Method Development Guidelines:

Solid Phase Extraction Using ISOLUTE® SCX-2 Sorbents for the Extraction of Aqueous Samples

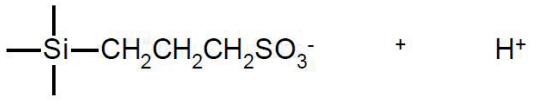


Figure 1. Structure of ISOLUTE® SCX-2 sorbent.

ISOLUTE® Cation Exchange Sorbents

SCX, SCX-2, SCX-3 and CBA

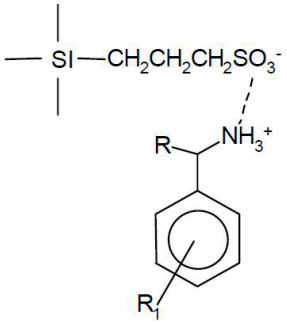
The ISOLUTE® family of cation exchange sorbents are used to extract organic cations (basic compounds capable of exhibiting a positive charge) from both aqueous and non-aqueous matrixes. Although extraction is by the same mechanism, each sorbent has properties that influence the way they are used.

Cation exchange SPE can be accomplished by strong (low pK_a) and weak (higher pK_a) ion exchangers. ISOLUTE SCX-2 (a propylsulfonic acid phase–see structure above) ISOLUTE SCX and ISOLUTE SCX-3 (benzenesulfonic acid and ethylbenzenesulfonic acid phases respectively) are strong cation exchangers. They maintain a permanent negative charge over the whole pH range (pH 1–14). ISOLUTE 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.

ISOLUTE SCX and SCX-3 shows more non-polar characteristics than ISOLUTE 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.

ISOLUTE 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



pK of analyte = 9

ELUTION

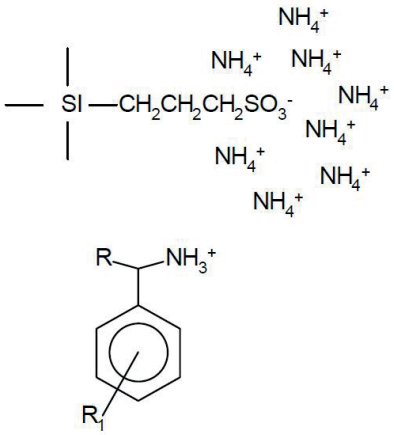


Figure 2. The retention and elution characteristics of ISOLUTE SCX-2.

RETENTION: At pH < 7 the analytes are essentially 100 % charged. Retention is due to both ionic interactions and non-polar interactions.

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

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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 ISOLUTE 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):

$Li^+ < H^+ < Na^+ < NH_4^+, RNH_3^+ < K^+ < Mg^{2+} < Ca^{2+}$

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

ISOLUTE 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 ISOLUTE SCX-2 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 ISOLUTE 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 ISOLUTE 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/solvent mixture or solvent adjusted to two (2) pH units above the pK_a of the analyte.

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Appendix

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.

Table 1. Effect of pH on the dissociation of a weak acid with a pK_a value of 4.0.

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

Table 2. 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

ISOLUTE® SCX-2 is available in a range of column and 96-well plate formats, **see www.biotage.com for details**.

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