Instrument, Method, and Sample Optimizations to Get the Most from Agilent InfinityLab Poroshell 120, 1.9 µm Columns

Application Note

General Analysis

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Abstract

Six compounds were analyzed through isocratic elution to demonstrate the effect of several instrument, method, and sample variables on column performance. A very efficient Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column capable of generating nearly 14,000 plates was used in this work. The impact on column performance of system capillaries, detector flow cells, data collection rates, injection volumes, sample solvents, and sample concentrations was studied.

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Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns generate high efficiency at lower pressure compared to their totally porous particle column counterparts [1]. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column [2]. The current trend with superficially porous particles is to reduce particle size for further efficiency improvements. The higher efficiency can be used to speed up analysis, or improve results by increasing resolution and sensitivity.

Small-dimension LC columns packed with small particles deliver increased productivity with faster analyses or greater resolution, reduced solvent usage, and better LC/MS and ELSD compatibility, compared to larger bore columns with 4.6 or 3 mm internal diameters, which 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 instrument, method, and sample must all be optimized.

Previous work 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.

However, the impact of extra-column volume will be more noticeable with small particle columns. 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 compared to a same-dimension 1.8 µm column, when data are normalized to account for percent efficiency decrease as a function of additional system volume [3].

Finally, the impact of reducing the LC system volume with small-dimension totally porous sub-2 µm particle columns is demonstrated [4-5]. This work includes optimizing the LC instrument, method, and sample for use with a highly efficient superficially porous Agilent InfinityLab Poroshell 120 EC-C18, 1.9 µm column.

Experimental

An Agilent 1290 Infinity LC System was used in this experiment. Table 1 shows the configuration details. For some experiments, the instrument was modified from this configuration. This information is provided as necessary and appropriate throughout this work. One Agilent LC column was also used in this experiment, and is listed in Table 1.

The LC method parameters for most analyses are shown in Table 2. In several experiments performed throughout this work, one parameter was varied to demonstrate the effect on chromatography; the data are labeled to indicate where modifications were made.

Table 1. UHPLC System Configuration

Agilent 1290 Infinity LC System Configuration	
Agilent 1290 Infinity Binary Pump (G4220A)	35 µL Solvent mixer: Jet weaver, 35 µL/100 µL (G4220-60006)
Agilent 1290 Infinity High Performance Autosampler (G4226A)	Seat assembly, ultra low dispersion, for Agilent 1290 Infinity Autosampler (G4226-87030) Autosampler → Heater: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784) Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (5182-0717) Vial insert, 250 µL, glass with polymer feet, 100/pk (5181-1270)
Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)	Heat exchanger, low dispersion, 1.6 µL, double (G1316-60005) Heater → Column: Agilent InfinityLab quick-connect assembly, 105 mm, 0.075 mm (5067-5961) Column → Flow cell: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell, 10 mm (G4212-60038)
Agilent OpenLAB CDS ChemStation Edition Revision C.01.05 [35]	G4220A: B.06.53 [0013] G4226A: A.06.50 [003] G1316C: A.06.53 [002] G4212A: B.06.53 [0013]
Agilent LC Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm (699675-902)

All analyses used instrument configuration shown above, unless stated otherwise.

Table 2. UHPLC Method Parameters

Column	Mobile phase	Flow rate (mL/min)	Elution	Injection volume (µL)	Sample	Thermostated Column Compartment (°C)	Diode Array Detector
Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm	20 mM sodium phosphate pH 7 in water with acetonitrile premixed 40/60	0.5	Isocratic	0.5	Uracil, butyl paraben, amitriptyline, naphthalene, dipropyl phthalate, acenaphthene see Table 3 for more information	25	254 nm, 80 Hz

All analyses used method parameters shown above, unless stated otherwise.

Six compounds were analyzed in this work, shown in Table 3. The test standard was prepared according to the concentrations in Table 3, unless stated otherwise. All analytes were purchased from Sigma-Aldrich. Sodium phosphate was also purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Table 3. Sample Information

Compound	Classification	Concentration in mobile phase	Retention factor (k')
Uracil	Void marker	n/a	n/a
Butyl paraben	Weak acid	0.05 mg/mL	1.3
Amitriptyline	Base	0.25 mg/mL	2.2
Naphthalene	Neutral	0.25 mg/mL	3.3
Dipropyl phthalate	Polar neutral	0.5 mg/mL	3.8
Acenaphthene	Neutral	0.5 mg/mL	6.1

All analyses used the sample shown above, unless stated otherwise.

Results and Discussion

All optimization experiments were done with the same standard, method, and system, except where noted otherwise. For each experiment, one variable was changed at a time to demonstrate the effect of that parameter on the efficiency of an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm column. For each experiment, efficiencies for butylparaben (k′ = 1.3), naphthalene (k′ = 3.3), and acenaphthene (k′ = 6.1) are illustrated to show the impact on performance of an early, mid, and late-eluting compound.

Instrument optimizations

Low-volume instrument configurations should be used with small-volume columns. The system volume of an Agilent 1290 Infinity LC was optimized in two steps. First, standard red 0.12 mm id capillaries were replaced with black 0.08 mm id capillaries. Second, the standard 1.0 µL flow cell was replaced with a 0.6 µL flow cell. Table 4 shows the details regarding the differences in system configuration. Figure 1 shows the results. In Figure 1A, there is a small shift in retention time observed for all peaks when the capillary volume is reduced. When the flow cell volume was reduced, all peaks became taller, narrower, and more efficient. Efficiency values are plotted in Figure 1B. System volume has a large impact on the efficiency of early eluting compounds. Both the capillaries and the flow cell substantially contribute to the LC system's volume. Therefore, both can individually impact the performance of a small-volume, high-efficiency column such as the InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 1.9 µm.

Subsequent experiments in this work used the smaller LC system volume configuration in Table 4.

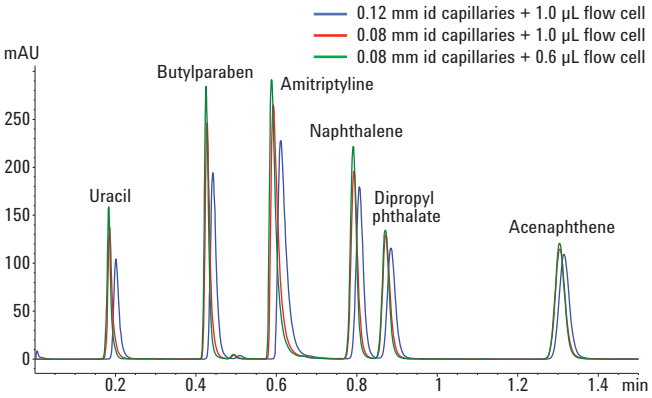

Figure 1A. The performance of an Agilent InfinityLab Poroshell 1.9 µm column is improved when LC system volume is reduced by using smaller internal diameter capillaries and a smaller volume detector flow cell.

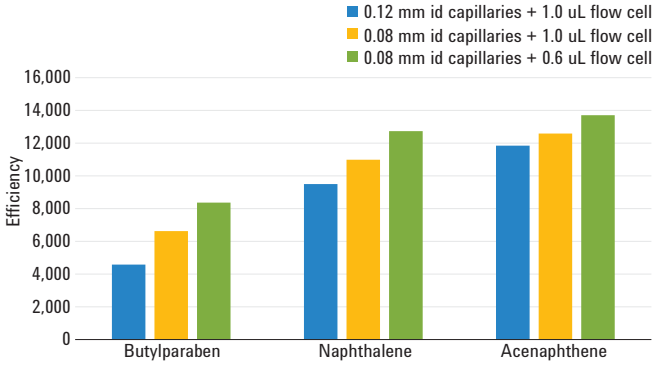

Figure 1B. The performance of an Agilent InfinityLab Poroshell 1.9 µm column is improved when LC system volume is reduced by using smaller internal diameter capillaries and a smaller volume detector flow cell.

Table 4. Instrument Modifications for Capillary and Flow Cell Comparisons

Agilent 1290 Infinity LC system configuration modifications	Larger system volume	Smaller system volume
Agilent 1290 Infinity High Performance Autosampler (G4226A)	Autosampler → Heater: Capillary, stainless steel, 0.12 × 340 mm (5067-4659)	Autosampler → Heater: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)	Heat exchanger, low dispersion, 1.6 µL, double (G1316-60005) Heater → Column: Agilent InfinityLab quick-connect assembly, 105 mm, 0.075 mm (5067-5961) Column → Flow cell: Capillary, stainless steel, 0.12 × 340 mm (5067-4659)	Heat exchanger, low dispersion, 1.0 µL, long, down (G1316-80012) Heater → Column: Agilent InfinityLab quick-turn fitting (5067--5966) Column → Flow Cell: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Agilent Standard Max-Light Cartridge Flow Cell, 1.0 µL, 10 mm (G4212-60008)	Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell, 0.6 µL, 10 mm (G4212-60038)

Method optimizations

After optimizing your LC system hardware, consideration should also be given to optimizing your method. When it comes to high efficiency columns such as the 1.9 µm InfinityLab Poroshell, detector data collection rate and its impact on column performance is commonly discussed. Data collection rate is the frequency at which the instrument takes measurements throughout an analysis. It is critical that a sufficient number of data points is measured across a chromatographic peak to accurately reflect the efficiency of that column. Too few data points will show artificially broad peaks, as shown in Figure 2A with butylparaben. In this example, we test the data collection rate of a diode array detector; however, other LC detectors also have a sampling rate that needs to be set in the method. Note that a default method in ChemStation has the diode array detector data collection rate set to 2.5 Hz; when building a new method for the 1.9 um Poroshell, it is imperative that the rate is increased to reflect the performance of the column. According to Figure 2B, this method should use a rate of at least 40 Hz; higher data collection rates could increase baseline noise and reduce method sensitivity.

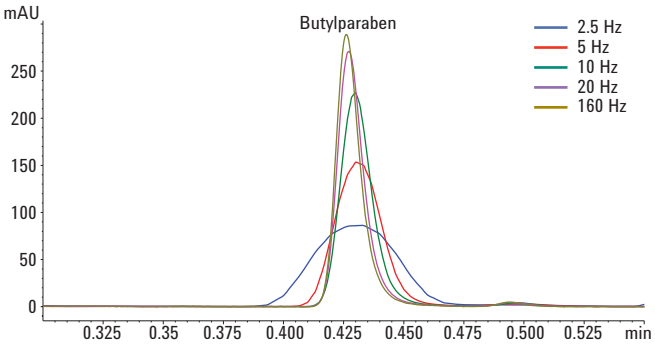

Figure 2A. Fast data collection rates must be used with Agilent InfinityLab Poroshell 1.9 µm columns to accurately measure the efficiency of the column, especially for early eluting compounds such as butylparaben (k′ = 1.3).

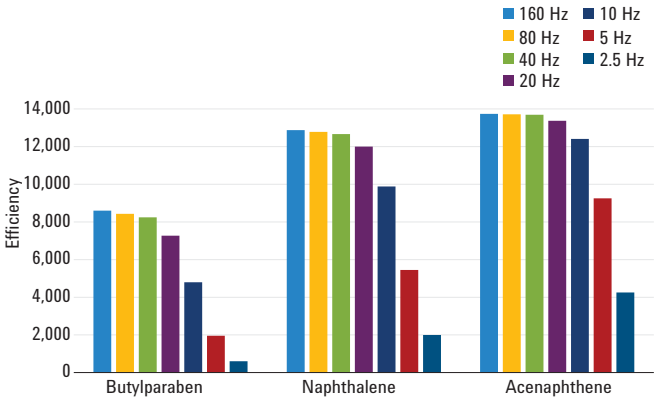

Figure 2B. Fast data collection rates must be used with Agilent InfinityLab Poroshell 1.9 µm columns to accurately measure the efficiency of the column, especially for early eluting compounds such as butylparaben (k′ = 1.3).

Another method parameter that can be used to optimize performance of a small-volume column is injection volume. The injection volume can be considered with the overall system volume, and should be kept as small as reasonably possible for your analysis. Figure 3A shows the effect that injection volume can have on chromatography. Comparing a 0.5 µL injection to a 16 µL injection with this 2.1 × 50 mm column shows a big difference in peak width and chromatographic performance. Efficiency values (Figure 3B) demonstrate the same trend that we saw with LC system volume. Larger injection volumes lead to broader peaks, and this effect is more pronounced with early eluting compounds. To isolate the impact of injection volume from possible effects of sample loading, the same amount of sample was injected onto the column throughout this experiment. The original sample was serially diluted with mobile phase, while the injection volume was appropriately scaled. Figure 4 illustrates that the same amount of sample was injected onto the column regardless of the injection volume being tested; this is shown through constant area counts for each compound.

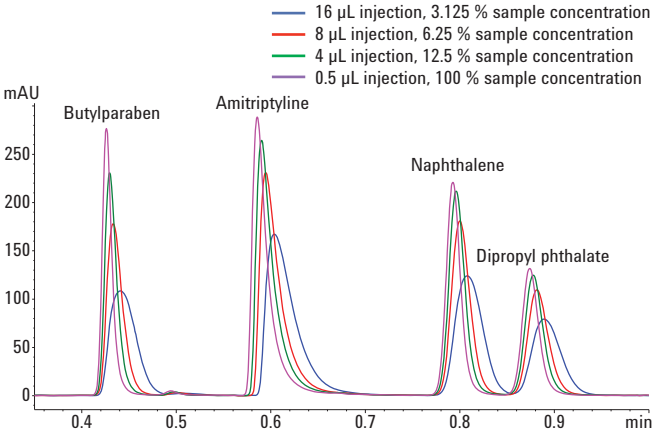

Figure 3A. Injection volumes contribute to overall system volume, and must be kept small to preserve the performance of high-efficiency columns such as a 1.9 µm Agilent InfinityLab Poroshell.

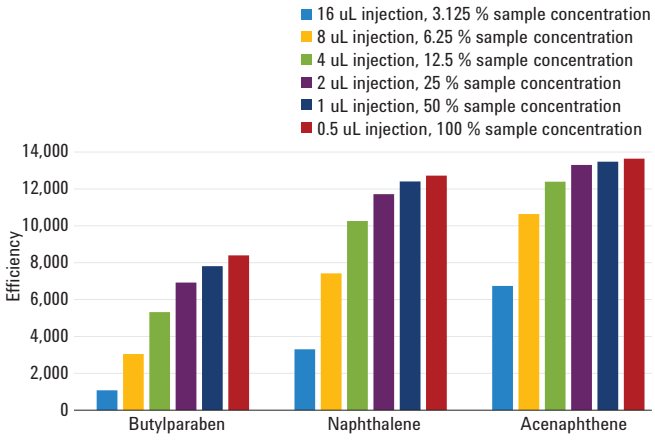

Figure 3B. Injection volumes contribute to overall system volume, and must be kept small to preserve the performance of high-efficiency columns such as a 1.9 µm Agilent InfinityLab Poroshell.

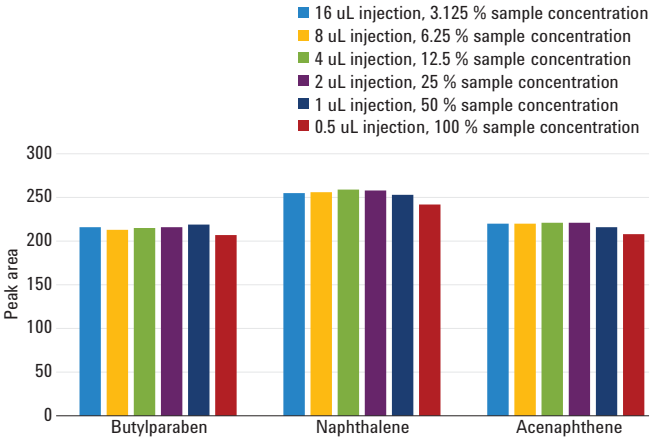

Figure 4. Measuring peak areas shows that the same amount of sample was injected onto the column in this injection volume comparison, reducing the possibility of other variables such as sample loading, and allowing the impact of injection volume to be isolated and studied.

Sample optimizations

In addition to instrument and method optimizations, samples can also be optimized to improve the observed performance of a column. The solvent in which your sample exists can affect chromatographic performance. In Figure 5A, samples were diluted 1:10 in various solvents, then 1.5 µL was injected. Some solvents, such as THF and DMSO, create large baseline disturbances at 254 nm, which could be problematic for very early eluting compounds. The larger issue with sample solvent is the potential for peak shape distortion, as seen with dipropyl phthalate when the sample solvent is THF or IPA. THF also greatly alters the peak shape for the remaining peaks. To prevent peak shape issues related to sample solvent, ensure that your sample is prepared in a solvent of equal or lesser strength than the mobile phase. Figure 5B demonstrates the effect of sample solvent on efficiency. Strong solvents such as THF, DMSO, and IPA greatly reduce efficiency by broadening the chromatographic peaks. The best performance comes from samples made up in mobile phase or water. Interestingly, water sample gave the best results. This is likely due to the sample being focused on the column. One problem with water, however, was that solubility was an issue for some analytes in this sample. The chromatograms in Figure 5A show low peak heights for naphthalene and acenaphthene, as they have poor solubility in water.

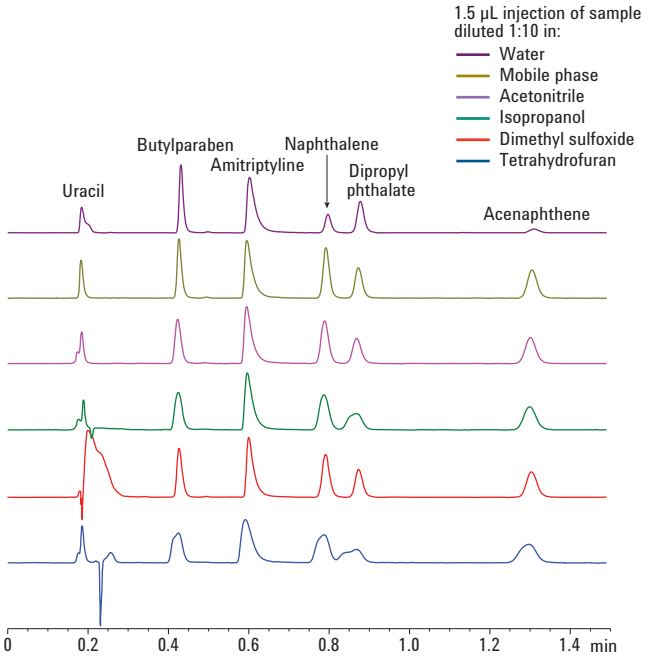

Figure 5A. Sample solvents should be of equal or lesser strength than the mobile phase, otherwise poor peak shape can occur, resulting in poor efficiency. Choosing the correct sample solvent strength becomes more critical as injection volume increases especially for low-volume columns such as an Agilent InfinityLab Poroshell 2.1 × 50 mm, 1.9 µm column.

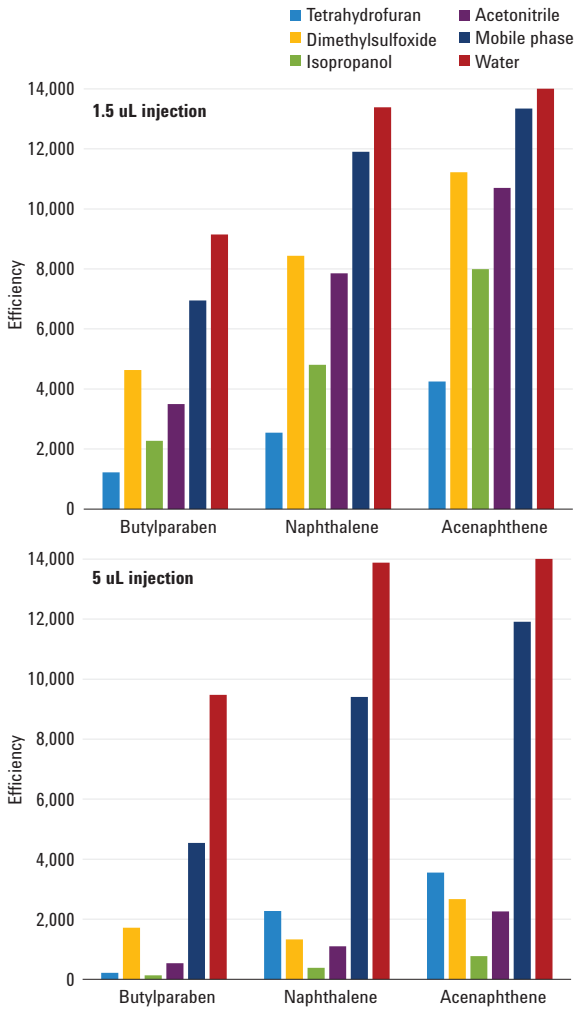

Figure 5B. Sample solvents should be of equal or lesser strength than the mobile phase, otherwise poor peak shape can occur, resulting in poor efficiency. Choosing the correct sample solvent strength becomes more critical as injection volume increases especially for low-volume columns such as an Agilent InfinityLab Poroshell 2.1 × 50 mm, 1.9 µm column.

The concentration of the analytes in the sample can also affect method performance. For the experiment shown in Figure 6, amitriptyline was prepared in mobile phase at various concentrations; all other parameters were held constant. Figure 6A illustrates how peak shape and retention time vary as the sample is loaded onto the column. In this case, performance was measured by efficiency and peak tailing, which are plotted in Figure 6B. There is no clear best concentration of amitriptyline to load onto the column. Ideally, one would choose a concentration at which efficiency was high and tailing low. All analytes will have their own unique behavior for sample loading, so it is important to consider compounds individually.

For the best performance from an InfinityLab Poroshell 2.1 × 50 mm, 1.9 µm column, sample solvent and analyte concentration should be optimized as best as possible for a given analysis. However, some analyses may not have the ability to change sample characteristics such as these.

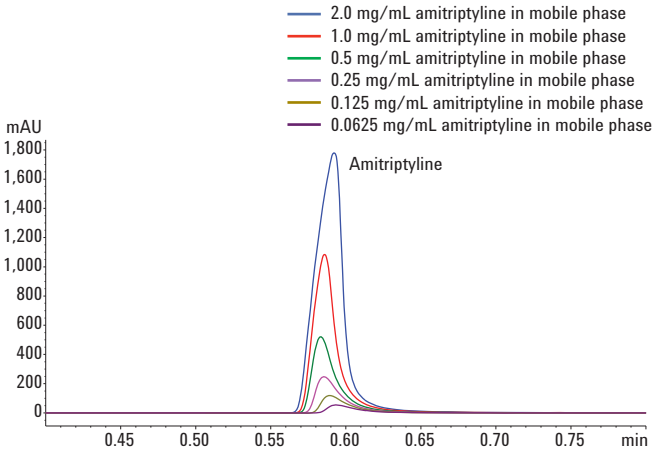

Figure 6A. Method performance can vary by analyte and by how much sample is loaded onto the column.

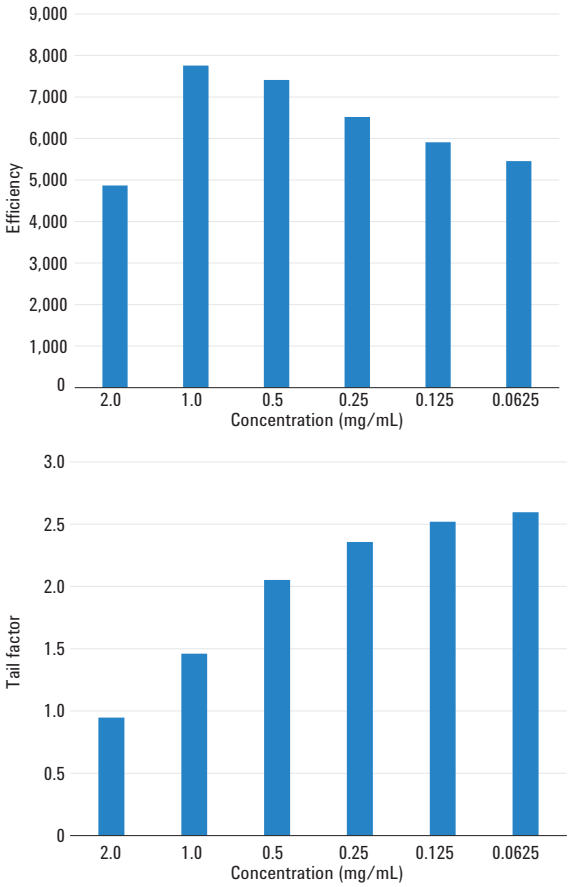

Figure 6B. Method performance can vary by analyte and by how much sample is loaded onto the column.

Conclusions

The highly efficient Agilent InfinityLab Poroshell 1.9 µm column is a power chromatographic separation tool. However, care should be taken to optimize the LC instrument, method, and sample to ensure that the full resolving power is realized. Instruments should be configured with low-volume capillaries and flow cells. Method data collection rates should be set sufficiently high, and injection volumes should be kept as low as feasible. Sample solvents should be of equal or lesser strength compared to the mobile phase, and analyte concentration should be low enough that it does not overload the column and impair peak shape.

References

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