Bioanalytical adherence monitoring also using alternative matrices

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VORWORT

Die nachfolgende Arbeit entstand unter der Anleitung von Herrn Univ.-Prof. Dr. rer. nat. Markus R. Meyer in der Abteilung für Experimentelle und Klinische Toxikologie der Fachrichtung 2.4. Experimentelle und Klinische Pharmakologie und Toxikologie der Universität des Saarlandes in Homburg in der Zeit von Januar 2019 bis August 2023.

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- Evaluation and analytical applicability of a novel volumetric absorptive microsampling strategy for adherence monitoring of antihypertensive drugs by means of LC-HRMS/MS, Jacobs et al., Anal Chim Acta., 2021. (DOI: 10.1016/j.aca.2021.339137)
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„There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle. “

Albert Einstein

ZUSAMMENFASSUNG

Diese Dissertation zielt darauf ab, einen umfassenden Überblick über verschiedene analytische Methoden zur Überwachung der Therapietreue zu bieten und ihre Bedeutung, Herausforderungen und Anwendungen bei verschiedenen Arzneistoffgruppen und chronischen Krankheiten zu beleuchten. Durch die Untersuchung verschiedener Probennahmestrategien, die Erläuterung von Herausforderungen und die Diskussion der Auswirkungen der Therapietreue-Überwachung auf die Gesundheitsversorgung strebt diese Dissertation an, ein tieferes Verständnis für die komplexe Beziehung zwischen der Therapietreue-Überwachung und dem Wohlbefinden der Patienten zu fördern.

Um diese Ziele zu erreichen, wurden vier verschiedene Strategien zur Therapietreue-Überwachung entwickelt, validiert und umgesetzt. Darüber hinaus wurden volumetrisch absorbierte Mikroprobennahmen von Blut aus der Fingerbeere und Speichel als alternative Probenmatrizes mit konventionellen Probenmatrizes wie Plasma und Urin hinsichtlich quantitativer Ergebnisse und der Klassifizierung der Therapietreue verglichen. Die alternativen Probennahmestrategien streben eine patientenzentrierte Versorgung an, allerdings bestehen weiterhin Herausforderungen wie die Definition von matrixspezifischen Grenzwerten für eine genaue Beurteilung der Therapietreue. Dennoch waren die alternativen Strategien zuverlässig und haben auf Akzeptanz bei den Patienten getroffen. Deswegen dienen diese als Grundlage für eine verbesserte Patientenversorgung.

SUMMARY

This thesis aimed to provide a comprehensive overview of different analytical strategies used for adherence monitoring, significance, challenges, and applications across different pharmacological compounds and chronic diseases. By examining different sampling tools and strategies for adherence monitoring, elucidating challenges, and discussing the implications of adherence monitoring on healthcare delivery, this thesis seeks to foster a deeper understanding of the intricate relationship between adherence monitoring and patient well-being.

In pursuit of these objectives, four different strategies for adherence monitoring were developed, validated, and implemented. Furthermore, the alternative sample matrices volumetric absorptive microsampling of finger prick blood and OF were compared to the conventional sample matrices plasma and urine for quantitative results and adherence classification. The evaluated alternative sampling strategies offered patient-centric care, yet challenges persist in defining matrix-specific cut-off concentrations for accurate adherence evaluation. Nonetheless, the alternative strategies have demonstrated reliability and patient acceptance, serving as foundation for enhanced patient care.

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1. GENERAL PART

1.1. ADHERENCE MONITORING

Adherence, often described as the extent to which a patient correctly follows medical advice, is a critical determinant of treatment success. Hereby, adherence is a multi-layered concept including pharmacological adherence, meaning taking medication as prescribed, or pharmacological abstinence in the context of substance use disorder, but also executing healthcare guidelines, including lifestyle and behavior modifications [1, 2]. In the forthcoming dissertation the central emphasis will be on the assessment of pharmacological adherence, particularly in the context of chronic diseases. Patients can exhibit non-adherence in several ways, including refraining from initiating prescribed medications (non-initiation), delaying prescription refills, discontinuing treatment (non-persistence), taking doses below prescribed levels (e.g., pill-splitting), acquiring more medication than needed (e.g., stockpiling), and administering medication incorrectly (e.g., incorrect timing of doses). Collectively, these behaviors are encompassed under the term “medication non-adherence” [3].

The treatment of chronic illnesses typically entails long-term use of pharmacotherapy and successful disease management as well as the optimization of the corresponding therapeutic outcomes depend significantly on the patient’s degree of adherence [3, 4]. Nonetheless, a high rate of non-adherence remains a pervasive challenge that can compromise the quality of patient care and place a substantial economic burden on the healthcare system since inadequate adherence is linked to preventable emergency department admissions and higher inpatient rates [5].

The pharmacological rate of adherence tends to exhibit higher levels among patients with acute conditions than patients dealing with chronic conditions. After the initial six months of therapy for a chronic disease, there is frequently a notable decline in adherence [6]. Key contributing factors to non-adherence include the occurrence of side effects, the patient’s general aversion to medication, or the absence of noticeable symptoms [7].

The multifaced nature of adherence monitoring involves diverse assessment techniques, ranging from patient self-reporting via questionnaires to more objective measures such as electronic pill-counting systems and bioanalytical monitoring [8, 9]. Hereby, self-reporting adherence through questionnaires is cost-effective, non-invasive, and provides prompt results; nevertheless, this subjective approach is susceptible to overestimating adherence levels. On the other hand, prescription records, pharmacy refill data, and electronic pill counting systems are objective; however, none of these methods can conclusively verify the actual medication intake. Consequently, direct monitoring of patient adherence becomes imperative. This is typically achieved through bioanalytical measurements of drugs, metabolites, or biological markers in different biospecimens [2].

However, all these methods provide valuable insights into patient adherence behavior, enabling the tailoring of interventions to address barriers to adherence, ultimately leading towards enhanced patient care.

1.2. LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY BASED ADHERENCE MONITORING

Briefly, biological samples, e.g., blood or urine are collected from patients and targeted drugs and/or their metabolites are extracted from the biospecimen by preanalytical sample preparation. Diverse sample preparation techniques, including but not limited to precipitation, liquid-liquid extraction, and solid-phase extraction, play a crucial role in the outcome of the analytical process [2]. Using liquid chromatography (LC), the extracted compounds are separated based on their chemical properties. This step is pivotal for isolating the targeted drugs and/or metabolites from complex biological matrices. The isolated compounds are then introduced into a mass spectrometer, where they are ionized and analyzed based on their mass-to-charge ratio. The generated data can be used for objective adherence assessment.

LC-mass spectrometry (MS) based bioanalytical techniques are recognized as reference analytical techniques [10], and the LC-MS based adherence monitoring represents therefore

a state-of-the-art and essential approach to addressing the challenge of patient non-adherence. Hereby, the presence of a prescribed drug, its metabolite, or biomarker in biological specimens such as blood, urine, or oral fluid (OF) can be assessed unbiased, and the corresponding concentration can be determined [10]. Deviations from expected drug concentration levels can indicate patient non-adherence. It is mandatory to ensure the accuracy and reproducibility of the analysis by a comprehensive analytical method validation [2].

However, due to required specialized equipment and expertise, LC-MS based adherence monitoring may be relatively expensive compared to other adherence monitoring methods like self-reporting or using medication refill data provided by the pharmacy. Furthermore, “white-coat-adherence”, where patients only take their prescribed medication in anticipation of a scheduled visit with their physician cannot be excluded by LC-MS based adherence assessment [2]. Nevertheless, it has been demonstrated that LC-MS based adherence monitoring improves overall adherence [11].

1.3. ALTERNATIVE SAMPLE MATRICES

The use of alternative sample matrices for adherence monitoring involves exploring biological specimens other than the conventionally used sample matrices blood plasma and urine. Hereby, e.g., dried blood spots (DBS), volumetric absorptive microsampling (VAMS), and OF, have become increasingly popular as sample matrices in recent years.

The best-known alternative to venous blood sampling, dating back to 1963, is the collection of DBS [12]. A small drop of capillary blood can be obtained by, e.g., a finger prick with a lancet. The blood drop of unknown volume is transferred by free falling or touching to a filter paper, which absorbs the blood rapidly and the sample is allowed to air dry [13]. The uncomplicated and minimal invasive sample collection can be performed by the patients themselves, making it well-suited for at-home sampling, consequently increasing the patients’ comfort [14]. The use of DBS relies on the principle that the dried samples contain the analytes of interest in a stable

form. This enables storage and transport at ambient temperatures, reducing logistical challenges associated with traditional venous blood sampling [14]. Several challenges are associated with using DBS, including the need for highly sensitive analysis techniques due to the limited sample volume available. Furthermore, issues related to sample collection may occur, even with well-trained medical staff or patients [14]. Variations in hematocrit levels can have an impact on both sample homogeneity and spreading behavior of blood, consequently affecting the analytical outcomes [15]. Moreover, it is important to note that drug reference concentrations are typically established for plasma as a sample matrix. Therefore, it is imperative to investigate the relationship between capillary blood and plasma concentrations, considering variations in hematocrit levels [13, 14]. VAMS are expected to overcome some limitations of DBS while retaining advantages such as cost-effectiveness, minimal invasiveness, and suitability for at-home sampling. VAMS devices consist of a porous hydrophilic tip attached to a plastic sample handler. The tip can absorb a specific, predetermined volume of, e.g., finger prick blood (FPB) through capillary action and absorption is claimed to be hematocrit independent [16].

A further alternative sample matrix of interest for adherence monitoring is OF. The collection is non-invasive, easy to perform and suitable for self-sampling at home [2]. The composition of OF closely mirrors that of blood by reflecting the free fraction of the circulating drug [17]. However, OF composition can be influenced by many factors, e.g., diet, hydration, and time of day, introducing variability in analyte concentration. Furthermore, drugs which are mainly protein bound may not be detectable or have very low concentrations within OF [18]. Moreover, the passage of a drug from blood to OF is influenced by the pH of OF as well as the pK_a, molecular weight, and lipid solubility of the drug [17]. As the pH of OF tends to be lower than that of plasma, basic dugs can be trapped in OF, leading to elevated concentrations [17]. Stimulation of salivary flow can increase the pH of OF and dilute the analyte, resulting in a change in drug concentration. A further challenge of OF sampling can be the dry mouth syndrome, which can be caused by drugs that block parasympathetic or increase sympathetic activity, but also by cigarette smoking [18]. As for DBS and VAMS, reference concentrations

for drugs in OF are barely available and must be established by investigation of the relationship between OF and plasma concentrations.

Alternative sample matrices can offer unique advantages but also challenges in tracking medication adherence. Their use broadens the scope of adherence monitoring by providing new options to suit different clinical needs and patient preferences, potentially leading to an increase in patients' cooperation to participate in monitoring events [19]. Ongoing research and advancements in analytical procedures contribute to an evolving landscape of adherence monitoring.

1.4. NON-ADHERENCE TOWARDS DRUG-CLASSES IN CHOSEN CHRONIC DISEASES

1.4.1. ANTIPSYCHOTICS

Worldwide, more than 65 million people live with schizophrenia or bipolar disorder [20]. Antipsychotic medications are primarily used to manage symptoms of these psychiatric disorders [21, 22]. Consistent and timely medication intake is crucial for controlling and stabilizing symptoms, preventing relapses, and improving overall mental health. However, low adherence is well known among patients suffering from psychotic symptoms, amongst others, due to a lack of insight into their illness and treatment [23-25]. A meta-analysis came to the result that 56 % of schizophrenia and 44 % of bipolar disorder patients are non-adherent to their psychotropic medication [26]. Another study confirmed these results by showing that only 53.6 % of schizophrenia and 52.4 % of bipolar disorder patients were adherent to their antipsychotic medication. Furthermore, among schizophrenia patients, 22.5 % switched medications and 15.1 % stopped therapy; among bipolar disorder patients, 15.8 % switched medication and 15.1 % stopped medication [27]. However, non-adherence and switching of antipsychotics have been linked to acute healthcare utilization [28, 29]. Consequently, adherence monitoring for antipsychotic drugs is crucial to ensure the optimal management of psychiatric disorders.

1.4.2. ANTIHYPERTENSIVE DRUGS

About 1.28 billion adults aged 30-79 years worldwide have hypertension, with hypertension being a major cause of premature death [30]. Herby, hypertension is defined by a blood pressure ≥140 mm Hg systolic or ≥90 mm Hg diastolic [31]. Current guidelines recommend, amongst others, pharmacotherapy with antihypertensive drugs (AHD) to reduce blood pressure and the risk of cardiovascular events like heart failure, coronary heart disease, and stroke [32, 33]. Hereby, two major factors contribute to the control of hypertension in treated patients; namely, the prescription of an adequate number and dose of prescribed AHD and adherence to therapy [31]. Nevertheless, adherence towards AHD is typically reported less than 50 % already one year after initiation of pharmacotherapy even though non-adherence to AHD is associated with uncontrolled blood pressure, poor clinical outcomes, and an increase in disease burden [31, 34]. Hence, medication's full benefit cannot be realized at the currently achievable adherence levels [35]. A study showed that adherence monitoring in non-adherent patients improved their level of adherence, resulting in a meaningful reduction in blood pressure [11]. Therefore, regulatory adherence monitoring is fundamental in the treatment of hypertension.

1.4.3. ORAL ENDOCRINE BREAST CANCER THERAPIES

In 2020, 2.3 million women worldwide received a diagnosis of breast cancer and there were 685,000 deaths. By the year's end, 7.8 million women who had been diagnosed in the previous five years were still alive, establishing breast cancer as the most prevalent cancer globally [36]. For patients diagnosed with hormone receptor positive breast cancer, clinical recommendations favor adjuvant oral endocrine therapy (OET) for a five-year treatment period. Studies indicated an improved disease-free and overall survival rate among patients receiving OET [37]. While it is generally anticipated that cancer patients would exhibit fewer challenges with non-adherence due to heightened motivation stemming from the gravity of their disease, research indicates contrasting reality. Despite the proven benefits of OET, studies show that

only half of breast cancer patients adhere to their prescribed regimen, and two-thirds discontinue therapy before completing the recommended five-year treatment duration [37, 38]. This poor adherence is linked to various factors, including patients' perception of an unfavorable risk/benefit ratio of the therapy, experienced adverse events, depressive symptoms, and the financial burden of medication costs [39]. The precise evaluation of adherence to OET is crucial in order to ensure the effectiveness of patient treatment [1, 2]. Consequently, adherence should be monitored on a regular basis.

2. AIMS AND SCOPES

Adherence to prescribed medication is crucial for successful disease management. This thesis aimed to provide different analytical methods suitable for objective adherence monitoring while focusing on innovative sampling tools and alternative sample matrices to enable patient-centric care.

Therefore, the following steps should be conducted:

- Evaluation of different sampling devices suitable and samples matrices suitable also for at-home sampling
- Evaluation of sample extraction procedures prior to bioanalysis
- Development of multi-analyte LC high resolution (HR) MS bioanalytical methods for selected drugs classes
- Validation of the LC-HRMS methods according to international guidelines including proof of concept studies

3. PUBLICATIONS OF THE RESULTS

The results of this dissertation were published in the following papers:

3.1. Development, validation, and application of a quantitative volumetric absorptive microsampling-based method in finger prick blood by means of LC-HRMS/MS applicable for adherence monitoring of antipsychotics [40]

(DOI: 10.1007/s00216-020-03143-0)

Author contribution

C.M.J.: conceptualization, methodology, validation, formal analysis, writing original draft; L.W.: conceptualization, methodology, writing review & editing; M. R. M.: conceptualization, methodology, resources, writing review & editing, supervision.

3.2. Evaluation and analytical applicability of a novel volumetric absorptive microsampling strategy for adherence monitoring of antihypertensive drugs by means of LC-HRMS/MS [19]

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3.3. Closing the gap – development of an analytical methodology using volumetric absorptive microsampling of finger prick blood followed by LC-HRMS/MS for adherence monitoring of antihypertensive drugs [41]

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3.4. Towards clinical adherence monitoring of oral endocrine breast cancer therapies by LC-HRMS – Method development, validation, comparison of four sample matrices, and proof of concept [42]

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4. DISCUSSION

Presented studies contribute to expanding the knowledge of the applicability of alternative sampling methods for bioanalytical adherence monitoring of different classes of drugs. In the first study, a strategy for adherence monitoring of 13 frequently prescribed antipsychotics was developed and validated using FPB sampled by VAMS. The study focused on the monitoring of parent compounds, except for paliperidone (9-hydroxyrisperidone), which required inclusion into the monitoring panel due to its dual role as either an active metabolite of risperidone or a prescribed drug. In general, the monitoring of the parent compound or a metabolite is considered sufficient for the purpose of adherence monitoring. To assess adherence, determined concentrations in FPB sampled by VAMS were compared to therapeutic reference concentrations available in literature, considering a ± 15 % tolerance limit to compensate for measurement uncertainty and inter-individual variabilities. A comparative quantitative analysis of 17 antipsychotic intakes was carried out using matching samples of FPB sampled by VAMS and plasma. Hereby, determined concentrations were comparable between matrices, which is consistent with existing literature [43, 44]. Additionally, patient adherence assessment using FPB samples by VAMS or plasma demonstrated equivalence for 16 out of 17 monitored antipsychotic intakes, affirming the suitability of the alternative sampling strategy for adherence assessment.

The second study focused on developing and validating a strategy for adherence assessment of 11 AHD in FPB sample by VAMS, later extended in a third study to include seven additional AHD. Due to the short half-life times of enalapril, losartan, ramipril, and spironolactone the respective metabolites enalaprilat, losartan-carboxylic acid, ramiprilat, and canrenone were included in the strategies instead of the parent compounds. This should prevent a misjudgment of non-adherence due to rapidly metabolized parent compounds. Patient-specific cut-off concentrations for adherence assessment of ADH were determined using dose-related concentration factors. This approach incorporates individual variabilities such as the dosing interval of the medication and pharmacokinetic properties. Even though this approach may be

a useful tool for adherence assessment, it remains a theoretical calculation that needs to be clinically validated due to interindividual influence on pharmacokinetics like age and comorbidity [45]. Moreover, a comparative analysis of determined drug concentrations in FPB sampled by VAMS and matching plasma samples was carried out. Therefore, 55 intakes of AHD were monitored in the second study and 35 intakes of AHD were monitored in the third study. Contrary to the antipsychotics, the comparative analysis of AHD concentrations revealed discrepancies between the matrices. This is consistent with existing literature and can be explained by the composition of FPB. FPB consists of whole blood, where total drug levels are measured, whereas measurements in plasma do not include intracellular drug levels [46, 47]. For amlodipine, e.g., a distribution up to 70 % into red blood cells is described [48]. As a result, quantitative results in FPB sampled by VAMS and plasma cannot be used interchangeably and specific reference concentrations need to be established for FPB sampled by VAMS. Nevertheless, the adherence assessment towards AHD was coincident between the two sample matrices in most cases. In conjunction with the adherence assessment in the second study, a survey evaluating the acceptability of the VAMS sampling procedure was conducted. Results demonstrated a general acceptance amongst patients, with the majority expressing a willingness to perform the sampling procedure themselves at home. This enables the possibility of home-based sampling, improving patient comfort and aligning with the principle of patient-centric care. Moreover, a notable proportion of patients were willing to regularly monitor their medication levels using the VAMS strategy, suggesting a potential avenue for enhancing medication adherence over time.

In the fourth study, a strategy for evaluating adherence to eight OET across four distinct sample matrices was developed and validated and adherence assessment was compared within the different matrices. Unlike the first three studies, where the quantification of drugs relied on calibration curves, this study employed relative response factors derived from corresponding isotope-labeled internal standards for the quantification of OET. Although the acquisition of corresponding isotope-labeled internal standards for each OET may incur expenses, their utilization can mitigate challenges within the assay, such as matrix effects or ion suppression

and expedites the analytical process. For adherence monitoring of OET, only parent compounds were included in the monitoring panel, except for endoxifen. Endoxifen is a metabolite of tamoxifen resulting from cytochrome (CYP) P450 2D6-dependent biotransformation. The metabolic activity of CYP2D6 exhibits considerable inter-individual variations, and individuals categorized as poor metabolizers of tamoxifen tend to exhibit lower levels of endoxifen, thereby resulting in an unfavorable clinical outcome in comparison to those classified as CYP2D6 extensive metabolizer [49, 50]. Moreover, endoxifen is currently being investigated as an independent anti-cancer drug [49, 50]. Considering these factors, both tamoxifen and endoxifen were incorporated into the monitoring panel. Given the generally prolonged half-lives of most OET, the approach to calculate individual dose-related concentration factors as in study two and three is deemed unnecessary. Adherence evaluation was conducted using available trough or steady-state plasma concentrations for OET. As in the preceding three studies, reference concentrations were exclusively available for plasma as a sample matrix. Determined concentrations in plasma were compared to matching OF, VAMS, and urine samples across 41 intakes of OET. As for the AHD, disparities in determined concentrations emerged between matrices, necessitating the establishment of specific reference concentrations for OF, VAMS, and urine. Additionally, the available reference concentrations in plasma need to be clinically validated for the purpose of adherence monitoring to perform a reliable assessment. The observation of elevated urine concentrations of abemaciclib, palbociclib, and ribociclib is noteworthy since these drugs are mainly excreted via feces [51]. However, the utility of urinary drug concentrations remains controversial due to the challenging interpretation of results caused by factors such as varying urinary flow, frequency of bladder emptying, and urinary pH [2]. Moreover, to the best of my knowledge, this study is the first to report the detectability of OET in OF. Like urine, the interpretation of results from OF is challenging. OF is considered to reflect the free fraction of circulating drug. However, its concentration is subject to influences such as contaminations in the oral cavity, salivary flow dynamics, and the pH of OF.

The four conducted studies are well suited to assess objective adherence to pharmacotherapy across different chronic diseases. Nevertheless, it is crucial to also discuss the limitations inherent in these approaches to avoid misinterpretation of results. Bioanalytical adherence monitoring cannot exclude “white coat adherence”, where patients only take their medication in anticipation of a monitoring event. Hence, regular monitoring without prior notification becomes essential. Additionally, the detectability of drugs in sample matrices presents challenges. Due to pharmacokinetic or chemical properties, some drugs are barely excreted via urine or may not transit into a matrix, leading to potential misclassification of non-adherence.

Moreover, drugs with short half-lives might be eliminated from the body before the next dosing event. To avoid misclassification of non-adherence, the sampling time must be carefully chosen considering the supposed time of drug intake or metabolites with longer half-lives can be included in the monitoring panel. However, not only drugs with short half-lives may cause misinterpretation in classification. Drugs with long half-lives may not exhibit significant changes in concentrations within different matrices even with occasional skip days, potentially leading to misclassification as adhered. Nevertheless, bioanalytical based adherence monitoring can still detect more extended periods of non-adherence or complete non-adherence.

Establishing appropriate cut-off concentrations for adherence assessment proves challenging. While reference concentrations for drugs are primarily available for plasma as a sample matrix, the conducted studies showed that determined drug concentrations in matching sample matrices can significantly differ. Therefore, specific cut-off concentrations must be evaluated and clinically validated for each sample matrix to evaluate adherence accurately.

Although the sampling devices used in the studies are user-friendly, patients require training for their proper use. In the case of VAMS, inadequate filling of the sampling tip with FPB was occasionally observed, leading to inaccuracies in quantification. However, laboratory personnel can visually identify an incomplete filled VAMS tip, rendering the sample invalid. Unlike VAMS, the OF sampling device lacks a visual assessment of filling, necessitating active

documentation by the patient. Moreover, in the case of at-home sampling, samples are dispatched via mail to the laboratory, exposing them to varying temperatures and humidity during transport, potentially impacting sample stability. Despite analyte stability testing in the studies, not every conceivable scenario has been accounted for, underscoring the importance of acknowledging these potential pit falls to prevent misinterpretation of results.

5. CONCLUSION

In conclusion, adherence monitoring is as an indispensable facet of modern healthcare, intertwined with the pursuit of improved patient outcomes and the sustainable management of healthcare resources. Alternative sampling methods have attracted attention to facilitate at-home sampling, thereby enhancing patient comfort and prioritizing patient-centered care. Although four strategies have been successfully developed, validated, and implemented, challenges persist in defining matrix-specific cut-off concentrations for accurate adherence evaluation. Nonetheless, these alternative sampling strategies demonstrated their reliability and have garnered favorable reception among patients, paving the path for improved patient care.

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7. ABBREVIATIONS

AHD	Antihypertensive drugs
CYP	Cytochrome
DBS	Dried blood spots
FPB	Finger prick blood
LC	Liquid chromatography
MS	Mass spectrometry
OET	Oral endocrine therapy
OF	Oral fluid
VAMS	Volumetrie absorptive microsampling