Application Note

Metabolomics

Analysis of Choline Metabolites by Hydrophilic Interaction Chromatography (HILIC) with LC/MS/MS

Authors

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Abstract

Choline and its metabolites were analyzed using Agilent InfinityLab Poroshell 120 HILIC-Z and Agilent InfinityLab Poroshell 120 HILIC-OH5 columns. The Poroshell 120 HILIC-Z provided better peak shape and a little higher sensitivity than the Poroshell 120 HILIC-OH5, but with weaker retention. The effect of varying mobile phase additives was explored using the Poroshell 120 HILIC-Z column. It was found that the best peak shape was achieved by adding 10 mM ammonium acetate to the mobile phase.

Introduction

Choline and its metabolites, betaine, acetylcholine, and glycerophosphocholine, are small, highly polar quaternary amine compounds that are not retained on a reversed-phase column. Newly developed HILIC chemistries with superficially porous particle technology are ideal for the retention of this class of compound. In this Application Note, choline and its metabolites are separated using the InfinityLab Poroshell 120 HILIC-OH5 and Agilent InfinityLab Poroshell 120 HILIC-Z chemistries. The influence of different mobile phase additives was explored when using the Poroshell 120 HILIC-Z column. An Agilent 6460 triple quadrupole LC/MS was used for low level detection of the sample.

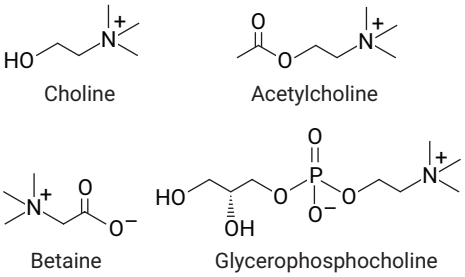

Figure 1. Choline and its metabolites.

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher. HPLC grade acetonitrile was bought from J. T. Baker (Center Valley, PA, USA). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Formic acid, acetic acid, ammonium acetate, and ammonium hydroxide were from J&K Scientific (Beijing, China). Choline, betaine, acetylcholine, and glycerophosphocholine were from Sigma-Aldrich (St. Louis, MO, USA). The standards solution was produced in 90:10 acetonitrile:water to a concentration of 0.5 nM.

Equipment and materials

- **Column inlet:** Agilent InfinityLab Quick Connect LC fitting (p/n 50675965)
- **Column outlet:** Agilent InfinityLab Quick Turn LC fitting (p/n 5067-5966)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size (p/n 5190-5265)
- Agilent vial, screw top, amber, write-on spot, certified, 2 mL (p/n 5182-0716)
- Agilent bonded screw cap, bonded blue, PTFE/red silicone septa (p/n 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three ports, one vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater
- Sonicator (VWR, Radnor, PA, USA)

Instrumentation

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II MCT (G7116B)
- Agilent 6460 triple quadrupole LC/MS (G6460A)
- Agilent MassHunter LC/MS data acquisition software, version B.08.00

Software

Agilent MassHunter qualitative analysis software, version B.07.00

HPLC conditions	
Column	InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (p/n 685775-924) InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm (p/n 685775-601)
Mobile phase A	Water with acid or buffers (shown in Figure 2)
Mobile phase B	Acetonitrile
Gradient	0 to 5 minutes, 10 to 50 %A; 5 to 6 minutes, 50 to 60 %A; 6 to 8 minutes, 60 %A; Stop: 8 minutes
Flow rate	0.30 mL/min
Column temperature	30 °C
Injection volume	1 µL
MS conditions	
Ion mode	ESI/Jet Stream ESI, Positive
Drying gas temperature	250 °C
Drying gas flow	5 L/min
Nebulizer pressure	45 psi
Sheath gas temperature	250 °C
Sheath gas flow	11 L/min
Capillary voltage (+)	3,500 V
Nozzle voltage (+)	0 V
MRM condition	ΔEMV, 500 V

Results and discussion

Different mobile phase additives were explored, as shown in Figure 2. With an increase in mobile phase pH, acetylcholine and choline were retained much more strongly on the Poroshell 120 HILIC-Z column, but their signals decreased slightly. When using either a neutral or basic mobile phase, retention remained unchanged. However, the mid pH mobile phase provided higher signals of acetylcholine and betaine than in basic conditions. Therefore, the addition of 10 mM ammonium acetate to the mobile phase provides the best separation of all four compounds.

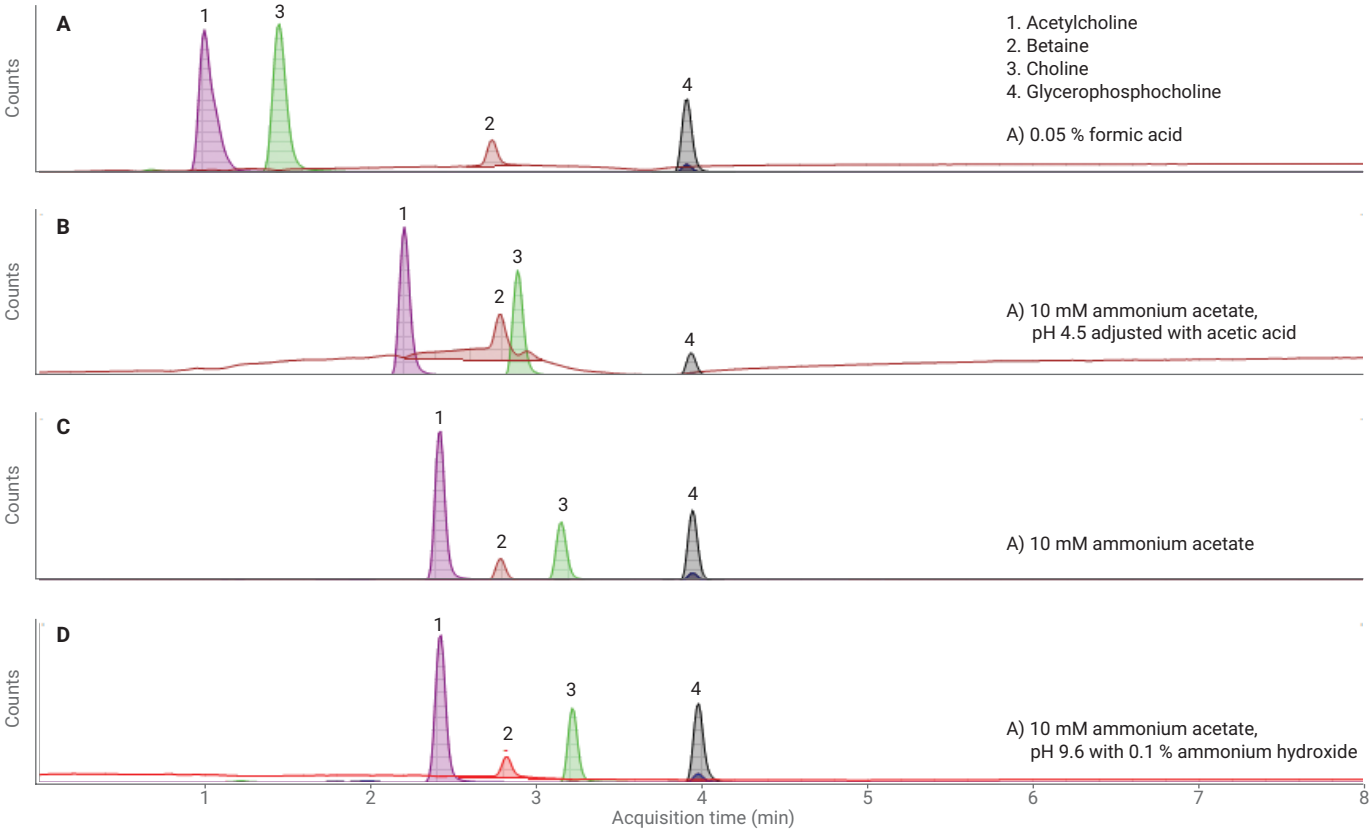

Figure 2. Effect of different mobile phase additives when using an InfinityLab Poroshell 120 HILIC-Z column with LC/MS/MS.

Separation was compared between two HILIC columns using the same mobile phase with neutral conditions. The Poroshell 120 HILIC-OH5 column showed increased retention of all compounds, shifted the peak order, and showed higher sensitivity for betaine. However, the Poroshell 120 HILIC-Z column gave better peak shape for the critical acetylcholine and choline analytes.

Conclusions

In general, the best separation of choline and its metabolites was achieved using an ammonium acetate-supplemented mobile phase at mid pH with the InfinityLab Poroshell 120 HILIC-Z column. An alternative HILIC chemistry, the InfinityLab Poroshell 120 HILIC-OH5, was also evaluated for this separation. While the sensitivity of betaine improved on the HILIC-OH5 column, the acetylcholine and choline peak shape and sensitivity was superior on the HILIC-Z column.

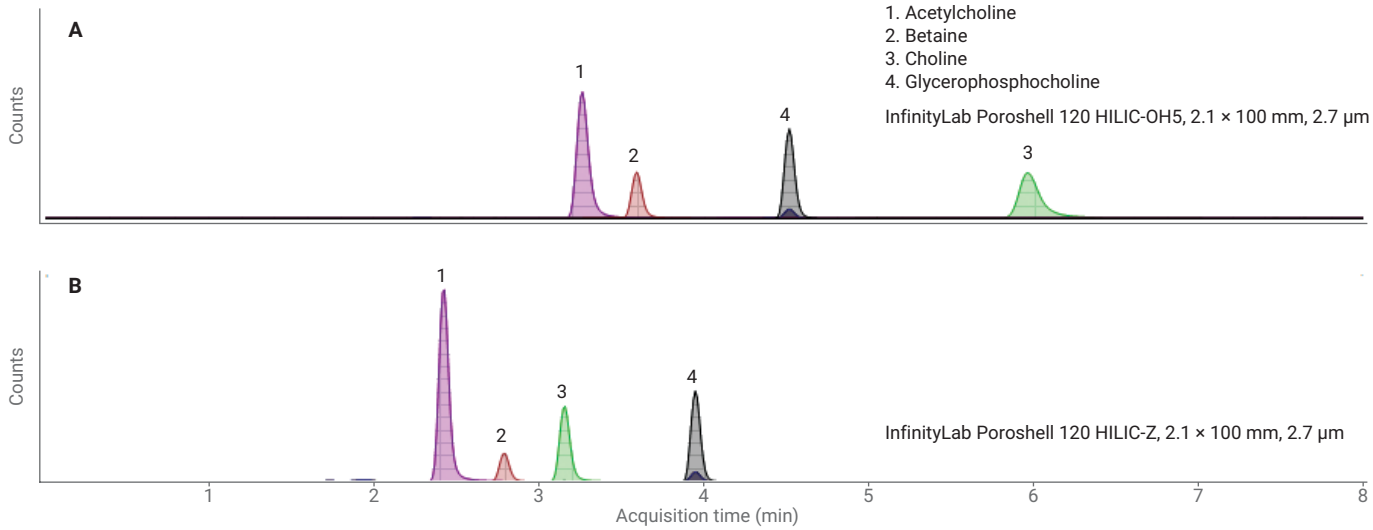

Figure 3. Comparison between InfinityLab Poroshell 120 HILIC-OH5 and InfinityLab Poroshell 120 HILIC-Z columns with 10 mM ammonium acetate in water as mobile phase A.

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