THE CHROMATOGRAPHIC DETERMINATION OF DUBINIDINE

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The polarographic determination of the alkaloids in the total bases isolated from the epigeal part of Haplophyllum foliosum Vved. has been reported previously [1]. In view of the fact that another six bases have been isolated from this plant in recent years [2], the necessity has arisen for improving the method of analysis.

We have developed a chromatopolarographic determination of dubinidine in a chloroform extract of the plant raw material. For its preparation, 10 g (accurately weighed) of the air-dried raw material moistened with 10 ml of 8% ammonia solution was exhaustively extracted with chloroform in a Soxhlet apparatus (6-7 overflows). The extract was evaporated to dryness and the residue was dissolved in 20 ml of chloroform. A plate with dimensions of 13 × 18 cm with a nonfixed layer of alumina (Brockmann activity grade III) was first impregnated with a 1 N solution of hydrochloric acid and was dried at 60°C for 30-35 min. Then 0.3-0.5 ml of the chloroform solution was deposited in it and chromatography was carried out in the chloroform–methanol (7 : 3) system. After chromatography, the plates were again dried at 90-100°C for 30-35 min. Part of the plate with a "marker" was treated with Dragendorff's reagent. The layer of sorbent with the dubinidine (R_f 0.30-0.35) was transferred to a Schott No. 4 funnel and neutralized with 25% ammonia, and the dubinidine was eluted with 50 ml of chloroform. The solvent was evaporated to dryness and the residue was dissolved in 2 ml of supporting electrolyte – 0.1 N $(C_2H_5)_4NOH$ in 80% ethanol – and subjected to polarography as described previously [1]. The concentration of the alkaloid was determined by the method of standard solutions; as the stardard the eluate obtained on chromatographing 0.3-0.5 ml of a solution of dubinidine with a concentration of 1-2 mg/ml was used. Elution with chloroform gave 95-98% desorption. The calculation was performed by means of the formula

$$X = \frac{10 \cdot H_x \, C_{st} \cdot V \cdot b}{H_{st} \cdot p \cdot a \cdot (100 - h)},$$

where X is the content of dubinidine in % of the weight of the absolutely dry raw material;

H_X is the height of the wave of the substance undergoing determination, mm;

H_{st} is the height of the wave of the solution of the standard sample, mm;

C_{st} is the concentration of the solution of the standard sample, mg/ml;

p is the weight of raw material, g;

h is the moisture content of the raw material, %;

V is the volume of the extract, ml;

a is the volume of the extract deposited on the chromatogram, ml; and

b is the volume of solution for polarography, ml.

The deviation from the mean of two independent determinations does not exceed 1%, which shows that the reproducibility of the method was checked by analyzing model mixtures of alkaloids and extracts with

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the addition of a crystalline preparation to them. The relative error does not exceed ±5%. Using this method, we have determined the amount of dubinidine in the epigeal part of H. foliosum. For comparison we carried out an analysis of the same raw material by the method described previously [1]. The similarity of the results obtained shows the applicability of both methods, but the second is preferable since it does not involve the laborious operation of isolating the total alkaloids.

The system of solvents that we used makes it possible to carry out a qualitative determination of dubinidine at all the stages of the process of the isolation and separation of the mixture of alkaloids.

LITERATURE CITED

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2. S. Yu. Yunusov, Alkaloids [in Russian], Tashkent, 1968, p. 121.