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Simultaneous Analysis of Water- and Fat-Soluble Vitamins in Beverages Using an ODS-Modified Metal-Doped Column

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Introduction

Vitamins play vital roles in the metabolic activities of the body. However, because they all cannot be synthesized in sufficient quantities in the body, they must be ingested either in our meals or by other means, such as tablets. Vitamins can be classified into two groups, water-soluble and fat-soluble, and their analysis is generally conducted by high-performance liquid chromatography. The water-soluble type consists mainly of highly polar basic components which exhibit weak retention in analysis by reversed-phase liquid chromatography. Since they exhibit weak retention, the analysis is typically conducted using ion-pair reagents. That makes it difficult to apply gradient elution. On the other hand, the fat-soluble type consists of hydrophobic components. They are usually analyzed by reversed-phase liquid chromatography with organic solvent mobile phases or normal-phase liquid chromatography.

In this study, a Shim-pack MAqC-ODS I was used to analyze water- and fat-soluble vitamins in a single method. This column contains ODS-modified and metal-doped stationary phase, so it was able to retain the weakly-retained components without ion-pairing reagents. In addition, by using a 100% organic solvent mobile phase after the water-soluble vitamins were eluted, fat-soluble vitamins were also able to be analyzed.

Materials and Method

Reagents and standards

Reagents: Ascorbic acid, thiamine hydrochloride, riboflavin, nicotinic acid, nicotinamide, D-pantothenic acid hemicalcium salt, pyridoxine hydrochloride, biotin, folic acid, cyanocobalamine, retinol, retinyl acetate, retinyl palmitate, α-tocopherol, DL-α-tocopherol acetate, ergocalciferol, cholecalciferol, phytonadione, menaquinone, phosphoric acid, sodium phosphate monobasic, sodium hydroxide, ethanol, and chloroform were purchased from Sigma-Aldrich. Water was made in house using a Millipore Milli-Q Advantage A10 Ultrapure Water Purification System. Acetonitrile was purchased from Honeywell.

Stock solutions: Ascorbic acid, thiamine hydrochloride, nicotinic acid, nicotinamide, D-pantothenic acid hemicalcium salt, pyridoxine hydrochloride, and cyanocobalamine were separately dissolved in water. Riboflavin, biotin, and folic acid were separately dissolved in 20 mmol/L sodium hydroxide aqueous solution. Retinol, α-tocopherol, DL-α-tocopherol acetate, ergocalciferol, and cholecalciferol were separately dissolved in ethanol. Retinyl acetate, retinyl palmitate, phytonadione, and menaquinone were dissolved in chloroform. They were all made to 1000 mg/L.

Standard solutions: The stock solution of water-soluble vitamins (ascorbic acid, thiamine hydrochloride, nicotinic acid, nicotinamide, D-pantothenic acid hemicalcium salt, pyridoxine hydrochloride, cyanocobalamine, riboflavin, biotin, and folic acid) was mixed equally and diluted to 50 mg/L and transferred to a 1.5 mL vial for analysis. The stock solution of fat-soluble vitamins (retinol, α-tocopherol, DL-α-tocopherol acetate, ergocalciferol, cholecalciferol, retinyl acetate, retinyl palmitate, phytonadione, and menaquinone) was mixed equally and diluted to 50 mg/L and transferred to a 1.5 mL vial for analysis.

Sample solution: Vitamin-fortified water was purchased at a local supermarket. The sample was directly transferred to a 1.5 mL vial for analysis.

Methods

Samples were injected to a Shimadzu Prominence-i integrated system (PDA model). Water- and fat- soluble vitamins were separated on the Shim-pack MAqC-ODS I column and detected by the PDA detector incorporated in the integrated system. Table1 shows analytical conditions.

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Table 1 Analytical conditions

System	: Prominence-i PDA model (LC-2030 3D)
Column	: Shim-pack MAqC-ODS I (150 mm L. x 4.6 mm I.D., 5 µm)
Guard Column	: Shim-pack GVP-ODS (10 mm L. x 4.6 mm I.D., 4.6 µm)
Mobile Phase A	: 10 mmol/L phosphate (sodium) buffer (pH 2.6)
Mobile Phase B	: Acetonitrile
Time Program	: 0 % (0-2.5 min) – 40 % (10 min) – 100 % (25-45 min) – 0 % (45.01-55 min)
Flow Rate	: 1.2 mL/min
Column Temperature	: 40 °C
Injection Volume	: 10 µL
Detection	: 220 nm

Results

Analysis of standard solution

Fig. 1 shows spectra of water- and fat-soluble vitamins. We set 220 nm as the detector wavelength in this study. Fig. 2 shows chromatograms of water- and fat-soluble vitamin standard solutions. The Shim-pack MAqC-ODS I contains an ODS-modified metal-doped stationary phase, so it was able to retain the weakly-retained components without ion-pairing reagents. In addition, by using a 100% organic solvent mobile phase after the water-soluble vitamins were eluted, fat-soluble vitamins were also able to be analyzed. All the water- and fat-soluble vitamins were well separated in a single method.

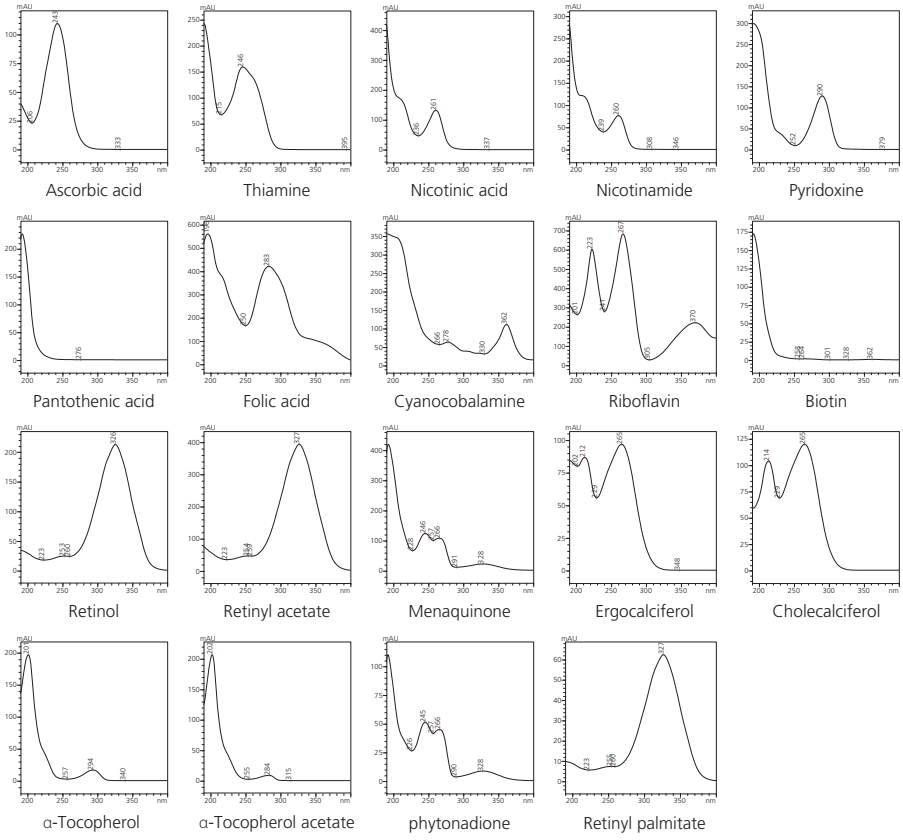

Fig.1 Spectra of analytes

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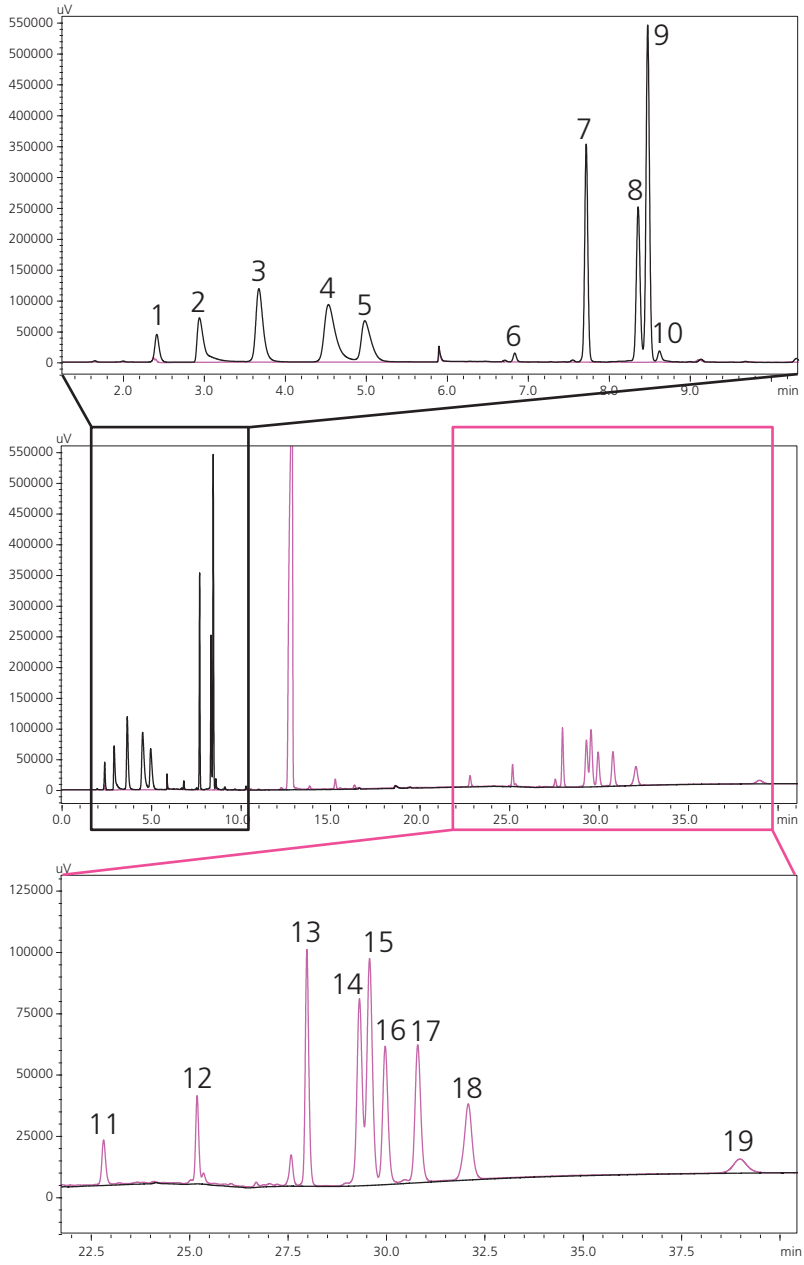

■ Peaks

1. Ascorbic acid (Vitamin C)
2. Thiamine (Vitamin B1)
3. Nicotinic acid (Vitamin B3)
4. Nicotinamide (Vitamin B3)
5. Pyridoxine (Vitamin B6)
6. Pantothenic acid (Vitamin B5)
7. Folic acid (Vitamin B9)
8. Cyanocobalamine (Vitamin B12)
9. Riboflavin (Vitamin B2)
10. Biotin (Vitamin B7)
11. Retinol (Vitamin A)
12. Retinyl acetate (Vitamin A acetate)
13. Menaquinone (Vitamin K2)
14. Ergocalciferol (Vitamin D2)
15. Cholecalciferol (Vitamin D3)
16. α-Tocopherol (Vitamin E)
17. α-Tocopherol acetate (Vitamin E acetate)
18. Phytonadione (Vitamin K1)
19. Retinyl palmitate (Vitamin A palmitate)

Fig.2 Chromatogram of standard solutions

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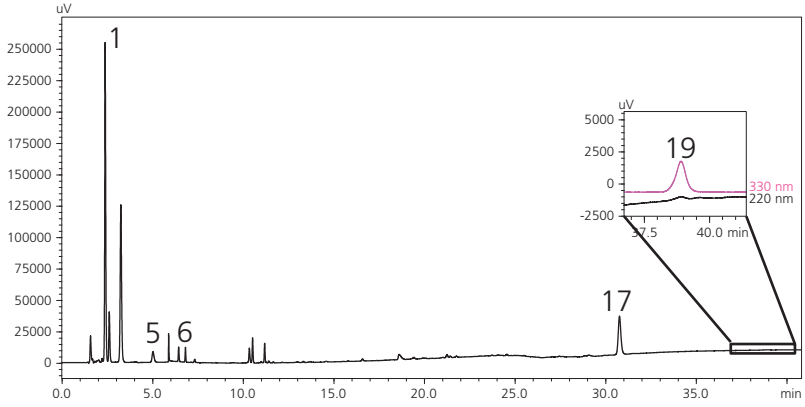
Analysis of sample solution

Fig. 3 shows a chromatogram of vitamin fortified water. All the water- and fat-soluble vitamins were able to detected in a single run. It was difficult to detect retinyl palmitate at 220 nm because of the low concentration, but it was able to be detected at 330 nm, which is a wavelength of maximum absorption.



■ Peaks

1. Ascorbic acid (Vitamin C)
5. Pyridoxine (Vitamin B6)
6. Pantothenic acid (Vitamin B5)
17. α-Tocopherol acetate (Vitamin E acetate)
19. Retinyl palmitate (Vitamin A palmitate)

Fig.3 Chromatogram of vitamin fortified water

Conclusions

- 10 water-soluble vitamins and 9 fat-soluble vitamins were successfully analyzed on a Shim-pack MAqC-ODS I in a single method.
- Water- and fat- soluble vitamins in a vitamin fortified water were successfully analyzed in a single run.

Reference

Shimadzu application news No. L459: Simultaneous Analysis of Water-Soluble Vitamins by a Newly Developed "Shim-pack MAqC-ODS I" Column

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