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Review Article

Extraction of Drugs and Metabolites from Biological Matrices

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ABSTRACT

Extraction of drugs and their metabolites from biological matrices such as blood, plasma, urine, or tissues is a fundamental process in pharmaceutical, biomedical, and toxicological analysis, enabling accurate drug quantification, pharmacokinetic studies, and clinical monitoring. Biological samples contain a variety of interfering substances, including proteins, lipids, and salts, which can impede analytical detection and instrument efficiency. The extraction techniques serve to isolate analytes from these matrix components, thereby improving specificity, sensitivity, and reproducibility of results. Common extraction procedures include protein precipitation (removal of proteins using acids or solvents), liquid-liquid extraction (partitioning between immiscible solvents), and solid-phase extraction (adsorbent-based separation), along with advanced methods like solid-phase micro extraction and membrane-based approaches. These procedures often include concentration steps to enhance detection limits and sample clean-up to reduce damage to analytical instruments. Pre-treatment processes such as hydrolysis or homogenization may be necessary depending on sample type and analyte characteristics. Factors influencing extraction efficiency include pH, solvent polarity, temperature, and the physicochemical properties of the drug and its metabolites.

INTRODUCTION

Bioanalysis is a crucial subfield of analytical chemistry that focuses on extracting medications and their metabolites from biological matrices like blood, plasma, urine, or tissues, enabling quantitative evaluation for pharmacokinetics, toxicokinetics, and bioequivalence. It also plays a role in forensic investigations and anti-doping tests. Common methods employed include various chromatographic techniques and mass spectrometry. Reliable bioanalytical methods face challenges due to the complexity of biological samples, requiring careful extraction techniques such as solid phase extraction and liquid-liquid extraction. Sample preparation is vital, as the presence of endogenous substances and proteins

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can complicate the analysis of drugs at low concentrations. The choice between plasma and serum for testing is debated, with plasma generally favored unless specific interferences necessitate serum use. Ultimately, successful drug analysis hinges on thorough sample preparation and purification processes.[1]

Sample collection

Sample collection methods include blood, plasma, urine, serum, and saliva. Blood is collected using evacuated tube systems with color-coded stoppers to prevent cross-contamination. Plasma is obtained by separating cellular components of anti-coagulated blood. Urine can be collected under various protocols such as first morning specimens for analyte concentration, fractional specimens for comparison with blood concentrations, and timed collections for excretion patterns, ideally kept refrigerated. Serum isolation does not require anticoagulants and should occur promptly to minimize contamination, aiding studies of antibodies and nutrients. Saliva can be collected using a mucus hoover cleaner or directly into sterile tubes.[2]

Sample preparation

Sample preparation is essential for maximizing system sensitivity by minimizing endogenous interferences and enriching the analyte within the sample. This phase is often the most critical and challenging due to the difficulties in isolating the analyte from various matrices, each presenting unique challenges—plasma has phospholipids, urine has high salt content, and whole blood contains red blood cells. The extraction technique is influenced by the properties of the matrix and analyte. Proper sample collection practices are vital for maintaining sample integrity from collection to analysis. While plasma is commonly used for LC-MS/MS bioanalysis, blood or serum may be more appropriate depending on the drug and its metabolism. Additionally, urine analysis can provide insights into drug activity, particularly for drugs excreted unchanged. Rapid collection and storage at appropriate temperatures are important for preserving unstable medicines and ensuring proper labeling of samples.[3]

Commonly used extraction techniques

Liquid liquid extraction

Sample preparation is crucial in analytical processes for maximizing system sensitivity and enriching the sample for analyte analysis, with each matrix posing unique challenges. Proper collection and handling of samples, particularly in bioanalysis, is essential to preserve integrity. Common extraction techniques include liquid-liquid extraction (LLE), which separates compounds using two immiscible solvents based on solubility differences. This method effectively isolates analytes from biological matrices like plasma, urine, and serum, allowing for good recovery and clean samples with reduced solvent volume in modern applications.[4]

Solid phase extraction

Solid Phase Extraction (SPE) is a widely utilized method for extracting analytes from complex materials, particularly in analytical laboratories. It offers advantages such as automation, parallel processing, and high recovery rates, making it ideal for preparing samples like blood, plasma, and urine. SPE operates by adsorbing analytes onto a solid phase from a liquid sample, distinguishing it from Liquid-Liquid Extraction (LLE), which interacts between two liquid phases. SPE improves quantitative recovery yields and minimizes issues encountered in LLE. Typically, a disposable cartridge filled with sorbent material (e.g., C18

silica) is used, allowing effective purification and reduced interference during analysis.

Types of SPE

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

Procedure for solid phase extraction

1) Conditioning: Wet the bound functional groups using methanol.
2) Equilibration: Optimize retention by treating the sorbent with a polar, pH-matching solution identical to the sample matrix.
3) Sample load: Introduce the sample with an aqueous solvent for analyte extraction.
4) Washing: To prevent elution of target chemicals, employ the strongest aqueous solution while adjusting ionic strength and pH, followed by cartridge drying.
5) Elution: Use the weakest organic solvent in the order of polarity (Ethyl acetate > acetone > THF > methanol > acetonitrile) to ensure complete analyte removal, adjusting pH and ionic strength accordingly.
6) Solvent exchange: Reconstitute the sample in the initial mobile phase after evaporating the organic solvent, using methanol for GC analysis, maintaining the same volume.[5]

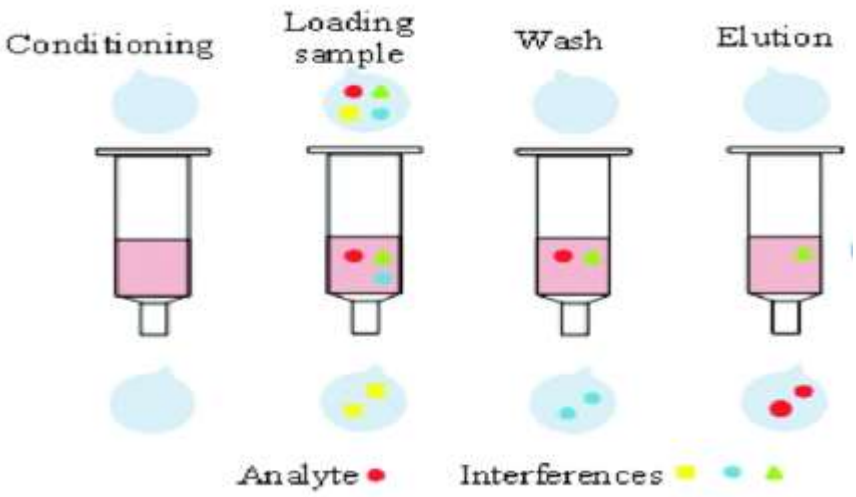

Figure 1:steps involved in solid phase extraction

Protein precipitation

Protein precipitation is a simple and cost-effective extraction technique, preferable to LLE and SPE. It utilizes organic solvents like acetonitrile and methanol for their ability to fully precipitate proteins and enhance analyte solubility. Acetonitrile is favored due to its complete protein precipitation capabilities, while methanol produces a clear supernatant and flocculent precipitate. After precipitation, the supernatant can be directly injected into HPLC or evaporated for reconstitution. This method effectively removes matrix interference and is particularly useful for samples with high protein content, such as plasma and saliva. Considerations for reagents include trichloroacetic acid, tungstic acid, and perchloric acid, alongside the analyte's characteristics and extraction matrix requirements.

Types of protein precipitation

1) Salting out

Primarily uses ammonium sulphate due to its high solubility and ionic strength, which is significantly

greater than that of sodium chloride. Ammonium sulphate's ions do not strongly interact with proteins, preventing destabilization. This salt can be easily removed from concentrated solutions via centrifugation, as it is cost-effective, maintains consistent solubility across temperatures, and has a lower density than proteins even in concentrated solutions.

2) Solvent precipitation

occurs when water-miscible solvents like ethanol or acetone are added to protein solutions, leading to protein aggregation due to a decrease in the dielectric constant. This process enhances interactions between charged protein groups, as the solvents hydrate more strongly than proteins, causing protein surfaces to dehydrate and bind through van der Waals forces. If proteins are near their isoelectric points, removing surrounding water increases charge interactions. Generally performed at low temperatures (0°C for proteins and -20°C for solvents), this method can improve protein purification when controlled effectively. Polyethylene glycol, at concentrations of 5% to 15%, can also facilitate this process without the need for such low temperatures. The resulting precipitate can be separated through centrifugation, allowing for further purification or immediate analysis via HPLC.[6]

Sample pre-treatment

Serum, plasma and whole blood: Techniques to break down protein binding in biological fluids include

- adjusting pH with acids or bases to extremes
- using a polar solvent (2:1 ratio) for protein precipitation
- applying acids or inorganic salts to precipitate proteins with potential pH adjustment
- sonicating the fluid followed by centrifugation.

Urine: heating for 15-20 minutes, cooling, diluting with buffer, and adjusting pH is recommended.[7]

Physicochemical properties of drug and their extraction from biological fluid

1) Molecular phenomena for solubility and miscibility

To dissolve a drug, a solvent must disrupt the chemical bonds between the drug and its neighbors (ionic, hydrogen, van der Waals forces) while maintaining sufficient solvent-molecule interactions. The bond-breaking process is endothermic and requires energy, leading to an increase in enthalpy. Additionally, two solvents may not be fully miscible if the entropy gained from their interaction does not compensate for the reduction in intermolecular bonding.

2) Water miscibility and water immiscibility

Alcohols are miscible with water due to hydrogen bonds and dipole-dipole interactions, while alkyl groups reduce solubility through dispersive forces. Hydrocarbons dissolve mainly via dispersive forces due to their hydrophobic nature, whereas halogenated hydrocarbons use dipolar interactions and dispersive forces for dissolution as they are more polar.

3) Distribution coefficient

Drugs in ionised forms are hydrophilic, making them harder to extract into organic solvents, while unionised forms can easily dissolve and be extracted into these solvents.

4) Choice of solvent

A variety of factors such as selectivity, density, toxicity, volatility, reactivity, physical hazards, and miscibility with water are important when choosing a solvent for extracting medication from its matrix and dissolving the required chemicals.

5) Mixed solvents

In some cases, pure solvents are insufficient for chemical extraction. While alcohols are effective solvents, their varying boiling points and solubility in water can be limiting. A solution is to use mixed solvents like a 1:1 combination of tetrahydrofuran and dichloromethane, which effectively removes polar substances from aqueous solutions.

6) Plasma proteins and emulsions

Proteins can hinder the removal of medication from plasma, leading to partialprecipitation that conceals the interface between layers and often results in emulsification. To precipitate the proteins, one can add five litres of a water-miscible solvent like acetonitrile, or use 10–20% trichloroacetic acid.

7) Role of pH for solvent extraction

Organic acids and bases have lower water solubility compared to salts. Bases are typically extracted into organic solvents at a high pH, usually two pH units above their pKa, while acids are extracted at low pH. Pharmaceuticals may be back extracted from organic solvents into acid or basified for re-extraction if they are suitably non-polar bases, and the reverse applies for acidic drugs.[8]

Analytical Method Validation

Validation is the process of ensuring that procedures, strategies, and instruments operate according to predefined criteria, emphasizing product conformity and analysis. It significantly impacts the pharmaceutical industry by ensuring the reliability, accuracy, and consistency of test results. During testing, validation can reveal issues and external interferences that need to be addressed to achieve these objectives.[9]

Types of Validation

1) Process Validation
2) Analytical Method validation
3) Cleaning Validation
4) Computerized System Validation

Key parameters of analytical method validation

- Accuracy
- Precision
  - Repeatability
  - Intermediate precision
  - Reproducibility
  - Specificity/Selectivity
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- Linearity
- Range
- Robustness
- Ruggedness
- System suitability testing.

Accuracy

Accuracy of an analytical method is defined by how closely test results align with the true value, assessed in terms of percentage recovery of a known analyte amount within the linearity range.

Precision

Analytical method precision is defined as the closeness of agreement between measurements obtained from multiple samplings of the same standardized sample under prescribed conditions.

Specificity

The specificity of an assay is its capacity to accurately and exclusively measure an analyte despite the presence of potentially interfering substances, as defined by ICH. A method that elicits a response for a single analyte is termed specific.

Limit of detection

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, although it may not always be quantifiable under specific experimental conditions. It indicates whether a sample is above or below a certain threshold, influenced by the type of instrument and the analysis process used.

Limit of quantitation

The LOQ (Limit of Quantification) refers to the smallest amount of analyte in a sample that can be quantitatively identified with satisfactory precision and accuracy, influenced by the procedure and sample makeup. It is primarily used for identifying contaminants or degradation products.

Linearity

The capacity of a method to yield test results that accurately reflect analyte concentration over a defined range is termed linearity. Assessing a linear relationship is essential in analytics, and it is recommended to utilize a minimum of five different concentrations to evaluate linearity.

Range

The range of an analytical technique is defined by the upper and lower analyte concentrations that demonstrate an adequate level of linearity, precision, and accuracy. This range is typically established through linearity experiments and varies based on the intended use of the process.

Robustness

It assesses the analytical method's capacity to tolerate small, deliberate alterations in process, providing insights into its variability in standard laboratory conditions.

Ruggedness

Repeatability of test results is assessed by examining the same sample across various testing scenarios, which include differences in analysts, instruments, testing days, reagents, and columns or TLC plates.

System suitability testing

Testing for system appropriateness is vital in analytical processes, focusing on the fitness of a technique or system post-validation. System suitability tests assess the entire apparatus, including electronics and samples, according to the validated procedure. A fundamental example is comparing a chromatogram trace with a standard in HPLC system suitability testing.[10]

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