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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

Preparation of macleaya cordata extract and leaves

(FEED -2021-2410; CRL/210072)

European Union Reference Laboratory
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Dossier related to:	**FEED-2021-2410 - CRL/210072**
Name of Product :	***Preparation of macleaya cordata extract and leaves***
Active Agent (s):	***Sanguinarine***
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Zigmas Ezerskis** **14/07/2023**
Report approved by: Date:	**Christoph von Holst** **14/07/2023**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for a *preparation of macleaya cordata extract and leaves* under the category / functional group (4 d) "zootechnical additives"/"other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the *feed additive* is sought to be used for used for sucking and weaned piglets and other growing suidae.

The *feed additive* (Sangrovit® extra) is a *preparation of macleaya cordata extract and leaves* containing a minimum *sanguinarine* content of 4000 mg / kg which according to the Applicant is the active substance of Sangrovit® extra. The *feed additive* is intended to be incorporated directly into *compound feed* or through *premixtures* to supply *sanguinarine* contents ranging from 0.6 to 0.75 mg / kg *compound feed*.

For the determination of *sanguinarine* in Sangrovit® extra the Applicant submitted a single-laboratory validated and further verified method based on reversed-phase high performance liquid chromatography (HPLC) coupled to fluorescence detection (FLD).

For the determination of *sanguinarine* in *premixtures* and *compound feed* the Applicant submitted another single-laboratory validated and further verified method based on HPLC coupled to tandem mass spectrometry (MS/MS).

Based on the acceptable performance characteristics presented the EURL recommends for official control (i) the single-laboratory validated and further verified method based on reversed-phase high performance liquid chromatography coupled to fluorescence detection (HPLC-FLD) for the determination of *sanguinarine* in Sangrovit® extra and (ii) the single-laboratory validated and further verified method based on reversed-phase high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) for the determination of *sanguinarine* in *premixtures* and *compound feed*.

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

Preparation of macleaya cordata extract and leaves, Sangrovit® extra, *sanguinarine*, zootechnical additives, sucking and weaned piglets and other growing suidae.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (authorisation of a new feed additive) for a *preparation of macleaya cordata extract and leaves* under the category / functional group (4 d) "zootechnical additives"/"other zootechnical additives", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, the *feed additive* is sought to be used for sucking and weaned piglets and other growing suidae [1].

The *feed additive* (Sangrovit® extra) is a reddish-orange dry granular *preparation* of *macleaya cordata extract and leaves* containing a minimum *sanguinarine* (active substance) content of 4000 mg / kg [2].

According to the Applicant the *feed additive* is intended to be incorporated directly into *compound feed* or through *premixtures* to supply *sanguinarine* contents ranging from 0.6 to 0.75 mg / kg *compound feed* [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 a *preparation of macleaya cordata extract and leaves* 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, compound feed and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the determination of *sanguinarine* in Sangrovit® extra (the *feed additive*), the Applicant submitted a single-laboratory validated [4] and further verified [5] method based on reversed-phase high performance liquid chromatography (HPLC) coupled to fluorescence detection (FLD) [6].

The Sangrovit® extra sample is shaken during 1 h with the extraction mixture and then filtered. The obtained filtrate, is further diluted with methanol and the clean extract directly analysed by HPLC. The target analyte is detected by FLD at wavelengths of 330 nm

(excitation) and 570 nm (emission). The quantification of *sanguinarine* is performed by an external standard calibration [6].

The performance characteristics reported for *sanguinarine* in the frame of the validation [4] and verification [5] studies for Sangrovit® extra (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 reversed-phase HPLC-FLD for the determination of *sanguinarine* in Sangrovit® extra.

For the determination of *sanguinarine* in *compound feed* the Applicant submitted another single-laboratory validated [8] and further verified method [9] based on HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) [10].

The sample of *compound feed* is spiked with the appropriate volume of the internal standard, then is shaken during 1 h with the extraction mixture and filtered. The clear extract obtained, is further diluted with the dilution solution and it is directly injected in the chromatographic system. The identification of the analyte is based on the comparison of the retention times, ion ratio and signal-to-noise ratio between the standards and the sample. The quantification of *sanguinarine* is performed by using the standard addition method or by using a recovery rate correction (using a blank feed sample spiked with a *sanguinarine* standard solution) [10].

Table 1 The performance characteristics of the single-laboratory validated and verified HPLC-FLD method for the determination of *sanguinarine* in Sangrovit® extra

	Sanguinarine	
	Validation	**Verification**
	[4]	[5]
Mass fraction, mg / kg	4782	
RSD_r %	1.8*	0.4*
RSD_{ip} %	2.1*	0.4*
R_{rec} %	95 - 100	97

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{rec}: *recovery* rate. * Recalculated by EURL after removal of outliers [7].

Table 2 The performance characteristics of the single-laboratory validated and verified HPLC-MS/MS method for the determination of *sanguinarine* in *compound feed.*

	Sanguinarine	
	Validation	**Verification**
	[8]	**[9]**
Mass fraction, mg/kg	0.23 - 0.87	0.38
RSD_r, %	6.7 - 9.3*	5.9*
RSD_{ip}, %	6.9 - 10.2*	5.9*
R_{rec}, %	95 - 108	109

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{rec}: *recovery* rate. * Recalculated by EURL [11].

The performance characteristics reported in the frame of the validation [8] and verification [9] studies for the determination of *sanguinarine* in *compound feed* are presented in Table 2.

Furthermore, the Applicant reported for the method described above a limit of detection (LOD) of 0.01 mg *sanguinarine* / kg *compound feed* and a limit of quantification (LOQ) of 0.02 mg *sanguinarine* / kg *compound feed* [10].

Regarding *premixtures*, the Applicant has reported low recovery rates for *sanguinarine*, which were justified by strong matrix interferences [12]. However, these interferences are not observed when analysing *sanguinarine* in *compound feed* in which Sangrovit® Extra is introduced via the vitamin-mineral *premixture* [13]. Consequently, the Applicant proposed the determination of *sanguinarine* in *premixtures* according to the method proposed for *compound feed* (prior solid dilution with blank feed). The EURL considers it as a suitable approach.

Based on the acceptable performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method based on reversed-phase HPLC-MS/MS for the determination of *sanguinarine* in *premixtures* and *compound feed*.

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)

The Applicant provided a method based in ultra-high performance liquid chromatography coupled to tandem mass spectrometer (UPLC-MS/MS) [14] for the determination of *sanguinarine* in animal tissues and verified it in poultry tissues (muscle, kidney, skin/fat and liver) [15].

However, the Applicant did not propose any MRLs thus, the EURL considers that the evaluation of the 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 (i) the single-laboratory validated and further verified method based on reversed-phase high performance liquid chromatography coupled to fluorescence detection (HPLC-FLD) for the determination of *sanguinarine* in Sangrovit® extra (the *feed additive*) and (ii) the single-laboratory validated and further verified method based on reversed-phase HPLC-MS/MS for the determination of *sanguinarine* in *premixtures* and *compound feed*.

Recommended text for the register entry (analytical method)

For the determination of *sanguinarine* in the *feed additive*:

- reversed-phase high performance liquid chromatography coupled to fluorescence detection (HPLC-FLD)

For the determination of *sanguinarine* in *premixtures* and *compound feed*:

- reversed-phase high performance liquid chromatography coupled to tandem mass spectrometry detection (HPLC-MS/MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of the *preparation of macleaya cordata extract and leaves* 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] *Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – https://webgate.ec.europa.eu/esfc/#/applications/4430 https://open.efsa.europa.eu/questions/EFSA-Q-2022-00357

[2] *Technical dossier: Identity_characterisation

[3] *Technical dossier: Conditions of use

[4] *Technical dossier, Annex_II.6.2

[5] *Technical dossier, Annex_II.6.12

[6] *Technical dossier, Annex_II.6.1

[7] Supplementary information: eurl-anova-fa.pdf

[8] *Technical dossier, Annex_II.6.4

[9] *Technical dossier, Annex_II.6.11

[10] *Technical dossier, Annex_II.6.3

[11] Supplementary information: eurl-anova-fs.pdf

[12] *Technical dossier, Annex_II.6.5

[13] *Technical dossier, Annex_II.4.2

[14] *Technical dossier, Section II – Annex_II.6.6

[15] *Technical dossier, Section II – Annexes_II.6.7 to II.6.10

*Refers to Dossier no:-FEED-2021-2410

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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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)