"New Mechanisms of Solid Phase Extraction to Improve Your Analytical Results"

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Types of Sorbent-Analyte Interactions

- Polar
- Non-polar
- Ion-exchange
- Covalent
- Copolymeric

Polar Extractions

- Also called hydrophilic or normal phase
- Unequal distribution of electrons
- Involves hydrogen bonding, pi-pi and dipole/dipole interactions
- Sorbents - silica, diol, diethylamino, cyanopropyl
- Applications - lipids, oil additives, carbohydrates, phenols, oil soluble vitamins
- Analytes - amines, hydroxyls, carbonyls, aromatic rings, heteroatoms (O, S, N, P)
- Matrix - non-polar, organic
- Elution solvents - medium to high polarity

Non-Polar Extractions

- Also called hydrophobic or reverse phase
- Interactions between sorbent C-H bonds and analyte C-H bonds
- Involves van der Waals / dispersion forces
- Sorbents - C2, C3,C4, iC4, tC4, C5, C6, C7, C8, C10, C12, C18, C20, C30 phenyl and cyclohexyl
- Applications - drugs of abuse, TDM, pesticides
- Analytes - protonated / neutral state, aromatics & alkyl chains
- Matrix - biologicals, water, aqueous buffers
- Elution solvents - typically non-polar to moderately polar

Ion Exchange Mechanisms

- Ionic interactions occur between charged sorbent & analyte of opposite charge
- pH is manipulated to ionize analytes functional group
- Ionic bonds are strong & retain analyte
- Hydrophobic interferences washed away with organic solvents
- Polar interferences removed with aqueous or weak aqueous / organic washes
- Elute solvents containing stronger counterions or by changing pH
- For ionic/hydrophobic analytes, elute by simultaneously disrupting both interactions

Cation Exchange Extractions

- Cation exchange sorbents negatively charged
- Basic analytes manipulated to carry positive charge
- Opposites attract forming strong bonds
- Sorbents
  - Benzenesulfonic acid (strong)
  - Propylsulfonic acid (strong)
  - Carboxylic acid (weak)
- Applications include basic drugs, catecholamines, pharmaceuticals, herbicides
- Analytes
  - Amines
  - Pyrimidines (cations)
- Matrix - aqueous
- Basic elution solvents to neutralize analyte

Anion Exchange Extractions

- Anion exchange sorbents positively charged
- Acidic analytes manipulated to carry negative charge
- Opposites attract forming strong bonds
- Sorbents
  - 1°, 2° amine
  - Aminopropyl (weak)
  - Quaternary amine (strong)
  - Diethylamino (weak)
- Applications include phosphates, acidic drugs, organic acids, fatty acids, vitamins
- Analytes
  - Phosphates
  - Carboxylic acids
  - Sulfonic acids (cations)
- Matrix - aqueous
- Acidic elution solvents to neutralize analyte

Copolymeric Extractions

- Hydrophobic & ionic retention mechanisms
- Reverse phase sorbent with cation OR anion exchange
- Acidic, basic & neutral analyte applications
- Matrix - aqueous
- Selective washes
- Elution solvents mixture of organics with acid or base
- Superior sample clean up

pKa, pH & Ionization

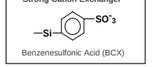
% of Compound in Ionic State

Functionality	Ionization State	pH units away from pKa 2<	1<	at pKa	1>	2>
Acid	Anion (-)	1	9	50	91	99
Base	Cation (+)	99	91	50	9	1

Relative Counter ion Selectivity

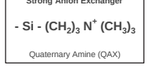
Larger numbers reflect greater ability of the ion to displace other ionic materials from the bonded surfaces

Strong Cation Exchanger



Benzenesulfonic Acid (BCX)

Strong Anion Exchanger



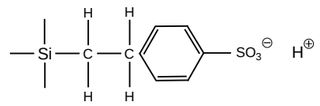
Quaternary Amine (QAX)

Cations		Anions	
Ba^{2+}	8.7		
Ag^{2+}	7.6	Benzene Sulfonate	500
Pb^{2+}	7.5	Citrate	220
Hg^{2+}	7.2	I^-	175
Cu^+	5.3	Phenate$^-$	110
Sr^{2+}	4.9	HSO_4^-	85
Ca^{2+}	3.9	ClO_3^-	74
Ni^{2+}	3.0	NO_3^-	65
Cd^{2+}	2.9	Br^-	50
Cu^{2+}	2.9	CN^-	28
CO^{2+}	2.8	HSO^-	27
Zn^{2+}	2.7	BrO	27
Cs^{2+}	2.7	NO_2	24
Rb^+	2.6	Cl^-	22
K^+	2.5	HCO_3^-	6.0
Fe^{2+}	2.5	IO_3^-	5.5
Mg^{2+}	2.5	Formate$^-$	4.6
Mn^{2+}	2.3	Acetate$^-$	3.2
NH_4^+	1.9	Propionate$^-$	2.6
Na^+	1.5	F^-	1.6
H^+	1.0	OH^-	1.0
Li^+	0.8		

Standard cation exchange counter ion

SPE Amine Scavenger

Purification of Small Molecule Libraries by Pharmasil® Ion Exchange SPE



UCT Column Part Number: CUBCX156
Sorbent Amount: 500mg
Column Volume: 6 mL

Sample Pre-treatment
Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized.

Column Conditioning
Condition the column with the appropriate solvents. (ethylacetate/hexane, methanol/ethylacetate, methanol, often times the elution solvent makes an excellent conditioning solvent).

Column Equilibration
Equilibrate the column with the same solvent you pretreat the sample with.(buffer, ethylacetate/hexane, etc.)

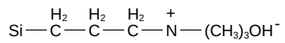
Sample Application
Apply the sample to the column under gravity. Positive pressure or vacuum can also be used just be certain the application rate does not exceed 1-2 ml per min. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the compound of interest exceeds the capacity of the sorbent you will not get the highest recovery of your compound. If you think this is a problem use a larger bed mass.

Product Purification
Elute neutral and polar reagents and byproducts with ethyl acetate,
25% methanol/ethylacetate, or buffers. (Caution: when using buffer washes be sure the pH of the buffer remains 2 pH units below the pKa of the compounds of interest you want to retain on the column)

Product Elution
Elute compound of interest with ethylacetate/ammonium hydroxide, ethylacetate/triethylamine, or ethylacetate/methanol/ammonium hydroxide. (the important factor is to be sure the pH of the elution solvent is 2 pH units above the pKa of your compound of interest. These solutions can be easily dried down to remove unwanted solvents before analysis.

SPE TFAA Removal

Purification of Small Molecule Libraries
TFAA Removal by Pharmasil® Ion Exchange SPE



UCT Column Part Number: CHQAX156
Sorbent Amount: 500mg
Column Volume: 6 mL

Sample Pre-treatment
Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of a pH 7 buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized... acid catalysts are strong anions and are charged across the complete pH range.

Column Conditioning
Condition the column with 1 ml of methanol followed by 1 ml of DI water.

Column Equilibration
Condition the column with pH 7 buffer.

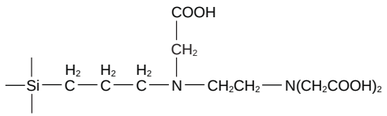
Application
Apply the sample to the column under gravity. The TFAA will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the TFAA exceeds the capacity of the sorbent you will not get the highest removal of TFAA. If you think this is a problem use a larger bed mass.

Product Purification
Wash the column with 1ml of buffer used in column equilibration.

Product Elution
Elute compound of interest with 1ml of methanol.

SPE Metal Removal

Purification of Small Molecule Libraries Palladium (Pd) Removal by Pharmasil® Ion Exchange SPE



UCT Column Part Number: CUTAX156
Sorbent Amount: 500mg
Column Volume: 6 mL

Sample Pre-treatment
Samples may or may not require pretreatment before addition. The primary concern using ion exchangers is to adjust the pH of the compound of interest so that it is totally ionized. This may require the addition of an acid or buffer. Ion exchange can be done out of organic solvents such as methanol or ethyl acetate as long as the compound of interest is ionized... Palladium catalysts are strong cations and are charged across the complete pH range. Adjust the sample to pH 9 with buffer or ammonium hydroxide.

Column Conditioning
Condition the column 1 ml of Methanol followed by 1 ml of water.

Column Equilibration
Condition the column with buffer of pH 9.

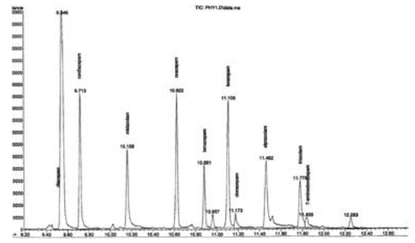
Sample Application
Apply the sample to the column under gravity. The palladium will stick to the column. The volume of the sample is not important and will probably be dictated by the equipment you use. The critical factor is concentration and capacity of the sorbent. If the concentration of the palladium exceeds the capacity of the sorbent you will not get the highest removal of palladium. If you think this is a problem use a larger bed mass.

Product Purification
Wash the column with 1ml of buffer used in column equilibration.

Benzondiazepines By Polar Reverse Phase

DEPARTMENT OF HEALTH

Compliments of Lisa Mundy (Philadelphia Dept. of Health)



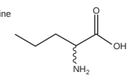
Chiral Solid Phase Extraction

Joint venture between UCT and ENS
ENS nanostructured sorbent does not require thousands of theoretical plates to get an effective separation

Chiral Enrichment of DL-Norvaline as a Function of pH Using ENS 1 mL Polar Cationic SPE Cartridges

Extraction Protocol Analyte samples were prepared at a concentration of 10 mg/mL (m/v) in 50:50 Ethanol/Water pH adjusted to values 2 through 6 extract a total volume of 1 mL analyte solution through an ENS 1mL Polar Cationic SPE cartridge. Each cartridge was then aspirated with Nitrogen to flush out any residual analyte solution. A centrifugal evaporator was used to concentrate the samples, which were then reconstituted in 70:30 Water/Methanol mobile phase to be analyzed using the Chirobiotic™ TAG column.

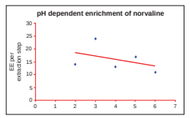
Analyte: DL-Norvaline
CAS#: 760-78-1



Concentration: 10 mg/mL
Cartridge Loading: 10 mg
Extraction Solvent: 50:50 Ethanol/Water pH adjusted using Acetic Acid or Triethylamine
Cartridge Flow Rate: 0.33 mL/min

Analysis Method: HPLC
Chirobiotic™ TAG 4.6 x 250 mm
Mobile Phase: 70:30 Water/Methanol
Flow Rate: 1.0 mL/min

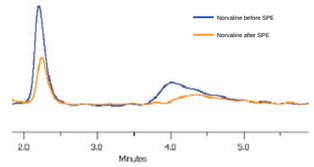
The chiral enrichment of norvaline is enhanced by lowering the pH of the extraction solvent. At a low pH acids on the surface of the sorbent are not charged, and the hydrophobic amino acid side chain drives adsorption and extraction.



pH	% EE	Conc. (mg/mL)	Loading (mg)	Cartridge Flow Rate (mL/min)
2	14 ± 5	10	10	0.33
3	25 ± 5	10	10	0.33
4	13 ± 5	10	10	0.33
5	17 ± 5	10	10	0.33
6	11 ± 5	10	10	0.33

Experimental errors in %EE are measurement errors arise from chromatographic noise and peak tailing.

Chiral Chromatograph - Norvaline before and after enantioenrichment



	Retention Time		Peak Area Ratio	EE
	Enantiomer 1	Enantiomer 2		
Before SPE	2.2067	4.0192	50/50	0
After SPE	2.2508	4.3708	62/38	24

Note: The difference in peak height for the first enantiomer results from different concentrations of norvaline injected onto the column.

For additional information: e-mail mnorman@unitedchem.com or call 215-781-9255 ext. 180