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NOTE

Spectroscopic Determination of Riboflavin in Formulations

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Two simple, sensitive and reproducible spectroscopic methods are developed for the determination of riboflavin in pharmaceutical formulations. Method **A** is based on formation of ion association complex between riboflavin and bromothymol blue to produce a bluish green coloured species with λ_{max} 440 nm and its absorbance is linear with concentration over the range of 40-200 µg/100 mL. Method **B** is based on a similar principle involving treatment of riboflavin with congo red to produce a pale yellow coloured species that shows maximum absorption at 430 nm and exhibits linerarity in the concentration range of 40-200 µg/100 mL. Results of analysis were validated statistically and by recovery studies. This method could be successfully employed for routine determination of riboflavin in formulations.

Key Words: Beer's law, Extractive spectrophotometry, Analysis, Validation, Riboflavin.

A thorough review of literature revealed only a few HPLC[1-5], LC-MS[6-10], general spectrophotometric methods[11,12] and fluorimetric estimations[13,14] for the determination of riboflavin. It was also analyzed that no extractive spectrophotometric methods involving reactions with dyes were found in the literature and thus the author has made an attempt to exploit the available functional groups and ring systems in riboflavin to develop two extractive spectrophotometric methods for routine quality control analysis of riboflavin in formulations.

After due calibration of the instrument, spectral and absorbance measurements are made using UV-visible spectrophotometer, Elico-EL 170. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Fresh solutions of bromo thymol blue (0.2 % w/v) and chloroform for method **A** and aqueous solution of congo red (0.2 % w/v) and chloroform for method **B** were prepared.

Standard and sample solution of riboflavin: About 100 mg of riboflavin was accurately weighed on a digital single pan balance and dissolved in 100 mL of water in a volumetric flask to prepare a solution that has a concentration equal 1 mg/mL. Further dilutions are made with the same solvent to 100 µg/mL standard solution and are used for method **A** and method **B**.

Assay procedure

Method A: Aliquots of standard riboflavin solution (1 mL = 100 µg) ranging from 0.4-2.0 mL were transferred into a series of 250 mL separating funnels. To that 2 mL of bromothymol blue (0.2 %) was added and the total volume of the aqueous phase was made upto 10 mL with distilled water. About 10 mL of chloroform was added to each funnel and the contents were shaken for 2 min the two phases were allowed to separate and the absorbance of the chloroform layer was measured 440 nm against the corresponding reagent blank. The amount of riboflavin present in the sample solution was computed from its calibration curve.

Method B: Aliquots of standard riboflavin solution (1 mL = 100 µg) ranging from 0.4-2.0 mL were transferred into a series of 250 mL separating funnels. To that 2 mL of congo red (0.2 %) was added and the total volume of the aqueous phase was made upto 10 mL with distilled water. About 10 mL of chloroform was added to each funnel and the contents were shaken for 2 min the two phases were allowed to separate and the absorbance of the chloroform layer was measured 430 nm against the corresponding reagent blank. The amount of riboflavin present in the sample solution was computed from its calibration curve.

The proposed methods **A** and **B** are based on the formation of ion association complex with riboflavin. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and sandell's sensitivity are presented in Table-1. The regression analysis using the method of least squares was made for the slope (a), intercept (b) and correlation coefficient (γ) obtained from different concentrations was summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1. The accuracy of the method was ascertained by comparing

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Parameters	Method **A**	Method **B**
λ_{max} (nm)	440	430
Beer's law limit (µg/mL)	4-20	4-25
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.06	0.0546
Molar absorptivity (L mol^{-1} cm^{-1})	0.6272×10^4	0.69×10^4
Correlation coefficient (r)	0.9998	0.998
Regression equation (Y)*		
Slope (a)	0.1226	0.3178
Intercept (b)	0.00081	0.000555
RSD** (%)	1.83	1.638
Range of errors (95 % confidence limits) (%)		
0.05 significance level	± 1.5302	± 1.3696
0.01 significance level	± 2.2638	± 2.0263

*: Y = a + bx, where 'Y' is the absorbance and x is the concentration of riboflavin in µg/mL.
**: For six replicates.

the results obtained with the proposed and reference methods in the case of formulations and are presented in Table-2. As an additional check on the accuracy of the method, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed formulations and per cent recovery values obtained are listed in Table-2. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

TABLE-2
ESTIMATION OF RIBOFLAVIN IN PHARMACEUTICAL FORMULATIONS

Formulations	Labelled amount (mg/vial)	Recovery by proposed methods (%)	
		Method **A**	Method **B**
Tablet 1	100 mg	98.17	98.36
Tablet 2	100 mg	98.57	98.66
Tablet 3	100 mg	99.01	98.96
Tablet 4	100 mg	99.14	99.11

Thus the proposed method is simple and sensitive with reasonable precision and accuracy. This can be used for the routine determination of lercanidipine in quality control analysis.

Method A: The results obtained in this method were based on extractive spectrophotometry and the final colour developed is due to ion association complex between bromo thymol blue (BTB) and riboflavin resulting in the formation of a bluish green solution that exhibited maximum absorption at a wavelength of 440 nm against the corresponding reagent blank.

Method B: The results obtained in this method were based on extractive spectrophotometry and the final colour developed is due to ion association complex between congo red (CR) and riboflavin resulting in the formation of a pale yellow coloured solution that exhibited maximum absorption at a wavelength of 430 nm against the corresponding reagent blank.

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