Method Development Guidelines: Solid Phase Extraction Using ISOLUTE® ENV+ for the Extraction of Aqueous Samples

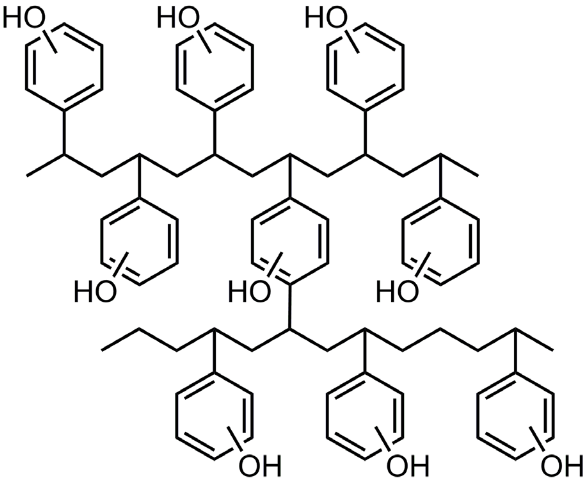


Figure 1. Structure of ISOLUTE® ENV+ polymeric non-polar sorbent.

ISOLUTE® Non-polar Sorbents:

C4, C8, C8(EC), C18, C18(EC), MFC18, PH, ENV+ and 101

The ISOLUTE® family of non-polar sorbents is used to extract organic compounds from aqueous matrices.

ISOLUTE® ENV+ and 101

These are the most hydrophobic of all of the sorbents. They are used primarily where the analytes are very water soluble, and extraction is difficult using a silica based sorbent. These high capacity, highly cross-linked polystyrene based polymeric sorbents are capable of retaining analytes of a wide range of polarities. The very accessible high surface areas of these non-polar sorbents provides retention of very polar and water soluble analytes. The optimized surface area / pore structure and the absence of fines provide high recoveries at high flow rates for many analytes.

The absence of monomers ensures compatibility of ISOLUTE ENV+ and 101 products with today's demanding environmental applications.

ISOLUTE® C18, C18(EC), MFC18

The non-endcapped trifunctional ISOLUTE C18 sorbent has enhanced secondary silanol interactions (which can be very useful for example in the extraction of basic compounds from aqueous solution) compared to ISOLUTE C18(EC). Non-endcapped C18 has a lower carbon loading than the endcapped sorbent. ISOLUTE C18 (EC) is also based on trifunctional silane chemistry, and the residual silanols on the silica surface are subsequently end capped to minimize secondary silanol interactions. ISOLUTE MF C18 (produced using monofunctional octadecyl silane) is non-endcapped and like the non-endcapped trifunctional C18, provides useful secondary silanol interactions. The accessibility of these silanol groups to analytes and solvents is increased in the monofunctional C18, compared to the trifunctional C18 sorbents.

ISOLUTE® C8, C8(EC), C4

The non-polar characteristics of these sorbents decreases with carbon chain length. This can be advantageous when extracting non-polar analytes from aqueous matrices. Large, very non-polar analytes, although well retained on C18 sorbents, can be difficult to elute as the non-polar interactions between analyte and sorbent are very strong. If a less retentive phase (such as C8 or C4) is used, the analytes will still be retained, but can be eluted more easily, in minimal elution volumes. Sorbents which are endcapped (C8(EC)) have fewer secondary interactions due to silanol groups than their non-endcapped versions, and are therefore not recommended for the extraction of basic compounds.

ISOLUTE® PH

This sorbent is generally considered to be less retentive than C18 sorbents, but exhibits different selectivities when extracting aromatic and non-aromatic analytes.

1 mL ISOLUTE® ENV+ Column.

In method development using ISOLUTE® ENV+, the following points are important:

Sample Pre-treatment

Due to the very retentive nature of ISOLUTE® ENV+, sample pre-treatment is often not necessary. For very viscous samples, deionized distilled water can be used to reduce the viscosity.

The extraction efficiency of polar ionizable molecules using ISOLUTE ENV+ columns is normally enhanced by suppressing ionization of the analytes by pH control of the sample. The "2 pH unit rule" is normally applied here. For example, for the extraction of an acidic herbicide such as 2,4-D (pK_a 3.05), extraction efficiency can be optimized by adjusting the pH of the sample to 2 units lower than the pK_a, i.e. adjust to pH 1.05 or lower. Similarly, for basic analytes, recoveries can often be enhanced by adjusting the pH at least 2 pH units above the pK_a value of the analyte(s).

For the extraction of non-ionizable analytes, pH control of the sample may still be advantageous if retention of ionizable polar interferences can be reduced by adjusting the sample pH to ensure the interferences are ionized and retained less strongly by the column.

Column Solvation and Equilibration

ISOLUTE ENV+ columns can be used without solvation or equilibration. For analyses requiring high sensitivity where it is necessary to ensure that there will be no interferences from the sorbent, the columns can be rinsed with a water miscible solvent (e.g., methanol, acetone, acetonitrile) prior to sample loading. The rinse should be followed with distilled water to remove excess solvent.

Sample Loading

When developing a method using ISOLUTE ENV+, good starting points for 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. It is likely that loading rates can be increased after method chemistry is established. For some analytes, loading rates as high as 120 mL/min using a 6 mL column have been demonstrated to yield good recoveries.

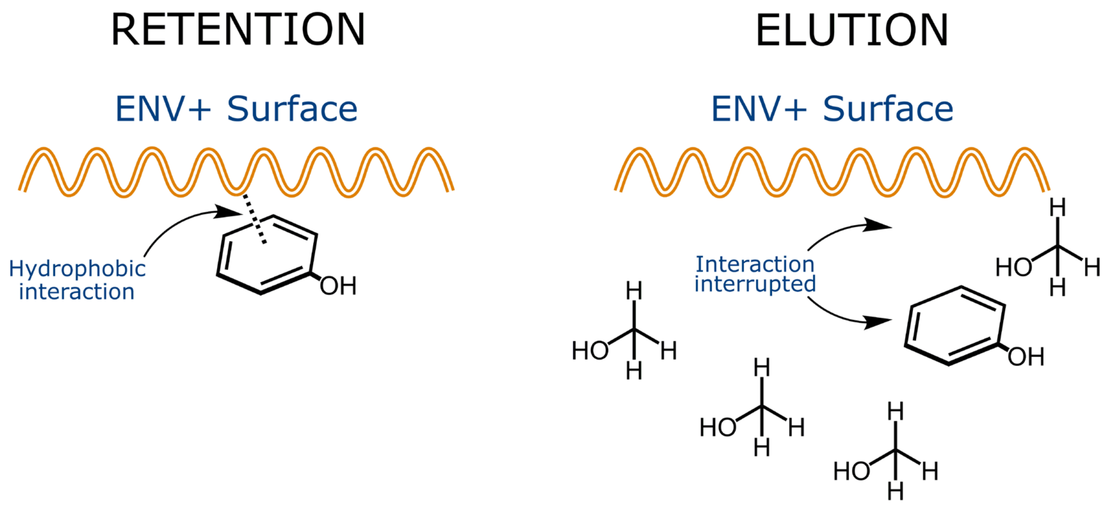
Evaluation of recovery vs. increasing flow rate is a useful exercise to maximize sample throughput.

Interference Elution

A typical solvent for interference elution is deionized distilled water.

If control of sample pH is necessary to maximize recoveries of the analytes, maintaining the pH of the interference elution solvent at the same pH is often necessary to prevent analyte breakthrough during this step. To improve the purity of the extract, it is sometimes possible to add a water miscible organic solvent such as methanol to the aqueous interference elution solvent without eluting the analyte(s).

For polar analytes that are not ionizable, pH control of the interference elution solvent may be appropriate if it is possible to minimize retention of these ionizable interferences by elution solvent pH adjustment that encourages ionization of interfering compounds.



Retention: Hydrophobic interactions between the analyte and sorbent retain the analyte during loading.

Elution: A solvent that can interrupt hydrophobic interactions, such as methanol, will compete for interaction with the surface, and elute the analyte.

Figure 2. Retention and elution using ISOLUTE® ENV+.

Analyte Elution

Analytes can be eluted using an organic solvent such as methanol, tetrahydrofuran (THF), isopropanol, acetonitrile, acetone or ethyl acetate. Adding a few percent of an organic acid to the elution solvent has been shown to improve the recoveries of many acidic compounds. A variety of applications have been developed using either 5% acetic acid in ethyl acetate, 0.2% TFA in acetone and 0.1% formic acid in methanol. The combination of acetone and TFA results in a final extract that is easy to blow down to dryness and reconstitute.

N.B. The use of dichloromethane (methylene chloride) with ISOLUTE® ENV+ is not recommended.

The ability of the ISOLUTE ENV+ sorbent to retain some of the most polar water soluble pesticides / herbicides means that for most method development, a poor recovery of an analyte is more often a function of difficulties of elution rather retention. These problems are particularly apparent when attempting to elute analytes from the column with water immiscible solvents such as hexane or ethyl acetate without drying the ISOLUTE ENV+ sorbent bed thoroughly using a vacuum manifold or positive pressure. Poor reproducibility of the elution step because of variability in column drying is perhaps best overcome by incorporating a water miscible mixed solvent such as acetone: ethyl acetate (1:1, v/v) as a substitute for ethyl acetate. This can dramatically improve the robustness of the procedure[1].

For some acidic compounds such as the chlorinated phenols, efficient elution with ethyl acetate prior to GC analysis can be achieved by adding glacial acetic acid to the ethyl acetate as a substitute for the acetone/ethyl acetate mixtures. Alternatively, elution with a water miscible solvent such as methanol is generally effective. Evaluate mixed solvents such as methanol / acetonitrile to meet the elution requirements for multiple analytes.

To minimize the analyte elution solvent volume, evaluate letting the elution solvent "soak" the column for a period of time. For users of SPE automation equipment, this soak step can be programmed into the method[1]. Determine analyte recovery verses elution solvent flow rate through the column to maximize recovery. Gravity flow of some elution solvents is sometimes a practical option.

References

1. Factors affecting the reliability of automated solid phase extraction for environmental analysis.

M Raisglid, MF Burke, KC Van Horne.

Literature Number: TN109.v4