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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-2010-0115
EURL/100086

Product Name: Chemically Defined Flavourings 30
(Miscellaneous substances)

Active Substance(s): Glycyrrhizic acid, ammoniated

Rapporteur Laboratory: European Union Reference Laboratory
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EXECUTIVE SUMMARY

In the current application authorisation is sought for *Glycyrrhizic acid, ammoniated* (Flavis No. 16.060) 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. *Glycyrrhizic acid, ammoniated* belongs to the *Chemically Defined Flavourings 30 (Miscellaneous substances)*, according to the Annex I of Commission Regulation (EC) No 1565/2000. Authorisation is sought for the use of the *feed additive* for all species and categories. The *feed additive* has a minimum purity of 98 %. The *feed additive* is intended to be incorporated only into *feedingstuffs* or *drinking water*, in combination with other flavouring substances as constituents of *flavouring mixtures*. The Applicant suggested no minimum or maximum levels for the *Glycyrrhizic acid, ammoniated*.

For the identification of *Glycyrrhizic acid, ammoniated* in the *feed additive* and in *flavouring mixtures*, the Applicant proposed the internationally recognised European Pharmacopoeia method 01/2008:1772, based on liquid chromatography. This method was proved by two independent laboratories to identify the *Glycyrrhizic acid, ammoniated*. Furthermore, the same European Pharmacopoeia describes a potentiometric titration with perchloric acid, to quantify total *Glycyrrhizic acid, ammoniated*.

Therefore, the EURL recommends for official control the European Pharmacopoeia method 01/2008:1772, for the qualitative identification of the *Glycyrrhizic acid, ammoniated* in the *feed additive* and *flavouring mixtures*.

As no experimental data were provided by the Applicant for the determination of the product in *feedingstuffs* and *water*, the EURL could not evaluate nor recommend the method for official control to determine *Glycyrrhizic acid, ammoniated* in *feedingstuffs* and *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

Glycyrrhizic acid ammoniated, Chemically Defined Flavourings, flavouring mixtures, sensory additives, all 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 additives already authorised under Directive 70/524/EC) for *Glycyrrhizic acid, ammoniated* (Flavis No. 16.060) 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. *Glycyrrhizic acid, ammoniated* belongs to the *Chemically Defined Flavourings 30 (Miscellaneous substances)*, according to the Annex I of Commission Regulation (EC) No 1565/2000 [2]. Authorisation is sought for the use of the *feed additive* for all species and categories [3].

The *feed additive* is the ammoniated salt manufactured from the naturally occurring triterpenoid saponin glycyrrhizic acid (Flavis No. 16.012) which is extracted from the roots and rhizomes of the liquorice plant *Glycyrrhiza glabra* [4]. The *feed additive* is a mixture of ammonium 18 α - and 18 β -glycyrrhizate, the β -isomer being the main component [4]. The *feed additive* has a minimum purity of 98 % [3].

The *feed additive* is intended to be incorporated only into *feedingstuffs* or drinking water, in combination with other flavouring substances as constituents of *flavouring mixtures* [4]. The Applicant suggested no minimum or maximum levels for the *Glycyrrhizic acid, ammoniated* [3], but normal contents of the flavouring compound 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 *Glycyrrhizic acid, ammoniated*, 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 *Glycyrrhizic acid, ammoniated* in the *feed additive* and in *flavouring mixtures*, the Applicant proposed the internationally recognised European Pharmacopoeia method 01/2008:1772 [6], based on High Performance Liquid Chromatograph (HPLC) coupled to ultraviolet detection. This method was proved by two independent laboratories to identify the *Glycyrrhizic acid, ammoniated* in *flavouring mixtures* [7,8] containing several compounds (i.e. sweeteners, botanical extracts and chemically defined flavourings). The method should work for all similar mixtures, however co-elution with another compound cannot be excluded.

For the determination of total *Glycyrrhizic acid, ammoniated* (sum of the two isomers 18 α - and 18 β -glycyrrhizate) in the *feed additive*, the same European Pharmacopoeia prescribes a potentiometric titration with perchloric acid.

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method 01/2008:1772, for the qualitative identification of *Glycyrrhizic acid, ammoniated* in the *feed additive* and in *flavouring mixtures*.

As no experimental data were provided by the Applicant for the determination of the product in *feedingstuffs* and *water*, the EURL could not evaluate nor recommend the method for official control to determine *Glycyrrhizic acid, ammoniated* in *feedingstuffs* and *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 Pharmacopeia 6.0, method 01/2008:1772 to determine *Glycyrrhizic acid, ammoniated* in the *feed additive* and in *flavouring mixtures*.

The Applicant provided no experimental data for *feedingstuffs* and *water*, therefore the EURL is unable to recommend a method for the determination of *Glycyrrhizic acid, ammoniated* in *feedingstuffs* or in *water*.

Recommended text for the register entry (analytical method)

For the identification of the *Glycyrrhizic acid, ammoniated* in the *feed additive* and *flavouring mixtures*:

- European Pharmacopeia 6.0, method 01/2008:1772

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Glycyrrhizic acid, ammoniated* 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. 1831/075-2010
 - [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_7_PhEu 2010 Ammonium glycyrrhizate
 - [7] *Technical dossier, Section II – AnnexII16Ph EUR Method in premixes – A
 - [8] *Technical dossier, Section II – Annex_II_17_Ph EUR Method in premixes – B
- * Refers to Dossier No. FAD-2010-0115

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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- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer (DE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)