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European Union Reference Laboratory for Feed Additives

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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2010-0344 CRL/100314
Feed additive:	Patent Blue V (E 131)
Active Substance(s):	Patent Blue V
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 4(1) and 10(2) for *Patent Blue V* under the category/functional group group 2(a)i "sensory additives"/"colourants - substances that add or restore colour in feedingstuffs", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories.

Patent Blue V is a synthetic blue powder or granules, soluble in water, consisting of a minimum of 90 % of total colouring matters content calculated as the calcium or sodium salts. *Patent Blue V* is intended to be incorporated directly in *feedingstuffs* as a solution in *water* (either added directly as a solid to the *feedingstuffs* in the presence of water or by addition of an aqueous solution), with a maximum content of 50 mg/kg *feedingstuffs*.

For the determination of total colouring matters content of *Patent Blue V* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph for food additives. Identification and quantification of total colouring matters content of *Patent Blue V* is based on spectrophotometry at 638 nm in aqueous solution at pH 5, as recommended by Commission Directive 2008/128/EC laying down specific purity criteria concerning colours for use in foodstuffs. The total colouring matters content is quantified using JECFA Procedure 1. The Applicant applied this JECFA method to quantify the total colouring matters content of *Patent Blue V* in the *feed additive* and reported acceptable performance characteristics.

The Applicant applied the JEFCA method mentioned above for the quantification of total colouring matters content of *Patent Blue V* in *feedingstuffs*. The following performance characteristics were reported for a *Patent Blue V* concentration in *feedingstuffs* ranging from 2 to 25 mg /kg: - RSD_r ranging from 3.4 to 5.9 %; - a *recovery* rate (R_{rec}) ranging from 83 to 97 %. The lowest concentration analysed by the applicant was set by the EURL as the limit of quantification (LOQ), to derive a LOQ of 2 mg/kg *feedingstuffs*.

Based on the experimental evidence and the performance characteristics provided by the Applicant, the EURL recommends for official control the JECFA monograph method - recommended by Commission Directive 2008/128/EC - based on spectrophotometry at 638 nm - for the quantification of total colouring matters content of *Patent Blue V* in the *feed additive* and *feedingstuffs*. However, other colouring substances present in the *feedingstuffs* may absorb/interfer in the 600-700 nm range, thus influencing the determination of total colouring matters content of *Patent Blue V*. Whenever the spectrophotometric reported values are above the maximum content, an additional chromatographic separation may be required for confirmation.

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

Patent Blue V, sensory additives, colourants, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use) and 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *Patent Blue V* under the category/functional group group 2(a)i "sensory additives"/"colourants - substances that add or restore colour in feedingstuffs" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all animal species and categories [2].

Patent Blue V is a synthetic blue powder or granules, soluble in water [3], consisting of a minimum of 90 % of total colouring matters content calculated as the calcium or sodium salts [2]. The Applicant states that the purity criteria set in the Commission Directive 2008/128/EC for the food additive apply to the requirement for the *feed additive* [3]. *Patent Blue V* is intended to be incorporated directly in *feedingstuffs* as a solution in *water* (either added directly as a solid to the feedingstuffs in the presence of water or by addition of an aqueous solution), with a maximum content of 50 mg/kg *feedingstuffs* [2].

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 *Patent Blue V*, 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, mycotoxins, and dioxins) are available from the respective European Union Reference Laboratories [4].

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

For the determination total colouring matters content of *Patent Blue V* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph for food additives [5], where:

- Identification of *Patent Blue V* is based on (i) spectrophotometry at 638 nm in water at pH 5 and (ii) Thin Layer Chromatography (TLC) with Retention factors (R_f) determined using several chromatographic conditions for confirmation, while
- Quantification of total colouring matters content of *Patent Blue V* is based on spectrophotometry at 638 nm in aqueous solution at pH 5, as recommended by Commission Directive 2008/128/EC. Total colouring matters content is quantified using JECFA Procedure 1 [5].

The Applicant applied this JECFA method to quantify the total colouring matters content of *Patent Blue V* in the *feed additive* and reported a relative standard deviation for *repeatability* (RSD_r) of 0.5 % and a minimum total colouring matters content of 90 % [6].

The Applicant applied the JEFCA method mentioned above for the quantification of total colouring matters content of *Patent Blue V* in *feedingstuffs* [5]. The sample is extracted with purified water. The aqueous solution is then filtered. The filtrate is adjusted to pH 5 with sodium hydroxide and acetic acid buffer solutions for further analysis. Total colouring matters content of *Patent Blue V* is determined by spectrophotometry at 638 nm using the reference absorption value ($E_{1cm}^{1\%} = 2000$) of total colouring matters content of *Patent Blue V* solution.

The following performance characteristics were reported for a *Patent Blue V* concentration in *feedingstuffs* ranging from 2 to 25 mg /kg [7]:

- RSD_r ranging from 3.4 to 5.9 %; and
- a *recovery* rate (R_{rec}) ranging from 83 to 97 %.

The lowest concentration analysed by the applicant was set by the EURL as the limit of quantification (LOQ), to derive a LOQ of 2 mg/kg *feedingstuffs*.

Based on the experimental evidence and the performance characteristics provided by the Applicant, the EURL recommends for official control the JECFA monograph method - recommended by Commission Directive 2008/128/EC - based on spectrophotometry at 638 nm - for the quantification of total colouring matters content of *Patent Blue V* in the *feed additive* and *feedingstuffs*.

However, other colouring substances present in the *feedingstuffs* may absorb/interfer in the 600-700 nm range, thus influencing the determination of total colouring matters content of *Patent Blue V*. Whenever the spectrophotometric reported values are above the maximum content, an additional chromatographic separation may be required for confirmation.

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 identification tests and the spectrophotometric method (at 638 nm) recommended by Commission Directive 2008/128/EC and described in the JECFA monographs No. 1 (Vol. 4), Combined Compendium for Food Additive Specifications, for the determination of total colouring matters content of *Patent Blue V* in *feed additive* and *feedingstuffs*.

However, other colouring substances present in the *feedingstuffs* may absorb/interfer in the 600-700 nm range, thus influencing the determination of total colouring matters content of *Patent Blue V*. Whenever the spectrophotometric reported values are above the maximum content, an additional chromatographic separation may be required for confirmation.

Recommended text for the register entry (analytical method)

For the quantification of total colouring matters content of *Patent Blue V* in the *feed additive* and *feedingstuffs:*

- Spectrophotometry at 638 nm (Commission Directive 2008/128/EC referring to FAO JECFA monographs No. 1, Vol. 4)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Patent Blue V* 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

[1] *Application, Reference SANCO/D/2: Forw. Appl. 1831/(00176) (10501)-2010

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis

[4] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[5] FAO JECFA monographs No. 1 (Vol. 4), Combined Compendium for Food Additive Specifications

[6] *Supplementary information – E131 pure dye content

[7] *Supplementary information – validation report_feedingstuffs

*Refers to Dossier No. FAD-2010-0344

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)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)