Empore™

Solid Phase Extraction Cartridges

Instructions for Use

Product Description

Empore™ Solid Phase Extraction Cartridges are designed for sample pretreatment to remove or minimize sample matrix and other interferences to "cleanup" a sample prior to analysis. This procedure can also concentrate an analyte to achieve the desired sensitivity range of an analytical method. Compounds are isolated from complex mixtures by proper selection of a variety of sorbent chemistries.

The cartridge is molded from a polypropylene resin. An Empore extraction disk is secured in place at the bottom of each cartridge with a sealing ring. A proprietary prefilter is placed above the Empore disk. This prefilter aids in preventing particulates and macromolecules from reaching the underlying membrane and improves the flow of biological samples, such as serum and plasma, through the cartridge.

The prefilter is composed of polypropylene microfiber layers of graded densities. Three different densities are used, with the coarsest one on top and the finest at the bottom. The top two microfiber layers are individual layers of material. The third microfiber layer, having the smallest effective pore size, is on the bottom of the prefilter and contains five individual layers of material. A porous polypropylene support membrane comprises the final layer.

Product Characteristics

The Empore SPE cartridges are available in 1 mL, 3 mL and 6 mL volumes. The effective SPE membrane diameters are specified as 4, 7 and 10 mm, respectively.

Sorbents Available

Sorbent	Size	Suggested Applications	Product Number
C8-SD (Standard Density)	4 mm / 1 mL	Moderately nonpolar analytes	4114SD
C8-SD (Standard Density)	7 mm / 3 mL	Moderately nonpolar analytes	4214SD
C8-HD (High Density)	4 mm / 1 mL	Moderately nonpolar analytes	4114HD
C18-SD (Standard Density)	4 mm / 1 mL	Strongly nonpolar analytes	4115SD
C18-SD (Standard Density)	7 mm / 3 mL	Strongly nonpolar analytes	4215SD
C18-SD (Standard Density)	10 mm / 6 mL	Strongly nonpolar analytes	4315SD
SDB-XC (High Density poly[styrenedivinylbenzene])	10 mm / 6 mL	Moderately nonpolar analytes plus pi-pi interactions	4340HD
UR-SD (Universal Resin Standard Density)	7 mm / 3 mL	Mixed phase suitable for hydrophilic (acidic or basic) and moderately hydrophobic analytes	4245SD

Empore™ Disk Technology

Empore™ Solid Phase Extraction Disks are produced by trapping sorbent particles within an inert matrix of polytetrafluoroethylene (PTFE). The resulting particle-loaded membrane yields a denser, more uniform extraction bed than can be achieved with traditional loosely packed SPE particles. The result is improved mass transfer kinetics with consistent performance in solid phase extraction methods.

The dense particle packing and uniform distribution within Empore disks offer outstanding sample preparation efficiency and reproducibility of results. Since the diffusion distance between particles is minimized, adsorption is more efficient, and extraction can be accomplished using low sorbent mass. The following performance gains can be realized:

- Reduced solvent volumes
- Small elution volumes
- Reduced time for eluate evaporation
- Potential elimination of eluate evaporation
- High throughput
- Channeling effects eliminated
- Excellent reproducibility/low CVs

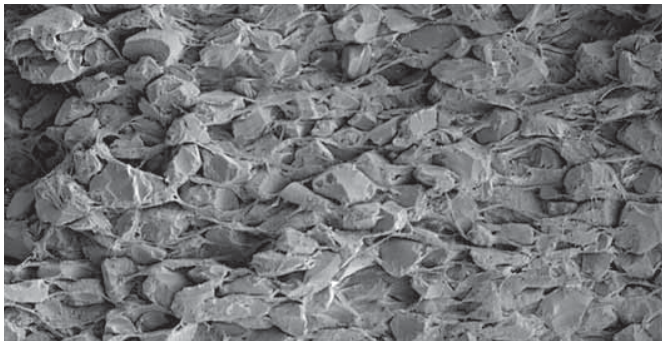
The Empore C8, C18 and Universal Resin cartridges are available in standard density (SD) membranes which are composed of chromatographic particles commonly referred to as from 40-60 µm in size. The standard density membrane has been optimized for improved flow rates for samples processed in most bioanalytical applications.

The Empore C8 and SDB-XC cartridges are available in high density (HD) membranes which are composed of chromatographic particles commonly referred to as from 10-12 µm in size. The high density membranes are designed for maximum extraction efficiency with minimal elution volumes of samples that have less matrix interference.

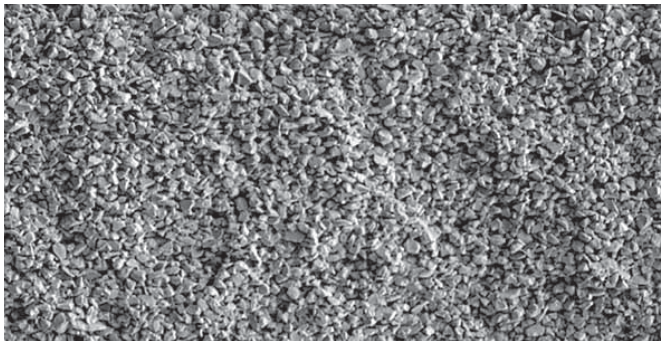
Volume Guidelines

Reversed Phase Extractions

The small bed mass of sorbent in the disk cartridge allows for the use of small solvent volumes. A general guide to solvent volumes for a disk cartridge SPE method using reversed phase sorbents (C18, C8, SDB-XC and UR) is listed in the table below. Each assay will need some further optimization in terms of selecting the best wash solvent composition (10% methanol as shown in the example will not be optimal for all assays) and the particular elution solvent (commonly methanol or acetonitrile).



Standard density (SD) Empore™ Membrane (40-60 µm particle size)



High density (HD) Empore™ Membrane (10-12 µm particle size)

Volume Guidelines: Reversed Phase (C18, C8, SDB-XC, UR) Empore Extraction Disk Cartridges

Step	Solvent	4mm/1mL	7mm/3mL	10mm/6mL
Condition	Methanol	150 µL	250 µL	500 µL
	Water	300 µL	500 µL	1000 µL
Load	Sample	250 µL	1000 µL	2000 µL
	Buffer/IS	250 µL	1000 µL	2000 µL
Wash	Water	300 µL	500 µL	1000 µL
	Organic/Aqueous	300 µL	500 µL	1000 µL
Elute	Organic	100-150 µL	200-300 µL	600-800 µL

Important Notes: It is recommended to optimize the volume of elution solvent to ensure that the minimum volume is used that will elute the analyte reproducibly from the sorbent phase. Due to disruption of ionic interactions, which can be stronger than reversed phase interactions, slightly more elution solvent volume may be required for mixed phase disks than for a reversed phase sorbent.

Product Selection

When developing a solid phase extraction method using Empore™ Extraction Disk Cartridges, a number of choices must be made. These choices refer to

1. **Membrane Density**
2. **Cartridge Size**
3. **Sorbent Chemistry**

Informed and proper choices lead to successful SPE methods. Note that each analytical compound is unique and only the analyst can be aware of the critical physicochemical factors influencing a specific analysis. The following general information is presented to familiarize you with the choices available in selecting Empore Extraction Disk Cartridges.

1. Membrane Density

The C8 bonded silica sorbent in Empore Extraction Disk Cartridges is available in both a standard density (SD) and a high density (HD) membrane format. The C18 and Universal Resin sorbents are available in SD membrane and the SDB-XC is available in the HD membrane. Both SD and HD membrane formulations provide the same unique features of uniform particle distribution and dense packing, but vary with respect to particle size and membrane thickness.

The standard density format is designed for use with biological matrices and is recommended as the first choice for most applications. The high density format is reserved for situations when an even smaller elution volume is desired, and when the sample matrix is relatively clean (e.g., water or filtered serum).

2. Cartridge Size

SPE cartridges have traditionally been defined by sorbent mass and reservoir volume (e.g., 100mg/1mL). Empore™ Extraction Disk Cartridges are defined by disk diameter and reservoir volume. Three Empore™ Extraction Disk Cartridge sizes are available and are designated as 4mm/1mL, 7mm/3mL and 10mm/6mL. The selection of cartridge size for an application typically depends on three factors: (a) sample volume, (b) sample viscosity, and (c) elution volume requirements. A general guide to cartridge size selection is shown below.

3. Sorbent Chemistry

The ideal extraction closely matches the physicochemical properties of the analyte (pKa; acidic, neutral or basic characteristics; functional groups) with the sorbent chemistry and its solid support (silica or copolymer). In many cases, the sample matrix and the surrounding environment (pH and/or salt concentration) play an important role in matching analyte with sorbent.

Reversed Phase Extraction Using Bonded Silica C18 and C8) Reversed phase extraction is the most common type of SPE performed. In this case, an aliphatic hydrocarbon chain (C18 or C8) is bonded to irregularly shaped silica particles. Analytes are retained by a combination of nonpolar interactions, Van der Waals forces, or secondary interactions (e.g., hydrogen bonding to silica silanols). C18 is strongly nonpolar and nonselective, so it tends to be used most often with success. C8 is moderately nonpolar and can be more selective than C18 for analytes.

Reversed Phase Extraction Using SDB-XC Copolymer
A frequently used alternative to a bonded silica sorbent for reversed phase extraction is based on a copolymer of poly(styrenedivinylbenzene), designated SDB-XC. This copolymer sorbent displays the following advantages over bonded silica sorbents:

- No secondary interactions
- No pH limitations
- Greater capacity
- Improved selectivity for moderately polar, water-soluble analytes

Multi-Mode Extractions Using Universal Resin

The Universal Resin (UR) sorbent is a terpolymer based on styrenedivinylbenzene and designed to provide good retention of a wide range of analytes. A single reversed phase sorbent can often be used to isolate and concentrate a variety of acidic, basic and neutral compounds. Method development time is saved by eliminating the need to screen a variety of sorbents.

4mm/1mL Extraction Disk Cartridge	• Miniaturizes SPE • Ideal for 0.05 to 0.5 mL sample volumes • Fast throughput using automation	• Elution volumes are small and range from 100-200 mL* • Small disk surface area results in slow flow characteristics if using vacuum • Centrifugation recommended as processing method
7mm/3mL Extraction Disk Cartridge	• Most commonly used and versatile size • Typically used for 0.5 to 2 mL sample volumes • Fast throughput using automation	• Elution volumes range from 200-400 mL* • Interchangeable with 100mg/1mL packed SPE columns
10mm/6mL Extraction Disk Cartridge	• Used for larger sample volumes of several milliliters • Higher capacity	• Elution volumes range from 600-1000 mL* • Faster flow characteristics due to larger disk surface area

*Elution volume will vary depending on the analyte, its affinity for the chosen sorbent, and the strength of the eluting solvent.

Performing an Extraction Method

Five Basic Steps of Solid Phase Extraction

1. **Condition the Disk**
2. **Load and Extract Sample**
3. **Wash out Interferences**
4. **Elute Analyte(s)**
5. **Prepare Eluate for Analysis**

1. Condition the Disk

It is necessary to first wet a reversed phase sorbent by adding methanol (or acetonitrile). Pass most of the methanol through the disk but leave the surface of the disk wetted. Remove residual methanol by adding DI water (use a volume of water greater than that used for methanol). Pass most of the water through the disk but leave the surface of the disk wetted. Common processing methods (vacuum, positive displacement, centrifugation, automated liquid handling workstations) to pass liquids through the disk are explained in a separate section.

It is important that the disk not be allowed to dry prior to sample addition. If the disk does become dry, repeat the conditioning procedure.

Note: When using solvents or other chemicals, be sure to read and follow the manufacturer's precautions and directions for use.

2. Load and Extract Sample

Carefully transfer the sample into the extraction disk cartridge. Add internal standard (IS) and/or adjust sample pH as the method requires. Dilution of sample with an equal volume of buffer may improve flow, in addition to maintaining sample pH, and is suggested. Pass the sample/buffer solution through the disk.

3. Wash out Interferences

The goal of the wash step after sample loading is to remove co-extracted substances that could potentially interfere with the subsequent analysis. Water and buffers are commonly used as wash solvents for reversed phase extractions. They are effective at removing adsorbed proteins remaining on the surface of the sorbent bed. It is recommended to always use an aqueous wash initially after sample loading, rather than using only a single organic/aqueous mixture. Pass the entire volume of water through the disk.

Water or buffer alone may not provide sufficient clean-up in each assay. A second wash should contain a small percentage of organic solvent (commonly 5 to 20% organic in an aqueous mixture) to more efficiently remove potential interfering substances. Note that the secondary wash should be chosen so that it removes as many interfering substances as possible without adversely affecting retention of the analyte(s) of interest. Pass the entire volume of wash solvent through the disk. Remove as much residual wash solvent as possible before the elution step is performed.

4. Elute Analyte(s)

Use a clean sample collection tube or vial for analyte elution. Note that the small bed volume of the disk allows for reduced elution volumes. Add to the extraction cartridge the proper volume of elution solvent necessary, depending on disk diameter, for analyte recovery (see Volume Guidelines section).

Pass the elution solvent through the disk. Add a second aliquot, and repeat the elution. Briefly vortex mix the eluate solution before analysis so that it is homogeneous (the most concentrated portion of the eluate will be on the bottom of the tube or vial).

5. Prepare Eluate for Analysis

The ability to elute in small volumes from the disk may mean that the evaporation and reconstitution step may be eliminated. If the eluate is LC mobile phase or a compatible solvent, no additional steps are necessary. If organic solvent is used for elution, and further sample concentration is required to achieve the sensitivity limits of the assay, a typical procedure involves evaporation of the eluate under nitrogen (sometimes with heat applied). Reconstitution is then performed by adding a known volume of an appropriate solution (compatible with instrumental analysis) to the collection tube and vortex mixing before analysis.

Sample Processing Options

The dense particle packing of the disk prevents sample from flowing freely under gravity. Some type of vacuum or positive displacement is always required to force liquids through the disk. The following methods have proved successful in processing liquids through the membrane:

1. **Vacuum**
2. **Positive Displacement**
3. **Centrifugation**
4. **Automated Liquid Handling Workstations**

1. Vacuum

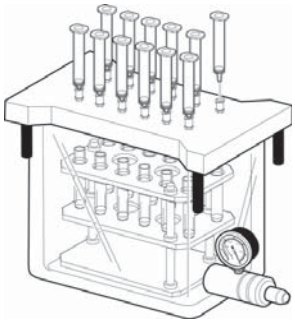
A vacuum manifold is commonly used to pass liquids through the disk. These manifolds are available from many vendors and generally hold from 12 to 24 cartridges at a time. Test tubes are placed below each cartridge position to collect liquids during each step. Note that these manifolds are not designed to effectively use the low volumes made possible by Empore™ Extraction Disk Cartridges (but were designed for packed column SPE cartridges that require much larger solvent volumes for processing).

Vacuum Recommendations

Empore™ Extraction Disk Cartridges (standard density, SD) generally require from 10-15 inHg (0.34 to 0.51 bar) to process biological fluids such as plasma and serum when using vacuum. High density disk cartridges require maximum vacuum, about 20 inHg (0.68 bar). Note that if the sample matrix is relatively clean and of a small volume, a lower vacuum may be used.

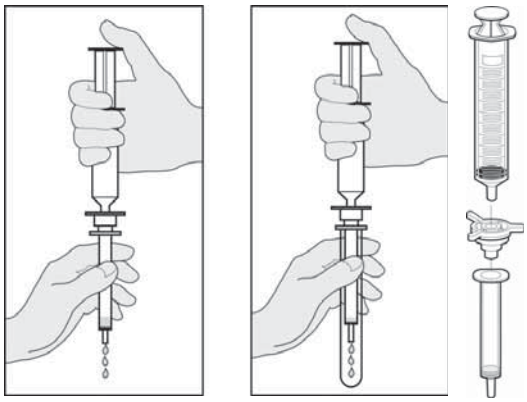
A general guide is to open the vacuum source to about 15 inHg (0.51 bar) for all steps, with two exceptions:

1. During method optimization, try loading the sample matrix at both a low vacuum (5-7 inHg; 0.17 to 0.24 bar) and a high vacuum (about 17-20 inHg; 0.58 to 0.68 bar). If an analyte has a low affinity for the sorbent, it may need to pass through the sorbent bed more slowly during the load step for sufficient attraction to occur.
2. A lower vacuum is generally desirable during the elution step to prevent splashing in the collection device. A lower vacuum may also be beneficial when eluting from MPC sorbent, as a slower flow rate will allow more time to disrupt ionic interactions (which are stronger than reversed phase interactions).



2. Positive Displacement

Positive air displacement can be used in a manual mode with disk cartridges. In this manner, one sample at a time can be processed by attaching a syringe to an adapter that fits between the cartridge and the syringe. Air is forced through the cartridge and displaces liquids.



A single piece device, the Supelco® Visi-1™ (Catalog #57080), is a similar approach that eliminates the need for a separate adaptor. It also provides for a more finely controlled positive displacement, resulting in tighter flow control.

Note that a specific type of manifold delivers positive pressure instead of using vacuum for processing liquids. Multiple cartridges can be processed at a time. It is available from Varian™ Sample Preparation Products.

3. Centrifugation

Positive pressure via centrifugation is another option for processing liquids through disk cartridges. Centrifugation can be preferable to vacuum as it requires less manipulation and permits more complete volume collection. Often, centrifugal forces of 75-120g are used; or from 1200-2750 RPM in general terms. Forces greater than these numbers may be used, but first examine the effect on analyte recovery. With centrifugation, the disk cartridge is suspended in a test tube and placed into a carrier tray that fits into the centrifuge.

Conditioning can be done off-line manually (see Positive Displacement section) or as part of the centrifugation method.

4. Automated Liquid Handling Workstations

Several different liquid handling workstations are commercially available to automate the extraction process. They use either positive displacement or vacuum to move liquids through the disk, utilizing unique methods (e.g., plunger, cap insertion, sealing plate). These systems, in combination with the disk format, can be an ideal combination to offer improved throughput. The possibility for human pipetting error and/or procedural error is eliminated.

Product Specifications

	Standard Density (SD)	High Density (HD)
Applications	For a wide range of samples and volumes including plasma and urine	Suggested for clean samples and when lower elution volumes are desired
Sorbent Particle Size	40-60 µ	10-12 µ
Membrane Thickness	0.75 mm (nominal)	0.50 mm (nominal)
Vacuum Requirements	5-10 inHg, (0.17-0.34 bar), depending on sample viscosity	Requires strong vacuum of 15-20 inHg, (0.51-0.68 bar) or greater

Note: Empore Sample Preparation Products are intended for solid phase extraction during scientific research only. These products are not intended for use in medical devices or in assessment and treatment of clinical patients.

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