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QUANTITATIVE DETERMINATION OF THE TOTAL IRIDOIDS IN PLANTS OF THE GENUS *Lagochilus*

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Chromatophotocolorimetric and accelerated photocolorimetric methods of determining 8-O-harpagide and harpagide in the epigeal parts of the plants L. platycalyx *and* L. setulosus *have been developed using 8-O-acetylharpagide as the standard substance.*

The isolation from the herbage of plants of the genus *Lagochilus* of iridoid glycosides — 8-O-acetylharpagide and harpagide — has been reported previously [1]. The presence of pronounced cholagogic activity in them [2] has induced us to study the amounts of total iridoids in the epigeal parts of the plants under investigation.

Analysis of literature information showed that it is mainly spectral methods that are used for the quantitative determination of iridoids in plant raw material: photocolorimetry, densitometry, spectrophotometry, and chromatospectrophotocolorimetry [3-7].

In the development of a method for the quantitative estimation of total iridoids, we took as a basis the method of determining 8-O-acetylharpagide (1) and harpagide (2) proposed in [7].

During the development of a chromatophotocolorimetric method, we studied the following stages: extraction of the total iridoids from the raw material, separation of the total extractive substances with the aid of thin-layer chromatography, the elution of compounds (1) and (2) from the sorbent, and the photometry of colored products.

To determine the degree of comminution of the raw material we analyzed samples of the herbage of *L. platycalyx* with different degrees of grinding (particle sizes 1-5 mm). It was established that the optimum degree of grinding the raw material from the point of view of completeness of extraction and reproducibility of the results is grinding to particles passing through a sieve with apertures having a diameter of 2 mm.

For the extraction of the total iridoids from the plant material, we tested a number of solvents: water, ethyl alcohol (80% and 95%), and methyl alcohol, and also various methods of extraction: shaking, boiling in a flask with a reflux condenser, and extraction in a Soxhlet apparatus. The results of the experiments (Table 1) showed that complete extraction of the total iridoids from the raw material was achieved by extraction for three hours in a Soxhlet apparatus, by shaking for five hours, or by boiling with 80% ethyl alcohol in a flask with a reflux condenser three times for 30 minutes each. The completenes of extraction was monitored chromatographically. For further work we selected the fastest and most economical method — fractional thermal extraction.

In selecting a system for thin-layer chromatography it was found that the best separation of the iridoids from the accompanying substances was achieved in the solvent system chloroform—methanol—acetone (6:2:1) using as the solid phase silica gel L 5/40 μm (Czech Republic) (N61 sieve, 150-200 mesh). Compounds (1) and (2) were detected with a 1% solution of vanillin in concentrated sulfuric acid, which revealed the iridoids in the form of crimson spots. The iridoids were eluted by boiling in a flask with a reflux condenser in 15 ml of 80% alcohol for 1 h. The degree of desorption was established by chromatographing a solution of a standard sample of a 8-O-acetylharpagide (mp 154-156°C) followed by its quantitative determination in the eluate.

Subsequent determination was carried out as described in [7].

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TABLE 1. Selection of the Optimum Conditions for the Extraction of Iridoid Glycosides from the Herbage of *L. platycalyx*

Method of extraction	Ratio of raw material and extractant (80% alcohol)	Extraction time, h	Total iridoids, %
Shaking at room temperature	1:10	1	0.25
		2	0.37
		3	0.44
		4	0.50
		5	0.54
Extraction in a Soxhlet apparatus	1:15	1	0.43
		2	0.50
		3	0.54
		4	0.54
Boiling in a flask with a reflux condenser	I 1:10	0.5	0.23
	II 1:10	0.5	0.45(I+II)
	III 1:10	0.5	0.54(I+II+III)

TABLE 2. Metrological Characteristics of the Method

f	$\bar{X}$	S	P, %	$t(p, f)$	$\Delta\bar{X}$	E, %
5	0.340	$\pm 1.42 \times 10^{-2}$	95	2.57	± 1.48	± 2.74

TABLE 3. Results of the Quantitative Determination of the Total Iridoids in an Extract of *L. platycalyx* (experiments with additives)

Amount in 1 ml of extract, mg	Added, mg	Found, mg	Relative error, %
0.080	0.056	0.136	0
0.080	0.056	0.134	+ 1.49
0.080	0.077	0.160	- 2.56

In the study of the plant raw material it was found that the absorption spectra of the products of the interaction of the substances accompanying the iridoids in the herbage of the plants under investigation did not give with the vanillin reagent the absorption maximum at a wavelength of about 530 nm that is characteristic for iridoids. This enabled the time of analysis to be shortened and the desired components to be determined directly in the plant extracts.

To determine the metrological characteristics of the photocolorimetric method of analysis we analyzed a sample of the epigeal part of *L. platycalyx* in sextuplicate (Table 2). The error of a single determination with 95% probability amounted to ±2.74%.

A trial of the method by adding 8-O-acetyloharpagide to an extract of *L. platycalyx* (Table 3) showed that the relative error of the determination was within the range of the random error of the method and, consequently, there were no losses of the desired components during the process of analysis.

Samples of the epigeal parts of the plants *L. platycalyx* Schrenk., *L. inebrians* Bunge., and *L. setulosus* Red. were analyzed by the accelerated photocolorimetric mthod. The results of the analyses are given in Table 4.

TABLE 4. Results of the Determination of the Amounts of Iridoid Glycosides in Seeds of the Plants of the Genus *L. lagochilus*

Plant	Site and time of collection	Amount of total iridoids, %
Lagochilus platycalyx	Brichmulla, June, 1984	0.41
"	Brichmulla, June, 1988	0.54
Lagochilus inebrians	Samarkand province, June, 1993	0.19
Lagochilus setulosus	Brichmulla, June, 1986	0.22

EXPERIMENTAL

Analysis of the Epigeal Part of *L. platycalyx* by the Chromatophotocolorimetric Method. An accurately weighed 10.0 g sample of the air-dried comminuted raw material (particle size 2 mm) was extracted with 100 ml of 80% alcohol in the boiling water bath with a reflux condenser for 30 min. Then the mixture was left for 15 min and the liquid was poured off through a POR 100 glass filter with avoidance of the passage of particles of the raw material on to the filter. The extraction was repeated three times. The combined extracts were evaporated in a rotary evaporator to a volume of about 10 ml and were then transferred quantitatively into a 25-ml measuring flask and the volume was made up to the mark with the same solvent (solution A).

With a micropipette, 0.1 ml of solution A was deposited on each of the first three bands of a 20 × 20 cm plate with a fixed layer of silica gel divided into five bands, and on the fourth band 0.1 ml of a solution of a standard sample of 8-O-acetylharpagide, while the fifth band was left for a control experiment. The plate was dried in the air for 1 h and chromatographed by the ascending method in a chamber with a mixture of the solvents chloroform, methanol, and acetone (6:2:1). Then the plate was dried in the air to eliminate the solvents.

The zones of the iridoids were detected by spraying the first band on the plate with a 1% solution of vanillin in concentrated sulfuric acid. The iridoid zones turned crimson. After the revelation of the spots, the zones of the iridoids in the other bands were marked and they and the zone from the control experiment were removed and each was eluted with 15 ml of 80% alcohol with heating in the water bath for 1 h.

To 1.5 ml of the filtered eluate was added 1.5 ml of a 2% alcoholic solution of vanillin and 4 ml of concentrated orthophosphoric acid. After 20 min, the optical density of the colored solution obtained was determined on a photocolorimeter against a background of the control experiment at a wavelength of about 530 nm. The amount of total alkaloids in the raw material (X, %), calculated as 8-O-acetylharpagide was determined from the formula

$$X = \frac{m_0 \cdot D \cdot 100 \cdot 100}{m \cdot D_0 (100 - W)},$$

where m_0 is the weight of 8-O-acetylharpagide, g;

m is the weight of raw material, g;

D_0 is the optical density of the solution of the standard sample of 8-O-acetylharpagide;

D is the optical density of the solution under investigation; and

W is the loss in weight on drying, %.

Preparation of the Standard Solution of 8-O-Acetylharpagide. An accurately weighed 0.050 g sample of 8-O-acetylharpagide was dissolved in 95% alcohol in a 25-ml measuring flask and the solution was made up to the mark with the same alcohol and mixed.

Analysis of the Herbage of *L. platycalyx* by the Photocolorimetric Method. A 25-ml measuring flask was charged with 0.25 ml of solution A, the volume was made up to the mark with 95% alcohol, and the mixture was stirred. The further procedure was as described above. The percentage of iridoids was determined from a calibration graph.

Construction of the Calibration Graph. Amounts of from 0.25 to 1.0 ml of the solution of the standard sample of 8-O-acetylharpagide were added to 25-ml measuring flasks. The color reaction was carried out as described above and the calibration graph was plotted from the results obtained.

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