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CRL Evaluation Report on the Analytical Methods submitted in connection with Section II – 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2007-0008 EFSA-Q-2007-067
Product name:	Coxidin®25%
Active Substance(s):	Monensin sodium
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium
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EXECUTIVE SUMMARY

Coxidin®25% is a feed additive authorised [Regulation (EC) No 109/2007] in EU (Registration number E 1701) under the category "zootechnical additives", functional group "coccidiostats and histomonostats" according to Annex I of Regulation (EC) No 1831/2003. The additive is authorised for use in compound feedingstuffs for chickens for fattening and for turkeys at concentrations of 100 to 125 mg/kg and 90 to 100 mg/kg, respectively.

In the current application a modification of the authorisation is sought for reducing the authorised minimum dose, within the conditions of use for turkeys, from current 90 to 60 mg/kg complete feedingstuffs. In accordance with Article 13 (3) of Regulation (EC) No. 1831/2003, only the technical documentation supporting this request has been submitted by the applicant. Therefore, the same methods already evaluated by the CRL in relation to the Dossier No. FAD-2005-003 (*Coxidin®*) [Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for Coxidin®, 20 July 2005[1]] have been considered in this report.

The conclusions drawn in the report issued by the CRL on 20 July 2005 as regards the analytical methods for the determination of the active substance (monensin) in the *additive*, *premixtures* and *feedingstuffs* are valid and applicable for the purpose of this application. Therefore, the methods are considered suitable for official control.

For the determination of *residues* in chicken and turkey tissues in the frame of the implementation of the provisional Maximum Residue Limits (MRLs) for monensin sodium, the FEEDAP Panel proposed a method [Opinion on the Maximum Residue Limits for monensin sodium for chickens and turkey for fattening, The EFSA Journal (2006) 413, 1-13] available in literature [Chéneau *et al.*, J. Chromatography B, 850 (2007) 15-23]. This method is based on liquid chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS) and uses the authorised feed additive narasin as internal standard. Furthermore, the mass spectrometry detection of monensin is based on one precursor ion and *one* ion transition (688.4 >635.3). The method has been validated for liver, muscle and fat. Since monensin belongs to group B of Annex I of Council Directive 96/23/EC[2], analytical methods for the determination of this substance in the target matrices for official control purposes have to comply with the criteria specified in Commission Decision 2002/657/EC[3]. Applying these criteria indicates acceptable validation results in terms of sensitivity, precision and trueness. However, the application of the method in the frame of official control is limited due to the following two aspects. The proposed

[1] Available at http://www.irmm.jrc.be/crl-feed-additives
[2] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues there of in live animals and animal products
[3] Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC)

method can *only* be considered suitable as a *quantitative* method to determine residues of monensin in target tissue samples at or around the provisional MRL values in cases where the presence of narasin can be *excluded*. In addition, the proposed protocol does not allow for the *unequivocal* identification of monensin in the case of a suspected non-compliant result, i.e. when the analytical results indicate exceeding the provisional MRLs. This is due the fact that the protocol uses one precursor ion and *one* transition thereby obtaining 2.5 identification points whereas at least *three* identification points would be required for identification of these substances according to Commission Decision 2002/657/EC. The CRL assumes that this method could slightly be modified by measuring a second transition in order to fulfil the criteria of the Commission Decision. However, validation data for this modification have not been provided.

Therefore, the CRL considers the proposed method only suitable for official control if (1) its application is limited to the target tissues in the frame of this authorisation, (2) the sample does not contain narasin, *and* (3) the measured concentration of monensin is below the provisional MRLs. In all other cases the proposed method is not suitable for official purposes.

As an alternative, another LC-MS/MS method using an internal standard which is different than the currently authorised coccidiostats has been identified and is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety[4]. The detection mode used grants *four* identification points obtained by one precursor ion and *two* transitions and the method is routinely used by official control laboratories in the EU. The method was successfully in-house validated in accordance with the requirements of Commission Decision 2002/657/EC in liver and muscle of calf, lamb, chicken, and turkey with acceptable performance characteristics. The CRL therefore recommends this method for official control purposes in the frame of this authorisation.

[4] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany

KEYWORDS

Coxidin®, monensin sodium, zootechnical feed additive, coccidiostat.

BACKGROUND

Coxidin®25% is a feed additive authorised in the EU (Registration number E 1701) under the category "zootechnical additives", functional group "coccidiostats and histomonostats" according to Annex I of Regulation (EC) No 1831/2003 [1]. The additive is authorised for use in compound feedingstuffs for chickens for fattening and for turkeys at concentrations of 100 to 125 mg/kg and 90 to 100 mg/kg, respectively.

The current application (*cf.* EFSA-Q-2007-067) is for a modification of the authorisation, according to Article 13 (3) of Regulation (EC) No 1831/2003, namely for a reduction of the authorised minimum dose, within the conditions of use for turkeys, from current 90 to 60 mg/kg complete feedingstuffs.

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 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. In accordance with Article 13 (3) of Regulation (EC) No. 1831/2003, only the technical documentation supporting this request has been submitted by the applicant. Therefore, the same methods of analysis already evaluated by the CRL in relation to the Dossier No. FAD-2005-003 (*Coxidin®*) [2] have been considered in this report.

EVALUATION

The evaluation of the analytical methods described under chapter 6 of the report issued by the CRL on 20 July 2005 [2] is still applicable and the conclusions are still valid for the purpose of this application, except for paragraph 6.3.3 "*Descriptions of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products*".

Descriptions of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products

Coxidin® is currently authorised with the following provisional Maximum Residue Limits (MRLs): 25 μg monensin sodium/kg of wet skin+fat and 8 μg monensin sodium/kg of wet liver, kidney and muscle [1]. These values are in accordance with the provisional MRLs proposed by EFSA's Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) [3].

The FEEDAP Panel also proposed a validated method for the determination of residues, based on liquid chromatography coupled to low resolution tandem mass spectrometry (LC-MS/MS). This method is available in literature and briefly described hereunder [4].

Five samples of each tissue (muscle, fat, and liver) containing 0.5 to 100 μg monensin/kg, 2.5 to 200 μg monensin/kg, and 1 to 100 μg monensin/kg respectively were extracted, after addition of narasin as internal standard (50 μg/kg), with a mixture of methanol/water 87:13 (v/v) followed by a clean up on a C_{18} solid-phase extraction cartridge. The extracts were injected into the LC-MS/MS system and eluted by applying a water/acetonitrile gradient on a C_{18} column. Mass spectrometry detection of monensin was based on one transition (688.4 > 635.3) using the ammonium adduct as precursor ion. The method was validated showing a relative repeatability standard deviation (RSD %) ranging from 2.8 to 10.9 % for muscle, from 1.9 to 6.5 % for fat, and from 3.5 to 11.8 % for liver. The relative standard deviation (RSD %) for the intermediate precision was: 4.6 to 10.9 % for muscle, 2.8 to 8.1 % for fat, and 3.5 to 11.8 % for liver. The percentage recovery rate was: 59 to 102 % for muscle, 93 to 107 % for fat, and 92 to 105 % for liver. The limit of quantification (LOQ) was 2.5 μg/kg. These performance characteristics are considered acceptable.

However, it should be noted that the method uses the ionophore narasin (authorised as feed additive) as an internal standard. Therefore, the method can only be considered suitable as quantitative method to detect residues of monensin in tissue samples at or around the provisional MRL values in cases where the presence of narasin can be excluded.

Moreover, the method uses a mass spectrometry detection of monensin based on one precursor ion and one transition (688.4 > 635.3) thereby obtaining 2.5 identification points, while for confirmatory[5] purposes for official control, a minimum of three identification points are required for substances listed in Group B of Annex I of Directive 96/23/EC. This criterion has been set by Commission Decision 2002/657/EC, Annex, par. 2.3.3.2. Thus, the method is not suitable for the confirmation of monensin residues in target tissues samples within the

[5]Commission Decision 2002/657/EC, Annex, definition 1.10: Confirmatory methods means methods that provide full or complementary information enabling the substance to be unequivocally identified and if necessary quantified at the level of interest.

frame of official control. The CRL assumes that this method could slightly be modified by measuring a second transition in order to fulfil the criteria of the Commission Decision. However, validation data for this modification have not been provided.

Another LC-MS/MS method [7] is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety[6], and is routinely used by official control laboratories in EU. This method uses an internal standard which is different than the currently authorised coccidiostats and the mass spectrometry detection is based on four identification points. The method was in-house validated in liver and muscle of calf, lamb, chicken, and turkey within a concentration range of 0.75 to 2.75 µg/kg and in accordance with the requirements of Commission Decision 2002/657/EC. The decision limit (CC_α) and the detection capability (CC_β) are 1.06 µg/kg and 1.32 µg/kg respectively, with underlying α- and β- error probabilities of 1 % and 5 % respectively.

This method is recommended by the CRL for official control purposes.

CONCLUSIONS AND RECOMMENDATIONS

The conclusions, drawn in the report issued by the CRL on 20 July 2005 as regards the analytical methods for the determination of the active substance (monensin) in the *additive*, *premixtures* and *feedingstuffs* are valid and applicable for the purpose of this application. The methods are therefore, considered suitable for official control.

For the determination of *residues* in chicken and turkey tissues the FEEDAP Panel proposed a specific LC-MS/MS method for which validation parameters have been provided. Since monensin belongs to group B of Annex I of Council Directive 96/23/EC[7], analytical methods for the determination of this substance in the target matrices for official control purposes have to comply with the criteria specified in Commission Decision 2002/657/EC. Evaluating the proposed method against this Commission Decision shows that this method is only suitable for official control if (1) its application is limited to the target tissues in the frame of this authorisation, (2) the sample does not contain narasin which is used as internal standard, *and* (3) the measured concentration of monensin is below the provisional MRLs. In all other cases the proposed method is not suitable for official purposes

The CRL recommends another LC-MS/MS method for official control purposes, which is available at the Community Reference Laboratory for Residues of Veterinary Drugs in Berlin.

[6] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany
[7] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues there of in live animals and animal products

The method was in-house validated in liver and muscle of calf, lamb, chicken, and turkey and complies with the requirements of Commission Decision 2002/657/EC.

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 post-column derivatisation and UV detection (λ= 520 nm)

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, samples of *Coxidin®* 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 109/2007

[2] Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for Coxidin®, 20 July 2005

[3] Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the Maximum Residue Limits for monensin sodium for chickens and turkey for fattening, The EFSA Journal (2006) 413, 1-13.

[4] Chéneau, E., Henri, J., Pirotais, Y., Abjean, J-P., Roudaut, B., Sanders, P., Laurentie, M. and Sanders, P. Determination of the ionophoric coccidiostat monensin in chicken tissues by liquid chromatography-tandem mass spectrometry after withdrawal of medicated feed. Fifth International symposium on Hormone and Veterinary Drug Residue Analysis (Antwerp, Belgium). May 2006. Submitted by AFSSA (Agence française de sécurité sanitaire des aliments)

[5] Henri, J., Chéneau, E., Diop, M., Abjean, J-P., Roudaut, B., Sanders, P. and Laurentie, M. Monensin residues and bioavailability studies to develop a physiologically based pharmacokinetic (PBPK) model in chicken. Tenth International Congress of the European Association for Veterinary Pharmacology and Toxicology (Torino, Italy). September 2006. Submitted by AFSSA (Agence française de sécurité sanitaire des aliments)

[6] Estelle Chéneau, Jérôme Henri, Yvette Pirotais, Jean-Pierre Abjean, Brigitte Roudaut, Pascal Sanders and Michel Laurentie, Liquid chromatography-electrospray tandem mass spectrometric method for quantification of monensin in plasma and edible tissues of chicken used in pharmacokinetic studies: Applying a total error approach, J. Chromatography B, 850 (2007) 15-23.

[7] Confirmatory method for the determination of nicarbazin, monensin, salinomycin, lasalocid, narasin and maduramycin in muscle and liver with LC-MS/MS, Community Reference Laboratory for Residues of Veterinary Drugs

RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additives (CRL-FA), Geel, Belgium.

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- Veterinary Faculty-National Veterinary Institute, Unit for Pathology of Animal Nutrition and Environmental Hygiene, Ljubljana, Slovenia

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