Syringe Filter Efficiency and Effect of Filtration on HPLC Column Life

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Introduction

Injection of particulate-laden samples will plug High Performance Liquid Chromatography (HPLC) columns, shorten injector life, and result in extensive maintenance on pumps. Particulate finding its way into a column results in increased column backpressure, disrupted nominal band shape, and reduced plate number, consequently shortening column life and making analytical results difficult to interpret. Using Pall Acrodisc® PSF syringe filters with GHP (hydrophilic polypropylene) membrane is the most efficient way to remove particulate and prolong the life of HPLC system components. Pall Acrodisc PSF syringe filters with GHP membrane are widely used for this purpose. The average retention efficiency of 0.45 µm rated Acrodisc PSF syringe filters with GHP membrane at removing 0.45 µm average diameter latex spheres is 94.9%. Compared to filters from two other manufacturers, Pall Acrodisc PSF syringe filters significantly prolonged HPLC column life with virtually no backpressure increase after 972 injections.

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Background on Column Failure

Of the four common causes for HPLC column failure (plugging, voids, adsorbed sample, and chemical attack), plugging is the most frequently encountered by analytical chemists or analysts. Injection of samples containing particulate will eventually block the column inlet, cause high column backpressure, and shorten the normal lifetime of the column. Operations of pump components, injectors, and detectors can be expected to be less troublesome when fluids are filtered. For HPLC applications, the 0.45 µm pore size filter is typically selected for removal of particulates. Although there are several seemingly equivalent such products on the market, lack of knowledge about the differences between filters leads to ore frequent column replacement and extensive operation downtime.

Filtration as a preventative maintenance tool for HPLC analyses is well documented. It is commonly taken for granted that column life will be extended if samples are filtered prior to injection, but the extension of the column life has not been well quantified. It is the intent of this work to demonstrate that filter efficiency must be considered when choosing an HPLC sample-prep filter and that filtration will lengthen the life of a column.

In this paper, retention efficiency of three effectively equivalent 0.45 µm rated syringe filters was examined using 0.45 µm average diameter latex spheres. This work was conducted with latex spheres to offer the best possible reproducibility in both sample preparation and filter efficiency measurements.

In order to correlate the retention of spheres to the actual application, the quantitative effect of filtration on HPLC column life was investigated. This involved examining column life without filtration compared to column life when samples were filtered. It should be recognized that extending the column life is dependent on the particulate within the sample and actual column life extension may vary.

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Test Methods

Materials

- Latex spheres and surfactant were purchased from Sigma. 0.45 µm average diameter latex spheres: L/N 31K1123, Cat #: LB-5, Triton X-100 L/N: 121K0090, Cat #: X-100
- 25 mm syringe filters with 0.45 µm pore size ratings were obtained from Pall, Company A, and Company B
- Pall Acrodisc PSF syringe filters with GHP membrane: P/N AP-4557, L/N: A224102521, A224227411, and A22422044
- Company A syringe filters with PVDF membrane: L/N: F2EN42569, F0BN28908, and F2NN83751
- Company B syringe filters with PVDF membrane: L/N: 11067, 11146, and 99297

Instrumentation

- High Performance Liquid Chromatography (HPLC)
- Waters (Milford, MA, USA) 616 Pump
- Waters 600s Controller
- Waters 717plus Autosampler
- Ultraviolet/Visible Spectrophotometer (UV/VIS), HP 8452A Diode Array

Columns

- Luna 5 µm C18(2), columns were purchased from Phenomenex (Torrance, CA, USA): Size: 30 x 3.0 mm, P/N: 00A-4252-Y0, S/N: 160111-3, 159485-4, 159742-3, and 159485-3

Testing

The UV/VIS Spectrophotometer was used for measuring absorbance of latex-sphere solutions. The maximum absorbance of the latex-sphere solution was observed at 272 nm, which was used to correlate latex sphere concentrations with absorbance. The surfactant solution, 0.1% Triton X-100 that is free of latex spheres, was measured as the blank at 272 nm. A series of standard solutions of 0.0025%, 0.0050%, 0.0075%, and 0.01% 0.45 µm latex sphere concentrations were made and used for creating the calibration curve. The linear relationship between latex sphere concentrations and absorbance was established, which is in accordance with Beer's law. A correlation coefficient of 0.9999 was obtained. The 0.01% 0.45 µm average diameter latex spheres solution was used for the retention efficiency study. The challenge solution was passed through each individual syringe filter and a 3 mL eluted aliquot was collected and analyzed at 272 nm. Three different filters from each of the three lots were tested (i.e., nine filters from each manufacturer were individually analyzed).

The HPLC was utilized for the column-plugging study. Column life was evaluated by comparing initial backpressure to backpressure after injections. A new LUNA* C18(2) 00A-4252-Y0 column (S/N: 160111-3) was installed. The outlet of the column was disconnected from the detector and allowed to run to drain. This modification allowed quicker injections for a more efficient determination of column backpressure. Acetonitrile:Water (35:65, percentage by volume) was used as mobile phase, with a flow rate of 1 mL/min. Column temperature was controlled at 25 °C. The system was set to automatically inject 50 µL each time. The column-plugging solution consisted of 0.05% (by weight) 0.45 µm average diameter latex spheres in 0.002% Triton X-100 solution. The first step was to inject this solution without any filtration to see how long the column would last without filtration. After the column was plugged (i.e., column backpressure reached 3500 psig, 241.3 bar, 507.6 kPa), a new LUNA C18(2) 00A-4252-Y0 column (S/N: 159485-4) was installed. This time the same latex-sphere solution was subjected to filtration using Company A filters. Thirty samples were generated with 30 Company A filters (ten from each of the three lots). The injections were carried out from sample vial one through vial 30 and then repeated in this sequence. The column backpressure was recorded with the number of injections. This procedure was repeated with new columns, S/N 159742-3 and 159485-3, for studies with Company B and Pall filters, respectively.

Test Results and Discussion

Latex Sphere Retention for Filters From Three Manufacturers

Tables 1 to 3 list latex sphere retention capabilities of Pall, Company A, and Company B syringe filters. Pall Acrodisc PSF syringe filters with GHP membrane are able to retain an average of 94.9% of the 0.45 µm average diameter latex spheres. By comparison, other syringe filters can only remove averages of 90.5% and 33.0% of the 0.45 µm average diameter latex spheres. Pall syringe filters show greater lot-to-lot consistency with a relative standard deviation (RSD) of 3.5%.

Table 1

0.45 µm Latex Sphere Retention Efficiency (in percentage) with 0.45 µm Pall GHP Filters

Filters	L/N: A224102521	L/N: A224227411	L/N: A22422044

Filter 1	98.2	95.8	92.5
Filter 2	97.8	97.1	90.5
Filter 3	97.0	93.8	91.0
Average from each lot	97.7	95.6	91.3
Average from three lots/RSD		94.9/3.5	

Table 2
0.45 μm Latex Sphere Retention Efficiency (in percentage) with 0.45 μm Company A Filters

Filters	L/N: F2EN42569	L/N: F0BN28908	L/N: F2NN83751
Filter 1	30.1	39.5	33.3
Filter 2	28.2	38.6	29.0
Filter 3	28.4	33.6	36.2
Average from each lot	28.9	37.2	32.8
Average from three lots/RSD		33.0/12.7	

Table 3
0.45 μm Latex Sphere Retention Efficiency (in percentage) with 0.45 μm Company B Filters

Filters	L/N: 11067	L/N: 11146	L/N: 99297
Filter 1	91.7	86.7	96.6
Filter 2	88.6	86.9	95.0
Filter 3	87.0	85.5	96.1
Average from each lot	89.1	86.4	95.9
Average from three lots/RSD		90.5/5.4	

These syringe filters from different manufacturers may appear similar and are all assigned a pore size rating of 0.45 μm from the manufacturer, but the test results show that they perform quite differently.

Function of Filtration to Extend HPLC Column Life

Figure 1 depicts the relationship between column backpressure and number of injections. Without filtration, the column failure due to plugging occurred after only 21 injections. After the 0.05% latex-sphere solution was filtered with competitive filters, the columns were plugged after 37 and 487 injections, respectively. In addition, the effluents from both competitive filters were hazy, indicating the presence of a significant number of latex spheres.

When the 0.05% latex-sphere solution was filtered through Pall Acrodisc PSF syringe filters with GHP membrane and injected to the HPLC system, the column backpressure did not increase after even 972 injections. The clear effluents from the filters suggested a more successful retention of latex spheres.

Figure 1
Effects of Filters on HPLC Column Life
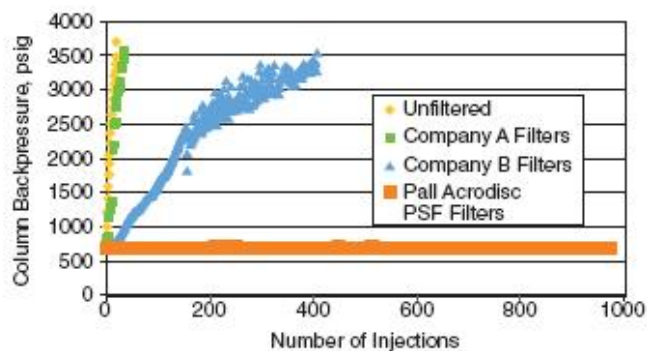
Conclusion

Among 0.45 µm rated filters from three manufacturers, the Pall Acrodisc PSF syringe filters with GHP membrane have the highest average retention efficiency (94.9%) of latex spheres, as well as greater lot-to-lot consistency.

We have shown that it is imperative that samples be filtered prior to their introduction into an HPLC system. Apparently, equivalent filters from various manufacturers with the same removal rating differ in capabilities. Using Pall Acrodisc PSF syringe filters with GHP membrane prolonged the column life 46 times with no increase in column backpressure.

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