METHOD FOR QUANTITATIVE DETERMINATION OF ALLAPININE IN *Aconitum leucostomum*

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Lappaconitine together with accompanying alkaloids obtained from the epigeal part of *Aconitum leucostomum* Worosch. (family Ranunculaceae) is used in the form of hydrobromides (allapinine) in medical practice as an antiarrhythmic drug [1, 2]. Allapinine consists of the hydrobromides of the alkaloids lappaconitine and N-deacetyllappaconitine, the amount of the latter being not more than 10%. The presence of not more than 2% of foreign substances of unestablished nature is permitted. The amount of allapinine in the drug is not less than 96% [3].

We have developed a method for the quantitative determination of lappaconitine with accompanying alkaloids in plant raw material which consists in obtaining the total alkaloids, their separation by TLC, and the determination of the total lycoctonine alkaloids in the eluate by spectrophotometry [4]. To eliminate the accompanying alkaloids, the total material was chromatographed in a fixed layer of silica gel of type KSK or LS 5/40 (Czechoslovakia) in the chloroform–benzene–ethanol (95%)–ammonia (25%) (40:40:10:0.2) system. Elution was performed with 95% ethanol [5].

It was established that the optical density of the residual alkaloids not forming components of allapinine was zero at λ 308 nm, i.e., they did not interfere with the determination of lappaconitine with its accompanying alkaloids at the given wavelength. This provided us with the possibility of determining the amount of lappaconitine and accompanying alkaloids without their chromatographic separation by dissolving the total alkaloids and a standard (allapinine – VFS [Temporary Pharmacopoeial Paper] 42-1667-86) in 95% ethanol and determining the optical density on a spectrophotometer.

Identical results were obtained in a determination of the amounts of lappaconitine with accompanying alkaloids in 14 samples of *A. leucostomum* by the method of VFS 42-1666-86 and the method without chromatographic separation.

The method without chromatographic separation of the total alkaloids permitted a halving of the time of analysis.

Below we give the metrological characteristics of the method for the analysis of *A. leucostomum* gathered in 1985 in Issyk-Kul' province:

n	f	$\bar{X}$	S	t	P	ΔX	ε
6	5	0.150	$1.73 \cdot 10^{-3}$	2.57	95	$4.4 \cdot 10^{-3}$	2.9

Results of the analysis in percentages of the air-dry mass of the raw material: 1) 0.149; 2) 0.151; 3) 0.148; 4) 0.148; 5) 0.151; 6) 0.152.

In this method, about 20 g of comminuted raw material is wetted with 20 ml of a 5% solution of sodium carbonate and is extracted exhaustively with chloroform in a Soxhlet apparatus. The extract is concentrated to a volume of 20-25 ml and is quantitatively transferred to a separatory funnel, and the alkaloids are exhaustively extracted with a 5% solution of sulfuric acid. The sulfuric acid extract of the alkaloids is alkalinized to pH 8-9 with sodium carbonate solution and the alkaloids are extracted with chloroform until the reaction with tungstosilicic acid is negative. The chloroform extracts are dewatered with anhydrous sodium sulfate and evaporated to dryness. The dry residue is dissolved quantitatively in 50 ml of ethanol; 2.5 ml of this solution is made up to 25.0 ml and is measured in a spectrophotometer in a cell with a layer thickness of 10 mm at a wavelength of

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308 nm using 95% ethanol as solvent. The optical density of an ethanolic solution of allapinine with a concentration of 0.05 mg/ml is measured in parallel.

The amount of lappaconitine with accompanying alkaloids, reckoned as lappaconitine, is calculated as a percentage (X) on the absolutely dry raw material by means of the formula:

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

where D_0 is the optical density of the allapinine solution;

D_1 is the optical density of the solution under investigation;

m_0 is the weighed amount of allapinine;

m is the mass of the raw material;

C_0 is the amount of allapinine in the standard sample, %;

w is the loss in mass drying, %; and

0.89 is a calculation factor equal to the ratio of the molecular masses of lappaconitine base and lappaconitine hydrobromide.

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ANALYSIS OF THE HEMORRHAGIC PRINCIPLE OF THE VENOM OF *Echis multisquamatus*

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The presence in snake venoms of a large number of interfering components substantially complicates the identification of their active principles [1]. Thus, according to various authors a hemorrhagic lesion may be caused by specific proteinases and by phospholipase A_2 interacting with cytotoxins and, probably, with independent hemorrhagins of protein nature present in snake venoms [2, 3].

The venom of the Central Asian viper Echis multisquamatus, from which individual proteinases and phospholipases A_2 have recently been obtained [4, 5], acts hemorrhagically, but the nature of its hemorrhagic principle is unknown. In the present work we have analyzed the distribution of the hemorrhagic, proteolytic, and phospholipase activities in the fractionation of the venom with the aid of gel filtration and ion-exchange chromatography.

Fig. 1, A, shows the distribution of the whole viper venom in gel filtration on a column of Sephadex G-75. The results obtained indicate differences in the molecular dimensions of the phospholipase A_2 ($PL\text{-}A_2$) and the hemorrhagically acting factor (H) of the venom, which appeared in the same fraction as a casein-hydrolyzing proteinase (or caseinolytic enzyme, CLE). With respect to its molecular mass, H of the venom differs from the specific

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