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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorised as Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2009-0011 CRL/090034
Name of Additive:	Monteban G100
Active Substance(s):	Narasin
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

Monteban (*Narasin*) is a product already authorised as *feed additive* by Commission Regulation (EC) No 545/2006, amending the conditions for authorisation of a feed additive belonging to the group of coccidiostats, according to the classification system of Annex I of Regulation (EC) No 1831/2003.

The active substance of *Monteban* is *narasin* and the proposed inclusion level of this compound in complete feedingstuffs is 60 mg/kg for the minimum content and 70 mg/kg for the maximum content.

In the current application submitted according to Article 13(3) (modification of authorisation) of Regulation (EC) No 1831/2003, the reduction of the withdrawal time in chickens for fattening from one to zero days is requested.

For the determination of *narasin* in *feed additive*, *premixtures* and *feedingstuffs,* the applicant submitted an in-house validated method, based on ISO 14183:2005, using high-performance liquid chromatograph (HPLC) operated with post-column derivatization. The performance characteristics determined by the applicant in *feed additive*, *premixtures* and *feedingstuffs* are in good agreement with those reported by the ISO 14183:2005 method listed hereafter:

- a relative standard deviation for *repeatability* (RSD_r) of 4.5 %;
- a relative standard deviation for *reproducibility* (RSD_R) of 13 %;
- a *recovery* rate ranging from 90 % to 110 % and
- a limit of quantification (LOQ) of 2 mg/kg.

Based on the above mentioned performance characteristics, the CRL recommends for official control, the ISO 14183:2005 method, for the determination of *narasin* in *feed additives*, *premixtures* and *feedingstuffs*.

Regarding **residues** of *narasin* in tissue, a maximum residues limit (MRL) has been established at 50 μg/kg for all tissues from chicken for fattening by Commission Regulation (EC) No 545/2006. In addition, the Authority informed the CRL about the recommendation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of setting two different MRLs depending on the target tissue, namely 50 μg/kg for liver and fat and 15 μg/kg for kidney and muscle. Furthermore, the Authority asked the CRL to evaluate analytical methods against their suitability for official control purposes to enforce the limits proposed by JECFA.

Based on the evaluation of available analytical methods, the CRL recommends a method developed and validated by the EU Reference Laboratory for Pharmacologically Active

Substances (Berlin, Germany). This method utilises liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and allows for the enforcement of both, the MRL established by Community legislation and MRLs proposed by JECFA in muscle and liver. In addition, the method complies with the criteria established by Commission Decision 2002/657/EC[1]. However, validation data of this method are not available for the matrices kidney and fat. Therefore, the CRL is not able to recommend a method for the official control of MRLs in kidney and fat.

Further testing or validation is not considered necessary.

[1] Narasin belongs to group B of Annex I of Council Directive 96/23/EC. 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)

KEYWORDS

Narasin, coccidiostats, chickens for fattening

1. BACKGROUND

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

The grey-brown material of *Monteban* contains 100 g of *narasin* activity per kg produced by *Streptomyces aureofaciens* (*NRRL 8092).* Strain *NRRL 8092* has been deposited in the stock culture collection of the Northern Marketing and Nutrition Research Division, U. S. Department of Agriculture, Agricultural Research Service, Peoria, Illinois 61604. Other components of the feed additive are rice hulls, antidusting oil and verxite fine [2].

In the current application submitted according to Article 13(3) (modification of authorisation) of Regulation (EC) No 1831/2003, the reduction of the withdrawal time in chickens for fattening from one to zero days is requested [3].

The target concentration of the active substance in complete feedingstuffs is 60 mg/kg for the minimum content and 70 mg/kg for the maximum content [4].

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 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 *Monteban* (EFSA-Q-2008-00502), 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, mercury and lead) are available at the respective Community Reference Laboratories (Commission Regulation (EC) No 776/2006).

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

For the determination of *narasin* in *feed additive*, *premixtures* and *feedingstuffs,* the applicant submitted an in-house validated method [5, 6]. *Narasin* is extracted from the *feed additive* or *feeds* with 90:10 methanol:water (v/v) and is quantified using a high-performance liquid chromatograph (HPLC) equipped with a reactor for post-column derivatization. *Narasin* is reacted with vanillin, and the resulting products are measured by a variable wavelength detector at 520 nm. The reported performance characteristics are presented in Tab. 1.

Furthermore, a standard international method, based on the experimental protocol submitted by the applicant, was established by the ISO Committee [7]. In the ISO 14183:2005, *narasin* is extracted using methanol:water (90:10) with mechanical shaking for 1 h, and filtered. The target analyte is determined by reverse-phase HPLC using post-column derivatization with vanillin and detection at 520 nm. This method was ring-trial validated for the *feed additive* and for *feedingstuffs*. The tested *feed additive* was *Maxiban* – previously authorised [8] – having a composition and *narasin* content similar to the ones in *Monteban* (80 and 100 g/kg *narasin*, respectively). The performance characteristics are presented in Tab. 1.

Tab.1. Performance characteristics of analysis methods for the determination of narasin in feed additive (FA) and in feedingstuffs (FS).

	FA		FS	
	[6]	ISO [7]	[5]	ISO [7]
RSD_r, %	1.4 – 4.4	–	1.1 – 6.2	4.5
RSD_{ip}, % (*)	3.3 – 5.0	–	2.4 – 5.6	–
RSD_R, %	–	13	–	13
Recovery, %	94 – 98	90 – 110	94 – 98	90 – 110
LOQ, mg/kg	0.05 – 0.1	2	0.05 – 0.1	2

(*) relative standard deviation for *intermediate precision.*

Based on the satisfactory agreement of performance characteristics reported by the applicant and the ISO method (cf. Tab.1), the CRL recommends for official control the ISO 14183:2005 method [7], for the determination of *narasin* in *feed additive* and *feedingstuffs*.

No experimental data were submitted by the applicant for the determination of *narasin* in premixtures. However, the CRL recommends to perform a solid dilution of the *premixtures* samples with blank feedingstuffs material (resulting in an appropriate *narasin* concentration, as in *feedingstuffs*) and to apply the corresponding method for feedingstuffs [7], mentioned above.

Methods of analysis for the determination of the residues of the additive or its metabolites in food.

For the determination of *narasin* in various tissues the applicant proposed a method which is comprised of three steps, namely (1) the extraction with iso-octane:ethyl acetate, (2) isolation of *narasin* by the means of silica solid phase extraction cartridges and (3) the determination by high performance liquid chromatography (HPLC), followed by post-column derivatization with vanillin reagent, resulting in a product measured at 520 nm. The method has been validated on all target matrices (i.e. kidney, muscle, liver and fat) at three concentrations, namely 25 μg/kg (which is half of the currently established MRL of 50 μg/kg), 50 μg/kg and twice the MRL (which corresponds to 100 μg/kg), obtaining acceptable values for the trueness and the precision [9]. However, the validation has not been conducted according to Commission Decision 2002/657/EC, since the selected detection system does not provide the required specificity. Therefore, the CRL does not consider this method suitable to be used within the frame of official control.

Another method based on LC-MS/MS is developed and validated by the EU Reference Laboratory for Pharmacologically Active Substances (CRL Berlin) [10]. The method uses one precursor ion (787.6 m/z) and two diagnostic product ions (431.0 and 531.4 m/z), thus fulfilling the required specificity criteria according to Commission Decision 2002/657/EC. The method has been validated on muscle and liver at concentrations ranging from 0.75 to 2.75 μg/kg obtaining acceptable values for the trueness expressed as percentage recovery ranging from 96 and 109 % and for the percentage precision varying between 13 and 18.2 %. The target concentrations selected for the validation exercise where much lower compared to the target MRL's from this report (i.e. 15 and 50 μg/kg), since at the time when the validation has been conducted these MRL's were not known. Nevertheless, it can be assumed that the method will also show an acceptable performance profile, when using it for the control of

MRL's at the higher levels of 15 and 50 μg/kg, respectively. Therefore the CRL recommends this CRL Berlin method for the official control in muscle and liver. However, since the validation experiments have not included kidney and fat, the CRL cannot conclude on the suitability of the method for official control of the target MRL's in the latter matrices.

Further testing or validation is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control the ISO 14183:2005 method, for the determination of *narasin* in *feed additive*, *premixtures* and *feedingstuffs*.

For the determination of *narasin* in *muscle* and *liver,* the CRL recommends for official control the validate method, according to Commission Decision 2002/657/EC, by the CRL Berlin.

Recommended text for the register entry (analytical method)

For the determination of *narasin* in *feed additive*, *premixtures* and *feedingstuffs*:

- reversed-phase high performance liquid chromatography (HPLC) using post-column derivatization with vanillin and detection at 520 nm - ISO 14183:2005

For the determination of *narasin* in muscle and liver:

- LC-MS/MS using one precursor ion and two diagnostic product ions.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Monteban* 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] Commission Regulation (EC) No 545/2006 of 31 March 2006, amending the conditions for authorisation of a feed additive belonging to the group of coccidiostats

[2] * Technical dossier, Section II – 2.2. Characterisation of the active substance

[3] * Application, Reference SANCO/D/2 Forw. Appl. 1831/007-2009 and annex I

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

[5] * Technical dossier, Section II – II Section II - Attachment 11.pdf "Determination of Narasin in animal feeds by HPLC using post-column derivatization (Method B01794)"

[6] * Technical dossier, Section II – II Section II - Attachment 11.pdf "Determination of potency, identification, and factor composition of narasin in narasin granulations and type a medicated articles by HPLC using post-column derivatization (Method B01795)"

[7] ISO 14183:2005(E), "Animal feeding stuffs — Determination of monensin, narasin and salinomycin contents — Liquid chromatographic method using post-column derivatization"

[8] Commission Regulation (EC) No 2430/1999 of 16 November 1999, "linking the authorisation of certain additives belonging to the group of coccidiostats and other medicinal substances in feedingstuffs to persons responsible for putting them into circulation"

[9] * Technical dossier, Section II – II Section II - Attachment 12.pdf "Determination of Narasin in the Edible Tissues of Chickens by HPLC"

[10] Confirmatory method for the determination of nicarbazin, monensin, salinomycin, lasalocid, narasin and maduramicin in muscle and liver with LC-MS/MS, available for official control from EU Reference Laboratory for Pharmacologically Active Substances, Berlin

* Refers to Dossier No. FAD-2009-0011

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, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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