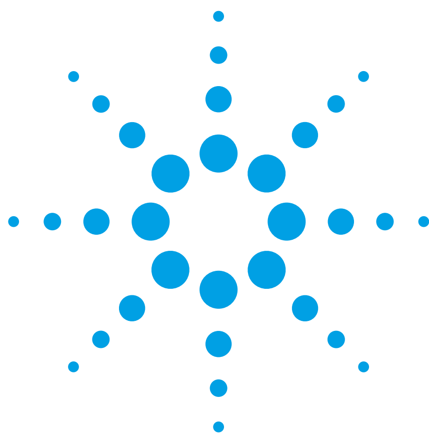

Analysis of Water Soluble Vitamins in Multivitamin Tablets Using Poroshell 120 EC-C18

Application Note

Pharmaceutical

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Abstract

A method separating nine multivitamin compounds originally developed on Agilent ZORBAX Eclipse Plus C18 columns of various sizes is transferred to an Agilent Poroshell 120 EC-C18 4.6 mm × 75 mm, 2.7 µm column using an Agilent 1260 Rapid Resolution LC. The gradient and flow rate are scaled, maintaining retention index with the objective of determining the optimal flow rate for each column evaluated. The new separation keeps the analysis time constant at 5 minutes and allows the use of a longer column with a higher flow rate and still maintains the pressure under 400 bar. Sample preparation used is dissolution of a tablet in water followed by either filtration through a 0.45 micron syringe filter or centrifugation. Over 800 injections were made without substantially increasing pressure.

Introduction

A compound is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained through diet. Vitamins have diverse biochemical functions. Many vitamins function as precursors for enzyme cofactors, that help enzymes in their work as catalysts in metabolism. Vitamins may also be less tightly bound to enzyme catalysts as coenzymes, detachable molecules that function to carry chemical groups or electrons between molecules.

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The two types of vitamins are classified by the materials in which they will dissolve. Fat-soluble vitamins, such as vitamins D, or E, dissolve in fat before they are absorbed in the blood stream to carry out their functions. Excesses of these vitamins are stored in the liver. Because they are stored, they are not needed every day in the diet.

By contrast, water-soluble vitamins dissolve in water and are not stored; they are eliminated in urine. We need a continuous supply of them in our diets. The water-soluble vitamins are the B-complex group and vitamin C.

The B-complex group: thiamin, riboflavin, niacin, pyridoxyl phosphate, folic acid, cobalamin, pantothenic acid and, in addition, ascorbic acid, or vitamin C, are widely distributed in foods. Riboflavin, for example, can be obtained from liver, milk, dark green vegetables, whole and enriched grain products, eggs. Their influence is felt in many parts of the body. They function as coenzymes that help the body obtain energy from food. They also are important for normal appetite, good vision, healthy skin, healthy nervous system and red blood cell formation.

Until the mid-1930s, when the first commercial yeast-extract and semi-synthetic vitamin C supplement tablets were sold, vitamins were obtained solely through food intake, and changes in diet (following a bad harvest) can alter the types and amounts of vitamins ingested. Vitamins have been produced as commodity chemicals and made widely available as inexpensive synthetic-source multivitamin dietary supplements, since the middle of the 20th century [1].

In addition to containing vitamins of interest, other excipient materials such as cellulose, maltodextrin, dextrin, gelatin, dextrose, soy lecithin are formulated into the tablets. Given the large size of the tablet and relatively small quantities of many of the vitamins, clogging problems have plagued this analysis when it has been attempted on small particle size columns.

Reverse Phase HPLC is well suited for vitamin analysis. Qualitative and Quantitative analysis of vitamins is important for clinical, food and pharmaceutical applications. Using a previously described method developed on an Agilent ZORBAX Eclipse Plus C18, 1.8 µm column, a fast separation method is converted to be used on an Agilent Poroshell 120 EC-C18 column [2]. The new method remains fast, but is less prone to clogging by excipients found in multivitamins.

In this work,a method using an Agilent Eclipse Plus C18 4.6 mm × 50 mm, 1.8 µm column is converted to use an Agilent Poroshell 120 EC-C18 4.6 mm × 75 mm column. Injection volume is increased and the risk of clogging is reduced.

Experimental

An Agilent 1260 Rapid Resolution LC (RRLC) system was used for this work:

- G1312B Binary Pump SL with mobile phase A: 25 mM Sodium Phosphate pH 2.5 in Water and B: Methanol. The gradient started at 1% B, held at that concentration, then ramped to 12% B and finally 30% B, held at that concentration, and then re-equilibrated to the initial condition. The system is configured with the pulse damper and standard mixer installed.
- G1367E Automatic Liquid Sampler (ALS) SL. Injection volume of 5 µL was used.
- G1316B Thermostatted Column Compartment (TCC) SL with temperature set to 35 °C.
- G4212C Diode Array Detector (DAD) SL with the signal set to 230, 4 nm and reference not used, using a G4212-60008 micro flow cell (10-mm path, 1-µL variance).
- ChemStation version B.04.02 was used to control the HPLC and process the data.
- Agilent Poroshell 120 EC-C18, 4.6 mm × 75 mm, 2.7 µm, p/n 697975-902

The compounds of interest are shown in Reference 2, with their respective structures. Compounds were dissolved in water at 1 mg/mL and used for identification. The following compounds were purchased from Sigma Aldrich: thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin, vitamin B_6, folic acid, vitamin B_{12}, biotin, pantothenic acid, and ascorbic acid. Additionally purchased from Sigma Aldrich (Bellefonte, PA) was the Sodium Monophosphate and Phosphoric Acid. Methanol was purchased from Honeywell, Burdick and Jackson High Purity, (Muskegon, MI). Water used was 18 MΩ WMilli-Q water (Bedford, MA). A multivitamin tablet was purchased at a local pharmacy. (One a Day Women's Active Metabolism, Bayer HealthCare, Morristown NJ.).

Tablets were dissolved by grinding them individually using a mortar and pestle and transferring the entire amount (about 1.6766 g/tablet) with 100 mL water to a 150 mL plastic coated bottle. The bottle is then sealed and shaken vigorously for 5 min. A cloudy solution is produced which is clarified by either filtration (using a 0.45 µm, 30 mm regenerated cellulose filter (p/n 5061-3364) and a 10 mL syringe or by centrifugation in a polypropylene tube (6000 rpm for 5 min). The resulting clear solution is then transferred to an Agilent MS Analyzed Write-On Vial (p/n 5190-2278).

Results and Discussion

In transferring this method from the original method, a longer column was chosen and used at a proportionally higher flow rate. Previous work has shown that higher peak capacity for an Agilent Poroshell 120 EC-C18 (75 mm instead of the previously used 50 mm column) can be obtained at higher linear velocity (1.5 mL/min instead of 1 m/min) [3]. In addition, a larger injection volume, which should be proportional to column volume, would be possible on the larger Poroshell column. The selectivities of an Agilent ZORBAX Eclipse Plus C18 (75 mm instead of the previously used 50 mm column) and Poroshell 120 EC-C18, have been shown to be very similar in previous work [4,5]. In addition, a larger column would be less affected by extra column effects such as additional tubing [6] required if a cooled autosampler was used, as recommended in a previous note on analysis of water soluble vitamins by Huber [7]. In this work, a slight change in elution order is noted at the beginning of the gradient. This could be the result of the change in column porosity (from fully porous to superficially porous), the dwell volume of the instrument, or the equilibration time between runs. Figure 1 shows a representative sample chromatogram, scaled to the largest peak. In the upper left corner, an expanded chromatogram is shown that reveals the lower intensity peaks. The chromatographic conditions, as well as the list of components in their elution order, are found on the right.

Over 800 injections of a vitamin tablet extract were made on this column without an increase in pressure. This is due in part to the 2 µm column inlet frit, which is less likely to clog than the smaller porosity frits used on sub 2-micron and totally porous 3 µm columns [8]. At least 100 injections were made using the centrifuge sample clarification method without any change in system pressure. In this case, filtration was found to be a faster method, requiring fewer steps in producing a final sample. The 0.45 µm regenerated cellulose filter presents less resistance than a 0.2 µm filter required for use with a sub 2-micron column. A plot describing pressure changes per injection is shown in Figure 2. An increase in pressure from 177 to 179 bar is noted over 3 days and 6 L of phosphate buffer.

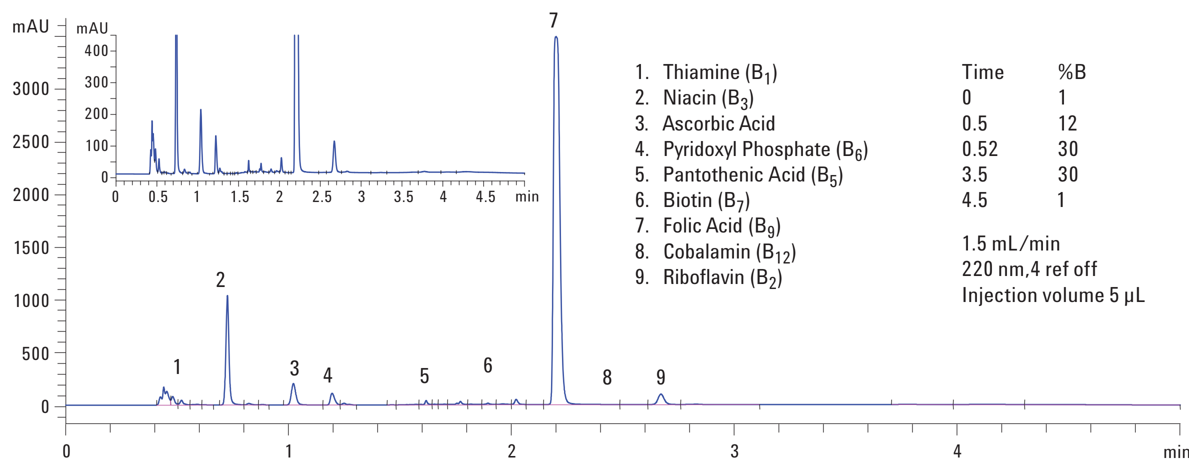

Figure 1. Elution order water soluble vitamins.

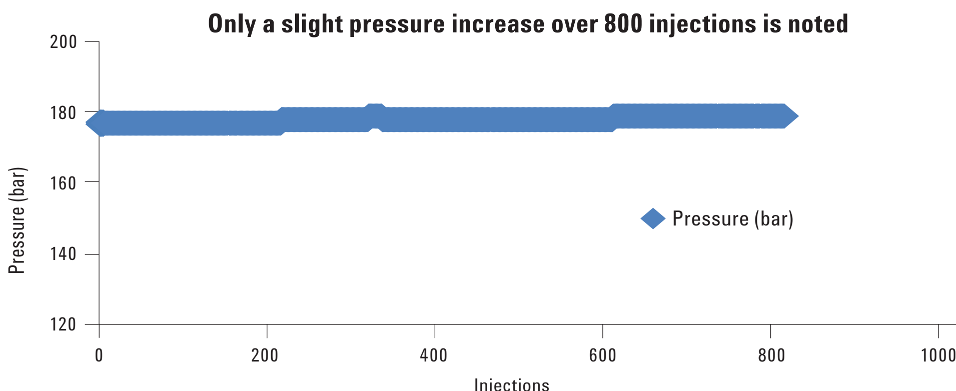

Figure 2. Agilent Poroshell 120 EC-C18 pressure remains nearly constant after 800 injections water soluble vitamin tablet.

Other wavelengths could also be used for this analysis, as several of these compounds form yellow or even red solutions in water, indicating absorbance in the visible region of the spectra [9]. Care should be taken when using a reference wavelength as an improper choice could lead to higher reference absorbance than in the analytical wavelength and to negative peaks.

Conclusion

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The superficially porous 2.7-µm Agilent Poroshell 120 EC-C18 offers similar efficiency and selectivity to the 1.8 µm Agilent ZORBAX Eclipse Plus C18 column, without the high back pressure. The 2 µm frit has demonstrated a resistance to clogging through the analysis of over 800 samples.

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