DETERMINATION OF NEVIRAPINE LEVELS IN HAIR AND PLASMA SAMPLES OF PEOPLE LIVING WITH HIV IN KENYA USING HPLC-UV AND LC-MS/MS TECHNIQUES

CHEPKIRUI C. PHILOMENA

I56/CTY/PT/37734/2016

A Thesis Submitted in Partial Fulfillment of the Requirement for the Award of the Degree of Master of Science in Applied Analytical Chemistry the School of Pure and Applied Sciences of Kenyatta University

JANUARY, 2025

DECLARATION

I declare that this thesis is my original work and it has never been presented for a degree in any other university.

Signature: …… Date: ……

Chepkirui C. Philomena

I56/CTY/PT/37734/2016

SUPERVISORS

We confirm that the candidate carried out the work reported in this thesis under our supervision and has been submitted with our approval as university supervisors.

Signature: …… Date: ……

Dr. Margaret M. Nganga

Department of Chemistry

Kenyatta University

Signature: …… Date: ……

Dr. Beatrice Irungu

Centre for Traditional Medicine and Drug Research

Kenya Medical Research Institute

Signature: …… Date: ……

Dr. Elizabeth Kigondu

Centre for Traditional Medicine and Drug Research

Kenya Medical Research Institute

DEDICATION

I dedicate this work to my husband, Samwel Bett; my children, Nicole Cheptoo, Lucas Kipchirchir, Elsie Lizz and Shawn Kiptoo; and my parents, Selina Cheruiyot and the late Richard Cheruiyot. I appreciate them so much for their encouragement, support, love, care and prayers.

ACKNOWLEDGEMENTS

First and foremost, I wish to thank the Almighty God for giving me the gift of life and good health throughout my MSc study. My profound thanks also goes to Kenyatta University for giving me a chance to pursue my MSc study.

I sincerely thank my research supervisors, Dr. Margaret Nganga, Department of Chemistry, Kenyatta University, Dr. Elizabeth Kigondu and Dr. Beatrice Irungu of Kenya Medical Research Institute, Centre for Traditional Medicine and Drug Research. I made it this far with their assistance, understanding and dedicated involvement in every step of my MSc study.

My gratitude goes to the Director-General of Kenya Medical Research Institute (KEMRI) for allowing me to undertake my MSc research project under a sponsored mother project entitled ''Monitoring Antiretroviral Adherence in HIV-infected Individuals on Treatment," with principal investigators being Dr. Bhavna Chohan and Dr. Musa Ngayo Otieno. I wish to acknowledge the government of Kenya for funding this project through the Kenya Medical Research Institute, Internal Research Grant (KEMRI/GRG/15).

I will not forget to express my gratitude to the laboratory technicians, namely John Kimathi, Josephine Ouma and Martin Muirigi of Chemistry Department Analytical Laboratories, Jomo Kenyatta University of Agriculture and Technology (JKUAT), for their assistance and support in analysing my samples in their laboratories.

Finally, I must express my gratitude to my husband, children and parents for providing me with unfailing support and continuous encouragement throughout my years of study and through researching and writing this thesis. This accomplishment would not have been possible without them.

TABLE OF CONTENTS

LIST OF TABLES

LIST FIGURES

ABBREVIATIONS AND ACRONYMS

ARVs:	Antiretroviral drugs
3TC:	Lamivudine
AIDS:	Acquired Immune Deficiency Syndrome
ART:	Antiretroviral Therapy
CD4:	Cluster of differentiation
CVR:	Centre for Virus Research
HAART:	Highly Active Antiretroviral Therapy
HIV:	Human Immunodeficiency Virus
LC/MS/MS:	Liquid Chromatography/Tandem Mass Spectrometry
Liq-liq:	Liquid-liquid extraction
TDM:	Therapeutic drug monitoring
NVP:	Nevirapine
RNA:	Ribonucleic Acid
Rpm:	Rotations per minute
UV:	Ultra- Violet
LLOD:	Lower limit of Detection
LLOQ	Lower limit of quantification
PPT:	Blood Preparation Tubes
MRM:	Multiple Reaction Monitoring
TFA:	Tri-fluoro acetic acid
QC	Quality control
PLWH:	People living with HIV

ABSTRACT

Monitoring the response to antiretroviral drugs (ARVs) in patients is crucial for effective HIV treatment. Antiretroviral therapy (ART) is pivotal in mitigating the epidemic of human immunodeficiency virus (HIV), particularly in the prevention of vertical transmission of the virus. Monitoring of the adherence of the patients to ART is imperative for gauging their response to antiretroviral drugs (ARVs) and identifying treatment inadequacies. Methods previously used in monitoring adherence, like measuring ARV drug concentrations in blood and urine, are limited by their ability to only reflect doses taken within 1 to 2 days before the day of sampling. In response to these limitations, hair testing has emerged as a preferred tool for assessing chronic exposure to various substances, owing to its extended detection window. Therefore, this study determined the viability of hair samples for adherence monitoring, serving as an alternative to blood-based ARV analysis. Furthermore, it investigated the potential of using high-performance liquid chromatography with a UV detector (HPLC-UV) as a cost-effective substitute of liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique, particularly in resource-limited settings like Kenyan hospitals. The research focused on nevirapine (NVP), a fundamental component of Kenya's first-line ART regimen. Hair and blood samples were collected from consenting patients with varying viral loads. NVP levels in these samples were compared using HPLC-UV and LC-MS/MS instruments. Significantly, the findings reveal no substantial difference between hair and plasma NVP concentrations, as verified by statistical tests. A robust positive association between the two measurement methods further validates the utility of HPLC-UV for monitoring ARV drug concentrations in both hair and blood in resource-limited settings. Quantitatively, the median (IQR) NVP levels in hair and blood samples were 67.80 ng/mL and 706.50 ng/mL for HPLC-UV, and 36.80 ng/mL and 19.32 ng/mL for LC-MS/MS, respectively. The Wilcoxon signed-rank test, yielding a statistical result of ($Z = -0.93$, $p > 0.05$), confirms no significant difference between hair and plasma NVP concentrations. The Spearman rank test indicates a significant positive association between NVP concentrations analysed using both LC-MS/MS and HPLC-UV ($r^2 = 0.995$, $p < 0.05$) for hair samples, and ($r^2 = 0.966$, $p < 0.05$) for plasma samples. This research demonstrated the potential of using hair testing as a non-invasive, cost-effective means to quantitatively monitor ARV adherence among people living with HIV, particularly in regions lacking traditional laboratory facilities and skilled personnel for blood sampling. Importantly, it does not suggest replacing plasma testing with hair testing in all scenarios; instead, these methods can complement each other for a comprehensive assessment of adherence to antiretroviral therapy.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The prevalence rate of Human immune Deficiency virus (HIV) in Kenya stands at approximately 6%, encompassing an estimated 1.6 million individuals grappling with HIV/AIDS. Kenya ranks among the top six nations in Africa in terms of its HIV-afflicted population (Kenya HIV County Profiles, 2016). Currently, 1.4 million people are living with HIV, 78% of those people are on antiretroviral treatment HIV remains one of the chronic illnesses that contributes significantly to a high mortality rate in the country (Scriven *et al*., 2021). Specifically, Nairobi County exhibits a prevalence rate of approximately 3.8% and Kisumu County records a substantially higher rate of 17.5%. Notably, these counties are among the top 10 counties in Kenya with the most elevated HIV prevalence levels (Onyango *et al.*, 2021).

The human immunodeficiency virus (HIV) operates by undermining white blood cells, specifically CD4+ cells, through a process of internal replication. HIV accomplishes this replication by inserting its genetic material, known as Ribonucleic Acid (RNA), into the host's genetic material, thereby enabling it to reproduce (Hemelaar, 2013). Since the virus contains RNA, it relies on the activity of a reverse transcriptase enzyme to convert its genetic material into DNA, a crucial step for its integration and replication within an individual's body (Makoae, 2011). Reverse transcriptase is only found in human cells, of people living with HIV (PLWH) (Bennett *et al*., 2008). The virus progressively weakens a person's immune system as it kills CD4+ cells and makes more copies of itself, leading to acquired immunodeficiency syndrome (AIDS) (World Health Organization, 2007).

ARVs were first introduced in the 1980s, aiming to attack HIV and prevent it from multiplying in the infected person's body, thus preventing the immune system's weakening (Adewumi *et al*., 2015). HIV epidemic reduction depends heavily on antiretroviral therapy (ART). The research and treatment cascade for HIV has advanced globally. As of the end of 2019, 400 million (67%) of the 38 million people living with HIV (PLWH) worldwide had been treated with antiretroviral medication (UNAIDS, 2020). By the end of 2019, 74% (1,112,254) of Kenya's adult population and 73% (71,500) of the country's youngsters in need of antiretroviral therapy (ART) were receiving ARVs. Surprisingly, 68% of these individuals had viral suppression. (UNAIDS, 2020). At the time of this study, first-line antiretroviral therapy (ART) regimen in Kenya recommended for children, youth, and adults included one non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTIs) plus. The common NRTIs are zidovudine [AZT] (1), lamivudine [3TC] (2), tenofovir [TDF] (3), stavudine [d4T] (4), (Figure 1.1) as a combination of one NNRTI and two NRTIs. The common NNRTIs were nevirapine (NVP) (5) and efavirenz (EFV) (6) (Figure 1.2), (NASCOP, 2018).

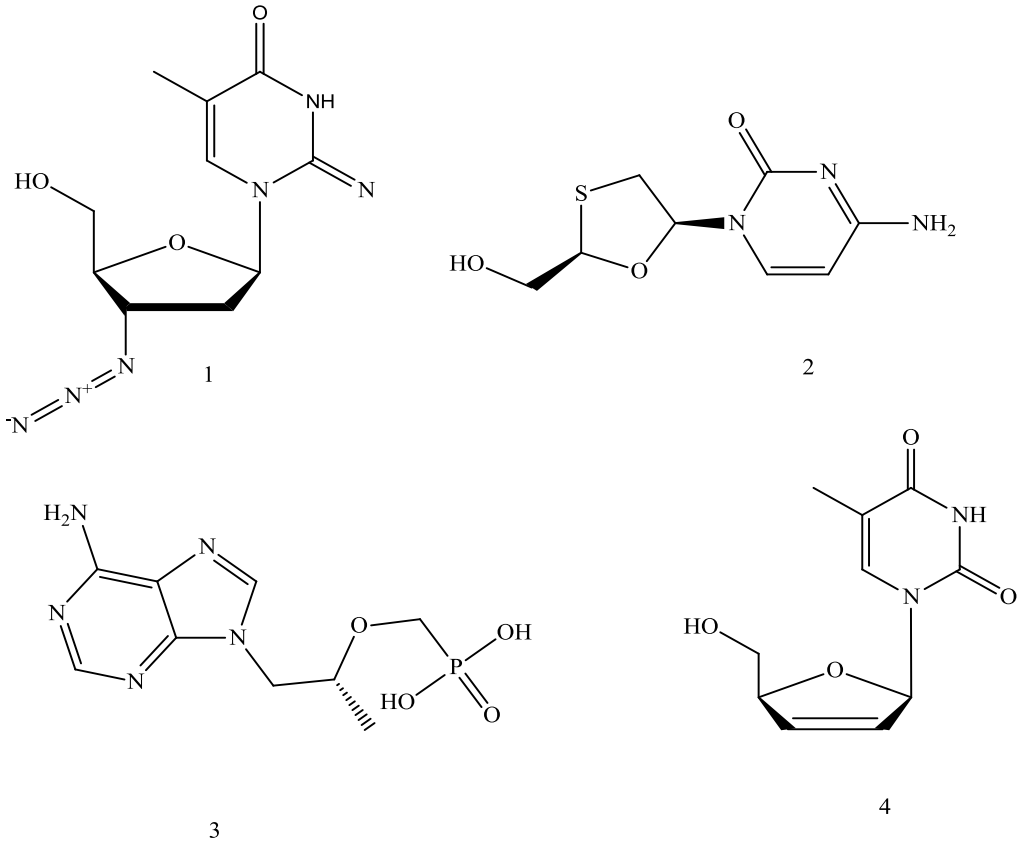


Figure 1.1: Structures Nucleoside reverse transcriptase inhibitors (NRTI's); zidovudine [AZT] (1), lamivudine [3TC] (2), tenofovir [TDF] (3), stavudine [d4T] (4).

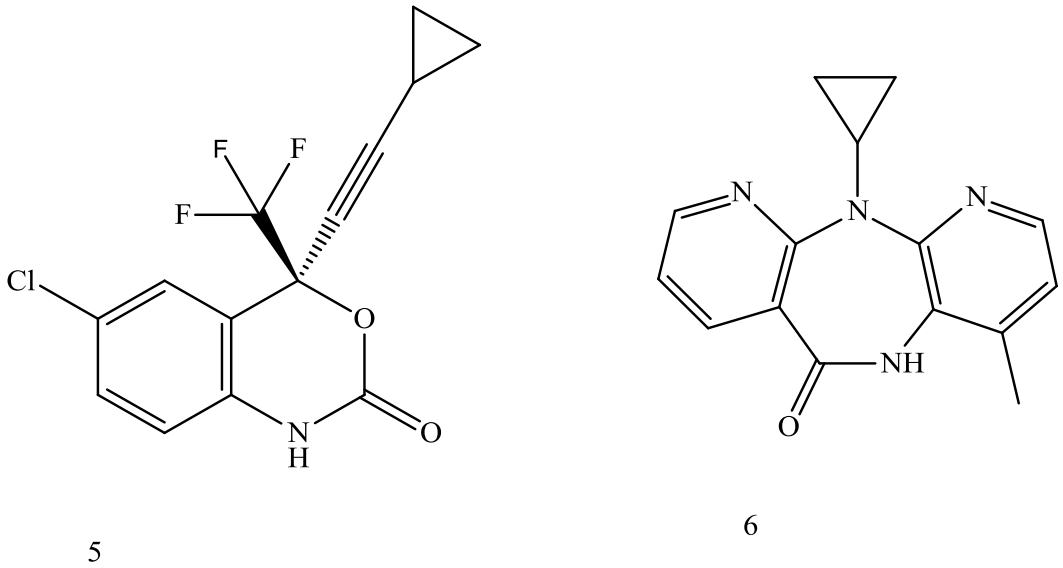


Figure 1.2: Structures of non-nucleoside reverse transcriptase inhibitors (NNRTIs); efavirenz (5) and NVP (6).

Antiretroviral therapy (ART) adherence is crucial to achieving a satisfactory clinical outcome in the complex process of managing HIV. Antiretroviral therapy (ART), has been instrumental in drastically lowering the death and morbidity rates among individuals living with HIV. Nevertheless, the intricacies associated with lifelong treatment, as well as factors such as recreational drug use, depression, and medication side effects, contribute to instances of non-adherence to ART, thereby posing a significant risk to treatment effectiveness and potentially leading to the emergence of HIV variants resistant to drugs (Gandhi *et al*., 2009; Yan *et al.,*2016). Monitoring ART adherence holds critical importance as it allows healthcare providers to evaluate individual responses to treatment, an essential consideration given the variation in drug dosage requirements for different patients (Gandhi *et al*., 2009).

As HIV replicates within an individual's body, a multitude of new copies emerge, carrying mutations that distinguish them from the original strain of HIV that initially infected the individual (Hemelaar, 2013). These mutant viruses continue to increase within the infected person's body, even when receiving antiretroviral (ARV) medication. To mitigate the development of these drug-resistant viruses, it is imperative to utilize either combined antiretroviral therapy (CART) or the highly active antiretroviral therapy (HAART) and maintain strict adherence to the prescribed ARV drug regimen (D'Ettorre *et al*., 2011). This approach is crucial in preventing the development of HIV mutants that are resilient to treatment.

Studies have shown that when a patient is on antiretroviral therapy (ART) for more than six months and has a continuously high viral load of > 1000 copies/mL, including a CD4+ count of < 200 cells/mm^3, the patient has failed to respond to

treatment (Kasaie *et al*., 2020). Most treatment failure in Kenya is mainly associated with poor or non-adherence to ART, leading to drug-resistant mutation (Hamers *et al*., 2011).

Monitoring the quantities of drugs in biological samples is known as therapeutic drug monitoring (TDM) (Kimulwo, 2018). Numerous research studies have suggested that TDM can enhance the effectiveness of Highly Active Antiretroviral Therapy (HAART). For instance, Panichsillapakit *et al*., 2016 concluded that TDM could be effectively employed to regulate the concentrations of antiretroviral drugs (ARVs) and improve the management of HIV.

Monitoring HIV infections is essential for understanding the reasons behind treatment failures, even when patients adhere to their prescribed regimens. It has been noted that blood tests alone may not be entirely reliable indicators of an individual's adherence to antiretroviral therapy (ART), as demonstrated by Yang *et al*., 2015). and Gandhi *et al*. 2009. This is partly due to the short elimination half-life of certain ARVs, such as 3TC (lamivudine), which is as brief as 3-4 hours. Due to its short half-life, 3TC blood concentrations can fluctuate significantly, making it difficult for a single measurement to correctly represent a patient's usual adherence patterns. (Yan *et al*., 2016).

LC-MS/MS and HPLC-UV have been previously used to analyse NVP and other ARV drugs in biological matrixes like blood and hair samples (Jung *et al*., 2007; Gandhi *et al.*, 2014); however, the comparison of the performance of the two instruments has never been made. This study, therefore, validated and compared the performance of the two instruments in the analysis of NVP levels in plasma and hair

samples with the primary purpose of determining whether HPLC-UV is sensitive enough to be used as a substitute for LC-MS/MS instruments in resource-limited settings or in cases where LC-MS/MS is not available.

1.2 Statement of the Problem

Adherence monitoring to antiretroviral therapy (ART) and therapeutic drug monitoring are often not part of the clinical management of HIV patients in Kenya. Adherence to ART is typically assessed through indirect methods, such as self-reports, monitoring patients' clinic attendance, and pill counts (Kenya-HIV-County-Profiles, 2016). However, these indirect methods can sometimes yield inaccurate estimations of patients' adherence levels. On the other hand, direct methods offer a more precise means of monitoring an individual's adherence to ART, as highlighted by Yan *et al*., 2016. Among the direct techniques employed, the analysis of antiretroviral drug levels in the blood of people living with HIV (PLWH) on ART stands out as a crucial step in evaluating adherence to ARV medication (Rakhmanina *et al*., 2007).

Regrettably, the entire process involving the analysis of drugs in the blood is prohibitively expensive and often inaccessible in resource-limited settings. Consequently, many individuals affected by HIV will lack access to this type of testing. The collection of blood samples necessitates skilled personnel and the use of sterile equipment. Blood samples can also become compromised if not stored at the appropriate temperature, as evidenced by their susceptibility to thermal denaturation at room temperature but not at -80^{O}C, underscoring the delicate nature of handling blood (Calcagno *et al*., 2012).

The LC-MS/MS instrument is more costly than the HPLC-UV. Additionally, maintenance and support services are limited and expensive. The study thus sought to compare HPLC-UV and LC-MS/MS methods' performance in quantifying ARVs since resource-limited settings can afford HPLC-UV. There were no studies carried out that sought to compare the performance of HPLC-UV and LC-MS/MS in the analysis of drugs in hair or blood; that being a gap in this area formed the basis of this study.

1.3 Justification of the study

HIV remains a major public health challenge in Kenya, with a burden on individuals and the healthcare system. Effective antiretroviral therapy (ART) is critical for managing the disease, improving patient outcomes, and preventing the transmission of the virus. However, accurate and reliable monitoring of ART adherence is essential to ensure therapeutic efficacy and prevent the development of drug resistance. While indirect methods of assessing adherence, such as self-reporting and pill counts, are commonly used, they are often unreliable and fail to provide an accurate picture of a patient's actual adherence behavior. This limitation poses a significant challenge to the effective management of HIV in resource-limited settings in Kenya, where patients may face various barriers to consistent medication adherence, including social stigma, economic constraints, and lack of access to healthcare services.

While LC-MS/MS equipment is known for its precision, it can be costly to procure maintain and requires technical expertise, limiting its suitability in resource-limited settings, such as clinical laboratories in low end hospitals. On the other hand, High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) is a less expensive, more widely available technology that has been used in various areas of

pharmaceutical and clinical research (Lamorde *et al*., 2014). While it has the potential to provide a feasible and cost-effective alternative for monitoring antiretroviral drug levels, there has been limited research comparing its performance to LC-MS/MS, particularly in the context of ART adherence monitoring in low and middle-income countries (LMICs) like Kenya. This lack of comparison creates a knowledge gap that impedes the optimal of HPLC-UV into routine clinical practice.

By addressing this gap, this study will provide valuable evidence on the relative efficacy and applicability of HPLC-UV compared to LC-MS/MS in quantifying antiretroviral drug levels in blood and hair samples. This research will contribute to improving ART monitoring practices, offering a practical solution for healthcare settings where resources are limited. Moreover, the findings may lead to more accessible, cost-effective methods of therapeutic drug monitoring, enhancing the management of ART and ultimately improving health outcomes for people living with HIV (PLWH).

The study's results will not only benefit healthcare providers and patients in Kenya but also have broader implications for other LMICs facing similar resource constraints. The ability to implement reliable, low-cost drug monitoring techniques will facilitate better clinical decision-making, reduce the risks of drug resistance, and improve the quality of life for individuals affected by HIV globally.

In conclusion, this study is justified by its potential to fill a critical research gap and its broader public health implications for improving HIV treatment adherence and management in resource-limited settings.

1.4 Objectives

1.4.1 General Objective

The main objective of this study was to determine the level of nevirapine of people living with HIV in Kenya in hair and plasma samples using HPLC-UV and LC-MS/MS.

1.4.2 Specific Objectives

i. To determine the levels of NVP in hair and matched blood samples.

ii. To determine the performance of HPLC-UV and LC-MS/MS in the analysis of blood and hair samples for NVP.

1.5 Hypothesis

i. There is no significant difference between the level of NVP in hair and matched blood samples of PLWH.

ii. There is no significant difference between the performance of HPLC-UV and LC-MS/MS techniques in the analysis of NVP levels in hair and plasma samples.

1.6 Significance of the Study

The monitoring of adherence to antiretroviral therapy (ART) and therapeutic drug levels is critical for effective HIV treatment and management. In Kenya, as in many resource-limited settings, adherence is often assessed through indirect methods such as self-reporting, pill counts, and clinic attendance, all of which can be prone to inaccuracies and biases. This study aims to address the gap in ART adherence monitoring by exploring more accurate, direct methods specifically through the comparison of the levels of nevirapine in hair and matched plasma samples: This

study also compared the performance of High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in the analysis of nevirapine in hair and plasma samples.

While LC-MS/MS has been shown to offer precise quantification of antiretroviral drugs in biological samples, its high cost and the need for specialized equipment and trained personnel make it impractical in many low-resource settings like Kenya. In contrast, HPLC-UV, which is less expensive and more accessible, offers a promising alternative for quantifying drug levels in blood or hair samples. However, limited research has directly compared the performance of these two methods in the context of ART drug monitoring in resource-constrained settings.

By investigating the comparative efficacy of HPLC-UV and LC-MS/MS in quantifying antiretroviral drugs, this study will contribute important insights into how cost-effective and accessible alternatives could be integrated into routine clinical practice in Kenya and similar low- and middle-income countries (LMICs). The findings could lead to improved ART adherence monitoring, facilitating more personalized and effective treatment regimens. This, in turn, could help reduce the incidence of treatment failure and drug resistance among people living with HIV (PLWH), ultimately improving health outcomes and the quality of life for individuals affected by HIV.

Furthermore, the study will address an important knowledge gap, as no prior studies have directly compared the use of HPLC-UV and LC-MS/MS in the analysis of ARVs in biological samples like blood or hair, especially within the context of

resource-limited settings. The results of this research could thus lay the foundation for future studies and inform policy decisions regarding the implementation of ART monitoring in similar settings globally.

CHAPTER TWO

LITERATURE REVIEW

2.1 Management of HIV using antiretroviral drugs

Antiretroviral drugs (ARVs) are the most effective approach for managing HIV, facilitating viral suppression, and reducing the associated morbidity and mortality linked to infection (Kantor *et al*., 2009). Although it's important to note that ARVs do not eliminate the infection, long-term treatment to achieve and maintain viral suppression is necessary. These drugs operate by curbing the replication of the virus, consequently bolstering the immune response in people living with HIV (PLWH) (Raffi *et al*., 2013).

Due to the high replication rate of the virus, mutations of HIV frequently occur. To counter this, a combination of various types of ARVs is employed to make therapy more effective (Baril *et al.,* 2016). Combined antiretroviral therapy (CART) and highly active antiretroviral therapy (HAART) are used to implement this strategy. HAART, for instance, combines a minimum of three different classes of ARVs, leveraging the synergistic effects of these drugs to suppress the replication of HIV (Nikanjam *et al*., 2012). Introduced in the late 1990s, HAART marked a turning point, transforming HIV from a once-fatal disease into a disease that can be managed. These regimens typically involve a lower pill burden, offer enhanced efficacy, and require less frequent dosing than initial protease inhibitor-based treatment regimens (Mouton *et al*., 2016).

2.2 Therapeutic Drug Monitoring (TDM)

TDM entails quantifying drug concentrations in various biological matrices, including urine, blood, saliva, and hair. This practice is instrumental in overseeing an

individual's adherence to medications administered over an extended period (Back *et al.,* 2002). TDM has found applications in an array of drug categories, such as antimicrobial agents, anticonvulsants, and psychotropic drugs, ensuring that the prescribed dosage is therapeutically effective and unlikely to cause harmful side effects (Raffi *et al*., 2014).

Studies have consistently indicated significant variability in the drug responses between different patients, particularly in the case of antiretroviral drugs (ARVs) 2012). Furthermore, research has established substantial relationships between the concentration of drugs in blood and the effectiveness of treatment as well as the risk of toxicities (Mouton *et al*., 2016). Notably, implementing TDM in clinical settings has shown tangible improvements in the management of medical conditions, especially in patients initially experiencing treatment failures (Kimulwo, 2018).

In Kenya, Therapeutic Drug Monitoring (TDM) and adherence monitoring for Antiretroviral Therapy (ART) are not currently conducted. The prevailing approach to monitoring ART adherence relies on indirect methods, encompassing self-reports, tracking clinic attendance, and pill counts (Kenya HIV County Profiles, 2016). However, these indirect methods have proven to be unreliable in monitoring ART adherence, potentially leading to either overestimations or underestimations of patients' adherence (Wu *et al.,* 2018). In contrast, direct methods offer greater accuracy when assessing adherence to ART in people living with HIV (PLWH). These direct techniques involve analysing drug concentrations within an individual's biological matrices (Rakhmanina *et al*., 2007).

One such direct approach is the analysis of antiretroviral drugs (ARVs) in the blood of HIV patients to monitor their adherence to ART. Unfortunately, obtaining blood samples necessitates phlebotomy for collection and the controlled shipment of samples to centralized laboratory testing facilities, making it a relatively cumbersome process (Gandhi *et al*., 2014; Yan *et al*., 2016). Moreover, the concentration of ARVs in biological matrices like blood and urine only reflects the doses of ARVs administered within a narrow window of 1 to 2 days before the sampling, rendering blood samples an unreliable indicator of an individual's overall ART adherence.

While many drugs are incorporated into hair as it grows, hair sample testing has traditionally been confined to forensic applications (Nielsen *et al*., 2014). The concentration of drugs in hair reflects drug absorption from the blood stream in the body over an extended period of weeks to months. This extended detection window has made hair testing a valuable tool for determining chronic exposure to certain drugs. Therefore, measuring drug levels in hair offers a distinct advantage over single blood drug concentrations when estimating an individual's average medication exposure level. Additionally, the hair matrix exhibits a high degree of resistance to decay and remains stable even at room temperature, contributing to the reliability of hair samples for drug testing (Kintz, 2004). Additionally, hair samples are advantageous economically because they may be obtained at a low cost and don't require intrusive procedures like phlebotomy. This makes hair testing a practical and accessible method for monitoring drug exposure and adherence, particularly in the context of long-term therapies like antiretroviral treatment for HIV (Gandhi *et al*., 2014; Rakhmanina *et al*., 2007; Hickey *et al*., 2014). TDM is performed in the USA under toxicities, pregnancy and treatment failure. TDM is

crucial especially for pregnant women are most susceptible to medical specialist failure and the subsequent transmission of viruses from mother to kid. (Reddy *et al*., 2016). Additionally, when the patients have been treated with ARVs heavily but still do not respond to the treatment, TDM is also performed.

However, as low blood concentration suggests recent poor medication exposure, the combination of ARV analysis in matching hair samples and blood can be used for both TDM and adherence monitoring. On the other hand, a low concentration of ARVs in the hair indicates a poor drug exposure average for the preceding month (Hickey *et al*., 2014).

2.3 Monitoring Adherence to Antiretroviral Therapy (ART)

ART plays a crucial role in effectively suppressing HIV, significantly reducing the risk of developing resistance to antiretroviral drugs, improving overall health, enhancing their norm of living, extending survival, and reducing the transmission of HIV (World Health Organization, 2007). It's important to note that for certain chronic diseases like diabetes or hypertension, medication can continue to be beneficial even after the temporary interruption of treatment, which is not the case for HIV infection. However, failing to follow ART prescriptions can result in the virus developing HIV mutants that are resistant to ARVs and restricting available ARVs treatment options in the future. Antiretroviral drug (ARV) use by HIV-positive people has greatly lowered rates of morbidity and death, improving health outcomes (Günthard *et al*., 2016). Nevertheless, the lack of adherence to antiretroviral therapy (ART) has been a notable issue, leading to treatment failures that could potentially undermine the positive impact of ARV treatment, particularly in cases where treatment monitoring and adherence tracking are inadequate (Hassan *et al*., 2012).

To address the challenges associated with adherence, it is imperative for all members of the treatment team to identify and address adherence-related issues. Implementing appropriate strategies to enhance patient compliance is a fundamental responsibility in the complete medical attention of individuals living with HIV (World Health Organization, 2007). This multifaceted approach encompasses not only the provision of antiretroviral medications but also the support and resources necessary to ensure that patients can consistently adhere to their treatment regimens, thereby promoting their health and well-being.

A study conducted by Barth *et al*. (2010) showed that after a year of commencing treatment, a significant percentage of patients in settings with minimal resources had become resistant to HIV medications. Nevirapine (NVP) is one often given element of highly active antiretroviral treatment (HAART), which is used alongside other viral inhibitors to achieve maximal viral suppression among patients in Kenya. According to Ochieng *et al*. (2015), in their trial, NVP was administered in conjunction with nucleoside reverse transcriptase inhibitors to 81% of the patients. Notably, therapeutic drug monitoring (TDM) is an effective technique utilized in the US to optimize NVP dosage. (L'Homme *et al*., 2008). Nevertheless, TDM is rarely performed in situations with limited resources because there aren't any simple or affordable ways to measure drug levels in different biological matrices like blood and saliva. (L'Homme *et al*., 2008). This presents a challenge in ensuring optimal dosing and treatment effectiveness in such settings.

Hair analysis emerged as a technique in the late 1970s, initially used to detect illicit drug abuse in forensic toxicology and for therapeutic monitoring purposes (Pego *et al*., 2017). Interestingly, indinavir, a popular HIV protease inhibitor, has also been measured in hair samples taken from AIDS patients. Research, including that carried out by Ekouevi *et al*. (2014), has demonstrated that the level of indinavir in hair is a valuable marker. It was notably higher among patients who adhered to antiretroviral therapy (ART) compared to those who did not adhere to the treatment regimen. This study shows that hair analysis can be used to track and evaluate HIV-positive people's adherence to their drug regimens.

Indeed, drug analysis in hair has found applications in therapeutic drug monitoring (TDM) not only for antiretroviral drugs (ARVs) but also for anticonvulsant and psychotropic medications. These drugs, like ARVs, are typically administered over extended periods, making adherence a critical factor in determining their effectiveness (Marchei *et al*., 2011).

Age, race, the degree of pigmentation, hair texture, cosmetic procedures, growth pace, and the anatomic site of hair collection are some of the variables that can affect the amounts of drugs in hair (Gandhi *et al.*,2009). Understanding and accounting for these factors is essential when utilizing hair analysis for TDM, as they can impact the interpretation of drug levels in hair and their relationship to treatment adherence.

It is important to collect hair samples close to the scalp since the occipital scalp has fewer varying hair growth rates and usually has the most significant drugs concentrations in hair (Liu *et al*., 2014). While the analysis of biological fluids such

as blood and urine are commonly used to detect drug presence or exposure, these methods have limitations, as there is often a poor correlation between the degree of addiction or medication adherence and the actual drug concentration in these fluids (van Zyl *et al*., 2011).

Hair possesses the advantage of stable drug-protein complex formation, and immediate processing of hair samples after collection is not required, as this complex remains stable at room temperature, offering advantages over plasma samples (Huang *et al*., 2011). These characteristics make hair a valuable method for monitoring adherence and determining exposure to drugs like nevirapine (NVP), especially in resource-limited settings. Hair samples can be easily collected on-site and submitted for analysis. This process is non-invasive and does not require highly skilled personnel to obtain samples, unlike plasma samples. Furthermore, obtaining hair samples lowers the likelihood of HIV transmission incidents, unlike for the collection of plasma samples, which involves a risk of needle injury and, as a result, the possibility of HIV transmission (Van Oosterhout *et al*., 2007). Consequently, the literature has suggested that ARV levels in hair can provide greater value than single plasma concentrations for monitoring and assessing treatment adherence (Johnston *et al.*, 2019).

This study has shown that the ARVs hair level is the most robust independent adherence determiner in HIV-infected individuals, surpassing single plasma levels. Though some studies have shown that it is possible to use hair to monitor drug adherence, the use of hair in our resource-limited settings has yet to be demonstrated in similar studies, which is the basis of the study.

2.4 Incorporation of drugs into the hair

The drugs get into the hair through multiple mechanisms during the hair formation process. These mechanisms include drug transfer from the blood through sweat and sebum. Sweat contains trace amounts of drugs from the bloodstream, and due to hair's porosity and its ability to absorb liquids, drugs can be easily transported into the hair via sweat (Johnston, 2017). Once in the hair, drugs bind to the melanin present in the hair structure. Notably, treatments such as hair relaxers and certain cosmetic products can destroy or modify melanin, reducing the binding capacity of drugs (Gandhi & Greenblatt, 2014).

There was a proposal to use hair samples obtained from pubic areas for adherence monitoring; however, this proposal was rejected. This rejection was based on the fact that the hair growth rate and behavior of pubic hair differ from that of hair on the head. Pubic hair ceases to grow after reaching a certain length, affecting the concentration of drugs within these hair structures, making them less suitable for monitoring drug exposure and adherence (Baxi *et al*., 2015).

A number of variables, such as lipophilicity, basicity, and melanin content can influence drug absorption into hair. Lipophilic molecules, which are not readily eliminated by the kidneys, are primarily released through sweat, tears, skin, and hair. They also can move swiftly across cell membranes, diffusing across concentration gradients. The pH of keratinocytes and melanocytes, where drug binding occurs, typically ranges from approximately 3 to 6, making it more acidic than blood (pH 7.3). Consequently, alkaline drugs are more likely to be incorporated into hair than drugs that are neutral or acidic (Johnston, 2017). Alkaline drugs tend to exhibit a

higher binding affinity to melanin, and this effect is particularly prominent in darker hair with higher melanin content (Saitoh *et al*., 2007).

2.5 Nevirapine

One of the most frequent antiretroviral drugs used in conjunction with other drugs as part of first-line antiretroviral therapy (ART) regimens to halt the development of HIV infection is nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor (NNRTI). Adherence to ART has a positive impact, leading to increased CD4+ cell counts, bolstering the immune system, stabilizing CD4+ T-cell levels, and reducing HIV viral loads (Adewumi *et al*., 2015).

NNRTIs, such as NVP, work by attaching to the reverse transcriptase enzyme's active site, which prevents HIV DNA synthesis and stops viral replication. HIV's genetic code may more easily be inserted into the host cell's genetic material thanks to the reverse transcriptase enzyme, which is responsible for transforming viral RNA into DNA (Ekouevi *et al*., 2014). After ingestion, NVP is absorbed into the bloodstream from the stomach walls, and its bioavailability exceeds 90%, with peak concentrations typically occurring around four hours post-ingestion. NVP exhibits a binding affinity to blood proteins of approximately 60% (Baxi *et al*., 2015). It undergoes extensive transformation under the catalysis of internal cytochromes, resulting in hydroxylated metabolites. In comparison to its metabolites, NVP lacks one hydrophilic group, rendering it less polar and highly lipophilic (Yang *et al*., 2020). This unique lipophilicity significantly influences NVP's incorporation into hair, as lipophilic substances can easily penetrate cell membranes driven by concentration gradients. Hence, this study primarily analysed NVP, rather than its metabolites, for its role in

hair incorporation. Importantly, Hair often has substantially higher quantities of NVP than its metabolites (Yang *et al.,* 2020)

2.6 Analytical Techniques for Quantifying NVP

2.6.1 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and High-performance Liquid chromatography with Ultra Violet Detection (HPLC-UV)

LC-MS/MS is considered the gold standard for the quantification of drugs like NVP in biological samples due to its high sensitivity, specificity, and ability to analyze complex matrices. LC-MS/MS combines the high-resolution separation capabilities of liquid chromatography with the precise and sensitive detection capabilities of mass spectrometry, enabling the detection of trace amounts of analytes in complex biological matrices (Jung *et al*., 2007).

HPLC-UV is a more accessible and less expensive analytical technique compared to LC-MS/MS. It relies on the separation of compounds based on their interaction with the stationary phase of the column and the detection of compounds using UV light. HPLC-UV has been applied to the analysis of ARV drugs, including NVP, with the advantage of being easier to operate and more widely available as compared to LC-MS/MS in many clinical and research laboratories (Jung *et al*., 2007; Gandhi *et al.,* 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The study employed a cross-sectional study strategy to collect samples. Consent was obtained from participants recruited at two regular HIV care clinics in Nairobi and Kisumu Counties. The study focused on patients with available health records who had been receiving antiretroviral therapy (ART) for a minimum of 12 months and were considered to have achieved steady-state drug plasma concentrations. Among the people living with HIV (PLWH) included in the study, 81.7% had viral loads exceeding 1000 copies/mL, while 62.2% had viral loads below 1000 copies/mL after 12 months on a first-line ART regimen. In total, 268 participants were recruited from Nairobi (41) County and 145 from Kisumu County (60), resulting in a combined sample size of 413 participants. The levels of HIV viral loads were retrieved from the medical folders of these participants.

3.1.1 The inclusion criteria for cases and controls

PLWH over the age of 18 who had been taking nevirapine (NVP) for more than a year, attended one of the two HIV treatment care clinics in Nairobi or Kisumu, and were willing to provide written informed permission voluntarily were eligible study participants. Furthermore, at the 12-month mark of treatment, the viral load results of the qualified trial participants were mandatory. After receiving therapy for a minimum of 12 months, the participants included: those with non-viral suppression whose viral load (VL) >1000 copies per mL of blood) and those with suppressed viral loads (VL <1000 copies). Participants with hair lengths of at least one centimetre who had not used permanent hair colour or other hair products within the preceding three months were asked to provide samples of their hair.

3.1.2 Ethical consideration

The KEMRI Scientific and Ethics Review Committee (SERU) approved this study under Protocol Number 3214. Prior authorization was obtained from the selected clinics in the two County Governments of Nairobi and Kisumu where the sample collection was conducted. Participants received guarantees that any information collected about them would be handled in the strictest confidence and that their names would not appear in any reports. After being gathered in biohazard bags, all biological wastes were incinerated.

3.2 Sample Collection, Transportation and Preservation

3.2.1 Collection of Blood Sample

A vein inside the participant's elbow was used to extract blood. Plasma preparation tubes (PPT) containing Ethylenediaminetetraacetic acid (EDTA) were filled with approximately 5 mL of extracted whole blood. After the blood was centrifuged to obtain the plasma, it was refrigerated to -80°C. Following their transfer to the KEMRI Centre of Microbiology Laboratory for testing and storage on dry ice, the plasma samples were kept at -80°C in the laboratory until NVP extraction (Jung *et al*., 2007).

3.2.2 Collection of Hair samples

The participant's hair samples were cut from the human occipital scalp using scissors. After being wrapped in aluminum foil, the hair samples were put in labeled zip-lock plastic bags. They were brought in sealed envelopes at room temperature to the KEMRI HIV laboratory, where they were batch-tested (Gandhi *et al*., 2009).

3.3 Materials and Reagents

The internal standard (IS) of carbamazepine and the NVP drug standard were kindly given by Jomo Kenyatta University of Agriculture and Technology. Sigma Aldrich supplied the analytical grade trifluoroacetic acid (TFA), methanol (MeOH), HPLC grade acetonitrile, Di-Methyl ether, ethyl acetate, and ammonium acetate.

3.4 Preparation of Standard Solution

A stock solution of 1 mg/mL of both nevirapine and internal standard carbamazepine was prepared in methanol. The stock solution was diluted with methanol to yield a 100 ng/mL solution, which was then used to create the internal standard solution. Standard solutions were made by adding 100 uL of internal standard solution to each solution after the stock solution had been diluted with methanol to acetonitrile (20:80, v/v) at concentrations that ranged from 10 ng/mL to 500 ng/mL.

3.5 Extraction of NVP from hair samples

10 mg of hair samples were separately weighed, cut into pieces around 3 mm in size, and put into glass test tubes that were 16 mm in diameter and 125 mm in height as part of the sample preparation procedure. Every sample was treated with an internal standard (IS) which had a concentration of 100 ng/mL. Two mL of a 9:1 (v/v) methanol and trifluoroacetic acid solution were added to the samples to extract nevirapine (NVP) from the hair. After that, the samples were put in a reciprocal shaker and given 16 minutes of shaking (Gandhi & Greenblatt, 2014). After that, nitrogen gas was used to dry the sample. A liquid-liquid (liq-liq) extraction procedure was utilized to further clean up the extracted drugs after drying. The sample was first mixed with 0.5 mL of a 0.2 M sodium phosphate buffer (pH 9.4) using a vortex mixer for 30 seconds. Next, 3 mL of a 1:1 dimethyl ether and ethyl acetate solution was added, and

vortexed three times, each for a period of one minute. This was followed by a 10-minute centrifugation of the mixture at 3000 revolutions per minute (rpm). Following the sample's freezing in dry ice, the supernatant layer was transferred to a fresh test tube measuring 100 mm in height by 13 mm in diameter. The sample was dried using nitrogen gas after 100 µL of a solution containing 1% trifluoroacetic acid in methanol was added to this supernatant layer. Reconstitution was done using 0.5 mL of acetonitrile/water in a 1:1 ratio after 30 seconds of vortex mixing. Following filtration via 0.45 µm microfilters, the collected contents were placed into auto-sampler vials. Aliquots were introduced into liquid chromatography-tandem mass spectrometry (LC-MS/MS) and high-performance liquid chromatography with a UV detector (HPLC-UV) for analysis in volumes of 10 µL and 20 µL, respectively.

3.6 Extraction of NVP from blood samples

A modified version of the protein precipitation method was adopted for extraction of NVP from blood samples, followed by a liquid-liquid extraction reported by (Jung *et al*., 2007). Blood samples (0.2 mL) were pipetted to 2.0 mL of Eppendorf tubes and 0.2 mL of 50 mM of solution of ammonium acetate solution and acetonitrile in the ratio1: 6 was added for protein precipitation and then vortexed for 3 minutes. One mL (1.0 mL) of ethyl acetate was added to the samples to extract analytes into the organic layer and hold the endogenous blood materials water-soluble in the aqueous layer to decrease the matrix effect. Vortex mixing for 5 min was then performed and centrifuged for 10 minutes at 4 °C at 12,000 rpm. The supernatant layer was transferred into a clean tube and evaporated using nitrogen gas under a temperature of 37 °C. A reconstitution solution of 500 µl made up of acetonitrile and deionized water in a ratio of 1:1 was used to reconstitute the residue and 10 µl and 20 µl aliquot were

introduced into the LC-MS/MS and HPLC-UV systems, respectively (Jung *et al*., 2007).

3.7 NVP quantification using LC-MS/MS

The study employed an Agilent 1100 HPLC system with temperature control for every analyte, a column compartment, an autosampler, a binary pump, and a solvent degasser. Kinetex Evo C-18 (3 mm x 100 mm) particle size for hair samples and C18 column (4.6 mm by 150 mm) particle size for blood samples were the columns utilized for separation. The flow rate of the mobile phases was 0.45 mL/minute. The autosampler had a temperature of 15 °C and an injection volume of 10 µl. 0.1% formic deionized water (mobile phase A) and 0.1% formic acetonitrile (mobile phase B) made up the mobile phase. The temperature of the column was adjusted to 30°C while the isocratic mode was utilized in a 20:80 ratio. For LC, the entire run time was ten minutes. Internal standards (IS) and analytes were detected using a triple quadrupole mass spectrometer outfitted with a positive ion mode and electro-ion spray ionization. Software called Masslynx was utilized to regulate every parameter on the LC and MS. Precursor and product ions of NVP and IS were quantified using multiple reaction monitoring (MRM), and the atmospheric technique was employed to directly inject 1000 ng/mL solutions into the MS within a suitable mass range for both positive and negative polarity modes. For both NVP and IS, the maximum intensity of $[M+H]^+$ ions was seen in positive mode. Table 3.1 lists the compounds' relative molecular mass, precursor, and product ions. Because the product ions of the analyte of interest monitored by MRM mode supported the precursor m/z and its fragment m/z (MRM transition) for each analyte, the LC-MS/MS approach provides excellent selectivity.

Table 3.1: Transition ions and optimal conditions used to obtain a relative abundance of product ions.

	Channel	Parent peak	Daughter ion	Cone voltage	Collision Temperature
NVP	+	267	226	36	25
Carbamazepine	+	237	197	32	20

3.8 NVP quantification using high-performance liquid chromatography with a UV detector (HPLC-UV).

Shimadzu HPLC equipment with the Chem Solution software was used to analyze NVP. The HPLC apparatus had a quaternary pump, an online degasser, an automated injector kept at 15 °C, and a 2998 photodiode array detector calibrated to detect analytes at a wavelength of 283 nm. The temperature of a waters column C18 (4.6 mm x 150 mm, five μm; Waters) was maintained at 30 °C. Methanol (solvent B) and HPLC-grade water (solvent A) were used as the mobile phase. The mixture was delivered at a 50:50 flow rate of one mL per minute for a total run period of ten min.

3.9 Validation of the LC-MS/MS and HPLC-UV protocols for analysis of blood samples

A highly sensitive, selective and reproducible HPLC-UV method developed by Charbe *et al*. (2016) and LC-MS/MS method developed by Jung *et al*. (2007) were validated and used in the analysis of plasma samples. The LC/MS/MS ion mass and retention times were used to identify the analytes. The analysis was done under the positive MRM mode. The validation tests for specificity, sensitivity, accuracy and precision were carried out.

3.9.1 Lower limit of detection (LLOD) and Lower limit of quantification (LOQ)

In a sample, the lowest analyte concentration that can be consistently detected is known as the lower limit of detection (LLOD). The lowest analyte concentration that can be accurately and precisely quantified with sufficient reliability is known as the lower limit of quantification (LLOQ). LLOQ is mostly taken as the lowest calibration standard. The peak-to-peak noise surrounding the analyte retention time was determined using the signal-to-noise approach. Next, an estimate is made of the analyte concentration required to produce a signal with a noise-to-signal ratio of a particular value. The instrument's auto-integrator determined the level of noise. A signal-to-noise ratio (S/N) of 3 has been accepted for determination of the LLOD while a signal-to-noise ratio of 10 is often used for determining the LLOQ. Nevirapine injections at concentrations ranging from 5 ng/mg to 100 ng/mg in the LC-MS/MS instrument and from 12.5 ng/mL to 100 ng/mL in the HPLC-UV instrument were used to calculate the LLOD and LLOQ of the drug (Phung *et al*., 2018).

3.9.2 Preparation of calibration standards and quality control

The NVP standard solutions were spiked with 200 µl of blank human plasma samples to create the spiked quality control (QC) samples which were used to make a calibration curve. The calibration curves were prepared over a linear range of 10–500 ng/mL for the LC-MS/MS system and 50-1000 ng/mL for the HPLC-UV system. Analyte concentrations (x) were plotted against the analyte peak area (y). For the calibration curves, a least-squares linear regression model was used (Wu *et al*., 2018).

3.9.3 Recovery (extraction efficiency)

To evaluate recovery, QC samples were injected directly into the column to represent 100% extractions. They were then compared to samples that were spiked in plasma and taken to NVP extraction process using the protein precipitation method. The peak regions were compared with those of QC samples that were directly injected to the column to compute recovery (Jung *et al*., 2007). Six plasma sample duplicates at three quality control (QC) concentrations were examined to determine the NVP extraction efficiency: Low QC 10 ng/mL, Medium QC (250 ng/mL), High QC with a concentration of 500 ng/mL for LC/MS/MS, and Low QC with a concentration of 50 ng/mL, Medium QC with a concentration of 250 ng/mL, and High QC with a concentration of 1000 ng/mL for HPLC-UV. The protein precipitation approach was used to extract ARVs from blank plasma, and the recovery was quantified by comparing their peak (Charbe *et al*., 2016).

3.9.4 Accuracy and precision

The determination of intraday accuracy involved analysing six samples each at three concentration levels, namely the LLOQ, medium QC, and high QC, in a single run. The interday accuracy was determined by processing the accuracies and precision of the LLOQ, low, medium, and high QC samples from three runs, using the formula:

% CV = standard deviation/mean……Equation 3.1

3.10 Validation of HPLC-UV and LC-MS/MS methods for the analysis of Hair sample

A very sensitive, selective and reproducible HPLC-UV method developed by Gandhi *et al.* (2014) and the LC-MS/MS method developed by Huang *et al.* (2011) were

validated and used to analyse hair samples. Using ion mass and retention durations, the analytes were identified during the positive MRM mode LC/MS/MS analysis. Three validation studies were conducted: sensitivity, specificity, and accuracy and precision.

3.10.1 Preparation of calibration standards and QCs

A stock solution of NVP (1 mg/mL) and internal standard (IS) carbamazepine were prepared in methanol. The internal standard solution (1000 mL) of 1000 ng/mL was prepared by diluting the stock solution with methanol to 100 ng/mL (10 ng/mg). The calibration curves were prepared for LC-MS/MS and HPLC-UV by spiking the NVP working solutions to 10 mg of cut blank human hair samples in glass tubes (16 × 125 mm) over a linear range of 1-25 ng/mg and 2-100 ng/mg, respectively. The analyte to the internal standard peak area ratio (y) was plotted against the analyte concentrations (x-axis). The calibration curves were fitted with a least-squares linear regression model.

3.10.2 Accuracy and precision

A pooled cut hair sample from a patient taking NVP medication was spiked with three concentration levels across the calibration curve range: the LLOQ, medium QC, and high QC. This was done since spiked hair QC samples might not perfectly replicate actual hair samples. To verify the interday accuracy, low, medium, and high QC samples from the three runs that were analysed on two consecutive days were assessed. The coefficient of variation (% CV) represented the precision, and while percentage of the nominal concentration (% Accuracy) represented the accuracy of the analyte concentration. Through calculating the accuracy and precision statistics

over the intraday and interday using the formula (Equation 3.2), the overall accuracy and precision of the validation was determined.

$$\%V = \frac{\text{standard deviation}}{\text{mean}} \times 100\% \ldots\ldots\ldots\ldots \text{Equation 3.2}$$

For the quality control (QC) samples, the mean concentration was supposed to be within 15% of the nominal values; the LLOQ, on the other hand, was supposed to be within 20% of the nominal values.

3.10.3 Specificity

Six healthy volunteers provided blank human hair samples weighing 10 mg each. The samples were cut into pieces measuring approximately 1 mm in length and the pieces were then placed into a glass test tube with dimensions of 16 mm in diameter by 125 mm in height. NVP was extracted from the hair samples using methanol/trifluoroacetic acid (9/1, v/v ratio) solution and shaking in a reciprocal shaker for 16 hours (Gandhi & Greenblatt, 2014). The sample was dried by evaporating the extraction solvent using using nitrogen gas. Cleaning of the extracted samples was done by liquid-liquid (liq-liq) extraction followed by centrifugation at 3000 rotations per minute (rpm) for a period of 10 minutes. The sample was frozen in the freezer and the supernatant layer was pipetted to a fresh test tube (13 mm diameter x 100 mm height). One hundred microliters (100 µL) of 1% trifluoroacetic acid in methanol were added to the supernatant layer and the sample dried by evaporating the solvent using using nitrogen gas. Reconstitution was done with 0.5 mL of acetonitrile/water (1:1) and followed by vortex mixing, each for 30 sec. The extracts were filtered using 0.45 µm microfilters and then transferred into auto-sampler vials. For analysis, a 20 µL and 10 µL of the sample was injected into the HPLC-UV and LC-MS/MS respectively (Makita-Chingombe *et al.,* 2019).

3.10.4 Extraction efficiency and recovery

The NVP drug concentrations of the hair samples that were taken through the extraction process after spiking (spiked)and NVP concentrations in hair samples that were spiked after the extraction process (blank) were compared to determine the recovery of the method and how efficient the method of extraction of NVP from hair is. The spiked hair samples were prepared by following these steps: Glass test tubes (16 mm diameter x 125 mm height) were filled with the six blank hair samples (10 mg each participant), which had been weighed and roughly cut into 3 mm pieces. An internal standard (IS) of 100 ng/mL was added to every sample. Subsequently, drugs were added to the blank samples at low, medium, and high QC levels. Duplicate samples of each concentration were made. The addition of 2 mL of methanol/trifluoroacetic acid (9/1, v/v ratio) solution to the samples was done and 16 hours of reciprocal shaking were required for the extraction of NVP from hair (Gandhi & Greenblatt, 2014). Nitrogen gas was then used to evaporate the organic solvent until it was completely dried. Liquid-liquid (liq-liq) extraction was used to further purify extracted NVP. To sum up, 30 seconds were spent vortex mixing the material after adding 0.5 mL of 0.2 M sodium phosphate buffer (pH 9.4). A three-minute vortexing was performed on the mixture after adding three (3) milliliters of a 1:1 dimethyl ether/ethyl acetate ratio. The centrifuge was run for ten minutes at 3000 revolutions per minute (rpm). Before transferring the supernatant layer to a new test tube with dimensions of 100 mm in height by 13 mm in diameter and freezing the sample in dry ice, this was done. The addition of 100 microliters (100 μL) of 1% trifluoroacetic acid in methanol was made and vortex mixed for 30 seconds. Drying was done using nitrogen and reconstitution was done using 0.500 mL of acetonitrile/water.

These were the methods used to prepare the non-extracted samples: Similar to the spiked hair samples above, 10 mg of six blank hair samples were taken through the extraction process. Duplicate samples of each concentration were made. After that, NVP was added to the residues at varying amounts to reestablish them. Stream of nitrogen gas was passed through the sample until it was dry. Thirty minutes of vortex mixing were done after reconstitution using 0.500 mL of acetonitrile/water in 1:1 ratio. After analysing the NVP levels in both extracted and non-extracted samples, the following formula was used to calculate the % recovery:

$$\%\ \text{recovery} = \frac{\text{measured concentration}}{\text{true concentration}} \times 100\% \ldots\ldots \text{Equation. 3}$$

3.10.5 Statistical analysis

Data was be presented in tables, bar graphs and linear graphs. Data was analyzed using Wilcoxon signed-rank test, Spearman's rank test, Shapiro-Wilk and Kolmogorov-Smirnov test. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the normality of the data. The Spearman's rank test was used to determine whether a strong correlation of results was obtained using HPLC-UV and LC-MS/MS instruments The Wilcoxon signed-rank test was used to test the association between levels of ARV drug (nevirapine) in plasma and matched hair sample.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Demographic characteristics of the study population

Study Population (Table 4.1) shows the demographic characteristics of study participants. A total of 413 participants on NVP as part of their ARV regimen were recruited for the study. The women participants contributed 58.400% of the study sample size. The median age was 41 years while the median duration of treatment was 6 (the range was 3-11) years. Hair samples of 223 participants were collected and presented to the laboratory. Only 105 participants donated enough hair quantity >10 mg since some were willing to donate their hair samples, but their hair was very short. The 328 plasma samples were collected and submitted to the laboratory to quantify nevirapine (NVP). However, only 308 plasma samples were analyzed for NVP level because some participants who donated blood samples were reluctant to grant their hair samples. Some donated hair and were unwilling to donate blood samples. Therefore, participants with the matched plasma and hair samples were only 94, which represented 22.000% of the participants. Only 51.400% of participants who donated their samples for analysis did not take their ARVs for a whole day or more (table 4.1).

Table 4.1: Demographic characteristics of participants of the MAAT study.

Variable		Frequency	Percentage
Age (years)	**Median (range)**	41 (34-49)	
	20-30	69	16.500
	31-40	135	32.600
	41-50	128	30.800
	>51	81	20.100
Gender	Female	242	58.400
	Male	171	41.600
Viral load	>1000 copies/ML	210	50.800
	<1000 copies/Ml	47	11.200
Samples types	Plasma samples	328	74.600
	Hair samples	223	25.400
	Matched hair and plasma samples	94	22.000
Donated samples	Donated only one sample type	329	78.000
Hair samples >10mg	Yes	105	47.100
	No	118	52.900
Plasma samples in good condition for analysis	Yes	308	93.900
	No	20	6.100
Duration on treatment	**Median (IQR)**	6	(3 - 11)
	1 - 5 Years	201	48.500
	5 - 10 Years	104	25.100
	>11 Years	108	26.400
Current ARV type	lamivudine, nevirapine, tenofovir	340	82.200
	lamivudine, nevirapine, zidovudine	72	17.300
	lamivudine, nevirapine, stavudine	1	0.500
Did not take ARV for a day or more	Yes	224	54.100
	No	189	45.900

4.2 Validation of the HPLC-UV and LC-MS/MS methods for analysis of plasma samples

4.2.1 Linearity and Sensitivity

For LC-MS/MS and HPLC-UV, the lower detection limit (LLOD) was 5 and 20 ng/mL, respectively. The HPLC-UV and LC-MS/MS lower limits of quantification (LLOQ) were 25 ng/mL and 10 ng/mL, respectively. The NVP calibration curves for the HPLC-UV and LC-MS/MS systems were linear throughout a range of 20–1000 ng/mL (Figure 4.1) and 5-500 ng/mL (Figure 4.2), respectively. Correlation coefficients greater than 0.995 indicate a strong link between the peak area and the NVP concentration (r^2 = 0.999 for HPLC-UV and r^2 = 0.9988 for LC-MS/MS).

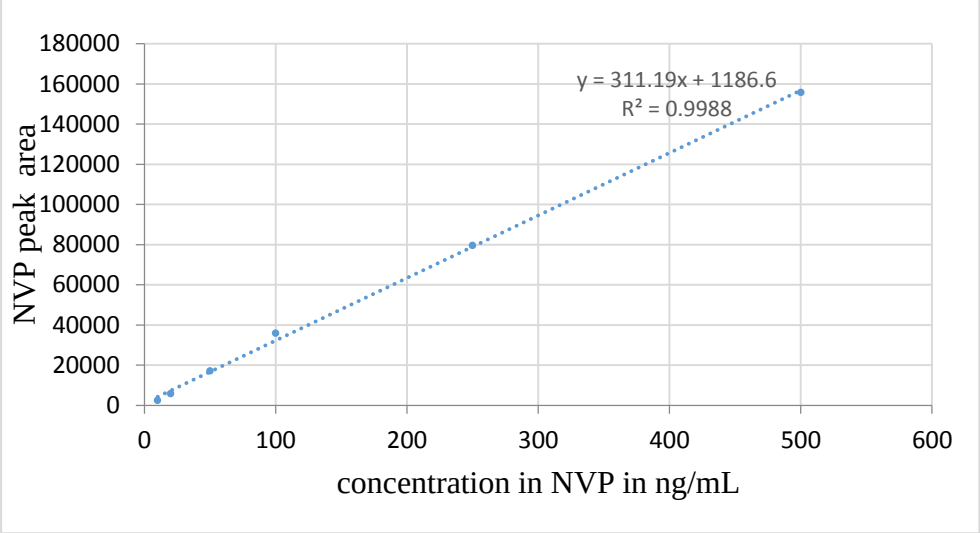


Figure 4.1: Calibration curve of LC-MS/MS for analysis of plasma.

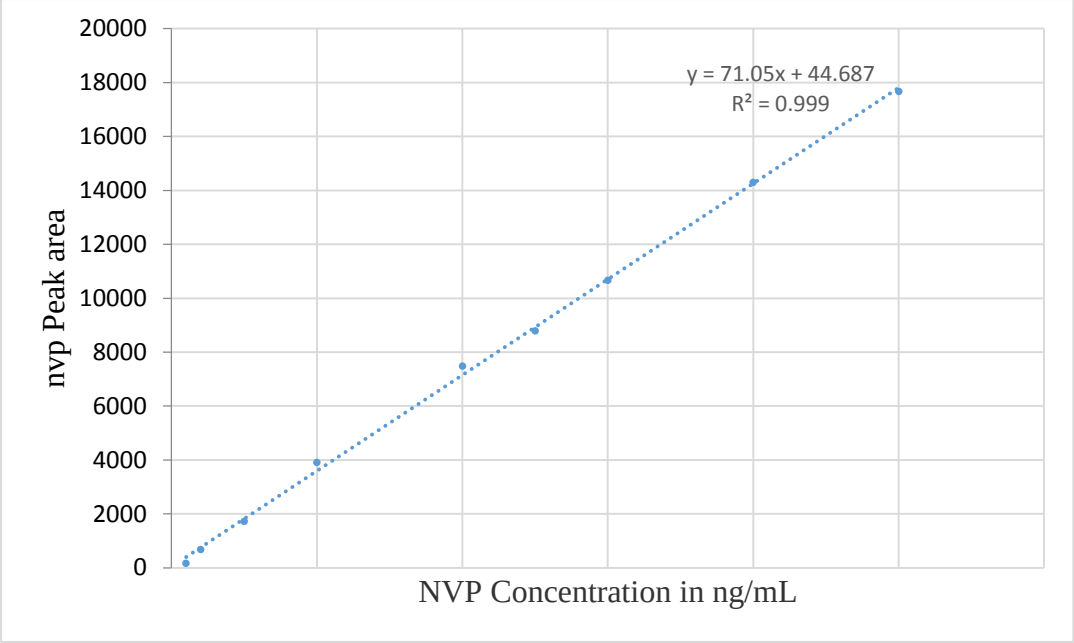


Figure 4.2: Calibration curve of LC-MS/MS for analysis of plasma.

4.2.2 Accuracy and precision

Appendix II and Appendix III, respectively, display the findings of the intraday and interday precision and accuracy for the quality controls for HPLC-UV and LC-MS/MS. With the HPLC-UV method, the accuracy ranged from 97.506 to 100.667, and the inter-day and intra-day percentage CV was less than 10%. Intra-day and inter-day percentage CVs for the LC-MS/MS technique were less than 10%, and the accuracy percentage ranged from 96.700 to 100.107%. The intra-day and inter-day accuracy and precision findings for the QCs of the LC-MS/MS and HPLC-UV procedures were found to be within acceptable limits (85-115%) of FDA (Medicines Agency, 2011). The accuracy of the LC-MS/MS apparatus mirrored results published in 2007 by Jung *et al*., (2007) who found an accuracy of 94.300%, falling between the advised range of 85–115%. As a result, they were deemed accurate and precise enough to analyze plasma sample NVP levels. The intra-day and inter-day accuracy and precision findings for the QCs of the LC-MS/MS and HPLC-UV procedures were

found to be within acceptable levels of FDA (Medicines Agency, 2011).

4.2.3 Specificity and selectivity

The standards that were analyzed using LC-MS/MS and HPLC-UV instruments did not exhibit any interfering peak during the NVP retention times. This, therefore, showed high selectivity towards NVP in plasma. Our data were consistent with (Ranaware *et al.*, 2013), which analyzed NVP in plasma using HPLC-UV. The resolution between NVP and endogenous plasma compounds was satisfactory and no peak from endogenous substances was present. Compared to the HPLC-UV instrument, the LC/MS/MS technique had a strong specificity since only the target analytes' precursor ions reached the MS. This is because there is no significant direct interference in the MRM channel for the analytes at the expected retention time, as shown by the representative chromatograms (Figure 4.3 and Figure 4.4).

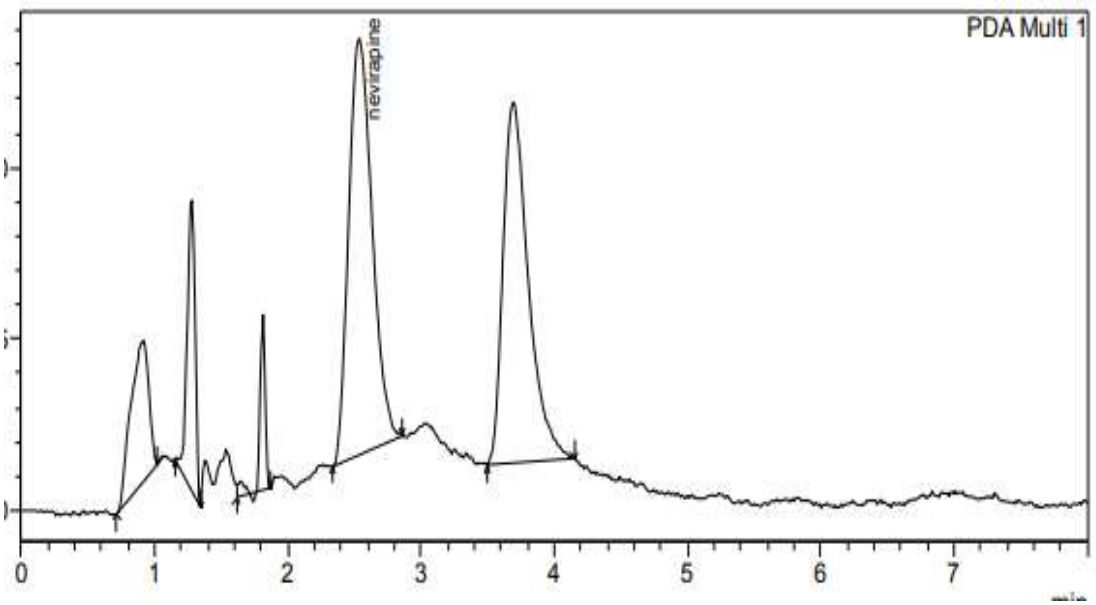


Figure 4.3: Incurred plasma sample HPLC-UV Chromatogram.

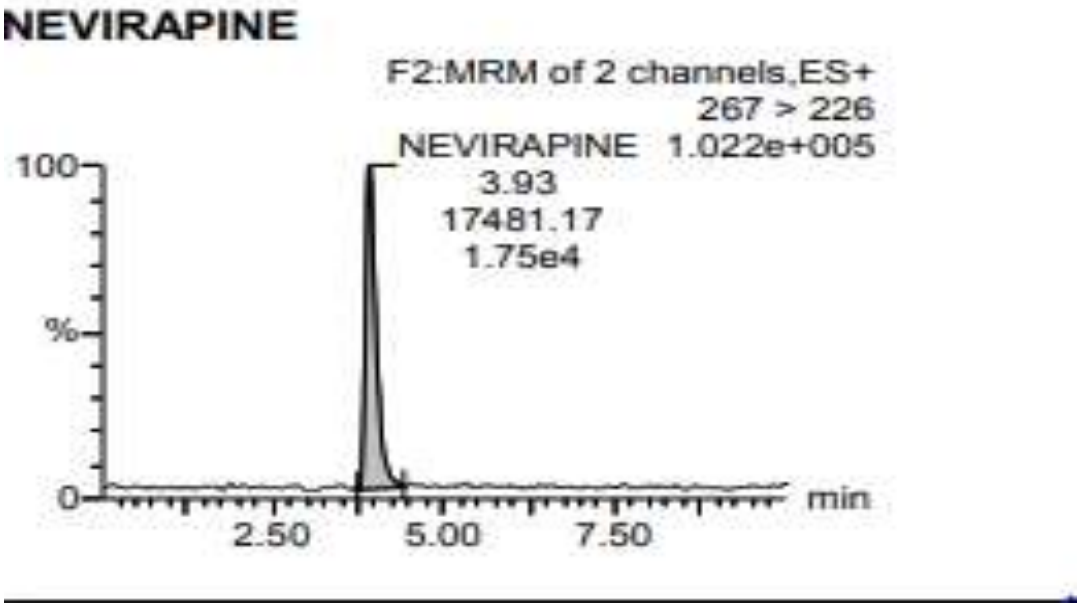


Figure 4.4: LC-MS/MS chromatogram for spiked blank (10 ng/mL).

4.2.4 Extraction Recovery

NVP's recovery in QC concentrations low, medium and high samples analyzed using HPLC-UV was relatively high at 97.130%, 97.210 % and 94.620% compared to 97.570%, 87.650% and 99.250%, recovery percentage of LC-MS/MS.

4.3 Validation of the HPLC and LC-MS/MS protocols for analysis of hair samples

4.3.1 Linearity

Lower detection limits (LLODs) were 2 ng/mg and 0.5 ng/mg for HPLC-UV and LC-MS/MS, respectively. Lower limits of quantification (LLOQ) for LC-MS/MS and HPLC-UV, on the other hand, were 10 ng/mL and 25 ng/mL, respectively. NVP concentration for LC-MS/MS ranged between 0.500-25.000 ng/mg, while the NVP concentration for HPLC-UV ranged between 2.000-50.000 ng/mg. The correlation coefficients (r^2) of LC-MS/MS and HPLC-UV's calibration curves were 0.9991 and 0.999, respectively (Figures 4.5 and Figure 4.6). The results showed that these linearity range methods are broad and suitable for studying clinical samples. Both

techniques were used successfully to identify antiretroviral drug concentrations in PLWH.

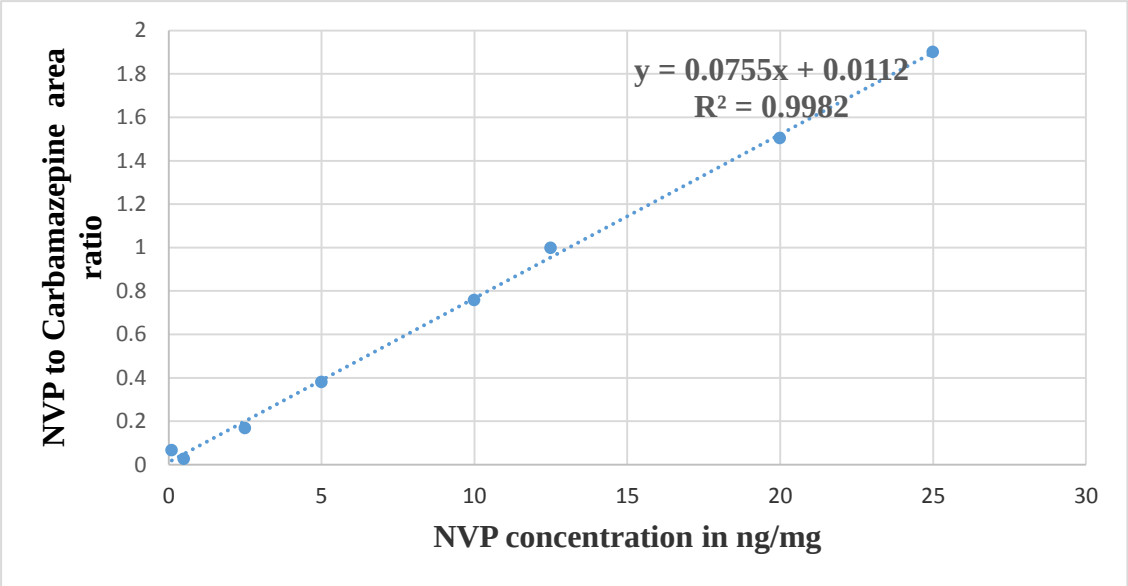


Figure 4.5: LC-MS/MS linear curve for Hair samples analysis.

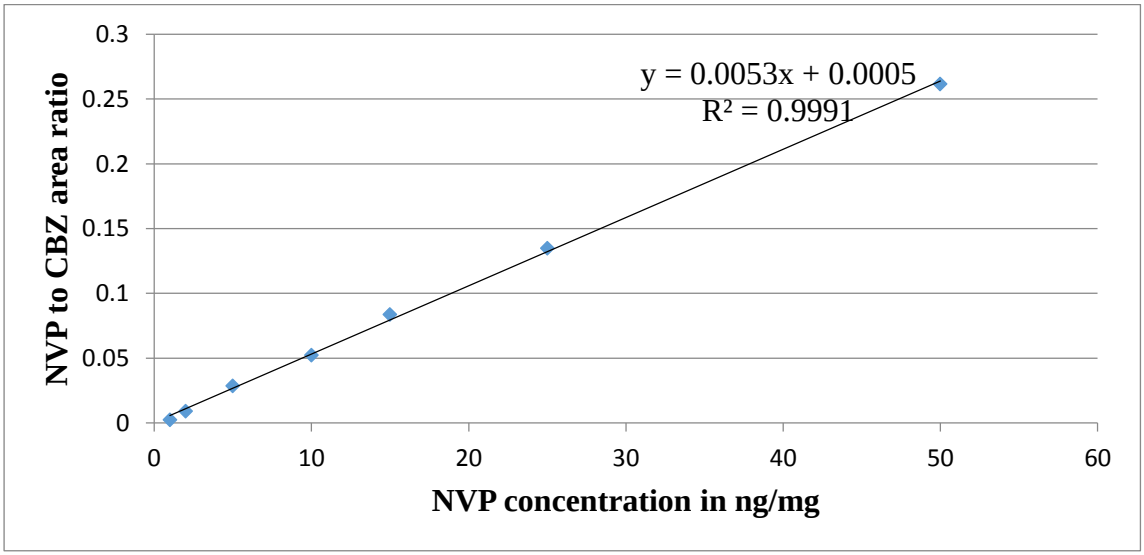


Figure 4.6: HPLC-UV calibration curve for Hair samples analysis.

4.3.2 Precision and Accuracy

Appendix IV and Appendix V provide a summary of the spiked quality control (QC) samples' interday and intraday accuracy and precision. The accuracy of the low, medium, and high QC concentrations for NVP using the HPLC-UV approach was

97.980-100.280% and the coefficients of variation (CV) were all less than 10% (Appendix IV). These findings show that the standard technique for determining NVP in human hair samples obtained from incurred hair is accurate. The intraday percentage CV for the LC-MS/MS techniques was less than 10%, and the accuracy ranged from 99.140 to 95.560% (Appendix V). According to FDA recommendations Medicines Agency, (2011), the interday and intraday accuracy and precision findings for NVP for HPLC-UV and LC-MS/MS QCs fell between 85 and 115% of permissible ranges. Huang *et al.* (2011) found that the accuracy of the low, medium, and high QC concentrations for NVP was 98.300–105.90% with coefficients of variation (CV) less than 10% in a study that was similar and employed the LC-MS/MS technology. These findings show that the existing techniques for measuring NVP in both human and hair samples have acceptable precision.

4.3.3 Specificity and selectivity

The chromatographic methods, HPLC-UV and LC-MS/MS demonstrated excellent separation for NVP (Figure 4.7 and Figure 4.8). This is because there was no co-elution of peaks in the retention times of NVP. As seen in the chromatograms of the HPLC-UV (Figure 4.7) and LC-MS/MS MRM (Figure 4.8), the technique was very precise for NVP and carbamazepine (CBZ) analytes.

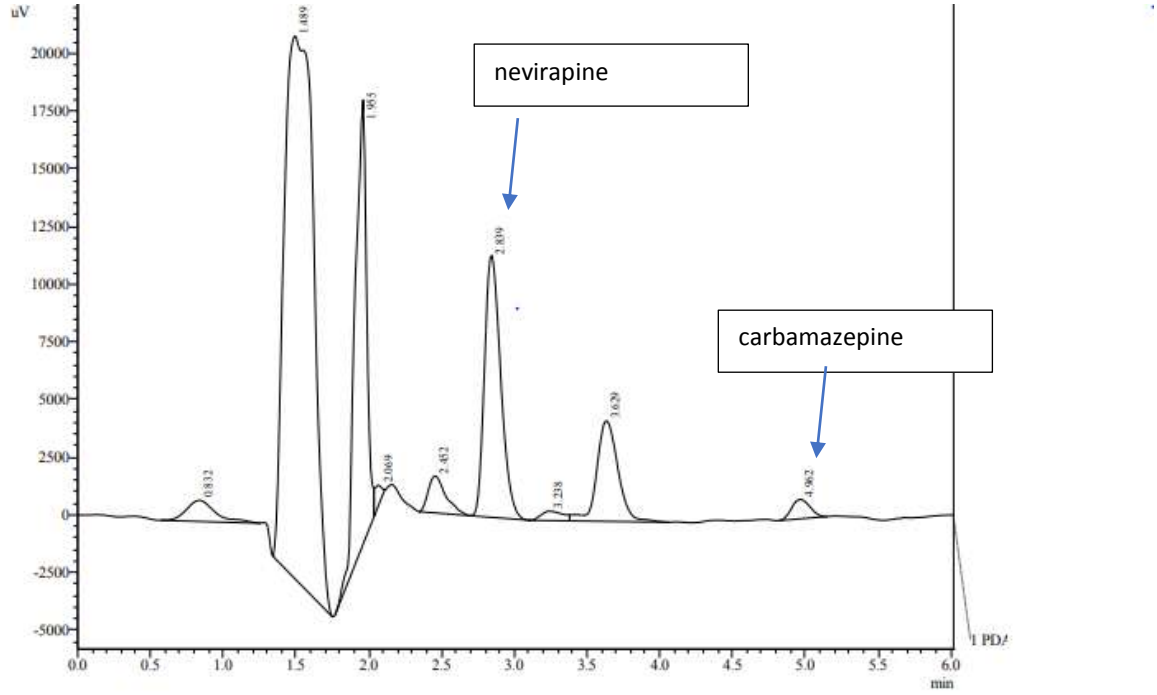


Figure 4.7: HPLC-UV Chromatogram of the spiked blank hair sample.

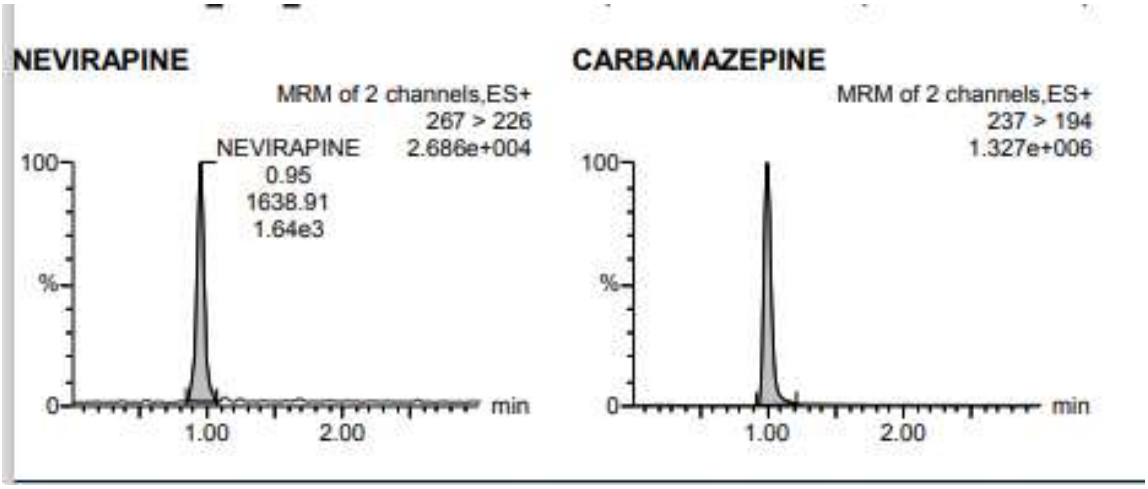


Figure 4.8: LC-MS/MS chromatogram of the spiked blank hair sample.

The methods were selective since no NVP or IS peaks were seen in the blank samples of HPLC-UV (Figure 4.9) and LC-MS/MS (Figure 4.10).

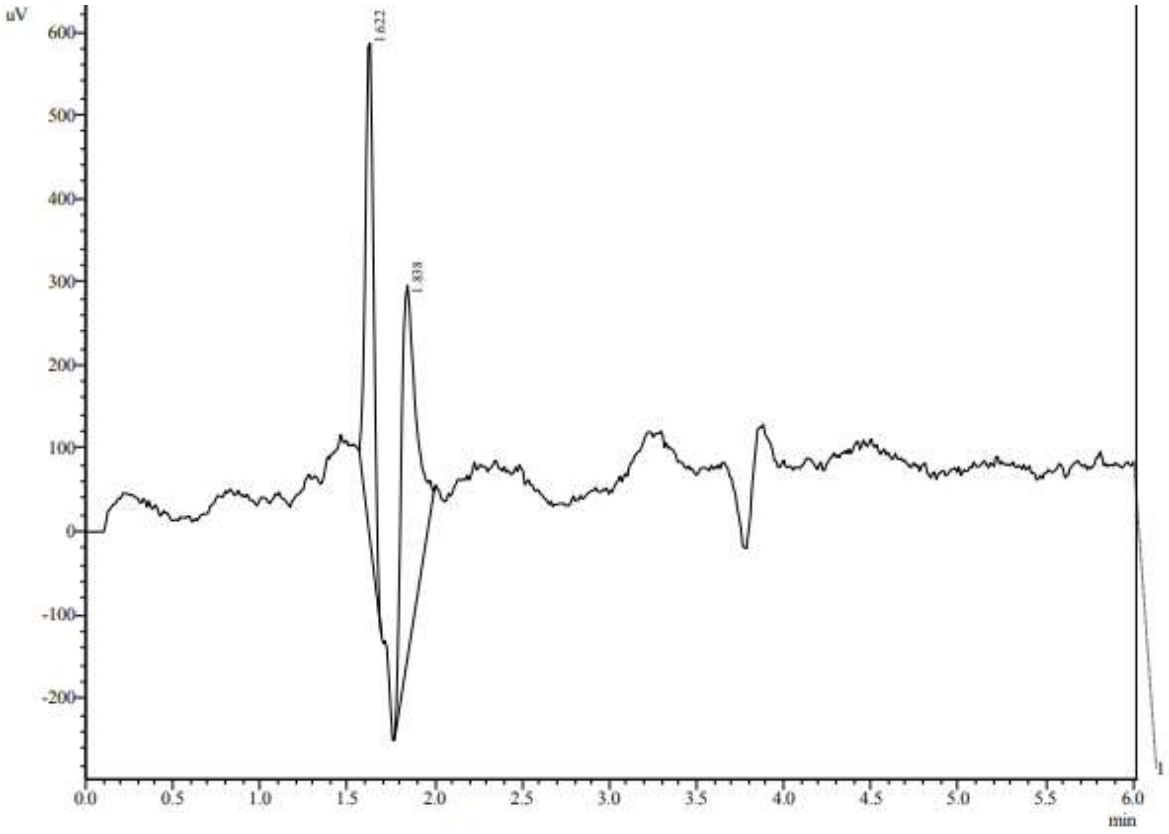


Figure 4. 9: HPLC-UV chromatogram of the blank hair sample

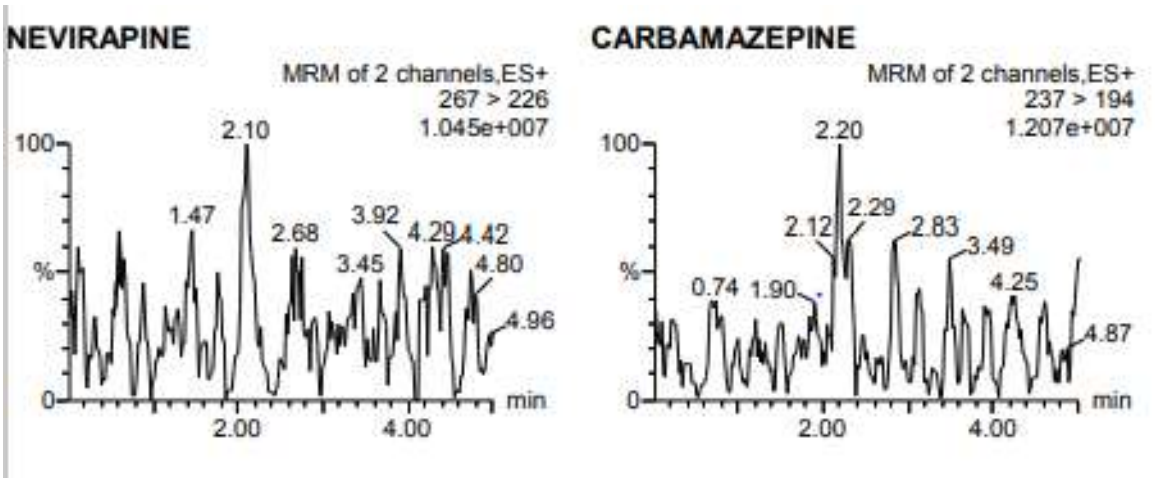


Figure 4. 10: LC-MS/MS chromatogram of the blank hair sample.

4.3.4 Extraction Efficiency

One of the hair samples was taken through three consecutive extraction processes of the NVP drug in hair samples. This was performed because the spiked NVP does not entirely get into the intact hair tissue. As the hair grows, the drug is integrated into the hair tissue. However, the extraction efficiency reflects the recovery procedure for Liquid-liquid extraction, not the first MeOH/TFA (v/v, 9:1) extraction part. After the first extraction of the sample, 810.000 ng/mL of nevirapine was extracted, the second extraction 51.700 ng/mL and 13.500 ng/mL were extracted during the second and the third extraction. The percentage of NVP in the extracted hair sample extracted in the second and the third extraction was 6.380% and 1.670% in the first extraction, respectively (Figure 4.11). This indicates that at least 92.000% of the NVP drug was extracted during the first extraction, with a small amount of NVP compound remaining in the extracted hair samples, proving that the extraction method was very effective.

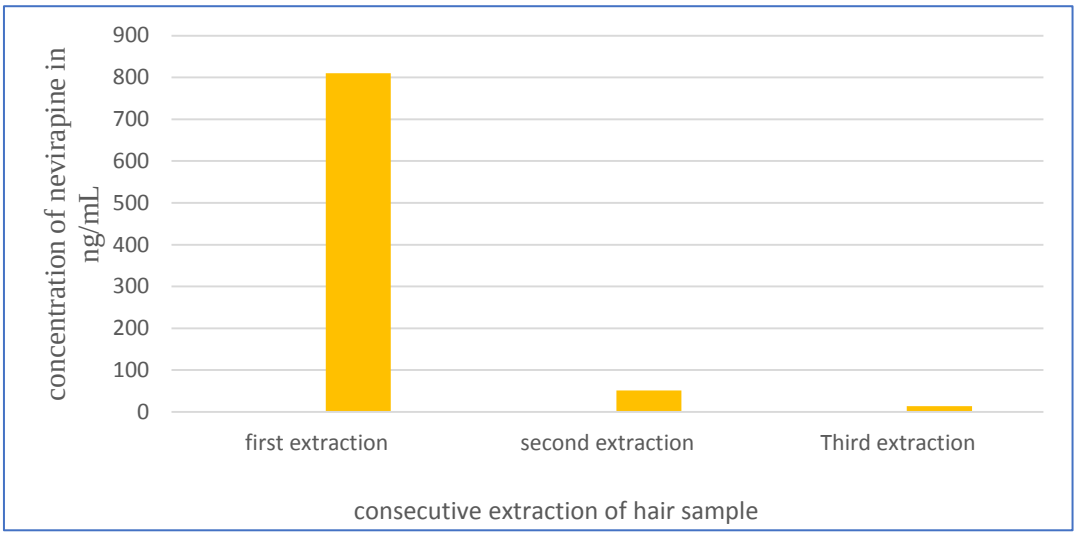


Figure 4. 11: Bar chart showing three consecutive extractions of NVP in the incurred hair sample.

4.4 Determination of nevirapine levels in the plasma and hair samples

The Shapiro-Wilk and Kolmogorov-Smirnov test p-values were all less than 0.05, indicating a significant departure from normality in the data.

The median hair NVP concentration was 19.300 ng/mL in the plasma sample and 36.8 ng/mL in the hair sample. The percentage of participants whose NVP plasma and hair concentrations were less than or equal to 17.100 and 5.230 ng/mL, respectively was 25%. Additionally, 75% of participants' NVP hair and plasma concentrations were less than or equal to 102.300 ng/mL and 562.650 ng/mL, respectively. The hair samples' interquartile range (IQR) was (17.100-102.300 ng/mL), which means that 50% of the hair NVP concentrations were between 17.100 and 102.300 ng/mL. The interquartile range (IQR) for the plasma samples was (5.225-562.650 ng/mL). This also means that 50% of the plasma NVP concentrations were between 5.230 and 562.650 ng/mL (Table 4.2). Nevirapine (NVP) is a medication that is extensively disseminated throughout tissues because it is very lipophilic. It is possible to identify NVP in even a single hair sample due to the high NVP level in human hair (Gandhi *et al*., 2014).

Table 4.2: Median and inter-quartile range (IQR 25-75) of hair and plasma samples.

Sample type	Number of samples	Median (ng/mL)	IQR range (ng/mL)
Hair samples	105	36.820	17.100-102.300
Plasma samples	308	19.320	5.230-562.650

The hair NVP concentration ranged between 5.400 to 1211.500 ng/mL while the plasma (Figure 4.12). NVP concentrations ranged between 0-5000 ng/mL (Appendix VI). The plasma concentration was extensively dispersed as compared to the hair concentration. This was in line with the report by Jung *et al*. (2007) that high upper concentration limits of NVP's plasma concentration extended up to 9000 ng/mL. Low

plasma NVP concentrations can arise due to poor adherence (Burger *et al*., 2006). However, some patients can still have low plasma levels of NVP, even while still adhering to the ART regimen. This could result from factors such as interpatient variability in exposure to NVP, drug-drug interactions and drug-food interactions (Burger *et al*., 2006).

The variance of NVP concentrations was found to be 197158.000 for plasma samples and 14793.360 for hair samples (Appendix VI). The variability of NVP levels across different patients could arise due to multiple variables: body mass index, age, race, rate of metabolism, different times of exposure to ARVs on the day of sampling and genetics (Yan *et al*., 2016).

4.5 Comparison of NVP levels in the plasma and matched hair samples

The goal of the analysis and comparison was to see if the amounts of NVP in the hair and matching plasma samples differed significantly from one another. The representative chromatograms of hair and matched plasma samples are shown in (Figures 4.12 and 4.13). The mean NVP concentration in hair was 10.490 ng/mg (104.900 ng/mL), while the mean NVP concentration in matched plasma samples was 525.350 ng/mL. The hypothesis was tested using the Wilcoxon signed-rank test. The statistic result was ($Z = -0.93$, $p > 0.05$), meaning there was no significant difference between the NVP hair concentration and the plasma NVP concentration (Table 4.3). These results matched what Gandhi and others found $p > 0.05$: a significant difference between concentrations of efavirenz in the hair and plasma samples (Gandhi *et al*., 2019). Johnson et al. similarly observed no statistically significant difference ($p > 0.05$) between the plasma and hair samples' efavirenz concentrations (Johnston *et al*., 2019). This showed that the drug deposition in the hair depends on its concentration

in the plasma and thus concluded that the hair's NVP concentrations can strongly measure the adherence, just like the plasma samples (Johnston *et al*., 2019). Hair analysis provides an advantage over blood or urine testing in assessing long-term adherence. The NVP level analysis process for hair has obvious cost and practicality advantages over blood analysis.

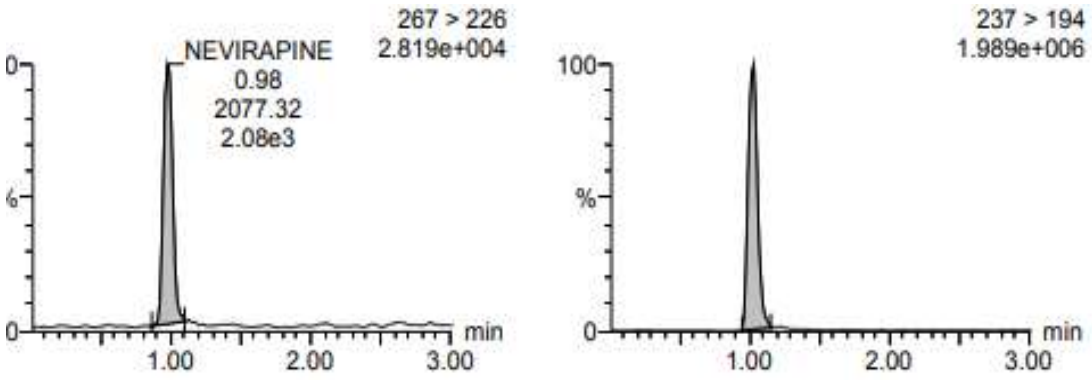


Figure 4.12: Representative chromatogram of hair sample analysed using LC-MS/MS.

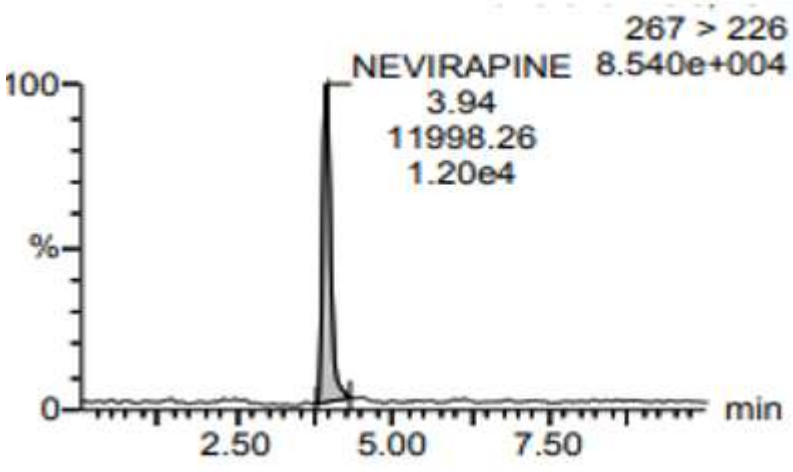


Figure 4.13: Representative chromatogram of matched plasma sample analysed using LC-MS/MS.

Table 4.3: Mean concentration of hair and plasma samples analysed using LC-MS/MS and HPLC-UV instrument.

	N	Mean ± SE (ng/mL)
Hair concentration LC-MS/MS	94	104.900 ± 19.700
Plasma concentration LC-MS/MS	94	525.350 ± 96.200
$Z = -0.93$, $p > 0.05$		

4.6 Performance of HPLC-UV and LC-MS/MS instruments in the analysis of NVP in hair and plasma samples

Comparison of the data from the two techniques was made to check whether the performance of the two instruments significantly differ. However, not all the samples analysed using LC-MS/MS were analysed using HPLC-UV. This is because the HPLC-UV detection limit was higher than the LLOQ of LC-MS/MS. A total of 123 and 54 hair and plasma samples were analysed using LC-MS/MS and HPLC-UV, respectively (Table 4.4). The mean of Hair and plasma samples analysed using HPLC-UV were 180.200 ng/mL and 878.930 ng/mL. The NVP concentrations of the plasma and hair samples that were analysed using LC-MS/MS were 978.890 ng/mL and 178.400 ng/mL, respectively. The HPLC-UV analysis of plasma and hair samples revealed greater mean NVP concentrations than LC-MS/MS; this difference could be due to co-elution, which is caused by the UV detector's reported lower selectivity (Jin *et al*., 2019).

The Spearman's rank test was used to determine whether a strong correlation of results was obtained using HPLC-UV and LC-MS/MS instruments. Spearman rank test statistics indicated a significant positive association between hair and plasma NVP concentrations samples analysed using both LC-MS/MS and hair and plasma NVP concentrations analysed using HPLC-UV respectively (R = 0.995 and R =

O.966, $p < 0.05$). These results matched with those of Jin and others, whereby a good correlation ($R = 0.936$) was reported between LTG analysis data analysed using HPLC-UV and UHPLC-MS/MS. The equality line and the correlation equation were nearly in line ($y = x$) (Jin *et al*., 2019). Our results also matched the report by Suneetha and Raja, stating that the LC-UV data was close to LC-MS/MS data (Suneetha & Raja, 2016). Since the performance of HPLC-UV and LC-MS/MS were almost the same, it proved that HPLC-UV could be used as a substitute for LC-MS/MS in the analyses ARVs, in resource-limited settings, or in cases where LC-MS/MS is unavailable.

Table 4.4: Mean of NVP levels in hair and plasma samples analysed using the LC-MS/MS and HPLC-UV.

	LC-MS/MS		**HPLC-UV**	
Sample type	N[1]	Mean ± SE (ng/mL)	N	Mean (ng/mL)
Plasma	123	978.890 ± 88.800	124	884.930 ± 82.100
Hair	55	178.380± 31.600	55	168.700 ± 29.000
$r^2 = 0.995$, $p < 0.05$) for hair samples r^2 = O.966, $p < 0$ .05) for plasma samples				

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Given that the amounts of NVP in matched plasma samples and hair did not differ significantly from one another. matching plasma samples, our study has shown that, in settings where laboratory facilities and trained staff to perform phlebotomy are scarce or lacking analysis of ARV drugs in the hair can determine drug exposure as an alternative to conventional plasma drug analysis. The analysis of ARVs in hair and plasma samples can be viewed as separate instruments with distinct benefits and drawbacks that can also be combined; nevertheless, it is not suggested that the results support substituting plasma testing with hair testing as a research measure in all circumstances. The outcomes that were reviewed and presented demonstrated how hair analysis may be used in Kenya to track ART adherence.

The comparison of LC-MS/MS and HPLC-UV techniques for the analysis of Nevirapine (NVP) concentrations in hair and plasma samples demonstrated that HPLC-UV is a viable alternative to LC-MS/MS. Despite differences in absolute concentrations due to the higher detection limit of HPLC-UV and potential co-elution issues with the UV detector, the Spearman's rank correlation analysis revealed a strong positive correlation between the results obtained from both methods ($R = 0.995$ for hair and $R = 0.966$ for plasma).

The high degree of agreement between the two methods supports the conclusion that HPLC-UV can provide reliable and comparable results to LC-MS/MS in the measurement of NVP concentrations. Given its lower cost and greater accessibility, HPLC-UV could be considered a suitable substitute for LC-MS/MS in resource-

limited settings or where LC-MS/MS is not available. Thus, HPLC-UV is a practical, cost-effective option for routine analysis of antiretroviral drugs (ARVs), such as Nevirapine, without compromising the accuracy or reliability of the results.

5.2 Recommendations

5.2.1 Recommendations from the study

i. Hair drug analysis can be used as a substitute for plasma drug analysis in monitoring adherence of people living with HIV in Kenya respect to determining the reproducibility of the method.

5.2.2 Recommendation for further research

i. Comparative analysis of ARVs in blood and other non-invasive samples like finger and toe nails should be performed in future studies to determine whether there is a relation between the levels of ARVs in nails and blood samples.

ii. Determination of levels of other types of ARV drugs in hair and blood-matched samples is highly recommended to ascertain whether hair samples can be used to monitor adherence to such drugs.

REFERENCES

Adewumi O. M., Odaibo G. N., & Olaleye O. D. (2015). Efficacy of generic highly active antiretroviral therapy in HIV-1 infected individuals in Nigeria. *Journal of Immunoassay and Immunochemistry*, ***36***, 464–477. https://doi.org/10.1080/15321819.2014.969436

Baril J-G, Angel JB, Gill MJ, Gathe J, Cahn P, van Wyk J, et al. (2016) Dual Therapy Treatment Strategies for the Management of Patients Infected with HIV: A Systematic Review of Current Evidence in ARV-Naive or ARV-Experienced, Virologically Suppressed Patients. PLoS ONE 11(2): e0148231. https://doi.org/10.1371/journal.pone.0148231

Barth R. E., van der Loeff M. F. S., Schuurman R., Hoepelman A. I., & Wensing A. M. (2010). Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. In *The Lancet Infectious Diseases* (**Vol. 10**, Issue 3, pp. 155–166). https://doi.org/10.1016/S1473-3099(09)70328-7

Baxi S. M., Greenblatt R. M., Bacchetti P., Jin C., French A. L., Keller M. J., Augenbraun M. H., Gange S. J., Liu C., Mack W. J., Gandhi M., Anastos K., Minkoff H., Young M., Tien P., Aouizerat B., Levine A., Cohen M., & Golub E. (2015). Nevirapine concentration in hair samples strongly predicts virologic suppression in a prospective cohort of HIV-infected patients. *PLoS ONE*, ***10***. https://doi.org/10.1371/journal.pone.0129100

Bennett M., Johns K., & Bondy, G. (2008). Current and future treatments of HIV-associated dyslipidemia. *Future Lipidology*, ***3***, 175–188. https://doi.org/10.2217/17460875.3.2.175

Burger D., Van Der Heiden I., La Porte, C., Van Der Ende, M., Groeneveld, P., Richter C., Koopmans P., Kroon, F., Sprenger, H., Lindemans, J., Schenk, P., & Van Schaik, R. (2006). Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: The effect of gender, raceand CYP2B6 polymorphism. *British Journal of Clinical Pharmacology*, ***61***, 148–154. https://doi.org/10.1111/j.1365-2125.2005.02536.x

Calcagno, A., D'Avolio A., Simiele M., Cusato J., Rostagno R., Libanore V., Baietto L., Siccardi M., Bonora S., & Di Perri G. (2012). Influence of CYP2B6 and ABCB1 SNPs on nevirapine plasma concentrations in Burundese HIV-positive patients using dried sample spot devices. *British Journal of Clinical Pharmacology*, ***74***, 134–140. https://doi.org/10.1111/j.1365-2125.2012.04163.x

Charb N., Baldelli S., Cozzi V., Castoldi S., Cattaneo D., & Clementi E. (2016). *Development of an HPLC–UV assay method for the simultaneous quantification of nine antiretroviral agents in the plasma of HIV-infected patients*. https://doi.org/10.1016/j.jpha.2016.05.008

D'Ettorre G., Forcina G., Ceccarelli G.andreotti M.andreoni C., Rizza C., Massetti A. P., Sarmati L., Mastroianni C. M., Vella S.andreoni M., & Vullo, V. (2011). Adherence and genotypic drug resistance mutations in HIV-1-infected patients failing current

antiretroviral therapy. *Journal of Chemotherapy*, ***23***, 24–27. https://doi.org/10.1179/joc.2011.23.1.24

Ekouevi D. K., Tchounga B. K., Coffie, P. A., Tegbe J.anderson A. M., Gottlieb, G. S., Vitoria, M., Dabis F., & Eholie S. P. (2014). Antiretroviral therapy response among HIV-2 infected patients: A systematic review. *BMC Infectious Diseases*, ***14.*** https://doi.org/10.1186/1471-2334-14-461

Gandhi M., Ameli, N., Bacchetti P., Gange S. J., Anastos K., Levine A., Hyman C. L., Cohen M., Young M., Huang Y., & Greenblatt R. M. (2009). Protease inhibitor levels in hair strongly predict virologie response to treatment. *AIDS*, ***23,*** 471–478. https://doi.org/10.1097/QAD.0b013e328325a4a9

Gandhi, M., & Greenblatt, R. M. (n.d.-a). Hair it is: The long and short of monitoring antiretroviral treatment. In *Annals of Internal Medicine* (pp. 674–6685). https://doi.org/10.7326/0003-4819-137-8-200210150-00016

Gandhi, M., Yang, Q., Bacchetti P., & Huang Y. (2014). Short communication: A low-cost method for analysing nevirapine levels in hair as a marker of adherence in resource-limited settings. *AIDS Research and Human Retroviruses*, ***30***, 25–28. https://doi.org/10.1089/aid.2013.0239

Günthard H. F., Saag M. S., Benson C. A., Del Rio, C., Eron J. J., Gallant J. E., Hoy J. F., Mugavero, M. J., Sax P. E., Thompson, M. A., Gandhi R. T., Landovitz R. J., Smith D. M., Jacobsen D. M., & Volberding, P. A. (2016). Antiretroviral drugs for treatment and prevention of HIV infection in Adults: 2016 recommendations of the international antiviral society-USA Panel. *JAMA - Journal of the American Medical Association*, ***316***, 191–210. https://doi.org/10.1001/jama.2016.8900

Hamers R. L., Wallis C. L., Kityo C., Siwale M., Mandaliya K., Conradie F., Botes M. E., Wellington, M., Osibogun A., Sigaloff K. C. E., Nankya I., Schuurman R., Wit F. W., Stevens W. S., van Vugt, M., & de Wit, T. F. R. (2011). HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: A multicentre observational study. *The Lancet Infectious Diseases*, ***11,*** 750–759. https://doi.org/10.1016/S1473-3099(11)70149-9

Hassan A. S., Fielding K. L., Thuo N. M., Nabwera H. M., Sanders E. J., & Berkley, J. A. (2012). Early loss to follow-up of recently diagnosed HIV-infected adults from routine pre-ART care in a rural district hospital in Kenya: a cohort study. *Tropical Medicine and International Health*, ***17***, 82–93. https://doi.org/10.1111/j.1365-3156.2011.02889.x

Hemelaar J. (2013). Implications of HIV diversity for the HIV-1 pandemic. *Journal of Infection*, ***66***, 391–400. https://doi.org/10.1016/j.jinf.2012.10.026

Hickey, M. D., Salmen, C. R., Tessler, R. A., Omollo, D., Bacchetti, P., Magerenge, R., Mattah, B., Salmen, M. R., Zoughbie, D., Fiorella, K. J., Geng, E., Njoroge, B., Jin, C., Huang, Y., Bukusi, E. A., Cohen, C. R., & Gandhi, M. (2014a). Antiretroviral concentrations in small hair samples as a feasible marker of adherence in rural Kenya. *Journal of Acquired Immune Deficiency Syndromes*, ***66***, *311–315. https://doi.org/10.1097/QAI.0000000000000154*

Huang, Y., Yang Q., Yoon K., Lei Y., Shi R., Gee W., Lin E. T., Greenblatt R. M., & Gandhi M. (2011). Microanalysis of the antiretroviral nevirapine in human hair from HIV-infected patients by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, ***401***, 1923–1933. https://doi.org/10.1007/s00216-011-5278-7

Johnston J. (2017). *Determination of biomarkers for toxicity and antiretroviral adherence in hair in South African patients*.

Johnston J., Wiesner L., Smith P., Maartens G., & Orrell C. (2019). Correlation of hair and plasma efavirenz concentrations in HIV-positive South Africans. *Southern African Journal of HIV Medicine*, ***20***. https://doi.org/10.4102/sajhivmed.v20i1.881

Jung B. H., Rezk N. L., Bridges A. S., Corbett A. H., & Kashuba A. D. M. (2007). Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry. *Biomedical Chromatography*, ***21***, 1095–1104. https://doi.org/10.1002/bmc.865

Kantor R., Diero L., Delong A., KamLe L., Muyonga S., Mambo F., Walumbe E., Emonyi W., Chan P., Carter J. E., Hogan J., & Buziba N. (2009). Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. *Clinical Infectious Diseases*, *49*(3), 454–462. https://doi.org/10.1086/600396

Kasaie P., Weir B., Schnure M., Dun C., Pennington J., Teng Y., Wamai R., Mutai, K., Dowdy D., & Beyrer, C. (2020). Integrated screening and treatment services for HIV, hypertension and diabetes in Kenya: assessing the epidemiological impact and cost-effectiveness from a national and regional perspective. *Journal of the International AIDS Society*, *23*(S1). https://doi.org/10.1002/jia2.25499

Kenya-HIV-County-Profiles-2016. (2016). *Kenya-HIV-County-Profiles*, 29–157.

Kimulwo M. J. (2018). *THERAPEUTIC DRUG LEVELS AND TREATMENT RESPONSIVENESS AMONG PATIENTS FROM HOMA BAY AND KISUMU COUNTY INFECTED WITH HIV TYPE 1*.

Kintz P. (2004). Value of hair analysis in postmortem toxicology. *Forensic Science International*, ***142,*** 127–134. https://doi.org/10.1016/j.forsciint.2004.02.027

Lamorde M., Fillekes, Q., Sigaloff, K., Kityo, C., Buzibye, A., Kayiwa, J., Merry, C., Nakatudde-Katumba, L., Burger, D., & de Wit, T. F. R. (2014). Therapeutic drug monitoring of nevirapine in saliva in Uganda using high performance liquid chromatography and a low cost thin-layer chromatography technique. *BMC Infectious Diseases*, ***14***. https://doi.org/10.1186/1471-2334-14-473

L'Homme R. F. A., Muro E. P., Droste J. A. H., Wolters, L. R., Van Ewijk-Beneken Kolmer, N. W. J., Schimana, W., & Burger, D. M. (2008). Therapeutic drug monitoring of nevirapine in resource-limited settings. *Clinical Infectious Diseases*, ***47***, 1339–1344. https://doi.org/10.1086/592694

Liu A. Y., Yang, Q., Huang Y., Bacchetti P.anderson P. L., Jin C., Goggin K., Stojanovski K., Grant R., Buchbinder S. P., Greenblatt R. M., & Gandhi M. (2014a). Strong

relationship between oral dose and tenofovir hair levels in a randomized trial: Hair as a potential adherence measure for pre-exposure prophylaxis (PrEP). *PLoS ONE*, ***9***, 18–24. https://doi.org/10.1371/journal.pone.0083736

Makita-Chingombe, F., Ocque, A. J., DiFrancesco, R., Maponga, C., Muzambi, F., Monera-Penduka, T. G., ... & Morse, G. D. (2019). Development and validation of a high performance liquid chromatography method to determine nevirapine in plasma in a resource-limited setting. *African Journal of Laboratory Medicine*, *8*(1), 1-7.

Makoae M. (2011). Ambiguous diagnosis, futile treatments and temporary recovery: Meanings of medical treatment among HIV/AIDS family caregivers providing care without ARVs. *SAHARA-J: Journal of Social Aspects of HIV/AIDS*, ***8***, 187–196. https://doi.org/10.1080/17290376.2011.9725003

Marchei E., Escuder D., Pallas C. R., Garcia-Algar O., Gómez A., Friguls B., Pellegrini, M., & Pichini, S. (2011). Simultaneous analysis of frequently used licit and illicit psychoactive drugs in breast milk by liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, ***55***, 309–316. https://doi.org/10.1016/j.jpba.2011.01.028

Mouton J. P., Cohen K., & Maartens G. (2016). Key toxicity issues with the WHO-recommended first-line antiretroviral therapy regimen. In *Expert Review of Clinical Pharmacology* (**Vol. 9,** pp. 1493–1503). Taylor and Francis Ltd. https://doi.org/10.1080/17512433.2016.1221760

NASCOP. (2018). *Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV in Kenya*.

Nielsen M. K. K., Johansen S. S., & Linnet K. (2014). Pre-analytical and analytical variation of drug determination in segmented hair using ultra-performance liquid chromatography-tandem mass spectrometry. *Forensic Science International*, ***234***, 16–21. https://doi.org/10.1016/j.forsciint.2013.10.029

Nikanjam M., Kabamba, D., Cressey T. R., Burger D., Aweek F. T., Acosta E. P., Spector S. A., & Capparelli E. V. (2012). Nevirapine exposure with WHO pediatric weight band dosing: Enhanced therapeutic concentrations predicted based on extensive international pharmacokinetic experience. *Antimicrobial Agents and Chemotherapy*, ***56*** , 5374–5380. https://doi.org/10.1128/AAC.00842-12

Ochieng W., Kitawi R., Nzomo T., Mwatelah, R., Kimulwo M., & Ochieng D. (2015). Implementation and Operational Research: Correlates of Adherence and Treatment Failure Among Kenyan Patients on Long-term Highly Active Antiretroviral Therapy. *J Acquir Immune Defic Syndr*, ***69.***

Onyango DO, van der Sande MAB, Musingila P, Kinywa E, Opollo V, Oyaro B, Nyakeriga E, Waruru A, Waruiru W, Mwangome M, Macharia T, Young PW, Junghae M, Ngugi C, De Cock KM, Rutherford GW. High HIV prevalence among decedents received by two high-volume mortuaries in Kisumu, western Kenya, 2019. PLoS One. 2021 Jul 1;16(7):e0253516. doi: 10.1371/journal.pone.0253516. PMID: 34197509; PMCID: PMC8248726.

Panichsillapakit T., Smith D. M., Wertheim J. O., Richman, D. D., Little, S. J., & Mehta, S. R. (2016). Prevalence of Transmitted HIV Drug Resistance among Recently Infected Persons in San Diego, CA 1996-2013. In *Journal of Acquired Immune Deficiency Syndromes* (**Vol. 71**, pp. 228–236). Lippincott Williams and Wilkins. https://doi.org/10.1097/QAI.0000000000000831

Pego, A. M. F., Roveri F. L., Kuninari R. Y., Leyton V., Miziara I. D., & Yonamine, M. (2017). Determination of cocaine and its derivatives in hair samples by liquid phase microextraction (LPME) and gas chromatography–mass spectrometry (GC–MS). *Forensic Science International*, ***274***, 83–90. https://doi.org/10.1016/j.forsciint.2016.12.024

Phung N., Kuncze K., Okochi H., Louie A., Benet L. Z., Ofokotun I., Haas D. W., Currier J. S., Chawana T. D., Sheth A. N., Bacchetti P., Gandhi M., & Horng H. (2018). Development and validation of an assay to analyse atazanavir in human hair via liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, ***32***, 431–441. https://doi.org/10.1002/rcm.8058

Raffi F., Babiker A. G., Richert L., Molina J. M., George E. C., Antinori A., Arribas J. R., Grarup J., Hudson F., Schwimmer C., Saillard J., Wallet C., Jansson P. O., Allavena C., van Leeuwen R., Delfraissy J. F., Vella S., Chêne G., & Pozniak, A. (2014). Ritonavir-boosted darunavir combined with raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults infected with HIV-1: 96-week results from the NEAT001/ANRS143 randomised non-inferiority trial. *The Lancet*, ***384***, 1942–1951. https://doi.org/10.1016/S0140-6736(14)61170-3

Raffi, F., Jaeger H., Quiros-Roldan E., Albrecht H., Belonosova E., Gatell J. M., Baril J. G., Domingo P., Brennan C., Almond S., & Min S. (2013). Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial. *The Lancet Infectious Diseases*, ***13,*** 927–935. https://doi.org/10.1016/S1473-3099(13)70257-3

Rakhmanina N. Y., Capparelli E. v., van den Anker J. N., Williams K., Sever J. L., Spiegel H. M. L., & Soldin S. J. (2007). Nevirapine concentration in nonstimulated saliva: An alternative to plasma sampling in children with human immunodeficiency virus infection. *Therapeutic Drug Monitoring*, ***29***, 110–117. https://doi.org/10.1097/FTD.0b013e31803258ed

Ranaware P. S., Ingle A. M., Ladke A., Madgulkar A. R., & DamLe M. C. (n.d.). Determination of nevirapine in human plasma by HPLC. *Available Online Www.Jocpr.Com Journal of Chemical and Pharmaceutical Research*, **6**, 3003–3009.

Reddy S., Thomas L., Santoshkumar K. S., Nayak N., Mukhopadhyay A., & Thangam, S. (2016). A LC–MS/MS method with column coupling technique for simultaneous estimation of lamivudine, zidovudineand nevirapine in human plasma. *Journal of Analytical Science and Technology*, ***7***. https://doi.org/10.1186/s40543-016-0097-2

Saitoh A., Sarles E., Capparelli E., Aweeka F., Kovacs, A., Burchett S. K., Wiznia A., Nachman, S., Fenton T., & Spector S. A. (2007). CYP2B6 genetic variants are

associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. *AIDS*, ***21***, 2191–2199. https://doi.org/10.1097/QAD.0b013e3282ef9695

Schoenenberger J. A., Aragones A. M., Cano S. M., Puig T., Castello, A., Gomez-Arbones X., & Porcel J. M. (2013). The Advantages of Therapeutic Drug Monitoring in Patients Receiving Antiretroviral Treatment and Experiencing Medication-Related Problems. *Therapeutic Drug Monitoring*, ***35***, 71–77. https://doi.org/10.1097/FTD.0b013e3182791f8c

Scriven YA, Mulinge MM, Saleri N, Luvai EA, Nyachieo A, Maina EN, Mwau M. Prevalence and factors associated with HIV-1 drug resistance mutations in treatment-experienced patients in Nairobi, Kenya: A cross-sectional study. Medicine (Baltimore). 2021 Oct 8;100(40):e27460. doi: 10.1097/MD.0000000000027460. PMID: 34622871; PMCID: PMC8500620.

Suneetha A., & Raja R. K. (2016). Comparison of LC-UV and LC–MS methods for simultaneous determination of teriflunomide, dimethyl fumarate and fampridine in human plasma: application to rat pharmacokinetic study. *Biomedical Chromatography*, *30*(9), 1371–1377. https://doi.org/10.1002/BMC.3694

UNAIDS. (2020). *Global AIDS update 2020. Seizing the Moment. Tackling entrenched Inequalities to end epidemics*.

UNAIDS report on the global AIDS epidemic shows that 2020 targets will not be met because of deeply unequal success; COVID-19 risks blowing HIV progress way off course UNAIDS. (n.d.). Retrieved March 23, 2021, from https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2020/july/20200706_global-aids-report

Van Oosterhout, J. J. G., Kumwenda J. J., Beadsworth M., Mateyu G., Longwe T., Burger D. M., & Zijlstra E. E. (2007). Nevirapine-based antiretroviral therapy started early in the course of tuberculosis treatment in adult Malawians. *Antiviral Therapy*, ***12***, 515–521.

Van Zyl G. U., van Mens T. E., McIlleron H., Zeier M., Nachega J. B., Decloedt E., Malavazzi, C., Smith P., Huang Y., Merwe L. van der., Gandhi M., & Maartens, G. (2011). Low Lopinavir Plasma or Hair Concentrations Explain Second-Line Protease Inhibitor Failures in a Resource-Limited Setting. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, ***56***, 333–339. https://doi.org/10.1097/QAI.0b013e31820dc0cc

Verdu C. F., Gatto J., Freuze, I., Richomme P., Laurens F., & Guilet D. (2013). Comparison of Two Methods, UHPLC-UV and UHPLC-MS/MS, for the Quantification of Polyphenols in Cider Apple Juices. *Undefined*, ***18***, 10213–10227. https://doi.org/10.3390/MOLECULES180910213

World Health Organization. (2007). *Addendum to the 2006 WHO Guidelines on Antiretroviral Therapy for HIV Infection in Adults and Adolescents*. (Issue 2009/09/02).

World Health Organization. (2007). *Addendum to the 2006 WHO Guidelines on Antiretroviral Therapy for HIV Infection in Adults and Adolescents.* (Issue 2009/09/02).

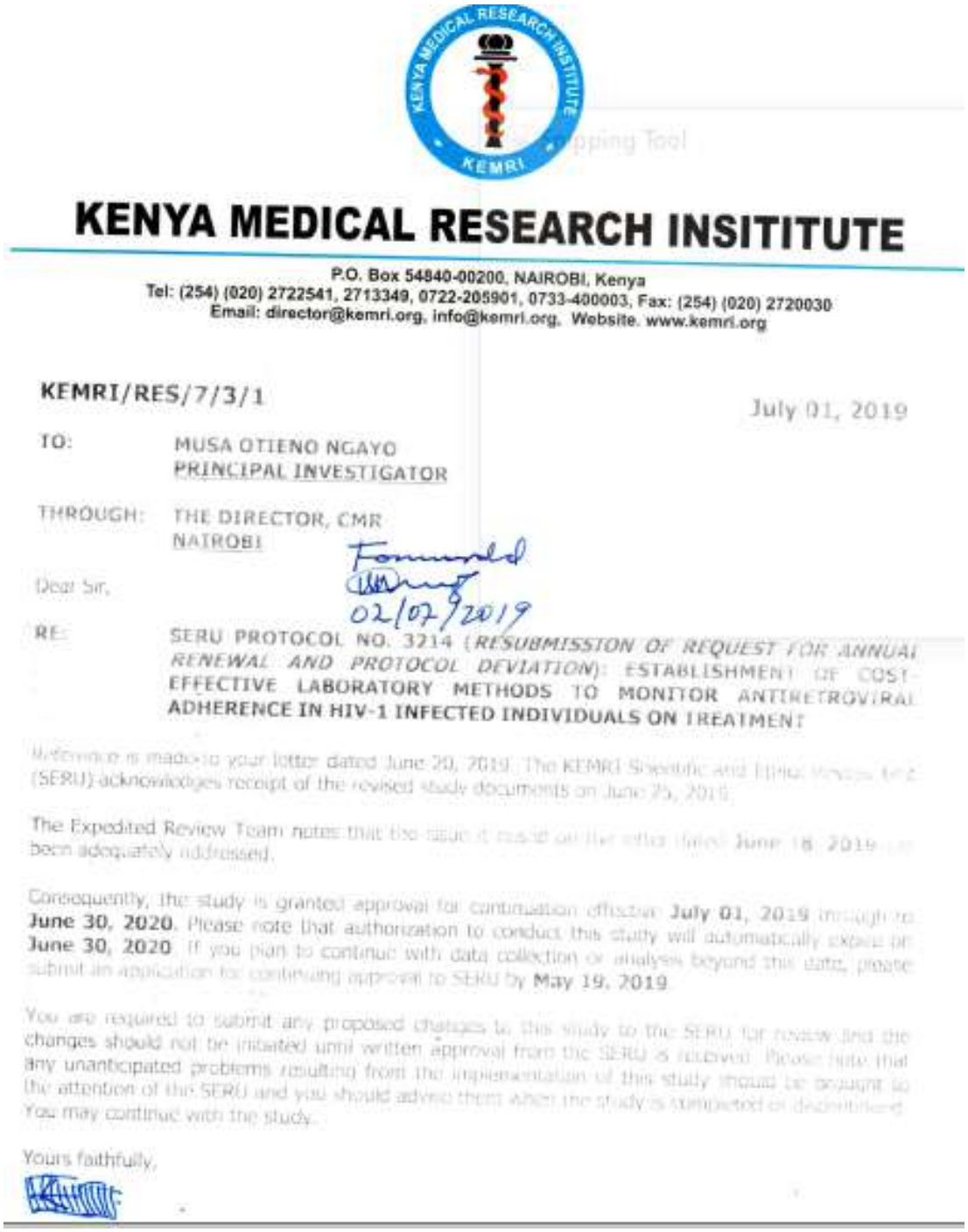
Wu Y., Yang J., Duan C., Chu L., Chen S., Qiao S., Li X., & Deng H. (2018). Simultaneous determination of antiretroviral drugs in human hair with liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, ***1083***, 209–221. https://doi.org/10.1016/j.jchromb.2018.03.021

Yan J., Liu J., Su B., Pan X., Wang Z., Wu J., Zhang J., Ruan Y., Hsi J., Liao L., Shao Y., & Xing H. (2016). Lamivudine Concentration in Hair and Prediction of Virologic Failure and Drug Resistance among HIV Patients Receiving Free ART in China. *PloS One*, ***11***, e0154421. https://doi.org/10.1371/journal.pone.0154421

Yang W. L., Kouyos R. D., Böni J., Yerly S., Klimkait T., Aubert V., Scherrer A. U., Shilaih M., Hinkley T., Petropoulos C., Bonhoeffer S., & Günthard H. F. (2015). Persistence of Transmitted HIV-1 Drug Resistance Mutations Associated with Fitness Costs and Viral Genetic Backgrounds. *PLoS Pathogens*, *11*, 1–13. https://doi.org/10.1371/journal.ppat.1004722

APPENDICES

APPENDIX I: LETTER OF ETHICAL APPROVAL



KENYA MEDICAL RESEARCH INSITITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1

July 01, 2019

TO: MUSA OTIENO NGAYO
PRINCIPAL INVESTIGATOR

THROUGH: THE DIRECTOR, CMR
NAIROBI

Forwarded
02/07/2019

Dear Sir,

RE: SERU PROTOCOL NO. 3214 (*RESUBMISSION OF REQUEST FOR ANNUAL RENEWAL AND PROTOCOL DEVIATION*): ESTABLISHMENT OF COST-EFFECTIVE LABORATORY METHODS TO MONITOR ANTIRETROVIRAL ADHERENCE IN HIV-1 INFECTED INDIVIDUALS ON TREATMENT

Reference is made to your letter dated June 20, 2019. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on June 25, 2019.

The Expedited Review Team notes that the issue it raised on the ethics dated June 18, 2019 has been adequately addressed.

Consequently, the study is granted approval for continuation effective **July 01, 2019** through to **June 30, 2020**. Please note that authorization to conduct this study will automatically expire on **June 30, 2020**. If you plan to continue with data collection or analyses beyond this date, please submit an application for continuing approval to SERU by **May 19, 2019**.

You are required to submit any proposed changes to this study to the SERU for review and the changes should not be initiated until written approval from the SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the SERU and you should advise them when the study is completed or discontinued. You may continue with the study.

Yours faithfully,

APPENDIX II: INTRADAY AND INTERDAY ACCURACY AND PRECISION FOR PLASMA NVP QUALITY CONTROLS ANALYSED USING LC-MS/MS

Intraday				**Interday**		
	LOQ	MLOQ	HLOQ	LOQ	MLOQ	HLOQ
Nominal concentration (ng/mL)	50.000	250.000	500.000	50.000	250.0000	500.000
Average measured concentration (ng/mL)	48.980	250.270	499.470	49.570	250.099	499.848
N	6	6	6	18	18	18
STDEV	1.144	0.361	1.397	0.512	0.276	0.345
%CV	0.023	0.0014	0.0028	1.030	0.100	0.070
% Accuracy	97.967	100.107	99.893	99.140	99.970	100.039
N- Number of run CV- Coefficient of variation STDEV- Standard Deviation						

APPENDIX III: INTRADAY AND INTERDAY ACCURACY AND PRECISION FOR PLASMA NVP QUALITY CONTROLS ANALYSED USING HPLC-UV

Intraday				Interday		
	LOQ	MLOQ	HLOQ	LOQ	MLOQ	HLOQ
Nominal concentration ng/mg	0.100	12.500	25.000	1.000	12.500	25
Average measured concentration (ng/mg)	0.980	12.512	24.808	0.980	12.535	25.001
N	6	6	6	18	18	18
STDEV	0.250	0.744	2.095	0.115	0.614	2.194
%CV	2.556	0.594	0.844	1.200	0.490	0.880
% Accuracy	97.983	100.097	99.233	98.000	100.280	100.004
N- Number of run CV- Coefficient of variation STDEV- Standard Deviation						

APPENDIX IV: INTRADAY AND INTERDAY ACCURACY AND PRECISION FOR HAIR NVP QUALITY CONTROLS ANALYSED USING LC-MS/MS

Intraday				Interday		
	LimiOQ	MLOQ	HLOQ	LOQ	MLOQ	HLOQ
Nominal concentration	50.000	600.000	1000.000	50.000	600.000	1000.000
Average measured concentration (ng/mL)	49.500	585.000	1000.700	50.200	593.000	1000.350
N	5	5	5	10	10	10
STDEV	0.001	0.018	0.016	0.001	0.011	0.004
%CV	2.032	3.096	1.633	1.877	1.904	0.451
% Accuracy	99.133	97.506	100.667	100.467	98.836	100.347
N- Number of run CV- Coefficient of variation STDEV- Standard Deviation						

APPENDIX V: INTRADAY AND INTERDAY ACCURACY AND PRECISION FOR HAIR NVP QUALITY CONTROLS ANALYSED USING HPLC-UV

Intraday				**Interday**		
	LLOQ	MLOQ	HLOQ	LOQ	MLOQ	HLOQ
Nominal concentration	1.000	2.500	50.000	10.000	2.500	50.000
Average measured concentration (ng/mg)	0.996	24.874	49.578	0.997	24.891	49.641
N	6	6	6	12	12	12
STDEV	0.047	1.123	5.658	0.017	0.236	1.987
%CV	0.475	0.451	1.141	0.170	0.0947	0.400
% Accuracy	99.562	99.497	99.136	99.800	99.629	99.430
N- Number of run CV- Coefficient of variation STDEV- Standard Deviation						

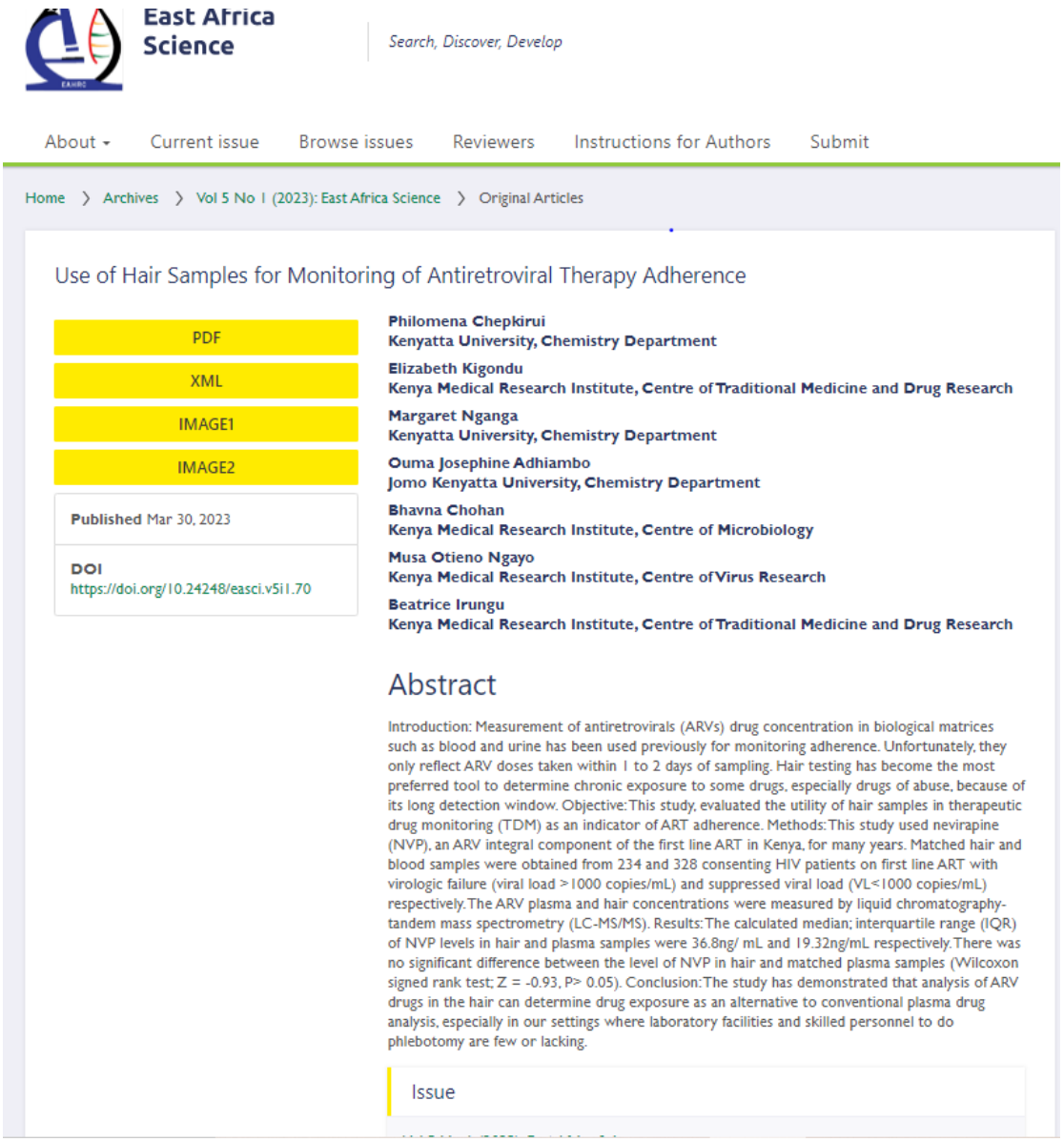
APPENDIX VI: DESCRIPTIVE STATISTICS OF ANALYSED HAIR AND PLASMA SAMPLES

		Hair concentration LC-MS/MS	Plasma concentration LC-MS/MS	Hair concentration HPLC-UV	Plasma concentration HPLC-UV
N		105	308	55	124
Mean		106.075	430.224	180.218	878.928
Median		36.800	19.300	67.800	706.500
Variance		34566.143	671273.194	52903.806	835846.200
Range		1210.500	5276.200	984.100	4546.700
Minimum		1.400	0.000	16.900	20.300
Maximum		1211.900	5276.200	1001.000	4567.000
Percentiles	25	17.100	5.225	38.900	88.425
	50	36.800	19.300	67.800	706.500
	75	102.250	562.650	200.000	1233.500

N- number of samples

APPENDIX VII: PUBLISHED PAPER

https://easci.eahealth.org/easci/article/view/70
https://ir-library.ku.ac.ke/handle/123456789/25598



East Africa Science

Search, Discover, Develop

About ▾ Current issue Browse issues Reviewers Instructions for Authors Submit

Home › Archives › Vol 5 No 1 (2023): East Africa Science › Original Articles

Use of Hair Samples for Monitoring of Antiretroviral Therapy Adherence

PDF
XML
IMAGE1
IMAGE2

Published Mar 30, 2023

DOI
https://doi.org/10.24248/easci.v5i1.70

Philomena Chepkirui
Kenyatta University, Chemistry Department

Elizabeth Kigondu
Kenya Medical Research Institute, Centre of Traditional Medicine and Drug Research

Margaret Nganga
Kenyatta University, Chemistry Department

Ouma Josephine Adhiambo
Jomo Kenyatta University, Chemistry Department

Bhavna Chohan
Kenya Medical Research Institute, Centre of Microbiology

Musa Otieno Ngayo
Kenya Medical Research Institute, Centre of Virus Research

Beatrice Irungu
Kenya Medical Research Institute, Centre of Traditional Medicine and Drug Research

Abstract

Introduction: Measurement of antiretrovirals (ARVs) drug concentration in biological matrices such as blood and urine has been used previously for monitoring adherence. Unfortunately, they only reflect ARV doses taken within 1 to 2 days of sampling. Hair testing has become the most preferred tool to determine chronic exposure to some drugs, especially drugs of abuse, because of its long detection window. Objective: This study, evaluated the utility of hair samples in therapeutic drug monitoring (TDM) as an indicator of ART adherence. Methods: This study used nevirapine (NVP), an ARV integral component of the first line ART in Kenya, for many years. Matched hair and blood samples were obtained from 234 and 328 consenting HIV patients on first line ART with virologic failure (viral load >1000 copies/mL) and suppressed viral load (VL<1000 copies/mL) respectively. The ARV plasma and hair concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results: The calculated median; interquartile range (IQR) of NVP levels in hair and plasma samples were 36.8ng/ mL and 19.32ng/mL respectively. There was no significant difference between the level of NVP in hair and matched plasma samples (Wilcoxon signed rank test; Z = -0.93, P> 0.05). Conclusion: The study has demonstrated that analysis of ARV drugs in the hair can determine drug exposure as an alternative to conventional plasma drug analysis, especially in our settings where laboratory facilities and skilled personnel to do phlebotomy are few or lacking.

Issue

APPENDIX VIII: 13-KASH-Abstract-Book

https://www.kemri.go.ke/wp-content/uploads/2023/02/13-KASH-Abstract-Book.pdf?cv=1

Abstract 160

Title: UTILITY OF HAIR SAMPLES IN MONITORING ANTIRETROVIRAL THERAPY ADHERENCE OF PERSONS LIVING WITH HIV IN KENYA

Authors: Philomena Chepkirui (Kenyatta University)*; Beatrice N Irungu (Kenya Medical Research Institute); Elizabeth Kigondu (Kenya Medical Research Institute); Margaret Nganga (Kenyatta University); Musa Otieno (Kenya Medical Research Institute); Bhavna Chohan (Kenya Medical Research Institute); Josephine Ouma (Jomo Kenyatta University of Agriculture and Technology)

Background of the study: Antiretroviral therapy (ART) is critical in reducing the human immunodeficiency virus (HIV) epidemic by preventing vertical transmission. Routine monitoring of of patient adherence in receiving antiretroviral therapy (ART) is essential as it guides in tracking response to ARVs treatment and assesses treatment failures. Measurement of ARVs drug concentration in biological matrices such as blood and urine has been used previously for monitoring adherence. Unfortunately, they only reflect ARV doses taken within 1 to 2 days of sampling. Hair testing has become the most preferred tool to determine chronic exposure to some drugs, especially drugs of abuse, due to its long detection window. This study, , evaluated the utility of hair samples in therapeutic drug monitoring (TDM) as an indicator of ART adherence t as an alternative method to ARVs analysis in blood. This study used nevirapine (NVP), an ARV integral component of the first-line ART in Kenya, for many years.

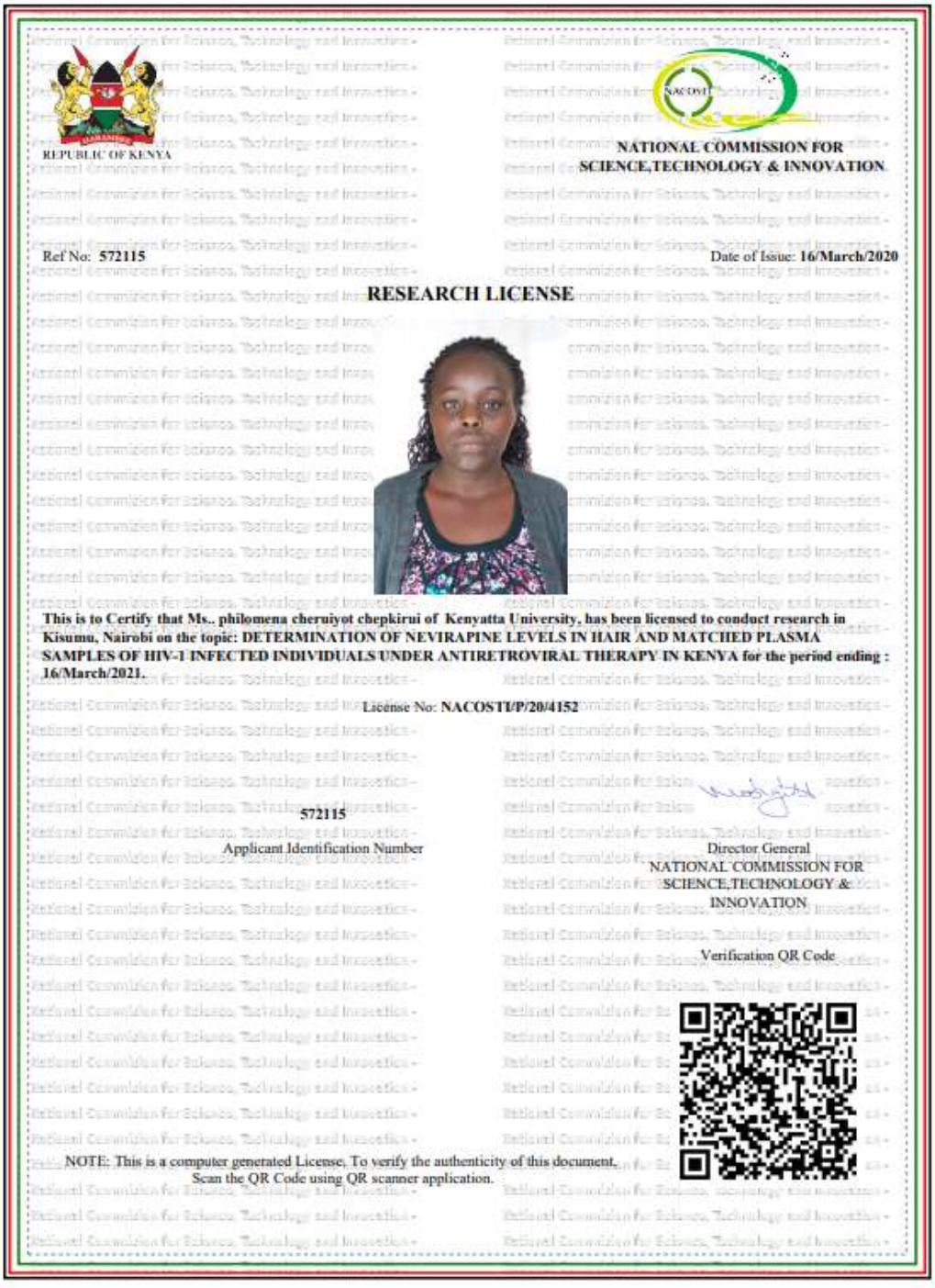
Methods Matched hair and blood samples were obtained from 234 and 328 consenting HIV patients with virologic failure (viral load >1000copies/ml) and suppressed viral load (VL<1000 copies/ml) first-line antiretroviral therapy (ART), respectively. The ARV plasma concentration was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: The calculated median (IQR) of NVP levels in hair and blood samples was 36.80 ng/ml and 19.32 ng/ml respectively. Wilcoxon signed-rank test was used to test the hypothesis; there is no significant difference between the level of NVP in hair and matched plasma samples. The Wilcoxon signed-rank test statistical result was, ($Z = -0.93$, $p > 0.05$) meaning there was no significant difference between the NVP hair concentration and the plasma.

Conclusion: Since there was no significant difference between the levels of NVP in hair and matched plasma samples, this study has demonstrated that analysis of ARV drugs in the hair can determine drug exposure as an alternative to conventional plasma drug analysis, especially in our settings where laboratory facilities and skilled personnel to do phlebotomy are few or lacking. However, it is not implied that the results support replacing plasma testing with hair testing as a research measure in all cases since the analysis of ARVs in hair and plasma samples can be taken as different tools that can also be used in combination to predict adherence to antiretroviral therapy.

Keywords: NVP concentration; hair samples; plasma samples

APPENDIX IX: RESEARCH LICENCE



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **572115**

Date of Issue: **16/March/2020**

RESEARCH LICENSE

This is to Certify that Ms.. philomena cheruiyot chepkirui of Kenyatta University, has been licensed to conduct research in Kisumu, Nairobi on the topic: DETERMINATION OF NEVIRAPINE LEVELS IN HAIR AND MATCHED PLASMA SAMPLES OF HIV-1 INFECTED INDIVIDUALS UNDER ANTIRETROVIRAL THERAPY IN KENYA for the period ending : 16/March/2021.

License No: **NACOSTI/P/20/4152**

572115

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.