A SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF URSOLIC ACID

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Ursolic acid belongs to the group of pentacyclic triterpenoids. Its structure resembles those of hormones physiologically important for the organism [1]. Ursolic acid possesses definite biological activity [2], and it is used as an effective emulsifying agent in perfumery and in the pharmaceutical industry [3].

We have shown the possibility of obtaining it industrially in the form of a technical preparation from wastes from the processing of the fruit of Oxycoccus quadripetalus Gilib. (cranberry) [4, 5].

In the development of a method for the analysis of ursolic acid, we made use of its property for giving in the UV region a distinct maximum at 310 nm [6] (Fig. 1).

EXPERIMENTAL

The measurements were performed on an SF-4A spectrophotometer in a cell with a layer thickness of 10 mm. A sample of ursolic acid with mp 281-282°C was used.

Determination of the Specific Absorption Index of Ursolic Acid and Construction of a Calibration Curve. An accurately weighed 20-mg sample of ursolic acid was transferred quantitatively into a 100-ml measuring flask, 80 ml of concentrated sulfuric acid (sp. gr. 1.835) was added, and the mixture was thermostated at 70°C for 1 h [7]. After cooling, the solution was made up to the mark with concentrated sulfuric acid. From the initial solution so obtained, portions of 0.2, 0.4, 0.6 ... 1.2 ml were taken, and each was made up to 10 ml with concentrated sulfuric acid, giving dilutions containing 4, 8, 12 ... 24 μg of ursolic acid per ml, respectively. Then the optical densities of the dilutions were measured on an SF-4A spectrometer relative to sulfuric acid at a wavelength λ_{max} 310 nm. The statistically treated results of the determinations are given below.

Concentration of ursolic acid in the solution, %	Optical density at λ_{max} 310 nm	Specific absorption index
0.0004	0.132	330.0
0.0008	0.265	331.3
0.0012	0.403	335.8
0.0016	0.528	330.0
0.0020	0.668	334.0
0.0024	0.788	328.3
$\bar{x} = 331.6$ $S_{\bar{x}} = \pm 1.48$	$\sum = \pm 0.9$	$A = \pm 0.27\%$

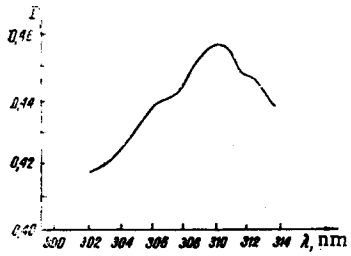

Fig. 1. Absorption spectrum of ursolic acid in concentrated sulfuric acid.

The relative error of the specific absorption index found is very low (0.27%) which shows that the Lambert–Beer law is observed for solutions of ursolic acid in concentrated sulfuric acid in the range of

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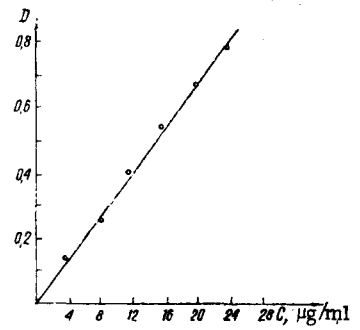

Fig. 2. Calibration curve for the determination of ursolic acid spectrophotometrically.

concentrations from 4 to 24 μg/ml. A calibration curve was plotted from the results obtained (Fig. 2).

Determination of Ursolic Acid in a Typical Preparation from Cranberry Wastes. An accurately weighed sample of the preparation (about 50 mg) was placed in a 50-ml measuring flask, 30 ml of concentrated sulfuric acid was added, and the mixture was thermostated at 70°C for 1 h. After cooling, the solution in the flask was made up to the mark with concentrated sulfuric acid (solution A). To measure the optical density, 0.6 ml of solution A was transferred to a 50-ml measuring flask and was made up to the mark with concentrated sulfuric acid (solution B). The absorption of solution B was determined in the spectrophotometer at 310 nm with a layer thickness of 10 mm. The comparison solution was concentrated sulfuric acid.

The amount of ursolic acid in the preparation was calculated from the formula

$$X = \frac{D \cdot P}{E^{1\%}_{1cm} a \cdot b},$$

where D is the optical density of the solution investigated at λ_{max} 310 nm; P is the dilution factor; $E^{1\%}_{1cm}$ is the specific absorption index; a is the weight of the sample of technical preparation, mg; and b is the volume of the sulfuric acid solution of the technical preparation taken for analysis, ml.

We give the statistically treated results of the spectrophotometric determination of ursolic acid in a technical preparation from industrial cranberry wastes:

Optical density		Ursolic acid found, %	Deviation from the arithmetic mean
0.268		67.30	+0.17
0.267		67.09	−0.04
0.268		67.30	+0.17
0.266		66.85	−0.28
0.267		67.09	−0.04
$\bar{x} = 67,13$	$S_{\bar{x}} = \pm 0,0773$	$\varepsilon = \pm 0.21$	$A = \pm 0,31\%$

To determine the percentage of ursolic acid directly in the cranberry wastes, we made use of the following formula:

$$x_1 = \frac{x \cdot D \cdot P}{E^{1\%}_{1cm} a \cdot b\ 100},$$

where x is the percentage of the technical preparation (ursolic acid) in the cranberry wastes; and D is the optical density of a solution of the technical preparation in concentrated sulfuric acid.

SUMMARY

A spectrophotometric method has been developed for the quantitative determination of ursolic acid in industrial cranberry wastes and in the technical preparation obtained from them.

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