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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

Camelia oleifera extract

(FAD-2021-0071; CRL/210020)

European Union Reference Laboratory
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Dossier related to:	**FAD-2021-0071 - CRL/210020**
Name of Product:	***Camelia oleifera extract***
Active Agent (s):	***Total saponins***
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Zigmas Ezerskis** **20/04/2022**
Report approved by: Date:	**Christoph von Holst** **20/04/2022**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for a *camelia oleifera extract* under the category / functional group (1 c) "technological additives"/"emulsifiers", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the *feed additive* to be used for all animal species (excluding fish).

The *feed additive* (Cosap®) is a liquid extract from tea seed meal of *camelia oleifera* containing a minimum content of 10.5 % (w / w) *total saponins*, which according to the Applicant is the active substance of Cosap®. The *feed additive* can be used in its liquid form or adsorbed on mineral supports or dried. Cosap® is intended to be incorporated directly into *feedingstuffs* or through *premixtures* at a recommended inclusion levels ranging from 30 to 750 mg / kg *feedingstuffs*.

For the determination of the *total saponins* content in Cosap® (the *feed additive*), the Applicant submitted a single-laboratory validated and further verified spectrophotometric method.

Based on the acceptable performance characteristics presented the EURL recommends for official control the single-laboratory validated and further verified spectrophotometric method for the determination of the *total saponins* content in the *feed additive* (Cosap®).

The Applicant did not provide any experimental data or an analytical method for the determination of the *total saponins* in *premixtures* and *feedingstuffs*.

Instead, the Applicant proposed two single-laboratory validated and further verified chromatographic methods for the determination of *camelliaside A* (the phytochemical marker of Cosap® as claimed by the Applicant) in *premixtures* and *feedingstuffs*.

Based on the acceptable performance characteristics presented, the EURL considers the two single-laboratory validated and further verified chromatographic methods suitable for the determination of *camelliaside A* in *premixtures* and *feedingstuffs*.

Moreover, as the unambiguous determination of the *feed additive* (Cosap®) added to *premixtures* and *feedingstuffs* is not achievable experimentally the EURL cannot recommend any method for official control for the determination of Cosap® 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

Camelia oleifera extract, Cosap®, *total saponins*, technological additives, all animal species (excluding fish)

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (authorisation of a new feed additive) for a *camelia oleifera extract* under the category / functional group (1 c) "technological additives"/"emulsifiers", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, the authorisation is sought for the *feed additive* to be used for all animal species (excluding fish) [2].

The *feed additive* (Cosap®) is a liquid extract from tea seed meal of *camelia oleifera* containing a minimum content of 10.5 % (w / w) of *total saponins*, which according to the Applicant is the active substance of the *feed additive* [3].

According to the Applicant the *feed additive* can be used in its liquid form or adsorbed on mineral supports or dried. Cosap® is intended to be incorporated directly into *feedingstuffs* or through *premixtures* at a recommended inclusion level ranging from 30 to 750 mg / kg *feedingstuffs* [2,4].

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 *camelia oleifera 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 determination of the *total saponins* content in Cosap® (the *feed additive*), the Applicant submitted a single-laboratory validated [5] and further verified [6] spectrophotometric method [7].

Total saponins are extracted from the *feed additive* (Cosap®) sample with a methanol in an ultrasonic bath followed by a centrifugation. The supernatant is then submitted to a chromogenic reaction using para-anisaldehyde and sulphuric acid, and the reaction products are measured by spectrophotometry at a wavelength of 600 nm. The quantification of the *total saponins* is performed by an external standard calibration and the content of the *total saponins* is expressed as *escin* equivalents [7].

The performance characteristics reported for the *total saponins* in the frame of the validation [5] and verification [6] studies for Cosap® (the *feed additive*) are presented in Table 1.

Based on the acceptable performance characteristics presented the EURL recommends for official control the single-laboratory validated and further verified method based on spectrophotometry for the determination of the *total saponins* in the *feed additive* (Cosap®).

The Applicant did not provide any experimental data or an analytical method for the determination of the *total saponins* in *premixtures* and *feedingstuffs*.

Instead, the Applicant submitted another two single-laboratory validated [8-9] and further verified chromatographic methods [10-11] for the determination of *camelliaside A* (the phytochemical marker of Cosap® as claimed by the Applicant) in *premixtures* and *feedingstuffs*.

For the *premixtures*, the sample (3 g) is treated with a mixture of water: methanol 50 : 50 (v / v) in an ultrasonic bath at room temperature, then the mixture is centrifuged and the supernatant is directly injected in the high performance liquid chromatography coupled to a photometric detection (HPLC-UV). The quantification of *camelliaside A* is performed by an external standard calibration curve [12].

Table 1 Performance characteristics of the single-laboratory validated and verified spectrophotometric method for the determination of *total saponins* in Cosap®

	Total saponins	
	Validation	**Verification**
Mass fraction, %	12.5	12.5
RSD_r %	4.2	2.1
RSD_{ip} %	4.2	2.1
R_{rec} %	96	95
Reference	[5]	[6]

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

Table 2 Performance characteristics of the single-laboratory validated and verified chromatographic methods for the determination of *camelliaside A* in *premixtures* (HPLC-UV) and *feedingstuffs* (UHPLC-MS/MS).

	Camelliaside A			
	Premixtures (HPLC-UV)		**Feedingstuffs (UHPLC-MS/MS)**	
	validation	**verification**	**validation**	**verification**
Mass fraction, mg/kg	819		3	
RSD_r, %	2.7	2.2	3.6	4.3
RSD_{ip}, %	3.4	5.1	7.4	4.3
R_{rec}, %	98	99	98	97
Reference	[8]	[10]	[9]	[11]

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

In the case of *feedingstuffs*, the sample is spiked with eriocitrin (used for the recovery calculation) before the extraction. Then, the sample is treated with methanol in an ultrasonic bath, filtered and evaporated until dryness. The obtained residue is re-solubilised in methanol and centrifuged. Aliquots of the supernatant are further spiked with known amounts of *camelliaside A* and eriocitrin, and finally injected in the ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) system. The content of *camelliaside A* is determined using the standard addition method [13].

The performance characteristics reported in the frame of the validation [8-9] and verification [10-11] studies for the determination of *camelliaside A* in *premixtures* and *feedingstuffs* are presented in Table 2.

Furthermore, the Applicant reported for the UHPLC-MS/MS method a limit of detection (LOD) of 0.017 mg *camelliaside A* / kg *feedingstuffs* and a limit of quantification (LOQ) of 0.057 mg *camelliaside A* / kg *feedingstuffs* [9].

Based on the acceptable performance characteristics presented, the EURL considers the single-laboratory validated and further verified chromatographic methods submitted by the Applicant suitable for the determination of *camelliaside A* in *premixtures* and *feedingstuffs*.

Moreover, as the unambiguous determination of the *feed additive* (Cosap®) added to *premixtures* and *feedingstuffs* is not achievable experimentally the EURL cannot recommend any method for official control for the determination of Cosap® 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.

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 single-laboratory validated and further verified method based on spectrophotometry for the determination of the *total saponins* in Cosap® (the *feed additive*).

As the unambiguous determination of the *feed additive* (Cosap®) added to *premixtures* and *feedingstuffs* is not achievable experimentally the EURL cannot recommend any method for official control for the determination of Cosap® in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *total saponins* in the *feed additive*:

- spectrophotometry at 600 nm expressing the *total saponins* content as escin equivalents

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *camelia oleifera 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, Reference SANTE/E5: FORW. APPL. 1831-0048-2021
[2] *Application, Annex I - Submission number 1616663226054
[3] *Technical dossier, Section II: 2.1 Identity of the additive
[4] *Technical dossier, Section II: 2.5 Conditions of use
[5] *Technical dossier, Section II – Annex_II.68
[6] *Technical dossier, Section II – Annex_II.69
[7] *Technical dossier, Section II – Annex_II.66
[8] *Technical dossier, Section II – Annex_II.72
[9] *Technical dossier, Section II – Annex_II.74

[10] *Technical dossier, Section II – Annex_II.73

[11] *Technical dossier, Section II – Annex_II.74-1

[12] *Technical dossier, Section II – Annex_II.52

[13] *Technical dossier, Section II – Annex_II.60

*Refers to Dossier no: FAD-2021-0071

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)
- Instytut Zootechniki - Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Wageningen Food Safety Research (WFSR)1 (NL)
- Laboratoire de Rennes (SCL L35), Service Commun des Laboratoires DGCCRF et DGDDI, Rennes (FR)

[1] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.