Application Note

Food and Beverage Testing

Analysis of organic acids on an Agilent InfinityLab Poroshell 120 HILIC-Z column

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Abstract

Ten organic acids were baseline separated in four minutes on an Agilent InfinityLab Poroshell 120 HILIC-Z column. The column was a 2.1 × 100 mm format with 2.7 µm superficially porous particles. Isocratic elution with a phosphate buffer and acetonitrile mobile phase was used to accomplish the separation on an Agilent 1290 Infinity LC.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns generate high efficiency at lower pressure compared to their totally porous particle column counterparts. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

Superficially porous particles have primarily focused on reversed-phase separations. With the maturation of superficially porous particle technology, applications for further chemistries and chromatographic techniques, such as hydrophilic interaction liquid chromatography (HILIC), are becoming available. HILIC is well suited for the analysis of polar analytes, which are often difficult to retain and separate in reversed-phase mode. This Application Note demonstrates the UHPLC performance of an Agilent InfinityLab Poroshell 120 HILIC-Z, 2.7 µm column, and its ability to baseline resolve 10 organic acids. Figure 1 shows these compounds.

Experimental

An Agilent 1290 Infinity LC configured for low dispersion was used for this work. Table 1 shows the details. Table 2 shows the chromatographic method that was used. All compounds were injected as individual standards, and Table 3 lists the concentrations and sample solvents.

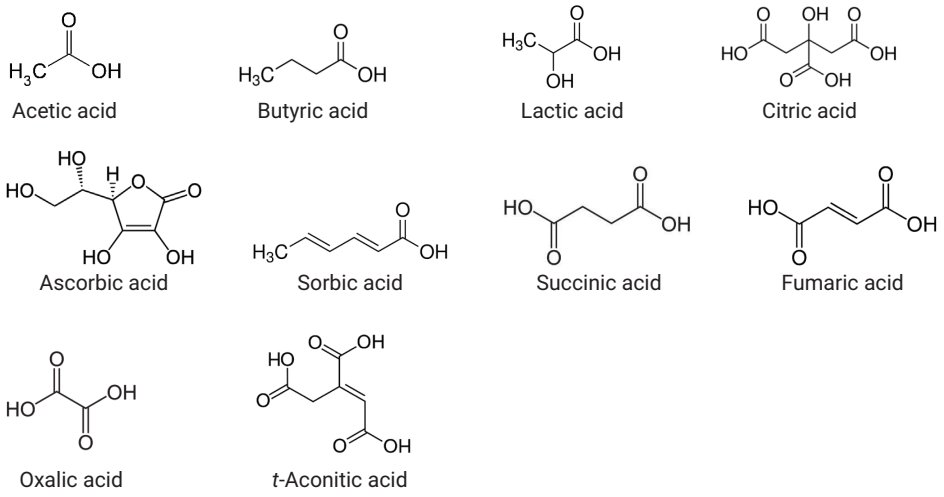

Figure 1. Compounds of interest.

Table 1. LC system configuration.

Agilent 1290 Infinity LC configuration	
Agilent G4220A binary pump	35 µL solvent mixer: Jet Weaver, 35 µL/100 µL (G4220-60006)
Agilent G4226A high performance autosampler	• Seat assembly, ultralow dispersion, for Agilent 1290 Infinity autosampler G4226A (G4226-87030) • Autosampler → Heater: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784) • Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (5182-0716) • Cap, screw, blue, PTFE/red silicone septa, 100/pk (5182-0717) • Vial insert, 250 µL, glass with polymer feet, 100/pk (5181-1270)
Agilent G1316C thermostated column compartment	• Heat exchanger, low dispersion, 1.6 µL, double (G1316-60005) • Heater → Column: InfinityLab Quick Connect assembly, 105 mm, 0.075 mm (5067-5961) • Column → Flow Cell: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent G4212A diode array detector	Ultralow dispersion Max-Light Cartridge flow cell, 10 mm (G4212-60038)
Agilent OpenLAB CDS ChemStation Edition Revision C.01.05 [35]	G4220A: B.06.53 [0013] G4226A: A.06.50 [003] G1316C: A.06.53 [002] G4212A: B.06.53 [0013]
Agilent LC column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (685775-924)

Table 2. LC Method parameters.

Mobile phase	Flow rate (mL/min)	Mobile phase composition	Injection volume (µL)	Thermostated column compartment (°C)	Diode array detector
A) 30 mM Sodium phosphate + 0.075 % phosphoric acid (pH ~6.7)	0.5	Isocratic elution	0.1	30	214 nm, 80 Hz
B) Acetonitrile		Premix mobile phase: 700 mL B + 300 mL A	For sample information, see Table 3		

The 10 organic acids analyzed were purchased from Sigma-Aldrich. Sodium phosphate and phosphoric acid were also from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered, 18 molecular weight, from a Milli-Q system (Millipore).

Important: Phosphate salts have low solubility in acetonitrile-rich solutions, and can precipitate when aqueous phosphate buffers are mixed with acetonitrile. Therefore, phosphates are not typically recommended for HILIC analyses. However, to allow for adequate detection of the organic acids at low wavelengths with a diode array detector, phosphate was necessary because formate and acetate salts produced significant baseline noise. To prevent salt from precipitating in the LC system, premixing the mobile phase, as detailed in Table 2, is highly recommended. The premixed mobile phase should be inspected and routinely monitored for salt precipitation. Preparing the mobile phase with more salt or more acetonitrile than indicated in this Application Note results in salt precipitation. If more mobile phase flexibility is desired, use alternate detectors (such as MSD, ELSD, or RID) with formate or acetate buffers. Use of these buffers may result in different selectivity, thus requiring extra method development.

Table 3. Sample preparation.

Organic acid	Concentration (mg/mL)	Sample solvent
Acetic acid	8.7	CH_3CN/H_2O (2:1)
Butyric acid	7.9	CH_3CN/H_2O (2:1)
Lactic acid	6.6	CH_3CN/H_2O (2:1)
Citric acid	7.5	CH_3CN/H_2O (2:1)
Ascorbic acid	8.3	CH_3CN/H_2O (2:1)
Sorbic acid	7.1	CH_3CN/H_2O (2:1)
Succinic acid	8.2	CH_3CN/H_2O (2:1)
Fumaric acid	7.4	CH_3CN/H_2O (8:5)
Oxalic acid	7.9	CH_3CN/H_2O (2:1)
t-Aconitic acid	7.8	CH_3CN/H_2O (2:1)

Results and discussion

The chromatogram in Figure 2 shows that 10 organic acids were baseline resolved on an Agilent InfinityLab Poroshell 120 HILIC-Z column. The separation was achieved in four minutes, with baseline resolution for all compounds.

The InfinityLab Poroshell 120 HILIC-Z phase uses a novel zwitterionic stationary phase bonded to a robust hybrid particle. It is stable up to 80 °C and up to pH 12. This phase offers excellent peak shape for charged compounds, and is capable of separating analytes over a wide range of polarity. The previously mentioned features of the HILIC-Z phase make it well suited for this challenging mid-pH separation of organic acids.

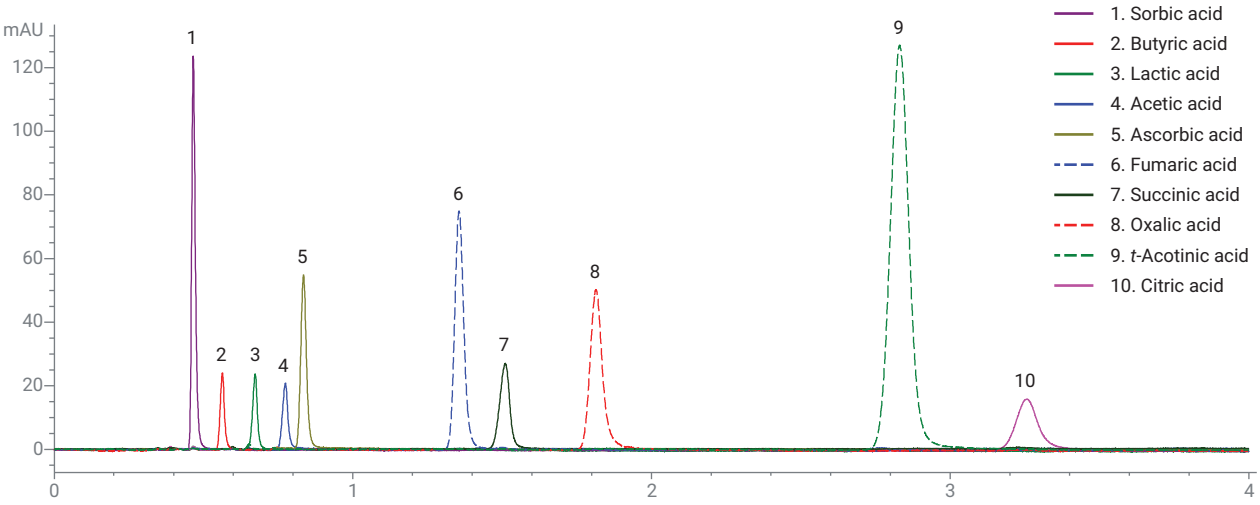

Figure 2. Separation of organic acids on an Agilent InfinityLab Poroshell 120 HILIC-Z column.

Conclusions

The Agilent InfinityLab Poroshell 120 HILIC-Z column provides a robust method for the separation of organic acids. This column offers good resolution and peak shape for all compounds.

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