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European Union Reference Laboratory for Feed Additives

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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0215** **CRL/100050**
Name of Feed Additive:	**Taurine**
Active Substance(s):	**Taurine**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report revised by: Date:	**Piotr Robouch (EURL-FA)** **07/03/2012**
Report approved by: Date:	**Christoph von Holst** **08/03/2012**

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for *Taurine* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *Taurine* for all animal species and categories. *Taurine* is a white crystalline powder with a minimum purity of 98 %. It is intended to be incorporated directly in *feedingstuffs* or through *premixtures* or directly in *water.* However, the Applicant did not specify any minimum or maximum concentrations of the *feed additive* in *feedingstuffs* or *water*.

For the determination of *Taurine* in the *feed additive* the Applicant proposed the Japanese Pharmacopoeia method, based on infrared absorption and potentiometric titration with sodium hydroxide. The EURL identified instead the European Pharmacopoeia method (01/2010:20256) for the determination of amino acids, based on ion-exchange chromatography with post column ninhydrin derivatisation and spectrophotometric detection at 570 nm. The Applicant provided experimental evidence of the applicability of the European Pharmacopoeia method for the determination of *Taurine* in *feed additive* in the frame of the CDG 34 dossier (FAD-2010-0107). Based on the experimental evidence presented, the EURL recommends for official control the European Pharmacopoeia method for the determination of *Taurine* in *feed additive*.

The Applicant proposed the dedicated ring-trial validated AOAC method (AOAC 999.12) for the determination of *Taurine* in pet food and the ring-trial validated Community method (Commission Regulation (EC) No 152/2009 – Annex III, F) for determination of *amino acids* in *premixtures* and *feedingstuffs,* based on ion-exchange chromatography with post column ninhydrin derivatisation and photometric detection at 570 nm. *Taurine* is a derivative of cysteine, an amino acid which contains a sulfhydryl group. Structurally related to amino acids, *Taurine* could be analyzed by analytical methods developed for the determination of amino acids. Therefore, the Applicant applied the Community method, slightly modified, and proved that the method is suitable for the determination of *Taurine* in *premixtures* and *feedingstuffs*. Based on the performance characteristics presented, the EURL recommends for official control, the AOAC and the modified Community method for the determination of *Taurine* in *premixtures* and *feedingstuffs.*

For the determination of *Taurine* in *water* the Applicant proposed the ring-trial validated AOAC method (AOAC 997.05) for determination of *Taurine* in powdered milk and powdered infant formula, based on liquid chromatography. Based on the performance characteristics presented and rationale that water is simpler matrix than milk, the EURL recommends for official control, the AOAC method for the determination of *Taurine* in *water*.

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
Taurine, nutritional additive, vitamins, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water) and 10(2) (re-evaluation of the additive already authorised under provisions of Council Directive 70/524/EEC) for *Taurine* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003 [1]. According to the Applicant, *Taurine* is a white crystalline powder with a minimum purity of 98 % [2, 3]. Specifically, authorisation is sought for the use of *Taurine* for all animal species and categories [2]. The *feed additive* is intended to be incorporated directly in *feedingstuffs* or through *premixtures* or directly in *water*. No minimum or maximum concentrations of the *feed additive* in *feedingstuffs* or *water* are proposed by the Applicant [2], as previously set in the regulation [4]. However, a typical concentration range from 400 to 500 mg/kg in *feedingstuffs* is suggested by the Applicant [5].

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 particular dossier, the methods of analysis submitted in connection with *Taurine*, 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 [6].

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

For the determination of *Taurine* in the *feed additive* the Applicant proposed the Japanese Pharmacopoeia method [7], based on infrared absorption and potentiometric titration with sodium hydroxide. The EURL identified instead the European Pharmacopoeia method (01/2010:20256) for the determination of amino acids [8], based on ion-exchange chromatography with post column ninhydrin derivatisation and spectrophotometric detection at 570 nm. The internal standard used for analysis is norleucine. The Applicant provided experimental evidence of the applicability of this method for the determination of *Taurine* in *feed additive* in the frame of the CDG 34 dossier (FAD-2010-0107) [9]. Based on the experimental evidence presented, the EURL recommends for official control the European Pharmacopoeia method for the determination of *Taurine* in *feed additive*.

The Applicant proposed the ring-trial validated AOAC method (AOAC 999.12) [10] for the determination of *Taurine* in pet food. The method is based on the Reverse Phase High Performance Liquid Chromatography (RP-HPLC) with fluorescence detector. The samples are hydrolysed with 6M HCl in oven at 110 °C for 16 hours, cooled to room temperature, diluted with water, filtered through 0.2 µm syringe filter and evaporated to dryness. Samples are then dissolved in water and a Na_2CO_3 solution and a dansyl chloride working solution are added. Samples are then heated at 65 °C for 30 minutes. After cooling to room temperature, 2% H_3PO_4 solution is added. This is followed by the addition of 0.5M phosphate buffer and water. The samples are then analysed by RP-HPLC. The following performance characteristics were reported for pet food (wet and dry dog and cat food) [10], for a total taurine content ranging from 170 to 2250 mg/kg:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 3.2 to 10 %; and
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 6.1 to 16 %.

Furthermore, the Applicant submitted the ring-trial validated Community method (Commission Regulation (EC) No 152/2009 – Annex III, F) [11] for the determination of

amino acids in *premixtures* and *feedingstuffs*. The method is based on ion-exchange chromatography combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. 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 after post column derivatisation with ninhydrin by photometric detection at 570 nm. The following performance characteristics were provided (for *amino acids* other than *Taurine*) [11]:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 1.9 to 3.4 %; and
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.0 to 13 %.

Taurine is a derivative of cysteine, an amino acid which contains a sulfhydryl group. Structurally related to amino acids, *Taurine* could be analyzed by analytical methods for the determination of amino acids. Therefore, upon the request by the EURL, the Applicant applied the Community method to the determination of *Taurine* in *premixtures* [12] and *feedingstuffs* [13-15], introducing slight modifications in the extraction conditions. The Applicant used 0.2M Li-Citrate buffer (pH 2.2)/H_2O 50/50 as extraction liquid instead of 0.2% Thiodiglycol in 0.1M HCl, while the extraction time is extended from 1 to 3 hours [13]. The Applicant confirmed experimentally the applicability of the Community method for the deteremination of *Taurine* for *premixtures* and *feedingtuffs* and the EURL calculated a recovery rate of 105% and 111% in milk replacer and piglet feed, respectively.

Based on the performance characteristics presented, the EURL recommends for official control, the AOAC and the modified Community method for the determination of *Taurine* in *premixtures* and *feedingstuffs.*

For the determination of *Taurine* in *water* the Applicant proposed a ring-trial validated AOAC method (AOAC 997.05) [16] for determination of *Taurine* in powdered milk and powdered infant formula. The method is based on pre-column derivatisation using dansyl chloride as derivatisation agent followed by liquid chromatography with UV or fluorescence detection. The following performance characteristics were reported for powdered milk and powdered infant formula [16], for a total taurine content ranging from 35 to 600 mg/kg:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 2.2 to 13.4 %; and
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 4.5 to 21.9 %.

The method was tested for powdered milk samples (complex matrix with proteins and fat-soluble compounds); therefore it should apply to a simpler water matrix without any major interfering substances. Based on the performance characteristics presented, the EURL

recommends for official control, the AOAC 997.05 method for the determination of *Taurine* in *water*.

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 method, based on ion-exchange chromatography with post-column ninhydrin derivatisation and spectrophotometric detection at 570 nm, to determine *Taurine* in *feed additive*;
- the ring-trial validated Community method (Commission Regulation (EC) No 152/2009 – Annex III, F), based on ion-exchange chromatography with post-column ninhydrin derivatisation and photometric detection at 570 nm, to determine *Taurine* in *premixtures* and *feedingstuffs*;
- the ring-trial validated AOAC method (AOAC 999.12), based on Reverse Phase High Performance Liquid Chromatography (RP-HPLC) with fluorescence detector, to determine *Taurine* in *premixtures* and *feedingstuffs*;
- the ring-trial validated AOAC method (AOAC 997.05), based on liquid chromatography, to determine *Taurine* in *water*.

Recommended text for the register entry (analytical method)

For the determination of *Taurine* in *feed additive*:

- Ion-exchange chromatography with post column ninhydrin derivatisation (Ph. Eur. 6.6 – 2.2.56 – Method 1);

For the determination of *Taurine* in *premixtures* and *feedingstuffs*:

- Ion-exchange chromatography with post-column ninhydrin derivatisation and photometric detection: based on Commission Regulation (EC) No 152/2009 (Annex - III, F);
- Reverse Phase High Performance Liquid Chromatography (RP-HPLC) coupled to fluorescence detector (AOAC 999.12);

For the determination of *Taurine* in *water*:

- Liquid Chromatography coupled to UV or fluorescence detector (AOAC 997.05).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Taurine* 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, Reference SANCO/D/2 Forw. Appl. 1831/00122 (10205)-2010

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II – Identity, characterisation and conditions of use of the additive; Methods of analysis

[4] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs

[5] *Technical dossier, Section II, Ref. II.2.1 Vitamins in animal nutrition

[6] 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

[7] *Technical Dossier, Section II, Annex_II_4: Monograph Taurine

[8] European Pharmacopoeia Monograph 01/2010:20256 – *Amino acid analysis*

[9] #Technical dossier, Section II – Sect_II_Identity: 2.1. Identity of the additives - 2.5. Conditions of use of the additive – 2.6. Method of analysis and reference samples

[10] *Technical dossier, Section II, Annex_II_19: Method of analysis AOAC 999.12

[11] Commission Regulation (EC) No 152/2009 *laying down the methods of sampling and analysis for the official control of feed* – Annex III-F, Official Journal L54 (26.02.2009) p.23-32

[12] *Technical dossier, Section II, Annex_II_21: Method of analysis in premixtures – Verification study report

[13] *Supplementary information, FAD-2010-0215_Answer to EURL-FA_2012-01-11-final

[14] *Supplementary Information, FAD-2010-0215_Answer to EURL-FA_2011-11-25

[15] *Supplementary Information, EURL_Annex_B_Experimental-data-for-the-extension-of-scope-of-Community-Method

[16] *Technical dossier, Section II, Annex_II_20_Method_of_analysis_AOAC_997_05

*Refers to Dossier No. FAD-2010-0215

#Refers to Dossier No. FAD-2010-0107

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)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
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