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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-2009-0046 CRL/090016
Name of Additive:	Beta-Carotene
Active Agent (s):	*Beta-Carotene*
Rapporteur Laboratory:	Laboratório Nacional de Investigação Veterinária (LNIV) Lisboa, Portugal
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Date:	20/05/2010
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Date:	28/05/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Beta-Carotene* 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. Specifically, authorisation is sought for the use of *Beta-Carotene* for all animal species and categories. The active substance and the feed additive is *Beta-Carotene*, which is a crystalline powder with a purity of at least 96%. It is produced by chemical synthesis and by fermentation from a strain of *Blakeslea trispora*. It is intended to be used in *premixtures*, *feedingstuffs* and *water* as a formulated product. According to the applicant typical formulations contain a minimum of 10% *Beta-Carotene*. The applicant does not propose any minimum or maximum concentration of the feed additive in feedingstuffs or water.

For the determination of the purity of *Beta-Carotene* (i.e. the percentage mass fraction of *Beta-Carotene* in the feed additive), the applicant proposed the European Pharmacopoeia method (Ph.Eur.3rd, monograph 1069). The CRL-FA considers this method suitable to be used within the frame of official control.

For the determination of *Beta-Carotene* in *premixtures* and *feedingstuffs* the applicant proposed a High Performance Liquid Chromatography (HPLC) method, tested for *premixtures* and *feedingstuffs* with concentration ranging from 100 to 2000 mg/kg for *premixtures* and from 10 to 100 mg/kg in *feedingstuffs*. The following performance characteristics derived from a three-laboratories intercomparison study were reported by the applicant for *premixtures* and *feedingstuffs*:

- a recovery rate of circa 100%,
- a repeatability relative standard deviation (RSD_r) ranging from 2.9 to 8.9 %,
- a reproducibility relative standard deviation (RSD_R) ranging from 3 to 11.5 %, and
- a limit of detection (LOD) of 0.05 mg/kg for *feedingstuffs*.

Based on these acceptable performance characteristics the CRL considers this method suitable for the determination of *Beta-Carotene* in *premixtures* and *feedingstuffs* within the concentration range covered by the collaborative study. Therefore the CRL recommends for

official control the HPLC method submitted by the applicant, for the determination of *Beta-Carotene* in *premixtures* and *feedingstuffs*.

For the determination of *Beta-Carotene* in *water* the applicant did not submit any analytical method nor experimental data, therefore the CRL cannot evaluate nor recommend any method for the determination of the active substance in this matrix.

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

Beta-Carotene, nutritional additive, vitamins, all species

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 authorized additive under council directive 70/524/EEC) for *Beta-Carotene* 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]. Specifically, authorisation is sought for the use of *Beta-Carotene* for all animal species and categories [1]. *Beta-Carotene* is a crystalline powder with a purity of at least 96% and a maximum content of 3% of other colouring matters [3]. It is produced by chemical synthesis and by fermentation from a strain of *Blakeslea trispora* [2]. According to the applicant typical formulations contain a minimum of 10% *Beta-Carotene* [2]. Preparations of *Beta-Carotene* include solutions or suspensions in edible fats or oils, emulsions and water dispersible powders. The applicant suggested no minimum or maximum levels for the feed additive in *feedingstuffs* and *water* [3], but typically the content is in the range of 10 to 125 mg/kg in *feedingstuffs* and *water*, depending on the animal species [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 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 (EFSA) for each application. For this particular dossier, the methods of analysis submitted in connection with *Beta-Carotene* and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification/Characterization 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, dioxins and PCBs) are available at the respective Community Reference Laboratories [5].

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

For the determination of the purity of the *Beta-Carotene* (i.e. the percentage mass fraction of *Beta-Carotene* in the feed additive), the applicant proposed the European Pharmacopeia [6] and JECFA Monographs [7]. The sample is dissolved in an organic solvent and the concentration in the solution is determined by visible absorption spectrophotometry at 455 nm.

For the determination of total *Beta-Carotene* in powder formulations [8], protease digestion is used followed by a liquid-liquid extraction using a mixture of dichloromethane and ethanol. In oily formulations the test solution is prepared by direct dissolution in the same mixture.

The substance is then determined by spectrophotometry after dilution of the extracts with cyclohexane.

The CRL-FA considers these methods suitable to be used within the frame of official control for the determination of the purity of *Beta-Carotene* and for the determination of *Beta-Carotene* in the various formulations.

For the determination of the active substance in *premixtures* and *feedingstuffs* the applicant proposes a High Performance Liquid Chromatography (HPLC) method [9]. After an enzymatic digestion the sample is extracted with ethanol and dichloromethane. The extract is then injected in a reverse phase high performance liquid chromatography system (RP-HPLC) using an ultraviolet (UV) detector for the determination of *Beta-Carotene*. A collaborative trial including three laboratories was organised for *premixtures* and *feedingstuffs* containing 10 to 2000 mg *Beta-Carotene*/kg .The following performance characteristics were reported:

- a recovery rate of about 100%,
- a repeatability relative standard deviation (RSD_r) ranging:

  from 4.7 to 8.9 % for *premixtures* and

  from 2.9 to 8.3 % for *feedingstuffs*,
- a reproducibility relative standard deviation (RSD_R) ranging:

  from 3.3 to 8.2 % for *premixtures* and

  from 3.0 to 11.5 % for *feedingstuffs*, and
- a limit of detection (LOD) of 0.05 mg/kg for *feedingstuffs* containing less than

  20 mg/kg *Beta-Carotene*.

Based on these acceptable performance characteristics the CRL considers this method suitable for the determination of *Beta-Carotene* in *premixtures* and *feedingstuffs* within the concentration range covered by the validation study. Therefore the CRL recommends for official the HPLC method submitted by the applicant for the determination of *Beta-Carotene* in *premixtures* and *feedingstuffs*.

For the determination of *Beta-Carotene* in *water* the applicant did not submit any experimental data nor analytical method, therefore the CRL cannot evaluate nor recommend any method for the determination of the active substance in this matrix.

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 authorization the CRL recommends for official control:

- the European Pharmacopoeia method (Ph.Eur. 3rd edition, monograph 1069) using spectrophotometric method for the determination of purity of *Beta-Carotene;*
- the reverse phase High Performance Liquid Chromatography (RP-HPLC) coupled to UV detector submitted by the applicant, for the determination of *Beta-Carotene* in *premixture*s and *feedingstuffs.*

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

For the determination of *Beta-Carotene* in the *feed additive*:

- spectrophotometric method based on the European Pharmacopoeia (Ph. Eur. 3rd edition, monograph 1069)

For the determination of *Beta-Carotene* in *premixtures* and *feedingstuffs*:

- Reverse phase High Performance Liquid Chromatography (RP-HPLC) coupled to UV detector

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

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

6. REFERENCES

[1] *Application/Ref:SANCO/D/2:Forw.Appl.1831/039-2009

[2] *Technical dossier, Section II: Identity

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

[4] *Technical dossier, Section II, 2.5. Condition of use of the additives

[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] *Ref 2.6.02: European Pharmacopoeia, 3rd Edition: Monograph 1069

[7] 2.6.01: FAO JECFA Monographs: Combined compendium of food additive specifications, Volume 4: Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications (2006)

[8] *Ref 2.6.03: FEFANA: Method of Analysis of vitamins, provitamins and chemically defined substances having a similar biological effect, 1st part "Tel Quel" (2006)*Ref

[9] *Ref 2.6.04: FEFANA draft 2007: Determination of Stabilised Beta-Carotene in Premixes and Feedstuffs

*Refers to Dossier no: FAD-2009-0046

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Laboratório Nacional de Investigação Veterinária (LNIV) Lisboa, Portugal. 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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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
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