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

Subject: Addendum to the EURL evaluation report

Reference:

FAD-2010-0154+FAD-2010-0187+FAD-2010-0357 (*citric acid, trisodium citrate dehydrate* and *tripotassium citrate monohydrate*) – JRC.DG.D.6/CvH/GB/ag/Ares(2011)991812

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, under the frame of article 5 of Regulation (EC) No 378/2005 [3], the EURL considered appropriate to perform a new evaluation of the methods of analysis for official control of *citric acid* in the *feed additives*, *premixtures, feedingstuffs* and *water*, in the frame of the above-mentioned *feed additive* dossier.

For the determination of *citric acid* (as total *citric acid*) in the *feed additives*, *premixtures, feedingstuffs* and *water* the EURL evaluated the 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 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].

The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method for the quantification of *citric 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 *citric acid*/kg *feedingstuffs* is reported [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 *citric acid* in *premixtures* and *feedingstuffs* (feed materials, complementary feed and compound feed) and *water*.

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	29889–110276	6756-33155	205
RSD_r, %	3.1-10.9	1.3-5.5	1.9
RSD_R, %	7.6-16.0	5.2-17.3	6.4
Reference	[1]		

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

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 *citric acid* (as total *citric acid*) in the *feed additives*, *premixtures* *feedingstuffs* and *water*.

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

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

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

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 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-FAD-CitricGroup.pdf

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Addendum
- Prepared by Stefano Bellorini
- Reviewed and approved by María José González de la Huebra and Christoph von Holst (EURL-FA), respectively, Geel, 21/05/2021

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

JRC.DG.D.6/CvH/GB/ag/ARES(2011)991812

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-2010-0154 - CRL/100226 FAD-2010-0187 - CRL/100138 FAD-2010-0357 - CRL/100160
Product Name:	citric acid (E 330) trisodium citrate dihydrate (E 331) tripotassium citrate monohydrate (E 332)
Active Substance(s):	citric acid trisodium citrate dihydrate tripotassium citrate monohydrate
Rapporteur Laboratory:	European Reference Laboratory for Feed Additives, IRMM, Geel, Belgium
Report prepared by:	Gerhard Buttinger
Report revised by: Date:	Piotr Robouch (EURL-FA) 19/09/2011
Report approved by: Date:	Christoph von Holst 19/09/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *trisodium citrate dihydrate (E 331)*[1] and *tripotassium citrate monohydrate (E 332)*[2] and under articles 4(1) and 10(2) for *citric acid (E330)*[3] under the category of "technological additives" functional group 1a (Preservatives), according to the classification system of Annex I of Regulation (EC) No 1831/2003.

Trisodium citrate dihydrate is a white granular crystals or crystalline powder with a minimum purity of 99 %. *Tripotassium citrate monohydrate* is a colourless, white powder or granulate with a minimum purity of 99 %. *Citric acid* (used in either anhydrous or monohydrate form) is a colourless crystals or white crystalline powder with a minimum purity of 99.5 % based on the anhydrous form.

Authorisation is sought for the use of the *trisodium citrate dihydrate* and *tripotassium citrate monohydrate* for dogs and cats, while authorisation is sought for the use of the *citric acid* for all categories and species.

Trisodium citrate dihydrate and *tripotassium citrate monohydrate* are intended to be mixed into *premixture*s and *feedingstuff,* whereas *citric acid* is also intended to be mixed into *water.* However, the Applicants suggested no minimum or maximum levels as set in the previous regulations.

For the quantification of *trisodium citrate dihydrate* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method (Monograph 0412), based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, as suggested by the Applicant.

For the quantification of *tripotassium citrate* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method(Monograph 0400), based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, as suggested by the Applicant.

For the quantification of *citric acid* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method (Monograph 0455 & 0456), based on acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator, as suggested by the Applicant.

[1] FAD-2010-0154
[2] FAD-2010-0187
[3] FAD-2010-0357

For the quantification of *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (as *total citric acid* content) in *premixtures, feedingstuffs* and *water* Applicant [3] proposed a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV). This method does not distinguish between *citric acid* and its salts. This HPLC-UV/RI method was ring trial validated with four laboratories and a relative standard deviation for *reproducibility* (RSD_R) ranging from 14.5 % to 21.1 % was reported for *premixtures* and *feedingstuffs* containing from 12 to 66 g *citric acid*/kg together with a limit of quantification of 0.43 g/kg *feedingstuff*.

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-UV method to determine *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (expressed as *total citric 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
citric acid, trisodium citrate dihydrate, tripotassium citrate monohydrate, all categories and species, technological, preservatives, cats and dogs

1. BACKGROUND

In the current application authorisation is sought under article 10(2) (re-evalutation of *feed additives* already authorized under provisions of Council Directive 70/524/EEC) for *trisodium citrate dihydrate (E 331)*[1] and *tripotassium citrate monohydrate (E 332)*[2] and under articles 4(1) (new use in water) and 10(2) for *citric acid (E330)*[3] under the category of "technological additives" functional group 1a (Preservatives) [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant[1], *trisodium citrate dihydrate* is a white granular crystals or crystalline powder with a minimum purity of 99 % [2].

According to the Applicant[2], *tripotassium citratemonohydrate* is a colourless, white powder or granulate with a minimum purity of 99 % [3].

According to the Applicant[3], *citric acid* is (used either in the anhydrous or monohydrate form) a colourless crystals or white crystalline powder with a minimum purity of 99.5 % based on the anhydrous form [4].

Specifically, authorisation is sought for the use of *trisodium citrate dihydrate* and *tripotassium citrate monohydrate* for dogs and cats, while authorisation is sought for the use of *citric acid* for all categories and species [1].

The *trisodium citrate dihydrate* and *tripotassium citrate monohydrate* is intended to be mixed into *premixture*s and *feedingstuff,* whereas *citric acid* is also intended to be mixed into *water*. However, the Applicants suggested no minimum or maximum levels [2, 3, 4] as set in the previous regulations [5].

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 *'citric acid', 'trisodium citrate dihydrate'*

[1] FAD-2010-0154
[2] FAD-2010-0187
[3] FAD-2010-0357

and *'tripotassium citrate monohydrate'*, 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

For the identification of *trisodium citrate dihydrate* in *feed additive* the EURL recommends the methods described in the European Pharmacopoeia monograph 0412 [6], based on selective precipitation reactions of sodium and a selective colour reaction of citrate, as suggested by the Applicant.

For the identification of *tripotassium citrate monohydrate* in *feed additive* the EURL recommends the methods described in the European Pharmacopoeia monograph 0400 [7], based on selective precipitation reactions of potassium and a selective colour reaction of citrate, as suggested by the Applicant.

For the identification of *citric acid* in *feed additive* the EURL recommends the methods described in the European Pharmacopoeia monograph 0455 and European Pharmacopoeia monograph 0456 [8, 9], dependent on the actual form, based among others on infrared absorption spectrophotometry, as suggested by the Applicant.

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 [10].

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

For the quantification of *trisodium citrate dihydrate* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method [6], based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, as suggested by the Applicant.

For the quantification of *tripotassium citrate* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method [7], based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, as suggested by the Applicant.

For the quantification of *citric acid* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method [8, 9], based on acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator, as suggested by the Applicant.

For the quantification of *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (as *total citric acid* content) in *premixtures, feedingstuffs* and *water* Applicant [3] proposed a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV) [11]. This method does not distinguish between *citric acid* and its salts.

The sample is extracted with 0.005 M sulphuric acid at a pH ranging from 2 to 3.5. The solution is then centrifuged or filtered and used for the HPLC measurement. After ion-exclusion chromatography, *citrate* is quantified as citric acid by spectrophotometry at 217 nm or by the refractive index, using external calibration.

The following performance characteristics for the quantification of total citrate, expressed as *total citric acid*, were derived from the single-laboratory validation study [12]:

- a relative standard deviation for *repeatability* ranging from 3 % to 19 % (for concentrations down to 0.43 g/kg);
- a limit of quantification (LOQ) of 0.43 g *citric acid*/kg *feedingstuffs;* and
- a *recovery* rate (R_{rec}) ranging from 87 to 102 % when UV detection is used; or ranging from 50 to 104% when RI detection is used. The Applicant explains that phosphoric acid can interfere with the detection of citric acid with RI.

Furthermore, the HPLC-UV method was ring trial validated with four laboratories and a relative standard deviation for *reproducibility* (RSD_R) ranging from 14.5 % to 21.1 % was determined for *premixtures* and *feedingstuffs* containing 12 to 66 g *citric acid*/kg, respectively [12].

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-UV method to determine *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (expressed as *total citric 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 Monograph 0412 for the identification and the quantification of *trisodium citrate dihydrate* in *feed additive;*
- the European Pharmacopoeia Monograph 0400 for the identification and the quantification of *tripotassium citrate monohydrate* in *feed additive;*
- the European Pharmacopoeia Monograph 0455 and 0456 for the identification and the quantification of *citric acid* (monohydrate and anhydrous) in *feed additive;*
- the ring trial validated method based on ion-exclusion HPLC-UV method to quantify *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (expressed as *total citric acid*) in *premixtures, feedingstuffs* and *water.*

Recommended text for the register entry (analytical method)

For the quantification of the *trisodium citrate dihydrate* in the *feed additive*:

- titration with perchloric acid (European Pharmacopoeia Monograph 0412)

For the quantification of the *tripotassium citrate monohydrate* in the *feed additive*:

- titration with perchloric acid (European Pharmacopoeia Monograph 0400)

For the quantification of the *citric acid* in the *feed additive*:

- titration with sodium hydroxide (Eur. Pharm. Monograph 0455 and 0456)

For the quantification of the *citric acid, trisodium citrate dihydrate* and *tripotassium citrate monohydrate* (expressed as *total citric acid)* in the *premixtures, feedingstuffs and water*:

- ion exclusion High Performance Liquid Chromatography with UV detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *citric acid, trisodium citrate dihydrate and tripotassium citrate monohydrate* 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. 1831/SANCO D2/BVP/eu (2011)500211

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

[3] +Application, Proposal for Register Entry – Annex A

[4] ±Application, Proposal for Register Entry – Annex A

[5] Official Journal of the European Union, C 50 of 25.2.2004, p. 1, *List of the authorised additives in feedingstuffs (1) published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs*

[6] European Pharmacopoeia Monograph 0412

[7] European Pharmacopoeia Monograph 0400

[8] European Pharmacopoeia Monograph 0455

[9] European Pharmacopoeia Monograph 0456

[10] 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

[11] ±Technical dossier, Section II

[12] ±Technical dossier, Section II – Annex-II-4

* Refers to Dossier No. FAD-2010-0154

+ Refers to Dossier No. FAD-2010-0187

± Refers to Dossier No. FAD-2010-0357

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

The following National Reference Laboratories contributed to this report:

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