JRC.F.5/CvH/MGH/AS/Ares

Subject: Addendum to the EURL evaluation report

Reference:
FAD-2010-0133 (*lactic acid* and *calcium lactate*) –
JRC.DG.D.5/CvH/ZE/AG/Ares(2012)197882

Upon the recent publication of new ring-trial validated methods EN 17294 [1] and EN 17298 [2] for the analysis of organic acids in feed additives, premixtures, feed materials, compound feed and water, the EURL, under the frame of article 5 of Regulation (EC) No 378/2005 [3], considered appropriate to perform a new evaluation of the methods of analysis for official control of *lactic acid* and *calcium lactate* in the *feed additives*, *premixtures, feedingstuffs* and *water*, in the frame of the above-mentioned *feed additive* dossier. In this line, aiming to recommend the available analytical methods complying with the highest requirements as stated in Annex II of Regulation (EC) No 429/2008 [4], the EURL also updates in this amendment the relevant methods for *calcium*.

For the determination of *lactic acid* and *calcium lactate* (as total *lactic acid*) in the *feed additives*, *premixtures, feedingstuffs* and *water* the EURL evaluated ring-trial validated EN 17294 method based on ion chromatography coupled to conductivity detection (IC-CD) [1]. This method is designed for the determination of formic, lactic, propionic, citric, fumaric, malic and acetic acids and their salts (as total individual acids) in feed additives, premixtures, feed materials, compound feed and water [1].

According to the method, 5 g of sample is mixed with 100 ml of water and the mixture is stirred for 60 min (or sonicated for 30 min). The resulting extract (after adjustment to ambient temperature in case of fumaric acid) is filtered using ash free paper filter or centrifuged at 5000 *g* for 3 min. The filtrate or the supernatant after the dilution is filtered through a membrane filter before the chromatographic analysis. The individual analytes are detected by ion conductivity detection and the quantification is performed using an external standard calibration curve prepared from the standard solutions of the above-mentioned acids [1].

Table 1. The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method [1] for the quantification of *lactic acid* in *premixtures, feedingstuffs* (feed materials, complementary feed and compound feed) and *water*.

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	28055 – 55908	2667 – 90601	993
RSD_r, %	3.5 – 4.1	1.3 – 4.6	0.7
RSD_R, %	11.3 – 12.8	4.9 – 11.2	4.7
Reference	[1]		

RSD_r and RSD_R: relative standard deviations for *repeatability and reproducibility, respectively*.

The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method for the quantification of *lactic acid* in *premixtures*, *feedingstuffs* (feed materials, complementary feed, compound feed) and *water* are presented in Table 1. In addition, a limit of quantification (LOQ) of 200 mg for *lactic acid*/kg *feedingstuffs* is reported [1].

Based on the performance characteristics presented and the scope of the method in terms of matrices, the EURL recommends for official control the ring-trial validated EN 17294 method based on ion chromatography coupled to conductivity detection (IC-CD) for the determination of *lactic acid* and *calcium lactate* (as total *lactic acid*) in the *feed additives*, *premixtures, feedingstuffs* and *water*.

In addition, in the frame of a similar organic acid dossier [5], the EURL has evaluated and recommended for official control for the determination of total *calcium* in the *feed additive* the two ring-trial validated methods, namely (i) EN ISO 6869 based on atomic absorption spectrometry (AAS) [6] and (ii) EN15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) [7]. These recommendations are also valid in the frame of this addendum.

Recommended text for the registry entry (analytical methods) (replacing the previous recommendations)

For the determination of *lactic acid* and *calcium lactate* (as total *lactic acid*) in the *feed additives*, *premixtures*, *feedingstuffs* and *water*:

- Ion chromatography with conductivity detection (IC-CD) – EN 17294

For the determination of total *calcium* in the *feed additive* (*calcium lactate*):

- Atomic absorption spectrometry (AAS) – EN ISO 6869; or
- Inductively coupled plasma-atomic emission spectrometry (ICP-AES) – EN15510

References

[1] EN 17294 Animal feeding stuffs: Methods of sampling and analysis – Determination of organic acids by Ion Chromatography with Conductivity Detection (IC-CD) – Complementary element

[2] EN 17298 Animal feeding stuffs: Methods of sampling and analysis – Determination of benzoic and sorbic acid by High Performance Liquid Chromatography (HPLC)

[3] Commission Regulation (EC) No 378/2005 of 4 March 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, OJ L 059 5.3.2005, p. 8

[4] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisations of feed additives, OJ L 133 22.5.2008, p. 1

[5] EURL evaluation report: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FormateGroup.pdf

[6] ISO 6869:2000 – Animal feeding stuffs – Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc — Method using atomic absorption spectrometry

[7] EN 15510:2017 – Animal feeding stuffs: Methods of sampling and analysis – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-AES

Addendum
- Prepared by María José González de la Huebra
- Reviewed and approved by Zigmas Ezerskis, and Christoph von Holst (EURL-FA), respectively, Geel, 26/05/2021

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.DG.D.5/CvH/ZE/AG/ARES(2012)197882

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-2010-0133 - CRL/100046
Feed additive Name:	Lactic acid (E 270) Calcium lactate (E 327)
Active Substance(s):	Lactic acid Calcium lactate
Rapporteur Laboratory:	European Reference Laboratory for Feed Additives (EURL-FA), IRMM, Geel, Belgium
Report prepared by:	Zigmas Ezerskis (EURL-FA)
Report revised by: Date:	Piotr Robouch (EURL-FA) 21/02/2012
Report approved by: Date:	Christoph von Holst 21/02/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) and 10(2) for *lactic acid (E 270)* and *calcium lactate (E 327)* under the category/functional group 1(a) "technological additives"/"preservatives", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, *lactic acid* is a liquid consisting of a minimum of 72 % of lactic acid and a maximum of 8 % of other organic acids, the rest being water. *Calcium lactate* is a solid consisting of a minimum of 97 % (on dry matter) of calcium lactate and a maximum of 3 % of water.
Authorisation is sought for the use of the two *feed additives* for all animal species and categories. Both *feed additives* are to be used in *premixtures* and *feedingstuffs,* whereas *lactic acid* is also intended to be mixed into *water* for drinking, with no recommended minimum or maximum concentration levels. However typical concentration levels of 30 g/L for *water* or 30 to 50 g/kg *feedingstuffs* are suggested by the Applicant.

For the determination of *lactic acid* in *feed additive* the Applicant proposed the European Pharmacopoeia monographs 0458 and the internationally recognised FAO JECFA monograph for food additives, based on: - the tests for acid and lactates; - acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator. For the determination of *calcium lactate* in *feed additive* the Applicant proposed a set of European Pharmacopoeia monographs for the various forms of calcium lactate (2118 for calcium lactate, anhydrous; 2117 for calcium lactate monohydrate; 0468 for calcium lactate, pentahydrate; 0469 for calcium lactate trihydrate) together with the internationally recognised FAO JECFA monograph for food additives based on: - the tests for calcium and lactates; and - the complexometric titration of calcium with sodium ethylenediaminetetraacetate in aqueous solution. Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia monographs and the FAO JECFA methods for the determination of *lactic acid* and *calcium lactate* in the *feed additives*.

For the quantification of *lactic acid* and *calcium lactate* (as total *lactic acid* content) in *premixtures, feedingstuffs* and *water* the Applicant proposed a single laboratory validated method based on high performance liquid chromatography with UV or refractive index detection (HPLC-UV/RI). This method does not distinguish between *lactic acid* and its salts. The following performance characteristics for the quantification of total *lactate*, expressed as total *lactic acid*, are reported for concentrations ranging from to 1 to 1000 g/kg: - a relative standard deviations for *repeatability* (RSD_r) ranging from 1.8 to 3.6 %; - a *recovery* rate (R_{rec}) ranging from 89 to 107 %; and - a limit of quantification (LOQ) of 0.46 g *lactic acid*/kg *feedingstuffs*. The HPLC-UV/RI method was further ring trial validated by five laboratories,

and a relative standard deviation for *reproducibility* (RSD_R) ranging from 10.7 to 14.7 % was determined for *premixtures* and *feedingstuffs* containing from 7.1 to 53.3 g *lactic acid*/kg.

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-UV/RI method to determine *lactic acid* and *calcium lactate* (expressed as *total lactic acid*) in *premixtures, feedingstuffs* and *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

Lactic acid, *calcium lactate*, technological additives, preservatives, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under article 4(1) and 10(2) for *lactic acid (E 270)* and *calcium lactate (E 327)* under the category/functional group 1(a) "technological additives"/"preservatives" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, *lactic acid* is a liquid consisting of a minimum of 72 % of lactic acid and a maximum of 8 % of other organic acids, the rest being water [2]. *Calcium lactate* is a solid consisting of a minimum of 97 % (on dry matter) of calcium lactate and a maximum of 3 % of water [2].

Authorisation is sought for the use of the two *feed additives* for all animal species and categories [1, 2]. Both *feed additives* are to be used in *premixtures* and *feedingstuffs*, whereas *lactic acid* is also intended to be mixed into *water* for drinking, with no recommended minimum or maximum concentration levels [2]. However typical concentration levels of 30 g/L for *water* or 30 to 50 g/kg *feedingstuffs* are suggested by the Applicant [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 *lactic acid* and *calcium lactate* 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, PAHs 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 and feedingstuffs

For the identification of *lactic acid* in *feed additive* the Applicant proposed the European Pharmacopoeia monographs 0458 for lactic acid [5] based on the tests for acid and lactates. For the quantification of *lactic acid* in *feed additive* the EURL recommends the above mentioned European Pharmacopoeia monographs and the internationally recognised FAO JECFA monograph for food additives [6], based on acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator.

For the identification of *calcium lactate* in *feed additive* the Applicant proposed a set of European Pharmacopoeia monographs for the various forms of calcium lactate (2118 for calcium lactate, anhydrous; 2117 for calcium lactate monohydrate; 0468 for calcium lactate, pentahydrate; 0469 for calcium lactate trihydrate) [7-10], all based on the tests for calcium and lactates. For the quantification assay of *calcium lactate* in *feed additive* the EURL recommends the above mentioned European Pharmacopoeia monographs and the internationally recognised FAO JECFA monograph for food additives [11], based on complexometric titration of calcium with sodium ethylenediaminetetraacetate in aqueous solution.

Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned European Pharmacopoeia monographs and the FAO JECFA methods for the determination of *lactic acid* and *calcium lactate* in the *feed additives*.

For the quantification of *lactic acid* and *calcium lactate* (as total *lactic acid* content) in *premixtures, feedingstuffs* and *water* the Applicant proposed a method based on high performance liquid chromatography with UV or refractive index detection (HPLC-UV/RI) [12]. This method does not distinguish between *lactic acid* and its salts.

The sample is extracted with 0.1 M or 10 M sodium hydroxide solution at a pH higher than 11 or 13 (depending on the expected initial concentration of lactic acid or lactates in the sample) for 30 min. After cooling the alkaline solution is adjusted to pH ranging from 2 to 3.5 with 2 M sulphuric acid. The acidified solution is then centrifuged or filtered and used for the HPLC measurement. After ion-exclusion chromatography, *lactate* is quantified as *lactic acid* by spectrophotometry at 217 nm or by the refractive index, using external calibration.

The following performance characteristics for the quantification of total *lactate*, expressed as total *lactic acid*, were derived from the single-laboratory validation study for concentrations ranging from to 1 to 1000 g/kg [12]:

- a relative standard deviations for *repeatability* (RSD_r) ranging from 1.8 to 3.6 %;
- a *recovery* rate (R_{rec}) ranging from 89 to 107 %; and
- a limit of quantification (LOQ) of 0.46 g *lactic acid*/kg *feedingstuffs*.

The HPLC-UV/RI method was further ring trial validated with five laboratories and a relative standard deviation for *reproducibility* (RSD_R) ranging from 10.7 to 14.7 % was determined for *premixtures* and *feedingstuffs* containing from 7.1 to 53.3 g *lactic acid*/kg [12].

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-UV/RI method to determine *lactic acid* and *calcium lactate* (expressed as *total lactic acid*) in *premixtures, feedingstuffs* and *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 European Pharmacopoeia monographs 0458 and FAO JECFA *lactic acid* monograph No. 1 (2006), Combined Compendium for Food Additive Specifications, for the determination of *lactic acid* in the *feed additive*;
- the European Pharmacopoeia monographs (2118; 2117; 0468 and 0469) and the FAO JECFA *calcium lactate* monograph No. 1 (2006), Combined Compendium for Food Additive Specifications, for the determination of *calcium lactate* in the *feed additive*; and
- the ring-trial validated method based on ion-exclusion HPLC-UV/RI method to determine *lactic acid* and *calcium lactate* (expressed as total *lactic acid*) in *premixtures, feedingstuffs* and *water*.

Recommended text for the register entry (analytical method)

For the determination of *lactic acid* in the *feed additive*:

- European Pharmacopoeia Monographs 0458 and the FAO JECFA *lactic acid* monograph No. 1 (2006)

For the determination of *calcium lactate* in the *feed additive*:

- European Pharmacopoeia Monographs (2118; 2117; 0468 and 0469), and the FAO JECFA *calcium lactate* monograph No. 1 (2006)

For the determination of the *lactic acid* and *calcium lactate* (expressed as total *lactic acid)* in the *premixtures, feedingstuffs and water*:

- ion-exclusion high performance liquid chromatography with UV or refractive index detection (HPLC-UV/RI).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *lactic acid* and *calcium lactate* 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. 0097 (9913)/1831/-2010

[2] *Application, Proposal for Register Entry - Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use

[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] European Pharmacopoeia monographs 0458

[6] FAO JECFA Combined Compendium of Food Additive Specifications, *Lactic acid*, Monograph No. 1 (2006)
http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-247.pdf
(last visited on 21/02/2012)

[7] European Pharmacopoeia monographs 2118

[8] European Pharmacopoeia monographs 2117

[9] European Pharmacopoeia monographs 0468

[10] European Pharmacopoeia monographs 0469

[11] FAO JECFA Combined Compendium of Food Additive Specifications, *Calcium lactate*, Monograph No. 1 (2006)
http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-088.pdf
(last visited on 21/02/2012)

[12] *Technical dossier, Section II - Annex II_2.1.3_1

* Refers to Dossier No. FAD-2010-0133

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European 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)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- 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)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)