Application Note

Food Testing & Agriculture

Separation of Organic Acids on an Agilent Polaris C18-A Column

Authors

Rongjie Fu
Agilent Technologies (Shanghai) Co. Ltd.

Cuiling Wu
Agilent Technologies (China) Co. Ltd.

Abstract

Six organic acids were analyzed with an Agilent Polaris C18-A column (4.6 × 250 mm, 5 µm) using an isocratic HPLC method with a highly aqueous mobile phase. The method was also scaled to a smaller particle and a shorter column (Agilent Polaris C18-A, 3.0 × 150 mm, 3 µm). All the organic acids were well resolved on both columns.

Introduction

Many organic acids, such as tartaric and malic acid, are highly polar and difficult to retain on a reversed-phase column. This often requires a high aqueous or a buffered-water mobile phase to achieve the desired separation. Use of the high-water content mobile phase with many reversed-phase columns leads to a dramatic decrease in retention over time. This loss in analyte retention in a high aqueous mobile phase is referred to as "phase collapse".[1] Polar modified reversed-phase columns are becoming popular for polar compounds analysis as a result of phase collapse. Agilent Polaris HPLC columns offer many polar phase chemistries designed to separate mixtures of polar and nonpolar analytes reliably and with excellent peak shapes. The polar-embedded C8 and C18 bonded phases are compatible with 100% water and offer alternative selectivity while still complying with USP L1 and USP L7.

In this application note, a Polaris C18-A (4.6 × 250 mm, 5 µm) column was used for analysis of organic acids according to the Chinese method GB5009.157-2016.[2] The method was also run on a smaller particle and a narrow bore Polaris C18-A column (3.0 × 150 mm, 3 µm).

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher. HPLC-grade methanol was bought from Merk (Billerica, MA, USA). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Phosphoric acid was purchased from Sigma-Aldrich. Standards were purchased from Anpel Laboratory Technologies (Shanghai, China). The standard mixture solution was made by dissolving the organic acids in water. The separate concentrations of the individual compounds are as follows: tartaric acid, 50 µg/mL; malic acid, 100 µg/mL; lactic acid, 50 µg/mL; citric acid, 50 µg/mL; succinic acid, 250 µg/mL; fumaric acid, 0.25 µg/mL)

Equipment and materials

- **Column inlet:** Agilent InfinityLab Quick Connect LC fitting (part number 5067-5965)
- **Column outlet:** Agilent InfinityLab Quick Turn LC fitting (part number 5067-5966)
- Agilent vial, screw top, amber, writeon spot, certified, 2 mL (part number 5182-0716)
- Agilent bonded screw cap, bonded blue, PTFE/red silicone septa (part number 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1 L (part number 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three-port, one-vent valve (part number 5043-1219)

Instrumentation

An Agilent 1260 Infinity II LC system, consisting of the following modules, was used:

- Agilent 1260 Infinity II quaternary pump (G7111B)
- Agilent 1260 Infinity II vialsampler (G7129A)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A), installed with InfinityLab Quick Change valve head, 4-column selector, 800 bar (5067-4279)
- Agilent 1260 Infinity II diode array detector (G4212B)

Table 1. HPLC conditions.

Column	Mobile Phase Composition	Flow Rate (mL/min)	Injection Volume (µL)	Thermostatted Column Compartment (°C)	Diode Array Detector
Agilent Polaris C18-A, 4.6 × 250 mm, 5 µm (p/n A2000250X046)	97.5% of 0.1% H_3PO_4 in water/2.5% methanol or 100% of 0.1% H_3PO_4 in water	1.0	20	40	210 nm, 10 Hz
Agilent Polaris C18-A, 3.0 × 150 mm, 3 µm (p/n A2001150X030)		0.425	5	40	210 nm, 20 Hz

Results and discussion

Figure 1 shows the method run on an Agilent Polaris C18-A column for the analysis of organic acids with a simple, high aqueous isocratic method. The chromatograms in Figure 1 were achieved following a regulated method under an isocratic mobile phase using 97.5% of 0.1% phosphoric acid in water and 2.5% methanol. All six compounds were well retained and separated. A beverage sample was tested with this method. Citric acid was detected in the sample.

This column is a polar-embedded C18 bonded phase with 100% aqueous compatibility without the risk of phase collapse. This analysis was able to run under 100% of 0.1% phosphoric acid in water and some target analytes, like malic acid and citric acid, were better resolved in the sample shown in Figure 2. Ten consecutive injections demonstrate a good reproducibility under 100% aqueous mobile phase, shown in Figure 3.

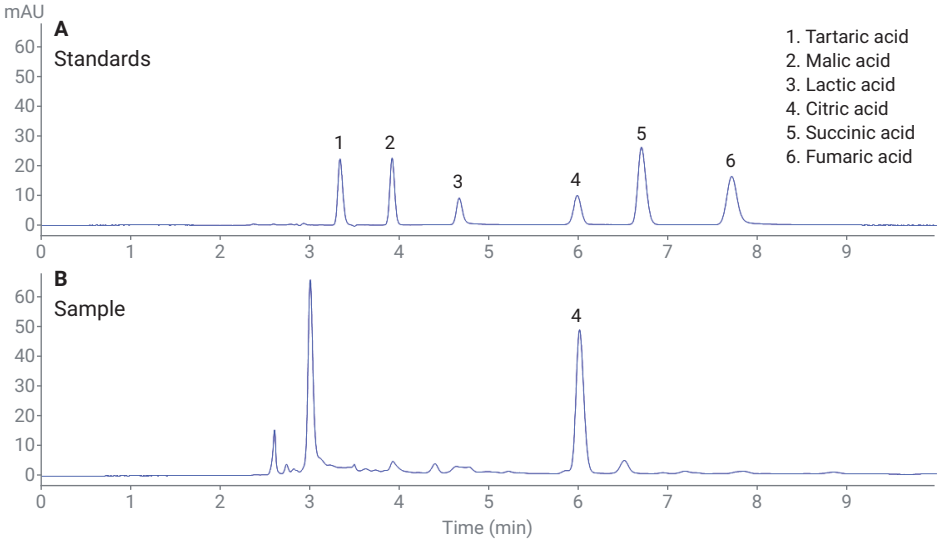

Figure 1. Analysis of standards and sample with an Agilent Polaris C18-A column (4.6 × 250 mm, 5 µm) using a mobile phase of 97.5% of 0.1% H_3PO_4 in water/2.5% methanol.

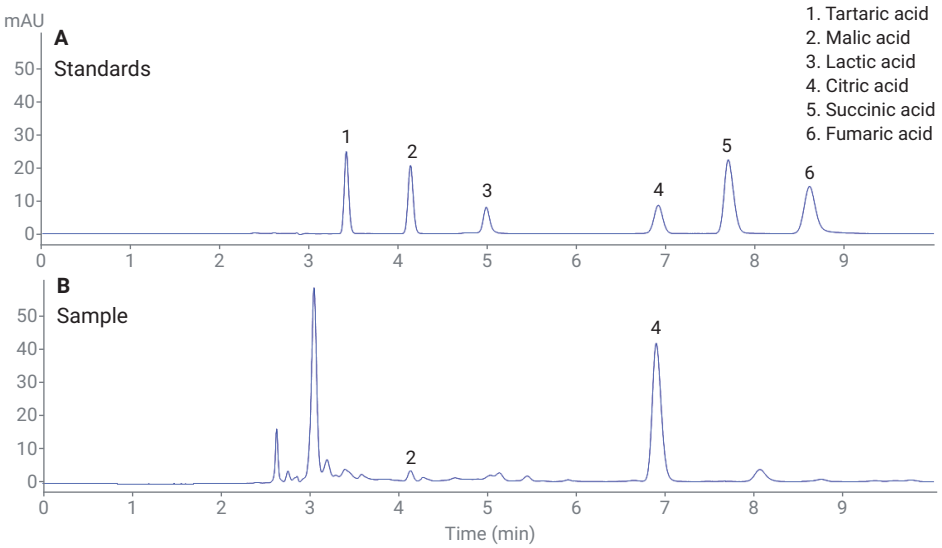

Figure 2. Analysis of standards and sample with an Agilent Polaris C18-A column (4.6 × 250 mm, 5 µm) using a mobile phase of 100% of 0.1% H_3PO_4 in water.

To save analysis time and solvent consumption, smaller particle columns are often used for HPLC analysis, while maintaining the same separation. Agilent offers Polaris C18-A columns in the 3 µm size as well as 5 µm. In this application note, the method with a 5 µm Polaris C18-A column was transferred to a Polaris C18-A, 3.0 × 150 mm, 3 µm column, shown in Figure 4. Both columns baseline-separated all six compounds, but the Polaris C18-A, 3.0 × 150 mm, 3 µm column saved 40% of the analysis time and 74.5% of the solvent consumption.

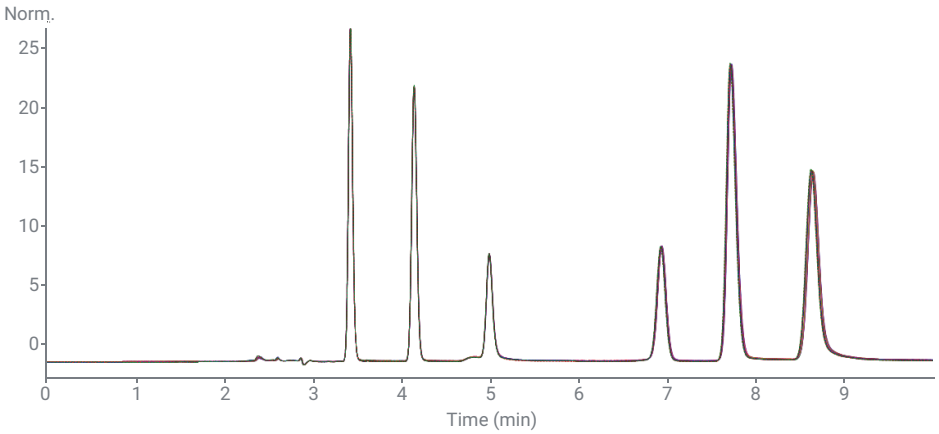

Figure 3. Overlay of consecutive injections of six organic acid standards with an Agilent Polaris C18-A column (4.6 × 250 mm, 5 µm) using a mobile phase of 100% of 0.1% H_3PO_4 in water.

Conclusion

The Agilent Polaris C18-A column was used to analyze six organic acids present in many food samples. The polar-embedded C18 bonded phase can retain and separate polar compounds like organic acids well without phase collapse. The smaller particle column (3 µm) saves time and solvent consumption when maintaining the same separation with a 5 µm column.

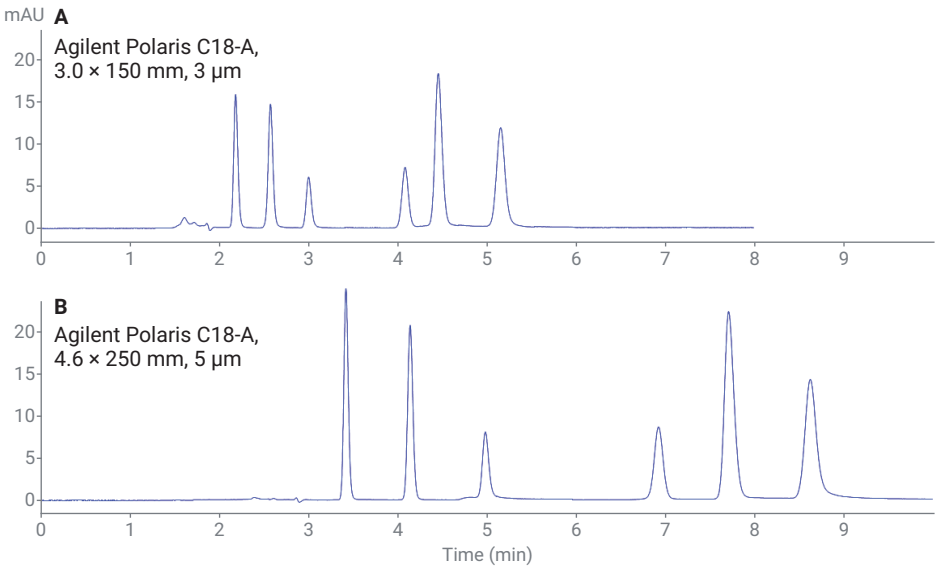

Figure 4. Analysis using an Agilent Polaris C18-A, 4.6 × 250 mm, 5 µm column compared to a Polaris C18-A, 3.0 × 150 mm, 3 µm column, using a mobile phase of 100% of 0.1% H_3PO_4 in water.

References

1. Bidlingmeyer, B. A.; Broske, A. D. The Role of Pore Size and Stationary Phase Composition in Preventing Aqueous-Induced Retention Time Loss in Reversed-Phase HPLC, *Journal of Chromatographic Science* **2004**, *42*.
2. Determination of Organic Acids in Food, *National Standards of Food Safety*, GB5009.157-2016.

www.agilent.com/chem

DE.9669675926

Printed in the USA, July 30, 2020
5994-2244EN

Agilent
Trusted Answers