

## A review of novel analytical methods for the determination of Vitamin B2 (Riboflavin)

HJ Salunkhe \*, GS Bhunje and KH Surnavar

*Department of Chemistry, Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati, Pune, MS, India.*

World Journal of Advanced Research and Reviews, 2025, 25(01), 333-338

Publication history: Received on 19 November 2024; revised on 30 November 2024; accepted on 01 January 2025

Article DOI: <https://doi.org/10.30574/wjarr.2025.25.1.4055>

### Abstract

This review highlights recent advancements in analytical techniques used for the determination of Vitamin B2 (riboflavin) in pharmaceuticals, foods, and biological samples. Various methods, including UV-Vis. spectrometry, fluorimetry, chromatography, mass spectrometry, and electrochemical techniques, are discussed. These methods leverage the unique properties of riboflavin, such as its fluorescence and redox potential, for accurate quantification. The review emphasizes the importance of these techniques in ensuring quality control and compliance with regulatory standards, while also exploring emerging trends like automation and green analytical chemistry.

**Keywords:** Riboflavin; Vitamin B2; Analytical Methods; Spectrometry; Chromatography; Fluorimetry

### 1. Introduction

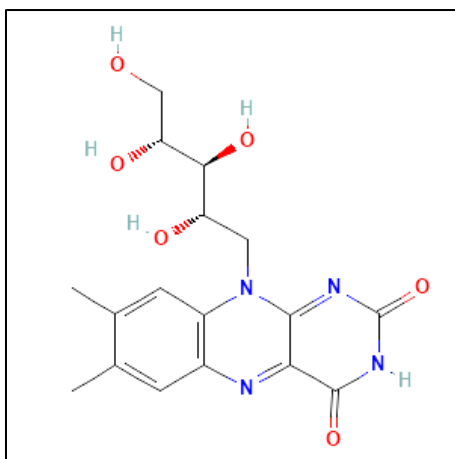
Riboflavin, commonly known as Vitamin B2, is a water-soluble vitamin essential for human health. It plays a crucial role in metabolic processes by acting as a precursor for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), coenzymes involved in numerous oxidation-reduction reactions. These coenzymes are critical for energy production and the metabolism of carbohydrates, fats, and proteins. Riboflavin is naturally found in various food sources, including dairy products, eggs, meat, leafy vegetables, and fortified cereals. Despite its dietary availability, deficiencies can occur, leading to conditions such as ariboflavinosis, characterized by symptoms like cracked lips, sore throat, and skin disorders.

The importance of riboflavin extends beyond its physiological roles. It is also widely used in pharmaceutical formulations and food fortification, making its accurate determination essential for quality control and regulatory compliance. The stability of riboflavin, however, is a major concern. It is highly sensitive to light, heat, and alkaline pH, which can lead to degradation and loss of bioactivity. This sensitivity necessitates the use of precise and reliable analytical methods to ensure the accuracy of riboflavin quantification in various matrices.

#### 1.1. Significance of Riboflavin Analysis

Riboflavin analysis is crucial for several reasons. First, it ensures that pharmaceutical products meet regulatory standards and contain the correct dosage of the vitamin. Second, it helps monitor and maintain the nutritional quality of fortified foods and beverages. Finally, riboflavin analysis is vital in clinical settings to assess vitamin B2 status in patients and diagnose deficiencies. Given these diverse applications, robust analytical methods are needed to accommodate different sample types and concentration ranges.

\* Corresponding author: HJ Salunkhe



**Figure 1** Chemical Structure of Vitamin B2 (Riboflavin)

### 1.2. Challenges in Riboflavin Analysis

Several challenges are associated with riboflavin analysis. The primary challenge is its instability, particularly under exposure to light and alkaline conditions. Riboflavin degrades into photoproducts such as lumichrome and lumiflavin, which can interfere with its accurate quantification. Additionally, riboflavin often exists in complex matrices, such as pharmaceutical formulations and biological samples, alongside other vitamins and compounds. This complexity requires analytical methods with high specificity and sensitivity to distinguish riboflavin from potential interferents.

The low concentrations of riboflavin in biological samples present another challenge. Detecting such trace levels necessitates highly sensitive techniques, such as liquid chromatography coupled with mass spectrometry (LC-MS). Furthermore, the need for rapid and cost-effective methods is growing, especially in industries where high-throughput analysis is required for quality control.

### 1.3. Analytical Techniques for Riboflavin Determination

Various analytical methods have been developed to overcome these challenges and ensure accurate riboflavin quantification. These methods can be broadly categorized into spectrometric, chromatographic, fluorometric, electrochemical, and microbiological techniques. Each method leverages the unique properties of riboflavin, such as its fluorescence and redox activity, to facilitate its detection and quantification.

- **Spectrometric Methods:** UV-Vis. spectrophotometry is one of the earliest techniques used for riboflavin analysis. It measures absorbance at specific wavelengths, typically around 444 nm. Although simple and cost-effective, this method can suffer from interference by other absorbing species in complex samples. Advanced spectrometric techniques, such as derivative and multivariate methods, have improved accuracy and precision by minimizing interference.
- **Fluorometric Methods:** Riboflavin's natural fluorescence makes fluorimetry a highly sensitive technique for its determination. Synchronous fluorometric methods allow simultaneous detection of riboflavin and other vitamins in pharmaceuticals and nutritional products. Fluorometric methods are particularly useful for analysing low concentrations of riboflavin in biological fluids.
- **Chromatographic Methods:** High-performance liquid chromatography (HPLC) is widely regarded as the gold standard for riboflavin analysis. HPLC methods can separate riboflavin from its photoproducts and other coexisting compounds, ensuring accurate quantification. Reversed-phase HPLC with UV or fluorescence detection is commonly used for routine analysis. Capillary electrophoresis (CE) and thin-layer chromatography (TLC) are also employed in specific applications.
- **Mass Spectrometry:** The combination of HPLC with mass spectrometry (LC-MS) has revolutionized riboflavin analysis by offering unparalleled sensitivity and specificity. LC-MS allows for the detection of riboflavin in trace amounts and provides structural information about its photoproducts and metabolites.
- **Electrochemical Methods:** Electrochemical techniques utilize the redox properties of riboflavin to facilitate its detection. These methods are simple and cost-effective but may require careful calibration to avoid interference from other electroactive species.

- **Microbiological Assays:** Although less common, microbiological methods can be used to measure riboflavin by assessing its effect on the growth of specific microorganisms. These assays are primarily used in research settings or when other analytical methods are not feasible.

#### 1.4. Emerging Trends and Future Directions

The field of riboflavin analysis is continuously evolving, driven by advancements in analytical instrumentation and the need for sustainable practices. Green analytical chemistry is gaining prominence, with a focus on minimizing solvent use and reducing waste. Portable and automated systems are also being developed to enable real-time, on-site riboflavin analysis in clinical and industrial settings. The integration of artificial intelligence (AI) and machine learning in data analysis is expected to enhance method development and improve the accuracy of riboflavin quantification.

The accurate determination of riboflavin is essential for ensuring the quality and efficacy of pharmaceuticals, fortified foods, and nutritional supplements. Despite the challenges posed by its instability and complex sample matrices, advancements in analytical techniques have significantly improved the reliability of riboflavin analysis. Continued innovation and the adoption of sustainable practices will further enhance the efficiency and accessibility of these methods in the future.

---

## 2. Analytical Methods for Riboflavin Determination

The accurate determination of riboflavin (Vitamin B2) is essential for ensuring the quality and safety of pharmaceuticals, fortified foods, and clinical samples. A variety of analytical techniques have been developed, each leveraging the unique physico-chemical properties of riboflavin, such as its fluorescence and redox activity. These methods are categorized into spectrometric, chromatographic, fluorometric, electrochemical, and microbiological techniques, each with specific advantages and limitations depending on the application and matrix complexity.

### 2.1. Spectrometric Methods

Spectrometric methods, including UV-Vis. spectrophotometry, are among the earliest and most commonly used techniques for riboflavin analysis. Riboflavin exhibits an absorption peak at 444 nm, which allows for its quantification in pharmaceutical formulations and pure solutions. Advanced techniques, such as derivative spectrophotometry and multivariate analysis, have been developed to overcome challenges related to matrix interference and overlapping signals. These methods improve accuracy and precision by isolating riboflavin's spectral features from those of other compounds.

Spectrometric methods are simple, cost-effective, and non-destructive but are less sensitive compared to other advanced techniques, especially in complex samples where interference can affect results.

### 2.2. Fluorometric Methods

Riboflavin's natural fluorescence makes fluorimetry a highly sensitive and selective method for its analysis. Fluorometric techniques detect riboflavin at low concentrations and are widely used in pharmaceutical and food industries. Synchronous fluorescence methods enable the simultaneous determination of riboflavin along with other vitamins, such as folic acid and pyridoxine, in complex mixtures. Fluorometric methods offer excellent sensitivity but can be affected by quenching agents and require careful control of experimental conditions.

### 2.3. Chromatographic Methods

Chromatographic techniques, particularly high-performance liquid chromatography (HPLC), are considered the gold standard for riboflavin determination. Reversed-phase HPLC with UV or fluorescence detection provides high sensitivity and specificity, allowing for the separation of riboflavin from its photoproducts and other vitamins in complex matrices.

In addition to HPLC, thin-layer chromatography (TLC) and capillary electrophoresis (CE) are also used for riboflavin analysis. These methods are simpler and less expensive but may not offer the same level of sensitivity and reproducibility as HPLC.

### 2.4. Mass Spectrometry

Coupling HPLC with mass spectrometry (LC-MS) significantly enhances riboflavin analysis by providing both quantitative and qualitative data. LC-MS allows for the detection of riboflavin in trace amounts and offers insights into

its structural properties and metabolites. This technique is particularly useful for analysing biological samples and fortified foods where precise quantification is critical. Despite its high sensitivity, LC-MS requires sophisticated instrumentation and expertise, which can be a limiting factor in some settings.

### **2.5. Electrochemical Methods**

Electrochemical techniques, such as voltammetry and polarography, utilize riboflavin's redox properties for detection. These methods are cost-effective and require minimal sample preparation, making them suitable for routine analysis. However, they can be affected by interference from other electroactive species in complex matrices.

### **2.6. Microbiological Assays**

Microbiological methods involve assessing riboflavin's impact on the growth of specific microorganisms. These assays are useful for determining bioavailable riboflavin in complex samples but are less commonly used due to their time-consuming nature and reliance on biological variability.

The choice of analytical method for riboflavin determination depends on factors such as sensitivity, specificity, cost, and the complexity of the sample matrix. Spectrometric and fluorometric methods are suitable for routine analysis, while HPLC and LC-MS offer high precision and are preferred for complex samples. Emerging trends focus on automation, sustainability, and the development of portable devices for on-site testing, enhancing the efficiency and accessibility of riboflavin analysis across various industries.

---

## **3. Challenges in Riboflavin Analysis**

The analysis of riboflavin (Vitamin B2) presents several challenges, primarily due to its chemical properties and the complexity of sample matrices. One of the most significant issues is riboflavin's instability. It is highly sensitive to light, heat, and alkaline pH, leading to degradation and the formation of photoproducts such as lumichrome and lumiflavin. These degradation products can interfere with riboflavin quantification, necessitating careful handling and storage to preserve sample integrity.

Another challenge is the presence of complex matrices in samples, such as pharmaceutical formulations, fortified foods, and biological fluids. These matrices often contain other vitamins, proteins, and excipients, which can cause interference and affect the accuracy of analytical methods. For example, spectrometric techniques may suffer from overlapping absorption spectra, while electrochemical methods may encounter interference from other redox-active species. Advanced separation techniques like HPLC are often required to isolate riboflavin from these interfering substances.

Low concentrations of riboflavin in biological samples pose another challenge, requiring highly sensitive methods such as LC-MS for accurate detection. However, these techniques are expensive and require specialized equipment and expertise, limiting their accessibility in routine analysis.

The need for rapid, high-throughput methods is also a pressing challenge, particularly in industrial settings where large sample volumes need to be analysed efficiently. Balancing sensitivity, accuracy, and speed remains a critical consideration in method development.

In summary, overcoming these challenges requires the use of advanced analytical techniques, careful sample handling, and innovations in automation and miniaturization to improve efficiency and reduce costs.

---

## **4. Future Trends and Sustainable Practices**

The future of riboflavin analysis is shaped by advancements in technology and a growing emphasis on sustainability. One key trend is the adoption of green analytical chemistry practices, focusing on reducing solvent usage, minimizing waste, and utilizing biodegradable materials. Techniques that align with these principles not only lower environmental impact but also improve cost-efficiency in routine analyses.

Automation and high-throughput screening are becoming increasingly prevalent, enabling faster and more reproducible analyses. Automated systems for sample preparation and analysis reduce human error and enhance productivity, especially in industrial settings. The integration of portable devices and point-of-care testing (POCT) technologies is also gaining traction, allowing for on-site, real-time monitoring of riboflavin in clinical and field applications.

The incorporation of artificial intelligence (AI) and machine learning is revolutionizing data analysis by improving pattern recognition and predictive capabilities. AI-driven models can optimize method development and enhance accuracy in complex sample matrices.

The Future methods will prioritize sustainability, efficiency, and accessibility, ensuring that riboflavin analysis remains effective and environmentally responsible across various industries.

---

## 5. Conclusion

The accurate analysis of riboflavin (Vitamin B2) is essential for ensuring the quality of pharmaceuticals, fortified foods, and nutritional supplements. As a critical micronutrient involved in metabolic processes, riboflavin's quantification is necessary for both regulatory compliance and clinical assessment. Despite its importance, riboflavin analysis presents challenges due to its sensitivity to light, heat, and pH, as well as the complexity of sample matrices. Advanced analytical techniques, such as HPLC, fluorimetry, and LC-MS, have proven effective in overcoming these obstacles, offering high sensitivity and specificity for riboflavin detection. Spectrometric and fluorometric methods are valuable for routine analysis due to their simplicity and cost-effectiveness, while chromatographic and mass spectrometric techniques are ideal for complex samples requiring high precision. Electrochemical methods offer a cost-efficient alternative for redox-based detection, though they require careful calibration to avoid interference. Looking ahead, the integration of sustainable practices and automation will play a pivotal role in enhancing analytical efficiency. Green analytical techniques that minimize solvent use and reduce waste are gaining importance, aligning with global sustainability goals. Additionally, advances in portable devices and AI-driven data analysis will enable real-time, on-site testing and improve accuracy and throughput. Continued innovation in analytical methods and sustainable practices will ensure the reliable and efficient determination of riboflavin across diverse industries, supporting public health and regulatory compliance.

---

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

---

## References

- [1] Abbas, S. H., Mustaan, N., Khan, M. F., Bano, R., Anwar, Z., & Ahmad, I. (2020). Methods of Analysis of Riboflavin. *Baqai Journal of Health Sciences*, 23(1), 1-8.
- [2] British Pharmacopoeia. (2016). Her Majesty's Stationery Office, London.
- [3] Warburg, O., & Christian, W. (1932). A New Oxidation Enzyme and Its Absorption Spectrum. *Biochemische Zeitschrift*, 254, 438-458.
- [4] O'Neil, M. J. (2013). *The Merck Index*. 13th Edition, Merck and Co. Inc.
- [5] Shiri, S., Khezeli, T., & Sajjadifar, S. (2013). A Novel Method for Determination of Vitamin B2 in Pharmaceutical Samples Using Aqueous Two-Phase Extraction. *Journal of Chemistry*, 10, 1155.
- [6] López-de-Alba, P. L., Martínez, L., & Hernández, J. (2006). Simultaneous Determination of Water-Soluble Vitamins by UV-Vis Spectrometry and Multivariate Analysis. *Journal of the Brazilian Chemical Society*, 17, 5053-5056.
- [7] Rocha, F. R. P., Filho, O. F., & Reis, B. F. (2003). A Multicommuted Flow System for Spectrophotometric Determination of Vitamins. *Talanta*, 57, 200-256.
- [8] Niazi, A., & Yazdanipour, A. (2006). Determination of Riboflavin in Human Plasma by Multi-Way Analysis. *Journal of the Chinese Chemical Society*, 53, 503-510.
- [9] Ahmad, I., & Hussain, W. (1992). Multicomponent Spectrometric Assay of Cyanocobalamin and Riboflavin. *Pakistan Journal of Pharmaceutical Sciences*, 5, 121-127.
- [10] Chen, P., & Atkinson, R. L. (2009). Single Lab Validation of LC/UV/FLD/MS Method for Vitamins. *Journal of AOAC International*, 92, 680-688.
- [11] Aranda, M., & Morlock, G. (2006). Simultaneous Determination of Vitamins in Energy Drinks by Planar Chromatography. *Journal of Chromatography A*, 1154, 268-275.

- [12] Mohamed, A. M., & Marwa, Z. R. (2011). Chemometric Methods for Simultaneous Determination of Vitamins. *Journal of AOAC International*, 94, 467-481.
- [13] Parveen, S., Yasmin, A., & Khan, K. M. (2009). Quantitative Estimation of Vitamins in Nutraceuticals by HPLC. *Open Analytical Chemistry Journal*, 3, 1-5.
- [14] Esteve, M. J., Farre, R., & Garcia-Cantabella, J. M. (2001). Determination of Thiamin and Riboflavin in Mushrooms by LC. *Journal of Agricultural and Food Chemistry*, 49, 1450-1454.
- [15] Sánchez-Machado, D. I., López-Cervantes, J., & Paseiro-Losada, P. (2004). Determination of Vitamins in Marine Seaweeds by HPLC. *Journal of Chromatographic Science*, 42, 117-120.
- [16] Tang, X., Cronin, D. A., & Brunton, N. P. (2006). Determination of Vitamins in Meat Products by RP-HPLC. *Journal of Food Composition and Analysis*, 19, 831-837.
- [17] Gliszczynska-Świgło, A., & Koziołowa, A. (2000). Chromatographic Determination of Riboflavin and Derivatives. *Journal of Chromatography A*, 881, 285-297.
- [18] Chatzimichalakis, P. F., & Papadoyannis, I. (2004). Development of HPLC Method for B-Complex Vitamins. *Journal of Separation Science*, 27, 1181-1188.
- [19] Kozik, A. (1996). Microtitre-Plate Enzyme-Linked Assay for Riboflavin in Plasma and Urine. *Analyst*, 121, 333-337.
- [20] Knobloch, E. (1967). Polarographic Study of Riboflavin Photolysis. *Biochimica et Biophysica Acta*, 141, 19-26.