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DETERMINATION OF WATER-SOLUBLE VITAMINS CONTENT IN YOGURT USING THE HPLC METHOD

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Abstract

In this study, a high-performance liquid chromatography (HPLC) method based on the HPLC technique was developed for the determination of water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}, PP, and C) in yogurt. Standard vitamin solutions were prepared in 0.1 N hydrochloric acid (HCl) and 0.025% sodium hydroxide (NaOH) solutions and analyzed using a Shimadzu LC-40 Nexera Lite system equipped with a Shim-pack GIST C18 column under gradient elution conditions. The yogurt sample extract was prepared by ultrasonic-assisted extraction at 60°C for 20 minutes with 0.1 N HCl, followed by filtration through a 0.22 µm syringe filter prior to analysis.

The results indicated that, per 100 g of yogurt, vitamin B_6 was present at the highest concentration (11.515 mg), while vitamin C was found at 47.435 mg; vitamin B_{12} was not detected. The method demonstrated high reproducibility and sensitivity, making it suitable for use in food quality control applications.

Keywords: yogurt; water-soluble vitamins; HPLC method; high-performance liquid chromatography; vitamin content determination

Introduction

Reagents and Equipment

Vitamin B_{12} was sourced from *Rhydburg Pharmaceuticals* (Germany), vitamin C from *Carl Roth GmbH* (Germany), vitamin B_9 from *DSM Nutritional Products GmbX* (Germany), and vitamins B_1, B_2, B_3, B_6, and PP from *BLDPharm* (China). HPLC-grade water, acetonitrile, glacial acetic acid, and sodium hydroxide were used as reagents.
The quantification of water-soluble vitamins in the yogurt extract was performed using a high-performance liquid chromatography (HPLC) system, LC-40 Nexera Lite, manufactured by Shimadzu Corporation (Japan) [1].

Preparation of Standard Solutions

Standard solutions of vitamins C (CAS 50-81-7), B_1 (CAS 59-43-8), B_6 (CAS 58-56-0), B_3 (CAS 59-67-6), B_{12} (CAS 68-19-9), and PP (CAS 98-92-0) were prepared by dissolving 5 mg of each vitamin in 50 mL of 0.1 N hydrochloric acid (HCl) to achieve a concentration of 100 mg/L. Standard solutions of vitamins B_2 (CAS 83-88-5) and B_9 (CAS 59-30-3) were prepared by dissolving 5 mg of each in 50 mL of 0.025% sodium hydroxide (NaOH) solution. Subsequently, 200 µL aliquots of the B_1, B_6, B_3, B_{12}, and PP vitamin standard solutions were combined to prepare a mixed standard with a concentration of 14.286 mg/L for each vitamin.

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Further dilutions were performed to obtain standard solutions with concentrations of 7.143, 3.571, and 1.786 mg/L.

Similarly, vitamin C standard solutions were prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Distilled water was used as the blank (0 mg/L) for calibration curve construction.

Preparation of Sample Extract

For the extraction of water-soluble vitamins, 1.0 g of the yogurt sample was accurately weighed into a 50 mL conical flask, and 25 mL of 0.1 N HCl solution was added. The mixture was subjected to ultrasonic extraction using a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. After extraction, the mixture was cooled to room temperature, filtered, and the volume was adjusted to 25 mL with distilled water in a volumetric flask. An aliquot of 1.5 mL of the extract was then filtered through a 0.22 µm syringe filter into a vial and used for HPLC analysis.

Chromatographic Conditions

Determination of Vitamins. The standard solutions and sample extracts were analyzed using a high-performance liquid chromatography (HPLC) system comprising the LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, operated with LabSolutions software version 6.92 (Shimadzu Corporation, Japan). Separation was performed on a reversed-phase Shim-pack GIST C18 column (150 × 4.6 mm; 5 µm particle size, Shimadzu, Japan) using a gradient mobile phase consisting of acetonitrile (solvent A) and 0.25% aqueous acetic acid solution (solvent B), as detailed in Table 1.

The injection volume was set at 10 µL, the flow rate at 0.6 mL/min, and the column oven temperature was maintained at 40°C.
The analytical signals (peak areas) for each vitamin were recorded at three detection wavelengths: 265 nm, 291 nm, and 550 nm (Figures 1–3).
For the determination of vitamin C, a specific 15-minute gradient program was applied (Table 2), with detection performed at a wavelength of 265 nm.

Table 1. Gradient Program for Vitamin Determination

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

Table 2. Mobile phase gradient program for vitamin C quantification.

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	

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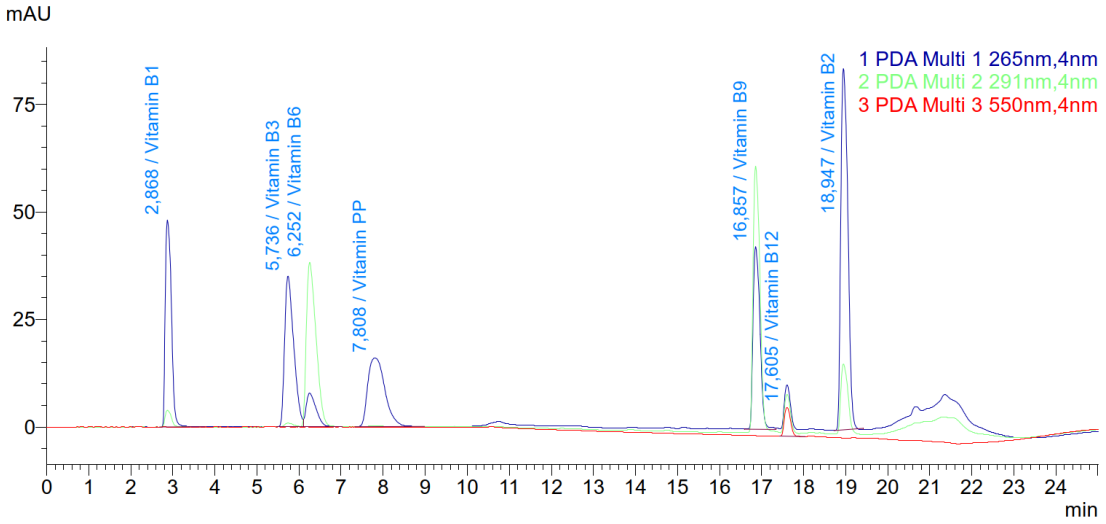

Figure 1. Chromatogram of a standard solution of vitamins.

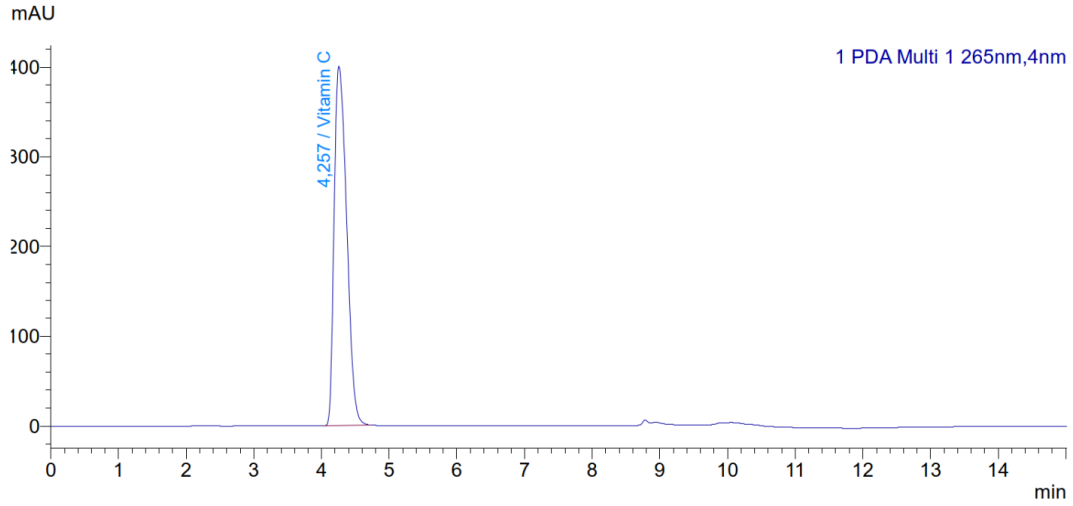

Figure 2. Chromatogram of a vitamin C standard solution.

Results

Determination of vitamins in the sample extract. A chromatogram of the sample extract (Figures 3-4) was obtained and based on the results, the amounts of vitamins in 100 g of the sample were calculated using the following formula and presented in Table 3.

$$X = \frac{C_{vit} \cdot V_{extract}}{m_{sample}} \cdot 100\ g$$

Here,

X – the amount of vitamins in 100 grams of the sample, expressed in mg; C_{vit} – the concentration of the vitamin in the extract determined by the HPLC method, expressed in mg/L;

$V_{extract}$ – the volume of the sample extract, expressed in liters (L);

$m_{samplem}$ – the mass of the sample used for extraction, expressed in grams (g).

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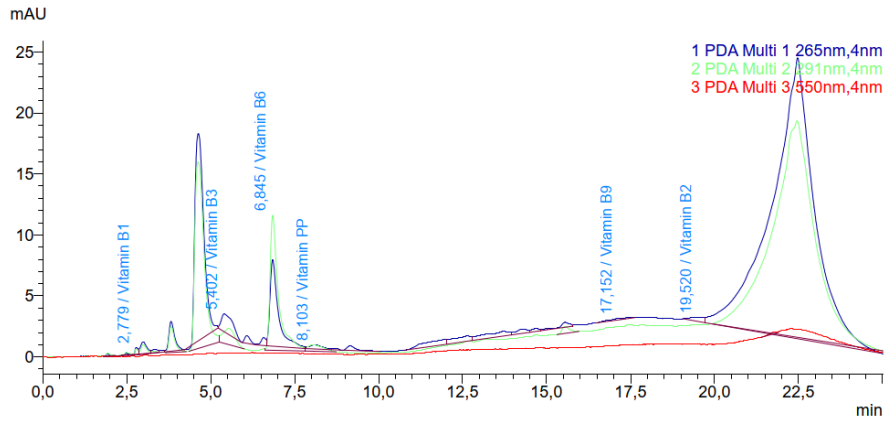

Figure 3. Chromatogram of the determination of vitamins in the sample extract.

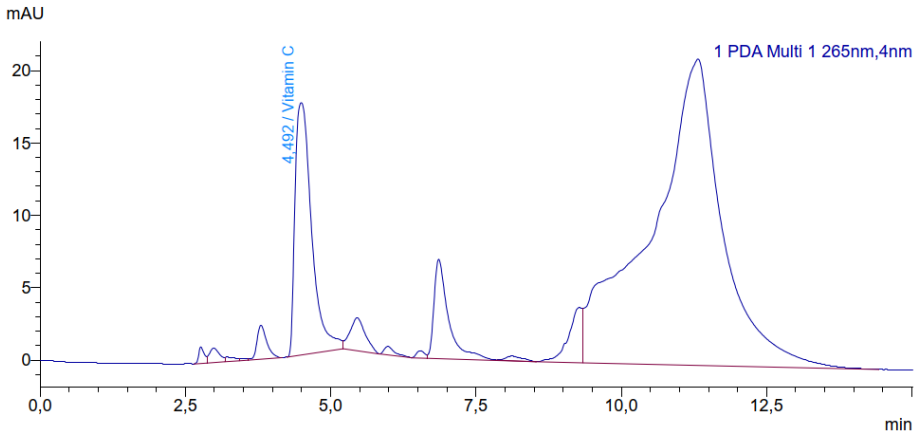

Figure 4. Chromatogram of vitamin C in the sample extract.

Table 3. Amount of vitamins in the extract and retention times.

Vitamin	Capture time, sec	Concentration, mg/l	Amount in 100 g of sample, mg
Vitamin B_1	2.779	0.197	0.493
Vitamin B_3	5.402	0.943	2.358
Vitamin PP	8.103	0.305	0.763
Vitamin B_9	17.152	0.113	0.283
Vitamin B_2	19.52	0.398	0.995
Vitamin B_6	6.845	4.606	11.515
Vitamin B_{12}	Not specified	0	0.000
Vitamin C	4.492	18.974	47.435

Conclusions

In this study, a high-performance liquid chromatography (HPLC) method was successfully developed and applied for the determination of water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}, PP, and C) in yogurt. The sample preparation involving ultrasonic-assisted extraction with 0.1 N hydrochloric acid, followed by HPLC analysis using a reversed-phase C18 column under gradient elution, proved to be effective for vitamin detection.

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The results demonstrated that vitamin B_6 (11.515 mg/100 g) and vitamin C (47.435 mg/100 g) were present in significant amounts, while vitamin B_{12} was below the detection limit. The method showed good reproducibility, sensitivity, and reliability, suggesting its suitability for quality control of food products containing water-soluble vitamins.

Future research is recommended to expand the method's application to different dairy matrices and to investigate the stability of the detected vitamins under various storage and processing conditions.

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