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DEVELOPMENT OF A METHOD FOR QUANTITATIVE DETERMINATION OF TRITERPENE SAPONINS IN THE GRASS OF THE TURKESTAN DESERT

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Abstract: An analysis of the current state of research in the field of studying the representatives of the family of lucidaceae - (Lami-aceae) showed that they have a wide spectrum of biological activity and are used both in scientific and folk medicine. These representatives include species of the genus Leonurns L. In many species of this genus, various groups of biologically active substances have been found: flavonoids (rutin, genquanin, quercetin), tannins, saponins, nitrogen-containing compounds (leonurin, stachydrin). Different species of the genus motherwort are official and are included in the National Pharmacopoeias of many countries of the world. Different species of the genus motherwort are official and are included in the National Pharmacopoeias of many countries of the world. The State Pharmacopoeia of the XI edition includes motherwort and five-lobed motherwort, known for their pronounced sedative effect (2-3 times stronger than that of valerian tincture). Pharmacological studies carried out abroad have also revealed hormone-like, antitumor, antimicrobial, anti-inflammatory and antioxidant effects of motherwort.

To achieve this goal, it was necessary to solve the following tasks:

- to study the current state of research in the field of studying terpenoid-containing plants used in domestic medical practice and drugs developed on their basis;
- to investigate the terpenoid composition of Turkestan motherwort herb;
- to propose methods of qualitative and quantitative analysis of terpenoid compounds of Turkestan motherwort raw materials.

Key words: triterpene glycosides, (triterpene saponins), potentiometric method, oleanolic acid, gravimetric method, UV spectrum, Fisher's criterion, Turkestan motherwort herb.

摘要：对灵芝科 (Lami-aceae) 代表研究领域的研究现状分析表明，它们具有广泛的生物活性，可用于科学和民间医学。这些代表包括Leonurns L属的物种。在该属的许多物种中，已经发现了多种生物活性物质：类黄酮 (芦丁、genquanin、槲皮素) 、单宁、皂苷、含氮化合物 (Leonurin、水苏苷) 。益母草属的不同物种是官方认可的，并被列入世界许多国家的国家药典。益母草属的不同物种是官方认可的，并被列入世界许多国家的国家药典。XI版国家药典包括益母草和五叶益母草，以其显著的镇静作用 (比缬草酊剂强2-3倍) 而闻名。国外开展的药理研究也揭示了益母草具有激素样、抗肿瘤、抗菌、抗炎和抗氧化作用。

为了实现这一目标，有必要解决以下任务：

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- 研究国内医疗实践中使用的含萜类植物和在其基础上开发的药物领域的研究现状；
- 研究土耳其斯坦益母草的萜类成分；
- 提出了土耳其斯坦益母草原料萜类化合物的定性和定量分析方法。

关键词：三萜苷，（三萜皂苷），电位法，齐墩果酸，重量法，紫外光谱，Fisher 判据，土耳其斯坦益母草。

Introduction

The drug policy of the Republic of Uzbekistan is based on the principles of providing medical institutions and the population with effective, safe and affordable drugs.

Medicinal plants occupy an important place in pharmaceutical practice. In the total nomenclature of medicines, approximately 40% are herbal preparations. Compared with synthetic, herbal preparations, along with a mild multilateral action, are characterized, as a rule, by the absence of unwanted side effects. The study and introduction into scientific medicine of not only new, but also well-known plant objects is one of the most important tasks of modern pharmacognosy.

It is known that the physiological activity of preparations based on plant raw materials is determined by the synergism of the biologically active substances contained in them. Among the latter, special attention is attracted by terpenoids, which have anti-inflammatory, antiulcer, antiviral, antitumor and other activity.

A deep chemical study of plants in the flora of Uzbekistan, provided with a sufficient raw material base, the development of methods for the qualitative and quantitative content of substances, as well as the development of a scientifically grounded technology for obtaining dosage forms is an urgent problem of pharmaceutical science.

Motherwort Turkestan is one of the most promising plants in this respect. This plant is

currently approved for widespread use in medical practice in the form of infusion, decoction, liquid extract and tincture.

Taking into account the above, it is obvious that a comprehensive study of the Turkestan motherwort growing in Uzbekistan in order to create new effective medicines based on its terpenoid compounds is an urgent task.

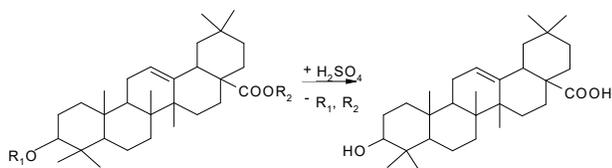
Purpose of the study. The purpose of this work was to study the possibility of using Turkestan motherwort as a source of triterpene saponins and the prospects of its use in traditional medicine along with other triterpene-containing plants.

Materials and research methods. Quality control of medicinal products containing triterpene glycosides (triterpene saponins) is currently difficult due to the lack of acceptable methods for their quantitative determination in plant raw materials and products made from them, as well as the absence of standard samples of these substances. The analysis is complicated by the specific physicochemical properties of saponins, in particular, the tendency to colloid formation and conjugation with lipid compounds of plant material. Determination of glycosides is also hampered by the fact that the composition of plant raw materials and phytopreparations obtained from it, as a rule, does not include an individual substance, but the sum of triterpene saponins, similar in structure, having a common aglycone and differing only in the number of sugar residues. Since the main methods for

determining the content of triterpene saponins are based on the physicochemical properties of their aglycones - the most reactive component of molecules that determines their biological activity, it would be rational to use aglycones as standard samples when developing methods for the quantitative determination of saponins. This approach is widely used to solve problems of practical analysis of various natural objects of complex composition. Methods for the quantitative determination of saponins through their aglycone have been introduced into the ND for medicinal plant raw materials of Manchurian aralia [1], herbal remedies obtained from this type of raw material [2]. Aglycone is determined titrimetrically after hydrolysis of saponins of the analyzed object, which makes the methods time-consuming and laborious.

There is no single method for the quantitative determination of saponins in medicinal plant materials. Physicochemical methods are most often used [3,4]:

1. Potentiometric method. Based on the determination of the change in electromotive force (EMF) as a result of titration. The method is used to determine the amount of aralosides in the roots of Manchurian aralia. Determination steps: extraction of aralosides with methyl alcohol and their acid hydrolysis:

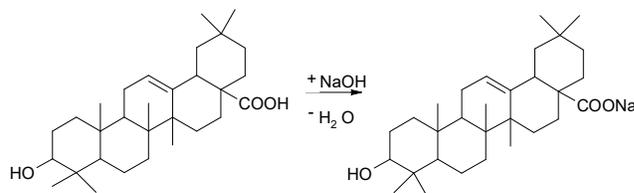


R₁, R₂ – Sahara

Oleanolic acid

- cleaning from accompanying substances: precipitation of oleanolic acid as a result of

- changing the solvent (diluting the alcohol extraction with water and cooling);
- dissolution of oleanolic acid in a hot mixture of methyl and isobutyl alcohols (1:1,5);
- quantitative determination: titration with a solution of sodium hydroxide (0.1 mol / l) in a mixture of methyl alcohol and benzene:



The equivalence point is determined potentiometrically.

2. Spectrophotometric method [5,6]: Based on the ability of saponins and their colored complexes to absorb monochromatic light at a certain wavelength. The method is proposed for determining the content of saponins in the following types of raw materials:

a) rhizomes with roots of Nippon Dioscorea. Acid hydrolysis of saponins is carried out, followed by the reaction of free aglycon (diosgenin) with p-dimethylaminobenzaldehyde. A colored complex is formed;

b) licorice roots. The precipitation of glycyrrhizic acid is carried out with a concentrated solution of ammonia. The precipitate is dissolved and the optical density of the resulting solution is determined.

3. Gravimetric method - determination of extractives. The method is based on the determination of the dry residue after drying, the sum of substances extracted from the raw material with the appropriate extractant. The method is proposed for assessing the quality of raw materials of ginseng, kidney tea, cyanosis, licorice.

We have studied the possibility of direct UV spectrophotometric determination of triterpene saponins having trisubstituted unsaturated bonds in the structure of aglycones. A similar structural fragment is present in the aglycone of saponins - derivatives of oleanolic acid at positions 12, 13, which suggests the possibility of using UV spectrophotometry to determine this substance in solutions.

Results

In the course of the work carried out, the possibility of direct quantitative UV-spectrophotometric determination of the isolated substance was investigated using its aglycone - oleanolic acid as a standard sample. The optimal conditions for the spectrophotometric analysis were selected. The possibility of using the technique for the determination of saponins in medicinal plant raw materials is shown.

Oleanolic acid, which contains 98% of the main substance, was used as a working standard sample (RSO).

To obtain additional information about the used PCO, a quantitative determination of the content of oleanolic acid was carried out by potentiometric titration in a medium of 95% ethyl alcohol. The control of purity (impurities of saponins) was carried out by TLC in solvent systems: petroleum ether - chloroform - acetone, 20: 20: 5 (I), n-butanol - ethanol - ammonia, 7: 2: 5 (II). The developer is a 20% alcohol solution of phosphotungstic acid. The chromatograms obtained showed one bright crimson spot with R_f (I) = 0.62, R_f (II) = 0.81, corresponding to oleanolic acid.

In the IR spectrum of the obtained PCO of oleanolic acid, there are no absorption bands in the frequency range 1120–1040 cm^{-1} , assigned to the stretching vibrations of the ether C – O– and hydroxyl – OH groups characteristic of

glucopyranoses, which are part of the carbohydrate moiety of the initial saponin molecules. The absorption band at 1726 cm^{-1} , inherent in the ester group (glucuronoside bond), is also absent in the spectrum of the oleanol band. The melting point (solvent - ethanol) 301 ° C and specific rotation (solvent - chloroform) + 79 ° of the aglycone saponin sample were determined. The correlation of the obtained results with the literature data allows one to classify oleanolic acid as a standard sample and use it in the development of a method for the determination of triterpene saponins (Table 1.).

Spectrophotometric measurements were carried out using an SF-46 spectrophotometer. A saponin solution was prepared with a concentration of 0.1 ml / ml (with different ratios of ammonia buffer - water) to record the absorption spectrum in the wavelength range of 180–260 nm.

The initial solutions of oleanolic acid were prepared by dissolving an accurate weighed portion of the samples, followed by the preparation of a series of working solutions by diluting the initial solutions to the required concentration with an ammonia buffer, based on the ratio of the aqueous phase - buffer 1: 1.

Table 1.
Physical and chemical characteristics of a standard sample saponins - oleanolic acid

Criterion	Analysis method	results research	Literature data
Molecular weight	UV spectrophotomerism	456.1 g / mol	456 g / mol
Appearance		White crystalline	White crystalline

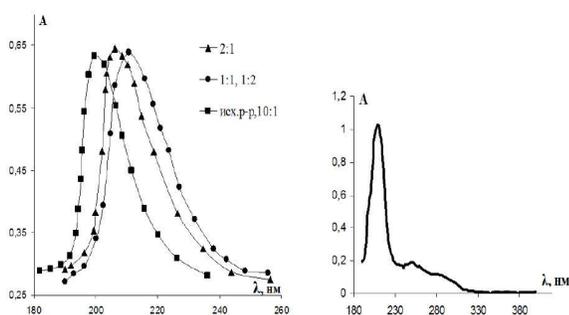
		powder, odorless and tasteless	powder, odorless and tasteless
Dissolve bridge -		Easily soluble in chloroform, alcohol, acetone, benzene and the air. Insoluble in water.	Easily soluble in chloroform, alcohol, acetone, benzene and ether. Insoluble in water.
Quality composition	IR spectroscopy	Bands 1726 cm^{-1} , 1120-1140 cm^{-1} are absent	No bands 1726 cm^{-1} , 1120-1140 cm^{-1}
	melting temperature (solvent - ethanol)	301 $^{\circ}\text{C}$	300-304 $^{\circ}\text{C}$
	specific rotation (solvent - chloroform)	+79 $^{\circ}$	+74-+80 $^{\circ}$
Main content	titrimetry	98%	98-100%
Availability impurities	TLC (Sorbfil 10 × 10 plates)	one zone Rf = 0.89 one zone Rf = 0.62	butanol - water - acetic acid (4: 1: 5) Rf = 0.89

		one zone Rf = 0.68	petroleum ether chloroform-acetone (20: 20: 5) Rf = 0.62 butanol - ethanol - ammonia (7: 2: 5) Rf = 0.68
Definiton purity	UV spectrophotometry	Water solution ammonium salt has a max at $\lambda = 210 \text{ nm}$ ($\epsilon = 6822 \text{ dm}^3 / (\text{mmol} \cdot \text{cm})^{-1}$).	Alcohol solution aglycone has a maximum at $\lambda = 204 \pm 2 \text{ nm}$ ($\epsilon = 6500-7000 \text{ dm}^3 / (\text{mmol} \cdot \text{cm})^{-1}$)

The working range of oleanolic acid concentrations was 0.01–0.4 mg / ml. A mixture of distilled water - ammonia buffer (1: 1) was used as a reference solution.

The authors of [7] note that the absorption region of compounds containing double bonds, as well as oxygen-containing carbohydrate rings and carboxyl groups conjugated with them, should be outside the working range of conventional spectrophotometers (165–200 nm). It was found experimentally that the absorption band of solutions of the studied saponins in

aqueous and aqueous-alcoholic solvents is located at $\lambda = 198\text{--}200$ nm, ie. in an area of little use for quantitative analysis. For compounds with multiple-bonded heteroatoms in the molecular structure, the shift of the absorption maximum to the long-wavelength region of the spectrum is possible with a change in the pH of the medium [7]. Since carboxyl groups were present in the molecular structure of the studied saponins, optimal results could be achieved by providing an alkaline reaction of the solvent medium. This problem was solved by adding ammonia buffer with pH = 10 to the solutions of saponins and oleanolic acid, in which the substances under study were converted into a salt (ammonium) form. Preliminary experiments to determine the optimal ratio of ammonia buffer and saponin solution (Fig. 1) made it possible to achieve a bathochromic shift of the absorption maximum from 199 to 210 nm at a ratio of 1:1 saponin solution to ammonia buffer. led to a shift of the absorption maximum to the long-wavelength region, as the optimal ratio was chosen 1:1, which allows you to obtain a stable position in the spectrum and intensity of the absorption maximum at $\lambda = 210$ nm. Under similar conditions (the ratio of the analyzed solution - buffer 1:1), we recorded the spectrum of the aglycone solution of the studied saponins - oleanolic acid and found that the absorption maximum of aglycone is also at $\lambda = 210$ nm (Fig. 2).



Rice. 1. UV spectra of solutions of saponin with ammonia buffer in the ratio: initial solution; 10:1; 2: 1; 1: 1, 1: 2, respectively, (concentration) of saponin - 0.1 mg / ml	Rice. 2. UV spectrum of a solution of oleanolic acid, C - 0.09 mg / ml
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The presence of a general, stable under the selected conditions, absorption maximum in the UV spectra of saponin and its aglycone indicates that only the aglycone of saponin is responsible for light absorption in this region of the spectrum, the sugars in it are optically transparent. The quantitative content of saponin in this case can be determined in terms of its aglycone.

Quantitative analysis method: 0.2 g of air-dry raw material was exhaustively extracted with a mixture of ethanol - acetone 1: 4 (control of the completeness of extraction was carried out by TLC under the conditions described above). To 50 ml of the obtained extract was added water acidified with HCl to pH = 1.0. The precipitate that formed was filtered off, washed on a filter with water, dried, dissolved in 25 ml of a mixture of ammonia buffer - water (1: 1) in a 25 ml volumetric flask and kept for 10 min. The optical density was determined in the region $\lambda = 210$ nm on a spectrophotometer in a cuvette with a layer thickness of 10 mm.

The results of determining the content of triterpene saponins in the herb of Turkestan motherwort and the metrological characteristics of the analysis method are presented in Table 2.

Table 2.

Results of determining the accuracy of the SF method

№	Content of triterpene saponins, %	di	Metrological characteristics
1	0,201	0,02	$\bar{X} = 0,2\%$; $f=4$; $t(P;f)=2,78$; $S^2=0,0000025$; $S=0,00158$; $\Delta \bar{X} =$ $0,0019$; $\pm \mathcal{E}$ $=0,98\%$
2	0,202	0,001	
3	0,198	0,00	
4	0,199	0,001	
5	0,200	0,002	

Validation of the developed method for the quantitative analysis of triterpene saponins

In accordance with modern requirements for the production of drugs, it is necessary to use validated analytical methods.

Validation (assessment of suitability) of an analysis method is an experimentally substantiated proof of its suitability, which includes an interconnected system of characteristics, specificity, suitability of a chromatographic system, linearity, correctness and reproducibility to obtain results with sufficient accuracy and precision. New analytical methods are subject to validation, as well as those that are used in the development and determination of indicators and quality standards for pharmaceutical products.

According to modern requirements, all methods for the quantitative determination of substances should be tested for such validation characteristics as: Specificity, Precision, Linearity, Accuracy, Range of Application (scope, Range).

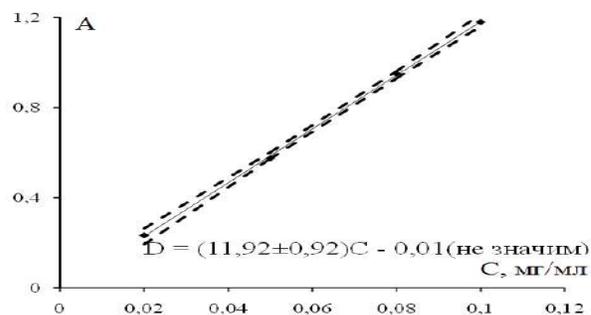
1. The specificity of the method is the ability (reliably) to determine the analyte in the composition of the Lf, in the presence of other accompanying substances and other related compounds.

To confirm the specificity of the developed technique, we carried out a quantitative determination of: a) the test solution, b) the PCO solution of the substance, c) the sample solvent (blank), e) the solution of the model mixture.

Preparation of model mixtures: 0.01 g (accurately weighed) of PCO of oleic acid was added to the alcoholic extract, dissolved and analyzed.

Mix No. 2-8. To the tested samples of the alcoholic extract, 70, 80, 90, 100, 110, 120, 130% oleic acid were added, in relation to the declared content of triterpene saponins in the medicinal plant material, dissolved and analyzed according to the above method.

2. The linear dependence of the developed technique lies in the direct proportionality of the increase in the peak area in the chromatogram with an increase in the amount of the analyte in the test samples. This validation characteristic was investigated on model mixtures No. 2-8 in the range of 70-130% of the declared content of triterpene saponins in the alcoholic extract of Turkestan motherwort (content of triterpene saponins 0.24%). The dependence of the analytical signal on the content of analytes (in $\mu\text{g} / \text{ml}$) is shown graphically in Fig. 3.



Rice. 3. Calibration curve of the dependence of the optical density of solutions of oleanolic acid on the concentration with regression bands

$$y = bx + a \quad (1)$$

$$y = 11,916 \cdot x - 0,0101$$

$$a = \frac{\sum_i^m y_i - b \sum_i^m x_i}{m} \quad (2)$$

where, y is the optical density, x is the concentration, mg / ml.

Результаты изучения зависимости между площадью пиков олеановой acid from its concentration showed that it is linear within the concentration range from 0.02 to 0.1 $\mu\text{g} / \text{ml}$. The detection sensitivity is 0.01mg / ml. The linearity correlation coefficient was 0.997 (formula 3), which is acceptable for the methods developed for medicinal products of natural origin.

$$r = \frac{m \sum_i^m x_i * y_i - \sum_i^m x_i \sum_i^m y_i}{\sqrt{\left[m \sum_i^m x_i^2 - \left(\sum_i^m x_i \right)^2 \right] \left[m \sum_i^m y_i^2 - \left(\sum_i^m y_i \right)^2 \right]}} \quad (3)$$

For statistical processing, at the first stage, the significance of the coefficient b (formula 4) of the calibration function was checked.

$$b = \frac{m \sum_i^m x_i * y_i - \sum_i^m x_i \sum_i^m y_i}{m \sum_i^m x_i^2 - \left(\sum_i^m x_i \right)^2} \quad (4)$$

According to the calculation results, the coefficient $b = 0.0101$ is insignificant, therefore, instrumental and experimental noises, as well as other random influences, do not bring a noticeable effect to the signal. Homogeneous values of the standard deviation of the

measurement results of each concentration made it possible to calculate the residual standard deviation and assess the adequacy of the calibration model by comparing the variance of adequacy and the variance of reproducibility by Fisher's test. The difference is not significant, which confirms that the chosen model is adequate [3].

The application of the principle of propagation of errors made it possible to estimate the influence of the variance of the values of D on the values of a and b and to calculate the variance of the constants. The obtained values suggest the existence of a confidence interval for the coefficient a , on the basis of which the regression band (corridor) for the calibration curve of the technique was built (Fig. 4.3.). To assess the quality of the analytical model, the metrological characteristics were calculated, the values of which are given in Table 3.

Table 3.
Metrological characteristics of the method for the quantitative determination of triterpene saponins

Время анализа, ч	Метрологические характеристики						
	S	S _y	S _c ·10 ³	r	a	Предел обнаружения сапонинов, мг/см ³	Рабочий диапазон концентрации сапонинов, мг/см ³
1,0	0,020	0,017	1,5	0,99	11,92	0,010	0,02-0,20

Legend: S - standard deviation of results (reproducibility variance), mg / ml; S_y — residual standard deviation (variance of adequacy), mg / ml; S_c is the standard deviation of the method S_c = S_y / a, mg / ml;

r is the correlation coefficient; a - sensitivity coefficient, conventional units (optical density) / (mg / ml).

3. The correctness (accuracy) of the method shows the systematic errors of the method and is expressed as the percentage of regeneration of an accurately weighed amount of

the analyzed sample. The correctness of the proposed method was established by the placebo method according to the results of the analysis of the test sample using the PCO of oleanic acid for 3 repeated determinations of 7 analytical concentrations. The results of the studies carried out are presented in table 4.

In this case, a given weight of 100% of the test sample is 100 g; the content of triterpene saponins in the test extract is 0.20 g / 100 g.

Table 4.
Results of assessing the correctness of the methodology

The specified number of active substances from the declared in the test sample, %	Oleanic acid content, g	Found, gr	Regeneration *, %
70	0,140	0,139	99,30
80	0,160	0,158	98,75
90	0,180	0,178	98,8
100	0,200	0,198	99,5
110	0,220	0,219	99,8
120	0,240	0,239	99,5
130	0,260	0,258	99,3

* Average of 3 definitions.

According to the data presented in Table 4, the technique has satisfactory accuracy. The average regeneration value is 99.3%. All the data obtained are in the range from 98.75 to 99.8%.

The narrow range of the regression band, the relatively small value of the standard deviation of the method, the maximum correlation coefficient indicate the high accuracy and correctness of the results obtained for the analysis of saponins in terms of oleanolic acid.

The results obtained confirm the advisability of using a standard sample, aglycone, in the practice of analyzing saponins, which makes it possible to increase the accuracy and reliability of the corresponding analysis method.

4. Intralaboratory repeatability (convergence). In accordance with the ICH recommendation for validation of analytical methods, the next step is to prove the repeatability of the quantitation results within the laboratory. Convergence is characterized by obtaining comparable results when the same analyst is working on the same equipment within a short period of time. [8]

To assess the intralaboratory convergence of the results of quantitative determination in one laboratory, at least 9 test solutions were prepared in one day under the same conditions from one batch of dry extract, covering the concentration range normalized by the method. 3 test solutions were prepared at each of three concentration levels - 50, 100, 150% (of the concentrations of the standard sample). For each test solution (i), at least 3 chromatograms were obtained and the content of oleanic acid X_i , the average result X_{av} , the standard deviation - SD, the relative standard deviation - RSD of the individual result with a confidence interval $P = 95\%$ of the mean result, and the coefficient variations - CV and confidence range for mean value at $P = 95\%$ - ΔX . At the same time, $RSD < 2.0\%$.

$$SD = \sqrt{\sum_{i=1}^m (X_i - \bar{X})^2 - (m-1)}$$

$$\bar{X} = \frac{\sum_{i=1}^n X}{n}$$

$$CV = \frac{SD}{\bar{X}} \cdot 100 \%$$

The tests were carried out according to the above method. The test results are presented in table 5.

5. Intra-laboratory reproducibility (precision).

The next validation indicator is the within-laboratory reproducibility (precision) of the analysis method. It is characterized by the reliability of the analysis according to the degree of coincidence of the results of individual determinations with repeated use. In this case, the results were obtained in the same laboratory by different analysts on different instruments for 2 days when analyzing the same sample. Each of the solutions was prepared independently of other solutions in accordance with the procedure and chromatographed at least 3 times. Reproducibility was assessed by standard deviation - SD or relative standard deviation - RSD in% for a series of measurements (instrumental reproducibility criterion - coefficient of variation - 2%).

Table 5.
Determination of within-laboratory repeatability of analysis results

№	Alcohol extraction weight, gr	Found oleanic acid,		Statistical characteristics, %
		mg	%	
1	2,010	4,8	0,238	X=0,253; ΔX=0,0645; S ² =0,000225; SD=0,015; CV=1,9
2	2,002	5,1	0,254	
3	1,900	5,1	0,268	
4	2,504	6,0	0,239	X=0,239; ΔX=0,0172; S ² =0,000016; SD=0,004; CV=1,67,
5	2,505	6,1	0,243	
6	2,510	5,9	0,235	

7	3,000	7,2	0,240	X=0,247; ΔX=0,0276; S ² =0,00642; SD =0,0000415; CV=1,68
8	2,900	7,3	0,250	
9	2,850	7,2	0,252	

The relative error of the determination was calculated by repeating the analysis with the addition of precise weighed portions of the standard - oleanolic acid (the additive was introduced at the stage of purification of the extraction during reprecipitation in acidified water in order to exclude the possibility of a background effect of compounds coextracted with saponins). The relative determination error in the case of the analysis of plant raw materials was on average 0.98%, which is acceptable for the analysis of such a complex object.

Thus, the method developed by the SF for the validation characteristics is specific for the quantitative determination of triterpene saponins in the aboveground part of the Turkestan motherwort. It is characterized by correct accuracy and reproducibility, linear dependence in the analytical area in relation to the declared content of triterpene saponins, which makes it possible to use it for a reliable assessment of the quality of Turkestan motherwort. [9]

The results of the determination of triterpene saponins, derivatives of oleanolic acid, using the developed UV spectrophotometric technique, make it possible to predict the possibility of its application for other types of plant raw materials and phytopreparations obtained from it.

Conclusion

1. For the first time, an individual triterpenoid compound was isolated from the aboveground part of the Turkestan motherwort,

which, based on the study of physicochemical constants and chemical transformations (alkaline hydrolysis), the isolated substance was identified as 3 β -O-palmitoyl-28-[3'-palmitoyl- β -D-glucopyranosyl]-olean-12-en-28-oic acid.

2. The possibility of direct quantitative UV spectrophotometric determination of triterpenoid saponins using its aglycone - oleanolic acid as a standard sample - has been investigated. The optimal conditions for the spectrophotometric analysis were selected, the possibility of using the technique for the determination of saponins in a medicinal product and plant raw materials was shown.

3. The optimal conditions for obtaining a stable in the position in the spectrum and the intensity of the absorption maximum at $\lambda = 210$ nm of tri-terpene saponins and oleanolic acid (the ratio of ammonia buffer - test solution is 1: 1). Under the established conditions, a calibration dependence of the optical density of oleanolic acid solutions on concentration was constructed, and the metrological characteristics were calculated. The relative analysis errors were 6 and 3%, respectively. Using the developed technique, the content of triterpene saponins (0.2%) was determined, and the proposed technique was validated.

4. Determination of biological activity of dry extract obtained from the aboveground part of Turkestan motherwort was carried out. It has been shown to have a pronounced anti-inflammatory effect.

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