

STANDARDIZATION OF NEW HERBAL COMPOSITION "FLUCAM"

Komila Z. Alimkulova¹, Flyura F. Urmanova²

¹ Head of the Scientific Center Med Standard LLC, Karasaray St. 343, Almazar district, Tashkent, Uzbekistan, Email: komila.yusupova@mail.ru, Orcid: 0000-0002-7806-937X

² Doctor of Pharmaceutical Sciences, Professor, Head of the Department of Pharmacognosy, Tashkent Pharmaceutical Institute, Oybek St., 45, Tashkent, Uzbekistan. Email: flyura.urmanova@mail.ru

Abstract: *Research objective: to standardize a new herbal product "Flucam" for its introduction into a medical practice.*

Materials and methods. *The study included the samples of the "Flucam" herbal product, consisting of specific species of plant raw materials, approved for medical use and provided with a sufficient raw material base on the territory of Uzbekistan. The development of standardization methods was carried out on five series of "Flucam" in accordance with the instructions of the SP XI edition, as well as the recommendations of the WHO, the International Conference on the Harmonization of Technical Requirements for Medicines Registration (ICH) and the requirements of the technical regulation on the safety of medicines.*

Results and discussion. *As a result of the research, the principles of standardization of the new herbal product "Flucam", recommended as the effective immunomodulatory drug, were proposed and substantiated. The characteristics of identification and indicators of the species purity were determined. Besides, we developed and introduced procedural methods for the qualitative and quantitative determination of the main active substances - the tannins and glycyrrhizic acid.*

Conclusion. *During the research, characteristics of identification and quality indicators of the product, as well as the optimal terms of its storage were experimentally determined and substantiated. Based on the study of microbiological purity and the content of hazardous contaminants - toxic heavy metals, radionuclides and pesticides, the possibility of safe use of "Flucam" was established.*

Key words: *"Flucam", standardization, tannins, glycyrrhizic acid, identification, quantitative determination, contaminants, microbiological purity, regulatory documentation.*

INTRODUCTION

In recent years, with an increase of ecological and social pressure, a significant reduction of the body's resistance is noted, which associated with the weakening of the immune, detoxification and other adaptive mechanisms.

In such conditions, the development of new immunomodulatory drugs has a particular relevance. They can be applied for the prevention of diseases in people with reduced general condition and those who have suffered serious illnesses. Especially it concerns the elderly and senile aged people, as well as the general population in order to increase the body's resistance, prevent fatigue during physical and mental stress and improve productivity. In this aspect, we consider very promising the use of medicinal plants and, especially, their optimal combinations – herbal products that have a complex effect on the body due to the harmonious interaction of various groups of biologically active substances.

Taking into consideration the noted circumstances, we have developed a new multicomponent herbal product with the provisional name "Flucam".

As it known, the standardization of new medicinal products, including herbal remedies, followed by the development of regulatory documentation is an essential condition for their implementation into medical practice and industrial production [1,2].

RESEARCH OBJECTIVE

To standardize a new herbal product "Flucam" for its introduction into a medical practice.

MATERIALS AND METHODS

The objects of the study were the samples of the "Flucam" herbal product, which included Blue Chamomile (*Matricaria Recutita*) flowers, leaves of garden sage (*Salvia officinalis*), Circassian walnut (*Tuglans regia*), urtica dioica (*Urtica dioica*), common St. John's wort (*Hypericum perforatum* L), Lemon balm herb (*Melissa officinalis*), Spanish licorice root (*Glycyrrhiza glabra*), burdock (*Lappa officinalis*), milk-govan (*Taraxacum officinale*) and oak bark (*Quercus robur*). All of these species are approved for medical use and provided with a sufficient raw material base on the territory of our country.

The development of standardization methods was carried out on five series of "Flucam" in accordance with the instructions of the SP XI edition, as well as the recommendations of the WHO, the International Conference on the Harmonization of Technical Requirements for Medicines Registration of (ICH) [3,5] and the requirements of the technical regulation on the safety of medicines [4]. The identification of the herbal product was established on the basis of the study of external, anatomical and diagnostic signs and the determination of the main active substances by qualitative reactions.

RESULTS AND DISCUSSION

The first stage included the analysis of external signs of the recommended multicomponent composition. The herbal product is a mixture of inhomogeneous particles (pieces of leaves, stems, flowers, bark and roots) of yellowish-green color with brownish speckles, which can pass through a sieve with hole diameter 7mm. The scent is pleasant. The taste of the aqueous extract is slightly spicy, bitterly-astringent.

For microscopic investigation pieces of each component of the herbal composition were selected under a stereomicroscope, and then slides were prepared from them.

Microscopic analysis was carried out on both fresh and fixed (cold soaking in a mixture of glycerin-water-ethanol, 1: 1: 1) material in accordance with the requirements of the SP XI articles "Leaves", "Herbs", "Flowers", "Bark", "Roots", "Rhizomes, tubers, bulbs" and "Technique of microscopic and micro-chemical research of medicinal raw materials" [3].

Microscopic investigation of leaves and herbs studied the surface micro-sections of leaves. For this purpose, an attention was paid to the structure of the epidermis, the type of stomas, the nature of trichomes (hairs, glands), the presence and shape of crystalline inclusions, mechanical tissue, pycnium, secretory canals, etc.

During the microscopic analysis of the bark, attention was paid to the thickness and number of cork layers and the presence of foreign matters in it, as well as to the nature and location of ray cell walls, mechanical elements and crystalline inclusions.

When examining the sections of the roots, we studied the nature of the external protective tissue (epidermis, cork) and the main parenchyma, the shape and structure of the pith rays, the presence of secretory formations (receptacles, laticifers, secretory passages, etc.), crystalline inclusions, storage cell products (starch grains, inulin spherical crystals,

aleurone granules).

It should be noted that during the microscopic investigation of the herbal product, all diagnostically significant anatomical signs were fully manifested, their visualization was not difficult.

For identification of the recommended product, we applied method of reaction of complex formation of tannins with ferric ammonium sulfate and chromatographic determination of glycyrrhizic acid.

Methods:

1) 0.1 g of the sample herbal product was boiled during 2-3 minutes with 10 ml of water, cooled and filtered. To 1 ml of filtrate we added 2-3 drops of ferric ammonium sulfate: a black-green staining was observed (tannins).

2) 5.0 g of raw material, crushed to the size of particles passing through a sieve with hole diameter 0.5 mm, was placed in a conical flask with a ground joint with a capacity of 100 ml, adding 10 ml of 96% alcohol - water (1:1) mixture, and boiled under reflux in a water bath during 10 min. After cooling to room temperature, the extract was filtered through a filter paper (test solution).

Preparation of a standard sample solution. About 0.005 g of CO monoammonium salt of glycyrrhizic acid is dissolved in 1 ml of 96% alcohol - water mixture (1:1 v/v).

On the start line of the chromatographic sheet "Silufol UV-254", with dimensions of 13cm x 5 cm, 0.01 ml of the test solution was applied with a micropipette. The sheet with the applied samples was dried in air for 3-5 minutes, then placed in a chamber with a chloroform-acetic acid solvent system (1:1). When the solvents front passed about 10 cm from the starting line of the sheet, it was taken out of the chamber, dried in air for 2-3 minutes (until the scent of solvents was eliminated) and viewed in UV light at a wavelength of 254 nm. At the level of the CO spot of glycyrrhizic acid, a dark adsorption zone (glycyrrhizic acid) should appear as a band.

When developing criteria for the purity of "Flucam", the following indicators were determined:

- content of active ingredients;
- humidity;
- content of total ash and ash insoluble in 10% hydrochloric acid solution;
- crude drug granulation and content of permissible impurities;
- microbiological purity.

The quantitative content of tannins was identified by accepted methods of permanganometric titration [3].

The results of identification are shown in the Table 1.

Table 1
Results of quantitative determination of tannins in the "Flucam" herbal product

X_{av}	P, %	n	S	S_x	S^2	f	ΔX	ΔX_{av}	ΔE	ΔE_{av}
18,51	95	5	0,25812	0,11543	0.06662	4	0,71656	0,32045	3,89351	1,74123
18,48										
18,44										
18,63										
17,96										
18,51										

As seen from the data, the content of tannins ranges from 17.96 to 18.63%. The relative determination error is $\pm 1.74\%$. Based on the results obtained, the standard for the tannins content in the product is at least 17.0%.

The quantitative content of glycyrrhizic acid was determined by high performance liquid

chromatography using LC-20 prominence chromatograph (Shimadzu Corp., USA) according to the method [7].

Chromatography conditions: column 3.0x150 mm, 4.6 mm, ZORBAX SBC18, the flow rate of the mobile phase (3.85 g of ammonium acetate is dissolved in 720 ml of water, 5 ml of acetic acid (conc.) and 280 ml of acetonitrile are added) was set in such way, that the retention time of glycyrrhizic acid was about 10 minutes; identification was carried out at a wavelength of 254 nm; column temperature - 25 ° C; analysis time - 15 min.

Preparation of the test solution. The analytical sample was ground finely to a particle size passing through a 1 mm sieve.

About 10.0 g (accurately weighed sample) of the crushed raw material was placed in a flask with a ground joint with a capacity of 250 ml, adding 100 ml of 50% alcohol. The flask was connected to a reflux condenser and heated in a boiling water bath for 30 minutes, shaking occasionally to wash off the particles of raw material from the walls of the flask.

The hot extraction was filtered through cottonwool into a 200 ml volumetric flask so that particles of the raw material did not fall on the filter. The cottonwool was placed in the extraction flask and added 50 ml of 50% alcohol. The extraction was repeated twice more under the conditions described above, filtering the extraction into the same volumetric flask. After cooling, the volume of extraction was diluted to the mark with 50% alcohol and mixed. The resulting solution was placed in a centrifuge tube with a lid, centrifuged for 5 min at 3000 rpm, after which the supernatant liquid was removed.

Preparation of a standard solution. The precisely weighed amount of the glycyrrhizic acid standard (0.025 g) was dissolved in 50% ethanol and brought up to 100 ml with the same solvent.

The analysis under the above conditions was performed 5 times. The relative standard deviation of the peak area of glycyrrhizic acid did not exceed 2.0%.

20 µL of the test solution and the CO solution of glycyrrhizic acid were alternately developed on the chromatograph, obtaining at least 5 chromatograms (Fig. 1, 2).

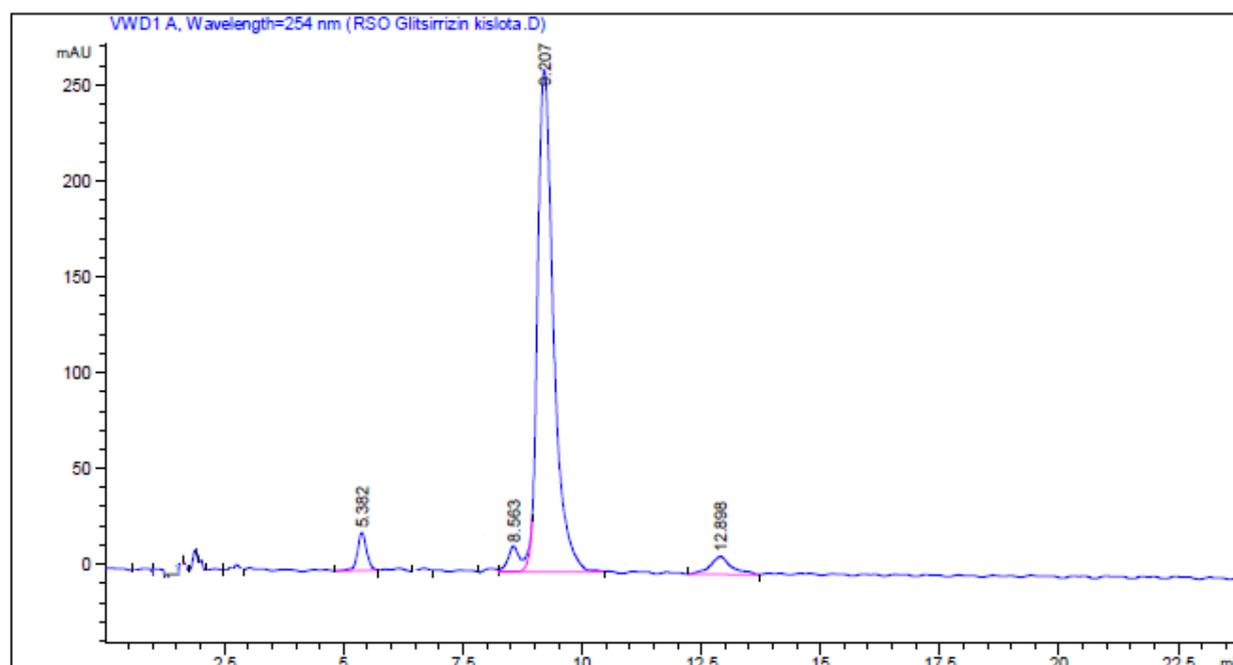


Fig. 1. Chromatogram of the standard solution of glycyrrhizic acid

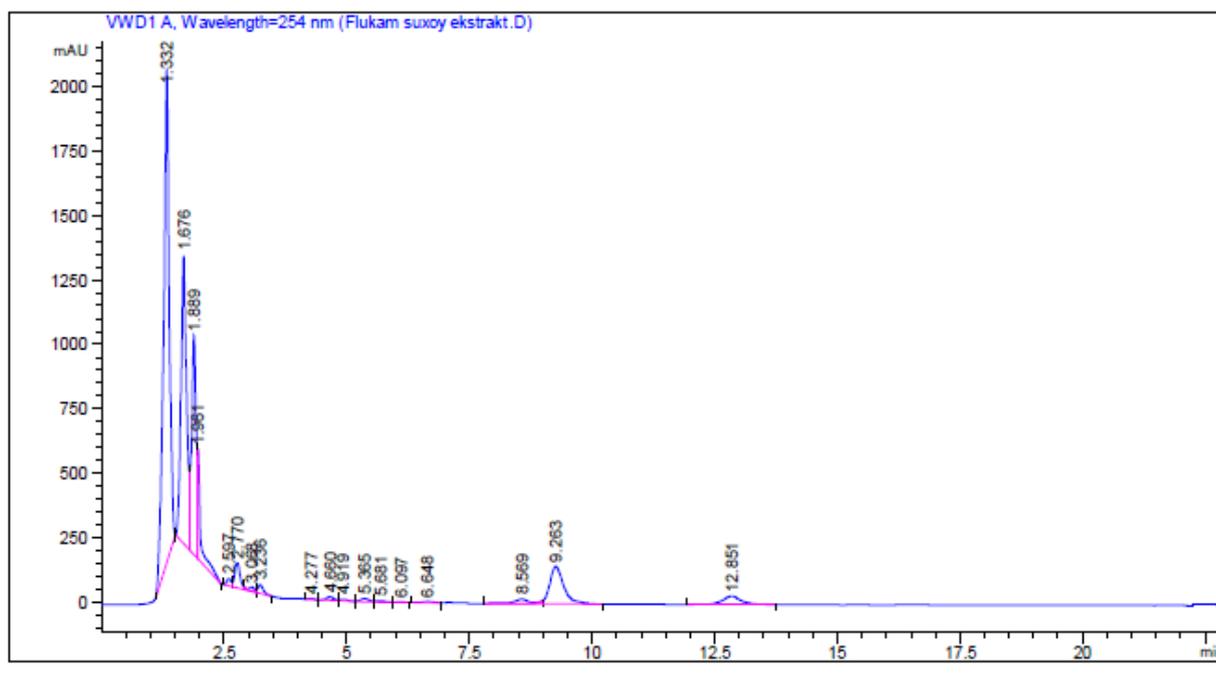


Fig. 2. Chromatogram of the test solution of the "Flucam" herbal product

The glycyrrhizic acid content was calculated by the formula:

$$X = \frac{S_1 \times m_0 \times 200 \times P \times 100}{S_0 \times m_1 \times 100 \times (100 - W)}$$

where S_1 is the average value of the peak areas of glycyrrhizic acid, calculated from the chromatograms of the test solution; S_0 is the average value of the peak areas of glycyrrhizic acid, calculated from the chromatograms of the standard solution; P is the content of glycyrrhizic acid in work standard in percent; m_1 is the mass of the sample of the herbal product in milligrams; m_0 is the weight of the work standard sample of glycyrrhizic acid in milligrams; W is the humidity of the test herbal product.

The results of identification are presented in the Table 2.

Table 2

The results of the quantitative determination of glycyrrhizic acid in "Flucam"

X_{av}	P, %	n	S	S_x	S^2	f	ΔX	ΔX_{av}	ΔE	ΔE_{av}
0,345	95	5	0,00649	0,00290	0.00004	4	0,01803	0,00806	5,35431	2,39452
0,329										
0,337										
0,341										
0,332										

The presented data shows that the content of glycyrrhizic acid in the herbal product is in the range of 0.329-0.345%. Based on this, the norm for the content of glycyrrhizic acid is at least 0.3%.

The other numerical indicators listed above were identified according to the methods described in the SP XI [3].

The results of the chemical and merchandising analysis of the product are summarized in the Table 3.

Table 3 Results of merchandising and chemical analysis of "Flucam"

	The content of tannins, %	The content of glycyrrhizic acids, %	Weight loss on drying, %	Ash total, %	Ash, insoluble in 10% hydrochloric acid solution, %	Particles that do not pass through the 7mm sieve, %	Particles passing through the 0.25 mm sieve, %	Organic impurity, %	Mineral impurity, %
1.	18,51	0,271	6,64	9,56	1,80	4,48	2,27	1,81	0,997
2.	18,48	0,296	6,89	9,39	1,76	4,94	2,53	1,92	0,974
3.	18,44	0,310	7,53	9,64	1,93	4,37	2,19	1,87	0,981
4.	18,63	0,284	7,71	9,59	1,12	4,74	2,78	1,91	0,994
5.	17,96	0,305	6,83	9,61	1,59	4,83	2,76	1,83	0,997

Based on the data obtained, we have established the norms of numerical indicators that normalize the quality of "Flucam" (Table 4).

Table 4 Numerical indicators of "Flucam"

The name of indicators	Product rate
Tannins, %, not less	17,0
Glycyrrhizic acid, %, not less	0,3
Humidity, %, no more	8,0
Total ash, %, no more	11,0
Ash insoluble in 10% hydrochloric acid solution, %, no more	3,0
Particles that do not pass through the 7mm sieve, %, no more	5,0
Particles passing through the 0.25mm sieve, %, not more	3,0
Organic impurity, %, no more	2,0
Mineral impurity, %, no more	1,0

Besides, the herbal composition safety indicators were also determined: microbiological purity, content of toxic heavy metals, radionuclides and pesticides [4,6]. It is established that "Flucam" fully complies with the requirements for medicinal plant raw materials in relation to its microbiological purity. It was also revealed that the content of hazardous contaminants in the product did not exceed the accepted maximum permissible concentration. The data obtained indicated the ecological purity and safety of the test product [4,6].

During storage, the external signs and numerical indicators of the herbal product did not change significantly within 2.5 years. Therefore, it is recommended to use this product within 2 years from the manufactured date.

CONCLUSION

As a result of the study, the system for standardizing the recommended herbal composition was proposed:

- methodological techniques for the qualitative and quantitative determination of the main active

substances were developed;

- the characteristics of identification and quality indicators of the product were determined, as well as the optimal terms of its storage were experimentally substantiated.

- data were obtained to substantiate the level of requirements and indicators characterizing the quality of the proposed product, which served as the basis for the development of the corresponding regulatory documentation.

REFERENCES

1. T.A. Sokolskaya. The role of standards in pharmaceutical analysis of medicinal plant raw materials and phytopreparations // T.A. Sokolskaya, T.D. Dargaeva // Issues of biological, medical and pharmaceutical chemistry. - 2009. -№3. - P.10-13.
2. V.L. Bagirova. Collection of guidelines for the standardization of medicines / V.L. Bagirova, G.N. Gildeeva, V.V. Kosenko and others –M.: Pelican, 2006.-P.392.
3. State Pharmacopoeia of the USSR: Issue 1. General methods of analysis / Ministry of Health of the USSR. 11th ed. - M.: Medicine, 1987. – P.336.
4. General technical regulations on the safety of medicines. Appendices to the Resolution of the Cabinet of Ministers of the Republic of Uzbekistan dated October 27, 2016 No. 365.
5. International Conference on Harmonization of Technical requirements for registration of Pharmaceuticals for Human USE. ICH. Proceedings of the Second International Conference on Harmonization.-Brussels, 2008.
6. Alimkulova K.Z., Urmanova F.F. "To the issue of ecological purity and safety of the new herbal composition "FLUCAM"" // Pharmaceutical journal. –Tashkent 2020, No. 1. –P.16-18.
7. The Japanese Pharmacopoeia XVII edition, Official from April 1, 2016.
8. Natural Health Products Directorate - Health Canada. Natural Health Product Tracking Survey-2010 Final Report. Ipsos-Reid; Toronto, ON, Canada: 2011.
9. Bhope SG, Nagore DH, Kuber VV, Gupta PK, Patil MJ. Design and development of a stable polyherbal formulation based on the results of compatibility studies. *Pharmacognosy Research*. 2011;3(2):122–129.
10. Gomase PV, Shire PS, Nazim S, Choudhari AB. Development and evaluation of polyherbal formulation for anti-inflammatory activity. *Journal of Natural Product and Plant Resources*. 2011;1(1):85–90.
11. Alamgir M, Uddin SJ. Recent advances on the ethnomedicinal plants as immunomodulatory agents. In: Chattopadhyay D, editor. *Ethnomedicine: A Source of Complementary Therapeutics*. Kerala, India: Research Signpost; 2010. pp. 227–244.
12. Lau A, Holmes MJ, Woo S, Koh H. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectrometry-mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*. 2003;31(2):401–406.
13. Mallavadhani UV. *Standardization and Quality Control of Multiherbal Formulations*. Bhubaneswar, India: Herbal Drugs and Bio-Remedies Cell Regional Research Laboratory; 2011.
14. Holistic Herbalist. Herbal formulation: why the balance and synergy of Herbs are the life of any herbal formulation. 2013, <http://www.holistic-herbalist.net/herbal-formulation.html>.