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GREEN MICROEXTRACTION TECHNIQUES IN THERAPEUTIC DRUG MONITORING

Kousi Maria and Samanidou Victoria*

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece

*samanidu@chem.auth.gr

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ABSTRACT

Therapeutic drug monitoring (TDM) is crucial for optimizing drug therapy, particularly for medications with narrow therapeutic windows. By measuring drug concentrations in biological fluids, TDM aids in dose individualization, adherence assessment, and the prevention of adverse effects. Recent advancements focus on miniaturized and eco-friendly sample preparation techniques, such as microextraction methods, which are efficient and low-cost. These include liquid- and solid-phase microextraction, offering benefits such as automation and high-throughput performance which have significantly improved TDM efficiency. Additionally, the emergence of microsampling offers a less invasive alternative to traditional blood collection for TDM and pharmacokinetic studies. Coupling these innovations with sensitive analytical techniques, like liquid chromatography-tandem mass spectrometry, enables accurate and reliable drug quantification from minimal sample volumes. These developments collectively enhance the clinical utility of TDM and contribute to improved patient outcomes. This paper provides a comprehensive review of recent advancements in green microextraction techniques as applied to therapeutic drug monitoring.

KEYWORDS

Therapeutic drug monitoring; microextraction techniques; Green Analytical Chemistry; sample preparation; drug analysis

1. INTRODUCTION

Therapeutic drug monitoring (TDM), which involves measuring drug levels or active metabolites and drug antibodies, is a promising strategy that can be used to optimize therapeutic treatments. It is based on the assumption of two facts 1. There is a relationship between drug exposure and outcomes and 2. There is significant inter-individual variability in how patients metabolize a drug (pharmacokinetics) and in the magnitude and duration of the response to treatment (pharmacodynamics). **[1]** TDM is an interdisciplinary clinical specialty aimed at improving patient care through individualizing drug dosing for which clinical experience or clinical trials have shown to improve outcomes in general or specific populations. It can be based on prior pharmacogenetic, demographic, and clinical information or on the subsequent measurement of drug concentrations

in the blood (pharmacokinetic monitoring) or biomarkers (pharmacodynamic monitoring).The three main reasons justifying TDM are: i. an experimentally determined and validated relationship between the plasma drug concentration and the pharmacological effect (efficacy & toxicity), ii. The significant variability of pharmacokinetics between and/or within the patient, and iii. The difficulty of monitoring the clinical effect of a specific drug. **[2]**

The criteria involved in a study during therapeutic drug monitoring include: 1. The ratio of the drug concentration in the blood to toxicity. 2. The ratio between the drug concentration in the blood and the drug dose. 3. Clinical indications relating to incompatibility, potential toxicity, and lack of response of the organism to the therapeutic treatment. Therapeutic drug monitoring (TDM) is a valuable tool for optimizing drug therapy by individualizing dosing based on a patient's unique pharmacokinetic and pharmacodynamic characteristics. By measuring drug concentrations in biological fluids, TDM helps to ensure that patients receive the optimal therapeutic dose, minimizing adverse effects and maximizing efficacy. This approach is particularly beneficial for drugs with a narrow therapeutic index, where small changes in drug concentration can have significant clinical consequences. The rationale behind TDM is grounded in the understanding that there is substantial inter-individual variability in drug response due to genetic, environmental, and physiological factors. Consequently, a one-size-fits-all dosing approach may not be suitable for all patients. TDM aims to address this variability by providing a more personalized approach to drug therapy. **[3]**

In recent years, analytical chemistry has witnessed a paradigm shift towards the development of environmentally sustainable methodologies. This transition aligns closely with the twelve principles of green chemistry, which advocate for minimizing sample preparation, reducing sample quantities, conducting in situ analyses, integrating analytical processes, automating methods, avoiding derivatization, minimizing waste generation, employing multi-analyte techniques, conserving energy, utilizing renewable resources, eliminating toxic substances, and ensuring operator safety.**[4]** By adhering to these principles, analytical chemists are actively contributing to a more sustainable future. Modern approaches focus on creating methods that are not only eco-friendly but also maintain high performance standards, including accuracy, repeatability, sensitivity, and selectivity. A key area of innovation lies in the pretreatment of samples. Although the ideal scenario would be to eliminate the need for sample pretreatment, it remains essential

due to the presence of interfering substances that complicate the detection of target analytes. Thus, optimizing sample pretreatment is a critical step in chemical analysis, with the goal of adhering to green chemistry principles. Additionally, there is a growing trend towards the development of advanced analytical instruments capable of accurately and sensitively detecting both organic compounds and metal ions. In clinical contexts, measuring drug concentrations in biological fluids is vital for personalizing therapy and monitoring patient adherence, thereby reducing the risk of drug interactions and toxicity. The quantification of these substances, whether for routine toxicological screening or advanced monitoring methodologies, plays a crucial role in public health and patient care. Furthermore, innovative extraction techniques that use minimal sample and solvent volumes are gaining attention for their potential to streamline analyses while minimizing environmental impact. **[5], [6]** This review discusses the application of microextraction techniques in sample preparation for therapeutic drug monitoring. In this review, the papers on microextraction techniques have been selected in such a manner that they cover a time window ranging from 2016 to 2024.

2. AUTOMATION STRATEGIES IN GREEN MICROEXTRACTION TECHNIQUES

The automation of green microextraction techniques marks a transformative development in analytical chemistry, enabling laboratories to achieve greater efficiency, precision, and sustainability in their processes. Traditionally, analytical methods have focused on sensitivity, selectivity, and precision, but recent trends emphasize the importance of minimizing environmental impact and reducing the use of toxic chemicals. Green microextraction techniques align with the principles of Green Analytical Chemistry (GAC) by minimizing the use of hazardous solvents, decreasing energy consumption, and reducing waste. The automation of these techniques further amplifies these benefits by streamlining sample preparation processes, enhancing reproducibility, and minimizing human error. For instance, the integration of robotic systems in sample handling automates labor-intensive tasks like sample transfer, vortexing, centrifugation, and dilution. This automation not only ensures consistency and accuracy across samples but also significantly increases throughput by allowing multiple samples to be processed simultaneously. Additionally, by reducing human intervention, robotic sample handling minimizes

the risk of errors such as contamination or incorrect pipetting, which are common in manual processes.

In the realm of automated extraction, devices such as those employed in Solid-Phase Microextraction (SPME) and Liquid-Liquid Microextraction (LLME) have been optimized to function with minimal human input. These devices often feature robotic arms or automated sample injection systems, which allow them to perform complex extraction procedures with high precision and reproducibility. Automated systems can precisely control critical parameters such as temperature, extraction time, and agitation, ensuring that the extraction conditions are optimal for each specific analyte. This level of control leads to more reliable analytical results and reduces variability between experiments. Moreover, automated extraction devices are often integrated with data acquisition and analysis software, enabling real-time monitoring of the extraction process. This integration not only speeds up the analytical workflow but also enhances the accuracy of the data by reducing the likelihood of human error during data interpretation. Consequently, automated extraction systems not only improve the efficiency of the analytical process but also contribute to the development of greener and more sustainable laboratory practices.

The adoption of advanced automation strategies, such as microfluidic devices and lab-on-a-chip (LOC) systems, represents a cutting-edge approach to further refining green microextraction techniques. These miniaturized systems allow multiple analytical steps—such as sample collection, extraction, separation, and detection—to be performed within a single, compact device. This integration not only reduces the need for manual intervention but also significantly lowers the consumption of reagents and solvents, aligning with the goals of Green Analytical Chemistry. Microfluidic devices, for example, utilize very small volumes of solvents and samples, which not only reduces waste but also enhances the efficiency of the extraction process. Lab-on-a-chip systems go even further by incorporating all necessary analytical steps into a single platform, which can be automated to operate continuously or in a stand-alone mode. The potential for coupling these systems with advanced detection methods, such as mass spectrometry, allows for rapid and highly sensitive analysis with minimal environmental impact.

The integration of advanced data acquisition and analysis tools further streamlines the analytical process and improves the accuracy and reliability of results. These tools allow for real-time data

processing, reducing the risk of human error in data interpretation and accelerating the analytical workflow. By automating data analysis, laboratories can obtain timely and reliable results, supporting informed decision-making. As these technologies continue to advance, the automation of green microextraction techniques will likely play an increasingly pivotal role in addressing the growing demand for efficient, sustainable, and accurate analytical methods in a variety of fields, including environmental monitoring, food safety, and pharmaceuticals.**[7-23]**

3. MICROEXTRACTION TECHNIQUES

3.1 Electromembrane Extraction – "EME"

In contrast to traditional liquid-liquid extraction (LLE), where the analyte is separated through diffusion, electro-membrane extraction (EME) utilizes electrokinetic migration to facilitate mass transfer. EME employs a three-phase system that includes two aqueous solutions separated by a supported liquid membrane (SLM). As depicted in figure 1, the system is connected to an external power supply, with electrodes placed in both the donor (sample) and acceptor aqueous solutions. For the extraction of basic analytes, the positively charged electrode (anode) is positioned in the donor solution, while the negatively charged electrode (cathode) is in the acceptor solution. For acidic analytes, the polarity of the electrodes is reversed. EME requires the target analyte to be ionized and carry a charge to be effectively extracted. **[24]**EME is characterized by rapid extraction kinetics, effective matrix removal, environmental friendliness, and superior selectivity attributed to the membrane and applied electric field.**[24,25]**

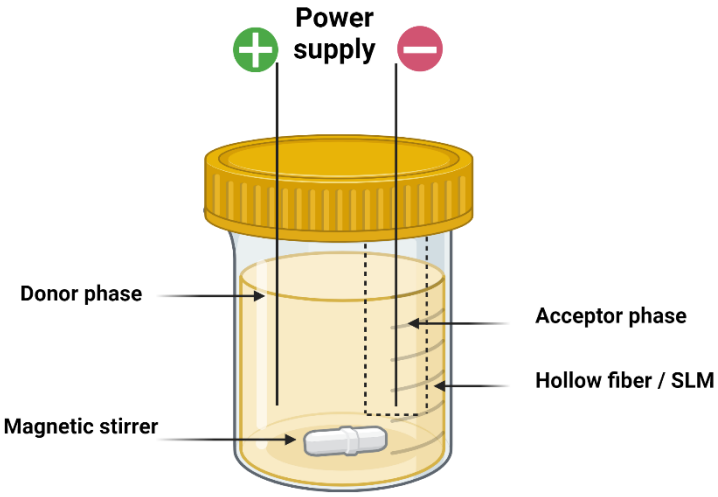

Figure 1 Electromembrane extraction (created with BioRender.com)

Table1 Bioanalytical procedures using EME for TDM

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Tofacitinib (TFB) and Cyclosporin A (CsA)	plasma	• **TFB**: Donor solution 300 µL diluted plasma spiked with 5 µg/mL TFBc (pH 2.1), SLM: 10 µL of 6MethylCoumarin:Thymol, Acceptor solution: 300 µL of 100 mM Formic Acid (pH 2.4),Voltage: 30 V, Agitation: 750 RPM • **CsA**: Donor solution: 250 µL of 20 µg/mL (5 µg) CsA: in 10 mM NaOH (pH 12), SLM: 10 µL 1 octanol, Acceptor solution:250 µL of 10 mM NaOH (pH 12),Agitation: 750 RPM, Extraction time: 15 min	HPLC-UV	TFB:0.05 µg/mL and not mentioned CsA: not mentioned and 2 µg/mL	TFB: 0.05-5 µg/mL CsA: 0.2-50 µg/mL.	**[25]**
Alimemazine, Amitriptyline, Atomoxetine, Clomipramine, Doxepin, Duloxetine, Fluvoxamine, Levomepromazine, Nortriptyline and Trimipramine and metabolites Desmethyl Clomipramine and Desmethyl Doxepin	serum	• 50 V for 15 min from serum samples (100 µL) diluted 1:3 with formic acid (0.1% v/v), using 2-nitrophenyl octyl ether as the supported liquid membrane (SLM), and formic acid (0.1% v/v, 300 µL) as acceptor phase	UHPLC-MS/MS	Estimated LODs ranged from 0.2 to 2 nM. The lowest calibration concentration represented LLOQ and ranged from 5 to 80 nM	not mentioned	**[26]**
Phenytoin (PHT)	plasma	• DBS punch (6 mm) • Internal standard (5 µL) added, nitrogen evaporation (25 °C, 10 min) • Microwave derivatization • Centrifugation (4 °C, 5 min, 13,000 rpm) • Reconstitution (100 µL injection solvent) • GC-MS analysis (1 µL injection)	CE-DAD	0.005 µg/mL and 0.03 µg/mL	0.03–4 µg/mL	**[27]**

Tofacitinib (TFB) and cyclosporine A (CsA) are immunosuppressants that can have toxic side effects if not dosed appropriately. Therapeutic drug monitoring (TDM) is crucial to ensure safe and effective treatment with these drugs. Electromembrane extraction (EME) is a promising sample preparation technique for TDM due to its efficiency, selectivity, and low solvent consumption. As presented in table 1, in **[25],** Sætrang investigated the use of EME for the sample preparation of CsA and TFB. For CsA, challenges arose due to its low solubility and detection limit using HPLC-UV. Despite attempts to extract CsA as an anion under alkaline conditions, successful extraction was

not achieved. However, for TFB, a suitable EME method was developed. By optimizing conditions such as pH, supported liquid membrane (SLM) composition, voltage, and extraction time, a 100% recovery of TFB was achieved from diluted plasma samples using a specific SLM and extraction conditions. While EME shows promise as a sample preparation technique for TFB, further optimization is necessary to determine its viability for CsA.

Regarding serum samples, Skallvik et al. **[26]** developed a novel conductive vial electromembrane extraction (EME) platform for the analysis of lipophilic basic drugs in serum. This approach innovatively replaces conventional platinum electrodes with conductive polymer-based sample and acceptor vials, thereby enhancing the system's portability and disposability. Coupled with UHPLC-MS/MS, the method demonstrated high analytical performance for a panel of psychoactive drugs and their metabolites. Extraction efficiency was optimized using 2-nitrophenyl octyl ether as the supported liquid membrane and formic acid as the acceptor phase. The method exhibited excellent linearity, precision, and accuracy, with near-complete extraction of target analytes. Successful application to clinical samples and comparable results to a reference method validate the potential of conductive vial EME for routine drug monitoring.

Seyfinejad et al. **[27]**, developed and validated a novel electromembrane extraction (EME) method for the quantification of free phenytoin (PHT) concentration in human plasma. Given the clinical significance of therapeutic drug monitoring and the growing demand for accurate free drug levels in drug development, a reliable method for determining unbound drug fractions is essential. This study introduces an EME-based approach that effectively overcomes limitations associated with traditional methods by employing a water-immiscible membrane and leveraging the efficiency of an electric field. The method demonstrated precise protein binding assessment for PHT, enabling accurate determination of the free drug fraction.

3.2 Parallel Artificial Liquid Membrane Extraction – “PALME”

Parallel artificial liquid membrane extraction (PALME) is a microextraction technique characterized by a planar membrane configuration. As depicted in figure 2, a supported liquid membrane (SLM) is immobilized within this membrane, separating the sample and acceptor phases housed in a 96-well plate format. Sample preparation involves pipetting the sample into a well, applying a small volume of organic solvent to the membrane, and subsequently adding the acceptor phase to the

opposite well before plate assembly. **[28]**PALME offers superior efficiency and enables high-throughput extraction of up to 96 samples simultaneously.**[29]**

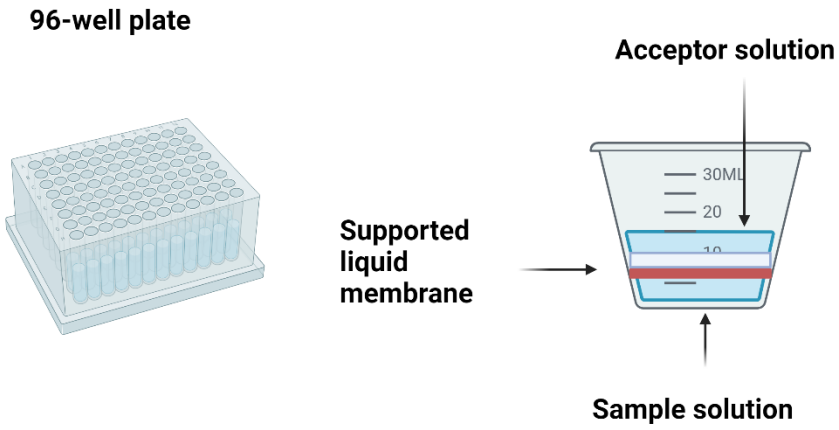

Figure 2 Parallel Artificial Liquid Membrane Extraction (Created with BioRender.com)

Table 2 Bioanalytical procedures using PALME for TDM

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Amitriptyline,Quetiapine, Ketoprofen, Fenoprofen, Flurbiprofen, Ibuprofen	whole blood	• **Amitriptyline and quetiapine**: DBS were initially subjected to desorption using 250 µL of 10 mM sodium hydroxide solution. The resulting desorbate was transferred to a corresponding well in a sond 96-well plate containing a supported liquid membrane (SLM) composed of 4 µL of 1% trioctylamine in dodecyl acetate. An acceptor phase consisting of 50 µL of 20 mM formic acid was added to the opposite well • **Ketoprofen, fenoprofen, flurbiprofen, ibuprofen**: Analytes were initially desorbed from the DBS matrix using a 20 mM formic acid solution. Subsequently, the desorbed analytes underwent extraction across a supported liquid membrane (SLM) composed of dihexyl ether into an acceptor phase consisting of 25 mM ammonia. This extraction process was conducted for a duration of 60 min, resulting in the efficient transfer of target analytes from the DBS matrix into the acceptor solution, which was subsequently introduced directly into the analytical instrument.	UHPLC-MS/MS	amitriptyline: 0.9 and 2.9 ng/mL ,quetiapine: 0.2 and 0.7 ng/mL , ketoprofen: not evaluated, fenoprofen: not evaluated, flurbiprofen: not evaluated, ibuprofen: not evaluated	not evaluated	**[30]**
O-desmethyl-venlafaxine, Venlafaxine, Citalopram, Paroxetine, Fluvoxamine, Fluoxetine, Norfluoxetine, Sertraline	plasma	• Plasma sample (125 µL) spiked with internal standard (10 µL), pH adjusted to 13 (115 µL 40 mM NaOH) • Donor plate: Plasma sample loaded • Acceptor plate: 1% trioctylamine in dodecyl acetate (5 µL), acceptor phase (50 µL 20 mM formic acid) • Plate assembly, agitation (900 rpm)	UHPLC–MS/MS	LOD was not evaluated for the analytes, O-desmethyl-venlafaxine: 0.23 ng/mL, Venlafaxine: 0.17 ng/mL, Citalopram: 0.23 ng/mL, Paroxetine: 0.16 ng/mL, Fluvoxamine: 0.13 ng/mL, Fluoxetine: 0.28 ng/mL, Norfluoxetine: 1.11 ng/mL, Sertraline: 0.29ng/mL	5-1000 ng/mL for every analyte	**[31]**
Repaglinide (RPG)	plasma	• 96-well donor plate: plasma, 50 mM phosphate buffer (pH 8.0), cetirizine • SLM: PP membrane, dodecyl acetate (1% trioctylamine) • Acceptor phase: DMSO:formic acid (50:50, v/v)	UPLC-ESI-MS/MS	not evaluated and 0.1 ng/mL	0.1–100 ng/mL	**[32]**

According to the summarized data of table 2, in **[30]**, this study introduces a novel approach using parallel artificial liquid membrane extraction (PALME) for the sample preparation of dried blood spots (DBS) prior to ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis. The aim was to develop a streamlined and efficient method for analyzing both basic and acidic model analytes in DBS samples. The PALME procedure involved a two-step process: desorption of analytes from the DBS and extraction through a supported liquid membrane (SLM). For basic model analytes, sodium hydroxide solution was used for desorption, while formic acid was used for acidic model analytes. The SLM consisted of either trioctylamine in dodecyl acetate or dihexyl ether, depending on the analyte type. The extracted analytes were then transferred to an acceptor solution for subsequent analysis. The PALME method demonstrated effective desorption and extraction of both basic and acidic model analytes from DBS samples within 60 minutes. High recoveries were achieved for all analytes, ranging from 63 to 85%. The PALME process also effectively removed phospholipids from the DBS samples, ensuring clean acceptor solutions for analysis. The method exhibited excellent linearity, with r^2 values greater than 0.99 for five of the six analytes. Additionally, the accuracy, precision, and matrix effects were found to be in accordance with European Medicines Agency guidelines. The results of this study demonstrate the potential of PALME as a promising sample preparation technique for DBS analysis. The method offers a rapid, efficient, and clean approach for processing DBS samples, enabling the analysis of a wide range of analytes. The ability to process up to 96 DBS samples within 60 minutes highlights the potential for high-throughput analysis using this technique.

It should be noted that in **[31]**, Olsen et al., employed parallel artificial liquid membrane extraction (PALME) for the extraction of serotonin and serotonin-norepinephrine reuptake inhibitors from human plasma samples. This study aimed to develop a semiautomated approach for this extraction technique. The method demonstrated efficient extraction of eight model analytes from 125 µL plasma samples, with recovery rates ranging from 72 to 111% and relative standard deviations (RSDs) below 12.8%. Successful implementation of a semiautomated pipetting system significantly reduced manual labor time. The method was validated using real patient samples, yielding accurate results. These findings highlight the feasibility of semiautomated PALME for the extraction of serotonin and serotonin-norepinephrine reuptake inhibitors from human plasma.

In **[32]**, Ahmed et al., present a novel method for the rapid and efficient therapeutic drug monitoring of repaglinide (RPG) in diabetic patients. The method combines parallel artificial liquid membrane extraction (PALME) with ultraperformance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS). PALME was performed using a 96-well plate containing plasma samples, phosphate buffer, and cetirizine as an internal standard. A polypropylene membrane served as the support for the liquid membrane, which consisted of dodecyl acetate and trioctylamine. The extracted analytes were transferred to an acceptor solution containing DMSO and formic acid. The developed method demonstrated high efficiency and reproducibility, with a linear calibration range, low limit of quantitation, and high recovery. The method allowed for the analysis of 198 samples per hour, highlighting its high throughput capabilities. Additionally, the use of a minimal amount of solvents contributed to the method's eco-friendly profile. The PALME-UPLC-ESI-MS/MS method was successfully applied to monitor RPG levels in diabetic patients following tablet administration, demonstrating its effectiveness for therapeutic drug monitoring.

A novel method for the extraction of polar basic drugs from human plasma was developed by Pilařová et al. **[33]**, utilizing parallel artificial liquid membrane extraction (PALME). Hydralazine, ephedrine, metaraminol, salbutamol, and cimetidine served as model compounds for method development and validation. The extraction process involved the transfer of analytes from alkalinized plasma across a supported liquid membrane into an aqueous acceptor phase. Ion-pair complexation between the hydrophilic analytes and a carrier within the liquid membrane facilitated extraction efficiency. Eliminating the need for solvent evaporation, the direct aqueous-based extraction streamlined sample preparation. Optimized extraction conditions yielded recovery rates ranging from 50 to 89% within a 45-minute extraction period. Comprehensive method validation demonstrated robust performance, aligning with regulatory expectations, and establishing PALME as a promising platform for high-throughput bioanalysis of polar basic drugs.

3.3 Dispersive Liquid-Liquid Microextraction – ‘DLLME’

Dispersive liquid-liquid microextraction (DLLME) involves the rapid injection of a mixture of water-immiscible extractant and water-miscible disperser solvent into an aqueous sample. Figure 3

illustrates this action that generates a fine dispersion of the extractant within the sample matrix. Following extraction, the resulting heterogeneous mixture is subjected to centrifugation to facilitate phase separation, with the extractant phase forming a distinct layer at the bottom of the tube. **[34]** DLLME offers several advantages, including operational simplicity, low cost, high preconcentration factors, excellent extraction efficiency, and minimal sample preparation requirements. **[35]**

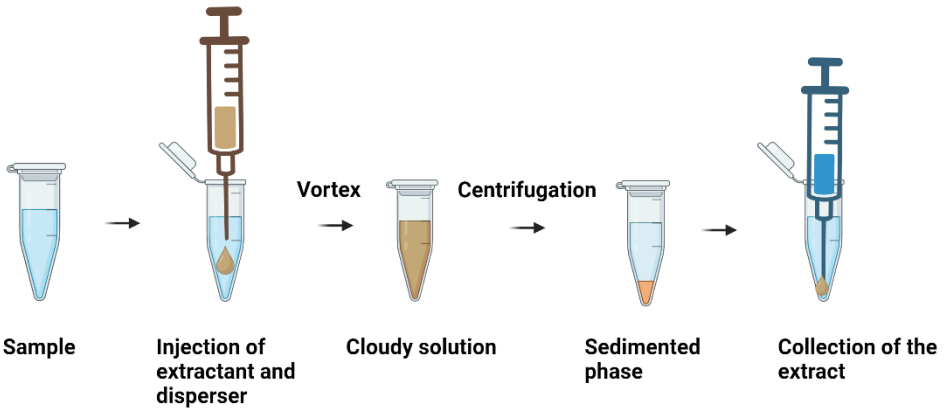

Figure 3 Dispersive Liquid-Liquid Microextraction (Created with BioRender.com)

Table 3 Bioanalytical procedures using DLLME for TDM

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Amitriptyline (AMP), Imipramine (IMP), Sertraline (SER), and Fluoxetine (FLX)	blood and urine	• 10 µL DES added to biological sample (1.5 mL tube) • Vortexing, 10 mg magnetic particles added • Magnetic gel formation, separation • Desorption with 100 µL ethanol • 20 µL eluate for LC-UV analysis	LC-UV	IMP: 0.028 µg/mL and 0.092 µg/mL, FLX: 0.020 µg/mL and 0.068 µg/mL, AMP: 0.027 µg/mL and 0.075 µg/mL, SER: 0.025 µg/mL and 0.080 µg/mL	0.1-10 µg/mL for every analyte	**[36]**
Risperidone and its metabolite 9-Hydroxyrisperidone	plasma	• DLLME parameter optimization: solvent types, volumes, ionic strength, vortex time • 3.0 mL aqueous sample, 500 µL acetone (disperser), 80 µL chlorobenzene (extractor) • Centrifugation, evaporation, reconstitution (200 µL acetonitrile-water) • LC-MS/MS analysis	LC-MS/MS	LOD was not evaluated, LOQ values were 5.0 ng/mL for both analytes	5.0-80.0 ng/mL for every analyte	**[37]**
Palbociclib, Ribociclib, Abemaciclib, Anastrozole, Letrozole and Fulvestrant	plasma	• Protein precipitation (PPT): 200 µL ACN added to 50 µL plasma • Supernatant evaporation • Reconstitution in aqueous phase (50-500 µL) • Disperser solvent (0-1000 µL) and extractant solvent (50-500 µL) addition • Mixture vortexed, centrifuged (5 min) • Organic layer collected, evaporated	HPLC-DAD-FLD	LOD and LOQ values were not mentioned	Palbociclib: 0.08-1.92 µg/mL, Ribociclib: 0.25-5.95 µg/mL, Abemaciclib: 0.11-2.61 µg/mL,	**[38]**

		• Residue reconstituted in 40 µL 65% methanol			Anastrozole: 2.51- 60.30 µg/mL, Letrozole: 0.04-1.01 µg/mL and Fulvestrant: 0.50-12.04 µg/mL	

As shown in table 3,noteworthy is the work carried out by Han et al. **[36]**, who proposed a green and rapid analytical method termed deep eutectic solvent dispersed liquid-liquid microextraction with magnetic particle-assisted retrieval (DES-DLLME-MPAR) for the extraction of antidepressants from biological samples**.** This approach utilized vortex-assisted extraction for efficient analyte transfer into the deep eutectic solvent (DES) and employed magnetic particles for rapid and convenient phase separation. Optimal extraction conditions were established, including the composition of the DES, sample pH, extraction and desorption times, and the amount of magnetic particles. The method demonstrated high extraction recoveries, excellent linearity, low limits of quantification, and satisfactory accuracy and precision. Furthermore, the green nature of the method was confirmed through greenness assessment metrics. The proposed DES-DLLME-MPAR method offers a promising alternative to traditional extraction techniques for the analysis of hydrophobic drugs in biological matrices.

There are some applications of DLLME techniques used for pretreatment of biological samples, mainly plasma. In 2020, Alcantara et al. **[37]**, developed a novel analytical method combining dispersive liquid-liquid microextraction (DLLME) with liquid chromatography-mass spectrometry (LC-MS) for the quantification of the active fraction of antipsychotic drugs in human plasma. Chromatographic separation was achieved using a C_{18} column with a gradient elution of ammonium acetate and acetonitrile. The DLLME procedure involved the use of chlorobenzene as the extractant and acetone as the disperser solvent, with sample pH adjusted to 12.0 and 10% NaCl added for enhanced extraction efficiency. The method underwent full validation according to regulatory guidelines and demonstrated successful application in the therapeutic drug monitoring of schizophrenic patients.

In **[38]**, Turković et al., present the development of a novel dispersive liquid-liquid microextraction (DLLME) method for the simultaneous extraction of six cyclin D-dependent kinase 4/6 (CDK 4/6)

inhibitors from human plasma. These inhibitors are used in combination with endocrine therapy for breast cancer treatment, and their monitoring in patient plasma is crucial for effective therapy. The study explored three different DLLME modes: organic sample DLLME (OrS-DLLME), aqueous sample DLLME (AqS-DLLME), and a modified air-assisted DLLME (AA-DLLME). These modes were optimized to achieve high extraction recoveries while minimizing sample and solvent consumption.The developed DLLME method demonstrated excellent linearity, precision, and accuracy, as validated according to established criteria. The method also achieved high extraction recoveries for all six drugs, with sample volumes as low as 50-100 µL and minimal organic solvent usage. The greenness of the method was confirmed by a high AGREE score, indicating compliance with green analytical chemistry principles. The validated DLLME method was successfully applied to analyze breast cancer patient plasma samples, demonstrating its suitability for the therapeutic drug monitoring of CDK 4/6 inhibitors. This study highlights the potential of DLLME as a promising sample preparation technique for the analysis of these important anticancer drugs.

3.4 Solid Phase Microextraction- 'SPME'

Solid-phase microextraction (SPME**)** is a solvent-free extraction technique widely employed in various fields, including pharmaceuticals, forensics, and environmental science. SPME integrates sampling, extraction, and sample introduction into a single process. Figure 4 demonstrates a fused silica fiber coated with a stationary phase (liquid or solid) which is exposed to the sample matrix, allowing for analyte partitioning between the sample and the adsorbent. This equilibrium-based extraction can be performed through direct immersion, headspace sampling, or membrane-protected configurations **[39]**. This technique exhibits high speed and sensitivity, enabling simultaneous extraction and preconcentration of target analytes. Additionally, it offers potential for automation **[3]**.

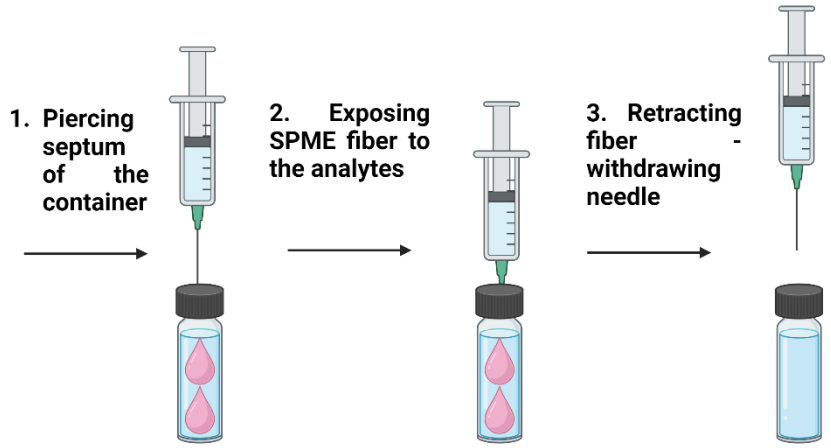

Figure 4 Solid Phase Microextraction (Created with BioRender.com)

Table 4 Bioanalytical procedures using SPME for TDM

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference

Tranexamic Acid	plasma and urine	• **Plasma:** The SPME device was initially conditioned by exposure to 1.5 mL of a 50:50 methanol:water (v/v) solution for a duration of 10 minutes. Subsequently, the device was immersed in 1 mL of plasma sample solution, prepared as a 1:3 ratio of sample to phosphate-buffered saline (PBS) containing an internal standard, for a 5-minute extraction period. Following extraction, the device was rinsed with 1 mL of a 90:10 water:methanol (v/v) solution for 10 seconds under static conditions. Finally, the analytes were desorbed from the SPME fiber into 1 mL of a 3:3:4 methanol:acetonitrile:water (v/v/v) solution through a 10-minute desorption step. • **Urine:** The SPME device was initially conditioned in 1.5 mL of a 50:50 methanol/water solution for 10 minutes. Subsequently, the device was immersed in 1 mL of urine sample, diluted 1:3 with 0.5 M phosphate buffer containing an internal standard, for a 5-min extraction period. Following extraction, the device was rinsed in 1 mL of water under agitated conditions for 10 seconds to remove residual salts. Finally, the analytes were desorbed into 1 mL of a 90:10 water/methanol solution through a 10-min desorption step.	LC-MS	LOD values are not mentioned, LOQ: urine: 25 µg/mL and plasma: 10 µg/mL	25 µg/mL-1000 µg/mL in urine, and 10 µg/mL-1000 µg/mL in plasma	**[40]**
Doxorubicin	Lung tissue and perfusate	• 3 Bio-SPME fibers/sampling site • Nitinol wire coated with sorbent particles • Tissue insertion (20 min) • Water rinse, wiping (5 s) • Snap freezing in dry ice	HPLC-MS/MS	Not specified; LOQ: 0.1 µg/mL (perfusate), 5 µg/g (lung)	0.1 - 10 µg/mL (perfusate), 5 - 500 µg/g (lung)	**[41]**
Folinic acid (FOL), 5-Fluorouracil (F), and Oxaliplatin (OX) (FOLFOX)	Lung tissue and perfusate	• SPME fiber sterilization (50:50 methanol:water, 30 min) • Fiber insertion into lung tissue (3 locations, 1-2 cm apart) • Water rinse (5 s), wiping • Storage in 300 µL polypropylene vials, snap-frozen in dry ice (-80 °C)	LC-HRMS	25 µg/g and 50 µg/g were achieved for both F and FOL	50 µg/g - 1000 µg/g for F and FOL	**[42]**

Looby et al. **[40]**, as shown in table 4, employed Solid Phase Microextraction (SPME) methodology to optimize tranexamic acid (TXA) therapeutic drug monitoring in plasma and urine samples from patients with chronic renal dysfunction (CRD). Employing hydrophilic-lipophilic balance (HLB)-coated SPME fibers significantly enhanced sample throughput, enabling the analysis of TXA levels in 96 samples within 25 min. The method demonstrated robust analytical performance with low

limits of quantification, excellent accuracy, and precision. Successful implementation in a clinical setting facilitated the adjustment of TXA dosing regimens for CRD patients undergoing cardiac surgery. The proposed SPME approach eliminates the need for complex sample pretreatment, rendering it a green and efficient analytical solution for TXA biomonitoring in both plasma and urine.

A novel in vivo lung perfusion (IVLP) technique has been developed, by Bojko et al. **[41]**, for targeted delivery of high-dose doxorubicin (DOX) to residual pulmonary micrometastases. To enable precise monitoring of drug distribution, a biocompatible nitinol wire coated with a sorbent material (Bio-SPME) was employed for in vivo extraction and quantification of DOX and its metabolites. Preclinical and clinical investigations demonstrated the feasibility of real-time DOX level assessment within lung tissue during IVLP. The Bio-SPME approach facilitated the acquisition of pharmacokinetic data and provided insights into the metabolic profile of the lung during drug administration. This innovative methodology offers the potential for personalized treatment strategies by enabling dynamic monitoring of drug exposure and tissue response.

In vivo lung perfusion (IVLP) is a novel technique for delivering high-dose chemotherapy directly to the lungs to treat metastatic lung cancer. The FOLFOX regimen, consisting of folinic acid, 5-fluorouracil, and oxaliplatin, is commonly used for various solid tumors. However, monitoring 5-fluorouracil levels is crucial due to its variability in plasma concentration. Since plasma levels may not accurately reflect tissue drug concentrations, sample preparation methods specifically designed for target organs are essential. In **[42]**, Looby et al., propose in vivo solid-phase microextraction (in vivo SPME) as a valuable tool for quantitative therapeutic drug monitoring of FOLFOX in porcine lungs during pre-clinical IVLP and intravenous (IV) trials. By simultaneously extracting other small molecules from the lung and analyzing them using liquid chromatography-high resolution mass spectrometry (LC-HRMS), this approach allows for an assessment of FOLFOX's impact on the lung's metabolic profile and the identification of metabolic pathways associated with the route of administration. The study demonstrates the importance of immediate instrumental analysis of metabolomic samples, as long-term storage can lead to changes in metabolite content. In vivo SPME provides a valuable tool for monitoring drug levels in the lung and assessing the metabolic impact of FOLFOX therapy.

3.5 Microextraction by Packed Sorbent – 'MEPS'

Microextraction by packed sorbent (MEPS) is a miniaturized solid-phase extraction technique designed to optimize sample throughput and reduce solvent consumption. By significantly decreasing sample volume and eliminating the need for extensive sample preparation, MEPS enables direct injection into chromatographic systems. This method offers enhanced efficiency and compatibility with automation protocols **[27-31]**. Microextraction by packed sorbent (MEPS) is a versatile sample preparation technique that employs a diverse array of sorbent materials, including silica-based phases, ion-exchange resins, polymeric sorbents, and molecularly imprinted polymers. The method encompasses a sequential process involving sorbent preparation, sample loading, washing, and analyte elution, as presented in figure 5. MEPS excels in its ability to efficiently extract and concentrate analytes from complex matrices while minimizing solvent consumption and sample volume. Moreover, the technique's compatibility with automation platforms enhances its throughput and reproducibility. These attributes, coupled with its broad applicability to various analytes and matrices, position MEPS as a valuable tool in analytical chemistry **[43]**.

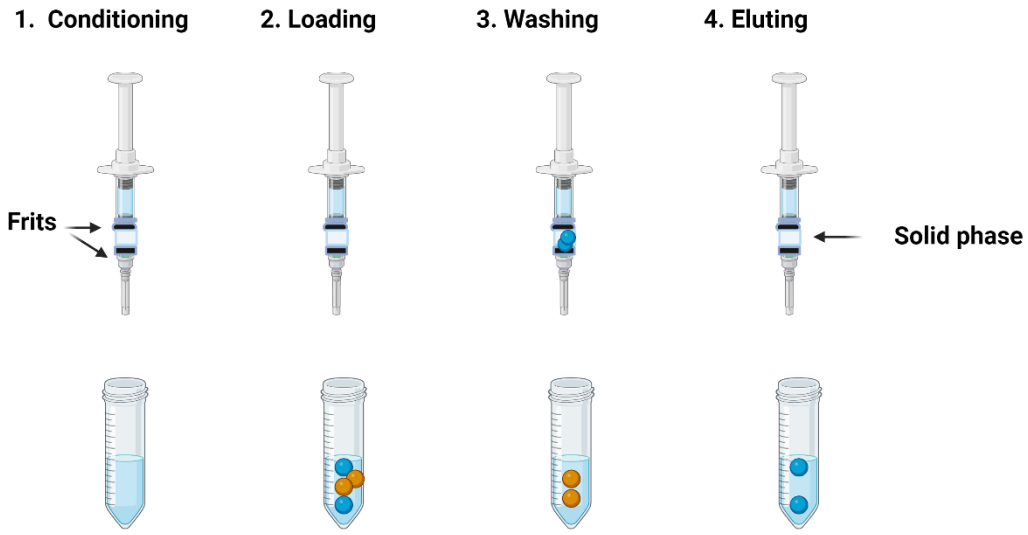

Figure 5 Microextraction by Packed Sorbent (Created with BioRender.com)

Table 5 Bioanalytical procedures using MEPS approaches for TDM

Analyte	**Sample**	**Sample Pretreatment and Extraction Procedure**	**Analytical technique**	**LOD;LOQ**	**Linear range**	**Reference**
Chlorpromazine (CLOR), Clozapine (CLOZ), Olanzapine (OLA) and Quetiapine (QUET)	plasma	• Plasma sample (150 µL) passed through RACNT (restricted acess carbon nanotube) bed (3 times) • Impurity removal (150 µL ultrapure water) • Analyte elution (2 x 100 µL acetonitrile), drying, reconstitution (50 µL mobile phase) • Sorbent reconditioning (10 x 200 µL acetonitrile, 2 x 200 µL ultrapure water)	UHPLC-MS/MS	LOD values are not mentioned; LOQ: 10 ng/mL for every analyte	200 ng/mL for OLA and 700 ng/mL for CLOR, CLOZ, and QUET	**[44]**
Fluoxetine (FLU), Norfluoxetine (NFLU), Paroxetine (PAR)	plasma	• Plasma (500 µL) spiked; acetonitrile (1.5 mL) added • Residue reconstituted in phosphate buffer (pH 4.0, 500 µL) • MEPS: C_8 cartridge activation, sample loading (3x) • Impurity removal (2 x 200 µL ammonium hydroxide) • Analyte elution (5 x 200 µL methanol-formic acid) • Eluate drying, reconstitution	LC–FLD	LOD: FLU and NFLU: 5 ng/mL, PAR: 1 ng/mL; LOQ: FLU and NFLU: 20 ng/mL, PAR: 5 ng/mL	FLU and NFLU: 20–750 ng/mL, PAR: 5–750 ng/mL	**[45]**

Lamotrigine	plasma and saliva	• Plasma/saliva (100 µL) spiked, acetonitrile (400 µL), centrifugation • Residue reconstituted (0.3% triethylamine-water, 200 µL) • C_{18} sorbent activation (methanol, water, 3 cycles each) • Sample drawn through sorbent (3 times) • Impurity removal (water, 200 µL) • Analyte elution (methanol, 2 x 30 µL), dilution, injection • Sorbent reconditioning (methanol, water)	HPLC-DAD	LOD values are not mentioned; LOQ: 0.1 µg/mL	0.1–20 µg/mL	**[46]**

Table 5 indicates that MEPS has been applied for the extraction of drugs in several biological specimens such as plasma and saliva. In **[44]**, Cruz et al., established a restricted access carbon nanotube (RACNT)-based microextraction by packed sorbent (MEPS) method for the quantification of antipsychotic drugs in untreated plasma samples from schizophrenic patients using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The RACNT sorbent, prepared by coating multi-walled carbon nanotubes with bovine serum albumin, exhibited exceptional protein rejection capabilities, maintaining consistent performance over multiple assays. Method optimization, encompassing sample pH, extraction cycles, desorption conditions, and cleanup steps, resulted in enhanced sensitivity and selectivity. The established MEPS-UHPLC-MS/MS method demonstrated robust analytical performance, characterized by excellent linearity, precision, and accuracy, with minimal matrix effects and carryover. Successful application to therapeutic drug monitoring in schizophrenic patients underscores the clinical utility of this innovative approach.

In **[45]**, Magalhães et al., present a novel liquid chromatography (LC) method for the simultaneous quantification of fluoxetine (FLU), norfluoxetine (NFLU), and paroxetine (PAR) in human plasma. The method utilizes microextraction by packed sorbent (MEPS) as a sample preparation technique and fluorescence detection (FLD) for quantification. The LC method employs a reverse-phase C_{18} column and an isocratic mobile phase consisting of phosphate buffer and acetonitrile/methanol. The method demonstrates excellent linearity, precision, and accuracy within specific concentration ranges for all three analytes. The developed method was successfully applied to the analysis of authentic plasma samples, demonstrating its suitability for therapeutic drug monitoring and clinical studies involving these antidepressant drugs. The MEPS/LC-FLD assay offers a robust,

low-cost, and efficient approach for the simultaneous quantification of FLU, NFLU, and PAR in human plasma.

A novel high-performance liquid chromatography-diode array detection (HPLC-DAD) method incorporating microextraction by packed sorbent (MEPS) was developed, by Ventura et al. **[46]**, for the quantitative determination of lamotrigine (LTG), a therapeutic drug with a narrow therapeutic index, in human plasma and saliva. Method optimization, including meticulous control of sample volume (100 μL) and chromatographic conditions, yielded exceptional selectivity and sensitivity. Isocratic elution on a C_{18} column facilitated rapid analysis of LTG and the internal standard, chloramphenicol, within five min. The method demonstrated robust linearity, precision, and accuracy, with a limit of quantification of 0.1 μg/mL in both biological matrices. Successful application to authentic human plasma and saliva samples from epileptic patients underscores the method's suitability for therapeutic drug monitoring.

3.6 Stir bar sorptive extraction – 'SBSE'

Stir bar sorptive extraction (SBSE) is a sample preparation technique that leverages the principle of analyte partitioning between an aqueous sample and a static phase. In figure 6 it can be clear that in contrast to SPME, SBSE employs a magnetic stir bar coated with a thicker layer of sorbent material, typically polydimethylsiloxane (PDMS), to enhance analyte adsorption capacity. The increased surface area of the stir bar facilitates efficient mass transfer and analyte enrichment during the extraction process. The magnetic core of the stir bar enables its continuous agitation within the sample matrix, promoting analyte diffusion into the PDMS coating. Following extraction, the stir bar is removed from the sample and subjected to thermal or liquid desorption to release the concentrated analytes for subsequent chromatographic analysis. This solvent-free approach, coupled with the high extraction efficiency and compatibility with various analytical platforms, has rendered SBSE a valuable tool for a wide range of analytical applications **[47]**. SBSE capitalizes on an extensive sorbent material, providing a substantial surface area that significantly enhances analyte enrichment and sensitivity. Ongoing research focuses on developing novel coated SBSE media, incorporating advanced materials and nanostructures, to further optimize extraction efficiency **[48]**.

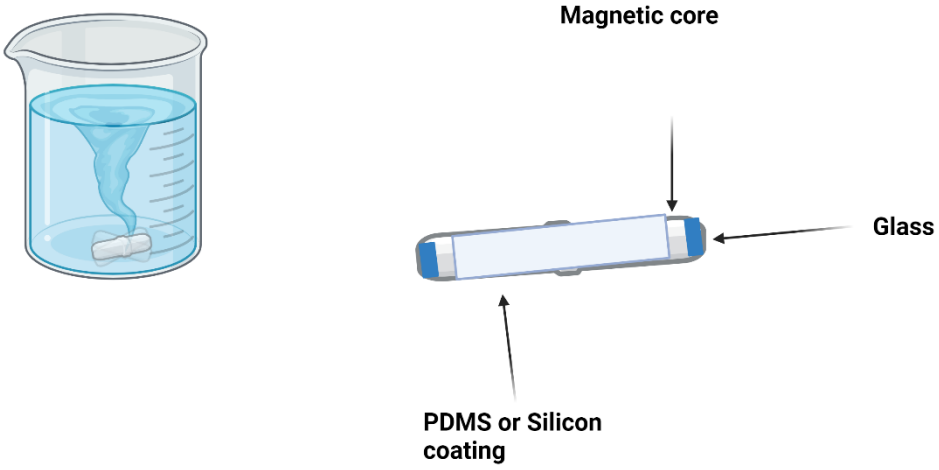

Figure 6 Stir Bar Sorptive Extraction (Created with BioRender.com)

Table6 Bioanalytical procedures using SBSE approaches for TDM

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Losartan (LOS) and Valsartan (VAS)	plasma	• Plasma (1 mL) spiked with LOS, VAS, diluted to 5 mL • Sodium chloride added • Stir bar extraction (1000 rpm, controlled conditions) • Desorption (250 µL solvent, ultrasound) • 20 µL aliquot injected for HPLC analysis	HPLC-UV	LOD; LOS: 7 ng/mL and VAS: 27 ng/mL LOQ; LOS: 24 ng/mL and VAS: 91 ng/mL	24 to 1000 ng/mL for LOS, and 91 to 1200 ng/mL for VAS	**[49]**

Fluoxetine (FLU)	plasma	• Plasma (240 µL) spiked with FLU standard (10 µL) • Sodium borate buffer (3750 µL, pH 9.00) added • Stirring (840 rpm) • Desorption (methanol-acetonitrile, 4000 µL, 50 °C, 50 min) • Evaporation, reconstitution (250 µL desorption solution) • HPLC-FD analysis	HPLC–FD	9.80 ng/mL and 32.67 ng/mL	25.00–1000.00 ng/mL	**[50]**
Sertraline, Mirtazapine, Fluoxetine, Citalopram, Paroxetine, Imipramine, Nortriptyline, Amitriptyne, and Desipramine	plasma	• Plasma sample (1 mL) diluted with borate buffer (pH 9.0, 4 mL) • Stir bar extraction (1000 rpm, 50 °C, 45 min) • Desorption (acetonitrile, 15 min, 50 °C) • HPLC analysis	HPLC-UV	LOD values are not mentioned; LOQ: Sertraline: 35 ng/mL, Mirtazapine: 40 ng/mL, Fluoxetine: 25 ng/mL, Citalopram: 10 ng/mL, Paroxetine: 40 ng/mL, Imipramine: 35 ng/mL, Nortriptyline: 15 ng/mL, Amitriptyne: 15 ng/mL, and Desipramine: 35 ng/mL	LOQ-1000.0 ng/mL for every analyte	**[51]**

Hypertension constitutes a major risk factor for morbidity and mortality among patients with cardiovascular diseases (CVDs). Angiotensin II receptor antagonists (ARAs) have emerged as effective and well-tolerated alternatives to traditional angiotensin-converting enzyme (ACE) inhibitors in the management of hypertension and heart failure. Losartan and valsartan, as prominent representatives of the ARA class, exhibit superior specificity, selectivity, and prolonged pharmacological effects compared to their peptide-based predecessors. Therefore, Babarahimi et al. **[49]**, as displayed in table 6, developed a novel stir bar coated with a polymeric material for the simultaneous determination of losartan and valsartan in human plasma samples using ultrasound-assisted liquid desorption coupled with high-performance liquid chromatography-ultraviolet

detection (HPLC-UV). The proposed stir bar exhibited superior extraction efficiency compared to commercially available polydimethylsiloxane and polyacrylate stir bars. Method optimization, including extraction and desorption conditions, resulted in excellent linearity, sensitivity, accuracy, and precision. The method demonstrated successful application in the analysis of patient plasma samples, offering a rapid and sensitive alternative to previously reported stir bar sorptive extraction techniques. The use of appropriate sample preparation effectively mitigated the selectivity limitations of UV detection, enabling reliable quantification of target analytes.

Depression is a significant global health issue, affecting millions of people. While antidepressants are effective for many, individual responses can vary. Monitoring plasma levels of antidepressants is crucial for effective treatment, but it presents analytical challenges. In **[50],** Marques et al., aim to develop a robust and efficient method for determining fluoxetine levels in human plasma using stir bar sorptive extraction (SBSE) coupled with high-performance liquid chromatography-fluorescence detection (HPLC-FD). The study optimized the SBSE process, including sorption and desorption steps. Factors such as temperature, time, stirring speed, desorption kinetics, and stirring mode were evaluated to achieve optimal extraction conditions. The developed method was validated and found to be robust, linear, precise, and accurate within a specific concentration range. The method also demonstrated excellent recovery and the ability to quantify fluoxetine without interference from other substances. The validated SBSE-HPLC-FD method was successfully applied to analyze real plasma samples, demonstrating its suitability for therapeutic drug monitoring and clinical studies. SBSE-HPLC-FD represents a promising alternative for the analysis of fluoxetine in low-volume samples. This method offers a cost-effective and efficient approach for therapeutic drug monitoring and can contribute to studies correlating plasma fluoxetine levels with clinical response.

In **[51]**, a sensitive and reproducible stir bar sorptive extraction (SBSE) coupled with liquid chromatography-ultraviolet detection (LC-UV) method was developed for the simultaneous determination of multiple antidepressants in human plasma. The method optimization encompassed extraction time, pH, ionic strength, protein effects, and desorption conditions to achieve optimal extraction efficiency. The SBSE/LC-UV method demonstrated excellent linearity, sensitivity, and precision, with quantification limits ranging from 10.0 to 40.0 ng/mL. Successful application of the method to the analysis of plasma samples from elderly depressed patients highlights its potential for clinical use in therapeutic drug monitoring.

3.7 Microwave Assisted Extraction – 'MAE'

Microwave-assisted extraction (MAE) represents a significant advancement in sample preparation methodologies for extracting analytes from solid matrices. As illustrated in figure 7, this technique leverages the application of microwave energy to create a dynamic extraction environment characterized by rapid and efficient heating, leading to enhanced mass transfer and analyte dissolution. Unlike conventional heating methods, MAE induces direct and homogeneous heating of the sample-solvent mixture, resulting in accelerated extraction kinetics and improved analyte recovery. The simplicity of the MAE process is a key advantage, as it typically involves minimal sample preparation steps. Researchers can readily introduce the target sample and appropriate solvent into a suitable extraction vessel and subject the mixture to microwave irradiation for a specified duration. Following the extraction, the resulting extract is collected and prepared for subsequent analysis. The streamlined workflow, coupled with the potential for automation, makes MAE a highly attractive and versatile technique for a wide range of analytical applications **[52]**. MAE offers significant advantages including accelerated extraction kinetics, reduced solvent consumption, and increased sample throughput. The technique's capacity for simultaneous multi-sample processing further enhances its efficiency **[53]**.

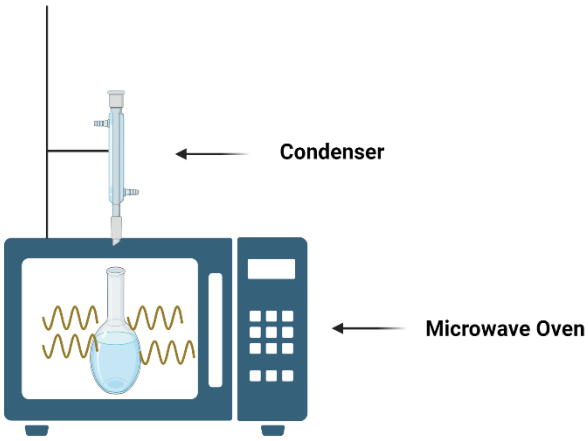

Figure 7 Microwave Assisted Extraction
(Created with BioRender.com)

Table 7 Bioanalytical procedures using MAE approaches for TDM

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD; LOQ	Linear range	Reference
Amitriptyline (AMI), Citalopram (CIT), Imipramine (IMI), Tetrazepam (TET), Zolpidem (ZOL)	whole blood	• Whole blood added, mixture centrifuged • Dried blood spots (DBS) prepared • DBS extraction (MAE, 55°C, 2.5 min) • Solvent extraction, centrifugation • Residue reconstitution, CE-MS analysis • Total time: ~3 h.	CE-MS	LOD: AMI: 1.76 ng/mL, CIT: 9.15 ng/mL, IMI: 2.01 ng/mL, TET: 14.7 ng/mL, ZOL: 6.26 ng/mL, LOQ; AMI: 5.27 ng/mL, CIT: 27.50 ng/mL, IMI: 6.70 ng/mL, TET: 49.0 ng/mL, ZOL: 20.9 ng/mL	LOQ-300 ng/mL	**[54]**
Levofloxacin, Ciprofloxacin, Moxifloxacin	Dried Plasma Spots	• Plasma (15 µL) deposited on paper; air dried (2 h) • 6 mm DPS punched, placed in Eppendorf tube • Extraction solution (90% methanol, IS) added, vortexed • Microwave extraction (400 W, 40 s) • Centrifugation (15,000 x g, 15 min) • LC-MS analysis (4 µL injection)	UHPLC-MS/MS	LOD values are not mentioned; LOQ: 0.2 µg/mL for every analyte	0.2–20 µg/mL	**[55]**
Gamma-hydroxybutyric acid (GHB) and Gabapentin	Dried Blood Spots	• DBS punch (6 mm) • Internal standard (5 µL), nitrogen evaporation (25 °C, 10 min) • Microwave derivatization • Centrifugation (4 °C) • Solvent reconstitution (100 µL) • GC-MS analysis (1 µL injection)	GC–MS	LOD values are not mentioned; LOQ: 10 µg/mL for GHB and 1 µg/mL for Gabapentin	10–100 µg/mL for GHB and 1–30 µg/mL for gabapentin	**[56]**

The summarized data from table 7 indicate that Świądro et al. **[54]**, developed an analytical methodology combining dried blood spots (DBS), microwave-assisted extraction (MAE), and capillary electrophoresis-mass spectrometry (CE-MS) for the comprehensive analysis of a broad spectrum of therapeutic drugs in human blood. The method demonstrated the successful quantification of tricyclic antidepressants, selective serotonin reuptake inhibitors, benzodiazepines, and hypnotics within a single analytical workflow. Rigorous optimization of MAE parameters, including extraction solvent, temperature, and time, was conducted to achieve maximal analyte recovery and matrix removal. The resulting method exhibited exceptional analytical performance, characterized by low limits of detection and quantification, excellent

linearity, precision, and accuracy. Moreover, the method demonstrated robust stability and minimal matrix effects, ensuring reliable and reproducible quantification of target analytes. Successful application to both post-mortem and clinical samples validated the method's suitability for forensic toxicology and therapeutic drug monitoring, highlighting its potential as a valuable tool for clinical and forensic laboratories.

Another possible application of the MAE extraction procedure is the work of Brahmadhi et al.**[55]**, who developed an analytical method for the quantitative determination of levofloxacin, ciprofloxacin, and moxifloxacin in dried plasma spots (DPS) utilizing microwave-assisted extraction (MAE) coupled with ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The method involved the deposition of a defined volume of plasma onto a protein saver card to create dried blood spots, followed by microwave-assisted extraction of the analytes from the punched-out spots. The optimized MAE procedure ensured efficient recovery of the target fluoroquinolones. Subsequent UHPLC-MS/MS analysis demonstrated excellent analytical performance, characterized by wide linear ranges, low limits of quantification, and high precision and accuracy. A robust validation study confirmed the method's reliability and suitability for quantitative bioanalysis. Direct comparison of quantification results obtained from DPS with those from conventional plasma samples revealed strong correlation, highlighting the potential of this method for therapeutic drug monitoring of fluoroquinolone antibiotics in tuberculosis patients. The integration of dried blood spot technology with microwave-assisted sample preparation and sensitive mass spectrometric detection offers a promising approach for streamlined and efficient bioanalysis of fluoroquinolones in clinical settings.

Dried blood spot (DBS) sampling is becoming increasingly popular in bioanalysis due to its advantages. However, the limited amount of sample material often requires highly sensitive detection techniques. Derivatization can enhance sensitivity in gas chromatography-mass spectrometry (GC-MS) analysis. In **[56],** Sadones et al., introduce a novel derivatization method, microwave-assisted on-spot derivatization, to minimize sample preparation time for DBS analysis. The study evaluated the applicability of microwave-assisted on-spot derivatization for the determination of gamma-hydroxybutyric acid (GHB) and gabapentin in DBS using GC-MS. The method was validated and found to be robust, with acceptable imprecision and bias values. Calibration lines were linear within specific concentration ranges for both analytes. Stability studies demonstrated the stability of GHB and gabapentin in DBS samples stored at room

temperature. The study also evaluated the impact of DBS-specific parameters, such as hematocrit and volume spotted, on the analytical performance. The method was found to be reliable and applicable for routine toxicology analysis. Microwave-assisted on-spot derivatization represents a promising approach for the analysis of polar low molecular weight compounds in DBS samples. This method offers a rapid, efficient, and reliable alternative for the determination of analytes such as GHB and gabapentin, as well as other compounds of interest in clinical and forensic toxicology.

4. Conclusions and future perspectives

The trajectory of therapeutic drug monitoring (TDM) research should prioritize the development of eco-friendly, high-throughput, and miniaturized extraction techniques that incorporate innovative sorbent materials. A concerted effort to integrate cutting-edge analytical instrumentation, such as high-resolution mass spectrometry, is essential to enhance the sensitivity, selectivity, and overall analytical performance of TDM assays. Along with pursuing entirely novel methodologies, the focus should be also on optimizing existing approaches to address their inherent limitations and expand their applicability. By refining current techniques and disseminating knowledge about these advancements, the field can significantly improve patient care through more precise and individualized therapeutic interventions. Ultimately, the integration of these technological advancements will enable the routine application of advanced TDM strategies in clinical practice, thereby facilitating optimal drug therapy and improved patient outcomes. **[57]**

The integration of microfluidic platforms with advanced sample preparation techniques presents a promising avenue for the development of more efficient and sustainable therapeutic drug monitoring (TDM) workflows. Microfluidic devices, including lab-on-a-chip systems, offer the potential for miniaturization, automation, and increased analytical throughput. Complementary technologies such as dried blood spot sampling and innovative extraction methods, including solvent-assisted microwave extraction, solid-phase microextraction, supercritical fluid extraction, and single-drop microextraction, can significantly enhance sample preparation efficiency and reduce solvent consumption. Moreover, the incorporation of nanostructured materials into these

platforms holds promise for the development of novel and environmentally friendly sample preparation approaches. By combining these technologies, researchers can create integrated analytical systems capable of handling complex biological matrices, improving sensitivity and selectivity, and ultimately enabling more precise and personalized patient care.

Abbreviations

Abbreviation	Full Name
LOD	Limit of Detection
LOQ	Limit of Quantification
HPLC-UV	High Performance Liquid Chromatography - Ultraviolet
UHPLC-MS/MS	Ultra-High-Performance Liquid Chromatography - Tandem Mass Spectrometry
CE-DAD	Capillary Electrophoresis - Diode Array Detector
UPLC-ESI-MS/MS	Ultra-Performance Liquid Chromatography - Electrospray Ionization - Tandem Mass Spectrometry
LC-UV	Liquid Chromatography - Ultraviolet
LC-MS/MS	Liquid Chromatography - Tandem Mass Spectrometry
HPLC-DAD-FLD	High Performance Liquid Chromatography - Diode Array Detector - Fluorescence Detector
LC-MS	Liquid Chromatography - Mass Spectrometry
HPLC-MS/MS	High Performance Liquid Chromatography - Tandem Mass Spectrometry
HPLC-HRMS	High Performance Liquid Chromatography - High Resolution Mass Spectrometry
LC-FLD	Liquid Chromatography - Fluorescence Detector
HPLC-DAD	High Performance Liquid Chromatography - Diode Array Detector
HPLC-FD	High Performance Liquid Chromatography - Flame Detector
CE-MS	Capillary Electrophoresis - Mass Spectrometry
GC-MS	Gas Chromatography - Mass Spectrometry

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