Bulletin 781E

How to Protect Your HPLC Column and Prolong Its Life

Noneluting sample components accumulating in HPLC columns can alter a column's chromatographic properties. Large particles (>2µm) in mobile phases or samples accumulate on the frit at the column inlet, disrupting the uniform flow of samples onto the packing bed. Smaller particles enter the column and obstruct spaces in the packing bed, increasing back pressure. This bulletin describes sample and in-line filters, guard columns, and other protective devices an HPLC analyst should use to protect high performance analytical columns.

Key Words:
● guard columns ● HPLC column care ● mobile phase

Only materials that will be eluted by the mobile phase, within a specified time, should (ideally) be introduced onto an analytical HPLC column. Yet, samples, mobile phases, and even normal wear on the system can introduce particles and noneluting compounds onto a column. These materials degrade column performance.

To keep an analytical column performing at its best, protective devices must be incorporated into the HPLC system. This bulletin will first evaluate ways to protect a column from contaminants introduced by the system itself (including the mobile phase), then consider those that originate in the sample.

Routine Operation of an HPLC System Can Introduce Impurities Onto the Column

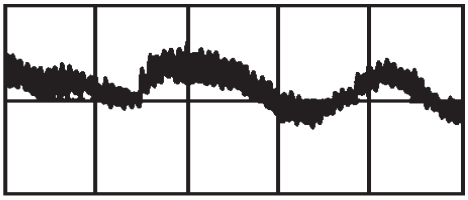
Particles and Dissolved Air in the Mobile Phase

Particles in the mobile phase can accumulate in the column, increasing back pressure. They can also damage pump seals and check valves. Consequently, only HPLC grade solvents and reagents should be used, and all water and buffers should be filtered through a 0.45µm filter (as shown in Apparatus 1 or 2 on page 7) before they are introduced into the system reservoir. If the system can be used to mix mobile phases from components in two or more reservoirs, you should determine in advance that the components will not form emulsions or precipitates on mixing. Furthermore, air might be more soluble in the separate liquids than in the mixture. If so, gas bubbles can form as the components mix, or after the mobile phase has passed through the column and is no longer under pressure. In the detector, bubbles increase noise or cause spikes in the signal. Figure A clearly indicates the importance of filtering and degassing mobile phases. Both air and particles can be conveniently removed from the mobile phase by passing the material through a 0.45µm filter into a vacuum flask.

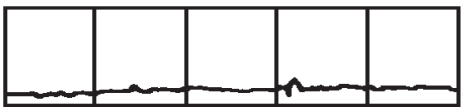
Figure A. Filtering the Mobile Phase Reduces Baseline Noise

Column:	**SUPELCOSIL LC-8, 15cm x 4.6mm, 5µm particles**
Cat. No.:	**58220-U**
Temp.:	30°C
Mobile Phase:	methanol:water (50:50) (v/v)
Flow Rate:	2.5mL/min
Det.:	254nm UV, 0.005 AUFS

A1 — Without Filtration



A2 — With Filtration



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A filtered, degassed mobile phase may reacquire particles from the air, through interaction with the reservoir, or through slow crystallization of its components. Thus, the mobile phase should be refiltered immediately before it passes through the pump. A typical mobile phase filter is a porous stainless steel element attached to the reservoir end of the tube between the mobile phase reservoir and the pump inlet (suction side). Filters designed for this purpose exclude most particles larger than 2µm and can be cleaned by periodic backflushing. Examples of these filters appear on page 8.

High pH Mobile Phases

Silica-based HPLC columns generally are most stable in the pH range of 2-7. More strongly acidic mobile phases may remove a bonded phase from its silica base, whereas alkaline mobile phases tend to dissolve silica. Contact between the mobile phase and the silica is minimized if the bonded phase uniformly coats the silica surface. When an alkaline mobile phase must be used, the analytical column may be further protected by saturating the mobile phase with silica (1,2). This is accomplished by inserting a 7.5cm x 4.6mm precolumn filled with porous silica particles (18µm diameter, 500m^2/g surface area) between the pump and the sample injection valve. Silica saturator kits are found on page 12.

Particles from the Injection Valve and Pump Seals

Normal wear of the injection (sampling) valve rotor seal and pump seals generates particles of various sizes. Small particles that pass through the pores in the column inlet frit will eventually clog the packing bed, causing high back pressures. Such material should be trapped in a guard column (detailed later in this bulletin.) Particles larger than the pores accumulate on the frit, causing symptoms that mimic column failure.

SUPELCOSIL™ HPLC columns filled with 3µm and 5µm packings have 0.5µm and 2µm frits, respectively, to confine the packing bed. The column end fittings are designed to distribute the stream over the entire frit. However, in extended experiments we have found that large particles derived from the injection valve rotor seal accumulate on the center of the inlet frit. Back pressure does not always increase when the frit is partially blocked, but the uniform distribution of samples onto the top of the column bed is disrupted. As a result, peak asymmetry increases and column efficiency is reduced.

The effects of partially obstructing the inlet frit are illustrated in Figure B. Figure B1 shows the sharpness and symmetry of a toluene peak eluted from a new SUPELCOSIL LC-18 column. After 3000 simulated sample injections, the shape of the peak had deteriorated (Figure B2), suggesting that column efficiency had been reduced. The column was then removed from the system and reversed. The former column outlet was connected to the injection valve, and the column was briefly flushed with mobile phase at a high flow rate. The column was not connected to the detector during this operation, to prevent accumulated particles on the frit from being flushed into the detector. Subsequently, the column was reinstalled in its original orientation. Injection of a test sample revealed (Figure B3) that much of the peak distortion evident in Figure B2 was due to disruption of the mobile phase flow profile, not to column damage *per se.*

Any well-packed column (i.e., one having ≥70,000 plates/meter for a 5µm packing or ≥120,000 plates/meter for a 3µm packing) should be operable with flow in either direction. We recommend periodically reversing the flow direction to keep the frits clean. This procedure is preferable to changing frits because the top of the column bed can be damaged during frit replacement.

Particles larger than 2µm can be prevented from reaching the frit in the first place by introducing a zero dead volume filter with a replaceable screen between the sample injection valve and the column inlet. As shown in Table 1, a zero dead volume filter does not detract from column efficiency, as measured in terms of N (theoretical plates) or AF (asymmetry at 10% of peak height, calculated for toluene in our standard reversed phase test mixture). Furthermore, it is far easier to change a filter or a screen than it is to backflush a column. Filters for this purpose are described on the products pages of this bulletin.

Figure B. Large Particles Obstructing the Column Inlet Frit Can Imitate Column Deterioration

Column: **SUPELCOSIL LC-18, 15cm x 4.6mm, 5µm particles**
Cat. No.: **58230-U**
Temp.: 30°C
Mobile Phase: methanol:water (66:34) (v/v)
Flow Rate: 2mL/min
Sample: 10µL of test mixture (Cat. No. **58278**) for Figures B1, B2, and B3; 3000 injections of 500µL mobile phase (60 injections/min. at 2mL/min., at 60°C) between Figures B1 and B2

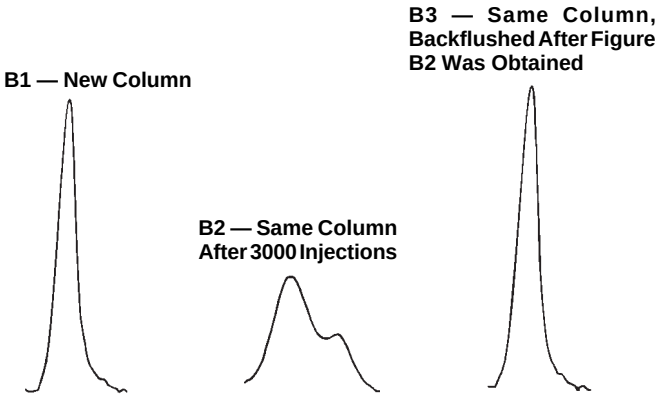

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Table 1. A Replaceable Screen Filter Does Not Affect Column Efficiency (N) and Peak Asymmetry (AF) as Functions of Flow Rate▲

	Column Alone		Column + Filter	
Flow Rate	N△	AF■	N	AF
1mL/min	10340	1.00	10525	0.98
2mL/min	8320	1.01	8310	1.01
3mL/min	6885	0.97	6740	1.03

▲ Mean for three measurements

△ N = theoretical plates = $5.54 \left(\frac{t_R}{W}\right)^2$, where t_R = retention time and w = peak width at 50% of peak height.

■ Asymmetry factor for toluene (width of right-hand part of peak/width of left-hand part of peak at 10% of peak height.

Pressure Surges

Routine operation of a sample injection valve produces transient interruptions in flow to the column. Pressure falls at the head of the column after flow is interrupted, then surges when the flow is reinstated. When the column is operated at relatively high flow rates and pressures, and with a slow injection valve (0.5-1 sec. switching time), repeated surges can eventually cause disruptions in the packing bed. This potential problem can be avoided by installing a Rheodyne model 7725 or similar valve, which incorporates Rheodyne's patented pressure relief technology. Alternatively, you can use a fast, automatic switching valve (<0.1 seconds).

Figure C. Refillable 5cm Guard Column and Connecting Hardware

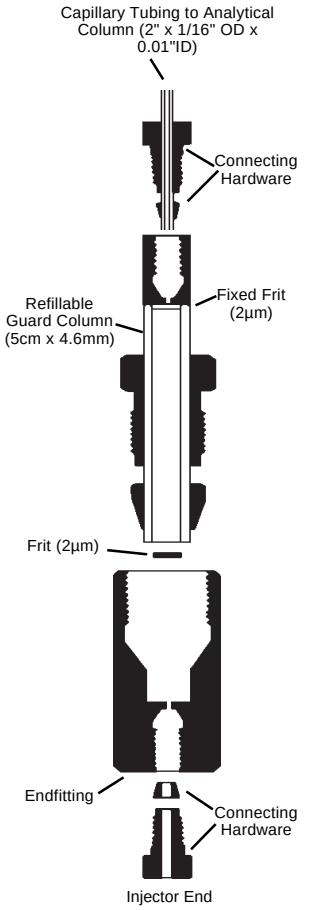


Figure D. Disposable 2cm Cartridge Guard Column, Cartridge Holder, and Connecting Hardware

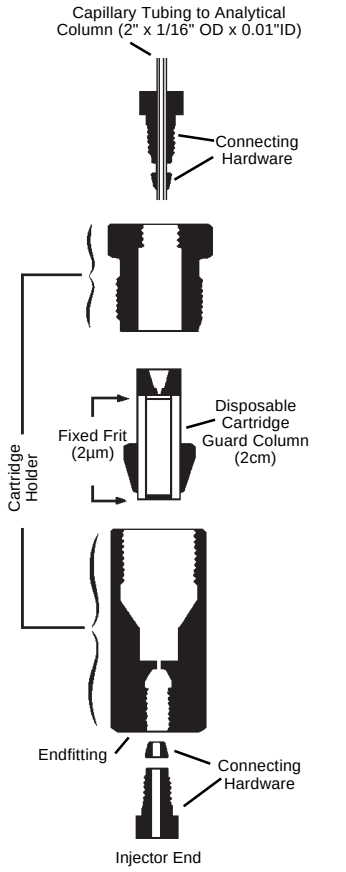


Table 2. Efficiency of an HPLC Column without and with a 5cm Guard Column Filled with a Pellicular Packing

Analytical Column	Column Efficiency (plates/15cm)[O]		
	Without Guard Column	With Guard Column	Difference
SUPELCOSIL LC-8	11,020	10,580	-4%
SUPELCOSIL LC-18	11,530	10,850	-6%

Column: **SUPELCOSIL LC-8 (58220-U) or LC-18 (58230-U), 15cm x 4.6mm, 5µm particles, with Pelliguard™ LC-8 (59643) or LC-18 (59644), 5cm x 4.6mm, 40µm packing**
Temp.: ambient
Mobile Phase: methanol:water (60:40) (for LC-8) or (66:34) (for LC-18)
Flow Rate: 1mL/min
Sample: 10µL test mix (Cat. No. **58278**)

[O] Calculated for toluene (k' approximately 3.0).

The Sample as a Contributor to Column Damage

Particles Larger Than 2µm

Measures to protect the analytical column from particles include carefully cleaning the samples through filtration, solid phase extraction (SPE), or other procedures. Sometimes, organic solvents are used to extract a sample from the original material, after which the sample is subjected to centrifugation to remove particles. Often the sample volume is small, and this complicates particle removal. SPE generally gives cleaner samples than comparable liquid-liquid extractions. The extracts, however, may need to be filtered to remove traces of the SPE packing. A small volume, disposable 2µm filter conveniently traps particles in samples obtained by either method. The filters can be used with samples from less than a milliliter to several milliliters in volume. Disposable 0.2µm or 0.45µm pore disk-type filters are useful for removing fine impurities from samples. This type of filter usually has a void volume of 0.1mL, so large samples are needed.

Late-Eluting and Noneluting Sample Components

To minimize run time, analysts prefer to have sample components elute from the column at a k'[❑] of between 1 and 10 (i.e., t_R for the last compound of interest = 2-11 x t_0). If k' for an analyte is less than 1, the peak usually will be too close to the solvent front (t_0 peak), and if k' is greater than 10, the analysis time is too long.

Very rapidly eluted compounds (k'< 0.5) that are not important usually have no undesirable effects on the analysis. On the other hand, late-eluting materials from one sample may appear as broad peaks or baseline drift in subsequent analyses. To minimize this problem, you can use gradient analysis or periodically flush the column with a stronger solvent, preferably with the direction of flow reversed. Alternatively, you can use a guard column to isolate undesirable sample fractions, via a column switching procedure described later in this bulletin.

Noneluting materials progressively cover the surface of the packing at the column inlet, decreasing sample retention and capacity. These changes may eventually alter the chromatographic properties of the packing. For purposes of column protection, small (<2µm) particles and noneluting sample components can be considered together. These materials, like late eluters, can be flushed from the column by reversing the direction of flow and rinsing with a strong solvent. It is more convenient to trap all of these materials on a guard column. Either the entire guard column or the packing can be changed periodically.

[❑] $k' = (t_R - t_0)/t_0$, where t_0 = elution time for an unretained peak and t_R = elution time for the peak of interest.

Guard Columns as Protective Devices

Guard Columns with Pellicular Packings

High pressure equipment and advanced technology are required for filling analytical or guard columns with 3 and 5µm packings. Consequently, analysts have had a strong incentive to prepare guard columns from pellicular (38-44µm) packings. Such materials can be poured in dry form into a guard column blank, then vibrated and tapped into a densely packed bed. Guard columns filled with pellicular packings are available commercially as refillable 5cm x 4.6mm columns and disposable 2cm x 4.6mm cartridges.

Refillable 5cm guard columns, filled with pellicular packings, have been widely used and are suitable for many purposes. A refillable guard column has a fixed frit at the end facing the analytical column and a removable frit at the end facing the injector. The guard column is connected to the analytical column, as illustrated in Figure C. When the guard column becomes contaminated, it is usually sufficient to remove only the top 2-5mm of packing and add fresh material. By maintaining an inventory of interchangeable guard columns, you can reduce down time when the guard column in the system must be cleaned and refilled. A drawback to using guard columns with pellicular packings is the accompanying small (about 5%) loss in apparent theoretical plates. This loss is brought about by the large size of the particles and by the extra connections and fittings required to add the guard column to the system (Table 2). A test sample should be analyzed before and after any guard column is installed in the system, to ensure that performance remains within acceptable limits.

In many cases disposable cartridge-type guard columns (Figure D) are more convenient and economical to use than refillable guard columns. Disposable 2cm x 4.6mm cartridge-type guard columns filled with a pellicular packing minimize the loss of operating time during replacement. These 2cm columns, like the 5cm refillable columns with pellicular packings, reduce the number of theoretical plates observed by about 5% (Table 3). Peak asymmetry also increases slightly. These effects are independent of flow rate.

Table 3. Effect of a 2cm Pellicular Guard Column on Analytical Column Efficiency and Peak Symmetry

	Column Alone		Column + Guard Column	
Flow Rate	**N**	**AF**	**N**	**AF**
1mL/min	10525	0.98	10035	1.11
2mL/min	8310	1.01	7830	1.11
3mL/min	6740	1.03	6650	1.10

Column: **SUPELCOSIL LC-8 (58220-U), 15cm x 4.6mm, 5µm particles, with Pelliguard™ LC-8 (59643), 5cm x 4.6mm, 40µm packing**
Temp.: ambient
Flow Rate: 1, 2, or 3mL/min
Mobile Phase: methanol:water (60:40)
Sample: 10µL test mix (Cat. No. **58278**)
Data represent mean for 3 measurements.

Table 4. Effects of a Pellicular Guard Column on t_0,Capacity Factors (k'), and Selectivity (α) of an Analytical Column

Parameter	Analytical column	Analytical Column + Guard Column
t_0 Uracil (min.)	1.67	1.85
k' Acetophenone	0.80	0.73
k' Benzene	1.59	1.45
k' Tolune	2.85	2.61
α (Benzene/ Acetophe none)	1.98	1.99
α (TolueneBenzene)	1.80	1.80

Column: **SUPELCOSIL LC-8 (58220-U), 15cm x 4.6mm, 5µm particles, with Pelliguard™ LC-8 (59643), 5cm x 4.6mm, 40µm packing**
Temp.: 30°C
Mobile Phase: methanol:water (60:40)
Flow Rate: 1mL/min.
Sample: 10µL test mixture (Cat. No. **58278**)

Because the primary function of a guard column is to retain materials that the mobile phase will not elute from the analytical column, the retention characteristics of the analytical and guard column packings should be similar (i.e., match silica with silica or octadecyl bonded phase with octadecyl bonded phase). However, a 38-44µm pellicular packing has only 1/100th of the surface area a porous 5µm analytical packing has, making the capacity of the pellicular packing much smaller than that of the analytical packing. Consequently, all sample components transverse the guard column in essentially equal time. Coupling a 2cm pellicular guard column to a 15cm analytical column increases t_0 and the elution times for retained compounds almost equally. As a result, k' for retained compounds decreases, but selectivity or separation factor ($\alpha = k'_2/k'_1$) is unaffected (Table 4 and Figure E). Because a guard column with a pellicular packing has little or no effect on analyte retention, the chromatographic properties of the guard column and analytical column packings need not match perfectly.

Guard Columns with Five Micron Packings

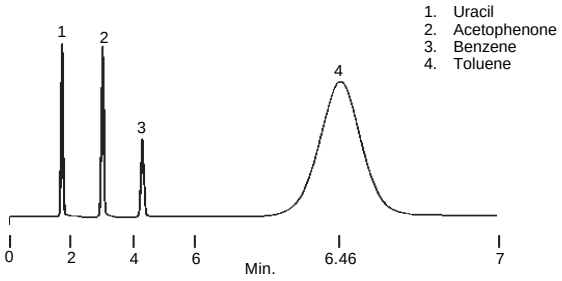
Guard columns filled with 5µm packings offer distinct advantages, compared to those filled with pellicular packings. For instance, a perfect match can be obtained with the analytical packing, and the sample-resolving capability of the system is enhanced. When a 2cm disposable, cartridge-type guard column with a 5µm packing is used, the theoretical plate loss attributed to guard column connections and fittings is more than offset by the theoretical plates the packing contributes (Table 5 and reference 3).

As is the case with pellicular guard columns, a small increase in peak asymmetry might be observed when adding a 5µm packing guard column to the system. Operating pressure will increase in proportion to the increase in total column length, i.e., 17/15 or 27/25, respectively, for a 15 or 25cm analytical column with a 2cm guard column. Since t_0 and t_R for retained compounds increase proportionately (Figure F), there will be no observable change in k' and α values. Poorly packed or improperly connected guard columns, however, reduce column efficiency and increase peak asymmetry (4).

Figure E. A Guard Column Containing a Pellicular Packing Has Little Effect on Analyte Retention

Column: **SUPELCOSIL LC-8 (58220-U) , 15cm x 4.6mm, 5µm particles (Figure E1), with Pelliguard LC-8 guard column (59643) , 2cm x 4.6mm, 40µm packing (Figure E2)**
Temp.: ambient
Mobile Phase: methanol:water (60:40)
Flow Rate: 1mL/min
Chart Speed: 1cm/min for 6 min., then 10cm/min
Sample: 10µL of test mixture (Cat. No. **58278**)

E1 — Analytical Column



E2 — Analytical Column plus Pellicular Guard Column

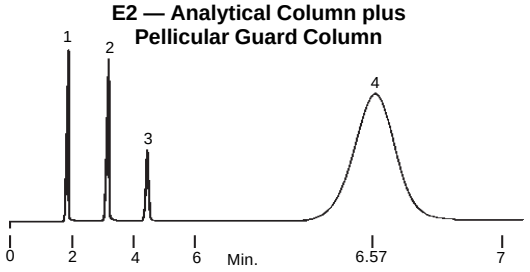


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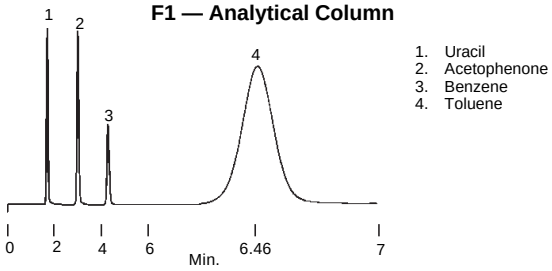
Figure F. A Guard Column Containing a 5µm Packing Increases t_0 and t_R Proportionately

Column: **SUPELCOSIL LC-8 (58220-U), 15cm x 4.6mm, 5µm particles (Figure F1), with Supelguard™ LC-8 guard column (59554), 2cm , 5µm packing (Figure F2)**
Temp.: ambient
Mobile Phase: methanol:water (60:40)
Flow Rate: 1mL/min
Chart Speed: 1cm/min for 6min., then to 10cm/min
Sample: 10µL of test mixture (Cat. No. **58278**)

F1 — Analytical Column



F2 — Analytical Column plus 5µm Guard Column

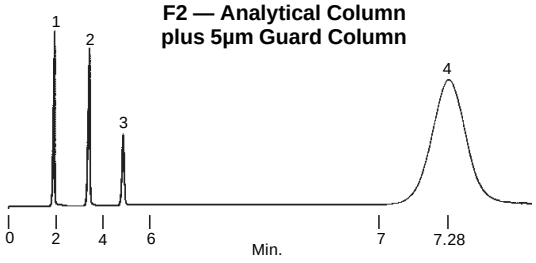


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Table 5. Effects of a 2cm Guard Column on Efficiency and Peak Symmetry of an Analytical Column Filled with the Same 5µm Packing

	Column Alone		Column + Guard Column	
Flow Rate	**N**	**AF**	**N**	**AF**
1mL/min	10525	0.98	10785	1.15
2mL/min	8310	1.01	8850	1.13
3mL/min	6740	1.03	7245	1.16

Column: **SUPELCOSIL LC-8 (58220-U), 15cm x 4.6mm, 5µm particles, with Supelguard LC-8 guard column (59562), 2cm, 5µm packing**
Temp.: ambient
Flow Rate: 1, 2, or 3mL/min
Mobile Phase: methanol:water (60:40)
Sample: 10µL test mix (Cat. No. **58278**)
Data represent mean for 3 measurements.

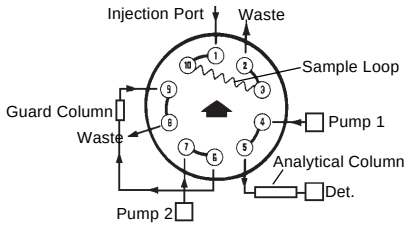
Our experience shows that extra-column effects and dead volumes introduced by pellicular packing guard columns, although small, reduce the efficiency of an analytical column with a 3µm packing. For this reason, only 5µm guard columns should be used with 3µm analytical columns. Basic chromatographic principles explain the problem. The height equivalent of a theoretical plate (HETP) varies with particle diameter, whereas operating pressure varies with the inverse of the square of the particle diameter. Compared to a 5µm packing, therefore, a 3µm packing offers a 1.67 fold (5/3) increase in plates at a 2.78 fold ($5^2/3^2$) increase in operating pressure. Pressure on 3µm packings is usually kept within acceptable limits by restricting analytical column length to 7.5 or 15cm. The higher efficiency of columns filled with 3µm packings, combined with short column length, produce smaller peak volumes for sample components than are obtained from columns filled with 5µm packings. Thus, factors peripheral to the column, such as those contributed by a guard column, have a greater effect on the efficiency of columns with 3µm packings.

In addition, 0.5µm frits are used to confine 3µm packings, so the guard column must efficiently retain small (<2µm) particles that otherwise will obstruct the inlet frit. Pellicular packings cannot do this. In contrast, only a small reduction in theoretical plates and increase in peak asymmetry are observed when a 2cm x 4mm disposable guard column with a 5µm packing is used in conjunction with a 7.5cm x 4.6mm, 3µm packing analytical column.

Supelco HPLC guard columns are available in internal diameters of 2.1mm, 3.0mm, and 4.0mm. The 4.0mm ID guards are designed to protect analytical columns of 4.0 and 4.6mm ID.

Figure G. A Sampling Valve Two Pump System for Backflushing a Guard Column

G1 — Fill Sample Loop



G2 — Inject Sample Onto Analytical Column

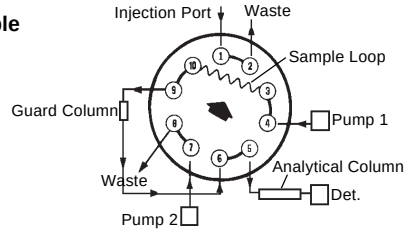


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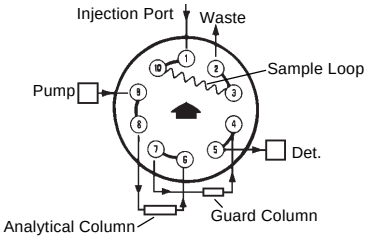
Figure H. A Sampling Valve Single-Pump System for Backflushing a Guard Column

H1 — Fill Sample Loop



H2 — Inject Sample Onto Analytical Column

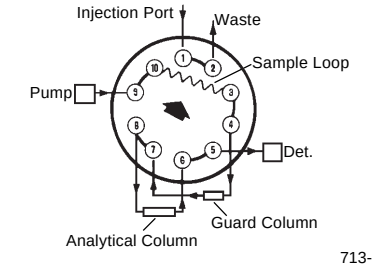


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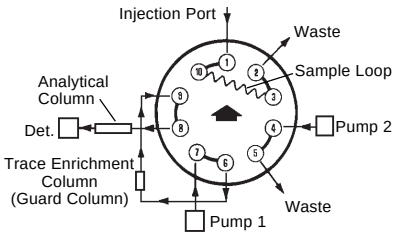
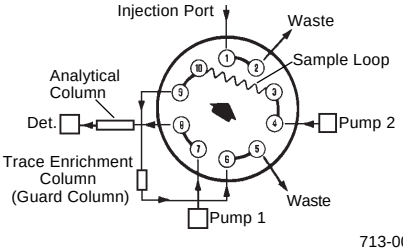
Figure I. A Sample Enrichment System Employing a Sampling Valve and a Guard Column

I1 — Fill Sample Loop (previous sample moves from enrichment column to analytical column)



I2 —Inject Sample Onto Enrichment Column



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Using Multiport Valves in Conjunction with Guard Columns

Multiport sampling valves are used to make and break connections between two or more columns and a detector. These valves are equally valuable for backflushing a guard column (to clean it) without manually disconnecting it from the analytical column.

A 10-port sampling valve and an auxiliary high pressure pump, incorporated into the HPLC system along with a guard column, conveniently allows you to protect the analytical column and prolong its useful life. The complete system is illustrated in Figure G. A sample is injected into the sample loop (Figure G1), then the valve is switched so that the sample moves through the guard column into the analytical column (Figure G2). Once the analytes of interest have entered the analytical column, the valve is switched again, isolating the guard column from the system (Figure G1). While the analysis continues, the guard column is backflushed by the second pump. In a variation of this procedure, a specific fraction of the sample is directed from the guard column to the analytical column, while the early portion of the eluant is vented to waste. Similarly, a 10-port valve and one pump can be set up to clean the guard column, but in this system material flushed from the guard column is voided through the detector (Figure H).

A system that includes a guard column (5µm porous packing), a 10-port valve, and two pumps can also be used to concentrate trace analytes (5,6). Generally , the sample is flushed from the sample loop onto the guard column in a weak mobile phase (e.g., water, in reversed phase HPLC). Compounds of interest are retained on the guard column, while less retentive materials are eluted to waste. When the valve is switched, the second pump flushes the analytes from the guard column onto the analytical column, in a stronger mobile phase. The system is illustrated in Figure I. In this application, the guard column does not protect the analytical column from particles and noneluting sample components. A second guard column should be used for this purpose.

When very complex samples must be analyzed, a guard column can be used to separate compounds by classes for more discriminate separations on the analytical column. In this situation, a sampling valve is used to sequentially apply the peaks from the guard column to a single analytical column, or to divert them to different columns.

Additional uses for multiport sampling valves are described in references 6-8. In all of these procedures the underlying goal remains the same. So far as is practical, only analytes that will be eluted by the specific mobile phase, in an appropriate timespan, should be allowed to enter the analytical column.

References

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2. Atwood, J.G., G.J. Schmidt, and W. Slavin, J. Chromatogr., *171*, 109 (1979).
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References not available from Supelco.

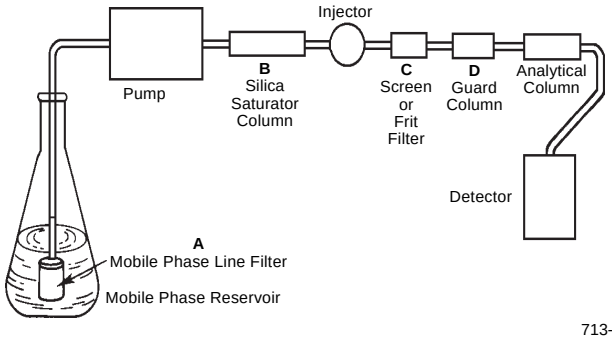
Rheodyne® multi-port sampling valves are available from Supelco in manual, electrical, or air-actuated designs.

Protection for Your HPLC Columns

Your HPLC column is valuable! To maintain highest column efficiency you should (refer to Figure J):

- Filter all samples before injection
- Filter and de-gas solutions used to prepare mobile phases
- Use a filter (A) in the mobile phase inlet line
- Use pH 2-7 mobile phases with silica-based packings, OR use a saturator column (B) for higher pHs
- Use a screen or frit filter (C) after the injector
- Use a guard column (D) between the filter and the analytical column
- Reverse the analytical column routinely to remove particles from the inlet frit

Figure J. Complete Protection for Your HPLC Column



713-0031

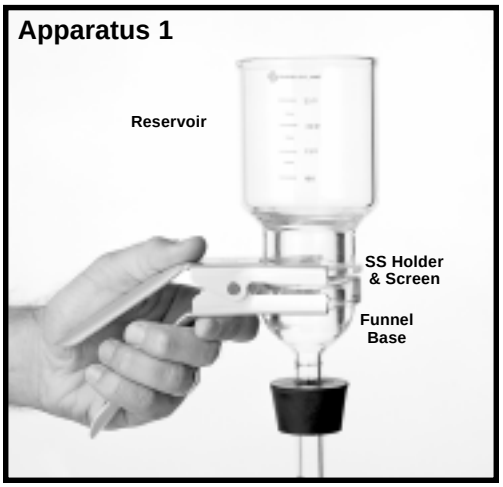
Ordering Information:

Supelco Solvent Filtration Apparatus

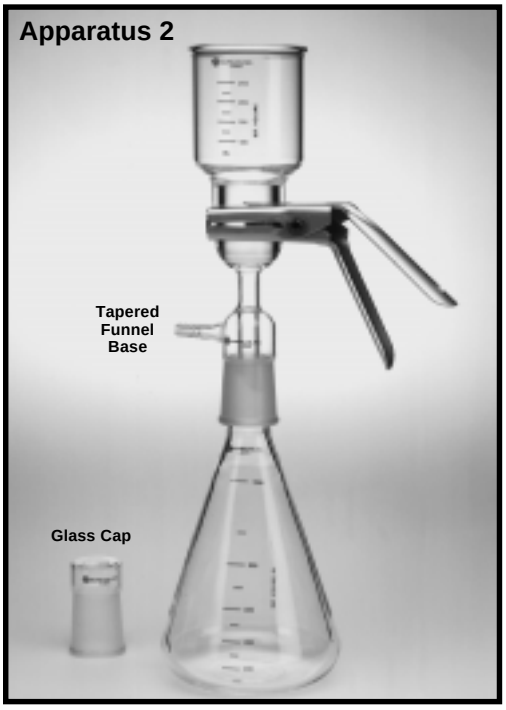
To protect your instrument and columns, remove particles and gases from solvents, buffer solutions, ion pairing reagents, and other mobile phase components. Apparatus 1 can be connected to a 1000mL side-arm flask for vacuum filtration. Apparatus 2 can be connected to a vacuum line and includes a tapered top (⅋34/45) 1000mL receiving flask. Nylon-66 membrane filters (0.45µm) used in either apparatus are compatible with all solvents commonly used in HPLC.

For other options, refer to the current Supelco catalog.

Description	Cat. No.
Filtration Apparatus 1 (250mL glass reservoir, funnel base and stopper, clamp, SS holder and screen, Teflon® gasket, 50 Nylon-66 filters)	**58061**
Replacement Parts:	
Glass Reservoir, 250mL	**58063**
Funnel Base and Stopper	**58064**
SS Filter Holder and Screen	**58065**
Teflon Gaskets (pk. of 10)	**58066**
Nylon-66 Membranes, 0.45µm (pk. of 50)	**58067**
Filtration Apparatus 2 (250mL glass reservoir, ⅋34/45 tapered funnel base, ⅋34/45 tapered-top 1000mL flask and glass cap, clamp, SS holder and screen, Teflon gasket, 50 Nylon-66 filters)	**58062-U**
Replacement Parts:	
Glass Reservoir, 250mL	**58063**
Tapered Funnel Base, ⅋34/45	**58068**
Tapered-Top Flask, ⅋34/45, 1000mL	**58070-U**
Glass Cap for Flask	**58071**
SS Filter Holder and Screen	**58065**
Teflon Gaskets (pk. of 10)	**58066**
Nylon-66 Membranes, 0.45µm (pk. of 50)	**58067**



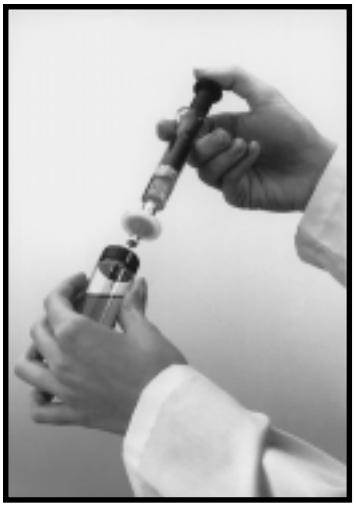
910-0549



910-0113

Iso-Disc™ Syringe-Tip Filter Units for Organic or Aqueous Solvents and Gases

- Unique angled thread on luer fitting provides a more reliable connection than filters with tabs
- No seams to fail under pressure
- Sample cannot bypass the membrane
- Solvent-resistant, autoclavable membranes and housings
- Extractable-free construction



910-0118

Specifications

Housing:	polypropylene
Membrane:	nylon - use with aqueous solvents PTFE - for gases or with organic solvents cellulose acetate - use with aqueous solvents
Pressure rating:	3mm - 60psi 25mm - 75psi
Process volume :	3mm - ≤2mL 25mm - 2mL to 100mL
Holdup volume:	3mm - <10µL (with air purge) 25mm - <60µL (with air purge)
Connections:	female luer lock inlet male luer taper outlet
Sterilization:	compatible with ethylene oxide, autoclavable

Iso-Disc Syringe-Tip Filter Units

Iso-Disc Filter Unit	Membrane	Diameter (mm)	Pore Size (µm)	Color Coding	Quantity	Cat. No.
N-254	Nylon	25	0.45	green	50	**59230-U**
N-252	Nylon	25	0.2	purple	50	**59231-U**
N-34	Nylon	3	0.45	green	100	**59238**
N-32	Nylon	3	0.2	purple	100	**59239**
P-255	PTFE	25	5.0	gray	50	**59244**
P-254	PTFE	25	0.45	yellow	50	**59234-U**
P-252	PTFE	25	0.2	blue	50	**59235-U**
P-34	PTFE	3	0.45	yellow	100	**59240-U**
P-32	PTFE	3	0.2	blue	100	**59241**
CA-254	Cellulose acetate	25	0.45	red	50	**59242**
CA-252	Cellulose acetate	25	0.2	white	50	**59243**

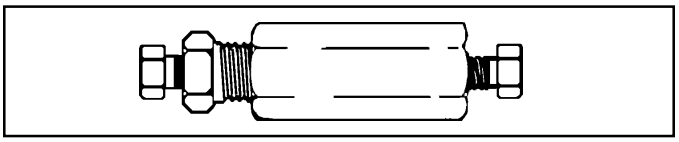
Mobile Phase Filter

910-0114

Protect your expensive HPLC column and pumping system against particles in your solvents. Attach a low pressure mobile phase filter to the reservoir end of the tube carrying mobile phase to the suction side of the pump. Stainless steel filter with a 2µm porous filter element. Easily cleansed by backflushing.

Description	Cat. No.
Mobile Phase Filter, 1/8" Swagelok® Tube Connection	**58268**

Valco Frit and Screen Filters*



713-0561

Efficient, low dead volume in-line filters protect your columns from particles without reducing column performance. The replaceable 1/8" frit has 0.5µm pores to protect 3µm or 5µm column packings. Replaceable screen has 2µm pores. Choose the frit filter for higher filtration capacity (most applications) or the screen filter for less dead volume (e.g., with microbore columns).

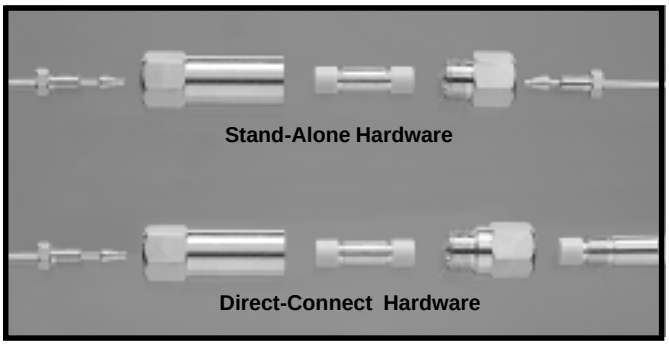
1/16" fittings included. Use with 1/16" OD tubing.

Description	Cat. No.
Frit Filter	**58420-U**
Replacement Frits (pk. of 10)	
0.5µm pores	**59037**
2.0µm pores	**59129**
Screen Filter	**58279-U**
Replacement Screens (pk. of 10)	**58284**

***Note:** Frits and screens should not be interchanged in these filters.

Guard Cartridges for Discovery, SUPELCOSIL, and SUPELCOGEL Columns

Supelguard guard column cartridges contain a Discovery™, SUPELCOSIL, or SUPELCOGEL™ packing, in a 2cm stainless steel body, enclosed by PEEK encapsulated stainless steel frits (2µm porosity). A **Supelguard kit** (one cartridge, a stand-alone holder, tubing, and 2 nuts and ferrules) enables you to use the cartridge with any analytical column. A **direct-connect guard cartridge holder** can directly connect a Supelguard cartridge to a Supelco analytical column. 3.0mm cartridges are available in packs of two only – purchase a stand-alone or modular holder separately. Low volume 3.0mm ID cartridges are a good choice for protecting an analytical column containing 3µm particles.



897-0038

2cm Supelguard Cartridges with 5µm Discovery, SUPELCOSIL, or SUPELCOGEL Packings

Supelguard Phase	Use To Protect	4.0mm ID Cartridge[1] Kit Cat. No.	4.0mm ID Cartridge[1] Pk. of 2 Cat. No.	3.0mm ID Cartridge Pk. of 2 Cat. No.	2.1mm ID Cartridge Kit Cat. No.	2.1mm ID Cartridge Pk. of 2 Cat. No.
Discovery RP-AmideC16	Discovery RP-AmideC16	**505080**	**505099**	**custom**	**505102**	**505110**
ABZ⁺Plus	ABZ⁺Plus	**59534-U**	**59535-U**	**59535-C30**	**59604**	**59605**
LC-ABZ	LC-ABZ	**59544-U**	**59545-U**	**59545-C30**	**59610**	**59611**
Suplex™ pKb-100	Suplex pKb-100	**59531-U**	**59541-U**	**59541-C30**	**59608**	**59609**
Discovery C18	Discovery C18	**505129**	**505137**	**custom**	**505161**	**505188**
LC-18	LC-18, LC-PAH	**59554**	**59564**	**59564-C30**	**59612**	**59613**
LC-318	LC-318	**59502**	**59512**	**custom**	**custom**	**custom**
LC-18-DB	LC-18-DB	**59555**	**59565**	**59565-C30**	**59616**	**59617**
LC-18-S	LC-18-S	**59629**	**59630**	**59630-C30**	**59161**	**59162**
LC-18-T	LC-18-T, LC-DABS	**59620**	**59621**	**59621-C30**	**custom**	**custom**
Discovery C8	Discovery C8	**59589-U**	**59590-U**	**custom**	**59587-U**	**59588-U**
LC-8	LC-8	**59552**	**59562**	**59562-C30**	**59614**	**59615**
LC-308	LC-308	**59501**	**59511**	**custom**	**custom**	**custom**
LC-8-DB	LC-8-DB	**59553**	**59563**	**59563-C30**	**59618**	**59619**
LC-DP	LC-DP	**59556**	**59566**	**59566-C30**	**custom**	**custom**
LC-3DP	LC-3DP	**custom**	**59513**	**custom**	**custom**	**custom**
LC-F	LC-F	**59520**	**59521**	**59521-C30**	**custom**	**custom**
LC-304	LC-304	**59591**	**59592**	**custom**	**custom**	**custom**
Discovery Cyano	Discovery Cyano	**59585-U**	**59586-U**	**custom**	**59583-U**	**59584-U**
LC-CN	LC-CN	**59557**	**59567**	**59567-C30**	**custom**	**custom**
LC-PCN	LC-PCN	**59504**	**59514**	**59514-C30**	**custom**	**custom**
LC-1	LC-1	**59551**	**59561**	**59561-C30**	**custom**	**custom**
TPR-100	TPR-100	**59570-U**	**59571**	**59571-C30**	**custom**	**custom**
ODP-50	ODP-50	**59312**	**59313-C40**	**custom**	**custom**	**custom**
LC-Si	LC-Si, LC-3Si	**59550**	**59560**	**59560-C30**	**custom**	**custom**
LC-NH_2	LC- NH_2	**59558**	**59568**	**59568-C30**	**custom**	**custom**
LC-NH_2-NP	LC- NH_2-NP	**59515**	**59516**	**59516-C30**	**custom**	**custom**
LC-Diol	LC-Diol, LC-3Diol	**59559**	**59569**	**59569-C30**	**custom**	**custom**
LC-SAX1	LC-SAX1	**59536-U**	**59537-U**	**59537-C30**	**custom**	**custom**
LC-SCX	LC-SCX	**59509**	**59519**	**59519-C30**	**custom**	**custom**
Hisep™	Hisep	**59639**	**59640-U**	**59640-C30**	**custom**	**custom**

[1] For 4.0mm ID and 4.6mm ID analytical columns.

Guard Columns for SUPELCOGEL Ion Exclusion Columns (5cm x 4.6mm column, 9µm particles)

Supelguard Phase	SUPELCOGEL Column Protected	Cat. No.
H	H, C-610H	**59319**
Ca	611, Ca	**59306-U**
K	K	**59344**
Pb	Pb	**59345**
Ag	Ag	**59316**

HELPFUL HINTS

To protect microbore (1mm ID) columns, use an in-line screen filter (Cat. No. 58279-U).

To protect preparative columns (6.2mm–50.8mm ID), use a 5cm column packed with the same particles and bonded phase as in the preparative column.

To protect other brands of columns, see our general catalog.

Guard Column Holders

Stand-Alone Holder for 2cm Guard Column - Holds 2.1mm, 3.0mm, or 4.0mm ID Supelguard cartridge. Includes 1/16" nuts and ferrules and 2"/5cm of 0.01" ID x 1/16" OD tubing.

Direct-Connect Guard Column Holder - Directly connects a 2cm Supelguard cartridge to a Supelco™ analytical cartridge after the column endfitting is removed.

Description	Cat. No
Guard Column Holders	
Stand-Alone	**59660-U**
Direct-Connect for 2.1mm ID analytical column	**504254**
Direct-Connect for 3.0/4.0/4.6mm ID analytical column	**55205**

Pelliguard Guard Columns

Packed Cartridges

997-0192

2cm x 4.6mm ID guard columns with 40µm pellicular packings.

- Ready-to-use, disposable
- Low cost
- Large particles for negligible pressure drop
- Sufficient capacity for most samples
- Use with 5µm SUPELCOSIL columns, other columns having corresponding phases

Each kit contains one cartridge guard column (filled with a 40µm Pelliguard packing), a reusable column holder, and hardware for connecting the holder to 1/16" tubing. Replacement cartridges come in packages of four.

Chromatography Mode	5µm Column to be Protected	Recommended Pelliguard Column Kit	Cat. No.
Normal Phase	Silica	2cm LC-Si Kit	**59641**
		Replacement Cartridges (pk. of 4)	**59651**
Polar Bonded Phase	Cyano	2cm LC-CN Kit	**59645-U**
		Replacement Cartridges (pk. of 4)	**59655**
	Amino	2cm LC-NH_2 Kit	**59646**
		Replacement Cartridges (pk. of 4)	**59656**
Reversed Phase	C8	2cm LC-8 Kit	**59643**
		Replacement Cartridges (pk. of 4)	**59653**
	C18	2cm LC-18 Kit	**59644**
		Replacement Cartridges (pk. of 4)	**59654**

Bulk Pellicular Packing Kits

5cm guard column hardware and 40µm pellicular packing.

Dry pack your own guard columns for maximum economy.

- Reusable 5cm x 4.6mm ID column hardware
- Sufficient capacity for most samples

Each column kit contains an empty 5cm x 4.6mm ID column, 10g of Pelliguard packing, 10 frits, and hardware for connecting the column to 1/16" tubing. About 1.3 grams of packing is needed to pack one 5cm x 4.6mm column.

Chromatography Mode	10µm Column to be Protected	Recommended Pelliguard Packing Kit	Cat. No.
Normal Phase	Silica	5cm LC-Si Kit	**58202**
		Pelliguard LC-Si Packing, 10g	**58291**
Polar Bonded Phase	Cyano	5cm LC-CN Kit	**58234**
		Pelliguard LC-CN Packing, 10g	**58235**
Reversed Phase	C8	5cm LC-8 Kit	**58222-U**
		Pelliguard LC-8 Packing, 10g	**58293**
	C18	5cm LC-18 Kit	**58232**
		Pelliguard LC-18 Packing, 10g	**58294**

Guard Column Hardware Kit

5cm column, endfittings, 2 frits (2µm porosity), and 2" (5cm) of 0.01" ID x 1/16" OD SS tubing

Description	Cat. No.
Guard Column Hardware Kit	**58319**
Replacement Frits (pk. of 10)	**58264**

Funnel and Tubing for Easier Column Filling

Pour Pelliguard packing easily into your guard columns with this convenient plastic funnel. Connects to column with the Tygon tubing included.

Description	Cat. No.
Funnel and Tubing	**20390-U**

910-0004

Evaluation Test Mixes for HPLC Columns

Well defined test mixes enable you to troubleshoot chromatographic problems, optimize system efficiency, and evaluate columns. We ship these test mixes in amber ampuls to prevent photodegradation, and we include instructions for proper use and interpretation of results.

Choose from column-specific or application-specific mixes. The amino phase test mix (sugars) calls for refractive index detection; all others call for UV detection. For additional information, request *HPLC Troubleshooting Guide* (Bulletin 826).

HPLC Column Test Mixes

1mL unless otherwise specified.

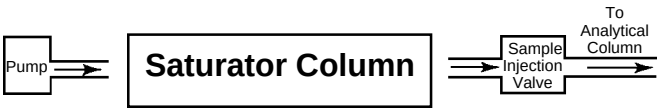
Test Mix	Use to Test	Solvent	Components (conc./mL)	Cat. No.
Amino Phase	LC-NH_2 columns	acetonitrile:water, 25:75	D-fructose (25mg) α-D-glucose (25mg) sucrose (25mg) maltose (25mg) lactose (25mg)	**58424**
Cyano Phase	LC-CN, LC-PCN columns, any weakly hydrophobic phase	acetonitrile:water, 40:60	uracil (7µg) acetophenone (7µg) benzene (750µg) toluene (775µg)	**58299**
Diphenyl Phase	LC-DP columns	methanol:water, 50:50	uracil (7µg) benzene (750µg) toluene (775µg)	**58421**
Normal Phase Mix 1	LC-Si (silica) columns	methylene chloride: methanol:water, 99:9:1	benzene (600µg) benzaldehyde (200µg) acetaldehyde (200µg)	**58281**
Normal Phase Mix 2	LC-Si, LC-CN, LC-NH_2 columns	ethanol:hexane, 5:95	toluene (1mg) diethyl phthalate (1mg) dimethyl phthalate (1mg)	**47640-U**
Nucleosides	LC-18-S columns	water, 10mg/mL sodium formate	12 nucleosides (10-100µg)	**47310-U**
LC-PAH	LC-PAH columns	methanol: methylene chloride, 50:50	16 PAHs (100-2000µg)	**48743**
Peptide Standard	reversed phase columns used for peptide separations (e.g., 300Å phases)	none (dried film)	Gly-Tyr (~0.125mg) Val-Tyr-Val (~0.5mg) Met enkephalin (~0.5mg) Leu enkephalin (~0.5mg) angiotensin II (~0.5mg)	**H2016**
Reversed Phase Mix 1	hydrophobic RP columns (e.g., LC-8, LC-18, ABZ⁺Plus)	methanol:water, 60:40	uracil (7µg) acetophenone (7µg) benzene (750µg) toluene (775µg)	**58278**
Reversed Phase Mix 2	hydrophobic RP columns (e.g., LC-8, LC-18, ABZ⁺Plus)	acetonitrile:water, 58:42	uracil (5µg) phenol (700µg) N,N-diethyl-m-toluamide (600µg) toluene (4mg)	**47641-U**
Chiral 1	chiral phases	hexane:ethyl acetate, 80:20	toluene (70µg) (+) TFAE* (25µg) (–) TFAE* (25µg)	**48250-U**
Chiral 2	chiral phases	chloroform	benzene (500µg) (+) N-PDBA** (50µg) (–) N-PDBA** (50µg)	**48251**

*2,2,2-trifluoro-1-(9-anthryl)ethanol
**N-(1-phenylethyl)-3,5-dinitrobenzamide

Custom Test Mixes

For information on made-to-order standards and test mixes, call our Technical Service chemists, or request our Custom Chemical Reference brochure (Publication No. 196905).

Saturator Column Kits



713-0580

Use a silica saturator column with alkaline mobile phases, to prevent dissolution of the silica packing base. Use a C18 saturator column to prevent stripping of phase by acidic mobile phases. Each kit contains a 7.5cm x 4.6mm column, 10g of porous silica (18µm spherical), two frits, and two 1/16" nuts and ferrules.

Description	Cat. No.
Silica Saturator Column Kit	**58410**
Spherical Silica Packing (18µm), 10g	**58411**
C18 Saturator Column Kit	**58418**
C18 Packing, 10g	**58419**
Funnel and Tubing	**20390**

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Supelco produces a wide variety of technical literature, all of which is free. You may request this literature several different ways:

- Use the business reply card located at the back of our catalog.
- Call our Ordering and Customer Service department (800-247-6628 or 814-359-3441).
- Use our ChromFax™ service.

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1. To use ChromFax you must dial from a touch-tone phone. If you do not have a touch-tone phone, call our Ordering and Customer Service department and we will Fax the publication to you.
2. Have the phone number of your Fax machine ready, and dial **800-652-8069 or 814-359-5748.** Remember you must have the *complete* Fax number (011 + country/city code + Fax number) when you are calling from outside the US or Canada, otherwise the document cannot be transmitted.
3. When ChromFax answers, follow the simple recorded instructions. Select the **ChromFax number** of the document you want. Be certain you order documents by their ChromFax numbers and NOT their literature numbers.
4. Enter your Fax number and hang up. The requested information will be sent to you within minutes. To report a problem with the ChromFax system contact our Technical Service department.

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