Hyphenated mass spectrometry techniques for assessing medication adherence: advantages, challenges, clinical applications and future perspectives

Abstract

Nonadherence to prescribed pharmacotherapy is an understated public health problem globally and is costing many patients their chance to return to good health and healthcare systems billions. Clinicians need an accurate assessment of adherence to medications to aid the clinical decision-making process in the event of poor patient progress and to maximize the patient health outcomes from the drug therapies prescribed. An overview of indirect and direct methods used to measure medication adherence is presented, highlighting the potential for accurate measuring of drugs in biological samples using hyphenated mass spectrometry techniques to provide healthcare professionals with a reliable evidence base for clinical decision making. In this review we summarise published applications of hyphenated mass spectrometry techniques for a diverse range of clinical areas demonstrating the rise in the use of such direct methods for assessing medication adherence. Although liquid chromatography-tandem mass spectrometry methods using plasma, serum and urine samples are the most popular, in recent years increased attention has been given to liquid chromatography high-resolution mass spectrometry methods and alternative biosample matrices including hair, saliva and blood microsamples. The advantages and challenges of using hyphenated mass spectrometry techniques to address this healthcare problem are also discussed alongside future perspectives.

Keywords

Medication adherence; hyphenated techniques; liquid chromatography-tandem mass spectrometry; liquid chromatography-high resolution mass spectrometry; quantitation; bioanalysis

List of abbreviations

ADHD	Attention deficit hyperactivity disorder
APCI	Atmospheric pressure chemical ionisation
ART	Antiretroviral therapy
CVD	Cardiovascular disease
DBS	Dried blood spot
EI	Electron impact
ESI	Electrospray ionisation
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HIV	Human immunodeficiency virus
HRMS	High resolution mass spectrometry
IT	Ion trap
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LC-HRMS	Liquid chromatography-high resolution mass spectrometry
LMIC	Low- and middle-income country
LOD	Limit of detection

LOQ	Limit of quantification
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
m/z	Mass to charge ratio
PCR	Polymerase chain reaction
qqq	Triple quadrupole
TDM	Therapeutic drug monitoring
ToF	Time of flight
UHPLC	Ultra-high-pressure liquid chromatography
VAMS	Volumetric absorptive microsampling
WHO	World Health Organisation

1. Introduction

Medicines are the most common intervention in global healthcare and are crucial in managing chronic conditions, curing communicable diseases and generally maintaining health and preventing illness. Getting the most from medicines is becoming increasingly important as people are living longer and are suffering from more than one long-term condition and therefore require multiple medications [1]. However, according to the World Health Organisation (WHO), only 50% of medicines in developed countries are taken as recommended and this figure is reported to be lower in low income countries [2]. Failure to take a prescribed medication regimen in the way recommended by their healthcare provider is termed “medication nonadherence” and is documented to be a worldwide problem of striking magnitude [2, 3]. Nonadherence to prescribed medicines results in blood drug levels outside of the therapeutic window which can result in treatment failure, side effects and

complications and worsening of health for patients. For healthcare services, the cost of not taking prescribed medicines correctly are staggering and growing and this healthcare problem results in medicines wastage and additional use of scarce healthcare resources such as avoidable doctor visits, unnecessary additional treatments, laboratory tests and unplanned hospital admissions. In cases of poor clinical outcomes the clinician needs to know if the patient has followed the prescribed regimen. Improving medication adherence, potentially the most effective route to improving the therapeutic benefit of pharmacotherapy and improving clinical outcomes, remains a challenge for healthcare systems worldwide. In low- and middle-income countries (LMIC) high levels of substandard and falsified medicines may exacerbate this healthcare problem by giving rise to unintentional nonadherence [4].

Currently, there is no “gold standard” method for assessing medication adherence in routine clinical practice but a multitude of methods have been explored [1,5,6]. These methods have generally been divided into two groups; indirect and direct assessment methods. Indirect assessment methods including patient self reports, patient questionnaires, pill counts, electronic monitors, prescription refill rates and an assessment of the patient’s clinical response are the most commonly used due to their simplicity and relative ease of use. However, such indirect methods cannot confirm if the patient has taken their medication correctly and are proxy measures of medication adherence [7,8]. For instance, pill counts simply confirm the number of tablets removed from their original container but cannot confirm if these tablets have been consumed by the patient. Furthermore, this method provides no information about the time a dose was taken which may be crucial in establishing clinical outcomes [1]. Direct assessment methods include direct patient observation, determination of drug or metabolite level in urine or blood, measurement of biomarker in blood or urine, and

the detection of an ingestible medication marker, added to the dosage form, in the blood [9]. Direct methods are the most accurate approaches for assessing medication adherence but are expensive and sometimes result in “white coat adherence”. Directly assessing drug, metabolite or biomarker levels in blood and urine samples provides an objective measure but levels may vary due to differences in patient metabolism and pharmacogenetics. Due to the ease of collection and non-invasiveness among other advantages, hair and saliva biosamples have been explored in medication adherence studies [10,11]. For therapeutic drugs there are well documented pharmacokinetic relationships between the drug dose given and concentration of the drug in the blood stream. Tanna and Lawson [9] discuss the potential for assessing adherence to medication by determining drug or metabolite levels in blood microsamples as the more suitable approach to ensure the presence of drug(s) within the required therapeutic window. This approach was also corroborated by other studies [6,12].

Considering the negative impact of medication nonadherence on the patient and healthcare providers, the need for more information regarding direct drug or metabolite measurements in biosamples cannot be overemphasized. Quantitative data confirming the presence of the medication in the patient’s body is evaluated based on the anticipated therapeutic window and this information can then be used by the clinician to assess adherence, rightness of the prescribed dosage and medication suitability.

The assessment of medication adherence by monitoring drug concentrations in biosamples has been performed using immunoassays but it is reported that immunoassays can suffer from interferences or metabolite effects, cross-reaction problems and there is a likelihood of obtaining a considerable number of false positive results. In this situation patients are identified as adherent, while they are not [13-15].

Furthermore, immunoassay kits may not be applicable to specimens other than those identified by the manufacturers and a traditional immunoassay quantifies only one target analyte [16]. In some studies immunoassay results were considered presumptive until confirmed via gas chromatography-mass spectrometry (GC-MS) methods [17]. Peat [18], suggests that with GC-MS confirmation, positive immunoassay results seemed to drop by 10% over a ten-year period from 18% to 8% further suggesting false positive results with the initial immunoassay tests. These limitations of immunoassays have promoted the use of hyphenated mass spectrometry (MS) techniques in clinical laboratories for nearly two decades with recent applications focused more on “personalised” or “precision” medicine. It is on this note that this review explores hyphenated MS techniques as a valuable option in the direct assessment of medication adherence for a range of clinical conditions. This review provides insight into the general capabilities of MS for the analyses of biosamples for adherence studies and particularly highlights advantages, challenges and future prospects of these MS-based techniques in the direct assessment of medication adherence for a diverse range of clinical areas.

2. Hyphenated mass spectrometry techniques for assessing medication adherence

Hyphenated mass spectrometry techniques combining chromatography and mass spectrometry (MS) have revolutionised the analysis of biosamples for clinical applications. In Table 1 the different hyphenated combinations of chromatography and MS reported in the literature related to assessment of medication adherence studies include:

- LC-MS (liquid chromatography-mass spectrometry), LC-MS/MS (liquid chromatography-tandem mass spectrometry), LC-HRMS (liquid chromatography-high resolution mass spectrometry)
- GC-MS (gas chromatography-mass spectrometry)

These hyphenated MS techniques provide acceptable specificity towards the target analyte by giving a separation capability prior to MS, MS/MS or HRMS detection. For the quantification of drugs in complex matrices such as biosamples, for medication adherence assessment, the mass spectrometer is an ideal detector providing data which is characteristic of the analyte coupled with sensitivity. For liquid samples the most commonly used LC is HPLC, however, UHPLC is being increasingly used since it uses narrow bore LC columns and offers shorter run times of approximately 2 minutes. In GC-MS applications the electron impact ionisation process provides sufficient energy to both ionise and fragment the molecule(s) under investigation to produce a fragmentation pattern from which the molecule could be independently identified. This capability is lost in LC-MS systems since the low energy electrospray ionisation (ESI) process produces little or no fragment ions [19] and suffers from matrix effects. ESI is an effective method for converting target analyte in solution into gas phase ions suitable for analysis by the processes of desolvation and ion desorption. This is especially good for polar analytes and as can be seen from Table 1 is the most popular source used for pharmaceutical bioanalysis. Another ionisation technique that is used is atmospheric pressure chemical ionisation (APCI). This uses ion-molecule reactions, at atmospheric pressure, to transfer charges originating from the action of a corona discharge on a spray of the mobile phase. APCI gives a more selective ionisation and for some analytes it has been shown to have much lower matrix effects.

The mass spectrometers used with both GC-MS and LC-MS systems fall into three broad categories: low-resolution scanning instruments, tandem MS systems, and high-resolution scanning systems. All MS systems measure the mass/charge ratio (*m/z*) of ions of interest and in the simplest form, MS provides some type of a molecular fingerprint of the analyte of interest. For a low-resolution scanning MS or linear quadrupole, the quadrupole mass filter has a mass range of around 3000 with a resolution up to 3000. A major challenge with GC-MS analyses is that all analytes must be volatile. Consequently, most clinical assays would require multiple extraction steps including chemical derivatization so the analytes are sufficiently volatile for analysis. The lengthy sample preparation steps involved in GC-MS analyses, resulting in high cost and low throughput, has limited its extensive use in clinical medication adherence studies as is evident from Table 1. However, the main advantage of this approach is the data-rich fingerprint electron impact mass spectrum for each compound eluting from the GC column. These can be compared with international databases and in combination with a calibrated retention time will provide the necessary specificity for target analyte recognition. LC-MS systems have the advantage that, unlike GC-MS systems, it is not necessary to derivatise the samples prior to analysis. Because the ionisation is a low-energy process, the most abundant and possibly the only significant ion formed is usually the MH+ and so the information-rich fragmentation data is not available and therefore these low-resolution scanning MS systems are not sufficiently selective. This issue is overcome by low resolution tandem MS systems including triple quadrupole (qqq) and ion traps (IT). The tandem MS/MS reproduces the fragmentation process by passing only the ions of the pre-selected (MS1) analyte into a collision cell, where collision induced fragmentation occurs, and the products of these collisions are monitored by MS2. This means of producing a fragmentation pattern is referred to as

multiple reaction monitoring (MRM). Before any analysis can be carried out, the appropriate *m/z* values for MS1 and MS2 have to be determined. To do this, target drug reference compound is used to determine the appropriate *m/z* values for MS1 and MS2. This is done by presetting MS1 to transmit the *m/z* value of the molecular ion and scanning MS2 to identify the *m/z* values of the resulting fragment ions. Once MS1 and MS2 are set accordingly the MS/MS instrument will only respond to that specific compound. Ion traps are referred to as “tandem in time” and can operate as tandem mass spectrometers by alternating between mass selective and non mass selective modes of operation. An alternate approach to molecular specificity is offered by the high-resolution mass measurement capabilities of the Time of Flight (ToF) and Orbitrap instruments. These high resolution mass spectrometry (HRMS) instruments are being increasingly used in quantitative bioanalysis [20]. High resolution implies the ability to measure the *m/z* value accurately to within a few parts per million of the mass via a calculation or direct measure of the accurate mass, typically to four decimal places of the target analyte. This approach provides an alternative to the MRM fragmentation based fingerprint of the target analyte. The ability of the HRMS instruments to determine accurate mass means that the level of selectivity from the LC component of the analysis can be reduced thus producing a time saving with no consequent loss in the value of the results. The data rich information acquired using HRMS analyses provides more freedom to analyse for metabolites with small mass differences. Further advantages of using an HRMS system include the fact that post acquisition data mining is inherent with this approach and all mass spectral information from the sample analysed can be recorded in full scan mode and this data covering the whole mass range can provide information when monitoring co-eluting interfering ions for ion suppression, adducts and other target analytes [16,19, 21].

From Table 1 it is evident that LC-MS based techniques have been widely used for the objective assessment of medication adherence in various clinical areas. Due to the high sensitivity and specificity, LC-MS/MS has been the main instrument of choice for the quantitative determination of therapeutic drugs in biosamples for this healthcare application. However, the monopoly of the tandem (MS/MS) system is now being challenged by hyphenated HRMS systems with several studies using this approach to determine drug concentrations in biosamples to assess adherence to prescribed drug therapy [11, 21-31].

Table 1. Hyphenated MS techniques for the quantification of therapeutic drug in medication adherence studies

Cardiovascular therapy drugs					
Analytical Method	Biosample	Analyte(s)	Ionisation Mode	LOD/LOQ	References
UHPLC-HRMS (ToF)	DBS	Cardiovascular therapy drugs - atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, simvastatin, valsartan	ESI	LOQ: atenolol 10 ng/ml atorvastatin 0.5 ng/ml bisoprolol 0.1 ng/ml diltiazem 0.5 ng/ml lisinopril 0.1 ng/ml simvastatin 0.1 ng/ml valsartan 50 ng/ml	[22]
LC-MS/MS (qqq)	Urine	29 cardiovascular therapy drugs	ESI	-	[32]
LC-MS/MS	Plasma	Dabigatran	-	-	[33]
LC-MS/MS (qqq)	Serum	Antihypertensive drugs - amlodipine, canrenone, hydrochlorothiazide, metoprolol	ESI	LOQ: amlodipine 0.15 µg/l canrenone 8.54 µg/l hydrochlorothiazide 0.05 µg/l metoprolol 0.23 µg/l	[34]
LC-MS/MS (IT)	Serum	Antihypertensive drugs	ESI	-	[35]
LC-HRMS/MS (Orbitrap)	Oral fluid	78 cardiovascular therapy drugs	ESI	LOD: 0.1-100 µg/l	[11]
LC-MS/MS (qqq)	Serum	263 prescription and over the counter medicines used for acute and chronic conditions	-	-	[36]
GC-MS	Urine	Antihypertensive drugs - ramipril, enalapril, benazepril, valsartan, irbesartan, lorsartan, metoprolol, bisoprolol, propranolol, lercanidipine,	EI	-	[37]

		amlodipine, felodipine, nitrendipine, verapamil, urapidil, torasemid, furosemide, piretanid, hydrochlorothiazide, xipamid, triamteren, eplerenone, spironolactone			
UHPLC-MS/MS (qqq)	Plasma	Antihypertensive drugs - amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril, telmisartan	ESI	LOQ: amlodipine 0.156 ng/ml atenolol 7.812 ng/ml clonidine 0.078 ng/ml chlortalidone 39.062 ng/ml doxazosin 0.078 ng/ml hydrochlorothiazide 0.132 ng/ml nifedipine 0.781 ng/ml olmesartan 0.781 ng/ml ramipril 0.781 ng/ml telmisartan 0.781 ng/ml	[38]
LC-MS/MS (qqq)	DBS	Amlodipine	ESI	LOQ: 0.5 ng/ml	[39]
UHPLC-MS/MS (qqq)	Serum	Antihypertensive drugs – candesartan, carvedilol, diltiazem, enalaprilat, irbesartan, lisonopril, ramiprilat, valsartan, verapamil, amlodipine, atenolol, bendroflumethiazide, bisoprolol, canrenone, doxazosin, lercanidipine, losartan, metoprolol, nifedipine, propranolol, hydrochlorothizide	ESI	LOQ: candesartan 0.55 ng/ml carvedilol 0.51 ng/ml diltiazem 0.52 ng/ml enalaprilat 0.44 ng/ml irbesartan 10.71 ng/ml lisonopril 0.24 ng/ml ramiprilat 0.19 ng/ml valsartan 5.44 ng/ml verapamil 0.57 ng/ml amlodipine 0.25 ng/ml	[40]

				atenolol 2.66 ng/ml bendroflumethiazide 0.11 ng/ml bisoprolol 0.81 ng/ml canrenone 0.85 ng/ml doxazosin 0.56 ng/ml lercanidipine 0.02 ng/ml losartan 1.09 ng/ml metoprolol 1.3 ng/ml nifedipine 3.5 ng/ml propranolol 0.32 ng/ml hydrochlorothizide 0.37 ng/ml	
LC-MS/MS (qqq)	Serum	Antihypertensive drugs	-	-	[41]
UHPLC-MS/MS	Plasma	Antihypertensive drugs - benazepril hydrochloride, fosinopril sodium, captopril, hydrochlorothiazide	-	-	[42]
LC-MS/MS	Urine	Antihypertensive drugs	ESI	-	[43]
LC-MS/MS (qqq)	Plasma	Antihypertensive drugs - amlodipine, lercanidipine, nebivolol, metoprolol, bisoprolol, perindoprilat, valsartan, candesartan, olmesartan, irbesartan, hydrochlorothiazide	-	LOQ: 1.0 ng/ml for all, except lercanidipine and nebivolol 0.25 ng/ml valsartan and irbesartan 50 ng/ml	[44]
UHPLC-HRMS (ToF)	VAMS, DBS	Cardiovascular therapy drugs - atenolol, atorvastatin, bisoprolol, diltiazem, lisinopril, simvastatin, valsartan	ESI	LOQ: atenolol 10 ng/ml atorvastatin 0.5 ng/ml bisoprolol 0.1 ng/ml diltiazem 0.5 ng/ml lisinopril 0.1 ng/ml simvastatin 0.1 ng/ml	[23]

				valsartan 50 ng/ml	
UHPLC-HRMS (ToF)	DBS	Cardiovascular therapy drugs - atenolol, atorvastatin, bisoprolol, diltiazem, doxazosin, lisinopril, losartan, ramipril, simvastatin, valsartan	ESI	LOQ: atenolol 10ng/ml atorvastatin 0.5ng/ml bisoprolol 0.1 ng/ml diltiazem 0.5 ng/ml doxazosin 0.1 ng/ml lisinopril 0.1 ng/ml losartan 5 ng/ml ramipril 0.1 ng/ml simvastatin 0.1 ng/ml valsartan 50 ng/ml	[24]
UHPLC-MS/MS (qqq)	Urine	Antihypertensive drugs - amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril, telmisartan	ESI	LOQ: amlodipine 7.81 ng/ml atenolol 78.12 ng/ml clonidine 13.90 ng/ml chlortalidone 39.06 ng/ml doxazosin 7.81 ng/ml hydrochlorothiazide 156.25 ng/ml nifedipine 78.12 ng/ml olmesartan 78.12 ng/ml ramipril 0.78 ng/ml telmisartan 156.25 ng/ml	[45]
LC–MS/MS (qqq)	Urine, Serum	Antihypertensive drugs – enalapril, lisinopril, periodopril, ramipril, quinalapril, trandolapril, candesartan, irbesartan, valsartan, losartan, telmisartan, olmesartan, atenolol, metoprolol, propranolol, labetolol, bisoprolol, nebivolol, amlodipine, felodipine, lercanidipine, lacidipine,	ESI	-	[46]

		nifedipine, diltiazem, verapamil, bendroflumethiazide, hydrochlorothiazide, indapamide, furosemide, chlorthalidone, bumetanide, eplerenone, spironolactone, amiloride, hydralazine, doxazosin, prazosin, moxonidine, aliskiren, methyldopa			
UHPLC–MS/MS	Urine	Antihypertensive drugs	-	-	[47]
LC-MS/MS (qqq)	Plasma	Antihypertensive drugs - amlodipine, atenolol, carvedilol, clonidine, diltiazem, hydrochlorothiazide, hydralazine, lisinopril, losartan, metoprolol, nifedipine, ramipril, valsartan, verapamil	ESI	LOQ: amlodipine 0.5 ng/ml atenolol 10 ng/ml carvedilol 1 ng/ml clonidine 1 ng/ml diltiazem 1 ng/ml hydrochlorothiazide 5 ng/ml hydralazine 25 ng/ml lisinopril 5 ng/ml losartan 0.5 ng/ml metoprolol 1 ng/ml nifedipine 1 ng/ml ramipril 1 ng/ml valsartan 10 ng/ml verapamil 1 ng/ml	[48]
LC-MS/MS (IT)	Serum	38 medications across broad range of chronic diseases	-	-	[49]
UHPLC–MS/MS	Plasma	Antihypertensive drugs	ESI	LOQ: amlodipine 0.5 µg/l canrenone 1.0 µg/l enalapril 0.2 µg/l enalaprilate 0.8 µg/l hydrochlorothiazide 40 µg/l	[50]

				losartan 0.5 µg/l losartan carboxylic acid 2.0 µg/l nifedipine 4.0 µg/l perindopril 0.5 µg/l perindoprilate 0.5 µg/l spironolactone 2.0 µg/l valsartan 5.0 µg/l	
UHPLC-MS/MS (qqq)	Plasma	Antihypertensive drugs - amlodipine, atenolol, clonidine, chlortalidone, doxazosin, hydrochlorothiazide, nifedipine, olmesartan, ramipril, telmisartan	ESI	LOQ: amlodipine 0.156 ng/ml atenolol 7.812 ng/ml clonidine 0.078 ng/ml chlortalidone 39.062 ng/ml doxazosin 0.078 ng/ml hydrochlorothiazide 0.132 ng/ml nifedipine 0.781 ng/ml olmesa 0.781 ng/ml telmisartan 0.781 ng/ml	[51]

LC-MS/MS (qqq)	Urine	Antihypertensive drugs – enalapril, lisinopril, periodopril, ramipril, quinalapril, trandolapril, candesartan, irbesartan, valsartan, losartan, telmisartan, olmesartan, atenolol, metoprolol, propranolol, labetolol, bisoprolol, nebivolol, amlodipine, felodipine, lercanidipine, lacidipine, nifedipine, diltiazem, verapamil, bendroflumethiazide, hydrochlorothiazide, indapamide, furosemide, chlorthalidone, bumetanide, eplerenone, spironolactone, amiloride, hydralazine, doxazosin, prazosin, moxonidine, aliskiren, methyldopa	ESI	-	[52]
UHPLC–MS/MS (qqq)	Urine	Antihypertensive drugs - alisikiren, amiloride, amlodipine, benazepril, benazeprilat, bisoprolol, candesartan, canrenone, carvedilol, chlorthalidone, clonidine, diltiazem, doxazosin, enalapril, enalaprilat, felodipine, furosemide, hydrochlorothiazide, irbesartan, lercanidipine, lisinopril, losartan, metoprolol, minoxidil,	ESI	-	[53]

		moxonidine, nebivolol, nifedipine, nitrendipine, olmesartan, perindopril, piretanide, prazosin, ramipril, ramiprilat, spironolactone, telmisartan, torasemide, triamterene, urapidil, valsartan, verapamil			
LC-HRMS/MS (Orbitrap)	Urine, plasma	Antihypertensive drugs	ESI	-	[25]
LC-MS/MS (qqq)	Serum	Antihypertensive drugs - amlodipine, atenolol, bisoprolol, carvedilol, clonidine, diltiazem, enalapril, furosemide, hydrochlorothiazide, indapamide, lacidypine, lisinopril, losartan, metoprolol, perindopril, propranolol, quinapril, ramipril, telmisartan	-	LOQ: 0.7-10 ng/ml	[54]
LC-MS/MS (qqq)	Urine	Antihypertensive drugs	-	-	[55]
LC-HRMS/MS (Orbitrap)	Urine	Antihypertensive drugs	ESI	LOD: 1–20 mg/l	[26]
LC-MS/MS (qqq)	Urine	Antihypertensive drugs	ESI	LOQ: 1-25 µg/l	[56]
UHPLC-HRMS (ToF)	DBS	Cardiovascular therapy drugs – amlodipine, atenolol, bisoprolol, doxazosin, ramipril, simvastatin, valsartan	ESI	LOQ: amlodipine 1 ng/ml atenolol 25 ng/ml bisoprolol 0.5 ng/ml doxazosin 1 ng/ml ramipril 1 ng/ml simvastatin 5 ng/ml valsartan 50 ng/ml	[27]

LC-MS/MS (qqq)	Urine	Antihypertensive drugs – enalapril, lisinopril, periodopril, ramipril, quinalapril, trandolapril, candesartan, irbesartan, valsartan, losartan, telmisartan, olmesartan, atenolol, metoprolol, propranolol, labetolol, bisoprolol, nebivolol, amlodipine, felodipine, lercanidipine, lacidipine, nifedipine, diltiazem, verapamil, bendroflumethiazide, hydrochlorothiazide, indapamide, furosemide, chlorthalidone, bumetanide, eplerenone, spironolactone, amiloride, hydralazine, doxazosin, prazosin, moxonidine, aliskiren, methyldopa	ESI	-	[57]
LC-MS/MS (qqq)	Serum, Plasma	Cardiovascular therapy drugs	ESI	LOQ: 0.2-250 ng/ml	[58]
LC-HRMS (ToF)	Urine	Atenolol	ESI	-	[28]
UHPLC-HRMS (ToF)	DBS	Cardiovascular therapy drugs – bisoprolol, ramipril, simvastatin	ESI	LOQ: bisoprolol 0.5 ng/ml ramipril 1 ng/ml simvastatin 5 ng/ml	[29]
LC-MS/MS (qqq)	Serum	Antihypertensive drugs - amlodipine, verapamil, betaxolol, bisoprolol, metoprolol, doxazosine, losartan, telmisartan,	ESI	LOQ 1.0 µg/l -75.0 µg/l	[59]

		hydrochlorothiazide, perindoprilate, ramiprilate, canrenoate, furosemide			
LC-MS/MS (qqq)	Plasma	Antihypertensive drugs	ESI	LOQ: 1ng/ml	[60]
UHPLC-HRMS (ToF)	DBS	Atenolol	ESI	LOQ: 25 ng/ml	[21]
LC-MS/MS (IT)	Serum	Antihypertensives - betaxolol, metoprolol, bisoprolol, amlodipine, nitrendipine, verapamil, losartan, telmisartan, hydrochlorothiazide, chlorthalidone, furosemide, doxazosin, rilmenidine, urapidil	ESI	LOQ: 0.5 -1 ng/ml	[61]
Antiretroviral therapy drugs					
LC-MS/MS (qqq)	Hair	Zidovudine, efavirenz, ritonavir, lopinavir	APCI	LOQ: zidovudine 36 pg/mg efavirenz 16 pg/mg ritonavir 12 pg/mg lopinavir 10 pg/mg	[62]
UHPLC–MS/MS (qqq)	Plasma	Sofosbuvir, sofosbuvir metabolite (GS-331007), daclatasvir	ESI	LOQ: sofosbuvir 11.71 ng/ml sofosbuvir metabolite (GS-331007) 19.53 ng/ml daclatasvir 11.71 ng/ml	[63]
LC-MS/MS (qqq)	DBS	Tenofovir, emtricitabine, lamivudine	ESI	LOQ: 100 fmol	[64]
LC-MS/MS (qqq)	Hair	Tenofovir, lamivudine, nevirapine	ESI	LOQ: tenofovir 416pg/mg lamivudine 12pg/mg nevirapine 39pg/mg	[10]
LC-MS/MS (qqq)	Urine	Tenofovir, disoproxil fumarate/emtricitabine	APCI	LOQ: tenovir 20 ng/ml emtricitabine 2 ng/ml	[65]
LC-HRMS/MS (Orbitrap)	Plasma	Efavirenz, lamivudine, nevirapine	-	LOD: 10 ng/ml LOQ: 2-20 ng/ml	[30]

LC-MS/MS (qqq)	Plasma, Hair	Antiretroviral therapy drugs	-	LOQ: 0.31 ng/ml	[66]
LC-MS/MS (qqq)	Plasma	Antiretroviral therapy drugs	-	-	[67]
LC-MS/MS (qqq)	Hair	Antiretroviral therapy drugs	ESI	LOQ: 0.01- 0.05 ng/mg	[68]
LC-MS/MS (qqq)	Hair	Antiretroviral therapy drugs	ESI	LOQ: 0.01- 0.05 ng/mg	[69]
LC-MS/MS	Plasma	Antiretroviral therapy drugs	-	-	[70]
LC-MS/MS (qqq)	Hair	Antiretroviral therapy drugs	ESI	LOQ: 0.12 ng/mg	[71]
LC-MS/MS (qqq)	Plasma	Antiretroviral therapy drugs	-	-	[72]
LC-MS/MS (qqq)	Plasma, saliva	Nevirapine, zidovudine, lamivudine	ESI	-	[73]
LC-MS/MS (qqq)	Plasma	Antiretroviral therapy drugs	-	-	[74]
LC-MS/MS (qqq)	Plasma	Antiretroviral therapy drugs	ESI	LOQ: 10 ng/ml	[75]
LC-MS/MS (qqq)	Hair	Ritonavir, lopinavir, atazanavir	ESI	LOD: ritonavir 0.01ng/mg lopinavir and atazanavir 0.05 ng/mg	[76]
LC-MS/MS (qqq)	Plasma, DBS	Atazanavir, darunavir, efavirenz, lopinavir, nevirapine, ritonavir	ESI	LOQ: atazanavir 0.0985 mg/l darunavir 0.0500 mg/l efavirenz 0.102 mg/l lopinavir 0.107 mg/l nevirapine 0.101 mg/l ritonavir 0.0546 mg/l	[77]
LC-MS/MS (qqq)	Plasma, DBS	Antiretroviral therapy drugs	ESI	LOQ: 41–102 ng/ml	[78]
Pain management drugs					
LC-MS/MS	Urine	Opioids	-	-	[79]

LC-MS/MS	Urine	Opioids	ESI	-	[80]
LC-MS/MS (qqq)	Hair	Alprazolam, amitriptyline, citalopram, clomipramine, clonazepam, delorazepam, diazepam, duloxetine, fluoxetine, flurazepam, levomepromazine, levosulpiride, lorazepam, lormetazepam, mirtazapine, paroxetine, quetiapine, sertraline, topiramate, trazodone, triazolam, venlafaxine, zolpidem	ESI	LOQ: 5.0–50.0 pg/mg	[81]
LC-MS/MS	Urine	Amphetamines, benzodiazepines/metabolite, buprenorphine, norbuprenorphine, benzoylecgonine, carisoprodol/metabolite, fentanyl/metabolite, methadone/metabolite, meperidine/metabolite, opiates, oxycodone/metabolites, propoxyphene, tapentadol, tramadol/ metabolite	ESI	LOQ: 2-100 ng/ml	[15]
LC-HRMS (ToF)	Urine	Opioids, benzodiazepines	ESI	LOD: 5-500 ng/ml	[31]
LC-MS/MS (qqq)	Serum	Opioids	-	LOQ: 0.2-20 ng/ml	[82]
LC-MS/MS (qqq)	Urine	Opioids	ESI	LOQ: 2-100 ng/ml	[83]
LC-MS/MS (qqq)	Urine	Opioids	-	LOQ: 50–100 ng/ml	[84]

LC-MS/MS (qqq)	Urine	Benzodiazepines	ESI	LOQ: 50–100 ng/ml	[85]
LC-MS/MS (qqq)	Urine, saliva	Opioids	ESI	LOQ: Saliva - 0.5-25 ng/ml Urine- 1-200 ng/ml	[86]
LC-MS/MS, GC-MS	Urine	Opioids	-	-	[87]
LC-MS/MS (qqq)	Urine, saliva	Opioids, benzodiazepines	ESI	LOQ: 0.1-25 ng/ml	[88]
LC-MS/MS, LC-MS, GC-MS	Urine	Opioids	-	-	[89]
LC-MS/MS (qqq)	Urine	Opioids	-	-	[90]
LC-MS/MS (qqq)	Urine	Opioids	ESI	LOQ: 50 ng/ml	[91]
LC-MS/MS (qqq)	Urine	Opioids, benzodiazepines	-	-	[92]
LC-MS/MS (qqq)	Urine	Opioids, benzodiazepines - amphetamine, methamphetamine, alphahydroxyalprazolam, lorazepam, nordiazepam, oxazepam, temazepam, cannabinoids, cocaine, methadone/metabolite, codeine, hydrocodone, hydromorphone, morphine, propoxyphene, norpropoxyphene.	-	LOQ: 10-100 ng/ml	[93]
LC-MS/MS (qqq)	Urine	Opioids	-	-	[94]
LC-MS/MS (qqq)	Urine	Benzodiazepines	ESI	LOQ: 40 ng/ml	[95]

LC-MS/MS (qqq)	Urine	Opioids, benzodiazepines	-	-	[96]
LC-MS	Plasma	Opioids - buprenorphine	-	-	[97]
GC-MS	Urine	Opioids	EI	LOD: 50 ng/ml	[98]
GC-MS	Urine	Opioids	-	-	[99]
GC-MS	Urine	Opioids	-	LOD: Oxycodone (Free) 100 µg/l Oxycodone (Total) 50 µg/l Oxymorphone (Free) - 100 µg/l Oxymorphone (Total)- 50 µg/l Noroxycodone (Free) - 50 µg/l	[100]
GC-MS	Urine	Benzodiazepines	-	-	[101]
Type 2 diabetes drugs					
LC-MS/MS (qqq)	Urine	Type 2 diabetes drugs	ESI	-	[102]
Oral anticancer drugs					
UHPLC-MS/MS (qqq)	DBS	Tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen, endoxifen	ESI	LOQ: 0.5 – 15 ng/ml	[103]
UHPLC-MS/MS (qqq)	Plasma	Imatinib	ESI	-	[104]
LC-MS	Urine	Anastrozole	-	LOD: 0.5 ng/ml LOQ: 2.5 ng/ml	[105]
LC-MS/MS (qqq)	Plasma	Anastrozole	-	LOQ: 5-25 ng/ml	[106]
LC-MS/MS (qqq)	Plasma	Erlotinib	-	-	[107]

LC-MS/MS (qqq)	Plasma	Imatinib	-	-	[108]
LC-MS/MS (qqq)	Plasma	Anastrozole, tamoxifen, letrozole	-	LOQ: 5-25 ng/ml	[109]
LC-MS/MS (qqq)	Plasma	Imatinib	APCI	LOQ: 4.0 ng/ml	[110]
Immunosuppression drugs					
LC-MS/MS (qqq)	DBS	Creatinine, tacrolimus, everolimus, sirolimus, cyclosporine A	ESI	LOQ: 1.0 µmol/l	[111]
LC-MS/MS (qqq)	DBS	Creatinine, tacrolimus	ESI	LOQ: 0.01 mg/dl	[112]
LC-MS/MS (qqq)	DBS	Cyclosporin A, tacrolimus	ESI	LOQ: cyclosporin A 8.5 g/l tacrolimus 2.3 g/l	[113]
LC-MS/MS (qqq)	Blood	Sirolimus, tacrolimus, everolimus	ESI	LOQ: 1.5 µg/l	[114]
Antipsychotic drugs					
UHPLC-MS/MS (qqq)	Urine	Risperidone, quetiapine, olanzapine, haloperidol	ESI	LOQ: risperidone 25 ng/ml quetiapine 25 ng/ml olanzapine 5 ng/ml haloperidol 25 ng/ml	[115]
LC-MS, LC-MS/MS	Serum	Risperidone, quetiapine, olanzapine, haloperidol	ESI	LOQ: 0.5-5ng/ml	[116]
Antidepressant drugs					
LC-MS/MS (qqq)	Urine	Amitriptyline, nortriptyline, imipramine	-	LOQ: 50 ng/ml	[117]
LC-MS/MS	Plasma	Citalopram, escitalopram, clomipramine	-	LOQ: 10-20 µg/l	[118]
Antiepileptic drugs					
GC-MS	DBS, Serum	Valproic acid, carbamazepine, phenobarbital, phenytoin	-	LOQ: 5.0 µg/ml	[119]
Asthma drugs					

LC-MS/MS (qqq)	Hair	Fluticasone propionate, fluticasone furoate, beclomethasone dipropionate, formoterol, salmeterol, vilanterol, umeclidinium, tiotropium, budesonide	-		[120]
LC-MS/MS (qqq)	Urine	Fluticasone propionate	-	LOQ: 10.3 pg/ml	[121]
LC-MS/MS (qqq)	Urine, blood	Beclomethasone, betamethasone, budesonide, flunisolide, dexamethasone, methylprednisolone, fluticasone propionate, prednisolone, predinisone	-	LOD: 0.03-0.3 g/dl	[122]
LC-MS/MS (qqq)	Urine	Fluticasone propionate	ESI	LOQ: 10.3 pg/ml	[123]
Antibiotics					
LC-MS/MS (qqq)	Urine	Mupirocin	ESI	LOQ: 5.0 ng/ml	[124]
LC-MS	DBS	Rifaximin	ESI	LOQ: 0.3 ng/ml	[125]
Tuberculosis drugs					
LC-MS/MS (qqq)	Serum	Moxifloxacin, prothionamide, cycloserine	ESI	-	[126]
LC-MS/MS (qqq)	Urine	isoniazid	-	LOQ: 0.125 mg/l	[127]
Arthritis drugs					
LC-MS/MS (qqq)	Plasma	Methotrexate	ESI	LOQ: 0.1 nM	[128]
LC-MS/MS (qqq)	DBS	Methotrexate polyglutamates	ESI	LOQ: 5 nmol/l	[129]
Inflammatory bowel disease drugs					

LC-MS/MS (qqq)	Urine	5-aminosalicyclic acid	-	-	[130]
Attention-deficit/hyperactivity disorder (ADHD) drugs					
LC-MS/MS (qqq)	Oral fluid	Lisdexamphetamine	ESI	LOQ: 7.2 pg/ml	[131]
LC-MS/MS (IT)	Hair	Lisdexamphetamine	-	-	[132]
LC-MS/MS (qqq)	Hair	Atomoxetine	ESI	LOQ: 0.21ng/mg	[133]

3. Biological sample collection

The collection of a suitable biosample from the patient presents a major challenge in the provision of objective drug concentration data using hyphenated MS techniques to assess adherence to prescribed pharmacotherapy. Patient age, drug dose, pharmacokinetics and the factors affecting the disposition of the drug in the body will affect the drug level in the biosample. The sample collection method must be acceptable to patients. As can be seen from Table 1, the most frequently used biosamples to objectively assess medication adherence are liquid blood (plasma or serum) and urine. The shipment costs of these standard matrices are often too high and these biosamples require cold storage. Furthermore, the collection of liquid blood samples requires a phlebotomist and therefore these factors can be a deterrent for widespread acceptance for routine medication adherence monitoring. The collection of urine samples is non-invasive and urine can provide a much larger detection window than blood samples, however, some patient groups may be reluctant to provide urine samples due to religious, cultural or ethical issues [134]. Additionally, photodegradation of light sensitive compounds was reported to be much higher in urine samples than in whole blood, due to lower turbidity and possible longer exposure to daylight [45]. More recently, moves away from urine and whole blood samples have been identified. The ease of sample collection, storage and transport provided by microsampling methods such as dried blood spot (DBS) cards and volumetric absorptive microsampling (VAMS) has seen the increased use of such methods in studies to assess medication adherence [21-24, 27, 29, 30, 39, 64, 77, 78, 103, 111-113, 119, 125, 129]. It is only through the increased sophistication and detection capabilities of MS instruments that the micro-volume DBS and VAMS sample can provide comparable data to a 1 mL blood sample. This enhanced analytical capability

has also spurned the investigation of alternate less invasive biosample matrices including hair and saliva for assessing medication adherence [10, 11, 62, 66, 68, 69, 71, 73, 76, 81, 86, 88, 120, 131-133]. The choice of the most appropriate biosample would depend on a number of factors including the ease of sample collection from the patient and knowing if the available biosample size contains sufficient target analyte to be detected. Notional sample volumes and speculative target drug amounts contained in these alternative sample formats, based on C_{max} and sample volume are provided in Table 2.

Table 2. Typical volumes/mass of biological samples [134]

Sample	Size	Drug mass
Urine	100-200 ml	0.1-10 µg
Liquid blood	5-10 ml	10-100 ng
Saliva	0.5-2 ml	0.1-10 ng
DBS and VAMS	10-50 µl	1-300 pg
Hair	20-100 mg	1-300 pg

Historically, saliva has been less used in medication adherence studies, due to various limitations, but the re-emergence of this minimally invasive sampling matrix is probably due to the increased MS instrumental detection capabilities coupled with the potential in saliva to directly measure the therapeutically active free non-protein bound drug and the ease of sampling collection [135, 136]. This sampling matrix was recently investigated to assess adherence to antihypertensive drugs where saliva produced comparable results to plasma except for acidic drug compounds [11].

The evaluation of drug concentrations in hair can provide information about past exposure and history of medication use. Assuming human hair grows at an average

of 1cm per month [137], each segment of hair can be related to a time-period. Therefore, drug determination in all hair segments can provide information of an average exposure to the drug over the longer term rather than a day-by-day assessment. Obtaining a hair sample from a patient is minimally invasive and sample(s) do not require cold storage and can be posted to the clinic for use in studies to assess medication adherence. From Table 1 it is apparent that novel LC-MS/MS assays to determine antiretroviral drug concentrations in hair have been used to measure drug adherence in human immunodeficiency virus (HIV) treatment and pre-exposure prophylaxis [10, 62, 66, 68, 69, 71, 76].

Although some LC-MS analyses on liquid biosamples can be carried out on samples directly using the “dilute-and-shoot” approach, for the majority of biological samples some initial sample preparation may be used to aid the analytical process by eliminating matrix effects, removing protein and interfering components or to prevent excess instrument downtime for column changes and cleaning. Matrix effects are the alteration of ionisation efficiency caused by co-eluting constituents such as phospholipids and salts. Salts are relatively easy to eliminate whereas phospholipids are difficult to remove even with sophisticated sample clean-up procedures. The sample clean-up techniques that are available include protein precipitation, liquid-liquid extraction and solid-phase extraction. The choice of the sample preparation will be dependent on the nature of the primary sample and whether an extract should be analysed or discarded. A simple sample preparation amenable for the quantitation of target analytes in biosamples would facilitate the widespread implementation of LC-MS-based assays for assessing medication adherence. To prepare a hair sample for analysis, hair should be rinsed/decontaminated and divided into sections of known length. The long, typically overnight, digestion period needed to extract the drug from

the hair structure means that this sample format is not appropriate from a practical viewpoint for routine assessment of adherence [134,136]. The sample preparation methods for different biological matrices are detailed by Tanna and Lawson [16,134] and Capiau et al [138].

4. Clinical applications of hyphenated mass spectrometry techniques for assessing medication adherence

Nonadherence to medications is documented to be a problem in situations where self-administration of oral medications is required [1,8]. Self-administration of drug therapy is common practice for chronic diseases which include cardiovascular disease (CVD), asthma, type 2 diabetes and depression and is also used for oral cancer therapies as well as for the treatment of communicable diseases such as HIV, tuberculosis and malaria. In order to ensure an effective and efficient treatment plan in which therapeutic relief is derived from the prescribed regimen, accuracy in the assessment of adherence is crucial [2].

The analysis of patient biosamples using LC-MS systems for monitoring adherence to cardiovascular therapy drugs is escalating as is evident from Table 1. The prevalence of non-adherence to cardiovascular therapy drugs is documented to be as high as 50% [139]. Currently, a combination of cardiovascular therapy medications are employed in the treatment of patients with CVD and these include antihypertensives, hypolipidemic drugs, anticoagulants and antiplatelet drugs. This low level of adherence to prescribed cardiovascular therapies is likely to contribute to poor blood pressure control and poor patient outcomes and is considered to be a major problem in patients diagnosed with resistant hypertension [8, 38, 46, 52, 56, 57]. Furthermore,

hypertension can be asymptomatic and can exacerbate the problem of nonadherence leading to worsening of the chronic problem. This uncontrolled hypertension may ultimately lead to adverse outcomes such as stroke, myocardial infarction and kidney disease [8,140]. Considering the negative consequences of nonadherence to cardiovascular medications, a simple and accurate objective test for direct assessment of adherence is crucial to enable clinicians to make an informed decision about the patient's course of treatment. In Table 1 the greatest number of reports objectively assessing medication adherence using LC-MS based assays is to cardiovascular therapy medicines and this is a pointer to the seriousness of the situation. However, assay of cardiovascular drugs in biological fluids has been a challenge for analytical scientists. This is as a result of the difficulty in simultaneously determining combined cardiovascular therapy drugs belonging to different families with varying physicochemical properties in biological fluids. Hyphenated MS techniques offer applicability for the analysis of a wide range of drugs and direct assessment of adherence to cardiovascular therapy drugs via assays in plasma, serum, urine, saliva, DBS and VAMS samples is reported (Table 1). Single measurements of drug levels in biosamples may not provide information about the duration of nonadherence or how long since the patient took the last dose. Lawson et al. [29] and Tanna et al. [27] report the use of self-collected DBS samples to address this issue. Self-collected DBS samples at different intervals during dosing were assessed using LC-HRMS for determination of CVD therapy drug levels. This approach will allow for detection of any pharmacokinetic variations or false negative due to drug-drug interactions. Gupta et al [46] show that nonadherent hypertensive patients respond to LC-MS/MS-based biochemical analysis with improved adherence to antihypertensives and associated blood pressure reduction and postulate that repeated LC-MS/MS-based analysis

should be considered as a potential therapeutic approach to nonadherence-driven resistant hypertension. A recent study by Wallbach et al [37] used a GC-MS based method for the determination of antihypertensive drugs in urine and found the level of nonadherence to antihypertensives to be 58% but a limitation of this GC-MS method was that four of the target analytes were detectable only at high concentrations.

Although HIV infection has no cure, adherence to antiretroviral therapy (ART) is vital in order to keep the virus under control by preventing its spread or multiplication which could lead to destruction of the immune system. Adherence rates required for optimal viral suppression must be at least 95% to achieve optimal viral suppression [141]. Although PCR techniques have been widely used to assess adherence to ART drugs by measuring the viral load in plasma or DBS once medication has been initiated, as can be seen from Table 1 there is a rise in the use of LC-MS/MS assays for the direct determination of target ART drug(s). The WHO reports that almost two thirds of the 38 million people living with HIV globally are based in Africa [142]. Sampling strategy will have to take into consideration the socioeconomic situation of the region where analysis is to take place and what biosample to be used. Therefore, plasma or serum might not be the best option for routine sampling in resource limited settings like sub Saharan Africa since trained personnel (phlebotomists) are required for collection of samples in clinics or hospitals. There is also the challenge with carrying out venous sampling in certain populations like paediatric patients. To address these issues, other more patient-friendly sampling methods such as saliva, hair and DBS have been investigated for ART therapy adherence assessment [10, 62, 64, 66, 68, 69, 71, 73, 76-78].

As is evident from Table 1 another growing area where hyphenated MS techniques are being used to objectively assess medication adherence is for pain management

drugs. Adherence monitoring of pain management medication is crucial especially when dealing with chronic non-malignant pain conditions. Current challenges confronting the clinician in this regard include abuse, overuse and diversion of controlled prescription drugs which include benzodiazepines and opioids [143]. Urine is the biological sample commonly used for these analyses as is evident from Table 1. Urine is preferred for these assays because it provides a long detection window due to the drug metabolite pharmacokinetics. A few studies have used plasma, serum, saliva and hair for assessing adherence to pain management drugs although drugs which are strongly protein bound (e.g. benzodiazepines) generally do not appear in high concentrations in saliva [81, 82, 86, 88, 97, 144]. Immunoassays are used for screening (qualitative) purposes which may identify drugs but with variable specificity. A major limitation of immunoassays for drug quantification is poor sensitivity compared to MS based methods. This is demonstrated by Mikel et al [96] who compared a urine opiate immunoassay to an LC-MS/MS assay and found that the immunoassay has a limit of detection of 300 ng/ml while the LC-MS/MS assay had a limit of detection of 50 ng/ml which resulted in approximately 69% of patients who were prescribed and taking an opiate having detectable drug concentrations from the LC-MS/MS assay but tested negative on the immunoassay. Hyphenated MS-based techniques (GC-MS, LC-MS/MS, LC-HRMS (ToF)) also offer more targeted (quantitative) confirmations. The LC-MS/MS has replaced the GC-MS for this analysis since it simplifies the sampling process by eliminating the chemical derivatization step in GC-MS.

Surprisingly very few reports have addressed the assessment of adherence to oral hypoglycaemic drugs in the management of Type 2 diabetes via direct MS-based bioanalytical assays. A very recent study [102] used an LC-MS/MS (qqq) based urine assay and found that 28.1% of patients were nonadherent to antidiabetic,

antihypertensive and/or lipid lowering medications. It is postulated that the paucity of data related to monitoring adherence to hypoglycaemic drugs may be because blood glucose levels are also routinely monitored by diabetic patients using simple fingerprick tests [1]. Notwithstanding, objectively monitoring adherence is paramount in order to improve clinical outcomes of this chronic illness.

The widespread problem of medication nonadherence has fuelled interest in the application of hyphenated MS-based (predominantly LC-MS/MS (qqq)) bioanalytical assays to adherence assessment studies in other clinical areas including cancer in situations where oral chemotherapy drugs are prescribed [103-110], immunosuppressant therapy [111-114], schizophrenia [115-116], depression [117-118], epilepsy [119], asthma [120-123], infectious diseases [124, 125], tuberculosis [126, 127], arthritis [128, 129], inflammatory bowel disease [130] and attention deficit hyperactivity disorder (ADHD) [131-133].

Even in the wealthy/industrialised countries where access to the most advanced instrumentation is not a major problem, healthcare costs are such that only medication adherence assessments for major chronic diseases such as cardiovascular disease are likely to be given priority. Infectious diseases like HIV and malaria are a global issue but pose a much bigger problem in countries where healthcare facilities are limited and so reliance on simpler manual methods would be expected. Collaboration between countries such that equipment or biological samples are sent to other countries for analysis might be the way forward in addressing the challenge of limited access to facilities in some countries and indeed this research group is currently collaborating with two of the largest hospitals in Nairobi, Kenya to address the problem of non-adherence to cardiovascular medicines since chronic diseases such as hypertension and type 2 diabetes are on the rise in Africa.

5. Advantages and challenges of using hyphenated mass spectrometry techniques for assessing medication adherence

Advances in MS technologies have revolutionised the analysis of biosamples for clinical studies and marked improvements in analytical specificity and sensitivity have augmented the use of hyphenated MS systems for objectively assessing medication adherence. Cost reduction has been another factor fuelling the potential use of MS for such routine clinical analyses. It can be argued that the initial capital cost of MS-based equipment is high, with skilled personnel needed for development, validation and application of the bioanalytical assays. However, Jannetto and Fitzgerald [17] suggest that MS-based systems can still be cost effective if laboratories develop in-house MS tests which will cut down on send-out costs for higher-volume tests. A major advantage of using MS-based systems is that it is possible to have a single MS method for a multianalyte assay thus saving time, effort and reagents. Simplifying and standardising sample processing makes MS-based methods even more appealing to smaller laboratories with less expertise especially in LMIC. Another advantage of using MS based techniques is that they are commonly used techniques across the world for a range of applications and this means they are well understood in terms of limitations and their applicability. The potential to provide medication adherence assessment tests for a wide range of clinical areas using MS-based techniques is evident from Table 1.

Although advancements with MS-based techniques have made them more attractive for potential use in routine clinical analysis, some substantial challenges have to be addressed before the wider adoption of an MS-based service for assessment of

medication adherence. These challenges include the need for skilled personnel, high cost of equipment and lack of automation, software and data handling and limitations on sample throughput [17,145,146].

The need for skilled personnel for MS instrument maintenance and running is paramount when using MS methods. While front end cleaning is required for all MS instruments, HRMS instruments need to be regularly calibrated to maintain the high mass accuracy and resolution. A certain level of expertise is required in the method development and validation process for bioanalytical applications. Generally, this skill set requires training and practical experience on the equipment for a substantial amount of time (months to years) to become proficient.

High initial capital cost of MS systems is another challenge that needs to be addressed to facilitate their use in the routine assessment of medication adherence especially in global regions were funds are scarce such as in LMIC. Cost is a significant challenge in obtaining LC-HRMS instruments for routine clinical applications. The price of an LC-HRMS instrument is often twice or more compared to a triple quadrupole (qqq) or single quadrupole instruments. From Table 1, it is evident that HRMS instruments are being used in clinical investigations for assessing medication adherence suggesting that their quantitative performance coupled with high specificity is acceptable for such analyses. If the costs of the LC-HRMS instruments could be reduced in the future then this could bring a shift of paradigm in LC-MS analyses because HRMS gives the most complete picture of what is in a biosample. This is very useful in medication adherence monitoring studies since the acquired HRMS data can be mined retrospectively to look for non-target analytes such as drug metabolites in instances where questionable results are obtained initially [29]. Versatility, excellent qualitative and quantitative determinations and global acquisitions are key criteria in the uptake of these

instruments. Working out return on investment might be a way of addressing this challenge bearing in mind factors like labour and training costs, service contracts, supplies, proficiency testing, time needed for development and validation of the method in addition to the cost of equipment. The quality and the amount of data required will help justify the use of MS for assessment of medication adherence. In LMICs where resources are limited, the approach might be to have these MS-based systems in zonal or national laboratories where biosamples from around the regions can be sent for analysis. In this situation, the feasibility and costs of transporting the biosamples will have to be considered as well.

The lack of an automated system that incorporates sample processing and preparation with the instrumentation is also a challenge especially when dealing with a large number of samples. This is usually the case when assessing medication adherence in patients taking multiple medications (polypharmacy). Typically, targeted quantitative LC-MS/MS analyses using gradient elution are carried out at a rate of a few minutes per injection and this can be reduced to about 2 minutes per injection using a UHPLC system. These runtimes can limit throughput and therefore developments are required to reduce run times more akin to that of direct injection methods but which include efficient chromatographic separation of target analytes and matrix components. Velghe et al [147] suggest multiplexing as an efficient option for further increasing throughput where multiple LC systems are coupled to one MS while ensuring the MS is used economically. Advances have also been made in the automation of processes related to therapeutic drug monitoring (TDM) which can be applied in the objective assessment of medication adherence. For instance, when DBS are employed, semi-automated punching devices are now available to replace the laborious manual punching process and sample extraction can be expedited using automated liquid

handling systems [148] while fully automated DBS analysers that can be coupled to standard LC-MS/MS systems are now commercially available [147].

Data file storage can also be a major challenge particularly when using LC-HRMS in full scan mode for large numbers of samples and data compression can address some of these concerns.

Managing data flow for clinical mass spectrometry testing can be challenging because of the lack of automated commercial solutions and data processing, analysis and reporting can be a time consuming part of LC-MS-based testing when quantitative data is required even for low volume clinical laboratories. It is therefore important to implement simplified, robust and optimised workflows for managing mass spectrometry data which begins with an order for a laboratory test and ends with an uploaded patient result.

Since the choice of instrumentation is dependent on the analyte of interest, another challenge will be deciding what instrument to purchase. The LC-triple-quadrupole mass spectrometer (qqq) has been identified as the instrument of choice for small molecule quantitative analysis [17,146].

6. Conclusion

Improving medication adherence, potentially the most effective route to improving the therapeutic benefit of pharmacotherapy, remains a challenge for healthcare systems worldwide. As can be seen from Table 1, researchers and healthcare professionals globally have been very enthusiastic about using hyphenated MS techniques for the

analysis of biosamples in a wide range of clinical areas to address this global healthcare problem. The results of such tests will provide the evidence base to aid the clinical decision making process and to maximise patient benefit from the prescribed drug therapies. These objective results could make a step change in allowing clinician-patient discussions to be focussed around which treatments are being taken, which not and for what reasons. Home or remote sampling will ease the burden on overstretched healthcare facilities globally and will augment this process. Assessing adherence to prescribed cardiovascular therapy drugs using hyphenated MS-based techniques is the fastest growing clinical area which is not surprising given that CVD is one of the biggest killers worldwide.

Analysts face challenges of assay robustness, reproducibility, specificity, sensitivity and accuracy of drug quantification and therefore optimisation of biosample, sample preparation, mass spectrometry method, LC and MS conditions is required for application in a clinical setting. The limitations of immunoassays promote the triple quadrupole LC-MS/MS systems as the current best technique for this application as it is a more robust and reliable method with superior specificity and sensitivity. LC-HRMS systems have great potential for quantitative bioanalysis and furthermore provide the most complete picture of what is in a biosample and have the inherent advantage of post-acquisition data mining. Healthcare providers face numerous challenges including instrument costs, instrument complexity, shortage of suitably trained staff, software and data handling and limitations on sample throughput in order to implement these adherence assessment methods in a wide clinical setting.

An appropriate analytical method for monitoring medication adherence should be one which is able to provide the required data with relative ease and simplicity. The miniaturisation of MS systems via the use of ambient MS-based techniques [149] or

the use of microfluidic technology [150] to monitor therapeutic drugs in biosamples will go a long way in providing more portable, easy-to-use equipment which will facilitate point-of-care assessment of medication adherence. Due to their portability and simplicity, these systems could then be used in various settings like clinician offices. Commercial availability of more approved MS-based kits, quality controls and calibrators is key in the drive towards adopting MS-based methods for routine monitoring of medication adherence. Further improvements in specificity and sensitivity as well as automation of various processes will make MS-based systems even more attractive for routine clinical use. With growth and advancement in technology, it is envisaged that hyphenated MS systems will become an essential component of clinical medicine for adherence assessment.

In conclusion, effective adherence to prescribed medications, for an individual, will be confirmed if the measured biosample drug levels are within the drug therapeutic windows. Thus the analysis of biosamples using hyphenated MS techniques is the way forward and will facilitate evidence-based and personalised therapy for the benefit of patients globally. The current moves to improve the objective assessment of the attainment of therapeutic levels of drugs by increasing adherence must continue and the benefits including the personalisation of healthcare, made obvious to all stakeholders.

Acknowledgments: Not applicable

Author contributions: All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared

Employment or leadership: None declared

Honorarium: None declared

Competing interests: None declared

In Memoriam: Dr Graham Lawson

Dr Graham Lawson sadly passed away on September 2nd 2019 and had co-authored this Review whilst he was in hospital. Graham was a pioneer in the mass spectrometry field and a well-respected analytical chemist internationally, whose significant contributions to the instrumental analysis field helped to advance the area in multiple ways.

Graham's insight was invaluable and he was a very passionate and enthusiastic researcher and a dedicated teacher with high standards and principles. He had conducted research in disparate areas such as environmental exposure in the polymer industry, the identification of migrants from food packaging and factors influencing drug delivery and clinical applications and he was also co-opted onto a NATO special studies group on standoff detection of radiation. More recently he had conducted research into novel analytical techniques applied to dried blood spot analyses for healthcare applications and to counterfeit drug detection.

Graham was a role model and a true example to many, both scientifically but also on a personal level. He will be remembered with gratitude and respect for being an immensely kind, generous and helpful person and a loyal friend, colleague and mentor. Over the years Graham guided, influenced and inspired thousands of students, colleagues and others he reached and his legacy will live on through the many scientists he has inspired.

References

1. Tanna S, Lawson G. Medication adherence. In: Thomas BF, editor. Analytical Chemistry for Assessing Medication Adherence. Amsterdam: Elsevier, 2016:1-21.

2. Sabaté E, editor. Adherence to long-term therapies: evidence for action. World Health Organization, 2003.

3. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005;353:487-97.

4. Lawson G, Ogwu J, Tanna S. Quantitative screening of the pharmaceutical ingredient for the rapid identification of substandard and falsified medicines using reflectance infrared spectroscopy. PloS One 2018;13:e0202059.

5. Kennedy J, Tuleu I, Mackay K. Unfilled prescriptions of medicare beneficiaries: prevalence, reasons, and types of medicines prescribed. J Manag Care Pharm 2008;14:553-60.

6. Brown MT, Bussell J, Dutta S, Davis K, Strong S, Mathew S. Medication adherence: truth and consequences. Am J Med Sci 2016;351:387-99.

7. Mathes T, Jaschinski T, Pieper D. Adherence influencing factors–a systematic review of systematic reviews. Arch Public Health 2014;72:37.

8. Gupta P, Patel P, Horne R, Buchanan H, Williams B, Tomaszewski M. How to screen for non-adherence to antihypertensive therapy. Curr Hypertens Rep 2016;18:89.

9. Tanna S, Lawson G. Dried blood spot analysis to assess medication adherence and to inform personalization of treatment. Bioanalysis 2014;6:2825-38.

10. Wu Y, Yang J, Duan C, Chu L, Chen S, Qiao S, et al. Simultaneous determination of antiretroviral drugs in human hair with liquid chromatography-electrospray ionization-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1083:209-21.

11. Richter LH, Jacobs CM, Mahfoud F, Kindermann I, Böhm M, Meyer MR. Development and application of a LC-HRMS/MS method for analyzing antihypertensive drugs in oral fluid for monitoring drug adherence. Anal Chimica Acta 2019;1070:69-79.

12. Morrison A, Stauffer ME, Kaufman AS. Defining medication adherence in individual patients. Patient Prefer Adherence 2015;9:893-7.

13. Tate J, Ward G. Interferences in immunoassay. Clin Biochem Rev 2004;25:105-120.

14. Adaway JE, Keevil BG. Therapeutic drug monitoring and LC–MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2012;883-884:33-49.

15. Snyder ML, Fantz CR, Melanson S. Immunoassay-based drug tests are inadequately sensitive for medication compliance monitoring in patients treated for chronic pain. Pain Physician 2017;20:SE1-9.

16. Tanna S, Lawson G. Analytical chemistry methods for the assessment of medication adherence. In: Thomas BF, editor. Analytical Chemistry for Assessing Medication Adherence. Amsterdam: Elsevier, 2016:51-86.

17. Jannetto PJ, Fitzgerald RL. Effective use of mass spectrometry in the clinical laboratory. Clin Chem 2016;62:92-8.

18. Peat MA. Financial viability of screening for drugs of abuse. Clin Chem 1995;41: 805-08.

19. Tanna S, Lawson G. Analytical methods used in conjunction with dried blood spots. Anal Meth 2011;3:1709-18.

20. Fung EN, Jemal M, Aubry AF. High-resolution MS in regulated bioanalysis: where are we now and where do we go from here? Bioanalysis 2013;5:1277-84.

21. Lawson G, Cocks E, Tanna S. Quantitative determination of atenolol in dried blood spot samples by LC–HRMS: a potential method for assessing medication adherence. J Chromatogr B Analyt Technol Biomed Life Sci 2012;897:72-9.

22. Alalaqi AD, Lawson G, Obaid Y, Tanna S. Non-adherence to cardiovascular pharmacotherapy in Iraq assessed using 8-items Morisky questionnaire and analysis of dried blood spot samples. Brit J Pharm 2019;4:doi: https://doi.org/10.5920/bjpharm.627.

23. Tanna S, Alalaqi A, Bernieh D, Lawson G. 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. Clin Mass Spectrom 2018;10:1-8.

24. Bernieh D, Lawson G, Tanna S. Quantitative LC–HRMS determination of selected cardiovascular drugs, in dried blood spots, as an indicator of adherence to medication. J Pharm Biomed Anal 2017;142:232-43.

25. Ewen S, Meyer MR, Cremers B, Laufs U, Helfer AG, Linz D, et al. Blood pressure reductions following catheter-based renal denervation are not related to improvements in adherence to antihypertensive drugs measured by urine/plasma toxicological analysis. Clin Res Cardiol 2015;104:1097-105.

26. Helfer AG, Michely JA, Weber AA, Meyer MR, Maurer HH. Orbitrap technology for comprehensive metabolite-based liquid chromatographic–high resolution-tandem mass spectrometric urine drug screening–exemplified for cardiovascular drugs. Anal Chim Acta 2015;891:221-33.

27. Tanna S, Bernieh D, Lawson G. LC-HRMS analysis of dried blood spot samples for assessing adherence to cardiovascular medications. J Bioanal Biomed 2015; 7:001-005.

28. Jung O, Gechter JL, Wunder C, Paulke A, Bartel C, Geiger H, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. J Hypertens 2013;31:766-74.

29. Lawson G, Cocks E, Tanna S. Bisoprolol, ramipril and simvastatin determination in dried blood spot samples using LC–HRMS for assessing medication adherence. J Pharm Biomed Anal 2013;81:99-107.

30. Zhang Y, Clarke W, Marzinke MA, Piwowar-Manning E, Beauchamp G, Breaud A, et al. Evaluation of a multidrug assay for monitoring adherence to a regimen for HIV preexposure prophylaxis in a clinical study, HIV Prevention Trials Network 073. Antimicrob Agents Chemother 2017;61:e02743-16.

31. McMillin GA, Marin SJ, Johnson-Davis KL, Lawlor BG, Strathmann FG. A hybrid approach to urine drug testing using high-resolution mass spectrometry and select immunoassays. Am J Clin Pathol 2015;143:234-40.

32. Burns AD, Lane D, Cole R, Patel P, Gupta P. Cardiovascular medication stability in urine for non-adherence screening by LC–MS-MS. J Anal Tox 2018;43:325-9.

33. Comuth WJ, de Maat MP, van de Kerkhof D, Malczynski J, Husted S, Kristensen SD, et al. Adherence to dabigatran etexilate in atrial fibrillation patients intended to

undergo electrical cardioversion. Euro Heart J Cardiovasc Pharmacother 2019;5:91-9.

34. Johannsen JO, Reuter H, Hoffmann F, Blaich C, Wiesen MH, Streichert T, et al. Reliable and easy-to-use LC–MS/MS-method for simultaneous determination of the antihypertensives metoprolol, amlodipine, canrenone and hydrochlorothiazide in patients with therapy-refractory arterial hypertension. J Pharm Biomed Anal 2019;164:373-81.

35. Pelouch R, Voříšek V, Furmanová V, Solař M. The Assessment of Serum Drug Levels to Diagnose Non-Adherence in Stable Chronic Heart Failure Patients. Acta Medica (Hradec Králové) 2019;doi: 10.14712/18059694.2019.46.

36. Sutherland JJ, Morrison RD, McNaughton CD, Daly TM, Milne SB, Daniels JS, et al. Assessment of patient medication adherence, medical record accuracy, and medication blood concentrations for prescription and over-the-counter medications. JAMA Netw Open 2018;1:e184196.

37. Wallbach M, Lach N, Stock J, Hiller H, Mavropoulou E, Chavanon ML, et al. Direct assessment of adherence and drug interactions in patients with hypertensive crisis—A cross-sectional study in the Emergency Department. J Clin Hypertens 2019;21:55-63.

38. Avataneo V, De Nicolò A, Rabbia F, Perlo E, Burrello J, Berra E, et al. Therapeutic drug monitoring-guided definition of adherence profiles in resistant hypertension and identification of predictors of poor adherence. Brit J Clin Pharmacol 2018;84:2535-43.

39. Chen G, Jirjees Sr F, Al Bawab A, McElnay JC. Quantification of amlodipine in dried blood spot samples by high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1072:252-8.

40. Gundersen PO, Helland A, Spigset O, Hegstad S. Quantification of 21 antihypertensive drugs in serum using UHPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1089:84-93.

41. de Jager RL, van Maarseveen EM, Bots ML, Blankestijn PJ. Medication adherence in patients with apparent resistant hypertension: findings from the SYMPATHY trial. Brit J Clin Pharmacol 2018;84:18-24.

42. Mahrouse MA. Simultaneous ultraperformance liquid chromatography/tandem mass spectrometry determination of four antihypertensive drugs in human plasma using hydrophilic–lipophilic balanced reversed-phase sorbents sample preparation protocol. Biomed Chromatogr. 2018;32:e4362.

43. Petit G, Berra E, Georges CM, Capron A, Huang QF, Lopez-Sublet M, et al. Impact of psychological profile on drug adherence and drug resistance in patients with apparently treatment-resistant hypertension. Blood Press 2018;27:358-67.

44. Sandbaumhüter FA, Haschke M, Vogt B, Bohlender JM. Medication adherence during laboratory workup for primary aldosteronism: pilot study. Patient Prefer Adherence 2018;12:2449-55.

45. De Nicolò A, Avataneo V, Rabbia F, Sciandra M, Tosello F, Cusato J, et al. UHPLC–MS/MS method with sample dilution to test therapeutic adherence through quantification of ten antihypertensive drugs in urine samples. J Pharm Biomed Anal 2017;142:279-85.

46. Gupta P, Patel P, Štrauch B, Lai FY, Akbarov A, Gulsin GS, et al. Biochemical screening for nonadherence is associated with blood pressure reduction and improvement in adherence. Hypertension 2017;70:1042-8.

47. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Routine urinary detection of antihypertensive drugs for systematic evaluation of adherence to treatment in hypertensive patients. J Hypertens 2017;35:1891-8.

48. McNaughton CD, Brown NJ, Rothman RL, Liu D, Kabagambe EK, Levy PD, et al. Systolic blood pressure and biochemical assessment of adherence: a cross-sectional analysis in the emergency department. Hypertension 2017;70:307-14.

49. Ryan TP, Morrison RD, Sutherland JJ, Milne SB, Ryan KA, Daniels JS, et al. Medication adherence, medical record accuracy, and medication exposure in real-world patients using comprehensive medication monitoring. PloS One 2017;12:e0185471.

50. van der Nagel BC, Versmissen J, Bahmany S, van Gelder T, Koch, BC. High-throughput quantification of 8 antihypertensive drugs and active metabolites in human plasma using UPLC–MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2017;1060:367-73.

51. De Nicolò A, Avataneo V, Rabbia F, Bonifacio G, Cusato J, Tomasello C, et al. UHPLC-MS/MS method with protein precipitation extraction for the simultaneous quantification of ten antihypertensive drugs in human plasma from resistant hypertensive patients. J Pharm Biomed Anal. 2016;129:535-41.

52. Patel P, Gupta PK, White CM, Stanley AG, Williams B, Tomaszewski M. Screening for non-adherence to antihypertensive treatment as a part of the diagnostic pathway to renal denervation. J Hum Hypertens 2016;30:368-73.

53. Schmieder RE, Ott C, Schmid A, Friedrich S, Kistner I, Ditting T, et al. Adherence to antihypertensive medication in treatment-resistant hypertension undergoing renal denervation. J Am Heart Assoc 2016;5:e002343.

54. Florczak E, Tokarczyk B, Warchoł-Celińska E, Szwench-Pietrasz E, Prejbisz A, Gosk M, et al. Assessment of adherence to treatment in patients with resistant hypertension using toxicological serum analysis. Pol Arch Med Wewn 2015;125:65-72.

55. Hamdidouche I, Jullien V, Billaud EM, Boutouyrie P, Azizi M, Laurent S. 7B. 06: Routine urinary detection of antihypertensive drugs for estimation of adherence to treatment a cross sectional study. J Hypertens 2015;33:e93.

56. Lawson AJ, Shipman KE, George S, Dasgupta I. A novel 'Dilute-and-shoot' liquid chromatography–tandem mass spectrometry method for the screening of antihypertensive drugs in urine. J Anal Toxicol 2015;40:17-27.

57. Tomaszewski M, White C, Patel P, Masca N, Damani R, Hepworth J, et al. High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. Heart 2014;100:855-61.

58. Dias E, Hachey B, McNaughton C, Nian H, Yu C, Straka B, et al. An LC–MS assay for the screening of cardiovascular medications in human samples. J Chromatogr B Analyt Technol Biomed Life Sci 2013;937:44-53.

59. Štrauch B, Petrák O, Zelinka T, Rosa J, Šomlóová Z, Indra T, et al. Precise assessment of noncompliance with the antihypertensive therapy in patients with resistant hypertension using toxicological serum analysis. J Hypertens 2013;31:2455-61.

60. Zoerner AA, Schroeder C, Kayacelebi AA, Suchy MT, Gutzki FM, Stichtenoth DO, et al. A validated, rapid UPLC–MS/MS method for simultaneous ivabradine,

reboxetine, and metoprolol analysis in human plasma and its application to clinical trial samples. J Chromatogr B Analyt Technol Biomed Life Sci 2013;927:105-11.

61. Ceral J, Habrdova V, Vorisek V, Bima M, Pelouch R, Solar M. Difficult-to-control arterial hypertension or uncooperative patients? The assessment of serum antihypertensive drug levels to differentiate non-responsiveness from non-adherence to recommended therapy. Hypertens Res 2011;34:87.

62. Chu L, Wu Y, Duan C, Yang J, Yang H, Xie Y, et al. Simultaneous quantitation of zidovudine, efavirenz, lopinavir and ritonavir in human hair by liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1097:54-63.

63. Notari S, Tempestilli M, Fabbri G, Libertone R, Antinori A, Ammassari A, et al. UPLC–MS/MS method for the simultaneous quantification of sofosbuvir, sofosbuvir metabolite (GS-331007) and daclatasvir in plasma of HIV/HCV co-infected patients. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1073:183-90.

64. Schauer AP, Sykes C, Cottrell ML, Prince H, Kashuba AD. Validation of an LC–MS/MS assay to simultaneously monitor the intracellular active metabolites of tenofovir, emtricitabine, and lamivudine in dried blood spots. J Pharm Biomed Anal 2018;149:40-5.

65. Koenig HC, Mounzer K, Daughtridge GW, Sloan CE, Lalley-Chareczko L, Moorthy GS, et al. Urine assay for tenofovir to monitor adherence in real time to tenofovir disoproxil fumarate/emtricitabine as pre-exposure prophylaxis. HIV Medicine 2017;18:412-8.

66. Baxi SM, Liu A, Bacchetti P, Mutua G, Sanders EJ, Kibengo FM, et al. Comparing the novel method of assessing PrEP adherence/exposure using hair samples to other pharmacologic and traditional measures. J AIDS 2015;68:13-20.

67. Kumar S, Rao PS, Earla R, Kumar A. Drug–drug interactions between anti-retroviral therapies and drugs of abuse in HIV systems. Expert Opin Drug Metab Toxicol 2015;11:343-55.

68. Olds PK, Kiwanuka JP, Nansera D, Huang Y, Bacchetti P, Jin C, et al. Assessment of HIV antiretroviral therapy adherence by measuring drug concentrations in hair among children in rural Uganda. AIDS care 2015;27:327-32.

69. Hickey MD, Salmen CR, Tessler RA, Omollo D, Bacchetti P, Magerenge R, et al. Antiretroviral concentrations in small hair samples as a feasible marker of adherence in rural Kenya. J AIDS 2014;66:311-315.

70. Bulgiba A, Mohammed UY, Chik Z, Lee C, Peramalah D. How well does self-reported adherence fare compared to therapeutic drug monitoring in HAART? Prev Med 2013;57:S34-6.

71. Shah SA, Mullin R, Jones G, Shah I, Barker J, Petroczi A, et al. Simultaneous analysis of antiretroviral drugs abacavir and tenofovir in human hair by liquid chromatography–tandem mass spectrometry. J Pharm Biomed Anal 2013;74:308-13.

72. Jones G, Hawkins K, Mullin R, Nepusz T, Naughton DP, Sheeran P, et al. Understanding how adherence goals promote adherence behaviours: a repeated measure observational study with HIV seropositive patients. BMC Public Health 2012;12:587.

73. Gras A, Schneider S, Karasi JC, Ternes AM, Sauvageot N, Karasi-Omes C, et al. Evaluation of saliva as an alternative matrix for monitoring plasma zidovudine, lamivudine and nevirapine concentrations in Rwanda. Curr HIV Res 2011;9:223-8.

74. Liu X, Ma Q, Zhang F. Therapeutic drug monitoring in highly active antiretroviral therapy. Expert Opin Drug Saf 2010;9:743-58.

75. Rakhmanina NY, van den Anker JN, Soldin SJ, van Schaik RH, Mordwinkin N, Neely MN. Can TDM Improve Pharmacotherapy of HIV Infection in Adolescents? Ther Drug Monit 2010;32:273-281.

76. Gandhi M, Ameli N, Bacchetti P, Gange SJ, Anastos K, Levine A, et al. Protease inhibitor levels in hair samples strongly predict virologic responses to HIV treatment. J AIDS 2009;23:471-8.

77. Ter Heine R, Rosing H, Van Gorp EC, Mulder JW, Van der Steeg WA, Beijnen JH, et al. Quantification of protease inhibitors and non-nucleoside reverse transcriptase inhibitors in dried blood spots by liquid chromatography–triple quadrupole mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008;867:205-12.

78. Koal T, Burhenne H, Römling R, Svoboda M, Resch K, Kaever V. Quantification of antiretroviral drugs in dried blood spot samples by means of liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2005;19:2995-3001.

79. Bodor GS. Pain Management Testing by Liquid Chromatography Tandem Mass Spectrometry. Clin Lab Med 2018;38:455-70.

80. Cua A, Rogers R, Lipnick P, Kilbourn K. Lower cutoffs for LC-MS/MS urine drug testing indicates better patient compliance. Pain Physician 2017;20:E1107-13.

81. Ferrari A, Licata M, Rustichelli C, Baraldi C, Vandelli D, Marchesi F, et al. Monitoring of adherence to headache treatments by means of hair analysis. Euro J Clin Pharmacol 2017;73:197-203.

82. Kipping K, Maier C, Bussemas HH, Schwarzer A. Medication compliance in patients with chronic pain. Pain Physician 2014;17:81-94.

83. Liu JC, Ma JD, Morello CM, Atayee RS, Best BM. Naltrexone metabolism and concomitant drug concentrations in chronic pain patients. J Anal Toxicol 2014;38:212-7.

84. Melanson SE, Tanasijevic MJ, Snyder ML, Darragh A, Quade C, Jarolim P. Significant cost savings achieved by in-sourcing urine drug testing for monitoring medication compliance in pain management. Clin Chim Acta 2013;422:10-14.

85. Melanson SE, Ptolemy AS, Wasan AD. Optimizing urine drug testing for monitoring medication compliance in pain management. Pain Med 2013;14:1813-20.

86. Heltsley R, DePriest A, Black DL, Crouch DJ, Robert T, Marshall L, et al. Oral fluid drug testing of chronic pain patients. II. Comparison of paired oral fluid and urine specimens. J Anal Toxicol 2012;36:75-80.

87. Christo PJ, Manchikanti L, Ruan X, Bottros M, Hansen H, Solanki DR, et al. Urine drug testing in chronic pain. Pain Physician 2011;14:123-43.

88. Heltsley R, DePriest A, Black DL, Robert T, Marshall L, Meadors VM, et al. Oral fluid drug testing of chronic pain patients. I. Positive prevalence rates of licit and illicit drugs. J Anal Toxicol 2011;35:529-40.

89. McCarberg BH. A critical assessment of opioid treatment adherence using urine drug testing in chronic pain management. Postgrad Med 2011;123:124-31.

90. Pesce A, West C, West R, Crews B, Mikel C, Rosenthal M, et al. Determination of medication cutoff values in a pain patient population. J Opioid Manag 2011;7:117-22.

91. Cone EJ, Zichterman A, Heltsley R, Black DL, Cawthon B, Robert T, et al. Urine testing for norcodeine, norhydrocodone, and noroxycodone facilitates interpretation and reduces false negatives. Forensic Sci Int 2010;198:58-61.

92. Manchikanti L, Malla Y, Wargo BW, Cash KA, Pampati V, Damron KS, et al. Protocol for accuracy of point of care (POC) or in-office urine drug testing (immunoassay) in chronic pain patients: A prospective analysis of immunoassay and liquid chromatography tandem mass spectrometry (LC/MS/MS). Pain Physician 2010;13:E1-E22.

93. Pesce A, Rosenthal M, West R, West C, Crews B, Mikel C, et al. An evaluation of the diagnostic accuracy of liquid chromatography-tandem mass spectrometry versus immunoassay drug testing in pain patients. Pain Physician 2010;13:273-281.

94. West R, Pesce A, West C, Crews B, Mikel C, Rosenthal M, et al. Observations of medication compliance by measurement of urinary drug concentrations in a pain management population. J Opioid Manag 2010;6:253-7.

95. West R, Pesce A, West C, Crews B, Mikel C, Almazan P, et al. Comparison of clonazepam compliance by measurement of urinary concentration by immunoassay and LC-MS/MS in pain management population. Pain Physician 2010;13:71-8.

96. Mikel C, Almazan P, West R, Crews B, Latyshev S, Pesce A, et al. LC-MS/MS extends the range of drug analysis in pain patients. Ther Drug Monitor 2009;31:746-8.

97. Vert P, Hamon I, Hubert C, Legagneur M, Hascoet JM. Infants of drug-addicted mothers: pitfalls of replacement therapy. Bull Acad Natl Med 2008;192:961-9.

98. Wasan AD, Michna E, Janfaza D, Greenfield S, Teter CJ, Jamison RN. Interpreting urine drug tests: prevalence of morphine metabolism to hydromorphone in chronic pain patients treated with morphine. Pain Med 2008;9:918-23.

99. Reisfield GM, Salazar E, Bertholf RL. Rational use and interpretation of urine drug testing in chronic opioid therapy. Ann Clin Lab Sci 2007;37:301-14.

100. Lee HK, Lewis LD, Tsongalis GJ, McMullin M, Schur BC, Wong SH, et al. Negative urine opioid screening caused by rifampin-mediated induction of oxycodone hepatic metabolism. Clin Chim Acta 2006;367:196-200.

101. Berndt S, Maier C, Schütz HW. Polymedication and medication compliance in patients with chronic non-malignant pain. Pain 1993;52:331-9.

102. Patel P, Gupta P, Burns A, Mohamed AA, Cole R, Lane D, et al. Biochemical Urine Testing of Adherence to Cardiovascular Medications Reveals High Rates of Nonadherence in People Attending Their Annual Review for Type 2 Diabetes. Diabetes Care 2019;42:1132-35.

103. Antunes MV, Raymundo S, de Oliveira V, Staudt DE, Gössling G, Peteffi GP, et al. Ultra-high performance liquid chromatography tandem mass spectrometric method for the determination of tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen in dried blood spots—development, validation and clinical application during breast cancer adjuvant therapy. Talanta 2015;132:775-84.

104. Sharma SK, Kumar S, Vijayakumar AR, Seth T, Mishra P, Mahapatra M, et al. Utility of the trough plasma imatinib level monitoring at two time points in patients with the chronic myeloid leukemia-chronic phase. J Cancer Res Ther 2014;10:305-8.

105. Clarke Hillyer G, Neugut AI, Crew KD, Kalinsky K, Maurer MA, Rotsides DZ, et al. Use of a urine anastrozole assay to determine treatment discontinuation among

women with hormone-sensitive breast cancer: a pilot study. J Oncol Pract 2012;8:e100-4.

106. Oberguggenberger AS, Sztankay M, Beer B, Schubert B, Meraner V, Oberacher H, et al. Adherence evaluation of endocrine treatment in breast cancer: methodological aspects. BMC cancer 2012;12:474.

107. Timmers L, Boons CC, Mangnus D, Moes JE, Swart EL, Boven E, et al. The use of erlotinib in daily practice: a study on adherence and patients' experiences. BMC Cancer 2011;11:284.

108. Yoshida C, Komeno T, Hori M, Kimura T, Fujii M, Okoshi Y, et al. Adherence to the standard dose of imatinib, rather than dose adjustment based on its plasma concentration, is critical to achieve a deep molecular response in patients with chronic myeloid leukemia. Int J Hematol 2011;93:618-23.

109. Beer B, Schubert B, Oberguggenberger A, Meraner V, Hubalek M, Oberacher H. Development and validation of a liquid chromatography–tandem mass spectrometry method for the simultaneous quantification of tamoxifen, anastrozole, and letrozole in human plasma and its application to a clinical study. Anal Bioanal Chem 2010;398:1791-1800.

110. Ishikawa Y, Kiyoi H, Watanabe K, Miyamura K, Nakano Y, Kitamura K, et al. Trough plasma concentration of imatinib reflects BCR–ABL kinase inhibitory activity and clinical response in chronic-phase chronic myeloid leukemia: A report from the BINGO study. Cancer Sci 2010;101:2186-92.

111. Koster RA, Greijdanus B, Alffenaar JW, Touw DJ. Dried blood spot analysis of creatinine with LC-MS/MS in addition to immunosuppressants analysis. Anal Bioanal Chem 2015;407:1585-94.

112. Koop DR, Bleyle LA, Munar M, Cherala G, Al-Uzri A. Analysis of tacrolimus and creatinine from a single dried blood spot using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2013;926:54-61.

113. Hinchliffe E, Adaway JE, Keevil BG. Simultaneous measurement of cyclosporin A and tacrolimus from dried blood spots by ultra high performance liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2012;883:102-7.

114. Sallustio BC, Noll BD, Morris RG. Comparison of blood sirolimus, tacrolimus and everolimus concentrations measured by LC-MS/MS, HPLC-UV and immunoassay methods. Clin Biochem 2011;44:231-6.

115. Miller J, Wehring H, McMahon RP, DiPaula BA, Love RC, Morris AA, et al. Urine testing for antipsychotics: a pilot trial for a method to determine detection levels. Hum Psychopharmacol 2015;30:350-5.

116. Grundmann M, Kacirova I, Urinovska R. Therapeutic drug monitoring of atypical antipsychotic drugs. Acta Pharm 2014;64:387-401.

117. Bordson SJ, Atayee RS, Ma JD, Best BM. Tricyclic antidepressants: is your patient taking them? Observations on adherence and unreported use using prescriber-reported medication lists and urine drug testing. Pain Med 2014;15:355-63.

118. Bosman J, ter Horst PG, Smit JP, Dijkstra JR, Beekhuis HR, Slingersland RJ, et al. Adherence of antidepressants during pregnancy: MEMS compared with three other methods. Ther Adv Psychopharmacol 2014;4:61-9.

119. Hidalgo PR, Antunes MV, Spaniol B, Peteffi GP, Da Silva CA, Linden R. Adherence to Antiepileptic Pharmacotherapy: Evaluation Using a Standardized

Questionnaire and Drug Concentration in Biological Samples. Lat Am J Pharm 2014;33:1481-6.

120. Hassall D, Brealey N, Wright W, Hughes S, West A, Ravindranath R, et al. Hair analysis to monitor adherence to prescribed chronic inhaler drug therapy in patients with asthma or COPD. Pulm Pharmacol Ther 2018;51:59-64.

121. Hagan JB, Netzel BC, Matthews MR, Korpi-Steiner NL, Singh RJ. Urinary fluticasone propionate-17beta-carboxylic acid to assess asthma therapy adherence. Allergy Asthma Proc 2012;33:e35-e39.

122. Hagan JB, Taylor RL, Singh RJ. Assessment of synthetic glucocorticoids in asthmatic sputum. Allergy Rhinol 2011;2:33-35.

123. Korpi-Steiner NL, Netzel BC, Seegmille JC, Hagan JB, Singh RJ. Liquid chromatography–tandem mass spectrometry analysis of urinary fluticasone propionate-17beta-carboxylic acid for monitoring compliance with inhaled-fluticasone propionate therapy. Steroids 2010;75:77-82.

124. Haidar EA, Lee TD, Barton JD, Coates AR, Mantle PG. HPLC/MS/MS methodology for sensitive quantitation of monic acid A, the metabolic product of the antibiotic mupirocin. Biomarkers 2011;16:422-5.

125. Nageswara Rao R, Vali RM, Ramachandra B, Maurya PK. Rapid determination of rifaximin on dried blood spots by LC-ESI-MS. Biomed Chromatogr 2011;25:1201-7.

126. Lee SH, Seo KA, Lee YM, Lee HK, Kim JH, Shin C, et al. Low serum concentrations of moxifloxacin, prothionamide, and cycloserine on sputum conversion in multi-drug resistant TB. Yonsei Med J 2015;56:961-7.

127. Amlabu V, Mulligan C, Jele N, Evans A, Gray D, Zar HJ, et al. Isoniazid/acetylisoniazid urine concentrations: markers of adherence to isoniazid preventive therapy in children. Int J Tuberc Lung Dis 2014;18:528-30.

128. Bluett J, Riba-Garcia I, Verstappen SM, Wendling T, Ogungbenro K, Unwin RD, et al. Development and validation of a methotrexate adherence assay. Ann Rheum Dis 2019 Jun 5. doi: 10.1136/annrheumdis-2019-215446. [Epub ahead of print].

129. Hawwa AF, AlBawab A, Rooney M, Wedderburn LR, Beresford MW, McElnay JC. Methotrexate polyglutamates as a potential marker of adherence to long-term therapy in children with juvenile idiopathic arthritis and juvenile dermatomyositis: an observational, cross-sectional study. Arthritis Res Ther 2015;17:295.

130. Gifford AE, Berg AH, Lahiff C, Cheifetz AS, Horowitz G, Moss AC. A random urine test can identify patients at risk of mesalamine non-adherence: a prospective study. Am J Gastroenterol 2013;108:249-55.

131. Böttcher M, Kühne D, Beck O. Compliance testing of patients in ADHD treatment with lisdexamphetamine (Elvanse®) using oral fluid as specimen. Clin Mass Spectrom 2019 Apr 22. doi: 10.1016/j.clinms.2019.04.002. [Epub ahead of print].

132. Binz TM, Williner E, Strajhar P, Dolder PC, Liechti ME, Baumgartner MR, et al. Chiral analysis of amphetamines in hair by liquid chromatography–tandem mass spectrometry: compliance-monitoring of attention deficit hyperactivity disorder (ADHD) patients under Elvanse® therapy and identification after controlled low-dose application. Drug Test Anal 2018;10:254-61.

133. Papaseit E, Marchei E, Mortali C, Aznar G, Garcia-Algar O, Farrè M, et al. Development and validation of a liquid chromatography–tandem mass spectrometry

assay for hair analysis of atomoxetine and its metabolites: Application in clinical practice. Forensic Sci Int 2012;218:62-7.

134 Tanna S, Lawson G. Opportunities and challenges for analytical chemistry in the assessment of medication adherence. In: Thomas BF, editor. Analytical Chemistry for Assessing Medication Adherence. Amsterdam: Elsevier, 2016:23-49.

135 Patsalos PN, Berry DJ. Therapeutic drug monitoring of antiepileptic drugs by use of saliva. Ther Drug Monitoring 2013;35:4-29.

136. Avataneo V, D'Avolio A, Cusato J, Cantu M. LC-MS application for therapeutic drug monitoring in alternative matrices. J Pharm Biomed Anal 2019;166:40-51.

137. LeBeau MA, Montgomery MA, Brewer JD. The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair. Forensic Sci Int 2011; 210:110-6.

138. Capiau S, Alffenaar J-W, Stove CP. Alternative sampling strategies for therapeutic drug monitoring. In: Clarke W, Dasgupta A, editors. Clinical challenges in therapeutic drug monitoring. Amsterdam: Elsevier, 2016:279-336.

139. Kronish IM, Ye S. Adherence to cardiovascular medications: lessons learned and future directions. Prog Cardiovasc Dis 2013;55:590-600.

140. Durand H, Hayes P, Morrissey EC, Newell J, Casey M, Murphy AW, et al. Medication adherence among patients with apparent treatment-resistant hypertension: systematic review and meta-analysis. J Hypertens 2017;35:2346-57.

141. Hinkin CH, Hardy DJ, Mason KI, Castellon SA, Durvasula RS, Lam MN, et al. Medication adherence in HIV-infected adults: effect of patient age, cognitive status, and substance abuse. AIDS 2004;18:S19.

142. World Health Organisation – Fact Sheets. HIV/AIDS. https://www.who.int/en/news-room/fact-sheets/detail/hiv-aids; 2019 Accessed 22.07.19.

143. McMillin GA, Slawson MH, Marin SJ, Johnson-Davis KL. Demystifying analytical approaches for urine drug testing to evaluate medication adherence in chronic pain management. J Pain Palliat Care Pharmacother 2013;27:322-39.

144. Moore C. Drug testing and adherence monitoring in pain management: Oral fluid testing. J Opiod Manag 2015;11:69-75.

145. Zakaria R, Allen KJ, Koplin JJ, Roche P, Greaves RF. Advantages and challenges of dried blood spot analysis by mass spectrometry across the total testing process. EJIFCC 2016;27:288-317.

146. Tanna S, Lawson G. Medication adherence: where do we go from here? In: Thomas BF, editor. Analytical Chemistry for Assessing Medication Adherence. Amsterdam: Elsevier, 2016:129-142.

147. Velghe S, Capiau S, Stove CP. Opening the toolbox of alternative sampling strategies in clinical routine: a key-role for (LC-) MS/MS. TrAC-Trend Anal Chem 2016;84:61-73.

148. Johnson CJL, Christianson CD, Sheaff CN, Laine DF, Zimmer JSD, Needham SR. Use of conventional bioanalytical devices to automate DBS extractions in liquid-handling dispensing tips. Bioanalysis 2011;3:2303-2310.

149. Ferreira CR, Yannell KE, Jarmusch AK, Pirro V, Ouyang Z, Cooks RG. Ambient ionization mass spectrometry for point-of-care diagnostics and other clinical measurements. Clin Chem 2016;62:99-110.

150. Lazar IM. Microfluidic devices in diagnostics: what does the future hold? Bioanalysis 2015;7:2677-80.