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RECENT ADVANCES IN SAMPLE PREPARATION TECHNIQUES FOR DRUG BIOANALYSIS: CURRENT TRENDS AND FUTURE PERSPECTIVES

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Abstract
Bioanalytical sample preparation plays a crucial role in pharmaceutical, clinical, and biomedical research by enabling accurate isolation, purification, and quantification of drugs, metabolites, peptides, proteins, and biomarkers from complex biological matrices. Biological samples such as plasma, serum, urine, saliva, and tissues contain proteins, phospholipids, salts, and endogenous compounds that interfere with chromatographic and mass spectrometric analysis. Therefore, efficient sample preparation is essential to improve sensitivity, selectivity, extraction recovery, and matrix clean-up in LC–MS/MS and chromatographic bioanalysis. Conventional techniques, including protein precipitation (PPT), liquid–liquid extraction (LLE), solid-phase extraction (SPE), filtration, and ultrafiltration, remain widely employed in routine pharmaceutical analysis. Recent advancements have led to the development of modern extraction approaches such as supported liquid extraction (SLE), salting-out assisted liquid extraction (SALLE), solid-phase microextraction (SPME), dispersive liquid–liquid microextraction (DLLME), and microextraction by packed sorbent (MEPS). Nanotechnology-based extraction systems, green analytical chemistry approaches, automation, and AI-assisted sample preparation have further enhanced analytical efficiency and sustainability. Emerging techniques including electro membrane extraction (EME), dried blood spot (DBS), molecularly imprinted polymers (MIPs), and miniaturized extraction platforms are transforming modern bioanalysis. This review comprehensively summarizes recent advances, applications, advantages, limitations, and future trends in sample preparation techniques for drug bioanalysis.

Keywords: *Bioanalytical sample preparation, LC–MS/MS, Solid-phase extraction, Liquid–liquid extraction, Microextraction techniques, Nanotechnology-based extraction, Green analytical chemistry***.**

INTRODUCTION

Bioanalytical sample preparation has become an indispensable component of modern pharmaceutical and clinical research because it directly influences the accuracy, sensitivity, and reliability of analytical results. The increasing complexity of biological matrices and the growing demand for ultra-trace quantification of drugs, metabolites, peptides, and biomarkers have significantly accelerated the development of advanced extraction strategies. Conventional techniques such as protein precipitation (PPT), liquid–liquid extraction (LLE), and solid-phase extraction (SPE) continue to serve as the foundation of routine bioanalysis due to their simplicity and robustness. However, recent technological advancements have introduced highly efficient and miniaturized approaches including supported liquid extraction (SLE), solid-phase microextraction (SPME), microextraction by packed sorbent (MEPS), electromembrane extraction (EME), and nanotechnology-based extraction systems, which offer improved selectivity, higher sensitivity, reduced solvent consumption, and compatibility with automated high-throughput workflows [1,2]. The integration of LC–MS/MS with innovative extraction procedures has greatly enhanced pharmacokinetic, toxicokinetic, therapeutic drug monitoring, and biomarker studies. Furthermore, the emergence of green analytical chemistry principles, AI-assisted optimisation, microfluidic systems, and sustainable extraction technologies is reshaping the future of pharmaceutical bioanalysis. Despite continuous progress, challenges related to matrix effects, analyte instability, reproducibility, and regulatory compliance still require careful optimisation and validation. Overall, ongoing advancements in bioanalytical sample preparation are expected to further improve analytical performance, operational efficiency, environmental sustainability, and precision medicine applications in pharmaceutical sciences [3,4].

BIOLOGICAL MATRICES AND ASSOCIATED CHALLENGES

Biological matrices play a fundamental role in bioanalytical studies because they serve as the primary sources for the detection and quantification of drugs, metabolites, biomarkers, and endogenous compounds. Common biological matrices used in pharmaceutical and clinical bioanalysis include plasma, serum, urine, saliva, tissues, and whole blood. Among these, plasma and serum are the most widely utilized matrices in pharmacokinetic, bioavailability, and therapeutic drug monitoring studies due to their ease of collection and high drug concentration levels. Whole blood analysis is important for drugs that partition into blood cells, while urine is frequently employed for metabolite identification, toxicological investigations, and excretion studies. Saliva has gained attention as a non-invasive alternative matrix for therapeutic monitoring because sample collection is simple and painless. Tissue samples are mainly used in distribution and toxicokinetic studies to evaluate drug accumulation in specific organs and biological compartments. Despite their importance, these matrices possess highly complex compositions containing proteins, phospholipids, lipids, salts, enzymes, carbohydrates, and endogenous metabolites that can interfere with analytical measurements [5]. One of the major challenges associated with biological matrices is matrix interference, which significantly affects the accuracy, precision, and sensitivity of bioanalytical methods. Matrix interferences arise from endogenous substances that co-elute with analytes during chromatographic separation and interfere with analyte detection. In LC–MS/MS bioanalysis, these interferences commonly result in matrix effects such as ion suppression or ion enhancement, which alter analyte ionization efficiency and lead to inaccurate quantification. Phospholipids and proteins are considered the major contributors to matrix effects in plasma and serum samples. Matrix effects can also reduce method reproducibility, increase variability, contaminate analytical systems, and shorten instrument lifespan. Therefore, efficient sample preparation and matrix clean-up procedures are essential to minimize these interferences and improve assay reliability. Techniques such as protein precipitation (PPT), liquid–liquid extraction (LLE), solid-phase extraction (SPE), and phospholipid depletion methods are commonly employed to reduce matrix-related problems and enhance analytical performance. Another important challenge in bioanalysis is the stability and degradation of analytes within biological matrices. Drugs and metabolites may undergo chemical, enzymatic, thermal, oxidative, or photolytic degradation during sample collection, storage, transportation, and analysis. Enzymatic activity present in biological samples can rapidly degrade unstable compounds, while exposure to temperature fluctuations, light, oxygen, or repeated freeze–thaw cycles may further compromise analyte integrity. Such degradation can lead to inaccurate analytical results and poor reproducibility. Therefore, stability assessment is a critical component of bioanalytical method development and validation. Various stability studies, including bench-top stability, short-term stability, long-term stability, freeze–thaw stability, stock solution stability, and autosampler stability, are conducted to ensure analyte integrity throughout the analytical process. Appropriate storage conditions, stabilising agents, anticoagulants, and controlled handling procedures are necessary to preserve sample quality and obtain reliable bioanalytical data [6].

CONVENTIONAL SAMPLE PREPARATION TECHNIQUES

Protein Precipitation (PPT)

Protein precipitation (PPT) is one of the simplest and most widely used sample preparation techniques in pharmaceutical bioanalysis. The method is mainly employed to remove proteins from biological matrices, such as plasma and serum, before LC–MS/MS or HPLC analysis. In this procedure, a biological sample is transferred into a centrifuge tube, followed by the addition of an internal standard and an organic precipitating solvent such as acetonitrile or methanol. The mixture is vortexed thoroughly and centrifuged to separate precipitated proteins from the analyte-containing supernatant. The clear supernatant is then injected directly into the analytical system or evaporated and reconstituted before analysis. PPT is rapid, cost-effective, and highly suitable for high-throughput pharmacokinetic studies. Common examples include the extraction of antidiabetic drugs, antibiotics, anticancer agents, antiviral drugs, antiepileptic drugs, antidepressants, antihypertensive drugs, steroids, and analgesics from plasma samples using acetonitrile precipitation [7].

Examples: Metformin, Orforglipron, Tovorafenib, Olanzapine, Oxcarbazepine, Ciprofloxacin, Dexamethasone, Ritonavir, Warfarin, Paracetamol.

Liquid–Liquid Extraction (LLE)

Liquid–liquid extraction (LLE) is a conventional extraction technique based on partitioning of analytes between an aqueous biological matrix and an immiscible organic solvent. The procedure involves mixing plasma, serum, urine, or tissue homogenate with an extraction solvent such as ethyl acetate, methyl tert-butyl ether (MTBE), dichloromethane, or hexane after suitable pH adjustment. The sample is vortex-mixed and centrifuged to achieve phase separation, after which the organic layer containing the analytes is collected, evaporated, and reconstituted in mobile phase before

chromatographic analysis. LLE provides better sample cleanup and reduced matrix effects than protein precipitation and is particularly useful for extracting lipophilic and weakly polar drugs [8].

Examples: Diazepam, Carbamazepine, Tamoxifen, Diclofenac, Ketoconazole, Paclitaxel, Cyclosporine, Progesterone, Testosterone, Clozapine.

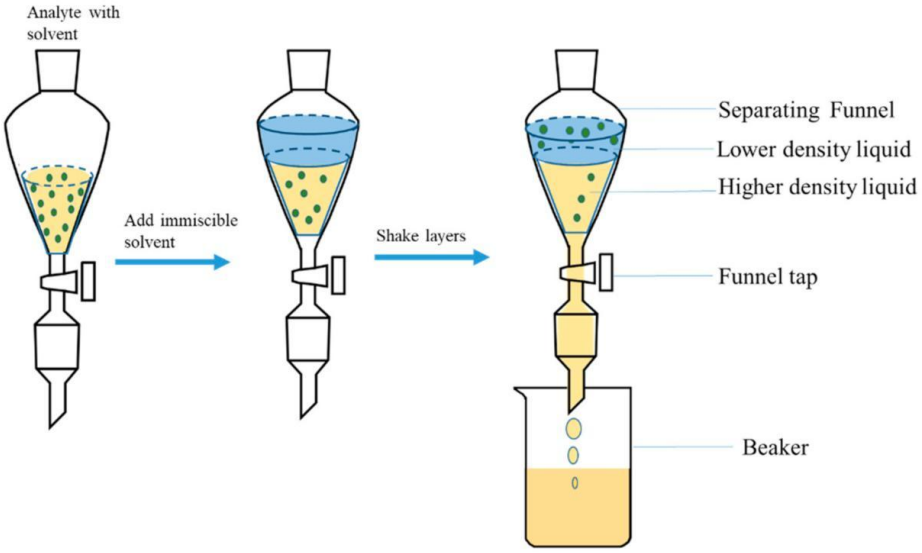

Figure 01: Liquid–Liquid Extraction Mechanism

SOLID-PHASE EXTRACTION (SPE)

Solid-phase extraction (SPE) is a highly selective sample preparation technique used for the purification and concentration of analytes from complex biological matrices. In SPE, a biological sample is loaded onto a cartridge or plate packed with sorbent material such as C18, C8, polymeric, or ion-exchange sorbents. Before sample loading, the cartridge is conditioned with methanol and equilibrated with water or buffer solution. The analytes are retained on the sorbent while interfering matrix components are washed away. Subsequently, analytes are eluted using suitable organic solvents, evaporated if necessary, and analysed using LC–MS/MS or HPLC systems [9].

Examples: Abaloparatide, Insulin analogues, Tacrolimus, Everolimus, Methotrexate, Imatinib, Rosuvastatin, Atorvastatin, Sildenafil, Vepdegestrant**.**

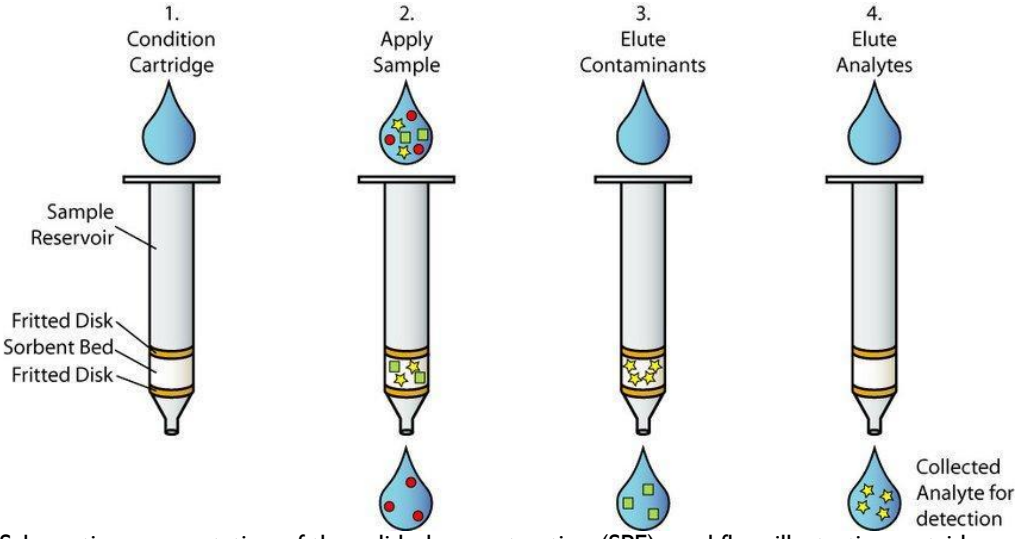

Figure 02: Schematic representation of the solid-phase extraction (SPE) workflow illustrating cartridge conditioning, equilibration, sample loading, washing, analyte elution, evaporation, and reconstitution before chromatographic analysis.

FILTRATION AND ULTRAFILTRATION METHODS

Filtration and ultrafiltration are membrane-based sample preparation techniques used for the removal of particulates, proteins, and macromolecules from biological samples. In filtration, biological samples are passed through syringe filters or membrane filters such as nylon, PTFE, PVDF, or cellulose acetate membranes to remove suspended particles before chromatographic analysis. Ultrafiltration utilises membranes with specific molecular weight cut-off values to separate

free drugs from protein-bound fractions under centrifugal force. The biological sample is added into an ultrafiltration device and centrifuged, allowing low molecular weight analytes to pass through the membrane while proteins and larger molecules are retained. These methods are commonly used in protein-binding studies, therapeutic drug monitoring, and bioanalysis of unstable compounds because they require minimal solvent use and provide rapid sample processing [10.11].

Examples: Vancomycin, Phenytoin, Digoxin, Theophylline, Lithium, Valproic acid, Gentamicin, Cyclosporine, Methotrexate, Levetiracetam.

Table 01: Comparative Table of Conventional Sample Preparation Techniques

PARAMETER	PPT	LLE	SPE	FILTRATION	ULTRAFILTRATION
Principle	Protein removal by organic solvents, acids, or salts	Partitioning of analytes between aqueous and organic phases	Selective adsorption of analytes onto sorbent material	Removal of particulates using membrane filters	Separation based on molecular weight cut-off membranes
Common Biological Matrices	Plasma, serum, whole blood	Plasma, serum, urine, tissues	Plasma, serum, urine, saliva	Plasma, urine, buffers	Plasma, serum
Main Objective	Protein removal	Analyte extraction and clean-up	Selective purification and enrichment	Clarification of samples	Separation of free drug from protein-bound drug
Common Solvents/Reagents	Acetonitrile, methanol, perchloric acid	MTBE, ethyl acetate, hexane	Methanol, water, buffers, elution solvents	Membrane filters	Ultrafiltration membranes
Advantages	Simple, rapid, inexpensive	Better clean-up and extraction efficiency	High selectivity and cleaner extracts	Simple and rapid	Suitable for protein-binding studies
Limitations	Incomplete matrix clean-up	Emulsion formation and solvent use	Expensive and multi-step process	Poor analyte enrichment	Membrane adsorption issues
Major Applications	Pharmacokinetics, LC–MS/MS screening	Lipophilic drug extraction, metabolite analysis	Trace-level bioanalysis, biomarker studies	Sample clarification	Free drug concentration analysis
Typical Examples	Metformin, Paracetamol	Diazepam, Tamoxifen	Tacrolimus, Methotrexate	Routine HPLC samples	Vancomycin, Phenytoin

ADVANCED AND MODERN EXTRACTION TECHNIQUES

Supported Liquid Extraction (SLE)

Supported liquid extraction (SLE) is an advanced sample preparation technique developed as an alternative to conventional liquid–liquid extraction (LLE). The method utilises an inert porous support material, commonly diatomaceous earth, to absorb the aqueous biological sample and facilitate analyte extraction using immiscible organic solvents. In a practical SLE procedure, the biological sample, such as plasma or serum, is first loaded onto the SLE cartridge or 96-well plate and allowed to disperse uniformly over the porous support material. After equilibration for a few minutes, an organic extraction solvent such as ethyl acetate, dichloromethane, or methyl tert-butyl ether is passed through the support bed. The analytes partition into the organic solvent while proteins, salts, and polar matrix components remain retained within the aqueous phase. The eluted organic layer is collected, evaporated under nitrogen, reconstituted with mobile phase, and analysed by LC–MS/MS or HPLC. SLE provides cleaner extracts, improved phospholipid removal, reduced emulsion formation, and better reproducibility compared to conventional LLE. It is widely used in pharmacokinetic studies and high-throughput pharmaceutical bioanalysis [12,13].

SALTING-OUT ASSISTED LIQUID EXTRACTION (SALLE)

Salting-out assisted liquid extraction (SALLE) is a hybrid extraction technique combining protein precipitation and liquid–liquid extraction principles for efficient analyte isolation from biological matrices. In this procedure, the biological sample is mixed with a water-miscible organic solvent such as acetonitrile, followed by the addition of inorganic salts, including ammonium sulfate, sodium chloride, or magnesium sulfate. The added salts decrease the solubility of the organic solvent in the aqueous phase, resulting in phase separation and transfer of analytes into the organic layer. Practically, plasma or urine samples are first vortex-mixed with acetonitrile and internal standard solution, followed by the addition of salt and centrifugation. The upper organic phase containing analytes is separated, evaporated if necessary, and injected into the chromatographic system. SALLE improves extraction efficiency, reduces matrix effects, minimises phospholipid interference, and provides excellent recovery for polar and semi-polar compounds. The method is particularly useful for LC–MS/MS bioanalysis of pharmaceuticals, metabolites, pesticides, and antibiotics [14].

SOLID-PHASE MICROEXTRACTION (SPME)

Solid-phase microextraction (SPME) is a solvent-free microextraction technique that integrates sampling, extraction, concentration, and sample introduction into a single step. The method utilises a fused silica fiber coated with an extracting stationary phase that selectively adsorbs analytes from biological samples. In practical SPME procedures, the coated fiber is exposed either directly to the liquid sample (direct immersion SPME) or to the vapor phase above the sample (headspace SPME). The analytes are adsorbed onto the fiber coating during a specified extraction time. After extraction, the fiber is transferred into the injection port of GC or desorption chamber of LC–MS systems where analytes are thermally or solvent desorbed for analysis. Common coatings include polydimethylsiloxane (PDMS), polyacrylate, and divinylbenzene materials. SPME significantly reduces solvent consumption, extraction time, and sample volume requirements while providing high sensitivity and automation capability. It is extensively applied in drug analysis, metabolomics, toxicology, and environmental bioanalysis [15].

DISPERSIVE LIQUID–LIQUIDMICROEXTRACTION (DLLME)

Dispersive liquid–liquid microextraction (DLLME) is a rapid and highly efficient microextraction technique based on the formation of a cloudy solution containing fine droplets of extraction solvent dispersed throughout the aqueous sample. In a practical DLLME procedure, a mixture of extraction solvent and disperser solvent is rapidly injected into the biological sample using a syringe. Common extraction solvents include chloroform or carbon tetrachloride, while methanol, acetone, or acetonitrile are used as disperser solvents. Rapid dispersion creates a large contact surface area between the extraction solvent and analytes, leading to rapid analyte transfer and equilibrium. After centrifugation, the fine droplets settle as a sedimented phase containing concentrated analytes. The sedimented extract is collected and analyzed using LC–MS/MS, HPLC, or GC–MS systems. DLLME offers high enrichment factors, rapid extraction, low solvent consumption, and excellent sensitivity. It is widely applied for extraction of pharmaceuticals, pesticides, environmental pollutants, and trace organic compounds [16].

MICROEXTRACTION BY PACKED SORBENT (MEPS)

Microextraction by packed sorbent (MEPS) is a miniaturized version of solid-phase extraction designed for extraction of analytes from very small sample volumes. In MEPS, small quantities of sorbent material are packed directly into a syringe barrel or needle assembly. The practical procedure begins by conditioning the sorbent with methanol and water. The biological sample is then aspirated and dispensed repeatedly through the packed sorbent bed, allowing analytes to adsorb onto the extraction material. Matrix impurities are removed using washing solvents, followed by elution of analytes with a small volume of organic solvent directly into an autosampler vial. The eluate is subsequently injected into LC–MS/MS or GC–MS systems for analysis. MEPS requires only microliter quantities of sample and solvent, making it highly suitable for pediatric and limited-volume samples. The technique provides rapid extraction, automation capability, reduced solvent consumption, and excellent sensitivity for pharmaceutical, forensic, and clinical bioanalysis [17].

Table 02: Comparative Table of Advanced and Modern Extraction Techniques

Parameter	SLE	SALLE	SPME	DLLME	MEPS
Principle	Partitioning using porous support material	Phase separation induced by salts	Adsorption of analytes onto coated fiber	Rapid dispersion of extraction solvent in sample	Miniaturized SPE using packed sorbent
Extraction Mechanism	Similar to liquid–liquid extraction	Combination of protein	Adsorption/absorption onto stationary phase	Micro-scaleliquid–liquid	Sorbent-based

Parameter	SLE	SALLE	SPME	DLLME	MEPS
		precipitation and LLE		extraction	analyte retention
Sample Volume	Moderate	Moderate	Very low	Low	Very low
Solvent Consumption	Moderate	Low to moderate	Minimal/solvent-free	Very low	Very low
Extraction Time	Short	Short	Moderate	Very rapid	Rapid
Major Sorbents/Materials	Diatomaceous earth	Salts and organic solvents	PDMS, polyacrylate fibers	Extraction and disperser solvents	C18, HLB, mixed-mode sorbents
Common Solvents Used	Ethyl acetate, MTBE	Acetonitrile with salts	Usually solvent-free	Chloroform, methanol, acetone	Methanol, acetonitrile
Advantages	Cleaner extracts, reduced emulsion formation	Reduced matrix effects and good extraction efficiency	Solvent-free and highly sensitive	Fast extraction with high enrichment	Requires very small sample volume
Limitations	Cartridge cost	Salt optimization required	Fiber fragility and limited lifetime	Limited solvent choices	Sorbent clogging may occur
Main Applications	Pharmacokinetic studies	Polar drug extraction	Drug analysis and metabolomics	Trace-level pharmaceutical analysis	Clinical and forensic bioanalysis
Typical Examples	Steroidal drugs, anticancer agents	Antibiotics, pesticides	Volatile drugs, metabolites	Trace pharmaceuticals, pollutants	Pediatric and limited-volume samples

NANOTECHNOLOGY-BASED SAMPLE PREPARATION APPROACHES

Nanotechnology-based sample preparation approaches have emerged as highly advanced and efficient strategies in modern bioanalysis due to their superior adsorption capacity, enhanced selectivity, large surface area, and rapid extraction performance. The incorporation of nanomaterials into extraction techniques has significantly improved analyte enrichment, sensitivity, and matrix clean-up in pharmaceutical and biomedical analysis. Nanomaterials such as magnetic nanoparticles, graphene, carbon nanotubes, nanofibers, and nanocomposite sorbents possess unique physicochemical properties, including a high surface-to-volume ratio, tunable surface functionality, chemical stability, and excellent adsorption efficiency. These characteristics enable efficient isolation of drugs, metabolites, peptides, proteins, and biomarkers from complex biological matrices, including plasma, serum, urine, and tissues. Nanotechnology-based extraction techniques also support miniaturisation, automation, reduced solvent consumption, and environmentally friendly analytical procedures, making them increasingly important in modern LC–MS/MS bioanalysis [18,19].

MAGNETIC NANOPARTICLE EXTRACTION

Magnetic nanoparticle extraction is a highly efficient sample preparation technique that utilizes magnetic nanomaterials for selective analyte extraction and rapid phase separation. Magnetic nanoparticles commonly consist of iron oxide cores coated with silica, polymers, graphene, or functional ligands to improve selectivity and adsorption capacity. In a practical procedure, magnetic nanoparticles are dispersed into the biological sample containing analytes of interest. The analytes adsorb onto the nanoparticle surface through hydrophobic, electrostatic, or hydrogen-bonding interactions. After extraction, an external magnetic field is applied to rapidly separate the nanoparticles from the sample matrix without centrifugation or filtration. The analytes are then desorbed using suitable solvents and analyzed by LC–MS/MS or HPLC. Magnetic nanoparticle extraction provides rapid extraction, high enrichment factors, minimal solvent use, and excellent matrix clean-up. It is widely used for the extraction of pharmaceuticals, peptides, proteins, antibiotics, and biomarkers from plasma and urine samples [20, 21].

SOLID-PHASE NANO-EXTRACTION (SPNE)

Solid-phase nano-extraction (SPNE) is an advanced nano-based extraction approach that employs nanoparticles as sorbent materials for analyte adsorption and enrichment. The technique is based on the strong affinity between analytes and nanoscale adsorbents possessing high surface area and multiple active binding sites. In practical SPNE procedures, nanoparticles such as silica nanoparticles, polymeric nanoparticles, magnetic nanoparticles, or metal oxide nanoparticles are added to the biological sample and mixed thoroughly to facilitate analyte adsorption. Following extraction, the nanoparticles are separated by centrifugation, magnetic field, or filtration, and the analytes are eluted using suitable solvents for chromatographic analysis. SPNE provides enhanced extraction efficiency, improved sensitivity, rapid equilibration, and excellent selectivity compared to conventional extraction methods. The technique has been extensively applied for the determination of drugs, pesticides, polycyclic aromatic hydrocarbons, heavy metals, and environmental contaminants in complex biological and environmental samples [21,22].

NANOFIBER AND NANOCOMPOSITE SORBENTS

Nanofiber and nanocomposite sorbents are innovative extraction materials developed to improve analyte retention, adsorption capacity, and extraction selectivity in modern bioanalysis. Nanofibers are ultra-fine fibrous materials produced using techniques such as electrospinning, while nanocomposite sorbents are hybrid materials combining nanoparticles with polymers, carbon materials, or silica supports. In practical extraction procedures, nanofiber membranes or nanocomposite-packed cartridges are used as sorbent materials in SPE, microextraction, or filtration-based systems. Biological samples are passed through the sorbent, where analytes interact with functionalized nanomaterials and are selectively retained. After washing to remove matrix impurities, analytes are eluted with organic solvents for LC–MS/MS analysis. These sorbents provide superior adsorption surface area, enhanced mechanical stability, rapid extraction kinetics, and high analyte recovery. Nanofiber and nanocomposite materials are increasingly utilised for the extraction of anticancer drugs, antibiotics, peptides, biomarkers, and trace pharmaceutical compounds from biological matrices [23].

GRAPHENE AND CARBON NANOTUBE-BASED EXTRACTION SYSTEMS

Graphene and carbon nanotube (CNT)-based extraction systems represent highly promising nanotechnology-based approaches in bioanalytical sample preparation. Graphene possesses a two-dimensional honeycomb carbon structure with exceptional adsorption capability, while carbon nanotubes exhibit cylindrical nanostructures with high mechanical strength and surface reactivity. In practical extraction procedures, graphene oxide, reduced graphene oxide, single-walled carbon nanotubes (SWCNTs), or multi-walled carbon nanotubes (MWCNTs) are incorporated into SPE cartridges, magnetic nanocomposites, or microextraction devices. Biological samples are exposed to these nanomaterials, allowing analytes to adsorb through π–π interactions, hydrophobic interactions, and hydrogen bonding. After adsorption, analytes are eluted using suitable solvents and analysed by LC–MS/MS or GC–MS systems. These extraction systems provide extremely high extraction efficiency, rapid analyte enrichment, excellent sensitivity, and effective matrix effect reduction. Graphene and CNT-based sorbents are widely applied for the extraction of steroids, anticancer agents, pesticides, antibiotics, hormones, and environmental pollutants from plasma, urine, and tissue samples [24].

Table 03: Comparative Table of Nanotechnology-Based Sample Preparation Approaches

Parameter	Magnetic Nanoparticle Extraction	Solid-Phase Nano-Extraction (SPNE)	Nanofiber and Nanocomposite Sorbents	Graphene and Carbon Nanotube-Based Extraction
Principle	Magnetic nanoparticles adsorb analytes and are separated using magnetic field	Nanoparticles act as nano-sorbents for analyte enrichment	Nanofibers/nanocomposites retain analytes through surface interactions	Graphene and CNTs adsorb analytes through π–π and hydrophobic interactions
Main Materials Used	Iron oxide nanoparticles, silica-coated magnetic particles	Silicananoparticles, polymericnanoparticles, metal oxide nanoparticles	Electrospun nanofibers, polymer nanocomposites	Graphene oxide, reduced graphene oxide, SWCNTs, MWCNTs
Surface Area	Very high	Extremely high	High	Extremely high

Parameter	Magnetic Nanoparticle Extraction	Solid-Phase Nano-Extraction (SPNE)	Nanofiber and Nanocomposite Sorbents	Graphene and Carbon Nanotube-Based Extraction
Solvent Consumption	Very low	Low	Low	Low
Separation Method	Magnetic separation	Centrifugation or magnetic separation	Filtration or SPE-based separation	SPE or dispersive extraction
Major Advantages	Fast separation without centrifugation	Large adsorption capacity and high enrichment	Enhanced mechanical stability and adsorption	Superior adsorption and ultra-trace sensitivity
Limitations	Nanoparticle aggregation possible	Complex synthesis procedures	Higher production cost	Possible nanomaterial toxicity concerns
Biological Matrices	Plasma, serum, urine	Plasma, urine, tissues	Plasma, serum, biological fluids	Plasma, urine, tissue samples
Examples of Target Analytes	Antibiotics, peptides, proteins	Pesticides, pharmaceuticals, heavy metals	Anticancer drugs, biomarkers	Steroids, hormones, anticancer agents

GREEN AND ECO-FRIENDLY SAMPLE PREPARATION METHODS

Solvent-Free Extraction Approaches

Solvent-free extraction approaches are advanced green analytical techniques designed to eliminate or drastically reduce the use of hazardous organic solvents during sample preparation. These methods rely on direct analyte adsorption, thermal desorption, or membrane-based extraction systems. Solid-phase microextraction (SPME) is one of the most widely used solvent-free techniques in which analytes are adsorbed onto a coated fiber and subsequently desorbed directly into chromatographic systems. In practical procedures, the SPME fiber is exposed either directly to the biological sample or to the headspace above the sample, allowing analytes to partition onto the coating material. After extraction, the fiber is transferred to the injection port of GC or LC–MS systems for desorption and analysis. Solvent-free extraction techniques significantly reduce chemical waste, improve laboratory safety, minimize sample handling, and support automation. These methods are extensively applied in pharmaceutical analysis, metabolomics, toxicology, food analysis, and environmental monitoring [25, 26].

CLOUD POINT EXTRACTION (CPE)

Cloud point extraction (CPE) is a green sample preparation technique based on phase separation of non-ionic surfactant solutions at elevated temperatures. In this method, surfactants such as Triton X-114 or Triton X-100 form micellar solutions that become cloudy above a characteristic cloud point temperature, resulting in separation into surfactant-rich and aqueous phases. In practical CPE procedures, the biological sample is mixed with surfactant solution and suitable reagents followed by incubation at controlled temperature to induce phase separation. The analytes partition into the surfactant-rich phase due to hydrophobic interactions, while interfering matrix components remain in the aqueous phase. Centrifugation is used to accelerate separation, and the concentrated surfactant phase is diluted with suitable solvent before chromatographic analysis. CPE provides high extraction efficiency, low solvent consumption, excellent enrichment capability, and environmentally friendly operation. The technique is commonly applied for extraction of drugs, vitamins, pesticides, metal ions, and biomarkers from biological and environmental samples [27].

AUTOMATION AND HIGH-THROUGHPUT BIOANALYSIS

96-WELL PLATE EXTRACTION SYSTEMS

The 96-well plate extraction system is one of the most widely used high-throughput sample preparation approaches in pharmaceutical bioanalysis. These systems allow simultaneous processing of multiple samples using specially designed extraction plates compatible with automated liquid-handling instruments. Various extraction techniques including protein precipitation (PPT), solid-phase extraction (SPE), supported liquid extraction (SLE), and phospholipid depletion methods can be performed using 96-well plate formats. In practical procedures, biological samples are dispensed into the wells followed by automated addition of solvents, buffers, internal standards, and extraction reagents. Vacuum manifolds or positive pressure systems facilitate washing and elution steps during SPE-based workflows. The processed samples are collected into receiver plates and directly analyzed using LC–MS/MS systems. The 96-well format

significantly reduces sample preparation time, increases throughput, improves reproducibility, and minimises manual handling errors. These systems are extensively used in pharmacokinetic screening, bioequivalence studies, and large-scale clinical bioanalysis [28, 29].

ONLINE SPE–LC–MS INTEGRATION

Online solid-phase extraction coupled with liquid chromatography–mass spectrometry (online SPE–LC–MS) is an advanced automated bioanalytical approach that integrates sample clean-up, analyte enrichment, chromatographic separation, and detection into a continuous workflow. In this system, biological samples are injected directly into an online SPE cartridge where analytes are selectively retained while matrix interferences are washed away. Subsequently, switching valves transfer the retained analytes onto the analytical LC column for chromatographic separation and MS detection. This automated integration eliminates several manual extraction steps, reduces sample preparation time, decreases solvent consumption, and minimises sample handling errors. Online SPE–LC–MS systems provide improved sensitivity, enhanced reproducibility, and efficient matrix removal, making them highly suitable for trace-level drug analysis and high-throughput pharmacokinetic studies. The technique is widely applied in therapeutic drug monitoring, metabolite analysis, peptide bioanalysis, and clinical research [30].

RECENT INNOVATIONS AND EMERGING TRENDS

Electromembrane Extraction (EME)

Electromembrane extraction (EME) is an advanced microextraction technique that utilises an electrically driven transport mechanism for selective analyte extraction across a supported liquid membrane. In practical EME procedures, the biological sample and acceptor solution are separated by a porous membrane impregnated with an organic solvent. When an electric potential is applied across the membrane, charged analytes migrate selectively from the donor solution through the liquid membrane into the acceptor solution. The extracted analytes are subsequently analysed using LC–MS/MS or capillary electrophoresis systems. EME provides excellent selectivity, rapid extraction, minimal solvent consumption, and high enrichment factors for ionic and polar compounds [31,32].

DRIED BLOOD SPOT (DBS) AND DRIED PLASMA SPOT (DPS) TECHNIQUES

DBS and DPS techniques are minimally invasive microsampling approaches increasingly used in clinical and pharmacokinetic studies. In DBS, small volumes of blood are spotted onto specialised filter paper cards and dried at room temperature before storage and analysis. DPS follows a similar principle using plasma samples. In practical procedures, punched discs from dried spots are extracted using suitable solvents and analysed by LC–MS/MS systems. These methods offer numerous advantages, including simplified sample collection, minimal biohazard risk, reduced storage requirements, improved analyte stability, and suitability for remote sampling. DBS and DPS are widely applied in neonatal screening, therapeutic drug monitoring, infectious disease testing, and pharmacokinetic studies [33].

MOLECULARLY IMPRINTED POLYMERS (MIPS)

Molecularly imprinted polymers (MIPs) are highly selective synthetic sorbents designed to recognise specific analytes based on molecular recognition principles. During MIP synthesis, template molecules are polymerised with functional monomers and cross-linkers to form selective binding cavities complementary to the target analyte. After removal of the template molecule, the polymer retains highly specific recognition sites capable of selectively rebinding the analyte from complex matrices. In practical bioanalysis, MIPs are incorporated into SPE cartridges, magnetic nanoparticles, or microextraction systems for selective analyte enrichment and purification. MIPs provide excellent selectivity, chemical stability, and reusability for the extraction of drugs, peptides, hormones, and biomarkers [34].

FUTURE PERSPECTIVES AND CHALLENGES

The future of bioanalytical sample preparation is expected to focus on automation, miniaturization, sustainability, and integration with advanced analytical technologies. Rapid growth in pharmaceutical research, personalized medicine, metabolomics, and biomarker discovery has increased the demand for highly sensitive, selective, and high-throughput extraction methods. Emerging approaches such as microfluidic devices, lab-on-a-chip systems, AI-assisted analytical optimization, and fully automated robotic extraction platforms are anticipated to revolutionize modern bioanalysis. Nanotechnology-based sorbents, molecularly imprinted polymers, and magnetic nanoparticles are likely to provide improved selectivity and ultra-trace analyte enrichment. Green analytical chemistry principles will continue to drive the development of solvent-free and eco-friendly extraction procedures with reduced waste generation and energy consumption [35, 36].

Despite significant progress, several challenges remain in bioanalytical sample preparation. Complex biological matrices continue to produce matrix effects, ion suppression, and analyte instability that may compromise analytical accuracy. Miniaturized techniques often require careful optimization and specialized instrumentation, which may increase operational costs. Reproducibility, scalability, and standardization of advanced extraction procedures also remain major concerns. Furthermore, integration of automated systems with regulatory compliance and method validation requirements presents additional difficulties. Future research should therefore focus on developing robust, cost-effective, environmentally sustainable, and universally applicable extraction techniques capable of supporting next-generation pharmaceutical and clinical bioanalysis [37].

CONCLUSION

Sample preparation remains one of the most critical steps in drug bioanalysis because it directly influences analyte recovery, matrix clean-up, sensitivity, selectivity, and overall analytical performance. Conventional extraction techniques such as protein precipitation, liquid–liquid extraction, and solid-phase extraction continue to be extensively utilized due to their reliability and broad applicability. However, recent advancements in microextraction technologies, nanotechnology-based sorbents, automation, green analytical chemistry, and AI-assisted systems have significantly improved extraction efficiency, reduced solvent consumption, and enhanced analytical throughput. Emerging techniques including electromembrane extraction, dried blood spot analysis, molecularly imprinted polymers, and miniaturized analytical platforms are transforming modern pharmaceutical and clinical bioanalysis. Continuous innovation in sample preparation technologies is essential to address challenges associated with complex biological matrices, analyte instability, and ultra-trace quantification. Future developments focusing on sustainable, automated, and highly selective extraction approaches will further strengthen the role of bioanalytical sample preparation in pharmaceutical research, therapeutic monitoring, toxicology, and precision medicine.

AUTHOR CONTRIBUTIONS

Vidya Sagar Pasumarty conceptualized the review topic, conducted the literature survey, organized the content, and drafted the manuscript. Anupkumar Dilipkumar Thakkar contributed to literature collection, critical review of the scientific content, and manuscript editing. Leena Alla contributed to scientific evaluation, manuscript revision, and final approval of the article. All authors read and approved the final manuscript

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