Application Note

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Separation of 11 Organic Acids on an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl Column with 2.7 µm Particle Size

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Abstract

This Application Note analyzed 11 organic acids with an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl (2.1 × 150 mm, 2.7 µm) column using a phosphate buffer and an acetonitrile gradient. The 11 structurally similar compounds were well resolved within three minutes, with a minimum resolution of 1.5.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns are more efficient at lower pressure compared to their totally porous particle column counterparts.[1] This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column.[2] The most popular particle size for superficially porous particle columns is 2.5 to 3 µm. The larger InfinityLab Poroshell 120 particles can easily be used in long column formats to maximize resolving power with UHPLC efficiency, while not exceeding pressure limitations.

This study demonstrates the UHPLC performance of a long (150 mm) InfinityLab Poroshell 120 Phenyl-Hexyl column with a 2.7 µm particle size, and its ability to resolve 11 closely related compounds.

Experimental

An Agilent 1290 Infinity II LC 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. Three Agilent LC columns were used in this experiment, and are listed in Table 1. Table 2 shows the LC method parameters.

Table 1. System configuration.

Agilent 1290 Infinity II LC System Configuration	
Agilent 1290 Infinity II Flexible Pump (G7104A)	Degasser
	Seal wash pump
	35 µL solventmixer: 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 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 (5190-0917)
	Firmware: D.07.23 [0009]
Agilent LC Columns	Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 × 150 mm, 2.7 µm (p/n 693775-912)
	Agilent InfinityLab Poroshell 120 PFP, 2.1 × 150 mm, 2.7 µm (p/n 693775-408)
	Agilent InfinityLab Poroshell 120 SB-Aq, 2.1 × 150 mm, 2.7 µm (p/n 683775-914)

Table 2. Method parameters.

Column	Mobile Phase	Flow Rate (mL/min)	Mobile Phase Composition	Injection Volume (µL)	Sample	Thermostated Column Compartment (°C)	Diode Array Detector
Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 ×150 mm, 2.7 µm	A) water B) acetonitrile C) n/a D) 200 mM sodium phosphate + 1% phosphoric acid (85%), pH ~2	0.4	**Gradient** 0 to 30% B in 3 minutes, hold 10% D throughout run, 4 minute re-equilibration	0.1	0.4 to 3 mg/mL each in water See Table 3 for exact concentrations See Figure 1 for structures	28	210 nm, 80 Hz

The 11 organic acids analyzed in this work were purchased from Sigma-Aldrich (St. Louis, MO, USA). Figure 1 shows their structures, and Table 3 displays the concentrations at which they were analyzed. Sodium phosphate and phosphoric acid were also purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson, Muskegon, MI, USA). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore, Burlington, MA, USA).

Table 3. Sample composition.

Analyte	Concentration (mg/mL) in Water
Acetic Acid	2.8
t-Aconitic Acid	0.044
Citric Acid	1.3
Fumaric Acid	0.046
Lactic Acid	3.2
Maleic Acid	0.043
Malic Acid	1.4
Malonic Acid	1.3
Oxalic Acid	0.45
Succinic Acid	1.8
Tartaric Acid	0.86

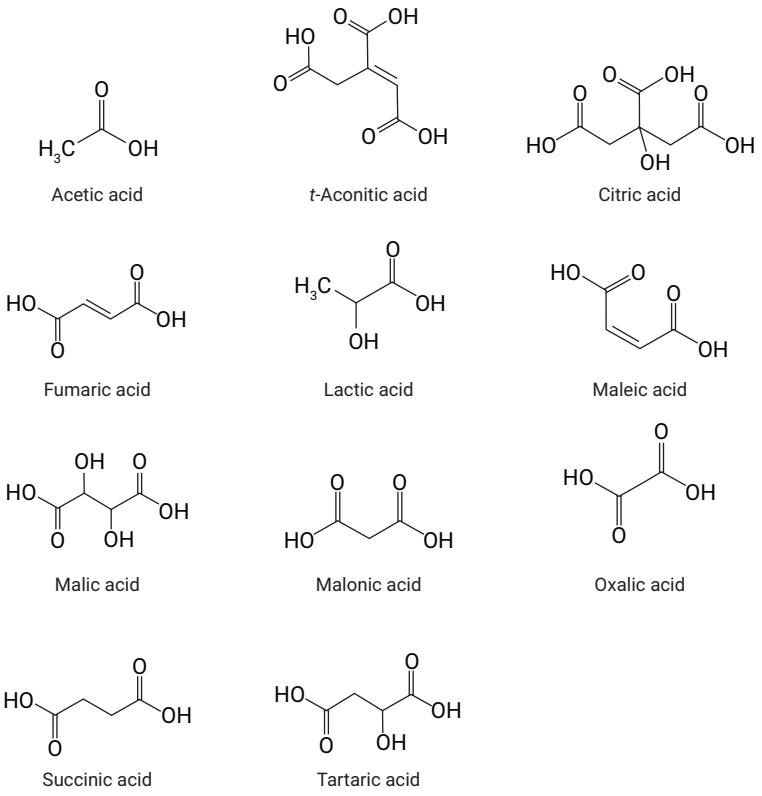

Figure 1. Compounds of interest.

Results and discussion

Figure 2 shows three InfinityLab Poroshell 120 columns screened for the analysis of organic acids with a simple aqueous isocratic method. These three phases were chosen due to their ability to be used with 100% aqueous mobile phases without the risk of dewetting. This feature can help to retain polar compounds, such as these organic acids, in reversed-phase LC mode. Phenyl-Hexyl provides the best separation of the early eluting acids, with the Agilent InfinityLab Poroshell 120 SB-Aq as a close second. Unfortunately, the SB-Aq phase struggles to separate malic acid and lactic acid.

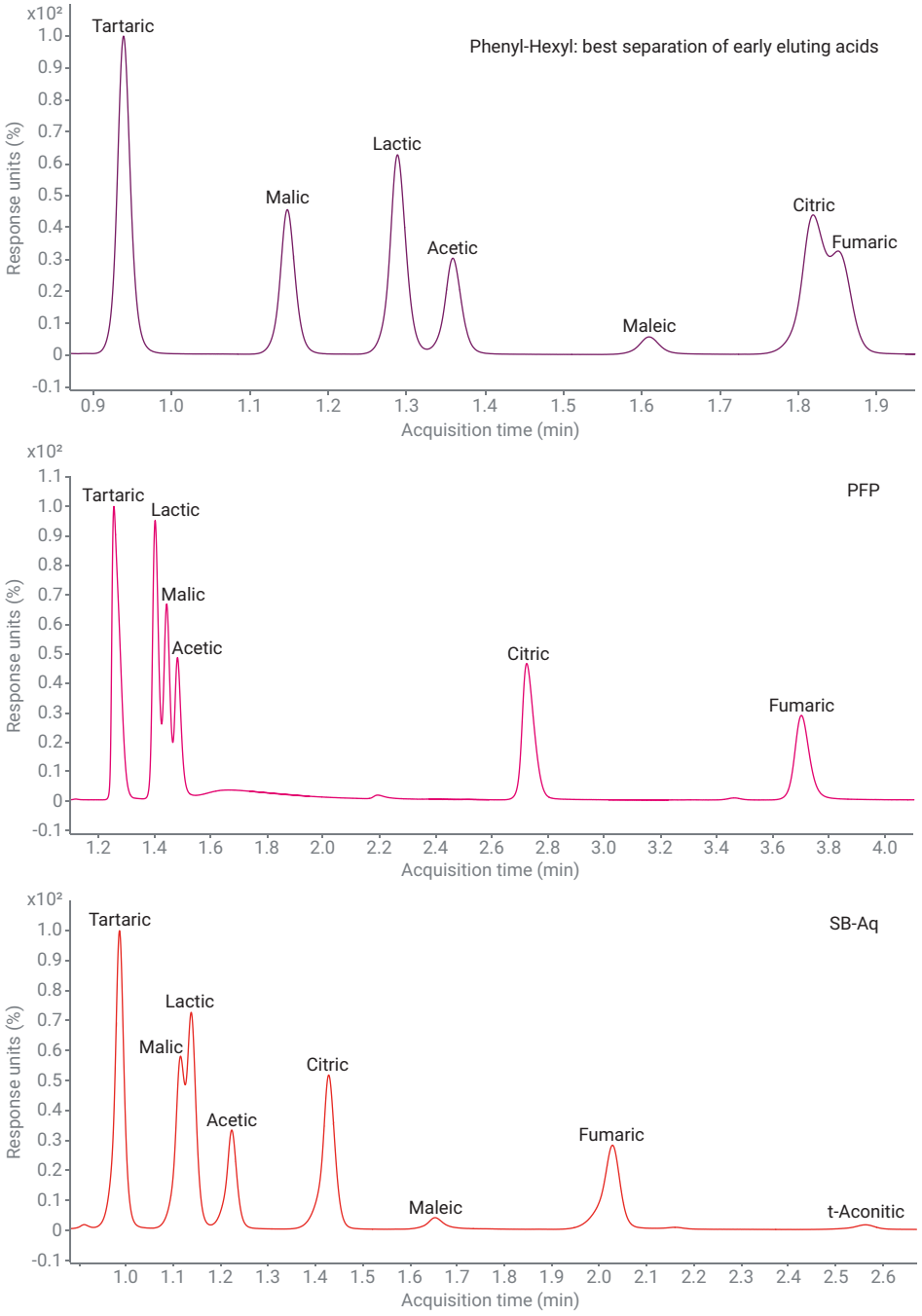

Figure 2. Alternate stationary phases on Agilent InfinityLab Poroshell 120 columns for organic acids. A: water, D: 200 mM sodium phosphate + 1% phosphoric acid (85%), pH ~2; isocratic: 90% A/10% D; 0.4 mL/min; 20 °C; 210 nm; 2.1 × 150 mm, 2.7 µm Agilent InfinityLab Poroshell 120 columns.

The partial coelution of citric and fumaric acid on Phenyl-Hexyl is solved by increasing the temperature of the column, as demonstrated in Figure 3.

Figure 4 shows the final separation of 11 organic acids on a 2.1 × 150 mm, 2.7 µm InfinityLab Poroshell 120 Phenyl-Hexyl column. All compounds are well resolved, with a minimum resolution of 1.5, in three minutes. The organic acids are difficult to retain because they are very polar, and are also difficult separate because they are structurally similar (Figure 1). However, the long, 150 mm, 2.7 µm Phenyl-Hexyl column has sufficient efficiency and resolving power to successfully retain and separate this sample.

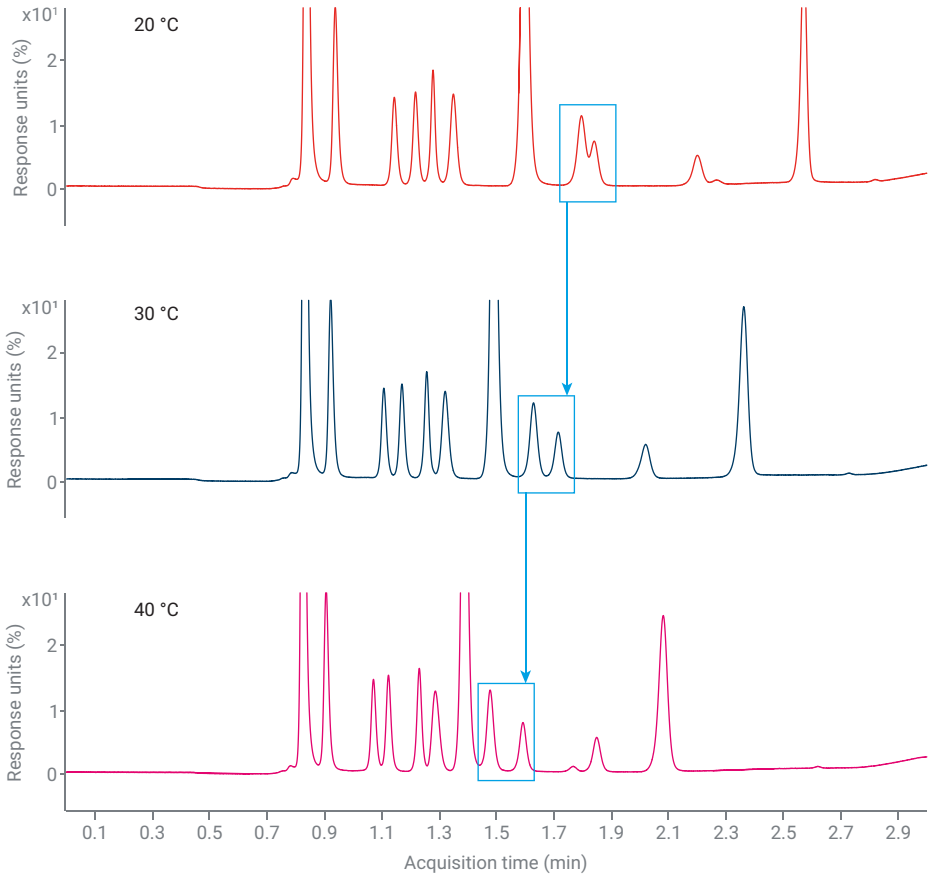

Figure 3. Column temperature screen for organic acids on Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column.

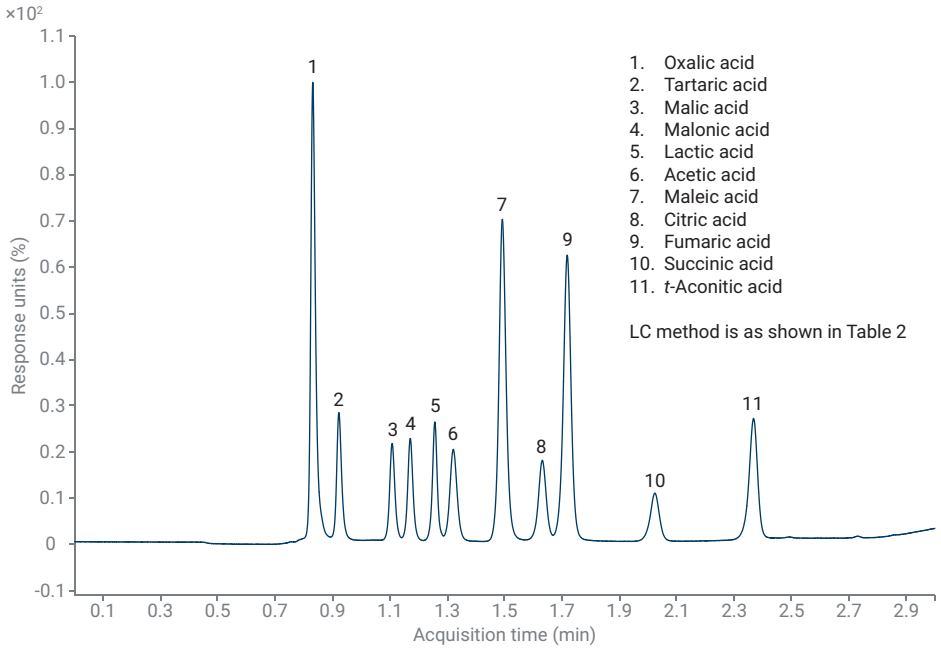

Figure 4. Separation of 11 organic acids on an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column.

Conclusion

The Agilent InfinityLab Poroshell 120, 2.7 µm column is used to accomplish a challenging separation of 11 organic acids. The high efficiency of this superficially porous particle column provides enough resolution to resolve the closely related compounds on a 2.1 × 150 mm column.

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**.

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Printed in the USA, June 26, 2019
5994-1082EN

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