EUROPEAN COMMISSION
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Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
European Union Reference Laboratory for Feed Additives

JRC F.5/CvH/ZE/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

Cashew nut shell liquid

(FAD-2016-0027; CRL/150013)

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-2016-0027 - CRL/150013**
Name of Product:	***Cashew nut shell liquid***
Active Agent (s):	**Cardol and Cardanol**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
Report prepared by:	**Zigmas Ezerskis**
Report checked by: Date:	**María José González de la Huebra** **16/12/2019**
Report approved by: Date:	**Christoph von Holst** **16/12/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *cashew nut shell liquid* under the category/ functional group 1(b) "technological additives"/" antioxidants", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for all animal species.

The *feed additive* is to be marketed as a preparation containing a minimum of 4 % (w/w) of *cardol* and a minimum of 10 % (w/w) of *cardanol* as active substances.

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs*. The Applicant proposed levels of the preparation of the *feed additive* ranging from 200 to 2000 mg/kg *feedingstuffs*, which correspond to *cardol* and *cardanol* levels in *feedingstuffs* ranging from 8 to 100 mg/kg and 40 to 400 mg/kg, respectively.

For the quantification of *cardol* and *cardanol* in the *feed additive* the Applicant proposed a single-laboratory validated and further verified method based on high performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

The following performance characteristics were reported by the Applicant in the frame of the validation and verification studies for the quantification of *cardol* and *cardanol* in the *feed additive*: a relative standard deviation for *repeatability* (RSD_r) ranging from 0.1 to 4.7 %; a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 1.5 to 6.7 %; and a *recovery* rate (R_{rec}) ranging from 94 to 106 %.

Based on the experimental evidence available the EURL recommends for the official control the above mentioned single-laboratory validated and further verified HPLC-UV method for the quantification of *cardol* and *cardanol* in the *feed additive.*

Since the determination of the content of *cashew nut shell liquid* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to determine *cashew nut shell liquid* in *feedingstuffs*. Furthermore, the EURL cannot recommend the above mentioned HPLC-UV method for official control for the quantification of *cardol* and *cardanol* in *feedingstuffs* at the proposed conditions of use.

However, the EURL considers as fit-for-purpose above mentioned single-laboratory validated and further verified HPLC-UV method for the quantification of *cardol* and *cardanol* (expressed as a sum) in *premixtures* at the validated and verified mass fraction ranges of the analytes.

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

Cardol, cardanol, cashew nut shell liquid, technological additives, antioxidants, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a feed additive) for *cashew nut shell liquid* under the category/ functional group 1(b) "technological additives"/" antioxidants", 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 [1,2].

The *feed additive* is to be marketed as a preparation containing a minimum of 4 % (w/w) of *cardol* and a minimum of 10 % (w/w) of *cardanol* as active substances [3]. In addition, it contains methyl cardanol, polymers and ash naturally present in the product together with castor oil and expanded vermiculite as carriers [3].

The *feed additive* is intended to be incorporated into *premixtures* and *feedingstuffs* [3]. The Applicant proposed levels of the preparation of the *feed additive* ranging from 200 to 2000 mg/kg *feedingstuffs*, which correspond to *cardol* and *cardanol* levels in *feedingstuffs* ranging from 8 to 100 mg/kg and 40 to 400 mg/kg, respectively [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 *cashew nut shell liquid* 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 *cardol* and *cardanol* in the *feed additive* the Applicant proposed a single-laboratory validated and further verified method based on high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [4].

The sample (0.5 to 2 g) is extracted with tetrahydrofuran (THF). The extraction of the same sample is repeated two times. The combined extracts are diluted with an acetonitrile solution containing THF (10 % (v/v)) before chromatographic analysis. The detection of the analytes is performed at 275 nm and *cardol* and *cardanol* are quantified using cashew nut shell liquid distillate (with known concentrations of *cardol* and *cardanol*) as a standard for the calibration [4], with certified mass fractions available on request from the Applicant.

The performance characteristics reported by the Applicant in the frame of the validation and verification studies [5,6] for the quantification of *cardol* and *cardanol* in the *feed additive* are presented in Table 1.

Based on the experimental evidence available the EURL recommends for the official control the above mentioned single-laboratory validated and further verified HPLC-UV method for the quantification of *cardol* and *cardanol* in the *feed additive*.

Since the determination of the content of *cashew nut shell liquid* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to determine *cashew nut shell liquid* in *feedingstuffs*.

Table 1 The performance characteristics of the single-laboratory validated and verified HPLC-UV method for the quantification of *cardol* and *cardanol* in the *feed additive*

	Cardol		***Cardanol***	
	Validation	**Verification**	**Validation**	**Verification**
Mass fraction, % (w/w)	2.9 – 13.7	4.5 – 10.9	11.1 – 95.6	54.9 – 95.5
RSD_r, %	0.3 – 2.2	0.9 – 4.7	0.1 – 1.3	3.4 – 3.7
RSD_{ip}, %	6.7	4.7	1.5	3.7
R_{rec}, %	97	106	99	94
Reference	[5,6]			

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

However, the Applicant applied the above mentioned HPLC-UV method for the quantification of *cardol* and *cardanol* (expressed as a sum) in *premixtures* and *feedingstuffs* [4].

The performance characteristics reported by the Applicant in the frame of the validation and verification studies [7,8,9] for the quantification of *cardol* and *cardanol* (expressed as a sum) in *premixtures* and *feedingstuffs* are presented in Table 2. In addition, limits of quantification (LOQ) of 260 mg and 500 mg of *cardol* and *cardanol* (expressed as a sum) /kg *feedingstuffs* were reported by the Applicant and the 2nd laboratory, respectively [9].

Because the levels of the sum of the *cardol* and *cardanol* content in *feedingstuffs* covered by the validation and verification studies (7000 – 56000 mg/kg) were much higher compared to the target levels in *feedingstuffs* (48 to 500 mg/kg), the EURL cannot recommend the above mentioned HPLC-UV method for official control for the quantification of *cardol* and *cardanol* in *feedingstuffs* at the proposed conditions of use.

However, the EURL considers as fit-for-purpose above mentioned single-laboratory validated and further verified HPLC-UV method for the quantification of *cardol* and *cardanol* (expressed as a sum) in *premixtures* at the validated and verified mass fraction ranges of the analytes.

Table 2 The performance characteristics of the single-laboratory validated and verified HPLC-UV method for the quantification of *cardol* and *cardanol* (expressed as a sum) in *premixtures* and *feedingstuffs*

	Premixtures		**Feedingstuffs**	
	Cardol & Cardanol	***Cardol & Cardanol***	***Cardol & Cardanol***	***Cardol & Cardanol***
	Validation	**Verification**	**Validation**	**Verification**
Mass fraction, mg/kg	81000 – 95000	66000 – 69000	8000 – 56000	7000 – 31000
RSD_r, %	1.2	0.8 – 2.7	3.1	1.1 – 12.9
RSD_{ip}, %	4.7	2.7	3.1	12.9
R_{rec}, %	80	86	98	85
Reference	[8]		[7,9]	

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

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)

Evaluation of corresponding methods of analysis is not considered necessary by the EURL.

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 single-laboratory validated and further verified HPLC-UV method from the Applicant for the quantification of *cardol* and *cardanol* in the *feed additive*.

Since the determination of the content of *cashew nut shell liquid* added to *premixtures* and *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to determine *cashew nut shell liquid* in *feedingstuffs*. Furthermore, the EURL cannot recommend the above mentioned HPLC-UV method for official control for the quantification of *cardol* and *cardanol* in *feedingstuffs* at the proposed conditions of use.

However, the EURL considers as fit-for-purpose the above mentioned single-laboratory validated and further verified HPLC-UV method for the quantification of *cardol* and *cardanol* (expressed as a sum) in *premixtures* at the validated and verified mass fraction ranges of the analytes.

Recommended text for the register entry (analytical method)

For the quantification of *cardol* and *cardanol* in the *feed additive*:

– high performance liquid chromatography with spectrophotometric detection (HPLC-UV)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *cashew nut shell liquid* 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_FWD. APPL. 1831-0025-2016

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the feed additive; methods of analysis

[4] *Technical dossier, Section II – Appendix 5-0-0_Conf

[5] *Technical dossier, Section II – Appendix-5-0-1-b_Conf

[6] *Supplementary information – Verification Report LCC

[7] *Technical dossier, Section II – Appendix 5 A-Validation Report_Conf

[8] *Supplementary information – Verification Report PREMIX

[9] *Supplementary information – Verification Report FEED

*Refers to Dossier no: FAD-2016-0027

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

The following National Reference Laboratories contributed to this report:

- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)