
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

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Date: 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<sub>R</sub>) 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*:


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. *Technical dossier, Section II: Identity, characterisation and conditions of use
4. European Pharmacopoeia monographs 0458
   (last visited on 21/02/2012)
6. European Pharmacopoeia monographs 2118
7. European Pharmacopoeia monographs 2117
8. European Pharmacopoeia monographs 0468
9. European Pharmacopoeia monographs 0469
    (last visited on 21/02/2012)
11. *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)
- Schwerpunkt labor 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)