Optimizing Performance of an Agilent ZORBAX RRHD Eclipse Plus C18 Column by Enhancing an Agilent 1290 Infinity LC System for Ultra-Low Dispersion

Application Note

General Analysis

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Abstract

An Agilent 1290 Infinity LC System is optimized for the lowest possible extra-column volume, to ensure minimal band broadening and optimal results from a small dimension, Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column. Agilent Technologies Ultra-Low Dispersion Tubing, Ultra-Low Dispersion Max-Light Cartridge Flow Cell (V(σ) = 0.6 µL) and LC System Rack decrease the 1290's extra-column volume by 60%. The effects on column performance are shown. Improvements to isocratic and gradient analyses are evident, with the early eluting peaks from the isocratic analysis showing the greatest improvement of up to a 58% increase in efficiency (k′ = 1.6). Gradient analyses are improved by 24 to 32% with respect to conditional peak capacity (k′ = 1.3 to 5.3). Additionally, there is no strong correlation between flow rate and the effect of extra-column volume, with respect to column performance for isocratic and gradient analyses.

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Introduction

Small dimension LC columns packed with small particles deliver increased productivity with faster analyses or more resolution, reduced solvent usage and better LC/MS and ELSD compatibility, as compared to larger bore columns with 4.6 or 3 mm internal diameters that require faster flow rates for equivalent linear velocities. Simply swapping a larger id column for a smaller one can yield these benefits. However, to take full advantage of small dimension columns, the LC system extra-column volume must be minimized; this can include connecting capillaries, needle seats, heat exchangers, and detector flow cells. Peak broadening occurs as soon as the sample is introduced into the LC system, as it travels through the autosampler, to the column, then to the detector, and finally through the detector flow cell. Minimizing this volume is especially critical for small dimension columns, as it will account for a higher percentage of the system's extra-column volume, compared to a larger volume column.

Previous work [1] shows the effect of extra-column volume on a variety of column dimensions and particle sizes. Extra-column volume is simplified in this experiment because the only variables are the diameter and length of the connecting capillary between the autosampler and column. We show that the effect of extra-column volume is dependent on column dimension, but is not dependent on particle size. For a 2.1 × 50 mm, 1.8 µm column, efficiency begins to decrease with as little as 2 µL of additional volume. In addition, we determined that larger 4.6 mm id columns are not significantly affected by extra volume ranging from 1.2 to 9.1 µL. Further work shows that a 5 µm column exhibits similar decreases in efficiency as compared to a same-dimension 1.8 µm column, when data is normalized to account for percent efficiency decrease as a function of additional system volume [1].

In this experiment, optimal column performance is obtained with an Agilent 1290 Infinity LC System, which is enhanced for minimal extra-column volume using Agilent Ultra-Low Dispersion Capillaries (0.08 mm id), an Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell (V(σ) = 0.6 µL) and an Agilent LC System Rack. Isocratic and gradient performance improvements are demonstrated using a set of alkylphenone compounds in the Agilent RRLC Checkout Sample. The column is an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm.

Experimental

Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phase A	H_2O
Mobile phase B	CH_3CN
Flow rate	0.4 mL/min, unless specified otherwise
Gradient	Isocratic (60% B) or gradient (25 to 95% B in 1.2 min, unless specified otherwise)
Sample	A 1-µL injection of Agilent RRLC Checkout Sample (p/n 5188-6529) spiked with 50 µL 2 mg/mL thiourea in water/acetonitrile (65:35). See Table 1 for compound names, elution order, and retention factors for isocratic and gradient analyses
Thermostatted Column Compartment (TCC)	26 °C
Diode Array Detector (DAD)	Sig = 254, 4 nm; Ref = Off
System	Agilent 1290 Infinity LC Agilent Ultra-Low Dispersion Capillary Kit (p/n 5067-5189) Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell, V(σ) = 0.6 µL (p/n G4212-60038) Agilent LC System Rack (p/n 5001-3726) MassHunter versions B.03.01, B.02.00 and B.03.01 were used for data acquisition, qualitative, and quantitative analyses respectively

Table 1. Retention Factors for all Alkylphenone Compounds for both Isocratic and Gradient Analyses

Compound	Isocratic k'	Gradient k'
Thiourea	0.0	0.0
Acetanilide	0.3	1.3
Acetophenone	0.9	2.4
Propiophenone	1.6	3.1
Benzophenone	2.6	3.6
Butyrophenone	3.0	3.8
Valerophenone	4.2	4.1
Hexanophenone	6.8	4.5
Heptanophenone	11.1	4.9
Octanophenone	18.2	5.3

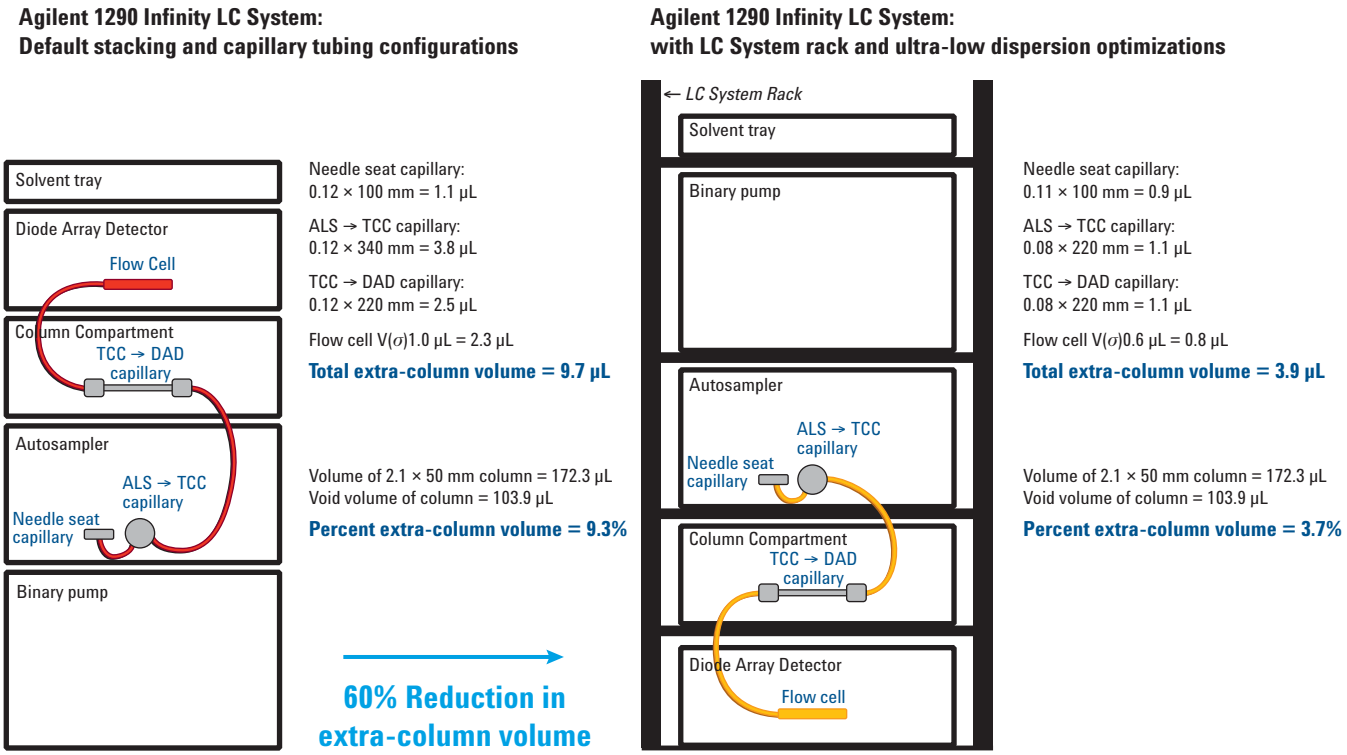


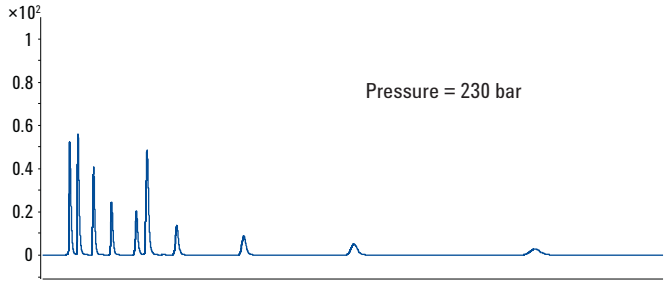
Figure 1. Comparison of extra-column volume on an Agilent 1290 Infinity LC System without (left) and with (right) ultra-low dispersion optimizations.

Results and Discussion

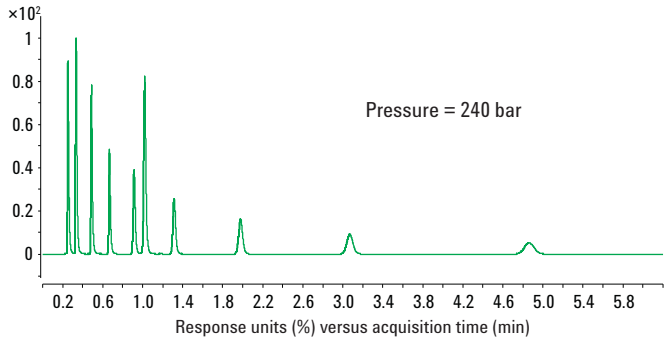
Narrow 0.08 mm id capillaries from the Agilent Ultra-Low Dispersion Capillary Kit replace the standard 0.12 mm id capillaries on the Agilent 1290 Infinity LC System (ALS→TCC capillary, TCC→DAD capillary and needle seat capillary [0.11 mm id]). Additionally, the V(σ) = 1.0 µL flow cell is replaced with the V(σ) = 0.6 µL (Ultra-Low Dispersion Max-Light cartridge) flow cell. The LC is further optimized by rearranging the modules with the LC System Rack. While the 1290 Infinity LC System is traditionally stacked with the binary pump on the bottom due to its weight, using an Agilent LC System Rack allows the pump to be safely located at the top of the stack (from top to bottom: solvent tray, binary pump, autosampler, column compartment, diode array detector); this permits use of the shortest possible capillaries. A shorter 220-mm length capillary connects the autosampler valve to the column inlet (as compared to 340 mm in the default configuration), and the same length capillary, 220 mm, is used to connect the column outlet to the detector flow cell. The result is a 60% reduction in extra-column volume from the default 1290 configuration (9.7 µL) to the optimized configuration (3.9 µL). See Figure 1 for a detailed illustrative and volumetric comparison of the extra-column volume in a default setup versus an optimized setup for the 1290 Infinity LC System.

The isocratic analyses in Figure 2 show a significant 3 to 65% improvement in efficiency with a 60% reduction in extra-column volume. Resolution is improved by 3 to 39% on the optimized Agilent 1290 Infinity LC System, with the critical pair, benzophenone (k′ = 2.6) and butyrophenone (k′ = 3.0), improved by 18%. Early eluting peaks are more affected than later eluting peaks. The bar chart below the chromatograms shows the percent improvement in peak width at half height, efficiency, and resolution for each peak, when comparing performance from the optimized LC to the default LC. Efficiency and resolution are functions of peak width, and so the trends for each of these three values is the same across the chart. These improvements in column performance for an isocratic analysis are possible with just a 5.8-µL (60%) decrease in extra-column volume.

Default Agilent 1290 Infinity LC System, 9.7 µL extra-column volume



Optimized Agilent 1290 Infinity LC System, 3.9 µL extra-column volume



Mobile phase A	H_2O
Mobile phase B	CH_3CN
Flow rate	0.4 mL/min
Isocratic	60% B
Sample	A 1-µL injection of RRLC checkout sample (p/n 5188-6529) spiked with 50 µL 2 mg/mL thiourea in water/acetonitrile
TCC	26 °C
DAD	Sig = 254, 4 nm; Ref = Off
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Analytes	1. Thiourea (v_0 marker) 2. Acetanilide 3. Acetophenone 4. Propiophenone 5. Butyrophenone 6. Benzophenone 7. Valerophenone 8. Hexanophenone 9. Heptanophenone 10. Octanophenone

% Improvement from default to optimized Agilent 1290 Infinity LC System with an isocratic analysis

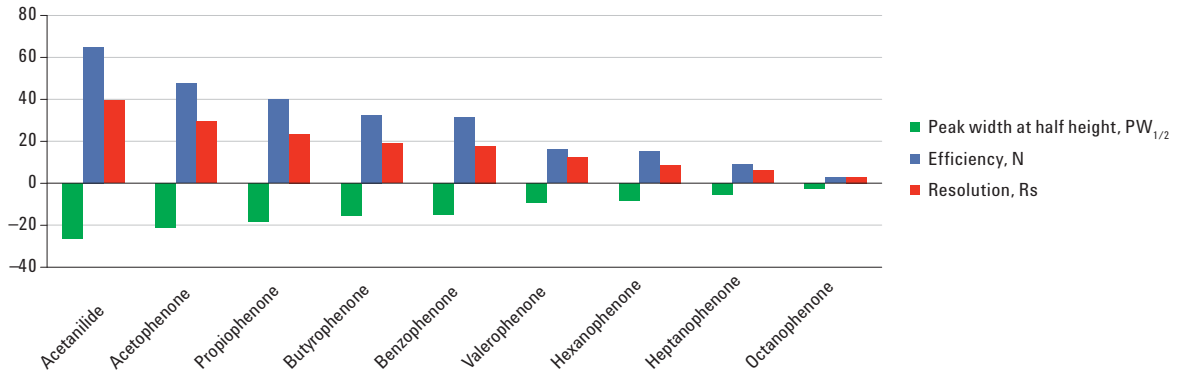


Figure 2. Effect of a 60% reduction in LC system extra-column volume on an isocratic analysis of alkylphenones.

% Improvement in efficiency with a 60% reduction in LC system extra-column volume with an isocratic analysis

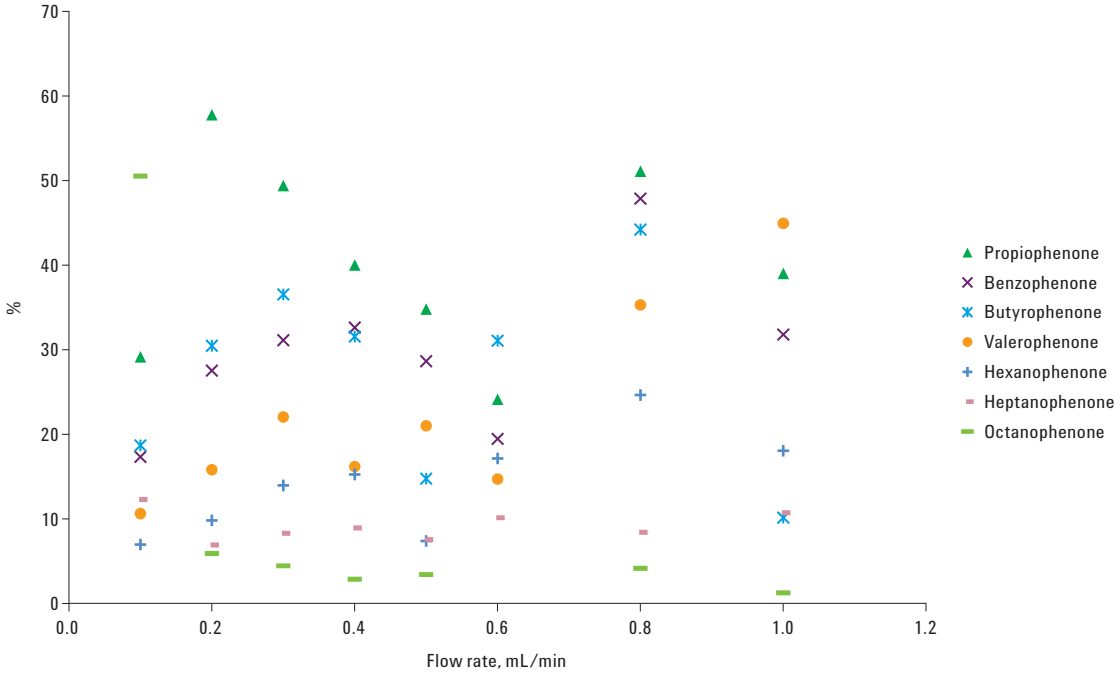


Figure 3. Scatter plot illustrating the effect of extra-column volume on efficiency with various flow rates for an isocratic analysis of alkylphenones.

Figure 2 shows example chromatograms and data from analyses run at 0.4 mL/min. The scatter plot in Figure 3 shows the percent improvement in efficiency for analyses ranging from 0.1 to 1 mL/min. The first two peaks, acetanilide and acetophenone, are poorly retained with k'<1. As a result, these data are not representative of good chromatography practice and are intentionally absent from this figure. Overall efficiency improvements range greatly from 1 to 58% (not including acetanilide and acetophenone). The flow rate of an isocratic analysis has little to no impact on the effects of extra-column volume with respect to column performance.

Gradient analyses for the same 10-component mixture are shown in Figure 4. Similar to Figure 2, the bar chart below the chromatograms depicts the percent improvement when comparing column performance on the optimized Agilent 1290 Infinity LC System to the default setup. Peak width at half height and resolution show the same trend across the chart, because resolution is dependent on peak width. Because efficiency is not a measure typically used to evaluate column performance of a gradient analysis, a line for the percent improvement in conditional peak capacity is displayed on the chart. Conditional peak capacity is the number of peaks that can be theoretically separated over a gradient time, and therefore is also dependent on peak width. See Equation 1.

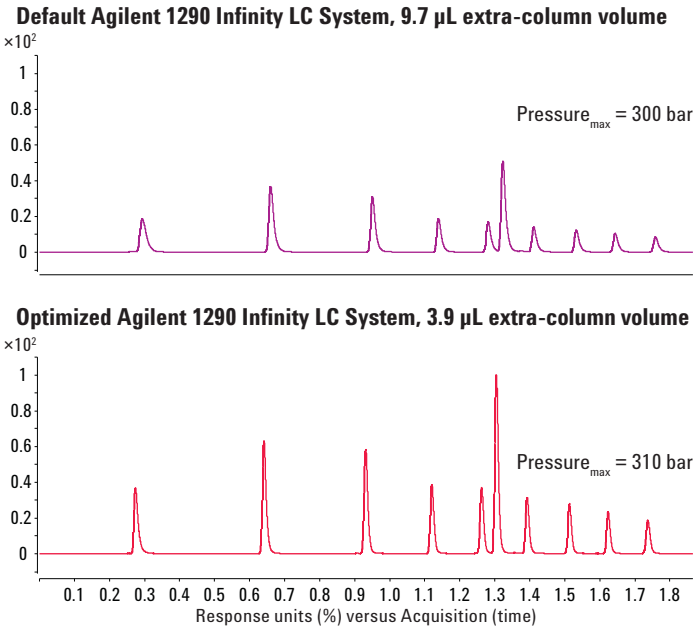
Equation 1. Conditional peak capacity.

$$\text{Conditional peak capacity} = n_c = \frac{t_{R,n} - t_{R,1}}{W}$$

$t_{R,n}$ and $t_{R,1}$: Retention times of the last and first eluting peaks

$$W: \frac{\overline{W_{½}}}{2.35} \times 4 \text{ (Average } 4\sigma \text{ peak width)}$$

$W_{½}$ is the average peak width at half height.



Mobile phase A	H_2O
Mobile phase B	CH_3CN
Flow rate	0.4 mL/min
Gradient	t (min) 0 1.2 %B 25 95
Sample	A 1-µL injection of RRLC checkout sample (p/n 5188-6529) spiked with 50 µL 2 mg/mL thiourea in water/acetonitrile
TCC	26 °C
DAD	Sig = 254, 4 nm; Ref = Off
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Analytes	1. Thiourea (v_o marker) 2. Acetanilide 3. Acetophenone 4. Propiophenone 5. Butyrophenone 6. Benzophenone 7. Valerophenone 8. Hexanophenone 9. Heptanophenone 10. Octanophenone

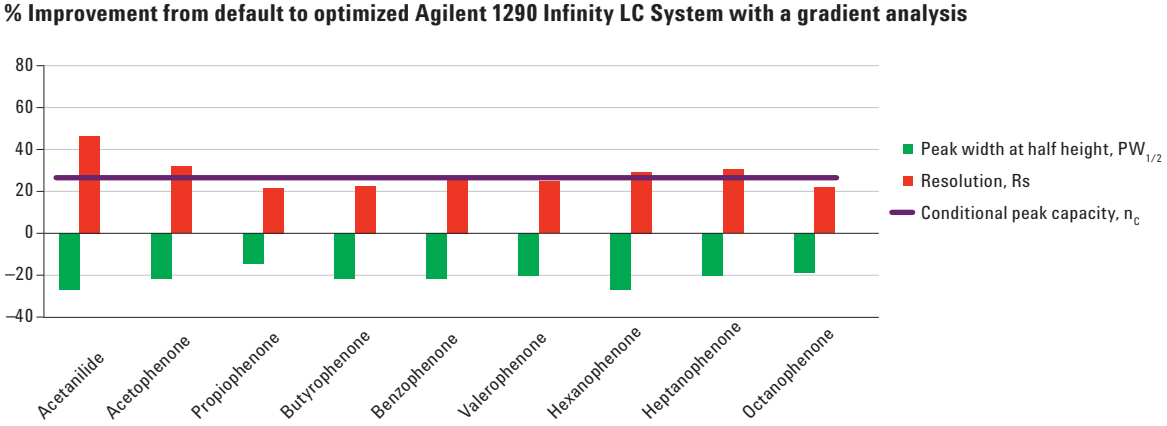


Figure 4. Effect of a 60% reduction in LC system extra-column volume on a gradient analysis of alkylphenones.

Reducing the extra-column volume of the LC system by 60% (5.8 µL) yields a 27% increase in conditional peak capacity of this gradient analysis. Furthermore, the narrower peaks improve the resolution of all peaks by >20%, including the critical pair (benzophenone and butyrophenone) by 27%.

Similar to Figure 3, Figure 5 is a scatter plot showing the percent improvement in resolution and conditional peak capacity for gradient analyses ranging from 0.1 to1 mL/min (gradient time is scaled according to column volume to maintain k' for all compounds). Once again, the first peak, acetanilide, elutes early and yields unusual values as compared to the other eight compounds and is therefore considered an outlier that is purposefully absent from this chart. Conditional peak capacity improves between 24 and 32%, while resolution improvements vary more and range from 18 to 37% (not including acetanilide). In general, it is evident that the flow rate of this fast gradient analysis has little to no impact on the effects of extra-column volume with respect to column performance.

When using the ultra-low dispersion 0.08 mm id capillaries with the Agilent 1290 Infinity LC System, system pressure increases, as shown in Figures 2 and 4. In this experiment, the observed pressure difference is 10 bar. However, if the Agilent LC System Rack was not used and longer 0.08 mm id capillaries (same length as the default setup) were required, the difference in system pressure would be greater than 10 bar. Because these capillaries are easily removable, and standard 0.12 mm id capillaries can be reinstalled, the Agilent 1290 Infinity LC System has significant flexibility. The 1200-bar pressure limit of the instrument can be used to accommodate very small 0.08-mm id capillaries for minimal sample band broadening, or the 0.12-mm id capillaries can be reinstalled and flow rates can be increased for higher sample throughput, whichever the method requirement.

% Improvement in resolution and peak capacity with a 60% reduction in LC System extra-column volume with a gradient analysis

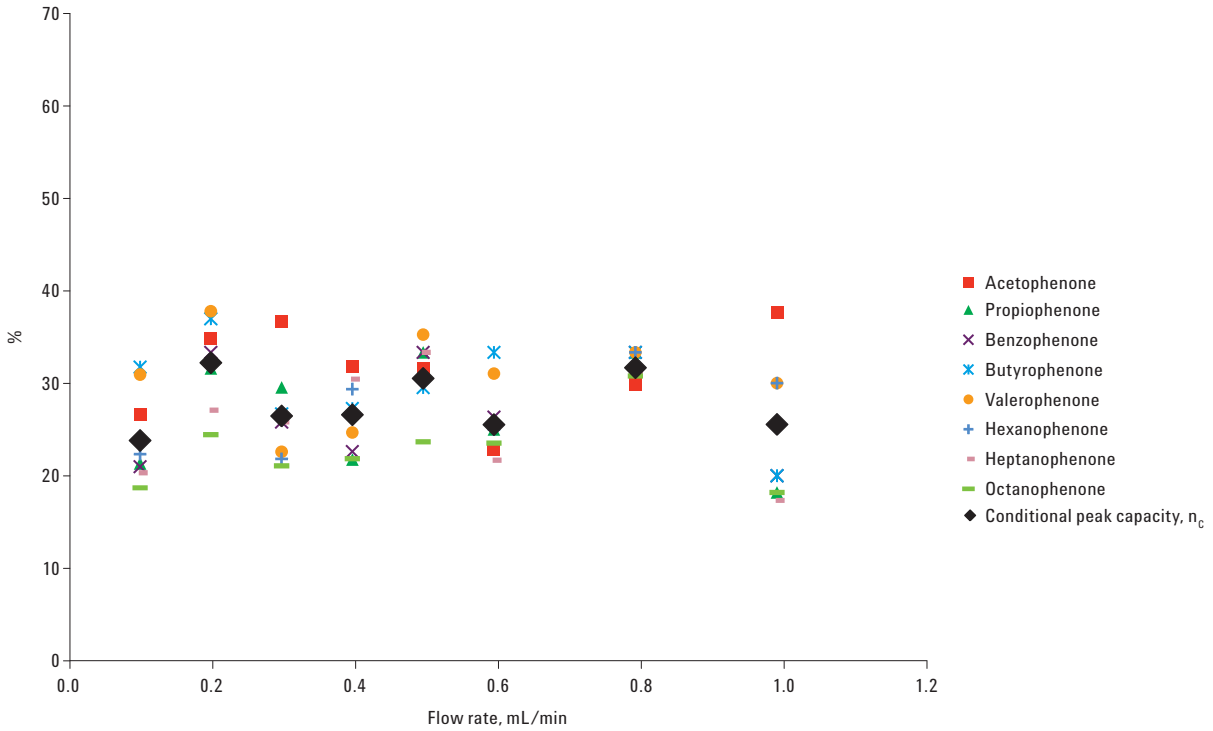


Figure 5. Scatter plot illustrating the effect of extra-column volume on resolution and conditional peak capacity over various flow rates for a gradient analysis of alkylphenones.

Conclusions

The performance of an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column is improved for isocratic and gradient analyses by using the new Agilent Ultra-Low Dispersion Capillaries, Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell (V(σ) = 0.6 µL), and Agilent LC System Rack to reduce extra-column volume in the Agilent 1290 Infinity LC System by 60% (5.8 µL). Conditional peak capacity in the gradient analysis increases by >24%. The efficiency of the isocratic analysis is more affected and increases by up to 58% for a compound with retention factor k' = 1.6. Additionally, the effects of extra-column volume are not further exacerbated by either increasing or decreasing flow rates.

Reference

1. W. Long and A. Mack. Reduce Tubing Volume to Optimize Column Performance. Agilent Publication 5990-4964EN. December 11, 2009. http://www.chem.agilent.com/Library/applications/5990-4964EN.pdf

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