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Feed Additives

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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2012-0017** **CRL/120009**
Name of Product:	**Coxiril®**
Active Substance(s):	**Diclazuril**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report revised by: Date:	**Piotr Robouch (EURL-FA)** **06/11/2012**
Report approved by: Date:	**Christoph von Holst** **07/11/2012**

EXECUTIVE SUMMARY

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

Coxiril® consists of 0.5 % of *diclazuril* (*active substance*), 1.5 % starch for granulation, 28 % calcium carbonate and 70 % wheat meal. The Applicant proposes 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 Chromatograph coupled to Ultraviolet detection (RP-HPLC-UV). The following performance characteristics were reported: - a relative standard deviation for *repeatability* (RSD_r) ranging from 0.2 to 0.3 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.2 to 0.5 %; and - a *recovery* rate (R_{Rec}) ranging from 98.7 to 102 %. Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method based on RP-HPLC-UV for the determination of *diclazuril* in the *feed additive*.

For the determination of *diclazuril* in *premixtures* and *feedingstuffs* the Applicant submitted the ring trial validated Community method based on RP-HPLC-UV. The following performance characteristics were reported for *premixtures*: - RSD_r ranging from 5.8 to 7.4 % and a relative standard deviation for *reproducibility* (RSD_R) around 7.5 %. Whereas for *feedingstuffs* the following values were reported: - RSD_r ranging from 1.9 to 17.3 %; - RSD_R ranging from 9.7 to 18.6 %, and - a limit of detection (LOD) and quantification (LOQ) of 0.1 and 0.5 mg/kg, respectively. Based on the performance characteristics presented, the EURL recommends for official control 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*.

The following MRLs for *diclazuril* in tissues are established for target poultry (*chickens for fattening*, turkeys for fattening and chickens reared for laying) by Commission Regulation (EC) No 1118/2010: 1500 µg/kg in liver, 1000 µg/kg in kidney and 500 µg/kg in skin/fat and in muscle. For the determination of *diclazuril residues* in tissues, the Applicant submitted a single laboratory validated and further verified method, based on RP-HPLC coupled to mass spectrometry detection (MS/MS). The performance characteristics reported comply with the confirmatory requirements set in the Commission Decision (2002/657/EC). Therefore, the EURL recommends the RP-HPLC-MS/MS method submitted by the Applicant for the official control of the above mentioned MRLs in tissues of target poultry.

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, chickens for fattening

1. BACKGROUND

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

Coxiril® consists of 0.5 % of *diclazuril* (*active substance*), 1.5 % starch for granulation, 28 % calcium carbonate and 70 % wheat meal [3]. *Diclazuril* is a pure synthetic substance incorporated in the *additive* with a minimum purity of 99 % [4]. *Coxiril*® is a pale beige powder intended to be incorporated in *feedingstuffs* as *premixture* [5,6]. The Applicant proposes a concentration of *diclazuril* in *feedingstuffs* ranging from 0.8 to 1.2 mg/kg [2]. Another *feed additive* containing *diclazuril* is already authorised for *chickens for fattening*, where the minimum and maximum level of the active substance in *feedingstuffs* is set at 1 mg/kg [7].

Furthermore the Applicant suggests the following Maximum Residue Limits (MRLs): 500 µg/kg in skin/fat, 500 µg/kg in muscle, 1500 µg/kg in liver and 1000 µg/kg in kidney [2] (as already established for *chickens for fattening* tissues by Commission Regulation (EC) No 1118/2010 [7]). As the above mentioned MRLs are not set up by Commission Regulation (EC) No 37/2010 [8], the correspondent methods of analysis have to be evaluated by the EURL [9].

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 [10].

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 Chromatograph coupled to Ultraviolet detection (RP-HPLC-UV) [11].

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 [12].

The following performance characteristics were reported for *diclazuril* in *Coxiril*® [13,14]:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 0.2 to 0.3 %;
- a relative standard deviation for *intermediate precision* (RSD_{ip}) ranging from 0.2 to 0.5 %;
- a *recovery* rate (R_{Rec}) ranging from 98.7 to 102 %.

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method based on RP-HPLC-UV for the determination of *diclazuril* in the *feed additive* (*Coxiril*®).

For the determination of *diclazuril* in *premixtures* and *feedingstuffs* the Applicant submitted the ring trial validated Community method based on RP-HPLC-UV [11,15].

Diclazuril is extracted from 1 g of *premixtures* sample (or 50 g of *feedingstuffs* sample) with acidified methanol after addition of an internal standard. The feed extract is purified by a C_{18} solid phase extraction cartridge. *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 [15].

The Community method was ring trial validated by 11 laboratories, using two *premixtures* containing 100 mg *diclazuril*/kg and three complete *feedingstuffs* samples containing 1 mg *diclazuril*/kg. The following performance characteristics were reported for *premixtures* [15]:

- RSD_r ranging from 5.8 to 7.4 %; and
- a relative standard deviation for *reproducibility* (RSD_R) around 7.5 % .

For *feedingstuffs*:

- RSD_r ranging from 1.9 to 17.3 %;
- RSD_R ranging from 9.7 to 18.6 %, and
- a limit of detection (LOD) and quantification (LOQ) of 0.1 and 0.5 mg/kg, respectively.

Based on the performance characteristics presented, the EURL recommends for official control 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*.

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.

Methods of analysis for the determination of the residues of the additive in food.

For the determination of *diclazuril* in target *tissues* the Applicant proposed a single laboratory validated and further verified method based on RP-HPLC coupled to a triple quadrupole mass spectrometer (MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards [16].

Acetonitrile is added to the minced sample, shaken and centrifuged. The supernatant is then transferred in a clean tube. The sample is extracted a second time and the supernatant is combined with the first extract. An aliquot is evaporated under nitrogen stream and the dried residue is reconstituted in a mixture of acetonitrile/water for the injection in RP-HPLC-MS/MS. The target matrices (i.e. kidney, muscle, liver and skin/fat) of various species (including chicken and turkeys) were investigated at different *diclazuril* concentrations (Table 1) [17,18]. The method was further verified by a second independent laboratory [18,19] and complies with the confirmatory requirements set by Commission Decision 2002/657/EC [20].

The performance characteristics derived from the validation and verification strudies are presented in Table 1. Furthermore the Applicant reported an LOQ for muscle, liver, kidney and for skin/fat ranging from 50 to 57 µg/kg [18,19].

Table 1. Performance characteristics of analytical method for the determination of the *residues* in food [17-19].

Tissues		µg/kg (#)	RSD_r (%)		RSD_{ip} (%)		R_{Rec} (%)	
			Valid	Verif	Valid	Verif	Valid	Verif
Chicken [17,19]	Muscle	250	6.2	---	4.7	---	94.2	---
		500	5.1	1.5-2.4	6.2	2.5	88.2	105
		1000	2.6	---	6.7	---	87.4	---
	Liver	750	5.1	---	6.2	---	80.1	---
		1500	3.9	1.0-3.4	5.9	2.7	99	102.1
		3000	4.9	---	5.9	---	101.6	---
	Skin/fat	250	5.2	---	13.8	---	94.2	---
		500	5.5	4.7-6.5	13	9.6	94.2	96
		1000	5.1	---	14.2	---	94.2	---
	Kidney	500	2.2	---	2.3	---	80.2	---
		1000	7.6	1.7-6.7	6.6	4.9	82.6	97.4
		2000	7.2	---	5.8	---	89.7	---
Turkey [18]	Muscle	500	1.3	2.6-3.2	2.3	3.2	102.9	98.2
	Liver	1500	1.3	6.3-7.5	4.8	7	95.1	93.7
	Skin/fat	500	1.8	4.2-5.3	3.2	4.7	103.2	97.3
	Kidney	1000	1.2	3.3-3.8	9	4.3	104.9	95.2

RSD_r: relative standard deviation for *repeatability* (%); RSD_{ip}: relative standard deviation for *intermediate precision* (%); R_{Rec}: *recovery* rate (%); # Fortified level.

Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC-MS/MS method proposed by the Applicant, to enforce the MRLs for *diclazuril* in the target *tissues*.

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* (*Coxiril*®);

- 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*.

Furthermore, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method for the determination of *diclazuril* in *target*

tissues. This method complies with the confirmatory requirements set by Commission Decision (2002/657/EC).

Recommended text for the register entry (analytical method)

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

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

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

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

For the determination of *diclazuril* in *target tissues*:

- Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) coupled to triple quadrupole mass spectrometer (MS/MS).

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. 1831/0036-2012

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

[3] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition

[4] *Technical dossier, Section II: 2.1.4. Purity

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

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

[7] Commission Regulation (EC) No 1118/2010 of 2 December 2010, concerning the authorisation of diclazuril as a feed additive for chickens for fattening (holder of authorisation Janssen Pharmaceutica NV) and amending Regulation (EC) No 2430/1999

[8] Commission Regiulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

[9] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives

[10] 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

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

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

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

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

[15] 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

[16] *Technical dossier, Section II: 2.6.2 Methods of analysis for the determination of the residues of the additive or of its metabolites in food

[17] *Technical dossier, Section II: Reference_II_22.pdf

[18] *Supplementary Information, verification turkeys.pdf

[19] *Technical dossier, Section II: Reference_II_25.pdf

[20] 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

* Refers to Dossier No. FAD-2012-0017

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:

- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Fødevarestyrelsen, Ringsted (DK)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)