Method Development Guidelines:

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

ISOLUTE® Polar Sorbents

SI, NH2, PSA, DIOL, CN

The ISOLUTE® family of polar sorbents are used to extract polar organic compounds from non-polar matrixes. See Figure 1 for structures of ISOLUTE® polar phases.

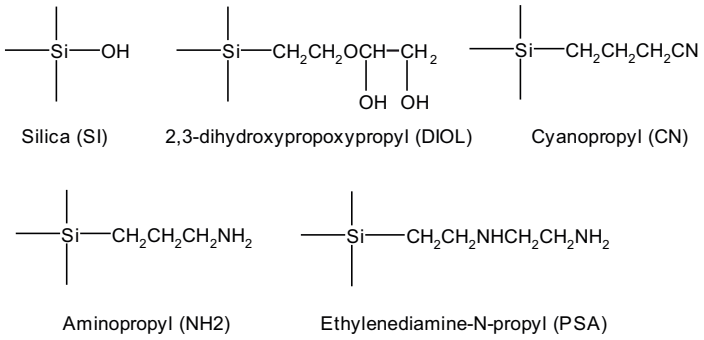


Figure 1. Structure of ISOLUTE® polar phases.

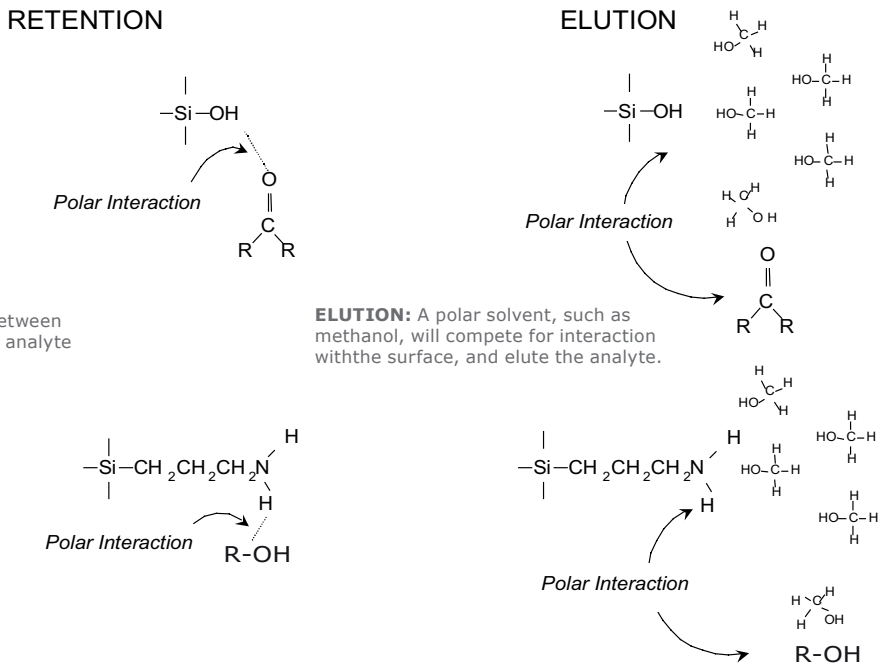
ISOLUTE® SI, NH2, PSA, DIOL, CN

Polar sorbents that vary in their ability to exert polar interactions.

There are many functional groups that can exhibit polar interactions, and they have different selectivities. Some of the polar interactions that exist include dipole-dipole, dipole-induced dipole and hydrogen bonding. Polar interactions are particularly useful for the separation of molecules that are very similar in structure (e.g. structural isomers).

ISOLUTE Silica (SI) has the greatest capacity for polar interactions, and is well suited for sample cleanup. SI is capable of exhibiting three different types of interactions: hydrogen bonding, dipole-dipole, and, in the presence of trace amounts of water, ion exchange. Aminopropyl (NH_2), ethylenediamine-N-propyl (a primary/secondary amine, PSA) and dihydroxypropoxypropyl (DIOL) sorbents can participate in hydrogen bonding and dipole-dipole interactions. Cyanopropyl (CN) can participate only in dipole-dipole, and is suitable for analytes that are difficult to elute from the other polar sorbents.

The method development guidelines in this technical note apply to other non-silica based polar ISOLUTE® sorbents, such as ISOLUTE Florisil and Alumina type materials.



RETENTION: Polar interactions between the analyte and sorbent retain the analyte during loading.

ELUTION: A polar solvent, such as methanol, will compete for interaction withthe surface, and elute the analyte.

Figure 2. Retention and elution characteristics of ISOLUTE polar sorbents. Above diagram shows retention and elution using ISOLUTE® SI and NH2. The remaining sorbents behave similarly.

Biotage®

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

Sample Pre-treatment

For optimum retention of polar analytes, the sample should be in a non-polar matrix. If the matrix contains a significant concentration of polar solvent (e.g., acetone or acetonitrile), a non-polar solvent such as hexane should be added to dilute the sample and maximize the non-polar character of the matrix. Other suitable solvents include dichloromethane, chloroform and isooctane.

For clean-up of samples originating from non-liquid matrices, the analytes should first be extracted into a non-polar solvent using a liquid extraction techniques such as soxhlet, ASE or similar.

Column Solvation

Polar columns should be solvated with a non-polar solvent similar to that used to dilute the sample. This will maximize the polar interactions between column and analyte.

Sample Loading

For polar 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. Loading rates may be increased after method chemistry is established.

Interference Elution

A typical solvent for interference elution is the equilibration solvent. Another good choice of solvent is one in which the interferences are soluble, but the analyte is not.

Analyte Elution

Analytes can be eluted using a mixture of polar (e.g. methanol, isopropanol, acetonitrile, acetone) and non-polar (e.g. hexaneethyl acetate) solvents. The polar fraction should be minimized to ensure the greatest selectivity, and hence, the cleanest extract. For some applications, the polar fraction can be as little as 1, 2, or 5% (v/v).

N.B. The activity of unbonded silica (SI) is moisture dependant and can be affected by atmospheric humidity conditions or solvents containing some moisture. It is very important to ensure that solvents used in polar SPE procedures are dry and do not contain any polar modifiers (e.g. ethanol stabilizer in DCM).

Bonded polar sorbents (NH2, PSA, CN and DIOL) are more resistant to changes in activity due to moisture, and SPE procedures using these sorbents can be more robust.

ISOLUTE Polar SPE sorbents are available in a wide range of column formats.

Sorbent	Sorbent Reference Number
SI	460
NH2	470
PSA	480
CN	420
DIOL	430
Florisil	712

Table 1. The range of ISOLUTE® polar sorbents.

See www.biotage.com for more information.

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: + 82 31 706 8500
Fax:+ 82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

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