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
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements
Community Reference Laboratory for Feed Additives

D08/FSQ/CVH/GS/D(2008)23901

CRL Evaluation Report on the Analytical Methods submitted in connection with the application for modification of authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	EFSA-Q-2008-80 FAD-2008-0001
Product name:	Avatec 150 G (Lasalocid A sodium 15g/100g)
Active Substance(s):	Lasalocid A sodium
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
Report prepared by:	Christoph von Holst (CRL-FA)
Report revised by: Date:	Giuseppe Simone (CRL-FA) 19/09/2008
Report approved by: Date:	Christoph von Holst (CRL-FA) 19/09/2008

EXECUTIVE SUMMARY

Avatec 150 G (Lasalocid A sodium 15g/100g) is a product already authorised as feed additive by Regulation (EC) No 2037/2005, under the category 'coccidiostats', according to the classification system of Annex I of Regulation (EC) No 1831/2003. The active substance of *Avatec 150 G* is lasalocid A sodium and the proposed inclusion level of this compound in complete feedingstuffs is 75 mg/kg for the minimum content and 120 mg/kg for the maximum content.

In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003 the extension of the use of *Avatec 150 G* for other animals, namely pheasants, partridges, quails, guinea fowl, ducks, geese is sought.

For the determination of the active substance (lasalocid A sodium) in the *feed additive* the applicant proposed a reverse-phase High Performance Liquid Chromatography (HPLC) method equipped with Ultraviolet (UV) detection measuring at 304 nm. The performance characteristics estimated on different formulations are considered acceptable, since the obtained percentage relative intermediate standard deviations were below 1.5 % and the percentage relative recovery rates were close to 100 %. The method is considered suitable for official control.

For the determination of lasalocid A sodium in *premixtures* and *feedingstuffs* the applicant proposes a HPLC method, which is very similar to the method utilised for the determination of the active substance in the feed additive. The method has been single-laboratory validation, showing an acceptable performance profile. For official control the CRL recommends the community method for the determination of lasalocid A sodium published in Commission Directive 1999/76/EC. The method is based on reverse-phase HPLC coupled to spectrofluorimetry applying an excitation wavelength of 310 nm and measuring at 419 nm. The method has been fully ring trial validated for premixtures and feeds at concentrations that are very close to the target level of lasalocid A of this application. The obtained values for the percentage relative standard deviation for *repeatability* varied between 2.12 and 5.37 % and for the percentage relative standard deviation for *reproducibility* varied between 5.03 and 10.7 %, depending on the matrix and concentration level included in the study.

Further testing or validation is not considered necessary.

KEYWORDS

Lasalocid A sodium 15g/100g, coccidiostats, pheasants, partridges, quails, guinea fowl, ducks, geese

BACKGROUND

Avatec 150 G (Lasalocid A sodium 15g/100g) is a product already authorised as feed additive for turkeys, chickens reared for fattening and chickens reared for laying under the category 'coccidiostats' [1], according to the classification system of Annex I of Regulation (EC) No 1831/2003.

Avatec 150 G is a product comprised of reddish, free flowing, non-dusty granules and contains *lasalocid A sodium* at a concentration of 150 mg/g as active substance produced by *Streptomyces lasaliensis subsp. lasaliensis (ATCC 31180)*. Other components of the feed additive are calcium sulphate dehydrate at a concentration of 809 mg/g, calcium lignosulphosphate at 40 mg/g and ferric oxide at 1 mg/kg [2]. The product also contains minor amounts of the lasalocid homologues B, C, D, E and the sum of these compounds in the active substance is equal or below 10 % [2]. The target concentration of the active substance in complete feedingstuffs is is 75 mg/kg for the minimum content and 120 mg/kg for the maximum content [2].

In the current application submitted according to Article 4(1) of Regulation (EC) No 1831/2003 the use of *Avatec 150 G* for pheasants, partridges, quails, guinea fowl, ducks, geese is sought [3].

TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the methods of analysis submitted in connection with *Avatec 150 G* (EFSA-Q-2008-80), and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

EVALUATION

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

Quantitative analysis of active substance (lasalocid A sodium) in the feed additive

For the determination of lasalocid A sodium in the *feed additive* the applicant proposed a method which is based on reverse-phase HPLC coupled to UV detection measuring at 304 nm. The target analyte is extracted from the feed additive by shaking 0.4g of the sample with methanol for 15 minutes followed by sonication for 5 minutes [4]. The extract is filtered and then subjected to HPLC analysis without further clean-up. The method has been single-laboratory validated obtaining the following method performance characteristics: The percentage relative standard deviation for *repeatability* (RSD_r) was 0.45 %, the percentage relative standard deviation for *intermediate* (RSD_{ip}) were below 1.5 % and the percentage relative recovery rate was 100.6 % [5]. Since the obtained values are acceptable, the method is considered fit for the intended purpose.

Description of the qualitative and quantitative analytical methods for the determination of the active substance in premixtures and feedingstuffs

For the determination of lasalocid A sodium in *premixtures and feedingstuffs* the applicant proposed the same method as utilised for the determination of the target analyte in the feed additive. The only adaptation of the method is related to the sample amount and the corresponding volumes of the extraction solvent, since 0.4 g of premixtures or 10 g of the feed samples are extracted, respectively. The results from the single-laboratory validation revealed an acceptable performance profile, since for both matrices the RSD_r was below 1.2 % and the percentage relative recovery rate was about 102 % [6].

For official control of the lasalocid A sodium in *premixtures* and *feedingstuffs* the CRL recommends the Community standard method as described in Commission Directive 1999/76/EC[1] [7]. The method protocol foresees that 2 g of the premixture sample or 5 to 10 g of the feedingstuffs sample are extracted with acidified methanol in an ultrasonic bath at approximately 40^{o}C for 20 minutes. The extract is filtered and subjected to HPLC analysis coupled to a spectrofluorimetric detector applying an excitation wavelength of 310 nm and measuring at 419 nm. The Community method also includes conditions of the Liquid Chromatography (LC) system, but allowing some flexibility to the laboratory, to modify the LC conditions. In this context a National Reference Laboratory reports on a different set of LC conditions that allows for a better separation from interfering substances, especially when analysing premixtures and mineral feed. The Community method places special emphasis on the investigation of possible matrix effects on the bias of the analytical result by including recovery tests on blank feed samples fortified with known amounts of the target analyte.

[1] The title of the method protocol refers to lasalocid sodium without specifying the target homologue, but the description of the method (chapter 3.10) clarifies that the target homologue is lasalocid A sodium.

Alternatively, the standard addition technique is recommended which is applied on the sample to be analysed. The method has been fully ring trial validated in various premixtures and feedingstuffs [9] containing lasalocid A sodium at concentrations which are close to the minimum and maximum contents of lasalocid in feedingstuffs as specified in the proposed register entry [2]. The obtained values for the percentage relative standard deviation for *repeatability* varied between 2.12 and 5.37 % and for the percentage relative standard deviation for *reproducibility* varied between 5.03 and 10.7 %, depending on the matrix and concentration level included in the study.

CONCLUSIONS AND RECOMMENDATIONS

The method proposed by the applicant for the determination of lasalocid A sodium in the *feed additive* having the characteristics described in the proposed annex entry is considered fit for the purpose by the CRL.

For official control of the lasalocid A sodium in *premixtures* and *feedingstuffs* at or around the proposed minimum and maximum content (75 to 120 mg active substance / kg complete feedingstuff) the CRL recommends the Community method as described in Commission Directive 1999/76/EC.

Recommended text for the register entry, fourth column (Composition, chemical formula, description, analytical method)

HPLC with spectrofluorimetry according to Commission Directive 1999/76/EC

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Avatec 150 G*, have been sent to the Community Reference Laboratory for Feed Additives.

The dossier has been made available to the CRL by EFSA.

REFERENCES

[1] Commission Regulation (EC) No 2037/2005 of 14 December 2005 amending the conditions for authorisation of a feed additive belonging to the group of coccidiostats

[2] Annex III. Proposal of Register entry

[3] Reference SANCO/D/2 Forw. Appl. 1831/004-2008

[4] Technical dossier, Section II – App_18.pdf "Lasalocid Assay by High Performance Liquid Chromatography (HPLC method TCA-020.04)"

[5] Technical dossier, Section II – App_23.pdf "Method development and validation for the quantitative determination of lasalocid by High Performance Liquid Chromatography (Report TC 4126), Table 27

[6] Technical dossier, Section II – App_24.pdf "Validation of an analytical method for the determination of lasalocid sodium A, B, C, D, and E in feed and vitamin/mineral premixtures (TNO report V5501)

[7] Comission Directive 1999/76/EC of 23 July 1999 establishing a Community method of analysis for the determination of lasalocid sodium in feedingstuffs

[8] Comment of the "Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit" (German NRL), 8th September 2008, file number: LV8-2662-111-1-V12-D5476/2008

[9] Determination of lasalocid sodium in poultry feeds and premixes. Analyst, 1995, 120, 2175 - 2180

RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Community 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.

ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Univerza v Ljubljani. Veterinarska fakulteta, Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana, Slovenia
- Plantedirektoratets Laboratorium, Lyngby, Lyngby, Denmark
- Laboratoire de Rennes, direction générale de la concurrence, de la consommation et de la répression des frauds (DGCCRF), Rennes, France
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL). Oberschleißheim, Germany
- Sächsische Landesanstalt für Landwirtschaft. Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig, Germany
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena, Germany

- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Germany
- Instytut Zootechniki w Krakowie, Krajowe Laboratorium Pasz, Lublin, Poland
- RIKILT- Instituut voor Voedselveiligheid, Wageningen, The Netherlands
- Foderavdelningen, Statens veterinärmedicinska anstalt (SVA), Uppsala, Sweden