RHEODYNE

TECHNICAL NOTE

Number 6

Protecting HPLC Columns from Particulate Contamination Using Column Inlet Filters

Abstract: A column inlet filter removes particles from the mobile phase before they reach the guard column and analytical column. It must have a filtering capacity large enough to prevent back pressure buildup, yet a volume small enough to prevent excessive sample dispersion (loss of resolution). Rheodyne has two filter geometries that meet these conflicting requirements. The two designs allow the filter and column to be matched, providing good capacity without unnecessary loss of resolution. This article describes these filters, tells which one to select for various applications, and shows conditions under which they will cause a loss of resolution.

A filter connected between the pump and injector removes suspended particles from the mobile phase before they reach the injector, where they can cause scratches in the seals, and before they reach the column, where they can cause increased back pressure and loss of efficiency. Sample doesn't flow through such pre-injector filters, so they need not have a small volume, and can have a high filtering capacity. However, they cannot remove particles contained in the sample or generated by the injector. For this a column inlet filter is needed, one that is connected between the injector and the column. Since sample does flow through this type, its design must be a compromise between two desirable but conflicting requirements, high capacity and low dispersion.

High capacity prevents the particles that are filtered out from rapidly increasing the back pressure. The faster the pressure increases, the more frequently the filter needs replacing. High capacity is achieved by increasing the surface area, which makes the volume larger.

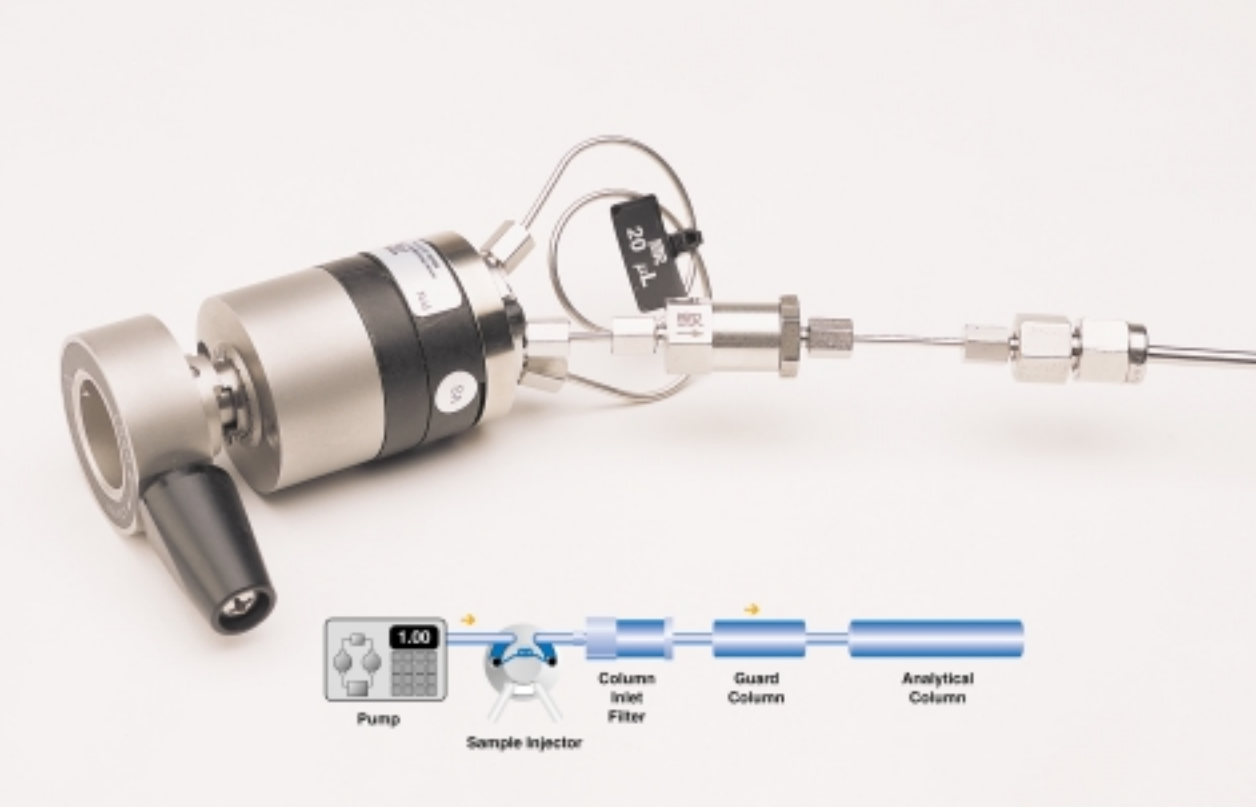

Fig. 1. Rheodyne Model 7335 Column Inlet Filter connected between a Rheodyne sample injector and a column. It has a 3 mm diameter filter frit. The Model 7315 Column Inlet Filter uses the same body, but contains a 1.5 mm diameter frit that causes less dispersion but has a lower filtering capacity. The two models can be interconverted by interchanging the frits. When frits become plugged they are easily replaced. Various types of filters can be used throughout a liquid chromatograph system. After the injector these filters must have a small volume in order to prevent excessive sample dispersion, which causes loss of resolution and sensitivity.

Table I. Minimum Peak Volumes for k′ = 1.

Column Diameter (mm)	Column Length (mm)	Packing Diameter (µm)	4σ Volume (µL)	σ² Variance (µL²)
4.6	250	10	223	3107
		5	158	1554
	100	5	100	621
		3	77	373
	30	3	42	112
2	250	10	42	112
		5	30	56
	100	5	19	22
		3	15	13
1	250	10	10.5	6.9
		5	8.3	4.3
		3	5.8	2.1
	100	5	4.7	1.4
		3	3.6	0.8

Column is assumed to be producing its theoretical maximum efficiency where the height equivalent to a theoretical plate H = 2d_p. In practice the peak volumes when k′= 1 are usually larger than the values in this table. For less retained peaks the volumes will be smaller, half as large in the limiting case of k′ = 0. For more retained peaks the volumes will be larger, twice as large in the case of k′ = 2. The peak volume can be calculated, under this assumption, from the expression $4\sigma = 3.33\ D^2 \sqrt{Ld_p}(1 + k')$, where the column diameter D, column length L, and particle diameter d_p are all in mm. The column porosity is assumed to be 0.75.

Low dispersion prevents the sample from diluting, which causes a loss of resolution and sensitivity. Low dispersion is achieved by decreasing the volume, and by careful control of the stream paths within the filter.

Rheodyne's Model 7315 and 7335 Column Inlet Filters allow the filter and column to be matched, providing adequate capacity, but avoiding excessive loss of resolution. Model 7335 provides moderate dispersion and high capacity with its 3 mm diameter filter frit. Model 7315 provides low dispersion and moderate capacity. Its 1.5 mm diameter filter frit has one fourth the capacity of the 3 mm frit. However, its dispersion is low enough to allow its use with some microbore columns. The characteristics of these products are discussed below.

Loss of Resolution

A column inlet filter will not affect resolution significantly if the dispersion it causes is small compared to the dispersion caused by the column and extra-column components. Stated differently, a particular filter will degrade resolution less when the volume of a peak is relatively large. Peak volumes have been decreasing recently due to the use of smaller packing particles, shorter column lengths, and smaller column diameters.

The volume of a peak is expressed as the microliters containing four standard deviations, 4σ. This is about 95% of the total volume when the peak is symmetrical. The peak can also be described by its variance, s^2, with units of square microliters. This term is useful because the dispersion caused by a series of components is the sum of the individual variances, and theoretical calculations can be made. Table I lists the peak volumes of representative columns of a relatively unretained peak, k′ = 1. Peak volumes are seen to vary widely.

We studied how filters affect resolution on these column types. This was done by comparing the chromatograms obtained without the filter to those obtained with the filter installed **(1)**. Neither of the two made a measurable change in resolution with conventional 4.6 mm ID X 250 mm long columns. But the filters did reduce resolution on columns that have smaller peak volumes. Even with microbore columns, the low-dispersion Model 7315 filter produced only a modest loss of performance. Some of these results are shown in Figure 2. The two filters were used with 0.13 mm diameter connection tubes, not the 0.18 mm tubes supplied as standard (see figure caption for details). The height of the valley between unresolved peaks 2 and 3 is an easily observed indicator of resolution. Peak height is another indicator. An additional measure of a filter's impact on column performance is the change it causes in the number of theoretical plates. The number on the chromatogram in Figure 2 is the percentage reduction in theoretical plates, %ΔN, caused by the inlet filter for peak 4 (k′ + 1.8).

Connecting Tube Characteristics

The tubes used to connect the injector to the filter and the filter to the column are each 6 cm long in these experiments as supplied with the filter. However, as discussed in the caption of Figure 2, three inside diameters are available. The dispersion caused by a tube in terms of square microliters of variance is roughly proportional to the length and the fourth power of the diameter. Theoretical calculations **(2)** yield variance values for the 0.13, 0.18, and 0.31 mm bore tubes of about 0.2, 0.6, and 5 mL^2 respectively, using the microbore conditions of Figure 2. These are significant when compared to the 12 mL^2 variance of the microbore column peak 4 in Figure 2. Our experiments show that although the 0.31 mm tubes affect resolution of 4.6 mm columns only a little, this diameter tube is ruled out for use with columns of smaller diameter.

As to the choice between 0.13 and 0.18 mm tubes, this depends on the desired tradeoff between better resolution and reduced risk of plugging. For example, using the same conditions as Figure 2, we found that the 0.13 mm tubes reduced the number of plates for peak 4 by 11%, compared to the control which used no filter and a 0.13 mm injector-column tube. The 0.18 mm tubes caused a 20% reduction, compared to the same control. Table II is a general

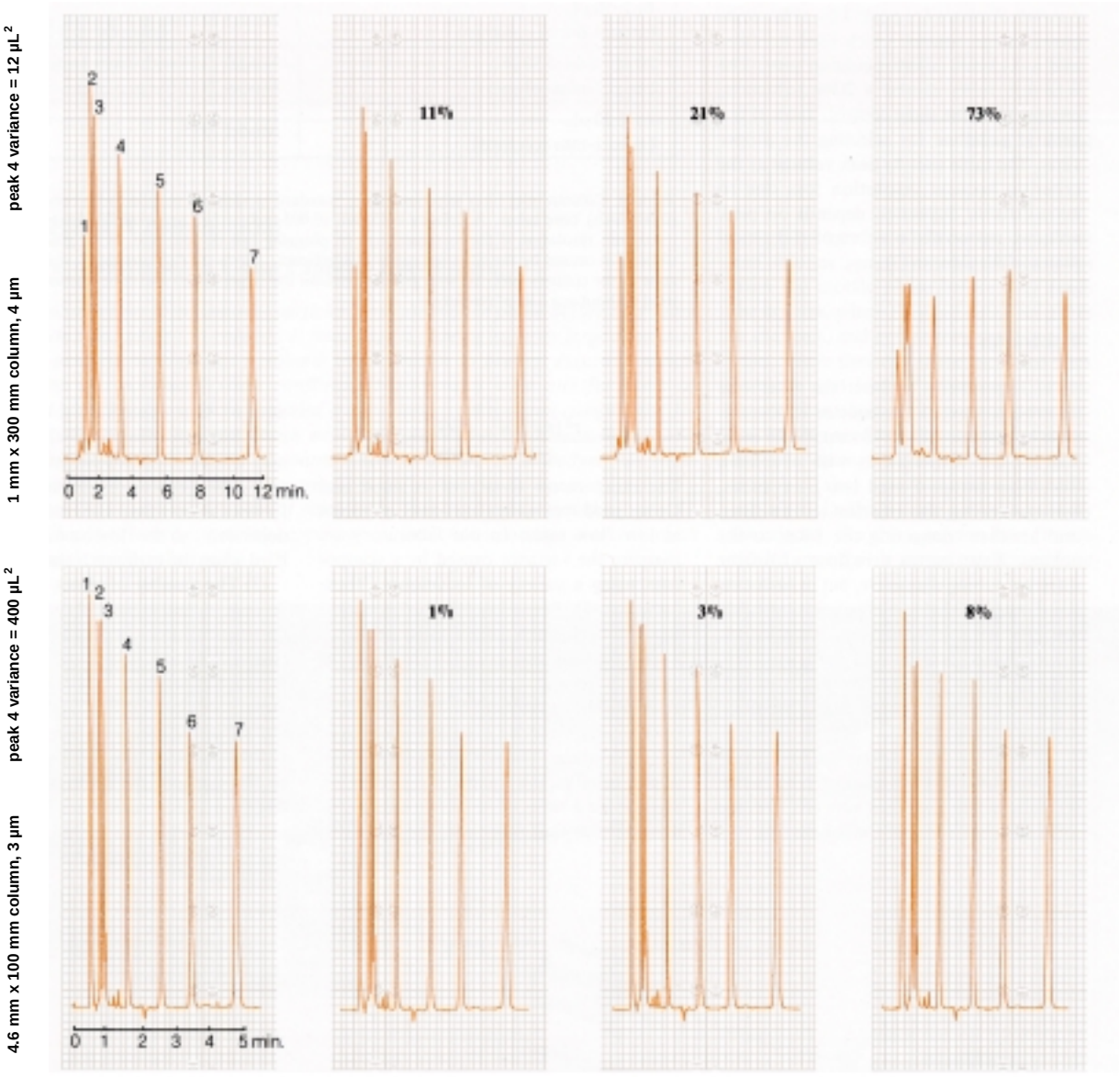

Fig. 2. Changes in performance caused by column inlet filters with two different columns. The bottom row of chromatograms was produced using a short high-performance column of 4.6 mm diameter. Peak 4 (k′= 1.8) has a 4σ volume of 80 µL, a σ^2 of 400 µL^2, and a theoretical plate number of 9700. These are smaller volumes than are encountered with most 4.6 mm columns, so it is a tough test for the filters used with conventional column diameters. Resolution changes are most easily observed in the height of the valley between peaks 2 and 3. Resolution changes are also indicated by the changes in peak height; note that the smaller volume, less retained peaks are affected the most. The number on each chromatogram is the percentage loss in theoretical plates caused by the inlet filter, as measured on peak 4. The loss decreases with increasing retention. For example, with Model 7335 filter the 3%ΔN of peak 4 drops to 1%ΔN for peak 7. The top row of chromatograms was produced using a high-performance microbore column of 1 mm diameter. Peak 4 (k′ = 1.8) has a 4σ volume of 14 µL, a σ^2 of 12 µL^2, and a theoretical plate number of 10,100. The filters' affects on resolution and plate number are much larger. The resolution loss caused by the low-dispersion Model 7315 is remarkably small, attesting to its excellent internal geometry. The connecting tubes used to attach the filter to the injector and column should be noted. The two filters, 7315 and 7335, were used with 0.13 mm (0.005 inch) bore connecting tubes. These are available as accessories. The filters are supplied with 0.18 mm (0.007 inch) tubes as standard; they do not plug as easily, yet increase the dispersion a tolerable amount in most cases, as discussed in the text. The old style filter was used with 0.31 mm (0.012 inch) tubes. The chromatogram shows the combined effect of the old filter design and large bore connecting tubes. The conditions in both rows of chromatograms were: JASCO Uvidec V detector with 1 µL flow cell connected to the column by a 0.13 mm X 50 mm tube, 210 nm wavelength. The peaks are in order of elution: unknown, caffeine, methyl parahydroxybenzoate, and C_3, C_5, C_6, and C_7 normal brominated paraffins. The conditions in the bottom row are: 3 mm C-18 packing, 4.6 mm X 100 mm column, 1.2 mL/min flow rate, 21°C temperature, 15 µL sample from a Rheodyne Model 7125 injector. In the top row: 4 mm C-18 packing, 1 mm X 300 mm column, 100 mL/min flow rate, 21°C temperature, 1 µL sample from a Rheodyne Model 7520 micro injector.

Table II. Column Inlet Filter Selection Guide.	
4σ Peak Volume at k′ = 1	**Recommended Filter**
4σ > 50 µL (most 4.6 mm columns)	Model 7335.
20 µL < 4σ < 50µL (most 2 mm columns)	Model 7335 with dirty samples Model 7315 for maximum resolution
4σ < 10 µL (most 1 mm columns)	Model 7315

Standard models are supplied with two 6 cm x 0.18 mm (0.007 inch) bore tubes. Accessory 0.13 mm (0.005 inch) tubes are available and can be used for maximum resolution if the increased risk of plugging can be tolerated. With gradient elution the dispersion caused by the filter is usually insignificant, due to the "condensing" of the initial sample zone at the column inlet. So with gradient elution the larger filters and tube diameters can often be used without any loss of resolution.

guideline for selecting the proper filter. The table uses 4σ peak volume as the criterion because resolution loss due to extra-column dispersion depends on peak volume, not on the number of theoretical plates in the column **(3)**.

Footnotes

(1) Experiments without the filter (the control) connected the injector to the column by a single tube 0.13 mm (0.005 inch) ID X 6 cm. Experiments with a column inlet filter installed used two 6 cm tubes, one connecting the injector to the filter, and another connecting the filter to the column. Experiments were done with three different tubing diameters, but in each case the diameter of the two connecting tubes was the same. Comparisons were always made to the control, with its single 0.13 mm connecting tube. The performance values for each set of conditions were the average of three runs.

(2) The classical expression for dispersion in a tube is a function of tube radius (r), tube length (L), flow rate (F), and the diffusion coefficient of the solutes (D):

$$s^2 = \frac{r^2 (\pi r^2 L)F}{24D} = \frac{r^2 VF}{24D} = \mu L^2$$

The theory of Atwood and Golay produces somewhat lower s^2 values at low flow rates. In our laboratory we measure the variance caused by a component using a variety of techniques. These include: 1) Inserting the component directly between a micro injector and micro detector, and using a computer to calculate central statistical moments, 2) Inserting the component in a system with a column and measuring the difference in σ^2 values produced under various conditions. A plot of σ^2 vs. the square of the retention volume for a series of retained peaks yields a y-intercept that represents the extra column σ^2. Our experimental data often has only fair agreement with theory, but this may be due to measurement errors. In any case, the general relationships are confirmed. Note that the variance is flow dependent, so the flow rate should be specified when the variance is stated.

(3) The number of plates produced by a column is not necessarily an indicator of the peak volumes, except when comparing two columns of the same diameter and length. For example, both the 4.6 mm and 1 mm columns in Figure 2 produce roughly the same number of plates for peak 7; 12,800 plates on the 4.6 mm column and 16,000 plates on the 1 mm column. But the 4σ volumes for peak 7 are 208 µL and 35 µL respectively.

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