Overcoming Barriers with UHPLC to Achieve Maximum Performance

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Abstract

UHPLC systems and columns improve laboratory productivity by enabling shorter analysis times and increased throughput. However, the benefits of higher efficiency, lower volume UHPLC columns can easily be negated by an inefficient instrument setup and poor connections. This work will show how maximum performance can be achieved using UHPLC columns, while overcoming common barriers to the technique. A discussion of extra column and delay volumes will be addressed, as well as the importance of proper tubing connections.

Introduction

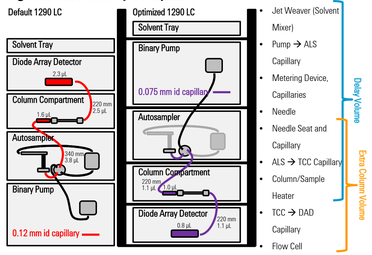
LC System Extra Column Volume

Extra column volume is considered the volume in the sample flow path from the point where the sample enters the LC system through the point of detection. The larger the system extra column volume is, the more dispersion and sample band broadening will occur causing wider, less efficient peaks. Minimizing this volume is critical, especially for small volume high efficiency LC columns to achieve optimal performance.

LC System Delay Volume

System delay volume is the volume between the point at which the solvents mix in the pump and the head of the analytical column. Delay volume is critical for fast gradient analyses, as this volume determines how long it takes the gradient to reach the column. Large delay volumes will cause peaks to elute later; this is particularly true for the early eluting compounds on small dimension columns.

How to Reduce Delay and Extra Column Volumes with an Agilent 1290 Infinity LC System



Experimental

Alkylphenones Analysis
System: Agilent 1290 Infinity LC
Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm, 959757-902
Supplies: Agilent Ultra Low Dispersion Kit, 5067-5189; Agilent Ultra Low Dispersion Max Light Cartridge Flow Cell, G4212-60038; Agilent LC System Rack, 5001-3726
Mobile Phase A: H_2O; B: CH_3CN
Flow Rate: 0.4 mL/min
Gradient: Time %B; or Isocratic: 60%B

0.0	25
1.2	95

Sample: 1 µL of Agilent RRLC Checkout Sample, 5188-6529 spiked with thiourea
Temperature: 26 C
Detection: 254 nm

Aromatic Acids Analysis
System: Agilent 1290 Infinity LC
Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm, 959757-902
Supplies: Agilent Ultra Low Dispersion Kit, 5067-5189; Agilent Ultra Low Dispersion Max Light Cartridge Flow Cell, G4212-60038; Agilent LC System Rack, 5001-3726
Mobile Phase A: 0.1% CH_3CO_2H in H_2O; B: CH_3CN
Flow Rate: 0.6 mL/min
Gradient: Time %B

0.0	2
5.0	60
5.5	60
5.6	2
6.0	2

Sample: 4 µL of 0.01 mg/mL protocatechuic acid, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, salicylic acid in H_2O
Temperature: 30 C
Detection: 280 nm

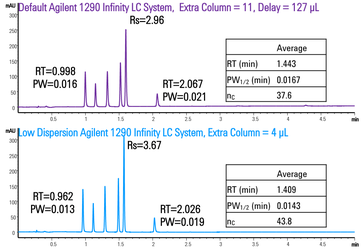
Catechins Analysis
System: Agilent 1290 Infinity LC with Agilent 6410A Triple Quadrupole LC/MS
Column: Agilent ZORBAX RRHD SB-C18, 2.1 x 100 mm, 1.8 µm, 858700-902
Mobile Phase A: 0.2% CH_3CO_2H in H_2O; B: CH_3CN
Flow Rate: 1.0 mL/min
Gradient: Time %B

0.0	10
0.5	15
2.0	27

Sample: 1 µL of 6 µg/mL gallic acid, gallocatechin, epigallocatechin, catechin, caffeine, epicatechin, epigallocatechin, gallocatechin gallate, epicatechin gallate, catechin gallate in H_2O
Temperature: 40 C
Detection: 210 nm; or MS Scan: 150-500, ESI+, 350 C, 10 L/min, 50 psi, 3500 V

Results and Discussion

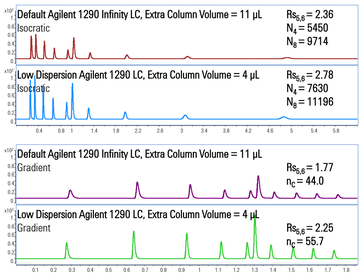
LC System Extra Column Volume Creates Dispersion, causing Increased Peak Widths and Lower Column Efficiency, Especially for Small Volume UHPLC Columns (2.1 x 50 mm)



The effects of extra column volume are shown above with an aromatic acids sample. The low dispersion system is achieved with short, small id connecting capillaries to reduce volume over the default configuration. Peak widths are narrower with the low dispersion system, thereby improving resolution of the critical pair and overall.

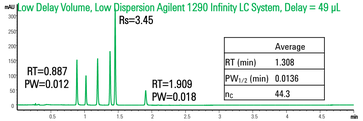
Both Isocratic and Gradient UHPLC Analyses can be Affected by Extra Column Volume

The alkylphenone examples below show that reducing extra column volume is beneficial for the 2.1 x 50 mm, 1.8 µm UHPLC column used, regardless of elution mode.



Reducing System Delay Volume is Necessary for Small Dimensions UHPLC Columns (2.1 x 50 mm) when Using Fast Gradient Analyses

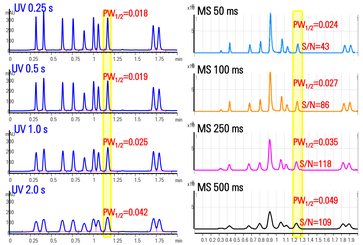
The system delay volume of Agilent Infinity Series LC's can be reduced using the ADVR (automatic delay volume reduction) software feature. In this mode, the autosampler valve is switched to bypass after injection, so that the mobile phase no longer flows through the sample loop, needle, metering device and capillaries in the module.



The result is a substantial reduction in delay volume over the default system (shown to the left), yielding shorter retention times for all peaks. The steeper the gradient, the more significant the impact system delay volume will have.

Choosing the Appropriate Data Collection Rate is Critical for Achieving the Best Possible Performance from High Efficiency UHPLC Columns

The example below uses a catechin sample to show how data collection rates with UV and MS can impact peak width, peak height and signal to noise.

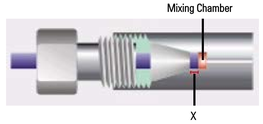


In this case, for MS detection, the data collection rate that results in the narrowest analyte peaks also results in the most baseline noise, negatively affecting sensitivity. Data collection rate should be optimized according to the needs of each specific method.

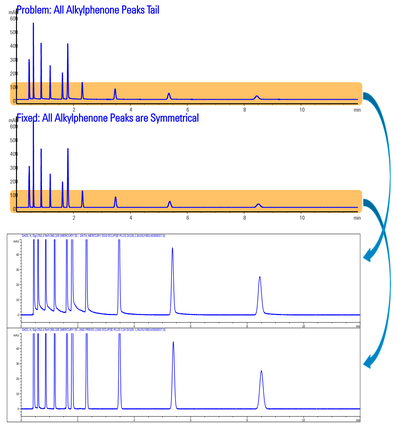
Results and Discussion

Improperly Installed Connecting Capillaries can also Lead to Sub-Par Chromatographic Results

In the fitting illustrated below, dimension X is too short, creating a dead volume or a mixing chamber.



One possible outcome of this dead volume is shown in the chromatograms below.



In this case, the capillary tubing connecting the autosampler and column was swaged improperly on the autosampler end. The ferrule was flush with the end of tubing, causing a void, which resulted in tailing peaks.

Referring back to the figure at the top of this column, if dimension X was too long, the most likely result would be leaking due to an inadequate seal at the ferrule.

Conclusions

UHPLC columns are capable of very high efficiency, but several instrument factors (both hardware and software) should be considered when using them.

Extra column volume is shown to contribute to peak width for both isocratic and gradient elution with UHPLC columns.

Extra column volume can be minimized using small id connecting capillaries in their shortest possible lengths.

Reducing extra column volume improves performance of high efficiency columns, like 2.1 x 50 mm, 1.8 µm formats.

Minimizing UHPLC system delay volume reduces retention times to fully take advantage of fast gradients.

Slow data collection rates can cause peaks to appear broad; fast data collection rates can increase peak height as well as baseline noise to reduce sensitivity.

Data collection rates should be optimized according to individual method needs (sensitivity, resolution, etc).

Care should be taken to properly install all capillaries, as poor seating can cause voids which will negatively affect chromatography.

References

1. 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.

2. Mack, Anne. *Improve a Waters Method for Aromatic Acids by Using an Agilent ZORBAX RRHD Column and an Agilent 1290 Infinity LC*. Agilent Publication 5991-1681EN. December 2012.

3. Mack, Anne. *Fully Using Agilent High Efficiency Columns with LC/MS*. Agilent Publication 5990-8623. January 2012.

4. Posters: *Tips for Optimizing your UHPLC Sensitivity and Throughput*. Agilent Publication 5991-1749EN, 5990-7594EN. January 2013, March 2011.

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