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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

Coxiril®

(FAD-2013-0042; CRL/130006)

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-2013-0042 - CRL/130006**
Name of Product:	***Coxiril®***
Active Agent (s):	**Diclazuril**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
Report prepared by:	**María José González de la Huebra**
Report checked by: Date:	**Piotr Robouch (EURL-FA)** **24/02/2014**
Report approved by: Date:	**Christoph von Holst** **25/02/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Coxiril®*, under article 4 (1), for the category "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for *rabbit*.

Coxiril® consists of 0.5 % of *diclazuril* (active substance), starch as binding agent and calcium carbonate and wheat meal as carriers. The Applicant proposed a concentration of *diclazuril* in *feedingstuffs* ranging from 0.8 to 1.2 mg/kg.

For the determination of *diclazuril* in the *feed additive (Coxiril®)*, the Applicant submitted a single-laboratory validated and further verified method based on Reversed-Phase High Performance Liquid Chromatography coupled to Ultraviolet detection (RP-HPLC-UV). The following performance characteristics were reported: - a precision ranging from 0.2 to 0.5 %; and - a *recovery* rate (R_{Rec}) ranging from 98 to 102 %.

For the determination of *diclazuril* in *premixtures* and *feedingstuffs* the Applicant submitted the ring trial validated Community method (Commission Regulation (EC) No 152/2009) based on RP-HPLC-UV. The following performance characteristics were reported: - a relative standard deviation for *repeatability* (RSD_r) ranging from 1.9 to 17.3 % and a relative standard deviation for *reproducibility* (RSD_R) ranging from 7.5 to 18.6 %, - R_{Rec} ranging from 89 to 115 % and - a limit of quantification (LOQ) of 0.5 mg/kg *feedingstuffs*. Furthermore, the Applicant provided experimental evidence demonstrating the applicability of the Community method to determine *diclazuril* in *premixture* and *feedingstuff samples* containing *Coxiril®*.

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method and the Community method based on RP-HPLC-UV for the determination of *diclazuril* in the *feed additive*, *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) is not considered necessary.

KEYWORDS

Diclazuril, *Coxiril®*, coccidiostat, *rabbit*

1. BACKGROUND

In the current application authorisation is sought for *Coxiril®*, under article 4(1), for the category "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for *rabbit* [1,2].

Coxiril® consists of 0.5 % of *diclazuril* (active substance), starch as binding agent and calcium carbonate and wheat meal as carriers [2]. *Coxiril®* is a pale beige powder intended to be incorporated into *premixture*s and *feedingstuffs* [3,4]. The Applicant proposed a concentration of *diclazuril* in *feedingstuffs* ranging from 0.8 to 1.2 mg/kg [2].

Furthermore MRLs for *diclazuril* in *rabbit* tissues are already set by the Commission Implementation Regulation (EU) No 1235/2013 [5], therefore the corresponding methods of analysis do not need to be evaluated by the EURL.

Note: The EURL previously evaluated the analytical methods for the determination of *diclazuril* in the frame of the FAD-2012-0017 and FAD-2013-0014 dossiers [6].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, 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 *Coxiril®* 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 [7]

Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the determination of *diclazuril* in the *feed additive*, the Applicant submitted a single-laboratory validated and further verified method based on Reversed-Phase High Performance Liquid Chromatography coupled to Ultraviolet detection (RP-HPLC-UV) [8].

The sample is weighed in a 100 ml volumetric flask. N, N-Dimethylformamide (DMF) is added and the *diclazuril* is extracted using an ultrasonic bath for 30 min with a temperature of 60°C ± 4°C. After cooling, the extract is filtrated and injected into the chromatographic column. The *diclazuril* content is determined by RP-HPLC-UV at 280 nm [9]. The reported performance characteristics are summarised in Table 1.

For the determination of *diclazuril* in *premixtures* and *feedingstuffs* the Applicant submitted the ring trial validated Community method (Commission Regulation (EC) No 152/2009) based on RP-HPLC-UV [12]., along with a method protocol [13] specifying how the Community method has been implemented in the Applicant's laboratory.

Diclazuril is extracted from 1 g of *premixtures* sample (or 50 g of *feedingstuff* sample) with acidified methanol after addition of an internal standard. The feed extract is purified by a C18 solid phase extraction cartridge (the Applicant performed the purification step on a Strata C18E 1ml/100mg [13]). *Diclazuril* is eluted from the cartridge with a mixture of acidified methanol and water. The extract is evaporated and the residues are dissolved in a mixture of DMF and water. The *diclazuril* content is then determined by RP-HPLC-UV at 280 nm. When analysing premixtures, the extract is measured by RP-HPLC-UV without any prior clean-up [12]. The reported performance characteristics are presented in Table 1. In addition, limits of detection (LOD) and quantification (LOQ) of 0.1 and 0.5 mg/kg, were also determined.

Table 1. Performance characteristics of analytical method for the determination of *diclazuril* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS) [10,11,12].

Matrices	Concentration (mg/kg)	RSDr (%)	RSDip(%)	RSD_R(%)	R_{Rec} (%)
FA [10,11]	5000	0.2 – 0.5	0.3 - 0.5	-	98 - 102
PM [12]	100	5.8 – 7.4	-	7.5	101* - 103*
FS [12]	1.0	1.9 – 17.3	-	9.7 – 18.6	89.0* – 115*

RSD_r: relative standard deviation for *repeatability* (%); RSD_{ip}: relative standard deviation for *intermediate precision* (%); RSD_R: relative standard deviation for *reproducibility* (%) R_{Rec}: *recovery* rate (%);
* Calculated by EURL from [12]

Furthermore, the Applicant provided experimental evidence demonstrating the applicability of the Community method to determine *diclazuril* in *premixture* and *feedingstuff samples* containing *Coxiril®* [13, 14].

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated Community method based on RP-HPLC-UV for the determination of *diclazuril* 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) 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 RP-HPLC-UV method for the determination of *diclazuril* in the *feed additive* and the ring trial validated Community method - Commission Regulation (EC) No 152/2009 based on RP-HPLC-UV for the determination of *diclazuril* in *premixtures* and *feedingstuffs*

Recommended text for the register entry (analytical method)

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

- Reversed-Phase High Performance Liquid Chromatography using Ultraviolet detection at 280 nm (RP-HPLC-UV)

For the determination of *diclazuril* in *premixtures* and *feedingstuffs:*

- Reversed-Phase High Performance Liquid Chromatography using Ultraviolet detection at 280 nm (RP-HPLC-UV) - Regulation (EC) No 152/2009.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Coxiril®* 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 SANCO/G1: Forw. Appl. 1831/0039-2013

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

[3] *Technical dossier, Section II: Reference_II_17.pdf

[4] *Technical dossier, Section II: 2.5. Conditions of use of the additive

[5] Commission Implementing Regulation (EU) No 1235/2013 of 2 December 2013, amending the Annex to Regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in feedstuffs of animal origin, as regards the substance diclazuril.

[6] EURL Evaluation Reports FAD 2012-0017 and FAD 2013-0014

http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2012-0017.pdf

http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2013-0014-Coxiril.pdf

[7] 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

[8] *Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance

[9] *Technical dossier, Section II: Reference_II_30.pdf

[10] *Technical dossier, Section II: Reference_II_19.pdf

[11] *Technical dossier, Section II: Reference_II_24.pdf

[12] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, O.J. L 54, 26.02.2009

[13] *Technical dossier, Section II: Reference_II_15.pdf & Reference_II_16.pdf

[14] *Supplementary information: SOP_Diclazuril_Coxiril_ HPLC.pdf & Representative chromatograms.pdf

*Refers to Dossier no: FAD-2013-0042

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 (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)
- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus[1] (DK)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)

[1] Name and address according to Regulation (EC) No 885/2009: Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby

– Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)

– Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL). Oberschleißheim[2] (DE)

– Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare ed animale, Roma (IT)

– Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya,Cabrils (ES)

– Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)

– Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)

[2] Name and address according to Regulation (EC) No 885/2009: Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim