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European Union Reference Laboratory for Feed Additives

JRC F.5/CvH/MGH/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**

Iron chelate of ethylenediamine
(FAD-2018-0086; CRL/180080)



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in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2018-0086 - CRL/180080**

Name of Product: ***Iron chelate of ethylenediamine***

Active Agent (s): **Iron**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

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Date: **29/05/2019**

Report approved by: **Christoph von Holst**
Date: **03/05/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for iron as an *iron chelate of ethylenediamine* preparation 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.

Iron chelate of ethylenediamine is a solid preparation for supplementing iron with a minimum content of 20 % (w/w) of *iron* and 20 % (w/w) of *ethylenediamine (EDA)*.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* according to the maximum levels of *total iron* in the *feedingstuffs* which range from 250 to 750 mg/kg depending on the animal species/category, as established by Regulation (EU) 2017/2330.

For the quantification of *total iron* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated CEN method EN 15621 based on inductively coupled plasma-atomic emission spectrometry ICP-AES after pressure digestion. This method together with the CEN method EN 15510 based on ICP-AES after ashing or wet digestion 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 iron 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).

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 iron* in the *feed additive*, *premixtures* and *feedingstuffs*; ii) EN 15510 for the quantification of *total iron* in *premixtures* and *feedingstuffs*; and iii) the Community method (Commission Regulation (EC) No 152/2009 – Annex IV-C) and EN 17053 for the quantification of *total iron* 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 (LC-MS/MS) detection using a hydrophilic interaction chromatography (HILIC) stationary phase. This method was previously evaluated by the EURL in the frame of the other *ethylenediamine chelate* dossiers for the characterisation of the ligand in the *feed additive* and it was considered as fit-for-purpose.

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

Iron, iron 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 iron as an *iron chelate of ethylenediamine* preparation 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].

Iron chelate of ethylenediamine is a solid preparation for iron with a minimum content of 20 % (w/w) of *iron* and 20 % (w/w) of *ethylenediamine (EDA)* [2,3,4].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [4] according to the following maximum levels of total iron in the *feedingstuffs* as established by Regulation (EU) 2017/2330: 250 mg/kg for piglets up to 1 week before weaning; 450 mg/kg for bovines and poultry; 500 mg/kg for ovine; 600 mg/kg for pet animals and 750 mg/kg for other species and categories [2,3,4,5].

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 *iron 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 iron* 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 [6].

This method together with the CEN method EN 15510 based on inductively coupled plasma-atomic emission spectrometry (ICP-AES) after ashing or wet digestion with hydrochloric acid [7] and the Community method based on atomic absorption spectrometry [8], which was further ring-trial validated by the UK Food Standards Agency (FSA) [9], were previously evaluated and recommended by the EURL in the frame of the iron group dossier (including FAD 2010-0068; FAD 2010-0095; FAD 2010-0236; FAD 2010-0295; FAD 2010-0296 and FAD 2010-0380) [10].

In addition, the EURL is aware of two ring-trial validated methods, namely ISO 6869 based on atomic absorption spectrometry (AAS) [11] and EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS) [12]. 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 iron* in the *feed additive, premixtures* and *feedingstuffs*; ii) EN 15510 for the quantification of *total iron* in *premixtures* and *feedingstuffs*; and iii) the Community method (Commission Regulation (EC) No 152/2009 – Annex IV-C) and EN 17053 for the quantification of *total iron* in *feedingstuffs*.

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

Table 1: Performance characteristics for the quantification of *total iron* 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)	277 - 15940	293 - 8182	198 - 340	362 - 31000	36 - 3114 ^(*)
RSD _r (%)	2.9 – 6.3	2.6 – 4.8	2.3 – 9.5	0.9 – 16.2	3.0 – 4.3
RSD _R (%)	9.6 – 12.4	5.2 – 10.3 ¹⁾	5.3 – 9.5	6.0 – 23.5	5.7 – 13.7
LOQ (mg/kg)	1	3	20	5	5
Reference	[6]	[7]	[9]	[11]	[12]

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

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 a hydrophilic interaction chromatography (HILIC) stationary phase [13].

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 an ethylene bridged hybrid (BEH) amide column and determined by mass spectrometry.

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 26.9 % (w/w) for *ethylenediamine*. A relative standard deviation for *repeatability* (RSD_r) of 1.4 % was obtained which is in agreement with the precision values reported in the frame of the validation study [13]. This method was previously evaluated by the EURL in the frame of the other *ethylenediamine chelate* dossiers [14] and considered as fit-for-purpose.

Consequently, the EURL recommends for the characterisation of the *feed additive* the determination of *ethylenediamine* by single-laboratory validated method based on high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) using a 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.

4. CONCLUSIONS AND RECOMMENDATIONS

For the characterisation of the ligand:

- the high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the determination of *ethylenediamine* in the *feed additive*.

For official control of *total iron*:

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

Recommended text for the register entry (analytical method)

For the characterisation of the ligand:

- High performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the determination of *ethylenediamine* in the *feed additive*

For the quantification of *total iron* 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 *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 *iron 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. 1831-0080-2018
- [2] *Application, Application Form – Annex 1 – Subm. No. 1543851058237-2326
- [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] Commission Implementing Regulation (EU) 2017/2330 concerning the authorisation of Iron(II) carbonate, Iron(III) chloride hexahydrate, Iron(II) sulphate monohydrate, Iron(II) sulphate heptahydrate, Iron(II) fumarate, Iron(II) chelate of amino acids hydrate, Iron(II) chelate of protein hydrolysates and Iron(II) chelate of glycine hydrate as feed additives for all animal species and of Iron dextran as feed additive for piglets and amending Regulations (EC) No 1334/2003 and (EC) No 479/2006
- [6] 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*
- [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*
- [8] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for official control of feed – Annex IV-C
- [9] Food Standards Agency – Information Bulletin on Methods of Analysis and Sampling for Foodstuffs, No 102; March 2010
- [10] EURL Evaluation Reports:
https://ec.europa.eu/jrc/sites/jrcsh/files/amended_finrep_iron_group_fad-2010-0095.pdf
- [11] 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*
- [12] 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)*
- [13] Technical dossier, Section II: Annex II_32
- [14] EURL Evaluation Reports:
<https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports>

*Refers to Dossier no: FAD-2018-0086

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:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- RIKILT Wageningen UR, Wageningen (NL)
- Istituto Superiore di Sanità. Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Roma (IT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ruokavirasto Helsinki (FI)
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