Low and High pH Stability of an Agilent Poroshell HPH C18

Application Note

Author

William Long

Agilent Technologies, Inc.

Introduction

The stability of an HPLC column is one of the critical factors that determines the success of a method. During the development of an RPLC analysis protocol, and anticipating its validation, chromatographers usually consider several issues. One of the most important is how long the column will last 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; end-capping protected the silica from dissolution, densely bonded phases increased column stability, and organic mobile phase buffers 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 covalent siloxane bonds. In principle, the chemical and thermal stability of RP columns can be enhanced by the improvement and development of substrates and bonding chemistry [1-4].

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. The residual silanols on the silica surface of the packing will be 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. An additional reason for using low pH is the poor stability of silica columns at high pH.

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As previously reported, two approaches have been made to achieve high pH stability in silica HPLC columns. One way to increase stability is to employ special bonding, as in the Agilent ZORBAX Extend C18 column [3]. 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. Using this approach, 2.7-µm and now 4-µm Poroshell particles are organically modified, making them less susceptible to attack at high pH.

In this work, the lifetime of an Agilent Poroshell HPH C18 is evaluated with a typical low pH mobile phase (0.1 % TFA) and a high pH mobile phase (10 mM ammonium bicarbonate pH 10) using gradient analysis with acetonitrile.

Materials and Methods

An Agilent 1290 Infinity system was used, consisting of:

- Agilent 1290 Infinity Binary Pump, capable of delivering up to 1,200 bar (G4220A), modified with a PEEK seal in the solvent bypass valve (Rotor Seal PEEK FL p/n 5068-0171)
- Agilent 1290 Infinity Thermostatted Column Compartment (TCC) (G1316C)
- Agilent 1290 Infinity High Performance Autosampler (G4226A) with PEEK rotor seal (5068-0170 Rotor Seal PEEK FL)
- Agilent 1260 Infinity Diode Array Detector (DAD) (G4220A) equipped with a 10-mm path length, 1-µL flow cell
- Agilent OpenLAB version C.01.05 was used to control the HPLC and process the data
- Agilent Poroshell HPH-C18, 2.1 × 50 mm, 4 µm (p/n 699770-702)

The samples contained quinine, nortriptyline, amitriptyline, hexanophenone, acetophenone, 4-chlorocinnamic acid, and 2-hydroxy-5-methyl-benzaldehyde, and were prepared in 50:50 water/acetonitrile at 1 mg/mL. Mobile phases that are commonly used in laboratories such as 0.1% trifloracetic acid (TFA) and ammonium bicarbonate buffer were used. The buffer was prepared by dissolving ammonium bicarbonate in water to produce a 10 mM solution prior to adjustment to the desired pH using concentrated base (ammonium hydroxide). Sodium phosphate dibasic and sodium phosphate monobasic used to produce buffers were purchased from Sigma-Aldrich, Corp. The acetonitrile was Burdick and Jackson, purchased from Honeywell, and water was Millipore 18 MΩ.

The UV detector was operated at 254 nm, 80 Hz.

Results and Discussion

Stability testing was carried out using a low pH mobile phase (0.1% TFA) and a high pH mobile phase (10 mM ammonium bicarbonate, pH 10).

Stability in 0.1% TFA

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 four minutes. The sample contained the typical acid, base, and neutral compound types found in almost all difficult samples. TFA at 0.1% is a commonly used chromatography mobile phase (pH ~2). It can be seen that the peaks were all retained at the same elution volume throughout the experiment. This demonstrated that the Poroshell HPH-C18 column was stable and usable for routine analyses at low pH (Figure 1). Peak shape remained excellent throughout the test. The run time of the method was approximately seven minutes per injection, requiring approximately 10 days and approximately 5 L each of acetonitrile and aqueous mobile phase.

Stability in 10 mM ammonium bicarbonate, pH 10

A second experiment was performed using 10 mM ammonium bicarbonate at pH 10 (Figure 2). 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 carbonate buffers damage silica columns far more than buffers such as glycine and borate [5]. The same materials used for the 0.1% TFA testing were used as a lifetest sample at high pH. A big difference between these two tests was the elution order. As shown in Figure 3, changing the pH of the mobile phase changed the elution order of analytes, indicating a drastic change in selectivity.

In this experiment, a Poroshell HPH-C18 column was evaluated with a gradient method using ammonium bicarbonate and acetonitrile at pH 10. As can be seen in Figure 2, the retention time of all compounds remained stable throughout the 2,000 injection run with the exception of nortriptyline. Similar results have been reported earlier with poorer nortriptyline peak shifting occurring on other vendor columns [6].

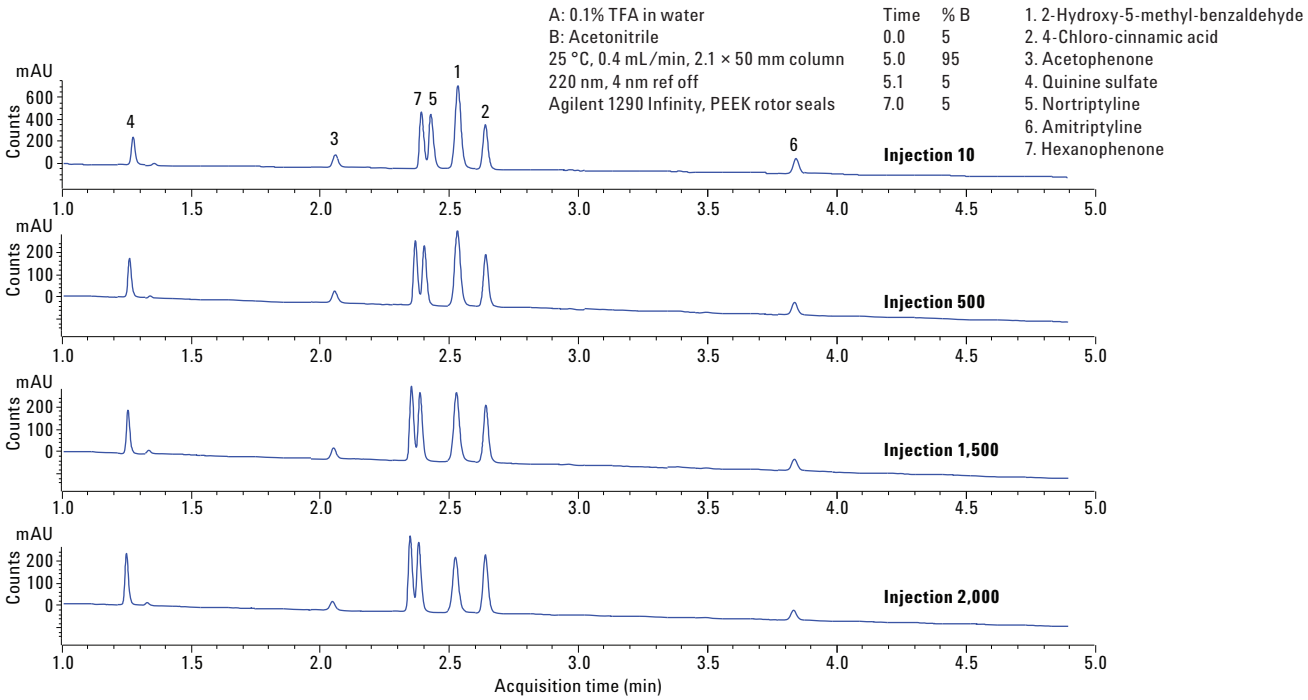

Figure 1. Agilent Poroshell HPH C18 lifetime in common acidic mobile phase, 2,000 injections 0.1% TFA.

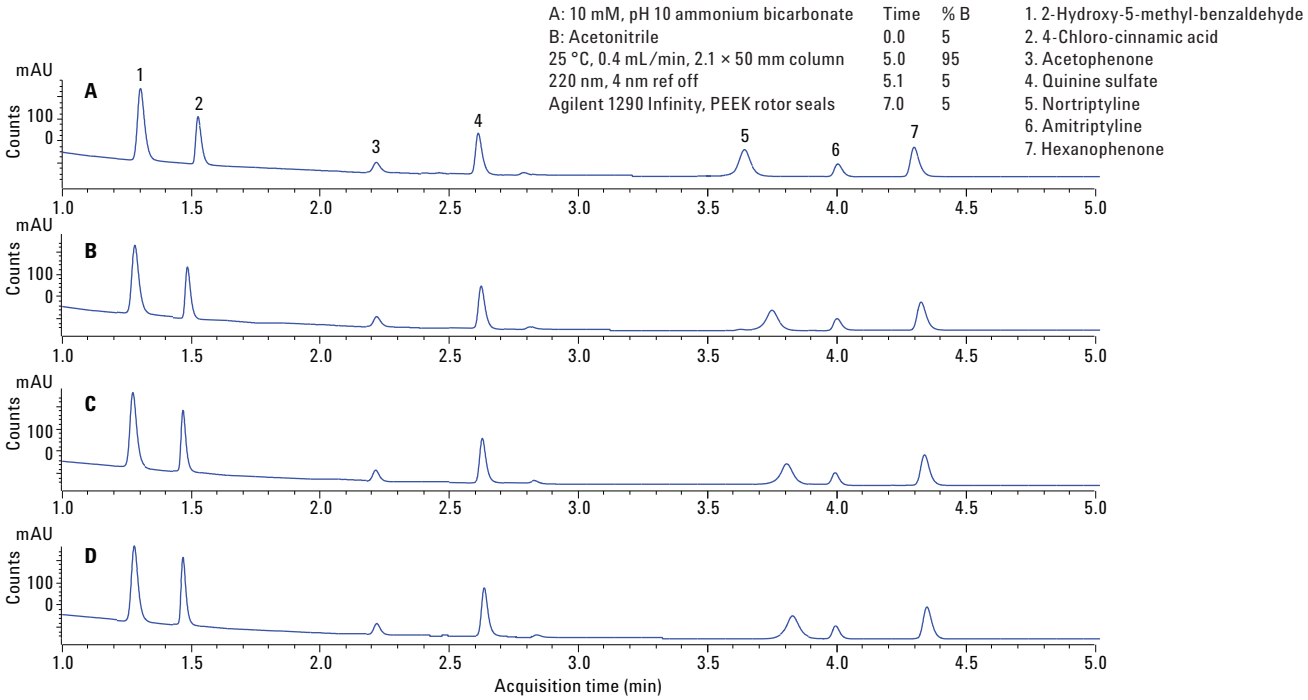

Figure 2. Agilent Poroshell HPH C18 lifetime in basic mobile phase, 2,000 injections 10 mM ammonium bicarbonate pH 10.

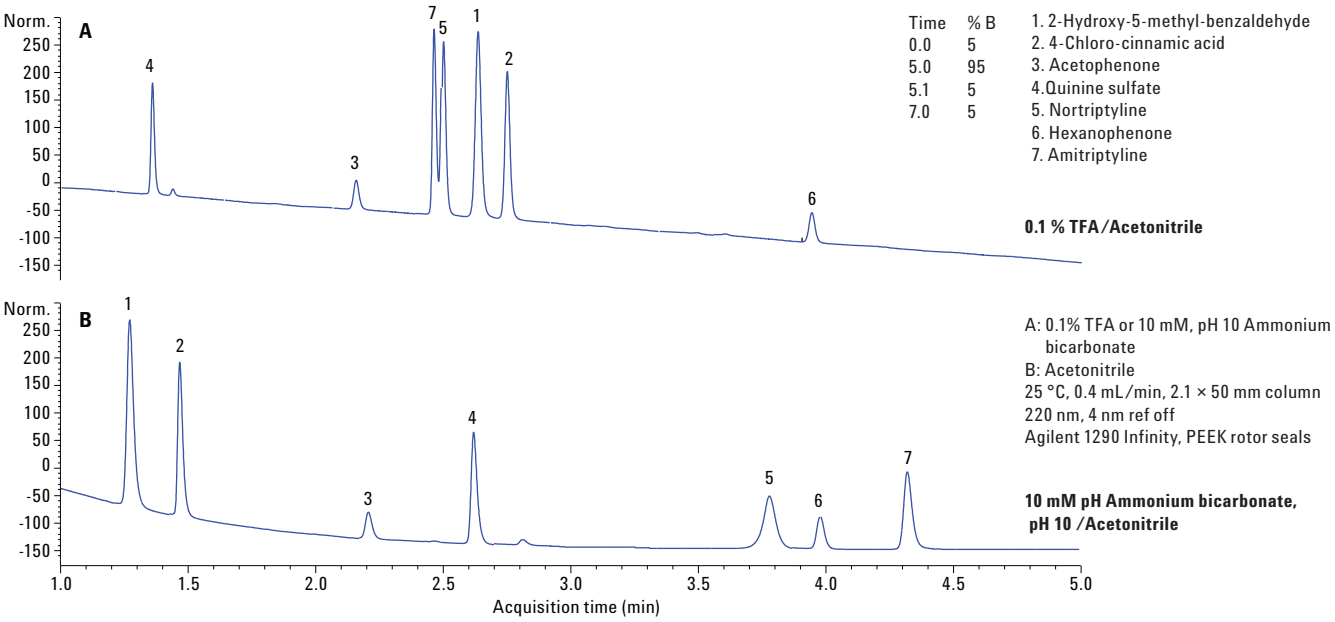

Figure 3. Agilent Poroshell HPH C18 can be used at low pH in 0.1% TFA or high pH in 10 mM ammonium bicarbonate pH 10 to achieve vastly different selectivity.

Conclusions

While elevated pH mobile phases such as ammonium bicarbonate buffer can be destructive to conventional silica HPLC columns, the Agilent Poroshell HPH-C18 column performed brilliantly in this mobile phase. TFA also has the potential be destructive to HPLC columns, removing the bonded phase by hydrolysis. This column technology will allow investigators to use the capabilities of hybrid particles together with superficially porous particles. Poroshell HPH particles maintain high performance with the high efficiency and low backpressure of superficially porous particles, as are used in other Poroshell 120 phases. The Poroshell HPH column not only maintains the advantages of superficially porous particles, but also provides chemical stability under high pH mobile phase conditions.

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