Method Development Guidelines: Solid Phase Extraction Using ISOLUTE® 101 SPE Columns for the Extraction of Aqueous Samples

ISOLUTE® Non-polar Sorbents:

C2, C2(EC), 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® 101 and ENV+ 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 area 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 the ISOLUTE 101 and ENV+ products with today's demanding low-level analytical applications.

ISOLUTE® 101 has an unmodified surface and therefore does not exhibit any secondary interactions. It must be conditioned before use, and analyte elution with unmodified (pure) solvents is possible. ISOLUTE 101 is ideal for the extraction of polar analytes that are not adequately retained on C18 or C8 sorbents.

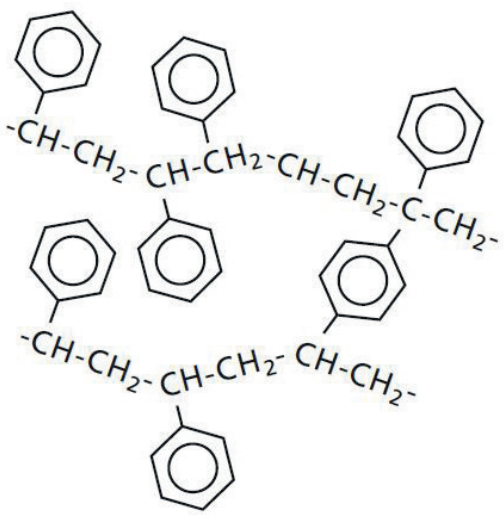


Figure 1. Structure of ISOLUTE 101.

ISOLUTE® ENV+

The sorbent has been specially derivatized to provide an easily wettable surface. It is used primarily where the analytes are very water soluble, and extraction is difficult using a silica based sorbent. This polymeric sorbent exhibits secondary interactions through the hydroxylated surface, which is particularly useful when extracting basic drugs from biological fluids, since modified elution solvents can be used, thus providing cleaner extracts.

Use of ISOLUTE ENV+ for many applications fields, in particular environmental analyses, is well documented.

ISOLUTE ENV+ is the sorbent of choice for extremely polar compounds.

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, with many of the residual silanols on the silica surface end capped to minimize secondary silanol interactions.ISOLUTE MF C18 (manufactured 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 MF C18, compared to the trifunctional C18 sorbents.

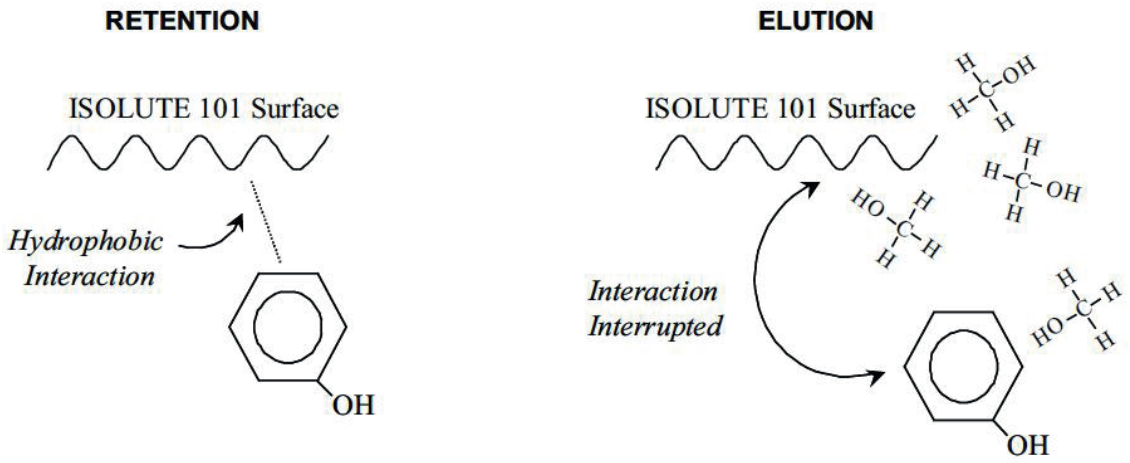
ISOLUTE® C8, C8(EC), C4, C2, C2(EC)

The non-polar characteristic of these sorbents decreases with carbon chain length. This can be advantageous when extracting non-polar analytes from aqueous matrices. Large, 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, C4, C2) is used, the analytes will still be retained, but can be eluted more easily, in minimal elution volumes. Sorbents which are endcapped (C2(EC), 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 exhibit different selectivities when extracting aromatic and non-aromatic analytes.

Biotage®



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® 101.

In method development using ISOLUTE® 101, the following points are important:

Sample Pre-treatment

Due to the extremely hydrophobic nature of ISOLUTE 101, sample pre-treatment is often unnecessary. For viscous samples, dilute with deionized distilled water to reduce the viscosity.

Suppressing ionization of the analytes by pH control can enhance the recovery of polar ionizable molecules using ISOLUTE 101 columns. Using the "2 pH unit rule", samples containing acidic compounds should be adjusted 2 pH units below the lowest pK, while samples containing basic compounds should be adjusted at least 2 pH units above the highest pK. (see Figure 2).

Column Solvation and Equilibration

ISOLUTE 101 should be solvated with a water miscible solvent (e.g., methanol, acetone, acetonitrile) prior to sample loading. The solvation step should be followed with a distilled water equilibration rinse to remove excess solvent. If pH control is required, the column should be equilibrated using a buffer of the same pH during the equilibration step.

Sample Loading

When developing a method using ISOLUTE 101, good starting points for flow rates are 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. Evaluation of analyte recovery versus increasing flow rate is a useful exercise to maximize sample throughput. It may be necessary to add 0.5 to 2% (v/v) wetting agent (e.g. methanol, isopropanol) to large volume samples (> 100 mL) to maintain an active sorbent surface.

Interference Elution

A typical solvent for interference elution is distilled deionized water.

If control of sample pH is necessary to maximize retention 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). When optimizing the concentration of organic solvent in the interference elution solvent, it is vitally important to monitor for losses in analyte recovery.

When analytes are non-ionizable, but interference compounds are ionizable, pH control of the interference elution step may be beneficial. To minimize retention of acidic interferences, use a high pH. To minimize retention of basic compounds, use a low pH.

Analyte Elution

This unmodified resin-based sorbent allows analyte elution using a pure organic solvent such as methanol, tetrahydrofuran (THF), isopropanol, acetonitrile, acetone or ethyl acetate.

To minimize the analyte elution solvent volume, allow the elution solvent to “soak” the sorbent bed for a period of time. For users of SPE automation equipment, this soak step can be programmed into the method. 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.

If eluting using a water immiscible solvent, ensure the column is dried before elution. Where pH control is necessary, it is recommended that the addition of a modifier (e.g. formic acid or ammonia) be added to improve the solubility of the analyte(s) in the chosen elution solvent.

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