

A method has been developed for the quantitative determination of triterpene saponins in the fruit of the hawthorn (haws) which is based on the formation of a complex of these compounds with tungstophosphoric acid on a sorbent with the use of the photodensitometric method. Advantages of the method are specificity, high sensitivity, and reproducibility.

To identify triterpenoids derived from  $\beta$ -amyrin (oleanolic and ursolic acids), many workers use their capacity for forming, on interaction with sulfuric acid, a carbocation with a characteristic absorption maximum at 310 nm [1-3]. However, chromatographic methods have recently come into use for the detection and identification of triterpenoids in a raw material, and these give the most reliable results [4, 5]. Gravimetric [6] and chromatophotometric [7] methods have been described for determining them quantitatively in a raw material.

Our aim was to study the conditions for the chromatographic identification of triterpene saponins in the fruit of the hawthorn (haws) and their quantitative estimation by the method of photodensitometry.

As the reagent for localizing the zones of the triterpene saponins on a chromatogram we used tungstophosphoric acid, since it ensures a more distinct coloration which is retained for a long time [4].

It was established by preliminary investigations that the minimum sensitivity of the detection of triterpene saponins with a 20% solution of tungstophosphoric acid in ethanol [8] is 0.5  $\mu$ g in the sorption zone. The crimson coloration formed as the result of thermostating at 120°C for 3 min is stable for 12 h.

Chromatography was carried out on Silufol UV<sub>254</sub> preprepared plates (Czechoslovakia), 15 × 15 cm. The solvent mixture described in the literature [4, 8] — benzene-acetone (8:2) — was used as the mobile phase. When acetone extracts of the raw material were chromatographed under conditions described (with oleanolic and ursolic acid as markers), zones with  $R_f$  values corresponding to the markers were detected on the chromatogram.

To evaluate the results of the separation quantitatively we made use of the photodensitometric method. The densitograms were recorded on a EPI-10 extinction-recording instrument (Carl Zeiss). We first studied the nature of the dependence of the logarithm of the area of the zone of adsorption of oleanolic acid on the logarithm of the concentration.

To plot the calibration graph given in Fig. 1, 0.0050 g (accurately weighed) of oleanolic acid was dissolved in 95% ethanol in a 25-ml measuring flask. The solution was made up to the mark with the same solvent and was carefully mixed. On a chromatographic plate, 0.005, 0.0125, 0.025, 0.0375, 0.050, 0.0625, and 0.075 ml of the 0.02% solution of oleanolic acid were deposited with the aid of a micropipette, these amounts corresponding to 1, 2.5, 5.0, 7.5, 10.0, 12.5, and 15  $\mu$ g of substance in the adsorption zone. After chromatography in the benzene-acetone (8:2) mobile phase and treatment of the chromatogram with the 20% solution of tungstophosphoric acid followed by thermostating at 120°C for 3 min, the resulting chromatogram was scanned in the EPI-10 extinction-recording densitometer using a No. 3 filter (effective center of filter — 510 nm) with a slit width of 9 mm and a scale of ordinates of 0.5. The areas of the peaks obtained were calculated by means of the appropriate formulas [9]. Each point on the calibration curve represents the mean of five determinations. It was established that this relationship was linear in the interval of 1-10  $\mu$ g of substance in the zone.

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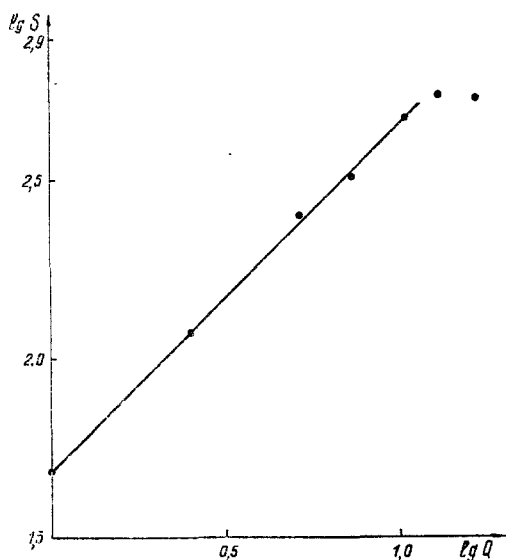


Fig. 1. Calibration graph of the dependence of the logarithms of the area of the adsorption of oleanolic acid on the logarithms of its amount in the adsorption zone.

Below we give the results of the quantitative determination of the triterpene saponins in haws and the results of statistical treatment:

$i$	1	2	3	4	5	6		
$X_i$	0,001523	0,001515	0,001452	0,001510	0,001497	0,001461		
$f$	$\bar{X}$ , %	$S^2$	$S$	$P$	$t(P, f)$	$\pm \Delta X$	$E$ , %	$\bar{E}$ , %
5	0,001493	$8,788 \cdot 10^{-10}$	$2,964 \cdot 10^{-5}$	95	2,57	$7,617 \cdot 10^{-5}$	5,1	2,1

A quantitative of the triterpene saponins in haws of different series was carried out by the procedure developed:

	Series	Amount, %
Teuchezhskaya RZK (Krasnodar krai)	261186	0.001495
Omsk ZSB	220986	0.001482
Rostov ZSB	40186	0.001503
Ryazan' ZSB	150586	0.001480

#### EXPERIMENTAL

**Analysis of the Raw Material.** An accurately weighed sample (5.0 g) of a comminuted powder of air-dry haws, after treatment with 50 ml of petroleum ether (70-100°C) was extracted with 95% ethanol at a ratio of raw material to extractant of 1:10 in the boiling water bath under reflux twice for 30 minutes. The ethanol was distilled off to dryness in vacuum, and the dry residue was treated fractionally with 30 ml of hot (40°C) acetone. The extracts were combined and evaporated to a volume of 1 ml. Of the acetone extract, 0.03 ml was deposited with the aid of a micropipette on a Silufol plate and was chromatographed by the ascending method in the benzene-acetone (8:2) system. After the zones of the saponins had been revealed, the results of the preparation were recorded under the same conditions as those described in the plotting of the calibration graph.

The amount of triterpene saponins in the haws ( $X$ , %) recalculated to the absolutely dry raw material, was found from the formula

$$X = \frac{Q \cdot 10^{-6} \cdot V_1 \cdot 100}{P \cdot V_2 \cdot (100 - h)} \cdot 100\%,$$

where  $Q$  is the amount of saponins found from the calibration graph (g);

$V_1$  is the volume of the total acetone extract, ml;  
 $V_2$  is the volume of extract deposited on the plate, ml;  
 $P$  is the weight of the raw material, g; and  
 $h$  is the loss in weight on the drying of raw material, %.

#### SUMMARY

A procedure has been developed for the selective quantitative determination of the triterpene saponins in hawthorn fruit (haws) with the aid of chromato-photodensitometry. The relative error does not exceed  $\pm 5.1\%$ .

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#### TOTAL SYNTHESIS OF THE RACEMIC ALKALOID DIPTOCARPAMINE

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A route for the synthesis of racemic diptocarpamine from hex-5-enoic acid has been developed.

Continuing work on the total synthesis of the alkaloid of *Dipthychocarpus strictus* [1-3], we have developed a short method of obtaining diptocarpamine (VIII) [4]. Our main attention was devoted to the synthesis of the key 7-thiaoctylamine (VI), which we had used previously in the preparation of the racemic alkaloids diptocarpidine and diptocarpiline [5].

Starting from hex-5-enoic acid,\* 7-thiaoctanoic acid (I) was prepared with quantitative yield by the thiylation reaction with methyl mercaptan. Its methyl ester (II) was converted under the action of lithium tetrahydroaluminate into the corresponding alcohol (III). The

\*The hex-5-enoic acid was supplied by E. K. Starostin.

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