BRIEF COMMUNICATIONS

SIMPLE METHOD FOR THE SEPARATE DETERMINATION OF DEOXY SUGARS IN CARBOHYDRATE-CONTAINING BIOPOLYMERS

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Wide use is made of the periodate-2-thiobarbituric acid (periodate-TBA) reaction for the determination of deoxy sugars – structural components of many carbohydrate-containing biopolymers [1, 2]. Here, neutral deoxy sugars (2-deoxyaldoses, 3,6-dideoxyhexoses) and acidic deoxy sugars (2-keto-3-deoxyaldoses and sialic acids), on being oxidized by periodate, give, respectively, malondialdehyde (MDA) and β-formylpyrotartaric acid (FPA), which, in the subsequent reaction with TBA, form colored compounds with similar optical properties. These chromophores cannot be determined separately by spectrophotometric methods.

This problem has been solved by indirect methods [3-5], and also by the densitometry of the chromophores after their separation on Silufol plates [6].

For the development of the following method, we propose to separate the chromophores on mini columns containing octadecyl silica gel (ODS) by means of reversed-phase chromatography. While retaining the same reproducibility of the results (rel. error < 3%), this method, as compared with [6], decreases the consumption of reagents and time for analyses by decreasing the number of operations requiring accurate performance in [6].

Procedure. After the necessary stages of the analysis (acid hydrolysis of sample, periodate oxidation, reaction with TBA, and determination of calibration factors) performed as described by Burtseva and Ovodov [4, 5] the whole of the colored solution (5 ml) or an aliquot part of it was force-filtered (MMC micropump, Czechoslovakia) at the rate of 1 ml/min through a column (0.4 × 4 cm) with ODS (grain size 10 μm) previously equilibrated with water. The chromophores were sorbed as a narrow crimson band in the top part of the column. They were eluted at the rate of 1 ml/min, separation being observed visually. First, water eluted the chromophore of FPA, and the eluate was collected in a 5-ml measuring flask to which 0.2 ml of 0.5 N sulfuric acid was added. The volume was made up to the mark with water and the A_{549} value was measured in a cell 1 cm long.

Then aqueous ethanol (1:1, v/v) eluted the MDA chromophore and the eluate was collected in another 5-ml measuring flask. The volume was made up to the mark with aqueous ethanol and the A_{532} value was measured.

The column was regenerated by the passage of 10 ml of ethanol and was equilibrated with water. The time for the elution of both chromophores did not exceed 8-10 min.

The proposed method is, as in [6], a direct method and its results do not depend on the qualitative and quantitative compositions of the accompanying components; it can be used as the concluding stage in the analysis of bipolymers of different compositions.

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