Application Note

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Separation of Water-Soluble Vitamins on the Agilent InfinityLab Poroshell 120 Aq-C18 Column

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Abstract

Ten vitamin B compounds plus ascorbic acid and caffeine were analyzed with an Agilent InfinityLab Poroshell 120 Aq-C18 column using a phosphate buffer and acetonitrile gradient. Good resolution and peak shape were achieved using this column.

Introduction

Ascorbic acid and most vitamin B compounds are water-soluble. These compounds are polar in nature and are not retained well with conventional C18 columns. A new InfinityLab Poroshell 120 Aq-C18 column has been developed based on superficially porous particles with optimized C18 ligands and proprietary end-capping surface. A larger pore size of 120 Å coupled with the newly optimized C18 chemistry enables column use with a highly aqueous mobile phase to avoid retention loss. Also, the column has stronger retention for the polar compounds than other superficially porous polar modified C18 columns that are commercially available, as well as a high-density C18 column.[1]

Experimental

Instruments and materials

An Agilent 1290 Infinity LC system with binary pump was used in this experiment, consisting of:

- Agilent 1290 Infinity binary pump (G4220A)
- Agilent 1290 Infinity autosampler (G4226A)
- Agilent 1290 Infinity thermostatted column compartment (G1316C)
- Agilent 1290 Infinity diode array detector (G4212A)

The LC column used was the Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (part number 695975-742)

All reagents and solvents were HPLC grade. Acetonitrile, sodium dihydrogen phosphate (NaH_2PO_4), phosphoric acid, sodium hydroxide, and the 12 standards were purchased from Anpel Laboratory Technologies, Shanghai, China. Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Ascorbic acid, thiamine, nicotinic acid, nicotinamide, D-(+)-pantothenic acid calcium salt, pyridoxine hydrochloride, aminobenzoic acid, caffeine, and cyanocobalamin were separately dissolved in water. Riboflavin, biotin, and folic acid were separately dissolved in 5 mmol/L sodium hydroxide aqueous solution. The standard solution was prepared by mixing the individual stock solutions and diluting with acetonitrile. Table 1 shows the individual concentration of all the components.

Table 1. Solutions for all the analytes.

Compound Name	Concentration (mg/mL)	Solvent	Concentration in Mixture (µg/mL)
Thiamine	1.0	Water	80
Ascorbic Acid	1.0	Water	40
Nicotinic Acid	1.0	Water	80
Nicotinamide	1.0	Water	80
Pyridoxine	1.0	Water	80
Pantothenic Acid	1.0	Water	160
Aminobenzoic Acid	1.0	Water	80
Folic Acid	1.0	5 mmol/L sodium hydroxide	40
Caffeine	1.0	Water	80
Cyanocobalamin	1.0	Water	80
Riboflavin	0.5	5 mmol/L sodium hydroxide	20
Biotin	1.0	5 mmol/L sodium hydroxide	160

Method parameters

The method parameters used in the experiment are displayed in Table 2.

Table 2. Method parameters.

Parameter	Value
Mobile Phase	A) 10 mM NaH_2PO_4 buffer with pH 2.5 B) Acetonitrile
Gradient	**Time (min)** **B%** 0 0 2 6 6 25 8 70 10 70
Stop Time	10 min; postrun: 2 min
Column Temperature	30 °C
Flow Rate	1.5 mL/min
Detector	UV 260 nm/210 nm
Injection Volume	1 µL

Results and discussion

The analysis was run on an InfinityLab Poroshell 120 Aq-C18 column. The gradient was started with 100% phosphate buffer to retain polar compounds such as thiamine and ascorbic acid. Figure 2 shows the separation of 10 vitamin B compounds, ascorbic acid, and caffeine on the Poroshell 120 Aq-C18 column. All 12 compounds were baseline separated with symmetrical peak shapes. The thiamine and ascorbic acid were retained and separated well on this column. Multiple UV wavelengths were used for the detection due to different levels of maximum UV absorption of these compounds. Pantothenic acid and biotin were not found under 260 nm due to lack of UV absorption, but they could be detected under 210 nm.

A reproducibility test was done by consecutive injections. The good results shown in Figure 2 demonstrate that the method is reproducible. This gradient is started with 100% buffer in aqueous solution, which is not recommended to be used with conventional C18 columns. The newly developed InfinityLab Poroshell 120 Aq-C18 column using the optimized C18 ligands with proprietary end-capping applied to 2.7 µm Poroshell particles with a pore size of 120 Å significantly improves the retention, peak shape, and reproducibility of polar analyte analysis with minimized pore dewetting phenomenon.

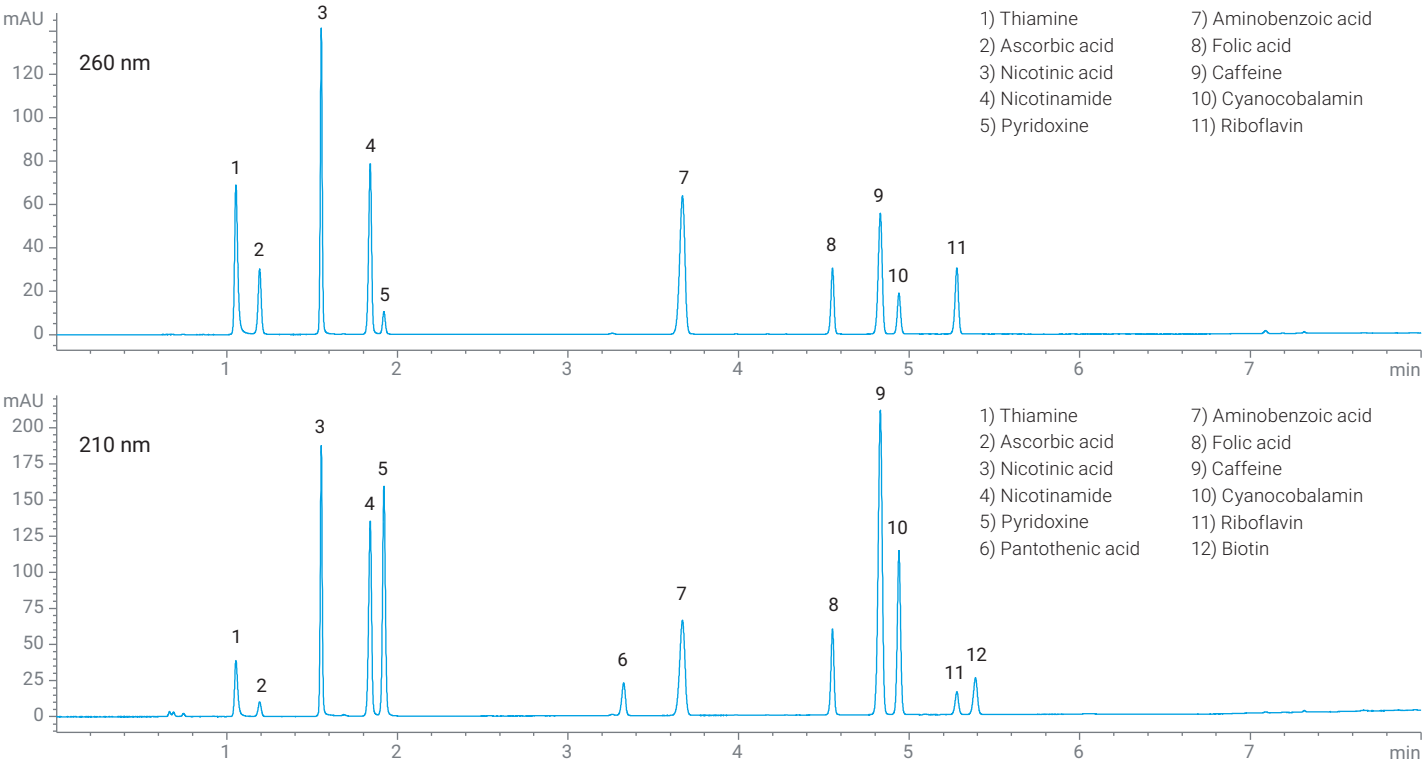

Figure 1. Water-soluble vitamin separation with the Agilent InfinityLab Poroshell 120 Aq-C18 under UV 260 and 210 nm.

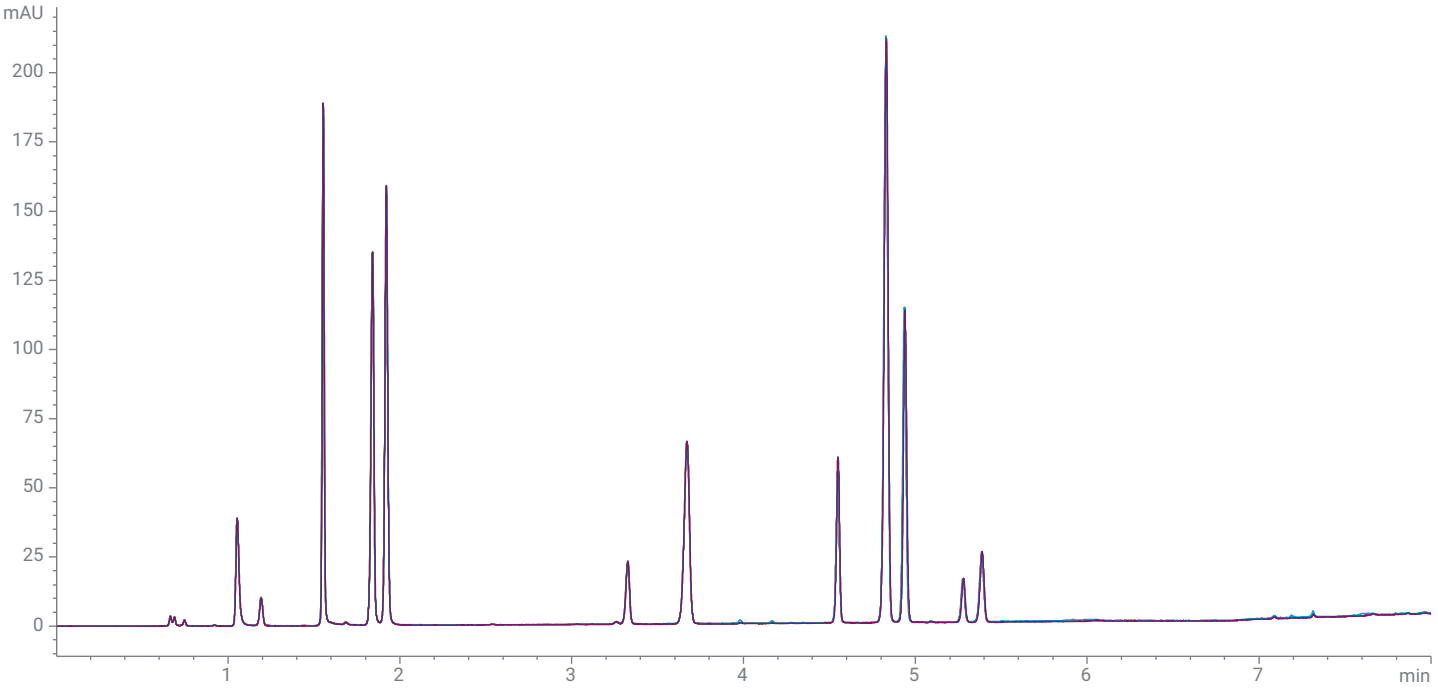

Figure 2. Overlaid chromatograms of six consecutive injections.

Conclusion

The separation of water-soluble vitamins was successfully achieved with an Agilent InfinityLab Poroshell 120 Aq-C18, 2.7 µm column. The column provided good resolution and peak shape for these polar compounds and reasonable retention for polar vitamins including thiamine and ascorbic acid. Reproducible results were also achieved with this column under the gradient starting with 100% aqueous buffer eluents.

References

1. Fu, R.-J.; Wei, T.-C. Analysis of Polar Compounds Using an Agilent InfinityLab Poroshell 120 Aq-C18 Column with Improved and Reliable Performance, *Agilent Technologies application note*, publication number 5994-5555EN, **2022**.

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