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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-2016-0002; CRL/150026)

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-2016-0002 - CRL/150026**
Name of Product:	***Carvacrol***
Active Agent (s):	**Carvacrol**
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)** **01/08/2016**
Report approved by: Date:	**Christoph von Holst** **01/08/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Carvacrol* under the category/functional group 4(d) ‘zootechnical additives’/‘other zootechnical additives’ according to the classification system of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for weaned piglets. The *feed additive* is to be marketed as a colourless solid product ("*Nimicoat*") consisting of 40 to 42 % *Carvacrol* (active substance) and a carrier containing fatty acids, silica and a surfactant. The *feed additive* is intended to be incorporated directly into *feedingstuffs* (not through *premixtures*) to obtain a dosage ranging from 250 to 1000 mg "*Nimicoat*"/kg complete *feedingstuffs*, which is equivalent to a *Carvacrol* content ranging from 100 to 420 mg/kg *feedingstuffs*.

For the quantification of *Carvacrol* in the *feed additive* and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on gas chromatography coupled with flame ionisation detection (GC-FID), and reported the following performance characteristics: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.8 to 3.8 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.9 to 9.6 %; - a *recovery* rate (R_{rec}) ranging from 78 to 108 %; and - a limit of quantification (LOQ) of 19 mg *Carvacrol*/kg *feedingstuffs*. Based on the experimental evidence available the EURL recommends for the official control this GC-FID method for the quantification of *Carvacrol* in the *feed additive* 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) is not considered necessary.

KEYWORDS

Carvacrol, *Nimicoat*, zootechnical additives, other zootechnical additives, weaned piglets

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new *feed additive*) for *Carvacrol* under the category/functional group 4(d) 'zootechnical additives'/'other zootechnical additives' according to the classification system of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additive* for weaned piglets [1,2].

The *feed additive* is to be marketed as a colourless solid product ("*Nimicoat*"), consisting of 40 to 42% *Carvacrol* (active substance) and a carrier containing fatty acids, silica and a surfactant [2,3].

The *feed additive* is intended to be incorporated directly into *feedingstuffs* (not through *premixtures*) to obtain a dosage ranging from 250 to 1000 mg "*Nimicoat*"/kg complete *feedingstuffs* [3], which is equivalent to a *Carvacrol* content ranging from 100 to 420 mg/kg *feedingstuffs*.

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

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 [4].

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

For the quantification of *Carvacrol* in the *feed additive* ("*Nimicoat*") and *feedingstuffs* the Applicant submitted a single-laboratory validated and further verified method based on gas chromatography coupled with flame ionisation detection (GC-FID) [5].

Samples (2.5 to 20 g) are extracted by Soxhlet with dichloromethane for 5 h. After addition of the internal standard (azulene), aliquots are diluted with pentane before chromatographic analysis. The quantification of *Carvacrol* is performed by matrix free calibration using the standard solutions containing the analyte of interest and the internal standard [5].

The performance characteristics determined in the frame of the validation [6] and verification [7] studies are presented in Table 1. Furthermore, the Applicant reported a limit of quantification (LOQ) of 19 mg *Carvacrol*/kg *feedingstuffs* [6], 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 method for the quantification of *Carvacrol* in the *feed additive* 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) is not considered necessary.

Table 1 The performance characteristics of the single laboratory validated and further verified GC-FID method for the quantification of *Carvacrol* in the *feed additive* ("*Nimicoat*") and *feedingstuffs*.

	Feed Additive		Feedingstuffs	
	Validation	**Verification**	**Validation**	**Verification**
Mass fraction, mg/kg	410800 – 429500	404840	78 – 343	91 – 401
RSD_r, %	1.3 – 3.8	-	0.8 – 1.9	-
RSD_{ip}, %	3.8	2.0	1.9	3.3 – 9.6
R_{rec}, %	103 – 108	101	78 – 90	81 – 102
Reference	[6]	[7]	[6]	[7]

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

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the single-laboratory validated and further verified method, based on gas chromatography with flame ionisation detection (GC-FID) for the quantification of *Carvacrol* in the *feed additive* ("*Nimicoat*") and *feedingstuffs*.

Recommended text for the register entry (analytical method)

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

- Gas Chromatography with Flame Ionisation Detection (GC-FID)

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/G1: Forw. Appl. 1831/0002-2016

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

[3] *Technical dossier, Section II

[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_8

[6] *Technical dossier, Section II – Annex II_9

[7] *Technical dossier, Section II – Annex II_10

*Refers to Dossier no: FAD-2016-0002

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

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- Fødevarestyrelsens Laboratorie Aarhus (kemisk) (DK)
- RIKILT Wageningen UR, Wageningen (NL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
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