QUANTITATIVE DETERMINATION OF THE COMBINED ALKALOIDS IN THE EPIGEAL PART OF *Heliotropium dasycarpum*

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A method is proposed for the quantitative determination of the combined alkaloids in the epigeal part of *Heliotropium dasycarpum* calculated as heliotrine which consists of nonaqueous titration with preliminary reduction of heliotrine N-oxide in an acid extract.

Heliotrine, isolated from the epigeal part of *Heliotropium dasycarpum* Ledeb ("downy-fruited heliotrope") [1] is used in medical practice for obtaining a model of hepatitis and cirrhosis of the liver in animals [2].

Methods are known for the quantitative determination of the pyrrolizidine alkaloids which consist in the repeated extraction of the alkaloids with ether and hydrofluoric acid followed by fractional titration [3], extraction, chromatography, and colorimetry [4], and polarographic [5] and adsorption [6] methods. Their disadvantages are the necessity for separating the combined alkaloids, the use of expensive and toxic substances, and the lengthiness of the analysis.

We have developed a method of nonaqueous titration [7] of heliotrine in the total material. The raw material was extracted by the steeping method with weak solutions of sulfuric acid. Good results were obtained on the use of a 10% solution. The steeping process was combined with the reduction of the N-oxide of the alkaloid [6], which considerably (more than double) increased the yield of alkaloid. The results of the analysis are given below:

Growth site of the plant, and year of collection	Heliotrine Content, %	
	Without the addition of zinc dust	With the addition of zinc dust
Fergana Province, 1980	0.27	0.60
Bukhara Province, 1980	0.23	0.60
Bukhara Province, 1981	0.29	0.55
Fergana Province, 1983	0.24	0.51

Simultaneously, we studied the influence of the time of steeping on the yield of heliotrine. The raw material was steeped for 3, 6, 12, 18, and 24 h. It was found that the optimum time of steeping was 16-18 h. In contrast to the adsorption method [6], we obtained the combined alkaloids directly from the acid extract by the usual method [8], thereby shortening the time of analysis and decreasing the losses of alkaloid.

The combined material was titrated with a 0.1 N solution of perchloric acid in a nonaqueous medium. Below, we given the results of a statistical treatment of determinations of heliotrine in the raw material collected in 1980 in Bukhara province:

n	$\bar{X}$, %	S^2	S	P	$t(P, f)$	ΔX	E, %	E_n, %
6	0,598	$8 \cdot 10^{-7}$	$8{,}94 \cdot 10^{-3}$	95	2,57	0,023	3,84	1,71

The relative error of the determinations does not exceed ±4%.

Using the method that we have developed, we determined the amounts of heliotrine in three other samples of the combined material obtained (see above), and also in all the stages of the technological process. This enabled us to establish the distribution and losses of alkaloid in the process of its isolation, in the meal, in the aqueous mother liquors, in the chloroform extracts, and in the finished product:

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Stage of the technological process	Amount of Heliotrine, %	
	On the weight of the raw material	On the amount in the raw material
Raw material	0.600	100.00
Meal	0.008	1.33
Washing chloroform	0.001	0.16
Aqueous liquor	0.062	10.33
Heliotrine satisfying Tu [Technical Specification] 6-09-50-2386-82	0.500	83.33

The quantitative determination of heliotrine as such was also carried out by the method of nonaqueous titration. The relative error of the determination does not exceed ±2%.

EXPERIMENTAL

Analysis of the Raw Material. An accurately weighed 20-g sample of the air-dry raw material was covered with 100 ml of a 1% solution of sulfuric acid, zinc dust in an amount of 20% on the weight of the raw material was added, and the mixture was left to steep for 16-18 h. Then the sulfuric acid extract was filtered through a paper filter to separate it from the meal, the meal was washed 2-3 times with 5-7 ml of 1% sulfuric acid, the sulfuric acid extracts were made alkaline with ammonia to pH 8-10, and the alkaloids were extracted completely with chloroform (until the reaction with tungstosilicic acid was negative). The solvent was evaporated off to dryness and the residue was brought to constant weight at 70°C. The resulting combined alkaloids were dissolved in 10 ml of glacial acetic acid and were titrated with 0.1 N perchloric acid solution in the presence of an indicator (Crystal Violet) until the color changed from violet to blue.

The amount of heliotrine in the raw material (X, %) calculated on the absolutely dry raw material was found from the formula

$$X = \frac{0{,}0313 \cdot V \cdot 100 \cdot 10}{P \cdot (100 - h)},$$

where V is the volume of perchloric acid consumed in titration, ml;

P is the weight of the raw material, g;

h is the loss in weight on the drying of the raw material, %.

1 ml of 0.1 N perchloric acid solution corresponds to 0.0313 g of heliotrine.

SUMMARY

A method has been developed for the quantitative determination of heliotrine in the eipgeal part of *Heliotropium dasycarpum* which consists of nonaqueous titration.

LITERATURE CITED

1. S. T. Akramov, F. Kiyalitdinov, and S. Yu. Yunusov, Dokl. Akad. Nauk. UzSSR, No. 4, 30 (1961); Khim. Prir. Soedin., 288 (1967); T. T. Shakirov, S. T. Akramov, and S. Yu. Yunusov, Khim. Prir. Soedin., 410 (1967).
2. M. N. Khanin, Experimental Heliotrope Hepatitis: 2nd All-Union Conference of Pathophysiologists. Abstracts of Lectures [in Russian], Kiev, (1963); N. Kh. Abdullaev, Vopr. Med. Khim., 6 (1966); V. M. Mal'tsev, Pathol. Fiziol., Éksp., Ter., 2, 53 (1971).
3. T. E. Gulimova, Apt. Delo, No. 4, 43 (1966).
4. V. E. Dauksha, Chemical Investigations in Pharmacy [in Russian], Kiev (1970), p. 86.
5. E. A. Vdoviko, O. R. Pryakhin, and S. A. Pokhmelkina, Khim. Prir. Soedin., 671 (1979).
6. V. E. Dauksha, Khim. Prir. Soedin., 831 (1976).
7. I. Gyenes, Titration in Nonaqueous Media, Van Nostrand, Princeton (1967).
8. A. P. Orekhov, The Chemistry of the Alkaloids [in Russian], Moscow (1955), p. 14.