EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
**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

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| Dossier related to: | FAD-2010-0200<br>CRL/100058 |
| Feed additive: | Vitamin A |
| Active Substance(s): | All- E -Retinol Acetate<br>All- E -Retinol Palmitate<br>All- E -Retinol Propionate |
| Rapporteur Laboratory: | European Union Reference Laboratory for Feed Additives (EURL-FA)<br>Geel, Belgium |
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| Report approved by:<br>Date: | Christoph von Holst (EURL-FA)<br>12/07/2011 |

## EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *Vitamin A* 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. According to the Applicant *Vitamin A* is in the form of a*ll-E-retinol acetate, all-E-retinol palmitate* and *all-E-retinol propionate.* The *feed additive* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water*. According to the European Pharmacopeia "the activity of Vitamin A is expressed in terms of Retinol Equivalents (RE) or in Internation Units (IU), where 1 mg RE is equivalent to 3333 IU and corresponds to the activity of 1 mg of *all-E-retinol*. The activity of the other retinols is calculated "stoichiometrically". The Applicant proposes a maximum content of the *feed additive* in complete *feedingstuffs* of 13500 IU/kg for most of the species; 25000 IU/kg for calves for fattening; while no maximum concentration is suggested for other species (such as chickens, turkeys, etc. as indicated in Annex A).

For the determination of *Vitamin A* the Applicant proposes the internationally recognised European Pharmacopoeia method (Ph.Eur. 6$^{th}$ Edition, monograph 0217), based on Thin Layer Chromatography and UV detection (TLC-UV). The method has no performance characteristics; however, the EURL considers this method suitable to be used within the frame of official control.

For the determination of *Vitamin A* in *premixtures* and *feedingstuffs* the Applicant proposed the ring trial validated Community Method, based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC) with UV or fluorescence detection. The methods includes a saponification step, to extract the target analytes from the matrix and to transform the *different* Vitamin A active substances present in the sample into Vitamin A *alcohol*, which is also used for calibration. The following performances characteristics were determined for Vitamin A concentrations ranging from $1.8x10^4$ to $1.7x10^7$ IU/kg:

- a relative standard deviation for *repeatability* ($RSD_r$) ranging from 3.0 to 8.1 %,
- a relative standard deviation for *reproducibility* ($RSD_R$) ranging from 6.2 to 20 % and
- a limit of quantification of 2000 IU/kg.

Based on the acceptable performance characteristics, the EURL recommends for official control the ring-trial validated Community Method based on RP-HPLC with UV or fluorescence detection to determine *Vitamin A* in *premixtures* and *feedingstuffs*.

For the determination of *Vitamin A* in the *water*, the Applicant proposed a method from the LFGB compendium (Foods, Consumer Goods and Feedstuffs Code), intended for aqueous solutions. However, no experimental data were provided by the Applicant for the determination of the product in *water*. Therefore, the EURL cannot evaluate nor recommend for official control this method to determine *Vitamin A* 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**

*Vitamin A, all-E-retinol acetate, all-E-retinol palmitate, all-E-retinol propionate*, nutritional additive, vitamins, all animal species, all 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 already authorised additive under council directive 70/524/EEC) for *Vitamin A* 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]. Authorisation is sought for the use of the *feed additive* for all animal species and categories [2]. According to the Applicant *Vitamin A* is in the form of *all-E-retinol acetate, all-E-retinol palmitate* and *all-E-retinol propionate* [2].

According to the European Pharmacopeia: "the activity of Vitamin A is expressed in terms of Retinol Equivalents (RE) or in Internation Units (IU), where 1 mg RE is equivalent to 3333 IU and corresponds to the activity of 1 mg of *all-E-retinol*. The activity of the other retinols is calculated stoichiometrically". *Retinol Acetate* is a yellowish crystalline powder, with a minimum activity of $2.8x10^6$ IU/g. *Retinol Palmitate* is a fat-like light yellow solid or oily liquid, with a minimum activity of $1.6x10^6$ IU/g. *Retinol Propionate* is a reddish-brown oily liquid with a minimum activity of $2.5x10^6$ IU/g [2, 3]. The *feed additive* is intended to be incorporated in *feedingstuffs* through *premixtures* or directly in *water*. The Applicant proposes a maximum content of the *feed additive* in complete *feedingstuffs* of 13500 IU/kg for most of the species; 25000 IU/kg for calves for fattening; while no maximum concentration is suggested for other species (such as chickens, turkeys, etc. as indicated in Annex A) [2, 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. For this dossier, the methods of analysis submitted in connection with and *Vitamin A* 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 $B_1$ 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 A* (*all-E-retinol acetate, all-E-retinol palmitate* and *all-E-retinol propionate*) the Applicant proposes the internationally recognised European Pharmacopoeia method (Ph.Eur. $6^{th}$ Edition, monograph 0217) [6], based on Thin Layer Chromatography and UV detection (TLC-UV). The method has no performance characteristics; however, the EURL considers this method suitable to be used within the frame of official control.

For the determination of *Vitamin A* in *premixtures* and *feedingstuffs* the Applicant proposed the ring trial validated Community Method, based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC) with UV or fluorescence detection [7].

The sample is hydrolysed with ethanolic potassium hydroxide solution and the vitamin A is extracted into light petroleum. The solvent is removed by evaporation and the residue is dissolved in methanol and, if necessary, diluted to the required concentration. The content of *vitamin A* expressed in IU is determined by Reversed Phase High Performance Liquid Chromatography (RP-HPLC) using a UV or a fluorescence detector and quantified against

Vitamin A alcohol. The chromatographic parameters are chosen so that there is no separation between the all-trans-vitamin A alcohol and its cis isomers.

The following performances characteristics are reported:

- a relative standard deviation for *repeatability* ($RSD_r$) ranging from 3.0 to 8.1 %,
- a relative standard deviation for *reproducibility* ($RSD_R$) ranging from 6.2 to 20%, and
- a limit of quantification of 2000 IU/kg.

Based on the acceptable performance characteristics, the EURL recommends for official control the Community Method based on RP-HPLC to determine *Vitamin A* in *premixtures* and *feedingstuffs*.

For the determination of *Vitamin A* in *water*, the Applicant proposed a method from the LFGB compendium (Foods, Consumer Goods and Feedstuffs Code), intended for aqueous solutions [8]. However, no experimental data were provided by the Applicant for the determination of the product in *water*. Therefore, the EURL cannot evaluate nor recommend for official control this method to determine *Vitamin A* 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 European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 0217), based on Thin Layer Chromatography and UV detection (TLC-UV) for the determination of *Vitamin A* in the *feed additive*; and the Community Method based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC) with UV or fluorescence detection to determine *Vitamin A* in *premixtures* and *feedingstuffs*.

For the determination of *Vitamin A* in *water*, the Applicant proposed a method from the LFGB compendium (Foods, Consumer Goods and Feedstuffs Code), intended for aqueous solutions. However, no experimental data were provided by the Applicant for the determination of the product in *water*. Therefore, the EURL cannot evaluate nor recommend for official control this method to determine *Vitamin A* in *water*.

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

For the determination of *Vitamin A* in the *feed additive:*

- Thin Layer Chromatography and UV detection (TLC-UV)
(Ph. Eur. 6th edition, monograph 0217)

For the determination of *Vitamin A* in *premixtures* and *feedingstuffs*:

- Reversed Phase High Performance Liquid Chromatography (RP-HPLC) with UV or fluorescence detection - Commission Regulation (EC) 152/2009

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Vitamin A* 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/Ref: SANCO/D/2: Forw. Appl. 1831/00131/2010

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

[3] *Technical dossier, Section II, 2.1.3

[4] *Technical dossier, Section II, 2.5.1

[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 Community Reference Laboratories

[6] *Technical Dossier, Section II, Annex_II_2PhEur_217

[7] *Technical Dossier, Section II, Annex_II_22_EEC_152_2009

[8] *Technical Dossier, Section II, Annex_II_23_method_water

* Refers to Dossier No. FAD-2010-0200

## 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


- Plantedirektoratet, Laboratorium for Foder og Gødning, Denmark
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- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit, Germany
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali, Italy
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- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Poland
- Österreichische Agentur für Gesundheit und Ernährungssicherheit, Austria