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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-2015-0001; CRL/140037)

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-2015-0001 - CRL/140037**
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)** **13/01/2016**
Report approved by: Date:	**Christoph von Holst** **15/01/2016**

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 authorisation under article 4 (1) of the Regulation (EC) No 1831/2003 is sought for *ducks*. *Monteban® G100* consists of 10 % (w/w) of *narasin* (active substance), rice hulls, mineral oil and verxite. The Applicant suggested a concentration of *narasin* in *feedingstuffs* ranging from 60 to 70 mg/kg. Furthermore the Applicant suggests Maximum Residue Limits (MRLs) 50 µg/kg for all relevant duck *tissues.*

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

For the quantification of *narasin* in *tissues* the Applicant submitted a single laboratory validated (in chicken *tissues*) and further verified (in a duck *tissue*) method based on 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 tissues of concern demonstrate that (i) the method proposed by the Applicant is equivalent to the BVL method, (ii) the Applicant method is also applicable to kidney and skin/fat tissues and (iii) the Applicant method is also applicable to duck *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, *ducks*

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 authorisation under article 4 (1) of the Regulation (EC) No 1831/2003 (new use) is sought for *ducks* [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 duck *tissues* (liver, kidney, muscle and skin/fat).

Note: The EURL previously evaluated the analytical methods for the determination of *narasin* in the frame of the FAD-2009-0011 & FAD-2013-0041 dossiers [7].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 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 spectrophotometric detection (HPLC-PCD-UV-Vis).

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. According to *Campbell & Nayeri*, potential interferences in the determination of *narasin* cannot be expected [12].

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 [11]:

- 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 applied the EN ISO method to the *feed additive (Monteban G100)*, using different sample sizes and extraction volumes and reported experimental data within the frame of the validation study [9]. These data were used by the EURL to calculate a precision (*repeatability* and *intermediate precision*) of 0.4 % [13].

Based on the performance characteristics available the EURL recommends for official control the HPLC-PCD-UV-Vis methods for the quantification of *narasin* in the *feed additive* [9] and *feedingstuffs* [11].

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

For the quantification of *narasin* in *tissues* the Applicant submitted a single laboratory validated (in chicken muscle, kidney, skin/fat and liver) [14] and further verified (in duck skin/fat) [15] method based RP-HPLC coupled to a triple quadrupole mass spectrometer (MS/MS) in electrospray ionisation (ESI) mode using matrix matched standards.

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 [14].

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 chicken *tissues* (Table 1) complying with the requirements of Commission Decision 2002/657/EC [16]. Four identification points were set for *narasin* using one parent and two daughter ions. Quantification is based on the transition m/z 787.5 > 431.2 while confirmation is based on the transition m/z 787.5 > 531.4 [14]. Furthermore the Applicant reported a recovery rate (R_{rec}) of 91.6 % and a detection limit (LOD) of 3.0 µg /kg in chicken muscle [17]. Following the EURL request the Applicant verified this method in one duck *tissue* (skin/fat) and reported similar performance characteristics [15].

Table 1 also presents the performance characteristics reported by BVL. 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*. Finally the acceptable results provided by the Applicant for the duck tissue (skin/fat) demonstrate the suitability –and therefore the extension of the scope – of the Applicant method to ducks.

Table 1. Performance characteristics for the quantification of *narasin* residues in chicken & duck 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 (chicken)	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
Liver (chicken)	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 (chicken)	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 (chicken)	Val.	25	2.8-7.9	9.4
		50	3.5-6.2	6.2
		75	3.8-6.9	6.1
Skin/Fat (ducks)	Ver	25	5.1-11	8.7
		50	5.1-11	9.9
		100	3.8-7.0	8.7

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

Consequently, the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-MS/MS method proposed by the Applicant or any equivalent other analytical methods complying with the requirements set by Commission Decision 2002/657/EC, to enforce the MRLs for *narasin* in the target duck *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-Vis methods for the quantification of *narasin* in the *feed additive* and *feedingstuffs* and the RP-HPLC-MS/MS single laboratory validated and further verified method proposed by the Applicant - or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC - for the quantification of *narasin* in duck *tissues*.

Recommended text for the register entry (analytical method)

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

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

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

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

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

– Reversed-Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer (RP-HPLC-MS/MS) or any equivalent methods complying with the requirements set by Commission Decision 2002/657/EC

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. 1831/0002-2015

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

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

[7] EURL Evaluation Reports FAD 2009-0011 & FAD 2013-0041
https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0041-monteban_0.pdf
https://ec.europa.eu/jrc/sites/default/files/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.30

[10] *Technical dossier, Section II: Annex II.32

[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] Harold Campbell et Gita Nayeri, Determination of Monensin, Narasin and Salinomycin in mineral premixes, supplements and animal feeds by liquid chromatography and post-column derivatization: collaborative study, J. of AOAC Int., 89, 5, 1229 – 1242, 2006

[13] *Supplementary Information, eurl_anova_monteban_fa.pdf

[14] *Technical dossier, Section II: Annexes II.33 & 34

[15] *Supplementary Information, Annex 1 - Validation of method narasin in duck Final report 211015.pdf

[16] 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

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

*Refers to Dossier no: FAD-2015-0001

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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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