

Using pH as a Method Development Tool with Agilent InfinityLab Poroshell 120 CS-C18

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Abstract

The low and high pH stability of the Agilent InfinityLab Poroshell 120 CS-C18 is demonstrated. The value of a broad range of mobile phase pH's is explored combining multiple buffers with a generic acetonitrile gradient of 5 to 95%. Two samples, including a pesticide mixture, are used to show how mobile phase pH affects analyte retention for acids, bases, and neutrals.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns are more efficient at lower pressure in comparison to their totally porous particle column counterparts.¹ This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution in the column.²

The most popular particle size for superficially porous particle columns is 2.5 to 3 μm . These particles produce similar efficiency to traditional sub 2 μm columns while generating approximately 50% of the backpressure. High efficiency can contribute to resolving closely eluting peaks, while low backpressure allows for flexibility with LC instrumentation.

Agilent recently developed a new bonded phase chemistry on their 2.7 μm Poroshell 120 particles. This phase was created by applying a positive charge to the silica surface and then

functionalizing the particle with a C18 bonded phase. The Agilent InfinityLab Poroshell 120 CS-C18 column provides enhanced loadability and peak shape for basic analytes under weak ionic strength mobile phase conditions, such as formic acid. This phase is also compatible with both low and high mobile phase pH, which is highly advantageous for controlling the retention and separation of ionizable compounds. The remarkably flexible 2.7 μm InfinityLab Poroshell 120 CS-C18 column can be used to effectively and robustly analyze various types of analytes, across many instrument and detector platforms.

This study demonstrates the performance of a charged surface superficially porous particle column, the Agilent InfinityLab Poroshell 120 CS-C18, and its ability to achieve excellent lifetime at both low and high pH, which is ideal for method development for ionizable compounds.

Experimental

An Agilent 1290 Infinity II LC with an Agilent Ultivo LC/TQ was used in this experiment. The system was modified from its standard configuration to have lower system volume and dispersion. Table 1 shows the configuration details. Two LC columns were used in this experiment and are listed in Table 1. Tables 2 to 4 show the LC and TQ method parameters.

The nine compounds analyzed in this work were purchased from Sigma Aldrich (St. Louis, MO, USA). The two pesticide standards were purchased from Agilent (p/n 5190-0469-1, 5190-0469-2). All samples are listed in Table 2.

Ammonium formate was purchased from Sigma-Aldrich. Formic acid (p/n G2453-85060) and LC/MS grade acetonitrile (p/n G2453-85050) were obtained from Agilent. Water was 0.2 μm filtered 18 molecular weight from a Milli-Q system (Millipore, Burlington, MA, USA).

Table 1. System configuration.

Agilent 1290 Infinity II LC System Configuration	
Agilent 1290 Infinity II flexible pump (G7104A)	Degasser Seal wash pump 35 μL solvent mixer: Agilent Jet Weaver, 35 $\mu\text{L}/100 \mu\text{L}$ (p/n G4220-60006) Firmware: B.07.23 [0009]
Agilent 1290 Infinity II vialsampler (G7129B)	Sample thermostat (p/n G7167-60101) Metering parameter: seat assembly PEEK 0.12 mm, sample loop 20 μL , analytical head 20 μL Autosampler \rightarrow heater: capillary, stainless steel, 0.12 \times 105 mm, SL/SL (p/n 5500-1238) Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717) Vial insert, 250 μL , glass with polymer feet, 100/pk (p/n 5181-1270) Firmware: D.07.23 [0009]
Agilent InfinityLab LC Series Integrated Column Compartment (G7130A)	Integral type: G7129B 3.0 μL heat exchanger Heater \rightarrow column: A-Line quick-connect assembly, 105 mm, 0.075 mm (p/n 5067-5961) Column \rightarrow flow cell: capillary, stainless steel, 0.075 \times 220 mm, SV/SLV (p/n 5067-4784) Firmware: B.07.23 [0009]
Agilent Ultivo LC/TQ (G6465A)	Agilent Jet Stream ESI Source
Agilent 1290 Infinity II diode array detector (G7117B)	Ultralow dispersion Max-Light cartridge flow cell, 10 mm, 0.60 μL (p/n G4212-60038) UV lamp (p/n 5190-0917) Firmware: D.07.23 [0009]
Agilent LC columns	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 \times 50 mm, 2.7 μm (p/n 699775-942) Agilent InfinityLab Poroshell 120 CS-C18, 2.1 \times 100 mm, 2.7 μm (p/n 695775-942)

Table 2. UHPLC method parameters.

Method	Column	Mobile Phases	Elution Conditions	Temperature	Injection/Sample	Detection
1 Low pH Lifetime	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 x 50 mm, 2.7 µm (p/n 699775-942)	A: 2% TFA in water B: methanol	0.3 mL/min, 50% B isocratic, 60 min run time Test Sequence: continuous injections	85 °C	1 µL of uracil, toluene	DAD: 254 nm, 80 Hz
2 High pH Lifetime	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 x 50 mm, 2.7 µm (p/n 699775-942)	A: water B: acetonitrile C: 10 mM ammonium bicarbonate pH 10	0.4 mL/min, QC: 60% B, 40% A; Stress Buffer: 100% C for 2 hr Test Sequence: 5x QC, Stress Buffer, repeat	50 °C	0.5 µL of uracil, phenol, 4-chloronitro benzene, naphthalene	DAD: 254 nm, 80 Hz
3 Low pH Method Development	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 x 100 mm, 2.7 µm (p/n 695775-942)	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 5 to 95% B in 10 min, with 5% C held constant throughout the analysis	30 °C	0.8 µL of uracil, amitriptyline, butyl paraben, dipropyl phthalate, acenaphthene	DAD: 254 nm, 80 Hz
					0.2 µL of Agilent Basic Pesticides (p/n 5190-0469-1) + Acidic Pesticides (p/n 5190-0469-2), mixed 1:3	MSD: See Tables 3 and 4
4 High pH Method Development	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 x 100 mm, 2.7 µm (p/n 695775-942)	A: water B: acetonitrile C: 200 mM ammonium formate pH 10	0.4 mL/min, 5 to 95% B in 10 min, with 5% C held constant throughout the analysis	30 °C	0.8 µL of uracil, amitriptyline, butyl paraben, dipropyl phthalate, acenaphthene	DAD: 254 nm, 80 Hz
					0.2 µL of Agilent Basic Pesticides (p/n 5190-0469-1) + Acidic Pesticides (p/n 5190-0469-2), mixed 1:3	MSD: See Tables 3 and 4

Table 3. LC/TQ sources method parameters.

MS Source	Set Point
Gas Temperature	150 °C
Gas Flow	12 L/min
Nebulizer	20 psi
Sheath Gas Temperature	250 °C
Sheath Gas Flow	5 L/min
Capillary Voltage	2,000 V

Table 4. LC/TQ SIM acquisition method parameters.

No.	Compound Name	Mass (m/z)	Dwell (ms)	Fragmentor (V)	Polarity
1	Aminocarb	209.3	10	135	Positive
2	Thiabendazole	202.1	10	135	Positive
3	Imazalil	297.1	10	135	Positive
4	Metazachlor	278.0	10	135	Positive
5	Carbofuran	222.1	10	135	Positive
6	Atrazine	216.1	10	135	Positive
7	Bentazon	239.1	10	135	Negative
8	2,4,5-T	252.9	10	135	Negative
9	Silvex	266.9	10	135	Negative
10	Dinoseb	239.1	10	135	Negative
11	Acifluorfen	360.0	10	135	Negative

Results and Discussion

Two separate Agilent InfinityLab Poroshell 120 LC columns were stressed, one with low pH and one with high pH conditions. The results are shown in Figure 1.

A high concentration solution of 2% trifluoroacetic acid (pH 1) was used to stress the CS-C18 column at an elevated temperature of 85 °C. The column was constantly exposed to these stressful conditions as repeat QC injections of toluene were made and tracked for performance changes. The column maintained its high performance, with little change to retention and peak shape, for more than 20,000 column volumes at pH 1 and 85 °C, demonstrating excellent low pH stability.

To test the high pH stability of the CS-C18, the column was alternately tested with a naphthalene sample and exposed to a high pH stress buffer of 10 mM ammonium bicarbonate (pH 10) for 2 hours. This was done repeatedly, for a total of 50,000 column volumes of the stress buffer, while column temperature was at an elevated 50 °C to further stress the column stationary phase. Again, the CS-C18 proved it is also robust under high pH conditions, as peak shape and retention were maintained through the duration of the test.

The exceptional range of pH stability for the CS-C18 makes it a great choice for method development with ionizable compounds. When operating in reversed-phase LC (RPLC) mode, analytes are most retained when they are in their neutral state. Acids are retained more at low pH and bases retain more at high pH. Neutral compounds are not impacted by mobile phase pH. Similarly, acids and bases that do not change ionization state will also not be affected by changes in mobile phase pH.

Figure 2 shows a simple example of pH being used as a method development tool. The sample contained one acid, one base, and three neutrals; it was run on a generic acetonitrile gradient of 5 to 95%, once with a low pH mobile phase, and then again with a high-pH mobile phase. The red arrow indicates the movement of the acidic compound (loses retention as pH increases), the blue arrow shows the movement of the basic analyte (gains retention with pH increase), while the black arrows demonstrate that the neutrals do not change retention according to mobile phase pH.

Figure 3 shows another generic acetonitrile gradient screen at low and high pH, but with a more complex sample of acidic and basic pesticides. The peak numbers in Figure 3 can be identified by the corresponding compound numbers in Table 4. Peak colors are consistent between the low and high pH chromatograms. As can be seen, there was substantial peak movement and very different selectivity between the different mobile phases at pH 2.7 and 10.

Figure 4 simplifies the peaks in Figure 3 so that they are color-coded as either red for acids or blue for bases. Now it is easy to observe that the acidic pesticides are more retained with formic acid at low pH, while the basic pesticides are better retained at high pH with the pH 10 ammonium formate buffer. Also noteworthy, are the two peaks shown in black. These are ionizable compounds, however their pKa's are outside of the tested pH range of 2.7 to 10. Therefore, these compounds do not change ionization state between the two mobile phases and behave similarly to neutral compounds and do not change retention.

The simple acetonitrile gradient used in the previously mentioned separations is a great starting point for method development. If ionizable compounds are present, screening your sample with a range of mobile phase pH's provides considerable selectivity changes, which can improve chances to retain and resolve all analytes.

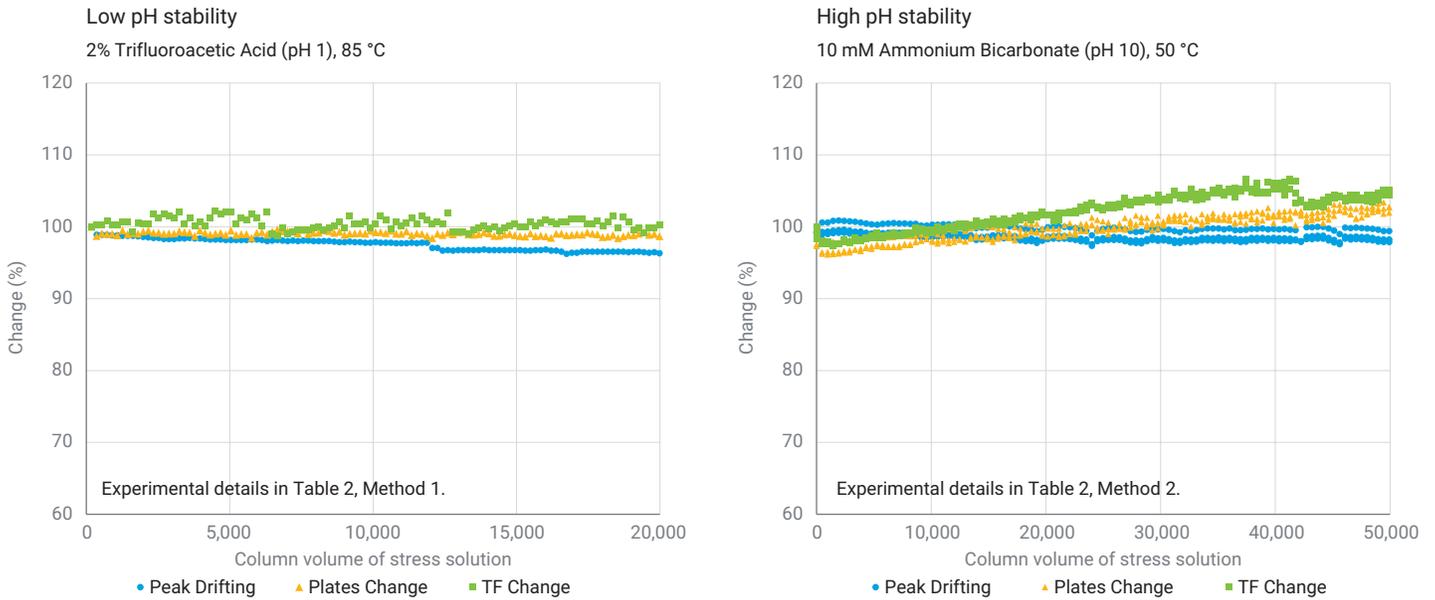


Figure 1. Agilent InfinityLab Poroshell 120 CS-C18 is stable for use with both low and high pH mobile phases, even during stressful high temperature analyses.

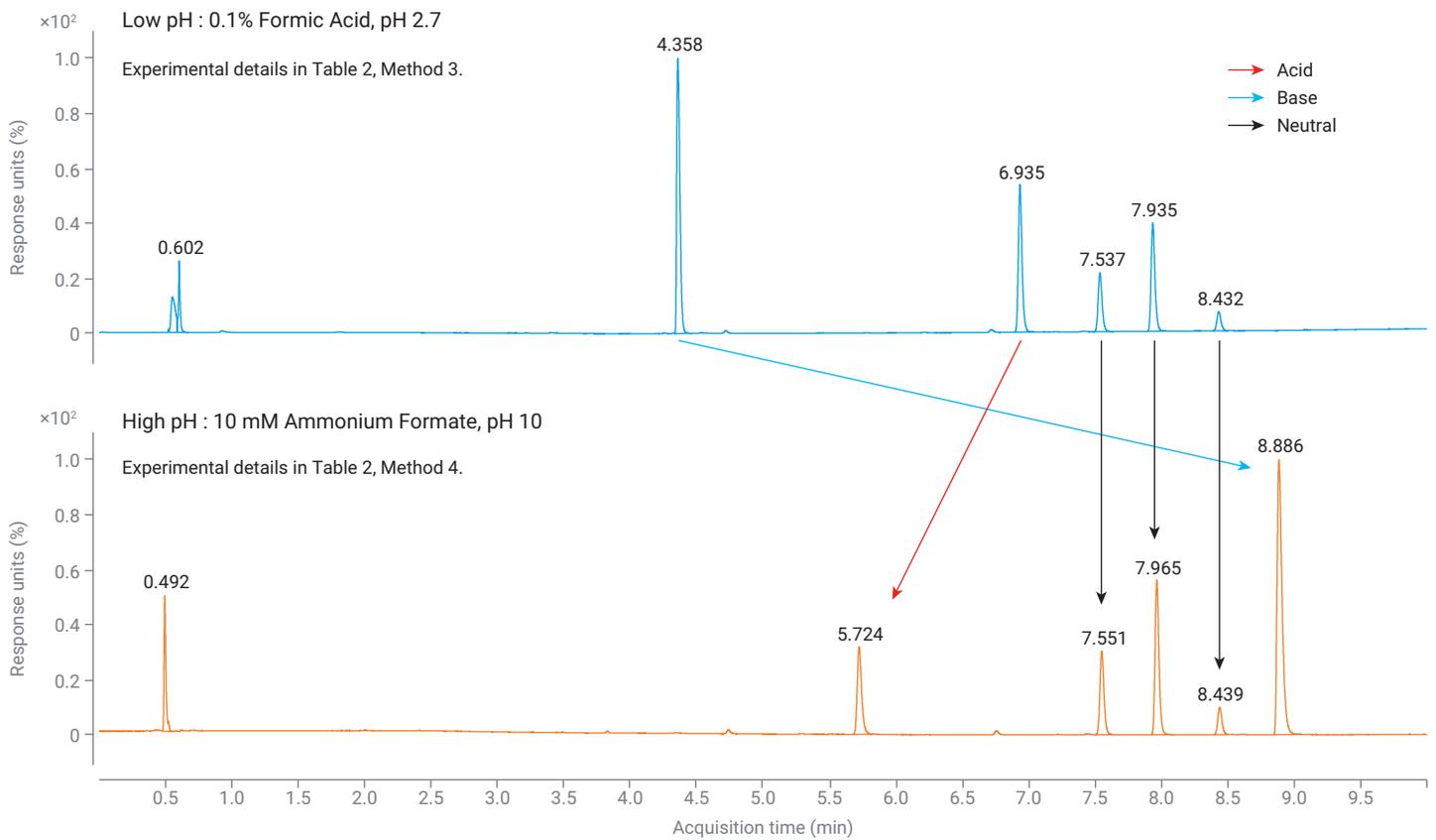


Figure 2. Mobile phase pH is a powerful method development tool for separating ionizable compounds with the Agilent InfinityLab Poroshell 120 CS-C18.

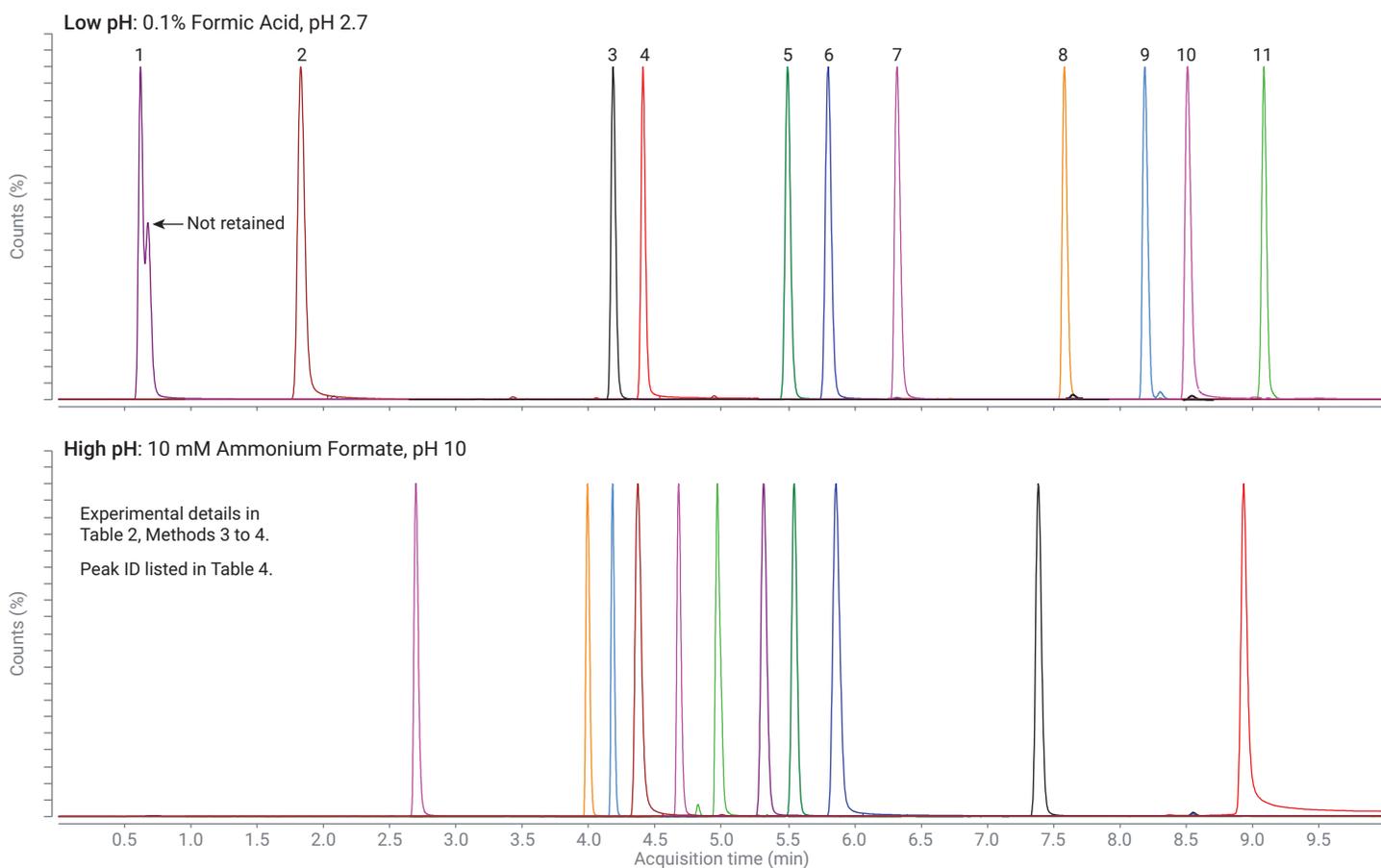


Figure 3. Changing mobile phase pH significantly alters selectivity for these acidic and basic pesticides on an Agilent InfinityLab Poroshell 120 CS-C18 column.

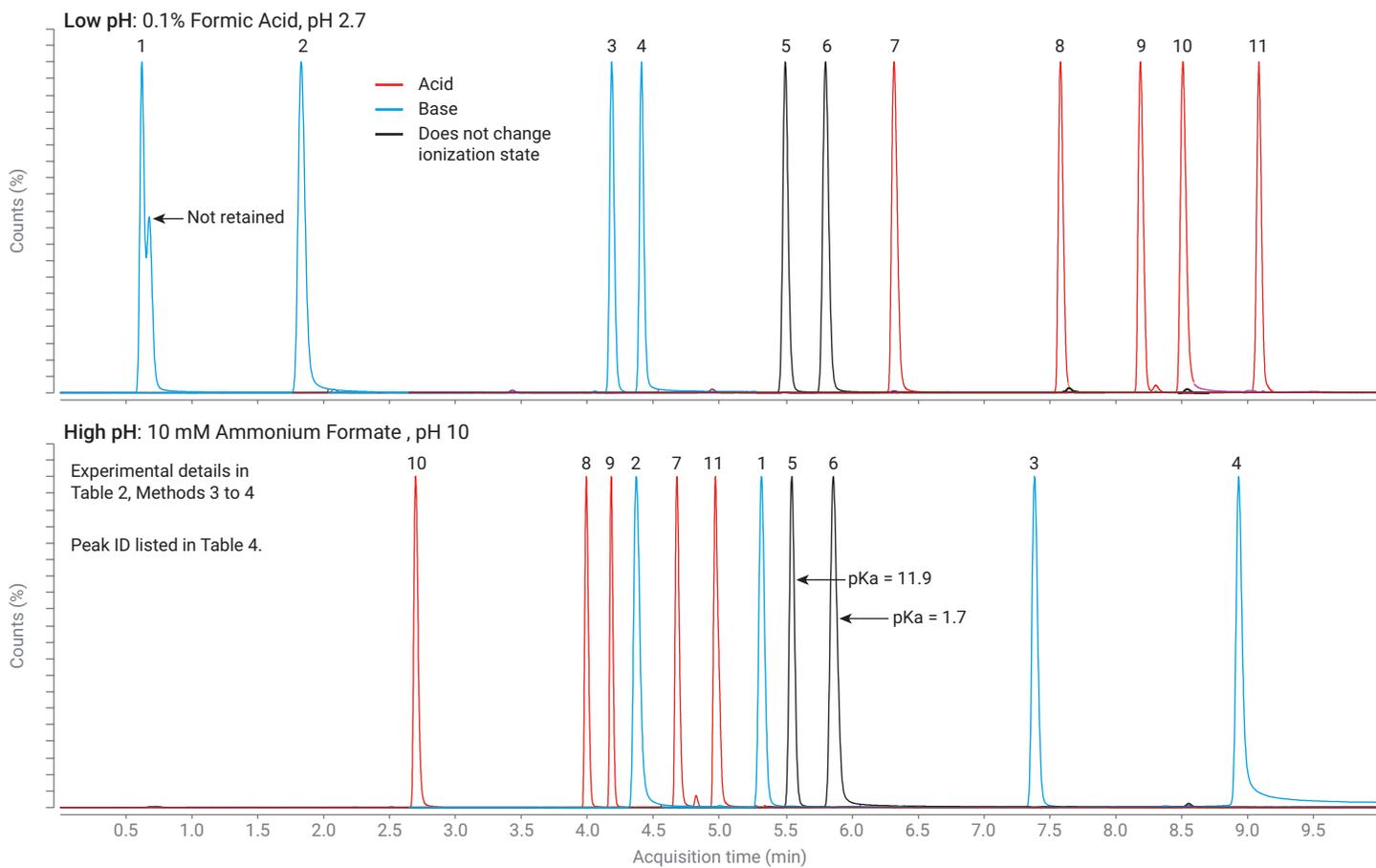


Figure 4. Peak colors are simplified from Figure 3 to show acidic (red) pesticides are more retained at low pH, while basic (blue) pesticides are more retained with high pH.

Conclusion

The charged surface Agilent InfinityLab Poroshell 120 CS-C18 column achieves excellent lifetime under both low and high mobile phase pH, even when stressful high temperature conditions are also applied. The effectiveness of using mobile phase pH as a method development tool is demonstrated with two sample mixtures. Analytes are more retained in RPLC mode when they are in their neutral state: acids are shown to be more retained at low pH, bases are more retained at high pH, and neutrals are unaffected by mobile phase pH changes. Using a column that is compatible across a broad pH range allows for exceptional flexibility during method development for ionizable compounds.

Reference

1. Gratzfield-Huguen, A.; Naegele, E. Maximizing Efficiency Using Agilent InfinityLab Poroshell 120 Columns. *Agilent Technologies Application Note*, publication number 5990–5602EN, **2016**.
2. Meyer, V. R. Practical High-Performance Liquid Chromatography. Fourth Edition, p. 34. Wiley, **2004**.

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