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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

Cumin Cyminum L. (Cumine Tincture)

(FAD-2014-0024; CRL/140001)

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-2014-0024 - CRL/140001**
Name of Product:	**Cumin Cyminum L. (Cumine Tincture)**
Active Agent (s):	**-**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **22/06/2016**
Report approved by: Date:	**Christoph von Holst** **23/06/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for the *Cumin Cyminum L. (Cumine Tincture)* 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. According to the Applicant the product is a brown liquid tincture obtained from *Cumin Cyminum L.*, containing 0.5 to 1.2% 4-*iso*-propylbenzaldehyde, later referred as the phytochemical marker. The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* and into *water* for drinking, with no proposed minimum or maximum concentration levels.

For the quantification of the phytochemical marker in the *feed additive*, the Applicant submitted a method based on gas chromatography coupled with flame ionisation detection (GC-FID). This method is derived from the ISO 9301 standard dedicated to the characterisation of oil from Cumin seed. The Applicant reported a relative standard deviation for *repeatability* (RSD_r) of 3.2% when analysing preparations of *Cumine Tincture* containing 24.2 mg/kg 4-*iso*-propylbenzaldehyde. Based on the experimental evidence provided the EURL recommends the GC-FID method for official control to quantify the phytochemical marker (4-*iso*-propylbenzaldehyde) in the *feed additive*.

The Applicant did not provide any experimental method or data for the quantification of added *Cumin Cyminum L. (Cumine Tincture)* in *premixtures*, *feedingstuffs* and *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Cumin Cyminum L. (Cumine Tincture)* in *premixtures*, *feedingstuffs* and *water*.

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

Cumin Cyminum L., *Cumine Tincture*, 4-*iso*-propylbenzaldehyde, 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 *Cumin Cyminum L. (Cumine Tincture)* 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 brown liquid tincture obtained from *Cumin Cyminum L.*, containing 0.5 to 1.2% 4-*iso*-propylbenzaldehyde, later referred as the phytochemical marker [2,3].

The *feed additive* is intended to be used directly into *feedingstuffs* or through *premixtures* and into *water* for drinking, with no proposed minimum or maximum concentration levels [2,3].

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 *Cumin Cyminum L. (Cumine Tincture)*, 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 substances in feed additive, premixtures, feedingstuffs and water

For the quantification of the phytochemical marker (4-*iso*-propylbenzaldehyde) in the *feed additive*, the Applicant submitted a method based on gas chromatography coupled with flame ionisation detection (GC-FID) [5]. This method is derived from the ISO 9301 standard dedicated to the characterisation of the oil from Cumin seed [6].

According to this method [5], the sample of the tincture preparation (1 ml) is extracted twice, each time with 2 ml of dicloromethane and combined organic phase. After addition of 0.2 ml of azulene internal standard (0.5 mg/ml), it is diluted with ethanol up to 10 ml for further chromatographic analysis. The phytochemical marker is quantified using 4-*iso*-propylbenzaldehyde standard calibration with the correction by the internal standard. The Applicant reported a relative standard deviation for *repeatability* (RSD_r) of 3.2% when quantifying preparations of *Cumine Tincture* containing 24.2 mg/kg 4-*iso*-propyl-benzaldehyde [5].

Based on the experimental evidence provided the EURL recommends the GC-FID method for official control to quantify the phytochemical marker (4-*iso*-propylbenzaldehyde) in the *feed additive*.

The Applicant did not provide any experimental method or data for the quantification of added *Cumin Cyminum L. (Cumine Tincture)* in *premixtures*, *feedingstuffs* and *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Cumin Cyminum L. (Cumine Tincture)* in *premixtures*, *feedingstuffs* and *water*.

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 detection (GC-FID) for the quantification of phytochemical marker (4-*iso*-propylbenzaldehyde) in the *feed additive*.

The Applicant did not provide any experimental method or data for the quantification of added *Cumin Cyminum L. (Cumine Tincture)* in *premixtures*, *feedingstuffs* and *water*. Therefore, the EURL cannot evaluate nor recommend any method for official control to quantify *Cumin Cyminum L. (Cumine Tincture)* in *premixtures*, *feedingstuffs* and *water*.

Recommended text for the register entry (analytical method)

For the quantification of the phytochemical marker (4-*iso*-propylbenzaldehyde) in the *feed additive*:

- gas chromatography coupled 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 *Cumin Cyminum L. (Cumine Tincture)* 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. 1831/0058-2014

[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] *Supplementary information – dosage cuminaldehyde teinture de cumin

[6] ISO 9301:2003 – *Oil of cumin seed (Cuminum cyminum L.)*

*Refers to Dossier no: FAD-2014-0024

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)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- RIKILT Wageningen UR, Wageningen (NL)
- 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)
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