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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-2011-0050 - CRL/110024**

Feed additive: **Lanthanide citrate**

Active Substance(s): **Lanthanum and cerium citrates**

Rapporteur Laboratory: **Centro di Referenza Nazionale per la
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EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *lanthanide citrate* under category/functional group 4(d) "zootechnical additives"/"other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for weaned piglets.

Lanthanide citrate (Lancer[®]) is a yellow crystalline powder consisting mainly of *lanthanum* and *cerium citrates* with a minimum purity of 65 % *lanthanide citrate*. The *feed additive* is intended to be incorporated through *premixtures* or directly in *feedingstuffs* to achieve a content of 250 mg Lancer[®]/kg *feedingstuffs*, which corresponds to 21 mg *added lanthanum* /kg *feedingstuffs* and 41 mg *added cerium* /kg *feedingstuffs*.

For the determination of *citrate* in the *feed additive*, the Applicant provided no experimental data or any analytical methods. However the EURL identified and recommends the European Pharmacopeia Monographs 0400 and 0412, based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, for the quantification of *citrate* in the *feed additive*.

The direct quantification of the rare-earth citrates (Lancer[®]) in *premixtures* and *feedingstuffs* is not experimentally achievable. The Applicant submitted instead a single-laboratory validated multi-analyte method based on Inductively Coupled-Mass Spectrometry (ICP-MS) for the quantification of the *total lanthanum* and *cerium* in the *feed additive* and in *feedingstuffs*. *Premixtures* samples can be diluted in blank feed and analysed as feed samples. Based on the performance characteristics presented and the experimental evidence available the EURL recommends for official control the single-laboratory validated ICP-MS method applying the sample preparation protocol described in the CEN standards (EN 15510 or CEN/TS 15621) for the quantification of *total lanthanum* and *total cerium* in the *feed additive*, *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

Lanthanide citrate, *lanthanum*, *cerium*, *citrate*, zootechnical additives, weaned piglets

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new authorisation) for *lanthanide citrate* under category/functional group 4(d) "zootechnical additives"/"other zootechnical additives" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for weaned piglets [2].

Lanthanide citrate (*Lancer*[®]) is a yellow crystalline powder consisting mainly of *lanthanum* and *cerium citrates* with a minimum purity of 65 % *lanthanide citrate* [2, 3]. According to the Applicant the product contains 8.53% lanthanum and 16.3% cerium [3]; while *praseodymium*, *neodymium* are considered as rear earth impurities [4]. The *feed additive* is intended to be incorporated through the *premixtures* or directly in *feedingstuffs* to achieve a content of 250 mg *Lancer*[®]/kg *feedingstuffs*, which corresponds to 21 mg added *lanthanum* /kg *feedingstuffs* and added 41 mg *cerium* / kg *feedingstuffs* [3].

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 *lanthanide citrate* (*Lancer*[®]), 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 [5].

For the analysis of the rear-earth impurities (such as *praseodymium* and *neodymium*) in the *feed additive* the Applicant submitted the multi-analyte method based on Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) [6] described here-after.

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

For the determination of *citrate* in the *feed additive*, the Applicant provided no experimental data or any analytical methods. However the EURL identified and recommends the European Pharmacopeia Monographs 0400 and 0412 [7, 8] based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, for the quantification of *citrate* in the *feed additive*.

The direct quantification of the rare-earth citrates (*Lancer*[®]) in *premixtures* and *feedingstuffs* is not experimentally achievable. The Applicant submitted instead a single-laboratory validated multi-analyte method based on Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for the quantification of *total lanthanum* and *total cerium* in the *feed additive* and in *feedingstuffs* – applicable also to *praseodymium* and *neodymium* [6].

Lancer[®] samples (50 mg) are weighed and dissolved in diluted nitric acid. Feed samples (20-30 g) are ashed at 550°C and the ash residue (100 mg) is fused with lithium metaborate at 1000°C. after cooling, the fused material is dissolved in a nitric and tartaric acid mixture. The digested samples are further diluted before analysis. Samples are then diluted to compare the obtained solutions to a calibration curve prepared in a similar acid matrix. Instrumental determination of the *lanthanides* is performed at the characteristic mass of each rare element (¹³⁹La, ¹⁴⁰Ce, ¹⁴¹Pr, ^{143&146}Nd) using indium (¹¹⁵In) as internal standard [6].

In order to avoid the use of lithium metaborate in the digestion procedure, the EURL recommends the apply for feed samples the samples treatment described in the CEN standards CEN/TS 15621 and EN 15510 [9, 10]). Furthermore, the EURL considers that *premixtures* samples could be diluted in blank feed and analysed as feed samples.

The performance characteristics obtained in the frame of the validation study [11] for *feed additive* and *feedingstuffs* samples are presented in Table 1. Similar content ratios (Ce/La) of *ca.* 1.8 are observed in *feed additive* and *feedingstuffs* samples. In addition, the Applicant reported limits of quantification of 2 mg/kg *feedingstuffs* for *lanthanum* and *cerium* [11], which are well below according to the recommended conditions of use [2].

Table 1: Performance characteristics of the ICP-MS method for the determination of *lanthanides* (*lanthanum* and *cerium*) in the *feed additive* (FA) and *feedingstuffs* (FS) [11]

Matrix	Element	Content (mg/kg)	RSD _r (%)	RSD _{ip} (%)	R _{Rec} (%)
FA	La	90 706	0.9	-	94.5 - 95.6
	Ce	167 562	1.1	-	94.6 - 96.7
	Ce/La ratio	1.85			
FS	La	23.4	4.5	4.6	94.5 - 103
	Ce	42.1	7.1	7.1	94.6 - 97.5
	Ce/La ratio	1.80			

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

However, the Applicant did not perform a verification study, claiming [12] that the laboratory chosen for the validation study is accredited for a wide range of analysis in the field of pharmaceutical and food, and provided certificates for Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP) analysis [13, 14]. Furthermore, the EURL is aware that ICP-MS techniques are commonly used in the geochemistry field for the determination of rare earths. Kent et al. [15] applied isotope dilution multi-collector inductively coupled plasma-mass spectrometry (MC-ICP-MS) for the analysis of eleven rare earth elements in geochemical reference materials, while Schwabe et al. [16] demonstrated the suitability of ICP-MS technique for the analysis of rare earth elements (*lanthanum*, *cerium*, *praseodymium*) in animal tissues and feed applying the international standard EN ISO 17294-2:2005-02.

Based on the performance characteristics presented and the experimental evidence available the EURL recommends for official control the single-laboratory validated ICP-MS method applying the sample preparation protocol described in the CEN standards (EN 15510 or CEN/TS 15621) for the quantification of *total lanthanum* and *total cerium* in the *feed additive*, *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 Pharmacopeia Monograph 0400 and 0412 methods based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator for the quantification of the *citrate* in the *feed additive*;
- the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) method submitted by the Applicant combined with the sample preparation described in the EN 15510 or EN 15621 for the determination of *total lanthanum* and *total cerium* in the *feed additive*, *premixtures* and *feedingstuffs*.

However, the direct quantification of the rare-earth citrates (Lancer[®]) in *premixtures* and *feedingstuffs* is not experimentally achievable.

Recommended text for the register entry (analytical method)

For the quantification of *citrate salts* in the *feed additive*:

- Titration - European Pharmacopeia Monographs 0400 and 0412

For the quantification of *total lanthanum* and *total cerium* in the *feed additive*:

- Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Note: In case that minimum and maximum levels for Lancer[®] are expressed in terms of content of *lanthanum* and *cerium* in *feedingstuffs* the following recommended text would apply:

For the quantification of *total lanthanum* and *total cerium* in *premixtures* and *feedingstuffs*:

- Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *lanthanide citrate* 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/G1: Forw. Appl. 1831/0006-2012
 - [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] *Supplementary Information – SIN-Lancer-FAD-2011-0050
 - [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] *Technical dossier, Section II – Annex 2-24: Signed method L67-02
 - [7] European Pharmacopoeia Monograph 6.0, 01/2008:0400
 - [8] European Pharmacopoeia Monograph 6.0, 01/2008:0412
 - [9] CEN/TS 15621:2007 – Animal feeding stuffs – Determination of cadmium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES
 - [10] EN15510:2007 – Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES
 - [11] *Technical dossier, Section II – Annex 2-25: Method validation report (ALS)
 - [12] *Technical dossier, Section II – Annex 2-28: Justification
 - [13] *Technical dossier, Section II – Annex 2-27: gmp certifikat 100222
 - [14] *Technical dossier, Section II – Annex 2-26: glp certifikat 100804
 - [15] A. J. R. Kent et al. *Geostandards and Geoanalytical Research*, 28 (2004), 417-429
 - [16] A. Schwabe et al. *Livestock Science*, 143 (2012) 5-14
- *Refers to Dossier No. FAD-2011-0050

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the Centro di Referenza Nazionale per la Sorveglianza ed il Controllo degli Alimenti per Animali (C.Re.A.A), Torino, Italy. 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)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- RIKILT - Instituut voor Voedselveiligheid, Wageningen (NL)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 - Landwirtschaftliches Untersuchungswesen, Leipzig (DE)