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AN INVESTIGATION INTO DRUG USE IN SUICIDE FATALITIES AT THE JOHANNESBURG FORENSIC PATHOLOGY SERVICE MEDICO-LEGAL MORTUARY

by

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A dissertation submitted to the Faculty of Health Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree:

MSc (Med) in Forensic Medicine

Supervisor:

Mrs. Ildiko Jean Wainer

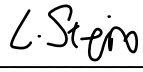
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Johannesburg, 2022

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not, necessarily, to be attributed to the NRF

Declaration

I Lauren Mandy Stein declare that this thesis is my own unaided work. It is being submitted for the degree of Master of Science (Medicine) in Forensic Medicine and Pathology at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or any examination in any other University.



(Signature of candidate)

Date: 2022/05/16

Presentations arising from this study

The 2nd National Forensic Science Conference, 10th – 13th February 2014, at the CSIR International convention Centre (poster presentation).

UNIPATH 2014 CONGRESS, 19th – 21st September 2104, at the CSIR International Convention Centre (poster presentation).

Wits Faculty of Health Sciences Biennial Research Day and Postgraduate Expo, 17th September 2014 (poster presentation).

Wits School of Clinical Medicine Research Day, 30th September 2015 (poster presentation).

Abstract

Drug use and abuse is a common occurrence worldwide, with significant effects on health, mortality, and morbidity of users. Mental illness being one such negative health effect associated with drug use, with drug users exhibiting signs of depression and anxiety related disorders. Depression has been recorded as a major risk factor for suicidal behaviour, and thus suicide has been identified as a leading contributor to death amongst drug users. South Africa has a high prevalence of suicide cases, however there is minimal data available on the extent and consequences of drug use within South Africa. Due to this high risk of suicide behaviour associated with illicit drug use it is important to assess the prevalence of these drugs in victims of suspected suicide within a South African population. The aim of this study is to describe the incidence of suspected suicide fatalities involving the use of prevalent drugs of abuse (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites) in Johannesburg, South Africa.

Blood and urine samples were collected from suicide decedents and were analysed using both screening and confirmatory testing. The initial drug screen was performed on an immunoassay-based test (Randox Biochip Array Technology immunoassay). Confirmatory testing was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS).

The study results found that 15% of cases had tested as a true positive result for one or more of the targeted drug groups of interest (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites). Positive results were noted in multiple methods of suicide such as overdose/poisonings, firearm related, hangings, and gassing/carbon monoxide related suicides. This pilot study has shown that drugs of abuse are present within all methods of suicide fatalities in Johannesburg South Africa. Future recommendations and future research as identified by the results suggests a need for a broader scope of toxicological evaluations within suicide fatalities presenting in Johannesburg South Africa.

Plagiarism declaration

I **Lauren Mandy Stein** (Student number: **0702831E**) am a student registered for the degree of **Master of Science in Medicine** in the academic year **2021**.

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Lastly, I would like to dedicate this research to my dear departed uncle Brian Feldman, Brian I hope this makes you proud.

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Abbreviations and nomenclature

6MAM	6-monacetylmorphine
AMPH	Amphetmaine
BAT	Biochip array technology
CAL	Calibrator
CDA	Central drug authority
DOA	Drugs of abuse
DOA I URN P	Drugs of Abuse Array I Urine plus
DOA I WB P	Drugs of Abuse Array I Whole Blood Plus
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
GC-MS	Gas chromatography mass spectrometry
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
JHB FPS-MLL	Johannesburg Forensic Pathology Service Medico-Legal Mortuary
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOD	Lower limit of detection
LLOQ	Lower limit of quantitation
LSD	Lysergic acid diethylamide
MAMP	Methamphetamine
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethylamphetamine
MDMA	3,4-methylenedioxy-methamphetamine (Ecstasy)
MS	Mass spectrometry
m/z	Mass to charge ratio
NDOH FCL JHB	National department of health Forensic Chemistry Laboratory Johannesburg
NIMSS	National Injury Mortality Surveillance System
NDMP	National Drug Master Plan
OUD disorder	Opioid use disorder
PCP	Phencyclidine

PMI	Postmortem interval
QC	Quality control
SACENDU	South African Community Epidemiology Network on Drug Use
SAPS	South African Police Service
SD	Standard deviation
SLE	Supported liquid extraction
UNDOC	United Nations Office on Drugs and Crime
UPLC	Ultra-performance liquid chromatography
URN	Urine
WB	Whole blood
WHO	World Health Organisation

Chapter 1: Introduction

1.1 Introduction

Drug use is a worldwide problem, with more than a quarter of a billion people said to use drugs globally, around 8% of which are located in Africa (United Nations Office on Drugs and Crime,2021). In 2019 drug use resulted in the death of least half a million individuals, with drug use disorders having great implications in health and mortality and morbidity (United Nations Office on Drugs and Crime, 2021). The most prevalent abused drugs globally include cannabis, opioids, amphetamines, and cocaine (United Nations Office on Drugs and Crime, 2021). There are many harmful effects associated with drug use, these can be subdivided into (i) chronic health problem i.e. cirrhosis of the liver, transmission of infectious agents such as hepatitis and HIV; (ii) Acute biological health effects such as an in cases of overdose and casualties or injuries sustained due to cognitive and behavioural impairments caused by drug of use including accidents and suicide; (iii) Adverse social consequences of use that affect both personal and work relations (WHO, 2004).

Illicit drug users have been shown to have higher mortality rates as compared to the general non-drug using population, with completed suicide being a leading contributor to death (Darke *et al*., 2010). Additionally, illicit substances are found to present in a fifth or more of completed suicide cases (Darke *et al*., 2010). The drug dependant population exhibit a higher rate of suicide attempts (20% - 40%) as compared to the general non-drug using population (5%) owing to high levels of depression associated with drug use, the intoxicating effects of illicit type drugs, as well as the negative social effects and additional stresses associated with an illicit drug dependant lifestyle, with between 5% and 10% of drug user deaths being attributable to suicide (Darke and Kaye, 2004 and Degenhardt and Hall, 2012). The increased risk of suicide amongst drug users may be amplified by social circumstances such as: unemployment, low education levels, social isolation, repeated incarceration, history of physical or sexual abuse, parental alcoholism, and divorce (Darke *et al*., 2010). Drugs are known to cause effects on mental health e.g., cocaine and methamphetamine use are associated with mental health effects such as anxiety, depression, hallucinations, delirium, violent agitation, and increased risky behaviour (Darke and Kaye, 2004). Depression is a major risk factor for suicide attempt and successful suicide attempt,

depression is rife amongst heroin users, with approximately a third of heroin users meeting the criteria for diagnoses of major depression (Darke and Ross, 2002). These effects on mental wellbeing may therefore all contribute and ultimately result in an increased risk of suicidal behaviour amongst drug using individuals (Darke and Kaye, 2004).

South Africa has a high prevalence of suicide cases accounting for estimated 11% of deaths of South Africa (Prinsloo *et al*., 2021), however there is minimal data available on the extent and consequences of drug use within South Africa. Due to this high risk of suicide behaviour associated with illicit drug use it is important to assess the prevalence of these drugs in victims of suspected suicide within a South African population.

1.2 Literature review

1.2.1 Drug use and abuse

Psychoactive substances, or drugs, are substances that when consumed effect a change in behaviour, consciousness, mood or thought process (WHO, 2004). A drug being a substance or medication that produces a physiological effect when introduced to the body, and where drug use is self-administration of a drug (National Drug Master Plan, 2020). Drugs can be classified into three different categories of use: that being medicinal (pain relief, relieve of mood disorders), licit or legal use (not related to the psychoactive property of the drug), and illicit or illegal drug use (use to benefit, or enjoy the psychoactive properties of the drug) whereby Illicit drugs are those drugs where use and trade is prohibited by law, and is often associated with problem drug usage (WHO, 2004).

Drug abuse is whereby the user continues to use the drug despite the known negative associations of use, that could be negative social effects, and physiological or physical issues experiences with use (WHO, 1994). Drug addiction, also referred to as problem drug use or a drug/substance use disorder, can be defined as an obsessive searching for and use of a substance (drug) despite the negative associations with use (Samaila and Abdulkadir, 2016). Certain indicators include: a strong desire for use with impaired

control over use; experience of withdrawal effects when drug usage is stopped; build-up of tolerance and a desire for larger doses to achieve the desired effect; and continued use even though adverse effects are experienced (Degenhardt and Hall, 2012). Drugs of abuse enact their reinforcing and addictive effects by stimulating the brains reward system, as well as conditioning the brains reward system to interpret drug effects as biologically rewarding stimulus (Crocq, 2007).

Illicit drugs/substances can be classified according to the following categories:

- Depressants of Central Nervous System: drugs that slow down brain activity, decrease anxiety, cause sedation e.g., benzodiazepines, barbiturate, ketamine.
- Stimulants of Central Nervous System: Strongly stimulate the brains activity, stimulate the brains reward system, raises heart rate, mood swings e.g., cocaine, amphetamine type stimulants (amphetamine, methamphetamines), nicotine.
- Opiate and Opioid (narcotic) analgesics: analgesia, drowsiness, sedation, causes physical and psychological dependence. These include natural opioids (e.g., morphine, codeine, papaverine), semi-synthetic opioids (e.g., heroin, oxycodone, oxymorphone), synthetic opioids (e.g., buprenorphine, methadone, fentanyl).
- Hallucinogens (Psychedelics): Cause changes in perception of reality, mood swings, hallucinations, delusions, chronic mental disorders e.g., LSD, MDMA, PCP (Samaila and Abdulkadir, 2016).

1.2.1.1 Global prevalence

In 2019, it was estimated that 5.5% of the global population (approx. 275 million people) aged between 15 - 64 years were reported to have used illegal drugs at least once in that year (United Nations Office on Drugs and Crime, 2021). There was shown to be growth in the prevalence of illicit drug usage globally, where within a 9-year period (2010 – 2019) illicit drug usage had grown by an estimated 22%, however this can in part be attributed to population growth (United Nations Office on Drugs and Crime, 2021). Within the illicit drug using population, there are those individuals who suffer from a drug use/substance use disorder. A drug use/substance use disorder is

a sign of problematic drug use which is harmful to the individual and which may lead to drug dependence and require some form of treatment. Individuals suffering from a substance use disorder are estimated to account for 0.7% of the global population aged between 15 - 64 years (United Nations Office on Drugs and Crime, 2021). The 2021 United Nations Office on Drugs and Crime (UNDOC) report notes the most commonly used illicit drugs as: cannabis (approx. 200 million users within 2019); opioids (opiates (heroin and opium) and pharmaceutical and/or synthetic opioids) (approx. 62 million users within 2019); amphetamine type stimulants (amphetamine, methamphetamine and pharmaceutical stimulants) (approx. 27 million users within 2019) and cocaine (approx. 20 million users within 2019) (United Nations Office on Drugs and Crime, 2021).

1.2.1.2 South African prevalence

There is minimal data available on the extent, nature and consequences of drug use and trends related to drug use within South Africa, largely in part due to the lack of historical comprehensive national population studies as well as efficient and compete data collection systems (CDA, 2012/2013). Until the late 1990's, information relating to substance use and trends in usage had largely come from police reports, ad hoc research studies and national surveys (Parry, 1998 and Van Heerden *et al*., 2009). In more recent years more reliable, community monitoring systems have been introduced to monitor and document trends in substance use trends within South Africa. The South African Community Epidemiology Network on Drug Use (SACENDU) is one such monitoring system, which monitors treatment admissions for drug and alcohol abuse within South Africa (Parry, 1998 and Van Heerden *et al*., 2009). Even with the implementation of such studies the data does not provide the full scope of substance abuse within the general population. As in the case of the SACENDU, information only relates to those individuals who are able to access appropriate treatment facilities and hence have their information recorded. In the 2019 SACENDU report, it was noted that in Gauteng the most used substances were heroin/opiates (36%), followed by cannabis (30%), alcohol (12%), and methamphetamine (11%) With cocaine accounting for 3% of patients during this period (Dada *et al*., 2020). From a demographic view, patients in substance abuse treatment facilities in Gauteng were

found to be predominantly male, Black individuals, with a mean age of 26 years with over half of the patients in this period being unemployed (Dada *et al*., 2020). In general White individuals were shown to have a higher prevelance of substance use compared to the rest of the population (Van Heerden *et al*., 2009).

There are multiple factors leading to the increased use of illict drugs within South Africa. Changes in the political, economic and social/cultural landscape since the end of Apartheid have made the population more vulnerable to substance use (Peltzer *et al*., 2010). Rapid modernization, the decline of cultural/traditional family structures and relations, and the transformation from rural-cultural society to a urban-westernised society, may place added pressures on individuals that lead to drug use (Peltzer *et al*., 2010 and Van Heerden *et al*., 2009). Additional factors may include high levels of unemployment, poverty, poor education, ease of availability and accessability to drugs, poor border control and policing, a greater tolerance of new ideas and limited enforcement of drug laws, as well as the diversity of drugs available (Peltzer *et al*., 2010 and Liebenberg, 2016). South Africa is also geographically located along world drug trafficking routes, with cocaine originating from Latin America transiting through South Africa onto destinations into Europe as well as heroin from the Far East passing through South Africa into Europe and the United States (Peltzer *et al*., 2010).

South Africa has legislation in place in an attempt to discourage, control and punish the use of illegal drugs. The South African Prevention of and Treatment for Substance Abuse Act No. 70 of 2008 governs the operations fo the Central Drug Authority, and outlines the social and legaslative response to drug use (Republic of South Africa, 2008). In accordance with this Act, the National Drug Master Plan (NDMP) was estabilished, which serves as a strategy to guide operational plans within those government departments tasked with reducing the demand for and supply of drugs (National Drug Master Plan, 2020). The South African Drugs and Drug Trafficking Act. 140 of 1992, defines the illegal activites relating to substances, and states penalties for drug use and possession, as well as stipulating law-enforcement roles and processes in cases of drug use and possession (Republic of South Africa, 1992 and National Drug Master Plan, 2020).

1.2.2 The impact of substance abuse on society

The high prevalence and chronic nature of illicit drug use places a large burden on society in terms of morbidity and mortality of the drug using population (Degenhardt and Hall, 2012 and Liebenberg *et al*., 2016). The health risks associated with illicit drug usage also increases as the frequency and the dose/quantity of drug usage increases (Degenhardt *et al*., 2004). Drug dependant users (or otherwise called "problem drug users") experience the most detrimental health effects from consistent drug use, with drug usage resulting in both physical harm to the user (e.g., organ damage), and/or psychological harm (drug-induced psychosis, depression) (Degenhardt *et al*., 2004). The harmful health effects from drug usage are broadly classified into four groups: acute toxic effects (overdose); acute effects of intoxication (accidental injury, violence); drug dependence; and adverse health effects of sustained chronic, regular use (chronic disease, blood-borne infections (HIV, Hepatitis), and mental disorders) (Degenhardt and Hall, 2012).

The most severe outcome for substance users is death. In 2019 the Global Burden of Disease Study estimated that 494,000 deaths worldwide were attributable to drug use (United Nations Office on Drugs and Crime, 2021). Drug related deaths can be both directly (overdose) and indirectly (e.g., liver cancer, hepatitis, HIV/AIDS, self-harm/suicide) associated with drug use. Deaths attributable to substance use disorder have been increasing over the past decade, most of which have been associated with opioid use disorder (United Nations Office on Drugs and Crime, 2021). The UNODC world drug report, 2018 document on the global overview of drug demand and supply, shows the leading causes of deaths attributable to drug use disorder in 2016 (Figure 1.1). With notably, drug use disorder comprises 31% of the deaths, also to note self-harm/suicide accounts for 8.3% of deaths. The remainder of deaths are attributable to disease (e.g., hepatitis, HIV/AIDS) (United Nations Office on Drugs and Crime, 2018).

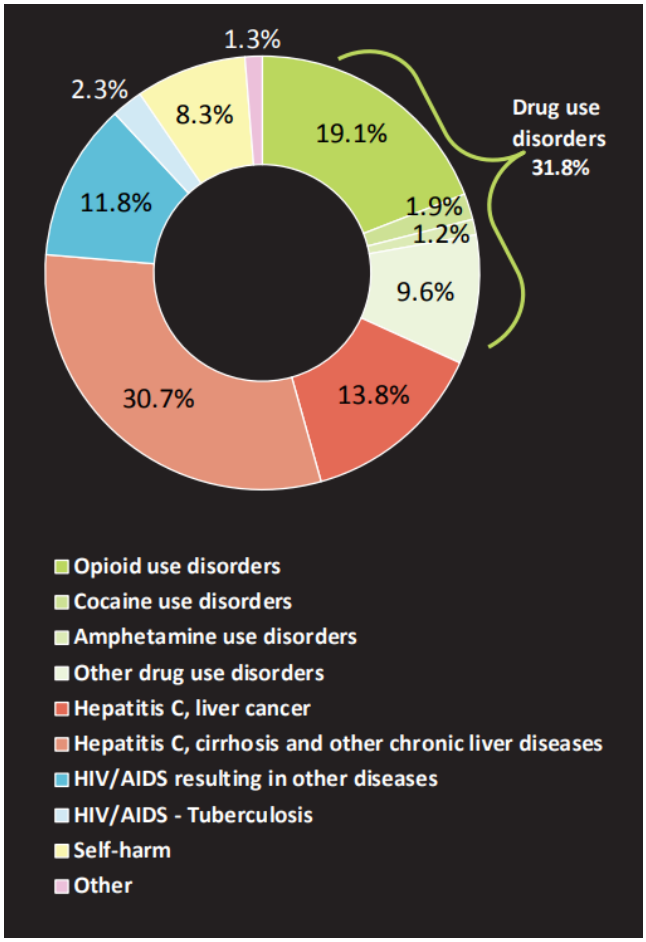


Figure 1.1 Leading causes of deaths attributable to drug use, 2016 (United Nations Office on Drugs and Crime, 2018)

Interestingly cannabis use is not shown in Figure 1.1. as a risk factor for drug related death, this is largely due to the opinion that cannabis use does not result in fatal overdoses (Degenhardt and Hall, 2012). Opioid, cocaine, and amphetamine use disorders have shown to contribute to drug related deaths (United Nations Office on Drugs and Crime, 2018 and Degenhardt and Hall, 2012). Usage of these drug groups are known to be associated with suicides, mental disorders, road-traffic accidents, and violence (United Nations Office on Drugs and Crime, 2018 and Degenhardt and Hall, 2012). The relationship between substance use disorder and suicide has been well investigated and established, with reported rates of suicide attempts within the opioid, cocaine and amphetamine use disorder population being higher than those in the general non-drug using population of the same age, sex, and socioeconomic status (Esang and Ahmed, 2018; Hesse *et al*., 2020; SAMHSA, 2016 and Degenhardt and

Hall, 2012). Cannabis use however, with a high prevalence use amongst drug users, does not show sufficient evidence if a causal relationship exists between use and potential suicide fatality (Degenhardt and Hall, 2012).

1.2.3 Drugs of abuse

1.2.3.1 Opiates

Opiates are drug compounds, either naturally occurring (alkaloids), or semi-synthetic, that are extracted from opium found within the seeds of the *Papaver somniferum* (opium poppy plant) (Dasgupta, 2010 and WHO, 2004). Opioids includes the “opiate” drug compounds, as well as synthetic drug compounds showing similar priorities and similar effects to the “opiate” drug compounds (WHO, 2004). Morphine is the most abundant natural alkaloid found in the *Papaver somniferum*, codeine being another alkaloid derived from the *Papaver somniferum* plant. Morphine serves as the structural building block for semi-synthetic opiate derivatives such as heroin, fentanyl, oxycodone, oxymorphone, hydromorphone, methadone, buprenorphine, and hydrocodone (Dasgupta, 2010 and Fareed *et al*., 2011). The molecular structure of morphine, codeine and heroin are shown in Figure 1.2.

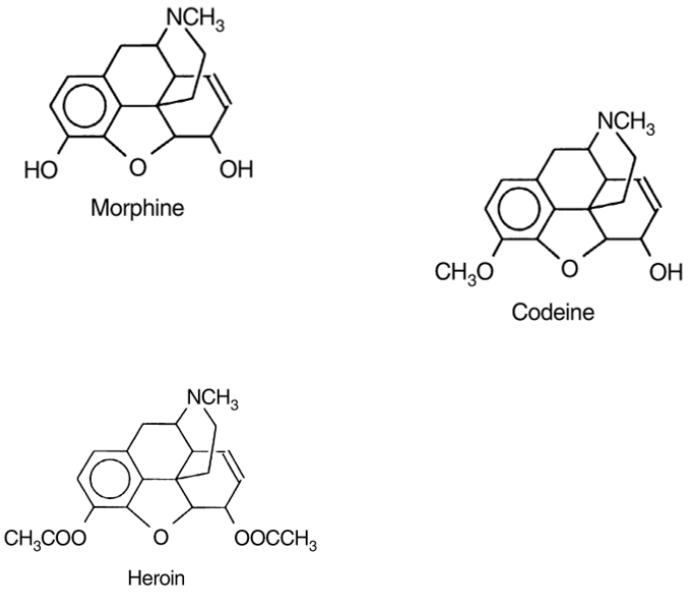


Figure 1.2 The molecular structure of morphine, codeine, and heroin (Stefanidou *et al*., 2010).

Opiates and Opioid have strong pain relief or analgesic effects, but also produce effects of sedation, euphoria, and respiratory depression (Dasgupta, 2010). Opiates act by binding to opiate cell surface receptors located primarily within the central nervous system (Fareed *et al*., 2011 and WHO, 2004). The effects experienced relate to the type of receptor involved, with three main opiate receptors: the mu, delta, and kappa receptors (Fareed *et al*., 2011; WHO, 2004 and Dasgupta, 2010). These receptors have a function in controlling the body's response to pain, regulating body temperature, respiration, mood, and motivation amongst others (Fareed *et al*., 2011). Opioid drugs act as agonists when binding the mu, delta, and kappa receptors producing effects such as inhibition of neuronal activity an analgesic response (WHO, 2004). Interaction with these receptors have shown to have positive reinforcing properties with feelings of euphoria and leading to further drug seeking behaviour (Fareed *et al*., 2011; WHO, 2004 and Dasgupta, 2010). The long-term use of these drugs leading to drug tolerance (that is a decreased responsiveness to the same drug concentration at the receptor site) and a high potential for drug dependence and abuse (Fareed *et al*., 2011; WHO, 2004 and Dasgupta, 2010).

Opiate testing is one of the most frequently requested screening tests in diagnosis of drug dependence and in drug death investigations. The interpretation of opiate positive results however can be complicated as both illicit opiates (heroin) and prescription opiates (codeine) are metabolized to morphine (Stefanidou *et al*.,2010). The rapid metabolism of 6MAM further complicates findings, as the presence or absence of this heroin metabolite cannot be used to reliably differentiate between illicit or therapeutic opiate use (Karch, 2002).

Morphine metabolism mainly occurs in the liver, mediated by the liver enzyme uridine diphosphate glucuronosyltransferase, where it is quickly converted into the principle metabolite morphine-3-glucuronide, and a slower conversion to the minor metabolite morphine-6-glucuronide. The glucuronide metabolites are then excreted via the kidneys in urine (Karch, 2002 and Dasgupta, 2010).

Codeine is metabolized in the liver where it undergoes glucuronidation and demethylation. The majority of codeine is converted to an in-active metabolite, codeine-6-glucuronide. A small percentage undergoes demethylation by the liver

enzyme CYP2D6 to form morphine. The morphine produced is further metabolized to morphine-3-glucuronide and morphine-6-glucuronide, and excreted within urine (Karch, 2002 and Dasgupta, 2010).

Heroin (diacetylmorphine) is rapidly metabolized by deacetylation to 6-monacetylmorphine (6MAM) and then morphine. The conversion to the metabolite 6MAM is completed within 10-15 minutes, and total conversion into morphine within a few hours (Karch, 2002). Heroin is mainly excreted in the urine as both free and conjugated form of morphine (Fareed *et al*., 2011).

Opiate dependant users have an increased risk of mortality from drug overdose, suicide, violence, and alcohol related causes (Hall *et al*., 2006). There are suggested to be three main factors contributing to mortality of individuals: the pharmacological action of the drug, infection, and environmental factors (Hulse *et al*., 1999). Mortality associated with pharmacological actions include respiratory failure or cardiac arrhythmias. Injecting drug users also have the additional risk of contracting blood-borne infections such as HIV/AIDS, hepatitis B and C, that results from sharing of needles and drug paraphernalia, as well as contracting of bacterial infections and tetanus from the use of non-sterile injecting equipment (Hulse *et al*., 1999 and Hall *et al*., 2006). Environmental factors such as poor standard of living associated with drug use and dependence, and an increased exposure to accidents, suicidal acts and acts of violence all contribute to rates of mortality (Hulse *et al*., 1999 and Hall *et al*., 2006). External signs of use include skin lesions, fresh needle punctures, atrophic scarring, abscesses and ulceration, track marks, necrotizing fasciitis, and lymphedema “puffy hands” syndrome (Karch, 2002).

Therapeutically opiates and opioids are used for the drugs analgesic proprieties, offering moderate to severe pain relief, this can be pain ranging from a headache (codeine) to chronic pain as well as pain associated with cancer (morphine, hydromorphone, oxycodone etc) (Karch, 2002).

1.2.3.2 Cocaine

Cocaine is a naturally occuring alkaloid found in the leaves of *Erythroxylon coca*, a tree that is found throughout parts of South America but is indigenous to Bolivia and

Peru. The coca leaf was chewed recreationally in South America for many centuries before cocaine was isolated from the leaf and proposed for medicinal use. Once the addictive properties of cocaine were realised, the drug was no longer fit for therapeutic/medicinal use (Dasgupta, 2010 and WHO, 2004). The molecular structure of cocaine is shown in Figure 1.3.

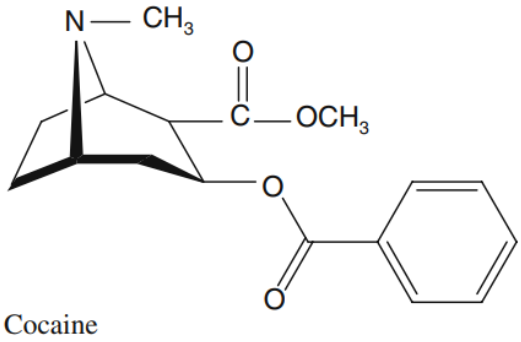


Figure 1.3 The molecular structure of cocaine (Dasgupta, 2010).

Cocaine acts as a nervous system stimulant that leads to feelings of euphoria and well-being, increasing alertness, energy levels, and motor activity, but subsequently leads to feelings of depression and discomfort ("drug crash") (Srinivasan *et al*., 2008 and WHO, 2004). There are multiple routes of administration, whereby cocaine can be taken intranasally (snorted as the hydrochloride salt), injected intravenously, or smoked (crack cocaine in form of crystal rocks) (Dasgupta, 2010 and WHO, 2004).

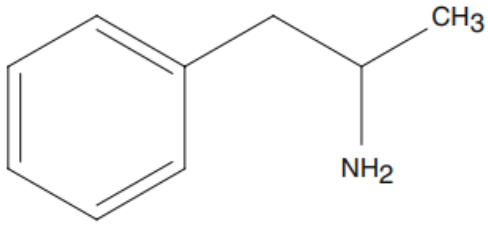
Cocaine acts by inducing the release of neurotransmitters such as dopamine and norepinephrine, resulting in feeling of euphoria, but also by blocking the re-uptake of these neurotransmitters by nerve cells and thereby extending the effect of euphoria (Srinivasan *et al*., 2000). Following this, the depletion of neurotransmitters results in a "crash", whereby users feel tired, dysphoric, sad, and irritable (Srinivasan *et al*., 2000; Dasgupta, 2010 and WHO, 2004). The ability of cocaine to act as a monoamine transport blocker (block the uptake of dopamine and norepinephrine), and the resultant increase in feelings of euphoria, creates positive reinforcing properties and leads to further cocaine seeking behaviour and potential addiction (WHO, 2004).

Cocaine is rapidly metabolized in plasma and has a short half-life (approx. 0.5–1.5 hours). There are two major metabolites of cocaine i.e., benzoylecgonine and ecgonine methyl ester (Srinivasan *et al*., 2000 and Dasgupta, 2010). Benzoylecgonine is formed by the spontaneous hydrolysis of cocaine and ecgonine methyl ester is formed by the action of esterases in plasma and in the liver (Dasgupta, 2010). Benzoylecgonine and ecgonine methyl ester are then further metabolized into ecgonine. These metabolites are excreted in urine and are useful for the identification of cocaine usage as they have longer half-lives (approx. 4-6 hours) than that of cocaine itself (Srinivasan *et al*., 2000; Karch, 2002 and Dasgupta, 2010). Norcocaine is also a metabolite of cocaine, produced in small amount by liver enzymes (Dasgupta, 2010). Cocaethylene is a metabolite produced in the liver, when cocaine and alcohol are used simultaneously (Dasgupta, 2010) and detectable for longer periods than cocaine (approx. 3 to 5 times longer than cocaine). Cocaethylene is also said to be more toxic than cocaine itself, due to its ability to block the uptake of neurotransmitters as effectively as cocaine itself, and due to the longer half-life than cocaine (that is present in the blood for longer than cocaine before metabolic breakdown) (Karch, 2002).

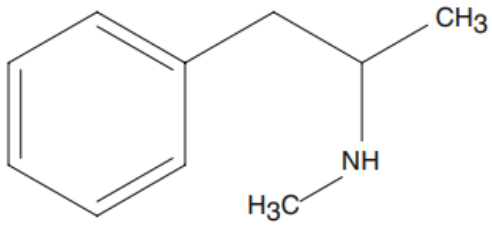
In excessive doses, cocaine may be harmful on both naïve cocaine users, and in cocaine tolerant users. Cocaine is a vasoconstrictor and causes strain on the body's physiological systems. Effects on the heart and cardiovascular system can range from chest pains to myocardial infarction and cardiac arrest, and neurological effects can cause stroke and seizures (Hall *et al*., 2006). Additional effects can include asthma, bronchitis, respiratory collapse, inability to control body temperature, vomiting and colitis (Hall *et al*., 2006). Cocaine users experience a high rate of psychiatric disorders and use is associated with negative psychological and behavioural effects such as depression, anxiety and affective disorders, and paranoia. Usage is also associated with risk of intentional self-harm (Hall *et al*., 2006 and WHO, 2004). External signs of use in chronic users include a perforated nasal septum (snorting cocaine users), dental erosions, crack thumb (callus formed on thumb from continuous lighting of crack pipe), cocaine track marks (repeated injection site marks and bruises) (Karch, 2002). Currently there is no prescription medication containing cocaine, and cocaine is only considered as an illicit drug (Dasgupta, 2010).

1.2.3.3 Amphetamine type stimulants

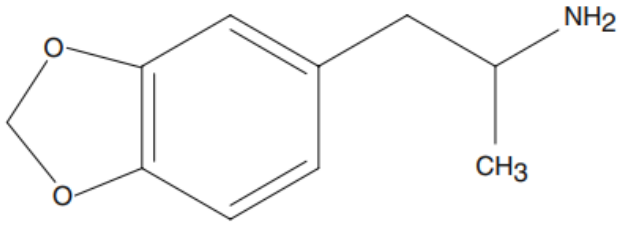
Amphetamine type stimulants are a group of synthetic stimulants and hallucinogens that are chemically related to phenylethylamine, and whose principal members include amphetamine, ephedrine, methamphetamine, methylphenidate, and pemoline (Dasgupta, 2010 and WHO, 2004). Amphetamine and methamphetamine exist as two enantiomers (D- and L-), each form having different pharmacological activities. The D-enantiomer acts on the central nervous system (stimulant), and L-enantiomer acts peripherally (appetite suppressant) (Cody, 2008 and Dasgupta, 2010). Throughout the years, substitutions have been made to the molecules in order to alter activity, mostly trying to lead to increased central nervous system stimulation. A “designer” amphetamines group of drugs have also been created which are not found to have legitimate medical use and generally found as an illicit abused drug. These include 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and 3,4-methylenedioxyethylamphetamine (MDEA) (Cody, 2008). The molecular structure of amphetamine, methamphetamine and MDMA/ecstasy is shown in Figure 1.4.



Amphetamine



Methamphetamine



3,4- Methylenedioxymethamphetamine

Figure 1.3 The molecular structure of amphetamine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA/ecstasy) (Dasgupta, 2010).

Amphetamine type stimulants stimulate the central nervous system and create effects such as increased alertness, energy, arousal, speech and motor activity, concentration and an overall feeling of well-being and euphoria (WHO, 2004). These drugs can also lead to restlessness, insomnia, confusion, dizziness, as well as induce psychotic episodes (WHO, 2004). Amphetamines act by increasing dopamine concentrations by stimulating the release of dopamine from nerve terminals, this action is in contrast with cocaine that blocks the reuptake of dopamine (WHO, 2004 and Dasgupta, 2010).

Amphetamine and methamphetamine are eliminated via hepatic and renal clearance, with a half-life of 6 to 12 hours. A significant proportion of the drugs are excreted unchanged in urine, with approx. 30% for amphetamines, and approx. 45% in methamphetamines (Dasgupta, 2010 and Cody, 2005). Amphetamines can diffuse through cell membranes and lipid layers into tissue and biological matrices and therefore can be detected in sweat, saliva, hair, and nails in addition to blood and urine specimens. Amphetamine is further metabolized to benzoic acid through aromatic hydroxylation to parahydroxyamphetamine and oxidative deamination (Dasgupta, 2010). Methamphetamine has a major metabolite i.e., amphetamine, which is formed by demethylation, additional metabolites include ephedrine derivatives (Cody, 2005 and Karch, 2002).

Amphetamine type stimulants can be taken orally, by smoking, sniffing, inhaling, and injecting. In Australia it is reported that 90% users (mostly methamphetamine) inject the drug. Injecting drug users are at high risk for transmission of blood-borne infections (WHO, 2004 and Hall *et al*., 2006). High doses of amphetamine, especially via injection, can cause episodes of paranoid psychosis, delusions, confusion, and hallucinations (Hall *et al*., 2006). Users have also reported feelings of depression and anxiety and panic attacks. Amphetamine use has negative effects on the cardiovascular system and can results in death by cardiac arrest. Death by hyperthermia has also been reported, whereby the drug affects thermoregulatory mechanisms, and a combination of sustained exertion, lack of fluid replacement and high ambient temperatures as experienced at party/dance venues can potentially lead to a rapid, fatal rise in body temperature (Hall *et al*., 2006).

Therapeutically amphetamine type stimulants can be used for alertness/wakefulness in cases of truck/lorry drivers, attention and concentration in students studying for exams and for treatment of attention deficit hyperactivity disorder as well as an appetite suppressant for weight loss (WHO, 2004).

1.2.4 Substance use and the link to suicide

1.2.4.1 Suicide

Suicide is a global phenomenon, occurring in all regions of the world. The WHO has defined suicide as “the act of deliberately killing oneself” (Parekh and Phillips, 2014). Wasserman *et al*. 2012, stated that the initial thought of suicide, to completion of successful suicide is a complex process developing over time. According to the World Health Organization (WHO) in 2012 approximately 800 000 individuals die from suicide every year, that is one suicide death occurring every 40 seconds. Globally, suicide is the second leading cause of death in those aged between 15 – 29 years (Parekh and Phillips, 2014). It has been noted that 77% of suicides occur in low- and middle-income countries, where majority of the world’s population live (WHO, 2021 and Bachmann, 2018). South Africa has a high prevalence of suicide cases accounting for estimated 11% of deaths of South Africa (Prinsloo *et al*., 2021) and approximately 12% of deaths in Gauteng (NIMSS, 2013).

Suicidal behaviour starts with thoughts of ending one’s life (suicidal ideation), followed by suicide attempts (self-inflicted harm/behaviour acted on with the intent of death, however non-fatal in outcome), and finally potentially resulting in a completed suicide fatality (Wasserman *et al*., 2012 and Nock *et al*., 2008). Research indicates that there are an estimated 10-40 attempted suicides for each completed suicide (Wasserman *et al*., 2012; Bachmann, 2018 and Zalar *et al*., 2018). It is to be noted that these estimates may be underrepresented as suicide attempts are not always reported (Zalar *et al*., 2018). Globally there is a male predominance seen in completed suicides, whereas females show a higher proportion for suicide attempts than males (Bachmann, 2018; Parekh and Phillips, 2014). This trend is also seen in South Africa where suicide rates are approximately five times higher in men than in women (Kootbodein *et al*., 2020). Research suggests that the higher proportion of completed suicided by males may be due to the lethality of method used. Males are shown to choose more high-risk lethal methods of suicide (e.g., firearms, hangings), whereas females tend to choose less lethal or less violent method of suicide (e.g., overdoes/poisoning, stabbing) (Bachmann, 2018; Parekh and Phillips, 2014; Wasserman *et al*., 2012; Zalar *et al*., 2018 and Lim *et al*., 2014).

Suicidal behaviour is complex with a multitude of factors contributing to the outcome of a completed suicide. These include psychological, personal, cultural, social, environmental, and biological factors (Parekh and Phillips, 2014). The majority of completed suicides are found in individuals with psychiatric or mental disorders, with the most relevant risk factors being depression, mood disorder, substance use disorders and psychosis/ personality disorders (Brådvik, 2018 and Wasserman *et al*., 2012). Depression and co-occurring substance use disorders are noted to be the most common diagnoses amongst suicide victims (Brådvik, 2018 and Baldessarini, 2020).

1.2.4.2 Suicide and substance use

Substance (alcohol and drugs) abuse is the second most frequent risk factor associated with suicidal behaviour; the risk is further increased when a substance use disorder co-occurs with depression and other mental health disorders such as anxiety and bipolar conditions (Centre for Suicide Prevention, 2014). Research suggests that those individuals suffering from depression, or mental health disorders are likely to turn to substance use as a form of coping mechanism (Centre for Suicide Prevention, 2014). Ongoing use of substances can lead to the development of substance use disorder which is problematic drug use. Substance use disorder can lead to suicidal ideation through loss of inhibition, impaired judgement, as well as cause changes in the brain over time that lead to depression (Centre for Suicide Prevention, 2014 and SAMHSA, 2016). Thus, those suffering from a substance use disorder are more susceptible to suicidal behaviours (Centre for Suicide Prevention, 2014 and SAMHSA, 2016). The Centre for Suicide Prevention (2014) presented that 40% of patients seeking treatment for a substance use disorder had attempted suicide at least once in their lifetime (Centre for Suicide Prevention, 2014). Further studies at addiction treatment facilities reported that between 50 – 75% of patients had some form of co-occurring mental health disorder. Also, studies at mental health facilities reported between 20 – 50% of patients presented with a co-occurring substance use disorder (Centre for Suicide Prevention, 2014). The Centre for Suicide Prevention (2014) has also stated that in 90% of completed suicide cases the victims suffer from either a mental health disorder and/or a substance use disorder, it is further noted that 40% of suicide victims were said to be intoxicated at the time of death, thus further reinforcing

the association between substance use, mental health, and suicide (Centre for Suicide Prevention, 2014).

As reported in the UNDOC 2018 report, suicide deaths accounted for 8.3% of global drug use related deaths (Figure 1.1). SAMHSA (2016) reported that within suicide deaths, opiates were found to present in 20% of cases, cocaine in 4% of cases, and amphetamines in 3% of cases. Opioid use disorder has shown association with suicide, with opioid related suicides having doubled over the period from 2003 to 2018, and with heroin use leading to a 13.5 times increased risk of suicide compared to non-opioid users (Esang and Ahmed, 2018 and Yuodelis- Flores and Ries, 2015). Cocaine dependence has also been noted as predictor of attempted suicide. Studies have also shown that approx. 40% of cocaine dependant patients have a history of suicide attempt, and the substance of abuse of cocaine is associated with an increased prevalence of suicidal thoughts and behaviours (Yuodelis-Flores and Ries, 2015 and Roy, 2009).

It was reported that 58% of polydrug dependant users in addiction treatment facilities in Norway had attempted suicide at least once within their lifetime, with a higher prevalence noted in female polydrug dependant users (70%) (Yuodelis-Flores and Ries, 2015). A study in China assessing amphetamine type stimulant users, found that 64% of study participants experienced depressive symptoms, 17% reported suicidal ideation, and 11% reported suicidal behaviours. Those groups expressing the highest prevalence of psychiatric symptoms were found in frequent drug users/drug dependent users (Du *et al*., 2014). The psychiatric symptoms and suicidal thoughts and behaviours found in the amphetamine stimulant users were found to be higher than that of the general non-drug using population of China (Du *et al*., 2014).

Postmortem examinations aid in establishing the self-inflicted basis of a suicide fatality, where pathologic and toxicologic findings may provide insight into events before completion of suicide, as well as the cause and manner of the death (Shields *et al*., 2006 and Dhossche, 2003). The toxicology results can shed light on possible factors at play with the decedent prior to suicidal act, be it medication mis-management or noncompliance in those suffering from mental health disorders, or reveal the presence of potentially abusable illicit substances, as well as the presence of prescription and over the counter medication and potential toxic levels, or merely if the

toxicological findings were purely incidental and had no role in the death and suicide outcome (Shields *et al*., 2006). Results can thus be used in combination with historical, familial and scene evidence to gather a greater understanding of the suicide decedent (Shields *et al*., 2006).

1.2.5 Postmortem investigations

The South African National Health Act (Act No. 61 OF 2003) (Republic of South Africa, 2003) makes provision for medico-legal mortuaries and medico-legal services as well as the provision for the rendering for services provided by Forensic Pathologists and related forensic pathology services (Republic of South Africa, 2003). Victims of unnatural or unknown causes of death require a comprehensive medico-legal investigation in order to determine the nature of the death, i.e., was this criminal, accidental, self-inflicted, or due to negligence. The South African Inquests Act (Act no. 58 of 1959) (Republic of South Africa, 1959) thereby governs the medico-legal investigation into an unnatural death. The primary purpose of the Forensic Pathology Service is to perform a medico-legal investigation of death service in cases where the cause of death is unnatural or unknown. Deaths involving unnatural causes include: death due to physical or chemical influence; deaths due to criminal nature; deaths where a medical procedure (e.g., anaesthesia, or surgical intervention) may have been a contributing factor; or where a sudden, unexplained death occurs whereby a doctor is unsure of the cause and there is no medical history to serve as explanation (Republic of South Africa, 2003), such deaths are reported to the South African Police Service (SAPS) which then in conjunction with the forensic pathology service investigate the death of the victim. The SAPS are responsible for the police investigation and the FPS for the medical evaluation. The medical evaluation for a comprehensive death investigation includes a postmortem examination (which includes autopsy) of the decedents body (macroscopic and microscopic) as well as the request on any ancillary laboratory tests in order to aid the evaluation process. The purpose of the postmortem examination is therefore to establish the cause and circumstance of death as well as any contributing factors (Republic of South Africa, 2003).

1.2.6 Forensic toxicology

A poisoning or intoxication can be defined as an individual being in a medical condition, or socially unacceptable condition that is a direct consequence of being under the influence of xenobiotics in a dose that is too high for the individual in question (Uges, 2001). In general, there are three possible routes of poisoning: accidental, experimental, or intentional (Uges, 2001; Dinis-Oliveira *et al*., 2010). For medical and legal purposes, it is important to ascertain which of these routes had led to the intoxicated state of the victim. A toxicological analysis will allow for the detection of substances present within biological specimens at the time of testing (Gill, 2005). Forensic toxicology is therefore concerned with the science of poisoning, the application of toxicological assessments and how these relate to established laws (Dinis-Oliveira *et al*., 2010 and Madea and Musshoff, 2004). Postmortem toxicology is thus a specialized subset of forensic toxicology relating to the toxicological study and assessment of deceased individuals. The purpose of which is to determine if the drugs or poisons present may have played a role in the cause of death, or in the events immediately preceding death (Madea and Musshoff, 2004 and Dinis-Oliveira *et al*., 2010). Factors to be considered in postmortem toxicological testing include:

- Biological specimen collection and accurate labelling of specimens collected: appropriate biological specimen collection at time of autopsy, the potential impact of any postmortem matrix-related specimen effects and the impacts on results upon analysis of sample thereof (EMCDDA, 2019 and SOFT/AAFS, 2006).
- Security and chain-of-custody: correct reporting of all personnel handling samples so that the chain-of-custody remains intact. Ensuring that access to the laboratory and samples is limited to only authorized personnel to ensure no tampering with samples (SOFT/AAFS, 2006).
- Analytical procedures: screening and confirmatory testing to confirm a positive or negative result. Two sets of tests to provide confirmation of a result. An initial preliminary screen to determine presence of specific drug classes, where results need to be confirmed by a secondary more sensitive method. A second analytical test method based off a different chemical technique is required to

confirm any results from the initial screen in order to deem the results as valid in terms of forensic analysis. The combined results of the screen and confirmatory test are used to determine if a biological specimen is confirmed as positive or negative from drug compounds. A “positive” result indicating that a substance/drug compound has been detected and identified in accordance with the laboratory protocols and test methodologies. A “negative” result indicating that no substance/drug compound has been detected within the limitations of the tests performed (SOFT/AAFS, 2006).

1.2.6.1 Specimen selection

There are a variety of specimens available for toxicological testing, and the choice of specimen depends on the type of application, for clinical and therapeutic drug monitoring the most common specimens chosen are blood (providing the most direct evidence of use), urine (indicates recent usage and large sample volume) and hair (provide history of exposure) (Drummer, 2010). Within postmortem toxicological assessments blood and urine are most collected, but also gastric contents and liver are collected as these specimens offer insights into recent usage and drug metabolism within the body (Drummer, 2010).

- **Blood**

Blood is the universally preferred specimen of choice for the quantification and interpretation of drug concentrations and the corresponding metabolites. A qualitative or positive blood result provides the most direct evidence for presence of a drug in the victim and depending on concentration levels may allow the toxicologist and forensic pathologist to deduce the potential pharmacological effects and role in cause and manner of death (Skopp, 2004 and Drummer, 2010). For qualitative analysis peripheral blood obtained from the femoral vein is the best source of blood for toxicological testing as this seems to provide a more representative concentration of drug/metabolite at time of death (TIAFT, 1999; Prahlow, 2010 and Drummer and Gerostamoulos, 2002). Postmortem blood collected from central blood (i.e., cardiac blood) may be affected by redistribution. Postmortem redistribution is a phenomenon

whereby drug levels within the samples will increase when previously bound drug in neighbouring tissues is released (as a result of disruption of cellular membranes) or diffuse from one tissue to another. That is the diffusion of drugs from surrounding tissues containing higher drug concentrations of drugs into blood vessels which have lower concentrations (Prahlow, 2010; Drummer, 2004 and Drummer and Gerostamoulos, 2002). The diffusion from the gastrointestinal tract and neighbouring organs are likely to have an influence on the concentration of cardiac blood, whereas peripheral blood is less effected by the redistribution phenomenon and therefore the preferential blood specimen (Drummer, 2004 and Drummer, 2010).

- **Urine**

Urine is most frequently used in screening tests due to ease of analysis and the biological renal clearance involved in many drugs, where the result can be useful in directing the laboratory to a certain drug or toxin. Drugs and metabolites are found to be present in higher concentration in urine as compared to blood, and dependent on the drug these may be present in urine for days or longer. Therefore, urine is used to reflect the degree of exposure, and historical use (Prahlow, 2010; TIAFT, 1999; Gill, 2005). However, urine cannot always provide evidence of an acute intoxication. This is because urine is stored in the bladder as a waste product and therefore dosage taken at time of use cannot be determined, as well as the effects of the drug at time of sampling may differ from that at time of use (Gill, 2005 and Drummer 2010). In the same regard a negative urine result may not necessarily indicate that no drug was used as in cases of very rapid deaths following exposure (TIAFT, 1999 and Skopp, 2004).

- **Liver**

Metabolism of most drugs and poisons takes place in the liver, as such both the parent drug and metabolite may be present in high concentrations (TIAFT, 1999). Liver specimens can therefore be used to supplement blood toxicology data, and are the favoured specimen when blood is not available, such as in instances where the body is decomposed or had been exposed to a fire, or exsanguination (Skopp, 2004). Liver

concentrations of certain drugs may help distinguish an acute intoxication from therapeutic ranges (Gill, 2005).

- **Gastric contents**

Gastric contents can be used to determine route of administration of drug i.e., if drugs are ingested, as well as the potential time of administration by assessing the state of digestion of the gastric contents (Drummer,2004 and Gil, 2005). Gastric contents may be used for a general screen of potential substance ingestion whereby whole tablets or plant materials may be found within the sample, and certain solvents and herbicides may have a particular odour alluding to presence within the gastric contents (Skopp, 2004). The total drug remaining in the specimen is more important than the concentration of the drug when assessing the case, as the amount of drug (in milligram) compared to the total amount of gastric contents (gram) can help determine the amount/number of drug/pills taken and if these were an intentional overdose amount or within a therapeutic range (Gill, 2005 and Skopp, 2004).

1.2.6.2 Postmortem matrix-related effects

Postmortem artifacts may affect drug concentration levels with both a qualitative and quantitative toxicological difference. A postmortem artifact is defined as any change caused in the body after death that may lead to a misinterpretation of significant result findings (Warushahennadi and Ruwanpura, 2017). Such causes of artifacts may include:

- Postmortem redistribution: the increase in concentration of drug levels seen in cardiac blood to diffusion through tissues and cell membranes.
- Improper storage and transportation of specimens: lack of preservative on sample collection, and inadequate refrigeration of sample leading to degradation of the specimen and hydrolysis of drugs within the specimen which may cause a negative sample test result.
- Stage of decomposition of decedent and postmortem interval: time between death and discovery of the corpse and subsequent medico-legal autopsy. The

stability of the drug in question may be affected due to the time from death to sample testing, drugs and metabolites will continue to metabolize after death and may not be detectable in specimens after a certain period (Gill, 2005; Skopp, 2004 and Drummer and Gerostamoulos, 2002).

These factors need to be taken into consideration when determining the implications of the forensic toxicological results, and as such the resultant toxicology test process cannot solely be relied upon to determine that an acute intoxication was the cause of death. Additional variables need to be considered in addition to a positive toxicological result (that is a drug concentration within range considered to be fatal) including the circumstances and history surrounding the death (i.e., history of drug use, drug paraphernalia at scene, external marks indicative of drug behaviour), and that following the postmortem examination no other cause of death due to disease or physical injury/harm that is attributable to death (Gill, 2005).

1.2.6.3 Analysis of samples

Due to the importance of results obtained in forensic toxicological investigations and the implications of such results in medico-legal cases, any substances suspected of being involved in the death of the decedent must be identified and taken into consideration when establishing cause of death. Forensic toxicological testing therefore requires that two independent tests are conducted in order to obtain a result in the drug identification that can be used for medico-legal purposes (Moffat *et al*., 2011 and Drummer, 2010). The initial stage of testing usually makes uses of a screening test that is used to provide an indication if a drug or metabolite is absent or present within a sample. It is important to note that a positive screening test does not provide unequivocal proof that the drug is present within the sample. A secondary, confirmatory test is thus required to confirm all results (Skopp, 2010 and Drummer, 2010). Only those samples with a positive result in both the screening and confirmatory test can then be deemed positive for presence of drug or metabolite. Immunoassays are often used as the initial screening test for presence of drug or metabolites, and then mass spectrometry (MS) is used for confirmation of the initial test and to identify the drug by spectral data (Drummer, 2010).

Immunoassays allow for a wide range of drugs to be tested for within a small amount of biological sample (normally blood or urine) volume (Moffat *et al*., 2011). The technique is based off of the concept of a competitive assay, where specific antibodies that show selectivity for a class of related drugs is bound to a medium and there is a competition between the analyte (drug of interest) and a labelled antigen for binding to these antibodies sites. The markers used for labelling of antigens include chemiluminescent substrates, fluorophores or enzymes that leads to a measurable result, where if the patient/decedent sample binds to the antibody site and thereby less labelled analyte can bind, which will result in a lesser signal/reaction response produced. This signal/reaction will then serve as a measure of the amount of drug or metabolite present in the sample (Jain *et al*., 2012 and Drummer, 2010).

The confirmatory test involves techniques of either gas chromatography (GC), or liquid chromatography (LC) coupled with a mass- spectrometry (GC-MS or LC-MS). These techniques involve the chromatographic separation of molecules, either through a gas or liquid phase, and the subsequent production of spectral data when the separation of molecules is coupled with a mass spectrometer. This allows for identification of molecules based on spectral peaks and fragmentation patterns of molecules of drugs of interest. The spectral peaks and patterns produced can then be compared to mass spectral data of known drugs and compounds to positively identify those found in the samples (Drummer, 2010 and Gergov, 2008).

Interpretation of “positive” and “negative” results are subjective to various factors, those being the limitations of the analytical techniques used, the nature of the specimens selected or available for testing, any postmortem influences on the body, quality of specimen collected (decomposition/purification of the body), sample storage and collection methods (Allan and Roberts, 2009 and Drummer, 2010). Decedent specific factors such a tolerance to drugs may also affect interpretation of concentrations of drugs found within a sample (Allan and Roberts, 2009).

1.2.7 Forensic toxicology application to suicide

Toxicological results can play a crucial role in the investigation of a suicide case. Results found can indicate if a drug of abuse was involved either directly in the death,

as in case of an overdose, or if drugs were found to be present but not involved in the direct manner of the death e.g., in hanging suicides. The results can then be used to assess potential contributing factors of drug use on the death such as impairment of behaviour, or assessment of psychological state (Allan and Roberts, 2009). As such toxicology results are to be used in combination with any evidence found at the scene of death, the decedents history indicating potential suicidal ideation, substance abuse and psychological struggles, in order to complete the postmortem examination and provide complete overview and assessment of the factors contributing to a successful suicide outcome (Shield *et al*., 2006).

1.3 Aims and objectives

The aim of this study is to describe the incidence of suspected suicide fatalities involving the use of opiates and metabolites (morphine, 6-monoacetylmorphine, codeine, morphine metabolites, hydromorphone, hydrocodone, dihydrocodeine), cocaine and metabolites (benzoylecgonine, cocaine, cocaethylene, norcocaine, AEME), and amphetamine type stimulants and metabolites (amphetamine, methamphetamine, MDMA, MDA, and MDEA) in Johannesburg, South Africa.

The objectives of this project are:

- To identify and describe the demographics of suspected suicide fatalities, including age, sex, and racial group in Johannesburg, South Africa.
- To determine the presence of opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites, and the occurrence thereof in suspected suicide fatalities in Johannesburg, South Africa.
- To describe the outcomes (true positive, true negative, and discordant results) of toxicological tests for cases of suspected suicide fatalities in Johannesburg, South Africa.

Chapter 2: Materials and methods

This study was a prospective, observational study consisting of a literature review of the proposed subject matter, as well as an assessment and identification of suspected suicide victims. Data of the selected victims was collected from the medico-legal autopsy reports at the Johannesburg Forensic Pathology Service Medico-Legal Mortuary (JHB FPS-MLL) between September 2013 and October 2014. Biological specimens were collected from the identified victims, analytical techniques were then utilized to test the samples for the presence of opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants. The study results were then analysed in terms of the aim and objectives of the project.

2.1 Site of study

This study took place at the JHB FPS-MLL located at 25a Hospital Street Braamfontein, 2001 (sample collection and phase I immunoassay testing) and at Randox Testing Services (Manchester, England) (phase II confirmatory testing).

The main service provided by the JHB FPS-MLL is to perform medico-legal investigation of unnatural deaths. This involves liaising with the South African Police Service in terms of their investigations, performing post-mortem examinations of the body including autopsy, collection and reporting of evidence collected from the body, generation of medico-legal reports. The purpose of which was to identify the cause of death and contribute information towards the possible circumstances leading to death in unnatural death cases. This service is governed by the South African Inquests Act 58 of 1959. Section 2 “If the body of a person who has allegedly died from other than natural causes is available, it shall be examined by the district surgeon or any other medical practitioner, who may, if he deems it necessary for the purpose of ascertaining with greater certainty the cause of death, make or cause to be made an examination of any internal organ or any part or any of the contents of the body, or of any other substance or thing” (Republic of South Africa, 1959).

The catchment area serviced by the JHB FPS-MLL is the greater Johannesburg area, comprising of the following areas: Alexandra, Booysens, Bramley, Brixton, Cleveland,

Douglasdale, Fairland, Hillbrow, Honeydew, Jeppe, Johannesburg Central, Lanlaagte, Linden, Midrand, Mondeor, Norwood, Parkview, Randburg, Rosebank, Sandringham, Sandton, Sophiatown, and Yeoville.

2.2 Sample: sourcing of suspected suicide victims

Suspected suicide cases were sourced by a daily review of the death register (that is the logbook of cases received at the JHB FPS-MLL) to ascertain the cases received at the mortuary. The case files were then assessed to obtain information relating to the history of the individual and circumstances surrounding the death. This information was sourced from the SAP 180 form, containing information: age; sex; racial group; particulars surrounding death (method of death, place of occurrence, medical history if available) and any possible circumstances surrounding the death such as suicide notes; family history and eyewitness accounts. The SAP 180 form is completed by the police investigating officer of the case, and this form was required by the FPS in order to conduct a medico-legal postmortem examination. The SAP 180 form would note if the case was suspected to be a suicide and use terms such as: jump from building/fall from height, gassed, hanging, overdose, shot etc. The FPS scene of death form was also used to gather information relating to the possible method of suicide.

2.2.1 Inclusion criteria

- Suspected suicide fatality victims as identified from SAP 180 documentation, FPS scene of death form, and the autopsy findings from cases autopsied from Monday-Friday (excluding public holidays) (Terminology assessed for in the death register included: fall from height, jump from height, fall from building gassing, hanging, overdoes, poisoning, shot, stabbing).
- Suspected suicide fatality victims found dead on arrival at hospitals, i.e., no medical intervention (administration of any medications or treatments) was received prior to death as noted in the SAP 180 document.
- Suspected suicide fatality victims from 18 years of age and older.

2.2.2 Exclusion criteria

- Suspected suicide fatality victims who had received medical intervention prior to death (i.e., resuscitation, or prolonged hospital stay following suicide attempt) as noted on the SAP 180, and hospital D28 and GW7/24 clinician's forms.
- Suspected suicide fatality victims autopsied over weekend periods.
- Any suspected suicide fatality victims showing stages of decomposition, i.e., reported signs of bloating, decay, putrefaction, and or rotting.
- Any victim in which an insufficient amount of biological specimen could be sampled

2.3 Assessment of files and paper-based data collection

A total of 103 victims were identified in this study. The method of suicide was noted prior to autopsy and included the following categories: carbon monoxide /gassing, fall from height, firearm/gunshot, hangings, overdose/poisoning (ingestion of toxic substance) and stabbing related suicides. Following the autopsy, the autopsy reports were collected and assessed for any further information pertaining to the case. The autopsy reports were compiled by the specialist pathologist who performed the medico-legal examination of the body. Permission to access the case files related to this study was granted to the researcher by the mortuary manager and head of department. The case files contained information about the mode and cause of death as ascertained by the pathologist and any other findings noted during examination of the body. All data collected was transferred to an excel data spreadsheet and saved according to the reference/labelling number allocated to the collected samples. Demographic and historical information was acquired as per Appendix I (see data collection sheet Appendix I).

2.4 Sample specimen collection

2.4.1 Blood

Blood sample specimens (n= 103) were obtained from the femoral vein/artery region, using a needle and hypodermic syringe. Blood was stored in glass BD Vacutainer®

EDTA (ethylenediaminetetraacetic acid) blood collection tubes. Approximately 2 – 4 mL of blood sample was collected from each victim. The samples were labelled with a reference number correlating to the recorded demographic data for each victim. All samples were stored under controlled conditions, at -20^{o}C in a freezer.

2.4.2 Urine

Urine sample specimens (n=51) were obtained, where possible, during dissection using a needle and hypodermic syringe directly into the bladder. Urine was stored in glass BD Vacutainer® no additive urine collection tubes. A maximum of 5 mL urine sample was collected. The samples were labelled with a reference number correlating to the recorded demographic data for each victim. All samples were stored under controlled conditions, at -20^{o}C in a freezer.

2.5 Testing

2.5.1 Overview of testing procedures

The testing procedure was split into two phases of testing. A phase I test which comprised of an Immunoassay preliminary screen and provided a semi quantitative result, i.e., an approximation of the analyte concentration within a sample. The preliminary screen therefore would indicate whether an analyte or drug group was present or not, however further testing was required to confirm this result.

A phase II test served as a confirmatory test to the phase I results. The confirmatory test used was a liquid chromatography tandem mass spectrometry (LC-MS/MS) test method.

All specimens were submitted to all phases of the testing process (i.e., a phase I negative result was still tested in phase II testing). The results of both the phase I and phase II tests were then used to determine the finalized result for any analytes/drug groups identified in the samples. The results were classified into three categories: true positive, true negative, and discordant. A sample testing positive for an analyte/drug group for both the phase I and phase II tests would be considered as a true positive

result. A sample testing negative for an analyte/drug group for both the phase I and phase II tests would be considered as a true negative result. A sample testing positive for an analyte/drug group for either the phase I test or the phase II test would then be considered as a discordant result. Figure 2.1 summarises the analytical process of the study.

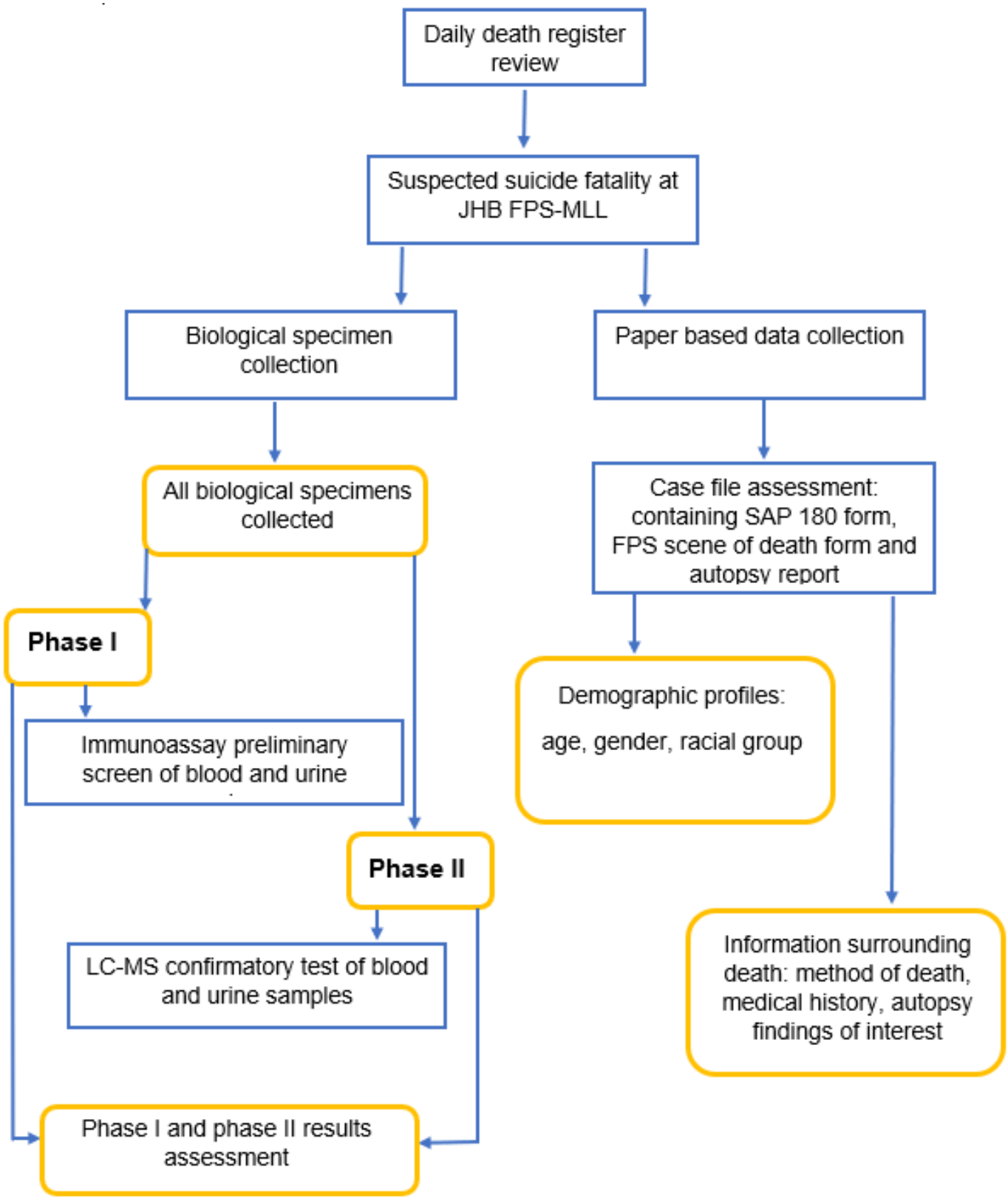


Figure 2.1 Flow diagram illustrating the analytical process of the research

2.5.2 Phase I test: Randox Biochip Array Technology Immunoassay

2.5.2.1 General principles of the test method

The immunoassay screening was performed using the Randox Biochip Array Technology (BAT) (Randox Laboratories Ltd, 2014). This test system consists of an Evidence Investigator™, a compact, semi- automated benchtop instrument, that works in conjunction with the biochip array technology. The technology allows for the simultaneous testing of multiple drug metabolites within in one sample. The Randox BAT system is kit based, with all necessary components provided for, these include controls, calibrators, biochips, chemicals and developing agents.

The biochips consist of nine biochips (9 x 9 mm wells) and are placed on a cassette (Figure 2.2). Within a single biochip there are discrete test regions containing immobilised antibodies that are specific to different drug metabolites, thus allowing for the simultaneous testing of multiple analytes in one test (Figure 2.3).



Figure 2.2 Randox biochip (nine, 9x9 mm wells) (Randox Laboratories Limited, 2021)

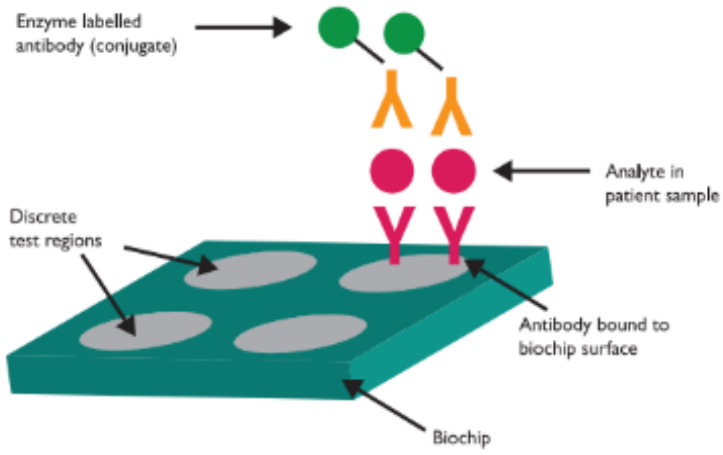


Figure 2.3 Randox BAT technology immunoassay (Randox Laboratories Limited, 2021)

This technology is based on a competitive chemiluminescent assay. The antibodies are attached to the surface of the biochips in the discrete test regions, the samples, and an enzyme-labelled conjugate (horseradish peroxidase (HRP)) are then added to each well. Any drug molecules present in the patient sample compete with the HRP conjugate (HRP-labelled drug molecules) for binding to the antibody sites (Figure 2.3). A signal reagent is then added, whereby a chemical light producing reaction (chemiluminescence) occurs on antibodies bound to the HRP conjugate. The chemiluminescent (light) signal created is inversely proportional to the concentration of the analyte/substance present within the test sample i.e., the lower the light output the higher the concentration of substance of interest (Randox Laboratories Ltd, 2014). Therefore, there will be no chemiluminescent reaction if the test sample outcompetes the HRP-labelled conjugate for antibody binding. The degree of chemiluminescence produced is detected with a digital camera within the Evidence Investigator™. The on-board software processes the image and compares the result to that of a stored calibration curve. The concentration of any analyte present within the samples are then calculated using the calibration curve (Randox Laboratories Ltd, 2014).

The Randox BAT test kits included calibrators and controls.

The kit consists of nine calibrators, that is nine vials of material containing the analytes for the full immunoassay screen test, at varying known concentration levels which cover the full test measurement range of the drugs being screened for. These

calibrators are then used to establish a calibration curve, which is stored on the system and applied to a sample test run. That is, the sample results (unknown) are compared to the known values on the established calibration curve to determine the amount/concentration of the analyte present within the sample.

The kits consist of two control components, control 1 (low concentration) and control 2 (high concentration). Both controls 1 and 2 contain the same analytes tested for within the kit, at two differing concentrations levels that cover the kit cut-off levels. Both controls will need to pass within the acceptable range (as noted in the respective kit instructions) for the test sample results to be accepted. The cut-off level is the minimum concentration (in ng/mL) of analyte in the test sample that can be considered as a significant/positive result.

For the purposes of this study the Randox BAT Drugs of Abuse (DOA) Array I test assay was selected. The drug panel (group/classes of drugs) tested for in the assay contained the drug metabolites of interest in this study, namely opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites. Table 2.1 below lists the drug classes screened for in DOA panel I, including the cut-off levels as per Randox recommendations. The analytes targeted in this study have been underlined for ease of reference.

Table 2.1 Drugs of Abuse array I Whole Blood PLUS panel and cut-off levels

Analyte	**Cut-off levels (ng/mL) ***
Amphetamine**	**25**
Barbiturates	50
Benzodiazepine	50
Buprenorphine	1
Cannabinoids	10
Benzoylecgonine**	**50**
3,4-Methylenedioxymethamphetamine MDMA**	**60**
Methadone	25
Methamphetamine**	**50**
Opiates**	**25**
Phencyclidine	5
Tricyclic antidepressants	60

**Randox Laboratories Ltd. Cut-off levels set according to manufacturer recommendations, as there are no set regulations to dictate cut-off levels in South Africa. Cut-off levels were applied to all samples tested.*

*** Analyte of interest in this research.*

Table 2.2 below lists the analytes/metabolites within each targeted analyte group that will test positive in the assay. This information has only been provided for the targeted drug groups in this study.

Table 2.2 The analytes/metabolites which will test positive in the Randox Drugs of Abuse assay (targeted drug analytes only) (Randox Laboratories Ltd, 2014)

Analyte	**Analytes/metabolites which will test positive in the assay**
Amphetamine type stimulants assay (methamphetamine/ amphetamine/ MDMA)	d-amphetamine, methamphetamine, dl-amphetamine, MDA, MDMA, MDEA, BDB, MBDB, PMMA, DL BDB Fenfluramine, phentermine, para-methoxy methamphetamine, TFMPP, 4-methoxymethamphetamine, mephedrone, methylone, methcathinone, flephedrone.
Cocaine metabolite assay	Benzoylecgonine, cocaine, cocaethylene, norcocaine
Opiates assay	Morphine, 6-monoacetylmorphine, codeine, morphine metabolites, hydromorphone, hydrocodone, dihydrocodeine.

2.5.2.2 Materials

Randox Evidence Investigator™ Instrument, Randox Evidence Investigator™ Drugs of Abuse Array I Whole Blood Plus (DOA I WB P) assay, and Randox Evidence Investigator™ Drugs of Abuse Array I Urine Plus (DOA I URN P) assay kits, Randox Evidence Investigator™ DOA I WB PLUS control, and Randox Evidence Investigator™ DOA I URN PLUS control kits were all purchased from Randox Laboratories Limited (County Antrim, United Kingdom). Double deionised water used from a Siemens LaboStar evoqua water purifier. Pipettes and tips: 200 µL; 1000 µL; 5000 µL, 1,5 mL plastic sampling tubes, 500 mL plastic water bottle, 500 mL plastic wash bottle, tin foil, and paper towel.

2.5.2.3 Blood assay components and sample preparation

The Randox Evidence Investigator™ DOA I WB P assay kit was used to screen the whole blood samples for the panel I drugs of interest, namely: amphetamine, methamphetamine, MDMA, benzoylecgonine and opiates (Table 2.1). DOA I WB P is a semi-quantitative test and provides a preliminary screening result.

The DOA I WB P kit consists of: assay diluent (DIL ASY, 20 mM phosphate buffer containing protein, detergents and preservatives); conjugate (CONJ, 20 mM Tris based buffer containing protein, preservatives and horse radish peroxidase-labelled drug derivatives); six biochip carriers containing 54 biochips; signal reagent-EV701 (comprised of luminol-EV701 and peroxide mixed in a 1:1 ratio); nine calibrators (CAL, lyophilised material containing analytes for the full DOA I WB P array); sample diluent (DIL SPE, phosphate buffer containing detergents and preservatives) and wash buffer (BUF WASH, 20 mM Tris buffered saline, containing surfactant and preservatives).

DOA I WB P quality control consists of two components: control 1 (low concentration) and control 2 (high concentration). These two controls contain a variety of analytes tested for within the kit at two different concentration levels which cover the cut-off range. With reference to this study the quality controls of interest were for opiates, benzoylecgonine, amphetamine, methamphetamine and MDMA.

The whole blood samples were prepared according to manufacturer kit requirements (Randox Laboratories Limited, 2014). This included: Samples were centrifuged at 20784 RCF for ten minutes. Prepared whole blood samples were diluted four-fold in sample diluent i.e., 50 µL of whole blood sample was added to 150 µL of sample diluent.

2.5.2.4 Urine assay components and sample preparation

The Randox Evidence Investigator™ DOA I URN P assay kit was used to screen the urine samples for the panel I drugs of interest, namely: amphetamine, methamphetamine, MDMA, benzoylecgonine and opiates (Table 2.1). DOA I URN P is a semi-quantitative test and provides a preliminary screening result.

The DOA I URN P kit consists of: assay diluent (DIL ASY, 20 mM phosphate buffer containing bovine serum albumin, bovine β-Lactoglobulin, detergents and preservatives); conjugate (CONJ, 20 mM Tris based buffer containing bovine serum albumin, preservatives and horse radish peroxidase-labelled drug derivatives); six biochip carriers containing 54 biochips; signal reagent-EV701 (comprised of luminol-EV701 and peroxide mixed in a 1:1 ratio); nine calibrators (CAL, lyophilised, 20 mM phosphate buffer, containing stabilizers, preservatives and drug concentrations) and

wash buffer (BUF WASH, 20 mM Tris buffered saline, containing surfactant and preservatives).

DOA I URN P quality control consists of two components: control 1 (low concentration) and control 2 (high concentration). These two controls contained a variety of analytes tested for within the same scheduled kit at two different concentration levels which cover the cut-off range. With reference to this study the quality controls of interest were for opiates, benzoylecgonine, amphetamine, methamphetamine and MDMA.

Urine samples did not require any pre-treatment before analysis.

2.5.2.5 Assay sample preparation (both blood and urine)

The sample preparation was carried out as per the manufacturer's instructions (Randox Laboratories Limited, 2014). Urine samples were removed from the freezer and left to thaw overnight at -4 ^{o}C. Whole blood samples were removed from the freezer and left to thaw overnight at -4 ^{o}C and prepared as per above procedure. Thawed/prepared samples, DOA I kit and controls (either WB or URN dependant on sample test to be run) were removed from the fridge and allowed to reach room temperature for 30 minutes prior to analysis. The calibrators and controls were reconstituted with 1 mL of deionised water into each bottle and placed on a rocker roller for 30 minutes. The biochip carriers were removed from the packaging, labelled, and numbered and then placed on the carrier holder.

2.5.2.6 Assay sample analysis (both blood and urine)

The sample analysis was carried out as per the manufacturer's instructions (Randox Laboratories Limited, 2014). A total of 190 uL of assay diluent was pipetted into each biochip well of the biochip carriers. This was followed by 10 µL of reconstituted calibrators, controls, and sample into each respective well i.e., calibrator one in well one and so on. A total of 120 uL conjugate was then pipetted into each biochip well. The biochip carrier holder containing the biochip carriers was then placed in the thermo shaker for incubation for 30 minutes at 33 ^{o}C and 330 rpm.

During this incubation period the signal reagent was prepared as follows: the luminol and peroxide were mixed in a 1:1 ratio (i.e., equal volumes of each reagent) and placed in a light protected container. The signal reagent was then placed on a rocker roller and left to mix for approximately 15 minutes.

Following the incubation period, the reagents in each biochip carrier were discarded and the biochip carriers were washed with the wash buffer solution. Each wash cycle consisted of two quick washes and four two-minute soaks. The purpose of the wash steps was to remove any unbound sample and conjugate. After the final wash the biochip carriers were filled with wash buffer solution to prevent the biochips from drying out.

Biochip carriers were individually inserted into the imaging platform for processing. The awaiting biochip carriers were covered with tin foil to protect from light. Prior to imaging the wash buffer was discarded and the biochip carrier was tapped onto paper towel to remove any residual wash buffer. Then 250 uL of working signal reagent was added to each biochip well and covered to protect from light. After 2 minutes of incubation the biochip carrier was placed into the Evidence Investigator™ for imaging.

2.5.2.7 Data processing

The images were automatically captured, and results were processed by the on-board software. The results were then printed and assessed.

A nine-point calibration curve was performed, and the results of the calibration curves were assessed according to the target curve fit (0.95) (See example Appendix II). Once verified, the established calibration curves for each drug/analyte group were applied to the test samples for the processing of results. The results of the quality control (control 1 (low concentration) and control 2 (high concentration)) were verified according to the manufacturer specified levels prior to the acceptance of the test sample results. The instrument was programmed with defined control levels as recommended by the manufacturer, which were kit specific. Appendix III provides an example of the cut off ranges for acceptance of the quality controls. To be verified as acceptable the analysed control values needed to be between the low and high

concentration ranges for both the control 1(low concentration) and control 2 (high concentration) as stipulated by the kit (Appendix III).

2.5.3 Phase II test: Liquid chromatography tandem mass spectrometry (LC-MS/MS)

2.5.3.1 General principles of the test method

A LC-MS/MS instrument was used for the confirmatory test. The LC-MS system comprises of a high-performance liquid chromatography (HPLC) system (for separation) coupled with a high-resolution mass spectrometer (for analyte detection). HPLC is a technique used to physically separate compounds in a mixture. A HPLC system is comprised of two components, a mobile liquid phase and a stationary phase consisting of a packed column. The packed column contains the chromatographic packing material that allows for the separation of compounds to take place. A high pressure is then applied to the HPLC system to generate the flow of the liquid mobile phase through the stationary phase. Compounds are then identified according to their peak retention times within the column compared to the retention times of reference standards (Waters Corporation, 2014).

Mass spectrometry (MS) follows from the HPLC separation to then identify and quantify these compounds/molecules. The mass spectrometry technique measures the mass of molecules, to do this molecule needs to be converted into a gas-phase ion. The eluent from the HPLC process enters the system where ionization occurs and converts the sample into the charged gas-phase ions. In this research Electrospray ionization (ESI) was used as the ionization technique, whereby a high voltage is applied to the eluent resulting in aerosol of charged ions. These ions are then separated, detected, and measured according to their respective mass-to-charge ratios (m/z), and according to the abundance of ions. In this way MS is both a qualitative and quantitative technique (Balogh, 2014).

Tandem quadrupole mass spectrometers were used in this research, and the technique is then referred to as MS/MS. In a MS system there is only a single quadrupole, whereas with the MS/MS system an additional quadrupole is added. In such an instrument the initial/parent ion will be further fragmented/broken down in the

second quadrupole creating a fragmentation pattern unique to that molecule (Balogh, 2014). The molecules can then be identified on comparison to known fragmentation patterns.

Internal standards were used in the sample testing process to aid in assessment of the results. The internal standard comprises of deuterated compounds that are the same analyte/drug group that the method is testing for. The deuterated internal standard has analytes of known concentration and thus is used to compare to the test sample results in terms of retention times, chromatographic peaks, and fragmentation pattern.

2.5.3.2 Materials

All materials and solutions were provided for by Randox Testing Services (Manchester, England). ISOLUTE® SLE+ were purchased from Biotage (Uppsala, Sweden). Methanol and HPLC grade water were purchased from Sigma-Aldrich (Texas, USA). Formic acid and acetonitrile were purchased from Honeywell Burdick and Jackson (New Jersey, USA). The LC-MS/MS column used was the ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 x 50 mm), from Waters (Milford, USA). Deuterated internal standard (containing each analyte that is tested for in the method) and elution mix (dichloromethane/ heptane/ isopropanol (90/10/4, v/v/v)) were prepared by Randox Testing Services (Manchester, England). The researcher was not granted permission to prepare the above solutions as these were working solutions used in Randox Testing Services day to day testing procedures, which included forensic testing services for police forces across the United Kingdom. The researcher was not granted access to the methodologies used to prepare the deuterated internal standards, quality controls, and calibrator stock solutions.

2.5.3.3 Quality control (QC) and calibration preparation

Randox Testing Services required the use of three quality controls for urine samples for the DOA method: QC 2.5 ng/mL; 10 ng/mL and 50 ng/mL, and four quality controls for the blood samples of the DOA method: 2.5 ng/ mL, 5 ng/ mL, 25 ng/mL, and 50 ng/

mL. Control stock solutions were provided and pre prepared by Randox Testing Services according to in house methodology. These controls contained the analytes tested for within the drugs of abuse panel I at differing concentration levels and cover the cut-off range. Two sets of full quality controls were run with each sample set, with one set of controls added before, and one set of controls added after each set. This was to ensure that no changes to the instrument occurred during the sample run.

Eight sets of calibrators (CAL) were used for both blood and urine samples (CAL 0.5 ng/mL; 1.0 ng/mL; 2.5 ng/mL; 5 ng/mL; 10 ng/mL; 25 ng/mL; 50 ng/mL and 100 ng/mL). Calibrator stock solutions were provided and pre prepared by Randox Testing Services according to in house methodology.

For blood preparation, working calibrators and QC's were prepared as follows: 40 µL of CAL/QC's and 400 µL of blank blood sample were pipetted into Eppendorf tubes and vortexed to ensure thorough mixing of solutions. 200 µL of internal standard (100 ng/mL) and 800 µL of methanol were added and solution was vortexed. The tubes were then centrifuged at 14000 rpm for 10 minutes. The supernatant was then diluted with 1.5mL of HPLC grade water.

For urine preparation, working calibrators and QC's were prepared as follows: 50 µL of CAL/QC's and 500 µL of blank urine sample were added to glass test tubes and vortexed to ensure thorough mixing of solutions. 200 µL of internal standard (100 ng/mL) and 1.5 mL of HPLC grade water was added. The solution was vortexed thoroughly.

2.5.3.4 Sample preparation: Supported liquid extraction (SLE) (Blood and urine)

Supported liquid extraction (SLE) is an organic sample preparation technique used to separate out compounds. A sample (containing both matrix components and analytes) were loaded onto the ISOLUTE SLE+ cartridge and are absorbed and immobilized on an inert support material. The analytes are then eluted from the cartridges with use of an immiscible organic solvent. This process allowed for the effective removal of matrix components such as proteins and phospholipids as these components are not soluble in the solvent and therefore are bound to the support material (Biotage, 2014).

Blood and urine samples were prepared according to the routine method followed at Randox Testing Services (Manchester, England) for extraction of drugs of abuse.

Blood samples: A total of 200 µL of deuterated internal standard; 200 µL of blood sample; 800 uL of methanol were added and solution was vortexed. The tubes were then centrifuged at 24104 RCF for 10 minutes. The supernatant was then diluted with 1.5mL of HPLC grade water.

Urine samples: A total of 200 µL of deuterated internal standard; 500 µL of urine sample and 1.5 mL of HPLC grade water were added to a glass test tube and vortexed thoroughly.

SLE extraction procedure was followed as per the routine method followed at Randox Testing Services (Manchester, England): Calibrators, quality controls and samples were loaded onto the preconditioned SLE cartridges (ISOLUTE SLE+ Cartridge, Biotage®) on a manifold. A vacuum was applied to start the process. Once all the liquid had passed through the cartridge, the cartridges were then left to stand for 10 minutes. An elution mix of dichloromethane: heptane: isopropanol (90:10:4, v/v/v) was added. The upper aqueous layer was removed as waste, and the eluent was evaporated to dryness at 40 ^{o}C under nitrogen gas. The eluent was reconstituted with 200 µL of HPLC grade water and vortexed thoroughly. Prepared samples were then transferred to HPLC vials and capped.

2.5.3.5 Analysis of samples

The Randox LC-MS/MS method tests for following 38 drug compounds: cocaine, benzoylecgonine, cocaethylene, AEME, amphetamine, methamphetamine, MDMA, MDEA, MDA, 6-MAM, morphine, codeine, dihydrocodeine, hydromorphone, hydrocodone, oxycodone, THC, THC-COOH, methadone, EDDP, diazepam, nordiazepam, 7-aminofluitrazepam, flunitrazepam, nitrazepam, 7-aminonitrazepam, temazepam, alprazolam, lorazepam, clonazepam, oxazepam, mephedrone, ketamine, norketamine, buprenorphine, PCP, LSD, and zolpidem

The test parameters used for the LC-MS/MS test methods for both blood and urine samples were identical to the routinely used method at Randox Testing Services

(Manchester, England). This method had a LLOD and LLOQ of 0.5 ng/mL for all compounds in the test method. The upper limit of quantification (ULOQ) was 100 ng/mL. That is any sample having a result over 100 ng/mL would need to be diluted and the sample would need to be rerun.

Blood samples, calibrators and control were analysed on the Waters (Milford, MA) UPLC system coupled to a Xevo TQ-S mass spectrometer for detection.

Urine samples, calibrators and control were analysed on the Waters (Milford, MA) Ultima HPLC coupled to a MS Triple Quadrople.

Chromatographic separation (HPLC) of analytes occurred on an ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 x 50 mm) (Waters Corp., Milford, MA). A gradient elution was performed using an aqueous Solvent A consisting of 0.1% formic acid in HPLC grade water, and organic Solvent B consisting of 0.1% formic acid in acetonitrile. The gradient was as follows: Starting at 100% A and 0% B and moving up to 70% B in seven minutes, followed by an increase up to 100% B by minute nine. 100% B was kept up to minute 11 to flush the column, from minute 11.01 up until minute 12 re-equilibration was established with 0% B and 100% A. This method has a total run time of 12 minutes, with an injection volume of 10 µL, a flow rate of 0.2 mL/min.

2.5.3.6 Data assessment and interpretation of LC-MS results

Waters MassLynx™ Mass Spectrometry Software with TargetLynx™ Application Manger (Waters Corp., Milford, MA) was used to automatically process and analyse the data obtained from the MS. The quantified sample data was then exported to an excel spreadsheet showing the name of compound, retention time, area of peak, area of internal standard, response, concentration (ng/mL), percentage deviation, coefficient of determination (R^2), isotope ratio and dilution factor. The deuterated internal standard was used to compare the peaks and retention times of the analytes of interest with that of blood and urine sample results. All data generated was assessed by the researcher, and the results were then reviewed and verified by the staff at Randox Testing Services (Manchester, England).

The quality controls needed to meet the following criteria to be accepted for the sample run: three out of four QCs needed to have passed within 20% of the target concentrations for the blood controls. Two out of three QCs needed to have passed within 20% of the target concentrations for the urine controls. For the calibration curve to be accepted a minimum of 4 points were required on the curve, with an R^2 of above 0.99.

2.6 Data analysis: Phase I and Phase II assessment of results

The results from the immunoassay and mass spectrometry analysis were used to classify the results into three subsets described below, as illustrated in Figure 2.4.

- True positive: The sample tested as positive for one or more of the targeted analytes of interest on **both test methods** (immunoassay positive and mass spectrometry positive). Results were reflected per victim and not specific to the sample specimen (i.e., results reflected as true positive in blood or urine, or in both blood and urine).
- True negative: The sample tested as negative for one or more of the targeted analytes of interest on **both test methods** (immunoassay positive and mass spectrometry positive). Results were reflected per victim and not specific to the sample specimen (i.e., results reflected as negative positive in blood or urine, or in both blood and urine).
- Discordant results: The sample tested as positive for one or more of the targeted analytes of interest on **only one test method** (immunoassay positive or mass spectrometry positive). Results were reflected per victim and not specific to the sample specimen (i.e., results reflected as true negative in blood or urine, or in both blood and urine)

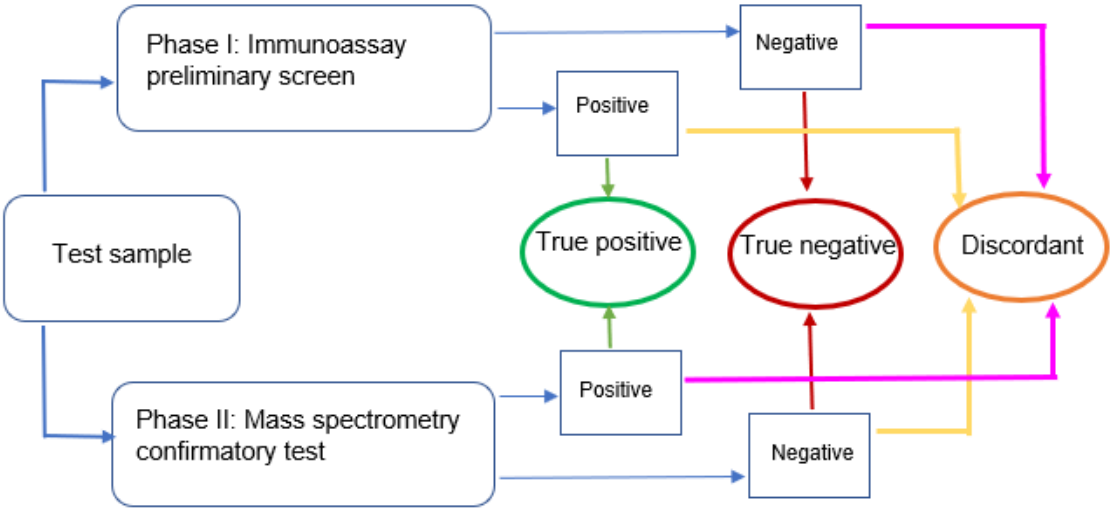


Figure 2.4 Flow diagram illustrating methodology of result outcomes

Only the targeted analytes of interest were assessed within the above-mentioned result subsets, that being opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites.

2.7 Ethical approval

Ethics clearance was approved unconditionally by the Human Research Ethics Committee (Medical), of the University of the Witwatersrand. Ethics Certificate number: M120504 (Appendix IV). Permission was also granted to the researcher by the Head Clinical Department (Chief Specialist) of the Forensic Pathology Service-Southern Gauteng, to conduct the study, and have access to the case files after the postmortem had been completed (Appendix V).

Chapter 3: Results

3.1 Introduction

This chapter will consist of the presentation of the results. This will include the sample demographics, and the results of the immunoassay and confirmatory LC-MS testing techniques in identifying the presence of the compounds and metabolites as listed in the objectives. A total of 103 suicide cases were analysed between September 2013 and October 2014 at the Johannesburg Forensic Pathology Service Medico-legal Mortuary. This was the total number of suspected suicide cases falling within the inclusion and exclusion criteria initially outlined. From the 103 decedents assessed, of these a blood specimen was obtained from all victims (n=103) however in only 51 of these victims was an additional urine specimen obtained. Therefore 51 decedents had 1 blood and 1 urine sample assessed. In the remaining 52 cases, urine samples were not available from the victim, as the bladder was either empty at time of death, or due to trauma to the bladder as a result of the method of suicide employed.

3.2 Demographics of the sample

The basic demographics of the sample suicide population were recorded as age, racial group, sex, and the method of suicide. The demographic data obtained is summarized in Table 3.1 below. The mean postmortem interval (PMI), that being the time between the date of death and the date of autopsy, was noted to be 2 days (SD= 1 day). Of the 103 suicide cases assessed only 12 (11%) cases were selected by the forensic pathologist for official toxicology testing at the NDOH FCL JHB. The 12 samples selected for formal toxicology testing comprised 83% (n=10/12) of overdose/poisoning suicide cases, and 17 % (n=2/12) hanging suicide cases.

Table 3.1 Summarised demographic information from sampled suspected suicide fatalities cases (n=103)

Category	Sub-category	Total sample (n (%))
Age Group (years)	18 - 28	31 (30%)
	29 - 38	33 (32%)
	39 - 48	13 (13%)
	49 - 58	13 (13%)
	59 - 68	9 (9%)
	69 - 78	4 (4%)
Racial group	Black	63 (61%)
	Coloured	1 (1%)
	Indian/Asian	7 (7%)
	White	32 (31%)
Sex	Male	84 (82%)
	Female	19 (18%)
Method of suicide	Gassing (CO)	4 (4%)
	Fall from Height	15 (15%)
	Firearm/gunshot	13 (13%)
	Hanging	46 (44%)
	Overdose/Poisoning	24 (23%)
	Stabbing	1 (1%)

3.2.1 Age in the overall suicide population

The largest number of cases observed fell within the 18 – 28 year and 29 – 38 year age ranges, accounting for 30% and 32% of population respectively. The least prevalent age range was noted in the 69 – 78 year age range accounting for 4% of the population sample.

Demographic analysis showed the overall mean age of the suicide population to be 38 ± 14 years. The mean ages and standard deviation (SD) of the suicide population were also assessed according to the method of suicide, as noted in Figure 3.1.

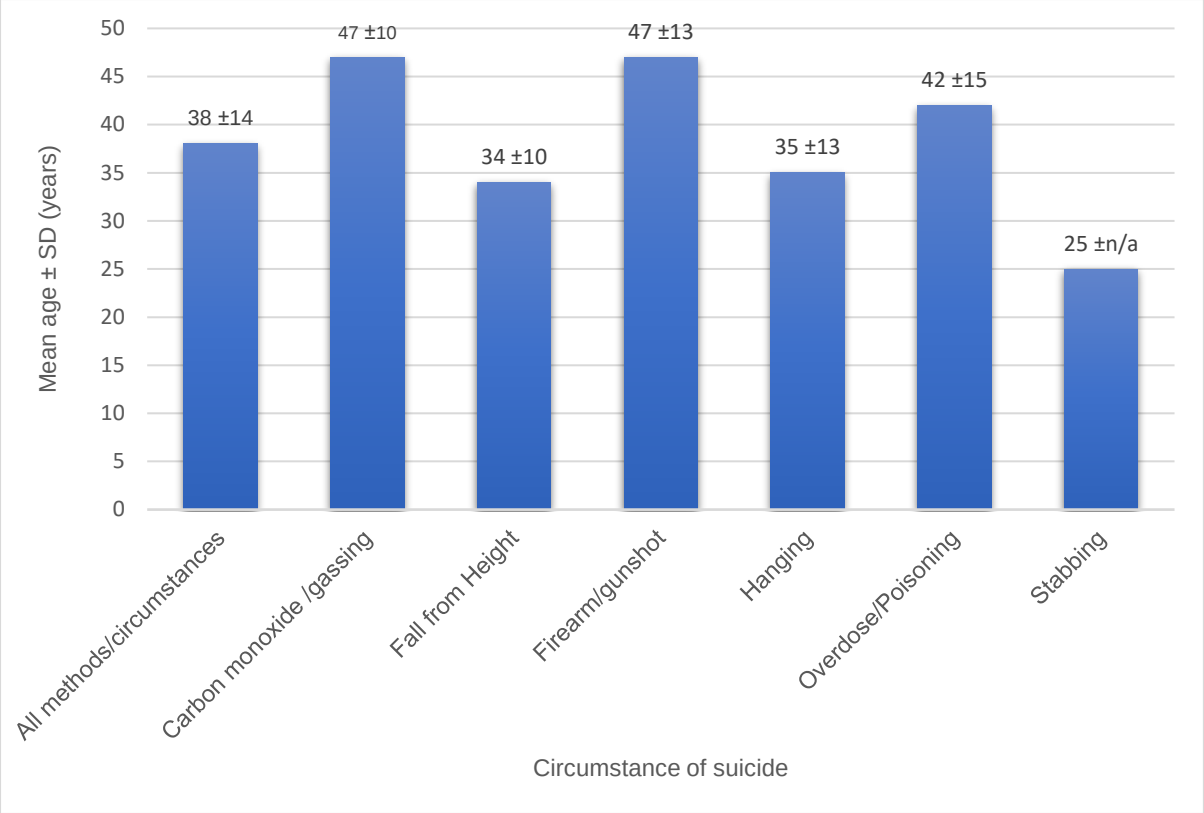


Figure 3.1 Mean age of the suicide population categorised by method of suicide (n=103)

3.2.2 Racial group in the overall suicide population

Of the 103 suicide cases collected for this research, most cases were noted in Black individuals accounting for 61% (n=63/103) of collected samples. The least represented group were Coloured individuals accounting for only 1% (n=1/103) of the collected samples. The racial group demographics are depicted in the Figure 3.2.

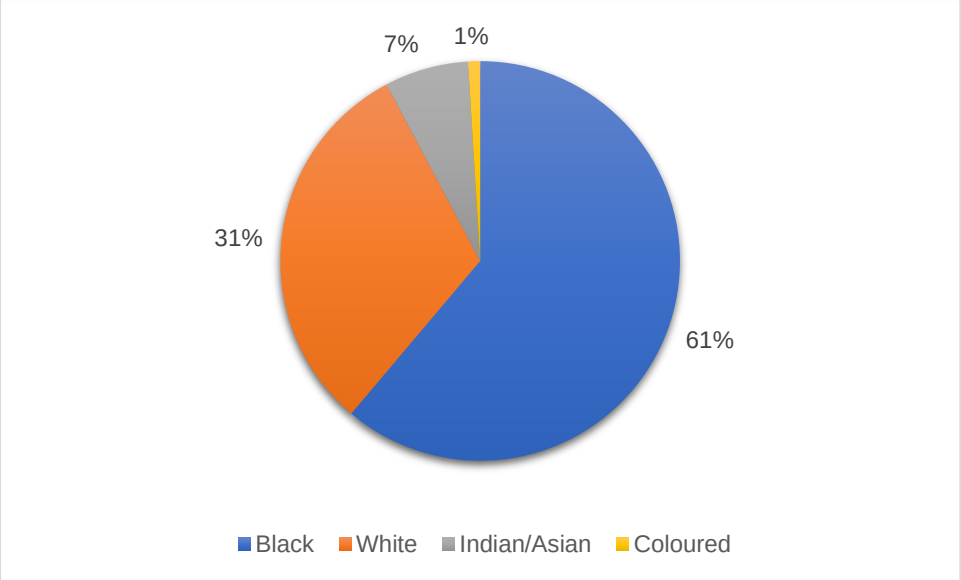


Figure 3.2 Racial group in overall suicide population (n=103)

3.2.3 Sex in the overall suicide population

Of the 103 suicide cases collected for this research, a majority of the cases comprised of male individuals with 84 cases (82%) and 19 female cases collected (18%).

3.2.4 Demographic profile within each method of suicide

Of the 103 suicide cases assessed suicide by hanging was found to be the most prevalent method of suicide, comprising almost half of the sample set (n=46/103, 44%). The least common method noted was stabbing related suicides (n=1/103, 1%). The age range, sex, and racial group was noted for each chosen method of suicide, these are further described in Table 3.2.

Table 3.2 Victim demographics within each method of suicide (n=103)

Victim Demographics		Method of suicide						
		Hanging (n=46 (44%))	Overdose/poisoning (n=24 (23%))	Firearm (n=13 (13%))	Fall from height (n=15 (15%))	Gassing (CO) (n=4 (4%))	Stabbing (n=1 (1%))	Total (n=103)
		n (%) of the total number of cases						
Mean age ± SD (years)		35 ± 13	42 ± 15	47 ± 13	34 ± 10	47 ± 10	25 ± n/a	38 ± 14
Age group (years)	18 - 28	17 (37%)	6 (25%)	2 (15%)	5 (33%)	0	1 (100%)	31 (30%)
	29 - 38	17 (37%)	6 (25%)	4 (31%)	5 (33%)	1 (25%)	0	33 (32%)
	39 - 48	3 (7%)	4 (17%)	1 (8%)	4 (27%)	1 (25%)	0	13 (13%)
	49 - 58	5 (11%)	4 (17%)	2 (15%)	1 (7%)	2 (50%)	0	13 (13%)
	59 - 68	4 (9%)	3 (12%)	1 (8%)	0	0	0	9 (9%)
	69 - 78	0	1 (4%)	3 (23%)	0	0	0	4 (4%)
Sex	Male	37 (80%)	17 (71%)	12 (92%)	14 (93%)	3 (75%)	1 (100%)	84 (82%)
	Female	9 (20%)	7 (29%)	1 (8%)	1 (7%)	1 (25%)	0	19 (18%)
Racial group	Black	30 (65%)	13 (54%)	6 (46%)	12 (80%)	1 (25%)	1 (100%)	63 (61%)
	White	11 (24%)	10 (42%)	6 (46%)	3 (20%)	2 (50%)	0	32 (31%)
	Coloured	1 (2%)	0	0	0	0	0	1 (1%)
	Indian/Asian	4 (9%)	1 (4%)	1 (8%)	0	1 (25%)	0	7 (7%)

3.2.4.1 Gassing (Carbon monoxide) related suicides

Carbon monoxide/gassing related suicides accounted for 4% of the total suicide cases assessed (n=4/103). The victims were on average 47 ± 10 years of age. Half of the cases occurred in the 49 – 58 year age group (n=2/4, 50%). A total of 75% of the cases were male (n=3/4). Gassing (CO) related suicides was noted to be more common in White individuals, comprising 50% of the sample collected (n=2/4).

3.2.4.2 Fall from Height related suicides

The cases comprising the fall from height related suicides accounted for 15% of the total suicide population assessed (n=15/103). The victims were on average 34 ± 10 years of age. Males accounted for 93 % of the cases (n=14/15). Black individuals (n=12/15, 80%) were the most prevalent within the sample subset.

3.2.4.3 Firearm/gunshot related suicides

Firearm/gunshot related suicides accounted for 13% (n=13/103) of the overall suicide cases collected. The victims were noted a predominantly male (n=12/13, 92%). Black and White individuals accounted for the same number of victims within this subset (n=6/13, 46%). Victims had an average age of 47 ± 17 years of age.

3.2.4.4 Hanging related suicides

Hanging was noted as the most prevalent method of (n=46/103, 44%) suicide choice. Hanging victims were on average 35 ± 13 years old of age. The victims were noted as mostly male (n=37/46, 80%) and Black (n=30/46, 65%).

3.2.4.5 Overdose/Poisoning related suicides

Overdose/poisoning related suicides was the second most common method of suicide noted within the victim group (n=24/103, 23%). The average victim age was noted as 42 ± 15 years of age. The victims were noted a predominantly male (n=17/24, 71%).

Black and White individuals comprised most of the victim profile (n=13/24, 54%, and n=10/24, 42%).

3.3 Drug Result outcomes: The presence and demographic profiles of the targeted analytes (opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites) within the sample

The results from the Randox BAT immunoassay and LC-MS/MS analysis were used to classify the result outcomes of the specific analytes of interest into three subsets, that is true positive results (positive on both screen and confirmatory test methods), true negative results (negative on both screen and confirmatory test methods) and discordant results (positive on one of the test methods, that being either the screen or the confirmatory test).

Of the 103 suicide cases assessed the results showed that 15% (n=15/103) of these cases tested as true positive, 40% (n=42/103) of cases had tested true negative and 45% (n=46/103) of cases had tested with discordant results. The demographic profiles of each victim were assessed according to the result outcome, as summarized in Table 3.3.

Table 3.3 Victim demographics within the true positive, true negative and discordant results for the analytes of interest (opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites) (n=103)

Victim Demographics		*True Positive cases (n=15 (15%))	*True Negative cases (n=42 (40%))	*/**Discordant cases (n=46 (45%))	Total (n=103)
		n (%) of the total number of cases			
Mean age ± SD (years)		40 ± 14	44 ± 16	33 ± 12	38 ± 14
Age group (years)	18 - 28	3 (20%)	14 (33%)	14 (30%)	31 (30%)
	29 - 38	6 (40%)	13 (31%)	15 (33%)	33 (32%)
	39 - 48	2 (13%)	6 (14%)	5 (11%)	13 (13%)
	49 - 58	3 (20%)	2 (4%)	8 (17%)	13 (13%)
	59 - 68	0	6 (14%)	3 (7%)	9 (9%)
	69 - 78	1 (7%)	2 (4%)	1 (2%)	4 (4%)
Sex	Male	12 (80%)	33 (79%)	39 (85%)	84 (82%)
	Female	3 (20%)	9 (21%)	7 (15%)	19 (18%)
Racial group	Black	4 (27%)	29 (69%)	30 (65%)	63 (61%)
	White	8 (53%)	11 (26%)	13 (28%)	32 (31%)
	Coloured	0	0	1 (2%)	1 (1%)
	Indian/Asian	3 (20%)	2 (5%)	2 (4%)	7 (7%)
Method of death	Hanging	3 (20%)	21 (50%)	22 (48%)	46 (44%)
	Overdose/Poisoning	6 (40%)	12 (29%)	6 (13%)	24 (23%)
	Firearm	4 (27%)	3 (7%)	6 (13%)	13 (13%)
	Fall from height	0	5 (12%)	10 (22%)	15 (15%)
	Gassing (CO)	2 (13%)	1 (2%)	1 (2%)	4 (4%)
	Stabbing	0	0	1 (2%)	1 (1%)

**Results shown only relate to the analytes of interest in this study (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites).*

***Discordant results: those results that were either positive on the immunoassay and negative on the mass spectrometry, or positive on the mass spectrometry and negative on the immunoassay.*

3.3.1 Assessment of the true positive results

Of the 15 true positive cases, 13 cases were true positive for opiates and metabolites, 2 cases were true positive for cocaine and metabolites, and 3 cases were true positive for amphetamine type stimulants and metabolites. There were 3 (20%) hanging related suicides within the true positive cases, all of which were noted as female individuals (research victim nos.1,8 and 15). The four firearm related suicides were noted to only test true positive for the opiates and metabolites drug group.

Of the true positive cases only two cases had notes in the SAP 180 form indicating potential opiate usage. Both cases were listed as overdose/poisoning related suicides and both had tested as true positive for opiates and metabolites. The notes accompanying the case for research victim no.7 stated that witnesses suggested that the decedent took a dose of heroin. Additionally, research victim no.7 was the only true positive case (1/15, 7%) that was noted to yield a true positive result for specifically 6-monoacetylmorphine, thus indicative of heroin use. The notes accompanying the case for research victim no.9 stated that the decedent had an overdose by means of Pethidine HCL (a synthetic opioid pain medication) injection.

Within these 15 cases, 13/15 (87%) were true positive for single drug use, and 2/15 (13%) were true positive for polydrug usage (these results were only within the targeted analytes of interest). The polydrug usage victims were noted to be both White males, aged 36 years, and each recorded as an overdose/poisoning related suicide. Case 1 (research victim no.13) tested true positive for: opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites. Case 2 (research victim no.14) tested true positive for: opiates and metabolites and amphetamine type stimulants and metabolites.

The analytes of interest identified within each true positive case, as well as the victim profile are listed in Table 3.4.

Table 3.4 Full victim profile within the true positive results, including analytes of interest identified, biological specimen indicating result, autopsy findings and official state toxicological testing status (n=15)

					Analytes of interest identified within the true positive cases					
Research no.	Method of suicide	Age (years)	*Race	**Sex	Opiates and metabolites	Cocaine and metabolites	Amphetamine type stimulants and metabolites	Biological specimen indicating result (Blood/ Urine)	History of drug use noted on autopsy report	Specimen sent for official toxicological testing at NDOH FCL JHB
1	Hanging	18	B	F	Morphine, Codeine			Both		Yes
2	Gassing	33	I/A	M	Codeine, Hydrocodone			Blood		
3	Overdose/poisoning	52	W	M	Morphine, Codeine			Both		
4	Firearm related	73	W	M	Morphine, Codeine			Both		
5	Firearm related	58	W	M	Dihydrocodeine, Oxycodone			Urine		
6	Firearm related	35	B	M	Codeine			Blood		
7	Overdose/poisoning	54	W	M	6MAM, Morphine, Codeine			Blood	Yes: heroin	Yes
8	Hanging	28	B	F	Morphine, Codeine, Dihydrocodeine			Blood		
9	Overdose/poisoning	42	A	M	Codeine, Dihydrocodeine, Hydrocodone			Blood	Yes: pethidine HCl	Yes
10	Overdose/poisoning	35	B	M	Codeine			Urine		
11	Firearm related	24	I/A	M	Morphine, Codeine			Urine		
12	Gassing	45	W	M		Cocaine, Benzoylecgonine, Cocaethylene		Blood		
13	Overdose/poisoning	36	W	M	Morphine, Codeine, Hydrocodone, Hydromorphone, Hydrocodone	Cocaine, Benzoylecgonine, Cocaethylene	Methamphetamine, Amphetamine, MDMA	Both: Opiates and metabolites, and Cocaine and metabolites Blood: Amphetamine type stimulants and metabolites		
14	Overdose/poisoning	36	W	M	Morphine, Codeine, Hydrocodone		Methamphetamine, Amphetamine	Blood		
15	Hanging	38	W	F			Methamphetamine	Blood		

** B= Black, W=White, I/A=Indian/Asian*
*** M=Male, F=Female*
Shaded blocks indicate negative/no findings per heading
NDOH FCL JHB: National department of health Forensic Chemistry Laboratory Johannesburg

3.3.1.2 Difference in test sample specimen: blood and urine specimens within the true positive results

Of the 103 cases, only 51 urine sample specimens were collected i.e., 51 cases had both blood and urine specimens collected and 52 cases only blood sample specimens collected. The blood and urine results were assessed to determine those samples that had tested as positive in blood only specimens, in urine only specimens and in both blood and urine specimens.

Within the true positive result set 20% (n=3/15) cases had tested true positive within urine only specimens, these three cases were found within the opiates and metabolites drug group. True positive blood only cases accounted for 53% (n= 8/15) cases, and 27% (n=4/15) cases had tested true positive within both blood and urine specimens. The true positive amphetamine type stimulants results were found within only the blood sample specimens. Figure 3.3 depicts the blood and urine results per target analyte group within the true positive sample set.

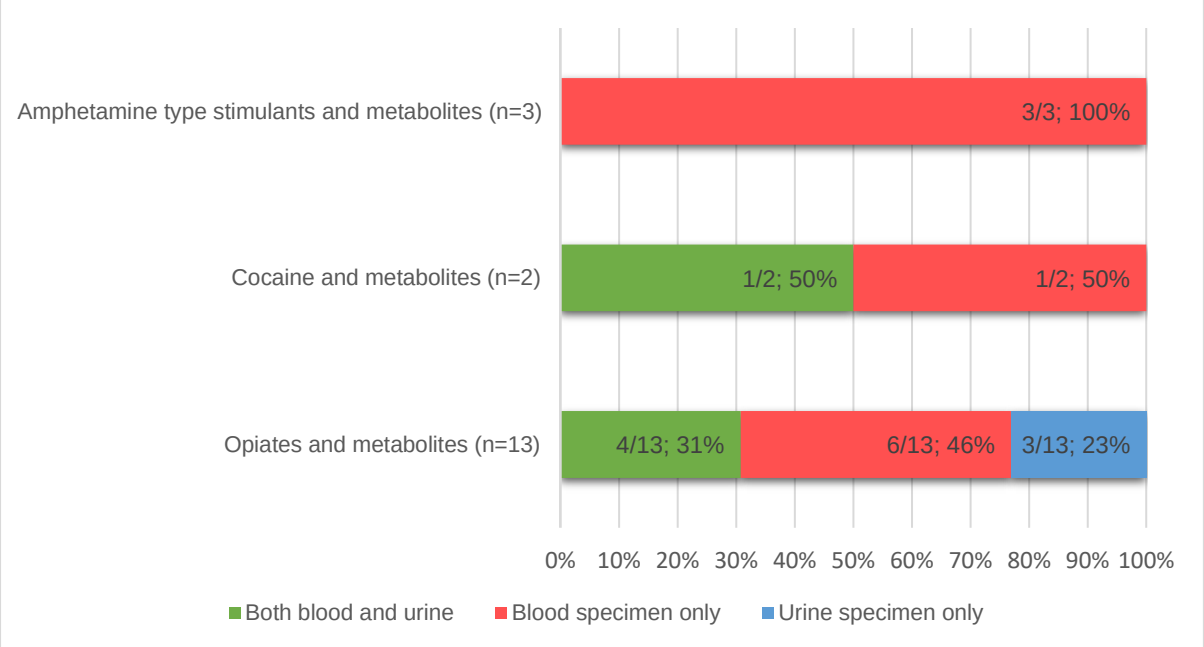


Figure 3.3 Analyte detection within the blood and urine sample specimens (True positive results) (n=15)

3.3.2 Assessment of the true negative results

The true negative cases, assessing only the targeted analytes of interest, accounted for 42/103 (40%) of cases (table 3.4). The victims were on average 44 years of age, predominantly Black (29/42, 69%) and male (33/42, 79%). Hanging related suicides comprised 50% (n=21/42) of the cases. Overdose/poisoning related suicides were noted in the true negative victim profiles (12/42, 29%), however as noted the results in this study only accounted for three sets of drug groups (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites). Therefore, a true negative result in terms of this study outcome did not indicate a definitive negative result for other drug groups.

3.3.3 Assessment of the discordant results

The discordant results are those samples that tested as either positive on the immunoassay screen and negative on the mass spectrometer; or tested as negative on the immunoassay screen and positive on the mass spectrometer. A total of 53 samples tested as discordant for one specific targeted analyte of interest in this study (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites). On a case level, 46 victims had a discordant result outcome (45%) (Table 3.3) for one or more target analyte of interest. The discordant cases consisted of predominately Black (30/46, 65%), male (39/46, 85%) individuals, with hanging related suicides accounting for 48% (n=22/46) of the cases. Overdose/poisoning related suicides were seen in 13% (6/46) of the discordant result cases.

The discordant results were assessed according to the different test method outcomes: results of the Randox BAT immunoassay screen and the results of the mass spectrometry (LC-MS/MS). Within the Randox DOA 1 array a total of 7% (n=7/103) of cases tested as positive only for one or more of the targeted range of analytes (opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants) on the immunoassay screen. With the LC-MS/MS test method 38% (n=39/103) of cases tested as positive only for one or more of the targeted range of analytes (opiates and metabolites, cocaine and metabolites, and amphetamine type

stimulants) on the mass spectrometer. The discordant results were therefore predominantly comprised of results from the LC-MS/MS test method. Assessing the target analyte groups separately (i.e., per analyte of interest not per case), there were in total 53/103 samples (51%) that tested as a discordant result for only one targeted analyte of interest. Table 3.5 summarizes the discordant results per drug/analyte group within each test method.

Table 3.5 Summary of the discordant results within each test method per analytes of interest (opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites) (n=53)

Targeted analyte of interest	*LC-MS/MS	**Randox BAT immunoassay	Total (n=53)
	n (%) of the total number of cases		
Opiates and metabolites	22 (42%)	2 (4%)	24 (45%)
Cocaine and metabolites	5 (9%)	1 (2%)	6 (11%)
Amphetamine type stimulants and metabolites	18 (34%)	5 (9%)	23 (43%)

** LLOD and LLOQ for test method: 0.5 ng/mL for each drug group*

*** Cut off levels applied to results for test method: Opiates: 25 ng/mL; Benzoylecgonine: 50 ng/mL; Amphetamine: 25 ng/mL; 3,4-Methylenedioxymethamphetamine MDMA: 60 ng/mL; Methamphetamine: 50 ng/mL (Table 2.1)*

3.3.3.1 Difference in test sample specimen: blood and urine specimens within the discordant results

Within the discordant result set, almost half the sample (29/53, 49%) had tested as a discordant result in urine only specimens. Urine only discordant results were found within the three targeted analyte groups of interest (opiates and metabolites, cocaine and metabolites, and amphetamine type stimulants and metabolites). Blood specimen only discordant results accounted for 42% (n=22/53) of the samples, and 9% (n=5/53) of the discordant samples were found both blood and urine specimens within the same case. Figure 3.4 depicts the blood and urine results per target analyte group within the discordant result sample set.

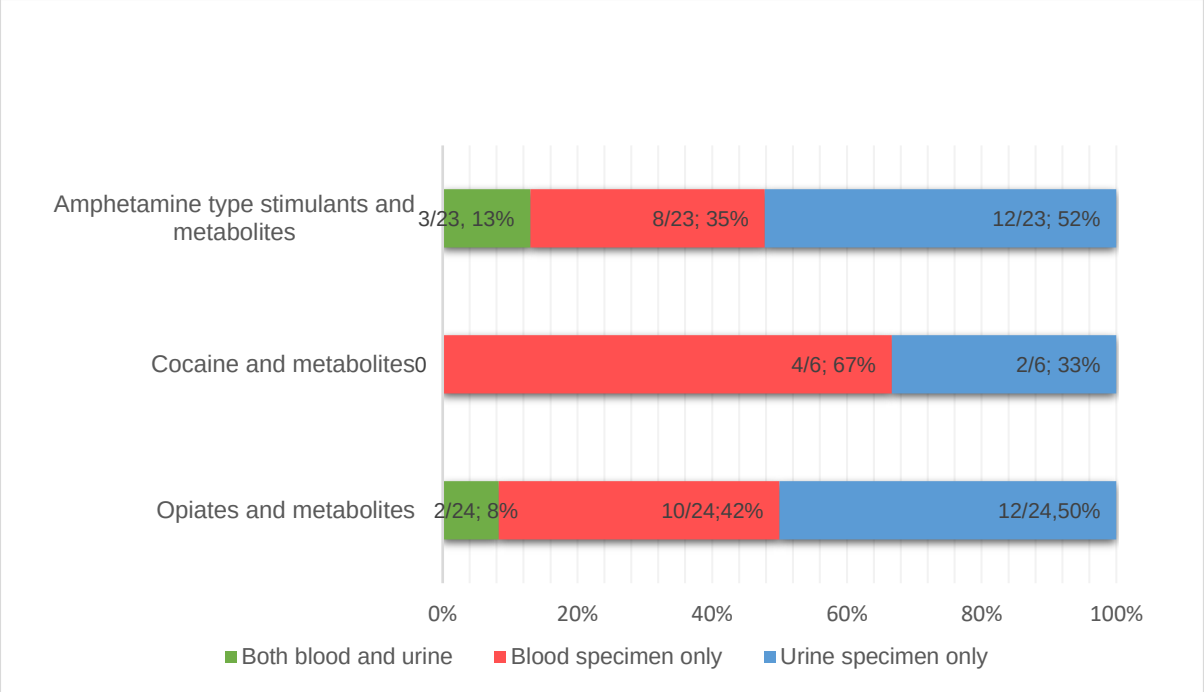


Figure 3.4 Analyte detection within the blood and urine sample specimens (Discordant results) (n=53)

3.4 Other drug group results (remaining analytes tested for on the LC-MS/MS test)

The LC-MS/MS test method tested for a total of 38 analytes (as discussed in the material and methods chapter). Positive results on the LC-MS/MS were noted for those analytes which did not form part of the targeted groups. There were 54 other analytes detected in the cases, these were discovered in both specimen types (blood and urine). The results are summarized in Table 3.6. It is noted that most of the additional positive analytes belonged to the benzodiazepine group of drugs.

Table 3.6 Additional LC-MS/MS positive results within the sample group (n=103)

Analyte	LC-MS positive result (n (%))
Zolpidem	5 (5%)
THC	3 (3%)
THC-COOH	7 (7%)
Alprazolam	9 (9%)
Clonazepam	5 (5%)
Norketamine	1 (1%)
Ketamine	1 (1%)
Mephedrone	1 (1%)
LSD	4 (4%)
7-Aminonitrazepam	2 (2%)
Flunitrazepam	2 (2%)
Methadone	1 (1%)
Oxazepam	3 (3%)
Diazepam	2 (2%)
Nordiazepam	2 (2%)
Temazepam	2 (2%)
Buprenorphine	1 (1%)
AEME	1 (1%)
Lorazepam	2 (1%)

A full assessment of the data was not carried out, i.e., true positive result and demographic profile, as these analytes did not form part of the study objectives.

Chapter 4: Discussion and Conclusion

4.1 Summary of results

The results of this study included the whole suspected suicide population managed at the JHB mortuary over the study time frame, as well as the illicit drug usage within the sample. The results noted that of the overall study population profile of the suspected suicide sample group, 82% (n=84/103) were male, 61% (n=63 /103) were Black and 32% (n=33/103) of decedents were between the ages of 29 – 38 years.

The 29 – 38 year age range was the most common age noted in decedents of the study, comprising 32% of the sample (n=33/103). Within the 29 – 38 year age range, 52% (n=17/33) comprised of hangings, 18% (n=6/33) comprising overdose/poisoning related suicides, 15% (n=5/33) comprising fall from height related suicides, and 12% (n=4/33) comprising firearm related suicides. Carbon monoxide related suicides fell into a higher age range of 49 – 58 years and was noted in 50% (2/4) of the suicide population sample.

Black individuals were noted as the prevalent racial group in this study, comprising 61% (n=63/103) of the overall suspected suicide sample. Black individuals comprised the highest proportion of hanging cases (n=30/46, 65%), overdose/poisoning related suicides (n=13/24, 54%) and fall from height related suicides (n=12/15, 80%). White individuals were found mostly in carbon monoxide related suicides comprising 50% (n=2/4) of the sample. There was an even representation of Black and White individuals within the firearm related suicides, each comprising 46% n=6/13).

Males were noted to be the prevalent sex, comprising 82% (n=84/103) of the sample. Males accounted for 80% (n=37/46) of hanging cases, 71% (n=17/24) of overdose/poisoning related suicides, 93% (n=14/15) of fall from height related suicides, 92% (n=12/13) of firearm related suicides and 75% (n=3/4) of carbon monoxide related suicides. There was only one (n=1/103, 1%) stabbing related suicide case noted, and this comprised of a Black male individual.

The results from this study have shown that there are suicide decedents autopsied at the Johannesburg FPS MLL who have engaged in illicit drug use prior to committing suicide. This study consisted of 103 suicide cases, of which 15 cases (15%) tested as

a true positive result for one or more of the illicit drug groups assessed (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites). It was noted that the positive cases were predominantly White (n=8/15, 53%) and male (n=12/15, 80%) individuals.

The study found that the most prevalent illicit drug group within the sample set were the opiates and metabolites group, comprising 13% (n=13/103) of the positive results. Cocaine and metabolites tested true positive in 2% (n=2/103) of cases, and amphetamine type stimulants tested true positive in 3% (n=3/103) of cases.

The results highlighted that single drug usage was more prevalent within the sample set, with 87% (n=13/15) of the positive result testing positive for only one illicit drug group. Two cases (n=2/15; 13%) had tested true positive for polydrug usage (more than one illicit drug group). It was noted that in both cases the decedents were White, male individuals, and the chosen method of suicide in each case was recorded as an overdose/poisoning.

The most common method of suicide in the true positive illicit drug sample was overdose/poisoning related suicides (n=6/15, 40%), all cases were male individuals. This was followed by firearm related suicides (n=4/15, 27%), hanging related suicide (n=3/15, 20%) and gassing related suicides (n=2/15, 13%). It was noted that the true positive hanging related suicides comprised of only female individuals (n=3/3, 100%).

The results highlighted that 44% of the samples had tested as a discordant result for one or more of the illicit drug groups assessed (opiates and metabolites, cocaine and metabolites and amphetamine type stimulants and metabolites), that is a result that tested as positive on only one of the test methods, either the Randox BAT preliminary screen, or with the LC-MS/MS test (i.e., neither a true positive nor true negative test result).

4.2 Suicide in the sample

4.2.1 Demographics of the suicide population

The results from this study identified Black males as the highest proportion of the suicide sample group, this coincides with the findings of South African suicide studies by Burrow and Laflamme (2006) and Kootbodien *et al*. (2020) with Black male individuals comprising the largest percentage of suicide cases. Black individuals comprise the majority of the South African population, the national Census of 2011 (www.statssa.gov.za) found that Black individuals comprised 79.2 % of the overall South African population, and 77.4% of the population within Gauteng. The Census results thus may provide an explanation as to why Black individuals are predominant in the suicide sample group. Data from Burrow and Laflamme (2006 and 2008) indicated that White individuals (both male and female) have a higher suicide rate than any other population group within South Africa (Burrow and Laflamme, 2006 and Burrow and Laflamme, 2008). Engelbrecht *et al*. (2017) performed a retrospective review of suicide cases in Pretoria, their results also indicated an over-representation of White males within the suicide population. The results from this research also suggests a similar outcome as White individuals showed a high proportion of the suicide group in the sample (31%) compared to White representation within the Gauteng province (15.6%) as noted in the Census of 2011 (www.statssa.gov.za) results. A possible reason suggested for the White individual suicide mortality rate includes a lack of source for blame for the hardships faced and the expectations for a high standard of life being unattainable (Burrow and Laflamme, 2006). However, further research is needed into why certain population groups in South Africa are more at risk of suicide than others.

Multiple South African studies have noted that the majority of suicides occur within the 20 – 39 year age range (NIMSS, 2013; Prinsloo *et al*., 2021; Engelbrecht *et al*., 2017 and Kootbodein *et al*., 2020) and similar outcomes were observed in this study whereby most of the suicide sample assessed fell within the 18 – 28 year and 29 – 38 year age ranges, accounting for 30% and 32% of the study population respectively. It has been reported that suicide rates are higher among young adults in low- and middle-income countries, as compared to high-income countries which show a higher suicide rate in individuals older than 70 years (Engelbrecht *et al*., 2017). Within the

South African context, it has been suggested that the rising unemployment levels are a contributing factor to the high suicide rates in males of working age (Kootbodein *et al*., 2020). Results from a Bloemfontein mortuary study found that 57% of suicide decedents were unemployed (Bantjes and Kagee, 2013) suggesting that unemployment could potentially be a risk factor for suicide. Unemployment leads to an increase in stressful life conditions, lack of social cohesion and is associated with risk of mental illness (Kootbodein *et al*., 2020). Thus, those individuals of working age who are unable to find employment in this tough economic environment may be a contributing to the prevalence of suicide in this age range.

The largest suicides rates have been noted in males, this was seen within the results of this study with males accounting for 82% of the study population (n=84/103). This male predominance in suicide is consistent with other South African studies, where it has been reported that male suicide rates are 4 to 5 times higher than that of female suicide rates (NIMSS, 2012; NIMSS, 2013; Matzopoulos *et al*., 2015; Burrow and Laflamme, 2006; Prinsloo *et al*., 2021; Engelbrecht *et al*., 2017; Scribante *et al*., 2004 and Kootbodein *et al*., 2020). It has been described that suicide attempts are more common in females; Tsirigotis *et al*, (2011) found female suicide attempts to be 3 times more frequent than male attempted suicide, however suicidal death (successful suicide attempt) is more common in males, being 2-3 more successful in completion (Tsirigotis *et al*., 2011). Reasons for the “less effective” suicide by females, and “more effective” suicide by males may include the method of suicide attempt used. Females have been shown to choose a low lethality/less violent method of suicide more commonly, whereas males tend to use more violent methods of suicides with a high level of lethality (Tsirigotis *et al*., 2011). For example, in patients with psychotic or substance-related disorders, in attempted suicides male patients had shown a preference for hanging, whereas female patients with similar conditions had shown a preference for self-poisoning in attempts (Tsirigotis *et al*., 2011 and Huisman *et al*., 2010). The less violent methods as seen in females may also be due to the fear of facial disfigurement. In certain violent methods such as death by firearm, research has shown that females are less likely to shoot themselves in the head due to aesthetic reasons (Tsirigotis *et al*., 2011). Another possible reason for gender difference is that males are less likely to seek help when suffering from major depression, whereas females more frequently seek treatment. As seen, there are multiple factors

contributing to the gender paradox noted in suicides and these can range from methodology, cultural bias, availability of means, and psychological conditions (Tsirigotis *et al*., 2011).

4.2.2 Methods of suicide noted in the suicide population

Hanging was found to be the common method of suicide, accounting for 44% of the sample of population. Similarly, multiple South African studies have also note that hangings comprise the vast majority of suicide cases within the Gauteng province (NIMSS, 2012; NIMSS, 2013; Burrow and Laflamme, 2006; Prinsloo *et al*., 2021; Engelbrecht *et al*., 2017 and Kootbodein *et al*., 2020). Data by Burrow and Laflamme (2006) and Engelbrecht *et al* (2017) found that firearm related suicides were the 2nd most common method. The NIMSS report (2012, 2013) and Prinsloo *et al* (2021) however found that following hanging, overdoes/poisoning related suicides were the 2nd most common method noted. Firearms were noted as the 3rd most common method (NIMSS, 2012; NIMSS, 2013; Prinsloo *et al*., 2021). These results were similar to those noted in this study, with overdose/poisoning related suicides being the 2nd most common method (23%), however fall from height related suicides (15%) were notes as the 3rd most common method, and closely followed by firearm related suicides (13%). The discrepancy between some of the results noted between this study and those reported may be due to the small sample size assessed (103 samples) as compared to the 1000+ cases in the mentioned studies. The 103 cases assessed were also limited in terms of exclusion criteria of the study, thus not every suspected suicide fatality presenting during the study period was assessed.

It is unclear as to why a certain method of suicide is more prevalent than others. Certain factors are suggested to play a role in this decision, such as availability of means to carry out the suicide and lethality of the method (Bantjes and Kagee, 2013). For example, the materials needed for hanging are generally common household items that are inexpensive and easy to access i.e., belts, rope, electrical cables. Overdose survivors have indicated that they selected a means of overdose for suicide attempt as drugs were readily available to them (Sarchiapone *et al*., 2011). Other than ease of access of material there are contributing factors to the choice of method of

suicide, these include media coverage whereby the suicide methodology is well described and sensationalized, symbolism behind the method for the individual, and possible cultural factors (Sarchiapone *et al*., 2011). Further research is required into factors that may influence an individual's choice in the means of suicide and the extent to which this is influenced by accessibility.

4.3 Illicit drug use in the suicide population

There is little data available on toxicology findings on all methods of suicide, other than only overdose/poisoning cases where routine toxicology testing is more commonplace. Research shows that most studies examining methods of committing suicide, other than overdose/poisoning, tend to focus on the psychological and social factors, decedent demographics and preventative measures of suicide, but typically don't assess the toxicological aspect of this type of death (San Nicolas and Lemos, 2015). This may be as the toxicology may not be the direct cause of death and therefore not deemed as a necessary additional test needed for medico-legal purposes.

Some international studies have investigated the relationship between drug use, toxicology findings, and non-overdose suicide. A study in Australia by Darke *et al*. (2009) examining the toxicology of death by violent suicide (that being methods of hanging, falling from a height, gunshot, stabbing, drowning, jumping in front of a vehicle, fire, and electrocution) found the most common methods of violent suicide to be hanging and falling from height (Darke *et al*., 2009). Darke *et al*. (2009) found hanging and fall from height related suicides to have victims associated with illicit drug usage. In contrast, these results did not match those found in this study where firearm related suicides were found as the most prevalent method of suicide with illicit drugs within the more violent suicide methods, and no fall from height cases had tested as true positive result. However, the discrepancy in results may be due to the limited sample number evaluated, and exclusion criteria further limiting case assessment.

The Darke study found that Illicit drugs were detected in 20% of violent suicide cases, with opioids accounting for 8.3%, methamphetamines 4.4% and cocaine /benzoylecgonine 1.7%. Polydrug use was detected in 3.6% of cases (Darke *et al*.,

2009). These results are similar to those noted in this study, with opiates showed to be the most prevalent drug group. It was also noted that polydrug usage was shown to account for a low percentage of the sample group, similar to the results in this study.

Darke *et al*. (2009) had shown that there was a strong association between violent manners of death and drug use. Pharmaceuticals (benzodiazepines, antidepressants, and antipsychotics) were noted as present in higher proportions than the illicit drugs groups within their study (Darke *et al*., 2009). The mentioned pharmaceutical types are prescribed therapeutic substances and did not form part of this research study. These drug types (pharmaceuticals) were identified within the results of the LC-MS/MS test in some of the cases (results not shown but provided in appendix 6). The presence of such therapeutic pharmaceuticals, in all methods of suicide could be investigated for future research into psychopathology, treatment with therapeutics and successful/completed suicide outcomes.

Sheehan *et al*. (2015) conducted a study in the United States assessing the postmortem presence of drugs and the method of violent suicide, with the aim to establish if there was a link between the violent method of suicide chosen (namely hanging and firearm related suicide) and potential drug use. These methods of suicide were chosen as they reflected the majority of suicide cases in the United States, 71% within the study sample set of Colorado, and 78% as reported by the U.S National Violent Death Reporting System sample (Sheehan *et al*., 2015). Similarly, to this study, the research results indicated hanging related suicides to be one of the most prevalent of the violent suicide methods. Sheehan *et al*. (2015) had found that opiate use was more associated with firearm related suicides compared to that of hanging, almost double the number of cases had tested positive for opiates in firearm related suicides than compared to hanging cases, and that their results suggested specific drugs rather than number of drugs (polydrug usage) was associated with a certain method. The results from this research differed in that opiate usage was predominate for both the hanging and firearm related suicide cases. Additionally, in this study results indicated that polydrug usage was only associated with non-violent method of suicide, namely overdose/poisoning. More research is required into this area within the South African landscape to assess what drugs groups may be present in suicide fatalities, and if there is any association between the drug of choice and method of suicide chosen.

There is very limited data available on suicide fatalities involving illicit drug use in South Africa, with little mention of the postmortem presence of illicit drugs in all methods suicide. A South African study by Liebenberg *et al*. (2010), conducted a retrospective analysis of all postmortem cases from the Pretoria Medico-Legal Laboratory over the period from 2003 – 2012, that were sent for state ancillary toxicological testing. Of the 22 566 medico-legal autopsies presented over the study, only 385 (1.7%) were sent for state toxicological testing. It should be noted that state toxicological testing is not routinely requested for in forensic practice in South Africa. This is due to a multitude of factors including a backlog of caseload work at the state laboratories, the backlog creates a delay in result outcomes which in turn delay the process of concluding the postmortem investigation (Liebenberg *et al*., 2016 and Maila, 2015). Resource constraints are also a factor as toxicological testing is both cost and labour intensive (Liebenberg *et al*., 2016). Additionally, only cases with a known historical evidence of drug use, and/or findings at autopsy suggesting drug use, will have specimens taken and submitted to the National department of health Forensic Chemistry Laboratory for testing. The Liebenberg study found that the official state tested samples, 40% had tested as positive for the presence of one or more illicit drug. The positive cases were found to be predominantly White (85.1%) males (90.35%) and aged between 20 – 30 years. Heroin (opiate) was found to be the most frequently detected drug (35.2%), this was followed by cocaine (19.9%), amphetamines were detected in 6.4% of the cases. Cannabis was detected in very few cases, only 4.6%, even though Cannabis has a high frequency of use it is known to contribute very little to mortality (Liebenberg *et al*., 2016). It is to be noted that these results are based off all manners of death presented to the Pretoria Medico-Legal Laboratory where the examining forensic pathologist deemed it necessary to request further toxicological testing. Thus, this will not be an indication of the illicit drug use in all manners of suicide but does give an indication of illicit drug use within a South African sample set. The study notes that the results are grossly under-represented due to limited number of samples sent for testing, and therefore highlights the need for more comprehensive toxicological testing of postmortem cases to fully assess the extent of illicit drug use in South Africa (Liebenberg *et al*., 2016). A prospective South African study by Auckloo and Davies, (2019) assessed the postmortem toxicology of violent fatalities in Cape Town, South Africa. The violent fatalities assessed comprised of homicides, non-overdose suicides,

and accidental deaths. The study found that 61% of cases had tested positive for drugs of abuse, with polydrug usage found in 49% of cases. Within the non-overdose suicide cases assessed decedents tested positive for antidepressants and opiates/opioids (Auckloo and Davies, 2019). The Auckloo and Davies study indicated a high prevalence of drugs of abuse in violent manners of death, and further highlights the need for more comprehensive and routine toxicological testing to better understand the role drugs play in manners of death (Auckloo and Davies, 2019).

4.3.1 The presence of opiates in suicide cases

A commonality noted between this research and other studies was that opiates form the most prominent illicit drug group found in suicide cases. As noted, opiates comprised 86% (n=13/15) of the true positive result, and thus accounted for 13% (12/103) of the overall suicide population sample assessed. The Substance Abuse and Mental Health Services Administration (2014) had noted that opiates were found to present in 20% of suicide cases in the United States (SAMHSA, 2016). In the United States those with opioid use disorder (OUD) show an increased suicide being six times greater than in the general population (Oquendo and Volkow, 2018). OUD is the misuse of opiates and synthetic forms of opiates as both illicit and as prescribed medication (Dydyk *et al*., 2021). OUD increased the suicide risk in men by 30% and doubled the suicide risk in women (Oquendo and Volkow, 2018). A study into the incidence and lethality of suicidal overdoes in the United States, identified opioids as being most commonly present in fatal suicide poisonings, opiates were also shown to have the greatest relative risk of death among those presenting to the emergency department with intentional opioid overdose/poisoning (Miller *et al*., 2020). This study also suggests that 75% - 87% of these suicide deaths could have been avoided had the decedents lacked access to opioids (Miller *et al*., 2020), thus further highlighting the role opioids play in suicide fatalities.

Opiates are said to play a multifaceted role in suicide. Opiates can cause an altered mental state leading to suicidal thoughts, as well as reducing rational thinking behind those thoughts (Pergolizzi *et al*., 2021). In comparison to healthy controls, patients with OUD showed impairments in their inhibitory decision-making abilities and control

of behaviour and emotions, these processing impairments, resulting in dysfunctional decision making, have been linked to an increased risk in suicidal behaviour (Rizk *et al*., 2021). Opiates also share a high rate of comorbidity with mental illness, approximately 75% of opioid dependant individuals meet the criteria for at least one comorbid psychiatric diagnosis, such as mood/anxiety disorders. It is suggested that opioid usage may mask/relieve symptoms of mental illness such as anxiety and depression (Rizk *et al*., 2021; Pergolizzi *et al*., 2021 and Ross and Peselow, 2012). These mental illnesses may be associated with a high suicidality which are partially relieved by opioid usage. Additionally, opioids are taken as a means to assist with pain relief. Chronic pain is also shown to be associated with suicidality, whereby individuals in states of constant pain may lead to feelings of hopelessness and suicidal thoughts (Pergolizzi *et al*., 2021). Other factors such as lack of family/support structure, homelessness and unemployment have high associations within individuals with OUD as well as in those individuals with suicidal tendencies (Rizk *et al*., 2021). The above mentioned factors could be suggested as possible reasons as to why opiates are so prevalent in suicide cases.

Most of the true positive opiate cases in this study identified the metabolites morphine and/or codeine (n=12/13 opiate true positive cases). Morphine and codeine are noted as the major metabolites of opiates and thus one cannot accurately identify the parent compound, and whether an illicit or prescription/therapeutic drug was taken. One case found a positive result for 6-MAM, thus indicative of heroin use. There was one case containing oxycodone. Oxycodone is a semisynthetic opioid derives from codeine and is prescribed for therapeutic use to assist with severe pain management in cases of cancer and skeletomuscular injury (Karch, 2002). Interpretation of opiate results are also difficult in that tolerance develops in opiate drug users, where the user may become tolerant of the respiratory depressant effect of the drugs. Tolerance also makes interpreting blood results difficult as ranges can differ widely between users and one cannot ascertain for certain the toxic range specific to an individual (Karch, 2002). In those suffering with an OUD disorder as drug tolerance develops, the user will continue to use higher concentrations of the drugs to try and achieve the desired effect, thus, a high concentration may not necessarily mean an acute intoxication (Allan and Roberts, 2009 and Drummer, 2010).

4.3.2 The presence of cocaine in suicides

Cocaine used had a low prevalence in the suicide cases assessed, with a positive result of 2% (n=2/103) of the overall sample. The results are similar to those noted in other studies, where a study conducted in Colorado USA, noted 4% of cases testing positive for cocaine (Sheehan *et al*., 2015), and in an Australian based study found Cocaine to be present in 2% of suicide cases (Darke *et al*., 2009). A retrospective study conducted in the UK (Bailey *et al*., 2021) assessed the toxicology of alcohol and cocaine use within suspected suicides cases between 2012 and 2016, which had been sent for state toxicological testing. This study assessed manners of suicide excluding that of self-poisoning and showed that 8% of suicide cases had tested positive for cocaine use over the 4-year period, and in cases of suicide in which alcohol was present, cocaine was detected in 16% of those cases (Bailey *et al*., 2021). The study notably found that a positive blood alcohol concentration level had a positive correlation with cocaine use, which could suggest a further need to assess the relationship of alcohol use in conjugation with other substance use and the effect and potential outcomes of successful suicide cases is known to increase (Bailey *et al*., 2021). The simultaneous use of cocaine and alcohol toxicity of the substances as compared to when alcohol or cocaine are abused alone (Dasgupta, 2010). This combination has shown to significantly increase rates of morbidity and mortality amongst users. Cocaethylene, a psychoactive substance, is formed as a biproduct in metabolism when cocaine and alcohol are used conjunction and is shown to markedly increase the risk of immediate death in cocaine users, and frequently found in large amounts in fatal overdose cases (Dasgupta, 2010). The two true positive cases for cocaine and metabolites both tested positive with cocaethylene as a metabolite, indicative of alcohol and cocaine abuse. It is to be noted blood alcohol concentrations were not available to the researcher to verify this result.

Garlow *et al*. (2003) had investigated cocaine use disorder and suicidal ideation in Georgia, USA and showed that even as cocaine rates in completed suicides were low, 40% of patients suffering from cocaine use disorder had expressed notions of suicidal ideation. The results of Garlow study were similar to those by Roy (2009), where cocaine dependant patients were interviewed to determine rates of suicidal ideation

and attempt. The Roy study found that 43% of cocaine using patients had attempted suicide. These individuals were noted to have comorbidities with other substance use disorders, as well as mood/mental health disorders (Roy, 2009). Research indicates that cocaine users show a high rate of suicidal ideation, however in completed suicides cocaine is found minimally in results, as noted similarly in the results of this study.

4.3.3 The presence of amphetamine type stimulants in suicide

Amphetamine type stimulants and cocaine are drugs with similar mechanisms of action, and thus thought to share common psychiatric and psychosocial effects of drug use (Glasner-Edwards *et al*., 2008). Methamphetamine acts as a stimulant, and hence users are shown to experience elevated rates of mood and anxiety disorders, as well as an increased risk of psychotic illness (Darke *et al*., 2019). Methamphetamine users also exhibit high rates of attempted suicide as compared to the non-drug using population, and thus are an at-risk group for successful suicide completion (Darke *et al*., 2019). The neurobiological and psychosocial effects experienced by methamphetamine users, as well as the effects of intoxication and withdrawal have shown to be associated with psychiatric illness and symptoms that increase risk behaviour, highly impulsive nature, and depressive mood which may also contribute to play a role in suicidality ideologies (Glasner-Edwards *et al*., 2008).

Methamphetamine use is fairly common in Gauteng accounting for an estimated 11% of patients in substance use treatment facilities (SACENDU, 2019), and hence there is a need to assess the risk of the users successful suicide attempts. Darke *et al*. (2019) conducted a national study on suicide among methamphetamines users in Australia and found that over a 6-year period 18.2% of all methamphetamine related deaths were noted as suicides. Additionally, methamphetamine related suicides accounted for 1.6% of all suicide cases over that period. The Darke study found that most of the suicide cases were hanging related, and that in cases of self-poisoning, decedents were likely to also test positive for antidepressants and opioids. In the current research there were three cases that tested true positive for amphetamine type stimulants (methamphetamine), of which one case was a hanging related suicide. The remaining two cases were both overdoes/self-poisoning related suicides, and in

common with the Darke study opioids were also found in these cases. Although the true positive amphetamine type stimulant cases accounted for 3% (n=3/103), the discordant results highlighted a high number of amphetamine type stimulants (22%, n=22/103). Differentiations in the analytical methods may have resulted in the discordant nature of the results however, possible future studies focusing on more quantitative techniques could be used to correctly determine the true extent of amphetamine type stimulant use and prevalence in suicide rates.

4.4 Limitations

4.4.1 Sample size

The preliminary, descriptive nature of the study, sample collection time frame, and resource constraints allowed for only a small sample size. The small sample size allows for only a limited evaluation of the results and possible true extent of drug usage within the suicide population. However, the preliminary data produced can be used to substantiate the need for further, more comprehensive forensic toxicological studies. Additionally, 12 out of 103 cases were selected by the pathologist for formal toxicological testing with the National department of health Forensic Chemistry Laboratory. Of the 15 true positive cases only three of those cases were within those 12 sent for formal state testing. This indicates that there is a proportion of drug positive cases that are missed for the presence of illicit substances, thus further substantiating the need for increased scope and sample size for future toxicological studies to fully understand the extent that illicit substances may play in suicide cases.

4.4.2 Analytical method

As per recommended forensic laboratory guidelines two independent test methods were used in this study (SOFT/AAFS, 2006; Moffat *et al*., 2011 and Drummer, 2010), a phase I immunoassay screen and a phase II confirmatory mass spectrometry technique. The purpose of the immunoassay screen is to indicate the presence of a drug class, but not to provide a measure of intoxication. Immunoassay screens allow for rapid on-site detection of drug classes and can therefore allow the analyst to focus

confirmatory testing to any specific drug classes identified as positive by the test. Screening of specimens can also lead to time and cost saving in cases where a negative screen result is found. This method does have downside in that certain drugs classes may be missed if not specific to the screen, and immunoassay tests also have lower sensitivity than a mass spectrometry technique and thus a “negative” may not mean that a drug is absent in the sample but that the concentration of drug is lower than the test cut off level (SOFT/AAFS, 2006). In order to confirm the initial screen results a mass spectrometry technique (LC-MS/MS) was used. Mass spectrometry allows for unequivocal identification of drugs based on their spectral peaks; thus, results can only be confirmed with use of a mass spectrometry technique (Drummer, 2010).

As noted in the findings of this study there was a high number of discordant results within the selected drugs of abuse. A large proportion of these results were found within the LC-MS/MS test method. This may have been due to the greater sensitivity and specificity of the technique, as well as the lower limit of detection thus possibly showing a positive result for samples with a lower quantity of drug than would be detected in the immunoassay screen. Additionally, there may be cross reactivity of compounds on the immunoassay screen leading to a false positive result for certain substances. It should be noted however that the aim of this study was to determine if there is the presence of drugs in all suicide cases presented at the JHB FPS MLL, and not to determine quantitative levels and whether these were within lethal/toxic ranges, and thus a direct cause of death.

There are additional differences in the analytical technique that need to be considered, that is the scope of the test panel and the costs involved. The Randox BAT immunoassay screens for a small variety of drugs on each test panel. In this study only the DOA I test panel was used, this was selected as this test panel covered the drugs of abuse selected for this research, however this is very limiting if further drugs groups are needed to be assessed, and in the case where a decedent may present with an unknown case history multiple test panels would need to be used to ensure that possible results have not been missed. The LC-MS/MS method tests for larger panel of drugs on one test method simultaneously thus allowing for a greater scope of testing per sample, and less chance of a potential positive result being missed.

The extent of costing involved in analytical processes and the more compounds required to be looked at, as well as improved sensitivity levels associated with certain methods will increase the costs of testing as well as take more time and scientific expertise. Thus, available resources need to be considered in any toxicological analysis in terms of appropriate test methods and goals of research before future studies can commence.

4.5 Future recommendations

As aforementioned there is limited research on this subject globally, and even more so in the South African context. The results suggest that more comprehensive research is needed into the topic of drug usage in all suicides. A larger sample volume, and a wider scope of test panels to include not only illicit substances but therapeutics as well could help provide a better understanding of the drug issues currently faced in South Africa, possible associations between different drugs and drug combinations and the any implications this may have on suicidality.

4.6 Conclusion

This pilot study provided a prospective assessment of postmortem toxicological data on drugs of abuse in all suicide deaths in Johannesburg South Africa. Due to the nature of the study no causative conclusions could be drawn. This study has shown that drugs of abuse are present within suicide cases, and these positive results extend beyond overdose/poisoning cases where one expects to see a positive drug result. This toxicologic information may have been useful in the medico-legal investigation assessing the manner and cause of death. These findings are therefore suggestive that regular toxicological screening should be performed, of both illicit and therapeutic (over the counter and prescription medication) drugs, on those cases of unnatural deaths with a potential suicide history presenting at the Johannesburg FPS MLL. Suicide is a complex topic with various factors contributing to the outcome of a completed suicide. Drug usage forming one of those risk factors. As research has suggested there is limited data available about drug usage in all methods of suicide,

and even less data from a South African standpoint. Toxicological samples are not readily taken from all presenting cases at the Johannesburg FPS MLL, in part as these results may not be deemed necessary in determination of cause of death for medico-legal purposes, and due to the current constraints within the forensic toxicology testing framework. However, it could be suggested that due to the strong associations between drug use and suicidal ideation, toxicological testing should become more common place in all manners of suicide fatalities so as to better understand the full scope and implications of illicit drug use, and the potential factors contributing the successful completion of a suicide attempt. This information could better enable support structures and government to spread awareness of the additional risks of illicit drugs on mental wellbeing, and thus play a role in suicide prevention strategies.

This pilot study highlights the need for further evaluations and assessments in toxicological outcomes, comprising a larger sample size, not only suicide fatalities, but for a more comprehensive assessment of potential drug associations in other manners of death presenting in Johannesburg South Africa.

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Appendix

Appendix I: Data collection sheet

DATA SHEET

Documentation of suspected suicide victims (FPS JHB)

Case Number: _____ Date of Autopsy: _____

Date of Death: _____ Age at Death: _____ (Estimated/Actual)

Race: _____ (B/W/C/A/?) Sex: _____ (M/F) Sample Number: _____

Name of Doctor: _____ Date of Analysis: _____

Description of scene of suspected overdose incident (Drug paraphernalia):

.....

.....

.....

Description of victim:

.....

.....

.....

Description of "circumstance" of death:

.....

.....

.....

Analytes present in specimens:

Sample Number: _____

Specimen	Analyte of interest	Immunoassay screen result (pos/neg)	Confirmatory test result (pos/neg)	Result summary (true pos/true/neg/discordant)
Blood				
Urine				

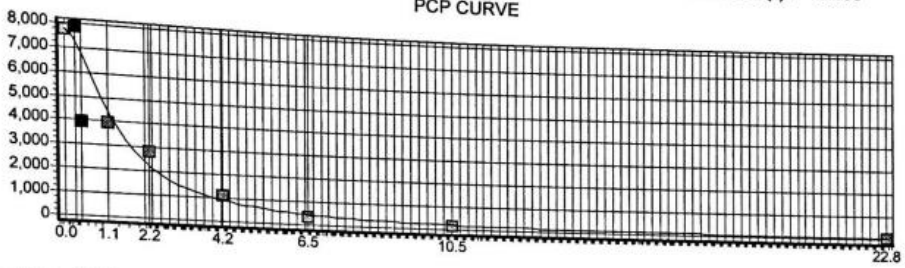
Appendix II: Example of Randox BAT calibration curve

Calibration Curves

Array Details:	Drugs of Abuse I WB PLUS
Calibration Ran By :	gillian craig
Calibration Id:	44
Date Of Calibration:	10/28/2014
Time of Calibration:	09:25 AM
Calibration Batch No:	6137
Calibrator Expiry Date:	05/31/2015

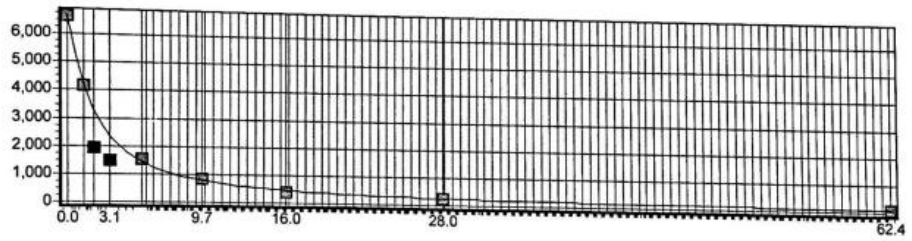
Analyte: PCP **Target Curve Fit(r):** 0.949 **Curve Fit(r):** 0.980

PCP CURVE



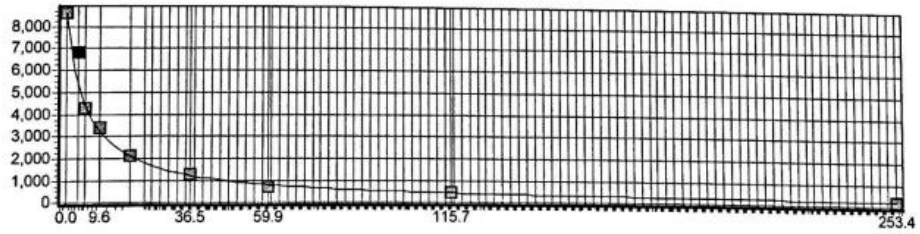
Analyte: BZG **Target Curve Fit(r):** 0.949 **Curve Fit(r):** 0.999

BZG CURVE



Analyte: MDMA **Target Curve Fit(r):** 0.949 **Curve Fit(r):** 0.998

MDMA CURVE



Appendix III: Example of Randox BAT control acceptance levels.

EVIDENCE INVESTIGATOR™

Level 1

Component	Target	Low Range	High Range	SD	Method
Methamphetamine	80.32 ng/ml	52.20	108.44	14.06	Investigator™
Barbiturates	31.92 ng/ml	20.74	43.10	5.59	Investigator™
Benzodiazepine1	8.75 ng/ml	5.69	11.81	1.53	Investigator™
Benzodiazepine2	20.23 ng/ml	13.15	27.31	3.54	Investigator™
Methadone	6.23 ng/ml	4.05	8.41	1.09	Investigator™
Opiates	15.16 ng/ml	9.86	20.46	2.65	Investigator™
PCP	2.86 ng/ml	1.46	4.26	0.70	Investigator™
BZG	23.73 ng/ml	15.43	32.03	4.15	Investigator™
MDMA	82.80 ng/ml	53.82	111.78	14.49	Investigator™
THC	7.41 ng/ml	4.81	10.01	1.30	Investigator™
TCA	24.94 ng/ml	16.22	33.66	4.36	Investigator™
Amphetamine	16.58 ng/ml	10.78	22.38	2.90	Investigator™
Buprenorphine	3.37 ng/ml	2.19	4.55	0.59	Investigator™

Level 2

Component	Target	Low Range	High Range	SD	Method
Methamphetamine	349.91 ng/ml	227.45	472.37	61.23	Investigator™
Barbiturates	118.03 ng/ml	76.73	159.33	20.65	Investigator™
Benzodiazepine1	29.16 ng/ml	18.96	39.36	5.10	Investigator™
Benzodiazepine2	50.90 ng/ml	33.08	68.72	8.91	Investigator™
Methadone	29.91 ng/ml	19.45	40.37	5.23	Investigator™
Opiates	44.96 ng/ml	29.22	60.70	7.87	Investigator™
PCP	17.52 ng/ml	11.12	23.92	3.20	Investigator™
BZG	61.14 ng/ml	39.74	82.54	10.70	Investigator™
MDMA	247.42 ng/ml	160.82	334.02	43.30	Investigator™
THC	48.39 ng/ml	31.45	65.33	8.47	Investigator™
TCA	161.92 ng/ml	105.24	218.60	28.34	Investigator™
Amphetamine	74.56 ng/ml	48.46	100.66	13.05	Investigator™
Buprenorphine	15.99 ng/ml	10.39	21.59	2.80	Investigator™

Appendix IV: Ethics clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Lauren Stein

CLEARANCE CERTIFICATE	**M120504**
PROJECT	An Investigation into Drug Use in Suicide Fatalities at the Johannesburg Forensic Pathology Service Medico-Legal Mortuary
	(revised title)
INVESTIGATORS	Ms Lauren Stein.
DEPARTMENT	Forensic Medicine/School of Pathology
DATE CONSIDERED	25/05/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07/06/2013 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr G Gordon

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix V: Approval of the head of department

Umnyango wezempilo no Kuthuthukiswa Komphakathi
Lefapha la Maphelo le Tshebeletso le Ntshetsopele ya Sechaba
Department of Health and Social Development
Departement van Gesondheid en Maatskaplike Ontwikkeling

Prof Jeanine Vellema
Chief Specialist & Head of Division
Forensic Pathology Service: Johannesburg
Tel: 011 - 489 1659/00
Cell: 082 777 0737
Fax: 086 656 8413
E-mail: vellema@telkomsa.net

2 May 2012

TO WHOM IT MAY CONCERN
RE: RESEARCH REQUEST BY MS. LAUREN STEIN

Permission is hereby granted for Ms. Lauren Stein to conduct her research as proposed. She is being granted access, pending ethics approval, to the autopsy suite to observe the autopsies as they are carried out as well as access to the scribe notes/ autopsy report with the understanding that the strictest confidentiality will be maintained and that all data will remain anonymous and unlinked.

Yours truly,

.....
Prof Jeanine Vellema
Professor, Chief Specialist & Head of Division
Forensic Pathology Service: Gauteng Southern Cluster

Appendix VI: LC-MS/MS raw data

Key:
Red: Blood positive result
Blue: Urine positive results
Black: Blood and urine positive results

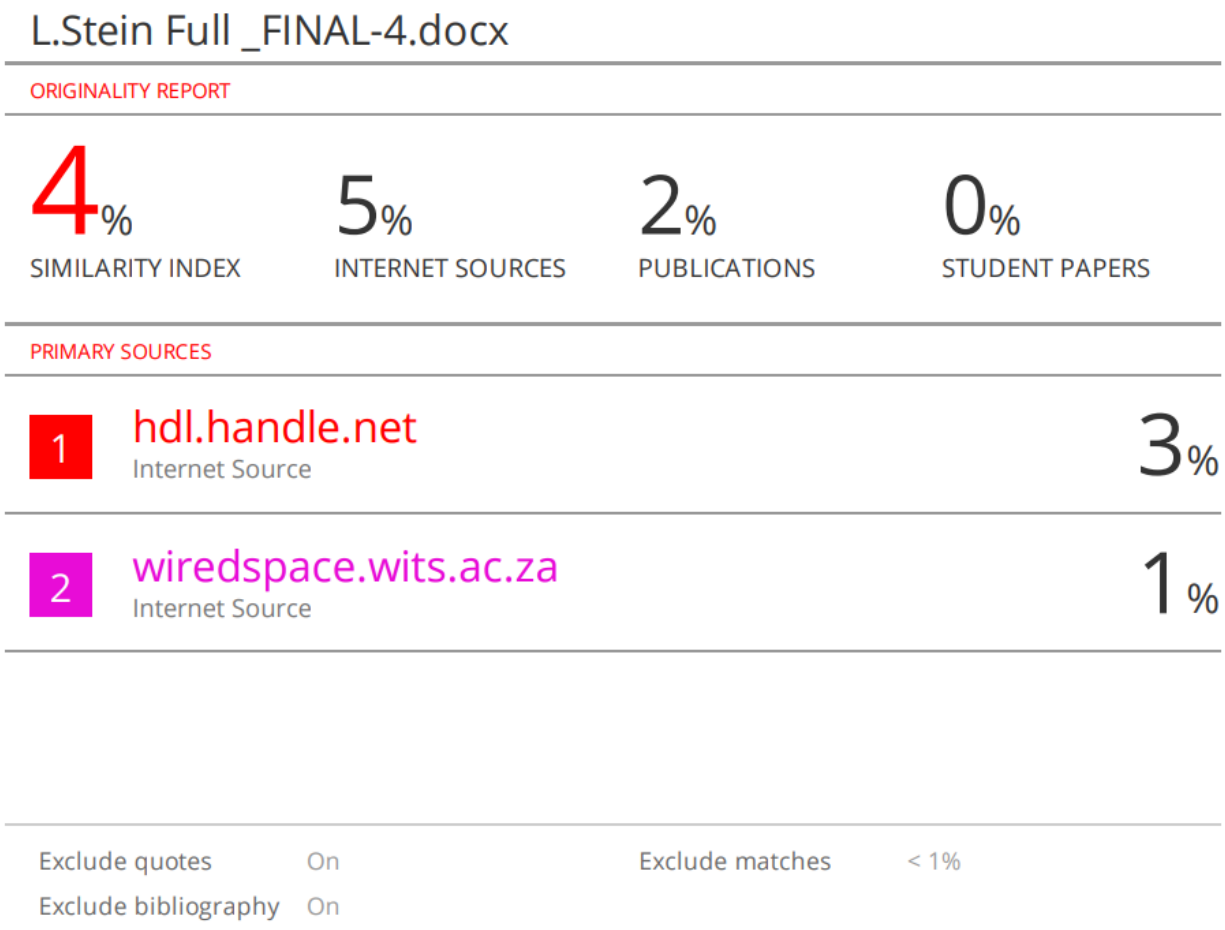
Victim no.	Method of death	Age	Race	Sex	LC-MS/MS results for full panel as per Randox laboratories DOA method (Manchester, UK)
1	Hanging	18	B	F	Morphine, codeine
2	Hanging	18	W	M	Methamphetamine
3	Hanging	54	W	M	Zolpidem, 6MAM
4	Fall from height	41	W	M	
5	Hanging	39	B	M	Amphetamine
6	Fall from height	38	B	M	THC, THC-COOH
7	Hanging	33	W	M	Alprazolam, Codeine
8	Hanging	27	C	M	Codeine, Amphetamine, Methamphetamine
9	Shot	30	B	M	
10	Overdose/poisoning	30	B	M	6MAM, Hydromorphone
11	Shot	33	B	M	Methamphetamine
12	Hanging	32	B	M	Codeine
13	Hanging	35	A	F	Cocaine, BZG, Cocaethylene, Methamphetamine, Amphetamine, MDMA, MDA
14	Overdose/poisoning	52	W	M	Codeine, Clonazepam
15	Hanging	27	B	M	Methamphetamine, Ketamine, Norketamine
16	Fall from height	23	B	M	Methamphetamine, Amphetamine
17	Fall from height	40	B	M	
18	Hanging	25	B	M	6MAM, Mephedrone
19	Fall from height	59	W	M	Cocaine, BZG
20	Hanging	18	B	F	Codeine
21	Hanging	31	B	M	
22	Hanging	27	B	F	Methamphetamine
23	Hanging	55	B	M	
24	Fall from height	23	B	M	
25	Shot	37	B	M	BZG, MDMA, Alprazolam
26	Shot	72	W	M	
27	Gassing (CO)	33	A	M	Codeine, Hydrocodone

28	Hanging	34	B	M	Codeine
29	Overdose/poisoning	72	W	F	Hyrdocodone, Alprazolam, Zolpidem
30	Overdose/poisoning	36	B	M	
31	Shot	69	W	F	
32	Overdose/poisoning	52	W	M	Morphine, Codeine, Dihydrocodeine, LSD
33	Overdose/poisoning	42	B	M	
34	Hanging	58	W	M	Morphine, Codeine, Hydrocodone, 7-Aminonitrazepam, Flunitrazepam
35	Shot	73	W	M	Morphine, Codeine, Dihydrocodeine
36	Gassing (CO)	53	W	F	
37	Hanging	25	B	M	THC, THC-COOH
38	Fall from height	31	B	M	Methamphetamine, Amphetamine
39	Overdose/poisoning	52	B	M	6MAM, Methadone, 7-Aminoflunitrazepam
40	Hanging	60	W	M	
41	Shot	58	W	M	Morphine, Codeine, Hydromorphone, Dihydrocodeine, Oxycodone, Clonazepam, Amphetamine, Zolpidem
42	Hanging	65	W	M	Alprazolam
43	Overdose/poisoning	28	B	M	
44	Shot	35	B	M	Codeine
45	Overdose/poisoning	59	W	F	Alprazolam
46	Fall from height	28	B	M	6MAM, Hydrocodone, Zolpidem
47	Hanging	33	B	M	
48	Hanging	23	W	M	Methamphetamine, Amphetamine
49	Shot	49	W	M	Methamphetamine, Oxazepam
50	Overdose/poisoning	54	W	M	6MAM, Morphine, Codeine, Dihydrocodeine, Clonazepam, THC-COOH,
51	Hanging	22	B	M	
52	Hanging	22	B	M	
53	Overdose/poisoning	18	B	F	
54	Hanging	30	B	M	
55	Fall from height	30	B	M	6MAM, THC-COOH
56	Overdose/poisoning	48	W	F	Diazepam, Nordiazepam, Temazepam, Alprazolam, Oxazepam
57	Hanging	33	A	F	
58	Hanging	28	B	F	Morphine, Codeine, Dihydrocodeine
59	Overdose/poisoning	29	B	M	
60	Overdose/poisoning	42	A	M	Morphine, Codeine, Dihydrocodeine, Amphetamine
61	Overdose/poisoning	35	B	M	Codeine, Hydromorphone

62	Hanging	35	B	M	THC, THC-COOH
63	Hanging	38	B	M	6MAM
64	Fall from height	35	B	M	Codeine
65	Overdose/poisoning	24	B	F	
66	Hanging	31	B	M	
67	Hanging	30	B	M	
68	Overdose/poisoning	27	B	M	
69	Overdose/poisoning	23	B	M	
70	Fall from height	24	B	M	Methamphetamine
71	Shot	44	B	M	Codeine, Hydrocodone, LSD
72	Hanging	27	B	M	Buprenorphine
73	Shot	60	W	M	Methamphetamine
74	Fall from height	40	B	M	Cocaine, Methamphetamine, THC-COOH
75	Shot	24	A	M	Morphine, Codeine, Dihydrocodeine, Hydromorphone, Hydrocodone, Zolpidem
76	Hanging	35	B	M	
77	Hanging	32	A	M	
78	Fall from height	44	B	M	Methamphetamine
79	Hanging	68	W	M	
80	Hanging	38	B	M	Methamphetamine
81	Overdose/poisoning	48	W	M	Alprazolam
82	Hanging	64	A	M	
83	Hanging	32	B	M	Morphine, Codeine
84	Stabbing	25	B	M	
85	Gassing (CO)	45	W	M	Cocaine, BZG, Cocaethylene
86	Hanging	49	W	M	Cocaine, BZG, Cocaethylene
87	Hanging	54	B	F	
88	Hanging	25	W	F	Codeine, Hydrocodone, Clonazepam
89	Gassing (CO)	56	B	M	Morphine, Codeine, Dihydrocodeine, Hydromorphone, LSD
90	Hanging	22	B	M	

91	Overdose/poisoning	36	W	M	6MAM, Morphine, Codeine, Dihydrocodeine, Hydromorphone, Hydrocodone, Cocaine, BZG, Cocaethylene, Methamphetamine, Amphetamine, Methamphetamine, AEME, Diazepam, Nordiazepam, Temazepam, Alprazolam, Lorazepam, Oxazepam, Flunitrazepam
92	Overdose/poisoning	36	W	M	Morphine, Codeine, Hydrocodone, Methamphetamine, Amphetamine, Alprazolam
93	Hanging	38	W	F	Codeine, Methamphetamine, Amphetamine, Lorazepam
94	Hanging	40	B	M	
95	Hanging	18	B	M	
96	Overdose/poisoning	25	B	F	
97	Overdose/poisoning	60	W	F	Methamphetamine, Clonazepam
98	Fall from height	35	B	M	6MAM, Methamphetamine
99	Shot	27	B	M	Methamphetamine, THC-COOH, Alprazolam, SD
100	Overdose/poisoning	68	B	M	
101	Hanging	23	B	M	
102	Fall from height	28	W	F	
103	Hanging	39	B	M	Amphetamine, Methamphetamine

Appendix VII: Turnitin report



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