
Deccox®
(FAD-2013-0034; CRL/130027)

Dossier related to: FAD-2013-0034 - CRL/130027
Name of Product: Decco®
Active Agent(s): Decoquinate
Rapporteur Laboratory: European Union Reference Laboratory for Feed Additives (EURL-FA) Geel, Belgium
Report prepared by: María José González de la Huebra
Report checked by: Piotr Robouch (EURL-FA)
Date: 03/12/2013
Report approved by: Christoph von Holst
Date: 03/12/2013
EXECUTIVE SUMMARY

*Deccox®* is a feed additive currently authorized in chickens for fattening by Commission Regulation (EC) No 1289/2004 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. *Deccox®* consists of 6 % *decoquinate*, 0.6 % colloidal silica, 2.85 % soya-bean oil on a wheat middlings carrier. The *Deccox®* active substance is *decoquinate*, a quinoline coccidiostat, with a minimum purity of 98%. *Deccox®* is a buff-coloured coarse powder formulation to be incorporated in *feedingstuffs* through *premixtures*. The Applicant suggested a concentration of *decoquinate* in *feedingstuffs* ranging from 20-40 mg/kg.

For the determination of *decoquinate* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated CEN standard method (EN 16162:2012) based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL). The experimental evidence provided, allows the EURL to recommend for official control this CEN standard method for the determination of *decoquinate* in the *feed additive*, in *premixtures* and *feedingstuffs*.

For the determination of *decoquinate residues* in *tissues*, the Applicant submitted a single laboratory validated and further verified method, based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer in electrospray ionisation mode (RP-HPLC-MS/MS) using matrix matched standards. The following performance characteristics are reported: precision (repeatability and/or intermediate precision) ranging from 0.9 to 7.2% and a recovery rate ranging from 95 to 110%. Based on the performance characteristics presented, the EURL recommends for official control the RP-HPLC-MS/MS method proposed by the Applicant to enforce the *decoquinate* 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

*Decoquinate, Deccox®, coccidiostat, chickens for fattening*
1. BACKGROUND

Deccox® is a feed additive currently authorized in chickens for fattening by Commission Regulation (EC) No 1289/2004 belonging to the group "Coccidiostats and other medicinal substances" listed in Chapter I of Annex B of Directive 70/524/EEC [1]. In the current application an authorisation of an existing product under article 10 (2) of the Regulation (EC) No 1831/2003 is requested [2,3].

Deccox® consists of 6 % decoquinate, 0.6 % colloidal silica, 2.85 % soya-bean oil on a wheat middlings carrier [4]. The Deccox® active substance is decoquinate, a quinoline coccidiostat, with a minimum purity of 98% [5]. Deccox® is a buff-coloured coarse powder formulation to be incorporated in feedingstuffs through premixtures [6]. The Applicant suggested a concentration of decoquinate in feedingstuffs ranging from 20-40 mg/kg [3].

The Applicant proposed the following MRLs for decoquinate in chicken for fattening tissues: 500 μg/kg in muscle, 800 μg/kg in kidney and 1000 μg/kg in skin/fat or in liver [3]. These MRLs are not covered by the Commission Regulation (EC) No 37/2010 [7], and corresponding methods of analysis need to be evaluated by the EURL.

Note: The EURL evaluated the methods of analysis for Deccox (decoquinate) in the report FAD-2013-0009 [8].

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 Deccox® 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 [9]
Description of the analytical methods for the determination of the active substance in feed additive, premixtures and feedingstuffs

For the determination of *decoquinate* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated CEN standard method (EN 16162:2012) based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL) [10]. This method is developed for the quantification of *decoquinate* in *feed additives*, *premixtures* and semi-liquid complete and complementary compound *feeds*. Furthermore, the Applicant provided additional data showing the applicability of the above mentioned CEN method, and determined *decoquinate* in *premixture* and *feedingstuffs* samples containing *Deccox*®.

*Decoquinate* is extracted from samples with a 1% calcium chloride solution in methanol using mechanical shaking or stirring for 60 min. After centrifugation or filtration, an aliquot is diluted with the extraction solvent and analysed by RP-HPLC-FL [11].

The performance characteristics reported in the EN16162 standard and in the verification reports submitted by the Applicant [12] are summarised in Table 1. Furthermore the EN16162 standard reported a limit of quantification (LOQ) of 1 mg/kg.

Based on the performance characteristics presented, the EURL recommends for official control the EN 16162 method based on RP-HPLC-FL for the determination of *decoquinate* in the *feed additive*, *premixtures* and *feedingstuffs*.

**Table 1.** Performance characteristics of analytical method for the determination of *decoquinate* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS) [11-12].

<table>
<thead>
<tr>
<th>Matrices</th>
<th>Concentration (mg/Kg)</th>
<th>RSDr (%)</th>
<th>RSDp (%)</th>
<th>RSDR (%)</th>
<th>RRec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>60000</td>
<td>2</td>
<td>1.90 – 3.21</td>
<td>2.94</td>
<td>6</td>
</tr>
<tr>
<td>FS</td>
<td>30</td>
<td></td>
<td>1.51 - 2.54</td>
<td>2.56</td>
<td>5.86</td>
</tr>
</tbody>
</table>

RSDr: relative standard deviation for *repeatability* (%); RSDp: relative standard deviation for *intermediate precision* (%); RSDR: relative standard deviation for *reproducibility* (%); RRec: recovery rate (%).

Ver: Verification
* Calculated by EURL from EN16132 Table A.2
** Recalculated by EURL after removal of an outlier.
Methods of analysis for the determination of the residues of the additive in food.

For the determination of *decoquinate* in poultry tissues (muscle, kidney, skin/fat and liver) the Applicant submitted a single laboratory validated and further verified method based on Reversed Phase High Performance Liquid Chromatography coupled to a triple quadrupole mass spectrometer in electrospray ionisation mode using matrix matched standards (RP-HPLC-MS/MS) \[13,14\].

Acetonitrile:water (80:20) is added to the homogenised tissue sample, mixed with an Ultra-Turrax and centrifuged. The decanted supernatant is transferred in a clean tube, while the remaining sample is extracted a second time. The supernatant is then combined with the first extract, further diluted with acetonitrile:water (80:20) and centrifuged. An aliquot of the extract undergoes a clean-up procedure using a solid phase extraction (SPE). The clean extract is then diluted with acetonitrile and quantified by RP-HPLC-MS/MS and using as internal standard a commercially available isotopically labelled *decoquinate*-d5 to correct for recovery losses and matrix interferences. Four identification points were set for *decoquinate* using one parent and two daughter ions. Quantification is based on the transition m/z 418 > 372 while confirmation is based on the transition m/z 418 > 204.

The validation and the verification studies were performed at two *decoquinate* concentrations and satisfactory performance characteristics are reported (Table 2) \[15-18\]. Furthermore the Applicant reported an LOQ of 10 μg/kg for all tissues. Even though the Applicant did not provide data at MRL levels, the EURL considers the submitted HPLC-MS/MS method - using the isotopically-labelled internal standard - suitable for official control to enforce *decoquinate* MRLs in the target tissues. Alternatively, official control laboratories may contact EURL Berlin (BVL) to use their multi-analytical LC-MS/MS technique for the determination of several coccidiostats in some of the target tissues.

**Table 2.** Performance characteristics of analytical method for the determination of the *decoquinate* residues in chicken tissues using internal standard calibration.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration μg/kg*</th>
<th>RSDr (%)</th>
<th>RSDip (%)</th>
<th>RRec (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Val</td>
<td>Ver</td>
<td>Val</td>
</tr>
<tr>
<td>Liver [15]</td>
<td>200</td>
<td>1.1-2.5</td>
<td>1.3-5.7</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>0.9-1.0</td>
<td>3.9-6.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Kidney [16]</td>
<td>200</td>
<td>1.8-1.9</td>
<td>1.2-1.4</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>1.8-2.5</td>
<td>3.4-4.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Muscle [17]</td>
<td>100</td>
<td>2.1-4.0</td>
<td>7.1-7.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>2.1-3.4</td>
<td>3.3-5.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Skin/Fat [18]</td>
<td>200</td>
<td>0.9-2.1</td>
<td>2.1-2.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>1.4-2.1</td>
<td>2.1-5.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

RSDr: relative standard deviation for *repeatability* (%); RSDip: relative standard deviation for *intermediate precision* (%); RRec: recovery rate (%);
Val: Validation, Ver: Verification
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 ring-trial validated EN 16162:2012 based on RP-HPLC-FL method for the determination of decoquinate in the feed additive, premixtures and feedingstuffs and the single-laboratory validated and further verified RP-HPLC-MS/MS method for the determination of decoquinate in tissues.

*Recommended text for the register entry (analytical method)*

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

- Reversed-Phase High Performance Liquid Chromatography with fluorescence detection (RP-HPLC-FL) – EN 16162

For the determination of *decoquinate* 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 *Deccox®* 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


[4] *Technical dossier, Section II: 2.1.3. Qualitative and quantitative composition*

[5] *Technical dossier, Section II: 2.2 Characterisation of the active substance(s)/agent(s). Purity*

[6] *Technical dossier, Section II: Table II.30*


[10] *Technical dossier, Section II: 2.6.1 Methods of analysis for the active substance


[13] *Technical dossier, Section II: 2.6.2 Methods of analysis for the determination of the residues of the additive or of its metabolites in food

[14] *Technical dossier, Section II: Annex II.6.2.1


[16] *Technical dossier, Section II: Annex II.6.2.4 & 5

[17] *Technical dossier, Section II: Annex II.6.2.6 & 7

[18] *Technical dossier, Section II: Annex II.6.2.8 & 9

*Refers to Dossier no: FAD-2013-0034

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)

   – Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
– Fødevarestyrelsen, Ringsted¹ (DK)
– Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Freistaat Sachsen, Nossen² (DE)
– Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
– Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare ed animale, Roma (IT)
– Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarnski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
– Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
– Kmetijski inštitut Slovenije, Ljubljana (SI)
– Państwowy Instytut Weterynaryjny, Puławy (PL)
– RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
– Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
– Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)

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