

QUANTITATIVE ANALYSIS OF VITAMINS B₁, B₂, B₃, B₆ A BY THE UHPLC METHOD IN VEGETABLES

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Abstract. The fastest and most accurate quantification of vitamins in food is due to the increasingly strict requirements of food regulatory agents. Quantification of B vitamins was done using a UHPLC with a DAD detector. The extraction was done using hexane as solvent. The study was conducted to determine vitamins B₁, B₂, B₃, and B₆ content from 4 vegetables: Spinach (*Spinacia oleracea*), Cabbage (*Brassica oleracea*), Broccoli (*Brassicaceae*), and Beans (*Phaseolus vulgaris*) by UHPLC. These vegetables can be exploited as a source of the vitamin B complex. The analysis of the B vitamin content of vegetables could serve as a means of calculating the dietary intake of B vitamins. Using this method, the complex of B vitamins studied, as shown in the chromatogram, were eluted with retention times between 0,667-5,992 min. The highest concentration of vitamin B₁ is found in beans (0,26mg/100g), vitamins B₂ and B₃ were found in spinach (0,196mg/100g, respectively 0,872 mg/100g), and vitamin B₆ in broccoli (0,247mg/100g).

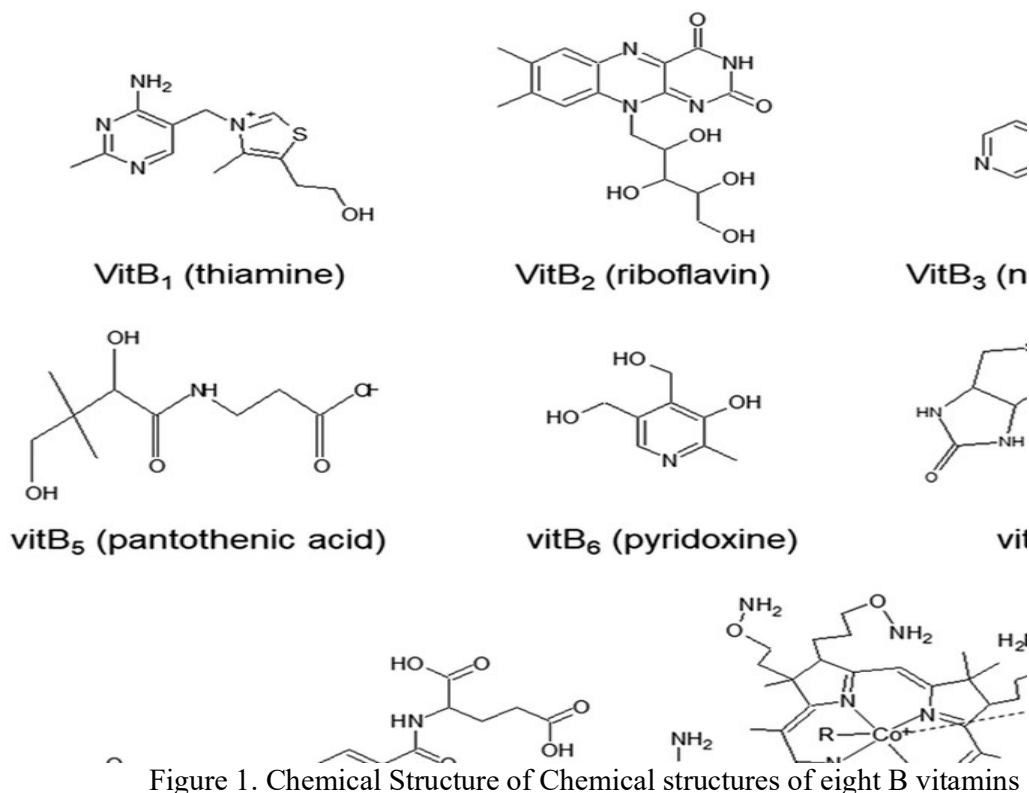
Keywords: B vitamins, vegetables, liquid chromatography, DAD detector

INTRODUCTION

Vegetables and fruits are best represented in the food pyramid as the foundation of a balanced diet, calling them the "base" for adequate nutrition and health. This group is considered the primary source of vitamins and minerals, once contributing to the daily intake of carbohydrates and dietary fibers [1,3] Mainly, vitamins serve as catalysts for specific reactions in our bodies. If these catalysts are missing, as in the case of vitamin deficiencies, the body's normal functions no longer work properly, and thus the increased risk of developing diseases appears. [2] The B vitamin complex is a mixture of vitamins B₁, B₂, B₃, B₆, B₆, B₇, B₉, and B₁₂. They are found in green vegetables, milk, eggs, and meat. These are a group of water-soluble vitamins that play an essential role in cellular metabolism. [4] B vitamins are necessary to support and increase the rate of metabolism, maintain healthy skin and muscle tone, to promote the growth and division of cells, including that of red blood cells, which help prevent anemia and reduce the risk of pancreatic cancer. [5] Most B vitamins must be supplemented from food. The chemical structure of the B vitamin complex is shown in figure 1.

Among the analytical methods, ultra-high performance liquid chromatography (UHPLC) is promising for its precision, reliability, high sensitivity, and different detectors.

The current stage was designed to analyze the content of thiamin (b₁), riboflavin (B₂), niacin (B₃), and pyridoxine (B₆) soluble in water from 4 vegetables: Spinach (*Spinacia oleracea*), Cabbage (*Brassica oleracea*), Broccoli (*Brassicaceae*), Beans (*Phaseolus vulgaris*) by UHPLC-UV.



MATERIALS AND METHODS

Reagents and standards: All solvents were HPLC grade from VWR, the ultra-pure water was obtained using the ULTRACLEAR UV UF EVOQUA Purification system, USA. All standards used were from Sigma-Aldrich.

Samples: Samples for four varieties of vegetables (spinach, cabbage, broccoli, and beans) were collected from local producers in Cluj County. The pieces were sprayed with water to maintain moisture, packed in dark polyethylene bags to prevent water loss and light damage, and then transported to the laboratory as soon as possible. The scientific names of the vegetables used in this study are presented in table 1.

Table 1

Plants source taken for estimation of B-vitamins (B₁, B₂, B₃ and B₆) content.

Serial	English name	Scientific name
1.	Spinach	Spinacia oleracea
2.	Cabbage	Brassica oleracea
3.	Broccoli	Brassicaceae
4.	Beans	Phaseolus vulgaris

Sample preparation: 5 g of the sample was extracted with 25 ml of Hexan for 30 minutes on an ultrasonic bath. The samples thus extracted were filtered through a glass fiber filter and passed through a 0.5 g Florisil column. The extract was concentrated to dryness on a rotary evaporator and returned with 1 ml of methanol.

Liquid Chromatography conditions: UHPLC Vanquisher H from Dionex, Thermo Fisher Scientific, Germany with DAD detector was used for the analysis of vitamins: Thiamine (B1), Riboflavin (B2), Nicotinamide (B3), Pyridoxine (B6). Mobile phase was composed of ultra-pure H₂O with 1 % acetic acid and MeOH in gradient with a flow of 0.3 ml/min. The chromatographic column used was a Accucore aQ 100x2.1 mm, 2.6 μ m, from Thermo Fisher, kept at 25 °C. The injection volume was 8 μ l and the detector was set at 270 nm.

RESULTS AND DISCUSSION

HPLC is the most expedient, and precise, analytical technique for estimation of B vitamin. The UHPLC method is the most practical and precise analytical technique for estimating B vitamins. To confirm the specificity of the method developed here, the concentration of working standard solution and retention times of vitamins varied (Table 2). All concentrations between each vitamin B compounds were <75 μ g * ml⁻¹, and the retention time (Rt) of vitamins B1, B2, B3, and B6 were 0,667, 5,992, 1,164, and 0,977 min, respectively.

Table 2

Retention time of B-vitamins standard at 270 nm

Vitamin	Injection volume (μ l)	Concentration (μ g * ml ⁻¹)	Retention time (min)
B1	8	20	0,667
B2	8	75	5,992
B3	8	60	1,164
B6	8	35	0,977

The chromatogram of the analyzed samples is presented in Figure 2. There was no matrix interference with the peak. The cleanup method, using a Florisil column, although it is simple, it has been proven to be efficient for the elimination of unwanted compounds found in the

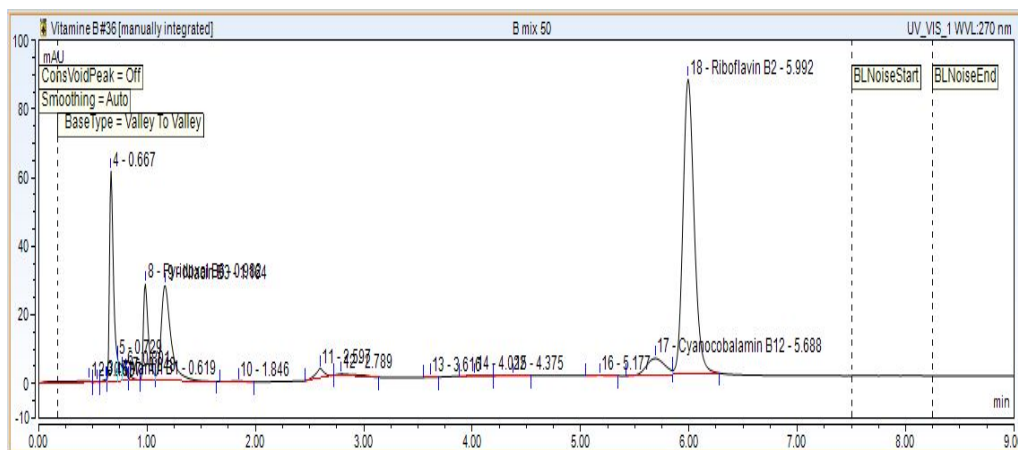


Figure 2. Chromatogram of the vegetables samples

The B vitamin (B1, B2, B3, and B6) content of the samples is shown in table 3, and it varies. All compounds had responses at 270 nm, where they were successfully separated.

Thiamine (B1) contributes to the proper functioning of the nervous system, being the coenzyme in the reactions of carbohydrate metabolism (with a role in the production of ribose) and several amino acids. The highest concentration of vitamin B1 is found in beans, providing 30% of the recommended daily dose.

Riboflavin(B2) is vital in cellular respiration; it is soluble in water, helps to treat anemia, and must be consumed every day because the body can only store this vitamin in small quantities, and the reserves are quickly exhausted. The highest concentration of vitamin B2 is found in spinach. The consumption of 100 g of spinach provides 20% of the recommended daily requirement of vitamin B2.

Riboflavin(B3) Vitamin B3 is essential for processing fat in the body, lowering cholesterol levels, and regulating blood sugar levels. Regular consumption of vegetables can provide significant amounts of B3 needed to maintain healthy body functions. The highest concentration of vitamin B3 is found in spinach (0,872mg/100g), and the lowest is in cabbage (0,254 mg/100g).

Pyridoxine (B6) is essential for the metabolism of proteins, fats, and carbohydrates; more precisely, this vitamin transforms food into energy and contributes to the creation of neurotransmitters, such as serotonin and dopamine. The highest concentration of vitamin B6 is found in broccoli (0,247mg/100g), and the lowest is in beans (0,12 mg/100g).

Table 3

Vitamin B₁, B₂, B₃ and B₆ contents in vegetables(mg/100g)

Samples	Vit-B1 (mg/100g)	Vit-B ₂ (mg/100g)	Vit B- ₃ (mg/100g)	Vit-B ₆ (mg/100g)
Spinach	0,095	0,196	0,872	0,214
Cabbage	0,067	0,005	0,254	0,132
Broccoli	0,12	0,13	0,61	0,247
Beans	0,26	0,11	0,67	0,12

CONCLUSIONS

A rapid, sensitive, and reliable method for quantifying B vitamins in vegetables was developed using a UHPLC, Vanquish H from Dionex, with a DAD detector. These vegetables can be exploited as a source of the vitamin B complex.

The analysis of the B vitamin content of vegetables could serve as a means of calculating the dietary intake of B vitamins. Using this method, the complex of B vitamins studied, as shown in the chromatogram, were eluted with retention times between 0,667-5,992 min. The highest concentration of vitamin B1 is found in beans (0,26mg/100g), vitamins B2 and B3 were found in spinach (0,196mg/100g, respectively 0,872 mg/100g), and vitamin B6 in broccoli (0,247mg/100g).

No matrix effect was observed for the compounds of interest in the analyzed samples; they have the same retention time as the standard solution.

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