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Current trends in bioanalytical sampling: Enhancing efficiency and accuracy

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Abstract
The evolution of bioanalytical sample preparation techniques has advanced to meet increasing demands for higher sensitivity, accuracy, and rapid analysis of complex biological matrices. Biological fluids often contain analytes at extremely low concentrations, necessitating efficient preconcentration approaches to minimize matrix effects. This review highlights modern, miniaturized, and green sample preparation technologies that enhance analytical speed, selectivity, and sensitivity. Significant progress in sorbent-based and microextraction methods is discussed, along with their applications in toxicology, clinical diagnostics, and pharmaceutical research.

Keywords: Bioanalytical sampling, microextraction, sample preparation, LC-MS/MS, solid-phase microextraction (SPME), microextraction by packed sorbent (MEPS), fabric phase sorptive extraction (FPSE)

1. Introduction
Bioanalytical sampling plays a vital part in pharmaceutical research, toxicology and clinical diagnostics. It involves collecting and processing biological matrices like blood, plasma, urine, saliva, and tissues for counting purposes of drugs, metabolites, and biomarkers [1]. Increasing demand for rapid, accurate quantification of trace analytes has accelerated the development of advanced sample preparation technologies. In recent years, significant advancements in miniaturized and solvent-reduced analytical techniques have transformed the landscape of bioanalytical sampling [2].

Novel methodologies such as hollow-fiber liquid-phase microextraction (HF-LPME), dispersive liquid-liquid microextraction (DLLME), single-drop microextraction (SDME), stir bar sorptive extraction (SBSE), solid-phase microextraction (SPME), and microextraction by packed sorbent (MEPS), and dried blood spot (DBS) sampling offer enhanced extraction efficiency, reduced sample and solvent requirements, and improved analyte stability. These techniques also conform to the green analytical chemistry tenets, making them attractive for modern laboratory applications [3, 4].

The incorporation of nanomaterials, advanced sorbents, aptamer-based recognition elements, and automated or in-vivo sampling devices has further expanded the capabilities of modern bioanalysis. Such innovations enable improved selectivity, faster mass transfer, and lower limits of detection, thereby supporting high-throughput workflows and real-time monitoring in clinical and pharmacokinetic studies [5].

This review provides a comprehensive overview of current trends in bioanalytical sampling, highlighting the evolution of traditional methods into modern microextraction technologies. It also discusses key developments, advantages, limitations, and future prospects that continue to shape analytical science.

2. Importance of Sample Preparation
Sample preparation enhances analytical performance by removing interferences and ensuring compatibility with instruments. Traditional methods are labor-intensive and solvent-consuming, prompting the shift toward eco-friendly, automated, and miniaturized techniques aligned with Green Analytical Chemistry (GAC) principles [6].

Sampling and sample preparation procedures account for more than 80% of the entire analysis time in bioanalytical workflows [2]. Improper sample processing compromises accuracy and reproducibility. Integrating online sample pretreatment is therefore essential for improving throughput, especially for LC-MS/MS-based analyses [7].

2.1 Common biological matrices

- **Blood and plasma:** Widely used in pharmacokinetics [8].
- **Urine and saliva:** Non-invasive alternatives for routine testing [9].
- **Tissues:** Require homogenization before analysis [8].

3. Advances in Sample Preparation Techniques

3.1 Solid-Phase Microextraction (SPME)

A solvent-free sampling method called solid-phase microextraction (SPME) combines sample input, extraction, concentration, and sampling into one step. It employs a fused silica fiber covered with polymer that adsorbs analytes based on biological matrices. SPME extracts analytes directly onto a coated fiber, enabling solvent-free enrichment of chemicals that are volatile and semi-volatile [10, 11]. Continued material innovations, including new sorbent coatings and in-tube formats, have expanded its application to pharmaceuticals and biological fluids [12]. However, fiber fragility, limited polarity range, and thermal instability remain challenges.

3.2 Stir Bar Sorptive Extraction (SBSE)

Stir bar sorptive extraction (SBSE) is a powerful enrichment technique within which analytes partition between A magnetic stir bar with a polymer coating and the sample matrix. Compared to SPME, SBSE offers significantly larger sorbent volumes, leading to higher extraction capacity and improved sensitivity SBSE utilizes a stir bar covered with sorbent to remove analytes with high capacity and reproducibility [13]. Advances in sorbent coatings-such as carbon nanotubes, graphene, MOFs, and ionic liquids-have improved extraction of hydrophobic and moderately polar analytes [14, 15]. It has been successfully applied to drugs, antibiotics, and contaminants in plasma, urine, milk, and environmental waters [16, 17].

3.3 Microextraction by Packed Sorbent (MEPS)

Microextraction by packed sorbent (MEPS) is a miniaturized version of the traditional Solid Phase Extraction, where the sorbent (1-2 mg) is incorporated into a needle or syringe barrel. This allows sampling, extraction as well as injection to be performed within only one gadget, significantly reducing solvent consumption and preparation time MEPS incorporates sorbent material inside a syringe barrel, enabling automation, minimal solvent use, and direct coupling to LC-MS/MS [18, 19]. MEPS is reusable for up to 100 extractions and suited for small volumes (10-1000 µL) [20]. It has been widely used for antidepressants, beta-blockers, and biomarkers in biofluids.

3.4 Fabric Phase Sorptive Extraction (FPSE)

Fabric Phase Sorptive Extraction uses sol-gel coated fabric substrates to remove analytes directly from intricate matrices without pretreatment. Benefits include high permeability, compatibility with green solvents, and broad applicability to drugs, hormones, and pollutants [21, 22]. It offers minimal solvent use and improved analytical accuracy. The benefits of equilibrium-based extraction are combined with sol-gel coating technology for microextraction sorbents in fabric phase sorptive extraction (FPSE), which also enhances the primary contact surface area (PCSA) for quick analyte sorbent interaction. Furthermore, just a small volume of elution solvent is needed because the fabric can be broken up into tiny fragments during desorption, which is consistent with the ideas of green analytical chemistry [23]. FPSE has been used to extract a variety of substances in a variety of fields, including food, biological samples, and environmental samples, because it solves the majority of the problems with existing microextraction techniques.

3.5 Dispersive Liquid-Liquid Microextraction (DLLME)

Dispersive Liquid-Liquid Microextraction offers quick extraction, high enrichment, and minimal solvent consumption [24]. It uses dispersive and extraction solvents to form fine droplets that capture analytes efficiently, forming a large surface area for analyte transfer. Selection of appropriate extractants is critical. The method is especially useful for environmental and pharmaceutical samples [25, 26]. Dispersive Liquid-Liquid Microextraction enhances increases the effectiveness of extraction by dispersing the solvent for extraction throughout the aqueous sample. Modifications such as vortex-assisted and air-assisted DLLME have reduced the use of toxic solvents The process of a solute in one liquid phase undergoing physical or chemical action in another liquid phase or being redistributed in two phases is known as liquid-liquid extraction. after the encounter of two liquid phases that are either totally immiscible or moderately miscible.

4. Emerging Microextraction Techniques

4.1 Single-Drop Microextraction (SDME)

Single-drop microextraction (SDME) involves exposing a micro drop of organic solvent or ionic liquid to a sample solution to extract analytes from liquid or headspace samples. It is inexpensive and green but limited by drop instability and small extraction volume. The first liquid-liquid microextraction method developed was the SDME. It focuses on the distribution of analytes between an aqueous solution and a tiny drop of extractant placed at the tip of a micro syringe needle. A syringe is used to inject a drop of an insoluble separating phase (less than 10 µL) into gaseous test media (headspace-single drop microextraction) or a fluid (direct immersion-single drop microextraction). After several extractions, the single drop is drawn into the micro syringe and sent to an analytical apparatus for analyte detection. In the awaiting drop, the intended analytes are separated from the test. Analyte partition coefficients have a major impact on recoveries and passive diffusion. The SDME kinds face difficulties including poor drop volume and erratic drops [27, 28].

4.2 Hollow-Fiber Liquid-Phase Microextraction (HF-LPME)

HF-LPME stabilizes the organic solvent inside hollow fibers, overcoming SDME drop instability. It offers excellent selectivity for polar analytes. Extraction occurs entering an acceptor phase within the fiber lumen via a supported liquid membrane. Although highly selective, long extraction times can occur. HF-LPME uses a porous fiber

containing organic solvent, forming a supported liquid membrane. It offers improved solvent stability and is effective for polar analytes. The analytes are separated into an acceptor solvent that is positioned within the fiber canal after first being separated into an aiding liquid membrane that is kept in the holes of a hydrophobic fibrous HF. This approach keeps the extractor liquid out of direct contact with an aqueous solution by covering it in microliters inside the lumen of a fibrous HF. This method's advantage is that, because the sample is manually protected, it can be agitated vigorously without losing the analyte. The organic layer is adsorbed in the HF pores by immersing the HF in an appropriate immiscible liquid before analysis. Within the HF's wall, a tiny layer of suitable solvent-less than 20 µL-forms. The HF is then loaded into a sample tube containing the desired aqueous solution. The test is stirred extensively to speed up the extraction. The analytes are subsequently extracted from the water system by passing them through the organic layer in the HF's pores and into an acceptor liquid within its lumen [29, 30].

4.3 Electro Membrane Extraction (EME)

Charged analytes are driven across a supported liquid membrane using an electric field in electro membrane extraction into an acceptor phase. It is ideal for highly polar drugs, peptides, and acidic/ basic compounds [31]. Examples include methadone, diclofenac, and buserelin extracted from urine and plasma. After being removed from the sample, the target analytes are transferred into an acceptor solution via a supported liquid membrane (SLM). An external electrical field maintained across the SLM facilitates this mass transfer through electrokinetic migration. An organic solvent (5-25 µL) immobilized by capillary forces in the pores of a porous polymeric membrane makes up the SLM. This porous polymeric support may take the form of a hollow fiber membrane or a flat sheet. In order to preserve the analytes in their protonated state during the extraction of cationic analytes (basic substances), the pH of the acceptor and sample must be either neutral or acidic. The target analytes are therefore vulnerable to electrokinetic migration because they are positively charged species. The anode is found in the sample, while the cathode is found in the acceptor solution. Anionic analytes are extracted by reversing the electrical field's direction and maintaining an alkaline or neutral pH in the sample and acceptor solution to keep the analytes negatively charged [32].

4.4 Solid-Phase Nano Extraction (SPNE)

Nanomaterials-including magnetic nanoparticles and graphene-enhance extraction surface area and affinity. SPNE is fast, solvent-efficient, and highly suitable for biofluid analysis [33, 34]. Magnetic nanoparticles are especially promising for biofluid analysis. Particularly in biological analysis, nano extraction lowered the amount of toxic solvent utilized and the overall time required for the extraction process. The positive effects of nanoparticles suggested that their use will have a significant future. These novel sorbents have the potential to advance analytical techniques and produce dependable and quick analytical techniques.

5. Microsample Preparation Strategies

5.1 Dried Sample Spotting (DBS, DPS, DSS, DUS): Dried blood spot (DBS) sampling is now a transformative approach in clinical and pharmacokinetic studies. A small volume of blood (10-50 µL) is applied onto filter paper, dried, and stored for subsequent analysis. Dried matrix spots reduce sample volume, improve stability, and simplify transport [35, 36]. DBS has gained importance in pharmacokinetics and clinical studies, though haematocrit effects can influence accuracy. Paper-spray MS enables direct detection of many drugs from dried spots [37]. These methods simplify sample storage and transport, using small dried volumes. They reduce biohazard risks and are increasingly applied in clinical and pharmacokinetic studies. The use of micro sampling in routine clinical pharmacology is still restricted today, primarily due to the requirement for equipment like liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which are only available in specialized facilities, that can quantify analytes in very small volumes with adequate sensitivity. Microsample drug assays are made possible by LC-MS/MS technology, which is sensitive enough to allow for their use in normal practice.

5.2 Volumetric Absorptive Micro sampling (VAMS)

Volumetric Absorptive Micro Sampling collects a fixed volume of the blood independent of haematocrit, reducing variability compared with DBS. The polymeric tip wicks exactly 10-20 µL, improving reproducibility and allowing automation [38, 39]. It supports automation and minimizes haematocrit-related variability. The haematocrit impacts the diffusion of spotted blood on the filter paper by determining the blood's viscosity. For additional investigation, a precise sub-punch is typically made inside the dried area.

5.3 Capillary Micro sampling (CMS): CMS collects 1-35 µL samples using glass capillaries, beneficial for animal studies and preclinical research. Enhanced injection systems improve precision and allow simultaneous analysis of anions and cations [40, 41]. A fixed volume of sample solution was manually injected using a syringe, and then another fixed volume of BGE solution was injected. A tiny amount of solution was fed into the capillary as the sample and BGE passed through the capillary's intake at a set pace since the micro-metering valve controlled the flow. Reproducibility and repeatability improved as a result.

6. Novel and Hybrid Approaches

6.1 Cloud Point Extraction (CPE)

CPE uses surfactants instead of organic solvents to extract analytes based on micelle formation above the cloud-point temperature. It is green, efficient, and suitable for biological fluids including saliva [42]. Formation of worm-like micelles enhances analyte enrichment. CPE uses surfactants to extract analytes without organic solvents. It is particularly effective for saliva analysis and environmentally friendly. The temperature at which a solution separates into two phases is known as the cloud point (CP). In separation science, cloud point pre concentration (CPP), which is based on the clouding phenomenon of surfactants, has received a lot of attention. Compared to conventional liquid-liquid extraction, CPP has several advantages. A salt solution and a surfactant solution that splits into immiscible surfactant-rich and surfactant-poor phases are the two fundamental elements required for CPP [43].

6.2 Micro dialysis: Micro dialysis is a method of *in vivo* sampling that monitor unbound analyte concentrations in

extracellular or interstitial fluids. A semi-permeable membrane facilitates passive diffusion of analytes into a perfusion fluid, enabling real-time monitoring. Microdialysis enables real-time sampling of extracellular fluid using semi-permeable membranes. Diffusion-based extraction allows continuous monitoring of neurotransmitters, drugs, and metabolites in tissues [44, 45].

6.3 Spin-Column Extraction (SCE)

SCE uses monolithic silica-packed columns for rapid, precise extraction. It accommodates a wide range of analytes including drugs, hormones, and pollutants. Centrifugal spin-column extraction in miniature uses porous mixed sorbents for rapid isolation of analytes [46]. Ionic liquid-modified sorbents enhance interaction through hydrophobic, electrostatic, or π-π interactions [47]. To accomplish rapid and efficient extraction, an adsorbent should display high permeability, rapid mass transfer, and superior adsorption efficiency.

7. Microfluidic and Aptamer-Based Devices

Microfluidic chips integrate sampling, extraction, and analysis into compact platforms for high-throughput bioanalysis [48]. Aptamers-synthetic oligonucleotides-offer exceptional specificity and stability for targeted extraction, sensing, and drug delivery. Their incorporation into bioanalytical sampling systems has enabled highly selective extraction and detection of biomolecules, proteins, and drugs. They are widely used in fluorescent biosensors, diagnostic probes, and targeted therapeutic systems [49, 50, 51].

8. Conclusion

Bioanalytical sampling continues to evolve toward miniaturization, automation, and greener methodologies. Techniques such as SPME, MEPS, FPSE, microfluidics, and aptamer-based systems offer improved precision, sensitivity, and sustainability. Future advancements will focus on integrated platforms, universal extraction sorbents, and real-time analytical technologies.

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