Application Note

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Syringe Filter Filtration Efficiency and Impact on LC Column Life

Author

Limian Zhao

Agilent Technologies, Inc.

Abstract

Agilent Captiva Premium syringe filters were tested for filtration efficiency by filtering a homogeneous solution of latex beads. The bead particle size selection was based on the syringe filter membrane pore size. Excellent and consistent filtration efficiency (removal of >90% particulates) was demonstrated for the Captiva Premium syringe filters. The filtration impact on an LC column was evaluated on sub-2 µm and superficially porous columns by monitoring the column backpressure with continuous injections of latex beads samples. The results showed that filtration can provide the best protection to LC columns and, thus, lead to considerably longer column lifetime.

Introduction

The most important function of syringe filters is to block and remove particulates from the sample matrix. It is critical to evaluate filtration efficiency to understand whether the filter performs to the expected standard. The particulate size that a filter can block is tightly linked to the pore size of the filter membrane.

Column plugging is the most frequently encountered source of LC column failure.[1] Injection of samples containing particulates will clog the column inlet frit, cause increased column backpressure followed closely by loss of efficiency, and subsequently shorten the column life. High column backpressure can cause the LC instrument to shut down if it unexpectedly reaches the pressure limit. It can also result in connection leaks and subsequently auto shut down for the LC system. These problems would interrupt analysis, reduce the number of samples run, waste more time than it would have taken to filter the samples in the first place, and potentially cause samples to be prepared again, incurring even more cost. These impacts can be more significant for sub-2 µm columns because these columns come with smaller size column inlet frits and are usually used under ultrahigh pressure for high-throughput analysis, and, thus, they are more susceptible to particulates in a sample. The accumulated particulates in column inlet frits can quickly increase column backpressure and subsequently shorten column lifetime, leading to frequent column replacement.

As modern LC detectors' sensitivity and selectivity improved, quick and simple sample preparation techniques have been widely used before LC and LC/MS to save time and cost. These techniques include direct injection, dilution followed by direct injection, protein precipitation (PPT) in bio-matrix sample processes, and QuEChERS in food sample processes. However, simple techniques usually do not clean sample matrix well. As discussed above, the introduction of particulates to the LC column can cause shortened column lifetime and LC instrument shut down. Therefore, it is critical to remove particulates from the sample matrix prior to injection, and filtration with an appropriate membrane pore size is the best way to prevent particulates from entering the LC system. For direct injection or dilution followed by direct injection, it is always recommended to filter samples prior to LC or LC/MS analysis. Other sample preparation techniques that use highly organic solvent to extract or elute target analytes from sample matrix require a solvent switch to highly aqueous solvent to obtain chromatographic separation integrity and sensitivity. However, solvent switching can cause some previously dissolved matrix components precipitated out from the sample. Because this step is usually applied at the end of sample preparation after extraction, the resulting samples are normally ready to run on the LC instrument. Therefore, it is also important to perform filtration to prevent the injection of particulates and, thus, preserve LC columns in these situations.

The intention of this study was to demonstrate the excellent filtration efficiency provided by Captiva premium syringe filters, and the sample filtration impact on extending the LC column lifetime. To correlate column lifetime extension to a practical application, PPT-treated plasma extracts were tested to compare samples with and without filtration, and with centrifugation.

Experimental

In this study, 0.3 µm latex beads were used for the 0.2 µm syringe filter test, and 0.46 µm latex beads were used for the 0.45 µm syringe filter test, with Captiva Premium syringe filters. Captiva Premium nylon 0.2 µm and regenerated cellulose 0.2 µm syringe filters were used to assess filtration impact on sub-2 µm column life, and nylon 0.45 µm syringe filters were used to test filtration impact on the life of superficially porous columns. Captiva Premium nylon 0.2 µm syringe filters were used to filter PPT extracts.

Chemicals and reagents

Latex beads, polystyrene LB3 (0.3 µm mean particle size), and LB5 (0.46 µm mean particle size) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.). Triton X-100 was from Sigma-Aldrich (St. Louis, MO, U.S.)

Solutions and standards

Triton X-100 0.1% solution was prepared by dilutingTriton X-100 stock 1,000 times with Milli-Q water. Triton X-100 0.002% solution was subsequently prepared by further dilution with Milli-Q water. These two solutions were free of latex beads and were used as blanks to prepare the latex solutions.

Latex solutions for the filtration efficiency test were prepared in 0.1% Triton X-100. Latex LB3 and LB5 solutions (0.01%) were prepared by diluting corresponding 10% stock solutions. Latex solutions for the column life test were prepared in 0.002% Triton X-100. Latex LB3 and LB5 solutions (0.05%) were prepared by diluting corresponding 10% stock solutions.

Sample preparation

About 2 mL of latex LB3 solution was filtered through a 0.2 µm syringe filter, and 2 mL of latex LB5 solution was filtered through a 0.45 µm syringe filter. The filtrate was collected into an autosampler vial for LC analysis. For each kind of syringe filter, ten replicates were performed. The filtered and unfiltered corresponding samples were then run on an HPLC system without column for filtration efficiency evaluation.

The pressure test was conducted on an HPLC or UHPLC system with appropriate column used, and the backpressure was recorded for each injection and then plotted against the injection number.

The unfiltered or filtered LB3 latex bead solutions were continuously injected to an UHPLC system with a new RRHD column until the column backpressure exceeded 1,000 bar (the recommended maximum operating pressure for this column) or by 1,000 injections. The unfiltered or filtered LB5 latex bead solutions were continuously injected to an HPLC system with a new Agilent Poroshell 120 column until the column backpressure exceeded 500 bar (the recommended maximum operating pressure for this column) or by 1,000 injections.

Human plasma extract, after protein precipitation, was used for the sub-2 µm column life application test. The plasma extract was prepared as follows.

1. 2 mL of human plasma was aliquoted into a test tube.
2. 10 mL of acetonitrile with 1% acetic acid was added.
3. The sample was vortexed vigorously and then centrifuged at 4,000 rpm for five minutes.
4. The supernatant was transferred into a clean test tube and blown dry with N_2 at 37 °C.
5. The dried sample was reconstituted in 2 mL of 10:90 MeOH/H_2O, vortex mixed, and sonicated.

The unfiltered, centrifuged, and filtered plasma extracts were run on an Agilent ZORBAX RRHD column for pressure monitoring. For the unfiltered plasma sample, the plasma extract was directly injected. For the centrifuged sample, the plasma extract was centrifuged at 4,000 rpm for three minutes, then injected. For the filtered sample, the plasma extract was passed through a Captiva Premium 0.2 µm nylon syringe filter prior to injection.

Instrumentation

Latex beads exhibit UV adsorption, and the maximum adsorption is at 272 nm. The filtered and unfiltered latex solutions were tested at 272 nm because the adsorption difference reflects concentration changes in the bead solution. An LC/UV system was used for automatic measurement of sample UV absorption. Because no separation was needed, no column was used to expedite the test. However, particulates were injected into the LC system, and so action was taken to prevent potential clogging. All of connection tubing was green (0.17 mm id) or blue (0.25 mm id). Stainless steel capillaries were used after the injector. A needle seat with 0.17 mm seat capillary and 10 mm standard flow cell was used.

The column life test used an Agilent 1200 SL Series fitted with a superficially porous Poroshell 120 HPLC column. To measure sub-2 µm column life, an Agilent 1290 Infinity UHPLC System was used with a RRHD column. Column backpressure was recorded for each injection. 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.

Instrument conditions for the filtration efficiency test

Parameter	Value
Mobile phase	Water
Injection volume	1 µL
Flow rate	1.0 mL/min, isocratic
Total run time	1 min
Detector	DAD SL, UV at 272 nm
HPLC	Agilent 1200 SL Series

Instrument conditions for the column life test

Parameter	Value
Columns	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902) Agilent Poroshell 120 EC-C18 Solvent Saver, 3.0 × 50 mm, 2.7 µm (p/n 699972-302)
Mobile Phase	35:65 acetonitrile:water (v/v)
Injection Volume	10 µL (for RRHD column), 50 µL (for Poroshell 120 column)
Flow Rate	0.4 mL/min (for RRHD column), 1.0 mL/min (for Poroshell 120 column)
Total Run Time	1 min
UHPLC	Agilent 1290 Infinity LC System (for RRHD column test)
HPLC	Agilent 1200 SL Series (for Poroshell 120 column test)

Agilent consumable supplies

Parameter	Value
Vials	Amber, write-on spot, 100/pk (p/n 5182-0716)
Vial Caps	Blue, screw cap, 100/pk (p/n 5182-0717)
Syringe	10 mL syringe, 100/pk (p/n 9301-6474)
Syringe Filters	Agilent Captiva Premium nylon 0.2 µm syringe filter, 15 mm (p/n 5190-5090); Agilent Captiva Premium nylon 0.45 µm syringe filter, 15 mm (p/n 5190-5091); Agilent Captiva Premium regenerated cellulose 0.2 µm syringe filter, 15 mm (p/n 5190-5108)

Results and discussion

Filtration efficiency

Filtered and unfiltered latex bead samples were tested on HPLC/UV, and the peak area was used for comparison of latex absorption. To correct the reagent blank contribution, the Triton X-100 reagent blanks were run before the filtered or unfiltered latex solution. The filtration efficiency was then calculated according to Equation 1. Ten replicates were run for each type of syringe filter. The filtration efficiency was then calculated and is summarized in Table 1.

The results show that Captiva Premium syringe filters provide excellent and consistent filtration efficiency.

Filtration impact on sub-2 µm column life

Sub-2 µm columns have gained increasing popularity in the past few years because of the significant advantages they can provide with up to 10 to 20 times faster analyses, higher resolution in equivalent or less time, and higher sensitivity compared to traditional HPLC columns with 3 to 5 µm particles. All of these advantages lead to lower costs because of the savings on laboratory time and equipment and, thus, will dramatically benefit high throughput analysis. Due to the smaller particle size, sub-2 µm columns produce much larger flow resistance or higher backpressure. At the same time, the introduction of particulates can easily plug the column inlet frit and cause unanticipated pressure changes. Therefore, extra precautions are necessary to use sub-2 µm columns successfully, including precautions on mobile phases, especially buffer preparation, column installation and equilibration, inline filter usage, and extra sample preparation. Agilent strongly recommends using an appropriate 0.2 µm filter to filter all samples before sample injection.

$$\text{Filtration efficiency (\%)} = \frac{(\text{PeakArea}_{\text{unfiltered LB solution}} - \text{PeakArea}_{\text{unfiltered blank}}) - (\text{PeakArea}_{\text{filtered LB solution}} - \text{PeakArea}_{\text{filtered blank}})}{(\text{PeakArea}_{\text{unfiltered LB solution}} - \text{PeakArea}_{\text{unfiltered blank}})} \times 100\%$$

Equation 1.

Table 1. The filtration efficiency (FE%) of Agilent Captiva Premium syringe filters.

	Agilent Captiva Premium 0.45 µm syringe filter						Agilent Captiva Premium 0.2 µm syringe filter					
	Nylon	PTFE	RC	PES	GF/NY	GF/PTFE	Nylon	PTFE	PES	CA	GF/NY	GF/PTFE
1	96.0	92.3	89.8	92.1	99.0	99.4	95.2	97.0	93.6	92.4	96.8	98.4
2	95.9	91.4	90.6	91.4	99.0	98.9	93.2	96.5	93.5	95.0	97.1	98.8
3	94.5	93.3	90.3	89.5	99.2	99.0	95.5	97.5	88.5	96.3	96.4	97.7
4	96.6	92.3	91.7	99.0	99.6	98.6	95.4	96.6	88.2	97.2	99.3	98.8
5	95.4	91.2	92.4	96.3	98.8	98.8	94.9	96.0	92.3	96.0	99.0	99.7
6	95.6	91.1	90.8	99.9	99.3	98.5	95.3	95.7	94.9	95.6	100.0	96.8
7	99.9	91.1	98.2	99.0	99.4	99.4	99.5	95.2	89.4	96.7	98.2	97.6
8	99.8	91.2	99.0	97.8	95.0	99.0	98.0	97.8	87.3	93.8	98.9	98.5
9	99.7	90.9	96.4	95.2	95.9	99.9	97.7	94.9	87.5	92.5	100.2	98.0
10	99.2	91.3	95.7	96.1	94.7	99.6	99.7	94.8	93.6	92.8	100.5	101.3
Average FE%	97.3	91.6	93.5	95.6	98.0	99.1	96.4	96.2	90.9	94.8	98.6	98.6
RSD (%)	2.2	0.8	3.7	3.7	2.0	0.5	2.2	1.1	3.3	1.9	1.5	1.3

The results in Figure 1 clearly show that the use of Captiva Premium 0.2 µm filter filtration can prevent column back pressure increase within 1,000 injections for the sub-2 µm column. This was expected as RRHD columns are specified to be able to withstand 5,000 continuous injections when clean samples are used. In contrast, when the latex sample was not filtered, column backpressure increased quickly and exceeded 1,000 bar within 100 injections.

Figure 2 shows the results of the sub-2 µm column life application test. The difference in pressure increase between the three sample preparation procedures was clear. The filtered plasma extract provided consistent and stable backpressure over 1,000 injections, whereas the injections of unfiltered and centrifuged plasma samples caused significant backpressure increase, which eventually would result in faster column failure. The test also demonstrated that filtration was more efficient than centrifugation in removing fine particles from the sample matrix.

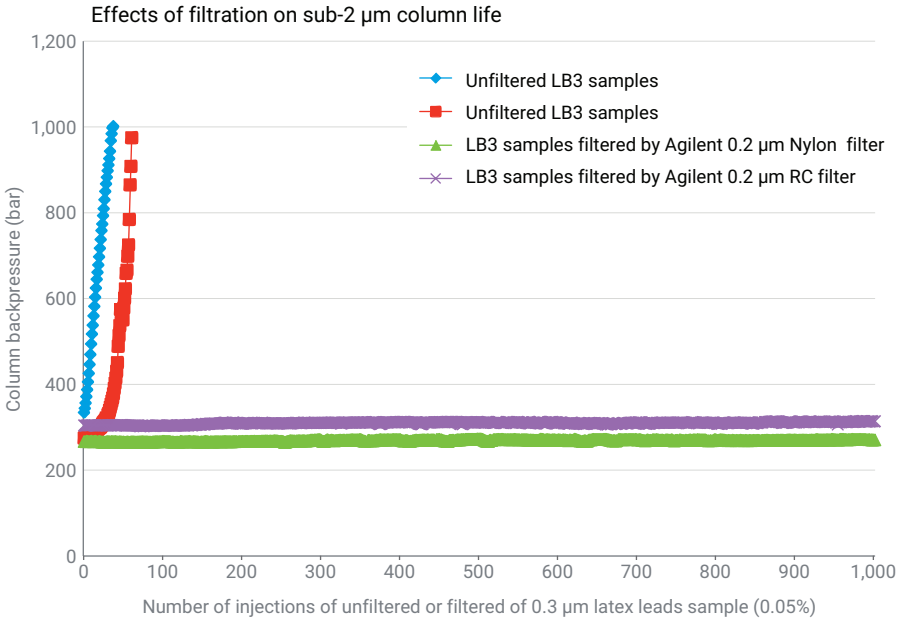

Figure 1. Filtration impact of Agilent Captiva Premium 0.2 µm syringe filters on sub-2 µm column life when filtering latex beads samples, 0.3 µm (LB3). Agilent 0.2 µm nylon and regenerate cellulose syringe filters were used. An Agilent RRHD C18 column was used.

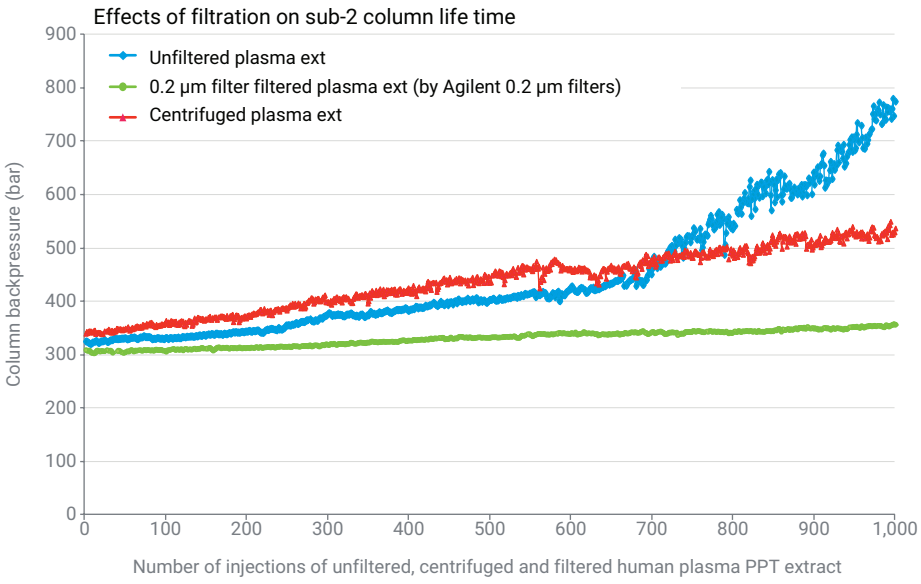

Figure 2. Filtration impact of Agilent Captiva Premium 0.2 µm nylon syringe filters on a sub-2 µm column when filtering human plasma extract. An Agilent RRHD C18 column was used.

Filtration impact on the lifetime of a superficially porous column

Superficially porous particle columns are an alternative to sub-2 µm particles for high speed analyses but at lower pressure. The column particle size is 2.7 µm consisting of a 1.7 µm solid core with a 0.5 µm porous silica shell. The 2.7 µm superficially porous column provides 40 to 50% lower backpressure and 80 to 90% the efficiency of a sub-2 µm column.[2] These columns are not as sensitive to fine particulates in samples as sub-2 µm columns with respect to back pressure, but presample filtration with a 0.45 µm filter is still highly recommended to achieve better column life.

The results shown in Figure 3 indicate that by using a 0.45 µm filter for sample filtration prior to injection, the lifetime of a superficially porous column can be extended 4 to 5 times.

Conclusion

Agilent Captiva Premium syringe filters were tested extensively for filtration efficiency to fully demonstrate their filtration capability. The impact of sample filtration on column lifetime was investigated on sub-2 µm and superficially porous LC columns. The results clearly demonstrate that appropriate sample filtration before injection on column can significantly extend column lifetime.

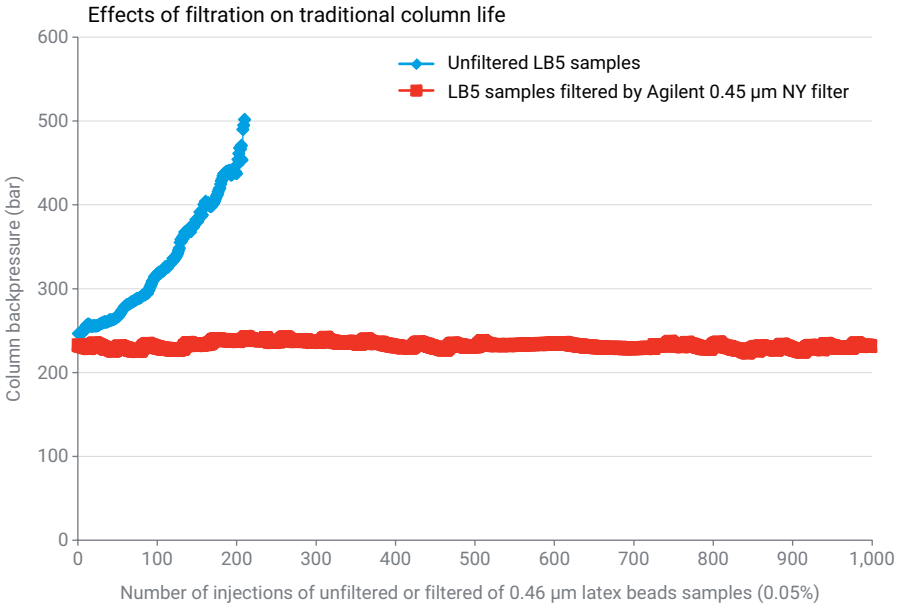

Figure 3. Filtration impact of Agilent Captiva Premium 0.45 µm nylon syringe filters on superficially porous column life when filtering latex beads, 0.46 µm (LB5). An Agilent Poroshell 120 EC-C18 column was used.

References

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