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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

Carvacrol

(FAD-2018-0020; CRL/180019)

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-2018-0020 - CRL/180019**
Name of Product:	***Carvacrol***
Active Agent (s):	**Carvacrol**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Stefano Bellorini** **25/10/2018**
Report approved by: Date:	**Christoph von Holst** **25/10/2018**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *Carvacrol* under the category/functional group (4a) "zootechnical additives"/"digestibility enhancers", 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 salmonids.

The *feed additive* consists of a minimum of 99 % (expressed as a relative peak area in the chromatogram) of *Carvacrol* as *active substance*.

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant proposed a minimum dose of 112 mg *Carvacrol*/kg *feedingstuffs.*

For the quantification of *Carvacrol* in the *feed additive* the Applicant submitted an in-house developed method based on gas chromatography coupled to flame ionisation detection (GC-FID), which is based on the generic international standard method ISO 7609 for analysis of essential oils.

The Applicant analysed five batches of the *feed additive* applying the above mentioned GC-FID method and a relative standard deviation for *repeatability* (RSD_r) of 0.1 % was derived for an average content of *Carvacrol* of 99.8 % (expressed as relative area).

Based on the experimental evidence available the EURL recommends for the official control the GC-FID method based on the ISO 7609 standard for the quantification of *Carvacrol* in the *feed additive.*

In addition, the EURL recommends for the official control the "Carvacrol" monograph of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *Carvacrol* in the *feed additive*.

For the quantification of *Carvacrol* in *premixtures* and *feedingstuffs* the Applicant submitted single-laboratory validated and verified methods based on GC coupled to FID and mass spectrometry (MS), respectively.

The performance characteristics reported in the frame of the validation and verification studies for the quantification of *Carvacrol* in *premixtures* and *feedingstuffs* are the following: i) *premixtures* (containing Carvacrol from 250 to 400 g/kg): RSD_r and *intermediate precision* (RSD_{ip}) ranging from 0.6 to 1.5 % and a recovery rate (R_{rec}) ranging from 97 to 110 %; ii) *feedingstuffs* (containing Carvacrol from 125 to 250 mg/kg): RSD_r and RSD_{ip} ranging from 2.7 to 10.2 %, R_{rec} ranging from 92 to 102 % and a limit of quantification (LOQ) of 35 mg Carvacrol/kg *feedingstuffs*.

Based on the experimental evidence available the EURL recommends for the official control the above mentioned single-laboratory validated and further verified GC-FID and GC-MS methods for the quantification of *Carvacrol* in *premixtures* and *feedingstuffs*, respectively.

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

Carvacrol, zootechnical additives, digestibility enhancers, salmonids

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (new *feed additive*) for *Carvacrol* under the category/functional group (4a) "zootechnical additives"/"digestibility enhancers", 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 salmonids [1,2].

The *feed additive* is a clear, slightly viscous, very light yellow liquid consisting of a minimum of 99 % (expressed as a relative peak area in the chromatogram) of *Carvacrol* as *active substance* [3,4].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures* [4]. The Applicant proposed a minimum dose of 112 mg *Carvacrol*/kg *feedingstuffs* [3,4].

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 *Carvacrol* 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 quantification of *Carvacrol* in the *feed additive* the Applicant submitted an in-house developed method based on gas chromatography coupled to flame ionisation detection (GC-FID) [5] which is based on the generic international standard method ISO 7609 for analysis of essential oils [6].

The sample is diluted with acetone and injected into the GC system. Individual components including the target analyte are separated by non-polar or polar capillary column and the substances are detected by FID. The quantification of *Carvacrol* is performed using relative area of *Carvacrol* versus the sum of the areas of all individual components [5].

The Applicant analysed five batches of the *feed additive* applying the above mentioned GC-FID method [5]. A relative standard deviation for *repeatability* (RSD_r) of 0.1 % was derived for an average content of *Carvacrol* of 99.8 % (expressed as relative peak area in the chromatogram) [4].

In addition, on request from the EURL, the Applicant provided the chromatogram of the *feed additive* analysis with clearly defined retention times of the analyte and of the residues [7]. Furthermore, for the purpose of the official control, the EURL advices to inject in parallel the reference standard substance in order to unambiguously identify *Carvacrol* in the *feed additive*.

Based on the experimental evidence available the EURL recommends for the official control the GC-FID method based on the ISO 7609 standard for the quantification of *Carvacrol* in the *feed additive.*

For the quantification of *Carvacrol* in oil *premixtures* the Applicant submitted a single-laboratory validated and verified method based on GC-FID [8].

The sample is dissolved in acetone and diluted with acetonitrile for further analysis. The quantification of *Carvacrol* is performed by using external standard calibration [8].

For the quantification of *Carvacrol* in fish *feedingstuffs* the Applicant submitted a single-laboratory validated and verified method based on gas chromatography coupled to mass spectrometry (GC-MS) [9].

The sample is treated with an hexane and acetone mixture by using accelerated solvent extraction (ASE). The extract is diluted with the same solvent mixture and injected into the GC-MS system. The quantification of the analyte is performed by using external calibration with standard solutions of *Carvacrol* [9].

Table 1 The performance characteristics of the single laboratory validated and further verified GC-FID and GC-MS methods for the quantification of *Carvacrol* in *premixtures* and *feedingstuffs*, respectively

	Premixtures		Feedingstuffs	
	Validation	Verification	Validation	Verification
Method	GC-FID		GC-MS	
Mass fraction, mg/kg	250000 – 400000	250000	250	125
RSD_r %	1.2 – 1.5	0.6 – 0.7	10.2	2.7 – 6.6
RSD_{ip} %	-	1.3	-	6.6
R_{rec} %	97 –110	101	92	102
Reference	[8]	[10]	[9]	[11]

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{rec}: *recovery* rate.

The performance characteristics reported in the frame of the validation [8,9] and verification [10,11] studies for the quantification of *Carvacrol* in *premixtures* and *feedingstuffs* are presented in Table 1. Furthermore, the Applicant reported a limit of quantification (LOQ) of 35 mg *Carvacrol*/kg *feedingstuffs* [9] which is well below the minimum content suggested by the Applicant.

Based on the experimental evidence available the EURL recommends for the official control the above mentioned single-laboratory validated and further verified GC-FID and GC-MS methods for the quantification of *Carvacrol* in *premixtures* and *feedingstuffs*, respectively.

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)

For the quantification of *Carvacrol* in fish fillet, liver, water and feces samples the Applicant submitted the in-house developed method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [12], which was further single-laboratory validated [13]. As there are no residue limits for *Carvacrol* in food, the evaluation of the corresponding methods by the EURL are not required.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

The EURL found the "Carvacrol" monograph of the Food Chemical Codex (FCC) where identification is based on infrared absorption [14].

The EURL recommends for the official control the Food Chemical Codex method for the identification of *Carvacrol* in the *feed additive*.

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:

- the "Carvacrol" monograph of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *Carvacrol* in the *feed additive*;
- the GC-FID method based on the ISO 7609 standard for the quantification of *Carvacrol* in the *feed additive*;
- the single-laboratory validated and further verified method based on GC-FID for the quantification of *Carvacrol* in *premixtures*; and
- the single-laboratory validated and further verified method based on GC-MS for the quantification of *Carvacrol* in *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification of *Carvacrol* in the *feed additive*:

- Food Chemical Codex "Carvacrol" monograph

For the quantification of *Carvacrol* in the *feed additive* and *premixtures*:

- Gas chromatography coupled to flame ionisation detection (GC-FID)

For the quantification of *Carvacrol* in *feedingstuffs*:

- Gas chromatography coupled to mass spectrometry (GC-MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Carvacrol* 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. 1831-0026-2018

[2] *Application, Annex I – submission number 1523860200190-2200

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

[4] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis

[5] *Technical dossier, Section II – Annex_II_17

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

[7] *Supplementary information – Chromatogram Carvacrol

[8] *Technical dossier, Section II – Annex_II_22

[9] *Technical dossier, Section II – Annex_II_24

[10] *Technical dossier, Section II – Annex_II_23

[11] *Technical dossier, Section II – Annex_II_25

[12] *Technical dossier, Section II – Annex_II_18

[13] *Technical dossier, Section II – Annex_II_19

[14] Food Chemical Codex monograph "Carvacrol", FCC 7 (2010), p.192

*Refers to Dossier no: FAD-2018-0020

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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