Application Note

Food Testing, Metabolomics

Methods for the Analysis of Underivatized Amino Acids by LC/MS

For Food, Life Science, and Metabolomics Applications

Authors

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Abstract

This Application Note presents a method optimized for the analysis of underivatized amino acids.

The polar nature of amino acids makes analysis by reversed-phase liquid chromatography challenging. Conversely, Hydrophilic Interaction Chromatography (HILIC) is capable of retaining and separating complex amino acid mixtures, while offering a similar workflow to traditional reversed-phase chromatography. The combination of HILIC with mass spectrometry offers a simple and powerful solution for amino acid analysis.

Introduction

Separation of underivatized amino acids using Hydrophilic Interaction Chromatography (HILIC) at low pH with positive mode LC/MS detection was found to give the best overall sensitivity and chromatographic performance. The Agilent InfinityLab Poroshell 120 2.7 µm HILIC-Z and HILIC-OH5 chemistries offer two excellent options for LC/MS of amino acids. Both give complete separation of the challenging leucine/isoleucine isobars, and fully resolve a wide range of amino acids.

Experimental

Reagents and Chemicals

All reagents were HPLC grade or higher. Ultra LC/MS grade acetonitrile was bought from J.T. Baker (Center Valley, PA, U.S.A.). Water was purified using an EMD Millipore Milli-Q Integral System (Darmstadt, Germany). Reagent-grade formic acid (FA) (p/n G2453-85060) was from Agilent Technologies. Ammonium formate, ammonium acetate, ammonium hydroxide, and amino acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amino acids were stored at –70 °C until day of use.

Equipment and Materials

- Agilent InfinityLab Fittings
  - Column front: InfinityLab Quick Connect LC fitting (p/n 5067-5965)
  - Column back: InfinityLab Quick Turn LC fitting (p/n 5067-5966)
- Agilent vial, screw top, amber, write-on spot, certified, 2 mL (p/n 5182-0716)
- Agilent bonded screw cap, PTFE/red silicone septa (p/n 5190-7024)
- Agilent vial insert, 250 µL, deactivated glass with polymer feet (p/n 5181-8872)
- Eppendorf pipettes and repeater
- Vortexer and multitube vortexers (VWR, Radnor, PA, USA)
- HDPE solvent bottles (VWR, Radnor, PA, USA)

Instrumentation

- Agilent 1290 Infinity II binary pump (G7120A)
- Agilent 1290 Infinity II vialsampler (G7129B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Ultralow dispersion kit for Agilent 1290 Infinity LC Series (5067-5189)
- Agilent MassHunter workstation software
- Agilent 6470 triple quadrupole LC/MS
- Agilent Jet Stream Electrospray ionization source

Sample Preparation

The amino acid standards were mixed to the concentrations listed in Table 1 in water, and analyzed with no further sample preparation.

Mobile Phase Preparation

A 200 mM ammonium formate stock was made in water, and adjusted to pH 3 with formic acid. Mobile phase A was made by diluting the stock solution 9:1 in water. Mobile phase B was made by diluting the stock solution 9:1 in ACN. The final concentration in both mobile phases was 20 mM of ammonium formate.

Extended exposure of the mobile phases to glassware was found to introduce ionic species that interfere with and suppress MS detection. Mobile phases stored in glass should be changed regularly, or the glass bottles should be replaced with HDPE bottles.

Instrument Conditions

Parameter	Value
HPLC	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm
Mobile phase A	10 % (200 mM ammonium formate in water at pH = 3), 90 % water
Mobile phase B	10 % (200 mM ammonium formate in water at pH = 3), 90 % acetonitrile
Flow rate	0.80 mL/min
Column temperature	30 °C
Injection volume	0.25 µL
Total run time	16 minutes
Gradient	Time (min) %B 0 100 10 70 11 100
MS	
Ionization mode	ESI Positive
Gas temperature	300 °C
Gas flow	7.0 L/min
Nebulizer	45 psi
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Capillary voltage	3,500 V
Nozzle voltage	0 V

Table 1. Amino acid standard concentration, retention, and dMRM values.

Analyte	Concentration (mM)	Agilent InfinityLab Poroshell 120 HILIC-Z RT (min)	Agilent InfinityLab Poroshell 120 HILIC-OH5 RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Dwell time (ms)
Phenylalanine	0.25	2.12	2.47	166.1	120.1	25	5	10
Leucine	0.25	2.34	2.74	132.1	86.1	25	8	10
Isoleucine	0.25	2.50	2.93	132.1	86.1	25	8	10
Methionine	0.25	2.71	3.09	150.0	104.0	75	8	10
Tyrosine	0.25	3.07	3.27	182.1	136.1	25	12	10
Valine	0.25	3.11	3.54	118.1	72.1	25	8	10
Proline	0.25	3.16	3.75	116.1	70.1	50	16	10
Alanine	0.25	3.73	4.13	90.1	44.1	25	8	10
Threonine	0.25	3.82	4.16	120.0	74.1	25	10	10
Glycine	0.25	4.08	4.51	76.0	30.1	25	5	10
Serine	0.25	4.27	4.56	106.1	60.1	25	8	10
Glutamic acid	0.25	4.71	5.43	148.1	84.1	75	16	10
Aspartic acid	0.25	5.25	6.14	134.0	74.0	50	15	10
Histidine	0.25	5.59	5.87	156.1	110.0	25	12	10
Arginine	0.25	6.09	5.91	175.1	70.1	75	28	10
Lysine	0.25	6.54	6.63	147.1	84.1	50	16	10

Note: All transitions used a cell accelerator voltage = 4.

Results

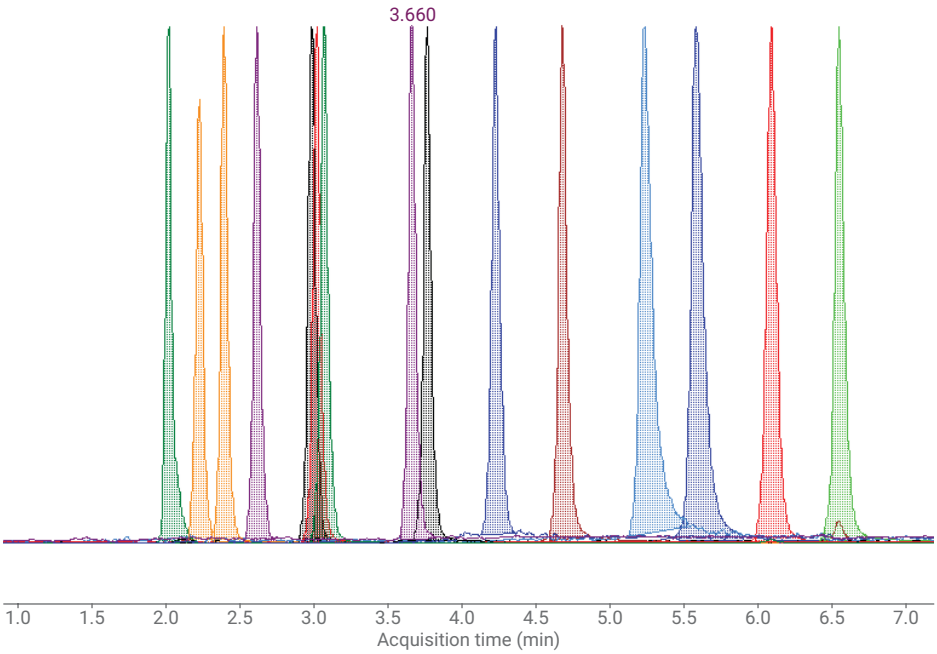

Figure 1. Separation of amino acids using the Agilent InfinityLab Poroshell 120 HILIC-Z column. Leucine/isoleucine selectivity – 1.08.

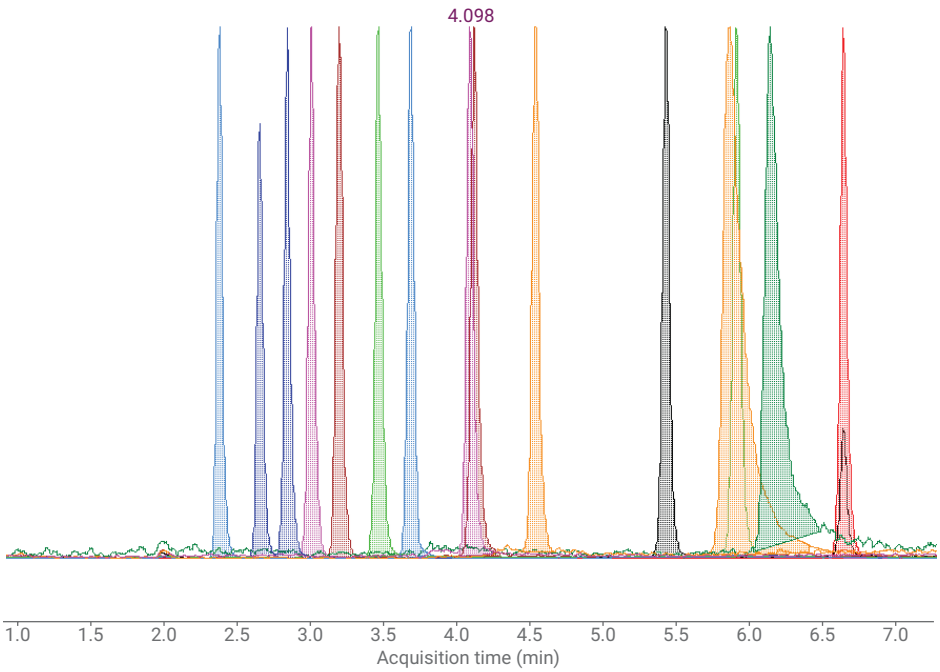

Figure 2. Separation of amino acids using the Agilent InfinityLab Poroshell 120 HILIC-OH5 column. Leucine/isoleucine selectivity – 1.08.

Conclusions

- Amino acids were successfully analyzed by positive mode LC/MS using HILIC.
- The Agilent InfinityLab Poroshell HILIC-Z showed better overall peak shape, especially for more basic compounds like aspartic acid and histidine.
- The Agilent InfinityLab Poroshell HILIC-OH5 showed better resolution of early eluting peaks, such as tyrosine, valine, and proline.
- The normally challenging leucine/isoleucine isobars were baseline separated by both columns.

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