Method Development Guidelines:

Solid Phase Extraction Using ISOLUTE® CBA Sorbents for the Extraction of Aqueous Samples

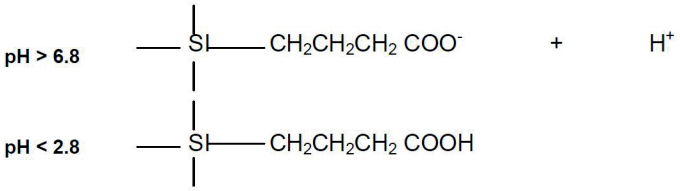

Figure 1. Structure of ISOLUTE® CBA sorbent.

ISOLUTE® Cation Exchange Sorbents

CBA, SCX, SCX-2 and SCX-3

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 weak (higher pK_a) or strong (very low pK_a) ion exchangers.

ISOLUTE CBA (a carboxy propyl phase–see structure above) is a weak cation exchanger, with a pK_a of 4.8. The charge on the sorbent is neutralized at a pH of 2.8 or less. ISOLUTE CBA should be used:

- For the extraction of analytes with a permanent positive charge, such as quaternary amines, which cannot be neutralized by pH control
- For the extraction of cations that exhibit a positive charge at pH 6.8 or higher
- When the analyte is not stable in the basic buffers required to elute from ISOLUTE SCX, SCX-2 or SCX-3

Both ISOLUTE SCX, (a benzenesulfonic acid phase) and SCX-3 (an ethylbenzenesulfonic acid phase) 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 show 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.

Retention and Elution Characteristics

Retention and elution characteristics of ISOLUTE CBA weak cation exchange sorbent are illustrated.

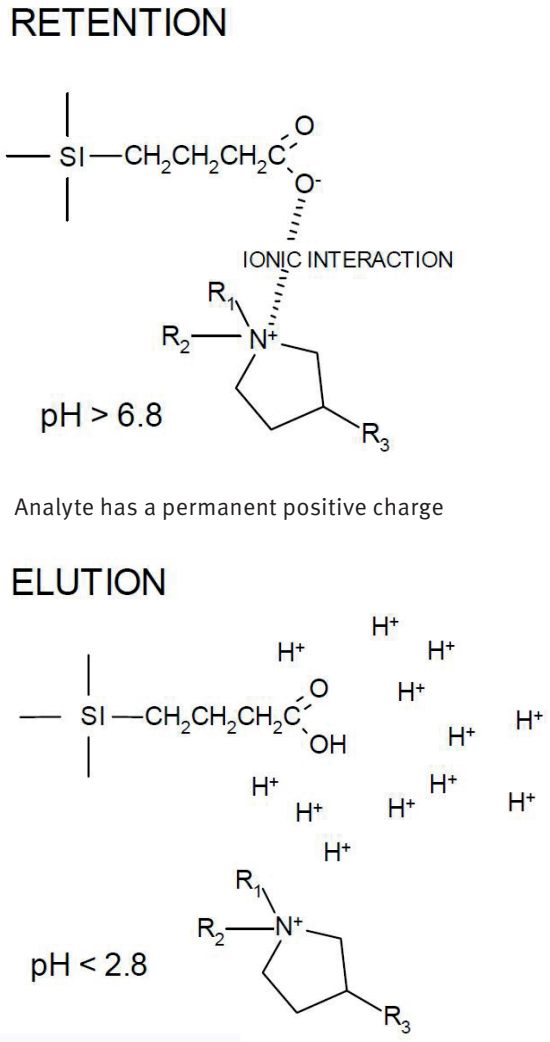

Figure 2. The retention and elution characteristics of ISOLUTE CBA weak cation exchange sorbent.

RETENTION: At pH ≥ 6.8, the sorbent is essentially 100% charged. Retention is due to ionic interactions.

ELUTION: At pH ≤ 2.8 the charge on the sorbent is neutralized, and elution can take place. Elution can also be facilitated by the addition of a high ionic strength elution solvent. In this case the analyte is displaced from the sorbent due to competition with other cations.

N.B. Weak cations can be eluted using a buffer with a pH two pH units higher than the pK_a of the analyte.

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In method development using ISOLUTE® CBA 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 CBA sorbent is approximately 0.6 mM/g. 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):

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

ISOLUTE CBA has a hydrogen counter ion as standard.

pH Control

To ensure that total ionization of the sorbent is maintained during loading, the pH of the sample should be adjusted to pH 6.8 or higher (two pH units above the pK_a of the sorbent) [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 CBA 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 sorbent 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 CBA 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 CBA 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 (>0.1 M) 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 CBA 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 an acid such formic or acetic acid (2–5%) are suitable.

Neutralization of the Charge on the Sorbent

Buffers with a pH ≤ 2.8 can be used for elution, as the charge on the sorbent is neutralized below pH 2.8. An appropriate organic solvent with the pH adjusted to ≤ 2.8 is also 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® CBA is available in a range of column and 96-well plate formats, **see www.biotage.com for details**.

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