
| Dossier related to: | FAD-2010-0261  
|                    | CRL/ 100260   |
| Name of Feed Additive: | L-Cystine   |
| Active Agent(s): | L-Cystine   |
| Rapporteur Laboratory: | European Union Reference Laboratory for Feed Additives (EURL-FA)  
|                    | Geel, Belgium |
| Report prepared by: | Stefano Bellorini (EURL-FA)  |
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| Date: | 08/05/2011  |
| Report approved by: | Christoph von Holst  |
| Date: | 08/05/2011  |
EXECUTIVE SUMMARY

In the current application authorisation is sought for L-Cystine under Articles 4(1), category 'nutritional additives' and functional group 3(c) 'amino acids, their salts and analogues' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of L-Cystine for all animal species and categories. The feed additive is intended to be mixed either in premixtures or added directly to complete feedingstuffs. The Applicant suggested no minimum or maximum L-Cystine concentrations in premixtures and feedingstuffs.

For the determination of the active substance in the feed additive the Applicant submitted the internationally recognised European Pharmacopoeia titrimetric method. No performance characteristics of this method are provided. However, the EURL considers this method suitable to determine L-Cysteine in the feed additive within the frame of official control.

For the determination of Cystine in premixtures and feedingstuffs the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009). The method applies for the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. However, only performance characteristics for the determination of total Cystine are reported:

- a relative standard deviation for repeatability (RSD_r) ranging from 1.7 to 4.6%;
- a relative standard deviation for reproducibility (RSD_R) ranging from 8.8 to 19%.

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 Cystine 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

L-Cystine, nutritional additives, amino acids, all animal species and categories.
1. BACKGROUND

*L-Cystine* is already authorised as *feed additive* as "sensory additive", functional group "flavouring compounds", under the category "natural or corresponding synthetic chemically defined flavouring" [1]. In the current application authorisation is sought for *L-Cystine* under Articles 4(1), category of 'nutritional additives' functional group 3(c) 'amino acids, their salts and analogues' according to Annex I of Regulation (EC) No 1831/2003 [2]. According to the Applicant, *L-Cystine* is produced by extraction from a natural source (i.e. keratin present in poultry feathers) [3]. The *feed additive* is a white solid crystalline powder with a minimum purity of 98.5% of the active substance [4, 5].

Specifically, authorisation is sought for the use of *L-Cystine* for all animal species and categories. The *feed additive* is intended to be mixed either in *premixtures* or added directly to complete *feedingstuffs* [6]. The Applicant suggested no minimum or maximum *L-Cystine* concentrations in *premixtures* and *feedingstuffs* [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-Cystine*, 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 and feedingstuffs.

For the determination of L-Cystine in the feed additive the Applicant proposed to apply the internationally recognised European Pharmacopoeia methods [9, 10]:

- for the identification, the optical rotation and infrared absorption spectrophotometry methods (test A & B respectively), and
- for the determination, the titrimetric method.

No performance characteristics of these methods are provided. However, the EURL considers these methods suitable to determine L-Cystine in the frame of official control.

For the determination of Cystine in premixtures and feedingstuffs the Applicant submitted the ring-trial validated Community method [11]. This method applies for the determination of free (synthetic and natural) 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 determined by post column derivatisation with ninhydrin and photometric detection at 570 nm. The procedure chosen for the determination of the total amino acids depends on the amino acids under investigation. Cystine must be oxidised to cysteic acid prior to hydrolysis. Oxidation is performed at 0° C with a performic acid/phenol mixture. Excess oxidation reagent is decomposed with sodium disulphite. Cysteic acid is hydrolysed with hydrochloric acid (6 mol/l) for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by ion exchange chromatography and determined after post column derivatisation with ninhydrin by photometric detection at 570 nm. The method doesn't distinguish between Cystine and Cysteine since both compounds are determined together as cysteic acid in hydrolysates of oxidised sample. Finally, the analytical result it is expressed as mass fraction of Cystine calculated from the measured cysteic acid and using a conversion factor (120.15 g/mol).

The Community method was ring trial validated using three different matrices listed in Table 1. This method was further ring-trial validated by twenty-three laboratories, resulting in the CEN EN ISO 13903:2005 method [12]. Furthermore, a limit of quantification of 0.35 g/kg feedingstuffs was derived for total Cystine. The performance characteristics reported for the determination of total Cystine are listed in Table 1.
Table 1: Method performance characteristics obtained in the frame of two different ring-trial validation exercises for the determination of Cystine in premixtures and feedingstuffs. The performance characteristics reported refer to total Cystine determination (peptide bound and free) and are a sum of Cystine and Cysteine (indicated as Cyst(e)ine).

<table>
<thead>
<tr>
<th>Intercomparison study</th>
<th>Matrix</th>
<th>Cyst(e)ine g/kg</th>
<th>RSDr (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broiler compound</td>
<td>4</td>
<td>2.8</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>Protein concentrate</td>
<td>5</td>
<td>2.6</td>
<td>12.3</td>
</tr>
<tr>
<td>ISO 13903:2005 [12]</td>
<td>Poultry meal</td>
<td>0.8</td>
<td>4.6</td>
<td>17.7</td>
</tr>
<tr>
<td>study carried out in 1994</td>
<td>Broiler finisher feed</td>
<td>0.3</td>
<td>3.1</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>Broiler starter feed</td>
<td>0.4</td>
<td>1.7</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Corn</td>
<td>0.2</td>
<td>3.9</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>Fishmeal</td>
<td>0.5</td>
<td>4.0</td>
<td>19.0</td>
</tr>
</tbody>
</table>

RSDr, RSDR - relative standard deviation for repeatability and reproducibility, respectively

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 Cystine in premixtures and feedingstuffs.

Furthermore, the EURL would like to underline that an additional interlaboratory comparison of the above-mentioned ISO-CEN method is scheduled for 2011 to prove its applicability to high content products (e.g. feed additive). When the corresponding validation results will be published, the EURL will consider this method suitable for the quantification of Cystine in the feed additive in the frame of official control.

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 European Pharmacopoeia 6.0, method 01/2008-0998, using titration to determine L-Cystine in feed additive;
– the ring trial validated Community method, using ion exchange chromatography coupled to post column derivatisation and photometric detection, to determine Cystine in premixtures and feedingstuffs.

**Recommended text for the register entry (analytical method)**

For the determination of L-Cystine in feed additive:

– Titrimetry, European Pharmacopoeia (Ph. Eur. 6.0, method 01/2008-0998)

For the determination of Cystine in premixtures and feedingstuffs:


5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of L-Cystine 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


[3] *Technical dossier, Section II: 2.3.1 Active substance(s)/agent(s)

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

[5] *Technical dossier, Section I: 1.1.2.b Identification of the additive

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


[9] *Technical dossier, Section II: 2.6.1 Method of analysis for the active substances


*Refers to Dossier no: FAD-2010-0261
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:

- 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)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer (DE)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Kmetijski inštitut Slovenije, Ljubljana (SI)