



JRC.F.5/CvH/MGH/AS/Ares

Subject: Addendum to the EURL evaluation reports

References:

FAD-2013-0009 - Deccox® (JRC.D.5/SFB/CvH/MGH/mds/Ares(2013)2926525)

FAD-2013-0034 - Deccox® (JRC.D.5/SFB/CvH/MGH/mds/Ares(2013)3639174)

FAD-2014-0014 - Deccox® 60 G (JRC.D.5/SFB/CvH/MGH/mds/Ares(2014)2704635)

Upon the publication of a new multi-analyte ring-trial validated method EN 17299 [1] for the analysis of coccidiostats the EURL, considered appropriate to include this standard method within the recommended methods of analysis for official control for the above-mentioned *feed additive* dossiers.

This addendum aims to provide an up-to-date EURL recommendations, including all the available analytical methods complying with the highest requirements as stated in Annex II of Regulation (EC) No 429/2008 [2] which will allow Member States official control laboratory full flexibility regarding the selection of method of analysis (single-analyte or multi-analyte method).

The recommendations included in this addendum apply for the *feed additives* containing *decoquinat*e as active substance that have been already evaluated by the EURL and/or are currently authorised by the related Regulations [3].

The EURL has developed and fully validated a multi-analyte method based on high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the determination of the various coccidiostats, including *decoquinat*e, in *compound feeds*.

According to the method the coccidiostats are extracted with a mixture of acetonitrile:methanol:water. The obtained extracts are centrifuged and supernatants are filtered. The analysis of samples is conducted by reversed-phase LC-MS/MS. The quantification of the detected target analytes is performed using a multi-level standard addition approach [1].

This method has been ring-trial validated for *decoquinat*e in different feed matrices at additive and at cross-contamination levels and published as CEN standard (EN 17299) [1].

Based on the obtained performance characteristics and the scope of the method in terms of matrices, the EURL considers the multi-analyte ring-trial validated EN 17299 method based on high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) fit for purpose for the determination of *decoquinate* in *compound feeds*.

Recommended text for the registry entry (analytical methods) (replacing the previous recommendations)

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

- High Performance Liquid Chromatography coupled with fluorescence detection (HPLC-FL) – EN 16162

For the determination of *decoquinate* in *compound feed*:

- High performance liquid chromatography coupled with fluorescence detection (HPLC-FL) – EN 16162 or
- High performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) – EN 17299

For the determination of *decoquinate* in *tissues*:

- High performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

References

- [1] EN 17299:2019 Animal feedingstuffs: Methods of sampling and analysis – Screening and determination of authorised coccidiostats at additive and 1 % and 3 % cross-contamination level, and of non-registered coccidiostats and of one antibiotic at sub-additive levels, in compound feed with High Performance Liquid Chromatography – Tandem Mass Spectrometry detection (LC-MS/MS)
- [2] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisations of feed additives, OJ L 133 22.5.2008, p. 1
- [3] Commission Implementing Regulation (EU) 2021/2094 of 29 November 2021 concerning the authorisation of decoquinate (Deccox and Avi-Deccox 60G) as a feed additive for chickens for fattening (holder of authorisation Zoetis Belgium SA) and repealing Regulation (EC) No 1289/2004 OJ L 427, 30.11.2021, p. 173

Addendum

- Prepared by María José González de la Huebra
- Reviewed and approved by Stefano Bellorini and Christoph von Holst (EURL-FA),
respectively, Geel, 26/01/2023



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European Union Reference Laboratory Feed Additives - Authorisation

JRC.D.5/SFB/CvH/MGH/mds/Ares

**EURL 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**

Deccox®

(FAD- 2013-0009; CRL/130013)



**EURL 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- 2013-0009 - CRL/130013**

Name of Additive: **Deccox®**

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 (EURL-
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Report checked by: **Piotr Robouch (EURL-FA)**
Date: **02/07/2013**

Report approved by: **Christoph von Holst**
Date: **23/08/2013**

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 a modification of the existing *Deccox[®]* authorisation is requested, under article 13(3), proposing new Maximum Residue Limits (MRLs) for *decoquinat*e in chicken tissues and requesting a modification of the withdrawal period from three to zero days. *Deccox[®]* consists of 6 % *decoquinat*e, 0.6 % colloidal silica, 2.85 % soya-bean oil on a wheat middlings carrier. The *Deccox[®]* active substance is *decoquinat*e, 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 *decoquinat*e in *feedingstuffs* ranging from 20-40 mg/kg. Furthermore the Applicant proposed the following MRLs for *decoquinat*e in poultry *tissues*: 500 µg/kg in muscle, 800 µg/kg in kidney and 1000 µg/kg in skin/fat or in liver. These MRLs are not covered by the Commission Regulation (EC) No 37/2010 and need therefore to be evaluated by the EURL.

For the determination of *decoquinat*e in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated CEN standard method (EN 16162:2012). Furthermore, the Applicant demonstrated the applicability of the CEN method to *premixtures* and *feedingstuffs* samples containing *Deccox* by performing a supplementary verification study. The performance characteristics provided are comparable to those of the EN 16162:2012 standard. Based on the experimental evidence provided, the EURL recommends for official control ring-trial validated CEN standard method (EN 16162:2012) based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL) for the determination of *decoquinat*e in the *feed additive*, in *premixtures* and *feedingstuffs*

For the determination of *decoquinat*e 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 using matrix matched standards (RP-HPLC-MS/MS). 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 *decoquinat*e 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

*Decoquinat*e, *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 to Directive 70/524/EEC [1]. In the current application a modification of the existing *Deccox*[®] authorisation is requested, under article 13(3), proposing new Maximum Residue Limits (MRLs) for *decoquinat*e in chicken tissues and requesting a modification of the withdrawal period from three to zero days [2, 3].

Deccox[®] consists of 6 % *decoquinat*e, 0.6 % colloidal silica, 2.85 % soya-bean oil on a wheat middlings carrier [4]. The *Deccox*[®] active substance is *decoquinat*e, 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 *decoquinat*e in *feedingstuffs* ranging from 20 to 40 mg/kg [3].

Furthermore the Applicant proposed the following MRLs for *decoquinat*e in poultry *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 need to be evaluated by the EURL.

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 [8].

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

For the determination of *decoquinat*e 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) [9]. This method is developed for the quantification of *decoquinat*e in *feed additives, premixtures* and semi-liquid complete and complementary compound *feeds*. Furthermore, the Applicant provided an additional data showing the applicability of the above mentioned CEN method, and determined *decoquinat*e in *premixture* and *feedingstuffs* samples containing Deccox[®].

*Decoquinat*e 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 [10].

The performance characteristics reported in the EN16162 standard and in the verification reports submitted by the Applicant [11] 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 *decoquinat*e in the *feed additive, premixtures* and *feedingstuffs*.

Table 1. Performance characteristics of analytical method for the determination of *decoquinat*e in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS) [10-11].

| Matrices | Concentration (mg/Kg) | RSD _r (%) | | RSD _{ip} (%) | RSD _R (%) | R _{Rec} (%) | |
|----------|-----------------------|----------------------|---------------|-----------------------|----------------------|----------------------|----------|
| | | EN16126 [10] | Ver [11] | Ver [11] | EN16126 [10] | EN16126 [10] | Ver [11] |
| FA | 60000 | 2 | 1.90 – 3.21 | 2.94 | 6 | 96.5* | 91.8 |
| PM | 6000 | 2 | 3.05** - 5.50 | 5.43** | 5 | 103* | 93.4 |
| FS | 30 | 2.63 | 1.51 - 2.54 | 2.56 | 5.86 | 89.4* | 90 |

RSD_r: relative standard deviation for *repeatability* (%); RSD_{ip}: relative standard deviation for *intermediate precision* (%); RSD_R: relative standard deviation for *reproducibility* (%) R_{Rec}: *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 *decoquinat*e 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) [12,13].

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 *decoquinat*e-d5 to correct for recovery losses and matrix interferences. Four identification points were set for *decoquinat*e 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 *decoquinat*e concentrations and satisfactory performance characteristics are reported (Table 2) [14-17]. 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 *decoquinat*e 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 *decoquinat*e residues in chicken *tissues* using internal standard calibration.

| Tissue | Concentration µg /kg* | RSD _r (%) | | RSD _{ip} (%) | | R _{Rec} (%) | |
|---------------|--------------------------|----------------------|---------|-----------------------|-----|----------------------|------|
| | | Val | Ver | Val | Ver | Val | Ver |
| Liver [14] | 200 | 1.1-2.5 | 1.3-5.7 | 2.3 | 4.2 | 110 | 106 |
| | 1600 | 0.9-1.0 | 3.9-6.4 | 1.0 | 5.3 | 107 | 97.2 |
| Kidney [15] | 200 | 1.8-1.9 | 1.2-1.4 | 2.3 | 1.6 | 107 | 108 |
| | 1600 | 1.8-2.5 | 3.4-4.8 | 2.7 | 4.0 | 103 | 100 |
| Muscle [16] | 100 | 2.1-4.0 | 7.1-7.2 | 3.4 | 6.9 | 95.5 | 97.2 |
| | 800 | 2.1-3.4 | 3.3-5.0 | 3.1 | 4.4 | 101 | 98.1 |
| Skin/Fat [17] | 200 | 0.9-2.1 | 2.1-2.5 | 1.6 | 2.2 | 107 | 107 |
| | 800 | 1.4-2.1 | 2.1-5.2 | 1.8 | 3.9 | 99.9 | 102 |

RSD_r: relative standard deviation for *repeatability* (%); RSD_{ip}: relative standard deviation for *intermediate precision* (%); R_{Rec}: *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-trail validated EN 16162:2012 based on RP-HPLC-FL method for the determination of *decoquinat*e in the *feed additive, premixtures and feedingstuffs*; and
- the single-laboratory validated and further verified RP-HPLC-MS/MS method for the determination of *decoquinat*e in *tissues*.

Recommended text for the register entry (analytical method)

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

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

For the determination of *decoquinat*e 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

- [1] Commission Regulation (EC) No 1289/2004 of 14 July 2004, concerning the authorisation the authorisation for 10 years of the additive *Deccox[®]* in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances.
- [2] *Application, Reference SANCO/G1: Forw. Appl. 1831/0006-2013
- [3] *Application, Proposal for Register Entry – Annex A
- [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.26

- [7] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin
 - [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: 2.6.1 Methods of analysis for the active substance
 - [10] EN 16162:2012 Animal feeding stuffs – Determination of decoquinat by HPLC with fluorescence detection
 - [11] *Technical dossier, Section II: Annex II.6.1.1 & 2 & 3
 - [12] *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
 - [13] *Technical dossier, Section II: Annex II.6.2.1
 - [14] *Technical dossier, Section II: Annex II.6.2.2 & 3
 - [15] *Technical dossier, Section II: Annex II.6.2.4 & 5
 - [16] *Technical dossier, Section II: Annex II.6.2.6 & 7
 - [17] *Technical dossier, Section II: Annex II.6.2.8 & 9
- * Refers to Dossier No. FAD-2013-0009

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:

- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)
- Laboratoire de Rennes, Service Commun des Laboratoires, Rennes (FR)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Freistaat Sachsen, Nossen (DE)
- Fødevarestyrelsen, Ringsted (DK)

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- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen, Jena (DE)
 - Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
 - Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare e animale, Roma (IT)

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