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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

L-histidine monohydrochloride monohydrate

produced by fermentation with strain *Escherichia coli* KCCM 80212 (H010)

(FAD-2020-0016; CRL/200007)

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-2020-0016 - CRL/200007**
Name of Product::	***L-histidine monohydrochloride monohydrate produced by fermentation with strain Escherichia coli* KCCM 80212 (H010)**
Active Agent (s):	**L-histidine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**María José González de la Huebra** **14/07/2020**
Report approved by: Date:	**Christoph von Holst** **14/07/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) for *L-histidine monohydrochloride monohydrate* produced by fermentation with the strain *Escherichia coli* KCCM 80212 (H010), under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for all animal species.

According to the Applicant, *L-histidine monohydrochloride monohydrate* has a minimum purity (mass fraction) of 98 %. The *feed additive* is intended to be added directly into *feedingstuffs* or through *premixtures*. However, the Applicant did not propose any minimum or maximum content of *L-histidine monohydrochloride monohydrate* in *feedingstuffs*.

For the quantification of *histidine* in the *feed additive* and *premixtures* the Applicant proposed and submitted the ring-trial validated method EN ISO 17180 originally dedicated for the determination of lysine, methionine and threonine in commercial amino acid products and premixtures. This standard method is based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD). It does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. The method is applicable for *products* and *premixtures* containing more than 10 % in mass fraction of the amino acid.

The extension of scope of the EN ISO 17180 method to another almost identical *feed additive* and *premixtures* containing *histidine* has been demonstrated by the Applicant in the frame of a recent *histidine* dossier.

For the quantification of *histidine* in *feedingstuffs* the Applicant submitted the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on IEC coupled to photometric detection (VIS). The method is designed for the analysis of amino acids in *premixtures* and *feedingstuffs* and does not distinguish between the salts and the amino acid enantiomers. This method was further ring-trial validated by 23 laboratories, resulting in the EN ISO 13903 method. The following performance characteristics were reported for the quantification of total *histidine*: a relative standard deviation for *repeatability* (RSD_r) ranging from 2.4 to 7.0 % and a relative standard deviation for *reproducibility* (RSD_R) ranging from 13 to 23 %.

Based on the performance characteristics available, the EURL recommends for official control: (i) the ring-trial validated method EN ISO 17180 based on IEC-VIS/FLD for the quantification of *histidine* in the *feed additive* and *premixtures*; and (ii) the ring-trial validated EU method, based on IEC-VIS for the quantification of *histidine* in *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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

L-histidine monohydrochloride monohydrate produced by fermentation with strain *Escherichia coli* KCCM 80212 (H010), nutritional additives, amino acids, all animal species

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (an authorisation of a new feed additive) for *L-histidine monohydrochloride monohydrate* produced by fermentation with the strain *Escherichia coli* KCCM 80212 (H010), under the category/functional group 3(c) 'nutritional additives'/'amino acids, their salts and analogues', according to Annex I of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for all animal species [1,2]. *L-histidine monohydrochloride monohydrate* produced by fermentation with *Escherichia Coli* is already authorised as *feed additive* under Commission Regulation (EC) No 244/2007 [3].

According to the Applicant, *L-histidine monohydrochloride monohydrate* has a minimum purity (mass fraction) of 98 % [4].

The *feed additive* is produced by fermentation with a genetically modified strain of *Escherichia coli* KCCM 80212 (H010) [5]. The production strain is deposited in the "Korean Culture Collection of Microorganisms" (KCCM) under accession number KCCM 80212 [6].

The *feed additive* is intended to be added directly into *feedingstuffs* or through *premixtures* [7]. However, the Applicant did not propose any minimum or maximum content of *L-histidine monohydrochloride monohydrate* in *feedingstuffs* [1,2,7].

Note: The EURL has previously evaluated the analytical methods for the determination of *L-histidine monohydrochloride monohydrate* in the frame of several dossiers [8-11].

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 *L-histidine monohydrochloride monohydrate* produced by fermentation with the strain *Escherichia coli* KCCM 80212 (H010) 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 *histidine* in the *feed additive* and *premixtures* the Applicant proposed [12] and submitted the ring-trial validated method EN ISO 17180 originally dedicated for the determination of lysine, methionine and threonine in commercial amino acid products and *premixtures* [13]. This standard method is based on the experimental protocol described in the ring-trial validated European Union (EU) method for the determination of amino acids in feed (including *histidine*) [14]. The EN ISO 17180 method does not distinguish between the salts of amino acids and cannot differentiate between enantiomers. The method is applicable for products and *premixtures* containing more than 10 % in mass fraction of the amino acid.

Following the EN ISO 17180 method, a free *histidine* is extracted with diluted hydrochloric acid and further diluted with sodium citrate buffer. After addition of norleucine as internal standard, the amino acids are separated by ion-exchange chromatography (IEC). *Histidine* is quantified (i) after post-column derivatisation with ninhydrine by Visible (VIS) detection at 440 nm and 570 nm or (ii) after post-column reaction with ortho-phthaldialdehyde by fluorescence detection (FLD) at an excitation wavelength of 330 nm and an emission wavelength of 460 nm [13].

The Applicant did not present proofs of an extension of scope of the above mentioned EN ISO 17180 method for the determination of *histidine* in the *feed additive* and *premixtures* in the frame of the current dossier. However, the extension of the scope of the EN ISO 17180 method, to another almost identical product and *premixtures* containing *histidine*, has been demonstrated recently in the frame of another dossier [8].

For the quantification of *histidine* in *feedingstuffs* the Applicant proposed [12] and submitted the above mentioned EU method [14]. This method is dedicated for the quantification of *free* (synthetic and natural) and *total* (peptide-bound and free) amino acids in *premixtures* and *feedingstuffs*, using IEC coupled to post-column derivatisation and VIS detection. It does not distinguish between the salts of amino acids and cannot differentiate between enantiomers.

Table 1: Method performance characteristics obtained in the frame of EN ISO 13903:2005 [15] for the determination of total *histidine* in *feed*.

Ring-Trial	Matrix	*histidine* content g/kg	RSD_r %	RSD_R %
[15]	Poultry meal	13.1	2.8	18.5
	Broiler finisher feed	5.0	4.0	19.8
	Broiler starter feed	6.5	2.8	15.4
	Corn	2.7	7.0	23.3
	Fishmeal	13.7	2.4	12.9

RSD_r, RSD_R: relative standard deviation for *repeatability* and *reproducibility,* respectively

Following the EU method, *free* amino acids are extracted with diluted hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The solution is filtered and adjusted to pH 2.2. The amino acids are separated by IEC and determined after post-column derivatisation with ninhydrin by photometric detection at 570 nm. The procedure chosen for the determination of the *total* amino acids depends on the amino acids under investigation. *Histidine* can be determined in either oxidised or non-oxidised samples. The oxidation is performed at 0 °C with a performic acid/phenol mixture. An excess of oxidation reagent is decomposed with sodium disulfite. The oxidised or non-oxidised sample is hydrolysed with hydrochloric acid (6 mol/l) for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and determined after post-column derivatisation with ninhydrin by photometric (VIS) detection at 570 nm [14].

The EU method was ring-trial validated using four different matrices for threonine, cyst(e)ine, methionine and lysine only [14]. This method was further ring-trial validated by 23 laboratories, resulting in the EN ISO 13903:2005 method (*histidine* included) [15]. The performance characteristics reported for the quantification of total *histidine* are listed in Table 1.

Based on the performance characteristics available, the EURL recommends for official control: (i) the ring-trial validated method EN ISO 17180 based on IEC-VIS/FLD for the quantification of free *histidine* in the *feed additive* and *premixtures*; and (ii) the ring-trial validated EU method, based on IEC-VIS to quantify *histidine* in *premixtures* and *feedingstuffs*.

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)

An evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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

In the frame of this authorisation the EURL recommends for official control (i) the ring-trial validated method EN ISO 17180 based on ion-exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD) for the quantification of *histidine* in the *feed additive* and *premixtures*; and (ii) the ring-trial validated European Union method based on IEC-VIS for the quantification of *histidine* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *histidine* in the *feed additive*:

- Ion-exchange chromatography coupled to post-column derivatisation and optical detection (IEC-VIS/FLD)

For the quantification of *histidine* in *premixtures*:

- Ion-exchange chromatography coupled to post-column derivatisation and optical detection (IEC-VIS/FLD) or
- Ion-exchange chromatography coupled to post-column derivatisation and photometric detection (IEC-VIS), Commission Regulation (EC) No 152/2009 (Annex III, F)

For the quantification of *histidine* in *feedingstuffs*:

- Ion-exchange chromatography coupled to post-column derivatisation and photometric detection (IEC-VIS), Commission Regulation (EC) No 152/2009 (Annex III, F)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *L-histidine monohydrochloride monohydrate* produced by fermentation with strain *Escherichia coli* KCCM 80212 (H010) 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. 1831/0021-2020 & Annex I – submission number 1581663638635-2552

[2] *Application, Proposal of Registry Entry – Annex A

[3] Commission Regulation (EC) No 244/2007 of 7 March 2007 concerning the authorisation of L-histidine monohydrochloride monohydrate as a feed additive, O.J. L 73/6, 13.03.2007

[4] *Technical dossier, Section II – II.1.3. Qualitative and quantitative composition

[5] *Technical dossier, Section II – II.2.1.2. Micro-organisms

[6] *Technical dossier, Section II – Annex II_2.1.1.2.a

[7] *Technical dossier, Section II – II.5.1. Proposed mode of use in animal nutrition

[8] #FAD-2018-0070, L-histidine monohydrochloride monohydrate produced using strain NITE BP-02526, Ref. Ares(2019)1958001 – 22/03/2019 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0070_l-histidine.pdf

[9] #FAD-2018-0040, L-histidine monohydrochloride monohydrate produced by Corynebacterium Glutamicum KCCM80179, Ref. Ares(2018)6592018 – 20/12/2018 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2018-0040-histidine.pdf

[10] #FAD-2018-0013, L-histidine monohydrochloride monohydrate produced by Corynebacterium glutamicum KCCM80172 Ref. Ares(2018) 5739711 – 09/11/2018 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0013_histidinehcl.pdf

[11] #EFSA-Q-2004-030, L-histidine monohydrochloride monohydrate, Ref. D.08/FSQ/CVH/GS/(2006) D13915 https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2006-0022.pdf

[12] *Technical dossier, Section II – II.6.1. Methods of analysis for the active substance

[13] EN ISO 17180:2013 – Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acid products and premixtures

[14] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, O.J. L 54, 26.02.2009

[15] EN ISO 13903:2005- Animal feeding stuffs – Determination of amino acids content

*Refers to Dossier no: FAD-2020-0016
#https://ec.europa.eu/jrc/en/eurl/feed-additives/evaluation-reports

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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- [2]Ruokavirasto Helsinki (FI)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)

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[2] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: Elintarviketurvallisuusvirasto/Livsmedelssäkerhetsverket (Evira), Helsinki/Helsingfors.