Methods for Improving Laboratory Productivity by Reducing UHPLC and LC/MS System Downtime

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Introduction

Recent improvements in HPLC columns, such as sub-2 µm totally porous and sub-3 µm superficially porous columns, have offered improvements in separation speed and performance through reduced analysis times and increased separation efficiency. With characteristics such as smaller frit porosity and smaller particle sizes, care must be taken to ensure that adopting these newer column technologies does not increase the frequency of instrument downtime. By utilizing some common techniques in HPLC analyses, one can help to prevent disruptions caused by dirty samples in the lab. In this work, we will demonstrate how to prevent common interruptions to sample workflow often caused by dirtier samples when coupled with newer column technologies. By adding sample filters, inline filters and guard columns to the analysis, column lifetime can be increase substantially. Data presented will demonstrate the improvement these accessories can have on column lifetime, detection sensitivity, and the overall reduction of instrument maintenance in the laboratory.

Experimental

Poroshell 120 Fast Guard Testing Conditions

System: Agilent 1200 Rapid Resolution LC
Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: Acetonitrile
Flow Rate: 0.65 mL/min
Gradient:

Time	%B
0.0	10
2.0	10
4.0	45
4.1	10
5.0	10

Sample: 10 µL of 1 µg/mL sulfachloropyridazine, sulfamethoxazole in dilute Similac (1:300 in water)
Column: Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm, P/N 695775-902
Guard Column: Agilent Poroshell 120 EC-C18 Fast Guard, 2.1 x 5 mm, 2.7 µm, P/N 821725-911
Temperature: 23 C
Detection: Sig = 254,4 nm; Ref = Off, 80 Hz

ZORBAX Fast Guard Testing Conditions

Similar experimental conditions to Poroshell 120 testing shown above, with the following exceptions:
System: Agilent 1290 Infinity Series LC with Ultra Low Dispersion Optimizations, P/N 5067-5189, G4212-60038, 5001-3726
Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm, P/N 959758-902
Guard Column: Agilent ZORBAX Eclipse Plus C18 Fast Guard, 2.1 x 5 mm, 1.8 µm, P/N 821725-901

Sample Filtration Testing Conditions

System: Agilent 1290 Infinity Series LC
Mobile Phase A: Water (65%)
Mobile Phase B: Acetonitrile (35%)
Flow Rate: 0.4 mL/min
Sample: 10 µL of human plasma extract: unfiltered, centrifuged at 4000 rpm for 5 min, or filtered through a 0.2 µm Agilent Captiva Premium nylon syringe filter, P/N 9301-6474, 5190-5090
Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm, P/N 959757-902
Temperature: ambient

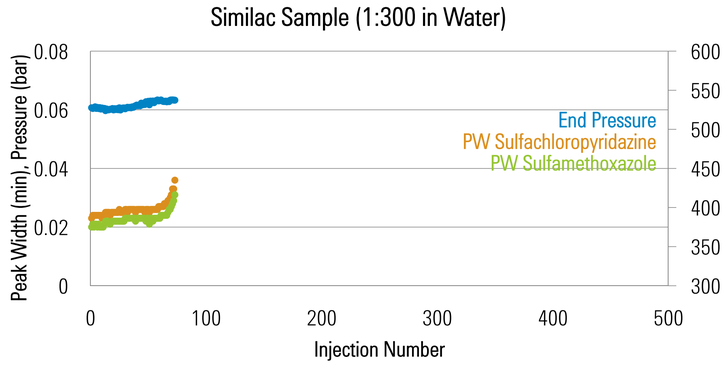
LC/MS Testing Conditions

System: Agilent 1200 Series Rapid Resolution LC with Agilent 6430 Triple Quadrupole LC/MS
Mobile Phase A: 0.1% Formic Acid in Water (20%)
Mobile Phase B: 0.1% Formic Acid in Methanol (80%)
Flow Rate: 0.5 mL/min
Sample: 10 µL of 100 ng/mL Vitamin D2 and D3 in urine, prepared via liquid-liquid extraction, solid phase extraction or protein precipitation
Column: Agilent Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm, P/N 699775-902
Temperature: 50 C
Detection: ESI+, 275 C, 10 L/min, 50 psi, 5000 V, MRM mode, 401→383, 401→159, 413→395, 413→355, 404→386, 416-398, Frag 106 V, Collision Energy 4 V (except 401→159, 24 V)

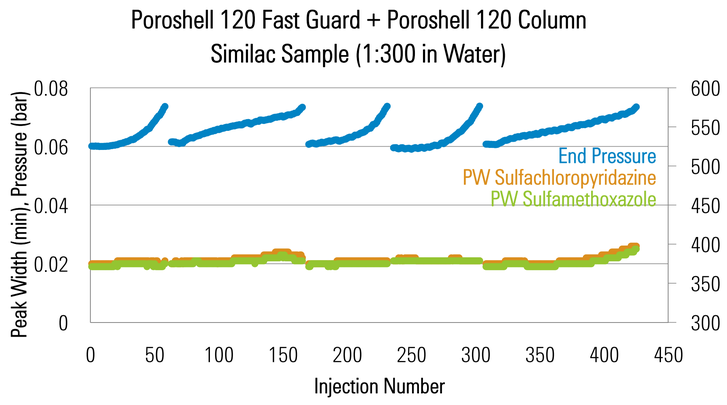
Results and Discussion

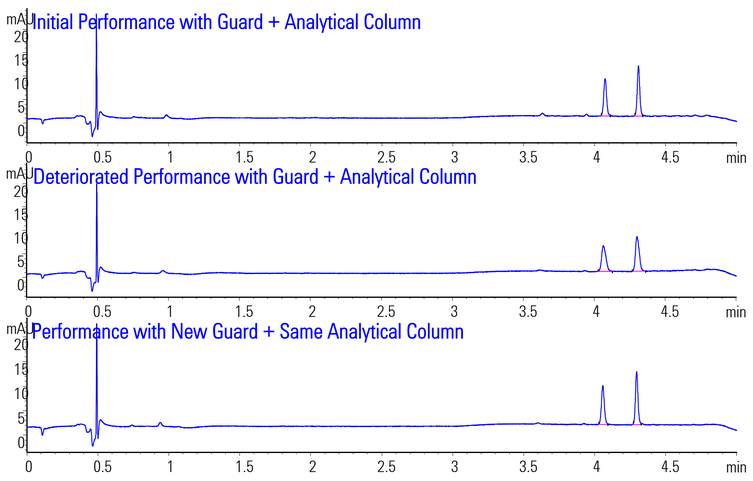
Agilent Poroshell 120 Fast Guards Extend Column Lifetime to Improve Productivity and Reduce Laboratory Costs

The results from the accelerated lifetime test below, show analytical column performance deteriorating after about 80 injections of a dirty sample (300:1 water/Similac).



Installing a guard column prior to the analytical column preserves the lifetime of the more expensive analytical column. Below, 5 guard columns are sequentially changed out to show consistent performance over more than 400 injections with the same analytical column.



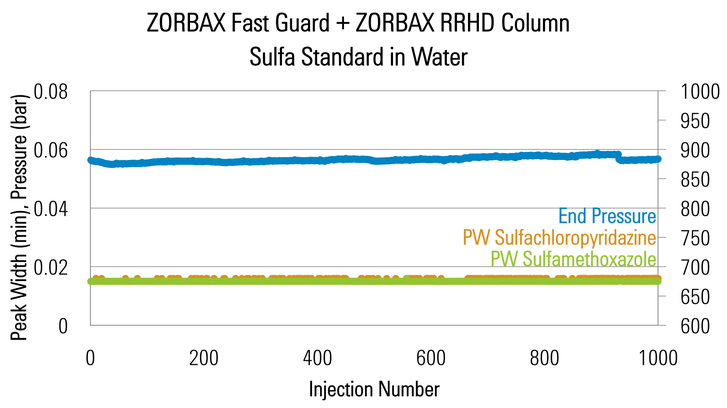


Chromatographically speaking, the dilute Similac sample causes the peaks to broaden and the pressure to increase, as proteins and particulates deposit at the head of the guard column and on the inlet frit. Replacing the old guard column with a new guard column restores performance back to its initial state.

Agilent Fast Guards are also Available in 1.8 µm Format for Pressures up to 1200 bar

While the Poroshell 120 Fast Guards are only stable to 600 bar, Fast Guards packed with 1.8 µm ZORBAX material are stable to 1200 bar (available in 2.1 and 3.0 mm id; 4.6 mm id stable to 600 bar).

Below the mechanical stability of a sub-2 µm ZORBAX Fast Guard is shown with a ZORBAX RRHD column. More than 1000 injections were made near 900 bar with a clean sulfonamide sample prepared in water, without any deterioration in performance.

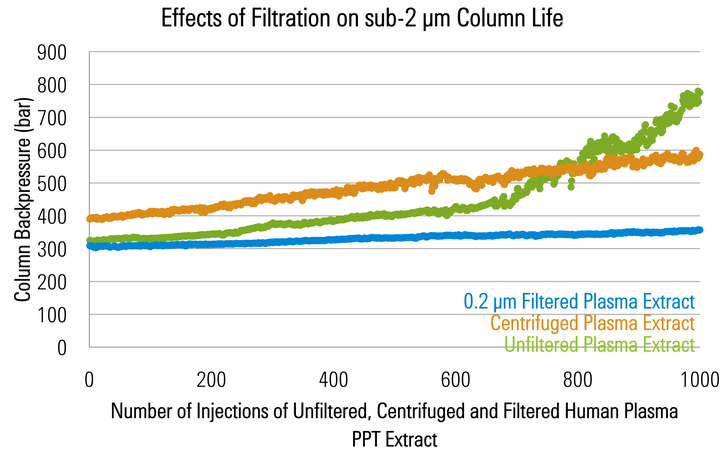


Chromatograms verify the consistent performance of the 2 analytes, sulfachloropyridazine and sulfamethoxazole, with no significant change in peak shape or pressure over the course of 1000 injections at 900 bar.

Results and Discussion

Sample Filtration with Agilent Captiva Premium Syringe Filters also Extend Analytical Column Lifetime

A comparison of repeated injections of human plasma extract (unfiltered, centrifuged, or filtered through a 0.2 µm Agilent Captiva Premium nylon syringe filter) shows that column lifetime can be extended with sample filtration. The data also illustrates that filtration is more efficient than centrifugation in removing fine particles from the sample matrix.



LC/MS Productivity can be Improved with Simple Sample Preparation Procedures to Prevent Instrument Downtime

Clean Source | After LLE/SPE | After PPT

The clean LC/MS source (left) provides the most sensitive, reliable data. Ideally the cleanliness of the source is maintained to prevent contamination or interferences such as ion suppression.

Minimal sample preparation, like protein precipitation, over time can result in salt deposits on the LC/MS sources, as shown on the right.

Further sample preparation, like liquid-liquid or solid-phase extraction, can help maintain an LC/MS source (middle) by removing additional components from dirty sample matrices and thereby preventing build up on the source and keeping the system running longer without interruptions.

Conclusions

Laboratory productivity can be improved by preventing downtime, this could include failure of the analytical LC column or the LC or LC/MS system.

Agilent Fast Guards can protect UHPLC analytical columns from premature failure by catching proteins and particulates that can cause poor chromatography over time or system shut downs due to clogged frits and increasing pressure.

Guard columns are relatively inexpensive and easy to replace; replacements can be done routinely or preemptively before an actual failure occurs and therefore prevent a loss of time and money.

Fast Guards are also available in sub-2 µm configurations for higher pressure limits, as shown at 900 bar for >1000 injections.

Sample filtration can also extend column lifetime by removing particulates from samples to prevent a clogged inlet frit on the analytical column.

Additional sample preparation procedures, like LLE and SPE can prevent unwanted salt deposits or other contaminants from collecting on the LC/MS source and consequently interfering with data collection and productivity.