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STANDARDIZATION OF SUBSTANCE BASED ON GINKGO BILOBA

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ABSTRACT

The substance is standardized in according to requirements of the European Pharmacopoeia (EP) for such an indicator as authenticity, loss in mass upon drying, sulfate ash, residual solvents and quantitative content of the sum of flavonoids (flavono glycosides).

KEYWORDS: *Gibkgo Biloba, European Pharmacopoeia, Identification, Assay, Total Flavonoids (Flavone Glycosides), Quercetin, Campferol, Isoramnetinetin, TLC And HPLC.*

INTRODUCTION

At present, one of the urgent problems of the modern pharmaceutical industry is the search for domestic biologically active compounds and the production of domestic drugs on their basis. One of these representatives is the leaves of the Ginkgo biloba trees (Ginkgo biloba).

For the development of domestic pharmacy and the expansion of the range of drugs based on local standardized substance, we carried out a study on the development of technology for a standardized, enriched dry extract of ginkgo biloba growing in the territory of Uzbekistan.

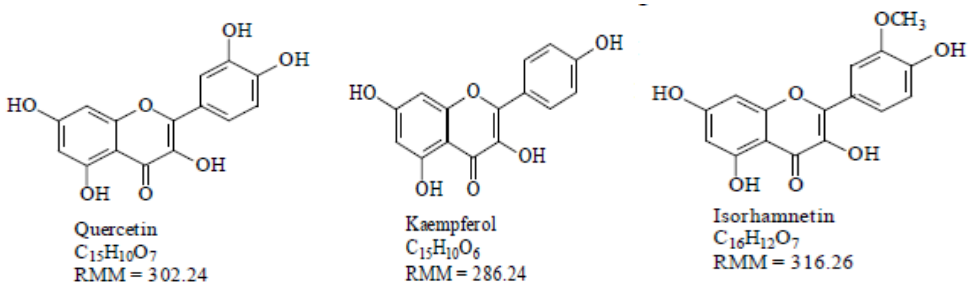
Ginkgo biloba leaf extract has a complex chemical composition; it includes more than 40 biologically active ingredients. A standardized extract from the leaves of Ginkgo biloba contains

three main groups of substances that determine its specific pharmacological activity and are indicators of the authenticity of raw materials: Flavonic glycosides, terpene lactones and ginkgolic acids [1]. Flavone glycosides have a wide range of effects: antioxidant, antiatherosclerotic and neurotransmitter effects. Terpene lactones (ginkgolides and bilobalide) are found only in the leaves of ginkgo biloba biloba, they have antioxidant activity, in addition, they inhibit the platelet activating factor and have anti-ischemic activity [2]. An important indicator of the quality of raw materials is the ratio of the flavone glycosides of kaempferol, quercetin and isorhamnetin. According to the provisions of the Pharmacopoeia of Europe and the United States, the total content of flavone glycosides in the extract should be in the range of 22-27% [3].

Quercetin is a flavonoid of the flavonol class, which has decongestant, antispasmodic, antihistamine, anti-inflammatory and diuretic effects; has antiviral and antitumor properties. It is included in the group of vitamin P. Quercetin is the most active of the flavonoids, has a pronounced antioxidant effect.

Kaempferol is a flavonoid of the flavonol class; it strengthens the walls of the vessels of the microvasculature and removes toxins from the body. This biologically active substance has a pronounced restorative, anti-inflammatory and tonic effect, it is also a diuretic.

Isoramnetin (3-methylquercetin) is a flavonoid of the flavonol class, a metabolite of quercetin. Less well studied compared to quercetin. In terms of pharmacological action, isorhamnetin is similar to quercetin and kaempferol. As an antioxidant, it protects the phospholipid membranes of brain cells from damage, prevents thrombus formation, strengthens the vascular wall, has the activity of vitamin P, is able to inhibit phosphodiesterase and hyaluronidase, protects adrenaline from oxidation and prevents the destruction of ascorbic acid.



a) **б)** **c)**

Rice. 1. Structural formulas of quercetin (a), kaempferol (b) and isorhamnetin (c)

The most popular preparations of ginkgo biloba extract are: tablets "Tanakan", "Memoplant", "Bilobil", "Ginos", "Ginkoum", "Vitrum memory" and are among the five best-selling [4]. The use of ginkgo preparations enhances concentration, increases energy, reduces distraction, relieves fatigue, depression, nervous conditions, headaches. The aging process of the body slows down, physical activity and efficiency increase.

The aim of the study is to standardize the obtained dry extract of ginkgo biloba growing in the territory of Uzbekistan.

Materials and methods. The following methods were used to standardize dry ginkgo biloba extract: TLC and HPLC

Experimental part

The object of the research is dry extract of ginkgo biloba leaves. (Ginkgo biloba).

Description.

The dry extract is characterized as a fine, light yellow brown powder.

Authenticity.

TLC (BP / EP 2.2.27).

Test solution: About 20 mg of the extract is dissolved in 10 ml of a mixture consisting of 2 ml of purified water and 8 ml of methanol.

Reference solution: Dissolve 1.0 mg chlorogenic acid and 3.0 mg rutin in 20 ml methanol.

Chromatography condition:

Plate: TLC silica gel (5-40 μm) or (2-10 μm)

Mobile phase: Anhydrous formic acid, glacial acetic acid, purified water, ethyl acetate (7.5: 7.5: 17.5: 67.5).

Application: 20 μl (or 5 μl).

Development: run length 17 cm (or 6 cm).

Drying: at a temperature of 100-105 ° C

Definition: The plate is initially sprayed with a 1% solution of diphenylboronic acid aminoethyl ester in methanol, and then with a 5% solution of macrogol 400 in methanol. The plate is air-dried for 30 min, viewed under UV light at 365 nm.

Results: The sequences of the zones in the chromatograms of the obtained reference solution and the test solution are shown below. Other areas of fluorescence can also be detected and can be detected on the chromatogram of the test solution.

Front plate	
	Blue fluorescent area
	Several light colored areas
----------	----------
	Brown fluorescent area
	Green fluorescent area
	Intense light blue fluorescent area, sometimes overlapping with a greenish brown fluorescent area
Chlorogenic acid: blue fluorescent zone	
	One or two green fluorescent zones
Rutin: yellowish brown fluorescent zone	One or two yellowish brown fluorescent areas
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	Several green and yellowish brown fluorescent areas
Reference solution	Test solution

Quantitation.

*Flavonoids (flavone glycosides): 22.0% to 27.0% (dry extract).*Метод ВЭЖХ (BP/EP 2.2.29.)

Test solution: 200.0 mg (so-called) extract is placed in a 100 ml volumetric flask. Add 20 ml of methanol and dissolve in an ultrasonic bath for 1 min. Add 15.0 ml of dilute hydrochloric acid (7.3%), 5 ml of purified water and 10 ml of methanol. Heat the flask in a water bath at 90 ° for 1 h 35 min. Up to 15 minutes before the end, 20 ml of methanol are added. After the end of heating, allow to cool and put on an ultrasonic bath for 3 minutes.

The resulting solution is transferred into a volumetric flask with a volume of 50 ml, rinsing the flask with methanol and the volume of the solution is brought to the mark with methanol, mixed and filtered through a Millipore filter with a pore size of 0.45 µm, discarding the first portions of the filtrate.

Quercetin dihydrate standard solution (CRS): 10.0 mg (so-called) quercetin dihydrate (CRS) is placed in a 50 ml volumetric flask and dissolved in 20 ml methanol. Add 15.0 ml of dilute hydrochloric acid (7.3%) and 5 ml of purified water and bring the volume of the solution to the mark with methanol, mix and filter through a Millipore filter with a pore size of 0.45 µm, discarding the first portions of the filtrate.

Chromatography conditions:

Mobile phase A: Adjust the pH of 1000 ml of purified water to pH 2.0 ± 0.05 with concentrated phosphoric acid.

Mobile phase B: Methanol.

Column: 125 x 4.0 mm, packed with octadecylsilyl C-18 sorbent, particle size 5.0 µm or similar.

Flow rate: 1.0 ml / min;

Detection: 370 nm;

Column temperature: 25 ° C;

Analysis time: 25 min;

Injection volume: 10 µL.

Note! Post time: 3 min.

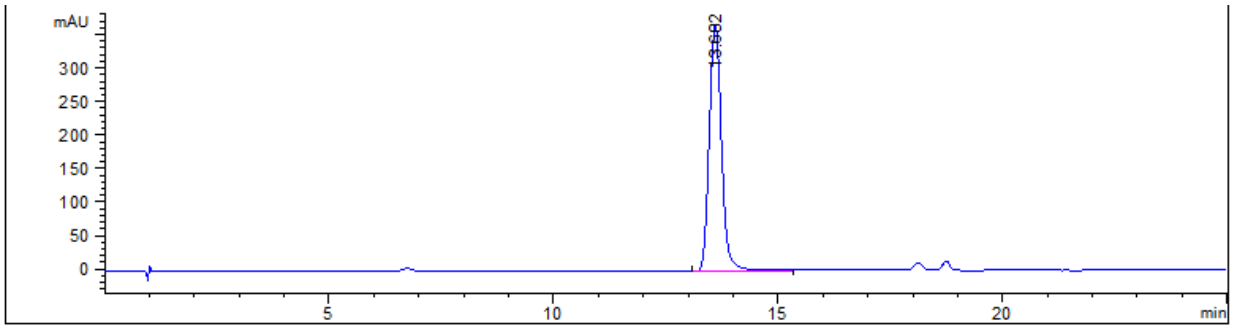
Time	Mobile phase A	Mobile phase B
0	60	40
1	60	40
15	45	55
24	0	100
25	0	100

Quercetin retention time: about 12.5 minutes,

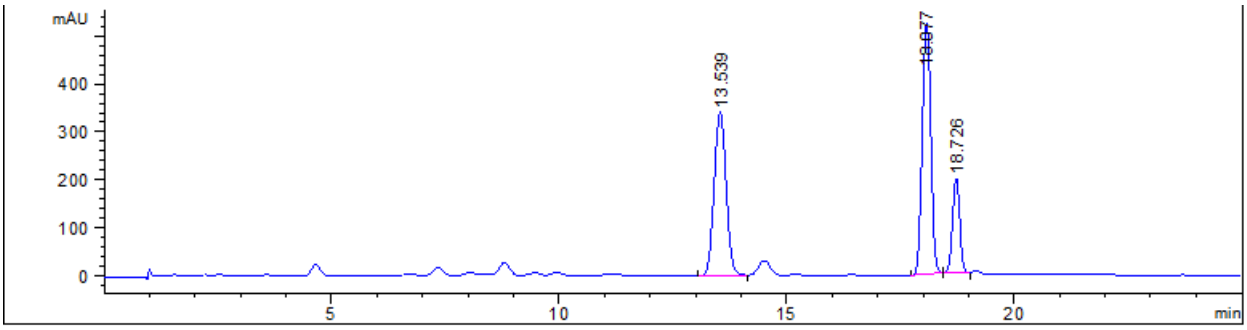
Relative retention time of kaempferol: about 1.4; isoramnetin: about 1.5.

Suitability of the chromatographic system: test solution:

- The separation between the peaks of kaempferol and isorhamnetin should be at least 1.5.



a)



б)

Rice. 2. Chromatograms from HPLC: a) chromatogram of the standard (quercetin),

b) chromatogram of the sample (ginkgo biloba).

The content of the sum of flavonoids (X), in%, in terms of flavone glycosides is calculated by the formula

X = (〖(S〗 _1 + S_2 + S_3) · m_st · 50 · 2.514 · P) / (S_st · m_1 · 50) = (〖(S〗 _1 + S_2 + S_3) · m_st · 2.514 · P) / (S_st · m_1)

where,

S1 is the area of the quercetin peak calculated from the chromatograms of the test solution;

S2 is the area of the peak of kaempferol calculated from the chromatograms of the test solution;

S3 is the isorhamnetin peak area calculated from the chromatograms of the test solution;

Sst is the peak area of quercetin calculated from the chromatograms of the standard solution;

mst is the weight of the quercetin dihydrate (CRS) sample, in mg;

m1 is the mass of the sample of the extract, in mg;

P - the actual content of anhydrous quartzin in the standard sample, in%;

2.514 is a coefficient associated with the average molecular weight of flavone glycosides.

Note. Preparation of 7.3% hydrochloric acid: 17 ml of concentrated hydrochloric acid is placed in a volumetric flask with a volume of 100 ml containing a small amount of purified water, stirred, the volume of the solution is brought to the mark with purified water and stirred.

The obtained chromatograms of the analysis of the quantitative content of dry extract of ginkgo biloba in Fig. 2.

The obtained results of the analysis of the quality of dry extract of ginkgo biloba are shown in table 1.

TABLE 1 RESULTS OF QUALITATIVE ANALYSIS OF DRY EXTRACT OF GINKGO BILOBA

Indicators	**Limits**	**Methods**	**results**
Description	Fine, light yellow brown powder.	Visually	Fine, light yellow brown powder.
Идентификация	ТСХ. На хроматограмме испытуемого раствора должны обнаруживаться следующие зоны: верхняя зона – синяя флуоресценции, коричневой флуоресценции, средняя зона– светло-голубой или overlapping with greenish-brown fluorescence and lower	ТСХ (BP/EP 2.2.27).	On the chromatogram of the test solution, the following zones were found: the upper zone - blue fluorescence, brown fluorescence, the middle zone - or overlapping with greenish-brown fluorescence and the lower zone - rutin: two yellowish-brown fluorescences.

	zone - rutin: one or two yellowish-brown fluorescences.		
Quantitative content	HPLC method Flavonoids (flavone glycosides): from 22.0% to 27.0% (dry extract).	(BP/EP 2.2.29).	24,63%

All materials and reagents used in the work are manufactured by Merck.

CONCLUSIONS

1. For the first time, the substance of dry extract of ginkgo biloba (ginkgo biloba) was standardized in accordance with the requirements of the European Pharmacopoeia (EF) for the content of the sum of flavonoids (flavone glycosides) growing in the territory of Uzbekistan.

2. For the first time, new methods of quantitative analysis of the amount of flavonoids (flavone glycosides) of dry extract of ginkgo biloba (ginkgo biloba) growing in the territory of Uzbekistan have been carried out.

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REFERENCES

1. OV Yatsevich Candidate of Pharmaceutical Sciences, Production Director of Tuscany Laboratory LLC, Moscow. Article ginkgo biloba Mesozoic relic - "silver apricot"

2. Kuznetsova S.M., Shulzhenko D.V. State Institution "Institute of Gerontology named after DF Chebotarev National Academy of Medical Sciences of Ukraine "Kiev. “Ginkgo biloba extract in a strategy for the treatment of chronic vascular diseases of the brain. No. 2 (72), 2015 p-111.

3. European Pharmacopoeia, ginkgo dry extract, refined and quantified, 04/2008: 1827

4. Onbysh T.E. Makarova L.M. Pogorely V.E. Scientific journal Modern high technologies. Issue of the journal No. 5 for 2005. Mechanisms for the implementation of the pharmacological activity of ginkgo biloba extract