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

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

<table>
<thead>
<tr>
<th>Method</th>
<th>EN 15621</th>
<th>EN 15510</th>
<th>UK FSA</th>
<th>ISO 6869</th>
<th>EN 17053</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass fraction (mg/kg)</td>
<td>277 - 15940</td>
<td>293 - 8182</td>
<td>198 - 340</td>
<td>362 - 31000</td>
<td>36 - 3114(*)</td>
</tr>
<tr>
<td>RSD_r (%)</td>
<td>2.9 – 6.3</td>
<td>2.6 – 4.8</td>
<td>2.3 – 9.5</td>
<td>0.9 – 16.2</td>
<td>3.0 – 4.3</td>
</tr>
<tr>
<td>RSD_R (%)</td>
<td>9.6 – 12.4</td>
<td>5.2 – 10.3</td>
<td>5.3 – 9.5</td>
<td>6.0 – 23.5</td>
<td>5.7 – 13.7</td>
</tr>
<tr>
<td>LOQ (mg/kg)</td>
<td>1</td>
<td>3</td>
<td>20</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

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

[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
[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
[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)

*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

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- Centro di referenza nazionale per la sorveglienza 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)
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- Ruokavirasto Helsinki (FI)
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