EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

JRC.D.5/FSQ/CvH/SB/ag/ARES(2012)765761

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

Dossier related to:	FAD-2010-0396 CRL/100364
Name of product:	Clove oil
Active Substance(s):	Clove oil
Rapporteur Laboratory:	European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by:	Stefano Bellorini (EURL-FA)
Report revised by: Date:	Piotr Robouch (EURL-FA) 18/06/2012
Report approved by: Date:	Christoph von Holst 25/06/2012

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Clove oil* under the category/functional group 4(d) "other zootechnical additives" according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use for chickens for fattening.

The Applicant intends to market the granulated product (Liderfeed®) containing 5 to 6 % *Clove oil* - an essential oil obtained from Syzygium aromaticum - containing 75 to 95 % *Eugenol*. The product is intended to be incorporated in complete *feedingstuffs* with a recommended dosage of *Eugenol* ranging from 4 to 50 mg/kg.

For the determination of *Eugenol* marker in the *feed additive* (*clove oil*) and in the product (Liderfeed®), the Applicant submitted a single-laboratory validated and further verified method based on Gas Chromatography coupled to a Flame Ionization Detector (GC-FID). The following performance characteristics were reported:

- a standard deviation for *repeatability* (RSD_r) ranging from 0.4 to 1.4 %;
- a standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.0 to 1.8 %;
- a *recovery* rate (R_{Rec}) ranging from 99 to 101 %.

For the determination of *Eugenol* (marker) in *feedingstuffs* the Applicant proposed another single-laboratory validated and further verified GC-FID method and reported the following performance characteristics for *Eugenol* concentrations ranging from 4.5 to 5.5 mg/kg *feedingstuff*: - RSD_r ranging from 1.2 to 3.2%; - RSD_{ip} = 3 %; - R_{Rec} ranging from 96 to 106%; and - a limit of quantification (LOQ) of 1 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control the two single-laboratory validated and further verified GC-FID methods for the determination of *Eugenol* in the *feed additive* and in *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) is not considered necessary.

KEYWORDS

Clove oil, *Eugenol*, *Liderfeed*®, zootechnical additives, other zootechnical additives, chickens for fattening.

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (new additive) for *Clove oil* under the category/functional group 4(d) "other zootechnical additives" according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for the use for chickens for fattening [1-2].

The Applicant intends to market a granulated product (Liderfeed®) consisting of 5 to 6 % *Clove oil* and 55% glyceryl polyethyleneglycol ricinoleate (E484) on a silica carrier. *Clove oil* is an essential oil obtained from Syzygium aromaticum - containing 75 to 95 % *Eugenol* [3-4]. The product is intended to be incorporated in complete *feedingstuffs* with a recommended dosage of *Eugenol* ranging from 4 to 50 mg/kg [2].

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 (EFSA) for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Clove oil (Eugenol)*, 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 quantification of the active substance in feed additive and feedingstuffs

For the determination of the *Eugenol* marker in the *feed additive* (*Clove oil*) and in the product (*Liderfeed*®), the Applicant proposed a single-laboratory validated [6-8] and further verified [9-10] method based on Gas Chromatography coupled to a Flame Ionization Detector (GC-FID). This method derives from the analytical methods proposed by ISO [11-13] and the European Pharmacopoeia [14] for the characterisation of *Clove oil*.

The sample is weighed in a 50ml amber volumetric flask. Acetone for *clove oil* (hexane for *Liderfeed*®) is added and the sample is dissolved using an ultrasonic bath and centrifuged. An aliquot from the upper phase is transferred into an amber volumetric flask containing an Azulene internal standard. The solution is then diluted with hexane and injected into the GC. *Eugenol* is separated from other volatile components by GC (with a retention time of 10 min) and detected using FID [6-8].

The following performance characteristics were reported for *Eugenol* in *Clove oil* and *Liderfeed*® for concentrations ranging from 5.2 to 92.5 % [7-10]:

- a standard deviation for *repeatability* (RSD_r) ranging from 0.4 to 1.4 %;
- a standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.0 to 1.8 %;
- a *recovery* rate (R_{Rec}) ranging from 98.8 to 100.9 %.

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method based on GC-FID for the determination of *Eugenol* in the *feed additive* (*Clove oil*).

For the determination of *Eugenol* in *feedingstuffs* the Applicant proposed another single-laboratory validated [6,15,16] and further verified [17] GC-FID method. The sample preparation consists of a Soxhlet extraction with n-hexane, in which 15/20g of *feedingstuff* is weighed directly into a cellulose extraction thimble. The extract is transferred into a volumetric flask and Azulene internal standard is added. Finally the extract is analysed with the GC-FID. *Eugenol* is separated from the other volatile components by GC, detected with FID and quantified against the internal standard [6,15,16].

The following performance characteristics were reported for *Eugenol* concentrations ranging from 4.5 to 5.5 mg/kg *feedingstuffs* [15-16]: - RSD_r ranging from 1.2 to 3.2%; - RSD_{ip} = 3 %; - R_{Rec} ranging from 95.8 to 105.5%; and - a limit of quantification (LOQ) of 1 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified GC-FID method for the determination of *Eugenol* 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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control the single-laboratory validated and further verified methods based on Gas Chromatography coupled to a Flame Ionization Detector (GC-FID) for the determination of *Eugenol* in the *feed additive (Clove oil)* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

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

- Gas Chromatography coupled to a Flame Ionization Detector (GC-FID)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Liderfeed*® 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. 1831/00179 (10443)-2010
[2] *Application, Proposal for Register Entry – Annex A
[3] European Pharmacopoeia 7.2, definition 01/2088/1091
[4] *Technical dossier, Section II, 2.1.3. Qualitative and quantitative composition
[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, 2.6.1 Methods of analysis for active substances
[7] *Technical dossier, Section II, Annex II-17
[8] *Technical dossier, Section II, Annex II-19
[9] *Technical dossier, Section II, Annex II-18
[10] *Technical dossier, Section II, Annex II-20
[11] Oil of clove leaves – ISO 3141:1997
[12] Oil of clove buds – ISO 3142:1997
[13] Oil of clove stems – ISO 3143:1997

[14] European Pharmacopoeia 7.2, tests 01/2088/1091
[15] *Technical dossier, Section II, Annex II-21
[16] *Technical dossier, Section II, Annex II-22
[17] *Technical dossier, Section II, Annex II-23
* Refers to Dossier No. FAD-2010-0396

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:

- Skúšobné laboratórium – Oddelenie analýzy krmív, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Bratislava (SK)
- Fødevarestyrelsen, Ringsted (DK)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)