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JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2008-0047** **CRL/080022**
Name of Additive:	**Vitamin E**
Active Substance(s):	**All-rac-alpha-Tocopheryl acetate,** **RRR-alpha-Tocopheryl acetate,** **RRR-alpha-Tocopherol**
Rapporteur Laboratory:	**University of Ljubljana, Veterinary Faculty, National Veterinary Institute (VF-NVI), Ljubljana, Slovenia**
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Date:	**01/02/2010**
Report approved by:	**Christoph von Holst (CRL-FA)**
Date:	**01/02/2010**

EXECUTIVE SUMMARY

In the current application authorisation is sought for *vitamin E* under the category nutritional additives, functional group '3a' vitamins, pro-vitamins and chemically well-defined substances having similar effect, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for use of vitamin E for all animal species and categories.

Vitamin E consists of a group of active substances. Three active substances are applied for in this dossier: (1) *all-rac-alpha-Tocopheryl acetate*, (2) RRR-*alpha-Tocopheryl acetate* and (3) *RRR-alpha-Tocopherol*. *RRR-alpha-Tocopherol acetate* and *RRR-alpha-Tocopherol* are derived from vegetable oil with the minimum purity of 40 % and 67 %, respectively. *All-rac-alpha-Tocopheryl acetate* is produced by chemical synthesis with the minimum purity of 93 %. Preparations of *vitamin E* consisting of these forms are variable depending on the producers, and are usually marketed in the form of adsorbate (powder) and spray-dried powder. No final concentrations of *feed additive* in *feedingstuffs* are proposed by the applicant.

For the determination of the three active substances mentioned above in the *feed additive*, the applicant proposed several European Pharmacopoeia (EP) methods, based on gas chromatography with flame ionisation detector (GC-FID): EP-0439 for *all-rac-alpha-Tocopheryl acetate* (oil), EP-0691 for *all-rac-alpha-Tocopheryl acetate* (powder form), and EP-1256 for *RRR-alpha-Tocopherol* (oil).

The CRL recommends for the determination of *vitamin E* (oil form) in *feed additives* the following GC-FID methods recommended by the European Pharmacopoeia: EP-0439 for *all-rac-alpha-Tocopheryl acetate*; EP-1257 for *RRR-alpha-Tocopheryl acetate* and EP-1256 for *RRR-alpha-Tocopherol*. For the determination of *vitamin E* (powder form) in *feed additives* the CRL recommends the following EP methods: EP-0691 for *all-rac-alpha-Tocopheryl acetate*; EP-1801 for *RRR-alpha-Tocopheryl acetate* and for *RRR-alpha-Tocopherol*.

For the determination of all three active substances in *premixtures* and in *feedingstuffs* the CRL recommends the Community method (Commission Regulation (EC) No 152/2009) at the validated concentration range (from 60 to 17000 mg *vitamin E*/kg), as proposed by the applicant.

In addition, the applicant reported that this method is also applicable to the determination of the target analyte in water (extension of scope). However, experimental data showing the validity of this approach have not been provided, thus the CRL cannot comment on the suitability of the Community method to the analysis of the matrix "water".

Further testing or validation is not considered necessary.

KEYWORDS

Vitamin E, nutritional additive, all-rac-alpha-Tocopheryl acetate, RRR-alpha-Tocopheryl acetate, RRR-alpha-Tocopherol, all species.

1. BACKGROUND

Vitamin E is a *feed additive* for which the authorisation is sought under the category nutritional additives, functional group '3a': vitamins, pro-vitamins and chemically well-defined substances having similar effect, according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. This dossier consists of a group of three active substances: (1*) all-rac-alpha-Tocopheryl acetate*, (2) *RRR-alpha-Tocopheryl acetate* and (3) *RRR-alpha-Tocopherol* – previously authorised (cf. OJ 2004/C 50/15 of 25/2/2004). The feed additive is intended to be used for all animal species and categories with no maximum limit [1, 2].

All three substances are viscous oils of either greenish-yellow or amber to reddish colour. *RRR-alpha-Tocopheryl acetate* and *RRR-alpha-Tocopherol* are derived from vegetable oil an have a minimum purity of 40% and 67%, respectively [3]. *All-rac-alpha-Tocopheryl acetate* is produced by chemical synthesis with the minimum purity of 93% [3].

The active substance can be administered to the animals via *feedingstuffs* (complete and complementary) or via *water*. When used in *feedingstuffs*, the active substance can be incorporated directly in oily form or it is formulated as a preparation, either as an adsorbate or as a spray-dried form. Both, the oil and the preparation can be either processed first in a *premixture* or added directly into the *feedingstuffs*. For the administration via *water* and inclusion via milk replacer, a spray-dried formulation containing emulsifiers and water soluble carriers is used.

Preparations are variable depending on the producers. According to the applicant, the typical preparation of *all-rac-alpha-Tocopheryl acetate*, *RRR-alpha-Tocopheryl acetate* and *RRR-alpha-Tocopherol* contains a minimum of 50% [4], 36.8% [5] and 33.5% [6] active substance, respectively.

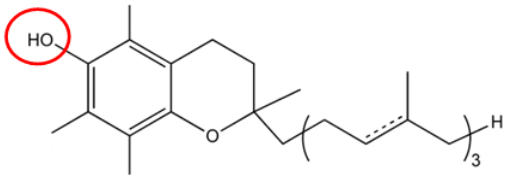
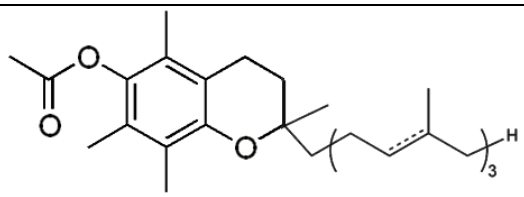
2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with *vitamin E* and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Editorial Note

Various nomenclatures are used by the applicant, the European Pharmacopea and in the community method. Two chemical functions are considered in this application: - the alcohol form (tocopherol) and the acetate one (tocopheryl acetate). The term combination "tocopherol acetate" is considered misleading and is not used in this manuscript.

Tocopherol $C_{29}H_{49}O$-OH	
Tocopheryl acetate $C_{29}H_{49}O$-$COOCH_3$	

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, mercury, lead, dioxine and PCBs) are available at the respective Community Reference Laboratories (Commission Regulation (EC) No 776/2206).

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

For the determination of the three active substances in the *feed additive* the applicant submitted [3] three European Pharmacopoeia (EP) methods (EP-0439; EP-0691; EP-1256).

For the determination of *vitamin E* in oil formulation, the test solution, the reference solution and the internal standard are prepared in cyclohexane. The substance is then determined by Gas Chromatography with Flame Ionization Detector (GC-FID) [7], using capillary poly(dimethyl)siloxane as stationary phase and helium as the carrier gas.

For the determination of *vitamin E* in the powder form, the reference solution and internal standard solution (dotriacontane) are prepared in hexane. The sample is treated with 1 M hydrochloric acid in ultrasonic bath at 70ºC. After addition of anhydrous ethanol and hexane containing internal standard, the mixture is shaken thoroughly for 30 minutes. The upper layer is used for the GC-FID measurements carried out with the column packed with diatomaceous earth silanised with dimethyldichlorosiloxane and impregnated with poly(dimethyl)siloxane and with nitrogen as the carrier gas.

For the determination of *vitamin E* (oil form) in *feed additive,* the CRL recommends the following European Pharmacopoeia methods [8]:

- EP-0439 for *all-rac-alpha-Tocopheryl acetate*;
- EP-1257 for *RRR-alpha-Tocopheryl acetate* and
- EP-1256 for *RRR-alpha-Tocopherol.*

For the determination of *vitamin E* (powder form) in *feed additives,* the CRL recommends the following European Pharmacopoeia methods [8]:

- EP-0691 for *all-rac-alpha-Tocopheryl acetate*;
- EP-1801 for *RRR-alpha-Tocopheryl acetate* and for *RRR-alpha-Tocopherol*.

For the determination of the three *active substances* in *premixtures* and in *feedingstuffs* the applicant proposed the Community method (Commission Regulation (EC) No 152/2009). The method is applicable for the determination of Tocopheryl acetate as well as for Tocopherol. The sample is hydrolysed with ethanolic potassium hydroxide solution under reflux and the *vitamin E* is extracted into light petroleum. The solvent is removed by evaporation and the residue is dissolved in methanol. If necessary the solution is diluted to the required concentration. The content of *vitamin E* is determined by reversed phase high performance liquid chromatography (RP-HPLC) using the fluorescence or UV detector. According to the procedure, the content of vitamin E is expressed as DL-alpha-Tocopherol acetate (*all-rac-alpha-Tocopheryl acetate*). 1 mg of Tocopheryl acetate corresponds to 0.91 mg Tocopherol.

The reported performance characteristics of this ring-trial validated method (obtained at concentrations ranging from 60 to 900 mg vitamin E / kg in *feedingstuffs;* and from 1100 to 17500 mg vitamin E /kg in *premixtures*) are:

- a repeatability relative standard deviation (RSD_r) ranging from 2.2 to 4.1% ;
- a reproducibility relative standard deviation (RSD_R) ranging from 4.8 to 13%; and
- a limit of quantification (LOQ) of 2 mg vitamin E / kg feedingstuffs, achieved with a fluorescence detector; while LOQ = 10 mg vitamin E /kg *feedingstuffs* when an UV detector is used.

Based on acceptable performance characteristics the CRL recommends for official controls the Commission Regulation (EC) No 152/2009 for the determination of *all-rac-alpha-Tocopheryl acetate*, *RRR-alpha-Tocopheryl acetate* and *RRR-alpha-Tocopherol* in *premixtures, feedingstuffs* at the validated concentration range.

In addition, the applicant reported that this method is also applicable to the determination of the target analyte in water (extension of scope). However, experimental data showing the validity of this approach have not been provided, thus the CRL cannot comment on the suitability of the Community method to the analysis of the matrix "water".

Further testing or validation is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for Official control:

- the European Pharmacopoeia methods [8] using gas chromatography for the determination of *all-rac-alpha-Tocopheryl acetate*, *RRR-alpha-Tocopheryl acetate* and *RRR-alpha-Tocopherol* in the *feed additive;*

- the Community Method (Commission Regulation (EC) No 152/2009) using HPLC-UV for the determination of *all-rac-alpha-Tocopheryl acetate*, *RRR-alpha-Tocopheryl acetate* and *RRR-alpha-Tocopherol* in *premixtures* and *feedingstuffs*;

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

- For the determination of *all-rac-alpha-Tocopheryl acetate*, *RRR-alpha-Tocopheryl acetate* and *RRR-alpha-Tocopherol* in the *feed additive*:

European Pharmacopoeia methods (Gas Chromatography)

- For the determination of *vitamin E* in *premixtures and feedingstuffs*:

Commission Regulation (EC) No 152/2009 (HPLC)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, Vitamin E samples have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] *Application, Reference SANCO/D/2 Forw.Appl. 1831/030-2009

[2] *Application, Annex III. Description and Condition of use of the additive as proposed by the applicant
[3] *Technical dossier, Section II - Identity
[4] *Technical dossier, Section 2.1.01
[5] *Technical dossier, Section 2.1.02
[6] *Technical dossier, Section 2.1.03
[7] European Pharmacopoeia Monograph 6.0 (01/2008) – Method 2.2.28 – Gas Chromatography
[8] European Pharmacopoeia Reference Standards (www.edqm.eu and www.edqm.eu/en/Knowledge-Database-707.html)

* Refers to Dossier No: FAD-2008-0047

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was University of Ljubljana, Veterinary Faculty, National Veterinary Institute (VF-NVI), Ljubljana, Slovenia. 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.

8. ACKNOWLEDGEMENTS

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- Österreichische Agentur für Gesundheit und Ernährungssicherheit, Wien, Austria
- Plantedirektoratets Laboratorium, Lyngby, Denmark
- Service commun des laboratoires, Laboratoire de Rennes, France
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig, Germany
- Thüringer Landesanstalt für Landwirtschaft, Abteilung Untersuchungswesen, Jena, Germany
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava, Slovakia
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