Application Note

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Analysis of Polar Compounds Using an Agilent InfinityLab Poroshell 120 Aq-C18 Column with Improved and Reliable Performance

Authors

Rongjie Fu and Ta-Chen Wei
Agilent Technologies, Inc.

Abstract

A group of polar compounds was separated on a newly developed Agilent InfinityLab Poroshell 120 Aq-C18 column. The retention loss due to pore dewetting was investigated by comparing the new InfinityLab Poroshell 120 Aq-C18 column to the current C18 phase and other superficially porous columns from various manufacturers.

Introduction

Analysis of polar analytes using reversed-phase columns can have problems with poor retention, distorted peak shapes, and coelution of early eluted peaks. Typically, a conventional C18 reversed-phase column is used for mid- to nonpolar compounds due to the hydrophobic characteristics of C18 ligands. Often, a highly aqueous mobile phase is used to improve the retention and separation of polar compounds with a nonpolar C18 stationary phase. However, it is not recommended to go directly with an organic composition of less than 5% or even 100% aqueous mobile phase. The use of a highly aqueous mobile phase leads to a dramatic loss in retention due to pore dewetting, and this phenomenon can be accelerated by turning off the flow for a short period of time.[1] In a previous investigation, the loss of retention in the aqueous mobile phase was found to be affected by particle pore size, bonded phase chemistry, and bonded stationary-phase density.

To better retain polar compounds and reduce pore dewetting when using a highly aqueous mobile phase with a C18 bonded phase, Agilent has developed a new InfinityLab Poroshell 120 Aq-C18 column. An optimized C18 ligand with proprietary endcapping is applied to 2.7 µm Poroshell particles with a pore size of 120 Å to significantly improve the retention, peak shape, and reproducibility of polar analyte analysis with minimized pore dewetting.

This application note demonstrates the increased retention and symmetrical peak shapes of six polar compounds separated under an isocratic method on an InfinityLab Poroshell 120 Aq-C18 column. The study measured the retention loss after the pump stopped for 10 minutes and compared it with other C18 columns from various manufacturers.

Experimental

Instruments and materials

An Agilent 1290 Infinity LC system with a binary pump was used in this experiment. The system includes:

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity autosampler (G4226A)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)
- Agilent 1290 Infinity diode array detector (G4212A)

All reagents and solvents were HPLC grade. Acetonitrile, ammonium formate, formic acid, and the six compounds were purchased from Anpel Laboratory Technologies, Shanghai. Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). The compounds in Figure 1 were all dissolved in water and mixed to achieve a concentration of 0.17 mg/mL respectively.

Method parameters for the experiment are detailed in Table 1.

Table 1. Method parameters.

Parameter	Value
Mobile Phase	A) 25% water, 75% acetonitrile B) 10 mM ammonium formate, pH 3.0
Column Dimensions	4.6 × 100 mm
Column Temperature	25 °C
Flow Rate	1.2 mL/min
Detector	UV 254 nm
Injection Volume	2 µL injection

The following procedure was used for all tests. Mobile phase A was pumped through each column for 20 minutes to ensure that the column was fully equilibrated. Mobile phase B was then pumped for 20 minutes followed by duplicate injections of the test sample. After the analysis was complete, the flow was turned off for 10 minutes. The flow was then turned back on for 2 minutes, followed by duplicate injections of the test sample. The ratio of retention time before and after the pump stop was calculated and compared.

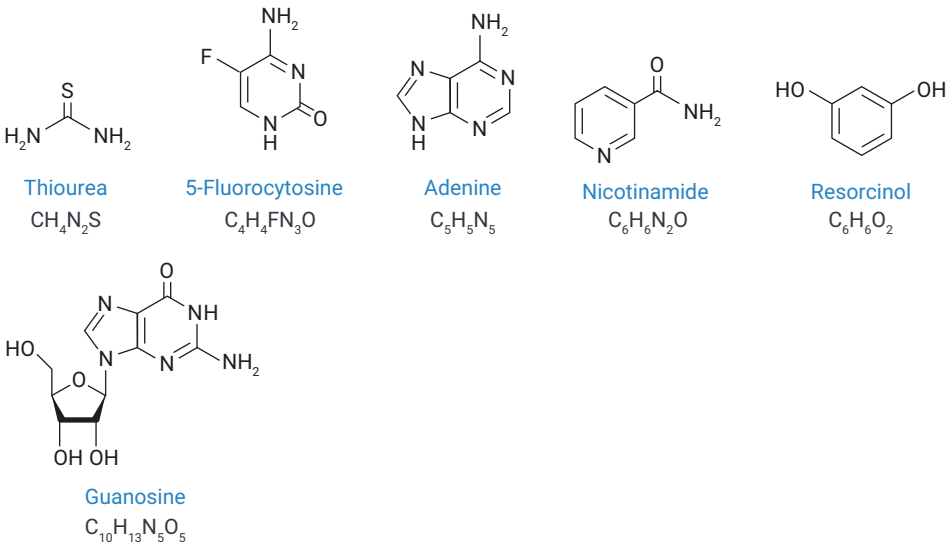

Figure 1. Compounds analyzed in this study.

Results and discussion

With an isocratic method using 100% formate buffer, the six polar compounds were separated on an InfinityLab Poroshell 120 Aq-C18 column, other superficially porous, polar-modified C18 columns from various vendors, and a high-density InfinityLab Poroshell 120 EC-C18 column. Table 2 shows the columns tested with packing material parameters.

Figure 2 shows the results of the separation of the six compounds on the selected columns. The chromatograms demonstrate that the InfinityLab Poroshell 120 Aq-C18 has stronger retention for these polar compounds than any of the other polar C18 columns tested. This improved retention may result in better peak characterization without peak-shape distortion, which is sometimes found for early eluted compounds. The InfinityLab Poroshell 120 Aq-C18 had more symmetrical peak shape than the other columns, especially for basic compounds such as adenine and nicotinamide.

Table 2. Columns tested in the study.

Column	Particle Size (µm)	Surface Chemistry	Pore Size (Å)	Retention Loss of Peak 6 (%)
Agilent InfinityLab Poroshell 120 Aq-C18	2.7	Optimized C18 and endcapped surface	120	–1.6
Agilent InfinityLab Poroshell 120 EC-C18	2.7	High-density, endcapped C18 surface	120	–41.1
Vendor A	2.7	Optimized C18 concentration	120	–11
Vendor B	2.6	Combined C18 and polar-modified surface	100	–4.2
Vendor C	2.6	C18 polar endcapped surface	80	–39.8

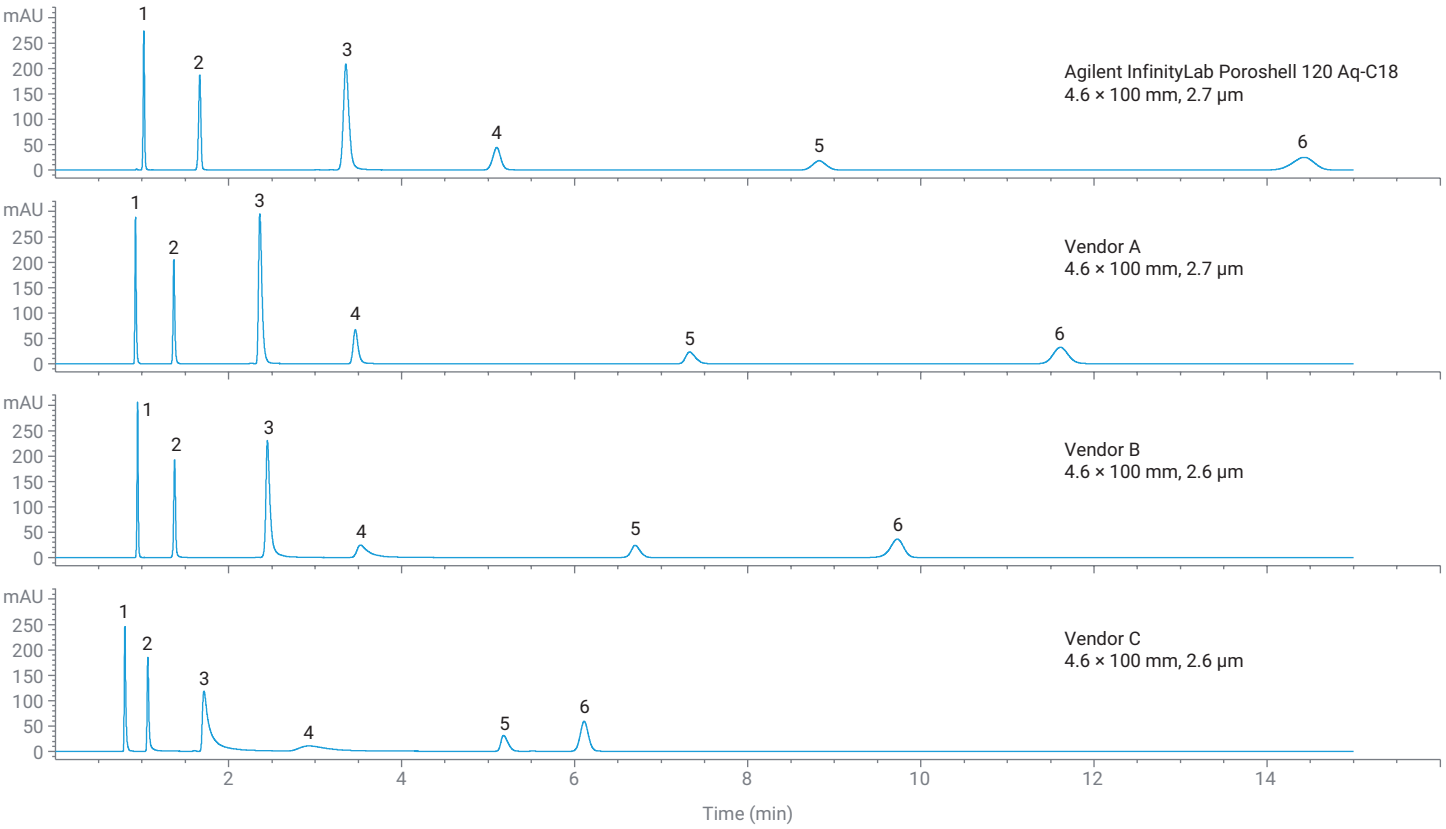

Figure 2. Comparison of the separation of six polar compounds under 100% aqueous buffer mobile phase.

Figures 3A to 3E compare the chromatograms before and after the 10-minute pump stop on the InfinityLab Poroshell 120 Aq-C18 and EC-C18 columns, and the three different vendor columns. The percentage of instant retention time lost on all columns was calculated from the retention time measured before and after the pump stop. The calculated data shown in Table 2 indicate the impact of pore dewetting. The InfinityLab Poroshell 120 Aq-C18 had the lowest retention loss compared to other superficially porous columns. However, the InfinityLab Poroshell 120 EC-C18 shows the highest retention loss because of the high-density C18 endcapped phase, which is not recommended for use with less than 5% organic mobile phase.

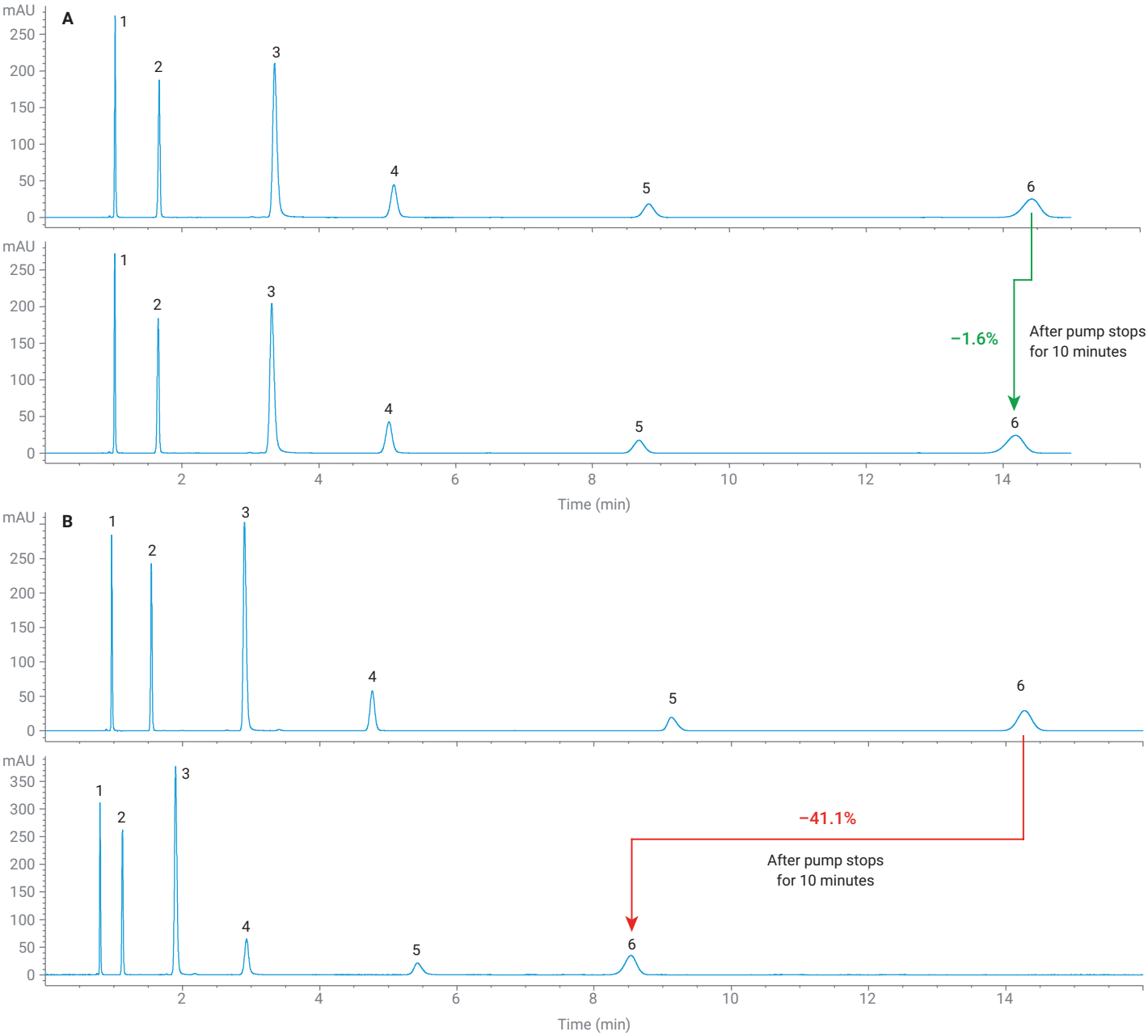

Figure 3A,B. Retention time loss after pump stop for 10 minutes on (A) Agilent InfinityLab Poroshell 120 Aq-C18 and (B) Agilent InfinityLab Poroshell 120 EC-C18 columns. Compounds: (1) thiourea, (2) 5-fluorocytosine, (3) adenine, (4) nicotinamide, (5) resorcinol, (6) guanosine.

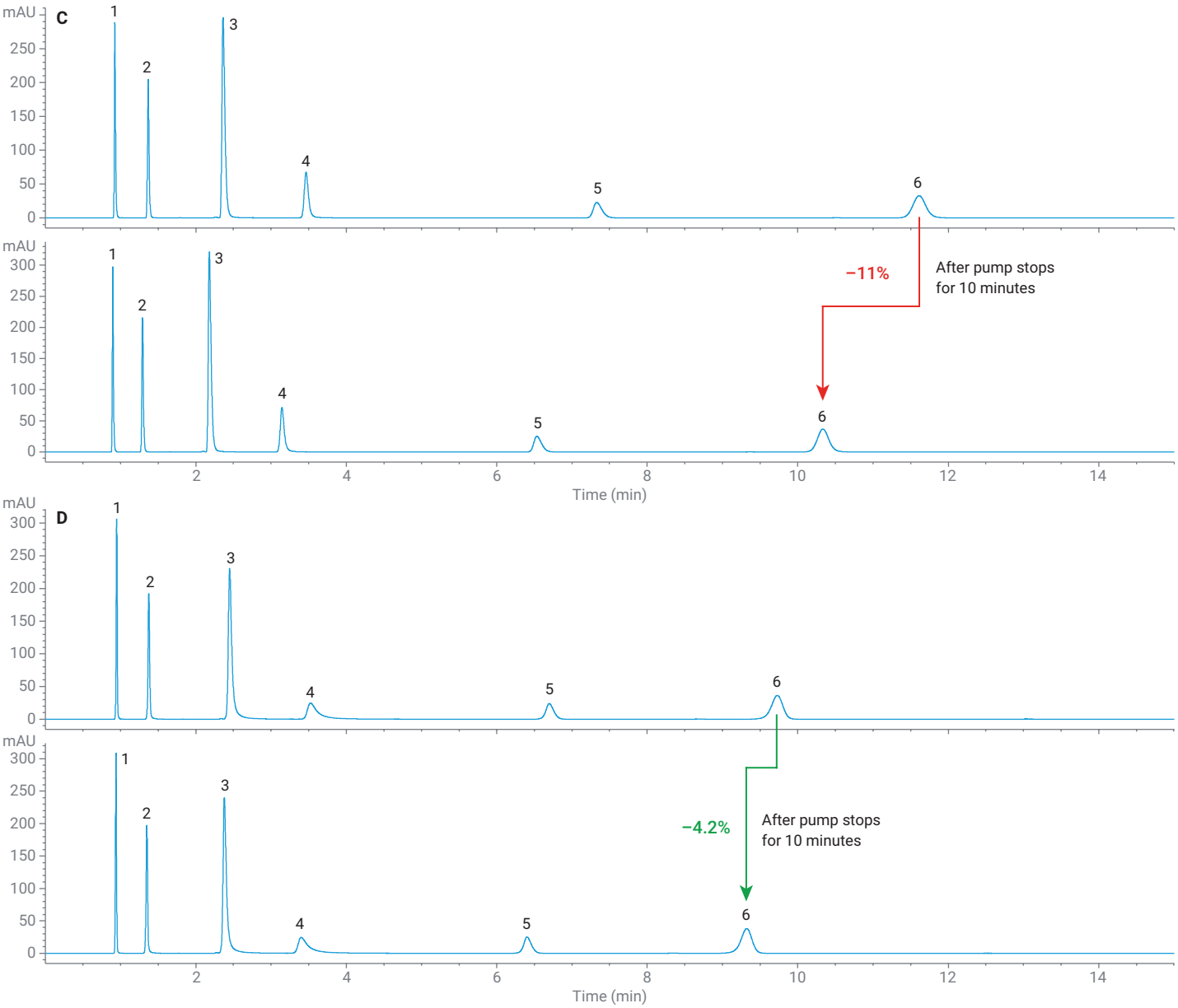

Figure 3C,D. Retention time loss after pump stop for 10 minutes on the (C) vendor A and (D) vendor B columns, under 100% aqueous buffer. Compounds: (1) thiourea, (2) 5-fluorocytosine, (3) adenine, (4) nicotinamide, (5) resorcinol, (6) guanosine.

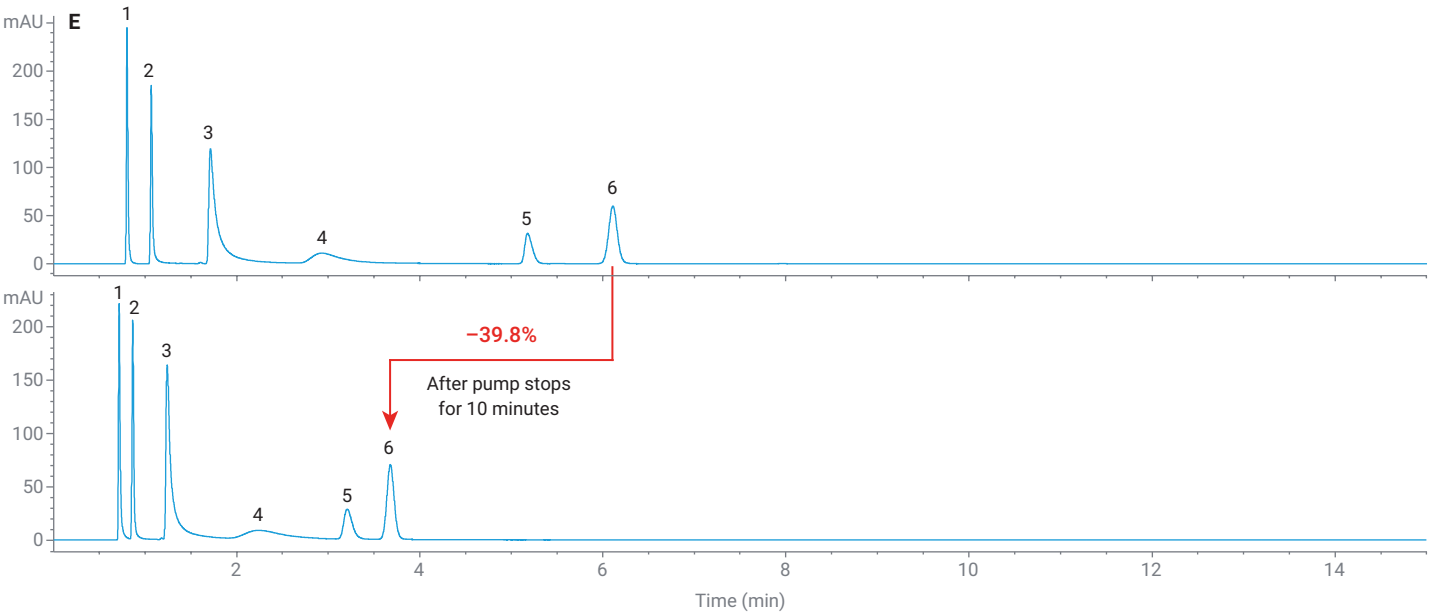

Figure 3E. Retention time loss after pump stop for 10 minutes on the vendor C column. Compounds: (1) thiourea, (2) 5-fluorocytosine, (3) adenine, (4) nicotinamide, (5) resorcinol, (6) guanosine.

Conclusion

With the optimized bonding chemistry and particle properties, the newly developed Agilent InfinityLab Poroshell 120 Aq-C18 column provides adequate retention for polar compounds with improved peak shape. This column is compatible with 100% aqueous mobile phase without significant retention loss from pore dewetting, which makes it a reliable choice for the analysis of polar analytes.

Reference

1. Bidlingmeyer, B. A.; Broske, A. D. The Role of Pore Size and Stationary Phase Composition in Preventing Aqueous-Induced Retention Time Loss in Reversed-Phase HPLC. *J. Chromatog. Sci*. **2004**, *42(2)*, 100–106. DOI: https://doi.org/10.1093/chromsci/42.2.100

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DE27725953

Printed in the USA, November 23, 2022
5994-5555EN

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