RAPID METHOD FOR THE IDENTIFICATION OF SOME ANTIBIOTICS OF THE POLYMYXIN GROUP

L. A. Baratova, G. S. Katrukha,
L. P. Belyanova, and M. N. Gael'

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The identification of polypeptide antibiotics of the polymyxin group is an extremely difficult problem, since they all possess well-defined basic properties and differ only slightly from one another in their content of neutral amino acids [1-3].

To separate the individual representatives of this group of antibiotics and their acid hydrolysis there are methods of paper and thin-layer chromatography [4-8], but these do not permit the separation of a number of antibiotics and require a long time.

The identification of a series of antibiotics of the polymyxin group can be performed by analyzing their acid hydrolyzates on an automatic amino-acid analyzer under two-column conditions for the analysis of protein hydrolyzates, which requires 5 h.

We have proposed a single-column variant of the method (Fig. 1). On using for elution a 0.8 N Na citrate buffer, pH 4.25*, after only 1 h we succeeded in separating and quantitatively determining the following amino acids that are included in the composition of the polymyxins: α,γ-DABA, phenylalanine, threonine, and leucine (isoleucine).

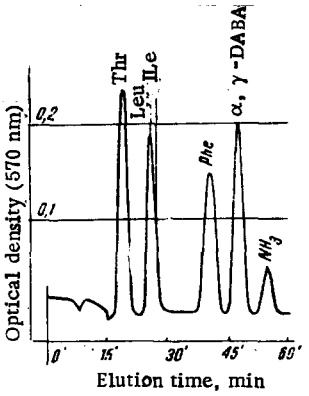

Fig. 1. Chromatographic separation of a standard mixture of amino acids on a Hitachi (Japan) KLA-3b amino-acid analyzer; rate of elution 60 ml/h; column temperature 55 ± 0.5°C; 0.8 N Na citrate buffer, pH 4.25 ± 0.02; 0.2 mmole of each amino acid.

Thus, the proposed method permits a fairly fast and accurate identification of the antibiotics polymyxins B, M, P, and E (colistin) [3, 9, 10]. Only 0.05-0.1 μmole (0.07-0.14 mg) of the initial antibiotic is required for the analysis. The method can be used for the rapid identification and quantitative determination of the amounts of the antibiotics mentioned in medicinal preparations, in their isolation from a culture liquid, and in the study of the processes taking place in their inactivation.

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* The buffer was prepared from standard 0.2 N Na^+ citrate buffer, pH 4.25, by the addition of NaCl to the required normality.

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