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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**

Oregano oil (*Oreganum vulgare*)
(FAD-2010-0381; CRL/100368)



**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-2010-0381 - CRL/100368**

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

Phytochemical markers: **carvacrol and thymol**

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

Report prepared by: **Stefano Bellorini**

Report checked by: **Zigmas Ezerskis**
Date: **27/02/2018**

Report approved by: **Christoph von Holst**
Date: **27/02/2018**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(2) for the botanically defined *Oregano oil (Origanum vulgare)* 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 poultry.

According to the Applicant, the phytochemical markers of the *feed additive* are *carvacrol* and *thymol* where the sum of the content of the two compounds is minimum 60 % expressed as a sum of the relative peak areas in the chromatogram. Therefore the characterisation of this *feed additive* is compliant with the definition of *Oregano oil* given in the monograph of the European Pharmacopoeia (Ph. Eur. 01/2008:1880). The *feed additive* is intended to be used in feed with a proposed level ranging between 12.3 and 18.4 mg/kg of complete *feedingstuffs*.

For the determination of the phytochemical markers in the *feed additive* the EURL identified 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 GC-FID method is presented. The Applicant identified instead the *Oregano* monograph of the European Pharmacopoeia (Ph. Eur. 01/2008:1880) where a specific test, equivalent to the method described in ISO 13171, is described for the determination of the two phytochemical compounds of interest. The Applicant analysed some samples by demonstrating satisfactory results applying the method described in the above mentioned monograph.

Based on the experimental evidence the EURL recommends for official control for the determination of the two phytochemical markers (*carvacrol* and *thymol*) in the *feed additive* the GC-FID method as indicated in the "European Pharmacopoeia monograph 01/2008:1880 for oregano" and equivalent to 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), carvacrol, thymol, sensory additives, poultry

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)* 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 poultry [2-4].

The *feed additive* is a clear yellowish-to-brown liquid [5]. According to the Applicant, the phytochemical markers of the *feed additive* are *carvacrol* and *thymol* [6]. The Applicant stated that, in agreement with the definition of *Oregano oil* as specified in the *Oregano* monograph of the European Pharmacopoeia 01/2008:1880, the "sum of the contents of *carvacrol* and *thymol*" in the product is "minimum 60 %". This parameter is expressed as a sum of the relative peak areas in the chromatogram determined via gas chromatography coupled with flame ionisation detection (GC-FID) according to the normalisation procedure described in the generic methods presented in the European Pharmacopoeia 2.2.28 for gas chromatography [6,7].

The *feed additive* is intended to be used in feed with a proposed level ranging between 12.3 and 18.4 mg/kg of complete *feedingstuffs* [3].

NOTE: The EURL has previously evaluated the analytical methods for the determination of the phytochemical marker *carvacrol* in *Oregano oil* in the frame of dossier FAD-2016-0004 [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)* 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 markers *carvacrol* and *thymol* in the *feed additive* the Applicant did not present a fit for purpose method for official control [9].

The EURL identified instead 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 gas chromatography coupled to flame ionisation detection (GC-FID), is presented [10,11]. Therefore, the EURL requested the Applicant to test the product with the GC-FID method described in ISO 13171 [7].

The Applicant submitted instead the *Oregano* monograph of the European Pharmacopoeia (Ph. Eur. 01/2008:1880) where a specific test, equivalent to the method described in ISO 13171, is described for the determination of the two phytochemical compounds of interest in the essential *Oregano oil* [6,7,12]. Furthermore, the requirements of the international standard proposed by the EURL are fully compatible with the definition of the European Pharmacopoeia monograph for *Oregano* [9,12].

According to the method described in the European Pharmacopoeia monograph, 0.2 µl of *Oregano oil* diluted with hexane are injected into the GC at a split ratio 1:100. Reference solutions of *carvacrol* and *thymol* are also analysed in order to identify the corresponding peaks from the sample solution [12]. The quantification is performed by FID using a normalisation approach for the estimation of the area percentage of individual components compared to the sum of the area of all peaks showing up in the chromatogram. The Applicant analysed some samples by demonstrating satisfactory results in which the sum of the relative peak areas of *carvacrol* and *thymol* was confirmed to be at least 60 % [6].

Based on the experimental evidence the EURL recommends for official control for the determination of the two phytochemical markers (*carvacrol* and *thymol*) in the *feed additive* the GC-FID method as indicated in the European Pharmacopoeia monograph 01/2008:1880 for *Oregano* and equivalent to 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 [9]. 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)

An evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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 markers *carvacrol* and *thymol* in the *feed additive* the GC-FID method as indicated in the European Pharmacopoeia monograph 01/2008:1880 or the equivalent 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 markers *carvacrol* and *thymol* in the *feed additive*:

- gas chromatography coupled to flame ionisation detection (GC-FID) – European Pharmacopoeia monograph 01/2008:1880 or 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)* 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. 1831-0009-2018
- [2] *Application form, Annex I, Submission No. 1288963611636-1462
- [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: 2.2 Characterisation of the active substance(s)/agent(s)
- [6] *Supplementary information: 181030_SIN answer.msg
- [7] *Supplementary information: cs_fad-2010-0381_oregano_oil_.pdf
- [8] 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
- [9] *Technical dossier, Section II: 2.6 Methods of analysis and reference samples
- [10] ISO 13171:2016 – Essential oil of oregano
- [11] ISO 11024:1998 – Essential oils – General guidance on chromatographic profiles
- [12] European Pharmacopoeia 01/2008:1880 – Oregano, p.2568

*Refers to Dossier no: FAD-2010-0301

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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