

A Sample Preparation Primer and Guide to Solid Phase Extraction Methods Development

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revised & adapted by Patrick D. McDonald, Ph.D.

from: *Solid Phase Extraction Applications Guide and Bibliography*

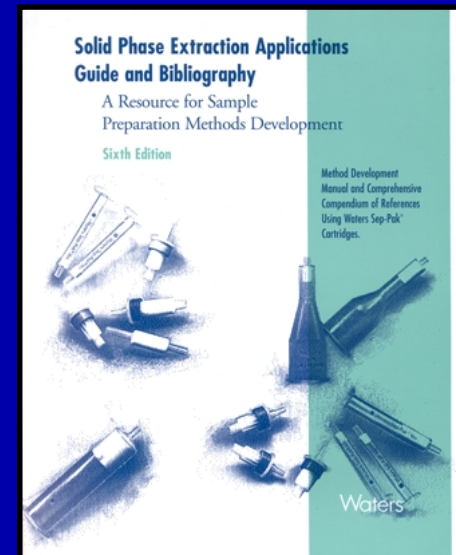
A Resource for Sample Preparation Methods Development, Sixth Edition

Editors: Patrick D. McDonald, Ph.D.; Edouard S.P. Bouvier, Ph.D. [1995]

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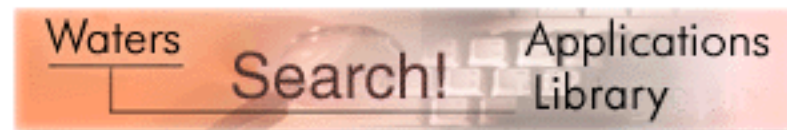
Preface

Perhaps more so than in any other discipline, those trained in chemistry turn first to the published literature when seeking ideas or solutions to problems at hand. Ideally, a synthetic or analytical scheme can be found that fills the need or triggers a discovery. But locating a critical piece of information may not be straightforward. Despite the abstracting efforts of organizations such as the American Chemical Society or the Royal Society of Chemistry, key word searches may not lead to appropriate articles. Although a few journals can now be searched in full-text format on-line, experimental details in the majority of sources remain inaccessible through common search mechanisms. Information seekers need to be skilled in deductive processes that would make Sherlock Holmes proud, or just lucky, to find the proverbial needle in the haystack.

To produce the *Waters Applications Library*, we scan the solid phase extraction literature on a daily basis. Information that typically may not appear in an abstract or may not be apparent from a title or a few keywords is extracted and indexed in a master database. Some references are obtained from sources not abstracted anywhere. Only those references that we, as editors, deem potentially useful to researchers are included here. The *Waters Applications Library* is the product of more than 20,000 hours of effort over a 23-year period. We hope that you will use the information contained therein as the foundation upon which to build your success in sample preparation methods development.

Of the various techniques used for sample preparation, solid phase extraction [SPE] now plays a prominent role. Also known as column solid-liquid extraction, SPE works by the same mechanisms as the premier analytical technique of today, HPLC. As testimony to the success of this method, over 3000 literature references by more than 9,000 authors describing the isolation, enrichment, and/or purification of more than 8,000 compounds are now available online in the *Waters Applications Library*.

[CLICK HERE](#) to perform a search:



Methods cited in our database cover a wide range of key applications in the Life Science, Pharmaceutical, Environmental, Forensic, Clinical, Food, Agricultural, and Industrial fields. Objectives range broadly from trace enrichment to sample clean-up. It is quite likely that you will find a method in one or more of these literature citations that you can use directly or adapt to your needs with little modification.

You can also use our database to generate ideas on how to approach your specific problem based on the experience of others who have solved similar problems. If a search for your particular compound or matrix of interest does not return any results, look for structurally related compounds, metabolites, derivatives, or matrices with similar characteristics. However, you should not use our database as a cookbook. We do not have the resources to verify all the procedures cited here and, therefore, cannot guarantee your success. Please be prudent and use your best scientific judgment. While some references describe officially sanctioned methods, most of these citations should be viewed merely as starting points or feasibility determinations. You may undoubtedly find a way to improve upon a specific protocol. To aid you in this process, the following *Primer* presents some background in sample preparation strategies and guidelines for developing methods using solid phase extraction.

Twenty-three years ago, the sale of the first Sep-Pak[®] Classic Cartridges triggered a revolution in streamlining sample preparation. Since then, Waters has continued to provide new tools to assist you in your sample preparation methods. Waters currently offers a broad line of devices to suit different sample preparation needs. Versatile Sep-Pak[®] Plus and Light Cartridges may be used with positive-pressure or vacuum-assisted flow in manual or automated applications. The Sep-Pak[®] Vac line of syringe-barrel-type cartridges are designed for use with vacuum manifolds and automated SPE instruments. Sep-Pak[®] Vac RC Cartridges are made for robotic applications as well as for use with vacuum manifolds, when larger liquid sample volumes need to be processed. Many of the methods in the literature were developed for manual techniques using a Sep-Pak[®] Classic Cartridge. You may need to use a different cartridge style. We have, therefore, included information that guides you in the conversion of methods from one Sep-Pak[®] Cartridge format to another.

We would like to thank all the people who have made this primer and the *Waters Applications Library* possible. In particular, Maureen Allegrezza, Joseph Arsenault, Carla Clayton, Dr. Zoubair El Fallah, Thomas Frado, Grace Lavallee, Debra Laviolette, Dr. Donna Martin, Patrick M. McDonald, Brian McDonald, Dr. Uwe Neue, Bridget O'Brien–Vaughan, and Dr. Michael Young have all contributed much time and effort to the on-line searching and procurement of papers, coding of each reference, data entry, database design, primer content, proofreading, and production of the printed book. Finally, a special thank you is extended to each of the authors who has contributed to the success of solid phase extraction by developing and using this technique, in often innovative ways, to solve a multitude of important problems. *Waters Applications Library* is, after all, a compendium of *your* work. We look forward to creating new, productive sample preparation tools for you to use in the future.

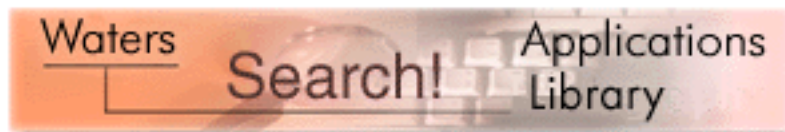
– Patrick D. McDonald, Ph.D. and Edouard S.P. Bouvier, Ph.D.
Milford, Massachusetts, March 17, 1995
revised by P.D.McD., March 17, 2001

Notes on References in *Waters Applications Library*

Reference File Number

Each reference has been assigned a unique alphanumeric file number. Type this file number in the box on the *Search page* for rapid access to the corresponding electronic record in the *Waters Applications Library*. Whenever you need to refer to any paper cited here in discussions or correspondence with Waters, please mention the appropriate file number.

NOTE: Any publication written primarily in a language other than English has its translated title shown in [brackets]. Also, occasionally the compounds, matrices, and techniques listed in each entry may not appear to be related to the title; they reflect the actual usage of the Sep-Pak[®] cartridge, not necessarily the primary theme of the publication.



Sep-Pak[®] Cartridge Configuration Icon Key



Sep-Pak[®] Plus cartridge, short body



Sep-Pak[®] Classic cartridge, short body



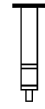
Sep-Pak[®] Plus cartridge, long body



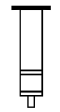
Sep-Pak[®] Classic cartridge, long body



Sep-Pak[®] Light cartridge



Sep-Pak[®] Vac cartridge, 1-cc



Sep-Pak[®] Vac cartridge, 3-cc



Sep-Pak[®] Vac RC cartridge

A Sample Preparation Primer

Importance of Sample Preparation

In the last decade, dramatic advances in analytical instrumentation and laboratory information management systems shifted the analyst's predominant tasks from assay measurements to sample preparation and data manipulation. As the stringency of requirements for higher sensitivity, selectivity, accuracy, precision, and number of samples to be processed has escalated, the corresponding increases in speed and sophistication of data collection and analysis have outpaced improvements in the many traditional techniques of sample collection and preparation. By some estimates, 75 to 80% of the work activity and operating cost in a contemporary analytical lab is spent processing and preparing samples for introduction or injection into an analytical separation and/or measurement device. Clearly, efforts directed and products designed to streamline sample preparation protocols are essential to future progress in analytical science.

Goals of Sample Preparation

Successful sample preparation for most analytical techniques (HPLC, GC, spectrophotometry, RIA, etc.) has a threefold objective: namely, to provide the sample component of interest

- ❑ in solution
- ❑ free from interfering matrix elements
- ❑ at a concentration appropriate for detection or measurement.

To accomplish these goals, a sample, or a representative portion thereof (not always easy to obtain), is prepared *via* traditional methods of dissolution, homogenization, extraction (liquid or solid phase), filtration, concentration, evaporation, separation, chemical derivatization, standardization (internal or external), etc. Usually such methods are used in combinations of multiple steps, which form a sample prep protocol. The fewer steps and methods used in any given protocol, the simpler, more convenient, cost effective, and less time consuming it is. Simpler protocols lend themselves more readily to automation and also lead to increased accuracy, reliability, reproducibility, and safety.

Innovation in Sample Preparation Methods

There are many ways to combine standard tools and techniques to accomplish the goals of sample prep. However, it is best to seek innovative means to streamline sample prep protocols:

- ❑ to combine the functions of several steps, if possible, into one operation,
- ❑ to eliminate needless sample transfers and manipulations,
- ❑ to reduce the scale as much as practicable (gaining economies of time, labor, and cost),
- ❑ to use new tools in creative ways.

Liquid-Liquid Extraction: a Classical Technique

One of the most useful tools that a chemist has for isolating a desired component from a mixture is liquid-liquid extraction [LLE]. Selective partitioning of the compound of interest into one of two immiscible (or partially miscible) phases occurs by the proper choice of extraction solvents. Often, however, it is not possible to find the optimum conditions that provide both high recovery and purity of the product in one extraction step. Low recoveries may necessitate further extractions to achieve acceptable yields. Reaching purity may require a second extraction procedure with a different solvent or pH. Each successive extraction increases the time required. Also, the resulting large volume of extraction solvent must be evaporated to recover the product. If the extraction requires many steps, techniques such as Craig countercurrent distribution can be used to increase recovery and purity. However, this countercurrent liquid-liquid extraction method is expensive, cumbersome, and time-consuming.

Solid Phase Extraction: a More Powerful Approach

As the name implies, *solid phase extraction* [SPE] is an extraction technique based on the selective partitioning of one or more components between two phases, one of which is a solid sorbent. The second phase typically is a liquid, but it may also be an emulsion, a gas, or a supercritical fluid. The components of interest may either preferentially adsorb to the solid, or they may remain in the second, non-solid phase. Once equilibrium has been reached, the two phases are physically separated by decanting, filtration, centrifugation or a similar process. If the desired analytes are adsorbed on the solid phase, they can then be selectively desorbed by washing with an appropriate solvent. If the components of interest remain in a liquid phase, they can be recovered *via* concentration, evaporation, chromatographic separation, and/or recrystallization. When SPE is performed in this single-step equilibrium batch mode, it is similar in practice to LLE, where the solid sorbent simply replaces one of the immiscible liquids.

Using solid sorbents in the traditional batch adsorption method just described still can lead to incomplete sample recovery and purification, due to unfavorable partition equilibria, insufficient sample capacity, liquid entrapment within the solid matrix, or incomplete desorption of the adsorbed species after filtration. The real benefits of SPE are gained when the sorbent is packed efficiently into a tube to form a uniform bed with good flow distribution characteristics.

By passing a liquid (or gas) through this bed, the liquid-solid phase extraction technique becomes a form of column chromatography, now commonly called *solid phase extraction* [SPE], that is governed by liquid chromatographic principles.

Advantages of Solid Phase Extraction Over Liquid-Liquid Extraction

One of the fundamental advantages of column liquid-solid extraction is that, by choosing suitably selective adsorbents, the partition equilibrium of specific sample components can be driven to effect nearly complete adsorption or desorption. In batch-mode SPE or in single-stage LLE, each extraction step is a single equilibration process, equivalent to one chromatographic “plate”. SPE operated in a packed bed mode, on the other hand, can typically generate 10-50 plates. This enables a *single-step* isolation process with promise of higher recoveries and greater enrichment of the desired components than could be obtained from a single step LLE or batch SPE. In addition, since the number of plates affects resolution of two components, SPE with 50 plates has a good chance of providing a more pure product than LLE with one or two extraction steps. To achieve similar results with LLE, one must perform several manual liquid extractions, or perform countercurrent LLE.

Effective Solid Phase Extraction

Effective separation by SPE depends primarily on the proper choice of sorbent and eluting solvents. After considering the chemical nature and chromatographic polarity of the sample components and the solvent in which they are dissolved, a suitable solid adsorbent of appropriate activity and particle size is chosen and packed into a column (fritted glass tube, syringe barrel, Pasteur pipet, etc.) . The chromatographic packed bed thus formed should be homogeneous and free of voids and channels to assure maximum efficiency of sample contact, separation, and recovery. The adsorbent, itself, must be reproducible in activity, selectivity, and retention properties. If necessary, a suitable solvent can be used to prewet, equilibrate, clean, and/or flush the bed. Then, the sample solution is applied to the inlet and allowed to flow into the bed under gravity, positive pressure, or vacuum at a controlled flow rate.

A series of solvents or solvent mixtures of successively increasing elution strength (*eluotropic series*) is used to selectively desorb desired components in an appropriate sequence. An aliquot of each eluting fraction may be taken for analysis, or, in some situations, further processing may be required. Elution strength is relative and depends upon the nature of the sorbent being used. In general, the chemical nature and polarity of a “strong” solvent matches

that of the solid surface while that of a “weak” solvent is at the opposite end of the spectrum. See [Table 6](#) for some guidelines.

Difficulties with column liquid-solid extraction usually arise in improper selection and standardization of the adsorbent, poor packed bed quality (inhomogeneity, void spaces, channeling, bed settling, etc.) and reproducibility, use of bed volume and capacity inappropriately matched to sample size and requirements, poor choice of sample loading and elution solvents, and excessive flow rates.

A variety of sorbents are available today, each offering a different selectivity. One can tailor carefully the sorbent to the needs of the application. When the second phase is a solid, unlike liquid-liquid extraction, problems of solvent phase miscibility and emulsion formation are obviated. Solid phase extraction therefore gives greater freedom in the choice of extraction solvent.

Sep-Pak[®] Cartridges for Rapid Sample Preparation

The convenient format and features of Sep-Pak[®] cartridges overcome many of the procedural difficulties of traditional column liquid-solid extraction and allow the enormous benefits of solid phase extraction to be realized. Adsorbent and packed bed quality, reproducibility, versatility, and ease of use are assured through intelligent design, production control, and quality testing.

One key to success in developing a rugged solid phase extraction method is the reproducibility of the solid phase sorbent. Waters takes significant steps to ensure that the solid phase chemistry does not change between batches. Each batch of Sep-Pak[®] cartridge stationary phase undergoes a variety of stringent analytical checks and functional chromatographic testing to ensure that each application will perform identically on every batch of sorbent. From start to finish, the production of Sep-Pak[®] cartridges is done in an *ISO 9002*-certified facility under strict *GLP* and *cGMP* guidelines.

Benefits of Sep-Pak[®] Cartridges

When compared to other sample preparation processes, solid phase extraction using Sep-Pak[®] cartridges offers:

- ❑ *Lower Cost*
 - less solvent consumption
 - less reagent consumption
 - less apparatus
- ❑ *Greater Recoveries*
 - minimal sample transfer
- ❑ *Faster Protocol*
 - fewer steps
- ❑ *Greater Safety*
 - less exposure to toxic agents
- ❑ *Greater Accuracy*
 - no cross contamination
- ❑ *No Emulsion Problems*
 - less sample handling
 - fewer steps
- ❑ *No Transporting of Samples to Lab*
 - direct field sampling
- ❑ *Reduced Harm to Labile Samples*
 - minimal evaporation
- ❑ *Minimal Glass Breakage*
 - less glassware used, less to wash

Sorbent chemistries

The wide variety of sorbent chemistries available from Waters lets you tailor a sample preparation step to the specific needs of your application. There are hydrophilic phases that range from high to intermediate to low polarity; nonpolar phases which vary from high to low degrees of hydrophobicity; strong and weak ion-exchangers; and specialty sorbents. For the adsorption of the analytes of interest, you can take advantage of a variety of adsorptive forces from the weaker van der Waals, hydrogen-bonding, and dipole-dipole interactions, to the progressively stronger hydrophobic-interaction and ion-exchange processes, to the extreme of chemical modification of the analyte by on-cartridge derivatization reactions.

Many sorbents can be used in more than one mode. For example, silica can be used for adsorption by hydrogen bonding or dipole-dipole interaction, for support of a stationary-phase liquid in liquid-liquid partition, and even for ion-exchange. Some sorbents, like Florisil or DNPH, have been designated, and are certified, for specific separations, but can be used in other applications as well. Aluminas are available with acidic [A], neutral [N], and basic [B] surfaces. Cation and anion exchangers are available in both strongly- and weakly-ionized forms, which

can be advantageously altered in function by changing pH. Non-polar sorbents can be used in reversed-phase mode to extract analytes from aqueous solvents and, then, in non-aqueous normal-phase mode for preferential elution of adsorbed analytes. Typical non-polar sorbents are C₁₈, C₈, or C₂ bonded-silica phases. HPLC users are familiar with these packings and their versatility.

Table 1 lists the general properties and some typical applications for sorbents currently available from Waters in Sep-Pak[®] cartridge format. Physicochemical characteristics and surface functionalities of packing materials are shown in **Table 2** and **Table 3**, respectively.

Table 1. Sorbent Properties & Typical Applications

Separation Mode:

Sorbent

Properties & Applications

Reversed-Phase:

C ₁₈	strongly hydrophobic silica-based bonded phase; used to adsorb analytes of even weak hydrophobicity from aqueous solutions; typical applications include drugs and their metabolites in serum, plasma or urine, desalting of peptides, trace organics in environmental water samples, organic acids in beverages; similar to reversed-phase HPLC columns in elution behavior.
tC ₁₈	strongly hydrophobic silica-based bonded phase; trifunctional bonding chemistry gives it increased hydrolytic stability over C ₁₈ ; applications similar to those of C ₁₈ .
C ₈	moderately hydrophobic silica-based bonded phase; use for methods requiring less retention than C ₁₈ ; typical applications include drugs and their metabolites in serum, plasma or urine, peptides in serum, plasma.
tC ₂	silica-based bonded phase with low hydrophobicity; use for methods requiring less retention than C ₈ ; applications are similar to C ₁₈ and C ₈ .
Porapak [®] Rdx	specialty cleaned, hydrophobic copolymer resin [poly(divinyl-benzene-vinylpyrrolidone)]. Can be used as an alternative to octadecyl-bonded silica for preparation of analytes that weakly adsorb to silica-based reversed phase sorbents. Compatible with sample or eluents at high and low pH. Specifically designed for the concentration of high explosives in aqueous samples.

Table 1 [continued]. Sorbent Properties & Typical Applications

Separation Mode:

Sorbent

Properties & Applications

*Normal- or
Reversed-Phase:*

Aminopropyl
[NH₂]

silica-based, moderately polar, bonded phase with weakly basic surface; can be used as a polar sorbent, like silica, with different selectivity for acidic/basic analytes or as weak anion exchanger in aqueous medium below pH 8; applications include phenols and phenolic pigments, petroleum fractionation, saccharides, drugs and drug metabolites.

Cyanopropyl
[CN]

silica-based bonded phase of low hydrophobicity; can be used as less polar alternative to silica in normal-phase applications or as less hydrophobic alternative to C₁₈ or C₈ in reversed-phase applications; typical applications include drugs, drug metabolites, and pesticides.

Diol

silica-based, moderately polar, bonded phase with neutral surface; can be used as an alternative to silica in normal phase applications, where the acidic character of silica is undesirable or as very weakly interacting hydrophobic phase in aqueous media; applications include antibiotics from cosmetics; isolation of proteins or peptides by hydrophobic interaction chromatography.

Table 1 [continued]. Sorbent Properties & Typical Applications

Separation Mode:

Sorbent

Properties & Applications

Normal-Phase:

Silica

polar sorbent, used primarily to adsorb analytes from non-polar solvents like hydrocarbons, chloro- or fluoro-substituted hydrocarbons or less polar esters and ethers; elution with more polar solvents like polar esters, ethers, alcohols, acetonitrile or water; the binding mechanism can be hydrogen bonding or dipole-dipole interaction; silica can also be used in aqueous medium as a cation exchanger of intermediate strength, or as a support for liquid-liquid partition separations with a polar stationary phase.

Alumina A, N, B

similar in use to silica; available in acidic [A], neutral [N], and basic [B] grades; highly active, polar surface; alumina also exhibits specific interactions with the pi-electrons of aromatic hydrocarbons, making it useful for applications like crude oil fractionation; acidic and basic grades can also be used as low-capacity ion-exchangers, which, unlike polymer-based exchangers, are unaffected by high energy, radioactive materials.

Florisil

highly active, polar sorbent with a slightly basic surface for adsorption of low to moderate polarity species from nonaqueous solutions; specifically designed for the adsorption of pesticides using official AOAC and EPA methods; other applications include polychlorinated biphenyls in transformer oil.

Table 1 [continued]. Sorbent Properties & Typical Applications

Separation Mode:

Sorbent

Properties & Applications

Ion Exchange:

Accell™ Plus QMA

silica-based, hydrophilic, strong anion-exchanger with large pore-size; extraction of anionic analytes in aqueous and non-aqueous solutions; due to the large pore-size, it is excellent for the isolation of anionic proteins, e.g., immunoglobulins, enzymes; other applications include the removal of acidic pigments from wines, fruit juices and food extracts, isolation of phenolic compounds, peptide pool fractionations.

Accell™ Plus CM

silica-based, hydrophilic, weak cation-exchanger with large pore-size; extraction of cationic analytes in aqueous and non-aqueous solutions; due to the large pore-size, it is excellent for the isolation of cationic proteins; other applications include pesticides, herbicides, steroids.

Specialty Packing:

DNPH-silica
and XPoSure™

contain acidified dinitrophenylhydrazine reagent coated on a silica sorbent. Used for quantitation of aldehydes and ketones in air samples by *in situ* reaction to form hydrazone derivative, which is then eluted and analyzed by HPLC. DNPH-silica is specified in several EPA procedures for the analysis of carbonyl compounds in air.

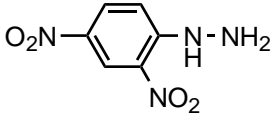
Table 2. Sep-Pak[®] Cartridge Packing Materials: Physicochemical Characteristics

Packing	Activity Grade	End-Capped?	% Carbon	Pore Size (nominal, Å)	Particle Size Range (µm)
C ₁₈	n/a	Yes	12	125	55–105
tC ₁₈	n/a	Yes	17	125	37–55
C ₈	n/a	Yes	9	125	37–55
tC ₂	n/a	Yes	2.7	125	37–55
NH ₂	n/a	No	3.5	125	55–105
CN	n/a	Yes	6.5	125	55–105
Diol	n/a	No	2	300	37–55
Silica	High*	n/a	n/a	125	55–105
Florisil	High**	n/a	n/a	60	50–200
Alumina A	1***	n/a	n/a	120	50–300
Alumina N	1***	n/a	n/a	120	50–300
Alumina B	1***	n/a	n/a	120	50–300
Accell [™] Plus CM	n/a	n/a	5.5	300	37–55
Accell [™] Plus QMA	n/a	n/a	6	300	37–55
Porapak [®] Rdx	n/a	n/a	95	200	125–150
DNPH-silica	n/a	n/a	n/a	125	55–105
XPoSure [™]	n/a	n/a	n/a	125	500–1000

* Moisture content ≤ 3.2%; ** Moisture content ≤ 2.5%

*** Moisture content ≤ 1.5%; activity ≤ 1 on Brockmann activity scale

Table 3. Sep-Pak[®] Cartridges: Chemistry of Bonded-Phase & Coated Packing Materials

Packing Material	Bonding Chemistry	Surface Functionality	Packing Material	Bonding Chemistry	Surface Functionality
C18	Monofunctional silane	-Si(CH ₃) ₂ C ₁₈ H ₃₇	CN	Difunctional silane	-Si(CH ₃)(CH ₂) ₃ CN
tC18	Trifunctional silane	-SiC ₁₈ H ₃₇	Diol	Trifunctional silane	-Si(CH ₂) ₃ OCH ₂ CH(OH)CH ₂ OH
C8	Monofunctional silane	-Si(CH ₃) ₂ C ₈ H ₁₇	Accell [™] Plus CM	Polymeric*	-CO ₂ ⁻ Na ⁺
tC2	Trifunctional silane	-SiC ₂ H ₅	Accell [™] Plus QMA	Polymeric**	-C(O)NH(CH ₂) ₃ N(CH ₃) ₃ ⁺ Cl ⁻
NH ₂	Trifunctional silane	-Si(CH ₂) ₃ NH ₂	DNPH-Silica	Coated***	

* An acrylic acid/acrylamide copolymer on Diol silica; ligand density: 350 μmoles/g; approximate protein binding capacity = 175 mg Cytochrome c/gm of packing or 63 mg/Accell[™] Plus CM Classic or Plus cartridge.

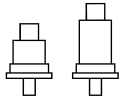
** An acrylamide copolymer on Diol silica; ligand density: 220 μmoles/g; approximate protein binding capacity = 200 mg BSA/g of packing or 72 mg/Accell[™] Plus QMA Classic or Plus cartridge.

*** Bare silica coated with 14 μmoles DNPH/g of packing or 5 μmoles/cartridge.

Choosing a Cartridge

Sep-Pak[®] cartridges are available in a variety of designs, each offering specific functional benefits. All cartridge types, however, are filled by weight with the same high-quality sorbents. As will be explained in a later section, this uniformity of packing chemistry makes transfer of methods from one design to another straightforward and predictable. All cartridges are sealed in special poly-foil pouches to maintain product integrity, packing activity, and purity until the package is opened.

Sep-Pak[®] Vac and Sep-Pak[®] Vac RC cartridges both contain integral solvent reservoirs and are principally used with vacuum manifolds and automated SPE instrumentation. Sep-Pak[®] Plus, Sep-Pak[®] Light, and Sep-Pak[®] Classic cartridges are the most versatile units. They attach easily to pumps and syringes for reliable, positive-pressure flow, but, if desired, can also be fitted with removable reservoirs and used on vacuum-assisted flow devices and certain automated systems. Waters patented technologies for radial compression [U.S. #4,250,035] and triaxial compression [U.S. #4,211,658] are used to form homogeneous packed beds, free of voids and channels, in Plus/Light and Classic cartridges, respectively.



Sep-Pak[®] Plus Cartridges: Maximum Versatility

Sep-Pak[®] Plus cartridges feature a state-of-the-art packing process as well as a precision design, suited to robotic applications, that also makes manual use even more convenient. Certain sorbents are available in short-body, and others in long-body, configurations. Both cartridge styles are molded from high-purity polyethylene with female-Luer inlet and male-Luer outlet fittings. Packing materials are retained by 20 micron frits made of a blend of HD and UHMW polyethylenes. Excellent solvent resistance and extremely low extractable levels are important characteristics of this design. A color-coded compression ring serves to seal the cartridge, radially compress the packed bed, and identify the type of sorbent. Connections can be made to different types of devices such as positive-flow pumps and syringes, vacuum manifolds, and some automated sample processors.

Outlet tips feature reduced internal volume for minimal sample holdup. The fittings also enable cartridges to be stacked in tandem: with cartridges of the same type for increased capacity; with cartridges of different types for sequential adsorption strategies; or with filter units for isolation or removal of sample particulates.

Sep-Pak[®] Plus cartridges can be used with liquid and gas samples and have the unique capability for reversing flow direction, a useful feature for trace enrichment applications. Interstitial volumes and weights of sorbents in the short- and long-body configurations match those of corresponding Classic cartridges. See [Table 4](#) for a complete list of weights of sorbents in each cartridge type.



Sep-Pak[®] Light Cartridges: Reduced Volume

Sep-Pak[®] Light cartridges are the same size and have the same features as, but about one-third the internal volume of, the short-body Sep-Pak[®] Plus cartridges. A distinctive "finned" outer body also features a color-coded compression ring. This cartridge format allows you to scale down your sample preparation protocols when sample size is limited or when excessive dilution is a concern. Fractions can be eluted in a minimal volume to improve sensitivity and reduce solvent consumption.



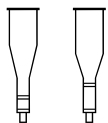
Sep-Pak[®] Classic Cartridges: Most Widely Referenced

Sep-Pak[®] Classic cartridges are the original Sep-Pak[®] cartridges introduced in January, 1978. They contain the same high quality packing materials as the other Sep-Pak[®] cartridge types and match the interstitial volumes of Sep-Pak[®] Plus cartridges with corresponding short (0.5 mL) and long (1 mL) bodies. This means that you can transfer published methods developed on Classic cartridges directly to the more versatile Plus cartridges.



Sep-Pak[®] Vac Cartridges: Economical Automation

Sep-Pak[®] Vac cartridges deliver the option of minimum cost sample preparation. The cartridges consist of molded polypropylene "syringe-barrel" bodies with two polyethylene frits to contain the packing material. A standard male-Luer tip permits use with multi-position vacuum manifolds. Cartridge sizes range from 1 cc to 35 cc and, as shown in [Table 5](#), contain sorbent weights from 50 mg up to 10 g. Color-coded printing on the syringe barrels identifies the sorbent.



Sep-Pak[®] Vac RC Cartridges: Larger Reservoir Capacity

Sep-Pak[®] Vac RC cartridges feature an integral 20-cc funnel-shaped reservoir and contain 100 or 500 mg of sorbent. They can be used with robotic sample preparation equipment or with vacuum manifolds. The larger reservoir permits convenient processing of greater volumes of samples or solvents. This reduces set-up time and lowers costs associated with the use of separate reservoirs. Sorbents are identified by color-coded printing on the cartridge bodies.

Table 4. Nominal Weight of Sorbent in Sep-Pak® Plus, Light and Classic Cartridges

Weight in mg [Icon indicates body type & length]


















<i>Sorbent Type</i>	<i>Plus</i>		<i>Classic</i>		<i>Light</i>	
C ₁₈		360		360		110
Environmental C ₁₈		820		n/a		n/a
tC ₁₈		390		n/a		125
Environmental tC ₁₈		870		n/a		n/a
C ₈		390		n/a		125
tC ₂		390		n/a		125
Aminopropyl [NH ₂]		360		360		110
Cyanopropyl [CN]		360		360		110

Table 4. continued.





























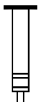





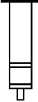


Diol		360		360		110
Silica		690		690		110
Florisil		900		900		125
Alumina A		1710		1850		260
Alumina B		1710		1850		260
Alumina N		1710		1850		260
Accell™ Plus QMA		360		360		115
Accell™ Plus CM		360		360		115
DNPH-silica		350		n/a		n/a
XPoSure™		350		n/a		n/a

Table 5. Nominal Weight of Sorbent in Sep-Pak[®] Vac Cartridges

Cartridge Type

Amount of Sorbent

<i>Cartridge Type</i>	<i>Amount of Sorbent</i>	<i>Amount of Sorbent</i>
Vac RC	 100 mg	 500 mg
1 cc	 50 mg	 100 mg
3 cc	 200 mg	 500 mg
6 cc	 500 mg	 1000 mg
12 cc	 2 g	
20 cc	 5 g	
35 cc	 10 g	

Solid Phase Extraction Methods Development

When you develop a new sample preparation protocol, the first thing to think about is the purpose of the method. What do you want the sample preparation to accomplish? This objective will determine the actual technique. Secondly, you should have some knowledge of the type of chemical "handle" you can use to isolate your analyte. What is the polarity of your analyte? Is it an acid or a base? Also, is the sample soluble in an aqueous or nonaqueous solvent? Answering these questions will help you to determine if solid phase extraction is feasible, and, if so, the type of sorbent chemistries to try.

Next, choose the size of the Sep-Pak[®] cartridge based on the size of the initial sample and/or the desired final volume of the analyte solution after the solid phase extraction step. Lastly, choose the design of the cartridge based on the equipment that it will be used with, for example, a vacuum manifold, a manually operated syringe, or a robot.

SPE *is* Chromatography

Keep in mind that solid phase extraction has the same fundamental basis as HPLC. Any knowledge of the chromatographic behavior of the analytes of interest, and of other matrix components, can help in choosing the proper sorbent and eluents. If, for example, you know that certain chromatographic conditions provide excellent separation between your analyte and interferences, then you may choose a similar SPE sorbent and solvent combination. Similarly, if you are trying to remove an interference that coelutes in HPLC, then you know *a priori* that similar SPE conditions will not be successful.

General Elution Protocols

There are two general strategies for isolating and cleaning up sample components of interest:

- ❑ adsorb matrix interferences while components of interest pass through the cartridge unretained.
- ❑ adsorb components of interest while matrix interferences pass through the cartridge unretained.

The first strategy is usually chosen when the desired sample component is present in high concentration. When components of interest are present at low levels, or multiple components of widely differing polarities need to be isolated, then the second strategy is generally employed. Trace enrichment of compounds present at extremely low levels and concentration of dilute samples are also achieved by the second strategy.

Sample Preparation Objectives

This section describes a few common sample preparation objectives and provides a general description of the techniques that best achieve these goals.

- ❑ *To remove sample constituents that elute after the analytes of interest or are strongly adsorbed:*
 - use solid phase extraction with sorbent surface chemistry that is the same as that in the analytical HPLC column
 - use a narrow solvent-strength window to elute analytes

This is one of the classical sample preparation situations, typically done when HPLC is the analytical technique. The removal of strongly adsorbed sample constituents protects the HPLC column from contamination that otherwise would result in either an unstable baseline after several runs and/or even a reduction in the useful life of the HPLC column. In this case, the sorbent surface chemistry of the solid phase extraction cartridge should be the same as, or very similar to, the surface chemistry of the HPLC column.

First, adsorb the sample onto the Sep-Pak[®] cartridge using a solvent of low eluting strength. Next, wash the cartridge with one or more solvents also of low eluting strength. Then elute the analytes from the cartridge using a solvent strength that is slightly higher than that of the mobile phase used in the HPLC separation. The contaminants should stay in the cartridge.

For reversed-phase separations of small molecules, one can use the rule of thumb that the retention of a compound changes about 2-fold for every 5-percent change in the concentration of the organic solvent in the mobile phase. Therefore, you probably want to use a wash solvent that contains about 15-percent less organic solvent than the HPLC mobile phase and an eluent that contains about 15-percent more organic solvent than your HPLC mobile phase. Narrower solvent-strength windows can be used with analytes of higher molecular weight. In all cases, you should verify that this procedure does not remove any analyte of interest in the washing process by analyzing the fractions by HPLC. You should also confirm that the analyte has been fully recovered from the cartridge by using an even stronger eluent and checking this fraction for any residual analyte. *Remember that, contrary to liquid-liquid extraction, you should not accept low sample recoveries in solid phase extraction.*

A complex matrix may be treated by a different elution strategy depending on the target analyte. For example, consider the strategies used in File #[SP87032](#) and #[SP88081](#). In both references, the matrix of interest is red wine. But the respective target analytes are organic acids/sugars and phenolic wine pigments. In the first case, the wine sample, at the appropriate pH, is passed through a C₁₈ reversed-phase cartridge, and the interfering wine pigments are removed by adsorption while the organic acids elute unretained. In the latter case, the authors isolate phenolic pigments, also with a C₁₈ cartridge. But here, the sugars and acids pass directly through the cartridge, while the pigments are retained and subsequently eluted with a stronger mobile phase. Of course, it is possible to combine two such applications into a single, multistep protocol to prepare several classes of compounds for analysis.

❑ *To remove sample constituents that coelute with an analyte of interest:*

- use solid phase extraction with sorbent surface chemistry different from that in the analytical column
- use a broad solvent strength window to elute analytes

This is a more difficult, yet common, sample preparation problem, most relevant when HPLC is the final analytical tool. To remove a contaminant that coelutes with an analyte on the HPLC column requires a solid phase extraction mechanism that is significantly different from that of the HPLC separation. This can be done either by significantly changing the mobile phase composition while keeping the surface chemistry of the Sep-Pak[®] cartridge and the HPLC column the same, or, more effectively, by using a Sep-Pak[®] cartridge with a sorbent surface chemistry different from that in the analytical column.

A change in the mobile phase can be useful only if the coeluting compounds respond very differently to this change. A good example is a change in pH, that causes one compound to change its ionic state of while leaving the other the same. However, changing the organic solvent rarely induces a large enough shift in selectivity to be useful for solid phase extraction.

When using a Sep-Pak[®] cartridge with a sorbent surface chemistry different from that of the analytical column, it is best to select a chemistry that is orthogonal to that in the analytical column. This means, for example, if the analytical column contains a C₁₈ phase, try a sample preparation procedure that uses normal-phase adsorption or ion-exchange rather than selecting another type of reversed-phase sorbent.

An example might be a basic compound that is in its cationic form at pH 7, typically analyzed by reversed-phase HPLC. It can be retained by ion exchange on a Sep-Pak[®] silica cartridge or on an Accell[™] Plus CM cartridge and then eluted with a high-salt concentration. The sample preparation and analytical techniques are orthogonal to each other, and the probability of coelution in SPE is very low. If a coelution occurs nevertheless, fine-tune the solid phase extraction technique by carefully investigating at which salt concentration the analyte and the interference elute. Narrow the solvent-strength window to remove selectively only the analyte of interest from the Sep-Pak[®] cartridge.

❑ *To enrich sample components present in low concentration:*

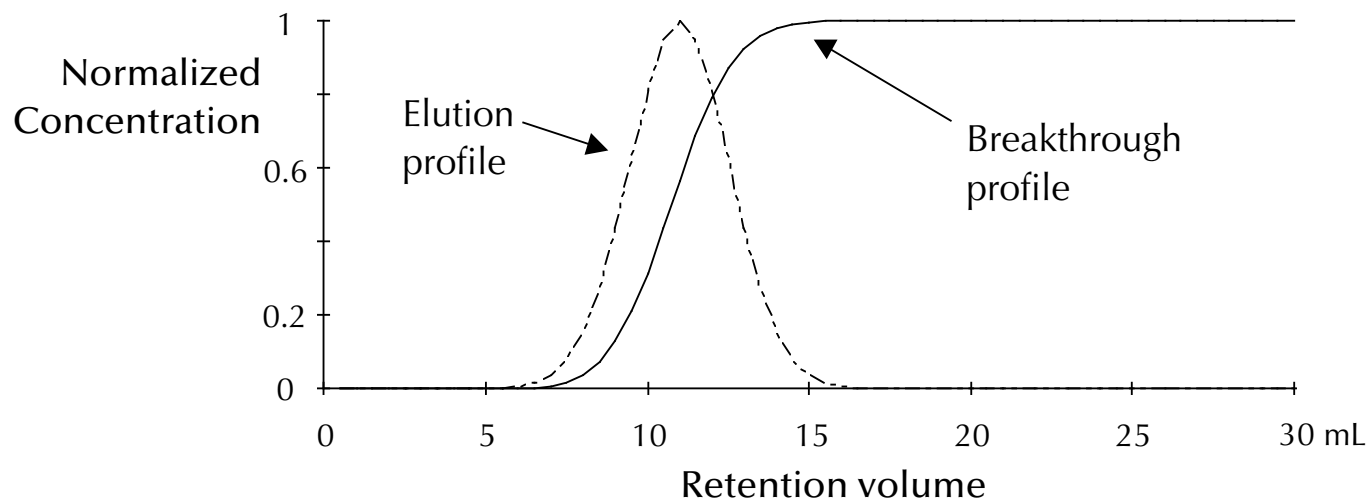
- use “large” sample volumes in adsorption-promoting solvent
- use “small” collection volume in desorption-promoting solvent
- use sorbent chemistry tailored to the analyte, independent of that in analytical column
- carefully choose chemistry of solid phase extraction column so further sample prep will be unnecessary

The need for enrichment of the sample is encountered in many analytical problems. A typical example is the analysis of trace contaminants in environmental water samples.

For this type of application, the amount of sorbent is a critical factor. This determines both the volume of the untreated sample that can be processed and the volume of the eluted sample after enrichment. The enrichment factor, the respective ratio of these two volumes, however, is also a function of the total amount of analytes present in the sample and the chemical interaction of the analytes with the sorbent. Therefore, it is most important to tailor the isolation chemistry to that of the analyte(s).

As an example, consider enriching a polar organic acid from water on a reversed-phase Sep-Pak[®] cartridge. The retention of any hydrophobic sample is greater on a C₁₈ surface than on a C₂ surface, so the C₁₈ cartridge is preferred over the Sep-Pak[®] C₂ cartridge. HPLC experiments determine that this analyte has a capacity factor (k') of about 10 in neat water on a C₁₈ HPLC column. This means that the maximum enrichment factor achievable is no more than 10, since the sample will start to break through after only 10 cartridge-volumes of sample have passed through the cartridge. Actually, breakthrough will occur somewhat earlier due to the low plate count of the Sep-Pak[®] cartridge, which broadens the elution profile. This is shown in [Figure 1](#).

Figure 1. Breakthrough Curve of Analytes on a Sep-Pak® Cartridge



To avoid premature breakthrough and to improve the enrichment factor, one needs to increase the capacity factor significantly. This is easily accomplished by suppressing the ionization of the acid by acidifying the sample with an inorganic acid to pH 2.0–2.5. In its neutral, more hydrophobic, state, the organic acid exhibits a dramatic increase in its capacity factor, perhaps to 500; the potential enrichment factor increases proportionally.

By tailoring to the needs of the application the chemical interaction of the analyte with the sorbent, sample recovery and enrichment can be significantly enhanced.

☐ *To desalt samples:*

- first, adsorb analytes on reversed-phase sorbent while salt breaks through unretained
- then, desorb using water-miscible organic solvent

Desalting is best accomplished using reversed-phase Sep-Pak[®] cartridges, unless the sample is a protein that could be denatured by organic solvents. A typical application is the desalting of peptides. Adsorb the sample from an aqueous medium with a high salt concentration on onto a Sep-Pak[®] C₁₈ cartridge. Then, wash the cartridge with a small amount of water, and, finally, desorb the peptides using either an organic solvent alone, or admixed with water. There are dozens of references in this bibliography to peptide isolation from biological sample matrices, see, for example, the work of [H.P.J. Bennett](#).

☐ *To exchange solvents:*

- adsorb the sample completely onto a strongly retentive sorbent and flush away the original solvent with a weaker eluent
- elute the analyte with the desired solvent

It is sometimes necessary to change the sample solvent, especially when the original solvent is incompatible with the final analytical method. Evaporating a sample to near dryness and, then, reconstituting it in a new solvent may harm a labile analyte or risk poor recovery. Solid phase extraction is a much safer and more convenient process for solvent exchange. For example, a sample dissolved in methylene chloride may be undesirable for gas chromatography with electron capture detection. By first diluting the sample 10-fold or more with a nonpolar hydrocarbon such as hexane, the analyte can be retained on a Sep-Pak[®] cartridge containing a polar sorbent, such as silica or alumina. Subsequently, elute the analyte with a small volume of a suitable, strong solvent, such as an ether or an ester.

A similar strategy can be executed using a reversed-phase sorbent to exchange an aqueous medium with an organic solvent or an undesired organic solvent/water mixture with a different, more suitable mixture. For example, dilute a sample dissolved in an aqueous-organic mixture at least 10-fold with water, being careful not to cause precipitation. Then, pass the diluted sample through a Sep-Pak[®] C₁₈ cartridge to adsorb the analyte. Elute the analyte with a more desirable organic solvent or organic solvent/water mixture.

When it is necessary to exchange two immiscible solvents, an intermediate wash with an appropriate co-solvent may be necessary. Or, as shown in File #[SP82011](#), it is possible to carefully dry a reversed-phase cartridge with clean, forced air, or an inert gas, after the adsorption step. Then, a new, water-immiscible, organic solvent, such as diethyl ether, may be used to elute selectively the analyte of interest.

❑ *To fractionate classes of compounds:*

- use a step-gradient sequence to divide a sample on the basis of hydrophobicity or polarity into fractions containing groups of analytes that share common properties

A simple example of this technique is the separation of synthetic oligonucleotides into ones with and without the nonpolar dimethoxytrityl (DMT) protecting group. The method is independent of the molecular weight of the oligomers and works for oligonucleotides of up to 100 base pairs in length. The oligonucleotide mixture is loaded onto a special reversed-phase Sep-Pak[®] cartridge with ammonia or buffer, and the oligonucleotides without the DMT protecting group are washed off using a triethylammonium acetate buffer. Then the oligonucleotides containing the intact protecting group can be eluted using a mixture of acetonitrile and buffer.

Similarly, you can fractionate petroleum into fractions of increasing polar functional group content. In this case, use a polar sorbent such as silica or alumina. An initial hexane wash removes aliphatic hydrocarbons. By sequentially increasing the solvent polarity, you can selectively elute unsaturated aliphatics, small aromatics, polynuclear aromatic hydrocarbons, heterocyclics, and other more polar fractions.

One of the most often practiced class fractionations is described in File #[SP85197](#). A Sep-Pak[®] silica cartridge is used to fractionate lipid samples into sterols (cholesterol), diglycerides, triglycerides, cholesteryl esters, free fatty acids, and phospholipids with a sequence of organic solvent mixtures.

❑ *To derivatize analytes using solid-phase reagents:*

- adsorb a derivatization reagent on the surface of the sorbent; then, collect the sample (usually a gas) under conditions that favor complete adsorption of the analyte; wait for the reaction to occur and then elute the derivative

One of the most novel applications of Sep-Pak[®] cartridges is their use as small chemical reactors. By coating the cartridges with a reagent, air or liquid samples can flow through the cartridge to scrub the sample of certain impurities or to generate a derivatized product that can then be subsequently eluted and analyzed.

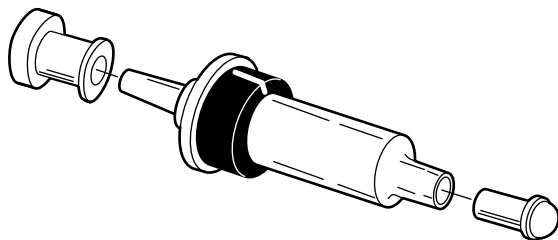
An example is Waters Sep-Pak[®] DNPH-Silica and XPosure[™] cartridges, which contain dinitrophenylhydrazine for the derivatization and enrichment of aldehydes and ketones from the gas phase. An important application of the DNPH cartridge is the determination of aldehydes and ketones in automobile exhaust (see File #[SP92111](#) or #[SP94018](#)). A known amount of exhaust is pumped through the cartridge, after which the aldehydes and ketone, as hydrazone derivatives, are eluted from the cartridge with acetonitrile and analyzed by HPLC.

Another example of using Sep-Pak[®] cartridges for derivatization is: coating a C₁₈ cartridge that has been treated with potassium hydroxide to trap carboxylic acids in a form of *in situ* ion exchange (File #[SP89137](#)). A related technique is dynamic ion exchange for paired-ion methods that have been used to isolate organic compounds. By coating a C₁₈ cartridge with alkaline cetrimide solution, the pesticides paraquat and diquat may be readily isolated (e.g., File #[SP90015](#) or #[SP83002](#)).

How to Use Sep-Pak® Cartridges

Sep-Pak® cartridges are available in a variety of configurations and sizes with Luer-compatible fittings to provide you with a convenient solution to your difficult sample preparation problems. In addition, several adapters are available to provide additional operational flexibility.

Luer male and female plugs are used to seal Sep-Pak® Plus, Light, and Classic cartridges for transport or storage.

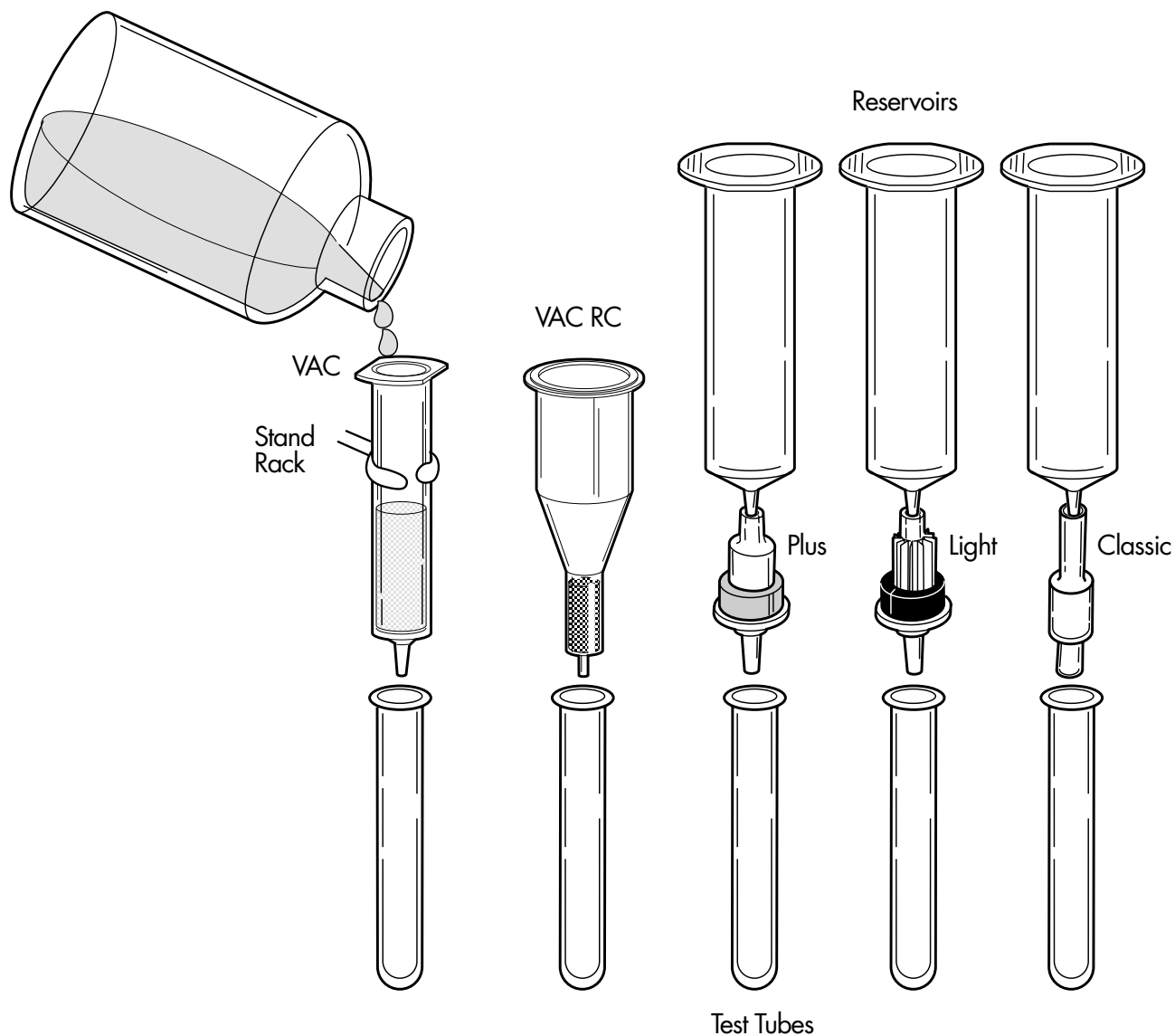


Sep-Pak® cartridges can be loaded and eluted by three flow mechanisms:

- Gravity
- Positive displacement
- Vacuum

▣ Gravity

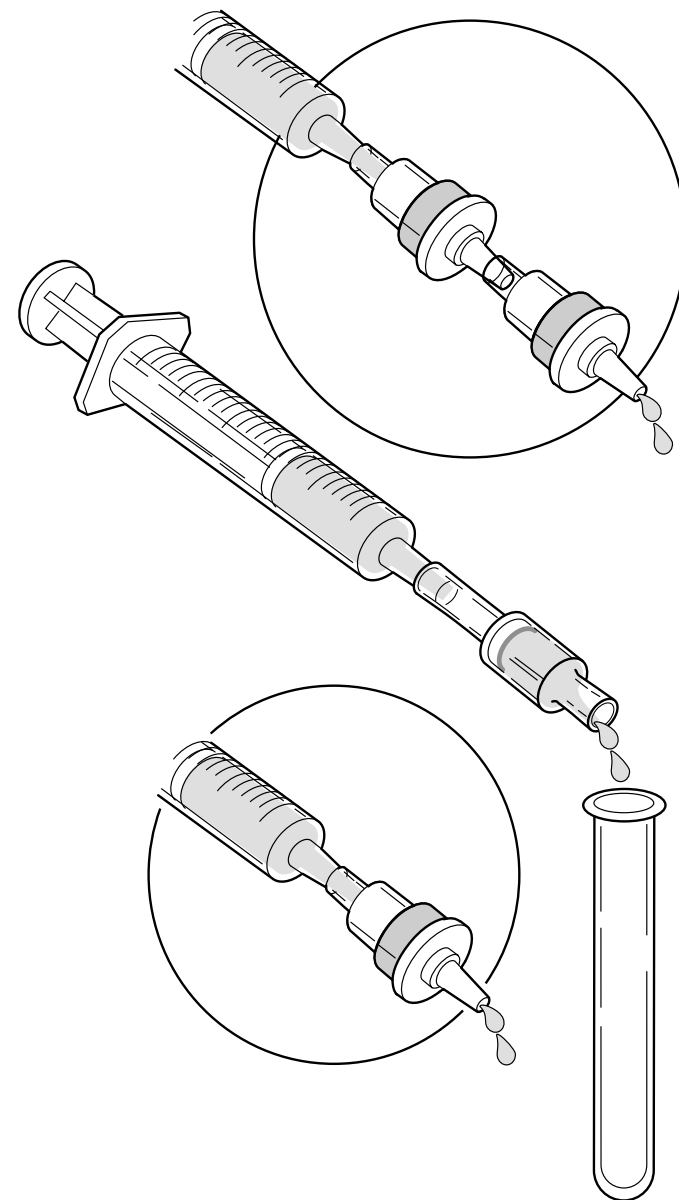
Gravity flow is perhaps the simplest way to use a Sep-Pak[®] cartridge, as it requires little additional equipment. However, the flow rate is very slow. Gravity will generate flow rates of less than 0.25 mL/min. To use gravity flow, set up a rack or stand to hold the cartridge above the liquid receiving vessel. Pour solvent or sample into the empty reservoir of a Sep-Pak[®] Vac or Vac RC cartridge or to a reservoir attached to a Sep-Pak[®] Plus, Light or Classic cartridge.



❑ *Positive Displacement*

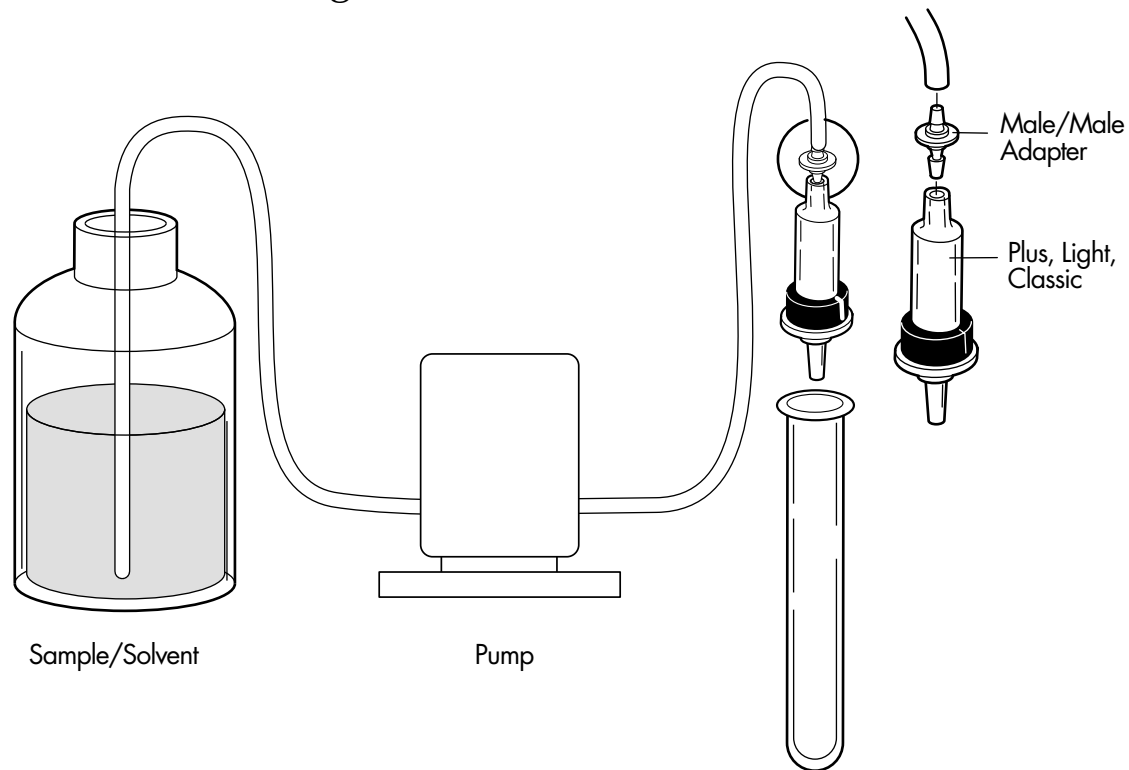
The original Sep-Pak[®] Classic cartridge, as well as the Sep-Pak[®] Plus and Sep-Pak[®] Light cartridges, is designed to be used with positive displacement by a Luer-tip syringe. Using positive displacement can greatly speed up the sample processing time compared to gravity flow.

The simplest way to perform positive displacement is to draw up a solvent or sample into a Luer-tip syringe using the plunger. Connect a Sep-Pak[®] Plus, Light, or Classic cartridge to the syringe using the Luer connector, then push your solvent or sample through the cartridge. (NOTE: Be sure the solvents you are using are compatible with the syringe and plunger.) Typical flow rates for maximum recovery are 1 to 10 mL/minute using the Sep-Pak[®] Classic and Plus cartridges and 0.3 to 3 mL/minute using the Sep-Pak[®] Light cartridges. However, depending on your specific application, you may be able to use faster flow rates.



NOTE: Sep-Pak[®] Plus and Light cartridges can be connected in series to achieve additional capacity. You can also connect cartridges of two different sorbent types to create a "mixed-mode" device. An example of this mixed-mode approach can be found in paper #**SP86001** for the separation of acidic, basic, and neutral analytes.

A pump may also be used with Plus, Light or Classic cartridges. A male/male adapter is used to make the connection to the cartridge (P/N **WAT024310**).

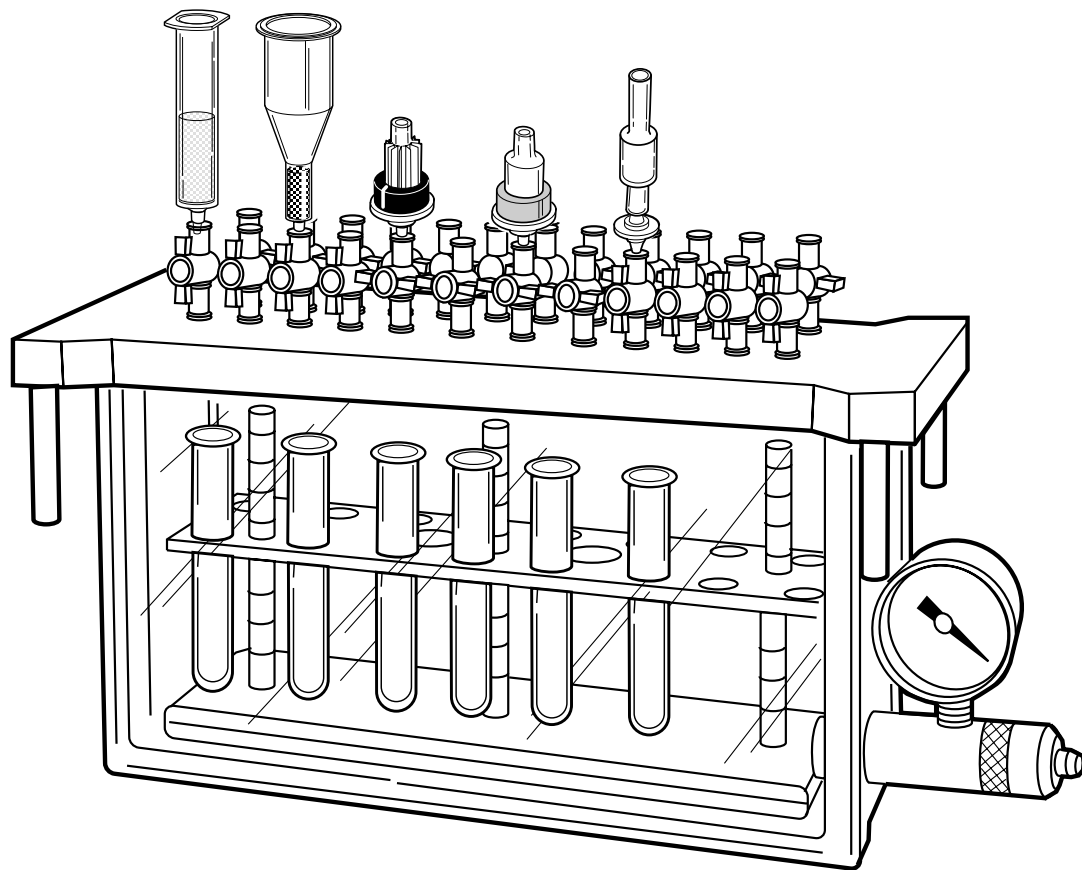


Many automated SPE instruments also can generate flow by positive displacement on Sep-Pak[®] cartridges. Depending on the instrument, Sep-Pak[®] Vac, Vac RC, Plus, or Light cartridges can be used.

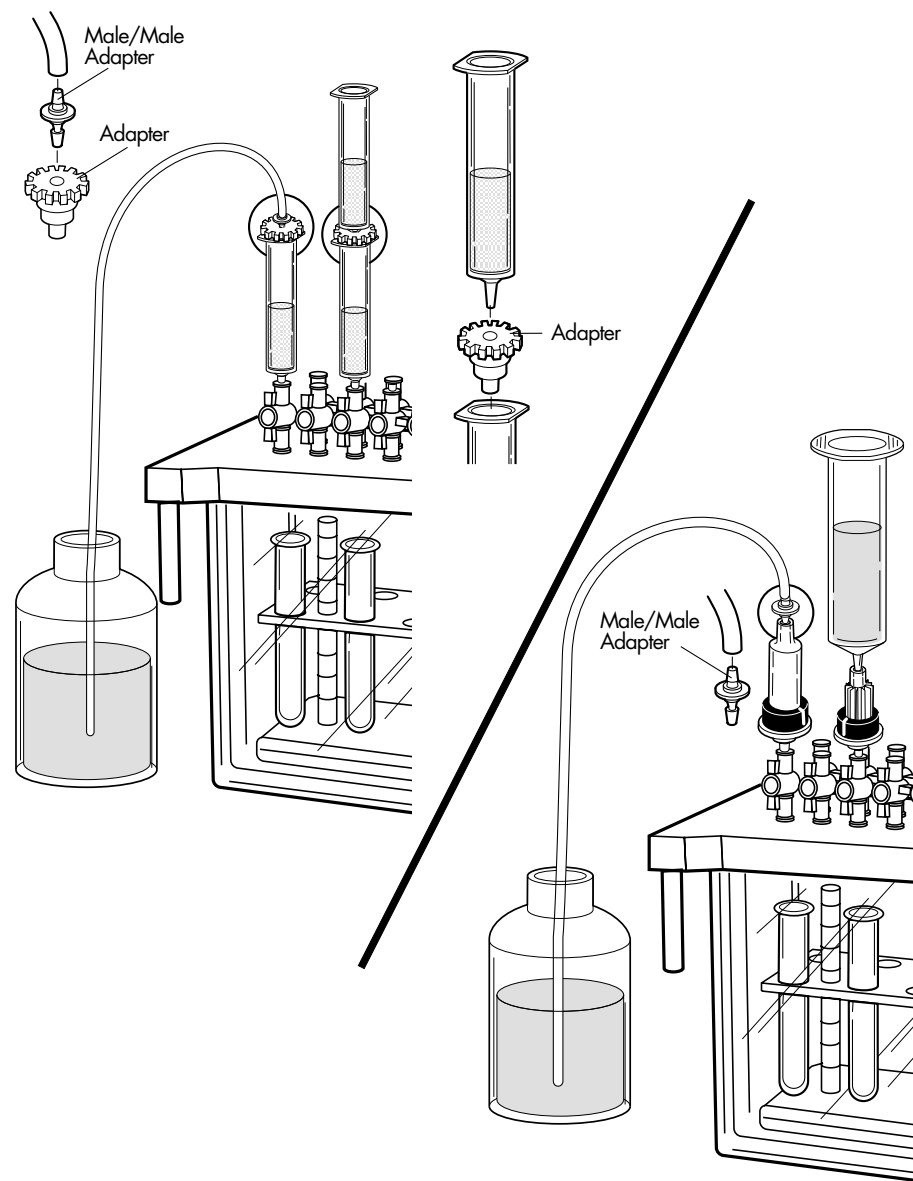
❑ Vacuum

When a large batch of similar samples need to be worked up, the use of a vacuum manifold allows you to run the SPE steps in parallel, thereby greatly reducing the processing time. Manifolds are available to accommodate 12, 16, or 24 samples at time.

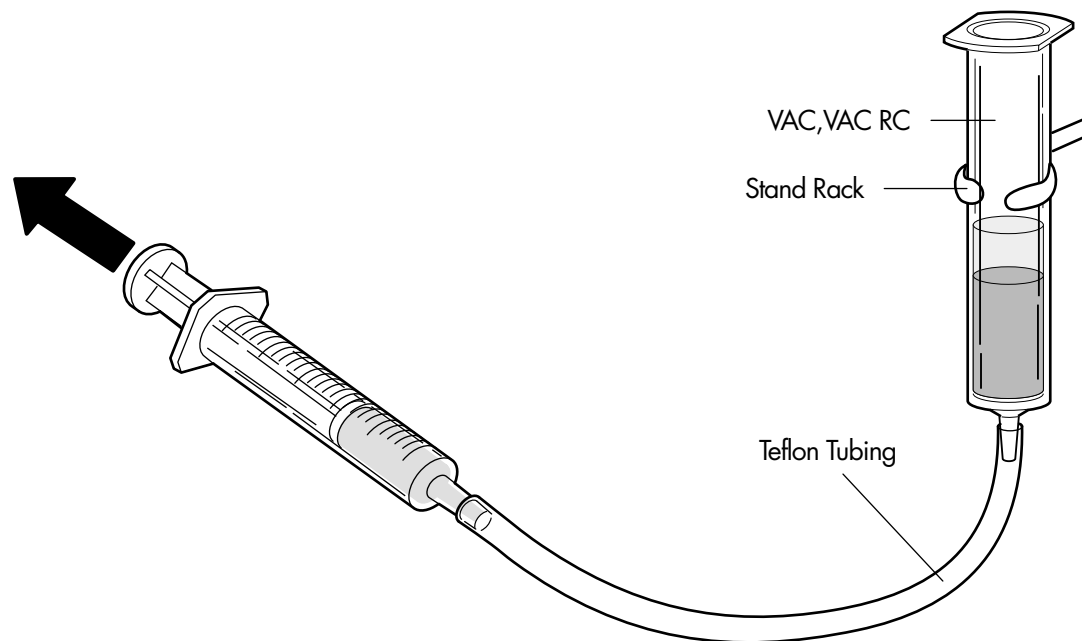
A collection vessel (test tube) held in a rack under each cartridge receives the sample or solvent to be collected. The manifold lid has female Luer fittings so that Sep-Pak[®] Vac, Vac RC, Plus, and Light cartridges can be used directly. Sep-Pak[®] Classic cartridges are used with a male/male adapter.



Sep-Pak[®] Vac RC cartridges can be used when you have volumes of sample or solvent to be processed that are larger than the capacity of the standard Sep-Pak[®] Vac cartridge tubes (up to 20 mL). Or, you can use a special adapter with the standard 1-cc, 3-cc, or 6-cc Sep-Pak[®] Vac cartridges to attach a reservoir, to connect two cartridges in series, or to connect a piece of tubing for drawing in larger sample volumes. Sep-Pak[®] Plus, Light, and Classic cartridges can be used on the vacuum manifolds either by attaching a reservoir directly to the cartridge, or by using a male/male adapter to connect to a piece of Teflon tubing which extends into a sample container.



For processing a small number of samples by vacuum, a Sep-Pak[®] Vac or Vac RC can be attached to a ring stand and an empty syringe with plunger can be used to draw the solvent or sample through the cartridge.



Steps of a Solid Phase Extraction Procedure

The following section describes the steps involved in a complete solid phase extraction procedure. In many applications, one or more of the steps, listed below and described here, can be omitted and the procedure simplified. Use the following explanation as a guideline in the development of your own procedure or when modifying literature procedures.

1. Pretreatment of the sample
2. Conditioning of the cartridge
3. Loading the sample
4. Elution of the fractions

1. Pretreatment of the sample

In many cases, the sample is in a solid form. Therefore, the first step in the pretreatment of the sample is either to dissolve or homogenize the solid, and extract the analyte in an appropriate solvent. Next, the sample has to be brought into a state that facilitates the adsorption of the analytes onto the solid phase extraction column. If, for example, the sample is dissolved in an organic solvent such as methanol or acetonitrile, and a reversed-phase method is to be used for sample cleanup, you can dilute the sample with water to promote the adsorption of the analytes onto the reversed-phase sorbent. Similarly, consider adjusting the pH of the sample to promote and control adsorption, if ionogenic compounds are involved. Unless the particulate load in the sample is exceptionally high, filtration is usually *not* necessary, since the Sep-Pak[®] cartridge itself contains filters. The pretreatment step may also include the addition of an internal standard for convenient quantitative analysis.

2. Conditioning of the cartridge

It is usually advisable to precondition the sorbent with the solvent used to load the sample. In some cases, this step can be omitted to streamline the process. However, you should carefully check the recovery of the analytes. In the case of reversed-phase sorbents, preconditioning of the sorbent with an organic solvent such as methanol, acetonitrile, isopropanol, or tetrahydrofuran is usually necessary to obtain reproducible results. Without this step, a highly aqueous solvent cannot penetrate the pores and wet the surface. Thus, only a small fraction of the surface area is available for interaction with the analyte. For the same reason, it is important not to let the Sep-Pak[®] cartridge dry out between the solvation step and the addition of the sample. A complete preconditioning of a reversed-phase cartridge includes the solvation step and an equilibration with a low-strength solvent such as water or buffer.

3. Loading the sample

The amount of sample that is applied should be controlled even if quantitation is not necessary or if an internal standard is used. Sample sizes must be scaled to suit the size of the cartridge bed and the separation mode and strategy to be employed. A typical normal- or reversed-phase Sep-Pak[®] Plus or Classic cartridge may have capacity for up to 100 mg of very strongly retained substances. Note that this quantity includes every substance that may be strongly retained in any given sample, not just the component(s) of interest. Less strongly held compounds may begin to elute before large volume samples have completely passed into the bed. Such breakthrough and capacity issues are described in more detail in File # [SP84177](#). Refer also to [Figure 1](#).

Similarly, the rate at which the sample is applied should be controlled. Appropriate flow rates must be used with Sep-Pak[®] cartridges so as to permit proper bed conditioning, sample loading, and elution, without the problem of incomplete equilibrium or sample breakthrough. In general, Sep-Pak[®] Plus or Classic cartridges may be conditioned at flow rates up to 25 mL/min. Sample loading and elution is best done at flow rates below 10 mL/min. Recovery may still be

adequate at flow rates up to 20 mL/min (testing should be done to verify this), but higher flow rates may not give acceptable results. For ion exchange applications, slower flow rates (1-2 mL/min) are recommended. For Sep-Pak[®] Light cartridges, which have smaller internal diameters, all the above flow rates should be reduced by a factor of three.

Keep in mind that it is actually linear velocity, μ , [flow rate/cross-sectional area of bed; e.g., 10 mL/min through a 1 cm diameter bed is equal to a linear velocity of 0.17 cm/sec] that is the important variable. Suggested flow rates are consistent with cartridge geometry. Thus, even higher volume flow rates may be used in very large diameter Sep-Pak[®] Vac cartridges, as long as the linear velocity is kept within the recommended range.

4. Elution of the fractions

In most cases, a first elution step removes sample constituents that are less retained on the sorbent than the analytes of interest. Subsequently, the analytes are eluted in a second step using a solvent of higher eluting strength while more strongly adsorbed sample constituents are left behind to be discarded with the sorbent. In both steps, the volumes and flow rates of the eluting solvents should be precisely controlled to ensure reproducible results.

In some cases, it may be desirable to perform a multi-step fractionation. In this case, the elution strength of the solvent is increased gradually in several steps. Step-wise elution is actually the procedure recommended during the development of a new solid phase extraction method to ensure that all analytes have been recovered quantitatively.

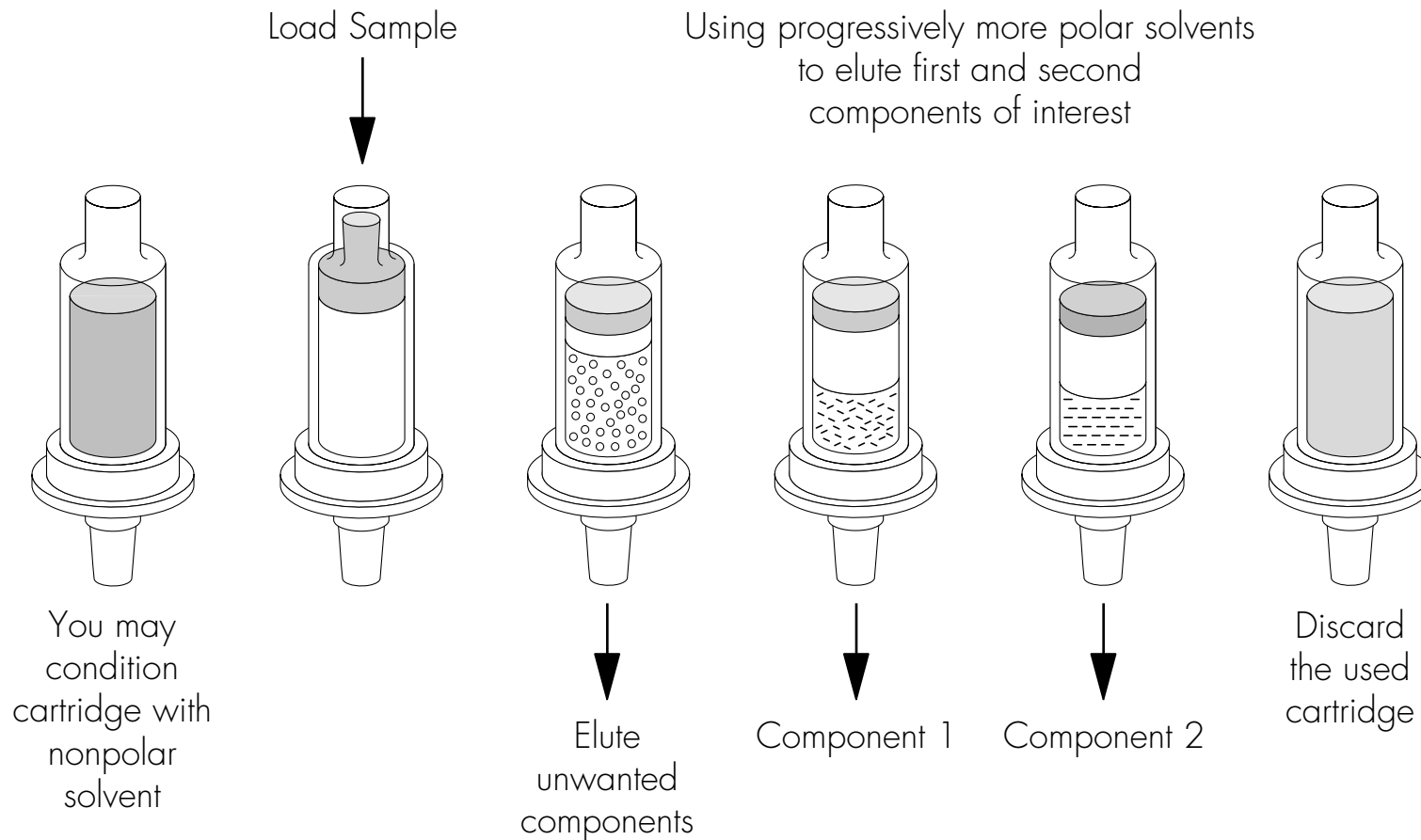
[Figures 2, 3, and 4](#) show general elution protocols for normal-phase, reversed-phase, and ion-exchange solid phase extraction. [Table 6](#) lists some guidelines for the various types of separation mechanisms.

Normal-Phase Chromatography

To perform normal-phase chromatography with Sep-Pak[®] cartridges, use a gradient of non-polar solvents with a polar packing material.

1. Condition the cartridge with six to ten hold-up volumes of non-polar solvent, usually the same solvent in which the sample is dissolved.
2. Load the sample solution into the cartridge.
3. Elute unwanted components with a non-polar solvent.
4. Elute the first component of interest with a more polar solvent.
5. Elute remaining components of interest with progressively more polar solvents.
6. When you recover all of your components, discard the used cartridge in an appropriate manner.

Figure 2. General Elution Protocol for Normal Phase Chromatography on Sep-Pak® Cartridges [Silica, Florisil, Alumina, Diol, NH₂]

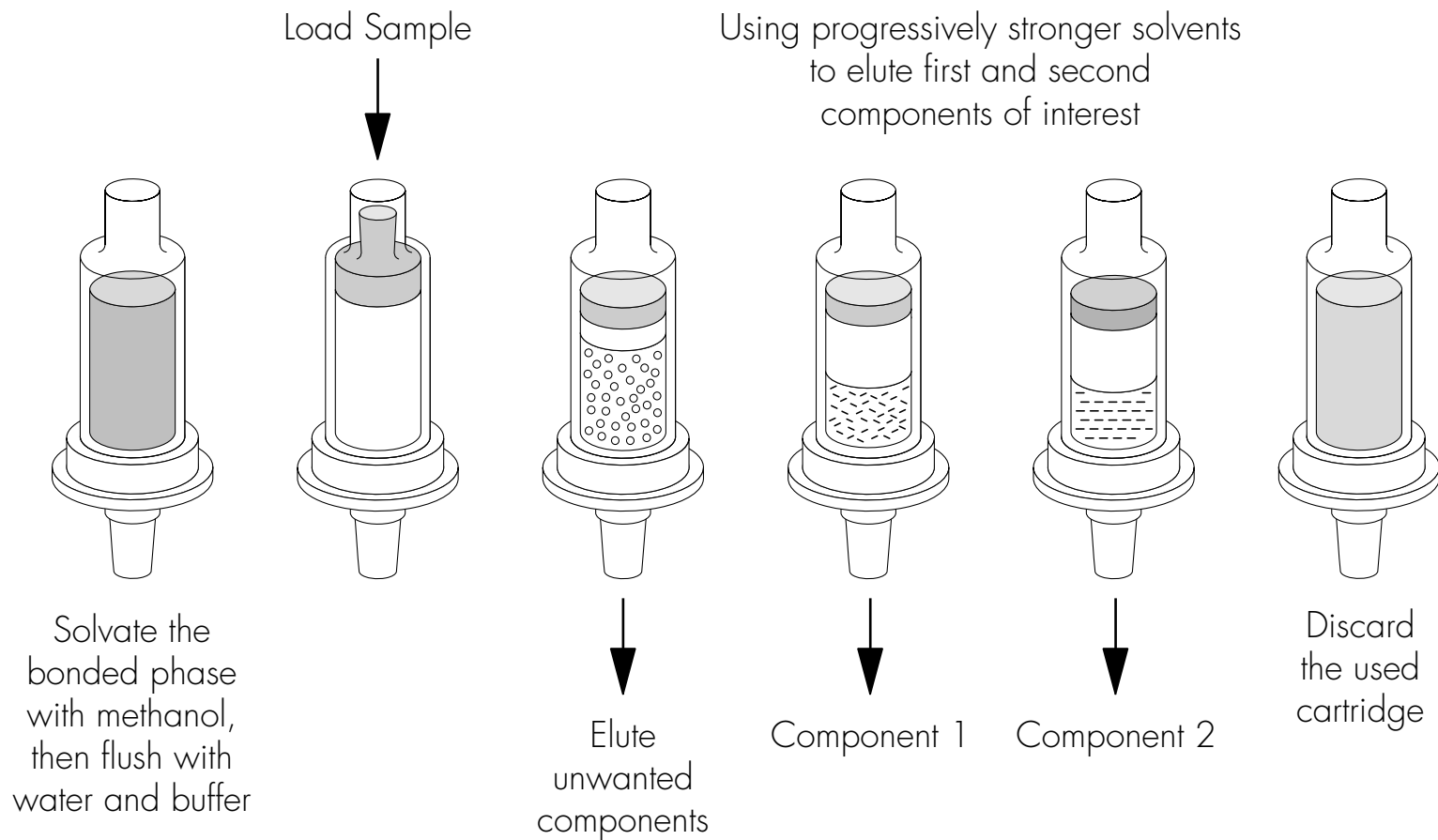


Reversed-Phase Chromatography

To perform reversed-phase chromatography with Sep-Pak[®] cartridges, use a gradient of strongly to weakly polar solvents with a non-polar packing material.

1. Solvate the bonded phase or polymer packing with six to ten hold-up volumes of methanol or acetonitrile. Flush the cartridge with six to ten hold-up volumes of water or buffer. Do not allow the cartridge to dry out.
2. Load the sample dissolved in a strongly polar solvent.
3. Elute unwanted components with a strongly polar solvent.
4. Elute weakly retained components of interest with a less polar solvent.
5. Elute more tightly bound components with progressively more non-polar solvents.
6. When you recover all of your components, discard the used cartridge in an appropriate manner.

Figure 3. General Elution Protocol for Reversed-Phase Chromatography on Sep-Pak® Cartridges [C₁₈, tC₁₈, C₈, C₂, CN, Diol, Porapak Rdx, NH₂]



Ion-Exchange Chromatography

To perform ion-exchange chromatography with Sep-Pak[®] cartridges, use a gradient of pH or ionic strength with an ion exchange packing material.

1. Condition the cartridge with six to ten hold-up volumes of deionized water or weak buffer.
2. Load the sample dissolved in a solution of deionized water or buffer.
3. Elute unwanted, weakly bound components with a weak buffer.
4. Elute the first component of interest with a stronger buffer (change the pH or ionic strength).
5. Elute other components with progressively stronger buffers.
6. When you recover all of your components, discard the used cartridge in an appropriate manner.

Figure 4. General Elution Protocol for Ion-Exchange Chromatography on Sep-Pak® Cartridges [NH₂, Accell Plus QMA, Accell Plus CM]

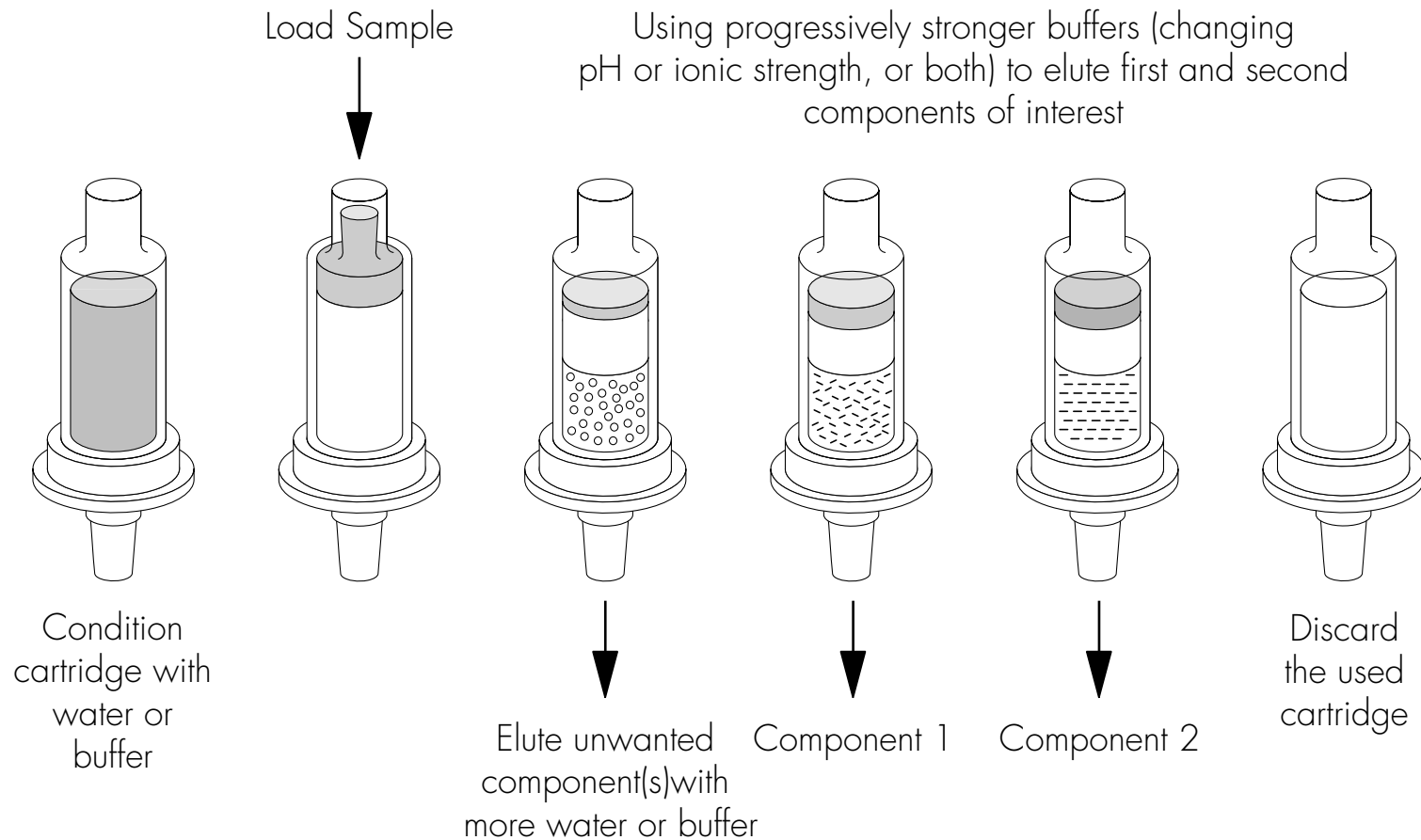


Table 6. Sep-Pak[®] Cartridge Separation Guidelines

Chromatographic Mode:	Normal-Phase	Reversed-Phase	Ion Exchange
	[Silica, Florisil, Alumina Diol, NH ₂ , CN]	[C ₁₈ , tC ₁₈ , C ₈ , Diol, Porapak [®] Rdx, NH ₂ , CN]	[Accell [™] Plus QMA, Accell [™] Plus CM, NH ₂]
Separation Characteristic:			
Packing Surface Polarity:	High	Low	High
Typical Solvent Polarity Range:	Low to Medium	High to Medium	High
Typical Sample Loading Solvent:	Hexane, Toluene, CH ₂ Cl ₂	H ₂ O, Buffers with low ionic strength	H ₂ O, Buffers
Typical Elution Solvent:	Ethyl acetate, acetone, CH ₃ CN	H ₂ O/CH ₃ OH, H ₂ O/CH ₃ CN	Buffers, salt solutions with high ionic strength
Sample Elution Order:	Least polar sample components first	Most polar sample components first	Most weakly ionized sample components first
Solvent Change Required to Elute Retained Compounds:	Increase solvent polarity	Decrease solvent polarity	Increase ionic strength or increase pH (AX) or decrease pH (CX)

Converting a Method from One Sep-Pak® Cartridge to Another

A method may have been originally developed on a Sep-Pak® Classic cartridge for a manual operation, but now needs to be converted to a Sep-Pak® Vac or Vac RC cartridge for automation. Or the amount of sample available dictates the use of a Sep-Pak® cartridge of a volume different from the one used originally. Whatever the reason, conversion of a protocol from one cartridge format to another is straightforward.

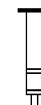
In all cases, the amount of sample and amounts of solvents used in the extraction process should be scaled in direct proportion to the amount of packing material in a cartridge. If you need to convert a method from a Sep-Pak® Classic to a Sep-Pak® Vac cartridge, you first need to know the amount of packing material in each cartridge. This information is found in [Tables 4 and 5](#).

The ratio of the respective sorbent weights in the cartridge you are converting *to* and the cartridge you are converting *from* is the conversion factor. Multiply all volumes in the original method by this conversion factor to obtain the volumes to be used in the new method.

Flow must also be scaled appropriately. For example, when converting to a Sep-Pak® Light cartridge, you should to reduce the appropriate flow rates by approximately three-fold to maintain the same *linear velocity*. (See the previous section: [3. Loading the Sample.](#))

Example:

Convert a method from:



Sep-Pak® C₁₈ Classic Cartridge

to

Sep-Pak® C₁₈ Vac 1-cc Cartridge

360 mg packing

100 mg packing

Conversion factor

$$100/360 = 0.3$$

1 mL sample

➤ reduce by 0.3x to

0.3 mL sample

3 mL wash buffer

➤ reduce by 0.3x to

0.9 mL wash buffer

1 mL elution solvent

➤ reduce by 0.3x to

0.3 mL elution solvent

On-Line Sample Preparation

The techniques of column liquid-solid extraction described earlier are performed *off-line* relative to the final analysis. This permits the greatest degree of flexibility in the use of various solvent combinations best suited to the isolation/cleanup/concentration/recovery protocol. When sample preparation is done prior to HPLC analysis and a less complex nature of the sample matrix and components of interest permit, it may be more convenient and time-efficient to perform the sample preparation *on-line*. In this mode, a miniature column sorbent bed is inserted in the fluid stream between the sample injection device and the analytical column. The use of one or more additional valves and pumps enables a more complex series of sample loading, solvent elution, and sample desorption steps to be performed. It is also possible to automate control of these devices for unattended operation of the entire sample prep/HPLC scheme.

Successful *on-line* sample prep requires careful consideration of the compatibility of sample prep and analytical column mobile phase solvents and of the retention characteristics of the various components in the sample matrix. Other considerations and precautions listed earlier for column liquid-solid extraction processes also must be heeded. To simplify the performance of *on-line* sample prep, Waters provides several innovative products. These are described in the Waters *Chromatography Columns and Supplies Catalog*; a PDF version may be accessed on [Waters website](#) or a printed copy of this catalog may be requested from the Waters office nearest to you.

Sample Preparation Educational Tools

Experiments

Those who teach chemistry may use Sep-Pak[®] cartridges to illustrate chromatographic separation techniques without making a capital investment in HPLC equipment (File #[SP84081](#)). Sep-Pak[®] cartridges also may be used in conjunction with TLC or HPLC techniques for student experiments (e.g., File #[SP79019](#), [SP79020](#), or [SP84083](#)). There are many Sep-Pak[®] cartridge applications that may be adapted for classroom demonstrations or laboratory experiments. For example, it would be interesting to see the FD&C dye separation protocol in File #[SP88183](#) or #[SP88190](#) developed into a student experiment using standards to identify unknown samples. A simple dye separation developed at Waters in 1978 has been used successfully at all grade levels from kindergarten through college (File #[SP90064](#)). A kit containing a grape drink mix, Sep-Pak[®] C₁₈ cartridges, and an experimental protocol is available from Waters.

Video

In addition, a new training video, *Experiments in Chromatography: Solid Phase Extraction Technology* (17 minutes), is also available from Waters. This videotape is designed for training laboratory personnel as well as chemistry students. This video tape includes information on:

- ❑ Chromatographic principles of solid phase extraction
- ❑ How to set-up and use Sep-Pak[®] cartridges
- ❑ Sample preparation strategies
 - Cleanup/purification
 - Fractionation
 - Trace enrichment/concentration
- ❑ Key steps in developing your application

A copy may be ordered from your nearest Waters office.

Part Numbers for Sep-Pak[®] Cartridge Accessories

Listed below are part numbers for Sep-Pak[®] cartridge accessories to help you with developing your Solid Phase Extraction application.

<i>Description</i>	<i>Part Number</i>
30-cc reservoir	WAT011390
60-cc reservoir	WAT024659
Reservoir adapter for 1-, 3-, 6-cc Sep-Pak [®] Vac cartridges	WAT054260
Male/male adapter	WAT024310
Male-Luer plugs	WAT044395
Female-Luer plugs	WAT044385

Training Video

NTSC format

WAT054295

PAL format

WAT054325

Chromatography Experiment Kit

WAT088253

(includes 2 Sep-Pak[®] C₁₈ cartridges,

1 package of grape beverage mix, and instructions)