



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

**References:**

FAD-2009-0027 (formic acid) – JRC.DDG.D.6/GS/PRO/mdS/Ares(2010)273538

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 *formic acid* in the *feed additive, premixtures, feedingstuffs* and *water* in the frame of the above-mentioned *feed additive* dossier. This is in agreement with the Annex II of the Regulation (EC) No 429/2008 [4], which states that the available analytical methods complying with the highest requirements have to be recommended for official control.

For the determination of *formic acid* in the *feed additive, 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 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 total *formic acid* in *premixtures, feedingstuffs* (feed material, complimentary feed and compound feed) and *water* are presented in Table 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 total *formic acid* in *premixtures*, *feedingstuffs* (feed material, complimentary feed and compound feed) and *water*.

	Premixtures	Feedingstuffs	Water
Mass fraction, mg/kg	39935 – 269745	5669 – 26309	999
RSD <sub>r</sub> , %	3.6 – 4.6	0.9 – 5.3	0.6
RSD <sub>R</sub> , %	8.5 – 9.5	4.5 – 21.2	5.9
Reference	[1]		

RSD<sub>r</sub> and RSD<sub>R</sub>: relative standard deviations for *repeatability* and *reproducibility*, respectively.

In addition, a limit of quantification (LOQ) of 200 mg for *formic acid* /kg *feedingstuffs* is reported [1].

Based on the performance characteristics available 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 total *formic acid* in the *feed additive*, *premixtures*, *feedingstuffs* and *water*.

### **Recommended text for the registry entry (analytical method) (replacing the previous recommendations)**

For the determination of total *formic acid* in the *feed additive*, *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

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Addendum

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- Reviewed and approved by María José González de la Huebra and Christoph von Holst (EURL-FA), respectively, Geel, 27/05/2021

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**JRC.DDG.D.6/GS/PRO/mds/ARES(2010)273538**

**CRL Evaluation Report on the Analytical Methods  
submitted in connection with the Application for the  
Authorisation of a Feed Additive  
according to Regulation (EC) No 1831/2003**

Dossier related to: FAD-2009-0027  
CRL/090012

Name of Additive: -

Active Substance(s): Formic acid

Rapporteur Laboratory: Central Institute for Supervising and  
Testing in Agriculture (CISTA)

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Date: 21/05/2010

## EXECUTIVE SUMMARY

*Formic acid* is a *feed additive* for which authorisation is sought under the category "technological additive", functional group 1(a) "preservatives" and 1(k) "silage additive", according to Annex I of Regulation (EC) No 1831/2003. In the current application submitted according to Article 4(1) (new use in water) and Article 10(2) of Regulation (EC) No 1831/2003, authorisation for all animal species is requested.

The additive is intended to be marketed as a liquid containing 84.5% *formic acid* and 15.5% water, and to be incorporated into *silages*, complete *feedingstuffs* or *water* (not in *premixtures*) at a maximum *formic acid* concentration of 10, 20 or 5 g/kg, respectively. However, no minimum content was proposed by the Applicant.

For the determination of *formic acid* in *feedingstuffs*, the Applicant suggested a single-laboratory validated method based on ion chromatography with electrical conductivity detection (IC-ECD). The following performance characteristics were reported: - a limit of detection (LOD) and quantification (LOQ) of 100 and 500 mg/kg *feedingstuffs*, respectively; - a recovery rate close to 100%; and - a relative standard deviation for *repeatability* ( $RSD_r$ ) of *ca.* 3.5%. The validation experiments were performed with a set of different feed samples covering a formate content ranging from 3.6 to 10 g/kg. These samples were also analysed by a second independent expert laboratory and all the results were in agreement. Furthermore, the validation report included summary information related to an inter-laboratory comparison organised by VDLUFA in 2006 for the determination of organic acids in *feedingstuffs*, including *formic acid*, to which six laboratories participated. The following performance characteristics were reported, for sample *formic acid* concentrations ranging from 7.2 to 506 g/kg *feedingstuffs*: -  $RSD_r$  ranging from 4 to 10 %; and - a *reproducibility* relative standard deviation ( $RSD_R$ ) ranging from 13 to 22 %. Based on these acceptable performance characteristics, the CRL recommends for official control purposes the method submitted by the Applicant for the determination of *formic acid* in *feedingstuffs* in the frame of authorisation.

Furthermore, the CRL considers that this method could apply to the determination of *formic acid* in the *feed additive* and *water*, in the frame of this authorisation.

The unambiguous determination of the content of exogenous *formic acid* added to *silages* is not achievable by analysis. The Applicant did not provide any experimental data nor suggested any methods of analysis. Therefore the CRL cannot evaluate nor recommend any method for official control to determine *formic acid* in *silages*.

Based on the several considerations presented, the CRL recommends for official control - in the frame of this authorisation - the single-laboratory validated (and further verified) method submitted by the Applicant for the determination of *formic acid* in *feed additive*, *feedingstuffs* and *water* (not in *silages*).

Further testing or validation is not considered necessary.

## KEYWORDS

*Formic acid*, technological additive, preservative, silage additive, all animal species

### 1. BACKGROUND

*Formic acid* is a *feed additive* for which authorisation is sought under the category "technological additive", functional group 1(a) "preservatives" and 1(k) "silage additive", according to Annex I of Regulation (EC) No 1831/2003 [1].

In the current application submitted according to Article 4(1) (new use in water) and Article 10(2) (re-evaluation of additives already authorised under Directive 70/524/EC) of Regulation (EC) No 1831/2003, authorisation for all species is requested [2].

The additive is intended to be marketed as a liquid containing 84.5% *formic acid* and 15.5% water, and to be incorporated into *silages*, complete *feedingstuffs* or *water* (not in *premixtures*) at a maximum *formic acid* concentration of 10, 20 or 5 g/kg, respectively [2]. However, no minimum content was proposed by the Applicant.

### 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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application or for each group of application. For this particular dossier, the methods of analysis submitted in connection with *Formic Acid*, were evaluated for their suitability for official controls.

### 3. EVALUATION

#### ***Identification/Characterisation of the feed additive***

##### *Quantitative and qualitative composition of impurities in the additive*

Several impurities generated during the production process are identified by the Applicant, such as methanol and methyl formate, or sulphuric acid and sodium formate [3]. The Applicant suggested for the quantification of impurities the following methods: gas chromatography (GC), ion chromatography method equipped with electrical conductivity detection (IC/ECD) and Atomic Emission Spectrometry (AES) [4]. The CRL considers these methods fit for the intended purpose.

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury and dioxins) are available from the respective Community Reference Laboratories [5].

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

For the determination of *formic acid* in *feedingstuffs*, the Applicant suggested a single-laboratory validated method based on ion chromatography with electrical conductivity detection (IC-ECD) [6]. Approximately 1g of sample is extracted with 80mL of water for 30 minutes and then filled up to 100 mL. After filtration through paper and membrane filters, the solution is injected into the ion chromatograph. External standard calibration is used for the quantification of the formate content. The measured formate content allows the calculation of the formic acid one. The following performance characteristics were reported: - a limit of detection (LOD) and quantification (LOQ) of 100 and 500 mg/kg *feedingstuffs*, respectively; - a recovery rate close to 100%; and - a *repeatability* relative standard deviation (RSD<sub>r</sub>) of ca. 3.5%. The validation experiments were performed with a set of different feed samples covering a formate content ranging from 3.6 to 10 g/kg. These samples were also analysed by a second independent expert laboratory and all the results were in agreement. Furthermore, the validation report included summary information related to an inter-laboratory comparison organised by VDLUFA in 2006 ([6], Annex B) for the determination of organic acids in *feedingstuffs*, including *formic acid*, to which six laboratories participated. The following performance characteristics were reported, for sample *formic acid* concentrations ranging from 7.2 to 506 g/kg *feedingstuffs* [6]: - RSD<sub>r</sub> ranging from 4 to 10 %; and - a *reproducibility*

relative standard deviation ( $RSD_R$ ) ranging from 13 to 22 %. Based on these acceptable performance characteristics, the CRL recommends for official control the method submitted by the Applicant for the determination of *formic acid* in *feedingstuffs* in the frame of authorisation.

Furthermore, the Applicant suggested using the method mentioned above for the determination of *formic acid* in water [7], with an appropriate dilution in water and without the preliminary extraction step. The CRL considers this approach fit for the intended use in the frame of authorisation.

The unambiguous determination of the content of exogenous *formic acid* added to *silages* is not achievable by analysis. The Applicant did not provide any experimental data nor suggested any methods of analysis. Therefore the CRL cannot evaluate nor recommended any method for official control to determine *formic acid* in silages.

For the determination of *formic acid* in the feed additive, the Applicant proposed the ISO 2114:2000 method based on a potentiometric titration [4,8] for the analysis of organic acids in "plastics (polyester resins) and paints and varnishes (binders)". As the *feed additive* of concern is very different from the matrices covered by this ISO standard (the *product* consists of 84.5% formic acid and 15.5% water), this ISO method is not considered suitable for the analysis of the *feed additive*. Another dedicated standard (ISO 731:1977 standard series) was previously available for the characterisation of "Formic acid for industrial use", but was withdrawn from the ISO catalogue in May 2002. Nevertheless, the CRL considers that the *product* could be analysed (as for the *water* matrix), using the method submitted by the Applicant for the analysis in *feedingstuffs* - with an appropriate dilution in water and without the preliminary extraction step.

Based on the several considerations presented, the CRL recommends for official control the single-laboratory validated (and further verified) method submitted by the Applicant for the determination of *formic acid* in the *feed additive*, *feedingstuffs* and *water* (not in *silages*).

Further testing or validation is not considered necessary.



#### 4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control - in the frame of this authorisation - the single-laboratory validated (and further verified) method submitted by the Applicant for the determination of *formic acid* in *feed additive*, *feedingstuffs*, and *water* (not in *silages*).

***Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)***

For the determination of *formic acid* in *feed additive*, *premixtures*, *feedingstuffs*, and *water*:

- Ion chromatography method equipped with electrical conductivity detection (IC-ECD)

#### 5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Formic acid* have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

#### 6. REFERENCES

- [1] \* Reference SANCO/D/2 Forw. Appl. 1831/018-2009.
- [2] \* Annex III: Proposal for Registry Entry
- [3] \* Section II, chapter 2.1. - Identity of the additive
- [4] \* Section II, chapter 2.6. - Methods of analysis and references samples
- [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] \* Annex Section II.4 – Analytical method
- [7] \* Section II, chapter 2.4.1 - Stability of formic acid in drinking water house validated methods
- [8] EN ISO 2114:2000 - "*Plastics (polyester resins) and paints and varnishes (binders) - Determination of partial acid value and total acid value*"

\* Refers to Dossier No. FAD-2009-0027

## **7. RAPPORTEUR LABORATORY**

The Rapporteur Laboratory for this evaluation was the National Reference Laboratory of Central Institute for Supervising and Testing in Agriculture, The Czech Republic (the NRL of CISTA). 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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- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer (DE)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
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