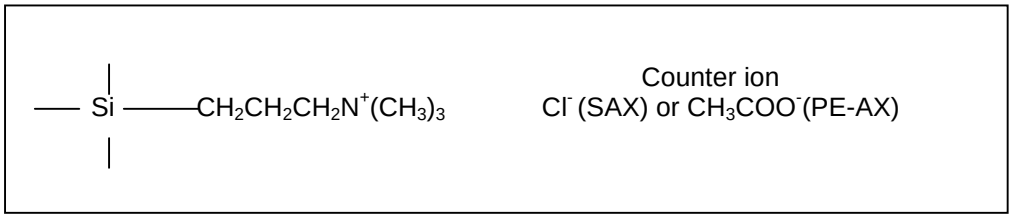
Method Development in Solid Phase Extraction using ISOLUTE® SAX and ISOLUTE PE-AX SPE Columns for the Extraction of Aqueous Samples

This technical note includes specifics on the use of ISOLUTE® SAX and PE-AX for the extraction of anionic analytes from aqueous samples (pages 1-3), ordering information (page 4) and a general discussion on anion exchangers (pages 5-6). For information on the extraction of acidic analytes from non-aqueous matrixes, contact Argonaut Technologies.

ISOLUTE Anion Exchange Sorbents: SAX, PE-AX, NH2 and PSA

The ISOLUTE family of anion exchange sorbents are used to extract organic anions (acidic compounds capable of exhibiting a negative charge) from both aqueous and non-aqueous matrixes.



Structure of the quaternary amine of ISOLUTE SAX and PE-AX anion exchange sorbents

In Method Development Using ISOLUTE SAX or ISOLUTE PE-AX, the Following Points are Important:

Sample Pre-treatment

Ionic Strength Control

Ionic strength of the sample should be reduced to <0.05 M by dilution with deionized water or low ionic strength buffer in order to facilitate maximum retention of the analytes. The capacity of a SAX or PE-AX column is approximately 0.6 milli-equivalents/g of sorbent. The analyte must compete with other anions in the sample for ion exchange sites, and so retention of the analyte is reduced when the ionic strength of the sample is high. Dilution may also reduce sample viscosity, which ensures a free-flowing sample. Analyte retention is facilitated by using buffers that contain anions of lower selectivity than the analyte. The selectivity of some common anions is as follows (ions on the right will displace those on the left):

$$OH^- < \text{acetate} < \text{formate} < HCO_3^- < Cl^- < HSO_3^- < CN^- < \text{citrate} < \text{benzene sulfonate}$$

ISOLUTE SAX has a chloride counter ion, whereas PE-AX is pre-equilibrated with an acetate counter ion, and therefore may be a better choice for certain acidic analytes.

pH Control

To ensure that total ionization of the analyte has occurred, the pH of the sample should be adjusted to two pH units above the pK_a of the analyte [see the two (2) pH unit rule in the appendix]. Buffering for pH control should be performed with the lowest strength buffer that will maintain the pH, usually 10-20 mM.

Column Solvation and Equilibration

SAX and PE-AX columns should be solvated with methanol, acetonitrile or THF.

For an aqueous matrix, both the pH and the ionic strength of the equilibration solvent must be optimized to ensure ionization of the analyte. Ionic strength should be the same as or very similar to that of the sample, ideally not higher than 0.05 M.

Counter Ion Exchange for SAX Columns Only

PLEASE NOTE THAT THIS STEP HAS ALREADY BEEN PERFORMED DURING THE MANUFACTURE OF PE-AX, WHICH IS PRE-EQULIBRATED WITH ACETATE COUNTER IONS

In order to improve retention for some analytes, it may be necessary to exchange the standard SAX chloride counter ion with one having lower selectivity. The standard protocol for this procedure is as follows.

1. Rinse the column with deionized water.
2. Apply an appropriate buffer. The buffer should contain phosphate, acetate, propanoate, carbonate or formate counter ions. For a 0.1 M solution of counter ions, 10-12 bed volumes should be applied (note, a 100 mg sorbent bed mass has a bed volume of 125 μL). For a 0.3 M solution, 6-8 bed volumes are required.
3. Equilibrate with a 10-20 mM solution of the same buffer.

Sample Loading

For SAX and PE-AX columns, typical flow rates are 1mL/min for 1 mL columns, 3 mL/min for 3 mL columns and 7 mL/min for 6 mL columns. The ion exchange process will not occur efficiently if the flow rate is too high.

Interference Elution

For SAX and PE-AX columns, ionic strength and pH control should be maintained to prevent analyte loss. The same buffer as the equilibration buffer is often suitable. Methanol or acetonitrile (10-30%) may be added to the buffer for removing lipophilic interferences. Test this rinse step for the presence of the analyte to ensure that there are no losses!

Analyte Elution:

Displacement of the Analyte by Mass Action

High ionic strength (>0.1 M) buffers can be used for elution. For analytes with two negative charges, buffers of >0.2 M should be used. Buffers containing ions with a higher affinity for the sorbent than the analyte can be used, but are not required if the ionic strength is sufficiently high. The high concentration of the anions in the buffer will compete with the analyte for the sites on the sorbent causing elution of the analyte. Since SAX and PE-AX exert very weak secondary (non-polar) interactions, the presence of an organic component may not be necessary for elution.

If a non-aqueous elution solvent is required, for example if the eluent is to be injected directly into a GC, evaporated to give a higher concentration of analyte, or derivatized prior to analysis, then organic solvents modified with an acid such formic or acetic acid (2-5%) are suitable.

Neutralization of the Charge on the Analyte (Weak Acids Only)

Weak acids can be eluted using a buffer or solvent adjusted to two (2) pH units below the pK_a of the analyte.

ISOLUTE SAX and PE-AX Product Ordering Information

ISOLUTE SAX SPE Columns Product Description	Columns / Box	Part Number
25 mg/1 mL	100	500-0002-A
50 mg/1 mL	100	500-0005-A
50 mg/10 mL	50	500-0005-G
100 mg/1 mL	100	500-0010-A
100 mg/3 mL	50	500-0010-B
100 mg/6 mL	30	500-0010-C
100 mg/10 mL	50	500-0010-G
200 mg/1 mL	100	500-0020-A
200 mg/3 mL	50	500-0020-B
200 mg/10 mL	50	500-0020-H
500 mg/3 mL	50	500-0050-B
500 mg/6 mL	30	500-0050-C
500 mg/10 mL	50	500-0050-H
1 g/3 mL	50	500-0100-B
1 g/6 mL	30	500-0100-C
2 g/15 mL	20	500-0200-D
5 g/25 mL	20	500-0500-E
10 g/70 mL	16	500-1000-F

ISOLUTE PE-AX SPE Columns Product Description	Columns / Box	Part Number
25 mg/1 mL	100	503-0002-A
50 mg/1 mL	100	503-0005-A
100 mg/1 mL	100	503-0010-A
100 mg/3 mL	50	503-0010-B
200 mg/3 mL	50	503-0020-B
500 mg/3 mL	50	503-0050-B
500 mg/6 mL	30	503-0050-C

Both ISOLUTE SAX and PE-AX sorbents are available in the high throughput 96-well SPE plates, ISOLUTE-96 and ISOLUTE Array. Please contact Argonaut Technologies for further information.

APPENDIX

ISOLUTE Anion Exchange Sorbents: SAX, PE-AX, NH2 and PSA

The ISOLUTE family of anion exchange sorbents are used to extract organic anions (acidic compounds capable of exhibiting a negative charge) from both aqueous and non-aqueous matrixes. Although extraction using these sorbents is by the same mechanism, each has properties which influence the way they are used.

Anion exchange SPE can be accomplished by strong (permanently charged) or weak (pH - dependent charge) ion exchangers.

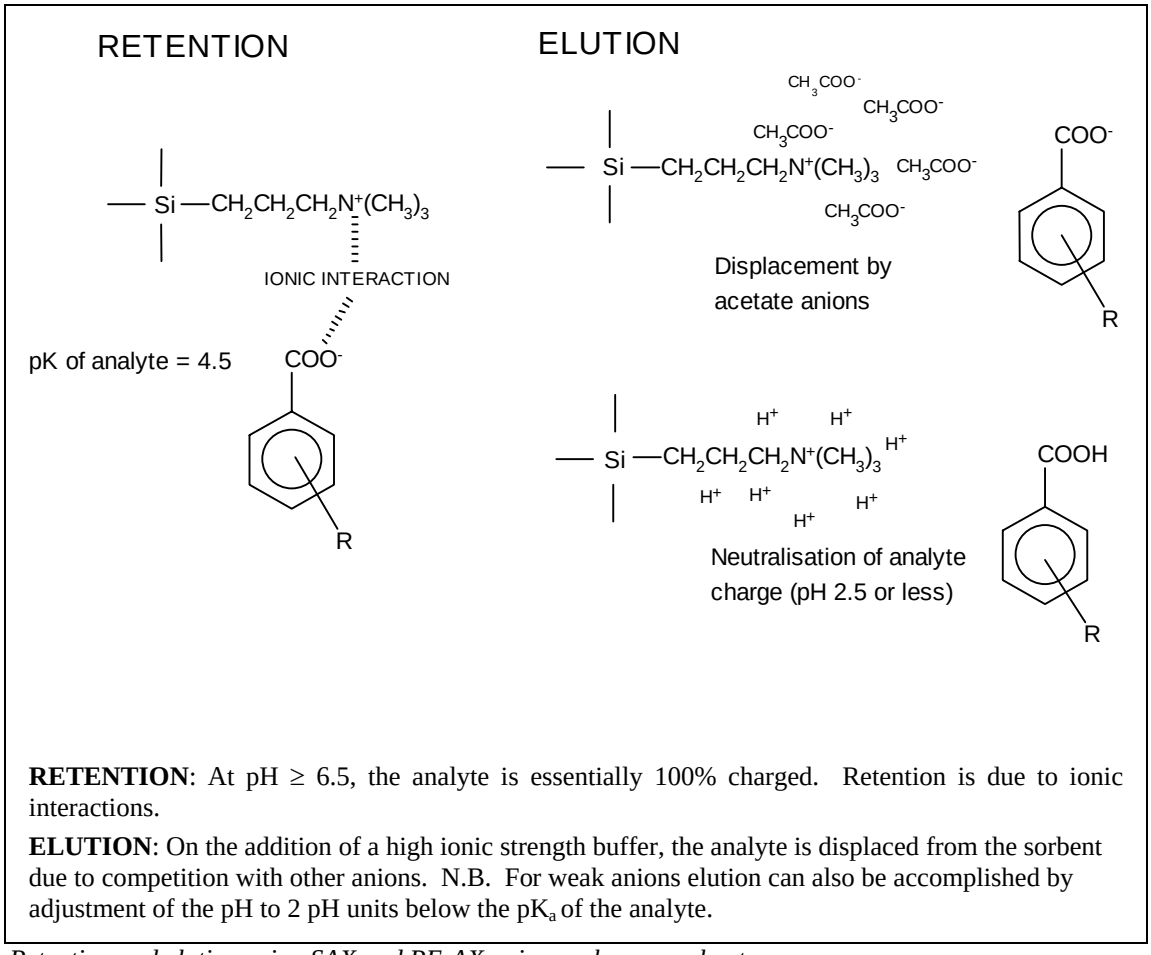
SAX and PE-AX, the strong anion exchangers, are quaternary amines (propyl-trimethyl amine – see structures on page 1) and therefore have a permanent positive charge under all pH conditions. Since this amine is tethered to the silica surface through a propyl linkage, these phases have limited non-polar character, and so secondary non-polar interactions with analytes are weak. This means that it may not be necessary to add an organic fraction to the elution solvent, which is often done when eluting from more hydrophobic sorbents in order to overcome non-polar retention mechanisms. These two phases differ only in that SAX has a chloride counter ion while PE-AX has an acetate counter-ion. It is easier to displace an acetate counter ion than a chloride counter ion, and so the extraction of carboxylic acids can be achieved without having to equilibrate the sorbent with a high concentration acetate buffer (as described for SAX later in this technical note).

NH2 (an aminopropyl phase) is a weak anion exchanger with a pK_a of 9.8. It is used for the extraction of anions that exhibit a negative charge at pH 7.8 or lower. The charge on the sorbent is neutralized at pH 11.8 or higher. This can be useful for the extraction of analytes with a permanent negative charge (strong acids) which cannot be neutralized by pH control.

PSA, (a primary/secondary amine phase) is a weak anion exchanger, with pK_a values of approximately 8 and 10. It is used for the extraction of anions that exhibit a negative charge at pH 8 or lower. The charge on the sorbent is neutralized at pH 12 or higher.

Retention and Elution Characteristics of ISOLUTE Strong Anion Exchange Sorbents

Retention and elution using SAX and PE-AX are illustrated on page 6.



RETENTION: At pH ≥ 6.5, the analyte is essentially 100% charged. Retention is due to ionic interactions.

ELUTION: On the addition of a high ionic strength buffer, the analyte is displaced from the sorbent due to competition with other anions. N.B. For weak anions elution can also be accomplished by adjustment of the pH to 2 pH units below the pK_a of the analyte.

Retention and elution using SAX and PE-AX anion exchange sorbents

The Two (2) pH Unit Rule

The pK_a of a molecular functional group is defined as the pH at which 50% of this group in solution are charged, and 50% are uncharged. Each pH unit change affects the percentage of charged or uncharged groups by a factor of 10, so it is sensible to perform extractions at a pH at least 2 pH units from the pK_a value, to ensure that 99.5% of the functional groups are in the desired state of ionization.

e.g. Effect of pH on the dissociation of a weak acid with a pK_a value of 4.0.

pH	% free acid (uncharged)	% dissociated (charged)
4.0	50	50
5.0	5.0	95
6.0	0.5	99.5

e.g. Effect of pH on the dissociation of the conjugate acid of a weak base with a pK_a value of 9.0

pH	% free base (uncharged)	% dissociated (charged)
9.0	50	50
8.0	5.0	95
7.0	0.5	99.5

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United States and Canada
Tel: + 1 434 979 2319
Toll-Free: + 1 800 446 4752
ordermailbox@biotage.com

United Kingdom, EIRE
Biotage
Tel: + 44 1443 811811
eurosales@eu.biotage.com

Sweden
Biotage
Tel: + 46 18 56 59 00
order@eu.biotage.com

Japan
Tel: + 81 422 281233
order.biotage.co.jp