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

Subject: Addendum to the EURL evaluation report

Reference:
FAD-2010-0161 (*acetic acid*, *calcium acetate* and *sodium diacetate*) –
JRC.DG.D.6/CvH/GB/ag/Ares(2011)459877

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 *acetic acid*, *calcium acetate* and *sodium diacetate* 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 the metals (*calcium* and *sodium*).

For the determination of *acetic acid*, *calcium acetate* and *sodium diacetate* (as total *acetic 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 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 *acetic acid* in *premixtures* and *feedingstuffs* (feed materials, complementary feed and compound feed) and *water*.

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	33726 – 120575	829 – 4904	316
RSD_r, %	1.9 – 7.4	0.8 – 5.9	1.6
RSD_R, %	5.9 – 12.8	2.1 - 18.0	5.5
Reference	[1]		

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

Note: Limitation occurs during simultaneous determination of high concentration of lactic acid and low concentration of acetic acid. If the ratio of concentration of lactic acid to acetic acid exceeds factor 20, the determination of acetic acid is not guaranteed [1].

The performance characteristics obtained in the frame of the ring-trial validation studies of the EN 17294 method for the quantification of *acetic 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 *acetic 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 *acetic acid, calcium acetate* and *sodium diacetate* (as total *acetic 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 *sodium* and *calcium* in the *feed additives* 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 *acetic acid, sodium diacetate* and *calcium acetate* (as total *acetic acid*) in the *feed additives*, *premixtures*, *feedingstuffs* and *water*:

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

For the determination of total *sodium* and *calcium* in the *feed additives* (*sodium diacetate* and *calcium acetate*):

- 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.6/CvH/GB/ag/ARES(2011)459877

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-2010-0161 EURL/100060
Product Name:	-
Feed additive/Active Substance(s):	Acetic Acid E260 Sodium Diacetate E262(ii) Calcium Acetate E263
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Gerhard Buttinger (EURL-FA)
Report checked by: Date:	Piotr Robouch (EURL-FA) 28/04/2011
Report approved by: Date:	Christoph von Holst 28/04/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1)*and 10(2) for three *feed additives*, namely *acetic acid**, *sodium diacetate* and *calcium acetate* under the category/functional group 1(a) 'technological additives'/'preservatives', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additives* for all animal species and categories. According to the Applicant: - *acetic acid* is a clear colourless liquid with a minimum purity of 99.8 %; - *sodium diacetate* is a white powder or crystals with a minimum content of 58 % acetic acid and 39 % sodium acetate; - *calcium acetate* is a white agglomerate or powder with minimum purity of 98.7 % (dried material) and a maximum water content of 6 %.

The *feed additives* are intended to be incorporated in *feedingstuffs* (including complete *feedingstuffs* or complementary *feedingstuffs*) through *premixtures* or directly in *water*. However, the Applicant did not specify any maximum or minimum concentration of the active substances in *feedingstuffs* or *water*, similarly to what was set in previous regulation.

For the determination of *acetic acid* in the *feed additive*, the Applicant proposes a selection of British Pharmacopoeia and ASTM methods. Nevertheless the EURL recommends the internationally recognised European Pharmacopoeia method (Ph.Eur. 6th Edition, monograph 0590), for official control to determine *acetic acid* in the *feed additive*.

For the determination of *sodium diacetate* in the *feed additive*, the Applicant proposes a selection of single laboratory validated methods. *Sodium diacetate* is a molecular compound of *sodium acetate* and *acetic acid*, and is therefore measured by these composite fractions. Therefore the EURL recommends the internationally recognised European Pharmacopoeia methods for determination of *acetic acid* and *sodium acetate* (Ph.Eur. 6th Edition, monograph 0590 and Ph.Eur. 6th Edition, monograph 0411, respectively), for official control to determine *sodium diacetate* in the *feed additive*.

For the determination of *calcium acetate* in the *feed additive*, the Applicant proposes a selection of single laboratory validated methods. Nevertheless the EURL recommends the internationally recognised European Pharmacopoeia method (Ph.Eur. 6th Edition, monograph 2128), for official control to determine *calcium acetate* in the *feed additive*.

For the determination of *acetic acid, sodium diacetate* and *calcium acetate* in *premixtures, feedingstuffs* and *water*, the Applicant proposes a ring-trial validated method based on ion exclusion HPLC with photometric and/or refractive index detection (HPLC-UV/RI). The method does not distinguish between the three *feed additives* as it only determines the acetate content. Based on the acceptable performance characteristics presented, the EURL

recommends for official control the ring trial validated ion-exclusion HPLC-UV/RI method to determine *acetic acid, sodium diacetate and calcium acetate* as *total acetic acid* content in *premixtures*, *feedingstuffs* and *water*, within the range of 0.4 g/kg to 1000 g/kg covered by the experimental data.

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
acetic acid, sodium diacetate, calcium acetate, technological additive, preservatives, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water*) and 10(2) (re-evaluation of additives already authorised under Council Directive 70/524/EEC) for *acetic acid**, *sodium diacetate* and *calcium acetate* 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. Authorisation is sought for the use of the *feed additives* for all animal species and categories [1].

According to the Applicant: - *acetic acid* is a clear colourless liquid with pungent characteristic odour, and a minimum purity of 99.8 % [3]; - *sodium diacetate* is a white powder or crystals, with a minimum content of 58 % acetic acid and 39 % sodium acetate [3]; - *calcium acetate* is a white agglomerate or powder, with a minimum purity of 98.7 % (dried material) and a maximum water content of 6 % [3].

The *feed additive* is intended to be incorporated in *feedingstuffs* (including complete *feedingstuffs* or complementary *feedingstuffs*) through *premixtures* or directly in *water*. However, the Applicant did not specify any maximum or minimum concentration of active substance in *feedingstuffs* or *water* [2], similarly to what was set in previous regulation [4].

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 *acetic acid, sodium diacetate* and *calcium acetate*, 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 *acetic acid* the EURL recommends the internationally recognised European Pharmacopoeia method [6], based on (i) the acidity of acetic acid and (ii) the selective colour reaction with lanthanum nitrate.

Sodium diacetate is a molecular compound of *sodium acetate* and *acetic acid.* Therefore, the EURL recommends for the identification of *sodium diacetate* the European Pharmacopoeia methods: - the above mentioned method for *acetic acid* [6]; and – the selective precipitation reaction with potassium pyroantimonate [7] for *sodium acetate*.

For the identification of *calcium acetate* the EURL recommends the European Pharmacopoeia method [8], based on (i) the acetate test with lanthanum nitrate, and – (ii) the selective precipitation reaction with potassium ferrocyanide and ammonium chloride.

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 [5].

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

For the determination of *acetic acid* in the *feed additive*, the Applicant proposes a selection of British Pharmacopoeia and ASTM methods [3]. Nevertheless the EURL recommends for official control the internationally recognised European Pharmacopoeia method (Ph.Eur. 6th Edition, monograph 0590, section assay) [6], based on acid/base titration with 1 M sodium hydroxide and phenolphthalein as indicator, to determine *acetic acid* in the *feed additive*.

For the determination of *sodium diacetate* in the *feed additive*, the Applicant proposes a selection of single laboratory validated methods [3]. The EURL recommends instead for official control the European Pharmacopoeia titration methods for the determination of *acetic acid* (mentioned above [6]) and *sodium acetate* [7], based on acid/base titration with 0.1 M perchloric acid and naphtholbenzein as indicator, to determine *sodium diacetate* in the *feed additive.*

For the determination of *calcium acetate* in the *feed additive*, the Applicant proposes a selection of single laboratory validated methods [3]. The EURL recommends instead for official control the European Pharmacopoeia method [8], based on complexometric titration of the calcium ion with 0.1 M sodium edetate (ethylenediaminetetraacetate) and methylthymol blue as indicator, to determine *calcium acetate* in the *feed additive*.

For the determination of *acetic acid, sodium diacetate* and *calcium acetate* in *premixtures, feedingstuffs* and *water*, the Applicant proposes a ring-trial validated method based on ion exclusion HPLC with photometric and/or refractive index detection (HPLC-UV/RI) [9]. This method does not distinguish between the three *feed additives*, as it only determines the *acetate* content.

The sample is extracted with 0.005 M sulphuric acid at a pH between 2 and 3.5. Thereafter the solution is either centrifuged or filtered and directly used for the HPLC measurement. The *acetate* is quantified as acetic acid using external calibration after ion-exclusion chromatography either photometrically at 217 nm and/or via the refractive index. The *acetic acid* content can be expressed as the corresponding salts as well.

The following performance characteristics were derived from the single-laboratory validation study [9]:

- a relative standard deviation for *repeatability* of 3.5 % and 21 % for concentrations ranging from 1 g/kg to 1000 g/kg and from 0.4 g/kg to 1 g/kg, respectively;
- a *recovery* rate (R_{rec}) ranging from 80 % to 110 %; and

- a limit of quantification (LOQ) of 0.4 g *acetic acid*/kg *feedingstuffs*.

The method was further verified in the frame of an inter-laboratory comparison with four laboratories and a relative standard deviation for *reproducibility* (RSD_R) ranging from 11 % to 17 % was determined for *premixtures* and *feedingstuffs* containing 5 and 50 g *acetic acid*/kg, respectively [9].

Based on the acceptable performance characteristics presented, the EURL recommends for official control the ring trial validated ion-exclusion HPLC-UV/RI method to determine *acetic acid, sodium diacetate and calcium acetate* in *premixtures*, *feedingstuffs* and *water,* within the concentration range covered by the experimental data.

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 method (Ph. Eur. 6th edition, monograph 0590) using titration with sodium hydroxide for the determination of *acetic acid* in *feed additive;*
- the European Pharmacopoeia methods (Ph. Eur. 6th edition, monograph 0590) using titration with sodium hydroxide and (Ph. Eur. 6th edition, monograph 0411) using titration with perchloric acid for the determination of *sodium diacetate* in *feed additive*;
- the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 2128) using titration with sodium edetate for the determination of *calcium acetate* in *feed additive;*
- the ring-trial validated method based on ion exclusion chromatography with UV and/or refractive index detection (HPLC-UV/RI) for the determination of *acetic acid, sodium diacetate* and *calcium acetate*, as <u>*total acetic acid*</u> content in *premixtures, feedingstuffs* and *water.*

Recommended text for the register entry (analytical method)

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

- Titration with sodium hydroxide (European Pharmacopoeia, monograph 0590)

For the determination of *sodium diacetate* in the *feed additive*:

- Titration with sodium hydroxide and titration with perchloric acid (European Pharmacopoeia, monograph 0590 and monograph 0411)

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

- Titration with sodium edetate (European Pharmacopoeia, monograph 2128)

For the determination of *acetic acid, sodium acetate* and *calcium acetate* as *total acetic acid* content in the *premixtures*, *feedingstuffs* and *water*:

- ion-exclusion chromatography coupled either with UV detection and/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 *acetic acid, sodium diacetate* and *calcium acetate* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCE

[1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/0099/2010
[2] *Application, Proposal for Register Entry – Annex A
[3] *Technical dossier, Section II
[4] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs
[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] European Pharmacopoeia, Monograph 0590
[7] European Pharmacopoeia, Monograph 0411
[8] European Pharmacopoeia, Monograph 2128
[9] *Technical dossier, Section II – Annex II-5

* Refers to Dossier No. FAD-2010-0161

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

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer (DE)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Kmetijski inštitut Slovenije, Ljubljana (SI)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)