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Community Reference Laboratory for Feed Additives Authorisation

CRL Feed Additives Authorisation

Addendum to CRL report (No. D08/FSQ/CVH/GS/(2006) D 221) on the dossier EFSA-Q-2005-168 (Elancoban®)

The suitability of the analytical method for official control purposes:

The analytical methods for the determination of monensin sodium in animal feed, namely AOAC Official Method 997.04 and the ISO standard 14183 are also suitable for official control at the *modified* dose of the active substance which ranges from 30 to 45 mg/kg of monensin sodium in animal feed.

CRL Evaluation Report on the Analytical Methods submitted in connection with Section II, 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	EFSA-Q-2005-168
Name of Additive:	Elancoban®
Active Substance(s):	Monensin sodium
Rapporteur Laboratory:	Central Institute for Supervising and Testing in Agriculture (CISTA), Praha, Czech Republic
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Date:	05/01/2006

EXECUTIVE SUMMARY

In the current application authorisation is sought for Elancoban® under the category 5. coccidiostats and histomonostats, according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought to use Elancoban® for the control of coccidiosis in calves for rearing and cattle for fattening.

The active substance in Elancoban® is monensin as sodium salt which contains the two major components monensin A contributing 97.6 % to the overall activity and monensin B. The monensin concentration in the feed additive, in premixtures and feedingstuffs is expressed in terms of monensin activity which is calculated from the measured concentration of both components. The target monensin activity in Elancoban® G 100 is 100 g/kg and the monensin activity in Elancoban G 200 is 200 g/kg. For both products the applicant proposed purity criteria expressed as range of monensin activity of 92.5 g/kg to 107.5 g/kg for Elancoban® 100 and of 185 to 215 g/kg for Elancoban® 200. The appearance is a dark brown, speckled with straw-coloured particles, free flowing meal, which contains rice hull or limestone granular, antidusting oil and monensin. The proposed dosage ranges from 40 to 120 mg of monensin /kg in the feedingstuff for calves and for cattle.

For the determination of the active substance (monensin) in the feed additive (Elancoban®)an isocratic High Performance Liquid Chromatography (HPLC) method with post-column derivatisation and Ultraviolet (UV) detection is proposed by the applicant. The method is also used to demonstrate the purity of monensin, which mainly consists of monensin A. The performance characteristics are considered acceptable, thus the method is considered suitable for official control purposes.

For the determination of the monensin in premixtures and feedingstuffs the applicant proposes a HPLC method, which is based on the same principle as mentioned above. The limit of quantification (LOQ) of the method for the determination of monensin is 4 mg /kg.

The method has also been validated by conducting an interlaboratory study (*J. of AOAC International 1997 80 693*) performed on various feed matrices including cattle feedingstuff. Acceptable precision data were obtained for the feedingstuffs, since the relative repeatability standard deviation (RSD_r) ranged from 6.1 to 15 % and the relative reproducibility standard deviation (RSD_R) ranged from 8.6 to 15 % .

The applicant's method has been adopted as AOAC Official method (AOAC Official Method 997.04). It is therefore considered suitable for official control purposes for the field of application that is sought. For official control purposes the CRL also recommends the ISO standard 14183:2005 which is a multi-analyte method, since it allows for the simultaneous determination of monensin, narasin and salinomycin in feedingstuffs. This method is based on the same principle as the method proposed by the applicant.

For determination of monensin in edible bovine tissues and milk a HPLC method based on the same principle as the method for the detection of the active substances in the other matrices has been submitted. The LOQ for monensin is 25 μg/kg in bovine muscle, liver, kidney and fat and 5 μg/kg in milk. The recovery rate of the target analyte in the four tissues types and milk ranged from 80 to 88% and the relative within-laboratory standard deviation for reproducibility varied from 3.6 to 9.1%. The obtained method performance characteristics are considered acceptable for the intended purpose. However, since there are no maximum residue levels (MRLs) for monensin fixed by European legislation, the suitability of the method for official control purposes cannot be evaluated.

Further testing or validation of the submitted methods is not considered necessary.

KEYWORDS

Elancoban®, monensin, coccidiostat, feed additive

TABLE OF CONTENTS

1. BACKGROUND 4
2. TERMS OF REFERENCE 5
3. EVALUATION 5
4. CONCLUSIONS AND RECOMMENDATIONS 9
5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL 9
6. REFERENCES 10
7. RAPPORTEUR LABORATORY 10

1. BACKGROUND

Elancoban® is a feed additive belonging to the category 5. coccidiostats and histomonostats. The active substance is monensin sodium. Elancoban® (E757) is already authorised as coccidiostat for chickens for fattening, chickens reared for laying and turkeys (Commission Regulation (EC) No 1356/2004) until 30.7.2014. Elancoban®contains rice hull or limestone granular, antidusting oil and monensin. The active substance is produced by an organism identified as *Streptomyces cinnamonensis*.

The intended use (*cf.* EFSA-Q-2005-168) of the current application is as an aid in the prevention of coccidiosis for calves for rearing and cattle for fattening. The proposed dosage ranges from 40 to 120 mg of monensin /kg of complete feedingstuff for calves and cattle.

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 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 suitability of the control methods and validation studies submitted in connection with Elancoban® (*cf*. EFSA-Q-2005-168), was evaluated.

3. EVALUATION

The numbering system under this point refers to that of Section II of the Annex of Commission Directive 2001/79/EC. The methods description for determination of the active substance in the feed additive, premixtures, feedingstuffs and in animal tissue and products and corresponding validation data are given in Section II (point 2.5 Control method) of the dossier.

Description of the methods used for the determination of criteria listed in Section II of the Annex point 2.5.1 of Commission Directive 2001/79/EC

Quantitative analysis of active substance (monensin) in the feed additive

The active substance of Elancoban® is monensin which contains the four compounds (factors) monensin A, B, C and D. The monensin concentration in the feed additive, in premixtures and feedingstuffs is expressed in terms of monensin activity which is calculated from the measured concentration of monensin A and B according to a formula. In detail, monensin A und B are quantified separately. The measured concentration of monensin A is multiplied with the factor of 1 and the measured concentration of monensin B is multiplied with the factor of 0.28, thereby obtaining the respective biopotency of each compounds. The monensin content equals the sum of both biopotencies.

For the determination of the monensin in the feed additive (Elancoban®) a High Performance Liquid Chromatography (HPLC) method with post-column derivatisation and Ultraviolet (UV)-detection detection is proposed by the applicant. Monensin is extracted from the samples with a mixture of methanol/water. After extraction the sample is diluted, filtered and measured with HPLC. The post column derivatisation involves acid reaction of monensin with vanillin and the resulting products are measured by the UV detector at 520 nm.

The method is able to separate monensin factor A, B, C and D. Confirmation of the factors was carried out on HPLC coupled to mass spectrometry. The obtained relative repeatability standard deviation was 3.2 % and the obtained relative recovery rate ranged from 98 to 102%. Therefore, this method is considered suitable for official control purposes.

Determination of dioxins

The production process of Elancoban® includes the use of clay, for which legal limits regarding the content of dioxins (sum of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)) expressed in World Health Organisation (WHO) toxic equivalents (Commission Directive 2003/57/EC) have been established.

Only the presence of 2,3,7,8 tetrachloro-p-dibenzoidioxin (TCDD) in clay is checked by the supplier of this product, but information on the presence of other congeners are not available. In addition, no method of analysis has been proposed.

For official control the selected method of analysis has to fulfil the criteria as established in Commission Directive 2002/70/EC. Special emphasis need to be placed on the selection of an appropriate extraction technique applied for analysis, since the extraction efficiency of dioxins from feed materials of mineral origin additives depends of the type of extraction technique [1]. The CRL recommends contacting the Expert Committee "Methods of Analysis in Feedingstuffs" established in DG Health and Consumer Protection of the European Commission, to obtain the most recent information on appropriate extraction techniques for this specific analyte/matrix combination.

Stability of the additive

The applied HPLC method for stability test is the same method as for the determination of the active substance in the feed additive and considered suitable for this purpose (see above in the chapter "Quantitative analysis of active substance in the feed additive").

Stability during the preparation and storage of the feed

The applied HPLC method for stability test is the same method proposed for the stability of the additive and is considered suitable for this purpose.

Description of the qualitative and quantitative analytical methods for routine control of the active substance in premixtures and feedingstuffs (Annex point 2.5.2 of Commission Directive 2001/79/EC)

For determination of monensin in premixtures and feedingstuffs a HPLC method with post-column derivatisation and UV detection is proposed by the applicant. The method is based on the same principle as the HPLC-UV method described for the determination of the active substance in the feed additive with some adaptation of the calibration curve. Sufficient specificity of the method is demonstrated since narasin and salinomycin are chromatographically separated from monensin. Other antibiotics such as lasalocid, tylosin, nicarbazin, bacitracin, lincomycin and bambermycin do not interfere with the analysis of monensin, since they give no response under the conditions of this analysis.

The applicant's method has also been validated in an interlaboratory study and the results of the study have been published in a peer reviewed journal [2]. The validation was performed

on various feed matrices including cattle feedingstuff. Acceptable precision data were obtained for the feedingstuffs, since the relative repeatability standard deviation ranged from 6.1 to 15 % and the relative reproducibility standard deviation ranged from 8.6 to 15 % . The limit of quantification (LOQ) is 4 mg/kg. The obtained method performance characteristics are considered acceptable.

The method has been adopted by AOAC as official method (No 997.04) [3] and is suitable for the determination of monensin in the range of 5 to 200 000 mg/kg in premixtures and feedingstuffs for official control purposes.

For official control purposes the CRL also recommends the ISO standard 14183:2005 [4] which is a multi-analyte method, since it allows for the simultaneous determination of monensin, narasin and salinomycin in feedingstuffs. This method is based on the same principle than the method proposed by the applicant.

Description of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products.

A HPLC method for the determination of monensin in edible bovine tissues and milk is proposed by the applicant which has been published in a peer reviewed journal [5]. This method is based on the same principle as for the determination of monensin in the feed additive, in premixtures and feedingstuffs. Monensin is extracted from the tissues with a mixture of methanol/water and the solution is then subjected to solid-phase extraction with silica gel. Finally, the sample is analysed by HPLC and measured after post-column derivatisation. The post column derivatisation involves acid reaction of monensin with vanillin and the resulting products are measured by UV detector at 520 nm.

The method has been validated in various bovine tissues and milk at three levels, which were 25, 50 and 100 μg/kg. Acceptable method performance characteristics were obtained, since the values for the recovery rate ranged from 80 to 88%, the relative within-laboratory standard deviation for reproducibility varied from 3.6 to 9.1% and the LOQ for monensin in bovine muscle, liver, kidney and fat was 25 μg/kg in and in milk was 5 μg/kg. The obtained method performance characteristics are considered acceptable for the intended purpose. However, since there are no maximum residue levels (MRLs) for monensin fixed by European legislation, the suitability of the method for official control purposes cannot be evaluated.

CHECKLIST

		Y	N	N/A	Comments
1.1	Is/Are the method(s) mentioned on Premixtures accompanied by information on:				
	- Sampling Method used		**X**		
	- Percentage Recovery	**X**			
	- Specificity	**X**			
	- Accuracy	**X**			
	- Precision	**X**			
	- Limit of detection		**X**		
	- Limit of quantification	**X**			
	- Validation procedure used	**X**			
1.2	Is/Are the method(s) mentioned on feedingstuffs accompanied by information on:				
	- 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**			
2.1	Is/Are the method(s) mentioned on Target tissues accompanied by information on:				
	- 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**			
2.2	Is/Are the method(s) mentioned on Animal products accompanied by information on:				
	- 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**			

4. CONCLUSIONS AND RECOMMENDATIONS

Concerning the determination of the active substance (monensin) in Elancoban® a HPLC method with post-column derivatisation and UV detection is proposed by the applicant. The method is also used to demonstrate the purity of monensin, which consists of the monensin factors A, B, C and D.

The method has been validated and the performance characteristics are considered acceptable. The method is therefore suitable for official control purposes.

For determination of monensin in premixtures and feedingstuffs a HPLC method with post-column derivatisation and UV detection is proposed by the applicant. The method is based on the same principle as the HPLC-UV method described for the determination of the active substance in the feed additive with some adaptation of the calibration curve. The method has been fully validated and adopted as AOAC Official method. It is therefore considered suitable for official control purposes for the field of application that is sought.

For official control purposes the CRL also recommends the ISO standard 14183:2005 which allows for the determination of monensin and other antibiotics, which are narasin and salinomycin.

The applicant proposes a HPLC method for the determination of monensin in edible bovine tissues and milk based on the same principle as for the determination of monensin in the feed additive, in premixtures and feedingstuffs. The method has been validated on cattle tissues, obtaining acceptable performance characteristics.

The validation data are considered sufficient to conclude that further testing or validation is not considered necessary.

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

According to the requirements of Regulation (EC) No 1831/2003, samples of Elancoban® have been sent to the Community Reference Laboratory for feed additives authorisation.

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

The dossier has been made available to the CISTA (Central Institute for Checking and Testing in Agriculture, Praha, Czech Republic).

6. REFERENCES

The dossier provided by the applicant is divided into various documents structured according to the Annex of Commission Directive 2001/79/EC.

[1] Summary Minutes of the Meeting of the Standing Committee on the Food Chain and Animal Health. Animal Nutrition Section. Brussels, 18 March 2005. European Commission, DG Health and Consumer Protection available via http://europa.eu.int/comm/food/committees/regulatory/scfcah/animalnutrition/summary29_en.pdf

[2] Coleman M.R. et al. Liquid Chromatographic Determination of Monessen in Premix and Animal Feeds: Collaborative study (1997). *J. of AOAC Int.* 80 693

[3] Official Methods of Analysis of AOAC INTERNATIONAL (Gaithersburg, Maryland 20877-2417 USA): AOAC Official Method 997.04 – Monensin in premix and animal feeds

[4] ISO 14183:2005 - Animal feeding stuffs -- Determination of monensin, narasin and salinomycin contents -- Liquid chromatographic method using post-column derivatisation

[5] Moren J.W. et al. Determination of Monensin in Edible Bovine Tissues and Milk by Liquid Chromatography (1995). *J. of AOAC Int.* 78 668

7. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was

CISTA, NRL RO Praha, The Czech Republic.