FLUORODENSITOMETRIC DETERMINATION OF MANGIFERIN AND ISOMANGIFERIN IN *Hedysarum flavescens* and *H. alpinum*

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The direct spectrophotometric determination of mangiferin and isomangiferin in the raw material is difficult because it contains a number of natural xanthones and flavonoids having similar spectral characteristics [1]. A chromatospectrophotometric method of determining mangiferin in *Hedysarum flavescens* Rgl. et Schmalh and *H. alpinum* has been developed previously, the error in this determination being ±4% [2]. In the present paper we propose a fluorodensitometric variant of the method, which is necessary for the rapid analysis of a large number of samples in order to determine the content of mangiferin and isomangiferin in raw material collected at different periods and from different growth sites and also in breeding investigations. This method of analysis possesses great sensitivity.

We used a Hitachi MPF-2A spectrofluorimeter equipped with a densitometric device enabling emission and absorption spectra of fluorescent substances to be obtained directly from chromatograms. The proposed method was compared with the spectrophotometric method [2] (%):

Plant	Spectrophotometric method	Fluorodensitometric Method
H. flavescens, 1922 harvest (Central Asia)	0,824	0,835
The same, 1974 harvest	1,224	1,219
H. alpinum, 1973 harvest (Siberia)	1,833	1,819
The same, 1975 harvest	1,910	1,925

As the figures given above show, the two methods have practically the same reproducibility and accuracy.

The fluorodensitometric method has been used for determining the amounts of isomangiferin in samples of *H. alpinum* (from 0.05 to 0.07%) and *H. flavescens* (from 0.08 to 0.1%).

EXPERIMENTAL

Chromatographically pure samples of mangiferin and isomangiferin with mp of 260° and 250°, respectively, were used as standards,

The comminuted herb (1 g, accurately weighed) with a particle size of about 1 mm was boiled with 30 ml of methanol for 2 h. Then the contents of the flask were cooled to room temperature and filtered through a paper filter.

The methanolic extract (10 ml) was evaporated to dryness. The dry residue was dissolved in 50 ml of a 10% solution of dioxane in ethanol and 3 µl of the resulting solution was deposited on a chromatogram (20 × 20 cm, cellulose). Solutions of standard substances in amounts of 0.2, 0.3, and 0.4 µg were deposited alongside, and chromatography was performed in the 15% acetic acid system. The chromatograms were dried in the air for 30 min and then under vacuum at 50°C for 1-1.5 h and were sprayed with a 3% ethanolic solution of aluminum chloride; after 30 min, densitometry was performed in the attachment to the spectrofluorimeter at a wavelength of the exciting light of 370 nm and with the emission monochromator adjusted to a wavelength of 470 nm. The sensitivity of the amplifier was ×1, and the width of the aperture slit 1 mm. The areas delimited by the curves on the paper strip were calculated by the triangle formula.

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To calculate the amounts of the substances being determined, we made use of the results obtained in the scanning of the spots containing the markers, and from these results (i.e., the areas under the curves) we plotted a calibration graph separately for each plate. The content of mangiferin and isomangiferin (as a percentage) was calculated from the formula

$$x = \frac{a \cdot y \cdot 100 \cdot 3}{b \cdot p \cdot 10^6}$$

where a is the content of mangiferin or isomangiferin in a spot of the chromatogram found from the calibration graph, μg; y is the volume of the solution obtained on dissolving the dry residue, ml; b is the volume of the solution deposited on the chromatogram, ml; and p is the weight of the sample of raw material, g.

Preparation of the Plates. A suspension formed by shaking 4 g of cellulose with 15 ml of ethanol was deposited on a glass plate with the aid of an automatic device for depositing thin layers from the firm Labor (Hungary). The plates were dried at room temperature for 2 h.

Plotting of the Calibration Graph. An accurately weighed sample of mangiferin or isomangiferin (0.005 g) was dissolved in 50 ml of a 10% solution of dioxane in ethanol. At the starting line of the plate were deposited 1, 2, 3, 4, 5, and 6 μl of the initial solution, containing 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 μg of the substance. The plates were chromatographed, dried, sprayed with a solution of $AlCl_3$, dried again, and subjected to densitometry. From the values of the areas under the curves on the paper strip obtained, calibration graphs were plotted with the amount of substance (μg) along the axis of abscissas and the areas under the curves along the axis of ordinates.

SUMMARY

A fluorodensitometric method has been proposed for determining mangiferin and isomangiferin in *Hedysarum flavescens* and *H. alpinum*. The relative accuracy of the method for the raw material is ±4.8%.

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