Extending Column Lifetime in Pharmaceutical Methods with High pH-Stable InfinityLab Poroshell HPH Chemistries

Technical Overview

Introduction

HPLC analyses play an important part in pharmaceutical method development and quality control. Recently, the inventions of sub-3 µm superficially porous particles, such as InfinityLab Poroshell 120, and hybrid particles were identified among the top three significant developments in pharmaceutical analysis. Combining these two innovations, a high-pH-stable superficially porous material is introduced by Agilent Technologies, Inc. In this work, we demonstrate the stability of a sub-3 µm organically modified superficially porous column across its operational pH range.

It is generally recommended that reversed-phase method development begin with low pH mobile phases, whether the analytes are acidic, neutral, or basic. There are several good reasons for following these guidelines. At low pH, acidic analytes will be neutral and well retained due to the residual silanols on the silica surface of the packing being protonated. Therefore, there will be fewer secondary interactions between acidic and basic analytes and the silica surface. Unfortunately, basic compounds, which carry a positive charge at low pH, will often be poorly retained, or have poor peak shape under these conditions. Historically, another reason for using low pH is the poor stability of silica columns at high pH.

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The stability of an HPLC column is one of the critical factors that determine the success of a method. During the development of an reversed phase LC (RPLC) analysis protocol, and anticipating its validation, chromatographers usually consider several issues. One of the most important is column lifetime under a specific set of analysis conditions. Silica has many properties that make it excellent as a support for reversed-phase HPLC columns. However, its solubility increases substantially as the mobile phase reaches pH 7-8 and above. In a study of high pH silica HPLC column stability at Rockland Technologies, several key findings were made; 1. end-capping protected the silica from dissolution, 2. densely bonded phases increased column stability, and 3. organic mobile phase buffers such as tris yielded significantly longer column life than phosphate buffers at similar pH. Studies have shown that bonded-phase packing degradation at pH 9-10 was mainly due to silica support dissolution, and did not primarily result from the hydrolysis of covalently siloxane bonds. In principle, the chemical and thermal stability of RP columns can be enhanced by the improvement of substrates and bonding chemistry [2,3].

Two approaches have been made to achieve high pH stability in silica HPLC columns. One way to increase stability is to employ special bonding chemistry, as in the Agilent ZORBAX Extend C18 column [4]. ZORBAX Extend C18 uses a bidentate bonding to protect the silica from dissolution at high pH. Another way to achieve high pH stability is to modify the silica itself, making it less soluble under those conditions. Using this approach, 2.7 µm InfinityLab Poroshell 120 particles are organically modified, making them less susceptible to attack at high pH.

Materials and Methods

An Agilent 1260 Infinity LC was used for this work.

- G1312B Binary Pump
- G1367C Automatic Liquid Sampler (ALS)
- G1316C Thermostatted Column Compartment (TCC) SL
- G4220A Diode Array Detector (DAD) (10-mm path, 1-µL volume)
- Open Lab version C.01.05 was used to control the HPLC and process the data
- Agilent InfinityLab Poroshell 120 HPH-C18, 2.1 × 50 mm, 2.7 µm (p/n 699775-702)
- Agilent InfinityLab Poroshell 120 HPH-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-702)
- Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (p/n 699775-902)
- Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)

Samples contained quinine, nortriptyline, amitriptyline, benzyl alcohol, phenol, hexanophenone, acetophenone, 4-chlorocinnamic acid, methyl salicylate, uracil, naphthalene, and butyl benzene. Mass-spec-compatible mobile phases, consisting of volatile buffers such as ammonium formate buffer and ammonium bicarbonate buffer, were used. These buffers were prepared by dissolving sufficient ammonium formate or ammonium bicarbonate in water to produce 10 mM solutions and adjusting the solutions to the desired pH with the appropriate concentrated formic acid or concentrated base (ammonium hydroxide). Solvents were made using only formic acid or ammonium hydroxide as mobile phase modifiers. A nonvolatile phosphate buffer was used as well. Sodium phosphate dibasic and sodium phosphate monobasic used to produce buffer were purchased from Sigma-Aldrich, Corp. Methanol and acetonitrile was Burdick and Jackson, purchased from Honeywell. Water was Millipore 18 MΩ.

The columns were tested with gradients at low pH with 0.2% formic acid/acetonitrile, and at high pH with 10 mM ammonium formate/acetonitrile at pH 10, designed to simulate normal column use, or 0.1% ammonium hydroxide [5,6]. An isocratic test at pH 10 was designed to stress the column aggressively by attacking the silica.

The Van Deemter curves were generated at different flow rates using a 4.6 × 50 mm, 2.7 µm InfinityLab Poroshell 120 HPH-C18 column. Mobile phase was 60% CH_3CN:40% H_2O, flow rate was 0.05 to 2.5 mL/min, injection was 1 µL naphthalene, and temperature was 25 °C. The UV detector operated at 254 nm, 80 Hz.

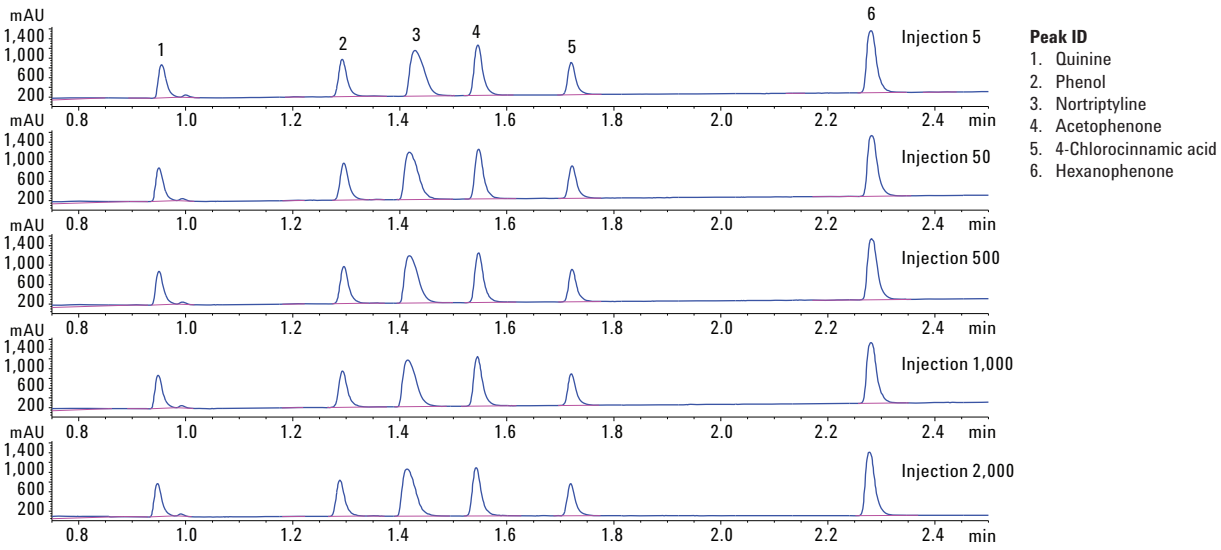
Results and Discussion

Stability testing was divided into two categories; normal mobile phases and stress mobile phases. Normal mobile phases included solvents and conditions that are regularly used in the laboratory. These included the formic acid, ammonium hydroxide and ammonium bicarbonate at 25 °C. These data give users of the column a basis for comparison to their own work. Stress mobile phase conditions were not typically used in the laboratory and are often considered extreme and detrimental to silica-based columns. In this case, ammonium bicarbonate at pH 10 at 50 °C and sodium phosphate, pH 8 at 65 °C, were designed to accelerate destruction of the column, to show the improvement of chemical stability of InfinityLab Poroshell 120 HPH-C18 within a reasonable time.

Normal HPLC conditions

Stability in 0.2% formic acid

In the first experiment, a new column was subjected to 2,000 injections. A sample containing quinine, phenol, nortriptyline, acetophenone, 4-chlorocinnamic acid, and hexanophenone was injected every 4 minutes. The sample contained the typical acid, base, and neutral compound types found in almost all difficult samples. Formic acid at 0.1% is one of the most ubiquitous chromatography solvents (pH ~2.8). Using more formic acid in the mobile phase (0.2% instead of 0.1%) lowers the mobile phase pH and places additional stress on the column. The formic acid in the acetonitrile was added to allow easier integration of the peaks. It can be seen that the baseline was flat and the peaks were all retained at the same elution volume throughout the experiment. This demonstrated that the InfinityLab Poroshell 120 HPH-C18 column was stable and usable for routine analyses at low pH (Figure 1).



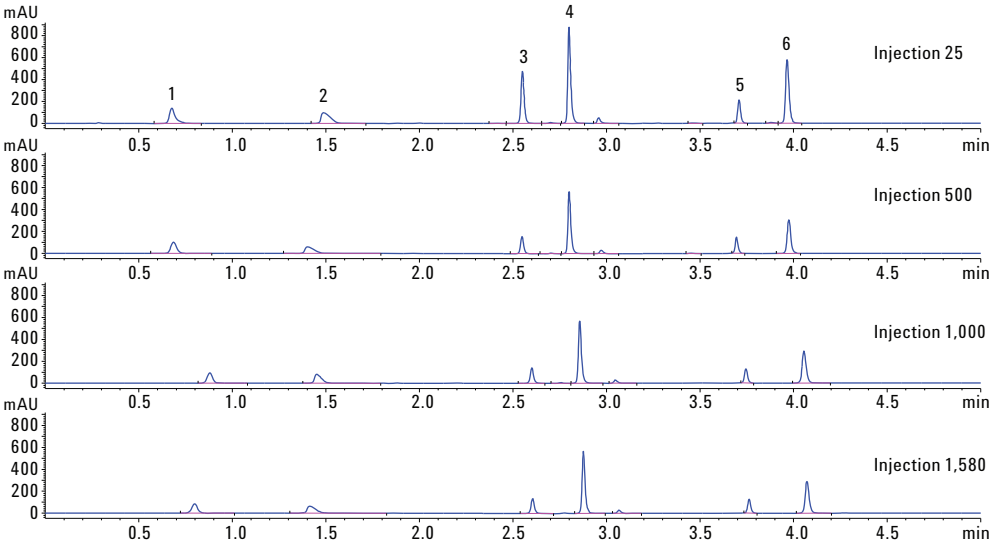
Conditions

Column:	Agilent InfinityLab Poroshell 120 HPH-C18, 2.1 × 50 mm, 2.7 µm
Eluent:	A) 0.2 % formic acid in water
	B) 0.15% acetonitrile
Flow rate:	0.4 mL/min
Gradient:	Time (min) % B
	0 5
	3 95
	3.5 5
Total run time:	4 min

Figure 1. Agilent InfinityLab Poroshell 120 HPH-C18 with a 0.2% formic acetonitrile gradient and 2,000 injections.

Stability in 0.1% ammonium hydroxide, pH 10.5

Ammonium hydroxide at pH 10.5 is another common mobile phase for LC/MS. And, unlike ammonium bicarbonate, it is compatible with silica columns. It is also compatible with preparative HPLC methods, as it is quite volatile. The samples used and elution order were similar to that achieved with an ammonium bicarbonate buffer. In this trial, over 1,500 injections were made on the column, over an eight-day period. The retention times of the peaks remained constant. However, the peak shapes of the first two peaks 5-methyl salicylaldehyde and 4-chlorocinnamic acid, were not as good as the peaks in the buffered ammonium bicarbonate mobile phase, mostly due to lower buffer capacity (Figure 2).



Peak ID

1. Methyl salicylate
2. 4-Chlorocinnamic acid
3. Acetophenone
4. Quinine
5. Nortriptyline
6. Heptanophenone
7. Amitriptyline

Conditions

Column:	Agilent InfinityLab Poroshell 120 HPH-C18, 2.1 × 50 mm, 2.7 µm
Eluent:	A) 10 mM ammonium hydroxide adjusted to pH 10.5 in water
	B) acetonitrile
Flow rate:	0.4 mL/min
Gradient:	Time (min) % B
	0 5
	3 95
	3.5 5
Total run time:	4 min

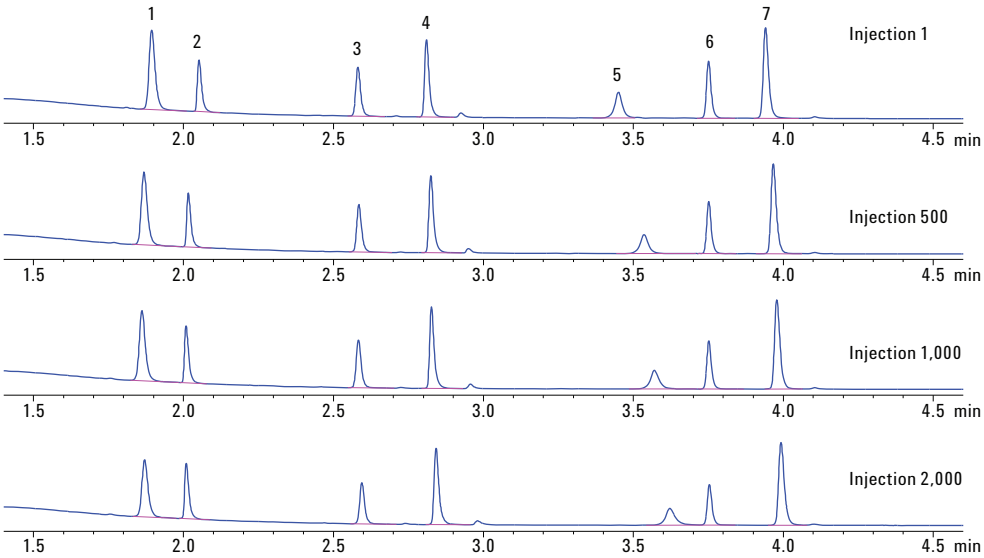
Figure 2. Agilent InfinityLab Poroshell 120 HPH-C18 with a 0.1% ammonium hydroxide gradient and 1,500 injections.

Stability in 10 mM ammonium bicarbonate, pH 10

A second experiment was performed using 10 mM ammonium bicarbonate at pH 10. This is a mobile phase commonly used with hybrid columns but not typically used with standard silica HPLC columns. This mobile phase helps to control pH as it has good buffering capacity and it allows use of MS detection as the buffer is volatile. It has been reported that phosphate and carbonate buffers damage silica columns far more than buffers such as glycine and borate [5]. In this case, methyl salicylate, 4-chlorocinnamic acid, acetophenone, quinine, nortriptyline, heptanophenone, and amitriptyline were used as test samples. Most of these test materials were also used in the formic acid test sample. A big difference between these two tests was the elution order. By changing the pH of the mobile phase, the elution order of analytes changed, indicating a drastic change in selectivity.

In this experiment, an InfinityLab Poroshell 120 HPH-C18 column was evaluated with a gradient method using ammonium bicarbonate and acetonitrile at pH 10. As can be seen, the retention time of all compounds remained stable throughout the 2,000 injection run with the exception of nortriptyline.

A second, non-Agilent column was subjected to the same experimental conditions. Most of the analytes remained at the same retention time throughout the 2,000 injections. However, nortriptyline moved rapidly to later elution times. Within 500 injections, nortriptyline began to coelute with the next compound, neutral hexanophenone. The nortriptyline peak continued to migrate through the hexanophenone peak, totally coeluting by injection 2,000. These results indicated greater degradation of the non-Agilent column compared to InfinityLab Poroshell 120 HPH-C18.

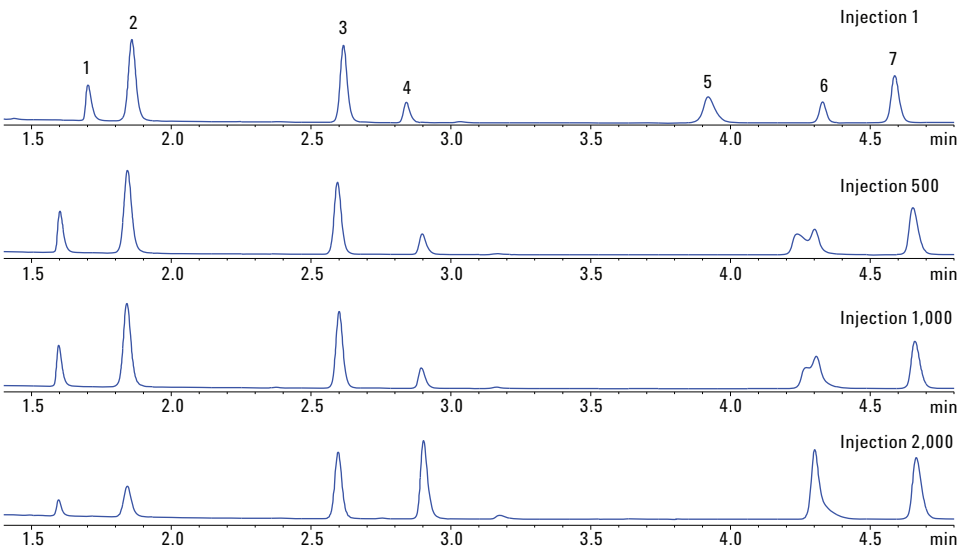


Peak ID
1. Methyl salicylate
2. 4-Chlorocinnamic acid
3. Acetophenone
4. Quinine
5. Nortriptyline
6. Heptanophenone
7. Amitriptyline

Conditions

Column:	Agilent InfinityLab Poroshell 120 HPH-C18, 2.1 × 50 mm, 2.7 µm	
Eluent:	A) 10 mM ammonium bicarbonate adjusted to pH 10.0 in water	
	B) acetonitrile	
Flow rate:	0.4 mL/min	
Gradient:	Time (min)	%B
	0	5
	5	95
	5.1	5
Total run time:	7 min	

Figure 3A. Agilent InfinityLab Poroshell 120 HPH-C18 on an ammonium bicarbonate gradient at pH 10.



Peak ID

1. Methyl salicylate
2. 4-Chlorocinnamic acid
3. Acetophenone
4. Quinine
5. Nortriptyline
6. Heptanophenone
7. Amitriptyline

Conditions

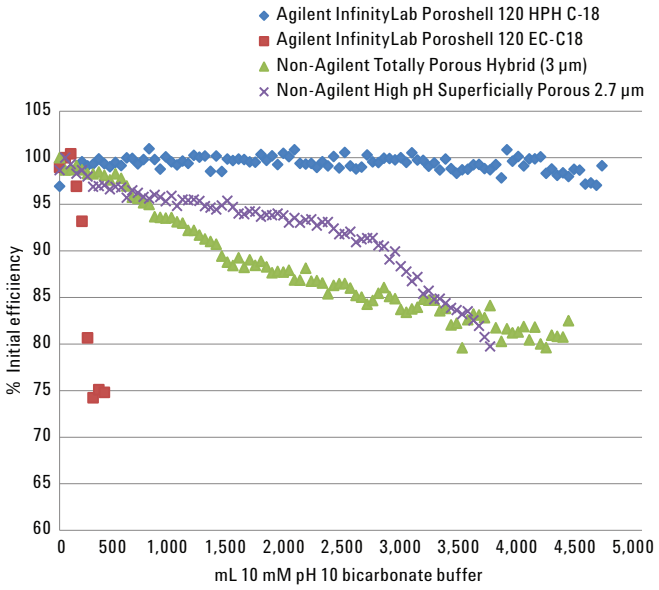
Column:	Other brand, 2.1 x 50 mm, 3 µm
Eluent:	A) 10 mM ammonium bicarbonate adjusted to pH 10.0 in water B) acetonitrile
Flow rate:	0.4 mL/min
Gradient:	Time (min) %B 0 5 5 95 5.1 5
Total run time:	7 min

Figure 3B. Other brand column on an ammonium bicarbonate gradient at pH 10.

Stress HPLC conditions

Stability in 10 mM ammonium bicarbonate, pH 10, isocratic

As the primary mode of stationary phase degradation is dissolution of the base silica, a two-step stress test was employed. In the first step, the efficiency of the column was determined by a simple isocratic test in 60:40 acetonitrile:water, using uracil and butyl benzene at 50 °C at a flow rate of 0.4 mL/min. The column and system pressure was approximately 90 to 100 bar. The test was repeated six times with the last three runs averaged. This allowed 15 minutes for the column to equilibrate fully and approximates 12 column volumes. A stress run of 2 hours of 100% 10 mM ammonium bicarbonate buffer was then directed through the column (48 column volumes). The volume of the stress buffer was plotted against the initial efficiency of the column. The column was considered damaged when its efficiency decreased by 10%. In this experiment, standard InfinityLab Poroshell 120 EC-C18 lost 25% efficiency within 500 mL of exposure to the stress buffer. At this elevated temperature, this mobile phase was extremely destructive to columns not designed for use at elevated pH. InfinityLab Poroshell 120 HPH-C18 took approximately 4 L of buffer or approximately 11 days for the column to lose 10% of its initial efficiency. Another brand of totally porous hybrid column lost 10% efficiency under these conditions after approximately 1,250 mL of buffer (approximately two days). A non-Agilent superficially porous column designed with improved bonding chemistry paralleled the performance of InfinityLab Poroshell 120 HPH-C18 for 2,750 mL, after which showed a sharp decline in performance. In this case a possible explanation for this drop in efficiency was exposure of the silica substrate.



Conditions

Columns:	2.1 × 50 mm, 2.7 µm
Eluent:	A) 0.1% ammonium hydroxide in water B) acetonitrile
Flow rate:	0.4 mL/min
Gradient:	Time (min) % B 0 5 3 95 3.5 5
Total run time:	4 min

Figure 4A. Two-step isocratic ammonium bicarbonate test, % initial efficiency.

In addition to the efficiency of the column, the retention time of the butyl benzene was also monitored and plotted. This plot again showed a rapid decay of standard InfinityLab Poroshell 120 EC-C18. However, the retention time of InfinityLab Poroshell 120 HPH-C18 stayed constant throughout the course of the test. The non-Agilent totally porous column lost 15% of its retention time during the test, potentially causing peaks to move sufficiently to become misidentified or not properly integrated. Finally, the retention time of the non-Agilent superficially porous column drifted 5% over the 3,500 mL of the test. This indicated that the bonding was stable, but more protection was needed for the silica.

Claessens *et al*. speculated that phosphate ions as well as carbonate could complex with the silica surface at intermediate and high pH, weakening surface silica-siloxane bonds. This would allow them to be more readily attacked by hydronium ions. The authors also suggested that only di- and tri-phosphate ions attacked the silica. It was shown that borate or glycine buffers, rather than commonly used phosphate and carbonate buffers, could improve column lifetime [7]. Even so, phosphate buffers are chosen in many cases, primarily due to their low UV noise.

In addition, so, while phosphate buffers are also considered a difficult buffer to use at pH 7 and above, they are still commonly used in many LC methods. In this part of the work, a 50 mM sodium phosphate dibasic/sodium phosphate monobasic buffer was made at pH 8 and diluted with 40% methanol to a final concentration of 30 mM. The column temperature was elevated to 65 °C. By raising the temperature, the rate of column degradation was accelerated. A sample containing naphthalene was injected every 10 minutes. As can be seen in Figure 5, the standard InfinityLab Poroshell 120 EC-C18 coped with approximately 200 mL in this mobile phase. At 1,000 mL the efficiency was reduced by 40%. When an InfinityLab Poroshell 120 HPH-C18 column was subjected to the same treatment, no degradation was noted at 2.5 L, and the column lost only 10% efficiency at 3 L.

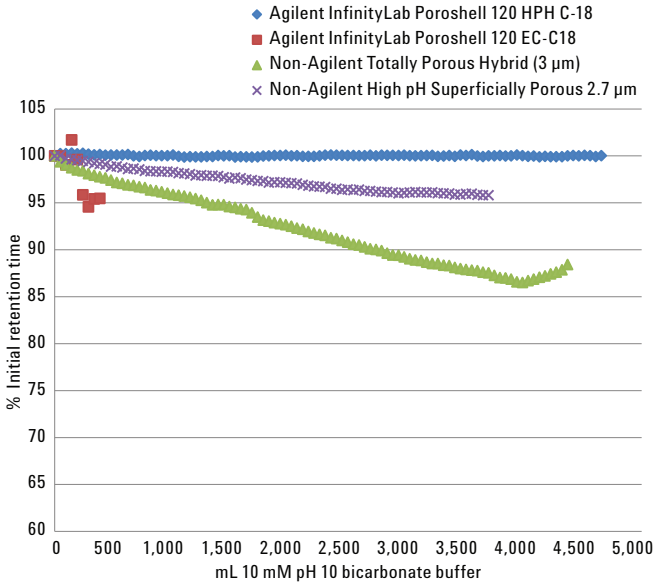


Figure 4B. Two-step isocratic ammonium bicarbonate test, retention time (all columns 2.1 × 50 mm).

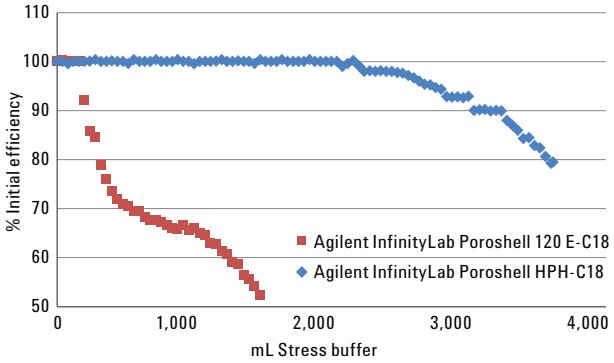


Figure 5. Lifetime of Agilent InfinityLab Poroshell columns in 40% MeOH 30 mM sodium phosphate buffer, pH 8 at 65 °C (both columns 2.1 × 50 mm).

Chromatographic performance

Superficially porous materials such as InfinityLab Poroshell 120 EC-C18 offer an alternative to sub-2 µm totally porous particles. They deliver 90% of the efficiency with only half the backpressure of a sub-2 µm particle. Since these columns use 2-µm frits instead of the 0.5-µm frits found on sub-2 µm columns, long lifetime can be achieved with minimal sample cleanup. InfinityLab Poroshell 120 HPH particles maintain high performance with the high efficiency and low back pressure of superficially porous particles such as InfinityLab Poroshell 120 particles. As can be seen in Figure 6, the van Deemter plots closely overlay and the reduced plate height of both columns was similar, indicating that for both 2.7-µm superficially porous materials the column efficiency was the same. Both columns are well packed with a reduced plate height of between 1.60 and 1.65.

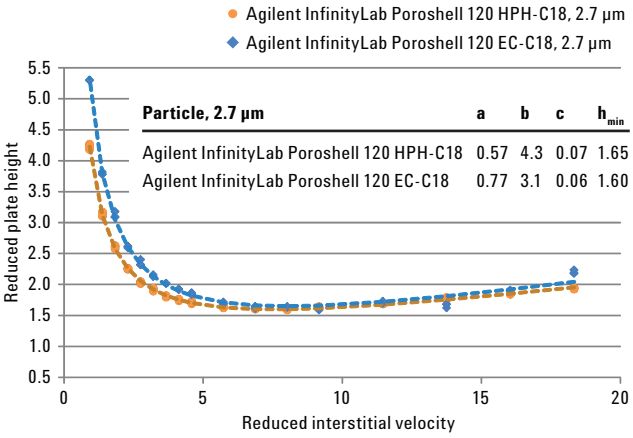


Figure 6. Reduced van Deemter plots of Agilent InfinityLab Poroshell 120 EC-C18 and Agilent InfinityLab Poroshell 120 HPH-C18.

Conclusions

While elevated pH mobile phases such as ammonium bicarbonate, ammonium hydroxide, and sodium phosphate dibasic/mono basic buffers can be destructive to conventional silica HPLC columns, the Agilent InfinityLab Poroshell 120 HPH-C18 column had excellent performance in these mobile phases. This work also demonstrated that InfinityLab Poroshell 120 HPH-C18 could be used in 0.1% ammonium hydroxide, a commonly used LC/MS solvent. The column can achieve substantially longer life (16x) in ammonium bicarbonate/ammonium hydroxide and similar lifetime increases in phosphate buffer at pH 8 and 65 °C, compared to the Agilent InfinityLab Poroshell 120 EC-C18. This column technology will allow investigators to use the capabilities of hybrid particles together with superficially porous particles. InfinityLab Poroshell 120 HPH particles maintain high performance with the high efficiency and low back pressure of superficially porous particles, as are used in other InfinityLab Poroshell 120 phases. The InfinityLab Poroshell 120 HPH column not only maintains the advantages of superficially porous particles, but also provides chemical stability under high pH mobile phase conditions.

References

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