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SUVDA ERUVCHAN VITAMINLAR MIQDORINI YUSSX USULIDA ANIQLASH METODIKASINI ISHLAB CHIQISH (CHILONJIYDA MISOLIDA)

РАЗРАБОТКА МЕТОДИКИ ОПРЕДЕЛЕНИЯ КОЛИЧЕСТВА ВОДОРАСТВОРИМЫХ ВИТАМИНОВ С ИСПОЛЬЗОВАНИЕМ МЕТОДА YSSX (НА ПРИМЕРЕ УНАБИ)

DEVELOPMENT OF A METHODOLOGY FOR DETERMINING THE AMOUNT OF WATER-SOLUBLE VITAMINS USING THE YSSX METHOD (CASE STUDY OF JUJUBE)

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Annotatsiya

Maqolada chilonjiyda mevasi misolida o‘simlik tarkibidagi suvda eruvchan B_1, B_2, B_3, B_6, B_9, B_{12}, C va PP vitaminlarning miqdorini YSSX metodi yordamida aniqlash metodikasini ishlab chiqish bo‘yicha olib borilgan tadqiqotlar natijalari bayon qilingan. O‘silik namunasini quritish, maydalash, ekstraktini olish, uni xromatografik analizga tayorlash va analizni bajarish sharoitlarining optimal parametrlari aniqlangan.

Osimlik namunasidan gidrofil vitaminlarni ekstraktsiya qilish jarayoni vaqti 20 minut bo‘lganda haroratning 20 °C dan 80 °C gacha ko‘tarish aniqlanayotgan vitaminlarning ekstraktdagi miqdorini ortib borishi tendensiyasi 60 °C gacha kuzatildi. Shuni uchun ekstraktsiyaning optimal temperaturasi etib 60 °C tanlandi. Ayni shu o‘zgarmas temperaturada vaqtini 10 minutdan 40 minutgacha oshirish ekstraktsiyaning optimal vaqtning 20 minut ekanligini ko‘rsatdi.

Standart eritmalar va analiz qilinuvchi namunalar tarkibidagi vitaminlarni aniqlash Shim pack GIST C18 (150 × 4,6 mm; 5 mkm, Shimadzu) teskari fazali kolonkasi bilan jihozlangan LC-40 Nexera Lite yuqori samarali suyuqlik xromatografida asetonitril va sirka kislotaning suvdagi 0,25 % li eritmasidan tashkil topgan gradientli harakatchan fazalaridan foydalananib amalga oshirildi. Xromatografik detektorlash 265, 291 va 550 nm to‘lqin uzunlikda bajarildi.

Olingan natijalarni matematik qayta ishlash asosida ishlab chiqilgan anliz metodikasining aniqligi yetarli darajada yuqori ekanligi tasdiqlandi. Chilonjiyda mevasida B_6, B_3, B_2 va C vitaminlari eng ko‘p miqdorda bo‘lib, B_{12} vitamini mavjud emasligi ko‘rsatildi.

Аннотация

В статье представлены результаты исследований, проведенных для разработки методики определения количеств водорастворимых витаминов B1, B2, B3, B6, B9, B12, C и PP в растительном составе плодов унаби с использованием метода YSSX. Были определены оптимальные параметры для сушки образца растения, измельчения, экстракции, подготовки к хроматографическому анализу и проведения анализа.

Установлено, что при времени экстракции гидрофильных витаминов из растительного образца в 20 минут повышение температуры с 20 °C до 80 °C приводит к увеличению количества определяемых витаминов в экстракте, причем эта тенденция наблюдается до 60 °C. Поэтому оптимальной температурой экстракции была выбрана 60 °C. Увеличение времени при этой постоянной температуре с 10 минут до 40 минут показало, что оптимальное время экстракции составляет 20 минут.

Определение витаминов в стандартных растворах и анализируемых образцах проводилось с использованием высокоэффективного жидкостного хроматографа LC-40 Nexera Lite, оснащенного обратнопоточной колонкой Shim pack GIST C18 (150 × 4,6 мм; 5 мкм, Shimadzu), с использованием градиентных подвижных фаз, состоящих из ацетонитрила и 0,25% водного раствора уксусной кислоты. Хроматографическое детектирование проводилось на длинах волн 265, 291 и 550 нм.

Математическая обработка полученных результатов подтвердила, что точность разработанной методики анализа достаточно высока. Показано, что в плодах унаби наибольшее количество витаминов B6, B3, B2 и C, в то время как витамин B12 отсутствует.

Abstarct

This article presents the results of research conducted to develop a methodology for determining the amounts of water-soluble vitamins B1, B2, B3, B6, B9, B12, C, and PP in the plant composition of Jujube fruit using the YSSX method. The optimal parameters for drying the plant sample, grinding, extracting, preparing for chromatographic analysis, and conducting the analysis were identified.

It was found that when the extraction time for hydrophilic vitamins from the plant sample is 20 minutes, raising the temperature from 20 °C to 80 °C leads to an increasing trend in the amounts of the identified vitamins in the extract, with this trend observed up to 60 °C. Therefore, the optimal extraction temperature was chosen to be 60 °C. Increasing the time at this constant temperature from 10 minutes to 40 minutes indicated that the optimal extraction time is 20 minutes.

The identification of vitamins in standard solutions and analyzed samples was carried out using an LC-40 Nexera Lite high-performance liquid chromatograph equipped with a Shim pack GIST C18 (150 × 4.6 mm; 5 µm, Shimadzu) reverse-phase column, utilizing gradient mobile phases consisting of acetonitrile and a 0.25% aqueous solution of acetic acid. Chromatographic detection was performed at wavelengths of 265, 291, and 550 nm.

The mathematical processing of the obtained results confirmed that the accuracy of the developed analysis methodology is sufficiently high. It was shown that in Jujube fruit, vitamins B6, B3, B2, and C are present in the highest amounts, while vitamin B12 is absent.

Kalit so'zlar: *Vitaminlar, chilonliyda, ekstraktsiya, YUSSX analiz, natija aniqligi.*
Ключевые слова: *витамины, плоды унаби, экстракция, анализ YSSX, точность результата.*
Key words: *vitamins, Jujube fruit, extraction, YSSX analysis, result accuracy.*

INTRODUCTION

Jujube (Ziziphus jujube.Mill., Unabi.rus) fruit is rich in proteins, carbohydrates, fats, amino acids, organic acids, vitamins, tannins, carotenes, trace elements, which are important in the treatment of various diseases. In addition, this fruit has been found to contain biologically active compounds such as sterols, coumarins, flavonoids, triterpenoids, betulin, betulinic acid, triterpene glycosides - A and B jujubosides, isoquinoline alkaloids [1, 2]. Therefore, the fruit of jujube is of great importance not only as a food, but also as a medicinal product [3-6].

Literature review. The amount of biologically active medicinal substances contained in this fruit has been thoroughly studied by scientists. Using solvents such as water, acetone, chloroform and ethylacetate, the dried leaves were isolated from biologically active compounds: mucilaginous substances (8.95%), saponins (0.44%), flavonoids (3.7%) and proanthocyanidins [7, 8]. The fruits contain up to 30% carbohydrates, up to 2.5% organic acids, up to 3.7% fats, up to 3% proteins, up to 10% tannins. Also vitamins (B, C, β-carotene), amino acids, trace elements, sterols, coumarins, flavonoids (kaempferol, myricetin, etc.), triterpenoids - oleanolic, ursolic, maslinic acids, betulin, betulinic acid, triterpene glycosides - A and B uubozides, isoquinoline alkaloids (stepharin, azimiloban), monosaccharides (glucose, arabinose, rhamnose, xylose, etc.) were found [9, 10].

The purpose of the research. The purpose of this research is to develop a methodology for determining the amount of water-soluble vitamins in jujube using a high-performance liquid chromatography method**.**

Experimental part. Determination of vitamins in standard solutions and analyzed samples was carried out on LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu [11]. The chromatograph was equipped with an LC-40D pump, a SIL-40 autosampler, and a SPD-M40 photodiode matrix detector (PDA), and mathematical processing of the results was carried out using LabSolutions ver 6.92 software. Separation of substances in the analyzed samples Shim pack GIST C18 (150 × 4.6 mm; 5 µm, Shimadzu) reversed-phase column [12] and acetonitrile (A) and 0.25% solution of acetic acid in water (B) gradient mobile phase (Table 1) was used. The injection volume was set at 10 µL, the flow rate at 0.6 mL/min, and the column thermostat temperature at 40 °C. The analytical signal (peak area) of each vitamin in the standard

mixture or the analyte mixture was measured at three wavelengths (265, 291 and 550 nm) (Figures 1-2).

Table 1. Mobile phase gradient software.

Time	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Termination	

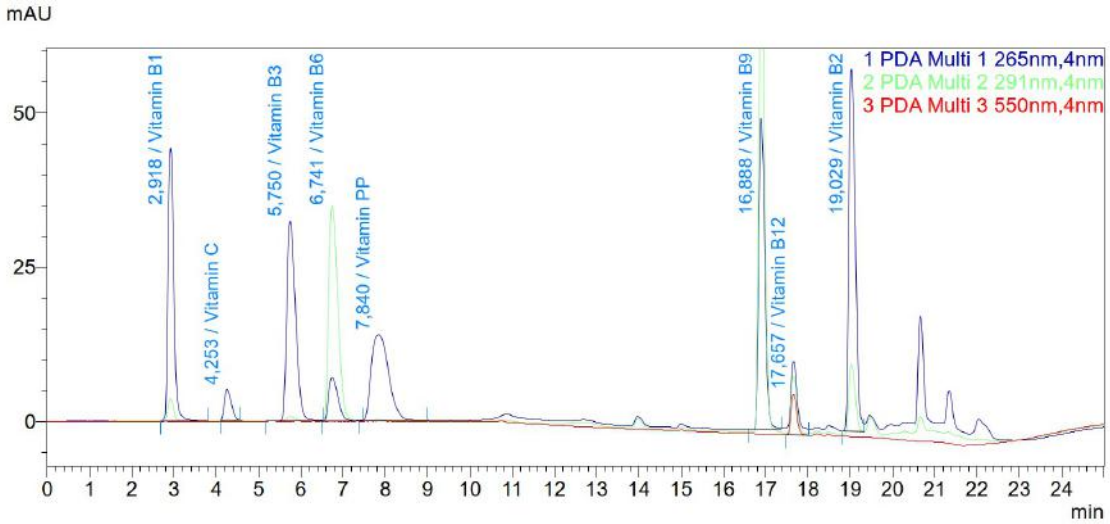

Figure 1. Chromatograms recorded at 265, 291 and 550 nm wavelengths of the standard solution of a mixture of vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, C, PP with a concentration of 12.5 mg/l

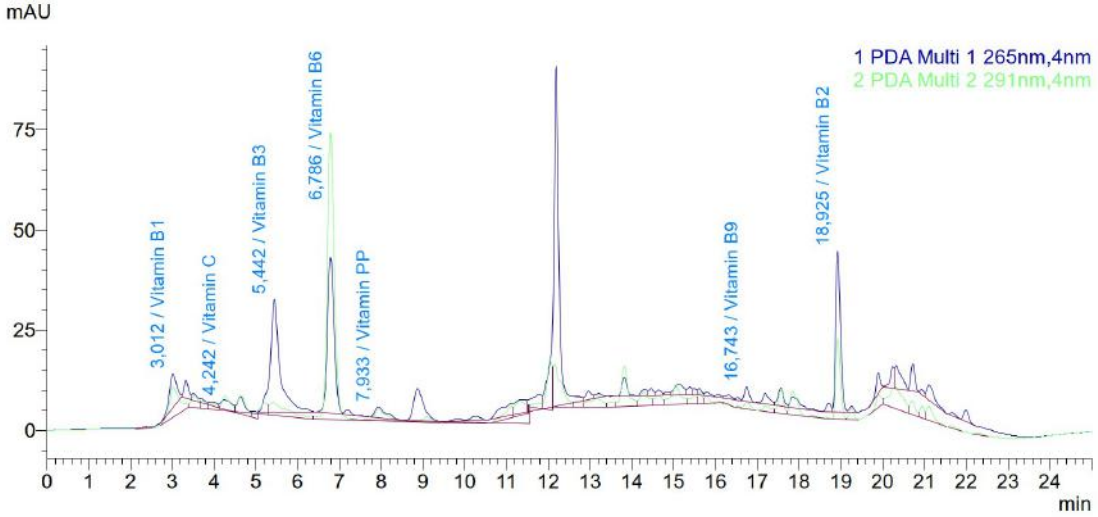

Figure 2. Chromatograms of jujube grown in the Fergana Valley at 265 and 291 nm wavelengths of extract taken at 60 °C for 20 minutes.

Reagents and equipment used. As standart samples; B_1, B_2, B_3, B_6, PP vitamins from BLDPharm [13], vitamin B_{12} from Rydburg Pharmaceuticals, vitamin C from Carl Roth GmbH [14] and DSM Nutritional Products GmbH [15] Vitamin B_9 was used. Chromatographic determinations also used HPLC grade purified distilled water, acetonitrile, and reagents of acetic acid, hydrochloric acid, and sodium hydroxide of a chemically pure grade.

Preparation of standard solutions. Vitamin C (CAS 50-81-7), Vitamin B_1 (CAS 59-43-8), Vitamin B_6 (CAS 58-56-0), Vitamin B_3 (CAS 59-67-6), Vitamin B_{12} (CAS 68– 19–9) and vitamin PP

(CAS 98-92-0), 5 mg of each were weighed on an analytical balance and dissolved in 50 ml of 0.1 N HCl solution. The same amount of vitamins B_2 (CAS 83-88-5) and B_9 (CAS 59-30-3) was dissolved in 50 ml of 0.025% sodium hydroxide solution. Then, 150 µl of each of the prepared solutions was taken to make the initial standard solution of the vitamin mixture. In this solution, the concentration of each vitamin was 12.5 mg/l. By diluting it, solutions with concentrations of 8.333 mg/l, 6.667 mg/l, 4.167 mg/l and 2.5 mg/l were prepared. Purified water was used as a vitamin-free standard (0 mg/l concentration) for the purpose of making a scaled graph.

Extraction of samples and preparation of solutions [16]. For the extraction of water-soluble vitamins, the seed saperated jujube fruit, which was initially analyzed, was ried for 48 hours at a temperature of 35 °C. The released moisture was 16.1-17.2%. 1.00 g of the obtained sample was placed in a 50 ml conical flask and 25 ml of 0.1 M concentrated HCl solution was added. In order to determine the completeness of the extraction of vitamins, the obtained mixture was extracted in a GT SONIC-D3 ultrasonic bath [17] at 20, 40, 60 and 80 oC and for 10, 20, 30 and 40 minutes. Then the mixture was cooled, placed in a measuring flask and made up to 25 ml with water. 1.5 ml of the extract was centrifuged at a speed of 7000 rpm in a "Bibse Mini-7" centrifuge, and the centrifuge was filtered through a 0.22 µm syringe filter and put into a chromatograph vial and analyzed.

Results and discussion. Graded graphs of the peak surface of each vitamin in the chromatogram of the prepared standard solutions and its dependence on concentration were made, mathematically processed, and the accuracy parameter R^2 of the dependence was calculated for each vitamin. As an example, Figure 3 shows the dependence of the surface of the chromatographic peaks measured at 265 nm wavelength of the standard solutions for vitamin B_3 on the concentration of the standard solution, the graph, the formula of the relationship with the correct output, and the experimental points. The R^2 value of the degree of closeness to the gray line is given. The closeness of the R^2=0.9999956 value to 1 indicates that the accuracy of this graded graph is high.

The R^2 values of the surface of the chromatographic peaks recorded at the appropriate wavelength for the standard solutions of other vitamins as a function of the concentration are presented in Table 2.

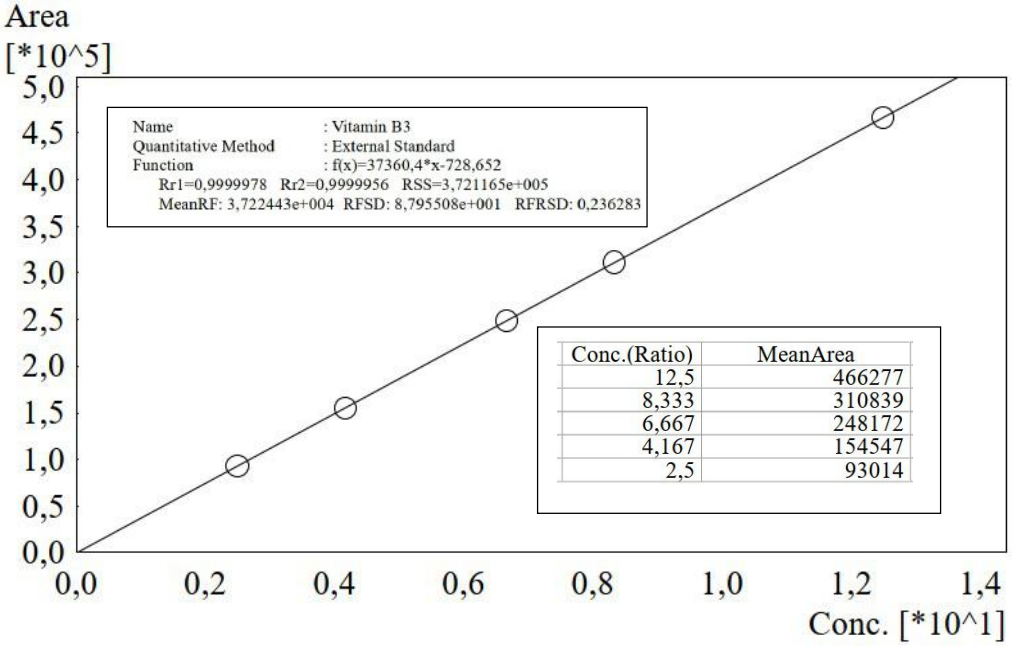

Figure 3. The results of dependence of the surface of the chromatographic peaks measured for vitamin B_3 on the concentration of the standard solution, the graph, the formula of the relationship with the correct output and the R^2 value (265 nm).

Table 2.

The retention times of the chromatographic peaks recorded at the appropriate wavelength for the standard solutions of vitamins and the R^2 values of the graphs of the surface of these peaks as a function of concentration.

#	Vitamin	Absornsion time, min	Wavelength, nm	R^2
	B_1	2,918	265	0.9997380
	B_2	19,029	291	0,9894794
	B_3	5,075	265	0,9999956
	B_6	6,741	265	0,9999922
	B_9	16,888	291	0,9998072
	B_{12}	17,657	265	0,9999780
	C	4,253	265	0,9983604
	PP	7,840	265	0,9999955

The results of Table 2 show that the accuracy of the graded graphs for all analyzed vitamins is high enough.

Table 3 lists the amounts of some vitamins determined by chromatography in the extracts taken at different temperatures and for different times in order to observe the completeness of the extraction process of water-soluble vitamins from the jujube fruit powder separated from the seed, dried and ground to a size of 2.0-3.5 mm[18].

Table 3.

Amounts of certain vitamins in extracts obtained from jujube fruit at different temperatures and times

№	Temperature, °C	Time, min	Amount of vitamins, mg/100g						
			B_1	B_2	B_3	B_6	B_9	C	PP
1.	20	20	7,92	16,68	34,71	54,19	1,11	7,01	1,91
2.	40	20	9,34	15,90	35,91	55,20	1,80	7,39	2,18
3.	60	10	6,91	18,92	34,80	48,34	1,83	9,57	1,27
4.		20	8,66	18,69	35,23	59,57	1,71	18,39	3,22
5.			9,66	19,05	37,13	62,63	1,79	19,93	4,94
6.			8,51	19,82	35,92	59,70	2,26	18,33	4,59
7.			9,62	19,09	37,07	62,94	1,68	19,41	5,01
8.			9,78	20,90	36,08	57,19	2,22	19,17	4,47
9.		30	8,94	21,21	30,06	62,45	2,48	16,31	3,31
10.		40	8,49	21,06	34,80	60,06	2,42	14,66	3,28
11.	80	20	9,81	21,75	38,44	62,43	2,51	14,34	3,22

From the analysis of the results presented in Table 3, when the time of the extraction process does not change, that is, when the temperature rises from 20 °C to 80 °C, it can be observed that the amount of almost all identified vitamins in the extract increases. Increasing the temperature from 60 °C to 80 °C almost did not increase the extraction yield of vitamins. Therefore, the optimal extraction temperature was chosen to be 60 °C. Without changing the extraction temperature (60 °C), increasing its time from 10 to 40 minutes showed that the optimal time was 20 minutes.

In order to evaluate the error of the chromatographic analysis, the extraction of vitamins contained in jujube was carried out 5 times in parallel for 20 minutes at a temperature of 60 °C, and the amount of extracted vitamins was determined (Table 3). Using the method of least squares [19, 20], the standard deviation of the results and the 95% reliable probability interval values of the experiment were estimated. The calculated results are presented in Table 4.

Table 4.

Results of chromatographic determination of water-soluble vitamins in jujube fruit (reliable probability 95%).

№	Vitamin	The average value of the determined amount, mg/100 g	Standard deviation, mg/100 mg	Reliable deviation, mg/100 mg
1.	B_1	9,25	0,61	±0,70
2.	B_2	19,51	0,88	±1,01
3.	B_3	36,29	0,80	±0,92
4.	B_6	60,41	2,39	±2,75
5.	B_9	1,93	0,28	±0,33
6.	C	19,05	0,68	±0,79
7.	PP	4,45	0,72	±0,83
8.	B_{12}	Not found	-	-

CONCLUSION

The obtained results confirm the possibility of chromatographic analysis of water-soluble vitamins in jujube fruit with sufficient accuracy using the methodology developed by us. For different vitamins, despite the fact that their amounts are different, with 95% probability, the reliable deviation of determining their amount per 100 grams did not exceed ±2.75 milligrams. It was shown that jujube fruit contains the most vitamins B_6, B_3, B_2 and C, and vitamin B_{12} is not found.

This developed methodology can be used to determine the amount of water-soluble vitamins in fruits and other parts of all plants.

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