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

Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for

AVATEC 150 G (Lasalocid sodium)
(Dossier No. FAD-2004-002)

1. EXECUTIVE SUMMARY

The current application for AVATEC 150 G is an extension of an already existing lasalocid formulation in which a new carrier, calcium sulphate, is proposed.

AVATEC 150 G is a new formulation for a feed additive containing 15% w/w of the active substance lasalocid sodium. Lasalocid sodium is a coccidiostat and the proposed formulation is intended for chickens (broilers), chickens (reared for laying) of a maximum of 16 weeks old and turkeys of a maximum 12 weeks old. A wide set of methods were applied for assessing the physical properties of the additive and of the active substance.

Concerning the determination of the active substance, two different HPLC methods were proposed by the applicant. The first one used an isocratic elution and UV detection and was applied for the analysis of the active substance (lasalocid A) in the additive, in premixtures and in animal feed whereas the second one used a gradient elution and fluorescence detection and was applied for the determination of residues of the active substance in animal tissues.

The CRL has some reservations about several of those methods which do not directly concern the determination of the additive, notably the lack of availability of a protocol for determination of the contents of heavy metals. However, the level of heavy metals in coccidiostats is minor.

While it was not clear from the initial dossier if the validation experiments were carried out on AVATEC 150 G or on the already authorised existing additive which uses corn cob as its carrier, the applicant, upon request, provided additional documentation to clarify this point.

The validation data regarding animal tissue were obtained from chicken tissue only, not turkey, but it should be noted that these types of food are relatively similar. Thus, regarding

residues, and taking into account the intended use of lasalocid, in the opinion of the CRL appropriate animal tissue was analysed. In the case of unintended usage of lasalocid e.g for laying hens, it could become relevant to ensure the validity of the method for animal products such as eggs. Regarding detection and validation of detection methods for possible metabolites in animal tissues/products no studies were submitted. To the knowledge of the CRL no established methods for such lasalocid metabolites are publicly available. Performance characteristics for the method to detect lasalocid in feed, described in the applicant's dossier for AVATEC 150 G, are within the ranges outlined in Commission Directive 1999/76/EC, which describes a similar method for the same purpose. It is therefore considered that the proposed method is satisfactory in this respect and for the proposed concentration range of the additive.

Although a MRL at European level might be proposed at some stage, currently no such MRL exists. The CRL therefore considers that further associated measurement uncertainty need not be provided by the applicant. Nevertheless, based on the performance characteristics of the analytical method provided by the applicant, with a limit of quantification of 20 ng/g the method for detection of active substance in animal tissue (chicken) is considered sufficiently sensitive for official control purposes at the present stage of legislation.

In summary, the CRL finds that both the proposed methods fulfil the requirements to quantitatively determine the presence of AVATEC 150 G in the proposed concentration range. On the basis of the supplied documentation, in the opinion of the CRL no supplementary experimental work (testing or method validation) is needed.

Date: 13 May 2005

2. KEYWORDS

AVATEC, lasalocid, coccidiostat, feed additive

3. TABLE OF CONTENTS

4. BACKGROUND

In accordance with Regulation (EC) No 378/2005 the CRL is required to submit an evaluation report to the European Food Safety Authority for applications for feed additives.
AVATEC 150 G is a new formulation for a feed additive containing 15% w/w of the active substance lasalocid sodium. The lasalocid sodium used in the additive is a mixture of at least 80% of the homologue A and a maximum of 10 % of the other analogues (B, C, D and E). Lasalocid sodium is a coccidiostat and the proposed formulation is intended for chickens (broilers), chickens (reared for laying) with a maximum age of 16 weeks and turkeys with a maximum age 12 weeks. A wide set of methods were applied for assessing the physical properties of the additive and of the active substance. This evaluation report concerns the control methods described in the Annex of the Commission Directive 2001/79/EC (Section II point 2.5).

The full information provided by the applicant is divided into a main body providing general information (volume 1 of the Section II of the dossier) and detailed information related to the characteristics of the methods of analysis, experimental data and/or validation in Annexes (Annex II.2; II.10, II.14, II.15; II.16; II.18; II.19; II.20; II.21; II.22; II.23; II.24; II.27; II.28; II.29;

II.32) compiled in four additional volumes (volumes 2, 3, 4 and 5). This evaluation report is specifically based on the information included in the point 2.5 related to control methods within the aforementioned Section II "Identity, characterisation and conditions of use of the additive, methods of control for AVATEC 150 G" of the dossier submitted for authorisation to the CRL for Feed Additives Authorisation.

It should be noted that additional documentation was provided by the applicant upon request.

5. 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 tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is required to submit a full evaluation report to the European Food Safety Authority for each application.

6. EVALUATION

The numbering system under this point refers to that of Commission Directive 2001/79/EC.

It should be noted that, unless otherwise stated, the results submitted via the dossier refer to total lasalocid contents, i.e. taking into account all five homologues (A-E). However, lasalocid A is by far the most significant, contributing on average 87% of the total lasalocid concentrations.

Section 2.5- Control Methods

2.5.1 – Analytical methods for:

2.5.1.1 - The qualitative and quantitative analysis of the active substance in the additive

An isocratic HPLC (High Pressure Liquid Chormatography) method using a C18 column with UV detection at a wavelength of 305 nm is used for the determination of the active substance (lasalocid sodium) in solid and liquid formulations (SOP No. TCA-020.04 in the Annex II.18). The method is detailed in Annex II.18. The composition of the granular mix is not specified in the Standard Operating Procedure (SOP), which suggests that the same method will be used for all the granular mix formulations (solid formulations) and it should therefore also be valid for AVATEC 150 G.

2.5.1.2 - The determination of the physical properties of the additive

Appearance, particle size, moisture content, bulk density, dusting and flowability are determined for the additive.

Regarding the moisture content the Dean and Stark method was used. This is a relatively time-consuming method which also involves the use of flammable solvents. It would therefore be advisable to select another method for the determination of the water content.

2.5.1.3 - The qualitative and quantitative analysis of the active substance

The qualitative and quantitative analysis of the lasalocid content is performed with an isocratic HPLC method using a C18 column and a UV detector set at 305 nm. The method is described in the Standard Analytical Procedure (SAP) 010-905 (Annex VMF-k) and is the same as the one used for the qualitative and quantitative analysis of the active substance in the additive described in SOP No. TCA-020.04 (Annex II.18). It would therefore be advisable to refer to the same document.

2.5.1.4 - Other properties of the active substance

As previously described with the additive, the appearance, particle size and water content were determined in the active substance.

Conversely to the determination of the water content in the additive, a Karl Fisher method was used in this case for the same purpose. A justification for using one or the other method should be given; otherwise a harmonisation of the methods would be advisable. The same comment applies for the different devices used for the particle size determination.

Moreover the heavy metals / fluoride content determination was performed by a “contract laboratory”. However, the description of the methods used is insufficiently detailed in SOP 010-718 (Annex VMF-g).

The stability of the additive (2.5.1.5), the premixtures and animal feed during preparation and storage (2.5.1.6.) and the homogeneity of the additive (2.5.1.7.), the incompatibilities between the additive and other materials (2.5.1.8.) and the qualitative/quantitative methods for routine control of the active substance in premixtures and animal feed (2.5.2.) were evaluated by the applicant using the chromatographic method previously mentioned (SOP No. TCA-020.04 in the Annex II.18). The method appears to be suitable for the aforementioned purposes.

2.5.3 - Qualitative/quantitative methods for determining the residues of the active substance in tissues and animal products

Another HPLC method, involving fluorescence detection, developed by an external laboratory (Inveresk), was used for the determination of residues of the active substance in broiler chicken tissues namely muscle, liver, kidney and skin with fat. A description of this method, Analytical Method No. 0231, is provided in appendix I of the IRI Report No 22690 (Annex II.22). Nevertheless some crucial information such as the excitation and the detection wavelengths used for the determination of the active substance is missing in the original application. Upon request this information has been submitted by the applicant.

The method reached a limit of quantification of around 20 ng/g in chicken tissue. It should be noted that no MRL has been established for lasalocid in the EU whereas in Canada and Australia MRLs of 350 and 1200 ng/g respectively have been established for lasalocid in poultry skin/fat.

2.5.4- Validation of the analytical methods used for the determination of the active substance in sections 2.5.1.1 (additive), 2.5.1.3 (active substance), 2.5.2 (premixtures and animal feed) and 2.5.3 (tissues and animal products)

In sections 2.5.1.1, 2.5.1.3 and 2.5.2 the method used was the HPLC method using UV detection at 305 nm, described in SOP No. TCA-020.04 (Annex II.18). The method development and the validation report in bulk drugs, fermentation broth and formulated products related to the aforementioned method were carried out by the applicant, and the conclusions are given in Report No TC4126 (Annex II.23). Nevertheless, conversely to the method stated in Annex II.18, in Annex II.23 a linear regression forced through zero was selected as calibration curves type. Even when both approaches can be justified, it would be advisable to use the same criteria whenever the method is applied.

Additionally the validation of the method SOP No. TCA-020.04 (Annex II.18) to analyse the active substance in vitamin/mineral premixtures and in animal feed was carried out by the applicant (Report No TC4192 (Annex II.29) and by the TNO institute (TNO Report V5501 (Annex II.24). The validation procedure followed by the applicant (Annex II.29) is based on USP XXIV, VICH (the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products) whereas the one followed by TNO (Annex II.24) is based on its internal protocols. A harmonised validation protocol would be advisable, preferably the IUPAC single lab validation protocol.

In section 2.5.3 (residue analysis in tissues and animal products) another chromatographic method was used, this time with a gradient elution and a fluorimetric detector. The method

has been developed and validated by Inveresk. The validation dossier was carried out by Inveresk (IRI report No 22690) namely “Establishment and validation of an analytical method for the determination of lasalocid A in broiler chicken tissues” (Annex II.22). The analytical method was validated with respect to system suitability (system precision, column efficiency and tailing factor), specificity, linearity, limit of detection, limit of quantification, inter-day accuracy and precision, matrix effects, stability studies in tissues and extracts. The validation study was originally intended for another formulated product, namely AVATEC 15%cc, which has the same composition than AVATEC 150 G in terms of active substance. Therefore as the matrix samples were quantified against a non-matrix-match calibration curve and as the matrix effects were assessed by spiking the extracted tissues samples with the active substance (lasalocid A), the conclusions reached in this validation dossier can be extrapolated to the target formulation AVATEC 150 G. The main remark about the procedure concerns the use of the parent active substance as the marker in the target tissues, which would only be acceptable if the parent compound is the only substance representing more than 10% of the total residue (Commission Directive 2001/79/EC point 4.1.3.3.). If the latter condition is not verified, the appropriate markers should be selected as the target analytes in this study.

A linear regression not forced through zero was used in this validation study for all the calibration curves. The validation report does not state if the assay in-house validation assay was performed following any international harmonised guidelines (e.g. IUPAC, ISO, USP, VICH). Moreover at least one acceptance criterion used in Annex II.22 (70-110%) differs from the one selected in Annex II.24 (80-110%). Therefore, and as commented before, harmonised criteria would be advisable or a justification for using different criteria should be provided.

The validation reports TC4126, TC4192 and TNO Report V5501 (Annexes II.23, II.29 and II.24 respectively) refer to the validation of the chromatographic method with UV detection described in SOP No. TCA-020.04 (Annex II.18) used in the sections 2.5.1.1 (additive), 2.5.1.3 (active substance) and 2.5.2 (premixtures and animal feed). The analyses were performed by spiking “blank” feed samples and premixtures with the active substance (lasalocid). In the same way the bulk drug was analysed by diluting it with a “blank” fermentation broth. As the matrices used as “blanks” are not affected by the composition of the new formulation the conclusions reached in these validation reports for the sections 2.5.2 and 2.5.1.3 can be extrapolated to the target formulation AVATEC 150 G.

On the contrary, in section 2.5.1.1 the validation of the analysis of the active substance in the additive is carried out by spiking a “blank” carrier (corn cob) with lasalocid. In this case

the composition of the new formulation AVATEC 150 G (using calcium sulphate as carrier) affects the matrix used as a blank. Therefore, even when it is likely that the new carrier introduced in the novel formulation would not produce any type of critical interference in the method, limited experimental evidence has been provided and therefore the validation data reported for this section proved to be insufficient to validate the qualitative / quantitative analysis of the active substance in the additive (AVATEC 150 G).

No data is provided to demonstrate lack of interference with other coccidiostats within the analytical methods submitted. However, as a rule, within feed applications coccidiostats are applied one at a time only. Furthermore, adequate specificity data have been provided.

While only a limited number of acceptance criteria to the validation study were provided by the applicant it was still possible to evaluate the dossier since a comparison was made with the official EU method to determine lasalocid, and the submitted methods were comparable to the EU method in this aspect.

It should also be noted that according to Commission Directive 2001/79/EC, regarding the analysis of the presence of the active substance in the feed additive, a validation of the method used is not explicitly requested.

CHECK LIST – Part I

			Y	N	N/A	Comments
1.	A.	Description of the Qualitative and Quantitative analytical method/s for routine control of the active substance in				Ann. II.18
		- Premixtures	X			
		- Feedingstuffs	X			
	B.	The method has been validated:				Ann. II.22 Ann. II.23
		- In a ring test involving at least four laboratories		X		
		- In-house following harmonised guidelines[1]	X			USP-VICH
	C.	The validation study contains the following parameters[2]:				Ann II.29
		- Applicability	X			Scope
		- Selectivity	X			Specificity
		- Calibration	X			
		- Accuracy	X			
		- Precision	X			
		- Range	X			
		- Limit of detection	X			
		- Limit of quantification	X			
		- Sensitivity		X		
		- Robustness	X			
		- Practicability		X		
	D.	Is there evidence available that the characteristics listed above have been assessed?	X			
2.	Description of the Qualitative and Quantitative analytical method/s to determine the marker residue(s) of the active substance:					
	- In target tissue/s		X			
	- In animal products			X		

[1] M. Thompson et al. : Harmonized Guidelines For Single Laboratory Validation Of Methods Of Analysis (IUPAC Technical Report) Pure Appl. Chem., Vol. 74, No. 5, pp. 835–855, 2002.
For some analytical methods, such as determining of enzymes and microorganisms, these guidelines and the parameters mentioned are not fully applicable. In these cases deviation from this document or the use of alternative internationally accepted guidelines can be accepted.
[2] Definition of parameters is given in Annex IV of this document

CHECK LIST – Part II

		Y	N	N/A	Comments
1.1	Is/Are the method(s) mentioned in Part I (1.- A. Premixtures) accompanied by information on:				Ann II.24
	- Sampling Method used		X		
	- Percentage Recovery	X			
	- Specificity		X		
	- Accuracy	X			
	- Precision	X			Repeatability
	- Limits of detection		X		
	- Limits of quantification		X		
	- Validation procedure used	X			
1.2	Is/Are the method(s) mentioned in Part I (1.- A. Feedingstuffs) accompanied by information on:				Ann II.29
	- Sampling Method used		X		
	- Percentage Recovery	X			
	- Specificity	X			
	- Accuracy	X			
	- Precision	X			
	- Limits of detection	X			
	- Limits of quantification	X			
	- Validation procedure used	X			USPXXIV VICH
2.1	Is/Are the method(s) mentioned in Part I (2. – Target tissues) accompanied by information on:				Ann II.22
	- Sampling Method used		X		
	- Percentage Recovery	X			
	- Specificity	X			
	- Accuracy	X			Defined as recovery
	- Precision	X			
	- Limits of detection	X			
	- Limits of quantification	X			
	- Validation procedure used	X			
2.2	Is/Are the method(s) mentioned in Part I (2. – Animal products) accompanied by information on:				Not applicable
	- Sampling Method used			X	
	- Percentage Recovery			X	
	- Specificity			X	
	- Accuracy			X	
	- Precision			X	
	- Limits of detection			X	
	- Limits of quantification			X	
	- Validation procedure used			X	
3.	If the method(s) has/have been devised, consideration has been given to the fact that their limits of quantification must be below the MRLs.			X	

7. CONCLUSIONS AND RECOMMENDATIONS

In the opinion of the CRL, and taking into account Commission Directive 1999/76/EC the proposed methods appear fit for purpose.

The CRL has some reservations about several of those methods which do not directly concern the determination of the additive, notably the lack of availability of a protocol for determination of the contents of heavy metals. However, the level of heavy metals in coccidiostats is minor.

The validation data regarding animal tissue were obtained from chicken tissue only, not turkey, but it should be noted that these types of food are relatively similar. Thus, regarding residues, and taking into account the intended use of lasalocid, in the opinion of the CRL appropriate animal tissue was analysed. In the case of unintended usage of lasalocid e.g for laying hens, it could become relevant to ensure the validity of the method for animal products such as eggs. Regarding detection and validation of detection methods for possible metabolites in animal tissues/products no studies were submitted. To the knowledge of the CRL no established methods for such lasalocid metabolites are publicly available. Performance characteristics for the method to detect lasalocid in feed, described in the applicant's dossier for AVATEC 150 G, are within the ranges outlined in Commission Directive 1999/76/EC, which describes a similar method for the same purpose. It is therefore considered that the proposed method is satisfactory in this respect and for the proposed concentration range of the additive.

Although a MRL at European level might be proposed at some stage, currently no such MRL exists. The CRL therefore considers that further associated measurement uncertainty need not be provided by the applicant. Nevertheless, based on the performance characteristicis of the analytical method provided by the applicant, with a limit of quantification of 20 ng/g the method for detection of active substance in animal tissue (chicken) is considered sufficiently sensitive for official control purposes at the present stage of legislation.

In summary, the CRL finds that both the proposed methods fulfil the requirements to quantitatively determine the presence of AVATEC 150 G in the proposed concentration range. On the basis of the supplied documentation, in the opinion of the CRL no supplementary experimental work (testing or method validation) is needed.

8. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

Samples of AVATEC 150 G have been made available to CRL by the applicant.
The dossier has been made available to the CRL by EFSA.

9. REFERENCES

Commission Directive 1999/76/EC (regarding a method to determine lasalocid)

10. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was the Community Reference Laboratory for Feed Additive Authorisation, IRMM, Geel, Belgium.
Responsible person for the evaluation is Christoph von Holst.

11. APPENDIX

Not applicable.