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JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
European Union Reference Laboratory for Feed Additives

JRC F.5/CvH/MGH/AS/Ares

**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**

Oregano oil (*Origanum vulgare* ssp.)
(FAD-2010-0245; CRL/100291)



**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-2010-0245 - CRL/100291**

Name of Feed Additive: ***Oregano oil (Origanum vulgare ssp.)***

Phytochemical marker(s): **Carvacrol**

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

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Date: **17/06/2019**

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Date: **18/06/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(2) for the botanically defined *Oregano oil (Origanum vulgare ssp.)* 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 all animal species and categories.

According to the Applicant, the phytochemical marker of the *feed additive* is *carvacrol*. The Applicant stated that the levels of *carvacrol* in *Oregano oil* may vary from 50 to 76 % (w/w). The *feed additive* is intended to be used in feed with a proposed dose up to 150 mg/kg of complete *feedingstuffs*.

For the determination of the phytochemical marker in the *feed additive* the Applicant proposed a gas chromatography coupled to flame ionisation detection (GC-FID) method based on the international standard "ISO 13171 – Essential oil of oregano" where a specific chromatographic profile derived from a GC-FID method is presented. Upon request of the EURL the Applicant provided experimental data for the analysis of *carvacrol* in two different batches of the *feed additive* applying the method described in ISO 13171.

Based on the experimental evidence the EURL recommends for official control for the determination of the phytochemical marker (*carvacrol*) in the *feed additive* the GC-FID method as indicated in the international standard "ISO 13171 – Essential oil of oregano".

The Applicant did not provide experimental data or an analytical method for the determination of *Oregano oil* in *premixtures* and *feedingstuffs* as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate or recommend any method for official control for the determination of *Oregano oil* 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Oregano oil (Origanum vulgare ssp.), *carvacrol*, sensory additives, all animal species and categories

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 *Oregano oil (Origanum vulgare ssp.)* 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 [1-3]. Specifically, the *feed additive* is sought to be used for all animal species and categories [2-4].

The *feed additive* is a colourless to pale yellow liquid [5]. According to the Applicant, the phytochemical marker of the *feed additive* is *carvacrol* [6]. The Applicant stated that, the levels of *carvacrol* in the *feed additive* may vary from 50 to 76 % (w/w).

The *feed additive* is intended to be used in feed at a proposed dose up to 150 mg/kg of complete *feedingstuffs* [3].

NOTE: The EURL has already evaluated the analytical methods for the determination of the phytochemical marker *carvacrol* in the frame of previous dossiers [7,8].

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 *Oregano oil (Origanum vulgare ssp.)* 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 characterisation of the phytochemical marker *carvacrol* in the *feed additive* the Applicant proposed a gas chromatography coupled to flame ionisation detection (GC-FID) method based on the international standard "ISO 13171 – Essential oil of oregano" where, in accordance with "ISO 11024:1998 Essential oils - General guidance on chromatographic profiles", a specific chromatographic profile, derived from a method based on GC-FID, is presented [9,10,11].

Furthermore, upon request of the EURL the Applicant provided experimental data for the analysis of *carvacrol* in two different batches of the *feed additive* applying the method described in ISO 13171 [12]. The quantification of the phytochemical marker in the *feed additive* is carried out with an external calibration using a *carvacrol* standard commercially available. According to the results provided by the Applicant in the frame of the verification study, the EURL calculated relative standard deviations for repeatability (RSD_r) and for intermediate precision (RSD_{ip}) ranging r from 1.8 to 2.7 % and from 2.2 to 2.7 %, respectively, for the quantification of the phytochemical marker (*carvacrol*) in the *feed additive* [13].

Based on the experimental evidence the EURL recommends for official control for the determination of the phytochemical marker (*carvacrol*) in the *feed additive* the GC-FID method as described in the international standard "ISO 13171 – Essential oil of oregano".

The Applicant did not provide experimental data or an analytical method for the determination of *oregano oil* in *premixtures* and *feedingstuffs*, as the unambiguous determination of the *feed additive* added to these matrices is not achievable experimentally. Therefore, the EURL cannot evaluate or recommend any method for official control for the determination of *oregano oil* in *premixtures* and *feedingstuffs*.

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 identification of the *feed additive* is pursued by the determination of the phytochemical marker *carvacrol* and the corresponding method of analysis is evaluated in chapter 2.6.1 of this report.

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 for the determination of the phytochemical marker *carvacrol* in the *feed additive* the GC-FID method as specified in the international standard "ISO 13171 – Essential oil of oregano".

Recommended text for the register entry (analytical method)

For the determination of the selected phytochemical marker *carvacrol* in the *feed additive*:

- gas chromatography coupled to flame ionisation detection (GC-FID) – ISO 13171

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Oregano oil (Origanum vulgare ssp.)* 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. 1831-0010-2018
- [2] *Application form, Annex I, Submission No. 1288116422339-1176
- [3] *Application, Proposal for Register Entry – Annex A
- [4] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition
- [5] *Technical dossier, Section II: 2.1.5 Physical state of each form of the product
- [6] *Technical dossier, Section II: 2.2 Characterisation of the active substance(s)/agent(s)
- [7] FAD-2016-0004, *Origanum vulgare* L., ssp. *hirtum* var. *Vulkan* (DOS 00001), Ref. Ares(2016)2755348 - 14/06/2016 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0004_oregano_oil.pdf
- [8] FAD-2010-0381, *Oregano oil (Origanum vulgare)*, Ref. Ares(2019)1345068 – 27/02/2019 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2010-0381_OreganoOil.pdf
- [9] K. Veres et al. *Chromatographia* (2003) 57:95-98 *Investigation of the composition and stability of the essential oils of origanum vulgare ssp. Vulgare L. and O. vulgare ssp. hirtum (link) letwaart.*
- [10] ISO 13171:2016 – Essential oil of oregano (*Origanum vulgare* L. subsp. *hirtum*) (Link) letsw
- [11] ISO 11024:1998 – Essential oils – General guidance on chromatographic profiles
- [12] *Supplementary information - Eurl-fa-technical-guide_sensory-additives 2018_Part_I_Nedlab-11-4-2019.pdf
- [13] *Supplementary information - EURL_ANOVA_verification

*Refers to Dossier no: FAD-2010-0245

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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