Application Note

Food Testing

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Separation of Organic Acids on an Agilent InfinityLab Poroshell 120 Aq-C18 Column

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Abstract

Nine organic acids were analyzed with an Agilent InfinityLab Poroshell 120 Aq-C18 column under an isocratic method using a highly aqueous mobile phase. Good resolution and reproducibility results were achieved using this column.

Introduction

Organic acids are an important group of components in many food samples, including fruit drinks, wine, and other aqueous samples. With a common reverse-phase condition, these organic acids are polar and difficult to retain. Some methods have been evaluated to analyze these compounds, such as using a hydrophilic interaction liquid chromatography (HILIC) column, which is well suited for the analysis of polar analytes.[1] However, HILIC has a limitation for direct injection of aqueous samples, which may cause distorted peak shape for early eluted peaks due to solvent effect.

A new InfinityLab Poroshell 120 Aq-C18 column has been developed based on superficially porous particles. An optimized C18 ligand with proprietary endcapping is applied to 2.7 µm Poroshell particles with a pore size of 120 Å to significantly improve the retention, peak shape, and reproducibility of polar analyte analysis, with minimized pore dewetting phenomenon. The column provides stronger retention for polar compounds than other superficially porous polar modified C18 columns that are commercially available, as well as a high-density C18 column.[2]

In this application note, an InfinityLab Poroshell 120 Aq-C18 column is used for the analysis of these organic acids under a highly aqueous mobile phase. Two mobile-phase additives were investigated, and the method was optimized separately.

Experimental

Instruments and materials

An Agilent 1290 Infinity LC system with binary pump was used in this experiment, consisting of the following elements:

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity autosampler (G4226A)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)
- Agilent 1290 Infinity diode array detector (G4212A)

The LC column used was an Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (part number 695975-742)

All reagents and solvents were HPLC grade. Methanol, sodium dihydrogen phosphate, phosphoric acid, and the nine standards were purchased from Anpel Laboratory Technologies, Shanghai. Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Oxalic, tartaric, malic, lactic, acetic, maleic, citric, fumaric, and succinic acids were all separately dissolved in water. The standard solution was prepared by mixing the individual stock solutions and diluting with water.

Table 1 shows the individual concentrations of all the components, and the method parameters are listed in Table 2.

Table 1. Solutions for the analytes.

Compound Name	Concentration of Stock Solution (mg/mL)	Solvent	Concentration in Mixture (mg/mL)
Oxalic Acid	20.0	Water	0.13
Tartaric Acid	10.0	Water	1.33
Malic Acid	2.6	Water	0.69
Lactic Acid	1.0	Water	2.67
Acetic Acid	20.0	Water	2.67
Maleic Acid	2.0	Water	0.053
Citric Acid	20.0	Water	2.67
Fumaric Acid	2.0	Water	0.067
Succinic Acid	20.0	Water	2.67

Table 2. Method parameters.

Parameter	Value
Mobile Phase	A) 97.5% (0.1% H_3PO_4)/2.5% methanol B) 99% 50 mM NaH_2PO_4 buffer with pH 2.5/1% methanol
Column Temperature	25 °C
Flow Rate	1.0 mL/min
Detector	UV 210 nm
Injection Volume	1 µL

Results and discussion

The analysis was performed on an InfinityLab Poroshell 120 Aq-C18 column. Both phosphoric acid and phosphate buffer were investigated as mobile-phase additives, and the composition of aqueous and organic phase was optimized to get ideal resolution for all the compounds. Figure 1 shows the separation of nine organic acids on the InfinityLab Poroshell 120 Aq-C18 column under both optimized mobile phases. All compounds were well retained and baseline separated. The two mobile phases provided different selectivity due to different pH values of the mobile phase. Usually, using buffers as mobile-phase additives helps improve peak shape. In this application, using a 50 mM phosphate buffer improved peak shape of several organic acids, like citric and fumaric acid.

A reproducibility test was done by consecutive injections. The results shown in Figure 2 demonstrate that the method is reproducible. This method used 99% aqueous phosphate buffer, which is not recommended to be used with conventional C18 columns. The combination of design features including large pore size and optimized C18 ligands and proprietary end-capping surface allows reproducible results when using low or no organic solvents and gives maximum retention.

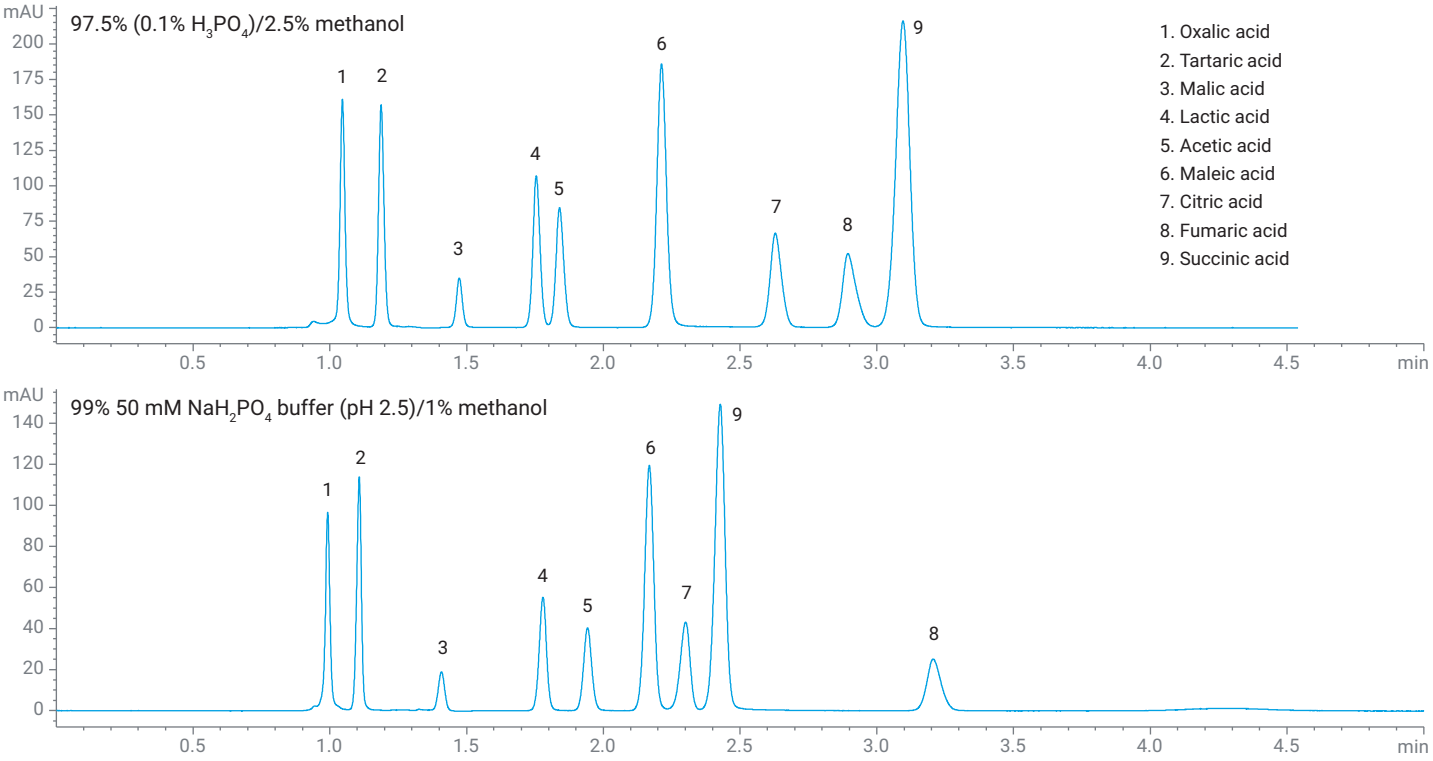

Figure 1. Organic acid separation with the Agilent InfinityLab Poroshell 120 Aq-C18 under different mobile-phase additives and compositions.

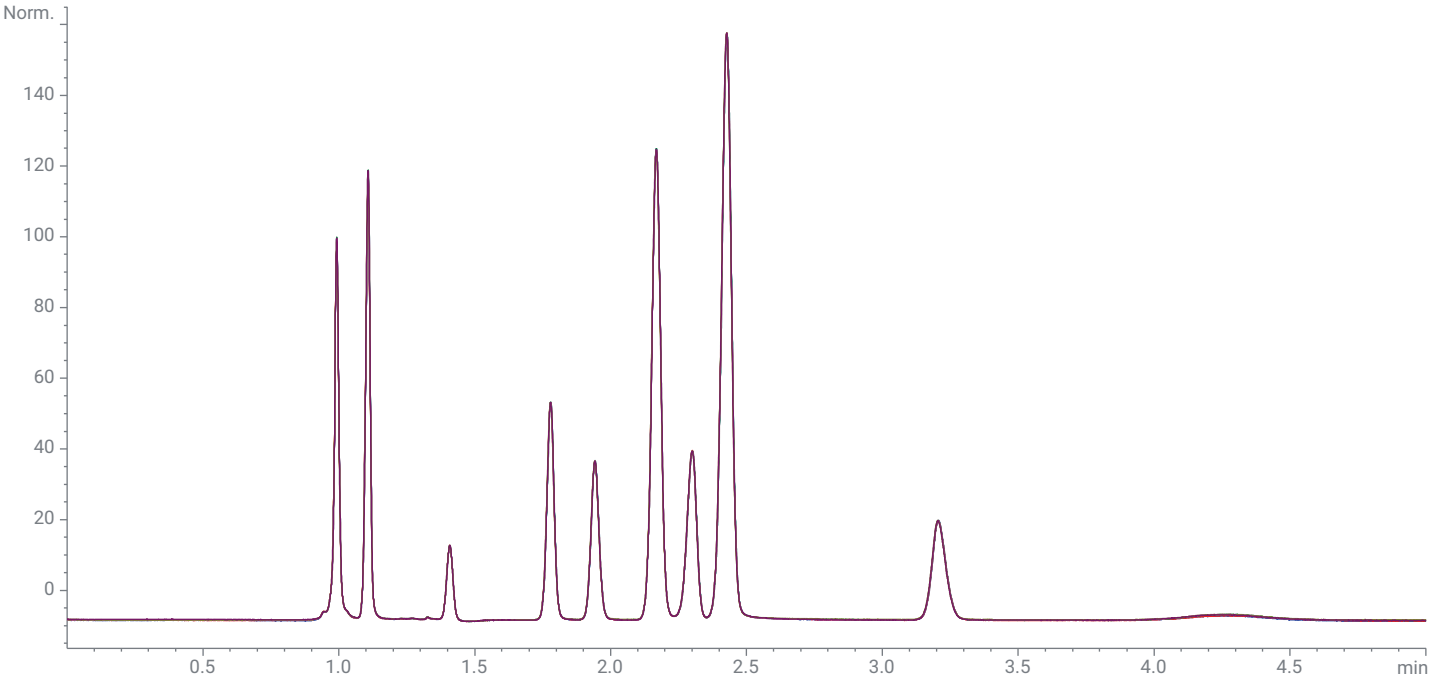

Figure 2. Overlay chromatograms of six consecutive injections under 99% phosphate buffer and 1% methanol.

Conclusion

The separation of nine organic acids was successfully achieved with an Agilent InfinityLab Poroshell 120 Aq-C18, 2.7 µm column. The column provided reasonable retention and good resolution for the polar compounds. Reproducible results were also achieved with this column under the isocratic mobile phase containing 99% aqueous phosphate buffer eluents.

References

1. Mack, A. Analysis of Organic Acids on an Agilent InfinityLab Poroshell 120 HILIC-Z Column, *Agilent Technologies application note*, publication number 5991-8985EN, **2018**.
2. Fu, R.-J.; Wei, T.-C. Analysis of Polar Compounds Using an Agilent InfinityLab Poroshell 120 Aq-C18 Column with Improved and Reliable Performance, *Agilent Technologies application note*, publication number 5994-5555EN, **2022**.

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