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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**

FLAMORE
(FAD-2020-0062; CRL/200045)



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in connection with the Application for Authorisation of a
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Dossier related to: **FAD-2020-0062 - CRL/200045**

Name of Product: ***FLAMORE***

Active Agent (s): **total polyphenols**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

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Date: **23/04/2021**

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Date: **23/04/2021**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4 for *FLAMORE* as *feed additive* 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, authorisation is sought for the use of the *feed additive* for dogs.

The *feed additive* is a blend of grape and blueberry extracts (*Vitis vinifera L.* and *Vaccinium angustifolium*), and it is to be used directly into *feedingstuffs*, or in complementary feed for dogs at a proposed level of 4 mg *FLAMORE* per kg of live body weight (BW) per day.

According to the Applicant, *polyphenols* are the main constituents of *FLAMORE*. Furthermore, the *total polyphenols* content expressed as catechin equivalent has been proposed by the Applicant as phytochemical marker for the characterisation of the *feed additive* (*FLAMORE*). As stated by the Applicant, typical minimum and maximum *total polyphenols* levels in *FLAMORE* are 20 and 40 % (w/w), respectively.

For the quantification of *total polyphenols* in the *feed additive* the Applicant proposed a spectrophotometric method based on the general European Pharmacopoeia Chapter 2.2.25. The *total polyphenols* are measured at 280 nm and expressed as catechin equivalent. However, the EURL identified instead a very similar ring-trial validated spectrophotometric method, also based on the European Pharmacopoeia Chapter 2.2.25, describing the quantification of *total polyphenols* expressed as catechin equivalent in a grape extract *feed additive* currently authorised. Upon request of the EURL the Applicant confirmed the equivalence of both analytical procedures.

Based on the experimental evidences the EURL recommends for official control for the characterisation of *FLAMORE* the ring-trial validated spectrophotometric method based on the European Pharmacopoeia Chapter 2.2.25 for the quantification of *total polyphenols* expressed as catechin equivalent.

Furthermore, the accurate quantification of added *FLAMORE* in *feedingstuffs* is not achievable experimentally. Consequently, the EURL cannot evaluate or recommend any method for official control to quantify *FLAMORE* in *feedingstuffs*.

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

FLAMORE, *Vitis vinifera L.*, *Vaccinium angustifolium*, total polyphenols, sensory additives, flavouring compounds, dogs

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *FLAMORE* 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]. Specifically, authorisation is sought for the use of the *feed additive* for dogs [1,2].

The Applicant described the *feed additive* as a powder composed by a blend of grape and blueberry extracts. The grape extract is obtained from grape seeds and grape skins (*Vitis vinifera L.*) while the blueberry extract is made from the fruits of wild blueberries (*Vaccinium angustifolium*) [3].

According to the Applicant, polyphenols are the main constituents of *FLAMORE*. Furthermore, the *total polyphenols* content expressed as catechin equivalent has been proposed by the Applicant as phytochemical marker for the characterisation of the *feed additive* (*FLAMORE*). As stated by the Applicant, typical minimum and maximum *total polyphenols* levels in *FLAMORE* are 20 and 40 % (w/w), respectively [2, 3].

The *feed additive* is to be used directly into *feedingstuffs* or in complementary feed for dogs at a proposed level of 4 mg *FLAMORE* per kg of live body weight (BW) per day [2,3].

Note: The EURL has previously evaluated an analytical method for the determination of *total polyphenols* content expressed as catechin equivalent in the frame of another dossier [4].

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 *FLAMORE* 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 quantification 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 proposed to characterise the *feed additive (FLAMORE)* through the quantification of the *total polyphenols* (phytochemical marker) expressed as catechin equivalent.

For the quantification of the *total polyphenols* in the *feed additive (FLAMORE)* the Applicant submitted a spectrophotometric method based on the European Pharmacopeia Chapter 2.2.25 [5]. Upon EURL request, the Applicant provided the standard operation procedure for applying this general method to the *feed additive* [6].

The *feed additive (FLAMORE)* is dissolved in ethanol and diluted with water (final content of ethanol should be below 1 % (w/w)). The mixture is then centrifuged. An aliquot of the supernatant is further diluted with water and the polyphenols content is measured at 280 nm and expressed as catechin equivalent [6]. Additionally, the Applicant applied this method to five different batches of the *feed additive* [7], leading to an average *total polyphenols* content of 37.5 % and a batch-to-batch variation (CV) of 4.4 %.

However, the EURL identified instead a very similar spectrophotometric method, also based on the European Pharmacopeia Chapter 2.2.25, describing the quantification of *total polyphenols* expressed as catechin equivalent in another grape extract *feed additive* that is currently authorised [8]. This method, suitable for total polyphenols above 20 %, was ring-trial validated for different grape sub-products rich in *polyphenols* i.e. with average *total polyphenol* content ≥ 63 % leading to a standard deviation for *reproducibility* (RSD_R) ranging from 1.2 to 4.5 % [4]. Following the request of the EURL the Applicant confirmed the equivalence of both analytical procedures.

Therefore, the EURL recommends for official control the ring-trial validated spectrophotometric method for the quantification of *total polyphenols* content expressed as catechin equivalent based on the European Pharmacopoeia Chapter 2.2.25 for the characterisation of *FLAMORE*.

The Applicant did not provide experimental data or an analytical method for the quantification of *FLAMORE* in *feedingstuffs*, as the unambiguous quantification of the *feed additive* added to this matrix is not achievable experimentally. Therefore, the EURL cannot evaluate or recommend any method for official control for the quantification of *FLAMORE* in *feedingstuffs*.

Methods of analysis for the quantification 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 further characterise the *feed additive (FLAMORE)* through its physical characterisation i.e. loss of drying and particle size.

For the loss of drying and particle size in the *feed additive* the Applicant proposed the use of the general methods described in the European Pharmacopeia Chapter 2.8.17 & Chapter 2.9.38, respectively [9, 10]. Furthermore, the Applicant applied these methods to different batches of the *feed additive* [7, 11].

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 characterisation of *FLAMORE* the quantification of the *total polyphenols* (phytochemical marker) expressed as catechin equivalent by using the ring-trial validated spectrophotometric method based on the European Pharmacopoeia Chapter 2.2.25.

Recommended text for the register entry (analytical method)

For the quantification of the *total polyphenols* (phytochemical marker) in the *feed additive*:

- spectrophotometry at 280 nm expressing the polyphenols as catechin equivalent

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *FLAMORE* 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: FORW. APPL. 1831-0053-2020 & Annex I – submission number 1595581066477-2650
- [2] *Application, Proposal for Register Entry - DescriptAdd.pdf
- [3] *Technical dossier, Section II, 2.1 Identity of the additive

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- [4] EURL Evaluation Report:
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0077-dry_grape_extract.pdf
- [5] European Pharmacopoeia Chapter 2.2.25 01/2008:20225 Absorption spectrophotometry, ultraviolet and visible
- [6] Supplementary information – DPQ42EN_Quantification of total polyphenols by UV spectrophotometry at DO280nm (catechin eq.)_V05.pdf
- [7] *Technical dossier, Section II, Annexes II.2; II.10; II.18; II.34 & II.36
- [8] Commission Implementing Regulation (EU) 2017/307 of 21 February 2017 concerning the authorisation of dry grape extract of *Vitis vinifera* spp. *vinifera* as a feed additive for all animal species except for dogs O.J. L 44, 11.2.2017
- [9] European Pharmacopoeia, Chapter 2.8.17 01/2008:202817 Loss on drying of extracts
- [10] European Pharmacopoeia, Chapter 2.9.38 01/2008:20938 Particle size distribution estimation by analytical sieving
- [11] *Technical dossier, Section II, Annexes II.40; II.41; II.42
- *Refers to Dossier no: FAD-2020-0062

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:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Wageningen Food Safety Research¹ (WFSR) (NL)
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)

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