## **METHODS**

## **Analysis of 5-Nitrofuran Derivatives** in **Biological Objects**

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 1, pp. 115-116, January, 2003 Original article submitted December 10, 2002

Original methods for identification and quantitative photometry of 5-nitrofurane derivatives based on the use of known rhodanine reagents were developed and used in clinical analysis.

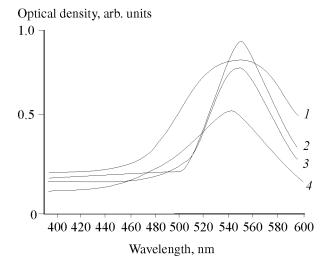
**Key Words:** 5-nitrofuranes; 3-α, γ-dicarboxypropylrhodanine; urine; exudate

Drugs of the 5-nitrofurane family are characterized by specific behavior under certain chemical conditions, for example low stability in alkaline medium. At the same time, there are just few reagents for their qualitative and quantitative evaluation, particularly in clinical practice. We developed new methods for identification and measurement of 5-nitrofurane compounds based on the use of reagents heretofore not used for this purpose.

Before measurements the biological specimens (urine, exudate) were filtered through paper filter wetted with distilled water and pH was tested by indicator paper. In case of alkaline pH, a universal Britton—Robinson buffer mixture (pH 7) was added to the sample, and the dilution was taken into consideration in further calculations [3]. The analyzed compound was 3 times extracted from the sample (10 ml) with ethylacetate. When needed, extraction was repeated from a new portion of the analyzed specimen with new portions of ethylacetate. The resultant ethylacetate extracts were pooled and evaporated in thermostable glass on a water bath (80°C). The content of the analyzed compounds was measured in dry residues dissolved in precise volume of distilled water.

Qualitative detection of furagine, furadonine, furasolidone, and nifuroxaside was carried out as follows: 1 ml water sample, 1 ml 1% 3-α,γ-dicarboxypropylrhodanine in 95% ethanol, and 0.15 ml 1 M aqueous solution NaOH were put into a tube; after 5 min the solution in the tube was crimson-colored.

For quantitative evaluation of furagine, furasolidone, and furadonine, 10 ml 1% 3- $\alpha$ , $\gamma$ -dicarboxypropylrhodanine in 95% ethanol and 1.6 ml (1 ml for furadonine) 1 M aqueous solution NaOH were added to the sample (10 ml), mixed, and after 5 min distilled water was added to a final volume of 25 ml. After thorough mixing optical density of stained solution



**Fig. 1.** Spectral curves of reaction of 5-nitrofurane derivatives with  $3-\alpha$ , $\gamma$ -dicarboxypropylrhodanine. 1) furagine; 2) furadonine; 3) furasolidone; 4) nifuroxaside.

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was measured on a spectrophotometer at  $\lambda$ =540 nm or on a photocolorimeter at  $\lambda$ =540±10 nm. The reference solution was a mixture of reagents. The drug content was estimated by calibration curve.

The light absorption peaks for furagine, furadonine, and furasolidone were observed at  $\lambda$ =540 nm (Fig. 1). Molar extinction of the analyzed compounds was below 1.27×10<sup>4</sup>, relative error of measurements was no more than 9.67% in all cases. The threshold level for 5-nitrofurane measurements by the reaction with 3- $\alpha$ , $\gamma$ -decarboxypropylrhodanine is 10  $\mu$ g/ml analyzed sample.

Methods for photometric measurement of 5-nitrofurane derivatives with other rhodanine derivatives for color reagents (3-carboxymethylrhodanine, 3-β-carboxyethylrhodanine, and unsubstituted rhodanine) were also developed, but their sensitivity is inferior to the above described.

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