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CRL 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-2009-0035** **CRL/090025**
Name of additive:	**Coxidin**
Active Agent (s):	***Monensin sodium***
Rapporteur Laboratory:	**Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium**
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Report approved by: Date:	**Christoph von Holst** **20/10/2010**

EXECUTIVE SUMMARY

Coxidin is a product already authorised as *feed additive*, under the category '*coccidiostats*', according to the classification system of Article 6 of Regulation (EC) No 1831/2003. In the current application a modification of the authorisation is sought according to Art. 13(3) of the above mentioned regulation. In particular, the composition of the additive is proposed to be changed from a formulation containing an organic carrier (wheat bran) to an inorganic one (calcium carbonate). The active substance of *Coxidin* is *monensin sodium*. It is authorised for chickens for fattening and for turkeys (up to 16 weeks of age) at concentrations in *feedingstuffs* of 100 to 125 mg/kg and 60 to 100 mg/kg, respectively.

For the determination of *monensin sodium* in *premixtures* and *feedingstuffs*, the CRL recommends the ring-trial validated method EN ISO 14183, a multi-analyte high performance liquid chromatography (HPLC) method with post-column derivatisation and UV-VIS detection, even though this method was not proposed by the Applicant. The performance characteristics reported for the CEN/ISO method are:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.6% to 5.2%
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.8% to 13%
- a *recovery* rate (R_{Rec}) ranging from 90.7% to 106.7% (recalculated by CRL), and
- a limit of quantification of 1 mg/kg *feedingstuffs.*

For the determination of the *monensin sodium* in the *feed additive* the Applicant re-submitted the data provided for the FAD-2005-0003 dossier. Upon request by the CRL, the Applicant provided additional experimental evidence applying the EN ISO 14183 method to the new product. From the reported experimental data, the CRL calculated RSD_r = 0.966 % and R_{Rec} = 108 %, thus confirming the applicability of the CEN/ISO method to the analysis of *Coxidin*.

Based on the above mentioned performance characteristics the CRL recommends for official control the ring-trial validated EN ISO 14183 method, based on HPLC method with post-column derivatisation and UV-VIS detection for the determination of *Coxidin* in *feed additive*, *premixtures and feedingstuffs*.

Regarding the analytical methods for *residues* of *monensin* in *tissue*s, the conclusions drawn in the CRL report FAD-2005-0003 (dated 01/08/2007) are valid and applicable for the purpose of the 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) is not considered necessary.

KEYWORDS

Coxidin, monensin sodium, coccidiostats, chickens for fattening, turkeys.

1. BACKGROUND

Coxidin is a product already authorised as *feed additive* [1], under the category '*coccidiostats*', according to the classification system of Article 6 of Regulation (EC) No 1831/2003. In the current application a modification of the authorisation is sought according to Art. 13(3) of the above mentioned regulation. In particular, the composition of the additive is proposed to be changed from a formulation containing an organic carrier (wheat bran) to an inorganic one (calcium carbonate) [2], [3].

Coxidin is a light beige to brown powder containing 25% of monensin, 15% to 20% perlite and a calcium carbonate matrix [4].

The active substance of *Coxidin* is *monensin sodium.* It is authorised for chickens for fattening and for turkeys (up to 16 weeks of age) at concentrations in *feedingstuffs* of 100 to 125 mg/kg and 60 to 100 mg/kg, respectively [1].

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 tasks of the Community Reference Laboratory concerning applications for authorizations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application or for each group of application. For this particular dossier, the methods of analysis submitted in connection with *Coxidin* and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification/Characterization 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, mercury, lead, dioxins and PCBs) are available at the respective EU Reference Laboratories [5].

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

For the determination of *monensin sodium* in *premixtures* and *feedingstuffs*, the CRL recommends the ring-trial validated method EN ISO 14183 [6], a multi-analyte high performance liquid chromatography (HPLC) method with post-column derivatisation and UV-VIS detection, even though this method was not proposed by the Applicant. The method consists in the extraction of *monensin* using methanol/water (90/10) followed by mechanical shaking for 1 h and filtration. The active substance is hence determined by reversed-phase HPLC using post-column derivatisation with vanillin, and detection at 520 nm. The performance characteristics reported for the CEN/ISO method are:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.6% to 5.2%
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.8% to 13%
- a *recovery* rate (R_{Rec}) ranging from 90.7% to 106.7% (recalculated by the CRL), and
- a limit of quantification of 1 mg/kg *feedingstuffs.*

For the determination of the *monensin sodium* in the *feed additive* the Applicant re-submitted the data provided for the FAD-2005-0003 dossier [7]. From this documentation it appears that the validation study was performed on a formulated product that significantly differs from the formulation of the current application in terms of matrix composition (wheat bran 55-60 % vs. mineral carrier 100% w/w) [4], [8], [9], [10]. Upon request by the CRL, the Applicant provided additional experimental evidence applying the EN ISO 14183 method to the new product. The experiments have been carried out by an external laboratory. Considering the high concentration level of the *active substance* in the *feed additive* itself a minor modification of the ISO standard method has been introduced (dilution step) [11], [12]. From the reported experimental data, the CRL calculated RSD_r = 0.966 % and R_{Rec} = 108 %, thus confirming the applicability of the CEN/ISO method to the analysis of *Coxidin*.

Furthermore, the CEN/ISO method, based on post-column derivatisation and VIS detection, is specific distinguishing between the narasin, salinomycin and *monensin* ionophores. Potential

interferences in the determination of *monensin* caused by the other components of the *feed additive*, *premixtures* or *feedingstuffs* are not expected.

Based on the above mentioned performance characteristics the CRL recommends for official control the ring-trial validated EN ISO 14183 method, based on HPLC method with post-column derivatisation and UV-VIS detection for the determination of *monensin sodium* in *feed additive*, *premixtures and feedingstuffs*.

Regarding the analytical methods for *residues* of *monensin* in *tissue*s, the conclusions drawn in the CRL report FAD-2005-0003 (dated 01/08/2007) are valid and applicable for the purpose of the application [13].

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 for the determination of *monensin sodium* in *feed additive*, *premixtures and feedingstuffs*:

- the HPLC method with post-column derivatisation and UV-VIS detection CEN/ISO standard method (EN ISO 14183:2008)

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

For the determination of *Coxidin* in the *feed additive, premixtures and feedingstuffs*:

- high performance liquid chromatography (HPLC) with post-column derivatisation and UV-VIS detection (EN ISO standard method 14183:2008)

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

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

6. REFERENCES

[1] Commission Regulation (EC) No 109/2007, amended by Commission Regulation (EC) No 156/2008 and Commission Regulation (EC) No 1095/2008.

[2] *Application/Ref:SANCO/D/2:Forw.Appl.1831/043-2009

[3] *Application, Proposal for Register Entry, Annex A

[4] *Technical dossier, Section II: 2.1 Identity of the additive

[5] 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

[6] EN ISO 14183:2008 – Animal feeding stuffs – Determination of monensin, narasin and salinomycin contents – Liquid chromatographic method using post-column derivatisation

[7] Evaluation Report of the Community Reference Laboratory Feed Additive Authorisation on the Method(s) of Analysis for Coxidin®, FAD-2005-0003, 20th July 2005

[8] *Technical dossier, Section II, Annex II.9, "Validation of the HPLC assay to determine monensin sodium (technical grade), the active principle of 'Coxidin 25% feed additive' "

[9] *Technical dossier, Section II, Annex II.10, "Validation of HPLC method for assay of monensin content in the feed additive Coxidin 25%"

[10] *Technical dossier, Section II, Annex II.11, " Coxidin – Monensin sodium 25% Validation of the HPLC-method"

[11] *Supplementary information, "FAD-2009-035-Answers.doc"

[12] *Supplementary information, "DOC200910.pdf"

[13] Evaluation Report of the Community Reference Laboratory Feed Additive Authorisation on the Method(s) of Analysis for Coxidin®25%, FAD-2007-0008, 1st August 2007

* Refers to Dossier No. FAD-2009-0035

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.

8. ACKNOWLEDGEMENTS

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