



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Article

Modern Chemistry Approaches Enhancing Blood Sample Preparation for Pharmaceutical Analysis

Pendyala Meghana*, Bobbili Sharanya, Moluguri Sandhya Rani

Department of Pharmaceutical Chemistry, University College of Technology, Osmania University, Hyderabad, Telangana, India-500007.

ARTICLE INFO

Published: 23 Nov 2025

Keywords:

Blood Sample Preparation, Paper Microfluidics (μ PADs), Microextraction Techniques, Green Extraction Techniques, Automation, Miniaturisation, Mass Spectrometry (MS), HPLC NMR

DOI:

10.5281/zenodo.17688342

ABSTRACT

This review explores the latest advancements in blood sample preparation, emphasizing the growing shift towards methods that are smaller in scale, automated [1], and eco-friendly. These innovations aim to overcome key challenges, such as the complexity of blood as a biological sample [1] and the difficulty of detecting substances present in very low amounts [7]. One major breakthrough is the use of paper-based microfluidic devices (μ PADs), which offer fast and portable point-of-care testing, which is especially valuable in areas with limited medical resources [1]. Another important development is the adoption of finger-prick capillary blood collection method [3]. This method is less invasive and has proven particularly useful in treatments such as radioiodine therapy [7], where it helps lower radiation exposure for healthcare workers. Significant progress in microextraction techniques, such as solid-phase microextraction (SPME), microextraction by packed sorbent (MEPS), and stir bar sorptive extraction (SBSE), alongside advanced solid-phase extraction (SPE) approaches, such as dispersive SPE (dSPE) and online SPE [7], have greatly improved the selectivity, sensitivity, and throughput of bioanalysis [5]. These methods, often coupled with high-performance liquid chromatography (HPLC) and mass spectrometry (MS), are vital for diverse applications in clinical diagnostics, metabolomics, and forensic analysis. However, achieving accurate, reliable, and comparable results necessitates rigorous standardization and meticulous control over preanalytical variables, including patient-specific factors, sample handling, processing, and storage conditions [1].

INTRODUCTION

Blood is an essential biological fluid composed of red and white blood cells, platelets, and plasma, and is extensively used for the diagnosis,

***Corresponding Author:** Pendyala Meghana

Address: Department of Pharmaceutical Chemistry, University College of Technology, Osmania University, Hyderabad, Telangana, India-500007.

Email ✉: meghanap1901@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



monitoring, and management of various diseases. However, directly analysing blood samples poses considerable challenges because of their complex matrix and the generally low levels of target analytes present.^[3] Therefore, sample preparation plays a crucial role in isolating, purifying, and concentrating the desired analytes while simultaneously minimizing unwanted matrix effects^[4]. Conventional sample preparation methods, such as liquid-liquid extraction (LLE), protein precipitation (PP), and solid-phase extraction (SPE), have certain drawbacks, such as excessive solvent use, low selectivity, and challenges in automation^[10]. Over the past decade, research has shifted toward greener, more compact, and efficient methods^[14]. Innovative approaches, such as microextraction techniques (e.g., SPME and MEPS), microfluidic systems (including lab-on-a-chip and droplet-based technologies), and direct or online processing methods, have emerged. These techniques offer clear benefits, including improved sensitivity, faster processing times, lower environmental impact, and better suitability for clinical use.

Key innovations contributing to this shift include:

- **Advanced Microextraction Techniques:** Significant progress has been made in methods such as solid-phase microextraction (SPME) and microextraction by packed sorbent (MEPS)^[15]. Additionally, stir bar sorptive extraction (SBSE) has been developed and applied for determining substances like polycyclic aromatic hydrocarbons in aqueous samples. These techniques, along with advanced solid-phase extraction (SPE) approaches like dispersive SPE (dSPE) and online SPE, have greatly improved the selectivity, sensitivity, and throughput of bioanalysis^[10]. These techniques are

frequently integrated with high-performance liquid chromatography (HPLC) and mass spectrometry (MS) to further improve their analytical performance and capabilities.^[11]

- **Microfluidic Systems, particularly Paper-Based Microfluidic Devices (μPADs):** These devices represent a major breakthrough, offering fast and portable point-of-care testing. They are especially valuable in areas with limited medical resources due to their low cost and simplicity^[1]. Recent advancements in paper microfluidics for blood analysis and diagnostics are being explored. μPADs are also capable of high-throughput screening. Examples include electrochemical microfluidic paper-based apt sensor platforms based on a biotin-streptavidin system for label-free detection of biomarkers^[12], patterned paper platforms for inexpensive, low-volume bioassays, and printed graphene-based electrochemical sensors integrated with paper microfluidics for rapid detection, such as lidocaine in blood. Combined methods like paper centrifugation–SERS immunoassay have also been developed for integration of blood separation and multiple-cytokine detection
- **Blood Micro-sampling:** The adoption of the finger-prick capillary blood collection method is a crucial development. This method is less invasive and has proven particularly useful in treatments such as radioiodine therapy^[5], where it helps to lower radiation exposure for healthcare workers by reducing the need for direct venous blood draws^[6]. Patterned dried blood spot (DBS) cards have also been introduced to enhance the accuracy and efficiency of whole blood sampling.

These innovative approaches offer clear benefits, including improved sensitivity, faster processing



times, and better suitability for clinical use ^[11], making them vital for diverse applications in clinical diagnostics and metabolomics ^[4]. For instance, sample selection, collection, and preparation are crucial for NMR-based metabolomics studies of blood. However, it is paramount to achieve accurate, reliable, and comparable results, which necessitates rigorous standardisation and meticulous control over preanalytical variables ^[3]. These variables include patient-specific factors, sample handling, processing, and storage conditions. Predicting and modeling pre-analytical sampling errors can also lead to improved accuracy and reliability in metabolomics data.

Purpose of Blood Sample Preparation:

Blood sample preparation is indeed the first and crucial step in the analysis of blood, ensuring the sample is in an optimal state for accurate testing. This process is essential due to the complex nature of blood and the often low concentrations of target analytes, which necessitate careful handling to achieve reliable and comparable results ^[4].

➤ **Removal of Interfering Substances**

- Blood contains various components (e.g., proteins, lipids, and salts) that can interfere with the detection of specific target analytes ^[1].
- These were removed to avoid false positives/negatives in the test results.
- For example, hemoglobin in lysed red blood cells can interfere with spectrophotometric analysis ^[1].

➤ **Concentration of Analytes**

- Some target compounds (e.g., drugs and biomarkers) are present at very low concentrations ^[1].

- Techniques such as solid-phase extraction or centrifugation are used to concentrate them, improving detection sensitivity ^[15].

➤ **Stabilization of Biomolecules**

- Biomolecules, such as proteins, DNA, RNA, and metabolites, are unstable and can degrade rapidly ^[3].
- Stabilization agents or temperature control are used to preserve them during processing and storage.

➤ **Compatibility with Analytical Instruments**

- Instruments such as LC-MS, GC-MS, and ELISA require samples in specific formats (e.g., free of particulates and specific solvents) ^[12].
- Sample preparation ensures that the sample meets the specific requirements of the analytical instrument, such as appropriate pH and viscosity.

➤ **Separation of Components**

- Blood contains plasma, cells, and other components.
- Techniques such as centrifugation help separate plasma/serum for targeted analysis.

➤ **Standardization for Analysis**

- Standardization ensures uniform processing of each sample.
- This improves reproducibility and allows for meaningful comparisons across multiple tests or with patients ^[3].

Purpose of the Blood Sample Analysis:



Once the sample is prepared, it undergoes analytical processes to extract useful clinical information.

➤ **Diagnosis of Disease**

- Key biomarkers (e.g., glucose, cholesterol, liver enzymes, and cancer antigens) are quantified to detect diseases.
- Example: High HbA1c = diabetes; Elevated CRP = inflammation.

➤ **Monitoring Health Status**

- Routine blood tests (such as CBC or metabolic panels) are used to monitor general health.
- It helps track organ function, immune response, and detect early signs of illness.

➤ **Therapeutic Drug Monitoring (TDM)**

- Monitors plasma drug concentrations to ensure therapeutic efficacy while avoiding toxicity by maintaining levels within the therapeutic window.
- Essential for drugs with narrow therapeutic windows (e.g., lithium, vancomycin, and warfarin).

➤ **Biomarker Identification**

- Biomarkers are measurable molecules that reflect a specific biological state, condition, or disease process in the body.
- Identifying these factors supports early disease detection, accurate prognosis, and personalized treatment strategies.

➤ **Monitoring Treatment Response**

- Blood tests indicate how well a patient responds to treatment.
- Example: decreases in cancer markers, such as CA-125, post-chemotherapy, indicates treatment success.

Combined Significance:

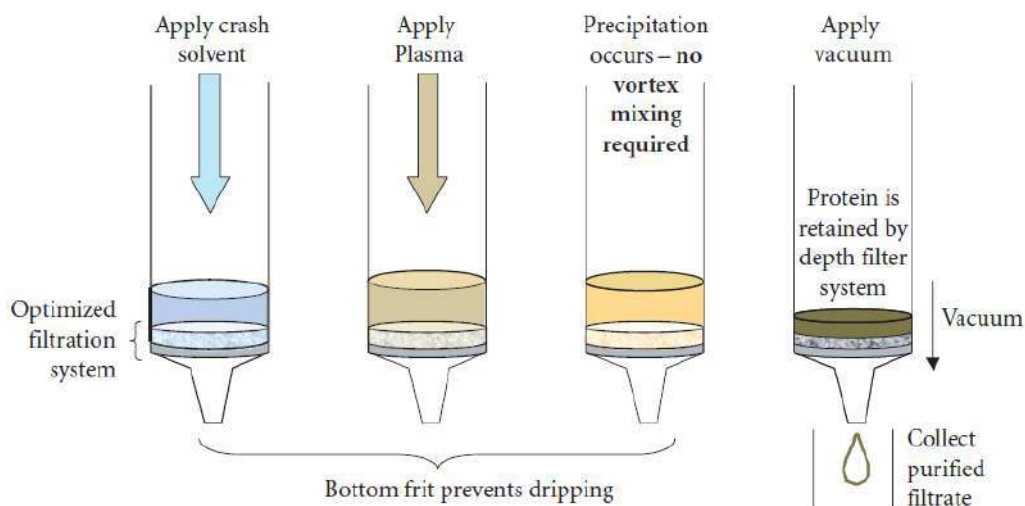
- Proper sample preparation ensures accurate and meaningful analyses.
- Together, these support diagnostics, personalized medicine, and healthcare decisions.

Traditional methods:

Traditionally, sample preparation in bioanalysis has relied on methods such as liquid-liquid extraction (LLE), protein precipitation (PP), solid-phase extraction (SPE), and simpler approaches such as direct dilution and injection (often called “dilute and shoot”). These techniques are essential for preparing blood samples, especially because blood is a complex fluid that typically contains only trace amounts of the analytes being measured. Proper preparation helps isolate, purify, and concentrate the target analytes while minimizing interference from other components, which even advanced analytical instruments can struggle with when dealing directly with unprocessed blood ^[14].

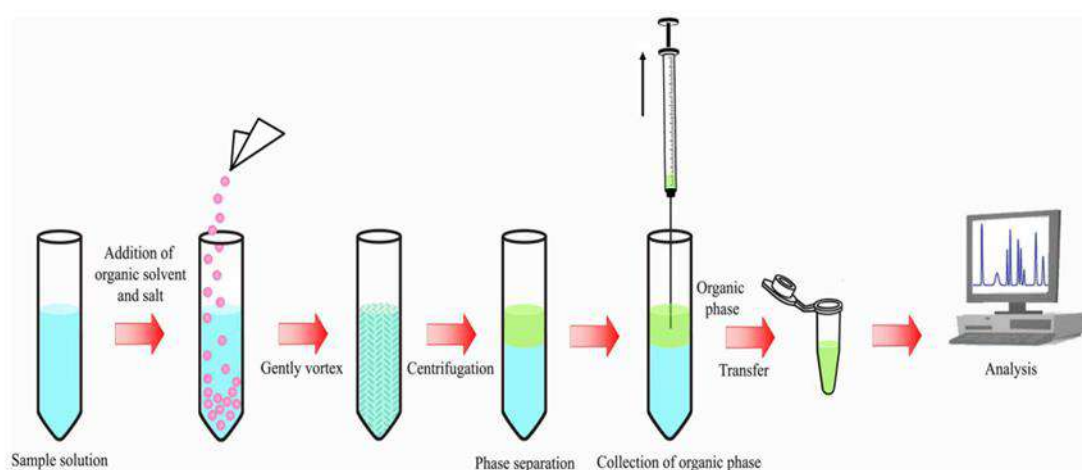
1. Protein Precipitation (PP): Protein Precipitation (PP) is one of the oldest and simplest techniques used for the preparation of biological samples. It works by breaking down or "denaturing" the proteins in the sample, usually by adding a strong acid or base, applying heat, or most commonly mixing in an organic solvent like acetonitrile or methanol. While this technique is quite effective at removing proteins, it can become time-

consuming when handling a large number of samples manually.



2. Liquid–liquid Extraction (LLE): This technique involves extracting analytes from a liquid sample into an immiscible organic solvent. Although simple, conventional LLE often requires large sample volumes, can lead to emulsion formation, requires manual handling, and may lack analyte-specific optimization. Salting-out assisted liquid–liquid extraction (SALLE) is an innovative technique that utilizes salts to induce phase

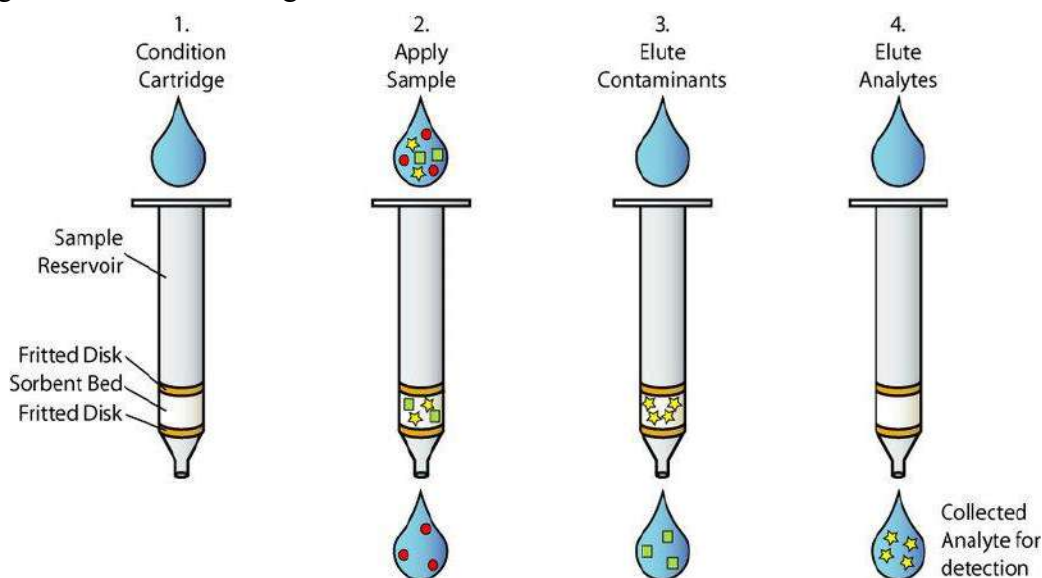
separation and enhance the partitioning of analytes into the extraction solvent. SALLE offers advantages such as applicability to a broad range of drugs, improved recovery, and the potential for automation and cost-effectiveness. However, the non-volatile nature of many salts used in SALLE can interfere with LC-MS/MS analysis, potentially causing ion suppression and contamination.



3. Solid-Phase Extraction (SPE): SPE was introduced to overcome the drawbacks of LLE

and PP. This technique relies on specific interactions between the analyte and sorbent,

aiming to capture and later release the analyte while removing unwanted components and enriching the sample. The goal is to retain and elute the analyte while washing away impurities and concentrating the sample. Although SPE has advantages, it also has



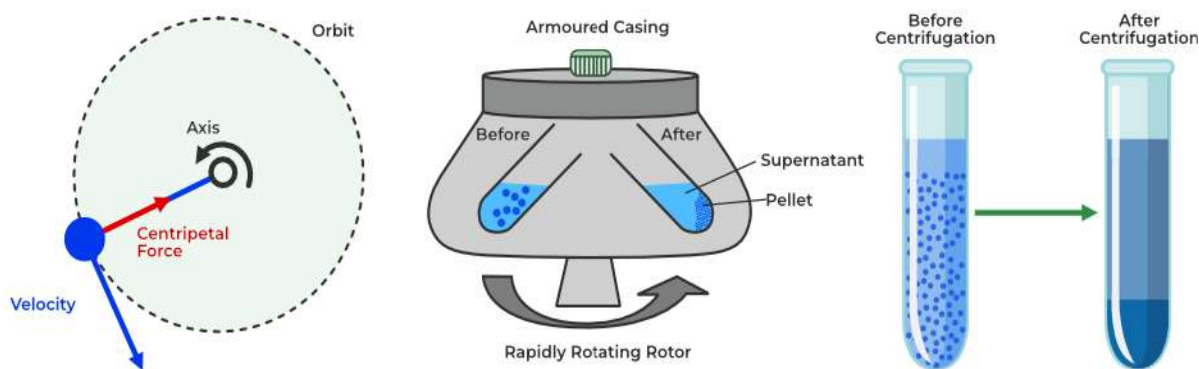
certain limitations. Sensitivity and selectivity can be issues, and co-adsorption of matrix components, especially polar compounds, can lead to matrix effects that affect the accuracy of LC-MS/MS analysis.

- 4. Filtration:** Traditionally, protein precipitation (PP) requires centrifugation to remove proteins; however, a newer development uses membrane-based PP filter plates. This allows filtration to take place in the same well immediately after precipitation, removing the need for separate centrifugation and transferring of the supernatant. These filter plates are commonly available in 96-well format, making them suitable for both manual and automated workflows. Interestingly, filtration has been used for sample processing for decades. For example, in the 1950s, the U.S. The Public Health Service uses carbon filters to extract organic compounds from surface water. Granulated activated carbon was packed into iron cylinders, transported to laboratories, dried, and then used to extract trapped chemicals, which is a foundational approach for modern filtration methods.
- 5. Dilution:** Dilution is a common pre-treatment step in blood sample processing. This method is particularly useful for reducing matrix effects in complex samples like whole blood. It enhances the extraction of important biomolecules such as DNA, RNA, and proteins. Microextraction by Packed Sorbent (MEPS), a miniaturized form of solid-phase extraction, typically incorporates a dilution step with water to enhance extraction efficiency and analyte recovery. Typically, plasma or serum is diluted at a 1:4 ratio, whereas whole blood is diluted at approximately 1:20. This was followed by centrifugation to help clean the sample before extraction.
- 6. Hemolysis:** Hemolysis, the breakdown of red blood cells (RBCs), is a significant issue in clinical research involving plasma or serum samples. When RBCs break open, their contents spill into the surrounding fluid,

significantly altering the levels of various metabolites in the blood. This usually results from mistakes during the pre-analytical phase, such as rough handling, incorrect centrifugation speeds or durations, temperature extremes, or even excessive mixing. These errors can mislead results, as changes in metabolite levels might be mistakenly interpreted as disease-related rather than artifacts of sample mishandling. For instance, studies have shown that hemolysis can reduce the levels of compounds such as acetate, citrate, and phenylalanine while increasing glucose levels. Hemolysis is often visible; mild cases give plasma or serum a pink tint, whereas severe cases appear red. It is best practice to discard visibly hemolyzed samples and report the percentage of such cases in any study, as this reflects the overall quality of sample handling. Moreover, the use of excessively high centrifugation speeds is a known contributor to hemolysis.

7. **Centrifugation:** Centrifugation is a core part of the traditional blood sample preparation. Its main application is the separation of blood components to obtain plasma or serum for subsequent analysis. To obtain serum, blood was first left to clot at room temperature for

approximately 30 min. The sample was then spun in a centrifuge at 1500–2000 RPM for about 10–15 minutes, allowing the serum to separate from the blood clot. In contrast, for plasma, blood is collected in tubes containing anticoagulants (such as EDTA, heparin, or citrate) [3] and should be centrifuged immediately, usually at 1600 RPM for 15 min. To obtain plasma free of platelets, a second spin at a higher speed (e.g., $15,000 \times g$ for 7 min) is often used [4]. Despite being essential, centrifugation has its downsides; it requires relatively large blood volumes, specialized laboratory equipment, and trained personnel, all of which can slow down the process. Any delay between blood collection and centrifugation may lead to degradation or alteration of metabolites, affecting the accuracy of analytical results. For example, glucose levels may drop, and lactate levels may rise due to red blood cell activity. Keeping samples at 4 °C before centrifugation can help preserve their integrity [3]. One concern in this research is that centrifugation details, such as spin speed, duration, and temperature, are often underreported in published studies, pointing to a broader issue of inconsistent laboratory practices and a lack of standardization in the field.



Analytical techniques:

- Over the past decade, major advancements in analytical technologies have transformed the

field of bioanalysis [2]. Instruments like LC-MS/MS are now regarded as the gold standard for both qualitative and quantitative analyses due to their exceptional sensitivity, specificity, and rapid performance. However, even powerful techniques such as LC-MS/MS can face challenges, particularly matrix effects from complex biological samples, which can reduce accuracy and sensitivity.

- Another key tool, Nuclear Magnetic Resonance (NMR), is valued for its high reproducibility, non-destructiveness, and minimal sample preparation. It is also highly compatible with automated systems and enables precise quantification of the results [3]. However, NMR lacks the sensitivity and wide metabolite coverage of mass spectrometry and often requires larger sample amounts.
- As biological samples, such as blood, plasma, and serum, contain many interfering substances (proteins, salts, and lipids), effective sample preparation is essential [3]. This helps isolate the analyte, remove interferences, and enhance detection. As analytical demands increase, sample preparation techniques are evolving to offer greater selectivity, automation, and compatibility with modern instruments.

Blood Sample Preparation:

1. Sample Collection

- Whole Blood is collected via venipuncture.
- Use appropriate tubes:
 - EDTA/Heparin → for plasma
 - Plain (no anticoagulant) → for serum

2. Centrifugation

- **Plasma:** The blood sample should be centrifuged right away at approximately 2,000 RPM for 10 to 15 minutes.
- **Serum:** Allow the blood to clot at room temperature for 30 to 45 minutes prior to centrifugation.

Outcome: Separates cells from plasma/serum, which contains most analytes.

3. Protein Precipitation (Deproteinization)

Used for: LC-MS, HPLC, NMR, UV-Vis

- Add cold organic solvent (e.g., acetonitrile, methanol) in a 3:1 to 5:1 solvent-to-sample ratio.
- Vortex mix and incubate on ice.
- Centrifuge at ~12,000–15,000g for 10 minutes.
- Collect supernatant (contains analytes).

Common solvents: Acetonitrile, methanol, ethanol (sometimes acidified).

4. Extraction / Cleanup

Option A: Liquid-Liquid Extraction (LLE)

Used for: GC-MS, LC-MS

- Add organic solvent (e.g., ethyl acetate, chloroform).
- Shake/mix to allow analytes to partition.
- Centrifuge and separate organic layer.

Option B: Solid-Phase Extraction (SPE)

Used for: HPLC, LC-MS, CE



- Load sample onto preconditioned SPE cartridge (C18, HLB, etc.).

- Wash and elute analytes using appropriate solvents.

5. Derivatization (If needed)

Used for: GC-MS (non-volatile analytes)

- Add derivatizing agents like BSTFA, MSTFA, IBCF to improve volatility/stability.
- Heat or incubate based on agent protocol.

6. Drying / Reconstitution (Optional)

- Evaporate sample under nitrogen or vacuum if required.
- Reconstitute in mobile phase or buffer compatible with analytical technique.

7. Filtration / Ultracentrifugation

Used for: NMR, HPLC, LC-MS

- Use 0.22 or 0.45 μm filters or centrifuge at high speed to remove debris.

8. Final Sample

- Aliquot final supernatant into vials or tubes for injection into:
 - HPLC/LC-MS/GC-MS
 - UV-Vis spectrophotometer
 - FTIR, NMR spectrometer
 - ELISA plate (after dilution)
- Various analytical techniques used to analyse blood samples include:

1. HPLC:

- **Samples:** Plasma or serum
- **Principle:** Separates molecules via interactions with C18 and UV-Vis or MS detection.
- **Solvents:** Acetonitrile, methanol; buffers like acetate, phosphate, formate
- **Examples:** methotrexate via acetonitrile PP + LLE + UV detection.
- **Advantages:** Quantitative, versatile, high-resolution
- **Limitations:** Costly equipment; matrix components may interfere

2. GC-MS:

- **Samples:** Plasma, serum
- **Principle:** Separates volatile or derivatized compounds, followed by mass-based identification
- **Solvents:** Methanol, acetonitrile, chloroform, ethyl acetate; common derivatization using MSTFA, IBCF
- **Examples:** Methanol detection in whole blood via pyran derivatization; GC-TOF-MS with methanol/chloroform for metabolome
- **Advantages:** High sensitivity/specificity (for small volatiles).
- **Limitations:** Requires derivatization; not suited for non-volatiles

3. LC-MS:

- **Samples:** Plasma, serum



- **Principle:** Combines HPLC separation with MS detection via ionization (e.g., ESI)
- **Solvents:** Organic (acetonitrile, methanol), aqueous buffers, acid modifiers; cleanup via SPE or microextraction
- **Examples:** Immunosuppressants, steroids, metabolomics profiling
- **Advantages:** Ultra-high sensitivity; minimal prep; broad analyte range
- **Limitations:** Matrix interference (ion suppression)
- **Examples:** Haemoglobin quantitation; routine liver function tests
- **Advantages:** Fast, inexpensive, easy
- **Limitations:** Applicable only to compounds that absorb UV/Vis light and offers lower specificity compared to more advanced detection methods

4. FT-IR:

- **Samples:** Serum or plasma (dried or liquid)
- **Principle:** Detects molecular vibrational bonds (e.g., –OH, –COOH)
- **Solvents:** None or minimal—all dry or liquid samples
- **Examples:** Functional profiling; disease biomarker screening
- **Advantages:** Rapid, non-destructive, minimal prep
- **Limitations:** Lower quantification accuracy; spectral overlap

5. UV-Visible Spectroscopy

- **Samples:** Plasma, serum
- **Principle:** Measures absorbance at specific wavelengths (e.g., Hb, bilirubin) via Beer–Lambert law
- **Solvents:** Buffer solutions; dilution or filtration

6. Enzyme-linked immunosorbent assay (ELISA):

- **Samples:** Serum or plasma
- **Principle:** Antigen–antibody binding with enzyme-based signal detection
- **Solvents:** Sample buffer dilutions (e.g., PBS + BSA)
- **Examples:** Hormones (insulin), cytokines (IL-6), infectious disease markers (HIV, HBsAg)
- **Advantages:** Highly specific, sensitive, multiplexable
- **Limitations:** Antibody cost; cross-reactivity

7. Capillary Electrophoresis (CE):

- **Samples:** Plasma, serum, sometimes whole blood
- **Principle:** Molecules are separated based on charge and size under high voltage
- **Solvents:** Dilution in buffer; filtration
- **Examples:** Hemoglobin variant separation; peptides, ionic metabolites
- **Advantages:** Fast, high resolution, low sample volume



- **Limitations:** Lower sensitivity than LCMS; requires conductive buffer

8. NMR:

- **Samples:** Plasma or serum with deuterated solvent
- **Principle:** Detects nuclear spin environments (structural/quantitative data)
- **Solvents:** D₂O or deuterated buffer, following protein removal
- **Examples:** Metabolomics, small-molecule structural studies
- **Advantages:** Structural insight; reproducible; non-destructive
- **Limitations:** Low sensitivity; expensive instruments

Upcoming Sample Preparation techniques: In recent years, several advanced and emerging techniques have been developed to address the limitations associated with traditional methods of sample preparation. These include advanced techniques such as microfluidics-based systems, solid-phase microextraction (SPME), magnetic solid-phase extraction (MSPE), fabric phase sorptive extraction (FPSE), and dispersive liquid-liquid microextraction (DLLME), among others [18]. These methods offer advantages such as reduced sample and solvent consumption, shorter processing times, increased automation potential, and improved analyte recovery from complex matrices.

Sorptive extraction techniques:

Sorptive extraction methods is like solid-phase microextraction (SPME) that rely on a sorbent phase to absorb analytes directly from a sample.

These techniques are valued for their ability to handle small volumes, reduce solvent usage, and improve sensitivity, especially for trace analysis in complex matrices.

1. **SPME (Solid Phase Microextraction):** It is a solvent-free technique used to extract chemicals (analytes) from a sample (like blood, water, or air) onto a special fiber which is then tested using instruments like GC-MS or HPLC [1].

It has 3 simple steps:

- **Extraction:** A special fiber coated with a solid material is exposed to the sample (liquid, gas, or solid), the analytes stick (adsorb or absorb) to the coating.
- **Desorption:** The fiber is then placed inside an instrument (like a gas chromatograph), where heat or solvent releases the analytes.
- **Analysis:** The analytes are analyzed by GC, GC-MS, or HPLC.

Advantages of SPME:

- No solvents needed so it is environmentally friendly
- Fast and simple
- Low cost and reusable fibers
- Compatible with GC, GC-MS, HPLC
- Good for volatile and semi-volatile compounds

Limitations:

- Fiber can wear out over time
- Only works for certain types of chemicals



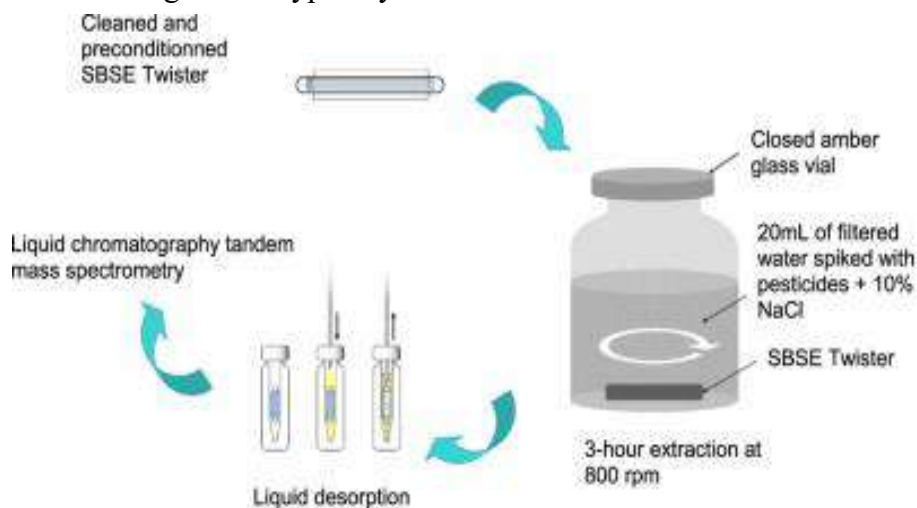
- Not suitable for very large molecules

Common Applications of SPME:

- Environmental - Detect pollutants in water and air
- Food - Analyze aroma compounds and flavors
- Clinical/Pharma- Detect drugs in blood, urine
- Forensics – Used to detect and identify poisons or drugs in biological samples

2. SBSE (Stir Bar Sorptive Extraction): First introduced in 1999, SBSE builds on the principles of SPME but enhances efficiency by using a stir bar coated with a Sorptive material most commonly polydimethylsiloxane (PDMS)^[1] to extract analytes during sample agitation.

Principle: A coated stir bar is placed into the prepared sample and stirred, enabling the analytes to move from the sample matrix into the extraction phase on the stir bar coating. SBSE typically uses



Limitations:

- A notable drawback is that commercially available SBSE stir bars typically only use

50–250 times larger volumes of PDMS compared to SPME fibres, theoretically affording greater extraction efficiency.

Modes of Extraction: Like SPME, SBSE can be performed in two ways:

- **Direct Immersion (DI-SBSE):** The stir bar is submerged directly in the liquid sample.
- **Headspace (HS-SBSE):** The sample is heated to release volatile compounds into the air above it, where the bar collects them.

Optimisation and Desorption:

- **Thermal Desorption (TD):** The bar is inserted into a heated GC injector, releasing the analytes.
- **Liquid Desorption (LD):** A small solvent volume is used to recover the analytes before analysis. Key parameters such as pH, speed of stirring, temperature, and extraction time must be optimized for the best performance.

PDMS, which is suitable mostly for hydrophobic compounds.

- This limits the technique's scope when dealing with polar or charged analytes.

Recent Advancements:

- Custom coatings: Researchers have created in-house stir bars with mixed phases (e.g., PDMS/carbon) to expand the technique's ability to extract polar compounds.
- Sequential SBSE: In cases where sample volume is limited or multiple analytes are present, several stir bars with different coatings or using altered sample conditions can be used in sequence to improve recovery and sensitivity.

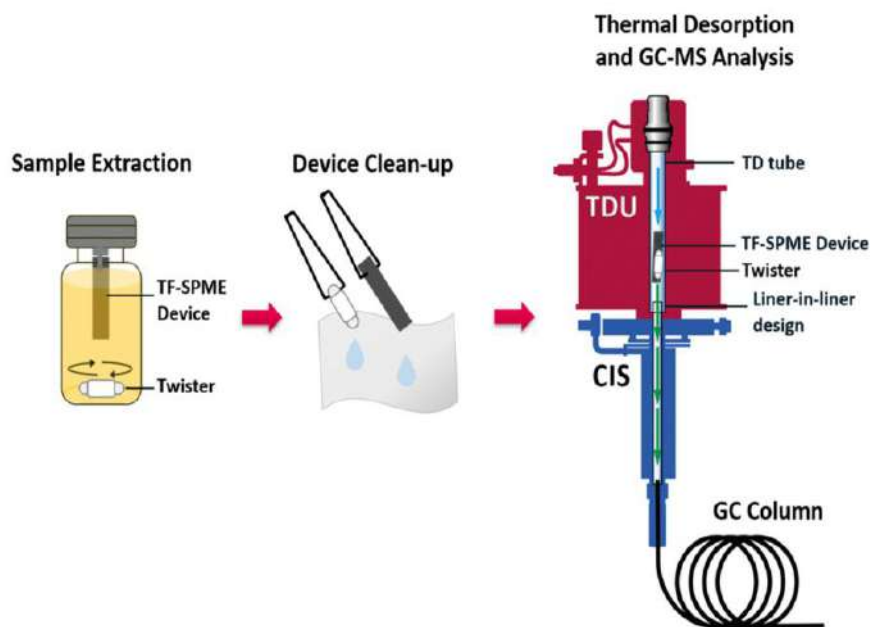
Advantages:

- Excellent extraction efficiency
- Reusable stir bars (after proper cleaning)

- Lower overall costs due to repeatable use
 - Improved sensitivity for trace-level detection
3. **TFME (Thin Film Microextraction):** TFME employs a flat, thin polymeric film rather than a fibre or stir bar. This film provides a **larger surface area**, allowing for faster and more efficient extraction of analytes.

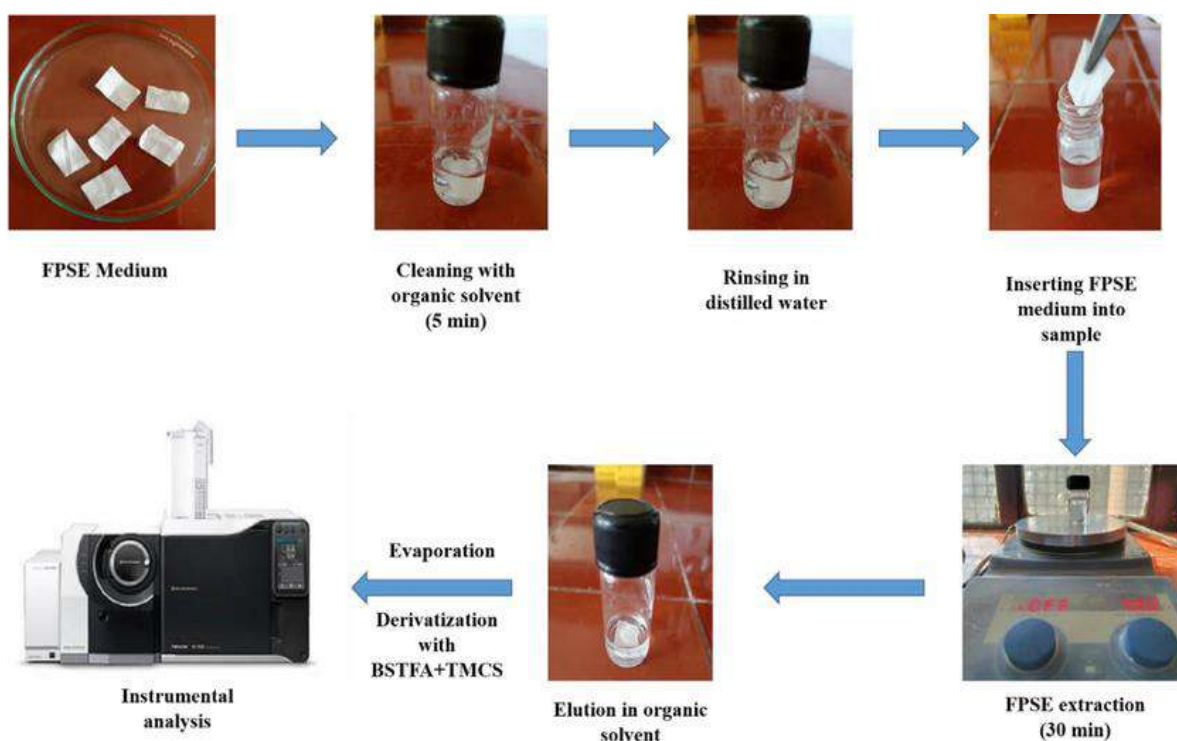
Key Benefits:

- Enhanced surface contact improves extraction rates
- Ideal for high-sensitivity applications
- This technique has proven highly effective for the detection of opioids in postmortem blood analysis



4. **Fabric-Phase Sorptive Extraction (FPSE):** FPSE is a newer, highly flexible method that uses **fabric substrates coated with sol-gel**

sorbent phases. These fabrics can be easily adapted to various sample types and are both reusable and environmentally friendly.



Why FPSE Stands Out:

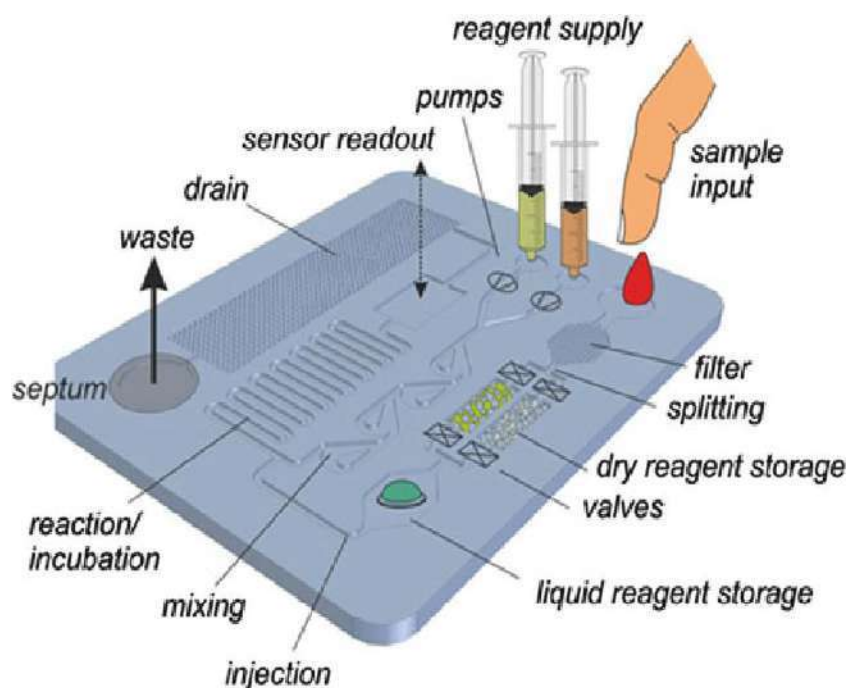
- Combines simplicity and durability
- The flexible fabric can be immersed directly into biological fluids
- Excellent compatibility with LC and GC systems
- Offers strong analyte recoveries from complex matrices like blood and urine

Microfluidic based techniques:

Paper-based microfluidics has revolutionized blood diagnostics by providing a lightweight, affordable, and easy-to-use alternative to conventional lab-based testing methods. These devices, known as microfluidic paper-based analytical devices (μ PADs) ^[1], typically feature hydrophobic barriers defining hydrophilic channels on paper matrix, facilitating capillary flow to distinct functional areas like sampling,

mixing, and detection zones. They are particularly appealing for point-of-care testing (POCT) in resource-limited settings.

The use of paper-based devices in blood diagnostics is generally categorized based on the type of blood sample used and whether any pretreatment steps are needed before analysis. Microfluidics-based techniques represent a cutting-edge approach to sample preparation, focusing on the precise manipulation of fluids at the microscale (typically less than 1000 μ L) through tiny channels or droplets. This miniaturisation enables a variety of sample preparation steps, including separation, mixing, lysis, purification, and detection, all integrated onto a single miniaturised chip or device. At this scale, fluids exhibit unique behaviours due to phenomena like laminar flow, capillary action, and surface tension, allowing for highly efficient control over various blood components such as cells, plasma, proteins, DNA, or drugs.



Advantages of Microfluidics in Blood Analysis:

- Requires only a small amount of blood or reagents
- Portable and compact—ideal for point-of-care testing (POCT)
- Speeds up workflows by integrating multiple steps (e.g., mixing, separation, detection)
- Supports automation and simultaneous multi-analyte analysis (multiplexing)
- Reduces costs and waste, aligning with green chemistry
- Compatible with modern detection tools, including smartphone-based readers

Limitations:

- Device fabrication can be complex
- Channels can clog or foul easily
- Throughput may be limited in some designs

- Results can be sensitive to surface chemistry
- Often needs specialized detection systems

1. Droplet-Based Microfluidics:

Principle: This technique involves the generation and manipulation of discrete microdroplets within immiscible carrier fluids. These individual droplets act as isolated reaction chambers, allowing for parallel reactions or isolations to be performed with tiny volumes.

2. Lab-on-a-Chip (LOC):

Principle: A Lab-on-a-Chip is a miniaturized device that integrates multiple laboratory functions such as sample preparation, reaction, separation, and detection onto a single microchip. For blood analysis, this can include a sequence of steps such as separation, lysis, extraction, and detection, all performed on the compact device. This integration aims to streamline the analytical workflow, offering speed and efficiency.

3. Paper-Based Microfluidics (μPADs):



Paper-based analytical devices (PADs) were first introduced by the Whitesides group in 2007, marking a significant step forward in low-cost, portable diagnostic tools, μ PADs have revolutionized blood diagnostics by offering a low-cost, portable, and easy-to-use alternative to traditional lab tests. They are especially valuable for POCT in resource-limited settings. μ PADs operate on capillary action, where fluids move through hydrophilic channels outlined by hydrophobic barriers on paper. These barriers can be created via techniques like printing, stamping, or photolithography.

Applications and Detection Techniques:

The applications of paper-based devices in blood diagnostics are diverse, covering various blood sample types and pretreatments:

- Electrochemical microfluidic paper-based aptasensor platforms, utilizing a biotin–streptavidin system, have been developed for label-free detection of biomarkers^[13].
- Additionally, printed graphene-based electrochemical sensors integrated with paper microfluidics have been designed for rapid and sensitive detection—such as the quantification of lidocaine in blood samples^[9].
- Recent Advances in Microfluidic Paper-Based Analytical Devices are geared toward High-Throughput Screening, enabling more efficient sample processing^[7].
- Patterned dried blood spot cards have been developed for the improved sampling of whole blood, representing a significant step in optimising blood collection for analysis^[11].
- An innovative approach involves the integration of blood separation and multiple-cytokine detection using a combined paper

centrifugation–SERS immunoassay method, highlighting the capacity for complex multi-step analyses on a single paper-based platform^[10].

Classification and Sample Processing:

A recent trend in the field is to classify μ PAD applications according to the type of blood sample used and whether any pretreatment is needed. This approach helps simplify and optimize both the design and development process.

a. Direct Testing of Serum/Plasma Samples

Plasma and serum, being free from cells, allow for straightforward testing. μ PADs have been used to detect TSAT, meningococcal infections, and Alzheimer's biomarkers using electrochemical platforms capable of multiplexing.

b. Processing and Analysis of Whole Blood Samples

Whole blood often needs pretreatment like dilution or separation:

- Paper-based Plasma Separation: Innovative low-cost centrifuges (e.g., electric yo-yo, hand-powered) have been repurposed to extract serum for biomarker analysis.
- Separation Membranes: LF1 and polycarbonate membranes offer efficient separation for analytes like triglycerides, ferritin, folic acid, vitamin B12, and Hb.
- Membrane-Free Techniques: Techniques using dielectrophoresis or functionalized paper (EDTA, chitosan, hydrogel) enable on-chip separation of plasma and analytes like glucose, cholesterol, and TGs.



- **Intracellular Extraction:** In-situ hemolysis methods allow direct hemoglobin detection. Some μ PADs tolerate cell debris, enabling simplified detection of compounds like glutathione and lidocaine without pretreatment.

c. Direct Whole Blood Analysis on Paper Microfluidics

This approach skips all pretreatment steps, enabling direct testing:

- **Blood Grouping:** Innovative tools like the FLIPPED card utilize QR codes and smartphone-based analysis to provide fast and precise ABO and RhD typing.
- **Hematocrit (Hct) Testing:** Foldable paper designs and blood-fingering pattern recognition allow fast anemia screening with mobile assistance.
- **Other Direct Tests:** μ PADs can directly measure fibrinogen levels via blood wicking distance, and alcohol levels using colorimetric assays with in-situ headspace separation.

4. Digital Microfluidics (DMF)

Principle: Manipulates droplets on a flat surface using electric fields (electrowetting) to move, mix, or split them.

Example: Automated lithium-ion detection in blood using colorimetry, with over 90% plasma separation efficiency in under 5 minutes.

Application:

- DMF platforms have been used for efficient plasma separation from whole blood, achieving over 90% efficiency in a short time

(e.g., 4 minutes), and can be integrated with preloaded

- Paper-based sensors have been developed to detect analytes such as lithium ions (Li^+) using colorimetric techniques.

5. Centrifugal Microfluidics (Lab-on-CD)

Principle: This technique utilises spinning discs (resembling compact discs, hence "Lab-on-CD") to move and separate blood components by harnessing centrifugal force. This principle is applied for tasks such as plasma separation and analyte isolation.

Application:

- Although not always referred to as "Lab-on-CD," the concept is effectively illustrated by low-cost innovations such as electric yo-yo centrifuges and hand-operated devices combined with paper-based microfluidic systems.
- These portable, cost-effective solutions perform serum/plasma separation from whole blood, enhancing decentralised blood testing in resource-poor regions.
- For instance, paper centrifugation technology has been combined with SERS immunoassay for detecting multiple cytokines in blood samples, enhancing signal sensitivity.

Green extraction techniques:

Green extraction techniques aim to reduce the environmental and health impacts of sample preparation by avoiding toxic solvents and enabling faster, cleaner, and more sustainable extraction processes. These techniques often rely on alternative energy sources, green solvents [16], and miniaturised formats [16].



Principle: The core principle behind green extraction techniques is the use of eco-friendly solvents, low-energy input, and minimal waste generation, while simultaneously maintaining or improving extraction efficiency. This is achieved by focusing on alternative energy sources, green solvents, and miniaturised formats.

Advantages: Green extraction techniques offer several significant benefits for sample preparation, especially in bioanalytical applications ^[16]:

- Lower sample and solvent volumes are required.
- They are energy efficient.
- They lead to high extraction efficiency.
- These technologies support the advancement of compact, fully automated platforms for efficient and high-throughput analysis.
- These systems offer sustained economic benefits through reduced operational expenses and improved resource efficiency.

Disadvantages: Despite their numerous advantages, green extraction techniques do have some limitations:

- The initial cost of equipment can be high.
- There may be limited analyte compatibility.
- They may require method development and validation.
- Scalability issues can arise.
- The options for eco-friendly solvents are currently limited and not widely accessible

Specific Green Extraction Techniques and Applications:

1. Microwave-Assisted Extraction (MAE):

- **Solvent Used:** Water, ethanol, or other green solvents.
- **Energy Source:** Microwave energy.
- **Sample Type:** Blood and plasma.
- **Application:** Rapid extraction of metabolites and hormones. Over the past 15–20 years, this technique has attracted considerable attention for its environmentally friendly and efficient extraction capabilities, notably cutting down both extraction time and solvent usage.

2. Supercritical Fluid Extraction (SFE):

- **Solvent Used:** Supercritical carbon dioxide (CO₂), sometimes with co-solvents like ethanol.
- **Energy Source:** Heat and pressure.
- **Sample Type:** Serum and plasma.
- **Application:** Lipid profiling and steroid analysis.

3. Ultrasound-Assisted Extraction (UAE):

- **Solvent Used:** Ethanol, water, or methanol (in low amounts).
- **Energy Source:** Ultrasonic waves.
- **Sample Type:** Whole blood and tissues.
- **Application:** Protein and enzyme extraction. UAE is known for its simplicity, effectiveness, and it offers higher extraction efficiency and faster reaction rates than traditional methods.

4. Pressurised/Accelerated Solvent Extraction (PLE/ASE):



- **Solvent Used:** Ethanol, acetone, or green organic solvents.
- **Energy Source:** Heat and pressure.
- **Sample Type:** Plasma and serum.
- **Application:** Extraction of drugs and bioactive compounds.

5. Deep Eutectic Solvents (DES) Extraction:

- **Solvent Used:** Choline chloride combined with urea or glycerol (Deep Eutectic Solvents).
- **Energy Source:** Mild heating or room temperature.
- **Sample Type:** Blood, serum, and urine.
- **Application:** Green solvent-based extraction of polar metabolites.

6. Matrix Solid-Phase Dispersion (MSPD):

- **Solvent Used:** Minimal solvent (ethanol, methanol) combined with a sorbent.
- **Energy Source:** Manual agitation and gentle pressure.
- **Sample Type:** Whole blood and tissue homogenates.
- **Application:** Simultaneous extraction and dispersion of analytes.

These techniques are part of a broader trend in blood sample preparation towards miniaturised, automated, and greener methods. They represent significant improvements in efficiency, sensitivity, and safety, especially for complex biological matrices and point-of-care diagnostics.

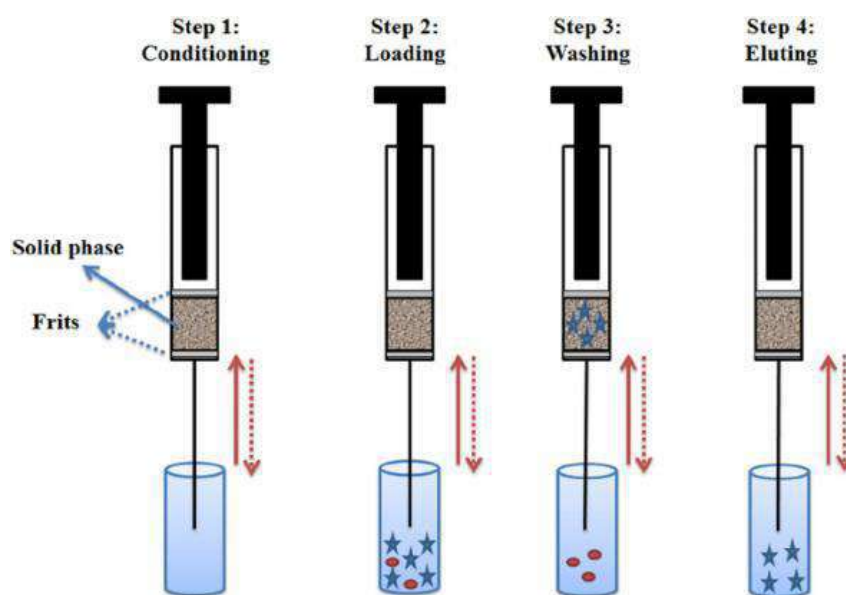
Microextraction techniques:

Microextraction techniques represent a class of sample preparation methods characterized by their simplicity, low solvent consumption, and high enrichment factors. These techniques involve the extraction of analytes from small sample volumes using a miniaturized extraction phase or solvent ^[1].

1. Microextraction by Packed Sorbent

(MEPS): This is a miniaturized version of solid-phase extraction, in which approximately 1 mg of sorbent material is packed into a syringe (100–250 μ L) or micro-column to retain analytes. MEPS is designed to handle a large sample volume range (from as low as 10–1000 μ L), reduce the number of steps involved in conventional SPE, and facilitate automation ^[1].

Principle: In MEPS (Microextraction by Packed Sorbent), the sample flows through a small cartridge where the target analytes bind to the sorbent material. To improve concentration, the sample can be repeatedly drawn in and dispensed. After washing away unwanted impurities, the analytes are eluted using a small amount of solvent. This eluted extract can be injected directly into analytical instruments like GC or LC for analysis.



Advantages:

- MEPS offers simplicity, ease of use, reduced solvent consumption [8], high enrichment factors, reduced sample preparation time [15], automation capability, and cost-effectiveness [8].
- The same syringe containing the sorbent bed can be reused multiple times after proper washing, which also helps minimize carry-over between samples.
- Moreover, MEPS contributes to the reduction of matrix effects by significantly lowering phospholipid content in the processed sample

Disadvantages:

- Microextraction techniques generally have limited analyte polarity spectrum, stronger matrix effects if not carefully controlled [1], heightened control requirements for extraction conditions, and potential fiber/coating issues specific to SPME.

Applications:

- MEPS can be applied to various biomatrices, including plasma, serum, urine, whole blood, hair, and saliva.
- It has been used for drug and metabolite screening using monolithic, polymer, and silica-based sorbents [1].

2. **Solid-Phase Microextraction (SPME):** Developed in 1990, SPME is a sample preparation method that utilizes a fused-silica fiber coated with a stationary phase. This syringe-like device streamlines the entire preparation process, minimizing time, solvent consumption, and waste, while enhancing detection sensitivity [1].

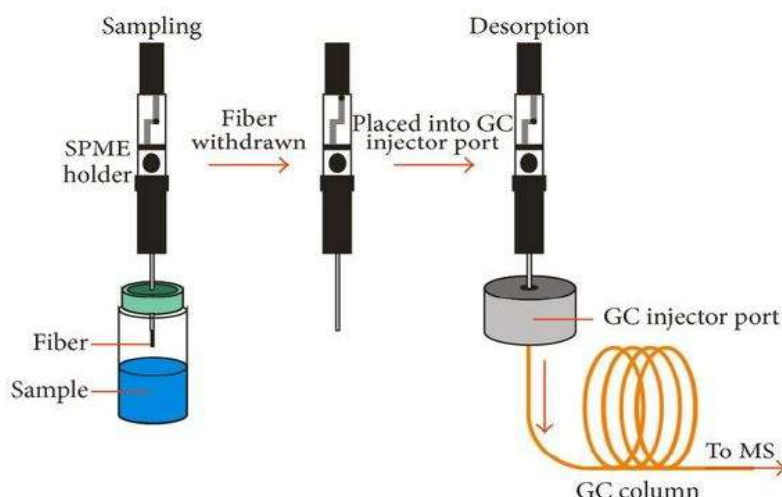
Modes of Extraction:

- **Direct Immersion (DI-SPME):** The SPME fibre is directly dipped into the liquid sample matrix.
- **Head-Space (HS-SPME):** The liquid sample matrix is heated to volatilise analytes, and the fibre is placed just above the sample matrix to adsorb them.

Fibre Coatings: Coatings can be tailored for different analyte types. For example:

- **PDMS** for non-polar analytes
- **PA** for polar compounds
- **CAR-PDMS** for low-molecular-weight volatiles
- **DVB-CAR-PDMS** for a wide analyte range

Extraction and Desorption: Optimisation of extraction conditions (pH, salt concentration, sample volume, agitation, temperature, time) is crucial. For desorption, SPME coupled with GC typically uses thermal desorption by exposing the fibre to a heated chamber in the GC injector, making it a "solvent-free" method. For LC, desorption can be achieved using a suitable volume of selective solvent/mobile phase, which can also be automated [6].



Derivatisation: SPME allows various derivatization strategies either before, during, or after extraction to enhance sensitivity and selectivity without adding much complexity or cost.

3. Liquid-Phase Microextraction (LPME)) is a widely used miniaturized technique that offers a greener and more efficient alternative to traditional Liquid-Liquid Extraction (LLE). It works by allowing the analytes to distribute between a very small volume of extraction solvent and the sample solution. This enables selective extraction and concentration of the target compounds from complex biological matrices. LPME techniques specifically extract analytes into a small volume of an immiscible solvent, significantly reducing the

amount of organic solvents required compared to traditional LLE methods.

LPME encompasses several different modes and techniques:

- **Dispersive Liquid-Liquid Microextraction (DLLME):** Dispersive Liquid-Liquid Microextraction (DLLME), introduced in 2006, is a fast, cost-effective, and efficient technique within the LPME (Liquid-Phase Microextraction) family. It offers high preconcentration efficiency using only microlitre amounts of both low- and high-density solvents. The process can also be automated by performing all extraction steps directly within a syringe setup.

- **Ultrasound-Assisted Emulsification Microextraction (USAEME):** It is a modified version of DLLME that uses ultrasound to disperse a tiny amount of water-immiscible extraction solvent directly into the sample eliminating the need for a separate dispersive solvent. This approach addresses some of the drawbacks of traditional DLLME, particularly the reliance on dispersive solvents and the use of environmentally harmful halogenated hydrocarbons.
- **Single-Drop Microextraction (SDME):** A small droplet of extraction solvent is suspended in the sample. It is simple but effective.
- **Hollow-Fiber Liquid-Phase Microextraction (HF-LPME):** It is a miniaturized technique that integrates extraction, pre-concentration, and cleanup into one simple step. It uses a hollow fiber membrane that acts as a protective barrier, shielding the acceptor phase from direct contact with interfering substances in the sample, which helps improve selectivity and sensitivity. It offers tolerance to a wide pH range and compatibility with multiple analytical instruments.
- **Solidified Floating Organic Drop Microextraction (SFODME):** This is a simple, fast, and inexpensive LPME method. A drop of extractant solvent floats on the sample and is solidified at low temperatures for easy collection.

Advantages of LPME:

- Simplicity and Ease of Use.

- Reduced Solvent Consumption, as LPME significantly decreases the amount of organic solvents needed.
- High Enrichment Factors.
- Reduced Sample Preparation Time.
- Automation Capability, particularly with techniques like DLLME.
- Cost-Effectiveness.
- Compatibility with various analytical instruments.
- High selectivity due to efficient phase partitioning

Disadvantages of LPME:

- Limited Analyte Polarity Spectrum.
- Can be affected by strong matrix effects.
- Requires heightened control of extraction conditions.

Applications: LPME has found diverse applications, particularly in forensic toxicology and environmental analysis:

➤ Forensic Toxicology:

- DLLME has been used for detecting pesticides (e.g., diazinon) and abused drugs in urine, blood, and hair.
- USAEME combined with UHPLC-MS/MS can detect anticoagulants and resolve false positives in drug screening (e.g., for suvorexant in oral fluids).

➤ Environmental Analysis:



- Advanced DLLME techniques can detect pollutants like fullerenes, herbicides, heavy metals, and endocrine disruptors in water samples.
- Ultrasound-assisted in-situ derivatization DLLME enables fast and sensitive screening of complex environmental samples.
- It is non-selective towards a wide range of basic, neutral, and acidic compounds.

Applications: Widely used for plasma phospholipid removal in LC-MS analysis of various analytes, including antidepressants, Verapamil and its metabolites, N-acylethanolamides, and sitagliptin.

Advanced SPE approaches:

Advanced SPE approaches focus on enhancing selectivity, mitigating matrix effects, and integrating sample preparation with analytical systems.

1. **Hybrid SPE-PPT:** This innovative method merges the simplicity of protein precipitation with the efficiency of selective filtration—specifically targeting phospholipids, which are common sources of matrix interference in blood samples.

Principle: Plasma or serum is initially acidified to precipitate proteins. The supernatant is then passed through a Hybrid SPE cartridge—often packed with zirconia sorbent, which has a strong affinity for phospholipids. There are also "in-well" formats available (e.g., 96-well plates), allowing precipitation and filtration to happen in a single step. Commercially available Hybrid SPE-PPT cartridges often contain a zirconia sorbent due to its high affinity for phospholipids.

Advantages:

- It significantly reduces matrix effects (especially phospholipid-based interference).
- It offers simplified sample preparation.
- It is compatible with automation, and provides good recovery and reproducibility.

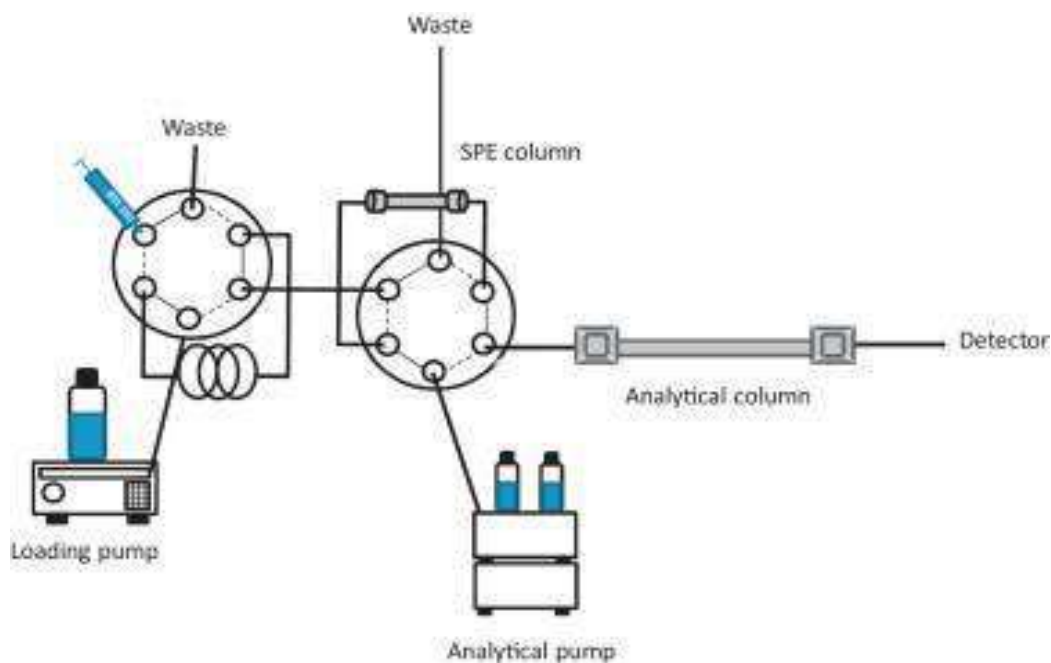
Disadvantages: Hybrid SPE-PPT cartridges are not reusable and have lower selectivity compared to some other methods.

2. **On-line SPE Coupled with HPLC:** This technique integrates sample cleanup (extraction) and chromatographic separation (HPLC) into a single, automated system.

Principle: It uses switching valves and pre-columns (SPE cartridges) to retain analytes on an SPE column. Matrix interferences are then washed away, and the analytes are directly eluted into the analytical HPLC column for separation and detection. Two main types of on-line SPE columns are commercially available: Restricted Access Material (RAM) columns and Turbulent Flow Chromatography (TFC) columns

- **RAM columns:** Designed for direct injection of samples like plasma or serum. They prevent large molecules (e.g., proteins) from entering the stationary phase while allowing small analytes to pass through for analysis.
- **TFC columns:** These utilise high flow rates (1.5–5 mL/min) and large particles (20–60 µm) to create turbulence. Small analytes diffuse into particle pores and bind, while larger matrix components (like proteins) are rapidly flushed to waste.





Advantages:

- On-line SPE coupled with HPLC is fully automated, offers improved reproducibility and accuracy, is effective for complex matrices, reduces sample loss, and is compatible with LC-MS/MS.
- Direct injection of biological samples using **RAM** or **TFC** columns streamlines sample preparation, boosts throughput, and significantly reduces or even eliminates matrix effects.
- Additionally, method development is faster, as these systems often rely on generic protocols that require minimal optimization.

Disadvantages: This method can involve high instrumentation costs and requires complex method development. It is also less flexible compared to off-line methods.

3. Dispersive Solid-Phase Extraction (dSPE): Popularly known as QuEChERS (quick, easy, cheap, effective, rugged, and safe), is

considered an advanced approach within Solid-Phase Extraction (SPE) techniques for sample preparation. It offers unique advantages over conventional methods.

Principle of dSPE: The dSPE approach combines principles from several sample preparation techniques. The process typically involves two main steps:

- **Solvent Extraction and Partitioning:** The sample is extracted using solvents like acetonitrile, and salts (e.g., magnesium sulfate) are added to promote phase separation. pH adjustments can be made to enhance extraction based on the analyte's chemical properties.
- **Clean-up:** After mixing and centrifugation, the clear supernatant is carefully transferred to a fresh tube. A key clean-up step follows, where a powdered sorbent is added to the supernatant to bind and remove interfering substances. Contaminants are retained on the sorbent material, allowing the analyte to remain in the liquid phase. A second centrifugation step is then performed to isolate the clean supernatant.

from the solid matrix. This purified supernatant can be evaporated, reconstituted, or directly injected into analytical instruments such as HPLC for analysis.

- In dSPE, unlike traditional SPE, the sorbent is mainly used to remove unwanted matrix

components rather than capture the analyte—though it can be tailored to retain the analyte if needed.

Sorbents Used: Graphitized carbon black (GCB), C18 silica, Primary-secondary amine (PSA), Acrylamides.



1 Choose the dispersive cleanup kit and add acetonitrile extract



2 Vortex for 1 minute



3 Centrifuge at 4000 rpm for 5 minutes



4 Take aliquot of supernatant and dry down or dilute as necessary



5 Place in autosampler vials for GC or LC analysis

Advantages of dSPE: dSPE offers numerous benefits, making it a promising alternative for routine bioanalytical applications:

- Simple, fast, and cost-effective
- Uses less solvent (greener method)
- Suitable for complex biological and environmental matrices
- High recovery rates
- Allows for high-throughput processing

- Reduces need for extra steps like filtration or centrifugation

Disadvantages of dSPE: Despite its advantages, dSPE also has some limitations:

- Sorbents are typically single-use
- May offer lower selectivity than advanced methods
- Automation options are limited
- Less suitable for large biomolecules

Applications: dSPE has been widely adopted in various analytical fields:

- Food & Pesticide Residue Analysis: Commonly used to detect pesticides and contaminants in fruits, vegetables, milk, and meats.
- Environmental Monitoring: Used for detecting pollutants (e.g., herbicides, psychoactive drugs) in water, soil, and sediment.
- Cosmetic & Additive Testing
- Bioanalysis: Growing interest in using dSPE for clinical and preclinical bioanalysis of drugs and metabolites, especially in challenging biological matrices.

Examples:

- Detecting drug residues (e.g., anthelmintics in milk, β -lactams in kidney)
- Analyzing psychoactive substances in blood and tissues
- Extracting drugs from lipid-rich liver samples
- Investigating drug overdoses and poisonings (e.g., new designer cathinones)

Future Outlook: The future of paper-based blood diagnostics looks highly promising, with several exciting directions for further advancement, including:

- **AI-Based Pre-Analytical Quality Control:** By integrating machine learning and artificial intelligence (AI) especially deep learning, paper-based diagnostic devices can significantly improve image analysis and result interpretation. These technologies help enhance accuracy and reliability by compensating for confounding factors such as

lighting variations, sample inconsistencies, and user handling.

- **Robotic and Fully Automated Sample Preparation:** The field is progressing toward fully automated lab-on-paper devices that seamlessly integrate blood separation, dilution, mixing, detection, and analysis—offering a streamlined, user-friendly, sample-to-report workflow.
- **Blood-on-Chip and Point-of-Care Integration:** Further integration of μ PADs with point-of-care testing (POCT) systems will enhance healthcare accessibility, especially in resource-limited settings.
- **Green and Sustainable Extraction Methods:** Continuous development of environmentally friendly extraction methods that reduce solvent usage and energy consumption.
- **Wearable and Minimally Invasive Sampling Devices:** Innovations in sample collection, such as patterned dried blood spot (DBS) cards and other minimally invasive methods, will continue to improve patient experience and sample quality.
- **Multiplexed and Multi-Omics-Compatible Techniques:** Development of techniques capable of simultaneously detecting multiple analytes and integrating with multi-omics approaches for a more comprehensive understanding of biological systems.
- **Smart Microfluidics with Real-Time Feedback:** Enhancing devices with real-time feedback mechanisms, possibly integrated with smartphone technology, to improve user experience and reliability in field settings.

CONCLUSION:



The field of blood sample preparation is advancing rapidly, marked by a strong shift toward miniaturised, automated, and eco-friendly technologies [17]. This aligns with the broader transformation toward miniaturized, automated, and environmentally friendly technologies, often utilizing Green Extraction Techniques (GreETs). The rapid advancements, particularly in paper-based microfluidics, also contribute to miniaturization and automation [1]. These emerging innovations bring significant improvements in efficiency, sensitivity, and safety are key benefits when dealing with complex biological samples, especially in point-of-care diagnostics (POCT) [17]. These advantages are central to the new technologies, with GreETs specifically designed to improve selectivity, sensitivity, and reduce harmful effects. Paper-based platforms are highlighted as inexpensive, low-volume, portable bioassays beneficial for POCT [16], and recent advancements in paper microfluidics are geared towards blood analysis and diagnostics [2].

Many of the challenges traditionally associated with blood sample preparation like time-consuming steps [15], limited multiplexing [1], and lack of portability [1] are now being addressed. Conventional techniques are frequently characterized by their time-consuming nature and high labor demands [15]. New paper-based platforms are designed for multiplexed detection and offer portability [2]. Yet, ensuring robust method development and maintaining strict control of pre-analytical variables remain essential to guarantee reliable, accurate, and reproducible results across various clinical and research settings [4]. The critical importance of controlling pre-analytical variables for high-quality, accurate, and reproducible analytical results is strongly emphasized, particularly for sensitive analyses like NMR-based metabolomics studies [4]. Sample

preparation is noted as a major source of experimental errors.

Bridging the gap between lab research and real-world applications is essential for the widespread adoption of paper-based blood testing technologies. Key factors include large-scale manufacturing, cost-effectiveness, and smooth integration into existing healthcare workflows [1]. These paper-based platforms are particularly valued for their low cost [17] and scalability, making them strong candidates for commercialisation. The incorporation of Green Extraction Techniques (GreETs) into clinical diagnostics further highlights their growing relevance in medical applications. Additionally, paper microfluidics continue to play a pivotal role in blood analysis and diagnostics, reinforcing their potential for seamless integration into future healthcare systems [1].

Abbreviations:

- LC-MS/MS – Liquid Chromatography–Tandem Mass Spectrometry
- HPLC – High-Performance Liquid Chromatography
- GC-MS – Gas Chromatography–Mass Spectrometry
- NMR – Nuclear Magnetic Resonance
- UV-Vis – Ultraviolet–Visible Spectroscopy
- ELISA – Enzyme-Linked Immunosorbent Assay
- CE – Capillary Electrophoresis
- FT-IR – Fourier Transform Infrared Spectroscopy
- PP – Protein Precipitation



- LLE – Liquid–Liquid Extraction
- SPE – Solid-Phase Extraction
- dSPE – Dispersive Solid-Phase Extraction
- SALLE – Salting-Out Assisted Liquid–Liquid Extraction
- MEPS – Microextraction by Packed Sorbent
- SPME – Solid-Phase Microextraction
- SBSE – Stir Bar Sorptive Extraction
- TFME – Thin Film Microextraction
- FPSE – Fabric-Phase Sorptive Extraction
- LPME – Liquid-Phase Microextraction
- DLLME – Dispersive Liquid–Liquid Microextraction
- USAEME – Ultrasound-Assisted Emulsification Microextraction
- SDME – Single-Drop Microextraction
- HF-LPME – Hollow-Fiber Liquid-Phase Microextraction
- SFODME – Solidified Floating Organic Drop Microextraction
- PLE/ASE – Pressurised/Accelerated Solvent Extraction
- DES – Deep Eutectic Solvents
- MSPD – Matrix Solid-Phase Dispersion
- μ PADs / μ PADs – Microfluidic Paper-Based Analytical Devices
- LOC – Lab-on-a-Chip
- DMF – Digital Microfluidics
- POCT – Point-of-Care Testing
- TDM – Therapeutic Drug Monitoring
- BSTFA – N,O-Bis(trimethylsilyl)trifluoroacetamide
- MSTFA – N-Methyl-N-(trimethylsilyl)trifluoroacetamide
- IBCF – Isobutyl Chloroformate
- PBS – Phosphate-Buffered Saline
- BSA – Bovine Serum Albumin
- SERS – Surface-Enhanced Raman Scattering
- RAM – Restricted Access Material
- TFC – Turbulent Flow Chromatography
- GreETs – Green Extraction Techniques

REFERENCES

1. Zhiyuan Yang, Yafei Lou, Linan Sun, Siyu Chen, Miaosi Li, Zhuoxuan Lu, Liming Zhang, Rong Cao, Junfei Tian, Recent advancements in paper microfluidics for blood analysis and diagnostics, *TrAC Trends in Analytical Chemistry*, Volume 190, 2025, 118289, ISSN 0165-9936, <https://doi.org/10.1016/j.trac.2025.118289..>
2. Emwas, AH., Zacharias, H.U., Alborghetti, M.R. et al. Recommendations for sample selection, collection and preparation for NMR-based metabolomics studies of blood. *Metabolomics* 21, 66 (2025). <https://doi.org/10.1007/s11306-025-02259-7>
3. Al Jabri, A., Cooke, J., Cournane, S., & Healy, M. L. (2024). Blood Micro-sampling: An alternative blood collection method for



- radioiodine therapy dosimetry. *Physica medica : PM : an international journal devoted to the applications of physics to medicine and biology : official journal of the Italian Association of Biomedical Physics (AIFB)*, 124, 103430. <https://doi.org/10.1016/j.ejmp.2024.103430>
4. Lassmann, M., Hänscheid, H., Chiesa, C., Hindorf, C., Flux, G., Luster, M., & EANM Dosimetry Committee (2008). EANM Dosimetry Committee series on standard operational procedures for pre-therapeutic dosimetry I: blood and bone marrow dosimetry in differentiated thyroid cancer therapy. *European journal of nuclear medicine and molecular imaging*, 35(7), 1405–1412. <https://doi.org/10.1007/s00259-008-0761-x>
5. Kanu A. B. (2021). Recent developments in sample preparation techniques combined with high-performance liquid chromatography: A critical review. *Journal of chromatography. A*, 1654, 462444. <https://doi.org/10.1016/j.chroma.2021.462444>
6. Ming, T., Cheng, Y., Xing, Y., Luo, J., Mao, G., Liu, J., Sun, S., Kong, F., Jin, H., & Cai, X. (2021). Electrochemical Microfluidic Paper-Based Aptasensor Platform Based on a Biotin-Streptavidin System for Label-Free Detection of Biomarkers. *ACS applied materials & interfaces*, 13(39), 46317–46324. <https://doi.org/10.1021/acsami.1c12716>
7. Kole, P. L., Venkatesh, G., Kotecha, J., & Sheshala, R. (2011). Recent advances in sample preparation techniques for effective bioanalytical methods. *Biomedical chromatography: BMC*, 25(1-2), 199–217. <https://doi.org/10.1002/bmc.1560>
8. Martinez, A.W., Phillips, S.T., Butte, M.J., & Whitesides, G.M. (2007). Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte Chemie International Edition*, 46, 1318–1320
9. Krishnakumar, A., Mishra, R. K., Kadian, S., Zareei, A., Rivera, U. H., & Rahimi, R. (2022). Printed graphene-based electrochemical sensor with integrated paper microfluidics for rapid lidocaine detection in blood. *Analytica chimica acta*, 1229, 340332. <https://doi.org/10.1016/j.aca.2022.340332>
10. Baillargeon, K.R., Brooks, J.C., Miljanic, P.R., & Mace, C.R. (2022). Patterned dried blood spot cards for the improved sampling of whole blood. *ACS Measurement Science Au*, 2, 31–38
11. Gu, Yingqiu & Chen, Yu & Zhang, Xue & Xu, Di & Shang, Yunsheng & Cao, Chengliang & Yang, Guohai & Qu, Lulu. (2023). Integration of blood separation and multiple-cytokine detection using a combined paper centrifugation–SERS immunoassay method. *Sensors and Actuators B: Chemical*. 396. 134597. [10.1016/j.snb.2023.134597](https://doi.org/10.1016/j.snb.2023.134597).
12. Abu M. Rustum, Determination of diltiazem in human whole blood and plasma by high-performance liquid chromatography using a polymeric reversed-phase column and utilizing a salting-out extraction procedure, *Journal of Chromatography B: Biomedical Sciences and Applications*, Volume 490, Pages 365-375, ISSN 0378-4347,
13. Brunius, C., Pedersen, A., Malmödin, D., Karlsson, B. G., Andersson, L. I., Tybring, G., & Landberg, R. (2017). Prediction and modeling of pre-analytical sampling errors as a strategy to improve plasma NMR metabolomics data. *Bioinformatics (Oxford, England)*, 33(22), 3567–3574. <https://doi.org/10.1093/bioinformatics/btx442>
14. Kolahgar, B., Hoffmann, A., & Heiden, A. C. (2002). Application of stir bar sorptive extraction to the determination of polycyclic aromatic hydrocarbons in aqueous samples. *Journal of chromatography. A*, 963(1-2), 225–



230. [https://doi.org/10.1016/s0021-9673\(02\)00361-8](https://doi.org/10.1016/s0021-9673(02)00361-8)
15. Boobphahom, S., Ly, M. N., Soum, V., Pyun, N., Kwon, O. S., Rodthongkum, N., & Shin, K. (2020). Recent Advances in Microfluidic Paper-Based Analytical Devices toward High-Throughput Screening. *Molecules* (Basel, Switzerland), 25(13), 2970. <https://doi.org/10.3390/molecules25132970>
16. Câmara, J. S., Perestrelo, R., Berenguer, C. V., Andrade, C. F. P., Gomes, T. M., Olayanju, B., Kabir, A., M. R. Rocha, C., Teixeira, J. A., & Pereira, J. A. M. (2022). Green Extraction Techniques as Advanced Sample Preparation Approaches in Biological, Food, and Environmental Matrices: A Review. *Molecules*, 27(9), 2953. <https://doi.org/10.3390/molecules27092953>
17. He, Y., & Concheiro-Guisan, M. (2019). Microextraction sample preparation techniques in forensic analytical toxicology. *Biomedical chromatography : BMC*, 33(1), e4444. <https://doi.org/10.1002/bmc.4444>
18. Tanasescu, E. C., Ene, A. G., Perdum, E., Iordache, O., & Secareanu, L. O. (2024). New fabric phase sorptive extraction for nondestructive analysis of heritage textile samples. *Heliyon*, 10(10), e31020. <https://doi.org/10.1016/j.heliyon.2024.e31020>

HOW TO CITE: Pendyala Meghana*, Bobbili Sharanya, Moluguri Sandhya Rani, Modern Chemistry Approaches Enhancing Blood Sample Preparation for Pharmaceutical Analysis, *Int. J. of Pharm. Sci.*, 2025, Vol 3, Issue 11, 3705-3734 <https://doi.org/10.5281/zenodo.17688342>

