SPECTROPHOTOMETRIC DETERMINATION OF PEUCEDANIN

B. A. Krivut and M. E. Perel'son

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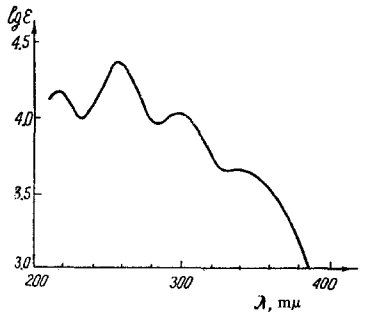
The furocoumarin peucedanin is used in medicine as an antitumoral and sensitizing agent [1-3]. A method for the quantitative determination of peucedanin based on its preparative isolation and subsequent titration has been described in the literature [4].

We propose a spectrophotometric method for determining peucedanin in a crystalline powder and in the roots of Peucedanum Morisoni Bess. (Morison's hogsfennel). In addition to peucedanin, the roots of this plant contain other natural coumarins [5, 6]. Consequently, in this method, thin-layer chromatography was used to separate the mixture of coumarin derivatives. Separation was carried out on a plate with a nonfixed layer of KSK silica gel in the petroleum ether–diethyl ether (1 : 2) system.

The UV spectrum of peucedanin (figure) has a series of strong bands due to transitions of the $\pi-\pi$ type [7]. For the quantitative determination of this substance, the most convenient band is that with a maximum at 298 mμ and a specific absorption coefficient of 401 ± 1.5 (mean of 20 independent determinations).



UV spectrum of peucedanin in 96 % ethanol.

In the range of working concentrations, the absorption of solutions of peucedanin obeys Beer's law.

We have performed experiments on the determination of peucedanin in the crystalline powder (Table 1). As follows from the table, the maximum relative error of the determination in this case is +2%.

Table 1. Results of Determination of Peucedanin

Amount taken, mg.	Amount found, mg (mean of two experiments)	Error	
		absolute, mg.	relative, %
1.091	1.105	+0.014	+1.28
1.110	1.102	−0.008	−0.72
0.926	0.931	+0.005	+0.54
1.002	0.987	−0.014	−1.32

To determine the completeness of the elution of peucedanin from the silica gel, experiments were carried out (in triplicate) involving the chromatography and subsequent quantitative determination of pure peucedanin (Table 2).

It can be seen from Table 2 that the relative error of a single determination is in the range from 0.8 to 3.2% and is always negative, which is due to the incomplete removal of the substance from the silica gel. The mean error of free determinations for four samples lies within fairly narrow limits (1.22–2.36%); this justifies the insertion into the formula for calculation of a correction factor of 1.018.

The exhaustive extraction of the total furocoumarins from the plant raw material took 3 hr.

Table 2. Results of the Quantitative Determination of Pure Peucedanin After Chromatography

Solution	Run	Amount taken, μg	Amount found, μg	Relative error, %: one determination	Relative error, %: mean from three determinations
1	1	10.087	9.880	−2.07	2.04
	2		9.980	−1.02	
	3		9.780	−3.04	
2	1	12.116	11.976	−1.15	1.22
	2		11.969	−1.21	
	3		11.958	−1.30	
3	1	20.196	19.760	−2.10	1.84
	2		200.005	−0.84	
	3		19.671	−2.59	
4	1	21.200	20.978	−1.52	1.99
	2		20.858	−1.61	
	3		20.596	−2.84	

The content of peucedanin in the roots of Morison's hogsfennel was determined in duplicate (Table 3). The deviation from the mean of two determinations did not exceed 2%, which shows the satisfactory reproducibility of the method.

Table 3. Results of the Determination of Peucedanin in the Roots of Morison's Hogsfennel, %

Number of the sample	Run	Peucedanin content	Mean	Deviation from the mean
1	1	1.33	1.335	−0.38
	2	1.34		+0.38
2	1	2.14	2.180	−1.83
	2	2.22		+1.83
3	1	1.82	1.855	−1.88
	2	1.89		+1.89

To determine the accuracy of the method we performed experiments with the addition of pure peucedanin to an extract of the roots of Morison's hogsfennel (Table 4). These experiments show that the mean relative error of three determinations does not exceed 2%.

Table 4. Results of Experiments with the Addition of Peucedanin to an Extract of the Roots of Morison's Hogsfennel

Amount added, mg	Nominal amount present, mg	Found (mean of three expts.), mg	Error: absolute, mg	Error: relative, %
—	20.86	20.86	—	—
0.58	21.42	21.20	0.22	1.02
1.57	22.43	22.30	0.13	0.50
3.42	24.28	23.88	0.40	1.64

It was interesting to compare the titration method [4] and the proposed spectrophotometric method for determining the content of peucedanin (Table 5).

As follows from the table, the titration method gives values 5–15% higher than those given by the spectrophotometric method, which is probably due to the inadequate separation of peucedanin from the other coumarin derivatives contained in the hogsfennel roots.

EXPERIMENTAL

A sample of peucedanin with mp 109° C (from CCl_4), the homogeneity of which was checked by chromatography,

was used.

Table 5. Results of a Comparison of the Spectrophotometric and the Titration Methods, %

Content of peucedanin		Difference, %
titration method	spectrophotometric method	
1.51	1.34	13.40
2.31	2.18	5.96
2.18	1.85	14.60
3.00	2.82	6.28

Determination of peucedanin in a powder. A 1-mg sample of peucedanin (accurately weighed) was dissolved in ethanol in 25-ml measuring flask and the solution was made up to the mark with ethanol (solution A). To 2 ml of solution A was added 10 ml of ethanol, and the optical density of the solution was determined on an SF-4A spectrophotometer in a 1-cm cell at a wavelength of 298 mμ. The percentage content of peucedanin was calculated from the formula

$$X = \frac{1000 \cdot V \cdot n \cdot D_{298}}{\left(D^{1\%}_{1\text{ cm}}\right)_{298} \cdot p \cdot l} = \frac{299 \cdot D_{298}}{p},$$

where V is the volume of solution A;

n is the dilution factor;

p is the weight of peucedanin, mg;

l is the thickness of the cell, cm.

Determination of peucedanin in hogsfennel roots. A 1-g sample of the comminuted hogsfennel roots (accurately weighed) was extracted with methanol in a Soxhlet apparatus for 3–3.5 hr (8–10 overflows). The methanolic extract was evaporated to dryness, and the dry extract was dissolved in 10 ml of methanol; 0.01–0.03 ml of this solution was chromatographed in a thin layer of KSK silica gel in the petroleum ether–diethyl ether (1 : 2) system. The silica gel was deposited on a plate by shaking it with chloroform. After chromatography (30–40 min) the chromatogram was observed in UV light. The peucedanin spot with R_f 0.49 (in UV light, peucedanin possesses a yellow fluorescence) was marked. This part of the silica gel was transferred to a 15–20 ml flask, treated with 10 ml of alcohol, and left for 12 hr; 5 ml of the resulting solution was filtered through dense filter paper into a cell with a layer thickness of 1 cm. The optical density of the solution was determined at a wavelength of 298 mμ against an eluate of an equal amount of silica gel from the same plate. The percentage content of peucedanin was calculated from the following formula:

$$X = \frac{1.018 \cdot V_1 \cdot V_3 \cdot D_{298}}{V_2 \cdot p \cdot \left(D^{1\%}_{1\text{ cm}}\right)_{298}},$$

where V_1 is the volume of the extract;

V_2 is the volume of the extract deposited on the chromatogram;

V_3 is the volume of the eluate;

1.018 is the correction factor; and

p is the weight of the raw material, g.

CONCLUSIONS

A spectrophotometric method has been proposed for determining peucedanin in a crystalline powder and in the roots of Peucedanum Morisoni Bess.

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