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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

Botanically Defined Flavourings Group BDG 12 – Gentianales

(FAD-2010-0321; CRL/100206)

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-2010-0321 - CRL/100206**
Name of Feed Additive:	***Gentian tincture from Botanically Defined Flavourings Group BDG 12***
Phytochemical marker(s):	**-**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Stefano Bellorini** **05/11/2020**
Report approved by: Date:	**Christoph von Holst** **05/11/2020**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4(1) and Article 10(2) for *gentian tincture* from *botanically defined flavourings group BDG 12 - Gentianales* 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. The authorisation is sought for the use of the *feed additive* for all animal species and categories.

In the updated dossier the Applicant described *gentian tincture* as brown aqueous/alcoholic preparation from *Gentiana lutea L.* roots, containing a mixture of chemical components naturally present in the plant such as polyphenols, flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside as major constituents.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or in combination with other flavouring substances (flavouring premixtures) with proposed maximum levels ranging from 50 to 600 mg *feed additive*/kg *feedingstuffs* (or *water* for drinking) depending on the target animal species.

The Applicant proposed to characterise the *feed additive* (*gentian tincture*) by the determination of the content of dry matter, ash, total polyphenols, total flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside. According to the Applicant the use of high performance thin-layer chromatography (HPTLC) profiles as a fingerprint for the identification of the *feed additive* is considered a reliable way to identify the *feed additive*.

For the identification and characterisation of the *feed additive* the EURL recommends the above mentioned methods based on gravimetry, spectrophotometry and HPTLC to determine the contents of dry matter, ash, total polyphenols, total flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside.

The Applicant did not provide experimental data or analytical method for the determination of *gentian tincture* in *premixtures* and *feedingstuffs*, as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

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

Botanically defined flavourings group BDG 12 – Gentianales, gentian tincture, sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new use of the *feed additive*) and Article 10(2) (re-evaluation of additives already authorised under the provisions of the Council Directive 70/524/EEC) for *gentian tincture* from *botanically defined flavourings group BDG 12 - Gentianales* 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]. The authorisation is sought for the use of the *feed additive* for all animal species and categories [1].

The request for authorisation of another *feed additive* of this grouped application, namely *cat's claw extract* [1], was withdrawn later by the European Commission on the request from the Applicant [2]. Therefore, the EURL will focus in this report exclusively on the evaluation of the suitability of analytical methods for official control of the remaining *feed additive*, namely *gentian tincture*.

According to the initially submitted dossier, the Applicant specified gentiopicroside and luteolin as phytochemical markers for the *feed additive* (*gentian tincture*), indicating also the content of the markers as criteria [3,4]. However, in the updated dossier the Applicant modified the description of the *feed additive* by excluding the above mentioned phytochemical markers and describing *gentian tincture* as brown aqueous/alcoholic preparation from *Gentiana lutea L.* roots, containing a mixture of chemical components naturally present in the plant such as polyphenols, flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside as major constituents [5].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or in combination with other flavouring substances (flavouring premixtures) with maximum levels proposed by the Applicant ranging from 50 to 600 mg *feed additive* /kg *feedingstuffs* (or *water* for drinking) depending on the target animal species [6].

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 *gentian tincture* from *Botanically Defined Flavourings Group BDG 12 – Gentianales* 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)

The Applicant did not provide a method for the determination of a phytochemical markeras no markers were specified in the updated dossier. Instead, the Applicant submitted other methods aiming at the identification and characterisation of the *feed additive* (*gentian tincture*).

Furthermore, the Applicant did not provide experimental data or an analytical method for the determination of *gentian tincture* in *premixtures* and *feedingstuffs*, as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally.

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)

An 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)

The Applicant proposed to characterise the *feed additive* (*gentian tincture*) by the determination of the content of dry matter, ash, total polyphenols, total flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside [5,7].

For the determination of dry matter and ash contents in the *feed additive* the Applicant proposed the use of gravimetric methods [8].

For the determination of total polyphenols the Applicant proposed a spectrophotometric method at 760 nm after derivatisation with Folin-Ciocalteu's reagent [8], which is based on a similar method described in the European Pharmacopoeia monograph for the determination of tannins in herbal drugs [9].

For the determination of total flavonoids and xanthones (gentisin and isogentisin) the Applicant proposed two methods based on high performance thin-layer chromatography (HPTLC) [8]. These methods are based on the general method of flavonoid analysis in plants [10].

For the determination of gentiopicroside the Applicant proposed another HPTLC method [8], which is based on a similar method described in the European Pharmacopoeia monograph for *gentian tincture* [11].

The Applicant has provided the results of the analysis of five different batches of the *feed additive* (*gentian tincture*) characterised by applying the methods mentioned above. These analyses led to average values of 4.3 % (w/w) for dry matter content [5]; 0.1 % (w/w) for ash

content [5]; 0.08 % (w/v) for total polyphenols [5,12]; 0.05 % (w/v) for total flavonoids [5,12,13]; 0.003 % (w/v) for xanthones (gentisin and isogentisin) [5,12,14]; and 0.002 % (w/v) for gentiopicroside [5,12,15].

According to the Applicant the use of the HPTLC profiles as a fingerprint for the identification of the *feed additive* is considered a more reliable way to identify the *feed additive* and thus preferred to the analysis of individual phytomarkers [7].

For the identification and characterisation of the *feed additive* the EURL recommends the above mentioned methods based on gravimetry, spectrophotometry and HPTLC to determine the contents of dry matter, ash, total polyphenols, total flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside.

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 the identification and characterisation of *feed additive* (*gentian tincture*) (i) the gravimetric methods for the determination of dry matter and ash content; (ii) the spectrophotometric method for the determination of total polyphenols content; and (iii) the methods based on high performance thin-layer chromatography (HPTLC) for the determination of contents of total flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside.

Recommended text for the register entry (analytical method)

For the identification and characterisation of the *feed additive* (*gentian tincture*):

- gravimetry for the determination of dry matter and ash content;
- spectrophotometry for the determination of total polyphenols content; and
- high performance thin-layer chromatography (HPTLC) for the determination of contents of total flavonoids, xanthones (gentisin and isogentisin) and gentiopicroside

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Botanically Defined Flavourings Group BDG 12 – Gentianales* 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: FWD. APPL. 00199(10275)/1831/-2010
[2] *Supplementary information – Partial withdrawal of applications for various botanically defined groups from FEFANA ASBL - Ares(2019)129932
[3] *Application, Proposal for Register Entry – Annex A
[4] *Technical dossier, Section II: 2.1. Identity of the additive
[5] *Supplementary information – Section II: 2.1.3 - Qualitative and quantitative composition (active substance(s), other components, batch to batch variation)
[6] *Supplementary information – Section II: 2.5 - Conditions of use of the additive
[7] *Supplementary information – Section II: 2.2 - Characterisation of the active substance(s)
[8] *Supplementary information – Annex_II_2_Methods of analysis
[9] European Pharmacopoiea monograph 2.8.14
[10] H. Wagner, S. Bladt. Plant Drug Analysis: A Thin Layer Chromatography Atlas (2nd ed., Springer, 1996)
[11] European Pharmacopoiea monograph 01/2008:1870
[12] *Supplementary information – Annex_II_3_Results of analysis
[13] *Supplementary information – Annex_II_10_Detailed report of flavonoides quantification
[14] *Supplementary information – Annex_II_8_Detailed report of xanthones quantification
[15] *Supplementary information – Annex_II_7_Detailed report of gentiopicroside quantification

*Refers to Dossier no: FAD-2010-0321

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

The following National Reference Laboratories contributed to this report:

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- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- [1]Wageningen Food Safety Research (WFSR) (NL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

[1] Name and address according to COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.