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SELECTIVE SPECTROPHOTOMETRIC DETERMINATION OF PURINE NUCLEOSIDES WITH PHLOROGLUCINOL

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ABSTRACT

A new absorptiometric procedure for the determination of purine nucleosides in pharmaceutical formulations is presented, based on the reaction of purine nucleosides with a mineral acid-containing phloroglucinol solution. Absorbance readings at 558 nm obey Beer's law within the concentration range from 2.00 to 21.00 $\mu g\ mL^{-1}$ (guanosine). The method is operationally simple, precise, and selective. A chemical mechanism is proposed.

INTRODUCTION

Most of the known chemical methods for the absorptiometric determination of purine nucleosides are based on chromo

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genic reactions which depend on the chemical properties of the carbohydrate fragment of the molecule (1-3).

In this publication, a new absortiometric method for the determination of purine nucleosides is presented. The procedure, which is sensitive and operationally simple since it does not require any chemical treatment prior to the chromogenic reaction, is based on the heating the sample compound with a solution of phloroglucinol in mineral acid at 100ºC. The furfuraldehyde produced by the acid hydrolysis of the purine nucleosides condenses directly with phloroglucinol, yielding a polycyclic dibenzopyran chromophore. The method has an adequate selectivity for the practical analysis of purine nucleosides (pyrimidine nucleosides do not interfere) and does not require sophisticated equipment.

EXPERIMENTAL

Reagents

All reagents (analytical grade) used as solvents and in the preparation of the solutions were obtained from E.Merck A.G. (Darmstad, West Germany). The purine nucleoside used (guanosine and inosine) were also from E. Merck.

Reagent Solution

The reagent solution used contains 0.30% phloroglucinol in aproximately 0.92 M hydrochloric acid, and is prepared by mixing concentrated hydrochloric acid (10 mL), glacial acetic acid (110 mL), and a methanolic 5% phloroglucinol dihydrate solution (10 mL). This solution must be used only when freshly prepared.

Equipment

Beckman (ACTA III) double-beam spectrophotometer, with 1.0 cm fused silica cells.

Electrically heated water bath, thermostated to ± 0.1°C.

Method

An aqueous solution (2.0 mL) of the purine nucleoside is transferred to a 10 mL volumetric flask; reagent solution (5.0 mL) is added and the contents are well mixed. The flask, well stoppered, is maintained in a boiling water bath for exactly the recommended time (Table I). It is then immersed for one minute in an ice-water bath to stop the reaction and to bring the contents to room temperature. Finally it is made up to the volume with unheated reagent solution. The absorbance of this solution is measured at the wavelenght of maximum absorption (Table I) in a 1.0 cm fused silica cell, against a reference sample obtained by subjecting distilled water (2.0 mL) to the same procedure.

RESULTS AND DISCUSSION

The chromogenic reaction with phloroglucinol is only possible with compounds containing a furan-like group, since its presence is critical for the condensation step of the procedure. The oxygen heteroatom of these compounds is linked to carbon atoms which, in most cases, are bonded to an hydroxy group in order to allow the dehydration step.

The mechanism for this reaction with the purinic nucleosides follows a complex path that includes an initial acid hydrolysis of the structure with a subsequent multistep dehy-

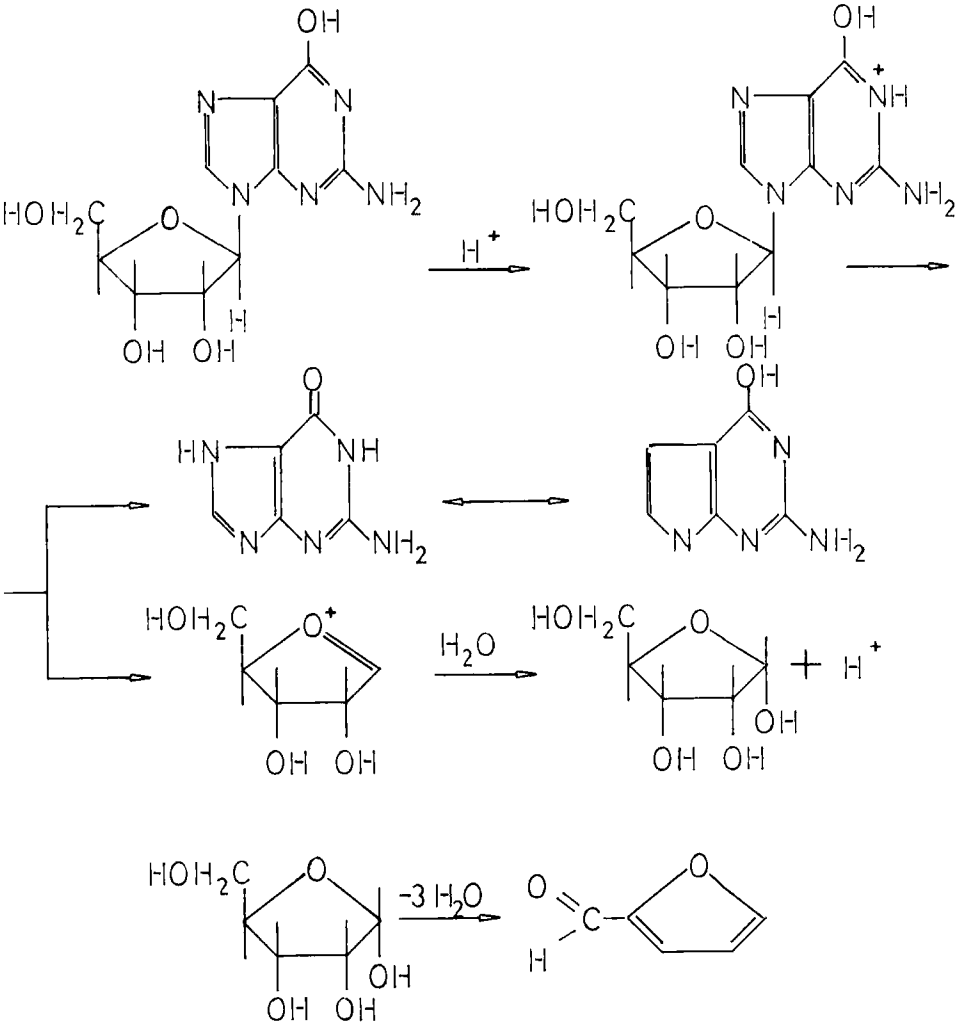

Fig. 1

dration of the resultant ribose going rise to furfuraldehyde (Fig. 1), and terminates with the condensation of this compound with phloroglucinol to yield a dibenzopyrane derivative (Fig. 2). This last step is facilitated by the absolute symmetry in the phloroglucinol molecule and the resultant high activating effect on its non-substituted carbon atoms.

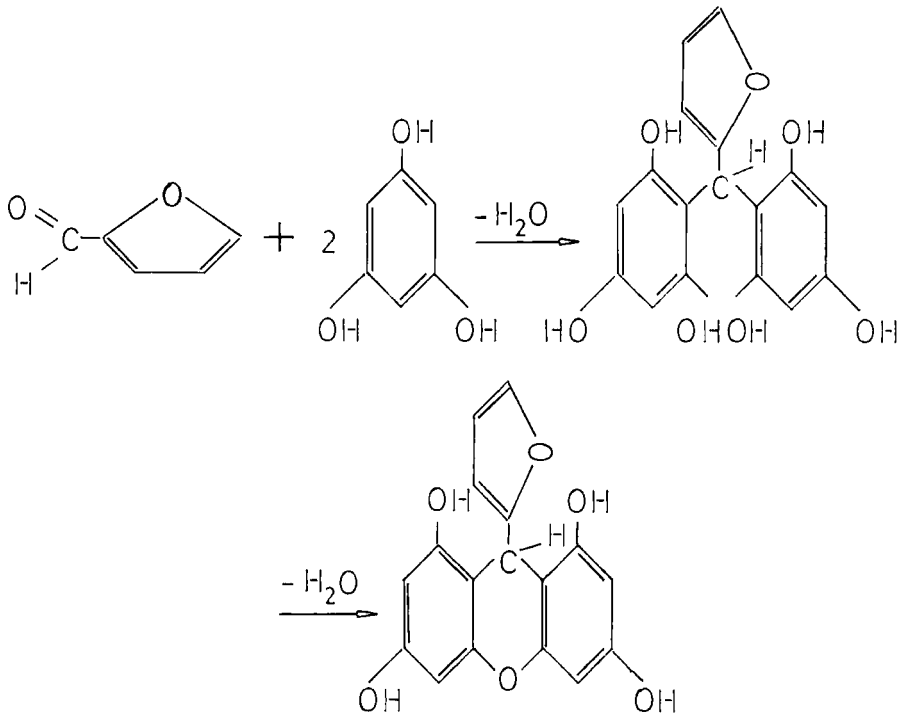

Fig. 2

Analysis of Guanosine and Inosine

Aqueous guanosine or inosine solutions processed in this method develop an intense colour, which is strongly dependent on the duration of the heating period. The corresponding spectra show two absorption bands, with maxima at the wavelengths recorded in Table I for each nucleoside.

Several series of standard solutions containing guanosine or inosine within the concentration ranges shown in Table I, were used to check adherence to Beer's law, at the wavelengths of maximum absorption. The concentration ranges of optimum accuracy were obtained from Ringbom's graphs (4) in accordance with standard practice.

T A B L E I

ANALYTICAL CONDITIONS AND APPLICABILITY RANGE.

Nucleoside	Heating time (min)	Wave lenght (nm)	Beer's interval ($Mx10^{-5}$)	Ringbom's interval (µg/mL)
Guanosine	30	450	2.01-15.4	15.6- 43.5
"	30	558	2.01-21.0	12.3- 43.8
Inosine	20	456	10.9-47.2	52.2-102.1
"	20	558	10.9-47.2	40.4-102.1

Good agreement was found in all cases over the studied ranges. When analysed by a least-squares procedure, the data fitted straight lines whose parameters are given in Table II, and gave very good correlation coefficients (signifiant to a P = 0.001 level (5)).

The coefficient of variation of the analytical results was in all cases less than 0.8% (Table III) and shows the suitability of the method for routine replicate analysis.

Selectivity of the reaction

In this study, the pyrimidine nucleosides were very resistant to undergo the colour-forming reaction with this low acidity phloroglucinol reagent solution, presumably due to the greater resistance of their N-C_1' bonds to acid hydrolysis (6). For this reason the interference caused by the presence of pyrimidine nucleosides in purine nucleoside solutions (at equal weigth) signifies a relative error of less than 1.0%. Further

T A B L E II

CALIBRATION DATA.

Nucleoside	Wave lenght (nm)	Number of results	Correl. Coef.	Slope (ΔA/ΔM)	Intercept (A)
Guanosine	450	10	0.998	1,440	−0.026
"	558	14	0.997	1,187	0.146
Inosine	456	9	0.998	504	0.015
"	558	9	0.995	602	0.317

T A B L E III

REPLICATE ANALYSIS DATA.

Nucleoside	Wave lenght (nm)	Number of results	Nominal (μg/mL)	Found (μg/mL)	Standard Deviation (μg/mL)	Coef. of Variation (%)
Guanosine	450	10	48	47–48	0.4	0.780
"	558	11	48	47–48	0.3	0.625
Inosine	456	8	148	148–150	1.1	0.765
"	558	7	148	146–150	0.9	0.650

studies of the analysis of pyrimidine nucleosides using more concentrated phloroglucinol solutions with a higher mineral acidity, are in progress.

The well-established selectivity of the phloroglucinol reaction for compounds which hydrolyze to substitued furan derivatives allows the determination of purine nucleosides in a variety of samples. In particular the reaction has been shown to be valid for the quantitative analysis of these compounds in complex galenical mixtures, since the results show that there is no interference from most of the usual components of geriatric formulations, such as pyridoxine derivatives, pyridinol, nicotinic acid, or xanthynol derivatives.

Neither do inorganic salts show any interference with the chromogenic reaction.

CONCLUSION

Operational simplicity, selectivity, sensitivity (10^{-5}M) and precision combine to make the new method most suitable for the rapid and accurate determination of purine nucleosides and in addition, it does not require sophisticated equipment.

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