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Community Reference Laboratory for Feed Additives

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CRL 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-2010-0010 CRL/100010
Name of Additive:	Clinacox 0.5%
Active Substance(s):	Diclazuril
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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Report approved by: Date:	Christoph von Holst 07/12/2010

EXECUTIVE SUMMARY

In the current application authorisation is sought under articles 10(2) (re-evaluation of the additive already authorised under council directive 70/524/EEC) for *Clinacox 0.5%* under the category "coccidiostats and histomonostats" according to Article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for *turkeys* for fattening. *Clinacox 0.5%* contains 0.5 % w/w of the active substance *diclazuril*. *Diclazuril* is a pure synthetic substance with a minimum purity of 98 %. Main components of the *feed additive* are protein poor soybean meal (99.25 % w/w), polyvidone K30 (0.2 % w/w) and sodium hydroxide (0.05 % w/w).

The Applicant proposes a concentration of *diclazuril* in *feedingstuffs* ranging from 0.9 to 1.1 mg/kg, whereas according to the current authorisation conditions the minimum and maximum levels of the active substance in feedingstuffs are set at 1 mg/kg.

For the determination of *diclazuril* in *premixtures* and *feedingstuffs,* the Applicant submitted a method based on the Community method (Commission Regulation (EC) No 152/2009), using Reversed-Phase High Performance Liquid Chromatograph (RP-HPLC) coupled to Ultraviolet detection. The performance characteristics determined by the Applicant are in good agreement with those reported by the ring trial validated Community method. The ring trial validated Community method included two *premixtures* containing 100 mg *diclazuril*/kg and three complete *feedingstuffs* containing 1 mg *diclazuril*/kg. The performance characteristics reported are:

For *premixtures*:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 5.8 to 7.4 %;
- 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.

The Applicant applied the Community method to determine *diclazuril* in the *feed additive,* obtaining an RSD_r of 3 % and a *recovery* rate of about 110 %.

Based on the above mentioned performance characteristics the CRL recommends for official control of *diclazuril* in *feed additive*, *premixtures* and *feedingstuffs* the Community method - Commission Regulation (EC) No 152/2009.

Regarding **residues** of *diclazuril* in edible tissues, the following MRLs were established for target poultry (chickens for fattening, *turkeys* for fattening and chickens reared for laying) tissues by Commission Regulation (EC) No 976/2008: 1500 μg/kg in liver, 1000 μg/kg in kidney, 500 μg/kg in skin/fat and in muscle.

For the determination of *diclazuril residues* in tissues, the Applicant submitted a single laboratory validated method, using RP-HPLC coupled to mass spectrometry detection (MS/MS). The method was further verified by a second independent laboratory. The performance characteristics reported comply with the confirmatory requirements set in the Commission Decision (2002/657/EC). Therefore, the CRL recommends the RP-HPLC-MS/MS method submitted by the Applicant for the official control of the above mentioned MRLs.

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, coccidiostat, turkeys for fattening

1. BACKGROUND

In the current application authorisation is sought under articles 10(2) (re-evaluation of the additive already authorised under council directive 70/524/EEC) for *Clinacox 0.5%* under the category "coccidiostats and histomonostats" according to Article 6 of Regulation (EC) No 1831/2003 [1]. Authorisation is sought for *turkeys* for fattening [2].

According to the Applicant, *Clinacox 0.5%* contains 0.5 % w/w of the active substance *diclazuril*. *Diclazuril* is a pure synthetic substance with a minimum purity of 98 % [3]. Main components of the *feed additive* are protein poor soybean meal (99.25 % w/w), polyvidone K30 (0.2 % w/w) and sodium hydroxide (0.05 % w/w) [4].

The Applicant proposes a concentration of *diclazuril* in *feedingstuffs* ranging from 0.9 to 1.1 mg/kg [2], whereas the current authorisation sets the minimum and maximum level of the active substance in feedingstuffs at 1 mg/kg [4].

The following MRLs have been established for *turkeys* for fattening tissues by Commission Regulation (EC) No 976/2008 [5]: 1500 μg/kg in liver, 1000 μg/kg in kidney, 500 μg/kg in skin/fat and in muscle.

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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Clinacox 0.5%*, 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 Community Reference Laboratories [6].

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

For the determination of *diclazuril* in *premixtures* [7] and *feedingstuffs* [8], the Applicant submitted methods based on the Community method [9], using Reversed-Phase High Performance Liquid Chromatograph (RP-HPLC) coupled to Ultraviolet (UV) detection. The performance characteristics determined in the frame of the validation study are: a relative standard deviation for *repeatability* (RSD_r) of 2.1 % and a *recovery* rate ranging from 88 to 97 % [8].

In the Community method [9], 1 g of *premixtures* sample (or 50 g of *feedingstuffs* sample) is extracted with acidified methanol after addition of an internal standard. The feed extract is purified by using a C18 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 dimethylformamide and water. The *diclazuril* content is determined by RP-HPLC/UV at 280 nm. When analysing *premixtures*, the extract is measured with the RP-HPLC/UV system without any clean-up of the extract.

The ring trial validated Community method included two *premixtures* containing 100 mg *diclazuril*/kg and three complete *feedingstuffs* samples for poultry containing 1 mg *diclazuril*/kg. The performance characteristics reported are:

For *premixtures*:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 5.8 to 7.4 %;
- 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.

The Applicant applied the Community method to the determine *diclazuril* in the *feed additive,* obtaining an RSD_r of 3 % and a *recovery* rate of about 110 % [10].

Based on the above mentioned performance characteristics the CRL recommends for official control of *diclazuril* in *feed additive*, *premixtures* and *feedingstuffs* the Community method - Commission Regulation (EC) No 152/2009 [9].

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 method [11] comprising three steps: extraction with acetonitrile; centrifugation of the combined extracts, followed by the evaporation of an aliquot of the supernatant to dryness and re-dissolution of the dry residue in a mixture of acetonitrile/water; and, the determination by Reversed-Phase High Performance Liquid Chromatograph (RP-HPLC) coupled to a triple quadrupole mass spectrometer (MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards. The method was validated on all target matrices (i.e. kidney, muscle, liver and skin/fat) at various concentrations (Table 1). The method was further verified by a second independent laboratory [12] and complies with the confirmatory requirements set by Commission Decision 2002/657/EC.

The performance characteristics, recalculated by CRL [13] based on the experimental data provided by the Applicant for validation [11]and verification data [12], are presented in Table 1. The limits of quantification for skin/fat, muscle and liver - kidney are 25, 20 and 15 µg/kg, respectively [11].

Table 1. Performance characteristics of analytical method for the determination of the *residues* in food [13].

Tissue	µg/kg (#)	Validation			Verification		
		RSD_r	RSD_{ip}	R_{Rec}	RSD_r	RSD_{ip}	R_{Rec}
Muscle	250	3.2	5.1	96-105	---	---	---
	500	3.2	4.9	93-101	5.2	5.2	85
	1000	4.5	6.1	95-103	---	---	---
Liver	750	3.5	3.8	105-109	---	---	---
	1500	4.4	4.7	96-99	7.1	9.7	87-96
	3000	4.7	4.7	96-99	---	---	---
Skin/fat	250	3.6	7.9	93-106	---	---	---
	500	3.2	5.0	95-102	7.8	7.9	87-91
	1000	3.4	8.1	94-108	---	---	---
Kidney	500	3.0	3.0	101-103	---	---	---
	1000	4.5	4.9	95-99	6.9	7.7	85-91
	2000	2.7	3.4	96-100	---	---	---

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 in Table 1, the CRL recommends for official control the RP-HPLC-MS/MS method proposed by the Applicant, to enforce the MRLs for *diclazuril* in *turkey 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

The CRL recommends for official control the Community method for the determination of *diclazuril* in *feed additive*, *premixtures* and *feedingstuffs*.

For the determination of *diclazuril* in *turkey tissues,* the CRL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant. The 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*, *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 *turkey tissues*:

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

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Clinacox 0.5%* have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/0002-2010

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

[3] *Technical dossier, Section II

[4] COMMISSION List of the authorised additives in feedingstuffs (1) published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01)

[5] Commission Regulation (EC) No 976/2008 of 6 October 2008, amending Regulations (EC) No 2430/1999, (EC) No 418/2001 and (EC) No 162/2003 as regards the terms of the authorisation of the feed additive "Clinacox", belonging to the group of coccidiostats and other medicinal substances

[6] 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

[7] *Technical Dossier, Section II, Annex_II_21_Val-200901

[8] *Technical Dossier, Section II, Annex_II_12_Validation

[9] Commission Regulation (EC) No 152/2009 of 29 January 2009 laying down the methods for sampling and analysis for the official control in feed

[10] #CRL Responses - Appendix 1 "Determination of diclazuril in Clinacox 0.5% oral premix and premixtures containing Clinacox 0.5% oral premix based on the Community method", supplementary provided by the applicant on request of the CRL

[11] *Supplementary Information, Validation

[12] *Supplementary Information, Verification

[13] *Additional Information – Precision data as recalculated by the CRL

* Refers to Dossier No. FAD-2010-0010
# Refers to Dossier No. FAD-2008-0053 and FAD-2009-0058

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community 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

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- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya,Cabrils (ES)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, (DE)
- Kmetijski inštitut Slovenije, Ljubljana (SI)
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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)