EUROPEAN COMMISSION
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European Union Reference Laboratory for Feed Additives

JRC.D.5/FSQ/CvH/DM/ago/ARES(2012)597741

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-2010-0105 - CRL/100077**
	FAD-2010-0271 - CRL/100179
Name of Feed Additive:	**Tocopherol-rich extracts of natural origin (E 306)**
	Synthetic alpha-tocopherol (E 307)
Active Agent (s):	**Alpha, beta, gamma, delta tocopherol, all-rac alpha tocopherol (dl-alpha-tocopherol)**
Rapporteur Laboratory:	**Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)**
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Date:	**16/05/2012**
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Date:	**21/05/2012**

EXECUTIVE SUMMARY

In the current grouped application (FAD-2010-0105 and FAD-2010-0271), authorisation is sought under Article 10(2) for two forms of *Tocopherol-rich extracts of natural origin* (E 306) including the *delta rich Tocopherol-rich extracts of natural origin* and *Synthetic alpha-tocopherol (E 307)* under the category/functional group 1(b) 'technological additives/antioxidants', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the two *Tocopherol-rich extracts of natural origin* and the *Synthetic alpha-tocopherol* for all animal species and categories. The *feed additives* are intended to be incorporated to *feedingstuffs* directly or through *premixtures*, with no recommended minimum or maximum concentration levels, as previously set in the regulation.

Tocopherol-rich extracts of natural origin contain *alpha, beta, gamma* and *delta tocopherols*, while the main component of *Synthetic alpha-tocopherol* is *all-rac alpha-tocopherol* (or *dl-alpha-tocopherol*).

According to the Applicants:

- *Tocopherol-rich extracts of natural origin* are viscous oils consisting of a minimum of 30% of *alpha, beta, gamma* and *delta tocopherol*;
- *Tocopherol rich extracts of natural origin/delta rich* is a viscous oil with minimal content of 70 % *delta tocopherol* and 80 % total *tocopherols*; and
- *Synthetic alpha-tocopherol* is a viscous oil with a minimal content of 96 % *all-rac alpha-tocopherol*.

For the determination of *all-rac alpha-tocopherol* in the *feed additive* (i.e. *Synthetic alpha-tocopherol*), both Applicants submitted the European Pharmacopoeia method (07/2011:0692), where identification is based on optical rotation, infrared absorption spectrophotometry and thin-layer chromatography with ultraviolet detection; while quantification is based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID). Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method for the determination of *all-rac alpha-tocopherol* in the *feed additive* (i.e. *Synthetic alpha-tocopherol*).

For the determination of the different *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol*) in the *feed additives* (i.e. two *Tocopherol-rich extracts of natural origin*) Applicant FAD-2010-0105 submitted the JECFA method (equivalent to the AOAC method 988.14). The method allows the quantification of each separate form of tocopherols (*alpha, beta, gamma* and *delta tocopherol*). The determination is based on Gas Chromatography

coupled to Flame Ionisation Detection (GC/FID), using the relative retention times of the corresponding propionate forms, where hexadecyl hexadecanoate is used as internal standard. Even though no performance characteristics are provided, the EURL recommends for official control the AOAC method 988.14 (equivalent to the JECFA method), based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID) for the determination of the *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol*) in the *feed additives* (i.e. two *Tocopherol-rich extracts of natural origin*).

For the determination of *all-rac alpha-tocopherol* in *premixtures* and *feedingstuffs* containing *Synthetic alpha-tocopherol*, both Applicants submitted the ring-trial validated Community method (Commission Regulation (EC) No142/2009), based on reverse phase High Performance Liquid Chromatography coupled to a UV or Fluorescence Detector (HPLC/UV or HPLC/FLD). Furthermore, the Community method allows the separate determination of the various *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)*, using a normal phase HPLC.

Additionally, the Applicant (FAD-2010-0105) applied the above mentioned Community method, without the saponification step, for the determination of the free/added *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)* in *premixtures* and *feedingstuffs* containing *Tocopherol-rich extracts of natural origin*. The Applicant reported performance characteristics determined in the frame of a verification study, demonstrating the applicability of the Community method for the determination of free/added *tocopherol forms* in *premixtures* and *feedingstuffs*, thus extending its original scope.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method for the determination of *all-rac alpha-tocopherol* (E 307) in *premixtures* and *feedingstuffs*. Furthermore, the EURL recommends the ring-trial validated Community method to quantify the content of free/added *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)* in *premixtures* and *feedingstuffs*. Provided that the composition of the specific feed additive (E 306) utilised in the preparation of these matrices is known, the results of analysis of the various tocopherol forms may allow for estimating the added amount of this additive to *premixtures* and *feedingstuffs*.

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

Tocopherol-rich extracts of natural origin (E 306), Synthetic alpha-tocopherol (E 307), technological additives, antioxidants, all animal species.

1. BACKGROUND

In the current grouped application (FAD-2010-0105 and FAD-2010-0271), authorisation is sought under Article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) [1, 2] for two forms of *Tocopherol-rich extracts of natural origin* (including the *delta rich Tocopherol-rich extracts of natural origin*) *(E 306)* and *Synthetic alpha-tocopherol (E 307)* under the category/functional group 1(b) 'technological additives/antioxidants', according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicants, the two *Tocopherol-rich extracts of natural origin* are produced by multiple extraction and refining steps from vegetable oils [3], while *Synthetic alpha-tocopherol* is manufactured by chemical synthesis from trimethyl hydrochinon and isophytol catalysed by zinc chloride/HCl [4].

Tocopherol-rich extracts of natural origin contain *alpha, beta, gamma* and *delta tocopherols* [5], while the main component of *Synthetic alpha-tocopherol* is *all-rac alpha-tocopherol* (or *dl-alpha-tocopherol*) [5,6].

According to the Applicants:

- *Tocopherol-rich extracts of natural origin* are viscous oils consisting of a minimum of 30% of *alpha, beta, gamma* and *delta tocopherol* [7];
- *Tocopherol rich extracts of natural origin/delta rich* is a viscous oil with minimal content of 70 % *delta tocopherol* and 80 % total *tocopherols* [7]; and
- *Synthetic alpha-tocopherol* is a viscous oil with a minimal content of 96 % *all-rac alpha-tocopherol* [7, 8].

Specifically, authorisation is sought for the use of the two *Tocopherol-rich extracts of natural origin* and the *Synthetic alpha-tocopherol* for all animal species and categories. The *feed additive* is intended to be incorporated to *feedingstuffs* directly or through *premixtures*, with no recommended minimum or maximum concentration levels [9, 10], as previously set in the regulation [11].

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 these dossiers, the methods of analysis submitted in connection with the *Tocopherol-rich extracts of natural origin* (including the *delta rich* form) and *Synthetic alpha-tocopherol*, 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) are available from the respective European Union Reference Laboratories [12].

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

For the determination of *all-rac alpha-tocopherol* in the *feed additive* (i.e. *Synthetic alpha-tocopherol*), both Applicants submitted the European Pharmacopoeia method [13], where:

- identification is based on optical rotation, infrared absorption spectrophotometry and thin-layer chromatography with ultraviolet detection; while
- quantification is based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID).

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method for the determination of *all-rac alpha-tocopherol* in the *feed additive* (i.e. *Synthetic alpha-tocopherol*).

For the determination of the *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol*) in the *feed additives* (i.e. two *Tocopherol-rich extracts of natural origin*) Applicant FAD-2010-0105 submitted the JECFA method [14]. This method is identical to the AOAC method for the determination of "Tocopherol forms in mixed tocopherol concentrate" [15]. The method

allows the quantification of each separate form of tocopherols (*alpha, beta, gamma* and *delta tocopherol*). Identification is based on a colour reaction with nitric acid, while quantification is based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID), using the relative retention times of the corresponding propionate forms, where hexadecyl hexadecanoate is used as internal standard.

Even though no performance characteristics are provided, the EURL recommends for official control the AOAC method (equivalent to the JECFA method), based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID) for the determination of the *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol*) in the *feed additives* (i.e. two *Tocopherol-rich extracts of natural origin*).

For the determination of *all-rac alpha-tocopherol* in *premixtures* and *feedingstuffs* containing *Synthetic alpha-tocopherol*, both Applicants submitted the ring-trial validated Community method [16], based on reverse phase High Performance Liquid Chromatography coupled to a UV or Fluorescence Detector (HPLC/UV or FLD). The following performance characteristics were reported, for *all-rac alpha-tocopherol* concentrations ranging from 61.3 to 17380 mg/kg:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.2 to 4.1 %; and
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 4.8 to 12.7 %.

Furthermore, the Community method allows the separate determination of the various *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)*, using a normal phase HPLC.

Additionally, the Applicant (FAD-2010-0105) applied the above mentioned Community method, without the saponification step, for the determination of the content of free/added *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)* in *premixtures* and *feedingstuffs* containing *Tocopherol-rich extracts of natural origin* or *Synthetic alpha-tocopherol*. Tocopheryl acetate is the standard used for external calibration. The Applicant provided a verification study [17]. The reported performance characteristics, presented in Table 1, demonstrate the applicability of the Community method for the determination of free/added *tocopherol forms* in *premixtures* and *feedingstuffs*, thus extending its original scope. Furthermore the Applicant reported a limit of quantification (LOQ) around 0.8 mg/kg *feedingstuffs*.

Table 1: Performance characteristics for the determination of *Tocopherol forms* in *premixtures (PM)* and *feedingstuffs (FS)* containing *Tocopherol-rich extracts of natural origin* or *Synthetic alpha-tocopherol* (*) [17]

		Content (mg/kg)	RSD_r (%)	R_{Rec} (%)
PM	total tocopherol	6910 – 13170	0.6 - 0.6	98.6 – 100.5
	alpha tocopherol	916- 1850	0.2 - 0.5	99.1 - 99.6
	beta tocopherol	94 - 177	2.1 - 4.1	100.1 - 100.6
	gamma tocopherol	4050 - 7560	0.7 - 0.9	98.6 – 101.1
	dlta tocopherol	1850 - 3580	0.9 – 1.2	98.3 - 99.8
FS	total tocopherol	30 - 46	3.5 - 7.7	98.5 – 103.7
	alpha tocopherol	3 - 16	8.1 - 14.1	92.9 – 100.0
	beta tocopherol	1	32.0	90.6 - 98.0
	gamma tocopherol	23 - 27	4.3 - 8.0	99.6 – 104.0
	delta tocopherol	2 - 3	17.2 - 17.7	99.7 – 104.3
PM	alpha tocopherol (*)	2500 - 9935	0.3 - 1.1	99.2 – 100.1
FS	alpha tocopherol (*)	55 - 180	0.5 – 2.1	99.3 – 99.8

RSD_r and R_{Rec} : relative standard deviation for *repeatability* and recovery rate

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method for the determination of *all-rac alpha-tocopherol* (E 307) in *premixtures* and *feedingstuffs*. Furthermore, the EURL recommends the ring-trial validated Community method to quantify the content of free/added *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)* in *premixtures* and *feedingstuffs*. Provided that the composition of the specific feed additive (E 306) utilised in the preparation of these matrices is known, the results of analysis of the various tocopherol forms may allow for estimating the added amount of this additive to *premixtures* and *feedingstuffs*.

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 (07/2011:0692), based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID) to determine *all-rac alpha-tocopherol* in the *feed additive* (i.e. *Synthetic alpha-tocopherol*);
- the AOAC method 988.14 (equivalent to the JECFA method), based on Gas Chromatography coupled to Flame Ionisation Detection (GC/FID), using hexadecyl hexadecanoate as internal standard to determine *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol*) in the *feed additives* (i.e. two *Tocopherol-rich extracts of natural origin*);
- the ring-trial validated Community method (Commission Regulation (EC) No 152/2009 – Annex IV, method B), based on normal or reverse phase High Performance Liquid Chromatography coupled to Ultra Violet Detection or Fluorescence Detection, HPLC/UV or FLD to determine the *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)* in the *premixtures* and *feedingstuffs* containing *Tocopherol-rich extracts of natural origin* or *Synthetic alpha-tocopherol.*

Recommended text for the register entry (analytical method)

For the determination of *all-rac alpha-tocopherol* in the *feed additive* (i.e. *Synthetic alpha-tocopherol*):

- Gas Chromatography coupled to Flame Ionisation Detection, GC/FID (Ph. Eur. 7.2 - 07/2011:0692), also including several identification tests;

For the determination of *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol*) in the *feed additives* (i.e. two *Tocopherol-rich extracts of natural origin*):

- Gas Chromatography coupled to Flame Ionisation Detection, GC/FID (AOAC 988.14)

For the determination of the *tocopherol forms* (*alpha, beta, gamma* and *delta tocopherol)* in the *premixtures* and *feedingstuffs* containing *Tocopherol-rich extracts of natural origin* or *Synthetic alpha-tocopherol*:

- High Performance Liquid Chromatography coupled to Ultra Violet Detection or Fluorescence Detection, HPLC/UV or FLD - (Commission Regulation (EC) 152/2009, Annex IV, B)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Tocopherol-rich extracts of natural origin, Tocopherol-rich extracts of natural origin/delta rich* and *Synthetic alpha-tocopherol* 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] [a]Application/Ref:SANCO/G/1: Forw.Appl. 1831/0070-2011
[2] [b]Application/Ref:SANCO/G/1: Forw.Appl. 1831/0071-2011
[3] [a] Technical dossier, Section II: 2.3. Manufacturing process
[4] [b] Technical dossier, Section II: 2.2. Characterisation of the active substance
[5] [a] Application, Proposal for Register Entry – Annex A
[6] [b] Application, Proposal for Register Entry – Annex A
[7] [a] Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition
[8] [b] Technical dossier, Section II: 2.1.4 Purity
[9] [a] Technical dossier, Section II: 2.5 Conditions of use
[10] [b] Technical dossier, Section II: 2.5 Conditions of use
[11] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs
[12] 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
[13] European Pharmacopoeia Monograph 07/2011:0692 – all-rac-α-Tocopherol
[14] [a] Supplementary Information, JECFA_method_additive_468_m4 (d-α-tocopherol, concentrate)
[15] AOAC method 988.14: Tocopherol isomers in mixed Tocopherol concentrate.
[16] Commission Regulation (EC) No 152/2009 *laying down the methods of sampling and analysis for the official control of feed* – Annex IV-B, Official Journal L54 (26.02.2009) p.66-71
[17] [a] Technical dossier, Section II, Annex 2.6.1.a

[a] Refers to Dossier no: FAD-2010-0105

[b] Refers to Dossier no: FAD-2010-0271

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha, The Czech Republic. 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:

- Fødevarestyrelsen, Ringsted (DK)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig (DE)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)