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

Subject: Addendum to the EURL evaluation reports

References:

FAD-04-002 - Lasalocid sodium 15% (Avatec®) (D08/FSQ/CVH/(2005) D 11466)

FAD-2008-0001 - Avatec® 150G (D08/FSQ/CvH/RL/D(2008)23901)

FAD-2008-0050 - Avatec® 150G (JRC.DDG06/FSQ/CvH/RMO/Mdr /Ares (2010) 56760)

FAD-2013-0040 - Avatec® 150G (JRC.D.5/SFB/CvH/MGH /mds/Ares(2014)375527)

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 of this addendum apply for the *feed additives* containing *lasalocid A sodium* as active substance that have been already evaluated by the EURL and/or are currently authorised by the related Regulations [3-5].

The EURL has developed and fully validated a multi-analyte method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the determination of the various coccidiostats, including *lasalocid A sodium,* 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 *lasalocid A sodium* 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 liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) fit for purpose for the determination of *lasalocid A sodium* in *compound feeds*.

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

For the determination of *lasalocid A sodium* in the *feed additive* and *premixtures*:

- High Performance Liquid Chromatography coupled with fluorescence detection (HPLC-FL) – Commission Regulation (EC) No 152/2009

For the determination of *lasalocid A sodium* in *compound feed*:

- High Performance Liquid Chromatography coupled with fluorescence detection (HPLC-FL) – Commission Regulation (EC) No 152/2009 or
- High Performance Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS) – EN 17299

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 Regulation (EC) No 1455/2004of 16 August 2004 concerning the authorisation for 10 years of the additive 'Avatec 15 %' in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances OJ L 269, 17.8.2004, p. 14

[4] Commission Regulation (EC) No 874/2010 of 5 October 2010 concerning the authorisation of lasalocid A sodium as a feed additive for turkeys up to 16 weeks (holder of authorisation Alpharma (Belgium) BVBA) and amending Regulation (EC) No 2430/1999 OJ L 263, 6.10.2010, p. 1

[5] Commission Implementing Regulation (EU) No 900/2011 of 7 September 2011concerning the authorisation of lasalocid A sodium as a feed additive for pheasants, guinea fowl, quails and partridges other than laying birds (holder of authorisation Alpharma (Belgium) BVBA) OJ L 231, 8.9.2011, p. 15

Addendum
- Prepared by María José González de la Huebra
- Reviewed and approved by Zigmas Ezerskis and Christoph von Holst (EURL-FA), respectively, Geel, 20/01/2023

EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate D: Institute for Reference Materials and Measurements
European Union Reference Laboratory for Feed Additives

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

Avatec® 150G

(FAD-2013-0040; CRL/130029)

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

Dossier related to:	**FAD-2013-0040 - CRL/130029**
Name of Feed Additive:	***Avatec® 150G***
Active Agent (s):	**Lasalocid A 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)** **13/02/2014**
Report approved by: Date:	**Christoph von Holst** **14/02/2014**

EXECUTIVE SUMMARY

In the current application authorisation is sought for *Avatec® 150G* under articles 10(2) and 13(3) for the category "coccidiostats and histomonostats" of Regulation (EC) No 1831/2003. Authorisation is sought for use for chickens for fattening and reared for laying, turkeys and pheasants, partridges, quails and guinea fowl. This *feed additive* is already authorized in chickens for fattening and reared for laying, in turkeys and in pheasants, partridges, quails and guinea fowl.

Avatec® 150G is a red-brown free flowing granular preparation formulated to contain - 15% *lasalocid A sodium*, 4% calcium lignosulphonate binder, 0.1% ferric oxide colouring agent, and a calcium sulphate dehydrate carrier. *Avatec® 150G* is meant to be incorporated in *feedingstuffs* through *premixtures*. The Applicant suggested a *lasalocid A sodium* content in *feedingstuffs* ranging from 75-125 mg/kg for all the animal species of concern.

MRLs for *lasalocid A* in poultry tissues and eggs are already set by the Commission Regulation (EC) No 37/2010, therefore the corresponding methods of analysis do not need to be evaluated by the EURL.

For the determination of *lasalocid A sodium* in the *feed additive*, *premixtures* and *feedingstuffs*, the Applicant submitted the European Community method published in Commission Regulation (EC) No 152/2009. In the frame of a previous dossier (cf. FAD-2008-0050) the Applicant demonstrated the applicability of the Community method to the *feed additive*. Furthermore, the Applicant provided additional experimental data obtained analysing the *feed additive*, *premixtures* and *feedingstuffs* containing *Avatec 150G*. Based on the experimental evidence provided, the EURL recommends for official control the European Community method published in Commission Regulation (EC) No 152/2009 based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL) for the determination of *lasalocid A sodium* in the *feed additive*, 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

KEYWORDS

Lasalocid A sodium, Avatec® 150G, coccidiostat, *chickens turkeys and minor avian species*

1. BACKGROUND

In the current application authorisation is sought for *Avatec® 150G*, under articles 10(2) (authorisation of an existing product) and 13(3) (modification of authorisation), for the category "coccidiostats and histomonostats" of Regulation (EC) No 1831/2003. Authorisation is sought for use for chickens for fattening and reared for laying, turkeys and pheasants, partridges, quails and guinea fowl [1, 2]. This *feed additive* is already authorized in chickens for fattening and reared for laying [3], in turkeys [4] and in pheasants, partridges, quails and guinea fowl [5].

Avatec® 150G is a red-brown free flowing granular preparation formulated to contain - 150 g/kg (15%) of the active substance *lasalocid A sodium* (a polyether ionophore produced by fermentation of *Streptomyces lasaliensis*), - 4% calcium lignosulphonate binder, - 0.1% ferric oxide colouring agent, and - a calcium sulphate dehydrate carrier [2]. *Avatec® 150G* is meant to be incorporated in *feedingstuffs* through *premixtures* [1]. The Applicant suggested a *lasalocid A sodium* content in *feedingstuffs* ranging from 75-125 mg/kg for all the animal species of concern [2].

MRLs for *lasalocid A* in poultry tissues and eggs are already set by the Commission Regulation (EC) No 37/2010 [6], therefore the corresponding methods of analysis do not 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 *Avatec®150G* 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 [7]

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

For the determination of *lasalocid A sodium* in *premixtures* and *feedingstuffs*, the Applicant submitted the ring-trial validated Community method based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL) [8].

Lasalocid A sodium is extracted from samples with 100ml of acidified methanol in an ultrasonic bath for 20 min followed by dilution (up to 250ml) with acidified methanol and mixed thoroughly. The mixture is then allowed to stand for 1h until the suspended material has settled and then an aliquot of the supernatant is filtered through a 0.45 μm membrane filter. The clear filtrate is then further diluted with acidified methanol to produce a final test solution containing about 4 μg/ml of lasalocid sodium and analysed by RP-HPLC-FL at an excitation wavelength of 310 nm and an emission wavelength of 419 nm [8].

In the frame of the FAD-2008-0050 dossier [9], the Applicant already demonstrated the applicability of the Community method for the determination of *lasalocid A sodium* in the feed additive [10]. Furthermore, the Applicant provided additional experimental data obtained analysing the *feed additive*, *premixtures* and *feedingstuffs* containing *Avatec 150G* [11]. The performance characteristics reported are in good agreement with those of the Community method (cf. Table 1). Furthermore the European Community method reported a limit of quantification (LOQ) of 10 mg/kg.

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

Table 1. Performance characteristics of analytical method for the determination of *lasalocid A sodium* in the *feed additive* (FA), *premixtures* (PM) and *feedingstuffs* (FS).

Matrices	RSD_r (%)		RDS_{ip} (%)	RSD_R (%)	R_{Rec} (%)	
	COM [8]	Verif [11]	Verif [11]	COM [8]	COM [8]	Verif [11]
FA	-	1.28-2.27	1.77	-	-	95.4
PM	2.12-2.52	0.64-0.94	0.87	5.45-5.66	101	96.1
FS	2.24-5.37	1.67-1.69	1.66	5.03-10.7	74.7-96.6	100

RSD_r, RSD_{ip} and RSD_R: relative standard deviation for *repeatability, intermediate precision* and *reproducibility,* respectively R_{Rec}: *recovery* rate (%); Verif: Verification; COM: Community Method

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 European Community method, based on Reversed Phase High Performance Liquid Chromatography coupled to fluorescence detection (RP-HPLC-FL) for the determination of *lasalocid A sodium* in the *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the determination of *lasalocid A sodium* in *feed additive*, *premixtures* and *feedingstuffs*:

– Reversed-Phase High Performance Liquid Chromatography with fluorescence detection (RP-HPLC-FL) – Commission 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 *Avatec® 150G* 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. 1831/0038-2013

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

[3] Commission Regulation (EC) No 1455/2004 of 16 August 2004, concerning the authorisation the authorisation for 10 years of the additive Avatec 15% in feedingstuffs, belonging to the group of coccidiostats and other medicinal substances

[4] Commission Regulation (EC) No 874/2010 of 5 October 2010, concerning the authorisation of lasalocid A sodium as feed additive for turkeys up to 16 weeks (holder of authorisation Alpharma (Belgium) BVBA) and amending Regulation (EC) No 2430/1999

[5] Commission Implementing Regulation (EU) No 900/2011 of 7 September 2011, concerning the authorisation of lasalocid A sodium as feed additive for pheasants, guinea fowl, quails and partridges other than laying birds (holder of authorisation Alpharma (Belgium) BVBA)

[6] 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

[7] 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

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

[9] EURL Evaluation Report FAD-2008-0050:

http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2008-0050.pdf

[10] *Technical dossier, Section II, Annex II.6.1.4.

[11] *Technical dossier, Section II, Annexes: Annex II.6.1.1-6.1.3.

*Refers to Dossier no: FAD-2013-0040

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:

- Federaal Laboratorium voor de Voedselveiligheid Tervuren (FLVVT – FAVV), Tervuren (BE)
- Fødevarestyrelsen, Laboratorierne, Ringsted og Aarhus[1] (DK)
- Sachgebiet Futtermittel des Bayrischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL). Oberschleißheim[2] (DE)
- Foderavdelningen, Statens Veterinärmedicinska Anstalt (SVA), Uppsala (SE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Istituto Superiore di Sanita' - Dipartimento di Sanita' alimentare ed animale, Roma (IT)
- Państwowy Instytut Weterynaryjny, Puławy (PL)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 - Labore Landwirtschaft. Nossen[3] (DE)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia i Pesca, Generalitat de Catalunya,Cabrils (ES)
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin (PL)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid[4] (ES)
- Thüringer Landesanstalt für Landwirtschaft (TLL), Abteilung Untersuchungswesen. Jena (DE)
- RIKILT-Instituut voor Voedselveiligheid, Wageningen (NL))

[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
[3] Name and address according to Regulation (EC) No 885/2009: Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, Labore Landwirtschaft, Leipzig
[4] Name and address according to Regulation (EC) No 885/2009: Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura, Pesca y Alimentación, Madrid