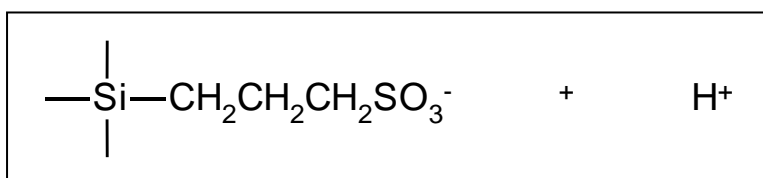


Method Development in Solid Phase Extraction using ISOLUTE® SCX-2 SPE Columns for the Extraction of Aqueous Samples

This technical note includes, specifics on the use of ISOLUTE® SCX-2 for the extraction of cationic analytes from aqueous samples (pages 1-2), ordering information (page 3) and a general discussion on cation exchanger sorbents (pages 4-5). For information on extraction of cationic analytes from non-aqueous matrixes, please contact Argonaut Technologies.



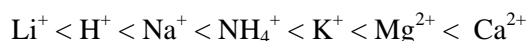
Structure of ISOLUTE SCX-2 sorbent

In Method Development Using ISOLUTE SCX-2, 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 SCX-2 column is approximately 0.5 mM/g of sorbent. The analyte must compete with other cations 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 cation chosen should be considered. Analyte retention is facilitated by buffers that contain cations of lower selectivity than the analyte. The selectivity of some common cations is as follows (ions on the right will displace those on the left):



ISOLUTE SCX-2 has a hydrogen counter ion as standard.

pH Control

To ensure that total ionization of the analyte has occurred, the pH of the sample should be adjusted to two pH units below 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 pH, usually 10-20 mM.

Column Solvation and Equilibration

SCX-2 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 at this stage. Ionic strength should be the same as or very similar to that of the sample, ideally not more than 0.05 M.

Sample Loading

For SCX-2 columns, typical flow rates are 1mL/min for 1mL 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 SCX-2 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 (10-20%) in buffer is often suitable for removing lipophilic interferences.

Analyte Elution

Displacement of the Analyte by Mass Action

High ionic strength (>0.1M) buffers can be used for elution. The high concentration of the cations in the buffer will compete with the cationic analyte for the anionic sites on the sorbent. This will cause elution of the analyte. For analytes with two positive 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 cationic analyte. As SCX-2 exerts very weak secondary (non-polar) interactions, the presence of an organic component is not 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 a volatile base such as tetramethyl ammonium hydroxide (2-5% v/v) are suitable.

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

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

ISOLUTE SCX-2 Product Ordering Information

ISOLUTE SCX-2 SPE Columns Product Description	Columns / Box	Part Number
25 mg/1mL	100	532-0002-A
50 mg/1mL	100	532-0005-A
50 mg/10 mL	50	532-0005-G
100 mg/1 mL	100	532-0010-A
100 mg/3 mL	50	532-0010-B
100 mg/10 mL	50	532-0010-G
200 mg/1 mL	100	532-0020-A
200 mg/3 mL	50	532-0020-B
200 mg/10 mL	50	532-0020-H
500 mg/3 mL	50	532-0050-B
500 mg/6 mL	30	532-0050-C
500 mg/10 mL	50	532-0050-H
1 g/3 mL	50	532-0100-B
1 g/6 mL	30	532-0100-C
2 g/15 mL	20	532-0200-D
5 g/25 mL	20	532-0500-E
10 g/70 mL	16	532-1000-F

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

APPENDIX

ISOLUTE Cation Exchange Sorbents: SCX-2, SCX and CBA

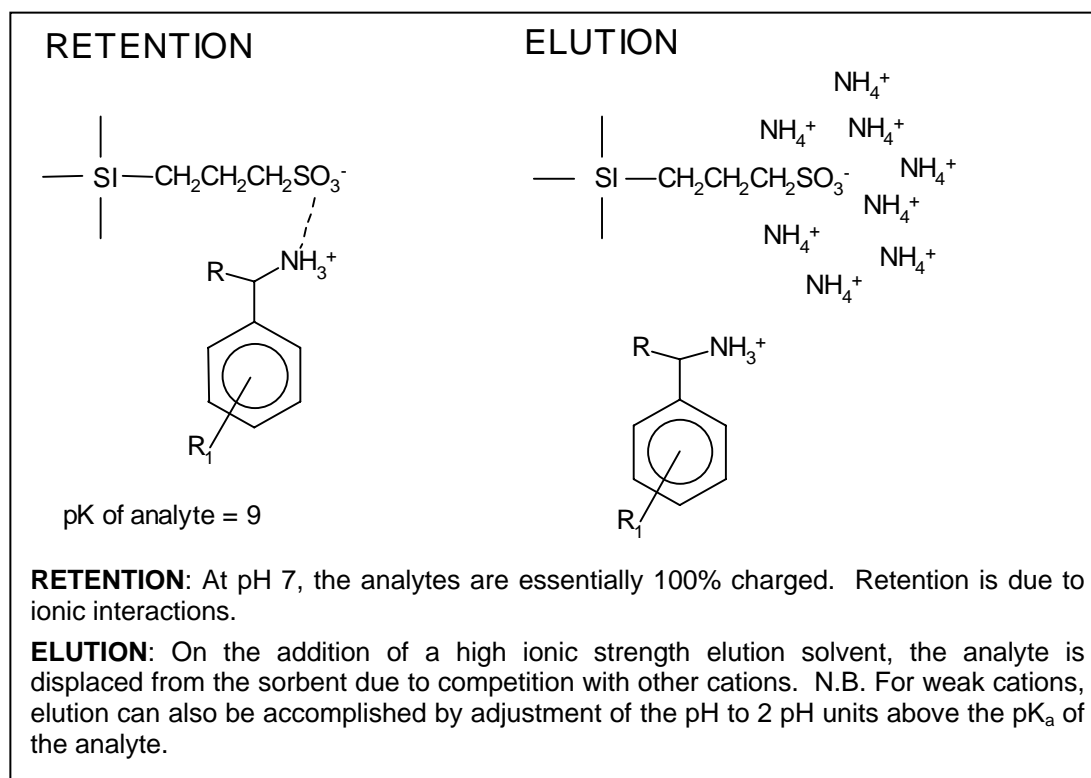
The ISOLUTE family of cation exchange sorbents are used to extract organic cations (basic compounds capable of exhibiting a positive charge) from both aqueous and non-aqueous matrixes. Although extraction is by the same mechanism, each sorbent has properties which influence the way they are used.

Cation exchange SPE can be accomplished by strong (low pK_a) and weak (higher pK_a) ion exchangers. Both **SCX-2** (a propylsulfonic acid phase – see structure on page 1) and **SCX** (a benzenesulfonic acid phase) are strong cation exchangers. They maintain a permanent negative charge over the whole pH range (pH 1-14). **SCX-2 has little non-polar character, so secondary non-polar interactions with analytes are very weak. This allows elution of analytes with a totally aqueous solvent if necessary.** SCX shows more non-polar characteristics than SCX-2 due to the aromatic ring, therefore secondary interactions are stronger. This can enhance recoveries, but a portion of organic solvent in the elution solvent is often necessary to overcome secondary interactions and elute analytes efficiently.

CBA (a carboxy propyl phase) is a weak cation exchanger, with a pK_a of 4.8. It is used for the extraction of cations that exhibit a positive charge at pH 6.8 or higher. The charge on the sorbent is neutralized at a pH of 2.8 or less. This can be useful for the extraction of analytes with a permanent positive charge, such as quaternary amines, which cannot be neutralized by pH control.

Retention and Elution Characteristics

The retention and elution characteristics of ISOLUTE SCX-2 are illustrated below.



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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