



Empore™ High Performance Extraction Disk Cartridges

October 1998

Answers to Frequently Asked Questions

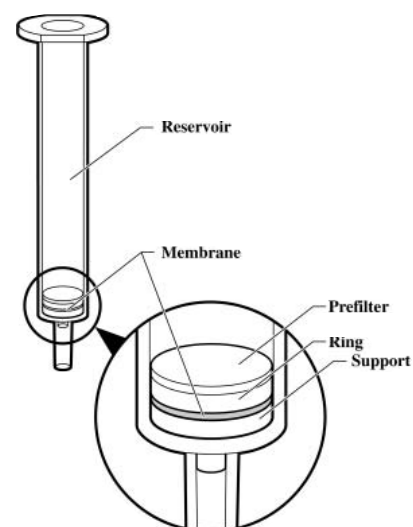
Product Description

What is the configuration within the Empore™ Extraction Disk Cartridge?

An Empore Disk Cartridge consists of an Empore membrane mounted into the bottom of a 1, 3 or 6 mL polypropylene syringe barrel. The effective membrane diameter is specified as 4, 7 or 10 mm, respectively. A sealing ring secures the membrane into place. Each cartridge contains a patented prefilter (graded density polypropylene) that improves flow with challenging sample matrices.

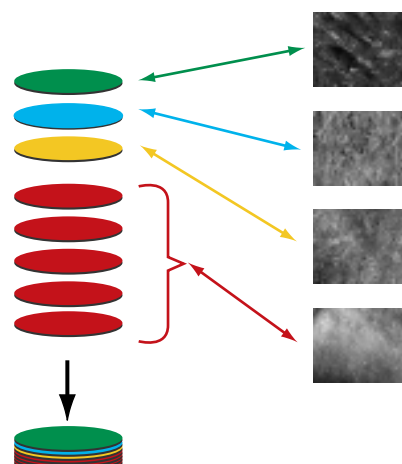
What are Empore Extraction Disk Cartridges used for?

Empore High Performance Extraction Disk Cartridges are used for the solid phase extraction (SPE) of analytes from liquid samples. This extraction results in a clean-up of a sample prior to analysis, and/or concentrates a compound 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. In addition, the disks can be used to remove impurities while allowing analytes to pass through.



What is the composition of the prefilter?

The patented prefilter is composed of polypropylene microfibers of graded densities. There are four densities represented, with the coarsest one on top and the finest at the bottom. Single layers of material represent the top three microfiber densities, from top to bottom. The fourth microfiber type, having the smallest effective fiber diameter, is on the bottom of the prefilter and contains five individual layers of material.



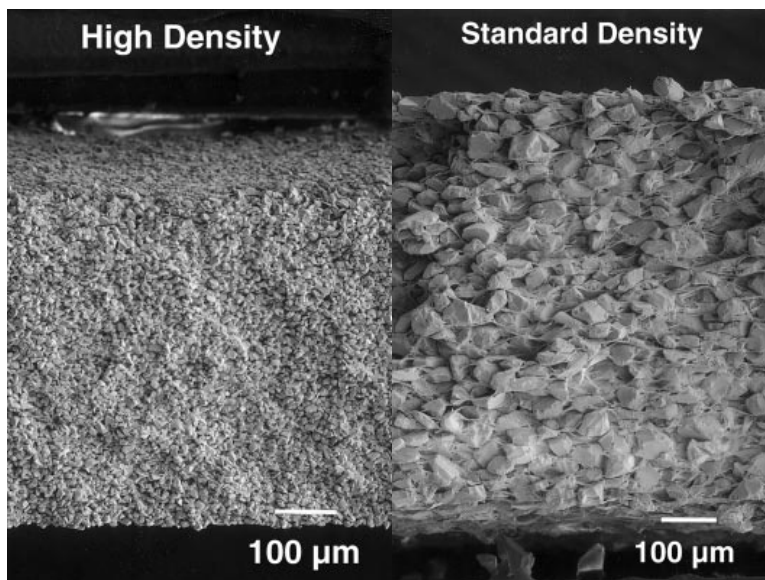
Empore Disk Technology

Explain the technology of the Empore Disk.

A 3M patented process entraps sorbent particles within an inert matrix of polytetrafluoroethylene (PTFE). The resulting particle-loaded membrane (90% particles: 10% PTFE, w/w) yields a denser, more uniform extraction bed than can be achieved in a traditional SPE cartridge made from loosely packed particles.

Describe what the "standard density" and "high density" membrane formats are, and which one should I use?

Four bonded silica sorbents (C18, C8, C2, MPC) in Empore Extraction Disk Cartridges are available in both a standard density (SD) and a high density (HD) membrane format. 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.



Micrograph of a cross section of a high density (HD) and a standard density (SD) Empore™ disk.

Standard density (SD) membranes are composed of chromatographic particles which are commonly referred to as from 40-60 µm in size (actual mean size is about 55µm). 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 the original membrane (disk) formulation and 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). Note that SDB-XC and SDB-RPS copolymer sorbents are available only in the high density formulation.

Why is the product referred to as "high performance"?

The dense particle packing and uniform distribution within Empore disks offer a great improvement in the efficiency and reproducibility of sample preparation techniques. The diffusion distance between particles is minimized, adsorption is more efficient, and extraction can be accomplished using less sorbent mass.

The following performance gains can be realized:

- Reduced solvent volumes
- Smaller elution volumes
- Reduced time for the evaporation step
- Ability to eliminate the evaporation step
- Higher throughput
- Minimal concerns with flow rate effects on recovery
- Significantly cleaner extracts with negligible fines

Sorbent Mass

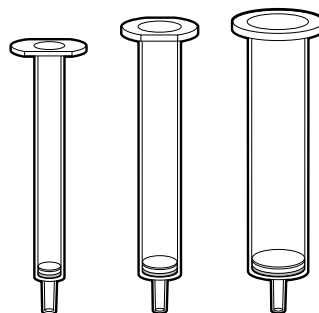
What is the sorbent mass contained within each disk cartridge?

The Empore disks mounted into cartridges are thin and 90% of the weight in each disk represents sorbent particles. Sorbent mass specifications for the three cartridge sizes are listed below. Note that bonded silica and copolymer sorbent particles have different densities that result in different sorbent masses within the same size cartridge.

Specifications (avg.)

Effective Membrane Diameter	Cartridge Volume	SD Sorbent Mass*	HD Sorbent Mass	
		Silica	Silica	Copolymer
4 mm	1 mL	5.5 mg	4 mg	2.2 mg
7 mm	3 mL	17 mg	12 mg	7.5 mg
10 mm	6 mL	35 mg	24 mg	15.0 mg

*Copolymer sorbent is not presently available in SD format



Processing Options

How are samples processed through the cartridge?

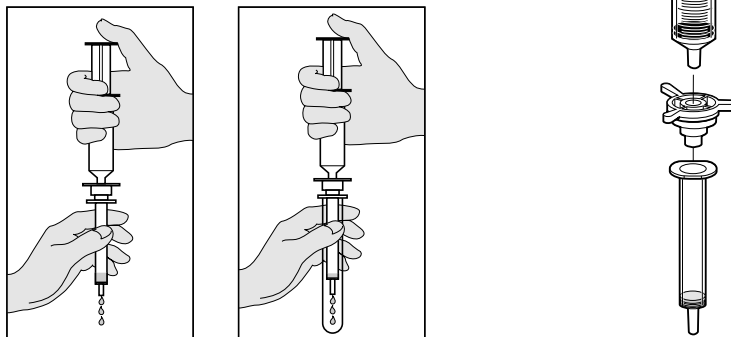
As a result of dense particle packing, Empore disks will not allow rapid flow under gravity. They require positive pressure, vacuum or centrifugation to process liquids through the disk. Semi-automated sample processing workstations can quickly process cartridges using vacuum or positive pressure techniques.

Can I extract samples manually, one at a time, using a syringe?

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

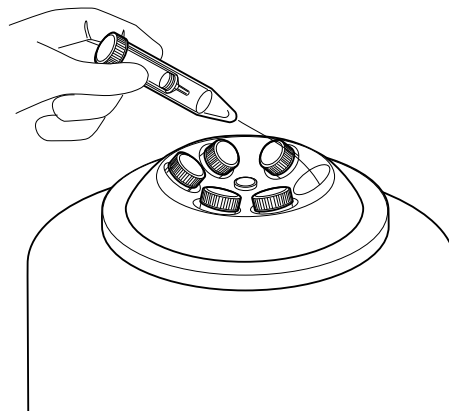
Processing Options (Continued)

A single piece device, the Visi-1 (Supelco 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.



Does centrifugation work? If so, what force should be applied to process liquids?

Positive pressure via centrifugation is an excellent 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. Use a time of 5 min and adjust either force or duration accordingly. 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 manually or as part of the centrifugation method.



Volume Guidelines

How much volume do I need for the conditioning, wash and elution steps?

The small bed mass of sorbent in the disk cartridge allows for the use of smaller solvent volumes compared with traditional SPE products. A general guide to solvent volumes for a disk cartridge SPE method using reversed phase sorbents (C18, C8, C2, SDB-XC) 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).

Important Note: It is recommended to optimize the volume of elution solvent, as it will vary depending on the analyte, its affinity for the chosen sorbent, and the strength of the eluting solvent.

Volume Guidelines (Continued)

Volume Guidelines: Reversed Phase (C18, C8, C2, SDB-XC) Empore Extraction Disk Cartridges (Standard Density)				
Step	Solvent	4mm/1mL	7mm/3mL	10mm/6mL
Condition	Methanol	150µL	250µL	500µL
	Water	300µL	500µL	1000µL
Load	Sample	250µL	1000µL	2000µL
	Buffer/IS	250µL	1000µL	2000µL
Wash	Water	300µL	500µL	1000µL
	Organic/Aqueous	300µL	500µL	1000µL
Elute	Organic	100-150µL	200-300µL	600-800µL

Notes: Elution volumes are provided as a range and should be optimized for each analyte; volumes required for high density disk cartridges would be slightly smaller

I am using a mixed phase sorbent (MPC or SDB-RPS). Do I need to use different volumes than for a reversed phase extraction method?

A general guide to solvent volumes for a disk SPE method using a mixed phase sorbent (both reversed phase and cation exchange bonded to same support) is listed in the table below. 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.

Volume Guidelines: Mixed Phase (MPC, SDB-RPS) Empore Extraction Disk Cartridges				
Step	Solvent	4mm/1mL	7mm/3mL	10mm/6mL
Condition	Methanol	150µL	250µL	500µL
	Water	300µL	500µL	1000µL
Load	Sample	250µL	1000µL	2000µL
	Buffer/IS	250µL	1000µL	2000µL
Wash	Water	300µL	500µL	1000µL
	Organic/Aqueous	300µL	500µL	1000µL
Elute	CH ₂ Cl ₂ /IPA/- NH ₄ OH (78/20/2)	150-200µL	300-400µL	800-1000µL

Notes: Elution volumes are provided as a range and should be optimized for each analyte; volumes required for high density disk cartridges would be slightly smaller

Vacuum Considerations

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

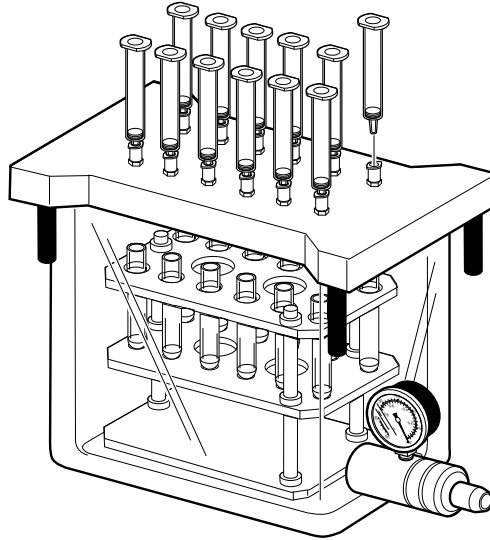
A general guide is to open the vacuum source to about 15 in Hg (0.51 bar) for all steps, with two exceptions.

1. During method optimization, try loading the sample matrix at both a low vacuum (5-7 in Hg; 0.17 to 0.24 bar) and a high vacuum (about 17-20 in Hg; 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.

Vacuum Considerations

(Continued)

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).



I have heard that I can use high vacuum or pressure without recovery loss. Is this true?

Empore Extraction Disk Cartridges utilize a patented technology that improves mass transfer kinetics and reduces channeling. The sample analytes make intimate contact with the immobilized particles in the disk. Solid phase extraction using HD cartridges can be performed at maximum vacuum without affecting recovery. In many cases, SD cartridges can also be used at high vacuum, except in cases when the analyte has a low affinity for the sorbent. In this instance, a lower vacuum allows a low affinity analyte more time to adsorb to the sorbent bed.

Selecting Disk Cartridges

How do I decide which disk cartridge to use for my assay development?

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 (SD or HD)
2. Cartridge Size (4, 7 or 10 mm diameter membranes)
3. Sorbent Chemistry (Bonded silica, mixed phase, copolymer)

Membrane Density

The standard density format is designed for use with biological matrices and is recommended as the first choice for most applications.

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. The sorbent mass is simply not so important in the selection of a disk cartridge size, since capacity has been shown to be more than sufficient for analyte concentrations in ng/mL and pg/mL ranges. The selection of cartridge size for an application typically depends on sample volume, sample viscosity, and elution volume requirements.

Most bioanalytical applications benefit from the 7mm/3mL disk cartridge size and this size is suggested as the first one to try.

Selecting Disk Cartridges

(Continued)

Note that three Empore™ Extraction Disk Cartridge sizes are available, designated as 4mm/1mL, 7mm/3mL and 10mm/6mL. A general guide to cartridge size selection is printed below.

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 µL*
- 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 µL*
- Interchangeable with 100mg/1mL packed SPE columns

10mm/6mL Extraction Disk Cartridge

- Used for larger sample volumes of several milliliters
- Higher capacity
- Faster flow characteristics due to larger disk surface area
- Elution volumes range from 600-1000 µL*

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

Sorbent Chemistry

Reversed phase extraction is the most common type of SPE performed. In this case, an aliphatic hydrocarbon chain (C18, C8 or C2) 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. C2 is weakly nonpolar and has been demonstrated to retain less interferences than a strongly nonpolar C18.

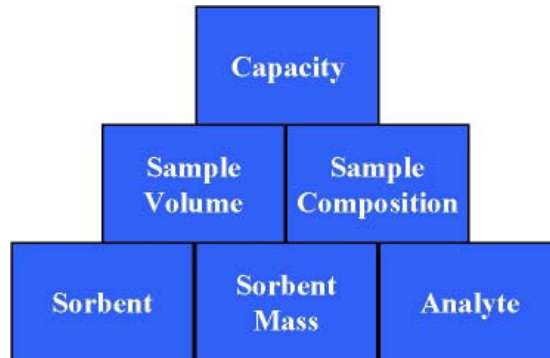
A frequently used alternative to a bonded silica sorbent for reversed phase extraction is based on a copolymer of poly(styrene-divinylbenzene), designated SDB-XC. Modification of SDB-XC by addition of sulfonic acid groups to the copolymer creates a different sorbent named SDB-RPS (Reversed Phase Sulfonated). The sulfonation imparts unique selectivity for organic analytes that are more polar, such as drug metabolites.

Mixed phase cation (MPC) sorbent is a silica-based particle that has been bonded with both a reversed phase group (octyl) and a strong cation exchange group (benzene sulfonic acid). This mixed phase chemistry allows for a more efficient and selective extraction of basic drugs compared with traditional reversed phase techniques.

Capacity

What is the capacity of disk cartridges? How can such small sorbent masses retain my analyte?

The capacity of a sorbent particle refers to the maximum amount of material (analytes, co-administered drugs and interfering substances) retained by a specific mass of sorbent from a particular sample matrix (water, plasma, serum, urine, etc.).



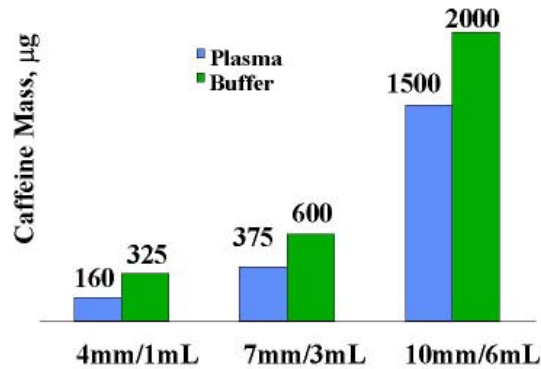
Capacity depends on the type of sorbent used and its mass, the sample matrix and its volume, and on the affinity of the analyte for the sorbent.

Empore Extraction Disk Cartridges contain less sorbent mass than equivalent reservoir sizes of packed SPE columns. Estimates of column capacity often appearing in the literature state that a sorbent's capacity is 1%, 5% or even 10% of the sorbent mass (reversed phase applications). For example, a 100mg/1mL packed column is estimated to have a capacity of 1mg (1% of the sorbent mass) to 10mg (10% of the sorbent mass). Similarly, a 7mm/3mL HD disk cartridge would be estimated to retain from 0.12 to 1.2 mg. However, since many variables are involved in determining capacity, a general answer is not usually predictive for actual situations. Capacity depends on the type of sorbent used and its mass, the sample matrix and its volume, and on the affinity of the analyte for the sorbent.

The best way to estimate capacity is to experimentally determine it for a particular analyte and sorbent in a given volume of sample matrix. A specific experiment addressing the capacity of HD disk cartridges was performed—extraction of caffeine from buffer and human plasma. Capacity was determined by applying known aliquots of radiolabeled drug and measuring the percent retention at different mass loadings. The breakthrough point was defined as when recovery dropped below 90%.

When buffer was the sample matrix and extracted using C18-HD disk cartridges, capacity for caffeine in 1 mL sample volume was about 325 μg (4mm/1mL), 600 μg (7mm/3mL) and 2000 μg (10mm/6mL). When plasma was the sample matrix, capacity was reduced somewhat to about 160 μg (4mm/1mL), 375 μg (7mm/3mL) and 1500 μg (10mm/6mL). See graph on next page. Since the upper limit of the therapeutic range of caffeine, and most drugs, is not greater than 20 $\mu\text{g}/\text{mL}$, even the smallest 4mm/1mL disk cartridge size demonstrates more than adequate capacity for typical drug levels.

Capacity (Continued)



Conditioning the Disk

Why do I need to condition the disk before adding my sample?

The sorbent particles and the PTFE disk are both hydrophobic when dry and an aqueous solution cannot properly wet the surface. Conditioning, or pre-treating, the disk with methanol (or acetonitrile) is necessary to reduce surface tension and solvate the hydrocarbon chains. An excess of water is next added to rinse out the organic solvent so that precipitation of sample proteins is prevented upon sample loading. If the surface of the disk becomes dry before the sample is added, repeat the conditioning procedure.

Wash Step Optimization

Is a single water wash adequate for effective removal of interferences?

The goal of the wash step after sample loading is to remove interfering substances, yet not remove the analyte(s) of interest. Water is commonly used as a wash solvent, and it is effective at removing adsorbed proteins remaining on the surface of the sorbent bed. It is recommended to always use a water wash first, so only an aqueous solvent passes through the disk after sample loading. Water alone as a wash solvent may not provide sufficient clean-up in each assay. A second aqueous wash solvent containing a small percentage of organic is usually used to further remove interfering substances.

These two wash steps are of critical importance in ensuring the performance of the membrane and the usability of the final eluate. If residual proteins are not removed with an aqueous wash, they may even precipitate during a low percentage organic wash and occlude flow through the disk. If protein is not adequately removed before elution, the eluate will be contaminated with miniscule amounts. These proteins can gradually build up on the LC column over time and raise the operating pressure.

How do I determine what percentage of organic in the wash solvent can be tolerated?

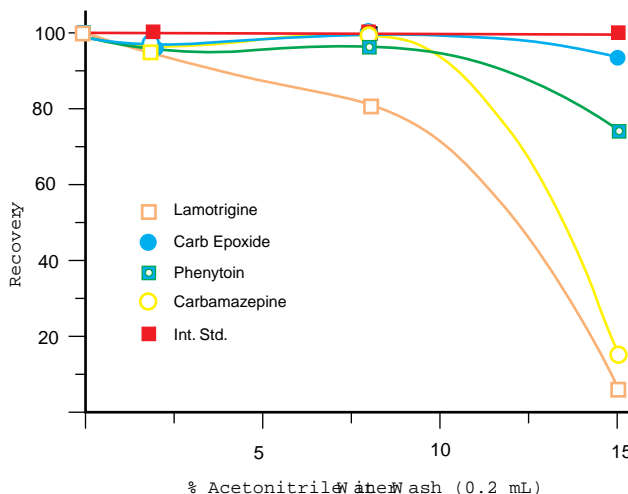
The influence of percent organic on analyte recovery can be evaluated by comparing recoveries obtained from different percentages of organic. For example,

1. Use varying percentages of organic in water, delivered at a constant volume
2. Determine recovery from each replicate
3. Plot analyte recovery vs. percent organic in water

An example is shown below for four anticonvulsant drugs, and internal standard, extracted from serum using a C8 Empore disk. The wash volume used was 200 µL. Lamotrigine could tolerate only 2.5% acetonitrile in the wash before elution occurred, while the other drugs could tolerate up to 8% organic. However, at 15% organic, the recovery of two more analytes dropped off. Thus, for this assay, 2.5% acetonitrile in water was optimal. If lamotrigine were excluded, then 8% acetonitrile in water was optimal.

Wash Step Optimization (Continued)

Influence of Organic Content of Wash on R



Elution Volume Optimization

Why is it important to optimize elution volume?

The reduced solvent volumes allowed by the disk format can yield great gains in throughput. These smaller volumes require less time to pass through the sorbent bed and generate more concentrated eluates. The elution volume should be closely examined to ensure maximal recoveries using the smallest practical volume. Elution volume may vary slightly depending on the particular analyte, its affinity for the chosen sorbent, and the strength of the elution solvent.

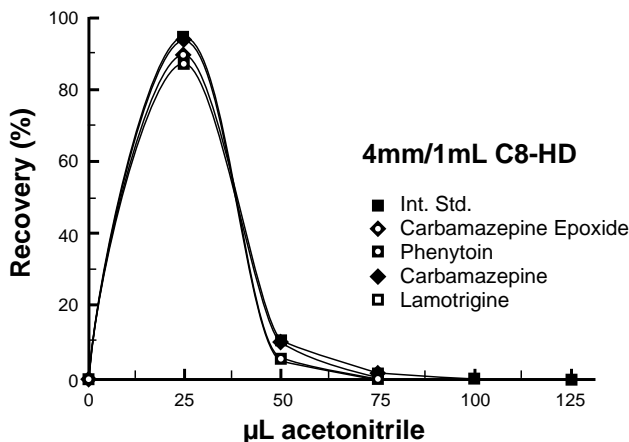
How do I optimize the elution volume required for my assay?

The influence of elution volume on analyte recovery can be evaluated by comparing recoveries. For example,

1. Deliver different volumes of elution solvent to different cartridges
2. Determine recovery from each cartridge
3. Plot analyte recovery vs. elution volume

The resulting percent recovery obtained from each elution volume can be evaluated and an appropriate volume can be selected. The graph below illustrates percent recovery for different volumes of acetonitrile in the elution of anticonvulsant drugs from an Empore C8-HD disk cartridge (4mm/1mL).

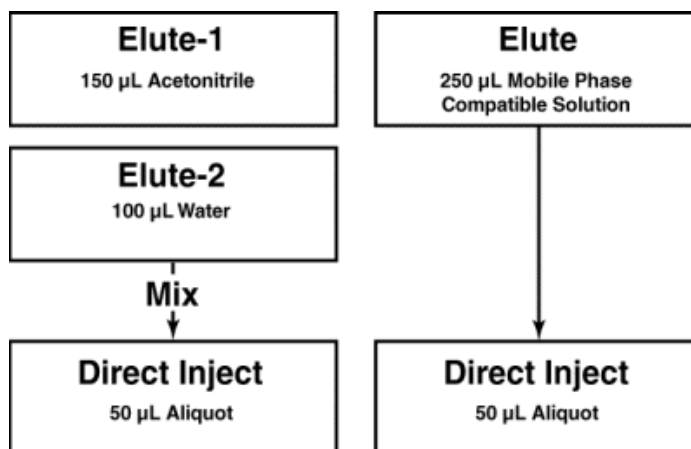
Elution Volume Profile



Eliminating the Evaporization Step

How can I eliminate the evaporation step in an SPE method?

The small bed volume of the Empore disk format allows for the use of reduced elution volumes. The need for a time-consuming evaporation and reconstitution step can often be eliminated. A common approach is to elute with a small volume of organic solvent, then add a volume of aqueous liquid so that the composition of the resulting solution is compatible with mobile phase. Another approach is to elute using a solvent with sufficient organic content to desorb analyte but which is also compatible with mobile phase for direct injection.



Automation Options

What factors influence throughput in automated SPE workstations?

In most automated SPE workstations, the time required to process a single sample is influenced by two factors—solvent volume and the speed at which that volume is processed through the extraction device. Traditional SPE packed columns contain a large mass of sorbent particles, which requires large volumes of solvent for processing. That large volume of solvent must flow at a very slow rate to ensure retention and avoid channeling. These factors together create slow throughput.

How can Empore High Performance Extraction Disk Cartridges increase throughput in automated SPE workstations?

Disk cartridges represent an advancement in SPE technology. A thin membrane, or disk, contains a reduced mass of sorbent particles and thus requires less solvent volumes for processing. Also, the patented technology of packing the particles into a membrane format eliminates the problem of channeling. Sample and solvent flow rates can be increased compared with traditional approaches. The abilities to use less solvent volumes and deliver those volumes at a faster rate increase throughput.

What are the optimal operating conditions for using disk cartridges with automated SPE workstations?

When disk cartridges are used according to the conditions applied to traditional SPE packed columns, the same limitations may occur and the disks may not work properly. However, once the new operating conditions for disks have been learned, new possibilities arise and better results can be obtained. Each brand of SPE workstation operates slightly differently than the others and the optimal operating conditions for disk cartridges vary between brands. Contact 3M technical service or the workstation manufacturer to obtain a template or program that represents optimized operating conditions.

Transferring Methods

Why would an analyte extract using a packed column SPE product but not when that same method is transferred to a disk cartridge product?

A packed column SPE product contains an excess of sorbent mass that fills up a column to a relatively large height. When an analyte has a low affinity for the sorbent chemistry chosen, it may still bind because of the excess mass. It may not be efficiently bound to the top 10% of the particle bed, but diffuse much lower into the bed and finally bind. A disk cartridge can contain the same sorbent chemistry but note that its bed height is much smaller (about 0.8 mm for the disk, compared with 12 mm or so for a 200mg/3mL packed bed). If the affinity of that analyte for the chosen sorbent is weak, it cannot compensate as well in a disk because there is not that same excess sorbent mass.

In order to transfer the method successfully, given that the analyte has a weak affinity for the sorbent, two suggestions are in order. The analyte should be loaded at a slow flow rate to increase the residence time for interaction with the sorbent. Another sorbent may be chosen that has a stronger affinity for the analyte. Often times, extraction methods are passed from user to user without the accompanying optimization data. Also, it should not always be assumed that the method was optimized in the first place. Consideration of these factors should allow you to examine some possible solutions to improve recovery when methods are transferred to disk cartridges.

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Detailed product and technical information, including *Instructions for Use* and *Answers to Frequently Asked Questions*, can be found on the Internet.

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