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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

Dossier related to:	FAD-2010-0373 CRL/100141
Feed Additive:	Capsanthin (E160c)
Active Substance(s):	Capsanthin
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Zigmas Ezerskis (EURL-FA)
Report revised by: Date:	Piotr Robouch (EURL-FA) 05/06/2012
Report approved by: Date:	Christoph von Holst 05/06/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *capsanthin (E160c)* under the category/functional group 2(a) 'sensory additives'/'colourants', for the following subgroups: - (i) substances that add or restore colour in *feedingstuffs*; - (ii) substances which, when fed to animals, add colours to food of animal origin; and - (iii) substances which favourably affect the colour of ornamental fish or birds, according to the classification system of Annex I of Regulation (EC) No 1831/2003.

The *feed additive* is a saponified paprika oleoresin, with a total carotenoids & xanthophylls content ranging from 35 to 130 g/kg, which corresponds to a capsanthin content ranging from 10 to 60 g/kg. The *feed additive* is to be marketed as solid or liquid formulations with a minimum total carotenoids & xanthophylls and capsanthin concentrations of 0.5 % and 0.15 %, respectively.

Specifically, authorisation is sought for the use of the *feed additive* for all poultry species, cats, dogs, ornamental fish and birds. The *feed additive* is intended to be incorporated in *premixtures, feedingstuffs* and *water*. While no maximum and minimum levels were proposed for cats, dogs, ornamental fish and birds, the Applicant proposed for all poultry species a maximum concentration of capsanthin or total carotenoids & xanthophylls in *feedingstuffs* and *water* of 80 mg/kg and 40 mg/L, respectively.

For the determination of capsanthin in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified method, based on normal phase High Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis). The following performance characteristics were reported:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.6 to 2.3%;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1 to 8.5%;
- a recovery rate ranging from 80 to 105%; and
- a limit of quantification of 1 mg/kg *feedingstuffs*

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified method, based on High Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis), submitted by the Applicant, to determine capsanthin in the *feed additive*, *premixtures* and *feedingstuffs*.

For the determination of total carotenoids & xanthophylls in the *feed additive* the Applicant proposed the internationally recognised FAO JECFA monograph for food additives, recommended by Commission Directive 2008/128/EC, where identification is based on several tests, including: - solubility; - colour reaction; - high performance liquid chromatography (HPLC), while the quantification of total carotenoids & xanthophylls is achieved by spectrophotometry at 462 nm. Even though no performance characteristics are provided, the EURL recommends for official control the methods recommended by Commission Directive 2008/128/EC and described in the above mentioned JECFA monograph for the determination of total carotenoids & xanthophylls in the *feed additive*.

For the determination of total carotenoids & xanthophylls (including capsanthin) in *feedingstuffs* the Applicant submitted the official method of Association of Analytical Communities (AOAC, 970.64) based on chromatographic separation (after saponification) of carotenes & xanthophylls and further detection of the different fractions by spectrophometry at 436 and 474 nm. This method could be applied on *premixtures* samples diluted in blank feed. Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned AOAC official method for the determination of total carotenoids & xanthophylls in *premixtures* and *feedingstuffs*.

The Applicant did not provide any analytical method or experimental data for the determination of capsanthin and total carotenoids & xanthophylls in *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine capsanthin and total carotenoids & xanthophylls 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
capsanthin, E160c, sensory additives, colourants, all poultry species, cats and dogs, ornamental fish and birds

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use of a feed additive) and 10(2) (re-evaluation of additives already authorised under provisions of Council Directive 70/524/EEC) for *capsanthin (E160c)* under the category/functional group 2(a) 'sensory additives'/'colourants', for the following subgroups: - (i) substances that add or restore colour in *feedingstuffs*; - (ii) substances which, when fed to animals, add colours to food of animal origin; and subgroups; and - (iii) substances which favourably affect the colour of ornamental fish or birds [1, 2], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, the *feed additive* (*capsanthin, E160c*) is a dark redish viscous saponified paprika oleoresin paste, obtained from *Capsicum annuum*, containing levels of total carotenoids & xanthophylls ranging from 35 to 130 g/kg, which corresponds to a capsanthin content ranging from 10 to 60 g/kg [2, 3]. The *feed additive* is to be marketed as solid or liquid formulations with a minimum total carotenoids & xanthophylls and capsanthin concentrations of 0.5 % and 0.15 %, respectively [3].

Specifically, authorisation is sought for the use of the *feed additive* for all poultry species, cats, dogs, ornamental fish and birds [2]. The *feed additive* is intended to be incorporated in *premixtures*, *feedingstuffs* and *water* [2].

While no maximum and minimum levels were proposed for cats, dogs, ornamental fish and birds [2], the Applicant proposed for all poultry species a maximum concentration of capsanthin or total carotenoids & xanthophylls in *feedingstuffs* and *water* of 80 mg/kg and 40 mg/L, respectively [2].

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 for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *capsanthin (E160c)*, 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, mycotoxins, and dioxins) are available from the respective European Union Reference Laboratories [4].

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

For the determination of capsanthin in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted a single laboratory validated and further verified method, based on normal phase High Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis) [5].

The grinded sample is extracted with a n-hexane:ethanol:acetone:toluene (10:6:7:7, v/v). The extract is injected into an isocratic normal phase HPLC system to resolve the geometrical isomers of capsanthin, zeaxanthin and lutein. All xanthophylls are quantified at 450 nm using β-carotene as the calibration standard. Experimentally determined relative response factors of different xanthophylls in relation to β-carotene are used for the correction of specific absorbances of the different xanthophylls. The performance characteristics determined for the three matrices are presented in Table 1. Furthermore, the Applicant reported a limit of quantification (LOQ) of 1 mg/kg *feedingstuffs*.

Table 1: Method performance characteristics for the determination of *trans*-capsanthin in *feed additive*, *premixtures* and *feedingstuffs* [6]

	Feed additive		Premixtures		Feedingstuffs	
	Validation	Verification	Validation	Verification	Validation	Verification
Content [mg/kg]	603 - 1238		618 - 964		10.9 - 27.3	
RSD_r (%)	0.6	0.9 - 1.9	0.4	1.7 - 1.8	0.2 - 1.8	0.1 - 2.3
RSD_{ip} (%)	0.7 - 4.9		2.6		1.7 - 8.5	
R_{rec} (%)	102 - 105	98 - 101	101 - 108	97 - 104	78 - 96	80 - 87

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

Based on the performance characteristics presented, the EURL recommends for official control, the single laboratory validated and further verified method, based on High Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis), submitted by the Applicant, to determine capsanthin in the *feed additive*, *premixtures* and *feedingstuffs*.

For the determination of total carotenoids & xanthophylls in the *feed additive* the Applicant proposed the internationally recognised FAO JECFA monograph for food additives [7], as recommended by Commission Directive 2008/128/EC, where identification is based on several tests, including: - solubility; - colour reaction; - high performance liquid chromatography (HPLC); while the quantification of total carotenoids & xanthophylls is achieved by spectrophotometry at 462 nm.

Even though no performance characteristics are provided, the EURL recommends for official control the methods recommended by Commission Directive 2008/128/EC and described in the above mentioned JECFA monograph for the determination of total carotenoids & xanthophylls in the *feed additive*.

For the determination of total carotenoids & xanthophylls (including capsanthin) in *feedingstuffs* the Applicant submitted the official method of Association of Analytical Communities (AOAC, 970.64) based on saponification, chromatographic separation of total carotenes & xanthophylls, and further detection of the different fractions by spectrophometry at 436 nm and 474 nm, respectively [8]. This method can be applied to *premixtures* samples after dilution with blank feed.

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned AOAC official method for the determination of total carotenoids & xanthophylls in *premixtures* and *feedingstuffs*.

The Applicant did not provide any analytical method or experimental data for the determination of capsanthin and total carotenoids & xanthophylls in *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine capsanthin and total carotenoids & xanthophylls 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 single laboratory validated and further verified method using High Performance Liquid Chromatography with visible wavelength detection (HPLC-Vis) for the determination of capsanthin in *feed additive*, *premixtures* and *feedingstuffs*.
- the methods described in FAO JECFA monograph *'paprika extract'* monograph No. 5 (2008), Combined Compendium for Food Additive Specifications - as recommended by Commission Directive 2008/128/EC - for the determination of total carotenoids & xanthophylls in the *feed additive,* where identification is based on: - solubility; - colour reaction; and - high performance liquid chromatography (HPLC); while quantification is based on spectrophotometry at 462 nm.
- the AOAC official method 970.64, based on chromatographic separation after saponification and further detection by spectrophometry at 436 nm and 474 nm, for the determination of total carotenoids & xanthophylls in *premixtures* and *feedingstuffs*.

The Applicant did not provide any analytical method or experimental data for the determination of capsanthin and total carotenoids & xanthophylls in *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine capsanthin and total carotenoids & xanthophylls in *water*.

Recommended text for the register entry (analytical method)

For the determination of capsanthin in the *feed additive*, *premixtures* and *feedingstuffs*:

- High Performance Liquid Chromatography with visible detection (HPLC-Vis)

For the determination of total carotenoids & xanthophylls in the *feed additive*:

- Commission Directive 2008/128/EC referring to FAO JECFA monograph *'paprika extract'* monograph No. 5 (2008), Combined Compendium for Food Additive Specifications

For the determination of total carotenoids & xanthophylls in *premixtures* and *feedingstuffs*:

- Liquid chromatography with visible detection (LC-Vis) - AOAC official method 970.64

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *capsanthin* 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. 1831/00159/(10171)/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] 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

[5] *Technical dossier, Section II, Annex_II_23

[6] *Technical dossier, Section II, Annexes Sect. II - EURLFA-Verification_form_Lutein, Zeaxanthin and Capsanthin_final

[7] FAO JECFA Combined Compendium of Food Additive Specifications, *'paprika extract'*, Monograph No. 5 (2008)
http://www.fao.org/ag/agn/jecfa-additives/specs/monograph5/additive-510-m5.pdf
(last visited on 05/06/2012)

[8] AOAC Official Method 970.64 Carotenes and Xanthophylls in dried plant materials and mixed feeds
http://www.aoac.org/omarev1/970_64.pdf
(last visited on 05/06/2012)

* Refers to Dossier No. FAD-2010-0373

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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- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)

- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Puławy (PL)