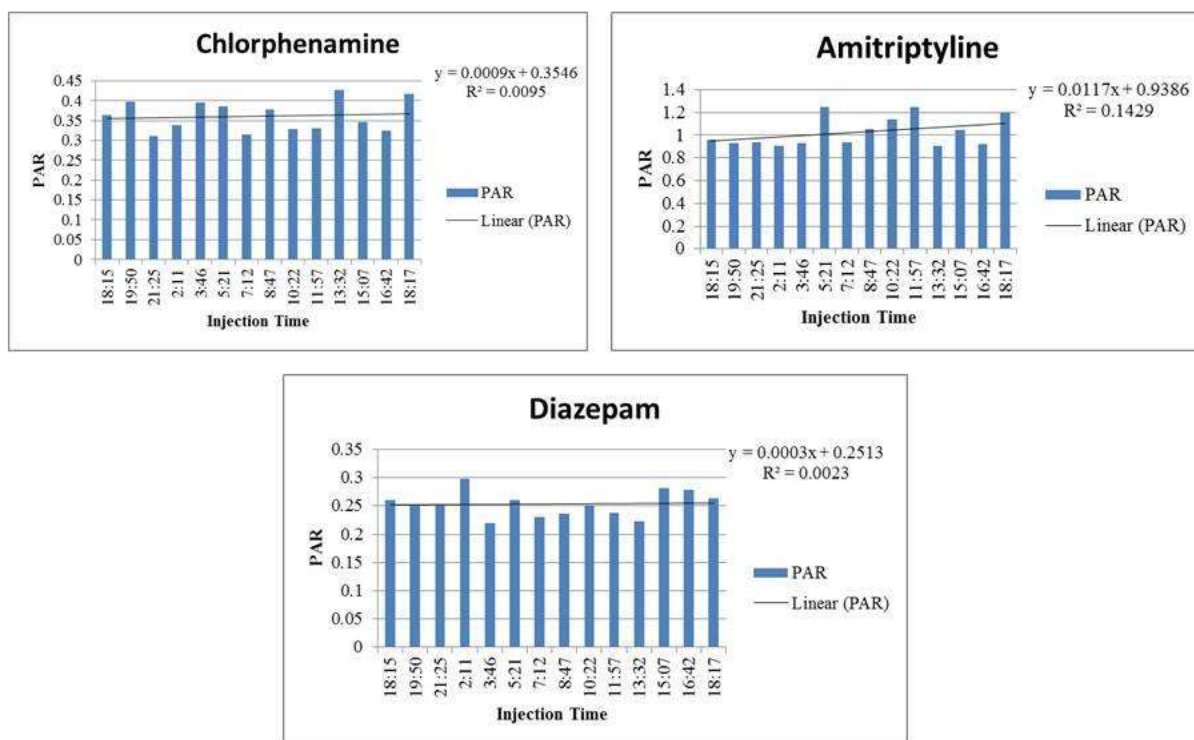


Figure 31: Graphs of auto-sampler stability for the individual underivatised drugs

In addition to derivatisation, the method used to extract the drugs from the sample matrix plays an important role in the reliable detection and confirmation of the drugs. Therefore, the next step in method optimisation was to develop and optimise a liquid-liquid extraction method.

4.2.9.3 Comparison of the Derivatised and Underivatised Drugs

The drugs or analytes requiring derivatisation i.e. antazoline and bromazepam, were subsequently removed from the study due to shortage of pharmaceutical stock in the country. However, antazoline was included in all the preliminary studies on derivatised drugs before it went out of stock. Based on the comparison between derivatised and underivatised drugs as discussed in sections 4.2.6, 4.2.7, 4.2.8 and 4.2.9.2., underivatised drugs gave better chromatographic and mass spectral data. Therefore all studies were to be repeated without the derivatisation step for chlorphenamine, amitriptyline and diazepam. However, due to unforeseen circumstances relating to a sudden lack of access to a GC-MS at the study institution and other local organisations during the process of conducting the underivatised drugs studies, the solvent selection and storage stability studies could not be completed with the underivatised drugs. Since the solvent selection and storage stability studies had already been carried out for the derivatised drugs, the data is presented in the following sections.

4.2.10 Sample Preparation

4.2.10.1 Solvent Selection: Analyte Extraction/Recovery from Alcoholic Beverages

The LLE method used was optimised by investigating the extraction solvents. The analysis of the derivatised samples was carried out in triplicate so as to validate the obtained results through measuring and comparing the relative differences between the data. The average peak area ratios of the triplicate samples were used to determine the %recovery with each solvent system. The results from the comparison of chloroform: isopropanol (CHCl₃:IPA, 2:1 v/v) and diethyl ether as extraction solvents are reported in Table 25 below.

Equation 1 (calculation of the %recovery) [110], was used to calculate the %recovery of the drugs after extraction. The unextracted sample, analysed alongside the extracted samples, represents 100 % recovery and has higher peak areas than the extracted samples [111].

Equation 1: Calculation of the %Recovery

$$\%Recovery = \left[\frac{\text{Peak Area Ratio of Extracted Sample}}{\text{Peak Area Ratio of Unextracted Sample}} \right] \times 100$$

Table 25: The average %recoveries for the analytes in the two solvent systems

Analyte	Average %Recovery ±SD	
	ClCH ₃ :IPA	Diethyl Ether
Chlorphenamine	20.80 ±0.15	0.81 ±0.01
Amitriptyline	75.78 ±0.02	NE
Antazoline	45.61 ±0.21	NE
Diazepam	79.57 ±0.44	16.44 ±0.13

NE: Not extracted

According to the data reported in Table 25, the extraction solvent system chloroform: isopropanol (2:1, v/v) gave better recoveries of the drugs of interest. All the drugs were extracted and detected

with chloroform: isopropanol while only chlorphenamine and diazepam were extracted and detected using diethyl ether as the extraction solvent. This may have been due to inability of the analytes to transfer to the organic phase, interferences from co-elution with other compounds either in the sample matrix or in the extraction solvent.

Diethyl ether yielded a recovery of 0.81% for chlorphenamine and 16.44% for diazepam. These poor recoveries may be due to these drugs having poor affinity to diethyl ether due to weak molecular interactions between the analytes and the solvent phase, thus limiting extraction [35], [64], [66], [112]. Also, the low recoveries of the extracted drugs could be that in addition to the aforementioned, the drugs underwent degradation reactions or hydrolysis in diethyl ether thereby changing into different compounds and consequently reducing the recovery [86]. According to [86], diethyl ether is also disadvantaged by its volatility as an extraction solvent and possible adsorption of the drugs of interest onto the glassware may have contributed to the low %recoveries. Chloroform:isopropanol (2:1) yielded recoveries of 20.80, 75.78, 45.61 and 79.57% for chlorphenamine, amitriptyline, antazoline and diazepam, respectively. The low recoveries of chlorphenamine and antazoline could have been due to analyte loss during sample preparation; when evaporating the solvent as well as when transferring samples between vials, or contributions to the analyte from the derivatising agent and incomplete reactions [71]. Antazoline and chlorphenamine according to published studies possibly undergo degradation reactions in acidic aqueous solutions [108], [113], and wine which was used to determine the %recovery or extraction efficiency had a pH of 3.50-3.56, which is acidic. Therefore some of the analyte could have been lost from sample preparation to analysis. In one study carried out to determine the degradation mechanisms of antazoline in acidic media, the overall degradation of antazoline was found to be dependent on the pH. In the pH range of 3.0–7.4, the formation of an antazoline hydrolysis product through three different pathways (acidic, non-catalysed, and semi-alkaline hydrolysis) was observed [114]. Diazepam and amitriptyline gave higher %recoveries in chloroform:isopropanol possibly because they have more affinity to the solvents compared to the other two drugs, especially chlorphenamine which gave the lowest %recovery (20.80%) in this solvent system.

The findings from this research correlate with published results as reported by other researchers, whereby different drugs have higher recoveries in the different solvents [115], [116]. So, chloroform: isopropanol (2:1, v/v) was selected as the best solvent system to use for this research as it was able to extract all the drugs with better %recovery than diethyl ether. In a reported study [86], chloroform

and 2-propanol (4:1, v/v) was a good solvent system for the extraction of benzodiazepines in beverages. The solvent system yielded recoveries between $89.8 \pm 0.3\%$ and $102.1 \pm 5.2\%$ for benzodiazepines using a high performance liquid chromatography method coupled with diode-array detection. Diazepam %recoveries in particular ranged from 84-99%, depending on the beverage from which it was extracted [86]. In addition, chloroform: isopropanol have shown to be a good solvent system for the extraction of most benzodiazepines, antihistamines and anti-depressants in other studies [37], [67], [86].

In addition, LLE conditions do not always favour all the drugs or analytes in a multianalyte method, therefore some analytes will have lower recoveries due to unfavourable pH conditions and solvent combinations [117]. As in this case the pKa values of chlorphenamine, amitriptyline and diazepam are different and thus the extraction efficiency would differ in the different beverages. In addition, the sample matrix, sample preparation and analyte type could also affect results. This could explain why the intra- and inter-assay precision studies also exhibited different RSDs values, with higher RSDs, indicating poor precision, especially for chlorphenamine. The factors affecting the %recovery of these drugs were not studied further in this research.

4.2.11 Storage Stability Studies

The stability of the three remaining drugs in the four alcoholic beverages was studied over 28 days and this subsection presents the results.

4.2.11.1 Drug Analytes Detection in the Alcoholic Beverages

The retention times and retention indexes of the derivatised analytes in the four alcoholic beverages were noted and in Table 26. Solvent blanks and unspiked alcoholic beverages were analysed to ensure there was no carryover between sample injections. The chromatograms showed that the drugs were not present in the solvent blanks and unspiked alcoholic beverages, indicating that the peaks of interest were only produced by the spiked analytes of interest, and that there was no matrix interference.

Table 26: Retention Times and Relative Retention Times of the Drugs in the Different Beverages

Beverage	Chlorphenamine RT ±SD	RRT	Amitriptyline RT ±SD	RRT	Diazepam RT ±SD	RRT
Derivatised mixed drug standard	6.870	1.05	7.814	1.19	9.032	1.38
Chibuku	6.996 ±0.005	1.03	7.922 ±0.007	1.18	9.109 ±0.004	1.36
Wine	6.996 ±0.005	1.03	7.922 ±0.007	1.18	9.103 ±0.004	1.36
Vodka	6.996 ±0.005	1.03	7.922 ±0.007	1.18	9.109 ±0.004	1.37
Savanna	6.996 ±0.006	1.03	7.910 ±0.006	1.18	9.114 ±0.005	1.36

Relative Retention Time (RRT) = $RT_{\text{analyte}}/RT_{\text{internal standard}}$; RT: Retention time

Table 26 shows that the retention times and relative retention times are **indicative** of successful detection of the drugs in the alcoholic beverages as they are similar to those observed in the mixed drug standard detection study, with slight variations of 0.01 and 0.02 from the RRTs (Tables 14 and 16). According to the WADA guidelines, the RRT of the analyte in the sample should be within ±0.5% of the same analyte in the reference [91]. The guidelines also state that the RT of an analyte should be within ±1%, or ±0.1 minute of the same analyte in the reference. The RTs of the analytes or drugs in the alcoholic beverages are within the stated criteria for all the drugs across all the beverages, not differing by more than 0.1 minute [91]. However, the RRTs of the analytes in the derivatised mixed drug standard slightly differ from those of the analytes in the alcoholic beverages as shown in Table 26. The RRTs in Table 26 deviated from the acceptance criteria for chlorphenamine, diazepam and amitriptyline. These slight deviations in RRTs may have been introduced by interferences due to extra compounds and peaks arising from the different alcoholic beverages. As the drugs were derivatised, Figure 32 showed extra peaks as it was also observed for the derivatised mixed drug standard in Figure 23B.

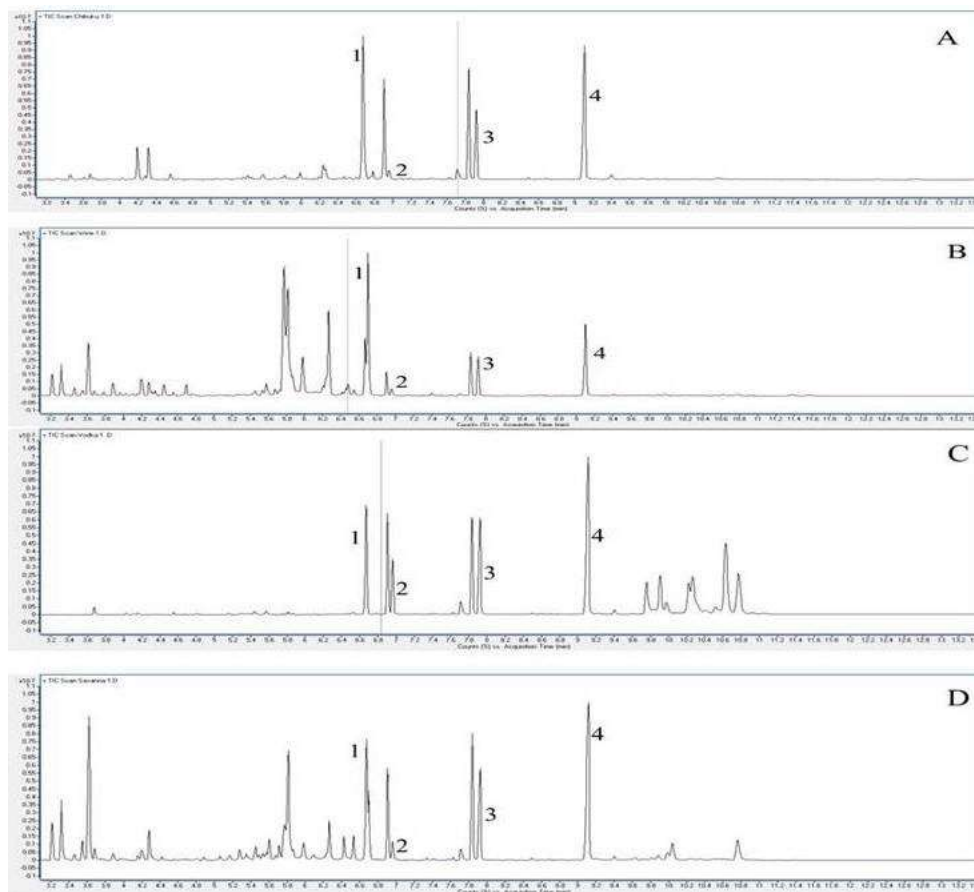


Figure 32: GC chromatograms of the analytes in the alcoholic beverages (1-Internal Standard, 2-Chlorphenamine, 3-Amitriptyline, 4-Diazepam)

A-Chibuku, B-Wine, C-Vodka, D-Savanna

The mass spectrometry data shown in Table 27 further confirmed the detection of the three drugs within the spiked alcoholic beverages as it correlates with the data in Tables 15-18. Most of the diagnostic ion intensities also met the acceptance stated by the WADA guidelines and the Standard for Mass Spectral Data Acceptance in Forensic Toxicology [91], [97], [98] as the relative abundances were within $\pm 20\%$ of the abundances obtained from the NIST Library spectra, except particularly

chlorphenamine (m/z 58), which exceeded the criteria limits significantly across the four beverages, which is also the case in the confirmation of the analyte in section 4.2.4.

Table 27: Mass Spectrometry Data for the Three Drugs in the Beverages

Drug	Diagnostic ions	NIST Library	Chibuku	Wine	Vodka	Savanna
Chlorphenamine	203	99.9	99.9	99.9	99.9	99.9
	167	26.0	18.2	18.0	17.9	18.3
	58	61.9	24.8	27.1	23.3	26.9
Amitriptyline	58	99.9	99.9	99.9	99.9	99.9
	202	5.0	6.3	8.3	8.8	6.9
	91	1.7	1.8	2.0	2.2	2.0
Diazepam	256	99.9	99.9	99.9	99.9	99.9
	283	94.9	94.0	94.8	94.4	94.7
	165	20.1	14.9	14.7	14.4	14.6

4.2.11.2 Drugs stability in the alcoholic beverages

Table 28 shows the stability of the derivatised drugs over the study period. The data shows that all the drugs were detected under both storage conditions, at room temperature and in the freezer (-18 °C), throughout the study period. The change in PARs from analysis time point day 0 to analysis time point day 28 was used to evaluate the stability of the drugs in the alcoholic beverages. Equation 2 was used to calculate the %loss of the analyte or drug over the 28-day period. A loss of <15% was considered stable, a loss of $\geq 15\%$ – $\leq 30\%$ was considered moderately stable and a loss of $>30\%$ was considered unstable [118]. The stable drugs were denoted 1, the moderately stable were denoted 2 and the unstable drugs were denoted 3.

Equation 2:

$$\%Loss = \left[\frac{[Average Peak Area Ratio (Day 0) - Average Peak Area Ratio (Day 28)]}{Average Peak Area (Day 0)} \right] \times 100$$

Table 28: Stability of the drugs in the different beverages at different temperatures

Drug	Beverage	Room Temperature (%loss)	Stability	Freezer (%loss)	Stability
Chlorphenamine	Chibuku	23.72	3	3.30	1
	Wine	80.34	3	37.32	3
	Vodka	79.35	3	53.41	3
	Savanna	-44.94	3	8.88	1
Amitriptyline	Chibuku	61.79	3	3.53	1
	Wine	45.32	3	22.64	2
	Vodka	8.46	1	3.63	1
	Savanna	8.87	1	1.92	1
Diazepam	Chibuku	71.75	3	12.28	1
	Wine	43.0	3	28.69	2
	Vodka	1.19	1	9.86	2
	Savanna	34.77	3	29.44	2

1-stable, 2-moderately stable, 3-unstable

All the three drugs were consistently detected and confirmed throughout the 28-day study period. The results in Table 28 show that there was a difference between the stability of the three drugs in different beverages at the two storage conditions. Amitriptyline and diazepam showed to be more stable than chlorphenamine. The drugs in the beverages stored at -18°C exhibited more stability than those stored at room temperature, as %losses at room temperature and in the freezer ranged from 1.19-80.34% and 1.92-53.41%, respectively. The %loss for chlorphenamine in the freezer ranged from 3.30-53.41%, while the %loss of amitriptyline ranged from 1.92-22.64%, while diazepam ranged from 12.28-29.44% in the freezer. Both amitriptyline and diazepam were stable to moderately stable when stored in the freezer, while chlorphenamine was unstable in wine and vodka, but stable in chibuku and savanna. In comparison to similar studies carried out to determine the stability of benzodiazepines in alcoholic [an alcopop (flavoured alcoholic drink), a beer, a white wine, a spirit] a and non-alcoholic beverages [fruit based non-alcoholic drink (J2O)], diazepam was found to be stable in all of the beverages over a 25-day study period, except in one non-alcoholic drink, under both storage conditions [37]. The other benzodiazepines, which were not included in this study, were

found to be unstable in all the beverages at both storage conditions throughout the study period. The difference in the stability of drugs in this study may be owing to uncertainties associated with instrumental variation and imprecision of the method or derivatised analyte [96]. In another study based on the detection and quantification of benzodiazepines in beverages using fast LLE with low temperature partitioning and paper spray mass spectrometry, the benzodiazepines were found to be stable in open air for 48 hours without significant changes to the drugs [119].

The %losses at room temperature ranges were 23.72-80.34% for chlorphenamine, 8.46-61.79% for amitriptyline and 1.19-71.75% for diazepam. Chlorphenamine showed instability at room temperature in all the four beverages, amitriptyline was stable in vodka and savanna but unstable in wine and chibuku whereas diazepam was stable in vodka, but unstable in chibuku, vodka and wine. Chlorphenamine in wine showed the most loss in concentration (80.34%) after 28 days when stored at room temperature, as well as diazepam in chibuku stored at room temperature with a loss of 71.75%. These high %losses may have been due to degradation reactions of chlorphenamine overtime, as well as hydrolysis of the drugs as they were stored at room temperature [113]. In addition, chlorphenamine yielded the lowest %recovery in the solvent system used as well as poor precision, and so could be present in trace concentrations thereby introducing fluctuations which may affect its stability in the beverages [103].

The three drugs were stable in beverages of low alcohol content, chibuku (4%) and savanna (6%), except diazepam which was moderately stable in savanna. In the beverages of higher alcohol content, the drugs were moderately stable to unstable, especially chlorphenamine. This trend was however not observed in the stability of drugs in alcoholic beverages stored at room temperature as all the drugs were unstable except amitriptyline in vodka and savanna, as well as diazepam in vodka. Generally, compounds are better preserved at colder temperatures than at room temperature [120], [121], and this holds for the data presented in this study. Therefore, as it was expected, the drugs were more stable in alcoholic beverages stored in the freezer than those stored at room temperature. The instability of the drugs may also be linked to the pH of samples, the alcohol content of the beverages and storage temperature.

According to published studies in which stability studies were conducted, the author is not aware of similar studies incorporating this combination of drugs or analytes in alcoholic beverages. While multiple studies on the stability of common date rape drugs in biological specimen are reported in literature, not much research has been carried out on the stability of sexual assault facilitating drugs in

alcoholic beverages. Therefore, more research has to be conducted on the detection and stability of these drugs in various alcoholic beverages. Research on their stability in biological specimen however show that they are stable for longer periods in different storage conditions [31], [105], [120], [121].

Increased concentrations of chlorphenamine were observed in savanna after storage at room temperature as it yielded a negative %loss (-44%). It was possible that the increases in concentration over the duration of the study indicate the presence of conjugated forms of chlorphenamine. This was also observed for some drugs at different analysis points during the study period. Chlorphenamine yielded the lowest %recovery compared to the other drugs, this suggests that chlorphenamine may be present in the samples at trace concentrations thus causing variation and instability in measurements [103]. Therefore, this may be the reason as to why it exhibited a high degree of instability in most beverages, especially savanna, at both storage conditions. In addition, chlorphenamine reportedly undergoes degradation reactions in acidic aqueous solutions and all the beverages studied are acidic [113]. Co-eluting analytes, from the sample matrix, were observed for the internal standard, chlorphenamine and amitriptyline in the unspiked beverages and this may have contributed to the supposed increase in peak area ratios of the drugs of interest overtime. The negative %losses or increase in concentrations of the drugs may also be a result of interactions between the spiked drugs and the sample matrix that vary with time as well as the presence of conjugate forms [119], [122], [123]. Moreover, the matrix enhancement effect and other reactions in the samples may have contributed to this increase, however further analysis would be required to evaluate this hypothesis [119], [123], [124]. Results from stability studies of multianalyte methods depend on the combination of drugs mixed together and hence these drugs may impact the stability of the other analytes or drugs [120]. The factors which may have affected the stability of the drugs in beverages are pH, storage time and temperature, alcohol concentration and inconsistencies with extraction and instrumental analysis [37], [77], [120], [124].

The trend in stability of the three drugs in the different beverages in both storage conditions is shown in Figure 33.

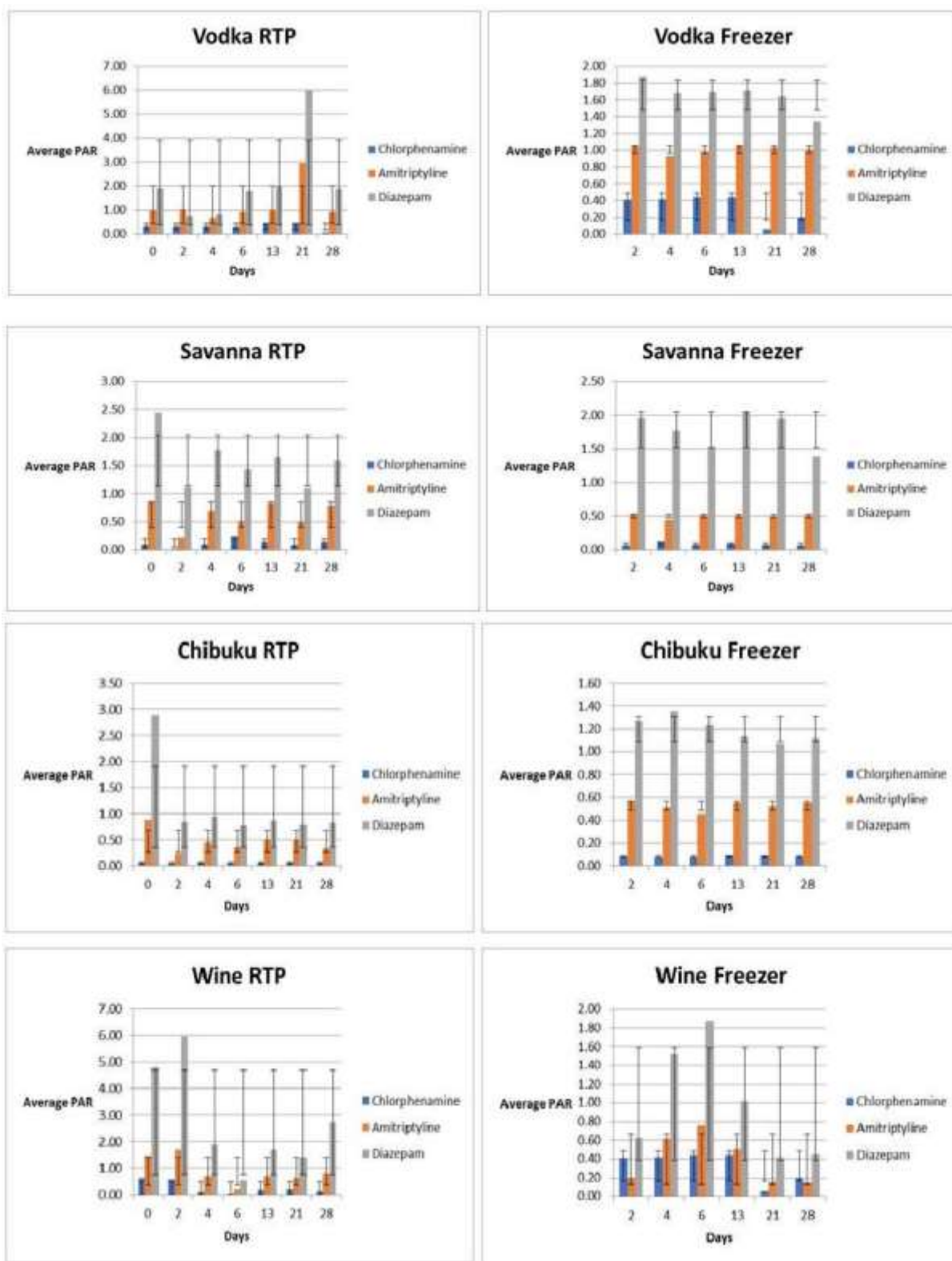


Figure 33: Graphs showing the stability of the individual drugs in alcoholic beverages at the two storage temperatures

Evidently, according to the results, the drugs in beverages stored at room temperature generally became unstable after a week as there was high change in PARs and therefore a significant loss of the drug from day six. The drugs in beverages stored in the freezer generally lost stability after two weeks as there were already high %losses on day 13. In addition, the graphs show that there was high fluctuations with the analyte measurements, especially in wine. This high fluctuation and variability is also depicted by the large error bars observed in Figure 33, possibly arising from interferences as explained in sections 4.2.5 and 4.2.6 [96]. Due to unavailability of the GC-MS instrument during the latter part of this research study at BIUST and at collaborator institutions in the country for 9 months, the stability of these drugs was only determined with the derivatisation step included, which showed that some uncertainties are introduced. This is supported by the unsatisfactory results obtained with derivatised drugs including the precision and auto-sampler stability study, implying that underivatised would give better results compared to the derivatised drugs. Therefore further studies determining the stability of these drugs underivatised would be sufficient as they showed better precision and auto-sampler stability without derivatisation. This is shown by the unreliable precision studies results obtained for the derivatised drugs compared to the underivatised drugs in section 4.2.8, as well as the fairly low %recoveries and instability in alcoholic beverages of some of the derivatised drugs, especially chlorphenamine.

CHAPTER 5

CONCLUSIONS

DFSA, as discussed in section 1.1, refers to a sexual activity in which the victim lacks capacity to give consent to the act due to intoxication by drugs of abuse, pharmaceutical compounds and/or alcohol. The aims of the research were to understand the true incidence of DFSA in the country along with the analysis of drug detectability and stability in common alcoholic beverages in Botswana. These were addressed through a cross-sectional study conducted through the use of an online survey on the knowledge, experiences and perceptions of DFSA in Botswana, which was shared via email and social media platforms. Research on the commonly dispensed benzodiazepines, anti-depressants and antihistamines (which are also potential compounds for DFSA) and common beverages consumed by college and university students was carried out, and the detection and stability of the drugs spiked in the alcoholic beverages was assessed using the GC-MS.

5.1 The Survey

This study indicated that sexual assault was a concern among tertiary students in Botswana and that there were cases of DFSA. This was the first such study in Botswana, to the author's knowledge, the low statistics may indicate underreporting of cases, as is the nature of DFSA cases. The findings from this study show that most of the study population has adequate knowledge of sexual assault and DFSA. Though majority of respondents knew about the topic, some respondents did not have knowledge and so there was need for increased access and awareness on DFSA. Alcohol was the most commonly used drug in DFSA cases, which according to this study typically occur at clubs, bars and entertainment events. A high number of victims do not report sexual assault and people generally believe that victims do not get the required support, especially from law enforcement. Therefore, the government, society and universities or colleges should act in reducing the prevalence of sexual assault and DFSA through changing policies within law enforcement, tightening laws for perpetrators and easing the reporting process.

5.2 Method Development for the Detection and Stability of Drugs in Alcoholic Beverages

In this study, a GC-MS method for the extraction and detection of four commonly dispensed and purchased pharmaceutical drugs (excluding bromazepam due to detectability issues) associated with DFSA was developed, optimised and validated for the analysis of drugs in alcoholic beverages. These

commonly dispensed benzodiazepines, anti-depressants and antihistamines were found to be diazepam, bromazepam, chlorphenamine, amitriptyline and antazoline, and they were reliably detected and confirmed using the GC-MS, except bromazepam. The method was developed and optimised taking into consideration the derivatising (antazoline) and non-derivatising drugs (diazepam, chlorphenamine and amitriptyline). The drugs were stable on the auto-sampler when derivatised and underivatised, except antazoline which was the only derivatising drug in the study. The underivatising drugs also exhibited good intra-assay and inter-assay precision when underivatised while derivatising the drugs resulted in poor intra-assay and inter-assay precision of the method, especially for chlorphenamine. However, the developed method for the derivatised and underivatised drugs demonstrates selectivity and specificity for the drugs included in this study, and yields good %recoveries of the drugs, except chlorphenamine which yielded a low recovery of 20.80%. This study shows that chlorphenamine, amitriptyline and diazepam can be detected in the alcoholic beverages- chibuku, wine, vodka and savannah over a 28 day period after storage at both room temperature and at -20°C. However, these derivatised drugs were not always stable in the beverages, especially after storage at room temperature in the four alcoholic beverages. Chlorphenamine particularly was unstable in most of the beverages at both storage conditions compared to the other drugs when derivatised. Even though better results were achieved without derivatising the drugs, derivatisation is more inclusive as it allows for the detection of more drugs that would otherwise not be detected when underivatised. The results of this study suggest that in cases where spiked beverages are found and collected, they should be analysed sooner, if possible within a week especially if they are stored at room temperature as they may be unstable depending on the storage temperature. Also, the spiked beverages may provide more evidence in the investigation of DFSA cases in Botswana, as detection in the alcoholic beverages matrix was consistent over longer periods in different storage conditions.

5.3 Recommendations

The recommendations from this study are that this study should be carried out on a larger scale for both the survey and method development for detection and stability studies of the drugs, with better implementation and instrumentation. This includes carrying out the cross-sectional study countrywide as well as studying more drugs in more alcoholic beverages, using a sensitive and rapid GC-MS. It is recommended that more validation and optimisation parameters should be carried out on the underivatised drugs for better comparison. According to the results obtained, drinks that are suspected

to be involved in DFSA cases should be collected and analysed as alternative types of evidence in DFSA cases, as the drugs are detectable over the 28 day period at both -20 °C and room temperature.

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APPENDICES

Appendix I

Comparison of Sociodemographic Factors with Knowledge, Experience and Perceptions on DFSA

Appendix I-a: Comparison of level of education and knowledge of DFSA

Level of Education	Knowledge of DFSA		Total	P value
	No	Yes		
BGCSE/IGCSE	6 (30.0%)	14 (70.0%)	20 100.0%	p=0.430 $\chi^2=4.887$
A-Level/Equivalent	1 50.0%	1 50.0%	2 100.0%	
Diploma/Equivalent	3 42.9%	4 57.1%	7 100.0%	
Degree/Equivalent	17 25.4%	50 74.6%	67 100.0%	
Master's Degree	2 10.0%	18 90.0%	20 100.0%	
Doctoral Degree	0 .0%	1 100.0%	1 100.0%	

Appendix I-b: Comparison of gender and opinions on the likelihood of experiencing DFSA

Gender	How Likely is it to Experience DFSA				Total	P value
	Not at all likely	Slightly likely	Very likely	Extremely likely		
Male	28 70.0%	9 22.5%	2 5.0%	1 2.5%	40 100.0%	p=0.002 $\chi^2=0.286$
Female	29 38.7%	32 42.7%	13 17.3%	1 1.3%	75 100.0%	
Prefer not to say	0 .0%	1 50.0%	1 50.0%	0 .0%	2 100.0%	

Appendix I-c: Comparison of college/university and how often respondents hear of DFSA cases

College/ University	Often Hear in College				Total	P value
	Never	Not Often	Often	Very Often		
BIUST	28 41.8%	30 44.8%	8 11.9%	1 1.5%	67 100.0%	p=0.282 χ^2 =27.501
UB	0 .0%	16 84.2%	3 15.8%	0 .0%	19 100.0%	
BU	2 50.0%	2 50.0%	0 .0%	0 .0%	4 100.0%	
BAC	2 5.9%	9 14.1%	3 18.8%	1 50.0%		
GUC	2 40.0%	1 20.0%	2 40.0%	0 .0%	5 100.0%	
IDM	0 .0%	2 100.0%	0 .0%	0 .0%	2 100.0%	
DDT	0 .0%	1 100.0%	0 .0%	0 .0%	1 100.0%	
BUAN	0 .0%	2 100.0%	0 .0%	0 .0%	2 100.0%	
GTC	0 .0%	1 100.0%	0 .0%	0 .0%	1 100.0%	

Appendix I-d: Comparison of gender and opinions on the main perpetrators of DFSA

Gender	Main Perpetrators			Total	P value
	Males	Females	Both		
Male	29 34.9%	2 20.0%	9 37.5%	40 34.2%	p=0.056 $\chi^2=9.205$
Female	54 65.1%	8 80.0%	13 54.2%	75 64.1%	
Prefer not to say	0 .0%	0 .0%	2 8.3%	2 1.7%	

Appendix I-e: Comparison of gender and experience on unwanted sexual advances

Gender	Unwanted Sexual Advances			Total	P value
	No	Yes	Prefer not to say		
Male	29 72.5%	6 15.0%	5 12.5%	40 100.0%	p=0.000 $\chi^2=28.721$
Female	23 30.7%	50 66.7%	2 2.7%	75 100.0%	
Prefer not to say	1 50.0%	1 50.0%	0 .0%	2 100.0%	

Appendix I-f: Comparison of gender and use of alcohol/drugs during the unwanted sexual advances

Gender	Using Alcohol/ Drugs			Total	P value
	No	Yes	Do not recall		
Male	6 13.0%	2 14.3%	1 100.0%	9 14.8%	p=0.187 $\chi^2=-0.171$
Female	39 84.8%	12 85.7%	0 .0%	51 83.6%	
Prefer not to say	1 2.2%	0 .0%	0 .0%	1 1.6%	

Appendix I-g: Comparison of gender and inability to consent to the unwanted sexual advances

Gender	Intoxicated/Unable to Consent			Total	P value
	No	Yes	Do not recall		
Male	8 15.4%	0 .0%	1 100.0%	9 14.8%	p= 0.584 $\chi^2=0.071$
Female	43 82.7%	8 100.0%	0 .0%	51 83.6%	
Prefer not to say	1 1.9%	0 .0%	0 .0%	1 1.6%	

Appendix I-h: Comparison of gender and the respondents being drugged unknowingly

Gender	Drugged Unknowingly			Total	P value
	No	Yes	Do not recall		
Male	7 13.5%	2 28.6%	0 .0%	9 14.8%	p= 0.693 $\chi^2=-0.052$
Female	44 84.6%	5 71.4%	2 100.0%	51 83.6%	
Prefer not to say	1 1.9%	0 .0%	0 .0%	1 1.6%	

Appendix I-i: Comparison of gender and how informed on DFSA respondents are

Gender	Informed on DFSA			Total	P value
	Not well informed	A bit well informed	Very well informed		
Male	13 32.5%	24 60.0%	3 7.5%	40 100.0% 34.8%	p=0.715 $\chi^2=0.672$
Female	15 20.5%	36 49.3%	22 30.1%	73 100.0% 63.5%	
Prefer not to say	0 .0%	2 100.0%	0 .0%	2 100.0% 1.7%	

Appendix II

The Chances and Risk Factors of Experiencing DFSA in Botswana

Appendix II-a: Respondents' Opinions on How Likely It Is To Experience Sexual Assault

How Likely Is It To Experience Sexual Assault	Frequency	%
Not at all likely	57	48.7
Slightly likely	42	35.9
Very likely	16	13.7
Extremely likely	2	1.7

Appendix III

Appendix III-a: Data Coding System

Survey Question	Responses/Themes	Numerical Code
Do you consent to take part in this survey?	Yes	1
	No	0
1. What is your age?	18-20	1
	21-24	2
	25-30	3
	31-40	4
	41-60	5
2. What gender do you identify as?	Male	1
	Female	2
	Prefer not to say	3
3. a) In which school/college/university are you or were you enrolled?	Botswana International University of Science and Technology-BIUST	1
	University of Botswana-UB	2
	Botho University-BU	3
	Botswana Accountancy College (BAC)	4

	Gaborone University College of Law and Professional Studies-GUC	5
	Institute of Development Management-IDM	6
	DDT College of Medicine- DDT	7
	Boitekanelo College-BC	8
	Botswana University of Agriculture and Natural Resources (BUAN)	9
	Gaborone Technical College (GTC)	10
3. b) Are you currently enrolled?	Yes	1
	No	0
4. What is your current education level?	International Certificate of Secondary Education (IGCSE)/Botswana General Certificate of Secondary Education (BGCSE)	1
	A-level or equivalent	2

	Diploma or equivalent	3
	Degree or equivalent	4
	Master's degree	5
	Doctoral degree	6
5. a) Did you know what sexual assault is before taking part in this research?	Yes	1
	No	0
5. b) Do you know what drug facilitated sexual assault is before taking part in this research?	Yes	1
	No	0
5. c) If yes, where did you learn about drug facilitated sexual assault (DFSA)?	At School	1
	Seminars/Workshops	2
	The Media	3
	The Internet/Research	4
	Through People	5
6. How well informed do you believe you are about drug facilitated sexual assault?	Not well informed	1
	A bit well informed	2
	Very well informed	3
7. Did you know that consent/permission is	Yes	1

required from both parties before a sexual act?	No	0
8. a) In your own opinion, how likely is it that you will experience drug facilitated sexual assault?	Not at all likely	1
	Slightly likely	2
	Very likely	3
	Extremely likely	4
8. b) In your own opinion, where are you most likely to experience drug facilitated sexual assault?	Parties	1
	Clubs and Bars	2
	Entertainment Events	3
	Homes or Campus Residences	4
	Others	5
9. How often do you hear of drug facilitated sexual assault cases in your school/university/college?	Never	1
	Not Often	2
	Often	3
	Very Often	4
10. In your view, when and where does drug facilitated sexual	Parties	1
	Clubs or Bars	2

assault typically occur?		
	Entertainment events	3
	Homes or Campus Residences	4
	Others	5
11. What substances/drugs do you think are involved in drug facilitated sexual assault cases?	Alcohol	1
	Sedatives or Date Rape Drugs	2
	Cocaine	3
	Marijuana	4
	Do not know	5
	Others	6
12. Who do you think are the main perpetrators of DFSA?	Males	1
	Females	2
	Both	3
13. a) Has someone ever made unwanted sexual advances to you?	Yes	1
	No	0
	Prefer not to say	2
13. b) At the time, what	Partner	1

was the person's relationship to you?	Ex-Partner	2
	Colleague	3
	Fellow College/University Student	4
	Friend/Acquaintance	5
	Stranger	6
	Family Member	7
	Other	8
13. c) Where did the incident take place?	My Residence	1
	His/her Residence	2
	Car/Other Vehicle	3
	Party	4
	Bar/Nightclub	5
	University Residence	6
	Hotel/Guesthouse	7

	Isolated Place	8
	Other	9
13. d) Were you using alcohol or drugs when the incident happened?	Yes	1
	No	0
	Do not recall	2
13. e) Before the incident, were you drunk, high, or passed out and unable to consent or give permission?	Yes	1
	No	0
	Do not recall	2
13. f) Do you think you were given alcohol/drugs without your knowledge?	Yes	1
	No	0
	Do not recall	2
14. a) Did you tell anyone or report the incident to the relevant authorities?	No	1
	Yes	0
	Do not recall	2
14. b) If you answered 'Yes', who was the incident reported to or who did you tell?	A friend	1
	Sibling	2
	Parent	3
	Colleague	4

	Others	5
15. c) If you answered 'No', what were the reasons for not reporting or telling anyone?	Fear or Confusion	1
	Feeling Ashamed	2
	Fear of not being believed	3
	Reporting is a tedious process	4
	Nothing happened	5
	Do not know/It was unnecessary	6
16. Do you know of anyone who has experienced drug facilitated sexual assault?	No	0
	Yes	1
	Do not know	2
17. a) Have you ever witnessed drink spiking, or heard of someone spiking anyone's drink in any setting?	No	0
	Yes	1
	Prefer not to say	2
17. b) Where did you witness this or hear of it happening?	The Media	1
	Clubs or Bars	2

	Entertainment Events	3
	Home or Residence	4
	Others	5
18. How informed are you about where to get help/support if you or someone you know experienced sexual assault?	Not informed	1
	A bit informed	2
	Very informed	3
19. a) Do you think enough support is available for victims?	No	0
	Yes	1
	Prefer not to say	2
18. b) If you answered 'No', what do you think can be done to improve the support system?	Increase education/ awareness	1
	Counselling	2
	Ease reporting process	3
	24hr Helpline	4
	Special Sexual Assault Centres	5
	Others	6
19. Based on experience, what happens when	Case is dismissed	1

one reports an incident of sexual assault at your college/university or to the relevant authorities?	Appropriate measures are taken	2
	No investigation/little is done	3
	Cases take too long	4
	Victim Blaming	5
	Do not know/ Never heard	6
20. In your opinion, who is most responsible person/organization for minimizing DFSA and sexual assault? Select all correct answers and give reasons or your answer.	Him/herself	1
	Police	2
	Government	3
	Family	4
	Society	5
21. Please include your reasons here;	Individual Responsibility	1
	Unity	2
	Legislation should be toughened	3
	Victim is never wrong	4
	Society can do better	5

Appendix IV

Appendix IV-a: Mass spectra of the underivatised drugs

