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CRL Evaluation Report on the Analytical Methods submitted in
connection with the Application for Authorised as Feed Additive
according to Regulation (EC) No 1831/2003

Dossier related to: FAD-2009-0055
CRL/090021

Name of Additive: FRESTA® F

Active Substance(s): Carvone

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EXECUTIVE SUMMARY

The current application is submitted according to Article 4(1) (new use) of Regulation (EC) No 1831/2003. The proposed inclusion level of *FRESTA® F* ranges from 250 to 400 mg/kg in complete *feedingstuffs*, which corresponds to 0.875 - 1.4 mg/kg of active substance (*carvone*) in complete *feedingstuffs*. The use of *FRESTA® F* for the weaned piglets (up to 120 days) is requested. *Carvone* is already authorised under Directive 70/524/EC as a “flavour, aromatic and appetising substance” subject to the provisions of Art. 10 (2) of Regulation No (EC) No 1831/2003.

For the determination of *carvone* in *feed additive*, *premixtures* and *feedingstuffs*, the applicant submitted an in-house validated method, using gas-chromatographic/mass-spectrometry (GC/MS). Furthermore, a verification study compliant with the CRL technical guide was submitted. The performance characteristics for the determination of *carvone* in *feed additive*, *premixtures* and *feedingstuffs*, re-calculated by CRL based on the experimental data provided by the applicant, are listed hereafter:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2 to 2.5 % for the *feed additive* and ranging from 4 to 11 % for *premixtures* and *feedingstuffs*;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) of 2.5 % for *feed additive* and ranging from 5.7 to 12 % for *premixtures* and *feedingstuffs*;
- a *recovery* rate ranging from 93 % to 107 % and
- a limit of detection (LOD) and quantification (LOQ) of 0.016 and 0.055 mg/kg of *feedingstuffs*, respectively.

Based on the above mentioned performance characteristics, the CRL recommends for official control, the method submitted by the applicant, for the determination of *carvone* in *feed additives*, *premixtures* and *feedingstuffs*.

Further testing or validation is not considered necessary.

KEYWORDS

Carvone, zootechnical additive, weaned piglets

1. BACKGROUND

FRESTA® F (carvone) is a product for which authorisation as *feed additive* is sought under the category 'zootechnical additives', functional group 4(d) 'other zootechnical additives' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003. Carvone is already authorised under Directive 70/524/EC as a “flavour, aromatic and appetising substance” subject to the provisions of Art. 10 (2) of Regulation No (EC) No 1831/2003.

FRESTA® F is a preparation of partially micro-encapsulated essential oils of caraway and lemon in an excipient based on mixed dried herbs & spices and other bulking and anti-caking agents [2], which contains 0.35% of the active substance (*carvone*) [3].

In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003, the authorisation for weaned piglets up to 120 days of age is requested [3].

The proposed inclusion level of *FRESTA® F* ranges from 250 to 400 mg/kg in complete *feedingstuffs* [3], thus corresponding to 0.87 - 1.4 mg/kg of active substance (*carvone*) in complete *feedingstuffs*.

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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with *FRESTA® F*, 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, mercury and lead) are available from the respective Community Reference Laboratories [4].

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

For the determination of *carvone* in *feed additive*, *premixtures* and *feedingstuffs*, the applicant submitted an in-house validated method [5,6]. *Carvone* is extracted from the sample with ethanol using ultrasonication. An internal standard (biphenyl) is added at the extraction step. The extract is centrifuged and filtered through a 0.45 µm membrane filter and subsequently analysed by gas-chromatographic/mass-spectrometry (GC/MS) in single ion modus (SIM). Furthermore, a verification study by a second laboratory was performed [7], according to the CRL technical guide [8]. The performance characteristics, recalculated by CRL [9] based on the experimental data provided by the applicant [6,7] are presented in Table 1. The higher values for the precision (RSD_r and RSD_{ip}) obtained during the verification study for *premixtures* were attributed to a potential particle segregation in the sample observed from the verification laboratory before the analysis.

Table 1 Performance characteristics of analytical methods for the determination of the *carvone* in *feed additive*, *premixtures* and *feedingstuffs*. Precision values were recalculated by the CRL [9] based on the experimental validation [6] and verification [7] data provided.

	RSD_r , % [9]		RSD_{ip} , % [9]		LOD mg/kg [6]	LOQ mg/kg [6]	Recovery rate % [6,7]
	Validation	Verification	Validation	Verification			
<i>Feed additive</i>	2.5	2	2.5	2.5	—	—	98 - 100
<i>Premixture</i>	4	11	5.7	11	—	—	99 - 104
<i>Mash feed</i>	7.3	5.5	12	5.5	0.016	0.055	93 - 101
<i>Pellet feed</i>	6.4	7.4	11	7.4	0.016	0.055	104 - 107

RSD_r : relative standard deviation for *repeatability*; RSD_{ip} : relative standard deviation for *intermediate precision*

LOD and LOQ: limit of detection and quantification;

Target values of *carvone* in the *feed additive*: 4842 mg/kg, *premix*: 24.20 mg/kg, *mash feed*: 1.51 mg/kg, and *pellet feed*: 1.34 mg/kg;

The performance characteristics of the in-house validated and further verified method for the determination of *carvone* in *feed additive*, *premixtures* and *feedingstuffs*, can be summarise as follows:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2 to 2.5 % for the *feed additive* and ranging from 4 to 11 % for *premixtures* and *feedingstuffs*;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) of 2.5 % for *feed additive* and ranging from 5.7 to 12 % for *premixtures* and *feedingstuffs*;
- a *recovery* rate ranging from 93 % to 107 % and
- a limit of detection (LOD) and quantification (LOQ) of 0.016 and 0.055 mg/kg of *feedingstuffs*, respectively.

Based on the above mentioned performance characteristics, the CRL recommends for official control, the method submitted by the applicant, for the determination of *carvone* in *feed additives*, *premixtures* and *feedingstuffs*.

Further testing or validation is not considered necessary

4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control the in-house method submitted by the applicant, for the determination of *carvone* in *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *carvone* in *feed additive*, *premixtures* and *feedingstuffs*:

- gas-chromatographic/mass-spectrometry (GC/MS) in single ion modus (SIM)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *FRESTA® F* have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

6. REFERENCES

- [1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/037-2009
 - [2] *Technical dossier, Section II – 2.2. Characterisation of the active substance
 - [3] *Application, Proposal for Register Entry – Annex A
 - [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_6a.pdf
"Detection and quantification of carvone in FRESTA® F, and in premixtures or feedingstuffs containing FRESTA® F, using GC/MS"
 - [6] *Technical dossier, Section II – Annex_II_6b.pdf
"Validation of method MA-1010a-01 (carvone detection and quantification by GC/MS)"
 - [7] *Technical dossier, Section II – Annex_II_6d.pdf
"Verification of Method MA-1010a-01 (carvone detection and quantification by GC/MS)"
 - [8] CRL-FA Technical Guide: Protocol for verification studies of single-laboratory/in-house validated methods
 - [9] *Additional Information – Precision data as recalculated by the CRL
- * Refers to Dossier No. FAD-2009-0011

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was the National Veterinary Institute (SVA), Uppsala, Sweden. 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

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