Simple and Rapid Analysis of Water-Soluble Vitamins

Introduction

The analysis of water-soluble vitamins in food products and nutritional formulations has been a challenge. In particular, the extreme polarity of several of typical components, such as thiamine (vitamin B1), pyridoxine (vitamin B6), and niacinamide (vitamin B3), can lead to their poor retention on most C18 phases. In this application, we have demonstrated a method to increase the retention of several highly polar analytes for full resolution of a generic water-soluble vitamin formulation. The method begins in a highly aqueous mobile phase composition, 97% buffer and 3% acetonitrile, and a polar-endcapped C18 phase, which has been utilized to avoid the "phase collapse" phenomenon associated with running alkyl-bonded phases under highly aqueous conditions.

"...this method was highly reproducible (RSD <3% for retention times)."

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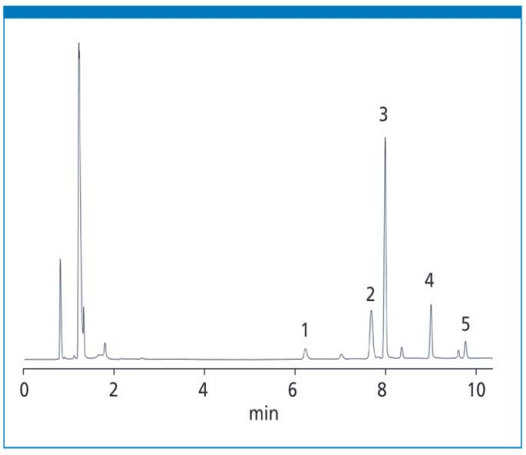

Figure 1: *A multivitamin tablet with 5 common vitamins was separated on the Synergi 4µ Hydro-RP 150 × 4.6 mm. 1 = pantothenic acid, 2 = p-aminobenzoic acid, 3 = pyridoxine, 4 = thiamine, 5 = riboflavin.*

Instrumentation/Equipment

Analysis was performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, California, USA) equipped with a quaternary pump, in-line degasser, multi-wavelength detector, autosampler and ThermaSphere™ TS-130 HPLC temperature controller (Phenomenex, Torrance, CA, USA) and HP Chemstation software (Version A. 06.04) was used for the data analysis. The HPLC columns used for the analyses were Synergi™ 4µ Hydro-RP and Synergi™ 4µ Polar-RP, both 150 x 4.6mm (Phenomenex, Torrance, California, USA). All of the reagents and chemicals used were of the highest grade obtainable, and generic, over-the-counter multivitamin tablets were purchased from a local convenience store.

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Experimental Method

Sample preparation: Ten water-soluble vitamin tablets were weighed and ground into a fine powder using a mortar and pestle. A fraction of this powder containing the equivalent of 10mg of niacinamide was transferred to a low-actinic volumetric flask (100mL) to which 60mL of a 1% glacial acetic acid solution (maintained at 70˚C) and 20mL of a 1% sodium thiosulfate solution had been added. After 45 min in a shaker bath, this solution was ultrasonicated for an additional 20 min and then allowed to come to room temperature. More of the 1% acetic acid solution (room temperature) was then added to bring the volume of the flask up to 100mL and this mixture was then filtered through a Phenex™ 0.45µm nylon membrane syringe filter (Phenomenex, Torrance, CA, USA).

Chromatographic conditions: Analysis was performed using a simple ion-pair, gradient method. The columns were initially equilibrated with the mobile phase for a period of time equivalent to approximately 15–20 column volumes. Mobile phase A

consisted of a mixture of 20mM Potassium phosphate and 0.1% Hexane sulfonate, adjusted to a pH of 3.0. Mobile phase B was acetonitrile. The columns were maintained at a temperature of 30˚C. The gradient program began with an isocratic hold (3 min at 97:3 A:B), followed by a linear gradient to 50:50 A:B in 15 min at a flow-rate of 1.5mL/min. A 5–10µL injection and a 10 min re-equilibration period was sufficient to assure reproducible analyses (RSD< 3% for the retention times of the active peaks).

Results

Comparisons of the retention times of the major peaks obtained from the vitamin tablets (Figure 1) with the retention times of the vitamin standards (Figure 2) show that this method can resolve the target analytes from interfering peaks. In addition, the use of an ion-pairing agent (hexane sulfonate) simply and effectively increases the retention times of thiamine, pyridoxine and niacinamide, all of which are extremely polar compounds poorly retained on reversed-phase columns and which typically elute close to the solvent front (and therefore may co-elute with polar excipient peaks). While ion-pair methods have been associated with reproducibility issues, with temperature control (30˚C) and an adequate re-equilibration period (10 min) this method was highly reproducible (RSD <3% for retention times).

To find an alternative column selectivity, we also screened several non-C18 phases using these same running conditions and found that Synergi Polar-RP, a polar-embedded ether-linked phenyl phase, provided a unique selectivity alternative to that of conventional or polar-endcapped C18 phases (Figure 3), with a reversal in the elution orders of p-aminobenzoic acid and pyridoxine.

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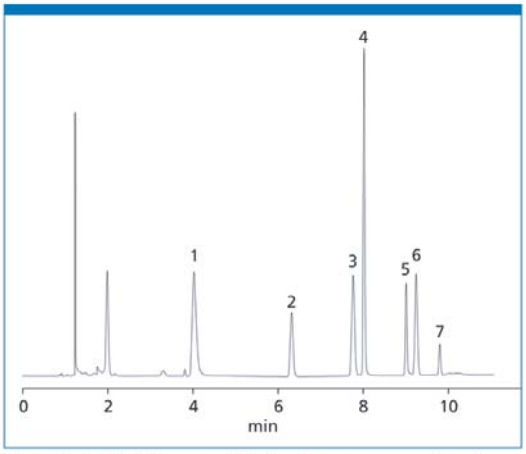

Figure 2: *A mix of 7 water-soluble vitamins were separated on the Synergi 4µ Hydro-RP 150 × 4.6 mm. 1 = niacinamide, 2 = pantothenic acid, 3 = p-aminobenzoic acid, 4 = pyridoxine, 5 = thiamine, 6 = vitamin B12 (cyanocobalamine), 7 = riboflavin.*

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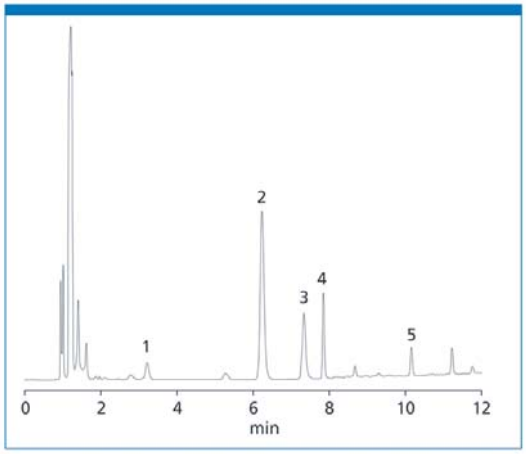

Figure 3: *The same 5 vitamins from Figure 1 were analysed on the Synergi 4µ Polar-RP 150 × 4.6 mm with a change in elution order. 1 = pantothenic acid, 2 = pyridoxine, 3 = p-aminobenzoic acid, 4 = thiamine, 5 = riboflavin.*

Ordering Information

Order Number	Description
00F-4375-E0	Synergi 4µ Hydro-RP 150 x 4.6mm
00F-4336-E0	Synergi 4µ Polar-RP 150 x 4.6mm
EH0-7057	ThermaSphere TS-130 HPLC Temperature Controller, 25-80˚C, 95-265VAC, 50/60Hz
AJ0-0414	Phenex 0.45µm nylon syringe filters, 100/box