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CRL Evaluation Report on the Analytical Methods submitted in connection with Section II – 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2006-0031 EFSA-Q-2006-317
Product name:	Lantharenol®
Active Substance(s):	Lanthanum carbonate octahydrate
Rapporteur Laboratory:	Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy
Report prepared by:	Maria Cesarina Abete (C.Re.A.A)
Report checked by:	Giuseppe Simone (CRL-FA)
Report approved by:	Christoph von Holst
Date:	1/08/2007

EXECUTIVE SUMMARY

Lantharenol® is a product for which authorisation is sought under the category "zootechnical additives", functional group "others zootechnical additives", according to the classification system of Annex I, of Regulation (EC) No 1831/2003. According to the applicant, *Lantharenol®* contains at least 85% lanthanum carbonate octahydrate as active substance.

In the current application authorisation is sought for use of *Lantharenol®* for cat feed. *Lantharenol®* is intended to be added to complete feed in concentration of 1500 to 7500 mg/kg expressed as concentration of lanthanum carbonate octahydrate and is not intended to be used in premixtures.

For the determination of the active substance in the *additive* the applicant proposed an inductively coupled plasma optical emission spectrometry (ICP-OES) method. The carbonate content of *Lantharenol®* was determined by electrochemical volumetric analysis. Both methods have been in-house validated. The performance characteristics are considered acceptable, therefore the methods are considered suitable for official control.

For *feed* analysis the applicant proposed the same ICP-OES method used to quantify lanthanum in the additive, but with a different extraction procedure. The method has been in house validated and a recovery study has been carried out on wet and dry cat feed, obtaining percentage recovery rate between 90.7% and 103%. The concentration of lanthanum carbonate octahydrate is calculated on the basis of the measured concentration of lanthanum. This is possible, since the applicant performed analyses to evaluate the background content of lanthanum in regular cat feed showing that the concentration of lanthanum in blank feed samples was less than 1/1000 of lanthanum present in the lowest recommended dose of lanthanum carbonate octahydrate. The results show that the method is robust and suitable for official control of lanthanum in cat feed.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

KEYWORDS

Lantharenol®, lanthanum carbonate octahydrate, zootechnical feed additive, phosphate binder, cats.

BACKGROUND

Lantharenol® is a product for which authorisation is sought under the category "zootechnical additives", functional group “other zootechnical additives” according to Annex I of Regulation (EC) No 1831/2003. According to the applicant, *Lantharenol®* contains at least 85% lanthanum carbonate octahydrate as active substance, the remaining being loosely-bound water.

The product is intended to be used as feed additive for cats feed, by mixing in complete feed to achieve a concentration of 1500 to 7500 mg/kg complete feed (*cf.* EFSA-Q-2006-317).

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 *Lantharenol®* dossier (EFSA-Q-2006-317) and their suitability to be used for official controls in the frame of the authorisation were evaluated.

EVALUATION

The numbering system under this point refers to that of Section II of the Annex of Commission Directive 2001/79/EC (2.5 Control methods).

Description of the methods used for the determination of the criteria listed under item 2.5.1 of Commission Directive 2001/79/EC

Degree of purity – qualitative and quantitative composition of the impurities

Impurities such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) were quantified by inductively coupled plasma mass spectrometry (ICP-MS). The content of each element was determined from an external multi-element standard curve. For all metals

limit of detection (LOD), limit of quantification (LOQ), linearity/correlation coefficient were tested. In all cases the linearity was over the required range (acceptance criteria r >0.9800). The LOQ was below the maximum specification values, which were: 2 ppm for As, 1 ppm for Cd, 2 ppm for Cr, 1 ppm for Pb and 0.2 ppm for Hg.

An electrochemical method was used to quantify fluoride levels. A linearity study was carried out over a range of 10% to 120% of the specification range and a correlation coefficient value of 0.99750 was found (acceptance criteria r >0.9800). Recovery was 112% with an acceptance criteria > 70% and the relative standard deviation (RSD%) was 1.4% (acceptance criteria < 2.5%). The LOQ was 10 ppm. The acceptance criterion set in the specifications was 100 ppm maximum [1].

Sodium levels were quantified by flame atomic absorption spectrometry method. A linearity study was carried out over a range of 100 to 1200 ppm and a correlation coefficient value of 0.9978 was found (acceptance criteria r >0.9800). Precision (repeatability) at 30 ppm was assessed by preparing a single sample six times, and resulted in a RSD% of 4% (acceptance criteria < 5%). The LOQ was 4.5 ppm. The acceptance criterion set in the specifications was 500 ppm maximum [1]. Ashes content was calculated using a thermal gravimetric method [2].

All these methods are considered suitable for the intended purposes.

Purity – Identification and quantification of microbial impurities and toxic substance

Microbial purity of five batches was tested and the method used complies with the requirements of European Pharmacopoeia 5th edition [3].

The applicant also provided results of analyses for the presence of dioxins (PCDD/PCDF). The measurement of the PCDD/PCDF was based upon isotopic dilution analysis by GC/MS quantification, with high resolution mass spectrometry. Samples were spiked with carbon -13 labelled compounds (used as internal standard) before the preparation procedures. The method used had been successfully validated in several inter-laboratory tests in accordance with European guidelines EN 1948 part II and III [4].

The mentioned methods are considered suitable for the intended purposes.

Qualitative and quantitative composition of the additive

Lanthanum content of *Lantharenol®* was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The sample (0.5 g) was dissolved with water and nitric acid and then diluted with water to obtain a specific volume. The determination of lanthanum was performed at 379.5 nm (alternatively at 408.7 nm) using a calibration curve which is based on calibration solutions of lanthanum. The corrected lanthanum concentration related to octahydrate was calculated considering the molecular weights and ashes percentage [7]. The

method was in house validated. A linearity study was carried out: five independent calibration solutions (from 5.0 to 20.0 mg/L) were tested and a correlation coefficient value of 0.9999 was found (acceptance criteria > 0.9800). Precision was assessed by analysing six independent sample preparations from the same batch. The RSD% found was 0.62% (acceptance criteria <2.5%) [1]. The identity of lanthanum was also confirmed using a solution test: lanthanum carbonate octahydrate was dissolved with water and nitric acid, then acid acetic solution and KJ/J2 solution were added with some drops of ammonia solution. Lanthanum was identified as a deep blue coloured deposit [6].

Carbonate content of *Lantharenol*® was determined by electrochemical volumetric analysis. The sample was blended with boric acid and carbonate free-water. Carbonate was driven off by adding acid in the reaction vessel of the carbon determination apparatus, using oxygen as carrier gas [7]. A linearity study, using six data points, was carried out and a correlation coefficient value of 0.9998 was obtained (acceptance criteria >0.9800). Accuracy and recovery were assessed and the percentage recovery rate was 99.2% (acceptance criteria not less than 90%) [1]. Alternatively, an identity test for carbonate using barium hydroxide, according to European Pharmacopoeia 5th edition [8] could be used

These methods are considered suitable for the intended purposes.

Description of the qualitative / quantitative analytical methods for the routine control of the active substance in cat feed (2.5.2. of the Guidelines)

The concentration of lanthanum carbonate octahydrate is determined on the basis of the measurement of lanthanum applying an ICP-OES method with sample pre-treatment. 5 g of cat feed are weighed into a platinum pan and first dry ashed over a burner and then in a muffle furnace at 550°C over night. The residue is fused together with 2 g of lithium meta-borate at 900°C in a melting furnace. The platinum pan content is then put into a beaker and water and hydrochloric acid is added to dissolve the melting. Afterwards, the dissolved sample is transferred into a graduate flask and subsequently the solution is diluted 1:10 with water. The determination of lanthanum is performed at 379.5 nm (alternatively at 408.7 nm) by ICP-OES, using a calibration curve based on calibration solution of lanthanum of e.g. 0 mg/L, 10 mg/L and 20 mg/L (matched to the matrix of lithium meta borate). This method was in house validated by assessing linearity, limit of detection and quantification (LOD, LOQ), precision, accuracy, and robustness. For linearity five independent calibration solutions (5 mg/L, 10 mg/L, 12,5mg/L, 15 mg/L, 20 mg/L) were tested and a correlation coefficient value of 0.9999 was obtained (acceptance criteria >0.9800). The LOD and LOQ were 0.0009 mg/L and 0.0033 mg/L (aqueous solution) respectively, corresponding to 0.18 mg/kg and 0.66 mg/kg respectively considering the initial amount of cat feed (5g). Repeatability was tested on six independent sample weights; the RSD% was 0.62% (acceptance criteria <2.5%). Accuracy of

the method was assessed by measuring the recovery at high and low dosage (7.5 g/kg and 1.5 g/kg) of lanthanum carbonate octahydrate in cat dry feed and in cat wet feed. The obtained recovery values, between 90.7% and 103%, are considered acceptable. Robustness was assessed by comparison of the results obtained by different operators using different ICP-OES instruments for independently prepared samples at high and low dosage. Thus, the robustness refers to the ICP-OES technique as well as to all sample preparation steps applied (weighing, dry ashing, wet digestion, dilution). The mean RSD% for robustness was 6% and is considered acceptable [9], [10]. The concentration of lanthanum carbonate octahydrate is stoechiometrically calculated from the measured lanthanum concentration.

Furthermore, the background content of lanthanum in regular cat feed was assessed by analysing 11 commercial cat feed (2 dry feed and 9 wet feed). Because of the expected very low contents, the samples were analyzed by ICP-MS. The content of lanthanum in the feed samples was found to be less than 1/1000 of lanthanum present in the lowest recommended dose of lanthanum carbonate octahydrate (1500 mg/kg) [10]. Therefore it can be concluded that the very low background concentration of lanthanum in cat feed does not interfere with the calculation of lanthanum carbonate octahydrate based on the measured lanthanum concentration in feed samples containing *Lantharenol®*.

This method is considered suitable for the determination of lanthanum carbonate octahydrate in cat feed for official control purposes.

CONCLUSIONS AND RECOMMENDATIONS

In general terms, the methods proposed are considered suitable for the intended purposes, as they are standard methods.

The ICP-OES method used for the assay of the active ingredient in *Lantharenol®* is considered suitable for the intended purpose.

The ICP-OES method for the control of lanthanum in cat *feed* was in house validated and the results show that the method is robust and suitable for official control of lanthanum in cat feed.

As the background levels of lanthanum in commercial cat feed are not significant, the product can be considered as the only relevant source of lanthanum.

The control methods submitted for determination of possible contaminants and impurities in the feed additive are classical methods and are considered suitable for the intended purposes

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

Inductively coupled plasma optical emission spectrometry (ICP-OES).

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

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

REFERENCES

The dossier provided by the applicant is divided into various documents structured according to the Annex of Commission Directive 2001/79/EC, containing the following files:

[1] Technical dossier, Section II, Appendix 2.26
[2] Technical dossier, Section II, Appendix 2.9
[3] Technical dossier, Section II, Appendix 2.16
[4] Technical dossier, Section II, Appendix 2.15
[5] Technical dossier, Section II, Appendix 2.4, Appendix 2.5
[6] Technical dossier, Section II, Appendix 2.6
[7] Technical dossier, Section II, Appendix 2.8
[8] Technical dossier, Section II, Appendix 2.7
[9] Technical dossier, Section II, Appendix 2.17
[10] Additional information of 25/05/2007 - mail

RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy.

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