EUROPEAN COMMISSION
DIRECTORATE-GENERAL
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
Community Reference Laboratory – Feed Additives Authorisation

Evaluation Report of the Community Reference Laboratory Feed Additives Authorisation on the Method(s) of Analysis for
Coxidin®
(Dossier No. FAD-2005-0003)

1. EXECUTIVE SUMMARY

Coxidin® is an ionophore coccidiostat additive used in feedingstuffs for chickens and turkeys for fattening. The formulation contains 25% w/w of the active substance monensin sodium. The proposed inclusion rates of the active substance in feedingstuff for chickens for fattening ranges from 100 mg/kg to 125 mg/kg, and for turkeys for fattening from 60 mg/kg to 100 mg/kg. The recommended withdrawal period is 3 days for chickens for fattening and 2 days for turkeys for fattening.

With respect to the methods, used to control the properties of the additive, these are either internationally recognised standards or methods developed by the applicant. In our opinion the internationally recognised procedures (ISO/DIS 14183.2) should be fit for purpose for routine analysis as well as for official controls. However, from the methods developed by the applicant descriptions about the determination of impurities (heavy metals), dioxins and microorganisms are not included in the dossier.

Concerning the determination of the active substance (monensin) in the additive, premixtures and feedingstuffs, different HPLC methods have been proposed. The principle of these methods is identical: Monensin is determined by means of an isocratic HPLC method with post-column derivatisation and UV-VIS detection at 520 nm.

For quantification of monensin in premixtures and feeds two different principles have been applied, *viz*. (i) potency, calculated with a formula and (ii) direct comparison of peak areas of monensin A and B, calculated relative to a reference standard. While the ratio of monensin A to monensin B is about the same in the Coxidin® and in the reference standard, it is found that both ways of calculations are acceptable.

The developed procedures are accurate and precise at the concentration levels in the additive Coxidin® (25 % monensin sodium), premixtures (2%, 10% and 20% monensin sodium) and feedingstuffs (100 mg monensin sodium/kg). The proposed methods to detect monensin fulfil the requirements of quantitative determination. The main performance characteristics of the methods are as follows: relative standard deviation: < 5%; recovery range: 95% – 105%. Figures for limit of quantification and limit of detection were only given for the method applied for the 2% premix. The limit of quantification was established as 1% monensin and the limit of detection was estimated as 0.01%.

An HPLC method has been developed to determine monensin in tissues from chickens and turkeys. The procedure has been validated to detect residues in tissues

(muscle, liver, kidney, skin+fat) at concentrations between 6 and 90 ng/g, with a limit of quantification of 6 ng/g. The method uses the ionophore additive salinomycin as an internal standard. According to the validation results the method can be considered as suitable for routine analysis of the determination of monensin in tissues of turkeys after use of Coxidin®.

However, the method is not suited for official control since the presence of salinomycin in unknown tissue samples cannot be excluded.

It is found that there is no need for further requirements regarding testing of validation.

Date: 20 July 2005

2. KEYWORDS

Coxidin®, monensin, coccidiostat, feed additive.

3. TABLE OF CONTENTS

4. BACKGROUND

Coxidin® is a coccidiostat additive that contains monensin sodium as the active ingredient. The additive may be used as an additive in feed according to Regulation (EC) No 1831/2003.
The additive Coxidin® is a brown product in powder form, which contains monensin sodium technical substance equivalent to monensin activity 25%, perlite from 15% to 20% and wheat bran added as diluent to 100%. The polyether ionophore monensin sodium represents a mixture of -related substances. According to the dossier the main substances are monensin A, monensin B, and monensin C in the ratio: monensin A ≥ 90%, sum of monensin A and monensin B ≥ 95%, and monensin C from 0.2 to 0.3%. Information about the content of monensin D is not given by the applicant.

Coxidin® is intended for chickens for fattening and turkeys for fattening. The proposed use of Coxidin® in mash and pelletised feedingstuffs is at a monensin content in feeds for chickens of 100 to 125 mg/kg and in feeds for turkeys of 60 to 100 mg/kg.
According to EU Directive 70/524/EC, the coccidiostat monensin sodium (E757) is currently registered in feeds for chickens for fattening at levels between 100 and 125 mg/kg and in feeds for turkeys for fattening at levels between 90 and 100 mg/kg (this information is only given for reference).

This evaluation report is specifically based on the information included in point 2.5 related to control methods within the before mentioned Section II “Identity, characterisation and conditions of use of the additive, methods of control” of the dossier Coxidin® submitted for authorisation to the CRL for feed additives. A wide set of methods was applied for assessing the physical properties of the additive and of the active substance.

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. For this particular dossier, the suitability of the control methods submitted in connection with the Coxidin ® dossier (FAD – 2005 – 0003) were evaluated.

6. EVALUATION

The evaluation under this point follows the structure of the requirements listed in Commission Directive 2001/79/EC.

Control methods.

Description of the methods used for the determination of the criteria listed (cf. pt. 2.5.1 of Commission Directive 2001/79/EC).

6.1 The determination of properties of the additive

All the control methods used are either internationally recognised methods (European Pharmacopoeia, EU Official Method, USP) or methods developed by the applicant.

- Content of chemical impurities.

The content of the chemical impurities arsenic, lead, cadmium and mercury in monensin sodium is determined by ICP-MS and FAAS quantitative analysis. Reference has been made to descriptions submitted to the dossier, corresponding to Commission Directive 2003/100/EC which describes the allowed maximum concentrations of undesirable substances in animal feed. A description of the used quantitative method for heavy metals (arsenic, lead, cadmium and mercury) is not submitted via the dossier.

- Content of dioxins.

For the method, applied for PCDD/PCDF, the applicant refers to Commission Directive 2002/70/EC.
This gives maximum allowed contents of dioxins and PCB's. Results of analyses for dioxins and PCB's in monensin sodium technical substance and wheat bran (Coxidin®) have been given. A description of the method is not given via the dossier.

- Absence of producing microorganism.

The absence of the producing organism is proven by carrying out a submerged cultivation on complex nutrient medium under conditions promoting the growth of monensin strain producer. However, a complete description of the used method for the presence/absence of living cells of the strain has not been given and the method is therefore not evaluated to this extent.

6.2 The quantitative analysis of the active substance in the additive (Ref. II.48, II.49, II.50).
The content of monensin in Coxidin® is determined by means of a validated HPLC method with post-column derivatisation and UV-VIS detection at 520 nm.
A sample is extracted with a mixture of methanol and water (9:1), sonicated, and finally diluted and filtered before HPLC analyses. The diluted extract is analysed and quantified by HPLC using a C18 column and post-column derivatisation. Monensin A and monensin B are detected separately by UV-VIS at a wavelength of 520 nm. The resolution of monensin A and monensin B peaks was calculated directly from the chromatographic data. Monensin C and D peaks are not detected in the standard monensin sodium used for the experiment.
The potency of monensin, in mg/g of feed additive, is calculated by the formula: A + 0.28 B + 1.5 C/D.

The method has been validated by 3 independent labs, as follows.
(See ref. II.48, II.49, II.50 of dossier)

Precision and repeatability:
Regarding repeatability an assay for six test solutions resulted in a mean value of 98.80% of declared value with a RSD of 1.11%, which is in line with Commission Directive 2002/657/EC. For the intermediate precision, the assay for the six test solutions gave a mean of 98.75% of declared value with a RSD of 0.33% which is found to be acceptable. Regarding the precision of method for the HPLC assay the coefficient of variation was determined to be 0.20% and RSD of 1.55% and 3.69% for monensin A and monensin B respectively, were calculated, which is in line with Commission Directive 2002/657/EC as well as applicant's own criteria.

Accuracy:
For the main component, monensin A, mean recovery of 100.10-101.60%, with a confidence interval of 1.13 was found. For monensin A and B the recovery is approximately 95%. This is considered acceptable.

Linearity:
The linearity of the HPLC assay method for both monensin A and monensin B were proven using six solutions of accurately weighed monensin Standard representing concentrations in the range from 50% to 150% of the typical standard concentration. The correlation coefficient is 0.99956 for monensin A and 0.99952 for monensin B, which is found to be acceptable.

Specificity:
The specificity of the HPLC method has been checked by comparing chromatograms of salinomycin, narasin A and narasin B with those obtained from the analysis of monensin. Monensin A eluted at approximately 8.1 min; monensin B eluted at approximately 7.5 min. No interfering peaks between monensin and the other ingredients of monensin sodium 25% were observed at the level of blank solution.

Degradation:
The results from forced degradation tests prove that the HPLC method for assay of monensin in the additive are able to detect degradation products, if any occur during storage of the additive, and therefore are also an indicator of stability.

Conclusions: The HPLC procedure of extraction and analysis of monensin in the additive Coxidin® 25% is described in a well recognised format. With respect to C/D quantification: it is assumed that this means C and/or D. Summary tables, chromatograms and final results of the experiments have been provided in addition to the description. The method has been validated for the analysis of monensin at the 250 µg/mg level in the additive Coxidin® 25%. On basis of the data provided it can be concluded that the precision of the analysis of monensin was acceptable and that the recovery was within acceptable range (95-105%). While parameters such as applicability, limit of detection, limit of quantification, robustness and practicability were not investigated, in our opinion is acceptable because the method is able to detect monensin at the 250 µg/mg level.
In conclusion the method can be considered as suitable for use as an analytical method for the determination of monensin in Coxidin® 25% feed additive.

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

Information has been submitted about HPLC methods used for the determination of monensin content in premixtures (2, 10 and 20% monensin) and feeds.
The descriptions and validations of these methods are evaluated and summarised below.

6.3.1 Descriptions of the quantitative analytical methods for routine control of the active substance in premixtures.

Information regarding premixtures with three different concentrations was submitted; containing 20%, 10% and 2% Coxidin® respectively.

6.3.1.1 Coxidin® 20% Premix (ref. II.74)
A sample of Coxidin® 20% premix is extracted with methanol, ultrasonicated, and finally diluted with HPLC mobile phase.
The diluted extract is analysed and quantified by HPLC using an X-Terra MS C18 column and post-column derivatisation with vanillin. Monensin A and monensin B are detected separately by UV-VIS at a wavelength of 520 nm.
The content of monensin sodium in Coxidin® 20% was calculated according to the peak area of monensin A and monensin B. An external calibration curve, prepared of standard monensin sodium (Sigma-Aldrich) in methanol, is used as reference.

Validation parameters have been tested. The following has been reported (the headings refer to paragraphs in ref. II.74 of the dossier):

Specificity:
The susceptibility of the method with respect to interferences by matrix components was checked with blank samples of single ingredients of Coxidin®. These did not interfere in the region corresponding to the retention time of monensin A and monensin B.

Chromatographic variability and system suitability:
The chromatographic variability and system suitability was tested by replicate injections of a test solution. The RSD found for mean peak area, mean plate number and mean symmetry was between 1.06% and 1.83%.

Linearity and accuracy:
In the range 1.5 – 3.6 mg/ml the analysis of variance showed an acceptable linearity with a correlation coefficient > 0.999. The accuracy of the method determined on injections of 1500, 3000 and 3600 µg/ml gave a mean recovery of 100.10% with a confidence interval of 1.48.

Precision:
Repeatability: Six independent test solutions (Coxidin® 20% preparations) and two reference solutions (standard monensin) were prepared and injected in duplicate. Found assay for the six test solutions ranged from 102.28% to 104.43% of declared value, with the mean value being 103.25% with a RSD of 0.97%.
Intermediate precision: Data of a second run of repeatability carried out on a different day by a different analyst were determined. Found assay for the six test solutions ranged from 102.33% to 104.98% of declared value. Mean value was 103.52% of declared value with a RSD of 1.14%
Analysis of variance did not demonstrate statistically significant differences between the two analytical runs.

6.3.1.2 Coxidin® 10% Premix (ref. II.75)
A sample of Coxidin® 10% premix is extracted with a mixture of methanol and water (9:1 v/v), ultrasonicated, and finally filtered before HPLC analysis.
The extract is analysed and quantified by isocratic HPLC using a C-18 Nucleosil 100 A column and post-column derivatisation with vanillin. Monensin A , monensin B, monensin C and monensin D are detected separately by UV-VIS at a wavelength of 520 nm.
An external calibration curve is used. The external calibration curve is prepared of standard monensin sodium (Biovet) in methanol/water (9:1 v/v).
The potency of monensin, in mg/g premix, is calculated by the formula: A + 0.28 B + 1.5 C/D
(A = quantity of monensin A; B = quantity of monensin B; C = quantity of monensin C/D).

Validation parameters have been tested. The following has been reported (the headings refer to paragraphs in ref. II.75 of the dossier):

Specificity:
The specificity of the HPLC applied has been determined by comparing the test results obtained with a blank containing all ingredients (active substance missing) and with the analyses of Coxidin® 10% premix. No interference peaks have been observed.

System suitability:
The system suitability was tested by injection of six replicates of a reference solution. The resolution between monensin A and monensin B peaks was 1.58. The RSD found for area of monensin A was 0.52% and monensin B 0.84%.

Linearity:
In the range 500 µg/ml to 1500 µg/ml, the response showed to be linear.
The correlation coefficient of 0.99988 indicates an acceptable linearity.

Precision:
The repeatability, expressed as coefficient of variation, of the HPLC method monensin content found by 10 independent determinations was 2.08% (mean concentration value being 101.3 µg/mg).
The intermediate precision, expressed as coefficient of variation, of the HPLC method monensin content found by five independent determinations by 2 different analysts in different days and conditions was 1.59% (mean concentration = 100.7 µg/mg) for the first analyst, and 1.81% (mean concentration = 100.7 µg/mg) for the second analyst.

Accuracy:
The accuracy of the HPLC method has been evaluated by preparing spiked samples of the active substance applied to a blank of the excipient to which known amounts of the active substance have been added above and below the specified level. Three independent determinations on each level (9 – 11 – 13%) obtained have been carried out through the complete analytical procedure from sample preparation to final test results. Found recovery ranged from 99.6% to 103.6%, with the mean value of 101.3%.

6.3.1.3 2% Premix (ref. II.76)
The ionophore monensin is extracted using methanol/water (90+10) with mechanical shaking for 1 h. The extract is then filtered. The ionophore is assayed by reversed-phase HPLC using post-column derivatisation with vanillin, and detection at 520 nm. The monensin content (monensin A plus monensin B) is determined by comparing the area of the sample peak with the average area of the peaks given by standards. Standards used: monensin sodium (Biovet: potency of monensin sodium µg/mg: 880. Content of monensin A 92.8%, content of monensin A+B 99.3%; monensin sodium usp: content 958 µg/mg of monensin sodium on as is basis: monensin A 94.4%).

Validation parameters have been tested. The following has been reported (the headings refer to paragraphs in ref. II.76 of the dossier):

Selectivity and specificity:
Lasalocid and salinomycin did not interfere with the determination of monensin. A blank premix showed a minor peak (peak is 0.2% of the peak given by monensin A at the target concentration) at the retention time corresponding to monensin A. The method is able to detect some degradation products of monensin.

Linearity, precision and accuracy:
Five concentrations were tested corresponding to 50, 75, 100, 125 and 150% of the target concentration (2% monensin in the premix).
The linearity was evaluated for both pure monensin in solution and monensin in premix after extraction and sample preparation. The relationship between peak area and monensin concentration was established with a sufficient level of statistical significance to allow determination of monensin content in the studied vitamins/minerals premixture in a range corresponding to 50 – 100% of the target concentration.
Precision was determined by repeating 7 assays daily at the target concentration (2% monensin in premix).
Repeatability and reproducibility (method precision) were assessed statistically by analysis of variance to detect any significant within-day and between-day variations. The mean values, standard deviations and confidence intervals obtained for each series are shown.
Results show that there was no within-day and between-day variation. The method may be considered as repeatable and reproducible, with an overall relative standard deviation of 1.8%.
Each day, a plot was constructed with pure monensin in solvent and Coxidin® in the vitamins/minerals premixture. This procedure was therefore employed to determine method accuracy.
Results (n = 21) show that the method was accurate as the 100% value was included in the 99% confidence interval. Average recovery was 98.84 ± 1.96% (confidence interval 97.62 – 100.1).

Limit of detection/quantification:
The limit of detection was determined by dilutions of a sample containing the equivalent of 2% monensin. The limit of detection was estimated on 0.01% monensin in the vitamin/minerals premixture.
The limit of quantification, i.e. that considered as the lowest concentration assayed with accuracy and precision was 0.5x target concentration, i.e. the equivalent of 1% monensin in the vitamins/minerals premix.

Conclusions on premixtures: The developed procedures are able to determine monensin in Coxidin® 20% premix, Coxidin 10% premix and 2% vitamins/minerals. The procedures are described in a well recognised format.
The monensin A and monensin B ratio is about the same in the premixtures as in standard. Therefore, in our opinion, the proposed calculation by means of external calibration with a reference standard is suitable.
Regarding validation summary tables, chromatograms and final results of the experiments have been provided. The methods have been validated for the analysis of monensin at the 2%, 10% and 20% level in the premix. Despite the omission of some validation parameters (applicability, robustness and practicability) the use of

the methods for determination of monensin in premixtures is agreed upon, because the determination is accurate and precise at the concentration level of 2%, 10% and 20% and because the methods have an acceptable recovery range (95-105%).

6.3.2 Description of the quantitative analytical method for routine control of the active substance in feed (ref. II.77).
Monensin is extracted from feed sample using methanol with sonication for 10 minutes. The extract is then filtered. Monensin is assayed by reversed-phase HPLC using post-column derivatisation with vanillin, and detection at 520 nm.
The monensin content is determined by comparing the sum area (monensin A plus monensin B) of the sample peaks with the area of the peaks given by standards.

The validation of the HPLC method is carried out in accordance with the CPMP Note for guidance: Validation of analytical procedures (CPMP/ICH/281/95, Final). The validation was carried out using samples containing monensin sodium at a concentration of 100 µg/g feed.
The following has been reported (the headings refer to paragraphs in ref. II.77 in the dossier):

Specificity:
The HPLC method is specific with regard to feed excipients. Salinomycin does not interfere with peaks for monensin A and B.

Linearity:
A linear relationship between the response and the concentration of monensin in the reference solution was observed. (mention figure to back up statement?)The HPLC-method for the assay of monensin is linear in the range from 50% to 150% of the theoretical amount of monensin in 100 µg/g feed.

Precision:
The following parameters were determined: the average, the standard deviation of six results (Std) and the variation coefficient of six results (RSD).
A concentration of monensin was obtained of 0.100102 mg/g with a RSD of 3.53% (confidence interval with α=0.05: 0.098467 – 0.101737 mg/g).

Accuracy:
10 g blank feed was weighed and monensin stock solution was added. The solution was prepared six times and injected at least three times each. The theoretical and measured concentrations of monensin in the spiked blank feed are then used to calculate the recovery.
Results: With an average recovery of 97.95% (range between 95.70 and 100.00%) and a RSD of 1.38%, it has been demonstrated that the HPLC-method is accurate for the assay of monensin.

Limit of detection:
The estimation of the limit of detection of monensin was based on the signal-to-noise approach (S/N = 3/1). The limit of detection for monensin A and B respectively is 0.103358 µg and 1.103358 µg of monensin per ml.

Conclusions: The procedure is described in a well recognised format. Although the intended use of the feed for which the method has been validated for analysis of monensin at the 100 mg/kg level is not specified, the feed composition that is given in ref. II.77 is typical for poultry feed. At the level of 100 mg/kg acceptable results were found for accuracy and precision. To be properly validated the study has to be carried out for chicken and turkey feeds in the proposed levels (100 to 125 mg/kg in feeds for chicken and 60 to 100 mg/kg in feeds for turkeys). Taking into account that the method proposed by the applicant is a well established method, and that it has been validated at 100 mg/kg poultry feed, it is considered that the method is suitable to quantify monensin sodium in feedingstuffs for chickens and turkeys at the appropriate levels.

6.3.3 Descriptions of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products (cf. pt. 2.5.3 of Commission Directive 2001/79/EC).

CHICKEN / TURKEYS (ref. II.78, II.79, II.81 and II.82)
Water was added to the tissue sample (ratio 2:1 w/w) and the mixture was homogenised with an Ultra-turrax. An aliquot of homogenate was extracted with acetonitrile containing 0.1% of formic acid. The clear supernatant (containing the test item and the internal standard salinomycin) obtained after centrifugation was analysed by reverse phase chromatography with MS/MS detection (LC-MS/MS, positive mode) after ionisation using an Electro-Spray Interface (ESI).
The ratio monensin sodium (monensin A) peak area/internal standard peak area was determined for each sample and the concentration of monensin sodium in the extract injected was calculated using the equation obtained from the calibration data.

The validation was carried out for each tissue (liver, kidney, muscle and skin+adhering fat). The following has been reported for chickens (ref. II.78 and II.81 of the dossier) and turkeys (ref. II.79 and II.82 of the dossier)

Specificity:
The specificity of the method was checked (for each tissue) by analysis of three independent sources of drug-free chicken and turkey tissue; chicken and turkey tissue spiked with monensin sodium and the internal standard (salinomycin); standard solutions of monensin sodium and internal standard.
There was no significant interference at the retention times of monensin sodium and internal standard.

Linearity:
A calibration curve was obtained by linear regression analysis of ratio monensin sodium peak area/internal standard peak area versus concentration of test item in extract of spiked tissue samples of chickens and turkeys (levels corresponding to 6, 12, 15, 30, 45, 60 and 90 ng/g in the tissue).

The calibration curve was linear over this concentration range. The assumption of linearity was confirmed by the coefficients of determination (r2) which were higher than 0.995.

Within-series precision and accuracy:
Within-series precision and accuracy were checked by replicate analysis of six tissue samples spiked at four concentration levels: 6, 15, 30 and 90 ng/g of monensin sodium in the tissue sample.
The within-series precision (expressed as coefficient of variation) obtained for chicken tissues (muscle, kidney, liver and skin+fat) was ≤ 15%. The accuracy (expressed as % recovery) ranged between 85% and 118%.
The within-series precision obtained for turkey tissues (muscle, kidney, liver and skin+fat) was ≤ 11%. The accuracy ranged between 86% and 116%.

Extraction efficiency of monensin sodium and salinomycin in chicken tissue:
The extraction efficiency was determined (in each tissue) from QC samples prepared for the within series precision and accuracy at 3 different concentrations i.e. 15, 30 and 90 ng/g of monensin sodium. Chickens: The mean value obtained for precision ranged between 3%-8% and for accuracy between 75%-97%.
The extraction efficiency was determined for the internal standard salinomycin (in each tissue), using the same preparation/calculation procedure, and the mean value obtained ranged for accuracy between 83%-95% and precision between 2%-15%.
Turkeys: The mean value obtained for precision ranged between 1%-6% and for accuracy between 77%-98%.
The extraction efficiency was determined for the internal standard salinomycin (in each tissue), using the same preparation/calculation procedure, and the mean value obtained ranged for accuracy between 83%-92% and precision between 2%-5%.

Limit of quantification:
The limit of quantification was established at 6 ng/g for monensin sodium in chicken and turkey tissue (muscle, kidney, liver and skin + adhering fat). At this level the precision and accuracy were in the intended range of ± 20%.

Conclusions: The developed procedure is able to determine residues of monensin in tissues (muscle, liver, kidney, skin+fat) of chickens and turkeys at concentrations between 6 and 90 ng monensin sodium / g tissue, with a limit of quantification of 6 ng/g. The procedure is described in a well recognised format.
The method can be considered as suitable for use as an analytical method for the determination (control) of monensin in tissues for chickens and turkeys after use of Coxidin®.
Regarding validation acceptable results were found for accuracy, precision and linearity according to the criteria as described in Commission Decision 2002/657/EC. However, since the presence of salinomycin in unknown tissue samples can not be excluded the method is not suitable for official control purposes.

CHECK LIST – Part I

Active substance in premixtures

			Y	N	N/A	Comments
1.	A.	Description of the Qualitative and Quantitative analytical method/s for routine control of the active substance in				
		- Premixtures	X			II.74, II.75, II.76
		- Feedingstuffs				
	B.	The method has been validated:	X			
		- In a ring test involving at least four laboratories		X		
		- In-house following guidelines	X			
	C.	The validation study contains the following parameters:				
		- Applicability		X		
		- Selectivity	X			
		- 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			

Active substance in feed

			Y	N	N/A	Comments
1.	A.	Description of the Qualitative and Quantitative analytical method/s for routine control of the active substance in				
		- Premixtures				
		- Feedingstuffs	X			II.77
	B.	The method has been validated:				
		- In a ring test involving at least four laboratories		X		
		- In-house following guidelines	X			
	C.	The validation study contains the following parameters:				
		- Applicability		X		
		- Selectivity	X			
		- 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			

Residues

			Y	N	N/A	Comments
2.	Description of the Qualitative and Quantitative analytical method/s to determine the marker residue (s) of the active substance:		X			II.78, II.79, II.81, II.82
	- In target tissue/s		X			
	- In animal products			X		

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:				II.74, II.75, II.76
	- 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			
1.2	Is/Are the method(s) mentioned in Part I (1.-A. Feedingstuffs) accompanied by information on:				II.77
	- 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 in Part I (2.- Target tissues) accompanied by information on:				II.78, II.79
	- 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 in Part I (2.- Animal products) accompanied by information on:			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	

N/A: Not applicable

7. CONCLUSIONS AND RECOMMENDATIONS

Concerning the determination of the active substance (monensin) in the additive, premixtures and feedingstuffs, different HPLC methods have been proposed. The principle of these methods is identical: Monensin is determined by means of an isocratic HPLC method with post-column derivatisation and UV-VIS detection at 520 nm.

For quantification of monensin in premixtures and feeds two different principles have been applied, *viz*. (i) potency, calculated with a formula and (ii) direct comparison of peak areas of monensin A and B, calculated relative to a reference standard. While the ratio of monensin A to monensin B is about the same in the Coxidin® and in the reference standard, it is found that both ways of calculations are acceptable.

The developed procedures are accurate and precise at the concentration levels in the additive Coxidin® (25 % monensin sodium), premixtures (2%, 10% and 20% monensin sodium) and feedingstuffs (100 mg monensin sodium/kg). The proposed methods to detect monensin fulfil the requirements of quantitative determination and can be considered as fit for purpose for routine analysis.
An HPLC method has been developed to determine monensin in tissues from chickens and turkeys. The procedure has been validated to detect residues in tissues (muscle, liver, kidney, skin+fat) at concentrations between 6 and 90 ng/g, with a limit of quantification of 6 ng/g. The method is considered sensitive enough. However, it is difficult to establish this with certainty since at present no MRL is set for monensin at European level.
The method uses an additive (salinomycin) as internal standard. According to the validation results the method can be considered as suitable for use as a routine analytical method for the determination of monensin in tissues of turkeys after use of Coxidin®.

However, since the presence of salinomycin in unknown tissue samples cannot be excluded, the method is not suitable for official control purposes.

8. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

The dossier has been made available to RIKILT – Institute of Food Safety by the Community Reference Laboratory (CRL).
Samples were provided to the CRL by the Applicant 14 December 2004.

9. REFERENCES

Commission Directive 2001/79/EC amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition.

Commission Directive 2002/657/EC concerning the performance of analytical methods and the interpretation of results.

ISO/DIS 14183.2: Animal Feeding stuffs – Determination of monensin, narasin and salinomycin contents – Liquid chromatographic method using post-column derivatisation. (2004-09-22).

10. RAPPORTEUR LABORATORY

The rapporteur Laboratory for this evaluation was RIKILT – Institute of Food Safety, Wageningen, The Netherlands. Responsible persons for the evaluation are Jacob de Jong and Wim Beek , as well as Christoph von Holst, Anne-Mette Jensen and Giuseppe Simone .

11. APPENDIX

Not applicable.