



**Subject:** Addendum to the EURL evaluation report

**Reference:**

FAD-2010-0143 (*malic acid* and *sodium and calcium malate*) –  
JRC.DG.D.6/CvH/GB/ag/Ares(2011)979440

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 *malic acid* and *sodium and calcium malate* 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 (*sodium* and *calcium*).

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

|                      | Premixtures   | Feedingstuffs | Water |
|----------------------|---------------|---------------|-------|
| Mass fraction, mg/kg | 17345 – 21167 | 1489 – 68618  | 295   |
| RSD <sub>r</sub> , % | 5.2 – 5.4     | 0.7 – 3.0     | 0.9   |
| RSD <sub>R</sub> , % | 10.6 – 15.3   | 4.2 – 19.9    | 8.6   |
| Reference            | [1]           |               |       |

RSD<sub>r</sub> and RSD<sub>R</sub>: 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 *malic 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 *malic 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 *malic acid* and *sodium and calcium malate* (as total *malic 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 *malic acid* and *sodium and calcium malate* (as total *malic 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 additive (sodium and calcium malate)*:

- 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

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## Addendum

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  - Reviewed and approved by Zigmantas Ezerskis and Christoph von Holst (EURL-FA), respectively, Geel, 26/05/2021
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**JRC.DG.D.6/CvH/GB/ag/ARES(2011)979440**

**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-2010-0143  
CRL/100044

Product Name: -

Active Substance(s): malic acid  
sodium calcium malate

Rapporteur Laboratory: European Reference Laboratory for Feed  
Additives, IRMM, Geel, Belgium

Report prepared by: Gerhard Buttinger

Report revised by: Piotr Robouch (EURL-FA)  
Date: 14/09/2011

Report approved by: Christoph von Holst  
Date: 14/09/2011

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## EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *malic acid* under the category of "technological additives" functional group 1a (preservatives) and 1j (acidity regulators), and under article 4(1) for *sodium calcium malate* under the category of "technological additives" functional group 1j (acidity regulators) according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicant, *malic acid* is a white crystalline or granular solid. The *feed additive* has a minimum purity of 99.5 %.

According to the Applicant, *sodium calcium malate* is a mixture of sodium and calcium salts of malic acid and is a white crystalline or granular solid. The *feed additive* has a minimum purity of 60 % expressed as malic acid content and a maximum sodium and calcium content of 4 % and 20 %, respectively.

Authorisation is sought for the use of *malic acid* as *preservative* for all categories and species and as *acidity regulator* for cats and dogs, while authorisation is sought for the use of *sodium calcium malate* for all categories and species.

Both *feed additives* are intended to be mixed into *premixtures* and *feedingstuffs*. The Applicant suggested no minimum or maximum levels as in the previous legislation.

For the quantification of *malic acid* in the *feed additive*, the EURL recommends for official control the method described in the European Pharmacopoeia Monograph 2080, based on potentiometrical, acid/base titration with 1 M sodium hydroxide.

For the quantification of *malic acid* in *premixtures* and *feedingstuff* the Applicant proposed a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV); this method does not distinguish between *malic acid* and its salts.

This HPLC- RI/UV method was further ring trial validated by five laboratories and a relative standard deviation for *reproducibility* (RSD<sub>R</sub>) ranging from 4.3 % to 7.5 % was determined for *premixtures* and *feedingstuffs* containing 10 to 72 g *malic acid*/kg, respectively.

The determination of sodium, calcium and malate is necessary for the characterisation of *sodium calcium malate* in *feed additive*.

For the quantification of *total sodium* and *total calcium* in the product, the EURL recommends for official control the internationally agreed EN ISO 6869:2000 method, based on atomic absorption spectrometry.

For the quantification of *total malate* in *feed additive*, *premixtures* and *feedingstuffs* the Applicant proposed the HPLC-UV/RI method mentioned above.

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated ion-exchange HPLC-UV method to determine *malic acid* (expressed as *total malic acid*) in *premixtures* and *feedingstuffs* and *sodium calcium malate* (expressed as *total malic acid*) in *feed additive*, *premixtures* and *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

malic acid, sodium calcium malate, all categories and species, 1a (Preservatives), 1j (acidity regulators), cats and dogs

## 1. BACKGROUND

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

According to the Applicant, *malic acid* is a white crystalline or granular solid. The *feed additive* has a minimum purity of 99.5 % [2]. A maximum impurity content is specified for *fumaric acid* and *maleic acid* of 1 % and 0.05 %, respectively.

According to the Applicant, *sodium calcium malate* is a mixture of sodium and calcium salts of malic acid and is a white crystalline or granular solid. The *feed additive* has a minimum purity of 60 % expressed as malic acid content and a maximum sodium and calcium content of 4 % and 20 %, respectively [2]. This corresponds to a mixture of 15 % sodium malate and 85 % calcium malate. A maximum impurity content is specified for *fumaric acid* and *maleic acid* of 1 % and 0.05 %, respectively.

Specifically, authorisation is sought for the use of *malic acid* as *preservative* for all categories and species and as *acidity regulator* for cats and dogs [2], while authorisation is sought for the use of *sodium calcium malate* for all categories and species [2].

The *feed additives* are intended to be mixed into *premixtures* and *feedingstuffs*. However, the Applicant suggested no minimum or maximum levels [2], as in previous legislation [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 "*malic acid*" and "*sodium calcium malate*", 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 *malic acid* the EURL recommends the internationally recognised European pharmacopoeia monograph methods [4], based on melting point determination and infrared absorption spectrometry.

### ***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, and PAHs) are available from the respective European Union Reference Laboratories [5].

For the quantification of *fumaric* and *maleic acid* impurities in *malic acid* the EURL recommends the internationally recognised European pharmacopoeia monograph method [4], based on high performance liquid chromatography with UV detection (HPLC-UV).

For the quantification of *fumaric* and *maleic acid* impurities in *sodium calcium malate* the EURL recommends the internationally recognised JECFA monograph method [6], based on high performance liquid chromatography with UV detection (HPLC-UV).

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

For the quantification of *malic acid* in the *feed additive*, the EURL recommends for official control the European Pharmacopoeia Monograph method [4], based on potentiometrical, acid/base titration with 1 M sodium hydroxide, to determine *malic acid* in the *feed additive*.

For the quantification of *malic acid* in *premixtures* and *feedingstuff* the Applicant proposed a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV) [7]. This method does not distinguish between *malic 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, *malate* is quantified as malic acid by spectrophotometry at 217 nm or by the refractive index, using external calibration.

The following performance characteristics for the quantification of *total malate*, expressed as *total malic acid*, were derived from the single-laboratory validation study [8]:

- a relative standard deviation for *repeatability* ( $RSD_r$ ) ranging from 3 % to 14%, for concentrations down to 0.1 g/kg;
- a limit of quantification (LOQ) of 0.1 g *malic acid*/kg *feedingstuffs*; and
- a *recovery* rate ( $R_{rec}$ ) ranging from 101 to 110% when UV detection is used; or ranging from 81 to 124% when RI detection is used. The Applicant did not explain whether the large scatter observed during the experiments with RI detection was due to potential interferences from the matrix, which was not evidenced using the UV detection.

Furthermore, the HPLC-UV/RI method was ring trial validated with five laboratories and a relative standard deviation for *reproducibility* ( $RSD_R$ ) ranging from 4.3 % to 7.5 % was determined for *premixtures* and *feedingstuffs* containing 10 to 72 g *malic acid*/kg, respectively [8].

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 *malic acid* (expressed as *total malic acid*) in *premixtures* and *feedingstuffs*.



For the determination of *sodium calcium malate* in *feed additive* the determination of sodium, calcium and malate is necessary.

For the quantification of *total sodium* and *total calcium* in *sodium calcium malate*, the EURL recommends for official control the internationally agreed EN ISO 6869:2000 method [9], based on atomic absorption spectrometry.

For the determination of *total malate* in *sodium calcium malate, premixtures* and *feedingstuffs* the Applicant proposed the HPLC-RI/UV method mentioned above [7]. This method does not distinguish between *malic acid* and its salts.

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 *sodium calcium malate* (expressed as *total malic acid*) in *feed additive, premixtures* and *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:

- the European Pharmacopoeia Monograph 2080 for the identification and the quantification of *malic acid* in *feed additive*;
- the ring trial validated method based on ion-exclusion HPLC-UV method to quantify *malic acid* (expressed as *total malic acid*) in *premixtures* and *feedingstuffs*;
- the EN ISO 6869:2000 method based on atomic absorption spectrometry to quantify *total sodium* and *total calcium* in *sodium calcium malate*;
- the ring trial validated on ion-exclusion HPLC-UV method to quantify *sodium calcium malate* (expressed as *total malic acid*) in *feed additive, premixtures* and *feedingstuffs*.

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***Recommended text for the register entry (analytical method)***

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

- titration with sodium hydroxide (European Pharmacopoeia Monograph 2080)

For the quantification of the *malic acid* (expressed as *total malic acid*) in the *premixtures and feedingstuffs*:

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

For the quantification of the *sodium* and *calcium* in *sodium calcium malate*:

- atomic absorption spectrometry, AAS (EN ISO 6869) – for the determination of total sodium and total calcium.

For the quantification of the *sodium calcium malate* (expressed as *total malic acid*) in the *feed additive, premixtures and feedingstuffs*:

- 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 *malic acid* and *sodium calcium malate* 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. 1831/0093-2010
- [2] \*Application, Proposal for Register Entry – Annex A
- [3] Council Directive 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs
- [4] European Pharmacopoeia Monograph 2080
- [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] FAO JECFA Monographs 1, Volume 4, 'Combined compendium of food additive specification', p. 82
- [7] \*Technical dossier, Section II
- [8] \*Technical dossier, Section II – Annex-II-4 Methods of analysis
- [9] EN 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

\* Refers to Dossier No. FAD-2010-143

## 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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- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena, DE
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby, DK
- Państwowy Instytut Weterynaryjny, Puławy, PL
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim, DE
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha, CZ
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin, PL