ANION EXCHANGE EXTRACTION: GENERAL GUIDELINES

SPE PROTOCOLS

A typical SPE extraction consists of 4 basic steps:

1. Activation. The sorbent bed is rinsed with a solvent designed to prepare the sorbent for interaction with the sample. This step is often called "conditioning" or "solvation".

2. Retention. The prepared sample is loaded onto the activated cartridge. Ideally only analyte would be retained while the matrix contaminants would pass through the cartridge to waste.

3. Washing. Additional matrix contaminants are rinsed away to waste by washing passing through the cartridge a solvent that is not strong enough to disrupt the sorbent/analyte interaction. To insure that all traces of washing solvent and contaminants are removed, the cartridge is typically dried under full vacuum to complete dryness.

4. Elution: A solvent designed to disrupt the chemical interaction between the sorbent and analyte is passed through the cartridge and collected for analysis.

Depending upon the nature of the sample, an additional specimen preparation step prior to the actual SPE extraction may be necessary. Like any sample preparation technique, they are performed in an effort to improve or facilitate further analysis. With SPE these specimen preparation steps are very simple steps designed to generate a strong sorbent-analtye interaction or to simply allow the sample to physically pass through the cartridge. Typically this involves simple straightforward techniques like dilution, pH adjustment, gross filtration, hydrolysis, etc..

ION EXCHANGE EXTRACTION METHOD DEVELOPMENT CONSIDERATIONS

Ion exchange extractions may be performed on analytes that posses ionizable functional groups. Sample composition is most commonly aqueous, although strongly ionized species may be extracted from organic samples. Ion exchange extractions are commonly performed solo or mixed with a non-polar sorbent to form so-called mixed phase SPE devices. Regardless of how they are used, ion exchange extractions are a powerful sample cleanup and concentration tool. Due to the very specific conditions under which most compounds are ionized, ion exchange extractions are the most complex and potentially least forgiving of all SPE extractions.

pH Effects:

Both sorbent and analyte must be quantitatively charged for effective, reproducible extraction. In practice this means that pH must be carefully controlled during all extraction steps.

Since a comprehensive discussion of acid/base chemistry is beyond the scope of this users guide, two very important factors or rules of thumb are:

1. Working definition of pKa:

pKa = the pH at which 50% of the species are protonated and 50% are unprotonated. For quantitative retention or elution, we need ionic species to be either 100% charged or uncharged. Common functional group pKa's are listed in table A.

Table A: Common Functional Group pKa's

Cation	pKa	Anion	pKa
R_4N+	>12.0	Benzene Sulfonic Acid	<1.0
R_2-NH+	11.0	R-COO-	4.8
R-NH2+	10.6	Phenol	10
R_3-NH+	9.8		
NH_4+	9.3		

2. "Rule of 2":

Quantitative ionization (99.9%) of a functional group occurs at a pH value 2.0 units above or below the pKa of the functional group.

To illustrate:

The typical pKa of a carboxylic acid functional group is approximately 4.5. At a pH of 4.5, 50% of the species in solution are protonated (neutral charge) and 50% are de-protonated (anionic). If we were to perform an anionic exchange extraction on the molecule at pH 4.5, the highest recovery we could expect would be 50%.

Disappointing, but understandable given the incomplete ionization of the analyte. Conversely, should we perform the extraction at pH 6.5 or higher (4.5 +2.0) the molecule would be quantitatively ionized so we could expect retention percentages in the high 90's. For elution, a solvent of pH 2.5 or lower (4.5 - 2.0=2.5), the carboxylic acid functional groups are protonated and neutrally charged effectively disrupting the ionic interaction between sorbent and analyte.

IONIZATION STATES AT VARIOUS pH's		
< pH 2.5	pH 4.5	pH > 6.5
R-COOH <--> R-COO⁻ >99% <1%	R-COOH <--> R-COO⁻ 50% 50%	R-COOH <--> R-COO⁻ <1% >99%
pH < 7.8	pH 9.8	pH > 11.8
$R\text{-}NH_2$ <--> $R\text{-}NH_3^+$ <1% > 99%	$R\text{-}NH_2$ <--> $R\text{-}NH_3^+$ 50% 50%	$R\text{-}NH_2$ <--> $R\text{-}NH_3^+$ >99% <1%

Counter Ion Effects:

Ions in the sample solution compete with the analyte for sorbent binding sites. Poor recovery of analyte can occur if these counter ions have a greater affinity for the sorbent than does the analyte. This occurs in two ways: high counter concentrations (ionic strength) and high counter ion chemical affinity for the bonded phase (high counter ion selectivity).

Retention of analyte is facilitated by loading the sample in a buffer or solvent system of the proper pH at low ionic strength (<0.010 M) buffer comprised of low selectivity ions. (E.g. 0.005 M Acetic Acid).

Elution is promoted by passing high ionic strength (>0.10 M) buffers of high selectivity counter ions. (e.g. 0.50 M Citric Acid).

Relative Counter Ion Selectivity	
Listed from strong to weak. Ions on the left displace ions on the right.	
Cations	Ba^{+2} > Pb^{+2} > Ag^{+} > Cu^{+} > Ca^{+2} > Fe^{+2} > K^{+} > NH_4^{+} > Na^{+} > H^{+} > Li^{+}
Anions	Benzene Sulfonate^{-} > Citrate^{-} > Br^{-} > Cl^{-} > HCO_3^{-} > Formate^{-} > Acetate^{-} > OH^{-} = Fl^{-}

Solvent Volumes:

The following table outlines suggested volumes for various SPE cartridge configurations. Note: solvent volume requirements are directly proportional to sorbent MASS and not the tube reservoir size (in ml or cc). More specifically, minimum solvent volume is determined by cartridge bed dead volume which is proportional to sorbent mass.

Sorbent Mass	**Minimum Wash and Elution Volume (2 bed volumes)**	**"Safe" Wash and Elution Volume (4 bed volumes)**
50 mg	125 ul	250 ul
100 mg	250 ul	500 ul
200 mg	500 ul	1 ml
360 mg	900 ul	1.8 ml
500 mg	1.25 ml	2.5 ml
1 g	2.5 ml	5 ml
2 g	5.0 ml	10 ml
5 g	12.5 ml	25 ml
10 g	25.0 ml	50 ml

I. Weak Anion Exchange:

Weak anion exchange is commonly performed on strongly anionic (acidic) compounds such as organic acids, phosphates and sulfates. Weakly acidic compounds containing carboxylic acids may also be retained on a weak anion exchanger although the pH range at which quantitative retention may occur is quite narrow and specific (pH 6-8) due to the requirement that both sorbent and analyte be quantitatively charged.

Weak anion exchange sorbents are typically organic primary, secondary or tertiary amines which are strongly ionized at neutral pH, but readily neutralized by moderately basic pH conditions.

Strong acids are typically eluted by neutralization of the sorbent charge, while weak acids may be eluted by neutralization of either the acidic analyte or the basic sorbent.

I. Specimen Preparation:

Procedure:

Dilute sample 1:1 with buffer or water ensuring that sample pH is at least 2 pH units above the pKa of the isolate.

Notes: Sample pH should be adjusted with buffer to ensure that both the analyte and sorbent are quantitatively charged.

2. Condition

Procedure:

Pass a minimum of 4 bed volumes of water miscible organic, followed by a minimum of 4 bed volumes of sample buffer through the cartridge at a flow rate of 2-4 ml / min.

Notes: Typical water miscible conditioning solvents are Methanol, Ethanol, ACN and IPA. The aqueous buffer used to condition the cartridge should be the same buffer used for the sample dilution/ pH adjustment. Low ionic strength (<0.10 M), low specificity buffers are preferred. Do not allow sorbent to dry under full vacuum for more than 1 minute before applying sample as this can cause the sorbent bed to be "de-conditioned". If the cartridges become de-conditioned, simply repeat the conditioning steps.

3. Load

Procedure:

Aspirate sample through the conditioned SPE cartridge at a rate of 2-4 ml per minute.

Notes: Ion exchange kinetics are relatively slow so do not pass the sample through the cartridge too rapidly as poor retention may occur.

4. Wash

Procedure:

Rinse the cartridge with 1 - 2 reservoir volumes of wash solution. Flow rate not critical.

Notes: Common wash solvents are sample dilution buffer and organic solvents. The primary criteria for wash solvents is that they not disrupt ionic interaction between

sorbent and analyte. Organic solvent washes have no acid/base properties and very effectively wash away non-polar matrix contaminants retained on the sorbent via secondary non-polar interactions.

5. Dry

Procedure:

Dry the cartridge under full vacuum for 2-5 minutes or until dry. Turn off the vacuum, and wipe tips of manifold needles or cartridge tubes to remove water droplets.

Notes: All traces of residual sample and wash solvents are removed from the sorbent ensuring a pure final extract. Dry cartridges look distinctly different than moist ones. Make note of the cartridge appearance before and after conditioning. Generally it is safer to slightly overdry the caridges, but beware of analyte volatility issues.

6. Elute

Procedure:

Apply 2-4 bed volumes of elution solvent to the cartridge, and then slowly aspirate through at a rate not to exceed 4 ml/mm.

Notes: Elution occurs via one of several methods: Neutralize the analyte. Neutralize the sorbent. Use counter ion strength or specificy to displace the analytic. Neutralize the charge on the isolate by eluting with a butter at least 2 pH units below the pKa of the acidic isolate. Neutralize the charge on the sorbent by eluting with a buffer at least 2 pH units above the pKa of the basic sorbent. Elute with a high ionic strength buffer (>0.1 M). Elute with a buffer containing a higher selectivity counter-ion.

Volatile elution solvents: Water miscible organic with 2% strong acid or base. E.g.: MeOH + 2% ammonium hydroxide, MeOH +2% Acetic Acid, Methylene chloride: Isopropyl alcohol: HCl (78:20:2).

Non-volatile elution solvents: Aqueous solutions of carefully determined pH, high ionic strength (>1.0M) or high selectivity counter-ion. Ion exchange kinetics are relatively slow. Rapid elution can cause poor recovery.

II. Strong Anion Exchange:

Strong anion exchange is commonly used for the extraction of weak organic acids from aqueous and organic solutions. Strong cation exchange functional groups are typically permanently charged quaternary amines.
Strong anion exchange sorbents are not recommended for the extraction of strong anions such as sulfonic acids. The interaction between the strong-acid and strong-base can not be disrupted making elution impossible. (However, this irreversible retention can be used to our advantage should our sample contain a strongly anionic contaminant whereby the contaminant can be irreversibly retained on the sorbent while the analyte passes through unretained)

I. Specimen Preparation:

Procedure:

> *Dilute sample 1:1 with buffer or water ensuring that sample pH is at least 2 pH units above the pKa of the isolate.*

Notes: Sample pH should be adjusted with buffer to ensure that analyte is quantitatively charged. Strong anion exchange sorbents are typically quaternary amines that are always charged irrespective of pH.

2. Condition

Procedure:

> *Pass a minimum of 4 bed volumes of water miscible organic, followed by a minimum of 4 bed volumes of sample buffer through the cartridge at a flow rate of 2-4 ml / min.*

Notes: Typical water miscible conditioning solvents are Methanol, Ethanol, ACN and IPA. The aqueous buffer used to condition the cartridge should be the same buffer used for the sample dilution/ pH adjustment. Low ionic strength (<0.10 M) buffers preferred. Do not allow sorbent to dry under full vacuum for more than 1 minute before applying sample as this can cause the sorbent bed to be "de-conditioned". If the cartridges become de-conditioned, simply repeat the conditioning steps.

3. Load

Procedure:

> *Aspirate sample through the conditioned SPE cartridge at a rate of 2-4 ml per minute.*

Notes: Ion exchange kinetics are relatively slow so do not pass the sample through the cartridge too rapidly as poor retention may occur.

4. Wash

Procedure:

Rinse the cartridge with 1 - 2 reservoir volumes of wash solution. Flow rate not critical.

Notes: Common wash solvents are sample dilution buffer and organic solvents. The primary criteria for wash solvents is that they not disrupt ionic interaction between sorbent and analyte. Organic solvent washes have no acid/base properties and very effectively wash away non-polar matrix contaminants retained on the sorbent via secondary non-polar interactions.

5. Dry

Procedure:

Dry the cartridge under full vacuum for 2-5 minutes or until dry.

Turn off the vacuum, and wipe tips of manifold needles or solvent through the cartridge at a rate not to exceed 4 ml/mm.

Notes: All traces of residual sample and wash solvent are removed from the sorbent ensuring a pure final extract. Dry cartridges look distinctly different than moist ones. Make note of the cartridge appearance before and after conditioning. Generally it is safer to slightly overdry the caridges, but beware of analyte volatility issues.

6. Elute

Procedure:

Slowly pass 2-4 bed volumes of elution solvent through the cartridge at a rate not to exceed 4 ml/min.

Notes: Elution from strong anion exchangers occurs via one of two methods: 1. Neutralize the analyte with strong acid. 2. Use high counter ion strength or specificity to displace the analtye. Volatile organic solvents with acidic modifiers are very effective elution solvents. Commonly used are MeOH +2% concentrated HCl, MeOH + 2% acetic acid, Methylene chloride: Isopropyl alcohol: concentrated HCl (78:20:2). Non-volatile options are strongly acidic aqueous buffers, high ionic strength (>1M) or high selectivity counter-ion. Ion exchange kinetics are relatively slow. Uncontrolled, rapid elution can cause poor, irreproducible recovery.