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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

Aviax 5%
(FAD-2014-0009; CRL/130030)

European Union Reference Laboratory
Feed Additives

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Dossier related to:	**FAD-2014-0009 - CRL/130030**
Name of Feed Additive:	***Aviax 5%***
Active Agent (s):	**Semduramicin sodium**
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)** **10/11/2014**
Report approved by: Date:	**Christoph von Holst** **10/11/2014**

EXECUTIVE SUMMARY

Aviax 5%, is a *feed additive* currently authorized for chickens for fattening by Commission Regulation (EC) No 1443/2006 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 authorisation under article 13(3) of Regulation (EC) No 1831/2003 is requested. *Aviax 5%* consists of 5% (w/w) *semduramicin*, 3-5% (w/w) mineral oil as dust control agent, 4% (w/w) sodium carbonate as stabilising agent, 2% (w/w) sodium alumino silicate as anticaking and soybean mill run as carrier. The *feed additive* is intended to be incorporated through *premixtures* or directly into *feedingstuffs*. The Applicant proposed a concentration of *semduramicin* in *feedingstuffs* ranging from 20 to 25 mg/kg.

For the quantification of *semduramicin* the Applicant submitted two single-laboratory validated methods based on High Performance Liquid Chromatography (HPLC) with: refractive index detection (RI) for the *feed additive*; and post-column derivatisation coupled to spectrophotometric detection (PCD-UV-Vis) for *premixes* and *feedingstuffs*. The EURL identified instead the ring-trial validated method EN 16158 for the quantification of *semduramicin* in *feedingstuffs*, based on reverse-phase HPLC using mass spectrometry detection (MS) or PCD-UV-Vis. This method was slightly adapted to quantify *semduramicin* in *premixtures* and in the *feed additive* (*Aviax 5%*) with similar method performance characteristics to the original EN 16158 method.

Based on the experimental evidence available the EURL recommends for official control the HPLC-PCD-UV-Vis and/or the HPLC-MS method for the quantification of *semduramicin* in the *feed additive*, *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.

KEYWORDS

Semduramicin, *Aviax 5%*, coccidiostat, chickens for fattening

1. BACKGROUND

Aviax 5%, is a *feed additive* currently authorized for chickens for fattening by Commission Regulation (EC) No 1443/2006 [1] 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 authorisation under article 13(3) of Regulation (EC) No 1831/2003 is requested, for a new *Aviax 5%* formulation containing myceliar *semduramicin* [2].

Aviax 5% consists of 5% (w/w) *semduramicin*, 3-5% (w/w) mineral oil as dust control agent, 4% (w/w) sodium carbonate as stabilising agent, 2% (w/w) sodium alumino silicate as anticaking and soybean mill run as carrier [3].

The *feed additive* is intended to be incorporated through *premixtures* or directly into *feedingstuffs* [4]. The Applicant proposed a concentration of *semduramicin* in *feedingstuffs* ranging from 20 to 25 mg/kg [3].

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 *Aviax 5%* 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 [5].

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

For the quantification of *semduramicin* in the *feed additive* the Applicant submitted a single-laboratory validated method based on High Performance Liquid Chromatography coupled to refractive index detection (HPLC-RI) [6]. *Semduramicin* is extracted from the *feed additive* in methanol and mechanically shaken for 30 min before centrifugation. The target analyte is determined by reverse-phase HPLC-RI [6]. The performance characteristics reported in the frame of the validation study are presented in Table 1. However, no verification data was provided by the Applicant.

For the quantification of *semduramicin* in *premixtures* and *feedingstuffs*, the Applicant submitted another single-laboratory validated method based on normal-phase HPLC with post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis) [7]. *Semduramicin* is extracted from the sample in a isooctane:methylene chloride (50:50 v:v) solution shaken mechanically for 30 min before centrifugation. Then a silica solid phase extraction cartridge is used for clean-up. The cleaned extract is evaporated to dryness, reconstituted with the mobile phase and filtered. The target analyte is determined by normal-phase HPLC-PCD with vanillin and detection at 522 nm [7]. The performance characteristics reported in the frame of the validation study are presented in Table 1. Furthermore, the Applicant reported a limit of quantification (LOQ) of 1 mg/kg in *feedingstuffs*. However, no verification data was provided.

The EURL identified instead the ring-trial validated EN 16158 method [8] for the quantification of *semduramicin* in *feedingstuffs*, based on reverse-phase HPLC using mass spectrometry detection (MS) or PCD-UV-Vis. Semduramicin is extracted using acetonitrile with mechanical shaking during 30 min. Extracts are filtered and diluted appropriately. *Semduramicin* is then determined by reverse-phase HPLC using single quadrupole mass spectrometry detection (MS) or PCD-UV-Vis with dimethylaminobenzaldehyde (DMAB) at 598 nm. Both methods allow the discrimination of *semduramicin* from *salinomycin, monensin, narasin* and *maduramicin* ionophores. The detection principles of post-column derivatisation coupled to spectrophotometric detection (HPLC-UV-Vis) or to mass spectrometry detection (HPLC-MS) are very selective. Potential interferences in the determination of *semduramicin* caused by other components of the *feed additive*, *premixtures* of *feedingstuffs*, are not expected. Table 1 presents the performance characteristics reported by the CEN standard method. LOQs in *feedingstuffs* of 1 and 3 mg/kg for MS and PCD-UV-Vis respectively were also reported.

This HPLC-PCD-UV-Vis method was also adapted for the quantification of *semduramicin* in *premixtures* and in the *feed additive* (*Aviax 5%*) by reducing the test portion and diluting the extract [9]. The corresponding method performance characteristics presented in Table 1 are in

good agreement with those reported in the EN 16158 standard thus demonstrating the applicability (extension of the scope) of the EN 16158 method to the quantification of *semduramicin* in the *feed additive (Aviax 5%)* and *premixtures*.

Table 1. Performance characteristics of analytical methods for the determination of *semduramicin* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS) [6-11] reported in the frame of the validation (Val), CEN standard method (EN) and the corresponding extension of scope study (Ext.EN).

Matrix	Concentration (mg/kg)	Method	Reference	RSD_r, %	RSD_{ip}, %	R_{Rec} (%)
FA	51300	HPLC-RI	Val [6]	0.8	1.2	95-102
		HPLC-PCD-UV-Vis	Ext. EN [9]	2.8	4.4	93
PM	3076	HPLC-PCD-UV-Vis	Ext. EN [9]	2.6	2.6	91
FS	10-42	HPLC-PCD-UV-Vis	Val[a] [7]	1.4-2.7	1.9-3.2	96-101
		HPLC-MS	EN [8,10,11]	2.0-9.0	8-18[b]	84-103
		HPLC-PCD-UV-Vis	EN [8,10,11]	2.0-10	11-16[b]	84-92
					[b]RSD_R,%	

RSD_r, RSD_{ip} & RSD_R: relative standard deviation for *repeatability*, *intermediate-precision* & *reproducibility,* respectively. R_{Rec}: *recovery* rate (%); [a] Recalculated by EURL

Based on the performance characteristics available, the EURL recommends for official control the HPLC-MS and/or HPLC-PCD-UV-Vis methods for the quantification of *semduramicin* in *feed additive*, *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.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation, the EURL recommends for official control the HPLC-MS and/or HPLC-PCD-UV-Vis methods for the quantification of *semduramicin* in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the quantification of *semduramicin* in the *feed additive and premixtures*:

– High Performance Liquid Chromatography using post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.)

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

- High Performance Liquid Chromatography using single mass spectrometry (HPLC-MS) or post-column derivatisation coupled to spectrophotometric detection (HPLC-PCD-UV-Vis.) – EN 16158

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Aviax 5%* 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 1443/2006 of 29 September 2006, concerning the permanent authorisation of certain additives and an authorisation for 10 years of a coccidiostat.

[2] *Application, Reference SANCO/G1: Forw. Appl. 1831/0005-2014

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

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

[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: Annex II.38 (p 606)

[7] *Technical dossier, Section II: Annex II.39

[8] EN 16158:2012 Animal feedingstuffs – Determination of semduramicin content – Liquid chromatography method using a "tree" analytical approach

[9] Vincent, U., Serano, F., González de la Huebra, M.J., von Holst, C. J. Pharm. Biomed. Anal. 61 (2012) 150-155

[10] González de la Huebra, M.J., Vincent, U, von Holst, C. Food Addit. Contam, 28 (2011) 35-43

[11] González de la Huebra, M.J., Vincent, U, Serano, F, von Holst, C. J. AOAC Int,. 95 (2012) 61-66

*Refers to Dossier no: FAD-2014-0009

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

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- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya,Cabrils (ES)
- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus[1] (DK)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL). Oberschleißheim[2] (DE)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare ed animale, Roma (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- 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: Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim