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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

Coxam®

(FAD-2016-0017; CRL/160017)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2016-0017 - CRL/160017**
Name of Product:	***Coxam®***
Active Agent (s):	**Amprolium hydrochloride**
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: Date:	**Piotr Robouch (EURL-FA)** **11/08/2016**
Report approved by: Date:	**Christoph von Holst** **16/08/2016**

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Coxam®*, under article 4(1), for the category "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for chickens for fattening and reared for laying. *Coxam®* consists of 250 g/kg of *amprolium hydrochloride* complemented by liquid paraffin as antidusting agent, and rice hulls as carrier. *Coxam®* is intended to be incorporated in *feedingstuffs* through *premixtures* at a content of *amprolium hydrochloride* of 125 mg/kg *feedingstuffs.*

For the quantification of *amprolium* in the *feed additive (Coxam®)*, the Applicant submitted a single-laboratory validated and further verified method based on Reversed-Phase High Performance Liquid Chromatography coupled to Ultraviolet detection (RP-HPLC-UV). The following performance characteristics were reported: - a precision ranging from 0.8 to 1.0 %; and - a *recovery* rate (R_{Rec}) ranging from 99 to 100 %.

For the quantification of *amprolium* in the *premixtures* and *feedingstuffs* the Applicant submitted the ring trial validated Community method (Commission Regulation (EC) No 152/2009) based on Cation Exchange High Performance Liquid Chromatography coupled to Ultraviolet detection IE-HPLC-UV. The following performance characteristics were reported: - a relative standard deviation for *repeatability* (RSD_r) ranging from 1.9 to 5.0 % and a relative standard deviation for *reproducibility* (RSD_R) ranging from 3.0 to 6.5 %, - R_{Rec} ranging from 91 to 103 % and - a limit of quantification (LOQ) of 5 mg/kg *feedingstuffs*. In addition, the Applicant provided experimental evidence demonstrating the applicability of the Community method for determining *amprolium* in *premixtures* and *feedingstuffs samples* containing *Coxam®*.

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

Amprolium, *Coxam®*, coccidiostat, chickens for fattening and reared for laying

1. BACKGROUND

In the current application authorisation is sought for *Coxam*®, under article 4(1), for the category "coccidiostats and histomonostats", according to the classification system of article 6 of Regulation (EC) No 1831/2003. Authorisation is sought for chickens for fattening and chickens reared for laying [1][2].

Coxam® consists of 250 g/kg of *amprolium* (active substance), liquid paraffin as antidusting agent and rice hulls as carrier [2][3]. *Amprolium* is a thiamine (Vitamin B1) analogue with a minimum purity of 95 % [3].

Coxam® is intended to be incorporated in *feedingstuffs* through *premixtures* at a content of *amprolium* of 125 mg/kg *feedingstuffs* [2][4].

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 *Coxam*® 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, mycotoxines and dioxins) are available from the respective European Union Reference Laboratories [5].

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

For the quantification of *amprolium* in the *feed additive*, the Applicant submitted a single-laboratory validated [6] and further verified [7] method based on Reversed-Phase High Performance Liquid Chromatography coupled to Ultraviolet detection (RP-HPLC-UV) [8].

The sample is weighed in a 500 ml iodine flask, 100 ml of methanol:water 1:1 are added and *amprolium* is extracted during 15 min using a shaker. The extract is then filtrated through a 0.45 μm Nylon filter and injected into the chromatographic system. The *amprolium* content is

determined by RP-HPLC-UV at 268 nm [8]. The reported performance characteristics are summarised in Table 1.

For the determination of *amprolium* in *premixtures* and *feedingstuffs* the Applicant submitted the ring trial validated Community method (Commission Regulation (EC) No 152/2009) based on Cation Exchange High Performance Liquid Chromatography coupled to Ultraviolet detection IE-HPLC-UV [9].

The *premixture* or *feedingstuffs* sample is weighed in a 500 ml volumetric flask and 200 ml of a methanol:water (2:1 v:v) mixture are added. *Amprolium* is extracted using an ultrasonic bath for 15 min followed by further mixing in a shaker for additional 60 minutes. An aliquot of the extract is then appropriately diluted with the mobile phase and mixed. The solution is then filtrated through a 0.45 μm polytetrafluorethylene (PTFE) membrane filter and injected into the chromatographic system. The *amprolium* content is determined by using a spectrophotometric detector at 264 nm. The reported performance characteristics are presented in Table 1. In addition, limits of detection (LOD) and quantification (LOQ) of 1 and 5 mg/kg, were also reported.

Furthermore, the Applicant provided experimental evidence demonstrating the applicability of the Community method to determine *amprolium* in *premixtures* and *feedingstuffs* samples containing *Coxam®* [10].

Based on the performance characteristics presented, the EURL recommends for official control the single-laboratory validated and further verified method based on RP-HPLC-UV for the determination of *amprolium* in the *feed additive* and the ring trial validated Community method based on IE-HPLC-UV for the determination of *amprolium* in *premixtures* and *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) is not considered necessary.

Table 1. Performance characteristics of analytical method for the determination of *amprolium* in the *feed additive* (FA), *premixtures* (PM) / *mineral feed* (MF) and *feedingstuffs* (FS) [6][9]

Matrices	Mass fraction (mg/kg)	RSDr (%)	RSDip(%)	RSD_R(%)	R_{Rec} (%)
FA [6]	248100	0.8	0.8	-	99
PM/MF [9]	5000-25000	2.2-3.5	-	3.0-5.2	101-103*
FS [9]	50-200	1.9-5.0	-	6.5	91-94*

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 (%);
* Calculated by EURL from [6][9]

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 for the quantification of *amprolium* in the *feed additive* and (ii) the ring trial validated Community method based on IE-HPLC-UV for the quantification of *amprolium* in *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

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

- Reversed-Phase High Performance Liquid Chromatography using Ultraviolet detection at 268 nm (RP-HPLC-UV).

For the quantification of *amprolium* in *premixtures* and *feedingstuffs*:

- Cation exchange High Performance Liquid Chromatography using Ultraviolet detection at 264 nm (IE-HPLC-UV) - Regulation (EC) No 152/2009.

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Coxam*® 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 SANCO/G1: Forw. Appl. 1831/0017-2016

[2] *Application, Proposal of Registry Entry – Annex A

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

[4] *Technical dossier, Section II: 2.5.1 Proposed mode of use in animal nutrition

[5] 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

[6] *Technical dossier, Section II, Annexes, Reference II.24

[7] *Technical dossier, Section II, Annexes, Reference II.25

[8] *Technical dossier, Section II, Annexes, Reference II.23

[9] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

[10] *Technical dossier, Section II, Annexes, Reference II.27

*Refers to Dossier no: FAD-2016-0017

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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- Fødevarestyrelsens Laboratorie Ringsted (DK)
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- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Centro di referenza nazionale per la sorveglienza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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