
DL-Methionyl-DL-Methionine

(\textit{FAD-2012-0034; CRL/120025})

Dossier related to: FAD-2012-0034 - CRL/120025

Name of Product: DL-Methionyl-DL-Methionine

Active Agent (s): DL-Methionyl-DL-Methionine

Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA)
Geel, Belgium

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EXECUTIVE SUMMARY

In the current application authorisation is sought under Articles 4(1) for DL-Methionyl-DL-Methionine, 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 the use of DL-Methionyl-DL-Methionine for all aquatic animal species. The feed additive is a white to cream powder having a minimum content of the active substance (Met-Met) of 93% and a maximum DL-Methionine content of 3%. It is intended to be mixed either in premixtures or to be added directly to complete feedingstuffs. The Applicant suggested no minimum or maximum feed additive concentrations in feedingstuffs.

For the quantification of DL-Methionyl-DL-Methionine in feed additive, premixtures and feedingstuffs the Applicant suggested at first the ring-trial validated Community method designed for the analysis of amino acids in premixtures and feedingstuffs. However, no experimental data were presented to demonstrate the applicability of the method to determine DL-Methionyl-DL-Methionine in the relevant matrices. Consequently, the Applicant was requested by the EURL to submit supplementary information, discussed hereafter.

For the quantification of DL-Methionyl-DL-Methionine in feed additive a single-laboratory validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to photometric detection (RP-HPLC-UV) was submitted. The following performance characteristics were calculated by the EURL based on the experimental data provided: relative standard deviation for repeatability (RSDr) ranging from 0.50 to 0.92%; relative standard deviation for intermediate precision (RSDip) of 0.63%; and recovery rate (RRec) of 100.1%. Based on these satisfactory performance characteristics, the EURL recommends for official control, the single-laboratory validated and further verified RP-HPLC-UV method to quantify DL-Methionyl-DL-Methionine in feed additive.

For the quantification of DL-Methionyl-DL-Methionine in premixtures and feedingstuffs the Applicant submitted experimental data obtained applying the ring-trial validated Community method based on HPLC (or amino acid analyser) equipped with ion exchange column, post column derivatisation and photometric detection at 570 nm (IEC-UV). The Applicant applied the protocol for the determination of free (synthetic and natural) amino acids. The following performance characteristics were reported for premixtures: RSDr ranging from 5.2 to 5.4%; RSDip ranging from 4.0 to 4.3%; and RRec ranging from 89.1 to 101.9%; and for feedingstuffs: RSDr ranging from 1.6 to 4.4%; RSDip ranging from 1.9 to 4.0%; and RRec ranging from 89.8 to 90.8%.

Based on these satisfactory performance characteristics, the EURL recommends for official control the ring-trial validated Community method, based on IEC-UV to quantify DL-Methionyl-DL-Methionine 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) is not considered necessary.

KEYWORDS

DL-Methionyl-DL-Methionine, nutritional additives, amino acids, fish, all aquatic animal species or categories

1. BACKGROUND

In the current application authorisation is sought under Articles 4(1) (authorisation of a new feed additive) for DL-Methionyl-DL-Methionine, 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].

According to the Applicant DL-Methionyl-DL-Methionine (Met-Met) is a dipeptide of the already authorised nutritional feed additive DL-Methionine [3,4]. It is produced through chemical synthesis including a controlled elimination of water [3]. The feed additive is a white to cream powder having a minimum content of the active substance (Met-Met) of 93 % and a maximum DL-Methionine content of 3% [2,5].

Specifically, authorisation is sought for the use of DL-Methionyl-DL-Methionine for all aquatic animal species ("fish" according to Annex IV Commission Regulation (EC) No 429/2008) [2,6]. The feed additive is intended to be mixed either in premixtures or to be added directly to complete feedingstuffs [7]. The Applicant suggested no minimum or maximum feed additive concentrations in feedingstuffs [1].

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 DL-Methionyl-DL-Methionine 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 substance in feed additive, premixtures and feedingstuffs.
For the quantification of DL-Methionyl-DL-Methionine in feed additive, premixtures and feedingstuffs the Applicant suggested at first the ring-trial validated Community method for the analysis of amino acids in feed, designed for the analysis of premixtures and feedingstuffs [9,10]. However, no experimental data were presented to demonstrate the applicability of the method to determine DL-Methionyl-DL-Methionine in the relevant matrices. Consequently, the Applicant was requested by the EURL to submit supplementary information, discussed hereafter [11].

For the quantification of DL-Methionyl-DL-Methionine in feed additive the Applicant decided to submit a single-laboratory validated and further verified method based on Reversed Phase High Performance Liquid Chromatography (HPLC) coupled to photometric detection (RP-HPLC-UV) [12,13]. The method allows the quantification of the two diastereomers of DL-Methionyl-DL-Methionine (DD/LL and DL/LD), DL-Methionine and Methionine Diketopiperazine in feed additive.

20 mg of sample are diluted in 100ml mobile phase and sonicated. A filtered aliquot of the extract is directly injected in the HPLC system. The different compounds are separated in gradient mode via a RP column and detected by UV at 205 nm [12]. The following performance characteristics were calculated by the EURL based on the experimental data provided [14]:
- a relative standard deviation for repeatability (RSDₜ) ranging from 0.50 to 0.92%;
- a relative standard deviation for intermediate precision (RSDᵦ) of 0.63%; and
- a recovery rate (RRec) of 100.1%

Based on these satisfactory performance characteristics, the EURL recommends for official control the single-laboratory validated and further verified RP-HPLC-UV method to quantify DL-Methionyl-DL-Methionine in feed additive.

For the quantification of DL-Methionyl-DL-Methionine in premixtures and feedingstuffs the Applicant applied the ring-trial validated Community method mentioned above [10]. The method is based on HPLC (or amino acid analyser) equipped with ion exchange column, post column derivatisation and photometric detection (IEC-UV). It does not distinguish between
the salts and the amino acid enantiomers. The Applicant applied the specific protocol for the determination of *free* amino acids [10,15,16].

*DL-Methionyl-DL-Methionine* is extracted with diluted hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and filtered. The pH of the clear solution is adjusted to 2.2. The amino acids are separated by Ion Exchange Chromatography (IEC) and determined by post column derivatisation with ninhydrin and photometric detection at 570 nm. Furthermore, the Applicant indicated that the *DL-Methionyl-DL-Methionine* peak could be interfered when analysing matrices containing 4-amino butyric acid (e.g. in soya) [17]. A slight modification in the chromatographic programme (e.g. buffer gradient) is advised to achieving the complete separation [18]. The following performance characteristics were reported for:

i) *premixtures* [19, 20]
- $\text{RSD}_r$ ranging from 5.2 to 5.4%;
- $\text{RSD}_{ip}$ ranging from 4.0 to 4.3%; and
- $\text{R}_{Rec}$ ranging from 89.1 to 101.9%;

ii) *feedingstuffs* [18, 21]:
- $\text{RSD}_r$ ranging from 1.6 to 4.4%;
- $\text{RSD}_{ip}$ ranging from 1.9 to 4.0%; and
- $\text{R}_{Rec}$ ranging from 89.8 to 90.8%.

Based on these satisfactory performance characteristics, the EURL recommends for official control the ring-trial validated Community method, based on IEC-UV to quantify *DL-Methionyl-DL-Methionine* 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) is not considered necessary.

### 4. CONCLUSIONS AND RECOMMENDATIONS

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

- the single-laboratory validated and further verified method based on reversed phase high performance chromatography (RP-HPLC-UV) to quantify *DL-Methionyl-DL-Methionine* in *feed additive*;
- the ring-trial validated Community method, using ion exchange chromatography coupled with photometric detection (IEC-UV) to quantify *DL-Methionyl-DL-Methionine* in *premixtures* and *feedingstuffs*. 

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Recommended text for the register entry (analytical method)
For the quantification of DL-Methionyl-DL-Methionine in feed additive:
- reversed-phase high performance liquid chromatography coupled to photometric detection at 205 nm (RP-HPLC-UV)

For the quantification of DL-Methionyl-DL-Methionine in premixtures and feedingstuffs:
- ion exchange chromatography coupled with post-column derivatisation and photometric detection at 570 nm (IEC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL
In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of DL-Methionyl-DL-Methionine 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
[3] *Technical dossier, Section II: 2.1.1 Name of the additive
[5] *Technical dossier, Section II: 2.2.1.1 Chemical substances
[7] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
[9] *Technical dossier, Section II: 2.6.1 Method(s) of analysis for the active substance
[12] *Supplementary information, AnnexII_29_Method Validation-HPLC-0550.pdf
[13] *Supplementary information, Method Validation and Verif in the Feed Additive.pdf
[14] *Supplementary information, Perf C Recalc.xlsx
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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- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Freistaat Sachsen, Nossen¹ (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratori Agroalimentari, Departament d’Agricultura, Ramadera i Pesca, Generalitat de Catalunya, Cabrils (ES)
- 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)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)

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