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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-valine produced by
Escherichia Coli K-12 (NITE BP-01755)
(FAD-2014-0015; CRL/140010)

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-2014-0015 - CRL/140010**
Name of Product:	**L-valine *produced by Escherichia coli K-12 (NITE BP-01755)***
Active Agent (s):	**L-valine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium**
Report prepared by:	**Stefano Bellorini**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **03/09/2014**
Report approved by: Date:	**Christoph von Holst** **11/09/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *L-valine produced by Eschericha Coli K-12 (NITE BP-01755)*, 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. *L-valine* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* and no minimum or maximum *feed additive* concentrations are suggested.

For the quantification of *valine* in *feedingstuffs* the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). The method was further ring-trial validated resulting in EN ISO 13903:2005. The method is based on ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS). This method does not distinguish between the salts and the amino acid enantiomers and it is designed for *feedingstuffs* and *premixtures*. The following performance characteristics were reported for the quantification of total *valine*: a relative standard deviation for *repeatability* (RSD_r) ranging from 1.7 to 3.8% and a relative standard deviation for *reproducibility* (RSD_R) ranging from 8.8 to 16.1%.

For the quantification of *valine* in the *feed additive* and *premixtures* the Applicant suggested to apply the EN ISO 17180:2013 method (also known as AOAC 999.13) specifically designed for the quantification of lysine, methionine and threonine - not *valine* - in feed grade amino acids and *premixtures*. The Applicant reported, for the analysis of *feed additive* only, precisions (*repeatability* and *intermediate*) in the order of 0.26%. Nevertheless, the EURL, as already suggested in previous *valine* reports and following the advice of several experienced NRLs, recommends the use of the Community method instead. Furthermore, for the characterisation of the *feed additive*, the EURL identified the methods described in the *"L-valine* monograph*"* of the Food Chemical Codex (FCC).

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method (equivalent to the EN ISO 13903:2005) based on IEC-VIS for the quantification of *valine* in the *feed additive*, *premixtures* and *feedingstuffs*, together with the *"L-valine* monograph*"* of the FCC for the characterisation of the *feed additive*.

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) is not considered necessary.

KEYWORDS

L-valine produced by Eschericha Coli K-12 (NITE BP-01755), L-valine, nutritional additives, amino acids, their salts and analogues, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *L-valine produced by Eschericha Coli K-12 (NITE BP-01755)*, 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 [1,2]. *L-valine* is already authorised under Commission Regulation (EC) No 403/2009 [3].

According to the Applicant, *L-valine* is a white crystalline powder with a minimum purity of 98% [1,4]. As described in the "*L-valine* monograph" of the Food Chemical Codex, the *feed additive* has a specific optical rotation ranging from +26.6º and +29.0º [5]. *L-valine* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs* [6]. The Applicant proposed no minimum or maximum *L-valine* concentrations in *feedingstuffs* [1].

Note: The EURL has previously evaluated the analytical methods in the frame of four similar applications, *L-valine* produced by: *Escherichia coli K-12 (FERM ABP-10640)* [7], *Escherichia Coli (NITE SD 00066)* [8], *Corynebacterium glutamicum (KCCM80058)* [9] and *Corynebacterium glutamicum (DSM25202)* [10].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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-valine produced by Eschericha Coli K-12 (NITE BP-01755)* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [11]

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the quantification of *valine* in *feedingstuffs* the Applicant submitted the Community method [12,13]. This method applies for the determination of *free* and of *total* (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography equipped with ion exchange column. Intended for *premixtures* and *feedingstuffs*, it does not distinguish between the salts and 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 ion exchange chromatography (IEC) and *free valine* is determined after post column derivatisation with ninhydrin by spectrophotometric detection at 570 nm (Visible – VIS).

For the quantification of the *total valine*, the sample is hydrolysed with hydrochloric acid (6 mol/l) containing 1g phenol/l for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and *total valine* is determined after post column derivatisation with ninhydrin by spectrophotometric detection at 570 nm.

The Community method was further ring-trial validated by twenty-three laboratories for the determination of total *valine* and resulted in the standard method EN ISO 13903:2005 [14]. The reported performance characteristics are listed in Table 1.

Table 1: Method performance characteristics reported in EN ISO 13903:2005 for the determination of total *valine* [14]

Matrix	*Valine* (g/kg)	RSD_r (%)	RSD_R (%)
poultry meal	28.2	3.2	12.8
broiler finisher feed	9.2	3.8	12.7
broiler starter feed	11.1	1.7	8.8
corn	3.8	2.4	16.1
fishmeal	27.8	2.3	11.2

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

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated Community method (equivalent to the EN ISO 13903:2005), based on IEC-VIS to quantify *valine* in *premixtures* and *feedingstuffs*.

For the quantification of *valine* in the *feed additive* and *premixtures* the Applicant submitted the ring-trial validated EN ISO method 17180:2013 (equivalent to the AOAC official method 999.13) based on IEC coupled with post-column derivatisation and photometric detection [12,15,16]. This method is applicable for "*the determination of free nonprotein-bound lysine, methionine and threonine* (not for *valine*) *in feed grade amino acids or in premixtures with more than 10% individual amino acid content*". It does not distinguish between the salts and the amino acid enantiomers. Free amino acids are extracted with diluted hydrochloric acid and further diluted with sodium citrate buffer. After addition of norleucine as internal standard, the analytes are separated by IEC. Free amino acids are quantified after post-column derivatisation with ninhydrine and VIS detection at 570 nm or by fluorescence detection (FD) after post column reaction with ortho-phthaldialdehyde with detector set at excitation wavelength 330 nm and emission 460 nm. The Applicant applied this ISO method to quantify *valine* in the *feed additive* and reported precisions (*repeatability* and *intermediate*) in the order of 0.26% [17].

Nevertheless, following the advice of several experienced NRLs, the EURL recommends the use of the above described Community method (equivalent to the EN ISO 13903:2005) for the quantification of *valine* in the *feed additive* [7-10].

Furthermore the EURL recommends, for the characterisation of *L-valine* in the *feed additive*, the *"L-valine* monograph*"* of the Food Chemical Codex (FCC), where identification is based on infrared absorption in combination with the analysis of the optical rotation, while quantification is based on titration with perchloric acid (0.1N) [5].

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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

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

- the FCC method for the characterisation of *L-valine* in the *feed additive* based on infrared absorption and optical rotation; and
- ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS), to quantify *valine* in *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the characterisation of *L-valine* in *feed additive*:

- Food Chemical Codex "L-valine monograph"

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

- ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS)

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

- ion exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS) – Commission Regulation (EC) No 152/2009

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *L-valine* 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 SANCO/G1: Forw. Appl. 1831/0019-2014

[3] Commission Regulation (EC) No 403/2009 of 14 May 2009 concerning the authorisation of a preparation of L-valine as a feed additive, O.J. L 120, 15.05.2009

[4] *Technical dossier, Section II: 2.1.5 Physical state of each form of the product

[5] Food Chemical Codex monograph "*L-Valine*", FCC 7 (2010), p. 1072

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

[7] FAD-2007-0015, L-valine, Ref. D08/FSQ/CvH/GS/D2008(5731) – 28/02/2008 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2007-0015.pdf

[8] FAD-2012-0023, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)181751 – 12/02/2013 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0023-L-Valine.doc.pdf

[9] FAD-2012-0028, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)998581 - 02/05/2013 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0028-L-Valine.doc_.pdf

[10] FAD-2011-0053, L-valine, Ref. JRC.D.5/SFB/CvH/SB/mds/Ares(2013)2914464 - 21/08/2013 https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2011-0053-L-Valine.doc_.pdf

[11] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

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

[13] 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 (Annex III, F)

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

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

[16] AOAC Official Method 999.13 – *Lysine, Methionine and Threonine in feed grade amino acids and premixes (1999)*

[17] *Technical dossier, Annex II_5_2

*Refers to Dossier no: FAD-2014-0015

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, 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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
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- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
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- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Freistaat Sachsen, Nossen[1] (DE)

[1] Name and address according to Regulation (EC) No 885/2009: Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig