EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

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

D08/FSQ/CvH/GS/D(2008)7879

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- 2007-111 FAD-2007- 0016
Product name:	MAXIBAN® G 160
Active Substance(s):	Narasin, Nicarbazin
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
Report prepared by:	Giuseppe Simone (CRL-FA)
Report revised by:	Christoph von Holst, Giuseppe Simone (CRL-FA)
Report approved by:	Christoph von Holst (CRL-FA)
Date:	26/03/2008

EXECUTIVE SUMMARY

Maxiban®G160 is a product already authorised as feed additive by Regulation (EC) No 2430/1999, amended by Council Regulation (EC) No 1756/2002 under the category 'coccidiostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agents of *Maxiban®G160* are narasin and nicarbazin and the authorised inclusion level is 80 to 100 mg/ kg complete feedingstuffs

In the current application a modification of the terms of authorisation is sought for *Maxiban®G160* according to Article 13(3) of Regulation (EC) No 1831/2003. Specifically, establishment of Maximum Residues Limits (MRLs) for nicarbazin (marker residue 4,4'-dinitrocarbanilide (DNC)) is sought. The proposed limit is 750 micrograms/kg liver of chickens for fattening

For the quantification of DNC in chicken liver the applicant proposed a liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method. Mass spectrometry detection of DNC is based on one precursor ion and one transition (300.8 m/z > 136.9 m/z). The CRL evaluated the method performance profile based on information provided in the dossier (first validation study) and on additional information provided by the applicant upon request (supplementary data). The following method performance characteristics were obtained from the first validation study: the limit of quantification (LOQ) and the limit of detection (LOD) were 50 and 4.06 micrograms/kg liver, respectively. Mean recovery, determined under repeatability conditions ranged from 85.9 to 104 %, with a coefficient of variation (CV) ranging between 3.2 and 10.8 %, depending on the fortification level. Mean recovery determined under intermediate precision conditions (different time and analyst) ranged between 93.8 and 101 %, with a CV ranging between 6.6 and 11.4 %, depending on the fortification level. Linearity was demonstrated over the range 50-750 micrograms/kg liver (target tissue). The specificity of the method for DNC was demonstrated by analysis of aliquots of each matrix without the addition of DNC and by addition of a number of commonly used ionophores coccidiostats (Narasin, Lasalocid, Salinomycin, Maduramicin, Monensin and Semduramicin) and other coccidiostats (Decoquinate, Diclazuril, Halofuginone and Robenidine). No interferences at the retention time of DNC were observed.

Supplementary data from a second in-house validation study covering a range which at least includes one-half and twice the proposed MRL were submitted by the applicant upon request of the CRL. Linearity was demonstrated over the range 50-1500 micrograms /kg liver. Mean recovery, determined under repeatability conditions ranged from 92.3 to 98.6 %, with a coefficient of variation (CV) ranging between 4.6 and 9.1 %, depending on the fortification level. Mean recovery determined under intermediate precision conditions (different time and analyst) at a fortification level of 1500 micrograms/kg was 99.4 %, with a CV 16.9 %.

The supplementary data submitted showed acceptable performance characteristics in terms of sensitivity, precision, and trueness. However, the application of the method in the frame of official control is limited because the proposed protocol does not allow for the *unequivocal* identification of nicarbazin in the case of a suspected non-compliant result, i.e. when the analytical results indicate exceeding the proposed MRL. 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[1]. The proposed method can *only* be considered suitable as a *quantitative* method to determine residues of nicarbazin in target tissue samples at or around the MRL value. 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 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[2]. The method was successfully in-house validated in accordance with the requirements of Commission Decision 2002/657/EC with acceptable performance characteristics. The CRL therefore recommends this method for official control purposes in the frame of this authorisation.

Further testing or validation is not considered necessary.

[1] Nicarbazin belongs to group B of Annex I of Council Directive 96/23/EC[1]. 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 of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC)

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

KEYWORDS

Maxiban®G160, narasin, nicarbazin, coccidiostats, chickens for fattening

1. BACKGROUND

Maxiban®G160 is a product already authorised as feed additive for chickens for fattening under the category 'coccidiostats' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

Maxiban®G160 contains 80g narasin /kg and 80 g nicarbazin /kg as the active agents and the authorised inclusion level is 80 to 100 mg/ kg complete feedingstuffs [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 *Maxiban®G160*. Specifically, setting of Maximum Residues Limits (MRLs) for nicarbazin (marker residue 4,4'-dinitrocarbanilide (DNC)) is sought [2]. The proposed limit is 750 micrograms/kg liver of chickens for fattening [3].

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 *Maxiban®G160* (EFSA-Q-2007-111), and their suitability to be used for official controls in the frame of authorisation, were evaluated.

3. EVALUATION

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

For the determination of nicarbazin (marker residue 4,4'-dinitrocarbanilide (DNC)) in the target tissue (liver) of poultry, the applicant proposed a liquid chromatography coupled with tandem mass spectrometry LC-MS/MS method [4]. The method is based on the principle that DNC is extracted from the target tissue by homogenisation with acetonitrile, diluted as appropriate, and analysed by a triple quadruple LC-MS/MS system. Briefly, 5 ml of acetonitrile are added to the sample which is then homogenised, centrifuged and the supernatant decanted. The pellet is re-suspended with 5 ml acetonitrile, mixed, centrifuged and the supernatant decanted. The two extracts are combined and volume is adjusted to 10 ml with acetonitrile. The sample is then diluted as appropriate with water and acetonitrile and analysed by LC-MS/MS [5], using one precursor ion (300.8 m/z) and one product ion (136.9 m/z).

The method has been in-house validated on different tissues including poultry liver, fortified with 100, 200, and 400 micrograms DNC/ kg [6]. The limit of quantification (LOQ) and the limit of detection (LOD) were 50 and 4.06 micrograms/kg liver respectively. Mean recovery, determined under repeatability conditions ranged from 85.9 to 104 %, with a coefficient of variation (CV) ranging between 3.2 and 10.8 %, depending on the fortification level. Mean recovery determined under intermediate precision conditions (different time and analyst) ranged between 93.8 and 101 %, with a CV ranging between 6.6 and 11.4 %, depending on the fortification level. Linearity was demonstrated over the range 50-750 micrograms/kg liver (target tissue). The specificity of the method for DNC was demonstrated by analysis of aliquots of each matrix without the addition of DNC and by addition of a number of commonly used ionophore-coccidiostats (Narasin, Lasalocid, Salinomycin, Maduramicin, Monensin and Semduramicin) and other coccidiostats (Decoquinate, Diclazuril, Halofuginone and Robenidine). No interferences at the retention time of DNC were observed [7].

By analogy with the requirements described in 'Notice to applicants and Guideline - Veterinary medicinal products - Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin' [8], the CRL

requested the applicant to submit validation data covering a range which at least includes one-half and twice the proposed MRL, i.e. 375, 750, and 1500 micrograms/kg. The applicant submitted the requested data with a preliminary draft validation report [9]. Linearity was demonstrated over the range 50-1500 micrograms /kg liver. Accuracy was determined on three fortification levels: 400, 750 and 1500 micrograms/ kg and is reported in the table below:

Analyte concentration level (micrograms/kg)	Actual fortification level (micrograms/kg)	Mean recovery (%)	Coefficient of variation (%)
400	400.8	98.6	9.1
750	744.3	96.8	4.6
1500[3]	1489	92.3	5.7
1500[1]	1489	107	4.7
1500[1]	1492	99.0	6.5

Mean recovery determined under intermediate precision conditions at fortification level of 1500 micrograms /kg was 99.6 % with a CV of 8.4 % [10].

The supplementary data submitted are sufficient and are considered acceptable by the CRL in order to conclude that the proposed method is suitable for quantitative determination of DNC residues at or around the MRL value.

However, mass spectrometry detection of DNC is based on one precursor ion and one transition (300.8 m/z > 136.9 m/z) only thereby obtaining 2.5 identification points, while for confirmatory[4] 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 DNC residues in target tissues samples within the frame of official control. The CRL assumes that this method could slightly be modified by measuring a

[3] In different days

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 [11] is available at the Community Reference Laboratory for Residues of Veterinary Drugs at the German Federal Office of Consumer Protection and Food Safety, which was validated at a concentration level of 1 microgram/ kg and in accordance with the requirements of Commission Decision (EC) No 657/2002. The method showed sensitivity expressed in terms of the decision limit (CCα) for DNC of 0.97 micrograms / kg. After an adaptation of the method protocol to the much higher concentration level of DNC in the frame of this application, the method is expected to be suitable for the determination of DNC at the proposed MRL. Based on this information, the CRL for Feed Additives recommends this method for official control.

4. CONCLUSIONS AND RECOMMENDATIONS

For the quantification of nicarbazin (marker residue 4,4'-dinitrocarbanilide (DNC)) in poultry liver, the applicant proposed an LC-MS/MS that has been in-house validated showing acceptable performance characteristics. However, since the proposed method was not validated in accordance with the requirements of Commission Decision (EC) No 657/2002, it cannot be considered suitable for unequivocal identification of the marker residue in the target tissue (poultry liver) for official control purposes. Another 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 for official controls in the frame of the authorisation.

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

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

[4] 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.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Maxiban®G160 have* been sent to the Community Reference Laboratory for Feed Additives.

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

6. REFERENCES

[1] Regulation (EC) No 2430/1999, amended by Council Regulation (EC) No 1756/2002

[2] Reference SANCO/D/2 Forw. Appl. 1831/13-2007

[3] Annex III. Proposal of Register entry

[4] Technical dossier, Section IV.3.4

[5] Technical dossier, Ref. 28, Appendix 1 (Cairns et al., 2006)

[6] Technical dossier, Ref. 28 (Cairns et al., 2006)

[7] Technical dossier, Ref. 28, par. 4.7 (Cairns et al., 2006)

[8] The rules governing medicinal products in the European Union (EudraLex) - Volume 8 - Notice to applicants and Guideline - Veterinary medicinal products - Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin, European Commission, October 2005

[9] Email communication of 30 January 2008

[10] Additional Validation for analytical Method No 0719 for the determination of DNC in edible tissues of chickens Study No. 207193 (Report No. 25477) – Unaudited draft report

[11] 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, Berlin, Germany. In: EuroResidue V, 10-12 May, 2004, Noordwijkerhout, The Netherlands.

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

The following National Reference Laboratories contributed to this report:

- Istituto Superiore di Sanità - Dipartimento di Sanità alimentare ed animale, Roma, Italy
- RIKILT - Institute of Food Safety, Wageningen UR, Wageningen, The Netherlands
- Service Commun des Laboratoires, Laboratoire de Rennes, France
- National Veterinary Institute, Dept of Animal Feed, Uppsala, Sweden
- Bavarian State institute for Health and Food Safety, Oberschleißheim, Germany
- Veterinary Faculty-National Veterinary Institute, Ljubljana, Slovenia
- Laboratory Agroalimentari - Department of Agriculture of The Generalitat of Catalonia, Cabrils, Spain
- National Veterinary Research Institute, Puławy, Poland
- Thuringian State Institute of Agriculture, Jena, Germany
- Central Institute for Supervising and Testing in Agriculture, Praha, Czech Republic
- C.Re.A.A. – National Reference Centre for the Surveillance and Monitoring of Animal Feed, Turin, Italy

The CRL for Feed Additives is also very grateful to the Community Reference Laboratory for Residues of Veterinary Drugs, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany for providing details on the analytical method for the detection of nicarbazin residues in tissues.