EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
European Union Reference Laboratory for Feed Additives

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

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

Manganese chelate of ethylenediamine

(FAD-2018-0067; CRL/180056)

European Union Reference Laboratory
Feed Additives

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

Dossier related to:	**FAD-2018-0067 - CRL/180056**
Name of Product:	***Manganese chelate of ethylenediamine***
Active Agent (s):	**Manganese**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**María José González de la Huebra** **25/01/2019**
Report approved by: Date:	**Christoph von Holst** **25/01/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *manganese chelate of ethylenediamine* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all categories and species.

Manganese chelate of ethylenediamine is a solid preparation with a minimum content of 21 % (w/w) of *manganese* and 21 % (w/w) of *ethylenediamine (EDA).*

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. In addition, the Applicant proposed maximum levels of *total manganese* in *feedingstuffs* complying with the limits set in Regulations (EC) No 1334/2003 and (EU) 2017/1490: 100 mg/kg for fish; and 150 mg/kg for other species.

For the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated CEN method EN 15621 based on ICP-AES after pressure digestion. This method together with the CEN method: EN 15510 based on inductively coupled plasma atomic emission spectrometry (ICP-AES) and the Community method based on atomic absorption spectrometry which was further ring-trial validated by the UK Food Standards Agency (FSA), were previously evaluated and recommended by the EURL in the frame of the Manganese group dossier.

In addition, the EURL is aware of two ring-trial validated methods, namely: ISO 6869 based on atomic absorption spectrometry (AAS) and EN 17053 based on inductively coupled plasma mass spectrometry (ICP-MS).

The following performance characteristics were reported for the five above mentioned CEN methods in the frame of the ring-trial validation studies for quantification of total *manganese* content ranging from 12 to 13200 mg/kg in matrices of the scope: a relative standard deviation for *repeatability* (RSD_r) ranging from 1 % to 6.4 %; and a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.4 % to 19.8 %.

Based on the acceptable method performance characteristics available, the EURL recommends for official control the five ring-trial validated methods: i) EN 15621 and ISO 6869 for the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs*; ii) EN 15510 and EN 17053 for the quantification of *total manganese* in *premixtures* and *feedingstuffs*; and iii) the Community method (Commission Regulation (EC) No 152/2009 – Annex IV-C) for the quantification of *total manganese* in *feedingstuffs*.

For the quantification of *ethylenediamine* in the *feed additive* the Applicant submitted a single-laboratory validated method based on high performance liquid chromatography coupled to mass spectrometry detection (LC-MS/MS) using hydrophilic interaction chromatography (HILIC) stationary phase.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Manganese, manganese chelate of ethylenediamine, nutritional feed additives, compounds of trace elements, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new *feed additive*) for *manganese chelate of ethylenediamine* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all categories and species [1,2,3].

Manganese chelate of ethylenediamine is a solid preparation with a minimum content of 21 % (w/w) of *manganese* and 21 % (w/w) of *ethylenediamine (EDA)* [2,3,4].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [4]. In addition, the Applicant proposed maximum levels of *total manganese* in *feedingstuffs* complying with the limits set in Regulations (EC) No 1334/2003 and (EU) 2017/1490: 100 mg/kg for fish; and 150 mg/kg for other species [2,3,4].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 *manganese chelate of ethylenediamine* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated CEN method EN 15621 based on ICP-AES after pressure digestion [5].

This method together with the CEN method: EN 15510 based on inductively coupled plasma atomic emission spectrometry (ICP-AES) after ash or wet digestion with hydrochloric acid [6] and the Community method based on atomic absorption spectrometry [7], which was further ring-trial validated by the UK Food Standards Agency (FSA) [8], were previously evaluated and recommended by the EURL in the frame of the manganese group dossier (including FAD-2010-0069; FAD-2010-0088 and FAD-2010-0235) [9].

In addition, the EURL is aware of two ring-trial validated methods, namely: ISO 6869 based on atomic absorption spectrometry (AAS) [10] and EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS) [11].

The performance characteristics reported for the five methods mentioned above are summarised in Table 1.

Based on the acceptable method performance characteristics available, the EURL recommends for official control the five ring-trial validated methods: i) EN 15621 and ISO 6869 for the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs*; ii) EN 15510 and EN 17053 for the quantification of *total manganese* in *premixtures* and *feedingstuffs*; and iii) the Community method (Commission Regulation (EC) No 152/2009 – Annex IV-C) for the quantification of *total manganese* in *feedingstuffs*.

Table 1: Performance characteristics for the quantification of *total manganese* in *premixtures* and *feedingstuffs*

	EN 15621	EN 15510	UK FSA	ISO 6869	EN 17053
Method	ICP-AES	ICP-AES	AAS	AAS	ICP-MS
Mass fraction (mg/kg)	127 – 10310	92.8 - 3527	30 - 128	16 - 13200	12 – 4603(*)
RSD_r (%)	2.1 – 3.5	1.9 – 6.3	2.7 – 4.3	1.0 – 4.2	3.1 - 6.4
RSD_R (%)	5.5 – 13.2	5.2 – 15.8	5.2 – 7.1	3.4 – 19.8	4.9 – 12.1
LOQ (mg/kg)	1	3	20	5	0.1
Reference	[5]	[6]	[8]	[10]	[11]

RSD_r and RSD_R: relative standard deviation for *repeatability* and *reproducibility*; LOQ: limit of quantification; (*) based on dry weight.

Even though the methods EN 15510 and EN 17053 were ring-trial validated at narrower range for *total manganese* content than the methods EN 15621 and ISO 6869, the first two ones still might be considered for the quantification of *total manganese* in the *feed additive* after appropriate dilution with the condition that the methods are proven as fit-for-purpose.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the quantification of *ethylenediamine* in the *feed additive* the Applicant submitted a single-laboratory validated method based on high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) using hydrophilic interaction chromatography (HILIC) stationary phase [12].

The *ethylenediamine* is extracted from the *feed additive* with water and diluted with acetonitrile containing 0.1 % formic acid before being injected into the LC-MS/MS system. The *ethylenediamine* is separated by HILIC on a BEH-amide column and determined by mass spectrometry [12].

The Applicant applied the above mentioned LC-MS/MS method for the analysis of five batches of the *feed additive* with an average content of 22.2 % (w/w) for *ethylenediamine* [4]. A relative standard deviation for *repeatability* (RSD_r) of 2.1 % was obtained which is in agreement with the precision values reported in the frame of the validation study [12].

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control:

- the EN 15621 and ISO 6869 for the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs*;
- the EN 15510 and EN 17053 for the quantification of *total manganese* in *premixtures* and *feedingstuffs*; and
- the Community method based on atomic absorption spectrometry (AAS) for the quantification of *total manganese* in *feedingstuffs* (only).

Recommended text for the register entry (analytical method)

For the quantification of *total manganese* in the *feed additive*, *premixtures* and *feedingstuffs*:

- Inductively Coupled Plasma-Atomic Emission Spectrometry after pressure digestion (ICP-AES) – EN 15621; or

- Atomic Absorption Spectrometry (AAS) – ISO 6869; or
- Inductively Coupled Plasma-Atomic Emission Spectrometry, (ICP-AES) – EN 15510 (for *premixtures* and *feedingstuffs* only); or
- Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) – EN 17053 (for *premixtures* and *feedingstuffs* only); or
- Atomic Absorption Spectrometry (AAS) – Commission Regulation (EC) No 152/2009 (for *feedingstuffs* only)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *manganese chelate of ethylenediamine* 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 SANTE/E5: FORW. APPL. 1831-0070-2018

[2] *Application, Application Form – Annex 1 – Subm. No. 1536570515268-2286

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

[4] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis

[5] EN 15621:2017 – *Animal feeding stuffs: Methods of sampling and analysis – Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES*

[6] EN 15510:2007 – *Animal feeding stuffs – Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES*

[7] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for official control of feed – Annex IV-C

[8] Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010

[9] EURL Evaluation Report (Manganese group): https://ec.europa.eu/jrc/sites/jrcsh/files/amendment-finrep-manganese.pdf

[10] 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*

[11] EN 17053:2018 *Animal feeding stuffs: Methods of sampling and analysis – Determination of trace elements, heavy metals and other elements in feed by ICP-MS (multi-method)*

[12] Technical dossier, Section II: Annex II_32

*Refers to Dossier no: FAD-2018-0067

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- RIKILT Wageningen UR, Wageningen (NL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors (FI)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural**.** Generalitat de Catalunya, Cabrils (ES)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft, Nossen (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)