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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 **Corynebacterium glutamicum KCCM80179**
(FAD-2018-0040; CRL/180027)

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-2018-0040 - CRL/180027**
Name of Product:	***L-histidine monohydrochloride monohydrate produced by fermentation with Corynebacterium glutamicum KCCM80179***
Active Agent:	**L-histidine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**Stefano Bellorini** **12/12/2018**
Report approved by: Date:	**Christoph von Holst** **14/12/2018**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4 for *L-histidine monohydrochloride monohydrate produced by fermentation with Corynebacterium glutamicum KCCM80179*, 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. 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 *L-histidine monohydrochloride monohydrate* in the *feed additive* the Applicant submitted an in-house validated analytical method based on reversed phase high performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). The Applicant reported in the frame of the validation study a relative standard deviation for repeatability (RSD_r) and intermediate precision (RSD_{ip}) ranging from 0.1 to 2.1 % and a recovery rate (R_{rec}) ranging from 98 to 102 %. Furthermore, the EURL calculated a RSD_r of 0.3 % from further analytical data presented by the Applicant.

For the quantification of *L-histidine* in *premixtures* and *feedingstuffs* the EURL identified the ring-trial validated Community method (Commission Regulation (EC) No 152/2009) based on ion exchange chromatography coupled with photometric detection (IEC-VIS). This method, designed for the analysis of amino acids in *premixtures* and *feedingstuffs*, does not distinguish between the salts and the amino acid enantiomers. The following performance characteristics were reported for the quantification of total *histidine*: RSD_r ranging from 2.4 to 7.0 % and RSD_R ranging from 13 to 23 %.

Based on the performance characteristics available, the EURL recommends for official control the in-house validated method based on HPLC-UV to quantify *L-histidine monohydrochloride monohydrate* in the *feed additive* and the ring-trial validated Community method based on IEC-VIS to quantify *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 Corynebacterium glutamicum KCCM80179, nutritional additives, amino acids, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *L-histidine monohydrochloride monohydrate produced by fermentation with Corynebacterium glutamicum KCCM80179*, 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. Authorisation is sought for all animal species [1-3]. *L-histidine monohydrochloride monohydrate* produced by fermentation with *Escherichia Coli* is already authorised as *feed additive* under Commission Regulation (EC) No 244/2007 [4].

According to the Applicant, the colourless/off-white crystalline powder *L-histidine monohydrochloride monohydrate* has a minimum purity (mass fraction) of 98 % [1,5].

The *feed additive* is produced by fermentation with a genetically modified strain of *Corynebacterium glutamicum* [6]. The production strain is deposited in the "Korean Culture Center of Microorganisms" (KCCM) under accession number KCCM80179 [7,8].

The *feed additive* is intended to be added directly into *feedingstuffs* or through *premixtures* [9]. However the Applicant did not propose any minimum or maximum content of *L-histidine monohydrochloride monohydrate* in *feedingstuffs* [1].

Note: The EURL has previously evaluated the analytical methods for the determination of *L-histidine monohydrochloride monohydrate* in the frame of dossiers related to EFSA-Q-2004-030 and FAD-2018-0013 [10,11].

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* 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 *L-histidine monohydrochloride monohydrate* in the *feed additive* the Applicant submitted an in-house validated analytical method based on reversed phase high performance liquid chromatography (HPLC) and ultraviolet detection (UV) [12,13].

The *feed additive* is diluted in distilled water and an aliquot is directly injected into the HPLC system equipped with a C18 column. The analyte is eluted with potassium phosphate buffer. *L-histidine monohydrochloride monohydrate* is detected at 210 nm and quantified via an external calibration curve.

In the frame of the validation study, the Applicant reported the following performance characteristics: relative standard deviations for repeatability (RSD_r) and intermediate precision (RSD_{ip}) ranging from 0.1 to 2.1 % and a *recovery rate* (R_{rec}) ranging from 98 to 102 %. Furthermore a second laboratory applied the above mentioned HPLC-UV method to different batches of the *feed additive* for the determination of *L-histidine monohydrochloride monohydrate*. Based on these results presented for five different batches of the same product [14], the EURL calculated a RSD_r of 0.3 %.

For the quantification of the *L-histidine monohydrochloride monohydrate* content in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method from the "Association of German Agricultural Analytical and Research Institutes" (VDLUFA, Germany – Method 4.11.6), based on ion exchange chromatography (IEC) coupled with post-column derivatisation and colorimetric or fluorescence detection [12,15]. This method was designed and ring trial validated only for the determination of free lysine, methionine and threonine in *feed additive* and *premixtures* at amino acid contents higher than 100 g/kg.

The EURL identified instead the ring-trial validated Community method [16]. This method applies for the determination of free (synthetic and natural) and of total (peptide-bound and free) amino acids (including *histidine*), using an amino acid analyzer or HPLC equipment provided with an ion exchange column. The method is intended for *premixtures* and *feedingstuffs*, it does not distinguish between the salts of amino acids and cannot differentiate the amino acid enantiomers.

The 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 free *histidine* is determined after post-column derivatisation with ninhydrin by spectrophotometric detection at 570 nm (visible – VIS).

Table 1: Method performance characteristics reported in EN ISO 13903:2005 for the determination of total *histidine* [17]

Matrix	histidine content (g/kg)	RSD_r (%)	RSD_R (%)
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 and RSD_R - relative standard deviation for *repeatability* and *reproducibility*, respectively.

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. Oxidation is performed at 0 °C with a performic acid/phenol mixture. The excess of the oxidation reagent is decomposed with sodium disulfite. The oxidised or non-oxidised sample is hydrolysed with hydrochloric acid (6 mol/l) containing 1 g phenol/l for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and total *histidine* is determined by post-column derivatisation with ninhydrin and photometric detection at 570 nm. The Community method was further ring-trial validated by twenty-three laboratories for the determination of total *histidine* and resulted in the equivalent standard method EN ISO 13903:2005 [17]. The reported performance characteristics are listed in Table 1.

Based on the performance characteristics available, the EURL recommends for official control the i) in-house validated method based on HPLC-UV to quantify *L-histidine monohydrochloride monohydrate* in the *feed additive* and ii) the ring-trial validated Community 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 the: i) in-house validated method based on high performance liquid chromatography and ultraviolet detection (HPLC-UV) to quantify *L-histidine monohydrochloride monohydrate* in the *feed additive* and ii) the ring-trial validated Community method based on ion exchange chromatography coupled to visible detection (IEC-VIS) for the quantification of *histidine* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *L-histidine monohydrochloride monohydrate* in the *feed additive*:

- high performance liquid chromatography coupled with photometric detection (HPLC-UV)

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

- ion exchange chromatography coupled with 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 Corynebacterium glutamicum KCCM80179* 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, Proposal of Registry Entry – Annex A

[2] *Application, Reference SANTE/E5: Forw. Appl. 1831/0044-2018

[3] Annex I – submission number 1528295981196-2228

[4] 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

[5] *Technical dossier, Section II: II.1. Identity of the additive

[6] *Technical dossier, Section II: II.1.3. Qualitative and quantitative composition

[7] *Technical dossier, Section II: II.1.1. Name of the additive

[8] *Technical dossier, Section II: II.2.2.2. Micro-organisms

[9] *Technical dossier, Section II: II.2.5.1. Proposed mode of use in animal nutrition

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

[11] 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

[12] *Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance

[13] *Technical dossier, Section II: Annex Annex_II_6_01 CJ L- His Method validation report

[14] *Technical dossier, Section II: Annex_II_1_03

[15] Bestimmung von Lysin, Methionin und Threonin in Aminosäurenhandelsprodukten und Vormischungen – 4.11.6, Methodenbuch III, 5. Erg. 2004, VDLUFA – Verlag, Darmstadt)

[16] 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

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

*Refers to Dossier no: FAD-2018-0040

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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