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Spectrophotmetric Determination of Riboflavin with Spermine-Copper Chloride Complexes in Pharmaceutical Preparations

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Abstract: A new rapid and sensitive Spectrophotometric method was developed for the determination of Riboflavin in both pure and dosage forms. The proposed method is based on the complex formation reaction between Cupric Chloride and amino groups in Spermine followed by reaction between Cupric-Spermine complex and Riboflavin that produces a colored compounds, which gives maximum absorbance at 520nm. The range of linearity is 0.1-1.5mg/mL. Interferences of several vitamins, amino acids and sugars have also been studied. From the literature review it is clear that the given method was not reported before. The proposed method is applicable for the determination of riboflavin in pharmaceutical formulation. The results demonstrated that the method is accurate and reproducible.

Key words: Riboflavin · Spermine · Cupric Chloride · Spectrophotometric determination · Pharmaceutical preparations

INTRODUCTION

Counterfeiting of pharmaceuticals and proliferation of substandard drugs is one of the faster growing economic crimes threatening both developed and developing world alike. The United States Food and Drug Administration estimates that counterfeits make up more than 10% of the global medicines market and are present in both industrialized and developing countries. It is estimated that up to 25% of the medicines consumed in poor countries are counterfeit or substandard [1].

For quantitative estimation of multivitamins, methodology, time, cost and instrumentation is substantial while elimination of counterfeits demand rapid and low cost field testing methods. A few reports are available on comparative rapid analytical methods e.g. color reactions etc [2].

Riboflavin, also known as vitamin B2 or additive E101 [3], is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. It plays an important role in energy metabolism and for the metabolism of fats, ketone bodies, carbohydrates and proteins.

Milk, cheese, leafy green vegetables, liver, kidneys, legumes, tomatoes, yeast, mushrooms and almonds [4] are good sources of vitamin B2, but exposure to light destroys riboflavin. Therefore Riboflavin is used in multivitamins tablets, so for there analysis the reported method has been developed.

Survey of the literature showed that there very few methods available for determination of riboflavin including HPLC [5], electrogenerated chemiluminescence (ECL) [6], spectrophotometric methods [7].

Most of the spectrophotometric methods reported suffer from the disadvantages of narrow range of determination, long duration for the completion of reaction, use of non-aqueous system, need of heating or extraction, stability of the colored product formed etc [8]. The objective of this study was to develop a sensitive spectrophotometric method to rapidly assess the quality of commercially available multivitamin tablets containing riboflavin.

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Experimental

Chemicals and Reagents: The chemicals were of analytical reagent grade and used without further purification. All the chemicals were purchased from E. Merck.

Equipment: UV-VIS spectrophotometer (Model 6305, Jenway, UK) with 1cm matched quartz cells was used for all absorbance measurements. pH meter for pH measurements.

Standard Solutions: Riboflavin 100 ppm (Merck) was prepared by dissolving 5g of it in distilled water. 0.1 ml of 0.1N NaOH solution was added to clear the solution. Final volume was made up to 50ml. Working solution were prepared by diluting the stock solution standard solution.

Synthesis Colour Producing Reagent (Cu-Spermine Complex): The colour producing reagent, Cu-Spermine complex was synthesized by dissolving 2g of Spermine (Fluka) were dissolved in 30ml of pure ethanol (Merck). Reflux the solution until Spermine dissolve. Add (1.275g $CuCl_2.2H_2O$/50mL ethanol) drop wise into the Spermine solution, Precipitate of brown color appeared on cooling the solution. Filter the precipitate, wash with and dry them. The melting point was 255°C.

The solution of Cu-Spermine complex, i.e; colour producing reagent was prepared by dissolving 0.02 g of it in 1ml of benzene and diluting to 10ml with methanol.

Analytical Procedure: To a sample of riboflavin (0.1-1.5mg/ml) was added 2ml of colour producing reagent, i.e; Copper-Spermine complex. On shaking yellowish green colour was appeared which absorbed at 520nm.

RESULTS AND DISCUSSION

Various analytical parameters i.e wavelength of maximum absorption ($ë_{max}$), pH, color stability, effect of reagent concentration and linear measuring range were studied.

Table 1: Absorption spectra of Cu-spermine reagent, $CuCl_2.2H_2O$, Riboflavin, Cu-spermine- riboflavin complex

Wavelength (nm)	Cu-Sp-Rbf Absorbance	Riboflavin Absorbance	$CuCl_2H_2O$ Absorbance	Cu-Spermine Absorbance
400	0.063	0.044	0.064	0.089
410	0.065	0.067	0.083	0.101
420	0.062	0.098	0.108	0.129
430	0.051	0.115	0.153	0.134
440	0.063	0.128	0.186	0.139
450	0.046	0.129	0.292	0.125
460	0.056	0.121	0.372	0.124
470	0.125	0.113	0.301	0.125
480	0.231	0.102	0.217	0.126
490	0.265	0.099	0.19	0.123
500	0.29	0.066	0.1	0.12
510	0.318	0.059	0.067	0.112
520	0.345	0.056	0.032	0.107
530	0.282	0.051	0.021	0.103
540	0.192	0.049	0.019	0.103
550	0.12	0.049	0.016	0.105
560	0.072	0.045	0.017	0.103
570	0.023	0.042	0.036	0.102
580	0.006	0.038	0.039	0.101
590	0.01	0.051	0.037	0.104
595	0.005	0.036	0.028	0.107
600	0.01	0.034	0.027	0.106
610	0.016	0.029	0.082	0.105
620	0.023	0.026	0.084	0.097
630	0.028	0.025	0.074	0.082
640	0.039	0.022	0.09	0.08
650	0.032	0.032	0.078	0.077
660	0.019	0.019	0.077	0.074
670	0.016	0.046	0.098	0.071
680	0.009	0.019	0.099	0.069
690	0.01	0.025	0.063	0.065
700	0.029	0.014	0.074	0.053

Table 2: Effect of pH on Absorbance

pH	Absorbance	pH	Absorbance	pH	Absorbance
2	0.476	4.65	0.527	7.5	0.724
2.5	0.521	5	0.529	8	0.756
3	0.523	5.5	0.582	8.5	0.772
3.5	0.524	6	0.615	9	0.796
4	0.524	6.5	0.674	10	0.797
4.5	0.526	7	0.697	11	0.796

Table 3: Effect of Concentration of colour producing reagent, i.e; Cu-Spermine complex

Conc.(ppm)	Absorbance	Conc.(ppm)	Absorbance
0.2	0.173	1.2	0.319
0.4	0.195	1.4	0.326
0.6	0.235	1.6	0.329
0.8	0.285	1.8	0.334
1	0.312	2	0.339

Table 4: Effect of time on intensity of colour

Time (min)	Absorbance	Time (min)	Absorbance	Time (min)	Absorbance
5	0.322	40	0.342	75	0.347
10	0.327	45	0.344	80	0.348
15	0.329	50	0.346	85	0.348
20	0.33	55	0.346	90	0.347
25	0.333	60	0.347	95	0.348
30	0.339	65	0.347	100	0.348
35	0.341	70	0.346		

Table 5: Calibration Curve

Conc.(ppm)	Absorbance	Conc.(ppm)	Absorbance
0.1	0.015	0.9	0.109
0.2	0.027	1	0.124
0.3	0.042	1.1	0.135
0.4	0.049	1.2	0.146
0.5	0.061	1.3	0.158
0.6	0.069	1.4	0.167
0.7	0.081	1.5	0.179
0.8	0.096		

Spectral Characteristics: Riboflavin was complexed with color producing reagent (Cu-Spermine complex) to produce the colored product of $ë_{max}$ 520nm. This wavelength was used for all absorbance measurements. The corresponding reagent blank showed negligible absorbance at this wavelength. The concentration of Riboflavin in the multivitamin tablets was calculated by knowing the absorbance at $ë_{max}$ using the Lambert's-Beer law.

UV- Visible spectra of $CuCl_2$, riboflavin Spermine, Cu-Spermine complex and Copper-Spermine-Riboflavin complex are shown in fig.1. From the figure it is clear that Cu-Spermine-Riboflavin complex absorb maximum at 520nm which is different from others. Therefore, all measurements of Cu-Spermine-Riboflavin complex were carried out at this wavelength.

Effect of pH: To a series of 10ml vials was added 2ml of 100ppm Riboflavin solution and 2ml of colour producing reagent, i.e; Cu-Spermine complex. The pH of solutions were varied from 2 to 11 with the help of 0.1N HCl solution. Final volume was made to 10ml with methanol. After shaking a while absorbance measurements were recorded & plotted in fig.2. From figure it is clear that a pH of 7.0 is required to have maximum absorbance.

Effect of Concentration of Colour Producing Reagent, i.e; Cu-Spermine Complex: To a series of 10ml measuring flasks was added 2ml of 100ppm Riboflavin solution. The concentration of colour producing reagent was varied from 0.25 to 2.00mg. The pH of each solution was adjusted to 7.0 with 0.1N HCl & final volume was made to 10ml with methanol. After shaking absorbance measurements were recorded & plotted in fig.3. From the figure it is clear that 1mg/10ml or 2ml of (0.02g/10ml) colour producing reagent is required for maximum absorbance of Cu-Spermine-Riboflavin complex.

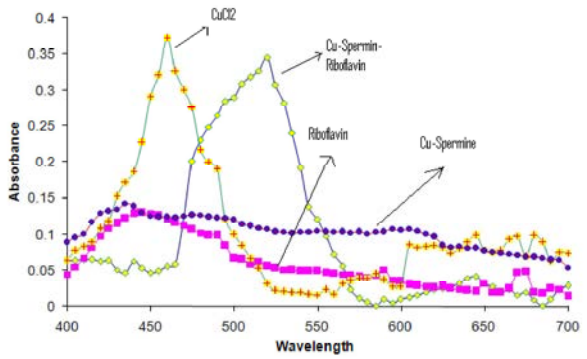

Fig. 1: Absorption spectrum of cupric chloride-spermine complex with riboflavin.

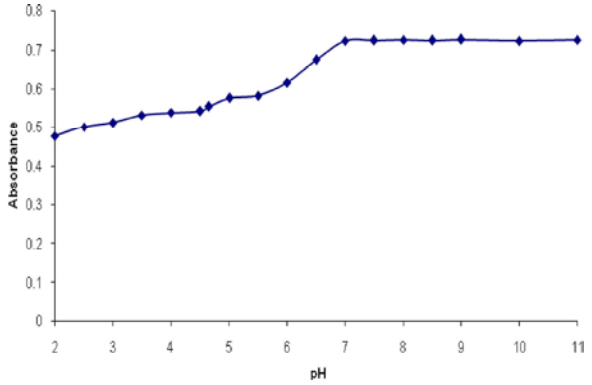

Fig. 2: Effect of pH on colour intensity.

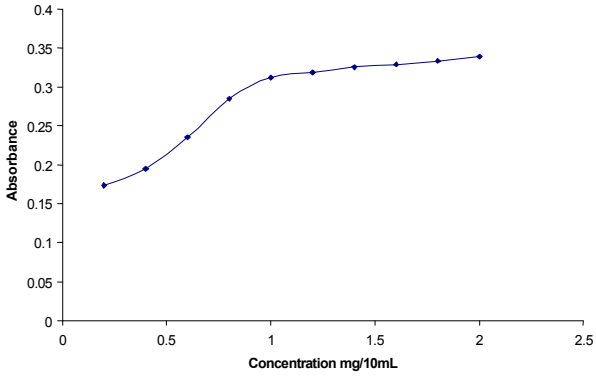

Fig. 3: Effect of concentration of color producing reagent on intensity of color.

Color Stability of the System: To a 10ml measuring flask was added 2ml of 100ppm Riboflavin solution and 2ml of colour producing reagent. pH of the solution was adjusted to 7.0 with 0.1N HCl & final volume was made to 10ml with methanol. Absorbance of the solution at 520nm was recorded at different intervals of time and plotted in fig. No.4. From the figure it is clear that Complex, i.e; Cu-Spermine-Riboflavin shows maximum absorbance after 15 minutes and remains so even after 1 $^{1}/_{2}$ hours.

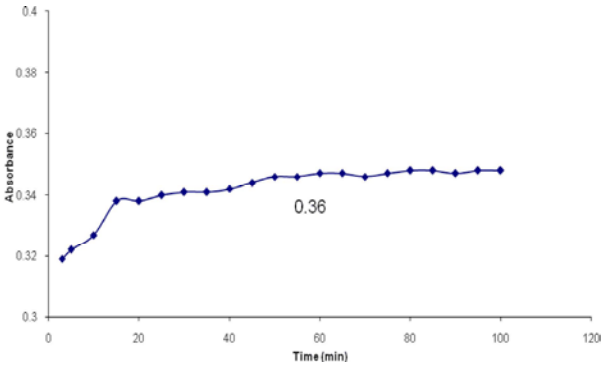

Fig. 4: Effect of time on intensity of color.

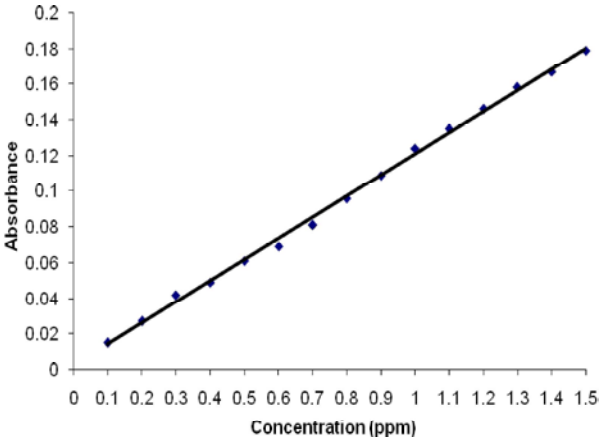

Fig. 5: Calibration Curve of Riboflavin.

Therefore, in future all absorbance measurements were carried out after 15 minutes of the synthesis of Cu-Spermine-Riboflavin complex.

Calibration Curve of Riboflavin: To a series of 10ml measuring flasks was added varying concentration of riboflavin, i.e; 0.1 to 1.5 mg/ml. to each flask was added 2ml of colour producing reagent. The pH of each solution was adjusted to 7.0 with 0.1N HCl. Final volume was made up to 10ml with methanol. Solutions were shaken for a while and absorbance measured after 15 minutes and plotted in fig. 5. From the figure it is clear that graph is linear from 0.1 to 1.5mg/ml of Riboflavin which is its therapeutic range of measurement.

Interferences: After the color development use of water should be avoided otherwise precipitation will cause which will interfere. Other problems of interference were not observed during this study. When the effects of other vitamins, amino acids and sugar were investigated in the formulations and pure forms using our developed method, no interference from all these common excipients and other substances was observed.

Table 6: Quantitative Assessment of Tolerable Amount of Different Vitamins, Sugars and Amino Acids

Compound	Maximum amount not interfering (mg)	Compound	Maximum amount not interfering (mg)
Vitamins		**Vitamins**	
Thiamine	>100	Glycin	>100
Ascorbic acid	>50	Hydroxyprolin	>100
Folic acid	>0.4	Lysine	>100
Pyrodoxine	>100	Methionine	>100
Sugars		Alanin	>100
Fructose	>100	Asparagin	>100
Glactose	>100	Arginin	>100
Glucose	>100	Tryptophan	>100
Maltose	>100	Glutamic acid	>100
Lactose	>100	Cystin	>100
Xylose	>100	Tyrosine	>100
		Phenylalanine	>100

Applications

Table 7: Determination of Riboflavin from pharmaceutical preparations.

Riboflavin from	Amount present	Amount found
Pure compound	µg/10mL	µg/10mL
	1.__	1.__
	2.__	2.__
	3__	3__
	4__	4__
	5__	5__
	mg/tablet	mg/tablet
Diabetone	5.00	4.8479
Biovit-M	8.50	8.2347
Engram	3.40	3.3192
Divasa	1.7	1.6469
Theragran-M	3.4	3.3192

CONCLUSION

The present methodology is found to be economical and more sensitive than the few reported spectrophotometric methods. The analysis of real samples containing riboflavin showed no interference from common excipients and additives. Hence the proposed method could be used for the determination of riboflavin in multivitamin tablets and other pharmaceutical formulations.

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