EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.DG.D.6/CvH/SB/AG/ARES(2011)707359

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

Dossier related to:	FAD-2010-0407 CRL/ 100347
Name of Product:	Carophyll® Red
Active Agent (s):	Canthaxantin
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 Article 4(1) for a *Canthaxanthin preparation, Carophyll® Red,* under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *Carophyll® Red* for turkeys and other poultry for breeding purposes. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs*. The Applicant suggested 2 and 6 mg/kg respectively as minimum and maximum *Canthaxanthin* concentration in *feedingstuffs*.

For the determination of the *active substance, Canthaxanthin,* in the *feed additive,* the Applicant submitted a ring trial validated method based on spectrophotometry. The following performance characteristics were reported:

- a standard deviation for *repeatability* (RSD_r) ranging from 0.2 to 0.8%;
- a standard deviation for *reproducibility* (RSD_R) ranging from 1.3 to 4.0%, and
- a *recovery* rate (R_{Rec}) ranging from 96.2 to 105%.

Based on the performance characteristics presented the EURL recommends for official control the ring trial validated spectrophotometric method, submitted by the Applicant, to determine *Canthaxanthin,* in the *feed additive*.

For the determination of *Canthaxanthin* in *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified method based on Normal Phase High-Performance Liquid Chromatography coupled to VIS detection (NP-HPLC-VIS). The following performance characteristics were reported:

- RSD_r ranging from 1.4 to 15%;
- a standard deviation for *intermediate precision* (RSD_{ip}) ranging from 2.1 to 14.8%, and
- R_{Rec} ranging from 85.5 to 107%.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified NP-HPLC-VIS method, submitted by the Applicant, to determine *Canthaxanthin* in *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

Canthaxanthin preparation, Carophyll® Red, Zootechnical additives, all poultry for breeding purposes.

1. BACKGROUND

Canthaxanthin is already authorised as *feed additive* under the category 'sensory additives' in the functional group 'colourants' [1]. In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for a *Canthaxanthin preparation, Carophyll® Red*, under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives', according to the classification system of Annex I of Regulation (EC) No 1831/2003 [2]. According to the Applicant *Canthaxanthin* is the *active substance* of the *preparation*. The registered trade name of the product is *Carophyll® Red* and can be referred exchangeably to three *Canthaxanthin preparations* as presented in Table 1 [3].

Canthaxanthin is obtained by chemical synthesis. It is included in the formulation with a minimum purity of 96%. The *feed additive* is a violet/red solid crystalline powder with a minimum of 10% of *active substance* in the preparation [4].

Specifically, authorisation is sought for the use of *Carophyll® Red* for turkeys and other poultry for breeding purposes. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs*. The Applicant suggested 2 and 6 mg/kg respectively as minimum and maximum *Canthaxanthin* concentration in *feedingstuffs* [5, 6].

Table 1: Percentage composition of the *feed additive* (*Canthaxanthin preparation*) as manufactured by the Applicant [7]

Ingredients	Carophyll ® Red 10%*	Carophyll ® Red	Carophyll ® Red 15%
Canthaxanthin	*10.00*	*10.00*	*15.00*
Ethoxyquin	2.20	1.50	3.00
Lignosulfonate	62.80	-	-
Dextrin Yellow	10.00	13.00	18.50
Corn Starch	15.00	28.38	-
Gelatin	-	33.12	42.50
Sucrose	-	13.00	18.50
Ascorbyl Palmitate	-	1.00	1.50
Silicon Dioxide	-	-	1.00

** presently marketed in the EU*

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 dossier, the methods of analysis submitted in connection with *Carophyll® Red*, 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 [8].

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

For the determination of *Canthaxanthin* in the *feed additive*, the Applicant submitted a spectrophotometric method [9]. This method applies for the analysis of powdery or water dispersible *Canthaxanthin preparations* and it is designed for concentrations of *active substance* ranging from 5 to 25%. The *feed additive* is enzymatically treated in water with protease in order to release the *active substance*. Following a dilution with acetone, the mass fraction of *Canthaxanthin* is determined photometrically at the isobestic wavelength of 426 nm. The mass fraction is calculated by an absolute measurement considering the specific absorbance of the *active substance* at this wavelength [10].

The analytical method was single laboratory validated and further verified by a collaborative study that involved six laboratories analysing seven samples in replicate.

The performance characteristics derived from the validation and the collaborative study are presented in Table 2.

Table 2: Method performance characteristics for the determination of *Canthaxanthin* in the *feed additive* (FA) [10]

FA	validation	intercomparison study
RSD_r (%)	0.3	0.2 - 0.8
RSD_{ip} (%)	0.3	-
RSD_R (%)	-	1.3 - 4.0
R_{rec} (%)	99.3	96.2 - 105

RSD_r, RSD_{ip} - relative standard deviation for *repeatability* and *intermediate precision*, respectively; RSD_R - relative standard deviation for *reproducibility*; R_{rec} - *recovery* rate.

Based on the performance characteristics presented, the EURL recommends for official control, the ring trial validated spectrophotometric method, submitted by the Applicant, to determine *Canthaxanthin* in the *feed additive*.

For the determination of *Canthaxanthin* in *premixtures* and *feedingstuffs*, the Applicant submitted a high performance liquid chromatographic (HPLC) method with VIS detection [9]. The method consists in an enzymatic digestion of the sample in order to release the active substance, followed by extraction with ethanol and dichlorometane. The extraction procedure differs in consideration of the nature of the sample and of the *Canthaxanthin* concentration declared. The extract is purified by open-column chromatography on silica gel. Finally, an aliquot is injected into an isocratic normal-phase HPLC system adjusted at 466 nm. The selected chromatographic conditions allow for a full resolution of the cis/trans isomers of *Canthaxanthin,* carotenes and other xanthophylls present in the feed. The *active substance* is expressed as the sum of the all-trans and cis isomers (total *Canthaxanthin*) [11]. The separated trans/cis isomers are invidually quantified against a standard solution prepared with the all-trans *Canthaxanthin*. Furthermore, the quantification of the cis isomers of *Canthaxanthin* includes the use of experimentally determined relative response factors, in order to compensate for the different absorbance coefficients of the cis isomers compared to all-trans *Canthaxanthin*.

The analytical method was single laboratory validated and further verified. The correspondent performance characteristics are presented in Table 3.

Table 3: Method performance characteristics for the determination of *Canthaxanthin* in *premixtures* (PM) and *feedingstuffs* (FS) [11]

PM & FS	Premix		Layer Feed		Broiler Feed		Fish Feed	
	Valid.	Verif.	Valid.	Verif.	Valid.	Verif.	Valid.	Verif.
Content, *mg/kg*	1017 - 3268		3.8 - 43.3		8.4 - 108.1		30.5 - 45.9	
RSD_r (%)	1.4 - 6.9	2.1 - 2.7	8.5 - 15.0	5.4 - 12.9	2.6 - 9.6	2.6 - 8.3	8.7 - 9.5	6.4 - 9.2
RSD_{ip} (%)	2.1	2.3	14.8	12.6	8.0	8.2	9.1	7.0
R_{rec} (%)	-	99.8 - 105	-	85.5 - 86.5	-	94.9 - 102	-	107
LOD (mg/kg)	-	20	0.03	0.3	0.01	0.3	0.01	0.3
LOQ (mg/kg)	-	60	1	1	1	1	1	1

RSD_r, RSD_{ip} - relative standard deviation for *repeatability* and *intermediate precision*, respectively;
R_{rec} - *recovery* rate; LOD – limit of detection; LOQ – limit of quantification

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified HPLC-VIS method, submitted by the Applicant, to determine *Canthaxanthin* in *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:

- a single laboratory validated and further ring trial verified method based on spectrophotometry to determine *Canthaxanthin* in the *feed additive*;
- a single laboratory validated and further verified method based on Normal Phase High-Performance Liquid Chromatography coupled to VIS detection (NP-HPLC-VIS) to determine *Canthaxanthin* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *Canthaxanthin* in *feed additive*:

- Spectrophotometry (426 nm),

For the determination of *Canthaxanthin* in *premixtures* and *feedingstuffs*:

- Normal Phase High-Performance Liquid Chromatography coupled to VIS detection (NP-HPLC-VIS, 466 nm)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Carophyll® Red* 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] List of authorised additives in feedingstuffs (2004/C50/01)
[2] *Application/Ref:SANCO/D/2:Forw.Appl.1831/(00208) (10511)-2010
[3] *Technical dossier, Section II: 2.1.1 Name(s) of the additive
[4] *Technical dossier, Section II: 2.2 Characterization of the active substance(s)
[5] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[6] *Application, (Annex A), FAD-2010-0407_Conditions of use_*Canthaxanthin preparation*
[7] *Technical dossier, Section II: 2.1.3.1 Active substance and all other components of the additive
[8] 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
[9] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substance
[10] *Technical dossier, Section II: Annex 2-25
[11] *Technical dossier, Section II: Annex 2-24
*Refers to Dossier no: FAD-2010-0407

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:

- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)