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Review paper

Current developments of bioanalytical sample preparation techniques in pharmaceuticals

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ABSTRACT

Sample preparation is considered as the bottleneck step in bioanalysis because each biological matrix has its own unique challenges and complexity. Competent sample preparation to extract the desired analytes and remove redundant components is a crucial step in each bioanalytical approach. The matrix effect is a key hurdle in bioanalytical sample preparation, which has gained extensive consideration. Novel sample preparation techniques have advantages over classical techniques in terms of accuracy, automation, ease of sample preparation, storage, and shipment and have become increasingly popular over the past decade. Our objective is to provide a broad outline of current developments in various bioanalytical sample preparation techniques in chromatographic and spectroscopic examinations. In addition, how these techniques have gained considerable attention over the past decade in bioanalytical research is mentioned with preferred examples. Modern trends in bioanalytical sample preparation techniques, including sorbent-based microextraction techniques, are primarily emphasized.

1. Introduction

The development of bioanalytical sample preparation techniques has become challenging over the decades because of the need to constantly accomplish higher sensitivity, accuracy, and speed of analysis in complex biofluids (e.g., blood, serum, plasma, saliva, feces, and urine). In addition, because of the minute concentration of analytes, samples are often required to be preconcentrated prior to analysis. However, this often increases the levels of interfering components, such as small molecules (e.g., drugs, salts, and metabolites) or large molecules (e.g., nucleic acids, proteins, and peptides). Consequently, highly specific sample cleanup actions are necessary for accurate and selective bioanalysis for regulatory purposes [1]. Subsequently, these studies support regulatory filings such as investigational new drug application, new drug application, and abbreviated new drug application [2]. Therefore, bioanalytical sample preparation techniques need to be thoroughly validated before they can be employed in actual sample analysis. In most biological samples, carbohydrates, proteins, lipids, salts, and other endogenous components are present in large amounts. They can hamper the preferred trace analytes via matrix effects, where their elimination is the primary purpose of sample preparation prior to analysis. In addition, more bioanalytical studies have been reported on liquid-liquid extraction (LLE) and solid-phase extraction (SPE). Recently, dispersive liquid-liquid microextraction (DLLME) and electromembrane extraction (EME) have become more acceptable due to their advantages in clinical investigations. Therefore, continuous improvement of novel sample preparation and microfluidics-based techniques is necessary to accelerate bioanalytical research.

In the present article, we review the current publications associated with sample preparation techniques in bioanalytics. This article does not intend to be inclusive but rather intends to address the principles, advantages and disadvantages, and capability for practice in bioanalytical laboratories based on the authors' collective knowledge and experiences.

2. Biological matrices relevant in bioanalysis

In bioanalytical studies, various types of biological matrices (e.g., blood, plasma, serum, urine, hair, human breast milk, saliva, sweat, cerebrospinal fluid (CSF), and tissue) need to be investigated. In

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addition, every matrix has unique challenges. For example, plasma contains more phospholipids, whereas urine contains a large amount of salt [3]. Conventionally, biofluids (e.g., blood, serum, plasma, saliva, sweat, urine, and tissue) are used extensively in bioanalysis [4]. Recently, hair, human breast milk, and feces have also been used as biological specimens. Hair is a stable and tough matrix that is easy to handle and hardly tampered with during collection, and it has a high degree of degradation in post-mortem studies [4]. Human breast milk is an excellent marker of drugs and ecological pollutants [5]. As has been known for a long time, drug and metabolite excretion in breast milk is a crucial issue for breastfeeding mothers. Similar to excretion in breast milk, some herbal medicines may be metabolized by intestinal microbiota and excreted in feces. Feces are nondigested, nonhomogeneous, complex, and laden with macromolecules and particulates, which can present problems for analytical systems. Global metabolic monitoring of feces demonstrates a challenge from both biochemical and analytical standpoints [6]. A short introduction to biological samples is provided below [7,8].

2.1. Blood, plasma, and serum

Blood is composed of various blood cells suspended in plasma. Plasma is composed of approximately 55% of blood fluid in humans and constitutes glucose, proteins, hormones, minerals, and blood cells. Serum is the fluid and solute component of blood without fibrinogens. It contains a variety of metabolites that can be used in the diagnosis of various clinical conditions and many severe disorders [9].

2.2. Urine

Urine is overwhelmingly composed of water (i.e., 95%), in addition to inorganic salts (e.g., sodium, phosphate, sulfate, and ammonia), urea, creatinine, proteins, and pigmented products of blood breakdown (e.g., urochrome). Urinary metabolomics approaches are likely to be used to monitor prior diagnostic and prognostic biomarkers of disorders such as urinary tract infection and chronic kidney diseases [10].

2.3. Hair

Hair is a hard and strong tissue. It is widely used in bioanalysis due to its ideal properties. Hair is a stable and strong matrix that is non-invasively collected, easy to handle and transport, and hardly tampered with during collection. In the case of drug addicts, most drugs are found in hair [4]. Hair analysis is also used to provide DNA evidence for criminal cases and investigation of heavy metals in the body, such as arsenic, mercury, and lead.

2.4. Human breast milk

Human breast milk is composed of certain levels of fat, proteins, lactose, and minerals. It is an excellent biomarker for the detection of drugs, metabolites, and environmental pollutants. Some drugs and metabolisms are excreted in breast milk, with lipophilic drugs having a higher tendency to excrete into breast milk [11], which can present a serious problem for breastfeeding mothers and the risk of infants receiving excreted drugs and metabolites. Therefore, it is advisable to cautiously feed mothers on any medicinal treatment during breastfeeding. Breast milk can be accumulated in a clinically recommended pump with glass vessels. The collected samples were transferred to polypropylene tubes for analysis.

2.5. Saliva

Saliva is composed of nearly 99% of water and compounds secreted by the salivary glands. Due to its easy collection and presence of significant biomarkers in many severe disorders, saliva has become a preferable biological fluid. Saliva contains a variety of electrolytes, including sodium, potassium, bicarbonate, magnesium, phosphate, and calcium. Saliva is an absolute medium that can be monitored for the examination of many disorders. In addition, saliva comprises a number of cytokines, enzymes, hormones, and antimicrobial components. Several biomarkers of heart diseases and cancers can be found in saliva. Saliva has been used as a diagnostic aid in clinical situations such as cystic fibrosis, Sjogren's syndrome, and adrenal cortex-related disorders. Compared to other biological fluids, saliva collection and handling is convenient, noninvasive, and economical [12].

2.6. Sweat and skin surface lipids

Sweat is comprised of approximately 99% water and sodium chloride as the main solute. Skin surface lipids constitute a combination of sebum and keratinocyte membrane lipids. Lipophilic drugs are prone to excretion through passive diffusion into sweat glands. Hence, it is important to be attentive to skin surface lipids and sweat samples [13]. Clinically, sweat is the gold standard for the diagnosis of cystic fibrosis and many other severe disorders.

2.7. Feces

Fecal matter as human body waste generally consists of indigestible food matter, inorganic substances (e.g., calcium and iron phosphate), and certain amounts of dead bacteria. Some medicines may be metabolized by intestinal microflora and excreted in the feces. The fecal sample is an ideal specimen for the investigation of herbal medicines metabolized by the intestinal microbiota. Typically, before sampling the fecal matter, the person is supposed to be fasting. The fecal samples are then collected and placed in normal saline until further processing [14]. Clinically, fecal analysis is primarily performed to identify diseases of the digestive tract, liver, and pancreas.

2.8. Tissue

Tissues are composed of a group of cells with similar functions and shapes. They can be categorized into three sections: soft tissues, tough tissues, and hard tissues. Soft tissues (e.g., lung, liver, kidney, brain, and spleen) are simple to handle. Tough tissues (e.g., stomach, intestine, colon, muscle, placenta, heart, and artery) require appropriate methods. Quantification of drugs in the skin is challenging due to low amounts that may be present, small sample volumes, and the rigid nature of skin itself [15]. Hard tissues (e.g., cartilage, skeletal muscle, nail, bones, and hair) undergo a typical defined process in terms of collection. Hence, all tissues require accurate sample preparation before proceeding to analysis [16]. Tissues play key roles in clinical diagnostic purposes, such as tumor and cancer detection.

2.9. CSF

CSF is the secretion fluid of the central nervous system (CNS), and approximately 80% is produced by the choroid plexus that occupies the ventricles of the brain, subarachnoid space, and spinal cord. Proper and accurate investigation of the CSF metabolome can offer many clinically important insights into critical CNS ailments (e.g., Parkinson's disease, multiple sclerosis, brain injury, and Guillain-Barre syndrome).

3. Sample preparation techniques in bioanalytics

Currently, the increasing demand for convenient and eco-friendly sample preparation techniques is indisputable. Solvent extraction methods, including LLE, liquid-phase microextraction (LPME), and associated perspectives, such as solid-liquid extraction (SLE), are ideal for bioanalytics. Such techniques lower the cost of drug development and yield interest in pharmaceutical manufacturing [17]. High sample throughput can be accomplished when automation is carried out, ensuring increased accuracy and low waste generation of harmful materials.

Recent studies have focused on the evolution of sample preparation techniques to achieve these benefits. The cybernation of extraction methods (e.g., SPE, LPME, and LLE) and other sample preparation methods using robotics has led to novel and elegant perceptions of bioanalytics [18].

In 1990, Arthur and Pawliszyn [19] introduced a solid-phase microextraction (SPME) technique. SPME demonstrates a non-exhaustive method, including sampling, preconcentration, and extraction in one step [20]. The benefits of this technique include simple and rapid operation, high accuracy, improvement in sample clean-up, and less solvent consumption. SPME provides concurrent preconcentration and separation of volatile and nonvolatile samples [21]. Meanwhile, SPME has been coupled with sophisticated analytical technologies such as two-dimensional (2D) gas chromatography (GC), surface-enhanced Raman scattering, and ambient mass spectrometry [22]. Recently, it has been applied to the investigation of metabolites and neurotransmitters in vivo [23,24] and in tissues (i.e., nails and skin) [25]. SPME has proven to be a dominant technique for the preconcentration of genotoxic impurities (i.e., aziridine, 2-chloroethylamine, and methyl and ethyl ester derivatives of sulfonic acids) in active pharmaceutical ingredients [26]. Recently, applications of the fluorescence-based SPME technique and a handy fluorometer have also offered prospects for the on-site examination of pharmaceuticals [27].

In contrast, LPME was established to downscale sample volumes to 100 μL or less per analysis [28]. LPME has been used in bioanalytical applications, as mentioned in numerous recent articles [29,30]. LPME suffers from some of the abovementioned LLE disadvantages, that is, tiresome chemical handling at the micro level or required skilled personnel for handling [18]. Theoretically, LPME is the same as SPME, except that the extraction phase consists of a small microliter solution. The foremost cutback of the utilization of organic solvents and preconcentration serves as the key reason for LPME progress. It may comprise automation, easy sample cleaning, and low price per sample [31]. Thus, further studies may be required for the use of LPME in bioanalytics.

3.1. Microextraction techniques

With the efforts of researchers, non-exhaustive microextraction techniques have developed appreciably in terms of sensitivity and accuracy from complex biological matrices. Microextraction techniques are based on the principle of using low volumes of solvents. Microextraction techniques are robust, versatile, solvent-free, and cost-effective. Automation of several microextraction techniques is appropriate for regular laboratory analyses. The primary microextraction techniques developed over the past decade are as follows:

3.1.1. Air-assisted liquid-liquid microextraction (AALLME)

In 2012, AALLME was initially developed for the study of phthalates with high extraction recovery and low solvent consumption [32]. Here, a non-polar organic solvent at a μL-concentration was dispersed in the sample solution. This technique has ample recognition among researchers because of its ease of handling, economical nature, and convenience in most bioanalytical laboratories [33]. AALLME has been used to determine six fluoroquinolone compounds in milk powder and eggs [34], triazole pesticides (e.g., penconazole) in edible oils [35], warfarin in biological samples [36], therapeutic lectin [37], and multiclass pesticide residues in vegetable and fruit juice samples [38].

3.1.2. Single drop microextraction (SDME)

SDME is a convenient and economical miniaturized tool for the isolation of several desired analytes from complex matrices. Liu and Dasgupta [39] first introduced SDME as another extraction method to eliminate the problem of solvent evaporation. In SDME, analyte distribution occurs among a microdrop of organic solvent (acceptor phase) and aqueous sample (donor phase). The extraction medium was a single drop, which is termed SDME. The microdrop typically consists of an organic solvent (e.g., 1–10 μL) [40]. A simple, economical, and eco-friendly microextraction technique lacks carry-over. The exercise of small solvent volumes renders SDME an eco-friendly analytical process that produces minimal or no waste. Nevertheless, the major drawbacks are the drop volume fluctuation and instability. In general, two major concepts can be applied to execute SDME, direct immersion (DI-SDME) and headspace mode (HS-SDME) [41] (Fig. 1). HS-SDME is identical to DI-SDME except that a microdrop of a higher boiling point extracting solvent is revealed to the sample headspace. DI-SDME is more appropriate for the analysis of non-polar to mid-polar analytes of high molecular weight. In contrast, HS-SDME is appropriate for semivolatile as well as volatile polar and non-polar small molecular weight compounds. SDME has been broadly utilized in the investigation of environmental samples, anesthetics, pyrethroid pesticides, ranitidine in water samples, multiclass pesticides in mangos [42], ethanol in wine [43], and chromium in biological fluids [44]. A major disadvantage of this method is its unsuitability for dirty samples.

3.1.3. Stir bar sorptive microextraction (SBSME)

In 1999, Baltussen et al. [45] introduced SBSME based on sorptive extraction. The only difference is that the deposition position differs from that in the SPME. Extraction and desorption are fundamental steps of SBSME. A polydimethylsiloxane-coated stir bar was deep in the sample solution during the analysis [46]. The quantity of sorbent used in commercial SBSME is approximately 125 μL, which is greater than that used in SPME. Owing to the hydrophobicity of the coating material, SBSME cannot be used for the analysis of highly hydrophilic compounds. To overcome this drawback, a number of investigators have projected the implementation of dual-phase stir bars, superior coating materials, molecularly imprinted polymers (MIPs), and monolithic materials. Newly promising materials (e.g., monoliths, metal-organic frameworks (MOFs), carbon nanotubes, graphene, graphene oxide, and porous organic polymers) are useful for coating purposes in SBSME [47]. In addition, novel high-performance materials such as nanomaterials, synthetic (e.g., electrospun fibers, and fabric phases), and natural polymers (e.g., cotton disks), as well as non-conventional solvents such as ionic liquids (ILs), magnetic ILs, deep eutectic solvents, and switchable solvents (e.g., secondary and tertiary amines) are used as effective extractants. These materials have several advantages such as the ability to process nano- to milliliter sample volumes for selective analysis and facile fabrication [48]. Currently, SBSME has become a valuable microextraction technique for the examination of semivolatile and volatile analytes from aqueous biological matrices because of its ease, accuracy, and cost-effectiveness. Recently, Peng et al. [49,50] determined naftopidil in urine and plasma samples as well as cefaclor and cefalexin in ecological water samples. SBSME was also used to monitor

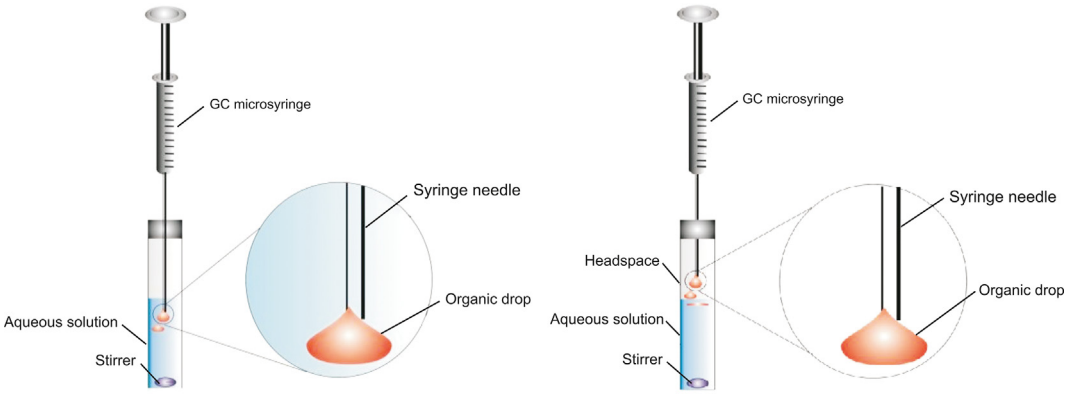

Fig. 1. Direct immersion-single drop microextraction and head space-single drop microextraction sample preparation techniques.

nitrosamines and chlorinated hydrocarbons in milk and polychlorinated biphenyl in serum samples.

3.1.4. Hollow fiber liquid-phase microextraction (HF-LPME)

HF-LPME is another microextraction technique with a small amount of extraction solvent (e.g., 10–20 μL) located inside a hollow tube made up of a hydrophobic porous fiber material (Fig. 2). The fiber is placed with an immiscible organic solvent, and therefore, the solvent is immobilized into the pores of the fiber to develop a supported liquid membrane (SLM). HF-LPME is an effective and economical microextraction method that can be easily programmed. It shows better solvent stability than SDME and no carry-over and memory effects because of the replaceable fibers. Other benefits include operation simplicity, swiftness, high reproducibility, clean extract, and a good enrichment factor. This method is currently popular but has a few disadvantages, such as adsorption of non-polar compounds on the fiber surface leading to blockage (e.g., plasma, blood, and urine samples) and air bubble generation on the fiber surface, which consequently decrease the transfer rate and lead to limited reproducibility [51].

SLM is an organic solvent found in the pores of fiber membranes; moreover, it preserves the acceptor inside the fiber lumen and thus circumvents leakage into the sample. This is a key reason for the implementation of this technique. However, the experimental framework for SLM is tedious, as it requires a peristaltic pump and a special unit machined from blocks of polytetrafluoroethylene [51,52]. Tahmasebi et al. [31] determined antidiabetic drugs (e.g., pioglitazone) in biological fluids. Bombana et al. [53] determined amphetamine, methamphetamine, fenproporex, 3,4-methylenedioxymethamphetamine, 3,4-methylene-dioxyamphetamine, and 3,4-methylene-dioxy-ethylamphetamine in whole blood samples. Ask et al. [54] developed a 96-well LPME technique for the determination of quetiapine, amitriptyline, nortriptyline, *o*-desmethylvenlafaxine, fluoxetine, and venlafaxine in human plasma.

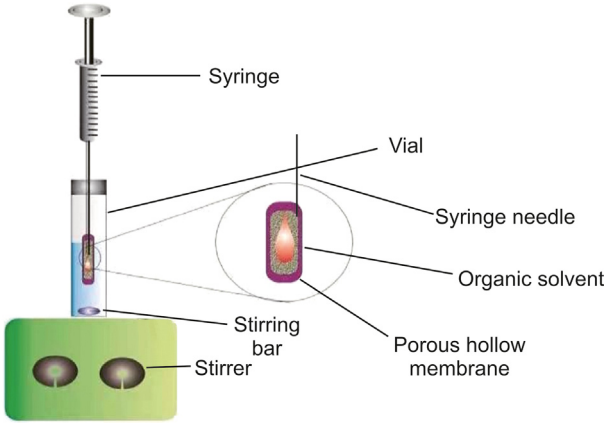

Fig. 2. Hollow fiber liquid-phase microextraction technique.

3.1.5. DLLME

In 2006, Berijani et al. [55] introduced DLLME as a new alternative for bioanalytics. DLLME resembles the application of dispersive mid-polar organic solvents that are compoundable with both extraction solvents and water [56]. In DLLME, the dispersion procedure dramatically increases the extraction kinetics by enlarging the exposure surface between the sample and the extractant. Subsequently, the formed emulsion was split by centrifugation, and the extractant was isolated [57]. DLLME has several advantages, including simple operation, minimum sample volume, cost-effectiveness with high recovery, quick processing, and no necessity for any particular appliance. The drawback is the exercise of harmful halogenated solvents, including carbon tetrachloride, chloroform, and chlorobenzene [58].

Numerous studies have verified the application of DLLME to highly polar metabolites (e.g., neurotransmitters and folate derivatives). DLLME has been used to determine hexachlorocyclohexane isomers and pyrethroid pesticides in milk [59], cobalt-60 in environmental water samples [60], suvorexant in urine [61], crystal violet and brilliant green dyes in fish [62], synthetic cannabinoids in oral fluid [63], and in the quantification of 60 drugs of abuse [64]. Recently, Zhou et al. [65] studied dozens of neurotransmitters in human urine samples. Hansen et al. [66] determined aripiprazole, pipamperone, flupentixol, haloperidol, zuclopenthixol, and trifluoroperazine in plasma and blood. For the purpose of extraction and dispersion, methanol or dichloromethane was used as a solvent with a 200 μL sample volume.

To reduce the use of harmful solvents in extraction, newer trends in DLLME have recently been developed, namely, binary solvent (BS)-DLLME, air-assisted DLLME, and vortex-assisted LLME, the last of which resembles the solidification of floating organic droplets (DLLME-SFO) [67]. DLLME can also be applied for the simultaneous determination of metronidazole, ciprofloxacin, meropenem, linezolid, and piperacillin in human plasma samples [68]. BS-DLLME has been reported for the quantification of tramadol in urine samples [69]. In DLLME-SFO, a high-density water-immiscible solvent (e.g., dodecanol and 2-dodecanol) was used as the extractant. It has been used to determine orthophosphate [70,71], suvorexant, and malathion in urine samples [72]. The additional benefits, disadvantages, and applications of DLLME-SFO are similar to those of DLLME. Recently, ILs

have been acknowledged as a potential choice for traditional DLLME techniques because of the exclusive properties shown by ILs from an environmental point of view, such as high thermal stability, nominal vapor pressure, and low flammability [73].

3.1.6. Solidified floating organic drop microextraction (SFODME)

The SFODME technique is dependent on the use of low-density organic solvents that easily solidify when placed in colder conditions and easily float on the surface of the sample solution (Fig. 3). SFODME has proven to be a method of choice for copper determination by flow injection flame atomic absorption spectrometry (FI-FAAS) and graphite furnace atomic absorption spectrometry [74]. SFODME has been used to determine indium [75], lead in food samples [76], cadmium [77], and glucocorticoids (e.g., cortisone acetate, prednisone, betamethasone, and dexamethasone) in animal-derived foods [78]. Recently, SFODME has been helpful for the identification of phenytoin and phenobarbital in plasma and urine samples [79]. Currently, an easy and fast syringe-to-syringe dispersive liquid-phase microextraction method with SFO drops has been developed and is useful for the analysis of ochratoxin-A [80], mercury in fish samples [81], and naproxen in plasma [82].

3.1.7. Thin film microextraction (TFME)

TFME is a technique developed to address the limiting uptake rate in fiber microextraction. Because of its suitability for integrated sample preparation, it was introduced as an alternative to SPME. The major advantage of TFME is that it reduces sample handling with lower detection limits for the desired analytes. TFME mainly facilitates fast extraction kinetics and high extractive capacity. Recently, two eco-friendly approaches relying on TFME for water examination were submitted to interlaboratory validation using the US-EPA method-8270 [83,84]. TFME has been used to determine trace amounts of polycyclic aromatic sulfur heterocycles in seawater [85], to extract quercetin from plasma and food samples [86] and to identify five estrogens in biological matrices. Laser-induced breakdown spectroscopy (LIBS-TFME) was initially used to identify Cu, Cr, Ni, and Pb in aqueous solutions. In this technique, analytes are extracted in a thin film of adsorbent material (e.g., graphene oxide) deposited on a solid support [87,88].

3.1.8. EME

EME is a microextraction strategy based on mass transfer across an SLM. EME was introduced to reduce the extraction time. Primarily, the extraction depends on electrokinetic migration in an electrical field. EME has an advantage over the HF-LPME technique because it decreases the equilibrium time during SLM. EME is mainly convenient for the extraction of basic analytes with high polarity. EME can remove either cations or anions and has been verified as a valuable fractionation method for charged metabolite complex matrices. Currently, EME is mostly applied to moderately lipophilic ionized compounds, such as organic ions, from various matrices. Drouin et al. [89] investigated cardiovascular biomarkers and hydrophilic analytes (e.g., choline, betaine, trimethylamine-*N*-oxide, 1-carnitine, and deoxy-l-carnitine) in plasma. Alternatively, EME has been applied to nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, salicylic acid, ketorolac, ketoprofen, naproxen, ibuprofen, barbiturates, anthracyclines, and tetracyclines in plasma [90]. Recently, aristolochic acid and antipsychotic drugs (e.g., risperidone, chlorprothixene, and haloperidol) were separated in whole blood and urine and analyzed by electromembrane extraction-liquid chromatography tandem-mass spectrometry (EME-LC-MS/MS) [91,92]. Mainstream pharmaceuticals are basic or acidic in nature, and therefore can be easily studied by EME.

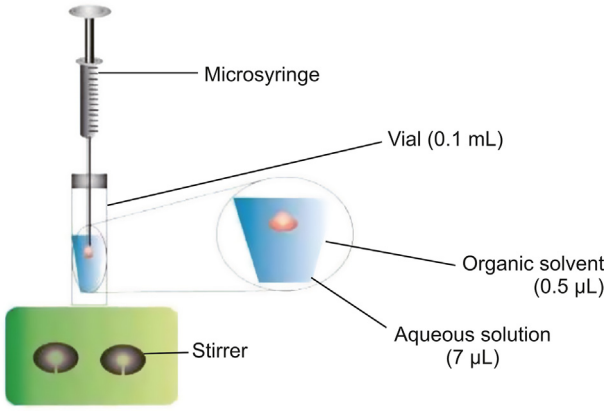

Fig. 3. Solidified floating organic drop microextraction technique.

3.2. Solid-phase nano-extraction (SPNE)

SPNE is another approach that depends on the sound affinity of analytes to adsorbents consisting of nanoparticles (NPs). SPNE retains the merits of SPME and shows novel advantages, such as a better adsorption area, several active sites for recognition of the analyte(s), modificative solid NP surfaces, and controllable surface states [93]. Due to several applications, SPNE is set as a green extraction practice. The SPNE technique has been used to determine polychlorinated biphenyls, carbaryl, and mercury ions in environmental water and polycyclic aromatic hydrocarbons in drinking water [94,95]. In addition, magnetic NPs have been recommended primarily for blood, serum, plasma, milk, tissue extracts, and urine sample preparations [96,97].

3.3. Microsample preparation techniques

Microsample preparation techniques involve sample preparation of small sample volumes (<50 µL) of biological fluids. They can improve animal welfare, that is, smaller numbers of animals are required in studies due to the reduction in the volume of biological matrices. Microsample preparation aligns with the 3R (reducing, reusing, and recycling) benefit strategy in nonclinical studies. Apart from this, microsample preparation possesses business considerations such as minimal inventory in animal husbandry, sample storage, shipment, and analysis [98].

3.3.1. Cloud point extraction (CPE)

In 1976, Miura et al. [99] established a CPE technique. CPE is based on surfactant solutions that become cloudy and separate into two isotropic phases. CPE has several merits over SPE, LLE, and SPME. CPE does not require the utilization of organic solvents; hence, the samples needed for the analysis were lower in amounts. The surfactants used in CPE are harmless and cheap, and have been utilized for the estimation of metals and several chemical moieties (e.g., drugs, vitamins, and pesticides) in diverse biological matrices [100] using green surfactants [101]. CPE is a method of choice for saliva analysis in bioanalytics [102–104]. CPE has advantages such as simplicity, low cost, higher extraction kinetics, and eco-friendly profile over other sample preparation techniques. However, there is little information on the function of CPE in bioanalysis. Antazoline was determined in human plasma by a surfactant (e.g., Triton X-114), followed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) [105]. Additionally, CPE was used to determine chlorogenic acid, deoxynojirimycin, rutin, isoquercitrin, and astragalin in mulberry leaves [106,107]. Thus, matrix effects should be evaluated when combined with LC-MS. Screening reveals that CPE-LC-MS is not related to notable

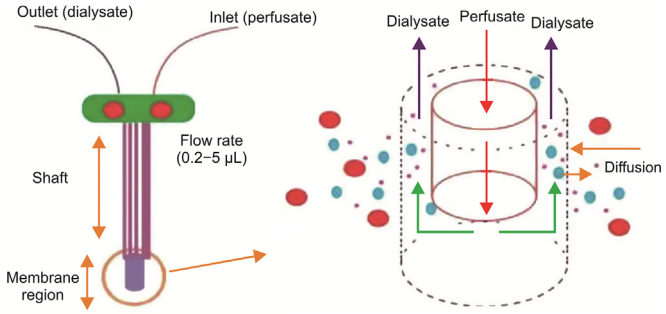

Fig. 4. Microdialysis assembly sample preparation technique.

matrix effects [108,109]. Wei et al. [110] reported a dual-cloud point extraction (d-CPE). In the first CPE process, the desired analytes were caught from the sample to the surfactant-rich phase and later extracted to the aqueous solution through the surfactant-rich phase. d-CPE was also used to determine an element in serum and saliva samples as well as sulfonamides in urine samples [111].

3.3.2. Microdialysis

Microdialysis is a well-known method that is usually useful in regular bioanalytical research, where a probe with a tiny semi-permeable membrane is positioned in the desired tissue. Microdialysis not only restricts dull sample preparation and cleaning protocols in bioanalysis, but also permits concurrent sampling at several sites with rapid and simple operation [73]. Microdialysis has been used to collect macromolecule-free samples in complex biological matrices [112]. A small microdialyser that ends with a perm-selective membrane was fixed into the human body to obtain a sample by perfusion (Fig. 4). Microdialysis has been proven to be a dominant technique for the pharmacokinetic (PK) study of an extensive choice of analytes from organs (e.g., brain, blood vessels, muscle, kidney, tumor, liver, skin, and gastrointestinal tract) to identify metabolites with xenobiotics from the interstitial space. Microdialysis has been used to determine serotonin, dopamine, methamphetamine, amphetamine, 4-hydroxymethamphetamine, 4-hydroxyamphetamine [113], and vancomycin in various biological fluids [114]. Currently, microdialysis possesses an extensive ability for in vivo investigations. It is capable of real-time analysis, but the analysis is restricted to polar molecules.

3.3.3. Cerebral microdialysis (CMD)

CMD is a sample preparation technique for brain extracellular fluid (ECF) [115]. CMD is a persistent method in which the interstitial fluid can be directly sampled. At the cellular level, one drawback is that it also accounts for unbound drug concentrations [116]. CMD has a probe-based mechanism that permits a discrete volume of the brain to be sampled for neurochemical investigation of biomarkers, neurotransmitters, metabolites, peptides, proteins, and chemotherapeutic drugs (e.g., temozolomide and methotrexate). CMD can be used to study brain tumors and is important for improving the cure of glioma [117], with respect to examining cerebral drainage in the jugular bulb [118] and diagnosing Japanese encephalitis virus [119].

3.3.4. Dried blood spot (DBS)

In 1963, Guthrie and Susi [120] introduced DBS to monitor the inborn metabolism defects in neonates. DBS is based on the principle of adsorption of biological components on the surface of a membrane carrier, followed by drying [121]. Recently, DBS has gained wide attention in bioanalytical and clinical laboratories. DBS resembles metabolomics examination, providing a dominant tool for studying new biomarkers and facilitating therapeutic drug monitoring [122,123].

DBS samples are easy to collect, store, and convey to places other than whole blood, serum, or plasma. DBS benefits clinical trial studies by reducing blood sample preparation volume. The rising exercise of DBS in preclinical studies resulted in a five-fold reduction in blood collection volume. DBS has a considerable optimistic impact on accuracy and data quality in animal studies (Fig. 5).

Because of the complex nature of whole blood, DBS specimens are more prone to matrix effects than the quantification of serum or plasma. Evaluation of the internal standard prior to sample preparation provides more benefits to compensate for matrix effects. Damen et al. [124] reported the simultaneous quantification of vincristine and actinomycin-D by DBS. Recently, Fischer et al. [125] reported DBS as a promising sample preparation technique for psycho-neuro-endocrinological research and for the screening of creatinine, cystathionine, guanidinoacetate, and carnitine in newborns [126]. In 2018, Bjornstad et al. [127] reported the capillary DBS technology for accurate glomerular filtration rate measurements. DBS has also been used for monitoring cocaine [128], acryl amide [129], and doping agents in blood samples [130].

3.3.5. Dried plasma spot (DPS)

Similar to DBS, DPS is a new emerging technique for the early diagnosis of neurodegenerative disorders. The DPS is a unique two-filter-paper-based remote blood collection tool [131]. It offers numerous benefits compared to conventional plasma collection methods. Dried spot collection on filter paper is easy, has no requirement for refrigeration, and can be transported with the least biohazard risk. These benefits offer significant flexibility to DPSs with respect to the classical methods of sample preparation. DPS has been used to determine fosfomycin, ritonavir, trimethoprim, and sulfamethoxazole in biological matrices [132–134]. Recently, DPS was shown to be suitable for the determination of amikacin, lithium, abiraterone, Δ(4)-abiraterone, lamotrigene, ceftolozane, fluoroquinolones, gabapentin, and caffeine in biological matrices [135,136]. DPS has proven to be a prominent technique when applied to PK studies, where plasma sample preparation is rapid and requires negligible plasma volumes [137].

3.3.6. Dried saliva spot (DSS)

In 2014, Abdel-Rehim et al. [138] introduced the DSS technique to monitor lidocaine as a model compound. DSS is a minimally invasive method for investigating salivary protein allocation in the oral cavity. DSS has several advantages, such as cost-effectiveness and easy and noninvasive sample collection [139]. DSS is promising and convenient in terms of sample preparation and extraction procedures compared to other techniques. Therefore, DSS increases

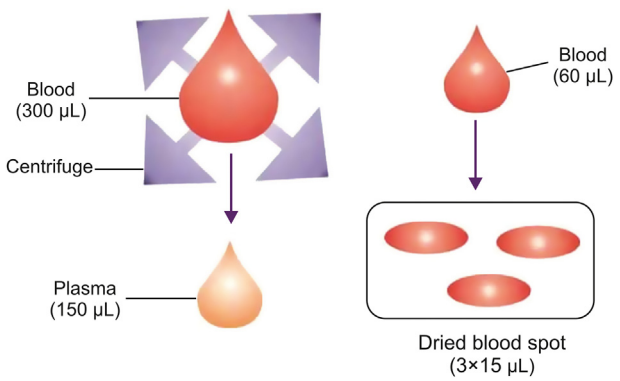

Fig. 5. Dried blood spot sample preparation technique.

the use of saliva to identify circulating biomarkers in the diagnosis of Alzheimer's disease [140], the detection of matrix metalloproteinase-1 as one of the most promising salivary biomarkers for oral squamous cell carcinoma (OSCC) [141], and the detection of (*dl*)-lactic acid for diabetes mellitus [142,143]. DSS has been used to determine methadone and its major metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in saliva [144] and 5-fluorouracil and six NSAIDs in the saliva of healthy volunteers [145]. DSS has gained advantages in evaluating the proteomes produced by salivary glands. DSS could be beneficial for the examination of local anesthetics (e.g., lidocaine) and for tracking a migraine drug compound and lactate in diabetic patients [146]. Recent studies have shown that paper microfluidic device can be applied to sense glucose and lactate in saliva samples [147] and cortisol and caffeine in biological matrices [148].

3.3.7. Dried urine spot (DUS)

DUS has been projected under various conditions to lower costs related to specimen collection, handling, storage, and transport in therapeutic investigations [149]. This technique enables the multianalyte detection of drugs of abuse in dried urine specimens. DUS was used to confirm the accumulation of methylcitrate, a biochemical hallmark of inborn errors in propionate metabolism [150]. It has also been used as an easy screening tool for congenital cytomegalovirus (CMV) infection in newborns [151]. DUS can also be used to determine several drugs (e.g., antidepressants, neuroleptics, opioids, benzodiazepines, cardiovascular drugs, and stimulants) as well as molybdenum, titanium, and organophosphate pesticides in urine samples [152]. DUS has the potential to be an alternative technique for drug and metabolite testing.

3.3.8. Volumetric absorptive microsample (VAMS) preparation technique

VAMS is a modern microsample preparation method applied to obtain dried specimens of biological matrices for bioanalytical purposes. VAMS is based on the same principle as DBS. VAMS has a few promising merits over DBS in terms of sample preparation, such as pretreatment, automation, volume accuracy, and hematocrit reliance. It allows reduction of volume from milliliters to microliters (sample volume approximately 10 μL). Microsample preparation devices (e.g., Mitra® and HemaPEN®) have overcome almost all the drawbacks of classical sample preparation with a few additional benefits. The VAMS device ensures homogeneity of the sample because a precise volume is absorbed onto the tip. This device enables ease of sample pretreatment as the centrifugation step of the liquid matrix, and the sub-punching step of DBS is eliminated. VAMS has been used to obtain dried specimens of blood and biological matrices. Recently, VAMS was applied to carry out LC-MS-based determination of vitamins, cefepime [153], and cocaine in human whole blood [154], and monitoring of antiepileptic drugs [155]. VAMS was proven to be an innovative strategy for monitoring tacrolimus levels in transplant recipients [156]. In the near future, VAMS may be recognized as a feasible alternative to DBS and other dried microsample preparation techniques [157].

3.3.9. Capillary microsample (CMS) preparation technique

CMS is a generic technique for the collection and handling of tiny volumes of liquid biological matrices. Fulfilling the objective of microsampling, CMS has proven to be superior to conventional sampling in terms of less sample volume, reduced processing steps, and stabilization of labile metabolites by quick sample dilution. The sample was collected in a glass capillary (1–35 μL). Therefore, CMS replaced classical large volume sample preparation techniques. CMS has been developed in response to the need for ethical use of animals in research because reanalysis of already diluted samples is possible, which reduces the requirement of blood samples from animals [158]. CMS provides an automated handling tool with less time for stabilization, which offers a new opportunity for labile analytes. As a technique that can provide refinement in blood sample preparation, CMS can be easily adapted to safety pharmacological studies [159]. CMS was used to determine NSAIDs (e.g., diclofenac, celecoxib, and tenoxicam) in Swiss albino mice [160].

3.3.10. Spin column extraction (SCE)

In 2015, Namera and Saito [161] initially established SCE as a sample preparation technique. Here, spin columns are utilized for monolithic silica disk packing, which utilizes the principle that target molecules bind to immobilized silica in the column. Several spin columns are manufactured and available in the market (e.g., C_{18}—C, C_{18}-SCX, and C_{18}—TiO). C_{18}-SCX is applied for the simultaneous determination of acidic and basic drugs with better accuracy and precision and shows no obstruction of endogenous substances [162]. SCE has several merits such as simple modus operandi, lesser elution volume, restricted solvent evaporation, and simultaneous processing of a number of analytes [7]. First-time users of SCE should pay attention to the flow rate and not dry the SPE column to achieve good accuracy. SCE was found to be helpful in the extraction of parabens in milk; beta-blockers, chlorophenols [163], fenitrothion, and amphetamines in human urine; eperisone in serum; and opiates, benzodiazepines, amines, and carboxylic acids in biological matrices [164].

3.4. Sorbent-based microextraction techniques

Currently, for the development of supramolecular microextraction [22], SPME fibers (ILs) [165], immuno-sorbents (ISs), metal nanoparticles (NPs), mesoporous-nanoporous silicates, carbo-nanomaterials, MIPs, electrospun fibers [166], and MOFs [167] have become a hot research field and will be discussed [110,168]. The main sample preparation methods include SPME as mentioned earlier, pressurized liquid extraction [169], microextraction by packed sorbent MIPs [170], carbon nanomaterials integrated MIPs [171], monolith spin extraction [172], turbulent flow chromatography, salting-out liquid-liquid extraction (SALLE), and stir bar sportive extraction (SBSE).

3.4.1. Restricted access materials (RAMs)

RAMs permit the clean-up of biological fluids through physicochemical diffusion barriers. RAMs are composed of a porous material with a restrictive and hydrophilic outer surface [173]. RAMs permit repetitive injection of complex biological matrices [1]. RAMs have been used for the rapid analysis of pyrethroids in whole urine samples [174]. RAMs are named as 'intelligent' sorbents due to their ability to retain analytes and exclude macromolecules. They have been applied to the resolution of organic and inorganic analytes from various biological matrices [48].

Additive manufacturing, also termed three-dimensional (3D) printing, has underwent a fabulous path over the past few years as a promising technology [175]. The application of 3D printing has grown in the field of bioanalytics to influence exclusive features. 3D printing offers major applications in sample preparation, optical sensing, and biosensing [48,176–178].

3.4.2. Microextraction by packed sorbent (MEPS)

Nowadays, MEPS is a popular miniaturized sample preparation technique in bioanalysis that adheres to the advantages such as automated, simple operation, and cost-effectiveness. In general, MEPS consists of four stages such as sample loading with washing, elution, and sorbent post-cleaning. In MEPS, the solvent volume used for the elution of the analytes is small (10–50 μL). The MEPS cartridge

Table 1
List of sample preparation techniques and compounds extracted from various biological matrices.

Sample preparation techniques	Biological matrices	Analytical techniques	Major merits	Compounds extracted	Refs.
SPE	Ovaries, testes, liver, serum, urine, plasma	UPLC-TOF/MS, LC-MS	Rapid, automatic, and quantitative	Ketamine, norketamine, venlafaxine, maleic acid, stiripentol, and retigabine	[7,195,196]
LLE	Serum, urine, CSF, saliva, plasma, tissue	GC-MS, LC-MS	Simple, high recovery, and automatic	Serum fatty, hormone, naproxen, metoclopramide, and ketamine	[7]
SPE/LLE	Tear, aqueous humor, vitreous body	HPLC-MS/MS, LC-MS/MS	Rapid and automatic via robotics technology	Kinase inhibitor and metabolite	[7]
AALLME	Biological fluids	LC-MS/MS	High extraction recovery, low solvent consumption, easy handling, and economical nature	Fluoroquinolone, penconazole, and warfarin	[34–36]
HS-SPME	Human urine, exhaled breath	GC-TOF/MS	Simple operation and cost effective	Amines, amides, hydrocarbons, aldehydes, ketones, esters, alcohols, ethers, carboxylic acids, nitriles, terpenoids, cycloalkanes, and heterocyclic compounds	[41]
SDME	Biological fluids	LC-MS	Simple, economical, ecofriendly, and no carryover	Anesthetics, pyrethroid pesticides, ranitidine, ethanol, and chromium	[42–44]
MEPS	Plasma, urine, saliva	GC-MS, LC-MS, LC-UV	Automated, simple operation, and cost effective	Zonisamide, meropenem, levofloxacin, statins, fluoxetine, beta-blocker, mandelic acid, antidepressants, lamotrigine, and local anesthetics	[48]
SBSME	Plasma, urine, milk, serum	HPLC-UV, HPLC-ICP-MS, LC-MS	Accuracy, cost-effective, and effective extractant phases	Naftopidil, cefaclor, cefalexin, nitrosamines, chlorinated hydrocarbons, and polychlorinated biphenyl	[49,50]
HF-LPME	Whole blood, plasma	GC-MS, LC-MS/MS	Better solvent stability, high reproducibility, clean extract, and good enrichment factor	Pioglitazone, amphetamine, methamphetamine, fenproporex, 3,4-methylenedioxymethamphetamine, 3,4-methylene-dioxy-ethylamphetamine,3,4-methylene-dioxyamphetamine, quetiapine, fluoxetine, venlafaxine, *O*-desmethylvenlafaxine, amitriptyline, and nortriptyline	[31,53,54]
DLLME	Milk, plasma, whole blood, urine	UHPLC-MS/MS, HPLC-UV,GC-MS/MS	Simple operation, minimum sample volume, cost-effective with high recovery, and quick processing	Hexachlorocyclohexane isomers, haloperidol, pyrethroid pesticides, malathion, suvorexant, aripiprazole, neurotransmitters, metronidazole, meropenem, ciprofloxacin, linezolid, flupentixol, piperacillin, trifluoroperazine, pipamperone, and zuclopenthixol	[54,59,61,65,66]
SFODME	Plasma, Urine	LC-MS	Simple operation and low solvent requirement	Cortisone acetate, prednisone, betamethasone, dexamethasone, phenobarbital, phenytoin, and naproxen	[78,79,82]
TFME	Plasma	UHPLC, LC-MS/MS	Reducing the sample handling with lower detection limits of analytes, economical	Estrogens and quercetin	[86]
EME	Plasma, whole blood, urine	UHPLC, LC-MS/MS	Low equilibrium time and compatible with analytical instruments	Choline, betaine, trimethylamine-*N*-oxide, 1-carnitine, deoxy-L-carnitine, diclofenac, salicylic acid, ketorolac, ketoprofen, naproxen, ibuprofen, anthracyclines, and tetracyclines	[89,90]
CPE	Plasma, serum, milk, and urine	LC-MS/MS	Harmless, cheap, simplicity, higher extraction kinetics, and ecofriendly	Antazoline, pesticides, and vitamins	[100,105]
Microdialysis	Biological fluids	LC-MS/MS	Rapid, simple operation, and selective technique for PK studies	Dopamine, serotonin, amphetamine, 4-hydroxyamphetamine, methamphetamine, 4-hydroxymethamphetamine, and vancomycin	[113,114]
Cerebral microdialysis	Interstitial fluid	GC-MS, LC-MS/MS	Highly selective technique	Neurotransmitters, metabolites, biomarkers, peptides, proteins, methotrexate, and temozolomide	[115]
DBS	Whole blood	LC-MS, UHPLC, GC-MS	Benefits preclinical testing, low sample requirement, and high accuracy	Vincristine, actinomycin-D, creatinine, cystathionine, guanidinoacetate, cocaine, acryl amide, and doping agents	[124,126,128–130]
DPS	Plasma	LC-MS, UHPLC, UHPLC-MS/MS	Easy operation, least biohazard risk, and no refrigeration required	Fosfomycin, ritonavir, trimethoprim and sulfamethoxazole, amikacin, lithium, abiraterone, Δ(4)-abiraterone, lamotrigene, ceftolozane, fluoroquinolones, gabapentin, and caffeine	[132–136]
DSS	Saliva	LC-MS, UHPLC, GC-MS	Cost effective, easy, and noninvasive sample collection	Metalloproteinase-1, DL-lactic acid, methadone, 5-fluorourasil, NSAIDs, lidocaine, cortisol, and caffeine	[141,142, 145,146,148]
DUS	Human urine	LC-MS, GC-MS	Low cost and easy screening	Methylcitrate, antidepressants, benzodiazepines, cardiovascular drugs, neuroleptics, opioids, stimulants, molybdenum, titanium, and organophosphate pesticides	[152]
VAMS	Biological fluids	LC-MS, UHPLC, GC-MS	Homogeneity of the sample, precise, and accurate	Vitamins, cefepime, cocaine, tacrolimus, and anti-epileptic drugs	[152–155]
CMS	Serum, blood	LC-MS, UHPLC, GC-MS	Low sample requirement, quick sample dilution, and rapid	Diclofenac, celecoxib, and tenoxicam	[160]

(*continued on next page*)

Table 1 (*continued*)

Sample preparation techniques	Biological matrices	Analytical techniques	Major merits	Compounds extracted	Refs.
SCE	Biological fluids, milk, urine, serum	LC-MS, UHPLC, GC-MS	Simple modus operandi, less elution volume, restricted solvent evaporation, and simultaneous processing of a number of analytes	Parabens, beta blockers, chlorophenols, fenitrothion amphetamines, eperisone, opiates, benzodiazepines, amines, and carboxylic acids	[161,164]

SPE: solid phase extraction; LLE: liquid-liquid extraction; HS-SPME: head space-single drop microextraction; AALLME: air-assisted liquid-liquid microextraction; SDME: single drop microextraction; MEPS: microextraction by packed sorbent; SBSME: stir bar sorptive microextraction; HF-LPME: hollow fiber-liquid phase microextraction; DLLME: dispersive liquid-liquid microextraction; SFODME: solidified floating organic drop microextraction; TFME: thin film microextraction; EME: electromembrane extraction; CPE: cloud point extraction; DBS: dried blood spot; DPS: dried plasma spot; DSS: dried saliva spot; DUS: dried urine spot; VAMS: volumetric absorptive microsample; CMS: capillary microsample; SCE: spin column extraction.

is packed with sorbent materials to provide suitability in operation. Several sorbents used in MEPS are cation exchanger, graphene oxide [179], reduced graphene oxide [180], organic monoliths, carbon NPs, ISs, and MIPs. Recently, MEPS has been applied in the determination of zonisamide, meropenem, levofloxacin, statins, and fluoxetine in plasma. In addition, MEPS is also beneficial for the determination of beta-blocker, mandelic acid, antidepressants, and asthma biomarkers in urine as well as psychoactive drugs, lamotrigine, and local anesthetics in saliva samples [48].

3.5. Other promising bioanalytical sample preparation techniques

Some recent developments with the advantages of well-known bioanalytical sample preparation techniques are briefly described here.

3.5.1. Biofluid sampling (BFS)

BFS has proven to be a novel technique for the investigation of whole blood samples. BFS is capable of whole blood sampling without converting it into plasma or serum. A sampler can hold a whole blood sample of 10–1000 μL. In contrast, BFS shares operational principles similar to DBS with the abolition of technical drawbacks. BFS offers a novel and extremely simplified approach for whole blood sample preparation. It is expected to renovate the existing practice of blood investigations. BFS has been used to determine ketoprofen, carprofen, and diclofenac in human whole blood [181].

3.5.2. Aptamers

Recently, aptamers have gained significant attention in scientific research. Aptamers are chemical oligonucleotides that bind to specific target molecules. Aptamers have gained substantial attention as molecular detection elements in the clinical field, therapeutics, and bioanalytics [182]. Aptamers have been adapted for the selective extraction of cocaine [183] and tetracyclines [184] in biological fluids and antibiotics in food matrices [185]. Recently, aptamers have been used as surface drug nanocarriers in anticancer therapy [186], and their clinical and bioanalytical applications are growing rapidly.

3.5.3. Microfluidic-based devices

The combination of automated and miniaturized techniques using microfluidic-based devices established a novel chip-based extraction approach, which offers a suitable tool for sampling in bioanalysis. This enables rapid analysis, thus diminishing related freeze-thaw issues. Lin and co-workers [187–190] have studied the majority of microfluidic-based devices as novel approaches. Subsequently, droplet microfluidic technology has been used as a powerful tool in bioanalysis. It has several advantages, including being rapid, portable, and reliable, and ideally without having the need for pretreatment steps [191,192]. In contrast, researchers have made significant efforts toward the development of paper-based analytical devices, which have gained significant interest in recent years [193]. These analytical devices have huge potential in developing portable and disposable platforms for rapid disease-relevant findings with predictions based on glucose, lactate, cortisol, caffeine, and uric acid biomarkers [194].

Table 1 [7,31,34–36,41–44,48–50,53,54,59,61,65,66,78,79,82,86,89,90,100,105,113,114,117,124,126,128–130,132–136,141,142,145,146,148,152–155,160,163,164,195,196] summarizes the sample preparation techniques and analytes extracted from biological matrices using sophisticated analytical techniques.

4. Conclusions and future perspectives

Bioanalysis is a key component of the discovery and development of pharmaceuticals. To combat the rising cost of drug development and increased sensitivity and specificity, new sample preparation and analyte detection techniques are being adopted. Moreover, many researchers are making the modification and improvement of classical techniques globally. Currently, laboratory automation is a key feature of easy, rapid, and eco-friendly methods. These newer techniques render miniaturization and rapid automatic high-throughput analysis possible. It is projected that these techniques for sample preparation will become mainstream in the near future. The current manuscript reveals dozens of promising bioanalytical sample preparation techniques, including SPME, LPME, AALLME, DLLME, CPE, SBSME, and CMD. The key features of several sample preparation techniques are summarized. A few sample preparation techniques (e.g., SPME, SDME, LPME, and DLLME) are easy, rapid, and economical but difficult to automate. Among them, SPME is the most accepted sample preparation technique for biological matrices but may be replaced by newer techniques. The DBS technique is well known and adopted for patients undergoing clinical trials, which require preparation of a large amount of sample from participating volunteers. Newer sample preparation techniques, such as capillary microsample preparation, VAMS, TFME, DSS, DUS, and dual-CPE have been found to be more precise, popular, and useful than classical techniques. Recently, the rapid increase in the application of ILs, aptamers, NPs, and microfluidic-based devices in bioanalysis has simplified analysis. Various available methodologies for sample preparation, especially when coupled with sophisticated analytical techniques, will greatly assist in the establishment of future drug metabolism and PK, pharmacodynamics, toxicokinetics, and bioequivalence studies of pharmaceutical discovery and development.

CRediT author statement

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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