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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:	FAD-2008-0037 CRL/080030
Product name:	MAXIBAN G160
Active Substance(s):	Narasin and Nicarbazin
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

Maxiban G160 is a product already authorised as feed additive by Regulation (EC) No 2430/1999, under the category 'coccidiostats and histomonostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active agents of *Maxiban G160* are narasin and nicarbazin. The authorised inclusion level in complete feed is 40 to 50 mg of narasin + 40 to 50 mg of nicarbazin per kilogram.

In the current application the re-authorisation is sought for *Maxiban G160* according to Article 10 (2) of Regulation (EC) No 1831/2003. Specifically, re-authorisation is sought to use *Maxiban G160* for the control of coccidiosis in chickens for fattening.

The *Maxiban G160* is a free-flowing mixture of tan-to-yellow particles and grey-brown particles, which contains granular narasin, granular nicarbazin, vegetable diluent, anti-dusting oil and microtracer.

The narasin concentration in the feed additive is expressed in terms of narasin 'g activity' which is calculated from the measured concentration of narasin components A, D and I.

The CRL recommends the standardized method EN 14183 for the determination of **narasin** in the *feed additive* (*Maxiban G160*), *premixtures* and *feedingstuffs* for official control purposes in the frame of the *Maxiban G160* authorisation. The method is based on high performance liquid chromatography (HPLC) with post-column derivatisation (PCD) and ultraviolet (UV) detection and its performance characteristics are: - a limit of quantification (LOQ) of 2 mg/kg; - a relative standard deviations for repeatability (RSD_r) ranging from 1.3 to 5.0 % and a relative standard deviations for reproducibility (RSD_R) ranging from 4.6 to 12.6 % depending on matrix and concentration level.

The CRL recommends the standardized method prEN 15782 suitable for the determination of **nicarbazin** in the *feed additive* (*Maxiban G160*), *premixture* and *feedingstuffs* for official control purposes in the frame of the *Maxiban G160* authorisation. The method is based on high performance liquid chromatography (HPLC) equipped with ultraviolet/visible (UV/VIS) detection and its performance characteristics are: - LOQ = 20 mg/kg; - RSD_r ranging from 2.6 to 10.2 % and - RSD_R ranging from 4.8 to 12.3 % depending on matrix and concentration levels.

Regarding **residues** in tissue the applicant proposed for the marker residue 4,4' dinitrocarbanilide (DNC) a maximum residues limit (MRL) of 750 µg/kg in liver of chicken for fattening. For official control of this level the CRL recommends a method of the Community Reference Laboratory for Residues of Veterinary Drugs (Berlin) based on liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The method has been validated in accordance with the requirements of Commission Decision (EC) No 657/2002[1]

Further testing or validation is not considered necessary.

KEYWORDS

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

1. BACKGROUND

Maxiban G160 is a feed additive belonging to the category 'coccidiostats and histomonostats', already authorised as the feed additive for chickens for fattening (registration number of additive E772) [1] according to the classification system of Annex I of Regulation (EC) No 1831/2003.

Maxiban G160 is a granulated mixture with active substances nicarbazin and narasin. Nicarbazin is a chemically synthesised product and consist of an equimolecular crystalline complex of 4,4-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethyl-pyrimidine (HDP). Narasin is a polyether ionophore produced by fermentation of *Streptomyces aureofaciens*, strain NRRL 8092 [2].

Maxiban G160 contains 80 g narasin and 80 g nicarbazin per kilogram as the active agents. The proposed concentration in complete feed is 40 to 50 of mg narasin + 40 to 50 mg of nicarbazin per kilogram [3].

A Maximum Residues Limit of 750 µg/kg [4] was proposed by the applicant for the marker residue 4,4'-dinitrocarbanilide (DNC) in liver of chickens for fattening.

[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)

In the current application a re-authorisation according to Article 10 (2) of Regulation (EC) No 1831/2003 is sought for *Maxiban G160* for the control of coccidiosis in chickens for fattening [5].

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, and their suitability to be used for official controls in the frame of authorisation, were evaluated.

3. EVALUATION

Description of the methods used for the determination of active substance in feed additive, premixtures and feedingstuffs

For the determination of the nicarbazin and narasin the applicant proposed a high performance liquid chromatography (HPLC) with post-column derivatisation (PCD) and ultraviolet (UV) detection for narasin [6] and HPLC-UV for nicarbazin [7].

Narasin is extracted from the *feed additive, premixtures or feedingstuffs* material with a mixture methanol:water (90:10 v/v) and is quantified using HPLC-PCD. Narasin is derivatized with vanillin in the presence of acid and heat and the resulting products are measured by a UV detector operating at 520 nm. The narasin concentration in the feed additive is expressed in terms of narasin 'g activity' which is calculated from the measured concentration of narasin A and narasin D + I, which are quantified separately. The measured concentration of narasin A is multiplied with the factor of 1,077. Biopotency conversion factor for narasin (D + I) is calculated from factor D (1,510) and factor I (0,012).

The following acceptable performance characteristics were reported [8]:

- *feed additive*: - a recovery rate ranging from 94 to 98 %; - RSD_r ranging from 0.7 to 2.0 % (as recalculated by CRL [9]) and - RSD_R ranging from 2.3 to 3.1 %.
- *feedingstuffs and premixtures*: - LOQ = 2 mg/kg; - a recovery rate ranging from 94 to 98 % determined at different concentration levels; - RSD_r ranging from 0.2 to 2.8 % and - RSD_R ranging from 1.7 to 3.9 %.

The performance characteristics of the standardized method EN 14183 are [13]: - LOQ = 2 mg/kg; - RSD_r ranging from 1.3 to 5.0 % and - RSD_R ranging from 4.6 to 12.6 % depending on matrix and concentration level.

The method submitted by the applicant is similar to the standardized EN 14183 method [13] and the reported performance characteristics are in agreement, thus confirming the applicability of the EN method in the frame of this authorisation, i.e. when narasin is measured in *Maxiban G160* or when narasin is introduced in premixtures and feed via *Maxiban G160*.

Nicarbazin is assayed and identified by detecting the 4, 4'-Dinitrocarbanilide moiety of nicarbazin. Appropriate quantity of sample is extracted by shaking with 400 ml of dimethylformamide (DMF) in the case of feed additive [7] or with 200 ml of mixture acetonitrile/water (80:20 v/v) in the case of feedingstuffs [10]. After dilution and filtration though a 0.45 micron filter the solution is inject into the chromatographic system.

The following acceptable performance characteristics were reported for:

- *feed additive* [11]: - a recovery rate ranging from 98 to 100 %; - RSD_r = 0.6 % and - RSD_R = 1.8 %.
- *premixtures and feedingstuff* [10]: - a recovery ranging from 98 to 103 % determined at different concentration levels; - RSD_r = 2.5 % for premixture and 3.3 to 4.4 % for feedingstuffs.

The performance characteristics of the standardized method pr EN 15782 are [14]: - LOQ = 20 mg/kg; - RSD_r ranging from 2.6 to 10.2 % and - RSD_R ranging from 4.8 to 12.3 % depending on matrix and concentration level.

In order to check if the standardized method [14] can be use for determination of nicarbazin in feed additive, the CRL asked the applicant for additional information about the extraction

procedure. The supplemental information provided by the applicant shows that the extraction with acetonitrile/water or acetonitrile/methanol gives equal or better results than the extraction with dimethylformamide [12]. The CRL therefore concludes that the method submitted by applicant complies with the procedure of the standardized method. The agreement on the performance characteristics confirms the applicability of prEN 15782 method within the frame of this authorisation, i.e. when nicarbazin is measured in *Maxiban G160* or when nicarbazin is introduced in premixtures and feed via *Maxiban G160*.

For official control purposes the CRL recommends the standardized method EN 14183 for the determination of narasin content [13] and prEN 15782 for the determination of nicarbazin content [14] in the frame of authorisation of *Maxiban G160*.

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 **residues in foodstuffs** of animal origin (in chicken liver, kidney, muscle, skin and fat) a liquid chromatography method coupled with tandem mass spectrometry (LC-MS/MS) [15] was proposed by applicant. This method was already evaluated in the CRL's report related to Question No EFSA-Q-2007-111 concluding that the applicant's 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. 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.

For official control the LC-MS/MS method of the Community Reference Laboratory for Residues of Veterinary Drugs, validated in accordance with the requirements of Commission Decision (EC) No 657/2002, is recommended in the frame of this authorisation [16].

4. CONCLUSIONS AND RECOMMENDATIONS

For official control purposes the CRL recommends standardized method EN 14183 for determination of narasin content and prEN 15782 for determination of nicarbazin content in the frame of this authorisation. These methods are based on the same principle as the method proposed by the applicant and fully fulfil the requirements of feed legislation.

For official control of residue of the nicarbazin in foodstuffs of animal origin the LC-MS/MS method of the Community Reference Laboratory for Residues of Veterinary Drugs, which was validated in accordance with the requirements of Commission Decision (EC) No 657/2002 is recommended in the frame of this authorisation.

Further testing or validation is not considered necessary.

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

The proposed dosage ranges from 40 to 50 mg narasin + 40 to 50 mg nicarbazin per kilogram of complete feed.

Determination of narazin - High performance liquid chromatography method with post-column derivatisation and ultraviolet detection (HPLC-PCD-UV).

Determination of nicarbazin - High performance liquid chromatography method and ultraviolet detection (HPLC-UV)

Determination of residue in of the nicarbazin in foodstuffs of animal origin - Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

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

[2] * Section I Summary of the Data, 2.2 Characterisation of the active substance

[3] * Section I Summary of the Data, 2.5 Conditions of use of the additive

[4] * Annex III. Proposal of Register entry

[5] * Reference SANCO/D/2 Forw. Appl. 1831/13-2007 ucel zadosti

[6] * Supplementary information – Method for narasin B01795.

[7] * Supplementary information – Method for nicarbazin B00271.

[8] * Supplementary information – in-house validation report for method B01795

[9] * Supplementary information – MiniTab calculation

[10] * Appendix 4 Quantitative analysis of Nicarbazin and in premix and in-feed samples (mash and pellets) Method Validation. Study No. T4HANL0602

[11] * Supplementary information – in-house validation report for method B00271

[12] * Supplementary information 2 – Explanatory note (15/07/2009)

[13] prEN 14183:2007 Animal feeding stuffs — Determination of monensin, narasin and salinomycin contents — Liquid chromatographic method using post-column derivatization

[14] prEN 15782:2008 Animal feeding stuffs — Determination of nicarbazin — High-performance liquid chromatographic method

[15] * Appendix 20 Determination of nicarbazin (marker residue DNC) in poultry liver, kidney, muscle, skin with fat and fat Inveresk, Analytical method No.0719, Study No. 207193, 19 May 2005 and Validation Report, Study No. 25477, 20 February 2006

[16] 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.

* Refers to Dossier No: FAD-2007-0051

7. RAPPORTEUR LABORATORY

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

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