Method Development Guidelines:

Solid Phase Extraction Using ISOLUTE® SCX and SCX-3 Sorbents for the Extraction of Aqueous Samples

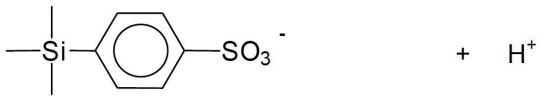

Figure 1. Structure of ISOLUTE® SCX sorbent. ISOLUTE SCX-3 is an ethylbenzene sulfonic acid.

ISOLUTE® Cation Exchange Sorbents

SCX, SCX-2 (PRS), 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. Both ISOLUTE SCX and SCX-3 (benzenesulfonic acid and ethylbenzene sulfonic acid phases respectively–see structure above) and ISOLUTE SCX-2 (PRS) (a propylsulfonic acid phase) are strong cation exchangers. They maintain a permanent negative charge over the whole pH range (pH 1–14). ISOLUTE SCX and SCX-3 have significant non-polar character due to the aromatic ring, so secondary non-polar interactions with analytes are present. This can enhance recoveries, but a proportion of organic solvent in the elution solvent is necessary to overcome secondary interactions and elute analytes efficiently. The additional strong secondary interaction of the ISOLUTE SCX-3 phase is particularly useful if the sample matrix is high or variable in ionic strength.

ISOLUTE SCX-2 has very little non-polar character, and is the strong cation exchanger of choice if the elution solvent must be totally aqueous. 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. See TN108 for additional information.

Retention and Elution Characteristics of ISOLUTE® SCX and SCX-3 Strong Cation Exchange Sorbents

Retention and elution using ISOLUTE SCX are illustrated below. The same mechanisms apply to the ISOLUTE SCX-3 sorbent (not shown).

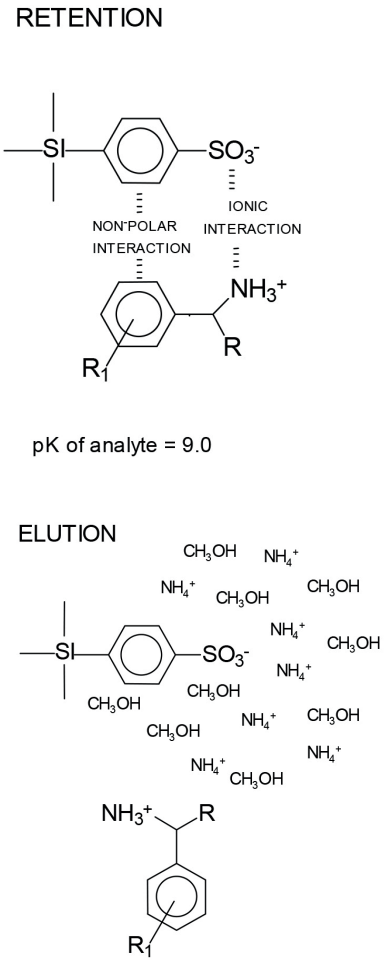

Figure 2. Retention and characteristics of ISOLUTE® SCX and SCX-3 cation exchange sorbents.

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

ELUTION: The elution solvent must overcome both retention mechanisms. On addition of an elution solvent containing both organic solvent and a high concentration of cations, the retention mechanisms are broken and the analyte is eluted. N.B. For weak cations, elution can also be accomplished using a solvent with the pH adjusted to 2 pH units above the pK_a of the analyte.

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In method development using ISOLUTE® SCX and SCX-3 sorbents, 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 the ISOLUTE SCX sorbent is approximately 0.35 mM/g of sorbent. The capacity of ISOLUTE SCX-3 is approximately 0.6 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. Buffers that contain cations of lower selectivity than the analyte facilitate analyte retention. 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 and SCX-3 have 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 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 and SCX-3 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 and SCX-3 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 and SCX-3 columns, equilibration buffer is often suitable. An initial wash with 0.01 M acid followed by acidified methanol or acetonitrile (up to 30%) in water is often suitable for removing lipophilic interferences. It should be noted that the pK_a of compounds can be altered in a solution containing organic solvent, so care should be taken to avoid losses of analytes in this way.

Analyte Elution

Displacement of the Analyte by Mass Action

To minimize elution volumes, the elution solvent must overcome both the primary ionic retention mechanism, and the secondary non-polar interactions. Unless the analyte is a very polar, water soluble compound, then some solvent component is essential. Buffers with an ionic strength of >0.1 M containing 25–50% organic solvent (e.g. methanol) are suitable. Methanol containing a soluble salt such as ammonium acetate (0.1 M) is also a suitable elution solvent.

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 unitsabove 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 and SCX-3 are available in a range of column and 96-well plate formats, **see www.biotage.com for details**.

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