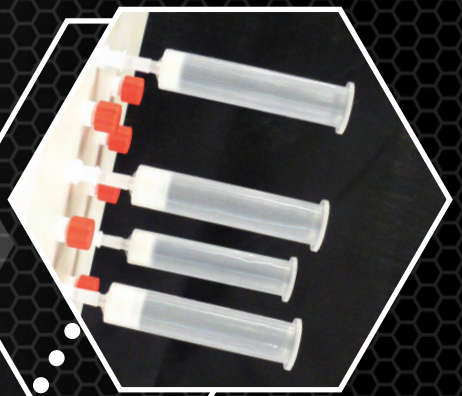
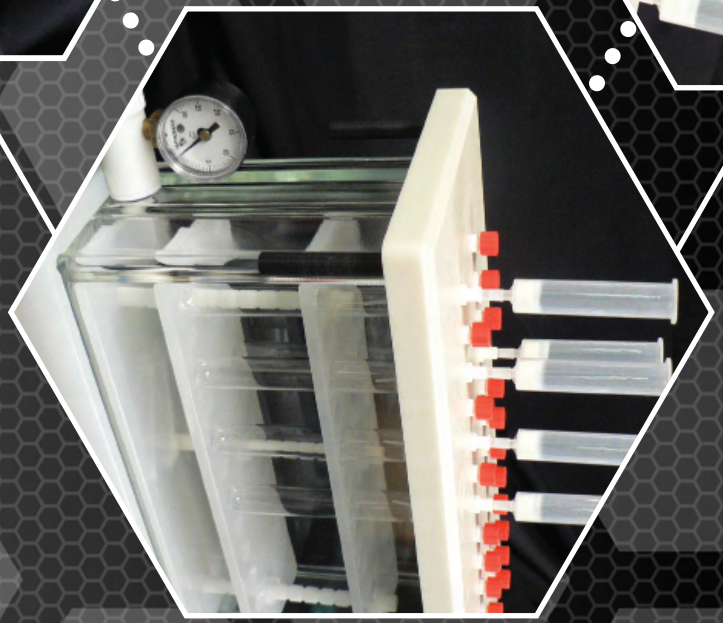
YOUR SPE STRATEGY

Material Solutions. Uncompromising Integrity.





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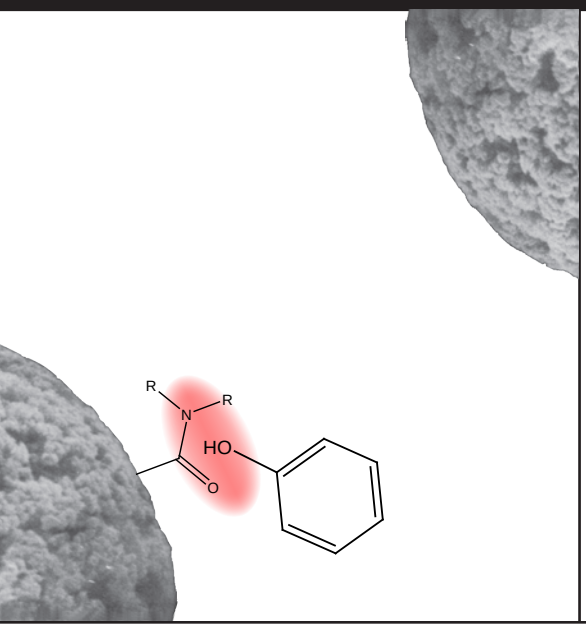

Normal-Phase

Normal-phase SPE retains sample analytes through polar/hydrophilic interactions between the analyte and resin. Typically, sample matrices include non-polar solvents (i.e. heptane, isooctane, hexane) containing a hydrophilic compound. Less polar or non-polar solvents maximize analyte retention, while more polar solvents cause elution.

Organic compounds having polar character are selectively retained on the resin through hydrogen bonding, dipole-dipole interactions or pi-pi interactions. Analytes with structural similarities can be separated based on their degree of polarity and type of interaction. Typical analytes for normal-phase SPE include those with hydroxyl groups, amines, carbonyls, or heteroatoms (O, N, S, P).

Normal-Phase Cartridges: Hydroclean RP™ (HRP)

Normal-Phase Concepts

Retention
Polar interactions including hydrogen bonding and dipole interactions

The Matrix
Non-polar samples, including: organic extracts, non-polar solvents and hydrocarbons

The Analyte
Polar analytes, including those with hydroxyl, amine or carbonyl functional groups or other heteroatoms (O, N, S, P)

Elution
Select a more polar solvent or solution to disrupt matrix/resin interactions (i.e. acetonitrile, methanol, isopropanol and buffer/solvent mixtures)

Applications
- Purification of organic extracts from environmental samples (i.e. soil samples)
- Removal of polar components from hydrocarbons

Methodology

1. ***Sample Preparation*** - Solid samples can be extracted using a non-polar solvent prior to SPE processing. It is common to extract or dilute with a non-polar or chlorinated solvent when preparing liquid samples.

Tips: A drying step may be necessary in the preparation of organic extract samples, as aqueous remnants may decrease the retention of analyte in normal-phase SPE.

2. ***Conditioning*** - Conditioning typically consists of adding 2 or more cartridge volumes of a non-polar solvent. It is preferable that the solvent used matches the solvent present in the sample matrix.

3. ***Sample Addition*** - Optimum sample retention can be achieved by adding the sample to the SPE tube and controlling the flow at a rate of ≤1mL/min. Strongly hydrophobic solvents promote sample retention.

Tips: Avoid using hydrogen bonding or strongly polar solvents such as methanol in this step, as these solvents are common elution solvents.

4. ***Washing*** - The wash step selectively removes interferences from the resin upon sample addition, while avoiding the elution of the analyte of interest. Typically, 1-2 cartridge volumes of a non-polar solvent are sufficient for washing.

Tips: Using sample preparation and conditioning solvents in the wash step is common practice.

5. ***Elution*** - Successful elution of sample components requires that the solvent disrupts the polar interactions between the sample and resin. Fractionation of various compounds is possible by adding solvents with increasing polarity to the resin bed. Typical elution solvents include methanol and acetonitrile.

Jordi Labs 4 Mill Street Bellingham, MA 02019 Phone: 1-508-966-1301 Fax: 1-508-966-4063 Email : www.jordilabs.com

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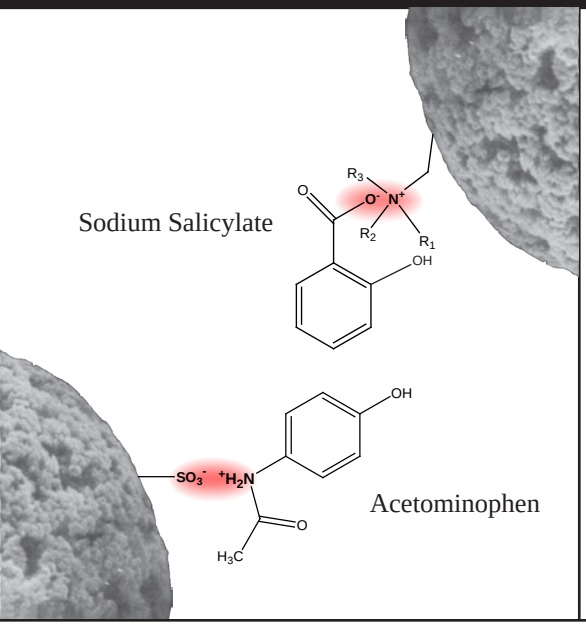

Ion Exchange

Ion exchange SPE uses the attractive forces between ions of opposite charge to achieve sample retention. Ion exchange SPE is typically used for the separation of charged species including amines, carboxylic acids, sulphonic acids and phosphates.

Hydroclean ion exchange resins have been designed to allow for mixed-mode separations, combining reverse phase with ion exchange properties. The primary factor determening this balance is the ionization state of compound as controlled through pH selection. Elution is ccontrolled by adjusting pH or adding counter ions to disrupt the favorable interactions between the analyte and resin functional groups.

** Anion exchange refers to the extraction of acidic compounds, whereas cation exchange includes extraction of basic compounds*

Ion-exchange Cartridges: HRP-CX, HRP-AX

Ion Exchange Concepts

Retention

Oppositely charged functional groups on the analyte and resin interact based on electrostatic attraction in ion exchange SPE. Mixed-mode separations incorporate reversed-phase interactions along with ion exchange.

The Matrix

Both aqueous and organic matrices apply to ion exchange and mixed-mode SPE (i.e. biological fluids). Ideally, these samples should have less than 50 mmol salt content.

The Analyte

Acidic or basic compounds

Elution

Disruption of electrostatic matrix/resin interactions is the foundational elution concept. Neutralization of ionizable groups in the analyte or resin functional groups aids in this process. Addition of salts may be used to disrupt sample resin interactions.

Tips: Counter-ion selectivity and increased salt concentration are two factors affecting successful elution.

Applications

- Acidic or Basic Compounds
- Drugs of Abuse (Opiates, Narcotics)

Methodology

1. ***Sample Preparation*** - Solution pH should be selected such that analyte and resin functional groups are ionized. This can be accomplished by adding an appropriate buffer in a 1:1 volume ratio. Typically, buffers of pH 7-9 are used to dilute acidic samples. Buffers of pH 3-6 are used to dilute basic samples. Commonly used buffer systems include phosphate, or acetates.

Tips: Mixed-mode SPE is preferable for sample matrices containing higher salt content or a larger number of interferences.

2. ***Conditioning*** - For samples in non-polar solvent, the same solvent should be used in the conditioning step. Adding 1-2 cartridge volumes of methanol or acetonitrile is appropriate for aqueous samples.

Tips: Add the same or a similar buffer as in the sample preparation step to equilibrate the resin

3. ***Sample Addition*** - The success of ion exchange SPE methods depends on a slow flow rate of ~1 mL/min. Maintaining this rate promotes better recovery.

Tips: Strong organic solvents such as acetonitrile and methanol often reduce analyte retention.

4. ***Washing*** - The wash step typically includes the addition of an appropriate buffer. Care should be taken to maintain an appropriate pH and ionic strength.

5. ***Elution*** - The primary methods used to achieve sample elution include increasing ionic strength or ajusting pH. Solutions of higher ionic strength (higher charge or higher concentration) are stronger eluents.

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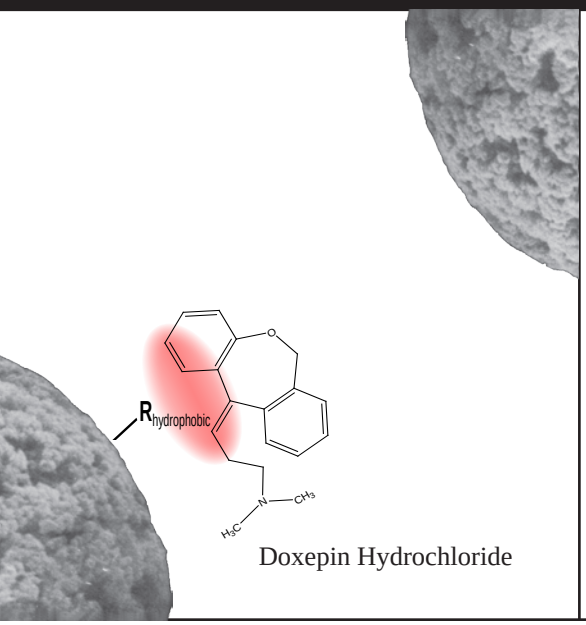

Doxepin Hydrochloride

Reverse-Phase

Reverse-phase SPE involves retention by hydrophobic or non-polar analyte-resin interactions. This type of SPE is the most broadly applicable as most analytes contain at least some potential for reverse phase interactions. It is also one of the simplest modes of chromatography to perform and is especially useful for removal of analytes from aqueous solutions. Thus, it is applicable for many biological matrices.

To encourage reverse phase retention, it is generally beneficial to select the sample pH such that the target analyte is neutralized. Hydrophobic character increases in neutral analytes, optimizing retention on the resin bed. Common reverse-phase analytes include: most organic compounds and compounds with aromatic, alkyl and alicyclic functionalities.

Reverse-Phase Cartridges: HRP DVB

Reversed-Phase Concepts

Retention
Non-polar or hydrophobic interactions

The Matrix
Aqueous samples, including: water samples, biological fluids (serum, urine, plasma), and aqueous tissue extracts

The Analyte
Most organic compounds, especially those with aromatic, alicyclic and alkyl functional groups that exhibit non-polar characteristics

Elution
Selecting non-polar solvent or solvent mixtures that disrupt matrix/resin interactions (i.e. methanol, ethyl acetate, dichloromethane and buffer/solvent mixtures)

Applications
- Drugs of abuse, pharmaceuticals and their metabolites in blood, plasma or urine
- Aqueous extracts
- Aqueous environmental samples

Methodology

1. ***Sample Preparation*** - Samples should be loaded using predominately aqueous solutions. Sample dilution with aqueous buffers can be used to thin viscous samples (i.e. serum) and to increase retention of ionizable compounds (neutral forms are more strongly retained). To effectively neutralize the analyte, adjust the pH ± (2) units from the compound's pKa.

Tips: Filtration or centrifugation may be necessary to avoid clogging the SPE tube.

2. ***Conditioning*** - Conditioning refers to the activation of the resin bed for sample reception. Typically, one or more cartridge volumes of an organic solvent such as methanol is used.

Tips: An equilibration step may be essential to optimize retention. Equilibration includes adding an "imitation sample solution" to the SPE cartridge, which has pH and solvent characteristics similar to the actual sample.

3. ***Sample Addition*** -To achieve ideal analyte retention, the flow rate should be maintained at approximately 1mL/min.

4. ***Washing*** - The wash step selectively removes interferences from the resin following sample addition. Typical wash solutions include dilute organic/water mixtures such as methanol/water.

5. ***Elution*** - Elution refers to the displacement of the analyte of interest. This is typically accomplished using an organic solvent such as methanol, ethyl acetate, dichloromethane, or an organic buffer such as methanol with ammonium acetate. Typically, a volume of nearly 4 times the bed volume is sufficient to elute the analyte (bed volume consists of the resin volume with the interstitial spaces).

Tips: Preventing ionization of the analyte by adjusting the pH during elution may help to avoid the risk of reduced recovery and decreased elution volumes.