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JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2010-0099 EURL/100156
Name of Additive:	Vitamin K_3 (Menadione)
Active Substance(s):	Menadione Sodium Bisulphite Menadione Nicotinamide Bisulphite
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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Report approved by: Date:	Christoph von Holst 07/02/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Vitamin K_3* (*Menadione*) 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. Specifically, authorisation is sought to use the active substances, *Menadione Sodium Bisulphite (MSB)* and *Menadione Nicotinamide Bisulphite (MNB)* for all animal species and categories, with a minimum content of 50 % and 44 % of *Menadione*, respectively. The *feed additive* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water* (only for *MSB*). However, the Applicant did not specify any maximum or minimum concentration of *MSB* and *MNB* in *feedingstuffs* or *water*.

For the determination of *Vitamin K_3* (*Menadione*) and its commercial derivatives *Menadione Sodium Bisulphite (MSB)* and *Menadione Nicotinamide Bisulphite (MNB)* in the *feed additive* the Applicant proposed a Method from the Association of German Agricultural Analytical Research Institutes (VDLUFA), validated for *premixtures* and *feedingstuffs*. The Applicant applied this method to the *feed additive* and reported a relative standard deviation of *repeatability* (RSD_r) of 1.7 % for *MSB* and *MNB* containing 50 % and 44 % of *Menadione*, respectively. Moreover, a *recovery* rate (R_{Rec}) ranging from 101 % to 104 % was calculated by EURL, based on the experimental data provided by Applicant.

Based on the performance characteristic presented, the EURL recommends for official control the validated method from VDLUFA (Bd.III 13.7.1) to determine *Vitamin K_3* in the *feed additive*.

For the determination of *Vitamin K_3* in *premixtures* and *feedingstuffs* the Applicant proposed the above mentioned VDLUFA method - validated for *premixtures* and *feedingstuffs*, for which an RSD_r ranging from 10 % to 20 % is reported.

An alternative ring-trial validated method for *premixtures* and *feedingstuffs* was published in the Italian Official Journal, based on Normal Phase High-Performance Liquid Chromatography (NP-HPLC) coupled to Ultraviolet (UV) detector, using a wavelength of 251 nm. The following performance characteristics were reported for *premixtures*, complementary *feedingstuffs* and complete *feedingstuffs* samples with a *Vitamin K_3* (*Menadione*) content ranging from 5.57 to 543 mg/kg:

- RSD_r ranging from of 2.9 to 6.8 %;
- a relative standard deviation of *reproducibility* (RSD_R) ranging from 7.0 to 11.7%;
- R_{Rec} ranging from 89.2 to 111.4 %, and

- a limit of detection (LOD) and quantification (LOQ) of 1.2 and 3.8 mg/kg, respectively.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated method, published in the Italian Official Journal, based on Normal Phase High-Performance Liquid Chromatography (NP-HPLC) coupled to UV detector to determine *Vitamin K_3* in *premixtures* and *feedingstuffs*, within the concentration range covered.

For the determination of the *Menadione Sodium Bisulphite (MSB)* in water, the Applicant applied the VDLUFA method. The following performances characteristics were reported for a concentration of 0.5 g *Menadione* /L:

- RSD_r ranging from 1.8 to 4.4 %, and
- R_{Rec} ranging from 97.9 to 99.2 %.

Based on the performance characteristics presented, the EURL recommends for official control the validated method from VDLUFA (Bd.III 13.7.1) to determine *Vitamin K_3* in *water*.

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

Menadione Sodium Bisulphite, Menadione Nicotinamide Bisulphite, Vitamin K_3, nutritional additive, vitamins, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water) and 10(2) (re-evaluation of the additive already authorised under council directive 70/524/EEC) for *Vitamin K_3* 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]. Two forms of *Vitamin K_3* are considered, namely: *Menadione Sodium Bisulphite (MSB)* and *Menadione Nicotinamide Bisulphite (MNB)*. Authorisation is sought for the use of the *feed additive* for all animal species and categories [2]. According to the Applicant, *MSB* is a free flowing white to yellowish crystalline powder with a minimum content of 50 % of *Menadione*, while *MNB* is a free flowing yellowish

powder with a minimum content of 44 % of *Menadione* [3]. The *feed additive* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water* (only for *MSB*). However, the Applicant did not specify any maximum or minimum concentration of *MSB* and *MNB* in *feedingstuffs* or *water* [2], similarly to what was set in previous regulation [4].

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 (EFSA) for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Vitamin K_3* (*Menadione Sodium Bisulphite* and *Menadione Nicotinamide Bisulphite*), 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 [5].

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

For the determination of *Vitamin K_3* (*Menadione*) and its commercial derivatives *Menadione Sodium Bisulphite (MSB)* and *Menadione Nicotinamide Bisulphite (MNB)* in the *feed additive* the Applicant proposed the VDLUFA Method Bd. III, 13.7.1 [6], validated for *premixtures* and *feedingstuffs*. The Applicant applied this method to the *feed additive* [7] and reported a relative standard deviation of *repeatability* (RSD_r) of 1.7 % for *MSB* and *MNB*, with a concentration of 50 % and 44 % of *Menadione*, respectively. Moreover, a *recovery* rate (R_{Rec}) ranging from 101 % to 104 % was calculated by EURL, based on the experimental data provided by Applicant [7].

Based on the performance characteristics presented, the EURL recommends for official control the validated method from the VDLUFA (Bd.III 13.7.1) to determine *Vitamin K_3* in *feed additive*.

For the determination of *Vitamin K_3* in *premixtures* and *feedingstuffs* the Applicant proposed the above mentioned VDLUFA method - validated for *premixtures* and *feedingstuffs* [6].

An alternative ring-trial validated method for *premixtures* and *feedingstuffs* was published in the Italian Official Journal [8]. The sample (5-10 g) is extracted with chloroform and stirred for 5 minutes. An ammonium hydroxide solution is added and stirred for 3 minutes; then 10 grams of celite/anhydrous sodium sulfate are added and the solution is stirred for 10 minutes. The solution is neutralized with acetic acid resulting in two separated phases. The organic phase is filtered and an aliquot is dried using a rotatory evaporator, then dissolved in 1,2-dichloroethane, before injection in the Normal Phase High-Performance Liquid Chromatograph (NP-HPLC). The *Vitamin K_3* concentration is measured at 251 nm and quantified against an external standard calibration curve. As *Vitamin K_3* is very sensitive to light and heat, light protected brown glasses were used during analysis. The following performance characteristics were reported for *premixtures*, complementary *feedingstuffs* and complete *feedingstuffs* samples with a *Vitamin K_3* (*Menadione*) content ranging from 5.57 to 543 mg/kg:

- RSD_r ranging from 2.9 to 6.8 %;
- a relative standard deviation of *reproducibility* (RSD_R) ranging from 7.0 to 11.7%;
- R_{Rec} ranging from 89.2 to 111.4 %, and
- a limit of detection (LOD) and quantification (LOQ) of 1.2 and 3.8 mg/kg, respectively.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated method published in the Italian Official Journal [8], based on Normal Phase High-Performance Liquid Chromatography (NP-HPLC) coupled to UV detector to determine *Vitamin K_3* in *premixtures* and *feedingstuffs*, within the concentration range covered.

For the determination of the *Menadione Sodium Bisulphite (MSB)* in water, the Applicant applied the VDLUFA method [6,7]. The following performances characteristics were reported for a concentration of 0.5 g *Menadione* /L:

- RSD_r ranging from 1.8 to 4.4 %, and
- R_{Rec} ranging from 97.9 to 99.2 %.

Based on the performance characteristics presented, the EURL recommends for official control the validated method from VDLUFA (Bd.III 13.7.1) to determine *Vitamin K_3* in *water*.

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 VDLUFA Method Bd. III, 13.7.1, using a spectrophotometer to determine *Menadione Sodium Bisulphite* and *Menadione Nicotinamide Bisulphite* in *feed additive*;
- the Italian official method, using Normal Phase High-Performance Liquid Chromatography coupled to ultraviolet detector, to determine *Menadione Sodium Bisulphite* and *Menadione Nicotinamide Bisulphite* in *premixtures* and *feedingstuffs*;
- the VDLUFA Method Bd. III, 13.7.1, using a spectrophotometer to determine *Menadione Sodium Bisulphite* in *water*.

Recommended text for the register entry (analytical method)

For the determination of *Menadione Sodium Bisulphite* and *Menadione Nicotinamide Bisulphite* in *feed additive*:

- a spectrophotometric method using a visible detector at 635 nm (VDLUFA - Bd. III, 13.7.1)

For the determination of *Menadione Sodium Bisulphite* and *Menadione Nicotinamide Bisulphite* in *premixtures* and *feedingstuffs*:

- Normal Phase High-Performance Liquid Chromatograph coupled to ultraviolet detector – decree 29/04/2010, Official Italian Journal n. 120 25/5/2010

For the determination of *Menadione Sodium Bisulphite* in *water*:

- a spectrophotometric method using a visible detector at 635 nm (VDLUFA - Bd. III, 13.7.1)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Menadione Sodium Bisulphite* and *Menadione Nicotinamide Bisulphite* 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. 1831/0062/2010

[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] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs

[5] 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 European Union Reference Laboratories

[6] *Technical Dossier, Section II, Annex II 17 VDLUFA Method K3

[7] *Technical Dossier, Section II, Annex II 19 Extending of scope K3-Method

[8] Decree 29/04/2010, Official Italian Journal n. 120 25/05/2010. Approvazione del metodo ufficiale di analisi per la determinazione della vitamina K_3 negli alimenti per animali

* Refers to Dossier No. FAD-2010-0099

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

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- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
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