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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

Dry Grape Extract

(FAD-2010-0077; CRL/100081)

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-2010-0077 - CRL/100081**
Name of Feed Additive:	**Dry Grape Extract**
Active Agent (s):	-
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report checked by: Date:	**Piotr Robouch (EURL-FA)** **29/02/2016**
Report approved by: Date:	**Christoph von Holst** **02/03/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 10(2) for *Dry Grape 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, authorisation is sought for the use of the *feed additive* for all animal species and categories. The *feed additive* is a purple to brown free flowing powder, consisting of a grape seed and grape skin extracts *(Vitis vinifera L.)*. The applicant reported on results of analysis indicating a minimum of 80 % of *total polyphenols* (determined by spectrophotometry at 280 nm and expressed as catechin equivalent) in the *feed additive*. The *feed additive* is to be used directly into *feedingstuffs* or through *premixtures*, with recommended concentration levels ranging from 2.5 to 100 mg *Dry Grape Extract* /kg complete *feedingstuffs*.

For the identification of the *feed additive* the Applicant suggested using *gallic acid* as the phytomarker and submitted a single-laboratory validated method based on high performance liquid chromatography coupled to UV detection (HPLC-UV) similar to the official method INA 111.02 published by National Sanitation Foundation (NSF) International.

For the quantification of *total polyphenols* in the *feed additive* the Applicant submitted a single-laboratory validated method based on spectrophotometry at 280 nm, using external calibration with catechin standards, and reported a relative standard deviation for the *intermediate precision* (RSD_{ip}) ranging from 0.6 to 6.1%. This method was further ring-trial validated by five different laboratories resulting in a relative standard deviation for *reproducibility* (RSD_R) ranging from 1.2 to 4.5 %, and recovery rates (R_{rec}) up to 130%.

Based on the experimental evidence presented the EURL considers (i) the HPLC-UV method to identify the *gallic acid* phytomarker, and (ii) the ring-trial validated method based on spectrophotometry at 280 nm with external calibration using catechin standards fit-for-purpose to quantify *total polyphenols* content in the *feed additive*.

Since no criteria for the characterisation of the product were specified by the Applicant in the proposed registry entry, the EURL is unable to evaluate the suitability for official control of the submitted methods.

Since the accurate quantification of added *Dry Grape Extract* in *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to quantify *Dry Grape Extract* in these matrices.

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

Dry Grape Extract, *Vitis vinifera L.*, *total polyphenols*, *gallic acid*, sensory additives, flavouring compounds, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under article 10(2) (re-evaluation of the already authorised additives under provisions of Council Directive 70/524/EEC) for *Dry Grape 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]. Specifically, authorisation is sought for the use of the *feed additive* for all animal species and categories [1,2].

The Applicant described the *feed additive* as a purple to brown free flowing powder, consisting of a grape seed and skin extract *(Vitis vinifera L.)*. While no information about the *feed additive* composition was provided in "Annex A" [2], the Applicant reported on results of analysis in Section II 2.1.3 [3] indicating a minimum of 80 % of *total polyphenols* (determined by spectrophotometry at 280 nm and expressed as catechin equivalent) in the *feed additive* [3].

Furthermore, the Applicant recalled the sulphite maximum levels of 1000 mg/kg in dried fruits set by the Codex Alimentarius [4], or 2000 mg/kg in dried grapes set by the European Parliament and Council Directive No 95/2/EC [5], where the sulphite limits expressed as sulphur dioxide.

The *feed additive* is to be used directly into *feedingstuffs* or through *premixtures*, with recommended concentration levels ranging from 2.5 to 100 mg *Dry Grape Extract* /kg complete *feedingstuffs* [2,3].

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 *Dry Grape Extract*, 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, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [6].

For the identification of the *feed additive* the Applicant suggested using *gallic acid* as the phytomarker [3] and submitted a single-laboratory validated method based on high performance liquid chromatography coupled to UV detection (HPLC-UV) [7] similar to the official method INA 111.02, published by National Sanitation Foundation (NSF) International [8].

The Applicant submitted the Franz-Paul method commonly used for the determination of total sulphur dioxide (SO_2) in winery products. SO_2 is extracted by gas in acid condition (air or nitrogen). It is then transformed into sulphuric acid in an oxidizing solution of H_2O_2 and quantified by acidimetry [9].

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

For the quantification of *total polyphenols* in the *feed additive* the Applicant submitted the single-laboratory validated method based on "direct" spectrophotometry at 280 nm [10] and determined a relative standard deviation for the *intermediate precision* (RSD_{ip}) ranging from 0.6 to 6.1% [11] for *total polyphenols* contents ranging from 80.5 to 96.2% in the product (expressed as catechin equivalent). This method was further ring-trial validated by five different laboratories (Applicant included) which analysed three samples containing 68, 84 and 115 % *total polyphenols*, expressed as catechin equivalent [12]. Somewhat scattered data were reported resulting in a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.9 to 9.4 %. Subsequently, the Applicant modified the original SOP including an additional sonication step in the sample preparation to improve the extraction process [13]. According to this modified protocol, the sample (1 g) is sonicated with 100 ml of deionised water for 5 min. After decantation an aliquot (0.5 ml) is diluted with water by factor of 50 or 100. The absorbance of this solution is measured by spectrophotometry at 280 nm. The *total polyphenols* content is quantified by external calibration using catechin standard aqueous solutions [12]. This method was further ring-trial validated by the five laboratories mentioned above using samples containing from 63 to 130 % total polyphenols (expressed as catechin equivalent) and smaller RSD_R were reported, ranging from 1.2 to 4.5 % [14].

Based on the experimental evidence presented the EURL considers (i) the HPLC-UV method to identify the *gallic acid* phytomarker; and (ii) the ring-trial validated method based on spectrophotometry at 280 nm with external calibration using catechin standards fit-for-purpose to quantify the *total polyphenols* content in the *feed additive* (*Dry Grape Extract*).

Since the Applicant did not specify the criteria for the characterisation of the product in the proposed registry entry, the EURL cannot evaluate the suitability of this method for official control.

For the quantification of *Dry Grape Extract* in *premixtures* and *feedingstuffs* the Applicant suggested the determination of a specific marker by high performance liquid chromatography coupled to triple quadruple mass spectrometry (HPLC-MS/MS) [3]. However, only few results were provided for complete feed samples containing 5 mg/kg *Dry Grape Extract*, while no results were presented for *premixtures*. Furthermore, the accurate quantification of added *Dry Grape Extract* in *premixtures* and *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Dry Grape Extract* added in *premixtures* and *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) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL considers (i) the HPLC-UV method to identify the *gallic acid* phytomarker, and (ii) the ring-trial validated method based on spectrophotometry at 280 nm with external calibration using catechin standards fit-for-purpose to quantify *total polyphenols* content in the *feed additive*.

Since the Applicant did not specify the criteria for the characterisation of the product in the proposed registry entry, the EURL cannot evaluate the suitability of this method for official control.

The accurate quantification of added *Dry Grape Extract* in *premixtures* and *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Dry Grape Extract* in these matrices.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Dry Grape Extract* 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, Ref. SANCO/G/1: Forw. Appl. 1831/0006-2014

[2] *Application, Proposal for Register Entry - Annex A

[3] *Technical dossier, Section II

[4] *Technical dossier, Section II – Annex_II_99_Codex Alimentarius.pdf

[5] *Technical dossier, Section II – Annex_II_100_Council Directive 95-2-EC.pdf

[6] 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

[7] *Technical dossier, Section II – Annex II_151_Protocol gallic acid.pdf

[8] National Sanitation Foundation (NSF) International http://www.nsf.org/

[9] *Technical dossier, Section II – Annex_II_96_Sulphur dioxide dose_conf.pdf

[10] *Technical dossier, Section II – Annex_II_144_Protocol Total Polyphenol.pdf

[11] *Technical dossier, Section II – Annex_II_5_polyphenol content.pdf

[12] *Technical dossier, Section II – Annex_II_150_Ring Test_conf.pdf

[13] *Supplementary information – SIN1-new_SOP_Total_Phenols.pdf

[14] *Supplementary information – SIN2-RingTrial-2(2012).pdf

*Refers to Dossier no: FAD-2010-0077

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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Fødevarestyrelsens Laboratorie Aarhus (kemisk) (DK)
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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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