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SPECTROPHOTOMETRIC DETERMINATION OF SPHAEROPHYSINE BENZOATE BY THE REACTION WITH DINITROBINDONE

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A method for the quantitative determination of sphaerophysine benzoate that is highly sensitive and simple in performance has been developed which may find wide use in pharmaceutical analysis.

Sphaerophysine is an alkaloid isolated from *Sphaerophysa* Pall. DC [salt globe pea]. In medicine, sphaerophysine in the form of its benzoate is used to lower arterial pressure, increase tonus, and to enhance the contraction of the musculature of the uterus [1].

In spite of its wide use, sphaerophysine benzoate has been studied inadequately. Methods of acid–base titration in a nonaqueous medium [2] and of polarographic [3] and bromatometric [4] determination have been described. These methods are characterized by low sensitivity, inconvenience in performance, and lengthiness of the analysis.

Our aim was to develop a highly sensitive method simple in use for the quantitative determination of sphaerophysine benzoate. We have used the reaction with dinitrobindone. It is established that dinitrobenzone reacts with sphaerophysine benzoate in dioxane to form an orange-red product. The intensity of the coloration is directly proportional to the sphaerophysine content of the sample under investigation and obeys Beer's law within the range of concentrations of 1.2-2.4 mg of substance in 100 ml of solution. The reaction was carried out at room temperature using a 0.5% solution of dinitrobindone. Dioxane of ch.d.a. ["pure for analysis"] grade was used as the solvent for the reagent and the compound being determined. It is assumed that in the reaction a salt of dinitrobindone with the sphaerophysine cation is formed.

We may give the following spectral characteristics for the reaction that we have developed:

Analytical index	Numerical value
Absorption maximum, nm	492
Molar absorption coefficient	19,550
Specific absorption, $cm^2/\mu g$	0.04417
Sandell coefficient	0.02264
Koch and Koch-Dedic coefficient	1.13
Limits of detection, μg/ml	1.13

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The quantitative determination of sphaerophysine benzoate was carried out in the following way. An accurately weighed sample of 0.01-0.3 g of the preparation was dissolved in 0.5 ml of water in a 50-ml measuring flask and the solution was made up to the mark with dioxane. From the resulting solution 1 ml was transferred to a 25-ml flask, and then 1 ml of a 0.5% solution of dinitrobindone in dioxane was added and the mixture was made up to the mark with the same solvent. A solution of a sample of sphaerophysine benzoate (0.02 g in 50 ml of dioxane) and a blank solution were prepared in parallel in exactly the same way. The optical densities of the colored solutions were measured relative to the blank on a SF-26 spectrophotometer at 492 nm using cells with a layer thickness of 1 cm.

The percentage content of sphaerophysine benzoate was determined from the formula

$$C = \frac{D \cdot 1250 \cdot C_0}{D_0 \cdot p \cdot l},$$

where D is the optical density of the solution being analyzed at 492 nm; D_0 is the optical density of the solution of a standard sample of sphaerophysine benzoate; C_0 is the concentration of the solution of the standard sample, g/ml; p is the weight of the sample, g; and l is the layer thickness, cm.

The results of the determination at p = 0.95 and n = 6 are given below:

Weight of sample, g	Found, %	Metrologic characteristics
0,0152	100,02	$\bar{X}=100,27$
0,0178	99,80	$S=\pm 0,396$
0,0226	100,16	$Sr=\pm 1,018$
0,0252	99,89	$St=\pm 1,02$
0,0275	100,94	$\bar{x}\pm St=100,27\pm 1,02$
0,0300	100,79	$t_{calc}=1,67$

The results of the analysis indicate the reliability of the method and the absence of any systematic error: The calculated Student's coefficient (1.67) is smaller than the tabular value (2.57).

An advantage of the proposed method of analysis as compared with the known methods is its high sensitivity (1.13 μg/ml) and the simplicity of its performance, which excludes the use of aggressive and concentrated acids — in particular, acetic and perchloric acids. The determination is performed with respect to the physiologically active part of the molecule. The time of performance of an analysis by the proposed method is 5-7 min. This method for the quantitative determination of sphaerophysine benzoate may find wide use in pharmaceutical analysis.

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