

УДК 615.322: 615.076.8

**STABILITY TESTING OF THE MEDICINAL PLANT
“CRATAEGUS ALMAATENSIS POJARK FRUITS”****ИЗУЧЕНИЕ СТАБИЛЬНОСТИ ЛЕКАРСТВЕННОГО РАСТИТЕЛЬНОГО СЫРЬЯ
ПЛОДОВ БОЯРЫШНИКА АЛМАТИНСКОГО
(CRATAEGUS ALMAATENSIS POJARK)****E.N. Bekbolatova¹, Z.B. Sakipova¹, A.T. Kabdenova²
Э.Н. Бекболатова¹, З.Б. Сакипова¹, А.Т. Кабденова²**¹) Asfendiyarov Kazakh National Medical University,
Republic of Kazakhstan, 050000, Almaty, Tole bi St., 88²) RSE “National Center for expertise of medicines, medical devices and equipment” MoH RK,
Republic of Kazakhstan, 050004, Almaty, Ablai khan Ave., 63¹) Казахский национальный медицинский университет имени С.Д. Асфендиярова,
Республика Казахстан, 050000, Алматы, ул. Толе би, 88²) РГП на ПХВ “Национальный центр экспертизы лекарственных средств, изделий медицинского назначения
и медицинской техники» МЗ РК,
Республика Казахстан, 050004, Алматы, пр. Абылай хана, 63

E-mail: elmirajumagulova@gmail.com

Resume. The results of stability testing in the frame of long-term programme of the medicinal plant “*Crataegus almaatensis* Pojark (*C. almaatensis*) fruits” at temperature 25°C±2°C and humidity 60%±5% storage conditions are provided in this article. The study was carried out in accordance to stability testing specifications and ICH Stability guidelines Q1A-Q1F. The three experimental-industrial batches of fruits were undergone stability testing in the packages intended for storage and sale. The compliance of obtained results to the numerical criteria of stability specification and absence of significant changes during 12 months of testing, gives us an opportunity to conclude that herbal substance is stable. At the present time stability testing is being continued.

Аннотация. В настоящей работе представлены результаты исследования стабильности лекарственного растительного сырья “плоды боярышника алматинского” (*C. almaatensis*) в режиме долгосрочных испытаний при температуре 25°C±2°C и относительной влажности 60%±5% в соответствии со спецификацией стабильности. Испытания стабильности проводились на опытно-промышленных сериях в упаковках, предназначенных для хранения и реализации. Соответствие полученных результатов числовым критериям спецификации стабильности и отсутствие значительных отклонений в период 12 месяцев позволяют установить стабильность сырья. В настоящее время испытания стабильности продолжаются.

Keywords: stability, long-term testing, storage conditions, herbal substance, hawthorn, *Crataegus almaatensis*.

Ключевые слова: стабильность, долгосрочные испытания, условия хранения, растительное сырье, боярышник, боярышник алматинский.

Introduction

Nowadays medicines derived from herbal substances are becoming popular in the medicinal practice [Журкин и др., 2014; Митрофанова и др., 2012]. Medicines of herbal origin has several advantages in comparison to chemical analogs, such as complex impact on the patient organism, low toxicity, possibility of prolonged application, low degree of inducing allergic reactions, which is particularly important in the cases of need of long-term treatment [Морозова и др., 2015; Jennifer and et., 2012].

The scientific work on the full-scale study of the endemic medicinal plant *C. almaatensis* is being carried out at the Asfendiyarov Kazakh National Medical University together with the LLP “FitOleum” and School of Pharmacy and pharmaceutical sciences, Trinity College Dublin, Ireland. The aim of the research work is the development of the new phytomedicines from *C. almaatensis* herbal substance with a goal to substitute imported medicines and expand the share of local market.



One of the important indicators of the product's quality is its stability during the storage process. Stability testing of the medicinal plant *C. almaatensis* is an integral part of the pharmaceutical development of new medicines on its basis.

Results of the stability studies can be used to determine following parameters: storage conditions, storage period, acceptable packaging material for the dosage form [ICH stability guideline, 2003].

Aim

The aim of the scientific work is the stability testing of the medicinal plant *C. almaatensis* fruits and confirmation the absence of significant quality changes under the influence of a variety of environmental factors during the storage process.

Materials and methods

Subject of the study – three batches of the *C. almaatensis* fruits, processed and prepared according to the WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants [Bekbolatova et., 2016].

The tree experimental industrial batches were manufacture and analytical normative document (AND RK) was developed (table 1). This batches were undergone long-term stability testing under temperature $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$ and relative humidity $60\%\pm 5\%$. The frequency of the control testing of samples is every three months for the first year. For the second year testing of the samples will be carried out every six months and annually afterwards. The compendial methods and verified techniques are used during stability studies [Государственная Фармакопея Республики Казахстан I., 2008].

The packaging material used during stability represents those which are intended for storage and sale. The materials are three-layered bags from extra-strong (kraft) paper complying with GOST 2228-81.

Table.1
Таблица 1

General properties of the experimental-industrial scale batches of the *C. almaatensis* fruits
Общая характеристика опытно-промышленных серий плодов *C. almaatensis*

Batch	Manufacturing date	Batch size, kg
20.09.2015	20.12.2015	10.2
21.09.2015	20.12.2015	10.5
22.09.2015	20.12.2015	10.5

The stability specification includes quality parameters which are susceptible to changes during storage of the herbal substance (table. 2).

Table. 2
Таблица 2

Stability specification of the herbal substance *C. almaatensis* fruits
Спецификация стабильности растительного сырья плоды *C. almaatensis*

Quality parameters	Acceptance criteria	Methods
Description	Dried fruits harvested from wild or cultivated shrubs or small trees of <i>Crataegus almaatensis</i> Pojark, Rosaceae family. The drug is collected when ripe.	State Pharmacopoeia RK I, v. 1
Identification	A. Fruits from obovate to globular shape, 11–14 mm long and 5–8 mm wide, purple-dark. The surface is pitted and reticulated. Pulp is reddish juicy and contains 3 to 5 triangular, shiny, smooth seeds. Odorless. With sweetish taste. B. <i>Epidermis</i> : on the surface it shows quadrangular – hexagonal cells with uniformly thickened yellowish-brown walls. <i>Pulp</i> : globular to oval shape cells, containing yellowish-brown coloring matters, small cluster crystals of calcium oxalate. Collateral fascicles through the inner layer of the pulp	State Pharmacopoeia RK I, v. 1



Окончание табл. 2

Qualitative reaction - anthocyanidin and leucoanthocyanidin	Solution A is placed into two tubes. Add 2 ml of hydrochloric acid (conc) R into the first tube, add 2 ml of water R into the second tube. Test tubes are heated in a water bath for 5 minutes; solution in a first tube turns into red color when compared with the second tube.	In compliance with AND RK
- flavonoids	Add 10 ml of solution A into evaporating flask and evaporate to dryness on a water bath. Dissolve residue with 4 ml 70% ethanol R, add 0.05 g magnesium R, 0.5 ml of hydrochloric acid (conc) R, mix well and heat in a water bath at 45-50°C temperature for 30 minutes. Cool, transfer to 5 ml flask; solution gradually changes into brown-reddish color.	In compliance with AND RK
- sugars	Add to 5 ml of solution A 0.3 ml of sodium hydroxide R, 3 ml of copper-tartrate solution R. Heat to boiling. Red precipitate is formed.	In compliance with AND RK
Foreign matters: - deteriorated fruits	Not more than 5 %	State Pharmacopoeia RK I, v. 1, 2.8.2
- other foreign matter	Not more than 2 %	State Pharmacopoeia RK I, v. 1, 2.8.2
- presence of other <i>Crataegus</i> species, characterized by the presence of different quantity of seeds.	Not permitted	State Pharmacopoeia RK I, v. 1, 2.8.2
Loss on drying	Not more than 12.0%	State Pharmacopoeia RK I, v. 1, 2.2.32
Microbiological test	Drug has to comply with State Pharmacopoeia RK I, v. 1, 5.1.4, category A requirements. The total bacterial count does not exceed 10 ⁷ cfu per g, the total combined molds and yeasts count does not exceed 10 ⁵ cfu per g, <i>Escherichia coli</i> does not exceed 100 cfu per g.	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13
Assay - procyanidins, expressed as cyanidin chloride (C ₁₅ H ₁₁ ClO ₆ ; Mr 322.7) (dried drug)	Not less than 1.0 %.	Spectrophotometry, State Pharmacopoeia RK I, v. 1, 2.2.25

Results and discussions

The results of the physico-chemical and microbiological analysis during stability testing of three batches of *C. almaatensis* fruits are provided in tables 3, 4, 5, 6, 7, 8. Dependence of “Assay” parameter on time of storage is illustrated as figures 1, 2, 3.

Table. 3
Таблица 3

Results of stability testing of *C. almaatensis* fruits (Batch 20.09.2015) Результаты испытаний стабильности плодов *C. almaatensis* (Серия 20.09.2015)

Quality parameters	Acceptance criteria	Methods	Testing period (months)				
			0	3	6	9	12
Description	In compliance with stability specification	State Pharmacopoeia RK I, v. 1	+	+	+	+	+
Identification	A. In compliance with stability specification B. In compliance with stability specification	State Pharmacopoeia RK I, v. 1	+	+	+	+	+



Окончание табл. 3

Qualitative reaction - anthocyanidin and leucoanthocyanidin - flavonoids - sugars	In compliance with stability specification	In compliance with AND RK In compliance with AND RK In compliance with AND RK	+	+	+	+	+
Foreign matters: - deteriorated fruits - other foreign matter - presence of other <i>Crataegus</i> species, characterized by the presence of different quantity of seeds.	Not more than 5 % Not more than 2 % Not permitted	State Pharmacopoeia RK I, v. 1, 2.8.2 State Pharmacopoeia RK I, v. 1, 2.8.2 State Pharmacopoeia RK I, v. 1, 2.8.2	1.36 0.28 +	1.42 0.33 +	1.45 0.33 +	1.42 0.30 +	1.44 0.35 +
Loss on drying	Not more than 12.0%	State Pharmacopoeia RK I, v. 1, 2.2.32	8.3%	8.5%	7.8%	8.1%	8.0%
Assay - procyanidins, expressed as cyanidin chloride (C ₁₅ H ₁₁ ClO ₆ ; Mr 322.7) (dried drug)	Not less than 1.0 %.	Spectrophotometry, State Pharmacopoeia RK I, v. 1, 2.2.25	1.30	1.29	1.28	1.26	1.24

Note: "+" - complies

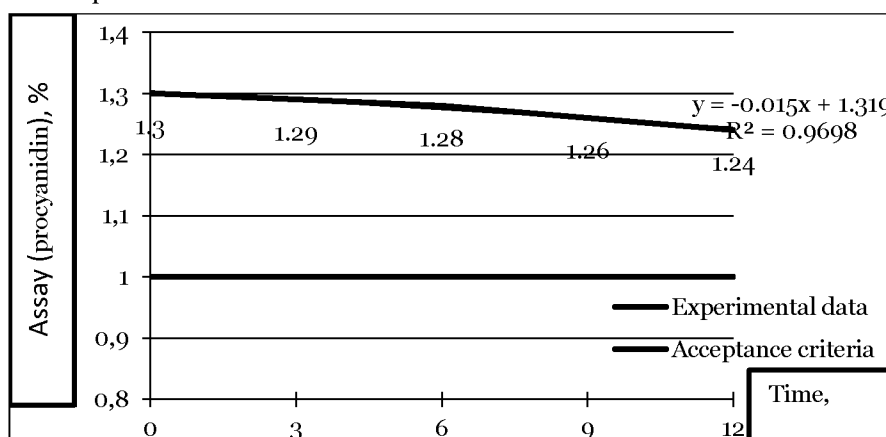


Fig. 1. Dependence of "Assay" parameter on storage time (Batch 20.09.2015)

Рис. 1. Зависимость показателя «Количественное определение» от времени испытания (Серия 20.09.2015)

Table. 4
Таблица 4

Results of microbiological testing of *C. almaatensis* fruits during storage (Batch 20.09.2015)
Результаты испытаний микробиологической чистоты сырья – плоды боярышника алматинского в процессе хранения (Серия 20.09.2015)

Quality parameters	Acceptance criteria	Methods	Experimental data
The total bacterial count, cfu per g	does not exceed 10 ⁷	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	1×10 ³
<i>Escherichia coli</i> , cfu per g	does not exceed 100	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	<1×10 ²
total combined molds and yeasts, cfu per g	does not exceed 10 ⁵	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	1×10 ³

Note. "Microbiological test" analysis was conducted in the beginning of stability studies and is planned to repeat at 24 months time of stability testing.

Table. 5
Таблица 5

Results of stability testing of *C. almaatensis* fruits (Batch 21.09.2015)
Результаты испытаний стабильности плодов *C. almaatensis* (Серия 21.09.2015)

Quality parameters	Acceptance criteria	Methods	Testing period (months)				
			0	3	6	9	12
Description	In compliance with stability specification	State Pharmacopoeia RK I, v. 1	+	+	+	+	+
Identification	C. In compliance with stability specification D. In compliance with stability specification	State Pharmacopoeia RK I, v. 1	+	+	+	+	+
Qualitative reaction - anthocyanidin and leucoanthocyanidin - flavonoids - sugars	In compliance with stability specification	In compliance with AND RK In compliance with AND RK In compliance with AND RK	+	+	+	+	+
Foreign matters: - deteriorated fruits - other foreign matter - presence of other <i>Crataegus</i> species, characterized by the presence of different quantity of seeds.	Not more than 5% Not more than 2% Not permitted	State Pharmacopoeia RK I, v. 1, 2.8.2 State Pharmacopoeia RK I, v. 1, 2.8.2 State Pharmacopoeia RK I, v. 1, 2.8.2	1.32 0.30 +	1.36 0.33 +	1.36 0.26 +	1.42 0.26 +	1.43 0.35 +
Loss on drying	Not more than 12.0%	State Pharmacopoeia RK I, v. 1, 2.2.32	8.1%	8.5%	7.9%	7.5%	7.5%
Assay - procyanidins, expressed as cyanidin chloride (C ₁₅ H ₁₁ ClO ₆ ; Mr 322.7) (dried drug)	Not less than 1.0%	Spectrophotometry, State Pharmacopoeia RK I, v. 1, 2.2.25	1.38	1.35	1.30	1.31	1.30

Note: "+" - complies

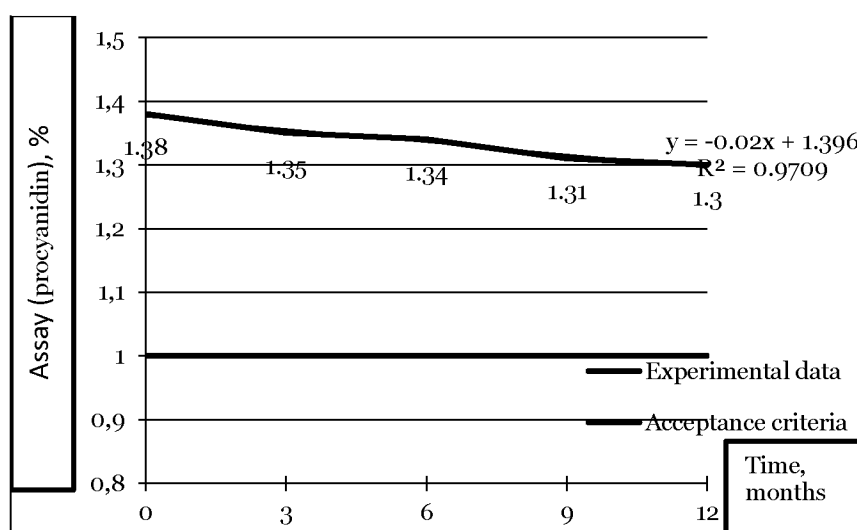


Fig. 2. Dependence of "Assay" parameter on storage time (Batch 21.09.2015)

Рис. 2. Зависимость показателя «Количественное определение» от времени испытания (Серия 21.09.2015)

Table. 6
Таблица 6

Results of microbiological testing of *C. almatensis* fruits during storage (Batch 21.09.2015)
Результаты испытаний микробиологической чистоты сырья – плоды боярышника алматинского в процессе хранения (Серия 21.09.2015)

Quality parameters	Acceptance criteria	Methods	Experimental data
The total bacterial count, cfu per g	does not exceed 10^7	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	1×10^3
<i>Escherichia coli</i> , cfu per g	does not exceed 100	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	$<1 \times 10^2$
total combined molds and yeasts, cfu per g	does not exceed 10^5	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	1×10^3

Note. "Microbiological test" analysis was conducted in the beginning of stability studies and is planned to repeat at 24 months time of stability testing.

Table. 7
Таблица 7

Results of stability testing of *C. almatensis* fruits (Batch 22.09.2015)
Результаты испытаний стабильности плодов *C. almatensis* (Серия 22.09.2015)

Quality parameters	Acceptance criteria	Methods	Testing period (months)				
			0	3	6	9	12
Description	In compliance with stability specification	State Pharmacopoeia RK I, v. 1	+	+	+	+	+
Identification	E. In compliance with stability specification F. In compliance with stability specification	State Pharmacopoeia RK I, v. 1	+	+	+	+	+
Qualitative reaction - anthocyanidin and leucoanthocyanidin - flavonoids - sugars	In compliance with stability specification	In compliance with AND RK In compliance with AND RK In compliance with AND RK	+	+	+	+	+
Foreign matters: - deteriorated fruits - other foreign matter - presence of other <i>Crataegus</i> species, characterized by the presence of different quantity of seeds.	Not more than 5% Not more than 2% Not permitted	State Pharmacopoeia RK I, v. 1, 2.8.2 State Pharmacopoeia RK I, v. 1, 2.8.2 State Pharmacopoeia RK I, v. 1, 2.8.2	1.28 0.32 +	1.33 0.32 +	1.36 0.30 +	1.26 0.23 +	1.35 0.30 +
Loss on drying	Not more than 12.0%	State Pharmacopoeia RK I, v. 1, 2.2.32	7.9%	8.3%	7.7%	7.7%	7.8%
Assay procyanidins, expressed as cyanidin chloride (C ₁₅ H ₁₁ ClO ₆ ; Mr 322.7) (dried drug)	Not less than 1.0 %	Spectrophotometry, State Pharmacopoeia RK I, v. 1, 2.2.25	1.43	1.40	1.38	1.38	1.35

Note: "+" - complies

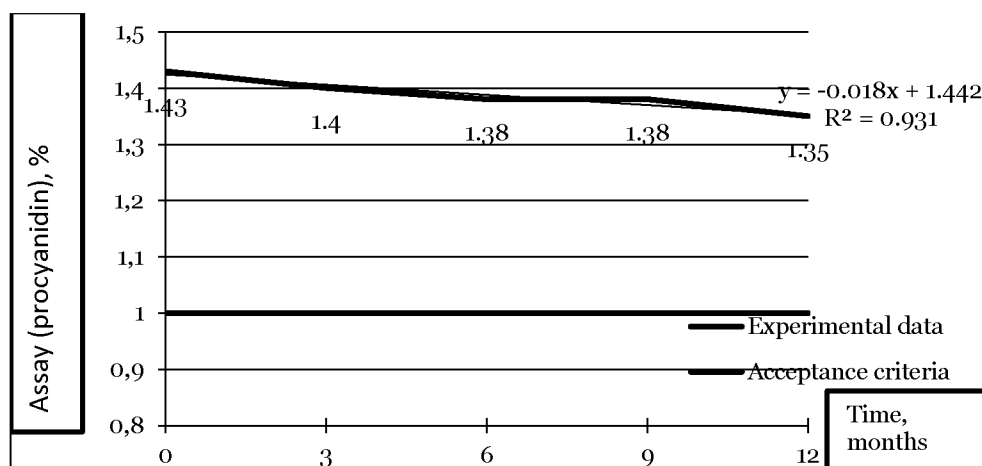


Fig. 3. Dependence of “Assay” parameter on storage time (Batch 22.09.2015)

Рис. 3. Зависимость показателя «Количественное определение» от времени испытания (Серия 22.09.2015)

Table. 8
Таблица 8

Results of microbiological testing of *C. almaatensis* fruits during storage (Batch 22.09.2015)

**Результаты испытаний микробиологической чистоты сырья –
плоды боярышника алматинского в процессе хранения (Серия 22.09.2015)**

Quality parameters	Acceptance criteria	Methods	Experimental data
The total bacterial count, cfu per g	does not exceed 10 ⁷	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	1×10 ³
<i>Escherichia coli</i> , cfu per g	does not exceed 100	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	<1×10 ²
total combined molds and yeasts, cfu per g	does not exceed 10 ⁵	State Pharmacopoeia RK I, v. 1, 2.6.12 State Pharmacopoeia RK I, v. 2, 2.6.13	1×10 ³

The results of long-term stability testing provided in tables 3, 4, 5, 6, 7, 8 and a curve for “Assay” as a function of testing time (figures 1, 2, 3) have revealed stability of controlled parameters over the 12 months time. The microbiological test is within specified limits according to State Pharmacopoeia RK.

During the process of storage the appearance of the drug substance corresponds to the requirements of stability specification, no yields and fungi have developed, no other changes were observed. The content of active substance is within accepted limits.

Conclusion

Thus, as a result of experimental work the stability of herbal substance *C. almaatensis* fruits was studied. During the testing no significant changes of controlled indicators were revealed. At this stage due to obtained results we can conclude that drug substance *C. almaatensis* fruits can be stored for the period of 12 months. The long term stability studies are still undergoing. It is recommended to store drug substance at temperature below 25°C in a dry place.

References

Список литературы

1. Государственная Фармакопея Республики Казахстан I. 2008. Астана. 1: 569.
Gosudarstvennaja Farmakopeja Respubliki Kazahstan I [State Pharmacopoeia of the Republic of Kazakhstan I]. 2008. Astana. 1: 569. (in Russian)
2. Государственная Фармакопея Республики Казахстан I. 2008. Астана. 1: 563
Gosudarstvennaja Farmakopeja Respubliki Kazahstan I [State Pharmacopoeia of the Republic of Kazakhstan I]. 2008. Astana. 1: 563. (in Russian)
3. Государственная Фармакопея Республики Казахстан I. 2008. Астана. 1: 226.
Gosudarstvennaja Farmakopeja Respubliki Kazahstan I [State Pharmacopoeia of the Republic of Kazakhstan I]. 2008. Astana. 1: 226. (in Russian)



4. Государственная Фармакопея Республики Казахстан I. 2008. Астана. 1: 91.
Gosudarstvennaja Farmakopeja Respubliki Kazahstan I. 2008 [State Pharmacopoeia of the Republic of Kazakhstan I]. Astana. 1: 91. (in Russian)
5. Государственная Фармакопея Республики Казахстан I. 2008. Астана. 1: 176–181.
Gosudarstvennaja Farmakopeja Respubliki Kazahstan I [State Pharmacopoeia of the Republic of Kazakhstan I]. 2008. Astana. 1: 176–181. (in Russian)
6. Государственная Фармакопея Республики Казахстан I. 2008. Астана. 1: 66.
Gosudarstvennaja Farmakopeja Respubliki Kazahstan I [State Pharmacopoeia of the Republic of Kazakhstan I]. 2008. Astana. 1: 66. (in Russian)
7. Куркин В.А., Петрухина И.К. 2014. Актуальные аспекты создания импортозамещающих лекарственных растительных препаратов. *Фундаментальные исследования*, 11: 366–372.
Kurkin V.A., Petruhina I.K. 2014. Aktual'nie aspekti sozdaniya impotozameshauishih lekarstvennix rastitel'nih preparatov. [Relevant aspects of developing importsubstituting herbal preparations]-Fundamental'nyie issledovanya, 11; 366–372. (in Russian)
8. Митрофанова И.Ю., Яницкая А.В., Бутенко Д.В. 2012. Многокритериальная оптимизация поиска растительных объектов и создание новых лекарственных средств на основе современных информационных технологий. *Научные ведомости Белгородского государственного университета. Серия: Медицина. Фармация*, 16 (135): 132–135.
Mitrofanova I.U., Yanitskaya A.V., Butenko D.V. 2012. Mnogokriteriy'l'naya optimizasya poiska rastitel'nix ob'ektov I sozdanie novyih lekarstvennyih sredstv na osnove sovremennykh informacionnykh tehnologii. [Multicriteria optimization of plants' search and new drugs development based on modern information technology]- Nauchnie vedomosti Belgorodskogo gosudarstvennogo universiteta. Seria:Meditsina. Pharmasya, 16 (135): 132–135. (in Russian)
9. Морозова Т.В., Куркина А.В., Правдивцева О.Е., Дубищев А.В., Куркин В.А., Зайцева Е.Н. 2015. Фармакогностическое и фармакологическое исследование сырья боярышника. *Известия Самарского научного центра Российской академии наук*, №5-3 (17): 959–963.
Morozova T.V., Kurkina A.V., Pravdivtseva O.E., Dubishchev A.V., Kurkin V.A., Zaitseva E.N. 2015. Farmakognosticheskoe I farmakologicheskoe issledovanie sirya boyarishnika. [Pharmacognostic and pharmacological study of hawthorn's raw material] – Izvestiya Samarskogo nauchnogo tsentra Rosiiskoi akademii nauk, №5-3 (17): 959–963.
10. Bekbolatova E., Sakipova Z., Ibragimova L., Malikova N. 2016. Technology of harvesting, primary processing and storage of the herbal drug *Crataegus almaatensis* Pojark fruits. *Вестник Казахского Национального медицинского университета*, 4: 316–319.
11. ICH HARMONISED TRIPARTITE GUIDELINE Q1A (R2). 2003. Stability Testing of New Drug Substances and Products. *Federal Register*, 68(225): 65717–18.
12. Jennifer E. E., Paula N. B., Nadia T. 2012. A review of the chemistry of the genus *Crataegus*. *Phytochemistry*, 79:5–26.