LC/MS Analysis of Free Amino Acids on Agilent InfinityLab Poroshell 120 HILIC 1.9 µm Columns

Application Note

Agriculture, Food Testing, Small Molecule Pharmaceuticals

Author

Anne Mack
Agilent Technologies, Inc.
Wilmington, DE, USA

Abstract

Twelve free amino acids were analyzed by LC/MS with an Agilent InfinityLab Poroshell 120 HILIC 2.1 × 150 mm, 1.9 µm column using an ammonium formate and acetonitrile gradient. The 550 bar analysis was accomplished in 11 minutes, with baseline resolution (Rs = 1.9) of isobaric leucine and isoleucine.

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Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. Superficially porous particle columns generate high efficiency at lower pressure relative to their totally porous particle column counterparts [1]. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column [2]. The current trend with superficially porous particles is reducing particle size for further efficiency improvements. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

This application note demonstrates the UHPLC performance of an Agilent InfinityLab Poroshell 120 HILIC 1.9 µm column, and its ability to analyze 12 free amino acids through LC/MS, including baseline resolution of an isobaric pair.

Experimental

An Agilent 1290 Infinity LC system with an Agilent 6460 triple quadrupole LC/MS was used in this experiment. The system was modified from its standard configuration to have low system volume and dispersion. Table 1 shows the configuration details and the Agilent LC column used in this experiment. Table 2 shows the LC method parameters, Table 3 shows the MS parameters, and Table 4 shows the MS SIM parameters.

The 12 amino acids analyzed in this application note were purchased as a mixed component solution from Agilent (5061-3330); the sample was diluted 1:10 in acetonitrile prior to injection. Ammonium formate and formic acid were purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Table 1. UHPLC System Configuration

Agilent 1290 Infinity LC system configuration	
Agilent 1290 Infinity Binary Pump (G4220A)	35 µL solvent mixer: Jet Weaver, 35 µL/100 µL (G4220-60006)
Agilent 1290 Infinity High Performance Autosampler (G4226A)	Seat assembly, ultralow dispersion, for Agilent 1290 Infinity Autosampler (G4226-87030) Autosampler to 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 1290 Infinity Thermostatted Column Compartment (G1316C)	Heat exchanger, low dispersion, 1.0 µL, long, down (G1316-80012) InfinityLab Quick Turn fitting (5067-5966) Column to MS source: capillary, stainless steel, 0.075 × 340 mm, SV/SLV (5067-4783)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Ultralow dispersion Max-Light cartridge flow cell, 10 mm (G4212-60038)
Agilent 6460 Triple Quadrupole LC/MS (G6460A)	Agilent Jet Stream technology
Agilent MassHunter Workstation Software LC/MS Data Acquisition for 6400 Series Triple Quadrupole Version B.07.01 Build 7.1.7112.0	G4220A: B.06.72 [0002] G4226A: A.06.54 [006] G1316C: A.06.53 [002] G4212A: B.06.72 [0002]
Agilent LC Column	Agilent InfinityLab Poroshell 120 HILIC, 2.1 × 150 mm, 1.9 µm (693675-901)

Table 2. UHPLC Method Parameters for Free Amino Acid Analysis

Parameter	**Value**
Mobile phase A	10 mM ammonium formate pH 3 in water
Mobile phase B	10 mM ammonium formate pH 3 in acetonitrile:water (9:1)
Flow rate	0.4 mL/min
Gradient	100–95 %B in 5 minutes, then 95–60 %B in 6 minutes
Post run	4 minutes
Injection	5 µL
Autosampler temperature	4 °C
Column temperature	15 °C
Diode array detector	Off
Column	Agilent Poroshell 120 HILIC, 2.1 × 150 mm, 1.9 µm (693675-901)
Sample	Agilent AA standard, 1 nmol/µL (5061-3330), diluted 1:10 in acetonitrile

Results and Discussion

Figure 1 shows the separation of 12 free amino acids on an InfinityLab Poroshell 120 HILIC, 2.1 × 150 mm, 1.9 µm column. With LC/MS detection, baseline chromatographic resolution is not necessary for all compounds, as the detector resolves the analytes by their specific mass fragments. However, when isobaric compounds are present, baseline chromatographic resolution is necessary. The chromatographic resolution for leucine and isoleucine was 1.9, shown in Figure 2, which provides for good integration and quantitation of these two isobars.

Table 3. MS Method Parameters

MS Source parameters		MS Acquisition parameters	
Gas temperature	300 °C	Scan type	MS2 SIM
Gas flow	5 L/min	Mass	See Table 4
Nebulizer	45 psi	Fragmentor	See Table 4
Sheath gas temperature	400 °C	Cell accelerator	7 V
Sheath gas flow	11 L/min	Polarity	Positive
Capillary	3,500 V	Time segment 1	3.6 minutes
		Time segment 2	6.6 minutes

Table 4. MS SIM Method Parameters

Amino acids (in elution order)	MW	M+H	Fragmentor voltage	Dwell time (ms)	Time segment
L-Phenylalanine	165.19	166	25	5	1
L-Tyrosine	181.19	182	25	5	1
L-Iso/leucine	131.17	132	25	1	1
L-Methionine	149.21	150	75	5	1
L-Valine	117.15	118	25	5	2
L-Threonine	119.12	120	25	5	2
L-Alanine	89.09	90	25	5	2
L-Serine	105.09	106	25	5	2
L-Proline	115.13	116	50	5	2
Glycine	75.07	76	25	5	2
L-Glutamic acid	147.13	148	75	5	2

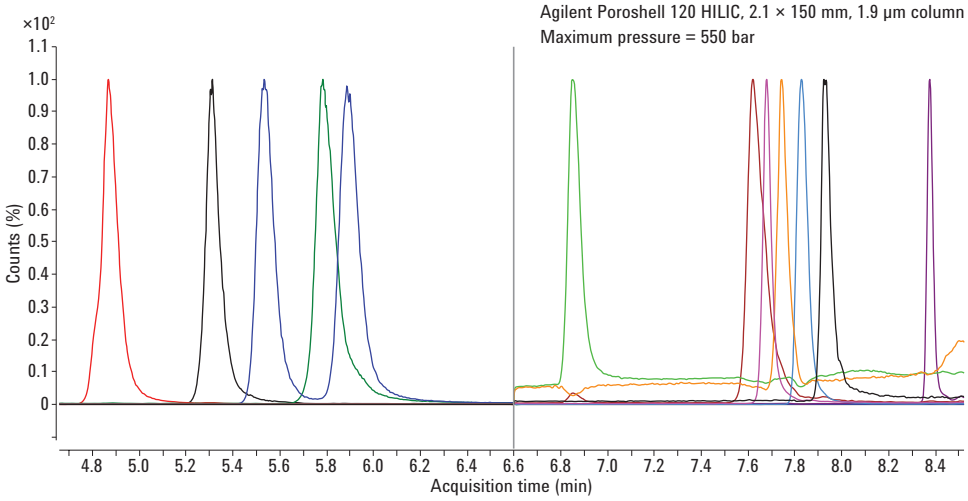

Figure 1. Separation of 12 free amino acids on an Agilent Poroshell 120 HILIC 1.9 µm column.

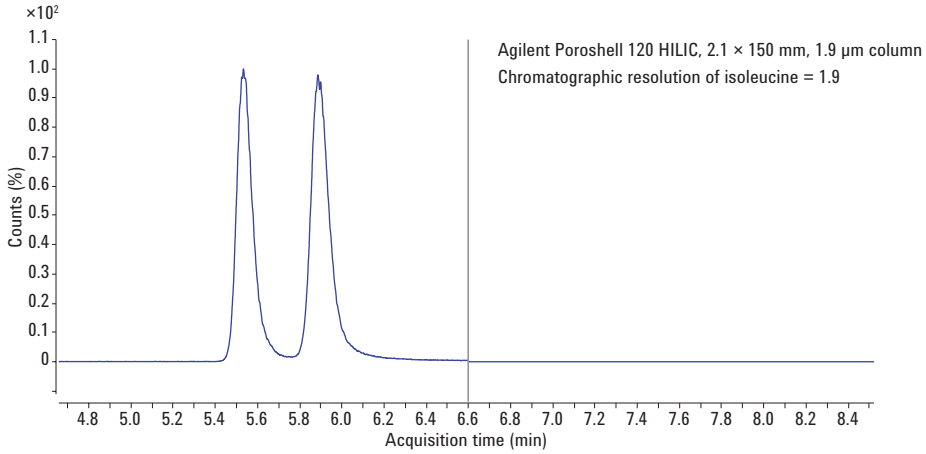

Figure 2. Separation of isobaric leucine and isoleucine on an Agilent Poroshell 120 HILIC, 1.9 µm column.

Conclusions

An Agilent InfinityLab Poroshell 120 HILIC 1.9 µm column was used to accomplish a separation of free amino acids by LC/MS. The high efficiency of this small superficially porous particle column provided sufficient resolution to baseline resolve the isobaric pair.

References

1. A. Gratzfield-Hugsen, E. Naegele. Maximizing efficiency using Agilent Poroshell 120 Columns. *Agilent Technologies Application Note*, publication number 5990-5602EN, **2016**.
2. V. R. Meyer. Practical High Performance Liquid Chromatography. Fourth Edition, p. 34. Wiley (2004).

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