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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-2010-0123 CRL/100090
Feed additive:	*Tannic acid (Flavis No. 16.080)*
Active Substance(s):	*Tannic acid*
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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Report approved by: Date:	Christoph von Holst 07/11/2011

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for the *feed additive Tannic acid (Flavis 16.080)* under the "sensory additives", functional group 2(b) "flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. *Tannic acid* belongs to the *Chemically Defined Flavourings - Group 30 (CDG 30)* - described in Annex I of Commission Regulation (EC) No 1565/2000 as – "*miscellaneous substances*". Authorisation is sought for the use of the *feed additive* for all species and categories.

Tannic acid is a yellow brown granular powder with a minimum purity of 93 %. *Tannic acid* is intended to be incorporated in *feedingstuffs* or drinking *water*, only in combination with other flavouring substances as *mixture of flavouring compounds*. The Applicant suggested no minimum or maximum, but normal contents of flavouring compound in *feedingstuffs* range from 0.1 to 100 mg/kg.

For the identification of *Tannic acid* in the *feed additive*, the Applicant proposed the internationally recognised European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 1477), based on colorimetric or precipitation tests. Additionally, the Applicant proposed for the determination of *Tannic acid* in the *feed additive* the internationally recognised FAO JECFA monograph for food additives, in which: (i) identification is based on Thin Layer Chromatography (TLC), with Retention factors (R_f) of the sample and reference standard have to be the same; and (ii) quantification is based on gravimetric method.

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 1477) and the FAO JECFA monograph for the determination of *Tannic acid* in the *feed additive*.

For the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*, the Applicant proposed a qualitative method based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC) coupled to an UV detector measuring at 270 nm. In order to demonstrate the transferability of the proposed analytical method, the qualitative method was tested successfully in a second independent laboratory using four commercial premixtures, in the frame of a verification study.

Based on the satisfactory experimental evidence provided, the EURL recommends for official control the method submitted by the Applicant, based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC), for the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*.

The Applicant did not provide any experimental method or data for the identification of *Tannic acid* in *feedingstuffs* and *water*. Therefore the EURL cannot evaluate nor recommend any method for official control to identify *Tannic acid* in *feedingstuffs* or *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
Tannic acid, sensory additive, flavouring compounds, 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 additives already authorised under the provisions of the Council Directive 70/524/EEC) for the *feed additive Tannic acid (Flavis 16.080)* under the "sensory additives", functional group 2(b) "flavouring compounds" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. *Tannic acid* belongs to the *Chemically Defined Flavourings - Group 30 (CDG 30)* - described in Annex I of Commission Regulation (EC) No 1565/2000 as – "*miscellaneous substances*". Authorisation is sought for the use of the *feed additive* for all species and categories [2].

Tannic acid is a yellow brown granular powder, consisting of a minimum purity of 93 % on dry matter basis [2,3]. *Tannic acid* is intended to be incorporated in *feedingstuffs* or drinking *water*, only in combination with other flavouring substances as *mixture of flavouring compounds* [3]. The Applicant suggested no minimum or maximum, but normal contents of flavouring compound in *feedingstuffs* range from 0.1 to 100 mg/kg [3].

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 *Tannic acid (Flavis 16.080)*, 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, mycotoxins, dioxins and PAHs) are available from the respective European Union Reference Laboratories [4].

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

For the identification of *Tannic acid* in the *feed additive*, the Applicant proposed the internationally recognised European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 1477) [5], based on colorimetric or precipitation tests.

Additionally, the Applicant proposed for the determination of *Tannic acid* in the *feed additive* the internationally recognised FAO JECFA monograph for food additives [6], in which:

- Identification of *Tannic acid* in the *feed additive* is based on Thin Layer Chromatography (TLC), with Retention factors (R_f) of the sample and reference standard have to be the same;
- Quantification of *Tannic acid* in the *feed additive* is based on a gravimetric method.

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 1477) and the FAO JECFA monograph for the determination of *Tannic acid* in the *feed additive*.

For the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*, the Applicant proposed a qualitative method based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC) coupled to an UV detector measuring at 270 nm [7]. Tannic acid is analysed after removal of lipophilic substances with n-hexane and after an enzymatic hydrolysis to gallic acid using tannase. The enzymatic reaction is stopped with caffeic acid. The peak identification is carried out by retention time comparison with a standard gallic acid solution.

In order to demonstrate the transferability of the proposed analytical method, the qualitative method was tested successfully in a second independent laboratory using four CRINA® premixtures, in the frame of a verification study [8].

Based on the satisfactory experimental evidence provided, the EURL recommends for official control the method submitted by the Applicant, based on Reversed Phase High Performance Liquid Chromatography (RP-HPLC), for the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*.

The Applicant did not provide any experimental method or data for the identification of *Tannic acid* in *feedingstuffs* and *water*. Therefore the EURL cannot evaluate nor recommend any method for official control to identify *Tannic acid* in *feedingstuffs* or *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 (Ph. Eur. 6th edition, monograph 1477) and the FAO JECFA monograph using colorimetric or precipitation qualitative tests and a gravimetric method for the determination of *Tannic acid* in the *feed additive*;
- the qualitative method submitted by the Applicant, based on Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV) for the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds.*

The Applicant did not provide any experimental method or data for the identification of *Tannic acid* in *feedingstuffs* and *water*. Therefore the EURL cannot evaluate nor recommend any method for official control to identify *Tannic acid* in *feedingstuffs* or *water*.

Recommended text for the register entry (analytical method)

For the determination of *Tannic acid* in the *feed additive*:

> Qualitative colorimetric or precipitation tests (Ph. Eur. 6th edition, monograph 1477) and quantitative gravimetric method (FAO JECFA Tannic acid monograph)

For the identification of *Tannic acid* (as gallic acid) in the *mixture of flavouring compounds*:

> Reversed Phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Tannic acid* 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. 1831/0094(9865)-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] 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

[5] *Technical dossier, Section II – Annex_II_20_Ph. Eur

[6] *Technical dossier, Section II – Annex_II_13_FAO 2009 Tannic acid monograph

[7] *Technical dossier, Section II – FFAC Tannic acid Valid. MoA premix Rep. 9937

[8] *Technical dossier, Section II – FFAC Tannic acid Verif. MoA_premix_Rep. 10316

* Refers to Dossier No. FAD-2010-0123

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)
- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, (DE)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)