SUMMARY

The presence of a 2"-hydroxypropenyl grouping at $C_{5'}$ of sachalinin appreciably changes its fragmentation as compared with columbianetin, which contains a hydroxypropyl radical in the same position.

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SPECTROPHOTOMETRIC DETERMINATION OF MANGIFERIN

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Mangiferin – 2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone – possesses a high biological activity [1]. At the present time a pharmacological study of mangiferin is being performed, in view of which the necessity has arisen for a method of determining it quantitatively in raw materials and powders.

We have developed a spectrophotometric method for determining mangiferin in a crystalline powder and in the epigeal part of two species of sweetvetch: Hedysarum alpinum L. and H. flavescens Rgl. et Schmalh. In addition to mangiferin, the epigeal part of the sweetvetch contains a number of other xanthone derivatives, including isomangiferin, and therefore we used thin-layer chromatography to separate the mixture of xanthones. This was performed on plates coated with layers of Merck and Filtrak celluloses and prepared by the KhNIKhFI [Khar'kov Scientific-Research Institute of Pharmaceutical Chemistry] method [2] with 15% acetic acid as the mobile phase.

The UV spectrum of mangiferin has a number of strong absorption bands. The most convenient for the quantitative determination of the substance is a band with its maximum at 369 nm and a specific adsorption coefficient of 295 ± 0.92 (mean of 20 independent determinations). In the region of working concentrations, the absorption of mangiferin solutions obeys the Lambert–Beer law.

Experiments were also performed on the determination of mangiferin in a crystalline powder. The number of replicates n = 12; we found for mangiferin m = 99.28%, and the relative error $\varepsilon = \pm 0.34$ at $\Sigma m^2 = 3.0999 \cdot 10^4$, $S = 15.32 \cdot 10^{-2}$.

In this case, the relative accuracy is not less than ± 0.34%. To obtain a result with a relative accuracy of ± 1%, two determinations are sufficient.

The results of a determination of mangiferin in the epigeal part of the sweetvetch in duplicate are given below (%):

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Raw Material	Mangiferin content	Mean	Deviation from the mean
H. flavescens	1.390	1.410	−1.41
	1.430		+1.41
	1.370	1.375	−0.36
	1.380		+0.36
H. alpinum	0.830	0.840	−1.19
	0.850		+1.19
	1.200	1.180	+1.69
	1.160		−1.69

The deviation from the mean for two determinations does not exceed ± 1.69%, which shows the satisfactory reproducibility of the method.

To determine the relative accuracy of the method we performed a quantitative determination of a sample of sweetvetch. The number of replicates n = 6, the amount of mangiferin found was m = 27.46 mg, and the relative error ε was ± 2.63 at $\Sigma m^2 = 2.3736$, S = 0.2812.

The relative accuracy of the method at a confidence level of 95% is ± 2.63%. Three determinations are sufficient to obtain a result with a relative accuracy of 4%.

To determine the accuracy of the method we performed experiments with additions of pure mangiferin to an extract from sweetvetch herbage:

Amount of pure mangiferin added, mg	Nominal, mg	Found (mean of three determinations), mg	Relative error,
—	15.36	15.36	—
0.46	15.82	15.71	0.70
0.79	16.15	15.98	1.05
1.12	16.48	16.33	0.91

The mean relative error of three determinations does not exceed 1.05%.

EXPERIMENTAL

The specific absorption coefficient was determined for a chromatographically pure sample of mangiferin with mp 260°C.

Determination of Mangiferin in a Powder. A 1-mg sample of mangiferin (accurately weighed) was as dissolved in tetrahydrofuran–water (1 : 1) in a 25-ml measuring flask, and the solution was made up to the mark with the same mixture (solution A). To 1 ml of solution A was added 5 ml of tetrahydrofuran –water (1 : 1), and the optical density of the solution was determined in an SF-16 spectrophotometer in a cell 1 cm thick at a wavelength of 369 nm.

The percentage of mangiferin was calculated from the formula

$$x = \frac{1000 \cdot V \cdot n \cdot D_{369}}{(D_{1\,cm}^{1\%})_{369} \cdot P \cdot l},$$

where V is the volume of solution A, ml; n is the dilution factor; P is the weight of the mangiferin, mg; l is the layer thickness, cm; and $D_{1\,cm}^{1\%}$ is the specific absorption coefficient, 295.

Determination of Mangiferin in Sweetvetch Herbage. The comminuted herbage (1 g, accurately weighed) with a particle size of 1-2 mm was covered with 30 ml of methanol and the mixture was boiled under reflux for 2 h. The contents of the flask were cooled at 20°C, the solution was filtered through a paper filter, and 20 ml of the methanolic extract was evaporated to dryness. The dry residue was dissolved in 5 ml of dioxane–water (1 : 1), and 0.01-0.02 ml of the solution obtained was deposited on a chromatogram and chromatographed in a thin layer of cellulose in 15% acetic acid as mobile phase for 30-40 min.

The chromatogram was dried in the air for 30 min and then in a drying chest under vacuum at 50°C for 1-1.5 h. It was examined in UV light and the mangiferin spot with R_f 0.51 was outlined (in UV light mangiferin possesses a yellow-orange fluorescence). The outlined section of the cellulose was transferred quantitatively

to a 10- to 15-ml flask, 5 ml of tetrahydrofuran–water (1 : 1) was added, and the mixture was left at room temperature for 2 h. Then the solution was filtered through a paper filter and the optical density of the eluate was determined (as described above) against an eluate of an equal amount of cellulose from the same plate.

The amount of mangiferin corresponding to the optical density of the eluate was found from a calibration graph. The percentage of mangiferin was calculated from the formula

$$x = \frac{a \cdot V}{B \cdot P \cdot 10000},$$

where a is the amount of mangiferin in the chromatogram spot found from the calibration curve, μg; V is the volume of the solution obtained from the dissolution of the dry residue, ml; B is the volume of solution deposited on the chromatogram, ml; and P is the absolutely dry weight of the raw material, g.

Note. Plotting of the Calibration Curve. An accurately weighed sample of about 0.005 g of mangiferin was dissolved in 5 ml of dioxane–water (1 : 1) and 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, 0.04, 0.045, and 0.05 ml portions of this solution, corresponding to 10, 15, 20, 25, 30, 35, 40, 45, and 50 μg mangiferin, were chromatographed. The subsequent procedure was as described in the method for determining mangiferin in sweetvetch herbage.

Preparation of the Plates. A mixture of 2 g of cellulose and 10 ml of water was shaken and the suspension was poured in a uniform layer on a glass plate with dimensions of 17 × 12 cm. The plate was dried in the air for 15 h.

SUMMARY

A spectrophotometric method is proposed for determining mangiferin in a crystalline powder and in sweetvetch herbage. The relative accuracy of the method is ± 0.34% for the powder and ± 2.63% for the herbage.

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