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STAGEWISE CONTROL OF THE PRODUCTION OF ALLAPININ FROM THE HERB *Aconitum leucostomum*

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An express method has been developed for the analysis of intermediates and wastes in the production of allapinin, using a diffusional equilibrium followed by spectrophotometry.

The industrial source for the preparation of the antiarrhythmic drug allapinin [1] is the epigeal part of aconite monkshood *Aconitum leucostomum* Woroch (fam. Ranunculaceae). The production of allapinin consists of several stages. To create the optimum conditions for conducting the technical process and for the output of allapinin the materials entering the process and the final product must be strictly controlled.

The control of production must be carried out in short times, and the use of an express method of analysis in place of the lengthy and laborious methods used previously [2, 3] is an urgent matter. We have previously developed an accelerated method for the quantitative determination of allapinin in the herb aconite monkshood [4].

The task of the present work was to create and develop an express method of analysis for all stages of the technological process. As the objects of investigation we took intermediates obtained in the Experimental Factory of the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan.

We have selected a method of accelerating analysis by extracting the alkaloids incompletely, using the diffusional equilibrium on first contact of the phases. Calculation factors (K) were found experimentally for extraction by ethyl alcohol ($K = 2$) and by isopropyl alcohol ($K = 1.8$). The procedure for analyzing the intermediates was developed on the basis of extraction (herbage, meal) or dilution (and, in the case of the analysis of the concentrated alcoholic extract and the alcoholic mother liquor, without extraction) with ethyl or isopropyl alcohol until diffusional equilibrium has been achieved.

The subsequent treatment of an aliquot sample depends on the material being examined: in some cases purification by changing the solvent and the pH of the medium is used. After purification, the sample is dried and is diluted with ethyl alcohol to the necessary concentration (D from 0.3 to 0.7). Lappaconitine, with the accompanying alkaloids (N-deacetyllappaconitine, leuconine, N-acetylseppaconitine) is determined spectrophotometrically in an aliquot sample or the absence of alkaloids from the sample is established (when spent solutions are being examined).

EXPERIMENTAL

Quantitative Analysis of the Herb Aconite Monkshood. A weighed sample of raw material (20 g) was extracted by 80% ethyl (or isopropyl) alcohol with boiling under reflux for 10 min. The extract was cooled and filtered, and 20 ml of the filtrate was evaporated to an aqueous residue and was made alkaline to pH 9. The alkaloids were then extracted with chloroform until the reaction with tungstosilicic acid was negative. The chloroform extract was dried with anhydrous sodium sulfate and filtered. The chloroform was distilled off to dryness, the dry residue was dissolved in 50 ml of 95% ethyl alcohol, and the solution was filtered into a 50-ml measuring flask, the volume of the solution being made up to the mark with the same solvent. With a pipet, 2.5 ml of this solution was transferred to a 25-ml measuring flask, and the volume of the solution was made up to the mark with the same solvent.

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The optical density of the solution so obtained was measured in a spectrophotometer at a wavelength of 308 nm in a cell with a layer thickness of 10 mm. The comparison solution used was 95% ethyl alcohol. The optical density of a solution of a standard sample (SSS) of allapinin was measured in parallel.

The percentage of lappaconitine with accompanying alkaloids, referred to the absolutely dry weight of the raw material (X), was calculated from the formula

$$X = \frac{D_1 \cdot m_0 \cdot C_0 \cdot 0.89 \cdot 20 \cdot 100 \cdot K}{D_0 \cdot m \cdot (100 - W) \cdot 100},$$

where D_0 and D_1 are the optical densities of the SSS of allapinin and of the solution under investigation, respectively;

m_0 and m are weights of allapinin and the raw material, respectively;

C_0 is the concentration of the main substance in the standard sample of allapinin;

W is the loss in weight on the drying of the raw material; and

K is the calculation factor for diffusional equilibrium (2 or 1.8).

Analysis of the Meal. An analytical sample of meal was dried in the air until the smell of the alcohol had disappeared. Then a weighed sample of the meal (40 g) was analyzed as described above. The time of extraction was 20 min. Calculation was carried out by the formula given above.

Analysis of the Concentrated Alcoholic Extract. The volume of the alcoholic extract was measured, 1 ml of it was diluted with ethyl alcohol to 100 ml, 20 ml of this dilution was filtered into a 50-ml measuring flask, and the volume was made up to the mark with the same solvent. The further procedure was as described above.

Calculation was performed by means of the formula

$$X = \frac{D_1 \cdot m_0 \cdot C_0 \cdot 0.89 \cdot 20 \cdot 100}{D_0 \cdot 100 \cdot V \cdot 100},$$

where V is the volume of concentrated alcoholic extract, ml.

Analysis of the Spent Aqueous Extract. The volume of the aqueous extract was measured, and 100 ml of it was taken and was concentrated to 15-20 ml in a rotary evaporator, after which the pH was checked and, if necessary, was brought to 9. The alkaloids were exhaustively extracted with chloroform, and the extract was evaporated on the water bath to dryness. The dry residue was then treated as described above.

Calculation was performed by means of the formula

$$X = \frac{D_1 \cdot m_0 \cdot C_0 \cdot 0.89 \cdot 100 \cdot 100}{D_0 \cdot V \cdot 100},$$

where V is the volume of the spent aqueous extract, ml.

Analysis of the Spent Chloroform Extract. The volume of the chloroform extract was measured, and 200 ml was taken and was evaporated to dryness on the boiling water bath. The dry residue was treated as described above.

Calculation was performed by means of the formula

$$X = \frac{D_1 \cdot m_0 \cdot C_0 \cdot 200 \cdot 0.89 \cdot 100}{D_0 \cdot V \cdot 100},$$

where V is the volume of the spent chloroform extract, ml.

Analysis of the Washing Chloroform. The volume of washing chloroform was measured, and 200 ml was taken and was evaporated to dryness on the boiling water bath. The dry residue was treated as described above.

Calculation was performed by means of the formula given above, where V is the volume of washing chloroform.

Analysis of the Spent Sodium Carbonate Solution. The volume of the sodium carbonate solution was measured, and 100 ml was taken and was concentrated to 15-20 ml. The alkaloids were exhaustively extracted with chloroform, and the extract was evaporated on the boiling water bath. The dry residue was treated as described above.

Calculation was performed by means of the formula

$$X = \frac{D_1 \cdot m_a \cdot C_2 \cdot 20 \cdot 0.89 \cdot 100}{D_c \cdot 100 \quad V},$$

where V is the volume of the spent sodium carbonate solution.

Analysis of the Alcoholic Mother Solution. The volume of the alcoholic mother solution was measured, and 20 ml was taken and was treated as described above.

Calculation was performed by means of the formula given above, where V is the volume of alcoholic mother solution.

Analysis of the Methanolic Mother Solution. The volume of the methanolic mother solution was measured. The further procedure was as shown above, V being the volume of the methanolic mother solution.

Analyses of the final product were performed in accordance with VFS (Provisional Pharmacopeial Standard) 42-1667-94.

REFERENCES

1. Provisional Pharmacopeial Standard 42-1667-94.
2. Provisional Pharmacopeial Standard 42-1666-94.
3. A. U. Makhkamova, É. V. Safonova, A. Z. Sadikov, E. K. Dobronravova, and T. T. Shakirov, Khim. Prir. Soedin., 436 (1989).
4. A. Z. Sadikov, É. V. Safonova, and T. T. Shakirov, Khim. Prir. Soedin., 306 (1993).