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European Union Reference Laboratory
Feed Additives

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

# 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

**Quinox®**
***(FAD-2021-0049; CRL/210015)***

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

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| Dossier related to: | **FAD-2021-0049 - CRL/210015** |
| Name of Product: | ***Quinox®*** |
| Active Agent (s): | **Decoquinate** |
| Rapporteur Laboratory: | **European Union Reference Laboratory for Feed Additives (EURL-FA)**<br>**JRC Geel, Belgium** |
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| Report checked by:<br>Date: | **Zigmas Ezerskis**<br>**14/12/2021** |
| Report approved by:<br>Date: | **Christoph von Holst**<br>**14/12/2021** |

## EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *decoquinate (Quinox®)*, under the category (e) 'coccidiostats and histomonostats'/, according to Article 6 of Regulation (EC) No 1831/2003. The authorisation is sought for chickens for fattening and chickens reared for laying.

The *feed additive (Quinox®)* consists of *decoquinate* as an active substance with mass fraction of 180 g/kg *feed additive* and it is intended to be incorporated in *feedingstuffs* through *premixtures*. The Applicant suggested a minimum and a maximum content of *decoquinate* in *feedingstuffs* of 20 and 40 mg/kg, respectively.

For the determination of *decoquinate* in *Quinox®*, the Applicant submitted a single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography coupled to photometric detection (RP-HPLC-UV). Additionally, within the systematic NRLs review, the EURL was provided with experimental proofs of the suitability of the ring-trial validated CEN standard method (EN 16162) based on reversed phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL) for the determination of *decoquinate* in *Quinox®*.

Furthermore, for the determination of *decoquinate* in *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated CEN standard method (EN 16162) based on reversed phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL).

Additionally, the EURL is aware of another ring-trial validated method based on liquid chromatography coupled to mass spectrometry LC-MS/MS for the determination of various coccidiostats, including *decoquinate*, in *feedingstuffs* that has been published as CEN standard (EN 17299).

Based on the experimental evidence provided, the EURL recommends for official control: i) the single-laboratory validated and further verified method based on reversed phase high performance liquid chromatography coupled to photometric detection (RP-HPLC-UV) or the ring-trial validated standard method (EN 16162) based on reversed phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL) for the quantification of *decoquinate* in the *feed additive*; ii) the ring-trial validated standard method (EN 16162) based on reversed phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL) for the quantification of *decoquinate* in *premixtures* and iii) the ring-trial validated standard method (EN 16162) based on reversed phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL) or the

ring-trial validated method based on liquid chromatography coupled to mass spectrometry LC-MS/MS (EN 17299) for the quantification of *decoquinate* in *feedingstuffs*.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

**KEYWORDS**

*Decoquinate, Quinox®*, coccidiostat, *chickens for fattening* and *chickens reared for laying*

## 1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *decoquinate (Quinox®)*, under the category (e) 'coccidiostats and histomonostats', according to Article 6 (1) of Regulation (EC) No 1831/2003 [1]. The authorisation is sought for chickens for fattening and chickens reared for laying [1,2].

The *feed additive (Quinox®)* is a whitish powder consisting of *decoquinate* as active substance with mass fraction of 180 g/kg *feed additive*, silica colloidal hydrated as anticaking agent and wheat meal as carrier [3]. Another *feed additive* containing *decoquinate* is currently authorised by Commission Implementing Regulation 2021/2094 [4].

*Quinox®* is intended to be incorporated in *feedingstuffs* through *premixtures* [5]. The Applicant suggested a minimum and maximum content of *decoquinate* in *feedingstuffs* of 20 and 40 mg/kg, respectively, which is in-line with the existing *decoquinate* authorisation [4].

Note: The EURL has previously evaluated the analytical methods for the determination of *decoquinate* in the frame of several dossiers [6].

## 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 *Quinox®* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

## 3. EVALUATION

***Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

For the determination of *decoquinate* in *Quinox®*, the Applicant submitted a single-laboratory validated [7] and further verified method [8] based on reversed phase high performance liquid chromatography coupled to photometric detection (RP-HPLC-UV) [9].

*Decoquinate* is extracted from *Quinox®* with a mixture of methanol and calcium chloride using a mechanical stirring for 30 min. After letting the mixture to stand for another 10 min an aliquot of the supernatant is diluted with methanol, further filtrated through a 0.45 μm filter and analysed by RP-HPLC-UV [9]. The extraction solvent used in the method proposed by the Applicant for the determination of decoquinate in *Quinox* [9] is equivalent than the one used in the EN 16162 which suggested the suitability of the EN 16162 for *Quinox®*.

In addition, within the systematic NRLs review the EURL has been informed by one expert on the EN 16162 method that, in the frame of the robustness study performed during the ring-trial validation of this method, one participant used for the *feed additive* a sample size three times higher than the one stated in the protocol leading to a recovery of 93 % [10]. This will result in an equivalent *decoquinate* mass/extractant ratio than the one obtained when applying the EN 16162 protocol to *Quinox* samples, demonstrating thus the suitability of the EN 16162 for the determination of *decoquinate* in *Quinox®*. However, to increase solubility it would be recommended to increase appropriately the extraction volume by three or five times.

Table 1 shows the performance characteristics obtained in the frame of the single-laboratory validation [7] and verification [8] studies for the determination of *decoquinate* in *Quinox®* using the RP-HPLC-UV method [9].

**Table 1.** Performance characteristics for the determination of *decoquinate* in the *feed additive* (FA)

| Matrix | Mass fraction range (mg/kg) | $RSD_r$ (%) | | | $RSD_{ip}$ (%) | | $RSD_R$ (%) | $R_{Rec}$ (%) |
|---|---|---|---|---|---|---|---|---|
| | | Val [7] | Ver [8] | EN16162 [10] | Val [7] | Ver [8] | EN16162 [10] | Val [7] |
| FA (Quinox®) | 180000 | 1.4* | 2.0* | - | 1.5* | 2.0* | - | 99 - 101 |
| FA [10] | 60000 | - | - | 2 | - | - | 6 | 97* |

*$RSD_r$, $RSD_{ip}$,* relative standard deviation for *repeatability and intermediate precision.* *$R_{Rec}$: recovery rate (%); *Calculated by EURL;*

**Table 2.** Performance characteristics of the RP-HPLC-FL (EN 16162) for the determination of *decoquinate* in *premixture* (PM) and *feedingstuffs* - poultry compound feed at authorised level - (FS)

| Matrices | Mass fraction range (mg/kg) [12]/[10] | $RSD_r$ (%) | | $RSD_{ip}$ (%) | $RSD_R$ (%) | $R_{Rec}$ (%) |
|---|---|---|---|---|---|---|
| | | [12] | EN16162 [10] | [12] | EN16162 [10] | EN16162 [10] |
| PM | 4066 – 4209 /6000 | 2.9 | 2.0 | 3.3 | 5.0 | 103* |
| FS | 18- 22 / 30 | 2.9 - 4.9 | 2.7 | 4.7 - 7.2 | 5.9 | 89* |

*$RSD_r$, $RSD_{ip}$, $RSD_R$*: relative standard deviation for *repeatability, intermediate precision* and *reproducibility,* respectively*; $R_{Rec}$: recovery rate (%); * Calculated by EURL*

For the determination of *decoquinate* in *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated CEN standard method (EN 16162) based on reversed phase high performance liquid chromatography coupled to fluorescence detection (RP-HPLC-FL) [10]. This method was developed for the quantification of *decoquinate* in *feed additives*, *premixtures* and complete and complementary *compound feeds* and has been ring-trial validated in samples with *decoquinate* content ranging from 3 mg/kg (carry over level) to 60 g/kg (*feed additive* level).

*Decoquinate* is extracted from samples with a 1 % calcium chloride solution in methanol using mechanical shaking/stirring for 60 min. After centrifugation/filtration, an aliquot is diluted with the extraction solvent and analysed by RP-HPLC-FL [10].

Furthermore, in the frame of the stability [11] and homogeneity [12] studies the Applicant applied this method to *premixtures* and *feedingstuffs*, leading to similar performance characteristics (Table 2) and thus demonstrating the suitability of the EN 16162.

The various performance characteristics obtained are presented in Table 2. Furthermore, a limit of quantification (LOQ) ranging from 0.3 to 1 mg *decoquinate* /kg feed is specified in the EN 16162 standard method.

In addition, the EURL has developed and fully validated a multi-analyte method based on liquid chromatography coupled to mass spectrometry (LC-MS/MS) for the determination in *feedingstuffs* of all coccidiostats currently authorised as *feed additives* within the EU, including *decoquinate*. In this method the coccidiostats are extracted with acetonitrile:methanol:water (80:10:10, v:v:v). The extracts are centrifuged and supernatants filtered. After a first screening analysis, the analytes are determined by reversed phase liquid chromatography using electrospray ionisation with tandem mass spectrometry detection (LC-MS/MS). The quantification of the detected target analytes is performed using a multi-level standard addition approach. This method has been ring-trial validated and published as CEN standard (EN 17299) [13].

Based on the performance characteristics presented, the EURL recommends for official control the in-house validated and further verified method based on RP-HPLC-UV for the

quantification of *decoquinate* in the *feed additive*, the EN 16162 method based on RP-HPLC-FL for the quantification of *decoquinate* in the *feed additive*, *premixtures* and *feedingstuffs* and the EN 17299 method based on LC-MS/MS for the quantification of *decoquinate* in *feedingstuffs*.

***Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)***

In the frame of previous *decoquinate* dossiers the EURL has evaluated and recommended methods for the quantification of *decoquinate* in poultry *tissues* (muscle, kidney, skin/fat and liver) [6].

However taking into account that i) the Applicant did not propose any MRLs and ii) no MRLs have been set in the last *decoquinate* Commission Implementing Regulation [4] the EURL considers that the evaluation of corresponding methods of analysis is not relevant for the present application.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

## 4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control i) the single-laboratory validated and further verified method based on RP-HPLC-UV or the ring-trial validated EN 16162 method based on RP-HPLC-FL for the quantification of *decoquinate* in the *feed additive* ii) the ring-trial validated EN 16162 method based on RP-HPLC-FL for the quantification of *decoquinate* in *premixtures* and iii) the ring-trial validated EN 16162 method based on RP-HPLC-FL or the ring-trial validated method based on liquid chromatography coupled to mass spectrometry LC-MS/MS (EN 17299) for the quantification of *decoquinate* in *feedingstuffs*.

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

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

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

For the quantification of *decoquinate* in *premixtures*:

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

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

- Reversed Phase High Performance Liquid Chromatography with fluorescence detection (RP-HPLC-FL) – EN 16162 or
- Liquid Chromatography coupled to a triple quadrupole mass spectrometry (LC-MS/MS) – EN 17299

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Quinox*® 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] *Application, Reference SANTE/E5: FORW. APPL. 1831/0040-2021

[2] *Application, Annex I – Submission number 1616423811582-2934

[3] *Technical dossier, Section II: 2.1 Identity of the additive

[4] 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

[5] *Technical dossier, Section II: 2.5 Conditions of use of the additive

[6] EURL Evaluation Reports
https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2013-0009-Deccox.doc_.pdf
https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2013-0034-Deccox.doc_.pdf
https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0014-deccox60g.pdf

[7] *Technical dossier, Section II: Annex II.25

[8] *Technical dossier, Section II: Annex II.26

[9] *Technical dossier, Section II: Annex II.24

[10] EN 16162:2012 Animal feedingstuffs – Determination of decoquinate by HPLC with fluorescence detection

[11] *Technical dossier, Section II: Annex II.19, Annex II.20 & Annex II.21

[12] *Technical dossier, Section II: Annex II.22 & Annex II.23

[13] 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)

*Refers to Dossier no: FAD-2021-0049

## 7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural**.** Generalitat de Catalunya, Cabrils (ES)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft, Nossen (DE)
- Wageningen Food Safety Research (WFSR)[1] (NL)
- Istituto Superiore di Sanità. Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Roma (IT)
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- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)

[1] Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.