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DETERMINATION OF THE GUANOSINE CONTENT OF TECHNICAL PREPARATIONS

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UDC 547.963.32+543.42.062:543.8

A method is proposed for the quantitative determination of the guanosine contents of technical preparations using the Folin reagent. The sensitivity of the method is 2.5 μg/ml, and the error of the determination does not exceed ±2.3%.

The purine and pyrimidine components of nucleic acids are widespread in nature. Derivatives of purine and pyrimidine enter into the composition of many coenzymes, vitamins, and antibiotics. A number of these valuable substances are obtained from technical preparations of the components of nucleic acids isolated from hydrolysates of them. Thus, an unpurified guanosine preparation of "technical" grade is the initial raw material for obtaining medicinal preparations (virazole [ribavirin] and thioguanine).

In the isolation of guanosine from hydrolysates of ribonucleic acid, preparations are formed which, in addition to guanosine, contain traces of other nucleosides – adenosine, uridine, cytidine – and also free guanine, proteins, and amino acids. Methods for analytical control are therefore required which permit guanosine to be determined in the presence of these impurities. The known methods of determining guanosine by UV spectroscopy [1] and potentiometric titration [2] are insufficiently selective. The chromatographic separation of the components of a technical preparation followed by UV spectrometric determination is laborious and has an extremely high error of determination (10-15%).

We have developed a method for the analysis of preparations of technical guanosine which permits the selective determination of the desired product in the presence of other nucleosides and also enables the presence of guanine, proteins, and amino acids to be allowed for. The determination is based on the capacity of guanine, unlike other nucleic bases, for reducing molybdotungstophosphoric acid (the Folin reagent) in an alkaline medium, as a result of which a colored compound with an absorption maximum at 770 nm is formed [3, 4].

To determine guanosine, the preliminary cleavage of the N-glycosidic bond is required, which is achieved by hydrolysis in 1 N hydrochloric acid on the boiling water bath for 10 min [5]. We have established the dependence of the intensity of absorption of the product of the interaction of guanine with the Folin reagent at 770 nm on its concentration in the solution undergoing photometry. The Lambert–Beer law is observed for guanine concentrations of 2.5-25 μg/ml. We first carried out the determination of guanosine in the presence of other nucleosides in artificial mixtures.

All-Union Scientific-Research Institute of Applied Biochemistry, Olaine. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 504-507, July-August, 1983. Original article submitted June 15, 1982.

0009-3130/83/1904-0475$07.50

As can be seen from the figures given below, the presence of other nucleosides in various ratios does not affect the results of the determination of guanosine. The error of the determination does not exceed ±2.2%:

Components of the mixture	Amount taken, μg	Amount found, μg	Error %
Guanosine + adenosine	16.4 15.9	16.1	−1.8
Guanosine + adenosine	17.3 80.4	17.6	+1.2
Guanosine + cytidine	21.4 30.5	21.7	+1.4
Guanosine + adenosine + cytosine	18.5 22.3 26.2	18.8	+1.6
Guanosine + uridine	24.1 45.2	23.8	−1.2
Guanosine + adenosine + uridine + cytosine	18.3 20.4 22.4 25.6	18.7	+2.2

The influence of impurities of protein nature, of certain amino acids giving a coloration with the Folin reagent, and also of free guanine was taken into account in a control experiment before the performance of hydrolysis. The results of the determination of guanosine in the presence of free guanine, albumin, tyrosine, and tryptophan in artificial mixtures indicate that, when the check on impurities is taken into account, the method can be used successfully for the determination of guanosine in technical preparations containing, in addition to other ribonucleosides, impurities giving colored reaction products with the Folin reagent. The error of the determination of guanosine does not exceed ±2.1%:

Components of the mixture	Amount taken, μg	Amount found, μg	Error %
Guanosine + guanine	20.11 5.03	19.69	−2.1
Guanosine + albumin	25.04 5.05	25.32	+1.1
Guanosine + albumin	15.30 15.36	15.26	−0.3
Guanosine + tyrosine	15.00 2.99	14.93	−0.5
Guanosine + tryptophan	15.03 2.98	15.20	+1.1

The statistical treatment of the results of the determination of guanosine in five batches of the technical preparation (at $n = 6$, $\alpha = 0.95$) showed that the relative standard deviation does not exceed ±2.3%.

The sensitivity of the proposed method is 2.5 μg/ml. The time of analysis amounts to 1-2 h. The method permits the selective determination of guanosine in the presence of other nucleosides without preliminary separation of the components and does not require reagents in short supply or complicated apparatus.

EXPERIMENTAL

The Folin reagent was prepared as described by Folin and Ciocalteu [6]. The prepared solution was diluted with distilled water in a ratio of 1:1. To measure optical densities we used a SF-16 spectrophotometer and cells with a layer thickness of 1 cm.

In order to plot a calibration graph, a standard solution of guanine was prepared. As the standard we used a chromatographically homogeneous material the purity of which was checked by UV spectroscopy [1].

Guanine (25 mg calculated as 100% substance) was placed in 100-ml measuring flask and dissolved in 1 N hydrochloric acid solution, the volume was made up to the mark with 1 N hydrochloric acid solution, and the contents of the flask were mixed. To prepare working solutions, 1-5-ml portions were transferred from the standard solution into 50-ml measuring flasks and each was made up to the mark with 1 N hydrochloric acid solution and mixed. The working solutions contained 5-25 μg of guanine in 1 ml.

From each working solution, 2 ml was transferred into a test tube with a volume of 15-20 ml, and to each was added 1 ml of the Folin reagent and 2 ml of saturated sodium acetate solution. The contents of the tubes were stirred and they were kept in a thermostated water bath at 50°C for 20 min. After cooling to room temperature, the optical densities of the solutions were measured on the spectrophotometer against a control solution containing 2 ml of 1 N hydrochloric acid in place of the working solution, and the same reagents. A calibration graph was plotted in the coordinates of guanine concentrations, μg/ml, as abscissas against optical densities as ordinates.

To perform an analysis, 20-25 mg of the preparation was placed in a 50-ml measuring flask, dissolved in 1 N hydrochloric acid solution, and the contents of the flask were made up to the mark and were mixed. Two 5-ml portions of the resulting solution were transferred into two 50-ml measuring flasks. The volume of the solution in one flask was made up to the mark with 1 N hydrochloric acid solution, and the contents were mixed (solution A for checking the amount of impurities). To the other measuring flask was added 20-30 ml of 1 N hydrochloric acid and hydrolysis was carried out in the boiling water bath for 10 min. After cooling to room temperature, the volume was made up to the mark with 1 N hydrochloric acid and the contents were mixed (solution B). To one test tube was added an aliquot volume (2 ml) of solution A, and to another was added 2 ml of solution B, and then the reagents were added and the determination was carried out as in the plotting of the calibration graph. After the solutions had been cooled to room temperature, the optical densities were measured — D_1 for solution A and D_2 for solution B. From the difference in the optical densities $D_2 - D_1$ the corresponding guanine concentration (μg/ml) was found on the calibration graph.

The percentage of guanosine in the preparation being analyzed (X) was calculated from the formula

$$X = \frac{C \cdot M_1 \cdot V \cdot n \cdot 100}{m \cdot M_2 \cdot 1000},$$

where C is the concentration of guanine found from the calibration graph from the difference of the optical densities of solutions B and A, μg/ml; M_1 is the molar mass of guanosine (M_1 = 283.24); V is the volume of the flask taken for dissolving the preparation to be analyzed, ml; n is the dilution factor; 100 is the factor for calculating to percentages, m is the mass of the sample, mg, M_2 is the molar mass of guanine (M_2 = 151.13); and 1000 is the factor for recalculating μg to mg.

SUMMARY

It has been shown that the Folin reagent can be used to determine the guanosine content of technical preparations in the presence of other nucleosides and of other impurities (free guanine, proteins, some amino acids).

The method is distinguished by high sensitivity (2.5 μg/ml), and rapidity and simplicity of performance. The error of the determination does not exceed ±2.3%.

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