SPE Introduction

How to Choose an SPE Product

1. Characterize the Sample

Factors such as the analyte's polarity relative to the matrix, the presence of charged functional groups, solubility, molecular weight, etc., determine how strong the analyte is retained by the packed bed.

2. Select a Retention Strategy

There are two basic methods for sample treatment:

a. Select the packing bed to retain the desired analyte. The contaminants are washed off and the desired analyte is then eluted for analysis.

b. Select the packing bed to retain the contaminants and the desired analyte passes directly through.

3. Select the Proper Packing Type

Select the proper packing type for the cleanest extract with the highest recovery.

a. Reversed-phase packings are hydrophobic, silica-based materials that retain moderately polar to non-polar compounds from a polar matrix while washing off polar interferences. Or you can retain non-polar contaminants while the polar compounds pass through unretained.

b. Normal-phase packings are hydrophilic, silica-based materials that retain polar compounds from a non-polar matrix while washing off non-polar interferences. Or you can retain polar contaminants while non-polar compounds pass through unretained.

c. Ion-exchange resins retain charged compounds or remove ionic interferences.

4. Optimize Conditions for Best Results

Select proper bed size and suitable conditioning, wash and elution solvents.

a. Poor sample recovery often occurs when the packed bed dimensions are not optimized. An excessive bed weight results in incomplete elution while an insufficient bed weight results in incomplete retention.

b. Consider the solvent strength relative to the packing material. The final conditioning solvent should be weak so it doesn't act as an eluting solvent. Buffers should be used to control ionization of potentially charged compounds.

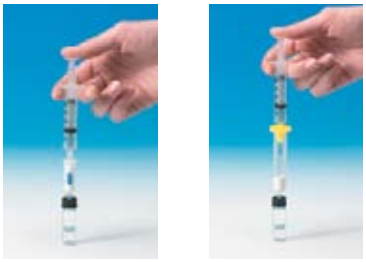
c. Wash solvents should remove weakly retained interferences without being strong enough to elute the analyte.

d. Elution solvents should be strong enough to completely elute an analyte in a small volume (1–2mL).

Solid-Phase Processing Methods

By Syringe

Process individual samples via luer hub syringe. Connect syringe directly to SPE cartridges or to SPE columns via adapters found on page 316.



4915

By Vacuum Manifold

Process multiple samples on a vacuum manifold using SPE columns. Extend volume via reservoirs. Products found on pages 314–316.

6275

By Filter Flask

Process large volume samples by placing SPE tube with an online filtration system. See page 130.

5953

For Environmental Applications

Recommended SW-846 Cleanup Methods

Analyte Group	EPA Method No.	Recommended SPE Sorbents
Aniline and Derivatives	3620	C18
Chlorinated Pesticides	3620	C18, Florisil®
Chlorinated Hydrocarbons	3620	C18, Phenyl
Haloethers	3620	C18, Florisil®
Nitroaromatics and Cyclic Ketones	3620	DVB, IC-RP
Nitrosamines	3610, 3620	C18
Organochlorine Pesticides	3620	C18, Florisil®
Organophosphorus Pesticides	3620	Florisil®, Carbograph
PCB's	3620, 3630	C18, SCX, Carbograph
Petroleum Waste	3611	Alumina
Phenols	3630	C18, Phenyl
Phthalate Esters	3610, 3620	C18, DVB
Polynuclear Aromatic Hydrocarbons	3630	C18, Phenyl

SPE Introduction

SPE Method Development

SPE method development typically contains four steps:

Step 1: Condition

The conditioning step is composed of two substeps; the first activates the sorbent ligands, the second equilibrates the sorbent bed.

Step 2: Load

In the load step, sample is applied to the SPE device. Matrix and flow rate are optimized to quantitatively retain target analytes.

Step 3: Wash

In the wash step, choose a solvent that elutes impurities but retains target analytes. Often the second conditioning solvent is a suitable wash solvent.

Step 4: Elute

The elution step ideally removes all target analytes with minimal solvent to maximize sensitivity. Sometimes this requires a combination of solvents to break both the primary and secondary interactions.

	General Method Development Procedures			
	Step 1—Condition	Step 2—Load	Step 3—Wash	Step 4—Elute
Reversed-Phase Extraction Procedure **Mechanism:** Bind moderately polar to non-polar compounds from a polar sample matrix	Methanol followed by water	Process sample at a flow rate of 1–5mL/min	Water or water: methanol (95:5)	Methanol or acetonitrile. May need to add strong acid or base to organic solvent to break secondary interactions.
Normal-Phase Extraction Procedure **Mechanism:** Bind polar compounds from a non-polar sample matrix	IPA followed by hexane	Process sample at a flow rate of 1–5mL/min	Hexane or hexane:IPA (98:2)	IPA, ethyl acetate, acetone, or hexane: IPA (50:50)
Ion-Exchange Extraction Procedure **Mechanism:** Bind charged (negative/anionic or positive/cationic) compounds	Methanol:water (50:50) followed by low ionic strength (0.1M) buffer	Apply slowly: less than or equal to 1mL/min ion exchange kinetics are slower than reversed- or normal-phase	Methanol:low ionic strength (0.1M) buffer (10:90)	High ionic strength (0.5M–1.0M) buffer or modify pH such that the analyte is uncharged. May need to add organic to break hydrophobic interactions.

Recommended Usage Guidelines*									
Bed Size:	50mg	100mg	200mg	500mg	500mg	1000mg	2000mg	5000mg	10,000mg
Sorbent Retention Capacity:	2.5mg	5mg	10mg	25mg	25mg	50mg	100mg	250mg	500mg
Condition Volume *(4-bed volumes)*	0.30mL	0.60mL	1.20mL	3.00mL	3.00mL	6.00mL	12.00mL	30.00mL	60.00mL
Wash Volume *(6-bed volumes)*	0.45mL	0.90mL	1.80mL	4.50mL	4.50mL	9.00mL	18.00mL	45.00mL	90.00mL
Minimum Elution Volume *(3-bed volumes)*	0.23mL	0.45mL	0.90mL	2.25mL	2.25mL	4.50mL	9.00mL	22.50mL	45.00mL

*Estimates only. Must optimize for each application.

tech tip

To calculate sorbent bed volume, use 150µL for every 100mg of sorbent.

tech tip

Retention capacity describes the total amount that an SPE sorbent will bind. This includes all compounds retained—analytes of interest as well as the contaminants.

tech tip

Minimum elution volume recommended in bed size chart above will offer best sensitivity, but more solvent may be required depending on application.

Solvent Polarity Chart

Less Polar (Stronger Reversed-Phase Elution)

Hexane
Isooctane
Cyclohexane
Carbon tetrachloride
Toluene
Chloroform
Methylene chloride
Tetrahydrofuran
Ethyl ether
Acetone
Acetonitrile
Pyridine
Isopropanol
Ethanol
Methanol
Water
Acetic acid

More Polar (Stronger Normal-Phase Elution)

SPE Introduction

Grace Davison® has been making Davisil® silica for over a quarter century. It is the foundation for all our silica-based SPE products, including Alltech®, Vydac®, and GracePure™ brands. Having complete control over the entire media process ensures highly consistent performance, and uninterrupted supply.

7377

Experts in Media Production

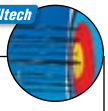
Using a consistent and pure silica base, and employing tightly controlled bonding techniques, insures predictable analyte-sorbent interactions. Both of these aspects also play equal importance in manufacturing a bonded phase with high and reproducible recoveries.

Highest Quality Control

Every part of our manufacturing process is carefully monitored. From silica production to final product, we perform multiple quality tests, and provide a comprehensive quality assurance certificate.

Grace SPE Product Lines

Alltech® Extract-Clean™ Columns

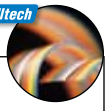


Format: SPE Columns

Sizes: 1.5, 4, 8, 15, 25, 75mL (the entire tube volume)

Summary: In production for over 25 years, with proven consistency, this is our most comprehensive SPE product line. It includes 30 media types in over 10 different bed weights. And with a complete offering of reversed, normal, and specialty medias exhibiting unique retention properties, you are sure to find the packing that delivers a cleaner, more concentrated sample. See pages 302–306.

Alltech® Maxi-Clean™ Cartridges



Format: SPE Cartridges

Sizes: 300, 600, 900mg (media amount, not device volume)

Summary: The Maxi-Clean™ line is offered in many of the same media as the Extract-Clean™ line, but slightly paired down, with over 20 chemistries available. This lure hub cartridge device is not as prevalent in the SPE industry, and while manual processing is most common, this format offers a number of other interesting processing options, including multimedia extractions. See page 307–310.

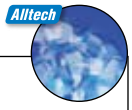
Vydac® Columns

Format: SPE Columns

Sizes: 1, 3mL (volume above the packing)

Summary: Ideal for extraction, concentration and cleanup of biological samples. This 300Å silica-based media has the same properties as the industry-leading Vydac® TP HPLC packing. Offered in C18 and C4, use for a variety of protein and peptide applications. See page 311 for details.

Alltech® Ultra-Clean™ Columns



Format: SPE Columns

Sizes: 4, 8mL (the entire tube volume)

Summary: Choose this ultra-low extractable version for very sensitive applications. Nine selected media are packed into highly inert fluorinated polypropylene tubes with PTFE frits. Less expensive than glass extraction devices, this durable format offers comparable inertness without the added concern of being easily broken. See pages 302–305.

Alltech® 96-Well Plates

Format: 96-Well Plates

Size: 2mL (the individual well volume)

Summary: Ideal for high-throughput SPE processing, this 8" x 12" standard format fits into traditional 96-well automated equipment. We offer six different medias in three bed weights packed in 2mL square wells. See page 311. Empty plates are also available on page 316.

GracePure™ Columns

Format: SPE Columns

Sizes: 1, 3, 6, 12, 20, 60mL (volume above the packing)

Summary: Our in-house capability to make everything from the silica particle to the finished good means we can deliver GracePure™ as the best value in SPE. With a concise offering of 11 sorbents in six bed weights, this high-quality SPE product line is a result of operational excellence that Grace Davison® is known for. See pages 312–313.

SPE Introduction

Device Options

Device Specifications		
Device	**Housing**	**Frit Material**
Extract-Clean™ Columns	Polypropylene	20µm Polyethylene
Ultra-Clean™ Columns	Treated Polypropylene	10µm PTFE
GracePure™ Columns	Polypropylene	20µm Polyethylene
Vydac® Columns	Polypropylene	Glass Fiber Filter Paper with Polyethylene Mesh Support
Maxi-Clean™ Cartridges	Polypropylene	20µm Polyethylene
96-Well SPE Plates	Polypropylene	20µm Polyethylene

Traditionally, differing nomenclature has been used to describe SPE column size. Sometimes columns are described in terms of full volume. Alternatively, the volume above the bed weight may also be used. Below is a cross-reference for your convenience.

Alltech®	*GracePure™/Vydac®*
1.5mL	*1mL*
4mL	*3mL*
8mL	*6mL*
15mL	*12mL*
25mL	*20mL*
75mL	*60mL*

SPE Columns

- Open top tubes with male luer bottom
- Process multiple samples with vacuum manifold or automated SPE instruments
- Process individual samples manually with use of adapter and syringe

75mL

25mL

15mL

8.0mL

4.0mL

1.5mL

SPE 96-Well Plates

- High throughput SPE devices
- 8" x 12" standard 96-well format
- 2mL square wells

SPE Cartridges

- Female luer top and male luer bottom
- Process single cartridge by syringe or multiple cartridges by vacuum
- Stack for multi-step extractions

900mg Bed

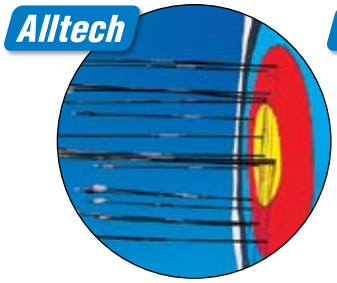
600mg Bed

300mg Bed

Alltech® SPE Sorbents

Alltech® SPE Sorbents are packed into three device types; columns, cartridges, and 96-well plates. See chart for availability.

Extract-Clean™
SPE Columns

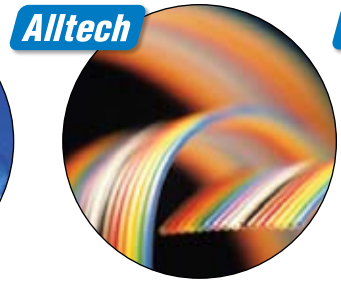


pages 302-306

Ultra-Clean™
Ultra-low Extractable SPE Columns

pages 302-305

Maxi-Clean™
SPE Cartridges



pages 307-310

96-Well Plates
High Throughput SPE Processing

page 311

Reversed-Phases (Non-Polar) Sorbent Specifications

Packing	Base	% Carbon	End-capped	Average Particle Size	Pore Size	Features	Benefits	Extract-Clean™	Ultra-Clean™	Maxi-Clean™	96-Well
Prevail™ C18	Silica	11.0%	Yes	50µm	60Å	100% water wettable	Hydrophilic/hydrophobic retention. Phase remains active even when completely dry. Can omit preconditioning step.	x		x	x
Standard C18	Silica	6.0%	Yes	50µm	60Å	Low carbon load C18	General purpose phase.	x	x	x	x
High-Flow C18	Silica	8.0%	Yes	100µm	60Å	Large particle	Less flow resistance for faster flow rates of large volume sample.	x			
High-Capacity C18	Silica	17.0%	Yes	50µm	60Å	High carbon load	Maximum capacity phase.	x		x	
Large Pore C18	Silica	14.0%	Yes	50µm	150Å	Larger than average pore size	Ideal for compounds >1500MW.	x		x	
Octyl (C8)	Silica	4.5%	Yes	50µm	60Å	Less hydrophobic than C18	Less retention of highly hydrophobic compounds. Use when C18 is too retentive.	x	x	x	
Phenyl (PH)	Silica	3.8%	Yes	50µm	60Å	Aromatic structure	Highly selective for aromatic compounds.	x		x	

Normal-Phases (Polar) Sorbent Specifications

Packing	Base	% Carbon	End-capped	Average Particle Size	Pore Size	Features	Benefits	Extract-Clean™	Ultra-Clean™	Maxi-Clean™	96-Well
Silica (SI)	Silica	—	—	50µm	60Å	Highly polar surface	Most common polar phase.	x	x	x	x
Aminopropyl (NH_2)	Silica	5.0%	No	50µm	60Å	Polar phase with slight anion exchange properties	Ideal for carbohydrates or generally with analyses containing hydroxyl functional groups.	x		x	
Cyanopropyl (CN)	Silica	6.0%	Yes	50µm	60Å	Unique selectivity	Can be used in normal-phase or reversed-phase modes.	x	x	x	
Diol (2OH)	Silica	4.0%	No	50µm	60Å	Polar surface with minor hydrophobic retention	Wets easily and offers more reproducibility.	x		x	
Florisil® (FL)	Magnesium Silicate	—	—	75–150µm	60Å	Highly polar surface	Referenced in many EPA methods. Ideally suited for pesticides and metals.	x	x	x	
Florisil® PR (FL-PR)	Magnesium Silicate	—	—	75–150µm	60Å	Specifically tested for chlorinated pesticides	Ensures most inert batches suitable for highly active compounds.	x	x	x	
Alumina Acidic (AL-A)	Aluminum Oxide	—	—	130µm	100Å	Alumina washed with acid surface	Increase capacity for acidic compounds.	x		x	
Alumina Basic (AL-B)	Aluminum Oxide	—	—	130µm	100Å	Alumina washed with base surface	Increase capacity for basic compounds.	x		x	
Alumina Neutral (AL-N)	Aluminum Oxide	—	—	130µm	100Å	Alumina washed with neutral surface	Interacts with highly aromatic compounds and neutral hydroxyls.	x		x	

Alltech® SPE Sorbents

Specialty Packings Specifications

Packing	Base	% Carbon	End-capped	Average Particle Size	Pore Size	Features	Benefits	Extract-Clean™	Ultra-Clean™	Maxi-Clean™	96-Well
DVB	100% DVB	—	—	40µm	—	100% DVB	Greater capacity than C18 for general SPE. Also free vinyl surface groups make a suitable solid-phase synthesis support.	x			
Carbograph	Graphitized Carbon	—	—	38–125µm	—	Graphitized Carbon	Retains polar organics in aqueous matrices. Ideally suited for acid, base-neutral extraction of pesticides and herbicides.	x	x		
AFT	C18, Silica, and Alumina	6	—	50–130µm	—	Unique blend of reversed and normal phases.	Ideal for Aflatoxins.	x			

General Ion-Exchange Sorbent Specifications

Packing	Base	Counter Ion	Particle Size	Functional Group	Exchange Capacity	Retains	Applications	Extract-Clean™	Ultra-Clean™	Maxi-Clean™	96-Well
SCX	Styrene-DVB	Hydrogen	50µm	Benzene Sulfonic Acid	2.0meq/mL	Cations, (+) charged compounds	Remove/concentrate basic compounds.	x	x	x	x
SAX	Styrene-DVB	Acetate	50µm	Tetramethyl Ammonium	1.0meq/mL	Anions, (–) charged compounds	Remove/concentrate acidic compounds.	x	x	x	x

Ion Chromatography Sorbent Specifications

Packing	Base	Counter Ion	Particle Size	Molecular Exclusion Limit	Exchange Capacity	Retains	Applications	Extract-Clean™	Ultra-Clean™	Maxi-Clean™	96-Well
IC-OH	Styrene-DVB	Hydroxide	50µm	1000 Daltons	1.0meq/mL	Anions	Exchanges anions for hydroxide. May be used to remove or concentrate anions from sample and to increase pH of acidic samples. Removes cations that form insoluble hydroxide salts.	x		x	
IC-H	Styrene-DVB	Hydronium	50µm	1000 Daltons	2.0meq/mL	Cations	Exchanges cations for H^+. May be used to remove or concentrate cations from sample and to reduce pH of basic samples.	x		x	
IC-Ag	Styrene-DVB	Silver	50µm	1000 Daltons	2.0meq/mL	Chloride Iodide Bromide	Removes excess halides through formation of Ag-halide salts.	x		x	
IC-Ba	Styrene-DVB	Barium	50µm	1000 Daltons	2.0meq/mL	Sulfate	Removes excess sulfate through formation of BaSO.	x		x	
IC-Na	Styrene-DVB	Sodium	50µm	1000 Daltons	2.0meq/mL	Cations	Exchanges cations for Na^+. May be used to remove or retain cations from sample without changing the pH of the sample.	x		x	
IC-Chelate	Styrene-DVB	Sodium	50µm	1000 Daltons	0.4meq/mL	Polyvalent metal ions	Exchanges transition metals and divalent cations for Na^+. May be used to remove or retain divalent cations and transition metals from sample.	x		x	
IC-RP	Polystyrene	—	550µm	—	—	Hydrophobic components	Removes surfactants, organic acids, and other organic substances. Inorganic ions pass through.	x		x	