T.C.
CUKUROVA UNIVERSITY
INSTITUTE OF ADDICTION AND FORENSIC SCIENCES
DEPARTMENT OF FORENSIC SCIENCES

FORENSIC ANALYSIS OF ABUSED DRUGS IN HAIR SPECIMENS OF PSYCHIATRIC PATIENTS AT BUTABIKA HOSPITAL IN UGANDA

MUGUME ISAAYA

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE A WARD OF THE DEGREE OF

MASTER OF FORENSIC SCIENCE OF CUKUROVA UNIVERSITY

SUPERVISOR

Assoc. Prof. Dr Fatma AYDINOGLU

ADANA - 2024

ACCEPTANCE AND APPROVAL

ACKNOWLEDGEMENT

First and foremost, I would like to thank the almighty Father for enabling me to settle for this work. Assoc. Prof. Dr Fatma Aydinoglu I extend my sincere thanks and regards for your unwavering support, guidance, and mentorship, you advised and corrected me, your guidance has helped a lot and inspired me to carry out my research with passion and love.

I warm-heartedly thank Prof. William Bazeyo, Prof. Dr Ayse Serin, ACP Andrew K Mubiru, Dr Kisitu Jaffar, Dr Pinar Özşeker, Dr Benedict Akimana, Lilian D Mutesi, Niwenshaba Nelson and Serrute Charles, your work towards this achievement can't be taken for granted, you tirelessly shared experience, assistance and knowledge which always drove my ambitions in accomplishing the academic research.

My appreciation still goes out to my facilitators, fellow students, workmates, family members, and friends, they relentlessly offered help, guidance, and support which kept me in motion to achieve this work.

Finally, thanks to the entire Cukurova University, and Makerere University, Uganda police force, Directorate of Forensic Services for the funding opportunity to pursue my studies at the Institute of Forensic Science and Addiction and also extend thanks to Butabika Mental National Referral Hospital for the hospitality I received during data collection.

May the good Lord bless you abundantly.

DEDICATION

This dissertation is dedicated to Prof Mete Korkut Gulmen who was the source of inspiration, may His Soul Rest in Eternal Peace.

I also dedicate this dissertation to Prof William Bazeyo and Assoc. Prof. Dr Fatma Aydinoglu for their continued support and advice to accomplish this dissertation.

Lastly, I dedicate this dissertation to my family members who encouraged me throughout the process.

TABLE OF CONTENTS

ACCEPTANCE AND APPROVAL i
ACKNOWLEDGEMENT i
DEDICATION ii
TABLE OF CONTENTS iii
LIST OF TABLES v
LIST OF FIGURES vi
ACRONYMS & ABBREVIATIONS vii
ABSTRACT ix
ÖZET x
1. INTRODUCTION 1
1.1. Introduction 1
1.2. Objectives 2
1.2.1. Aim 2
1.2.2. Specific Objectives 2
2.. GENERAL INFORMATION 3
2.1. Substance Abuse 3
2.2. Classes of Abused Substances 4
2.2.1. Opioids 4
2.2.2. Cannabis 5
2.2.3. Cocaine 5
2.2.4. Amphetamine-Type Stimulant 6
2.2.5. Khat 6
2.3. Substance Abuse and Psychiatric Disorders 6
2.4. Identification of Abused Substances 7
2.4.1. Urinalysis, ... 8
2.4.2. Blood analysis, ... 8
2.4.3. Oral fluid analysis 9
2.4.4. Hair analysis, ... 9
3.. MATERIALS & METHODS 11
3.1. Chemicals 11

3.2. Preparation of Standards and Solutions 11
3.3. Study Design 11
3.4. Sample Preparation 12
3.4.1. Collection of hair samples 12
3.4.2. Decontamination and Homogenization 12
3.5. Methanol Extraction and Analysis 13
3.6. Method Development 13
3.7. Validation Parameters 14
3.7.1. Limit of Detection (LOD) and Limit of Quantification (LOQ) 15
3.7.2. Evaluation of Method Linearity and Selectivity 15
3.8. Statistical Analysis 16
4. RESULTS 17
4.1. Demographic Parameters 17
4.2. Self-reported Substance Abuse 18
4.3. Substance Use in Hair Samples. 18
4.4. Concordance Between Hair Analysis and Self-report. 20
4.5. Concentration of Substances Use in Hair Samples. 21
4.6. The Association Between Substance Abuse and Psychological Disorders 27
5. DISCUSSION 30
5.1. Discussion 30
5.2. Limitations 33
5.3. Conclusion 33
6. REFERENCES 34
7. APPENDICES 40
Appendix A Some Chromatographic Peaks of some compounds detected in hair samples 40
Appendix B. *Consent Form* 42
Appendix C Questionnaire 43
RESUME 40

LIST OF TABLES

Table 1 Drug Transitions and Retention times monitored for each analyte. 14
Table 2 Instrument features of the method for determination of target analytes 16
Table 3 Participant Demographic Parameters 17
Table 4 Self-reported substance use. 18
Table 5 Percentage of patients with substance abuse detected /undetected in hair samples. 19
Table 6 Hair analyzes relative to self-reported substance use. 20
Table 7 Concentration of drug analytes. 22
Table 8 Hair sample- analytes determined below and above LoD and LoQ 27
Table 9 Psychological situation of patients. 27
Table 10 Substance Use and Psychological effects. 28

LIST OF FIGURES

Figure 1 Bar graph shows self-reported substance use. 18
Figure 2 Bar graph shows the percentage of hair samples with substance detection. .. 19
Figure 3 Bar graph shows detected substance use in hair samples relative to self-reported substance use. 21
Figure 4 Concentration of drug Analytes' distribution determined in hair. 26
Figure 5 Bar graph shows the psychological disorders of patients according to patient reports. 28
Figure 6 Bar graph shows the association between self-reported substance use and psychological effects. 29

ACRONYMS & ABBREVIATIONS

(CoVAB.MSC3.2023)	College of Veterinary Medicine, Animal Resources & Biosecurity, Makerere University, Research Ethics Committee
ADD	Attention Deficit Disorder
ADHD	Attention- Deficit/Hyperactivity Disorder
AED	Automated external defibrillator
ATS	Amphetamine-Type stimulus
BAC	Blood Alcohol Concentration
CBD	Cannabidiol
CBN	Cannabinol
CBRNe-A	Chemical Biological Radiological Nuclei Explosive Analysis
CE	Collision Energy
CPR	Cardiopulmonary Resuscitation
DFS	Directorate of Forensic Services
ESI	Electrospray ionization
GC	Gas Chromatography
HIV	Human immune Deficiency
HPLC	High-Performance Liquid Chromatography
ID	Identification
INST	Instrument
IS	Internal standard.
LC	Liquid Chromatography
LoD	Limit of Detection
LoQ	Limit of Quantification
MDA	-3,4 -methylenedioxyamphetamine
MDMA	methylenedioxyamphetamine
MS	Mass Spectrometry
NCCMH	National Collaborating Centre for Mental Health
NIDA	National Institute on Drug Abuse
NIMH	National Institute of Mental Health

NPS	New Psychoactive Substances
PCP	Phencyclidine,
PTSD	Post Trauma Stress Disorder
Rt	Retention time
UNODC	United Nations Office on Drugs and Crime
WI	Work Instruction
Δ^9 -THC	Delta-9-Tetrahydrocannabinol

ABSTRACT

Forensic Analysis of Abused Drugs in Hair Specimens of Psychiatric Patients at Butabika Hospital in Uganda

Drug abuse may cause adverse effects on an individual's health, relationships, and overall quality of life. In Uganda, there has been an increase in mental health illnesses associated with the abuse of drugs such as cannabis, opioids, amphetamine-type stimulants, khat, and cocaine among others. This study was designed to use hair samples to detect substances of abuse in psychiatric patients. For this purpose, the hair samples were collected from patients between 18- and 50 years old hospitalized in Butabika Hospital's Alcohol and Drug Unit. Also, the questionnaires for patients were performed to evaluate the sociodemographic characteristics of patients and obtain their self-report for substance use. A reliable analytical method was designed to identify these compounds, and hair samples obtained from the occipital region of patients were analyzed after methanolic extraction of the target analytes. A total of 154 patients were included in the study, 76.62% male and 23.38% female. The average age of the patients with hair analyzed was 29.77 years. Survey interviews revealed that 62.99% (n=97) of patients had been exposed to Cannabis. The experimental method showed a difference in some detected substances reported during the interview and included Cannabis, Khat, Pethidine, Tramadol, Heroin, MDMA, Cocaine, Amphetamine, MDA, Methamphetamine, Codeine, and Morphine. Furthermore, the relationship between substance abuse and psychological disorders was evaluated. Depression, anxiety, mood disorder, psychosis, and eating disorders were obtained in patients' records.
In conclusion, the present study suggests that hair analysis is an important technique for the screening of abused drugs in forensic and psychological units.

Keywords: substance abuse, hair analysis, questionnaire, psychological disorders

ÖZET

Uganda'daki Butabika Hastanesindeki Psikiyatri Hastalarının Saç Örneklerinde Kötüye Kullanılan İlaçların Adli Analizi

Uyuşturucu kullanımı bireyin sağlığı, ilişkileri ve genel yaşam kalitesi üzerinde olumsuz etkilere neden olabilir. Uganda'da esrar, opioidler, amfetamin tipi uyarıcılar, khat ve kokain gibi uyuşturucuların kötüye kullanılmasıyla ilişkili akıl sağlığı hastalıklarında bir artış oldu. Bu çalışma, psikiyatri hastalarında kötüye kullanılan maddeleri tespit etmek için saç örneklerini kullanmak üzere tasarlandı. Bu amaçla Butabika Hastanesi Alkol ve Uyuşturucu Ünitesinde yatan 18-50 yaş arası hastalardan saç örnekleri toplandı. Ayrıca hastaların sosyodemografik özelliklerini değerlendirmek ve madde kullanımına ilişkin öz bildirimlerini almak amacıyla hastalara anket uygulandı. Bu bileşikleri tanımlamak için güvenilir bir analitik yöntem tasarlandı ve hastaların oksipital bölgesinden alınan saç örnekleri, hedef analitlerin metanolik ekstraksiyonundan sonra analiz edildi. Çalışmaya %76,62'si erkek, %23,38'i kadın olmak üzere toplam 154 hasta dahil edildi. Saç analizi yapılan hastaların ortalama yaşı 29,77 idi. Anket görüşmelerinde hastaların %62,99'unun (n=97) esrara maruz kaldığı ortaya çıktı. Deneysel yöntem, görüşme sırasında bildirilen bazı tespit edilen maddelerde farklılık gösterdi ve bunlar arasında Esrar, Khat, Petidin, Tramadol, Eroin, MDMA, Kokain, Amfetamin, MDA, Metamfetamin, Kodein ve Morfin yer aldı. Ayrıca madde kullanımı ile psikolojik bozukluklar arasındaki ilişki değerlendirilmiştir. Hastaların kayıtlarından depresyon, anksiyete, duygudurum bozukluğu, psikoz ve yeme bozuklukları elde edildi. Sonuç olarak bu çalışma, saç analizinin, suistimal edilen ilaçların taranmasında önemli bir teknik olduğunu ortaya koymaktadır.adli tıp vePsikolojik birimler.

Anahtar Kelimeler: madde kullanımı, saç analizi, anket, psikolojik bozukluklar

1. INTRODUCTION

1.1 Introduction

In Uganda, there has been an increase in mental health illnesses associated with abuse of drugs like Cannabis, opioids, amphetamine-type stimulants, khat, and cocaine among others. Identifying these substances with the utilization of biochemical tests to lay out sufficient data about a person's use and treatment of the potential results is still ineffective.

Unknown exposure to adulterant drugs is also common practice among users of illegal drugs and has caused serious public health complications worldwide. Drug abuse causes various adverse effects on an individual's health, relationships, and overall quality of life, the substances bring up symptoms of psychiatric disorders and can trigger the onset of new symptoms, thus seeking help and support is crucial for those affected. Among the psychiatric disorders caused by these drugs include Anxiety, depression, attention-deficit hyperactivity disorders, bipolar disorders, personality disorders, and schizophrenia among others.

Given the association between substance abuse and psychiatric disorders, accurate estimation of health complications requires the use of appropriate diagnostic techniques. Self-report testing and biological screening are fundamental techniques used in drug screening and assessment, both techniques are utilized to enhance each other for adequate information about the investigated substances. The fact that self-reporting can't provide all information required for treatment about drug use due to individual perspectives like failure to remember information, giving false information, or not giving any, lays elective utilization of biological testing for objective and reliable information. For example, in circumstances where Clinicians had not recognized cocaine use among patients admitted to a psychiatric hospital after they tested positive for cocaine, using biochemical tests [4].

Categories of common biological samples used for the identification of abused substances include urine, blood, Oral fluid, sweat, and hair. Urine, saliva, and blood, specimens have limitations in providing enough information about the drugs consumed after a long period. This has influenced the application of hair analysis as an alternative to drug detection [30]**.** Like other biological techniques, hair analysis also helps to categorize abused substances and determine the amount of the substances using chromatographic

analytical instruments like Liquid Chromatography (LC) and Gas Chromatography (GC) with an MS detector.

The process of hair analysis consists of several pretreatment steps including hair collection, decontamination, pulverization, and extraction/digestion [18], and the Society of Hair Testing recommends producing reliable results at all stages of analysis [54]. Extraction of drugs from hair requires the utilization of an appropriate method that considers the chemical structure of the analyte of interest and its reaction with the substances. Efficient extraction of substances of abuse from the hair matrix involves using solvents (solid or Liquid extraction) supported by cutting hair into smaller segments and incubating in an ultrasonic bath. Generally, methanolic incubation for extraction of basic drugs was considered better for analyte stability than with alkaline digestion [64].

1.2 Objectives

1.2.1 Aim

This study is aimed at determining and evaluating substance abuse and use of non-medical prescription drugs in hair samples of patients hospitalized at Butabika Hospitals' alcohol and drug unit, this will provide new insights into the reliability of prevalence estimates for a variety of illicit substances and non-medically used prescription drugs, establish a laboratory analytic approach for testing substance abuse in hair in forensic and clinical units, a method that may be used by mental health hospitals to generate information about the drug/substance before medical treatment or in forensic investigations to provide evidence to support the criminal justice system.

1.2.2 Specific Objectives

1. Determine the prevalence estimates of substance abuse and non-medical prescription drug use.
2. Detect substance abuse in hair samples collected from psychiatric patients at Butabika Hospital Uganda using Liquid Chromatography-Mass spectrometry.
3. Evaluate the relationship between substance abuse and psychiatric disorders.

2. GENERAL INFORMATION

2.1 Substance Abuse

Substance abuse is described as the harmful or hazardous consumption of psychoactive compounds including alcohol and illicit drugs in a manner that negatively impacts a person's social and health problems,[26, 28]. Substance abuse refers to excessive use of a drug in a way that is detrimental to self, society, or both [27].

The global estimate for substance abuse has increased over the past years and according to the World Drug Report 2023 approximately 296 million people worldwide abused substances to the extent that one person was reported to abuse a drug(s) in every 17 people. The survey indicated that cannabis was the most abused substance in 219 million people, 60 million people had abused opioids, with a high prevalence of death resulting from opioid overdose, 36 million people abused Amphetamine-type stimulants (ATS), and 22 million abused cocaine substances and New Psychoactive substances (NPS). America, Western, and Central Europe had the highest prevalence of substance abuse, for cannabis and Cocaine while Asia largely used opioids and increased use of NPS was exhibited in Africa [57].

In Africa, many people under the age of 35 years present symptoms of substance use disorders, cannabis with a prevalence rate of approximately 10% (30 million people) and is the most treated drug problem. Non-medical use of tramadol, cocaine, and opioids (mainly heroin, codeine, and opium) also poses a threat in some parts of Africa. The use of Khat is predominant in East Africa, whereas most parts of Southern Africa use New Psychoactive substances (NPS) [57]. In African countries, among adolescent students, Tobacco, illegal drugs, and alcohol were reported most commonly used substances, and these substances are linked to mental distress, suicidal ideation, sleeping disorders, and Truancy [50].

Uganda has a relatively high prevalence of substance abuse and occurrences are more in males than females. Alcohol (23.3%) was the most abused substance, followed by Kuber (10.8%), khat (10.5%), Aviation fuel (10.1 %), cannabis (9.2%), and tobacco (5.9%) **(1)**. This is commonly observed among people within the age range of 15-24 years. Also, in psychiatric patients, cannabis was among the most abused substances in Uganda

and was used in combination with other substances like alcohol, cocaine, heroin, pethidine, Tobacco, Khat, and Kuber [6].

2.2 Classes of Abused Substances

Classes of substances most used in Uganda include opioids, cannabis, cocaine, amphetamine-type stimulants, and Khat.

2.2.1 Opioids

Derived from poppy seeds, opioids are substances used to alleviate pain by affecting the nervous system and include illegal drugs like Heroin and prescription pain medicines like Morphine, Codeine, Tramadol, Pethidine, Methadone, oxycodone, hydrocodone, and many others. Opioids are powerful analgesics that function by reducing the sensory, discriminatory, and emotional components of pain. However, when opioids, whether legally prescribed or available over the counter, are misused, they can give rise to various health problems and risks [45]. Opioids possess potent physical and psychological effects that carry significant risks of addiction and overdose. These effects manifest when the drug overwhelms the body, leading to respiratory depression and cardiac arrest. Cardiac arrest is associated with opioid overdose and is linked to the malfunction of the electricity of the heart causing irregular heartbeat (arrhythmia) or stopping the heart altogether. This sudden loss of heart function leads to the pulse becoming absent or extremely weak, resulting in the loss of consciousness, and cessation of breathing. Without immediate medical intervention, such as cardiopulmonary resuscitation (CPR) and the use of an automated external defibrillator (AED), cardiac arrest can be fatal within minutes [65]. Inappropriate use of opioids often leads to addiction, and the development of psychiatric disorders, such as depression, anxiety, bipolar disorder, and schizophrenia [21]. Prolonged opioid use can induce changes in the amygdala, a region of the brain involved in emotional processing, resulting in increased levels of anxiety and depression [2, 54 &35].

Methadone, an opioid medication, carries the risk of abuse and addiction, particularly when used incorrectly or more than prescribed doses. Misuse of methadone can lead to physical dependence, addiction, alteration of reward systems (the decreased

ability of the brain to experience pleasure from natural or any non-drug related activities), and the emergence of psychiatric disorders, including bipolar disorder, anxiety, depression, or psychosis. Discontinuing methadone use can result in anxiety, depression, insomnia, and flu-like withdrawal symptoms [40]. **Tramadol and Pethidine** are other synthetic opioid analgesics used for pain management including labour pains and as adjuncts to anaesthesia [32]. These drugs possess the potential for abuse and the onset of psychiatric disorders such as substance use disorder, depression, anxiety, and psychosis after prolonged or excessive use [16].

2.2.2 Cannabis

This is a hallucinogenic drug made from the cannabis plant, commonly called marijuana. It is the most used illegal drug in the world. Studies indicate that Δ9-tetrahydrocannabinol is a key component responsible for cannabis' psychoactive effects [62]. Cannabis can also cause psychosis, especially when used frequently and in high doses [17]. Psychiatric disorders influenced by cannabis use include schizophrenia, depression, anxiety, and bipolar disorder [58]. Caution and moderation are required to minimize the risk of side effects of cannabis when it is prescribed for medical purposes.

2.2.3 Cocaine

Cocaine is an addictive central nervous system stimulant made from the coca plant, used primarily in the form of cornstarch-like cocaine powder snorted through the nose, or the crack rock inhaled as vapours of the heated cocaine crystals. This substance is associated with both substance abuse disorders and psychiatric disorders. It is a highly addictive substance that puts a person’s life at risk by impairing the brain's ability to produce serotonin and dopamine, two chemicals that regulate mood and cause substance-related mood disorders characterized by mood swings [43, 5]. Continuous cocaine use reduces the brain's ability to produce these chemicals, which can lead to depression [46]. Cocaine users experience psychotic symptoms such as anxiety, delusions, and hallucinations, a condition known as cocaine-induced psychosis. Some of these symptoms are particularly severe during cocaine withdrawal [3].

2.2.4 Amphetamine-Type Stimulant

Amphetamine along with methamphetamine, dexamphetamine, and ecstasy, appear as synthetic substances with cocaine-like properties that affect the central nervous system and pose serious health risks [45]. Amphetamine use causes negative physical and psychological symptoms such as depression, anxiety disorders, bipolar disorder, schizophrenia, and psychosis. Also, continued use of amphetamines leads to memory impairment, and sleep disturbances [38].

2.2.5 Khat

The origin of the Khat substance is believed to be from East Africa and the Arabian Peninsula, khat is also known as the *Catha edulis* plant. Cathinone and Cathine are the main psychoactive compounds of Khat leaves, stimulants that generate sociability, excitement, loss of appetite, and mild euphoria effects and they are responsible for addictive effects [51]. Khat abuse has been associated with psychotic effects such as hallucinations, delusions, and paranoia [47]. Khat can disrupt normal sleep patterns and cause insomnia, and excessive daytime sleepiness. Chronic khat use may also contribute to the development or exacerbation of depressive symptoms and can lead to major depressive disorder [42].

2.3 Substance Abuse and Psychiatric Disorders

It is essential to understand that substance abuse is a complex issue influenced by various factors, including the addictive properties of certain drugs, psychological factors, social environments, and personal circumstances. Illegal use of drugs causes various adverse effects on an individual's health, relationships, and overall quality of life, thus seeking help and support is crucial for those affected. Abused substances bring up symptoms of psychiatric disorders and can trigger the onset of new symptoms. Among the psychiatric disorders caused by these drugs include Anxiety, depression, attention-deficit hyperactivity disorders, bipolar disorders, personality disorders, and schizophrenia, *National Institute of Mental Health* (*NIMH), 2023*. A higher prevalence of substance abuse and psychiatric disorders such as depression, anxiety, bipolar disorder,

and schizophrenia were found common in people with issues of substance abuse [13]. Drugs are always used to calm peoples' nerves and numb their negative emotions leading to drug abuse and addiction that eventually elevate to anxiety and depression disorders. Stimulant drugs like cocaine or prescription amphetamines are used to improve focus and concentration, and drugs and alcohol used to cope with traumatic memories and flashbacks lead to Attention- Deficit Disorders (ADD) and Post Traumatic Stress Disorders (PTSD)respectively [8]. 80% of 538 adolescents suffered from various types of psychiatric symptoms, 60% of both sexes had anxiety symptoms where 51% of girls had depression and 47% of boys had ADD [53]. The relationship between substance use disorders and personality disorders shows that people with substance use disorders are significantly more likely to have comorbid personality disorders, particularly antisocial and borderline personality disorders [56]. For psychiatric patients who used controlled prescription drugs for non-medical purposes, out of 1,275 participants, 145 patients had used drugs, especially benzodiazepines, and 36 used illicit drugs [30].

2.4 Identification of Abused Substances

Identification of abused substances comprises screening and evaluating drug use, this involves using techniques like self-reporting tests, biological screening techniques, or both which act as fundamental techniques in illicit drug screening and assessment, both techniques can be utilized to enhance each other for adequate data about the explored substances. Self-report can provide information about the drug history, gestation, and level of drug abuse while biological testing involves the use of body fluids (urine, blood sweat and saliva) or tissues (hair and nails) to determine the presence or absence of substance abuse [64]. Biological testing can be used to validate the history of drug use or detect unreported drug use based on self-report [12]. The purpose of screening is to establish the abused substance, measure the degree of repetitive use, determine the complexity of substance abuse, investigate risky behaviors, identify medical, social, and mental health problems, determine the need for treatment and the extent of change, as well evaluate the relevance for the alternative medication [45]. Given the association between substance abuse and psychiatric disorders, accurate estimation of health complications requires the use of appropriate diagnostic techniques. The fact that self-reporting can't provide all

information required for treatment about drug use due to individual perspectives like failure to remember information, individuals choosing to give false information or not giving any, lays elective utilization of biological testing where reliable information obtained evenhanded regarding the sample utilized. Biological techniques help to categorize abused substances and determine the amount of the substances using chromatographic analytical instruments like Liquid Chromatography (LC) [36], Gas Chromatography (GC) with an MS detector, instruments that are susceptible to even detecting trace amounts of a compound in a substance and have been used worldwide as the best techniques in the detection of drugs and their metabolites due to their powerful rates of sensitivity and specificity to identify individual components in a class of drugs[64]. Categories of common biological samples used for identification of drugs of abuse include.

2.4.1 Urinalysis,

Urine testing is currently regarded as the best strategy for detecting substances of abuse. Urine is excreted in sufficient quantities, is easy to collect, and contains large amounts of drugs and metabolites after immediate consumption [14, 64]. However, urine collection has more chances of tampering with the sample by dilution or addition of other substances if the exercise is not carefully supervised and is also limited by the short time window for drug detection for less than 3 days [14].

2.4.2 Blood analysis,

Like urine, blood is used for determining drugs and their metabolites days after the last intake. This method is suitable for quantitative analysis in forensic toxicology laboratories for cases of drink-drive to ascertain the blood alcohol concentration (BAC), sudden death, toxicity, or when there is suspected poisoning and can be used in therapeutic drug monitoring to determine treatment overdose of drugs. Besides that, blood carries risks of transmitting pathogenic risks like hepatitis and HIV in the event of sample collection, it requires difficult storage conditions, and its analytical techniques are complex for unskilled or trained personnel [65].

2.4.3 Oral fluid analysis

This approach uses saliva as the sampling tool for the detection of abused substances that have just been used for less than 48 hours [60, 64]. The technique is commonly used in occupational areas such as workplace substance abuse and drug driving [63]. Oral fluid is easy to collect, has moderate supervision, and is less invasive which makes it a first-line screening technique for drugs of abuse. Oral fluid testing is limited by the short window of detection, requires enough supervision whereby a person is not supposed to eat or drink before sample collection and the mode of drug consumption limits the presence of the drugs if the person used drug injection [14].

2.4.4 Hair analysis,

Hair one of the biological samples used in the detection of drugs, can provide useful information about substance abuse. In hair, deposited drugs are detected after long-term uptake, and segmental analysis of hair can detect single or multiple drug deposits over time. Hair analysis has been used around the world by an increasing number of forensic laboratories and in various areas of clinical toxicology particularly in psychiatric patients as a unique material for retrospective determination of chronic or unintentional drug use [7]. In circumstances of knowing the history of past drug use, hair analysis was used to verify the limitations of self-report tests [34]. Hair is less invasive and easier to collect and store. Collection requires less skills, and supervision, and does not need complicated storage conditions compared to other biological samples [59, 18]. In other fields, hair analysis has been utilized in various areas including,

Workplace drug testing, like in recent studies, amphetamine substances, cocaine, and metabolites were established in workplace groups that were tested for drug abuse. The results of drug tests for amphetamine-type stimulants of a working group were compared with those of drug users under rehabilitation and established a very close similarity for both results [22, 9-10].

Driving license renewal, fitness assessment to grant or renew driving license in Germany, Italy, and Spain, drug addicts and former addicts were denied licenses following the examination system of drug abuse using hair analysis. The employment and driver aptitude tests conducted among people using urine and hair, and hair analysis

showed higher sensitivity in the detection of abused drugs compared to urine testing [34, 11 & 19].

Compliance with drug substitution/ maintenance therapy, treatment programs, and therapeutic drug monitoring helps to discover the illegal use of other drugs. Reduction of heroin injection examined by hair testing in prisoners was observed as a sign of an effective methadone treatment program, also hair testing was used to determine the absence of codeine in a controlled heroin maintenance program. For instance, morphine and heroin consumption can be distinguished by heroin metabolite (6-MonoAcetylMorphine) incorporated into hair [14].

Drug-facilitated crimes, in circumstances where the substances used to facilitate crimes are not found or obtained in blood and urine or for single-used substances, the substances can be found in hair by segmental analysis. The technique has been used in the investigation of sexual assault cases, chemical abuse in the elderly, and poison cases of methadone in children. It was discovered that the concentration of drug exposure determined in hair for drug-facilitated cases was higher in reported casework than in the controlled doses, [66].

In doping control, in the examination of anabolic steroids, hair analysis plays a significant role in circumstances of large detection windows to provide improved results of the drug test. However, urine and blood are better approaches, and hair analysis also acts as a better complement in the field of doping control [22, 15].

In light of the available studies, hair analysis can be reliably used for the detection of abused substances to provide valuable information that can be used in clinical and forensic investigations. Our study therefore intends to utilize hair specimens to detect misused substances in psychiatric patients admitted to Butabika Hospital's alcohol and drug unit in Uganda.

3. MATERIALS & METHODS

3.1 Chemicals

Amphetamines, cannabinoids, cannabinol, Delta-9-THC, morphine, cocaine, codeine, MDA, MDMA, methamphetamine, cathine, cathinone, tramadol, and pethidine were supplied from Lipomed Services for health. Acetone, dichloromethane, distilled water, methanol, ammonium formate, and formic acid were used as solvents. All solvents were mass spectrometric grade. Chlorpheniramine was provided by Kampala Pharmaceutical Industries as a donation.

MYSPIN 12 model thermos-scientific centrifuge from China, filtered pipettes, and a GT Sonic D13 model professional ultrasonic cleaner from China were among the equipment and apparatus used.

3.2 Preparation of Standards and Solutions

A working solution of 250ng/l of 14 target compounds (amphetamines, cannabinoids, cannabinol, Delta-9-THC, morphine, cocaine, tramadol, pethidine, MDA, MDMA, and methamphetamine) was prepared in methanol from a stock solution of 1000ng/l stored at -20^{o}C, chlorpheniramine (internal standard) was also prepared at a concentration of 25ug/l.

3.3 Study Design

The present study was purposed to evaluate the association between substance abuse and mental health outcomes by analyzing the hair samples of psychiatric patients. For this purpose, the hair samples were collected from patients staying in Butabika Hospital's Alcohol and Drug Unit. Also, questionnaires for patients were performed to evaluate the sociodemographic characteristics of patients and obtain their self-report for substance use. The present study was conducted under ethical approval by the Ethical Commission of Cukurova University (17-02-2023- 21948), Makerere University, Research Ethics Committee (CoVAB.MSC3.2023) and Butabika Hospital Research Administration. Patients were informed about the study and for the collection of hair

samples and questionnaire, all consent forms were obtained from patients. The data collected was strictly for this study and not for any other purpose. All information was kept private and treated with the confidentiality it deserved. Hair drug testing focused on assessing the concordance between the hair analyzed at the time of self-report, and collateral informants' reports.

3.4 Sample Preparation

Sample preparation and extraction were carried out according to previous studies and Guidelines (Society of Hair Testing [19], the guidelines for testing drugs under international control in hair, sweat, and oral fluid [19].

3.4.1 Collection of hair samples

Hair samples were collected from 154 patients between 18- and 50 years old hospitalized at Butabika Hospital's Alcohol and Drug Unit and met the inclusion criteria of the study. Patients who had undergone hair treatment or hair colouration were excluded from the study. The consent forms were provided for each patient before the hair collection. Patients that were recruited with hair of at least 1cm, were provided 50 to 100 mg of hair, this was collected from the occipital region of the participants' heads onto an aluminium foil using cleaned scissors. The samples were wrapped into the foil, put in an envelope, and labelled with sample ID. The drug-free hair specimens for the preparation of control samples were obtained from drug-abstinent volunteer forensic scientists at the Uganda Police Forensic Laboratory.

3.4.2 Decontamination and Homogenization

Hair samples (50mg) were washed with 2ml of dichloromethane three times. After the last wash, the samples were dried with absorbent paper (filter paper). Hair samples were homogenized/pulverized by cutting into small segments 1-3mm using scissors.

3.5 Methanol Extraction and Analysis

In the present study, the homogenized hair samples were put in 1.2 ml of methanol. After that, samples were incubated in the ultrasonic bath at 40^{o}C for 18 hours to remove substances from the hair matrix into the solvent. Then samples were centrifuged at 4000rpm for 15 minutes. The supernatants were removed into a vial and evaporated to dryness.

The analyte was then reconstituted in 800ul of methanol in an autosampler vail of 1.5ml for analysis.

Analyte samples were analyzed on a Shimadzu HPLC-hyphenated to an 8060-NX Mass spectrometer. The reconstituted analytes were vortexed for 5 seconds after adding an internal standard. Each sample was analyzed in replicates, and a blank solution with an IS was analyzed before each analyte sample. The data of the runs was analyzed using Shimadzu LabSolutions Insight Software. Slopes of the calibrations for spiked analytes were used for calculating the unknown concentration of the analyzed samples.

3.6 Method Development

The Laboratory's existing method for drug analysis was used to develop the method specifically for testing substances reported during the survey. The mass spectrometer was operated in Flow injection analysis mode to optimize the transitions of the target compounds. The analytical method had a run time of 10 minutes with a mobile phase program starting with 95% A that is decreased to 70 % A in 2.5 minutes and then to 100% B in the next 1.5 minutes. This was maintained for 1 minute and then dropped back to 5% B in the next 0.1 minutes. This mixture was maintained for the next 3 minutes before the next injection started. The mass spectrometric instrument used had the capability of switching between positive and negative modes although all analytes were detected in positive mode. The mobile phase had formic acid and ammonium formate as organic modifiers and the mass spectrometry instrument was operated in the ESI mode. Optimization of each target compound for the analytical method consisted of product ions, collision energies, and Retention time determined in. (*Table 1*)

Table 1. Drug Transitions and Retention times monitored for each analyte

Compound	***MSMS-Transitions ([2]m/z)***		***[1]CE***	***[2]Rt***
	Precursor ion	***Product ions***		
Codeine	*300.15*	*165.1, 44.1*	*-41, -30*	*3.4*
Morphine	286.15	165.1,153.1	*-40, -45*	*1.8*
Amphetamine	*136.1*	*91.05,119.05*	*-17, -15*	*3.9*
Methamphetamine	*150.1*	*91.05,119.1*	*-19, -10*	*3.5*
MDA	*180.1*	*105.1,77*	*-23, -40*	*3.8*
MDMA	*194.1*	*163.05,105.1*	*-13, -25*	*4.2*
Cocaine	*304.15*	*182.25,82.1*	*-21, -35*	*3.4*
Cannabinol	*311.25*	*223.1,238.25*	*-22, -18*	*5.9*
Cannabidiol	*315.2*	*259.2,193.2,203.05*	*-18, -22, -21*	*6.0*
Delta-9-THC	*315.22*	*193.3,259.2,203.15*	*-24, -20, -21*	*5.9*
Cathine	*152.1*	*134.05,117*	*-13, -15*	*2.7*
Cathinone	*150.08*	*132.05,117.05*	*-16, -25*	*2.7*
Pethidine	*248.16*	*70.05,174.1*	*-32, -20*	*4.7*
Tramadol	*264.19*	*58.2,246.185*	*-25, -25*	*4.6*
Heroin	*370.16*	*58.15, 44.1*	*-30, -40*	*4.6*
Chlorpheniramine (IS)	*275.12*	*230, 167.05*	*-15, -40*	*4.9*

3.7 Validation Parameters

The method was validated according to the guidelines provided by the Society of Toxicological and Forensic Chemistry (GTFCh), which gives acceptable performance standards for defining analytical methods. The following performance parameters were evaluated: Limit of Detection (LoD), Limit of Quantification (LoQ), Selectivity, Linearity, and Recovery using drug-free hair spiked in methanol at 0.5, 5, 10, and 20,100

ng/ml for Delta-9-THC, cannabinol, cannabidiol, codeine, amphetamine, MDMA, methamphetamine and MDA.

3.7.1 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The instrument detection and quantification limits for target compounds were determined by using the mean peak area ratio of the noise at the retention time of each analyte, obtained from spiked matrix samples, the standard deviation from the analysis of the figures from the blank injections, and the slope of the calibration curve. The parameters were used in the formulas below.

$$LoD = \frac{\bar{A}A/IS + 3.\delta n}{b} \qquad Eqn.1$$

$$LoQ = \frac{\bar{A}A/IS + 10.\delta n}{b} \qquad Eqn.2$$

Where ĀA/IS is the mean peak area ratio of the noise at the retention time of each analyte, δn is the standard deviation of the peak area ratio from the analysis of the figures from the blank injections and b is the slope of the calibration curve.

3.7.2 Evaluation of Method Linearity and Selectivity

Five blanks were analyzed to determine the method selectivity and interfering peaks were then crosschecked at the respective retention time windows of the target analytes for verification of the analyte identity. Calibration graphs for target analytes were also fitted for the linearity of the method. Carryover of the analyte was eliminated by injecting a blank solvent before analyzing a real sample.

The analytical methods' limits of detection (LoD) and limits of Quantification (LoQ) used for quantifying cannabidiol (CBD), cannabinol (CBN), Delta-9-THC, codeine and amphetamine, methamphetamine, MDMA and MDA in hair samples ranged from 27.1pg/mg to 125.2pg/mg and 43.8pg/mg to 265.1pg/mg, respectively (Table 2).

Table 2. Instrument features of the method for determination of target analytes

Compound	[1]LoD (pg/mg)	[2]LoQ (pg/mg)	[3]r	Recovery (%)			
				5ng/ml	10 ng/ml	20 ng/ml	100 ng/ml
Cannabidiol	28.4	61.6	0.997	94	99	100	101
Delta-9-THC	27.1	43.8	0.996	96	99	100	100
Cannabinol	30.2	48.0	0.999	97	99	100	102
Codeine	113.3	198.4	0.999		98	101	101
Amphetamine	125.2	265.1	0.999		100	99	110
MDMA	109.8	178.6	0.999		99	101	103
Methamphetamine	96.5	165.0	0.995		98	100	101
MDA	108.7	250.4	0.997		98	100	100

[1]Limit of Detection, [2]Limit of Quantification, [3]r- regression coefficient

3.8 Statistical Analysis

The statistical analyses were performed using Microsoft Excel, for determining the number and percentage of demographic parameters of patients involved in this study, calculating the concentration of substances in hair from extrapolated values of the calibration graphs and graphical representation. Also, GraphPad Prism version 9 was used for estimating the graphical presentation of the distribution of analyte concentration in hair samples.

4. RESULTS

4.1 Demographic Parameters

The sociodemographic characteristics of patients were evaluated by a survey and are presented in Table 3. In total 154 patients participated in the present study. The patients were 76.62% male and 23.38% female. The average age of the patients with hair analyzed was 29.77 years (SD= 8.299). According to the education levels, patients were categorized into four categories: none (1.95%), high school (47.40%), tertiary (22.73%), and graduates (27.92). The marital status of the patients was, married 17.53% and 82.47 % not married.

Table 3. Participant Demographic Parameters

	Total participants (n=154) **% (*n*)**
Gender	
Male	76.62 % (*n= 118*)
Female	23.38% (*n= 36*)
Marital status	
Married	17.53% (*n= 27*)
Not married	82.47% (*n= 127*)
Education level	
Graduates	27.92% (*n= 43*)
Diploma/Tertiary	22.73% (*n= 35*)
High School	47.40% (*n= 73*)
None	1.95% (*n= 03*)
Age Bracket (*Years*)	
<20	9.09% (*n= 14*)
20-25	24.68% (*n= 38*)
26-30	33.77% (*n= 52*)
31 -35	7.79% (*n= 12*)
36 -40	11.69% (*n= 18*)
>40	12.99% (*n= 20*)

4.2 Self-reported Substance Abuse

The self-report about the drug use history of patients was obtained. All patients (n=154) reported and revealed exposure to substances including cannabis, cocaine, heroin, khat, pethidine, tramadol, amphetamine, and MDMA. The prevalence of these drugs was determined in (Table 4. and Figure 1). Cannabis use was reported higher at (62.99%), whereas amphetamine use and cocaine use were reported lower at 0.65%.

Table 4. Self-reported substance use

Substance Use	Cannabis	Cocaine	Heroin	Khat	Pethidine	Tramadol	Amphetamine	MDMA
[1]Self-report (+)	62.99% (n=97)	0.65% (n=1)	3.32% (n=5)	31.82% (n=49)	12.34% (n=19)	8.44% (n=13)	0.65% (n=1)	2.6% (n=4)
[2]Self-reported (-)	37.01% (n=57)	99.35% (n=153)	96.75% (n=149)	68.18% (n=105)	87.66% (n=135)	91.56% (n=141)	99.35% (n=153)	97.4% (n=150)

[1] refers to patients who self-reported substance use, and [2] refers to patients who self-reported non-substance use.

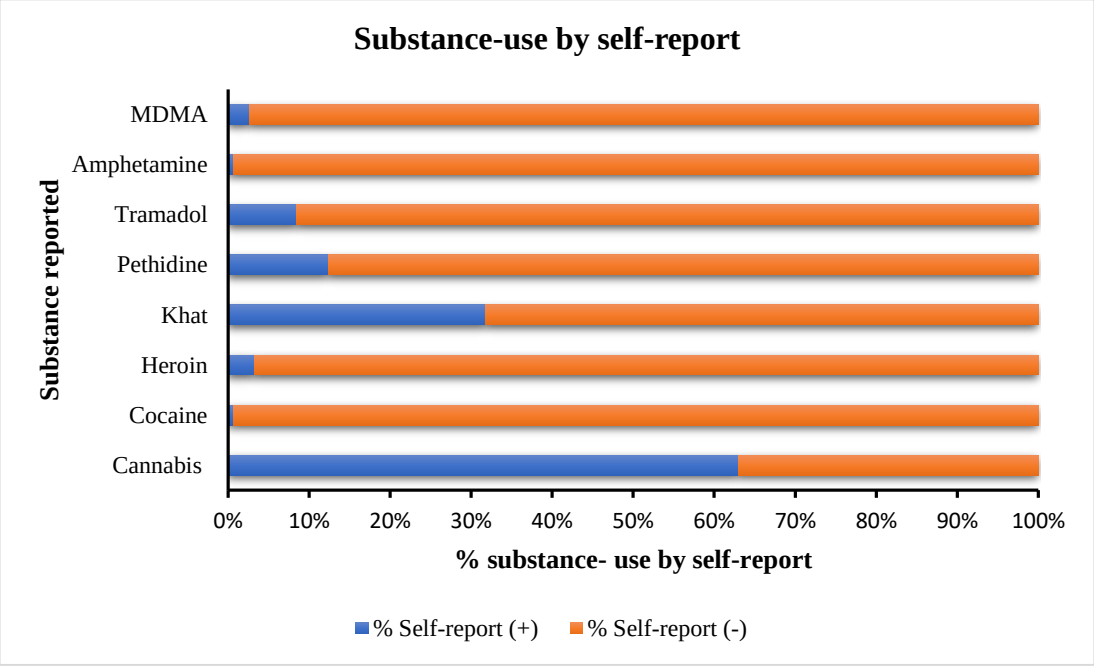


Figure 1. Bar graph shows self-reported substance use.

4.3 Substance Use in Hair Samples.

The substance detections in hair samples were performed in 154 samples. Cannabis was detected higher in hair samples, in 82.47% of patients. Also, the use of

morphine, codeine, MDA, or methamphetamine was determined in the hair samples of patients (Table 5, Figure 2)

Table 5. Percentage of patients with substance abuse detected /undetected in hair samples

	Cannabis	**Cocaine**	**Heroin**	**Khat**	**Pethidine**	**Tramadol**	**Amphetamine**	**MDMA**	**Morphine**	**Codeine**	**MDA**	**Meth**
[1]Hair (+)	82.47 % (n=127)	3.9 (n=6)	11.69% (n=18)	57.14% (n=88)	24.67 % (n=38)	26.62 % (n=41)	22.08% (n=34)	14.93% (n=23)	6.49% (n=10)	11.69 % (n=18)	50 % (n=77)	20.13% (n=31)
[2]Hair (-)	17.53 % (n=27)	96.1 % (n=148)	88.31% (n=136)	42.86% (n=66)	75.32 % (n=116)	73.38 % (n=113)	77.92% (n=120)	85.09% (n=131)	93.51 % (n=144)	88.31 % (n=136)	50 % (n=77)	79.87% (n=123)

[1] *refers to patients in whom the substance was detected, and* [2] *refers to patients in whom the substances were undetected.*

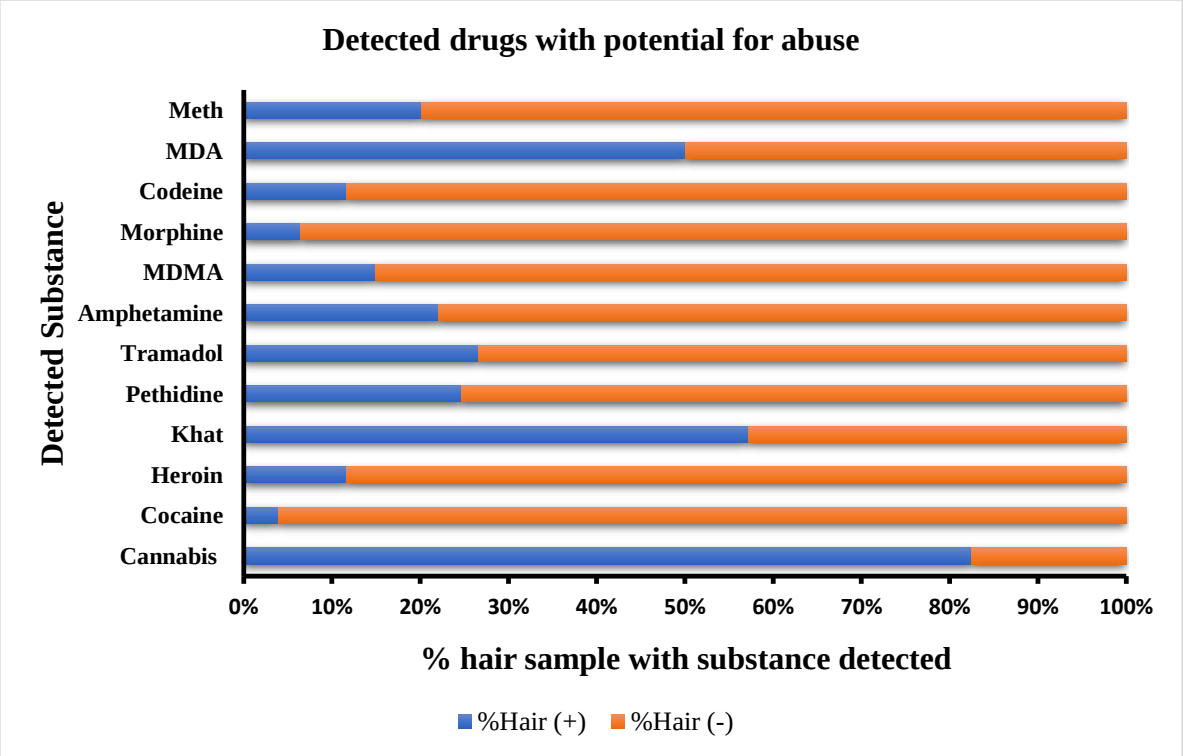


Figure 2. Bar graph shows the percentage of hair samples with substance detection

4.4 Concordance Between Hair Analysis and Self-report.

The survey results about the substance abuse declaration were evaluated by hair analysis.

Also, the detected substances in hair samples and self-reported substance-use were compared in (Table 6 and Figure 3).

Table 6. Hair analyzes relative to self-reported substance use.

	Cannabis	**Cocaine**	**Heroin**	**Khat**	**Pethidine**	**Tramadol**	**Amphetamine**	**MDMA**	**Morphine**	**Codeine**	**MDA**	**Meth**
[1]Hair (+)	82.47% (n=127)	3.9% (n=6)	11.69% (n=18)	57.14% (n=88)	24.67% (n=38)	26.62% (n=41)	22.08% (n=34)	14.93% (n=23)	6.49% (n=10)	11.69% (n=18)	50% (n=77)	20.13% (n=31)
[2]Self-report (+)	62.99% (n=97)	0.65% (n=1)	3.32% (n=5)	31.82% (n=49)	12.34% (n=19)	8.44% (n=13)	0.65% (n=1)	2.6% (n=4)	0% n=0	0% n=0	0% n=0	0% n=0
[3]Hair (-)	17.53% (n=27)	96.11% (n=148)	88.31% (n=136)	42.86% (n=66)	75.32% (n=116)	73.38% (n=113)	77.92% (n=120)	85.09% (n=131)	93.51% (n=144)	88.31% (n=136)	50% (n=77)	79.87% (n=123)
[4]Self-reported (-)	37.01% (n=57)	99.35% (n=153)	96.75% (n=149)	68.18% (n=105)	87.66% (n=135)	91.56% (n=141)	99.35% (n=153)	97.4% (n=150)	100% n=154	100% n=154	100% n=154	100% n=154

[1] *refers to patients in whom the substance was detected,* [2] *refers to patients that self-reported substance use,* [3] *refers to patients in whom the substances were undetected and* [4] *refers to patients that self-reported substance non-use.*

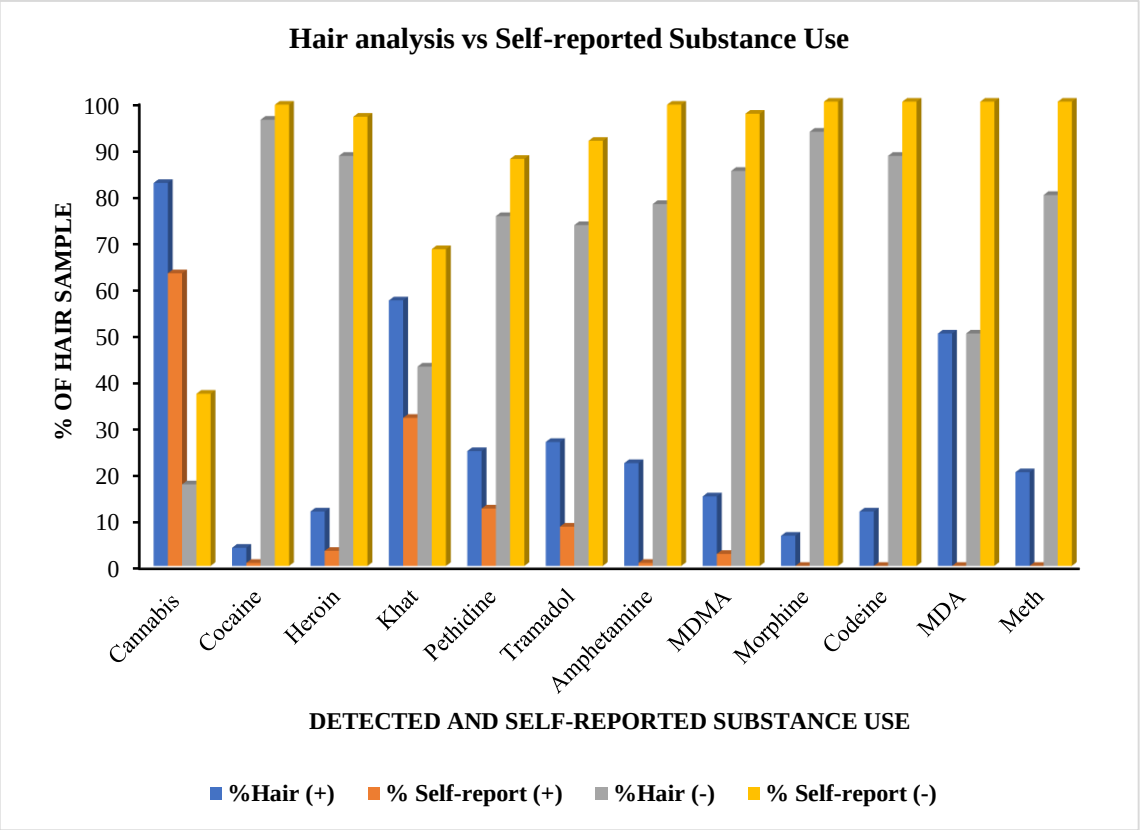


Figure 3. Bar graph shows detected substance use in hair samples relative to self-reported substance use.

4.5 Concentration of Substances Use in Hair Samples

In hair samples obtained from the participants, the concentration of cannabinol (59.7 – 1045 pg/mg), cannabidiol (61.7 - 665.7 pg/mg), Delta-9-THC (46.9 - 928.2 pg/mg), codeine (217.1 - 1845.5 pg/mg), amphetamine (266.9 - 512.2pg/mg), MDMA (187.5 – 489 pg/mg), methamphetamine (169.3 - 788.6 pg/mg) and MDA (256 – 1008 pg/mg) substances in hair above the limit of quantification was determined, 1845.5 pg/mg of codeine was the highest amount detected (Table 7). Also, the number of samples below and above the limits of detection and Quantification were determined (Table 8),

Table 7. Concentration of drug analytes

Sample	Concentration of analytes - pg/mg of hair							
	CBD	**THC**	**CBN**	**Codeine**	**Amphetamine**	**MDMA**	**Methamphetamine**	**MDA**
S-1	237.4	120.0	192.2			204.4		
S-2				898.8				
S-3								
S-4	301.0	134.6	344.2	346.8				272.0
S-5	86.3	168.9	64.2	802.0				296.0
S-6								168.0
S-7	229.0	54.2	1045.5				139.4	928.0
S-8								164.0
S-9	61.7	383.0						800.0
S-10	396.3	894.6	894.4				141.7	168.0
S-11	195.5					286.1		
S-12	53.3	185.0						638.4
S-13		40.3						
S-15		55.7			340.4			
S-16								
S-17								
S-18							205.2	
S-19	183.0	200.4	254.4					
S-20	278.4	536.5	803.7	476.6				246.4
S-21	54.6	58.6	99.7	217.1			623.5	
S-22	200.6	456.1	305.1				379.4	
S-23	99.3	402.0	636.6					
S-24	96.8		442.0	886.4	292.6	139.1	246.7	280.8
S-25					280.4			
S-26	285.6		508.5					
S-27	61.7				358.7			617.6
S-28	513.5		41.1					
S-29	400.5		766.4	171.7				
S-30	413.1	141.9	490.8		71.1		195.1	60.0
S-31	68.4	190.1	406.4					
S-32	151.6	332.6	99.7					
S-33	29.5		237.5					
S-34	75.1	0.9	593.1		275.5			
S-35	443.2	195.3	213.5					
S-36	314.8	112.7	944.2		155.6		169.3	480.8
S-37	145.8	84.9	431.3		311.0		195.1	261.6
S-38			10.0		138.4	163.0	91.9	12.8
S-39								464.0
S-40		331.9	156.6		266.9	163.0		25.6
S-41		25.0						616.0
S-42	229.0	163.8	23.3		71.1			360.0

S-43					254.7			688.0
S-44	61.7					59.1		448.0
S-45								540.0
S-46		98.1				145.5		213.6
S-47								231.2
S-48								768.0
S-49								
S-50	204.7	5.3	263.3	1267.7				
S-51								
S-52		32.3						
S-53	99.3	215.0	355.3					
S-54	414.3	17.7	814.4					
S-55	509.3	274.9	93.5				102.2	245.6
S-56	254.1	25.0	316.6					
S-57	82.6	317.3	975.3					
S-58	54.1	346.5	565.5					
S-59	178.4	1.6	757.3				48.0	
S-60	145.3	60.8	181.5					
S-61	203.9	159.4	190.4		46.6			376.0
S-62	64.6	346.5	489.1					
S-63	195.5	456.1	40.2		169.0			608.0
S-64					156.8			
S-65	95.1	317.3						
S-66							298.3	
S-67			512.2					
S-68	429.8	478.0	122.0	716.6				
S-69	257.4	68.8	220.6					
S-70	300.1	346.5	94.4		58.9		218.3	832.0
S-71	157.9	635.9	942.4					
S-72	76.8	310.0	88.2				102.2	269.6
S-73	86.8	244.2	156.6					1008.0
S-74	95.1	54.2	106.0			9.5		
S-75	1.4	602.3	361.1				143.5	
S-76	229.0	310.0	620.6					
S-77	643.2	83.4	183.3				298.3	
S-78	95.1	32.3	106.0					
S-79	454.9	207.7	915.7		426.0			
S-80					415.0			
S-81		17.7			125.0			763.2
S-82	99.3	163.8	24.2					
S-83	61.7	310.0	512.2		71.1			
S-84	30.8	8.8	174.4					
S-85	145.8	25.0						
S-86	57.5	353.8	334.4		405.2			472.0
S-87	284.2	200.4	73.1					

S-88	336.5	3.1	108.6	156.6				478.4
S-89		46.9	743.3		230.2		50.6	69.6
S-90					379.5	462.3		316.0
S-91		83.4						
S-92		456.1				1.4		536.0
S-93				1845.5		28.9		32.0
S-94		3.1				187.5		259.2
S-95								
S-96	114.4	317.3	352.2					
S-97	42.4	25.0	521.1				73.8	
S-98	402.6	602.3	217.1				109.9	
S-99		331.9						240.0
S-100	116.0	602.3	253.5					
S-101	605.5	478.0	42.0					
S-102	153.7	200.4	257.1					
S-103	329.4	63.0	150.4		423.6			192.0
S-104	93.5	35.2	532.6		204.5			75.2
S-105					46.6			
S-106								
S-107	199.7		78.4				633.8	
S-108	48.7	46.9	244.6			227.8		82.3
S-109								
S-110	149.5	928.2	42.8		107.8			496.0
S-111							401.5	
S-112	316.9	163.8	547.7					288.0
S-113	580.8	690.0	202.0				290.6	
S-114	236.1	83.4	85.5					
S-115					203.4			688.0
S-116	54.1				225.3	1.4		448.0
S-117	224.8	236.9	698.8				788.6	82.4
S-118	78.4	61.5	61.5		22.2		91.9	168.0
S-119	124.4		156.6		512.9			264.8
S-120	160.8		933.5			23.5		341.6
S-121	80.1		378.8			40.5		249.6
S-122	36.6		99.7					236.0
S-123	425.6		616.2					
S-124	243.2		193.1					68.9
S-125				110.4		30.7	151.2	256.0
S-126	12.3							448.0
S-127	22.8	178.4	2.8					288.0
S-128	580.4	10.4	393.1	165.5				192.0
S-129	85.5	46.9	130.8					
S-130	513.5	68.8	202.8					216.8
S-131	237.0	190.1	743.3					
S-132	206.0	624.9	416.2				249.3	220.0

S-133	351.6	368.4	257.1	343.3	443.1		
S-134	68.8	624.2	593.1				528.0
S-135	418.1	487.5	744.2	138.8			246.4
S-136						205.4	174.4
S-137	195.5	10.4	77.5				288.0
S-138	195.5	10.4	77.5				
S-139	583.3	25.0	15.3			218.3	
S-140	329.4	98.1	172.6		23.0	582.2	768.0
S-141	173.4	46.9	548.7				498.4
S-142	511.4	163.8	171.7				888.0
S-143	296.8	310.0	343.3				792.0
S-144	216.0	646.1	716.6				88.0
S-145	655.7	405.0	896.2		114.6		24.0
S-146	293.0	543.8	120.2				260.8
S-147	36.6	280.7	374.4				226.4
S-148	104.8	127.3	59.7				
S-149	237.0	25.0	325.5				
S-150				503.3			128.0
S-151	208.9	17.7	172.6	1756.6			160.0
S-152					489.8		376.0
S-153	53.3	98.1	45.5				608.0
S-154	69.2	171.1	442.0				448.0

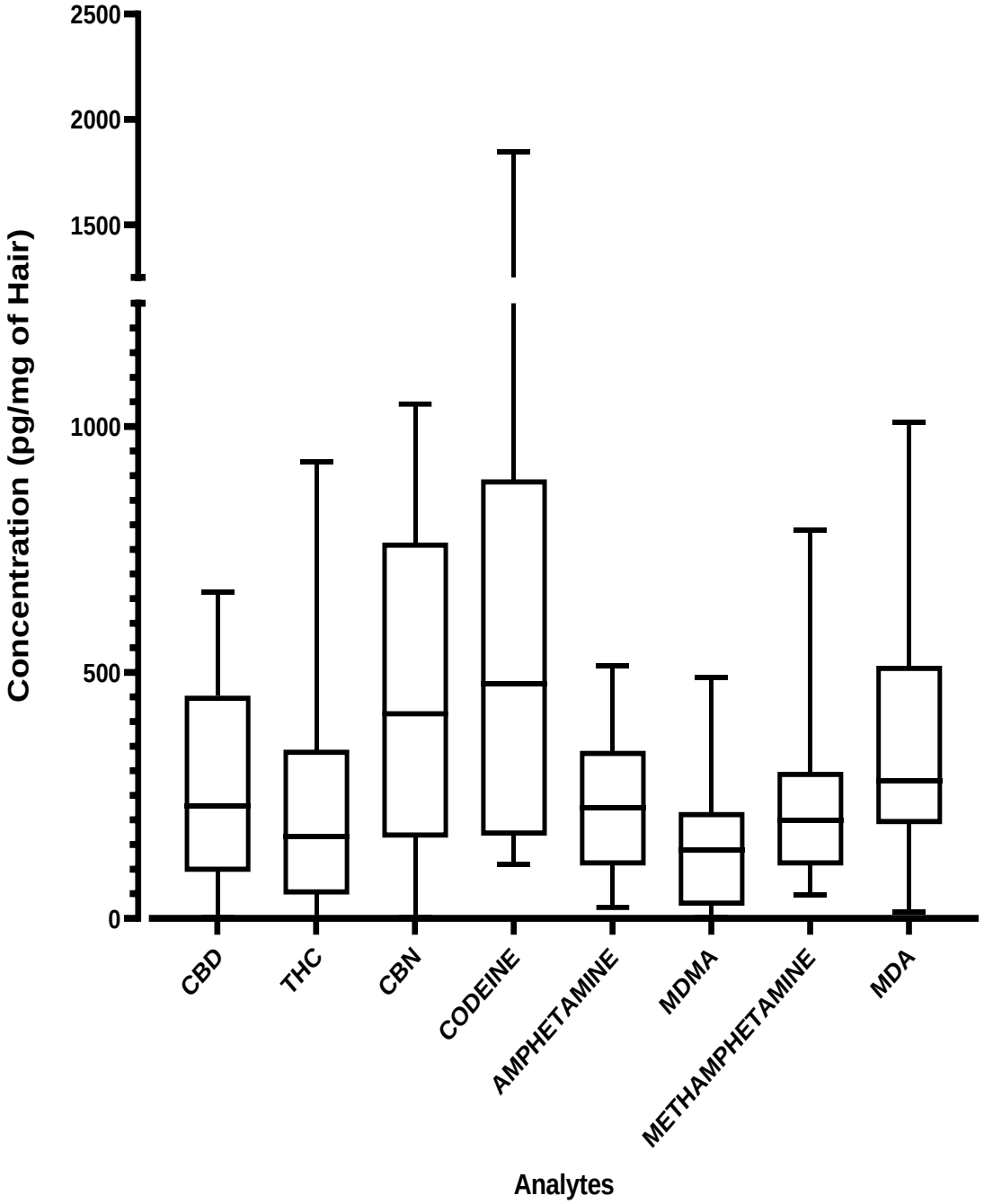


Figure 4. Concentration of drug Analytes' distribution determined in hair

Table 8. Hair sample- analytes determined below and above LoD and LoQ

	CBD	THC	CBN	Codeine	Amphetamine	MDMA	Methamphetamine	MDA
<= LoD[a]	3	18	5	1	9	9	5	11
<=LoQ[b]	15	22	10	5	18	14	12	31
>=LoD[c]	106	86	99	16	22	12	25	66
>=LoQ[d]	94	82	94	12	13	7	18	46

[a] represents hair sample concentration below the limit of detection, [b] represents hair sample concentration below the limit of quantification, [c] represents hair sample concentration above the limit of detection and [d] represents hair sample concentration above the limit of quantification.

4.6 The Association Between Substance Abuse and Psychological Disorders

In the present study, the psychological situations of patients were determined, and anxiety, depression, psychosis, eating disorders, mood and personality disorders were revealed (Table 9, figure 5). Also, the association between substance abuse and psychological disorders were evaluated for patients (Table 10, Figure 6).

Table 9. Psychological situation of patients

Psychological effects	0	1	2	3	4	5	6
Patients. n=154 (%)	5 (3.2)	48 (31.2)	44 (28.6)	41 (26.6)	16 (10.4)	0	0

	Anxiety	**Depression**	**Psychosis**	**Eating Disorder**	**Mood**	**Personality disorder**
Positive	54.55% n=84	64.94% n=100	21.43% n=33	13.64% n=21	40.91% n=63	14.29% n=22
Negative	45.45% n=70	35.06% n=54	78.57% n=121	86.36% n=133	59.09% n=91	85.71% n=132

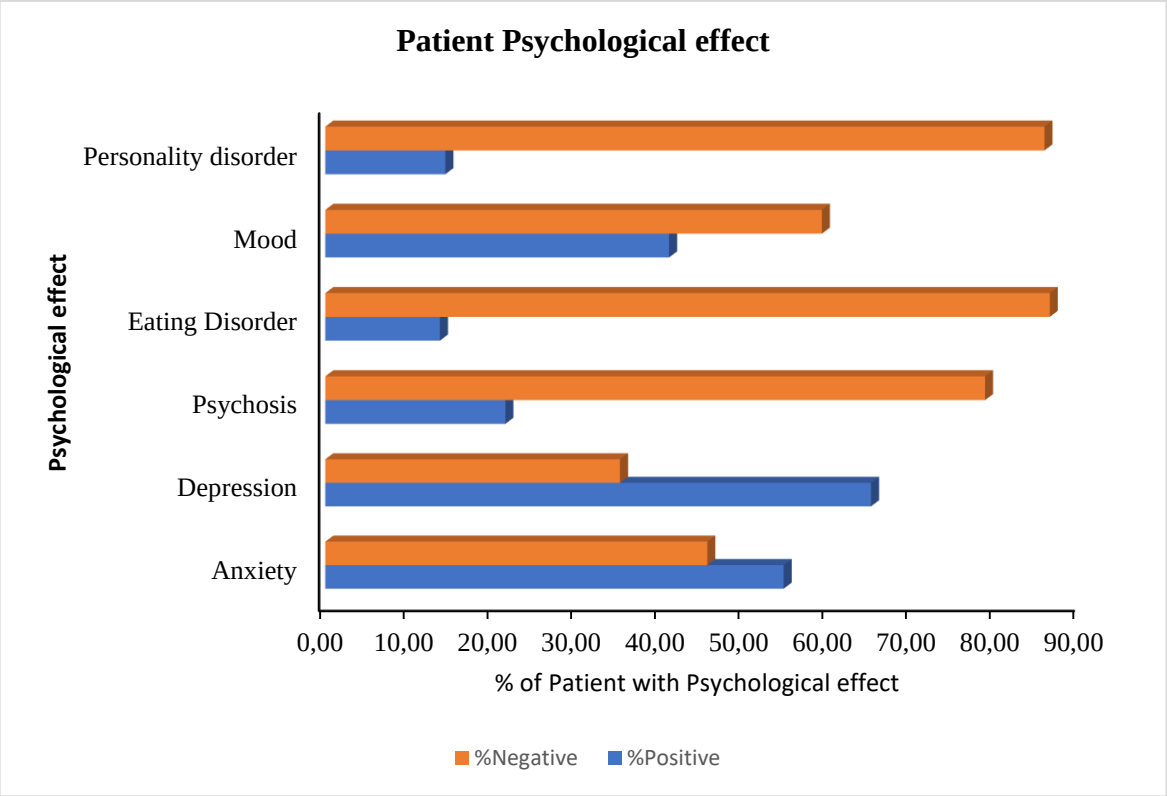


Figure 5. Bar graph shows the psychological disorders of patients according to patient reports

Table 10. Substance Use and Psychological effects

Psychological effects	Cannabis	Cocaine	Heroin	Khat	Pethidine	Tramadol	Amphetamine	MDMA
Anxiety	60	1	5	29	11	9	1	3
Depression	59	1	5	32	7	6	1	1
Psychosis	23	1	3	14	4	4	1	1
Eating Disorder	12	0	0	8	1	3	0	1
Mood	50	1	5	18	12	4	1	4
Personality disorder	19	0	0	11	6	8	0	0

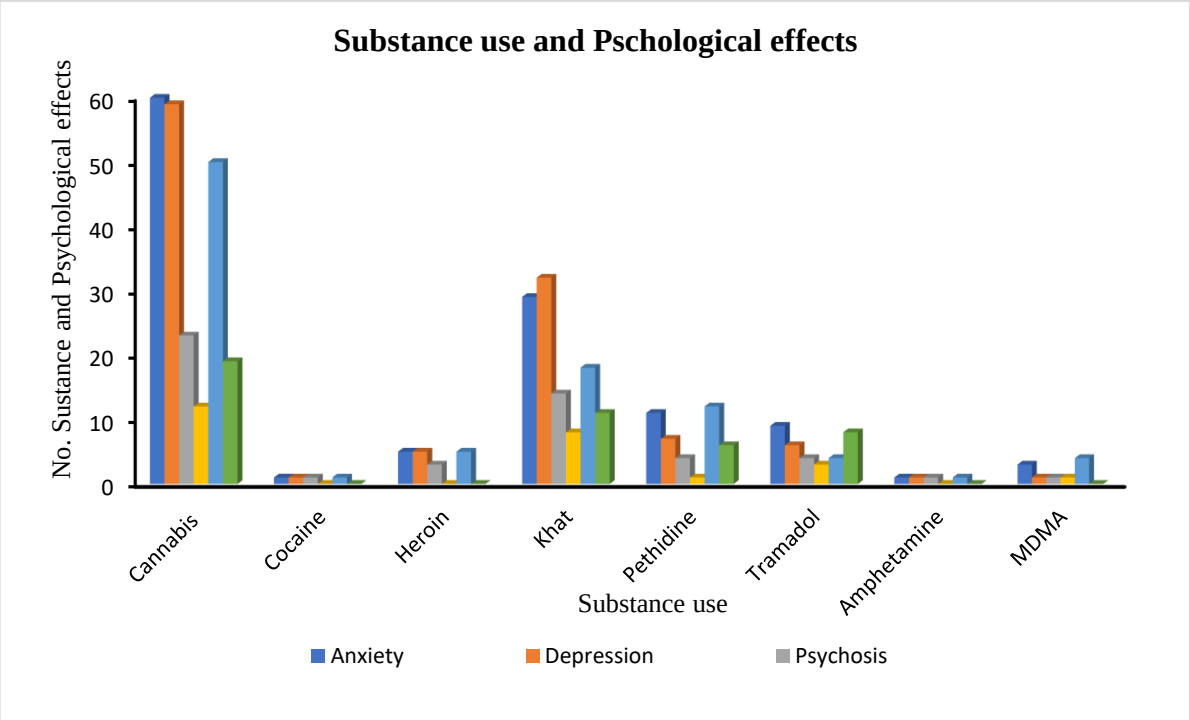


Figure 6. Bar graph shows the association between self-reported substance use and psychological effects

5. DISCUSSION

5.1 Discussion

In the present study, substance abuse and psychiatric disorders of patients in Butabika Hospital's Alcohol and Drug Unit were evaluated. Substance abuse of patients was determined by hair samples and their self-report about the substance use history. Also, the sociodemographic characteristics of patients were determined by a survey. The results of the present study demonstrated the following: I) substance abuse was evaluated to sociodemographic characteristics such as gender, marital status, education level and age, II) the rate of substance abuse by hair analysis results was higher than the rate of survey results III) most of the patients demonstrated psychological disorders.

The sociodemographic characteristics such as age, gender, education level, and marital status of patients were evaluated by questionnaire. The number of male patients was higher compared to females. Consistent with our findings, it has been stated that men are more likely to use almost all kinds of illicit drugs than women [18]. Also, it has been reported that men use cannabis at higher rates than women [24, 6]. Substance use disorders are characterized by greater prevalence in men [37]. In the present study, 18 and 50-year-old patients were included in the study, the average age was found to be 29.78 years. Also, a study [6], demonstrated that those between 20-29-year-old patients were the largest age group for Cannabis use in Uganda Mental Hospitals. Marital status and education level were among other parameters that were evaluated. Most substance users were not married 82.47% and 17.53% were married. Most participants were in high school 47.04%, graduates 27.92%, diplomas 22.73% and the least 1.95 had not been to school before. Across most substance users, age, gender and employment status are associated with risky substance use, with a higher likelihood of risk reported by males, unemployed and younger [55].

In the present study, the self-report about the drug use history of patients was obtained. All patients reported exposure to substances including cannabis, cocaine, heroin, khat, pethidine, tramadol, amphetamine and MDMA, the prevalence of these drugs was determined. Self-reported substance use is more likely to be influenced by underreporting bias compared to biological markers. For this reason, it is recommended that self-report and biological indicators be used for more accurate evaluation in

substance use studies [31, 44]. It was reported that hair analysis is more proper compared to urine, blood, and salvia for detecting psychoactive substances exposure over a long time[29]**.** In our study detection of substance abuse in hair was performed using a developed analytical method. The optimization of the chromatographic separation of the target compounds was by mass spectrometry, a detection technique that offers the advantage of being able to extract individual analyte transitions even for closely eluting compounds. The parameters in the ionization source and mass spectrometer were optimized to give the highest sensitivity for the analytes. The mass spectrometric instrument used had the capability of switching between positive and negative modes although all analytes were detected in positive mode. The optimization procedure gave the transitions for each compound and at least two transitions were chosen i.e. the quantifier and the qualifier. The quantifier transitions were those giving the highest intensity and were used for quantifying the analytes, while the qualifier transitions were used for confirming their identity [(41, 32).]

The Limit of detection (LoD) and limit of quantification (LoQ) were assessed by spiking blank hair with the analytes in decreasing concentrations (0.5 - 100 ng/ml). The LOD and LOQ were calculated at a signal-to-noise ratio of 3 and 10, respectively, they ranged from 27.1pg/mg to 125.2pg/mg and 43.8pg/mg to 265.1pg/mg, respectively. The other validation parameters included the recovery at four different concentrations representing the low (5 ng/ml), medium (10 ng/ml, and 20 ng/ml) and high (100 ng/ml) and at three different concentrations of low (10 ng/ml), medium (20 ng/ml) and high (100 ng/ml) analyte concentrations. For the selected analytes, the recoveries were above 90% irrespective of the spike concentration although generally higher at the highest spike concentration. The recoveries were lower for the cannabinoids at the lower spike concentrations but increased to achieve 100% for the medium and high spike concentrations save for cannabinol. For the other test analytes, recoveries ranged between 98% - 110% for all spike concentrations. For the assessment of the method linearity range, the regression coefficients of all analytes were always higher than 0.995 within the tested concentration range of 1.0 -150 ng/ml and the results of intermediate precision, and values of accuracy were within the acceptable limits [41]. Concordance of self-report and analysis results were investigated. It was also discovered that cannabis and khat were the most prevalent substances both in the survey and experiment, cannabis plants and khat are at

times cultivated by some farmers in their private gardens for medicinal purposes and the light regulations imposed on these substances have eased their access to abuse. The abuse of prescription substances, such as pethidine (24.68%) and Tramadol (26.62%), was observed. Also, heroin, morphine, codeine, amphetamine, MDA, MDMA, and Methamphetamine were determined in hair samples. The analytical method revealed the use of substances more than the substances reported in the interview, approximately twice the percentage. Consistent with our data, it has shown low concordance between hair results and self-report, in adolescents [44]. The prevalence of substance use based on urine tests was found higher compared to self-reported [31]. This can be attributed to patients' unwillingness to provide correct information some patients feel uncomfortable disclosing during interviews or patients' failure to know and recall the substances used. The concentration substances determined in hair samples were in the range of cannabinol (59.7 – 1045 pg/mg), cannabidiol (61.7 - 665.7 pg/mg), Delta-9-THC (46.9 - 928.2 pg/mg), codeine (217.1 - 1845.5 pg/mg), amphetamine (266.9 - 512.2pg/mg), MDMA (187.5 – 489 pg/mg), methamphetamine (169.3 - 788.6 pg/mg) and MDA (256 – 1008 pg/mg) above their respective limits of quantification, codeine had the highest positive value 1845.5 pg/mg of (Table 7, Figure 4)). Codeine has a relatively better binding capacity in pigmented hair [23] and being a therapeutic drug that can be accessed from pharmacies enabling repeated use. In the present study, diagnosed psychological effects were reported in patients and some appeared in combination, four symptoms were reported present at the same time in patients (10.4%), two or three symptoms were almost equally experienced while most patients (31.2%) experienced one symptom, some (3.2%) did not experience any psychological effects. Also, the association between substance abuse and psychological disorders of patients was also investigated., Depression and anxiety were highly observed with few participants showing eating disorders Table (9). MDMA, Amphetamine, Heroin, and cocaine users equally experienced high Anxiety and mood disorders, in the same manner, khat, cannabis, amphetamine, cocaine, and heroin users experienced anxiety and depression disorders. Almost all disorders were determined in cocaine, amphetamine, and heroin users. Substance abuse or use of mixed substances has influence on the psychological effects experienced with in the patients.

5.2 Limitations

Insufficient analyte standards, i.e. Pethidine, Heroin, cocaine, Tramadol, cathine and cathine were less in stock hence unable to use enough amounts for quantification, however, information about these analytes in hair specimens was determined qualitatively using the analytical instruments.

In the present study, only hair samples were utilized in the analysis method, but this method can be improved for utilization by doing a comparison study for analysis of substance abuse in hair and other biomarkers like urine, blood or saliva.

5.3 Conclusion

The study demonstrated the prevalence of the most used substance using both survey and experiment methods. The experimental method discovered the use of substances like MDA, Methamphetamine, and Codeine, which informs about the need for continued monitoring. Overall, our study suggests that hair analysis is an important technique for the screening of abused drugs in clinical and forensic units to provide information left out alongside contemporary testing methods for past drug use, in circumstances where the person has forgotten the substances they have been exposed to or when self-reported information is insufficient for an investigation or treatment of a health complication caused by drugs and the fact that hair analysis is unable to detect drugs in recent use, applying more than one technique during drug screening can provide good data for treatment.

Finally, Intervention to boost prevention and treatment programs is required to reduce the increasing prevalence of drug abuse and more research can focus on testing emerging drug products and their effects.

6. REFERENCES

1. **Abbo, C., Okello, E., Muhwezi, W., Akello, G., & Ovuga, E.** Alcohol, Substance Use and Psychosocial Competence of Adolescents in Selected Secondary Schools in Uganda: A Cross-Sectional Survey. International Neuropsychiatric Disease Journal, **2016;** 7(2), 1–14. https://doi.org/10.9734/indj/2016/25387

2. **Ahmadi, J., Toobaee, S., Kharras, M., & Radmehr, M.** Psychiatric disorders in opioid dependents. Journal of Chemical Dependency Treatment, **2002;** 8(1), 37–43. https://doi.org/10.1300/J034v08n01_03

3. **Alexander Morton, W.** Cocaine and psychiatric symptoms. Primary Care Companion to the Journal of Clinical Psychiatry, **1999;** 1(4), 109–113. https://doi.org/10.4088/pcc.v01n0403

4. **Andrew Shaner., M. Elena Khalsa., Roberts L., et al.** Unrecognized cocaine use among schizophrenic patients. Am J Psychiatry. **1993;** 150:758-62. [PubMed: 8480822]

5. **Arias, F., Szerman, N., Vega, P., Mesias, B., Basurte, I., Morant, C., Ochoa, E., Poyo, F., & Babin, F.** Cocaine abuse or dependency and other psychiatric disorders. Madrid study on dual pathology. Revista de Psiquiatría y Salud Mental (English Edition), **2013;** 6(3), 121–128. https://doi.org/10.1016/j.rpsmen.2012.09.003

6. **Awuzu, Epaenetus & Kaye, Emmanuel & Vudriko, Patrick.** Prevalence of Cannabis Residues in Psychiatric Patients: A Case Study of Two Mental Health Referral Hospitals in Uganda. Substance abuse: research and treatment. **2014;** 8. 1-5. 10.4137/SART.S13254.

7. **Balíková M.** Hair analysis for drugs of abuse. Plausibility of interpretation. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. **2005;** Dec;149(2):199-207. PMID: 16601756.

8. **Cacciola, J. S., Koppenhaver, J. M., Alterman, A. I., & McKay, J. R.** Posttraumatic stress disorder and other psychopathology in substance abusing patients. *Drug and Alcohol Dependence*, **2009;** *101*(1–2), 27–33. https://doi.org/10.1016/j.drugalcdep.2008.10.018

9. **Cairns, T., Hill, V., Schaffer, M., & Thistle, W.** *Amphetamines in washed hair of demonstrated users and workplace subjects*. **2004;** *145*, 137–142. https://doi.org/10.1016/j.forsciint.2004.04.028

10. **Cairns, T., Hill, V., Schaffer, M., & Thistle, W.** *Levels of cocaine and its metabolites in washed hair of demonstrated cocaine users and workplace subjects*. **2004;** *145*, 175–181. https://doi.org/10.1016/j.forsciint.2004.04.033

11. **Castro, A. De, Cruz, A., Lo, M., Lendoiro, E., & Concheiro, M.** Target screening and confirmation of thirty-five licit and illicit drugs and metabolites in hair by LC–MSMS. *Forensic Science International*, **2012;** *217*(1–3), 207–215. https://doi.org/10.1016/j.forsciint.2011.11.006

12. **Crome, I. B., Bloor, R., & Thom, B. 2006;** Screening for illicit drug use in psychiatric hospitals: Whose job, is it? *Advances in Psychiatric Treatment*, *12*(5), 375–383. https://doi.org/10.1192/apt.12.5.375

13. **Degenhardt, L., Coffey, C., Hearps, S., Kinner, S. A., Borschmann, R., Moran, P., & Patton, G.** Associations between psychotic symptoms and substance use in young offenders. Drug and Alcohol Review, 34(6), 673–682. https://doi.org/10.1111/dar.12280

14. **Dolan, K., Rouen, D., & Kimber, J. (2004).** An overview of the use of urine, hair, sweat, and saliva to detect drug use. *Drug and Alcohol Review*, **2015;** *23*(2), 213–217. https://doi.org/10.1080/09595230410001704208

15. **Dumestre-Toulet V, Cirimele V, Ludes B, Gromb S, Kintz P.** Hair analysis of seven bodybuilders for anabolic steroids, ephedrine, and clenbuterol. J Forensic Sci. **2002;** Jan;47(1):211-4. PMID: 12064656.

16. **El-Hadidy, M. A., & Helaly, A. M. N.** Medical and psychiatric effects of a long-term dependence on a high dose of tramadol. Substance Use and Misuse, **2015;** 50(5), 582–589. https://doi.org/10.3109/10826084.2014.991406

17. **Fankhauser, K., Marino, M., & Mccarty, D.** Related disorders are documented in electronic health records. **2021;** 52(3), 228–236. https://doi.org/10.1080/02791072.2020.1747665.

18. **Fattore L, Marti M, Mostallino R, Castelli MP.** Sex and Gender Differences in the Effects of Novel Psychoactive Substances. Brain Sci. **2020;** Sep 3;10(9):606. doi: 10.3390/brainsci10090606.).

19. **For, M., By, U. S. E., Drug, N., & Laboratories, A. (n.d.).** Guidelines for Testing Drugs under International control in Hair, Sweat and Oral Fluid C.

20. **Forsman, M., Ka, K., Kronstrand, R., & Nystro, I.** *Hair analysis for drugs in driver's license regranting. A Swedish pilot study*. **2010;** *196*, 55–58. https://doi.org/10.1016/j.forsciint.2009.12.036

21. **G.A.A. Cooper, et al.,** Society of Hair Testing guidelines for drug testing in hair, Forensic Sci. Int. dio: 10.1016/j.forsciint.2011.10.024 **2011.**

22. **Gallardo, E., & Vieira, D. N. (n.d.).** *Hair: a complementary source of bioanalytical information in forensic toxicology*. 67–79.

23. **Gygi SP, Joseph RE Jr, Cone EJ, Wilkins DG, Rollins DE.** Incorporation of codeine and metabolites into hair. Role of pigmentation. **1996;** Apr;24(4):495-501. PMID: 8801066.

24. **Hemsing N, Greaves L.** Gender Norms, Roles and Relations and Cannabis-Use Patterns: A Scoping Review. Int J Environ Res Public Health. **2020;** Feb 4;17(3):947. doi: 10.3390/ijerph17030947

25. **Herlinger, K., & Lingford-Hughes, A.** Opioid use disorder and the brain: a clinical perspective. Addiction, **2022;** 117(2), 495–505. https://doi.org/10.1111/add.15636

26. Https://www.afro.who.int/health-topics/substance-abuse

27. https://www.ncbi.nlm.nih.gov/books/NBK319/

28. **Jaber, J. Al, Holt, D., & Johnston, A.** Method Development for the Detection of Basic / Weak Basic Drugs in Hair by LCMSMS: Comparison between Methanolic and Alkaline Extraction on Real Samples. **2012;** (July), 263–274.

29. **Joseph J. Palamar & Alberto Salomone** On the challenges of hair testing to detect underreported substance use in research settings, The American Journal of Drug and Alcohol Abuse, https://doi.org/10.1080/00952990.2023.2166414. **2023.**

30. **Kamba, P. F., Mulangwa, J., Kageni, P., Balikuna, S., Kengo, A., Mutamba, B. B., Sewankambo, N., Adome, R. O., & Byakika-Kibwika, P.** Predictors of controlled prescription drug non-medical and lifetime use among patients accessing public mental health services in Uganda: A cross-sectional study. BMJ Open, **2021;** 11(3). https://doi.org/10.1136/bmjopen-2020-037602

31. **Khalili, P., Nadimi, A.E., Baradaran, H.R. et al.** Validity of self-reported substance use: research setting versus primary health care setting. Subst Abuse Treat Prev Policy. 16, 66. **2021;** https://doi.org/10.1186/s13011-021-00398-3,

32. **Kim, J., Ji, D., Kang, S., Park, M., Yang, W., Kim, E., Choi, H., & Lee, S.** Journal of Pharmaceutical and Biomedical Analysis Simultaneous determination of 18 abused opioids and metabolites in human hair using LC–MS / MS and illegal opioids abuse proven by hair analysis. *Journal of Pharmaceutical and Biomedical Analysis*, **2014;** *89*, 99–105. https://doi.org/10.1016/j.jpba.2013.10.041

33. **Kintz, P.** Hair analysis in forensic toxicology. WIREs Forensic Science, **2019;** 1(1), 1–11. https://doi.org/10.1002/wfs2.1196

34. **Kintz, P., Villain, M., & Cirimele, V.** Hair analysis for drug detection. Therapeutic Drug Monitoring, **2006;** 28(3), 442–446. https://doi.org/10.1097/01.ftd.0000211811.27558.b5

35. **Kissiwaa, S. A., Patel, S. D., Winters, B. L., & Bagley, E. E.** Opioids differentially modulate two synapses important for pain processing in the amygdala. *British Journal of Pharmacology*, **2020;** *177*(2), 420–431. https://doi.org/10.1111/bph.14877

36. **Kronstrand, R., Nyström, I., Strandberg, J., & Druid, H.** Screening for drugs of abuse in hair with ion spray LC-MS-MS. *Forensic Science International*, **2004;** *145*(2–3), 183–190. https://doi.org/10.1016/j.forsciint.2004.04.034

37. **McHugh RK, Votaw VR, Sugarman DE,** Greenfield SF. Sex and gender differences in substance use disorders. Clin Psychol Rev. **2018;** Dec; 66:12-23. doi: 10.1016/j.cpr.2017.10.012.

38. **McKetin, R., Leung, J., Stockings, E., Huo, Y., Foulds, J., Lappin, J. M., Cumming, C., Arunogiri, S., Young, J. T., Sara, G., Farrell, M., & Degenhardt, L.** Mental health outcomes associated with the use of amphetamines: A systematic review and meta-analysis. EClinical Medicine, **2019;** 16(xxxx), 81–97. https://doi.org/10.1016/j.eclinm.2019.09.014

39. **McLellan, A. T.** Substance Misuse and Substance Use Disorders: Why do they matter in healthcare? **2017;** 128, 112–130. PMID: 28790493; PMCID: PMC5525418.

40. **McPherson, M. L., Walker, K. A., Davis, M. P., Bruera, E., Reddy, A., Paice, J., Malotte, K., Lockman, D. K., Wellman, C., Salpeter, S., Bemben, N. M., Ray, J. B., Lapointe, B. J., & Chou, R.** Safe and Appropriate Use of Methadone in Hospice and Palliative Care: Expert Consensus White Paper. In Journal of Pain and Symptom Management (Vol. 57, Issue 3, **2019;** American Academy of Hospice and Palliative Medicine. https://doi.org/10.1016/j.jpainsymman.2018.12.001

41. **Milena, M., Markus, R., Scholz, C., Madry, M. M., Kraemer, T., & Baumgartner, M. R.** LC-MS / MS analysis of Δ9-THC, CBN and CBD in hair contamination. Published by Oxford University Press on behalf of Society of Forensic. **2022;** 46, 504–511.

42. **Mossie, A., Kindu, D., & Negash, A.** Prevalence and Severity of Depression and Its Association with Substance Use in Jimma Town, Southwest Ethiopia. Depression Research and Treatment, **2016;** https://doi.org/10.1155/2016/3460462

43. **Narvaez, J. C. M., Jansen, K., Pinheiro, R. T., Kapczinski, F., Silva, R. A., Pechansky, F., & Magalhães, P. V.** Psychiatric and substance-use comorbidities associated with lifetime crack cocaine use in young adults in the general population. Comprehensive Psychiatry, **2014;** 55(6), 1369–1376. https://doi.org/10.1016/j.comppsych.2014.04.021

44. **Natasha E. Wade, Ryan M. Sullivan, Susan F. Tapert, William E. Pelham III, Marilyn A. Huestis, Krista M. Lisdahl & Frank Haist.** Concordance between substance use self-report and hair analysis in community-based adolescents, The American Journal of Drug and Alcohol Abuse, **2023;** 49:1, 76-84, DOI: 10.1080/00952990.2023.2164931

45. **National Collaborating Centre for Mental Health.** Drugs Misuse, Psychosocial interventions National Clinical Practice Guideline **2008;** 51. In Health (San Francisco) (Issue 51). www.nice.org.uk%5Cnwww.nccmh.org.uk%5Cnwww.rcpsych.ac.uk

46. **NIDA.** Cocaine Research Report What is Cocaine? National Institute on Drug Abuse, May. **2016.**

47. **Odenwald, M., Neuner, F., Schauer, M., Elbert, T., Catani, C., Lingenfelder, B., Hinkel, H., Häfner, H., & Rockstroh, B.** Khat use as a risk factor for psychotic disorders: A cross-sectional and case-control study in Somalia. BMC Medicine, **2005;** 3, 1–10. https://doi.org/10.1186/1741-7015-3-5

48. **Paul, A. L. D., Mußhoff, F., & Determination, S.** Quality requirements for the determination of special analytes from biological matrices including annex of tables (prevailing specifications for detection limits). **2009.**

49. **Paul, A. L. D., Musshoff, F., & Establishment, S.** Guideline for quality control in forensic-toxicological analyses Purview (Validity) **2018.**

50. **Peltzer, K.** Prevalence and correlates of substance use among school children in six African countries. International Journal of Psychology, **2009;** 44(5), 378–386. https://doi.org/10.1080/00207590802511742

51. **Pendl, E., Pauritsch, U., Kollroser, M., & Schmid, M. G.** Determination of cathinone and cathine in Khat plant material by LC–MS/MS: Fresh vs. dried leaves. *Forensic Science International*, **2021;** *319*, 110658. https://doi.org/https://doi.org/10.1016/j.forsciint.2020.110658

52. **Ritter, M. L., Bohr, A. D., & McQueen, M. B.** Association of Cardiac Arrest with Opioid Overdose in Transport. Substance Abuse: Research and Treatment, **2022;** 16. https://doi.org/10.1177/11782218221103582

53. **Shrier, L. A., Harris, S. K., Kurland, M., & Knight, J. R.** "Substance use problems and associated psychiatric symptoms among adolescents in primary care." ("Psychosocial and Clinical Correlates of Substance Use Disorder in an ...") ("Psychosocial and Clinical Correlates of Substance Use Disorder in an ...") Pediatrics, **2003;** 111(6 Pt 1). https://doi.org/10.1542/peds.111.6.e699

54. **Šimić, G., Tkalčić, M., Vukić, V., Mulc, D., Španić, E., Šagud, M., Olucha-Bordonau, F. E., Vukšić, M., & Hof, P. R.** Understanding emotions: Origins and roles of the amygdala. Biomolecules, **2021;** 11(6), 1–58. https://doi.org/10.3390/biom11060823

55. **Stevens MWR, Harland J, Alfred S, Ali RL.** Substance use in the emergency department: Screening for risky drug use, using the ASSIST-Lite. Drug Alcohol Rev. **2022** Nov;41(7):1565-1576. doi: 10.1111/dar.13513.

56. **Trull, T. J., Sher, K. J., Minks-Brown, C., Durbin, J., & Burr, R.** (two thousand). Borderline personality disorder and substance use disorders: A review and integration. *Clinical Psychology Review*, **2000;** *20*(2), 235–253. https://doi.org/10.1016/S0272-7358(99)00028-8

57. **UNODC.** *Executive Summary*. (n.d.). www.unodc.org/unodc/en/data-and-analysis/world-drug-report-2023.html **2023.**

58. **Urits, I., Gress, K., Charipova, K., Li, N., Berger, A. A., Cornett, E. M., Hasoon, J., Kassem, H., Kaye, A. D., & Viswanath, O.** Cannabis Use and its Association with Psychological Disorders. Psychopharmacology Bulletin, **2020;** 50(2), 56–67.

59. **Usman, M., Naseer, A., Baig, Y., Jamshaid, T., Shahwar, M., & Khurshuid, S.** Forensic toxicological analysis of hair: a review. *Egyptian Journal of Forensic Sciences*, **2019;** *9*(1). https://doi.org/10.1186/s41935-019-0119-5

60. **Verstraete, A. G.** *Detection Times of Drugs of Abuse in Blood, Urine, and Oral Fluid*. http://journals.lww.com/drug-monitoring **2004.**

61. **Vogliardi, S., Tucci, M., Stocchero, G., Ferrara, S. D., & Favretto, D.** Sample preparation methods for determination of drugs of abuse in hair samples: A review. Analytica Chimica Acta, **2015;** 857, 1–27. https://doi.org/10.1016/j.aca.2014.06.053

62. **Volkow, N. D., Baler, R. D., Compton, W. M., & Weiss, S. R. B.** Adverse Health Effects of Marijuana Use. *New England Journal of Medicine*, **2014;** *370*(23), 2219–2227. https://doi.org/10.1056/nejmra1402309

63. **Wille, S. M. R., Baumgartner, M. R., Di Fazio, V., Samyn, N., & Kraemer, T.** Trends in drug testing in oral fluid and hair as alternative matrices. Bioanalysis, **2014;** 6(17), 2193–2209. https://doi.org/10.4155/bio.14.194

64. **Wolff, K.** A Brief Overview of the Detection of Drug and Alcohol Use: The Matching of Purposes and Procedures Characterization of MDMA tablets and biomarkers of use View project Driving under the influence of Psychoactive Drugs View project. https://www.researchgate.net/publication/315111674 **2017.**

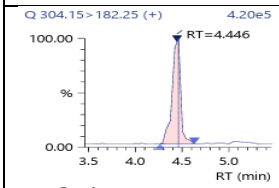
65. **Wolff, K., Farrell, M., Marsden, J., Monteiro, M. G., Ali, R., Welch, S., & Strang, J.** A review of biological indicators of illicit drug use, practical considerations, and clinical usefulness. Addiction, **1999;** 94(9), 1279–1298. https://doi.org/10.1046/j.1360-0443.1999.94912792.x

66. **Xiang, P., Shen, M., & Drummer, O. H.** Review: Drug concentrations in hair and their relevance in drug-facilitated crimes. *Journal of Forensic and Legal Medicine*, **2015;** *36*, 126–135. https://doi.org/10.1016/j.jflm.2015.09.009

7. APPENDICES

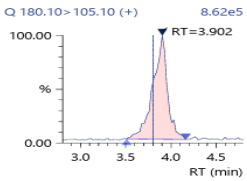
Appendix A Some Chromatographic Peaks of some compounds detected in hair samples

S-4



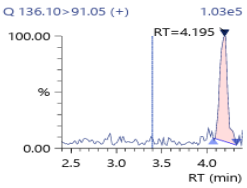
Cocaine
Modified: Yes
Manual: Yes
Start RT: 4.262 min
End RT: 4.632 min
Area: 2648292
Height: 418852

S-8



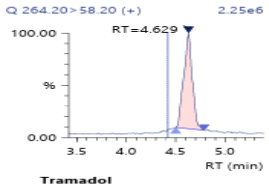
MDA
Modified: Yes
Manual: Yes
Start RT: 3.509 min
End RT: 4.162 min
Area: 9580625
Height: 857166

S-6



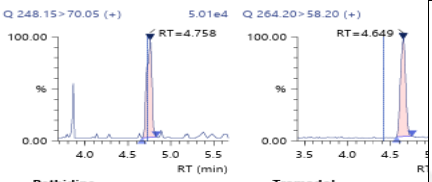
Amphetamine
Modified: Yes
Manual: Yes
Start RT: 4.069 min
End RT: 4.326 min
Area: 605232
Height: 99850

S-25



Tramadol
Modified: Yes
Manual: Yes
Start RT: 4.501 min
End RT: 4.787 min
Area: 12694006
Height: 2118895

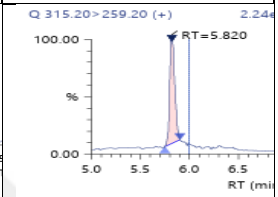
S-28



Pethidine
Modified: Yes
Manual: Yes
Start RT: 4.664 min
End RT: 4.832 min
Area: 202994
Height: 49773

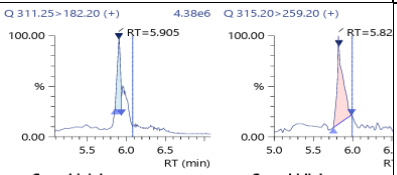
Tramadol
Modified: Yes
Manual: Yes
Start RT: 4.572 min
End RT: 4.750 min
Area: 2268068
Height: 552055

S-29



Cannabidiol
Modified: Yes
Manual: Yes
Start RT: 5.748 min
End RT: 5.902 min
Area: 7626795
Height: 2094515

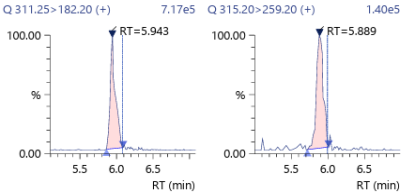
S-21



Cannabiniol
Modified: No

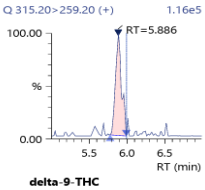
Cannabidiol
Modified: Yes
Manual: Yes
Start RT: 5.754 min
End RT: 5.988 min
Area: 3290444
Height: 658833

S-55



Cannabiniol
Modified: Yes
Manual: Yes
Start RT: 5.866 min
End RT: 6.091 min
Area: 3423635
Height: 706819

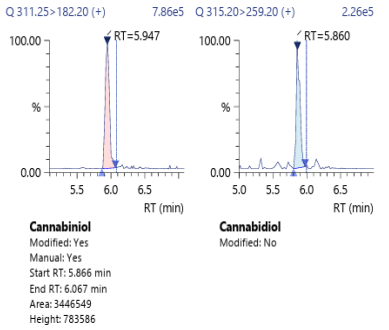
Cannabidiol
Modified: Yes
Manual: Yes
Start RT: 5.722 min
End RT: 6.011 min
Area: 904041
Height: 137778



delta-9-THC
Modified: Yes
Manual: Yes
Start RT: 5.777 min
End RT: 5.990 min
Area: 635532
Height: 115863

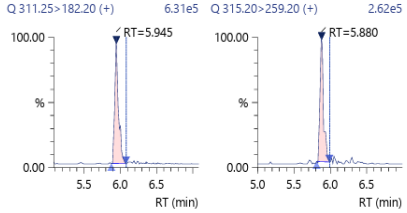
S-59

S-60



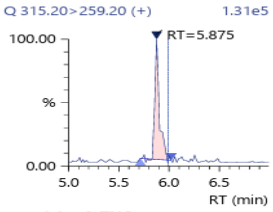
Cannabiniol
Modified: Yes
Manual: Yes
Start RT: 5.866 min
End RT: 6.067 min
Area: 3446549
Height: 783586

Cannabidiol
Modified: No



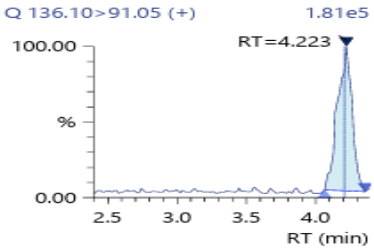
Cannabiniol
Modified: Yes
Manual: Yes
Start RT: 5.876 min
End RT: 6.077 min
Area: 2185202
Height: 629372

Cannabidiol
Modified: Yes
Manual: Yes
Start RT: 5.812 min
End RT: 5.986 min
Area: 874537
Height: 257003



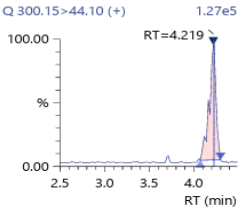
delta-9-THC
Modified: Yes
Manual: Yes
Start RT: 5.714 min
End RT: 6.018 min
Area: 404878
Height: 128373

S-75

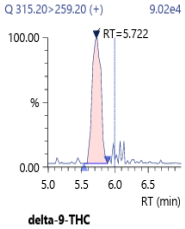


Amphetamine
Modified: No

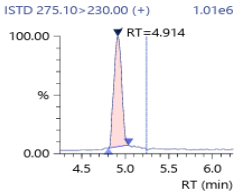
S-79



Codeine
Modified: Yes
Manual: Yes
Start RT: 4.071 min
End RT: 4.297 min
Area: 634723
Height: 124645



delta-9-THC
Modified: Yes
Manual: Yes
Start RT: 5.535 min
End RT: 5.888 min
Area: 831615
Height: 90188



Chlorpheniramine (dexchlorpheniramine)
Modified: Yes
Manual: Yes
Start RT: 4.805 min
End RT: 5.036 min
Area: 6059534
Height: 978347

Appendix B.*Consent Form*

CONSENT FORM

Title

"Forensic analysis of drugs in hair specimens of psychiatric patients at Butabika Hospital in Uganda".

Description of the study

This study involves the collection of data and hair samples. The study is part of the master's program course as a thesis which is aimed at the analysis and evaluation of drugs of abuse from hair samples of patients hospitalised in Butabika Hospital. Hair analysis is a useful tool for detecting drugs in clinical and forensic units. Screening and identifying abused drugs help to lay out sufficient data about a person's use of drugs, exposure, and treatment of the potential results.

The results will be used primarily for scientific purposes, your personal information will be kept confidential. You have the right not to participate in this study, which does not require you to pay and for which no payment is made, and you have the right to withdraw after participating. If you request additional information, it will be given verbally.

If you agree to participate in our research, please write your name and surname, date and sign in the section below. Thank you.

The researcher interviewed the participant.

_____

I AGREE TO PARTICIPATE IN THE RESEARCH WITHOUT ANY PRESSURE OR FORCE UNDER THE ABOVE-STATED TERMS.

1. Name

……

Signature/thumbprint of participant

……..

Date

…….....

2. Name

……

Signature of interviewer/Person obtaining informed consent

…….....

Date

…….....

3. Name

……

Signature of witness

Appendix C Questionnaire

FORENSIC ANALYSIS OF DRUGS OF ABUSE IN HAIR OF PSYCHIATRIC PATIENTS AT BUTABIKA HOSPITAL

Demographics/ Participant Details

1. Sample ID ?
CU/MFS/000/2023

_____

2. Gender ?

- ◯ Male
- ◯ Female

3. Age ?

_____

4. Marrital status?

- ☐ Married
- ☐ Not married
- ☐ others

5. Education ?

- ◯ Yes
- ◯ No

6. Level of Education?

_____

7. Occupation?

_____

Drug details

8. Drug screening

- ◯ Yes
- ◯ No

9. Method of identification?

- ☐ Self-report
- ☐ Urinalysis
- ☐ Blood Analysis

Others

_____

10. Psychiatric Behaviour/ Disorder

- ☐ Anxiety (feeling nervous and fearful)
- ☐ Depression (loss of interest in activites)
- ☐ Psychosis (loss of contact with reality)
- ☐ Eating disorder (abnormal eating behaviour)
- ☐ mood disorder (Distorted emotional state)
- ☐ personality (disruptive patterns of thinking,behaviour)

11. Psychoactive substance reported/screened ?

- ☐ Alcohol
- ☐ Cannabis
- ☐ Cocaine
- ☐ Heroin
- ☐ Kuber
- ☐ khat
- ☐ Amphetamine
- ☐ Tobacco
- ☐ Pethidine
- ☐ Tramadol
- ☐ Crystal Meth.

Others specify

_____

8. RESUME

Mugume Isaaya. I completed High school in the year 2010 at Masaka Secondary School and a Bachelor of Science with education in Chemistry and Mathematics at Mbarara University of Science and Technology (MUST) in 2014.

After joining the Uganda Police Force in 2015, I served under the Directorate of Operations from 2016 to 2019, from 2020 to date, I have been under the Directorate of Forensic Services in the Department of Quality Assurance and Research, My roles and responsibilities include inspection and evaluation of laboratory operations, establishment, and promotion of the Quality management system in coordination with the lab departments, development and reviewing policies, standard operating procedures, work instructions and other Quality instruments, promotion of the occupational health and safety within the lab, preparation and conduction of internal audits, and coordination of research activities.

I also practice in the Forensic Chemistry lab. and I have gained experience and skills in crime scene management (identification, collection, and preservation of evidence), analysis of forensic (chemistry and toxicological) evidence using analytical instruments (TLC, GC and LC-MSMS) and presentation of findings. This has enabled me to conduct research for my master's thesis titled "Forensic *Analysis of Abused Drugs in Hair Samples of Psychiatric Patients at Butabika Hospital in Uganda*."

Referees

Prof. William Bazeyo
Chair of the Board of Directors, Uganda Cancer Institute
Kampala, Uganda

Dr Kisitu Jaffar
Head of Forensic Toxicology
Directorate of Forensic Services

Immaculate Ivy Atuhaire

Head of Department Quality Assurance & Research