Application Note

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Scaled Separation of Nine Water-Soluble Vitamins on the Agilent InfinityLab Poroshell 120 SB-Aq Column

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Abstract

Nine vitamin B compounds were analyzed with Agilent InfinityLab Poroshell 120 SB-Aq columns using a phosphate buffer and acetonitrile gradient. The separation was geometrically scaled to preserve selectivity across 1.9, 2.7, and 4 µm particle sizes, while maintaining L/dp.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns are highly efficient at lower pressures compared to their porous particle column counterparts[1] because of their shorter mass transfer distance and substantially narrower particle size distribution.[2]

The most popular particle size for superficially porous particle columns is 2.5 to 3 µm. With the 2.7 µm InfinityLab Poroshell 120 particles, columns can easily be used to maximize resolving power with UHPLC efficiency, while not exceeding pressure limitations. However, the current trend with superficially porous particles is reducing particle size for further efficiency improvements. The higher efficiency can be used to speed up testing or improve results by increasing resolution and sensitivity. Larger 4 µm particles are also available, and can easily be used to improve traditional 5 µm methods.

This work shows how the selectivity of Poroshell particles is maintained across particle sizes, allowing easy method and instrument flexibility.

Experimental

An Agilent 1290 Infinity LC was used in this experiment. The system was modified from its standard configuration to have lower system volume and dispersion. Table 1 shows the configuration details. Three Agilent LC columns are also used in this experiment and are listed in Table 1. Table 2 shows the LC method parameters.

Table 1. System configuration.

Agilent 1290 Infinity II LC System Configuration	
Agilent 1290 Infinity II Flexible Pump (G7104A)	• Degasser • Seal wash pump • 35 µL solvent mixer: Agilent Jet Weaver, 35 µL/100 µL (p/n G4220-60006) • Firmware: B.07.23 [0009]
Agilent 1290 Infinity II Vialsampler (G7129B)	• Sample thermostat (p/n G7167-60101) • Metering parameter: seat assembly PEEK 0.12 mm, sample loop 20 µL, analytical head 20 µL • Autosampler → heater: capillary, stainless steel, 0.12 × 105 mm, SL/SL (p/n 5500-1238) • Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) • Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717) • Vial insert, 250 µL, glass with polymer feet, 100/pk (p/n 5181-1270) • Firmware: D.07.23 [0009]
Agilent InfinityLab LC Series Integrated Column Compartment (G7130A)	• Integral type: G7129B • 3.0 µL heat exchanger • Heater → column: A-Line quick-connect assembly, 105 mm, 0.075 mm (p/n 5067-5961) • Column → flow cell: capillary, stainless steel, 0.075 × 220 mm, SV/SLV (p/n 5067-4784) • Firmware: B.07.23 [0009]
Agilent 1290 Infinity II Diode Array Detector (G7117B)	• Ultralow dispersion Max-Light cartridge flow cell, 10 mm, 0.60 µL (p/n G4212-60038) • UV lamp (5190-0917) • Firmware: D.07.23 [0009]
Agilent MassHunter LC/MS Data Acquisition for Ultivo LC/TQ	• Version 1.1 • Build 1.1.2222
Agilent LC Columns	• Agilent InfinityLab Poroshell 120 SB-Aq, 2.1 × 50 mm, 1.9 µm (p/n 689675-914) • Agilent InfinityLab Poroshell 120 SB-Aq, 3.0 × 100 mm, 2.7 µm (p/n 685975-314) • Agilent InfinityLab Poroshell 120 SB-Aq, 4.6 × 150 mm, 4.0 µm (p/n 683970-914)

Table 2. Method parameters.

Column	Mobile Phase	Flow Rate (mL/min)	Mobile Phase Composition	Injection Volume (µL)	Sample	Thermostated Column Compartment (°C)	Diode Array Detector
Agilent InfinityLab Poroshell 120 SB-Aq, 2.1 × 50 mm, 1.9 µm (p/n 689675-914)	A: water B: acetonitrile C: N/A D: 200 mM monosodium phosphate + 1% phosphoric acid (85%) v/v, pH ~2	0.5	Gradient: 0 to 30% B in 3 minutes, hold 2.5% D throughout run	0.1	0.1 to 0.8 mg/mL each in water See Table 3 for exact concentrations See Figure 1 for structures	70	260 nm, 80 Hz
Agilent InfinityLab Poroshell 120 SB-Aq, 3.0 × 100 mm, 2.7 µm (p/n 685975-314)		1.0	Gradient: 0 to 30% B in 6 minutes, hold 2.5% D throughout run	0.4			
Agilent InfinityLab Poroshell 120 SB-Aq, 4.6 × 150 mm, 4.0 µm (p/n 683970-914)		1.5	Gradient: 0 to 30% B in 12 minutes, hold 2.5% D throughout run	1.2			

The nine vitamin B compounds analyzed were purchased as powders from Sigma-Aldrich (Figure 1), and the concentrations at which they were analyzed are shown in Table 3. Sodium phosphate and phosphoric acid were also purchased 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

Figure 2 shows the separation of nine water-soluble B vitamins separated on three Agilent InfinityLab Poroshell 120 SB-Aq columns. The InfinityLab Poroshell 120 SB-Aq is available in three particle sizes. This gradient separation is scaled across 1.9, 2.7, and 4 µm SB-Aq particles. The flow rates were adjusted so that each column runs at its optimal linear velocity. Column dimensions were adjusted to ensure similar L/dp performance. Finally, the gradient times were scaled to ensure that the separation profile was similar. The result is the same separation and selectivity regardless of particle size.

Having the choice of particle size can be useful to adjust methods as needed. The 1.9 µm Poroshell 120 SB-Aq column gives the fastest separation and saves the most time, solvent, and cost. However, the 1.9 µm Poroshell 120 SB-Aq columns have smaller 0.5 µm inlet frits, which can be problematic with dirty samples and cause column clogging. 2.7 and 4 µm SB-Aq particles both have larger 2 µm inlet frits, making them more resistant to clogging with dirty samples. The LC instrument should be considered when choosing column dimensions. High efficiency, low volume columns (1.9 µm, 2.1 mm id) should be paired with low volume LC systems for optimal performance. Larger volume columns are more flexible for use with any LC system.

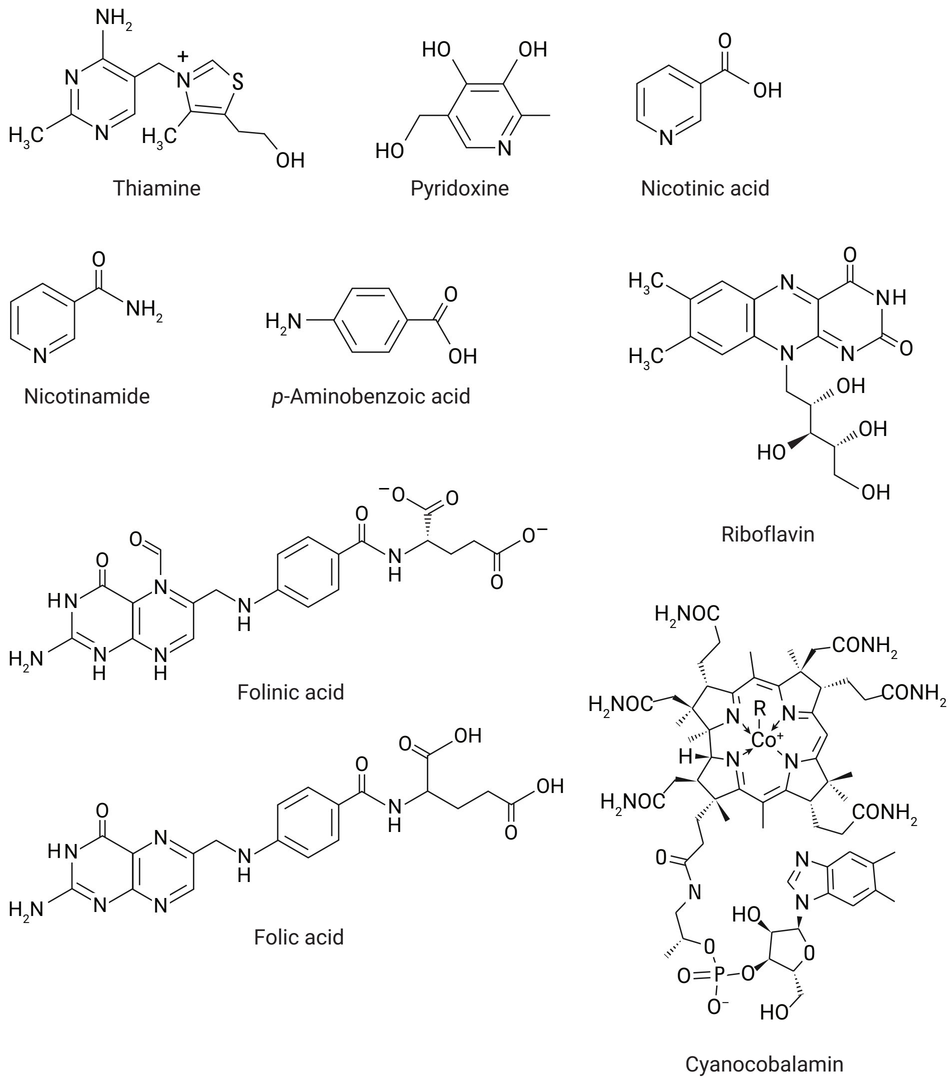

Figure 1. Compounds of interest.

Table 3. Sample composition.

B Vitamins (in Elution Order)	Concentration in Water (mg/mL)
Thiamine	0.2
Pyridoxine	0.8
Nicotinic acid	0.2
Nicotinamide	0.2
p-Aminobenzoic acid	0.2
Folinic acid	0.4
Folic acid	0.4
Riboflavin	0.1
Cyanocobalamin	0.8

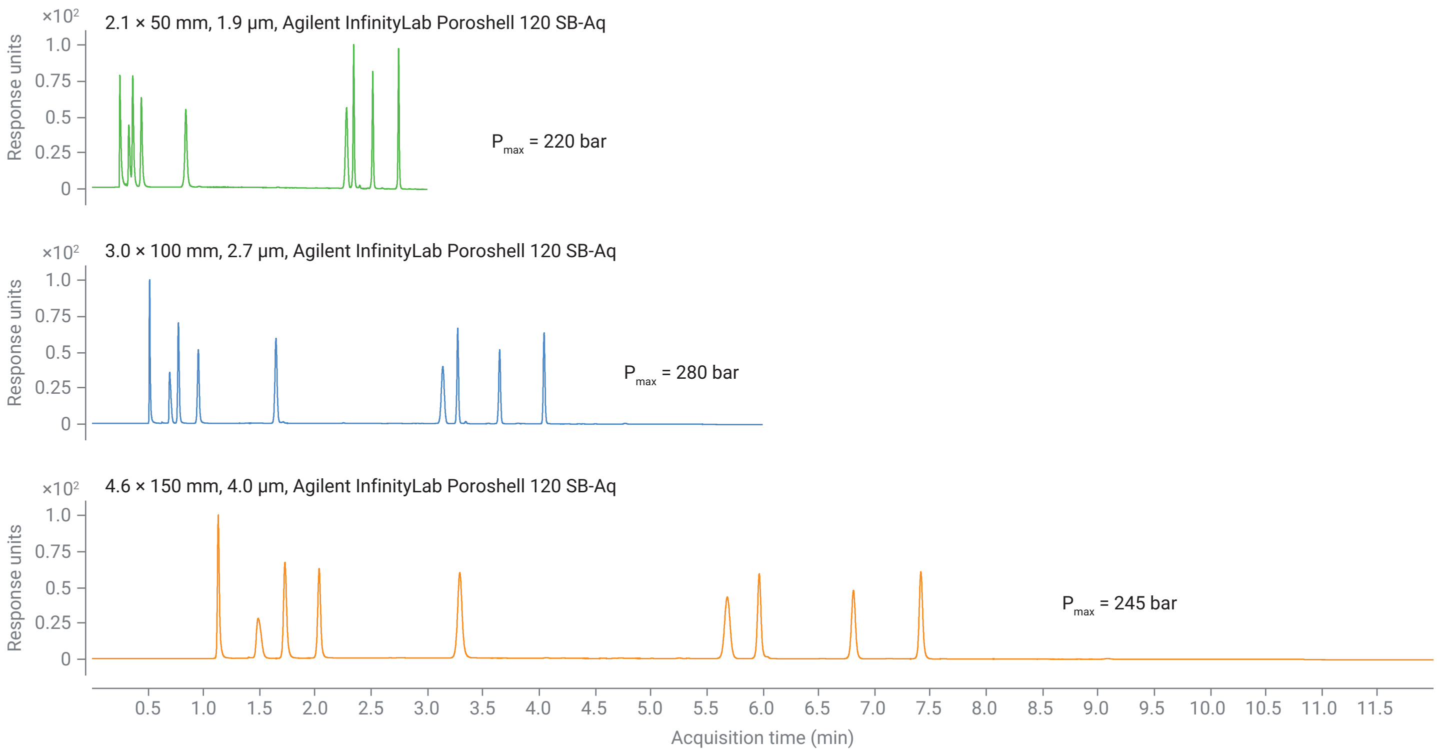

Figure 2. Scaling a separation of B vitamins on Agilent InfinityLab Poroshell 120 SB-Aq columns. Method parameters can be found in Table 2.

Conclusion

The separation of nine B vitamins is successfully scaled across 1.9, 2.7, and 4 µm Agilent InfinityLab Poroshell 120 SB-Aq particles. Column dimensions are adjusted to best preserve L/dp and uphold performance, while the gradient is geometrically scaled to maintain selectivity.

References

1. Gratzfield- Huesgen, A.; Naegele, E. Maximizing Efficiency Using Agilent Poroshell 120 Columns, *Agilent Technologies Application Note*, publication number 5990-5602EN. June 1, **2016**.
2. V. R. Meyer. Practical High-Performance Liquid Chromatography. Fourth Edition, Wiley: 2004; p. 34.

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Printed in the USA, November 5, 2019
5994-1494EN