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Standards for Food Bioscience
European Union Reference Laboratory for Feed Additive Authorisation

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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

Lenziaren

(FAD-2012-0026; CRL/120019)

European Union Reference Laboratory

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Dossier related to:	**FAD-2012-0026** **CRL/120019**
Name of product:	**Lenziaren**
Active Substance(s):	**Iron, -aqua, -carbonate, -hydroxy, -oxo starch sucrose complex**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Date:	**21/03/2013**
Report approved by:	**Christoph von Holst**
Date:	**21/03/2013**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Lenziaren* 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. Authorisation is sought for the use of the *feed additive* for cats.

Lenziaren is a synthetic reddish-brown complex polymer, consisting of iron (minimum of 19 %), starch, sucrose and sodium hydrogencarbonate. The *feed additive* is an intestinal phosphate binder for cats with a typical phosphate adsorption capacity ranging from 17 to 26 % w/w. The active substance in *Lenziaren* is *iron, aqua carbonate hydroxyl oxo starch sucrose complex*.

Lenziaren is to be mixed with cat feed to obtain *Lenziaren* concentration levels of 5 to 20 mg/kg *feedingstuffs*, equivalent to daily rations of 0.25 to 1g per cat.

For the characterisation of the *feed additive,* the Applicant suggested to record the infrared spectrum of the product, to quantify iron content and to determine phosphate adsorption capacity. The Applicant submitted Fourier transformed infrared spectroscopy (FTIR), described in generic European Pharmacopoeia monograph 01/2008:20224. However, this method is not specific enough to distinguish *Lenziaren* from starch and/or sucrose. Additionally, the EURL identified Community method (Commission Regulation (EC) No 152/2009, Annex IV-C) for the quantification of total iron in the *feed additive*. Finally, the Applicant submitted a single-laboratory validated and further verified method based on ion chromatography with conductivity detection for the determination of the phosphate adsorption capacity of *Lenziaren*. Based on the experimental evidence provided the EURL recommends all three methods mentioned above for official control to characterise the *feed additive*.

As the accurate determination of *iron, aqua carbonate hydroxyl oxo starch sucrose complex* in *feedingstuffs* is not achievable experimentally, the EURL does not recommend any methods for official control to determine *iron, aqua carbonate hydroxyl oxo starch sucrose complex* in *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

Lenziaren, Iron, -aqua, -carbonate, -hydroxy, -oxo starch sucrose complex, zootechnical additives, other zootechnical additives, cats

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new feed additive) for *Lenziaren* 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 [1]. Authorisation is sought for the use of the *feed additive* for cats [2].

Lenziaren is a synthetic reddish-brown complex polymer, consisting of iron (minimum of 19 %), starch, sucrose and sodium hydrogencarbonate [3]. The *feed additive* is an intestinal phosphate binder for cats with a typical phosphate adsorption capacity ranging from 17 to 26 % w/w [2]. The active substance in *Lenziaren* is *iron, aqua carbonate hydroxyl oxo starch sucrose complex* [3].

Lenziaren is supplied in single-use stickpacks [3]. The content of the stickpack is to be mixed with cat feed, to obtain *Lenziaren* concentration levels of 5 to 20 mg/kg *feedingstuffs* [2] equivalent to daily rations of 0.25 to 1g per cat [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 *Lenziaren*, 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

For the characterisation of the *feed additive* the Applicant suggested to record the infrared spectrum of the product, to quantify iron content and to determine the phosphate adsorption capacity.

- For the identification of the *feed additive*, the Applicant suggested Fourier transformed infrared spectroscopy (FTIR) in attenuated total reflection (ATR) and transmission modes at the wave range from 4000 to 600 cm^{-1} [5], described in generic European Pharmacopoeia monograph [6]. The positions and relative intensities of bands of the IR spectrum of the *feed additive* are compared to those of the reference substance. Nine bands are to be considered, with no additional major bands to be detected. The Applicant compared *Lenziaren* spectra to those obtained using sucrose and starch. No significant variation in peak positions and broadness of bands were observed between the *Lenziaren* and starch spectra, while sucrose displayed more IR absorption peaks [7]. These observations were confirmed experimentally at the Institute for Reference Materials and Measurements (IRMM). As stated by the Applicant, *"*[…] *IR spectroscopy is not enough specific to have a good discrimination of Lenziaren in comparison to starch and sucrose* […]*".*

- For the quantification of *total iron* in the *feed additive*, the Applicant submitted flame atomic absorption spectrometry (FAAS) method [8] based on the generic European Pharmacopoeia monograph [9]. The Applicant reported the following results, obtained in the frame of the validation study [10]: - an average total iron content of 20.1 % w/w; - RSD_r = 0.7 %; and - a recovery rate (R_{Rec}) of 97 %. However, the EURL identified instead the Community method based on FAAS ([11], Annex IV-C).

- For the determination of the phosphate adsorption capacity the Applicant performed the liquid/solid extraction experiment [12] to assess the ability of the *feed additive* to adsorb phosphate. The sample (365 to 385 mg) is suspended in a sodium hydrogenphosphate solution adjusted to pH 2 and incubated for 2 hours at 37 °C. The suspension (2 ml) is then filtered and diluted in 50 ml of water. The amount of free phosphate in solution determined by ion chromatography with conductivity detector is subtracted from the initial phosphate concentration to derive the phosphate adsorption capacity expressed in percentage (%) (mass of adsorbed phosphate / mass of *feed additive*). This method was single-laboratory validated [13] and further verified in the second independent laboratory [14]. The performance characteristics of the method are summarised in Table 1.

Table 1: Performance characteristics for the determination of adsorption capacity of phosphate by the *feed additive*

	Validation [14]	Verification [14]
PO_4^{3-} adsorption capacity (%)	18.8 - 20.2	19.2 - 20.5
RSD_r (%)	1.9 - 2.9	0.9 - 2.1
RSD_{ip} (%)	3.7	3.3

RSD_r and RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*

In addition , the Applicant submitted for the determination of *sucrose* in the *feed additive* an assay based on optical rotation [15] (derived from the generic European Pharmacopoeia monograph [16]) and reported the following results [17]: - an average total sucrose content of 29.9 % w/w and - a relative standard deviation for *repeatability* (RSD_r) of 0.4 %. The EURL identified as an alternative the Community method for the determination of sugar, such as sucrose, based on the Luff-Schoorl reaction by titration ([11], Annex III-J). However, both methods may lead to inaccurate/overestimated results due to the presence of starch in the sample.

Based on the experimental evidence provided the EURL recommends for official control to characterise the *feed additive* the *combined* application of the following methods: - the European Pharmacopoeia method based on FTIR for the identification of *feed additive*; - the Community method based on FAAS to quantify the total iron content; and - the single laboratory validated and further verified method based on determination of phosphate adsorption capacity by ion chromatography with conductivity detection.

As the accurate determination of *iron, aqua carbonate hydroxyl oxo starch sucrose complex* in *feedingstuffs* is not achievable experimentally, the EURL does not recommend any methods for official control to determine *iron, aqua carbonate hydroxyl oxo starch sucrose complex* in *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 to apply all three methods to characterise the *feed additive*:

- the European Pharmacopoeia Monograph (01/2008:20224) based on Fourier transformed infrared spectrometry (FTIR) for qualitative analysis of *Lenziaren*; and
- the Community method (Commission Regulation (EC) No 152/2009) for the quantification of total iron in *Lenziaren*; and
- single-laboratory validated and further verified method based on ion chromatography (IC) with conductivity detection for the determination of the *Lenziaren* phosphate adsorption capacity

As the accurate determination of *iron, aqua carbonate hydroxyl oxo starch sucrose complex* in *feedingstuffs* is not achievable experimentally, the EURL does not recommend any methods for official control to determine *iron, aqua carbonate hydroxyl oxo starch sucrose complex* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of the *feed additive*:

- Fourier transformed infrared spectrometry (FTIR) (European Pharmacopoeia monograph 20224) for qualitative analysis; and
- flame atomic absorption spectrometry (FAAS) for quantification of total iron (Commission Regulation (EC) No 152/2009); and
- ion chromatography (IC) with conductivity detection for quantification of the phosphate adsorption capacity

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lenziaren* 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. 1831/0047-2012

[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] 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_3

[6] European Pharmacopoeia Monograph 01/2008:20224

[7] *Technical dossier, Section II - Annex II_10

[8] *Technical dossier, Section II - Annex II_21

[9] European Pharmacopoeia Monograph 01/2008:20223

[10] *Technical dossier, Section II - Annex II_22

[11] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

[12] *Technical dossier, Section II - Annex II_4

[13] *Technical dossier, Section II - Annex II_11

[14] *Technical dossier, Section II - Annex II_12

[15] *Technical dossier, Section II - Annex II_23

[16] European Pharmacopoeia Monograph 01/2008:20207

[17] *Technical dossier, Section II - Annex II_24

*Refers to Dossier No. FAD-2012-0026

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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- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
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