Improving Laboratory Productivity by Preventing UHPLC and LC/MS System Downtime

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Introduction

Advancements in liquid chromatography columns, such as those with sub 2 µm and superficially porous particles, and UHPLC and LC/MS instrumentation allow for faster analyses and higher sample throughput than ever before. However, increased productivity is also dependent on reducing instrument downtime and predicting when you need to exchange a column. This work will address improved, but practical and valuable tools to maximize performance with UHPLC columns and prevent interruptions to laboratory productivity when using dirty samples, such as: sample filters and UHPLC guard columns. Data will show how and when these tools can improve LC and LC/MS system and column productivity by minimizing instrument maintenance and extending column lifetime.

Experimental

Instruments Used:

- Agilent 1290 Infinity LC System
- Agilent 1200 Series Rapid Resolution LC System
- Agilent 6430 Triple Quadrupole LC/MS System

Analytical UHPLC Columns Used:

- Agilent Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm, 695775-902
- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm, 959758-902
- Agilent Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm, 699972-302
- Agilent Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm, 699775-902

UHPLC Fast Guard Columns Used:

- Agilent Poroshell 120 EC-C18 Fast Guard, 2.1 x 5 mm, 2.7 µm, 821725-911
- Agilent ZORBAX Eclipse Plus C18 Fast Guard, 2.1 x 5 mm, 1.8 µm, 821725-901

Supplies Used:

- Agilent Ultra Low Dispersion Kit for 1290's, 5067-5189
- Agilent Ultra Low Dispersion Max Light Cartridge Flow Cell, G4212-60038
- Agilent LC System Rack, 5001-3726
- Agilent 10 mL syringe, 100/pk, 9301-6474
- Agilent Captiva Premium nylon 0.45 µm syringe filter, 15 mm, 5190-5091

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 uL of 1 ug/mL sulfachloropyridazine, sulfamethoxazole in dilute Similac (1:300 in water)
Column: Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm
Guard Column: Poroshell 120 EC-C18 Fast Guard, 2.1 x 5 mm, 2.7 µm
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
Column: ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm
Guard Column: ZORBAX Eclipse Plus C18 Fast Guard, 2.1 x 5 mm, 1.8 µm

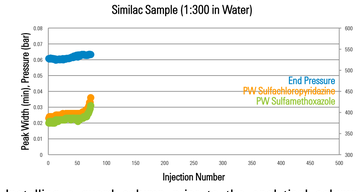
Sample Filtration Testing Conditions
System: Agilent 1200 Series Rapid Resolution LC
Mobile Phase A: Water (65%)
Mobile Phase B: Acetonitrile (35%)
Flow Rate: 1.0 mL/min
Sample: 50 µL of latex LB5 solution, with or without filtration through a 0.45 µm Agilent Captiva Premium nylon syringe filter
Column: Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm
Temperature: ambient
Detection: 272 nm

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: Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm
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)

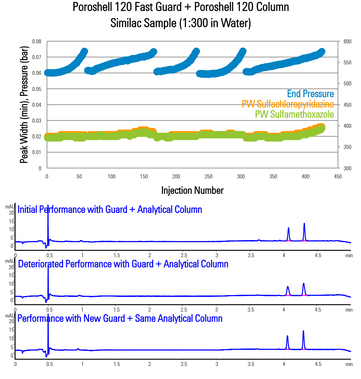
Results and Discussion

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.

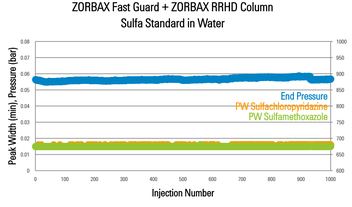


Chromatographically speaking, the dilute Similac sample causes the peaks to broaden and the pressure to increase, as proteins and particulates deposit at the head of the guard column and on the inlet frit. Replacing the old guard column with a new guard column restores performance back to its initial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

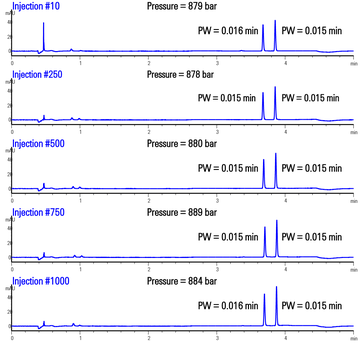
Agilent Fast Guards are also Available in 1.8 µm Format for Pressures up to 1200 bar

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.



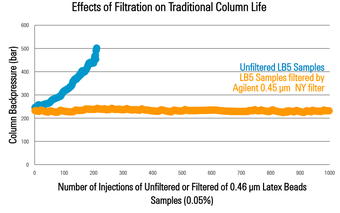
Chromatograms verify the consistent performance of the 2 analytes, sulfachloropyridazine and sulfamethoxazole, with no significant change in peak shape or pressure over the course of 1000 injections at 900 bar.



Results and Discussion

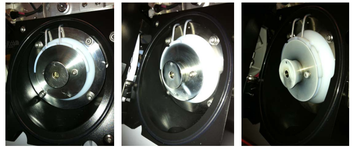
Sample Filtration with Agilent Captiva Premium Syringe Filters also Extend Analytical Column Lifetime

A comparison of repeated injections of a latex LB5 solution (0.46 µm beads), with and without filtration through a 0.45 µm Agilent Captiva Premium nylon syringe filter, shows that column lifetime can be extended 4 to 5 times with sample filtration.



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.

References

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2. Brochure: *Agilent Poroshell 120 Columns for HPLC and UHPLC*. Agilent Publication 5990-5951EN. October 2012.
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5. Mack, Anne. *Optimizing Performance of an Agilent ZORBAX RRHD Eclipse Plus C18 Column by Enhancing an Agilent 1290 Infinity LC System for Ultra Low Dispersion*. Agilent Publication 5990-9502EN. April 2012.

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