Assessment of non-adherence to cardiovascular medications in Iraq by 8-items Morisky Medication Adherence Scale and analysis of dried blood spots

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Declaration

This thesis contains the original work of the author except where otherwise indicated

Abstract

Medication non-adherence is common in chronic conditions such as cardiovascular diseases (CVD). According to the WHO, over 50% of patients are non-adherent to CVD medications, which results in poor health outcomes, hospital readmissions, high mortality rates and avoidable costs. The aim of this study was to assess medication non-adherence to target CVD medications via the eight-item Morisky Medication Adherence Scale (MMAS-8) and quantification of drug concentrations in blood microsamples collected on Whatman 903 cards and a volumetric absorptive microsampling device (VAMS) for the same patients using liquid chromatography–high resolution mass spectrometry (LC-HRMS). Iraqi patients who had been taking one or more of nine commonly prescribed cardiovascular medications (amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin, and valsartan) for at least six months were enrolled in this study. MMAS-8 scores for individual patients were determined, and whole blood microsamples assessed via LC-HRMS. To estimate overall medication non-adherence, MMAS-8 (score < 6) and the results of quantitative LC-HRMS analysis were compared. 303 patients were recruited for this study (mean age 54) taking an average of four CVD medications. Non-adherence assessed via MMAS-8 was 18.2%, as compared to the 49.2% determined via LC-HRMS analysis of blood microsamples. Both approaches showed no significant correlation between non-adherence and age or gender, but was significantly associated with the number of medications or tablets being taken daily. Quantitative LC-HRMS results obtained via the two microsampling methods (VAMS and 903 cards) were generally consistent and comparable, confirming good reproducibility. MMAS-8 was subject to overestimation and was unable to identify non-adherence to multiple medications in the regimens. Conversely, LC-HRMS gave valuable information about non-adherence to each medication in each patient's regimen. In subsequent clinician-led patient interviews the main reasons for medication non-adherence were side effects, dose frequencies, complicated regimens, medication cost, patient beliefs, patient knowledge/understanding, and forgetfulness. The impact of using a combination approach of patient MMAS-8 data and objective blood drug concentration data with face-to-face interviews conducted by the specialist in Iraq has the potential to provide Iraqi clinicians with a novel approach to improving patients' health and reducing the costs of treatment by monitoring and optimising CVD medications in routine clinical practice.

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Lastly, my appreciation also goes to Professor Donald E. Morisky for allowing us to use MMAS-8 in this study. Use of the MMAS is protected by US copyright laws. Permission for use is required. A license agreement is available from: Donald E. Morisky, ScD, ScM, MSPH, Professor, department of Community. Health Sciences, UCLA Fielding School of Public Health, 650 Charles E. Young Drive South, Los Angeles, CA 90095±1772, dmorisky@ucla.edu.

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Outputs from this Research

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List of Abbreviation

a.m.	Ante meridiem
ABPI	Association of the British Pharmaceutical Industry
ACE inhibitors	angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blocker
BNF	British National Formulary
C	Carbon
C max	The maximum blood concentration of a drug
CDC	Centres for Disease Control and Prevention
CE	Collision energy
CHD	Coronary heart disease
CLSI	Clinical and Laboratory Standards Institute
CV	Coefficient of variation
CVDs	Cardiovascular diseases
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
Dr	Doctor
EIC	Extracted ion chromatogram
EU	European Union
F	Female
FDA	Food and Drug Administration
FDC	Fixed-dose combination
GP	General practitioners
GS-MS	Gas chromatography mass spectrometry
H	Hydrogen
H2O	Water
HCT	Haematocrit
HPLC	High-performance liquid chromatography
Hz	Hertz
IS	Internal standard
KIMADIA	State Company for Marketing Drugs and Medical Appliances

L	Litre
LC	Liquid chromatography
LC-HRMS	liquid chromatography–high resolution mass spectrometry
LDL	Low density lipoprotein
LLOQ	Low limit of quantification
M	Male
m/z	Mass-to-charge ratio
M+H+	Molecular ion
M+Na+	Sodium adduct ion
MeOH	Methanol
mg	Milligram
MHRA	Medicines and Healthcare Products Regulatory Agency
min	Minute
ml	Millilitre
mm	Millimetre
MOH	Ministry of Health
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
N	Nitrogen
NaOH	Sodium hydroxide
NBS	Newborn screening
NCDCR	The National Centre for Drug Control and Research
NCDs	Communicable diseases
NEHI	New England Healthcare Institute
ng	Nanogram
NHS	National Health Services
NICE	National Institute for Health and Care Excellence
NSQAP	Newborn Screening Quality Assurance Program
O	Oxygen
OTC	Over-the-counter
p.m.	Post meridiem
PCR	Polymerase chain reaction

PHC	Primary health centres
PIL	Participants information leaflets
PK	Pharmacokinetic
ppm	Part per million
PPRS	Pharmaceutical Price Regulation Scheme
QC	Quality Control
R^2	Correlation coefficient
RE	Relative error
RMM	Relative molecular mass
S/N	Signal-to-noise ratio
SD	Standard deviation
SIM	Single ion monitoring mode
SLE	Solid-liquid extraction
SOP	The standard operation procedure
ß	Beta
$t_{1/2}$	Half- life
t_{max}	Time required for a drug to reach the maximum plasma concentration
TDM	Therapeutic drug monitoring
TIC	Total ion chromatogram
TK	Toxicokinetic (TK)
TOF	Time of Flight
TV	Television
UK	United Kingdom
US	United State
V	Voltage
v/v	Volume by volume
VAMS	Volumetric absorptive microsampling
WHO	World Health Organization
X-ray	Energetic High-Frequency Electromagnetic Radiation
µl	Microliter
ρ	Spearman's correlation coefficient

>	Greater than
<	Less than
≤	Less or equal to
~	Approximately
$	Dollar
%	Percentage
£	Pound
€	Euro
8-MMAS	Eight items Morisky Medication Adherence Scale

Chapter 1

Healthcare System in Iraq: An Overview

This chapter provides background information about the Iraqi healthcare system and highlights the global prevalence of, and mortality rates due to cardiovascular diseases. Current policies and action plans related to cardiovascular diseases in Iraq are also discussed. It provides information about types of healthcare systems, the healthcare workforce, and access to, and the prescription of medications in Iraq and the UK. The levels of quality control and the price regulations of medications in Iraq are documented. In order to assess where the Iraqi healthcare system stands in the global scheme, the Iraqi situation is outlined in relation to the healthcare system in the UK, where part of this research was conducted.

1.1. General Background

Iraq is one of the Middle Eastern countries, whose neighbours are Turkey to the north, Iran to the east, with Kuwait and Saudi Arabia to the south, and Syria to the west. Over the past 25 years, the population in Iraq has increased by 51.0%, reaching 35.8 million in 2015 (World Health Organization, 2017b).

1.2. Mortality and Chronic Disorders

According to the World Health Organization (WHO), the disease landscape in Iraq has undergone a drastic transformation over the years. In 2002, communicable, maternal, perinatal and nutritional deficiencies accounted for 44% of all deaths, whilst non-communicable diseases (NCDs) accounted for 56%. Of the NCDs, cardiovascular diseases (CVDs) accounted for 21% of the total deaths, injuries 13%, cancer 6%, diabetes 1%, and other chronic non-communicable diseases 15% (Figure 1.1) (World Health Organization, 2002). In 2014, 19% of all deaths were due to communicable, maternal, perinatal and nutritional deficiencies, while NCDs accounted for 81%. CVDs accounted for 33% of all deaths, injuries 19%, cancer 10%, diabetes 4%, and other chronic NCDs 15% (Figure 1.1) (World Health Organization, 2014). In the UK, only 7% of all deaths were due to communicable, maternal, perinatal and nutritional deficiencies, while NCDs accounted for 93% and CVDs accounted for 31% of all deaths, followed by cancer at 29%, other NCDs at 20%, chronic respiratory diseases at 8%, injuries at 4%, and diabetes at 1% (World Health Organization, 2014). This indicates that CVD represent a major

challenge to healthcare systems, whether in low- and middle-income countries such as Iraq or in developed nations such as the UK.

Despite these reports about high mortality rates due to CVDs in Iraq, there are no large-scale plans or guidelines for their management to reduce the devastating effects of such conditions in Iraq (Turk-Adawi et al., 2018). With regards to CVDs in particular, there have been operational policies, strategies, or action plans to reduce unhealthy diets and tobacco usage and in promoting increased physical activity, but these are not applied at the national level in Iraq. There are no insurance health care schemes or drug counselling centres for cardiovascular diseases (World Health Organization, 2017d). Thus, patients will have to bear the burden of the cost of healthcare services. The absence of drug counselling centres would mean extra responsibilities/workload for doctors who already have limited time for their patients.

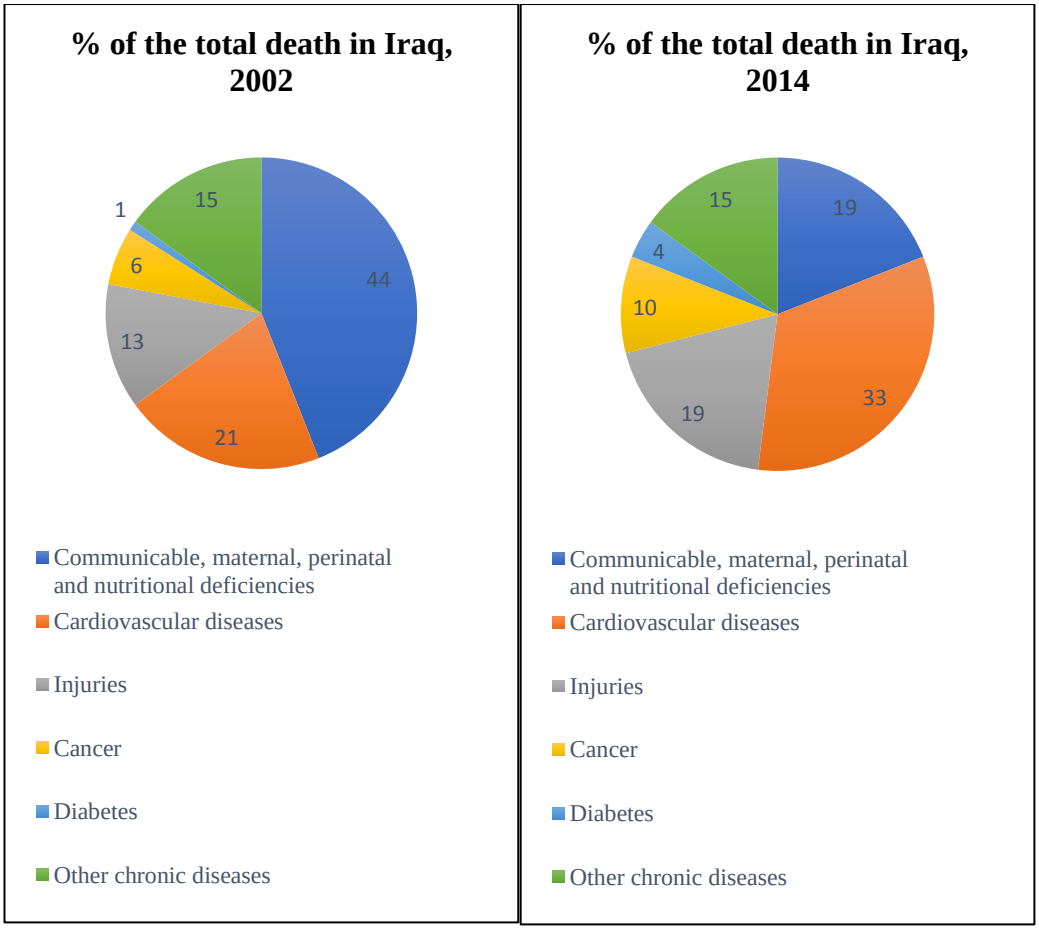

Figure 1.1. A comparison of causes of death in Iraq in 2002 and 2014.

1.3. Healthcare System in Iraq

The healthcare system in Iraq is mainly divided into the public, private and intermediate sectors. These existing healthcare systems in Iraq are discussed in the following subsections.

1.3.1. Public Healthcare Systems

In Iraq, the Ministry of Health (MOH) is responsible for the country's healthcare system. The public sector provides preventative and curative services through primary health centers (PHC), secondary care (hospital-based) and tertiary care (specialist hospitals) (Al Hilfi *et al.*, 2013). The public sector is funded by the MOH. The working hours for primary health care centres are between 8.00 a.m. and 3.00 p.m. Secondary and tertiary facilities are available for emergencies on a 24/7 basis. Despite the facilities provided by the primary, secondary and tertiary health care centres, there are many problems with the Iraqi public health system as pertaining to data management and security. The paucity of medical doctors and trained medical professionals in Iraq adversely affects health care services (Al Mosawi and Al Hasnawi, 2009). These services also suffer from shortages of medications and long waiting hours. Those citizens opting for the public health system due to their low costs typically live in poverty or, indeed, are unemployed (International Organization of Migration, 2018).

In the past 50 years, there has been a shift away from welfare financing in order to provide these healthcare services towards a greater focus on 'self-sustainability', increasing the financial burden on individuals seeking welfare services (World Health Organization, 2017a). The role of the MOH is to provide services that are primarily funded by the government. In the early 1980s, the MOH was charged with providing services for free, or at least for very low fees. In Baghdad, 1997, seven public hospitals began charging high fees for medical care and implemented a self-financing policy that quickly cascaded to other public hospitals and health centres. By 2003, the MOH had cracked down on these self-financing policies by restricting them and enforcing the re-adoption of the provision of free or low-fee services (Al Mosawi and Al Hasnawi, 2009).

Today, austerity measures, resource shortages, and the absence of a more widespread adoption of health insurance schemes, are driving up individual patient costs. Patients

today must pay for services such as consultations and treatment, although the cost of medication is relatively low in comparison to that in the private sector (International Organization of Migration, 2016; World Health Organization, 2018a). It is reported that 23% of Iraqis are under the poverty line with spending of less than $2.2 per person per day which is almost equal to £1.8 or 2618 Iraqi Dinars (United Nations Iraq, 2019). This could be a significant barrier to adherence to the prescribed medications.

In the UK, the National Health Service (NHS) is designed to provide medical treatment and support to everyone, regardless of their ability to pay, though people can and do choose to take out their own private health insurance policies. The public sector in the UK consists of primary (e.g., community care, general practitioners, dentists, pharmacists, etc.) secondary (hospital-based care accessed through GP referral) and tertiary care (specialist hospitals) in a similar way to in Iraq, even though the manner in which they operate differs (Grosios *et al.*, 2010). Access to medication in the Iraqi public health sector is discussed in Subsection 1.3.1.1.

1.3.1.1. Medication Supplies and Access to Medications in the Iraqi Public Health Sector

In Iraq, the State Company for Marketing Drugs and Medical Appliances (KIMADIA) was the primary service provider of drug and medical appliance import, storage, and distribution for both the private- and public-sector hospitals until 2003; thereafter, its services were concentrated purely on the public sector. KIMADIA acts as an intermediary between drug companies and the public health sector, as shown in Figure 1.2.

Patients can gain access to medications after visiting the public clinics and, based on their diagnoses, the clinician may refer them for laboratory tests or, if further intervention is required, they are sent to the hospital on admission. If a patient needs medication, the clinician writes a prescription and for patients to get medications from public sector pharmacies for the appropriate fee. Although, medicines are available in the public sector at a low price in comparison with the private sector, these medicines are frequently subject to shortages since demand typically exceeds supply. If medications are not available from public sector pharmacies, patients can instead obtain their medications from private sector pharmacies.

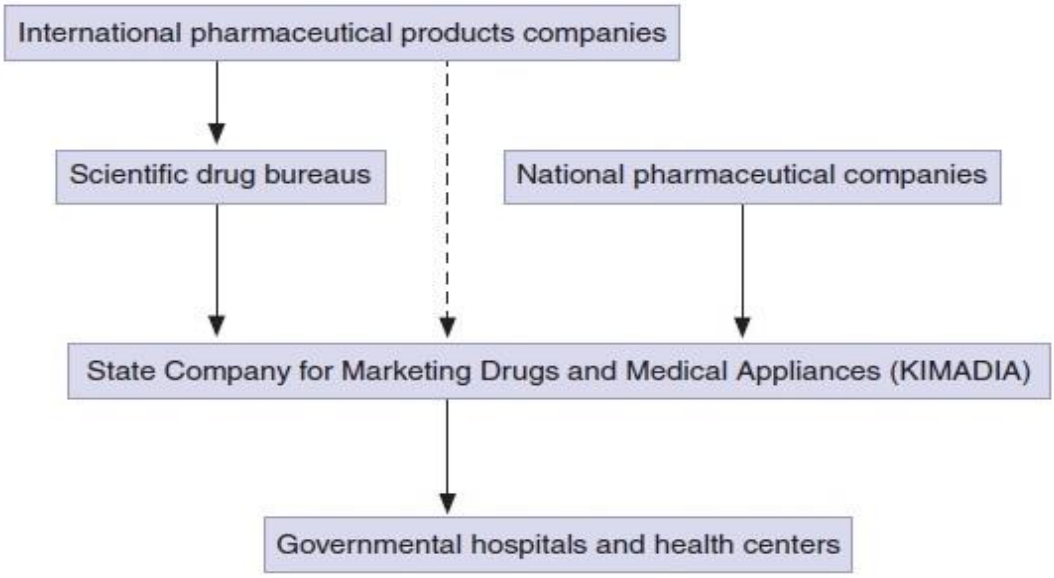

Figure 1.2. KIMADIA medication distribution scheme within the public sector (Al-Jumaili *et al.*, 2013)**.**

The procedure is slightly different in the UK, where the patient has to book an appointment with the clinic and medications are prescribed by appropriate healthcare practitioners such as doctors, nurses, dentists and optometrists. These prescriptions are only dispensed through pharmacies in community or hospital settings (National Health Service, 2017), where direct charges to the patient are made which include prescription charges, currently at £9.00 per item (National Health Service, 2019). However, there are certain situations that allow individuals to access free prescriptions in England, for example those who are under 16 or 60 and over, people with certain medical conditions (e.g., cancer, diabetes) and during pregnancy (Black, 2014).

1.3.2. Private Healthcare Systems in Iraq

The private sector in Iraq includes private clinics and private hospitals. Private clinics are owned and run by specialist physicians and, unlike public clinics, provide services to patients after 3.00 p.m. However, the medications prescribed by these physicians are only dispensed from community pharmacies of the private sector. Details of access to medicines through private healthcare systems are outlined in Subsection 1.3.2.1. A high number of private clinics are available in Iraq and are well distributed across the nation

in a geographical sense. The physicians who work in private clinics are either retired physicians or public sector physicians who finish work at 3 p.m. In the private sector, healthcare costs are covered by the individual requesting treatment.

Specialist private hospitals are mostly located in Baghdad and, to a lesser extent, in the outlying provinces. The quality of care provided by private sector services in Iraq is high in comparison to that provided by the public sector. Private hospitals and clinics are generally owned by individual or group practices and are headed by physicians or entrepreneurs (World Health Organization, 2006). This sector mainly provides surgical services, obstetrics/gynaecological beds, operative and labour theatres, and support services such as medical laboratories and X-ray units.

In the UK, the private healthcare sector is made up of hospitals and clinics which are run independently of the National Health Service (NHS). Private healthcare is directly funded by insurance schemes paid for directly by either individuals or major employer schemes (Grosios *et al.*, 2010; Chang *et al.*, 2011). Just as with the private sector in Iraq, patients using the private sector in the UK are responsible for the any fees that might be payable since the NHS itself does not support any of the associated costs.

1.3.2.1. Medication Supply and Access to Medications in the Iraqi Private Sector

Private wholesalers obtain medicines from scientific bureaus and national pharmaceutical companies. Wholesalers supply medicines to private pharmacies, as shown in Figure 1.3. The patient visits a private clinic and pays the consultation fee. The clinician prescribes medications which are only administered from private sector pharmacies. In this sector, the medications provided by such pharmacies are consistently of high quality and are readily available, though the associated costs are themselves quite high and must be covered by individuals seeking treatment (International Organization of Migration, 2016).

The government does not run an active national medication price monitoring system to track the retail prices of drugs in private healthcare facilities (World Health Organization, 2011). The combined effects of this lack of price regulation and the general lack of health insurance schemes has led to individuals incurring high costs when seeking health services in this sector (World Health Organization, 2017d).

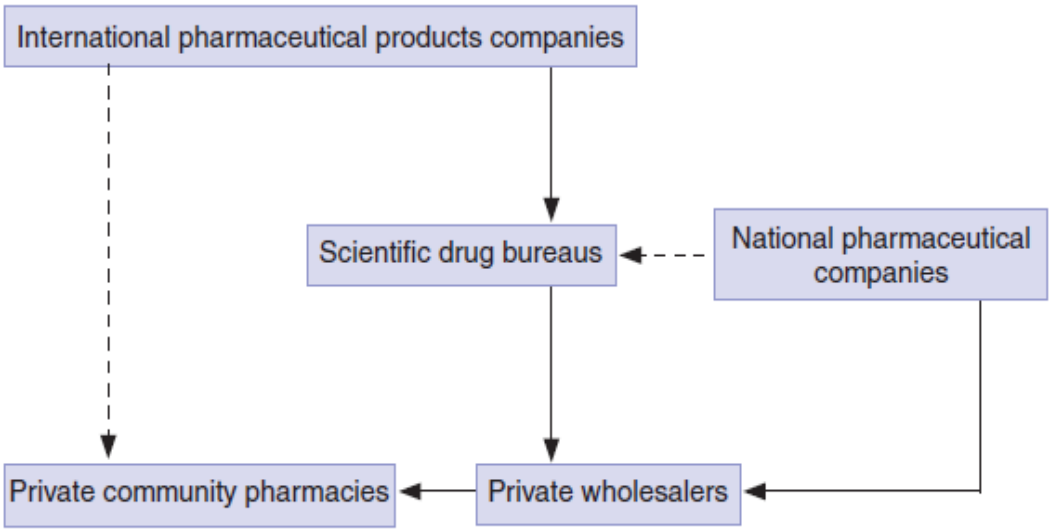

Figure 1.3. Distribution scheme for medicines distributed to the private sector (Al-Jumaili *et al.*, 2013)**.**

1.3.3. Intermediate Sector

The intermediate sector, as the name suggests, refers to the basic healthcare services available when primary public services cannot be accessed. The intermediate sector provides services via the public clinics. These public clinics embody the interaction between the public and private care facilities that operate in PHCs and provide curative care to the public beyond the official working hours of public facilities for a period of three hours per day (3-6 pm). The clinics recruit staff independently from MOH staff, retired professionals, and private practitioners (World Health Organization, 2006).

1.3.3.1. Medications Supply and Access to Medications in the Intermediate Sector

The patient pays for the consultation, laboratory analysis, and treatment in the intermediate sector; the total cost is higher than in the public sector but considerably less than would be charged in the private sector. The price of medications is regulated by the MOH. Medication in this sector is subject to shortages, just as with the public sector, where again demand exceeds supply.

1.3.4. Healthcare Workforce in Iraq

In Iraq, the average physician to population ratio is 7.5:10,000. The ratios in a sample of cities at the high and low end of the range are as follows: Erbil 10.5, Basra 9.0, Kerbala 9.2; and Thi-Qar 4.4, Diyala 4.3, and Misan 3.5 (World Health Organization, 2018a). A particular issue faced by the healthcare system in Iraq is that of a general shortage of nurses and support staff in rural areas and health centres (World Health Organization, 2018a). Staff shortages affect consultations and follow-up waiting periods. The average community pharmacist to population ratio is about 1:3; however, there is a limited number of clinical pharmacists, usually 1-2 clinical pharmacists for each hospital ward (Al-Jumaili *et al.*, 2013). Generally, a hospital ward in Iraq will cater for 66 patients, suggesting a higher workload for the pharmacists (about 1:30 patients) available.

In the UK, the doctor to population ratio was 21:10,000 in England and Wales in 2001 (Yar *et al.*, 2006), and of pharmacist to population ratio was 7.5:10,000 (The Centre for Workforce Intelligence, 2013). Consequently, these data obtained from both Iraq and the UK indicate that the health workforce to patient ratio may affect the health services provided and adds to the burden on both doctors and patients.

1.3.5. Medication Quality Control

All pharmaceutical products used in the healthcare sectors in Iraq (Section 1.3) must first be registered and licensed according to MOH Public Health Law regulations. This registration process falls under the auspices of the Technical Affairs Directorate and Registration Department. For prescription medications, full documentation is required, including bioavailability and bioequivalence studies. Over-the-counter (OTC) medicines require only reduced documentation. Registered products retain their legal status for five years, after which they must be re-registered. The National Centre for Drug Control and Research (NCDCR) is responsible for safety, quality and efficacy control in Iraq (World Health Organization, 2006). In the UK, the Medicines and Healthcare Products Regulatory Agency (MHRA) regulates medications, medical devices, and blood components for transfusion (Medicines & Healthcare products Regulatory Agency, 2018).

Until recently, KIMADIA was also charged with drug post-marketing surveillance in coordination with the MOH anti-poison centre. However, an investigation published in 2006 indicated that no serious post-marketing surveillance studies had been conducted over the preceding 15 years (World Health Organization, 2006). According to the WHO, it is estimated that 1 in 10 medical products is substandard/falsified in low and middle-income countries (World Health Organization, 2018c). The WHO defines substandard medicines as "authorized medical products that fail to meet either their quality standards or their specifications, or both" and falsified medicines as "medical products that deliberately/fraudulently misrepresent their identity, composition or source" (World Health Organization, 2017e). Ultimately, it is difficult to make an informed decision as to the availability of substandard and falsified medicines in the Iraqi market; however, a number of reports have surfaced about substandard and falsified medicines medications circulating in the country. The Iraqi parliament called on the MOH and the Syndicate of Iraqi Pharmacists to prevent the distribution of substandard and falsified medicines after confiscating 14 containers of such being smuggled into Umm Qasr Port (Alsumaria Iraqi Satellite TV Network, 2012). According to the Iraqi Centre of Pharmacovigilance, falsified cardiovascular medications such as amlodipine, clopidogrel and valsartan have been circulating in the Iraqi market (Syndicate of Iraqi Pharmacist, 2016). It is estimated that 30% of medications in the Kurdistan region in Iraq are substandard and falsified (Bahram, 2013). Just as quality control is important to ensuring patients are provided with the correct medication in the correct dosage, price regulation for medicines is also paramount in order to ensure access.

1.3.6. Medication Price Regulation

In Iraq, the price of medications prescribed to patients in the public (sections 1.3.1) and the intermediate sectors (section 1.3.3) is controlled by MOH; however, there are no legal requirements to control medication prices or retail prices for drugs sold in the private sector (World Health Organization, 2011), which has led to unstable and high retail prices for medications. On the other hand, in the UK, the Pharmaceutical Price Regulation Scheme (PPRS), which is a voluntary agreement between the government and pharmaceutical industry, has the dual aims of seeking:

- To create an environment that ensures safe and effective medications are available on reasonable terms to the National Health Services (NHS); and
- To maintain a strong, efficient, and profitable pharmaceutical industry (The Association of the British Pharmaceutical Industry (ABPI), 2018; Paul and Morgan, 2018).

The PPRS puts in place controls on the prices of branded drugs sold to the NHS and covers all licensed, branded, prescription medications sold to them. It does not cover products without a brand name (generics), nor does it cover those branded products available without prescription (OTC) (The Association of the British Pharmaceutical Industry (ABPI), 2018; Paul and Morgan, 2018).

The absence of price regulation, along with the lack of any health insurance scheme, facilitates the high prices being charged for health services in Iraq (World Health Organization, 2017d). Lack of sufficient documentation and government involvement and, indeed, oversight of Iraqi drug prices are a major point of concern. The cost of CVD medications in the private sector is significantly higher than those in the public sector. Table 1.1 shows the cost of 10 tablets of various forms of medication in the public and private sector. The cost is obtained from private sector pharmacies in Misan, Iraq.

Table 1.1. Estimated cost of 10 tablets of the target medications in the public and private sector in Iraq

Medication	Estimated cost (Iraqi Dinar) in the public sector	Estimated cost (Iraqi Dinar) in the private sector
Amlodipine	1000	2000-2500
Atenolol	500	1500-2000
Atorvastatin	Not available in this sector	3000-4000
Bisoprolol	500	1000-1500
Diltiazem	500	6000-8500
Lisinopril	500	1500-2000
Losartan	500	3000-5000
Valsartan	Not available in this sector	3000-5000
Simvastatin	Not available in this sector	2000-4000

1.3.7. Medical Guidelines for Management of Chronic Diseases

Medical guidelines provide outlines for clinical decisions and best practices and consist of statements and recommendations aimed at improving patient care and communication between patients and healthcare professionals. The outlines are informed by systematic

reviews of available evidence and assessments of the benefits and potential side effects associated with alternative care options (Mazrou, 2013). Such consensus guidelines enable greater consistency in the care provided at the local and national levels. Their implementation has also had an economic impact, reducing spending on hospitalisation, prescriptions, surgeries, and other procedures (Woolf *et al.*, 1999; Kredo *et al.*, 2016).

In Iraq, there is a guideline for the management of hypertension (Iraqi Ministry of Health, 2012), but it is not generally applied by physicians. On the other hand, there are no applicable national guidelines, protocols, or standards for the management of cardiovascular diseases and other major NCDs through primary care. A comparison with the UK health system's guidelines and standards for the management and treatment of cardiovascular diseases is given in Table 1.2.

This may suggest that improving guidelines is vital to the maintenance of good clinical care practices. The lack of medical guidelines in Iraq leads to a relatively wide variation in decision making as each doctor prescribes medications based on their personal experiences. This opens the door to numerous treatment options and overprescribing. Moreover, the absence of guidelines will result in undefined priorities.

Table 1.2. Comparison of health system policies and response to address cardiovascular diseases in Iraq and the UK (Grosios et al., 2010; Al Hilfi et al., 2013; World Health Organization, 2014).

	Iraq	**UK**
Guideline for management and treatment of CVD	Not available	Available
Guideline for management of medication non-adherence	Not available	Available
Operational CVD unit/branch or department within the Ministry of Health, or equivalent	Not available	Available
Operational multisectoral national policy, strategy or action plan that integrates several NCDs and shared risk factors	Not available	Available
Operational policy, strategy, or action plan to reduce physical inactivity and/or promote physical activity	Available (not applicable)	Available

Table 1.2 continued

Operational policy, strategy, or action plan to reduce the burden of tobacco use	Available (not applicable)	Available
Operational policy, strategy or action plan to reduce the harmful use of alcohol	Available (not applicable)	Available
Operational policy, strategy, or action plan to reduce unhealthy diet and/ or promote healthy diets	Available (not applicable)	Available
Evidence-based national guidelines, protocols, or standards for the management of CVD through primary care	Not Available	Available

1.4. Cardiovascular Diseases and Choice of Medications in Iraq

Cardiovascular diseases are the leading cause of death in Iraq (Ala'din, 2004). In Iraq, Ischemic heart diseases and stroke are the top two causes of death, with 27,500 deaths due to ischemic heart disease and 16,800 due to stroke in 2012 (Iraqi Ministry of Health, 2012; World Health Organization, 2015).

The increase in the prevalence of CVDs may be due to individual factors such as physical inactivity, age, unhealthy diet, alcohol consumption, smoking, economic wellbeing, and comorbidities such as diabetes, hypertension, dyslipidaemia, and obesity. Cultural changes such as globalisation, urbanisation, and population ageing may also contribute (Cooper *et al.*, 2006; Yu *et al.*, 2009; Murakami *et al.*, 2013; National Health Service, 2016; World Health Organization, 2018b).

Current clinical therapies for CVDs use certain combinations of medications to treat hypertension and lower cholesterol levels (Yusuf *et al.*, 2013). However, doctors do not follow any particular medical guidelines in Iraq in this regard, as mentioned earlier in section 1.3.7. CVD medications are prescribed based purely on past experience, thus leading to some considerable variation in the associated decision making. The most commonly prescribed CVD drugs in Iraq are amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, losartan, simvastatin, and valsartan. According to the annual medications need list in Iraq for 2016-2017, not all medications were available in KIMADIA such as atorvastatin, simvastatin and valsartan (KIMADIA, 2017). As KIMADIA provides the public sector in Iraq with its medications, atorvastatin, simvastatin and valsartan are not available in the public sector in any of the Iraqi

provinces, including Misan. However, these medications are prescribed by cardiologists and are only available in the private sector.

1.5. Thesis Outline

The following chapter outlines the definition of terms used to express medication-taking behaviour and factors associated with non-adherence to medication. Also, Chapter 2 outlines the prevalence and the consequences of medication non-adherence in addition to providing information on the approaches used for the assessment of non-adherence, identifying the gap in knowledge and the rationale behind the approach used in the study, as well as the specific aims and objectives of this research.

Chapter 3 describes the simulation and application of a previously validated liquid chromatography-high resolution mass spectrometry (LC-HRMS) assay for the simultaneous determination of nine commonly prescribed CVD medications in microvolume blood samples collected from 303 Iraqi on 903 cards and volumetric absorptive microsampling devices (VAMS) for the same volunteers. Volunteers were taking one or more of these CVD medications. The correlation between the analyte concentration collected on the 903 cards and VAMS was also investigated.

Chapter 4 detailed the application of the eight-item Morisky Medication Adherence Scale (MMAS-8) to assess non-adherence to the most commonly prescribed cardiovascular medications in 303 Iraqi volunteers, who were prescribed one or more of these medications and who provided blood samples on 903 cards and VAMS. The chapter investigates certain factors, such as gender, age, number of medications and number taken per day, which are associated with medication-taking behaviour.

Chapter 5 compares the results of non-adherence to prescribed CVD medications when using indirect and indirect methods for 303 Iraqi volunteers.

Chapter 6 outlines the clinical application of the results obtained from assessment of non-adherence to cardiovascular medications on the clinical practice in Iraq and suggests the proper intervention to improve adherence to medications in Iraqi volunteers based on the outcomes obtained from the above direct and indirect methods.

Chapter 7 summarises the overall conclusion and gives a number of recommendations for any future work.

1.6. Conclusion

Based on the information available on the Iraqi health system (as outlined in this chapter), the following can be inferred:

- Mortality due to chronic disorders including CVDs is considerably high and represents a challenge for the health system in both Iraq and the UK.
- In Iraq there are no effective regulatory systems in place with reference to the price of medication, prescription of medication, or management of chronic diseases such as CVDs in comparsion with the UK. This indicates that the Iraqi health system need to be upgraded to implement a multi-sector strategy to control cardiovascular diseases such as promoting healthy life style, reduce cardiovascular risks and ensuring the optimum use of CVD medications.

Chapter 2

Cardiovascular Diseases and Assessment of Medication Non-adherence

This chapter outlines cardiovascular diseases and their associated mortality rates, both worldwide and in Iraq. It also provides an overview of the problem of non-adherence to prescribed medication, the prevalence of non-adherence to medications and concepts and terms describing medication-taking behaviour. This chapter also highlights several factors that affect medication non-adherence in addition to the associated consequences. Furthermore, the current available methods of assessing patient medication non-adherence and their advantages and disadvantages will also be discussed. The direct method for assessment of non-adherence by microsampling analysis, and its advantages, challenges and the analytical techniques used in analysis of dried blood spots, namely immunoassay and liquid chromatography-mass spectrometry, are also discussed. Finally, the gaps in the literature and the aims and the objectives of this research are stated.

2.1. Introduction

Cardiovascular diseases (CVDs) refer to disorders of the heart and blood vessels such as hypertension, angina, heart attack, stroke, and heart failure (Tanna and Lawson, 2014a). This class of diseases accounts for the highest number of deaths worldwide at 17.9 million people in 2016 (World Health Organization, 2017a), wherein 7.4 million people are estimated to have died from coronary heart disease and 6.7 million from stroke (World Health Organization, 2017c).

CVDs are the leading cause of death in Iraq (Ala'din, 2004). Ischemic heart diseases and stroke are the top two causes of death, which resulted in 27,500 deaths due to ischemic heart diseases and 16,800 due to stroke in 2012, as mentioned in Chapter 1 Section 1.4 (Iraqi Ministry of Health, 2012; World Health Organization, 2015).

In the United Kingdom (UK), CVDs account for nearly 160,000 deaths per a year (British Heart Foundation, 2017). Currently, nearly 7 million UK residents endure some form of CVD, as equally divided between men and women (British Heart Foundation, 2017).

Current therapies for CVDs use various combinations of medications including ß-blockers and angiotensin converting enzyme (ACE) inhibitors to treat hypertension, and statins to lower cholesterol levels. Optimum clinical outcomes are not only dependent on choosing the proper treatment but also on the dose required to achieve the necessary

plasma concentration (Tanna and Lawson, 2016a). The required drug concentration can be achieved by adherence to the appropriate regime of medications. It is estimated that globally more than half of patients do not adhere to their medications (Sabaté, 2003; Kronish and Ye, 2013). This leads to poor clinical outcomes, increases health care expenditure and consequently affects labour force productivity and public health in general (Sabaté, 2003).

The World Health Organization (WHO) described non-adherence to prescribed medication as a "worldwide problem of striking magnitude" (Sabaté, 2003), which affects all disease states including cardiovascular, cancer and diabetes (Cutler *et al.*, 2018). There is evidence worldwide that more than 50% of prescribed CVD drugs are not taken by patients as recommended (Sabaté, 2003; Tanna and Lawson, 2016a; Ferdinand *et al.*, 2017). Non-adherence to medications results in increased morbidity, mortality, medicine wastage, and raised costs (Tanna and Lawson, 2016a; Giner-Soriano *et al.*, 2018). Patient's physical and psychiatric disabilities are exacerbated by poor adherence to medication, affecting family, work and social responsibilities.

Moreover, poor adherence to medication limits health funding that might otherwise be used more effectively elsewhere. The economic cost of medication non-adherence is not only due to waste of medications but also due to increased demand for healthcare related to rehospitalisation (Stuart *et al.*, 2009; pharmaphorum, 2018; Cutler *et al.*, 2018).

2.1.1. Definition of Terms Related to Patient Behaviour in Medicine Taking

Patient behaviour, as associated with taking, or indeed not taking, medication as prescribed has been discussed using different terms such as compliance, adherence, concordance, and persistence. Whilst these terms are often used interchangeably, they do however reflect different views on the relationship between patients and healthcare providers (Vrijens *et al.*, 2012).

2.1.1.1. Compliance

Compliance can be considered the oldest term to describe patients' medication-taking behaviour. Compliance is defined as "the extent to which the patient's behaviour matches the prescriber's recommendations" (Haynes, 1979).

Using the term compliance exaggerates the role of healthcare providers and imposes a paternalistic relationship. It suggests a one-sided interaction where the patient must comply with the prescribed medication, regardless of whether it is suitable for them or otherwise. The term "compliance" has been criticised since it conveys a negative relationship between patients and healthcare providers (Rafii *et al.,* 2014).

2.1.1.2. Adherence

The World Health Organization (WHO) defined adherence as "the extent to which a person's behaviour taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a healthcare provider" (Sabaté, 2003).

The terms 'adherence' and 'compliance' are often used interchangeably and, indeed, are considered to be synonymous (Cramer *et al.,* 2008). However, they reflect different views on the relationship between patients and healthcare providers. Adherence implies a certain level of cooperation and the sharing of perspectives and views between patient and caregiver in order to improve the patient's health, since patients are free to agree or otherwise with the medical plan proposed by the healthcare provider. The main difference between adherence and compliance is the agreement in terms of recommendations and sharing decisions (World Health Organization, 2002; Nguyen, 2016).

Furthermore, adherence focusses on patient autonomy and is patient-centred via the collaboration between patient and healthcare provider rather than being a paternalistic relationship, and indeed the patient actively participates in the treatment plan (Vermeire *et al.*, 2001). For proper medication adherence, six key factors should be involved which include taking the correct drug in the correct dose, at the correct time and on the correct schedule, under the correrct conditions whilst adopting the correct precautions (Tanna and Lawson, 2014b). This further corroborates the WHO definition mentioned earlier.

2.1.1.3. Concordance

Concordance is a new approach to the prescription and taking of medicines. It was introduced by the Royal Pharmaceutical Society of Great Britain in 1995 (Vrijens *et al.*, 2012). Concordance is a patient's medicine-taking behaviour as achieved after equal negotiation and agreement between the patient and healthcare professional to determine

when and how their medicine is taken (Blenkinsopp *et al.*, 1997). Concordance cannot be used as a synonym for adherence because it includes communication, shared decision and medical consultation (Cushing and Metcalfe, 2007).

2.1.1.4. Persistence

Persistence can be defined as the length of time between the initiation of the first dose of medication and the last dose preceding the discontinuation of the therapy (Vrijens *et al.*, 2012). Persistence is distinct to adherence because the former refers to how long patients continue taking medication, whereas adherence refers to how patients respect the medical regimen, thus the terms adherence and persistence cannot be used synonymously. It describes the medication-taking period, rather than consider the factors that might be associated with the decision to stop taking medication.

2.1.1.5. Medication Non-adherence

Non-adherence to medication can be defined as a patient's failure to follow the recommendations agreed with their doctor in terms of timing, dosage, and frequency (Aldeer *et al.*, 2018). It is a major problem in patients with chronic diseases (Bitton *et al.*, 2013; Palmer *et al.*, 2018). For instance, medication non-adherence increases the risk of heart disease-related hospitalisation and death in cardiovascular patients (Ho *et al.*, 2008; Hood *et al.*, 2018).

The National Institute for Health and Care Excellence (NICE) separates medication non-adherence into two overlapping categories, namely the intentional and the unintentional (Nunes *et al.*, 2009). Medication non-adherence can be intentional, unintentional, or both (Usherwood, 2017). In intentional medication non-adherence, the patient actively decides not to follow the treatment recommendations given due to associated beliefs and perceptions, skipping doses to avoid side effects, the opinions of friends and family or due to the cost of the medication (Lehane and McCarthy, 2007b; Usherwood, 2017). Considerable research has been carried out to understand the causes of intentional non-adherence to medication for a wide range of diseases, the results of which indicate similar causes (Laba *et al.*, 2015). Furthermore, it is evident that about half of medication non-adherence cases are intentional (Pound *et al.*, 2005; Mukhtar *et al.*, 2014).

Unintentional non-adherence is when the patient wants to follow the treatment recommendations but has practical problems in doing so. Unintentional medication non-adherence is an unplanned, poor medication-taking behaviour due to a condition which is out of the patient's control, and about which the patient can do nothing (Wroe, 2002). The following are the common causes of unintentional medication non-adherence: forgetfulness, misunderstanding of medical instructions, low level of education, inability to access medication, taking substandard/falsified medications, lack of reminders, complexity of the regimen (polypharmacy) and dose frequency, and physical problems such as poor eyesight (Morisky *et al.*, 1986; Wroe, 2002; Lowry *et al.*, 2005; Atkins and Fallowfield, 2006; Lehane and McCarthy, 2007a; Clifford *et al.*, 2008; Unni and Farris, 2011; Hugtenburg *et al.*, 2013; Tanna and Lawson, 2016a; Usherwood, 2017).

Studies on cardiovascular medication non-adherence in Iraq are very limited and all studies used indirect methods, typically though application of a questionnaire. It is estimated that non-adherence to CVD medications in Iraq was between 19.6% and 63% (Samer, 2008; Al-Dabbagh and Aswad, 2009; Hasan *et al.*, 2011; Bushra and Kameran, 2013; Jamal and Saleem, 2014; Safaa and Ali, 2015).

- A cross-sectional study on 322 diabetic and hypertensive patients in Iraq by the application of an eight-item Morisky questionnaire showed that 19.6% of patients were non-adherent (Jamal and Saleem, 2014).
- A cross-sectional study on 323 Iraqi hypertensive patients in using MMAS-8 combined with a socio-demographic information questionnaire showed that 42.3% of patients were non-adherent to antihypertensive medications (Safaa and Ali, 2015).
- A cross-sectional study based on a questionnaire designed by the researcher given to 100 hypertensive patients showed that 63% of participants were non-adherent to medication (Samer, 2008).
- A cross-sectional study to assess non-adherence to antihypertensive medication based on asking patients about their taking behaviour was carried out with 191 hypertensive patients with acute ischemic stroke and acute myocardial infarction (MI). The study reported that 61% of patients were non-adherent to antihypertensive medications (Hasan *et al.*, 2011).

- A cross-sectional study conducted in Iraq with 400 hypertensive patients using a questionnaire designed by the researcher showed that 58.8% of patients were non-adherent to antihypertensive medications (Bushra and Kameran, 2013).

The studies mentioned above further support the fact highlighted that there is currently more data related to the assessment of hypertension than there is for CVDs. It is therefore important that more studies related to CVDs are conducted, and hence the basis for this research. With reports (as mentioned in Section 2.2.5) suggesting that between 19.6% and 63% of prescribed CVD drugs are not taken by patients as recommended, it is important to consider factors related to medication adherence. An understanding of the reasons for non-adherence associated with each individual patient may allow for the required interventions to increase adherence (Monroe *et al.*, 2018)

2.1.2. Factors Associated with Medication Non-adherence

Factors affecting non-adherence to medication are different in different parts of the world. As highlighted in Chapter 1, Section 1.2, in Iraq, there are no insurance contributions and patients have to pay to see a clinician in the public and private sectors, and further pay the full cost of the prescription. Hence, the cost of treatment may be a significant determinant of non-adherence to medication in Iraq. The situation may be different to other countries, such as the UK, as the cost of treatment is subsidised through patients' health insurance. In view of this difference in health systems, interventions designed to improve adherence in Iraq may not necessarily be applicable in the UK because due to the underlying factors relevant in each case.

Non-adherence is not only influenced by individuals' behavioural factors, but also by the disease itself, the complexity and duration of the treatment, adverse drug reactions, cost of treatment, and social factors. The WHO further classifies factors affecting non-adherence into five subclasses: socioeconomic, healthcare system, condition-related, therapy-related and patient-related (Ferdinand *et al.*, 2017).

2.1.2.1. Socioeconomic-Related Factors

A review by Martin *et al*., (2018), on barriers and strategies to improve adherence showed that patients who are supported by their families, friends and healthcare providers in terms

of assisting with their medication showed better adherence. However, patients living in unstable environments, or who have limited access to healthcare, a lack of financial resources, or who are unable to get their medication due to cost showed high levels of non-adherence (Kalogianni, 2011).

2.1.2.2. Healthcare System-Related Factors

There is a relationship between non-adherence and communication between patients and healthcare providers. Martinez and Finken showed that patients who have good relationships with their clinicians were generally adherent to their medications, whilst on the other hand patients who were not happy with this relationship tended not to be adherent (Martinez and Finken, 2017)**.**

Clinicians' communication skills are important to the patient's understanding of their conditions, possible complications, and the importance of medication adherence (Schoenthaler *et al.*, 2017). Poor communication between healthcare providers and patients can sometimes in itself lead to poor adherence, medication errors and unnecessary hospital readmissions (Ferdinand *et al.*, 2017).

Long waiting times at the clinic or pharmacy have been identified as a barrier to patients' medication adherence (Vermeire *et al.*, 2001; Ferdinand *et al.*, 2017; Leslie *et al.*, 2018). It has been reported that a lack of support offered from healthcare providers to patients and a poor relationship between clinician and patient have been recognised as significant determinants of medication non-adherence (Khatib *et al.*, 2014; Leslie *et al.*, 2018). In addition, health systems that cannot provide patients with appropriate education on their treatment or follow-up on such will promote non-adherence to treatment. For instance, patient information leaflets are generally written using a high level of literacy and this may make it difficult for patients to understand the required information about the prescribed medications (Schoenthaler *et al.*, 2017).

Healthcare systems should implement the required system changes to ensure that assessment of medication non-adherence is properly considered in health practice and encourage a blame-free environment between patients and healthcare providers (Abbo *et al.*, 2008).

2.1.2.3. Condition-Related Factors

The adherence to medications which are taken as part of long-term treatments is associated with a marked decrease over time, especially with conditions that are asymptomatic. Absence of symptoms is a barrier to patients taking the appropriate medication. It is crucial that patients understand their diseases and the associated consequences of not taking medications (Kalogianni, 2011).

2.1.2.4. Therapy-Related Factors

Non-adherence to medication is associated with therapy-related factors such as the complexity of the medication regimen, duration of the medical course, side effects and the medication's route of administration (Kleeberger *et al.*, 2001; Gellad *et al.*, 2011, Toy *et al.*, 2011; Fawzi *et al.*, 2012; Laba *et al.*, 2012; Ruppar *et al.*, 2012; Schneider *et al.*, 2018). Substandard/falsified medications have also been associated with medication non-adherence rates (Tanna and Lawson, 2016a). Falsified and substandard medications contain raised, reduced or no active pharmaceutical ingredient. Using such medications compromises treatment, causing poor clinical outcomes with the possibility of serious side effects which consequently leads to or otherwise implies non-adherence to medications, since recommended dosages are not adhered to (Buckley and Gostin, 2013).

Prescription of a complex medication regimen, especially in patients with co-morbid conditions, leads to serious consequences and can worsen the condition (Monroe *et al.*, 2018). A high level of comorbidities is associated with CVD. One in every four patients who suffers from CVD has a high probability of the co-occurrence of other chronic diseases (Kendir *et al.*, 2018). A combination of drugs in one dosage form is associated with a higher rate of adherence in comparison with giving each medication separately (Sherrill *et al.*, 2011). Generally, adherence decreases as the number of doses taken per day increases (Assawasuwannakit *et al.*, 2015; Xu and Worden, 2016).

2.1.2.5. Patient-Related Factors

There are conflicting results regarding the association between gender and medication non-adherence. The majority of studies showed that the risk of non-adherence to medication regimes is higher in women than in men by 7-10% (Leslie *et al.*, 2018). However, other studies have showed that women adhere to medication regimes better

than men (Nielsen *et al.*, 2017), whilst others still have showed no association between gender and non-adherence (Lewey *et al.*, 2013).

Cross-sectional studies in Iraq based on a questionnaire designed by the researcher that was given to hypertensive patients showed that non-adherence to antihypertensive medications in men was higher than in women (Samer, 2008; Bushra and Kameran, 2013). However, another study showed that females showed greater non-adherence than males (Jamal and Saleem, 2014).

Other studies outside Iraq showed a significant relationship between age and adherence, where older patients were likely to be more adherent to antihypertensive medication than younger patients (Ramli *et al.*, 2012; Alhewiti, 2014; Meinema *et al.*, 2015; Assawasuwannakit *et al.*, 2015; Khayyat *et al.*, 2017). A similar result was obtained from a study in Iraq (Al-Dabbagh and Aswad, 2009). On the other hand, a systemic review by Yap *et al.* showed that older patients were more non-adherent to medications (Yap *et al.*, 2016). This could be because people begin to forget as they get older. A conflicting result from a study in Iraq (Bushra and Kameran, 2013) and other studies outside Iraq showed no relation between adherence to antihypertensive medications and age (Tomaszewski *et al.*, 2014; Krueger *et al.*, 2015; Pandey *et al.*, 2015; Jin *et al.*, 2016).

Lack of knowledge was frequently associated with medication non-adherence. Patients may discontinue treatment when they feel better due to a lack of, or a reduction in their symptoms or because they do not understand that their disease is chronic and requires long-term treatment (Rashid *et al.*, 2014; Lee *et al.*, 2018). It is estimated that over 80 million adults in the US have inadequate health literacy and that this leads to suboptimal clinical outcomes and consequently increases the risk of hospital readmission (Mayo-Gamble and Mouton, 2018).

In addition to patient's beliefs and attitudes, other factors such as previous treatment experiences, religious and cultural beliefs about the condition, mental health problems and lack of motivation may affect adherence (Atinga *et al.*, 2018; McQuaid and Landier, 2018; Kvarnstrom *et al.*, 2018)**.** A systematic review by Rashid *et al*. showed that the use of a self-reporting tool cited fears of dependence on cardiovascular medication as a barrier to adherence (Rashid *et al.*, 2014). Patients who believe that medication will control and manage their diseases show the highest adherence to medication in comparison with

patients with low motivation (Ross *et al.*, 2004; Osterberg and Blaschke, 2005; Joyner-Grantham *et al.*, 2009; Brunner *et al.*, 2009; Petrie *et al.*, 2012; Alhalaiqa *et al.*, 2012; Sjolander *et al.*, 2013; Berglund *et al.*, 2013; Horne *et al.*, 2013; Rajpura and Nayak, 2014). Physical factors such as visual, hearing and mobility impairment and swallowing problems are patient-related factors that have also been associated with medication non-adherence (Adult Medication, 2006).

2.1.3. Consequence of Non-adherence to Medication

2.1.3.1. Clinical Outcomes

Non-adherence to medication is associated with a significant impact on the efficacy of medicines, leading to treatment failure, progression of the disease (which worsens the patient's condition) and consequently increases both morbidity and mortality rates (Tanna and Lawson, 2016a). Physicians may incorrectly relate poor clinical outcomes to the prescribed medications and therefore look for an unnecessary alternative approach such as increasing the dose or prescribing medications that are more potent or more expensive. Physicians cannot assess non-adherence for each individual medication based on the clinical outcomes especially for patients who take many medication and the physician may increase the dose for a medication that patient is already adhering to, which could clearly lead to serious consequences such as poor clinical outcomes (Sokol *et al.*, 2005; Lam and Fresco, 2015).

Patients who are non-adherent to cardiovascular medications tend to show multiple poor health outcomes such as an increased risk of cardiac events in comparison with adherent patients (Gehi *et al.*, 2007; Addison *et al.*, 2011; Wu and Moser, 2018). Patients who adhere to antihypertensive medication are able to maintain control over their blood pressure (Hyre *et al.*, 2007; Macedo *et al.*, 2010). Non-adherence to antihypertensive medication leads to the development of coronary artery diseases and chronic heart failure in non-adherent patients (Tanna and Lawson, 2016a). Poor adherence to statins increases the risk of cardiovascular diseases by 1.2- to 5.3-fold and an increase in the risk of mortality by 2.5-fold in comparison with patients who take their medications as prescribed (De Vera *et al.*, 2014). There is a recent debate on the role of statins in cardiovascular diseases, in a recent study 50% of patients on long term statin therapy were

presented with a sub-optimal level of LDL cholesterol with risks of cardiovascular events (Akyea *et al*., 2019; Mahase, 2019).

According to a study by Gehi *et al*, cardiovascular events (coronary heart disease, MI, or stroke) in non-adherent patients with stable coronary artery disease increased more than two-fold (Gehi *et al.*, 2007). Choudhry *et al*. found that cardiovascular events in adherent patients with a previous history of a heart attack were less likely to happen in comparison with a control group (Choudhry *et al.*, 2014). This indicated that a patient who is adherent to medication has a lower risk of developing complications or the progression of cardiovascular disease in comparison with one who is non-adherent.

Non-adherence to cardiovascular medication is associated with a high mortality rate. The mortality rate in patients who discontinue their medication is higher than in those who continue taking their medication as prescribed. For example, in patients taking aspirin and statins, the mortality rate increases by almost two-fold in patients who discontinue taking aspirin and five-fold in patients who stop taking statins (Ho *et al.*, 2006). Rasmussen *et al*. found that the mortality rate in patients with acute MI who had been prescribed statins was 25% in poorly adherent patients and 12% in highly adherent patients (Rasmussen *et al.*, 2007). A study by Spertus *et al*. showed that there was a nine-fold increase in the mortality rate in patients who discontinued taking anti-platelet medications (thienopyridines) (Spertus *et al.*, 2006).

A study by Rieckmann *et al*. showed that poor adherence to aspirin increases the risk of mortality and cardiovascular events by almost two-fold in patients with acute coronary syndrome (Rieckmann *et al.*, 2011). Comparable results were seen in non-adherence to clopidogrel for patients receiving drug-eluting medication, where the mortality rate similarly increased by two-fold due to MI (Ho *et al.*, 2010).

2.1.3.2. Increased Healthcare Expenditure

Non-adherence imposes burden on medical resources (Cutler *et al.*, 2018). Rates of hospitalisation are considerably higher in patients with poor adherence to medications for chronic diseases such as diabetes, hypercholesterolemia, hypertension and congestive heart failure (Miura *et al.*, 2001; Sokol *et al.*, 2005). A total of 33-69% of instances of hospitalisation in the US are related to medication non-adherence (Ho *et al.*, 2006). A rise

in the trend of hospitalisation was also seen in patients with heart failure who were not adherent to digoxin (Miura *et al.*, 2001).

According to Sokol *et al*., the consequences of poor adherence to antihypertensive and anti-hypercholesterolemia drugs are those of increased hospitalisation and poor clinical outcomes (Sokol *et al.*, 2005). A systematic review of the literature by Bitton *et al.* (2013) showed a significant difference in the annual cost of medical care for coronary heart disease (CHD) in patients showing adherence and non-adherence. The annual cost of non-adherent per patient was $4940, as compared to $4040 for adherent patients (Bitton *et al.*, 2013). A systematic review of the economic impact of cardiovascular diseases as a group, showed that the annual CVD medication non-adherence cost per patient per year for cardiovascular diseases can be estimated at around $10,000 (Cutler *et al.*, 2018).

Optimal adherence represents an excellent opportunity for investment in the medical care sector. According to the New England Healthcare Institute (NEHI), the estimated avoidable medical costs in the US across the range of chronic diseases associated with medical-related problems was $290 billion (New England Health Institute (NEHI), 2009). The situation in the EU is not particularly different, with non-adherence being associated with an annual cost of €125 billion (Pefoyo *et al.*, 2015). According to the National Health Service (NHS), 5% of all emergency admissions in the UK were due to inappropriate use of medication, costing £500 million a year in England alone (Barnett, 2014). In Australia, 10% of hospitalisations were due to medication non-adherence, costing an extra $2000 per patient per year (Sokol *et al.*, 2005; Cutler *et al.*, 2018). In Iraq, there is no available data about non-adherence and health expenditure as the study of medication non-adherence is still effectively in its infancy.

2.1.3.3. Medicine Wastage

Suboptimal adherence to medication leads to therapeutic loss and waste of medications, which includes unused medication, either disposed of or returned to the pharmacy, or medication kept at home without being used. In the UK, it is estimated that £4 billion worth of medication prescribed by the NHS is not used as prescribed (Nunes *et al.*, 2009; Tanna and Lawson, 2016a) and almost $8 billion worth in the US, where 3-7% of medication is wasted (Tchen *et al.*, 2013). In Iraq, the cost of wasted medication is unknown because there are no studies that have, to date, focussed on medicine wastage

or on non-adherence. In addition to the effects that poor adherence has on health, it also has the potential to impact medication development and manufacturing, where the estimated losses incurred by US pharmaceutical companies in 2012 were $188 billion (Ken, 2015).

Considering the consequences of non-adherence already mentioned, it is very important that different methods, as applicable to different settings, are developed for assessment of medication non-adherence.

2.2. Assessment of Medication Non-adherence

Although various strategies have been employed to measure medication non-adherence, there is no gold standard for routine clinical practice (Kennedy *et al.*, 2008). Each method used for the assessment of non-adherence has its own strengths and weaknesses, with trade-offs between accuracy and practicality. Moreover, each method provides different information on medication (Vitolins *et al.*, 2000; Lehmann *et al.*, 2014). Methods used to measure adherence can be categorised into the indirect and the direct, as summarised below.

2.2.1. Indirect Methods

Indirect methods include patient diaries, patient interviews, pill counts, electronic monitoring, adherence questionnaires, and pharmacy refill prescription databases and clinical outcomes. These methods are widely used and easily carried out (Mathes *et al.*, 2014).

2.2.1.1. Patient Diaries

Patient diaries are the only self-report tool that documents how the patient follows their prescribed regime(s). The use of patients' diaries as a tool to assess adherence is optimistic and subject to overestimation; furthermore, assessment cannot be carried out if the patient does not return the diary (Lam and Fresco, 2015; Tanna and Lawson, 2016a). This approach cannot be used in Iraq to assess non-adherence because patients' diaries are not updated and are badly documented and stored.

2.2.1.2. Patient Interview

Patient interviews are a non-invasive, straightforward, low-cost subjective method by which to assess patient medication non-adherence (Farmer, 1999; Suzanne, 2011). Typically, the doctor or the pharmacist asks the patient to report the medication name, dose and time of drug intake. The doctor may ask the patient how often per week or per month they forget to take their medication; based on the answers, the level of medication non-adherence can be determined (Vik *et al.*, 2004; Lam and Fresco, 2015). Nevertheless, the assessment of non-adherence through interviewing patients is strongly affected by the communications skills of the interviewer (Farmer, 1999). Thus, patient interview is subject to overestimation, bias and cannot confirm that medications have been taken as prescribed (Osterberg and Blaschke, 2005).

2.2.1.3. Pill Counts

Pill counts involve counting the number of dosage units that have been taken between two scheduled appointments or clinic visits (Lam and Fresco, 2015). To calculate the percentage adherence, the number of pills taken by the patient is divided by the number of pills prescribed, the value of which is then multiplied by 100; patients are described as adherent when the percentage is 80% or more (Lam and Fresco, 2015). This provides an indication of the number of medication units taken by the patient within a given period of time (Neiheisel *et al.*, 2014). However, the accuracy of pill counts as a tool for estimating medication adherence is uncertain because some patients may not return their unused medication (Lawrence *et al.*, 2017). Pill counts is easy applicable and cheap; however, there is no confirmation that the patients actually took the medication (Tanna and Lawson, 2016a).

2.2.1.4. Electronic Monitoring

Electronic monitors consist of a microprocessor placed in the medication container. The microprocessor is activated and records the date and time at which the patient opens the cap of the medication container (Lehmann *et al.*, 2014). Additionally, an electronic monitoring device provides accurate information on the time at which the container was opened, and thus can provide information about daily adherence variation (Urquhart, 1997; van Heuckelum *et al.*, 2017). However, such technology is expensive, used more in clinical trials, and has limited use for patients taking multiple forms of medication

(Lam and Fresco, 2015; Sidorkiewicz *et al.*, 2016). The device is suitable for solid pharmaceutical dosage forms and again cannot confirm that the patient has actually taken the medication (Choo *et al.*, 1999; Rosen *et al.*, 2004; Lehmann *et al.*, 2014) as patients may open the container and then merely discard the medication.

There are different models of such electronic devices; some can provide information about medication adherence patterns to the provider by telephone or modem (Bosworth, 2014). Some more modern examples are equipped with adherence aids that can remind patients to take their medication as prescribed (Haberer *et al.*, 2012). There are various limitations to this kind of technology, however; for instance, a patient may take multiple doses at a given time when opening the cap just once, or possibility taking more than one dose each time they open the cap, especially when leaving home or travelling. Furthermore, the recorded data requires the accompanying technology to interpret the data collected, and it is a challenge to monitor all the individual forms of medication being taken by the patient (Lehmann *et al.*, 2014).

2.2.1.5. Questionnaires

A questionnaire can provide both quantitative and qualitative results. Answers that are obtained by closed-ended questions with multiple choice provide quantitative results and answers that are obtained by open-ended questions provide qualitative results (Research Methodology, 2019). Questionnaires can be done by face-to-face and telephone interview, on the web or by self-completion (Phillips and Stawarski, 2008).

Questionnaires are cheap, easily done. However, Questionnaires need that respondents must be able to read the questions and respond to them. Therefore, some outcomes by questionnaires may not be actual. Moreover, respondents may not able to express their other thoughts about a problem because of the absence of a related question (Research Methodology, 2019).

Questionnaires are the most commonly used in the clinical practice for assessing medication non-adherence because questionnaire is the cheapest and the simplest method in terms of routine care (Osterberg and Blaschke, 2005; Garfield *et al.*, 2011; Stirratt *et al.*, 2015). Some questionnaires (Table 2.1) provide information about factors associated with medication non-adherence such as forgetfulness and the adverse effects of

medications or beliefs (Ogedegbe *et al.*, 2003; Krousel-Wood *et al.*, 2004; Ogedegbe *et al.*, 2004). However, questionnaires cannot provide essential information associated with clinical outcomes, such as the timing of the doses (Tanna and Lawson, 2016a). Moreover, patients tend to overestimate their levels of adherence (Dunbar-Jacob *et al.*, 1991). Questionnaires are subject to social desirability and patient recall (Steiner and Prochazka, 1997; Choo *et al.*, 1999; Althubaiti, 2016).

A systemic review by Nguyen *et al.* identified 43 validated questionnaires, which have been validated with other approaches such as pharmacy records or electronic monitoring across different populations and showed significant correlation. These questionnaires can be grouped into five distinct sets:

- Questionnaires that assess medication-taking behaviour.
- Questionnaires that assess medication-taking behaviour and barriers to adherence.
- Questionnaires that assess only barriers to adherence.
- Questionnaires that assess barriers and beliefs.
- Questionnaires that assess beliefs only.

Among these validated questionnaires, only 21 valid questionnaires were used to assess adherence to medication for cardiovascular diseases (Nguyen *et al.*, 2014). There is no agreement as to the best form of questionnaire (Eskås *et al.*, 2016). The eight-item Morisky Medication Adherence Scale (MMAS-8) was used in this study because all studies in Iraq regarding non-adherence was done by questionnaires and this provide a chance to compare the results of the present study with previous studies in Iraq. MMAS-8 is short, easily scored, can assess non-adherence and identify some reason associated with non-adherence such side effects and forgetfulness. MMAS-8 also can define whether non-adherence is intentional or unintentional based on the patient response to the questions (Detailed information described in detail in Chapter 4).

Table 2.1. Validated questionnaires used to assess adherence to cardiovascular diseases (Nguyen et al., 2014).

Cardiovascular diseases	**Questionnaire**	**Application**
Hypertension/Dyslipidaemia	Adherence Self-Report Questionnaire	Medication-taking behaviour
	Medication Adherence Rating Scale - 5	Medication-taking behaviour
	Stages of Change for Adherence Measure	Medication-taking behaviour
	Brief Medication Questionnaire	Medication-taking behaviour and barriers
Hypertension/Dyslipidaemia	Choo et al. Questionnaire	Medication-taking behaviour and barriers to adherence
	Fodor Adherence Questionnaire	Medication-taking behaviour and barriers to adherence
	Hill-bone Compliance Scale - 10	Medication-taking behaviour and barriers to adherence
	Hill-Bone Compliance Scale - 14	Medication-taking behaviour and barriers to adherence
	Morisky Medication Adherence Scale (MMAS-8)	Medication-taking behaviour and barriers to adherence
Hypertension/Heart failure Dyslipidaemia/ Coronary heart disease	Reported Adherence to Medication Scale	Medication-taking behaviour and barriers to adherence
Hypertension/Heart failure Dyslipidaemia	Medication Adherence Questionnaire (4-items)	Barriers to adherence
	Medication Adherence Self-Efficacy Scale	Barriers to adherence
	Medication Adherence Self-Efficacy Scale-Revised	Barriers to adherence
Hypertension/Dyslipidaemia Coronary heart disease	Self-Efficacy for Appropriate Medication Use Scale	Barriers to adherence
Coronary heart disease	Gehi *et al.* Adherence Question	Medication-taking behaviour
Hypertension/Heart failure Coronary heart disease	Beliefs about Medicines Questionnaire	Beliefs
	Maastricht Utrecht Adherence in Hypertension	Barriers and beliefs
Heart failure	Adherence to Refills and Medications Scale	Medication-taking behaviour and barriers to adherence
	Adherence Starts with Knowledge - 12	Medication-taking behaviour and barriers to adherence
	Adherence Starts with Knowledge - 20	Medication-taking behaviour and barriers to adherence
	Medication Adherence Reasons Scale	Barriers to adherence

2.2.1.6. Pharmacy Refills and Claim Data

Clinicians or pharmacists may assess non-adherence to medication by reviewing pharmacy records. Pharmacy databases can be checked when prescriptions are initially filled out, repeated or prematurely discontinued. Repeat prescription records provide data on the quantity of the medications prescribed (Sidorkiewicz *et al.*, 2016). This approach can be used to assess non-adherence, especially in a large population, to multidrug regimens (Bosworth, 2014; Lam and Fresco, 2015). However, one of the recognisable limitations to this method is that adherence can only be estimated for patients who purchase their medication from a certain pharmacy in order to track medication refills (Osterberg and Blaschke, 2005).

The application of this approach depends on the availability of a computerised system, which is considered to be a major limitation in countries with limited infrastructure such as Iraq, with no confirmation that medications have been taken as prescribed and which consequently may result in medication adherence overestimation (Krousel-Wood *et al.*, 2015; Sidorkiewicz *et al.*, 2016).

Adherence according to pharmacy refills and claim data is defined as the number of refills obtained over time/number of months of follow-up. Patients are considered as adherent based on pharmacy repeat prescription data, when the number of repeat prescriptions obtained over time/number of months is 80% or greater. This percentage is considered as a cut-off point to categorise a patient as adherent or non-adherent (Ho *et al.*, 2009). However, there is evidence that blood pressure and LDL cholesterol are decreased with an adherence of more than 80%, and this suggested that optimal adherence is achieved beyond this point (Bryson *et al.*, 2007). This cut-off point, however, may be too low to be effective for other conditions (Ho *et al.*, 2009).

2.2.1.7. Clinical Outcomes

Clinical outcomes are a poor indicator of adherence since they may depend on other unrelated factors, for instance, patients who are adherent to their antihypertensive medications may have uncontrolled blood pressure due to a high dietary intake of salt, or increases in body weight, or due to alcohol consumption (Feldman *et al.*, 1998, Murray *et al.*, 2009).

Moreover, clinical outcomes cannot give an idea about non-adherence to each medication in the medical regimen for patients taking more than one medication. Healthcare providers may increase the number of medicines to add synergic action because they may think that the medication(s) are not efficient or may follow new strategies such as increasing the doses of medications that the patient already adheres to (Sokol *et al.*, 2005; Lam and Fresco, 2015). In addition, they may prescribe expensive medication without any real assessment of patient adherence. These factors ultimately may lead to uninformed decisions being made by physicians with potentially dangerous and expensive consequences.

2.2.2. Direct Methods

Direct methods involve the direct observation of the patient taking medicines or analysis of biological fluids (such as urine or blood) for the existence of the drugs or their metabolites, or the detection of biological markers added to medications (Lam and Fresco, 2015).

2.2.2.1. Direct Observation

Directly observed therapy (DOT) involves inviting patients to the clinic to ingest their medications under the direct supervision of a nurse. Hameed *et al*. (2015) and Gupta *et al*. (2016) reported that the assessment of adherence to antihypertensive medications via DOT showed that non-adherent patients were admitted to hospitals due to sudden drops in blood pressure because the previously avoided dose of antihypertension medications was now being taken (Hameed *et al.*, 2016; Gupta *et al.*, 2016).

Direct observation is accurate and non-invasive, but its main limitations are that direct observation is cost- and labour-intensive. This approach is inconvenient for patients and impractical in an outpatient setting, as patients may have to travel long distances to get to the hospital and may then have to spend half a day at the clinic. In addition, patient supervision has to be undertaken by appropriately trained personnel. Moreover, the patient can manipulate medication taking by hiding tablets in their mouths (Hawkshead and Krousel-Wood, 2007).

Another development in this field is the use of ingestible electronic sensors that are attached to pills to track medication ingestion (digital pills) (Abderrahman, 2018). However, patient security and privacy may be of concern when sensors are used in this manner (Aldeer *et al.*, 2018).

2.2.2.2. Therapeutic Drug Monitoring in Biofluids

Therapeutic drug monitoring (TDM) is defined as the management of a patient's medication regime based on the concentration of the target medication in serum, plasma, or whole blood (Clarke, 2016). Direct methods measure the concentration of drugs or their metabolites in biological fluids such as blood, urine, saliva, sweat or hair. The assessment of non-adherence by the direct method of using biological markers is limited as biomarkers are only available for a limited number of drugs (Lehmann *et al.*, 2014) such as low-density lipoprotein (LDL) levels of statins, platelet function tests for aspirin, and other anti-platelet medications. However, they cannot distinguish whether poor adherence can be attributed to the pharmacology of the drug or the biology of the patient (Kronish and Ye, 2013).

Measuring the concentration of drugs or metabolites in a biological fluid can be performed either at specified intervals or randomly. It provides an indication as to whether the patient has taken the medicine (Morrison *et al.*, 2015). Consequently, direct methods of assessment are the most accurate approach to measuring adherence (Aonuma *et al.*, 2017). Conventional direct methods using blood as a biosample require large volumes (1-10 ml) to obtain a sufficient volume of plasma or serum (Tanna and Lawson, 2016b), and can be carried out via liquid chromatography (LC)-tandem mass spectrometry (MS/MS) or LC-MS (Gonzalez *et al.*, 2010; Gonzalez *et al.*, 2011; Dias *et al.*, 2013; González *et al.*, 2015). In order to get the necessary volume, patients need to visit a phlebotomist. Conventional direct methods have special requirements associated with the collection of samples (use of a syringe, collection tube, etc.) and their transportation and storage (cooling box), thereby increasing the cost and making them unsuitable in routine clinical practice for therapeutic drug monitoring (Lawson *et al.*, 2013; Tanna and Lawson, 2016a; De Nicolò *et al.*, 2017).

Urine samples provide a non-invasive means by which to confirm that a particular medication has been ingested by detecting the drug either directly or through one of its

metabolites as it is eliminated from the body. However, this approach is limited to drugs that are predominantly metabolised and excreted in urine (Lehmann *et al.*, 2014). Also, in Iraq, using urine as a biosample for assessment of adherence may represent a challenge as some individuals might have reservations for cultural and religious reasons. Urine samples require sterile containers with boric acid to preserve the sample and prevent overgrowth of organisms during transport to the laboratory. Urine samples should be stored in a fridge when there is any delay in their transportation (National Health Service, 2018a). Urine analysis by LC-MS/MS has been used for screening adherence to antihypertensive medications for patients exhibiting resistant hypertension (Tomaszewski *et al.*, 2014; Hamdidouche *et al.*, 2015; De Nicolò *et al.*, 2017; Hamdidouche *et al.*, 2017).

Other biological samples such as saliva, sweat and hair have been used to assess adherence to antiretroviral therapy and pain management medications because of their advantages over blood and urine such as being non-invasive, painless and stress-free sampling methods, providing information about the long-term use of medications, their low costs and the lack of any need to visit a clinic or hospital to collect samples (Olds *et al.*, 2015; Moore, 2015; Ferrari *et al.*, 2017). However, validation and study of the analyte stability should be considered before sample collection.

2.2.2.3. Whole Blood Microsampling Analysis

Considering the large volumes of blood required for conventional direct methods (as mentioned in the preceding section), microsampling might provide a valuable alternative for such analysis. Microsampling requires very small volumes of biofluid samples such as blood, plasma, urine and milk for determination of the concentration of the target analyte or endogenous substance, the most commonly used of which is dried blood spots (DBS) (Zane and Emmons, 2013; Ayre *et al.*, 2018). DBS is a dried microsampling matrix which involves the collection of liquid whole blood as a dried sample on a paper-type substrate. DBS is an alternative matrix of measuring drug concentrations in the blood which requires the collection of a micro blood volume (< 30 µl) from a finger or heel prick, which has the potential to overcome the barriers associated with conventional methods which require blood collection using venepuncture.

DBS was first introduced in 1960 by Dr Robert Guthrie to screen for phenylalanine in the blood samples of newborns in order to diagnose phenylketonuria (Shah *et al.*, 2013). DBS

sampling is now used by public health laboratories for the screening of more than 95% of all newborns in the USA and 100% of UK newborns for metabolic disorders (Cavanagh and Coppinger, 2009; Deep *et al.*, 2012).

DBS sampling does not require highly skilled staff, and this enables samples to be collected by patients themselves or parents/guardians at home and then sent by standard mail services to a laboratory (Spooner, 2013). This allows for convenient drug monitoring at any time and during a routine check-up. Home sampling does not require patients to travel and thus provides them a potential means of money saving, and eliminates the need for specialised sample collection. Cost analysis by Martial *et al*. (2016) showed that DBS home sampling reduced the associated costs by 61% for renal transplant patients and 43% for haemato-oncology patients in comparison with conventional blood sampling methods (Martial *et al.*, 2016).

The advent of analytical instruments such as liquid chromatography–mass spectrometry (LC-MS), liquid chromatography–high resolution mass spectrometry (LC-HRMS) and immunoassay, has allowed the DBS technique to be used as an alternative to conventional methods. DBS sampling has been used in therapeutic drug monitoring for a wide range of medications as detailed in the Table 2.2.

Table 2.2. Examples of therapeutic drug monitoring by application of DBS.

Medications	**References**
Antiepileptic drugs	(Shah *et al.*, 2013; Shah *et al.*, 2013; Linder *et al.*, 2016; Das *et al.*, 2017; Linder *et al.*, 2018)
Immuno-suppressants	(Koop *et al.*, 2013; Koster *et al.*, 2017; Martial *et al.*, 2017; Veenhof *et al.*, 2017)
Antiretroviral medications	(Castillo-Mancilla *et al.*, 2013; Hoffman *et al.*, 2013; Zheng *et al.*, 2014; Alcaide *et al.*, 2017).
Cardiovascular medications	(Lawson *et al.*, 2012; Lawson *et al.*, 2013; Tanna and Lawson, 2014a; Bernieh *et al.*, 2017a)
Antibiotics	(Al-Ghazawi and AbuRuz, 2010; la Marca *et al.*, 2012; Hawwa *et al.*, 2014; Vu *et al.*, 2014; Barco *et al.*, 2017; Weber *et al.*, 2017)
Antidiabetic medications	(Scherf-Clavel and Högger, 2015)
Antimalarial drugs	(Blessborn *et al.*, 2010; Ippolito *et al.*, 2018)
Pharmacokinetic / toxicokinetic studies	(Kole *et al.*, 2017)
Forensic applications of drugs of abuse	(Chepyala *et al.*, 2017)
Sports for doping analysis	(Verplaetse and Henion, 2016)
Environmental analysis	(Provatas *et al.*, 2017)
Food safety	(Xue *et al.*, 2016)
Endocrinology and metabolism	(Heussner *et al.*, 2017)

2.2.3. Advantages of Blood Microsampling

Conventional methods of blood sampling require a large volume of blood to obtain the required plasma volume. However, from the patients' perspectives, conventional methods are painful, discomforting, frightening, and the blood collection requires a phlebotomist. On the other hand:

- Microsampling requires low blood volumes (< 30 µl) in comparison with conventional methods that require an amount of blood (1-10 ml) to obtain the required plasma (Tanna and Lawson, 2016a; De Nicolò *et al.*, 2016).
- Microsampling can be considered "patient friendly" with high patient acceptability because blood sampling onto DBS is collected via a minimally

invasive approach (Wilhelm *et al.*, 2014; Sharma *et al.*, 2014; Verhaeghe *et al.*, 2017).

- Microsampling offers huge savings in terms of cost of shipment. DBS samples are easily transported and stored, where DBS samples can be shipped by post without special treatment such as a cold box, dry ice or the special equipment at clinical sites required for liquid blood or plasma (Sharma *et al.*, 2014).
- Dried blood spot provides better analyte stability because analytes are held in a dried matrix rather than a liquid matrix (Waterman and Adami, 2005; Manicke *et al.*, 2016).
- Dried microsampling matrix reduced possibility of exposure of biohazards (Zane and Emmons, 2013; Sharma *et al.*, 2014; Nys *et al.*, 2017).
- One of the unique characteristics of microsampling is the possibility of self-sampling at home by patients (Spielberg *et al.*, 2000) at any desired sampling time (Tanna and Lawson, 2014a). Samples can be sent to a laboratory for analysis through the regular post (Spooner, 2013). Thus, there is no need for appointments, clinical visits and the services of a phlebotomist; consequently, this approach should yield significant savings (Martial *et al.*, 2016). The advantages of microsampling, including the small volume of blood required for any given analysis, have attracted research in the area such as the assessment of medication non-adherence, newborn screening (NBS), therapeutic drug monitoring (TDM), pharmacokinetic (PK) and toxicokinetic (TK) studies, paediatric studies, metabolism and pharmaceutical drug development.

2.2.4. Current Microsampling Methods

2.2.4.1. Card Microsampling

The popularity of dried blood spots has increased due to the small blood volume required, low associated costs and good adsorption properties of filter paper, which can be easily disposed of because DBS cards are biodegradable or otherwise easily incinerated (Pelton, 2009).

Commercially available blood sample collection cards can be grouped into two types, namely untreated and chemically treated cards (Wagner *et al.*, 2014**).** Cards are either

cellulose in nature (e.g., Whatman 903, Ahlstrom 226) or non-cellulose-based materials (e.g., Tomtec PDMS 7, polyester cards). The loading capacity and blood spreading on the filter paper are mainly determined by card thickness, pore size and particle retention (Quraishi *et al.*, 2013)**.**

Three filter paper sampling cards (Perkin-Elmer 226, Ahlstrom 226 and Whatman 903) are recommended by the Clinical and Laboratory Standards Institute (CLSI) as the conventional devices for blood sample collection. These (cellulose) cards are registered by the U.S. Food and Drug Administration (FDA) as *in vitro* Class II medical devices and approved by the Newborn Screening Quality Assurance Program (NSQAP) and the Centres for Disease Control and Prevention (CDC) (Wolff, 2017).

In card microsampling, a few drops of blood are collected on filter paper by a finger prick or heel prick, popularly known as DBS cards. Extraction of the target analytes from blood samples collected on DBS cards is achieved by punching a fixed size disk from the spot, which is then analysed (Figure 2.1). The fixed diameter disk punched from the spot is directly proportional to the volumetric measure used in quantitative analysis (Lawson *et al.*, 2013)**.** The size of the punched disk acts as volumetric measure of the spotted sample as long as the blood has been homogenously absorbed (Mei *et al.*, 2011). It is assumed that each punched disk contains the same fixed volume of blood. Haematocrit (HCT) level effects the way in which the blood spreads and, consequently, spot size, thickness and drying time (Timmerman *et al.*, 2011; Tanna and Lawson, 2016a). HCT represents the volume percentage of red blood cell in the blood and it is normally 47% ± 5% for men and 42% ± 5% for women (Walker *et al.*, 1990). HCT values may deviate from the normal range in certain diseases which affect the percentage volume of red blood cells, such as anaemia and polycythaemia, which is a condition in which an excessive number of red blood cells are produced by the bone marrow cells. Blood with high levels of HCT is more viscous and produces smaller spots on DBS cards. Thus, the HCT effect is significant when a punch is used (O'Mara *et al.*, 2011).

The problem associated with HCT can be overcome by analysis of the whole spot or pre-cut disks (Youhnovski *et al.*, 2011; De Kesel *et al.*, 2014; Bernieh, 2017b) or by application of volumetric absorptive microsampling (VAMS), which is a novel device that utilises a fixed volume of blood regardless of the HCT level. Other developments

include the HemaSpot–HF blood collection device, Noviplex cards, the Hemaxis-DB blood collection device, Ahlstrom 167L cards and Tomtec dry media spot slides (Tanna and Lawson, 2016a).

Figure 2.1. A Whatman 903 card containing four spots of dried blood and two spots punched from the marked sections.

2.2.4.2. Volumetric Absorptive Microsampling (VAMS)

Volumetric absorptive microsampling (VAMS) is a novel device for biological fluid collection, in particular whole blood. This device has been designed to overcome inhomogeneity and blood volume inconsistency, and HCT issues (Denniff and Spooner, 2014). It consists of a polymeric absorbent tip located on a moulded plastic handle.

Blood samples are collected by dipping the tip in the blood and waiting until the tip turns completely red, which takes 2-3 seconds. The tip of the sampler must not be completely submerged into the blood sampler, as this may cause overfilling (Tanna *et al.,* 2018). VAMS collects an accurate and precise volume of blood (10 µl or 20 µl) with less than ±5% volume variation directly from the finger, regardless of HCT level (Denniff and Spooner, 2014)**.** The entire sample is used for extraction because a precise volume is taken. VAMS come in two or four separated sampling devices in a clamshell which can be closed after sample collection, and which are then allowed to dry**.** On the clamshell, there is a label with which to register sample details and information. Figure 2.2 shows the VAMS device before application (white tip) and after application of the blood sample and use (red tip).

Figure 2.2. VAMS consists of a polymeric absorbent tip located on a moulded plastic handle. The unused device has a white tip, while the red tip indicates a used device as it contains dried blood (Denniff *et al.*, 2015).

2.2.5. Blood Sample Collection, Spotting and Storage on DBS and VAMS

Capillary blood collection is the conventional approach to microsampling in which blood can be collected from a finger (adults) or heel prick (children) as an alternative to venepuncture. The sampling kit consists of either a DBS card or VAMS, a disposable tractable sterile lancet, gauze, plaster, desiccant, and zipper storage bags for shipping.

To encourage blood flow, it is recommended that the fingertip or heel be gently massaged, after which the finger is pricked with a retractable lancet. The first drop of blood is wiped using sterile gauze and then gentle pressure is applied to deposit a few drops of blood onto the marked spotting area on a sampling card or onto the tip of a VAMS device. This is then labelled appropriately**.** DBS samples are left horizontally in a clean place at room temperature to dry for at least three hours before storage in the zipper bag with a desiccant.

2.2.6. Analytical Challenges to Using Dried Matrix Microsampling

Dried matrix microsampling involves the collection of microvolumes of biosamples such as plasma, urine and milk in a dried form onto a dried substrate, the most commonly used of which is dried blood spots (DBS) (Ayre *et al.*, 2018). The small sample size and the complexity of the matrix and lack of sophisticated detection techniques with sufficiently high sensitivity and selectivity resulted in the limited popularity and application of microsampling**.** With the advent of instrumentation such as LC and MS, however, the application of this approach has increased (Tanna and Lawson, 2011), and has allowed

the quantification of various target analytes in biosamples. Currently, HCT levels have been identified as a particularly significant parameter in terms of their influence in quantitative analysis in card microsampling methods, as detailed in Section 2.3.4.1.

Challenges still exist with the collection of microvolumes of blood on DBS cards due to problems with analyte recovery from the microsamples, interference from the DBS card and blood matrices which sometimes interfere with MS analysis. Sample aging with long-term storage prevents analyte recovery from paper (Wagner *et al.*, 2014). Moreover, issues relating to analyte stability for some compounds such as antiepileptic drugs have led to difficulties in recovering the sample from the card (Linder *et al.*, 2016).

2.2.7. Extraction of Target Analytes from Dried Blood Matrix

The extraction of DBS can be performed online using flow-through extractions or offline by punching DBS spots and placing them in microcentrifuge tubes or well plates before using extraction solvents (Heinig *et al.*, 2011).

Biosamples are complex in nature, and the target analyte is normally present at a lower concentration than other constituents (Gjelstad and Pedersen-Bjergaard, 2014). Therefore, a clean-up procedure by using of organic solvents such methanol or acetonitrile is required to eliminate unwanted materials from the blood and/or card matrix before concentration and analysis (Bylda *et al.*, 2014; Tanna and Lawson, 2016a). Moreover, the small volume of sample used requires a robust extraction protocol to ensure that the analyte is appropriately recovered and detected.

Dried blood spots are solid samples that must be extracted with an appropriate solvent to allow compatible analytical techniques to be used, such as LC-MS**.** The main extraction technique used for analyte extraction from dried blood matrix (DBS and VAMS) is solid-liquid extraction (SLE) (Cape *et al.*, 2017).

In solid-liquid extraction, the solid phase is the DBS punched disk or VAMS tip, whilst the liquid phase is the extraction solvent. The efficiency of the SLE depends on three factors: the target analyte solubility, the matrix effect of the card, and the matrix effect of the biosample (Alkhateeb, 2015).

Analytes which are tightly bound to proteins and retained on the card, such as amlodipine, are extracted by adding 0.5 M sodium hydroxide (NaOH) to the extraction solvent to hydrolyse the drug-protein bond to increase the efficiency of the extraction and recovery of the target analyte. Following extraction, the resulting supernatant is dried with nitrogen gas and reconstituted prior to analysis (Bernieh, 2017b).

2.2.8. Analytical Techniques Used in Conjunction with Dried Matrix Microsampling

Since the introduction of microsampling, several analytical techniques have been utilised for target analyte qualification and identification. For instance, immunoassay methods, polymerase chain reaction (PCR)-amplified DNA analysis, high-performance liquid chromatography (HPLC), and techniques such as gas chromatography mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and liquid chromatography-high resolution mass spectrometry (LC-HRMS) (Tanna and Lawson, 2011).

2.2.8.1. Immunoassay

Immunoassay has commonly been used for drug quantification since the 1980s in parallel with chromatography methods such as liquid chromatography before the implementation of MS detectors in bioanalysis (Cape *et al.*, 2017)**.** Immunoassay depends on the selectivity of antibodies in combining with the target medications or metabolites (antigens) and in providing signalling or labelling capabilities. The complex which results from the antigen-antibody bonding constitutes the selectivity step, and it is necessary for the complex to produce a measurable signal that can be directly correlated with level of the complex formation (Cox *et al.*, 2014). The availability of this method is restricted to kits which may be expensive and specific to a certain target (Tanna and Lawson, 2016a).

The assays require the handling radioactive materials, prolonged incubation times, and choosing the specific antibody for the analyte of interest (Shipkova *et al.*, 2017). However, there is possibility of false positives due to cross-reactivity with endogenous components that are similar in structure and/or reactivity to the drugs or metabolites themselves (Sturgeon and Viljoen, 2011; Cape et al., 2017; Harper et al., 2017).

This technique has been used to quantify biomarkers in DBS samples such as Apolipoprotein B (ApoB) and C-reactive proteins. ApoB is a biomarker of CVD risk (Eick *et al.*, 2017), which is the main protein found in low-density lipoproteins (LDL). LDL is amongst the most important causal agents of atherosclerotic cardiovascular disease (Shapiro and Fazio, 2017). C-reactive protein is indicative of the inflammatory processes related to the pathophysiology of CVD, where slight increases in CRP (> 1–2 mg/L) indicate a risk of developing cardiovascular problems (McDade *et al.*, 2004).

2.2.8.2. Liquid Chromatography–Mass Spectrometry (LC-MS)

Over the past twenty years, the key tool for qualitative and quantitative bioanalysis has been LC-MS (Xie *et al.*, 2012). LC-MS provides effective, rapid and specific quantitative and qualitative data for the determination of biomarkers in plasma to help in TDM, TK and PK studies, metabolism, drug discovery and neonatal screening. LC-MS, operating in single ion monitoring mode (SIM) and using a single mass analyser (a quadrupole mass filter), is shown in Figure 2.3.

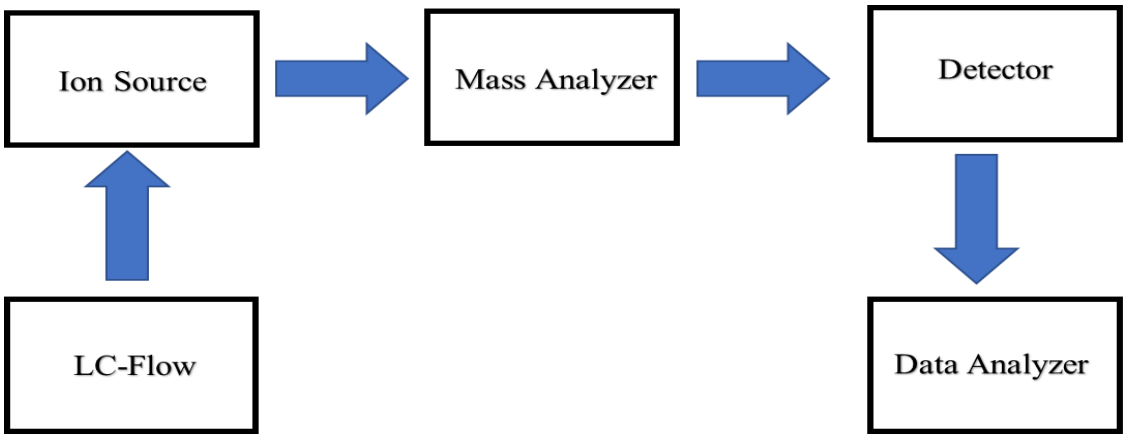

Figure 2.3. Schematic structure of a liquid chromatography coupled to a mass spectrometer.

The quadrupole mass filter has a relatively low resolution and is only able to measure the *m/z* ratio of an ion to the nearest integer value, and therefore cannot necessarily provide or distinguish the elemental composition of an ion (Breidinger and Woolf, 2017). This represents a challenge in specificity since there may be several compounds with the same,

or nearly the same mass-to-charge ratio (*m/z*) as the analyte of interest (Lawson *et al.*, 2012).

LC-MS also uses a soft ionisation mechanism that primarily yields molecular ions, that is, with very little or no fragmentation of the molecule. Therefore, it is unlikely that the molecular mass alone will make structural assignment possible. Hence, where there is a lack of any data on fragmentation patterns, dependence on retention time will not be sufficient to offer the required selectivity. However, the introduction of tandem mass spectrometry instruments has solved this problem (Tanna and Lawson, 2011).

2.2.8.3. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

LC coupled with MS/MS has various advantages that include ease of use, specificity, low detection limits and high throughput (Li and Lee, 2014; Zakaria *et al.*, 2016). LC-MS/MS has gained a considerable reputation in routine laboratories and its application has been extended to therapeutic drug monitoring (TDM), newborn screening (NBS), and toxicology and drug discovery.

The increase in the application of LC-MS/MS in DBS analysis is due to developments in analytical instrumentation which provide unique specificity, fast method development, and simultaneous analysis of various drugs and their metabolites in microsamples within a short timeframe (Zakaria *et al.*, 2016). For example, LC-MS/MS has been used for quantification of antihypertensive medications (Chernonosov, 2018), oncolytic agent (Wickremsinhe *et al.*, 2018), illegal drugs such as cocaine, benzoylecgonine, ecgonine methyl ester, norcocaine, *meta*-hydroxy-benzoylecgonine, cocaethylene (Ambach and Stove, 2019). LC-MS/MS also used for analysis of vitamin D (Heath *et al.*, 2014).

The basic design of an MS/MS includes two mass analysers (MS1) and (MS2) with a collision cell between them (Figure 2.4). The ion preselected by MS1 is allowed to enter the collision cell where dissociation occurs, the product ions from which are monitored by MS2. The target ion (precursor ion) of a specific mass-to-charge ratio (*m/z*) is selected by MS1 and the characteristic fragmentation pattern for that specific compound is determined by MS2. This combination of data provides a unique fingerprint through which to identify the MS1 precursor ion. Identification is achieved by tuning MS1 to a specific mass-to-charge ratio (*m/z*) and monitoring the characteristic *m/z* value via MS2.

Analysis by LC-MS/MS requires optimisation of MS/MS parameters such as choosing the appropriate precursor and product ions and collision energy (CE) optimisation, which are different for each compound of interest (Zhang *et al.*, 2009). In LC-MS/MS, only the preselected ions that are derived from the sample by MS1 enter the collision cell. Any data related to other ions in the sample will be thus be lost, hence there is no possibility of rechecking the data collected to look for information on other ions if so required. Application of LC-HRMS instruments (Bowen *et al.*, 2016) resolve most of these challenges because in HRMS all ions are recorded with the further possibility of revisiting data when required, as further detailed in Section 2.3.8.4.

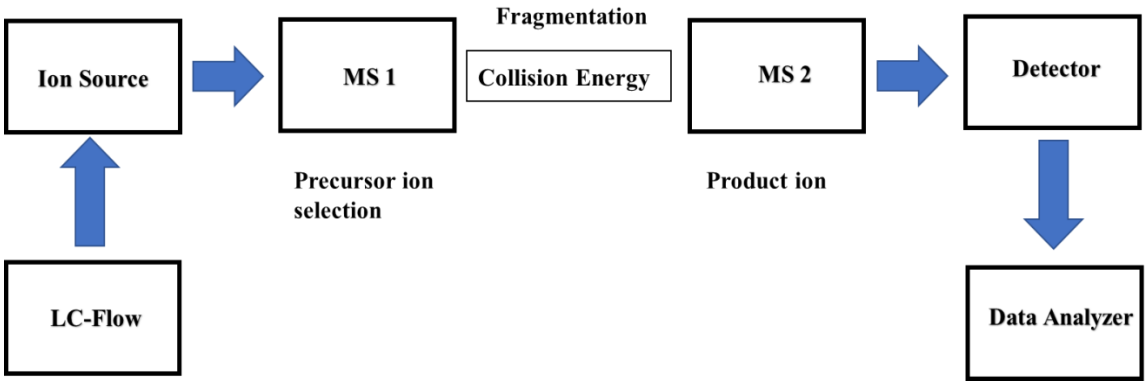

Figure 2.4. Schematic structure of liquid chromatography coupled to tandem mass spectrometry

2.2.8.4. Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS)

Although the standard technique for the quantitative bioanalytical assays is LC-MS/MS, there has been increased interest over the years within the bioanalytical society in other MS approaches with regards to solving certain bioanalytical challenges (Zhang *et al.*, 2009; Kaufmann *et al.*, 2011). One such alternative to traditional LC-MS/MS has been the use of LC coupled with high-resolution MS, that is, LC-HRMS. Thus, HRMS simply refers to a mass analyser with high resolving power. Resolving power refers to the ability to discriminate species with near-identical *m/z* values.

For years, HRMS was mainly used in a qualitative manner in drug metabolism and metabolite identification studies; the reason for this was due to the poor sensitivity of older HRMS instruments (Ramanathan and Korfmacher, 2012). However, the suitability of HRMS for use in quantitative analysis has recently been improved through advancements in instrumentation; for instance, accurate mass determination, where the mass of the molecular ion can be measured to better than 1.0 ppm of the relative molecular mass (RMM). Hence, this precise value can be used to determine the atomic composition based on, for example, C = 12.0000, H = 1.0078, N = 14.0031, O = 15.9949 and therefore the likely molecular structure. For example, considering three compounds of mass 266.3, where atenolol, a beta-blocker, ($C_{14}H_{22}N_2O_3$) = 266.3361, dienestrol ($C_{18}H_{18}O_2$) = 266.3340 and leptospermone ($C_{15}H_{22}O_4$) = 266.3330, nominal mass measurements would be unable to differentiate these species; however, the ability of HRMS to measure to the fourth decimal place would allow their differentiation.

The capability of full-scan acquisition has given HRMS the competitive edge. All mass spectral information from a sample is gathered, which offers the possibility to reinterrogate the data if such an approach were thought to be clinically important (Bernieh, 2017b). Additionally, as data is collected concurrently over a certain mass range, it is also likely that interfering ions produced by a sample matrix which interfere with the detection and quantification of the target compound will also be directly monitored and choosing of narrow extraction window will reduce interference and improve quantitative analysis (Meyer and Schilling, 2017). It has been argued that a paradigm would make HRMS in MS detectors the method of first choice (Rochat *et al.*, 2012).

There is now an increasing use of HRMS in the quantitative analysis in early drug discovery (Korfmacher and Ramanathan, 2016), therapeutic drug monitoring (Oliveira *et al.*, 2014), adherence to cardiovascular medications (Lawson *et al.*, 2012; Lawson *et al.*, 2013; Tanna and Lawson, 2014a; Bernieh *et al.*, 2017a), quantification of insulin and insulin analogues (Thomas and Thevis, 2018) and environmental studies (Krauss *et al.*, 2010). Figure 2.5 shows a diagram of the LC-QTOF-HRMS used for the analysis of dried blood spots in this study

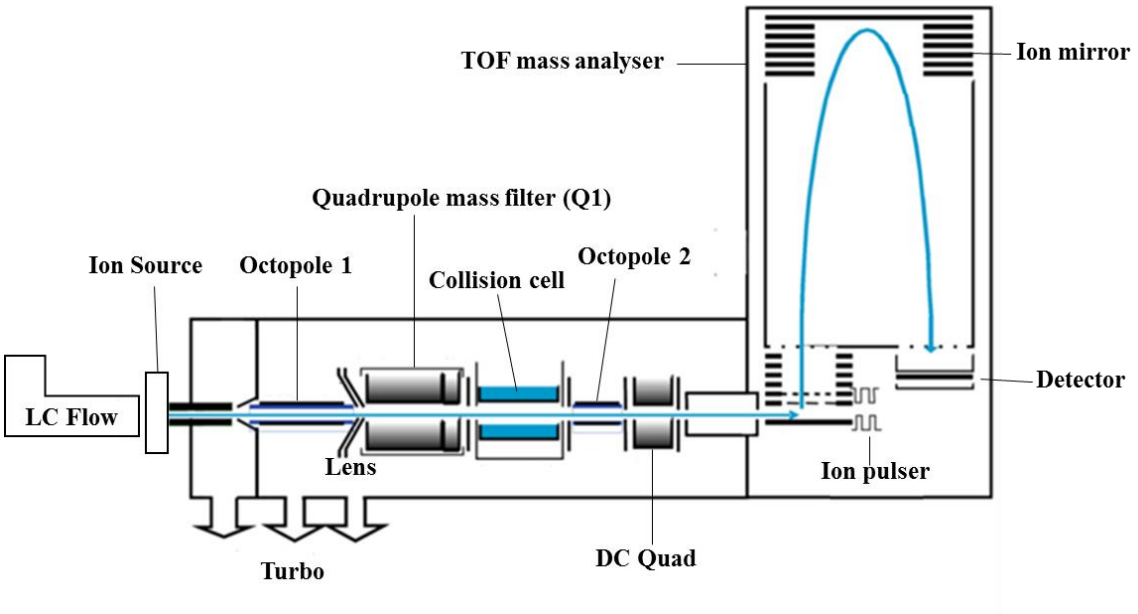

Figure 2.5. A schematic diagram of an LC-QTOF-HRMS (Agilent Technologies, 2014)

There are a number of HRMS platforms available to bioanalysts including the Q-TOF, TOF, Fourier transform ion cyclotron resonance and Orbitrap-based mass analysers (Sturm *et al.*, 2016). HRMS can provide rich information in a single run and is now applied to resolve the majority of bioanalytical challenges across various fields (Zhang *et al.*, 2009). For instance, it can provide multiple drug and metabolite profiling in a single run (Ma and Chowdhury, 2013; Ramakrishnan *et al.*, 2016). The data acquired can be used to improve patient care. The ability of the instrument to perform simultaneous analyses of multiple species decreases the sample volume required in comparison to other techniques (Shipkova and Svinarov, 2016).

High selectivity can eliminate interference in samples due to matrix effects, leading to a considerable increase in signal-to-noise ratio. LC-MS/MS assays may struggle with interference from matrix effects at lower detection limits in comparison with HRMS, and may require the use of complex sample preparation and chromatography to isolate the species of interest (Li and Tse, 2010). This high selectivity is derived from the fact that HRMS can separate mass fragments at *m/z* ratios that are accurate to the fourth decimal place.

There are still limitations that have prevented the wider acceptance of this technique across the bioanalytical community, whereas there is considerable enthusiasm for the use of HRMS to develop bioanalytical assays. However, Rago and Negahban argue that additional guidance is required from regulators regarding the acceptance of bioanalytical assays developed with HRMS (Rago and Negahban, 2016).

Another challenge with the extension of HRMS to clinical studies is the informed consent process, since full-scan HRMS analysis preserves all the data derived from the sample. Therefore, without ensuring proper informed consent on the part of the patient or volunteer, the advantages of a full HRMS data scan cannot be realised; furthermore, the data generated from full-scan acquisitions requires a much higher storage capacity. The advantages and disadvantages of HRMS are summarized in Table 2.3.

Table 2.3. Advantages and disadvantages of HRMS. (Hird et al., 2014; Colby et al., 2017; Rochat, 2018)

Advantages of HRMS	Disadvantages of HRMS
1. There is no need for selective molecular fragmentation for detection in HRMS except when using a Q-TOF instrument in MS/MS mode. 2. HRMS platforms can record various acquisitions at high resolution with accurate mass determination to better than 1 ppm of the RMM, which enables discrimination of interferences with highly similar *m/z* values. 3. HRMS is capable of full-scan acquisitions. 4. In HRMS, full-scan acquisition allows a better overview of the analysed extract, because coeluting compounds, contaminants, adducts and charge state can be monitored, which are useful during method development and troubleshooting.	1. Slow progress in the implementation of HRMS technology may be due to difficulties in changing the entire fleet of triple quadrupole MSs in clinical laboratories as the triple quadrupole can be replaced by an engineer. 2. Cost of HRMS instruments is often twice or more that of the triple quadrupole MS. 3. HRMS maintenance is more complex than standard triple quadrupole MS. 4. Lack of official guidelines for HRMS data analysis and acceptability criteria by regulatory authorities. 5. Problems associated with system robustness. HRMS requires calibration before running samples to maintain the high mass accuracy and resolution. 6. Running HRMS in full-scan mode for large samples requires a very large data storage capacity.

2.3. Gaps in the Literature

There is no gold standard method by which to assess medication non-adherence. However, the application of multi-approach measures through a combination of feasible self-reporting approaches and reasonable objective approaches is recommended by the

WHO (Sabaté, 2003) in order to gain results that are likely to be close to reality. The selection of two (or more) methods that will allow the strengths of one method to compensate for the weaknesses in the other will provide higher reliability, and disclose further reasons for non-adherence, are now recommended (Rapoff, 2010, Lam and Fresco, 2015, Forbes *et al.*, 2018). However, the complexity of analysis and interpretation of results should be considered when multi-measure approaches are applied (Modi *et al.*, 2006).

Multiple methods with similar sources of errors, such as using two indirect (subjective) methods, will not be helpful to the accurate prediction of the level of adherence (Llabre *et al.*, 2006). Also, cost and practicality should be considered (Lam and Fresco, 2015). Liu *et al.* (2001) showed that the application of multi-measures provides for the accurate predictive power of adherence measurement in comparison with using individual methods, confirms original findings, and minimises discrepancies (Liu *et al.*, 2001; Van Onzenoort *et al.*, 2010).

Studies documenting levels of non-adherence to medication – particularly to CVD medications – in Iraq are limited, and those that exist used only indirect methods, with no comparisons to true non-adherence as measured by direct methods; no previous study in Iraq has assessed non-adherence to CVD medication using a direct method, either by conventional means or by application of DBS analysis.

There is no previous study that assesses and compares non-adherence to the target cardiovascular medication using a combination of indirect methods by application of MMAS-8 and the application of less invasive direct methods via dried blood microsamples analysis onto 903 cards and VAMS via liquid chromatography high-resolution mass spectrometry (LC-HRMS).

The approach applied in this study gives information about medication-taking behaviour for each medication in the patient's regimen using the direct approach, and about the reasons underlying such behaviour using the indirect approach. This approach will enable adherence to be tracked for each individual medication, which is helpful for medication dose optimisation and understanding the causes of non-adherence. The outcomes will

help healthcare providers and stakeholders to implement appropriate strategies to improve adherence.

2.4. Aims and Objectives

The aim of this research is to assess non-adherence to selected cardiovascular medications prescribed to Iraqi patient volunteers by application of two different approaches, namely a practice-based approach (indirect method) and a laboratory-based approach (direct method). The indirect approach involves the application of a standardised and validated Arabic version of MMAS-8, while the laboratory-based approach (direct method) involves the collection of microvolumes of blood on a special substrate (Whatman 903 cards and a VAMS device).

Analysis of the collected whole blood samples via a validated liquid chromatography high-resolution mass spectrometry (LC-HRMS) method will be used to answer the following questions:

1. What is the level of non-adherence to cardiovascular medications among Iraqi cardiovascular patients as determined by application of an indirect method, namely the MMAS-8 questionnaire?

2. What is the level of non-adherence to cardiovascular medications among Iraqi cardiovascular patients as determined by application of a direct method of determination of the target drugs' concentrations in dried blood spots on 903 cards and using VAMS?

3. What is the agreement between the indirect and direct methods of assessment of non-adherence for the same volunteers?

4. Is there concordance between cardiovascular drug concentrations measured via DBS cards and in via the VAMS device?

5. What are the factors associated with non-adherence among Iraqi cardiovascular patients and what are the possible, successful and effective interventions which may tackle and improve adherence to cardiovascular medications in the target population?

6. How can this research be adopted in the clinical practice in Iraq?

2.5. Conclusion

Optimum clinical outcomes can be achieved by adherence to cardiovascular medication which will consequently reduce the morbidity, mortality and increased health expenditure due to waste of medications and increased demand on the health service. Different methods can be used to assess non-adherence to medications. Each method has its own strengths and weakness and there is no real agreement on the method of choice. The use of a questionnaire is popular in the clinical setting because of its low cost and ease of application. The eight-item Morisky Medication Adherence Scale (MMAS-8) is a standardised questionnaire which is widely used to assess non-adherence and some of the associated causes. Direct methods can assess non-adherence to each medication taken by the patient, but of course would be unable to determine the reasons associated with non-adherence. Direct methods are well documented in the literature and require large volumes of blood, as collected by venepuncture. Whole blood microsampling approach requires few drops of blood collected by finger prick on a substrate such as 903 cards or VAMS in dried form is a successful alternative to conventional methods. These collected samples are easily transported by post with minimum requirements and low biohazard risk in comparison with conventional methods.

A mixed approach between the direct method by analysis of blood microsamples using LC-HRMS and the indirect method through the use of MMAS-8 and integration of the outcomes from two approaches (MMAS-8 and LC-HRMS analysis) with face-to-face interview to assess non-adherence to CVD medications is considered a novel research through which to fill this gap in the literature. The rational to using this mixed approach is to provide more information about medication non-adherence based on individual medication and the associated reasons. The overall outcome of this research will help to improve the health care system in Iraq in terms of the patient's health and quality of life, reducing the number of hospital admissions and eventually mortality rate

Application of liquid chromatography coupled to high resolution mass spectrometry can achieve a full scan with high specificity using a precise mass to charge ratio for the target medication. The full scan method is useful for retrospective studies or can be used by the clinician if further information is required.

Chapter 3

Determination of Cardiovascular Medication Levels in Microsamples and Assessment of Non-adherence

This chapter details the application of a previously validated liquid chromatography-high resolution mass spectrometry (LC-HRMS) assay for the simultaneous determination of nine commonly prescribed CVD medications (amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan) in finger prick microvolume blood samples collected from 303 Iraqi volunteers who had been prescribed one or more of these CVD medications. This chapter also investigates the correlation between the analyte concentration determined on 903 cards and VAMS for extraction of the target medications for the same volunteers.

3.1. Introduction

Measurements of CVD medication concentrations in plasma and serum using liquid chromatography mass spectrometry (LC-MS) or liquid chromatography-tandem mass spectrometry tandem (LC-MS/MS) is well documented in the literature (Gonzalez *et al.*, 2010; Gonzalez *et al.*, 2011; Dias *et al.*, 2013; González *et al.*, 2015). However, as detailed in Chapter 2 Section 2.3.2.2, a relatively large volume of blood is needed when compared to microsampling. Microsampling involves the collection of only a very small volume (<30 µl) of blood to determine the concentration of the target analyte or endogenous substance (Zane and Emmons, 2013). Dried blood spot (DBS) is a type of microsample and is an alternative approach to conventional liquid blood sampling for drug quantification (Tanna and Lawson, 2014b).

Studies have demonstrated a link between poor clinical outcomes in patients with cardiovascular diseases and medication non-adherence (Zullig *et al.*, 2017). The prescribed medication should produce therapeutic drug levels in the blood. Medication adherence ensures that the prescribed drug concentration is within the therapeutic window so as to obtain the maximum benefits from medication and improve patient clinical outcomes (Tanna and Lawson, 2016a). Researchers and healthcare providers are therefore interested in medication adherence assessment to optimise treatment of patients and maximise drug efficacy.

The value of maximum drug concentration (C_{max},), the time required for a drug to reach the maximum plasma concentration (t_{max}) and time required for a drug concentration to decrease to one-half of the initial concentration ($t_{1/2}$) each play a key role in the

concentrations of medications in the blood (Flanagan *et al.*, 2008). Figure 3.1(a) simulates the pharmacokinetic profile of a single oral drug administration. Following the administration of the drug, the drug concentration in plasma increases as the drug is absorbed into the blood until it reaches a maximum concentration (C_{max}) after time (t_{max}), after which it starts to decline due to metabolism or excretion. The rate of concentration decrease is calculated according to its half-life ($t_{1/2}$) in the body, as follows (Shinya, 2011):

- The peak level decreases to 50% in the first $t_{1/2}$
- Further decrease to 25% by 2($t_{1/2}$)
- Further decrease to 12.5% by 3($t_{1/2}$)
- Further decrease to 6.25% by 4($t_{1/2}$)
- Further decrease to 3.125% by 5($t_{1/2}$)

In the instance of regular medication intake, the drug concentration fluctuates about the steady-state concentration as shown in Figure 3.1(b). The pharmacological action is proportional to the blood concentration of medication within a given range of concentrations, above which toxicity may occur and below which the drug is only present in sub-therapeutic concentrations. Patients who take medication as prescribed have blood concentrations that remain within the effective therapeutic window and thus the patient will gain the full benefits of the medications, leading to a better clinical response (Tanna and Lawson, 2016a; Keenan, 2017). On the other hand, if a patient misses a drug dose, the drug concentration may drop to sub-therapeutic concentrations, as indicated in Figure 3.1(a). In this case, the patient may experience serious consequences, leading to poor clinical outcomes (Tanna and Lawson, 2016a; Otto, 2017). The WHO stated that medications have no pharmacological effect after about 4.5 half-lives, which is equivalent to < 5% of the C_{max} (Moffat *et al.*, 2011), as the amount of drug present has reduced by almost 94% to 97% of the peak level (Shinya, 2011). In the present study, CVD patients will be classified as non-adherent by microsample analysis when one or more of their prescribed medication concentrations is < 5% of C_{max} or > $C_{max.}$ The PK parameters and dose information for these target CVD medications are derived from plasma samples for the selected target drugs (Table 3.1).

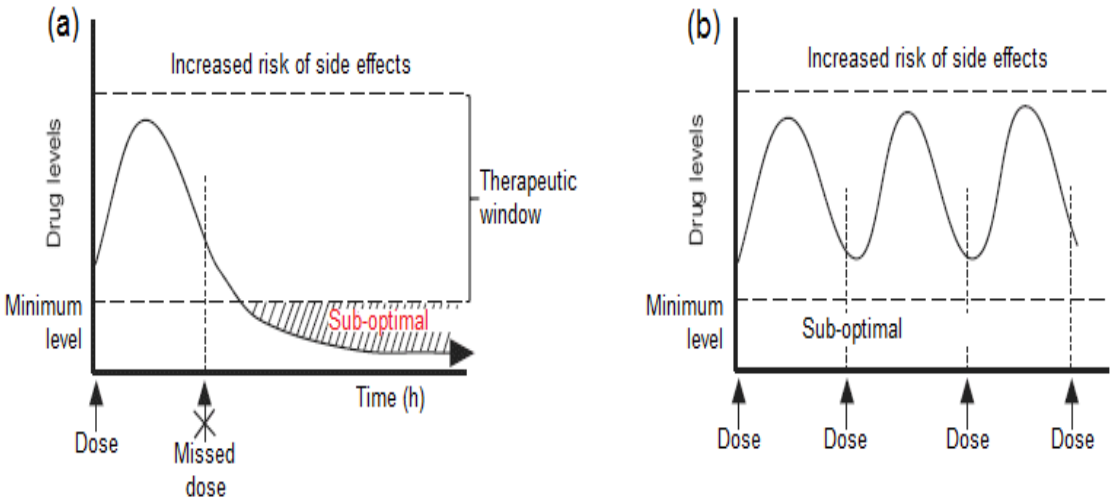

Figure 3.1. Pharmacokinetic profile of a drug concentration versus time profile in the instances of (a) non-adherence and (b) adherence. (Bernieh, 2017b).

Table 3.1. Pharmacokinetic information for the selected cardiovascular medications.

Medication	**C_{max} ng/ml**	**t_{max} hr.**	**$t_{1/2}$ hr.**	**References**
Amlodipine 5 mg	5-7.5	5-8	35-50	(Meredith and Elliott, 1992; Vincent *et al.*, 2000; Shah *et al.*, 2012)
Atenolol 50 mg	159-377	1.5-6	2.5-11	(Lewis *et al.*, 1988; de Abreu *et al.*, 2003; Chang and Shin, 2012)
Atenolol 100 mg	240-1370	2-4	5-9.1	(WU *et al.*, 2003; Najib *et al.*, 2005; Lawson *et al.*, 2012)
Atorvastatin 40 mg	5.53-28.57	0.38-1.37	7.18-17.15	(Koytchev *et al.*, 2004; Mendoza *et al.*, 2006)
Bisoprolol 5 mg	20.71-26.9	1.2-3	7.1-10.82	(Ding *et al.*, 2007; Tjandrawinata *et al.*, 2012)
Bisoprolol 10 mg	37-87	1.5-4	5-16	(Lewis *et al.*, 1988)
Diltiazem 60 mg	74.72-82.38	2.23-2.49	3.18-14.8	(Loffreda *et al.*, 1999; Yan *et al.*,2013)
Diltiazem 90 mg	105.65-150.87	10.05-12.25		
Lisinopril 10 mg	41.75-80.47	5.79-6.91	8.13-13.4	(Shin *et al.*, 2008; Zhou *et al.*,2008)
Lisinopril 20 mg	86.4-139	5.6-6.6		
Losartan 25 mg	43.6-125.4	0.5-1.1	0.94-4.02	(Ohtawa *et al.*, 1993; Zhou *et al.*, 2008; Salvadori *et al.*,2009)
Losartan 50 mg	89.1-306.1	0.5-2.2		
Losartan 100 mg	263.67-783.41	0.54-1.88		
Simvastatin 20 mg	4.88-5.86	1.98-2.52	1.3-3.06	(Selvan and Pal, 2009; Lilja *et al.*, 2004)
Simvastatin 40 mg	5-40	2-3		
Valsartan 80 mg	1010-2270	2	4.1-8.63	(Flesch *et al.*, 1997; Bindschedler *et al.*, 1997)
Valsartan 160 mg	1930-4000	1.5-3		

3.2. Materials and Methods

3.2.1. Ethical Statement

Ethical approval for the collection of blood microsamples from Iraqi volunteers with cardiovascular diseases was obtained from the Ethical Committee in Misan Health Directorate (Reference 244 in 11/4/2016) (Appendix 1). Ethical approval for the collection of blood samples had already been obtained from De Montfort University's Faculty of Health and Life Science Research Ethics Committee and updated to include the name of the PhD student Ahmed Alalaqi (Reference 1212 in 01/10/2013) (Appendix 2). All participants were required to read a study participant information leaflet and provide written consent before being able to participate in this research.

3.2.2. Recruitment, Inclusion and Exclusion Criteria

The sample size was calculated based on the Daniel equation (Daniel, 1999; Pourhoseingholi *et al.*, 2013).

$$n = \frac{Z^2 P (1 - P)}{d^2}$$

Where n is the sample size

Z = Z statistic, corresponding to level of confidence, taken as 1.96 for a 95% confidence interval.

P = expected prevalence or proportion

d = precision (as a proportion of one; if 5%, d = 0.05).

Since there is no literature available showing the prevalence of cardiovascular disease in Iraq, sample size calculation in this study was determined based on the assumption that the prevalence of cardiovascular diseases was 27%, which is close to the prevalence of hypertension at 26.5% (Al-Ghuzi and Al-Asadi, 2014). Accordingly, 303 patients were recruited in this study to assess non-adherence to selected cardiovascular medications. Cardiovascular patients were recruited from Alsader Teaching Hospital and Misan

Cardiac Centre in Iraq during a routine clinical visit between July 2016 and March 2018 according to the following inclusion criteria

(i) Had been prescribed at least one medication to treat cardiovascular disease including amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan.

(ii) Had been taking one or more cardiovascular medications for more than six months.

(iii) Were aged 18 years and above.

(iv) Were able to understand and communicate in Arabic.

(v) Had completed a written consent form.

Potential volunteers were provided with Arabic translations of:

- Participant information leaflets
- Consent form
- CVD drug prescription 'mini' questionnaire.

These are detailed in Appendices 3-5 overleaf. The translation of documents into Arabic was crucial to helping participants understand the aims of the study (Appendices 6-8). The CVD drug prescription 'mini-questionnaire', asked for information concerning the prescribed drug, the dose and time at which the last dose had been taken. Information regarding all medication taken by the patients is included in Appendix 9.

3.2.3. Sample Collection Kits

Prior to visits to the Alsader Teaching Hospital and Misan Cardiac Centre in Iraq, sample collection kits were prepared based on either the dried blood spot (DBS) or the volumetric adsorptive microsampling (VAMS) methodologies.

Kit 1 prepared for DBS sample collection comprised:

- DBS 903 Sample collection card } (Sigma–Aldrich, UK)
- 2 mm lancet } (Owen Mumford, UK)
- Gauze pad } (Shemond, UK)
- Plaster } (Reliance Medical, UK)
- Plastic resealable bag } (Fischer Scientific Loughborough, UK)
- Desiccant } (CelloExpress, UK)
- Alcohol Pad } (BSN Medical GmbH, Germany)

Kit 2 prepared for VAMS sample collection comprised:

- 1 clamshell pack (x4 Mitra ^{TM}) devices } (Neoteryx, Torrance, CA, USA)
- Plastic resealable bag } (Fischer Scientific, Loughborough, UK)
- Dessicant } (CelloExpress, UK)

It was anticipated that, where possible, both sampling methods would be used to test the same patient and therefore duplicate plasters, gauzes and lancets would not be required in the second kit.

3.2.4. Reference CVD Drug Samples

The most prescribed CVD medications in Iraq were amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan. Reference samples of all of these drugs (> 98% purity), amlodipine, atenolol (R-(+), (99%)), atenolol-d7, atorvastatin calcium salt, bisoprolol hemifumarate salt, diltiazem hydrochloride, lisinopril, losartan potassium salt, simvastatin and valsartan were obtained from Sigma Aldrich, Poole, Dorset, UK.

3.2.5. Solvents and Other Equipment for Sample Extraction and Analysis

For work of this nature, solvents of the highest purity are required. The necessary liquid chromatography mass spectrometry (LC-MS) grade solvents were:

- Methanol (Fisher Scientific Loughborough, UK)
- Water (Fisher Scientific Loughborough, UK)
- Acetonitrile (Fisher Scientific Loughborough, UK)
- Formic acid (Sigma Aldrich, Poole, Dorset, UK)

Microcentrifuge tubes (1.5 ml), volumetric pipettes, and pipette tips were purchased from Fisher Scientific, Loughborough, UK. Autosampler vials with 250 µl inserts and vial caps were obtained from Agilent Technologies (Cheshire, UK).

Drug calibration standards were prepared in freshly donated whole blood using lithium heparin-coated blood collection tubes. These tubes were obtained from International Scientific Supplies Ltd. (Bradford, UK).

3.2.6. Sample Collection Methods

Volunteers were recruited as part of their routine visits to the Alsader Teaching Hospital and Misan Cardiac Centre in Iraq. Blood samples were collected from volunteers taking one or more of the target CVD medications. A series of blank control samples were also obtained from healthy Iraqi volunteers who were not taking any of the target drugs. These control samples were collected using the same protocols as the trial samples. The protocol developed for the collection of 903 card and VAMS samples was as reported in Appendix 10.

3.2.7. Sample Transport and Storage

After collection, the DBS cards were left on the storage racks for at least 2 hours to dry. Once dry, they were sealed into labelled bags containing a desiccant pouch. VAMS samplers do not require a drying period and can be shipped once the clamshell package is closed and sealed in a bag containing a desiccant. The DBS card samples and the VAMS devices were transported to De Montfort University, Faculty of Health and Life Sciences under ambient conditions and delivered to the secure laboratory (00.15 Hawthorn Building).

3.2.8. Sample Extraction, Analysis and Quantification

Initial work by Bernieh (2017) provided information on both blood microsamples calibration/QC sample preparation, extraction and analysis (Bernieh, 2017b). Prior to the analysis of the volunteer samples, both the extraction and the analytical methodology were assessed under the same conditions as the previous validated method. This required the preparation of stock solutions of all the drugs, both individually and with one

containing all the drugs at known concentrations. These were used to test the separation capabilities of the LC-HRMS instrumentation and the extraction capabilities using dosed calibration/QC whole blood to provide known samples on both the 903 DBS card and the VAMS samplers.

3.2.8.1 Preparation of Stock Solutions to Test the Separation Capability of the LC-HRMS System

Standard stock solutions of amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan with concentrations of 1 mg/ml in methanol were prepared for each target medication after which an intermediate solution of 10,000 ng/ml was prepared from the standard stock for each medication. 100 ng/ml of a multicomponent working solution was prepared from the intermediate stock solutions using methanol: water (70:30, v/v) as a diluent, which was used for LC-HRMS analysis.

3.2.8.2 Preparation of Spiked Blood Calibration Samples for DBS

Standard stock and intermediate solutions of amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan were prepared for each target medication as prescribed in Section 3.2.8.1. Multicomponent working solutions for the nine CVD medications were prepared from individual stock solutions to cover the calibration range for each medication as detailed in Table 3.6. The standard operation procedure (SOP) for the preparation of blood calibration standards for the nine target medications is detailed in Appendix 11. The spiked blood standards were prepared by spiking 100 µl of one of each multicomponent working solution with 900 µl of blank blood and vortexed for 1 min to produce the final concentration. Volumetric pipettes were used to apply 30 µl of multicomponent blood standards onto the 903 sampling paper. Blank DBS standards were prepared by spiking 100 µl from a 70:30 MeOH: H_2O, v/v with 900 µl of blood that was then mixed thoroughly by vortexing for 1 min. Volumetric pipettes were used to apply 30 µl of blank bloods standards onto the 903-sampling paper. Spot sizes were ~9.5 mm after applying 30 µl of calibration standards and blanks on the sampling paper. Sampling cards were left to dry at room temperature for at least 3 hours and then stored individually in labelled plastic resealable bags containing desiccant.

3.2.8.3. Preparation of Quality Control Standards (QC) for DBS

Three concentrations were chosen independently at low, medium and high concentration levels for each target medication, as detailed in Table 3.6, as quality control standards. The spiked blood QC standards were prepared by spiking 100 µl of one of each multicomponent working solution with 900 µl of blood and vortexing for 1 min to produce the final concentration. Volumetric pipettes were used to apply 30 µl of QC standards directly onto the 903 sampling cards. Sampling cards were left to dry at room temperature for at least 3 hours prior to processing.

3.2.8.4. Preparation of Spiked Blood Samples for VAMS

The spiked blood standards for VAMS were prepared using the 10 µl tip size devices by dipping the upper part of VAMS device into a volume of spiked whole blood and blank and waiting for about 2 seconds till the tip had turned completely red. Care needed to be taken to avoid completely dipping the tip into the blood to avoid overfilling (Tanna *et al.*, 2018).

3.2.8.5. Preparation of Quality Control Standards (QC) for VAMS

QC samples at the low, medium and high concentration levels of the target analytes, as detailed in Table 3.2, were independently prepared by spiking 100 µl of one of each multicomponent working solution with 900 µl of blood and vortexing for 1 min to produce the final concentration. The spiked blood QC standards on VAMS were prepared by dipping the upper part of the VAMS into a volume of spiked whole blood and waiting for about two seconds until the tip turned completely red.

Table 3.2. Calibration range of the target medications in whole blood.

Drug	Calibration range ng/ml	Calibration standards (ng/ml)							
			LOW				MED		HIGH
Amlodipine	0.5-100		0.5	1	5	10	25	50	100
Atenolol	10-1500	10	20	50	100	200	500	1000	1500
Atorvastatin	0.5-100		0.5	1	5	10	25	50	100
Bisoprolol	0.1-100	0.1	0.5	1	5	10	25	50	100
Diltiazem	0.5-600	0.5	1	5	10	50	100	300	600
Lisinopril	0.1-100	0.1	0.5	1	5	10	25	50	100
Losartan	5-1000	5	10	25	50	100	250	500	1000
Simvastatin	0.1-100	0.1	0.5	1	5	10	25	50	100
Valsartan	50-4000	50	100	250	500	1000	2000	3000	4000

3.3 Results and Discussion

3.3.1. Operation of the LCMS System

The LC-HRMS system consisted of an Agilent 1290 LC which was coupled to an Agilent G6530A QTOF mass spectrometer using the TOF mode. Target drugs were analysed on a Zorbax Eclipse C18 rapid resolution HD column (Agilent Technologies, Cheshire, UK, 100 mm x 2.1 mm i.d., 1.8 µm particle pore size) which was preceded by a Security Guard Ultra guard column (Phenomenex, Macclesfield, UK).

100 ng/ml of a multicomponent working solution of the target cardiovascular medication was prepared from the intermediate stock and the injected volume of 2 µl. The mobile phase consisted of water containing 0.1% (v/v) formic acid (eluent A) and acetonitrile containing 0.1% (v/v) formic acid (eluent B), and was delivered at 0.6 ml/min with gradient elution. The mobile phase was initiated at 4% B and maintained for 0.5 min before increasing to 65% and then to 95% B for 1.5 min., and was then maintained for 2.5 min. before returning to 4% B. The gradient elution program was then held for 1.5 min. to re-equilibrate the column prior to the next injection. The overall run time was 4 min.

The operation of the mass spectrometer was in electrospray positive ion mode. The MS source and chamber conditions were as follows: fragmentor voltage: 165V; skimmer: 65 V; drying gas temperature: 350°C; dry gas flow: 10 L/min; nebuliser: 45.0 psig; sheath gas temperature: 400°C; sheath gas flow: 12 L/min. mass range: 100–1000 *m/z*; recording rate: 1 Hz. HRMS lock reference masses: 121.0508 *m/z* and 922.0097 *m/z*. MassHunter Workstation Acquisition Software for TOF/Q-TOF version B. 06.00 (Agilent Technologies, UK) was used to operate the system and acquire all data, which was processed using Qualitative Analysis B. 06.00 and Quantitative Analysis B. 06.00 software (Agilent Technologies). External calibration of the TOF mass spectrometer was performed daily before starting the analysis.

The mass to charge (*m/z*) ratios of the ionised species for the target medications were calculated based on their molecular formulae using the mass calculator in the qualitative analysis software version 6.00 and compared to the *m/z* ratios used in the previously

validated method. These *m/z* ratios were used because the ionised species for the target medications produced the highest signal intensities (Bernieh, 2017b).

The initial data obtained represents the total ion chromatogram (TIC), where all ions were recorded via TOF during the sample run (Figure 3.2). A mass window within 5 ppm was used to extract each drug in the multicomponent solution to produce an extracted ion chromatogram (EIC) using $[M+Na]^+$ for amlodipine *m/z* 431.1344 and simvastatin *m/z* 441.2611 and the protonated molecule $[M+H]^+$ for atenolol at *m/z* 267.1703, atorvastatin at *m/z* 559.2610, bisoprolol at *m/z* 326.2326, diltiazem at *m/z* 415.1686, lisinopril at *m/z* 406.2336, losartan at *m/z* 423.1695, and valsartan at *m/z* 436.2343, as shown in Figure 3.3.

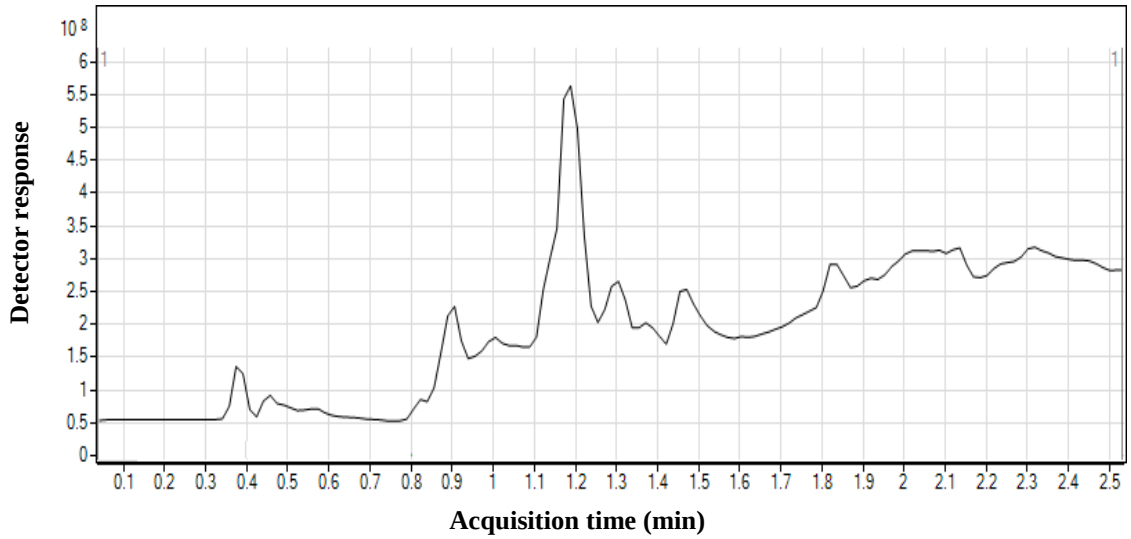

Figure 3.2. Representative LC-HRMS total ion chromatogram (TIC) of a 100 ng/ml multicomponent solution standard containing the selected target drugs.

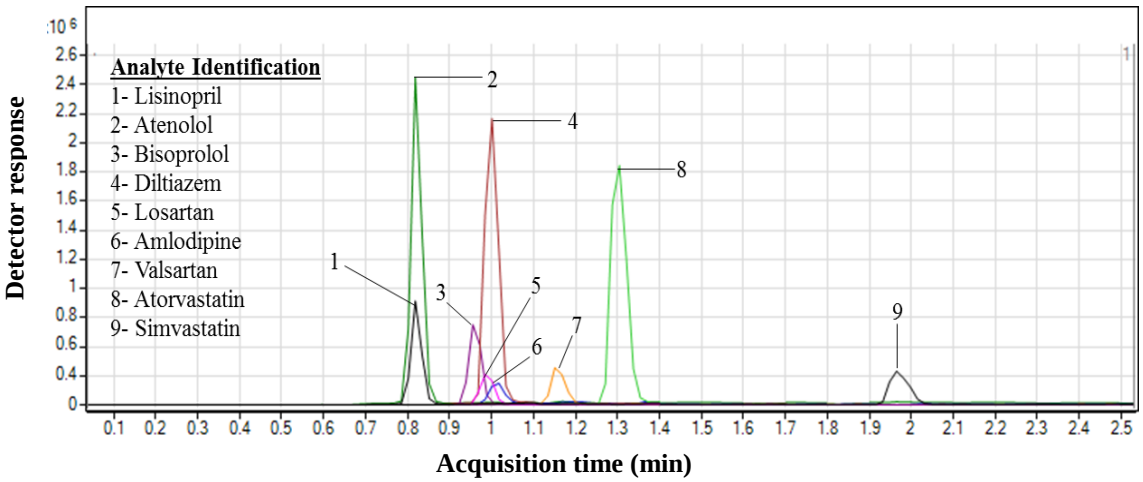

Figure 3.3. Representative LC-HRMS overlaid extracted ion chromatograms (EIC) of a 100 ng/ml multicomponent solution standard containing the selected target drugs.

The detector response was high for atenolol, atorvastatin and diltiazem (≥ 1,800,000 counts) (Figure 3.3). The detector responses for bisoprolol and lisinopril were about 700,000 and 900,000 counts, respectively. For amlodipine, losartan, valsartan and simvastatin, the detector responses were all almost 400,000. All the target medications were detected with good peak shapes.

3.3.2 Extraction of Target Analytes from Dried Blood Matrix

3.3.2.1. Preparation of Internal Standard and Extraction Solution

The internal standard (IS) stock solution of (atenolol-d7) was prepared at 1 µg/µl by dissolving 0.4 mg in 400 µl methanol. The standard operation procedure (SOP) for the preparation of IS is detailed in Appendix 11. The stock solution was further diluted with methanol/water (70:30, v/v) to produce an extraction solvent consisting of methanol containing 20 ng/ml of the internal standard (IS).

3.3.2.2. Extraction and Analysis of Target Medications from DBS

An 8-mm disc (approximately 20 µl of blood) was punched from each DBS spot into a 1.5 ml microcentrifuge tube. A 300 µl volume of extraction solvent consisting of methanol and 20 ng/ml atenolol-d7 as an internal standard (IS) was used for the extraction of the CVD medications from the DBS spot. Microcentrifuge tubes were vortexed for a minute then sonicated for 30 minutes at 40°C in a temperature-controlled ultrasonic bath. Afterwards, sonication tubes were centrifuged at 13,200 rpm for 10 minutes. 270 µl of

the liquid supernatants were transferred into new 1.5 ml labelled microcentrifuge tubes and dried using a gentle stream of nitrogen gas. The dried samples were reconstituted with 150 µl of methanol/water (40:60, v/v) containing 0.1% formic acid and vortexed for 1 minute. Finally, the liquid samples were transferred into autosampler LC vials with a 250 µl insert for analysis via LC–HRMS (Figure 3.4).

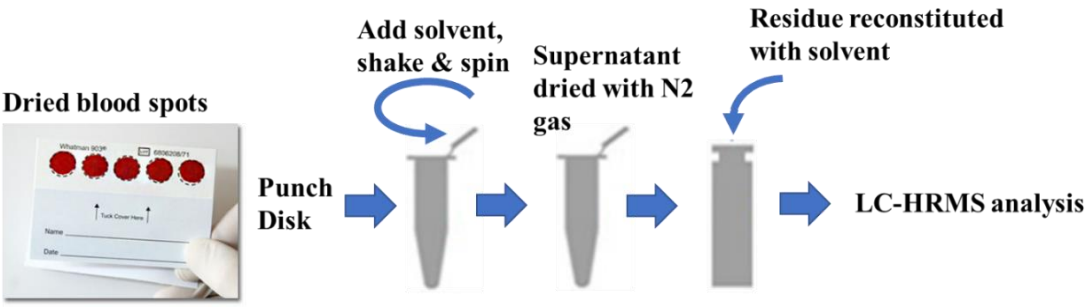

Figure 3.3. Extraction and analysis of dried blood samples on 903 cards.

Analysis of the spiked DBS standard showed that all the target analytes were detectable except amlodipine whereas amlodipine was detected in solution (Bernieh, 2017b). The application of the same extraction procedure of the previous validated study by Bernieh in the present study showed that amlodipine was not detected in the DBS extract, Figure 3.5 shows overlaid EICs from a spiked DBS standard containing the eight target analytes and the internal standard. Amlodipine was not detected in the DBS extract, which may be the result of poor extraction of amlodipine from the DBS. Card material and the complex matrix of blood may interfere with the extraction of amlodipine from filter paper, and this is considered to be a challenge during extraction. It has been reported that amlodipine has high degree of protein binding (98%) (Nirogi *et al.*, 2007). Thus, there is a possibility of amlodipine being retained on the DBS card and this may explain the reason behind its poor detection in the DBS extract.

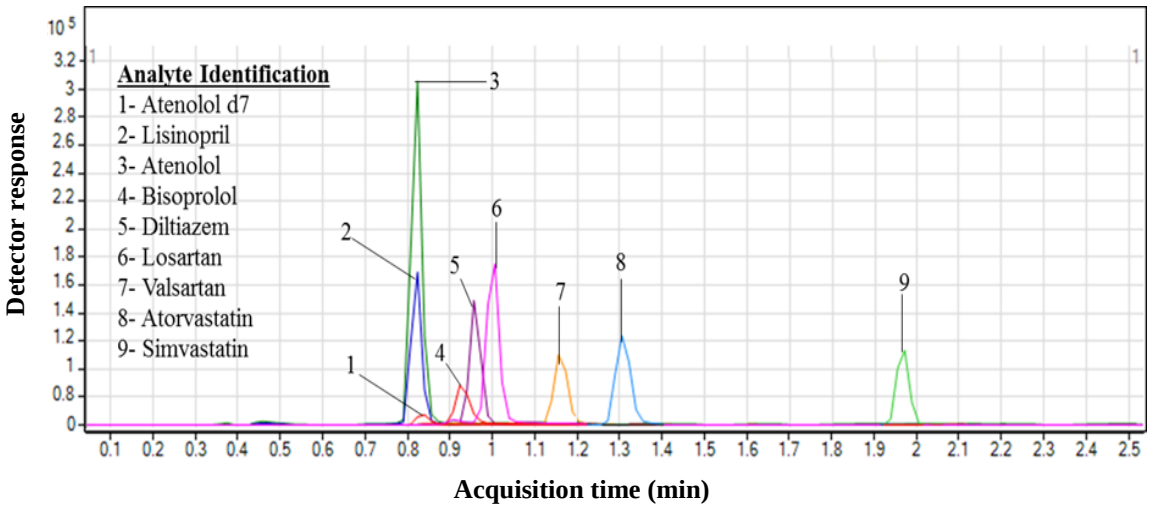

Figure 3.4. Representative LC-HRMS overlaid with an extracted ion chromatogram of the highest concentration DBS standard of the target drugs and 20 ng/ml of the IS.

Bernieh (2017) extracted amlodipine in DBS using 300 µl of acetonitrile containing 20 ng/ml atenolol-d7. 10 µl 0.5 M sodium hydroxide (NaOH) were added to the extraction solvent. The dried residue was reconstituted with 150 µl of acetonitrile/water (40:60, v/v) containing 0.1% formic acid, and was vortexed again for 1 minute, then transferred to an autosampler vial for analysis. Methanol containing 10 µl of 0.5 M sodium hydroxide (NaOH) could not be used to extract amlodipine from DBS because the addition of NaOH produced a dark supernatant that could not be used for extraction (Bernieh, 2017b). Amlodipine was identified and the EIC at *m/z* 431.1344 showed a good peak shape when acetonitrile was used as the extraction solvent (Figure 3.6).

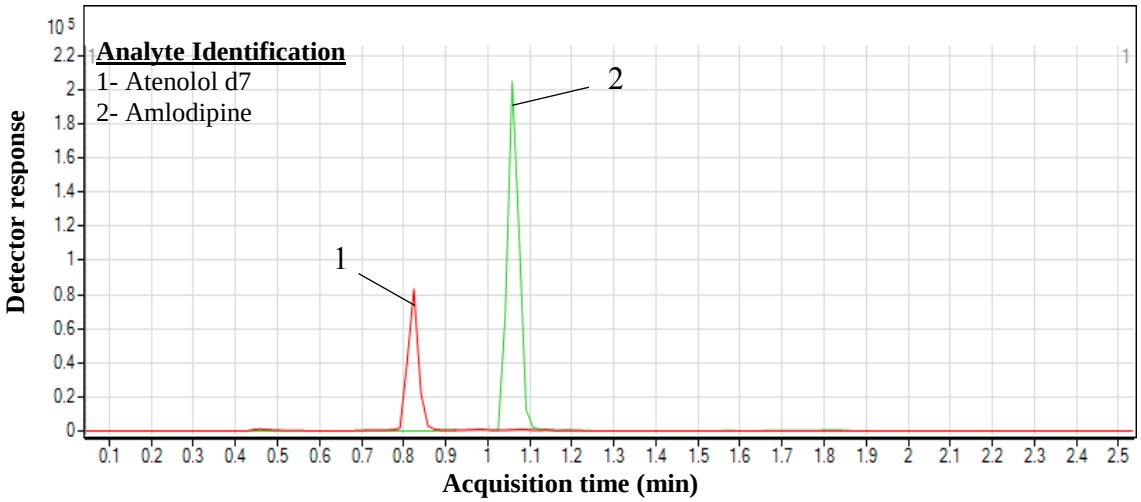

Figure 3.5. Representative LC-HRMS overlaid with an extracted ion chromatogram of the highest concentration DBS standard of amlodipine extracted using acetonitrile containing 20 ng/ml of the IS.

3.3.2.3. Extraction and Analysis of Target Medications from VAMS

Each VAMS whole tip was transferred to individual 1.5 ml labelled microcentrifuge tubes. 300 µl of extraction solvent consisting of methanol and 20 ng/ml atenolol-d7 was used for the extraction of the CVD medication VAMS samples. These tubes were vortexed for 1 minute then sonicated for 30 minutes at 40˚C in a temperature-controlled ultrasonic bath. After sonication, tubes were centrifuged at 13,200 rpm for 10 minutes. 270 µl of the liquid supernatants were transferred into new 1.5 ml labelled microcentrifuge tubes and dried by a gentle stream of nitrogen gas. The dried samples were reconstituted using 150 µl of methanol/water (40:60, v/v) containing 0.1% formic acid, and the tubes were vortexed again for 1 minute (Figure 3.7). Then, the liquid samples were transferred into autosampler LC vials with 250 µl inserts for analysis via LC–HRMS (Figure 3.8).

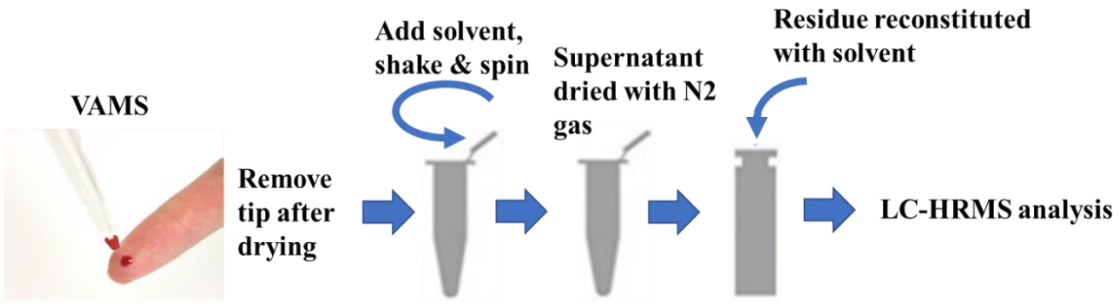

Figure 3.6. Extraction and analysis of dried blood samples on VAMS.

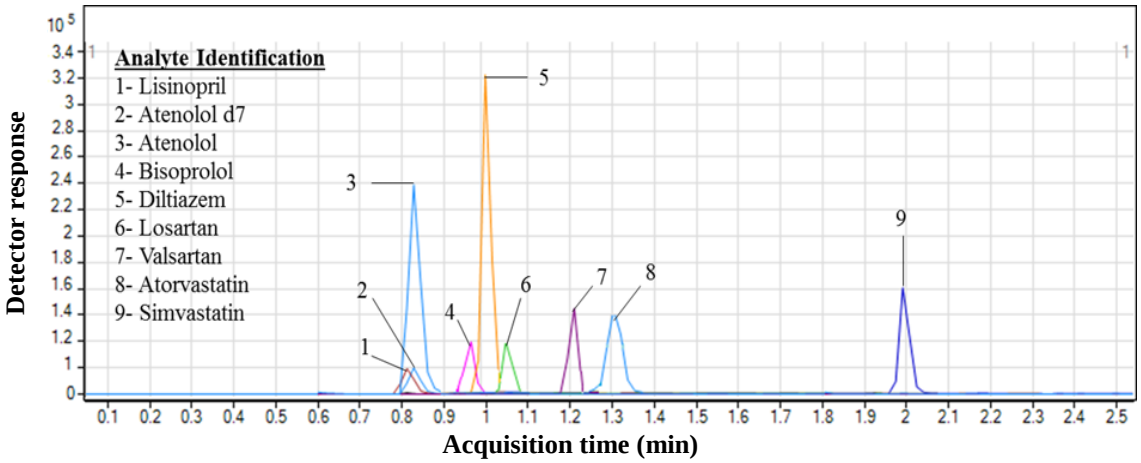

Figure 3.7. Representative LC-HRMS overlaid with the extracted ion chromatogram of the highest concentration VAMS standard of the target drugs and 20 ng/ml of the IS.

Amlodipine in VAMS was also extracted using 300 µl of acetonitrile containing 20 ng/ml atenolol-d7. 10 µl of 0.5 M sodium hydroxide (NaOH) was added to the extraction solvent and the dried residue was reconstituted with 150 µl of acetonitrile/water (40:60, v/v) containing 0.1% formic acid, which was then vortexed again for 1 minute and transferred to an autosampler vial for analysis. Amlodipine was identified and an EIC at *m/z* 431.1344 showed a good peak shape when acetonitrile was used as the extraction solvent (Figure 3.9).

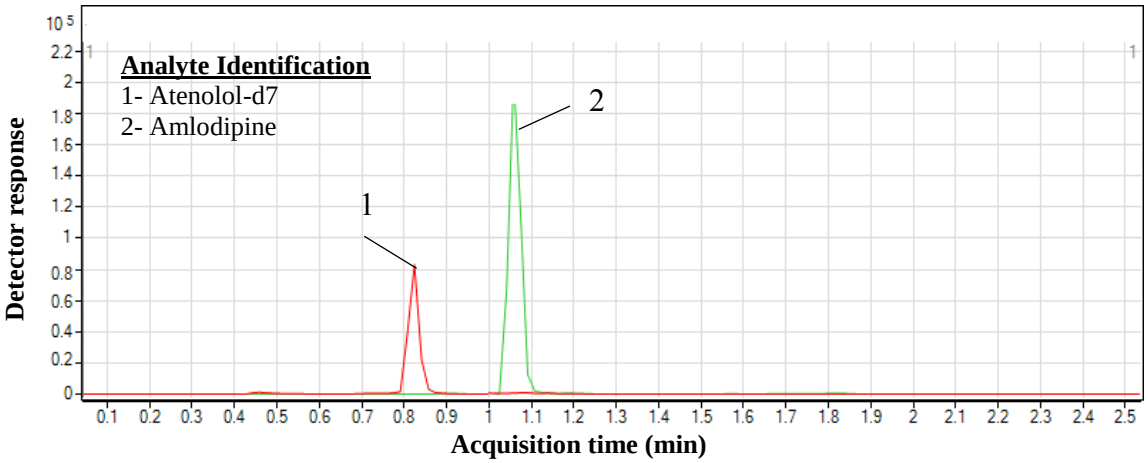

Figure 3.8. Representative LC-HRMS overlaid with the extracted ion chromatogram of the highest concentration VAMS standard of amlodipine extracted using acetonitrile containing 20 ng/ml of the IS.

Different ratios of methanol and acetonitrile (50:50, 75:25, 75:25 v/v) were tested to optimise the extraction procedure of the previous validated method by Bernieh (2017) to get a standard procedure for all CVD medications in a single run. However, the amlodipine peak didn't fulfil the validation criteria and further research to optimise the extraction for all CVD medications in the present study is recommended.

3.3.2.4. Collection of Calibration Data

To demonstrate that the previously validated method by Bernieh (2017) for the determination of the target cardiovascular medications in microsamples collected onto 903 cards and VAMS was capable of replicating and producing an acceptable level of performance, parameters such as LLOQ, selectivity precision and accuracy were checked

based on FDA guidelines for the method verification procedure (Food and Drug Administration, 2014).

3.3.2.4.1. Limit of Quantification

A minimum 7-point calibration curve that was prepared for the target medication as detailed in Section 3.2.8.2 was generated in replicate (n = 6) using the ratio of the target analyte/IS peak area against the nominal concentration for each analyte. The limit of quantification for the target analyte was determined as the lowest concentration that produced a signal-to-noise (S/N) ratio ≥ 10. The obtained limit of quantification (LOQ) for the present study using 903 paper and VAMS as a sampling substrate showed that results were in agreement with those obtained from the previous validated method in this laboratory.

3.3.2.4.2. Selectivity

Investigation of the possibility of interference from the matrix was undertaken by extracting and analysing blank blood obtained from three individuals with blood spiked with the target analyte onto 903 card and VAMS. EICs were obtained using the accurate masses for each target cardiovascular medication and internal standard using 5 ppm as the mass window at the limit of quantification. Qualitative analysis software version B. 06.00 (Agilent Technologies, UK) was used to extract EICs for the protonated molecule $[M+H]^+$ for atenolol at *m/z* 267.1703, atorvastatin at *m/z* 559.2610, bisoprolol at *m/z* 326.2326, diltiazem at *m/z* 415.1686, lisinopril at *m/z* 406.2336, losartan at *m/z* 423.1695, valsartan at *m/z* 436.2343, atenolol-d7 (internal standard) at *m/z* 274.2143, and the sodium adduct with amlodipine at *m/z* 431.1344 and simvastatin at *m/z* 441.2611. The applied method, which used a DBS- and VAMS-based LC-HRMS approach, showed good selectivity and no interfering peaks were seen at the retention times for any of the target drugs or IS. Appendix 12 shows the representative EICs for each analyte and internal standard.

3.3.2.4.3. Accuracy and Precision

The accuracy and precision were determined by analyses of replicates (n = 6) of blood spots spiked with the target analyte of the target CVD medications prepared on 903 cards

and VAMS separately, using the same conditions and extraction protocol used by (Bernieh 2017). Back calculation using the same equations as the previous validated method for DBS and VAMS – see Tables 3.3 and 3.4 (Bernieh, 2017b) – produced relative errors (RE %) ≤ 15% (between 2-6% for DBS and 3-5% for VAMS), which is considered acceptable with reference to international guidelines (Committee for Medicinal Products for Human Use, 2011; Food and Drug Administration, 2013). A summary of the results is shown in Table 3.5 and Table 3.6.

Table 3.3. Equations used for quantification of the target medications on 903 sampling paper for Iraqi volunteers (Bernieh, 2017b).

Drug	Range (ng/ml)	$y = ax + b$	R^2	LOQ (ng/ml)
Amlodipine	0.5 – 100	y = 0.004x + 0.043	0.993 ± 0.004	0.5
Atenolol	10 – 1500	y = 0.0044x – 0.047	0.997 ± 0.001	10
Atorvastatin	0.5 – 100	y = 0.0014x + 0.0244	0.986 ± 0.013	0.5
Bisoprolol	0.1 – 100	y = 0.019x + 0.034	0.994 ± 0.003	0.1
Diltiazem	0.5 – 600	y = 0.016x + 0.053	0.997 ± 0.002	0.5
Lisinopril	0.1 – 100	y = 0.002x + 0.031	0.978 ± 0.007	0.1
Losartan	5 – 1000	y = 0.004x + 0.0713	0.995 ± 0.002	5
Simvastatin	0.1 – 100	y = 0.013x + 0.081	0.996 ± 0.003	0.1
Valsartan	50 – 4000	y = 0.002x – 0.139	0.994 ± 0.003	50

Table 3.4. Equations used for quantification of the target medications in VAMS for Iraqi volunteers (Bernieh, 2017b).

Drug	Range (ng/ml)	$y = ax + b$	R^2	LOQ (ng/ml)
Amlodipine	0.5 – 100	y = 0.007x + 0.086	0.990 ± 0.002	1
Atenolol	10 – 1500	y = 0.0074x – 0.136	0.992 ± 0.001	10
Atorvastatin	0.5 – 100	y = 0.0033x + 0.023	0.997 ± 0.001	0.5
Bisoprolol	0.1 – 100	y = 0.0097x + 0.096	0.996 ± 0.002	0.1
Diltiazem	0.5 – 600	y = 0.008x + 0.224	0.995 ± 0.003	0.5
Lisinopril	0.1 – 100	y = 0.0013x + 0.021	0.985 ± 0.004	0.1
Losartan	5 – 1000	y = 0.0024x + 0.110	0.993 ± 0.007	5
Simvastatin	0.1 – 100	y = 0.016x + 0.215	0.988 ± 0.003	0.1
Valsartan	50 – 4000	y = 0.0006x + 0.125	0.992 ± 0.001	50

The following equation was used; $y = ax + b$, where y is the ratio of analyte to IS response, a is the gradient, x represents the concentration and b is the y-intercept

Table 3.5. Accuracy and precision data for the nine target cardiovascular drugs in 903 sampling paper extracts (n = 6).

Drug	Nominal conc. (ng/ml)	Mean conc. (ng/ml)	SD	Accuracy (RE) %	Precision (CV) %
Amlodipine	0.5	0.53	0.01	-5.33	1.18
	1	1.01	0.05	-0.67	5.28
	5	4.93	0.16	1.40	3.30
	10	10.07	0.03	-0.67	0.34
	25	24.54	0.76	1.85	3.09
	50	50.32	0.46	-0.64	0.91
	100	101.14	0.92	-1.14	0.91
Atenolol	10	9.91	0.06	0.93	0.63
	20	19.65	0.09	1.75	0.45
	50	51.13	1.02	-2.26	1.99
	100	101.84	1.57	-1.84	1.54
	200	200.64	0.45	-0.32	0.23
	500	498.98	5.75	0.20	1.15
	1000	1000.31	3.28	-0.03	0.33
	1500	1495.94	6.19	0.27	0.41
Atorvastatin	0.5	0.51	0.02	-1.52	3.56
	1	1.00	0.03	-0.33	3.39
	5	4.98	0.12	0.33	2.50
	10	9.96	0.05	0.43	0.50
	25	24.52	0.06	1.91	0.25
	50	50.65	0.69	-1.30	1.35
	100	101.13	1.50	-1.13	1.48
Bisoprolol	0.1	0.11	0.01	-6.33	9.10
	0.5	0.49	0.01	1.79	2.77
	1	1.06	0.01	-6.33	0.89
	5	5.11	0.16	-2.27	3.09
	10	9.79	0.11	2.05	1.17
	25	24.88	0.49	0.48	1.96
	50	49.81	0.44	0.37	0.88
	100	100.03	0.06	-0.03	0.06
Diltiazem	0.5	0.50	0.01	0.46	1.84
	1	1.06	0.01	-5.66	1.33
	5	5.25	0.02	-4.93	0.39
	10	10.05	0.03	-0.50	0.29
	50	49.57	0.48	0.86	0.97
	100	99.88	0.46	0.12	0.46
	300	300.04	0.78	-0.01	0.26
	600	599.67	0.55	0.05	0.09

Table 3.5 continued

Drug	Nominal conc. (ng/ml)	Mean conc. (ng/ml)	SD	Accuracy (RE) %	Precision (CV) %
Lisinopril	0.1	0.10	0.01	-2.67	5.11
	0.5	0.50	0.01	-0.34	1.10
	1	0.98	0.01	1.67	0.96
	5	5.02	0.01	-4.00	0.16
	10	9.95	0.03	0.47	0.33
	25	24.70	0.44	1.19	1.78
	50	49.90	0.09	0.19	0.17
	100	100.58	0.92	-0.58	0.91
Losartan	5	4.9	0.1	1.1	2.1
	10	9.9	0.1	1.3	1.1
	25	25.23	0.01	-1.00	0.05
	50	49.9	0.86	0.19	1.73
	100	100.25	0.45	-0.25	0.45
	250	250.21	0.48	-0.09	0.19
	500	500.65	0.84	-0.13	0.17
	1000	999.25	0.54	0.75	0.05
Simvastatin	0.1	0.10	0.01	-2.67	5.11
	0.5	0.51	0.01	-1.68	1.53
	1	1.00	0.01	0.33	0.95
	5	5.02	0.01	-0.40	0.16
	10	9.97	0.03	0.32	0.26
	25	25.23	0.02	-0.91	0.08
	50	50.24	0.55	-0.47	1.09
	100	99.96	0.05	0.04	0.05
Valsartan	50	49.87	0.13	0.26	0.26
	100	99.97	0.05	0.03	0.05
	250	249.10	0.25	0.36	0.10
	500	500.28	0.55	-0.06	0.11
	1000	999.78	1.52	0.02	0.15
	2000	2009.97	0.87	-0.50	0.04
	3000	3001.59	1.24	-0.05	0.04
	4000	4005.59	3.98	-0.14	0.10

Table 3.6. Accuracy and precision data for the nine target cardiovascular drugs in VAMS extracts (n = 6).

Drug	Nominal conc. (ng/ml)	Mean conc. (ng/ml)	SD	Accuracy (RE) %	Precision (CV) %
Amlodipine	0.5	0.50	0.01	0.67	1.26
	1	1.00	0.01	-1.00	0.82
	5	5.04	0.01	-0.07	0.25
	10	9.72	0.46	2.80	4.68
	25	24.87	0.31	0.52	1.23
	50	50.61	0.12	-1.22	0.23
	100	100.21	0.23	-0.21	0.23
Atenolol	10	9.96	0.02	0.42	0.20
	20	19.89	0.05	0.53	0.26
	50	50.38	0.40	-0.76	0.80
	100	101.37	1.04	-1.37	1.03
	200	200.14	0.04	-0.07	0.02
	500	500.48	0.78	-0.10	0.16
	1000	999.94	1.46	0.01	0.15
	1500	1499.19	1.63	0.05	0.11
Atorvastatin	0.5	0.50	0.01	-0.19	1.75
	1	0.99	0.01	0.67	1.26
	5	5.01	0.07	-0.13	1.33
	10	9.98	0.03	0.17	0.29
	25	25.52	0.06	-2.09	0.24
	50	50.32	0.21	-0.63	0.43
	100	99.47	0.27	0.53	0.27
Bisoprolol	0.1	0.11	0.01	-5.00	6.73
	0.5	0.49	0.01	1.79	2.77
	1	0.99	0.01	0.67	1.26
	5	5.10	0.15	-2	2.96
	10	9.86	0.10	1.39	1.04
	25	25.48	0.50	-1.92	1.97
	50	49.61	0.39	0.77	0.78
	100	99.03	0.76	0.97	0.77
Diltiazem	0.5	0.50	0.01	-0.88	2.12
	1	1.04	0.02	-3.67	1.82
	5	5.23	0.02	-4.53	0.39
	10	10.05	0.01	-0.47	0.12
	50	49.91	0.05	0.19	0.10
	100	99.83	0.42	0.17	0.43
	300	299.91	0.15	0.03	0.05
	600	600.31	0.50	-0.05	0.08

Table 3.6 continued

Drug	Nominal conc. (ng/ml)	Mean conc. (ng/ml)	SD	Accuracy (RE) %	Precision (CV) %
Lisinopril	0.1	0.10	0.01	-2.33	5.31
	0.5	0.51	0.01	-2.34	2.33
	1	1.01	0.03	-1.33	3.26
	5	5.02	0.01	-0.40	0.16
	10	9.99	0.02	0.13	0.21
	25	25.03	0.01	-0.12	0.06
	50	49.61	0.49	0.78	0.99
	100	100.11	0.10	-0.11	0.10
Losartan	5	4.93	0.05	1.13	1.10
	10	9.94	0.23	0.62	2.33
	25	24.59	0.50	1.65	2.02
	50	50.24	0.55	-0.47	1.09
	100	99.92	0.04	0.08	0.04
	250	249.95	0.18	0.02	0.07
	500	500.42	0.69	-0.08	0.14
	1000	999.59	0.52	0.04	0.05
Simvastatin	0.1	0.10	0.01	-2.67	5.11
	0.5	0.50	0.01	-0.34	1.89
	1	1.00	0.02	0.33	1.71
	5	5.02	0.01	-0.47	0.25
	10	9.98	0.04	0.15	0.38
	25	25.23	0.80	-0.91	3.16
	50	49.90	0.09	0.19	0.17
	100	99.63	0.44	0.37	0.44
Valsartan	50	49.91	0.15	0.19	0.29
	100	99.64	0.43	0.36	0.43
	250	249.77	0.72	0.09	0.29
	500	499.86	0.47	0.03	0.09
	1000	999.44	1.05	0.06	0.10
	2000	2005.97	4.22	-0.30	0.21
	3000	3000.25	0.37	-0.01	0.01
	4000	4006.59	4.92	-0.16	0.12

3.3.2.4.4. Accuracy and Precision of QC

The accuracy and precision of the quality control standards (QC) were determined by running replicate (n = 6) analyses of the QC standards for the target CVD medications (amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin

and valsartan). Accuracy was expressed in terms of relative error (RE %) and precision as the coefficient of variation (CV %). Back calculations produced accuracies and precisions within ≤ 15% with reference to international guidelines (Committee for Medicinal Products for Human Use, 2011; Food and Drug Administration, 2013). QC standards were run alongside the patients' samples in each batch for the Iraqi volunteers in order to determine the performance of the instrument.

3.3.3. Application of Method for Assessment of Non-adherence

3.3.3.1. Statistical Analysis

Descriptive statistical frequency distributions were obtained using the SPSS software (version 22. Armonk, NY: IBM Corp). Qualitative variables such as gender and medications were expressed in terms of frequencies and percentages. Spearman's correlation coefficient was used to determine the relationship between non-adherence assessed by blood microssamples analysis and age, number of CVD medications in patients' regimens and number of tablets taken by patients. A Chi-squared test was used to examine the relationship between medication adherence and gender. Mean and standard deviation were used to express the concentration of medications in the biological samples. A Bland-Altman plot was used to compare the results obtained from the two microsampling methods (DBS and VAMS).

3.3.3.2. Qualitative Analysis

After extraction of patient microsamples collected on DBS cards and VAMS, qualitative analysis was conducted using qualitative analysis software version B. 06.00 (Agilent Technologies, UK) by extracting EICs using the accurate mass values (*m/z*) of the target cardiovascular medications. An extraction window of 5 ppm was used for EIC extraction. Qualitative analysis was used to confirm the existence of the target medication in the (DBS and VAMS) blood microsamples, as per Appendix 13. Qualitative analysis for the target medications in DBS and VAMS showed good agreement between these two sampling methods.

3.3.3.3. Quantitative Analysis

Medications which were qualitatively identified in the blood microsamples (DBS AND VAMS) from 303 Iraq volunteers in Section 3.3.3.3 were quantified by quantitative analysis B. 06.00 software (Agilent Technologies). Quantitative analysis was undertaken by extracting EICs using the accurate masses for each target cardiovascular medication within a 5 ppm mass window. The ratio of the target analyte/IS peak area was used in the equation used for the previous validated method (Bernieh, 2017b). Patients were categorised as non-adherent when one or more of their prescribed medications concentration was < 5% of C_{max} or > C_{max}. The results of this analysis are summarised in Appendix 14.

The non-adherence to the target cardiovascular medications in the present study was not uniform. The average non-adherence to medications in the target sample was almost 41%; however, patients adhere in different ways to medications, as shown in Table 3.7.

Table 3.7. Percentage of adherence and non-adherence to the target cardiovascular medication in the Iraqi volunteer sample.

Medications	Adherence (%)	Non-adherence (%)	Total
Amlodipine	66.7	33.3	15
Atenolol	78.0	22.0	59
Atorvastatin	44.4	55.6	18
Bisoprolol	74.0	26.0	77
Diltiazem	58.8	41.2	34
Lisinopril	65.8	34.2	73
Losartan	46.8	53.2	47
Simvastatin	48.0	52.0	50
Valsartan	50.8	49.2	65

Amlodipine was taken by 15 Iraqi volunteers, to which 10 patients (66.7%) were adherent (Patient reference numbers …125, 126, 127, 128, 160,161, 162, 163, 197 and 222) and five patients (33.3%) were non-adherent (Patient reference numbers …195, 196, 198, 240 and 252). Figure 3.10 shows the representative LC-HRMS overlaid with the EIC of patients who were adherent and non-adherent to amlodipine. Patients who were suspected to be non-adherent to amlodipine reported the time since their last dose was less than 24 hrs (5-8 hrs). However, amlodipine was detected in the other 10 volunteers where the

ingestion time was similar to non-adherent patients, which was 5-8 hrs. A possible explanation is that those patients who did not take their medication was either due to forgetfulness or because they thought the medication has been taken but was not, which may have been due to them being on complex medical regimens. Another possible explanation is that volunteers did not tell the truth about their medication-taking behaviour. The $t_{1/2}$ of amlodipine is quite long at 35-50 hrs, and this may suggest that patients who were non-adherent to amlodipine and had not taken it for more than 5 days.

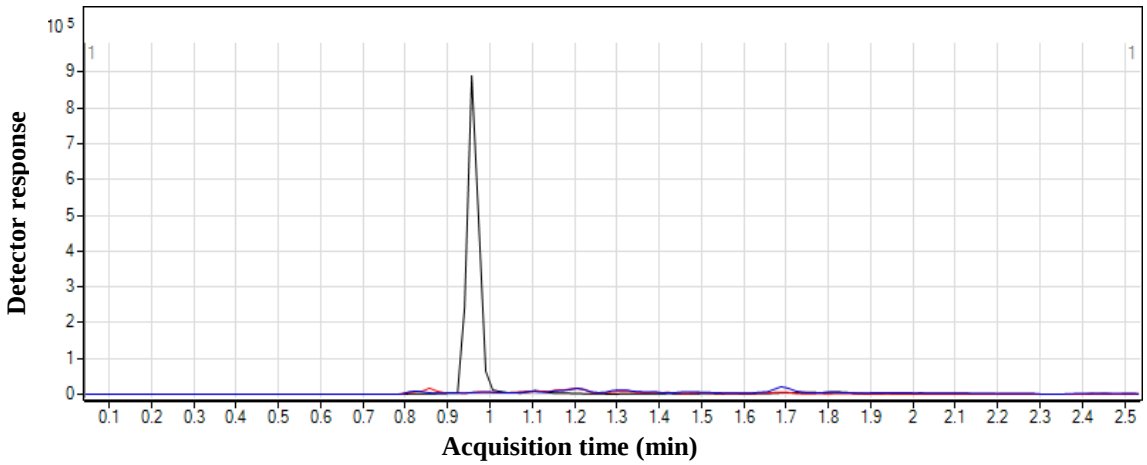

Figure 3.9. Comparison between LC-HRMS extracted ion chromatograms for amlodipine (*m/z* 431.1344) in DBS samples for patient reference number …125 who was adherent to amlodipine (black line) and patient reference number …195 who was non-adherent (blue line), with the red line showing the blank control.

For other calcium channel blockers, diltiazem was taken by 34 volunteers. Twenty patients (85.8%) were adherent to diltiazem (Patient reference numbers …61, 65, 72, 76, 86, 117, 118, 119, 120, 152,153, 154, 155, 186, 187, 188, 189, 213, 218, 219). On the other hand, 14 volunteers (41.2%) were non-adherent (Patient reference numbers …21, 60, 220, 236, 237, 238, 247, 248, 267, 269, 277, 282, 285 and 289). Figure 3.11 shows representative LC-HRMS overlaid with the EIC of patients who were adherent and non-adherent to diltiazem.

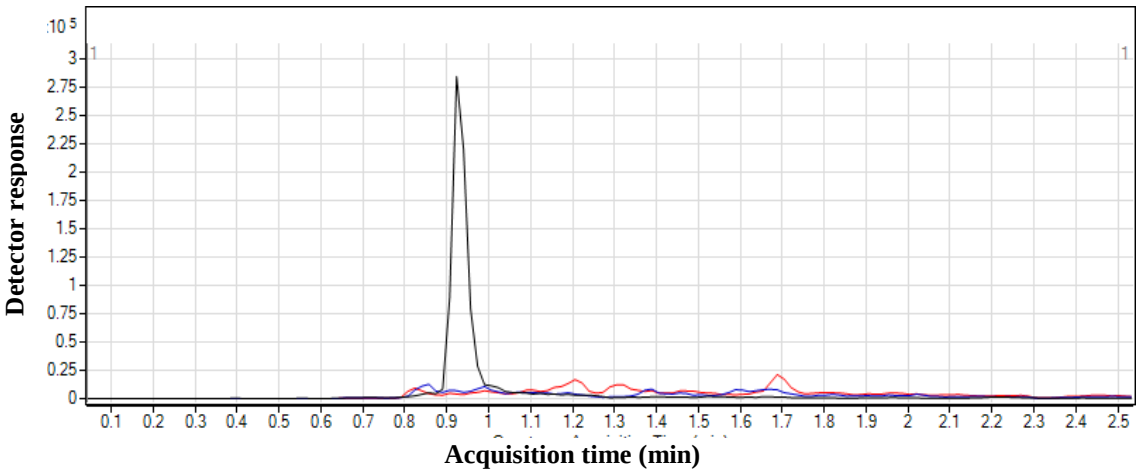

Figure 3.10. Comparison between LC-HRMS extracted ion chromatograms for diltiazem (*m/z* 415.1686) in DBS samples for patient reference number …61 who was adherent to diltiazem (black line) and patient reference number …21 who was non-adherent (blue line), with the red line showing a blank control.

Fifty-nine volunteers were taking atenolol and it was detected in 50 volunteers (Patient reference numbers …14, 23, 34, 37, 40, 42, 45, 51, 52, 53, 58, 67, 100, 101, 102, 103, 104, 105, 106, 107, 138, 139, 140, 141, 142, 143, 144, 169, 170, 171, 172, 173, 174, 175, 176, 206, 207, 208, 209, 210, 211, 226, 227, 228, 229, 244, 245, 256, 257, 258) and not detected in nine volunteers (Patient reference numbers …08, 264, 270, 274, 283, 287, 296, 307 and 310). Four of the patients taking atenolol were considered non-adherent even though atenolol was detected in their blood (Patient reference numbers …23, 45, 53, 58) because the measured concentration exceeded the corresponding C_{max} for the reported dose of atenolol (50 mg or 100 mg). Therefore, 46 patients (78%) were considered adherent and 13 patients (22%) were considered non-adherent in total. Figure 3.12 shows a representative LC-HRMS overlaid with the EIC of an adherent and non-adherent patient to atenolol.

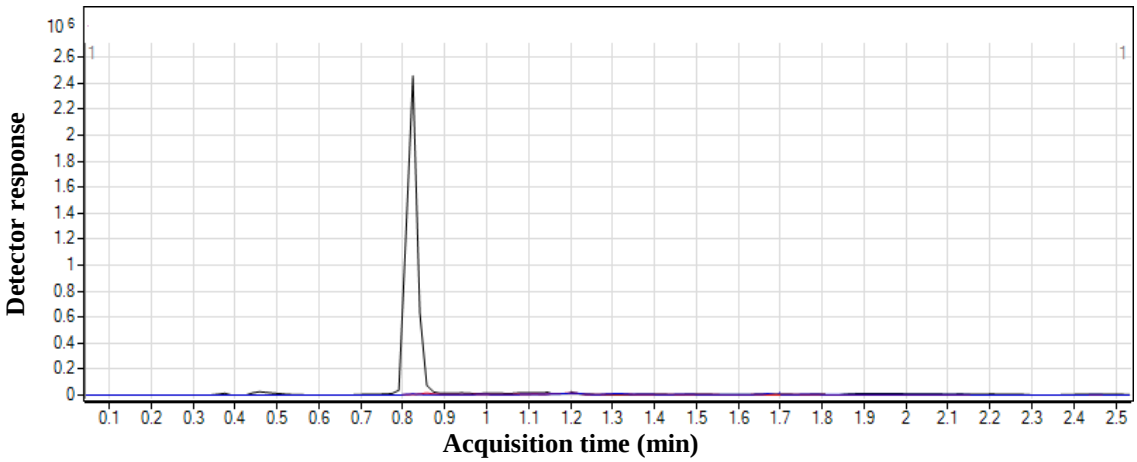

Figure 3.11. Comparison between LC-HRMS extracted ion chromatograms for atenolol (*m/z* 267.1703) in DBS samples for patient reference number …14 who was adherent to atenolol (black line) and patient reference number…08 who was non-adherent to atenolol (blue line), with the red line showing a blank control.

Patient reference numbers …23 and 45 reported that atenolol 50 mg had been prescribed to them; however, the concentrations measured exceeded the C_{max} for the reported dose of atenolol. This may indicate that patients took double the dose by mistake or there was a prescribing error (prescribing 100 mg atenolol instead of 50 mg) or possibly the patients incorrectly reported the dose of atenolol they had taken in the mini-DBS questionnaire. Since the information provided by patients was assumed to be correct, these patients were labelled as non-adherent.

For patient reference number …53, the concentration of atenolol measured in their DBS sample was higher than the C_{max} for a dose of 100 mg of atenolol even though the reported time since the last dose was 13 hrs, which may indicate that the patient took two 100 mg tablets of atenolol. The high concentration of atenolol in this patient may have been due to dosage errors. Further investigation by the doctor is required in this case to determine the cause of this high concentration. Atenolol is water soluble and it is highly dependent on renal elimination, and there is a possibility of drug accumulation in patients with chronic kidney diseases (CKD) (Faull and Lee, 2007), thus this patient needs further investigation with regards to adjusting the dose and overcoming possible drug side effects and toxicity.

The volunteer with reference number …58 was taking atenolol 50 mg and atorvastatin 40 mg and reported an ingestion time for both medications of 15 hrs. However, the atenolol

concentration exceeded the C_{max} for atenolol and atorvastatin was not detected. In the Iraqi market, there is increasing prevalence of generic medications which have similar shapes and colours, and patients may depend on the shape and/or colour of the pill to take their medication doses without knowing other characteristics of the medication, such as name and dosage, and consequently this might account for taking more than required dose (Lenahan *et al.*, 2013). This patient may have taken double the dose of atenolol and missed the dose of atorvastatin, for instance, which may explain why the concentration of atenolol was so high.

The results of the current study showed that statins group such as atorvastatin and simvastatin showed high level of non-adherence at 55.6% and 52% respectively. 18 volunteers were taking atorvastatin, to which eight patients (44.4%) were adherent (Patient reference numbers…70, 82, 291, 301, 311, 320, 330 and 331). On the other hand, 10 volunteers (55.6%) were not adherent to atorvastatin (Patient reference numbers …17, 55, 56, 58, 59, 62, 64, 72, 75, and 88). Figure 3.13 shows representative LC-HRMS overlaid with EIC of a patient adherent to atorvastatin and a patient who was non-adherent.

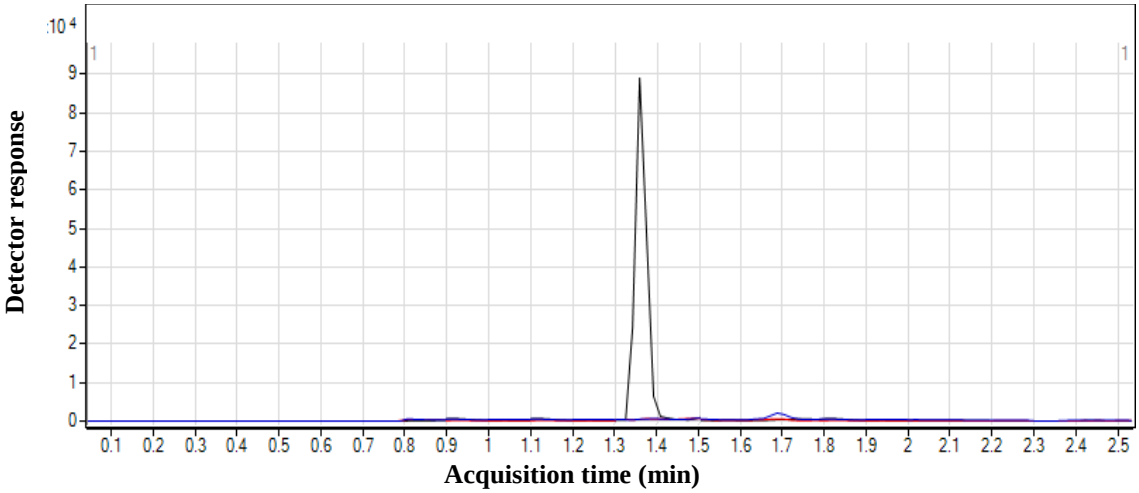

Figure 3.12. Comparison between LC-HRMS extracted ion chromatograms for atorvastatin (*m/z* 559.2610) in DBS samples for patient reference number…70 who was adherent to atorvastatin (black line) and patient reference number…17 who was non-adherent (blue line), with the red line showing a blank control.

Simvastatin was taken by 50 volunteers, 24 patients (48%) were adherent to simvastatin (Patient reference number…100, 101, 102, 103, 123, 132, 138, 139, 140, 141, 156, 167, 169, 170, 171, 172, 191, 192, 194, 206, 207, 208, 226, and 227). 26 volunteers (52%) were non-adherent (Patient reference numbers …07, 20, 121, 122, 124, 133, 157, 158, 159, 168, 190, 193, 202, 221, 239, 249, 250, 251, 314, 323, 324, 325, 326, 328, 334, and 336). Figure 3.14 shows representative LC-HRMS overlaid with EIC of a patient adherent to simvastatin and one who was non-adherent. Simvastatin has a relatively short $t_{1/2}$ (1.3-2.7 hrs) and there was a need to check the presence of one of its metabolites, simvastatin acid at *m/z* 436.5815, for adherent and non-adherent to confirm that volunteers were adherent or non-adherent. Since the LC-HRMS system operates in the full-scan mode, the data was revisited to look for the simvastatin metabolite without having to run a new sample. Simvastatin acid was detected in blood microsamples in adherent patients, however, it was not detected in non-adherent patients, and this confirmed that these patients were non-adherent to their prescription. Simvastatin is recommended to be taken in the evening. There is evidence showed that considerable increase in the level of total and low-density lipoprotein cholesterol occurred due to switching taking of simvastatin from the evening to the morning (Wallace *et al.*, 2003).

Side effects associated with statins such as muscle pain may account for high rates of non-adherence to these CVD medications. Patients with cardiovascular disease normally take medications from different therapeutic classes (complex regimen) (Anderson and Nawarskas, 2001) and this combined therapy consequently increases the risk of adverse drug effects and drug interactions (Abolbashari *et al.*, 2017).

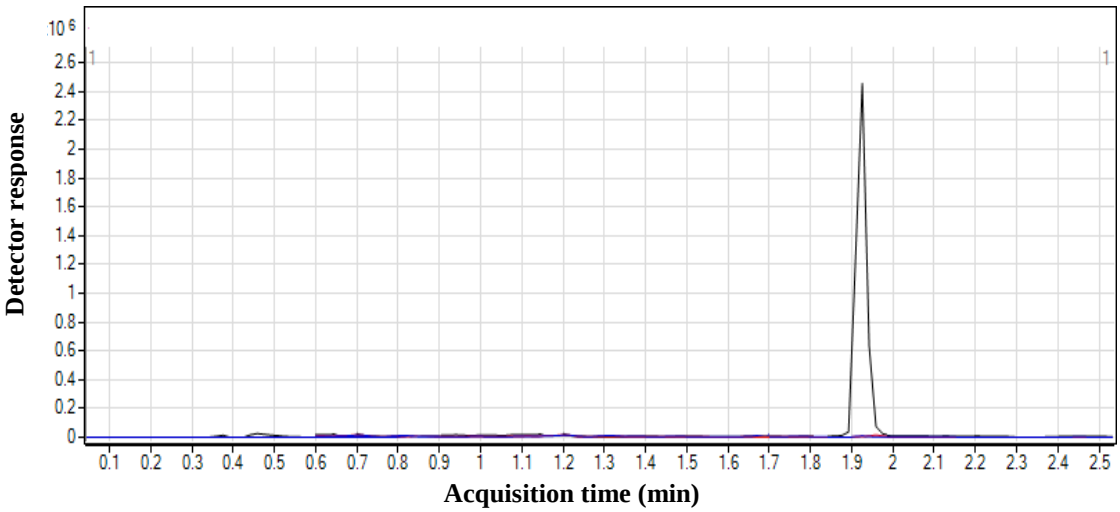

Figure 3.13. Comparison between LC-HRMS extracted ion chromatograms for simvastatin (*m/z* 441.2611) in DBS samples for patient reference number…100 who was adherent to simvastatin (black line) and patient reference number…07 who was non-adherent (blue line), with the red line showing a blank control.

Both atorvastatin and simvastatin were not available in the public sector in Iraq according to annual need for medication in 2016 (Chapter 1 Section 1.4). Patients need to pay from their own pockets to get these medications from private sector, and this extra cost may lead to high rates of non-adherence to these medications. Participants may decrease the number of doses in order to decrease their out of pocket expenses, and this will consequently lead to the poor clinical outcomes associated with poor adherence.

Bisoprolol was a very popular cardiovascular medication in the Iraqi samples collected, where it was taken by 77 volunteers. This regularity in the prescription of bisoprolol may be due to availability of the medication in both the public and private sectors, or possibly due to the relatively low cost of this medication compared to the other medications, as shown in Chapter 1 Section 1.3.6. Bisoprolol was detected in 57 patients (74%) (Patient reference numbers …15, 27, 30, 36, 38, 48, 57, 70, 87, 90, 91, 96, 99, 108, 109, 110, 111, 112, 113, 114, 115, 116, 145, 146, 147, 148, 149, 150, 177, 178, 180, 181, 182, 183, 184, 185, 212, 214, 215, 216, 217, 329, 230, 231, 232, 233, 234, 235, 246, 259, 260, 261, 262, 293, 297, 298, and 303). On the other hand, bisoprolol was not detected in 20 volunteers (Patient reference numbers …10, 19, 59, 95, 268, 272, 278, 281, 284, 290, 300, 304, 306, 315, 316, 319, 322, 327, 333, and 335). Figure 3.15 shows representative LC-HRMS overlaid with the EIC of a patient adherent to bisoprolol and a patient who was non-

adherent. Volunteers taking bisoprolol showed high rates of adherence to this medication in comparison to the non-adherent at 74% and 26%, respectively.

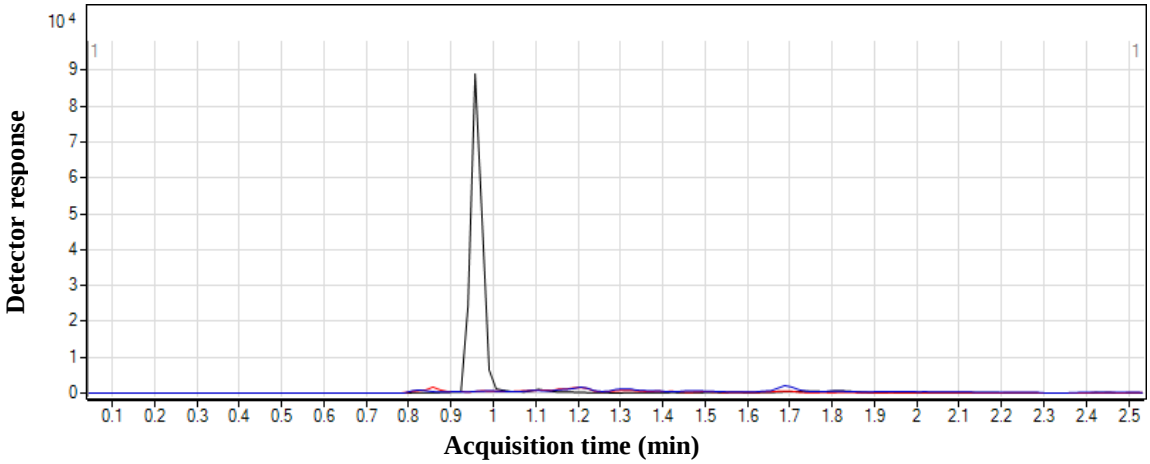

Figure 3.14. Comparison between LC-HRMS extracted ion chromatograms for bisoprolol (*m/z* 326.2326) in DBS samples for patient reference number…15 who was adherent to bisoprolol (black line) and patient reference number…10 who was non-adherent (blue line), with the red line showing a blank control.

Lisinopril was the second-most popular medication used by patients in the samples collected where 73 volunteers were found to be taking it and it was actually detected in 47 patients (64.4%) (Patient reference numbers …6, 32, 57, 66, 77, 91, 93, 104, 105, 106, 110, 111, 117, 118, 119, 142, 143, 144, 147, 152, 153, 154, 155, 173, 174, 175, 176, 178, 182, 187, 188, 209, 210, 213, 214, 219, 228, 229, 232, 244, 245, 246, 256, 257, 258, 259, and 260), whereas 26 volunteers (35.6%) (Patient reference numbers …56,107, 108, 109, 120, 145, 146, 177, 180, 181, 184, 186, 189, 211, 212, 220, 230, 231, 236, 237, 238, 247, 248, 294, 313, and 332) were non-adherent. Figure 3.16 shows representative LC-HRMS overlaid with the EIC of a patient adherent to lisinopril and a patient who was non-adherent.

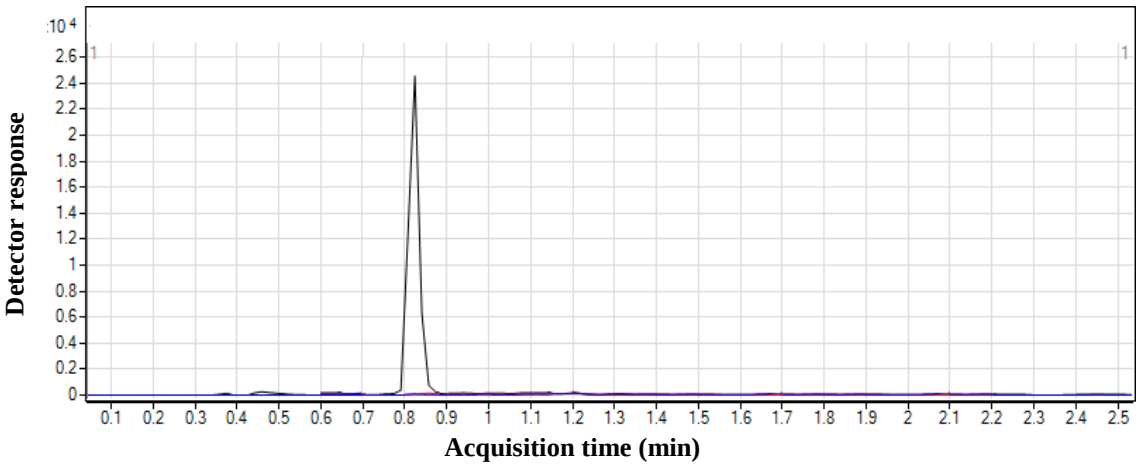

Figure 3.15. Comparison between LC-HRMS extracted ion chromatograms for lisinopril (*m/z* 406.2336) in DBS samples for the patient with reference number …6 who was adherent to lisinopril (black line) and the patient with the reference number …56 who was non-adherent (blue line) with the red line showing a blank control.

Losartan was prescribed for 47 patients. 22 patients (46.8%) were adherent (Patient reference numbers …25, 27, 50, 55, 69, 121, 122, 123, 129, 130, 131, 156, 164, 165, 166, 191, 192, 194, 199, 200, 201, and 223). Patients showed high levels of non-adherence, where 25 volunteers (53.2%) were non-adherent (Patient reference numbers ...33, 38, 41, 61, 124, 157, 158, 159, 190, 193, 221, 239, 249, 250, 251, 265, 271, 275, 279, 288, 295, 299, 305, 308, and 317). Figure 3.17 shows a representative LC-HRMS overlaid with EIC for the DBS microsample of a patient adherent to losartan and a patient who was non-adherent. Various factors may be the cause of the non-adherence, in this instance such as side effects. It was reported that vertigo is one of the more common side effects associated with the use of losartan (National Health Service, 2018c). However, other factors may have contributed to poor adherence, such as patients' attitudes and beliefs, and medication cost and availability. Losartan has a short $t_{1/2}$ (0.94-4.02 hrs) and to confirm that a given volunteer was non-adherent, data was revisited in order to look for losartan metabolites (losartan acid), as was checked at *m/z* 436.8941. Losartan acid was not detected in non-adherent patients but was detected in adherent patients, and this confirmed that non-adherent patients were indeed not adhering to their prescribed medication regimen.

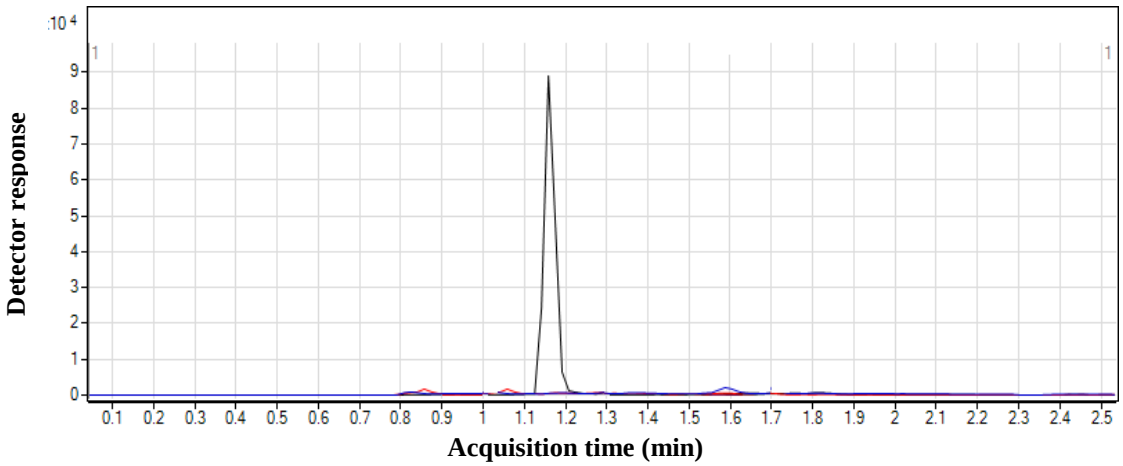

Figure 3.16. Comparison between LC-HRMS extracted ion chromatograms for losartan (*m/z* 423.1695) in DBS samples for patient reference number …25 who was adherent to losartan (black line) and patient reference number…33 who was non-adherent (blue line), with the red line showing a blank control.

Valsartan was taken by 65 volunteers, where adherence and non-adherence were almost equal. 33 volunteers (50.8%) were adherent (Patient reference numbers ...12, 16, 17, 24, 30, 68, 78, 84, 89, 113, 114, 115, 136, 137, 148, 149, 150, 183, 184, 185, 205, 215, 216, 225, 233, 234, 243, 254, 255, 292, 302, 312, and 321); conversely, 32 volunteers (49.2%) were non-adherent (Patient reference numbers …11, 31, 36, 48, 61, 72, 73, 74, 80, 82, 99, 112, 116, 134, 135, 204, 217, 224, 235, 241, 242, 253, 261, 262, 263, 266, 273, 276, 280, 286, 309, and 318). Figure 3.18 shows a representative LC-HRMS overlaid with the EIC in DBS for a patient adherent to valsartan and a patient who was non-adherent. To confirm adherence and non-adherence to valsartan, data was revisited to look for valsartan metabolites (4-hydroxy valsartan at *m/z* 535.2790). 4-hydroxy valsartan was seen in adherent volunteers but not seen in non-adherent group of volunteers. Valsartan is not available in the public sector in Iraq, and this may be the cause of the high prevalence of valsartan non-adherence.

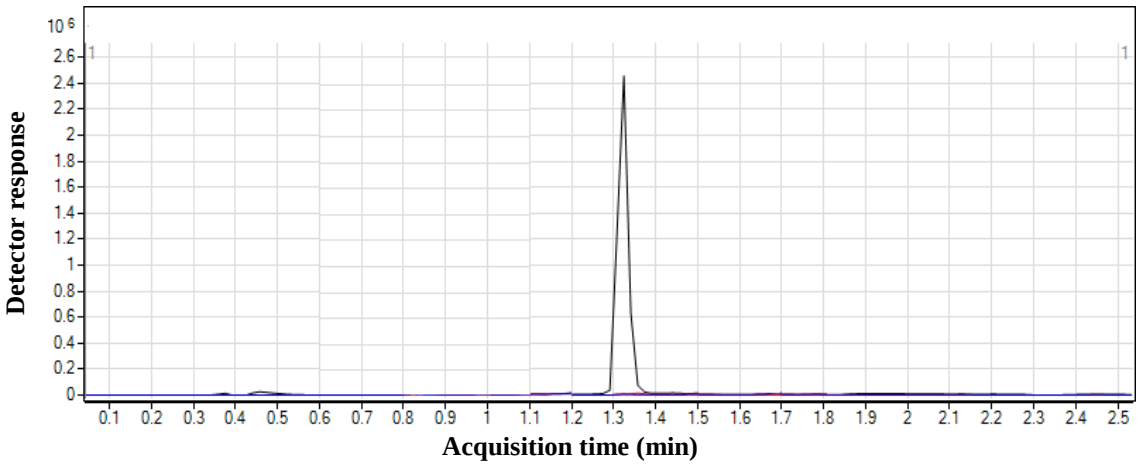

Figure 3.17. Comparison between LC-HRMS extracted ion chromatograms for valsartan (*m/z* 436.2343) in DBS samples for patient reference number …12 who was adherent to valsartan (black line) and patient reference number …11 who was non-adherent (blue line), with the red line showing a blank control.

Assessment of non-adherence by the direct, objective method of microsamples analysis in the present study showed that 154 patients (50.8%) were adherent to medications (82 males and 72 females) and 149 patients (49.2%) were non-adherent (68 males and 81 females). This level of non-adherence is almost close to 50%, which was essentially the figure reported for non-adherence in the case of chronic illnesses in developed countries by the WHO (Sabaté, 2003). These results are based on cumulative data from analyses using DBS and VAMS since results using either sampling method were in good agreement (as highlighted in 3.4.4). All previous adherence studies in Iraq used indirect methods only, and hence this is the first research to use a direct method to assess non-adherence to medications and, in particular, cardiovascular medication in Iraq. Thus, there is no previous data about the level of non-adherence found by direct methods to compare with in Iraq. This study is considered novel in this regard, and an original contribution to knowledge regarding assessment of levels of non-adherence to medications in Iraq. As mentioned in Chapter 1 Section 1.3.5, the quality of medicines is also an important issue to consider, with reports of high incidences of substandard/falsified medicines in the markets of developing countries such as Iraq. Assessment of non-adherence to the target medication in this study was based on the associated concentrations measured in the microsamples. On the other hand, there is a possibility of patients being identified as non-adherent as a result of taking substandard and falsified medicines which contain little or no active pharmaceutical ingredients. In

these cases, non-adherence would not be due to the patient's failure to take the medication as prescribed but rather as a result of treatment-related factors where the drugs actually being administered are not as they should be.

The current results indicated a huge difference in levels of non-adherence in comparison with a previous study conducted in the UK, which showed that 10% of volunteers were suspected to be non-adherent to CVD medications (Bernieh, 2017b). A possible explanation for this difference in results may be due to the relatively poor approach adopted by the Iraqi health system in comparison with that in the UK, as highlighted in Chapter 1 Section 1.3.7. In Iraq, there is no fully applicable operational multisectoral national policy, strategy or action plan to deal with cardiovascular diseases, there are no evidence-based national guidelines, protocols, or standards for the management of CVD disease or non-adherence of CVD medications through primary care in Iraq (World Health Organization, 2014; Turk-Adawi *et al.*, 2018).

Moreover, there is no insurance-based healthcare, free CVD medications schemes or medication counselling centres for cardiovascular patients in Iraq (World Health Organization, 2017d). The availability of suitable insurance or some other programme that might provide a degree of protection factor non-adherence is essentially non-existent (Schneider *et al.*, 2018). However, medication non-adherence is affected by various factors such as patients' attitudes towards medication, differential regimen complexity, patient knowledge and education, social biases hindering amicable patient-doctor relationships and access to medications. All these are considered challenges to healthcare systems worldwide. The differences in these factors might accounted for difference in non-adherence levels observed specifically between Iraq and the UK.

The differences in design between studies may be responsible for the considerable difference in non-adherence levels since for volunteers in the study conducted in the UK, patients had prior knowledge that blood microsamples would be collected in the clinic to assess adherence to the target medication, so the 'white coat' effect could be anticipated (Bernieh, 2017b). However, the Iraqi samples were collected from patients during routine visits to the clinic, that is, without prior knowledge. Moreover, the assessment of non-adherence to medication is not applicable in routine clinical practice in Iraq.

The cost of the medications prescribed may account for the high levels of non-adherence in Iraq. Patients in Iraq have to pay for medication, some of which are not available in the public sector such as atorvastatin, simvastatin and valsartan (KIMADIA, 2017) and which instead must be procured from the private sector. Paying out of pocket increases the burden on patients and consequently impacts on their adherence to medications.

Shortage of staff in the Iraqi hospitals and clinics could affect the level of non-adherence. In Iraq, the average health care worker to population ratio is 7.5:10,000 (World Health Organization, 2018a) in comparison with 21:10,000 in the UK (Yar *et al.*, 2006). Staff shortages lead to long waiting times and shorter consultation times/discussion between doctor and patient. Long waiting periods in hospital are associated with a high prevalence of medication non-adherence (Ibrahim and Deleu, 2018). Time spent with the doctor has a significant impact on the degree to which the patient engages in discussion about prescribed medications (Albaz, 1997; Brown and Bussell, 2011).

The research showed no significant correlation between non-adherence determined via blood microsamples analysis and patient gender in the current sample (Chi-squared value = 1.707, df = 1, p value = 0.185); a similar result was obtained from another study (Jones *et al.*, 2017). However, a further study indicated contradictory results, showing that non-adherence to medications was correlated with gender (Pandey *et al.*, 2015). The outcomes showed no significant correlation between level of non-adherence to the prescribed CVD medications and age ($\rho = 0.025$, p value > 0.05). Non-adherence to CVD medications in this study affects all age groups, and this is possibly due to the absence of any form of action plan to treat cardiovascular diseases, or guidelines to manage poor adherence to CVD medications. In addition, there is the absence of a free health scheme to obtain free CVD drugs, and indeed of social support programmes for CVD patients, as detailed in Chapter 1 Section 1.3.6.

There was a significant positive correlation between the level of non-adherence measured by the direct method and the number of medications in the regimen ($\rho = 0.636$, p value < 0.05) and the number of tablets taken by patients ($\rho = 0.674$, p value < 0.05). The mean (± SD) of medications in the non-adherent group was 5.46 ± 2.15, compared to 2.5 ± 1.40 in the adherent group. Polypharmacy is a common problem for patients with multiple comorbidities, where the risk of adverse drug reactions is exacerbated by an increase in

the number of the medications used (Dagli and Sharma, 2014). A similar result was seen in other studies (Ryan *et al.*, 2017; Schneider *et al.*, 2018). In contrast, other studies showed no correlation between the level of adherence and number of medications (Tomaszewski *et al.*, 2014; Pandey *et al.*, 2015; Lee *et al.*, 2018; Ulley *et al.*, 2019).

The disagreement, and thus conflicting results regarding the correlation between non-adherence and gender and number of medications show that adherence is a complex issue associated with different medication-taking behaviours such as attitudes towards medication, differential regimen complexities, patient education and knowledge, social biases that hinder amicable patient-doctor relationships, and access to medications.

Microsample-based LC-HRMS analyses were successfully used to quantify the target cardiovascular medications in blood microsamples from 303 Iraqi volunteers. Thus, this method is able to track non-adherence to each medication in the regimen, which is helpful to clinicians in terms of monitoring patient adherence to prescribed drug therapy and in guiding clinicians towards the personalisation and optimisation of patients' medications. For example, patient reference number …99 was taking bisoprolol 5 mg and valsartan 80 mg once daily, but with poor clinical outcomes. The physician referred this patient for assessment of medication non-adherence by microsampling blood analysis via the validated LC-HRMS method, the results of which indicated that this patient was only adherent to bisoprolol and non-adherent to valsartan. Figure 3.19(a) shows the LC-HRMS extracted ion chromatograms for patient reference number …99, whilst Figure 3.19(b) shows the LC-HRMS extracted ion chromatograms for patient reference number…114 who, by contrast, was adherent to both bisoprolol and valsartan. The objective blood drug concentration data provided an evidence-base to the clinician to initiate a friendly discussion with the patient ….99 to establish the causes of non-adherence and to plan the next steps in the treatment to improve the patient outcomes.

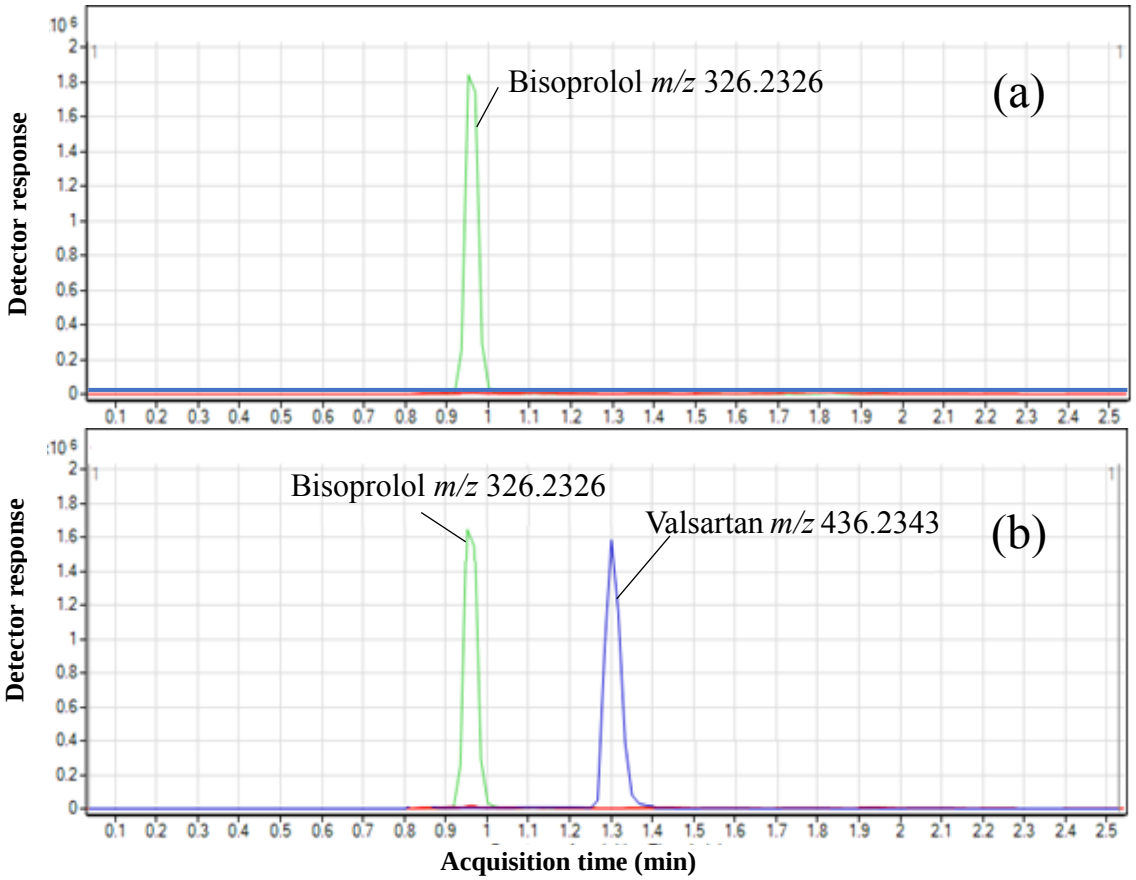

Figure 3.18. LC-HRMS extracted ion chromatograms (a) in patient reference number …99, who was adherent to bisoprolol but non-adherent to valsartan, and (b) patient reference number …114, who was adherent to both medications (green line – bisoprolol; blue line – valsartan; red line - blank).

Patient reference number…122 was taking losartan 50 mg and simvastatin 40 mg, for whom assessment of non-adherence showed adherence to losartan and non-adherence to simvastatin. Figure 3.20 shows the EIC of this patient in comparison with patient reference number…156, who was adherent to both medications.

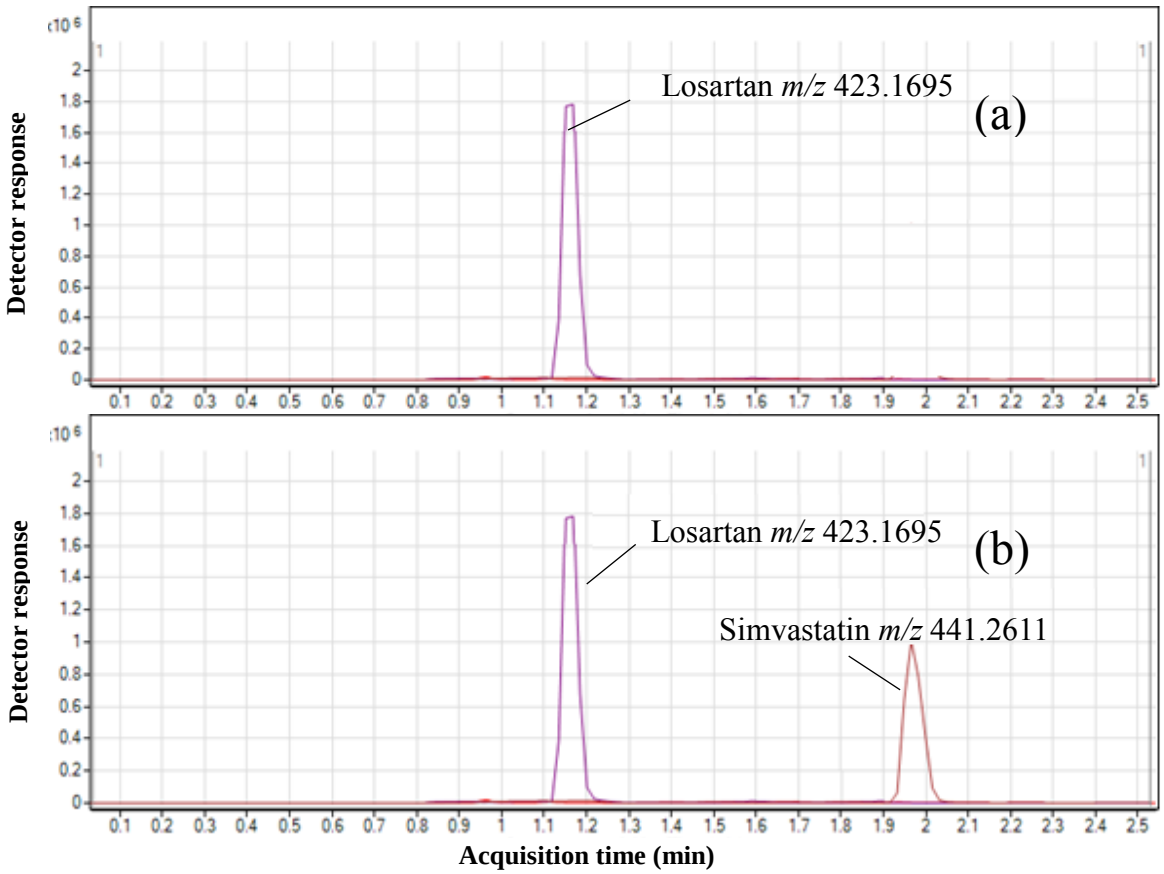

Figure 3.19. Comparison between LC-HRMS extracted ion chromatograms (a) in patient reference number …122, who was adherent to losartan but non-adherent to simvastatin, and (b) patient reference number …156, who was adherent to both medications (purple line – losartan; brown line – simvastatin; red line – blank).

Patient reference number …38 was taking bisoprolol 5 mg and losartan 50 mg. Assessment of non-adherence showed that this patient was adherent to bisoprolol and non-adherent to losartan. Figure 3.21 shows the EIC of this patient in comparison with patient reference number …27, who was adherent to both medications.

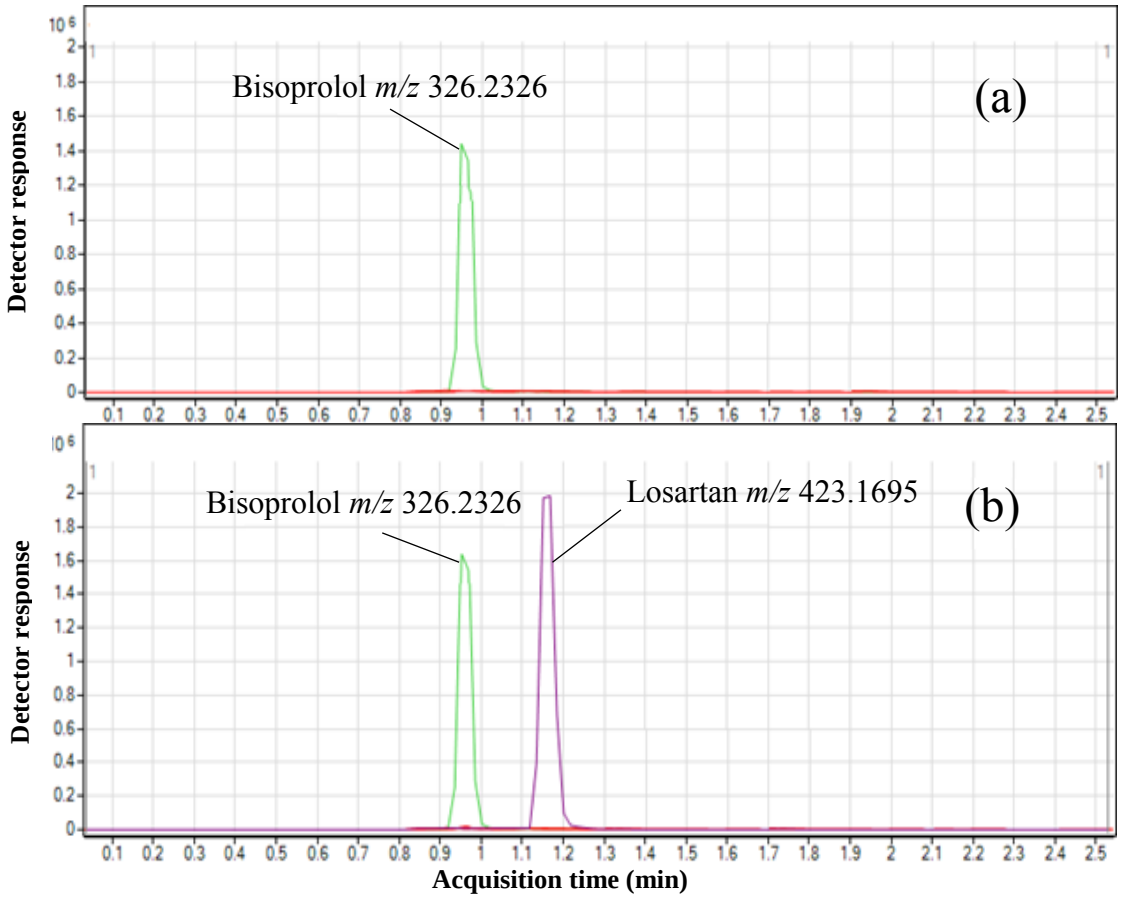

Figure 3.20. LC-HRMS extracted ion chromatograms (a) in patient reference number …38, who was adherent to bisoprolol but non-adherent to losartan, and (b) patient reference number …27, who was adherent to both medications (green line – bisoprolol; purple line – losartan; red line – blank).

The information obtained by LC-HRMS approach can individualise information for each medication for each patient and this will help to optimise medication and improve patient safety. For example, for patient reference number…99, who had poor clinical outcomes, the clinician may unknowingly increase the dose for both bisoprolol and valsartan; this in turn would increase the concentration of valsartan, which the patient was already adherent to, increasing the possibility of associated side effects.

The outcomes of this research have confirmed the possibility of the application of microsampling-based LC-HRMS in the monitoring of the CVD drugs used in routine clinical practice in Iraq. Application of such a convenient analysis method will improve adherence among CVD patients and allow self-sampling at home without need to visit a clinic.

Previous work in the literature used urine analysis as a qualitative tool for assessment of medication adherence, which provide yes/no-type answers for the existence/absence of a drug or its metabolites in the urine (Hamdidouche *et al.*, 2015, Hamdidouche *et al.*, 2017, Tomaszewski *et al.*, 2014). Microsample analysis via LC-HRMS provided quantitative data based on the concentration of the target medication measured in the blood microsamples. Moreover, HRMS provides full-scan mass spectrometry for the sample, which can provide more information if required without re-running the sample. This information should provide an evidence base for clinicians in the instance of poor patient progress, as the effectiveness of the treatment is related to blood drug concentration. Hence it will help the clinician to tailor each individual treatment to each patient.

Moreover, when urine samples are used to assess adherence, the relationship between ingestion time, ingested dose, and the amount of drug in blood cannot be established. Secondly, quantitative blood concentration data provides information on concentrations for each medication and each patient; this can help to monitor and personalise treatment, which is not otherwise possible with urine samples.

White coat syndrome is a major limitation to assessing medication adherence via direct methods, where patients take the dose before visiting the clinic. White coat syndrome is reported to be very common when urine samples are used for analysis (MacLaughlin *et al.*, 2005). However, the method used in this research can identify such a situation when it is anticipated by analyses of DBS samples collected from the same volunteers several hours apart. When the drug concentration in the second sample is significantly less than in the first sample, this would indicate that the dose was taken because the test has been anticipated, whereas in adherent patients the drug concentration would be at a comparable level, that is, indicative of the steady state.

The main limitation of the microsampling-based LC-HRMS assay developed in the current study is that the extraction of amlodipine from DBS or VAMS samples requires a separate extraction procedure to that used for the other eight CVD drugs (atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan). That means four DBS or VAMS samples are required from each patient on amlodipine and any medication from the other eight CVD medications for the analysis of volunteers' dried blood samples.

For medications which were not detected in patients with reference numbers between 263 and 290, and between 304 and 336, to ensure that LC-QTOF was working properly and that these results were not due to some malfunction or problem with extraction, data were rechecked where the internal standard and target medications in the QC samples were detected. For example, in volunteer reference number… 268, bisoprolol was not detected in their sample but both the IS and bisoprolol were in the QC samples (Figure 3.22). In another example, volunteer reference number 30, losartan was not detected in the blood microsamples, but both the IS and losartan were detected in the QC samples (Figure 3.23).

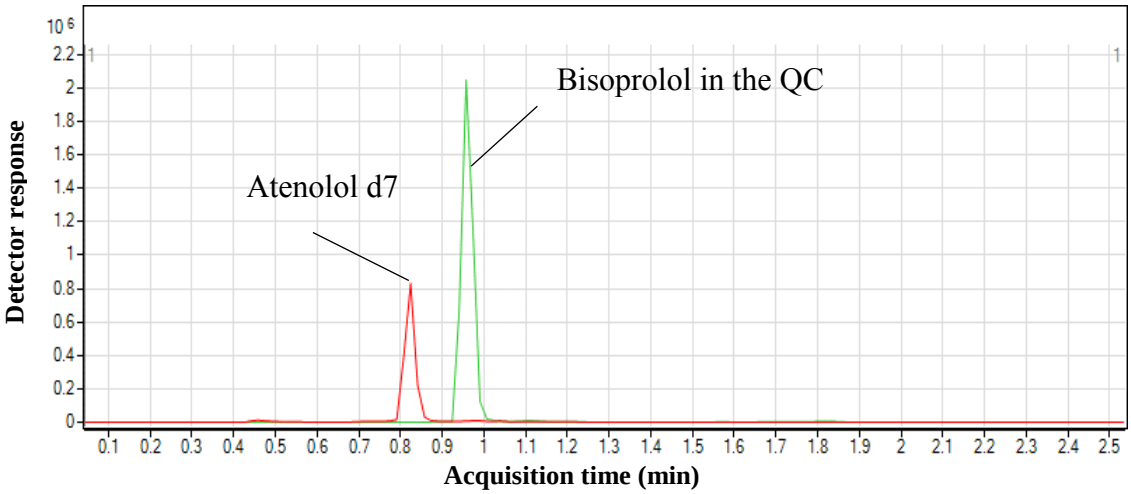

Figure 3.21. LC-HRMS extracted ion chromatograms showing the performance of the instrument in patient reference number…263 who was taking bisoprolol even though it was not detected in DBS sample, despite this medication and the internal standard (atenolol-d7) being detected in the QC.

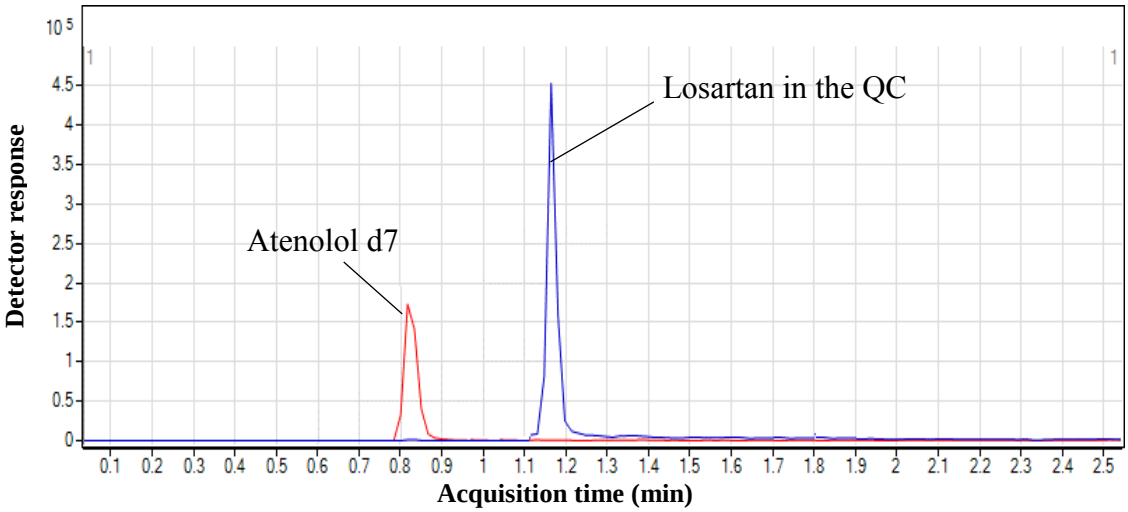

Figure 3.22. LC-HRMS extracted ion chromatograms showing the performance of the instrument in patient reference number… 265, who was taking losartan even though it was not detected in DBS sample, despite this medication and the internal standard (atenolol-d7) being detected in the QC.

3.3.3.4. Comparison between DBS and VAMS

Assessing the agreement between two methods is often one of the requirements made of medical laboratories to confirm method integrity. Correlation studies using linear relationship is not recommended, and instead a Bland-Altman plot can be used to assess the agreement between two quantitative methods by studying the mean difference, a method which now is recommended for assessment of agreement (Giavarina, 2015).

Bland-Altman plot was used to compare the results obtained from 903 sampling cards and VAMS for 75 volunteers. The *x*-axis represents the average concentration found by DBS and VAMS, whilst the *y*-axis represents the difference in concentration between DBS and VAMS. The upper limit of agreement was defined as the mean difference + two standard deviations, whilst the lower limit was defined as the mean difference - two standard deviations. The value of two is an approximation to 1.96, which is the z-value for a 95% confidence interval (Giavarina, 2015).

Bland-Altman plot showed that the measured concentration of the target medication on 903 cards and VAMS were scattered around the mean with good concordance in the concentrations found using VAMS and DBS (Figure 3.24), where the associated differences were less than 2 SD from the mean. The results confirmed acceptable reproducibility and agreement between the two microsampling methods and demonstrated that microsampling methodologies can produce comparable quantitative results and may thus be used interchangeably. A bridging study to determine the drug concentrations in 903 cards and VAMS also confirmed the integrity and accuracy of the original method.

However, there was one outlier point for patient reference number …17. The valsartan concentration measured for this patient on 903 cards was 147.34 ng/ml, but was 160.21 ng/ml on VAMS with a mean difference of -12.87 ng/ml. This difference in concentration may be due to sampling error resulting from overfilling the VAMS tip (Tanna *et al.*, 2018).

The results showed significant agreement between 903 cards and VAMS in the determination of the concentrations of selected cardiovascular medications in the blood of Iraqi volunteers. However, taking blood samples on 903 cards is more difficult than

VAMS for some volunteers as a sufficient volume of blood has to be deposited on the predetermined circles located on the card. Sometimes, blood drops fall outside the sampling area when the finger is directed towards the spotting area. 903 cards require more time to complete sampling and additional assistance is sometimes required. Care is required to avoid touching the blood spot on the card as this leads to sample contamination.

In contrast, VAMS is designed to absorb a fixed volume of blood until the substrate is full, making VAMS easier and quicker, and for which assistance is not required; in comparison with DBS, this method facilitates patient self-sampling. Moreover, there is no need for drying racks or the use of a puncher with VAMS as the entire tip is extracted and analysed, saving time and effort. Sometimes clamshell may cause inconvenience. DBS cards are easier to label as VAMS have no suitable labelling surface on the clamshell or plastic holder. Although VAMS has many advantages over 903 cards, the cost of VAMS must also be considered.

VAMS appears to be more promising than DBS due to ease of use and, most importantly, the fact that it overcomes HCT bias issues and sample inhomogeneity. Cost, it seems, is the only disadvantage of the VAMS microsampler in comparison to 903 cads; one VAMS sampler is almost five times more expensive than a 903 card. Therefore, the cost of a VAMS microsampler is, unfortunately, a significant consideration, especially in areas with limited resources (Kip *et al.*, 2017).

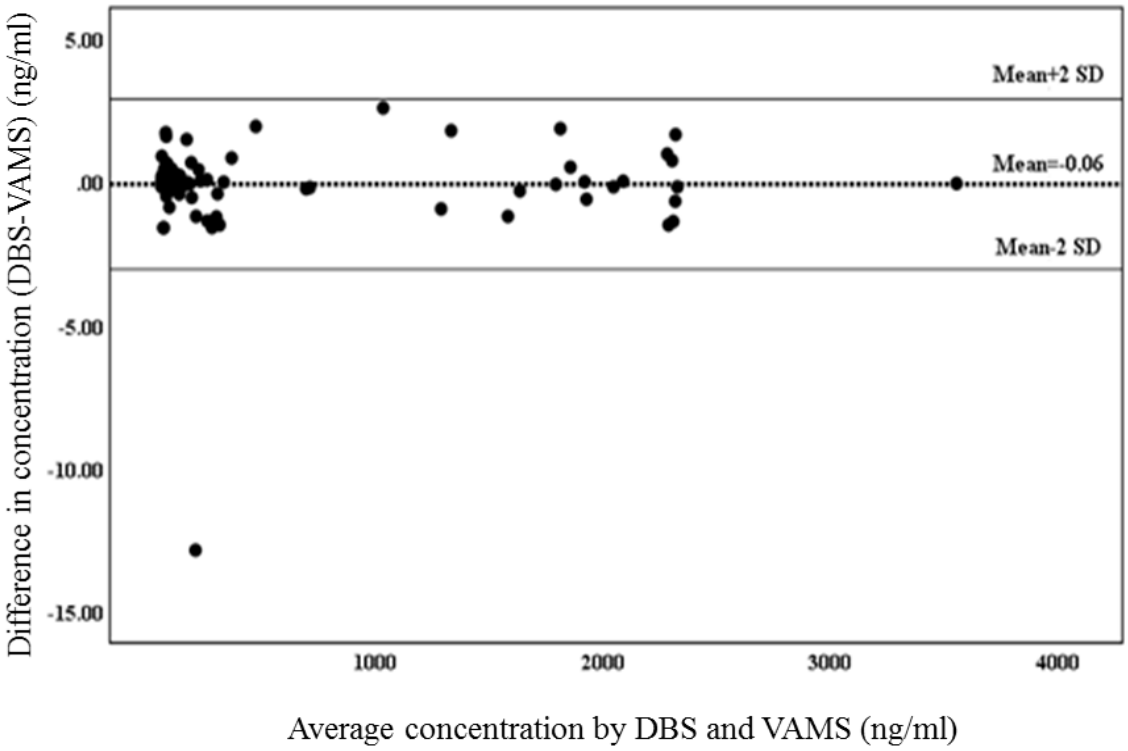

Figure 3.23. Bland-Altman plot comparing DBS and VAMS concentrations for the target medications in volunteer samples.

3.4. Conclusion

The application of microsampling-based LC-HRMS analyses is a potential alternative to conventional methods of monitoring CVD drugs in routine clinical practice, providing objective and specific information for each individual medication in any given patient's regimen. All the mass spectral data from the sample is collected in one run with no need to run the sample again, as data can be revisited at some later point if there is need for more clinical assessment to help manage the patient's condition. For example, the patient may be taking medications in addition to the prescribed medications without having informed their doctor of such, where such medications may be responsible for poor clinical outcomes. In the case of poor patient progression to medications, this will provide an evidence base for clinicians that could help determine whether this is due to medication non-adherence, incorrect diagnosis, or poor selection of medication.

Therapeutic drug monitoring (TDM) in routine clinical practice is currently limited because obtaining blood samples is highly invasive (venepuncture) and requires s phlebotomist and prior booking of clinical appointments. Therapeutic drug monitoring using microvolume blood samples collected by a less invasive approach can help clinicians to optimise and personalise a patient's medication. This approach is more convenient to patients because it is less invasive and allows self-sampling at home, and so without the need to visit a clinic. Medication individualisation and optimisation of the use of medication allows for huge savings due to better patient outcomes, reducing avoidable hospital readmissions, and lowering associated mortality rates.

The application of the microsampling approach may be more feasible and economic to both patient and healthcare providers in comparison with the conventional approach. The former approach has its advantages for patients in term of saving patient time and costs as it eliminates the need to book an appointment with a phlebotomist or to travel to a clinic to provide the blood sample. The ease of transportation and reduced storage requirements of DBS samples – without the need for cooling, ice boxes, or dry ice – will reduce the cost in comparison with conventional methods, making it is easy to collect DBS samples in remote areas where there is limited infrastructure. They can then be sent via standard postal services to a hospital laboratory for analysis in less affluent regions. In addition, the DBS and VAMS sample collection methods do not require the use of syringes or collection tubes. Thus, disposal of DBS and VAMS is easy in comparison with the disposal of liquid samples such as plasma or indeed the disposal of containers or syringes.

The level of non-adherence to the target cardiovascular medication by measurement of drug concentration in the DBS samples was 49.2%. However, non-adherence to CVD medication was not uniform, and patients may adhere differently to each medication in their prescribed regimens. This high rate of non-adherence could explain the high mortality rate in Iraq from cardiovascular diseases especially in the absence of strategies to manage and control the risk factors associated with cardiovascular diseases. The outcomes of the study showed no significant relationship between non-adherence to cardiovascular medications and gender or age. However, there was significant positive

correlation between non-adherence and number of medications and number of tablets taken per day.

Measuring the concentration of the target cardiovascular medications in dried blood samples from Iraqi volunteers on 903 cards and VAMS showed both reliable and comparable data with no significant bias, which confirmed the integrity of the outcomes and showed the acceptability of the validated microsampling-based LC-HRMS method for the quantitative determination of CVD drugs when using DBS or VAMS. Nevertheless, of the two VAMS is considerably more patient friendly and convenient, enabling self-sampling at home rather than requiring a visit to the clinic and the services of the appropriate medical professional(s).

The main limitation of the microsampling-based LC-HRMS assay developed in the current study is that the extraction of amlodipine from DBS or VAMS samples requires a separate extraction procedure.

As the direct method cannot provide information about factors associated with non-adherence, Chapter 4 assesses the non-adherence amongst the same patient sample as in the current chapter by application of the Morisky Medication Adherence Scale (MMAS-8) to understand the causes associated with non-adherence and to allow comparison with the objective data gathered in this chapter.

Chapter 4

Assessment of Non-adherence to Cardiovascular Medications by the Eight-item Morisky Medication Adherence Scale (MMAS-8).

This chapter focusses on the application of the eight-item Morisky Medication Adherence Scale (MMAS-8) to assess non-adherence to the cardiovascular medications (Chapter 1 Section 1.4) most frequently prescribed to Iraqi volunteers who were prescribed one or more such medications. In addition, a number of factors associated with medication-taking behaviour such as gender, age, number of different medications and number of tablets taken daily is discussed.

4.1. Introduction

Indirect methods for the assessment of medication non-adherence such as the use of a validated questionnaire have been found to be more popular used in clinical practice (Garfield *et al.*, 2011p Moon *et al.*, 2018). However, there is no agreement regarding the questionnaire of choice (Eskås *et al.*, 2016).

Morisky *et al.*(2008) developed a self-reported scale with four items with respect to common medication-taking behaviour that could lead to drugs not being taken, and indeed this scale has been used widely (Morisky *et al.*, 2008). However, in order to overcome some of its limitations, four additional items addressing the circumstances surrounding such behaviour were used to supplement the original four items (Morisky *et al.*, 1986; Shalansky *et al.*, 2004; Thorpe *et al.*, 2009). This updated scale was named the eight-items Morisky Medication Adherence Scale (MMAS-8) which is probably the most well-known and recognized self-report questionnaire to be used as a non-adherence screening tool across a range of circumstances (Lam and Fresco, 2015) including cardiovascular diseases (Kassab *et al.*, 2013; Park *et al.*, 2014; Vinluan *et al.*, 2015; Granger *et al.*, 2015; Kharameh *et al.*, 2018; Kosobucka *et al.*, 2018), diabetes mellitus (Bramlage *et al.*, 2014; Chan and Hassali, 2014; Arora *et al.*, 2014; Cummings *et al.*, 2014; Guo *et al.*, 2014; Tabasi *et al.*, 2014; Katalenich *et al.*, 2015; Almadhoun and Hala, 2018), neoplasm (Berry *et al.*, 2015) and chronic kidney diseases (Kefale *et al.*, 2018).

The MMAS-8, as mentioned, consists of eight items, the first seven of which require yes/no answers, while question 8 is rated according to a five-point Likert scale rating (strongly disagree to strongly agree) (Table 4.1). The total score that can be awarded in the MMAS-8 ranges from 0 to 8. Scores of less than 6 indicate low adherence, scores of

6 to 8 indicate moderate adherence, and a score of 8 indicate high adherence (Morisky *et al.*, 2008).

Table 4.1. Questions constituting the eight-item Morisky Medication Adherence Scale (MMAS-8).

Questions	**No=1**	**Yes=0**
1. Do you sometimes forget to take your cardiovascular medication(s)?		
2. People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your cardiovascular medication(s)?		
3. Have you ever cut back or stopped taking your medication(s) without telling your doctor, because you felt worse when you took it?		
4. When you travel or leave home, do you sometimes forget to bring along your cardiovascular medication(s)?		
5. Did you take your cardiovascular medication(s) yesterday?		
6. When you feel like your cardiovascular disease is under control, do you sometimes stop taking your medication(s)?		
7. Taking medication(s) every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your cardiovascular treatment plan?		
8. How often do you have difficulty remembering to take all your medication(s)? **(Please circle your answer below)** Never/Rarely **4** Once in a while **3** Sometimes **2** Usually **1** All the time **0**		

MMAS-8 has been translated into more than 50 languages because of the simplicity of its application and scoring (Morisky *et al.*, 1986; Oliveira-Filho *et al.*, 2012). An Arabic version of MMAS-8 is used in Arabic countries for the assessment of adherence to

medications for a range of diseases (Sa'ed *et al.*, 2013; Aljumah *et al.*, 2014; Alkatheri *et al.*, 2014; Ashur *et al.*, 2015; Alhalaiqa *et al.*, 2016). As Arabic is the official language in Iraq, MMAS-8 can be used there without further adaptation. MMAS-8 is protected by copyright and prior permission to use it is required from its owner, Professor Donald Morisky (Appendix 15).

Little attention has been paid to non-adherence to medications in Iraq. There are very limited studies that have used MMAS-8 to assess medication non-adherence in Iraq; Jamal and Saleem (2014) used it for assessment of adherence amongst diabetic and hypertensive patients (Jamal and Saleem, 2014), for instance, and Al-Tukmagi and AL-Auqbi (2015) used MMAS-8 to assess non-adherence to oral hypoglycaemic agents in a sample of Iraqi patients with type 2 diabetes mellitus (Al-Tukmagi and Al-Auqbi, 2015). There have been other studies in Iraq which used non-standardised questionnaires prepared by the researchers involved in these studies as indirect methods of assessing non-adherence to cardiovascular medications (Samer, 2008; Hasan *et al.*, 2011; Bushra and Kameran, 2013). Therefore, the outcomes of this study can be compared to at least few previous studies since MMAS-8 is employed. There has been no known use of direct methods to assess medication non-adherence in Iraq. Thus, the application of the direct method used in this research to assess medication non-adherence is novel research in Iraq.

The MMAS-8 scale can be used to assess both medication-taking behaviour and some of the reasons for such non-adherence, such as gaining an understanding of the medication regimen, reasons for non-adherence, patient's attitudes and beliefs toward medicines, and other factors (Nguyen *et al.*, 2014; Menditto *et al.*, 2015). Each of the eight questions in MMAS-8 assesses a specific medication-taking behaviour (Morisky *et al.*, 2008; Bae *et al.*, 2015; Bae *et al.*, 2016). The questions associated with intentional non-adherence include:

- Have you ever cut back or stopped taking your medication(s) without telling your doctor, because you felt worse when you took it?
- When you feel like your cardiovascular disease is under control, do you sometimes stop taking your medication(s)?
- Taking medication(s) every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your cardiovascular treatment plan?

The questions associated with unintentional non-adherence include:

- Do you sometimes forget to take your cardiovascular medication(s)?
- People sometimes miss taking their cardiovascular medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your cardiovascular medication(s)?
- When you travel or leave home, do you sometimes forget to bring along your cardiovascular medication(s)?
- How often do you have difficulty remembering to take all your medication(s)?

A patient is labelled as an intentional non-adherent patient when the majority of their answers infer intentional non-adherence. On the other hand, the patient is labelled as unintentionally non-adherent when the majority of their answers infer unintentional non-adherence (Menditto *et al.*, 2015).

One of the objectives of this study was to assess non-adherence to cardiovascular medications in adult Iraqi patients who had been prescribed one or more of CVD medication. This was achieved through the application of a standardized Arabic version of the eight-item Morisky Medication Adherence Scale (MMAS-8) to help understand some of the reasons associated with poor adherence. The results of this section of the study will be compared with the findings from previous Iraqi studies and literature.

4.2. Material and Methods

4.2.1. Ethics Statement

Ethical approval for the application of an MMAS-8 questionnaire was obtained from the Ethical Committee in Misan Health Directorate (Appendix 1) and from De Montfort University's Faculty of Health and Life Science Research Ethics Committee (Appendix 16). Written informed consent was obtained from each participant.

Recruited patients, as detailed in Chapter 3 Section 3.2.2, who provided blood samples as detailed in Chapter 3 Section 3.2.6, were provided with Arabic translations of:

- Participant information leaflets
- Consent form

- Eight-item Morisky Medication Adherence Scale (MMAS-8).

The participant information leaflet (PIL) and the consent form are detailed in Appendices 17 and 18, with the MMAS-8 as previously detailed in Table 4.1. The Arabic version of PIL, the consent form and the Arabic version of the MMAS-8 are detailed in Appendices (19-21). Consent forms and questionnaire papers were transported to the UK and stored in a secure place at De Montfort University, Faculty of Health and Life Sciences, Room 00.15.

4.2.2. Evaluation of Medication Non-adherence Using MMAS-8

Medication non-adherence to CVD medications was assessed using a validated Arabic version of MMAS-8 (Morisky *et al*., 2008; Krousel-Wood *et al*., 2009; Morisky and DiMatteo, 2011). The scale consists of eight standardized questions; questions 1 to 7 require an answer of YES (assigned a score of 0) or No (assigned a score of 1) while question 8 is a Likert scale-type question which has a five-item rating scale (scores of 0-4). Regarding item 5, the response is reversed in a positive direction where Yes = 1 and No = 0.

Item 8 uses a five-point Likert scale and can take one of five values (0-4) which has to be divided by 4 to get the summated score. The level of adherence is determined by summating the scores for items 1–7, and then adding the result of the summation of item 8 (De las Cuevas and Peñate, 2015).

4.2.3. Statistical Analysis

Statistical analyses were conducted using the SPSS software (version 22. Armonk, NY: IBM Corp). Qualitative variables such as gender and medications were expressed in terms of frequencies and percentages. The Spearman's correlation coefficient was used to determine the relationship between levels of non-adherence as measured by the MMAS-8 and patients' ages, number of CVD medications in patients' regimens and numbers of tablets taken. A Chi-squared test was used to examine the relationship between levels of non-adherence and gender. A P-value less than 0.5% was considered significant**.**

4.3. Results and Discussion

4.3.1. Patient Characteristics

303 Iraqi patients were recruited in this study: 150 males (49.5%) and 153 females (50.5%) with a mean age of 53.93 (SD = ±8.97). Patients were prescribed one or more CVD medications, where the mini-DBS questionnaire enabled the identification of which CVD medication(s) each volunteer had been prescribed. The prescribed CVD medications, the number of CVD medications per regimen, and the number of tablets taken per day, as detailed in Tables 4.2 and 4.3, were extracted from the mini-DBS questionnaire, as detailed in chapter 3 section 3.2.2. Patients' ages were obtained from their clinical records, where patient characteristics and medication(s) prescribed to patients are summarized in Table 4.2 and Table 4.3.

Table 4.2. Patient population sample characteristics (n = 303).

Variables	**Total number of participants = 303**	
	N	%
Gender		
Male	150	49.5
Female	153	50.5
Age	Mean (SD) 53.93 (8.97)	
30-39	25	8.3
40-49	87	28.7
50-59	100	33
60-69	91	30
Number of medications	Mean (SD) 3.95 (2.33)	
1-2	130	42.9
3-4	57	18.8
5-6	53	17.5
>6	63	20.8

Table 4.3. Medications Prescribed for the treatment of CVD in the Iraqi sample.

Medication type	N (%) of patients prescribed medication
β blockers	
Atenolol	59 (13.5)
Bisoprolol	77 (17.5)
ACE inhibitor	
Lisinopril	73 (16.7)
Angiotensin II Receptor Blockers	
Valsartan	65 (14.8)
Losartan	47 (10.7)
Statins	
Atorvastatin	18 (4.1)
Simvastatin	50 (11.4)
Calcium Channel Blockers	
Amlodipine	15 (3.4)
Diltiazem	34 (7.8)
Total	438 (100)

4.3.2. Medication Non-adherence

Patients were categorized into three groups based on their responses to the MMAS-8 questions: low adherence (MMAS-8 score < 6), medium adherence (MMAS-8 score 6 to < 8) or high adherence (MMAS-8 score of 8). The current study found that 54.1% (164 participants) showed high adherence, 27.7% (84 participants) showed medium adherence and 18.2% (55 participants) showed low adherence (Table 4.4). Responses to MMAS-8 for all 303 participants are summarized in Table 4.5. For the purposes of this analysis, patients were classified as adherent or non-adherent rather than low, medium and high using a score of 6 as the cut-off point (Morisky *et al.*, 2008; Khayyat *et al.*, 2017). Thus, 248 participants (81.8%) were adherent of which 125 were male (50.4%) and 123 were female (49.6%). By contrast, 55 participants (18.2%) were non-adherent (25 males, 45.5%, and 30 females, 54.5%).

The proportion of non-adherent patients, as determined by MMAS-8 in the current study, was 18.2%. This result almost matches that observed in an earlier study in Iraq, where the reported level of non-adherence was 19.6% (Jamal and Saleem, 2014). However, the level

of non-adherence to CVD medication in this research was significantly lower than that reported in other studies in Iraq (Al-Dabbagh and Aswad, 2009; Hasan *et al.*, 2011; Bushra and Kameran, 2013) and less than the non-adherence reported for other developing countries such as Lebanon (22.4%) (Yassine *et al.*, 2015) Saudi Arabia (33.7%) (Altuwairqi, 2016) and Iran (54%) (Moharamzad *et al.*, 2015).

Table 4.4. Adherence amongst Iraqi cardiovascular disease patients based on MMAS-8

Adherence level (score)	**Total study population (N = 303)**	
	N	%
Low adherence	55	18.2
Medium adherence	84	27.7
High adherence	164	54.1
Total	303	100

The difference in the level of non-adherence found in the current study and the literature may suggest that adherence is a complex and dynamic psychological behaviour issue. Non-adherence can be affected by many variables such as differences in the study populations, or other factors such as patient knowledge, the complexity of the medical regimen, and patients' health conditions. It is possible that patients overestimated their adherence in the current study to a greater degree than in previous studies. The possibility of such overestimation can be confirmed through a comparison with direct methods of assessment of medication adherence by determining drug levels in dried blood spots obtained from the same study participants (Chapter 3).

The results of the present study revealed no significant relationship between the level of non-adherence assessed by MMAS-8 and gender (Chi squared value = 0.441, df = 1, p value = 0.507). In the literature, there are conflicting results about the correlation between adherence and gender. Results from the USA, Saudi Arabia, Hong Kong and the UK showed greater levels of non-adherence in females than males (Irvin *et al.*, 2012; Pandey *et al.*, 2015; Kang *et al.*, 2015; Safaa and Ali, 2015; Khayyat *et al.*, 2017; Gohar *et al.*, 2008). However, other studies showed higher levels of non-adherence in males than females (Al-Dabbagh and Aswad, 2009: Jamal and Saleem, 2014; Sandoval *et al.*, 2018). Discrepancies between the level of non-adherence to cardiovascular medications and gender in such studies may indicate complex psychological behavioural factors and

sociological gender-based dynamics are at play. Such factors may include social biases that hinder amicable patient-doctor relationships due to social, cultural or religious issues.

Table 4.5. Responses for each question in the MMAS-8 scale.

Questions	**Study population (N = 303)**				
	Yes (%)	No (%)			
1. Do you sometimes forget to take your cardiovascular medication(s)?	59 (19.5)	244 (80.5)			
2. People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past-two weeks, were there any days when you did not take your cardiovascular medication(s)?	45 (14.9)	258 (85.1)			
3. Have you ever cut back or stopped taking your medication(s) without telling your doctor, because you felt worse when you took it?	75 (24.8)	228 (75.2)			
4. When you travel or leave home, do you sometimes forget to bring along your cardiovascular medication(s)?	17 (5.6)	286 (94.4)			
5. Did you take your medication(s) yesterday?	260 (85.8)	43 (14.2)			
6. When you feel like your cardiovascular disease is under control, do you sometimes stop taking your medication (s)?	73 (24.1)	230 (75.9)			
7. Taking medication(s) every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your cardiovascular treatment plan?	43(14.2)	260 (85.8)			
8. How often do you have difficulty remembering to take all your medication(s)?	All the time 0 (0%)	Never/Rarely 286 (94.4)	Sometimes 8 (2.6)	Once in a while 5 (1.7)	Usually 4 (1.3)

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Bivariate correlation using Spearman's correlation coefficient (ρ) showed no significant correlation between age and non-adherence to CVD medications ($\rho = 0.092$, p value > 0.05). This result was in line with the findings reports in several different studies (Bushra and Kameran, 2013; Krueger *et al.*, 2015; Pandey *et al.*, 2015; Jin *et al.*, 2016). However, this was contrary to other studies which suggested a significant correlation between age and non-adherence to antihypertensive medication (Ramli *et al.*, 2012; Alhewiti, 2014; Meinema *et al.*, 2015; Yap *et al.*, 2016; Khayyat *et al.*, 2017). Results indicating a lack of correlation between age and levels of non-adherence are agreeable due to certain factors highlighted in Chapter 1(Table 1.2).

Bivariate correlation using Spearman's correlation coefficient (ρ) indicated a significant positive correlation between non-adherence and number of medications taken ($\rho = 0.966$, p value < 0.05) and the number of tablets of different medications taken ($\rho = 0.976$, p value < 0.05). As the number of medications taken by a patient increased, the possibility of non-adherence correspondingly increased. In the non-adherent group, the mean number of cardiovascular medications taken by patients was 6.53 ± 1.63 in comparison with 3.38 ± 2.07 in the adherent group. The need to take a larger number of medications can lead to non-adherence because this can lead to errors in dosing and administration. Furthermore, medications may be missed on a daily basis and, thus, increase the possibility of adverse drug events and impose a treatment-related burden on patients. All the above can lead to medication non-adherence (Marcum and Gellad, 2012; Kvarnstrom *et al.*, 2018). There are also conflicting results between this study and others regarding the correlation between number of medications in a regimen and subsequent adherence. Some of these studies indicate that low adherence to CVD medications is associated with the number of medications taken (Shalansky and Levy, 2002; Choudhry *et al.*, 2011; Bazargan *et al.*, 2017). By contrast, other studies have showed that regimen complexity and number of medications may not influence the level of non-adherence (Stange *et al.*, 2013; Jamal and Saleem, 2014). The responses to the MMAS-8 in the non-adherent group (n = 55 patients) are summarized in Table 4.6 where the responses of non-adherent patients to MMAS-8 are:

- 85.5% of non-adherent participants, according to MMAS-8, answered YES to the question "*When you feel like your cardiovascular disease is under control, do you*

sometimes stop taking your medication (s)?". This response may indicate that patients do not understand their disease well enough to realise that it is a chronic condition (Oliveira-Filho *et al.*, 2014; Alhalaiqa *et al.*, 2016) which thus requires continuous treatment, and that medications should not be stopped even when the patient feels better.

- 81.8% of non-adherent participants, according to MMAS-8, answered YES to the question "Do *you sometimes forget to take your cardiovascular medication(s)?*" where this response indicates that the major cause of non-adherence was forgetfulness (Al-Ramahi, 2015; Alhalaiqa *et al.*, 2016; Miyazaki *et al.*, 2018).

- 81.8% of non-adherent participants, according to MMAS-8, answered YES to the question "*Have you ever cut back or stopped taking your medication(s) without telling your doctor, because you felt worse when you took it?*" which indicates that medication-based side effects accounted for considerable non-adherence among CVD patients (Al-Ramahi, 2015; Alhalaiqa *et al.*, 2016).

- 70.9% of non-adherent participants, according to MMAS-8, answered YES to the question "*People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past-two weeks, were there any days when you did not take your cardiovascular medication (s)?*". Patients gave no reasons for not taking medications. This indicates that the questionnaire itself may need improvement in order to better assess the reasons for non-adherence. For instance, other reasons for non-adherence which are not addressed in the questionnaire may be the financial cost of the medications, or patients' beliefs about their use.

Table 4.6. The responses to the eight-item Morisky Medication Adherence Scale (MMAS-8) in the non-adherent group of patients.

Questions	**Study population (N = 55 patients)**				
	Yes (%)	No (%)			
1. Do you sometimes forget to take your cardiovascular medication(s)?	45 (81.8)	10 (18.2)			
2. People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past-two weeks, were there any days when you did not take your cardiovascular medication(s)?	39 (70.9)	16 (29.1)			
3. Have you ever cut back or stopped taking your medication(s) without telling your doctor, because you felt worse when you took it?	45 (81.8)	10 (18.2)			
4. When you travel or leave home, do you sometimes forget to bring along your cardiovascular medication(s)?	14 (25.5)	41 (74.5)			
5. Did you take your medication(s) yesterday?	17 (30.9)	38 (69.1)			
6. When you feel like your cardiovascular disease is under control, do you sometimes stop taking your medication (s)?	47 (85.5)	8 (14.5)			
7. Taking medication(s) every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your cardiovascular treatment plan?	34 (61.8)	21 (38.2)			
8. How often do you have difficulty remembering to take all your medication(s)?	All the time	Never/Rarely	Sometimes	Once in a while	Usually
	0 (0%)	50 (90.9)	2 (3.6)	0 (0%)	3(5.5)

- 61.8% of non-adherent participants, according to MMAS-8, answered YES to the question "*Taking medication(s) every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your cardiovascular treatment plan?*". This shows that non-adherent patients felt sticking to their medications to be an imposition (Al-Ramahi, 2015; Alhalaiqa *et al.*, 2016). The tools applied could not provide information about the sources of this inconvenience. However, reasons for such feelings of inconvenience could be the dose frequencies, prescription refills, the need to take with or without food, etc.

- 25.5% of non-adherent participants, according to MMAS-8, answered YES to the question "*When you travel or leave home, do you sometimes forget to bring along your cardiovascular medication(s)?*" According to the answers received, patients forgetting to bring their medications while traveling or leaving home accounted for their non-adherence (Alhalaiqa *et al.*, 2016). The majority of non-adherent participants (90.9%) have no problem with remembering to take their medications.

This research is one of the few studies in Iraq to have assessed non-adherence to cardiovascular medications. This study identified five risk factors associated with CVD medication non-adherence. These are: poor understanding of the diseases, forgetfulness, side effects, medication inconvenience and travelling and leaving home.

Several limitations to the present study in using MMAS-8 to assess medication non-adherence need to be acknowledged:

- The sample size in this study was determined based on the prevalence of hypertension because there was no documentation about the prevalence of cardiovascular diseases in Iraq. Thus, the results may not have precisely determined the level of non-adherence to CVD medications amongst all CVD patients.
- The samples were taken at two hospitals in Misan, where patient characteristics may vary from other regions in Iraq. Moreover, the study excluded patients who were unable to read and write and those with cognitive impairment, which may limit the generalisability of this study.

- The assessment of medication adherence by application of MMAS-8 may be subject to a degree of overestimation. Patients may not answer questions truthfully in an attempt to please their doctors, instead claiming what they believe their doctors want to hear from them.
- There is a possibility of bias in the responses known as the "Acquiescence response", where the participants give affirmative answers regardless of the content of the question, and this possibility for bias might be expressed within the self-reported questionnaire (Watson, 1992).
- MMAS-8 provides a single qualitative adherence assessment depending on the total score of a patient's responses to the questions and it thus unable to differentiate non-adherence to multiple medications in the prescribed pharmacotherapy regimens. For example, a patient subject to polypharmacy may unintentionally take a double dose of one medication and miss the other. In such scenarios, the medication was taken but the patient took the wrong dose and therefore this patient was nevertheless non-adherent.
- MMAS-8 categorizes patients as adherent or non-adherent based on their medication-taking behaviour as defined by the answers to the eight questions. However, there are differences between females and males in terms of their body compositions, physiologies (e.g., influence of hormones during the menstrual cycle, menopause, pregnancy), pharmacokinetics (e.g., reduction in renal and liver functions due to aging) and pharmacodynamic parameters (Jochmann *et al.*, 2005; Sera and McPherson, 2012; Rosano *et al.*, 2015; Tamargo *et al.*, 2017). Thus, the efficacy and safety of medications can differ depending on the sex of the patient (Rosano *et al.*, 2015; EUGenMed *et al.*, 2016).
- The application of MMAS-8 is not helpful in personalizing CVD treatments. For example, a patient may have a genetic difference in the production of enzymes responsible for drug metabolism, leading to either unusually slow metabolism of medications (e.g., clopidogrel) or unusually rapid metabolism (e.g., Warfarin) (Vermeire *et al.* 2001). Consequently, patients may have different therapeutic outcomes despite taking the same dose. Clopidogrel is a prodrug that is absorbed through the intestines and then metabolized in the liver to form its active metabolites (Savi *et al.*, 1992; Lins *et al.*, 1999). Slow metabolism of clopidogrel

will lead to the reduced biotransformation of clopidogrel to its active metabolite. On the other hand, warfarin is metabolized into inactive metabolites (Cavallari and Limdi, 2009) which will lead to variation in clinical outcomes among patients taking the same dose and thus increase the possibility of cardiovascular events.

- The MMAS-8 can only capture a few of the reasons associated with non-adherence such as forgetfulness, patient knowledge and medication-based side effects. Thus, it is difficult to develop interventions to improve adherence based purely on the results of the MMAS-8 scale (Unni and Farris, 2015).

4.4. Conclusion

The application of MMAS-8 as a tool to assess adherence indicated that 81.8% of patients were adherent to the target cardiovascular medications and accordingly that 18.2% of patients were non-adherent. The main causes of non-adherence were a limited understanding of the medication regimen, medication-based side effects and forgetfulness. However, some patients gave no reasons for their poor adherence which may indicate that the MMAS-8 needs further development to gain a better assessment of the reasons behind non-adherence.

The use of MMAS-8 as the sole method of assessment of medication non-adherence has certain drawbacks, such as overestimation and inability to track non-adherence for each medication in multiple drug regimens. MMAS-8 can assess medication-taking behaviour but cannot identify pharmacokinetic differences between individual patients, which can lead to variations in clinical outcomes for a given dose in different patients. MMAS-8 can simply generate yes or no results, and it is well-known that self-report questionnaires can produce a certain bias in results in terms of assessment of non-adherence. This indicates the need for a reliable and practical approach to assess medication non-adherence. The desired clinical outcomes for Iraqi patients can be achieved by optimising the use of medicines through application of a feasible, time efficient and objective therapeutic drug monitoring method that can allow the adherence to each medication in the regimen to be assessed with due consideration for patient-to-patient pharmacokinetic and pharmacodynamic variations. The results based on MMAS-8 also showed that there was no correlation between the level of non-adherence to CVD medication and gender or age.

On the other hand, MMAS-8 showed a significant positive correlation between non-adherence and number of medications in the patient's regimen and the number of tablets taken by the patient.

The next chapter compares the assessment of medication non-adherence by MMAS-8 and by analysis of DBS samples via LC-HRMS to study the agreement and disagreement between the two approaches.

Chapter 5

Comparative Assessment of Non-adherence as Determined by the Eight-item Morisky Medication Adherence Scale (MMAS-8) and Determination of Drug Concentrations in Blood Microsamples

This chapter compares the results of non-adherence in 303 Iraqi volunteers using two different approaches, namely the indirect method of using a standardized Arabic version of the eight-item Morisky Medication Adherence Scale (MMAS-8) and the direct method of analysis of blood microsamples via liquid chromatography-high resolution mass spectrometry (LC-HRMS). The combination of these two methods was expected to help to confirm whether volunteers were adherent or otherwise to the prescribed CVD medications. Additionally, some of the causes of non-adherence were expected to be obtained by the application of MMAS-8.

5.1. Introduction

Although various strategies have been employed to measure prescription adherence, to date no consensus has been reached as to an appropriate 'gold standard' for such for application in routine clinical practice (Kennedy *et al.*, 2008). As detailed in chapter 2 Section 2.6, the various methods used for assessment of non-adherence each have their particular strengths and weaknesses, with trade-offs between accuracy and practicality, which makes their acceptability subjective. Moreover, each method (direct or indirect) provides different information on medication-taking behaviour (Vitolins *et al.*, 2000; Lehmann *et al.*, 2014).

According to a report by the World Health Organization (WHO) entitled "Adherence to Long-Term Therapies", a multi-measure approach, as applied by combining feasible self-reporting and reasonable objective approaches, is recommended (Sabaté, 2003). Selecting two or more methods allows one method's strengths to compensate for the weaknesses in another, improving the quality of information used to determine adherence levels.

In the current research, a combination of the indirect and direct methods should provide more comprehensive information about non-adherence and its causes, facilitating more effective efforts towards improving adherence by the clinician.

There are different statistics to measure the agreement between the two methods, such as Cohen's kappa (for two raters) and Fleiss kappa (for three or more raters). Poor agreement is considered to have occurred when kappa is less than 0.40, fair to good agreement in the range 0.40 to 0.75, whilst higher than 0.75 represents excellent agreement (Landis and Koch, 1977). For clinical studies, it is recommended that a kappa

of 0.8 should be taken as the *minimum* acceptable value for agreement (McHugh, 2012), as will be used in the present study.

5.2. Participants

The agreement between the MMAS-8 and the determination of drugs concentration in DBS was assessed for the same sample of Iraqi volunteers who consented to provide blood samples as described in section 3.2.2 in chapter 3 and completed the MMAS-8 as described in chapter 4 Section 4.2.1.

5.3. Statistical Analysis

Descriptive statistical frequency distributions were obtained using the SPSS software (version 22. Armonk, NY: IBM Corp). Qualitative variables, such as gender and medications, were expressed using frequencies and percentages. Spearman's correlation coefficient was used to determine the relationship between non-adherence level measured by the MMAS-8 questionnaire or via blood microsamples analysis and number of CVD medications and number of tablets taken daily. A Chi-squared test was used to examine the relationship between level of non-adherence and gender. Means and standard deviations were used to express the concentration of medications in the biological samples. The kappa concordance test was used to measure the degree of agreement between the non-adherence classified by the Morisky questionnaire and the blood microsample analyses, where a P-value of less than 0.5% was considered significant.

5.4. Results and Discussion

5.4.1. Assessment of Adherence to Target CVD Medications Using MMAS-8 and Blood Microsample Analyses for 303 Iraqi Volunteers

The blood microsampling analysis using LC-HRMS and its integration with MMAS-8 is the first study to attempt to assess non-adherence to cardiovascular medications in Iraq. The assessment of non-adherence to CVD medications by MMAS-8 was based on the cut-off point of a score of 6. On the other hand, patients were classified as non-adherent through blood microsample analysis when one or more of their prescribed medications concentration was < 5% of C_{max} or was > C_{max} for that prescribed medication. The

concentration of the target CVD medications determined in the blood microsamples, as collected onto 903 cards and VAMS from the 303 Iraqi volunteers in the present study, showed significant agreement, as detailed in Chapter 3 Section 3.4.4. Thus, the assessment of non-adherence in the blood microsamples represents the results obtained from both 903 cards and VAMS.

MMAS-8 showed that 248 (81.8%) participants were adherent, namely 125 males (50.4%) and 123 females (49.6%), whilst 55 (18.2%) participants were non-adherent, namely 25 male (45.5%) and 30 females (54.5%); by comparison the assessment of non-adherence by determination of the target drugs' concentrations in blood microsamples from the same volunteers indicated that 154 patients (50.8%) were adherent to medications (82 males and 72 females) and 149 patients (49.2%) were non-adherent (68 males and 81 females). The detailed agreement, or indeed disagreement, between these two methods of assessment for this sample of 303 volunteers is summarized in Table 5.1.

Table 5.1. The results of assessment of adherence to target CVD medications using MMAS-8 and blood microsample analyses for 303 Iraqi volunteers.

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-280716-AA-06	Female	YES	YES
903-310716-MT-07	Male	YES	NO
903-310716-AA-08	Female	NO	NO
903-310716-AA-10	Male	NO	NO
903-310716-AA-11	Female	NO	NO
903-310716-AA-12	Female	YES	YES
903-310716-AA-14	Male	YES	YES
903-310716-AA-15	Female	YES	YES
903-010816-AA-16	Female	YES	YES
903-010816-AA-17	Male	YES	NO
903-030816-AA-19	Female	NO	NO
903-030816-AA-20	Male	NO	NO
903-030816-AA-21	Female	YES	NO
903-030816-AA-23	Male	YES	NO
903-030816-AA-24	Female	YES	YES
903-030816-AA-25	Female	YES	YES
903-030816-AA-27	Female	YES	YES
903-040816-AA-30	Male	YES	YES
903-040816-AA-31	Male	NO	NO
903-040816-AA-32	Female	YES	YES
903-040816-AA-33	Male	NO	NO
903-050816-AA-34	Male	YES	YES
903-050716-AA-36	Female	YES	NO
903-050816-AA-37	Male	YES	YES
903-050816-AA-38	Male	YES	NO
903-050816-AA-40	Female	YES	YES
903-050816-AA-41	Female	YES	NO
903-050816-AA-42	Male	YES	YES
903-060816-AA-45	Male	YES	NO
903-060816-AA-48	Male	NO	NO
903-060816-AA-50	Male	YES	YES
903-060816-AA-51	Male	YES	YES
903-060816-AA-52	Male	YES	YES
903-060816-AA-53	Male	YES	NO
903-070816-AA-55	Female	YES	NO
903-070816-AA-56	Female	YES	NO

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-070816-AA-57	Female	YES	YES
903-070816-AA-58	Male	YES	NO
903-070816-AA-59	Female	YES	NO
903-070816-AA-60	Female	YES	NO
903-070816-AA-61	Male	NO	NO
903-070816-AA-62	Male	NO	NO
903-070816-AA-64	Male	YES	NO
903-070816-AA-65	Male	YES	YES
903-080816-AA-66	Female	YES	YES
903-080816-AA-67	Male	YES	YES
903-080816-AA-68	Male	YES	YES
903-080816-AA-69	Male	YES	YES
903-080816-AA-70	Male	YES	YES
903-080816-AA-72	Female	YES	NO
903-090816-AA-73	Female	YES	NO
903-090816-AA-74	Male	NO	NO
903-090816-AA-75	Female	YES	NO
903-090816-AA-76	Male	YES	YES
903-090816-AA-77	Male	NO	YES
903-090816-AA-78	Male	YES	YES
903-100816-AA-80	Male	NO	NO
903-100816-AA-82	Male	YES	NO
903-100816-AA-84	Male	NO	YES
903-100816-AA-86	Female	YES	YES
903-100816-AA-87	Female	YES	YES
903-100816-AA-88	Male	YES	NO
903-100816-AA-89	Female	NO	YES
903-110816-AA-90	Male	YES	YES
903-120816-AA-91	Male	YES	YES
903-120816-AA-93	Male	YES	YES
903-150816-AA-95	Male	YES	NO
903-150816-AA-96	Male	YES	YES
903-150816-AA-99	Female	YES	NO
903-200717-AA-100	Male	YES	YES
903-200717-AA-101	Male	YES	YES
903-200717-AA-102	Male	YES	YES

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-200717-AA-103	Male	YES	YES
903-200717-AA-104	Male	YES	YES
903-200717-AA-105	Male	YES	YES
903-200717-AA-106	Male	YES	YES
903-200717-AA-107	Male	YES	NO
903-200717-AA-108	Male	YES	NO
903-200717-AA-109	Male	YES	NO
903-200717-AA-110	Male	YES	YES
903-200717-AA-111	Male	YES	YES
903-200717-AA-112	Male	YES	NO
903-200717-AA-113	Male	YES	YES
903-200717-AA-114	Male	YES	YES
903-200717-AA-115	Female	YES	YES
903-200717-AA-116	Female	YES	NO
903-200717-AA-117	Female	YES	YES
903-200717-AA-118	Male	YES	YES
903-200717-AA-119	Male	YES	YES
903-200717-AA-120	Male	YES	NO
903-210717-AA-121	Male	YES	NO
903-210717-AA-122	Female	YES	NO
903-210717-AA-123	Male	YES	YES
903-210717-AA-124	Female	YES	NO
903-210717-AA-125	Female	YES	YES
903-210717-AA-126	Male	YES	YES
903-210717-AA-127	Female	YES	YES
903-210717-AA-128	Male	YES	YES
903-210717-AA-129	Female	NO	YES
903-210717-AA-130	Male	NO	YES
903-220717-AA-131	Male	YES	YES
903-220717-AA-132	Male	NO	YES
903-220717-AA-133	Male	YES	NO
903-220717-AA-134	Male	YES	NO
903-220717-AA-135	Male	YES	NO
903-220717-AA-136	Male	YES	YES
903-220717-AA-137	Female	YES	YES
903-220717-AA-138	Female	YES	YES
903-220717-AA-139	Male	YES	YES

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-220717-AA-140	Female	YES	YES
903-230717-AA-141	Male	YES	YES
903-230717-AA-142	Female	YES	YES
903-230717-AA-143	Male	YES	YES
903-230717-AA-144	Male	YES	YES
903-230717-AA-145	Male	YES	NO
903-230717-AA-146	Male	YES	NO
903-230717-AA-147	Female	YES	YES
903-230717-AA-148	Male	YES	YES
903-230717-AA-149	Female	YES	YES
903-230717-AA-150	Female	YES	YES
903-240717-AA-152	Male	YES	YES
903-240717-AA-153	Male	YES	YES
903-240717-AA-154	Female	YES	YES
903-240717-AA-155	Female	YES	YES
903-240717-AA-156	Male	YES	YES
903-240717-AA-157	Female	YES	NO
903-240717-AA-158	Female	YES	NO
903-240717-AA-159	Female	YES	NO
903-240717-AA-160	Male	YES	YES
903-250717-AA-161	Female	YES	YES
903-250717-AA-162	Female	YES	YES
903-250717-AA-163	Male	YES	YES
903-250717-AA-164	Male	NO	YES
903-250717-AA-165	Male	YES	YES
903-250717-AA-166	Female	YES	YES
903-250717-AA-167	Male	NO	YES
903-250717-AA-168	Male	YES	NO
903-250717-AA-169	Female	YES	YES
903-250717-AA-170	Male	YES	YES
903-270717-AA-171	Female	YES	YES
903-270717-AA-172	Male	YES	YES
903-270717-AA-173	Female	YES	YES
903-270717-AA-174	Female	YES	YES
903-270717-AA-175	Female	YES	YES
903-270717-AA-176	Male	YES	YES
903-270717-AA-177	Female	YES	NO

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-270717-AA-178	Male	YES	YES
903-270717-AA-180	Female	YES	NO
903-280717-AA-181	Male	YES	NO
903-280717-AA-182	Female	YES	YES
903-280717-AA-183	Female	YES	YES
903-280717-AA-184	Male	YES	YES
903-280717-AA-185	Male	YES	YES
903-280717-AA-186	Female	YES	NO
903-280717-AA-187	Male	YES	YES
903-280717-AA-188	Male	YES	YES
903-280717-AA-189	Female	YES	NO
903-280717-AA-190	Female	YES	NO
903-290717-AA-191	Female	YES	YES
903-290717-AA-192	Female	YES	YES
903-290717-AA-193	Female	YES	NO
903-290717-AA-194	Male	YES	YES
903-290717-AA-195	Male	YES	NO
903-290717-AA-196	Male	YES	NO
903-290717-AA-197	Male	YES	YES
903-290717-AA-198	Male	YES	NO
903-290717-AA-199	Female	YES	YES
903-290717-AA-200	Male	YES	YES
903-300717-AA-201	Male	YES	YES
903-300717-AA-202	Male	YES	NO
903-300717-AA-204	Male	YES	NO
903-300717-AA-205	Male	YES	YES
903-300717-AA-206	Female	YES	YES
903-300717-AA-207	Male	YES	YES
903-300717-AA-208	Female	YES	YES
903-300717-AA-209	Female	YES	YES
903-300717-AA-210	Female	YES	YES
903-300717-AA-211	Female	YES	NO
903-300717-AA-212	Female	YES	NO
903-300717-AA-213	Male	YES	YES
903-300717-AA-214	Male	YES	YES
903-300717-AA-215	Male	YES	YES
903-300717-AA-216	Female	YES	YES

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-300717-AA-217	Male	YES	NO
903-300717-AA-218	Female	YES	NO
903-300717-AA-219	Female	YES	YES
903-300717-AA-220	Female	YES	NO
903-310717-AA-221	Female	YES	NO
903-310717-AA-222	Male	YES	YES
903-310717-AA-223	Female	YES	YES
903-310717-AA-224	Female	YES	NO
903-310717-AA-225	Male	YES	YES
903-310717-AA-226	Female	YES	YES
903-310717-AA-227	Female	YES	YES
903-310717-AA-228	Female	YES	YES
903-310717-AA-229	Female	YES	YES
903-310717-AA-230	Male	YES	NO
903-100817-AA-231	Male	YES	NO
903-100817-AA-232	Male	YES	YES
903-100817-AA-233	Female	YES	YES
903-100817-AA-234	Female	YES	YES
903-100817-AA-235	Female	YES	NO
903-100817-AA-236	Female	YES	NO
903-100817-AA-237	Female	YES	NO
903-100817-AA-238	Female	YES	NO
903-100817-AA-239	Male	YES	NO
903-100817-AA-240	Male	YES	NO
903-020817-AA-241	Male	NO	NO
903-020817-AA-242	Male	YES	NO
903-020817-AA-243	Male	YES	YES
903-020817-AA-244	Female	YES	YES
903-020817-AA-245	Female	YES	YES
903-020817-AA-246	Male	YES	YES
903-020817-AA-247	Female	YES	NO
903-020817-AA-248	Female	YES	NO
903-020817-AA-249	Female	YES	NO
903-020817-AA-250	Female	YES	NO
903-030817-AA-251	Female	YES	NO
903-030817-AA-252	Female	YES	NO
903-030817-AA-253	Male	YES	NO

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-030817-AA-254	Female	YES	YES
903-030817-AA-255	Female	YES	YES
903-030817-AA-256	Female	YES	YES
903-030817-AA-257	Female	YES	YES
903-030817-AA-258	Male	YES	YES
903-030817-AA-259	Female	YES	YES
903-030817-AA-260	Female	YES	YES
903-040817-AA-261	Male	YES	NO
903-040817-AA-262	Male	YES	NO
903-040817-AA-263	Male	NO	NO
903-040817-AA-264	Male	NO	NO
903-040817-AA-265	Male	NO	NO
903-040817-AA-266	Male	NO	NO
903-040817-AA-267	Female	NO	NO
903-040817-AA-268	Female	NO	NO
903-040817-AA-269	Female	NO	NO
903-040817-AA-270	Female	NO	NO
903-050817-AA-271	Female	NO	NO
903-050817-AA-272	Male	NO	NO
903-050817-AA-273	Female	NO	NO
903-050817-AA-274	Male	NO	NO
903-050817-AA-275	Female	NO	NO
903-050817-AA-276	Female	NO	NO
903-050817-AA-277	Female	NO	NO
903-050817-AA-278	Female	NO	NO
903-050817-AA-279	Female	NO	NO
903-050817-AA-280	Female	NO	NO
903-100817-AA-281	Female	NO	NO
903-100817-AA-282	Female	NO	NO
903-100817-AA-283	Male	NO	NO
903-100817-AA-284	Female	NO	NO
903-100817-AA-285	Female	NO	NO
903-100817-AA-286	Female	NO	NO
903-100817-AA-287	Female	NO	NO
903-100817-AA-288	Female	NO	NO
903-100817-AA-289	Female	NO	NO
903-100817-AA-290	Female	NO	NO

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-210318-AA-291	Male	YES	YES
903-210318-AA-292	Male	YES	YES
903-210318-AA-293	Female	YES	YES
903-210318-AA-294	Male	NO	NO
903-210318-AA-295	Female	YES	NO
903-210318-AA-296	Male	YES	NO
903-210318-AA-297	Male	YES	YES
903-210318-AA-298	Female	YES	YES
903-210318-AA-299	Male	YES	NO
903-210318-AA-300	Female	YES	NO
903-240318-AA-301	Female	YES	YES
903-240318-AA-302	Female	YES	YES
903-240318-AA-303	Female	YES	YES
903-240318-AA-304	Male	NO	NO
903-240318-AA-305	Female	YES	NO
903-250318-AA-306	Male	YES	NO
903-250318-AA-307	Female	YES	NO
903-250318-AA-308	Female	YES	NO
903-250318-AA-309	Male	YES	NO
903-250318-AA-310	Female	YES	NO
903-260318-AA-311	Female	YES	YES
903-260318-AA-312	Female	YES	YES
903-260318-AA-313	Female	NO	NO
903-260318-AA-314	Male	YES	NO
903-260318-AA-315	Female	NO	NO
903-260318-AA-316	Male	YES	NO
903-260318-AA-317	Male	YES	NO
903-260318-AA-318	Female	YES	NO
903-260318-AA-319	Female	YES	NO
903-260318-AA-320	Female	YES	YES
903-260318-AA-321	Male	YES	YES
903-260318-AA-322	Female	NO	NO
903-260318-AA-323	Female	YES	NO
903-260318-AA-324	Male	YES	NO
903-260318-AA-325	Female	YES	NO
903-260318-AA-326	Female	YES	NO
903-260318-AA-327	Male	YES	NO

Table 5.1 continued

Patient reference number	Sex	Adherence assessment by MMAS-8	Adherence assessment by microsampling analysis
903-260318-AA-328	Female	YES	NO
903-270318-AA-329	Female	YES	YES
903-270318-AA-330	Female	YES	YES
903-270318-AA-331	Male	YES	YES
903-270318-AA-332	Female	NO	NO
903-270318-AA-333	Male	YES	NO
903-270318-AA-334	Female	YES	NO
903-270318-AA-335	Female	YES	NO
903-270318-AA-336	Male	YES	NO

To assess the agreement and disagreement between MMAS-8 and blood microsample analysis approaches, the measurement of drug concentration in the blood microsamples was considered to represent the 'true' classification of non-adherence. Thus, 248 participants were classified as adherent by MMAS-8 (Score > 6). However, blood microsample analyses showed that only 146 (58.9%) of these 248 patients were actually adherent because the CVD concentrations measured were between 5% of C_{max} and C_{max}; the other 102 patients (41.9%) were non-adherent. This suggests the likely overestimation of adherence to medication by the 102 patients identified as being non-adherent via DBS analysis or possibly this result was related to the quality of medicines used.

On the other hand, 55 patients were categorized as non-adherent by MMAS-8, with 47 (85.5%) of these 55 patients confirmed as being non-adherent by subsequent blood microsample analysis. The other eight patients (14.5%) patients were defined as being adherent by blood microsample analysis. This discrepancy may be explained by the acquiescence bias response where the participants give affirmative answers regardless of the content of the question, and where the chances of this form of bias becoming apparent in self-reported questionnaires is quite high (Watson, 1992). Affirmative answers in MMAS-8 take a value of zero. Thus, the total score will classify patients as being non-adherent.

The agreement between the two approaches to assessing CVD medication adherence was tested via the kappa test, which showed significantly poor agreement (kappa = 0.28, P-value < 0.05). This result is different to those reported in other, studies which showed that

questionnaires are generally highly concordant with drug level measurements (Garber *et al.*, 2004; Warren *et al.*, 2013; Fabbiani *et al.*, 2016). Other studies have showed limited concordance between questionnaires and drug concentrations (Pandey *et al.*, 2015; Dawood *et al.*, 2018). However, these studies either used statistical analysis, such as the Pearson coefficient, which is not recommended for assessment of agreement between two approaches, or using an arbitrary cut-off point in the kappa test. For example, some considered 0.3 to represent good concordance (Hidalgo *et al.*, 2014) while other studies considered this value to represent only weak concordance (Warren *et al.*, 2013). As noted previously, for clinical studies it is recommended that a kappa of 0.8 should be used as the minimum acceptable value for agreement (McHugh, 2012).

As shown in Table 5.2, agreement and disagreement between MMAS-8 and blood microsample analyses for each medication showed high agreement for atenolol and bisoprolol at 88.1 and 87%, respectively, and high disagreement for atorvastatin and simvastatin, at 50 and 52%, respectively. The average agreement was 67% in comparison with 33% disagreement. However, as mentioned earlier, the overall agreement for patient non-adherence as assessed by the kappa test showed significant weak agreement between the two approaches (kappa = 0.28, P-value < 0.05).

Table 5.2. Agreement and disagreement of non-adherence assessment between MMAS-8 and blood microsamples analysis.

Medication (n)	Agreement between MMAS-8 and blood microsamples analysis (%)	Disagreement between MMAS-8 and blood microsamples analysis (%)
Amlodipine (15)	10(66.7)	5(33.3)
Atenolol (59)	52(88.1)	7(11.9)
Atorvastatin (18)	9(50)	9(50)
Bisoprolol (77)	67(87)	10(13)
Diltiazem (34)	25(73.5)	9(26.5)
Lisinopril (73)	49(67.1)	24(32.9)
Losartan (47)	26(55.3)	21(4.7)
Simvastatin (50)	24(48)	26(52)
Valsartan (65)	44(67.7)	21(32.3)
Average	67.0	33.0

Where (n) = number of patients taking medication)

5.4.2. Face-to-Face Interview with Non-adherent Volunteers by the Clinician

Despite the discrepancy between MMAS-8 and blood microsample analysis in the assessment of non-adherence, the insights generated by the responses to the MMAS-8, when validated by the blood microsample analysis, showed that 93.6% of validated non-adherent patients answered YES to the question "*Have you ever cut back or stopped taking your medication(s) without telling your doctor because you felt worse when you took it?*"

It has been reported that medication side effects mainly affect patients with cardiovascular diseases that require polypharmacy (Abolbashari *et al.*, 2017). Face-to-face interviews between the clinician and patients showed that patients taking statins reported that the associated side effects, such as muscle pain and weakness, was the cause of non-adherence (Appendix 22 Section 1).

[… Muscle pain…] [Patient reference number…17, 323,334]
[...Feel tired…weakness in muscle….] [Patient reference number…59]
[…Feel worse… and complicated regimen…] [Patient reference number…88]
[…I sometimes do not take medication because I feel not good…] [Patient reference number…314]

Other non-adherent patients stated that the side effects associated with taking losartan, such as vertigo, was the cause of their non-adherence (Appendix 22 Section 1).

[…Losartan makes me ill……] [Patient reference number…190]
[…Feeling bad taking losartan …dizziness…] [Patient reference number…305]

This may indicate that frequent follow-ups by clinics are important to monitor the side effects patients are experiencing and to adjust prescriptions as needed to alleviate them. Patients' fears and concerns about adverse drug reactions should be considered by the health care professionals to prevent them if possible.

91.5% of validated non-adherent patients answered YES to the question "*When you feel like your cardiovascular disease is under control, do you sometimes stop taking your medication(s)?*"

Face-to-face interviews between the clinician and patients indicated that some patients had little or no knowledge about or understanding of their particular cardiovascular diseases (Appendix 22 Section 3).

[I think some medications are used as needed….] [Patient reference number…157]
[I feel OK…. I did not take medications….] [Patient reference number…168]
[…feel that this condition is under control, no need for medications] [Patient reference number…220]

This may indicate that patients do not understand their diseases well enough to realize when they represent chronic conditions that require continuous treatment. Inadequate knowledge about medications and their usage can leave the patient unconvinced as to the need for treatment, and consequently affect their adherence.

85.1% of validated non-adherent patients answered YES to the question "*Taking medication(s) every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your cardiovascular treatment plan?*"
This question is difficult to address because the source of this inconvenience is not directly assessed by MMAS-8. Reasons for feelings of inconvenience could be related to patient-physician discordance which will lead to patient dissatisfaction; it has been reported that 40%-60% of patients misunderstand the directions for use of any medicine prescribed immediately after visiting their doctors (Jimmy and Jose, 2011). Face-to-face interviews between the clinician and patients showed that some non-adherent patients were too embarrassed to ask their clinician how to take their medications (Appendix 22 Section 6).

[…. I was shy to ask….] [Patient reference number…285,296]
[…. I did not understand …. I was shy…] [Patient reference number…300]

Inconvenience may also be the result of the required dose frequencies, complicated regimens, or improper time of administration. A significant number of non-adherent patients reported that taking many tables a day is distracting and disturbs their daily routines and was ultimately the reason they stopped taking their medications (Appendix 22 Section 1).

[It is inconvenient to me to take many medications...] [Patient reference number…8]
[Taking many medications disturbs my life…work] [Patient reference number…10]
[Daily life disturbed by taking many medications…] [Patient reference number…11]

85.1% of validated non-adherent patients also answered YES to the question "*Do you sometimes forget to take your cardiovascular medication(s)?*" This indicates the major role of forgetfulness in non-adherence. Face-to-face interviews between the clinician and patients provided more information about the source of this forgetfulness, where some non-adherent patients reported that this was due to having a busy life and long working hours (Appendix 22 Section 4).

[We are old…forgetfulness is common in our age group…] [Patient reference number…198]
[Busy life…. forget medications….] [Patient reference number…240]
[…Missed medications….] [Patient reference number…274]
[…Forget medications….] [Patient reference number…283]
[...Work made me forget my medications….] [Patient reference number…310]

70.9% of validated non-adherent patients gave no reasons for not taking their medications. This indicates that the MMAS-8 provides only limited information about the reasons associated with levels of non-adherence and may need to be improved or further developed to allow for better assessment of the associated reasons. Triangulation with other methods such as face-to-face interviews between the clinician and patients was helpful in gaining additional information in this regard. For instance, other reasons for non-adherence that were not addressed in the MMAS-8 were the financial cost of medications or patient's belief. Some patients who were non-adherent to atorvastatin, simvastatin and valsartan reported that the cost of these medications was their primary reason for non-adherence. These patients could not afford the price as these medications are not always available in the public sector, requiring instead that they be purchased from the private sector (Appendix 22 Section 2).

[I cannot find these medications in the hospital] [Patient reference number…17, 55, 62, 134, 204]
[Medications are expensive…I am jobless...] [Patient reference number…64]
[I cannot afford the price…….] [Patient reference number…75]

[I did not take medicines…I could not find medicines in the hospital...] [Patient reference number…121, 24,133,159]

Other non-adherent patients did not believe in medications, whilst some believed that taking medications would harm them. On the other hand, some patients believed that taking high doses of medication would lead to improved health outcomes (Appendix 22 Section 5).

[We think if we continue using medications, we you cannot stop; your body will get used to it] [Patient reference number…242]
[…. will addict on these medications…….] [Patient reference number…252]
[In my opinion, these tablets cannot improve my diseases, so I decided to stop it] [Patient reference number…263]
[This dose may not be enough …….] [Patient reference number…23]
[Taking high dose is better….] [Patient reference number…45]
[Taking two tables will not harm…. better….] [Patient reference number…53]

12.7% of non-adherent patients indicated that forgetting to bring their medications while traveling or when leaving home accounted for their non-adherence (Appendix 22 Section 4).

[Forget my medications when I travelled ….] [Patient reference number…224]
[Forget taking medicine when travelling….] [Patient reference number…238]

This research proposes a convenient, rapid, cost-effective, specific, and sensitive method for directly detecting drug concentrations for use alongside such questionnaires as MMAS-8. It should be possible to apply this method to assess non-adherence to a wide range of CVD medications.

Intentional non-adherence was noticed in almost 75% of patients and may indicate that the problem may arise from the beliefs, attitudes and expectations that influence them and a lack of motivation to continue their treatment regimen (Horne *et al.*, 2005). The required interventions for addressing both intentional and unintentional non-adherence are detailed in chapter 6.

Assessment of non-adherence to a medication regimen is crucial to optimising the clinical use of medications and to preventing unnecessary increases in dose or adding more medications to the medical regimen. In cases of poor clinical outcomes, the clinician may incorrectly consider these to be due to the previous treatment being ineffective, and thus may add more medications, or intensify the dose(s) of those already being taken. This increases the possibility of introducing or exacerbating side effects, thus increasing the associated number of hospital visits. Clinicians should, therefore, take the prevalence of non-adherence seriously and seek to develop relationships of mutual trust with patients in order to limit false reports of high adherence. Simultaneously, standardised direct measurement methods of adherence using the convenience of DBS samples should be incorporated into routine clinical practices to gain an accurate understanding of adherence. Clinicians must be adequately informed when making the decision to alter a prescription.

Blood microsample analysis by LC-HRMS, can provide information about levels of non-adherence for each medication taken and would be helpful to clinicians in terms of optimizing and individualising each medication in the regimen for each patient. For example, patient reference number …121 was taking losartan and simvastatin and was categorized as adherent according to MMAS-8. However, blood microsample analysis of this patient showed that he was only adherent to losartan but not simvastatin.

MMAS-8 alone cannot determine whether patients took the correct dose at the correct time. For example, patient reference numbers …23, 45, and 53 were taking atenolol and were categorised as adherent based on MMAS-8; however, they were considered non-adherent based on blood microsample analysis as the measured concentrations exceeded the corresponding C_{max} for the reported dose of atenolol (50 mg or 100 mg). Face-to-face interviews between the clinician and these patients, however, revealed very important information which both blood micosample analysis and MMAS-8 were unable to obtain (Appendix 22). When the clinician asked them about their non-adherence to their medications, the patients believed that taking more than the prescribed dose would lead to an improved clinical outcome than the recommended dose. Another example was patient reference number… 58 who was prescribed atenolol 50 mg and atorvastatin 40

mg; this patient was categorised as adherent by MMAS-8, but the concentration of atenolol in their blood sample was high for their prescription and atorvastatin did not appear to be present. After reporting this result, the clinician interviewed this patient and found that patient had mistakenly inserted the atenolol blister pack in the atorvastatin packaging, and so had been taking a double dose of atenolol whilst missing their dose of atorvastatin (Appendix 22). This explained the high concentration of atenolol and lack of atorvastatin in the blood sample taken from this patient.

Nonadherence to cardiovascular medications was not uniform (Figure 5.1). Non-adherence can be influenced by the medication group prescribed (Lane *et al.*, 2019). A study by Gupta *et al.* showed that non-adherence to statins was higher than other cardiovascular medications such as β-blockers, ACE inhibitors and calcium channel blockers (Gupta *et al.*, 2018). Medication side effects may be associated with these differences in levels of non-adherence (Lane *et al.*, 2019). However, other factors could have accounted for these differences, such patient-related factors or the health system, as detailed in Chapter 2 Section 2.2.5.1.

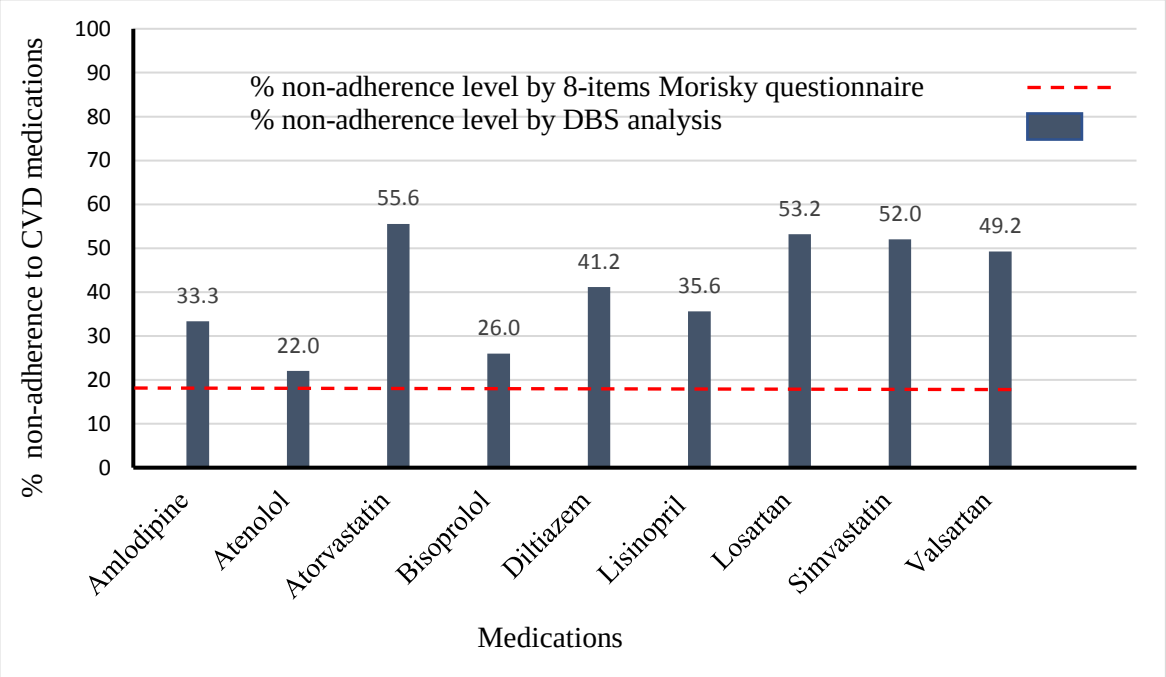


Figure 5.1. Comparison of the levels of non-adherence based on individual medication between MMAS-8 and blood microsample analyses

5.5. Conclusion

The integrated approach adopted to assess non-adherence to CVD medications by determination of the concentration of the target medications in blood microsample via LC-HRMS and by MMAS-8 is a novel research that can provide information on the levels of each medication in the patients' blood and provide at least some of the reasons behind poor patient progress. This information can help clinicians to track adherence for each medication and determine the reasons for poor adherence, such as incorrect dose or poor choice of medication, and can also help the clinician to apply an appropriate strategy to improve adherence. Since MMAS-8 provides limited information about the causes of non-adherence, integration of results obtained from blood microsample analysis and MMAS-8 with face-to-face interviews between the clinician and patients providing additional crucial information related to medication non-adherence such as polypharmacy, cost, patient's beliefs and patient-clinician relationships.

Both approaches showed no significant correlation between the level of non-adherence and either age or gender. Moreover, the levels of non-adherence could be significantly associated with the number of medications in the regimen and the number of tablets taken per day. The agreement between the two approaches for assessment of non-adherence was found to be relatively poor. Despite the discrepancy in outcomes found for the two methods, the insights generated by the responses to MMAS-8, when validated by the blood microsample analyses, demonstrated that 72.3% of patients were intentionally non-adherent to their medications, the main causes of which were the associated side effects, complicated regimens, dose frequency, patient-physician discordance, cost of medication and patients' beliefs. By contrast, 27.3% of patients were unintentionally non-adherent, the most common reasons for which were a lack of understanding of the disease, forgetfulness, and travelling and leaving home.

MMAS-8 is unable to assess non-adherence to multiple medications in the prescribed pharmacotherapy regimens. The assessment of non-adherence by MMAS-8 is subject to overestimation because this is dependent on the total score obtained from a given patient's responses to the questions. By contrast, blood microsamples analysis can accurately assess non-adherence for each form of medication. However, blood microsample analysis clearly cannot provide any information about the causes of non-adherence. The main

limitation to face-to-face interviews in the present study was that the clinician was unable to meet all the volunteers due to time constraints and contact details, such as email or phone numbers, not being available to the clinician.

Chapter 6

Implications of This Research for the Assessment of Non-adherence to Cardiovascular Medications on Clinical Practice in Iraq

6.1. Introduction

Each country has its own perspectives on the construction of their healthcare systems and delivery policies (Lloyd *et al.*, 1999). The proficiency of the healthcare system of any given country is representative of the future of that same country. A highly efficient healthcare system can help people to improve their health and quality of life. Healthcare regulations and policies must seek to improve both the health environment of the associated populace and promote awareness of health problems.

Two extreme examples of healthcare systems are the US's provisions and the National Health Service (NHS) in the UK. Healthcare in the US is almost totally in the hands of private companies who have the leverage to control healthcare system delivery within the country (Ridic *et al.*, 2012). In other words, to gain access to healthcare, patients need to pay out of their own pockets. Thus, the quality of the US healthcare service will always depend on how much the patient pays. The US drug formulary represents the focal point for the prescription of medications to promote cost-effective prescription and includes lists of developed, and approved generic and brand medications and pharmaceutical products to ensure efficient dispensing of prescription drugs without sacrificing quality. The inclusion of these medications is based on recommendations from a committee of doctors, pharmacists, and other medical experts on the basis of drug efficacy, safety, and cost-effectiveness. The prescription of these medication is usually covered by health insurance plans (Fox, 2003).

As detailed in chapter 1 Section 1.3.1.1, the UK's NHS can be considered a social healthcare system that is 'free at the point of delivery' in the majority of circumstances (National Health Service, 2019). Examples in the UK where direct charges are made as a matter of course include prescription charges, currently set at £9.00 per item (National Health Service, 2019). Medications are prescribed by appropriate healthcare practitioners such as doctors, dentists in the UK. These prescriptions are only dispensed through pharmacies in either community or hospital settings (National Health Service, 2017). A range of exemptions by which people can obtain free prescriptions is available in England, for example those under 16, those who are 60 or over, people with certain medical conditions (e.g., cancer, diabetes) and during pregnancy (Black, 2014).

The British National Formulary (BNF) contains a wide spectrum of information and advice about prescribing and pharmacology, along with specific facts and details about the medicines available from the UK National Health Service (Barbour, 2001). Private healthcare is available in the UK and is directly funded by insurance schemes that are paid for directly by either individuals or by major employer schemes.

As described in chapter 1 Section 1.3, the healthcare system in Iraq is a combination of public and private supply. In Iraq, the Ministry of Health (MOH) is responsible for the country's healthcare system and funds the public health sector (Al Hilfi *et al.*, 2013). Private healthcare is delivered by entrepreneurs. In Iraq, if patients are checked by doctors in the public sector, the doctors can prescribe medications which patients can get from hospital pharmacies after paying the appropriate fee. This is comparable to the NHS, where the price for such is low in comparison with their real-term costs or indeed their cost in the private sector. If their medications are not available in public sector pharmacies, patients have to pay extra in order to get them from private sector pharmacies instead. The cost of medications is high, especially for brand medications. In Iraq, there are no applicable guidelines, such as those given by the BNF, which can lead to arbitrary decisions at the time of prescription and which is considered one of the major weaknesses of the Iraqi healthcare system.

Today, the growth of the internet has allowed people to get their medications from other sources, such as buying them online. There has been an increased use of the internet to gain access to medicines in Iraq since 2003. However, many online pharmacies worldwide are unregistered, and this increases the possibility of buying potentially unsafe, substandard or falsified medications (Jackson *et al.*, 2012; National Health Service, 2018b; Food and Drug Administration, 2018). Substandard or falsified medicines are now a significant problem worldwide. The WHO estimates that around 10% of all medicines currently reaching developing countries fall into this category (World Health Organization, 2017f). In the UK, MHRA reported that the number of substandard medicines within the country had increased ten-fold between 2001 and 2011 (Almuzaini *et al.*, 2013). Similar concerns are prevalent in the US, but more frequently for 'lifestyle drugs.' Buying prescription-only medicines from unauthorized sources significantly increases the risk of getting substandard medicines (Almuzaini *et al.*, 2013).

The potential negative effects of using substandard or falsified medications is that of using such without actually getting the desired clinical benefit, increasing the risk of disease progression, side effects, or the need to change the treatment plan. This accounts for poor patient outcomes and increased costs (Johnston and Holt, 2014).

In Iraq, there is no current information about the distribution of substandard or falsified medicines from online sources. However, reports have recently surfaced about substandard or falsified medications circulating in the country. The Iraqi parliament called on the Ministry of Health and the Syndicate of Iraqi Pharmacists to prevent the distribution of substandard or falsified medications (Alsumaria Iraqi Satellite TV Network, 2012).

6.2. Assessment of Medication Non-adherence

Perhaps somewhat surprisingly, the NHS considers questioning a patient's adherence to medication to be unethical and this is therefore not generally undertaken. Exceptions include immunosuppressant therapy in organ transplant patients, lithium determinations and therapeutic drug monitoring in patients with persistent hypertension (Tanna and Lawson, 2016a). The reverse is true in the US, with drug monitoring in patients being a prerequisite to the continued supply of certain forms of pain medication (Tanna and Lawson, 2016a). As discussed previously in chapter 2 Section 2.1.1, the WHO defines adherence as "the extent to which a person's behaviour taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a healthcare provider" (Sabaté, 2003). The methods used to assess adherence can be categorized as either direct or indirect. Indirect methods include patient interviews, patient diaries, pill counts, questionnaires, electronic monitoring, patient self-reports, prescription-refill databases and clinical outcomes. Direct assessment methods are based either on measuring the concentration of drugs or metabolites in a biological sample (such as urine, blood or saliva), the presence of a biological marker, or by direct observation of the patient taking their medicines (Lam and Fresco, 2015).

Although indirect methods are cheap and easily applied in clinical settings, the application of approaches such as prescription refills depend on the availability of computerised systems, but this is considered a major limitation in Iraq because of the country's limited infrastructure. Prescription refill cannot confirm whether patients have

taken or thrown away their medications and consequently this may result in the overestimation of medication adherence. Furthermore, it is rather challenging for the researcher to obtain information regarding barriers to medication adherence in terms of individual patients (Krousel-Wood *et al.,* 2015). Patient interviews to assess non-adherence are also highly dependent on the communication skills of the interviewers (Lam and Fresco, 2015, Farmer, 1999).

The accuracy of pill counts as a tool for estimating medication adherence is uncertain because some patients may not return their unused medications (Lawrence *et al.*, 2017). Electronic monitoring is of only limited use in patients taking multiple medications (Sidorkiewicz *et al.*, 2016). The pill count method is optimistic as it cannot confirm whether the patient has taken their medication as, for instance, the patient may open the container and merely discard the medication (Aldeer *et al.*, 2018).

Questionnaires can assess general medication-taking behaviour but cannot provide essential information about specific medications or the concentrations of drugs in the blood (Mathes *et al.*, 2014; Tanna and Lawson, 2016a). Also, this approach may be subject to bias because it depends on patient recall and perceptions (Choo *et al.*, 1999; Althubaiti, 2016). Patients may unintentionally overestimate their adherence to medications.

Direct methods involve either the observation of the patient taking their medicines or the collection and analysis of a biosample (such as urine or blood) to measure the concentration of drugs or metabolites eliminated (Lam, Fresco 2015). Assessment of adherence as based on the detection of biomarkers is limited, as biomarkers are only available for a limited number of drugs (Lehmann *et al.*, 2014). Furthermore, direct methods clearly cannot identify the reasons for non-adherence to medications (Lam, Fresco 2015), although concentration data may suggest confusion when taking multiple medications.

The observation of the patient taking their medicines is inconvenient for patients and impractical in an out-patient setting as the patients involved may have to travel a considerable distance to get to a hospital and/or may have to spend half their day at the clinic, which will clearly be costly to them (Alipanah *et al.*, 2018). In addition, supervision of patients may have to be undertaken by trained personnel. Moreover,

patients can manipulate medication-taking observations by hiding the medication in their mouths (Hawkshead and Krousel-Wood, 2007).

Direct methods of measuring the concentration of drugs or metabolites in biosamples requires complex and costly instrumentation based either on immunoassay or LC-MS techniques. Analyses can be undertaken at specified intervals or randomly when appropriate. Direct methods of assessment are the most informative approach to measuring adherence (Aonuma *et al.*, 2017). They provide direct confirmation that the patient has taken the medicine (Morrison *et al.*, 2015). The application of either LC-MS or immunoassay for the determination of drug concentration is well documented in the literature (González *et al.*, 2015, Gonzalez *et al.*, 2011, Gonzalez *et al.*, 2010, Dias *et al.*, 2013). However, some assays require large volumes (1-10 ml) of blood (Tanna and Lawson, 2016a), and are time consuming and costly as they require special tubes for the collection of blood samples and centrifugation to obtain the required volume of plasma or serum, plus refrigeration to maintain sample stability. This complexity is clearly unsuitable for routine drug monitoring (Lawson *et al.*, 2013, De Nicolò *et al.*, 2017). All these steps for the collection and preparation of samples and their subsequent analysis will delay the time between collection and results being made available.

Dried blood spots represent an alternative matrix for measuring blood drug concentrations and require the collection of only a few drops (< 30 µl) of blood. This approach is helpful to overcoming the barriers associated with blood collection using venepuncture. Blood samples are easily collected and transported with no associated special requirements, such as refrigeration. There is no need for facilities to store DBS samples. Sample collection requires less time in comparison with conventional approaches and even offers the possibility of self-collection at home.

6.3. Benefits and Implications of this Research.

6.3.1. Starting Collaboration with Health Directorate in Misan-Iraq

Arrangements were made with Dr Yaseen Obaid to provide access to the CVD Clinic at the Alsader Teaching Hospital and Misan Cardiac Centre after getting the required ethical approval and permission. Assessment of adherence to CVD medications by comparing of MMAS-8 and blood microsampling analysis and the response of the clinician to the

outcomes of this research can be considered the first such study of clinical practice in Iraq. Moreover, this collaboration represents the first of its type between De Monfort University and Misan Health Directorate in Iraq.

6.3.2. Transfer of Experiences in Sample Collection and Analysis

All aspects of volunteer recruitment were arranged by Mr Alalaqi, as detailed earlier in chapter 3. Samples were collected by Mr Alalaqi, which was helpful in improving skills in sample collection. The researcher will be transferring the experience regarding the application of LC-HRMS in the clinical analysis and will be training lab staff and other professionals on collection of microsamples using DBS cards and VAMS using the same protocol applied in this study.

6.3.3. Individualisation of Medication and Clinician Responses

The applied approach can determine the concentration of nine cardiovascular medications in one run, which represents cost-effectiveness for patients by helping to decrease patient spending on health and facilitating drug optimisation within clinical practice. Patients differ in their responses to medication, and thus the resultant clinical benefits differ accordingly. Integration the outcomes of DBS analysis with the outcomes from MMAS-8 will be helpful to the clinician in terms of finding the required interventions and response to improve adherence.

Assessment of medication non-adherence can help in the selection of a more effective drug or in suggesting the use of a combination of medications. DBS analysis via LC-HRMS can measure the concentration for each individual drug in a patient's blood. Physicians can identify more appropriate drugs or doses for individual patients, and indeed personalize patients' treatments. For example, patient reference number …114 was taking bisoprolol 5 mg and valsartan 80 mg once daily. This patient had poor clinical outcomes, despite the assessment of non-adherence by MMAS-8 showing they were adherent. DBS analysis also showed that the patient was adherent to both medications. The clinician may choose to increase the patient's dose or add other medications to improve their progression, or indeed consider whether the patient may need other

interventions. The applied approach can help the clinician to determine the required steps as based on the data available.

Assessment of medication non-adherence can help in the personalization of patients' data for each medication. Take, for example, patient reference number …99, who was taking bisoprolol 5 mg and valsartan 80 mg once daily. This patient was categorized as adherent based on his response to the MMAS-8 questions. However, their clinical outcomes were not optimum. LC-HRMS extracted ion chromatograms that showed the patient was only adherent to bisoprolol. Without detailed data about each medication in the regimen, the clinician may have chosen to increase the dose of bisoprolol and valsartan, which would of course mean increasing the concentration of bisoprolol to which the patient was already adherent – which would likely have increased the possibility of bisoprolol-related adverse effects.

In this study, 72.3% of non-adherent patients were found to be intentionally non-adherent, where the most common reasons for such were due to side effects at 93.6%, for example, patients taking statins reported that the associated side effects such as muscle pain ultimately led to non-adherence. Other patients stated that the side effects associated with taking losartan, such as vertigo, led to non-adherence. The collaborating clinician in Iraq started friendly discussions with non-adherent patients who reported side effects as a barrier to medication adherence and explained to them the possible and significant side effects associated with medications such as statins on muscles, and of losartan such as vertigo, and patients were engaged in their treatment plan, such as being made aware of the possibility of switching to another medicine that offered a reduced risk or severity of side effects. Moreover, patient concerns about medicines were considered. A systematic review by Kuntz *et al.* showed that patient education would improve patient knowledge and thus improve adherence (Kuntz *et al.*, 2014). However, shortage of staff and time pressures can limit the application of such in routine clinical practice. (Foster *et al.*, 2016). The clinician may prescribe medication with fewer or less severe side effects such as prescribing angiotensin receptor blockers (ARB) as an alternative to ACE inhibitors in patients experiencing the associated dry cough. Side effects associated with statins can be managed by prescribing non-statin-based medications such as ezetimibe, which works by limiting the absorption of cholesterol (Vavlukis and Vavlukis, 2018), or evolocumab,

which inhibits proprotein convertase subtilisin–kexin type 9 inhibitor (PCSK9i) and reduces levels of low-density lipoprotein (LDL) (Sabatine *et al.*, 2017). A study that compared the non-adherence to statins and PCSK9i showed figures of 79.4% and 30.9%, respectively (Gragnano *et al.*, 2017).

85.1% of non-adherent patients reported that medication-related inconvenience was associated with non-adherence, where the reasons for such feelings of inconvenience could be associated with the complexity of the regimen and the dose frequencies, as reported by non-adherent patients. Iraqi volunteers in the present study reported that taking a large number of medications each day interrupted the patient's normal routine, and therefore that prescribers should simplify the medical regimen as much as possible. Adherence may be improved by reducing the frequency of administration or through the introduction of combination medicines (Usherwood, 2017). Patients may prefer medications that must be administered once daily, prescribing the maximum number of doses possible at one time and thus limiting the frequency at which treatment is required. Physicians should prescribe a fixed-dose combination (FDC) of pills if possible. FDC combinations can be helpful for patients on multiple medicines and may improve adherence in some settings (Webster *et al.*, 2016). The clinician simplified the medication regimen if possible.

Swapping medications may cause confusion and is certainly inconvenient, and may consequently impair adherence (Usherwood, 2017). Swapping medications is very common in clinical practice in Iraq because there are no guidelines for the prescription of cardiovascular medications. Pharmacists are responsible for patients' and carers' education if they swap their medication (Usherwood, 2017). Inconvenience may be associated with the inability to swallow tablets (Cooper *et al.*, 2015). Discussion between patients and their pharmacist may be helpful in tailoring appropriate preparations or formulations.

Another reason for feelings of inconvenience could be the result of patient-physician discord. Some non-adherent patients stated that they were too embarrassed to ask their clinician how to take their medication correctly. Improving communication between the physician and patient with due consideration for patient's beliefs is a key and effective strategy for improving adherence (Palacio *et al.*, 2016). In this study, the clinician

initiated friendly discussions with patients to improve communication and encourage them to ask about their diseases and medications.

70.9% of non-adherent patients gave no reason for their refusal to take their medications. This indicates that the questionnaire itself may need improvement in order to better assess such reasons. Subsequent face-to-face discussion, as detailed in chapter 5 Section 5.4.2, provided additional information which was not captured by the MMAS-8. For instance, other reasons for non-adherence which are not addressed in the questionnaire may be the cost of medications or patients' beliefs. The most easily recognised barrier to accessing medicines is their out-of-pocket cost (Sinnott *et al.*, 2013). Doctors should consider the cost of a medication prior to its prescription to Iraqi patients. The pharmacist can help in this regard by prescribing generic or lower-cost brand medicines when appropriate (Usherwood, 2017). Iraqi health providers should improve patient access to cardiovascular medications in the public sector. Some cardiovascular medications are not available in the public sector in Iraq, and this will increase the burden on patients by forcing them to obtain them from the private sector. The clinician in Iraq responded to this outcome in patients who reported that non-adherence was related to medication cost by prescribing them less expensive medications, as available in the public sector, to reduce the associated costs.

Patient attitude and beliefs are important factors associated with medication adherence, where the clinician explained the rationale behind prescribing particular medicines and the possibility of adverse drug reactions and toxicity if the patient takes a higher dose than prescribed. The clinician should discuss patient beliefs without imposing his/her beliefs or values on them, as the definition of adherence is agreement regarding the proposed medical plan between the clinician and the patient. Patient beliefs about their diseases and medications can be improved through the use of patient-centred counselling techniques such as motivational interviews to inspire behavioural change by supporting positive intentions and challenging negative ideas. It has been shown that interviews by pharmacy staff, by both face-to-face and/or by telephone, can improve adherence (Usherwood, 2017, van Buskirk and Wetherell, 2014, Hill and Kavookjian, 2012).

27.7 % of non-adherent patients in the present study were non-intentional, where the most common reasons for non-adherence were due to a poor understanding of the disease

(91.5%), forgetfulness (85.1%) and travelling and leaving home (12.7%). The clinician in Iraq started discussions with patients to improve patient knowledge about cardiovascular diseases, particularly as being chronic in nature and which thus require continuous treatment; ultimately, patients should not stop taking their medications even if they begin to feel well and explained the correct use of each medication using everyday language. Jargon should not be used, making information more accessible and understandable.

The clinician prepared cards for non-adherent patients, including information about the prescribed medication, its benefits, its expected side effects, how to use the medicine, and what to do if a dose is missed. The clinician advised these patients to use available technology, such as mobile phones, to alert them to take their next dose. Friendly discussions about life balance and the importance of being healthy were initiated with patients who forgot medications due to being busy or who worked long hours, or because of travelling or leaving home.

6.4. Discussion

Assessment of non-adherence to cardiovascular medications has a potential impact on clinical practice in Iraq. This assessment is helpful to patients, clinicians and the healthcare system in general. Assessment of medication non-adherence has a potential impact on patients through the optimisation and personalisation of required doses, maximising the benefit of the prescribed medication, preventing unnecessary interventions such as being prescribed an increased dose or adding further medications to the regimen, thus improving patients' clinical outcomes and their quality of life. Medication non-adherence may lead to increased mortality and morbidity and increase costs due to rehospilisation and medication wastage.

The data obtained from the MMAS-8 and blood microsampling analysis and integration of the outcome with face-to-face interviews can help the clinician to individualise patient care and understand why patients are not adherent to a particular medication, which will consequently help the clinician in terms of finding the interventions required to improve adherence. For instance, the clinician may respond to non-adherence related to medication side effects by educating such patients about the possible and the significant

side effects associated with medications prior to prescription or by prescribing medications with a low risk of, or less severe side effects. Moreover, the clinician can simplify the medication regimen to reduce the possibility of medication side effects.
Non-adherence to medication due to cost can by managed by prescribing less expensive brand medicines when appropriate or prescribing generic medications which are accessible and available in the public sector to reduce out of pocket expenses to the patient. The pharmacist can advise the clinician about the availability of low-price medications or by prescribing generic, rather than brand, medications.

Unintentional non-adherence due to patients' lack of knowledge about the nature of chronic diseases, such as cardiovascular diseases, can be managed by the clinician improving patient education about the diseases and the rational use of medication using everyday language, and making such information more accessible and understandable. Non-adherence due to forgetfulness also can be managed by preparation of cards that includes information about the prescribed medication, its benefits, its expected side effects, how to use it, and what to do if a dose is missed. Patients can be advised to use available technology, such as mobile phones, to alert them that it is time to take their medication. The patient–provider relationship is crucial to improving adherence, where the key related point is ensuring a blame-free and friendly environment for discussions with patients in which they are encouraged to ask about their conditions and the associated medications.

Some patients intentionally do not adhere to their medications due to their beliefs. The clinician should explain to such patients the rationale behind prescribing particular medicines and the possibility of adverse drug reactions if a higher dose than prescribed is taken. Patients' beliefs about their diseases and medications can be improved through the use of patient-centred counselling techniques, such as motivational interviews, to challenge negative ideas. The assessment of non-adherence represents an excellent investment in clinical practice that may improve quality of patients' lives and reduce the cost of treatment.

6.5. Conclusion

Assessment of non-adherence can help the clinician to apply the required interventions to improve medication adherence, individualise patient medication, and to facilitate drug optimisation within clinical practice. Different factors are associated with non-adherence, so understanding the underlying causes is crucial to the adoption of the required interventions. Adherence can be improved by educating patients about their diseases, expected side effects and the medication regimen; it can also be improved by prescribing less expensive medications which are available from the public sector. Patient beliefs and attitudes can be improved through the use of patient-centred counselling techniques, such as motivational interviews, to inspire behavioural change and challenge negative ideas. Patients who forget to take medication can be advised to use various forms of technology to remind them to take their dose.

Chapter 7

Overall Conclusion and Future Work

This chapter summarises the general findings of this study and also highlights future prospects for related research.

7.1 Introduction

Globally, there has been a definite increase in patients requiring polypharmacy for chronic diseases (Mangin *et al*., 2018). For instance, in the UK, it is estimated than 24% of adults taking more than three medications (Moody and Mindell, 2017). Associated non-adherence to medication is accordingly common and could result in poor clinical outcomes or increased mortality and morbidity. Thus, reduced medication adherence could imply adverse events, and increased costs to healthcare systems due to the increased need for rehospitalisation. Measures to improve medication adherence are urgently needed worldwide in order to increase general life expectancy. Optimising adherence to medications may represent a powerful means of reducing morbidity and mortality. Medication adherence has a positive effect on the healthcare sector and indeed most healthcare providers.

Research into medication adherence and investigating the reasons for non-adherence to prescribed pharmacotherapy in Iraq is still in its infancy. To date, there has been no previous application of direct methods for the assessment of non-adherence, and indeed only limited studies using indirect methods through the use of questionnaires. The majority of such studies have used non-standard questionnaires.

The current research assessed non-adherence to certain target cardiovascular medications and further attempted to identify some of the causes of non-adherence to these same medications; it further proposed a number of interventions since CVD is one of the top killers in Iraq. Two different methods (direct and indirect) were used to assess non-adherence to selected CVD medications in 303 Iraqi patients who took one or more of these medications: the indirect method, by application of the eight-item Morisky Medication Adherence Scale (MMAS-8), and the direct method, through determination of the concentrations of these medications in dried blood spots by application of microsampling-based LC-HRMS assay. This study is, to the best of our knowledge, the first to use the direct method of analysis of dried blood spots (DBS) analysis by LC-HRMS in Iraq and further, again to the best of our knowledge, there has been no previous study that integrates DBS analysis by the previously validated LC-HRMS method and

MMAS-8 to assess non-adherence to cardiovascular diseases. This demonstrates a novel research approach. The outcomes determined for the present study suggested interventions to improve adherence to CVD medication after commencing friendly discussions between the clinician and patient, which would also represent novel clinical practice in Iraq.

The outcomes from the integrated approaches in the present study produced the following conclusions:

Significant weaknesses in the Iraqi health system with regard to dealing with chronic diseases and, particularly, cardiovascular diseases, have been noticed. Despite the fact that cardiovascular diseases are the top killer in Iraq, there is no documentation about their prevalence in the general population. Moreover, there are no guidelines for the treatment or management of cardiovascular medications, which has led to arbitrary decision making regarding their prescription.

The mortality rate associated with cardiovascular diseases in Iraq is very high, and there are no applicable action plans or strategies enacted at the national level to control CVD. This mortality rate may in part be due non-adherence to CVD medications. The study showed that 49.2% of Iraqi volunteers were non-adherent to one or more of their prescribed CVD medications, thus the assessment of medication non-adherence should be considered a priority and should be enforced in routine follow-up visits in clinical practice in Iraq.

Not all medications used for the treatment of cardiovascular diseases are available and accessible to patients in the public sector, which may be due to poor assessments of patients' annual needs for CVD medications. The lack of availability of CVD medications within the public sector will place additional economic burdens on patients and consequently result in poor adherence to medication. This may suggest that the assessment of the annual need of CVD medications should be improved and provide a free medication scheme to patients with cardiovascular diseases.

Different methods of assessment of non-adherence can produce different outcomes. Huge differences and discrepancies in the assessment of non-adherence to the selected

cardiovascular medications in the Iraqi volunteers was apparent through the application of the indirect method, using the eight-item Morisky Medication adherence scale (MMAS-8), and the direct method of measurement of drug concentrations in DBS samples for the same volunteers. MMAS-8 indicated that 18.2% of volunteers were non-adherent. However, the level of non-adherence to the target cardiovascular medications as determined by DBS analysis was 49.2%. The results showed that 72.3% of patients were intentionally non-adherent to their medication, the main causes of which were side effects and the inconvenience associated with taking the medication. However, 70.9% of non-adherent patients did not specify a reason for their non-adherence. MMAS-8 was not able to provide information about the source of inconvenience or non-specified reasons, which suggests that MMAS-8 needs further development or can be combined with face-to-face interviews to provide accurate information about the sources of inconvenience or other reasons.

Only 27.3% of patients were unintentionally non-adherent, the main causes of which were a poor understanding that cardiovascular diseases are chronic and require that medication be taken for the rest of their lives, forgetfulness, and travelling and leaving home. Otherwise, this may also possibly indicate that the problems arise from patients' beliefs, attitudes and expectations and a lack of motivation to continue their treatment regimen; all this requires additional study.

The application of indirect methods such as MMAS-8 is unable to track non-adherence to each medication in the regimen. Moreover, tracking both dosing error and prescription error is not possible. Patients may take the wrong medication or the wrong dose at the wrong time, and in this case whilst the medication-taking behaviour is present the patient will lose the benefits of their medications or may experience adverse side effects. Moreover, MMAS-8 cannot determine patient-to-patient variation in pharmacokinetics, pharmacodynamics and pharmacogenetics, which affect bioavailability and drug concentrations in the blood. On the other hand, the direct method using DBS analysis can track non-adherence to each medication taking into consideration all variables and individual patient's data.

Microsampling analysis can individualise patient data by providing information on the levels of each medication in the patient's blood. Thus, in case of a poor patient response

to treatment, this information can help clinicians to assess adherence to each medication and the determination of which medication(s) in the regimen the patient is non-adherent to. The results of this DBS assay can provide objective data on blood drug levels to enable the clinician to make an informed decision about future treatment, i.e., this method provides a valuable evidence base. The results could represent a useful approach to improving patients' health through dose optimisation and individualisation and reduce costs by reducing hospital readmission and medications wastage. The application of the MMAS-8 and microsampling analysis in conjunction with clinician led face-to-face interview where questionable results are obtained can help healthcare providers to accurately assess non-adherence and identify barriers associated with non-adherence, and thus improve individual patient outcomes.

The outcomes of this study demonstrated no significant relation between non-adherence to the target medication and either gender or age. Also, the results showed a significant positive relationship between non-adherence to cardiovascular medication and the number of such medications being taken by individual patients.

The validated and developed method, through the application of microsampling-based LC-HRMS, was able to simultaneously determine a number of cardiovascular medications in a given volunteer's blood sample in a single run. This offers a reliable, cost-effective method for assessment of different cardiovascular medications. The applied method could represent a feasible alternative to traditional blood sampling (venepuncture) for the TDM of cardiovascular drugs, which is less invasive. The sample can be collected by patients at home and sent to the laboratory by post, which may enable the implementation of routine TDM for CVD medications in everyday clinical practice. This is considered to represent a novel approach in Iraq.

The developed microsampling-based LC-HRMS assay can be adapted and extended to assess adherence to other medications used for cardiovascular diseases or, indeed, other chronic diseases such as diabetes, depression and cancer. The full mass scan offered by LC-HRMS is useful in TDM. In cases of poor patient progression, it is still possible to revisit data at a later time to provide additional clinical data if required with need for further testing.

The results showed that 903 cards and VAMS provide comparable quantitative results for assessing non-adherence to target medications. However, VAMS overcomes some of the limitations to the use of conventional DBS cards since there is no need to use a puncher with VAMS. Eliminating card punching from the process would save considerable time and effort. Sampling using VAMS is quick and does not require assistance. Sometimes, however, the VAMS clamshell may cause some inconvenience. Labelling a DBS card is more convenient in comparison with VAMS since spaces are provided to record information on the DBS cards while this is not the case with VAMS. VAMS is also significantly more expensive than DBS cards.

It was observed in this study, as highlighted in chapter 6 Section 6.3.3.7, that the clinician reported improved adherence to medications after appropriate discussions were initiated with the patients.

7.2 Future Work

7.2.1. Analytical Aspects

The extraction procedure used for atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan in microvolume blood samples collected on VAMS and DBS cards was not suitable for amlodipine. Thus, a different extraction procedure needs to be developed to enable analysis of all compounds, including amlodipine, in a single LC-HRMS analytical run.

Furthermore, since plasma is considered to represent the gold standard matrix for TDM, a study to determine the ratio between drug concentrations in DBS and plasma for the selected cardiovascular drugs should be undertaken in future research.

Currently, the extraction procedure requires a considerable amount of time, especially when running hundreds of patient samples. The extraction procedure involves the manual punching of 903 cards, followed by the addition of solvents to the punched disk, vortexing, centrifuging, and the evaporation of the supernatant and reconstitution of the dried residue in solvent for analysis. In addition, although efforts towards the miniaturisation of mass spectrometers have been explored over the last three decades, these instruments, as are currently available, require a considerable amount of space and hence would not be ideal for a hospital ward. However, this instrument could be placed

in one regional central laboratory where all samples could be sent for analysis. Funds will therefore be needed to procure such instruments as well as to provide the necessary training for the technicians required to operate the equipment. Thus, automation is a particular requirement to reducing processing time and increasing analytical throughput. Automation of the instrument is further required in everyday practice, such as-in sample preparation, performing analyses and producing reports. Automation of the equipment will, of course, enhance its applicability to clinical practice.

Considering the potential of this research, it is important to explore the application and development of microsampling-based LC-HRMS assay for screening other cardiovascular medications which were not included in this research. This will help to provide more efficient clinical practice and, consequently, decrease mortality and morbidity of cardiovascular diseases in Iraq and decrease medication waste.

7.2.2. Technology Transfer and Implementation of Microsample Analysis in Iraq

The microsampling-based LC-HRMS assay developed in this study has drawn considerable interest from the Iraqi government and, dependent on its final outcomes, is being considered for implementation in clinical practice. There is a serious desire on the part of the Misan health directorate to transfer this technology for application in its laboratories (Appendix 23). Analysis of large patient samples requires extensive memory space/storage, and this could possibly slow down data processing; software upgrades will also be required. Long waiting times for the results may delay the proper response to patients' cases in the worst case leading to patient death.

Medicine optimisation is critical to ensuring the effectiveness of medications in the management and treatment of chronic diseases such as cardiovascular diseases and diabetes. The Iraqi Ministry of Health should set appropriate guidelines to manage the treatment of cardiovascular diseases and regulate the costs of treatment. Quality of medicines should also be assessed since if the medicine is falsified/substandard and a patient takes it, this could lead to unintentional non-adherence (amongst other poor patient outcomes). The MOH should apply such a policy by facilitating the availability of cardiovascular medications from the public sector, prevention or control of tobacco use through stringent policy making, increased taxes for foods high in fat, salt, and sugar,

the construction of walking and cycling paths, running awareness campaigns regarding the importance of physical activity, and should implement strategies to reduce the harmful effects of alcohol consumption.

Other socioeconomic factors, such as the cost of medication, level of education, and route to accessing medications, could be considered to develop further ideas related to factors associated with poor adherence. Qualitative methods can also be explored to gain patients' insights to understand more of the causes associated with non-adherence. Triangulation of quantitative and qualitative methods in future research to assess non-adherence could reveal additional associated causes.

References

ABBO, E.D., ZHANG, Q., ZELDER, M. and HUANG, E.S., 2008. The increasing number of clinical items addressed during the time of adult primary care visits. *Journal of General Internal Medicine,* **23**(12), pp. 2058-2065.

ABDERRAHMAN, B., 2018. Digital ingestion tracking system in pills and medication adherence: future advantages and current limitations. *Lung Cancer,* **15**, pp. 1-5.

ABOLBASHARI, M., MACAULAY, T.E., WHAYNE, T.F., MUKHERJEE, D. and SAHA, S., 2017. Polypharmacy in Cardiovascular Medicine: Problems and Promises. *Cardiovascular & Hematological Agents in Medicinal Chemistry,* **15**(1), pp. 31-39.

ADDISON, C.C., JENKINS, B.W., SARPONG, D., WILSON, G., CHAMPION, C., SIMS, J. and WHITE, M.S., 2011. Relationship between medication use and cardiovascular disease health outcomes in the Jackson Heart Study. *International Journal of Environmental Research and Public Health,* **8**(6), pp. 2505-2515.

ADULT MEDICATION, 2006. Improving Medication Adherence in Older Adults Overview of medication adherence where are we today? Available: http://adultmeducation.com/OverviewofMedicationAdherence_4.html [Accessed 16/01/ 2018].

AGILENT TECHNOLOGIES, 2014. Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide. Available: https://www.agilent.com/cs/library/usermanuals/public/G3335-90173_TOF_Q-TOF_Concepts.pdf [Accessed 15/03/2019].

AKYEA, R.K., Kai, J., QURESHI, N., IYEN, B., WENG, S.F., 2019. Sub-optimal cholesterol response to initiation of statins and future risk of cardiovascular disease. *Heart*, 105(13), pp. 975-981.

AL HILFI, T.K., LAFTA, R. and BURNHAM, G., 2013. Health services in Iraq. *The Lancet*, **381**(9870), pp. 939-948.

AL MOSAWI, A. and AL HASNAWI, S., 2009. Iraq health care system: An overview. *The New Iraqi Journal of Medicine,* **5**(3), pp. 5-13.

ALA'DIN, A., 2004. *Health in Iraq. The Current Situation, Our Vision for the Future and Areas of Work.* Iraq: Ministry of Health.

ALBAZ, R.S., 1997. Factors affecting patient compliance in Saudi Arabia. *Journal of Social Sciences,* **25**(6), pp. 5-8.

ALCAIDE, M.L., RAMLAGAN, S., RODRIGUEZ, V.J., COOK, R., PELTZER, K., WEISS, S.M., SIFUNDA, S. and JONES, D.L., 2017. Self-report and dry blood spot

measurement of antiretroviral medications as markers of adherence in pregnant women in rural South Africa. *AIDS and Behavior,* **21**(7), pp. 2135-2140.

AL-DABBAGH, S. and ASWAD, S., 2009. Compliance of hypertensive patients to management in Duhok governorate using Morisky-green test. *Duhok Medical Journal,* **4**, pp. 28-39.

ALDEER, M., JAVANMARD, M. and MARTIN, R.P., 2018. A Review of Medication Adherence Monitoring Technologies. *Applied System Innovation,* **1**(14), pp. 1-27.

AL-GHAZAWI, M. and ABURUZ, S., 2010. Determination of ciprofloxacin in dried blood spots for therapeutic drug monitoring. *Chromatographia,* **71**(11-12), pp. 999-1005.

AL-GHUZI, A.A.S. and AL-ASADI, J.N., 2014. Prevalence and socio-demographic determinants of hypertension in Thi-Qar Governorate: a household survey. *Open Journal of Advanced Drug Delivery*, **2**(6), pp. 802-815.

ALHALAIQA, F., DEANE, K., NAWAFLEH, A., CLARK, A. and GRAY, R., 2012. Adherence therapy for medication non-compliant patients with hypertension: a randomised controlled trial. *Journal of Human Hypertension,* **26**(2), pp. 117-126.

ALHALAIQA, F., OMARI, O., WYNADEN, D., ALMUSALLAMI, N., BASHTAWY, M., MORISKY, D.E., BATIHA, A. and ALKHAWALDEH, A., 2016. Adherence to Antipsychotic Drug Measured by an Arabic Version of Morisky Scale. *Issues in Mental Health Nursing,* **37**(12), pp. 975-982.

ALHEWITI, A., 2014. Adherence to Long-Term Therapies and Beliefs about Medications. *International Journal of Family Medicine,* **2014**, pp. 1-8.

ALIPANAH, N., JARLSBERG, L., MILLER, C., LINH, N., FALZON, D., JARAMILLO, E. and NAHID, P., 2018. Adherence interventions and outcomes of tuberculosis treatment: A systematic review and meta-analysis of trials and observational studies. *PLoS Medicine,* **15(**7), pp. 1-44.

ALJUMAH, K., AHMAD HASSALI, A. and ALQHATANI, S., 2014. Examining the relationship between adherence and satisfaction with antidepressant treatment. *Neuropsychiatric Disease and Treatment,* **10**, pp. 1433-1438.

AL-JUMAILI, A.A., HUSSAIN, S.A. and SOROFMAN, B., 2013. Pharmacy in Iraq: history, current status, and future directions. *American Journal of Health-System Pharmacy*, **70**(4), pp. 368-372.

ALKATHERI, A.M., ALYOUSIF, S.M., ALSHABANAH, N., ALBEKAIRY, A.M., ALHARBI, S., ALHEJAILI, F.F., ALSAYYARI, A.A., QANDIL, A.M. and QANDIL, A.M., 2014. Medication adherence among adult patients on hemodialysis. *Saudi Journal of Kidney Diseases and Transplantation,* **25**(4), pp. 762-768.

ALKHATEEB, F., 2015. *Novel Strategies for Rapid Analytical Preparation of Solid Samples*, (Doctoral thesis, University of Calgary).pp.3-35.

ALMADHOUN, M. and HALA, A., 2018. Assessment of Medication Adherence and its Association with Glycemic Control among Type-2 Diabetes Mellitus Patients in Gaza–Palestine. *Journal of Clinical and Experimental Pharmacology,* **8**(3), pp. 1-10.

ALMUZAINI, T., SAMMONS, H. and CHOONARA, I., 2013. Substandard and falsified medicines in the UK: a retrospective review of drug alerts (2001-2011). *British Medical Journal Open,* **3**(7), pp. 1-9.

AL-RAMAHI, R., 2015. Adherence to medications and associated factors: A cross-sectional study among Palestinian hypertensive patients. *Journal of Epidemiology and Global Health,* **5**(2), pp. 125-132.

ALSUMARIA IRAQI SATELLITE TV NETWORK, 2012. Counterfeit medications in Iraqi markets, an alarming occurrence [Homepage of Alsumaria Iraqi Satellite TV Network], [Online]. Available: https://www.alsumaria.tv/news/56380/counterfeit-medications-in-iraqi-markets-an-alarmi/en# [Accessed 24/06/2018].

ALTHUBAITI, A., 2016. Information bias in health research: definition, pitfalls, and adjustment methods. *Journal of Multidisciplinary Healthcare*, **9**, pp. 211-217.

AL-TUKMAGI, H.F. and AL-AUQBI, T.F., 2015. Assessment of Morisky Medication Adherence scale (8-MMAS) in a sample of Iraqi type 2 diabetic patients. *Iraq Journal of Community Medicine,* **28**(4), pp. 212-215.

ALTUWAIRQI, H.B., 2016. Barriers to medication adherence among cardiac patients following at King Fahad Medical City, Riyadh, Saudi Arabia. *Saudi Journal for Health Sciences,* **5**(1), pp. 20-24.

AMBACH, L. and STOVE, C., 2019. Determination of Cocaine and Metabolites in Dried Blood Spots by LC-MS/MS. In: S.C. LANGMAN L., ed, *LC-MS in Drug Analysis.* New York, NY: Humana Press, pp. 261-272.

ANDERSON, J.R. and NAWARSKAS, J.J., 2001. Cardiovascular drug-drug interactions. *Cardiology Clinics,* **19**(2), pp. 215-234.

AONUMA, K., SHIGA, T., ATARASHI, H., DOKI, K., ECHIZEN, H., HAGIWARA, N., HASEGAWA, J., HAYASHI, H., HIRAO, K. and ICHIDA, F., 2017. Guidelines for therapeutic drug monitoring of cardiovascular drugs clinical use of blood drug concentration monitoring (Jcs 2015). *Circulation Journal,* **81**(4), pp. 581-612.

ARORA, S., PETERS, A.L., BURNER, E., LAM, C.N. and MENCHINE, M., 2014. Trial to examine text message–based mHealth in emergency department patients with diabetes (TExT-MED): A randomized controlled trial. *Annals of Emergency Medicine,* **63**(6), pp. 745-754.

ASHUR, S., SHAMSUDDIN, K., SHAH, S., BOSSERI, S. and MORISKY, D., 2015. Reliability and known-group validity of the Arabic version of the 8-item Morisky Medication Adherence Scale among type 2 diabetes mellitus patients. *Eastern Mediterranean Health Journal,* **21**(10), pp. 722-728.

ASSAWASUWANNAKIT, P., BRAUND, R. and DUFFULL, S., 2015. A model-based meta-analysis of the influence of factors that impact adherence to medications. *Journal of Clinical Pharmacy and Therapeutics,* **40**(1), pp. 24-31.

ATINGA, R.A., YARNEY, L. and GAVU, N.M., 2018. Factors influencing long-term medication non-adherence among diabetes and hypertensive patients in Ghana: A qualitative investigation. *PloS One,* **13**(3), pp. 1-15.

ATKINS, L. and FALLOWFIELD, L., 2006. Intentional and non-intentional non-adherence to medication amongst breast cancer patients. *European Journal of Cancer,* **42**(14), pp. 2271-2276.

AYRE, A., CHAUDHARI, P., SHAIKH, J., JAGDALE, S. and AGRAWAL, O., 2018. DRIED MATRIX SPOTING-AN INNOVATIVE SAMPLE PREPARATION TOOL IN BIOANALYSIS. *International Journal of Pharmaceutical Sciences and Research,* **9**(9), pp. 3597-3607.

BAE, S.G., JEON, H.J., YANG, H.S., KIM, B.K. and PARK, K.S., 2015. Medication adherence and its predictors in community elderly patients with hypertension. *Korean Journal of Health Promotion,* **15**(3), pp. 121-128.

BAE, S.G., KAM, S., PARK, K.S., KIM, K.Y., HONG, N.S., KIM, K.S., LEE, Y.M., LEE, W.K. and CHOE, M.S., 2016. Factors related to intentional and unintentional medication nonadherence in elderly patients with hypertension in rural community. *Patient Preference and Adherence,* **10**, pp. 1979-1989.

BAHRAM, R., 2013. Reinventing Iraqi Pharmaceuticals [Homepage of invest in group], [Online]. Available: https://investingroup.org/interview/5 [Accessed 18/02/2019].

BARBOUR, V., 2001. Imatinib for chronic myeloid leukaemia: a NICE mess. *Lancet,* **358**(9292), pp. 1478.

BARCO, S., CASTAGNOLA, E., MOSCATELLI, A., RUDGE, J., TRIPODI, G. and CANGEMI, G., 2017. Volumetric adsorptive microsampling-liquid chromatography tandem mass spectrometry assay for the simultaneous quantification of four antibiotics in human blood: method development, validation and comparison with dried blood spot. *Journal of Pharmaceutical and Biomedical Analysis,* **145**, pp. 704-710.

BARNETT, N., 2014. Medication adherence: where are we now? A UK perspective. *European Journal of Hospital Pharmacy,* **21**(3), pp. 181-184.

BAZARGAN, M., SMITH, J., YAZDANSHENAS, H., MOVASSAGHI, M., MARTINS, D. and ORUM, G., 2017. Non-adherence to medication regimens among older African-American adults. *BMC Geriatrics,* **17**(163), pp. 1-12.

BERGLUND, E., LYTSY, P. and WESTERLING, R., 2013. Adherence to and beliefs in lipid-lowering medical treatments: a structural equation modeling approach including the necessity-concern framework. *Patient Education and Counseling,* **91**(1), pp. 105-112.

BERNIEH, D., LAWSON, G. and TANNA, S., 2017a. Quantitative LC-HRMS determination of selected cardiovascular drugs, in dried blood spots, as an indicator of adherence to medication. *Journal of Pharmaceutical and Biomedical Analysis*, **142**, pp. 232-234.

BERNIEH, D., 2017b. *Application of microsampling methods to cardiovascular medication adherence assessment*, (Doctoral thesis, De Montfort University), p.p. 163-164.

BERRY, D.L., BLONQUIST, T.M., HONG, F., HALPENNY, B. and PARTRIDGE, A.H., 2015. Self-reported adherence to oral cancer therapy: relationships with symptom distress, depression, and personal characteristics. *Patient Preference and Adherence,* **9**, pp. 1587-1592.

BINDSCHEDLER, M., DEGEN, P., FLESCH, G., DE GASPARO, M. and PREISWERK, G., 1997. Pharmacokinetic and pharmacodynamic interaction of single oral doses of valsartan and furosemide. *European Journal of Clinical Pharmacology,* **52**(5), pp. 371-378.

BITTON, A., CHOUDHRY, N.K., MATLIN, O.S., SWANTON, K. and SHRANK, W.H., 2013. The impact of medication adherence on coronary artery disease costs and outcomes: a systematic review. *The American Journal of Medicine,* **126**(4), pp. 7-27.

BLACK, L., 2014. Prescriptions: Costs and charges in the UK and Republic of Ireland [Homepage of Northern Ireland Assembly], [Online]. Available: http://www.niassembly.gov.uk/globalassets/Documents/RaISe/Publications/2014/general/6114.pdf [Accessed 11/12/2018].

BLENKINSOPP, A., BOND, C., BRITTEN, N., FEELY, M., GREEN, P., GEORGE, C., HARRIS, C., HURST, L., MAGGS, C. and MARINKER, M., 1997. *From Compliance to Concordance: Achieving Shared Goals in Medicine Taking.* London: Royal Pharmaceutical Society of Great Britain.

BLESSBORN, D., ROMSING, S., BERGQVIST, Y. and LINDEGARDH, N., 2010. Assay for screening for six antimalarial drugs and one metabolite using dried blood spot sampling, sequential extraction and ion-trap detection. *Bioanalysis*, **2**(11), pp. 1839-1847.

BOSWORTH, H.B., 2014. *Enhancing medication adherence: The public health dilemma.* Durhum, NC: Springer Science & Business Media.

BOWEN, C.L., KEHLER, J., BORAM, S., ABBERLEY, L., SZAPACS, M. and EVANS, C., 2016. Modify on the fly: triple quad to high resolution in support of a dermal clinical study requiring an ultra low LLOQ. *Bioanalysis*, **8**(3), pp. 205-214.

BRAMLAGE, P., KETELHUT, R., FRONK, E., WOLF, W., SMOLNIK, R., ZEMMRICH, C. and SCHMIEDER, R.E., 2014. Clinical impact of patient adherence to a fixed-dose combination of olmesartan, amlodipine and hydrochlorothiazide. *Clinical Drug Investigation*, **34**(6), pp. 403-411.

BREIDINGER, S. and WOOLF, E., 2017. Regulated Bioanalysis—Historical Aspects and Key Concepts Related to Its Use. *Regulated Bioanalysis: Fundamentals and Practice.* Springer, pp. 87-102.

BRITISH HEART FOUNDATION, 2017. CVD STATISTICS–BHF UK FACTSHEET [Homepage of British Heart Foundation], [Online]. Available: https://www.bhf.org.uk/research/heart-statistics [Accessed 04/07/2018].

BROWN, M.T. and BUSSELL, J.K., 2011. Medication adherence: WHO cares? *Mayo Clinic Proceedings*, **86**(4), pp. 304-314.

BRUNNER, R., DUNBAR-JACOB, J., LEBOFF, M.S., GRANEK, I., BOWEN, D., SNETSELAAR, L.G., SHUMAKER, S.A., OCKENE, J., ROSAL, M. and WACTAWSKI-WENDE, J., 2009. Predictors of adherence in the women's health initiative calcium and vitamin D trial. *Behavioral Medicine*, **34**(4), pp. 145-155.

BRYSON, C.L., AU, D.H., YOUNG, B., MCDONELL, M.B. and FIHN, S.D., 2007. A refill adherence algorithm for multiple short intervals to estimate refill compliance (ReComp). *Medical Care*, **45**(6), pp. 497-504.

BUCKLEY, G.J. and GOSTIN, L.O., 2013. *Countering the Problem of Falsified and Substandard Drugs.* Washington, D.C.: National Academies Press.

BUSHRA, B. and KAMERAN, H., 2013. Uncontrolled Hypertension In A Group Of Hypertensive Patients In Erbil. *Journal of Kufa for Nursing Science*, **3**(2), pp. 126-132.

BYLDA, C., THIELE, R., KOBOLD, U. and VOLMER, D.A., 2014. Recent advances in sample preparation techniques to overcome difficulties encountered during quantitative analysis of small molecules from biofluids using LC-MS/MS. *Analyst*, **139**(10), pp. 2265-2276.

CAPE, S.S., MENG, M., KOUPAEI-ABYAZANI, M.R. and FAST, D.M., 2017. Liquid Chromatography–Mass Spectrometry Assays in the Regulated Bioanalytical Laboratory. *Regulated Bioanalysis: Fundamentals and Practice.* Springer, pp. 121-153.

CASTILLO-MANCILLA, J.R., ZHENG, J., ROWER, J.E., MEDITZ, A., GARDNER, E.M., PREDHOMME, J., FERNANDEZ, C., LANGNESS, J., KISER, J.J. and BUSHMAN, L.R., 2013. Tenofovir, emtricitabine, and tenofovir diphosphate in dried

blood spots for determining recent and cumulative drug exposure. *AIDS Research and Human Retroviruses,* **29**(2), pp. 384-390.

CAVALLARI, L.H. and LIMDI, N.A., 2009. Warfarin pharmacogenomics. *Current Opinion in Molecular Therapeutics,* **11**(3), pp. 243-251.

CAVANAGH, C. and COPPINGER, C., 2009. Newborn blood spot sampling. *Infant,* **5**(3), pp. 168-171.

CHAN, H. and HASSALI, M.A., 2014. Modified labels for long-term medications: influences on adherence, comprehension and preferences in Malaysia. *International Journal of Clinical Pharmacy,* **36**(5), pp. 904-913.

CHANG, J., PEYSAKHOVICH, F., WANG, W. and ZHU, J., 2011. The UK Health Care System. Available: http://assets.ce.columbia.edu/pdf/actu/actu-uk.pdf [Accessed 03/13/2019].

CHANG, M. and SHIN, W., 2012. Comparative Pharmacokinetics and Bioequivalence of Two 50 mg Atenolol Tablet Formulations in Healthy Korean Male Volunteers. *Arzneimittelforschung,* **62**(09), pp. 410-413.

CHEPYALA, D., TSAI, I., LIAO, H., CHEN, G., CHAO, H. and KUO, C., 2017. Sensitive screening of abused drugs in dried blood samples using ultra-high-performance liquid chromatography-ion booster-quadrupole time-of-flight mass spectrometry. *Journal of Chromatography A,* **1491**, pp. 57-66.

CHERNONOSOV, A., 2018. The Use of Dried Blood Spots for the Quantification of Antihypertensive Drugs. *International Journal of Analytical Chemistry,* **2018**, pp. 1-12.

CHOO, P.W., RAND, C.S., INUI, T.S., LEE, M.T., CAIN, E., CORDEIRO-BREAULT, M., CANNING, C. and PLATT, R., 1999. Validation of patient reports, automated pharmacy records, and pill counts with electronic monitoring of adherence to antihypertensive therapy. *Medical Care,* **37**(9), pp. 846-857.

CHOUDHRY, N.K., FISCHER, M.A., AVORN, J., LIBERMAN, J.N., SCHNEEWEISS, S., PAKES, J., BRENNAN, T.A. and SHRANK, W.H., 2011. The implications of therapeutic complexity on adherence to cardiovascular medications. *Archives of Internal Medicine,* **171**(9), pp. 814-822.

CHOUDHRY, N.K., GLYNN, R.J., AVORN, J., LEE, J.L., BRENNAN, T.A., REISMAN, L., TOSCANO, M., LEVIN, R., MATLIN, O.S. and ANTMAN, E.M., 2014. Untangling the relationship between medication adherence and post–myocardial infarction outcomes: medication adherence and clinical outcomes. *American Heart Journal,* **167**(1), pp. 51-58.

CLARKE, W., 2016. Overview of Therapeutic Drug Monitoring. *Clinical Challenges in Therapeutic Drug Monitoring.* 1 edn. New York: Elsevier, pp. 1-15.

CLIFFORD, S., BARBER, N. and HORNE, R., 2008. Understanding different beliefs held by adherers, unintentional nonadherers, and intentional nonadherers: application of the necessity–concerns framework. *Journal of Psychosomatic Research,* **64**(1), pp. 41-46.

COLBY, J.M., THOREN, K.L. and LYNCH, K.L., 2017. Optimization and validation of high-resolution mass spectrometry data analysis parameters. *Journal of Analytical Toxicology,* **41**(1), pp. 01-05.

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE, 2011. Guideline on bioanalytical method validation [Homepage of European Medicines Agency], [Online]. Available: www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500109686. [Accessed 08/16/2018].

COOPER, R.S., ORDUNEZ, P., IRAOLA FERRER, M.D., MUNOZ, J.L.B. and ESPINOSA-BRITO, A., 2006. Cardiovascular disease and associated risk factors in Cuba: prospects for prevention and control. *American Journal of Public Health,* **96**(1), pp. 94-101.

COOPER, J.A., CADOGAN, C.A., PATTERSON, S.M., KERSE, N., BRADLEY, M.C., RYAN, C. and HUGHES, C.M., 2015. Interventions to improve the appropriate use of polypharmacy in older people: a Cochrane systematic review. *British Medical Journal Open,* **5**(12), pp. 1-11.

COX, K.L., DEVANARAYAN, V., KRIAUCIUNAS, A., MANETTA, J., MONTROSE, C. and SITTAMPALAM, S., 2014. Immunoassay methods. In Assay Guidance Manual [Internet]. Bethesda, USA: Eli Lilly & Company and the National Centre for Advancing Translational Sciences.

CRAMER, J.A., ROY, A., BURRELL, A., FAIRCHILD, C.J., FULDEORE, M.J., OLLENDORF, D.A. and WONG, P.K., 2008. Medication compliance and persistence: terminology and definitions. *Value in Health,* **11**(1), pp. 44-47.

CUMMINGS, D.M., LUTES, L., LITTLEWOOD, K., DINATALE, E., HAMBIDGE, B., SCHULMAN, K. and MORISKY, D.E., 2014. Regimen-related distress, medication adherence, and glycemic control in rural African American women with type 2 diabetes mellitus. *Annals of Pharmacotherapy,* **48**(8), pp. 970-977.

CUSHING, A. and METCALFE, R., 2007. Optimizing medicines management: From compliance to concordance. *Therapeutics and Clinical Risk Management,* **3**(6), pp. 1047-1058.

CUTLER, R.L., FERNANDEZ-LLIMOS, F., FROMMER, M., BENRIMOJ, C. and GARCIA-CARDENAS, V., 2018. Economic impact of medication non-adherence by disease groups: a systematic review. *British Medical Journal,* **8**(1), pp. 1-13.

DAGLI, R.J. and SHARMA, A., 2014. Polypharmacy: a global risk factor for elderly people. *Journal of International Oral Health,* **6**(6), pp. i-ii.

DANIEL, W., ed, 1999. *Biostatistics: A Foundation for Analysis in the Health Sciences.* 7th ed. edn. New York: John Wiley & Sons.

DAS, S., FLEMING, D.H., MATHEW, B.S., WINSTON A, B., PRABHAKAR, A.T. and ALEXANDER, M., 2017. Determination of serum carbamazepine concentration using dried blood spot specimens for resource-limited settings. *Hospital Practice,* **45**(2), pp. 46-50.

DAWOOD, R., TWINER, M., MCNAUGHTON, C., BRODY, A., REED, B., DAWOOD, K., MANGO, L., FLACK, J. and LEVY, P., 2018. 106 Estimation of Medication Adherence by Morisky Medication Adherence Scale-4 Construct Compared to Liquid Chromatography Mass Spectrometry in Emergency Department Patients with Chronic Hypertension. *Annals of Emergency Medicine,* **72**(4), pp. S45.

DE ABREU, L., DE CASTRO, S. and PEDRAZZOLI, J., 2003. Atenolol quantification in human plasma by high-performance liquid chromatography: Application to bioequivalence study. *The American Association of Pharmaceutical Scientists,* **5**(2), pp. 116-122.

DE KESEL, P.M., CAPIAU, S., LAMBERT, W.E. and STOVE, C.P., 2014. Current strategies for coping with the hematocrit problem in dried blood spot analysis. *Bioanalysis,* **6**(14), pp. 1871-1874.

DE LAS CUEVAS, C. and PEÑATE, W., 2015. Psychometric properties of the eight-item Morisky Medication Adherence Scale (MMAS-8) in a psychiatric outpatient setting. International Journal of Clinical and Health Psychology, 15(2), pp. 121-129.

DE NICOLÒ, A., AVATANEO, V., RABBIA, F., BONIFACIO, G., CUSATO, J., TOMASELLO, C., PERLO, E., MULATERO, P., VEGLIO, F. and DI PERRI, G., 2016. UHPLC–MS/MS method with protein precipitation extraction for the simultaneous quantification of ten antihypertensive drugs in human plasma from resistant hypertensive patients. *Journal of Pharmaceutical and Biomedical Analysis,* **129**, pp. 535-541.

DE NICOLÒ, A., AVATANEO, V., RABBIA, F., SCIANDRA, M., TOSELLO, F., CUSATO, J., PERLO, E., MULATERO, P., VEGLIO, F. and DI PERRI, G., 2017. UHPLC–MS/MS method with sample dilution to test therapeutic adherence through quantification of ten antihypertensive drugs in urine samples. *Journal of Pharmaceutical and Biomedical Analysis,* **142**, pp. 279-285.

DE VERA, M.A., BHOLE, V., BURNS, L.C. and LACAILLE, D., 2014. Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review. *British Journal of Clinical Pharmacology,* **78**(4), pp. 684-698.

DEEP, A., KUMAR, P., KUMAR, A. and THAKKAR, A., 2012. Dry blood spot technique: A review. *International Journal of Pharmaceutical Sciences Review and Research,* **15**, pp. 90-94.

DENNIFF, P. and SPOONER, N., 2014. Volumetric absorptive microsampling: a dried sample collection technique for quantitative bioanalysis. *Analytical Chemistry,* **86**(16), pp. 8489-8495.

DENNIFF, P., PARRY, S., DOPSON, W. and SPOONER, N., 2015. Quantitative bioanalysis of paracetamol in rats using volumetric absorptive microsampling (VAMS). *Journal of Pharmaceutical and Biomedical Analysis,* **108**, pp. 61-69.

DIAS, E., HACHEY, B., MCNAUGHTON, C., NIAN, H., YU, C., STRAKA, B., BROWN, N.J. and CAPRIOLI, R.M., 2013. An LC–MS assay for the screening of cardiovascular medications in human samples. *Journal of Chromatography B,* **937**, pp. 44-53.

DING, L., ZHOU, X., GUO, X., SONG, Q., HE, J. and XU, G., 2007. LC–ESI-MS method for the determination of bisoprolol in human plasma. *Journal of Pharmaceutical and Biomedical Analysis,* **44**(2), pp. 520-525.

DUNBAR-JACOB, J., DWYER, K. and DUNNING, E.J., 1991. Compliance with antihypertensive regimen: a review of the research in the 1980s. *Annals of Behavioral Medicine,* **13**(1), pp. 31-39.

EICK, G.N., KOWAL, P., BARRETT, T., THIELE, E.A. and SNODGRASS, J.J., 2017. Enzyme-Linked Immunoassay-Based Quantitative Measurement of Apolipoprotein B (ApoB) in Dried Blood Spots, a Biomarker of Cardiovascular Disease Risk. *Biodemography and Social Biology,* **63**(2), pp. 116-130.

ESKÅS, P.A., HEIMARK, S., EEK MARIAMPILLAI, J., LARSTORP, A.C.K., FADL ELMULA, FADL ELMULA M and HØIEGGEN, A., 2016. Adherence to medication and drug monitoring in apparent treatment-resistant hypertension. *Blood Pressure,* **25**(4), pp. 199-205.

EUGENMED, CARDIOVASCULAR CLINICAL STUDY GROUP, REGITZ-ZAGROSEK, V., OERTELT-PRIGIONE, S., PRESCOTT, E., FRANCONI, F., GERDTS, E., FORYST-LUDWIG, A., MAAS, A.H. and KAUTZKY-WILLER, A., 2016. Gender in cardiovascular diseases: impact on clinical manifestations, management, and outcomes. *European Heart Journal,* **37**(1), pp. 24-34.

FABBIANI, M., DI GIAMBENEDETTO, S., CINGOLANI, A., FANTI, I., COLAFIGLI, M., TAMBURRINI, E., CAUDA, R., NAVARRA, P., DE LUCA, A. and MURRI, R., 2016. Relationship between self-reported adherence, antiretroviral drug concentration measurement and self-reported symptoms in patients treated for HIV-1 infection. *Infectious Diseases,* **48**(1), pp. 48-55.

FARMER, K.C., 1999. Methods for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. *Clinical Therapeutics*, **21**(6), pp. 1074-1090.

FAULL, R. and LEE, L., 2007. Prescribing in renal disease. *Australian Prescriber*, **30**(1), pp. 17-20.

FAWZI, W., MOHSEN, M.Y.A., HASHEM, A.H., MOUSSA, S., COKER, E. and WILSON, K.C., 2012. Beliefs about medications predict adherence to antidepressants in older adults. *International Psychogeriatrics*, **24**(01), pp. 159-169.

FELDMAN, R., BACHER, M., CAMPBELL, N., DROVER, A. and CHOCKALINGAM, A., 1998. Adherence to pharmacologic management of hypertension. *Canadian Journal of Public Health*, **89**(5), pp. I16-I18.

FERDINAND, K.C., SENATORE, F.F., CLAYTON-JETER, H., CRYER, D.R., LEWIN, J.C., NASSER, S.A., FIUZAT, M. and CALIFF, R.M., 2017. Improving medication adherence in cardiometabolic disease: practical and regulatory implications. *Journal of the American College of Cardiology*, **69**(4), pp. 437-451.

FERRARI, A., LICATA, M., RUSTICHELLI, C., BARALDI, C., VANDELLI, D., MARCHESI, F., PALAZZOLI, F., VERRI, P. and SILINGARDI, E., 2017. Monitoring of adherence to headache treatments by means of hair analysis. *European Journal of Clinical Pharmacology*, **73**(2), pp. 197-203.

FLANAGAN, R., BROWN, N. and WHELPTON, R., 2008. Therapeutic drug monitoring (TDM). *CPD Clinical Biochemistry*, **9**(1), pp. 3-21.

FLESCH, G., MÜLLER, P. and LLOYD, P., 1997. Absolute bioavailability and pharmacokinetics of valsartan, an angiotensin II receptor antagonist, in man. *European Journal of Clinical Pharmacology*, **52**(2), pp. 115-120.

FOOD AND DRUG ADMINISTRATION, 2013. Draft guidance for industry: bioanalytical method validation. [Homepage of Food and Drug Administration], [Online]. Available: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm36 [Accessed 08/16/2018].

FOOD AND DRUG ADMINISTRATION, 2014. Methods, methods Verification and validation [Homepage of FDA], [Online]. Available: https://www.fda.gov/downloads/ScienceResearch/FieldScience/LaboratoryManual/UCM092147.pdf [Accessed 02/13/2019].

FOOD AND DRUG ADMINISTRATION, 2018. How to Buy Medicines Safely from an Online Pharmacy [Homepage of FDA], [Online]. Available: https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048396.htm [Accessed 11/02/2018].

FORBES, C.A., DESHPANDE, S., SORIO-VILELA, F., KUTIKOVA, L., DUFFY, S., GOUNI-BERTHOLD, I. and HAGSTRÖM, E., 2018. A systematic literature review comparing methods for the measurement of patient persistence and adherence. *Current Medical Research and Opinion,* **34**, pp. 1613-1625.

FOSTER, J.M., SMITH, L., USHERWOOD, T., SAWYER, S.M. and REDDEL, H.K., 2016. General practitioner-delivered adherence counseling in asthma: feasibility and usefulness of skills, training and support tools. *Journal of Asthma,* **53**(3), pp. 311-320.

FOX, P.D., 2003. Prescription drug benefits: cost management issues for Medicare. *Health care financing review,* **25**(2), pp. 7-21.

GARBER, M.C., NAU, D.P., ERICKSON, S.R., AIKENS, J.E. and LAWRENCE, J.B., 2004. The concordance of self-report with other measures of medication adherence: a summary of the literature. *Medical Care,* **42**(7), pp. 649-652.

GARFIELD, S., CLIFFORD, S., ELIASSON, L., BARBER, N. and WILLSON, A., 2011. Suitability of measures of self-reported medication adherence for routine clinical use: a systematic review. *BMC Medical Research Methodology,* **11**(149), pp. 1-9.

GEHI, A.K., ALI, S., NA, B. and WHOOLEY, M.A., 2007. Self-reported medication adherence and cardiovascular events in patients with stable coronary heart disease: the heart and soul study. *Archives of Internal Medicine,* **167**(16), pp. 1798-1803.

GELLAD, W.F., GRENARD, J.L. and MARCUM, Z.A., 2011. A systematic review of barriers to medication adherence in the elderly: looking beyond cost and regimen complexity. *The American Journal of Geriatric Pharmacotherapy,* **9**(1), pp. 11-23.

GIAVARINA, D., 2015. Understanding bland altman analysis. *Biochemia Medica,* **25**(2), pp. 141-151.

GINER-SORIANO, M., SOTORRA FIGUEROLA, G., CORTES, J., PERA PUJADAS, H., GARCIA-SANGENIS, A. and MORROS, R., 2018. Impact of Medication Adherence on Mortality and Cardiovascular Morbidity: Protocol for a Population-Based Cohort Study. *Journal of Medical Internet Research,* **7**(3), pp. e73.

GJELSTAD, A. and PEDERSEN-BJERGAARD, S., 2014. Challenges and new directions in analytical sample preparation. *Analytical and Bioanalytical Chemistry,* **406**, pp. pp.375–376.

GOHAR, F., GREENFIELD, S.M., BEEVERS, D.G., LIP, G.Y. and JOLLY, K., 2008. Self-care and adherence to medication: a survey in the hypertension outpatient clinic. *Complementary and Alternative Medicine,* **8**(4), pp. 1-9.

GONZALEZ, O., IRIARTE, G., RICO, E., FERREIRÓS, N., MAGUREGUI, M.I., ALONSO, R.M. and JIMÉNEZ, R.M., 2010. LC–MS/MS method for the determination of several drugs used in combined cardiovascular therapy in human plasma. *Journal of Chromatography B,* **878**(28), pp. 2685-2692.

GONZALEZ, O., ALONSO, R.M., FERREIRÓS, N., WEINMANN, W., ZIMMERMANN, R. and DRESEN, S., 2011. Development of an LC–MS/MS method for the quantitation of 55 compounds prescribed in combined cardiovascular therapy. *Journal of Chromatography B,* **879**(3-4), pp. 243-252.

GONZÁLEZ, O., FERREIRÓS, N., BLANCO, M.E. and ALONSO, R.M., 2015. Cardiovascular drug determination in bioanalysis: an update. *Bioanalysis,* **7**(18), pp. 2399-2417.

GRAGNANO, F., CESARO, A., CRISCI, M., SPERLONGANO, S., FIMIANI, F., NATALE, F., PEZZULLO, E., CALABRO, R., RUSSO, M. and CALABRO, P., 2017. P1513Adherence to PCSK9 inhibitors in high cardiovascular risk patients in real-world setting: results from a single-center experience and comparison with statin therapy. *European Heart Journal,* **38**(suppl_1), pp. 134.

GRANGER, B.B., EKMAN, I., HERNANDEZ, A.F., SAWYER, T., BOWERS, M.T., DEWALD, T.A., ZHAO, Y., LEVY, J. and BOSWORTH, H.B., 2015. Results of the Chronic Heart Failure Intervention to Improve MEdication Adherence study: a randomized intervention in high-risk patients. *American Heart Journal,* **169**(4), pp. 539-548.

GROSIOS, K., GAHAN, P.B. and BURBIDGE, J., 2010. Overview of healthcare in the UK. *European Association for Predictive Preventive & Personalized Medicine,* **1**(4), pp. 529-534.

GUO, X.H., JI, L.N., LU, J.M., LIU, J., LOU, Q.Q., LIU, J., SHEN, L., ZHANG, M.X., LV, X.F. and GU, M.J., 2014. Efficacy of structured education in patients with type 2 diabetes mellitus receiving insulin treatment. *Journal of Diabetes,* **6**(4), pp. 290-297.

GUPTA, P., PATEL, P., HORNE, R., BUCHANAN, H., WILLIAMS, B. and TOMASZEWSKI, M., 2016. How to screen for non-adherence to antihypertensive therapy. *Current Hypertension Reports,* **18**(89), pp. 1-8.

GUPTA, P., MOHAMED, A., PATEL, P., BURNS, A., SAEED, M., LANE, D., SEIDU, S. and KHUNTI, K., 2018. High rates of non-adherence to antidiabetic, antihypertensive and lipid lowering treatment in patients with type 2 diabetes revealed liquid chromatography-tandem mass spectrometry (lc-ms/ms) urine analysis. *Atherosclerosis Supplements,* **34**, pp. e1-e2.

HABERER, J.E., ROBBINS, G.K., YBARRA, M., MONK, A., RAGLAND, K., WEISER, S.D., JOHNSON, M.O. and BANGSBERG, D.R., 2012. Real-time electronic adherence monitoring is feasible, comparable to unannounced pill counts, and acceptable. *AIDS and Behavior,* **16**(2), pp. 375-382.

HAMDIDOUCHE, I., JULLIEN, V., BILLAUD, E.M., BOUTOUYRIE, P., AZIZI, M. and LAURENT, S., 2015. 7b.06: Routine Urinary Detection of Antihypertensive Drugs for Estimation of Adherence to Treatment: A Cross Sectional Study. *Journal of Hypertension,* **33**(1), pp. e93-e93.

HAMDIDOUCHE, I., JULLIEN, V., BOUTOUYRIE, P., BILLAUD, E., AZIZI, M. and LAURENT, S., 2017. Routine urinary detection of antihypertensive drugs for systematic evaluation of adherence to treatment in hypertensive patients. *Journal of Hypertension,* **35**(9), pp. 1891-1898.

HAMEED, M., TEBBIT, L., JACQUES, N., THOMAS, M. and DASGUPTA, I., 2016. Non-adherence to antihypertensive medication is very common among resistant hypertensives: results of a directly observed therapy clinic. *Journal of Human Hypertension,* **30**(2), pp. 83-89.

HARPER, L., POWELL, J. and PIJL, E.M., 2017. An overview of forensic drug testing methods and their suitability for harm reduction point-of-care services. *Harm Reduction Journal,* **14**(52), pp. 1-13.

HASAN, Z.N., HUSSEIN, M.Q. and HAJI, G.F., 2011. Hypertension as a risk factor: is it different in ischemic stroke and acute myocardial infarction comparative cross-sectional study? *International Journal of Hypertension,* **2011**, pp. 1-5.

HAWKSHEAD, J. and KROUSEL-WOOD, M.A., 2007. Techniques for measuring medication adherence in hypertensive patients in outpatient settings. *Disease Management & Health Outcomes,* **15**(2), pp. 109-118.

HAWWA, A.F., ALBAWAB, A., ROONEY, M., WEDDERBURN, L.R., BERESFORD, M.W. and MCELNAY, J.C., 2014. A novel dried blood spot-LCMS method for the quantification of methotrexate polyglutamates as a potential marker for methotrexate use in children. *PloS one,* **9**(2), pp. e89908.

HAYNES, R.B., 1979. Determinants of compliance: the disease and the mechanics in treatment. In: R.B. HAYNES, D.L. SACKETT and D.W. AND TAYLOR, eds, *Compliance in health care.* Baltimore, MD: Johns Hopkins University Press, pp. 3-18.

HEATH, A.K., WILLIAMSON, E.J., EBELING, P.R., KVASKOFF, D., EYLES, D.W. and ENGLISH, D.R., 2014. Measurements of 25-hydroxyvitamin D concentrations in archived dried blood spots are reliable and accurately reflect those in plasma. *The Journal of Clinical Endocrinology & Metabolism,* **99**(9), pp. 3319-3324.

HEINIG, K., WIRZ, T., BUCHELI, F. and GAJATE-PEREZ, A., 2011. Determination of oseltamivir (Tamiflu®) and oseltamivir carboxylate in dried blood spots using offline or online extraction. *Bioanalysis,* **3**(4), pp. 421-437.

HEUSSNER, K., RAUH, M., CORDASIC, N., MENENDEZ-CASTRO, C., HUEBNER, H., RUEBNER, M., SCHMIDT, M., HARTNER, A., RASCHER, W. and FAHLBUSCH, F.B., 2017. Adhesive blood microsampling systems for steroid measurement via LC–MS/MS in the rat. *Steroids,* **120**, pp. 1-6.

HIDALGO, P.R., ANTUNES, M.V., SPANIOL, B., PETEFFI, G.P., DA SILVA, C.A. and LINDEN, R., 2014. Adherence to Antiepileptic Pharmacotherapy: Evaluation Using

a Standardized Questionnaire and Drug Concentration in Biological Samples. *Latin American Journal of Pharmacy,* **33**(9), pp. 1481-1486.

HILL, S. and KAVOOKJIAN, J., 2012. Motivational interviewing as a behavioral intervention to increase HAART adherence in patients who are HIV-positive: a systematic review of the literature. *AIDS Care,* **24**(5), pp. 583-592.

HIRD, S.J., LAU, B.P., SCHUHMACHER, R. and KRSKA, R., 2014. Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food. *TrAC Trends in Analytical Chemistry,* **59**, pp. 59-72.

HO, P.M., SPERTUS, J.A., MASOUDI, F.A., REID, K.J., PETERSON, E.D., MAGID, D.J., KRUMHOLZ, H.M. and RUMSFELD, J.S., 2006. Impact of medication therapy discontinuation on mortality after myocardial infarction. *Archives of Internal Medicine,* **166**(17), pp. 1842-1847.

HO, P.M., MAGID, D.J., SHETTERLY, S.M., OLSON, K.L., MADDOX, T.M., PETERSON, P.N., MASOUDI, F.A. and RUMSFELD, J.S., 2008. Medication nonadherence is associated with a broad range of adverse outcomes in patients with coronary artery disease. *American Heart Journal,* **155**(4), pp. 772-779.

HO, P.M., BRYSON, C.L. and RUMSFELD, J.S., 2009. Medication adherence: its importance in cardiovascular outcomes. *Circulation,* **119**(23), pp. 3028-3035.

HO, P.M., TSAI, T.T., MADDOX, T.M., POWERS, J.D., CARROLL, N.M., JACKEVICIUS, C., GO, A.S., MARGOLIS, K.L., DEFOR, T.A., RUMSFELD, J.S. and MAGID, D.J., 2010. Delays in filling clopidogrel prescription after hospital discharge and adverse outcomes after drug-eluting stent implantation: implications for transitions of care. *Circulation: Cardiovascular Quality and Outcomes,* **3**(3), pp. 261-266.

HOFFMAN, J.T., ROSSI, S.S., ESPINA-QUINTO, R., LETENDRE, S. and CAPPARELLI, E.V., 2013. Determination of efavirenz in human dried blood spots by reversed-phase high-performance liquid chromatography with UV detection. *Therapeutic Drug Monitoring,* **35**(2), pp. 203-208.

HOOD, S.R., GIAZZON, A.J., SEAMON, G., LANE, K.A., WANG, J., ECKERT, G.J., TU, W. and MURRAY, M.D., 2018. Association Between Medication Adherence and the Outcomes of Heart Failure. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy,* **38**(5), pp. 539-545.

HORNE, R., WEINMAN, J., BARBER, N., ELLIOTT, R., MORGAN, M. and CRIBB, A., 2005. *Concordance, adherence and compliance in medicine taking.* London: National Coordinating Centre for NHS Delivery and Organisation.

HORNE, R., CHAPMAN, S.C., PARHAM, R., FREEMANTLE, N., FORBES, A. and COOPER, V., 2013. Understanding patients' adherence-related beliefs about medicines prescribed for long-term conditions: a meta-analytic review of the Necessity-Concerns Framework. *PloS One,* **8**(12), pp. e80633.

HUGTENBURG, J.G., TIMMERS, L., ELDERS, P., VERVLOET, M. and VAN DIJK, L., 2013. Definitions, variants, and causes of nonadherence with medication: a challenge for tailored interventions. *Patient Prefer Adherence,* **7**, pp. 675-682.

HYRE, A.D., KROUSEL-WOOD, M.A., MUNTNER, P., KAWASAKI, L. and DESALVO, K.B., 2007. Prevalence and predictors of poor antihypertensive medication adherence in an urban health clinic setting. *The Journal of Clinical Hypertension,* **9**(3), pp. 179-186.

IBRAHIM, F. and DELEU, D., 2018. Assessment of Non-adherence of Multiple Sclerosis Patients to Medication and Follow up Clinic. *Health Science Journal,* **12**(2), pp. 1-5.

INTERNATIONAL ORGANIZATION OF MIGRATION, 2016, COUNTRY FACT SHEET IRAQ-BAGHDAD [Homepage of International Organization of Migration], [Online]. Available: germany.iom.int/sites/default/files/ZIRF_downloads/2016/Iraq_CFS_2016_EN.pdf [Accessed 25/06/2018].

IPPOLITO, M.M., HUANG, L., SIAME, M., THUMA, P., SHAPIRO, T.A. and AWEEKA, F.T., 2018. Semi-quantitative measurement of the antimalarial lumefantrine from untreated dried blood spots using LC–MS/MS. *Journal of Pharmaceutical and Biomedical Analysis,* **155**, pp. 241-246.

IRAQI MINISTRY OF HEALTH, 2012. *National Guidelines for Primary Health Care Physicians Hypertension: Prevention , Diagnosis , and Treatment.* Baghdad: Ministry of Health.

IRVIN, M.R., SHIMBO, D., MANN, D.M., REYNOLDS, K., KROUSEL-WOOD, M., LIMDI, N.A., LACKLAND, D.T., CALHOUN, D.A., OPARIL, S. and MUNTNER, P., 2012. Prevalence and Correlates of Low Medication Adherence in Apparent Treatment-Resistant Hypertension. *The Journal of Clinical Hypertension,* **14**(10), pp. 694-700.

JACKSON, G., PATEL, S. and KHAN, S., 2012. Assessing the problem of counterfeit medications in the United Kingdom. *International Journal of Clinical Practice,* **66**(3), pp. 241-250.

JAMAL, K. and SALEEM, S., 2014. Drug Adherence among Diabetic and Hypertensive Patients in Association with Demographic and Healthy Behavior . *International Journal of Science and Research,* **3**(12), pp. 1998-2003.

JIMMY, B. and JOSE, J., 2011. Patient medication adherence: measures in daily practice. *Oman Medical Journal,* **26**(3), pp. 155-159.

JIN, H., KIM, Y. and RHIE, S.J., 2016. Factors affecting medication adherence in elderly people. *Patient Preference and Adherence,* **10**, pp. 2117-2125.

JOCHMANN, N., STANGL, K., GARBE, E., BAUMANN, G. and STANGL, V., 2005. Female-specific aspects in the pharmacotherapy of chronic cardiovascular diseases. *European Heart Journal,* **26**(16), pp. 1585-1595.

JOHNSTON, A. and HOLT, D.W., 2014. Substandard drugs: a potential crisis for public health. *British Journal of Clinical Pharmacology,* **78**(2), pp. 218-243.

JONES, E., LESOSKY, M., BLOCKMAN, M., CASTEL, S., DECLOEDT, E., SCHWAGER, S., STURROCK, E., WIESNER, L. and RAYNER, B., 2017. Therapeutic drug monitoring of amlodipine and the Z-FHL/HHL ratio: Adherence tools in patients referred for apparent treatment-resistant hypertension. *South African Medical Journal,* **107**(10), pp. 887–891.

JOYNER-GRANTHAM, J., MOUNT, D.L., MCCORKLE, O.D., SIMMONS, D.R., FERRARIO, C.M. and CLINE, D.M., 2009. Self-reported influences of hopelessness, health literacy, lifestyle action, and patient inertia on blood pressure control in a hypertensive emergency department population. *The American Journal of the Medical Sciences,* **338**(5), pp. 368-372.

KALOGIANNI, A., 2011. Factors affect in patient adherence to medication regimen. *Health Science Journal,* **5**(3), pp. 157-158.

KANG, C.D., TSANG, P.P., LI, W.T., WANG, H.H., LIU, K.Q., GRIFFITHS, S.M. and WONG, M.C., 2015. Determinants of medication adherence and blood pressure control among hypertensive patients in Hong Kong: a cross-sectional study. *International Journal of Cardiology,* **182**, pp. 250-257.

KASSAB, Y., HASSAN, Y., AZIZ, N.A., ISMAIL, O. and ABDULRAZZAQ, H., 2013. Patients' adherence to secondary prevention pharmacotherapy after acute coronary syndromes. *International Journal of Clinical Pharmacy,* **35**(2), pp. 275-280.

KATALENICH, B., SHI, L., LIU, S., SHAO, H., MCDUFFIE, R., CARPIO, G., THETHI, T. and FONSECA, V., 2015. Evaluation of a remote monitoring system for diabetes control. *Clinical Therapeutics,* **37**(6), pp. 1216-1225.

KAUFMANN, A., BUTCHER, P., MADEN, K., WALKER, S. and WIDMER, M., 2011. Quantitative and confirmative performance of liquid chromatography coupled to high-resolution mass spectrometry compared to tandem mass spectrometry. *Rapid Communications in Mass Spectrometry,* **25**(7), pp. 979-992.

KEENAN, J., 2017. Improving adherence to medication for secondary cardiovascular disease prevention. *European Journal of Preventive Cardiology,* **24**(3), pp. 29-35.

KEFALE, B., TADESSE, Y., ALEBACHEW, M. and ENGIDAWORK, E., 2018. Management practice, and adherence and its contributing factors among patients with chronic kidney disease at Tikur Anbessa Specialized Hospital: A hospital-based cross-sectional study. *PloS One,* **13**(7), pp. 1-21.

KEN, C., 2015. Pharma Needs A Medication Adherence Game Plan. Available: http://www.pharmaceuticalonline.com/doc/pharma-needs-a-medication-adherence-game-plan-0001 [Accessed 21/07/2018].

KENDIR, C., VAN DEN AKKER, M., VOS, R. and METSEMAKERS, J., 2018. Cardiovascular disease patients have increased risk for comorbidity: A cross-sectional study in the Netherlands. *European Journal of General Practice,* **24**(1), pp. 45-50.

KENNEDY, J., TULEU, I. and MACKAY, K., 2008. Unfilled prescriptions of medicare beneficiaries: prevalence, reasons, and types of medicines prescribed. *Journal of Managed Care Pharmacy,* **14**(6), pp. 553-560.

KHARAMEH, Z.T., KHOSHRAVESH, S., NOORI, R., ABDOLMALAKI, M. and BAKHSHI, M., 2018. Determinants of Medication Adherence Among Iranian Elderly Patients with Chronic Diseases. *Jundishapur Journal of Chronic Disease Care,* **7**(3), pp. 1-6.

KHATIB, R., SCHWALM, J., YUSUF, S., HAYNES, R.B., MCKEE, M., KHAN, M. and NIEUWLAAT, R., 2014. Patient and healthcare provider barriers to hypertension awareness, treatment and follow up: a systematic review and meta-analysis of qualitative and quantitative studies. *PloS one,* **9**(1), pp. e84238.

KHAYYAT, S.M., KHAYYAT, S.M.S., ALHAZMI, R.S.H., MOHAMED, M.M. and HADI, M.A., 2017. Predictors of Medication Adherence and Blood Pressure Control among Saudi Hypertensive Patients Attending Primary Care Clinics: A Cross-Sectional Study. *PloS One,* **12**(1), pp. 1-12.

KIMADIA, 2017. List of Needs Annual Drug Items For 2016 [Homepage of the state company for drug and medical appliances], [Online]. Available: http://kimadia.iq/en/article/detail/7125 [Accessed 20/07/2018].

KIP, A.E., KIERS, K., ROSING, H., SCHELLENS, J., BEIJNEN, J. and DORLO, T.P., 2017. Volumetric absorptive microsampling (VAMS) as an alternative to conventional dried blood spots in the quantification of miltefosine in dried blood samples. *Journal of Pharmaceutical and Biomedical Analysis,* **135**, pp. 160-166.

KLEEBERGER, C.A., PHAIR, J.P., STRATHDEE, S.A., DETELS, R., KINGSLEY, L. and JACOBSON, L.P., 2001. Determinants of heterogeneous adherence to HIV-antiretroviral therapies in the Multicenter AIDS Cohort Study. *JAIDS Journal of Acquired Immune Deficiency Syndromes,* **26**(1), pp. 82-92.

KOLE, P., RAO, A., KURAWATTIMATH, V. and MANDLEKAR, S., 2017. LC–MS determination of triazolam and its hydroxy metabolites in mouse dried blood spots: application to transgenic mouse pharmacokinetic studies. *Bioanalysis,* **9**(13), pp. 987-1000.

KOOP, D.R., BLEYLE, L.A., MUNAR, M., CHERALA, G. and AL-UZRI, A., 2013. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid

chromatography tandem mass spectrometry. *Journal of Chromatography B,* **926**, pp. 54-61.

KORFMACHER, W. and RAMANATHAN, R., 2016. HRMS in DMPK. *Bioanalysis,* **8**(16), pp. 1635-1637.

KOSOBUCKA, A., MICHALSKI, P., PIETRZYKOWSKI, Ł, KASPRZAK, M., OBOŃSKA, K., FABISZAK, T., FELSMANN, M. and KUBICA, A., 2018. Adherence to treatment assessed with the Adherence in Chronic Diseases Scale in patients after myocardial infarction. *Patient Preference and Adherence,* **12**, pp. 333–340.

KOSTER, R.A., VEENHOF, H., BOTMA, R., HOEKSTRA, A.T., BERGER, S.P., BAKKER, S.J., ALFFENAAR, J.C. and TOUW, D.J., 2017. Dried blood spot validation of five immunosuppressants, without hematocrit correction, on two LC–MS/MS systems. *Bioanalysis,* **9**(7), pp. 553-563.

KOYTCHEV, R., OZALP, Y., ERENMEMISOGLU, A., VAN DER MEER, MIKE JOHN and ALPAN, R.S., 2004. Bio equivalence study of atorvastatin tablets. *Arzneimittelforschung,* **54**(09), pp. 573-577.

KRAUSS, M., SINGER, H. and HOLLENDER, J., 2010. LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Analytical and Bioanalytical Chemistry,* **397**(3), pp. 943-951.

KREDO, T., BERNHARDSSON, S., MACHINGAIDZE, S., YOUNG, T., LOUW, Q., OCHODO, E. and GRIMMER, K., 2016. Guide to clinical practice guidelines: the current state of play. *International Journal for Quality in Health Care,* **28**(1), pp. 122-128.

KRONISH, I.M., RIECKMANN, N., HALM, E.A., SHIMBO, D., VORCHHEIMER, D., HAAS, D.C. and DAVIDSON, K.W., 2006. Persistent depression affects adherence to secondary prevention behaviors after acute coronary syndromes. *Journal Of General Internal Medicine,* **21**(11), pp. 1178-1183.

KRONISH, I.M. and YE, S., 2013. Adherence to cardiovascular medications: lessons learned and future directions. *Progress in Cardiovascular Diseases,* **55**(6), pp. 590-600.

KROUSEL-WOOD, M., THOMAS, S., MUNTNER, P. and MORISKY, D., 2004. Medication adherence: a key factor in achieving blood pressure control and good clinical outcomes in hypertensive patients. *Current Opinion in Cardiology,* **19**(4), pp. 357-362.

KROUSEL-WOOD, M., HOLT, E., JOYCE, C., RUIZ, R., DORNELLES, A., WEBBER, L.S., MORISKY, D.E., FROHLICH, E.D., RE, R.N., HE, J., WHELTON, P.K. and MUNTNER, P., 2015. Differences in cardiovascular disease risk when antihypertensive medication adherence is assessed by pharmacy fill versus self-report: the Cohort Study of Medication Adherence among Older Adults (CoSMO). *Journal of Hypertension,* **33**(2), pp. 412-420.

KROUSEL-WOOD, M., ISLAM, T., WEBBER, L.S., RE, R.N., MORISKY, D.E. and MUNTNER, P., 2009. New medication adherence scale versus pharmacy fill rates in seniors with hypertension. The American Journal of Managed Care, 15(1), pp. 59-66.

KRUEGER, K., BOTERMANN, L., SCHORR, S.G., GRIESE-MAMMEN, N., LAUFS, U. and SCHULZ, M., 2015. Age-related medication adherence in patients with chronic heart failure: A systematic literature review. *International Journal of Cardiology,* **184**, pp. 728-735.

KUNTZ, J.L., SAFFORD, M.M., SINGH, J.A., PHANSALKAR, S., SLIGHT, S.P., HER, Q.L., LAPOINTE, N.A., MATHEWS, R., O'BRIEN, E. and BRINKMAN, W.B., 2014. Patient-centered interventions to improve medication management and adherence: a qualitative review of research findings. *Patient Education and Counseling,* **97**(3), pp. 310-326.

KVARNSTROM, K., AIRAKSINEN, M. and LIIRA, H., 2018. Barriers and facilitators to medication adherence: a qualitative study with general practitioners. *British Medical Journal Open,* **8**(1), pp. 1-8.

LA MARCA, G., GIOCALIERE, E., VILLANELLI, F., MALVAGIA, S., FUNGHINI, S., OMBRONE, D., FILIPPI, L., DE GAUDIO, M., DE MARTINO, M. and GALLI, L., 2012. Development of an UPLC–MS/MS method for the determination of antibiotic ertapenem on dried blood spots. *Journal of Pharmaceutical and Biomedical Analysis,* **61**, pp. 108-113.

LABA, T., BRIEN, J. and JAN, S., 2012. Understanding rational non-adherence to medications. A discrete choice experiment in a community sample in Australia. *Family Practice,* **13**(1), pp. 61.

LABA, T., ESSUE, B., KIMMAN, M. and JAN, S., 2015. Understanding patient preferences in medication nonadherence: a review of stated preference data. *The Patient-Patient-Centered Outcomes Research,* **8**(5), pp. 385-395.

LAM, W.Y. and FRESCO, P., 2015. Medication Adherence Measures: An Overview. *BioMed Research International,* **2015**, pp. 1-12.

LANDIS, J.R. and KOCH, G.G., 1977. The measurement of observer agreement for categorical data. *Biometrics,* , pp. 159-174.

LANE, D., PATEL, P., KHUNTI, K. and GUPTA, P., 2019. Objective measures of non-adherence in cardiometabolic diseases: a review focused on urine biochemical screening. *Patient Preference and Adherence,* **13**, pp. 537-547.

LAWRENCE, D., MILLER, H. and FLEXNER, C., 2017. Medication Adherence. *The Journal of Clinical Pharmacology,* **57**(4), pp. 422-427.

LAWSON, G., COCKS, E. and TANNA, S., 2012. Quantitative determination of atenolol in dried blood spot samples by LC–HRMS: a potential method for assessing medication adherence. *Journal of Chromatography B*, **897**, pp. 72-79.

LAWSON, G., COCKS, E. and TANNA, S., 2013. Bisoprolol, ramipril and simvastatin determination in dried blood spot samples using LC–HRMS for assessing medication adherence. *Journal of Pharmaceutical and Biomedical Analysis*, **81**, pp. 99-107.

LEE, S., RAAMKUMAR, A.S., LI, J., CAO, Y., WITEDWITTAYANUSAT, K., CHEN, L. and THENG, Y., 2018. Reasons for Primary Medication Nonadherence: A Systematic Review and Metric Analysis. *Journal of Managed Care & Specialty Pharmacy*, **24**(8), pp. 778-794.

LEHANE, E. and MCCARTHY, G., 2007a. An examination of the intentional and unintentional aspects of medication non-adherence in patients diagnosed with hypertension. *Journal of Clinical Nursing*, **16**(4), pp. 698-706.

LEHANE, E. and MCCARTHY, G., 2007b. Intentional and unintentional medication non-adherence: a comprehensive framework for clinical research and practice? A discussion paper. *International Journal of Nursing Studies*, **44**(8), pp. 1468-1477.

LEHMANN, A., ASLANI, P., AHMED, R., CELIO, J., GAUCHET, A., BEDOUCH, P., BUGNON, O., ALLENET, B. and SCHNEIDER, M.P., 2014. Assessing medication adherence: options to consider. *International Journal of Clinical Pharmacy*, **36**(1), pp. 55-69.

LENAHAN, J.L., MCCARTHY, D.M., DAVIS, T.C., CURTIS, L.M., SERPER, M. and WOLF, M.S., 2013. A drug by any other name: patients' ability to identify medication regimens and its association with adherence and health outcomes. *Journal of Health Communication*, **18**(1), pp. 31-39.

LESLIE, K., MCCOWAN, C. and PELL, J., 2018. Adherence to cardiovascular medication: a review of systematic reviews. *Journal of Public Health [Online]: Available from: doi.org/10.1093/pubmed/fdy088* [Accessed 21/01/2019].

LEWEY, J., SHRANK, W.H., BOWRY, A.D., KILABUK, E., BRENNAN, T.A. and CHOUDHRY, N.K., 2013. Gender and racial disparities in adherence to statin therapy: a meta-analysis. *American Heart Journal*, **165**(5), pp. 665-678.

LEWIS, R., MACLEAN, D., IOANNIDES, C., JOHNSTON, A. and MCDEVITT, D., 1988. A comparison of bisoprolol and atenolol in the treatment of mild to moderate hypertension. *British Journal of Clinical Pharmacology*, **26**(1), pp. 53-59.

LI, W. and TSE, F., 2010. Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. *Biomedical Chromatography*, **24**(1), pp. 49-65.

LI, W. and LEE, M., eds, 2014. *Dried Blood Spots: Applications and Techniques.* John Wiley & Sons. New Jersey: Hoboken.

LILJA, J.J., NEUVONEN, M. and NEUVONEN, P.J., 2004. Effects of regular consumption of grapefruit juice on the pharmacokinetics of simvastatin. *British Journal of Clinical Pharmacology,* **58**(1), pp. 56-60.

LINDER, C., WIDE, K., WALANDER, M., BECK, O., GUSTAFSSON, L.L. and POHANKA, A., 2016. Comparison between dried blood spot and plasma sampling for therapeutic drug monitoring of antiepileptic drugs in children with epilepsy: a step towards home sampling. *Clinical Biochemistry,* **50**(7-8), pp. 418-424.

LINDER, C., HANSSON, A., SADEK, S., GUSTAFSSON, L.L. and POHANKA, A., 2018. Carbamazepine, lamotrigine, levetiracetam and valproic acid in dried blood spots with liquid chromatography tandem mass spectrometry; method development and validation. *Journal of Chromatography B,* **1072**, pp. 116-122.

LINS, R., BROEKHUYSEN, J., NECCIARI, J. and DEROUBAIX, X., 1999. Pharmacokinetic profile of 14C-labeled clopidogrel. *Seminars in Thrombosis and Hemostasis,* **25**(2), pp. 29-33.

LIU, H., GOLIN, C.E., MILLER, L.G., HAYS, R.D., BECK, C.K., SANANDAJI, S., CHRISTIAN, J., MALDONADO, T., DURAN, D. and KAPLAN, A.H., 2001. A comparison study of multiple measures of adherence to HIV protease inhibitors. *Annals of Internal Medicine,* **134**(10), pp. 968-977.

LLABRE, M.M., WEAVER, K.E., DURÁN, R.E., ANTONI, M.H., MCPHERSON-BAKER, S. and SCHNEIDERMAN, N., 2006. A measurement model of medication adherence to highly active antiretroviral therapy and its relation to viral load in HIV-positive adults. *AIDS Patient Care & STDs,* **20**(10), pp. 701-711.

LLOYD, P., BRAITHWAITE, J. and SOUTHON, G., 1999. Empowerment and the performance of health services. *Journal of Management in Medicine,* **13**(2), pp. 83-94.

LOFFREDA, A., MATERA, M., VACCA, C., MOTOLA, G., DE SANTIS, D., RUGGIERO, A., RUSSO, F., FICI, F., CANTONI, V. and LAMPA, E., 1999. Bioequivalence assessment of two different tablet formulations of diltiazem after single and repeated doses in healthy subjects. *Current Medical Research and Opinion,* **15**(1), pp. 53-61.

LOWRY, K.P., DUDLEY, T.K., ODDONE, E.Z. and BOSWORTH, H.B., 2005. Intentional and unintentional nonadherence to antihypertensive medication. *The Annals of Pharmacotherapy,* **39**(7-8), pp. 1198-1203.

MA, S. and CHOWDHURY, S.K., 2013. Data acquisition and data mining techniques for metabolite identification using LC coupled to high-resolution MS. *Bioanalysis,* **5**(10), pp. 1285-1297.

MACEDO, A.F., MORGADO, M., CASTELO-BRANCO, M., ROLO, S. and PEREIRA, L., 2010. Predictors of uncontrolled hypertension and antihypertensive medication nonadherence. *Journal of Cardiovascular Disease Research*, **1**(4), pp. 196-202.

MACLAUGHLIN, E.J., RAEHL, C.L., TREADWAY, A.K., STERLING, T.L., ZOLLER, D.P. and BOND, C.A., 2005. Assessing medication adherence in the elderly. *Drugs &Aging*, **22**(3), pp. 231-255.

MAHASE, E., 2019. Half of patients prescribed statins fail to hit target reduction in LDL cholesterol. *British Medical Journal*,365(1838), pp.1-1.

MANGIN, D., BAHAT, G., GOLOMB, B.A., MALLERY, L.H., MOORHOUSE, P., ONDER, G., PETROVIC, M. and GARFINKEL, D., 2018. International Group for Reducing Inappropriate Medication Use & Polypharmacy (IGRIMUP): position statement and 10 recommendations for action. *Drugs & aging*, **35**(7), pp.575-587

MANICKE, N.E., BILLS, B.J. and ZHANG, C., 2016. Analysis of biofluids by paper spray MS: advances and challenges. *Bioanalysis*, **8**(6), pp. 589-606.

MARCUM, Z.A. and GELLAD, W.F., 2012. Medication adherence to multidrug regimens. *Clinics in Geriatric Medicine*, **28**(2), pp. 287-300.

MARTIAL, L.C., AARNOUTSE, R.E., SCHREUDER, M.F., HENRIET, S.S., BRÜGGEMANN, R.J. and JOORE, M.A., 2016. Cost evaluation of dried blood spot home sampling as compared to conventional sampling for therapeutic drug monitoring in children. *PloS One*, **11**(12), pp. 1-17.

MARTIAL, L.C., HOOGTANDERS, K.E., SCHREUDER, M.F., CORNELISSEN, E.A., VAN DER HEIJDEN, J., JOORE, M.A., VAN MAARSEVEEN, E.M., BURGER, D.M., CROES, S. and BRÜGGEMANN, R.J., 2017. Dried Blood Spot Sampling for Tacrolimus and Mycophenolic Acid in Children: Analytical and Clinical Validation. *Therapeutic Drug Monitoring*, **39**(4), pp. 412-421.

MARTIN, L.R., FEIG, C., MAKSOUDIAN, C.R., WYSONG, K. and FAASSE, K., 2018. A perspective on nonadherence to drug therapy: psychological barriers and strategies to overcome nonadherence. *Patient Preference and Adherence*, **12**, pp. 1527-1532.

MARTINEZ, J. and FINKEN, G., 2017. Improved Medication Adherence with Pharmacist Interventions for Patients with Diabetes in Phase 3 NIGLM Clinical Trials. *Diabetes*, **66**, pp. A178-A179.

MATHES, T., JASCHINSKI, T. and PIEPER, D., 2014. Adherence influencing factors–a systematic review of systematic reviews. *Archives of Public Health*, **72**(37), pp. 1-9.

MAYO-GAMBLE, T.L. and MOUTON, C., 2018. Examining the Association Between Health Literacy and Medication Adherence Among Older Adults. *Health Communication,* **33**(9), pp. 1124-1130.

MAZROU, S.H.A., 2013. Expected benefits of clinical practice guidelines: Factors affecting their adherence and methods of implementation and dissemination. *Journal of Health Specialties,* **1**(3), pp. 141.

MCDADE, T.W., BURHOP, J. and DOHNAL, J., 2004. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clinical Chemistry,* **50**(3), pp. 652-654.

MCHUGH, M.L., 2012. Interrater reliability: the kappa statistic. *Biochemia medica,* **22**(3), pp. 276-282.

MCQUAID, E.L. and LANDIER, W., 2018. Cultural issues in medication adherence: disparities and directions. *Journal of General Internal Medicine,* **33**(2), pp. 200-206.

MEDICINES & HEALTHCARE PRODUCTS REGULATORY AGENCY, 2018. The Medicines and Healthcare products Regulatory Agency regulates medicines, medical devices and blood components for transfusion in the UK [Homepage of Medicines & Healthcare products Regulatory Agency], [Online]. Available: https://www.gov.uk/government/organisations/medicines-and-healthcare-products-regulatory-agency/about#who-we-are [Accessed 06/24/2018].

MEI, J.V., LI, L., RASMUSSEN, S.A., COLLIER, S., FRIAS, J.L., HONEIN, M.A., SHAW, G.M., LOREY, F., MEYER, R. and CHAING, S., 2011. Effect of specimen storage conditions on newborn dried blood spots used to assess Toxoplasma gondii immunoglobulin M (IgM). *Clinica Chimica Acta,* **412**(5-6), pp. 455-459.

MEINEMA, J.G., VAN DIJK, N., BEUNE, E.J., JAARSMA, D.A., VAN WEERT, H.C. and HAAFKENS, J.A., 2015. Determinants of adherence to treatment in hypertensive patients of African descent and the role of culturally appropriate education. *PLoS One,* **10**(8), pp. 1-14.

MENDITTO, E., GUERRIERO, F., ORLANDO, V., CROLA, C., DI SOMMA, C., ILLARIO, M., MORISKY, D.E. and COLAO, A., 2015. Self-assessment of adherence to medication: a case study in Campania region community-dwelling population. *Journal of Aging Research,* **2015**, pp. 1-6.

MENDOZA, L., HAJDUCH, M., PLAUŠINAITIS, R., PLATILOVA, V., EMRITTE, N. and SVOBODA, M., 2006. Pharmacokinetic and bioequivalence testing of atorvastatin formulations in healthy male volunteers. *Die Pharmazie-An International Journal of Pharmaceutical Sciences,* **61**(9), pp. 805-806.

MEREDITH, P.A. and ELLIOTT, H.L., 1992. Clinical pharmacokinetics of amlodipine. *Clinical Pharmacokinetics,* **22**(1), pp. 22-31.

MEYER, J.G. and SCHILLING, B., 2017. Clinical applications of quantitative proteomics using targeted and untargeted data-independent acquisition techniques. *Expert Review of Proteomics,* **14**(5), pp. 419-429.

MIURA, T., KOJIMA, R., MIZUTANI, M., SHIGA, Y., TAKATSU, F. and SUZUKI, Y., 2001. Effect of digoxin noncompliance on hospitalization and mortality in patients with heart failure in long-term therapy: a prospective cohort study. *European Journal of Clinical Pharmacology,* **57**(1), pp. 77-83.

MIYAZAKI, M., NAKASHIMA, A., NAKAMURA, Y., SAKAMOTO, Y., MATSUO, K., GOTO, M., UCHIYAMA, M., OKAMURA, K., MITSUTAKE, R. and URATA, H., 2018. Association between medication adherence and illness perceptions in atrial fibrillation patients treated with direct oral anticoagulants: An observational cross-sectional pilot study. *PloS One,* **13**(9), pp. 1-13.

MODI, A.C., LIM, C.S., YU, N., GELLER, D., WAGNER, M.H. and QUITTNER, A.L., 2006. A multi-method assessment of treatment adherence for children with cystic fibrosis. *Journal of Cystic Fibrosis,* **5**(3), pp. 177-185.

MOFFAT, A.C., OSSELTON, M.D., WIDDOP, B. and WATTS, J., 2011. *Clarke's analysis of drugs and poisons in pharmaceuticals, body fluids and post mortem materials (Vol. 3).* 4th edn. London: Pharmaceutical press.

MOHARAMZAD, Y., SAADAT, H., NAKHJAVAN SHAHRAKI, B., RAI, A., SAADAT, Z., AERAB-SHEIBANI, H., NAGHIZADEH, M.M. and MORISKY, D.E., 2015. Validation of the Persian Version of the 8-Item Morisky Medication Adherence Scale (MMAS-8) in Iranian Hypertensive Patients. *Global Journal of Health Science,* **7**(4), pp. 173-183.

MONROE, A., PENA, J., MOORE, R., RIEKERT, K., EAKIN, M., KRIPALANI, S. and CHANDER, G., 2018. Randomized controlled trial of a pictorial aid intervention for medication adherence among HIV-positive patients with comorbid diabetes or hypertension. *AIDS Care,* **30**(2), pp. 199-206.

MOODY, A., & MINDELL, J., 2017. Health Survey for England 2016: Prescribed medicines. Health and Social Care Information Centre/NHS Digital Available: http:// healthsurvey.hscic.gov.uk/media/63790/HSE2016-pres-med.pdf [Accessed 18/09/ 2019]

MOON, S.J., LEE, W., HWANG, J.S., HONG, Y.P. and MORISKY, D.E., 2018. Correction: Accuracy of a screening tool for medication adherence: A systematic review and meta-analysis of the Morisky Medication Adherence Scale-8. *PloS one,* **12**(11), pp. 1-18.

MOORE, C., 2015. Drug testing and adherence monitoring in pain management: Oral fluid testing. *Journal of Opioid Management,* **11**(1), pp. 69-75.

MORISKY, D.E., GREEN, L.W. and LEVINE, D.M., 1986. Concurrent and predictive validity of a self-reported measure of medication adherence. *Medical Care,* **24**(1), pp. 67-74.

MORISKY, D.E., ANG, A., KROUSEL-WOOD, M. and WARD, H.J., 2008. Predictive validity of a medication adherence measure for hypertension control. *The Journal of Clinical Hypertension,* **10**(5), pp. 348-354.

MORISKY, D.E. and DIMATTEO, M.R., 2011. Improving the measurement of self-reported medication nonadherence: Final response. *Journal of Clinical Epidemiology*, **64**, pp. 258-263.

MORRISON, A., STAUFFER, M. and KAUFMAN, A., 2015. Defining medication adherence in individual patients. *Patient Preference and Adherence,* **9**, pp. 893-897.

MUKHTAR, O., WEINMAN, J. and JACKSON, S.H., 2014. Intentional non-adherence to medications by older adults. *Drugs & aging,* **31**(3), pp. 149-157.

MURAKAMI, Y., OKAMURA, T., NAKAMURA, K., MIURA, K. and UESHIMA, H., 2013. The clustering of cardiovascular disease risk factors and their impacts on annual medical expenditure in Japan: community-based cost analysis using Gamma regression models. *British Medical Journal,* **3**(3), pp. 1-6.

MURRAY, M., TU, W., WU, J., MORROW, D., SMITH, F. and BRATER, D., 2009. Factors associated with exacerbation of heart failure include treatment adherence and health literacy skills. *Clinical Pharmacology & Therapeutics,* **85**(6), pp. 651-658.

NAJIB, N.M., IDKAIDEK, N., ADEL, A., MOHAMMED, B., AL-MASRI, S., ADMOUR, I., ALAM, S.M. and DHAM, R., 2005. Comparative bioavailability of two brands of atenolol 100 mg tablets (Tensotin and Tenormin) in healthy human volunteers. *Biopharmaceutics & Drug Disposition,* **26**(1), pp. 1-5.

NATIONAL HEALTH SERVICE, 2016. Cardiovascular diseases [Homepage of NHS, UK], [Online]. Available: https://www.nhs.uk/conditions/cardiovascular-disease/ [Accessed 05/26/2018].

NATIONAL HEALTH SERVICE, 2017. Who can write a prescription? [Homepage of NHS], [Online]. Available: https://www.nhs.uk/common-health-questions/medicines/who-can-write-a-prescription/ [Accessed 11/12/2018].

NATIONAL HEALTH SERVICE, 2018a. The Collection, Handling and Transport of Specimens. Policy Infection Prevention and Control. Available: http://www.leicspart.nhs.uk/Library/TheCollectionHandlingandTransportofSpecimensPolicyexpAug21.pdf [Accessed 29/08/2018].

NATIONAL HEALTH SERVICE, 2018b. Dangers of buying medicines online [Homepage of NHS], [Online]. Available: https://www.nhs.uk/using-the-nhs/nhs-services/pharmacies/dangers-of-buying-medicines-online/ [Accessed 11/02/2018].

NATIONAL HEALTH SERVICE, 2018c. LOSARTAN POTASSIUM-side effects [Homepage of NICE], [Online]. Available: https://bnf.nice.org.uk/drug/losartan-potassium.html#sideEffects [Accessed 08/29/2018].

NATIONAL HEALTH SERVICE, 2019. NHS charges from April. Available: https://www.gov.uk/government/speeches/nhs-prescription-charges-from-april-2019 [Accessed 11/12/2019].

NEIHEISEL, M.B., WHEELER, K.J. and ROBERTS, M.E., 2014. Medication adherence part one: Understanding and assessing the problem. *Journal of the American Association of Nurse Practitioners,* **26**(1), pp. 49-55.

NEW ENGLAND HEALTH INSTITUTE (NEHI), 2009. *Thinking Outside the Pillbox A System-wide Approach to Improving Patient Medication Adherence for Chronic Disease.* Cambridge: New England Health Institute (NEHI).

NGUYEN, T., CAZE, A.L. and COTTRELL, N., 2014. What are validated self-report adherence scales really measuring?: a systematic review. *British Journal of Clinical Pharmacology,* **77**(3), pp. 427-445.

NGUYEN, T., 2016. *Improving medication adherence in patients with chronic disease using a targeted and tailored approach*, (Doctoral thesis, University of Queensland). pp.1-2.

NIELSEN, J.Ø, SHRESTHA, A., NEUPANE, D. and KALLESTRUP, P., 2017. Non-adherence to anti-hypertensive medication in low-and middle-income countries: a systematic review and meta-analysis of 92443 subjects. *Journal of Human Hypertension,* **31**(1), pp. 14-21.

NIROGI, R., MUDIGONDA, K. and KANDIKERE, V., 2007. Chromatography–mass spectrometry methods for the quantitation of statins in biological samples. *Journal of Pharmaceutical and Biomedical Analysis,* **44**(2), pp. 379-387.

NUNES, V., NEILSON, J., O'FLYNN, N., CALVERT, N., KUNTZE, S., SMITHSON, H., BENSON, J., BLAIR, J., BOWSER, A. and CLYNE, W., 2009. Clinical guidelines and evidence review for medicines adherence: involving patients in decisions about prescribed medicines and supporting adherence [Homepage of National Institute for Health and Clinical Excellence], [Online]. Available: https://www.nice.org.uk/guidance/cg76/chapter/1-Guidance#reviewing-medicines [Accessed 08/12/2018].

NYS, G., KOK, M.G., SERVAIS, A. and FILLET, M., 2017. Beyond dried blood spot: current microsampling techniques in the context of biomedical applications. *Trends in Analytical Chemistry,* **97**, pp. 326-332.

O'MARA, M., HUDSON-CURTIS, B., OLSON, K., YUEH, Y., DUNN, J. and SPOONER, N., 2011. The effect of hematocrit and punch location on assay bias during quantitative bioanalysis of dried blood spot samples. *Bioanalysis,* **3**(20), pp. 2335-2347.

OGEDEGBE, G., HARRISON, M., ROBBINS, L., MANCUSO, C.A. and ALLEGRANTE, J.P., 2004. Barriers and facilitators of medication adherence in hypertensive African Americans: a qualitative study. *Ethnicity & Disease,* **14**(1), pp. 3-12.

OGEDEGBE, G., MANCUSO, C.A., ALLEGRANTE, J.P. and CHARLSON, M.E., 2003. Development and evaluation of a medication adherence self-efficacy scale in hypertensive African-American patients. *Journal of Clinical Epidemiology,* **56**(6), pp. 520-529.

OHTAWA, M., TAKAYAMA, F., SAITOH, K., YOSHINAGA, T. and NAKASHIMA, M., 1993. Pharmacokinetics and biochemical efficacy after single and multiple oral administration of losartan, an orally active nonpeptide angiotensin II receptor antagonist, in humans. *British Journal of Clinical Pharmacology,* **35**(3), pp. 290-297.

OLDS, P.K., KIWANUKA, J.P., NANSERA, D., HUANG, Y., BACCHETTI, P., JIN, C., GANDHI, M. and HABERER, J.E., 2015. Assessment of HIV antiretroviral therapy adherence by measuring drug concentrations in hair among children in rural Uganda. *AIDS Care,* **27**(3), pp. 327-332.

OLIVEIRA, R.V., HENION, J. and WICKREMSINHE, E.R., 2014. Automated high-capacity on-line extraction and bioanalysis of dried blood spot samples using liquid chromatography/high-resolution accurate mass spectrometry. *Rapid Communications in Mass Spectrometry,* **28**(22), pp. 2415-2426.

OLIVEIRA-FILHO, A.D., BARRETO-FILHO, J.A., NEVES, S.J.F. and LYRA JUNIOR, DIVALDO PEREIRA DE, 2012. Association between the 8-item Morisky Medication Adherence Scale (MMAS-8) and blood pressure control. *Arquivos Brasileiros de Cardiologia,* **99**(1), pp. 649-658.

OLIVEIRA-FILHO, A.D., MORISKY, D.E., COSTA, F.A., PACHECO, S.T., NEVES, S.F. and LYRA-JR, D.P., 2014. Improving post-discharge medication adherence in patients with CVD: a pilot randomized trial. *Arquivos Brasileiros de Cardiologia,* **103**(6), pp. 503-512.

OSTERBERG, L. and BLASCHKE, T., 2005. Adherence to medication. *New England Journal of Medicine,* **353**(5), pp. 487-497.

OTTO, C.M., 2017. Heartbeat: Achieving better medication adherence. *Heart,* **103**(14), pp. 1057-1058.

PALACIO, A., GARAY, D., LANGER, B., TAYLOR, J., WOOD, B.A. and TAMARIZ, L., 2016. Motivational interviewing improves medication adherence: a systematic review and meta-analysis. *Journal Of General Internal Medicine,* **31**(8), pp. 929-940.

PALMER, M.J., BARNARD, S., PEREL, P. and FREE, C., 2018. Mobile phone-based interventions for improving adherence to medication prescribed for the primary prevention of cardiovascular disease in adults. *The Cochrane Library,* (6), pp. 1-56.

PANDEY, A., RAZA, F., VELASCO, A., BRINKER, S., AYERS, C., DAS, S.R., MORISKY, D.E., HALM, E.A. and VONGPATANASIN, W., 2015. Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment–resistant hypertension. *Journal of the American Society of Hypertension,* **9**(6), pp. 420-426.

PARK, L.G., HOWIE-ESQUIVEL, J., CHUNG, M.L. and DRACUP, K., 2014. A text messaging intervention to promote medication adherence for patients with coronary heart disease: a randomized controlled trial. *Patient Education and Counseling,* **94**(2), pp. 261-268.

PAUL, R. and MORGAN, L., 2018. Medicines pricing and reimbursement: EU and UK [Homepage of Thomson Reuters], [Online]. Available: https://uk.practicallaw.thomsonreuters.com/w-005-2025?transitionType=Default&contextData=(sc.Default)&firstPage=true&comp=pluk&bhcp=1 [Accessed 06/24/2018].

PEFOYO, A.J.K., BRONSKILL, S.E., GRUNEIR, A., CALZAVARA, A., THAVORN, K., PETROSYAN, Y., MAXWELL, C.J., BAI, Y. and WODCHIS, W.P., 2015. The increasing burden and complexity of multimorbidity. *British Medical Journal of Public Health,* **15**(1), pp. 1-11.

PELTON, R., 2009. Bioactive paper provides a low-cost platform for diagnostics. *TrAC Trends in Analytical Chemistry,* **28**(8), pp. 925-942.

PETRIE, K.J., PERRY, K., BROADBENT, E. and WEINMAN, J., 2012. A text message programme designed to modify patients' illness and treatment beliefs improves self-reported adherence to asthma preventer medication. *British Journal of Health Psychology,* **17**(1), pp. 74-84.

PHARMAPHORUM, 2018. Non-adherence: pharma's final financial frontier [Homepage of pharmaphorum media limited], [Online]. Available: https://pharmaphorum.com/views-and-analysis/non-adherence-pharmas-final-financial-frontier/ [Accessed 10/10/2018].

PHILLIPS, P.P. and STAWARSKI, C.A., 2008. Data collection: Planning for and collecting all types of data. San Francisco: Pfeiffer.

POUND, P., BRITTEN, N., MORGAN, M., YARDLEY, L., POPE, C., DAKER-WHITE, G. and CAMPBELL, R., 2005. Resisting medicines: a synthesis of qualitative studies of medicine taking. *Social Science & Medicine,* **61**(1), pp. 133-155.

POURHOSEINGHOLI, M.A., VAHEDI, M. and RAHIMZADEH, M., 2013. Sample size calculation in medical studies. *Gastroenterology and Hepatology from Bed to Bench,* **6**(1), pp. 14-17.

PROVATAS, A.A., KING, C.A., KOLAKOWSKI, S.L., STUART, J.D. and PERKINS, C.R., 2017. Quantification of Polycyclic Aromatic Hydrocarbons in Avian Dried Blood

Spots by Ultra-performance Liquid Chromatography with Simple Liquid Extraction and Phospholipid Solid-phase Extraction Preparation. *Analytical Letters,* **50**(16), pp. 2649-2660.

QURAISHI, R., JAIN, R. and AMBEKAR, A., 2013. The use of dried blood spot samples in screening drugs of abuse. *Pharmacology & Pharmacy,* **4**(2), pp. 152-159.

RAFII, F., FATEMI, N.S., DANIELSON, E., JOHANSSON, C.M. and MODANLOO, M., 2014. Compliance to treatment in patients with chronic illness: A concept exploration. *Iranian journal of nursing and midwifery research,* **19**(2), pp. 159-167.

RAGO, B. and NEGAHBAN, A., 2016. Outsource-ability of high-resolution MS-based DMPK assays. *Bioanalysis,* **8**(16), pp. 641-1644.

RAJPURA, J.R. and NAYAK, R., 2014. Role of illness perceptions and medication beliefs on medication compliance of elderly hypertensive cohorts. *Journal of Pharmacy Practice,* **27**(1), pp. 19-24.

RAMANATHAN, R. and KORFMACHER, W., 2012. The emergence of high-resolution MS as the premier analytical tool in the pharmaceutical bioanalysis arena. *Bioanalysis,* **4**(5), pp. 467-469.

RAMAKRISHNAN, P., NAIR, S. and RANGIAH, K., 2016. A method for comparative metabolomics in urine using high resolution mass spectrometry. *Journal of Chromatography A,* **1443**, pp. 83-92.

RAMLI, A., AHMAD, N.S. and PARAIDATHATHU, T., 2012. Medication adherence among hypertensive patients of primary health clinics in Malaysia. *Patient Preference and Adherence,* **6**, pp. 613-622.

RAPOFF, M.A., 2010. Measurement issues: Assessing adherence and disease and health outcomes. *Adherence to Pediatric Medical Regimens.* Springer, pp. 69-113.

RASHID, M.A., EDWARDS, D., WALTER, F.M. and MANT, J., 2014. Medication taking in coronary artery disease: a systematic review and qualitative synthesis. *Annals of Family Medicine,* **12**(3), pp. 224-232.

RASMUSSEN, J.N., CHONG, A. and ALTER, D.A., 2007. Relationship between adherence to evidence-based pharmacotherapy and long-term mortality after acute myocardial infarction. *The Journal of the American Medical Association,* **297**(2), pp. 177-186.

RESEARCH METHODOLOGY, 2019-last update, Questionnaires. Available: https://research-methodology.net/research-methods/survey-method/questionnaires-2/ [09/19, 2019].

RIDIC, G., GLEASON, S. and RIDIC, O., 2012. Comparisons of health care systems in the United States, Germany and Canada. *Materia socio-medica,* **24**(2), pp. 112-120.

RIECKMANN, N., BURG, M.M., KRONISH, I.M., CHAPLIN, W.F., SCHWARTZ, J.E. and DAVIDSON, K.W., 2011. Aspirin adherence, depression and one-year prognosis after acute coronary syndrome. *Psychotherapy and Psychosomatics,* **80**(5), pp. 316-318.

ROCHAT, B., KOTTELAT, E. and MCMULLEN, J., 2012. The future key role of LC–high-resolution-MS analyses in clinical laboratories: a focus on quantification. *Bioanalysis,* **4**(24), pp. 2939-2958.

ROCHAT, B., 2018. Quantitative and Qualitative LC-High-Resolution MS: The Technological and Biological Reasons for a Shift of Paradigm. *Recent Advances in Analytical Chemistry.* London: IntechOpen, pp. 1-19.

ROSANO, G.M., LEWIS, B., AGEWALL, S., WASSMANN, S., VITALE, C., SCHMIDT, H., DREXEL, H., PATAK, A., TORP-PEDERSEN, C. and KJELDSEN, K.P., 2015. Gender differences in the effect of cardiovascular drugs: a position document of the Working Group on Pharmacology and Drug Therapy of the ESC. *European Heart Journal,* **36**(40), pp. 2677-2680.

ROSEN, M.I., RIGSBY, M.O., SALAHI, J.T., RYAN, C.E. and CRAMER, J.A., 2004. Electronic monitoring and counseling to improve medication adherence. *Behaviour Research and Therapy,* **42**(4), pp. 409-422.

ROSS, S., WALKER, A. and MACLEOD, M., 2004. Patient compliance in hypertension: role of illness perceptions and treatment beliefs. *Journal of Human Hypertension,* **18**(9), pp. 607-613.

RUPPAR, T.M., DOBBELS, F. and DE GEEST, S., 2012. Medication beliefs and antihypertensive adherence among older adults: a pilot study. *Geriatric Nursing,* **33**(2), pp. 89-95.

RYAN, T.P., MORRISON, R.D., SUTHERLAND, J.J., MILNE, S.B., RYAN, K.A., DANIELS, J.S., MISRA-HEBERT, A., HICKS, J.K., VOGAN, E. and TENG, K., 2017. Medication adherence, medical record accuracy, and medication exposure in real-world patients using comprehensive medication monitoring. *PloS One,* **12**(9), pp. 1-19.

SA'ED, H.Z., AL-JABI, S.W., SWEILEH, W.M. and MORISKY, D.E., 2013. Relationship of treatment satisfaction to medication adherence: findings from a cross-sectional survey among hypertensive patients in Palestine. *Health and Quality of Life Outcomes,* **11**(191), pp. 1-7.

SABATÉ, E., 2003. Adherence to long-term therapies: evidence for action [Homepage of World Health Organization], [Online]. Available: http://www.who.int/chp/knowledge/publications/adherence_report/en/ [Accessed 12/12/ 2018].

SABATINE, M.S., GIUGLIANO, R.P., KEECH, A.C., HONARPOUR, N., WIVIOTT, S.D., MURPHY, S.A., KUDER, J.F., WANG, H., LIU, T. and WASSERMAN, S.M.,

2017. Evolocumab and clinical outcomes in patients with cardiovascular disease. *New England Journal of Medicine,* **376**(18), pp. 1713-1722.

SAFAA, H. and ALI, A., 2015. Hypertensive Patients Compliance with Medications in Marjan Teaching Hospital/ Babylon . *Medical Journal of Babylon,* **12**(3), pp. 592-602.

SALVADORI, M.C., MOREIRA, R.F., BORGES, B.C., ANDRAUS, M.H., AZEVEDO, C.P., MORENO, R.A. and BORGES, N.C., 2009. Simultaneous determination of losartan and hydrochlorothiazide in human plasma by LC/MS/MS with electrospray ionization and its application to pharmacokinetics. *Clinical and Experimental Hypertension,* **31**(5), pp. 415-427.

SAMER, R., 2008. Assessment of Patients' Compliance with Essential Hypertension. *Scientific Journal of Nursing,* **21**(2), pp. 1-10.

SANDOVAL, D., NAZZAL, C. and ROMERO, T., 2018. Clinical, Socioeconomic, and Psychosocial Factors Associated with Blood Pressure Control and Adherence: Results from a Multidisciplinary Cardiovascular National Program Providing Universal Coverage in a Developing Country. *International Journal of Hypertension,* **2018**, pp. 1-10.

SAVI, P., HERBERT, J., PFLIEGER, A., DOL, F., DELEBASSEE, D., COMBALBERT, J., DEFREYN, G. and MAFFRAND, J., 1992. Importance of hepatic metabolism in the antiaggregating activity of the thienopyridine clopidogrel. *Biochemical Pharmacology,* **44**(3), pp. 527-532.

SCHERF-CLAVEL, M. and HÖGGER, P., 2015. Analysis of metformin, sitagliptin and creatinine in human dried blood spots. *Journal of Chromatography B,* **997**, pp. 218-228.

SCHNEIDER, A.P.H., GAEDKE, M.Â, GARCEZ, A., BARCELLOS, N.T. and PANIZ, V.M.V., 2018. Effect of characteristics of pharmacotherapy on non-adherence in chronic cardiovascular disease: A systematic review and meta-analysis of observational studies. *International Journal of Clinical Practice,* **72**(1), pp. 1-14.

SCHOENTHALER, A., KNAFL, G.J., FISCELLA, K. and OGEDEGBE, G., 2017. Addressing the Social Needs of Hypertensive Patients: The Role of Patient-Provider Communication as a Predictor of Medication Adherence. *Circulation: Cardiovascular Quality and Outcomes,* **10**(9), pp. 1-9.

SELVAN, P.S. and PAL, T., 2009. Chromatography–tandem mass spectrometry method for the simultaneous quantitation of metoprolol succinate and simvastatin in human plasma. *Journal of Pharmaceutical and Biomedical Analysis,* **49**(3), pp. 780-785.

SERA, L.C. and MCPHERSON, M.L., 2012. Pharmacokinetics and pharmacodynamic changes associated with aging and implications for drug therapy. *Clinics in Geriatric Medicine,* **28**(2), pp. 273-286.

SHAH, S., ASNANI, A., KAWADE, D., DANGRE, S., ARORA, S. and YENDE, S., 2012. Simultaneous quantitative analysis of olmesartan medoxomil and amlodipine besylate in plasma by high-performance liquid chromatography technique. *Journal of Young Pharmacists,* **4**(2), pp. 88-94.

SHAH, M., HAWWA, F., MILLERSHIP, S., COLLIER, S. and MCELNAY, C., 2013. A simple bioanalytical method for the quantification of antiepileptic drugs in dried blood spots. *Journal of Chromatography B,* **923**, pp. 65-73.

SHAH, N.M., HAWWA, A.F., MILLERSHIP, J.S., COLLIER, P.S., HO, P., TAN, M.L., PEAKE, D., TIRUPATHI, S., BOTHWELL, J. and BAILIE, N., 2013. Adherence to antiepileptic medicines in children: A multiple-methods assessment involving dried blood spot sampling. *Epilepsia,* **54**(6), pp. 1020-1027.

SHALANSKY, S.J. and LEVY, A.R., 2002. Effect of number of medications on cardiovascular therapy adherence. *Annals of Pharmacotherapy,* **36**(10), pp. 1532-1539.

SHALANSKY, S.J., LEVY, A.R. and IGNASZEWSKI, A.P., 2004. Self-reported Morisky score for identifying nonadherence with cardiovascular medications. *Annals of Pharmacotherapy,* **38**(9), pp. 1363-1368.

SHAPIRO, M.D. and FAZIO, S., 2017. Apolipoprotein B-containing lipoproteins and atherosclerotic cardiovascular disease. *F1000Research,* **6**, pp. 134-142.

SHARMA, A., JAISWAL, S., SHUKLA, M. and LAL, J., 2014. Dried blood spots: concepts, present status, and future perspectives in bioanalysis. *Drug Testing and Analysis,* **6**(5), pp. 399-414.

SHERRILL, B., HALPERN, M., KHAN, S., ZHANG, J. and PANJABI, S., 2011. Single-pill vs free-equivalent combination therapies for hypertension: a meta-analysis of health care costs and adherence. *The Journal of Clinical Hypertension,* **13**(12), pp. 898-909.

SHIN, M.C., KIM, J. and KIM, C., 2008. Bioequivalence evaluation of two brands of lisinopril tablets by in vitro comparative dissolution test and in vivo bioequivalence test. *Arzneimittelforschung,* **58**(01), pp. 11-17.

SHINYA, I., 2011. Pharmacokinetics 101. *Paediatrics & Child Health,* **16**(9), pp. 535-536.

SHIPKOVA, M. and SVINAROV, D., 2016. LC–MS/MS as a tool for TDM services: Where are we? *Clinical Biochemistry,* **49**(13-14), pp. 1009-1023.

SHIPKOVA, M., RAPP, S., RIGO-BONNIN, R., WIELAND, E. and PETER, A., 2017. Therapeutic Drug Monitoring of Everolimus: Comparability of Concentrations Determined by 2 Immunoassays and a Liquid Chromatography Tandem Mass Spectrometry Method. *Therapeutic Drug Monitoring,* **39**(2), pp. 102-108.

SIDORKIEWICZ, S., TRAN, V.T., COUSYN, C., PERRODEAU, E. and RAVAUD, P., 2016. Development and validation of an instrument to assess treatment adherence for each individual drug taken by a patient. *British Medical Journal,* **6**(5), pp. 1-9.

SINNOTT, S., BUCKLEY, C., O'RIORDAN, D., BRADLEY, C. and WHELTON, H., 2013. The effect of copayments for prescriptions on adherence to medicines in publicly insured populations: a systematic review and meta-analysis. *PloS One,* **8**(5), pp. e64914.

SJOLANDER, M., ERIKSSON, M. and GLADER, E.L., 2013. The association between patients' beliefs about medicines and adherence to drug treatment after stroke: a cross-sectional questionnaire survey. *British Medical Journal Open,* **3**(9), pp. 1-8.

SOKOL, M.C., MCGUIGAN, K.A., VERBRUGGE, R.R. and EPSTEIN, R.S., 2005. Impact of medication adherence on hospitalization risk and healthcare cost. *Medical Care,* **43**(6), pp. 521-530.

SPERTUS, J.A., KETTELKAMP, R., VANCE, C., DECKER, C., JONES, P.G., RUMSFELD, J.S., MESSENGER, J.C., KHANAL, S., PETERSON, E.D. and BACH, R.G., 2006. Prevalence, predictors, and outcomes of premature discontinuation of thienopyridine therapy after drug-eluting stent placement results from the PREMIER registry. *Circulation,* **113**(24), pp. 2803-2809.

SPIELBERG, F., CRITCHLOW, C., VITTINGHOFF, E., COLETTI, A.S., SHEPPARD, H., MAYER, K.H., METZGER, D., JUDSON, F.N., BUCHBINDER, S. and CHESNEY, M., 2000. Home collection for frequent HIV testing: acceptability of oral fluids, dried blood spots and telephone results. *Aids,* **14**(12), pp. 1819-1828.

SPOONER, N., 2013. A dried blood spot update: still an important bioanalytical technique? *Bioanalysis,* **5**(8), pp. 879-883.

STANGE, D., KRISTON, L., VON-WOLFF, A., BAEHR, M. and DARTSCH, D.C., 2013. Reducing cardiovascular medication complexity in a German university hospital: effects of a structured pharmaceutical management intervention on adherence. *Journal of Managed Care Pharmacy,* **19**(5), pp. 396-407.

STEINER, J.F. and PROCHAZKA, A.V., 1997. The assessment of refill compliance using pharmacy records: methods, validity, and applications. *Journal of Clinical Epidemiology,* **50**(1), pp. 105-116.

STIRRATT, M.J., DUNBAR-JACOB, J., CRANE, H.M., SIMONI, J.M., CZAJKOWSKI, S., HILLIARD, M.E., AIKENS, J.E., HUNTER, C.M., VELLIGAN, D.I. and HUNTLEY, K., 2015. Self-report measures of medication adherence behavior: recommendations on optimal use. *Translational behavioral medicine,* **5**(4), pp. 470-482.

STURGEON, C.M. and VILJOEN, A., 2011. Analytical error and interference in immunoassay: minimizing risk. *Annals of Clinical Biochemistry,* **48**(5), pp. 418-432.

STUART, B., SIMONI-WASTILA, L., ZHAO, L., LLOYD, J.T. and DOSHI, J.A., 2009. Increased persistency in medication use by U.S. Medicare beneficiaries with diabetes is associated with lower hospitalization rates and cost savings. *Diabetes care,* **32**(4), pp. 647-649.

STURM, R.M., JONES, B.R., MULVANA, D.E. and LOWES, S., 2016. HRMS using a Q-Exactive series mass spectrometer for regulated quantitative bioanalysis: how, when, and why to implement. *Bioanalysis,* **8**(16), pp. 1709-1721.

SUZANNE, A.:., 2011. The Pharmacist's Role in Medication Adherence. *US Pharmacist,* **36**(5), pp. 45-48.

SYNDICATE OF IRAQI PHARMACIST, 2016. falsified medicines. Available: http://www.iraqipharm.com/?paged=9 [Accessed 03/13/2019].

TABASI, H.K., MADARSHAHIAN, F., NIKOO, M.K., HASSANABADI, M. and MAHMOUDIRAD, G., 2014. Impact of family support improvement behaviors on anti diabetic medication adherence and cognition in type 2 diabetic patients. *Journal of Diabetes & Metabolic Disorders,* **13**(113), pp. 1-6.

TAMARGO, J., ROSANO, G., WALTHER, T., DUARTE, J., NIESSNER, A., KASKI, J.C., CECONI, C., DREXEL, H., KJELDSEN, K. and SAVARESE, G., 2017. Gender differences in the effects of cardiovascular drugs. *European Heart Journal,* **3**(3), pp. 163-182.

TANNA, S. and LAWSON, G., 2011. Analytical methods used in conjunction with dried blood spots. *Analytical Methods,* **3**(8), pp. 1709-1718.

TANNA, S. and LAWSON, G., 2014a. Cardiovascular drug medication adherence assessed by dried blood spot analysis. *Journal of Analytical and Bioanalytical Techniques,* (S12), pp. 001-003.

TANNA, S. and LAWSON, G., 2014b. Dried blood spot analysis to assess medication adherence and to inform personalization of treatment. *Bioanalysis,* **6**(21), pp. 2825-2838.

TANNA, S. and LAWSON, G., 2016a. *Analytical Chemistry for Assessing Medication Adherence.* 1 edn. New York, USA: Elsevier.

TANNA, S. and LAWSON, G., 2016b. Opportunities and challenges for analytical chemistry. *Analytical Chemistry for Assessing Medication Adherence.* 1st Edition edn. New York, USA: Elsevier, pp. 13-16.

TANNA, S., ALALAQI, A., BERNIEH, D. and LAWSON, G., 2018. Volumetric absorptive microsampling (VAMS) coupled with high-resolution, accurate-mass (HRAM) mass spectrometry as a simplified alternative to dried blood spot (DBS) analysis for therapeutic drug monitoring of cardiovascular drugs. *Clinical Mass Spectrometry*, **10**, pp. 1-8.

TCHEN, J., VAILLANCOURT, R. and POULIOT, A., 2013. Wasted medications, wasted resource. *Canadian Pharmacists Journal,* **146**(4), pp. 181-182.

THE ASSOCIATION OF THE BRITISH PHARMACEUTICAL INDUSTRY (ABPI), 2018. Pharmaceutical Price Regulation Scheme (PPRS) [Homepage of The Association of the British Pharmaceutical Industry], [Online]. Available: https://www.abpi.org.uk/what-we-do/pricing-regulation/pharmaceutical-price-regulation-scheme-pprs/ [Accessed 06/24/2018].

THE CENTRE FOR WORKFORCE INTELLIGENCE, 2013. A strategic review of the future pharmacist workforce: Informing pharmacist student intakes. Available: https://www.gov.uk/government/publications/strategic-review-of-the-future-pharmacist-workforce [Accessed 03/18/2019].

THOMAS, A. and THEVIS, M., 2018. Analysis of insulin and insulin analogs from dried blood spots by means of liquid chromatography–high resolution mass spectrometry. *Drug Testing and Analysis,* **10**(11-12), pp. 1761-1768.

THORPE, C.T., BRYSON, C.L., MACIEJEWSKI, M.L. and BOSWORTH, H.B., 2009. Medication acquisition and self-reported adherence in veterans with hypertension. *Medical Care,* **47**(4), pp. 474-481.

TIMMERMAN, P., WHITE, S., GLOBIG, S., LÜDTKE, S., BRUNET, L. and SMERAGLIA, J., 2011. EBF recommendation on the validation of bioanalytical methods for dried blood spots. *Bioanalysis,* **3**(14), pp. 1567-1575.

TJANDRAWINATA, R.R., SETIAWATI, E., YUNAIDI, D.A., SANTOSO, I.D., SETIAWATI, A. and SUSANTO, L.W., 2012. Bioequivalence study of two formulations of bisoprolol fumarate film-coated tablets in healthy subjects. *Drug Design, Development and Therapy,* **6**, pp. 311-316.

TOMASZEWSKI, M., WHITE, C., PATEL, P., MASCA, N., DAMANI, R., HEPWORTH, J., SAMANI, N.J., GUPTA, P., MADIRA, W., STANLEY, A. and WILLIAMS, B., 2014. High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. *Heart,* **100**(11), pp. 855-861.

TOY, E.L., BEAULIEU, N.U., MCHALE, J.M., WELLAND, T.R., PLAUSCHINAT, C.A., SWENSEN, A. and DUH, M.S., 2011. Treatment of COPD: relationships between daily dosing frequency, adherence, resource use, and costs. *Respiratory Medicine,* **105**(3), pp. 435-441.

TURK-ADAWI, K., SARRAFZADEGAN, N., FADHIL, I., TAUBERT, K., SADEGHI, M., WENGER, N.K., TAN, N.S. and GRACE, S.L., 2018. Cardiovascular disease in the Eastern Mediterranean region: epidemiology and risk factor burden. *Nature Reviews Cardiology,* **15**(2), pp. 106-119.

ULLEY, J., HARROP, D., ALI, A., ALTON, S. and DAVIS, S.F., 2019. Deprescribing interventions and their impact on medication adherence in community-dwelling older adults with polypharmacy: a systematic review. *BMC Geriatrics,* **19**(1), pp. 15.

UNITED NATIONS IRAQ, 2019. Country Profile. Employment and Poverty. Available: http://www.uniraq.org/index.php?option=com_k2&view=item&layout=item&id=941&Itemid=472&lang=en [Accessed 17/09/2019].

UNNI, E.J. and FARRIS, K.B., 2011. Unintentional non-adherence and belief in medicines in older adults. *Patient Education and Counseling,* **83**(2), pp. 265-268.

UNNI, E.J. and FARRIS, K.B., 2015. Development of a new scale to measure self-reported medication nonadherence. *Research in Social and Administrative Pharmacy,* **11**(3), pp. 133-143.

URQUHART, J., 1997. The electronic medication event monitor. *Clinical pharmacokinetics,* **32**(5), pp. 345-356.

USHERWOOD, T., 2017. Encouraging adherence to long-term medication. *Australian Prescriber,* **40**(4), pp. 147-150.

VAN HEUCKELUM, M., VAN DEN ENDE, CORNELIA HM, HOUTERMAN, A.E., HEEMSKERK, C.P., VAN DULMEN, S. and VAN DEN BEMT, BART JF, 2017. The effect of electronic monitoring feedback on medication adherence and clinical outcomes: A systematic review. *PloS One,* **12**(10), pp. 1-18.

VAN ONZENOORT, H.A., VERBERK, W.J., KESSELS, A.G., KROON, A.A., NEEF, C., VAN DER KUY, PAUL-HUGO M and DE LEEUW, P.W., 2010. Assessing medication adherence simultaneously by electronic monitoring and pill count in patients with mild-to-moderate hypertension. *American Journal of Hypertension,* **23**(2), pp. 149-154.

VANBUSKIRK, K.A. and WETHERELL, J.L., 2014. Motivational interviewing with primary care populations: a systematic review and meta-analysis. *Journal of Behavioral Medicine,* **37**(4), pp. 768-780.

VAVLUKIS, M. and VAVLUKIS, A., 2018. Adding ezetimibe to statin therapy: latest evidence and clinical implications. *Drugs in Context,* **7**, pp. 1-9.

VEENHOF, H., KOSTER, R.A., ALFFENAAR, J.C., BERGER, S.P., BAKKER, S.J. and TOUW, D.J., 2017. Clinical Validation of Simultaneous Analysis of Tacrolimus, Cyclosporine A, and Creatinine in Dried Blood Spots in Kidney Transplant Patients. *Transplantation,* **101**(7), pp. 1727-1733.

VERHAEGHE, T., DILLEN, L., STIELTJES, H., ZWART, L.D., FEYEN, B., DIELS, L., VROMAN, A. and TIMMERMAN, P., 2017. The application of capillary microsampling in GLP toxicology studies. *Bioanalysis,* **9**(7), pp. 531-540.

VERMEIRE, E., HEARNSHAW, H., VAN ROYEN, P. and DENEKENS, J., 2001. Patient adherence to treatment: three decades of research. A comprehensive review. *Journal of Clinical Pharmacy and Therapeutics,* **26**(5), pp. 331-342.

VERPLAETSE, R. and HENION, J., 2016. Quantitative determination of opioids in whole blood using fully automated dried blood spot desorption coupled to on-line SPE-LC-MS/MS. *Drug Testing and Analysis,* **8**(1), pp. 30-38.

VIK, S.A., MAXWELL, C.J. and HOGAN, D.B., 2004. Measurement, correlates, and health outcomes of medication adherence among seniors. *The Annals of Pharmacotherapy,* **38**(2), pp. 303-312.

VINCENT, J., HARRIS, S.I., FOULDS, G., DOGOLO, L.C., WILLAVIZE, S. and FRIEDMAN, H.L., 2000. Lack of effect of grapefruit juice on the pharmacokinetics and pharmacodynamics of amlodipine. *British Journal of Clinical Pharmacology,* **50**(5), pp. 455-463.

VINLUAN, C.M., WITTMAN, D. and MORISKY, D., 2015. Effect of pharmacist discharge counselling on medication adherence in elderly heart failure patients: A pilot study. *Journal of Pharmaceutical Health Services Research,* **6**(2), pp. 103-110.

VITOLINS, M.Z., RAND, C.S., RAPP, S.R., RIBISL, P.M. and SEVICK, M.A., 2000. Measuring adherence to behavioral and medical interventions. *Controlled Clinical Trials,* **21**(5), pp. S188-S194.

VRIJENS, B., DE GEEST, S., HUGHES, D.A., PRZEMYSLAW, K., DEMONCEAU, J., RUPPAR, T., DOBBELS, F., FARGHER, E., MORRISON, V. and LEWEK, P., 2012. A new taxonomy for describing and defining adherence to medications. *British Journal of Clinical Pharmacology,* **73**(5), pp. 691-705.

VU, D., KOSTER, R., BOLHUIS, M., GREIJDANUS, B., ALTENA, R., NGUYEN, D., BROUWERS, J., UGES, D. and ALFFENAAR, J., 2014. Simultaneous determination of rifampicin, clarithromycin and their metabolites in dried blood spots using LC–MS/MS. *Talanta,* **121**, pp. 9-17.

WAGNER, M., TONOLI, D., VARESIO, E. and HOPFGARTNER, G., 2014. The use of mass spectrometry to analyze dried blood spots. *Mass Spectrometry Reviews,* **35**(3), pp. 361-438.

WALKER, H., HALL, W. and HURST, J., eds, 1990. *Clinical Methods, The History, Physical and Laboratory examinations.* 3rd edn. Boston, Butterworths. Chapter 151, p 718-719.

WALLACE, A., CHINN, D. and RUBIN, G., 2003. Taking simvastatin in the morning compared with in the evening: randomised controlled trial. *British Medical Journal*, **327**(7418), p.p.788

WARREN, S.R., RAISCH, D.W., CAMPBELL, H.M., GUARINO, P.D., KAUFMAN, J.S., PETROKAITIS, E., GOLDFARB, D.S., GAZIANO, J.M., JAMISON, R.L. and VETERANS AFFAIRS SITE INVESTIGATORS, 2013. Medication adherence assessment in a clinical trial with centralized follow-up and direct-to-patient drug shipments. *Clinical Trials,* **10**(3), pp. 441-448.

WATERMAN, K.C. and ADAMI, R.C., 2005. Accelerated aging: prediction of chemical stability of pharmaceuticals. *International Journal of Pharmaceutics,* **293**(1-2), pp. 101-125.

WATSON, D., 1992. Correcting for acquiescent response bias in the absence of a balanced scale: An application to class consciousness. *Sociological Methods & Research,* **21**(1), pp. 52-88.

WEBER, J., OBERFELD, S., BONSE, A., TELGER, K., LINGG, R. and HEMPEL, G., 2017. Validation of a dried blood spot method for therapeutic drug monitoring of citalopram, mirtazapine and risperidone and its active metabolite 9-hydroxyrisperidone using HPLC–MS. *Journal of Pharmaceutical and Biomedical Analysis,* **140**, pp. 347-354.

WEBSTER, R., PATEL, A., SELAK, V., BILLOT, L., BOTS, M.L., BROWN, A., BULLEN, C., CASS, A., CRENGLE, S. and ELLEY, C.R., 2016. Effectiveness of fixed dose combination medication ('polypills') compared with usual care in patients with cardiovascular disease or at high risk: a prospective, individual patient data meta-analysis of 3140 patients in six countries. *International Journal of Cardiology,* **205**, pp. 147-156.

WICKREMSINHE, E.R., CALLIES, S., SCHMALZ, C.A., LEE, L.B., LABELL, E.S. and SATONIN, D.K., 2018. Incorporating dried blood spot LC–MS/MS analysis for clinical development of a novel oncolytic agent. *Bioanalysis,* **10**(5), pp. 341-356.

WILHELM, A.J., DEN BURGER, J.C. and SWART, E.L., 2014. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. *Clinical Pharmacokinetics,* **53**(11), pp. 961-973.

WOOLF, S.H., GROL, R., HUTCHINSON, A., ECCLES, M. and GRIMSHAW, J., 1999. Clinical guidelines: potential benefits, limitations, and harms of clinical guidelines. *British Medical Journal,* **318**(7182), pp. 527-530.

WOLFF, K., 2017. *Detection of Drug Misuse: Biomarkers, Analytical Advances and Interpretation.* London: Royal Society of Chemistry. pp. 120-130.

WORLD HEALTH ORGANIZATION, 2002a. The impact of chronic disease in Iraq. Available: www.who.int/chp/chronic_disease_report/media/impact/iraq.pdf [Accessed 13/07/2018].

WORLD HEALTH ORGANIZATION, 2002b. The world health report 2002: reducing risks, promoting healthy life. Available: http://www.who.int/whr/2002/en/ [Accessed 30/11/2016].

WORLD HEALTH ORGANIZATION, 2006. Health System Profile-Iraq [Homepage of World Health Organization], [Online]. Available: http://apps.who.int/medicinedocs/en/d/Js17295e/ [Accessed 20/06/2018].

WORLD HEALTH ORGANIZATION, 2011. Iraq pharmaceutical country profile [Homepage of World Health Organization], [Online]. Available: http://apps.who.int/medicinedocs/en/d/Js19734en/ [Accessed 21/06/2018].

WORLD HEALTH ORGANIZATION, 2014. Noncommunicable diseases country profiles 2014 [Homepage of World Health Organization], [Online]. Available: http://www.who.int/nmh/publications/ncd-profiles-2014/en/ [Accessed 12/12/2018].

WORLD HEALTH ORGANIZATION, 2015. Iraq: WHO statistical profile. [Homepage of WHO], [Online]. Available: http://www.who.int/gho/countries/irq/country_profiles/en/ [Accessed 06/02/2018].

WORLD HEALTH ORGANIZATION, 2017a. Cardiovascular diseases (CVDs). Key facts [Homepage of WHO], [Online]. Available: https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) [Accessed 02/05/2019].

WORLD HEALTH ORGANIZATION, 2017b. Iraq health profile 2015 [Homepage of World Health Organization], [Online]. Available: http://apps.who.int/iris/handle/10665/254984 [Accessed 27/05/2018].

WORLD HEALTH ORGANIZATION, 2017c. Media centre Cardiovascular diseases (CVDs). Fact sheet. Available: http://www.who.int/mediacentre/factsheets/fs317/en/# [Accessed 03/12/2018].

WORLD HEALTH ORGANIZATION, 2017d. Noncommunicable Diseases Progress Monitor 2017 [Homepage of World Health Organization], [Online]. Available: http://www.who.int/nmh/publications/ncd-progress-monitor-2017/en/ [Accessed 27/05/2018].

WORLD HEALTH ORGANIZATION, 2017e. A study on the public health and socioeconomic impact of substandard and falsified medical products [Homepage of WHO], [Online]. Available: https://www.who.int/medicines/regulation/ssffc/publications/se-study-sf/en/ [Accessed 29/04/2019].

WORLD HEALTH ORGANIZATION, 2017f. WHO urges governments to take action [Homepage of WHO], [Online]. Available: https://www.who.int/news-room/detail/28-11-2017-1-in-10-medical-products-in-developing-countries-is-substandard-or-falsified [Accessed 05/25/2019].

WORLD HEALTH ORGANIZATION, 2018a. Iraq Public Sector Modernization (I-PSM) [Homepage of World Health Organization], [Online]. Available: http://www.emro.who.int/irq/programmes/iraq-public-sector-modernization-i-psm.html [Accessed 28/05/2018].

WORLD HEALTH ORGANIZATION, 2018b. Noncommunicable diseases: Key facts. Available: http://www.who.int/en/news-room/fact-sheets/detail/noncommunicable-diseases [Accessed 13/07/2018].

WORLD HEALTH ORGANIZATION, 2018c. Substandard and falsified medical products [Homepage of WHO], [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products [Accessed 29/04/2019].

WROE, A.L., 2002. Intentional and unintentional nonadherence: a study of decision making. *Journal of Behavioral Medicine*, **25**(4), pp. 355-372.

WU, F.L., CHEN, P., LEE, Y. and CHEN, R.R., 2003. Comparative pharmacokinetics of two atenolol products. *Journal of Food and Drug Analysis,* **11**(1), pp. 4-7.

WU, J. and MOSER, D., 2018. Impact of HF Medication Adherence on Hospitalization and Mortality Outcomes. *Journal of Cardiovascular Nursing,* **33**(1), pp. 40-46.

XIE, C., ZHONG, D., YU, K. and CHEN, X., 2012. Recent advances in metabolite identification and quantitative bioanalysis by LC–Q-TOF MS. *Bioanalysis,* **4**(8), pp. 937-959.

XU, Y. and WORDEN, C., 2016. Adherence, compliance, and persistence with lipid-lowering therapies: a systematic review. *Value in Health,* **19**(3), pp. A50.

XUE, K.S., CAI, W., TANG, L. and WANG, J., 2016. Aflatoxin B1-lysine adduct in dried blood spot samples of animals and humans. *Food and Chemical Toxicology,* **98**, pp. 210-219.

YAN, X., CHEN, Z., WANG, R., YANG, J., AI, F., PAN, Y., QIU, P. and WANG, D., 2013. Pharmacokinetics of diltiazem hydrochloride delay-onset sustained-release pellet capsules in healthy volunteers. *Brazilian Journal of Pharmaceutical Sciences,* **49**(1), pp. 29-38.

YAP, A.F., THIRUMOORTHY, T. and KWAN, Y.H., 2016. Systematic review of the barriers affecting medication adherence in older adults. *Geriatrics & Gerontology International*, **16**(10), pp. 1093-1101.

YAR, M., DIX, D. and BAJEKAL, M., 2006. Socio-demographic characteristics of the healthcare workforce in England and Wales–results from the 2001 Census. *Health Statistics Quarterly,* **32**, pp. 44-55.

YASSINE, M., AL-HAJJE, A., AWADA, S., RACHIDI, S., ZEIN, S., BAWAB, W., ZEID, M.B., EL HAJJ, M. and SALAMEH, P., 2015. Evaluation of medication adherence in Lebanese hypertensive patients. *Journal of Epidemiology and Global Health,* **6**, pp. 157-167.

YOUHNOVSKI, N., BERGERON, A., FURTADO, M. and GAROFOLO, F., 2011. Pre-cut dried blood spot (PCDBS): an alternative to dried blood spot (DBS) technique to overcome hematocrit impact. *Rapid Communications in Mass Spectrometry,* **25**(19), pp. 2951-2958.

YU, D., HUANG, J., HU, D., CHEN, J., CAO, J., LI, J. and GU, D., 2009. Association between prehypertension and clustering of cardiovascular disease risk factors among Chinese adults. *Journal of cardiovascular pharmacology*, **53**(5), pp. 388-400.

YUSUF, S., ATTARAN, A., BOSCH, J., JOSEPH, P., LONN, E., MCCREADY, T., MENTE, A., NIEUWLAAT, R., PAIS, P. and RODGERS, A., 2013. Combination pharmacotherapy to prevent cardiovascular disease: present status and challenges. *European heart journal,* **35**(6), pp. 353-364.

ZAKARIA, R., ALLEN, K.J., KOPLIN, J.J., ROCHE, P. and GREAVES, R.F., 2016. Advantages and Challenges of Dried Blood Spot Analysis by Mass Spectrometry Across the Total Testing Process. *International Federation of Clinical chemistry and Laboratory Medicine,* **27**(4), pp. 288-317.

ZANE, P. and EMMONS, G., 2013. Advantages and challenges of microsampling. In: P. ZANE and G. EMMONS, eds, *Microsampling in pharmaceutical bioanalysis.* London: Future Medicine, pp. 3-97.

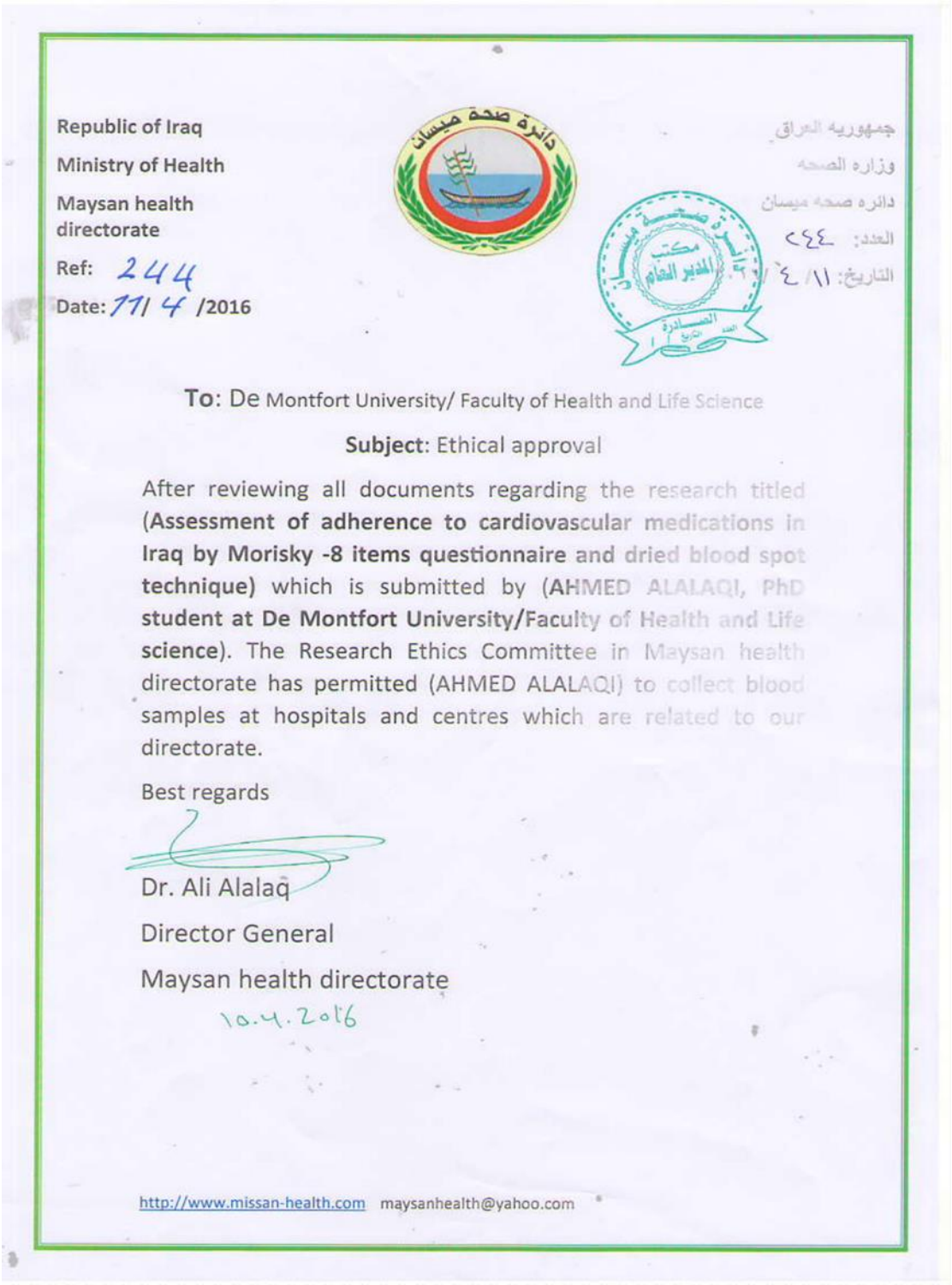
ZHANG, N., YU, S., TILLER, P., YEH, S., MAHAN, E. and EMARY, B., 2009. Quantitation of small molecules using high-resolution accurate mass spectrometers–a different approach for analysis of biological samples. *Rapid Communications in Mass Spectrometry*, **23**(7), pp. 1085-1094.

ZHENG, J., GUIDA, L.A., ROWER, C., CASTILLO-MANCILLA, J., MEDITZ, A., KLEIN, B., KERR, B.J., LANGNESS, J., BUSHMAN, L. and KISER, J., 2014. Quantitation of tenofovir and emtricitabine in dried blood spots (DBS) with LC–MS/MS. *Journal of Pharmaceutical and Biomedical Analysis,* **88**, pp. 144-151.

ZHOU, N., LIANG, Y.Z., CHEN, B.M., WANG, P., CHEN, X. and LIU, F.P., 2008. Development and validation of LC-MS method for the determination of lisinopril in human plasma and its application in a bioequivalence study. *Journal of Chromatographic Science,* **46**(10), pp. 848-853.

ZULLIG, L.L., RAMOS, K. and BOSWORTH, H.B., 2017. Improving medication adherence in Coronary heart disease. *Current Cardiology Reports,* **19**(11), pp. 113-126.

Appendix 1. Ethical Approval for the Application of the MMAS-8 and Collection of Biosamples from Misan Health Directorate



Republic of Iraq
Ministry of Health
Maysan health directorate
Ref: 244
Date: 11/ 4 /2016

جمهوريه العراق
وزاره الصحه
دائره صحه ميسان
العدد: ٢٤٤
التاريخ: ١١/ ٤/ ٢٠١٦

To: De Montfort University/ Faculty of Health and Life Science

Subject: Ethical approval

After reviewing all documents regarding the research titled (**Assessment of adherence to cardiovascular medications in Iraq by Morisky -8 items questionnaire and dried blood spot technique)** which is submitted by (**AHMED ALALAQI, PhD student at De Montfort University/Faculty of Health and Life science**). The Research Ethics Committee in Maysan health directorate has permitted (AHMED ALALAQI) to collect blood samples at hospitals and centres which are related to our directorate.

Best regards

Dr. Ali Alalaq
Director General
Maysan health directorate
10.4.2016

http://www.missan-health.com maysanhealth@yahoo.com

Appendix 2. Ethical Approval for the Collection of Blood Samples Obtained from De Montfort University's Faculty of Health and Life Science Research

Ethics Application - Novel methods for drug monitoring using microanalysis (dried blood spot analysis)

Ethics Application Ref: 1212

Sangeeta Tanna, Graham Lawson, Dennis Bernieh

Leicester School of Pharmacy, Faculty of Health and Life Sciences

Addition of new PhD student: Ahmed Alalaqi

Start Date: 1st October 2013

End Date: 31st December 2019

This project is on the development of a simple non-invasive test to assess adherence to cardiovascular therapy in primary and secondary care.

Cardiovascular disease is one of the biggest killers worldwide affecting 1 in 3 people in the UK. Current care of such patients and increasingly for patients over 50 years old is the prescription of a combination of a beta blocker (BB), an ace inhibitor (AI) and a statin (ST). Good patient recovery depends on the combination of clinical skills and adherence with drug therapy. Non-adherence to cardiovascular medication is a growing concern to clinicians and other healthcare professionals because of mounting evidence that it is prevalent. There is evidence that up to 60% of patients prescribed cardiovascular drugs do not adhere to their prescribed regimen leading to increases in le morbidity, mortality and higher costs of care. In cases of poor clinical outcomes, it is essential to the clinical decision-making process that adherence is assessed. A simple test to monitor adherence would therefore be highly valued.

In this non-patient project, we present a compliance assay test for beta blockers, ace inhibitors and statins, to help the clinical decision-making process. Sample collection for such a test would be via dried blood spots (DBS) in which a drop of blood is collected on a card non-invasively by a simple finger prick procedure. Sampling can be carried out by

the volunteer and the card then posted to the laboratory for analysis. The assay of the target drugs or metabolites would be carried out using mass spectrometry techniques.

The Project would be divided into the following stages:

1. Identifying the use of the principal cardiovascular drugs (BB, AI, ST) drugs based on current pharmacy practice.
2. Developing a DBS based simultaneous analytical method for the cardiovascular drugs identified.
3. Fully validating the developed DBS based simultaneous method for cardiovascular drugs.
4. Applying the validated analytical method to DBS samples from volunteers in order to inform medication taking behaviour (or adherence). Volunteers on the target cardiovascular drugs would be recruited from the University, the Square Mile, and from clinics in Iraq. All samples will be anonymous and there will be no means of identifying the sampled volunteer. The only information requested from the volunteer will be:
    - **a.** Cardiovascular drug prescribed
    - **b.** Date/time the last dose of the prescribed CVD drug(s) was taken
    - **c.** Dose prescribed
5. Initial assessment of results on a YES/NO basis and comparison with data supplied above.

Appendix 3. Patient Information Leaflet (English Version).

PARTICIPANT INFORMATION LEAFLET

Dried blood spot analysis to assess adherence to cardiovascular medications

Faculty of Health and Life Sciences

What is the study?

This project is on the development of a simple minimally invasive test to assess adherence to cardiovascular therapy in primary and secondary care. Cardiovascular disease is one of the biggest killers worldwide affecting 1 in 3 people in the UK. Current care of such patients and increasingly for patients over 50 years old is the prescription of a combination of cardiovascular therapy drugs including beta blockers (BB), ACE inhibitors (AI) and statins (ST). There is evidence that up to 60% of patients prescribed cardiovascular drugs do not adhere to their prescribed regimen leading to increases in morbidity, mortality and higher costs of care. The estimated cost of unused prescription medicines in the UK is ~£4 billion annually. A simple test to monitor prescription drug levels would therefore be highly valued.

What will happen?

The programme for this study will involve testing the developed and validated of a dried blood spot (DBS) based analytical method for the principal cardiovascular drugs identified. This analytical method will be used to test DBS samples obtained from participants who are currently taking cardiovascular medication(s) to confirm the successful detection of these drugs in their blood.

How will you be involved?

After reading this Participant Information Leaflet you will be asked to sign a consent form prior to giving a blood spot sample and you will also be asked to complete a small questionnaire. Information requested in this questionnaire will be:

1. Cardiovascular drug(s) prescribed
2. Time since the last dose of the prescribed CVD drug(s) was taken
3. Dose prescribed

The blood spot collection card or device and the questionnaire will remain anonymous.

How is a blood spot sample collected?

The general approach for the collection and uses of DBS is as follows: One or two drop(s) of blood are obtained minimally invasively by a simple finger prick or thumb prick procedure. This small volume of blood (~25 µl) is applied to a sample collection card or other blood sampling device and dried at room temperature for at least 2-3 hours. The sampling can be done almost anywhere. For example, in a laboratory or at home by the participant; in a clinic by a nurse; or in a pharmacy by the community pharmacist. The dried blood spot sample will then be sent to our laboratory for analysis.

How is the blood spot analysed?

In the laboratory, a fixed area of the DBS is extracted, either directly or as a disk punched from the DBS, and the presence of the drug in question is identified by mass spectrometry.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept on a password protected database and is strictly confidential. Your sample will be given a reference code which will be used instead of your name. Any identifiable information you may give will be removed and anonymised.

What will happen to the results of the research study?

The results will be an essential part of PhD thesis in clinical pharmacy practice at De Montfort University, Leicester

Who is organising and funding the research?

The research is for a PhD studentship at De Montfort University Leicester and is funded by the Iraqi Ministry of Health, Misan Health Directorate.

Who has reviewed the study?

This study has been reviewed and approved by De Montfort University, Faculty of Health and Life Sciences Research Ethics Committee.

Directions for collection of dried blood sample (DBS) on a sample collection card

Kit contents:

- DBS sample collection card (1)
- Alcohol Prep Pad (1)
- Lancet (1)
- Gauze Pad (1)
- Plaster (1)
- Plastic re-sealable bag (1)

1. Fill out the participant reference number on the DBS sample collection card.

2. Warm the skin on a finger or thumb by gentle rubbing.

3. Clean sample site with the alcohol pad provided and allow site to AIR DRY.

4. Lance the sample site and wipe away the first blood drop with sterile gauze.

5. Gently apply intermittent pressure near the puncture site to obtain the blood sample on the finger.

6. Allow blood to accumulate on the finger or thumb tip and drop onto the sampling card in the circled area. The blood drop(s) should fall freely to the sampling card.

7. AVOID TOUCHING the sampling card and DO NOT spread/smear/smudge blood to cover the circled area as this will render the DBS sample invalid.

8. Allow multiple drops to fall on the same circled area until this area is COMPLETELY covered and soaked.

9. Once a circled area is covered, start on the next one. At least 2 circles on the DBS card must be filled for each sample – this would be from the same finger prick. Over spotting or layering can give rise to erroneous results and the sample will be rejected.

10. The participant will be supplied with a gauze pad and plaster.

11. Sample cards must then be dried for 2-3 hours at room temperature. Sample cards should be kept apart (i.e., not stacked with each other if there is more than one card) and away from heat.

12. After drying the sample cards must be stored in individual a plastic resealable bag and are ready for collection or postage to the laboratory with the accompanying completed consent form and adherence questionnaire.

Directions for collection of dried blood sample (DBS) on a Mitra™ blood sampling device

Kit contents:

- Mitra™ (1 clamshell pack containing 4 samplers)
- Alcohol Prep Pad (1)
- Lancet (1)
- Gauze Pad (1)
- Plaster (1)
- Plastic resealable bag (1)
- Desiccant

1. Open sealed packaging and remove clamshell package.

2. Label samplers with participant reference number (see Quick Start Guide provided).

3. Uncover the samplers by pulling apart the clamshell and pressing the sides together to create a handle (Quick Start Guide step 2).

4. Clean sample site (side or tip of finger) with the alcohol pad provided and allow site to AIR DRY.

5. Lance the sample site and wipe away the first blood drop with sterile gauze.

6. Gently apply intermittent pressure near the puncture site to obtain the blood sample on the finger.

7. Apply sampler tip to surface of blood sample at an angle as shown in Steps 3 and 4 on the Quick Start Guide.

8. Wait for the tip to go fully red and then count 2 additional seconds. Slowly remove the sampler tip from the blood.

9. Repeat 7 and 8 above with the remaining three samplers in the four–pack.

10. Unfold clamshell to cover sampler tips and press closed.

11. The participant will be supplied with a gauze pad and plaster.

12. The covered sampler tips can be immediately placed in the bag with the desiccant.

13. The sampler is now ready for collection or postage to the laboratory with the accompanying completed consent form and adherence questionnaire.

Dr Sangeeta Tanna	Dr Graham Lawson	Ahmed Alalaqi
Leicester School of Pharmacy	Leicester School of Pharmacy	Leicester School of Pharmacy
De Montfort University	De Montfort University	De Montfort University
The Gateway	The Gateway	The Gateway
Leicester LE1 9BH	Leicester LE1 9BH	Leicester LE1 9BH
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Appendix 4. English Version of Patient Consent Form for DBS Collection

Dried blood spot analysis to assess adherence to cardiovascular medications

Participant Reference Number:

(To be completed by research team)

Name of Researchers: Dr Sangeeta Tanna, Dr Graham Lawson & Ahmed Alalaqi

Please initial box

I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information and ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected.	☐
I understand that the data collected during the study, may be looked at by responsible individuals from the research team or from individuals from regulatory authorities.	☐
I agree to take part in this study	☐

_____	_____	_____
Name of Participant	Signature	Date
_____	_____	_____
Name of person taking consent	Signature	Date

1 copy to participant; 1 copy for research file

Appendix 5. English Version of Mini-DBS Questionnaire

ADHERENCE QUESTIONNAIRE

Dried blood spot analysis to assess adherence to cardiovascular medications

Participant Reference Number:

Q1. Have you read the participation information sheet and signed the consent form?

Q2 Gender: M or F

Q3. Are you prescribed any cardiovascular (heart disease) medications? **Y/N**

If **Yes** to Q3, please complete Table 1.

Table 1: Names of prescribed medicines

Approved name	**Prescribed (√)**	**Dose (mg)**	**Frequency (x daily)**	**Approximate time since last dose (hours)**
Amlodipine				
Atenolol				
Atorvastatin				
Bisoprolol				
Diltiazem				
Doxazosin				
Lisinopril				
Losartan				
Ramipril				
Simvastatin				
Valsartan				
(Not listed) *				

* Other cardiovascular medicines? Please give name

Appendix 6. Participant Information Leaflet for Collection of Biosamples from Iraqi Volunteers (Arabic Version).

DE MONTFORT UNIVERSITY LEICESTER

Faculty of Health and Life Sciences

تحليل بقعة الدم لتقييم الالتزام أدوية القلب والأوعية الدموية

ما هي الدراسة؟

هذا المشروع يعتمد على اليه قليله الايذاء للمريض لتقييم التزام المريض بادويه القلب والاوعيه الدمويه في مراكز تقديم الخدمه الاوليه والثانويه

أمراض القلب والأوعية الدموية هي واحدة من أكبر أسباب الوفاة التي تؤثر في جميع أنحاء العالم في 1 من 3 أشخاص في المملكة المتحدة. لرعاية الحالية لهؤلاء المرضى وعلى نحو متزايد للمرضى الذين اعمارهم أكثر من 50 عاما ويتضمن العلاج مزيج من الأدوية بما في ذلك حاصرات بيتا (BB)، مثبطات ACEوالادويه الخافظه للدهنيات في الدم. وهناك ادله على ان هناك ما يصل إلى 60٪ من المرضى لا يلتزمون باخذ عقاقير القلب والاوعيه الدمويه كما منصوص عليه مما يودي الى زياده نسبه الاعتلال والوفيات وارتفاع تكاليف الرعايه الطبيه . التكاليف المتوقعه لعدم استخدام الادويه في المملكه المتحده هي تقريبا 4 بليون باوند سنويا . اختبار بسيط لمراقبه تركيز الادويه في الدم سيكون ذو قيمه عاليه.

ماذا سيحدث؟

برنامج الدراسه يتضمن اختبار متقدم ومصادق عليه معتمدا على مبدا التحليل لبقعه الدم الجافه للكشف ومعرفه تراكيز الادويه المستخدمه في علاج امراض القلب والاوعيه الدمويه. وسوف تستخدم هذه الطريق التحليليه لاختبار عينات الدم والتي يتم الحصول عليها من المشاركين الذين يتناولون ادويه القلب والاوعيه الدمويه لتاكيد الاكتشاف الناجح لهذه الادويه في الدم.

كيف لك أن تشارك؟

بعد قراءه المعلومات عن البحث سيطلب منك التوقيع على استماره المشاركه قبل اعطاء وكما يطلب منك مليء استبيان صغير وستكون المعلومات في هذا الاستبيان مايلي

1.الادويه المستخدمه من قبلك

2. اخر وقت تم اخذ اخر جرعه من ادويه القلب والاوعيه الدمويه

3. الجرعه الموصوفه

جميع المعلومات عن فيما يتعلق بالعينه والاستبيان ستكون محفوظه وتمتع بالخصوصيه

كيف يتم جمع عينة بقعة الدم؟

النهج العام في هذا الاختبار يتضمن الحصول على قطره او قطرتين من الدم بواسطه اجراء بسيط يتضمن وخز الاصبع وتوخذ كميه صغيره تعادل 25 مايكرومل وسيتم وضع هذه العينه على ورقه خاصه او عن طريق تقنيات اخرى وتترك العينات لتجف في درجه حراره الغرفه لمده 2-3 ساعات على الاقل .اخذ العينات يمكن ان يكون في اي مكان على سبيل المثال في المنزل او المختبر او في عياده التمريض او في الصيدليه وبعدها يتم ارسال العينات الى المختبر.

كيف يتم تحليل بقعه الدم؟

في المختبر يتم اخراج منطقه معينه من العينه بشكل مباشر او عن طريق قص منطقه من العينه الموجوده على الورقه الخاصه .

هل ستبقى مشاركتي في البحث سريه؟

وستبقى جميع المعلومات التي يتم جمعها عنك أثناء البحث في قاعدة بيانات محمية بكلمة مرور وسرية تامة .سيتم إزالة رمز إشارة التي سيتم استخدامها بدلا من اسمك وأية معلومات تعريفية قد تعطي.

ماذا سيحدث لنتائج الدراسة؟

وسوف تكون نتائج الدراسه جزءا أساسيا من رسالة الدكتوراه في الصيدلة السريرية في جامعة دي مونتفورت، ليستر

من يمول هذا البحث

هذا البحث هو لدراسه لدرجة الدكتوراة في جامعة دي مونتفورت , ليستر وتموله وزارة الصحة العراقية، دائره صحة ميسان.

من يتابع ويشرف على الدراسه؟

وقد استعرضت هذه الدراسة والموافقة عليها من قبل جامعة دي مونتفورت، كلية الصحة وعلوم الحياة ولجنة أخلاقيات البحث في دائره صحة ميسان.

ارشادات جمع العينه

المحتويات :

1.بطاقات جمع العينات

2. وساده كحول للتعقيم

3. لانسيت

4. شاش

5.بلاستر

6. كيس من البلاستك لحفظ العينات

1. تعبئه الرقم المرجعي للمشارك على بطاقه جمع العينات

2. تدفئه جلد الاصبع بالدلك الخفيف

3. تعقيم موقع اخذ النموذج بالكحول وتركه ليجف

4. وخز الاصبع ومسح اول قطره بالشاش المعقم

5. اضغط ضغطا خفيفا بالقرب من موقع الوخز من اجل الحصول على العينه

6.السماح لقطره الدم الاصبع للسقوط الحر على الورقه الخاصه في المنطقه المخصصه للعينه

7. تجنب لمس بطاقه اخذ العينات او نشر العينه حيث ان ذلك يودي الى جعل العينه غير صالحه

8.السماح لقطرات متعدده من السقوط على منطقه العينه حتى يتم تغطيتها بالكامل

9.عند اكمال عينه معينه يتم الانتقال الى العينه الاخرى وعلى الاقل يتم مليء دائرتين من الورقه وهذا يكون من نفس مكان الوخز.يتم رفض العينه في حاله تكون طبقات في العينه

10. يتم تزويد المشارك بالشاش والبلاستر

11.تترك العينات لتجف من 2-3 ساعات ولا يتم حفظ العينات الا بعد التاكد من جفافها وان تحفظ بعيدا عن الحراره

12.بعد جفاف العينات يتم حفظ العينات في الاكياس البلاستيكيه وتكون مهياه للنقل عن طريق البريد العادي مع استماره الموافقه على المشاركه والاستبيان

ارشادات جمع العينه بواسطه Mitra™

1.وساده كحول

2. لانسيت

3. شاش

4. بلاستر

5.كيس من البلاستك لحفظ العينات

6.مجففات الرطوبه

1. افتح مختومة التعبئة والتغليف

2. عينات تسمية مع الرقم المرجعي للمشارك

3. سحب العينات

4.تنظيف موقع العينه وتركه ليجف

5. وخز الاصبع ومسح اول قطره بالشاش

6. الضغط الخفيف قرب موضع اخذ العينه للحصول على الدم

7. تطبيق راس اخذ العينه بزاويه وكما موضح في الخطوات 3 و 4

8. انتظر حتى يصبح راس اخذ العينه اخر ثم انتظر ثانيتين اخريتين وبسرعه ارفع الراس من من الدم

9. كرر الخطوات 7و8 مع العينات المتبقيه

10.حفظ العينات المسحوبه

11. يجهز المريض بالشاش والبلاستر

12. راس اخذ العينات يتم وضعه في الكيس مع مانع الرطوبه

13. العينات الان جاهزه للتجميع والنقل عن طريق البريد مع استماره الموافقه والاستبيان

بمن يمكن الاتصال للمزيد من المعلومات؟

د. سانكيتا تانا
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Appendix 7. Consent Form for Blood Microsamples Collection (Arbic Version)

CONSENT FORM

استماره الموافقه

بقعه الدم الجافه لتقييم الالتزام بادويه القلب والاوعيه الدمويه

الرقم المرجعي للمشارك:

يملىء من قبل فريق البحث

اسماء الباحثين احمد العلاق د.سانكيتا تانا د.كراهام لاوسن

أؤكد أنني قد قرأت وفهمت ورقة المعلومات للدراسة المذكورة أعلاه. وقد أتيحت لي الفرصة للنظر في المعلومات، وطرح الأسئلة، وكان هذه الإجابة مرضية. ☐

وأنا أفهم أن مشاركتي طوعية وأنا حر في الانسحاب في أي وقت دون إبداء أي سبب، دون تاثر حقوقي القانونية ☐

وأنا أفهم أن البيانات التي تم جمعها خلال هذه الدراسة، يمكن النظر فيها من قبل الأفراد المسؤولين عن فريق البحث أو من الأفراد من السلطات التنظيمية. ☐

أنا أوافق على المشاركة في هذه الدراسة ☐

اسم المشارك **التوقيع** **التاريخ**

اسم الشخص الذي اخذ الموافقه **التوقيع** **التاريخ**

نسخه الى المشارك ونسخه الى الملف

Appendix 8. Mini Adherence Questionnaire (Arabic Version).

ADHERENCE QUESTIONNAIRE

استبيان الالتزام

بقعه الدم الجافه لتقييم الالتزام بادويه القلب والاوعيه الدمويه

الرقم المرجعي للمشارك:

س 1. هل قراءت معلومات المشاركه بالبحث ووقعت استماره الموافقه نعم لا

س 2.الجنس ذكر او انثى

س 3. هل تصرف لك ادويه القلب والاوعيه الدمويه نعم لا

في حاله الاجابه بنعم يرجى مليء الجدول رقم 1

جدول رقم 1 اسماء الادويه الموصوفه

اسم الدواء	موصوف	الجرعه ملغم	عدد الجرعات اليوميه (يوميا)	الوقت التقريبي بالساعات لاخر جرعه
Amlodipine				
Atenolol				
Atorvastatin				
Bisoprolol				
Diltiazem				
Doxazosin				
Lisinopril				
Losartan				
Ramipril				
Simvastatin				
Valsartan				
اخرى *				

اخرى يرجى ذكر اسم الدواء

Appendix 9. All Medications Prescribed to the Iraqi Volunteers.

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-280716-AA-01	Control	-	-	-
903-280716-AA-02	Control	-	-	-
903-280716-AA-03	Control	-	-	-
903-280716-AA-04	Control	-	-	-
903-280716-AA-05	Control	-	-	-
903-280716-AA-06	Lisinopril 10mg		1	1
903-310716-AA-07	Simvastatin 20mg	Aspirin 100mg	3	3
		Enalapril 20mg		
903-310716-AA-08	Atenolol 50 mg	Aspirin 100mg	8	13
		Clopidogrel 75mg		
		Metformin 500mg		
		Chlordiazepoxide 5mg		
		Multivitamin nd		
		Ranitidine 150mg		
		Diclofenac 50mg		
903-310716-AA-09	Control	-	-	-
903-310716-AA-10	Bisoprolol 10mg	Clopidogrel 75mg	8	11
		Lorazepam 2mg		
		Ranitidine 150mg		
		Indomethacin 20mg		
		Multivitamin nd		
		Aspirin 100mg		
		Chlordiazepoxide 5mg		
903-310716-AA-11	Valsartan 80mg	Clopidogrel 75mg	8	11
		Aspirin 100mg		
		Naproxen 500mg		
		Ranitidine 150mg		
		Chlordiazepoxide 5mg		
		Gemfibrozil 600mg		
		Multivitamin nd		
903-310716-AA-12	Valsartan 160mg		1	1
903-310716-AA-13	Control	-	-	-
903-310716-AA-14	Atenolol 100mg	Enalapril 20mg	2	2
903-310716-AA-15	Bisoprolol 5mg	Rosuvastatin 40mg	2	2
03-010816-AA-16	Valsartan 80mg		1	1
903-010816-AA-17	Atorvastatin 40mg	Carbamazepine 200mg	6	6
	Valsartan 80mg	Amiodaron 200mg		
		Aspirin 100mg		
		Lorazepam 2mg		
903-010816-AA-18	Control	-	-	-

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-030816-AA-19	Bisoprolol 5mg	Clopidogrel 75mg	8	11
		Warfarin 2mg		
		Ibrufen 200mg		
		Carbamazepine 200mg		
		Lorazepam 2mg		
		Furosemide 40mg		
		Gemfibrozil 600mg		
903-030816-AA-20	Simvastatin 20mg	Candesartan 8mg	5	6
		Furosemide 40mg		
		Aspirin 100mg		
		Clopidogrel 75mg		
903-030816-AA-21	Diltiazem 90mg	Rosuvastatin 20mg	7	11
		Aspirin 100mg		
		Clopidogrel 75mg		
		Metformin 500mg		
		Lorazepam 2mg		
		Famotidine 20mg		
903-030816-AA-22	Control	-	-	-
903-030816-AA-23	Atenolol 50mg	Aspirin 100mg	8	11
		Clopidogrel 75mg		
		Metformin 500mg		
		Carbamazepine 200mg		
		Bplex		
		Lorazepam 2mg		
		Furosemide 40mg		
903-030816-AA-24	Valsartan 80mg	-	1	1
903-030816-AA-25	Losartan 25mg	Clopidogrel 75mg	2	3
903-030816-AA-26	Control			
903-030816-AA-27	Bisoprolol 5mg	-	2	2
	Losartan 100mg			
903-030816-AA-28	Control			
903-030816-AA-29	Control			
903-040816-AA-30	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-040816-AA-31	Valsartan 80mg	Amiodarone 200mg	5	8
		Clopidogrel 75mg		
		Metformin 500mg		
		Carbamazepine 200mg		
903-040816-AA-32	Lisinopril 10mg	Rosuvastatin 20mg	2	2

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-040816-AA-33	Losartan 25mg	Metoprolol 50mg	8	9
		Telmisartan 80mg		
		Spironolactone 50mg		
		Rosuvastatin 40mg		
		Chlordiazepoxide 5mg		
		Aspirin 100mg		
		Lorazepam 2mg		
903-050816-AA-34	Atenolol 50mg	Gemfibrozil 600mg	2	2
903-050816-AA-35	Control	-	-	-
903-050816-AA-36	Bisoprolol 5mg	Lorazepam 2mg	5	7
	Valsartan 160mg	Metformin 500mg		
		Chlordiazepoxide 5mg		
903-050816-AA-37	Atenolol 50mg	Aspirin 100mg	3	3
		Isosorbide 10mg		
903-050816-AA-38	Bisoprolol 5mg	Lorazepam 2mg	5	7
	Losartan 50mg	Metformin 500mg		
		Chlordiazepoxide 5mg		
903-050816-AA-39	Control	-	-	-
903-050816-AA-40	Atenolol 50mg	-	1	1
903-050816-AA-41	Losartan 25mg	-	1	1
903-050816-AA-42	Atenolol 50mg	Aspirin 100mg	2	2
903-060816-AA-45	Atenolol 50mg	Gemfibrozil 300mg	4	6
		Lorazepam 2mg		
		Metformin 500mg		
903-060816-AA-46	Control	-	-	-
903-060816-AA-47	Control	-	-	-
903-060816-AA-48	Bisoprolol 5mg	Clopidogrel 75mg	8	12
	Valsartan 89mg	Aspirin 100mg		
		Famotidine 20mg		
		Lorazepam 2mg		
		Metformin 500mg		
		Chlordiazepoxide 5mg		
903-060816-AA-49	Control	-	-	-
903-060816-AA-50	Losartan 50mg	Aspirin 100mg	2	2
903-060816-AA-51	Atenolol 100mg	Candesartan 16mg	4	5
		Aspirin 100mg		
		Famotidine 20mg		
903-060816-AA-52	Atenolol 50mg	Aspirin 100mg	4	6
		Clopidogrel 75mg		
		Famotidine 20mg		
903-060816-AA-53	Atenolol 100mg	Enalapril 10mg	5	8
		Diclofenac 50mg		
		Gabapentin 300mg		
		Metformin 500mg		
903-060816-AA-54	Control	-	-	-

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-070816-AA-55	Losartan 100mg	Aspirin 100mg	5	7
	Atorvastatin 40mg	Famotidine 20mg		
		Clopidogrel 75mg		
903-070816-AA-56	Atorvastatin 40mg	Clopidogrel 75mg	3	4
	Lisinopril 20mg			
903-070816-AA-57	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-070816-AA-58	Atenolol 50mg	Isosorbide 10mg	8	12
	Atorvastatin 40mg	Famotidine 20mg		
		Diclofenac 50mg		
		Gabapentin 300mg		
		Metformin 500mg		
		Aspirin 100mg		
903-070816-AA-59	Atorvastatin 40mg	Aspirin 100mg	7	9
	Bisoprolol 5mg	Ranitidine 150mg		
		Diclofenac 50mg		
		Folic acid 1mg		
		Lorazepam 2mg		
903-070816-AA-60	Diltiazem 90mg	Rosuvastatin 20mg	8	11
		Candesartan 20mg		
		Aspirin 100mg		
		Metformin 500mg		
		Aspirin 100mg		
		Ranitidine 150mg		
		Lorazepam 2mg		
903-070816-AA-61	Diltiazem 60mg	Aspirin 100mg	8	10
	Losartan 50mg	Ranitidine 150mg		
	Valsartan 160mg	Lorazepam 2mg		
		Folic acid 1mg		
		Diclofenac 50mg		
903-070816-AA-62	Atorvastatin 40mg	Candesartan 16mg	5	6
		Metoprolol 100mg		
		Furosemide 40mg		
		Aspirin 100mg		
903-070816-AA-64	Atorvastatin 40mg	Aspirin 100mg	8	12
		Isosorbide 10mg		
		Enalapril 10mg		
		Ranitidine 150mg		
		Lorazepam 2mg		
		Diclofenac 50mg		
		Metformin 500mg		
903-070816-AA-65	Diltiazem 60mg	Rosuvastatin 20mg	2	2
903-070816-AA-66	Lisinopril 10mg	-	1	1
903-080816-AA-67	Atenolol 100mg	-	1	1
903-080816-AA-68	Valsartan 80mg	Metoprolol 50mg	4	5
		Fluvastatin 20mg		
		Aspirin 100mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-080816-AA-69	Losartan 50mg	Metoprolol 50mg	2	3
903-080816-AA-70	Atorvastatin 40mg	-	1	1
903-080816-AA-71	Control	-	-	-
903-090816-AA-72	Atorvastatin 40mg	-	3	3
	Diltiazem 60mg			
	Valsartan 160mg			
903-090816-AA-73	Valsartan 80mg	Metoprolol 50mg	3	4
		Gemfibrozil 600mg		
903-090816-AA-74	Valsartan 80mg	Aspirin 100mg	7	12
		Clopidogrel 75mg		
		Famotidine 20mg		
		Diclofenac 50mg		
		Gabapentin 300mg		
		Metformin 500mg		
903-090816-AA-75	Atorvastatin 40mg	Chlortalidone 25mg	3	4
		Metoprolol 50mg		
903-090816-AA-76	Diltiazem 90mg	-	1	1
903-090816-AA-77	Lisinopril 10mg	Aspirin 100mg	3	4
		Clopidogrel 75mg		
903-090816-AA-78	Valsartan 160mg	Trimetazidine 35mg	2	2
903-090816-AA-79	Control	-	-	-
903-100816-AA-80	Valsartan 80mg	Rosuvastatin 20mg	7	9
		Aspirin 100mg		
		Ranitidine 150mg		
		Diclofenac 50mg		
		Folic acid 1mg		
		Lorazepam 2mg		
903-100816-AA-81	Control	-	-	-
903-100816-AA-82	Atorvastatin 40mg	Metoprolol 50mg	4	5
	Valsartan	Aspirin 100mg		
903-100816-AA-83	Control			
903-100816-AA-84	Valsartan 160mg	Aspirin 100mg	4	6
		Ranitidine 150mg		
		Clopidogrel 75mg		
903-100816-AA-85	Control	-	-	-
903-100816-AA-86	Diltiazem 60mg	Hydrochlorothiazide 50mg	2	2
903-100816-AA-87	Bisoprolol 5mg	-	1	1
903-100816-AA-88	Aspirin 100mg	Atorvastatin 40mg	3	4
		Metoprolol 50mg		
903-100816-AA-89	Valsartan 160mg	Metoprolol 50mg	4	6
		Aspirin 100mg		
		Ranitidine 150mg		
903-110816-AA-90	Bisoprolol 5mg	Candesartan 8mg	2	2
903-120816-AA-91	Bisoprolol 5mg		2	2
	Lisinopril 10mg			
903-120816-AA-92	Control	-	-	-
903-120816-AA-93	Lisinopril 10mg	Metoprolol 50mg	2	3

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-120816-AA-94	Control	-	-	-
903-150816-AA-95	Bisoprolol 5mg	Verapamil 40mg	3	3
		Aspirin 100mg		
903-150816-AA-96	Bisoprolol 5mg	Aspirin 100mg	2	2
903-150816-AA-97	Control	-	-	-
903-150816-AA-98	Control	-	-	-
903-150816-AA-99	Bisoprolol 5mg	-	2	2
	Valsartan 160mg			
903-200717-AA-100	Atenolol 50mg	-	2	2
	Simvastatin 40mg			
903-200717-AA-101	Atenolol 50mg	-	2	2
	Simvastatin 40mg			
903-200717-AA-102	Atenolol 100mg	-	2	2
	Simvastatin 40mg			
903-200717-AA-103	Atenolol 50mg	Aspirin 100mg	3	3
	Simvastatin 40mg			
903-200717-AA-104	Atenolol 50mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-105	Atenolol 50mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-106	Atenolol 100mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-107	Atenolol 100mg	Amiodarone 200mg	3	3
	Lisinopril 10mg			
903-200717-AA-108	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-109	Bisoprolol 5mg	Aspirin 100mg	3	3
	Lisinopril 10mg			
903-200717-AA-110	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-111	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-112	Bisoprolol 5mg	Famotidine 20mg	3	4
	Valsartan 80mg			
903-200717-AA-113	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-200717-AA-114	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-200717-AA-115	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-200717-AA-116	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-200717-AA-117	Diltiazem 60mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-118	Diltiazem 90mg	-	2	2
	Lisinopril 10mg			
903-200717-AA-119	Diltiazem 90mg	Lorazepam 2mg	4	5
	Lisinopril 10mg	Ranitidine 150mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-200717-AA-120	Diltiazem 90mg	Aspirin 100mg	4	4
	Lisinopril 10mg	Warfarin 2mg		
903-210717-AA-121	Losartan 50mg	Aspirin 100mg	4	4
	Simvastatin 40mg	Furosemide 40mg		
903-210717-AA-122	Losartan 50mg	Aspirin 100mg	4	5
	Simvastatin 40mg	Ranitidine 150mg		
903-210717-AA-123	Losartan 50mg	-	2	2
	Simvastatin 40mg			
903-210717-AA-124	Losartan 50mg	-	2	2
	Simvastatin 40mg			
903-210717-AA-125	Amlodipine 5mg	Clopidogrel 75mg	2	3
903-210717-AA-126	Amlodipine 5mg	Aspirin 100mg	2	2
903-210717-AA-127	Amlodipine 5mg	Aspirin 100mg	2	2
903-210717-AA-128	Amlodipine 5mg	Aspirin 100mg	2	2
903-210717-AA-129	Losartan 50mg	Aspirin 100mg	3	3
		Furosemide 40mg		
903-210717-AA-130	Losartan 50g	Clopidogrel 75mg	2	3
903-220717-AA-131	Losartan 50mg	Aspirin 100mg	2	2
903-220717-AA-132	Simvastatin 40mg	Clopidogrel 75mg	2	3
903-220717-AA-133	Simvastatin 40mg	Aspirin 100mg	4	5
		Furosemide 40mg		
		Clopidogrel 75mg		
903-220717-AA-134	Valsartan 80mg	Aspirin 100mg	2	2
903-220717-AA-135	Valsartan 80mg	Aspirin 100mg	2	2
903-220717-AA-136	Valsartan 80mg	Clopidogrel 75mg	2	3
903-220717-AA-137	Valsartan 80mg	-	1	1
903-220717-AA-138	Atenolol 50mg	Aspirin 100mg	4	4
	Simvastatin 40mg	Gemfibrozil 600mg		
903-220717-AA-139	Atenolol 100mg	-	2	2
	Simvastatin 40mg			
903-220717-AA-140	Atenolol 50mg	-	2	2
	Simvastatin 40mg			
903-230717-AA-141	Atenolol 100mg	-	2	2
	Simvastatin 40mg			
903-230717-AA-142	Atenolol 50mg		2	2
	Lisinopril 10mg			
903-230717-AA-143	Atenolol 50mg		2	2
	Lisinopril 10mg			
903-230717-AA-144	Atenolol 50mg	Aspirin 100mg	3	3
	Lisinopril 10mg			
903-230717-AA-145	Bisoprolol 5mg	Aspirin 100mg	4	5
	Lisinopril 10mg	Ranitidine 150mg		
903-230717-AA-146	Bisoprolol 15mg	-	2	2
	Lisinopril 10mg			
903-230717-AA-147	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-230717-AA-148	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-230717-AA-149	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-230717-AA-150	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-240717-AA-152	Diltiazem 60mg	-	2	2
	Lisinopril 10g			
903-240717-AA-153	Diltiazem 60mg	Isosorbide 10mg	5	7
	Lisinopril 10mg	Paracetamol 500mg		
		Chlordiazepoxide 5mg		
903-240717-AA-154	Diltiazem 60mg	Amiodaron 200mg	3	3
	Lisinopril 10mg			
903-240717-AA-155	Diltiazem 90mg	-	2	2
	Lisinopril 10mg			
903-240717-AA-156	Losartan 100mg	-	2	2
	Simvastatin 40mg			
903-240717-AA-157	Losartan 50mg	-	2	2
	Simvastatin 40mg			
903-240717-AA-158	Losartan 50mg	-	2	2
	Simvastatin 40mg			
903-240717-AA-159	Losartan 100mg	-	2	2
	Simvastatinn 40mg			
903-240717-AA-160	Amlodipine 5mg	Clopidogrel 75mg	3	4
		Aspirin 100mg		
903-250717-AA-161	Amlodipine 5mg	-	2	2
	Aspirin 100mg			
903-250717-AA-162	Amlodipine 5mg	-	2	2
	Aspirin 100mg			
903-250717-AA-163	Amlodipine 5mg	Aspirin 100mg	5	7
		Isosorbide 10mg		
		Paracetamol 500mg		
		Chlordiazepoxide 5mg		
903-250717-AA-164	Losartan 50mg	Aspirin 100mg	7	11
		Clopidogrel 75mg		
		Famotidine 20mg		
		Lorazepam 2mg		
		Paracetamol 500mg		
		Chlordiazepoxide 5mg		
903-250717-AA-165	Losartan 50mg	Aspirin 100mg	2	2
903-250717-AA-166	Losartan 50mg	Aspirin 100mg	2	2
903-250717-AA-167	Simvastatin 40mg	Isosorbide 10mg	7	11
		Aspirin 100mg		
		Metformin 500mg		
		Diclofenac 50mg		
		Lorazepam 2mg		
		Naproxen 500mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-250717-AA-168	Simvastatin 40mg	Aspirin 100mg	2	2
903-250717-AA-169	Atenolol 50mg	-	2	2
	Simvastatin 40mg			
903-250717-AA-170	Atenolol 100mg		2	2
	Simvastatin 40mg			
903-270717-AA-171	Atenolol 50mg		2	2
	Simvastatin 40mg			
903-270717-AA-172	Atenolol 100mg	Aspirin 100mg	4	4
	Simvastatin 40mg	Isosorbide 10mg		
903-270717-AA-173	Atenolol 50mg	Famotidine 20mg	4	5
	Lisinopril 10mg	Aspirin 100mg		
903-270717-AA-174	Atenolol 50mg	Aspirin 100mg	4	4
	Lisinopril 10mg	Clopidogrel 75mg		
903-270717-AA-175	Atenolol 100mg		2	2
	Lisinopril 10mg			
903-270717-AA-176	Atenolol 100mg	Carbamazepine 200mg	6	8
	Lisinopril 10mg	Warfarin 2mg		
		Ranitidine 150mg		
		Naproxen 500mg		
903-270717-AA-177	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-270717-AA-178	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-270717-AA-180	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-280717-AA-181	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-280717-AA-182	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-280717-AA-183	Bisoprolol 5mg	Aspirin 100mg	6	6
	Valsartan 80mg	Gemfibrozil 300mg		
		Diazepam 5mg		
		Amiodarone 200mg		
903-280717-AA-184	Bisoprolol 5mg	Aspirin 100mg	3	3
	Valsartan 80mg			
903-280717-AA-185	Bisoprolol 5mg	Aspirin 100mg	4	4
	Valsartan 80mg	Lansoprazole 30mg		
903-280717-AA-186	Diltiazem 60mg	Clopidogrel 75mg	5	7
	Lisinopril 10mg	Ranitidine 150mg		
		Mefenamic acid 250mg		
903-280717-AA-187	Diltiazem 90mg	-	2	2
	Lisinopril 10mg			
903-280717-AA-188	Diltiazem 90mg	Ibrufen 200mg	5	6
	Lisinopril 10mg	Carbamazepine 200mg		
		Lorazepam 2mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-280717-AA-189	Diltiazem 90mg	-	2	2
	Lisinopril 10mg			
903-280717-AA-190	Losartan 50mg	Aspirin 100mg	5	5
	Simvastatin 40mg	Furosemide 40mg		
		Lansoprazole 30mg		
903-290717-AA-191	Losartan 100mg		2	2
	Simvastatin 40mg			
903-290717-AA-192	Losartan 100mg	Aspirin 100mg	6	8
	Simvastatin 40mg	Warfarin 2mg		
		Ranitidine 150mg		
		Naproxen 500mg		
903-290717-AA-193	Losartan 100mg	Clopidogrel 75mg	5	6
	Simvastatin 40mg	Warfarin 2mg		
		Amiloride 5mg		
903-290717-AA-194	Losartan 100mg		2	2
	Simvastatin 40mg			
903-290717-AA-195	Amlodipine 5mg		2	2
	Aspirin 100mg			
903-290717-AA-196	Amlodipine 5mg		2	2
	Aspirin 100mg			
903-290717-AA-197	Amlodipine 5mg		2	3
	Clopidogrel 75mg			
903-290717-AA-198	Amlodipine 5mg	Aspirin 100mg	8	10
		Metformin 500mg		
		Warfarin 2mg		
		Ranitidine 150mg		
		Naproxen 500mg		
903-290717-AA-199	Losartan 50mg	-	2	3
	Clopidogrel 75mg			
903-290717-AA-200	Losartan 50mg	-	2	2
	Aspirin 100mg			
903-300717-AA-201	Losartan 50mg	Warfarin 2mg	5	7
		Ranitidine 150mg		
		Naproxen 500mg		
		Aspirin 100mg		
903-300717-AA-202	Simvastatin 40mg	Aspirin 100mg	5	8
		Ranitidine 150mg		
		Naproxen 500mg		
		Clopidogrel 75mg		
903-300717-AA-203	Control	-	-	-
903-300717-AA-204	Valsartan 80mg	Clopidogrel 75mg	7	10
		Diclofenac 50mg		
		Ranitidine 150mg		
		Mefenamic acid 250mg		
		Aspirin 100mg		
		Amiloride 5mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-300717-AA-205	Valsartan 80mg		2	2
	Aspirin 100mg			
903-300717-AA-206	Atenolol 50mg	Aspirin 100mg	4	5
	Simvastatin 40mg	Clopidogrel 75mg		
903-300717-AA-207	Atenolol 100mg	Aspirin 100mg	5	8
	Simvastatin 40mg	Metformin 500mg		
		Diclofenac 50mg		
903-300717-AA-208	Atenolol 50mg	Aspirin 100mg	7	8
	Simvastatin 40mg	Furosemide 40mg		
		Naproxen 500mg		
		Omeprazole 40mg		
		Lorazepam 2mg		
903-300717-AA-209	Atenolol 100mg	Aspirin 100mg	5	6
	Lisinopril 10mg	Ranitidine 150mg		
		Naproxen 500mg		
903-300717-AA-210	Atenolol 1oomg		2	2
	Lisinopril 10mg			
903-300717-AA-211	Atenolol 100mg	Clopidogrel 75mg	6	9
	Lisinopril 10mg	Alprazolam 0.5mg		
		Ibrufen 200mg		
		Isosorbide 10mg		
903-300717-AA-212	Bisoprolol 5mg	Famotidine 20mg	6	10
	Lisinopril 10mg	Metformin 500mg		
		Clopidogrel 75mg		
		Lansoprazole 30mg		
903-300717-AA-213	Diltiazem 90mg	-	2	2
	Lisinopril 10mg			
903-300717-AA-214	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-300717-AA-215	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-300717-AA-216	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-300717-AA-217	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-300717-AA-218	Diltiazem 60mg	Aspirin 100mg	6	8
	Lisinopril 10mg	Omeprazole 20mg		
		Lorazepam 2mg		
		Metformin 500mg		
903-300717-AA-219	Diltiazem 90mg	-	2	2
	Lisinopril 10mg			

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-300717-AA-220	Diltiazem 90mg	Aspirin 100mg	7	8
	Lisinopril 10mg	Ranitidine 150mg		
		Gabapentin 300mg		
		Folic acid 1mg		
		Chlordiazepoxide 5mg		
903-310717-AA-221	Losartan 100mg	Aspirin 100mg	4	5
	Simvastatin 40mg	Diclofenac 50mg		
903-310717-AA-222	Amlodipine 5mg	-	2	2
	Aspirin 100mg			
903-310717-AA-223	Losartan 50mg	-	2	2
	Aspirin 100mg			
903-310717-AA-224	Valsartan 80mg	-	2	2
	Aspirin 100mg			
903-310717-AA-225	Valsartan 80mg	Aspirin 100mg	3	4
		Ranitidine 150mg		
903-310717-AA-226	Atenolol 100mg		2	2
	Simvastatin 40mg			
903-310717-AA-227	Atenolol 50mg	Clopidogrel 75mg	5	7
	Simvastatin 40mg	Ibrufen 200mg		
		Lansoprazole 30mg		
903-310717-AA-228	Atenolol 100mg	-	2	2
	Lisinopril 10mg			
903-310717-AA-229	Atenolol 100mg	Omeprazole 20mg	4	6
	Lisinopril 10mg	Naproxen 500mg		
903-310717-AA-230	Bisoprolol 5mg	Carbamazepine 200mg	5	6
	Lisinopril 10mg	Diazepam 2mg		
		Naproxen 200mg		
903-010817-AA-231	Bisoprolol 5mg	Aspirin 100mg	5	5
	Lisinopril 10mg	Amiodarone 200mg		
		Isosorbide 10mg		
903-010817-AA-232	Bisoprolol 5mg	-	2	2
	Lisinopril 10mg			
903-010817-AA-233	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-010817-AA-234	Bisoprolol 5mg	-	2	2
	Valsartan 80mg			
903-010817-AA-235	Bisoprolol 5mg	Aspirin 100mg	5	6
	Valsartan 80mg	Furosemide 40mg		
		Diclofenac 50mg		
903-010817-AA-236	Diltiazem 60mg	Aspirin 100mg	5	6
	Lisinopril 10mg	Clopidogrel 75mg		
		Lorazepam 2mg		
903-010817-AA-237	Diltiazem 90mg		2	2
	Lisinopril 10mg			

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-010817-AA-238	Diltiazem 90mg	Aspirin 100mg	3	3
	Lisinopril 10mg			
903-010817-AA-239	Losartan 100mg	Aspirin 100mg	4	4
	Simvastatin 40mg	Chlordiazepoxide 5mg		
903-010817-AA-240	Amlodipine 5mg	Aspirin 100mg	5	7
		Clopidogrel 75mg		
		Furosemide 40mg		
		Diclofenac 50mg		
903-020817-AA-241	Valsartan 80mg	Aspirin 100mg	7	8
		Furosemide 40mg		
		Chlordiazepoxide 5mg		
		Omeprazole 40mg		
		Alprazolam 0.5mg		
		Meloxicam 7.5mg		
903-020817-AA-242	Valsartan 80mg		2	2
	Aspirin 100mg			
903-020817-AA-243	Valsartan 80mg		2	2
	Aspirin 100mg			
903-020817-AA-244	Atenolol 100mg	Amiloride 5mg	4	4
	Lisinopril 10mg	Omeprazole 40mg		
903-020817-AA-245	Atenolol 100mg	Nifedipine 20mg	3	4
	Lisinopril 10mg			
903-020817-AA-246	Bisoprolol 5mg		2	2
	Lisinopril 10mg			
903-020817-AA-247	Diltiazem 90mg	Omeprazole 20mg	4	5
	Lisinopril 10mg	Mefenamic acid 250mg		
903-020817-AA-248	Diltiazem 90mg	Clopidogrel 75mg	3	4
	Lisinopril 10mg			
903-020817-AA-249	Losartan 100mg	Aspirin 100mg	5	5
	Simvastatin 40mg	Warfarin 2mg		
		Furosemide 40mg		
903-020817-AA-250	Losartan 100mg	Aspirin 100mg	7	10
	Simvastatin 40mg	Warfarin 2mg		
		Furosemide 40mg		
		Ranitidine 150mg		
		Paracetamol 500mg		
903-030817-AA-251	Losartan 100mg	Aspirin 100mg	3	3
	Simvastatin 40mg			
903-030817-AA-252	Amlodipine 5mg	Clopidogrel 75mg	5	8
		Lorazepam 2mg		
		Ranitidine 150mg		
		Diclofenac 50mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-030817-AA-253	Valsartan 80mg	Clopidogrel 75mg	5	7
		Furosemide 40mg		
		Amiodarone 200mg		
		Famotidine 20mg		
903-030817-AA-254	Valsartan 80mg	Clopidogrel 75mg	2	3
903-030817-AA-255	Valsartan 80mg	Furosemide 40mg	3	3
		Aspirin 100mg		
903-030817-AA-256	Atenolol 100mg	Amiodarone 200mg	4	5
	Lisinopril 10mg	Famotidine 20mg		
903-030817-AA-257	Atenolol 100mg	Aspirin 100mg	4	5
	Lisinopril 10mg	Famotidine 20mg		
903-030817-AA-258	Atenolol 100mg		2	2
	Lisinopril 10mg			
903-030817-AA-259	Bisoprolol 5mg		2	2
	Lisinopril 10mg			
903-030817-AA-260	Bisoprolol 5mg		2	2
	Lisinopril 10mg			
903-040817-AA-261	Bisoprolol 5mg	Aspirin 100mg	3	3
	Valsartan 80mg			
903-040817-AA-262	Bisoprolol 5mg	Warfarin 2mg	3	3
	Valsartan 80mg			
903-040817-AA-263	Valsartan 80mg	Aspirin 100mg	5	7
		Clopidogrel 75mg		
		Famotidine 20mg		
		Diazepam 2mg		
903-040817-AA-264	Atenolol 100mg	Aspirin 100mg	7	11
		Ranitidine 150mg		
		Diclofenac 50mg		
		Metformin 500mg		
		Furosemide 40mg		
		Lansoprazole 30mg		
903-040817-AA-265	Losartan 100mg	Aspirin 100mg	6	7
		Isosorbide 10mg		
		Paracetamol 500mg		
		Furosemide 40mg		
		Lansoprazole 30mg		
903-040817-AA-266	Valsartan 80mg	Aspirin 100mg	8	13
		Metformin 500mg		
		Lorazepam 2mg		
		Diclofenac 50mg		
		Folic acid 1mg		
		Isosorbide 10mg		
		Paracetamol 500mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-040817-AA-267	Diltiazem 90mg	Aspirin 100mg	8	11
		Metformin 800mg		
		Paracetamol 500mg		
		Lorazepam 2mg		
		Folic acid 1mg		
		Clopidogrel 75mg		
		Lorazepam 2mg		
903-040817-AA-268	Bisoprolol 5mg	Clopidogrel 75mg	7	11
		Lorazepam 2mg		
		Omeprazole 40mg		
		Aspirin 100mg		
		Famotidine 20mg		
		Ibrufen 200mg		
903-040817-AA-269	Diltiazem 90mg	Aspirin 100mg	6	8
		Famotidine 20mg		
		Ibrufen 200mg		
		Lorazepam 2mg		
		Amiodarone 200mg		
903-040817-AA-270	Atenolol 100mg	Aspirin 100mg	8	8
		Hydrochlorothiazide 50mg		
		Lorazepam 2mg		
		Mefenamic acid 500mg		
		Metoclopramide 5mg		
		Lorazepam 2mg		
		Amiodarone 200mg		
903-050817-AA-271	Losartan 50mg	Aspirin 100mg	6	6
		Famotidine 40mg		
		Carbamazepine 200mg		
		Lorazepam 2mg		
		B-complex		
903-050817-AA-272	Bisoprolol 5mg	Paracetamol 500mg	6	9
		Furosemide 40mg		
		Ibrufen 200mg		
		Lansoprazole 30mg		
		Diazepam 2mg		
903-050817-AA-273	Valsartan 80mg	Aspirin 100mg	6	7
		Ibrufen 200mg		
		Isosorbide 10mg		
		Amiodarone 200mg		
		Folic acid 1mg		
903-050817-AA-274	Atenolol 50mg	Furosemide 40mg	7	8
		Isosorbide 10mg		
		Lansoprazole 30mg		
		Aspirin 100mg		
		Ibrufen 200mg		
		Folic acid 1mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-050817-AA-275	Losartan 50mg	Aspirin 100mg	6	7
		Furosemide 40mg		
		Isosorbide 10mg		
		Ibrufen 200mg		
		Folic acid 1mg		
903-050817-AA-276	Valsartan 80mg	Clopidogrel 75mg	6	10
		Aspirin 100mg		
		Ibrufen 200mg		
		Gabapentin 300mg		
		Famotidine 20mg		
903-050817-AA-277	Diltiazem 90mg	Aspirin 100mg	8	10
		Clopidogrel 75mg		
		Amiodarone 200mg		
		Lansoprazole 30mg		
		Metoclopramide 5mg		
		Ibrufen 200mg		
		Gabapentin 300mg		
903-050817-AA-278	Bisoprolol 5mg	Clopidogrel 75mg	5	7
		Ranitidine 150mg		
		Carbamazepine 200mg		
		Furosemide 40mg		
903-050817-AA-279	Losartan 50mg	Aspirin 100mg	8	12
		Omeprazole 20mg		
		Gabapentin 300mg		
		Indomethacin 20mg		
		Clopidogrel 75mg		
		Ranitidine 150mg		
		Lorazepam 2mg		
903-050817-AA-280	Valsartan 80mg	Aspirin 100mg	6	7
		Gemfibrozil 600mg		
		Furosemide 40mg		
		Ibrufen 200mg		
		Lorazepam 2mg		
903-100817-AA-281	Bisoprolol 5mg	Aspirin 100mg	5	6
		Clopidogrel 75mg		
		Lorazepam 2mg		
		Furosemide 40mg		
903-100817-AA-282	Diltiazem 60mg	Aspirin 100mg	7	9
		Amiodarone 200mg		
		Metformin 500mg		
		Meloxicam 7.5mg		
		Lorazepam 2mg		
		Furosemide 4mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-100817-AA-283	Atenolol 100mg	Gabapentin 300mg	7	10
		Naproxen 500mg		
		Omeprazole 40mg		
		Metformin 500mg		
		Domperidone 10mg		
		Ibrufen 200mg		
903-100817-AA-284	Bisoprolol 5mg	Aspirin 100mg	4	4
		Gabapentin 300mg		
		Chlordiazepoxide 5mg		
903-100817-AA-285	Diltiazem 90mg	Lorazepam 2mg	8	10
		Omeprazole 40mg		
		Domperidone 10mg		
		Ibrufen 200mg		
		Metformin 500mg		
		Aspirin 100mg		
		Omeprazole 40mg		
903-100817-AA-286	Valsartan 80mg	Aspirin 100mg	6	10
		Clopidogrel 75mg		
		Metformin 500mg		
		Diclofenac 50mg		
		Lorazepam 5mg		
903-100817-AA-287	Atenolol 50mg	Aspirin 100mg	5	7
		Metformin 500mg		
		Furosemide 40mg		
		Gabapentin 300mg		
903-100817-AA-288	Losartan 50mg	Aspirin 100mg	5	5
		Isosorbide 10mg		
		Lorazepam 5mg		
		Furosemide 40mg		
903-100817-AA-289	Diltiazem 60mg	Aspirin 100mg	5	5
		Mefenamic acid 500mg		
		Carbamazepine 200mg		
		Omeprazole 40mg		
903-100817-AA-290	Bisoprolol 5mg	Aspirin 100mg	6	9
		Metformin 500mg		
		Diclofenac 50mg		
		Lorazepam 5mg		
		B-complex		
903-210318-AA-291	Atorvastatin 40mg	Aspirin 100mg	3	4
		Clopidogrel 75mg		
903-210318-AA-292	Valsartan 80mg	Aspirin 100mg	2	2

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-210318-AA-293	Bisoprolol 5mg	Aspirin 100mg	3	3
		Lorazepam 2mg		
903-210318-AA-294	Lisinopril 10mg	Clopidogrel 75mg	7	11
		Aspirin 100mg		
		Famotidine 20mg		
		Lorazepam 2mg		
		Metformin 500mg		
		Chlordiazepoxide 5mg		
903-210318-AA-295	Losartan 25mg	Lorazepam 2mg	6	8
		Clopidogrel 75mg		
		Aspirin 100mg		
		Famotidine 20mg		
		Gemfibrozil 600mg		
903-210318-AA-296	Atenolol 50mg	Aspirin 100mg	8	11
		Clopidogrel 75mg		
		Lorazepam 2mg		
		Omeprazole 20mg		
		Folic acid 1mg		
		Ibrufen 200mg		
		Chlordiazepoxide 5mg		
903-210318-AA-297	Bisoprolol 5mg	Aspirin 100mg	6	10
		Metformin 500mg		
		Isosorbide 10mg		
		Diclofenac 50mg		
		Famotidine 20mg		
903-210318-AA-298	Bisoprolol 5mg	Aspirin 100mg	8	13
		Clopidogrel 75mg		
		Lorazepam 2mg		
		Famotidine 20mg		
		Chlordiazepoxide 5mg		
		Metformin 500mg		
		Diclofenac 25mg		
903-210318-AA-299	Losartan 50mg	Aspirin 100mg	6	12
		Ibrufen 200mg		
		Famotidine 20mg		
		Lorazepam 2mg		
		Metformin 500mg		
903-240318-AA-300	Bisoprolol 5mg	Aspirin 100mg	7	12
		Clopidogrel 75mg		
		Famotidine 20mg		
		Diclofenac 50ng		
		Gabapentin 300mg		
		Metformin 500mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-240318-AA-301	Atorvastatin 40mg	Aspirin 100mg	2	2
903-240318-AA-302	Valsartan 80mg	Aspirin 100mg	3	4
		Famotidine 20mg		
903-240318-AA-303	Bisoprolol 5mg	Naproxen 500mg	3	5
		Famotidine		
903-240318-AA-304	Bisoprolol 5mg	Aspirin 100mg	6	8
		Ranitidine 150mg		
		Diclofenac 50mg		
		Folic acid 1mg		
		Lorazepam 2mg		
903-240318-AA-305	Losartan 50mg	Aspirin 100mg	6	8
		Ranitidine 150mg		
		Diclofenac 50mg		
		Folic acid 1mg		
		Lorazepam 2mg		
903-250318-AA-306	Bisoprolol 5mg	Aspirin 100mg	6	8
		Clopidogrel 75mg		
		Famotidine 20mg		
		Lorazepam 2mg		
		Folic acid 1mg		
903-250318-AA-307	Atenolol 50mg	Clopidogrel 75mg	6	9
		Famotidine 20mg		
		Lorazepam 2mg		
		Ibrufen 200mg		
		Folic acid 1mg		
903-250318-AA-308	Losartan 25mg	Aspirin	8	12
		Clopidogrel		
		Famotidine		
		Lorazepam		
		Ranitidine		
		Indomethacin		
		Multivitamin		
903-250318-AA-309	Valsartan	Aspirin 100mg	8	12
		Clopidogrel 75mg		
		Metformin 500mg		
		Chlordiazepoxide 5mg		
		Multivitamin		
		Ranitidine 150mg		
		Diclofenac 50mg		
903-250318-AA-310	Atenolol 50mg	Clopidogrel 75mg	7	9
		Lorazepam 2mg		
		Ranitidine 150mg		
		Indomethacin 20mg		
		Multivitamin		
		Aspirin 100mg		
903-260318-AA-311	Atorvastatin 40mg	Aspirin 100mg	2	2

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-260318-AA-312	Valsartan 80mg	Aspirin 100mg	3	3
		Furosemide 40mg		
903-260318-AA-313	Lisinopril 20mg	Clopidogrel 75mg	6	8
		Aspirin 100mg		
		Ranitidine 150mg		
		Lorazepam 2mg		
		Furosemide 40mg		
903-260318-AA-314	Simvastatin 40mg	Aspirin 100mg	7	9
		Naproxen 500mg		
		Ranitidine 150mg		
		Lorazepam 2mg		
		Furosemide 40mg		
		Folic acid 1mg		
903-260318-AA-315	Bisoprolol 5mg	Clopidogrel 75mg	7	10
		Aspirin 100mg		
		Naproxen 500mg		
		Ranitidine 150mg		
		Chlordiazepoxide 5mg		
		Gemfibrozil 600mg		
903-260318-AA-316	Bisoprolol 5mg	Clopidogrel 75mg	8	10
		Warfarin 2mg		
		Ibrufen 200mg		
		Carbamazepine 200mg		
		Lorazepam 2mg		
		Furosemide 40mg		
		Gemfibrozil 600mg		
903-260318-AA-317	Losartan 50mg	Aspirin 100mg	5	5
		Warfarin 2mg		
		Carbamazepine 5mg		
		Bplex		
903-260318-AA-318	Valsartan 80mg	Aspirin 100mg	8	11
		Clopidogrel 75mg		
		Metformin 500mg		
		Carbamazepine 200mg		
		Bplex		
		Lorazepam 2mg		
		Furosemide 40mg		
903-260318-AA-319	Bisoprolol 5mg	Clopidogrel 75mg	7	12
		Aspirin 100mg		
		Ibrufen 200mg		
		Carbamazepine 200mg		
		Metformin 500mg		
		Omeprazole 20mg		
903-260318-AA-320	Atorvastatin 40mg	Clopidogrel 75mg	2	3

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-260318-AA-321	Valsartan 80mg	Aspirin 100mg	3	4
		Clopidogrel 75mg		
903-260318-AA-322	Bisoprolol 5mg	Aspirin 100mg	6	9
		Lorazepam 2mg		
		Diclofenac 50mg		
		Ranitidine 150mg		
		Clopidogrel 75mg		
903-260318-AA-323	Simvastatin 20mg	Clopidogrel 75mg	6	9
		Diclofenac 25mg		
		Lorazepam 2mg		
		Aspirin 100mg		
		Ranitidine 150mg		
903-260318-AA-324	Simvastatin 20mg	Warfarin 2mg	7	8
		Aspirin 100mg		
		Gemfibrozil 600mg		
		Famotidine 20mg		
		Lorazepam 2mg		
		Carbamazepine 200mg		
903-260318-AA-325	Simvastatin 40mg	Aspirin 100mg	8	12
		Lansoprazole 30mg		
		Mefenamic acid 500mg		
		Chlordiazepoxide 5mg		
		Clopidogrel 75mg		
		Furosemide 40mg		
		Ibrufen 200mg		
903-260318-AA-326	Simvastatin 20mg	Aspirin 100mg	7	12
		Diazepam 2mg		
		Famotidine 20mg		
		Furosemide 40mg		
		Metformin 500mg		
		Alprazolam 0.5mg		
903-260318-AA-327	Bisoprolol 5mg	Aspirin 100mg	8	12
		Clopidogrel 75mg		
		Furosemide 40mg		
		Metformin 500mg		
		Alprazolam 0.5mg		
		Famotidine 20mg		
		Folic acid 1mg		

Patient Ref.	Target CVD Medication	Other medications	Total number of medications	No. of tablet taken per a day
903-260318-AA-328	Simvastatin 20mg	Aspirin 100mg	7	10
		Clopidogrel 75mg		
		Furosemide 40mg		
		Gemfibrozil 600mg		
		Ibrufen 200mg		
		Famotidine		
903-260318-AA-329	Bisoprolol 5mg	Aspirin 100mg	2	2
903-260318-AA-330	Atorvastatin 40mg	Aspirin 100mg	3	3
		Omeprazole 20mg		
903-260318-AA-331	Atorvastatin 40mg	Aspirin 100mg	2	2
903-260318-AA-332	Lisinopril 20mg	Aspirin 100mg	8	11
		Clopidogrel 75mg		
		Furosemide 40mg		
		Gabapentin 300mg		
		Isosorbide 10mg		
		Paracetamol 500mg		
		Chlordiazepoxide 5mg		
903-270318-AA-333	Bisoprolol 5mg	Carbamazepine 200mg	7	9
		Amiodaron 200mg		
		Aspirin 100mg		
		Lorazepam 2mg		
		Isosorbide 10mg		
		Paracetamol 500mg		
903-270318-AA-334	Simvastatin 40mg	Amiodaron 200mg	6	7
		Aspirin 100mg		
		Lorazepam 2mg		
		Isosorbide 10mg		
		Omeprazole 20mg		
903-270318-AA-335	Bisoprolol 5mg	Amiodaron 200mg	8	11
		Isosorbide 10mg		
		Paracetamol 500mg		
		Chlordiazepoxide 5mg		
		Aspirin 100mg		
		Lorazepam 2mg		
		Omeprazole 20mg		
903-270318-AA-336	Simvastatin 40mg	Aspirin 100mg	6	6
		Metformin 500mg		
		Clopidogrel 75mg		
		Lorazepam 2mg		
		Diclofenac 50mg		

Appendix 10. Collection Protocol for DBS and VAMS Samples.

1. Provide volunteer participant information leaflet, consent form and adherence questionnaire before sample collection.

2. The researcher should prepare the lab for sample collection by covering lab bench with tissue roll paper.

3. Wear safety spectacles, a laboratory coat, and disposable gloves.

4. Welcome volunteer and introduce yourself and be ready to answer volunteer's questions.

5. Ask the patient if he/she has read the patient information leaflet. After reading this Participant Information Leaflet, volunteer will be asked to sign two copies of the consent form and to complete a short questionnaire prior to giving a blood spot sample. One copy of the signed consent form is given to volunteer before leaving the lab.

6. Put the volunteer reference number on two copies of the consent form, the 'mini-questionnaire' and the DBS sample collecting card and resealable bag using the following format: 903-date-initials of the person taking sample-sample number starting 01 (**903-ddmmyy-XX-01**) where XX is the initials of the person taking the sample.

7. Ask volunteer to warm their finger by gentle rubbing to increase the blood flow.

8. Clean the site with an alcohol pad and allow the site to dry.

9. The sampling should be carried out in a clean tray.

10. Lance the sample site and wipe away the first blood drop with sterile gauze.

11. If the volunteer is not bleeding well, ask them to gently apply intermittent pressure near the puncture site to increase the blood flow.

12. Allow blood to accumulate on the finger or thumb tip and drop onto the sampling card in the circled area. The blood drop(s) should fall freely to the sampling card.

13. Apply the accumulated blood on the finger or the thumb tip to the circled area on the sampling card.

14. Avoid touching the sampling site on the card and do not spread/smear/smudge blood to cover the circled area. The sample in such cases is considered as invalid.

15. Allow multiple drops to fall on the same circled area until the area is fully covered and soaked.

16. After covering the circled area with a blood sample, start filling the next area. At least two circles must be filled for each volunteer. Overspotting and layering sample is invalid, and the sample should be excluded.

17. After collecting sample, put the sampling card on the drying rack and leave to dry at room temperature for at least 2-3 hours. This should be done immediately to prevent contamination. Sample cards should be kept apart from each other and away from heat.

18. If the volunteer bleeds well, label a Mitra sampler and package with the volunteer number in the following format: **Mitra-ddmmyy-XX-01.** Start numbering with 01 and then number consecutively.

19. Remove the Mitra sampling device by opening the sealed pack and remove the clamshell package. Pull apart the clamshell and open the sides to form a handle.

20. Apply sampler tip to drop of blood at an angle.

21. Wait till the tip goes fully red then count 2 additional seconds.

22. Remove the tip slowly.

23. Complete sampling of at least two tips.

24. Refold clamshell to cover tips and press close and label with volunteer reference number.

25. Supply patient with a gauze and plaster.

26. After drying, sampling cards and Mitra devices must be stored in individual plastic re-sealable bags.

27. Label resealable bag with volunteer reference number and date.

28. These are ready to be sent to the analytical laboratory with the completed consent form and short questionnaire.

29. Record information in a record book and keep it in a secure place. This information includes volunteer reference number, date of collection, number of spots on each card and comments.

30. Disposal of the lancet, gauze and alcohol pads should be via the clinical waste bin in the locally approved manner.

31. Change gloves before taking sample from each new volunteer.

32. If there is a spillage on the tray you should clean it with proper laboratory disinfectant.

33. After completing sample collection, remove and dispose of gloves and take off lab coat and safety spectacles and wash your hands.

Appendix 11. The Standard Operation Procedure (SOP) for Preparation of Multicomponent Cardiovascular Medications in DBS

1. Introduction

The aim of this document is to prepare a protocol for the preparation of calibration and quality control standards for 9 selected cardiovascular medications and internal standard (Atenolol d7) in human whole blood. The target medications are Amlodipine Besylate salt, Atenolol, Atorvastatin Calcium salt, Bisoprolol Hemifumarate salt, Diltiazem Hydrochloride, Lisinopril, Losartan Potassium salt, Simvastatin, Valsartan and Atenolol-d7 (internal standard). Concentration range of QC in whole blood for the selected cardiovascular drugs in the previously validated method are summarized in Table 1

Table 1. Concentration range of the calibration and quality control standard in whole blood for the target medications

Drug	Calibration range ng/ml	Calibration standards (ng/ml)							
		LOW				MED			HIGH
Amlodipine	0.5-100		0.5	1	5	10	25	50	100
Atenolol	10-1500	10	20	50	100	200	500	1000	1500
Atorvastatin	0.5-100		0.5	1	5	10	25	50	100
Bisoprolol	0.1-100	0.1	0.5	1	5	10	25	50	100
Diltiazem	0.5-600	0.5	1	5	10	50	100	300	600
Lisinopril	0.1-100	0.1	0.5	1	5	10	25	50	100
Losartan	5-1000	5	10	25	50	100	250	500	1000
Simvastatin	0.1-100	0.1	0.5	1	5	10	25	50	100
Valsartan	50-4000	50	100	250	500	1000	2000	3000	4000

2. Safety

To prepare the QC standards, personal protective equipment must be worn as per the appropriate risk assessment and completed COSHH for each drug. Laboratory coat and disposable gloves must be worn. Hands should be washed and disinfected before and after handling blood samples. Care must be taken in case of any cut on the hands by covering the cut by waterproof dressing or plasters. Spillage should be cleaned by a suitable disinfectant and disposable paper towels. Lab benches should be wiped by a suitable disinfectant after each work session.

3. Blood samples storage

Blood samples in specimen tubes should be stored in number or code labelled re-sealable polythene bags in refrigerator in lab HB 00. 15.

4. Disposal

Discarded blood spots sampling paper and any contaminated materials used to clean spillages in the clinical waste bag (yellow plastic bag). Discarded specimens in micro-centrifuge tubes, Specimen tubes and contaminated pipets tips and LC vial should be put into the clinical waste bags. Sharp contaminated materials such as needles should be disposed in the yellow rigid walled container.

5. Equipment

Analytical balance, volumetric pipets, Eppendorf tubes, volumetric flasks, 903 DBS cards and volumetric absorptive microsampling (VAMS) device.

6. Materials

Amlodipine besylate salt, atenolol, atorvastatin calcium salt, bisoprolol hemifumarate salt, diltiazem hydrochloride, lisinopril, losartan Potassium salt, simvastatin, valsartan, atenolol- D7 (Internal Standard), acetonitrile (LC-MS grade), methanol (LC-MS grade), and water (LC-MS grade).

7. Preparation of diluent (70:30 MeOH: H2O v/v)

To prepare 500 ml of diluent, transfer 350 ml of methanol in a 500 ml measuring cylinder and add LC-MS grade water to 500 ml. Transfer into 500 ml plain glass bottle and shake it well. Label the bottle with the date and dilution ratio.

8. Preparation of standard stock solutions of the target medications

Weigh 5 mg of amlodipine, atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, losartan, simvastatin and valsartan in 5 ml volumetric flask. Dissolve each drug in suitable volume of methanol to get 1 mg/ml for each medication see Table 2.

Table 2. Preparation of stock solution of the 9 target medications

Drugs	Weight in mg	Volume of 100% MeOH (ml)	Concentration mg/ml
Amlodipine	5	5	1
Atenolol	5	5	1
Atorvastatin	5	5	1
Bisoprolol	5	5	1
Diltiazem	5	5	1
Lisinopril	5	5	1
Losartan	5	5	1
Simvastatin	5	5	1
Valsartan	5	5	1

9. Preparation of intermediate stock solution with concentration 10000 ng /ml

Intermediate stock solutions of the target medications amlodipine, bisoprolol, diltiazem, lisinopril, atorvastatin, and simvastatin were prepared by Pipetting 100 µl of the 1 mg/ml standard stock solution of drug into a 10 ml volumetric flask and dilute with 70:30 MeOH: H_2O, v/v to the mark. Shake well and keep solution refrigerated.

10. Preparation of Internal Standard stock solution (Atenolol- d7 10000 ng/ml)

Weigh 0.4 mg of Atenolol d7 and dissolve it 400 µl methanol shake well to get 1 µg/µL primary stock of atenolol-D7. The stock solution was diluted to 10,000 ng/mL. Further dilution was with methanol/water (70:30, v/v) to produce internal standard (atenolol-D7) concentration of 20 ng/ml and keep solution refrigerated.

11. Preparation of extraction solvent with 20ng/ml concentration of internal standard (Atenolol d_7)

Pipette 20 µl of the 10,000 ng/ml stock solution of (atenolol d_7) into a 10 ml volumetric flask and make to the mark with 70:30 MeOH: H_20, v/v. Shake well and keep solution refrigerated.

12. Preparation of multicomponent solution for the target medications

Tables 3-11 shows the final concentration of medications in solutions.

Table 3. Final concentration of amlodipine in solution

	Amlodipine concentration (ng/ml)	Volume of 10000 ng/ml stock required(µl)	Total volume (ml)
Solution 1	1	1	10
Solution 2	5	5	10
Solution 3	10	10	10
Solution 4	50	50	10
Solution 5	100	50	5
Solution 6	250	125	5
Solution 7	500	250	5
Solution 8	1000	500	5

Table 4. Final concentration of atenolol in solution

	Atenolol concentration (ng/ml)	Volume of 1 mg/ml stock required (µl)	Total volume (ml)
Solution 1	100	1	10
Solution 2	200	2	10
Solution 3	500	5	10
Solution 4	1000	10	10
Solution 5	2000	10	5
Solution 6	5000	25	5
Solution 7	10000	50	5
Solution 8	15000	75	5

Table 5. Final concentration of atorvastatin in solution

	Atorvastatin concentration (ng/ml)	Volume of 10000 ng/ml stock required (µl)	Total volume (ml)
Solution 1	1	1	10
Solution 2	5	5	10
Solution 3	10	10	10
Solution 4	50	50	10
Solution 5	100	50	5
Solution 6	250	125	5
Solution 7	500	250	5
Solution 8	1000	500	5

Table 6. Final concentration of bisoprolol in solution

	Bisoprolol concentration (ng/ml)	Volume of 10000 ng/ml stock required(µl)	Total volume (ml)
Solution 1	1	1	10
Solution 2	5	5	10
Solution 3	10	10	10
Solution 4	50	50	10
Solution 5	100	50	5
Solution 6	250	125	5
Solution 7	500	250	5
Solution 8	1000	500	5

Table 7. Final concentration of diltiazem in solution

	Diltiazem concentration (ng/ml)	Volume of 10000 ng/ml stock required(µl)	Total volume (ml)
Solution 1	5	5	10
Solution 2	10	10	10
Solution 3	50	50	10
Solution 4	100	100	10
Solution 5	500	250	5
Solution 6	1000	500	5
Solution 7	3000	1500	5
Solution 8	6000	3000	5

Table 8. Final concentration of lisinopril in solution

	Lisinopril concentration (ng/ml)	Volume of 10000 ng/ml stock required(µl)	Total volume (ml)
Solution 1	1	1	10
Solution 2	5	5	10
Solution 3	10	10	10
Solution 4	50	50	10
Solution 5	100	50	5
Solution 6	250	125	5
Solution 7	500	250	5
Solution 8	1000	500	5

Table 9. Final concentration of losartan in solution

	Losartan concentration (ng/ml)	Volume of 10000 ng/ml stock required (µL)	Total volume (ml)
Solution 1	50	50	10
Solution 2	100	100	10
Solution 3	250	250	10
Solution 4	500	500	10
Solution 5	1000	500	5
Solution 6	2500	1250	5
Solution 7	5000	2500	5
Solution 8	10000	5000	5

Table 10. Final concentration of simvastatin in solution

	Simvastatin Concentration (ng/ml)	Volume of 10000 ng/ml stock required (µl)	Total volume (ml)
Solution 1	1	1	10
Solution 2	5	5	10
Solution 3	10	10	10
Solution 4	50	50	10
Solution 5	100	50	5
Solution 6	250	125	5
Solution 7	500	250	5
Solution 8	1000	500	5

Table 11. Final concentration of valsartan in solution

	Valsartan concentration (ng/ml)	Volume of 1mg/ml stock required(µl)	Total volume (ml)
Solution 1	500	5	10
Solution 2	1000	10	10
Solution 3	2500	25	10
Solution 4	5000	50	10
Solution 5	10000	50	5
Solution 6	20000	100	5
Solution 7	30000	150	5
Solution 8	40000	200	5

From Tables 3-11 there will be 8 multicomponent solutions (1, 2, 3, 4, 5, 6, 7, and 8).

12. Preparation of multicomponent calibration standards and quality control standards (QC) for the target medications in whole blood

For each multicomponent solution (3-11) solutions, pipet 100 µl to an Eppendorf tube and add 900 µl of fresh human blood. Vortex for 1 min to produce the final calibration concentration in Tables 13-21. For blank samples pipettes 100 µl of extraction solvent containing 10 ng/ml internal standard and add 900 µl of blood and mix well by vortexing for 1 min. Table 12 – 20 show the final calibration concentrations of each target drug in whole blood.

Table 12. Final QC concentration of Amlodipine in whole blood

	Concentration of amlodipine standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of amlodipine in whole blood (ng/ml)
Standard A	1	100	0.1
Standard B	5	100	0.5
Standard C	10	100	1
Standard D	50	100	5
Standard E	100	100	10
Standard F	250	100	25
Standard G	500	100	50
Standard H	1000	100	100

Table 13. Final concentration of atenolol in whole blood

	Concentration of atenolol standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of atenolol in whole blood (ng/ml)
Standard A	100	100	10
Standard B	200	100	20
Standard C	500	100	50
Standard D	1000	100	100
Standard E	2000	100	200
Standard F	5000	100	500
Standard G	10000	100	1000
Standard H	15000	100	1500

Table 14. Final concentration of atorvastatin in whole blood

	Concentration of atorvastatin standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of atorvastatin in whole blood (ng/ml)
Standard A	1	100	0.1
Standard B	5	100	0.5
Standard C	10	100	1
Standard D	50	100	5
Standard E	100	100	10
Standard F	250	100	25
Standard G	500	100	50
Standard H	1000	100	100

Table 15. Final concentration of Bisoprolol in whole blood

	Concentration of bisoprolol standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of bisoprolol in whole blood (ng/ml)
Standard A	1	100	0.1
Standard B	5	100	0.5
Standard C	10	100	1
Standard D	50	100	5
Standard E	100	100	10
Standard F	250	100	25
Standard G	500	100	50
Standard H	1000	100	100

Table 16. Final concentration of diltiazem in whole blood

	Concentration of diltiazem standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of diltiazem in whole blood (ng/ml)
Standard A	5	100	0.5
Standard B	10	100	1
Standard C	50	100	5
Standard D	100	100	10
Standard E	500	100	50
Standard F	1000	100	100
Standard G	3000	100	300
Standard H	6000	100	600

Table 17. Final concentration of lisinopril in whole blood

	Concentration of lisinopril standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of lisinopril in whole blood (ng/ml)
Standard A	1	100	0.1
Standard B	5	100	0.5
Standard C	10	100	1
Standard D	50	100	5
Standard E	100	100	10
Standard F	250	100	25
Standard G	500	100	50
Standard H	1000	100	100

Table 18. Final concentration of losartan in whole blood

	Concentration of losartan standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of losartan in whole blood (ng/ml)
Standard A	50	100	5
Standard B	100	100	10
Standard C	250	100	25
Standard D	500	100	50
Standard E	1000	100	100
Standard F	2500	100	250
Standard G	5000	100	500
Standard H	10000	100	1000

Table 19. Final concentration of simvastatin in whole blood

	Concentration of simvastatin standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of simvastatin in whole blood (ng/ml)
Standard A	1	100	0.1
Standard B	5	100	0.5
Standard C	10	100	1
Standard D	50	100	5
Standard E	100	100	10
Standard F	250	100	25
Standard G	500	100	50
Standard H	1000	100	100

Table 20. Final concentration of valsartan in whole blood

	Concentration of valsartan standard solution (ng/ml)	Volume of standard to be added to whole blood (µl)	Final concentration of valsartan in whole blood (ng/ml)
Standard A	500	100	50
Standard B	1000	100	100
Standard C	2500	100	250
Standard D	5000	100	500
Standard E	10000	100	1000
Standard F	20000	100	2000
Standard G	30000	100	3000
Standard H	40000	100	4000

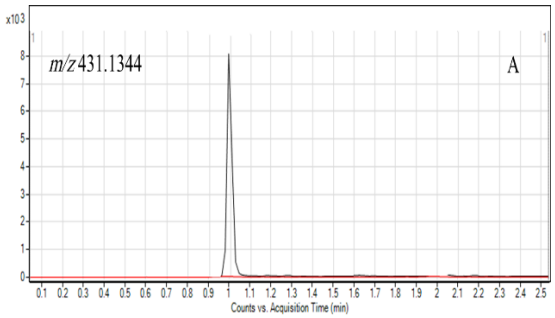
From Tables 12 – 20 there will be 8 calibration standards in whole blood (A, B, C, D, E, F, G, H and the blank). Standard C, F and H are chosen to represent low, medium and high

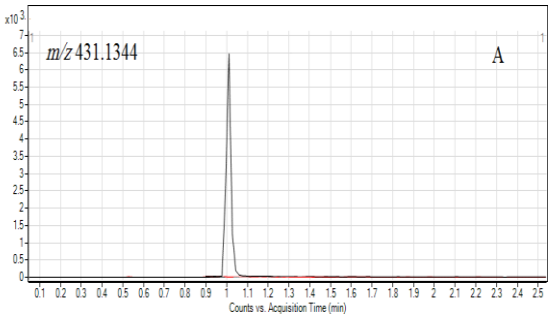
13. Spotting of blood target on DBS cards and VAMS

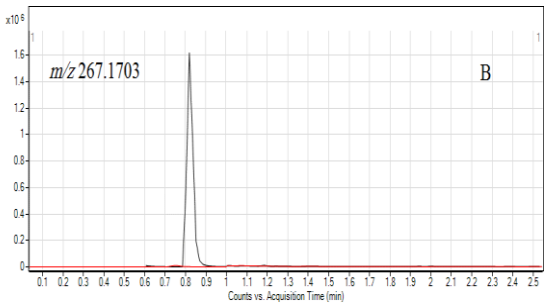
Pipette 30 µl of blood from low, medium and high standards on Whatman 903 cards and left at least 2 hrs to dry at ambient temperature and after drying keep each card in coded labelled re-sealable polythene bags in a secure cabinet at lab 00.15. For VAMS dip the blank VAMS tips at angle of about 45 degrees into each blood standard (A, B, C, including blank sample. Wait for 2 second till the tip becomes fully red and then count for more two (2) seconds. This confirms that the VAMS substrate precisely samples 10 µL of blood. Remove slowly remove tip from the microcentrifuge tube and close the clamshell.

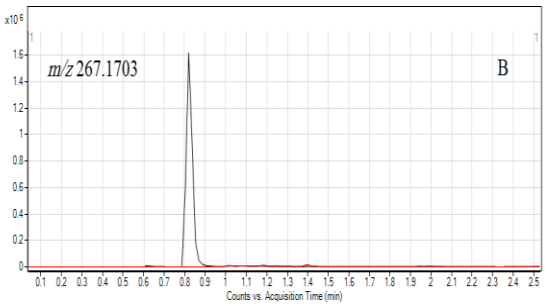
Appendix 12. Side by Side LC-HRMS Representative (EICs) of an Extracted Blank Blood Sample (red) and a Calibration Standard at the LOQ Spiked with the Nine Target Drugs (black) and Atenolol-d7 (The Internal Standard) on 903 Sampling Paper and VAMS.

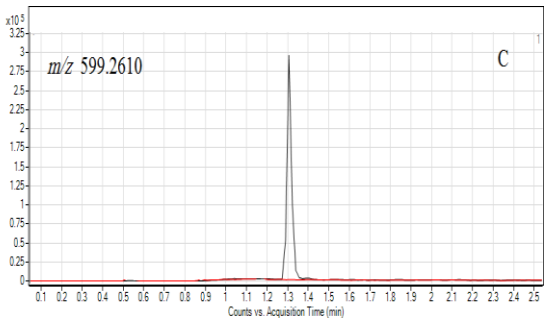
903 sampling paper (DBS) **VAMS**

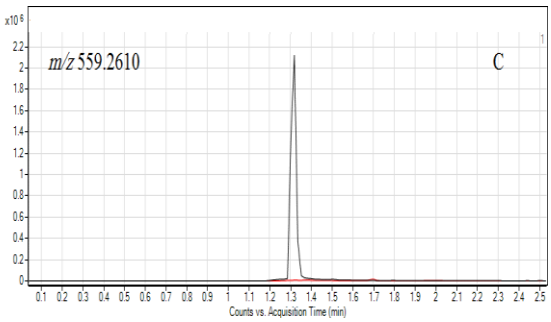


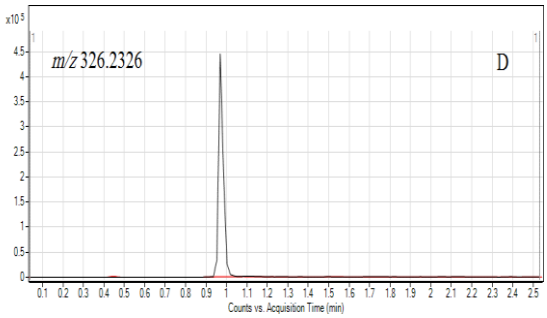


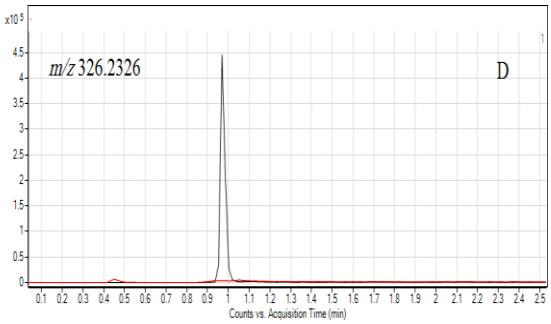


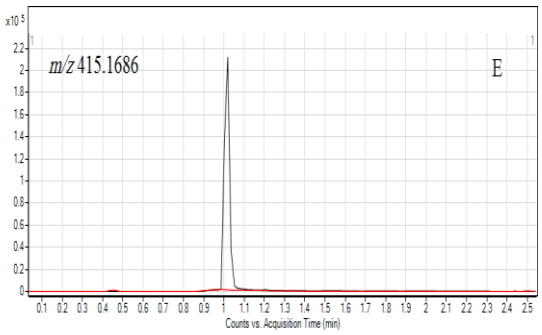








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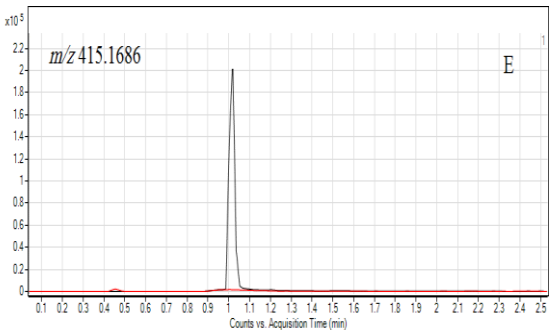
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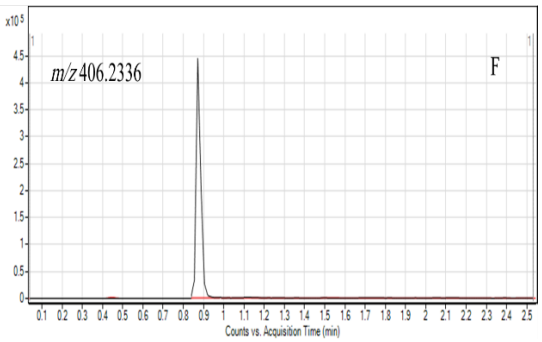
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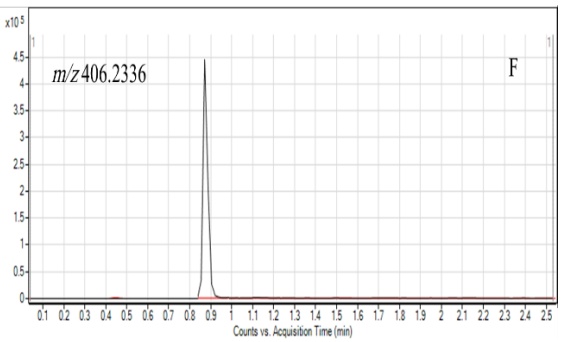
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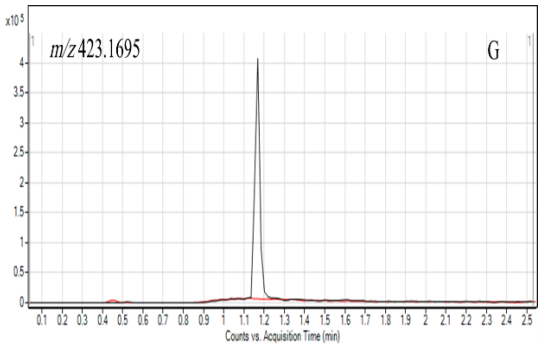
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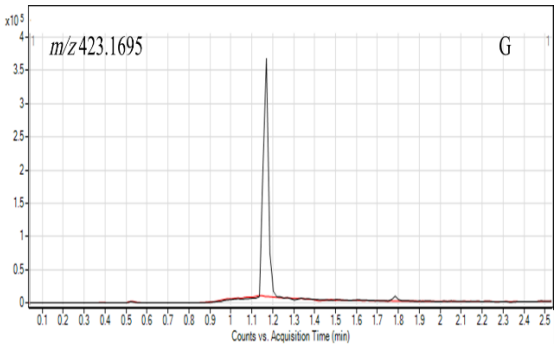
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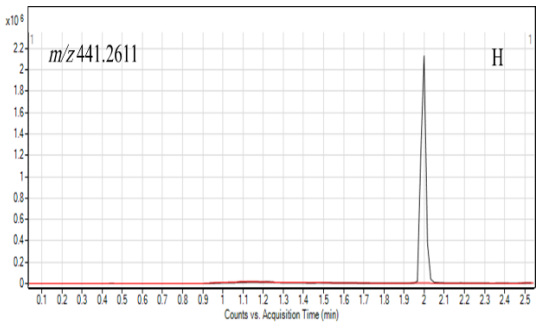
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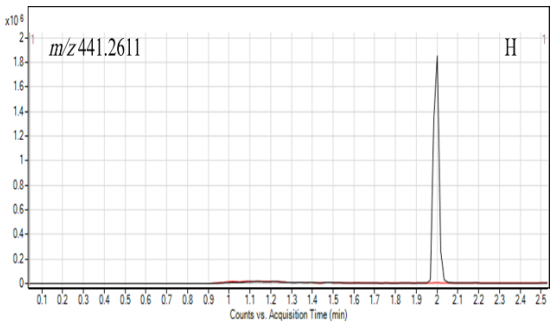
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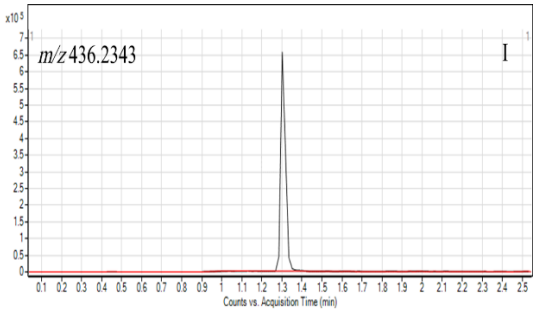
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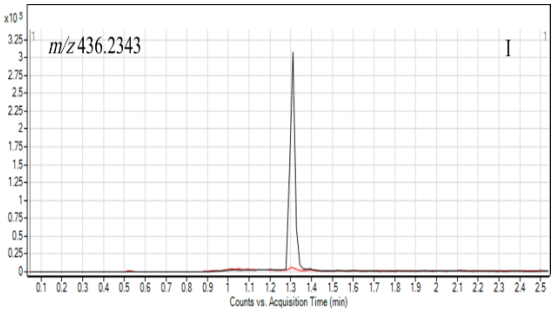
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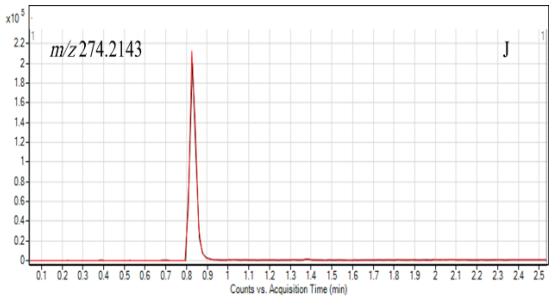
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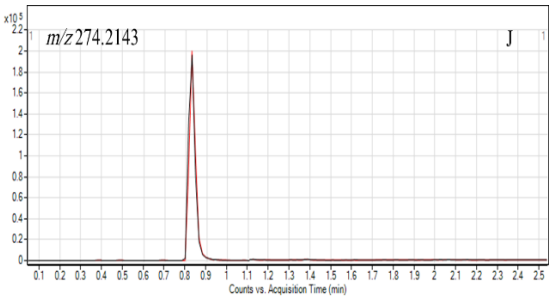
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Counts vs. Acquisition Time (min)

Appendix 13. Qualitative Analysis of Blood Microsamples on 903 Card and VAMS for the Cardiovascular Drugs Studied in Iraqi Volunteers

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-280716-AA-01	M	Control	DBS	-
			VAMS	
903-280716-AA-02	F	Control	DBS	-
			VAMS	
903-280716-AA-03	M	Control	DBS	-
			VAMS	
903-280716-AA-04	F	Control	DBS	-
			VAMS	
903-280716-AA-05	M	Control	DBS	-
			VAMS	
903-280716-AA-06	F	Lisinopril	DBS	YES
903-310716-AA-07	M	Simvastatin	DBS	NO
903-310716-AA-08	F	Atenolol	DBS	NO
903-310716-AA-09	F	Control	DBS	-
903-310716-AA-10	M	Bisoprolol	DBS	NO
903-310716-AA-11	F	Valsartan	DBS	NO
903-310716-AA-12	F	Valsartan	DBS	YES
903-310716-AA-13	M	Control	DBS	-
			VAMS	
903-310716-AA-14	M	Atenolol	DBS	YES
			VAMS	YES
903-310716-AA-15	F	Bisoprolol	DBS	YES
903-010816-AA-16	F	Valsartan	DBS	YES
			VAMS	YES
903-010816-AA-17	M	Valsartan	DBS	YES
			VAMS	YES
		Atorvastatin	DBS	NO
			VAMS	NO
903-010816-AA-18	F	Control	DBS	-
			VAMS	
903-030816-AA-19	F	Bisoprolol	DBS	NO
			VAMS	NO
903-030816-AA-20	M	Simvastatin	DBS	NO
903-030816-AA-21	F	Diltiazem	DBS	NO
			VAMS	NO
903-030816-AA-22	M	Control	DBS	-
			VAMS	
903-030816-AA-23	M	Atenolol	DBS	YES
903-030816-AA-24	F	Valsartan	DBS	YES
			VAMS	YES
903-030816-AA-25	F	Losartan	DBS	YES
			VAMS	YES
903-030816-AA-26	F	Control	DBS	-
			VAMS	
903-030816-AA-27	F	Bisoprolol	DBS	YES
			VAMS	YES
		Losartan	DBS	YES
			VAMS	YES
903-030816-AA-28	F	Control	DBS	-
			VAMS	

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-030816-AA-29	M	Control	DBS	-
			VAMS	
903-040816-AA-30	M	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-040816-AA-31	M	Valsartan	DBS	NO
			VAMS	NO
903-040816-AA-32	F	Lisinopril	DBS	YES
			VAMS	YES
903-040816-AA-33	M	Losartan	DBS	NO
			VAMS	NO
903-050816-AA-34	M	Atenolol	DBS	YES
903-050816-AA-35	M	Control	DBS	-
			VAMS	
903-050816-AA-36	F	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	NO
			VAMS	NO
903-050816-AA-37	M	Atenolol	DBS	YES
			VAMS	YES
903-050816-AA-38	M	Bisoprolol	DBS	YES
		Losartan	DBS	NO
903-050816-AA-39	F	Control	DBS	-
			VAMS	
903-050816-AA-40	F	Atenolol	DBS	YES
			VAMS	YES
903-050816-AA-41	F	Losartan	DBS	NO
903-050816-AA-42	M	Atenolol	DBS	YES
903-050816-AA-43	F	Control	DBS	-
			VAMS	
903-050816-AA-44	M	Control	DBS	-
			VAMS	
903-060816-AA-45	M	Atenolol	DBS	YES
903-060816-AA-46	F	Control	DBS	-
			VAMS	
903-060816-AA-47	M	Control	DBS	-
			VAMS	
903-060816-AA-48	M	Bisoprolol	DBS	YES
		Valsartan	DBS	NO
903-060816-AA-49	M	Control	DBS	-
903-060816-AA-50	M	Losartan	DBS	YES
903-060816-AA-51	M	Atenolol	DBS	YES
903-060816-AA-52	M	Atenolol	DBS	YES
903-060816-AA-53	M	Atenolol	DBS	YES
903-060816-AA-54	F	Control	DBS	-
903-070816-AA-55	F	Losartan	DBS	YES
		Atorvastatin	DBS	NO
903-070816-AA-56	F	Atorvastatin	DBS	NO
		Lisinopril	DBS	NO

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-070816-AA-57	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-070816-AA-58	M	Atenolol	DBS	YES
		Atorvastatin	DBS	NO
903-070816-AA-59	F	Atorvastatin	DBS	NO
		Bisoprolol	DBS	NO
903-070816-AA-60		Diltiazem	DBS	NO
903-070816-AA-61	M	Diltiazem	DBS	YES
			VAMS	YES
		Losartan	DBS	NO
			VAMS	NO
		Valsartan	DBS	NO
			VAMS	NO
903-070816-AA-62	M	Atorvastatin	DBS	NO
903-070816-AA-63	F	Control	DBS	-
903-070816-AA-64	M	Atorvastatin	DBS	NO
903-070816-AA-65	M	Diltiazem	DBS	YES
903-080816-AA-66	F	Lisinopril	DBS	YES
			VAMS	YES
903-080816-AA-67	M	Atenolol	DBS	YES
			VAMS	YES
903-080816-AA-68	M	Valsartan	DBS	YES
			VAMS	YES
903-080816-AA-69	M	Losartan	DBS	YES
			VAMS	YES
903-080816-AA-70	M	Atorvastatin	DBS	YES
			VAMS	YES
		Bisoprolol	DBS	YES
			VAMS	YES
903-080816-AA-71	F	Control	DBS	-
			VAMS	
903-090816-AA-72	F	Atorvastatin	DBS	NO
		Diltiazem	DBS	YES
		Valsartan	DBS	NO
903-090816-AA-73	F	Valsartan	DBS	NO
			VAMS	NO
903-090816-AA-74	M	Valsartan	DBS	NO
			VAMS	NO
903-090816-AA-75	F	Atorvastatin	DBS	NO
			VAMS	NO
906-090816-AA-76	M	Diltiazem	DBS	YES
			VAMS	YES
903-090816-AA-77	M	Lisinopril	DBS	YES
			VAMS	YES
903-090816-AA-78	M	Valsartan	DBS	YES
			VAMS	YES
903-090816-AA-79	F	Control	DBS	-
			VAMS	
903-100816-AA-80	M	Valsartan	DBS	NO

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-100816-AA-81	F	Control	DBS	-
			VAMS	
903-100816-AA-82	M	Atorvastatin	DBS	YES
			VAMS	YES
		Valsartan	DBS	NO
			VAMS	NO
903-100816-AA-83	M	Control	DBS	-
903-100816-AA-84	M	Valsartan	DBS	YES
			VAMS	YES
903-100816-AA-85	F	Control	DBS	-
903-100816-AA-86	F	Diltiazem	DBS	YES
			VAMS	YES
903-100816-AA-87	F	Bisoprolol	DBS	YES
903-100816-AA-88	M	Atorvastatin	DBS	NO
903-100816-AA-89	F	Valsartan	DBS	YES
			VAMS	YES
903-110816-AA-90	M	Bisoprolol	DBS	YES
903-120816-AA-91	M	Bisoprolol	DBS	YES
			VAMS	YES
		Lisinopril	DBS	YES
			VAMS	YES
903-120816-AA-92	F	Control	DBS	-
903-120816-AA-93	M	Lisinopril	DBS	YES
903-120816-AA-94	M	Control	DBS	-
903-150816-AA-95	M	Bisoprolol	DBS	NO
903-150816-AA-96	M	Bisoprolol	DBS	YES
903-150816-AA-97	M	Control	DBS	-
			VAMS	
903-150816-AA-98	F	Control	DBS	-
			VAMS	
903-150816-AA-99	F	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	NO
			VAMS	NO
903-270717-AA-100	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-270717-AA-101	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-270717-AA-102	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-200717-AA-103	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-200717-AA-104	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-105	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-106	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-107	M	Atenolol	DBS	YES
		Lisinopril	DBS	NO

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-200717-AA-108	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-200717-AA-109	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-200717-AA-110	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-111	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-112	M	Bisoprolol	DBS	YES
		Valsartan	DBS	NO
903-200717-AA-113	M	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-200717-AA-114	M	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-200717-AA-115	F	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-200717-AA-116	F	Bisoprolol	DBS	YES
		Valsartan	DBS	NO
903-200717-AA-117	F	Diltiazem	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-118	M	Diltiazem	DBS	YES
		Lisinopril	DBS	YES
903-200717-AA-119	M	Diltiazem	DBS	YES
			VAMS	YES
		Lisinopril	DBS	YES
			VAMS	YES
903-200717-AA-120	M	Diltiazem	DBS	YES
			VAMS	YES
		Lisinopril	DBS	NO
			VAMS	NO
903-210717-AA-121	M	Losartan	DBS	YES
		Simvastatin	DBS	NO
903-210717-AA-122	F	Losartan	DBS	YES
		Simvastatin	DBS	NO
903-210717-AA-123	M	Losartan	DBS	YES
			VAMS	YES
		Simvastatin	DBS	YES
			VAMS	YES
903-210717-AA-124	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-210717-AA-125	F	Amlodipine	DBS	YES
903-210717-AA-126	M	Amlodipine	DBS	YES
903-210717-AA-127	F	Amlodipine	DBS	YES
			VAMS	YES

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-210717-AA-128	M	Amlodipine	DBS	YES
			VAMS	YES
903-210717-AA-129	F	Losartan	DBS	YES
			VAMS	YES
903-210717-AA-130	M	Losartan	DBS	YES
			VAMS	YES
903-220717-AA-131	M	Losartan	DBS	YES
			VAMS	YES
903-220717-AA-132	M	Simvastatin	DBS	YES
			VAMS	YES
903-220717-AA-133	M	Simvastatin	DBS	NO
			VAMS	NO
903-220717-AA-134	M	Valsartan	DBS	NO
903-220717-AA-135	M	Valsartan	DBS	NO
			VAMS	NO
903-220717-AA-136	M	Valsartan	DBS	YES
			VAMS	YES
903-220717-AA-137	F	Valsartan	DBS	YES
			VAMS	YES
903-220717-AA-138	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-220717-AA-139	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-220717-AA-140	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-230717-AA-141	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-230717-AA-142	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-230717-AA-143	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-230717-AA-144	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-230717-AA-145	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-230717-AA-146	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-230717-AA-147	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-230717-AA-148	M	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-230717-AA-149	F	Bisoprolol	DBS	YES
		Valsartan	DBS	YES
903-230717-AA-150	F	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-230717-AA-151	F	Control	DBS	-
			VAMS	

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-240717-AA-152	M	Diltiazem	DBS	YES
		Lisinopril	DBS	YES
903-240717-AA-153	M	Diltiazem	DBS	YES
		Lisinopril	DBS	YES
903-240717-AA-154	F	Diltiazem	DBS	YES
			VAMS	YES
		Lisinopril	DBS	YES
			VAMS	YES
903-240717-AA-155	F	Diltiazem	DBS	YES
			VAMS	YES
		Lisinopril	DBS	YES
			VAMS	YES
903-240717-AA-156	M	Losartan	DBS	YES
			VAMS	YES
		Simvastatin	DBS	YES
			VAMS	YES
903-240717-AA-157	F	Losartan	DBS	NO
		Simvastatin	DBS	NO
903-240717-AA-158	F	Losartan	DBS	NO
		Simvastatin	DBS	NO
903-240717-AA-159	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-240717-AA-160	M	Amlodipine	DBS	YES
903-240717-AA-161	F	Amlodipine	DBS	YES
903-250717-AA-162	F	Amlodipine	DBS	YES
			VAMS	YES
903-250717-AA-163	M	Amlodipine	DBS	YES
			VAMS	YES
903-250717-AA-164	M	Losartan	DBS	YES
			VAMS	YES
903-250717-AA-165	M	Losartan	DBS	YES
			VAMS	YES
903-250717-AA-166	F	Losartan	DBS	YES
			VAMS	YES
903-250717-AA-167	M	Simvastatin	DBS	YES
			VAMS	YES
903-250717-AA-168	M	Simvastatin	DBS	NO
			VAMS	NO
903-250717-AA-169	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-250717-AA-170	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-270717-AA-171	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-270717-AA-172	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-270717-AA-173	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-270717-AA-174	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-270717-AA-175	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-270717-AA-176	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-270717-AA-177	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-270717-AA-178	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-270717-AA-179	M	Control	DBS	-
903-270717-AA-180	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-280717-AA-181	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-280717-AA-182	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-280717-AA-183	F	Bisoprolol	DBS	YES
		Valsartan	DBS	YES
903-280717-AA-184	M	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-280717-AA-185	M	Bisoprolol	DBS	YES
		Valsartan	DBS	YES
903-280717-AA-186	F	Diltiazem	DBS	YES
		Lisinopril	DBS	NO
903-280717-AA-187	M	Diltiazem	DBS	YES
		Lisinopril	DBS	YES
903-280717-AA-188	M	Diltiazem	DBS	YES
			VAMS	YES
		Lisinopril	DBS	YES
			VAMS	YES
903-280717-AA-189	F	Diltiazem	DBS	YES
			VAMS	YES
		Lisinopril	DBS	NO
			VAMS	NO
903-280717-AA-190	F	Losartan	DBS	NO
		Simvastatin	DBS	NO
903-290717-AA-191	F	Losartan	DBS	YES
			VAMS	YES
		Simvastatin	DBS	YES
			VAMS	YES
903-290717-AA-192	F	Losartan	DBS	YES
			VAMS	YES
		Simvastatin	DBS	YES
			VAMS	YES
903-290717-AA-193	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-290717-AA-194	M	Losartan	DBS	YES
			VAMS	YES
		Simvastatin	DBS	YES
			VAMS	YES

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-290717-AA-195	M	Amlodipine	DBS	NO
903-290717-AA-196	M	Amlodipine	DBS	NO
			VAMS	NO
903-290717-AA-197	M	Amlodipine	DBS	YES
			VAMS	YES
903-290717-AA-198	M	Amlodipine	DBS	NO
			VAMS	NO
903-290717-AA-199	F	Losartan	DBS	YES
			VAMS	YES
903-290717-AA-200	M	Losartan	DBS	YES
			VAMS	YES
903-300717-AA-201	M	Losartan	DBS	YES
			VAMS	YES
903-300717-AA-202	M	Simvastatin	DBS	NO
			VAMS	NO
903-300717-AA-203	M	Control	DBS	-
903-300717-AA-204	M	Valsartan	DBS	NO
			VAMS	NO
903-300717-AA-205	M	Valsartan	DBS	YES
			VAMS	YES
903-300717-AA-206	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-300717-AA-207	M	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-300717-AA-208	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-300717-AA-209	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-300717-AA-210	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-300717-AA-211	F	Atenolol	DBS	YES
		Lisinopril	DBS	NO
903-300717-AA-212	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-300717-AA-213	M	Diltiazem	DBS	YES
		Lisinopril	DBS	YES
903-300717-AA-214	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-030717-AA-215	M	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-030717-AA-216	F	Bisoprolol	DBS	YES
		Valsartan	DBS	YES
903-030717-AA-217	M	Bisoprolol	DBS	YES
		Valsartan	DBS	NO
903-300717-AA-218	F	Diltiazem	DBS	YES
		Lisinopril	DBS	NO
903-300717-AA-219	F	Diltiazem	DBS	YES
		Lisinopril	DBS	YES

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-300717-AA-220	F	Diltiazem	DBS	NO
			VAMS	NO
		Lisinopril	DBS	NO
			VAMS	NO
903-310717-AA-221	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-310717-AA-222	M	Amlodipine	DBS	YES
903-310717-AA-223	F	Losartan	DBS	YES
			VAMS	YES
903-310717-AA-224	F	Valsartan	DBS	NO
903-310717-AA-225	M	Valsartan	DBS	YES
			VAMS	
903-310717-AA-226	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-310717-AA-227	F	Atenolol	DBS	YES
		Simvastatin	DBS	YES
903-310717-AA-228	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-310717-AA-229	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-310717-AA-230	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-010817-AA-231	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	NO
903-010817-AA-232	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-010817-AA-233	F	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-010817-AA-234	F	Bisoprolol	DBS	YES
			VAMS	YES
		Valsartan	DBS	YES
			VAMS	YES
903-010817-AA-235	F	Bisoprolol	DBS	YES
		Valsartan	DBS	NO
903-010817-AA-236	F	Diltiazem	DBS	NO
		Lisinopril	DBS	NO
903-010817-AA-237	F	Diltiazem	DBS	NO
		Lisinopril	DBS	NO
903-010817-AA-238	F	Diltiazem	DBS	NO
			VAMS	NO
		Lisinopril	DBS	NO
			VAMS	NO
903-010817-AA-239	M	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-010817-AA-240	M	Amlodipine	DBS	NO
			VAMS	NO

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-020817-AA-241	M	Valsartan	DBS	NO
			VAMS	NO
903-020817-AA-242	M	Valsartan	DBS	NO
			VAMS	NO
903-020817-AA-243	M	Valsartan	DBS	YES
			VAMS	YES
903-020817-AA-244	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-020817-AA-245	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-020817-AA-246	M	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-020817-AA-247	F	Diltiazem	DBS	NO
			VAMS	NO
		Lisinopril	DBS	NO
			VAMS	NO
903-020817-AA-248	F	Diltiazem	DBS	NO
			VAMS	NO
		Lisinopril	DBS	NO
			VAMS	NO
903-020817-AA-249	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-020817-AA-250	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-030817-AA-251	F	Losartan	DBS	NO
			VAMS	NO
		Simvastatin	DBS	NO
			VAMS	NO
903-030817-AA-252	F	Amlodipine	DBS	NO
			VAMS	NO
903-030817-AA-253	M	Valsartan	DBS	NO
			VAMS	NO
903-030817-AA-254	F	Valsartan	DBS	YES
			VAMS	YES
903-030817-AA-255	F	Valsartan	DBS	YES
			VAMS	YES
903-030817-AA-256	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-030817-AA-257	F	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-030817-AA-258	M	Atenolol	DBS	YES
		Lisinopril	DBS	YES
903-030817-AA-259	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-030817-AA-260	F	Bisoprolol	DBS	YES
		Lisinopril	DBS	YES
903-030817-AA-261	M	Bisoprolol	DBS	YES
		Valsartan	DBS	NO
903-040817-AA-262	M	Bisoprolol	DBS	YES
		Valsartan	DBS	NO

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-040817-AA-263	M	Valsartan	DBS	NO
903-040817-AA-264	M	Atenolol	DBS	NO
903-040817-AA-265	M	Losartan	DBS	NO
903-040817-AA-266	M	Valsartan	DBS	NO
903-040817-AA-267	F	Diltiazem	DBS	NO
903-040817-AA-268	F	Bisoprolol	DBS	NO
903-040817-AA-269	F	Diltiazem	DBS	NO
903-040817-AA-270	F	Atenolol	DBS	NO
903-050817-AA-271	F	Losartan	DBS	NO
903-050817-AA-272	M	Bisoprolol	DBS	NO
903-050817-AA-273	F	Valsartan	DBS	NO
903-050817-AA-274	M	Atenolol	DBS	NO
903-050817-AA-275	F	Losartan	DBS	NO
903-050817-AA-276	F	Valsartan	DBS	NO
903-050817-AA-277	F	Diltiazem	DBS	NO
903-050817-AA-278	F	Bisoprolol	DBS	NO
903-050817-AA-279	F	Losartan	DBS	NO
903-050817-AA-280	F	Valsartan	DBS	NO
903-100817-AA-281	F	Bisoprolol	DBS	NO
903-100817-AA-282	F	Diltiazem	DBS	NO
903-100817-AA-283	M	Atenolol	DBS	NO
903-100817-AA-284	F	Bisoprolol	DBS	NO
903-100817-AA-285	F	Diltiazem	DBS	NO
903-100817-AA-286	F	Valsartan	DBS	NO
903-100817-AA-287	F	Atenolol	DBS	NO
903-100817-AA-288	F	Losartan	DBS	NO
903-100817-AA-289	F	Diltiazem	DBS	NO
903-100817-AA-290	F	Bisoprolol	DBS	NO
903-210318-AA-291	M	Atorvastatin	DBS	YES
903-210318-AA-292	M	Valsartan	DBS	YES
903-210318-AA-293	F	Bisoprolol	DBS	YES
903-210318-AA-294	M	Lisinopril	DBS	NO
903-210318-AA-295	F	Losartan	DBS	NO
903-210318-AA-296	M	Atenolol	DBS	NO
903-210318-AA-297	M	Bisoprolol	DBS	YES
903-210318-AA-298	F	Bisoprolol	DBS	YES
903-210318-AA-299	M	Losartan	DBS	NO
903-240318-AA-300	F	Bisoprolol	DBS	NO
903-240318-AA-301	F	Atorvastatin	DBS	YES
903-240318-AA-302	F	Valsartan	DBS	YES
903-240318-AA-303	F	Bisoprolol	DBS	YES
903-240318-AA-304	M	Bisoprolol	DBS	NO
903-240318-AA-305	F	Losartan	DBS	NO
903-240318-AA-306	M	Bisoprolol	DBS	NO
903-250318-AA-307	F	Atenolol	DBS	NO
903-250318-AA-308	F	Losartan	DBS	NO
903-250318-AA-309	M	Valsartan	DBS	NO
903-250318-AA-310	F	Atenolol	DBS	NO
903-250318-AA-311	F	Atorvastatin	DBS	YES
903-260318-AA-312	F	Valsartan	DBS	YES
903-260318-AA-313	F	Lisinopril	DBS	NO
903-260318-AA-314	M	Simvastatin	DBS	NO
903-260318-AA-315	F	Bisoprolol	DBS	NO
903-260318-AA-316	M	Bisoprolol	DBS	NO

Patients Ref.	Sex	CVD medication	Sampling device	(YES) Detected/ (NO) non-detected
903-260318-AA-317	M	Losartan	DBS	NO
903-260318-AA-318	F	Valsartan	DBS	NO
903-260318-AA-319	F	Bisoprolol	DBS	NO
903-260318-AA-320	F	Atorvastatin	DBS	YES
903-260318-AA-321	M	Valsartan	DBS	YES
903-260318-AA-322	F	Bisoprolol	DBS	NO
903-260318-AA-323	F	Simvastatin	DBS	NO
903-260318-AA-324	M	Simvastatin	DBS	NO
903-260318-AA-325	F	Simvastatin	DBS	NO
903-260318-AA-326	F	Simvastatin	DBS	NO
903-260318-AA-327	M	Bisoprolol	DBS	NO
903-260318-AA-328	F	Simvastatin	DBS	NO
903-260318-AA-329	F	Bisoprolol	DBS	YES
903-260318-AA-330	F	Atorvastatin	DBS	YES
903-260318-AA-331	M	Atorvastatin	DBS	YES
903-260318-AA-332	F	Lisinopril	DBS	NO
903-270318-AA-333	M	Bisoprolol	DBS	NO
903-270318-AA-334	F	Simvastatin	DBS	NO
903-270318-AA-335	F	Bisoprolol	DBS	NO
903-270318-AA-336	M	Simvastatin	DBS	NO

Abbreviation: M: Male; F: Female.

Appendix 14. Drug Concentrations on 903 Cards and VAMS of the Cardiovascular Drugs Studied in Iraqi Samples from Patients Prescribed One or More of the CVD Drugs Under Consideration

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-280716-AA-01	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-280716-AA-02	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-280716-AA-03	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-280716-AA-04	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-280716-AA-05	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-280716-AA-06	F	Lisinopril	10 mg	DBS	32.87± 4.80	3	41.75-80.47	5.79-6.91	1	1	YES
903-310716-AA-07	M	Simvastatin	20 mg	DBS	<LLOQ	10	4.88-5.86	1.98-2.52	3	3	NO
903-310716-AA-08	F	Atenolol	50 mg	DBS	<LLOQ	36	159-377	1.5-6	8	13	NO
903-310716-AA-09	F	Control	-	DBS	-	-	-	-	-	-	
903-310716-AA-10	M	Bisoprolol	10 mg	DBS	<LLOQ	48	37-87	1.5-4	8	11	NO
903-310716-AA-11	F	Valsartan	80 mg	DBS	<LLOQ	240	1010-2270	2	8	11	NO
903-310716-AA-12	F	Valsartan	160 mg	DBS	2000.57± 10.33	5	1930-4000	1.5-3	1	1	YES
903-310716-AA-13	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-310716-AA-14	M	Atenolol	100 mg	DBS VAMS	204.87±2.09 206.22±1.55	5	240-1370	2-4	2	2	YES
903-310716-AA-15	F	Bisoprolol	5 mg	DBS	11.28± 0.69 12.87± 0.66	10	16.64-26.9	1.2-3	2	2	YES
903-010816-AA-16	F	Valsartan	80 mg	DBS VAMS	147± 3.35 160.21± 2.95	12	1010-2270	1.5-3	1	1	YES
903-010816-AA-17	M	Valsartan	80 mg	DBS VAMS	655.34± 2.22 655.52± 2.10	12	1010-2270	2	8	6	YES
		Atorvastatin	40 mg	DBS VAMS	<LLOQ <LLOQ	48	5.53-28.57	0.38-1.37			NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-010816-AA-18	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-030816-AA-19	F	Bisoprolol	5 mg	DBS VAMS	<LLOQ	27	16.64-26.9	1.2-3	8	11	NO
903-030816-AA-20	M	Simvastatin	20 mg	DBS	<LLOQ	12	4.88-5.86	1.98-2.52	5	6	NO
903-030816-AA-21	F	Diltiazem	90 mg	DBS VAMS	<LLOQ <LLOQ	72	105.65-150.87	10.05-12.25	7	11	NO
903-030816-AA-22	M	Control		DBS VAMS	-	-	-	-	-	-	-
903-030816-AA-23	M	Atenolol	50 mg	DBS	900.06±22.75	9	159-377	1.5-6	8	11	NO (>CMAX)
903-030816-AA-24	F	Valsartan	80 mg	DBS VAMS	1231.40± 0.88 1232.33± 0.55	4	1010-2270	2	1	1	YES
903-030816-AA-25	F	Losartan	25 mg	DBS VAMS	19.04± 3.66 19.27± 2.50	10.5	43.6-125.4	0.5-1.1	2	3	YES
903-030816-AA-26	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-030816-AA-27	F	Bisoprolol	5 mg	DBS VAMS	15.96±3.12 15.5±2.24	13	16.64-26.9	1.2-3	2	2	YES
		Losartan	100 mg	DBS VAMS	120.30± 1.60 120.31± 1.30	13	263.67-783.41	0.54-1.88			YES
903-030816-AA-28	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-030816-AA-29	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-040816-AA-30	M	Bisoprolol	5 mg	DBS VAMS	23.60± 4.82 23.90± 3.75	3	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	277.13± 2.27 277.12± 2.15	3	1010-2270	2			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-040816-AA-31	M	Valsartan	80 mg	DBS VAMS	<LLOQ <LLOQ	20	1010-2270	2	5	8	NO
903-040816-AA-32	F	Lisinopril	10 mg	DBS VAMS	55.65± 5.92 55.68± 4.65	8	41.75-80.47	5.79-6.91	2	2	YES
903-040816-AA-33	M	Losartan	25 mg	DBS VAMS	<LLOQ <LLOQ	48	43.6-125.4	0.5-1.1	8	9	NO
903-050816-AA-34	M	Atenolol	50 mg	DBS	211.04±21.64	6	159-377	1.5-6	2	2	YES
903-050816-AA-35	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-050816-AA-36	F	Bisoprolol	5 mg	DBS VAMS	6.74± 0.702 5.83± 0.66	3	16.64-26.9	1.2-3	5	7	YES
		Valsartan	160 mg	DBS VAMS	<LLOQ <LLOQ	32	1930-4000	1.5-3			NO
903-050816-AA-37	M	Atenolol	50 mg	DBS VAMS	86.11±2.05 85.9±2.02	11	159-377	1.5-6	3	3	YES
903-050816-AA-38	M	Bisoprolol	5 mg	DBS	11.90± 0.67	15	16.64-26.9	1.2-3	5	7	YES
		Losartan	50 mg	DBS	<LLOQ	15	89.1-306.1	0.5-2.2			NO
903-050816-AA-39	F	Control		DBS VAMS	-	-	-	-	-	-	-
903-050816-AA-40	F	Atenolol	50 mg	DBS VAMS	82.91±5.77 82.7±5.67	20	159-377	1.5-6	1	1	YES
903-050816-AA-41	F	Losartan	25 mg	DBS	<LLOQ	51	43.6-125.4	0.5-1.1	1	1	NO
903-050816-AA-42	M	Atenolol	50 mg	DBS	159.25±18.26	3.5	159-377	1.5-6	2	2	YES
903-050816-AA-43	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-050816-AA-44	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-060816-AA-45	M	Atenolol	50 mg	DBS VAMS	639.18±17.9 639.4±17.85	10	159-377	1.5-6	4	6	NO (>CMAX)

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-060816-AA-46	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-060816-AA-47	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-060816-AA-48	M	Bisoprolol	5 mg	DBS VAMS	5.13± 0.20 5.29± 0.17	21	16.64-26.9	1.2-3	8	12	YES
		Valsartan	80 mg	DBS VAMS	<LLOQ <LLOQ	36	1010-2270	2			NO
903-060816-AA-49	M	Control		DBS	-	-	-	-	-	-	-
903-060816-AA-50	M	Losartan	100 mg	DBS	37.68± 1.10	12	263.67-783.41	0.54-1.88	2	2	YES
903-060816-AA-51	M	Atenolol	100 mg	DBS	706.98± 20.46	10	240-1370	2-4	4	5	YES
903-060816-AA-52	M	Atenolol	50 mg	DBS VAMS	81.89± 0.69 82.3± 0.62	8	159-377	1.5-6	4	6	YES
903-060816-AA-53	M	Atenolol	100 mg	DBS VAMS	1524.11±10.53 1525.3±10.44	13	240-1370	2-4	5	8	NO (>CMAX)
903-060816-AA-54	F	Control	-	DBS	-	-	-	-	-	-	-
903-070816-AA-55	F	Losartan	100 mg	DBS	297.80± 1.98	2	263.67-783.41	0.54-1.88	5	7	YES
		Atorvastatin	40 mg	DBS	<LLOQ	12	5.53-28.57	0.38-1.37			NO
903-070816-AA-56	F	Atorvastatin	40 mg	DBS	<LLOQ	12.5	5.53-28.57	0.38-1.37	3	4	NO
		Lisinopril	10 mg	DBS	<LLOQ	23.5	41.75-80.47	5.79-6.91			NO
903-070816-AA-57	F	Bisoprolol	5 mg	DBS	14.86± 1.30	2.5	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	34.33± 6.55	2.5	41.75-80.47	5.79-6.91			YES
903-070816-AA-58	M	Atenolol	50 mg	DBS	491.15± 33.77	15	159-377	1.5-6	8	12	NO (>CMAX)
		Atorvastatin	40 mg	DBS	<LLOQ	15	5.53-28.57	0.38-1.37			NO
903-070816-AA-59	F	Atorvastatin	40 mg	DBS VAMS	<LLOQ <LLOQ	76	5.53-28.57	0.38-1.37	7	9	NO
		Bisoprolol	5 mg	DBS VAMS	<LLOQ <LLOQ	76	16.64-26.9	1.2-3			NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-070816-AA-60	F	Diltiazem	90 mg	DBS	<LLOQ	38	105.65-150.87	10.05-12.25	8	11	NO
903-070816-AA-61	M	Diltiazem	60 mg	DBS VAMS	25.17± 0.57 25.66± 0.32	1	74.72-82.38	2.23-2.49	8	10	YES
		Losartan	50 mg	DBS VAMS	<LLOQ <LLOQ	13	89.1-306.1	0.5-2.2			NO
		Valsartan	160 mg	DBS VAMS	<LLOQ <LLOQ	72	1930-4000	1.5-3			NO
903-070816-AA-62	M	Atorvastatin	40 mg	DBS	<LLOQ	48	5.53-28.57	0.38-1.37	5	6	NO
903-070816-AA-63	F	Control	-	DBS	-	-	-	-	-	-	-
903-070816-AA-64	M	Atorvastatin	40 mg	DBS	<LLOQ	25.5	5.53-28.57	0.38-1.37	8	12	NO
903-070816-AA-65	M	Diltiazem	60 mg	DBS	38.22± 2.58	7.5	74.72-82.43	2.23-2.49	2	2	YES
903-080816-AA-66	F	Lisinopril	10 mg	DBS VAMS	38.61±6.30 39.48±5.27	13	41.75-80.47	5.79-6.91	1	1	YES
903-080816-AA-67	M	Atenolol	100 mg	DBS VAMS	1276.61±23.27 1274.8±21.88	10	240-1370	2-4	1	1	YES
903-080816-AA-68	M	Valsartan	80 mg	DBS VAMS	115.93±2.12 114.43±2.11	11	1010-2270	2	4	5	YES
903-080816-AA-69	M	Losartan	50 mg	DBS VAMS	37.66±1.40 37.93±1.55	8	89.1-306.1	0.5-2.2	2	3	YES
903-080816-AA-70	M	Atorvastatin	40 mg	DBS VAMS	14.04±4.13 15.63±3.12	12	5.53-28.57	0.38-1.37	1	1	YES
		Bisoprolol	5 mg	DBS VAMS	24.72±3.15 24.70±3.10	2	16.64-26.9	1.2-3			YES
903-080816-AA-71	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-090816-AA-72	F	Atorvastatin	40 mg	DBS	<LLOQ	32	5.53-28.57	0.38-1.37	3	3	NO
		Diltiazem	60 mg	DBS	51.56±2.20	8.5	74.72-82.43	2.23-2.49			YES
		Valsartan	160 mg	DBS	<LLOQ	22.5	1930-4000	1.5-3			NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-090816-AA-73	F	Valsartan	80 mg	DBS VAMS	<LLOQ <LLOQ	7.5	1010-4501	1.55-2.2	3	4	NO
903-090816-AA-74	M	Valsartan	80 mg	DBS VAMS	<LLOQ <LLOQ	11	1010-2270	2	7	12	NO
903-090816-AA-75	F	Atorvastatin	40 mg	DBS VAMS	<LLOQ <LLOQ	27.5	5.53-28.57	0.38-1.37	4	4	NO
906-090816-AA-76	M	Diltiazem	90 mg	DBS VAMS	87.26±3.43 87.42±2.39	11	105.65-150.87	10.05-12.25	1	1	YES
903-090816-AA-77	M	Lisinopril	10 mg	DBS VAMS	33.30±2.87 32.71±2.75	10	41.75-80.47	5.79-6.91	3	4	YES
903-090816-AA-78	M	Valsartan	160 mg	DBS VAMS	225.22±4.82 226.80±4.77	6	1930-4000	1.5-3	2	2	YES
903-090816-AA-79	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-100816-AA-80	M	Valsartan	80 mg	DBS	<LLOQ	26	1010-2270	2	7	9	NO
903-100816-AA-81	F	Control		DBS VAMS	-	-	-	-	-	-	-
903-100816-AA-82	M	Atorvastatin	40 mg	DBS VAMS	8.78±1.31 8.89±1.22	13	5.53-28.57	0.38-1.37	4	5	YES
		Valsartan	80 mg	DBS VAMS	<LLOQ <LLOQ	20	1010-2270	2			NO
903-100816-AA-83	M	Control	-	DBS	-	-		-	-	-	-
903-100816-AA-84	M	Valsartan	160 mg	DBS VAMS	3493.72 ± 8.78 3493.76±4.30	3.5	1930-4000	1.5-3	4	6	YES
903-100816-AA-85	F	Control	-	DBS	-	-		-	-	-	-
903-100816-AA-86	F	Diltiazem	60 mg	DBS VAMS	8.86±0.34 8.77±0.30	18	74.72-82.43	2.23-2.49	2	2	YES
903-100816-AA-87	F	Bisoprolol	5 mg	DBS	3.82±0.34	13	16.64-26.9	1.2-3	1	1	YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-100816-AA-88	M	Atorvastatin	40 mg	DBS	<LLOQ	26.5	5.53-28.57	0.38-1.37	3	4	NO
903-100816-AA-89	F	Valsartan	160 mg	DBS VAMS	419.41±2.89 417.45±2.77	15.5	1930-4000	1.5-3	4	6	YES
903-110816-AA-90	M	Bisoprolol	5 mg	DBS	3.97±0.32	19	16.64-26.9	1.2-3	2	2	YES
903-120816-AA-91	M	Bisoprolol	5 mg	DBS VAMS	18.13±0.09 17.88±0.07	1	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS VAMS	24.97±2.05 24.88±2.15	1	41.75-80.47	5.79-6.91			YES
903-120816-AA-92	F	Control	-	DBS	-	-	-	-	-	-	-
903-120816-AA-93	M	Lisinopril	10 mg	DBS	45.86±2.87	10	41.75-80.47	5.79-6.91	2		YES
903-120816-AA-94	M	Control	-	DBS	-	-	-	-	-	-	-
903-150816-AA-95	M	Bisoprolol	5 mg	DBS	<LLOQ	48	16.64-26.9	1.2-3	3	3	NO
903-150816-AA-96	M	Bisoprolol	5 mg	DBS	3.38±0.13	18	16.64-26.9	1.2-3	2	2	YES
903-150816-AA-97	M	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-150816-AA-98	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-150816-AA-99	F	Bisoprolol	5 mg	DBS VAMS	11.39±0.36 11.10±0.31	4.5	16.64-26.9	1.2-3	3	2	YES
		Valsartan	160 mg	DBS VAMS	<LLOQ <LLOQ	15	1930-4000	1.5-3			NO
903-270717-AA-100	M	Atenolol	50 mg	DBS	72.72±0.28	10	159-377	1.5-6	2	2	YES
		Simvastatin	40 mg	DBS	2.73±0.33	12	5-40	2-3			YES
903-270717-AA-101	M	Atenolol	50 mg	DBS	107.53±0.36	9	159-377	1.5-6	2	2	YES
		Simvastatin	40 mg	DBS	2.23±0.18	12	5-40	2-3			YES
903-270717-AA-102	M	Atenolol	100 mg	DBS	396.83±1.08	11	240-1370	2-4	2	2	YES
		Simvastatin	40 mg	DBS	1.26±0.19	11	5-40	2-3			YES
903-200717-AA-103	M	Atenolol	50 mg	DBS	198±1.73	11	159-377	1.5-6	3	3	YES
		Simvastatin	40 mg	DBS	2.96±0.37	11	5-40	2-3			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-200717-AA-104	M	Atenolol	50 mg	DBS	131.72±1.14	11	159-377	1.5-6	2	2	YES
		Lisinopril	10 mg	DBS	36.29±0.60	12	41.75-80.47	5.79-6.91			YES
903-200717-AA-105	M	Atenolol	50 mg	DBS	246.27±1.43	16	159-377	1.5-6	2	2	YES
		Lisinopril	10 mg	DBS	20.48±0.88	16	41.75-80.47	5.79-6.91			YES
903-200717-AA-106	M	Atenolol	100 mg	DBS	508.05±3.06	12	240-1370	2-4	2	2	YES
		Lisinopril	10 mg	DBS	20.62±1.06	12	41.75-80.47	5.79-6.91			YES
903-200717-AA-107	M	Atenolol	100 mg	DBS	596.14±2.40	5.5	240-1370	2-4	3	3	YES
		Lisinopril	10 mg	DBS	<LLOQ	12.5	41.75-80.47	5.79-6.91			NO
903-200717-AA-108	M	Bisoprolol	5 mg	DBS	4.32±0.13	15	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	<LLOQ	15	41.75-80.47	5.79-6.91			NO
903-200717-AA-109	M	Bisoprolol	5 mg	DBS	12.17±0.25	10	16.64-26.9	1.2-3	3	3	YES
		Lisinopril	10 mg	DBS	<LLOQ	14	41.75-80.47	5.79-6.91			NO
903-200717-AA-110	M	Bisoprolol	5 mg	DBS	21.68±0.49	2	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	22.99±0.82	12	41.75-80.47	5.79-6.91			YES
903-200717-AA-111	M	Bisoprolol	5 mg	DBS	17.86±0.29	2.5	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	27.76±0.86	11	41.75-80.47	5.79-6.91			YES
903-200717-AA-112	M	Bisoprolol	5 mg	DBS	13.70±0.14	7	16.64-26.9	1.2-3	3	4	YES
		Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2			NO
903-200717-AA-113	M	Bisoprolol	5 mg	DBS VAMS	24.51±0.11 24.14±0.10	2	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2259.01±6.23 2259.67±1.63	2	1010-2270	2			YES
903-200717-AA-114	M	Bisoprolol	5 mg	DBS VAMS	22.83±0.13 22.23±0.33	3	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2268.49±3.14 2268.65±1.80	3	1010-2270	2			YES
903-200717-AA-115	F	Bisoprolol	5 mg	DBS VAMS	21.32±0.13 21.15±0.18	3	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2229.06±9.17 2230.55±3.41	3	1010-2270	2			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-200717-AA-116	F	Bisoprolol	5 mg	DBS	26.09±0.16	2.5	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2			NO
903-200717-AA-117	F	Diltiazem	60 mg	DBS	10.62±0.64	11.5	74.72-82.43	2.23-2.49	2	2	YES
		Lisinopril	10 mg	DBS	43.68±2.07	15	41.75-80.47	5.79-6.91			YES
903-200717-AA-118	M	Diltiazem	90 mg	DBS	128.30±1.23	9	105.65-150.87	10.05-12.25	2	2	YES
		Lisinopril	10 mg	DBS	23.61±1.26	9	41.75-80.47	5.79-6.91			YES
903-200717-AA-119	M	Diltiazem	90 mg	DBS VAMS	54.48±1.24 54.39±1.22	15	105.65-150.87	10.05-12.25	4	5	YES
		Lisinopril	10 mg	DBS VAMS	54.88±1.52 54.51±0.27	15	41.75-80.47	5.79-6.91			YES
903-200717-AA-120	M	Diltiazem	90 mg	DBS VAMS	128.30±1.23 128.36±1.85	9.5	105.65-150.87	10.05-12.25	4	4	YES
		Lisinopril	10 mg	DBS VAMS	<LLOQ <LLOQ	15	41.75-80.47	5.79-6.91			NO
903-210717-AA-121	M	Losartan	50 mg	DBS	125.08±4.23	5	89.1-306.1	0.5-2.2	4	4	YES
		Simvastatin	40 mg	DBS	<LLOQ	15	5-40	2-3			NO
903-210717-AA-122	F	Losartan	50 mg	DBS	132.20±4.67	2.5	89.1-306.1	0.5-2.2	4	5	YES
		Simvastatin	40 mg	DBS	<LLOQ	12	5-40	2-3			NO
903-210717-AA-123	M	Losartan	50 mg	DBS VAMS	45.33±1.20 44.88±0.08	3	89.1-306.1	0.5-2.2	2	2	YES
		Simvastatin	40 mg	DBS VAMS	5.33±0.04 5.28±0.01	11	5-40	2-3			YES
903-210717-AA-124	F	Losartan	50 mg	DBS VAMS	<LLOQ	2.5	89.1-306.1	0.5-2.2	2	2	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	11	5-40	2-3			NO
903-210717-AA-125	F	Amlodipine	5 mg	DBS	6.67±0.19	5.5	5-7.5	5-8	2	3	YES
903-210717-AA-126	M	Amlodipine	5 mg	DBS	4.32±0.07	6	5-7.5	5-8	2	2	YES
903-210717-AA-127	F	Amlodipine	5 mg	DBS VAMS	5.26±0.23 5.19±0.02	6.5	5-7.5	5-8	2	2	YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-210717-AA-128	M	Amlodipine	5 mg	DBS VAMS	5.18±0.23 5.17±0.03	8.5	5-7.5	5-8	2	2	YES
903-210717-AA-129	F	Losartan	50 mg	DBS VAMS	51.46±0.14 51.68±0.77	5	89.1-306.1	0.5-2.2	3	3	YES
903-210717-AA-130	M	Losartan	50 mg	DBS VAMS	49.53±1.99 49.08±1.46	5	89.1-306.1	0.5-2.2	2	3	YES
903-220717-AA-131	M	Losartan	50 mg	DBS VAMS	99.57±3.01 99.69±2.92	2.5	89.1-306.1	0.5-2.2	2	2	YES
903-220717-AA-132	M	Simvastatin	40 mg	DBS VAMS	5.86±0.35 5.96±0.25	11	5-40	2-3	2	3	YES
903-220717-AA-133	M	Simvastatin	40 mg	DBS VAMS	<LLOQ	11.5	5-40	2-3	4	5	NO
903-220717-AA-134	M	Valsartan	80 mg	DBS	<LLOQ	10	1010-2270	2	2	2	NO
903-220717-AA-135	M	Valsartan	80 mg	DBS VAMS	<LLOQ	8	1010-2270	2	2	2	NO
903-220717-AA-136	M	Valsartan	80 mg	DBS VAMS	1869.35±4.33 1869.94±4.29	5.5	1010-2270	2	2	3	YES
903-220717-AA-137	F	Valsartan	80 mg	DBS VAMS	1577.27±4.00 1577.57±2.1	6	1010-2270	2	1	1	YES
903-220717-AA-138	F	Atenolol	50 mg	DBS	120.53±1.34	10.5	159-377	1.5-6	4	4	YES
		Simvastatin	40 mg	DBS	2.87±0.05	13	5-40	2-3			YES
903-220717-AA-139	M	Atenolol	100 mg	DBS	502.29±1.10	10	240-1370	2-4	2	2	YES
		Simvastatin	40 mg	DBS	4.97±0.25	10	5-40	2-3			YES
903-220717-AA-140	F	Atenolol	50 mg	DBS	218.80±0.9	6	159-377	1.5-6	2	2	YES
		Simvastatin	40 mg	DBS	4.49±0.23	12	5-40	2-3			YES
903-230717-AA-141	M	Atenolol	100 mg	DBS	517.23±1.45	6	240-1370	2-4	2	2	YES
		Simvastatin	40 mg	DBS	4.73±0.38	10.5	5-40	2-3			YES
903-230717-AA-142	F	Atenolol	50 mg	DBS	145.55±2.63	10.5	159-377	1.5-6	2	2	YES
		Lisinopril	10 mg	DBS	26.64±1.00	10.5	41.75-80.47	5.79-6.91			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-230717-AA-143	M	Atenolol	100 mg	DBS	504.20±2.77	12	240-1370	2-4	2	2	YES
		Lisinopril	10 mg	DBS	24.44±1.02	12	41.75-80.47	5.79-6.91			YES
903-230717-AA-144	M	Atenolol	100 mg	DBS	462±3.75	6	240-1370	2-4	3	3	YES
		Lisinopril	10 mg	DBS	39.05±0.52	11.5	41.75-80.47	5.79-6.91			YES
903-230717-AA-145	M	Bisoprolol	5 mg	DBS	6.60±0.20	16	16.64-26.9	1.2-3	4	5	YES
		Lisinopril	10 mg	DBS	<LLOQ	16	41.75-80.47	5.79-6.91			NO
903-230717-AA-146	M	Bisoprolol	5 mg	DBS	9.82±0.27	10	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	<LLOQ	10	41.75-80.47	5.79-6.91			NO
903-230717-AA-147	F	Bisoprolol	5 mg	DBS	22.11±0.67	3	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	24.74±0.82	12	41.75-80.47	5.79-6.91			YES
903-230717-AA-148	M	Bisoprolol	5 mg	DBS VAMS	25.96±0.16 24.35±0.22	3	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2224.41±4.90 2223.42±1.55	3	1010-2270	2			YES
903-230717-AA-149	F	Bisoprolol	5 mg	DBS	11.44±0.31	7.5	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS	546.67±9.29	14	1010-2270	2			YES
903-230717-AA-150	F	Bisoprolol	5 mg	DBS VAMS	25.50±0.18 25.57±0.18	2.5	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2030.86±2.77 2030.82±1.14	2.5	1010-2270	2			YES
903-230717-AA-151	F	Control	-	DBS VAMS	-	-	-	-	-	-	-
903-240717-AA-152	M	Diltiazem	60 mg	DBS	49.30±0.54	9.5	74.72-82.43	2.23-2.49	2	2	YES
		Lisinopril	10 mg	DBS	23.82±0.69	9.5	41.75-80.47	5.79-6.91			YES
903-240717-AA-153	M	Diltiazem	60 mg	DBS	18.14±0.82	9.5	74.72-82.43	2.23-2.49	5	7	YES
		Lisinopril	10 mg	DBS	24.82±0.44		41.75-80.47	5.79-6.91			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-240717-AA-154	F	Diltiazem	60 mg	DBS VAMS	53.42±1.74 53.52±0.67	5.5	74.72-82.43	2.23-2.49	3	3	YES
		Lisinopril	10 mg	DBS VAMS	28.04±1.52 27.88±0.10	15.5	41.75-80.47	5.79-6.91			YES
903-240717-AA-155	F	Diltiazem	90 mg	DBS VAMS	135.50±1.78 134.81±1.46	9	105.65-150.87	10.05-12.25	2	2	YES
		Lisinopril	10 mg	DBS VAMS	25.04±1.33 25.02±0.99	15	41.75-80.47	5.79-6.91			YES
903-240717-AA-156	M	Losartan	100 mg	DBS VAMS	46.10±0.14 45.65±0.09	3.5	263.67-783.41	0.54-1.88	2	2	YES
		Simvastatin	40 mg	DBS VAMS	5.31±0.04 5.33±0.01	12	5-40	2-3			YES
903-240717-AA-157	F	Losartan	50 mg	DBS	<LLOQ	3	89.1-306.1	0.5-2.2	2	2	NO
		Simvastatin	40 mg	DBS	<LLOQ	12	5-40	2-3			NO
903-240717-AA-158	F	Losartan	50 mg	DBS	<LLOQ	5	89.1-306.1	0.5-2.2	2	2	NO
		Simvastatin	40 mg	DBS	<LLOQ	12.5	5-40	2-3			NO
903-240717-AA-159	F	Losartan	100 mg	DBS VAMS	<LLOQ	4	263.67-783.41	0.54-1.88	2	2	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	12	5-40	2-3			NO
903-240717-AA-160	M	Amlodipine	5 mg	DBS	6.23±0.10	5.5	5-7.5	5-8	3	4	YES
903-240717-AA-161	F	Amlodipine	5 mg	DBS	6.39±0.22	5	5-7.5	5-8	2	2	YES
903-250717-AA-162	F	Amlodipine	5 mg	DBS VAMS	3.41±0.27 3.40±0.02	6	5-7.5	5-8	2	2	YES
903-250717-AA-163	M	Amlodipine	5 mg	DBS VAMS	5.28±0.20 5.23±0.02	8.5	5-7.5	5-8	5	7	YES
903-250717-AA-164	M	Losartan	50 mg	DBS VAMS	204.72±1.13 204.63±1.33	3	89.1-306.1	0.5-2.2	7	11	YES
903-250717-AA-165	M	Losartan	50 mg	DBS VAMS	250.10±0.58 250.50±0.54	2.5	89.1-306.1	0.5-2.2	2	2	YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-250717-AA-166	F	Losartan	50 mg	DBS VAMS	175.19±0.74 175.14±1.25	4.5	89.1-306.1	0.5-2.2	2	2	YES
903-250717-AA-167	M	Simvastatin	40 mg	DBS VAMS	6.14±0.28 6.25±0.20	11.5	5-40	2-3	7	11	YES
903-250717-AA-168	M	Simvastatin	40 mg	DBS VAMS	<LLOQ <LLOQ	11.5	5-40	2-3	2	2	NO
903-250717-AA-169	F	Atenolol	50 mg	DBS	148.55±2.19	11	159-377	1.5-6	2	2	YES
		Simvastatin	40 mg	DBS	2.48±0.29	12.5	5-40	2-3			YES
903-250717-AA-170	M	Atenolol	100 mg	DBS	555.30±2.28	10.5	240-1370	2-4	2	2	YES
		Simvastatin	40 mg	DBS	2.70±0.14	10.5	5-40	2-3			YES
903-270717-AA-171	F	Atenolol	50 mg	DBS	218.63±1.17	5	159-377	1.5-6	2	2	YES
		Simvastatin	40 mg	DBS	3.43±0.15	12	5-40	2-3			YES
903-270717-AA-172	M	Atenolol	100 mg	DBS	524.55±1.82	6.5	240-1370	2-4	4	4	YES
		Simvastatin	40 mg	DBS	2.48±0.28	12.5	5-40	2-3			YES
903-270717-AA-173	F	Atenolol	50 mg	DBS	112.38±1.24	10.5	159-377	1.5-6	4	5	YES
		Lisinopril	10 mg	DBS	24.37±0.59	10.5	41.75-80.47	5.79-6.91			YES
903-270717-AA-174	F	Atenolol	50 mg	DBS	200.61±2.94	16	159-377	1.5-6	4	4	YES
		Lisinopril	10 mg	DBS	19.02±0.33	16	41.75-80.47	5.79-6.91			YES
903-270717-AA-175	F	Atenolol	100 mg	DBS	494.09±2.21	12	240-1370	2-4	2	2	YES
		Lisinopril	10 mg	DBS	22.69±1.33	12	41.75-80.47	5.79-6.91			YES
903-270717-AA-176	M	Atenolol	100 mg	DBS	503.30±1.91	6.5	240-1370	2-4	6	8	YES
		Lisinopril	10 mg	DBS	21.76±0.48	10	41.75-80.47	5.79-6.91			YES
903-270717-AA-177	F	Bisoprolol	5 mg	DBS	4.63±0.18	15.5	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	<LLOQ	15.5	41.75-80.47	5.79-6.91			NO
903-270717-AA-178	M	Bisoprolol	5 mg	DBS	22.99±0.42	3	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	23.44±0.85	11.5	41.75-80.47	5.79-6.91			YES
903-270717-AA-179	M	Control	-	DBS	-	-	-	-	-	-	-
903-270717-AA-180	F	Bisoprolol	5 mg	DBS	5.47±0.18	15	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	<LLOQ	15	41.75-80.47	5.79-6.91			NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-280717-AA-181	M	Bisoprolol	5 mg	DBS	8.36±0.20	8	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	<LLOQ	12	41.75-80.47	5.79-6.91			NO
903-280717-AA-182	F	Bisoprolol	5 mg	DBS	25.30±0.17	1.5	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	27.54±0.84	12.5	41.75-80.47	5.79-6.91			YES
903-280717-AA-183	F	Bisoprolol	5 mg	DBS	18.59±0.28	10	16.64-26.9	1.2-3	6	6	YES
		Valsartan	80 mg	DBS	538.56±9.28	15	1010-2270	2			YES
903-280717-AA-184	M	Bisoprolol	5 mg	DBS VAMS	24.56±0.46 24.30±0.29	2.5	16.64-26.9	1.2-3	3	3	YES
		Valsartan	80 mg	DBS VAMS	1988.35±2.12 1988.51±1.58	2.5	1010-2270	2			YES
903-280717-AA-185	M	Bisoprolol	5 mg	DBS	24.77±0.72	3	16.64-26.9	1.2-3	4	4	YES
		Valsartan	80 mg	DBS	531.77±5.12	15	1010-2270	2			YES
903-280717-AA-186	F	Diltiazem	60 mg	DBS	20.41±0.96	10.5	74.72-82.43	2.23-2.49	5	7	YES
		Lisinopril	10 mg	DBS	<LLOQ	15	41.75-80.47	5.79-6.91			NO
903-280717-AA-187	M	Diltiazem	90 mg	DBS	134.03±1.24	9	105.65-150.87	10.05-12.25	2	2	YES
		Lisinopril	10 mg	DBS	27.55±1.97	9	41.75-80.47	5.79-6.91			YES
903-280717-AA-188	M	Diltiazem	90 mg	DBS VAMS	57.88±0.90 57.59±0.86	15	105.65-150.87	10.05-12.25	5	6	YES
		Lisinopril	10 mg	DBS VAMS	28.55±1.88 27.88±1.22	15	41.75-80.47	5.79-6.91			YES
903-280717-AA-189	F	Diltiazem	90 mg	DBS VAMS	83.33±0.65 83.08±0.93	9	105.65-150.87	10.05-12.25	2	2	YES
		Lisinopril	10 mg	DBS VAMS	<LLOQ <LLOQ	12	41.75-80.47	5.79-6.91			NO
903-280717-AA-190	F	Losartan	50 mg	DBS	<LLOQ	5	89.1-306.1	0.5-2.2	5	5	NO
		Simvastatin	40 mg	DBS	<LLOQ	12	5-40	2-3			NO
903-290717-AA-191	F	Losartan	100 mg	DBS VAMS	259.02±3.44 260.51±1.56	3	263.67-783.41	0.54-1.88	2	2	YES
		Simvastatin	40 mg	DBS VAMS	3.68±0.35 3.80±0.10	11.5	5-40	2-3			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-290717-AA-192	F	Losartan	100 mg	DBS VAMS	312.79±2.52 311.94±2.46	3	263.67-783.41	0.54-1.88	6	8	YES
		Simvastatin	40 mg	DBS VAMS	5.61±0.29 5.47±0.02	12.5	5-40	2-3			YES
903-290717-AA-193	F	Losartan	100 mg	DBS VAMS	<LLOQ	4.5	263.67- 783.41	0.54-1.88	5	6	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	15	5-40	2-3			NO
903-290717-AA-194	M	Losartan	100 ng	DBS VAMS	244.74±2.64 245.95±2.05	3.5	263.67-783.41	0.54-1.88	2	2	YES
		Simvastatin	40 mg	DBS VAMS	6.35±0.25 6.27±0.09	12	5-40	2-3			YES
903-290717-AA-195	M	Amlodipine	5 mg	DBS	<LLOQ	5.5	5-7.5	5-8	2	2	NO
903-290717-AA-196	M	Amlodipine	5 mg	DBS VAMS	<LLOQ	5	5-7.5	5-8	2	2	NO
903-290717-AA-197	M	Amlodipine	5 mg	DBS VAMS	6.22±0.17 5.99±0.19	8	5-7.5	5-8	2	3	YES
903-290717-AA-198	M	Amlodipine	5 mg	DBS VAMS	<LLOQ <LLOQ	8	5-7.5	5-8	6	10	NO
903-290717-AA-199	F	Losartan	50 mg	DBS VAMS	155.70±1.72 156.89±1.11	5	89.1-306.1	0.5-2.2	2	3	YES
903-290717-AA-200	M	Losartan	50 mg	DBS VAMS	137.29±2.04 137.82±2.25	3	89.1-306.1	0.5-2.2	2	2	YES
903-300717-AA-201	M	Losartan	50 mg	DBS VAMS	167.60±1.73 167.16±1.67	3.5	89.1-306.1	0.5-2.2	5	7	YES
903-300717-AA-202	M	Simvastatin	40 mg	DBS VAMS	<LLOQ	11	5-40	2-3	5	8	NO
903-300717-AA-203	M	Control	-	DBS	-	-	-	-	-	-	-
903-300717-AA-204	M	Valsartan	80 mg	DBS VAMS	<LLOQ	8.5	1010-2270	2	7	10	NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-300717-AA-205	M	Valsartan	80 mg	DBS VAMS	978.56±3.49 975.96±1.95	6.5	1010-2270	2	2	2	YES
903-300717-AA-206	F	Atenolol	50 mg	DBS	147.32±1.15	8	159-377	1.5-6	4	5	YES
		Simvastatin	40 mg	DBS	2.62±0.19	14	5-40	2-3			YES
903-300717-AA-207	M	Atenolol	100 mg	DBS	507.74±1.94	11	240-1370	2-4	5	8	YES
		Simvastatin	40 mg	DBS	4.35±0.46	11	5-40	2-3			YES
903-300717-AA-208	F	Atenolol	50 mg	DBS	202.57±1.80	5.5	159-377	1.5-6	7	8	YES
		Simvastatin	40 mg	DBS	2.44±0.23	11.5	5-40	2-3			YES
903-300717-AA-209	F	Atenolol	100 mg	DBS	195.07±2.23	16.5	240-1370	2-4	5	6	YES
		Lisinopril	10 mg	DBS	18.33±0.87	16.5	41.75-80.47	5.79-6.91			YES
903-300717-AA-210	F	Atenolol	100 mg	DBS	522.22±1.74	10	240-1370	2-4	2	2	YES
		Lisinopril	10 mg	DBS	19.30±0.70	10	41.75-80.47	5.79-6.91			YES
903-300717-AA-211	F	Atenolol	100 mg	DBS	517.49±1.73	5.5	240-1370	2-4	6	9	YES
		Lisinopril	10 mg	DBS	<LLOQ	12	41.75-80.47	5.79-6.91			NO
903-300717-AA-212	F	Bisoprolol	5 mg	DBS	5.41±0.21	16	16.64-26.9	1.2-3	6	10	YES
		Lisinopril	10 mg	DBS	<LLOQ	16	41.75-80.47	5.79-6.91			NO
903-300717-AA-213	M	Diltiazem	90 mg	DBS	137.58±1.14	9	105.65-150.87	10.05-12.25	2	2	YES
		Lisinopril	10 mg	DBS	25.13±1.42	9	41.75-80.47	5.79-6.91			YES
903-300717-AA-214	M	Bisoprolol	5 mg	DBS	21.66±0.37	2.5	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	22.36±0.67	12.5	41.75-80.47	5.79-6.91			YES
903-030717-AA-215	M	Bisoprolol	5 mg	DBS VAMS	25.68±0.29 25.39±0.22	3	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2249.4±5.1 2250.77±2.67	3	1010-2270	2			YES
903-030717-AA-216	F	Bisoprolol	5 mg	DBS	26.28±0.25	1.5	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS	2261.12±5.37	1.5	1010-2270	2			YES
903-030717-AA-217	M	Bisoprolol	5 mg	DBS	20.36±0.26	2	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2			NO
903-300717-AA-218	F	Diltiazem	60 mg	DBS	11.94±0.65	10.5	74.72-82.43	2.23-2.49	6	8	YES
		Lisinopril	10 mg	DBS	<LLOQ	10.5	41.75-80.47	5.79-6.91			NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-300717-AA-219	F	Diltiazem	90 mg	DBS	102.93±2.17	9	105.65-150.87	10.05-12.25	2	2	YES
		Lisinopril	10 mg	DBS	26.68±2.16	9	41.75-80.47	5.79-6.91			YES
903-300717-AA-220	F	Diltiazem	90 mg	DBS VAMS	<LLOQ	9.5	105.65-150.87	10.05-12.25	7	8	NO
		Lisinopril	10 mg	DBS VAMS	<LLOQ	13	41.75-80.47	5.79-6.91			NO
903-310717-AA-221	F	Losartan	100 mg	DBS VAMS	<LLOQ	3.5	263.67-783.41	0.54-1.88	4	5	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	12	5-40	2-3			NO
903-310717-AA-222	M	Amlodipine	5 mg	DBS	5.28±0.07	5.5	5-7.5	5-8	2	2	YES
903-310717-AA-223	F	Losartan	50 mg	DBS VAMS	49.53±1.99 49.08±1.46	5	89.1-306.1	0.5-2.2	2	2	YES
903-310717-AA-224		Valsartan	80 mg	DBS	<LLOQ	16	1010-2270	2	2	2	NO
903-310717-AA-225	M	Valsartan	80 mg	DBS VAMS	1861.79±3.59 1861.77±2.44	7.5	1010-2270	2	3	4	YES
903-310717-AA-226	F	Atenolol	100 mg	DBS	506.76±1.22	11	240-1370	2-4	2	2	YES
		Simvastatin	40 mg	DBS	1.56±0.27	11	5-40	2-3			YES
903-310717-AA-227	F	Atenolol	50 mg	DBS	142.59±1.39	4.5	159-377	1.5-6	5	7	YES
		Simvastatin	40 mg	DBS	2.64±0.19	11	5-40	2-3			YES
903-310717-AA-228	F	Atenolol	100 mg	DBS	465.20±1.45	11	240-1370	2-4	2	2	YES
		Lisinopril	10 mg	DBS	21.37±1.24	11	41.75-80.47	5.79-6.91			YES
903-310717-AA-229	F	Atenolol	100 mg	DBS	426.05±3.53	7	240-1370	2-4	4	6	YES
		Lisinopril	10 mg	DBS	20.52±0.47	12.5	41.75-80.47	5.79-6.91			YES
903-310717-AA-230	M	Bisoprolol	5 mg	DBS	5.30±0.12	15.5	16.64-26.9	1.2-3	5	6	YES
		Lisinopril	10 mg	DBS	<LLOQ	15.5	41.75-80.47	5.79-6.91			NO
903-010817-AA-231	M	Bisoprolol	5 mg	DBS	12.65±0.21	9	16.64-26.9	1.2-3	5	5	YES
		Lisinopril	10 mg	DBS	<LLOQ	12	41.75-80.47	5.79-6.91			NO
903-010817-AA-232	M	Bisoprolol	5 mg	DBS	23.61±0.27	3	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	20.38±0.98	11	41.75-80.47	5.79-6.91			YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-010817-AA-233	F	Bisoprolol	5 mg	DBS VAMS	23.82±0.39 23.51±0.22	2.5	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2261.36±3.18 2259.69±3.03	2.5	1010-2270	2			YES
903-010817-AA-234	F	Bisoprolol	5 mg	DBS VAMS	24.45±0.49 22.71±0.26	1.5	16.64-26.9	1.2-3	2	2	YES
		Valsartan	80 mg	DBS VAMS	2245.79±5.46 2245.03±3.10	1.5	1010-2270	2			YES
903-010817-AA-235	F	Bisoprolol	5 mg	DBS	24.66±0.16	2.5	16.64-26.9	1.2-3	5	6	YES
		Valsartan	80 mg	DBS	<LLOQ	14	1010-2270	2			NO
903-010817-AA-236	F	Diltiazem	60 mg	DBS	<LLOQ	11.5	74.72-82.43	2.23-2.49	5	6	NO
		Lisinopril	10 mg	DBS	<LLOQ	15	41.75-80.47	5.79-6.91			NO
903-010817-AA-237	F	Diltiazem	90 mg	DBS	<LLOQ	9.5	105.65-150.87	10.05-12.25	2	2	NO
		Lisinopril	10 mg	DBS	<LLOQ	9.5	41.75-80.47	5.79-6.91			NO
903-010817-AA-238	F	Diltiazem	90 mg	DBS VAMS	<LLOQ	9.5	105.65-150.87	10.05-12.25	3	3	NO
		Lisinopril	10 mg	DBS VAMS	<LLOQ	12	41.75-80.47	5.79-6.91			NO
903-010817-AA-239	M	Losartan	100 mg	DBS VAMS	<LLOQ	3.5	263.67-783.41	0.54-1.88	4	4	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	15	5-40	2-3			NO
903-010817-AA-240	M	Amlodipine	5 mg	DBS VAMS	<LLOQ	8	5-7.5	5-8	5	7	NO
903-020817-AA-241	M	Valsartan	80 mg	DBS VAMS	<LLOQ	16.5	1010-2270	2	7	8	NO
903-020817-AA-242	M	Valsartan	80 mg	DBS VAMS	<LLOQ	7.5	1010-2270	2	2	2	NO
903-020817-AA-243	M	Valsartan	80 mg	DBS VAMS	1734.91±2.93 1734.98±1.25	7	1010-2270	2	2	2	YES

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-020817-AA-244	F	Atenolol	100 mg	DBS	415.62±1.29	8	240-1370	2-4	4	4	YES
		Lisinopril	10 mg	DBS	22.32±0.67	12.5	41.75-80.47	5.79-6.91			YES
903-020817-AA-245	F	Atenolol	100 mg	DBS	392.32±0.77	8	240-1370	2-4	3	4	YES
		Lisinopril	10 mg	DBS	21.32±0.57	11.5	41.75-80.47	5.79-6.91			YES
903-020817-AA-246	M	Bisoprolol	5 mg	DBS	20.80±0.20	3	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	15.43±0.74	11.5	41.75-80.47	5.79-6.91			YES
903-020817-AA-247	F	Diltiazem	90 mg	DBS VAMS	<LLOQ	9.5	105.65-150.87	10.05-12.25	4	5	NO
		Lisinopril	10 mg	DBS VAMS	<LLOQ	12.5	41.75-80.47	5.79-6.91			NO
903-020817-AA-248	F	Diltiazem	90 mg	DBS VAMS	<LLOQ	9	105.65-150.87	10.05-12.25	3	4	NO
		Lisinopril	10 mg	DBS VAMS	<LLOQ	14	41.75-80.47	5.79-6.91			NO
903-020817-AA-249	F	Losartan	100 mg	DBS VAMS	<LLOQ	4	263.67-783.41	0.54-1.88	5	5	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	12	5-40	2-3			NO
903-020817-AA-250	F	Losartan	100 mg	DBS VAMS	<LLOQ	4	263.67-783.41	0.54-1.88	7	10	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	12	5-40	2-3			NO
903-030817-AA-251	F	Losartan	100 mg	DBS VAMS	<LLOQ	4	263.67-783.41	0.54-1.88	3	3	NO
		Simvastatin	40 mg	DBS VAMS	<LLOQ	15	5-40	2-3			NO
903-030817-AA-252	F	Amlodipine	5 mg	DBS VAMS	<LLOQ	6.5	5-7.5	5-8	5	8	NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-030817-AA-253	M	Valsartan	80 mg	DBS VAMS	<LLOQ	7	1010-2270	2	5	7	NO
903-030817-AA-254	F	Valsartan	80 mg	DBS VAMS	1756.12±3.84 1754.24±3.68	8	1010-2270	2	2	3	YES
903-030817-AA-255	F	Valsartan	80 mg	DBS VAMS	1799.53±3.89 1799±1.33	8.5	1010-2270	2	3	3	YES
903-030817-AA-256	F	Atenolol	100 mg	DBS	445.50±1.75	5.5	240-1370	2-4	4	5	YES
		Lisinopril	10 mg	DBS	22.32±0.55	11.5	41.75-80.47	5.79-6.91			YES
903-030817-AA-257	F	Atenolol	100 mg	DBS	446.17±1.54	6	240-1370	2-4	4	5	YES
		Lisinopril	10 mg	DBS	21.12±0.55	13	41.75-80.47	5.79-6.91			YES
903-030817-AA-258	M	Atenolol	100 mg	DBS	486.90±2.02	11.5	240-1370	2-4	2	2	YES
		Lisinopril	10 mg	DBS	19.46±1.26	11.5	41.75-80.47	5.79-6.91			YES
903-030817-AA-259	F	Bisoprolol	5 mg	DBS	21.56±0.23	3	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	15.53±0.56	11.5	41.75-80.47	5.79-6.91			YES
903-030817-AA-260	F	Bisoprolol	5 mg	DBS	21.52±0.29	2.5	16.64-26.9	1.2-3	2	2	YES
		Lisinopril	10 mg	DBS	22.44±0.80	11	41.75-80.47	5.79-6.91			YES
903-030817-AA-261	M	Bisoprolol	5 mg	DBS	21.75±0.20	3	16.64-26.9	1.2-3	3	3	YES
		Valsartan	80 mg	DBS	<LLOQ	15	1010-2270	2			NO
903-040817-AA-262	M	Bisoprolol	5 mg	DBS	23.03±0.19	2	16.64-26.9	1.2-3	3	3	YES
		Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2			NO
903-040817-AA-263	M	Valsartan	80 mg	DBS	<LLOQ	8.5	1010-2270	2	5	7	NO
903-040817-AA-264	M	Atenolol	100 mg	DBS	<LLOQ	30	240-1370	2-4	7	11	NO
903-040817-AA-265	M	Losartan	100 mg	DBS	<LLOQ	28	263.67-783.41	0.54-1.88	6	7	NO
903-040817-AA-266	M	Valsartan	80 mg	DBS	<LLOQ	36	1010-2270	2	8	13	NO
903-040817-AA-267	F	Diltiazem	90 mg	DBS	<LLOQ	25	105.65-150.87	10.05-12.25	8	11	NO
903-040817-AA-268	F	Bisoprolol	5 mg	DBS	<LLOQ	33	16.64-26.9	1.2-3	7	11	NO
903-040817-AA-269	F	Diltiazem	90 mg	DBS	<LLOQ	36	105.65-150.87	10.05-12.25	6	8	NO
903-040817-AA-270	F	Atenolol	100 mg	DBS	<LLOQ	30	240-1370	2-4	8	8	NO
903-050817-AA-271	F	Losartan	50 mg	DBS	<LLOQ	12	89.1-306.1	0.5-2.2	6	6	NO
903-050817-AA-272	M	Bisoprolol	5 mg	DBS	<LLOQ	15	16.64-26.9	1.2-3	6	9	NO
903-050817-AA-273	F	Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2	6	7	NO

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903-050817-AA-274	M	Atenolol	100 mg	DBS	<LLOQ	36	240-1370	2-4	7	8	NO
903-050817-AA-275	F	Losartan	50 mg	DBS	<LLOQ	14	89.1-306.1	0.5-2.2	6	7	NO
903-050817-AA-276	F	Valsartan	80 mg	DBS	<LLOQ	33	1010-2270	2	6	10	NO
903-050817-AA-277	F	Diltiazem	90 mg	DBS	<LLOQ	16	105.65-150.87	10.05-12.25	8	10	NO
903-050817-AA-278	F	Bisoprolol	5 mg	DBS	<LLOQ	13	16.64-26.9	1.2-3	5	7	NO
903-050817-AA-279	F	Losartan	50 mg	DBS	<LLOQ	12	89.1-306.1	0.5-2.2	8	12	NO
903-050817-AA-280	F	Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2	6	7	NO
903-100817-AA-281	F	Bisoprolol	5 mg	DBS	<LLOQ	30	16.64-26.9	1.2-3	5	6	NO
903-100817-AA-282	F	Diltiazem	90 mg	DBS	<LLOQ	20	105.65-150.87	10.05-12.25	7	9	NO
903-100817-AA-283	M	Atenolol	100 mg	DBS	<LLOQ	11	240-1370	2-4	7	10	NO
903-100817-AA-284	F	Bisoprolol	5 mg	DBS	<LLOQ	36	16.64-26.9	1.2-3	4	4	NO
903-100817-AA-285	F	Diltiazem	90 mg	DBS	<LLOQ	12	105.65-150.87	10.05-12.25	8	10	NO
903-100817-AA-286	F	Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2	6	10	NO
903-100817-AA-287	F	Atenolol	100 mg	DBS	<LLOQ	15	240-1370	2-4	5	7	NO
903-100817-AA-288	F	Losartan	50 mg	DBS	<LLOQ	14	89.1-306.1	0.5-2.2	5	5	NO
903-100817-AA-289	F	Diltiazem	60 mg	DBS	<LLOQ	20	74.72-82.43	2.23-2.49	5	5	NO
903-100817-AA-290	F	Bisoprolol	5 mg	DBS	<LLOQ	15	16.64-26.9	1.2-3	6	9	NO
903-210318-AA-291	M	Atorvastatin	40 mg	DBS	20.5±1.89	12	5.53-28.57	0.38-1.37	3	4	YES
903-210318-AA-292	M	Valsartan	80 mg	DBS	2726.80±17	11.5	1010-2270	2	2	2	YES
903-210318-AA-293	F	Bisoprolol	5 mg	DBS	23.27±0.47	11.5	16.64-26.9	1.2-3	3	3	YES
903-210318-AA-294	M	Lisinopril	10 mg	DBS	<LLOQ	31	41.75-80.47	5.79-6.91	7	11	NO
903-210318-AA-295	F	Losartan	50 mg	DBS	<LLOQ	12.5	89.1-306.1	0.5-2.2	6	8	NO
903-210318-AA-296	M	Atenolol	100 mg	DBS	<LLOQ	15	240-1370	2-4	8	11	NO
903-210318-AA-297	M	Bisoprolol	5 mg	DBS	22.13±0.41	11.5	16.64-26.9	1.2-3	6	10	YES
903-210318-AA-298	F	Bisoprolol	5 mg	DBS	22.45±0.33	11.5	16.64-26.9	1.2-3	8	13	YES
903-210318-AA-299	M	Losartan	50 mg	DBS	<LLOQ	15	89.1-306.1	0.5-2.2	6	12	NO
903-240318-AA-300	F	Bisoprolol	5 mg	DBS	<LLOQ	12.5	16.64-26.9	1.2-3	7	12	NO
903-240318-AA-301	F	Atorvastatin	40 mg	DBS	19.37±1.89	11.5	5.53-28.57	0.38-1.37	2	2	YES
903-240318-AA-302	F	Valsartan	80 mg	DBS	941.67±3.55	12	1010-2270	2	3	4	YES
903-240318-AA-303	F	Bisoprolol	5 mg	DBS	25.86±3.32	12	16.64-26.9	1.2-3	3	5	YES

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903-240318-AA-304	M	Bisoprolol	5 mg	DBS	<LLOQ	30	16.64-26.9	1.2-3	6	8	NO
903-240318-AA-305	F	Losartan	50 mg	DBS	<LLOQ	12	89.1-306.1	0.5-2.2	6	8	NO
903-240318-AA-306	M	Bisoprolol	5 mg	DBS	<LLOQ	11	16.64-26.9	1.2-3	6	8	NO
903-250318-AA-307	F	Atenolol	100 mg	DBS	<LLOQ	15	240-1370	2-4	6	9	NO
903-250318-AA-308	F	Losartan	50 mg	DBS	<LLOQ	11	89.1-306.1	0.5-2.2	8	12	NO
903-250318-AA-309	M	Valsartan	80 mg	DBS	<LLOQ	12	1010-2270	2	8	12	NO
903-250318-AA-310	F	Atenolol	100 mg	DBS	<LLOQ	12.5	240-1370	2-4	7	9	NO
903-250318-AA-311	F	Atorvastatin	40 mg	DBS	22.18±0.35	12.5	5.53-28.57	0.38-1.37	2	2	YES
903-260318-AA-312	F	Valsartan	80 mg	DBS	2013±7.05	11.5	1010-2270	2	3	3	YES
903-260318-AA-313	F	Lisinopril	10 mg	DBS	<LLOQ	40	41.75-80.47	5.79-6.91	6	8	NO
903-260318-AA-314	M	Simvastatin	40 mg	DBS	<LLOQ	11.5	5-40	2-3	7	9	NO
903-260318-AA-315	F	Bisoprolol	5 mg	DBS	<LLOQ	13	16.64-26.9	1.2-3	7	10	NO
903-260318-AA-316	M	Bisoprolol	5 mg	DBS	<LLOQ	12	16.64-26.9	1.2-3	8	10	NO
903-260318-AA-317	M	Losartan	50 mg	DBS	<LLOQ	16	89.1-306.1	0.5-2.2	5	5	NO
903-260318-AA-318	F	Valsartan	80 mg	DBS	<LLOQ	15	1010-2270	2	8	11	NO
903-260318-AA-319	F	Bisoprolol	5 mg	DBS	<LLOQ	12.5	16.64-26.9	1.2-3	7	12	NO
903-260318-AA-320	F	Atorvastatin	40 mg	DBS	27.45±1.99	11.5	5.53-28.57	0.38-1.37	2	3	YES
903-260318-AA-321	M	Valsartan	80 mg	DBS	2108±3.32	12	1010-2270	2	3	4	YES
903-260318-AA-322	F	Bisoprolol	5 mg	DBS	<LLOQ	35	16.64-26.9	1.2-3	6	9	NO
903-260318-AA-323	F	Simvastatin	40 mg	DBS	<LLOQ	12.5	16.64-26.9	1.2-3	6	9	NO
903-260318-AA-324	M	Simvastatin	40 mg	DBS	<LLOQ	13	5-40	2-3	7	8	NO
903-260318-AA-325	F	Simvastatin	40 mg	DBS	<LLOQ	13	5-40	2-3	8	12	NO
903-260318-AA-326	F	Simvastatin	40 mg	DBS	<LLOQ	13	5-40	2-3	7	12	NO
903-260318-AA-327	M	Bisoprolol	5 mg	DBS	<LLOQ	12.5	16.64-26.9	1.2-3	8	12	NO
903-260318-AA-328	F	Simvastatin	40 mg	DBS	<LLOQ	12	5-40	2-3	7	10	NO
903-260318-AA-329	F	Bisoprolol	5 mg	DBS	18.67±0.74	12.5	16.64-26.9	1.2-3	2	2	YES
903-260318-AA-330	F	Atorvastatin	40 mg	DBS	23.62±2.47	11.5	5.53-28.57	0.38-1.37	3	3	YES
903-260318-AA-331	M	Atorvastatin	40 mg	DBS	20.15±0.12	12.5	5.53-28.57	0.38-1.37	2	2	YES
903-260318-AA-332	F	Lisinopril	10 mg	DBS	<LLOQ	33	41.75-80.47	5.79-6.91	8	11	NO
903-270318-AA-333	M	Bisoprolol	5 mg	DBS	<LLOQ	12.5	16.64-26.9	1.2-3	7	9	NO

Patients Ref.	Sex	CVD medication	Dose (mg)	Sampling device	Concentration (ng/ml) ± (sd)	Time since last dose (h)	C_{max} (ng/ml)	t_{max} (h)	Total number of medications	Total number of tablets per day	Adherence results
903-270318-AA-334	F	Simvastatin	40 mg	DBS	<LLOQ	12	5-40	2-3	6	7	NO
903-270318-AA-335	F	Bisoprolol	5 mg	DBS	<LLOQ	12	16.64-26.9	1.2-3	8	11	NO
903-270318-AA-336	M	Simvastatin	40 mg	DBS	<LLOQ	12.5	5-40	2-3	6	6	NO

Abbreviation: M: Male; F: Female.

Appendix 15. MMAS-8 License Contract and Copyright Agreement

MMAS-8 License Contract and Copyright Agreement

Required citations and copyright acknowledgement for the MMAS-8 item scale are available on the final license contract and copyright agreement

In consideration for the right to use certain Morisky proprietary psychometric tools and intellectual property, the undersigned researcher (hereunder "Licensee" or "you") agrees to the following:

A. Ownership and Fees: All psychometric products as well as their translations, adaptations, computer programs, and scoring algorithms, trade secrets, and any other related documents and information (including those in electronic form) which embody or are related to the MMAS tools (including without limitation the Morisky Medication Adherence Scale 4- and 8-item versions, 4-item Morisky Adherence Questionnaire, and any documentation thereof) are intellectual property of Donald E. Morisky, ScD, ScM, MSPH. ("Owner") Professor of Community Health Sciences, UCLA Fielding School of Public Health, Los Angeles, CA 90095-1772 (the address for all payments and communications related to this agreement).

B. Translations: Permission will only be granted to translate the MMAS tools subject to the following requirements: all new translations must be made by contracting with the MAPI Institute and final translations must be approved by the Owner. The MAPI Institute employs the most rigorous standards in the translation process using two native linguistic experts to independently conduct forward and backwards translation; the Owner is actively involved in validating each item in the scale and grants use of the translated scale through a separate license agreement that is linked to the License Agreement Contract/Copyright Agreement. Languages that have already been translated and validated by the MAPI Institute can be requested through the Owner/Developer, Dr. Donald E. Morisky.

C. Use: Licensee understands and agrees that

1) Changes to the wording or phrasing of any Morisky scale, tool or document require written permission. If any changes made to the wording or phrasing of any MMAS item or other Morisky document without permission, the result cannot be considered the MMAS, and subsequent analyses and/or comparisons to other MMAS data may violate Owner's rights.

2) Coding and scoring criteria of the MMAS-8 are trade secrets of the Owner and as such cannot be divulged in any publication or report without the Owner's prior written permission;

3) Permission to use the trademarks "Morisky," "MORISKY SCALE" or "MMAS" is not and will not be granted for any unauthorized use or translations of the MMAS or other MORISKY intellectual property, in whole or in part. No analyses, research results or publications based on unauthorized changes or translated versions, or results thereof, will use MORISKY, MMAS or confusingly similar attributions.

4) The MORISKY SCALE intellectual property legend on the documents provided to you must be included on the first page of a MORISKY SCALE questionnaire in study documents, and in any reproductions for manuscript or other publication purposes. The footnote must be noted at the end of the first Table or Figure that displays the MMAS-8 items.

5) In case of scientific, administrative or intellectual property misconduct in using the MORISKY SCALE system of questionnaires or the Morisky name or MMAS names, Owner reserves the right to withdraw permission for use and to pursue all legal remedies. Licensee agrees to the jurisdiction in and venue of the State and Federal Courts in Los Angeles County.

6) Further specific requirements, e.g., citations required in publications, may be obtained from the Owner via <dmorisky@ucla.edu>. If you publish your work, you must acknowledge the use of the MMAS-8 in the acknowledgement section of your manuscript

by indicating: I have obtained written permission from copyright owners for any excerpts from copyrighted works that are included and have credited the sources in the Article or the Supplemental Materials. The credit footnote is located in the copyright agreement.

Please print, sign, and scan (PDF) and email *this agreement to* dmorisky@ucla.edu

Please sign and return this contractual agreement in a PDF format, to Professor Morisky and he will provide you (upon receipt of the payment invoice) with pages listing the MMAS-8 items, scoring and re-coding criteria and signature authorizing full use of this copyrighted scale. I agree to use only the English version of the MMAS-8 unless I purchase a validated translation of the MMAS-8 through Professor Morisky. I understand that it is a violation of international copyright laws to either use your own translation and call it the "MMAS-8" or use an existing MMAS-8 scale that has been translated and used for another study. The validated translation is non-transferrable and is linked to a specific license agreement and cannot be reproduced, copied, distributed, placed on the internet, published, or used by another individual. If the licensee violates any copyright laws contained in this licensing agreement, they will be solely responsible for a $5000.00 penalty and any associated legal costs.

Name and Contact Information of Licensee: AHMED DAYER ALWAN ALALAQI

Title of Study: ADHERENCE TO CARDIOVASCULAR MEDIATIONS IN IRAQ.

Total number of administrations: 426, one time only.
Signature of developer/owner of the MMAS-8:
Donald E. Morisky, ScD, Developer/Owner of the MMAS-8
Date Signed:

Signature of Licensee: /AHMED DAYER ALWAN ALALAQI

Date Signed:

LICENSURE AGREEMENT

The following shall constitute a contract for use of the © MORISKY MEDICATION ADHERENCE SCALE (MMAS-8) made on February 17, 2016, between AHMED DAYER ALWAN ALALAQI, Licensee, and Donald E. Morisky, ScD. ScM, MSPH, herein referred to developer/owner of the MMAS- 8.

SECTION 1. USE OF THE MORISKY MEDICATION ADHERENCE SCALE

Client hereby uses the Morisky Medication Adherence Scale on the terms set forth in this contract.

SECTION 2. FEES AND TERMS OF USAGE

In consideration of the owner's intellectual property, client agrees to pay owner a fee of $426($1.00 x 426 participants administered the MMAS-8 for one time). The license fee is in effect for a one-year period or the duration of the study, whichever is shorter.

SECTION 3. DUTIES OF OWNER

Owner shall provide the client with a listing of the © 8-item Morisky English scale along with a description of how each item is to be coded and summed to give a total score, ranging from 0 to 8. Psychometric properties of the scale (reliability and validity) will also be provided upon request.

SECTION 4. DUTIES OF THE CLIENT

Client agrees not to publish, distribute, copy or divulge the contents of the © Morisky Scale or its coding methodology to any individual. Transfer of this intellectual property is prohibited under copyright law.

SECTION 5. TERMS and TERMINATION

The license contract is in effect for a one-year or the duration of the study, whichever is shorter. This contract shall automatically terminate without further notice at the end of the term of usage as specified in SECTION 2.

This contract shall automatically terminate without further notice at the end of the term of usage as specified above.

If the Licensee terminated contract the owner will be entitled to the full amount of the contract terms.

SECTION 6. PAYMENT OF FEES

Client shall pay owner the amount of fees calculated based on the terms stated under SECTION 2 at the time of contract signature. Payment shall be made out to: Dr. Donald E. Morisky, Professor, UCLA School of Public Health, 650 Charles E. Young Drive South, Los Angeles, CA 90095-1772. Payment must be made at least 45 days after to the signing of this contract. A 10% late payment will be assessed on all late payments. Written notification must be sent to the Owner prior to the payment deadline date if Licensee needs additional time processing the invoice, otherwise a late fee will be assessed.

MMAS-8 License Contract and Copyright Agreement

Please print, sign, and scan (PDF) and email *this agreement to* dmorisky@ucla.edu

Please sign and return this contractual agreement in a PDF format, to Professor Morisky and he will provide you (upon receipt of the payment invoice) with pages listing the MMAS-8 items, scoring and re-coding criteria and signature authorizing full use of this copyrighted scale. I agree to use only the English version of the MMAS-8 unless I purchase a validated translation of the MMAS-8 through Professor Morisky. I understand that it is a violation of international copyright laws to either use your own translation and call it the "MMAS-8" or use an existing MMAS-8 scale that has been translated and used for another study. The validated translation is non-transferrable and is linked to a specific license agreement and cannot be reproduced, copied, distributed, placed on the internet, published, or used by another individual. If the licensee violates any copyright laws contained in this licensing agreement they will be solely responsible for a $5000.00 penalty and any associated legal costs.

Name and Contact Information of Licensee: AHMED DAYER ALWAN ALALAQI

Title of Study: ADHERENCE TO CARDIOVASCULAR MEDIATIONS IN IRAQ .

Total number of administrations: 426, one time only.

Signature of developer/owner of the MMAS-8: Donald E. Morisky
Donald E. Morisky, ScD, Developer/Owner of the MMAS-8

Date Signed: March 4, 2016

this contract is now extended to March 3, 2018 with a total of 426 administrations.

Signature of Licensee: /AHMED DAYER ALWAN ALALAQI

Date Signed:
4. 3. 2016

Appendix 16. Ethical Approval for Application of 8-item Morisky Questionnaire from De Montfort University's Faculty of Health and Life Science Research Ethics Committee

DE MONTFORT UNIVERSITY LEICESTER

HLS FREC Ref: 1747

15th July 2016

Ahmed Alalaqi
PhD Candidate

Dear Ahmed,

Re: Ethics application – Assessment of adherence to cardiovascular medications in Iraq by Morisky - 8 items questionnaire and dried blood spot technique (ref: 1747)

I am writing regarding your application for ethical approval for a research project titled to the above project. This project has been reviewed in accordance with the Operational Procedures for De Montfort University Faculty of Health and Life Sciences Research Ethics Committee. These procedures are available from the Faculty Research and Commercial Office upon your request.

I am pleased to inform you that ethical approval has been granted by Chair's Action for your application. This will be reported at the next Faculty Research Committee.

Should there be any amendments to the research methods or persons involved with this project you must notify the Chair of the Faculty Research Ethics Committee immediately in writing. Serious or adverse events related to the conduct of the study need to be reported immediately to your Supervisor and the Chair of this Committee.

The Faculty Research Ethics Committee should be notified by e-mail to hlsfro@dmu.ac.uk when your research project has been completed.

Yours sincerely,

Professor Martin Grootveld
Chair
Faculty Research Ethics Committee
Faculty of Health & Life Sciences
De Montfort University

Email: hlsfro@dmu.ac.uk
Web: http://www.dmu.ac.uk/research/ethics-and-governance/faculty-specific-procedures/health-and-life-sciences-ethics-procedures.aspx

Faculty of Health and Life Sciences, Edith Murphy House, The Gateway, Leicester LE1 9BH.
T: (0116) 255 1551 **F:** (0116) 257 7135

Appendix 17. Participant Information Leaflet for the Application of MMAS-8 (English Vesion).

Version 1
DATE / /

Faculty of Health and Life Sciences

Assessment of adherence to cardiovascular medications in Iraq by Morisky -8 items questionnaire

What is the study?

Cardiovascular disease (CVD) covers disorders of the heart and blood vessels, namely hypertension, angina, heart attack, stroke and heart failure. It is one of the biggest killers worldwide and in 2012 accounted for one in three of all deaths. According to the 2006 Iraqi national survey for chronic disease risk factors, 40.4% of the Iraqi adult population have elevated blood pressure. Ischemic heart diseases and stroke take positions one and two, respectively, in the top 10 causes of death in Iraq. The current medical care of CVD patients uses a combination of cardiovascular therapy drugs including beta-blockers and ace inhibitors to treat hypertension, and statins to lower cholesterol. There is evidence that, worldwide, as many as 50% of prescribed CVD drugs are not taken by patients as recommended. This non-adherence to medications results in morbidity, mortality, medicine wastage and higher costs of care.

What will happen?

The programme for this study will involve testing the adherence to cardiovascular medication in volunteers who are able to read and write in Arabic with no visual or cognitive impairment by using a standardized and validated Arabic version of the Morisky eight-item questionnaire (MMAS-8 questionnaire) which consists of eight standardized questions. Patient adherence profile will be determined according to the adherence drug index. Response choices for questions 1 to 7 are “Yes” or “No”. Question No. 8 is a Likert-type question. In addition to the MMAS-8, a checklist to gather demographic data as well as variables about other diseases or medications the patients were taking will also be used.

Do have to take part?

It is up to you to decide whether or not to take part, and if you decide to take part you are still free to withdraw at any time and without giving any reason.

What if I agree to take part and then change my mind?

You can withdraw from the study at any time without giving any reason.

How will you be involved?

After reading this Participant Information Leaflet, recruited volunteers will be asked to complete the questionnaire which consists of eight standardised questions.

How is the questionnaire analysed?
Patient adherence profiles will be determined according to the adherence drug index. Response choices for questions 1 to 7 are "Yes" or "No". Question No. 8 is a Likert-type question. The total score ranges from 0 to 8. Scores of less than 6 indicate low adherence, scores of 6 to < 8 indicate moderate adherence, and a score of 8 indicates high adherence.

What if something goes wrong/who can I complain to?

If you have a complaint regarding anything to do with this study, you can initially approach the lead investigator and, if a satisfactory outcome is not achieved, then you can contact the ethical committee in Misan Health Directorate, Misan, Amara or the Administrator for the Faculty Research Ethics Committee, Research & Commercial Office, Faculty of Health & Life Science, 1.25 Edith Murphy House, De Montfort University, The Gateway, Leicester, LE1 9BH or hlsfro@dmu.ac.uk

Will my taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept on a password-protected database and is strictly confidential. A reference code will be used instead of your name and any identifiable information you may give will be removed and anonymized.

What will happen to the results of the research study?
The results will form an essential part of a PhD thesis in clinical pharmacy practice at De Montfort University, Leicester.

Who is organizing and funding the research?
The research is for a PhD studentship at De Montfort University Leicester and is funded by the Iraqi Ministry of Health, Misan Health Directorate.

Who has reviewed the study?
This study has been reviewed and approved by De Montfort University, Faculty of Health and Life Sciences Research Ethics Committee and the ethical committee of the Misan Health Directorate.

Who should I contact if I have further questions?

Dr Sangeeta Tanna
Leicester School of Pharmacy
De Montfort University
The Gateway
Leicester LE1 9BH
T: 0116 2078274
E: stanna@dmu.ac.uk

Dr Graham Lawson
Leicester School of Pharmacy
De Montfort University
The Gateway
Leicester LE1 9BH
T: 0116 2577129
E: glawson@dmu.ac.uk

Ahmed Alalaqi
Leicester School of Pharmacy
De Montfort University
The Gateway
Leicester LE1 9BH
T:07714714552
E: 14018429@my365.dmu.ac.uk

Appendix 18. English Version of Patient Consent Form for the Eight-Item Morisky Medication Adherence Scale MMAS-8.

Morisky Medication Adherence Scale MMAS-8 to assess adherence to cardiovascular medications

Participant Reference Number:

(To be completed by research team)

Name of Researchers: Dr Sangeeta Tanna, Dr Graham Lawson & Ahmed Alalaqi

	Please initial
I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information and ask questions and have had these answered satisfactorily.	☐
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected.	☐
I understand that the data collected during the study, may be looked at by responsible individuals from the research team or from individuals from regulatory authorities.	☐
I agree to take part in this study.	☐

_____	_____	_____
Name of Participant	Signature	Date
_____	_____	_____
Name of person taking consent	Signature	Date

1 copy to participant; 1 copy for research file

Appendix 19. Patient Information Leaflet for the Application of MMAS-8 (Arabic Version).

PARTICIPANT INFORMATION LEAFLET

استماره معلومات للمشارك في البحث

نموذج 1

التاريخ :

تقييم الالتزام بادويه القلب والاوعيه الدمويه بواسطه استبيان مورسكي ذو الثمان نقاط

ما هي الدراسة؟

أمراض القلب والأوعية الدموية ويشمل اضطرابات في القلب والأوعية الدموية، وهي ارتفاع ضغط الدم والذبحة الصدرية والنوبات القلبية والسكتة الدماغية وقصور القلب. وهو واحد من أكبر أسباب الوفاة في جميع أنحاء العالم وفي عام 2012 مثل واحد من كل ثلاثة اشخاص يموتون بسبب هذا المرض. ووفقا للمسح الوطني العراقي عام 2006 لعوامل خطر الإصابة بأمراض مزمنة، 40.4٪ من السكان البالغين في العراق يعانون من ارتفاع ضغط الدم.

أمراض القلب والسكتة الدماغية اخذت المرتبه الاولى والثانيه على التوالي في أعلى 10 اسباب للوفاة في العراق. الرعاية الطبية لمرضى الأمراض القلبية الوعائية تستخدم توليفة من الأدوية في علاج القلب والأوعية الدموية بما في ذلك حاصرات بيتا ومثبطات ايس لعلاج ارتفاع ضغط الدم، والستاتين لخفض الكولسترول. هناك أدلة على أن ما يصل إلى 50٪ من الأدوية القلبية الوعائية المنصوص عليها لا تؤخذ من قبل المرضى على النحو الموصى به. هذا عدم الالتزام النتائج الأدوية في المراضة والوفيات، والأدوية الهدر وارتفاع تكاليف الرعاية.

ماذا يحدث في البحث

البرنامج لهذه الدراسة تشمل اختبار الالتزام في دواء القلب والأوعية الدموية في المتطوعين القادرين على القراءة والكتابة باللغة العربية مع عدم وجود ضعف في البصر وباستخدام النسخة العربية من Morisky 8 بنود الاستبيان (MMAS-8 الاستبيان) والذي يتألف من ثمانية أسئلة موحدة. وسيتم تحديد ا التزام المريض وفقا لمؤشر الالتزام.. اختيارات الإجابة عن الأسئلة 1-7 هي "نعم" أو "لا". والسوال الثامن هو ليكرت

يجب أن يشارك؟
الأمر متروك لك لتقرر ما إذا كانت أو عدم المشاركة وإذا قررت المشاركة لا تزال تتردد في الانسحاب في أي وقت ودون إبداء أي سبب

كيف تشارك في البحث؟

بعد قراءة المعلومات عن البحث في ورقه مشاركه المريض سيطلب المتطوعين اكمال الاستبيان الذي يتكون من ثمانية أسئلة موحدة.

كيفيه تحليل الاستبيان؟

وسيتم تحديد التزام المريض وفقا لمؤشر الالتزام. اختيارات الإجابة عن الأسئلة 1-7 هي "نعم" أو "لا". والسؤال رقم 8 هو من نوع ليكرت . وتتراوح الدرجة الكلية من 0 إلى 8. أقل من 6 تشير إلى التزام منخفضة، و من 6 إلى <8 تشير إلى التزام المعتدل، والنتيجة = 8 يدل على التزام عالي

ماذا لو حدث خطأ ما؟ / يمكنني تقديم شكوى ؟

إذا كان لديك أي شكوى يمكنك الاتصال مع اللجنة الأخلاقية في ميسان مديرية الصحة أو اللجنة الأخلاقية في كلية الصحة وعلوم الحياة 1.25 إديث ميرفي دي مونتفورت hlsfro@dmu.ac.uk

هل ستبقى مشاركتي في البحث سريه؟

وستبقى جميع المعلومات التي يتم جمعها عنك أثناء البحث في قاعدة بيانات محمية بكلمة مرور وسرية تامة. سيتم إزالة رمز إشارة التي سيتم استخدامها بدلا من اسمك وأية معلومات تعريفية قد تعطي.

ماذا سيحدث لنتائج الدراسة؟

وسوف تكون نتائج الدراسه جزءا أساسيا من رسالة الدكتوراه في الصيدلة السريرية في جامعة دي مونتفورت، ليستر.

من يمول هذا البحث

هذا البحث هو لدراسه لدرجة الدكتوراة في جامعة دي مونتفورت يستر وتموله وزارة الصحة العراقية، دائره صحة ميسان.

من يتابع ويشرف على الدراسه؟

وقد استعرضت هذه الدراسة والموافقة عليها من قبل جامعة دي مونتفورت، كلية الصحة وعلوم الحياة ولجنة أخلاقيات البحث في دائره صحة ميسان

بمن يمكن الاتصال لمعرفه مزيد من المعلومات؟

الدكتور سانكيتا تانا	الدكتور جراهام لونسون	أحمد العلاق
مدرسه ليستر للصيدله	مدرسه ليستر للصيدله	مدرسة ليستر الصيدلة
جامعه دي مونت فورت	جامعه دي مونت فورت	جامعة دي مونت فورت
Leicester LE1 9BH	Leicester LE1 9BH	ليستر LE1 9BH
T: 0116 2078274	T: 0116 2577129	T: 07714714552
stanna@dmu.ac.uk	glawson@dmu.ac.uk	14018429@myemail.dmu.ac.uK

Appendix 20. Consent Form for the Application of MMAS-8 (Arabic Version).

استماره الموافقه

Consent form

تقييم الالتزام بادويه القلب والاوعيه الدمويه في العراق بواسطه استبيان مورسكي ذو الثمان نقاط

الرقم المرجعي للمشارك:

يملىء من قبل فريق البحث

اسماء الباحثين احمد العلاق د.سانكيتا تانا د.كراهام لاوسن

☐ **أؤكد أنني قد قرأت وفهمت ورقة المعلومات للدراسة المذكورة أعلاه. وقد أتيحت لي الفرصة للنظر في المعلومات، وطرح الأسئلة، وكان هذه الإجابة مرضية.**

☐ **وأنا أفهم أن مشاركتي طوعية وأنا حر في الانسحاب في أي وقت دون إبداء أي سبب، دون تاثر حقوقي القانونية**

☐ **وأنا أفهم أن البيانات التي تم جمعها خلال هذه الدراسة، يمكن النظر فيها من قبل الأفراد المسؤولين عن فريق البحث أو من الأفراد من السلطات التنظيمية.**

☐ **أنا أوافق على المشاركة في هذه الدراسة**

اسم المشارك **التوقيع** **التاريخ**

اسم الشخص الذي اخذ الموافقه **التوقيع** **التاريخ**

نسخه الى المشارك ونسخه الى الملف

Appendix 21. The 8-items Morisky questionnaire (MMAS-8) (Arabic Version).

Arabic Translation 5-10-2013 مقياس مورسكي للالتزام بالعلاج

الاشخاص لديهم سلوك مختلف تجاه اخذهم للأدويه ونحن مهتمون بتجرُبَتك الشخصيه. لا توجد اجابه صحيحه او خاطئه. الرجاء ان تجيب على هذه الاسئله بناء على تجربتك الشخصيه في تناول العلاج.

السؤال	نعم (0)	لا(1)
1.هل تنسى في بعض الاحيان ان تتناول علاجك الخاص ؟(من غير قصد)		
2. الناس أحياناً لا يأخذون أدويتهم لسبب آخر غير النسيان , فهل كان هناك ايام لم تأخذ فيها أدويتك خلال الاسبوعين الماضيين ؟(عن قصد)		
3. هل سبق لك ان توقفت أو أنقصت جرعه علاجك بدون إخبار الطبيب لانك شعرت بسوء أو تعب عند اخذك للدواء ؟(عن قصد)		
4. عندما تغادر البيت او تسافر, هل تنسى في بعض لاحيان ان تحضر علاجك الخاص معك؟ (من غير قصد)		
5.هل تناولت علاجك في الامس؟ (من غير قصد)		
6. عندما تشعر بان وضعك الصحي تحت السيطره او هل تتوقف في بعض الاحيان عن تناول علاجك ؟ (عن قصد)		
7.ان تناول العلاج بشكل يومي هو هوأمر مزعج بالنسبة للبعض. هل شعرت يوما بالانزعاج من الإلتزام بخطه علاجك ؟ (عن قصد)		

8. إلى أي مدى تجدت صعوبه في تذكر اخذ جميع أدويتك ؟ الرجاء وضع دائرع حول ما يناسبك: (من غير قصد):

- **مطلقا/ابدا 4**
- **من حين لاخر 3**
- **احيانا.....2**
- **عادة.....1**
- **كل الاوقات.....0**

Appendix 22. The Response of Iraqi Patients to Their Clinician in Iraq After Reporting the Results to Their Clinician

Introduction

The obtained data about the non-adherence level for 303 Iraqi volunteers by the research conducted by Ahmed Alalaqi is specific for each patient and provide specific information about each medication in the regimen and this helps physician to track the problem of non-adherence for each patient in order to improve clinical outcomes, prevent the deterioration of the diseases and saving cost. Saving of cost come from decreasing waste of medications and prevent unnecessary rehospitalisation.

I started blame free discussion and encouraged patient to explain they were non-adherent to medications to find out the reason and this will help me to apply the required intervention. I asked non-adherent patients open-ended questions (*why you not adhere to ……medication?*), to reveal more reasons associated with non-adherence and to increase patients' involvement in the treatment plan. I explained to patients the purpose of asking these questions. The following are samples of patients' response and the required intervention. This approach is considered as novel and this is the first research in Iraq that is helpful for physician to track poor adherence in cardiovascular diseases.

1. **Medication side effect and complex regimen and**

Most of non-adherent patients reported that they feel worse when taking cardiovascular medications. For examples, non-adherent patients to statins reported that side effect and complicated regimen were the main cause of non-adherent

[… muscle pain…] [patient reference number…17, 323,334]

[feel tired…weakness in muscle….] [patient reference number…59]

[……Feel worse…… and complicated regimen….] [patient reference number…88]

[……I sometimes do not take medication because I feel not good…….] [patient reference number…314]

Some patients taking losartan reported they feel bad after taking losartan

[…Losartan makes me ill……] [patient reference number…190]

[…Feeling bad taking losartan …dizziness…] [patient reference number…305]

I explained to them the possible and significant side-effect of statins on muscle and side effect of losartan such as vertigo. However, I explained the benefits of using medications.

I also discuss with the possibility of switching to another medicine with less risk of side effects and this may improve patients' adherence. I also discussed with patients their concerns about medicines, and whether they believe they need them. I told my patients that he can report this, and I will be happy to listen to him and looking for solutions.

Significant number of non-adherent patients reported that taking many tables a day is distracting and disturb daily routine and sometimes they stop taking some medicines.

[It is inconvenient to me to take many medications...] [patient reference number…8]

... [Taking many medications disturb my life……work….] [patient reference number…10]

….. [Daily life disturbed by taking many medications….] [patient reference number…11]

I started simplifying the medical regimen or prescribing of a fixed-dose combination (FDC) of pills if possible.

2. Cost of medications

Some non-adherent patients to atorvastatin, simvastatin and valsartan stated that medications are expensive and are not always available in the public sector, and we can't afford the price.

Some patients stated that they intentionally stopped taking some medications because they don't have job and tried to reduce out pocket expenditure.

… [I cannot find these medications in the hospital……] [patient reference number…17, 55, 62, 134, 204]

… [Medications are expensive…I am jobless...] [patient reference number…64]

… [I cannot afford the price…….] [patient reference number…75]

…. [I did not take medicines …. I could not find medicines in the hospital...] [patient reference number…121, 124,133,159]

I discussed with the patients the possibility of prescribing less expensive medications and for some patient I changed the medical regimen to include medicines available in the public sector to reduce the cost.

3. **Patient knowledge**

Most of non-adherent patients stated that they do not know that they need to take medications even if they feel better and they do not believe taking all medications is necessary.

… [I think some medications are used as needed….] [patient reference number…157]

… [I feel OK…. I did not take medications….] [patient reference number…168]

[……feel that this condition is under control and no need for medications ….] [patient reference number…220]

I tried to improve patients' knowledge about cardiovascular diseases as chronic diseases and explained the rational use of each medication using everyday language avoiding jargon language to make information accessible and understandable

4. **Forgetfulness**

Some patients who took amlodipine stated that the main cause of non-adherence to medication is forgetfulness

… [We are old ……. forgetfulness is common with our age group …….] [patient reference number…198]

… [Busy life…. forget medications….] [patient reference number…240]

The same response was obtained from patients took atenolol, patient with reference number

… […Missed medications….] [patient reference number…274]

… […Forget medications….] [patient reference number…283]

… […Doing works made me forgot medications….] [patient reference number…310]

I encouraged patients to ask about their treatment to find out their preferences. I prepared cards for my patients including:

- what the medicine is
- how the medicine is likely to affect their condition (that is, its benefits), likely or significant adverse effects and what to do if they think they are experiencing them
- how to use the medicine
- what to do if they miss a dose

Also, I advise them to use their mobile phone to alert for the next dose.

Some patients stated that they are busy and working for long time and sometime forget taking medications when travelled or leaving home. I started friendly discussion about balance of life and being healthy will be important for career and family

….. [Forget my medications when I travelled ….] [patient reference number…224]

[Forget taking medicine when travelling….] [patient reference number…238]

5. Patients attitude and beliefs

Some non-adherent patients stated that continuing taking medications will harm them and some expressed that they do not believe in medicines.

[……we think if we continue using medications we you cannot stop it; your body will get used to it]. [patient reference number…242]

[…. will addict on these medications…….] [patient reference number…252]

Some patients stated [……, in my opinion, these tablets cannot improve my diseases, so I decided to stop it….] [patient reference number…263]

Regarding patients 23, 45, 53, 58 showed high concentration of atenolol in their blood for the reported dose of atenolol (50 mg or 100 mg).

Patient 23, 45, 53 thinks if they would take higher dose this will be better for them.

[This dose may not enough …….] [patient reference number…23]

[Taking high dose is better….] [patient reference number…45]

[Taking two tables will not harm…. better….] [patient reference number…53]

For patients 58 when I checked the medicines I found that patient by mistake inserted the strip of atenolol in the package of atorvastatin and in this case, patient took double dose from atenolol and miss the atorvastatin dose.

I discussed this issue with them by explaining the reason of prescribing medicines and possibility of getting adverse drug reaction and toxicity if patient has taken high dose.

One patient stated that he did not want his family worry about that

[…. I did not tell his family that I have cardiac problem because I do not want them to worry so I did not take medication at home and kept them at office ...] [patient reference number…283]

I stared blame free discussion with this patient and told him that patients need support from the family to use medications effectively also his health is important for his family and if you are ill the family will be unhappy. I explain to him the possible complication of cardiovascular diseases.

Patients should be supported to use medications effectively

6. **Patient–provider relationship**

Some patients stated that they were unable to understand how they receive the medicine, but they were shy to say that.

[…. I was shy to aske….] [patient reference number…285,296]

[…. I did not understand …. I was shy…] [patient reference number…300]

7. **Outcomes**

There was significant improvement the health outcomes after starting blame free environment to address all issues raised by non-adherent patients and this may indicate improvement in level of adherence in patients. A Comparative study is recommended to compare the level of non-adherence before and after interventions for future.

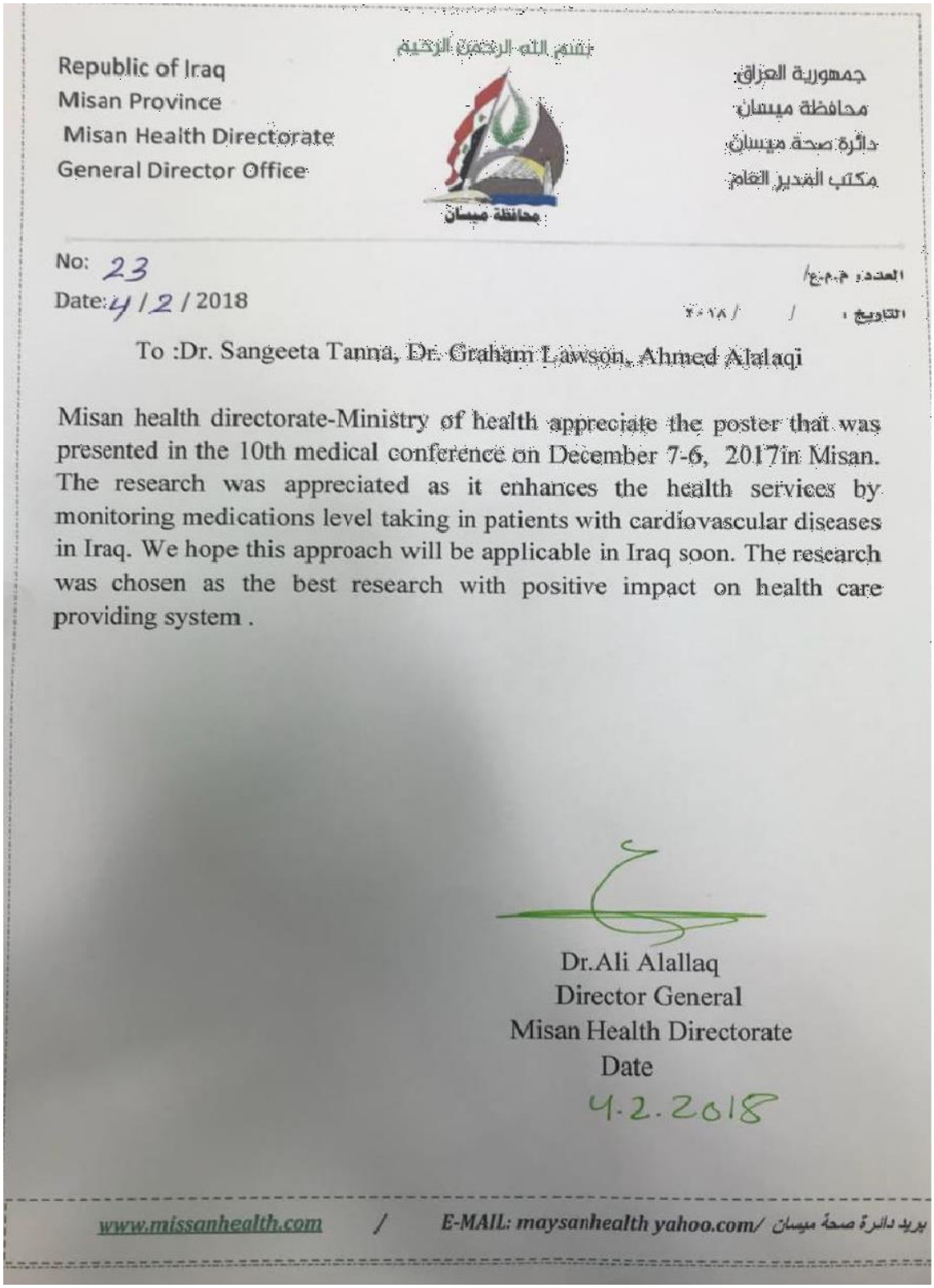
The results of current study provide an evidence that the health system in Iraq should be improved and apply a guideline for management and supporting of patients' adherence to cardiovascular disease in Iraq.

Professor Yaseen Obaid Yaseen

Dean of college of medicine

University of Misan

Appendix 23. Letter from Misan Health Directorate Showed the Desire to Transfer This Technology for Application in Its Laboratories.



بسم الله الرحمن الرحيم

Republic of Iraq
Misan Province
Misan Health Directorate
General Director Office

جمهورية العراق
محافظة ميسان
دائرة صحة ميسان
مكتب المدير العام

محافظة ميسان

No: 23
Date: 4 / 2 / 2018

العدد: م.م.ع/
التاريخ: / / ٢٠١٨

To :Dr. Sangeeta Tanna, Dr. Graham Lawson, Ahmed Alalaqi

Misan health directorate-Ministry of health appreciate the poster that was presented in the 10th medical conference on December 7-6, 2017in Misan. The research was appreciated as it enhances the health services by monitoring medications level taking in patients with cardiovascular diseases in Iraq. We hope this approach will be applicable in Iraq soon. The research was chosen as the best research with positive impact on health care providing system .

Dr.Ali Alallaq
Director General
Misan Health Directorate
Date
4.2.2018

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