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SPE for biological fluids

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Introduction

Sample preparation is a critical step prior to LC or GC analysis. Due to their nature, biological fluids such as serum, plasma, blood, and urine present a difficult challenge.

Solid Phase Extraction (SPE) can be used for three important purposes:

- Removal of interfering matrix components. This can help protect analytical instrumentation and columns from “dirty” biological samples
- Concentration of the sample to increase sensitivity of the analysis
- Transfer of analytes into a suitable solvent for analysis. This can help make the sample more compatible for analysis.

Important notes

- For effective analyte retention, pre-treat and optimize the sample, ensure analytes are free in solution or choose an appropriate hydrolysis procedure, and remove excessive particulates via filtration or centrifugation.
- Dilute sample with an equal volume of water or suitable buffer prior to applying the sample to the SPE column/96-well plate. Buffer choice and pH considerations are dependent upon the compound of interest in the sample. If diluting in buffer, ensure the sample is at a proper pH for optimum retention.
- Capacity of the SPE devices are approximately between 1–5% of the sorbent bed weight. This needs to be verified with your individual sample.

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Materials required

- Appropriate solvents and buffers required for conditioning and washing the SPE columns/96-well plates
- Vacuum manifold, positive pressure manifold, or centrifuge
- Tubes, vials, or 96-well plates for collection of filtrates

Reversed phase protocol for acidic, basic, and neutral compounds

1. Condition SPE device with 2–5 column volumes of a strong solvent like methanol
2. Equilibrate with 2–5 column volumes of a weak solvent like water. Do not let packing bed dry before application of sample.
3. Apply sample and push or draw sample through the packing bed at a flowrate of about 1 mL/min
4. Wash off any weakly retained interferences with a weak polar solvent such as methanol/water; 5/95 (v/v)
5. Elute using a strong elution solvent such as methanol and collect for analysis

Mixed mode cation exchange procedure for basic ionizable compounds

1. Condition SPE device with 2–5 column volumes of a strong solvent like methanol
2. Equilibrate with 2–5 column volumes of water with 1% formic acid. Do not let packing bed dry before application of sample.
3. Apply sample and push or draw sample through the packing bed at a flowrate of about 1 mL/min
4. Wash off any weakly retained interferences using water with 1% formic acid
5. Elute using a strong elution solvent such as methanol with 1% ammonium hydroxide and collect for analysis

Mixed mode anion exchange procedure for acidic ionizable compounds

1. Condition SPE device with 2–5 column volumes of a strong solvent like methanol
2. Equilibrate with 2–5 column volumes of water with 1% ammonium hydroxide. Do not let packing bed dry before application of sample.
3. Apply sample and push or draw sample through the packing bed at a flowrate of about 1 mL/min
4. Wash off any weakly retained interferences using water with 1% ammonium hydroxide
5. Elute using a strong elution solvent such as methanol with 1% formic acid and collect for analysis

Recommended wash and elution volumes

Sorbent Bed Weight	Recommended Wash and Elution Volume
2 mg	0.2 mL
10 mg	0.5 mL
30 mg	1–2 mL
60 mg	2–3 mL
200 mg	3–4 mL
500 mg	5–6 mL

Related products

Description	Part Number
Thermo Scientific™ Hypersep™ Universal SPE Vacuum Manifold, for 96-well plate or 24/48 cartridges	60104-233
Thermo Scientific™ Hypersep™ Vacuum Pump, European version	60103-351
Thermo Scientific™ Hypersep™ Vacuum Pump, North American version	60104-243

Current versions of product instructions are available at **separatedbyexperience.com/chromexpert**

Learn more about HyperSep SPE extraction and clean-up products at **separatedbyexperience.com/chromexpert**

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