BULLETIN OF NATIONAL ACADEMY OF SCIENCES
OF THE REPUBLIC OF KAZAKHSTAN
ISSN 1991-3494
Volume 5, Number 387 (2020), 48 – 55 **https://doi.org/10.32014/2020.2518-1467.142**

UDC: 615.072: 543.544.5.068.7:615.32

I. Kriukova[1], S. Gubar[1], I. Vladymyrova[1], T. Tishakova[2]

[1]National University of Pharmacy, Kharkiv, Ukraine;
[2]Kharkiv National Medical University, Kharkiv, Ukraine.
E-mail: kriukova92@gmail.com

STUDY OF AMINOACID COMPOSITION OF THE HARICOT BEAN SHELLS

Abstract. Commonly, the production of herbal medicines is more advantageous economically than chemical synthesis. Agricultural crops, having sufficient raw material base, are of main interest.

In this respect the shells of haricot bean (*Phaseolus vulgaris L.* (Fabaceae)), that are waste products of beans, are of special interest. Due to the absence of regulatory documents for this kind of drug raw material in Ukraine the development of standardization parameters for the raw material of haricot bean is a very topical. Pharmacotherapeutic action of this drug raw material is caused by the presence of amino acids, proteins, macro- and microelements as well as flavonoids.

There are medicines at the pharmaceutical market of Ukraine that was produced from the haricot bean: «Sadifit» (ZAO «Liktravy», Ukraine), «Arphazetin» (ZAO Pharmaceutical plant «Viola», Ukraine), «Hepatophyt» (LLC «Research and production pharmaceutical company «AIM», Ukraine), «Shells of haricot bean» (ZAO «Liktravy», Ukraine).

Medicines are introduced to the market in the form of medicinal herbs mixture that's why main method of application is a preparation of restorative drinks, tinctures, teas. In light of this, study of amino acid composition in the water extracts from raw material is reasonable.

Research of qualitative and quantitative amino acid composition of 5 lots of the shells of haricot bean was carried out by HPLC method. This raw material was harvested in different regions of Ukraine over a period of 2014 – 2016. 16 amino acids were identified in the water extracts of the shells of haricot bean. Glutamic acid (1.60 – 5.56 ´µg/100 mg), L-alanine (1.41 – 2.23 µg/100 mg), L-arginine (0.65 – 2.12 µg/100 mg) had the greatest content in the investigated samples. D-serine (0.04 – 0.13µg/100 mg) and D,L-methionine (0.02 – 0.43 µg/100 mg) had the lowest quantity in the investigated samples.

Sufficiently high content of amino acids makes it possible to consider them as potential substances-markers at the development of assay procedure for the standardization of raw material.

Key words: amino acids, shells of the haricot bean, chromatography-mass spectrometry.

Nowadays quality control of herbal raw material used for the manufacture of medicines is a key point of pharmaceutical segment of health service [1,2,3]. It makes necessary to develop universal procedures enabling to control content of biologically-active substances in the raw material as well as in medicines on its base [4,5].

That's why haricot bean is of research interest. This plant has sufficient raw materials base but there are not regulatory documents on the territory of Ukraine [6,7].

Haricot bean (*Phaseolus vulgaris L. (Fabaceae)*) is a crop that has been used in medicine for many years. Preparations, containing haricot bean as active substance, normalize carbohydrate metabolism and reduce sugar level due to the presence of amino acids, proteins macro- and microelements, flavonoids in this plant [8,9,10].

The study of the chemical composition of various types of beans was carried out by scientists of the National University of Pharmacy, Kovalev S.V. and Kovalev V.M. Scientists have determined

quantitative composition of phenolic compounds (3.64 ± 0.09%), flavonoids (2.78 ± 0.12%), hydroxycinnamic acids (2.50 ± 0.13%), polyphenol compounds (3.68 ± 0.12%), ascorbic acid (0.023 ± 0.001%) and organic acids (1.12 ± 0.02%) in the herb of scarlet bean. Besides they studied qualitative and quantitative composition of amino acids and mineral components in the lots of common bean, black beans, sieva bean, scarlet bean, red mung, green mung [11]. 16 amino acids, 7 of which are essential, were identified in accordance with the results of abovementioned research. It was found that glutamic and asparaginic acids, phenilalanine, valine, methionine, alanine, glycine, leucine had the highest level of content in the shells of bean [12,13]. The presence of 19 macro- and microelements was also established in the herb of bean; among which calcium, potassium, magnesium and silicon prevailed. Obtained data was used to get the substance with hypoglycemic activity that is called glyphasine and obtained from the herb and shells of bean [14].

It is known from literature sources that amino acids are structural components of vegetable protein, cellular fluid and take part in the synthesis of enzymes, vitamins and alkaloids. In humans, amino acids play an important role at the synthesis of antibodies, enzymes, hormones, hemoglobin, provide energy for muscular tissue, enhance metabolic processes, reduce level of cholesterol, have powerful antioxidant action, restore tissues of liver and kidney [15,16].

Generally, methods used for amino acid analysis are based on the chromatographic separation of amino acids presented in the test sample (GC-MS and HPLC-MS) [17,18].

Table 1 includes medicines on the base of the shells of haricot bean that exist on the pharmaceutical market of Ukraine [19].

Table 1 – Haricot bean shells-based medicines

Sl.No.	Name of the medicine	Manufacturer	Dosage form	Pharmacological class
1	«Sadifit»	ZAO «Liktravy», Ukraine	Medicinal herbs mixture	Diabetes medication
2	«Arphazetin»	ZAO Pharmaceutical plant «Viola», Ukraine	Medicinal herbs mixture	Diabetes medication
3	«Hepatophyt»	LLC «Research and production pharmaceutical company «AIM», Ukraine	Medicinal herbs mixture	Medication for hepatic disorders and bile ducts disorders
4	«The shells of haricot bean»	ZAO «Liktravy», Ukraine	Shells of bean in the packs with internal package	Mild to moderately severe diabetes mellitus type I (as part of complex therapy)

According to the data given in the table 1, the shells of haricot bean are used to prepare medicinal herbs mixture, tinctures, and teas. Considering the method of application, it is rational to conduct a quality assessment in water extracts [20,21].

The aim of this research is the study of amino acid composition in the water extracts from the shells of haricot bean, growing on the territory of Ukraine, as one of the step of the standardization of raw material.

Materials and methods. The study was carried out on 5 lots of raw material from the shells of haricot bean, harvested in different regions of Ukraine during 2014-2016, using high performance liquid chromatography (HPLC) with mass spectrometric detection. High-performance liquid chromatograph, model Acquity H-class UPLC system (Waters, USA), equipped with triple-quadrupole mass-spectrometer (Xevo, Waters, USA) was used for qualitative and quantitative amino acid analysis.

Preparation of test solutions. Place about 100 mg (precisely weighed amount) of powdered raw material, poured through the sieve with the diameter of holes 355 μm, in the 20 ml volumetric flask, add 15 mL of *water P*. Ultrasonicate at the temperature 45°C for 10 minutes and leave it to cool. Bring the

final solution volume to the mark with the addition of *water P*, mix and filter through the Q-max Syringe membrane filter (13 mm, 45 мкм, Nylon) into the vials.

Preparation of standard solutions. Standard sample (SS) of amino acids was used. This sample contained 17 amino acids dissolved in 0.1M hydrochloric acid solution: L-Alanine, L-Arginine, DL-Aspartic acid, L-Cystine, Glutamic acid, Glycine, L-Tryptophan, Isoleucine, DL-Leucine, L-Lysine, DL-Methionine, DL-Phenylalanine, L-Proline, D-Serine, L-Threonine, L-Tyrosine, Valine. Content of every amino acid was 2.5±0.01 mmol/L (Sigma-Aldrich, lot number BCBQ0950V, expiry date - 27.07.2019).

Contents of 1 vial (2 mL) of SS is shaked, bringed the temperature to 20°C and diluted 2-fold, 5-fold, 10-fold, 20-fold, 50- and 100-fold with water *P*, mixed and filtered through the Q-max Syringe membrane filter (13 mm, 45 μm, Nylon) in to the vial.

Chromatographic procedure. High-performance liquid chromatograph is equipped with triple-quadrupole mass-spectrometer (Xevo, Waters, USA) with electrospray source of ionization (ESI). Separation of amino acids in the samples was carried out using ZIC-HILIC column 150 mm × 2.10 mm, packed with silica gel for chromatography, octadecylsilyl P with size particle 3.5 μm.

Chromatographic conditions:

- Column temperature – 40°C;
- Injection volume – 1 μl;
- Flow Rate – 0.5 mL/min;

Triple quadrupole mass spectrometry detection

- Source Temperature – 150°C;
- Desolvation Temperature –400°C;
- Cone Gas Flow – 20 (L/Hr);
- Desolvation Gas Flow –1000 (L/Hr);
- Capillary – 3.50 (kV).

Gradient elution is performed using mobile phase A– 0.050 M ammonium acetate buffer solution with a pH 4.5± 0.05 which was adjusted using diluted acetic acid; mobile phase B – acetonitrile.

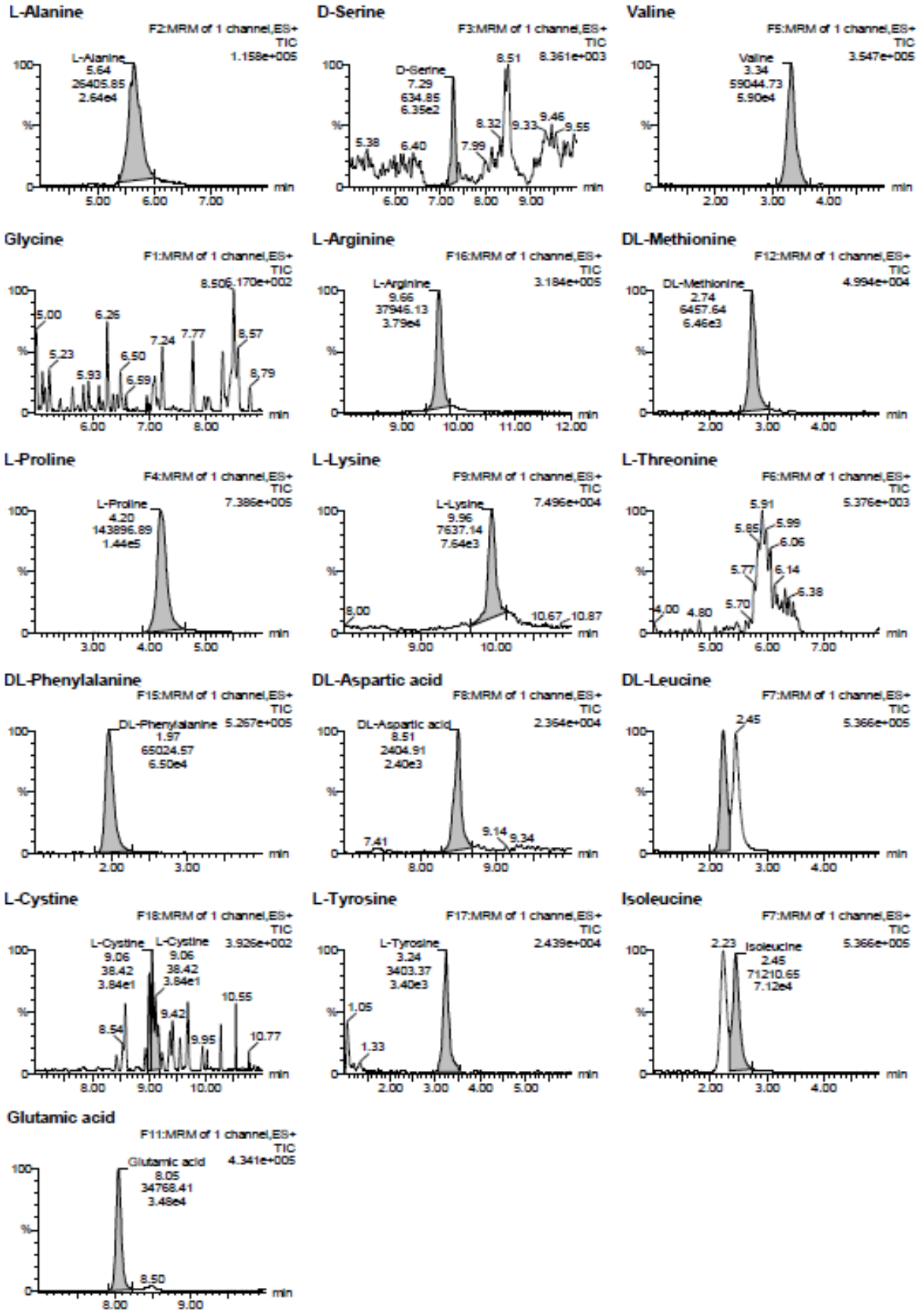
Chromatography is performed at the following gradient program (table 2).

Table 2 – Gradient program for chromatography

Time(min)	Mobile phase A (%, v/v)	Mobile phase B (%, v/v)
0-5	20%	80%
5-6	20→35%	80→65%
6-10	35→50%	65→50%
10-11	50%	50%
11-11,2	50→20	50→80%

Results. Identification of every substance was done by mass-spectra given on the following chromatograms (figure 1).

Quantitative composition of amino acids was calculated using calibration curves (method of calibrated function). Results are given in the table 3.



Example of chromatogramms of amino acid composition of the shells of haricot bean obtained by mass-spectrometry

Table 3 – Amino acid composition of water extracts of the shells of haricot bean (μg/100 mg)

Sl.No.	Name of amino acid	Content of amino acids, μg/100 mg				
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	L-Alanine	1.57	2.23	2.05	2.11	1.41
2	D-Serine	0.12	0.13	0.04	0.13	0.06
3	Valine*	0.34	1.24	1.00	1.91	1.57
4	Glycine	–	–	–	–	–
5	L-Arginine	2.12	2.09	–	0.65	1.28
6	DL-Methionine*	0.02	0.11	–	0.05	0.43
7	L-Proline	0.33	1.22	1.46	1.46	0.32
8	L-Lysine*	0.54	0.42	0.19	0.60	0.74
9	L-Threonine	–	–	–	–	–
10	DL-Phenylalanine*	0.14	0.57	0.42	0.92	0.94
11	DL-Aspartic acid	1.63	2.08	0.96	1.79	2.79
12	DL-Leucine*	0.28	0.77	0.55	1.04	1.24
13	L-Cystine	0.06	0.01	0.00	0.00	0.00
14	L-Tyrosine	0.14	0.23	0.27	0.38	0.60
15	Isoleucine*	0.21	0.76	0.32	1.10	1.22
16	L-Tryptophan	–	–	–	–	–
17	Glutamic acid	3.04	5.56	2.76	4.22	1.60
Sum of essential amino acids		1.53	3.88	2.48	5.63	6.16
Sum of nonessential amino acids		9.01	13.57	7.54	10.75	8.07
Total sum of amino acids		10.54	17.45	10.02	16.38	14.23
Note: «*» – essential amino acids.						

Discussion. As a result of research 16 amino acids were identified, among which there are 6 nonessential amino acids: valine, DL-methionine, L-lysine, DL-phenylalanine, DL-leucine, isoleucine. Valine has a dominant content which varies from 0.34 to 1.91 μg/100 mg. Glutamic acid (1.60 - 5.56 μg/100 mg), L-alanine (1.41 - 2.23 μg/100 mg), L-arginine (0.65 - 2.12 μg/100 mg) prevailed over all samples of raw material according to the quantitative composition. Minor amino acids were D-serine (0.04 - 0.13 μg/100 mg), DL-methionine (0.02 – 0.43 μg/100 mg).

Biological activity of identified amino acids was thoroughly studied. In accordance with data of scientific literature L-alanine is an amino acid that takes part in energetic metabolism, stimulates immunity, and regulates level of sugar in the blood. Arginine takes part in the regulation of synthesis and secretion of hormones such as: insulin, prolactin, thyroid hormone, parathyroid hormone, adrenal hormones, and hormones of fertility cycle.

Sufficiently high content of discussed amino acids affords an opportunity to consider their as potential substances-markers at the development of assay procedure for standardization of raw materials.

Conclusion. The present study has been undertaken to determine the qualitative and qualitative analysis of the shells of haricot bean by the HPLC method. The three amino acids, glutamic acid, L-alanine and L-arginine, were found at the highest content: 1.60 – 5.56 μg/100 mg, 1.41 – 2.23 μg/100 mg, 0.65 – 2.12 μg/100 mg, respectively. The obtained results indicate the possibility of usage of the shells of haricot bean as an alternative raw material for preparation of easily digested forms of amino acids that regulate metabolic processes in the human body.

Due to sufficiently high content of found amino acids can be considered as substances-markers at the development of assay method for standardization of raw materials.

А. И. Крюкова, С. Н. Губарь, И. Н. Владимирова, Т. С. Тишакова

[1]Ұлттық фармацевтика университеті, Харьков, Украина;
[2]Харьков ұлттық медицина университеті, Харьков, Украина

КӘДІМГІ ҮРМЕБҰРШАҚ ЖЕМІСІ ЖАРМАСЫНЫҢ АМИНҚЫШҚЫЛДЫ ҚҰРАМЫН ЗЕРТТЕУ

Аннотация. Көптеген жағдайларда өсімдік шикізаттан дәрілік құралдарды алу химиялық синтезбен салыстырғанда экономикалық тұрғыдан тиімдірек болады. Аса көп сұранысқа жеткілікті шикізаттық қоры бар ауылшаруашылық дақылдар ие болады.

Бұл аспектіде кәдімгі үрмебұршақ – Phaseolus vulgaris L. (Fabaceae) өндірісіндегі қалдық болып саналатын бұршақ жемісі жармасы назар аударады. Украинада осы ДӨШ түріне нормативтік құжаттаманың жоқ болуы кәдімгі үрмебұршақ шикізатына стандарттау параметрлерін әзірлеу өзектілігін айқындайды. Осы дәрілік шикізаттың фармакотерапиялық әсері құрамында амин қышқылдары, ақуыздар, макро және микроэлементтердің, сондай-ақ флаваноидтердің болуы арқылы түсіндіріледі.

Украинаның фармацевтика нарығында құрамында кәдімгі үрмебұршақ шикізаты бар дәрілік құралдар ұсынылған: «Садифит» («Лектравы» ЖАҚ, Украина), «Арфазетин» («Виола» фармацевтика фабрикасы» ЖАҚ, Украина), «Гепатофит» («ЭЙМ» ғылыми-өндірістік фармацевтика компаниясы» ЖШҚ, Украина), «Үрмебұршақ жемісі жармасы» («Лектравы» ЖАҚ, Украина).

Препараттар жинақ түрінде ұсынылған, сондықтан да негізгі қолдану әдісі – қайнатпа, тұнба, шай дайындау. Осыған байланысты шикізаттан нақты сулы бөлінділерінің аминқышқылды құрамын зерттеу ұтымды болып саналады. Масс-спектрометриялық детекторлау арқылы жүргізілетін ТЖСХ (ВЭЖХ) әдісі арқылы Украинаның түрлі аймағында 2014-2016 жж. аралығында дайындалған кәдімгі үрмебұршақ жемісі жармасындағы амин қышқылдарының 5 сериясын сапалық және сандық анықтау бойынша зерттеулер жүргізілді. Кәдімгі үрмебұршақ жемісі жармасының су сығындыларында 16 амин қышқылдары сәйкестендірілді. Зерттелетін үлгілерде құрамы жағынан басым болып глутамин қышқылы (1,60 бастап 5,56 мкг/100 мг дейін), L-аланин (1,41 бастап 2,23 мкг/100 мг дейін), L-аргинин (0,65 бастап 2,12 мкг/100 мг дейін) анықталды. Минорлық амин қышқылы ретінде D-серин (мөлшері 0,04 бастап 0,13 мкг/100 мг дейін) және DL-метеонин (мөлшері 0,02 бастап 0,43 мкг/100 мг дейін) есептеледі.

Аталған амин қышқылдарының жеткілікті жоғары мөлшері оларды шикізатты стандарттау үшін сандық анықтау әдістемесін әзірлеу кезінде ықтималды маркер-заттар ретінде қарастыру мүмкіндігін береді.

Түйін сөздер: амин қышқылдары, кәдімгі үрмебұршақ жемісі жармасы, хромато-масс-спектрометрия.

А. И. Крюкова, С. Н. Губарь, И. Н. Владимирова, Т. С. Тишакова

[1]National University of Pharmacy, Kharkiv, Ukraine;
[2]Kharkiv National Medical University, Kharkiv, Ukraine

ИССЛЕДОВАНИЕ АМИНОКИСЛОТНОГО СОСТАВА СТВОРОК ПЛОДОВ ФАСОЛИ ОБЫКНОВЕННОЙ

Аннотация. Получение лекарственных средств из растительного сырья в большинстве случаев экономически выгоднее химического синтеза. Наибольший интерес вызывают сельскохозяйственные культуры, обладающие достаточной сырьевой базой.

В данном аспекте внимание привлекают створки плодов фасоли обыкновенной – Phaseolus vulgaris L. (Fabaceae), которые являются отходом производства бобов фасоли. Отсутствие нормативной документации на данный вид ЛРС в Украине определяет актуальность разработки параметров стандартизации на сырье фасоли обыкновенной. Фармакотерапевтическое действие данного лекарственного растительного сырья обусловлено наличием аминокислот, белков, макро- и микроэлементов, а также флавоноидов.

На фармацевтическом рынке Украины представлены лекарственные средства, содержащие сырье фасоли обыкновенной: «Садифит» (ЗАО «Лектравы», Украина), «Арфазетин» (ЗАО Фармацевтическая фабрика «Виола», Украина), «Гепатофит» (ООО «Научно-производственная фармацевтическая компания «ЭЙМ»», Украина), «Фасоли створки плодов» (ЗАО «Лектравы», Украина).

Препараты представлены в виде сборов, поэтому основной способ применения – это приготовление отваров, настоек, чаев. Исходя из этого, рациональным является изучения аминокислотного состава именно водных извлечений сырья.

Методом ВЭЖХ с масс-спектрометрическим детектированием проведены исследования качественного и количественного определения аминокислот 5 серий створок плодов фасоли обыкновенной, заготовленной в разных регионах Украины на протяжении 2014-2016 гг. В водных экстрактах створок плодов фасоли обыкновенной идентифицировано 16 аминокислот. В исследуемых образцах доминирующими по содержанию установлены глутаминовая кислота (от 1,60 до 5,56 мкг/100 мг), L-аланин (от 1,41 до 2,23 мкг/100мг), L-аргинин (от 0,65 до 2,12 мкг/100 мг). Минорными аминокислотами являются D-серин (содержание от 0,04 до 0,13 мкг/100 мг) и DL-метеонин (содержание от 0,02 до 0,43 мкг/100 мг).

Достаточно высокое содержание данных аминокислот дает возможность рассматривать их как потенциальные вещества-маркеры при разработке методики количественного определения для стандартизации сырья.

Ключевые слова: аминокислоты, фасоли обыкновенной створки плодов, хромато-масс-спектрометрия.

Information about authors:

Kriukova Anna I., PhD, Assistant Professor of the Drug Technology Department NUPh, Kharkiv, Ukraine; kriukova92@gmail.com; https://orcid.org/0000-0002-9866-0976

Gubar Svitlana M., PhD, Associate Professor of the Department of pharmaceutical chemistry, head of state scientific-research laboratory of NUPh for medicinal substances quality control, Kharkiv, Ukraine; gubarsn@ukr.net; https://orcid.org/0000-0002-5434-9502

Vladymyrova Inna V., PhD, Associate Professor of the Department of Pharmacognosy, Head of scientific methodological (scientific research) laboratory on pharmaceutical education of NUPh, Kharkiv, Ukraine; inna.vladimirova2015@gmail.com; https://orcid.org/0000-0002-6584-4840

Tishakova Tetyana S., PhD, Associate professor, Medical and bioorganic chemistry department, Kharkiv National Medical University, Kharkiv, Ukraine; https://orcid.org/0000-0002-0257-7757

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