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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROMETRIC METHOD FOR ESTIMATION OF WATER SOLUBLE VITAMIN RIBOFLAVIN

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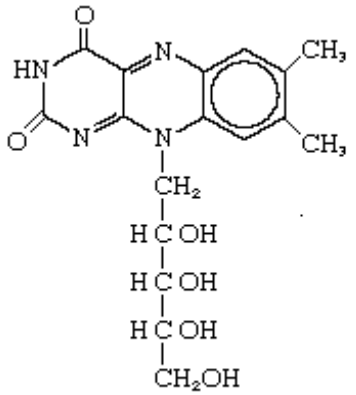
ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-Visible spectrophotometric method for the estimation of Riboflavin raw material. The Riboflavin is water soluble vitamin, so the solvent used throughout the experiment was 0.1N NaOH, the absorption maxima of drug was found at 445 nm. Beer's law was obeyed in the range of 5ppm-30ppm. the developed method was successfully validated with respect to linearity, accuracy and precision. The method was validated and shown linearity in mentioned concentration. The correlation coefficient for Riboflavin was 0.999. The percentage relative standard deviation of inter-day precision range 0.66-1.04% and intra-day precision 1.05-1.39% both should be less than 2%. Hence proposed method was precise, accurate and cost effective, simple and rapid. This validated method can be applicable for quantitative determination of the titled drug with respect to assay from or for their solid dosage forms.

INTRODUCTION: Vitamin B_2 (Riboflavin or vitamin G) is chemically, 3, 10-dihydro-7, 8-dimethyl-10-[2S, 3S, 4R)-2, 3, 4, 5 tetrahydroxypentyl]-benzopteridine-2, 4-dione (**fig. 1**).



Vitamin B_2 (riboflavin)

It is a yellow to orange-yellow crystalline compound. Action and physiological role of vitamin B_2, flavinadenine dinucleotide (FAD) and flavin mononucleotide (FMN) are coenzyme forflavoproteins involved in many oxidation-reduction reactions. Characteristic lesions of vitamin B_2 deficiency are angular stomatitis; sore and raw tongue, lips, throat, ulcers in mouth; vascularization of cornea. Dry scaly skin, loss of hair; anemia and neuropathy develop later.

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Biologically Literature survey revealed that few analytical methods are available for the estimation of Riboflavin by HPLC method and few methods were reported for estimation by fluorescence spectroscopy, some method in visible region by UV-Visible spectrophotometer but they are complicated and time consuming.

As the Riboflavin is water soluble vitamin, this study is carried out on the same property of water solubility of Riboflavin. Yet there is no method reported in the literature for the estimation of Riboflavin on the basis of water solubility property.

The aim of the work was to develop and validate an analytical method by using UV-Visible spectrophotometer for the estimation of Riboflavin by using aqueous solvent system.

MATERIAL AND METHOD:

Instrumentation: UV spectrophotometric method was performed on double beam UV-Visible spectrophotometer (Shimadzu, model 1700) having two matched quartz cells with 1 cm path length.

Method Development:

Solubility: Soluble in water, 0.1 N NaOH, practically insoluble in chloroform, ether, acetone.

Determination of λ_{max}:

Preparation of Stock Solution: Standard stock solution of Riboflavin was prepared by dissolving 100mg of Riboflavin in 100ml of 0.1N NaOH which gives 1000ppm concentration. 10ml of this stock solution was taken and was diluted up to 100ml by using 0.1N NaOH to produce a concentration of 100ppm solution

Preparation of Working Standard: From the above stock solution 1.5ml pipetted in to 10ml volumetric flask and the volume was made up with 0.1N NaOH to produce concentration of 15ppm. The solution was scanned in UV-Visible spectrophotometer in the range 600nm-400nm using 0.1N NaOH as a blank. The wavelength corresponding to maximum absorbance (λ_{max}) was found at 445.0 nm.

Preparation of Calibration Curve: 0.5ml solution of 100ppm was diluted to 10ml to produce 5ppm solution. 1ml, 1.5ml, 2ml, 2.5ml, 3ml of 100ppm solution were diluted to 10ml with 0.1N NaOH to produce 10ppm, 15ppm, 20ppm, 25ppm, 30ppm respectively. Thus calibration curve was constructed by taking solution concentrations ranged from 5ppm-30ppm. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (**Fig. 2**).

The curve showed linearity in the concentration range of 5ppm-30ppm. This straight line obeyed linearity in the concentration range of 5ppm-30ppm.the method was validated and shown linear in the mentioned range. The correlation co-efficient for riboflavin was 0.999 (Fig. 2).

Method Validation: Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result. The validation for UV method development was performed using parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, and Limit of detection (LOD), Limit of quantification (LOQ).

1. **Linearity:** Various aliquots were prepared from the secondary stock solution (100 ppm) ranging from 5ppm-35ppm. The samples were scanned in UV-Visible Spectrophotometer against 0.1N NaOH as blank. It was found that the selected drug shows linearity between the ranges of 5ppm-35ppm (**table 1**).

2. **Accuracy:** Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration using Riboflavin working standard as per the test method and taken absorbance of each solution in triplicate. The recovery results showed that the proposed method has an acceptable level of accuracy for Riboflavin which is from 80%-120% of test concentration is 99.51% -100.01% (**table 5**).

3. **Precision:** Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study nine different solutions of same concentration 5ppm, 15ppm, 25ppm were analyzed three times in a day i.e. from

morning, afternoon and evening and the absorbance is noted. From the absorbance result mean, standard deviation and %RSD was calculated and given in (**table 2**). In the inter-day variation studies, solution of same concentration 5ppm, 15ppm, 25ppm were analyzed three times for the three consecutive days and the absorbance result mean, standard deviation and %RSD was calculated and given in (**table 3**).

4. **Robustness:** Robustness of the method was determined by carrying out the analysis under different temperature condition i.e. at room temperature (25°C) and at (20°C). The respective absorbance of 20ppm were noted and the result was indicated as %RSD and given in (**table 4**).

5. **Ruggedness:** Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 20ppm was noted. The result was indicated as %RSD and given in (table 4).

6. **Limit of Detection (LOD):** The limit of detection (LOD) was determined by preparing solutions of different concentrations ranging from 0.1-0.5ppm. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantification as an exact value (**table 5**).

7. **Limit of Quantification (LOQ):** The LOQ is the concentration that can be quantification reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (**table 5**).

RESULT AND DISCUSSION:

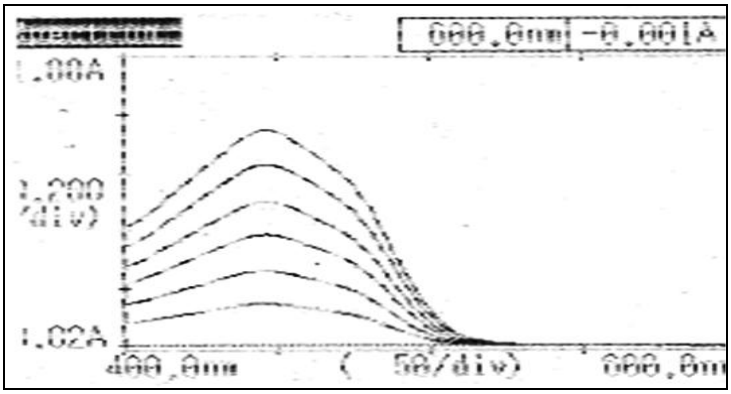

FIG 1: OVERLAY SPECTRA OF RIBOFLAVIN BY UV-VIS SPECTROSCOPY

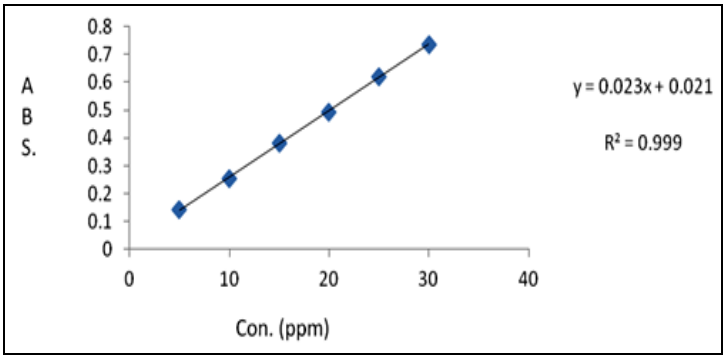

FIG 2: CALIBRATION CURVE OF RIBOFLAVIN BY UV-VISIBLE SPECTROSCOPY

TABLE 1: CALIBRATION DATA OF RIBOFLAVIN BY UV-VIS SPECTROSCOPY

S. No	Concentration (ppm)	Absorbance mean S.D (n=3)	% C.V.
1	05	0.144(±0.0012)	0.83
2	10	0.255(±0.0015)	0.58
3	15	0.380(±0.0010)	0.26
4	20	0.493(±0.0024)	0.49
5	25	0.619(±0.0040)	0.65
6	30	0.736(±0.0052)	0.71

TABLE 2: INTER-DAY PRECISION OF RIBOFLAVIN

Concentration	Absorbance	% C.V.
05	0.144(±0.0015)	1.04
15	0.380(±0.0025)	0.66
25	0.619(±0.0060)	0.96

TABLE 3: INTRA-DAY PRECISION OF RIBOFLAVIN

Concentration	Absorbance Mean abs. of (0, 3, 6 hrs.)	% C.V.
05	0.144(±0.0020)	1.13
15	0.380(±0.0045)	1.18
25	0.619(±0.0065)	1.05

TABLE 4: ROBUSTNESS AND RUGGEDNESS OF THE METHOD BY UV-VIS SPECTROPHOTOMETER OF RIBOFLAVIN

	Analyst-1		Analyst-2	
	Absorbance	Absorbance	Absorbance	Absorbance
	25°C	20°C	25°C	20°C
	0.491	0.492	0.492	0.490
	0.493	0.491	0.491	0.494
	0.491	0.490	0.494	0.492
Mean	0.4917	0.491	0.4923	0.492
%RSD	0.4881	0.4889	0.4875	0.4878

TABLE 5: SUMMARY OF VALIDATION PARAMETERS OF SIMPLE UV SPECTROSCOPY

Sr no	Parameters	Result
1	**λ_{max}**	445nm
2	**Regression line equation**	y=0.023+0.021
3	**Correlation coefficient(R^2)**	0.999
4	**Slope, b**	0.023
5	**Intercept,c**	0.021
6	**Precision (%RSD)**	
	Inter-day precision	0.66-1.04%
	Intra-day precision	1.05-1.39%
7	**Accuracy**	99.9%
8	**LOD**	0.215ppm
9	**LOQ**	0.652ppm
10	**Robustness**	0.4885%
11	**Ruggedness (20°C)**	0.4878%
	(25°C)	0.4884%

CONCLUSION: Unlike the gas chromatographic and HPLC procedures, the instrument is simple and affordable. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component.

The reagents utilized in the proposed methods are cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as reagent concentration, temperature.

The wide applicability of the new procedure for routine quality control is well established by the assay of vitamin B_2 (Riboflavin). Thus,the proposed validated method can be use for quality control of title material as well as its formulation.

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REFERENCES:

1. Tripathi K.D, Essentials of Medical Pharmacology, Jaypee Brother Medical Publication (P) Ltd, New Delhi,6th edition, 2008:873,874.
2. Barar F.S.K, Essentials of Pharmacology., S. Chand and Company Ltd., New Delhi ,4th edition, 2008:376-382.

3. Martindala, The Complete Drug Reference., Pharmaceutical Press,34th edition;1996.
4. The Marck Index,Monograph No. 9366, Marck and Co., White House Station, NJ, USA,13th edition, 2001 :1657.
5. The Marck Index, Monograph No. 8284, Marck and Co., White House Station, NJ, USA,13th edition, 2001 :1470.
6. Indian Pharmacopoeia. Government of India, Ministry of Health and Family welfare, The controller of publication, Civil Lines, Delhi, India,16th edition,2010:657.
7. United States Pharmacopoeia; The US Pharmacopoeial Convention, Inc. 12601 Twin brook Parkway, Rockville MD,24th edition,2007: 1480.
8. Analytical Methods Committee; Recommendations for the definition, estimation and use of the detection limit. Analyst112:1987: 199- 204.
9. ICH; draft Guidelines on Validation of Analytical Procedures, Definitions and Terminology, Federal Register, 60, IFPMA, Switzerland, 1995:1260.
10. ICH; Guidelines Q2BValidation of Analytical Procedures: Methodology. International Conferences on Harmonization. Geneva, 1996.
11. Bender, David A.; Nutritional biochemistry of the vitamins. Cambridge, U.K.: Cambridge University Press, 2003.
12. Sethi P.D.; Quantitative Analysis Drug Pharmaceutical Formulation. CBS Publishers and Distributors, Darya Ganj, New Delhi, 2006:578
13. Satyanarayan U., Chakrapani U., Biochemistry, Books and Allied (p) Ltd; 3rd edition:137-138.

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