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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

Coxidin®
(FAD-2016-0009; CRL/150035)

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-2016-0009 - CRL/150035**
Name of Feed Additive:	***Coxidin®***
Active Agent (s):	**Monensin Sodium**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **JRC Geel, Belgium**
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Report checked by: Date:	**Piotr Robouch (EURL-FA)** **27/02/2017**
Report approved by: Date:	**Christoph von Holst** **28/02/2017**

EXECUTIVE SUMMARY

Coxidin® is a *feed additive* currently authorized for turkeys and chickens for fattening by Commission Regulation (EC) No 109/2007 for the category (e) "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. This authorisation was further amended by Commission Regulations (EC) No 156/2008 and No 1095/2008 and by Commission Implementing Regulation (EC) No 495/2011. In the current application a renewal of the *feed additive* authorisation under article 14 of the Regulation (EC) No 1831/2003 is requested. *Coxidin®* is a light beige to brown powder containing *monensin sodium* technical substance (active substance) equivalent to a 25 % (w/w) of *monensin* activity, perlite as binding agent and wheat bran as carrier. *Coxidin®* is intended to be incorporated into *feedingstuffs* through *premixtures*. The Applicant proposed a content of *monensin* ranging from 60 to 125 mg/kg complete *feedingstuffs* depending on the target species. Furthermore the Applicant suggested Maximum Residue Limits (MRLs) for *monensin sodium* in *tissues* of turkeys and chickens for fattening of 8 µg/kg (in liver, muscle and kidney), and of 25 µg/kg (in skin/fat), as already established by Commission Regulation (EC) No 1095/2008.

For the quantification of *monensin* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted single-laboratory validated methods based on Reversed Phase High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (RP-HPLC-PCD-UV-Vis). In addition, the EURL identified another ring-trial validated method (EN ISO 14183) using a similar experimental protocol. Based on the performance characteristics available the EURL recommends for official control the EN ISO 14183 method for the quantification of *monensin* in the *feed additive*, *premixtures* and *feedingstuffs*.

For the quantification of *monensin sodium* in chicken and turkey *tissues* the Applicant submitted a single-laboratory validated method based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in an electrospray ionisation mode without providing a verification study. The EURL found instead a similar single-laboratory validated and further verified method submitted by the same Applicant in the frame of another *monensin* dossier. Based on the performance characteristics available, the EURL recommends for official control this single-laboratory validated and further verified method or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the *monensin sodium* MRLs in the relevant *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.

KEYWORDS

Monensin sodium, *Coxidin®*, coccidiostat, turkeys and chickens for fattening

1. BACKGROUND

Coxidin® is a *feed additive* currently authorized for turkeys and chickens for fattening by Commission Regulation (EC) No 109/2007 [1] for the category (e) "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. This regulation was further amended by Commission Regulations (EC) No 156/2008 and No 1095/2008 [2][3] and by Commission Implementing Regulation (EC) No 495/2011[4]. In the current application a renewal of the *feed additive* authorisation under article 14 of the Regulation (EC) No 1831/2003 is requested for turkeys and chickens for fattening [5][6].

Coxidin® is a light beige to brown powder containing *monensin sodium* technical substance (active substance) equivalent to a 25 % (w/w) of *monensin* activity, 15 - 20 % (w/w) perlite (binding agent) and 55 – 60 % (w/w) wheat bran (carrier) [6][7]. *Coxidin®* is intended to be incorporated into *feedingstuffs* through *premixtures* [6]. The Applicant proposed a content of *monensin* ranging from 60 to 125 mg/kg complete *feedingstuffs* depending on the target species [6][8].

Furthermore the Applicant suggested the following Maximum Residue Limits (MRLs) for *monensin sodium* in *tissues* of turkeys and chickens for fattening: 8 µg/kg in liver, muscle and kidney, and 25 µg/kg in skin/fat, as established by Commission Regulation (EC) No 1095/2008 [3].

Note: The EURL previously evaluated the analytical methods for the quantification of *monensin* in the frame of several dossiers [9].

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 *Coxidin®* 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 quantification of *monensin* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted single-laboratory validated methods [11][12][13] using Reversed Phase High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (RP-HPLC-PCD-UV-Vis), based on the same principle as the ring-trial validated EN ISO 14183 [14] method.

In the EN ISO 14183 method, *monensin* is extracted using methanol:water (90:10) with mechanical shaking for 1 h, filtered and subjected to analysis without further clean-up. The target analyte is determined by Reversed Phase HPLC using post-column derivatisation with vanillin and detection at 520 nm. According to *Campbell & Nayeri*, relevant interferences in the determination of *monensin* cannot be expected [15].

This method was ring-trial validated for *premixtures* and *feedingstuffs* at a mean *monensin* content ranging from 3.1 to 178000 mg/kg leading to the following performance characteristics [14]:

- a relative standard deviation for *repeatability* (RSD_r) ranging from 2.6 to 9.5 % ;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.7 to 13.1 %; and
- a limit of quantification (LOQ) of 1 mg/kg in *feedingstuffs*.

Furthermore, in the frame of previous dossiers [9] the Applicant applied the EN ISO 14183 method to quantify *monensin* in other *feed additives* with the same *monensin* content and/or with similar carrier components (cf. FAD-2009-0035, FAD-2012-0027) and reported precision values in agreement with those presented above, thus confirming the applicability of this method to the analysis of the *feed additive per se*.

Based on the performance characteristics available the EURL recommends for official control the EN ISO 14183 method [14] for the quantification of *monensin* in the *feed additive*, *premixtures* and/or *feedingstuffs*.

Note: The EN ISO 14183 method was ring-trial validated for a *drug premixture* (Rumensin premix) with a *monensin* content of 17.8 % w/w which is close to the *monensin* content in *Coxidin®* (25 % w/w) [7].

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

For the quantification of *monensin sodium* in chicken and turkey *tissues* the Applicant submitted a single-laboratory validated method based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) with electrospray ionisation [16], without providing a verification study.

The EURL found a similar single-laboratory validated and further verified method submitted by the same Applicant in the frame of another *monensin* dossier (FAD-2012-0027) [9] for the quantification of *monensin sodium* in chicken and turkey *tissues*, where *monensin* is extracted from the minced tissues with acetonitrile in an ultrasonic bath followed by centrifugation. The supernatant is then evaporated under nitrogen and the dried residue is reconstituted in acetonitrile, filtered and injected into the HPLC system. *Monensin* is determined by RP-HPLC-MS/MS with electrospray ionisation (ESI) using matrix matched standards.

The relevant *tissues* were investigated at different *monensin* content levels (ranging from MRL/2 to 2MRL). The method was further verified by a second independent laboratory. Four identification points were set for *monensin* using one parent and two daughter ions. Quantification is based on the transition m/z 693.3 > 675.3 while confirmation is based on the transition m/z 693.3 > 461.3 thus complying with the confirmatory requirements set by Commission Decision 2002/657/EC [17]. The corresponding validation and verification studies led to the following performance characteristics [9]:

- RSDr ranging from 1.4 to 8.3 %;
- RSD_R ranging from 1.4 to 15 %; and
- R_{Rec} ranging from 92 to 107 %.

Furthermore the Applicant estimated a LOQ of 0.5 µg/kg for muscle, liver, kidney and skin/fat *tissues* [9].

Based on the performance characteristics available the EURL recommends for official control the single-laboratory validated and further verified method based on RP-HPLC-MS/MS or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC to enforce the *monensin sodium* MRLs in the relevant *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: (i) the ring-trial validated RP-HPLC-PCD-UV-Vis method (EN ISO 14183) for the quantification of *monensin* in the *feed additive, premixtures* and *feedingstuffs* and (ii) the single-laboratory validated and further verified RP-HPLC-MS/MS method or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC to enforce the *monensin sodium* MRLs in the relevant *tissues*.

Recommended text for the register entry (analytical method)

For the quantification of *monensin* in the *feed additive*, *premixtures* and *feedingstuffs*:

– Reversed Phase High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (RP-HPLC-PCD-UV-Vis) – EN ISO 14183

For the quantification of *monensin sodium* in chicken and turkey *tissues*:

– Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Coxidin®* 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] Commission Regulation (EC) No 109/2007 of 5 February 2007, concerning the authorisation of monensin sodium (Coxidin) as feed additive.

[2] Commission Regulation (EC) No 156/2008 of 21 February 2008, amending Regulation (EC) No 109/2007 as regards the minimum content of the feed additive monensin sodium (Coxidin).

[3] Commission Regulation (EC) No 1095/2008 of 6 November 2008, amending Regulation (EC) No 109/2007 as regards the terms of authorisation of the feed additive monensin sodium (Coxidin).

[4] Commission Implementing Regulation (EC) No 495/2011 of 20 May 2011, amending Regulation (EC) No 109/2007 as regards the composition of the feed additive monensin sodium.

[5] *Application, Reference SANTE/E5: Forw. Appl. 1831/0009-2016

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

[7] *Technical dossier, Section II: II.1 Identity of the additive

[8] *Technical dossier, Section II: II.5 Conditions of use of the additive

[9] EURL Evaluation Reports FAD 2012-0027, FAD 2009-0035
https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2012-0027-monimax.pdf
https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2009-0035.pdf

[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: Annex II.44, 45 & 46

[12] *Technical dossier, Section II: Annex II.48

[13] *Technical dossier, Section II: Annex II.49

[14] EN ISO 14183:2008 Animal feedingstuffs – Determination of monensin, narasin and salinomycin contents – Liquid chromatography method using post-column derivatisation (ISO 14183:2005)

[15] Campbell, H., Nayeri, G. J. AOAC Int. 89 (2006) 1229-1242

[16] *Technical dossier, Section II: Annex II.50, 51 & 52

[17] Commission Decision 2002/657/EC 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-2016-0009

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

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- Fødevarestyrelsens Laboratorie Ringsted (DK)
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- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- RIKILT Wageningen UR, Wageningen (NL)

- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural**.** Generalitat de Catalunya, Cabrils (ES)
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