Sample Filtration Impact on LC Column Lifetime

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Introduction

Column plugging is the most frequently encountered source of LC column failure. The injection of samples containing particulates will clog the column inlet frit, and result in the following issues:

- Increased column backpressure
- Loss of efficiency
- Shortened column life
- Unexpected LC instrument shut down

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. Thus, they are more susceptible to particulates in a sample.

As modern LC detectors' sensitivity and selectivity improved, quick and simple sample preparation techniques have been widely used prior to LC and LC/MS to save time and cost. However, these fast and simple sample preparation techniques usually do not clean samples very well. Therefore, the chances for the particulates being introduced to LC columns are higher.

Therefore, it is important to perform sample filtration to prevent the injections of particulates and preserve LC columns. This is especially true for applications using high efficiency small particle size columns and/or the injection of complicate samples with no sample cleaning or simpler preparation techniques.

It is an intent of this study to demonstrate that sample filtration will extend the life of LC column using Latex Beads solutions. The appropriate testing particle sizes were selected based on membrane pore size. In order to correlate the column life extension to the actual application, the plasma extracts by protein precipitation treatment were also tested for the comparison of samples without filtration, samples with centrifugation, and samples with filtration.

Experimental

Filtration Efficiency Test

Chemicals and reagents:

- Latex Bead LB3 (0.3 µm mean particle size) for 0.2 µm syringe filters
- Latex Bead LB5 (0.46 µm mean particle size) for 0.45 µm syringe filters

Sample preparation:

- About 2 mL of Latex Bead solution was filtered through a syringe filter, and filtrate was collected for HPLC analysis.

LC conditions (Agilent 1200 SI series)

- Mobile phase: Water @ 1.0 mL/min, isocratic
- Injection volume: 1 µL. No column used
- Detector: DAD SL, UV at 272 nm

Column Life Test

The unfiltered or filtered LB3 or LB5 latex bead solutions were continuously injected into a UHPLC system with a new RRHD column until the column backpressure exceeded 1,000 bar or by 1,000 injections, or to an HPLC system with a new Poroshell 120 column until the column backpressure exceeded 500 bar or by 1,000 injections.

LC conditions (column used):

- Columns: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (for 0.2 µm filters test);
  Agilent Poroshell 120 EC-C18 Solvent Saver, 3.0 × 50 mm, 2.7 µm (for 0.45 µm filters test).
- 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)
- UHPLC: Agilent 1290 Infinity LC System (for RRHD column test),
- HPLC: Agilent 1200 SL Series (for Poroshell 120 column test).

Human plasma extract, after protein precipitation, was used for the sub 2 µm column life application test. The unfiltered, centrifuged, and filtered plasma extracts were run on an Agilent ZORBAX RRHD column for pressure monitoring.

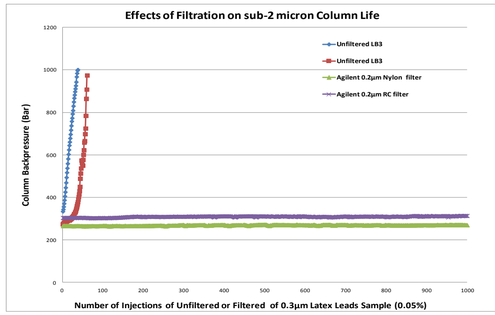
Results and Discussion

Filtration Efficiency Results

Filtration Efficiency	Agilent Premium 0.45 µm syringe filter						Agilent Premium 0.2µm 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

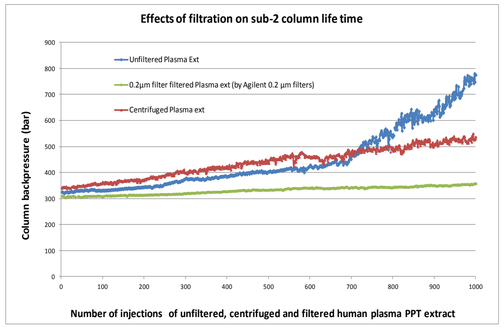
Results and Discussion

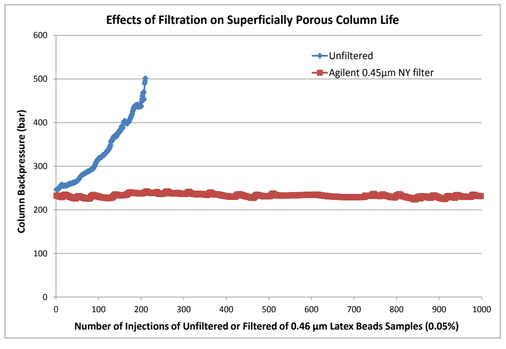
Column Lifetime Impact by Sample Filtration



- Latex Bead LB3 samples (0.05%)
- Agilent 0.2 µm Nylon and Regenerate Cellulose syringe filters
- Agilent RRHD C18 column

- Unfiltered, filtered and centrifuged human plasma extract
- Agilent 0.2 µm Nylon syringe filters
- Agilent RRHD C18 column





- Latex Bead LB5 samples (0.05%)
- Agilent 0.45 µm Nylon syringe filters
- Agilent Poroshell 120 EC-C18 column

Conclusions

- All of Agilent's premium syringe filters were demonstrated to provide excellent and consistent efficiency on blocking particulates from sample solutions.
- Appropriate sample filtration prior to injection on a LC column can significantly extend LC column lifetime.

To learn more about Agilent Capitiva syringe filters, visit us online at www.Agilent.com/chem/SamplePrep/Filtration