METHODS OF DETERMINING FERUTIN AND FERUTININ

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A considerable number of esters of phenolcarboxylic acids and sesquiterpene alcohols has been isolated from plants of the genus Ferula: some of them possess valuable biological properties [1-6]. In connection with the determination of their amounts in plant raw material, the necessity has arisen for the development of methods for their analysis. The group of substances mentioned is based on residues of vanillic, isovanillic, and p-hydroxybenzoic acids. These are responsible for intense absorption in the 260-280 nm region, which enables UV spectrometry to be used for analytical purposes.

The amounts of these compounds can, in principle, be determined in several ways – by direct spectrophotometry, colorimetrically after the diazo coupling reaction, etc.

We have developed three methods of determining two representatives of this group – ferutin and ferutinin.

The UV spectrum of ferutin (Fig. 1) has a maximum at 260 nm (log ε 4.38; $E^{1\%}_{1cm}$ 2750 ± 127), and that of ferutinin (Fig. 2) has a maximum at 261 nm (log ε 4.21; $E^{1\%}_{1cm}$ 4780 ± 234).

In the range of working concentrations from 0.01 to 0.1 γ/ml, the intensity of absorption of these substances obeys the Bouguer–Lambert–Beer law.

In order to determine the amounts of ferutin and ferutinin in a raw material they must first be separated chromatographically. We have tested a number of sorbents and systems, the best results having been obtained in thin-layer chromatography on KSK silica gel in the petroleum ether–ethyl acetate (3:1) system (R_f for ferutin 0.25 and for ferutinin 0.35). After the separation of the substances, they were eluted from the adsorbent by steeping in ethanol for 15 min at room temperature and were determined by method I. The relative error of the method amounted to ±3-3.5%.

The amounts of the substances mentioned can also be determined without chromatographic separation on the basis of a difference in the physicochemical properties of these substances. The first promising variant in this connection was the use of differences in their UV spectra in an alkaline medium.

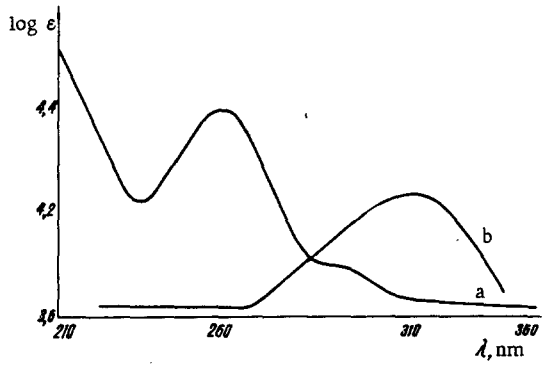

Fig. 1. UV spectra of ferutin in ethanol (a) and in the presence of KOH (b).

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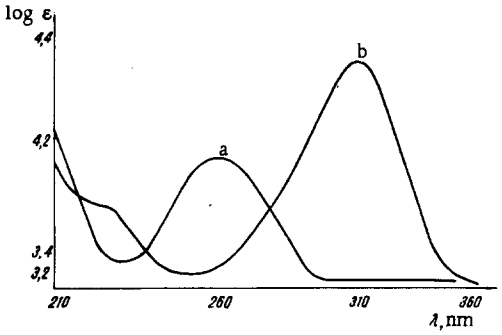

Fig. 2. UV spectra of ferutinin in ethanol (a) and in the presence of KOH (b).

The absorption maxima of the two substances in the presence of alkali undergo bathochromic shifts and are displaced to 310 nm. In this process, for ferutinin, as for a para-substituted phenol, the specific absorption coefficient rises from $(E^{1\%}_{1\,cm})^{260}_{B}$ 4780 to $(E^{1\%}_{1\,cm})^{310}_{B}$ 7714 ± 394.8, while for ferutin (meta substitution with respect to the carbonyl group) it falls from $(E^{1\%}_{1\,cm})^{260}_{A}$ 2750 to $(E^{1\%}_{1\,cm})^{310}_{A}$ 2386 ± 114. On the basis of these figures it is possible to draw up equations with two unknowns:

$$(E^{1\%}_{1\,cm})^{260}_{A} \cdot C_A + (E^{1\%}_{1\,cm})^{260}_{B} \cdot C_B = D^{260},$$

$$(E^{1\%}_{1\,cm})^{310}_{A} \cdot C_A + (E^{1\%}_{1\,cm})^{310}_{B} \cdot C_B = D^{310},$$

where $(E^{1\%}_{1\,cm})^{260}_{A}$ and $(E^{1\%}_{1\,cm})^{310}_{A}$ are the specific absorption coefficients of ferutin at the two wavelenths, C_A is its concentration, and $(E^{1\%}_{1cm})^{260}_{B}$ and $(E^{1\%}_{1cm})^{310}_{B}$ and C_B are the same for ferutinin. On solving these equations, we find

$$C_A = \frac{D^{260} - (E^{1\%}_{1cm})^{260}_{B} \cdot C_B}{(E^{1\%}_{1cm})^{260}_{A}} \quad (1)$$

$$C_B = \frac{D^{310}(E^{1\%}_{1cm})^{260}_{A} - (E^{1\%}_{1cm})^{310}_{A} \cdot D^{260}}{(E^{1\%}_{1cm})^{310}_{B} \cdot (E^{1\%}_{1cm})^{260}_{A} - (E^{1\%}_{1cm})^{310}_{A}(E^{1\%}_{1cm})^{260}_{B}}. \quad (2)$$

Thus, by determining the optical densities of an ethanolic solution in a neutral medium at 260 nm and in an alkaline medium at 310 nm and solving the system of equations it is possible to find the amount of each substance without having recourse to chromatographic separation.

This method (see [2]) was checked in model experiments with additions to a raw material of calculated amounts of the substances, and showed high reproducibility. The error of the method is ±4.2%.

A possible variant of the determination of ferutin is its colorimetric determination in the visible region (method III). In the ferutin molecule, the para position to the hydroxyl is free; it reacts with diazotized sulfanilamide forming a product with an orange coloration (λ_{max} 485 nm, $E^{1\%}_{1\,cm}$ 21.23 ± 1).

In the range of concentrations from 0.2 to 1.25 γ/ml the intensity of the coloration obeys the Bouguer–Lambert–Beer law and is suitable for analytical purposes. Ferutinin does not give this reaction.

Method I. A weighed sample of about 0.15 g of the air-dried comminuted raw material was covered with 25 ml of ethanol and heated on the water bath under reflux for 40-45 min. The flask with its contents was cooled, weighed, brought to the original weight with ethanol, and stirred, and the extract was filtered off through a No. 4 porous glass filter. A 0.2-ml portion of the filtrate was deposited on a 18 × 24-cm plate coated with type KSK silica gel applied in the form of an aqueous suspension (15 g of silica gel to 40-42 ml of water). Chromatography was performed in the petroleum ether–ethyl acetate (3 : 1) system. Control spots of ferutin and ferutinin were revealed with a 1% solution of vanillin in sulfuric acid (yellow coloration).

The adsorbent located at a distance corresponding to a control spot was transferred into a 25-ml flask, covered with 5 ml of ethanol, and left at room temperature for 15-20 min with occasional shaking. Then the liquid was filtered through a No. 4 porous glass filter. By pipette, 1 ml of the filtrate was transferred to a 25-ml flask to which 4 ml of ethanol was added. The solution was placed in the cell of a spectrophotometer (thickness of the layer of liquid 1 cm) and its optical density was measured at a wavelength of 260 nm.

As a control, in each case, the eluate obtained by treating the silica gel obtained from the same plate at the level of the control spot was used. The amounts of ferutin and ferutinin were calculated from the formula

$$X = \frac{D \cdot V_1 \cdot V_2 \cdot V_4}{E^{1\%}_{1cm} \cdot a \cdot V_3}.$$

Method II. Extraction and thin-layer chromatography were performed as in method I.

Both components were eluted, and in each case the optical density of the eluate was determined at 260 nm. Then a drop of a saturated ethanolic solution of KOH was added to the solution and after 15 min the optical density of the liquid at 310 nm was found, using as comparison solution alcohol to which the same amount of alkali had been added. The amount of ferutin was determined from formula (1).

Method III. Extraction, chromatographic separation, and elution were performed as in method I. To the eluate was added a drop of the Kutáček reagent and a drop of a saturated ethanolic solution of KOH. After 10-15 min, the solution obtained was placed in the cell of a spectrophotometer, and the optical density was measured at a wavelength of 485 nm.

The amount of ferutin was calculated from the following formula:

$$X = \frac{D \cdot V_1 \cdot V_2}{E_{1\,\text{cm}}^{1\%} \cdot a \cdot V_3},$$

where X is the amount of ferutin in the raw material, %; D is the optical density of the eluate at 260 nm; V_1 is the volume of extract from the raw material, ml; V_2 is the volume of solvent taken for elution, ml; V_3 is the volume of extract deposited on the chromatogram; and a is the weight of the sample of raw material, g.

SUMMARY

Methods have been developed for the spectrophotometric determination of ferutin and ferutinin in a plant raw material.

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