Extraction of Drugs and Metabolites from Biological Matrices

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Basic Idea

- Bioanalytical methods are used to detect, identify, and measure drugs, metabolites, and biomarkers in biological samples.
- Biological matrices are complex materials obtained from living organisms. Common biological matrices include blood, plasma, serum, urine, saliva, tissues, cerebrospinal fluid, and breast milk.
- Drugs and their metabolites are usually present in very low concentrations within these matrices.
- Biological samples contain many endogenous substances such as proteins, lipids, salts, carbohydrates, and cellular components that can interfere with analysis.
- Sample preparation is an essential step in modern bioanalytical techniques because it removes interfering substances and concentrates the analyte of interest.

General Need for Sample Preparation

Why Sample Preparation is Required

- Biological matrices are highly complex and can interfere with analytical measurements.
- Sample preparation improves the selectivity and sensitivity of bioanalytical methods.
- It removes proteins, phospholipids, and other endogenous compounds that may affect analysis.
- It protects analytical instruments such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS) from contamination.
- It improves the accuracy, precision, and reproducibility of analytical results.
- It helps concentrate analytes that are present at low concentrations.
- It reduces matrix effects that can suppress or enhance analytical signals.

General Principle of Drug and Metabolite Extraction

- The main principle of sample preparation is to separate the target analyte from interfering substances present in the biological matrix.
- The extraction method is selected according to the chemical and physical properties of the analyte.
- Factors such as solubility, polarity, pKa, molecular weight, stability, and protein binding influence the choice of extraction method.
- The selected method should provide high recovery, minimal matrix interference, and good reproducibility.

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General Procedure in Bioanalytical Sample Preparation

1. Sample Collection

- Biological samples are collected using appropriate procedures to prevent contamination.
- Proper labeling and documentation are essential.

2. Sample Storage

- Samples are stored under suitable conditions to prevent degradation of the analyte.
- Low temperatures are commonly used to maintain stability.

3. Sample Preparation

- Interfering substances are removed using an appropriate extraction technique.
- The analyte may be concentrated during this step.

4. Analysis

- The processed sample is analyzed using suitable analytical instruments such as HPLC, LC-MS/MS, GC-MS, or immunoassays.

5. Data Interpretation

- The concentration of the analyte is calculated and interpreted according to study requirements.

First Hope

Protein Precipitation

- Protein precipitation is the simplest and most commonly used sample preparation technique in bioanalysis.
- The method removes proteins from biological samples by causing them to lose their solubility and form solid precipitates.
- This technique is frequently used for plasma and serum samples.

Principle

- Organic solvents or strong acids disrupt the structure of proteins and reduce their solubility.
- The precipitated proteins are separated from the liquid phase by centrifugation.
- The analyte remains dissolved in the supernatant and is collected for analysis.

Common Precipitating Agents

- Acetonitrile.
- Methanol.
- Ethanol.

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- Perchloric acid.
- Trichloroacetic acid.

Procedure

1. Sample Preparation

- A measured volume of biological sample is transferred to a suitable container.

2. Addition of Precipitating Agent

- An organic solvent or acid is added to the sample.

3. Mixing

- The sample is mixed thoroughly to ensure complete precipitation of proteins.

4. Centrifugation

- The sample is centrifuged to separate the precipitated proteins.

5. Collection of Supernatant

- The clear supernatant containing the analyte is collected.

6. Analysis

- The processed sample is injected into the analytical instrument.

First Hope

Advantages

- The procedure is simple and rapid.
- The method requires minimal sample handling.
- The technique is inexpensive.
- The method is suitable for high-throughput analysis.

Limitations

- The method provides limited sample clean-up.
- Matrix effects may still occur.
- Recovery may be poor for highly protein-bound drugs.
- The technique may not be suitable for very sensitive analyses.

Liquid-Liquid Extraction (LLE)

- Liquid-liquid extraction separates analytes based on their different solubilities in two immiscible liquids.
- One liquid is usually an aqueous biological sample, and the other is an organic solvent.

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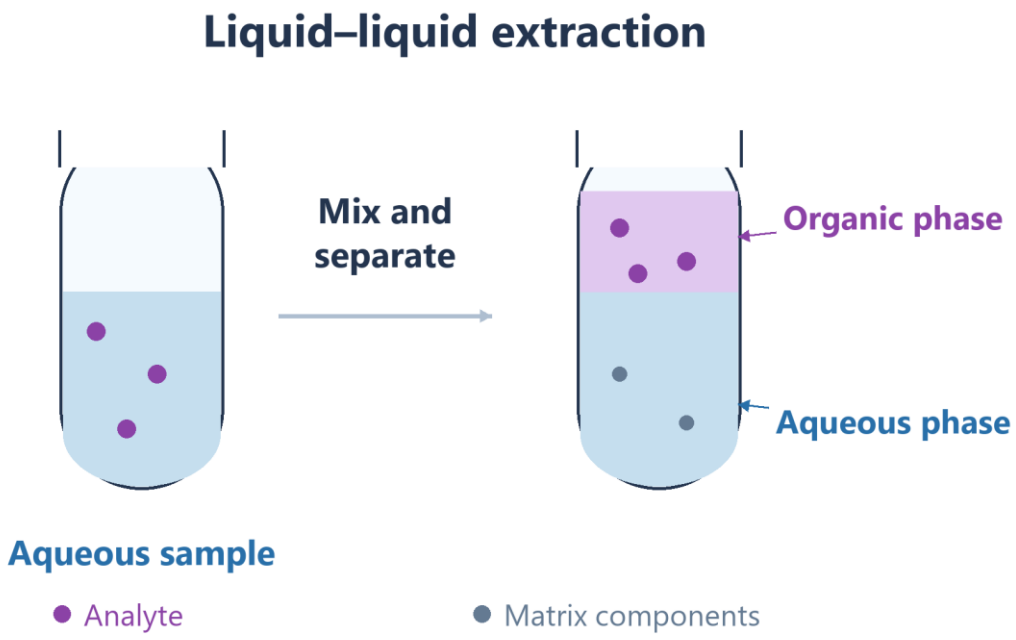
Principle

- The analyte distributes between the aqueous phase and the organic phase according to its partition coefficient.
- The extraction efficiency depends on the analyte's polarity, pKa, and ionization state.
- Adjusting the pH of the sample can improve extraction by converting the analyte into its non-ionized form.

Common Organic Solvents

- Ethyl acetate.
- Methyl tert-butyl ether.
- Dichloromethane.
- Hexane.
- Diethyl ether.

Procedure



The analyte partitions between immiscible phases; the organic phase is lighter in this illustrative example

1. Sample Preparation

- The biological sample is placed in an extraction tube.

2. pH Adjustment

- The pH is adjusted when necessary to optimize analyte extraction.

3. Addition of Organic Solvent

- A suitable organic solvent is added to the sample.

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4. **Mixing**
   - The mixture is shaken or vortexed to allow analyte transfer into the organic phase.
5. **Phase Separation**
   - The sample is centrifuged to separate the aqueous and organic layers.
6. **Collection of Extract**
   - The organic layer containing the analyte is collected.
7. **Evaporation and Reconstitution**
   - The solvent is evaporated, and the residue is reconstituted in a suitable solvent.
8. **Analysis**
   - The prepared sample is analyzed.

Advantages

- The method provides cleaner extracts than protein precipitation.
- The technique improves analyte concentration.
- The method is suitable for many lipophilic compounds.

Limitations

- The procedure is time-consuming.
- Large volumes of organic solvents are required.
- Emulsion formation may occur.
- The method is difficult to automate.

Solid Phase Extraction (SPE)

Basic Idea

- Solid phase extraction is a selective sample preparation technique that uses a solid sorbent material to isolate analytes from biological samples.
- This technique provides excellent sample clean-up and concentration.

Principle

- The analyte is selectively retained on a solid sorbent while unwanted matrix components are removed.
- The retained analyte is later eluted using an appropriate solvent.
- Separation is based on interactions such as hydrophobic, polar, ionic, or mixed-mode interactions.

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