
Vitamin B₂ (Riboflavin Sodium Phosphate)  
*(FAD-2011-0051; CRL/100356)*

Dossier related to: FAD-2011-0051 - CRL/100356
Name of Product: Vitamin B2
Active Agent (s): Riboflavin Sodium Phosphate
Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 10(2) for Vitamin B$_2$ (Riboflavin sodium phosphate) under the category-functional group 3(a) ‘nutritional additives’/‘vitamins, pro-vitamins and chemically well-defined substances having similar effect’ according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the feed additive for all animal species and categories. The feed additive is a yellow orange, crystalline hygroscopic powder produced by chemical synthesis consisting mainly of the riboflavin sodium phosphate (73-79 % of riboflavin). The feed additive is intended to be used in water for drinking through liquid or soluble powder premixtures. While no maximum dosage in water is provided, the Applicant recommends not to exceed the 3 % of Vitamin B2 in the final solution, due to its solubility.

For the characterisation of Riboflavin sodium phosphate in the feed additives the Applicant proposed the European Pharmacopoeia method and the FAO JECFA monograph recommended by Commission Regulation EU No 231/2012, where identification is based on specific optical rotation and ultraviolet and visible absorption spectrophotometry, while quantification of the total colouring matter content of the Riboflavin sodium phosphate is based on spectrophotometry at 444 nm. Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia and the FAO JECFA methods to characterise the Riboflavin sodium phosphate salt.

For the quantification of Riboflavin sodium phosphate in the liquid and soluble powder form premixtures the Applicant proposed an in-house developed High Performance Liquid Chromatography coupled to an UV detector (HPLC-UV) method, derived from the method described in the European Pharmacopoeia. Based on the satisfactory performance characteristics provided, the EURL considers the HPLC-UV method submitted by the Applicant suitable for official control to quantify riboflavin sodium phosphate in liquid and soluble powder form premixtures.

The Applicant did not present experimental data of analysis of the riboflavin 5’-phosphate sodium in water, however the EURL considers the method derived from the ring-trial validated CEN method (EN 14152) suitable for the quantification of riboflavin sodium phosphate in water, based on acid hydrolysis followed by enzymatic dephosphorylation and using HPLC with fluorimetric detection (HPLC-FL).

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.
KEYWORDS

Vitamin B₂, Riboflavin sodium phosphate, nutritional additives, vitamins, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 10(2) (re-evaluation of the already authorised additives under provisions of Council Directive 70/524/EEC) for Vitamin B₂ (Riboflavin sodium phosphate, also called riboflavin-5'-sodium hydrogen phosphate) under the category/functional group 3(a) ‘nutritional additives’/‘vitamins, pro-vitamins and chemically well-defined substances having similar effect’ according to Annex I of Regulation (EC) No 1831/2003 [1,2]. Authorisation is sought for the use of the feed additive for all animal species and categories [1,2].

The feed additive is a yellow orange, crystalline hygroscopic powder produced by chemical synthesis consisting mainly of the riboflavin sodium phosphate (73-79 % of riboflavin). It also contains other structural isomers of Riboflavin sodium mono-phosphate and riboflavin sodium di phosphate, and riboflavin dehydrated on ribose in 1-2 position and in 3-4 position [3,4].

The feed additive is intended to be used in water for drinking through liquid or soluble powder premixtures [5]. While no maximum dosage in water is provided, the Applicant recommends not to exceed the 3 % of Vitamin B2 in the final solution, due to its solubility [2].

Note: The EURL previously evaluated the analytical methods for the quantification of Riboflavin sodium phosphate in the frame of the FAD-2010-0304 dossier.

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with Vitamin B₂ (Riboflavin sodium phosphate) and their suitability to be used for official controls in the frame of the authorisation were evaluated.
3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [6]

Description of the analytical methods for the determination of the active substance in feed additive, soluble premixtures and water

For the characterisation of Riboflavin sodium phosphate in the feed additives the Applicant proposed the European Pharmacopoeia method [7] and the FAO JECFA monograph [8] recommended by Commission Regulation EU No 231/2012, where identification is based on specific optical rotation and ultraviolet and visible absorption spectrophotometry, while quantification of the total colouring matter content of the Riboflavin sodium phosphate is based on spectrophotometry at 444 nm.

Even though no performance characteristics are available, the EURL recommends for official control the European Pharmacopoeia and the FAO JECFA (recommended by Commission Regulation EU No 231/2012) methods to characterise the Riboflavin sodium phosphate salt.

For the quantification of Riboflavin sodium phosphate in the liquid and soluble powder form premixtures the Applicant proposed an in-house developed High Performance Liquid Chromatography coupled to an UV detector (HPLC-UV) method [9], based on the method described in the European Pharmacopoeia.

The sample (5 mg) is transferred in a volumetric flask of 200 mL to which 100 mL of water are added. Ultrasonic bath for 10 minutes is required for the powder form. The resulting solution is diluted to 200 mL with mobile phase (phosphate buffer: methanol, 80:20), shaken and filtered with syringe filters directly into the vial, before analysis by an HPLC-UV at 266 nm. Quantification is performed using external calibration [9].

The method was single-laboratory validated [10] and further verified [11]. The Applicant reported a relative standard deviation for repeatability (RSDr) ranging from 0.5 to 2 % and a recovery rate (Rrec) ranging from 97 to100.5 % [10,11]. Based on the experimental data provided, the EURL considers the HPLC-UV method submitted by the Applicant suitable for official control to quantify riboflavin sodium phosphate in liquid and soluble powder form premixtures.
The Applicant did not present experimental data of analysis of the riboflavin sodium phosphate in water, however the EURL already evaluated and recommended in the frame of the FAD-2010-0304 dossier [12] a method derived from the ring-trial validated CEN method (EN 14152) [13] for the quantification of riboflavin sodium phosphate in water, based on acid hydrolysis followed by enzymatic dephosphorylation and using HPLC with fluorimetric detection (HPLC-FL).

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- The European Pharmacopoeia methods, using a spectrophotometry at 444 nm to quantify Riboflavin sodium phosphate in the feed additives (Ph. Eur. 6.0, 01/2008:0786 or FAO JECFA "Riboflavin sodium phosphate" monograph);

- the High-Performance Liquid Chromatography coupled to UV detector (HPLC-UV), to quantify Riboflavin sodium phosphate in soluble premixtures;

- the method based on acidic hydrolysis and enzymatic dephosphorylation followed by High Performance Liquid Chromatographic with a Fluorimetric detection (HPLC-FL), to quantify Riboflavin sodium phosphate (as total Vitamin B₂) in water.

**Recommended text for the register entry (analytical method)**

For the quantification of Riboflavin sodium phosphate in *feed additive*:

- Spectrophotometry method at 444 nm - Ph. Eur. 6.0, method 01/2008:0786 or FAO JECFA "Riboflavin sodium phosphate" monograph

For the quantification of Riboflavin sodium phosphate in *premixtures*:

- High-Performance Liquid Chromatography coupled to UV detector (HPLC-UV)

For the quantification of Riboflavin sodium phosphate (as total Vitamin B₂) in *water*:

- High Performance Liquid Chromatography with Fluorescence detection (HPLC-FL)
5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Vitamin B\textsubscript{2} (Riboflavin sodium phosphate) have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[2] *Application, Proposal for Registry Entry – Annex A
[3] *Technical dossier, Section II, 2.1. Identity of the additive
[4] *Technical dossier, Section II, 2.2. Characterisation of the active substance(s) / agent(s)
[5] *Technical dossier, Section II, 2.5. Conditions of use of the additive
[9] *Technical dossier, Section II, 2.6.1.1. Methods of analysis used for the determination of Riboflavin sodium phosphate in premixture (liquid and soluble powder form)

*Refers to Dossier no: FAD-2011-0051

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.
8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratori Agroalimentari, Departament d’Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Cabrils (ES)
- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare ed animale, Roma (IT)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarni inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
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