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General reversed phase SPE optimization

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Introduction

Solid Phase Extraction (SPE) can be used to make samples compatible with analysis, to concentrate or dilute the sample in the appropriate solvent, or to remove matrix interferences. Proper sample preparation improves analytical results and reduces instrument downtime. Reversed phase SPE sorbents can be polymeric or silica-based C18, and elution is performed with organic solvent. This document describes a general procedure for optimizing reversed phase SPE clean-up methods.

Important notes

- Reversed Phase (RP) SPE is typically used with moderately polar to non-polar analytes that are uncharged. Consider ion exchange SPE for acidic or basic analytes.
- The method optimization instructions in this protocol are for a 1 mL column Thermo Scientific™ HyperSep™ Retain PEP cartridge with a 30 mg bed weight. Solvent volumes should be adjusted accordingly if different bed weights are used.
- Do not allow the sorbent to dry between conditioning steps or before sample application. Prior to elution, fully dried cartridges will ensure optimal analyte recovery.
- Analytes that are not in their correct ionization state (i.e., neutral or charged) will not effectively bind to the sorbent and may result in erratic recoveries. For charged compounds, a pH at which the compound is not charged should be used.

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Materials required

- Reversed Phase SPE Columns
- Solvents for conditioning, loading, wash and elution
- Vacuum or positive pressure SPE manifold

Protocol

1. Condition column with 1 mL methanol followed by 1 mL water.
2. Load 1 mL of 500 ng/mL sample in water. Collect any flow-through eluate.
3. Wash sequentially with increasing strengths of methanol in water, collecting the eluent 0% methanol/100% water to 90% methanol/10% water increasing the methanol content by 10% each time.
4. Elute with three volumes of 100% methanol, collecting each as an individual fraction.
5. Analyze each fraction and use the following guidelines to optimize the method.

Method optimization

1. If the analyte is present in the Load fraction, the bed weight is too small relative to the sample matrix volume and breakthrough has occurred. Reduce the sample load or increase sorbent bed weight. Typical bed capacity is a maximum of 5% (silica-based C18) to 10% (polymeric) of the bed weight, including buffers, salts, and interferences in addition to the analyte of interest.
2. The wash fraction immediately before the analyte begins to appear in the analyzed fractions represents the optimum wash composition.
3. The optimum elution solvent is determined by the fraction at which the total recovery is close to 100%.
4. If 100% of the loaded analyte is not recovered after three washes with 100% methanol, a stronger elution solvent is required. Suggested elution solvents in order of increasing elution strength are: Methanol, Acetonitrile, THF, and Ethyl Acetate.
5. Optimization of interference removal can be performed by varying the pH of the wash and elution solvents.

Related products

Description	Part Number
HyperSep Retain PEP Cartridge, 30mg 1 mL, 100/pk	60107-201

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

Learn more about Solid Phase Extraction products at
separatedbyexperience.com/chromexpert

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