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CRL Evaluation Report on the Analytical Methods submitted in connection with the application for re-evaluation of an authorised Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2008-0050 CRL/080027
Name of Additive:	Avatec ® 150G
Active Substance(s):	Lasalocid sodium A
Rapporteur Laboratory:	Community Reference Laboratory for Feed Additives (CRL-FA) Geel, Belgium
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EXECUTIVE SUMMARY

AVATEC (Lasalocid sodium A) 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* is *lasalocid sodium A* and the proposed inclusion level of this compound in complete feedingstuffs is 75 mg/kg for the minimum content and 125 mg/kg for the maximum content.

In the current application according to Article 10(2) of Regulation (EC) No 1831/2003 the re-evaluation of the use of *AVATEC* for Turkeys (up to 16 weeks) is sought.

For the determination of *lasalocid sodium A* in *premixtures* and *feedingstuffs*, the applicant submitted a single-laboratory validated method derived from the community method published in Regulation (EC) No 152/2009, based on reverse-phase HPLC. The performance characteristics determined by the applicant are in good agreement with those reported by the ring trial validated community method listed hereafter (for Turkey, in *feedingstuffs* and *premixtures*):

- a relative standard deviation for *repeatability* ranging from 2.2 and 2.5 %;
- a relative standard deviation for *reproducibility* ranging from 5.0 and 5.7 %;
- a limit of detection and a limit of quantification of 5 and 10 mg/kg *feedingstuffs*, respectively, and
- a *recovery* rate of at least 80 % for *feedingstuffs*, and great than 90 % for *premixtures*.

Upon request by the CRL, the applicant provided - for the determination of the *lasalocid sodium A* in the *feed additive* - experimental evidence showing that the above mentioned community method applies also to the *feed additive* matrix. From the experimental data reported, the CRL evaluated the following performance characteristics: - a standard deviation for *repeatability* of 4.4 %; - a relative standard deviation for *intermediate precision* of 5.7 % and – a recovery rate of 98.2 %.

Based on the above mentioned performance characteristics, the CRL recommends for official control, the community method published in Regulation (EC) No 152/2009, for the determination of *lasalocid sodium A* in *feed additives*, *premixtures* and *feedingstuffs*.

Further testing or validation is not considered necessary.

KEYWORDS

Lasalocid sodium A, coccidiostats, turkeys (16 weeks)

1. BACKGROUND

AVATEC 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.

The reddish granules of *AVATEC* contain 15% w/w of *lasalocid sodium A* produced by *Streptomyces lasaliensis subsp. lasaliensis (ATCC 31180)*. Other components of the feed additive are calcium sulphate dihydrate (carrier) at a concentration of 809 mg/g, calcium lignosulphonate (binder) at 40 mg/g and ferric oxide (colouring agent) 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].

In the current application submitted according to Article 10(2) of Regulation (EC) No 1831/2003 the use of *AVATEC* is sought for turkeys (up to 16 weeks) [3].

The target concentration of the active substance in complete feedingstuffs is 75 mg/kg for the minimum content and 125 mg/kg for the maximum content [4].

2. 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* (EFSA-Q-2008-0050), 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, mercury and lead) are available at the respective Community Reference Laboratories (Commission Regulation (EC) No 776/2006).

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

For the determination of *lasalocid sodium A* in *premixtures* and *feedingstuffs*, the applicant submitted an in-house validated method [5] derived from the community method described in the Regulation (EC) No 152/2009 [6]. Minor modifications were implemented by the applicant in the operating procedure, related to sample amount and chromatographic conditions [5]. *Lasalocid sodium* is extracted from the sample into methanol and determined by reversed-phase high performance liquid chromatography (HPLC) using a UV detector. The following performance characteristics were reported by the applicant [7]: - a relative standard deviation for *repeatability* (RSD_r) of 0.39 %; - a relative standard deviation for *intermediate precision* (RSD_{ip}) of 0.44 %; a recovery rate of about 100 %; and a limit of detection and a limit of quantification of 0.32 and 1.07 mg/kg *feedingstuffs*, respectively.

In the community method *lasalocid sodium* is extracted from the sample into acidified methanol and determined by reversed-phase high performance liquid chromatography (HPLC) using a spectrofluorometric detector. This method was ring-trial validated and the performance characteristics reported are: - a relative standard deviation for *repeatability* ranging from 2.2 and 2.5 %; - a relative standard deviation for *reproducibility* ranging from 5.0 and 5.7 %; - a minimum *recovery* rate of 80% for *feedingstuffs* and 90 % for *premixtures*; - a limit of detection and quantification of 5 and 10 mg/kg *feedingstuffs* [6], respectively.

The data provided by the applicant demonstrates the applicability of the community method on the *premixtures* and *feedingstuffs* containing *lasalocid sodium A*. Therefore the CRL recommends for official control the community method (Regulation (EC) No 152/2009, [6]) for the determination of *lasalocid sodium A* in *premixtures* and *feedingstuffs*.

Upon request of the CRL, the applicant applied the community method on the *feed additive per se* and provided supplementary information [8]. Only minor experimental modifications were applied, in compliance with the community method recommendations (i.e ultrasonication performed at room temperature, and chromatographic conditions were adapted). From the experimental data reported [8], the CRL evaluated the following performance characteristics: - a relative standard deviation of *repeatability* of 4.4 %; - a relative standard deviation of *intermediate precision* of 5.7 %. Furthermore, a recovery rate of 98.2 % was reported by the applicant [8].

Based on these acceptable performance characteristics - demonstrating the applicability of the community method to the product - the CRL recommends for official control the community method (Regulation (EC) No 152/2009) for the determination of *lasalocid sodium A* in the *feed additive*.

Further testing or validation is not considered necessary.

CONCLUSIONS AND RECOMMENDATIONS

The CRL recommends for official control the community method published in Regulation (EC) No 152/2009, for the determination of *lasalocid sodium A* in *feed additive*, *premixtures* and *feedingstuffs*.

Recommended text for the registrer entry (analytical method)

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

- reversed-phase high performance liquid chromatography (HPLC) with spectrofluorometric detector (Regulation (EC) No 152/2009)

DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *AVATEC* 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] * Technical dossier, Section II – 2.2. Characterisation of the active substance

[3] * Reference SANCO/D/2 Forw. Appl. 1831/032-2008"

[4] * Technical dossier, Section II – 2.5. Conditions of use of the additive

[5] * Technical dossier, Section II – II_18 Lasalocid Assay.pdf "Lasalocid Assay by High Performance Liquid Chromatography (HPLC method TCA-020.04)"

[6] Commission Regulation No 152/2009 of 27 January 2009, laying down the methods of sampling and analysis for the official control of feed – Determination of Lasalocid Sodium

[7] * Technical dossier, Section II – II_29 Validation HPLC lasalocid.pdf "Method Validation for the Quantitative Determination of Lasalocid by High Performance Liquid Chromatography, Part 11; Validation in Animal Feed Matrices"

[8] * Supplementary Information – 2009_CER_Avatec_validation_report.pdf "Validation of an analytical method for the determination of lasalocid in *AVATEC*"

* Refers to Dossier No. FAD-2008-0050

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.

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