Ref. Ares(2016)2755348 - 14/06/2016

EUROPEAN COMMISSION
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JRC.D.5/CvH/ZE/mds/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

Origanum vulgare L., ssp. hirtum var. Vulkan (DOS 00001)

(FAD-2016-0004; CRL/150012)

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-2016-0004 - CRL/150012**
Name of Product:	**Origanum vulgare L., ssp. hirtum var. Vulkan (DOS 00001)**
Active Agent (s):	**Oregano essential oil**
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)** **03/06/2016**
Report approved by: Date:	**Christoph von Holst** **13/06/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for natural essential oil from *Origanum vulgare L., ssp. hirtum var. Vulkan (DOS 00001)* 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 Applicant defined the product as "natural essential oil from *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)*", containing the following main constituents: Carvacrol (60 – 65 %); *para*-Cymene (5 – 10 %); *gamma*-Terpinene (4 – 9 %); *beta*-Caryophyllene (2.0 – 5.0%); Thymol (1.0 – 3.5 %); Linalool (less than 5.0%); *alpha*-Terpinene and Terpinen-4-ol (less than 2.0 % each). The Applicant suggested using Carvacrol as the phytochemical marker. The *feed additive* is to be used in *feedingstuffs* with no proposed minimum or maximum concentration levels. However, recommended inclusion levels of the *feed additive* are ranging from 15 to 150 mg /kg complete *feedingstuffs*.

For the characterisation of the *feed additive*, the Applicant submitted a gas chromatography coupled with flame ionisation and masspectrometric detection (GC-FID/MS) method - derived from the ISO 11024 and the European Pharmacopoeia monograph 8.0, 2.2.28 - to identify and quantify the main constituents. The Applicant reported a relative standard deviation for *intermediate precision* (RSD_{ip}), ranging from 0.8 to 6.1% when quantifying Carvacrol and Thymol in the pure essential oil. Based on the experimental evidence provided the EURL recommends the GC-FID/MS method for official control to identify the major constituents and quantify the phytochemical marker (Carvacrol), in the *feed additive.*

For the quantification of Oregano essential oil in *premixtures* the Applicant submitted a method based on volumetric analysis after water-steam distillation - derived from the ISO 6571 standard and European Pharmacopeia monograph 8.0, 2.8.12. The Applicant applied this method for the analysis of the essential oil in three different types of *premixtures* containing the essential oil on silica and fatty plant oil carriers and reported satisfactory recovery rates (R_{rec}) ranging from 89.7 to 90.9% for the volatile oil content. As for the *feed additive*, the EURL suggests applying the GC-FID/MS for the identification of the obtained essential oil. Based on the experimental evidence available the EURL considers these methods suitable for determination of Oregano essential oil in the *premixtures* investigated.

The accurate quantification of added essential oil from *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)* in *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)* 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) is not considered necessary.

KEYWORDS

Origanum vulgare L., ssp. hirtum var. Vulkan (DOS 00001), Oregano essential oil, Carvacrol, sensory additives, flavouring compounds, all animal species and categories

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new feed additive) for the "natural essential oil from *Origanum vulgare L., ssp. hirtum var. Vulkan (DOS 00001)*" 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].

According to the Applicant the product is a yellow-green to dark brown natural essential oil obtained from *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)*. While no information about the *feed additive* composition is provided in "Annex A" [2], the Applicant provided the following specifications in Section II 2.1.3 and Annex_II_6 [3,4]:

Profile	GC area (in %)
Carvacrol	60 – 65 %
para-Cymene	5 – 10 %
gamma-Terpinene	4.0 – 9.0 %
beta-Caryophyllene	2.0 – 5.0 %
Linalool	< 5.0 %

Profile	GC Area (in %)
Thymol	1.0 – 3.5 %
alpha-Terpinene	< 2.0 %
Terpinen-4-ol	< 2.0 %
trans-Sabinene hydrate	> 0.3 %

The Applicant suggested using *Carvacrol* as the phytochemical marker [3].

The *feed additive* is intended to be used in *feedingstuffs* with no proposed minimum or maximum concentration levels [2]. However, the Applicant suggested inclusion levels of the *feed additive* in complete *feedingstuffs* ranging from 15 to 150 mg /kg [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 natural essential oil from *Origanum vulgare L., ssp. hirtum var. Vulkan (DOS 00001)*, 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 [5].

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

For the characterisation of the *feed additive*, the Applicant suggested gas chromatography coupled with flame ionisation and masspectrometric detection (GC-FID/MS) [6] to identify and quantify the major constituents specified earlier. This method is derived from the ISO 11024 standard [7] and the European Pharmacopeia monograph 8.0, 2.2.28 [8].

According to the method, Oregano essential oil is diluted 100 times with *iso*-octane and 1 µl of the solution is injected into GC at a split ratio of 1:60. The quantification is performed by FID using the normalisation approach for the estimation of the area percentage of individual components. Consequently, the major constituents are identified using gas chromatography - mass spectrometry (GC-MS). The Applicant reported a relative standard deviation for *intermediate precision* (RSD_{ip}), ranging from 0.8 to 6.1% when quantifying Carvacrol and Thymol in the pure essential oil [6].

Based on the experimental evidence provided the EURL recommends the GC-FID/MS method for official control to identify the major constituents and quantify the phytochemical marker (Carvacrol), in the *feed additive*.

Furthermore, the Applicant submitted another GC-FID / GC-MS method [9] - for the full characterisation of the *feed additive* - based on the internationally recognised ISO 7609 standard method for the analysis of essential oils [10]. Identification is performed using GC-MS, while quantification of individual components is assessed as area percentage using GC-FID [9]. Five batches of the *feed additive* were analysed and the composition of each individual constituent was reported [9].

For the quantification of Oregano essential oil in *premixtures* the Applicant submitted a method based on volumetric analysis after water-steam distillation [11,12,13] – derived from the ISO 6571 standard [14] and European Pharmacopeia monograph 8.0, 2.8.12 [15]. The Applicant applied this method to analyse three different types of *premixtures* containing the essential oil on silica and fatty plant oil carriers, and reported satisfactory recovery rates (R_{rec}) ranging from 89.7 to 90.9 % for the volatile oil content [11,12,13]. As for the *feed additive*, the EURL suggests applying GC-FID/MS [6] for the identification of the obtained essential oil. Based on the experimental evidence available the EURL considers these methods suitable for determination of Oregano essential oil in the *premixtures* investigated.

The accurate quantification of added Oregano essential oil in *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)* in *feedingstuffs*.

However, the Applicant submitted a single-laboratory validated GC-MS method [16], for the quantification of Carvacrol (phytochemical marker) in *feedingstuffs*. The Applicant analysed two sets of feed spiked with Carvacrol and Oregano essential oil at the concentration levels of Carvacrol in feed ranging from 11.1 to 16.4 mg/kg, and reported a relative standard deviation of *repeatability* (RSD_r) ranging from 1.5 to 1.9%; a recovery rate (R_{rec}) of 99% and a limit of quantification (LOQ) of 5 µg Carvacrol /kg feed [16].

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 gas chromatography coupled with flame ionisation and masspectrometric detection (GC-FID/MS) for the identification of the major constituents and for the quantification of the phytochemical marker (Carvacrol) in the *feed additive*.

The accurate quantification of added essential oil from *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)* in *feedingstuffs* is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification of the major constituents and for the quantification of the phytochemical marker (Carvacrol) in the *feed additive*:

- gas chromatography coupled with flame ionisation and masspectrometric detection (GC-FID/MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of natural essential oil from *Origanum vulgare L. ssp. hirtum var. Vulkan (DOS 00001)* 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. SANTE/G1: Forw. Appl. 1831/0008-2016

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

[3] *Technical dossier, Section II

[4] *Technical dossier, Section II – Annex_II_06

[5] 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

[6] *Technical dossier, Section II – Annex II_09

[7] ISO 11024-1:1998 – *Essential oils – General guidance on chromatographic profiles – Part 1: Preparation of chromatographic profiles for presentation in standards*

[8] European Pharmacopeia monograph 8.0, 2.2.28

[9] *Technical dossier, Section II – Annex_II_02

[10] ISO 7609:1985 – *Essential oils – Analysis by gas chromatography on capillary columns – General method*

[11] *Technical dossier, Section II – Annex_II_20

[12] *Technical dossier, Section II – Annex_II_21

[13] *Technical dossier, Section II – Annex_II_22

[14] ISO 6571:2008 – *Spices, condiments and herbs – Determination of volatile oil content (hydrodistillation method)*

[15] European Pharmacopeia monograph 8.0, 2.8.12

[16] *Technical dossier, Section II – Annex_II_10

*Refers to Dossier no: FAD-2016-0004

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

The following National Reference Laboratories contributed to this report:

- Fødevarestyrelsens Laboratorie Aarhus (kemisk) (DK)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)