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JRC F.5/CvH/MGH/AS/Ares

**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**

Lemon extract
(FAD-2010-0130; CRL/090032)

**Revised
version**



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Dossier related to: **FAD-2010-0130 - CRL/090032**

Name of Feed Additive ***Lemon extract***

Phytochemical marker(s): **total polyphenols**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
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Date: **16/12/2020**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10(2) for *Lemon extract* 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.

The *feed additive* is a dark orange-to-yellow liquid extract obtained from the peel and the seeds of the *Citrus limon* (L.) Burm. (CoE 139). *Lemon extract* is to be used through *premixtures* or directly into *feedingstuffs* and *water* at proposed maximum levels of 1000 mg /kg *feedingstuffs* and 0.25 ml / l *water*. According to the Applicant, the phytochemical markers proposed for the characterisation of the *feed additive* (*Lemon extract*) are *total polyphenols* and *eriocitrin*. *Lemon extract* is a natural product and thus the *total polyphenols* content varies from harvest to harvest, ranging generally from 1 to 4 % (w/w).

For the quantification of the *total polyphenols* in the *feed additive* (*Lemon extract*) the Applicant proposed a colourimetric method described in the European Pharmacopoeia monograph 2.8.14. Upon request of the EURL, the Applicant provided experimental data for the analysis of the *total polyphenols* content in two different batches of *Lemon extract*.

Based on the acceptable performance characteristics presented the EURL recommends for official control the colourimetric method described in the European Pharmacopoeia 2.8.14 monograph for the quantification of the *total polyphenols* content in the *feed additive*.

For an additional identification/characterisation of the *feed additive*, the EURL considers a method based on high performance liquid chromatography with spectrophotometric detection (HPLC-UV) suitable for the determination of *eriocitrin* in the *feed additive*.

The Applicant did not provide experimental data or analytical methods for the determination of *Lemon extract* in *premixtures* and *feedingstuffs*, as the unambiguous determination of the *feed additive* added to the matrices is not achievable experimentally. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of *Lemon extract* in *premixtures* 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Lemon extract, *total polyphenols*, *eriocitrin*, sensory additives, flavouring compounds, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 10(2) (authorisation of an existing product) for *Lemon extract* 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 [1,2].

The Applicant described the *feed additive* as a dark orange-to-yellow liquid extract obtained from the peel and the seeds of the *Citrus limon* (L.) Burm. (CoE 139) fruit [3].

According to the Applicant, the phytochemical markers proposed for the characterisation of the *feed additive* (*Lemon extract*) are *total polyphenols* and *eriocitrin* [4]. *Lemon extract* is a natural product and thus the *total polyphenols* content, primarily based on flavonoids, coumarins and limonoids, varies from harvest to harvest, ranging generally from 1 to 4 % (w/w) [3].

Lemon extract is to be used through *premixtures* or directly into *feedingstuffs* and *water* at proposed maximum levels of 1000 mg /kg *feedingstuffs* and 0.25 ml / l *water* [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 *Lemon extract* 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 the *total polyphenols* in the *feed additive* (*Lemon extract*) the Applicant proposed a colourimetric method described in the European Pharmacopoeia monograph 2.8.14. The polyphenols are extracted with water and mixed with a mixture of phosphomolybdate and phosphotungstate solutions (Folin–Ciocalteu reagent (FCR)). The coloured complex formed with the polyphenols is then measured at 760 nm and the *total polyphenols* content is expressed as *pyrogallol* equivalent [5].

Upon request of the EURL, the Applicant provided experimental data for the analysis of *total polyphenols* content in two different batches of *Lemon extract* [6]. According to the results provided by the Applicant in the frame of the analysis, the EURL calculated relative standard deviations for *repeatability* (RSD_r) and for *intermediate precision* (RSD_{ip}) ranging from 2.1 to 5.2 % and from 3.3 to 6.5 %, respectively [7]. Furthermore, the Applicant applied this method to five different batches of the *feed additive* leading to *total polyphenols* content ranging from 1.4 to 3.5 % [5].

Based on the experimental evidences provided the EURL recommends for official control the colourimetric method described in the European Pharmacopoeia [5] for the quantification of *total polyphenols* (phytochemical marker) in the *feed additive*.

The Applicant did not provide experimental data or an analytical method for the determination of *Lemon extract* in *premixtures* and *feedingstuffs*, as the unambiguous determination of the *feed additive* added to these matrices is not achievable experimentally. Therefore, the EURL cannot evaluate or recommend any method for official control for the determination of *Lemon extract* in *premixtures* and *feedingstuffs*.

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)

An evaluation of corresponding methods of analysis is not relevant for the present application.

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

For further identification/characterisation of the *feed additive* (*Lemon extract*) the Applicant proposed the quantification of another phytochemical marker, namely *eriocitrin*.

For the quantification of the *eriocitrin* content in the *feed additive* (*Lemon extract*) the Applicant applied a modified ring-trial validated method from the “International Fruit and Vegetable Association” (IFU 580) dedicated for the determination of *hesperidin* and *naringin*. This method is based on high performance liquid chromatography with spectrophotometric detection (HPLC-UV) [8]. The Applicant provided experimental data for the analysis of the *eriocitrin* content in two different batches of *Lemon extract* [9]. According to the results provided by the Applicant in the frame of the analysis, the EURL calculated a RSD_r of 0.1 % and 1.8 %, and a RSD_{ip} of 8.1 % and 8.5 % [10].

The EURL considers the above mentioned HPLC-UV method fit-for-purpose for the identification/characterisation of 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 colourimetric method described in the European Pharmacopoeia monograph 2.8.14 for the quantification of the *total polyphenols* (phytochemical marker) in the *feed additive*.

For an additional identification/characterisation of the *feed additive*, the EURL considers the method based method based on HPLC-UV suitable for the determination of *eriocitrin* in the *feed additive*.

Recommended text for the register entry (analytical method)

For the quantification of the phytochemical marker (*total polyphenols*) in the *feed additive*:

- spectrophotometry at 760 nm expressing the *total polyphenols* content as *pyrogallol* equivalent (European Pharmacopoeia monograph 2.8.14)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Lemon extract* 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/E5: FORW. APPL. 1831-0045-2018 & Annex I Sub. No. 1285152699179-876
- [2] *Application, Proposal for Register Entry - Annex A
- [3] *Technical dossier, Section II
- [4] Supplementary information - EURL Supplementary information request
- [5] *Technical dossier, Section II, 2.6.1
- [6] Supplementary information - Annex_3_ validation report Polyphenols.pdf
- [7] Supplementary information - eurl_anova_polyphenols.pdf
- [8] Supplementary information - Annex_2_ SOP Eriocitrin IFU
- [9] Supplementary information - Annex_8_ validation report eriocitrin.pdf
- [10] Supplementary information - eurl_anova_eriocitrin.pdf

*Refers to Dossier no: FAD-2010-0130

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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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Wageningen Food Safety Research¹ (WFSR) (NL)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)

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