CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Review Article*

NOVEL APPROACH FOR ESTIMATION OF DRUG IN BIOLOGICAL SAMPLE RECENT TRENDS AND FUTURE PROSPECT

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Abstract:

Accurate estimation of drugs in biological matrices is fundamental to pharmaceutical development, clinical therapeutics, toxicological evaluation, and regulatory compliance. The increasing complexity of modern drug molecules and the demand for ultra-trace detection have driven significant advancements in bioanalytical methodologies. Conventional techniques such as UV spectroscopy and high-performance liquid chromatography have progressively evolved into highly sensitive and selective hyphenated systems, particularly liquid chromatography–tandem mass spectrometry, which is now considered the gold standard in bioanalysis. Emerging technologies including high-resolution mass spectrometry, microfluidic platforms, nanotechnology-assisted detection, biosensors, and miniaturized sampling strategies such as dried blood spot and volumetric absorptive microsampling have further transformed the analytical landscape. In parallel, novel sample preparation approaches and green analytical methodologies have improved efficiency while reducing environmental impact. These innovations collectively address challenges associated with matrix interference, low analyte concentration, and high-throughput requirements. This review critically discusses recent advancements in drug estimation techniques, their applications in pharmacokinetics, therapeutic drug monitoring, toxicology, forensic science, and anti-doping analysis, and explores future perspectives emphasizing automation, artificial intelligence integration, and sustainable bioanalytical practices.

Keywords:*Bioanalytical methods; LC–MS/MS; high-resolution mass spectrometry; pharmacokinetics; therapeutic drug monitoring; biosensors; microfluidics; nanotechnology; dried blood spot; green analytical chemistry; sample preparation; drug quantification.*

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Please cite this article in press ***Shubhada V. Bhopale*** *et al.,* ***Novel Approach For Estimation Of Drug In Biological Sample Recent Trends And Future Prospect*** *.,Indo Am. J. P. Sci, 2024; 11 (12).*

INTRODUCTION:

Accurate estimation of drugs in biological matrices represents a cornerstone of modern pharmaceutical analysis, clinical therapeutics, and translational research. Quantification of drugs and their metabolites in complex biological systems such as blood, plasma, urine, saliva, and tissues enables the characterization of absorption, distribution, metabolism, and excretion processes, thereby supporting evidence-based drug development and regulatory approval.[1-5] The reliability of bioanalytical data directly influences dose selection, safety assessment, and therapeutic optimization. In clinical practice, precise measurement of drug concentrations ensures appropriate exposure, minimizes adverse effects, and enhances treatment efficacy, particularly for drugs with narrow therapeutic windows. Consequently, robust bioanalytical methodologies are indispensable not only in preclinical and clinical investigations but also in post-marketing surveillance and real-world therapeutic monitoring.

Drug estimation plays a pivotal role in pharmacokinetic and pharmacodynamic investigations by establishing quantitative relationships between administered dose, systemic exposure, and pharmacological response.[6-8] Through concentration–time profiling, pharmacokinetic parameters such as C_max, T_max, area under the curve (AUC), clearance, and half-life can be accurately derived, forming the basis for dosage regimen design and bioequivalence evaluation. In pharmacodynamic assessments, correlation of plasma drug levels with therapeutic or toxic responses allows for exposure–response modeling and optimization of individualized therapy. Therapeutic drug monitoring further depends on precise bioanalysis to maintain plasma concentrations within target ranges, particularly for antiepileptics, immunosuppressants, anticancer agents, and certain antimicrobials. Beyond clinical pharmacology, drug estimation is integral to toxicological screening, forensic investigations, anti-doping control, and environmental exposure assessment, where detection sensitivity and analytical specificity are critical for legal and regulatory decision-making.[9-12]

Despite its importance, bioanalysis in biological matrices presents substantial analytical challenges. Biological fluids are inherently complex, containing proteins, lipids, endogenous metabolites, salts, and cellular components that can interfere with analytical detection. Matrix effects such as ion suppression or enhancement in mass spectrometric systems compromise quantitative accuracy and reproducibility. Additionally, many drugs are present at trace or sub-trace levels, often in the nanogram or picogram per milliliter range, necessitating highly sensitive and selective methodologies. Instability of analytes, protein binding, metabolic transformation, and sample degradation further complicate reliable quantification. Variability in matrix composition among individuals introduces additional analytical uncertainty, demanding rigorous validation protocols and carefully optimized sample preparation strategies.[13-15]

In response to these limitations, there is a growing demand for innovative, high-throughput, environmentally sustainable and ultra-sensitive analytical platforms. Advances in hyphenated techniques such as ultra-high-performance liquid chromatography coupled with tandem mass spectrometry have significantly enhanced detection capability and selectivity, while miniaturized sample preparation approaches reduce solvent consumption and improve efficiency. Emerging technologies including biosensor-based detection, microfluidic systems, nanomaterial-assisted extraction, and automation-driven analytical workflows are redefining the landscape of drug estimation. Simultaneously, the integration of green analytical chemistry principles promotes reduced environmental impact without compromising analytical performance. Collectively, these developments reflect a paradigm shift toward faster, more precise, and sustainable bioanalytical methodologies capable of meeting the evolving demands of pharmaceutical research and personalized medicine.[16-18]

Biological Matrices Used in Drug Estimation

Selection of an appropriate biological matrix is a critical determinant in bioanalytical method development, as it directly influences analytical sensitivity, clinical relevance, and interpretative accuracy. Different matrices provide distinct pharmacokinetic and toxicological information depending on drug distribution, metabolism, and elimination pathways. The choice of matrix must consider factors such as invasiveness of sampling, analyte stability, expected concentration range, and susceptibility to matrix interference. A comprehensive understanding of the advantages and limitations associated with each biological specimen ensures reliable quantification and meaningful interpretation of drug exposure.[19-20]

Blood (Plasma/Serum)

Blood, particularly plasma and serum, remains the primary matrix for quantitative drug estimation in pharmacokinetic and bioequivalence studies. Plasma reflects the circulating fraction of a drug and is directly correlated with systemic exposure, making it the gold standard for regulatory submissions and therapeutic drug monitoring. Its advantages include established validation guidelines, well-characterized reference ranges, and broad applicability across therapeutic classes.

However, blood sampling is invasive and requires trained personnel, sterile conditions, and controlled storage. High protein content and endogenous components may cause matrix effects, particularly in mass spectrometric analysis. Despite these challenges, plasma-based quantification remains indispensable in clinical pharmacology and regulatory bioanalysis.[21-22]

Urine

Urine is widely used in toxicological screening and drug abuse monitoring due to its non-invasive collection and relatively high concentrations of parent drugs or metabolites. It is particularly suitable for detecting excreted compounds and assessing recent exposure. The matrix generally contains fewer proteins than plasma, simplifying sample preparation. Nevertheless, urinary drug levels may not directly correlate with pharmacologically active plasma concentrations, limiting its utility in pharmacokinetic modeling. Variability in urine pH, dilution, and renal function can further affect analytical outcomes. Urine analysis is therefore most applicable in forensic investigations, compliance testing, and elimination studies.[23]

Saliva

Saliva has emerged as an attractive alternative matrix owing to its non-invasive, stress-free collection and minimal requirement for specialized equipment. Drug concentrations in saliva often reflect the free, pharmacologically active fraction present in plasma, providing clinically relevant information for certain compounds. It is particularly advantageous in pediatric and geriatric populations. However, saliva production rate, pH variability, and contamination can influence drug levels. Additionally, lower analyte concentrations compared to plasma necessitate highly sensitive detection techniques. Saliva-based testing is increasingly explored in roadside drug screening, therapeutic monitoring, and point-of-care applications.[24]

Cerebrospinal Fluid (CSF)

Cerebrospinal fluid offers unique insight into central nervous system (CNS) drug penetration and is essential for evaluating drugs targeting neurological disorders. Measurement of drug concentrations in CSF helps assess blood–brain barrier permeability and central pharmacokinetics. The principal limitation is the invasive nature of sample collection via lumbar puncture, which restricts routine use. Small sample volumes and low analyte concentrations demand highly sensitive analytical platforms. Consequently, CSF analysis is primarily employed in specialized clinical research and neuropharmacological investigations.[25]

Dried Blood Spots (DBS)

Dried blood spot sampling represents a miniaturized and patient-friendly alternative to conventional venous sampling. Small volumes of capillary blood are collected on filter paper, dried, and transported without stringent cold-chain requirements. DBS reduces biohazard risk, simplifies logistics, and is well suited for remote or resource-limited settings. Nonetheless, hematocrit variation can affect spot homogeneity and quantification accuracy. Extraction efficiency and matrix effects must be carefully validated. DBS is increasingly adopted in pediatric pharmacokinetics, large-scale epidemiological studies, and decentralized clinical trials.[26-28]

Hair and Nails

Hair and nails provide a long-term record of drug exposure, as compounds are incorporated into keratinized matrices during growth. These specimens are particularly useful in forensic toxicology and retrospective exposure assessment. They allow detection of chronic or past drug use over extended periods. However, external contamination, variable growth rates, and complex extraction procedures pose analytical challenges. Quantitative interpretation can also be complicated due to differences in pigmentation and cosmetic treatments. Despite these limitations, keratin-based matrices are valuable for historical drug profiling.[29-30]

Tissue Samples

Tissue analysis offers direct measurement of drug distribution at the site of action, providing essential information in preclinical pharmacokinetic and biodistribution studies. It enables evaluation of organ-specific accumulation and potential toxicity. However, tissue sampling is invasive and often limited to animal studies or biopsy samples in clinical settings. Homogenization procedures, matrix complexity, and low analyte recovery may complicate quantification. Tissue-based drug estimation is therefore primarily applied in translational research, oncology, and toxicological investigations.

Table 1: Comparison of Biological Matrices for Drug Estimation[31-35]

Matrix	Advantages	Limitations	Applications
Blood (Plasma/Serum)	Gold standard; direct correlation with systemic exposure; regulatory acceptance	Invasive sampling; matrix effects; storage requirements	Pharmacokinetics, bioequivalence, therapeutic drug monitoring
Urine	Non-invasive; higher metabolite concentration; easy collection	Poor correlation with plasma levels; variability in dilution and pH	Toxicology, forensic analysis, drug abuse testing
Saliva	Non-invasive; reflects free drug fraction; suitable for point-of-care	Low concentration; variability in flow rate and pH	Roadside testing, TDM, pediatric studies
CSF	Direct assessment of CNS penetration	Highly invasive; limited sample volume	Neuropharmacology, CNS drug research
Dried Blood Spots (DBS)	Minimal sample volume; easy storage and transport	Hematocrit effect; extraction variability	Pediatric PK, remote clinical trials
Hair and Nails	Long-term exposure assessment	External contamination; complex extraction	Forensic toxicology, retrospective analysis
Tissue Samples	Site-specific distribution data	Invasive; complex processing	Preclinical studies, biodistribution, oncology research

Conventional Analytical Techniques for Drug Estimation in Biological Samples[36-42]

Conventional analytical methodologies constitute the foundational framework of bioanalysis and have significantly contributed to the evolution of quantitative drug estimation in biological matrices. Prior to the emergence of highly sophisticated hyphenated systems, these techniques enabled reliable separation, detection, and quantification of drugs in plasma, urine, and other biological fluids. Although modern analytical demands increasingly require ultra-trace sensitivity and high-throughput performance, conventional approaches remain relevant due to their operational simplicity, cost-effectiveness, and well-established validation protocols. Their application is particularly valuable in routine laboratory settings, preliminary investigations, and resource-limited environments.

Ultraviolet–Visible Spectrophotometry

Ultraviolet–visible (UV–Vis) spectrophotometry is one of the earliest instrumental techniques used for drug estimation. It is based on the measurement of light absorption by chromophoric groups present in drug molecules at specific wavelengths. The technique offers rapid analysis, minimal sample preparation under controlled conditions, and relatively low instrumentation cost. In pharmaceutical quality control, UV–Vis analysis is frequently employed for assay determination and dissolution studies.

However, direct application of UV–Vis spectroscopy to biological samples is constrained by poor selectivity and significant interference from endogenous components such as proteins, lipids, and metabolites. Biological matrices often exhibit overlapping absorbance, leading to reduced specificity. To overcome these limitations, extensive sample cleanup, extraction, or derivatization procedures are typically required. Consequently, while UV–Vis spectroscopy provides simplicity and accessibility, its role in advanced bioanalysis is limited primarily to supportive or preliminary assessments.

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) represented a major advancement in analytical science by enabling efficient separation of complex mixtures prior to detection. The technique employs high-pressure pumping systems, specialized stationary phases, and detectors such as UV, fluorescence, or diode-array detectors. In bioanalysis, HPLC has been widely utilized for quantifying drugs and metabolites in plasma, serum, and urine samples, particularly in pharmacokinetic and therapeutic drug monitoring studies.

The principal advantages of HPLC include high reproducibility, good resolution, and adaptability to various chemical classes. Different column chemistries allow selective retention and separation of analytes from endogenous matrix components. Nonetheless, conventional HPLC coupled with non-mass-based detectors may exhibit limited sensitivity for drugs present at trace levels. Longer run times and significant solvent consumption can also reduce analytical throughput and increase operational costs. Despite these limitations, HPLC remains a reliable and widely accepted technique in regulated bioanalytical laboratories.

Gas Chromatography (GC)

Gas chromatography (GC) has been extensively applied for the analysis of volatile and thermally stable compounds. It provides excellent separation efficiency and high sensitivity when coupled with detectors such as flame ionization detectors (FID) or electron capture detectors (ECD). GC has historically been used in toxicological and forensic investigations for the detection of drugs of abuse, anesthetics, and environmental contaminants.

A significant limitation of GC in bioanalysis is the requirement for analytes to be volatile and thermally stable. Many pharmaceutical compounds are polar or thermolabile and require chemical derivatization to improve volatility and chromatographic behavior. This additional sample preparation step increases analysis time and may introduce variability. While GC remains valuable in specific applications, its use has declined in comparison with liquid chromatography-based techniques for modern drug molecules.

Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a simple, economical, and rapid separation technique commonly used for qualitative and semi-quantitative analysis. It involves migration of analytes over a stationary phase coated on a plate under the influence of a suitable solvent system. TLC is particularly useful for preliminary screening of drugs and identification of multiple analytes within a single sample.

Despite its advantages in simplicity and low cost, TLC lacks the precision, sensitivity, and automation required for rigorous pharmacokinetic or therapeutic drug monitoring studies. Quantitative reproducibility can be limited, and detection sensitivity is generally lower compared to instrumental chromatographic systems. Therefore, TLC is primarily applied in academic laboratories, preliminary investigations, and basic toxicological screening.

Immunoassay-Based Techniques
Immunoassays introduced enhanced specificity into drug estimation through antigen–antibody interactions. Techniques such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence polarization immunoassay (FPIA) allow rapid and high-throughput screening of drugs in biological fluids. These methods are extensively employed in clinical laboratories for therapeutic drug monitoring and detection of drugs of abuse.

The major advantage of immunoassays lies in their operational speed and minimal requirement for complex instrumentation. However, cross-reactivity with structurally related compounds and metabolites can compromise specificity. Quantitative accuracy may also be influenced by antibody variability and matrix interferences. As a result, positive findings obtained through immunoassay screening are often confirmed using chromatographic techniques.

Advanced and Novel Analytical Approaches[43-49]
The rapid expansion of pharmaceutical innovation, personalized medicine, and regulatory expectations has driven the transformation of bioanalysis from conventional chromatographic systems to highly sophisticated, sensitive, and miniaturized analytical platforms. Modern drug estimation in biological samples demands ultra-trace detection, superior selectivity, high throughput, and minimal matrix interference. Advanced hyphenated techniques, sensor-based platforms, nanotechnology-assisted detection, and green analytical strategies now constitute the core of contemporary bioanalytical science. These approaches collectively enhance sensitivity, reduce sample volume, improve analytical speed, and enable decentralized testing environments.

LC–MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)
Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is widely regarded as the gold standard in bioanalysis due to its exceptional sensitivity, selectivity, and robustness. The technique integrates chromatographic separation with mass-based detection, allowing precise quantification of drugs and metabolites even in highly complex biological matrices. The use of multiple reaction monitoring (MRM) enhances analytical specificity by monitoring predefined precursor-to-product ion transitions, thereby minimizing matrix interference and improving signal-to-noise ratio.

LC–MS/MS routinely achieves quantification in the nanogram per milliliter to picogram per milliliter range, making it suitable for pharmacokinetic profiling, bioequivalence studies, and therapeutic drug monitoring. The method also supports multiplex analysis, enabling simultaneous estimation of multiple analytes within a single run. Despite high capital and maintenance costs, LC–MS/MS remains indispensable in regulated bioanalytical laboratories due to its compliance with stringent validation guidelines and superior analytical performance.

UHPLC–MS/MS
Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) represents an advancement over conventional LC systems. By employing sub-2 µm particle columns and higher operating pressures, UHPLC significantly enhances chromatographic resolution and reduces run time. This results in sharper peak shapes, improved sensitivity, and increased throughput.

The integration of UHPLC with MS/MS detection facilitates rapid bioanalysis without compromising accuracy or reproducibility. Reduced solvent consumption and shorter analysis cycles align with both economic and environmental considerations. UHPLC–MS/MS is particularly advantageous in high-throughput clinical trials and large-scale pharmacokinetic studies where efficiency and precision are critical.

High-Resolution Mass Spectrometry (HRMS)
High-resolution mass spectrometry (HRMS) offers accurate mass measurement with superior resolving power, enabling precise identification of analytes and metabolites. Instrument platforms such as Orbitrap and quadrupole time-of-flight (Q-TOF) systems provide exact mass determination and enhanced structural elucidation capabilities.

HRMS is especially valuable in metabolite profiling, impurity characterization, and untargeted screening. Its ability to differentiate compounds with minimal mass differences improves confidence in qualitative and quantitative analysis. Although data processing and instrumentation complexity may be higher compared to triple quadrupole systems, HRMS plays a critical role in advanced pharmacokinetic research and comprehensive drug metabolism studies.

Capillary Electrophoresis–Mass Spectrometry (CE–MS)
Capillary electrophoresis coupled with mass spectrometry (CE–MS) offers efficient separation based on charge-to-size ratio under an applied electric field. The technique requires very small sample volumes and minimal reagent consumption, making it suitable for limited biological specimens. CE–MS demonstrates high separation efficiency and is particularly effective for polar, ionic, and

small molecular weight compounds. However, challenges related to interface stability and reproducibility have limited its widespread adoption. Despite these limitations, CE–MS remains promising for specialized bioanalytical applications and metabolomic investigations.

Supercritical Fluid Chromatography–Mass Spectrometry (SFC–MS)

Supercritical fluid chromatography (SFC) employs supercritical carbon dioxide as the primary mobile phase, often modified with organic solvents to enhance solubility. When coupled with mass spectrometric detection, SFC–MS provides rapid separation, reduced solvent usage, and improved efficiency for non-polar and moderately polar compounds.

SFC–MS combines the advantages of both gas and liquid chromatography, offering faster analysis and environmentally favorable operation. Its growing application in chiral separations and lipidomics highlights its potential in advanced drug analysis and metabolite characterization.

Biosensors for Drug Estimation

Biosensor-based detection has emerged as an innovative approach for rapid and on-site drug estimation. These devices rely on specific biological recognition elements such as enzymes, antibodies, or aptamers integrated with transducers to generate measurable signals.

Electrochemical biosensors convert biochemical interactions into electrical signals and are widely appreciated for their portability, sensitivity, and low cost. Optical biosensors, including fluorescence-based systems and surface plasmon resonance (SPR) platforms, enable real-time, label-free detection with high specificity. Paper-based analytical devices further expand accessibility by offering inexpensive, disposable, and user-friendly diagnostic tools.

Although biosensors may not always match the sensitivity of LC–MS/MS systems, their suitability for point-of-care testing and decentralized monitoring makes them increasingly important in therapeutic drug monitoring and rapid screening.

Nanotechnology-Based Detection

Nanomaterials have revolutionized bioanalysis by enhancing signal amplification and improving analyte capture efficiency. Gold nanoparticles are widely used for colorimetric and electrochemical detection due to their unique optical properties and biocompatibility. Quantum dots provide high fluorescence intensity and stability, enabling sensitive optical detection. Carbon nanotubes enhance electron transfer and increase electrode surface area in electrochemical sensing platforms.

Nanotechnology-based detection systems offer improved sensitivity, reduced detection limits, and innovative sensing mechanisms. However, reproducibility, stability, and large-scale standardization remain areas requiring further development.

Microfluidics and Lab-on-a-Chip Systems

Microfluidic platforms integrate sample preparation, separation, and detection within miniaturized devices, significantly reducing sample volume and reagent consumption. Lab-on-a-chip systems enable rapid bioanalysis and support automation, making them suitable for point-of-care applications.

These platforms enhance analytical efficiency while minimizing waste generation. Their portability and integration potential with biosensors or mass spectrometric detection create opportunities for decentralized therapeutic monitoring. Challenges related to fabrication complexity and device standardization are gradually being addressed through technological advancements.

Dried Blood Spot (DBS) and Volumetric Absorptive Microsampling (VAMS)

Miniaturized sampling strategies such as dried blood spots (DBS) and volumetric absorptive microsampling (VAMS) have gained significant attention in clinical bioanalysis. These approaches require minimal blood volume and simplify storage and transportation without stringent cold-chain requirements.

While DBS may be influenced by hematocrit variability, VAMS devices provide more consistent volumetric sampling and improved quantitative reliability. When coupled with LC–MS/MS detection, these techniques support decentralized clinical trials, pediatric pharmacokinetics, and remote patient monitoring.

Green Bioanalytical Methods[46-52]

The growing emphasis on environmental sustainability has encouraged the development of green bioanalytical strategies. These approaches focus on reducing solvent consumption, minimizing hazardous waste, and adopting energy-efficient instrumentation. Miniaturized extraction techniques, such as microextraction methods and reduced-scale chromatography, contribute to sustainable laboratory practices.

Green bioanalysis does not compromise analytical performance; instead, it aligns technological

innovation with environmental responsibility. As regulatory and institutional policies increasingly emphasize sustainability, eco-friendly analytical methods are expected to become integral to future bioanalytical research.

Novel Sample Preparation Techniques[53-56]

Sample preparation represents one of the most decisive steps in bioanalytical workflows, as it directly influences sensitivity, accuracy, reproducibility, and matrix interference control. Biological matrices such as plasma, urine, and tissue homogenates contain proteins, phospholipids, salts, and endogenous metabolites that can compromise chromatographic separation and mass spectrometric detection. Efficient extraction and cleanup are therefore essential to minimize matrix effects, enhance analyte recovery, and prolong instrument performance. In recent years, emphasis has shifted toward miniaturized, solvent-efficient, and high-throughput extraction strategies that align with both analytical rigor and green chemistry principles.

Solid Phase Extraction (SPE)

Solid phase extraction (SPE) is one of the most widely adopted sample preparation techniques in regulated bioanalysis. It involves retention of analytes on a solid sorbent packed in cartridges or plates, followed by selective washing and elution. SPE provides effective matrix cleanup, high reproducibility, and improved analyte concentration, making it particularly suitable for plasma and serum samples prior to LC–MS/MS analysis. The technique reduces matrix effects such as ion suppression and enhances method robustness. However, SPE can be relatively time-consuming and may require moderate solvent consumption. Automation using 96-well plate formats has significantly improved throughput and reproducibility in clinical laboratories.

Solid Phase Microextraction (SPME)

Solid phase microextraction (SPME) is a solvent-free extraction technique that employs a coated fiber to adsorb analytes directly from biological matrices. After equilibrium, the analytes are desorbed into a chromatographic system for analysis. SPME integrates sampling, extraction, and concentration into a single step, minimizing solvent usage and sample handling. It is particularly advantageous for volatile and semi-volatile compounds and for applications requiring minimal sample volume. Although equilibrium-based extraction may limit absolute recovery, SPME offers simplicity, environmental sustainability, and compatibility with both GC and LC systems.

Microextraction by Packed Sorbent (MEPS)

Microextraction by packed sorbent (MEPS) is a miniaturized adaptation of SPE in which a small amount of sorbent is integrated within a syringe or pipette tip. This configuration enables repeated extraction cycles using very small sample volumes, often less than 100 µL. MEPS reduces solvent consumption and allows direct coupling with chromatographic systems. It is particularly suitable for pediatric and microsampling studies where specimen availability is limited. While sorbent capacity is lower compared to conventional SPE, MEPS offers rapid processing and cost-effective operation.

QuEChERS

The QuEChERS method, originally developed for pesticide residue analysis, has been adapted for drug estimation in biological matrices. The technique involves salting-out liquid–liquid extraction followed by dispersive solid phase cleanup. It is characterized by simplicity, rapid execution, and reduced solvent use. QuEChERS is particularly effective for multi-residue analysis and high-throughput workflows. However, additional optimization may be required to achieve adequate selectivity in highly complex biological matrices. Its adaptability and efficiency make it a promising alternative for routine screening and large-scale studies.

Liquid–Liquid Microextraction (LLME)

Liquid–liquid microextraction (LLME) is a miniaturized version of traditional liquid–liquid extraction. It utilizes small volumes of extraction solvent to isolate analytes based on differential solubility between immiscible phases. Variants such as dispersive liquid–liquid microextraction (DLLME) enhance surface contact between phases, improving extraction efficiency and reducing equilibrium time. LLME offers high enrichment factors and reduced solvent consumption compared to classical extraction methods. Nevertheless, careful optimization of solvent type and extraction conditions is essential to ensure reproducibility and minimize analyte loss.

Magnetic Nanoparticle Extraction

Magnetic nanoparticle-based extraction represents a recent advancement in sample preparation technology. Functionalized magnetic nanoparticles selectively bind target analytes from biological matrices. Application of an external magnetic field enables rapid separation of analyte-bound particles without centrifugation or filtration. This technique provides high surface area for adsorption, rapid processing, and compatibility with automation. Magnetic extraction enhances sensitivity and reduces processing time while minimizing solvent usage. Challenges remain in terms of nanoparticle stability, reproducibility, and large-scale

standardization, but the approach demonstrates strong potential for future bioanalytical applications.

Table 2: Comparison of Modern Sample Preparation Techniques

Technique	Sample Volume	Solvent Use	Sensitivity	Application
Solid Phase Extraction (SPE)	Moderate (0.5–2 mL)	Moderate	High	Pharmacokinetics, therapeutic drug monitoring
Solid Phase Microextraction (SPME)	Very low	Minimal/solvent-free	Moderate to High	Volatile drugs, environmental and forensic studies
Microextraction by Packed Sorbent (MEPS)	Very low (<100 µL)	Low	High	Microsampling, pediatric PK studies
QuEChERS	Moderate	Low	Moderate to High	Multi-residue screening, high-throughput analysis
Liquid–Liquid Microextraction (LLME)	Low	Low	High (with enrichment)	Trace-level drug estimation
Magnetic Nanoparticle Extraction	Very low to moderate	Minimal	Very High	Advanced LC–MS/MS bioanalysis, selective extraction

Modern sample preparation strategies emphasize miniaturization, reduced solvent consumption, enhanced selectivity, and compatibility with high-sensitivity detection systems. The evolution from conventional extraction methods toward microextraction and nanotechnology-assisted approaches reflects the growing demand for efficient, sustainable, and high-performance bioanalytical workflows.

Comparative studies of the Evolution of Drug Estimation Techniques[52-59]

The progression of drug estimation methodologies reflects a clear transition from simple spectroscopic measurements to highly sophisticated, integrated analytical platforms capable of ultra-trace detection and real-time monitoring. Early techniques primarily focused on qualitative or semi-quantitative estimation with limited sensitivity and significant matrix interference. With the advent of chromatographic separation and detector advancements, analytical performance improved substantially in terms of accuracy and reproducibility. The introduction of hyphenated systems such as LC–MS/MS marked a paradigm shift, enabling highly selective and sensitive quantification in complex biological matrices.

Recent decades have witnessed the emergence of high-resolution mass spectrometry, microfluidics, nanotechnology-assisted detection, and biosensor-based platforms, emphasizing rapid analysis, minimal sample consumption, and decentralized testing. In parallel, green analytical chemistry principles have driven efforts toward solvent reduction and sustainable laboratory practices. The ongoing integration of automation, artificial intelligence, and miniaturized instrumentation suggests that future drug estimation technologies will be increasingly portable, environmentally responsible, and capable of real-time therapeutic monitoring.

Table 3: Evolution of Drug Estimation Techniques

Era	Technique	Sensitivity	Speed	Sample Volume	Future Potential
1960s–1980s	UV–Visible Spectrophotometry, TLC	µg/mL range	Moderate	High	Limited; mainly educational and routine QC applications
1980s–1990s	HPLC, GC	ng/mL range	Moderate	Moderate to high	Continued use with improved detectors
1990s–2000s	HPLC with fluorescence/advanced detectors, Immunoassays	ng/mL to low ng/mL	Faster	Moderate	Clinical screening and TDM expansion
2000s–2010s	LC–MS/MS	ng/mL to pg/mL	High	Low to moderate	Established gold standard for regulated bioanalysis
2010s–Present	UHPLC–MS/MS, HRMS (Orbitrap, Q-TOF)	pg/mL and sub-pg/mL	Very high	Low	Advanced metabolomics, precision medicine
Emerging Era	Biosensors, Nanotechnology-based detection, Microfluidics, Lab-on-a-Chip	pg/mL (application dependent)	Rapid/Real-time	Very low (microscale)	Point-of-care testing, wearable monitoring
Sustainable Era	Green bioanalytical methods, Microextraction techniques	Comparable to advanced systems	High	Minimal	Eco-friendly, automated, AI-integrated platforms

Applications of Advanced Drug Estimation in Biological Samples[48-66]

Accurate and sensitive drug estimation in biological matrices underpins multiple domains of pharmaceutical sciences, clinical medicine, and regulatory decision-making. The integration of advanced analytical technologies such as LC–MS/MS, high-resolution mass spectrometry, and biosensor-based platforms has expanded the scope of bioanalysis beyond routine quantification to comprehensive exposure assessment, safety evaluation, and legal enforcement. These applications collectively highlight the translational importance of modern bioanalytical methodologies.

Pharmacokinetic Studies

Pharmacokinetic studies rely fundamentally on precise quantification of drugs and their metabolites in biological fluids over time. Concentration–time data enable calculation of critical parameters such as maximum concentration (C_max), time to reach peak concentration (T_max), area under the curve (AUC), half-life, clearance, and volume of distribution. Advanced analytical platforms allow detection of drugs at trace levels, facilitating characterization of absorption, distribution, metabolism, and excretion even in early-phase clinical trials. High sensitivity is particularly essential for drugs administered at low doses, biologics with complex metabolism, and pediatric pharmacokinetic studies requiring minimal sample volume. Reliable bioanalysis ensures accurate dose optimization and supports regulatory submissions for new drug approval.

Bioequivalence Studies

Bioequivalence studies compare the rate and extent of drug absorption between test and reference formulations. Quantitative drug estimation in plasma is central to demonstrating equivalence in systemic exposure parameters such as AUC and C_max. Regulatory agencies require highly validated analytical methods with defined sensitivity, precision, and reproducibility. LC–MS/MS has become the preferred technique due to its ability to detect low concentrations with minimal interference. Robust bioanalytical performance reduces variability and enhances statistical confidence in equivalence assessment, thereby accelerating generic drug development and approval.

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) involves measurement of drug concentrations to maintain levels within a defined therapeutic range, particularly for medications with narrow safety margins. Drugs such as antiepileptics, immunosuppressants, anticancer agents, and certain antibiotics require individualized dosing based on measured plasma levels. Advanced analytical techniques provide rapid and accurate quantification, enabling clinicians to adjust dosage regimens and prevent toxicity or subtherapeutic exposure. Emerging biosensor and point-of-care technologies further support real-time monitoring, enhancing patient compliance and personalized medicine approaches.

Clinical Toxicology

In clinical toxicology, rapid detection and quantification of drugs, poisons, and metabolites are essential for diagnosis and management of overdose or poisoning cases. High-resolution mass spectrometry enables both targeted and untargeted screening, allowing identification of unknown or emerging substances. Sensitive analytical methods facilitate detection of drugs at toxicologically relevant concentrations, even in complex biological matrices. Accurate bioanalysis guides therapeutic interventions, supports medico-legal documentation, and contributes to pharmacovigilance activities.

Forensic Drug Analysis

Forensic applications demand highly specific and legally defensible analytical data. Drug estimation in blood, urine, hair, or other matrices assists in determining intoxication, substance abuse, and cause of death. Advanced chromatographic and mass spectrometric systems provide confirmatory evidence with high analytical confidence. The ability to detect trace amounts and differentiate between parent drugs and metabolites strengthens the reliability of forensic conclusions. Chain-of-custody procedures and validated analytical protocols are critical to ensuring admissibility in legal proceedings.

Anti-Doping Analysis

Anti-doping analysis focuses on detection of prohibited substances and performance-enhancing drugs in athletes. Sensitive bioanalytical methods are required to identify minute quantities of anabolic agents, stimulants, peptide hormones, and novel designer drugs. The World Anti-Doping Agency plays a pivotal role in establishing standardized testing protocols, updating prohibited substance lists, and promoting research into advanced detection technologies. Through accredited laboratories worldwide, the agency supports the development of high-resolution mass spectrometric methods, biological passport programs, and long-term metabolite detection strategies. Continuous advancements in analytical sensitivity and metabolite profiling are essential to counteract emerging doping strategies and ensure fairness in competitive sports.[61-66]

Overall, the applications of advanced drug estimation techniques extend across pharmaceutical development, clinical practice, toxicology, forensic science, and sports regulation. The ongoing refinement of analytical sensitivity, speed, and selectivity continues to strengthen the reliability of data used in therapeutic decision-making, regulatory approval, and legal enforcement.

FUTURE SCOPE

The estimation of drugs in biological samples is expected to advance significantly with the development of more sensitive, rapid, and efficient analytical techniques. Future bioanalytical methods will increasingly focus on miniaturized and automated systems capable of analyzing very small sample volumes with high accuracy. Technologies such as microfluidic devices and lab-on-a-chip platforms are anticipated to simplify sample handling and enable faster analysis, particularly in clinical and point-of-care settings.

Biosensor technology is also likely to expand in the coming years, offering rapid and selective detection of drugs directly in biological fluids. The integration of nanomaterials, including nanoparticles and carbon-based materials, may further improve analytical sensitivity and signal detection. These developments could support real-time monitoring of drug levels and contribute to improved therapeutic management.

In addition, the application of artificial intelligence and advanced data processing tools may assist in improving analytical efficiency and interpretation of complex bioanalytical data. At the same time, increasing attention will be given to environmentally sustainable approaches through the adoption of green analytical chemistry, reduced solvent consumption, and miniaturized extraction techniques.

Overall, continued progress in analytical instrumentation, sample preparation strategies, and digital technologies will enhance the reliability and efficiency of drug estimation methods. These advancements will support pharmaceutical research, therapeutic drug monitoring, and clinical decision-making while promoting more sustainable bioanalytical practices.

CONCLUSION:

The field of drug estimation in biological samples has undergone a substantial transformation from conventional spectroscopic and chromatographic methods to highly advanced, integrated analytical platforms. Modern bioanalysis demands exceptional sensitivity, specificity, and throughput to support pharmacokinetic evaluation, bioequivalence assessment, clinical toxicology, forensic investigations, and anti-doping surveillance. Techniques such as LC–MS/MS and high-resolution mass spectrometry have significantly improved detection capability, while innovations in microextraction, nanotechnology-based sensing, and microsampling have enhanced efficiency and minimized sample and solvent requirements. The incorporation of green analytical principles reflects a growing commitment toward sustainable laboratory practices.

Future advancements are expected to focus on miniaturized instrumentation, real-time monitoring systems, wearable biosensors, and artificial intelligence-assisted data processing to enable personalized therapeutic management and decentralized testing. Continued integration of technological innovation with regulatory compliance and environmental responsibility will define the next generation of bioanalytical science, ensuring precise, rapid, and sustainable drug estimation in increasingly complex biological matrices.

FUNDING:

Nil

ACKNOWLEDGMENT:

The author gratefully acknowledges the Department of Pharmaceutical Sciences, Jaipur National University, Jaipur, for providing the necessary facilities and support to carry out this review work successfully.

AUTHORS CONTRIBUTIONS:

All authors have contributed equally.

CONFLICTS OF INTERESTS:

All authors have declared no conflict of interest.

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