

Method Development Guidelines:

Solid Phase Extraction Using ISOLUTE® NH2 SPE Sorbents for the Extraction of Aqueous Samples

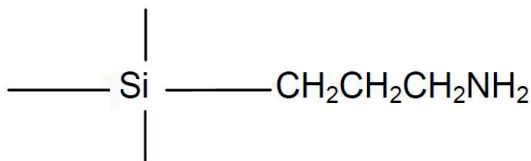


Figure 1. Structure of ISOLUTE® NH2 sorbent.

ISOLUTE® Anion Exchange Sorbents

NH2, PSA, SAX and PE-AX

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.

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

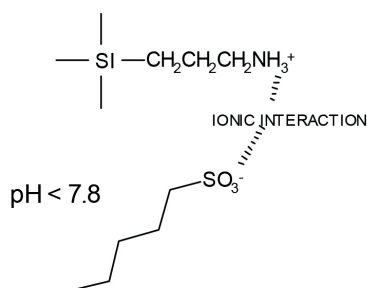
NH2 (an aminopropyl phase – see structure in figure 1) is a weak anion exchanger, with a pK_a of 9.8. It is used for the extraction of anions that exhibit a negative charge at pH 7.8 or lower. The charge on the sorbent is neutralized at pH 11.8 or higher. This can be useful for the extraction of analytes with a permanent negative charge (such as strong acids) which cannot be neutralized by pH control. ISOLUTE NH2 should also be used.

- » For analytes unstable at low pHs
- » For analytes containing sulfate or phosphate groups which are difficult to elute from SAX sorbents.

ISOLUTE PSA, (a primary/secondary amine phase) is a weak anion exchanger, with pK_a values of approximately 10.1 and 10.8. 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. See TN105 for additional information.

ISOLUTE SAX and PE-AX (quaternary amine phases) are strong anion exchangers with a chloride or acetate counter ion respectively. They maintain a permanent positive charge over the whole pH range (pH 1–14). ISOLUTE SAX and PE-AX have little non-polar character, so secondary non-polar interactions with analytes are weak. This means that organic solvent may not be necessary in the elution solvent to overcome retention mechanisms and elute the analytes. See TN103 for additional information.

RETENTION



Analyte has a permanent negative charge

ELUTION

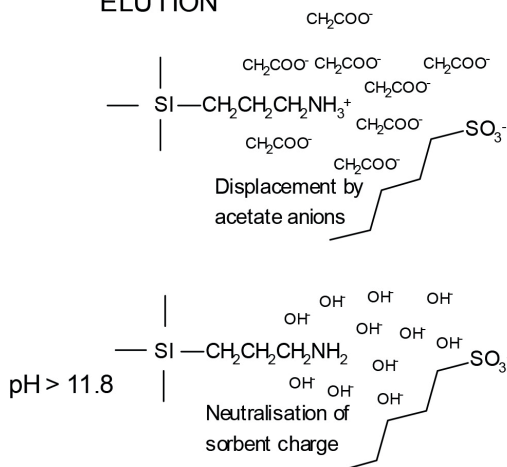


Figure 2. Retention and elution using ISOLUTE® NH2.

RETENTION: At pH < 7.8, 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 11.8 or above, which neutralizes the charge on the sorbent.

In method development using ISOLUTE® NH₂, 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 NH₂ sorbent is approximately 0.65 mM / g. 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 < formate < HCO₃⁻ < Cl⁻ < HSO₃⁻
< CN⁻ < citrate < benzene sulfonate

ISOLUTE NH₂ 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 7.8 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

ISOLUTE NH₂ 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 NH₂ 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 NH₂ 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 (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 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 >0.2M 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 ISOLUTE NH₂ 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

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 11.8 or above).

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.



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

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