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DEVELOPMENT OF A METHOD FOR THE QUANTITATIVE DETERMINATION OF NEFROTSIZIN (LUTEOLIN 7-O-β-D-GLUCOPYRANOSIDE) IN THE LEAVES OF Ferula varia

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A method is proposed for determining nefrotsizin (luteolin 7-O-β-D-glucopyranoside) in the radical leaves of Ferula varia. which is based on the extraction of nefrotsizin from the raw material, its identification by TLC, elution from the plate, and spectrophotometric determination.

Luteolin 7-O-β-D-glucopyranoside has been isolated in the Institute of the Chemistry of Plant Substances of the Uzbekistan Republic Academy of Sciences from radical leaves of Ferula varia (Schrenk.) Trautv, family Apiceae, and at the present time, under the name nefrotsizin (I), is undergoing clinical trials as a hyperazotemic agent [2].

Nefrotsizin is an odorless microcrystalline powder, yellow or grayish yellow with a greenish tinge. It is insoluble in water, very slightly and slowly soluble in alcohols, and most soluble in aqueous alcohols (60 and 80% ethanol, 80% methanol). The UV spectrum of (I) in 80% ethanol in the 250-400 nm region has absorption maxima at 256 and 253 nm and a shoulder at 268 nm.

The procedure that has been developed for the quantitative determination of (I) in the radical leaves of F. varia is based on spectrometry in combination with TLC. During the development of the method the following stages were studied: the extraction of (I) from the plant raw material; 2) its chromatographic separation from accompanying substances; and 3) the elution of (I) from the sorbent and its spectrophotometric determination.

For this study, as the standard substance we used (I) corresponding to the requirements of the draft Provisional Pharmaceutical Standard for this preparation.

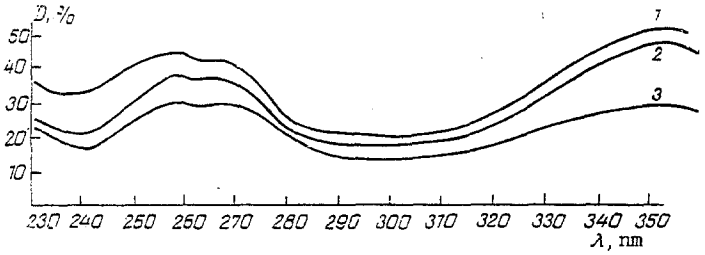

Fig. 1. UV spectrum of (I) in 80% ethanol: 1) solution of standard (I); 2) eluate of the zone of the standard sample of (I) from a plate; 3) eluate of the zone from a plant extract.

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TABLE 1. Dynamics of the Extraction of Nefrotsizin

Extraction time, min	Weight of raw material, g	Nefrotsizin content, %
30	4,13301	1,43
60	4,13301	1,65
90	4,13301	1,65
120	4,13301	1,65

TABLE 2. Metrological Characteristics of the Method at n = 6

f	X	$S_{\bar{x}}$	P	$t_{(P,f)}$	$\Delta\bar{X}$	E, %	E_1
5	1,65	0,039	95	2,57	0,1	±6,1	±3,53

TABLE 3. Results of Experiments with Additions of Nefrotsizin to an Extract of F. varia Leaves

Amount of nefrotsizin in the extract	Nefrotsizin added	Calculated	Mean value found from 3 determinations	Relative error
mg				
4,678	2,133	6,811	6,883	+1,05
	1,613	6,291	6,397	+1,67
	1,067	5,745	5,868	+2,14

The maximum at 353 nm is recommended as the analytical band. The high intensity of this band (log ε 4.44) ensures a high sensitivity of the method, and its shape – a fairly diffuse maximum – permits measurement to be made with the least error. Furthermore, solvatochromic effects are less pronounced in the long-wave absorption band of flavonoids [3]. In this analytical band, the absorption of a solution of (I) obeys Beer's law in the region of working concentrations of 2.5-10 mg/ml (D = 0.15-0.60).

We used TLC to separate the (I) from the accompanying substances. As the stationary phase we used commercial Silufol UV-254 (Czechoslovakia) and Sorbfil (Krasnodar) plates, with various solvent systems as the mobile phases. As a result of the investigations performed, we selected the stationary phase Sorbfil and the mobile phase chloroform–methanol–acetic acid (4:2:2). Under these conditions, the zone of (I) is fairly well separated from the accompanying phenolic compounds with R_f 0.62, and the time for running the chromatogram is 35 min. The individuality of the zone containing the (I) was shown by comparing the UV spectra of eluates from the zones of authentic pure (I) and the zone obtained on chromatographing the plant extract (see Fig. 1).

The extraction of the raw material was performed with boiling 80% ethanol in a flask with a reflux condenser. The ratio to solvent was 1:10. The dynamics of the extraction of (I) from the raw material by this method is shown in Table 1, from which it can be seen that the dynamic equilibrium between the liquid and solid phases was reached after 1 h.

The amount of (I) in the raw material was confirmed by extraction in a Soxhlet apparatus. The (I) content was 1.70%.

The optimum conditions for the elution of (I) from the sorbent were established by a study of the desorption of an authentic sample of (I) from the sorbent as functions of the time and the temperature. The results of the determination showed that a dynamic equilibrium between the solution and the sorbent was reached in 30 min, and the optimum temperature of the water bath was 60°C.

The metrological characteristics are given in Table 2. The error of a single determination at a confidence level of 95% and n = 6 does not exceed ±6.1, or, with analysis in triplicate, ±3.53. Experiments with addition of (I) to a plant extract showed the absence of a systematic error (Table 3).

EXPERIMENTAL

Procedure for Quantitative Determination. An analytical sample of the raw material was comminuted to particles passing through a No. 10 sieve with aperture dimensions of 1 mm according to GOST [State Standard] 214-77.

About 4 g (weighed to an accuracy of 0.01 g) was charged into a 100-ml round-bottomed flask and was covered with 40 ml of 80% ethanol. The flask was weighed with an error not exceeding 0.01 g and was attached to a reflux condenser and heated in the boiling water bath for 1 h from the moment of the boiling of the solvent in the flask. The flask with its contents was cooled to room temperature and reweighed, and its weight was brought up to the original level with 80% ethanol. The extract was filtered through a paper filter, the first 10 ml of filtrate being discarded.

A Sorbfil chromatographic plate (10 × 15 cm) was divided into four sections. At the starting lines of each of two of the sections 0.05 ml of the extract obtained was deposited in the form of a 3-cm band, and in the third section 0.05 ml (100 μg) of a solution of authentic (I), while the fourth section was left free (control). The plate with deposited samples was dried in air for 20 min and was chromatographed by the ascending method in a chamber previously saturated with the solvent mixture chloroform–methanol–acetic acid (4:2:2) for 30 min. When the solvent front had travelled 7 cm, the plate was removed from the chamber and dried in the air for 10-15 min, and the zones containing the (I) at the level of the spot of the marker were marked in UV light. The zones of the sorbent containing the (I) and the zone of the control experiment were transferred into 50-ml flasks with ground-in stoppers, each was covered with 10 ml of 80% ethanol, and elution was performed for 30 min with constant shaking in the water bath (t = 60°C). The eluates were filtered through glass filters (por 16), the first 10 ml of filtrate being discarded, and the optical densities of the solutions were determined against a background of the control sample in a spectrometer at a wavelength of 353 nm in a cell with a layer of thickness of 10 mm.

The percentage of (I) in the absolutely dry raw material was calculated from the formula

$$X = \frac{D_1 \cdot M_0 \cdot V_1 \cdot 100 \cdot 100}{D_0 \cdot M_1 \cdot V_0 \cdot (100 - W)},$$

where D_1 is the optical density of the solution under investigation;
D_0 is the optical density of the solution of the standard (I);
M_1 is the weight of the raw material;
M_0 is the weight of the (I), g;
W is the loss in weight of the raw material on drying;
V_1 is the total volume of the extract, ml; and
V_0 is the total volume of the solution of standard (I), ml.

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