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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-glutamine
produced using strain NITE BP-02524
(FAD-2018-0059; CRL/180039)

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-2018-0059 - CRL/180039**
Name of Product:	***L-glutamine produced using strain NITE BP-02524***
Active Agent:	**L-glutamine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA) JRC Geel, Belgium**
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Date:	**19/03/2019**
Report approved by:	**Christoph von Holst**
Date:	**19/03/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *L-glutamine produced using strain NITE BP-02524*, under the category/functional groups 3(c) 'nutritional additives'/'amino acids, their salts and analogues' and 2(b) 'sensory additives/flavouring compounds' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species.

According to the Applicant, *L-glutamine* has a minimum purity (mass fraction) of 98 %. As nutritional *feed additive*, *L-glutamine* is intended to be added directly into *feedingstuffs* or through *premixtures*. As sensory feed additive, *glutamine* is intended to be added into *feedingstuffs* through flavouring *premixtures*. The Applicant did not propose any minimum or maximum content *of glutamine* in *feedingstuffs* but, when authorised as sensory additive, recommended to use *glutamine* at a level of 25 mg/kg complete feed.

For the quantification of *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method EN ISO 17180:2013 specifically designed for lysine, methionine and threonine in products containing more than 10 % of amino acid. 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 cannot differentiate between enantiomers. The Applicant presented results from validation and verification studies demonstrating the extension of the scope of the above mentioned ISO method for the determination of *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs*. The following performance characteristics were reported: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.4 to 1.9 %, a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.7 to 2.2 %, a *recovery rate* – from 95 to 105 % and a limit of quantification (LOQ) of 300 mg/kg *feedingstuffs*.

However, the proposed method is not applicable for the determination of *glutamine* in *feedingstuffs* when the *feed additive* is used as sensory additive as the LOQ in this case is above the maximum recommended inclusion level of *glutamine* in *feedingstuffs*.

In addition, the EURL identified the "L-glutamine monograph" of the Food Chemical Codex (FCC) for the identification of *L-glutamine* in the *feed additive*.

In the frame of this authorisation the EURL recommends for official control (i) the "L-glutamine monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *L-glutamine* in the *feed additive*; (ii) the ring-trial validated method EN ISO 17180:2013 based on ion exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD) to quantify free *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs* (only as 'nutritional 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-glutamine produced using strain NITE BP-02524, nutritional additives, amino acids, all animal species and categories, sensory additives, flavouring compounds

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *L-glutamine produced using strain NITE BP-02524*, under the category/functional groups 3(c) 'nutritional additives'/'amino acids, their salts and analogues' and 2(b) 'sensory additives/flavouring compounds' according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species [1,2].

According to the Applicant, the white crystalline powder *L-glutamine* has a minimum purity (mass fraction) of 98 % [1,3,4]. The *feed additive* is produced by fermentation using a genetically modified strain of *Corynebacterium glutamicum* [5]. The production strain is deposited in the "National Institute of Technology and Evaluation" (NITE) with reference BP-02524. As nutritional *feed additive*, *glutamine* is intended to be added directly into *feedingstuffs* or through *premixtures* [6]. As sensory feed additive, *glutamine* is intended to be added into *feedingstuffs* through flavouring *premixtures* [6]. The Applicant did not propose any minimum or maximum content of *L-glutamine* in *feedingstuffs*. However, when authorised as sensory additive, it is recommended to use *glutamine* at a level of 25 mg/kg complete feed [1,6].

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-glutamine* 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 *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method EN ISO 17180:2013 - "Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acid products and premixtures" [7,8]. This standard method is based on the experimental protocol described in the Community method for the determination of amino acids in feed [9]. The method does not distinguish between the salts of amino acids and it cannot differentiate between enantiomers. It applies for products and *premixtures* containing more than 10 % of amino acid.

The Applicant presented validation and verification studies with the aim to demonstrate the extension of scope of the above mentioned ISO method for the determination of *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs* as *glutamine* is not in the scope of the abovementioned Community method [9-12]. Minor modifications from the original standard operating procedure are described in the corresponding documents [10-12].

Free *glutamine* 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 High Performance Liquid Chromatography (HPLC) with an Ion Exchange Column (IEC). Free *glutamine* is quantified either after post-column derivatisation with ninhydrine and Visible (VIS) detection at 440 nm and 570 nm or by fluorescence detection (FLD) after post-column reaction with ortho-phthaldialdehyde with a detector excitation wavelength at 330 nm and emission at 460 nm. The following performance characteristics were reported: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.4 to 1.9 %, a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.7 to 2.2 %, a *recovery rate* – from 95 to 105 % and a limit of quantification (LOQ) of 300 mg/kg *feedingstuffs* [10-12].

Based on the performance characteristics available, the EURL recommends for official control the ring-trial validated method EN ISO 17180:2013 based on IEC-VIS/FLD for the quantification of free *glutamine* as nutritional feed additive in the *feed additive*, *premixtures* and *feedingstuffs*.

However, the proposed method is not applicable for the determination of *glutamine* in *feedingstuffs* when the *feed additive* is used as sensory additive as the LOQ in this case is above the maximum recommended inclusion level of *glutamine* in *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)

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)

The EURL found the "L-glutamine monograph" of the Food Chemical Codex (FCC) where identification is based on infrared absorption [13].

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 "L-glutamine monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *L-glutamine* in the *feed additive*; (ii) the ring-trial validated method EN ISO 17180:2013 based on ion exchange chromatography coupled to visible or fluorescence detection (IEC-VIS/FLD) to quantify free *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs* (only as 'nutritional additive').

Recommended text for the register entry (analytical method)

For L-glutamine authorised under the category/functional groups 3(c) 'nutritional additives'/'amino acids, their salts and analogues':

For the identification of *L-glutamine* in the *feed additive*:

- Food Chemical Codex "L-glutamine monograph"

For the quantification of *glutamine* in the *feed additive*, *premixtures* and *feedingstuffs*:

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

For L-glutamine authorised under the category/functional groups 2(b) 'sensory additives/flavouring compounds':

For the identification of *L-glutamine* in the *feed additive*:

- Food Chemical Codex "L-glutamine monograph"

For the quantification of *glutamine* in the *feed additive* and *premixtures*:

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *L-glutamine produced using strain NITE BP-02524* 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. 1831-0066-2018 & Annex I – submission number 1533650610074-2278

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

[4] *Technical dossier, Section II: 2.1.3 Qualitative and quantitative composition

[5] *Technical dossier, Section II: 2.3 Manufacturing process, including any processing procedures

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

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

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

[9] 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

[10] *Technical dossier, Section II: An_II_54_Raterink_2018 _Triskelion_V21163_ Validation_Meth_in_CP.pdf

[11] *Technical dossier, Section II: An_II_56_Raterink_2018_Triskelion_V21164_ Validation_Meth_in_premix.pdf

[12] *Technical dossier, Section II: An_II_60_Raterink_2018_Triskelion_V21165_ Validation_Meth_GLN_Feed.pdf

[13] Food Chemical Codex monograph "L-glutamine", FCC 7 (2010), p.441

*Refers to Dossier no: FAD-2018-0059

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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