REVIEW ARTICLE

"METHODS OF ANALYSIS OF RIBOFLAVIN"

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Summary:
Vitamins are essential micronutrients required for normal growth and biochemical functions. Riboflavin (Vitamin B_2) is a water-soluble vitamin present in dairy products, vegetables, wheat, egg yolk, and meat. A number of techniques have been used for the analysis of riboflavin alone and in mixtures including UV and visible spectrometry, mass spectrometry, fluorimetry, chromatography and electrochemical methods. This article presents a mini review of the analytical methods that are used for the determination of riboflavin (vitamin B_2) in pharmaceutical products, food materials, and biological samples. These methods are based on spectral and electrochemical characteristics of riboflavin.

Keywords: Riboflavin, analysis, spectrometry, chromatography, fluorimetry.

1. INTRODUCTION :

The name vitamin is derived from a Latin word "vita" meaning life. Vitamins were also called vital amines because they were considered as amines on the basis of thiamin (vitamin B_1) and being essential component of diet. Vitamins usually act as catalysts or co-enzymes or often are essential parts of co-enzymes. These are organic molecules needed in small quantities for biochemical functions such as growth and repair of tissue. The structures of all the vitamins are known and synthetic vitamins are structurally similar to the natural vitamins. The sources of vitamins include dairy products, fish, rice, wheat, egg yolk, vegetables, fortified cereals, chicken and meat[1].

Vitamins have been considered to be necessary food factors. Humans either cannot synthesize vitamins or synthesize them in very small amounts and some amino acids which are characterized as dietary essentials[2]. The functions of vitamins are important for humans as they help in metabolism and vitamin deficiency

diseases. Avitaminosis leads to some form of disease which is associated with the type of the vitamin that is deficient[3]. Deficiency of vitamin D results in rickets and many other diseases of bones especially in children and new born. Anemia is caused by the deficiency of cyanocobalamin (vitamin B_{12}). Certain drugs required in some diseases may deplete some vitamins in the body which in turn must be supplied such as vitamin B_6 in Tuberculosis. Vitamin K deficiency leads to blood clotting problems and eye sight weakness and blindness is attributed to the deficiency of vitamin A. Vitamin E is known to be an antioxidant and its deficiency causes edema, thrombosis and hemolytic anemia[4]. Patients cannot tolerate over doses of vitamins and may respond with strain to gastrointestinal tract and nerve damage as in the case of pyridoxine overdose[5, 6]. Vitamins have found a limited use therapeutically but diseases caused by vitamin deficiencies find vitamins supplements as a first line cure. There are different

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diseases that are associated with the deficiency of each vitamin. These micro components of diet have also been indicated to be used in prophylactic measures. A balanced diet contains all the necessary nutrients but in some cases vitamin supplementation is required[7]. Vitamins are divided into water-soluble and fat-soluble vitamins on the basis of their solubility and chemical nature and the members of each group have some basic properties in common. The lipid-soluble vitamins are A, D, E and K and the water-soluble vitamins are B and C. After the structure determination of vitamins, they have been given the chemical names. The lipid-soluble vitamins are stored in liver whereas water-soluble vitamins are easily lost resulting in insignificant storage of their enzymes[8]. Therefore, daily intake is required of these vitamins to maintain everyday functions.

2. METHOD OF ANALYSIS OF RIBOFLAVIN:

This review deals with various methods used for the analysis of riboflavin in pharmaceuticals and biological fluids. Riboflavin [7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5 tetra-hydroxypentyl] benzo [*g*] pteridine 2,4(*3H*, *1OH*)-dione][9] was isolated as a coenzyme-enzyme complex from yeast by Warburg and Christian in 1932[10] and was designated as yellow oxidation ferment. It is synthesized by all green plants and by most bacteria and fungi. Although yeast is the richest source, eggs, dairy products, legumes, and meats are the major sources of riboflavin in the diet. The precursor is a guanosine phosphate derivative, but the exact synthetic steps leading to the vitamin are not understood completely[7]. The chemical structure of riboflavin (1) is shown in Fig. 1.

Riboflavin is a yellow to orange-yellow, crystalline powder with a slight odor. It is soluble in water 1:3000 to 1:20,000 mL, with the variation in solubility being due to differences in internal crystalline structure. It is more soluble in an isotonic solution of sodium chloride. It is soluble in benzyl alcohol (3%), gentisic acid (3%), and urea in varying amounts. Niacinamide is used to solubilize riboflavin when relatively high concentrations are needed for parenteral solutions. Gentisic ethanol amide and sodium 3-hydroxy-2-naphthoate also solubilize riboflavin effectively[11]. The following methods are used for the analysis of riboflavin.

2.1 UV and Vis Spectrometric Methods

The official British Pharmacopoeia[9] method for the assay of riboflavin is based on its absorption measurement at 444 nm and is applicable to pure solutions and single ingredient preparations. A novel and sensitive method has been described for the determination of riboflavin in urine and pharmaceutical samples and urine using an aqueous two-phase extraction (ATPE). An ATPE is formed mostly by water and does not require an organic solvent[11]. In another method, the separation of riboflavin, flavin mononucleotide (FMN) (2) (Fig.1) and flavin adenine dinucleotide (FAD) (3) (Fig.1) has been achieved in biological tissues by capillary zone electrophoresis using laser-induced fluorescence detection[13].

An enzymatic and spectroscopic determination of riboflavin using amylase phosphate is based on the formation of coloured species via the reaction of the enzyme, amylase phosphate, with ferric chloride, *n*-methyl benzothiazolone hydrazone (MBTH), and potassium ferricyanide in the presence of HCl and formation of a light green and blue coloured chromogen[14]. Soft independent modeling of class analogy and PLS (partial least-square) regression has been used for the identification and quantification of thiamine, riboflavin, nicotinamide and pyridoxine by UV-Vis spectrometry, without involving separation or preconcentration steps in the procedure [15].

A new spectrometric method has been proposed for the analysis of riboflavin and other water-soluble vitamins in pharmaceutical preparations using a multicommuted flow system. The concentrations between 5.00-50.00 mg/ml of riboflavin gave a linear response at the wavelength of 290 nm used for the assay[16].

Riboflavin along with vitamin B_1 and B_6 in pharmaceutical preparations has been assayed spectrometrically using PLS modeling and least-square support vector machines (LS-SVM). The

root mean square for riboflavin was found to be 0.3755 with PLS and 0.0318 with LS-SVM[17]. Multicomponent spectrophotometric assay of riboflavin and its photoproducts (formylmethylflavin, lumichrome and lumiflavin) have been reported. The photoproducts are separated by extracting with the chloroform. The method has been found to be specific, rapid and convinent for the assay of riboflavin, formylmethylflavin and other photoproducts[18].

The flex tolerance simplex method (FTSM) has been applied for the spectrometric determination of riboflavin, thiamine, niacin and pyridoxine. The recoveries of riboflavin have been found to be 100.1 ± 0.8 % [19]. A new method has been developed for the determination of riboflavin along with other water soluble vitamins by PLS regression method. The limit of detection has been found to be 0.09 µg/ml of riboflavin. The method can resolve complex mixture of compounds by strong over lapping signals[15].

Soft independent modeling of class analogy and PLS regression methods have been used for the identification and quantitation of thiamine, riboflavin, nicotinamide and pyridoxine by UV-visible spectrometry without separation or preconcentration steps in the procedure [20].

A group of workers have carried out two spectrophotometric methods, i.e. derivative and multivariate methods for the determination of riboflavin in multivitamin preparations. The methods have shown good accuracy and precision and linear calibration curves were obtained. The concentration of the solutions was in the range of 2.5-90 µg/ml and was measured in the wavelength range of 200-500 nm[21].

2.2. Mass spectrometric methods

Laser depletion mass spectrometry has been used for the assay of certain vitamins. The molecular fragmentation pattern of the vitamins have been found helpful in the analysis of the vitamins including vitamin A, D_3, C, B_1, B_2 and B_6[22].

A method has been developed for the analysis of water-soluble vitamins in multi-vitamin, multi-mineral dietary supplements using multiple reaction modes such as LC/UV/MS-MRM. The extraction of the vitamins is carried out with a 10 mM phosphate buffer at pH 2.5. The technique does not need any treatment before analysis[23].

A simple and reliable method has been reported for the determination of riboflavin along with vitamin B_3, B_6, caffeine and taurine in energy drinks by planar chromatography electro-spray ionization mass spectrometry (ESI-MS). The fluorescence measurement of riboflavin is performed at 366/>400 nm. All the calibrations are linear and the repeatabilities have been found to be between 0.8 and 1.5%[24].

2.3. Spectrofluorimetric methods

A fluorimetric method has been developed for the determination of riboflavin and pyridoxine in the acetate buffer (pH 6) in the presences of cyanocobalamin. The method has been found to be sensitive and has shown good precision with no interference from the pharmaceutical additives[25]. A new rapid synchronous spectrofluorimetric method for the simultaneous determination of riboflavin and folic acid in nutritional beverages has been found to give satisfactory results. The calibration curves are linear in the range of 1-250 µg/l and the detection limit is 0.014 µg/l[26]. Riboflavin along with thiamine and pyridoxine in a pharmaceutical preparation has been assayed spectrofluorimetrically by this method. The method has proved to be stability-indicating with the sensitivity ranging between 0.4-2.0 µg/ml[27].

2.4. Chromatographic methods

A rapid, precise and time saving validated HPLC method has been used for the simultaneous determination of riboflavin, pyridoxine, cyanocobalamin and folic acid in nutraceuticals. The method involves gradient elution of mobile phase through C_{18} discovery column in RP-HPLC with UV detection at 254 nm at ambient temperature[28]. A simple, fast, inexpensive, and reliable method for the simultaneous, routine

determination of thiamine and riboflavin in mushrooms has been developed using an extraction procedure followed by liquid chromatographic separation on a RP Spherisorb ODS column with methanol/water as a mobile phase[29]. Another method for the simultaneous determination of thiamine and riboflavin in edible marine seaweeds by HPLC has been developed. In this study samples were prepared by acid and enzymatic hydrolysis prior to analysis[30].

The separation of thiamine, riboflavin and niacin has been achieved using TLC and then fluorimetrically determined by a fibre-optic-based instrument. Under fluorimetric monitering riboflavin showed native fluorescence, but niacin and thiamine had to be pre-chromatographically converted to fluorescent derivatives for detection[31]. A simplified procedure for the determination of thiamine and riboflavin contents in meat products has been proposed. The vitamins ware extracted from the meats by digestion with 0.1 M HCl followed by the hydrolysis of phosphorylated forms of both vitamins using an acid phosphatase enzyme[32].

The determination of folic acid, nicotinamide, nicotinic acid, riboflavin, riboflavin-5'-phosphate, pyridoxine and thiamine in tablets has been successfully automated using a bench-top robotic system (Zymark Tablet Processing Workstation II) coupled to reversed-phase (RP-18) HPLC with UV-detection[27]. A new high-throughput method has been developed to detect simultaneously riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by multiple detection[33]. Three elution methods on two different RP C_{18}Êcolumns have been developed to determine flavin derivatives in raw egg white, raw egg yolk, egg powder, pasteurised milk, fermented milk products and liver[34]. An HPLC method has been used for the simultaneous determination of seven water-soluble vitamins, viz. thiamine, riboflavin, nicotinic acid, nicotinamide, pyridoxine, cyanocobalamin, and folic acid, in multivitamin pharmaceutical formulations and biological fluids[35]. The analysis of free riboflavin and its two coenzymes, FMN and FAD, is optimized using RP-HPLC with fluorescence detection[36].

2.5. Electrochemical methods

Riboflavin and its photoproducts, formylmethylflavin and lumichrome has also been determined by polarographic methods using half-wave potentials of -0.47V, -0.45V and -0.58V, respectively[37].

2.6. Enzymatic assay method

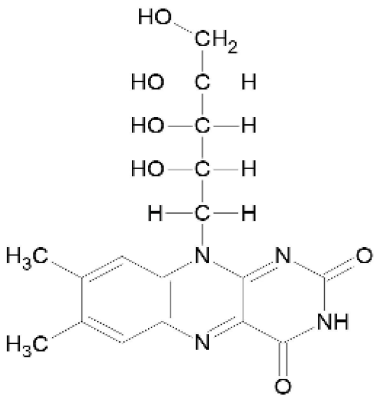
An enzyme-linked ligand-sorbent assay (ELLSA) of vitamin B_2 has been reported. The conjugates obtained by coupling 3-carboxymethylriboflavin and bovine serum albumin are adsorbed on the multi-well microtitre plates. The detection limit of the method has been found to be 0.8 pmol. The method can be used for the determination of riboflavin in human plasma and urine[38].

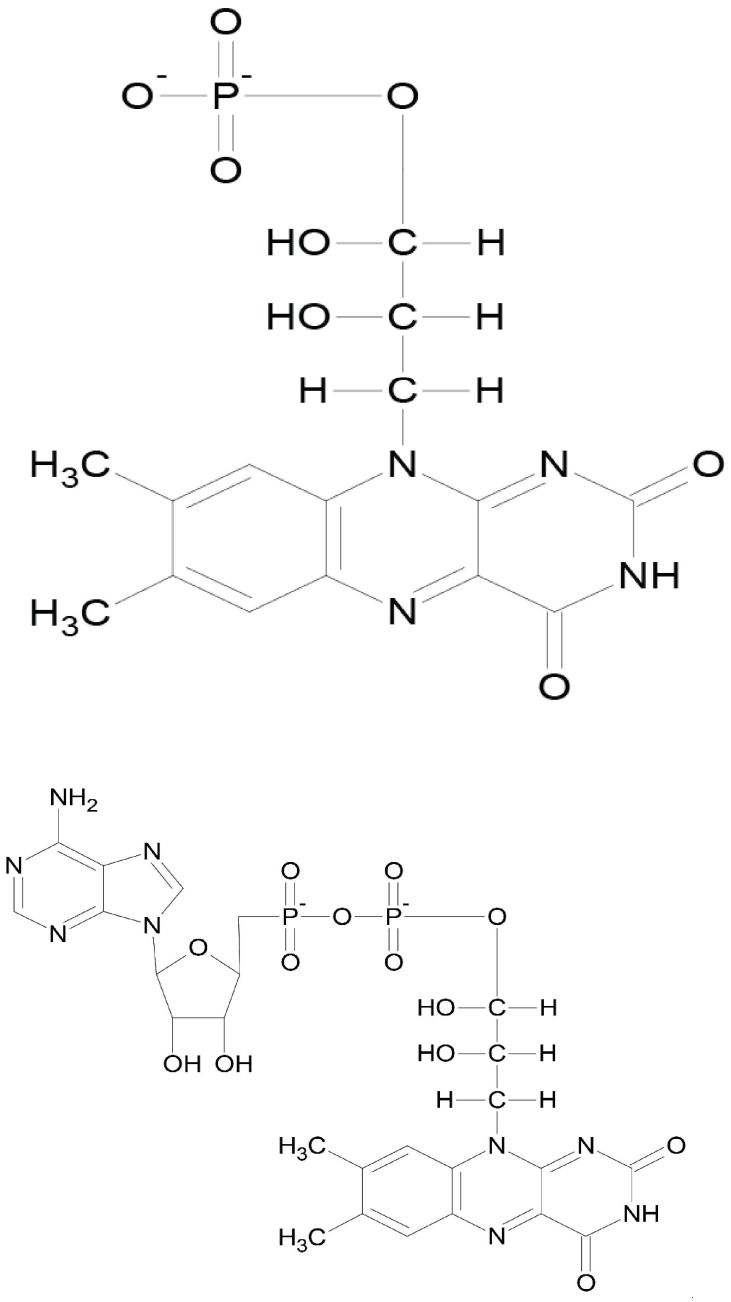
2.7. Microbiological methods

A microbiological method has been reported which can be used for the assay of riboflavin, thiamine, pyridoxine, cyanocobalamine, calcium pantothenate, nicotinic acid, pantothenol and folic acid. The cultures were prepared with separate automated systems, turbidity produced has been measured and the values of extinction has been printed at the rate of 300/hr[39].

3. CONCLUSION

Riboflavin is widely used as a constituent of vitamin preparations. Several analytical methods based on spectrometric, fluorimetric, electrochemical, chromatographic and electrophoretic techniques have been presented for the assay of riboflavin in foods, vitamin preparations and biological fluids. All of the techniques used have several advantages along with the accurate and precise results. The fluorimetric methods for the determination of riboflavin are more sensitive than the other methods.





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