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European Union Reference Laboratory

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

(FAD-2012-0023 ; CRL/120012)



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Dossier related to: **FAD-2012-0023
CRL/120012**

Name of Feed Additive: **L-valine**

Active Substance(s): **L-valine**

Rapporteur Laboratory: **European Union Reference
Laboratory for Feed Additives
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Date: **12/02/2013**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *L-valine* produced by *Escherichia Coli* (NITE SD 00066), 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*. The *feed additive* is intended to be mixed either in *premixtures* or added directly to *feedingstuffs*. The Applicant proposed no minimum or maximum *L-valine* concentrations in *feedingstuffs*, suggesting, however, typical levels of inclusion up to 2000 mg/kg complete feed.

For the determination of *L-valine* in *premixtures* and *feedingstuffs* the Applicant submitted the Community method - Commission Regulation (EC) No 152/2009 (further ring-trial validated - CEN EN ISO 13903:2005). The following performance characteristics were reported for the determination 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%.

For the determination of *L-valine* in the *feed additive*, the Applicant submitted the European Pharmacopoeia general method for the determination of amino acids, based on High Performance Liquid Chromatography (HPLC). The EURL recommends instead 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. Moreover, as already recommended in the report FAD-2007-0015, the EURL suggests the above mentioned Community method for the determination of *valine* in the *feed additive*.

Based on the performance characteristics presented, the EURL recommends for official control: - the Food Chemical Codex for the determination of *L-valine* in the *feed additive* and - the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and photometric detection to determine *valine* in *feed additive*, *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) is not considered necessary.

KEYWORDS

L-valine, nutritional additives, amino acids, their salts and analogues, 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-valine* produced by *Escherichia Coli* (NITE SD 00066), 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 [1,2]. Authorisation is sought for *all animal species* [2].

According to the Applicant, *L-valine* is a greyish crystalline powder or granules with a minimum purity of 98% [2,3]. As described in the "*L-valine* monograph" of the Food Chemical Codex, the *feed additive* has a specific optical rotation ranging from +26.6° and +29.0° [4].

L-valine is intended to be mixed either in *premixtures* or added directly to *feedingstuffs*. The Applicant proposed no minimum or maximum *L-valine* concentrations in *feedingstuffs*, however suggested typical levels of inclusion up to 2000 mg/kg complete feed [5].

L-valine produced by *Escherichia coli* (K-12 AG314) is already authorised as feed additive, without any restrictions and the correspondent methods have been evaluated by EURL (i.e. FAD-2007-0015) [6, 7].

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 (EFSA) for each application or group of applications. For this dossier, the methods of analysis submitted in connection with *L-valine* 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 [8].

Description of the analytical methods for the determination of the active substances in feed additive, premixtures, feedingstuffs.

For the determination of *L-valine* in *premixtures* and *feedingstuffs* the Applicant submitted the Community method [9,10]. 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 (HPLC) equipment. The method 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 and *free valine* determined by post column derivatisation with ninhydrin and photometric detection at 570 nm.

For the determination 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 ion exchange chromatography and the *total valine* determined after post column derivatisation with ninhydrin by spectrophotometric detection at 570 nm. If cyst(e)ine and/or methionine have to be quantified, an additional oxidation step is required.

This Community method was further ring-trial validated by twenty-three laboratories, resulting in the CEN EN ISO 13903:2005 method [11]. The corresponding performance characteristics are listed in Table 1.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated Community method, based on ion exchange chromatography coupled with post-column derivatisation and photometric detection to determine *valine* in *premixtures* and *feedingstuffs*.

Table 1: Method performance characteristics obtained in the frame of the CEN EN ISO 13903:2005 ring-trial validation exercises for the determination of *total valine* in *premixtures* and *feedingstuffs*.

Intercomparison study	Matrix	Valine g/kg	RSD _r (%)	RSD _R (%)
ISO 13903:2005 [11] study carried out in 1994	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

For the determination of *L-valine* in the *feed additive*, the Applicant suggested the use of HPLC [9]. The protocol proposed is based on the general methodology indicated in the European Pharmacopoeia [12]. No performance characteristics or supporting experiments were provided. The EURL proposed instead 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 by titration with perchloric acid (0.1N).

Even though no performance characteristics are presented, the EURL recommends for official control the FCC “*L-valine*” monograph based on the identification by infrared absorption and optical rotation and the quantification by titration with perchloric acid to determine *L-valine* in the *feed additive*. Moreover, as already recommended in the report FAD-2007-0015 [7], following the advice of the NRLs, the EURL suggests the above mentioned Community method for the determination of *valine* in *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.

4. CONCLUSIONS AND RECOMMENDATIONS

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

- the FCC monograph method based on the identification by infrared absorption and optical rotation and the quantification by titration with perchloric acid to determine *L-valine* in the *feed additive*;
- the ring trial validated Community method, using High Performance Liquid Chromatography (HPLC) coupled to post column derivatisation and photometric detection to determine *valine* in *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

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

- Food Chemical Codex monograph

For the determination of *valine* in *feed additive*, *premixtures* and *feedingstuffs*:

- ion exchange chromatography coupled with post-column derivatisation and photometric detection (HPLC/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/Ref: SANCO/G1: Forw.Appl.1831/0040-2012
- [3] *Technical dossier, Section II: 2.1 Identity of the additive
- [4] Food Chemical Codex monograph "*L-Valine*", FCC 7 (2010), p. 1072
- [5] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
- [6] 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
- [7] #FAD-2007-0015, *L-valine*, Ref. D08/FSQ/CvH/GS/D2008(5731) – 28/02/2008
- [8] 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.
- [9] *Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance
- [10] 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)
- [11] Animal feeding stuffs – Determination of amino acids content; CEN EN ISO 13903:2005
- [12] *Technical dossier, Section II: Annex II-1_HPLC method.pdf

* Refers to Dossier No FAD-2012-0023

http://irmm.jrc.ec.europa.eu/EURLs/EURL_feed_additives/authorisation/evaluation_reports/

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

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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
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