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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2010-0292 CRL/100235
Feed additive:	*Origanum heracleoticum L.*
Active Substance(s):	*oregano essential oil*
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 4(1) and 10(2) for the *feed additive oregano essential oil* under the "sensory additives", functional group 2(b) "flavouring compounds" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all species and categories.

The *oregano essential oil* mainly consists of carvacrol and thymol, two predominant compounds (approximately 85%) used as phytochemical markers to trace the *feed additive*. The *oregano essential oil* is intended to be incorporated in *premixtures*, *feedingstuffs* or drinking *water*, with no minimum or maximum levels.

For the identification and quantification of the two phytochemical markers carvacrol and thymol of *oregano essential oil*, the Applicant proposed the internationally recognised European Pharmacopoeia method – Ph. Eur. 7.0, method 07/2010:1880, based on Gas Chromatography coupled to flame ionisation detection (GC-FID). The two phytochemical markers are identified with the retention times from the chromatogram of the pure solutions of carvacrol and thymol. Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method – Ph. Eur. 7.0, method 07/2010:1880, for the identification and quantification of carvacrol and thymol as phytochemical markers of the *feed additive*.

The Applicant did not provide any experimental method or data for the identification of *oregano essential oil* in *premixtures*, *feedingstuffs* and *water*. Furthermore, the unambiguous identification of the content of *oregano essential oil* added to *premixtures*, *feedingstuffs* or *water* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *oregano essential oil* in *premixtures*, *feedingstuffs* and *water*.

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
Origanum heracleoticum L., oregano essential oil, sensory additive, flavouring compounds, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water) and 10(2) (re-evaluation of additives already authorised under council directive 70/524/EEC) for the *feed additive oregano essential oil* under the "sensory additives", functional group 2(b) "flavouring compounds" [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* for all species and categories [2].

Oregano essential oil is a steam distillate from flowers, leaves and upper stems of the plant *Origanum heracleoticum L.* [2,3]. The *oregano essential oil* mainly consists of carvacrol and thymol, two predominant compounds (approximately 85%) used as phytochemical markers to trace the *feed additive* [3]. *Oregano essential oil* is intended to be incorporated in *premixtures*, *feedingstuffs* or drinking *water*, with no minimum or maximum levels, but typical conditions of use are suggested by the Applicant for different animal species [3].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 essential oil (Origanum heracleoticum L.)*, 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, mycotoxins, and dioxins) are available from the respective European Union Reference Laboratories [4].

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

Two phytochemical markers (carvacrol and thymol) are suggested by the Applicant for the identification of *oregano essential oil*.

For the identification and quantification of carvacrol and thymol, the Applicant proposed the internationally recognised European Pharmacopoeia method – Ph. Eur. 7.0, method 07/2010:1880 [5], based on Gas Chromatography coupled to flame ionisation detection (GC-FID). The two phytochemical markers are identified with the retention times from the chromatogram of the pure solutions of carvacrol and thymol. The percentage content of the sum of carvacrol and thymol is determined using the normalisation procedure.

Even though no performance characteristics are provided, the EURL recommends for official control the European Pharmacopoeia method – Ph. Eur. 7.0, method 07/2010:1880, for the identification and quantification of carvacrol and thymol as phytochemical markers of the *feed additive*.

The Applicant did not provide any experimental method or data for the identification of *oregano essential oil* in *premixtures*, *feedingstuffs* and *water*. Furthermore, the unambiguous identification of the content of *oregano essential oil* added to *premixtures*, *feedingstuffs* or *water* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *oregano essential oil* in *premixtures*, *feedingstuffs* and *water*.

Additionally, the Applicant provided an experimental protocol for the identification and quantification of carvacrol and thymol in two formulations (Orego-Stim® Liquid&Powder) [6-7], based on High Performance Liquid Chromatography coupled to ultraviolet detection (HPLC-UV) at 280 nm. The EURL considers the method submitted by the Applicant, using HPLC-UV, suitable for identification of carvacrol and thymol in the two formulated products mentioned above, even though the Applicant did not provide any experimental data (i.e. chromatograms).

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 recommends for official control the European Pharmacopoeia method – Ph. Eur. 7.0, method 07/2010:1880, based on Gas Chromatography coupled to flame ionisation detection (GC-FID) for the identification and quantification of phytochemical markers carvacrol and thymol in *feed additive*.

The Applicant did not provide any experimental method or data for the identification of *oregano essential oil* in *premixtures*, *feedingstuffs* and *water*. Furthermore, the unambiguous identification of the content of *oregano essential oil* added to *premixtures*, *feedingstuffs* or *water* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine *oregano essential oil* in *premixtures*, *feedingstuffs* or *water*.

Additionally, the Applicant provided an experimental protocol for the identification and quantification of carvacrol and thymol in Orego-Stim® Liquid&Powder [6-7], based on High Performance Liquid Chromatography coupled to ultraviolet detection (HPLC-UV) at 280 nm. The EURL considers the method submitted by the Applicant, using HPLC-UV, suitable for identification of carvacrol and thymol in *water* and in the two formulated products mentioned above, even though the Applicant did not provide any experimental data.

Recommended text for the register entry (analytical method)

For the identification and quantification of the phytochemical markers carvacrol and thymol in the *feed additive*:

- Gas Chromatography coupled to flame ionisation detection
  (European Pharmacopoeia 7.0, method 07/2010:1880)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Origanum heracleoticum L.* 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 Forw. Appl. 1831/00134/(10163)/2010

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

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis

[4] 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

[5] *Technical dossier, Section II – Annex_II_1_2_01_PhEu_Oregano

[6] *Technical dossier, Section II – Annex_II_6_1_03_AssayMethLiquid

[7] *Technical dossier, Section II – Annex_II_6_1_04_AssayMethPowder

* Refers to Dossier No. FAD-2010-0292

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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)