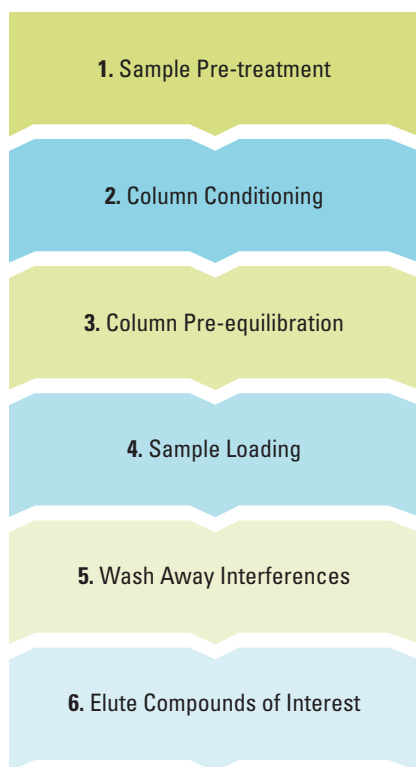


SPE Procedure – Six Steps for a Clean Extract



1. Sample Pre-treatment

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It is important to optimize the sample for effective analyte retention. The following should be considered:

- Sample volume/analyte concentration/matrix complexity
- Adjust sample/matrix composition for proper dilution/ionic strength
- Sample pH for optimum retention
- Confirm that analytes are free in solution
- Remove any unwanted particulates via filtration or centrifugation

2. Column Conditioning

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Prepare the sorbent for effective interaction(s) with the compounds of interest.

- Use appropriate solvent for column condition/activation
- Prevent sorbent drying during conditioning

3. Column Pre-equilibration

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Equilibrate with weakly eluting solvent to prepare the phase for sample addition.

- Use the same solvent as for sample pre-treatment
- Prevent sorbent drying during column equilibration

4. Sample Loading

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Analytes are retained on the sorbent.

- Apply samples at appropriate flow rate (1mL/minute typical)

5. Wash Away Interferences

For Reversed-Phase Interactions

- Neutral compounds are not affected by pH
- For charged compounds, a pH at which the compound is not charged is used. Neutralize the molecule according to the following:
 - For basic compounds, the neutral molecule exists at least 2pH units below the pK_a of the compound
 - For acidic compounds, the neutral molecule exists at least 2pH units above the pK_a of the compound

For Normal-Phase Interactions

- pH is not normally an issue in normal phase interactions, as the solvents used are typically non-polar organic solvents, rather than water
- There is no need to verify the sample application pH

For Ion-Exchange Interactions

- pH and pK_a are important considerations
- Acidic compounds are extracted from a sample solution at least 2pH units above the pK_a of the analyte
- Basic compounds are extracted from a sample solution 2 or more pH units below the pK_a of the analyte
- For second (organic) wash, choose the strongest solution where no compound breakthrough occurs
- For elution step, use a solution stronger than where all the compound of interest is eluted
- NB: when choosing these solutions allow some margin for error

5. Wash Away Interferences

Remove impurities bound less strongly than the compounds of interest.

- Select a strong enough wash solvent to remove interferences but weak enough to leave compounds of interest bound
- Selectively rinse away the less strongly bonded interferences
- Wash solvent selected according to phase mechanism/analyte properties

6. Elute Compounds of Interest

Selectively recover the analyte(s) by disrupting the analyte-sorbent interaction.

- Selectively elute analytes of interest using different solvents
- Smaller elution volume produces a more concentrated extract
- Select elution solvent that does not elute strongly retained impurities
- Select elution solvent according to phase mechanism/analyte properties

It is important to optimize the Wash and Elution steps in order to obtain maximum levels of recovery.

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