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DETERMINATION OF VITAMIN CONTENT IN EXTRACTS OF SELECTED MEDICINAL PLANTS GROWN UNDER THE CONDITIONS OF UZBEKISTAN

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Abstract. This study investigated the levels of water-soluble vitamins in extracts of medicinal plants cultivated under the conditions of Uzbekistan, including *Hypericum scabrum* and *Calendula officinalis* (3:1), as well as *Hippophae rhamnoides* and *Urtica dioica* (1:1). The research was motivated by the increasing interest in biologically active compounds of medicinal plants and the limited data on their vitamin composition in Uzbekistan-grown species.The selected plants are widely used in traditional medicine due to their anti-inflammatory, immune-supporting, and micronutrient-rich properties. High-performance liquid chromatography (HPLC) was employed for vitamin determination. Extracts were filtered prior to analysis, and chromatographic conditions were optimized to ensure accurate separation and reliable detection.The results demonstrated that the applied HPLC method provided stable and reproducible determination of water-soluble vitamins. The findings support the pharmaceutical and food-related potential of these medicinal plants and provide a scientific basis for quality control, identification of bioactive compounds, and further development of plant-based products.

Keywords: Medicinal plants, Water-soluble vitamins, *Hypericum scabrum*, *Calendula officinalis*, *Hippophae rhamnoides*, *Urtica dioica*, Plant extracts, High-performance liquid chromatography (HPLC), Biologically active compounds, Pharmaceutical and food applications.

Аннотация. В данном исследовании изучалось содержание водорастворимых витаминов в экстрактах лекарственных растений, выращенных в условиях Узбекистана, включая *Hypericum scabrum* и *Calendula officinalis* (3:1), а также *Hippophae rhamnoides* и *Urtica dioica* (1:1). Актуальность работы обусловлена возрастающим интересом к биологически активным соединениям лекарственных растений и недостаточностью данных

о витаминном составе растений, культивируемых в Узбекистане. Выбранные растения широко применяются в традиционной медицине благодаря их противовоспалительным, иммуномодулирующим и богатым микронутриентами свойствам. Для определения витаминов использовали метод высокоэффективной жидкостной хроматографии (ВЭЖХ). Перед анализом экстракты фильтровали, а хроматографические условия были оптимизированы для обеспечения точного разделения и надежного определения компонентов. Полученные результаты показали, что применённый метод ВЭЖХ обеспечивает стабильное и воспроизводимое определение водорастворимых витаминов. Полученные данные подтверждают перспективность использования исследуемых лекарственных растений в фармацевтической и пищевой промышленности, а также создают научную основу для контроля качества, идентификации биологически активных соединений и дальнейшей разработки растительных препаратов.

Ключевые слова: лекарственные растения, водорастворимые витамины, *Hypericum scabrum*, *Calendula officinalis*, *Hippophae rhamnoides*, *Urtica dioica*, растительные экстракты, высокоэффективная жидкостная хроматография (ВЭЖХ), биологически активные соединения, фармацевтические и пищевые применения.

Annotatsiya. Ushbu tadqiqotda O‘zbekiston sharoitida yetishtirilgan dorivor o‘simliklarning ekstraktlarida, jumladan Hypericum scabrum va Calendula officinalis (3:1), shuningdek Hippophae rhamnoides va Urtica dioica (1:1) tarkibidagi suvda eriydigan vitaminlar miqdori o‘rganildi. Ishning dolzarbligi dorivor o‘simliklarning biologik faol birikmalariga qiziqish ortib borayotganligi va O‘zbekistonda yetishtiriladigan o‘simliklarning vitamin tarkibi haqidagi ma’lumotlarning yetarli emasligi bilan izohlanadi. Tanlangan o‘simliklar yallig‘lanishga qarshi, immunomodullovchi va mikronutriyentlarga boy xususiyatlari tufayli an’anaviy tibbiyotda keng qo‘llaniladi. Vitaminlarni aniqlash uchun yuqori samarali suyuqlik xromatografiyasi (VEYX) usuliKalit so'zlar: dorivor o'simliklar, suvda eriydigan vitaminlar, Hypericum scabrum, Calendula officinalis, Hippophae rhamnoides, Urtica dioica, o'simlik ekstraktlari, yuqori samarali suyuqlik xromatografiyasi (HPLC), biologik faol birikmalar, farmatsevtika va oziq-ovqat ilovalari.takrorlanadigan aniqlashni ta’minlaydi. Olingan ma’lumotlar farmatsevtika va oziq-ovqat sanoatida o‘rganilayotgan dorivor o‘simliklardan foydalanish istiqbolliligini tasdiqlaydi, shuningdek, sifat nazorati, biologik faol birikmalarni aniqlash va o‘simlik preparatlarini yanada ishlab chiqish uchun ilmiy asos yaratadi.

Kalit so'zlar: dorivor o'simliklar, suvda eriydigan vitaminlar, Hypericum scabrum, Calendula officinalis, Hippophae rhamnoides, Urtica dioica, o'simlik ekstraktlari,

yuqori samarali suyuqlik xromatografiyasi (HPLC), biologik faol birikmalar, farmatsevtika va oziq-ovqat ilovalari.

INTRODUCTION.

In recent years, the investigation of biologically active compounds from natural sources has become an important research area in pharmaceutical sciences and biochemistry. In particular, determination of water-soluble vitamins in medicinal plants is essential for evaluating their physiological and therapeutic significance. These vitamins participate in energy metabolism, antioxidant protection, and immune system regulation (Abdurahmonov S.M., Toshpo‘latov A.R. 2019; Griffits L.A., Smith J.K. 2017).

Uzbekistan possesses rich floristic resources, and medicinal plants growing under local conditions are widely used in traditional medicine. However, quantitative analysis of water-soluble vitamins in these plants using modern analytical techniques remains insufficiently studied (Xudoyberdiyev A., Ergashev S. 2018). Environmental conditions, including climate and soil composition, significantly influence the biochemical profile of medicinal plants, making scientific standardization necessary before practical application (Rustamov B., Qodirov N. 2019).

Medicinal plants such as *Hypericum scabrum*, *Calendula officinalis*, *Hippophae rhamnoides*, and *Urtica dioica* are rich in flavonoids, phenolic compounds, carotenoids, vitamins, and essential микро- hamda makroelementlar, which determine their pharmacological activity (Karim M.R. et al., 2025). These plants are known for antioxidant, anti-inflammatory, regenerative, and immunomodulatory properties. Previous studies confirmed their therapeutic value; however, data on vitamin composition of combined extracts from plants grown in Uzbekistan remain limited (Bourgaud F. et al., 2001).

Recent preliminary studies demonstrated that combinations of *Hypericum scabrum* with *Calendula officinalis* (3:1) and *Hippophae rhamnoides* with *Urtica dioica* (1:1) exhibit enhanced antiradical activity, suggesting synergistic interactions among their bioactive compounds (Asqarov I.R., 2019).

Therefore, the present study aimed to quantitatively determine water-soluble vitamins in extracts of selected medicinal plants cultivated in Uzbekistan using high-performance liquid chromatography (HPLC). The obtained results may contribute to the development and standardization of plant-based pharmaceutical and nutraceutical products (Sharma P. et al., 2009).

MATERIALS AND METHODS.

Reference standards of cyanocobalamin (vitamin B_{12}), ascorbic acid (vitamin C), folic acid (vitamin B_9), thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2), nicotinic acid and nicotinamide (vitamin B_3/PP), and pyridoxine

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hydrochloride (vitamin B_6) were obtained from certified suppliers and used for analytical calibration. Chromatographic analyses were performed using analytical-grade ultrapure water, acetonitrile, glacial acetic acid, and sodium hydroxide.

Quantitative determination of water-soluble vitamins in plant extracts was carried out using a Shimadzu Nexera LC-40 Lite high-performance liquid chromatography (HPLC) system (Japan) (Asqarov I.R. et al., 2024). Standard vitamin solutions were prepared in appropriate solvents to construct calibration curves and determine concentration ranges. Ultrapure water was used as the blank reference.

3.1. Preparation of Sample Extracts

For analysis, 1 g of each plant sample was placed into a 50 mL conical flask, followed by addition of 25 mL of 0.1 N hydrochloric acid. Extraction was performed in a GT SONIC-D3 ultrasonic bath (China) at 60 °C for 20 min. After cooling, the mixtures were filtered and adjusted to 25 mL with deionized water. Finally, 1.5 mL of each extract was filtered through a 0.22 µm syringe filter into HPLC vials for chromatographic analysis.

HPLC Analysis

High-performance liquid chromatography (HPLC) was employed to quantify water-soluble vitamins in both the plant extracts and the corresponding standard solutions. HPLC conditions and detector settings were established according to the standard protocols of the Shimadzu LC-40 Nexera Lite system.

3.2. Chromatographic Conditions

Chromatographic conditions and solvent gradients were optimized to achieve efficient separation of water-soluble vitamins. Detailed operational parameters and gradient compositions are presented in Tables 2–4. Analyses were performed using a 15-minute gradient program with an injection volume of 10 µL, column temperature of 40 °C, and mobile phase flow rate of 0.6 mL/min. Detection was carried out at wavelengths of 265, 291, and 550 nm, while the 265 nm signal was used specifically for quantification of ascorbic acid (vitamin C).

The gradient program was developed according to the polarity differences of vitamins to ensure accurate separation and reliable peak resolution. Initially, the mobile phase contained 100% 0.5% acetic acid, allowing effective retention of polar vitamins. Subsequently, the acetonitrile concentration was gradually increased to facilitate elution of moderately polar and less polar compounds. At the final stage, the system was returned to the initial conditions to regenerate the column for subsequent analyses.

For vitamin C determination, a separate optimized gradient system was applied. During the initial stage, the mobile phase consisted entirely of 0.5% acetic acid to maintain retention of ascorbic acid, followed by gradual addition of

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acetonitrile for efficient elution. The system was then restored to the starting conditions to ensure reproducibility and stability of the chromatographic analysis.

RESULTS AND DISCUSSION.

Quantitative analysis of vitamins in the prepared plant extracts was performed based on HPLC chromatograms obtained from the analyzed samples (Figures 3–6). The vitamin content per 100 g of plant material was calculated using the following equation:

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

where:

X – vitamin content per 100 g of sample (mg);

Cvit – vitamin concentration in the extract determined by HPLC (mg/L);

Vextract – total extract volume (L);

msample – mass of plant material used for extraction.

The 3:1 extract of *Hypericum scabrum* and *Calendula officinalis* demonstrated distinct peaks corresponding to vitamins B_1, B_3, B_6, PP, B_9, and

B_2 at characteristic retention times. Polar vitamins eluted in the early chromatographic stage, whereas less polar vitamins appeared later as the proportion of organic solvent increased. The well-resolved peaks and low background noise confirmed the selectivity and sensitivity of the developed HPLC method. Analysis at wavelengths of 265, 291, and 350 nm further improved reliability of vitamin identification (Figure 3).

The HPLC–PDA chromatogram of the 3:1 combined extract showed a clear vitamin C peak at a retention time of 4.172 min detected at 265 nm, confirming the presence of ascorbic acid in the extract (Figure 4). Additional peaks observed between 9–10 min corresponded to other biologically active compounds such as flavonoids and phenolic acids, which did not interfere with vitamin C detection. The symmetrical peak shape and good baseline separation indicated high selectivity and reliability of the method.

Analysis of the 1:1 extract of *Hippophae rhamnoides* and *Urtica dioica* also confirmed the presence of vitamin C together with several B-group vitamins. Distinct peaks corresponding to vitamins B_1, B_3, B_6, PP, and B_9 were detected, demonstrating a rich vitamin composition of the extract (Figure 5). Simultaneous PDA detection at 265, 291, and 550 nm enhanced the accuracy of peak identification and confirmed the suitability of the chromatographic conditions.

In addition, vitamin C determination in the 1:1 extract showed a characteristic peak at 4.44 min, matching the retention time and UV spectrum of the ascorbic acid standard (Figure 6). Other bioactive compounds present in the

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extract did not interfere with its detection, confirming the selectivity and reproducibility of the HPLC–PDA method.

Overall, the obtained results demonstrated that the investigated medicinal plant extracts contain various water-soluble vitamins in biologically significant amounts. The determined retention times, extract concentrations, and vitamin contents per 100 g of plant material are presented in the following tables.

This study investigated water-soluble vitamins in extracts of medicinal plants cultivated in Uzbekistan, including *Hypericum scabrum*, *Calendula officinalis*, *Hippophae rhamnoides*, and *Urtica dioica*. Quantitative HPLC analysis enabled determination of vitamin concentrations and their contents per 100 g of plant material.

The results showed that the extracts were rich in B-group vitamins, particularly vitamins B_3, B_2, B_9, and B_1, while vitamin C and vitamin PP (niacin) were detected in lower amounts. Vitamin B_{12} was not identified in the analyzed extracts.

The obtained data confirm the biological and practical significance of the investigated medicinal plants for pharmaceutical and food-related applications. In addition, the HPLC method demonstrated high reliability, selectivity, and reproducibility for determining water-soluble vitamins in plant extracts.

Table 1

Concentrations of Standard Solutions

Vitamin	CAS Number	Solvent for Standard Solution Preparation	Prepared Concentrations (mg/L) (mg/L)
B1	59-43-8	0,1 N HCl	14,286; 7,143; 3,571; 1,786
B2	83-88-5	0,025% NaOH	14,286; 7,143; 3,571; 1,786
B3	59-67-6	0,1 N HCl	14,286; 7,143; 3,571; 1,786
B6	58-56-0	0,1 N HCl	14,286; 7,143; 3,571; 1,786
B9	59-30-3	0,025% NaOH	14,286; 7,143; 3,571; 1,786
B12	68-19-9	0,1 N HCl	14,286; 7,143; 3,571; 1,786

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PP	98-92-0	0,1 N HCl	14,286; 7,143; 3,571; 1,786
C	50-81-7	0,1 N HCl	286; 143; 71,5; 57,2
0 (blank)	–	Purified water	0

Table 2

Gradient elution scheme employed in HPLC for vitamin analysis

Elapsed time (minutes)	Acetonitrile proportion (A, %)	0.25% Acetic Acid in water (B, %)
0	5	95
5	10	90
10	20	80
15	25	75

Table 3

Solvent gradient scheme applied in HPLC for the analysis of vitamins

Elapsed time (minutes)	Acetonitrile proportion (A, %)	0.25% Acetic Acid in water (B, %)
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	End of gradien programm	

Table 4

Gradient elution profile of HPLC solvents employed for quantifying ascorbic acid (vitamin C)

Elapsed time (minutes)	Acetonitrile proportion (A, %)	0.25% Acetic Acid in water (B, %)
0	0	100
2	0	100
6	50	50
6,01	0	100
15	End of gradien programm	

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Table 5

Vitamin contents and retention times in the 3:1 combined extract of Hypericum scabrum and Calendula officinalis

Vitamin	Elution time of analyte, s	Measured concentration in the extract, mg/L	Vitamin amount corresponding to 100 g of plant material, mg
Thiamine (Vit B_1)	2,733	0,815	2,038
Niacin (Vit B_3)	5,874	7,724	19,310
Niacinamide (Vit PP)	7,864	0,775	1,938
Folic acid (Vit B_9)	16,879	0,186	0,465
Riboflavin (Vit B_2)	19,066	0,768	1,920
Pyridoxine (Vit B_6)	6,02	0,978	2,445
Cobalamin (Vit B_{12})	Not detected	0	0,000
Ascorbic acid (Vit C)	4,172	0,56	1,400

Table 6

Vitamin concentrations along with corresponding chromatographic retention times in an equimolar extract combination of *Hippophae rhamnoides* and *Urtica dioica*

Vitamin	Elution time of analyte, s	Measured concentration in the extract, mg/L	Vitamin amount corresponding to 100 g of plant material, mg
Thiamine (Vit B_1)	2,982	2,813	7,033
Niacin (Vit B_3)	5,758	4,43	11,075
Niacinamide (Vit PP)	8,028	1,385	3,463
Folic acid (Vit B_9)	16,772	3,796	9,490
Riboflavin (Vit B_2)	18,976	4,273	10,683
Pyridoxine (Vit B_6)	6,307	0,897	2,243
Cobalamin (Vit B_{12})	Not detected	0	0,000

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Ascorbic acid (Vit C)	4,44	1,201	3,003

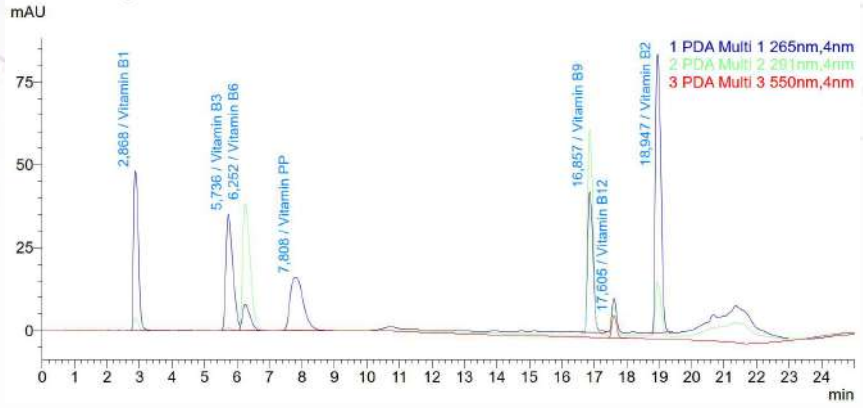


Figure 1. HPLC chromatographic profile of the prepared vitamin standard mixture

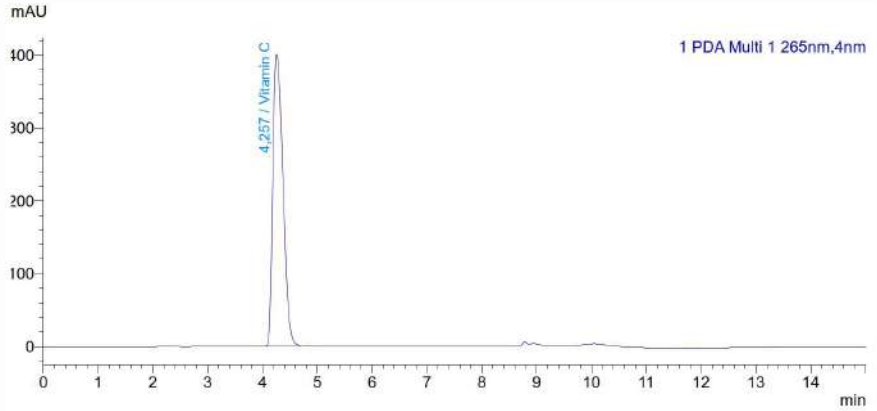


Figure 2. HPLC profile depicting the prepared standard solution of ascorbic acid (vitamin C)

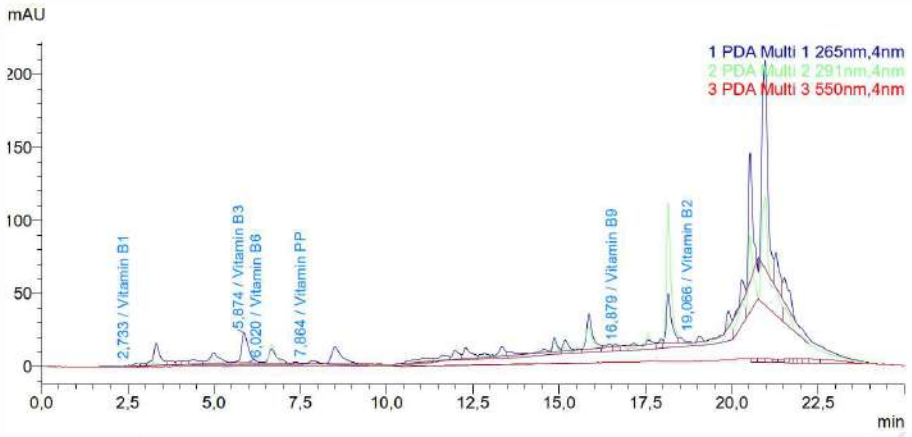


Figure 3. Chromatographic representation corresponding to the determination of vitamins in a 3:1 extract of *Hypericum scabrum* and *Calendula officinalis*

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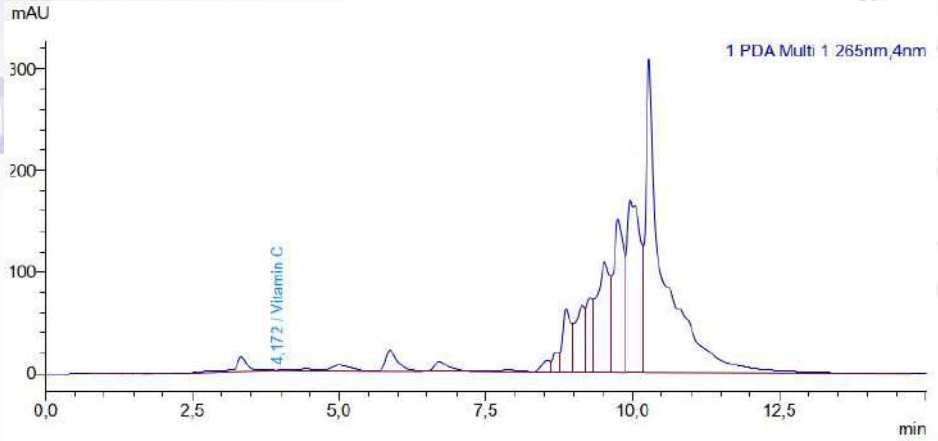


Figure 4. High-performance liquid chromatography (HPLC) profile illustrating the analysis of ascorbic acid in a 3:1 extract of *Hypericum scabrum* and *Calendula officinalis*

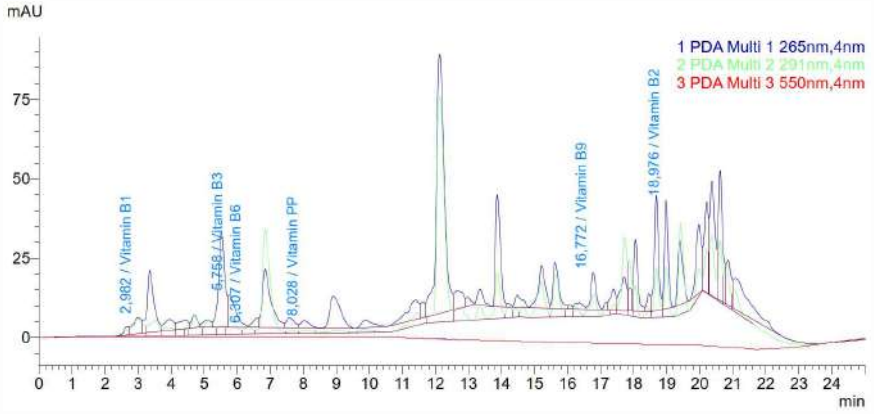


Figure 5. High-performance liquid chromatography (HPLC) profile depicting the determination of ascorbic acid in a 1:1 extract of *Hippophae rhamnoides* and *Urtica dioica*

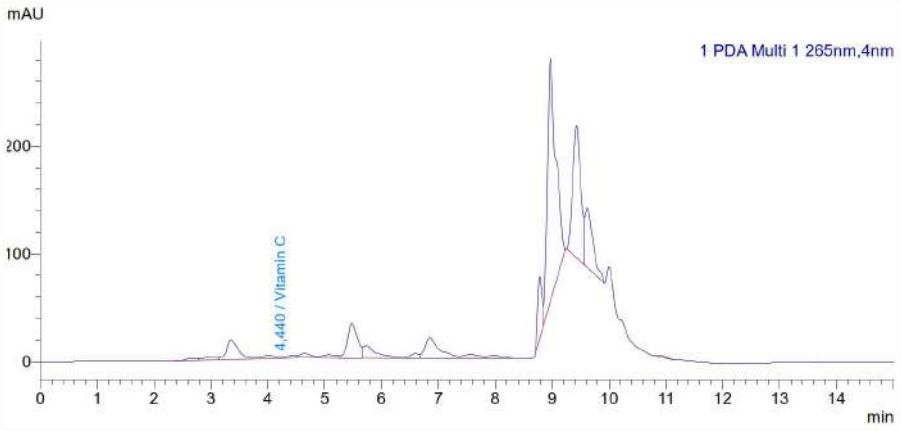


Figure 5. High-performance liquid chromatography (HPLC) profile depicting the determination of ascorbic acid in a 1:1 extract of *Hippophae rhamnoides* and *Urtica dioica*

CONCLUSION. This study investigated water-soluble vitamins in extracts of medicinal plants cultivated in Uzbekistan, including *Hypericum scabrum*, *Calendula officinalis*, *Hippophae rhamnoides*, and *Urtica dioica*. Quantitative

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HPLC analysis enabled determination of vitamin concentrations and their contents per 100 g of plant material.

The results showed that the extracts were rich in B-group vitamins, particularly vitamins B_3, B_2, B_9, and B_1, while vitamin C and vitamin PP (niacin) were detected in lower amounts. Vitamin B_{12} was not identified in the analyzed extracts.

The obtained data confirm the biological and practical significance of the investigated medicinal plants for pharmaceutical and food-related applications. In addition, the HPLC method demonstrated high reliability, selectivity, and reproducibility for determining water-soluble vitamins in plant extracts.

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