



Faculty of Science

Department of Chemical and Forensic Science

**INTERDISCIPLINARY APPROACH TO OBTAINING DRUG USE DATA:
ANALYSIS OF WASTEWATER USING GAS CHROMATOGRAPHY – MASS
SPECTROMETRY AND A SURVEY**

by

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LIST OF ABBREVIATIONS

ACS	American Chemical Society
ADHD	Attention Deficit Hyperactivity Disorder
ARV	Anti-retroviral
ATS	Amphetamine Type Stimulants
BIUST	Botswana International University of Science and Technology
BOMRA	Botswana Medicines Regulatory Authority
BOSASNet	Botswana Substance Abuse Support Network
BPS	Botswana Police Service
DOA	Drugs of Abuse
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GIMC	Gaborone International Music and Culture Festival
GV	Glen Valley
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
HIV	Human Immunodeficiency Virus
IS	Internal Standard
ISO	International Organization for Standardization
LC-MS	Liquid Chromatography-Mass Spectrometry
LLE	Liquid-Liquid Extraction
MS	Mass Spectrometry
NGO	Non-governmental organization
NIST	National Institute of Science and Technology

PET	Polyethylene terephthalate
PY	Palapye
RT	Retention Time
SE	Sewage Epidemiology
SIM	Selected Ion Monitoring
SM	Serowe Mabogopa
SPE	Solid-Phase Extraction
SR	Serowe-Radihemelo
TCM	Toxicology Checkout Mixture
THC	Tetrahydrocannabinol
TIC	Total Ion Chromatogram
UNODC	United Nations Office on Drugs and Crime
WBE	Wastewater-based epidemiology
WWTP	Wastewater Treatment Plant
WUC	Water Utilities Corporation

ABSTRACT

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**Title-Interdisciplinary approach to obtaining drug use data: analysis of wastewater using
Gas Chromatography-Mass Spectrometry and a survey.**

Wastewater-based epidemiology (WBE), as a means of obtaining drug use and abuse data, is increasingly becoming an alternative method for estimating drug usage and consumption in a population. The data obtained through WBE on drug usage and consumption is near real-time and it allows analysts to monitor current patterns and trends in novel psychoactive substances, illicit drugs, and pharmaceutical drugs. WBE can therefore be complemented with other methods of obtaining data on drug use and abuse such as survey questionnaires.

The survey questionnaire is administered in communities where wastewater samples are collected. The data obtained through WBE is compared with the data obtained from survey responses and establishes a correlation if any. Through survey responses, researchers can obtain the demographic information of participants, frequency of use, and mode of administration of drugs. This research reports the use of a Gas Chromatography-Mass Spectrometry (GC-MS) method for the detection of illicit drugs and pharmaceutical drugs in wastewater collected from different sites in Botswana, introducing the analysis for the first time in the country. Online survey questionnaire responses from the Botswana International University of Science and Technology (BIUST) community on the use, disposal, and knowledge on the fate of pharmaceutical

and illicit drugs in wastewater are also reported. Due to the increase in the use of illicit drugs and the misuse of pharmaceutical drugs in Botswana over the years, this research had to be done to help in the fight against illicit drug use and the misuse of pharmaceutical drugs.

Influent wastewater samples were collected once a month, i.e., during the initial visit to the wastewater treatment plant (WWTP), during the WWTP tour, after the last weekend of the month, after a public holiday, after a long weekend or after a major event such as a music festival or sports tournament. Eighteen influent wastewater samples were collected at Palapye- BIUST, Palapye-main village, Serowe-Radihemelo, Serowe-Mabogopa and Gaborone- Glen Valley WWTP in Botswana between July 2019 and October 2019. Samples were extracted by the Solid-Phase extraction (SPE) technique using Strata-X Drug B SPE cartridges before analysis by GC-MS. Drugs were identified in wastewater samples using scan and splitless injection. Ibuprofen and caffeine were detected and confirmed from the wastewater samples while efavirenz, prolintane, mefexamide, xylazine and amiphenazole were only identified and not confirmed due to a lack of drug standards.

An online survey questionnaire link was created via google forms and administered at BIUST between March 2021 and May 2021. A total of 120 people participated in the survey. A total of 80.5% of the respondents indicated that they used different pharmaceuticals. Painkillers such as ibuprofen and paracetamol were the most used pharmaceuticals. The results obtained also indicated that females used pharmaceuticals more than their male counterparts. Only 16.1% of the survey respondents indicated to have use different illicit drugs such as cannabis, cocaine, and methamphetamine. Hallucinogens such as cannabis were the most used class of illicit drugs.

The results obtained on illicit drug use indicated that males used illicit drugs more than their female counterparts. Most of the illicit drugs users indicated that they used the drugs every day, every weekend, and the last weekend of the month. Frequency of use of pharmaceutical drugs was not recorded. Majority of both pharmaceutical and illicit drug users were in the 18-24 years age group. For disposal of leftover/expired pharmaceutical and illicit drugs, most respondents indicated that they dispose them in litter bins and down the drain. Most of the respondents also indicated that it is through reading books and research articles that they have knowledge on the fate of both illicit and pharmaceutical drugs in wastewater.

Even though survey respondents indicated to have used different illicit and pharmaceutical drugs, these drugs were not detected in the Palapye-BIUST wastewater samples that were analyzed, as a result there was no correlation established between the BIUST online survey responses with the GC-MS results for the wastewater samples collected at the Palapye-BIUST wastewater treatment plant. Pharmaceuticals mentioned in the survey answers were not detected in wastewater samples from Palapye-BIUST WWTP, although ibuprofen was detected in other wastewater samples collected at Palapye-main village and Serowe-Mabogopa WWTP.

The aims and specific of the research were successfully achieved as a GC-MS method was used to detect ibuprofen, caffeine, efavirenz, prolantane, mefexamide, xylazine and amiphenazole in wastewater samples collected at different sites in Botswana. The use, disposal, and knowledge of the fate of illicit and pharmaceutical drugs in wastewater at BIUST was also successfully achieved through administration of an online survey questionnaire to the BIUST community. A comparison of the Palapye-BIUST GC-MS results for the wastewater samples and BIUST survey questionnaire responses obtained showed that there was no correlation established between the results obtained by the two means. The research questions were also answered as we were able to identify the different illicit and pharmaceutical drugs that are used by different communities in Botswana.

Results obtained from the research will help relevant stakeholders in Botswana in the fight against drug abuse as they provide the much-needed information on commonly used drugs, frequency of use, and demographic information of participants.

Keywords: Wastewater-based Epidemiology, Drugs of Abuse, Influent, Gas Chromatography-Mass Spectrometry, Solid-Phase Extraction

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

This chapter presents the foundation for this research from the perspective of drug use globally and locally, the sample collection, pre-treatment, preparation, and analytical techniques used.

1.1 Drugs

Drugs are synthetic, semi-synthetic or natural substances that when taken into the body induce psychological change [1]. There are various modes of administration of drugs into the body such as orally, parenterally or snorting [2]. The classification of the various drugs is based on their origin (natural, synthetic, or semi-synthetic), effect on the body (analgesics, depressants, hallucinogens, narcotics, and stimulants), use (date-rape, human performance, club drugs) and schedule [1].

Drugs are taken for different purposes such as medical reasons or taken for recreational purposes [3],[4]. Drugs and related substances are subject to regulations and laws because of their potential to abuse due to their harmful effects on the user and others [1]. The abuse of drugs can result in psychological and or physiological dependence [1]. Most of these drugs of abuse are normally manufactured in a clandestine laboratory or diverted from legitimate use[5].

Drugs of abuse have harmful effects on human beings therefore their use, possession and distribution are controlled in many countries [5]. Despite the government's restrictions on the possession, supply, manufacture, and use of illicit drugs, the use of drugs of abuse is still on the rise [5]. In 2020, about 275 million people were reported to have used drugs, which was up by 22% from 2010 [6]. Demographic factors project the number of people using drugs to rise by 11% globally and as much as 40% in Africa alone by 2030 [6].

Illicit drug use affects people's behaviour which can result in their health being compromised. The administration of drugs through unsafe injections can result in one being infected with viruses such as Human Immunodeficiency Virus (HIV) or Hepatitis C virus if they share the injection needles with a person infected with any of the viruses [6]. Hepatitis C virus results in hepatitis-related liver cancer and cirrhosis [6]. Overdosing on illicit drugs can also lead to the premature death of an individual if they do not get medical attention as soon as possible. The misuse of pharmaceutical drugs can result in respiratory complications, dependence, and premature death in the case of overdose [6]. According to the 2021 World Drug Report, almost half a million people died due to drug use in 2019 [6].

1.2 Commonly used drugs

According to the 2021 World Drug Report, over 200 million people used cannabis in 2019, making it the most used drug by far [20]. Between 2010 and 2019, the use increased by more than 18% worldwide. Still, in 2019, 27 million used Amphetamine Type Stimulants (ATS) such as methamphetamine, about 20 million people used ecstasy and 20 million people used cocaine [20]. For New Psychoactive Substances (NPS), there has been a decrease in their use in North America and Europe. An increase in the use of NPS has been noted in Africa with seizures increasing from 1kg to 828kg in 2015 and 2019 respectively [20]. Still, in 2019, about 62 million people used opioids for non-medical purposes [20]. Pharmaceutical opioids such as fentanyl and codeine are by far the most misused pharmaceutical drugs [20].

There is high stigma and discrimination associated with drug abuse resulting in users not being able to share their experiences freely [21]. Therefore, this can lead to limited information on drug use being obtained from drug users, making it difficult to know the actual or real-time current drug trends and patterns in the community. Reporting facts about drugs and promoting science-based interventions is vital to reducing the demand and supply of drugs, while also facilitating access to controlled medicines for those in need [6].

1.3 Drug use in Botswana

Botswana is not immune to the use of illicit drugs and the misuse of pharmaceutical compounds [7],[8],[9]. The use, possession, and distribution of illicit drugs in Botswana have increased over the years. In the past, Botswana was used as a transit for illicit drugs [7]. Some of these illicit drugs remained in Botswana where they were distributed and used by locals. According to the Narcotics, Fauna, and Flora Investigating unit of the Botswana Police Service (BPS) between 2010 and 2014, more Botswana compared to foreigners were leading in illicit drug dealings and more women than men dominated the drug trade in Botswana [7].

These drugs are used by different age groups in the country including students [10]. Some students in certain secondary schools in Botswana have admitted to the use of cannabis and drinking alcohol at an early age [10]. Drug dealers target students in schools and recruit some to be their distributors [11]. Some Botswana claim that due to the high unemployment rates they resort to the sale and distribution of illicit drugs such as cannabis to make ends meet [12],[13]. According to the BPS and the Botswana Substance Abuse Support Network (BOSASNet), the most commonly used drugs in Botswana include alcohol, cannabis, methcathinone, methamphetamine, cocaine, and heroin [14].

The BPS has indicated that illicit drug use is the root cause of societal ills and criminal activities such as burglary, shoplifting, robbery, rape, and murders, among others, in Botswana [14]. Violence associated with the use of illicit drugs in Botswana sometimes results in perpetrators raping or murdering their victims [15]. Rape cases have increased significantly around the country in which many victims suspect that they were drugged before rapists had their way with them [16]. Some perpetrators who murdered confess to having been under the influence of illicit drugs when committing the crime [15]. This goes on to show that most citizens one way or the other are exposed to illicit drugs.

The BPS has been fighting the use, possession, and distribution of illicit drugs in the country for many years. Despite the efforts by BPS to fight the drug battle, the country is still being targeted by international drug barons [7],[17]. The BPS conducts random drug raids at locations such as border posts, homes, and schools. They also hold campaigns, workshops, and press conferences in which they reach out to Botswana educating them on the dangers

of possessing, distributing, and using illicit drugs. BPS also partners with other relevant stakeholders such as teachers, social workers, community leaders, and Non-Governmental Organisations (NGOs) such as BOSASNet to come up with strategies to fight the national drug problem [7].

The increase in drug use in Botswana does not only concern illicit drugs but the misuse of pharmaceutical drugs as well [8]. The commonly misused pharmaceutical drugs include opioids that are used in pain treatment, depressants such as those used to treat anxiety and stimulants such as those used in treating Attention Deficit Hyperactivity Disorder (ADHD) [18]. According to a physician in the Ministry of Health and Wellness in Botswana, people in the 18-25 age range make up most of the abusers of pharmaceutical/prescription drugs such as sleeping pills, anti-anxiety medications and stimulants used to treat ADHD [8]. The misuse of these drugs can be associated with a cultural shift in which young people always resort to the use of medications whenever they encounter difficulties in life [8].

When pharmaceutical drugs are taken in high amounts than what was prescribed or mixed with other drugs, they become dangerous [8]. The risk of drug-drug interaction when the drugs are mixed can have long-term effects on vital organs such as the kidneys [19]. The misuse of medications such as sleeping pills can lead to cardiac and respiratory depression which can lead to death [8]. In some cases, these drugs can be overdosed which can lead to premature death if the person does not get medical assistance as soon as possible [6]. Chronic exposure to these drugs can also result in dependence and tolerance. This will result in the people experiencing withdrawal symptoms when they stop taking the drug.

There is limited information on illicit drug use and the misuse of pharmaceutical drugs in Botswana available online or publicly. The information available on illicit drug use is normally obtained through police drug raids, drug seizures, medical records, surveys, and questionnaires. To the author's knowledge, a multidisciplinary approach involving wastewater-based epidemiology and a survey for obtaining data on community drug usage has not been done in Botswana. This identifies a knowledge gap that this research aims to fill. Such research needs to be done in Botswana as the data obtained is near real-time and it can be used as a tool to monitor drug patterns and trends weekly, monthly, and seasonally. It can also be used to evaluate the effectiveness of anti-drug campaigns and drug raids conducted by law enforcement officers.

1.4 Wastewater-based Epidemiology

Everyday life processes such as bathing, toilet flushing, laundry, and dishwashing result in wastewater which ends up in sewer lines. This wastewater comes from residences, institutions, industries, groundwater, and rainwater [22]. The main components of wastewater are water, solid particles, organic substances, pathogens, pollutants, and nutrients such as phosphorous [23],[24]. Almost anything that is consumed by a certain community or disposed of in sewer lines is detected in wastewater. To come up with preventive measures to fight the use and abuse of illicit drugs and the misuse of pharmaceutical drugs, a clear understanding of the drugs that are used, how they are used, the quantities used, and the supply chain is needed.

Wastewater-based epidemiology (WBE) enables information about exposure to external agents and diseases and estimation of drug usage in a certain population to be obtained through the analysis of wastewater [4],[21],[25]. WBE gives near real-time data that can be used to monitor current drug trends, and drug patterns, and to identify emerging new psychoactive substances [4],[23],[26]. Through WBE weekly, monthly, annual and seasonal variations of target analytes of interest can be studied or monitored [27],[28],[29],[30],[31]. Monitoring the patterns and trends in drug use and abuse is vital for policy planning of measures aiming to deter drug abuse and in the fight against illicit drug smuggling/use or monitoring the effectiveness of anti-drug campaigns.

The data obtained through WBE allows for addressing emerging problems, identifying previously unknown drug threats, and assisting in assessing the effectiveness of drug demand reduction strategies and drug supply disruption strategies [32]. The data obtained is near real-time and specific to the community that is serviced by the wastewater plant where the samples are collected [23],[28],[33]. This can help researchers identify the spectrum of substances used in that community or even monitor certain diseases at that particular time [21],[34],[35].

WBE complements traditional methods of obtaining data on drug use by various communities such as medical records, criminal records, drug monitoring, drug seizures, population surveys and questionnaires [3],[4],[25]. The information obtained from self-report surveys and questionnaires can be misleading as consumers can be dishonest and they might not know the exact chemical composition of the drugs that they have taken [25]. Drug seizures and drug raids are subject to randomness and population surveys can provide

subjective data, therefore other forms of obtaining information on drug use and abuse are needed [26]. Combining the various methods to gather data on drug use and abuse may help in painting a more accurate picture of the drugs that are being used.

Administration of survey questionnaires is one of the many conventional/traditional methods used to obtain community drug use and abuse data [10]. Obtaining information on community drug use and abuse cannot only depend on one method and as a result, complementary methods such as WBE can also be used to evaluate drug abuse more comprehensively [35]. For this study, the data obtained from the survey responses were compared to the GC-MS results that were obtained from the wastewater samples collected at the Palapye-BIUST WWTP to find out if there is any correlation in the data obtained.

For law enforcement officials/policymakers and social workers to come up with effective measures in the fight against illicit drug use and the misuse of pharmaceutical drugs, data on drug use and abuse is needed. The localised data obtained from this survey is very crucial as BIUST management will be able to know the type of illicit drugs that are mostly used, their frequency of use, places where they are mostly used, age range and status of most drug users between March 2019 and May 2021. The data obtained can serve as a reference point to BIUST management as they develop policies on drug use and abuse and subsequent measures that will be put in place.

Different research groups in different countries such as South Africa, the United Kingdom, Italy, Belgium, and Turkey have analysed wastewater to estimate community drug usage [2],[3],[23],[26],[28],[36]. The different research groups have targeted different classes of drugs for different reasons. The analytical methods used also differs depending on the aim of the research. Comparison studies on drug usage patterns and trends have also been done in different regions like Europe [31]. WBE has also been adopted by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) as a tool to estimate community drug usage and monitor drug patterns [4].

Although WBE is anonymous and generalized, it can also be localized whereby sampling is done in a certain area such as schools, prisons, hospitals, military camps, or specific government offices. WBE is non-invasive monitoring, therefore it can be used to estimate drug use and consumption by a certain community as the samples collected cannot be traced back to individuals. There is no direct involvement of human beings and sampling is carried out on a population scale to avoid invading the privacy of people [25]. Influent is the wastewater that flows into the WWTP and is collected when estimating community drug

usage [37]. This allows analysts to estimate the number of drugs consumed by measuring the levels of illicit drugs and their metabolites [38]. When collecting samples to estimate community drug consumption/usage influent samples are mostly considered because some of the analytes of interest can be lost during the treatment process or their concentration can be reduced and end up not being detectable after treatment.

Wastewater treatment plants (WWTP) are more suitable than the water in the river/taps as sampling sites for estimating community drug usage because the river/taps would have undergone processing which can affect the analytes of interest. However, hospitals, manufacturers and clandestine laboratories can also dispose of drugs directly into sewer lines and mix them with those from other areas in the community [24],[32]. This can result in the overestimation of community drug usage. However, the separation of chiral compounds will help differentiate between illicit drug consumption and therapeutic use of prescribed pharmaceutical drugs as well as between consumption vs direct disposal of non-consumed drugs into the sewage lines [36].

Enantiomeric profiling can give valuable information on abuse trends and the potency of chiral drugs. It can also help with distinguishing between the legal and illicit use of drugs as well as indicate actual consumption vs disposal of non-consumed drugs. This is because drug synthesis is associated with different chiral signatures that depend on the route of synthesis [39]. Furthermore, chiral drugs undergo stereoselective disposition in humans leading to changes in their chiral signature [36].

Targeting both the parent drugs and metabolites, where possible, is preferred because the presence of parent drugs in wastewater does not indicate consumption and in some instances, parent drugs undergo complete metabolism and there is no trace of it. The major metabolites are the most preferred as they are eliminated at higher concentrations, therefore, increasing the chances of detection. Research is being done to determine the individual stability and the drug-drug interaction of the different drugs in wastewater. The stability of the drugs in a complex matrix such as wastewater is a function of storage conditions, the physiochemical properties of the target compounds, biochemical changes, the matrix itself and the containers utilised [40].

Analysis of wastewater for drugs of abuse, however, has its limitations. The drug users are unknown since there is no data about individual patterns of drug use, routes of administration, and frequency of use [4]. Uncertainties can also arise from sampling, metabolism, and back calculations. Population fluctuations due to music festivals, tourism peak seasons and sports tournaments are some of the limitations [27]. Some factors influence drug concentration in water such as the flow design of the wastewater system, wastewater treatments, temperature and pH of wastewater which makes comparison studies to be more complex [27].

Most studies which were done in WBE focused mainly on traditional illicit drugs such as cannabinoids, amphetamines, opioids, and cocaine [22]. Recently studies have included pharmaceutical compounds which are being diverted from legitimate use and NPS like piperazines and cathinone, anaesthetics, tranquillizers, antidepressants, and blood pressure medications [22],[25],[36],[41]. The period of sampling of the wastewater entirely depends on the aim of the research. Sampling can be done after a weekend, major festival, entertainment activity, sporting event, certain public holidays, etc. [27].

1.5 Sample Collection & Pre-treatment

Over the years different researchers working on determining community drug usage have collected samples from different water sources such as ground water, surface water, wastewater, potable water, sewage sludge and air [2],[5],[42],[43]. The type of water source chosen by various researchers depends on many reasons such as the aim of the research, comparative studies and detection, the expected concentration of the analytes of interest and accessibility to the water source [5]. For this research, influent wastewater samples were used for detection as this has been noted to have a higher concentration of illicit drugs and pharmaceuticals than effluent wastewater or surface water [44],[45].

Different sampling techniques such as composite sampling and grab sampling are used to collect the wastewater samples [44]. The sampling technique used depends on the aim of the research, the water source to be sampled, the nature and expected concentration of the analytes of interest as well as the analytical technique that will be employed [5]. Composite sampling is the most used sampling technique in WBE owing to local heterogeneity compared to grab sampling [46]. Composite samples give a time-weighted average (TWAS) as the collected aliquots are pooled into one sample after sampling [47]. As a result, composite samples are considered to give a better representation of possible analytes of interests that pass through a WWTP over a specified timeframe [5]. Even though grab sampling does not give TWAS it gives some useful information, and it has been used by some researchers in WBE [44],[48]. Grab sampling gives the concentration of the analytes of interest at the time of sampling. It can also be collected from the outflow tracks of most catchments as there is no need to set up special sampling equipment to collect the wastewater [49].

Sampling containers that are mostly used in this research are amber glass or plastic containers made of polyethylene terephthalate (PET) or high-density polyethylene (HDPE) [40],[50]. Sample volumes that are normally collected by other researchers range from 50 mL to 1500 mL [51],[52]. Once collected, the wastewater samples are transported to the laboratory where they are pre-treated and stored awaiting analysis. Samples are transported in cool dark containers and filtered immediately upon arrival. The samples are filtered to remove particulate matter which can clog the solid phase extraction (SPE) cartridge sorbent and the Gas Chromatogram (GC) column, injection needles and liners if not removed efficiently [5]. Filtration is followed by acidifying the sample to preserve the analytes and minimise decomposition within the sample due to microbiological activity before cold

storage [5],[53]. Acidifying also helps the analytes of interest to be retained on the SPE sorbent by various interactions such as hydrogen bonding, van der Waals forces as well as ion exchange [5]. When the samples are to be extracted within 7 days of collection, they are kept in the fridge at 4 °C or at 5 °C and samples that are to be extracted at a much later date are kept at -20 °C to prevent degradation of the analytes of interest [41],[54],[55]. The samples can be frozen for up to two months before extraction [41].

1.6 Sample Extraction

All the target compounds need to be extracted from the sample matrix before instrumental analysis. Various researchers have used different techniques such as solid-phase extraction (SPE), solid-phase micro-extraction (SPME) and liquid-liquid extraction (LLE) to extract the analytes of interest from the sample matrix [40],[45],[53],[56],[57]. LLE is mostly used in clinical and toxicological studies and has been used in the extraction of biological samples, organic contaminants and pharmaceuticals in wastewater [57],[58]. LLE is a solvent-based extraction technique, therefore, any improvements in the recovery of analytes of interest from the sample matrix rely on the different solvents used [59]. In WBE, SPE is the most used technique to extract pharmaceuticals and illicit drugs in wastewater [3],[4].

Unlike LLE, SPE relies on the use of extraction sorbents with varying properties and is selective for analytes therefore, optimisation of the extraction process not only relies on sorbents but also on the type of sorbents selected for use [5]. With LLE, the analytes of interest are isolated from the sample matrix through their ability to partition between two immiscible solvents. The polarity of the solvent used with respect to the polarity of the analytes of interest, sample pH with respect to the pK_a of the target analytes, the volume of the extraction solvent used and the number of times the extraction is repeated all play a role in improving the recovery of analytes of interest from the sample matrix [5]. The effectiveness of any given solvent in extracting a particular analyte is largely determined by how miscible with water the solvent is and its polarity [5].

In SPE, the analytes of interest are systematically retained onto the solid-phase sorbent material and subsequently extracted or eluted with a suitable solvent or a mixture of solvents [5]. This enables analytes to be isolated from the sample matrix and re-dissolved in the elution solvent. In most cases, the sample matrix comprises a mixture of target analytes and other compounds that can interfere or are undesired. The more the target analytes are

selectively separated from the other matrix components, the cleaner the extract will be with reduced interfering compounds [59].

Even though LLE is also used for the extraction of a wide range of compounds including drugs in the matrix such as wastewaters, SPE was the extraction technique used for this research. SPE has a higher enrichment factor compared to LLE which leads to increased selectivity and sensitivity [60],[61]. This is very vital for sample matrices where analytes of interest are present at trace levels like in wastewater and surface water [24],[45],[61]. Samples that are normally extracted in wastewater analysis for drugs of abuse range from 50 mL to over 1500 mL [51],[52]. The larger the initial volume used in extraction, not exceeding the breakthrough volume, the higher the chances of detecting and quantifying compounds which normally occur in trace amounts [56],[61]. However, SPE is not exclusively selective which may concentrate suspended solids and interferents within the matrix which could result in clogging of the column and signal suppression or enhancement [45],[62],[63]. The target analytes are then eluted with 4-10 mL of a suitable solvent and concentrated further for analysis.

The different SPE sorbents that are used come in different forms such as disks, cartridges, and syringe barrels [64],[65]. The format of the sorbent used for a particular extraction mainly depends on the nature of the sample matrix, sample volume, the expected concentration of the target analytes and the analytical technique employed [65]. For this research, syringe barrels were used. The sorbent material that is normally packed in the syringe barrel has different chemical properties. These sorbent materials are either silica-based or polymer-based and have reversed phase, normal phase, ion exchange and mixed-mode [5]. Different SPE sorbents such as Oasis MCX, Oasis HLB, Strata XC, and Strata-X Drug B are some of the widely used SPE cartridges extracting analytes of interest (drugs of abuse) from wastewater and potable water [4],[41],[43]. Phenomenex Strata X-Drug B cation mixed-mode polymer sorbent was used for this research as it allows for the simultaneous extraction of different classes of illicit drugs and pharmaceutical drugs.

1.7 Chemical Derivatization

Derivatization is a process in which a compound is chemically changed resulting in a new compound that has properties more amenable to a certain analytical method that will be used [66]. This process is done when GC and GC-MS are used [54], [60]. The main aim of chemical derivatisation is to reduce the polarity of a compound while increasing its volatility and

thermal stability. GC-MS uses a very high temperature, therefore thermal stability of the compounds is needed. The outcomes of derivatization include reduction of peak tailing and improved peak shape, detectability and selectivity and a more distinct mass spectrum resulting in less interference [54],[60].

Some compounds are still detectable even without derivatisation. Therefore, the nature and chemical properties of the target analytes, the constitution of the sample matrix and the presence of interfering substances that could affect the analytical results, as well as the methodological approach, are taken into consideration before the decision on whether to derivatise or not can be taken [5]. Most illicit drugs and pharmaceutical drugs have medium to high polarity due to different functional groups such as alcohols, phenols, carboxylic acids and amides [5],[35]. Therefore, a knowledge of the chemical structure allows for an informed decision on whether a compound can and should be derivatised as well as the most suitable derivatising reagents to use [60].

There are different categories of derivatising reagents for different functional groups. Some of the categories of derivatising reagents that are used are acylation, silylation and alkylation [60]. The two categories that are mostly used in WBE are silylation and acylation [60]. In silylation, a labile hydrogen is substituted with an alkyl silyl group and chemicals such as N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and trimethylsilyl (TMS) can be used. During acylation, labile hydrogen is replaced with an acyl group to give an ester/amide. Chemicals such as trifluoroacetic anhydride (TFAA) and pentafluoro propionic anhydride (PFPA) are used for acylation [41],[54]. For this research, chemical derivatisation was not done due to the lack of drug standards. Other researchers have also done similar work in which they analysed wastewater for illicit drugs and pharmaceutical drugs without derivatising the samples before GC-MS analysis [67],[68],[69].

1.8 Instrumental Techniques

Different analytical techniques have been used by different researchers for the detection of different compounds such as pharmaceutical drugs and illicit drugs from different complex sample matrices. Analytical techniques such as GC-MS and LC-MS that are capable of detecting target analytes at very low concentrations in complex matrices are mostly used [70],[71]. High-performance liquid chromatography (HPLC) with a diode array detector or a fluorescence detector, GC coupled with Electron Capture Detector (GC-ECD) as well as GC coupled with High-Resolution Mass Spectrometry (GC-HRMS) have also been used

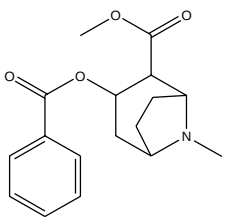
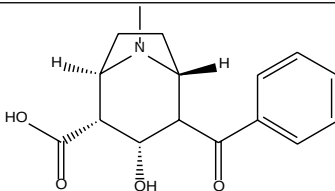
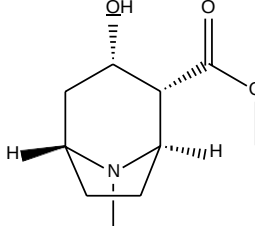
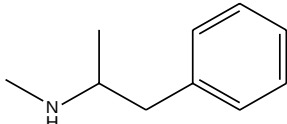
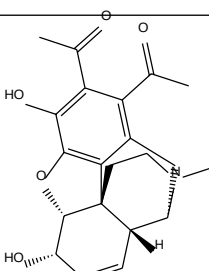
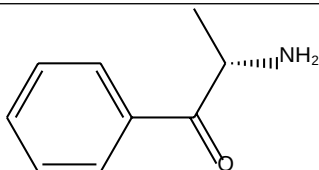
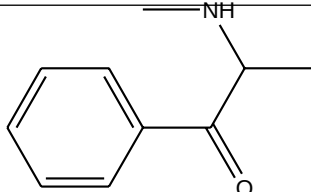
to a lesser extent [23],[34],[70],[71],[72],[73]. These techniques have been previously used to detect illicit drugs in biological samples such as blood and urine [74]. Illicit drugs normally occur at much lower concentrations in environmental samples compared to biologicals which result in the need for instrumental techniques capable of trace level detection [5].

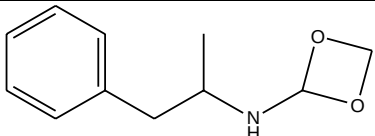
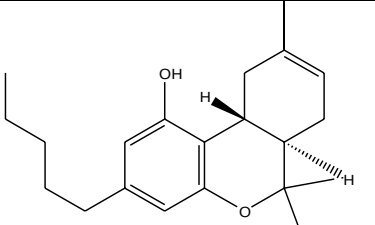
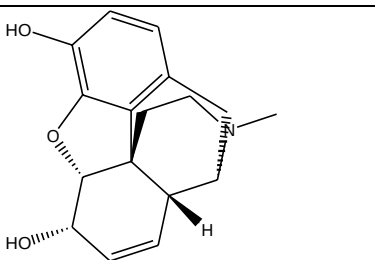
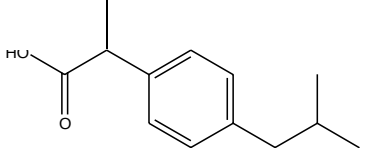
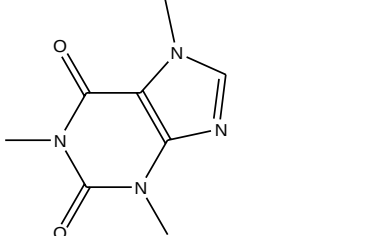
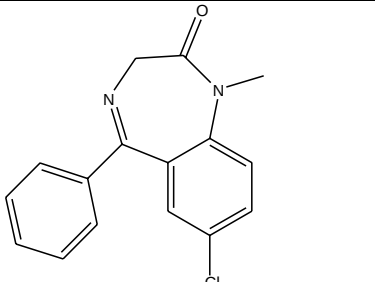
LC-MS with Electrospray Ionisation (ESI) is the most used technique for analysis of drugs in wastewater owing to the polarity nature of most of the drugs. Liquid Chromatography-Mass Spectrometry/ Mass Spectrometry (LC-MS/MS) is commonly used in the analysis of pharmaceutical drugs and their metabolites owing to its robustness and excellent reproducibility making it an ideal technique for analysis of illicit drugs and their metabolites in wastewater samples [35]. Even though LC-MS is popular in WBE for the analysis of drugs, GC-MS is still being used by other researchers as an instrument of choice [41],[70],[75]. GC-MS is very sensitive and has very low detection limits, which makes it suitable for the analysis of drugs which are mostly at trace levels in wastewater [5],[72]. Unlike LC-MS, GC-MS is less affected by matrix effects [5].

1.9 Selection of drugs for this research

The selection of target drugs and their metabolites included in this research was based on reports by the BPS, published reports on drug use and abuse in Botswana, and published reports on WBE and toxicological studies. Most data obtained from published WBE studies has been on classic drugs of abuse such as cocaine, amphetamine, and ecstasy and therapeutic drugs such as morphine, diazepam, efavirenz, ibuprofen and NPS [31],[41],[76]. Table 1.1 lists some of the parent drugs and metabolites that were targeted in this research. Of the drugs listed in Table 1.1, tetrahydrocannabinol, methcathinone, methamphetamine, cocaine, and heroin are the most commonly used drugs in Botswana according to the BPS and BOSASNet [14].

Table 1.1: List of some of the parent drugs and metabolites targeted in this research.

Compound Name	Structure	Empirical Formula	Molar mass (g/mol)	pKa
Illegal drugs and metabolites				
Cocaine		C ₁₇ H ₂₁ NO ₄	303.3	8.60
Benzoyllecgonine		C ₁₆ H ₁₉ NO ₄	289.3	3.35 and 10.82
Ecgonine methyl ester		C ₁₀ H ₁₇ NO ₃	199.3	9.57
Methamphetamine		C ₁₀ H ₁₅ N	149.2	9.87
Heroin		C ₂₁ H ₂₃ NO ₅	369.4	7.95
Cathinone		C ₉ H ₁₁ NO	149.2	7.55 and 18.89
Methcathinone		C ₁₀ H ₁₃ NO	163.2	8.02 and 18.76

MDMA		C ₁₁ H ₁₅ NO ₂	193.3	9.90
Tetrahydrocannabinol		C ₂₁ H ₃₀ O ₂	314.5	10.60
Pharmaceutical drugs				
Morphine		C ₁₇ H ₁₉ NO ₃	285.1	8.21
Ibuprofen		C ₁₃ H ₁₈ O ₂	206.3	4.91
Caffeine		C ₈ H ₁₀ N ₄ O ₂	194.2	14.0
Diazepam		C ₁₆ H ₁₃ ClN ₂ O	284.7	3.40
Chemical structures from ChemDraw Ultra 8.0. pKa values, empirical and molecular formulas from PubChem				

1.10 Problem Statement

The use of illicit drugs and the misuse of pharmaceutical drugs in Botswana have been increasing over the years. Not much is known about the trends and patterns in the use of illicit drugs and misuse of pharmaceutical drugs as limited information is available to the public. Illicit drug use is the root cause of societal ills and criminal activities such as rape, and theft in Botswana. The misuse of pharmaceutical drugs can result in respiratory complications, dependence, and death.

1.11 Research Aim and Specific Objectives

Aims

- To use a Gas Chromatography-Mass Spectrometry (GC-MS) method for the detection of illicit and pharmaceutical drugs in wastewater samples collected from different sites in Botswana.
- To determine the use, disposal, and knowledge of the fate of illicit and pharmaceutical drugs in wastewater at the Botswana International University of Science and Technology (BIUST) using a survey.

Specific Objectives

- To administer a survey questionnaire to the BIUST community.
- Analyse wastewater samples using solid-phase extraction and GC-MS for drugs.
- To compare the GC-MS results and the survey questionnaire data to see if there is any correlation.

1.12 Hypothesis

Different illicit and pharmaceutical drugs are present in wastewater samples collected from different wastewater treatment plants in Botswana.

1.13 Research Question(s)

The study aims at answering the questions:

- What are the different illicit and pharmaceutical drugs that are used by different communities in Botswana?
- Is there a correlation between the BIUST survey responses and the GC-MS results for the wastewater samples collected at BIUST?

1.14 Significance of the study

The research gave an idea of the type of drugs that are used in areas where the various wastewater samples were collected. The results obtained can allow relevant stakeholders to evaluate the effectiveness of drug raids, anti-drug campaigns or follow drug supply lines. The results obtained will also help relevant stakeholders in the fight against drug abuse as they provide the much-needed information on commonly used drugs, frequency of use, and demographic information of drug users. The findings of this research were also presented at two international conferences in February 2021 and February 2022, which resulted in knowledge and idea exchange that can contribute to this research positively in the future.

CHAPTER TWO: EXPERIMENTAL PROCEDURES

This chapter details the experimental protocols, materials and methods that were used for the detection of drugs in influent wastewater samples as well as the method used for the development and administration of the online survey questionnaire. The chapter is divided into subsections that highlight each process involved from sampling to analysis of the wastewater samples as well as the administration of the survey questionnaire.

2.1 GC-MS Experimental Procedure

2.1.1 Collection of wastewater samples

The influent wastewater samples used in this research were all collected by grab sampling from the Palapye-Botswana International University of Science & Technology (BIUST), Palapye-main village, Serowe-Mabogopa, Serowe-Radihemelo and Gaborone-Glen Valley WWTP in Botswana. Grab sampling was used as it was easy and convenient to use because of limited funds to purchase special equipment for 24 hours composite sampling which is mostly preferred in WBE.

BIUST is the university where the research was conducted. Palapye is a village in the central district of Botswana where BIUST is located. BIUST has a WWTP separate from the Palapye-main village WWTP. Serowe, which is about 40 km North-West of Palapye is also in the central district in Botswana and has two WWTP which are Serowe-Mabogopa and Serowe-Radihemelo. The Gaborone-Glen Valley WWTP is in Gaborone, the capital city of Botswana, and it is about 300 km South of Palapye. Figure 2.1 shows the areas where the wastewater samples were collected [77].



Figure 2.1: Map of Botswana, the circled areas are where the wastewater samples were collected for this research. Picture taken from Centre for Disease Control, Botswana Traveler View 2022.

For this research, 1000 mL of the influent wastewater samples were collected as it is considered sufficient [35],[40]. Polyethene terephthalate (PET) plastic bottles (1 litre) were used to store the wastewater samples. On the day of sampling, the samples were then transported to the Forensic Science laboratory at BIUST and stored in a fridge at 4 °C awaiting filtration, acidification, and storage at -20 °C. Samples were stored at -20 °C after two days of sampling to prevent degradation of the analytes of interest as the samples were extracted and analysed at a later date. Sampling for this research was done from July 2019 to October 2019. Sampling was done during the initial visit to the WWTP, after the last weekend of the month, after a long weekend/public holiday, or after a sporting event or major concert/festival. The details are listed in Table 2.1.

Samples were collected on the above-mentioned dates because most people in Botswana would have received their end-of-month salaries and students would also have received their monthly allowances or stipends and are likely to partake in activities that involve the use of more drugs such as clubbing and partying. During special events and weekends, the concentration of illicit drugs can increase [33],[78]. The availability of personnel from the WWTP and Water Utilities Corporation (WUC), who are the custodians of the Palapye-main village, Serowe-Mabogopa, Serowe-Radihemelo and Gaborone-Glen Valley WWTP, also influenced the date and time of sampling because sampling could only be done when they were free or available to assist at the wastewater ponds. Appointments for sampling were always made in advance.

A total of 18 influent wastewater samples were collected from the five different WWTP used for this research. These 18 samples were collected during the WWTP tour, initial visit to the WWTP, after the last weekend of the month, after a long weekend/public holiday, or after a sporting event or major concert/festival. Samples collected on the same day at the same WWTP were treated as one sample as they were mixed later at the laboratory. The samples were extracted using the Solid Phase Extraction(SPE) technique and analysed by Gas Chromatography-Mass Spectrometry as mentioned in sections 2.1.2 and 2.1.5 respectively.

Table 2.1:Details pertaining to collection of wastewater samples (date, volume and location, and reason for sampling on the particular date).

Sampling Site	Date of sampling	Sample amount collected in litre (L)				
		WWTP Tour	Initial visit to WWTP	After the last weekend of the month	After the long weekend/holiday	After a sporting event/concert/music festival
Palapye-BIUST	02/07/2019			2L		
	27/08/2019					2L
	02/10/2019				2L	
	28/10/2019				2L	
Palapye main village	03/07/2019			2L		
	03/09/2019			2L		
	02/10/2019				2L	
Serowe-Radihemelo	05/08/2019		2L			
	27/08/2019	1L				
	02/10/2019				2L	
	28/10/2019				2L	
Serowe-Mabogopa	27/08/2019	1L				
	02/10/2019				2L	
	28/10/19				2L	
Gaborone-Glen Valley	09/08/2019		4L			
	10/09/2019					3L
	03/10/2019				3L	
	29/10/2019				4L	

2.1.2 Sample Preparation

The wastewater samples were filtered by suction filtration within 1 day after sampling using 11 µm (pore size) Whatman cellulose filter papers from Sigma Aldrich. In WBE filter papers with varying pore sizes such as 0.2-0.7 µm are used [44],[79],[80]. The samples are filtered to remove particulate matter which can clog the solid phase extraction (SPE) cartridge sorbent and the Gas Chromatogram (GC) column, injection needles and liners if not removed efficiently [5]. The pH of the samples was then adjusted to 2.4-2.8 using 32% Hydrochloric acid. The pH meter used was always calibrated before use, using pH 4.0, 7.0 and 9.0 buffer solution. The acidified wastewater samples were then frozen at -20 °C before Solid Phase Extraction (SPE). This sample preparation process was adopted from a previous study on similar work [41].

2.1.2.1 Solid-Phase Extraction Technique

SPE was used to extract the analytes of interest from the sample matrix. The samples were extracted two months after collection. Samples were defrosted at room temperature 24 hours before extraction and re-filtered. Phenomenex Strata-X Drug B 33 µm, cation mixed-mode polymer sorbent in syringe barrels (60 mg, 3 mL) were used for extraction.

A total of 18 samples were collected from the WWTP (see Table 2.1) and 300 mL from each sample was extracted. The wastewater samples were mixed thoroughly to ensure homogeneity before the 300 mL was taken for SPE. Two SPE cartridges were used per sample and 150 mL of wastewater was loaded in each cartridge. Previously published SPE protocols as well as the Strata-X Drug B manufacturers protocols were used with modification (see Table 2.2). The eluate was collected in 8 mL amber glass vials and stored in a freezer at -20 °C after evaporating the eluents awaiting analysis.

Table 2.2: Modified Solid-Phase Extraction (SPE) Protocol for Strata -X Drug B 33µm cation mixed-mode polymer sorbent in syringes (60 mg, 3 mL)

Parameters	Conditions
Sample Loading	150 mL wastewater sample
Rinse	2 mL acidified distilled water (pH 2.5)
Drying	15 minutes under high vacuum
Elution 1	2 mL Ethyl acetate/isopropanol (85:15 v/v)
Elution 2	4 mL ethyl acetate/isopropanol/ammonium hydroxide (70:20:10 v/v/v)
Evaporation	Heating block at 40°C under Nitrogen

2.1.2.2 Preparation of extracted wastewater samples and drug standards for GC-MS analysis

Prior to GC-MS analysis, the dried extracted wastewater samples were removed from the freezer, brought to room temperature then reconstituted with 50 μ L ethyl acetate containing 10 μ g/mL internal standard (IS) and vortexed for 10 minutes at a speed of 600 rpm using a Stuart vortex mixer and then transferred into a GC-MS vial insert. The drug standard solutions were transferred directly from the glass vials in the 4 °C fridge into GC-MS vial inserts and brought to room temperature before analysis. The drug standard solutions were always analysed before the wastewater samples. Ethyl acetate was used as a solvent blank, and it was run between the standards as well as between the wastewater samples to avoid sample carry-over.

2.1.3 Solvents and standards used in this research

All the solvents and standards used in this research are listed in Table 2.3. Ethyl acetate, methanol, and isopropanol were of American Chemical Society (ACS) reagent grade (purity $\geq$ 99.5%). The 32% hydrochloric acid was also ACS grade while the 25% ammonium hydroxide was of International Organisation for Standardisation (ISO) grade. Docosane, caffeine and ibuprofen standards were analytical standards in powder form.

Table 2.3: List of all solvents and standards that were used for this research.

Solvents	Standards
Ethyl acetate	Docosane
Methanol	Caffeine
32% hydrochloric acid	Ibuprofen
25% ammonium hydroxide	Toxicology Checkout Mixture with the following compounds: d-amphetaminesulfate salt (as d-amphetamine), a, a-dimethyl phenethylamine, (+)- methamphetamine hydrochloride (as (+)-methamphetamine), nicotine, (±)-3,4-methylenedioxyamphetamine hydrochloride (as MDA), (±)-3,4-methylenedioxymethamphetamine hydrochloride (as MDMA), (±)-3,4- methylenedioxy-N-ethyl-amphetamine hydrochloride (as MDEA), meperidine hydrochloride (as meperidine), phencyclidine hydrochloride (as phencyclidine) (±)-methadone hydrochloride (as methadone), cocaine hydrochloride (as cocaine), proadifen hydrochloride (as proadifen), oxazepam, codeine, (±)-lorazepam, diazepam, hydrocodone (+)-bitartrate salt (as hydrocodone), THC, oxycodone hydrochloride (as oxycodone), temazepam, diacetylmorphine hydrochloride monohydrate (as heroin), nitrazepam, clonazepam, alprazolam, verapamil hydrochloride (as verapamil), strychnine, trazodone hydrochloride (as trazodone) and flunitrazepam
Isopropanol	
All the solvents and standards were from Sigma Aldrich except for the Toxicology Checkout Mixture which was from Agilent Technologies.	

2.1.4 Preparation of drug standards

Docosane: Internal Standard - a 0.5 mg/mL stock solution of docosane was prepared in ethyl acetate. A working solution of 10 µg/mL was prepared from the stock solution.

Toxicology Checkout Mixture- 2.5 mL of the 10 µg/mL docosane internal standard was added to 500 µL of 5 µg/mL Toxicology Checkout Mixture (TCM). The drugs in the TCM are listed in Table 2.3. The final concentrations of Docosane and the TCM were 5 µg/mL and 0.5 µg/mL, respectively.

Caffeine Standard - a 1 mg/mL caffeine stock solution was prepared in methanol. A working solution of 10 µg/mL was prepared from the stock solution and analysed with ethyl acetate as a blank.

Ibuprofen Standard - a 1 mg/mL ibuprofen stock solution was prepared in methanol. A working solution of 10 µg/mL was prepared from the stock solution and analysed with ethyl acetate as a blank.

All the drug standards solutions, caffeine and internal standard were put in glass vials after preparation and stored in a fridge at 4 °C. Ethyl acetate was used as a blank when the standards and wastewater samples were analysed by GC-MS.

2.1.5 Sample Analysis by Gas Chromatography-Mass Spectrometry

The GC-MS with Electron Ionisation (EI) was the instrument of choice as it was readily available in the university where this research was carried out. The EI allowed for a comparison of the results obtained with those obtained by other laboratories. The instrumental analysis was done on an Agilent Technologies Gas Chromatograph-Mass Spectrometer (GC-MS) with an HP-5MS 30 m x 250 µm x 0.25 µm column. The Mass Spectrometer (MS) was autotuned before the analysis was done. The GC parameters listed in Table 2.4 and the MS parameters listed in Table 2.5 that were used for this research were adapted from previous studies [5],[41].

Table 2.4:Modified Instrumental Parameters for Gas-Chromatography

GC Parameters	GC Conditions
Injector Temperature	250 °C
Injection Volume	4 µL
Injection Mode	Splitless
Oven Temperature Programme	50 °C held for 2 minutes, increased to 280 °C at 8 °C/min and held for 5 minutes
Run time	31.167 minutes
Solvent Delay	3.5 minutes
Transfer Line	280 °C

Table 2.5:Instrumental Parameters for Mass Spectrometer.

MS Parameters	MS Conditions
Ion Source	EI
Electron Energy	70 eV
Source Temperature	230 °C
Quad Temperature	150 °C
Mass Analyzer	Scan: 50-620 m/z

2.2 Online survey questionnaire Method

2.2.1 Study Design

The online survey questionnaire was administered to the BIUST community in Palapye from March 2021 to May 2021. The survey questionnaire was created via google forms. As of May 2021, BIUST had a population of two thousand five hundred and four (2504). Of the 2504 people, five hundred and twenty-five (525) were staff members while one thousand nine hundred and seventy-nine (1979) were students enrolled for the first semester of the academic year 2020/2021. Of the total number of students, 1565 were undergraduate and 414 were postgraduate.

The online survey questionnaire was administered to determine the use, disposal, and knowledge of the fate of pharmaceutical and illicit drugs in wastewater at BIUST. All the survey respondents were 18 years old and above and were part of BIUST when the survey

was administered. The Cochran formula (Equation 2.1) was employed for this study to determine the minimum sample size required. This formula allows for a calculation of the best possible size at a preferred confidence interval and precision [80].

Cochran Formula

$$\text{Equation 2.1} \quad n = \frac{Z^2 \times pq}{d^2}$$

Cochran Formula

$$\text{Equation 2.1} \quad n = \frac{Z^2 \times pq}{d^2}$$

From the Cochran formula in Equation 2.1, n is the required minimum sample size in a study. Z is the z-score or standard deviation (SD), p is the proportion of people who use drugs in the study area, $q=1-p$, whereas d is the acceptable error level. For this study, the acceptable error level (d) of 0.08 (8%) and confidence interval (CI) of 90% were employed. These are within the recommended CI and error levels for research in the social sciences [80]. The 90% CI corresponds to a z-score of 1.645. For BIUST, the number of people who use pharmaceutical drugs and or illicit drugs is not known, as a result, maximum heterogeneity with a 50:50 split in users and non-users of pharmaceutical and or illicit drugs was assumed with $p=0.5$.

Calculation of the sample size using Equation 2.1

$$n = \frac{Z^2 \times pq}{d^2}$$

$$n = \frac{1.645^2 \times (0.5 \times 0.5)}{0.08^2}$$

$$n = 105$$

The minimum sample size required for this study was therefore one hundred and five (105) respondents if 50% of the BIUST population used pharmaceutical drugs and/or illicit drugs in the previous 24 months before the survey.

2.2.2 Sampling Method

A convenience sampling technique was employed for this study. The online survey questionnaire link was created through google forms. The online survey questionnaire link was sent to students and staff members at BIUST through their BIUST email addresses. The same link was also circulated via BIUST students' private Facebook group and the BIUST postgraduate students' private WhatsApp group for participation. Each participant had to partake in the survey only once. Accompanying the link was a short message which briefly explained what the survey was about. The survey was anonymous therefore identification of participants could not occur. A total of one hundred and twenty (120) people participated in the survey. This value was above the required minimum sample size, $n = 105$, for this study as determined using Equation 2.1.

2.2.3 Administration and measurement of research instrument

A pilot study was carried out to validate the actual questionnaire before it went live. The pilot survey was administered to six participants who were chosen by the author, and it was conducted in 24 hours. Both the pilot and actual survey had the same questions. The pilot survey participants gave positive feedback on it, and they made minor suggestions on the wording of some of the questions for better understanding and comprehension. The survey questionnaire was self-administered on all three platforms: email, Facebook, and WhatsApp. Completing the survey needed about 5-10 minutes.

Both close-ended and open-ended questions were included in the survey questionnaire. Close-ended questions were used to collect the sociodemographic data of the participants which included their age range, gender, and status. Close-ended questions were also used to examine if participants had used pharmaceutical or illicit drugs between March 2019 and May 2021 and if they were aware that when both pharmaceutical and illicit drugs are consumed/flushed down the toilet/drain they end up in WWTP and can be detected thereafter. Another close-ended question asked participants if they thought that there was enough information on the misuse of pharmaceutical drugs and the dangers of illicit drug use in Botswana.

Open-ended questions were used to examine the frequency of use of illicit drugs, the modes of disposal for both illicit and pharmaceutical drugs, the location where both illicit and

pharmaceutical drugs were used, the categories/types of drugs taken as well as suggestions on what can be done to improve the lack of knowledge on the misuse of pharmaceutical drugs and dangers of illicit drug use in Botswana.

2.2.4 Data Analysis

Thematic analysis was used for the analysis of textual data obtained from open-ended questions. For open-ended questions, participants were allowed to give more than one answer. In such cases each answer was treated as an individual response, resulting in the number of responses exceeding the total number of responses in a category. The statistical package for the social sciences (SPSS V.18.0) was used for inferential and descriptive statistics. The comparison of sociodemographic factors and the use or non-use of pharmaceutical/illicit drugs was carried out using inferential statistics. The Pearson 2 test was used to examine the impact of independent sociodemographic variables i.e., gender, age and status on the use and non-use of both pharmaceutical and illicit drugs in the study and $p=0.5$ and 95% confidence interval.

2.2.5 Community Involvement

The BIUST community participated in this study by completing an online survey questionnaire with sixteen questions. The survey form is included in Appendix I. The pilot study was conducted to validate the questionnaire as well as test the feasibility of the survey on a larger scale before it went live online. The questions focused on the use of pharmaceutical and illicit drugs between March 2019 and May 2021. Prior knowledge and experience of the researchers were used when developing the survey questionnaire. For recruiting survey questionnaire participants, the convenience sampling technique was employed.

2.2.6 Consent

The permit to carry out this research was granted by the Ministry of Tertiary Education, Research, Science and Technology in Botswana. The application for the research permit also included the questions included in the survey. A consent form was provided at the beginning of the survey so that all the respondents could have a better understanding of the type of information that was required from them and how it would be used (see Appendix I). In addition, all respondents had to acknowledge being above the age of 18. Due to the nature of the survey, the answers could not be redacted once submitted. However, if the respondents wished to leave the survey at any time, they could leave the webpage before

their answers were submitted. If consent was provided by answering ‘Yes’ at the beginning of the survey, the respondents were automatically authorizing the researcher to use their answers in the research. If the answer to giving consent was ‘No’ the respondents could not proceed to the next stage.

CHAPTER THREE: RESULTS AND DISCUSSION

This chapter presents the GC-MS results for the Toxicology Checkout Mixture (TCM), ibuprofen standard, caffeine standard and influent wastewater samples as well as their discussion. The wastewater samples were collected by grab sampling once a month, between July 2019 and October 2019 at the Palapye-BIUST, Palapye-main village, Serowe-Radihemelo, Serowe-Mabogopa and Gaborone-Glen Valley wastewater treatment plants in Botswana. The TCM, caffeine standard, ibuprofen standard and all the wastewater samples were analysed using a GC-MS method mentioned in section 2.1.5.

This chapter also presents the results and discussion of the online survey questionnaire that was administered at BIUST in Palapye, Central District, Botswana from March 2021 to May 2021. The online survey questionnaire enabled the researcher to assess the respondents' age, gender, status, their knowledge about pharmaceutical and illicit drugs, including their fate in wastewater. This information helped with analysing differentiated knowledge and understanding based on the sociodemographic data of the respondents.

3.1 GC-MS Results and Discussion

Identification of the compounds present in the standards and wastewater samples was based on retention time (RT), relative retention factor (RRF), relative intensities, mass of ions, reverse match factor and mass spectral library search using the National Institute of Science and Technology (NIST) Mass Spectral (MS) Search 2.0 library. The reverse match factor compares similar peaks of the unknown mass spectrum to the peaks in the library mass spectrum. When the peaks in the unknown mass spectrum are not in the library mass spectrum (reference spectrum) they are ignored [81]. The reverse match factor score is useful when two compounds are co-eluting in the chromatogram [81]. The general guidelines for reverse match factor scores as suggested by NIST are listed in Table 3.1.

Table 3.1: Reverse match factor scores and corresponding mass spectral match for NIST mass spectral library search

Reverse Match Factor Score	Mass spectral match
>900	Excellent Match
800-900	Good Match
700-800	Fair Match
<600	Poor Match

3.1.1 Drug Standards Results

A TCM was used as a multi-drug standard for this research. The other standards that were used are ibuprofen and caffeine. The use of standards aid in establishing the accuracy and precision of the test method employed.

3.1.1.1 Toxicology Checkout Mixture Results

Figure 3.1 depicts the total ion chromatogram (TIC) of the TCM. The drugs that were contained in the original TCM are listed in Table 2.3. Amphetamine, the first compound to be detected in the TCM had a retention time of 6.67 minutes and heroin, the last compound to be detected had a retention time of 26.11 minutes. The internal standard (IS), docosane, was detected at 20.89 minutes.

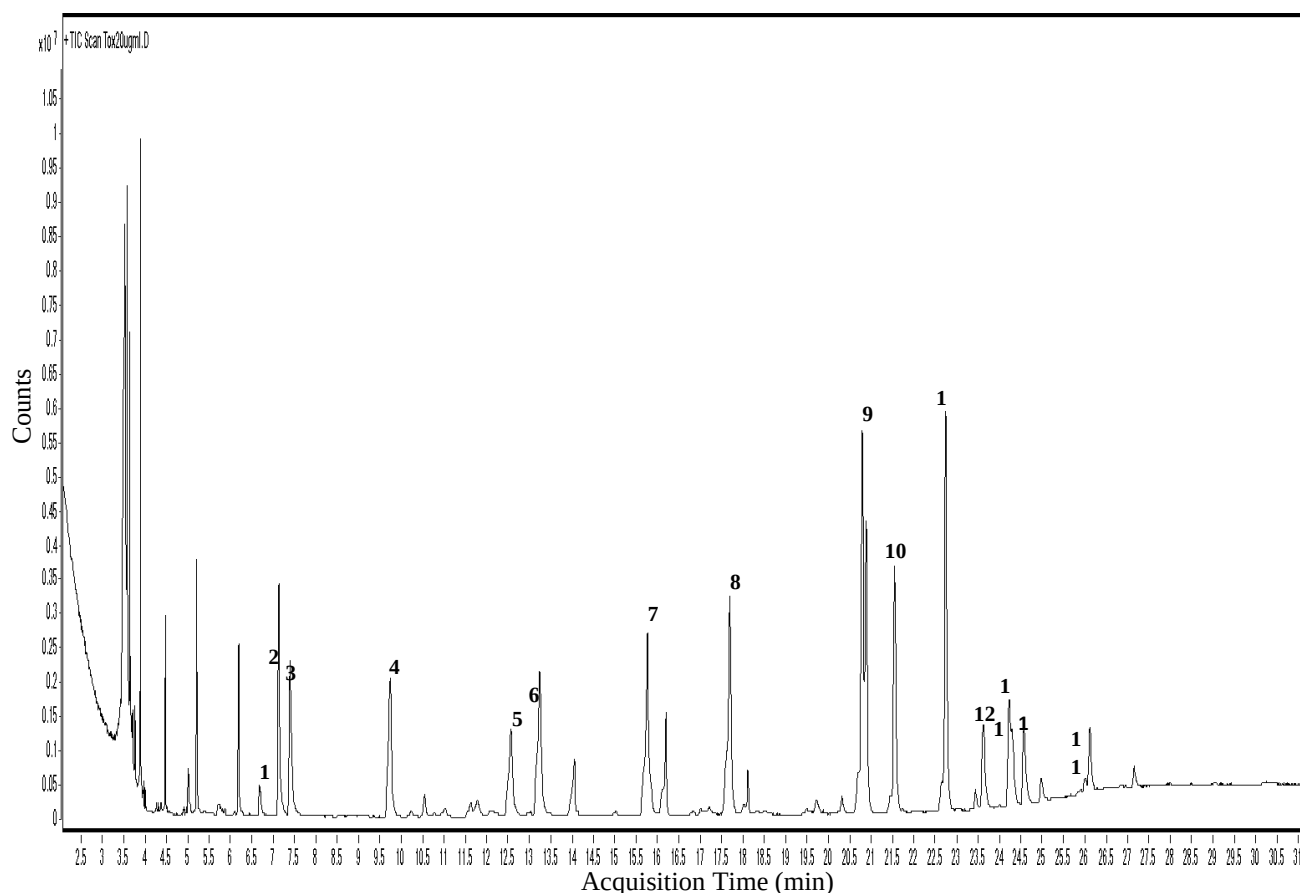


Figure 3.1: Total Ion Chromatogram of a toxicology checkout mixture showing various peaks including detected drugs (see Table 3.2). amphetamine (1), phentermine (2), methamphetamine (3), nicotine (4), MDMA (5), MDEA (6), meperidine (7), phencyclidine (8), methadone (9), cocaine (10), proadifen (11), codeine (12), diazepam (13), hydrocodone (14), THC (15), flunitrazepam (16) and heroin (17).

The relative intensities of the compounds are listed in Table 3.2. The relative intensities of amphetamine, cocaine and flunitrazepam differ between the TCM (unknown mass spectra) and the NIST library (reference/library spectra). The relative intensities of any of the diagnostic ions are not supposed to differ by more than the amount listed in Table 3.4 from the relative intensities of the same ions from a reference material that was analysed contemporaneously [82].

The difference in relative intensities for amphetamine could be due to the mass spectrometer start mass that was used when the sample was analysed. The start mass was set at 50 while the end mass was set to 620 (see Table 2.5). The start mass was set following the validated GC-MS method adopted for this research [41]. Amphetamine normally has a 44 m/z peak which is the base peak, however, the amphetamine spectrum that was collected for this research did not have the 44 m/z peak which resulted in amphetamine having a base peak of 91 m/z in the spectrum obtained for the TCM [126]. Normally, the 91 m/z peak in the amphetamine mass spectrum is the second tallest.

When comparing the mass spectrum of the unknown with the reference library it is important to compare like with like, that is, the same ionisation technique and instrumental technique used to obtain the reference mass spectrum should be the same one that was used for the sample. When performing the library search for this research, there were cases where the instrument matched the unknown mass spectrum obtained with the reference mass spectrum in which Electrospray Ionisation (ESI) was used as the ionization technique even though Electron Ionisation (EI) technique was used for this research. Therefore, whenever a match was made on the instrument, cross-checking of the reference mass spectrum had to be done to ensure that the ionisation technique used when generating the reference mass spectra was EI. This was to make sure that when comparing the unknown with the known spectrum, we are comparing like with like.

In EI a sample is volatilized directly in the ion source, that is in the vacuum system attached to the analyser. The molecules in the gaseous phase are then bombarded by a beam of electrons formed by heating a filament resulting in the electron beam ejecting an ion to produce a radical [83]. EI is considered a hard ionisation technique because it causes the ion to fragment. In ESI, the electrospray is created by putting a high voltage on a flow of liquid at atmospheric pressure. The created spray is then directed to an opening in the vacuum system of the MS, where the droplets are de-solvated by a combination of heat, vacuum, and acceleration into a gas by voltages [83]. The ions are then ejected from the droplets and accelerated into the mass analyser by voltages. Due to the natural use of a flowing liquid, it is easily adapted to Liquid Chromatography.

Ideally, when one has pure drug standards, they can create their library databases. This would allow confident identification and confirmation of compounds as a comparison will be made using the results that were obtained contemporaneously under the same conditions. Another identification point that was used for this research is the reverse match factor score. In some instances, a very high reverse match factor score was obtained while the relative intensities for the ions differed between the sample and the NIST library and vice-versa. The various compounds that were detected in the TCM are listed in Table 3.2.

Table 3.2: List of drugs in the toxicology checkout mixture with their retention times, relative retention factors, reverse match factors, ions of interest and ion ratios. The retention time of the internal standard was 20.89 minutes.

Compound Name	Retention Time (minutes)	Relative Retention Factor (RRF)	Reverse Match Factor	Ions of interest and their relative intensities from TCM (%)	Ions of interest and their relative intensities from NIST MS library (%)	Ion Ratio
Amphetamine	6.67	3.13	913	Q- 91m/z (99.9) C1-65m/z (45.9) C2-120m/z (16.2)	Q- 91m/z (39.8) C1-65m/z (12.8) C2-120m/z (6.5)	0.134
Phentermine	7.12	2.93	973	Q- 58m/z (99.9) C1-91m/z (12.9) C2-134m/z (6.2)	Q- 58m/z (99.9) C1-91m/z (10.3) C2-134m/z (4.2)	0.060
Methamphetamine	7.39	2.83	950	Q- 58m/z (99.9) C1-91m/z (10.0) C2-65m/z (4.6)	Q- 58m/z (99.9) C1-91m/z (8.4) C2-65m/z (3.8)	0.043
Nicotine	9.73	2.15	946	M ⁺ -162m/z (16.3) Q-84m/z (99.9) C1-133m/z (32.5)	M ⁺ -162m/z (11.9) Q-84m/z (99.9) C1-133m/z (42.0)	0.163
3,4-Methylenedioxymethylamphetamine (MDMA)	12.56	1.66	960	Q- 58m/z (99.9) C1-135m/z (6.2) C2-77m/z (6.1)	Q- 58m/z (99.9) C1-135m/z (7.3) C2-77m/z (7.0)	0.058
N-Ethyl-3,4-methylenedioxymethylamphetamine (MDEA)	13.23	1.58	944	Q- 72m/z (99.9) C1-135m/z (6.5) C2-77m/z (6.2)	Q- 72m/z (99.9) C1-135m/z (10.5) C2-77m/z (8.1)	0.059
Meperidine	15.76	1.33	946	M ⁺ -247m/z (38.4) Q-71m/z (99.9) C1-172m/z (29.0)	M ⁺ -247m/z (30.7) Q-71m/z (99.9) C1-172m/z	0.285

					(35.1)	
Phencyclidine	17.68	1.18	947	Q- 200m/z (99.9) C1-91m/z (37.0) C2-242m/z (31.4)	Q- 200m/z (99.9) C1-91m/z (40.5) C2-242m/z (30.2)	0.306
Methadone	20.78	1.01	938	Q-72m/z (99.9) C1-165m/z (2.7) C2-178m/z (2.5)	Q-72m/z (99.9) C1-165m/z (3.5) C2-178m/z (2.5)	0.019
Cocaine	21.54	0.97	954	M ⁺ -303m/z (18.0) Q-82m/z (99.9) C1-182m/z (99.0)	M ⁺ -303m/z (13.3) Q-82m/z (99.9) C1-182m/z (80.3)	0.182
Proadifen	22.74	0.92	946	Q- 86m/z (99.9) C1-99m/z (18.7) C2-58m/z (3.1)	Q- 86m/z (99.9) C1-99m/z (21.7) C2-58m/z (6.4)	0.030
Codeine	23.61	0.88	955	M ⁺ & Q-299m/z (99.9) C1-162m/z (42.9) C2-229m/z (27.5)	M ⁺ & Q-299m/z (99.9) C1-162m/z (50.4) C2-229m/z (26.9)	0.263
Diazepam	24.22	0.86	969	M ⁺ -284m/z (72.2) Q- 256m/z (99.9) C1-283m/z (92.6)	M ⁺ -284m/z (65.3) Q- 256m/z (99.9) C1-283m/z (94.9)	0.682
Hydrocodone	24.29	0.86	875	M ⁺ & Q-299m/z (99.9) C1-242m/z (53.3) C2-185m/z (29.0)	M ⁺ & Q-299m/z (99.9) C1-242m/z (56.5) C2-185m/z	0.223

					(24.6)	
THC	24.57	0.85	935	M ⁺ -314m/z (81.6) Q-299m/z (99.9) C1-231m/z (87.6)	M ⁺ -314m/z (79.5) Q-299m/z (99.9) C1-231m/z (78.9)	0.831
Flunitrazepam	26.00	0.80	762	Q-285m/z (80.9) C1-312m/z (88.4) C2-266m/z (50.0)	Q-285m/z (99.9) C1-312m/z (94.2) C2-266m/z (56.3)	0.261
Heroin	26.11	0.80	911	M ⁺ 369m/z (70.3) Q-327m/z (99.9) C1-268m/z (65.5)	M ⁺ 369m/z (80.1) Q-327m/z (99.9) C1-268m/z (61.1)	0.651
M ⁺ = Molecular ion, Q=Base peak, C1=Confirmation Ion 1 and C2=Confirmation Ion 2						
RRF=Retention Time of Internal Standard / Retention Time of Analyte						

Most of the compounds have reverse match factors above 900 which shows an excellent match on the NIST MS library. Hydrocodone has a reverse match factor of 875 showing a good match while flunitrazepam has a reverse match factor of 762, indicating a fair match on the NIST MS library. The match factor value is affected by the number of peaks in a mass spectrum

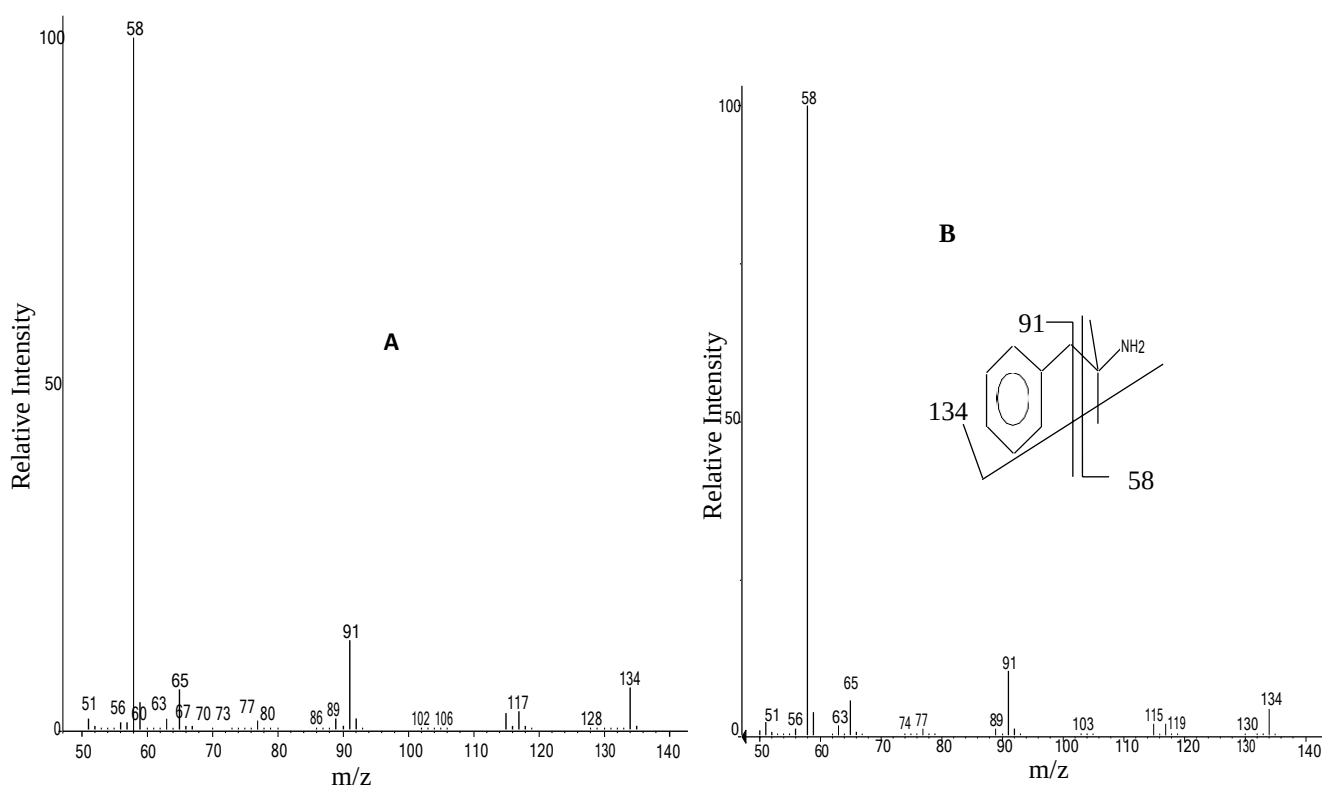


Figure 3.2: Mass spectrum of phentermine detected in the TCM (A) with mass spectrum and structure of phentermine from NIST MS library (B). Both A and B show Q of 58 m/z, C1 of 91 m/z and C2 of 134 m/z.

Figure 3.2 depicts the mass spectra of phentermine detected in the TCM (A) and from the NIST MS library database (B). The fragmentation patterns depicted in Figure 3.2 (B) and the rest of this document were done using Microsoft Office PowerPoint 365. Fragmentation patterns are also shown in all the mass spectra (which have structures) presented in this thesis. The diagnostic ions 58 m/z, 91 m/z, and 134 m/z in Figure 3.2, which were also used in the identification of phentermine, are present in both (A) and (B). For this research three diagnostic ions were monitored to improve the reliability in the identification and confirmation of the detected compounds [82].

For this research, the identification points used were diagnostic ions, retention time, relative retention factor, relative intensities, and reverse match factors. These identification points are all included in various Tables under the different subsections in this chapter. The RRF was calculated by dividing the retention time of the internal standard by that of the analyte of interest see (Equation 3.1).

$$\text{Equation 3.1} \quad \text{RRF} = \frac{\text{RT of internal standard}}{\text{RT of analyte}}$$

Example calculation of RRF

Equation 3.1 was used to calculate the RRF of phentermine detected in the TCM. The retention time (RT) of phentermine was 7.12 minutes (see Table 3.2) while that of the internal standard (docosane) was 20.89 minutes.

$$\text{RRF} = \frac{\text{RT of internal standard}}{\text{RT of analyte}}$$

$$\text{RRF} = \frac{20.89 \text{ minutes}}{7.12 \text{ minutes}}$$

$$\text{RRF} = 2.93$$

A total of 17 compounds that were detected in the TCM are listed in Table 3.2, however, only the mass spectrum of phentermine is included as an example. The mass spectra of the other 16 compounds in Table 3.2 are contained in Appendix II and all mass spectra results (selection ions and their relative intensities) are included in Table 3.2.

3.1.1.2 Ibuprofen Standard Results

Figure 3.3 depicts the TIC of the ibuprofen standard. Ibuprofen was detected at 14.02 minutes and the internal standard (IS) was detected at 20.87 minutes.

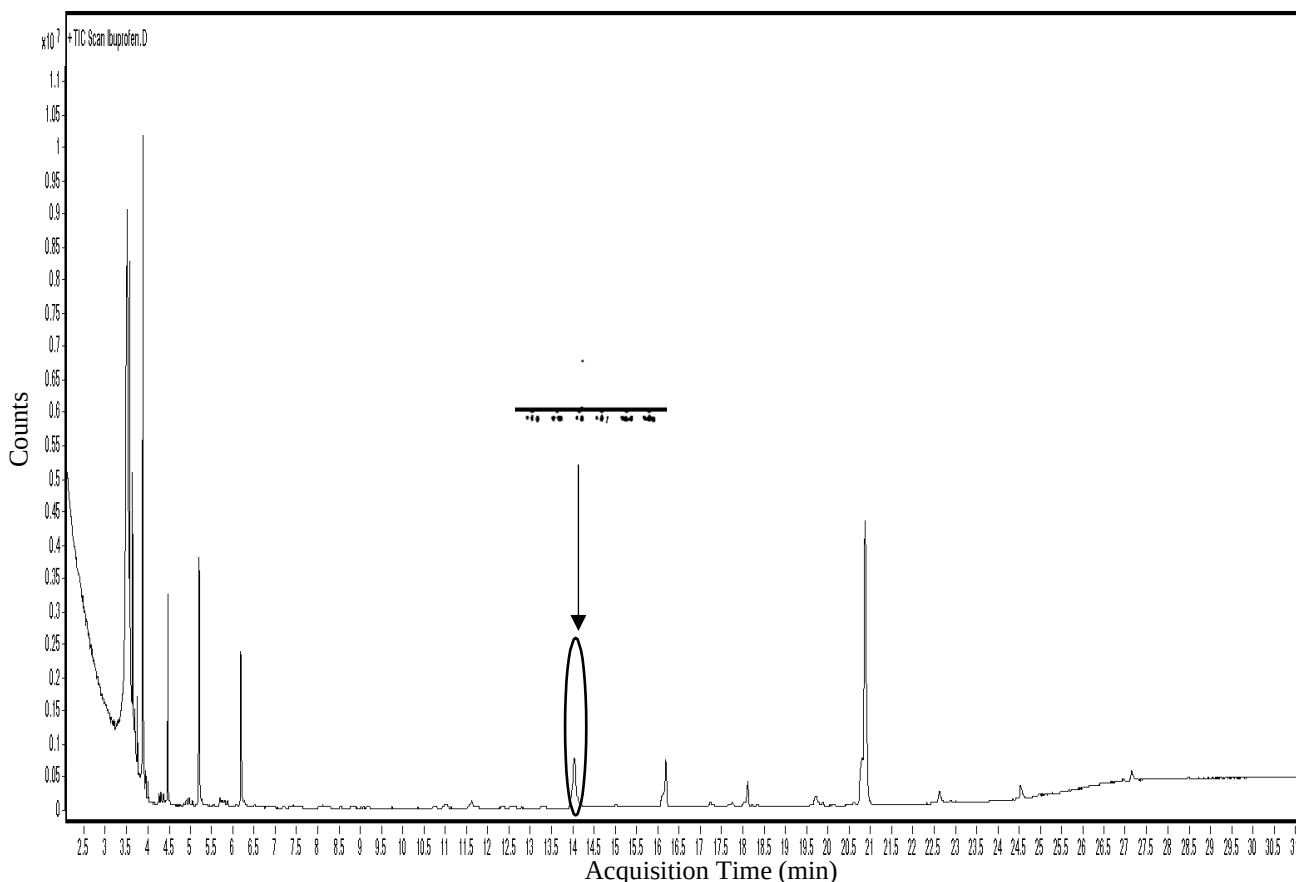


Figure 3.3: Total Ion Chromatogram of an Ibuprofen standard. The area highlighted shows the area where the Ibuprofen peak with a retention time of 14.02 minutes is found. The zoomed in peak is the ibuprofen peak.

Table 3.3 shows the reverse match factor of ibuprofen, which is 934, indicating an excellent match of the compound in the NIST MS library search.

Table 3.3: Ibuprofen standard with its retention time, relative retention factor, reverse match factor, ions of interest and ion ratio. The retention time of the internal standard was 20.87 minutes.

Compound Name	Retention Time (minutes)	Relative Retention Factor (RRF)	Reverse Match Factor	Ions of interest and their relative intensities from Ibuprofen standard (%)	Ions of interest and their relative intensities from NIST MS library (%)	Ion Ratio
Ibuprofen	14.02	1.49	934	Q-161m/z (96.6) C1-91m/z (60.0) M ⁺ 206m/z (45.4)	Q-161m/z (99.9) C1-91m/z (68.1) M ⁺ 206m/z (46.9)	0.550
M ⁺ = Molecular ion, Q=Base peak, and C1= Confirmation Ion 1						
RRF=Retention Time of Internal Standard/Retention Time of Analyte						

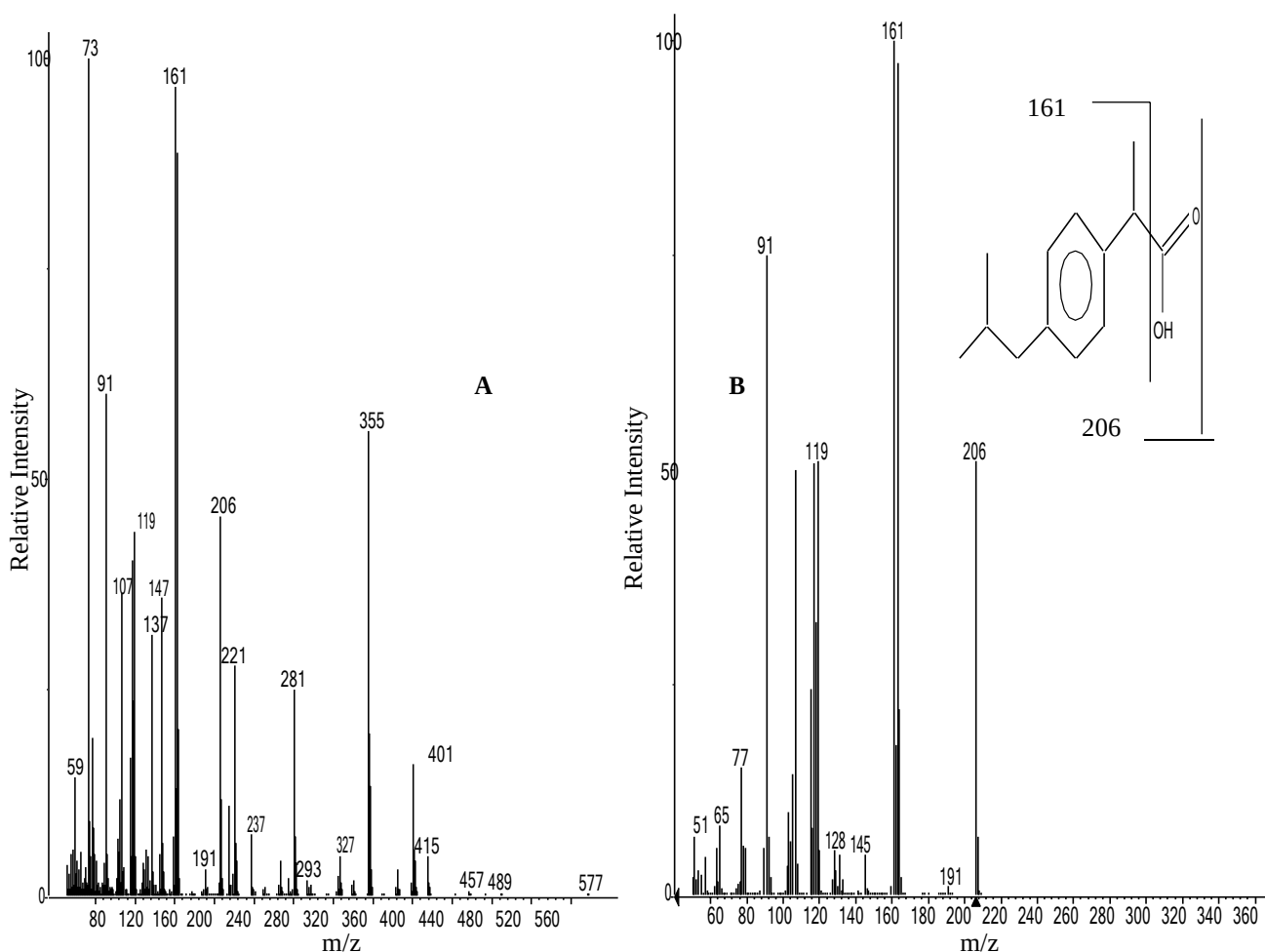


Figure 3.4: Mass spectrum of Ibuprofen detected in the ibuprofen standard (A). Mass spectrum and structure of ibuprofen from NIST MS library (B). Both A and B show Q of 161 m/z, C1 of 91 m/z and M⁺ of 206 m/z.

Figure 3.4 (A) shows the mass spectrum of the ibuprofen standard while Figure 3.4 (B) shows the mass spectrum and structure of ibuprofen from the NIST MS library. The detected ibuprofen had a retention time of 14.02 minutes and a relative retention factor of 1.49 as listed in Table 3.3. The diagnostic ions 161 m/z, 91 m/z and 206 m/z that are used to identify ibuprofen are present in both spectra. The relative intensities of all the three diagnostic ions are very similar (see Table 3.3) indicating the identification of the compound.

The ion ratio of ibuprofen is included in Table 3.3. The ion ratio is calculated as the peak area ratio of a less intense ion to that of a more intense ion for any given compound [84]. The peak areas that were used were all obtained from the instrument after the integration of the extracted chromatograms. In this research, 3 mass ions are listed for each compound whether standard or wastewater sample. The less intense ion of the 3 mass ions listed is the mass ion with the lowest relative intensity while the more intense ion of the 3 mass ions listed is the one with the highest relative intensity from a given compound in the sample/standard. The permitted tolerances for relative ion intensities depend on the mass spectrometric technique used and the percentage of the base peak [85].

Ion ratio calculation example

Using ibuprofen as an example, the ion ratio was calculated using Equation 3.2. The less intense ion is the mass ion with the lowest relative intensity while the more intense ion is the one with the highest relative intensity from a given compound in the sample. These relative intensities can be found in Tables 3.2, 3.3, 3.5, 3.6, 3.7 and 3.8.

$$\text{Equation 3.2} \quad \text{Ion ratio} = \frac{\text{Peak area of less intense ion}}{\text{Peak area of a more intense ion}}$$

- a) The peak areas for the ibuprofen ions in column 5, Table 3.3 were obtained after the integration of the extracted ion chromatograms.

$$\text{Ion Ratio} = \frac{422930.73}{769197.15}$$

$$\text{Ion ratio} = 0.550$$

Once the ion ratio of the sample and that of the reference standard have been calculated or are known, the compliance of the sample ion ratio can be assessed.

Equation 3.3 was used to check the compliance of the ion ratios in the samples (see Table 3.4).

Equation 3.3

$$\text{Rel.Diff} = \frac{\text{Ion Ratio}_{\text{sample}} - \text{Ion Ratio}_{\text{ref}}}{\text{Ion Ratio}_{\text{ref}}} \times 100\%$$

Equation 3.2 was used to calculate the ion ratios of the TCM, ibuprofen standard and caffeine standard in subsections 3.1.1.1, 3.1.1.2 and 3.1.1.3 respectively. Equation 3.3 could not be applied to subsections 3.1.1.1, 3.1.1.2 and 3.1.1.3 as they are standards, and they will be used to check the compliance of the ions in the samples. However, both Equations 3.2 and 3.3 were applied to all the wastewater samples in which ibuprofen or caffeine were detected.

Table 3.4, which was adapted from the World Anti-Doping Agency (WADA) Document TD2010IDCR, contains the tolerance limits for relative intensities which were used to ensure appropriate confidence in the identification of compounds [82]. The relative intensities of any given ion should not differ by more than the amount listed in Table 3.4 from the relative intensities of the same ions acquired from a reference material that was analysed contemporaneously [82].

Table 3.4: Maximum tolerance windows for relative Ion Intensities

Relative Abundance (% of base peak)	Electron Ionisation _GC-MS
> 50%	±10%
25% to 50%	± 20%
5% to <25%	±5%
<5%	± 50%

3.1.1.3 Caffeine Standard Results

Figure 3.5 depicts the TIC of the caffeine standard. Caffeine was detected at 16.82 minutes while the IS was detected at 20.87 minutes.

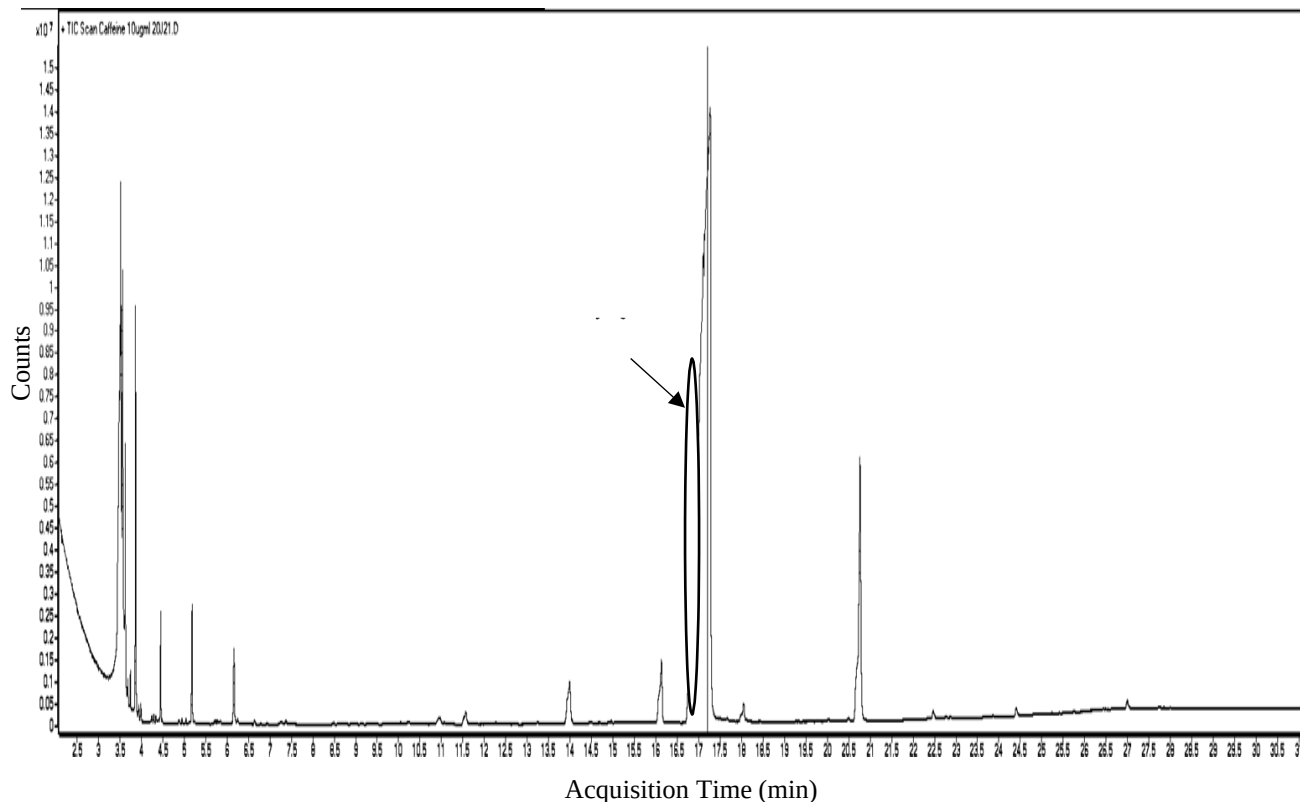


Figure 3.5: Total Ion Chromatogram of a caffeine standard. The area highlighted shows the area where the caffeine peak with a retention time of 16.84 minutes is found. The zoomed in peak is the caffeine peak.

Table 3.5 shows the reverse match factor of caffeine which is 963, indicating an excellent match of the caffeine in the standard to the NIST MS library search.

Table 3.5:Caffeine standard with its retention time, relative retention factor, reverse match factor, ions of interest and ion ratio. The retention time of the internal standard was 20.87 minutes.

Compound Name	Retention Time (minutes)	Relative Retention Factor (RRF)	Reverse Match Factor	Ions of interest and their relative intensities from caffeine standard (%)	Ions of interest and their relative intensities from NIST library (%)	Ion Ratio
Caffeine	16.84	1.24	963	Q & M ⁺ -194m/z (99.9) C1-109m/z (52.1) C2-82m/z (21.5)	Q & M ⁺ -194m/z (99.9) C1-109m/z (74.2) C2-82m/z (30.4)	0.199
M ⁺ = Molecular ion, Q=Base peak, C1=Confirmation Ion 1 and C2=Confirmation Ion 2 RRF=Retention Time of Internal Standard/Retention Time of Analyte						

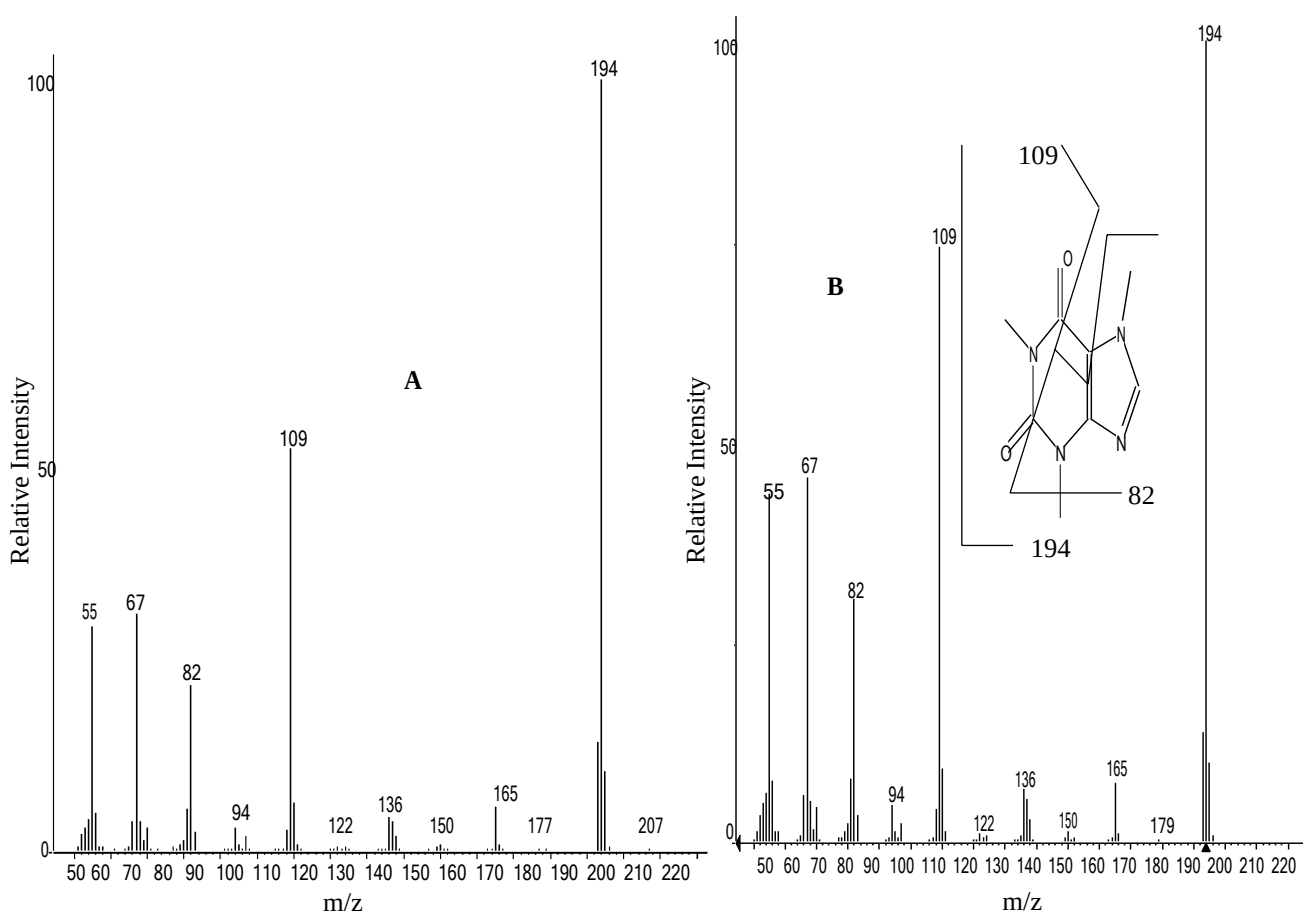


Figure 3.6:Mass spectrum of caffeine detected in the caffeine standard (A). Mass spectrum and structure of caffeine from NIST MS library (B). Both A and B show M⁺ & Q of 194 m/z, C1 of 109 m/z and C2 of 82 m/z

The mass spectrum depicted in Figure 3.6 (A) is that of caffeine that was detected in the caffeine standard while Figure 3.6 (B) shows the mass spectrum and structure of caffeine from the NIST MS library. The detected caffeine had a retention time of 16.84 minutes and a relative retention factor of 1.24 as listed in Table 3.5. The diagnostic ions 194 m/z, 109 m/z and 82 m/z that are used to identify caffeine can be seen in both spectra Figures 3.6 (A) and (B).

3.1.2 Serowe-Mabogopa Wastewater Treatment Plant

Three influent wastewater samples were collected at the Serowe-Mabogopa (SM) WWTP. The Serowe-Mabogopa WWTP serves different parts of Serowe including the Serowe abattoir, Serowe brigade, Serowe police, Serowe main mall and Serowe Engen mall. The six digits following SM denotes the day, month, and year of sampling, respectively. All the three samples collected had compounds of interest to this research.

3.1.2.1 SM021019 Wastewater sample

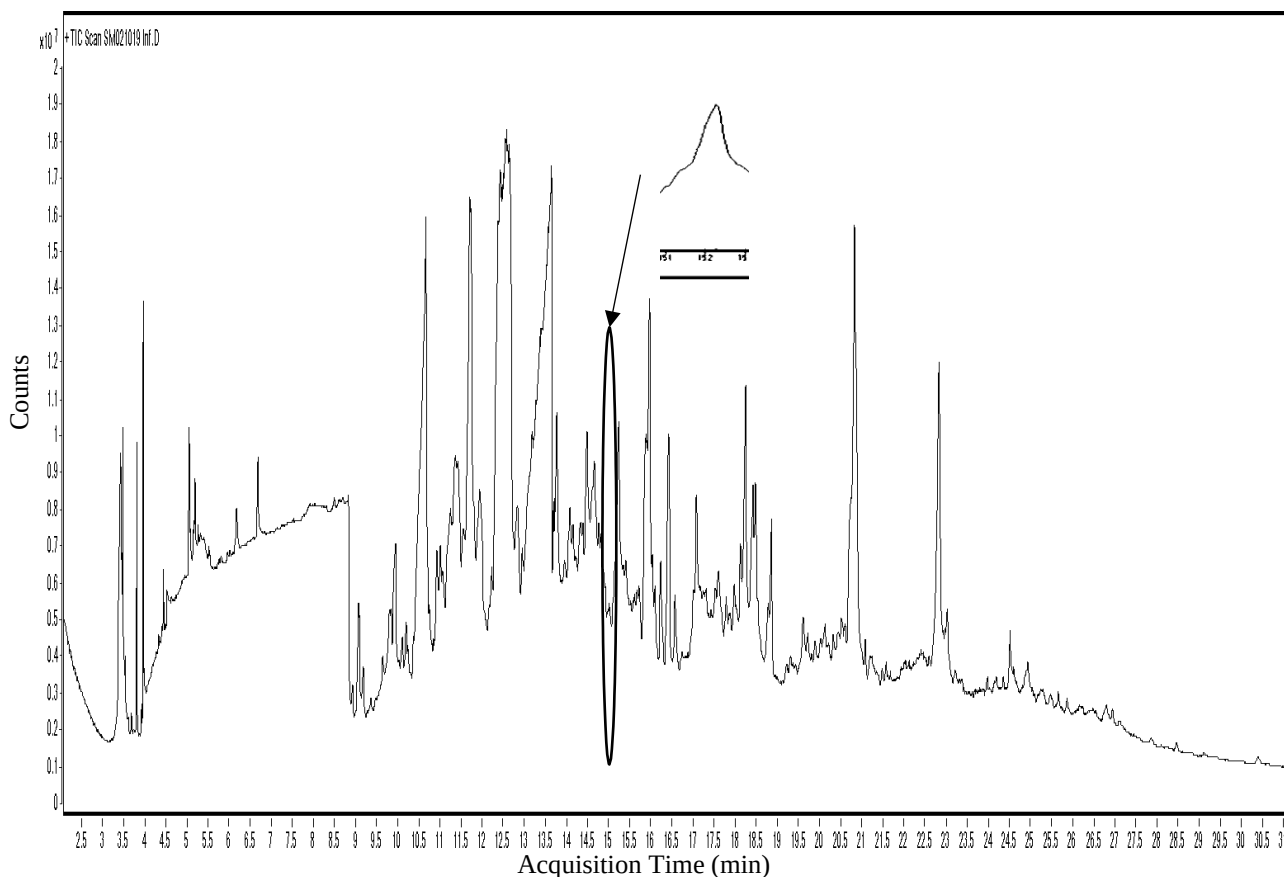


Figure 3.7: Total Ion Chromatogram of a SM WW influent sample collected on 02/10/19. The area highlighted show where a xylazine peak with retention time of 15.26 minutes is found. The zoomed in peak is the xylazine peak.

The TIC in Figure 3.7 is for the SM021019 wastewater sample. Two compounds of interest, xylazine and ibuprofen were detected in this wastewater sample (Figure 3.7). The area highlighted shows where one of the compounds, xylazine, with a RT of 15.26 minutes was detected. The internal standard, docosane was detected at 20.87 minutes.

The detected ibuprofen had a RT of 14.14 minutes and an RRF of 1.48 as listed in Table 3.6. The RT and RRF of the ibuprofen detected in the SM021019 sample are very close to those of the ibuprofen standard which were 14.02 minutes and 1.49 respectively (see Table 3.3). In Table 3.6 xylazine has a reverse match factor of 639 while ibuprofen has a reverse match factor of 746. The reverse match factor scores indicate a fair match on the NIST MS library.

Table 3.6:List of compounds detected in the SM021019 sample with their retention times, relative retention factors, ions of interest, reverse match factor, use and ion ratios. The retention time of the internal standard was 20.87 minutes.

Compound Name	Retention Time (minutes)	Relative Retention Factor (RRF)	Reverse Match Factor	Ions of interest and their relative intensities (%) from the SM021019 WW sample	Ions of interest and their relative intensities from NIST library (%)	Use	Ion Ratios
Ibuprofen	14.14	1.48	746	Q-161m/z (53.1) M ⁺ 206m/z (34.5) C1-91m/z (44.6)	Q-161m/z (99.9) M ⁺ 206m/z (50.7) C1-91m/z (74.7)	Non-steroidal anti-inflammatory drug [86].	0.454
Xylazine	15.26	1.37	639	M ⁺ 220m/z (20) Q-205m/z (99.9) C1-105m/z (6.9)	M ⁺ 220m/z (71.7) Q-205m/z (99.9) C1-105m/z (10.5)	A veterinary drug used for sedative and analgesic purposes. Also used in drug-facilitated sexual assault [87].	0.034
M ⁺ = Molecular ion, Q=Base peak, C1=Confirmation Ion 1 and C2=Confirmation Ion 2 RRF=Retention Time of Internal Standard/Retention Time of Analyte							

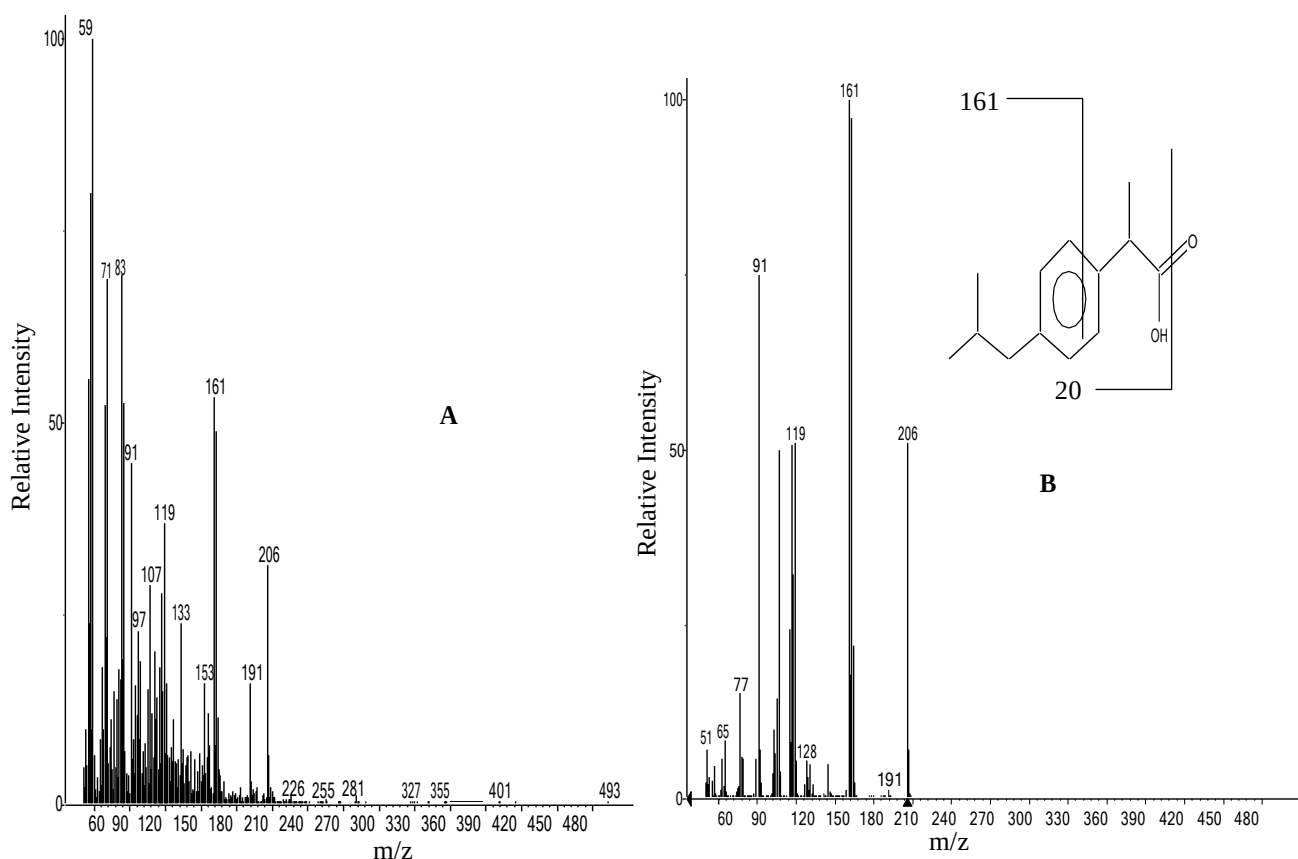


Figure 3.8: Mass spectrum of Ibuprofen detected in the SM021019 sample (A). Mass spectrum and structure of ibuprofen from NIST MS library (B). Both A and B show Q of 161 m/z , C1 of 91 m/z and M^+ of 206 m/z .

Figure 3.8 (A) shows the mass spectrum of ibuprofen that was detected in the SM021019 sample while Figure 3.8 (B) shows the mass spectrum and structure of ibuprofen from the NIST MS library. The mass spectrum of xylazine is in Appendix III. The diagnostic ions 161 m/z , 91 m/z and 206 m/z that are used to identify ibuprofen are present in the two spectra. One ion ratio of ibuprofen is included in Table 3.6. The ion ratio was calculated using Equation 3.2 and the compliance was found to be -0.2% using Equation 3.3 which falls within the set tolerance limit for ion ratios when the base peak's relative intensity is greater than 50% (see Table 3.4). Therefore, the ibuprofen detected in the SM021019 wastewater sample was further identified and confirmed using the ion ratio concept. This was done for all the samples in which ibuprofen and caffeine were detected.

The ibuprofen detected in different wastewater samples was identified and confirmed based on RT, RRF, relative intensities, ion ratios, and reverse match value. In capillary gas chromatography, the RT of the analyte of interest shall not differ by more than ± 0.1 minutes from that of the reference material analysed contemporaneously [82]. For RRF, the RRF of the analyte of interest shall not differ by more than $\pm 1\%$ from that of the reference material

analyzed contemporaneously [82]. The ibuprofen detected in the SM021019 wastewater sample and the caffeine that was detected in the GV090819 wastewater sample met these criteria.

An ibuprofen standard was used for further confirmation and identification of the ibuprofen. Ibuprofen is a common anti-inflammatory medication in Botswana and is registered with BOMRA [88]. The response obtained from the oral interviews conducted at pharmacies in Serowe and Palapye showed that ibuprofen is available in Botswana. In local pharmacies, it is sold as both prescription medication and over-the-counter depending on the dosage. Therefore, ibuprofen can be expected in wastewater samples in Botswana as it is a common medication in the country. To the authors' knowledge, there are no published reports on the detection of ibuprofen in wastewater samples collected in Botswana.

As mentioned earlier the Serowe-Mabogopa wastewater treatment plant serves the Serowe abattoir among other areas. Xylazine, a veterinary drug was detected in the SM021019 wastewater sample. Xylazine is used as a sedative, anaesthetic, tranquillizer, and muscle relaxant that is mainly used during surgeries in animals. In Botswana, xylazine was used in the immobilization of African wild dogs, one of the most endangered species, for research purposes [89]. Still in Botswana, xylazine is sold as a prescription medication and it is used as a sedative in veterinary clinics. Therefore, the presence of xylazine in wastewater samples in Botswana could be linked to veterinary clinics. Over the years xylazine has gained use as a recreational drug, in drug-facilitated sexual assault as well as suicide attempts worldwide [87],[90],[91].

3.1.3 Palapye-main village Wastewater Treatment Plant

Three influent wastewater samples were collected at the Palapye-main village (PY) WWTP. The Palapye-main village WWTP serves different parts of Palapye and surrounding villages in the Tswapong region such as Lecheng and Tamasane. Vacuum tankers collect wastewater from the villages surrounding Palapye occasionally and deposit their loads at the Palapye-main village WWTP. The six digits following PY denote the day, month, and year of sampling, respectively. Of the three samples collected, only two sample had compounds of interest to this research.

3.1.3.1 PY030919 Wastewater sample

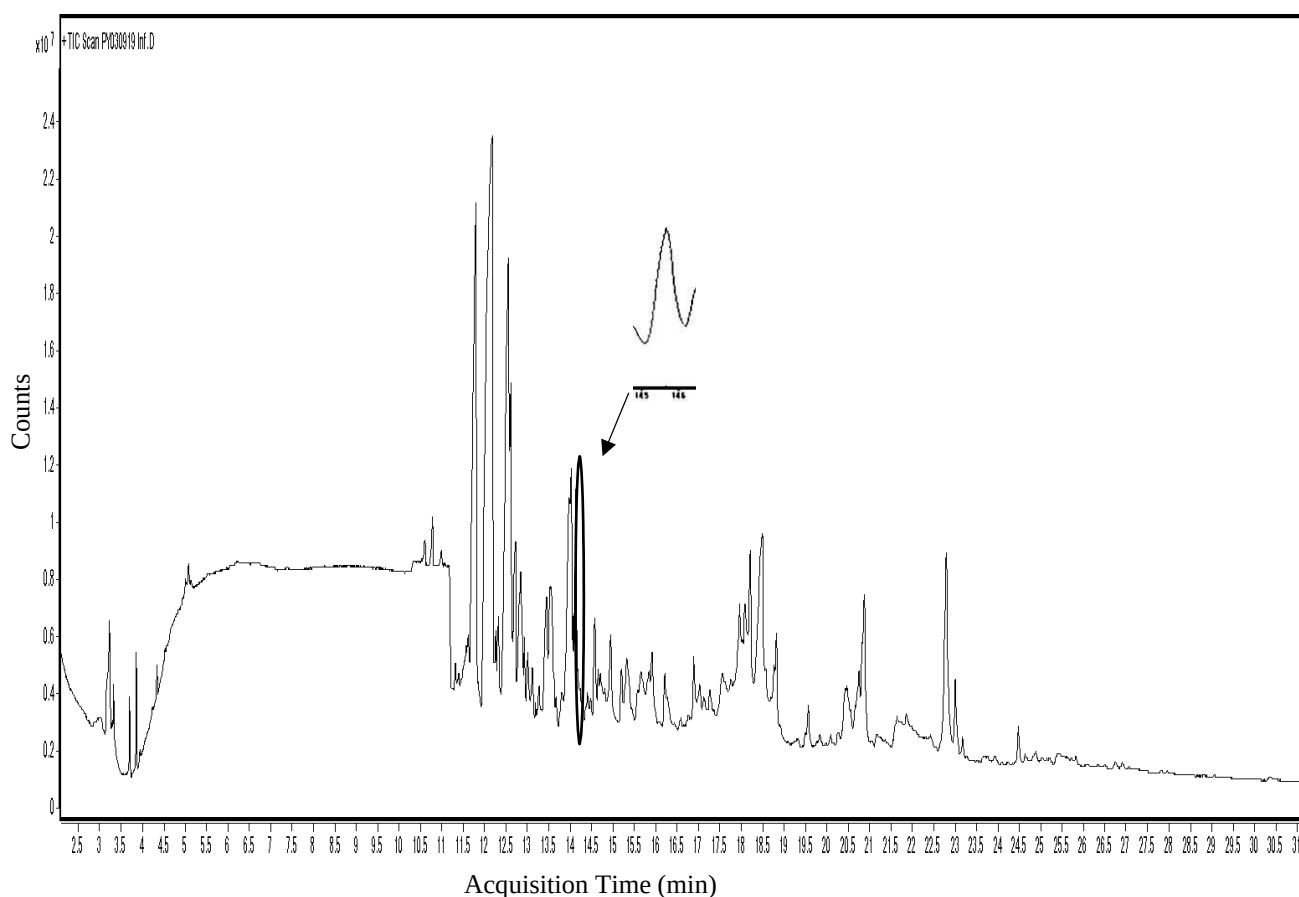


Figure 3.9: Total Ion Chromatogram of a PY030919 influent sample. The area highlighted shows where ibuprofen peak with a retention time of 14.57 minutes is found. The zoomed-in peak is the ibuprofen peak.

As mentioned in section 3.1.2.1, ibuprofen is a common anti-inflammatory medication in Botswana therefore it can be expected to be detected in wastewater samples collected at different wastewater treatment plants on different dates. The reverse match factor listed in Table 3.7 indicates a fair match of the ibuprofen on the NIST mass spectral library search.

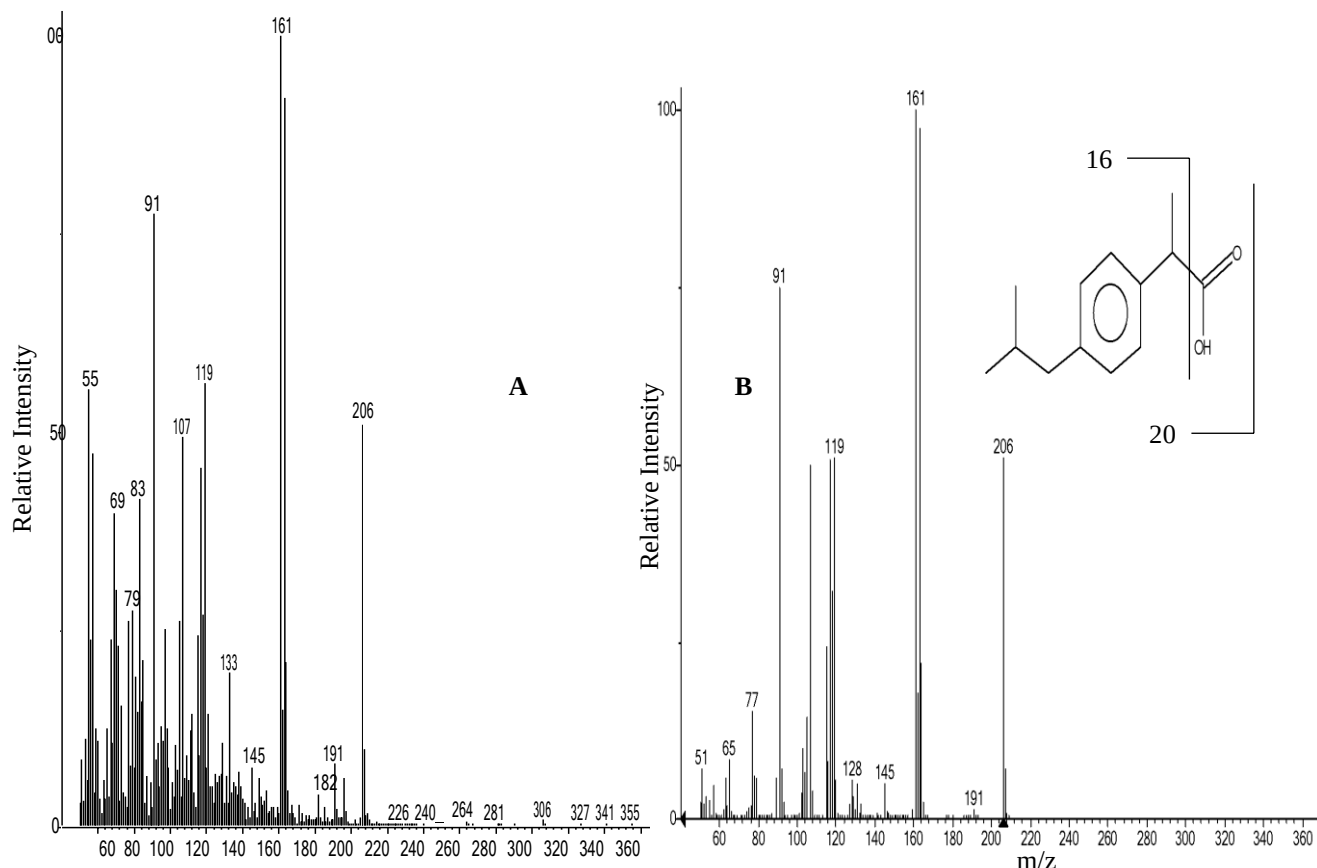


Figure 3.10: Mass spectrum of Ibuprofen detected in the PY030919 sample (A). Mass spectrum and structure of ibuprofen from NIST MS library (B). Both A and B show Q of 161 m/z , C1 of 91 m/z and M^+ of 206 m/z .

Figure 3.10 (A) shows the mass spectrum of ibuprofen that was detected in the PY030919 sample while Figure 3.10 (B) shows the mass spectrum and structure of ibuprofen from the NIST MS library. The diagnostic ions 161 m/z , 91 m/z and 206 m/z that are used to identify ibuprofen are present in the two spectra. One ion ratio of ibuprofen is included in Table 3.7. The ion ratio was calculated using Equation 3.2 and the compliance was found to be -0.55% using Equation 3.3 which falls within the set tolerance limit for ion ratios when the base peak's relative intensity is greater than 50% (see Table 3.4). Therefore, the ibuprofen detected in the PY030919 wastewater sample was further identified and confirmed using the ion ratio concept. This was done for all the samples in which ibuprofen and caffeine were detected.

Table 3.7:Compound detected in the PY030919 sample with its retention times, relative retention factors, ions of interest, reverse match factors, use and ion ratio. The retention time of the internal standard was 20.80 minutes.

Compound Name	Retention Time (minutes)	Relative Retention Factor	Reverse Match Factor	Ions of interest and their relative intensities from PY030919 (%)	Ions of interest and their relative intensities from NIST library (%)	Use	Ion Ratio
Ibuprofen	14.57	1.43	737	Q-161m/z (99.9) M ⁺ -206m/z (50.8) C1-91m/z (77.4)	Q-161m/z (99.9) M ⁺ -206m/z (50.7) C1-91m/z (74.7)	Non-steroidal anti-inflammatory drug [86].	0.547
M ⁺ = Molecular ion, Q=Base peak, C1=Confirmation Ion 1 and C2=Confirmation Ion 2 RRF=Retention Time of Internal Standard/Retention Time of Analyte							

3.1.4 Gaborone-Glen Valley Wastewater Treatment Plant

Four influent wastewater samples were collected at the Gaborone-Glen Valley (GV) WWTP. The Gaborone-Glen Valley WWTP serves the greater Gaborone area including neighbouring villages of Mmopane, Mogoditshane and Tlokweng including the Botswana Defence Force Glen Valley Barracks. Vacuum tankers also collect wastewater from the villages surrounding Gaborone occasionally and empty their loads into the wastewater treatment plant. The six digits following GV denotes the day, month, and year of sampling, respectively. Of the four samples collected, only three samples had compounds of interest to this research (Figure 3.11 and Table 3.9).

3.1.4.1 GV090819 Wastewater sample

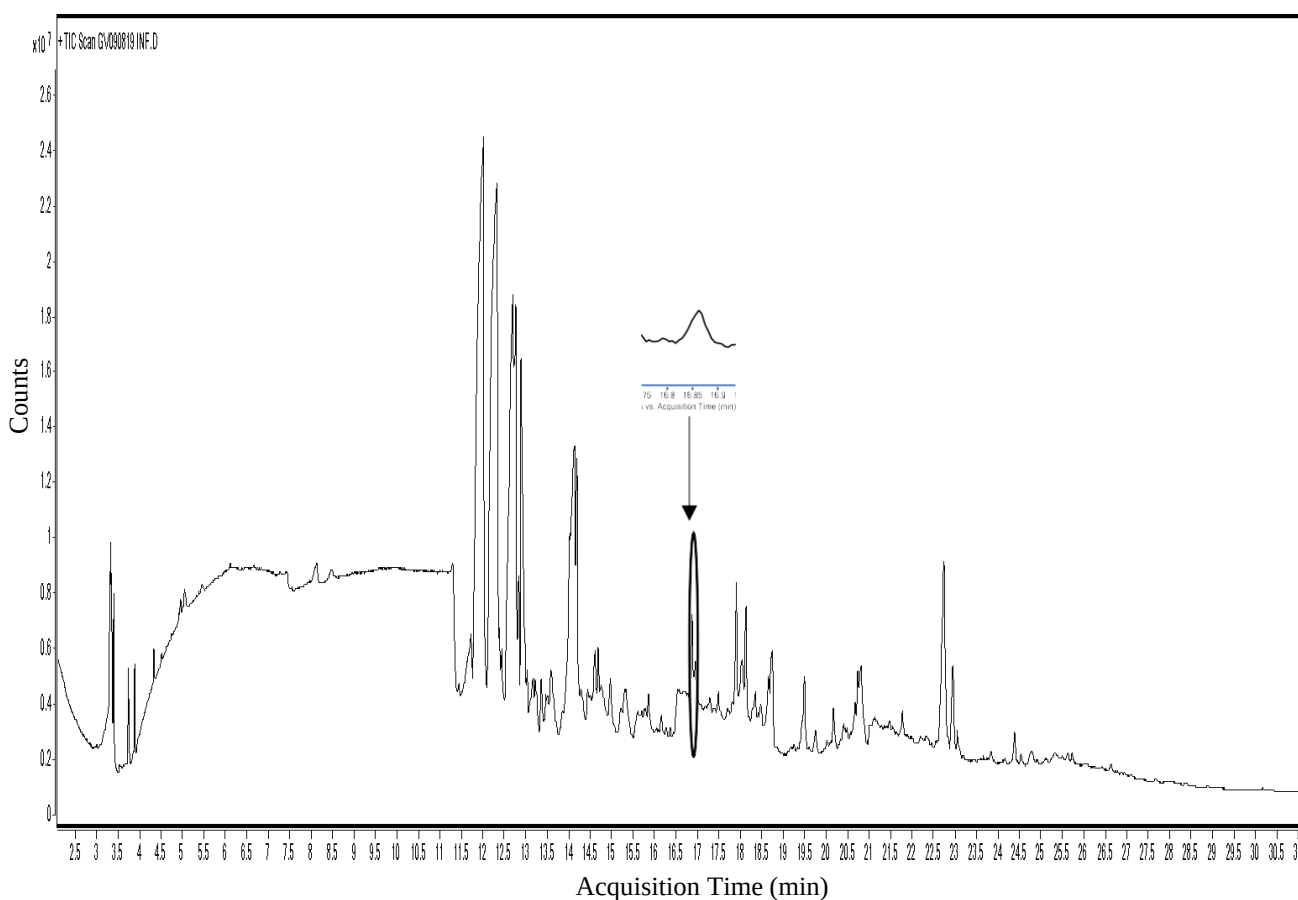


Figure 3.11: Total Ion Chromatogram of a GV090819 influent sample. The area highlighted shows where the caffeine peak with a retention time of 16.88 minutes is found. The zoomed-in peak is the caffeine peak.

Figure 3.11 is the TIC for the GV090819 wastewater sample. Caffeine with an RT of 16.88 minutes and RRF of 1.23 was detected in this sample. The RT and RRF of the caffeine detected in the GV090819 sample are close to those of the caffeine standard which were 16.84 minutes and 1.24 respectively. The IS was detected at 20.72 minutes. Caffeine has a reverse match factor score of 814 as listed in Table 3.8. A reverse match factor of 814 indicates a good match on the NIST MS library.

Table 3.8:Compound detected in the GV090819 sample with their retention times, relative retention factors, ions of interest, reverse match factors and use. The retention time of the internal standard was 20.72 minutes.

Compound Name	Retention Time (minutes)	Relative Retention Factor (RRF)	Reverse Match Factor	Ions of interest and their relative intensities (%) from GV090819	Ions of interest and their relative intensities from NIST library (%)	Use	Ion Ratio
Caffeine	16.88	1.23	814	Q & M ⁺ - 194m/z (99.9) C1-109m/z (46.8) C2-82m/z (23.6)	Q & M ⁺ - 194m/z (99.9) C1-109m/z (73.8) C2-82m/z (29.9)	A stimulant that is used in foods and medicines [92],[93].	0.212
M ⁺ = Molecular ion, Q=Base peak, C1=Confirmation Ion 1 and C2=Confirmation Ion 2 RRF=Retention Time of Internal Standard/Retention Time of Analyte							

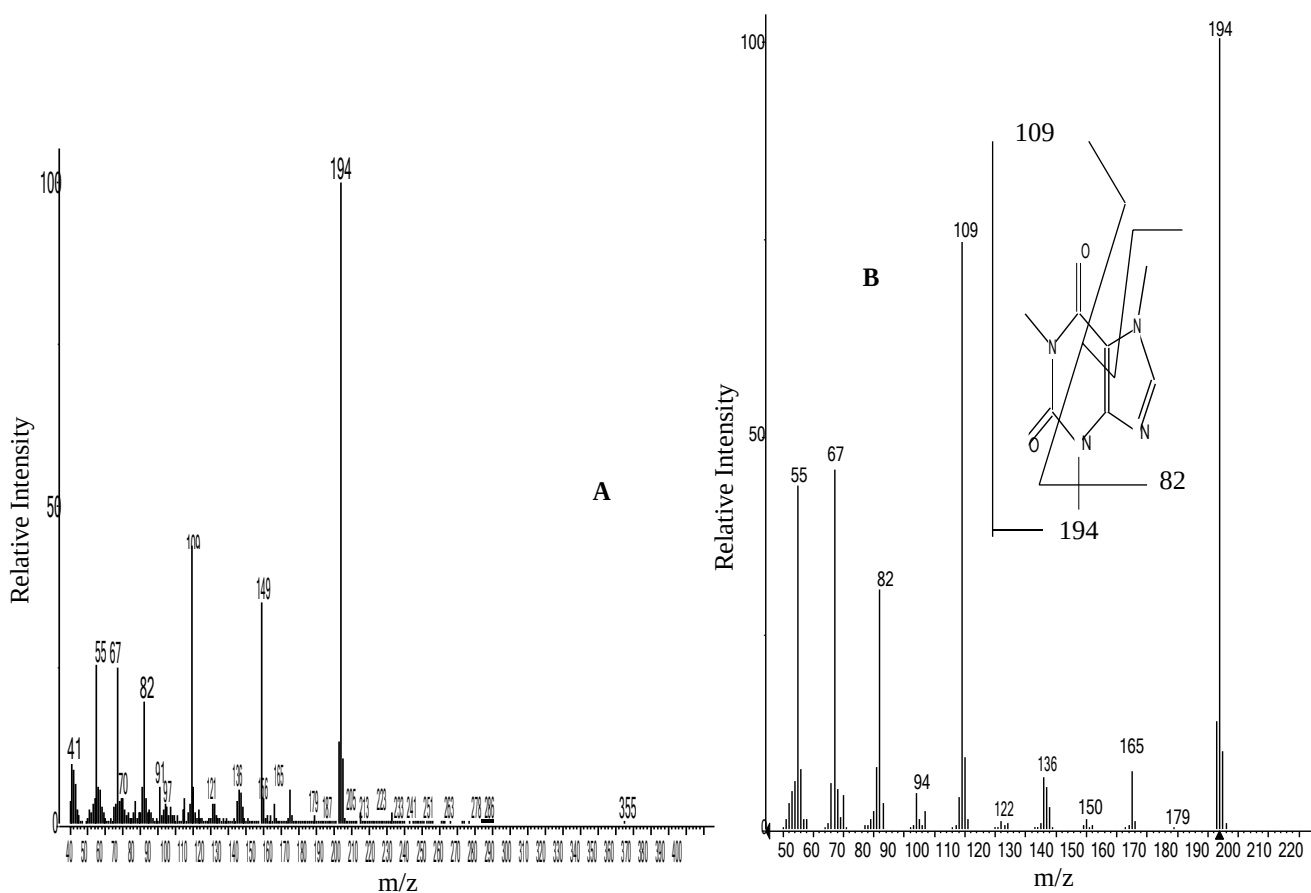


Figure 3.12:Mass spectrum of caffeine detected in the GV090819 sample (A). Mass spectrum and structure of caffeine from NIST MS library (B). Both A and B show Q of 161 m/z, C1 of 91 m/z and M⁺ of 206 m/z.

Figure 3.12 (A) shows the mass spectrum of caffeine that was detected in the GV090819 wastewater sample and Figure 3.12 (B) shows the mass spectrum and structure of caffeine from the NIST MS library. The diagnostic ions 194 m/z, 109 m/z and 82 m/z that are used to identify caffeine are present in spectra (A) and (B). One ion ratio of caffeine is included in Table 3.8. The compliance check for the ion ratio in Table 3.8 was done, and it was found to be 6%, which falls within the set tolerance limit (see Table 3.4).

It is worth noting that this is the first detection of caffeine in the wastewater samples for this research. To the author's knowledge, there are no previous published reports on the detection of caffeine in wastewater in Botswana. However, caffeine has been reported in groundwater in Botswana [94]. Caffeine is considered an emerging environmental contaminant and has previously been detected in wastewater in Brazil and San Diego [95],[96],[97].

Caffeine is the most ubiquitous of stimulants as it is found in beverages (coffee, tea, drinks), foodstuff, and medicines. Therefore, the source of caffeine in the wastewater sample could be linked to any of these. The caffeine in wastewater can be from the unconsumed coffee, tea and soft drinks poured down the drain. The sale, manufacturing and possession of caffeine are not prohibited by law in Botswana. Caffeine is used by different classes of people for different reasons. Energy drinks that contain caffeine are mostly common among students to aid them through their academic work [98],[99].

3.1.5 Palapye-BIUST Wastewater Treatment Plant

Four influent wastewater samples were collected at the Palapye-BIUST (BI) WWTP. The Palapye-BIUST WWTP serves the whole BIUST which comprises the academic area, cafeteria, multi-purpose hall, administration block, student hostels and staff houses. From the four wastewater samples, only three samples had a compound that was of interest to this research. Mefexamide is the only compound that was detected in the three samples. Due to the lack of the mefexamide standard, it could not be confirmed. However, the RT, RRF, reverse match factor and use of the mefexamide are listed in Table 3.9. This identification was been/compared with the NIST library only.

3.1.6 Serowe-Radihemelo Wastewater Treatment Plant

Four influent wastewater samples were collected at the Serowe-Radihemelo (SR) WWTP. The SR WWTP serves most parts of Serowe including the Sekgoma II Memorial Hospital, Serowe Institute of Health Sciences and Boiteko junction mall. Occasionally vacuum

tankers from Palapye village and Letlhakane town (about 196 km north of Serowe) in the Boteti sub-district also empty the wastewater at the Serowe-Radihemelo WWTP. The six digits following SR denotes the day, month, and year of sampling, respectively. Of the four samples collected, only three samples had compounds of interest to this research. Amiphenazole was detected in the SR050819 sample, efavirenz was detected in the sample SR270819 sample, while amiphenazole and mefexamide were detected in the SR281019. Due to the lack of the amiphenazole, efavirenz and mefexamide standards for this research, they could not be confirmed. However, the RT, RRF, reverse match factor and use of the amiphenazole, efavirenz and mefexamide are listed in Table 3.9. This identification was compared with the NIST library only.

Botswana is one of the countries that has a very high Human Immunodeficiency Virus (HIV) prevalence rate worldwide [100]. As a result, most HIV patients get free antiretroviral (ARV) medication such as efavirenz from government health facilities, or they buy from local private pharmacies. A similar study that was conducted in South Africa in which selected ARVs were determined in wastewater showed the presence of efavirenz in wastewater samples [76]. Therefore, the results obtained for this sample, are like those obtained by other researchers in South Africa.

3.1.7 Compounds that were tentatively identified in wastewater samples

The compounds, amiphenazole, efavirenz, mefexamide and prolintane listed in Table 3.9 were tentatively identified based on their RT, RRF, reverse match factor scores and NIST library search due to the unavailability of standards for these compounds. The reverse match factor of the other compounds detected in the wastewater samples were between 751 and 646 indicating a fair match (see Table 3.1). Only prolintane detected in the PY030719 sample and the mefexamide detected in the BI021019 sample had reverse match factor of 852 and 813 respectively, indicating a good match on the NIST library. Pure standards of the four compounds are needed to confirm these compounds. The mass spectra of the compounds in Table 3.9 are included in Appendix III, IV, V, VI and VII.

Table 3.9: Compounds that were tentatively identified from various wastewater samples collected at different wastewater treatment plants.

Sample Name	Compound Name	Retention Time of compound (minutes)	Retention Time of the Internal Standard	Relative retention factor	Reverse match factor	Use	Prescription/over-the-counter medicine (OTC)
SR050819	Amiphenazole	12.14	20.92	1.72	646	Respiratory stimulant [101]	Not for sale in Botswana.
SR270819	Efavirenz	21.50	20.87	0.97	721	An antiretroviral drug is used in the treatment of HIV [102].	Prescription-only
SR281019	Mefexamide	5.01	20.90	4.17	742	A psychostimulant drug [103]	Not for sale in Botswana.
	Amiphenazole	12.88	20.90	1.62	702	Respiratory stimulant [101]	Not for sale in Botswana.
SM270819	Amiphenazole	12.48	20.99	1.68	698	Respiratory stimulant [101]	Not for sale in Botswana.
SM021019	Mefexamide	5.04	20.87	4.14	746	A psychostimulant drug [103]	Not for sale in Botswana.

SM281019	Mefexamide	5.00	20.86	4.17	751	A psychostimulant drug [103]	Not for sale in Botswana.
PY030719	Mefexamide	5.04	20.86	4.14	737	A psychostimulant drug [103]	Not for sale in Botswana.
	Prolintane	5.08	20.86	4.11	852	Amphetamine-related central nervous stimulant [57]	Not for sale in Botswana.
BI281019	Mefexamide	5.01	20.82	4.15	737	A psychostimulant drug [103]	Not for sale in Botswana.
BI021019	Mefexamide	5.04	20.81	4.13	813	A psychostimulant drug [103]	Not for sale in Botswana.
GV100919	Mefexamide	5.01	20.74	4.14	739	A psychostimulant drug [103]	Not for sale in Botswana.
GV031019	Mefexamide	5.00	20.76	4.15	766	A psychostimulant drug [103]	Not for sale in Botswana.

Mefexamide is a central nervous stimulant drug that is no longer marketed as human medicine. Currently, it is still being sold and used for research purposes. The presence of mefexamide in the university wastewater samples could be due to it being used for research purposes. However, mefexamide was not detected in the Palapye-BIUST wastewater

samples only, it was detected across all the wastewater treatment plants. The continued use/availability of mefexamide-containing products such as mefexamide hydrochloride could also result in the compound continuing to be detected in wastewater in Botswana. However, mefexamide is not registered with the Botswana Medicines Regulatory Authority (BOMRA) [88].

Prolintane was detected in the PY030719 wastewater sample. Prolintane is an ATS that has been used as a treatment for narcolepsy and Attention Deficit Hyperactivity Disorder (ADHD) in Africa [57]. To the author's knowledge, there are no published reports in which prolintane was detected in wastewater samples from Botswana or other countries. Although prolintane has been known for medicinal use it is not registered with BOMRA. Another compound that is not registered with BOMRA but was detected in wastewater samples is amiphenazole. Amiphenazole is a respiratory stimulant that is used as an antidote to opiate and barbiturate overdose [101].

One-on-one interviews conducted with pharmacists as part of this research indicated that the presence of unregistered pharmaceutical drugs in wastewater does not necessarily indicate the smuggling of the pharmaceutical drugs into Botswana. People can seek medical assistance in other countries and have such medications prescribed for them. Also in special cases, when doctors need medicines not registered in Botswana, they can request special permission from BOMRA to import and use the medicine in Botswana [104]. However, the prevalence of unregistered drugs being used in Botswana is unknown at present and therefore a correlation cannot be made as to whether their presence in wastewater would be significant enough to be detected.

3.1.8 Comparison of results across different wastewater treatment plants

As mentioned in section 2.1.1, several influent wastewater samples were collected on different dates from five different WWTP across Botswana. Even though wastewater samples were collected on different dates and from various WWTP, a comparison was made on the GC-MS results obtained to establish if certain compounds listed in Table 3.10 were detected in samples from more than one wastewater treatment plant.

Table 3.10: Comparison of GC-MS results for wastewater samples collected at five different wastewater treatment plants in Botswana.

Compound Name	Serowe-Radihemelo	Serowe-Mabogopa	Palapye-main village	Palapye-BIUST	Gaborone-Glen Valley
Amiphenazole	√	√			
Efavirenz	√				
Mefexamide	√	√	√	√	√
Ibuprofen		√	√		
Prolintane			√		
Xylazine		√			
Caffeine					√

Amiphenazole, a prescription medication that is not sold in Botswana was detected in samples collected at Serowe-Radihemelo, Serowe-Mabogopa and Gaborone-Glen Valley wastewater treatment plants. Efavirenz, a very common ARV medication was detected in the wastewater sample collected at the Serowe-Radihemelo WWTP only. As mentioned earlier Botswana is one of the countries in the world with a very high HIV prevalence rate worldwide[100]. This means that most of the HIV patients across Botswana are on some ARV medication which can include efavirenz. Therefore, the detection of efavirenz in wastewater samples can be expected.

The results obtained in Table 3.10 show that mefexamide, which is a psychostimulant was detected in wastewater samples collected at all the wastewater treatment plants used for this research. The areas where the wastewater samples were collected all have different learning institutes and some have research institutes. BIUST as a university carries out different research. The Serowe-Radihemelo and Serowe-Maboga WWTP are located in Serowe where there is the Serowe Institute of Health Sciences (general nursing college).

The Gaborone-Glen Valley WWTP is located in the capital city of Botswana where there are universities including the University of Botswana which houses the Sir Ketumile Masire Teaching Hospital as well as research institutes such as the Botswana Institute for Technology Research and Innovation (BITRI). All the above-mentioned could be possible sources of mefexamide detected in the wastewater samples. A localised sampling of the

wastewater samples was not done for this research, as a result, it cannot be confirmed if the mefexamide is coming directly from learning institutes or some other areas where research is being conducted.

Ibuprofen is a very common anti-inflammatory drug that is sold over the counter and as a prescription medication depending on the dosage. It was detected in samples collected at the Serowe-Mabogopa and Palapye-main village WWTP. There are no published reports on the detection of ibuprofen in wastewater samples in Botswana. However, research was done in the Czech Republic in which ibuprofen and its metabolites hydroxyibuprofen and carboxyibuprofen were detected in wastewater samples for more than twelve months [105]. It can be expected that ibuprofen is detected in wastewater samples that were collected from different wastewater treatment plants at different times. Of all the compounds listed in Table 3.10, only ibuprofen, caffeine and efavirenz have been previously reported in wastewater in other countries [76],[95],[105]. To the authors' knowledge, there are no published reports on the detection of ibuprofen, caffeine and efavirenz in wastewater in Botswana.

3.1.9 Summary of results from wastewater analysis

One of the aims of this research was to use a GC-MS method for the detection of pharmaceutical and illicit drugs in wastewater samples collected at different WWTP in Botswana. The research aim was successfully achieved as a GC-MS method was adapted specifically for this research. Using the GC-MS method mentioned in section 2.1.5, amiphenazole, ibuprofen, caffeine, mefexamide, efavirenz, prolintane and xylazine were detected from complex wastewater sample matrix. This shows that the adapted GC-MS method can detect some drugs that are mostly at trace levels and low concentrations in wastewater samples.

A total of 18 influent wastewater samples were collected from five different wastewater treatment plants across Botswana. Most of the influent wastewater samples used were collected at the Palapye-BIUST WWTP, Serowe-Radihemelo and Gaborone-Glen Valley wastewater treatment plants. Three wastewater samples were collected from the Serowe-Mabogopa and the other three samples from the Palapye-main village WWTP separately. The use of Strata -X Drug B SPE cartridges enabled the successful extraction of analytes of interest from the wastewater sample matrix before analysis by GC-MS.

This research targeted illicit drugs, pharmaceutical drugs, and their metabolites. Even though most of the detected compounds could not be confirmed using drug standards, the

identification that was made on the results is worth noting as it paves a way for further research in this area in Botswana. In complex sample matrices such as wastewater, the analytes of interest are mostly at trace levels and chances of co-eluting interferences are high which can lead to only a few target analytes being detected.

Most of the compounds that were detected have known pharmaceutical use as they are used for the treatment of medical conditions such as HIV and as an anti-inflammatory medication. Many hospitals/clinics in Botswana do not have their separate wastewater treatment plants as a result they share with the rest of the communities. However, that does not imply that the presence of all the pharmaceutical drugs in the wastewater samples is linked to the hospital/clinics as some of the medications such as ibuprofen are prescribed or sold over the counter and can be taken at any place.

Of all the compounds that were detected in wastewater samples, it is worth noting that ibuprofen, efavirenz and xylazine are registered by the Botswana Medicines Regulatory Authority (BOMRA) and they are available in many health facilities including private pharmacies. Caffeine on the other hand is ubiquitous and occurs in many items such as tea, coffee, soft drinks, and medicines. The other three compounds, amiphenazole, prolintane and mefexamide, are not registered with BOMRA.

The detection of the unregistered pharmaceutical compounds in wastewater samples collected in Botswana does not necessarily mean they are being smuggled into the country and sold on black markets. Mefexamide, for example, can be present in wastewater due to research that is carried out in the country. Patients are also allowed to seek medical assistance outside Botswana in which they can be given such medications and bring them back into Botswana and end up being detected in wastewater samples. Also, doctors can request special permission to import such medications for their patients who might not have any other alternative medication to use. Through the analysis of influent wastewater samples, some of the drugs that were used by the different communities during the time of sampling were identified. WBE is very useful in identifying the broad spectrum of different substances (including illicit drugs and pharmaceutical drugs) that are used by a population served by a certain WWTP at any given time.

3.2 Online Survey Questionnaire Results and Discussion

3.2.1 Sociodemographic Characteristics of respondents

A total of 120 people gave valid consent to take part in the online survey questionnaire. Table 3.11-3.20 contains the exact questions as well as the multiple-choice answers that were given to the participants in the survey questionnaire. For some questions, participants could choose more than one answer. The option 'other' was also available so that participants could write down their answers in case they are not sure where their answer falls within the given options. The term medicinal was used to refer to pharmaceutical drugs in the survey questionnaire that was administered for this research.

Table 3.11 shows the sociodemographic characteristics of the respondents. As a result, for this research, we were able to analyse differentiative knowledge/experience based on the respondents' age, education, and status. For this study, there were more male respondents (50.0%) than female respondents (48.3%) and those who preferred not to reveal their gender (1.7%). Of the 2,504 people who make up the BIUST community, 1,591 are males while their female counterparts are 913. The results obtained indicated great participation of females than their male counterparts in this survey.

Table 3.11: Sociodemographic characteristics of survey respondents.

	Frequency	Percentage (%)
Gender		
Male	60	50.0
Female	58	48.3
Prefer not to say	2	1.7
Age (Years)		
18-24	64	53.3
25-30	29	24.2
31-40	18	15.0
41-50	4	3.3
51-60	4	3.3
61 and above	1	0.8
Status		
Undergraduate student	62	51.7
Postgraduate student	37	30.8
Staff	21	17.5

More than half (53.3%) of the survey respondents were in the 18-24 years age range. In Botswana, most people go for tertiary education after high school at the age of 17-19 years, i.e., most people go for further education/training before they can look for employment. The tertiary education participation age group is 18-23 years in Botswana [106]. The total number of undergraduate students is 1,565 in BIUST, and it exceeds the combined total of postgraduate students and staff members which is 939. The undergraduate students are mostly in the 18-23 years age group, therefore, having more respondents being in the 18-24 years age range can be expected for this study.

As depicted in Table 3.11, the 41-50, 51-60 and 61 and above age range groups had the least number of respondents. Since there were more students than staff members at BIUST and most of those students were below the age of 40, this implies that most participants above the age of 40 were most likely to be staff members. Therefore, for this study, most

participants were below the age of 40 years, and they were mainly students. Based on Table 3.11 most of the respondents were undergraduate students (51.7%). Postgraduate students and staff members who participated in the survey were 30.8% and 17.5%, respectively. With the number of undergraduate students exceeding that of both postgraduate students and staff members combined, having more undergraduate students partaking in this study can be expected.

3.2.2 Knowledge and use of pharmaceutical and illicit drugs

Considering the use of pharmaceutical drugs, 80.8% of the respondents used pharmaceutical drugs in the previous 24 months while 19.2% of the respondents did not use pharmaceutical drugs in the previous 24 months as indicated in Table 3.12. For the categories of pharmaceutical drugs used, 88.7% of the respondents indicated they used painkillers such as aspirin, ibuprofen, panado, tramadol, codeine etc. Previous studies conducted indicated that over-the-counter painkillers such as ibuprofen and aspirin are some of the painkillers that are mostly used by the public [107]. Painkillers, especially those that can be bought over-the-counter are mostly used as the first line of treatment as they are affordable and can be easily bought in retail shops [108],[109],[110].

Table 3.12: Knowledge and use of pharmaceutical drugs in the 24 months prior to the survey.

	Frequency	Percentage (%)
Used medicinal drugs in the last 24 months		
Yes	97	80.8
No	23	19.2
Category of medicinal drug used		
Painkillers such as aspirin, ibuprofen, panado, tramadol, codeine etc	86	88.7
Antidepressants such as diazepam, fluoxetine, olanzapine, amitriptyline etc.	4	4.1
Antihistamines such as allergex, cetirizine, loratadine etc.	35	36.1
Chronic diseases medication such as for asthma, heart diseases, diabetes etc.	6	6.2
Other	7	7.2
Place where medicinal drugs are mostly used		
Hostels	45	46.4
Office	11	11.3
Academic areas (classrooms/laboratories)	7	7.2
Home (off campus)	72	74.2
Other	1	1.0

A study conducted in Greece showed that over-the-counter painkillers, such as paracetamol, are widely used to treat pain and fever [108],[111]. These findings are in line with the findings from this study which indicated that painkillers were the most used pharmaceutical drugs in the 24 months before taking the survey. Also, ibuprofen, an anti-inflammatory drug and painkiller, was detected in some wastewater samples collected for this research (see Table 3.10). This shows that indeed painkillers are common medications in different places.

Chronic disease medications for conditions such as asthma, heart disease and diabetes were the least used pharmaceutical drugs with 6.2% of the respondents indicating to have used them in the previous 24 months. Non-communicable chronic diseases have been on the rise over the years in Botswana and are estimated to account for about 31% of all deaths in Botswana [112]. However, findings from this study, suggest that chronic disease medications were the least used compared to painkillers, antidepressants, and antihistamines in the 24 months before taking the survey. This survey was conducted at BIUST; therefore, the findings might not be used to make inferences about the general population in Botswana which also means this might not represent the overall use.

For the places where pharmaceutical drugs were mostly used, 74.2% of the respondents indicated that they use the pharmaceutical drugs at their homes (off-campus). Different pharmaceutical drugs have different side effects such as dizziness or drowsiness. As a result, people might opt to take such medications when they are in the comfort of their homes rather than in offices or classrooms/laboratories when they are working. About 45% of the respondents indicated that they took their pharmaceutical drugs at the hostels. As mentioned earlier most participants of this survey are undergraduate students and more than 90% of BIUST undergraduate students stay on-campus. Therefore, having a high number of respondents indicating that they take their pharmaceutical drugs at the hostels can be expected. Adhering to prescription instructions of pharmaceuticals is very important to avoid negative effects, therefore people should take their medications as prescribed and on time regardless of where they are.

Table 3.13: Knowledge and use of illicit drugs in the 24 months prior to the survey.

	Frequency	Percentage (%)
Used illegal drugs in the last 24 months		
Yes	19	15.8
No	101	84.2
Type of illegal drug used		
Opioids such as heroin.	0	0
Hallucinogens such as cannabis, LSD etc.	18	94.7
Sedative such as mandrax.	0	0
Stimulants such as cocaine, methamphetamine, amphetamine, ecstasy, methcathinone etc.	2	10.5
Other	1	5.3
Place where illegal drugs are normally used		
Hostels	5	26.3
Office	0	0
Academic areas (classrooms/laboratories)	1	5.3
Home (off campus)	7	36.8
Bars/nightclubs/liquor restaurants	1	5.3
Hotels/lodges/guest houses	1	5.3
Prefer not to say	7	36.8
Other	2	10.5
Frequency of use of illegal drugs		
Everyday	3	15.8
Every weekend	3	15.8
Last weekend of the month	3	15.8
Before/during/after a major sporting event	0	0
Before/during/after a major entertainment event	1	5.3
Before/after tests/exams/presentations	1	5.3

Before/after attending class/tutorial/laboratory session	0	0
Before/after working hours	2	10.5
Prefer not to say	1	5.3
Other	9	47.4

On the use of illicit drugs, only 15.8% of the respondents indicated to have used illicit drugs in the previous 24 months while 84.2% of the respondents have not used illicit drugs in the last 24 months as listed in Table 3.13. A cross-sectional study on alcohol and drug use that was conducted on 1936 secondary school students from 17 public secondary schools in Gaborone, Lobatse, Molepolole and Mochudi in Botswana indicated that 16.7% of the respondents had used illicit drugs [113]. These results correspond to the results which were obtained in this study in which only 15.8% of the respondents indicated having used illicit drugs in the 24 months prior to the survey. This survey questionnaire was administered to both students and staff members at BIUST, however, only 1 (0.8%) staff member indicated to have used illicit drugs in the 24 months before the survey.

Hallucinogens such as cannabis and LSD were the most used class of illicit drugs in the 24 months before the survey with 94.7% followed by stimulants such as cocaine, methamphetamine, amphetamine, ecstasy, methcathinone etc at 10.5% (Table 3.13). According to the Botswana Police Service (BPS), cannabis is the most common illicit drug in Botswana, [14],[114]. Also, published studies conducted in Botswana indicate that cannabis is the most common illicit drug used in Botswana including among secondary school students [113],[13].

The use of stimulants such as cocaine, methamphetamine and methcathinone is on the rise in Botswana [14]. As a result, it can be expected for respondents to indicate the use of stimulants. The findings for this study, therefore, correlate with the BPS statement and other published literature. In addition, in January 2021, two BIUST students were arrested by the BPS in Letlhakane town (about 234 km North of Palapye) with 1.5 kg of methcathinone and 2 kg of cannabis [115]. Although it is not known whether the students were going to bring these drugs back for sale at BIUST, it correlates with the findings from this research on the use of cannabis and methcathinone (stimulant category) among BIUST students.

On the frequency of use of illicit drugs, a total combined response of 47.4% of respondents indicated that they used the illicit drugs every day, every weekend, and the last weekend of the month. The use of illicit drugs is likely to increase over the weekend or during special events such as festivals. Other respondents indicated that they used illicit drugs occasionally such as during festivals. Illicit drugs have very well-known dangerous physical and psychological side effects on the user. Therefore, prolonged daily use might be an indication of addiction for the user.

Respondents indicated that they used illicit drugs in different places such as homes (36.8%) or hostels (26.3%). These findings correlate with the previous section where respondents indicated that pharmaceutical drugs are used mainly in homes and hostels (see Table 3.12). One respondent indicated that they used illicit drugs in the academic areas (classrooms/laboratories). Illicit drugs are known to affect/alter one's psychological state, therefore, when they are taken in laboratories the user does not only put his/her life in danger but that of their colleagues as well because they might be tempted to mishandle chemicals/solvents or equipment while under the influence of drugs.

When both illicit drugs and pharmaceutical drugs are consumed, they are excreted from the body through urine and faeces as parent drugs or metabolites ending up in WWTP [45], [32],[38]. Although findings from the survey questionnaire indicated the use of cannabis and stimulants by the BIUST community, there were no illicit drugs detected in the wastewater samples collected at Palapye-BIUST WWTP (see section 3.1.5). Furthermore, there were no illicit drugs or their metabolites that were detected in wastewater samples collected at other WWTP for this research.

3.2.3 Disposal of drugs

Table 3.14: Mode of disposal of leftover or expired pharmaceutical and illicit drugs.

	Frequency	Percentage (%)
Medicinal drugs disposal		
Throw them in a litter bin	74	76.3
Throw them in pit latrine toilets	9	9.3
Flush them down the drain (sink or toilet)	12	12.4
Return them to the clinic/hospital/pharmacy	3	3.1
Other	9	9.3
Illegal drugs disposal		
Throw them in a litter bin	10	52.6
Throw them in pit latrine toilets	0	0
Flush them down the drain (sink or toilet)	4	21.1
Other	6	31.6

From Table 3.14, 76.3% of the respondents disposed of expired or leftover medications by throwing them in litter bins together with other household litter. According to the Food and Drug Administration (FDA), if there are no community drug take-back programs or if there are no disposal instructions on the prescribed drug's labelling then the drugs can be thrown in household trash [116]. This corresponds to the findings of this study in which most people indicated that they throw leftover and/or expired medicines in litter bins.

A total of 12.4% of the respondents indicated that they flush expired/leftover medicines down the drain. The FDA advises against this practice unless there are clear instructions on the medicines' labels for flushing down the drain [116]. For disposal of illicit drugs, 52.6% of respondents indicated that when they are no longer going to use the illicit drugs, they also throw them in the litter bin while 21.1% of the respondents flush them down the drain. In Botswana, the disposal of unwanted medications should be conducted as per the regulations stated in the Medicines and Related Act, 8 of 2013, which state that expired pharmaceuticals should be returned to the pharmacy/hospital for disposal unless clearly

stated on the label [104]. From the findings of this study, it's a common practice for leftover/expired pharmaceutical drugs and illicit drugs to either be thrown with household trash or flushed down the drain.

The improper disposal of both pharmaceutical and illicit drugs can be harmful to the environment and human life. Drugs that are thrown in pit latrines or flushed down the toilet can end up in a WWTP. Drugs thrown in litter bins can end up in dumping sites and could be detected in soils and eventually leach into water systems. The presence of drugs in the water system does not only pose a threat to human lives but aquatic lives as well because they affect their endocrine system and physiology [117],[118].

Drugs that are also disposed of in household trash can pose a danger to young children if within their reach as they can mistake them for candy resulting in accidental poisoning. If available, communities should make use of 'drugs take-back programs' for expired/leftover medications where proper disposal techniques such as incineration can be employed to avoid contaminating the environment [116]. To the authors knowledge, there are no community drug-take-back programs. Therefore, the Ministry of Health and Wellness in conjunction with pharmacies, clinics and hospitals should establish drug-take-back programs and educate communities on them as well as have facilities where people can return leftover/expired pharmaceuticals for safe disposal.

3.2.4 Relationship between sociodemographic characters and the use of pharmaceutical and illicit drugs

The Pearson's chi-square test was used in this section to establish if there is a relationship between the use of pharmaceutical drugs and age, gender as well as status at a 95% confidence level. For establishing if there is a relationship between gender and the use of pharmaceutical drugs, the two respondents who preferred not to state their gender were excluded when the Pearson's chi-square test was performed. From Table 3.15 there was a significant difference in the number of females (93.1%) who used pharmaceutical drugs in the previous 24 months when compared with males (68.3%) ($P= 0.001$, $X^2 = 11.531$, $df = 1$). The findings of this study were consistent with published literature conducted in Southern Italy, in which the number of pharmaceutical drug prescriptions was higher in females compared to their male counterparts (55.6% for females' vs 44.4% for males) [119]. Studies conducted in other places have also indicated that females use pharmaceutical drugs more

than males [120],[121]. Pharmaceutical drugs are taken for different reasons such as treating or preventing a disease.

Table 3.15: Significance of socio-demographic characteristics on the use and non-use of pharmaceutical drugs.

Characteristics	Use of pharmaceutical drugs	Non-use of pharmaceutical drugs	Total	P-value, Chi-square value and degrees of freedom
Gender				
Male	41 (68.3%)	19 (31.7%)	60	P=0.001 $X^2 = 11.531$ df = 1
Female	54 (93.1%)	4 (6.9%)	58	
Total number of responses	95	23	118	
Age (years)				
18-24	51 (79.7%)	13 (20.3%)	64	P=0.426 $X^2 = 2.785$ df = 3
25-30	26 (89.7%)	3 (10.3%)	29	
31-40	14 (77.8%)	4 (22.2%)	18	
41 and above	6 (66.7%)	3 (33.3%)	9	
Total number of responses	97 (80.8%)	23 (19.2%)	120	
Status				
Undergraduate student	49 (79.0%)	13 (21.0%)	62	P= 0.604 $X^2 = 0.269$ df = 1
Postgraduate student and staff member	48 (82.8%)	10 (17.2%)	58	
Total number of responses	97	23	120	

There was no observed significant difference between the different age groups in pharmaceutical drug use ($P = 0.426$, $X^2 = 2.785$, $df = 3$). There were few respondents from other age groups (41-50 years and 60 years and above) as only one person from each of the two age groups participated in the survey. The findings recorded in this study were however

not consistent with the literature as it was observed in previous studies conducted that the use of prescription medicine increases with age [120],[121],[122]. As one ages, they may be faced with more health conditions depending on their lifestyles which will require the use of different medications. On the relationship between status and pharmaceutical drug use, there was no observed significant difference ($P = 0.604$, $X^2 = 0.269$, $df = 1$).

The Pearson's chi-square test was also used in this section to establish if there is a relationship between the use of illicit drugs and age, gender as well as status at a 95% confidence level. For establishing if there is a relationship between gender and the use of illicit drugs, the two respondents who preferred not to state their gender were excluded when the Pearson's chi-square test was performed. There was no significant relationship between gender and illicit drug use as depicted in Table 3.16 ($P = 0.094$, $X^2 = 2.798$, $df = 1$). The results of this study are not consistent with published literature as it has been indicated that males are more likely to use illicit drugs than females [123]. Also, the three-year substance abuse treatment program conducted in Botswana by BOSASNet showed that more participants were men (83%) compared to women (16.8%) [13].

Table 3.16: Significance of socio-demographic characteristics on the use and non-use of illicit drugs.

Characteristics	Use of illicit drugs	Non-use of illicit drugs	Total	P-value, Chi-square value and degrees of freedom
Gender				
Male	13 (21.7%)	47 (78.3%)	60	P=0.094 $X^2 = 2.798$ df = 1
Female	6 (10.3%)	52 (89.7%)	58	
Total number of responses	19	99	118	
Age (years)				
18-24	14 (21.9%)	50 (78.1%)	64	P= 0.076 $X^2 = 6.875$ df = 3
25-30	5 (17.2%)	24 (82.8%)	29	
31-40	0	18 (100.0%)	18	
41 and above	0	9 (100.0%)	9	
Total number of responses	19	101	120	
Status				
Undergraduate student	14 (22.6%)	48 (77.4%)	62	P= 0.036 $X^2 = 4.382$ df = 1
Postgraduate student and staff member	5 (8.6%)	53 (91.4%)	58	
Total number of responses	19	101	120	

There was no significant relationship between the different age groups and illicit drug use ($P= 0.076$, $X^2 = 6.875$, $df=3$). The results obtained in this study are not consistent with a study that was done in Botswana which showed that drug abuse was common in the age range of 21-30 years (43%) and least prevalent in the 40 years and above age range [13]. The results obtained in Table 3.16 indicate that there is a significant relationship between status and the use of illicit drugs ($P= 0.036$, $X^2 = 4.382$, $df=1$).

Abuse of illicit drugs was more prevalent among students (undergraduates and postgraduates) compared to members of staff at BIUST in the 24 months prior to the survey. A study conducted in Botswana showed that illicit drug use was more prevalent in students compared to the employed, unemployed, and retired people [13]. These results are consistent with the results of this study which indicates that of the total illicit drug users, more undergraduate students (73.7%, n=14) used illicit drugs compared to postgraduate student and staff members (26.3%, n=5) in the previous 24months.

3.2.5 Knowledge of the fate of pharmaceutical and illicit drugs in wastewater

The Pearson's chi-square test was used in this section to establish if there is a relationship between the knowledge of the fate of pharmaceutical and illicit drugs in wastewater, gender as well as status at a 95% confidence level. For establishing if there is a relationship between gender and knowledge of the fate of pharmaceutical and illicit drugs in wastewater, the two respondents who preferred not to state their gender were excluded when the Pearson's chi-square test was performed. There is no significant relationship between gender and knowledge of the fate of illicit and pharmaceutical drugs in wastewater ($P=0.190$, $X^2 = 1.714$, $df=1$) (see Table 3.17). There is no significant relationship between age ($P=0.358$, $X^2 = 3.229$, $df=3$) and knowledge of the fate of illicit and pharmaceutical drugs in wastewater. There is also no significant relationship between status ($P=0.521$, $X^2 = 0.411$, $df=1$) and knowledge of the fate of illicit and pharmaceutical drugs in wastewater.

Table 3.17: Knowledge of the fate of pharmaceutical and illicit drugs in wastewater.

	Yes	No	Total	P-value, Chi-square value and degrees of freedom
Gender				
Male	44 (73.3%)	16 (26.7%)	60	P= 0.190 $X^2 = 1.714$ df=1
Female	36 (62.1%)	22 (37.9%)	58	
Total number of responses	80	38	118	
Age (Years)				
18-24	47 (73.4%)	17 (26.6%)	64	P= 0.358 $X^2 = 3.229$ df = 3
25-30	16 (55.2%)	13 (44.8%)	29	
31-40	13 (72.2%)	5 (27.8%)	18	
41 and above	6 (66.7%)	3 (33.3%)	9	
Total number of responses	82	38	120	
Status				
Undergraduate student	44 (47.4%)	18 (47.4%)	62	P= 0.521 $X^2 = 0.411$ df = 1
Postgraduate student and staff member	38 (65.5%)	20 (34.5%)	58	
Total	82 (68.3%)	38 (31.6%)	120	

Table 3.18 depicts the different sources of information for respondents on the fate of pharmaceuticals and illicit drugs in wastewater. Most respondents (61.0%) indicated they found out through research articles or books that when illicit and pharmaceutical drugs are consumed/flushed down the drain, they end up in wastewater treatment plants and can be detected thereafter. BIUST is a learning and research institute therefore almost all students

and teaching staff read articles/books at some point. There are a lot of published articles on wastewater-based epidemiology for drug use and abuse, as a result, they were most likely to come across such articles in which they gained some knowledge.

Table 3.18: Sources of information on the fate of pharmaceutical and illicit drugs in wastewater.

	Frequency	Percentage (%)
Friends	2	2.4
Through research articles or books	50	61.0
Through radio or television	13	15.9
Through social media such as Facebook/WhatsApp/Twitter	12	14.6
Other	17	20.7

About 15.9% and 14.6% of the respondents indicated that they obtained information on the fate of illicit drugs from radio/television and social media (Facebook, WhatsApp, Twitter etc) respectively. Only 2.4% of the respondents indicated having obtained the information from a friend. The findings from our study show that there are different means that researchers can use to share information on drug-related topics and educate the nation or raise more awareness programmes required to increase knowledge on wastewater-based epidemiology for drugs of abuse and pharmaceutical drugs as well as on the safe disposal of pharmaceutical drugs.

Table 3.19: Adequate information on the dangers of misuse of pharmaceutical and illicit drug use in Botswana.

Is there adequate information on the dangers of misuse of medicinal and illegal drug use in Botswana?	Frequency	Percentage (%)
Yes	7	36.8
No	9	47.4
I do not know	3	15.8

Table 3.19 indicates the responses from participants when asked if they think there is enough information on the dangers of illicit drug use and misuse of pharmaceutical drugs in Botswana. The majority (47.4%) of the respondents indicated that there is not enough

information on the illicit drug use and misuse of pharmaceutical drugs in Botswana whereas 15.8% of the participants indicated that they do not know whether there is enough information on the dangers of illicit drug use and the misuse of pharmaceutical drugs in Botswana.

Table 3.20: Ways in which a lack of knowledge on the dangers of illicit drugs and the misuse of pharmaceutical drugs in Botswana can be improved.

Suggestions made by participants	Frequency	Percentage (%)
More Education	6	75
Public awareness	2	25
Public speaking	1	12.5
Radio & television documentaries	1	12.5
Research and publication on the topic	1	12.5
Posters in hostels, workplaces, and social media	1	12.5

Table 3.20 indicates that most of the respondents (75%) suggested that more education is needed to improve the lack of knowledge on the dangers of illicit drugs and the misuse of pharmaceutical drugs in Botswana. Some respondents suggested public awareness involving recovered addicts, among other things. The United Nations Office on Drugs and Crime (UNODC) also promotes school-based education for drug abuse prevention [124]. In Table 3.19 most respondents indicated that there is a lack of knowledge on the danger of misuse of pharmaceutical drugs and illicit drug use in Botswana. Therefore, the suggestions made in Table 3.20 can be implemented to bridge the gap in knowledge on the dangers of misuse of pharmaceutical drugs and the dangers of illicit drug use.

3.2.6 Summary of results from the survey questionnaire

The administered online survey questionnaire aimed at exploring the knowledge of the public on the use, disposal, and fate of pharmaceutical and illicit drugs in wastewater in Botswana. From the one hundred and twenty responses obtained it was evident that most of the respondents acknowledged using different pharmaceutical drugs (80.5%) compared to fewer respondents who admitted to using illicit drugs (16.1%).

The responses also show more females used pharmaceutical drugs than their male counterparts. However, it is worth noting that pharmaceutical drug use depends on various

health factors. From the findings of this study, it is evident that painkillers were the most used class of pharmaceutical drugs by the BIUST population between March 2019 and May 2021. The high prevalence of painkillers such as ibuprofen can be linked to them being easily available as over-the-counter medication and their affordability. Ibuprofen can be both over-the-counter or prescription depending on the dosage.

On the use of illicit drugs, it was shown that most of the illicit drug users are male compared to their female counterparts. The use of illicit drugs is also prevalent in the 21–30-year age group in Botswana, which is also consistent with the findings of this study. Respondents indicated the use of hallucinogens such as cannabis more than any other class of illicit drugs, which is consistent with the reports given by the Botswana Police Service in which they indicated that cannabis is the most common illicit drug used/found in Botswana.

Through analysis of the responses obtained it was shown that most people dispose of expired/leftover pharmaceutical drugs and illicit drugs which are no longer going to be used through the litter bin and down the drain. The improper disposal of leftover/expired pharmaceutical and illicit drugs can result in drugs ending up in soils via dumping sites and eventually leaching into the water system. The presence of pharmaceutical compounds in the water system poses a risk to aquatic life.

Most respondents indicated that it is through reading research articles and books that they were made aware of pharmaceutical and illicit drugs ending up in wastewater treatment plants after consumption and being detected thereafter. The survey participants are all in the education sector, at an institution of higher learning therefore it can be expected that they gained knowledge on the fate of drugs in wastewater through reading books and research articles compared to social media platforms and radio/television.

The fight against the misuse of pharmaceutical drugs and the use of illicit drugs requires a collective effort by all stakeholders to succeed. Therefore, this can be achieved in various ways which include knowledge exchange between communities and relevant stakeholders such as law enforcement officials. Some of the respondents indicated that there is a lack of information on the dangers of misuse of pharmaceutical drugs and the use of illicit drugs in Botswana. As a result, suggestions given by participants such as increasing public awareness campaigns and education can be implemented in society to bridge the gap in knowledge on the dangers of illicit drug use and misuse of pharmaceutical drugs.

3.3 Comparison of GC-MS results for wastewater samples and online survey questionnaire

An online survey questionnaire with sixteen questions was administered to the BIUST community from March 2021 to May 2021 and a total of 120 responses were obtained. One of the reasons for administering an online survey questionnaire was to determine if there could be a correlation between findings from the survey with those from the analysis of Palapye-BIUST wastewater samples using the GC-MS. Mefexamide, a central nervous system stimulant was the only compound that was detected in the wastewater samples collected at the Palapye-BIUST WWTP. None of the participants indicated in the survey responses that they had taken/used mefexamide. Mefexamide is no longer marketed as human medicine and is sold strictly for research purposes.

The GC-MS results for the Palapye-BIUST wastewater samples do not correlate with the survey responses. This is because survey respondents indicated the use of different pharmaceutical and illicit drugs which were not detected in the four wastewater samples that were collected at the Palapye-BIUST WWTP on different dates. The GC-MS results not correlating with the survey results could be due to various factors. The time of wastewater sampling affects the GC-MS results in that if the samples were collected when most people have not used the drug, or the wastewater has not yet reached the wastewater treatment plant then there was a very high chance of the drugs not being detected. Grab sampling was used for this research and grab samples are representative of the condition of wastewater at the time of sampling. The lack of correlation between the GC-MS results could also be linked to only a screening method used for this research.

Another challenge experienced with this research was the lack of drug standards to confirm some of the drugs detected. This resulted in the research being qualitative analysis instead of quantitative analysis. With the availability of pure drug standards, selected ion monitoring (SIM) could have been employed as it increases the sensitivity and specificity of the GC-MS method. This research was only qualitative, as a result, further method validation is needed to establish the sensitivity of the GC-MS used. Full validation of the method could have enabled the calculation of Limit of Detection (LOD), and Limit of Quantitation (LOQ). Derivatisation was not explored for this study due to a lack of drug standards, however, it reduces the polarity of the compound as well as increases its volatility and thermal stability which can increase the chances of such a compound being detected.

In addition, derivatisation improves the resolution between co-eluting compounds and overlapping peaks, which was frequently experienced in this research.

Wastewater is a very complex matrix in which drugs may be available at trace levels and be below the limit of detection of the instrument that was used. Therefore, more sensitive instruments such as LC-MS should be used. LC-MS is fast becoming the preferred method for drug testing than GC-MS as it has far greater sensitivity and can selectively detect analytes of interest in a complex sample matrix, thus making it easy to find and identify suspected analytes at trace levels [125]. Also, most illicit drugs and pharmaceutical drugs are polar compounds which makes LC-MS the most suitable instrument to use. In addition, this research was qualitative rather than quantitative due to the limited availability of drug standards, therefore, method validation was not able to be conducted.

WBE as means of obtaining data on community drug usage and consumption is fast becoming an alternative method as it gives near real-time data. It is a non-intrusive monitoring tool through which evidence-based real-time data on community drug usage can be obtained. This makes it the most preferred tool over other conventional methods of obtaining data on community drug usage and consumption.

Although WBE is increasingly becoming an alternative method, it has its limitations. Through WBE, demographic information of drug users, the frequency of use of drugs and the mode of administration cannot be obtained but can be obtained through the survey questionnaires. With WBE, there is no knowledge of the behaviour of each drug user as it gives the wide spectrum of drug use by a community serviced a WWTP where samples are collected. As a result, WBE is not a replacement but is rather a complementary technique for other conventional means of obtaining data on community drug use and abuse such as the administration of survey questionnaires and medical records. By combining survey-based monitoring of drugs used in each population and WBE, the issue of drug abuse can be evaluated more comprehensively and potentially support relevant stakeholders in the fight against drug abuse.

CHAPTER FOUR: CONCLUSION, LIMITATIONS AND FURTHER WORK

This chapter summarises the key research findings regarding the research aims and objectives and discusses the value and contribution, thereof. It will also review the limitations of the study and give recommendations for further work/future research.

4.1 CONCLUSION

Over the years, the analysis of wastewater for drugs of abuse has gained momentum worldwide owing to its non-intrusive nature as well as the near real-time data that can be obtained. The presence of pharmaceutical drugs and illicit drugs in wastewater has gained scientific interest as it can be used to determine the prevalence of the drugs in each geographical area as well as estimate the community drug usage and consumption. WBE does not only target traditional drugs of abuse. With the constant emergence of new psychoactive substances such as synthetic cannabinoids, new analytical methods which are quick, specific, and sensitive enough are needed for the detection of such compounds in complex matrices such as wastewater.

One of the aims of this research was to use a GC-MS method for the detection of pharmaceutical drugs and illicit drugs in wastewater samples collected from different WWTP in Botswana. This was achieved through the collection of 18 influent wastewater samples from Palapye-BIUST, Palapye-main village, Serowe-Radihemelo, Serowe-Mabogopa and Gaborone-Glen Valley WWTP in Botswana. The influent wastewater samples collected were successfully extracted by the SPE technique at pH 2.5–2.8 using Strata -X Drug B SPE cartridges. GC-MS was then used to analyse the samples for the analytes of interest. The results obtained indicate the presence of two confirmed compounds and five tentatively identified compounds from samples collected at the different WWTP.

The compounds that were identified and confirmed from the different wastewater samples were ibuprofen and caffeine. Both ibuprofen and caffeine had been previously reported in wastewater in other countries. The other compounds of interest; amiphenazole, efavirenz, mefexamide, prolintane and xylazine were tentatively identified using the NIST database owing to the lack of drug standards. Of the compounds that were tentatively identified, only efavirenz is registered with BOMRA and is sold/dispensed in pharmacies across the country. Ibuprofen which was detected and confirmed from the wastewater samples is registered

with BOMRA and is also sold/dispensed in pharmacies. This, therefore, answered one of the research questions which was to find out the different pharmaceutical and illicit drugs used by the various communities where wastewater samples were collected.

Efavirenz has also been previously reported in wastewater samples in South Africa. The detection of other compounds that are not registered with BOMRA or that are no longer marketed for human use may be linked to research carried out in Botswana or medical doctors importing such drugs for medicinal use after requesting permission. Also, patients are allowed to seek medical care outside Botswana and bring medications that are not registered with BOMRA in Botswana. Another possible source for the presence of unregistered medication in Botswana sewer can be linked to smuggling or the black market.

The other aim of this research was to determine the use, disposal, and knowledge of the fate of illicit and pharmaceutical drugs in wastewater at BIUST using a survey. This was achieved through administering an online survey questionnaire to the BIUST community, in which a total of one hundred and twenty (120) responses were recorded. The survey results obtained, indicated that 80.8% of the participants used pharmaceutical drugs, while only 15.8% of the participants indicated having used illicit drugs between March 2019 and March 2021.

From the survey responses obtained, based on the answer options given, there is high usage of painkillers such as panado, whereas hallucinogens such as cannabis were the most used class of illicit drugs. The findings of the survey also showed that females used pharmaceutical drugs more than their male counterparts, whereas males used illicit drugs more than their female counterparts, confirming findings from previously published literature.

Through this research, it was established that illicit drug use at BIUST between March 2019 and May 2021 was prevalent in the 18-24 years age range which confirms published literature on the age range of most illicit drug users in Botswana. The findings of the survey also indicated that expired or leftover pharmaceutical drugs are disposed of in household litter bins. The improper disposal of leftover/expired drugs can result in the contamination of the underground water system or aquatic life; therefore, proper measures should always be followed when disposing of such drugs.

Furthermore, 68.3% of the participants showed that they know the fate of both pharmaceutical and illicit drugs in wastewater. Most of these participants, stated that they obtained such knowledge through reading research articles and books, which is something that can be expected because the participants are in a learning institute. The participants also showed that they gained knowledge on WBE through social media platforms, as well as radio and television. This shows that there are many ways through which communities can be informed about the dangers of illicit drug use and the misuse of pharmaceutical drugs, as some of the respondents indicated that, there is a lack of information on the dangers of illegal drug use and misuse of pharmaceutical drugs in Botswana.

For many years, data on community drug usage and abuse has been obtained through various means, some more effective than others. Through this research, an interdisciplinary approach to the analysis of drugs of abuse was introduced in Botswana. WBE and survey questionnaires complement one another as they can be used together to evaluate drug use and abuse more comprehensively and potentially support the stakeholders in the fight against illicit drug use and the misuse of pharmaceutical drugs.

The data that is obtained through survey-based monitoring and WBE, can be compared to see if there is a correlation in the results obtained from the two means. However, for this research, the results of the wastewater samples for Palapye-BIUST WWTP did not match the survey responses from the BIUST community. The survey respondents indicated the use of both pharmaceutical and illicit drugs which were not detected in the wastewater samples collected. However, ibuprofen, a common pharmaceutical drug was detected in samples collected at other WWTP. This, therefore, answered the second research question for this study in which we were to find out if there is a similarity between the data obtained from the survey responses with the BIUST wastewater samples. Through WBE, near real-time data on the drugs that were used in the community of interest was obtained whereas, with the online survey questionnaire, the sociodemographic information of the drug users, frequency of drug use, the location where drugs are mostly used as well as the mode of disposal of the drugs by the various users was obtained.

The research gave an idea of the type of drugs that are used in areas where the wastewater samples were collected. The detected compounds mainly had known pharmaceutical use. It was established that ibuprofen is a common drug in Botswana as it was detected in samples from different wastewater samples collected on different dates. Mefexamide was detected

in samples from all the WWTP, while amiphenazole was detected in samples from two out of the five WWTP.

The findings of this study can be used to check if there is the illicit use of mefexamide, amiphenazole and other unregistered medications in Botswana. This is a study that can involve stakeholders such as BOMRA and the BPS. This research also shows that WBE can be effectively implemented in Botswana to monitor drug use patterns and trends as well as follow drug supply lines.

Through the survey questionnaire, it was established that there is illicit drug use among the BIUST community. These findings can be used by the university management to evaluate the effectiveness of drug raids, and anti-drug campaigns, follow drug supply lines and establish more effective measures against the use of illicit drugs. The results obtained will also help relevant offices in the fight against drug abuse as they provide some of the much-needed information on commonly used drugs, frequency of use, and demographic information of drug users.

Wastewater-based epidemiology has limitations like any other technique. WBE is an evolving method that needs further refinement before it can be standardised. Also, there is little knowledge on the stability of the various compounds in wastewater before they undergo analysis. Drugs that are used in very little quantities can also pose an analytical challenge in that they can be used in an area for years without being detected if they are below the LOD of the method.

The sociodemographic data, mode of administration of drugs, frequency of use and location of use, among others, cannot be obtained through WBE. Conventional techniques such as the administration of survey questionnaires, that were used in this study also have their limitations. Online survey questionnaires can be intrusive, subject to self-report bias, limited population coverage and therefore under-representing or over-representing the drug usage in a community. It can also take a long time before survey data can be obtained which can result in the data being irrelevant or outdated by the time it is obtained or published.

4.2 Suggestions for further work

The stability in wastewater of the compounds that were targeted for this research is unknown or has not been fully investigated. For further work, stability studies of the various compounds of interest in wastewater can be done. For collecting wastewater, 24 hours

composite sampling gives a better representation of the samples. Therefore, in the future, automated composite samplers should be employed to increase the chances of detection of the analytes of interest. In the extraction of wastewater samples, only one type of SPE sorbents was used, therefore, in the future various SPE cartridges can be employed to determine their performance. Quantitative analysis should also be done to determine the amount of the different compounds detected in wastewater samples.

The extracted samples were analysed by GC-MS only. In future, LC-MS can be used to be able to compare the results of the two instruments. Enantiomeric profiling of the drugs can also be done to differentiate between direct disposal and consumption of the drugs as well as distinguishing between recreational use and pharmaceutical use of the drugs.

In the future, survey questionnaires should be administered in all the areas where wastewater samples are collected to corroborate the findings of the GC-MS. Also, GC-MS analysis should be complemented by other conventional methods such as medical records, police drug raids, customs drug data reports as well as rehabilitation centres records. To come up with effective anti-drug campaigns, drug raids or amending of drug policies, all the data on drug-on-drug use and consumption should be obtained. This can only be achieved through the implementation of all the conventional as well as emerging methods at a national level.

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APPENDICES

Appendix I: Online Survey Questionnaire Form

PURPOSE OF THE STUDY

The questionnaire will allow the researcher to assess the participant's age, gender, employment status, their knowledge about illegal drugs and medicinal drugs including their fate in wastewater. The data that will be obtained from the survey will be compared with the GC-MS results that will be obtained from the analysis of wastewater samples to see if there is any correlation. This information will help us analyse differentiated knowledge and understanding based on respondents' age, gender, and employment status.

RESPONDENTS

Anyone willing to take part in this survey should be 18 years old and able to give valid consent.

CONSENT

If consent is provided by answering 'Yes' at the beginning of the survey, filling out and submitting your answers, you are authorizing the researcher to use your answers in the research. Due to the nature of the research, the answers cannot be redacted once submitted, however, if you wish to leave the survey at any time you can exit the webpage without your answers being submitted. If your answer is 'No', you will not be able to proceed to the next stage.

WHAT WILL THE SURVEY INVOLVE?

After consent is provided the respondents will be asked to complete 16 questions about illegal drugs and medicinal drugs. The survey should take 5-10 minutes to complete and only needs to be completed once. All the answers will be anonymous so identification of respondents cannot occur. The data gathered from the survey will be compiled and written up in a master's research project at the Botswana International University of Science and Technology (BIUST). The findings may also be presented at conferences and included in research journal articles. The online survey link will be shared via email and or social media platforms (WhatsApp and Facebook).

CONTACT DETAILS

If you have any questions about the study, please don't hesitate to contact Dr Ellen Mwenesongole at mwenesongolee@biust.ac.bw or Lorato Makanye at lorato.makanye@studentmail.biust.ac.bw.

MANDATORY DATA PROTECTION

For this research, no personal data will be asked at any given stage except for gender, age and employment status. The researchers: Lorato Makanye (MSc student), Dr Ellen Mwenesongole (supervisor) and Dr Lata Gautam (co-supervisor) have sole responsibility and control over the answers given.

PARTICIPANT CONSENT FORM

Research Title: Interdisciplinary approach to the analysis of drugs of abuse in wastewater using a survey and gas chromatography-mass spectrometry

Main Investigator: Lorato Makanye

Email address: lorato.makanye@studentmail.biust.ac.bw

Research team members: Lorato Makanye, Dr Ellen Mwenesongole & Dr Lata Gautam.

1. I agree to take part in the above research. I have read the Participant Information Sheet for the study.
2. I understand what my role will be in this research, and all my questions have been answered to my satisfaction.
3. I understand that I am free to withdraw from the research at any time, without giving a reason by the closing browser before submitting the questionnaire.
4. I am free to ask any questions at any time before and during the study by contacting researchers using their email addresses provided in the participant information sheet.
5. I understand what information will be collected from me for the study.
7. I understand what will happen to the data collected from me for the research.
8. I have been told about any disadvantages or risks regarding me taking part.
9. I have been informed how my data will be processed, how long it will be kept and when it will have destroyed.
10. I have been provided with a copy of this form and the Participant Information Sheet (add date and version number)

Consent

The following question is compulsory.

If your answer to the question below is 'Yes', please proceed to the next section.

If your answer is 'No', please submit the form and exit the page.

Do you consent to take part in this survey?

- ☐ Yes
☐ No

Required -*

1. What gender do you identify as? *

- ☐ Male
☐ Female
☐ Prefer not to say

2. How old are you? *

- ☐ 18-24 years
☐ 25-30 years
☐ 31-40 years
☐ 41-50 years
☐ 51-60 years
☐ 61-years and above

3. Are you a student or a member of staff at BIUST? *

- ☐ Undergraduate student
☐ Postgraduate student
☐ Staff

4. Have you used any medicinal drug(s) in the last 24 months? *

- ☐ Yes
☐ No

5. Which category of medicinal drug(s) did you use? (Multiple answers allowed) *

- ☐ Painkillers such as aspirin, ibuprofen, panado, tramadol, codeine etc.
☐ Antidepressants such as diazepam, fluoxetine, olanzapine, amitriptyline etc.

- ☐ Antihistamines such as allergex, cetirizine, loratidine etc.
- ☐ Chronic diseases medication such as for asthma, heart disease, diabetes etc
- ☐ Other

6. Where do you normally use the medicinal drug(s)? (Multiple answers are allowed) *

- ☐ Hostels
- ☐ Office
- ☐ Academic areas (classrooms/laboratories)
- ☐ Home (off campus)
- ☐ Other

7. How do you dispose of expired or leftover medicinal drugs? (Multiple answers are allowed).*

- ☐ Throw them in a litter bin
- ☐ Throw them in pit latrine toilets
- ☐ Flush them down the drain (sink or toilet)
- ☐ Return them to the clinic/hospital/pharmacy
- ☐ Other

8. Have you used any illegal drug(s) in the last 24 months? Illegal drugs examples are marijuana, cocaine etc.*

- ☐ Yes
- ☐ No

9. Which illegal drug did you use? (Multiple answers allowed) *

- ☐ Opioid such as heroin.
- ☐ Hallucinogens such as marijuana, LSD etc.
- ☐ Sedative such as mandrax.
- ☐ Stimulants such as cocaine, methamphetamine, amphetamine, ecstasy, methcathinone etc.
- ☐ Other

10. Where do you normally use the illegal drug(s)? (Multiple answers are allowed) *

- ☐ Hostels
- ☐ Office
- ☐ Academic areas (classrooms/laboratories)
- ☐ Home (off campus)
- ☐ Bars/ night clubs/ liquor restaurants

- ☐ Hotels/Lodges/ Guesthouses
- ☐ Prefer not to say
- ☐ Other

11. How often do you use the illegal drug(s)? (Multiple answers are allowed) *

- ☐ Everyday
- ☐ Every weekend
- ☐ Last weekend of the month
- ☐ Before/during/after major sporting event
- ☐ Before/during/after major entertainment event
- ☐ Before/after tests/examinations/presentations
- ☐ Before/after attending class/tutorial session/laboratory session
- ☐ Before/after working hours
- ☐ Prefer not to say
- ☐ Other

12. How do you dispose of illegal drug(s) when they are no longer going to be used? (Multiple answers are allowed)*

- ☐ Throw them in a litter bin
- ☐ Throw them in pit latrine toilets
- ☐ Flush them down the drain (sink or toilet)
- ☐ Other

13. Are you aware that both illegal and medicinal drugs, when consumed or flushed down the toilet/sink, can end up in a wastewater treatment plant (sewage) and be detected thereafter?*

- ☐ Yes
- ☐ No

14. How did you know that when medicinal and illegal drugs are consumed or flushed down the

toilet/sink, they end up in a wastewater treatment plant (sewage) and be detected thereafter? (Multiple answers are allowed)*

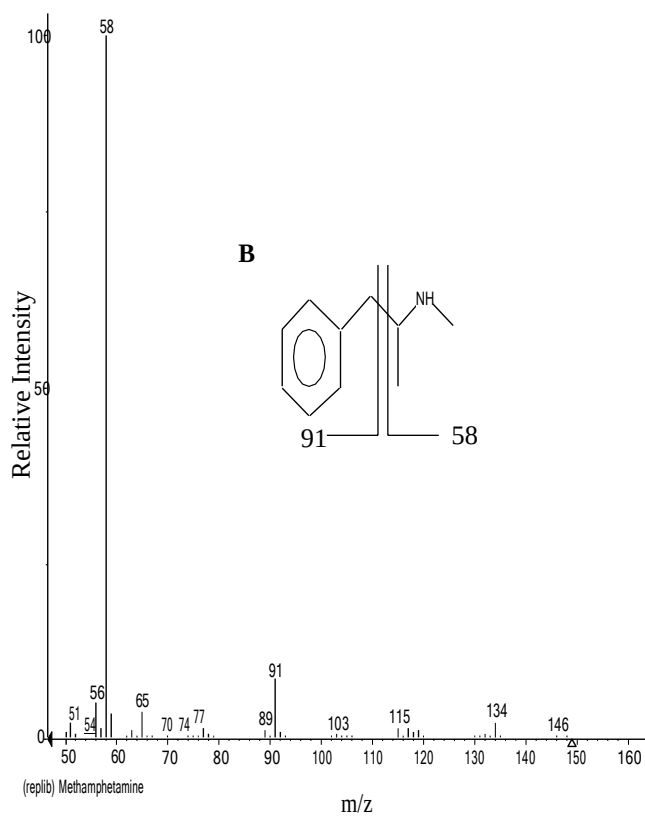
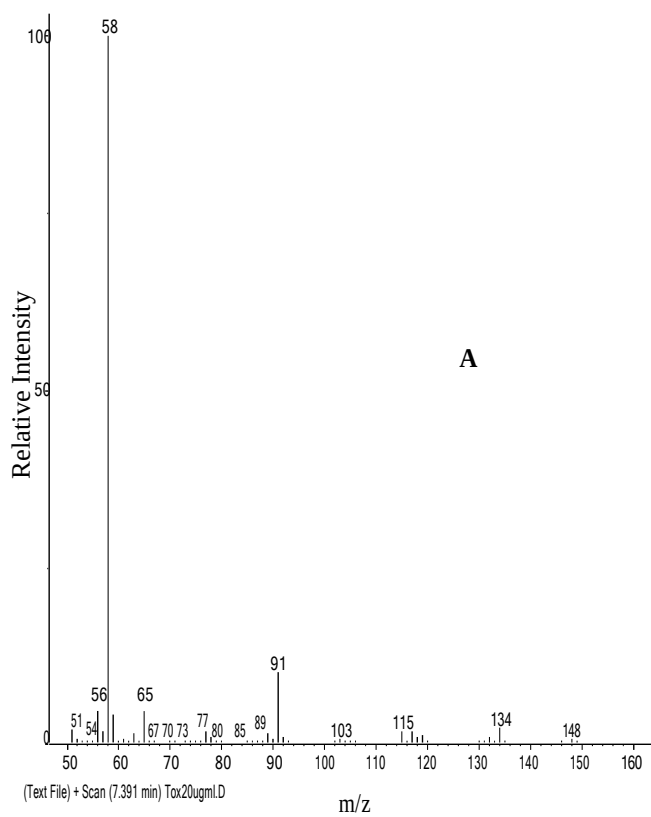
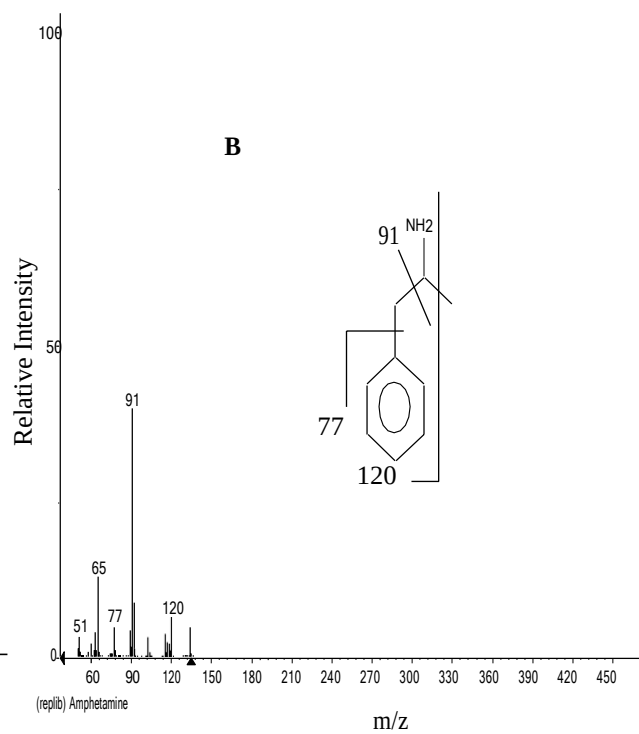
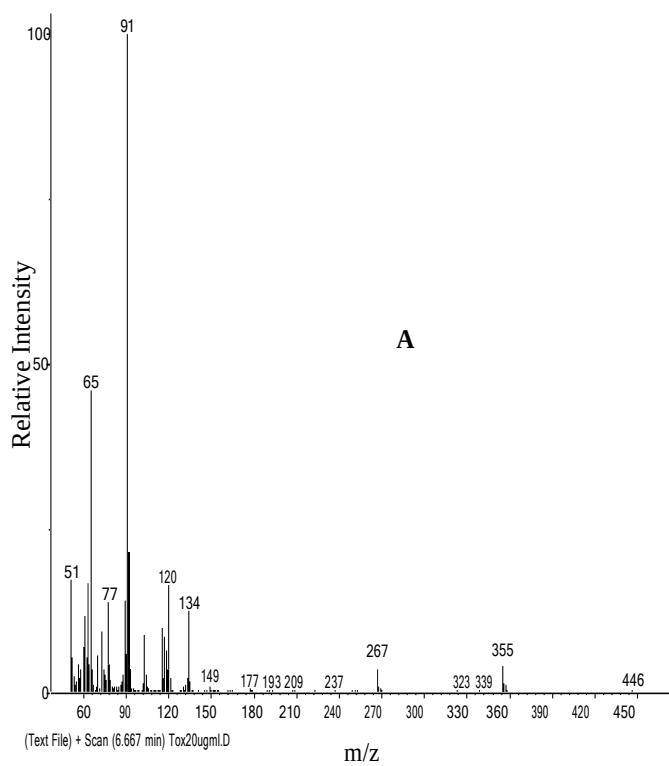
- ☐ through a friend
- ☐ through research articles or books.
- ☐ through radio or television.
- ☐ through social media e.g. Facebook, WhatsApp, Twitter etc.
- ☐ other

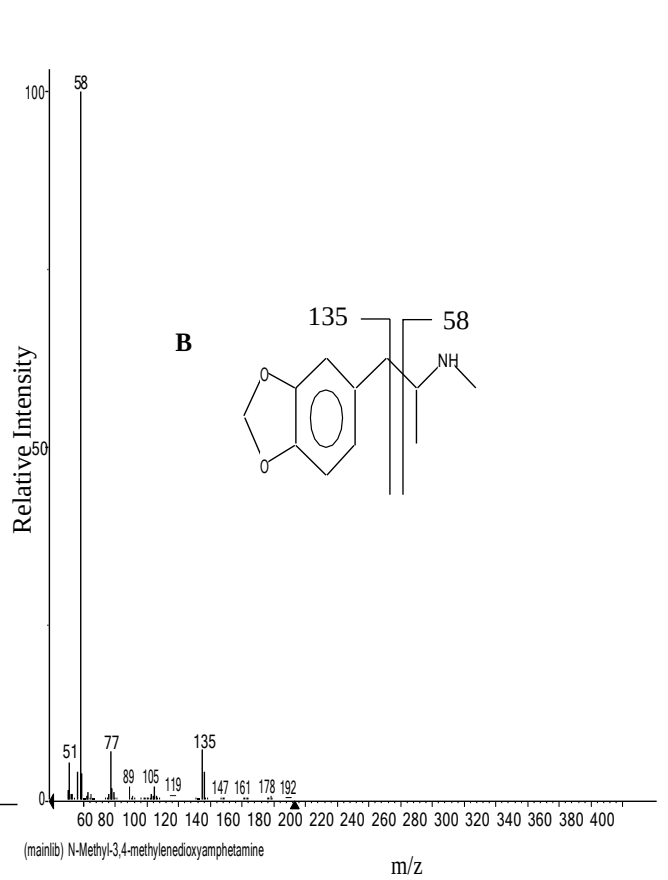
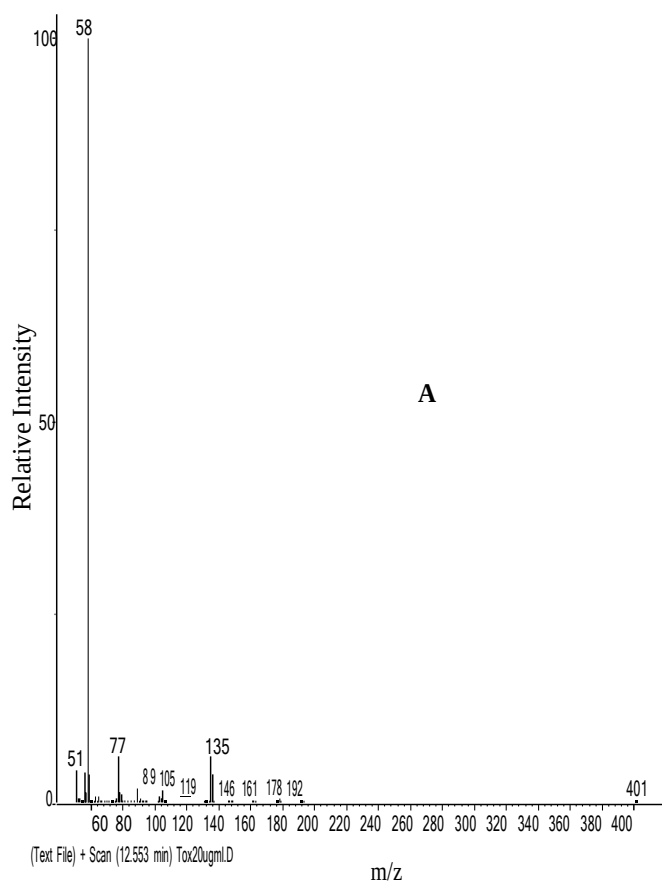
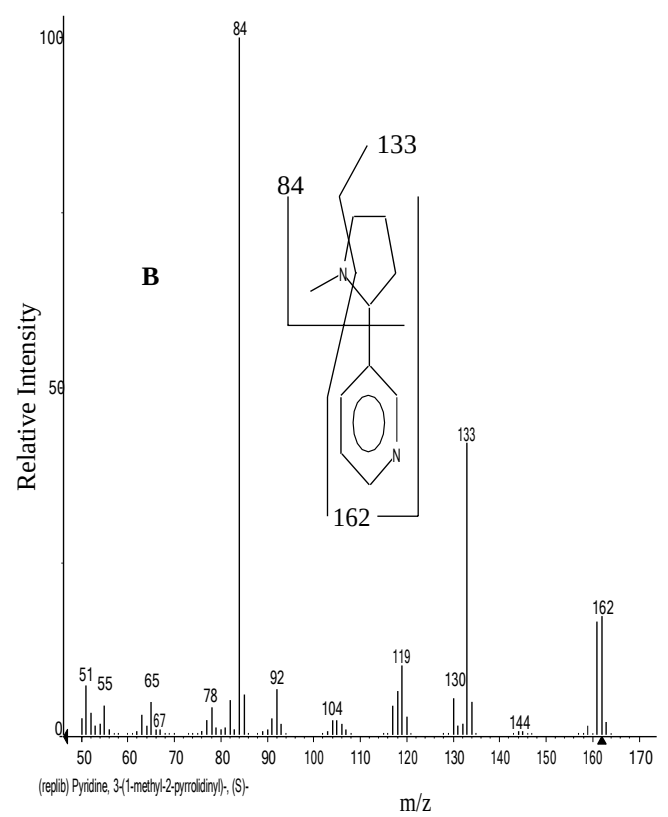
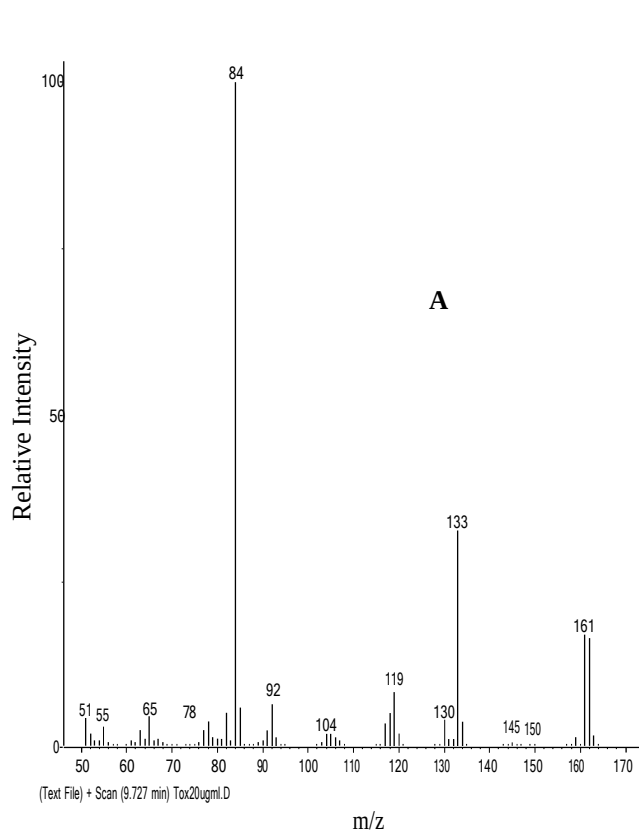
15. According to you, is there enough information on the dangers of using illegal drugs and the misuse of medicinal drugs in Botswana?*

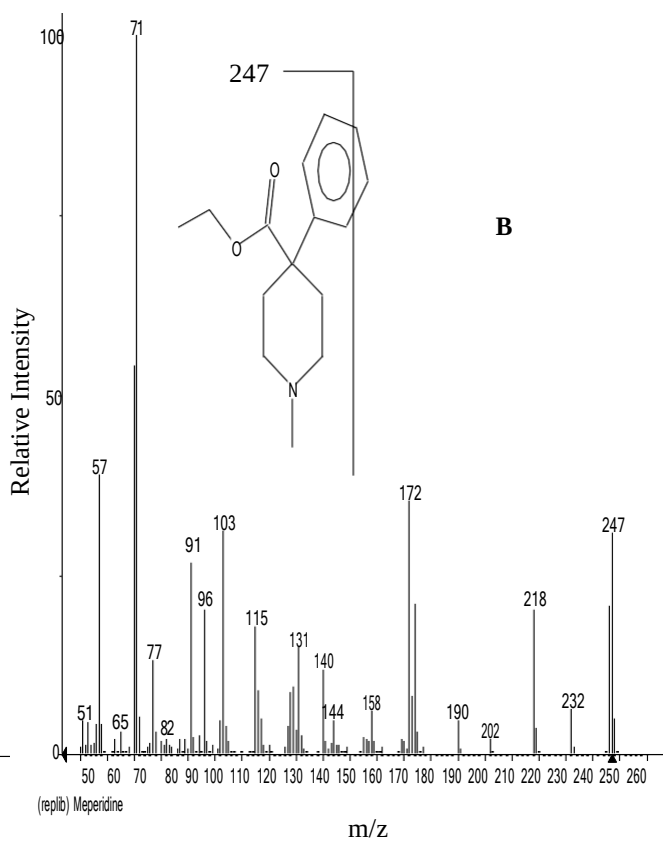
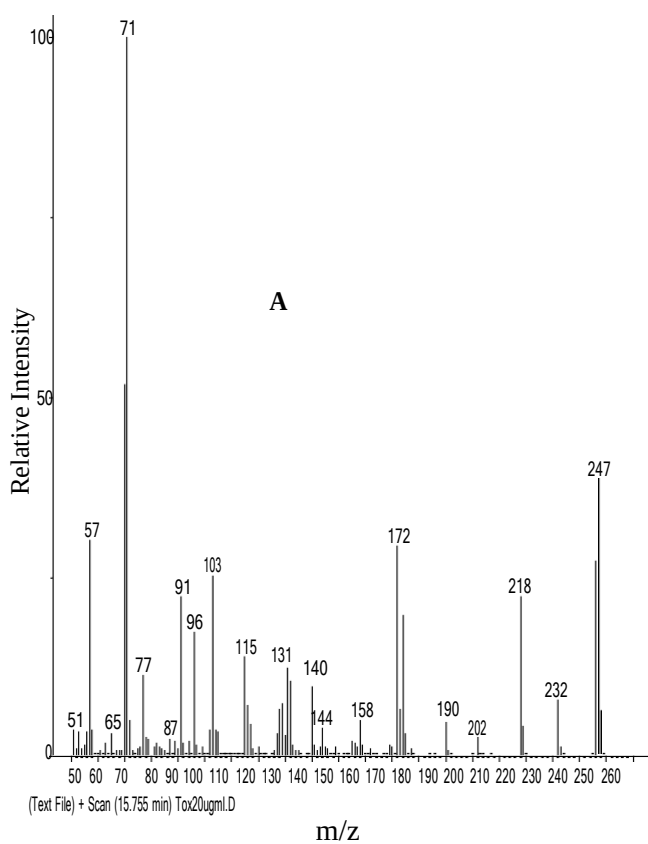
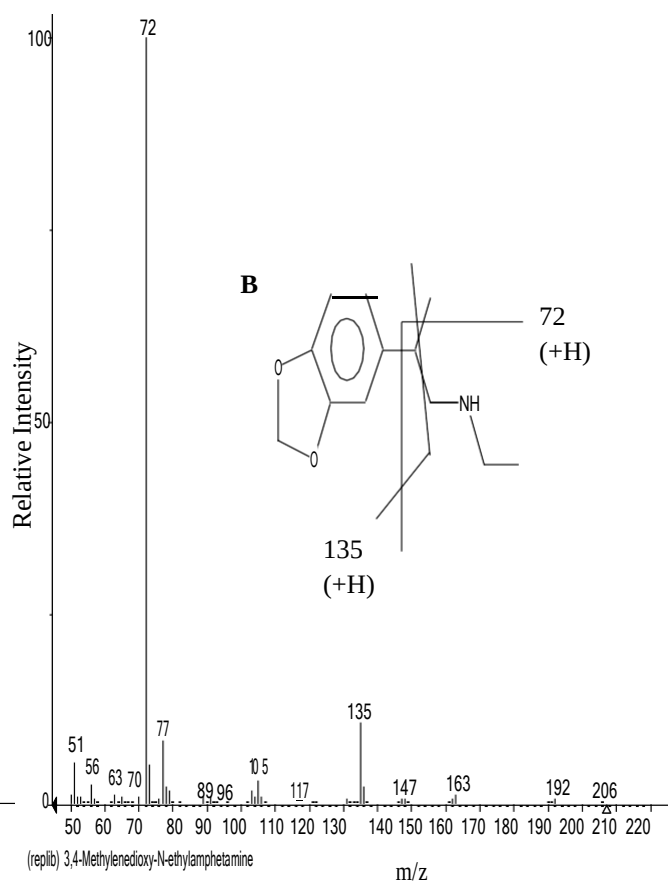
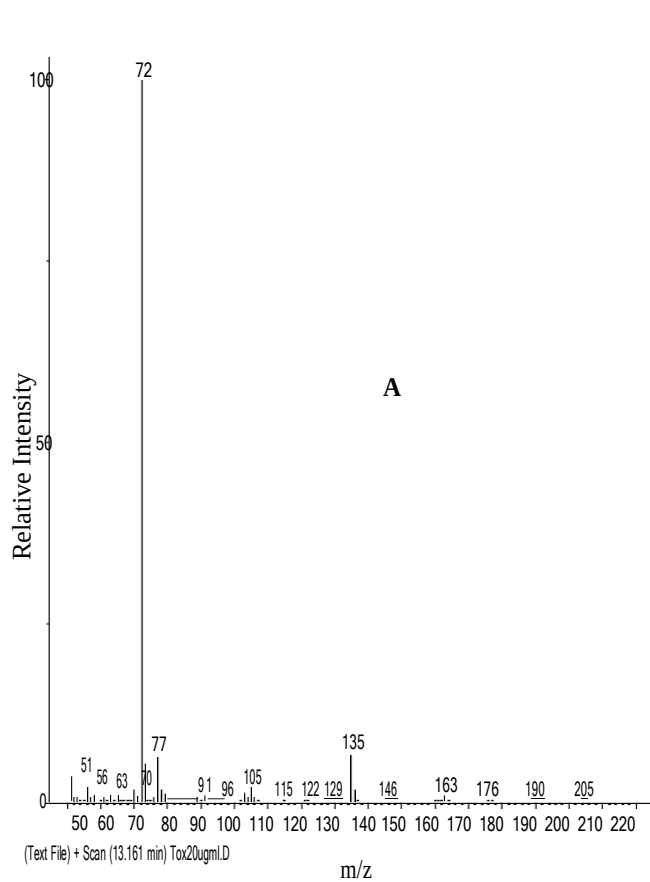
- ☐ Yes
- ☐ No
- ☐ I do not know

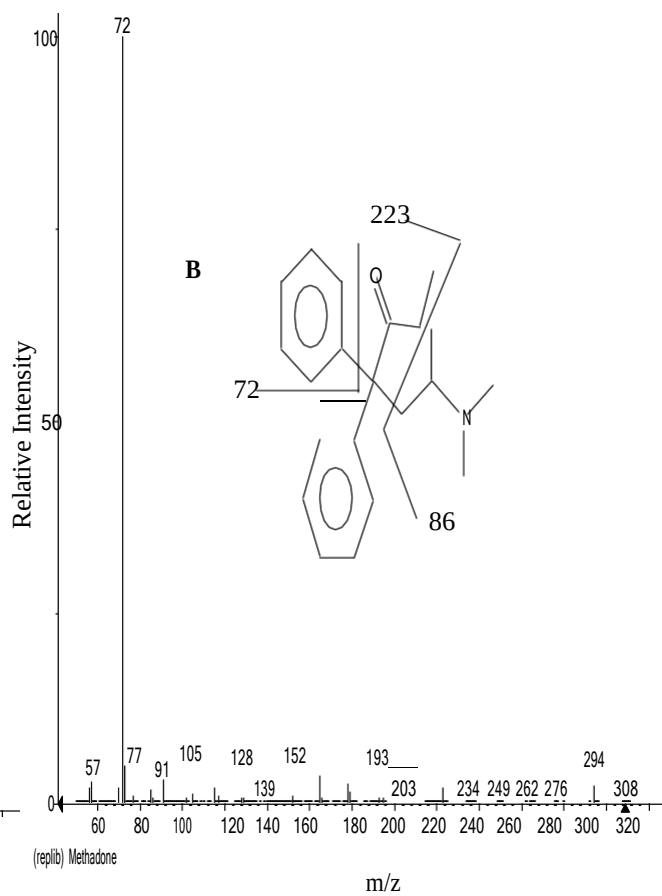
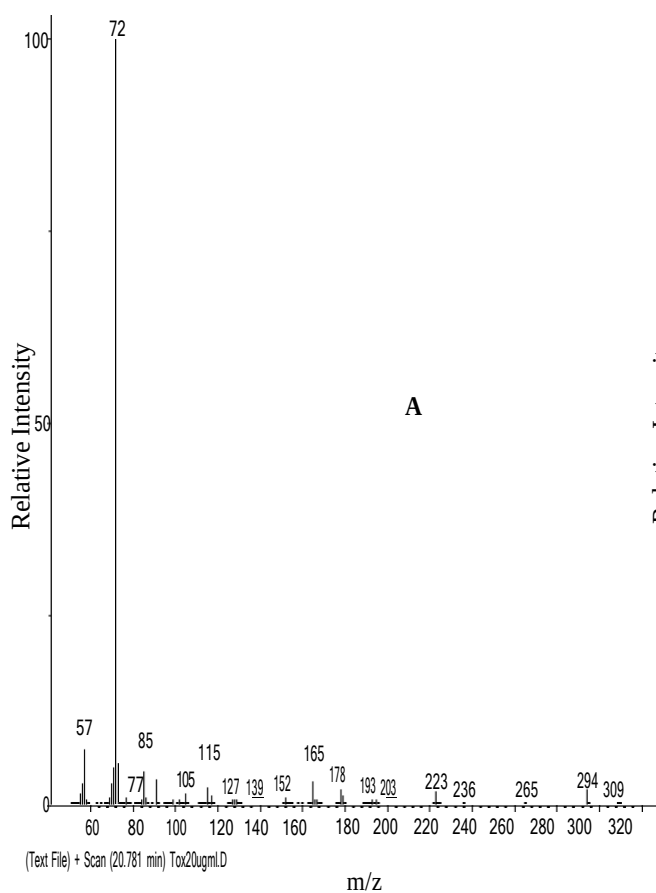
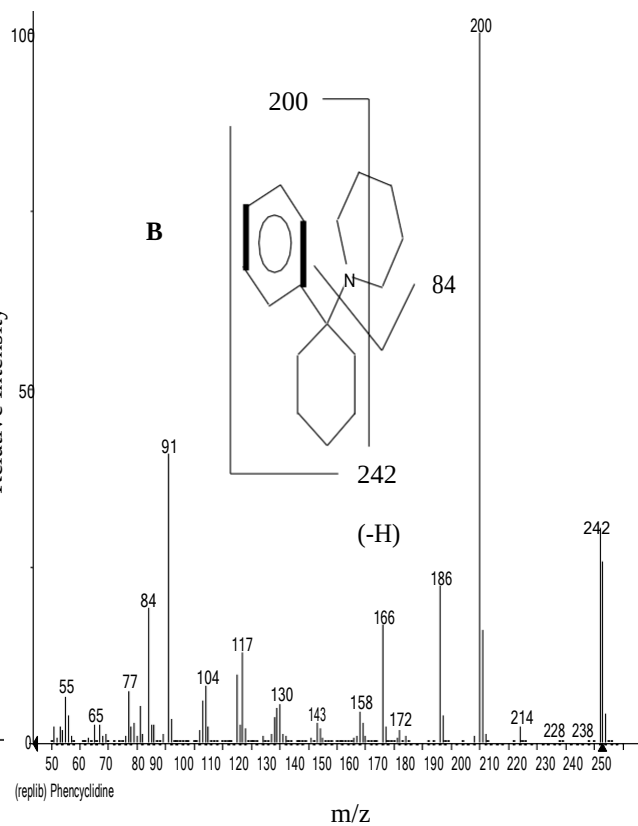
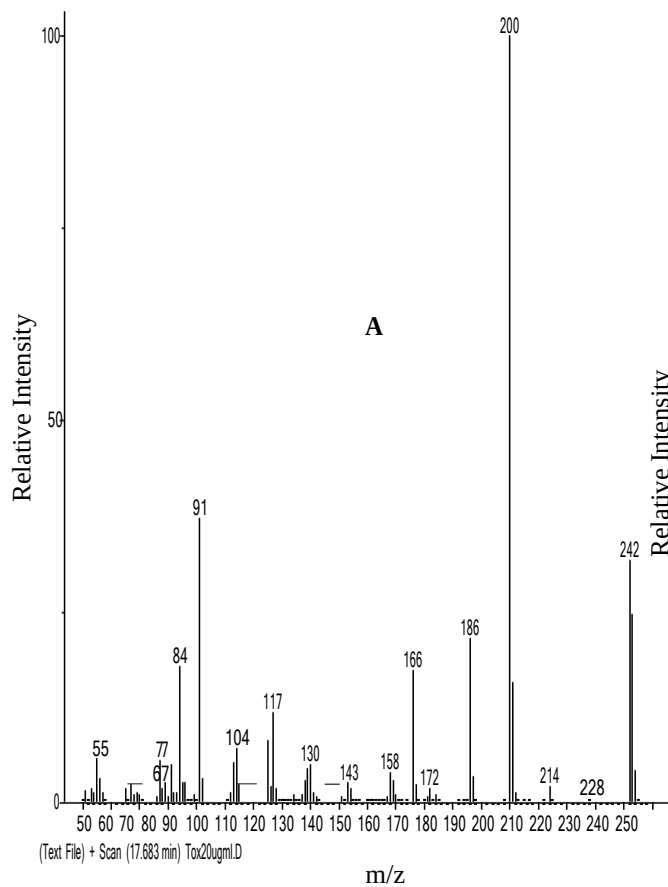
16. What do you think can be done to improve the lack of knowledge on the dangers of using illegal drugs and misuse of medicinal drugs.?

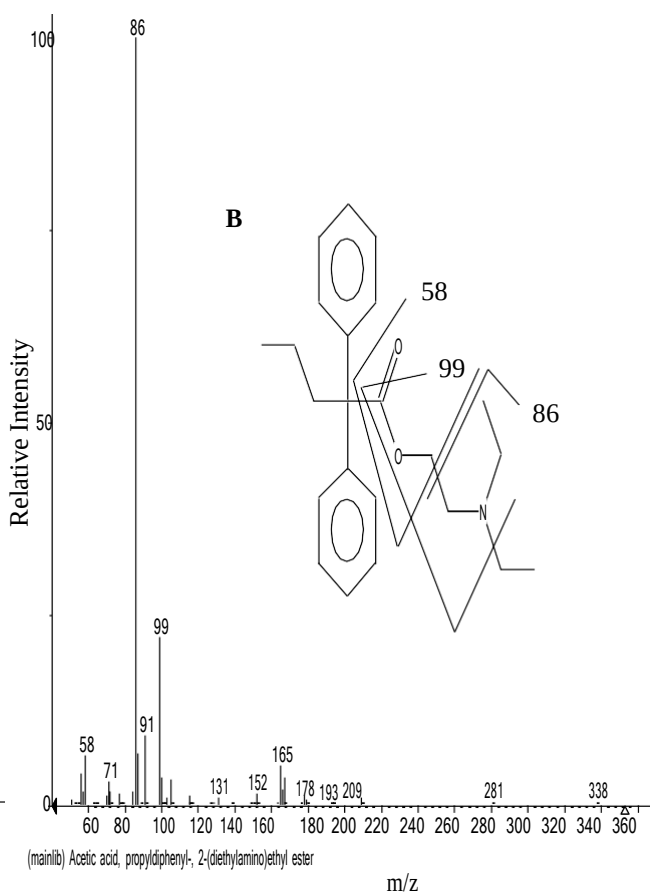
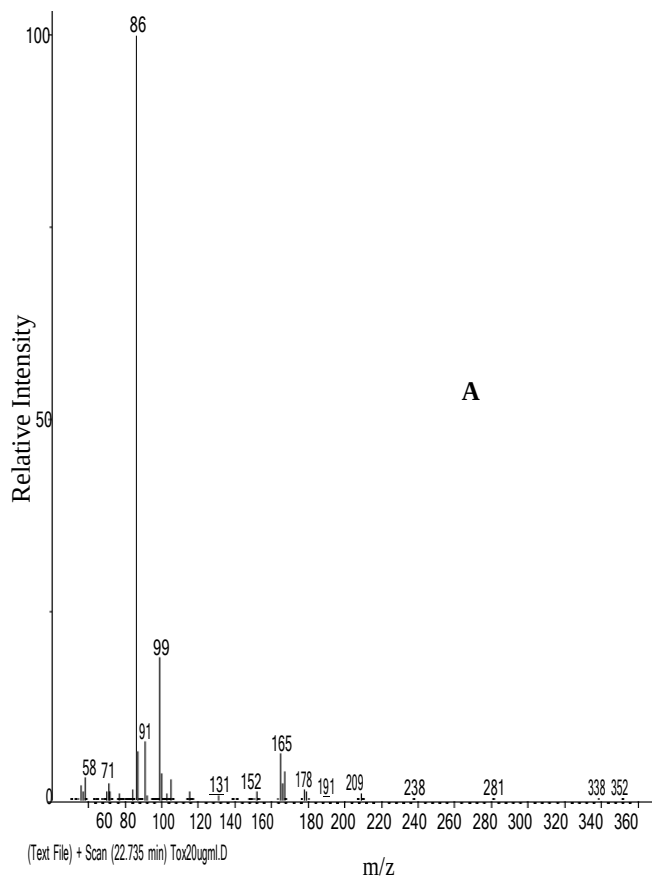
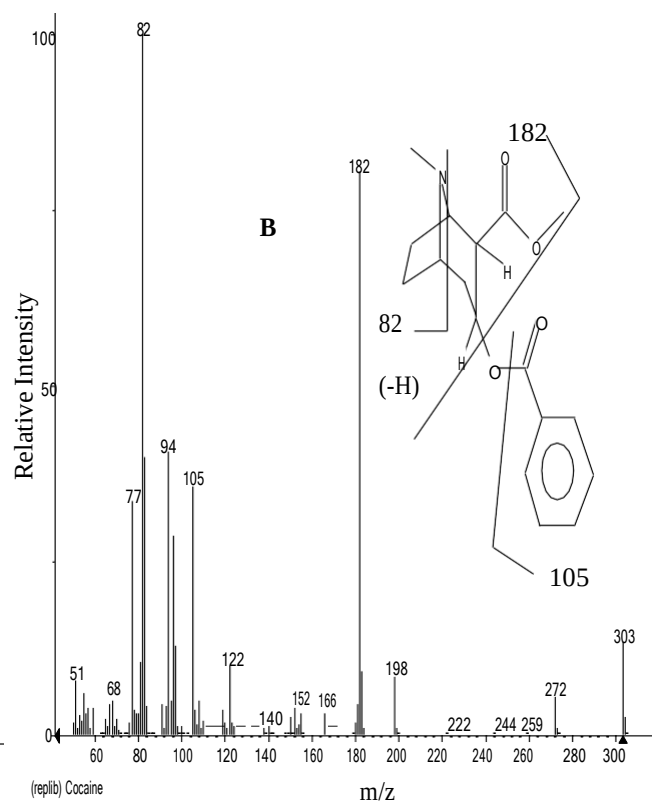
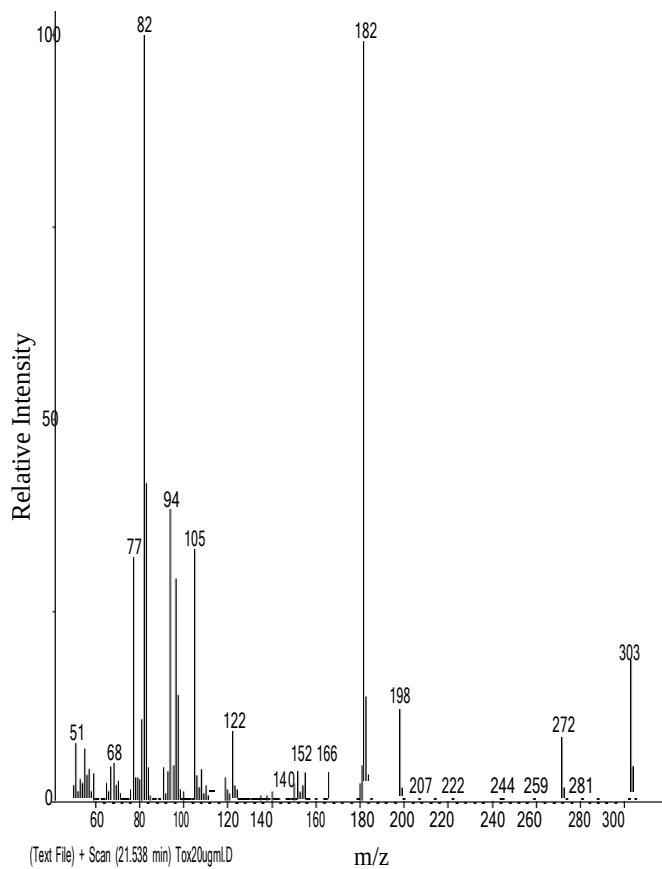
Appendix II: Toxicology Checkout Mixture Mass Spectra

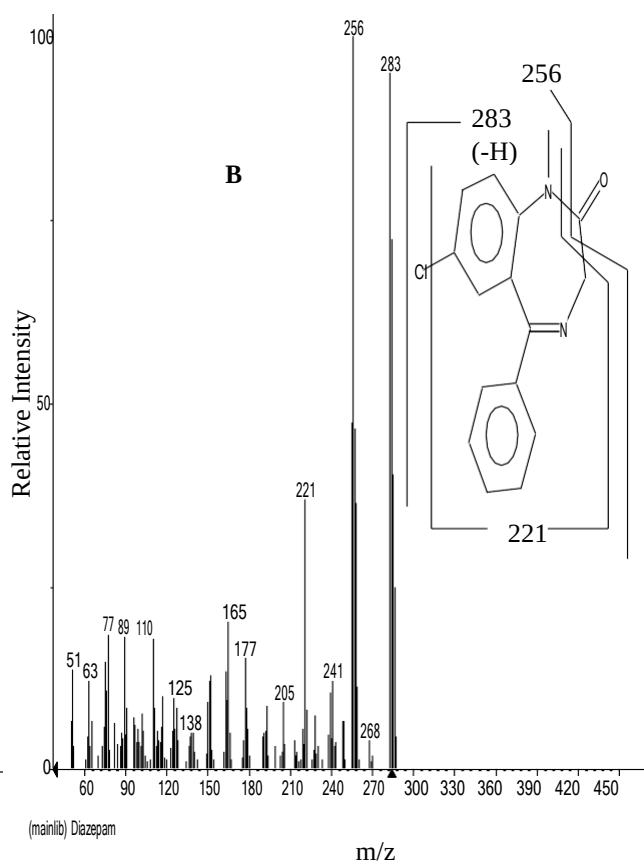
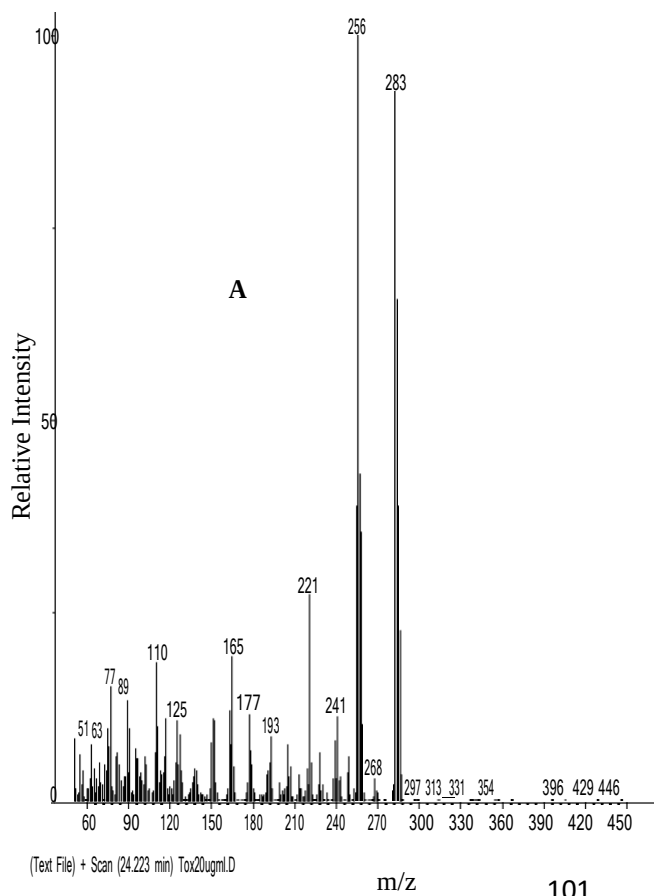
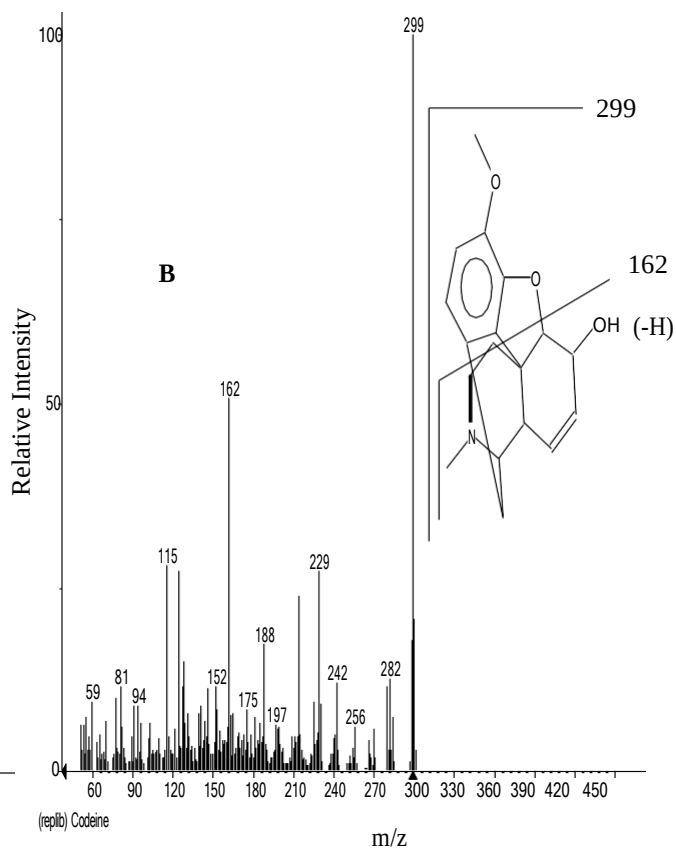
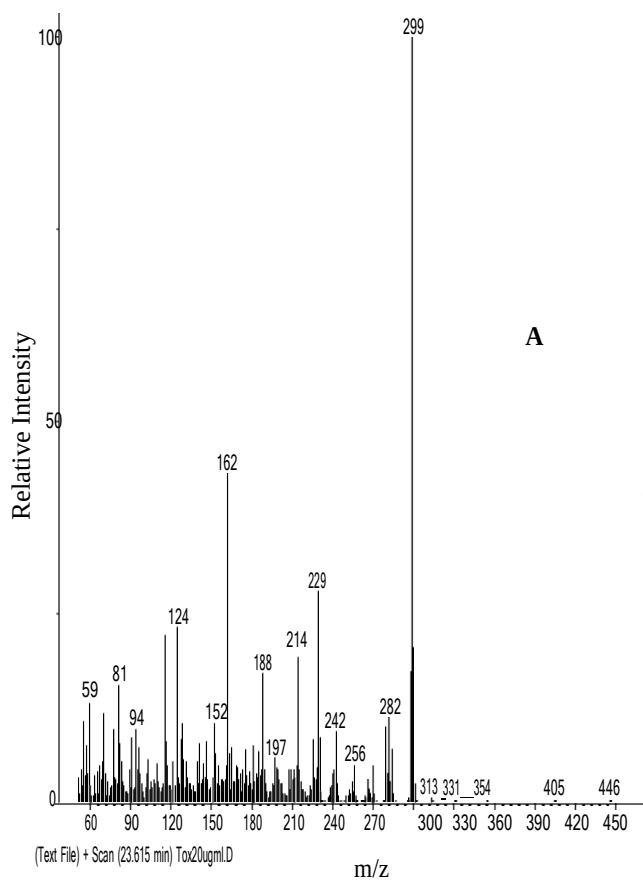


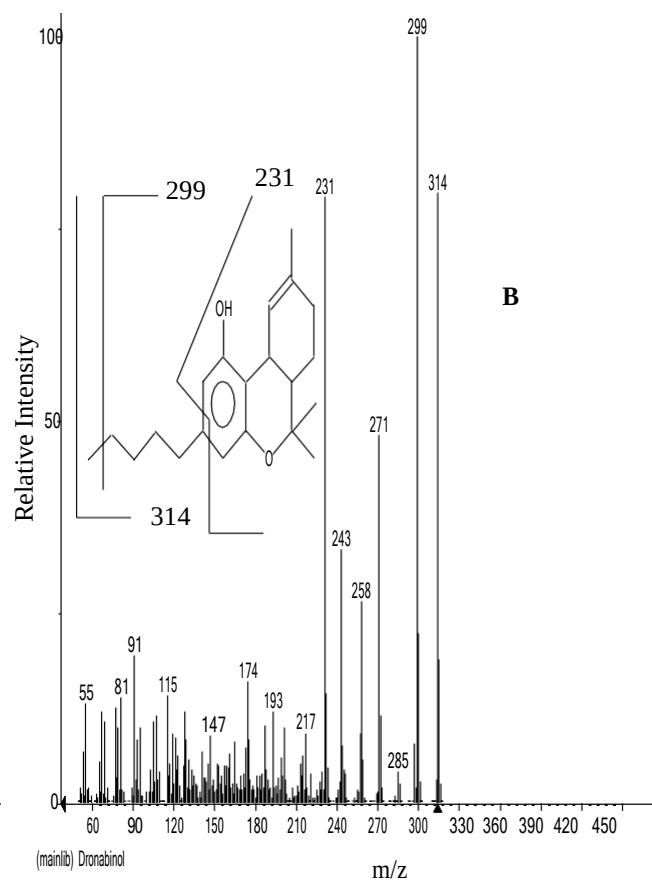
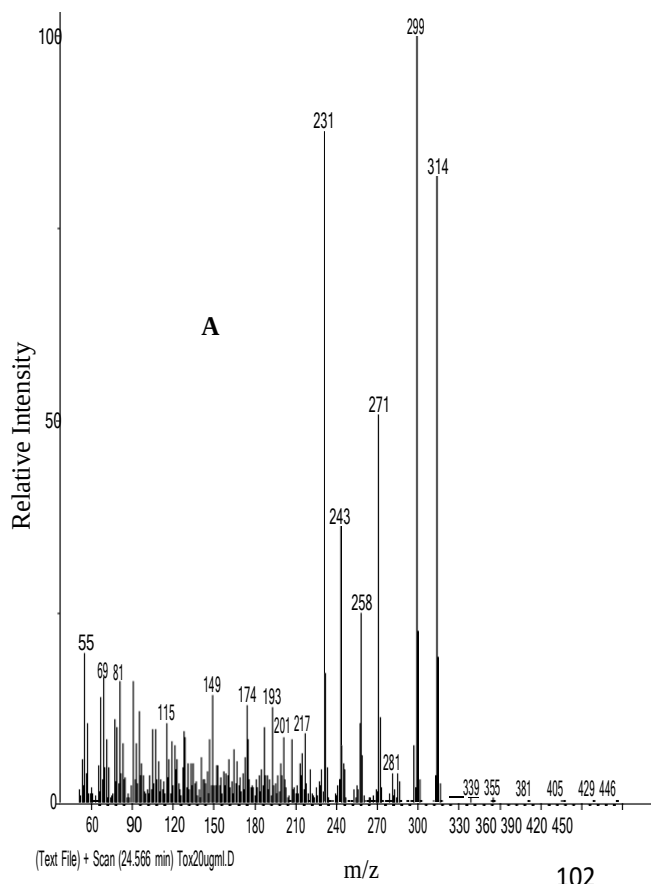
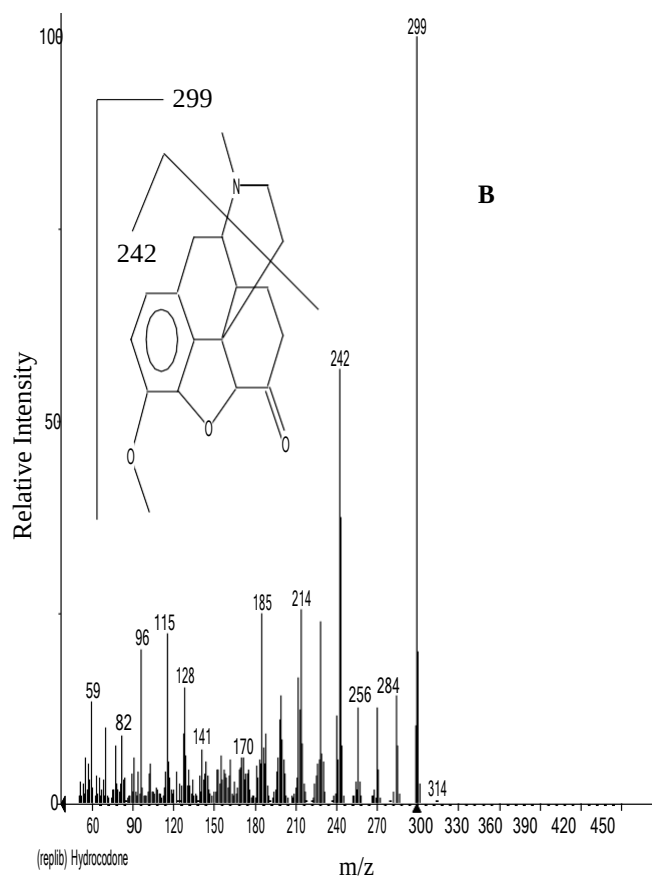
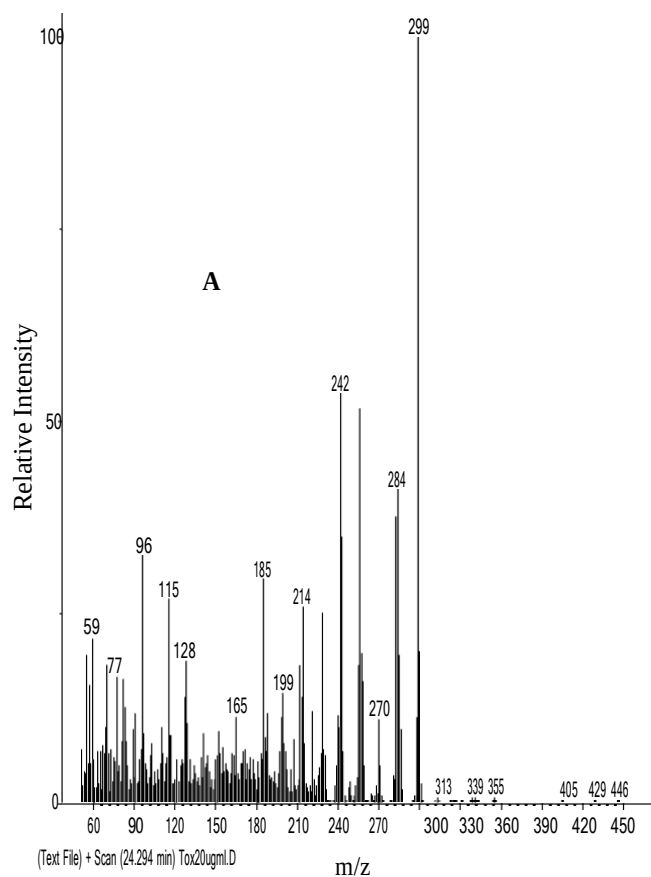


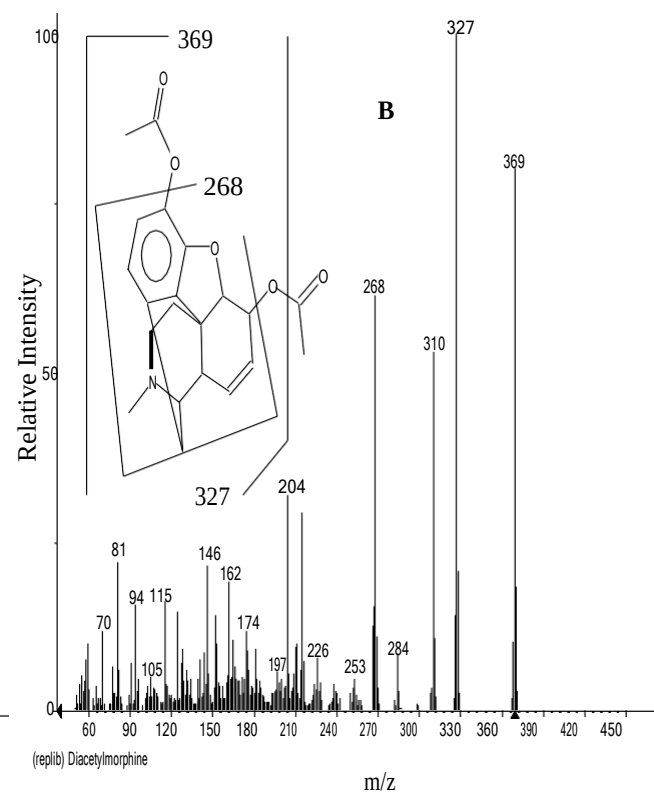
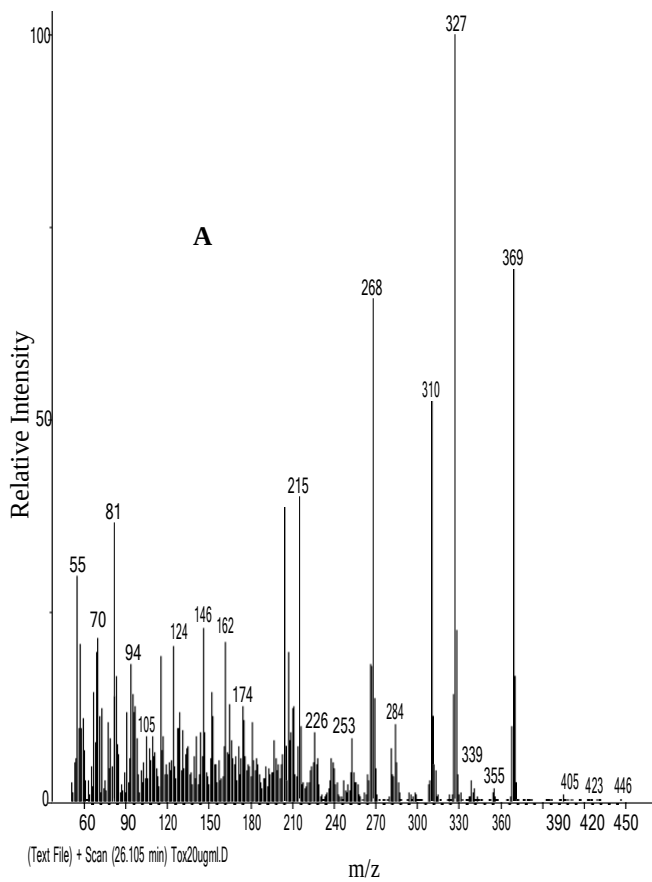
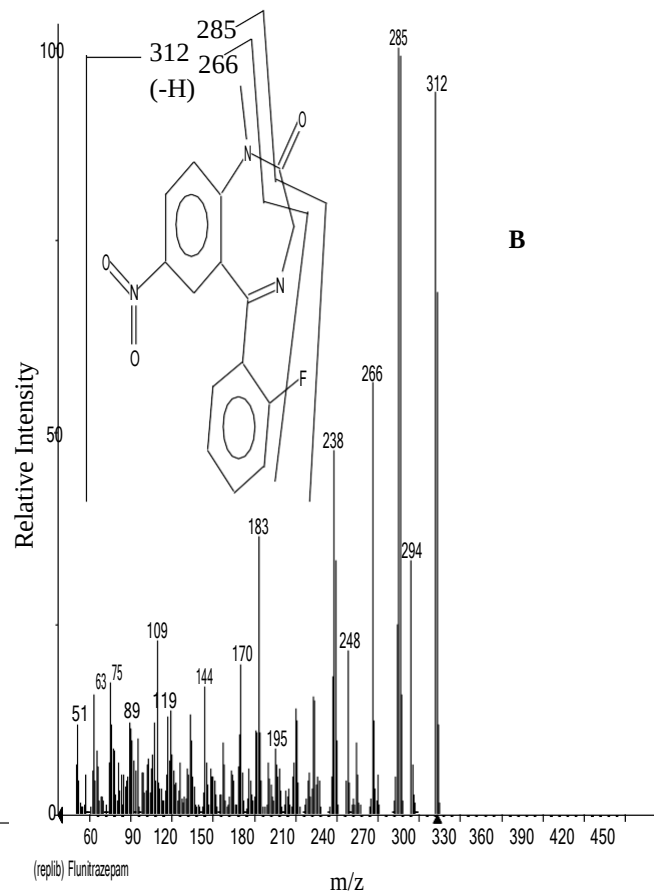
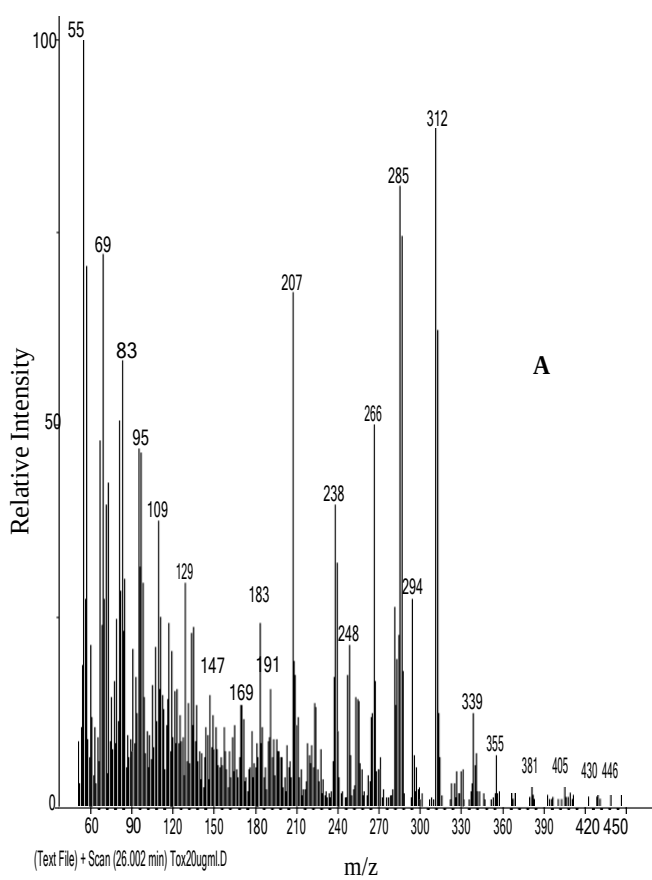




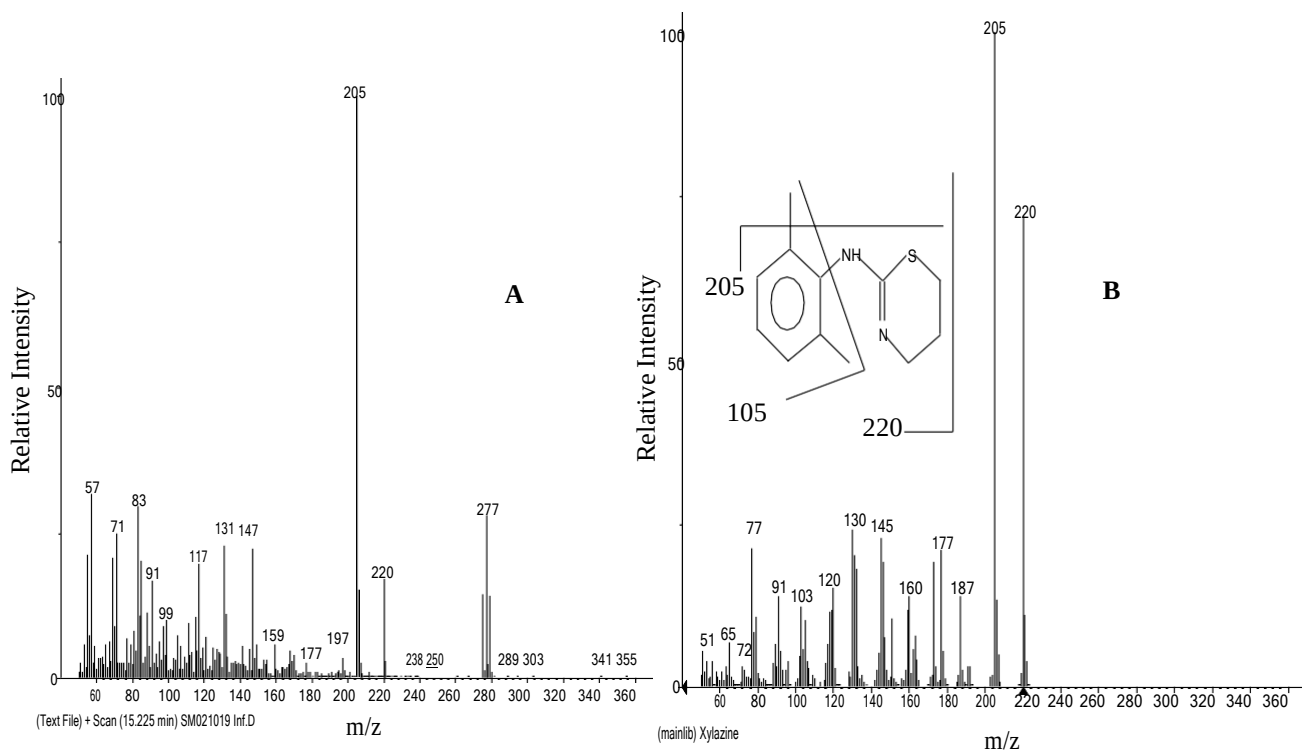
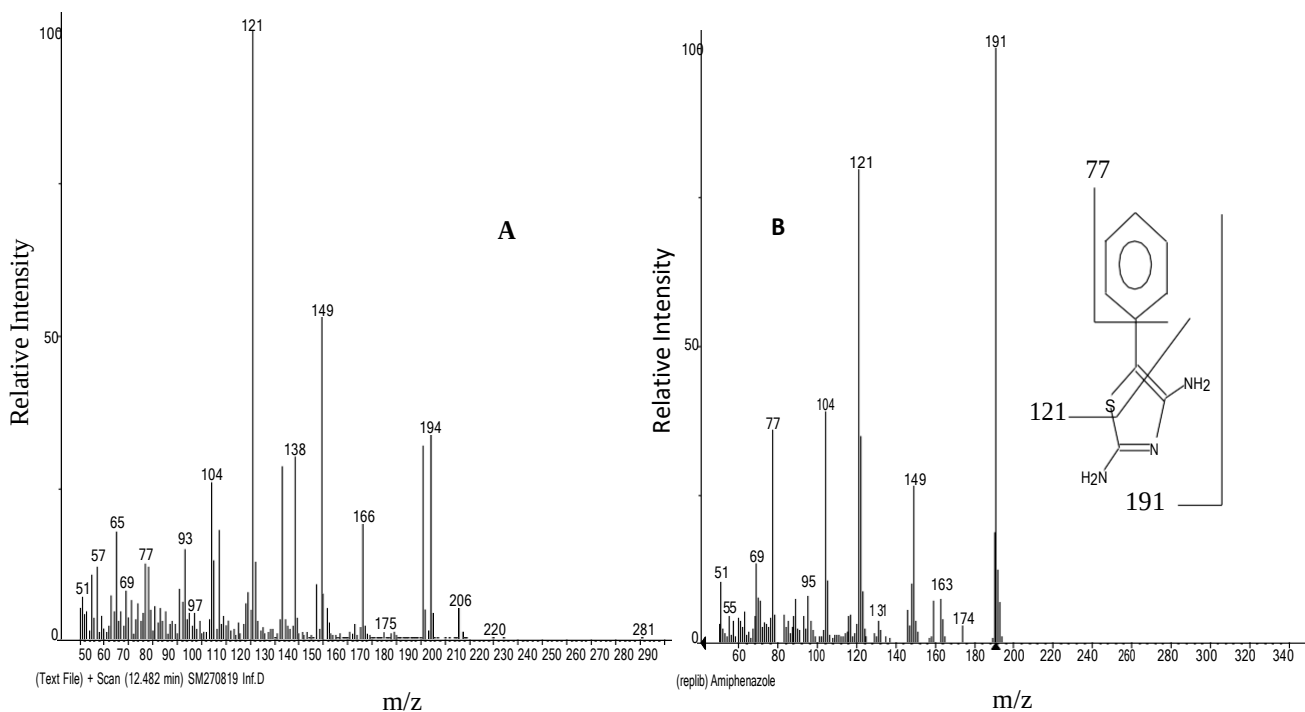


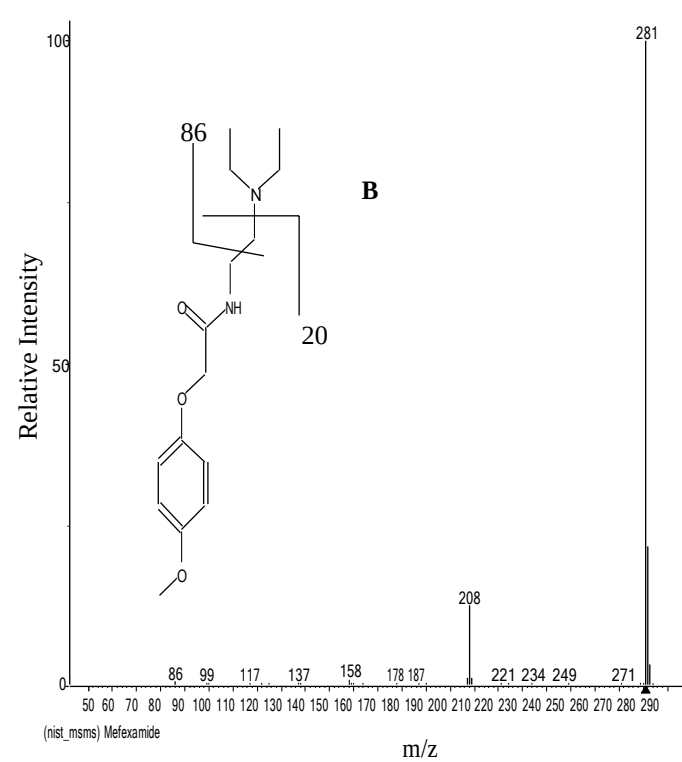
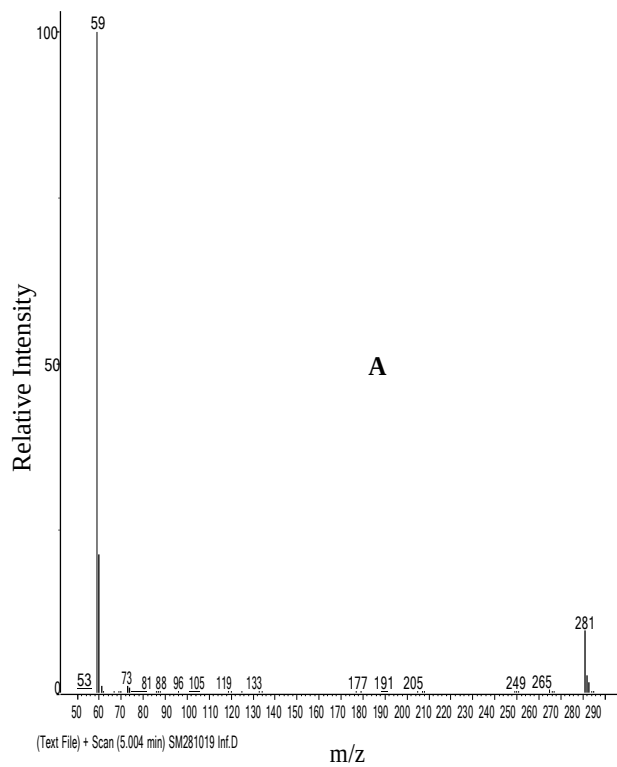
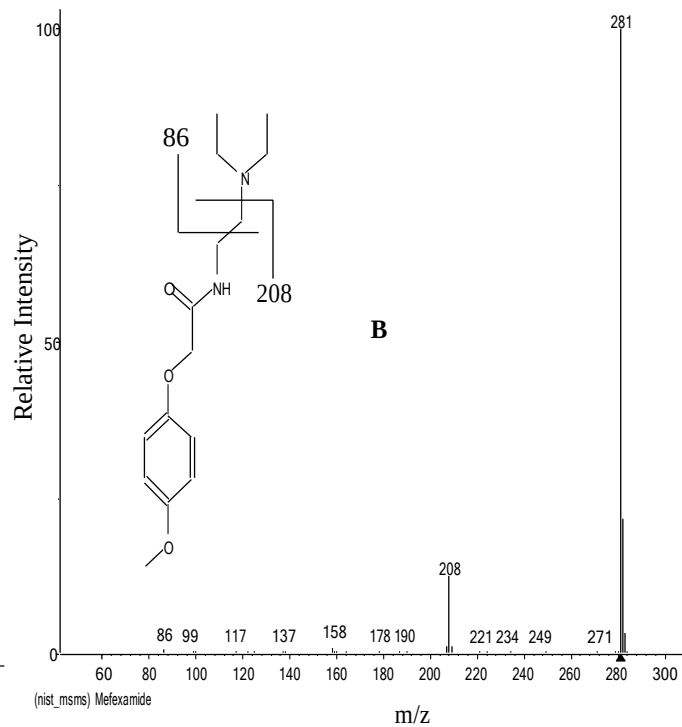
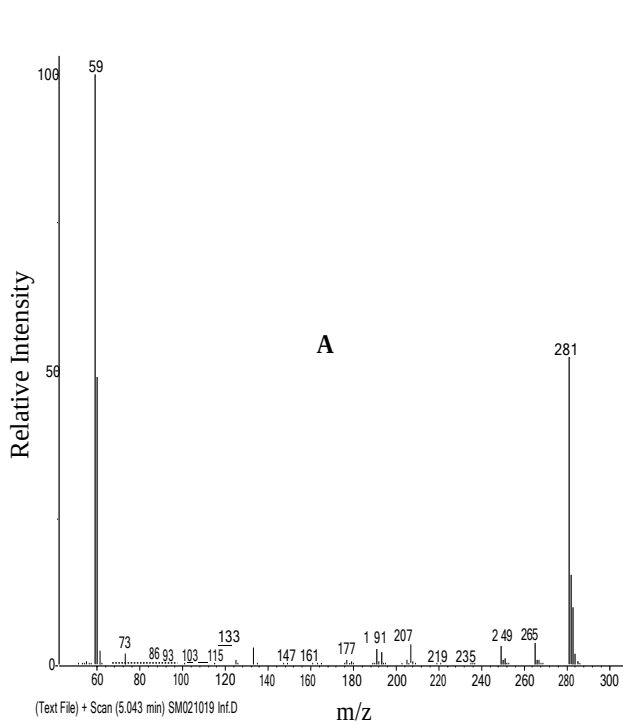




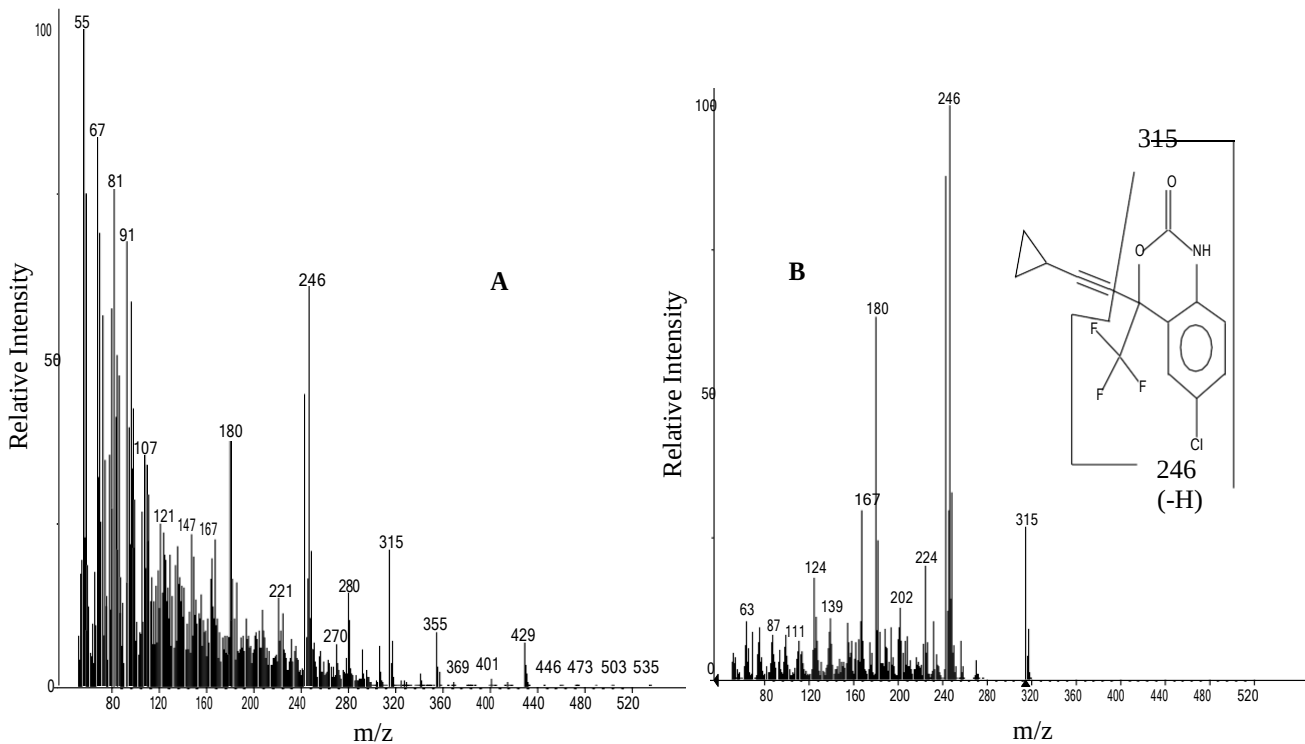
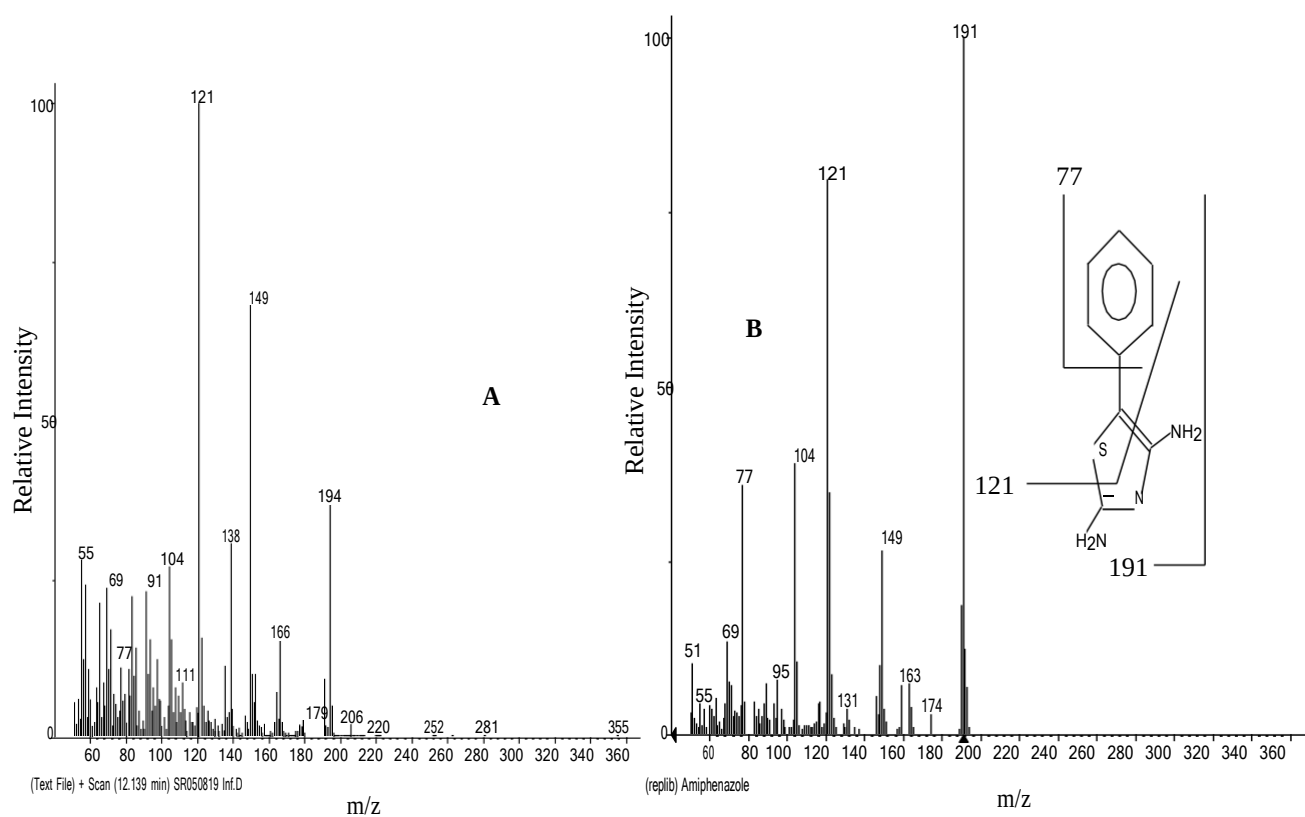


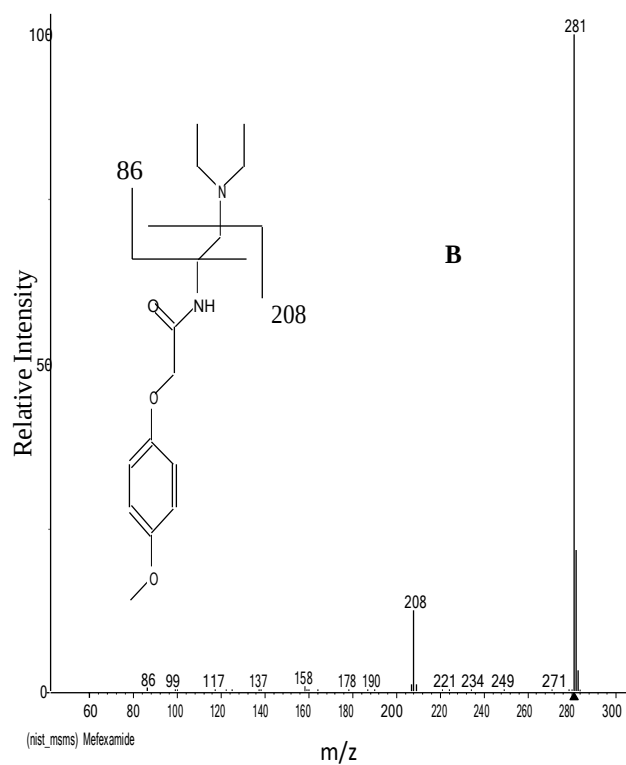
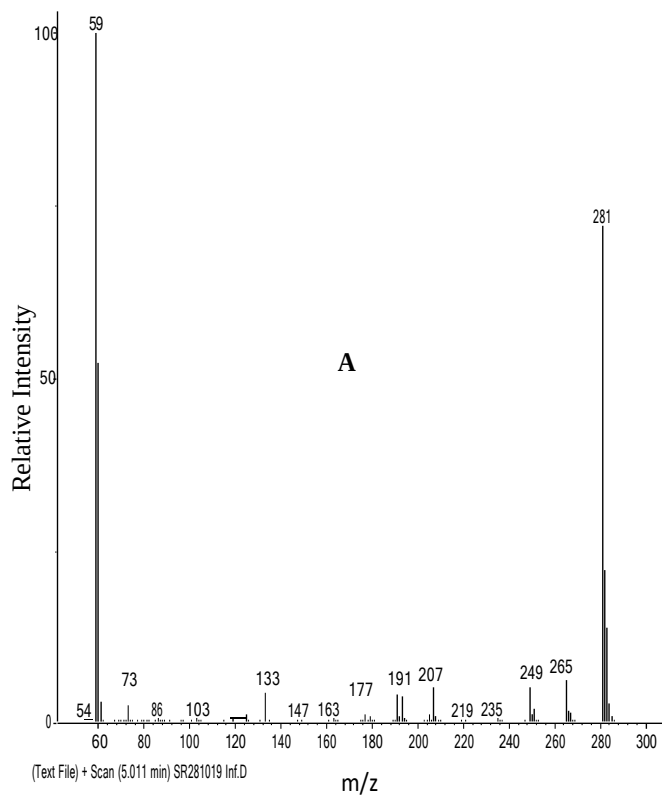
Appendix III: Serowe-Mabogopa WWTP Mass Spectra



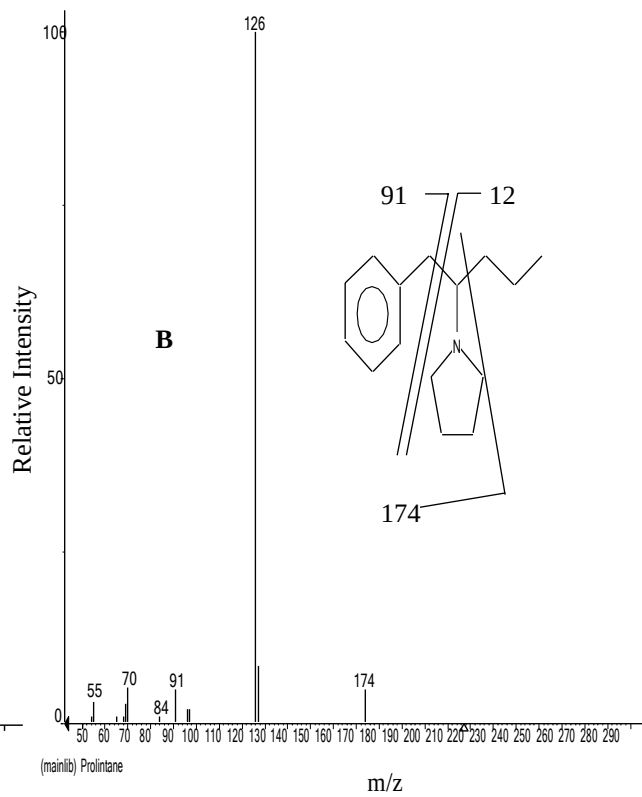
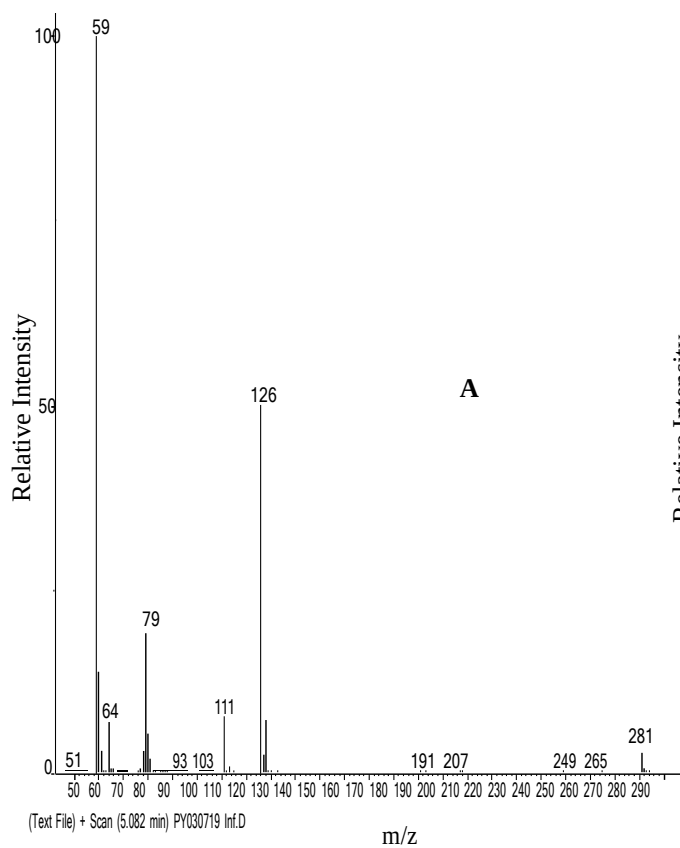
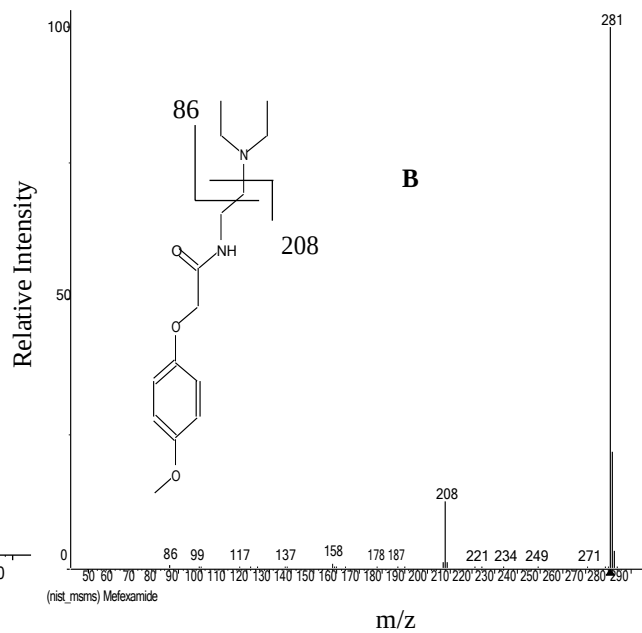
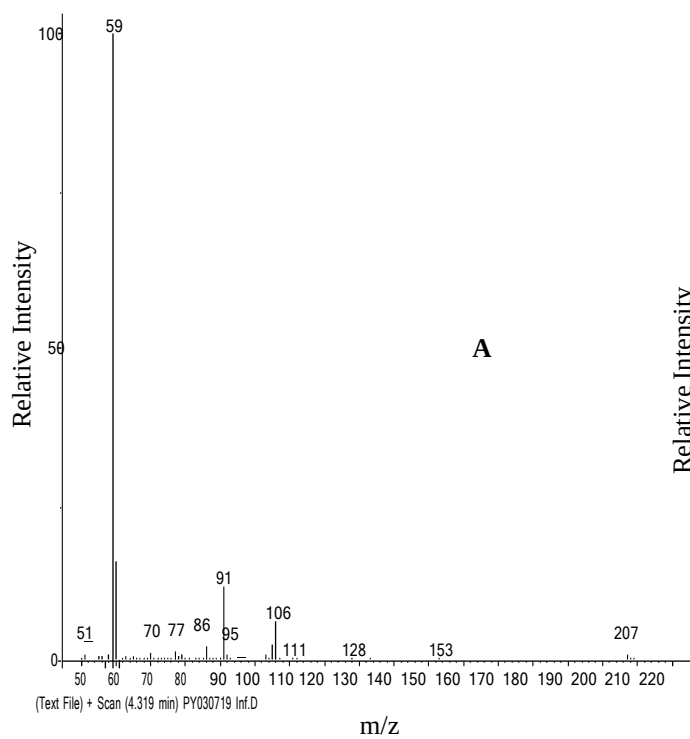


Appendix IV: Serowe-Radihemelo WWTP Mass Spectra

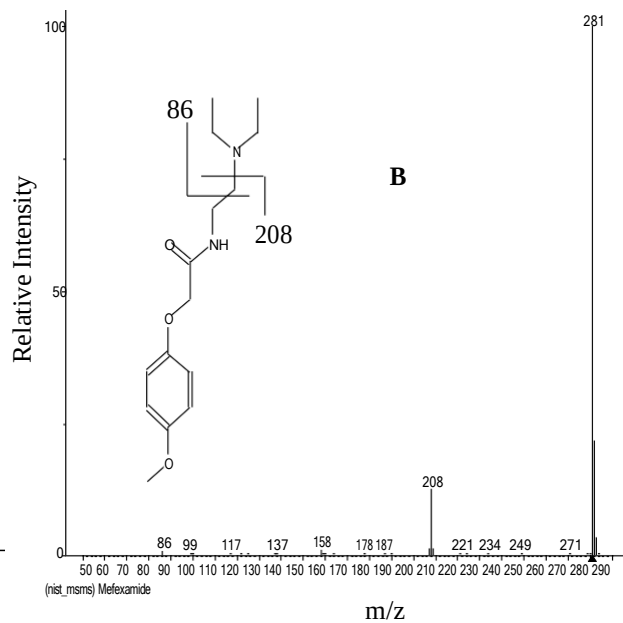
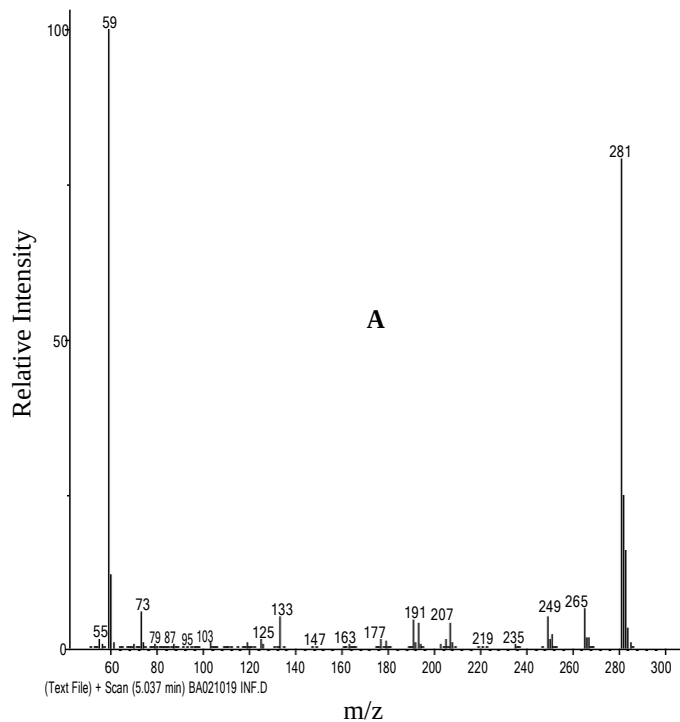
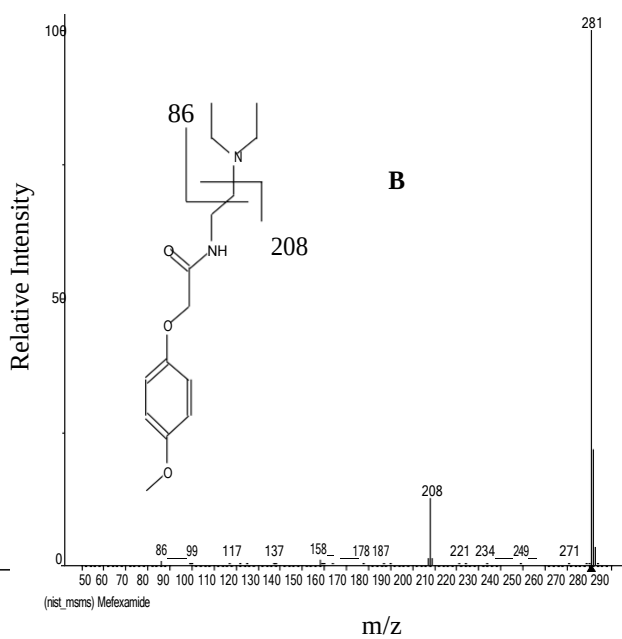
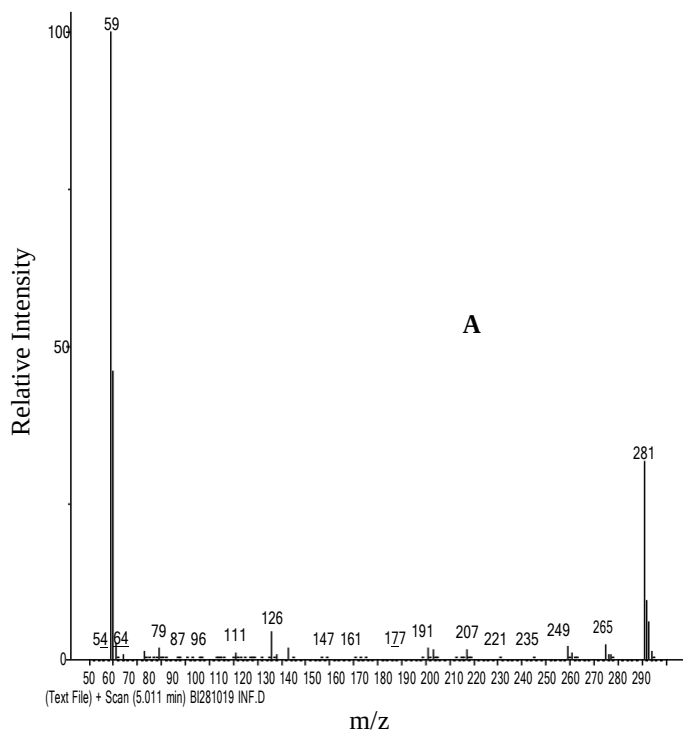




Appendix V: Palapye -main village WWTP Mass Spectra



Appendix VI: Palapye-BIUST WWTP Mass spectra



Appendix VII: Gaborone-Glen Valley WWTP Mass Spectra

