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JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

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CRL Evaluation Report on the Analytical Methods submitted in connection with the application for modification of authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	EFSA-Q- 2007-140 FAD-2007- 0030
Product name:	ELANCOBAN® G100/G200
Active Substance(s):	Monensin sodium
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

Elancoban®G100/G200 is a product already authorised as feed additive by Regulation (EC) No 1356/2004, amended by Regulation (EC) No 108/2007 under the category 'coccidiostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agent of *Elancoban®G100/G200* is monensin sodium and the authorised inclusion level is ranging from 60 to 125 mg active substance/ kg complete feedingstuffs, depending on the target animal species.

In the current application a modification of the terms of authorisation is sought for *Elancoban®G100/G200* according to Article 13(3) of Regulation (EC) No 1831/2003. Specifically, a reduction of the withdrawal time from 3 days to 1 day is sought.

For the determination of the active substance (monensin) in the *feed additive* an isocratic High Performance Liquid Chromatography (HPLC) method with post-column derivatisation and Ultraviolet (UV) detection is proposed by the applicant. The method is also used to demonstrate the purity of monensin, which mainly consists of monensin A. The performance characteristics are considered acceptable, and the method is considered suitable for official control.

For the determination of the monensin in *premixtures* and *feedingstuffs* the applicant proposes a HPLC method, which is based on the same principle as mentioned above. The limit of quantification (LOQ) of the method for the determination of monensin is 4 mg /kg.

The method has also been validated by conducting an interlaboratory study (*Coleman et al.*,1997) performed on various feed matrices including poultry feedingstuff. Acceptable precision data were obtained, since the repeatability relative standard deviation (RSD_r) ranged from 6.1 to 12 % for poultry feed and the reproducibility relative standard deviation (RSD_R) ranged from 2.8 to 3.4 % for premixtures and from 8.6 to 15 % for poultry feed. This method is also adopted as AOAC Official method (AOAC Official Method 997.04) and is considered suitable for the intended purpose.

For official control the CRL recommends the ISO standard 14183:2005 which is a multi-analyte method, since it allows for the simultaneous determination of monensin, narasin and salinomycin in feedingstuffs. This method is based on the same principle as the method proposed by the applicant.

Since monensin belongs to group B of Annex I of Council Directive 96/23/EC[1], analytical methods for the determination of *residues* in chicken and turkey tissues target matrices for official control have to comply with the criteria specified in Commission Decision 2002/657/EC[2]. A method based on liquid chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS) is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety[3]. The method was successfully in-house validated in accordance with the requirements of Commission Decision 2002/657/EC with acceptable performance characteristics and is recommended by the CRL for Feed Additives for official control.

Further testing or validation is not considered necessary.

KEYWORDS

Elancoban®, monensin, coccidiostats, chickens for fattening, chickens reared for laying, turkeys

[1] O.J. No L125, 03.05.1996, p. 10
[2] O.J. No L221, 17.08.2002, p. 8
[3] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany

BACKGROUND

Elancoban® G100/G200 is a product already authorised as feed additive for chickens for fattening, chickens reared for laying and turkeys under the category 'coccidiostats' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

Elancoban® is a dark brown granular product and contains monensin sodium as active substance produced by *Streptomyces cinnamonensis* (ATCC 15413) equivalent to monensin activity 10% w/w and 20% w/w for the formulation G100 and G200, respectively. The polyether ionophore monensin sodium represents a mixture of related substances. The main substances are monensin A, and monensin B, in the ratio: monensin A ≥ 90%, sum of monensin A and monensin B ≥ 95%. The authorised inclusion level is ranging from 60 to 125 mg active substance/ kg complete feedingstuffs, depending on the target animal species [1].

In the current application a modification of the terms of authorisation according to Article 13(3) of Regulation (EC) No 1831/2003 is sought for *Elancoban®G100/G200.* Specifically, a reduction of the withdrawal time from 3 days to 1 day is sought [2].

TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 for each application. For this particular dossier, the methods of analysis submitted in connection with *Elancoban®G100/G200* (EFSA-Q-2007-140), and their suitability to be used for official controls in the frame of authorisation, were evaluated.

EVALUATION

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

Quantitative analysis of active substance (monensin) in the feed additive

The active substance of *Elancoban®G100/G200* is monensin which contains the four compounds (factors) monensin A, B, C and D. The monensin concentration in the feed additive, in premixtures and feedingstuffs is expressed in terms of monensin activity which is calculated from the measured concentration of monensin A and B according to a formula. In detail, monensin A and B are quantified separately. The measured concentration of monensin A is multiplied with the factor of 1 and the measured concentration of monensin B is multiplied with the factor of 0.28, thereby obtaining the respective biopotency of each compounds. The monensin content equals the sum of both biopotencies [4].

For the determination of the monensin in the *feed additive* (*Elancoban®G100/G200*) a High Performance Liquid Chromatography (HPLC) method with post-column derivatisation and Ultraviolet (UV) detection is proposed by the applicant [4]. Monensin is extracted from the samples with a mixture of methanol/water. After extraction the sample is diluted, filtered and measured with HPLC. The post column derivatisation involves acid reaction of monensin with vanillin and the resulting products are measured by the UV detector at 520 nm.

The method is able to separate monensin factor A, B, C and D. Confirmation of the factors was carried out on HPLC coupled to mass spectrometry. The obtained relative repeatability standard deviation was 3.2 % and the obtained relative recovery rate ranged from 98 to 102%. Therefore, this method is considered suitable for the intended purpose.

Description of the qualitative and quantitative analytical methods for the determination of the active substance in premixtures and feedingstuffs

For determination of monensin in *premixtures* and *feedingstuffs* a HPLC method with post-column derivatisation and UV detection is proposed by the applicant [5]. The method is based on the same principle as the HPLC-UV method described for the determination of the active substance in the feed additive with some adaptation of the calibration curve. Sufficient

specificity of the method is demonstrated since narasin and salinomycin are chromatographically separated from monensin. Other antibiotics such as lasalocid, tylosin, nicarbazin, bacitracin, lincomycin and bambermycin do not interfere with the analysis of monensin, since they give no response under the conditions of this analysis.

The proposed method has also been validated in an interlaboratory study and the results of the study have been published in a peer reviewed journal [6]. The validation was performed on various feed matrices including poultry feed. Acceptable precision data were obtained both for premixtures and for feedingstuffs for poultry. The repeatability relative standard deviation ranged from 6.1 to 12 % for poultry feed. The reproducibility relative standard deviation ranged from 2.8 to 3.4 for premixtures and from 9.8 to 12 % for poultry feed. The limit of quantification (LOQ) is 4 mg/kg. The obtained method performance characteristics are considered acceptable.

The method is also adopted by AOAC as official method [7] and is suitable for the determination of monensin in the range of 5 to 200 000 mg/kg in premixtures and feedingstuffs for the intended purpose.

For official control purposes the CRL recommends the ISO standard 14183:2005 [8] which is a multi-analyte method, since it allows for the simultaneous determination of monensin, narasin and salinomycin in feedingstuffs. This method is based on the same principle as the method proposed by the applicant.

Description of the qualitative and quantitative methods for determining the marker residue(s) of the active substance in target tissues and animal product

Elancoban®G100/G200 is currently authorised with the following provisional Maximum Residue Limits (MRLs): 25 micrograms monensin sodium/kg of wet skin+fat and 8 micrograms monensin sodium/kg of wet liver, kidney and muscle [1].

For the determination of monensin *residues* in chicken and turkey tissues an analytical method based on LC-MS/MS which was validated in accordance with the requirements of Commission Decision 2002/657/EC is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety [9].

The method was validated for the simultaneous detection and determination of several coccidiostats, including monensin, in muscle and liver of lamb, chicken, turkey, and calf in a concentration range of 0.75 to 2.75 micrograms/kg. Validation was carried out with underlying α- and β- error probabilities of 1% and 5% respectively, and showed sensitivity expressed in terms of the decision limit (CCα) for monensin of 1.06 micrograms/kg. The detection capability (CC_β) was 1.32 micrograms/kg. The CRL for Feed Additives recommends this method for official control.

CONCLUSIONS AND RECOMMENDATIONS

For the determination of the active substance (monensin) in *Elancoban®G100/G200* a HPLC method with post-column derivatisation and UV detection is proposed by the applicant. The method is also used to demonstrate the purity of monensin, which consists of the monensin factors A, B, C, and D. The method has been validated showing acceptable performance characteristics and is considered suitable for official control.

For determination of monensin in *premixtures* and *feedingstuffs* a method based on the same principle as the HPLC-UV method described for the determination of the active substance in the feed additive is proposed by the applicant. The method has been fully validated, it is adopted as AOAC Official method, and it is considered suitable for the intended purpose. However, for official control the CRL recommends the ISO standard 14183:2005 which allows for the simultaneous determination of monensin, narasin, and salinomycin.

For the determination of monensin *residues* in chicken and turkey tissues a LC-MS/MS method which has been validated according to the legislation requirements is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety and is recommended by the CRL for Feed Additives for official controls.

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

Method for determination of the active substance: high performance liquid chromatography (HPLC) with postcolumn derivatisation and UV detection (λ = 520 nm).

Method for determination of residues in target tissues: Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Elancoban®G100/G200,* have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

REFERENCES

[1] Regulation (EC) No 1356/2004, amended by Regulation (EC) No 108/2007

[2] Reference SANCO/D/2 Forw. Appl. 1831/22-2007

[3] Annex III. Proposal of Register entry

[4] Technical dossier, Section II – Attachment 24

[5] Technical dossier, Section II – Attachment 26

[6] Coleman et al. (1997), J. of AOAC Int., 80, 693-703

[7] Official Methods of Analysis of AOAC International: AOAC Official Method 997.04 – Monensin in premix and animal feeds

[8] ISO 14183:2005 - Animal feeding stuffs - Determination of monensin, narasin and salinomycin contents - Liquid chromatographic method using post-column derivatisation

[9] Confirmatory method for the determination of nicarbazin, monensin, salinomycin, lasalocid, narasin, and maduramicin in muscle and liver with LC-MS/MS, Community Reference Laboratory for Residues of Veterinary Drugs, Berlin, Germany. In: EuroResidue V, 10-12 May, 2004, Noordwijkerhout, The Netherlands.

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.

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- VITO, Mol, Belgium
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- National Veterinary Institute, Uppsala, Sweden
- C.Re.A.A. – National Reference Centre for the Surveillance and Monitoring of Animal Feed, Turin, Italy
- Laboratório Nacional de Investigação Veterinária, Lisbon, Portugal

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