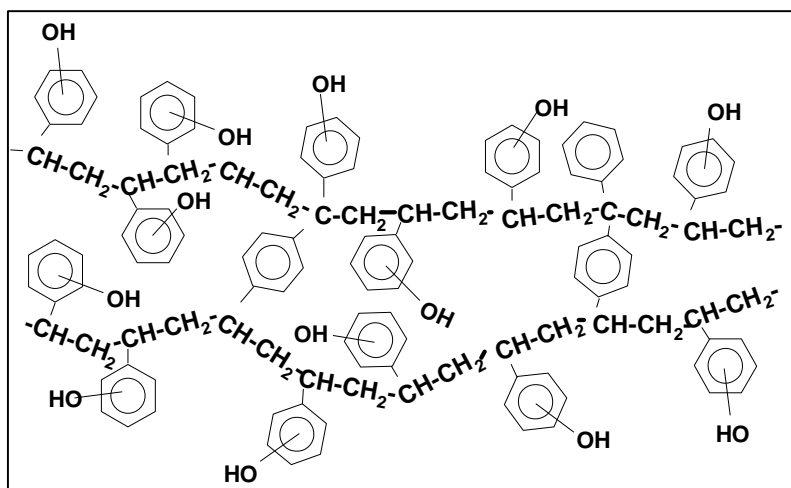


Method Development in Solid Phase Extraction using ISOLUTE[®] ENV+ SPE Columns for the Extraction of Aqueous Samples

This technical note includes a general discussion on non-polar sorbents (page 1), specifics on the use of ISOLUTE[®] ENV+ for the extraction of polar analytes from aqueous samples (page 2), and ordering information (page 5).



Structure of ISOLUTE ENV+ resin based non-polar sorbent

In Method Development Using ISOLUTE ENV+, the Following Points are Important:

Sample Pre-treatment

Due to the very hydrophobic nature of 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 (pKa 3.05), extraction efficiency can be optimized by adjusting the pH of the sample to 2 units lower than the pKa, 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 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

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 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 versus 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 ionisation of interfering compounds.

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 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 procedure¹.

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 method¹. Determine analyte recovery versus 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.

ISOLUTE ENV+ Product Ordering Information

ISOLUTE ENV+ SPE Columns Product Description	Columns / Box	Part Number
10 mg/1 mL ^{1,2}	100	915-0001-A
10 mg/10 mL ¹	50	915-0001-G
25 mg/1 mL ^{1,2}	100	915-0002-A
25 mg/10 mL ¹	50	915-0002-G
50 mg/1 mL ²	100	915-0005-A
50 mg/3 mL	50	915-0005-B
50 mg/10 mL	50	915-0005-G
100 mg/1 mL	100	915-0010-A
100 mg/3 mL ²	50	915-0010-B
100 mg/6 mL	30	915-0010-C
100 mg/10 mL	50	915-0010-G
200 mg/3 mL ²	50	915-0020-B
200 mg/3 mL ³	50	915-0020-BS
200 mg/6 mL	30	915-0020-C
200 mg/6 mL ³	30	915-0020-CS
200 mg/6 mL ⁴	30	915-0020-L
200 mg/10 mL	50	915-0020-H
500 mg/3 mL	50	915-0050-B
500 mg/6 mL	30	915-0050-C
500 mg/6 mL ⁴	30	915-0050-L
1 g/6 mL	30	915-0100-C
1 g/25 mL	20	915-0100-E

¹ The configuration of this product has been optimized to achieve minimum elution volumes of 100 μ L

² This column is also contained in the ENV+ Sorbent Mass Optimisation Kit, Part # 208-9150-AB

³ Fitted with stainless steel frits

⁴ Glass column fitted with PTFE frits

This sorbent is also available in the high throughput 96-well SPE plates, ISOLUTE-96 and ISOLUTE Array. Please contact Biotage for further information.

ISOLUTE Non-polar Sorbents: C2, C2(EC), C4, C6, C8, C8(EC), C18, C18(EC), MFC18, CH(EC), PH, PH(EC), ENV+ and 101

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

ISOLUTE ENV+ and 101 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. The resin based sorbents are high capacity, highly cross linked polystyrene based polymer columns 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 purity of the polystyrene based polymer represents a dramatic improvement when compared with the first generation of commercially available styrene divinyl benzene polymer products. The absence of monomers ensures compatibility of the ISOLUTE ENV+ products with today's demanding environmental applications.

C18, C18(EC), MFC18

The non-encapped trifunctional **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 **C18(EC)**. Non-encapped **C18** has a lower carbon loading than the encapped sorbent. **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. **MF C18** (produced using monofunctional octadecyl silane) is non-encapped and like the non-encapped 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.

C8, C8(EC), C6, C4, C2, C2(EC), CH(EC)

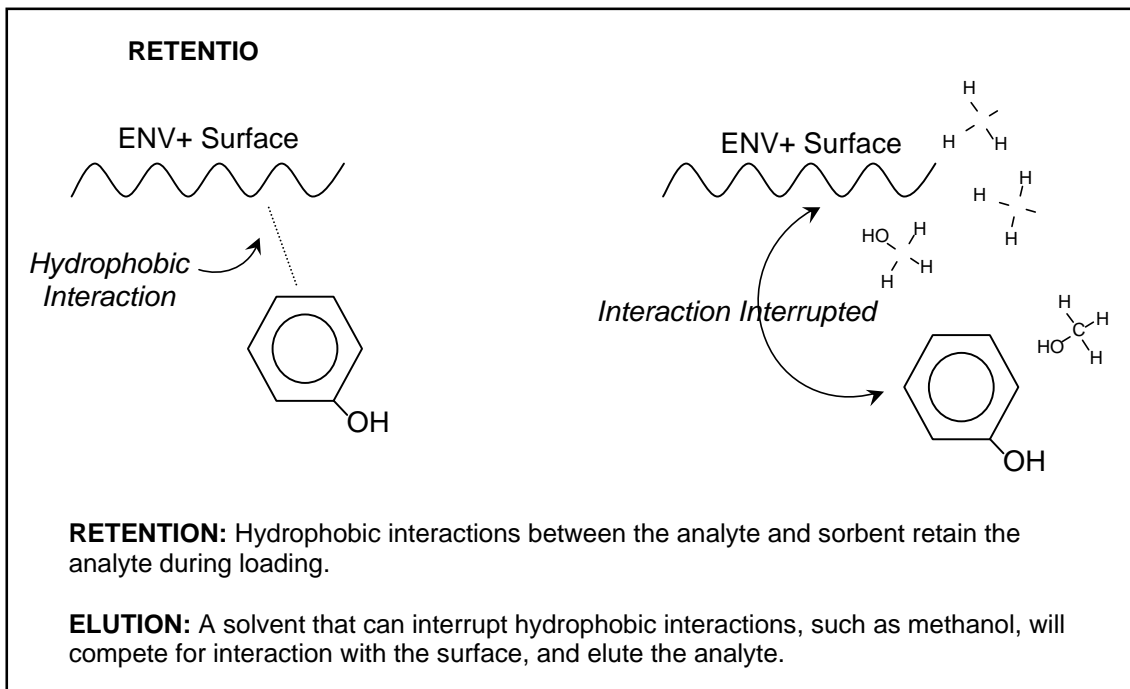
The non-polar characteristics of these sorbents decreases with carbon chain length. This can be advantageous when extracting non-polar analytes from aqueous matrices. Large, very non-polar analytes, although well retained on **C18** sorbents, can be difficult to elute as the non-polar interactions between analyte and sorbent are very strong. If a less retentive phase (such as **C8, C6, C4, C2**) is used, the analytes will still be retained, but can be eluted more easily, in minimal elution volumes. Sorbents which are encapped (**C2(EC), C8(EC)**) have fewer secondary interactions due to silanol groups than their non-encapped versions, and are therefore not recommended for the extraction of basic compounds.

PH, PH(EC)

These sorbents are generally considered to be less retentive than **C18** sorbents, but exhibit different selectivities when extracting aromatic and non-aromatic analytes.

Retention and Elution Characteristics

The retention and elution characteristics of ISOLUTE ENV+ are illustrated below.



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