Method Development Guidelines:

Solid Phase Extraction Using Non-Polar, Silica Based ISOLUTE® SPE Sorbents for the Extraction of Aqueous Samples

ISOLUTE® Non-polar Sorbents

C2, C2(EC), C4, C8, C8(EC), C18, C18(EC), MFC18, PH

The ISOLUTE® family of both endcapped and non-endcapped, non-polar, silica based sorbents are used to extract organic compounds from aqueous matrixes.

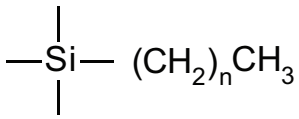

Figure 1. Structure of ISOLUTE® C2 to C18 non-polar sorbents (n=1, 3, 5, 7 or 17).

ISOLUTE® C18, C18(EC), MFC18

The non-endcapped trifunctional C18 sorbent (ISOLUTE C18) 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. 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 MFC18 (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, C2, C2(EC)

The non-polar characteristics of these sorbents decrease with carbon chain length (see structure, figure 1). 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 ISOLUTE C8, C4 or C2) is used, the analytes will still be retained, but can be eluted more easily, in minimal elution volumes. Sorbents which are endcapped (ISOLUTE 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 type sorbents, but exhibits different selectivities when extracting aromatic and non-aromatic analytes.

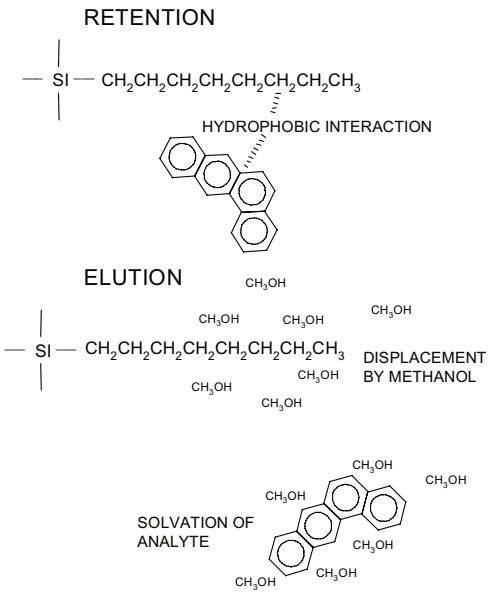

Figure 2. Retention and elution using non-polar ISOLUTE® sorbents.

RETENTION: As shown here, retention is primarily due to hydrophobic interactions.

ELUTION: Hydrophobic interaction can be disrupted with an organic solvent such as methanol which solvates the surface of the sorbent as well as the analyte.

ISOLUTE® ENV+

ISOLUTE ENV+ is the most hydrophobic of all of the sorbents. This polymeric sorbent is used primarily where the analytes are very water soluble, and extraction is difficult using a silica-based sorbent. For information on method development using ISOLUTE ENV+ please refer to Chemistry Data Sheet TN109.

In method development using ISOLUTE® non-polar sorbents, the following points are important:

Sample Pre-treatment

This stage ensures maximum interaction between the analyte and sorbent, and minimum interactions between analyte and matrix. There are various forms of sample pre-treatment, with their effectiveness dependant on the specific nature of the analytes of interest, and the matrix involved.

- It may be necessary to filter the sample if it contains particulate material (e.g. natural waters, wastewater). Care should be taken to ensure that no loss of analyte occurs due to association with the particular phase. This is most important for very non-polar analytes. For some samples, SPE columns containing integral depth filters can be used.
- For the extraction of ionizable, acidic compounds, it may be necessary to acidify the sample to 2 pH units below the pK_a of the analytes. This ensures that the acidic analyte is protonated, and therefore more readily retained by non-polar interactions.
- It may be necessary to add 0.5 to 2% wetting agent (e.g. methanol, isopropanol) to large volume samples (>100 mL) to maintain an active sorbent surface.
- Dilution may be necessary to reduce sample viscosity, to ensure a free-flowing sample. This is important for extractions from such matrices as urine or other biological fluids.
- For chlorinated water samples (drinking water) sodium sulfite can be used as a chlorine scavenger.

pH Control

pH control is very important in ensuring that a robust method is developed. Due to the presence of free silanol groups in nonpolar sorbents, a combination of non-polar and weak cation exchange retention mechanisms is often the norm when extracting from aqueous samples. For analytes with some basic characteristic, the secondary silanol interactions can be valuable in providing an extra retention mechanism. This can be exploited to produce cleaner extracts (refer to Chemistry Data Sheet TN112 for further details). pH control of the sample is essential to avoid problems due to variable matrix pH effects when utilizing secondary silanol interactions.

Column Solvation and Equilibration

Non-polar columns should be solvated with an organic solvent such as methanol, acetonitrile or THF.

The equilibration solvent should be similar to the pre-treated sample matrix with respect to pH and ionic strength. For example, if extracting from diluted urine, a low ionic strength buffer is a suitable equilibration solvent. It is important to maintain pHcontrol at this stage for the extraction of ionizable analytes.

Typical volumes for both solvation and equilibration solvents are 1–2 mL/100 mg sorbent.

Sample Loading

Optimization of loading flow rates is an important part of method development. A good starting point is 1 mL/min for a 1 mL cartridge, 3 mL/min for a 3 mL cartridge and 7 mL/min for a 6 mL cartridge (wider diameter cartridges yield lower linear velocities). The flow rate can be increased after the method chemistry is optimized. Flow rate is increased until some sample breakthrough is seen (as indicated by a drop in recovery). A flow rate slightly lower than the upper limit should be used. The optimum flow rate should be controlled and recorded to ensure reproducibility.

If the analytes of interest are known or likely to stick to the sides of the sample container (e.g. Oil and Grease samples) the sample container should be thoroughly rinsed after loading the bulk of the sample with a water miscible solvent in which the analytes are soluble. This rinse solvent should then be diluted with water (generally 1:10, v/v) and loaded onto the SPE column. This procedure will minimize losses to the container walls.

Biotage®

Interference Elution

The purpose of interference elution is to selectively remove undesired compounds from the sorbent without eluting the analytes. A typical volume of interference elution solvent is 1–2 mL/100 mg of sorbent. The flow rate should be adjusted such that thesolvent is in contact with the sorbent for 1–2 minutes.

Ionic strength and pH control should be maintained at this stage to prevent analyte loss. A good choice of solvent is often the equilibration buffer. A buffer containing 10–30% methanol or acetonitrile is often suitable for removing lipophilic interferences. Cartridge drying may be necessary to remove water if the elution solvent is water immiscible.

Drying can be performed by vacuum aspiration, N_2 or CO_2 flow, or centrifugation (useful if the analytes are volatile). Drying times depend on factors such as sorbent type (C18 dries in approximately half the time of C2, due to its relative hydrophobicity), bed dimensions and drying method. A typical range, depending on the degree of dryness required, is 30 seconds to 30 minutes. If a water miscible elution solvent is selected, the time required for cartridge drying can be reduced or eliminated. If the sample is to be concentrated to a smaller volume after elution, and the drying step was reduced or eliminated, care should be taken not to reduce the volume of solvent to where phase separation occurs, or the analytes precipitate.

Analyte Elution

The elution solvent should be one in which the analytes are soluble. It must often overcome primary and secondary retention mechanisms, and so a solvent or mixture of solvents offering multiple interactions is usually most effective. The elution solvent should also be compatible with the final analysis technique. A water miscible elution solvent may be used to elute analytes and minimize cartridge drying times (see interference elution in previous step).

For example, for an HPLC analysis, a solvent similar to the mobile phase is a good choice of elution solvent. A volatile solvent is generally selected for subsequent GC analysis. Other factors to consider include whether there will be a derivatization step, as well as volatility of the solvent if further concentration is required.

A minimum volume of elution solvent allows maximum concentration of the analytes. A typical minimum elution volume is 250 µL/100 mg of sorbent. Flow control is important to ensure reproducibility. The use of two small aliquots of solvent with a 1–4 minute soak step between elution volumes is often more efficient than one large aliquot. If a single elution is required, the flow rate of the elution solvent should be such that contact time between solvent and sorbent is 1–4 minutes.

Typical elution solvents are methanol, acetone or other solvents or solvent mixtures; solvent containing 1–5% formic or acetic acid, or solvent containing a volatile amine such as TEA or TMA **www.biotage.com for more information.**

ISOLUTE® Non-polar SPE sorbents are available in a wide range of both column and 96-well plate formats.

Sorbent	Sorbent Reference Number
C18	220
C18(EC)	221
MFC18	240
C8	290
C8(EC)	291
C4	390
C2	320
C2(EC)	321
PH	360

Table 1. The range of ISOLUTE® non-polar sorbents.

See www.biotage.com for more information.

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Distributors in other regions are listed on www.biotage.com

Part Number: TN101.V.1

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