

Analysis of Water-Soluble Vitamins on an Agilent InfinityLab Poroshell 120 HILIC-OH5 Column

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Abstract

Eight water-soluble vitamins are baseline separated in 4 minutes on an Agilent InfinityLab Poroshell 120 HILIC-OH5 column. The column is a 2.1 × 100 mm format with 2.7 μm superficially porous particles. An ammonium acetate and acetonitrile gradient was used to accomplish the separation on an Agilent 1290 Infinity LC.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns generate high efficiency at lower pressure compared to their totally porous particle column counterparts¹. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

To date, superficially porous particles have primarily focused on reversed-phase separations. With the maturation of superficially porous particle technology, applications for further chemistries and chromatographic techniques, such as hydrophilic interaction liquid chromatography (HILIC), are becoming available. HILIC is well suited for the analysis of polar analytes, which are often difficult to retain and separate in reversed-phase mode. This Application Note demonstrates the UHPLC performance of an Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.7 μm column, and its ability to baseline resolve eight water-soluble vitamins. Figure 1 shows these compounds.

Experimental

An Agilent 1290 Infinity LC configured for low dispersion was used for this work. Table 1 shows the details. Table 2 shows the chromatographic method that was used. All compounds were injected as individual standards, with concentrations and sample solvents listed in Table 3.

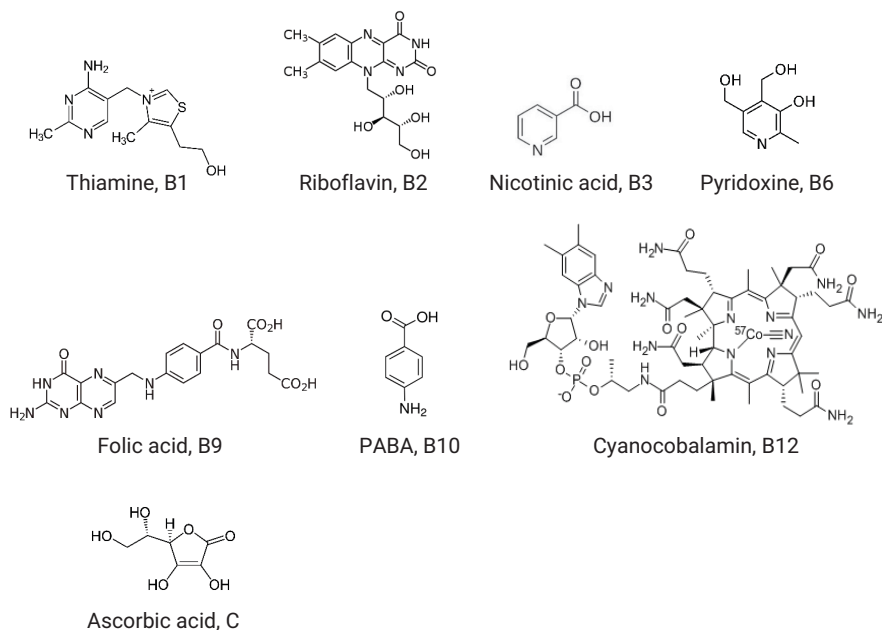


Figure 1. Water-soluble vitamins.

Table 1. Agilent 1290 Infinity LC System configuration.

Parameter	Value
Agilent 1290 Infinity Binary Pump (G4220A)	35 μL Solventmixer: Agilent Jet weaver, 35 $\mu\text{L}/100 \mu\text{L}$ (G4220-60006)
Agilent 1290 Infinity autosampler G4226A	<ul style="list-style-type: none"> • Seat assembly, ultra low dispersion, for Agilent 1290 Infinity autosampler G4226A (G4226-87030) • Autosampler \rightarrow Heater: Capillary, stainless steel, 0.075 \times 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	<ul style="list-style-type: none"> • Heat exchanger, low dispersion, 1.6 μL, double (G1316-60005) • Heater \rightarrow Column: Agilent InfinityLab Quick Connect assembly, 105 mm, 0.075 mm (5067-5961) • Column \rightarrow Flow cell: Capillary, stainless steel, 0.075 \times 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Agilent Ultra-Low Dispersion Max-Light Cartridge flow cell, 10 mm (G4212-60038)
Agilent OpenLAB CDS ChemStationEdition Revision C.01.05 [35]	<ul style="list-style-type: none"> • G4220A: B.06.53 [0013] • G4226A: A.06.50 [003] • G1316C: A.06.53 [002] • G4212A: B.06.53 [0013]
Agilent LC column	Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 \times 100 mm, 2.7 μm (685775-601)

Table 2. LC method parameters.

Column	Mobile phase	Flow rate (mL/min)	Mobile phase composition	Injection volume (μL)	Thermostated column compartment ($^{\circ}\text{C}$)	Diode array detector
Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 \times 100 mm, 2.7 μm	A) 100 mM ammonium acetate + 0.5 % acetic acid B) Acetonitrile	0.5	87 %B for 0.5 minutes, 87–50 %B in 3.5 minutes, 3 minutes re-equilibration	1.0 For sample information, see Table 2	40	260 nm, 80 Hz

The eight vitamins analyzed in this work were purchased as individual powders from Sigma-Aldrich. Ammonium acetate and acetic acid were also from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 μm filtered, 18 molecular weight, from a Milli-Q system (Millipore).

Results and Discussion

Eight vitamin compounds were baseline resolved on an Agilent InfinityLab Poroshell 120 HILIC-OH5 column, as shown in the chromatogram in Figure 2. The separation was achieved in 4 minutes, with a minimum resolution of 2.7 between the critical pair of vitamins B2 and B10.

Other HILIC phases are available from Agilent on 2.7 μm InfinityLab Poroshell 120 particles, including HILIC-Z and HILIC. These phases were also considered for the separation of these vitamin compounds. However, the performance was not as good as with the InfinityLab Poroshell 120 HILIC-OH5, as shown in Figure 3. The Agilent InfinityLab Poroshell 120 HILIC-Z column offers excellent peak shape for all eight compounds, but did not resolve vitamins B1 and B3 under these method conditions. The Poroshell 120 HILIC column has very orthogonal selectivity, however peak shape is not desirable for vitamins B9 and C.

Table 3. Sample preparation

Water-soluble vitamin (B,C)	Concentration (mg/mL)	Sample solvent
Thiamine (B1)	0.4	CH ₃ CN/H ₂ O (9:1)
Riboflavin (B2)	0.1	CH ₃ CN/DMSO (9:1)
Niacin/nicotinic acid (B3)	0.4	CH ₃ CN/H ₂ O (9:1)
Pyridoxine/pyridoxol(B6)	0.4	CH ₃ CN/H ₂ O (9:1)
Folate/folic acid (B9)	0.4	CH ₃ CN/DMSO (9:1)
4-Aminobenzoic acid/PABA (B10)	0.2	CH ₃ CN/H ₂ O (95:5)
Cyanocobalamin (B12)	0.4	H ₂ O
Ascorbic acid (C)	0.2	CH ₃ CN/H ₂ O (9:1)

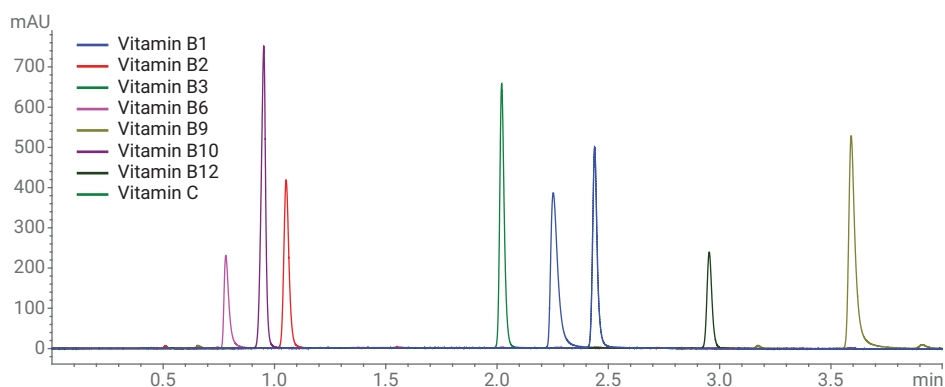


Figure 2. Separation of water-soluble vitamins on an Agilent InfinityLab Poroshell 120 HILIC-OH5 column.

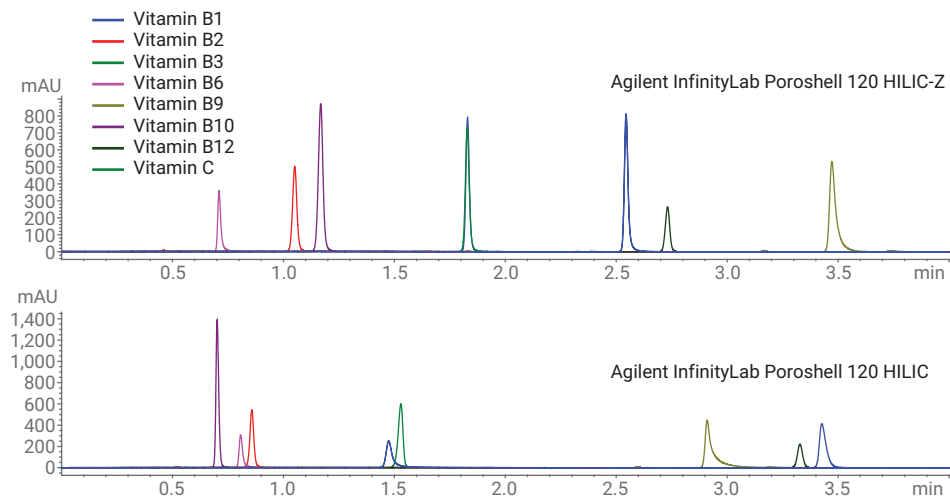


Figure 3. Separation of water-soluble vitamins on alternative Agilent InfinityLab Poroshell 120 HILIC phases.

Conclusion

The Agilent InfinityLab Poroshell 120 HILIC-OH5 column is well suited for the separation of water-soluble vitamins. This column offers good resolution and peak shape for all compounds. Other HILIC chemistries were also evaluated for this separation. However, the resolution on other phases was not adequate, and would require further method development to assess optimal conditions for a suitable separation.

References

1. Gratzfield-Huguen, A.; Naegele, E. Maximizing efficiency using Agilent InfinityLab Poroshell 120 Columns, *Agilent Technologies Application Note*, publication number 5990-5602EN, **2016**.

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