

QUANTITATIVE ANALYSIS OF PINOCEMBRIN

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A procedure has been developed for the chromato-spectrometric determination of pinocembrin in the substance isolated from the liquorice plant.

Pinocembrin is a flavonoid isolated from common liquorice, *Glycyrrhiza glabra* L., fam. Fabaceae [1, 2], that possesses pronounced biological activity [3].

At the present time a technology for the isolation of pinocembrin from the epigeal part of the liquorice plant has been developed in the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. The raw material supplies of liquorice in the Republic of Uzbekistan amount to hundreds of thousands of hectares [4, 5].

We set ourselves the task of developing a procedure for the quantitative determination of pinocembrin in the technological material, using a spectrophotometric method of analysis.

The quantitative of pinocembrin determination as such was carried out on a SF-26 spectrophotometer with cells having a layer thickness of 1 cm in the 250-350 nm region. The UV spectrum of a solution of the preparation in 96% ethanol has two characteristic absorption maxima, at 292 ± 2 and $362 \pm$ nm. Absorption maxima at 292 and 326 nm are also observed in the UV spectra of impurities, which are shown in Fig. 1. In the spectrophotometric determination of pinocembrin, therefore, the impurities must be eliminated. We have developed a chromato-spectrometric method for the determination of pinocembrin as such which permits the isolation of chromatographically pure pinocembrin and its quantitative determination by a spectrophotometric method.

An investigation of technological pinocembrin by preparative thin-layer chromatography on Silufol UV-254 in the chloroform-benzene-methanol (18:1:1) system showed that it contained, in addition to the main substance, with R_f 0.42, the accompanying substances glabranin and galangin, with R_f 0.45 and 0.32 ± 0.05 , and an unidentified flavonoid with R_f 0.28 ± 0.05 .

As standard pinocembrin, we used a sample obtained from the technical product by three recrystallizations from solution in 63% aqueous alcohol in the presence of type "A" activated carbon. When subjected to TLC analysis on a Silufol UV-254 plate, the standard pinocembrin, deposited in an amount of 100 μ g, did not show the presence of accompanying substances. The sample of standard pinocembrin consisted of white acicular crystals with mp 199-200°C.

The concentration of pinocembrin at which the Bouguer-Lambert-Beer law was satisfied (2-12 μ g/ml in ethanol) was found experimentally. The sensitivity of the determination was 1 μ g/ml.

To plot the calibration graph we used a solution of the standard pinocembrin with a concentration of 100 μ g/ml.

Preparation of the Standard Solution. In a 50-ml measuring flask, 0.005 g (accurately weighed) of standard pinocembrin was dissolved in 20 ml of 96% ethyl alcohol, and then the volume was made up of the mark with the same solvent and the solution was mixed.

Plotting the Calibration Graph. Seven 25-ml measuring flasks were charged with, respectively, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml of the standard solution obtained, and the contents of each were then made up to the mark with ethyl alcohol and were carefully mixed. The resulting pinocembrin concentrations were 0.8, 2, 4, 6, 8, 10, and 12 μ g/ml. After 120 min the optical density of each solution was measured in the spectrometer at a wavelength of 292 nm in a cell with a layer thickness of 1 cm, using ethyl alcohol as the standard of comparison.

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TABLE 1

Amount of preparation, μg	Found		Absolute error, %	Metrological characteristics
	μg	%		
20	18.6	93.0	-0.9	$\bar{X}=93.9\%$ $r=4$
20	18.9	94.5	+0.6	$Sx=0.29$
20	18.7	93.5	-0.4	0.95
				$\Delta X=\pm 0.81$
20	18.9	94.5	+0.6	$E_{\text{rel}}=0.63\%$
20	18.8	94.0	+0.1	

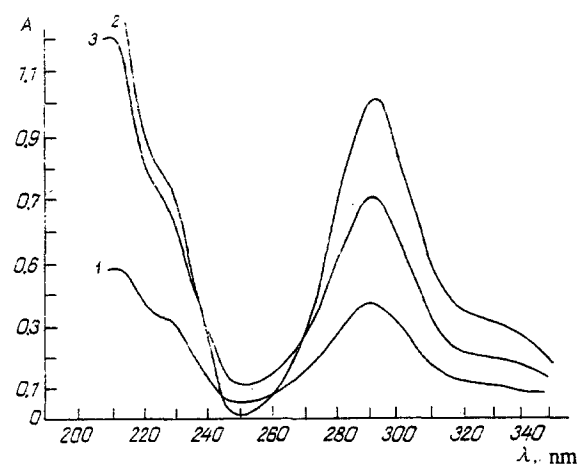


Fig. 1. Absorption spectra of alcoholic solutions of pinocembrin (2) and accompanying substances (1, 3).

Procedure for Determining Pinocembrin. A 0.01-sample of the preparation (accurately weighed) is dissolved in 96% ethyl alcohol in a 100-ml measuring flask, and the solution is made up to the mark with alcohol.

Two bands at a distance of 2 cm from one another are marked on a starting line 2 cm above the lower edge of a 7×14 cm Silufol plate. With a micropipet 0.3 ml (30 μg) of the standard pinocembrin solution is deposited on the first band and 0.3 ml of the solution of the pinocembrin under investigation on the second. After drying in the air, the plate is placed in a chromatography chamber with the solvent system chloroform-benzene-methanol (18:1:1). When the solvent front has risen 10 cm, the plate is removed from the chamber and, after the finishing line has been marked, it is left in the air for a few minutes for the solvent to evaporate. After this, the plate is irradiated with UV light. A pink spot appears at the level of the standard, with $R_f 0.42 \pm 0.05$. The spot is outlined on the chromatogram and is then cut out and eluted with 10 ml of ethyl alcohol. The solution is filtered, and its optical density is determined in the spectrophotometer at a wavelength of 292 nm, using 96% ethyl alcohol as the comparison solution. The amount of pinocembrin in the preparation, as a percentage (X, %) on the dry matter, is calculated from the formula

$$X = \frac{A \cdot m_0 \cdot 100 \cdot 100}{A_0 \cdot m \cdot (100 - W)},$$

where A is the optical density of the solution of the preparation under investigation;

A_0 is the optical density of the solution of the standard sample;

m_0 is the weight of the standard sample, g;

m is the weight of the sample of preparation under investigation, g;

W is the moisture content, on the dry matter, %;

100 is for conversion into percentages.

In Table 1, we give the results of a determination of pinocembrin in various series (mean results of three determinations) (\bar{X} is the arithmetic mean of the measurements; $S_{\bar{x}}$ is the standard deviation of the mean result; ΔX is the half-width of the confidence interval; and E_{rel} is the relative error:

It follows from the results obtained that a sufficiently accurate determination of the pinocembrin content of the technological substance and of medicinal forms is possible only after its preliminary separation from accompanying substances by TLC, followed by spectrophotometric determination. The method possesses adequate reproducibility and accuracy, and it can be used to determine the pinocembrin content of the technological substance.

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