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

Monteban® G100

(FAD-2013-0041; CRL/130011)

European Union Reference Laboratory
Feed Additives

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

Dossier related to:	**FAD-2013-0041 - CRL/130011**
Name of Feed Additive:	***Monteban® G100***
Active Agent (s):	**Narasin (E765)**
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report checked by: Date:	**Piotr Robouch (EURL-FA)** **29/04/2014**
Report approved by: Date:	**Christoph von Holst** **02/05/2014**

EXECUTIVE SUMMARY

Monteban® G100 is a feed additive currently authorized for *chickens for fattening* by Commission Regulation (EC) No 1464/2004 belonging to the group "Coccidiostats and other medicinal substances" listed in Chapter I of Annex B of Directive 70/524/EEC. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested. *Monteban® G100* consists of 10 % (w/w) of *narasin* (active substance), rice hulls as base material, mineral oil as anti-dusting oil and verxite as anti-caking agent. The Applicant suggested a concentration of *narasin* in *feedingstuffs* ranging from 60 to 70 mg/kg.

Furthermore the Applicant suggests Maximum Residue Limits (MRLs) of 50 µg/kg for all wet tissues from *chicken for fattening* as already established by Commission Regulation (EC) No 545/2006.

For the quantification of *narasin* in the *feed additive* and *feedingstuffs*, the Applicant submitted single-laboratory validated methods based on the EN ISO 14183 using High Performance Liquid Chromatography with post-column derivatisation coupled to Ultraviolet detection (HPLC-PCD-UV). Based on the provided performance characteristics the EURL recommends for official control the HPLC-PCD-UV method for the quantification of *narasin* in the *feed additive,* and the EN ISO 14183 for the quantification of *narasin* in *premixtures* and *feedingstuffs*.

For the quantification of *narasin* in chicken *tissues* the Applicant submitted a single laboratory validated (in muscle, kidney, skin/fat and liver) and further verified (in muscle) method based RP-HPLC coupled to a triple quadrupole mass spectrometer (MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards, similar to the one developed and validated by the European Union Reference Laboratory for Pharmacologically Active Substances (BVL). The satisfactory performance characteristics provided by the Applicant for the four tissues of concern demonstrate that (i) the method proposed by the Applicant is equivalent to the BVL method, and (ii) the Applicant method is also applicable to kidney and skin/fat tissues. Based on the performance characteristics presented, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant to enforce the *narasin* MRLs in the relevant *tissues*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

KEYWORDS

Narasin (E765), *Monteban® G100*, coccidiostat, *chickens for fattening*

1. BACKGROUND

Monteban® G100 is a feed additive currently authorized for *chickens for fattening* by Commission Regulation (EC) No 1464/2004, belonging to the group "Coccidiostats and other medicinal substances" listed in Chapter I of Annex B of Directive 70/524/EEC [1]. This regulation has been further modified according to Article 13(3) of Regulation (EC) No 1831/2003 by Commission Regulations (EC) No 545/2006 and No 884/2010 [2,3]. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested for *chickens for fattening* [4,5].

Monteban® G100 consists of 10 % (w/w) of *narasin* (active substance), rice hulls as base material, mineral oil as anti-dusting oil and verxite as anti-caking agent [5]. *Monteban® G100* is intended to be incorporated directly into *feedingstuffs* [6]. The Applicant proposed a concentration of *narasin* in *feedingstuffs* ranging from 60 to 70 mg/kg [5].

Furthermore the Applicant suggests Maximum Residue Limits (MRLs) of 50 µg/kg for all wet tissues from *chicken for fattening* as already established by Commission Regulation (EC) No 545/2006 [2].

Note: The EURL previously evaluated the analytical methods for the determination of *narasin* in the frame of the FAD-2009-0011 dossier [7].

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 European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Monteban® G100* 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, lead, mercury, aflatoxin B1 and dioxins) are available from the respective European Union Reference Laboratories [8]

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

For the quantification of *narasin* in the *feed additive* and *feedingstuffs* the Applicant submitted single-laboratory validated methods [9,10] based on EN ISO 14183 [11] using High Performance Liquid Chromatography with post-column derivatisation coupled to Ultraviolet detection (HPLC-PCD-UV). The scope of the EN ISO 14183 [11] includes *premixtures* and *feedingstuffs*.

Narasin is extracted using methanol:water (90:10) with mechanical shaking for 1 h, filtered and subjected to analysis without further clean-up. The target analyte is determined by reverse-phase HPLC using post-column derivatisation with vanillin and detection at 520 nm. This method was ring-trial validated for broiler *feedingstuffs* at a mean *narasin* content of 66.2 mg/kg leading to the following performance characteristics:

- a relative standard deviation for *repeatability* (RSD_r) of 4.5 %;
- a relative standard deviation for *reproducibility* (RSD_R) of 6.5 %; and
- a limit of quantification (LOQ) of 2 mg/kg.

The Applicant method, using different sample sizes and extraction volumes than the EN ISO method, was also applied to the *feed additive (Monteban G100)* and the following performance characteristics were reported:

- RSD_r ranging from 1.4 to 2.6 % and
- a relative standard deviation for *intermediate precision* (RSD_{ip}) of 2.4 %.

Based on the provided performance characteristics the EURL recommends for official control the HPLC-PCD-UV method [9] for the quantification of *narasin* in the *feed additive*, and the EN ISO 14183 [11] for the quantification of *narasin* in *premixtures* and *feedingstuffs*.

Methods of analysis for the determination of the residues of the additive in food.

For the quantification of *narasin* in chicken *tissues* the Applicant submitted a single laboratory validated (in muscle, kidney, skin/fat and liver) and further verified (in muscle) method based RP-HPLC coupled to a triple quadrupole mass spectrometer (MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards [12,13].

A solution of 1 % (v/v) acetic acid in acetonitrile is added to the tissue and homogenized with an appropriate dispersing device until the sample is fully dispersed. The extract is then dried -

by shaking it with anhydrous sodium sulphate- and further centrifuged. The obtained supernatant is then submitted to a clean-up step by shaking it with a mixed C_{18}/NH_2 packing material. An aliquot of the cleaned extract is finally vortex mixed and centrifuged before the injection in the RP-HPLC-MS/MS system [12].

A similar method has been previously developed and validated by the European Union Reference Laboratory for Pharmacologically Active Substances (BVL) for the determination of *narasin* in two target tissues (muscle and liver). The EURL already evaluated and recommended this method in the frame of the FAD-2009-0011 dossier [7].

The Applicant validated the RP-HPLC-MS/MS method at different concentration levels in the relevant *tissues* (Table 1) complying with the requirements of Commission Decision 2002/657/EC [14]. Additionally the Applicant provided a verification study in muscle *tissue* and reported a recovery rate (R_{rec}) of 91.6 % and a detection limit (LOD) of 3.0 µg /kg [13].

Table 1 presents the performance characteristics reported in the frame of the validation and verification studies together with those reported by BVL. Additionally, BVL reported a LOD of 1.5 µg /kg and R_{rec} ranging from 95.7 to 109 %.

Table 1. Performance characteristics for the quantification of *narasin* residues in chicken tissues obtained in the frame of the validation (Val.) and verification (Ver.) studies, compared to those reported by the European Union reference Laboratory Pharmacologically Active Substances (BVL).

Tissue		Conc. (µg /kg)	RSD_r (%)	RSD_{ip} (%)
Muscle	BVL	0.75-2.75	10-18	13-18
	Val.	7.5	1.9-2.7	4.2
		15	3.6-5.3	4.2
		22.5	4.5-5.0	5.8
	Ver.	50	3.9	8.5
Liver	BVL	0.75-2.75	10-18	13-18
	Val.	25	2.5-3.9	4.5
		50	2.8-4.5	4.3
		75	2.7-4.5	3.9
Kidney	Val.	7.5	1.8-4.8	9.0
		15	1.8-3.7	5.6
		22.5	2.6-3.8	4.6
Skin/Fat	Val.	25	2.8-7.9	9.4
		50	3.5-6.2	6.2
		75	3.8-6.9	6.1

RSD_r; RSD_{ip}: relative standard deviation for *repeatability* and *intermediate precision*

The satisfactory performance characteristics provided by the Applicant for muscle and liver *tissues* demonstrate that the BVL method was equivalent to the one proposed by the Applicant. Additionally the satisfactory results provided by the Applicant for kidney and skin/fat further demonstrate the applicability - and therefore extension of scope - of the Applicant method to these two additional *tissues*.

Consequently, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant for the determination of *narasin* in chicken *tissues*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control the HPLC-PCD-UV methods for the quantification of *narasin* in the *feed additive*, *premixtures* and *feedingstuffs* and (ii) the RP-HPLC-MS/MS single laboratory validated and further verified method proposed by the Applicant for the quantification of *narasin* in chicken *tissues*.

Recommended text for the register entry (analytical method)

For the quantification of *narasin* in *feed additive*:

- High Performance Liquid Chromatography using post-column derivatisation coupled to Ultraviolet detection (HPLC-PCD-UV)

For the quantification of *narasin* in *premixtures* and *feedingstuffs*:

- High Performance Liquid Chromatography using post-column derivatisation coupled to Ultraviolet detection (HPLC-PCD-UV) – EN ISO 14183

For the quantification of *narasin* in *tissues*:

- Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS).

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Monteban® G100* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] Commission Regulation (EC) No 1464/2004 of 17 August 2004, concerning the authorisation for 10 years of the additive 'Monteban' in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances

[2] Commission Regulation (EC) No 545/2006 of 31 March 2006, amending Regulation (EU) No 1464/2004 as regards the conditions for the authorisation of the additive 'Monteban', belonging to the group of coccidiostats and other medicinal substances

[3] Commission Regulation (EU) No 884/2010 of 7 October 2010, amending Regulation (EU) No 1464/2004 as regards the withdrawal time of the additive 'Monteban', belonging to the group of coccidiostats and other medicinal substances

[4] *Application, Reference SANCO/G1: Forw. Appl. 1831/0034-2013

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

[6] *Technical dossier, Section II: II.5 Conditions of use of the additive

[7] EURL Evaluation Report FAD 2009-0011
http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2009-0011.pdf

[8] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[9] *Technical dossier, Section II: Annex II.28

[10] *Technical dossier, Section II: Annex II.30

[11] EN ISO 14183:2008 Animal feedingstuffs – Determination of monensin, narasin and salinomycin contents – Liquid chromatography method using post-column derivatisation (ISO 14183:2005)

[12] *Technical dossier, Section II: Annexes II.31 & 32

[13] *Technical dossier, Section II: Annex II.35

[14] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

*Refers to Dossier no: FAD-2013-0041

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union 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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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya, Cabrils (ES)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)
- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus[1] (DK)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft. Nossen[2] (DE)
- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
- Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare ed animale, Roma (IT)
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- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)

[1] Name and address according to Regulation (EC) No 885/2009: Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby

[2] Name and address according to Regulation (EC) No 885/2009: Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig