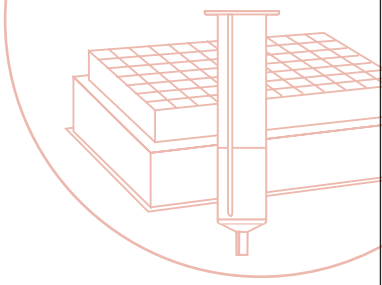


Normal Phase Extraction Procedure

In normal phase solid phase extraction (SPE), target analytes and other compounds with a large proportion of polar functional groups (amines, amides, hydroxyls, carbonyls, heteroatoms) are extracted from non-polar organic solvents (hexane or chlorinated solvents) The retention mechanism is based on hydrogen bonding, dipole-dipole and π–π interactions between polar analytes and polar stationary phases such as Strata™ Silica, NH_2, CN, and Florisil. ***Table 1*** gives the physical and chemical properties of these normal phase sorbents. Highly specific normal phase extractions can be obtained by carefully optimizing the polarity of the conditioning solvent and the solvent(s) used to dilute and load the sample/matrix. Elution occurs in the presence of polar solvents.

Table 1. Physical and chemical characteristics of the Strata normal phase sorbents

	Silica	Florisil	NH_2	CN
Silica Bonding	-	-	Monofunctional	Trifunctional
Endcapped?	-	-	No	No
% Carbon Load	-	-	5	10
Surface Area (m^2/g)	500	300	500	500
Particle Size (µm)	55	170	55	55
Pore Size (Å)	70	80	70	70

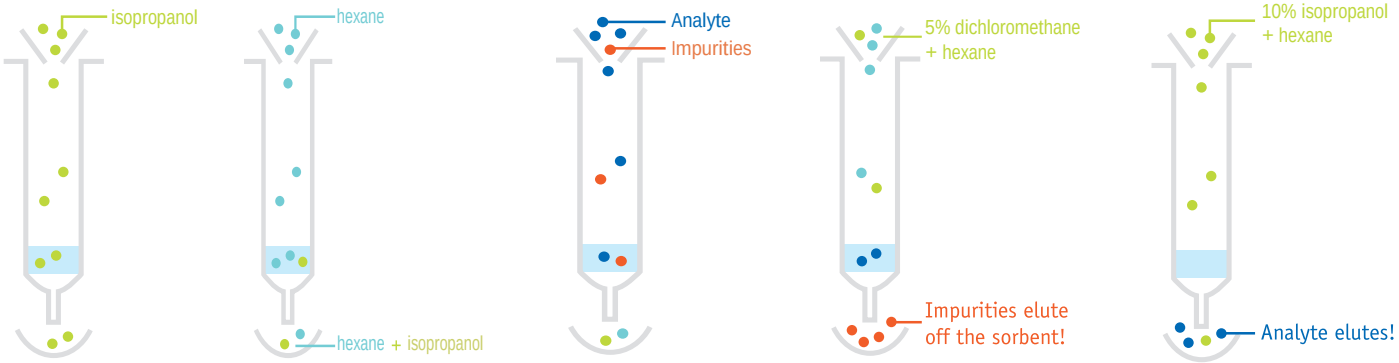
Specimen preparation

In general, the retention of polar analytes occurs only when the sample/matrix is composed almost entirely of a non-polar, water-immiscible solvent(s). As a result, the original sample/matrix should ideally be composed of solvents such as “alkanes” (hexane(s) or petroleum ether), aromatics (toluene or benzene) or alkyl halides (dichloromethane). Samples/matrices containing moderately polar organic solvents (such as alcohols) must be diluted extensively in non-polar organic solvents prior to sample loading. In fact, alcohols are extremely potent elution solvents for normal phase SPE. Aqueous samples/matrices need to be diluted first in a water-miscible, polar organic solvent (such as THF, acetone, or acetonitrile) and then diluted extensively in a non-polar organic solvent. Tetrahydrofuran (THF) is probably the best intermediate solvent for this purpose, since it is fully miscible with water as well as with non-polar organic solvents. Since aqueous samples/matrices require such extensive preparation and dilution prior to normal phase SPE, they are more often (and more easily) processed on reversed phase (or ion exchange) SPE sorbents.

The pH of the sample/matrix (and the mobile phases) may need to be adjusted with acid, base or buffered so the net charge on the analyte is zero. Viscous samples should be diluted further in order to improve the flow rate and prevent the column from clogging.

SPE method*

The volumes shown are for 100mg sorbent mass.



Condition
with 1mL isopropanol

Equilibrate
With 1mL nonpolar organic solvent

Load Sample

Wash Impurities
with 1mL 5% dichloromethane in hexane and dry 2-5 min at full vacuum

Elute Analyte!
with 1mL 10-20% polar organic in hexane

* This method is designed as a convenient starting point for further investigation. The following section contains optimization tips allowing you to tailor the method to your needs. Phenomenex makes no guarantee regarding the accuracy or completeness of the method.

Activation

Sorbent activation consists of "conditioning" and equilibration steps, which are solvent rinses designed to prepare the sorbent for interaction with the sample. The choice of the conditioning solvent is critical in normal phase SPE. An initial solvation step (which includes isopropanol and dichloromethane (1:1), either alone or in combination with hexane or another non-polar organic solvent) is beneficial, particularly on bonded silicas such as CN and NH_2. This will help remove trapped air and help to fully solvate the entire chromatographic surface. The equilibration solvent should be similar in composition to the diluted sample/matrix, but should contain as little polar solvent as possible, particularly water.

Solvent volumes between 2 to 4 times the sorbent bed volume are necessary to ensure proper activation. Conventional silica-based SPE products have a bed volume of approximately 150µL/100mg of sorbent. The flow rate is not particularly critical during conditioning; 3-5mL/min is typical.

Important! Do not allow the sorbent to dry under full vacuum before applying the sample since the loss of the solvating solvent deconditions the sorbent resulting in lower recoveries. If the tubes are inadvertently dried for too long, simply repeat the conditioning steps.

Sample Loading

Apply the sample to the tube with the vacuum or pressure turned off, then either aspirate the sample through the tube at a flow rate of approximately 1mL/min, or allow it to flow by gravity. A slow load rate is generally preferable since the kinetics of sorbent-analyte interactions are highly variable and strongly influenced by the composition of the sample/matrix and the nature of the target analyte(s). However, the flow rate should be balanced against the time constraints of passing the entire sample through the tube. For sample sizes of 0.5-1L (common for environmental analysis), a flow rate of 25-50mL/min can be used. To compensate for the faster flow rate, a larger bed mass is advised so that analyte breakthrough is minimized.

Wash/Dry

Rinse the tube with 1mL for every 100mg of bed mass (or 5 to 10 sorbent bed volumes) of wash solvent. Common wash solvents are non-polar organic solvents such as hexane containing a small amount of polar organic solvent (e.g. 1-2% isopropanol or methanol), or preferably, a slightly higher concentration of a moderately polar solvent (e.g. 2-8% dichloromethane, acetone, or THF), alone or in combination. The flow rate should be approximately 1-2mL/min. After the wash solvent has been passed through the sorbent bed, continue to apply vacuum or positive pressure. Dry the sorbent under full vacuum (or pressure) for 2-5 minutes. Turn off the vacuum, and wipe the tips of the manifold needles in order to remove any residual sample/matrix or wash solvent.

Elution

Insert the collection tubes into the manifold rack, and add a minimum of 2 to 4 sorbent bed volumes of elution solvent to the tube. Allow the elution solvent to remain or "soak" in the sorbent bed (without vacuum) for approximately one minute in order to optimize recovery. Slowly aspirate the elution solvent through the tube (1-2mL/min). Collect the analyte.

Elution of the analyte is facilitated with polar solvents that can disrupt the hydrogen bonding between functional groups on the analyte and the sorbent.

Typical elution solvents include low levels of polar organic solvents (such as isopropanol, acetonitrile or methanol) either alone or in combination with moderately polar organic solvents (such as dichloromethane, THF and acetone), all dissolved in non-polar organic solvents (such as hexane, toluene or petroleum ether). In order to improve recovery, use two or more aliquots containing a smaller volume of elution solvent rather than a single, larger volume.

Extraction Tips!

1. Silica is hydroscopic. Any water adsorbed to the surface can greatly reduce the retention of organic solutes. It is important to keep silica sorbents dry and free from water.
2. Very polar analytes containing hydroxy or amino groups are tightly bound to silica. The use of CN or NH_2 often permits recovery of compounds that interact too strongly with silica.

Questions? Please contact your local Phenomenex Technical Consultant

Strata reversed phase sorbents are available in numerous formats to meet all your sample preparation needs.

Tubes

	1mL	3mL		6mL		12mL		20mL	60mL	
Phase	**100mg**	**200mg**	**500mg**	**500mg**	**1g**	**500mg**	**2g**	**5g**	**10g**	**20g**
NH_2	8B-S009-EAK	8B-S009-FBJ	8B-S009-HBJ	8B-S009-HCH	8B-S009-JCH	—	8B-S009-KDG	8B-S009-LEG	8B-S009-MFF	—
CN	8B-S007-EAK	8B-S007-FBJ	8B-S007-HBJ	8B-S007-HCH	8B-S007-JCH	—	8B-S007-KDG	8B-S007-LEG	8B-S007-MFF	—
Silica	8B-S012-EAK	8B-S012-FBJ	8B-S012-HBJ	8B-S012-HCH	8B-S012-JCH	8B-S012-HDG	8B-S012-KDG	8B-S012-LEG	8B-S012-MFF	8B-S012-VFF
Florisil	—	—	8B-S013-HBJ	8B-S013-HCH	8B-S013-JCH	—	8B-S013-KDG	8B-S013-LEG	8B-S013-MFF	—

96-well plates

	25mg	50mg
NH_2	8E-S009-CGB	8E-S009-DGB
CN	8E-S007-CGB	8E-S007-DGB
Silica	8E-S012-CGB	8E-S012-DGB

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