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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

Elancoban® G200
(FAD-2013-0037; CRL/130012)

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-2013-0037 - CRL/130012**
Name of Feed Additive:	**Elancoban® G200**
Active Agent (s):	***Monensin sodium***
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)** **10/09/2014**
Report approved by: Date:	**Christoph von Holst** **11/09/2014**

EXECUTIVE SUMMARY

Elancoban®G200 is a *feed additive* currently authorized for turkeys, chickens for fattening and reared for laying by Commission Regulation (EC) No 1356/2004 belonging to the "Coccidiostats and other medicinal substances" group listed in Directive 70/524/EEC. The authorisation was further amended by Commission Regulation (EC) No 1096/2008. In the current application an authorisation according to article 10 (2) of Regulation (EC) No 1831/2003 is requested. *Elancoban®G200* consists of granular *monensin* (active substance) containing 20 % (w/w) *monensin*, anti-dusting oil, and rice hulls or limestone granular as base material. It is intended to be preferably incorporated into *feedingstuffs* through *premixtures.* The Applicant proposed a concentration of *monensin* in *feedingstuffs* ranging from 60 to 125 mg/kg, depending on the target species.

Furthermore the Applicant suggested the following Maximum Residue Limits (MRLs) in *tissues* of turkeys and chickens for fattening and reared for laying: 8 μg/kg in liver, muscle and kidney and 25 μg/kg in skin/fat *tissues*, as already established by Commission Regulation (EC) No 1096/2008.

For the quantification of *monensin* in the *feed additive*, *premixes* and *feedingstuffs* the Applicant submitted the AOAC ring-trial validated methods (AOAC 997.04) based on High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.). In addition, the EURL identified another multi-analyte ring-trial validated method (EN ISO 14183) using a similar experimental protocol. Based on the performance characteristics available the EURL recommends for official control both methods for the quantification of *monensin* in the *feed additive*, *premixtures* and/or *feedingstuffs*.

For the quantification of *monensin* in chicken and turkey *tissues* the Applicant submitted the ring trial validated method (AOAC 2011.24) based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode using matrix matched standards. Based on the performance characteristics available the EURL recommends for official control this AOAC ring-trial validated method or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the *monensin* 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, *Elancoban®G200*, coccidiostat, turkeys, chickens for fattening and reared for laying

1. BACKGROUND

Elancoban®G200 is a *feed additive* currently authorized for turkeys, chickens for fattening and reared for laying by Commission Regulation (EC) No 1356/2004 [1] belonging to the "Coccidiostats and other medicinal substances" group listed in Directive 70/524/EEC. This regulation was further amended by Commission Regulation (EC) No 1096/2008 [2]. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested for turkeys, chickens for fattening and reared for laying [3, 4].

Elancoban®G200 consists of granular *monensin* (active substance) containing 20 % (w/w) *monensin*, 1-3% (w/w) anti-dusting oil, and rice hulls or limestone granular as base material [4]. *Elancoban®G200* is intended to be preferably incorporated into *feedingstuffs* through *premixtures* [5]. The Applicant proposed a concentration of *monensin* in *feedingstuffs* ranging from 60 to 125 mg/kg depending on the target species [4, 5].

Furthermore the Applicant suggested the following Maximum Residue Limits (MRLs) in *tissues* of turkeys and chickens for fattening and reared for laying: 8 µg/kg in liver, muscle and kidney; or 25 µg/kg in skin/fat, as already established by Commission Regulation (EC) No 1096/2008 [2].

Note: The EURL previously evaluated the analytical methods for the determination of *monensin* in the frame of the FAD-2007-0030 dossier [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 *Elancoban®G200* 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 quantification of *monensin* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated method (AOAC 997.04) based on High Performance Liquid Chromatography with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis) [8].

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 reverse-phase HPLC-PCD with vanillin and detection at 520 nm.

The following performance characteristics were reported for *monensin* concentrations ranging from 30 mg/kg to 176 mg/g (nb: equivalent to 17.6 % w/w):

- a relative standard deviation for *repeatability* (RSD_r) from 6.1 to 12 %;
- a relative standard deviation for *reproducibility* (RSD_R) from 2.8 to 12 %;
- a recovery rate (R_{Rec}) ranging from 99 to 115 %; and
- a limit of quantification (LOQ) of 4 mg/kg in *feedingstuffs*.

Furthermore, the Applicant applied this AOAC method to quantify *monensin* in *Elancoban®G200* [9]. The precision values reported of 1% are in agreement with those presented above, thus confirming the applicability of this method to the analysis of the *feed additive per se*.

In the previous evaluation report related to FAD-2007-0030 [6], the EURL identified the multi-analyte ring-trial validated EN ISO 14183 [10] for the quantification of *monensin* in *premixtures* and *feedingstuffs*, based on a similar experimental protocol. Precisions (repeatability and reproducibility) were reported for target matrices to range from 4 to 7 %.

This method can distinguish between the main substances of the *narasin*, *salinomycin* and *monensin* ionophores. The detection principle of post-column derivatisation coupled to spectrophotometric detection (HPLC-UV-Vis) is specific. Potential interferences in the determination of *monensin*, caused by other components of the *feed additive*, *premixtures* of *feedingstuffs*, are not expected [11].

Based on the performance characteristics available the EURL recommends for official control the EN ISO 14183 [10] and/or the AOAC 997.04 [8] for the quantification of *monensin* in the *feed additive*, *premixtures* and/or *feedingstuffs*.

Note: The EN ISO 14183 and the AOAC 997.04 methods were ring-trial validated for a *drug premixture* (Rumensin premix) with a *monensin* content of 17.6 % w/w which is close to the *Elancoban®G200* content (20 % w/w) [4].

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

For the quantification of *monensin* in chicken and turkey *tissues* the Applicant submitted the AOAC ring-trial validated method (AOAC 2011.24) [12] based on reverse phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) in electrospray ionisation mode using matrix matched standards.

In order to comply with the requirements of Commission Decision 2002/657/EC [13] the Applicant verified the RP-HPLC-MS/MS method at different concentration levels (ranging from MRL/2 to 2MRL) in the relevant *tissues* [14]. Quantification of *monensin* is based on the transition "m/z 693>675" while confirmation is based on two transition ratios "m/z 693>501 : 693>675" and "m/z 693>479 : 693>675" [13]. Table 1 presents the performance characteristics reported for the various *tissues*.

Based on the performance characteristics available the EURL recommends for official control the AOAC ring-trial validated 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* MRLs in the relevant *tissues*.

Table 1 Performance characteristics for the quantification of *monensin* residues in tissues obtained in the frame of the verification [14] and the AOAC ring-trial validation [12] studies.

Assay	Tissue		Conc. (µg /kg)	RSD_r (%)	RSD_{ip} (%)	RSD_R (%)
Verification [14]	Muscle*	turkey	4-20	3.3-12	17-18	
		chicken		1.1-8.2	3.3-5.2	
	Liver*	turkey		0.9-9.5	12-14	
		chicken		2.7-16	9.5-11	
	Kidney*	turkey		1.7-6.3	4.0-4.4	
		chicken		3.8-9.2	6.2-14	
	Skin/fat**	turkey	13-51	1.5-5.5	3.9-4.5	
		chicken		2.6-13	5.9-16	
AOAC [12]	muscle	chicken	18.8	13		19
	Skin/fat		78.4	5.1		14

* MRL= 8 µg /kg; ** MRL= 25 µg /kg

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 HPLC-PCD-UV-Vis. methods (EN ISO 14183 or AOAC 997.04) for the quantification of *monensin* in the *feed additive*, *premixtures* and *feedingstuffs* and (ii) the RP-HPLC-MS/MS ring-trial validated method (AOAC 2011.24) or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC to enforce the *monensin* 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*:

- High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.) – EN ISO 14183 or AOAC 997.04

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

- Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) – AOAC 2011.24 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 *Elancoban®G200* 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 1356/2004 of 21 July 2004, concerning the authorisation for 10 years of the additive "Elancoban" in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances.

[2] Commission Regulation (EC) No 1096/2008 of 6 November 2008, amending Regulation (EC) No 1356/2004 as regards the terms of the authorisation of the feed additive "Elancoban", belonging to the group of coccidiostats and other medicinal substances.

[3] *Application, Reference SANCO/G1: Forw. Appl. 1831/0031-2013

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

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

[6] EURL Evaluation Report FAD 2007-0030
https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2007-0030.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] AOAC Official Method 997.04. Monensin in Premix and Animal Feeds, Liquid Chromatographic Method

[9] *Technical dossier, Section II: Annex II.23 & 32

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

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

[12] AOAC Official Method 2011.24. Narasin and Monensin in Chicken, Swine, and Bovine tissues LC-MS/MS

[13] 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

[14] *Technical dossier, Section II: Annex II. 27

*Refers to Dossier no: FAD-2013-0037

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

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- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus[1] (DK)
- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL). Oberschleißheim[2] (DE)
- Państwowy Instytut Weterynaryjny, Puławy (PL)

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

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

– RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)

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– Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)

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

[1] Name and address according to Regulation (EC) No 885/2009: Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig