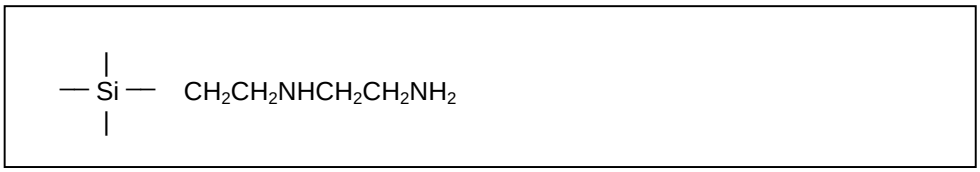
Method Development in Solid Phase Extraction using ISOLUTE® PSA SPE Columns for the Extraction of Aqueous Samples

This technical note includes), specifics on the use of ISOLUTE® PSA for the extraction of anionic analytes from aqueous samples (page 1-2), ordering information (page 3) and a general discussion on anion exchangers (pages 4-5). For information on extraction of anionic analytes from non-aqueous matrices, contact Argonaut Technologies.

ISOLUTE Anion Exchange Sorbents: PSA, NH2 and SAX

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. See appendix for more information on these sorbents.



Structure of ISOLUTE PSA sorbent

In Method Development Using ISOLUTE PSA, the Following Points are Important:

Sample Pre-treatment

Ionic Strength Control

Ionic strength of the sample should be reduced to 50 mM by dilution with deionized water or low ionic strength buffer in order to facilitate maximum retention of the analytes. The capacity of a PSA column is approximately 0.4 mM / g of sorbent. The analyte must compete with other anions in the sample for ion exchange sites, so retention of the analyte is reduced when the ionic strength of the sample is high. Dilution will also reduce sample viscosity, to ensure a free-flowing sample. The selectivity of the buffer anion chosen should be considered. Analyte retention is facilitated by 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^- < acetate, borate < formate < HCO_3^- < Cl^- < HSO_3^- < CN^- < citrate < benzene sulfonate

ISOLUTE PSA is shipped as the free base.

pH Control

To ensure that total ionization of the sorbent has occurred, the pH of the sample should be adjusted to 8 or below [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 pH, usually 10-20 mM.

Column Solvation and Equilibration

PSA columns should be solvated with methanol, acetonitrile or THF.

The pH of the equilibration solvent must be optimized to maintain ionization of the analyte upon loading. Ionic strength should be the same as or very similar to that of the sample, ideally not more than 0.05 M. In addition, the equilibrium buffer is used to ensure the presence of an appropriate counter ion on the column.

Sample Loading

For PSA columns, typical flow rates are 1 mL/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 PSA columns, ionic strength and pH control should be maintained at this stage to prevent analyte loss. The same buffer as the equilibration buffer is often suitable. Methanol or acetonitrile (5-30%) in buffer is often suitable for removing lipophilic interferences.

Analyte elution

Displacement of the Analyte by Mass Action

High ionic strength (>0.1 M) buffers can be used for elution. The high concentration of the anions in the buffer will compete with the anionic analyte for the cationic sites on the sorbent. This will cause elution of the analyte. 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 for elution by displacement of the anionic analyte. As PSA exerts very weak secondary (non-polar) interactions, the presence of an organic component is not necessary for elution.

Neutralization of the Charge on the Sorbent

Strong anions can be eluted using a buffer or solvent adjusted to two (2) pH units above the pK_a of the sorbent (i.e. to pH 12 or above).

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 trifluoroacetic acid (2-5%) are suitable.

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

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

ISOLUTE PSA Product Ordering Information

ISOLUTE PSA SPE Columns Product Description	Columns / Box	Part Number
25 mg/1mL	100	480-0002-A
50 mg/1 mL	100	480-0005-A
50 mg/10mL	50	480-0005-G
100 mg/1 mL	100	480-0010-A
100 mg/3 mL	50	480-0010-B
100 mg/6 mL	30	480-0010-C
100 mg/10 mL	50	480-0010-G
200 mg/1 mL	100	480-0020-A
200 mg/3 mL	50	480-0020-B
200 mg/10 mL	50	480-0020-H
500 mg/3 mL	50	480-0050-B
500 mg/6 mL	30	480-0050-C
500 mg/10 mL	50	480-0050-H
1 g/3 mL	50	480-0100-B
1 g/6 mL	30	480-0100-C
2 g/15 mL	20	480-0200-D
5 g/25 mL	20	480-0500-E
10 g/70 mL	16	480-1000-F

This sorbent in also available in the high throughput 96-well SPE plates, ISOLUTE-96 and ISOLUTE Array. Please contact Biotage for further information.

APPENDIX

ISOLUTE Anion Exchange Sorbents: PSA, NH2 and SAX

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 is by the same mechanism, each sorbent has properties which influence the way they are used.

Anion exchange SPE can be accomplished by strong (very high pK_a) or weak (lower pK_a) ion exchangers.

PSA (a primary/secondary amine phase – see structure on page 1) is a weak anion exchanger, with pK_as of approximately 10.1 and 10.9. 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. This can be useful for the extraction of analytes with a permanent negative charge (such as strong acids) which cannot be neutralized by pH control. PSA should also be used

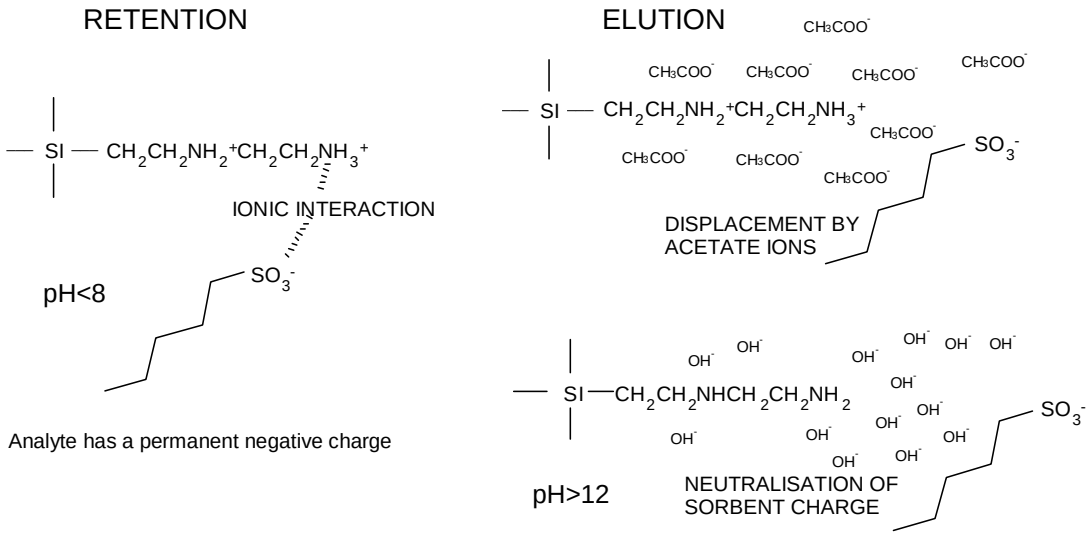
- **for analytes unstable at low pHs**
- **for analytes containing sulfate or phosphate groups which are difficult to elute from strong anion exchange (SAX) sorbents.**

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.

SAX (a quaternary amine phase) is a strong anion exchanger is manufactured with chloride as the counter ion. It maintains a permanent positive charge over the whole pH range (pH 1-14). SAX has limited non-polar character, so secondary non-polar interactions with analytes are minimal. This means that organic solvent is often unnecessary in the elution solvent to overcome secondary non-polar interactions.

Retention and Elution Characteristics

Retention and elution characteristics of ISOLUTE PSA are illustrated on page 5.



RETENTION: At pH ≤ 8, the sorbent 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. Elution can also be accomplished by application of a solvent of pH 12 or above, which neutralizes the charge on the sorbent.

Retention and elution characteristics of ISOLUTE PSA

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.

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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