HALO®

TECHNICAL REPORT: AMT-TR12_20_02

TITLE: **FAT- AND WATER-SOLUBLE VITAMIN ANALYSIS IN SUPPLEMENTS**

MARKET SEGMENT: VITAMINS AND SUPPLEMENTS

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ABSTRACT

Reversed phase HPLC methods for water- and fat-soluble vitamins are presented using a HALO® AQ-C18 and a HALO® C30 column. Analytical standards are used in order to optimize the methods followed by a comparison of two different multivitamin tablets. Both methods show high resolution between compounds of interest using superficially porous particle technology.

INTRODUCTION

Vitamins are a well-known group of organic compounds that regulate physiological functions of an organism, and are essential for human health. Vitamins are classified into two main groups, water-soluble and fat-soluble. Water-soluble vitamins include B group vitamins and ascorbic acid (vitamin C), while fat-soluble vitamins include A, E, D, and K. The main supply and intake of vitamins is governed by diet; however, vitamin content in foods can decrease after cooking, storing or further processing.[1-3] In an effort to increase vitamin intake, many people supplement their diet with a multivitamin, or consume vitamin infused beverages during the day. For example, a large amount of vitamin C is found in sports drinks, and common multivitamins contain many fat-soluble vitamins, such as vitamins A, E, D, K, and ß-carotene. Vitamin analysis can be challenging due to instability of the analyte, of which many factors can contribute, including exposure to heat, light and air as well as interactions with other food components. In 2020 the vitamin and supplement market was estimated with a value of $36.2 billion, with expectations for growth upwards of 10% in the next year, and very little regulation for quality control.[4] Therefore, it is critically important to screen for these compounds in multivitamins and beverages to ensure these products are representing their contents accurately. In this report, we present HPLC methods for fat- and water-soluble vitamin analysis along with a comparison of two multivitamins, utilizing HALO® columns, which allow high sensitivity, high resolution, and high-speed separations.

KEY WORDS:

Vitamins, fat-soluble vitamins, water-soluble vitamins, superficially porous particles, Fused-Core®

EXPERIMENTAL DATA:

All experiments were run on a Shimadzu Nexera HPLC instrument (Columbia, MD) using a UV diode array detector (1 µL flow cell), and LabSolutions software (Shimadzu). Vitamin standards were obtained from Millipore Sigma (St. Louis, MO). Methanol (HPLC grade), water (HPLC grade), phosphoric acid, and potassium phosphate were purchased from Millipore Sigma (Burlington, MA). Two reversed phase stationary phases with the following properties were tested: 4.6 × 150 mm column format, 2.7 micron (µm), / superficially porous particle packed column. The stationary phases used in this study were an 90 Å AQ-C18 and a 160 Å C30 HALO® column from Advanced Materials Technology, Inc. (Wilmington, DE).

Multivitamin tablets were obtained from a local grocery store. Tablets were crushed with a mortar and pestle and placed in water (water-soluble) and/ or methanol (fat-soluble). Samples were prepped daily at 30 mg/ml and sonication was avoided due to vitamin degradation, such as ascorbic acid. Samples were filtered using a 0.2 µm PTFE syringe filter (VWR International) and injected into the HPLC.

INSTRUMENT PARAMETERS AND GRADIENT (**Water-Soluble**)
Analytical Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-722
Mobile Phase A: 0.025 M, potassium phosphate in water, pH: 2.5
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	0
1.0	0
6.0	70
10.0	70

Flow Rate: 1.2 mL/min
Pressure: 243 bar
Temperature: 30 °C
Injection Volume: 2.0 µL
Sample Solvent: Water
Detection: 215 nm, VWD

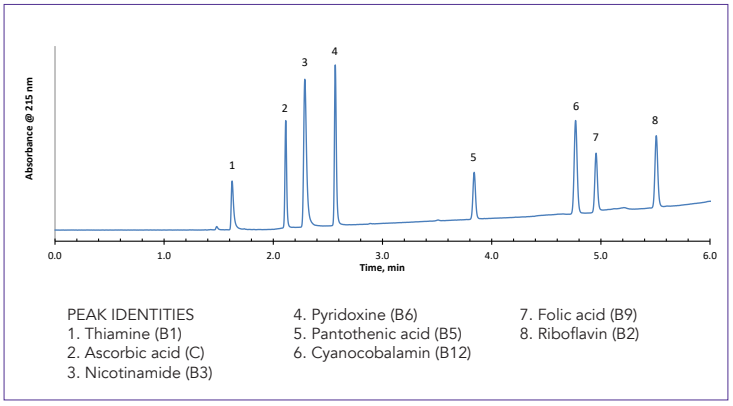
LC System: Shimadzu Nexera X2

INSTRUMENT PARAMETERS AND GRADIENT (Fat-Soluble)
Analytical Column: HALO 160 Å C30, 2.7 µm, 4.6 x 150 mm
Part Number: 92114-730
Isocratic: Methanol
Flow Rate: 1.5 mL/min
Pressure: 262 bar
Temperature: 30 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol
Detection: 280 nm, PDA
LC System: Shimadzu Nexera X2

RESULTS:

HALO® AQ-C18 columns can be used with high, or completely aqueous mobile phases making the column an ideal candidate for separating water-soluble vitamins. Analytical standards were used in order to optimize the method. In Figure 1, eight water-soluble vitamins are well-separated in under six minutes using a 100% aqueous isocratic hold

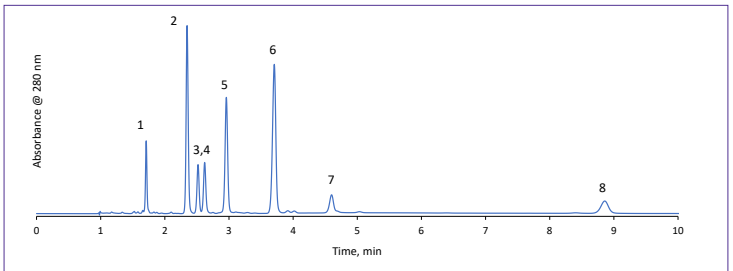
Figure 1: HALO® AQ-C18 water-soluble vitamin separation (standards):



PEAK IDENTITIES
1. Thiamine (B1)
2. Ascorbic acid (C)
3. Nicotinamide (B3)
4. Pyridoxine (B6)
5. Pantothenic acid (B5)
6. Cyanocobalamin (B12)
7. Folic acid (B9)
8. Riboflavin (B2)

An independent method that is fast, simple and achieves high resolution and sensitivity is the best alternative to having a compromised separation with both fat- and water-soluble vitamins in a single assay. Hence, the HALO® C30 was used for the analysis. Fat-soluble vitamins (D, E, K, and A) are stored in the liver and fatty tissue. These vitamins are essential to good health and contribute to several physiological functions, including bone growth, immune system regulation, cell division, and blood clotting, while vitamin E acts as an antioxidant[1-3]. HALO® C30 enables a fast, efficient separation of a typical fat-soluble vitamin panel in less than 9 minutes, while maintaining baseline resolution (R_s: 1.62) between vitamins D2 and D3. This simple 100% methanol isocratic separation uses analytical standards and can be seen in Figure 2.

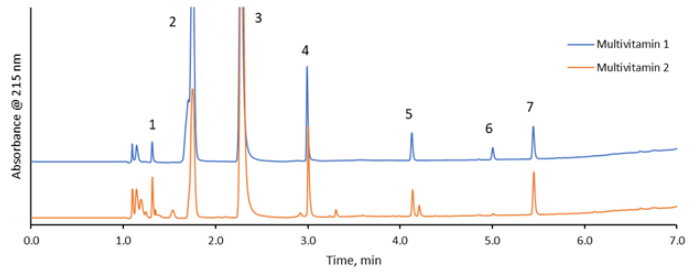
Figure 2: HALO® C30 fat-soluble vitamin separation (standards):



PEAK IDENTITIES
1. Retinyl acetate (A)
2. Delta tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. 2,3-*trans*-phylloquinone (K)
8. Retinyl palmitate (A)

Simultaneous analysis of multiple vitamins that are contained in multivitamin samples is challenging for a multitude of reasons. The variation in chemical properties between, and large disparity in concentrations of vitamins in the multivitamin are two prominent factors which contribute to limited sensitivity. In addition, vitamin degradation, matrix effects and solubility issues all further impact the analysis. It is for this reason that two methods were developed: one for water-soluble vitamins and one for fat-soluble vitamins. For comparative purposes, two different brands of multivitamins were procured from the local grocery store, one generic and one name brand. For water-soluble vitamins, the sample preparation consisted of crushing the multivitamin tablet and placing in water at 30 mg/mL followed by being vortexed. The sample was then filtered and injected into the system. See Figure 3 for the water-soluble vitamin separation results.

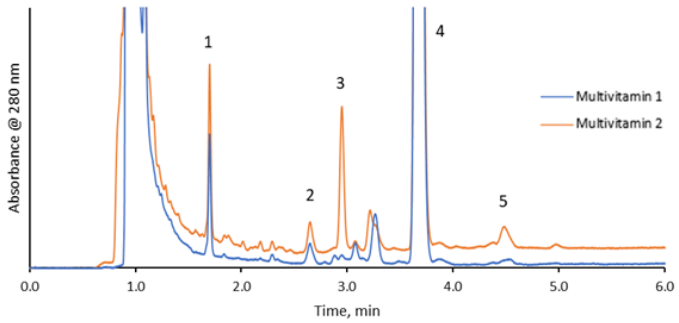
Figure 3: HALO® AQ-C18 water-soluble vitamin separation (two multivitamin tablets):



PEAK IDENTITIES
1. Thiamine (B1)
2. Ascorbic acid (C)
3. Nicotinamide (B3)
4. Pyridoxine (B6)
5. Pantothenic acid (B5)
6. Folic acid (B9)
7. Riboflavin (B2)

For the fat-soluble vitamins, multivitamin tablets were crushed, and then placed in methanol, vortexed, and filtered before injection. (30 mg/ml) The two multivitamins showed many similarities. However, one main difference is the presence of alpha tocopherol in one tablet and not in the other. This separation can be seen in Figure 4. Beta carotene was also detected using a wavelength of 450 nm.

Figure 4: HALO® C30 fat-soluble vitamin separation (two multivitamin tablets):



PEAK IDENTITIES
1. Retinyl acetate (A)
2. Cholecalciferol (D3)
3. Alpha tocopherol (E)
4. DL-alpha-tocopherol acetate (E)
5. 2,3-*trans*-phylloquinone (K)

CONCLUSION:

Vitamins are essential to our well-being for many reasons, and adding a daily multivitamin supplement may be helpful to ensure an appropriate balance is maintained. Analyzing both water- and fat-soluble vitamins simultaneously is difficult due to solubility issues along with matrix effects. Two different HPLC methods were developed for analyzing both water- and fat-soluble vitamins found in multivitamin tablets by Fused Core® technology. Since dietary supplements are less stringently regulated than pharmaceuticals, it is essential to have robust methods that can be applied in both qualitative and quantitative environments for multivitamin analysis.

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