Efficient Extraction Techniques for Bioanalysis : A Review of Current Methodologies

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ABSTRACT

Bioanalytical methods are commonly employed to quantify pharmaceuticals and their metabolites in plasma matricesand they play an essential part in toxicokinetic, pharmacokinetic, and pharmacodynamics research estimate and interpretation.Sample preparation is considered as the bottleneck step in bioanalysis because each biological matrix has its own unique challenges and complexity, to ensure high reliability, sensitivity, selectivity and reproducibility of an analytical method. This paper reviews the recent developments in bioanalysis sample preparation techniques and gives an update on modern trends in bioanalytical sample preparation techniques, including sorbent-based microextraction techniques, are primarily emphasized.Conventional liquid-liquid extraction (LLE), protein precipitation (PP) and solid-phase extraction (SPE) techniques are now been considered as methods of the past.

KEY WORDS: Bioanalysis, Biological matrices, Sample Preparation Techniques,

Micro extraction ,Solid Phase Extraction.

INTRODUCTION

The development of bioanalytical sample preparation techniques has become challenging over the decades because of the need to constantly accomplish higher sensitivity, accuracy, and speed of analysis in complex biofluids (e.g., blood, serum, plasma, saliva, faeces, and urine).(1) The main objective of sample preparation process is to provide a suitable sample, usually for chromatographic analysis, which will not contaminate the instrumentation and where the concentration in the prepared sample is reflective of that found in the original.The method of sample preparation selected is generally dictated by the analytical technique available and thephysical characteristics of the analytes under investigation(2).

Sample preparation is necessary for at least two reasons(10).

a. To remove as many of the endogenous interferences from the analyte as possible.

b. To enrich the sample with respect to the analyte, thus maximizing the sensitivity of the system.

It also serves to ensure that the injection matrix is compatible with the selected column andmobile phase. In simple terms, sample preparation is a process which aims at selective isolation of the analyte of interest from the matrix, minimization/elimination of matrix components in the processed sample and, if required, concentration of the analyte of interest.

Extraction techniques in bioanalysis

- **Protein Precipitation**

Principle: It depends on the solubility of analyte in particularsolvent present in biological matrix i.e., blood, plasma, serum.

Solvent used: Methanol, ACN etc. It is basically denaturing the proteins. Precipitation of proteinscan be done by using any one of the method mentioned below:

By changing the pH of sample- by mixing inorganicreagents like Perchloric acid, Trichloroacetic acid etc. In isoelectric pH, proteins have no net charge, which causes insolubility thus precipitates(1).

By addition of organic solvents- It decreases the dielectricconstant of the medium, leads to insolubility thus causeprecipitation, in another case high affinity for thehydrophobic surfaces of the protein leads to denaturing ofproteins. Example: Methanol, Acetonitrile etc.

By addition of Salts - salts used forprecipitation of proteinsare citrates, phosphates, acetates, etc.

Protein precipitation plates

One of the recent approaches to eliminating the disadvantages ofthe conventional manual PPmethod is development ofmembrane-based PP filter plates.PP plates have been developedso that, after precipitation of protein, filtration can be carried outin the same well without centrifugation and supernatant transfersteps.PP plates when compared with manualPP, offers several advantages such as, reduced processing time, Higher solvent recovery(2).

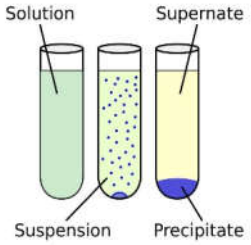


Fig No:01 Protein Precipitation

- **Liquid Liquid Extraction**

Principle: It is based on selective extraction of intended analytepresent in liquid sample through immiscible organic solvent.

Solvent used: Methyl ter-Butyl Ether (MTBE), Dichloromethane(DCM), Ethyl acetate (EC), Diethyl ether (DEE), Hexane etc.any solvent as individual or in combination with any othersuitable solvent can be used as an extraction solvent(1).

Liquid–liquid extraction (LLE) is a technique that consists in the transfer of the target compoundfrom their original medium to a water-immiscible solvent, namely extractant solvent, with which the sample is put in contact during a certain time. The contact between the two immiscible phases is enhanced mechanically, typically by agitation and, less frequently, by (magnetic) stirring or by creating turbulences with a cylinder/piston system Ideally, an efficient LLE will transfer quantitatively the target analytes to the extractant solvent, maintaining the potential interfering substances and particulate material in the aqueous phase, which is discarded. The water-organic phase partition coefficient of the target compound is the main parameter that rules this process(8).

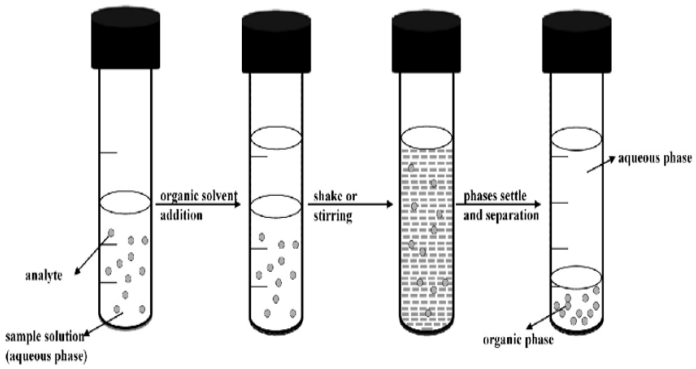


Fig no:02 LLE

- **Solid-phase Extraction Technique**

Principle: It is based on adsorption (or) Partitioning on to solid sorbent (Adsorbent)" Selective retardation of analyte using solidsorbent under specific condition. SPE is based on the selectiveadsorption mechanism. If the targeted analyte are adsorbed onthe solid phase, they can selectively be removed or eluted byusing an appropriate elution solvent6. Different cartridges areused in solid phase extraction(3).

Steps involved in SPE:-

1. Conditioning: All SPE tubes are required to beconditioned with appropriate solvents prior to sampleapplication.

2. Sample Application: Application of sample from the topof the cartridge at a slow flow rate without any brake by takingcare that no sample drop should remain on the inner wall of thecartridges, slow rate is necessary to allow analyte to interactwith adsorbent thus to achieve the retention of analytes becauseof temporary weak bonding.

3. Rinsing or Washing: It is intended for the removal ofmatrix components or other interferences by washing thecartridge with relative weak dilute solvent or solvent mixtures orbuffer and interferences that are weakly retained than theanalytes are drained out from the cartridge. Rinsing or washingsolvents are water, buffers of different pH(7).

4. Drying; Drying can be done by applying appropriatevacuum for recommended time period with help of vacuumpump, recommended drying time is 2-3 minutes.

5. Elution: It involves passing of strong solvent throughcartridge at a slow flow rate thus allowing more soak time onthe packing of reach maximum extraction efficiency, elutionsolvents are MeOH, ACN, acidic or alkaline MeOH or ACN (7).

Type of SPE:

1. Reversed phase SPE
2. Normal phase SPE
3. Ion exchange SPE

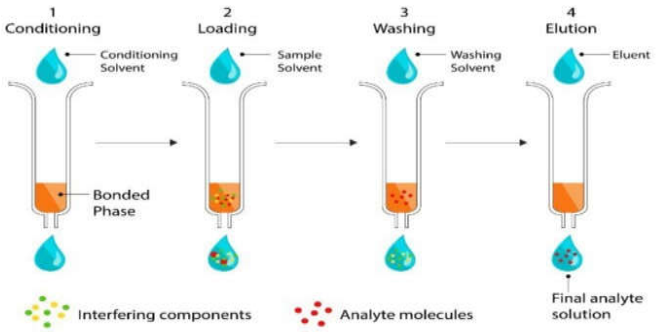


Fig No:03 SPE

- **Micro-extraction by packed sorbent**

Micro-extraction bypacked sorbent (MEPS) is a miniature SPE. The purpose ofMEPS is to reduce the sorbent bed volume, making it suitablefor large sample volume range (from as low as 10–1000 mL),reducing the number of steps typically involved in conventionalSPE and providing easy automation. Typical MEPS is designedin the syringe format, wherein approximately 1mg of the sorbentis packed inside a syringe (100–250 mL) as a plug or betweenthe barrel and the needle as a cartridge (1).

- **Solid phase micro extraction**

A new sample preparation technique using a fused-silica fibercoated on the outside with an appropriate stationary phase and istermed Solid phase micro-extraction (SPME).Physically, it isamodified syringe that contains stainless steel microtubing withinits syringe needle. This microtubing has about a 1 cm fusedsilicafiber tip which is coated with an organic polymer. Thiscoated silica fiber can be moved backwards and forwards withthe plunger of the syringe(5).The foremost advantage of the technique isimproved detection limits. There are two types of extractionmodes for SPME: first, direct immersion (DI) of SPME fiber into liquid sample matrix, simply termed as DI-SPME andsecond, head-space (HS) extraction in which the liquid samplematrix is heated in a vial to volatilize the analytes and the fiber is placed just above the sample matrix.Various commercially available fiber coatings arepolydimethylsiloxane (PDMS) for extraction of non-polaranalytes, polyacrylate (PA, thickness 85 mm) for extraction of polar analytes(2).

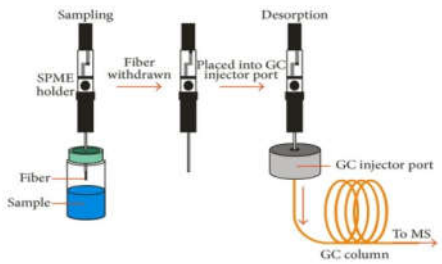
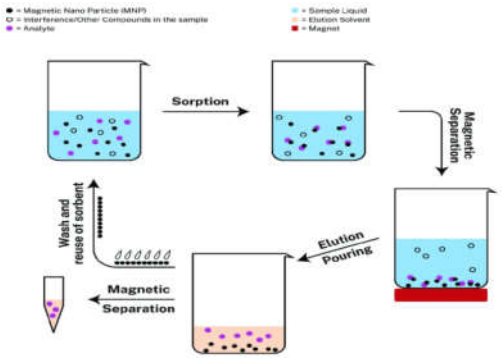


Fig No:04 SPME

- **Magnetic Solid Phase Extraction**

Magnetic solid phase extraction (MSPE) is a new technique that utilizes a magnetic adsorbent to a solution containing a certain analyte. The analyte is adsorbed into the magnetic sorbent and then separated magnetically (10).Finally, the analyte eluted from the recovered adsorbent.It involves the use of magnetic materials, such as magnetic nanoparticles or beads, functionalized with specific ligands that selectively bind to target molecules. The sample is mixed with these magnetic particles, allowing the analytes to be captured. After binding, an external magnet is applied to separate the solid phase from the liquid phase, followed by washing steps to remove impurities. Finally, the analytes are eluted from the magnetic phase for further analysis, such as chromatography or mass spectrometry. MSPE offers high sensitivity, efficiency, and minimal sample preparation(6).



FigNo:05 Magnetic Phase Solid Extraction

- **Super Critical Fluid Extraction**

Supercritical fluid extraction (SFE) is an analytical technique that uses a supercritical fluid (typically CO_2) to extract compounds from solid or liquid samples. The principle behind SFE relies on the unique properties of supercritical fluids, which have characteristics of both gases

and liquids. In the supercritical state, these fluids exhibit high diffusivity, low viscosity, and high solubility for a wide range of compounds, allowing efficient extraction(4).

SFE is particularly useful for bioanalysis, as it minimizes sample degradation due to the low temperatures involved and avoids the use of organic solvents, making it more environmentally friendly. Furthermore, it is highly efficient in extracting bioactive compounds such as lipids, alkaloids, and essential oils from biological matrices. The advantages of SFE in bioanalysis include high selectivity, minimal sample preparation, and the ability to handle sensitive or thermolabile substances. Additionally, it offers faster extraction times compared to traditional methods like solvent extraction, making it a valuable tool in modern bioanalytical laboratories(9).

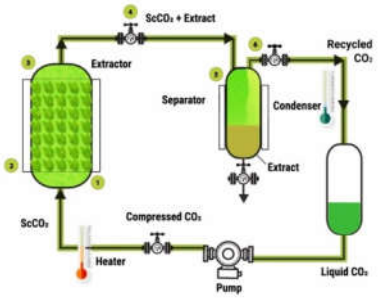


Fig No:06 SFE

- **Disposable pipette extraction**

DPX is an effective sample preparation technique for separationand extraction of analytes fromthe various matrices. In itssimplest original form, it is modified standard pipette tip (1/5mL),which is loaded with free flowing sorbent powder, whichis free to disperse. Being a standard tip, is it easy to take thesolvent in or out through this dispersive sorbent(9).In its modified form, the standard tip contains a dispersible sorbent looselyplaced between two frits (one frit placed on the lower end of thetip through which solvent can be taken up/down and the secondplaced at the upper end of the tip to avoid solvent contaminationin to the pipette).In case of conventional SPE cartridges which contain packedbed sorbent, sample is loaded from the top; every sorbentparticle is used once (as sample travels through the bed undergravity), thus much sorbent material is required to retainanalytes. Also, the success of conventional SPE depends on

flowcontrol of the sample loading, washing and elution to achieve good repeatabilities. This leads to fast and efficient extractionand not much material needed to retain analytes(3).

Application Of Bioanalytical Strategies

If a sample has been analysed with a separation method theresult will be in form of a chromatogram.(2) The eluting substances will produce signals in the detector separated bytime, creating a pattern of peaks. Calibration curves can beconstructed from a set of samples with different concentrationsand be used for predictions of concentrations in unknownsamples. Area or peak height can be used and the choicedepends upon the situation.The developed Bioanalytical method can be applied for variouspurposes:

a. pharmacokinetic methods

Pharmacokinetic deals with the changes of drug concentration inthe drug product and changes of concentration of a drug and/orits metabolite(s) in the human or animal body followingadministration of the drug product, i.e., the changes of drugconcentration in the different body fluid and tissues in thedynamic system of liberation, absorption, distribution, bodystorage, binding, metabolism, and excretion.Two major pharmacokinetic methods arePlasma level-Time studies and Urinary excretion studies(2).

b. pharmacodynamic studies-

It involves direct measurement of drug effect on a physiologicalprocess as function of time(1).

c. Toxicology and Forensic Science

d.Clinical Diagnostics and Disease Monitoring

Biomarker Detection: Identifying and quantifying biomarkers for the diagnosis of various diseases (e.g., cancers, infections, metabolic disorders).

CONCLUSION

The selection of an extraction technique in bioanalysis is a critical step that impacts the quality of data, efficiency of the process, and cost-effectiveness of the analysis. Factors such as analyte characteristics, sample matrix, sensitivity requirements, and regulatory compliance must be carefully considered to choose the optimal extraction method. Making the wrong choice can lead to poor recovery, interference from matrix effects, inaccurate results, and wasted resources, while a well-chosen method will streamline the analysis process, improve reliability, and ensure valid conclusions.Ultimately, the selection of an extraction method is guided by factors such as the physicochemical properties of the analytes, sample type, matrix complexity, time constraints, and the sensitivity needed for downstream analytical techniques. Advancements in extraction technologies, such as miniaturized and automated systems, continue to enhance the robustness and throughput of bioanalytical processes, enabling more precise and efficient analysis across a broad range of applications in drug development, clinical diagnostics, and environmental monitoring.

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