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

Ginkgo biloba L. extract

(FAD-2010-0328; CRL/100327)

European Union Reference Laboratory
Feed Additives

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

Dossier related to:	**FAD-2010-0328 - CRL/100327**
Name of Feed Additive:	***Ginkgo biloba L. extract***
Phytochemical marker(s)	**Flavonoids** **Ginkgolides A, B and C** **Bilobalides** **Ginkgolic acids**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Z. Ezerskis & C. von Holst** **20/08/2018**
Report approved by: Date:	**Christoph von Holst** **20/08/2018**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(2) for the botanically defined *Ginkgo biloba L. extract* under the category / functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the *feed additive* is sought to be used for cats and dogs.

The *feed additive* is a brown fine powder containing a mixture of chemical components naturally present, such as *flavonoids*: *flavonols (quercetin, kaempferol* and *isorhamnetin)* in the form of glycosides; diterpene lactones: *ginkgolides A, B, C, J, and M*; sesquiterpene lactone – *bilobalide*; and *ginkgolic acids*. The standardised extracts contain 22 to 27 % of the *flavonol* glycosides; 2.8 to 3.4 % of *ginkgolides A, B and C*; 2.6 to 3.2 % of *bilobalide*, and less than 5 mg of *ginkgolic acids*/kg extract. According to the Applicant, the phytochemical markers of the *feed additive* - as specified by European Pharmacopoeia Monograph 04/2008:1827 - are (1) the sum of various *flavonoids quantified against quercetin*, (2) *terpene lactones* (e.g. *ginkgolides A, B and C*, *bilobalide*) and (3) *ginkgolic acids*.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant proposed no minimum or maximum level of the *feed additive*; however, a maximum content of 40 mg *feed additive* /kg *feedingstuffs* was suggested by the Applicant.

For the determination of Ginkgo flavonoids in the *feed additive* the Applicant applied an in-house analytical method based on the European Pharmacopoeia Monograph 04/2008:1827. The mentioned European Pharmacopoeia Monograph is related to, refined and quantified dry extract produced from Ginkgo leaf and describes assays for identification, based on thin layer chromatography (TLC) and for quantification, based on high performance liquid chromatography (HPLC).

The EURL recommends for official control the methods included in the above mentioned European Pharmacopoeia Monograph for the quantification of the selected phytochemical markers in the *feed additive*.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Quercetin, ginkgolides A, B and C; *bilobalide* and *ginkgolic acid, ginkgo biloba L.*, sensory additives, cats and dogs

1. BACKGROUND

In the current application authorisation is sought under Article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for the botanically defined *Ginkgo biloba L. extract* under the category / functional group (2 b) "sensory additives"/"flavouring compounds", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1][2]. Specifically, the *feed additive* is sought to be used for cats and dogs [1][2][3].

The *feed additive* is a brown fine powder containing a mixture of chemical components naturally present, such as *flavonoids*: *flavonols (quercetin*, *kaempferol* and *isorhamnetin)* in the form of glycosides; diterpene lactones: *ginkgolides A, B, C, J, and M*; sesquiterpene lactone – *bilobalide*; and *ginkgolic acids*. The standardised extracts contain 22 to 27 % of the *flavonol* glycosides; 2.8 to 3.4 % of *ginkgolides A, B and C*; 2.6 to 3.2 % of *bilobalide*, and less than 5 mg of *ginkgolic acids*/kg extract [4]. According to the Applicant, the phytochemical markers of the *feed additive* - as specified by European Pharmacopoeia Monograph 04/2008:1827 - are (1) the sum of various *flavonoids* quantified against *quercetin*, (2) *terpene lactones* (e.g. *ginkgolides A, B and C, bilobalide*) and (3) *ginkgolic acids* [3][4].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant proposed no minimum or maximum level of the *feed additive*; however, a maximum content of 40 mg *feed additive* /kg *feedingstuffs* was suggested by the Applicant.

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 *Ginkgo biloba L* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the determination of Ginkgo flavone glycosides in the *feed additive* [5] the Applicant submitted an in-house analytical method [6] based on the European Pharmacopoeia Monograph 04/2008:1827 [7].

The above mentioned European Pharmacopoeia Monograph is dedicated to refined and quantified dry extract produced from ginkgo leaf with the qualitative and quantitative composition as previously specified. For the determination of the phytochemical markers the following three methods of European Pharmacopoeia Monograph 04/2008:1827 are used.

For the quantification of flavonoids the assay based on a high performance liquid chromatography (HPLC) coupled to spectrophotometric detection at 370 nm. The sample is mixed with a solution of methanol, hydrochloric acid and water. Then, the mixture is heated in a water bath and cooled down for further chromatographic analysis. The quantification is performed by summing up the areas of all flavonoids identified in the chromatogram under the conditions of this method and calibrating the obtained value against quercetin dihydrate used as a standard substance [7]. The content of flavonoids in expressed as flavone glycosides.

The assay of terpene lactones (e.g. bilobalide, ginkgolide A, B and C) is carried out by using HPLC coupled to a refractive index detector set at 35 ^{o}C. The sample is dissolved in phosphate buffer and transferred to a preparative chromatography column. An ethyl acetate is used for the elution and the eluent containing the analytes is collected, evaporated to dryness and reconstituted with the mobile phase before its injection into the chromatographic system. The quantification of the individual terpene lactones is performed using benzyl alcohol as a standard substance.

The assay of ginkgolic acids (e.g. C13, C15 and C17) is based on HPLC coupled to spectrophotometric detection at 210 nm. The sample is dissolved in methanol and centrifuged before its injection into the chromatographic system. The quantification of ginkgolic acids is performed by summing up the areas of the three ginkgolic acids and calibrating the obtained value against ginkgolic acid (C17) used as a standard substance.

In addition, the Applicant applied a single-laboratory validated method [6] based on the assay of flavonoids described in the above mentioned monograph [7] for the determination of Ginkgo flavone glycosides in seven different batches of the *feed additive* and a relative standard deviation for repeatability (RSD_r) of 7.2 % was derived [5].

Based on the available performance profile, the EURL recommends for official control the assays of flavonoids, terpene lactones and ginkgolic acids of the European Pharmacopoeia Monograph (04/2008:1827) for the quantification of the selected phytochemical markers in the *feed additive*.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

Evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the identification/characterisation of the *feed additive* the EURL considers the test based on thin layer chromatography (TLC) described in the above mentioned European Pharmacopeia monograph 04/2008:1827 fit for purpose [7].

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. as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the assays of flavonoids, terpene lactones and ginkgolic acids of European Pharmacopoeia Monograph (04/2008:1827) for the quantification of the selected phytochemical markers in the *feed additive*.

Recommended text for the register entry (analytical method)

For the quantification of the selected phytochemical markers (flavonoids, terpene lactones and ginkgolic acids) in the *feed additive*:

- High performance liquid chromatography (HPLC) – European Pharmacopoeia Monograph 04/2008:1827

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Ginkgo biloba L* 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 SANTE_E5_FWD. APPL. 1831-0019-2018

[2] *Application form, Annex I, Submission No. 1288803335697-1392

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

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

[5] *Technical dossier, Section II: Annex II.1.0.2

[6] *Technical dossier, Section II: Annex II.6.0.1

[7] European Pharmacopoeia Monograph 6.0, 04/2008:1827

*Refers to Dossier no: FAD-2010-0328

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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