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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-2010-0107 CRL/100171
Product Name:	Chemically defined flavourings from Chemical Group 34 – Amino acids
Active Substance(s):	Twenty chemically defined flavourings from Chemical Group 34
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA)
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EXECUTIVE SUMMARY

The *Chemically Defined Flavourings - Group 34* (*Amino acids*), in this application comprises twenty substances, for which authorisation as feed additives is sought under the category "sensory additives", functional group 2(b) "flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003.

In the current application submitted according to Article 4(1) and Article 10(2) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested. The flavouring compounds of interest have a purity ranging from 95% to 99%.

Mixtures of flavouring compounds are intended to be incorporated only into *feedingstuffs* or drinking *water*. The Applicant suggested no minimum or maximum levels for the different flavouring compounds in *feedingstuffs*.

For the identification of *amino acids* in the *feed additive*, the Applicant submitted the European Pharmacopeia method based on ion-exchange chromatography with post column ninhydrin derivatisation. Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the European Pharmacopeia method (Ph. Eur. 6.6 – 2.2.56) submitted by the Applicant for the qualitative identification of the individual (or mixture of) *flavouring compounds* of interest (listed in Table 1) in the *feed additive.*

For the qualitative identification of *amino acids* in *feedingstuffs* the Applicant suggested the ring-trial validated Community method for determination of *amino acids* using High Pressure Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. However, the method does not distinguish between the salts of amino acids and it cannot differentiate between D and L forms of amino acids. Furthermore, this method does not ensure the accurate identification/discrimination between (i) flavouring amino acids added at low concentration to the *feedingstuffs* and (ii) endogenous amino acids present at higher level of concentrations in the feed matrix. Therefore, EURL is unable to recommend a method for the official control to determine unambiguously *amino acids* added in *feedingstuffs*.

As no experimental data were provided by the Applicant for the identification of the *active substance(s)* in *water*, the EURL cannot evaluate nor recommend a method for the official control to identify the *active substance(s)* of interest (cf. Table 1) 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.

(*)Full list provided in EURL evaluation report, available from the EURL website.

KEYWORDS
Chemically Defined Flavourings - Group 34, *amino acids*, mixture of flavouring compounds, sensory additives, all species.

1. BACKGROUND

The *Chemically Defined Flavourings - Group 34* (*CDG 34*) is a grouped application for which authorisation as feed additive is sought under the category "sensory additives", functional group 2(b) "flavouring compounds" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. The *CDG 34* application contains twenty flavouring compounds (listed in Table 1) belonging to the group - described in Annex I of Commission Regulation (EC) No 1565/2000 [2] as – "*Amino acids*".

In the current application submitted according to Article 4(1) (new use in water) and Article 10(2) (re-evaluation of additives already authorised under Directive 70/524/EC) of Regulation (EC) No 1831/2003, the authorisation for all species and categories is requested [1].

The flavouring compounds of interest are produced by different routes of manufacturing, providing a purity ranging from 95% to 99% [3].

Mixtures of flavouring compounds are intended to be incorporated only into *feedingstuffs* or drinking *water* [4]. The Applicant suggested no minimum or maximum levels for the different flavouring compounds [3], but normal contents of single flavouring compounds in *feedingstuffs* range up to from 0.1 to 100 mg/kg [4].

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 *Chemically Defined Flavourings – Group 34,* and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

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, and dioxins) are available from the respective European Union Reference Laboratories [5].

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

For the identification of *amino acids* in the *feed additive* (listed in Table 1), the Applicant submitted the European Pharmacopeia method [6] based on ion-exchange chromatography with post column ninhydrin derivatisation and using norleucine as internal standard. The system suitability check was performed using an equal weight mixture of three amino acids (threonine, methionine and lysine). The obtained characteristics of the chromatogram - related to quantitative compositions, peak shapes and elution order - should be comparable with those of the reference chromatogram (Figure II.1 and Table II.8 [4]). The twenty *amino acids* of CDG 34 were analysed in a mixture containing the substances at equal concentration (1000 mg/kg). Each substance was identified by comparison with a corresponding amino acid standard. The Applicant provided the typical chromatogram for the *CDG 34* of interest (cf. Fig II.2-3 [4]). The method does not distinguish between the salts of amino acids, nor differentiates between D and L forms of amino acids.

Based on the satisfactory experimental evidence provided, the EURL recommends for official control for the European Pharmacopeia method submitted by the Applicant for the qualitative identification of the individual (or mixture of) *flavouring compounds* of interest (listed in Table 1) in the *feed additive.*

For the qualitative identification of *amino acids* in *feedingstuffs* the Applicant suggested the ring-trial validated Community method [7] for determination of *amino acids* using High Pressure Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. This method does not distinguish between the salts of amino acids, nor differentiates between D and L forms of amino acids. Furthermore, the Applicant applied this Community method to detect and identify *taurine* - an amino acid originally not included in the scope of the Community method [8]. As for *beta-Alanine*, the Applicant did not provide any experimental evidence.

However, this method does not ensure the accurate identification/discrimination between (i) flavouring amino acids added at low concentration to the *feedingstuffs* and (ii) endogenous amino acids present at higher level of concentrations in the feed matrix. Therefore, EURL is unable to recommend a method for the official control to determine unambiguously *amino acids* added in *feedingstuffs*.

Table 1. Retention Time for the flavouring compounds of *CDG 34* [4] - Ph. Eur. 6.6 – method 2.2.56. The method does not distinguish between the salts of amino acids, nor differentiates between D and L forms of amino acids; co-eluting compounds are highlighted.

FL-no	CAS-no	EU Register name	Retention time (min)
16.056	107-35-7	Taurine	6.4
17.001	107-95-9	beta-Alanine	81.6
17.002	56-41-7	L-Alanine	51.7
17.003	74-79-3	L-Arginine	127.4
17.005	56-84-8	L-Aspartic acid	20.7
17.008	71-00-1	L-Histidine	113
17.010	443-79-8	D,L- Isoleucine	73.7
17.012	61-90-5	L-Leucine	75.3
17.018	63-91-2	L-Phenylalanine	83.7
17.019	147-85-3	L-Proline	48.3
17.020	302-84-1	D,L-Serine	29.5
17.022	60-18-4	L-Tyrosine	79.6
17.023	516-06-3	DL- Valine	59.3
17.028	72-18-4	L-Valine	59.3
17.027	63-68-3	L-Methionine	68.1
17.033	52-90-4	L-Cysteine	45
-	7048-04-6	L-Cysteine HCl monohydrate	45
17.034	56-40-6	Glycine	50.3
-	142-47-2	Monosodium glutamate	35.1
-	56-86-0	L glutamic acid	35.1

FL-no: EU Flavour Number

As no experimental data were provided by the Applicant for the identification of the *active substance(s)* in *water*, the EURL cannot evaluate nor recommend a method for the official control to identify the *active substance(s)* of interest (cf. Table 1) 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

The EURL recommends for official control the European Pharmacopeia method submitted by the Applicant, based on ion-exchange chromatography with post-column ninhydrin derivatisation, for the qualitative identification of the twenty *flavouring compounds* of the *CDG 34* in the *feed additive* of the individual (or mixture of) *flavouring compounds* of interest. However, the method does not distinguish between the salts of amino acids and it cannot differentiate between D and L forms of amino acids.

The Applicant submitted the Community method for the determination of *amino acids* in *feedingstuffs*. However, this method does not ensure the accurate identification/discrimination between (i) flavouring amino acids added at low concentration to the *feedingstuffs* and (ii) endogenous amino acids present at higher level of concentrations in the feed matrix. Therefore, EURL is unable to recommend a method for the official control to determine unambiguously *amino acids* added in *feedingstuffs*.

The Applicant provided no experimental data for *water*, therefore the EURL is unable to recommend a method for the identification of the twenty *flavouring compounds* of the *CDG 34* in *water*.

Recommended text for the register entry (analytical method)

For the identification of twenty *flavouring compounds* in mixtures of flavourings:

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

(new) EURL website:
http://irmm.jrc.ec.europa.eu/EURLs/EURL_feed_additives/authorisation/evaluation_reports

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Chemically Defined Flavourings – Group 34* (*CDG 34*) 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. 1831/077-2010

[2] Commission Regulation (EC) No 1565/2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council

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

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

[5] 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

[6] *Technical dossier, Section II – Annex_II.04_European Pharmacopeia 6.6 – Method 2.2.56 (2009)

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

[8] *Technical dossier, Section II – Annex_II.20_Study report CRL

* 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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