
Monteban® G100
(FAD-2013-0041; CRL/130011)

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: Piotr Robouch (EURL-FA) 29/04/2014

Report approved by: 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


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 (RSDr) of 4.5 %;
- a relative standard deviation for reproducibility (RSDR) 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:

- RSDr ranging from 1.4 to 2.6 %
- a relative standard deviation for intermediate precision (RS دي) 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 C18/NH2 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 (Rrec) 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 Rrec 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).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Conc. (μg/kg)</th>
<th>RSDr (%)</th>
<th>RSDip (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BVL</td>
<td>0.75-2.75</td>
<td>10-18</td>
<td>13-18</td>
</tr>
<tr>
<td>Val.</td>
<td>7.5</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Ver.</td>
<td>50</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.75-2.75</td>
<td>10-18</td>
<td>13-18</td>
</tr>
<tr>
<td>Val.</td>
<td>25</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val.</td>
<td>7.5</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Skin/Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val.</td>
<td>25</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>6.1</td>
<td></td>
</tr>
</tbody>
</table>

RSDr; RSDip: 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


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

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


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


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


*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

The following National Reference Laboratories contributed to this report:

- 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 Aarhus¹ (DK)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft. Nossen² (DE)
- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
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- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)

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