Application Note

Food Testing & Agriculture

Taking Advantage of the Orthogonal Selectivities of Agilent InfinityLab Poroshell 120 C18 Columns for Method Development at High pH

Author

Anne Mack
Agilent Technologies, Inc.

Abstract

In this application note, resolution is briefly discussed with respect to LC method development, with selectivity being a key contributor. Using a simple ammonium formate and acetonitrile gradient, the unique selectivities of two high-pH stable Agilent InfinityLab Poroshell 120 C18 columns is demonstrated with a sample of pesticides at pH 10.

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 flexibility with LC instrumentation.

Agilent currently offers 12 bonded-phase chemistries on their 2.7 µm InfinityLab Poroshell 120 particles for use with reversed-phase LC separations. Four of these phases are C18s, each with unique separation abilities, two of which are stable with high-pH mobile phases. This study demonstrates that not all C18s are the same, and shows the value in having multiple selectivity options during LC method development.

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 two pesticide standards were purchased from Agilent (part numbers 5190-0469-1 and 5190-0469-2). Ammonium formate and ammonium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC/MS grade acetonitrile (part number G2453-85050) was 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 µL/100 µL (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 → heater: capillary, stainless steel, 0.12 × 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 → column: A-Line quick-connect assembly, 105 mm, 0.075 mm (p/n 5067-5961) • Column → flow cell: capillary, stainless steel, 0.075 × 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 × 100 mm, 2.7 µm (p/n 695775-942) • Agilent InfinityLab Poroshell HPH-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-702)

Table 2. UHPLC method parameters.

Mobile Phases	Elution Conditions	Column Temperature	Injection Volume	Sample	Detection
A) Water B) Acetonitrile C) 200 mM Ammonium formate in water, adjusted to pH 10 with 28 to 30% ammonium hydroxide	0.4 mL/min, 10 to 95% B in 10 min, with 5% C held constant throughout the analysis	30 °C	0.5 µL	Agilent Basic Pesticides (p/n 5190-0469-1) + Acidic Pesticides (p/n 5190-0469-2), mixed 1:2	LC/MS, ESI±, SIM: See Tables 3 to 4

Table 3. LC/TQ source 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

Results and discussion

Chromatographic resolution is a common separation criterion for LC method developers. Achieving baseline resolution of all analytes allows accurate integration and quantitation when using nonselective detectors such as diode array, fluorescence, refractive index, and evaporative light scattering. Even for more sophisticated detectors, such as mass spectrometers, achieving chromatographic resolution is still valuable in the event of isobaric pairs, or to prevent ion suppression from coeluting species.

A detailed discussion of resolution and the variables that drive it is included in Agilent application note "Taking Advantage of the Orthogonal Selectivities of Agilent InfinityLab Poroshell 120 C18 Columns for Method Development at Low pH" (publication number 5994-2358EN).[3] In this application note, it is demonstrated that selectivity is the largest contributing factor to chromatographic resolution.

To alter selectivity, the chemistry of the chromatographic system must change—that is, the mobile phase or the column stationary phase. For the mobile phase, the type of organic solvent, acetonitrile versus methanol, can impact selectivity. Mobile phase pH can also impact selectivity, as shown in Agilent application note "Using pH as a Method Development Tool with Agilent InfinityLab Poroshell 120 CS-C18" (publication number 5994-2274EN).[4]

Table 4. LC/TQ SIM acquisition method parameters.

	Compound Name	Mass (*m/z*)	Dwell (ms)	Fragmentor (V)	Polarity
1	Metosulam	418.1	10	135	Negative
2	Thiabendizole	202.1	10	135	Positive
3	Metoxuron	229.1	10	135	Negative
4	Acifluorfen	360.0	10	135	Negative
5	Aminocarb	209.3	10	135	Positive
6	Carbofuran	222.1	10	135	Positive
7	Atrazine	216.1	10	135	Positive
8	Imazalil	297.1	10	135	Positive
9	Malathion	331.0	10	135	Negative
10	Diazinon	305.2	10	135	Negative
11	Pyrachlostrobin	388.2	10	135	Negative
12	Hexaflumuron	459.0	10	135	Positive

Changing the column stationary phase is another way to alter selectivity and potentially improve resolution. Figure 1 demonstrates that even different variations of C18-bonded phases can be different enough to change elution order and impact chromatographic resolution.

Figure 1 demonstrates the orthogonal selectivity of two InfinityLab Poroshell 120 C18 columns with a sample of pesticides with a high-pH mobile phase. Screening multiple columns with a simple acetonitrile gradient, as done in this work, is a common way to initiate LC method development. The more complex

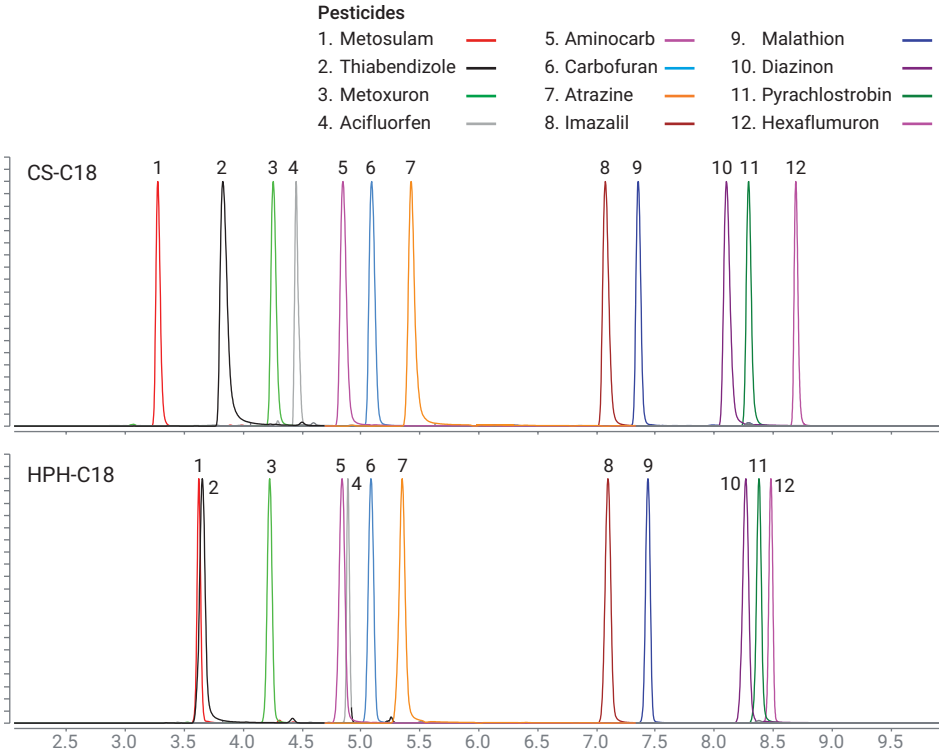

Figure 1. Two different high-pH stable Agilent InfinityLab Poroshell 120 C18 phases give unique selectivity for pesticides with a pH 10 mobile phase.

a sample is, the less likely it is that a column screen will immediately give you baseline resolution of all compounds of interest. Often, additional method development will be required. However, starting method development this way can systematically guide you towards an appropriate column choice for your analysis.

The pesticide sample shown in Figure 1 contains many basic analytes. In reversed-phase LC, basic analytes are more retained in their neutral state, with a high-pH mobile phase. However, most silica-based LC columns are not compatible with prolonged use at high pH. The Agilent InfinityLab Poroshell 120 family has two C18 phases that are intentionally designed for high pH stability, which can be beneficial for retaining and separating basic analytes. Both the CS-C18 and HPH-C18 are ideal columns to use for method development of basic analytes under high-pH conditions.

The InfinityLab Poroshell 120 CS-C18 gave the most desirable separation. Given that many of these pesticides are basic, this is not surprising. The charged surface chemistry on the CS-C18 is designed to provide the user with unique retention of bases, as well as excellent peak shape and loadability under both low and high-pH conditions.

Conclusion

The simple acetonitrile gradient used in the previously mentioned separations is an excellent starting point for method development. Combined with high-efficiency columns, such as those in the InfinityLab Poroshell 120 family, this simple method can be used to quickly evaluate multiple column stationary phases, which can improve chances to retain and resolve all analytes.

References

1. Gratzfield-Hugsen, 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.
3. Mack, A. Taking Advantage of the Orthogonal Selectivities of Agilent InfinityLab Poroshell 120 C18 Columns for Method Development at Low pH. *Agilent Technologies application note*, publication number 5994-2358EN, **2020**.
4. Mack, A. Using pH as a Method Development Tool with Agilent InfinityLab Poroshell 120 CS-C18. *Agilent Technologies application note*, publication number 5994-2274EN, **2020**.

www.agilent.com/chem

DE.0205671296

Printed in the USA, September 14, 2020
5994-2390EN

Agilent
Trusted Answers