European Commission

JRC SCIENTIFIC AND POLICY REPORTS

Determination of authorised coccidiostats in poultry compound feed

EURL Feed Additives Control
Proficiency test exercise 2012

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2012

Report EUR 25633 EN

Joint Research Centre

European Commission
Joint Research Centre
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JRC77456

EUR 25633 EN

ISBN 978-92-79-27781-8 (pdf)

ISSN 1831-9424 (online)

doi:10.2787/69470

Luxembourg: Publications Office of the European Union, 2012

Printed in Belgium

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1. Summary

The Institute for Reference Materials and Measurements (IRMM) of the Joint Research Centre, a Directorate General of the European Commission, operates the European Union Reference Laboratory for Feed additives (EURL-FA). This EURL is having two mandates, the authorisation of feed additives (according to Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 [1]) and the control activities (according to the Regulation (EC) No 882/2004 of the European Parliament and of The Council of 29 April 2004 [2]). In the frame of the latter, The EURL-FA Control has been mandated by the Directorate General for Health and Consumers (DG SANCO) to organise a proficiency test (PT) among appointed National Reference Laboratories (NRLs) in order to assess their capacity to correctly determine 11 authorised coccidiostats in feed matrices at maximum carry-over levels as established by European legislation. Other national laboratories from EU Member States and Switzerland expressed their interest in participating in this PT; this PT was opened to all these participants. This report presents the results of the PT organised by the EURL-FA Control focusing on the determination of authorised coccidiostats in poultry compound feed at cross-contamination level[1].

The test materials used in this exercise were produced at the EURL-FA Control by spiking milled blank poultry feed with the 5 selected coccidiostats standard solutions (monensin, narasin, salinomycin, lasalocid and diclazuril). The first material consisted of a blank poultry feed spiked with narasin and diclazuril (MAT 1). The second material was spiked with monensin, salinomycin and lasalocid (MAT 2) and the third material was left as a blank (MAT 3). The resulting spiked feed materials (MAT 1 and MAT 2) were homogenised and distributed in aluminium bags sealed under vacuum and stored at -20 °C. The blank materials MAT 3 were also distributed using the same type of bags sealed under vacuum and stored at -20 °C. All bags were labelled ensuring a random number encoding and dispatched to the participants during the second half of May 2012. Each participant received one bag of each material. Each bag contained approximately 20 g of test material. Thirty laboratories from 22 countries registered to the exercise. Thirty laboratories reported quantitative results for monensin, narasin and salinomycin, 29 for lasalocid and 24 for diclazuril. Depending on the scope of the method used, the laboratories also reported qualitative results as regards to the presence or absence of the other authorised coccidiostats in the test materials.

[1] according to Commission Regulation (EU) No 574/2011 of 16 June 2011 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, Ambrosia spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto [3]

The assigned values (x_a) for the concentration of monensin, narasin, lasalocid, diclazuril and salinomycin in the samples were calculated from the formulation, according to the IUPAC harmonized protocol [4]. The uncertainties for the assigned values (u_a) were calculated according to the ISO Guide for the Expression of Uncertainty in Measurement (GUM) [5]. Participants were invited to report the uncertainties of their measurements. This was done by most participants.

Laboratory results were rated using z- and ζ-scores (zeta-scores) in accordance with ISO 13528 [6]. The standard deviation for proficiency assessment (σ_p) for each assigned value, applying the modified Horwitz equation [7]]-[[8] for monensin, lasalocid, salinomycin, narasin and diclazuril. Z-scores were considered satisfactory if their absolute values were below 2.

Between 62% and 73 % of the laboratories reported satisfactory results for monensin, lasalocid, diclazuril and salinomycin. For narasin, only 57 % of the laboratories submitted satisfactory results. The results are summarised in the following Table 1. The laboratories also reported qualitative results as regards the presence of one or more of the other authorised coccidiostats. On the whole, the rate of false positive results was 3% for monensin and narasin, 4% for robenidine, 7% for lasalocid and 0% for all the others.

Table 1: Summary results of the proficiency test exercise

Analyte	x_a mg kg^{-1}	Number of satisfactory z-scores	Total number of z-scores	Relative number of satisfactory results (%)
Monensin	1.094	20	30	67
Lasalocid	1.191	18	28	62
Salinomycin	0.663	22	29	73
Narasin	0.678	17	29	57
Diclazuril	0.010	15	23	63

2. Introduction

Maximum carry-over levels for coccidiostats in feedingstuffs are established by Commission Regulation (EU) No 574/2011 [3], and National Reference Laboratories and Official Control laboratories are in charge of implementing the surveillance monitoring control plan in the Member States as regards these substances in animal feed.
The Directorate General for Health and Consumers (DG SANCO) requested the EURL-FA Control to organise a PT for the network of National Reference Laboratories (NRLs) to assess their performance on the determination of authorised coccidiostats at cross-contamination level in compound poultry feed samples. Other national laboratories from EU Member States and Switzerland expressed their interest in participating in this PT, and the PT was opened to all these participants.
The current exercise is the first one organised in this field in Europe.

This report summarises the outcome of the exercise.

3. Scope

As stated in Regulation No 882/2004 of the European Parliament and the Council [2], one of the core duties of the EURL-FA Control is to organise interlaboratory comparisons (ILCs) for the benefit of National Reference Laboratories. The scope of this PT is to assess the proficiency of the participating laboratories to correctly determine coccidiostats in potentially contaminated feed samples and report results in the specified units within a defined time frame.

Statistical assessment of the proficiency of laboratories is evaluated by calculating an individual dimensionless Z-score calculated according to ISO 13528 [6].

4. Time Frame

The proficiency test was agreed upon by the EURL-FA Control and the Directorate General for Health and Consumers (DG SANCO). Invitation letters were sent to the participants on 8th March 2012 (Annex 1). The samples were dispatched to the participants on 14th May 2012. The reporting deadline was 22nd June 2012.

5. Test material

5.1. Preparation

Blank poultry feed (commercial laying hens feed) available at the EURL-FA Control was used. The main ingredients of the feed were corn (40%), wheat (22%), sunflower byproduct (11%), soya (10%) and limestone (7%). The feed also contained 40% starch, 16% proteins, 4% fat and was of a moisture of 11%. The material was first tested at the EURL-FA Control laboratories using High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS) to ensure that no contamination by any coccidiostat was present. The feed was ground and sieved to obtain particle sizes ranging from 250 µm to 500 µm. The sample set was comprised of three different materials, where material 1 (MAT 1) contained narasin and diclazuril, material 2 (MAT 2) contained monensin, salinomycin and lasalocid and material 3 (MAT 3) was the same feed but without any coccidiostats (blank feed).

Sufficient blank feed material to cover the total required amount for the PT was taken for MAT 1 and MAT 2 respectively and spiked with appropriate standard solutions containing individually narasin and diclazuril for MAT 1, and monensin, salinomycin and lasalocid for MAT 2. The nominal concentrations of the selected coccidiostats were set close to the cross-contamination levels as defined in the legislation [3]. Homogeneous impregnation was ensured by thorough agitation of the feed. The solvents in excess were subsequently evaporated until the feed obtained had a "fluid" aspect with no aggregates. The bulk materials of MAT 1 and MAT 2 were subsequently homogenised using a tubular mixer and divided into 20 g sub-samples using a commercial sample divider. All sub-samples were filled into aluminium bags, sealed under vacuum, labelled and stored at -20 °C until further dispatch and/or analysis.

5.2. Homogeneity and stability

5.2.1. Homogeneity

To assess the homogeneity of the materials produced, 10 bags of each material were randomly selected. Two aliquots from each bag were extracted and further analysed in duplicate by HPLC-MS/MS. The mean values of the sub-sample duplicates were subjected to analysis of variance (ANOVA – F-test). The results obtained for each of the materials showed that the homogeneity of the materials was sufficient (Annex 2) to proceed with the PT exercise.

5.2.2. Stability

Experience at EURL-FA Control has shown that the materials prepared by spiking with standard solutions and kept at -20°C were stable for several months. It was therefore decided to proceed with the dispatch of the samples as such. At the end of the exercise, two bags of each material were analysed in triplicate. Each replicate was injected three times. The values obtained for the concentration of each measurand were compared with those obtained during the homogeneity study. The results show that the materials were stable during the time frame of this PT exercise (Annex 3).

5.3. Distribution

All samples were dispatched to participants by IRMM on 14th May 2012. The participants were warned by email on upcoming dispatch on 10th May 2012 (Annex 4). Each participant received:

a) One bag containing approximately 20 g of test material MAT 1,

b) One bag containing approximately 20 g of test material MAT 2,

c) One bag containing approximately 20 g of test material MAT 3,

d) An accompanying letter with instructions for sample handling and reporting (including the individual lab code) (Annex 5) and

e) A "Confirmation of receipt" form to be sent back to IRMM after receipt of the test materials (Annex 5).

f) The reporting sheet to be used to report the results was sent by email on 1st June 2012 to each participant (Annex 6).

6. Instructions to participants

Concrete instructions were given to all participants in a letter accompanying the test materials and in the reporting sheet (Annex 5).

Laboratories were asked to perform the analysis of the three different materials received, following their own in-house method in the same conditions as if performing official control. Presence or absence of the 11 authorised coccidiostats had to be reported as "detected", "not detected" or "no result" (if the feed was not analysed for this measurand). When a measurand was detected, participants were asked to report the content as the concentration of the detected coccidiostat in mg kg^{-1} of feed given with 3 decimals, together with the associated measurement uncertainty. In addition, details on the

analytical method (sample preparation, instrumental technique, limits of detection and of quantification) used to perform the measurements were also to be reported.

All results and additional information were to be reported in a special form developed at the EURL-FA Control. Each laboratory was assigned a unique code which was individually communicated in each instruction letter accompanying the test materials (Annex 5).

7. Reference values and their uncertainties

The assigned value for the total content in monensin, narasin, salinomycin and lasalocid was set as the nominal value calculated from the formulation, following the IUPAC protocol [4] and corrected for purity according to Equation (1).

$$x_a = \frac{m_{meas}}{M} \times \frac{V_2 \times V_4}{V_1 \times V_3} \times p \times 1000 \qquad \text{Eq. (1)}$$

where

x_a is the assigned concentration of the measurand monensin, salinomycin, narasin or lasalocid, in mg kg^{-1} of feed

m_{meas} is the weighed mass of the corresponding measurand pure reference standard, in mg

V_1 is the volume of solvent added for the stock standard solution, in ml

V_2 is the volume of stock standard solution of the measurand used to produce the spiking solution, in ml

V_3 is the volume of solvent added to V_2 to produce the spiking solution, in ml

V_4 is the volume of the spiking solution used for spiking the blank feed with the corresponding measurand, in ml

M is the mass of blank feed to be spiked, in g

p is the purity of the measurand standard substance as declared on the certificate of analysis provided by the supplier.

For diclazuril, an intermediate standard solution was to be used before producing the spiking solution due to the low value targeted as final concentration in the feed. The nominal value is therefore given by Equation (2).

$$x'_a = x_a \times \frac{V_a}{V_b} \qquad \text{Eq. (2)}$$

where

x'_a is the assigned concentration of the measurand diclazuril, in mg kg^{-1} of feed

V_a is the volume of stock standard solution of diclazuril, in ml

V_b is the volume of solvent added to V_a to produce the intermediate standard solution of diclazuril, in ml.

The associated standard uncertainty to the assigned values is given by Equation (3) according to the ISO Guide for the Expression of Uncertainty in Measurement (GUM) [5].

$$RSu_a = \sqrt{\sum RSu_i^2} \qquad \text{Eq. (3)}$$

where

RSu_a is the relative standard uncertainty associated to the assigned concentration, and

RSu_i is the relative standard uncertainty associated to each component of Equation (1) or Equation (2).

Note 1: The type B uncertainties on the volume, the mass and the purity are assumed to follow a rectangular distribution. Following the Eurachem guide, the calculations of the standard uncertainty u_i and the relative standard uncertainty $RSDu_i$ are done according to Eq. (4) and Eq. (5) respectively.

$$u_i = \frac{reported\_half\_range}{\sqrt{3}} \qquad \text{Eq. (4)}$$

and the relative standard uncertainty is

$$RSDu_i = \frac{u_i}{i} \qquad \text{Eq. (5)}$$

Table 2 displays the assigned values (x_a) with the associated uncertainties (u_a) and the standard deviations for PT assessment (σ_p). Overall mean concentrations obtained during the homogeneity study for each analyte are included for comparison.

Table 2: Assigned ranges in mg kg^{-1} of feed, expressed as mean value ± expanded uncertainty (k=2) and target standard deviations in %. The ranges ($C_{homogeneity}$) obtained during the homogeneity study are given for information.

Material	Measurand	$x_a \pm U_a$ (k=2) mg kg^{-1}	Target standard deviation σ_p (%)	$C_{homogeneity} \pm u_{homogeneity}$ (k=2) (mg kg^{-1})
		Assigned		**Observed (n=20)**
MAT 1	Narasin	**0.678 ± 0.006**	**16.8**	**0.782 ± 0.037**
	Diclazuril	**0.010 ± 0.0001**	**22**	**0.008 ± 0.0009**
MAT 2	Lasalocid	**1.191 ± 0.013**	**15.5**	**1.026 ± 0.156**
	Monensin	**1.094 ± 0.012**	**15.7**	**1.059 ± 0.053**
	Salinomycin	**0.663 ± 0.006**	**16.9**	**0.593 ± 0.039**
		Formulation		**Homogeneity**

8. Evaluation of results

8.1. General observations

Thirty laboratories from 22 countries registered to the exercise. Thirty laboratories reported quantitative results for monensin, narasin and salinomycin, 29 for lasalocid and 24 for diclazuril. All laboratories responded to the questionnaire included in the reporting form although some did not answer comprehensively. Three laboratories reported their results after the deadline of 22 June 2012; the last results were received on 28 June 2012 after agreement of the EURL-FA Control. All results were included in the final evaluation.

Figure 1 gives an overview of the geographical distribution of participating laboratories.

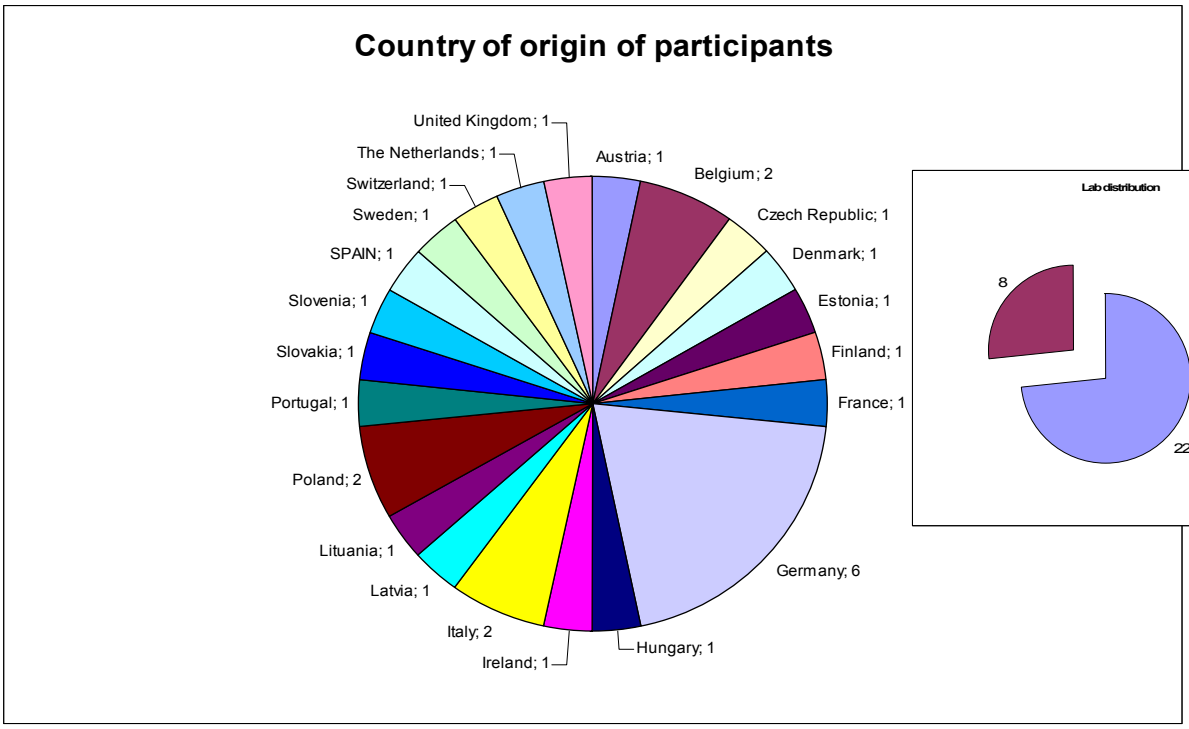


Figure 1: Country of origin of the participants – NRLs and non NRLs laboratories.

8.2. Scores and evaluation criteria

Individual laboratory performance is expressed in terms of z- and ζ-scores in accordance with ISO 13528 [6].

$$z = \frac{x_{lab} - x_a}{\sigma_p} \qquad \text{Eq. (6)}$$

$$\zeta = \frac{x_{lab} - x_a}{\sqrt{u_{lab}^2 + u_a^2}} \qquad \text{Eq. (7)}$$

where:

x_{lab} is the measurement result reported by a participant,

x_a is the target value (assigned value),

u_{lab} is the standard uncertainty reported by a participant,

u_a is the standard uncertainty of the reference value,

σ_p is the target standard deviation for proficiency assessment.

The assigned reference values (x_a), and their respective uncertainties are summarised in Table 2.

The interpretation of the z- and ζ-score is done as follows:

|score| ≤ 2 satisfactory result

2 < |score| ≤ 3 questionable result

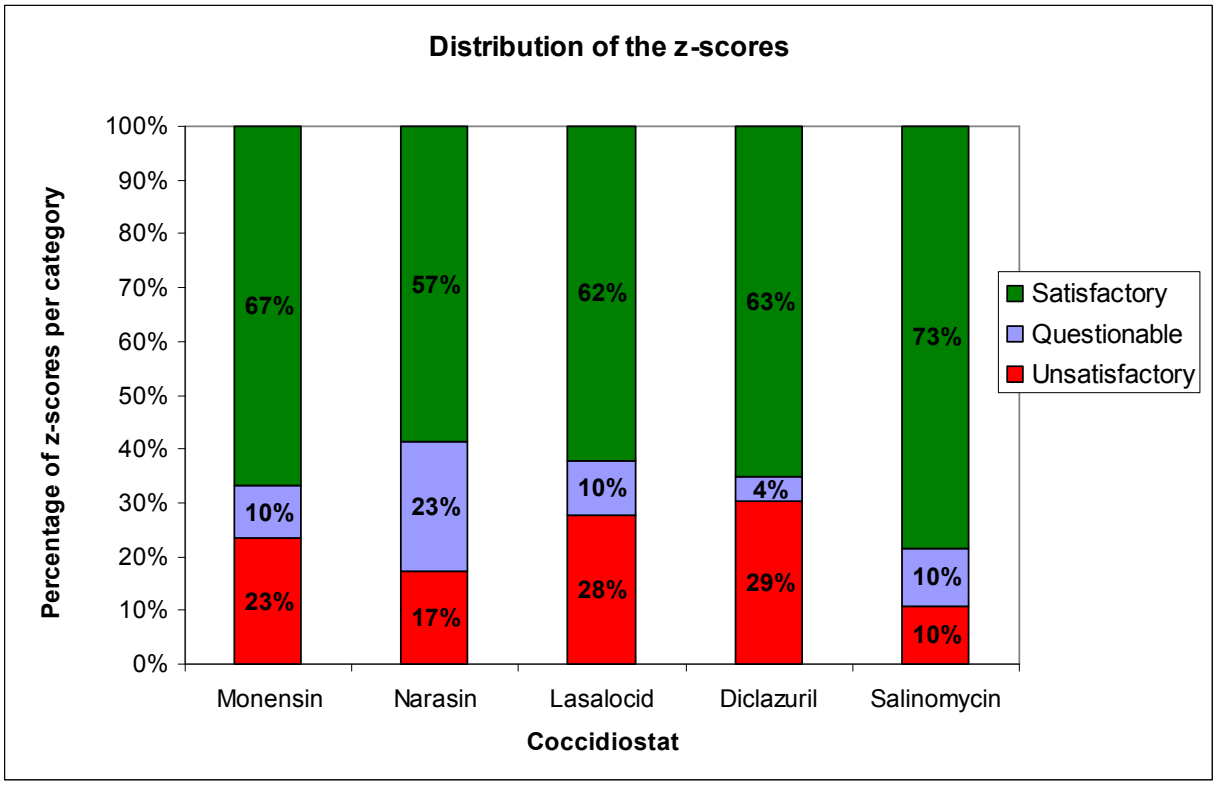
|score| > 3 unsatisfactory result

Table 3 and Figure 2 display the distribution of the z- and ζ-score values.

Table 3: Distribution of the z- and ζ-scores values

		z			ζ		
Coccidiostat	N	S	Q	U	S	Q	U
Monensin	30	67%	10%	23%	70%	13%	13%
Lasalocid	29	62%	10%	28%	55%	10%	34%
Narasin	30	57%	23%	17%	70%	13%	13%
Diclazuril	24	63%	4%	29%	46%	17%	33%
Salinomycin	30	73%	10%	10%	80%	-	17%

N: number of reporting laboratories; S: satisfactory score; Q: questionable score; U: unsatisfactory score.



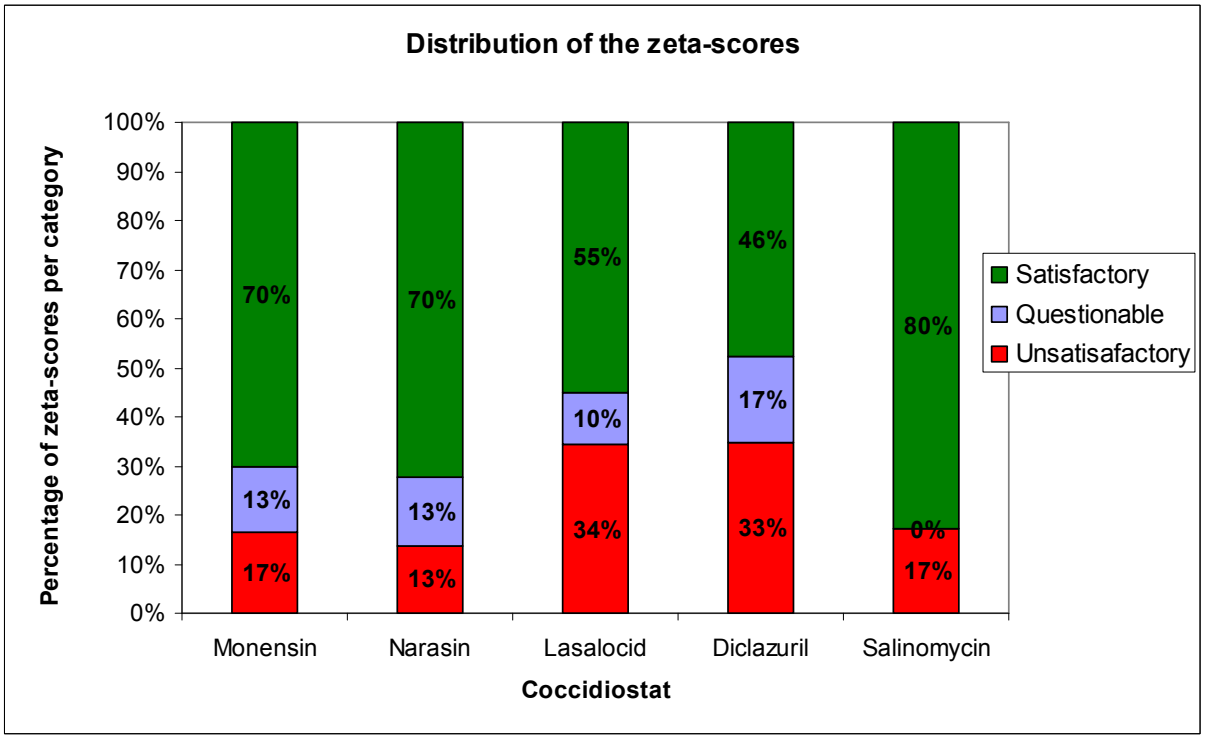


Figure 2: Complete distribution of the z- and ζ-scores values

The z-score compares the participant's deviation from the assigned value with the target standard deviation for proficiency assessment (σ_p) used as common quality criterion. σ_p is

defined by the PT organiser as the maximum acceptable standard uncertainty. For monensin, lasalocid, salinomycin and narasin, the target mass ratios are between 1.2 10^{-7} and 0.138; values for σ_p were therefore calculated applying the Horwitz equation (Eq. 8).

$$RSD_{R_{\text{Horwitz}}} = \sigma_p = 2 \times C^{-0.15} \qquad \text{Eq. 8}$$

where

$RSD_{R\ \text{Horwitz}}$ is the relative standard deviation predicted by Equation 8,

σ_p is the target standard deviation set for the PT exercise, and

C is the concentration ratio (1=100 g/100 g); 0.001=1000 mg kg^{-1}).

For diclazuril, the target concentration ratio is lower than 1.2 10^{-7}; σ_p was therefore set at 22% according to the modified Horwitz equation [8]. All σ_p values are summarised in Table 2. All participants were requested to report their estimation of the related uncertainies together with the values determined for the concentration of the analytes. Calculation of ζ-score was therefore also part of the evaluation. The ζ-score states if the laboratory result agrees with the assigned value within the respective uncertainty. The denominator of Eq. 7 is the combined uncertainty of the assigned value and the measurement uncertainty as stated by the laboratory. The ζ-score is therefore a relevant evaluation parameter, as it includes all parts of a measurement result, namely the expected value (assigned value), its uncertainty as well as the uncertainty of the reported values. An unsatisfactory ζ-score can either be caused by an inappropriate estimation of the concentration or of its uncertainty.

The standard uncertainty of the laboratory (u_{lab}) was estimated by dividing the reported expanded uncertainty by the reported coverage factor, k. When no uncertainty was reported, u_{lab} was set at 0 and when k was not specified and the laboratory specified its uncertainty as the standard deviation of the measurements, u_{lab} was set at the value reported by the laboratory.

Uncertainty estimation is not trivial; therefore an additional assessment was performed to indicate how reasonable the uncertainty estimate is. The standard uncertainty from the

laboratory (u_{lab}) is most likely to fall in a range between a minimum uncertainty (u_{min}), and a maximum allowed (u_{max}). u_{min} is set to the standard uncertainty of the assigned value. It is unlikely that a laboratory carrying out the analysis on a routine basis would measure the measurand with a smaller uncertainty compared to u_{min}, which is in our case free of any error contributions from a LC/MS experiment, than the expert laboratory establishing the assigned value. u_{max} is set to the target standard deviation (σ_p) accepted for the PT. If u_{lab} is smaller than u_{min}, the laboratory may have underestimated its uncertainty. If $u_{lab} > u_{max}$, the laboratory may have overestimated the uncertainty. An evaluation of this statement can be made when looking at the difference of the reported value and the assigned value: if the difference is small and the uncertainty is large, then overestimation is likely. If, however, the deviation is large but is covered by the uncertainty, then the uncertainty is properly assessed even if large [9].

8.3. Laboratory results and scorings

The results as reported by the participants for the total content of monensin, narasin, salinomycin, lasalocid and diclazuril in the analysed test samples together with the related uncertainties are displayed in Annex 7.

First, it should be noted that not all laboratories analysed all 5 measurands in the three materials and they therefore reported "no result" for one or more given measurand. More than 57% of the laboratories reported results with a IZI lower than 2 for the 5 measurands present in the samples (Table 3). However, some laboratories systematically reported x_{lab} values out of the assigned range for 3, 4 or 5 measurands (Table 4). The technical reasons for such biased results need to be further investigated.

Table 4: Laboratories having reported questionable or unsatisfactory results

Monensin	Narasin	Lasalocid	Diclazuril	Salinomycin
			L01	
L02	L02			
	L03			
L05	L05	L05	L05	L05
	L07	L07	L07	L07
L09	L09	L09		
			L10	
	L13	L13*		
				L14
			L16	
L17				
		L19		
		L20		
	L21*	L21		L21
	L22		L22	
L23	L23	L23		L23
L25			L25	
L26	L26		L26	L26
L27	L27	L27		L27
		L29		
L30		L30		
L31	L31			

*: IZI ≅ 2

The robust mean calculated from the results reported are statistically comparable to the corresponding assigned values of the target coccidiostats present in the materials (Table 5). Therefore, it can be concluded that the PT exercise is globally satisfactory.

Table 5: Statistical comparison of the robust mean with the assigned value for each measurand

		x_a mg kg^{-1}	σ_p %	Robust mean mg kg^{-1}	Standard deviation S_R mg kg^{-1}	RSD %
MAT 1	Narasin	0.678	16.8	0.78	0.25	32.0
	Diclazuril	0.010	22	0.010	0.004	34.7
MAT 2	Lasalocid	1.191	15.5	1.14	0.41	36.0
	Monensin	1.094	15.7	1.15	0.35	30.5
	Salinomycin	0.663	16.9	0.66	0.18	27.4

S_R: intermediate precision

8.3.1. z- and ζ-scores

The results for monensin, narasin, lasalocid, salinomycin and diclazuril for each laboratory are summarised in Annex 8. Figures 3-12 show a graphical presentation of the z- and ζ–scores calculated for each participant and for each of the measurand.

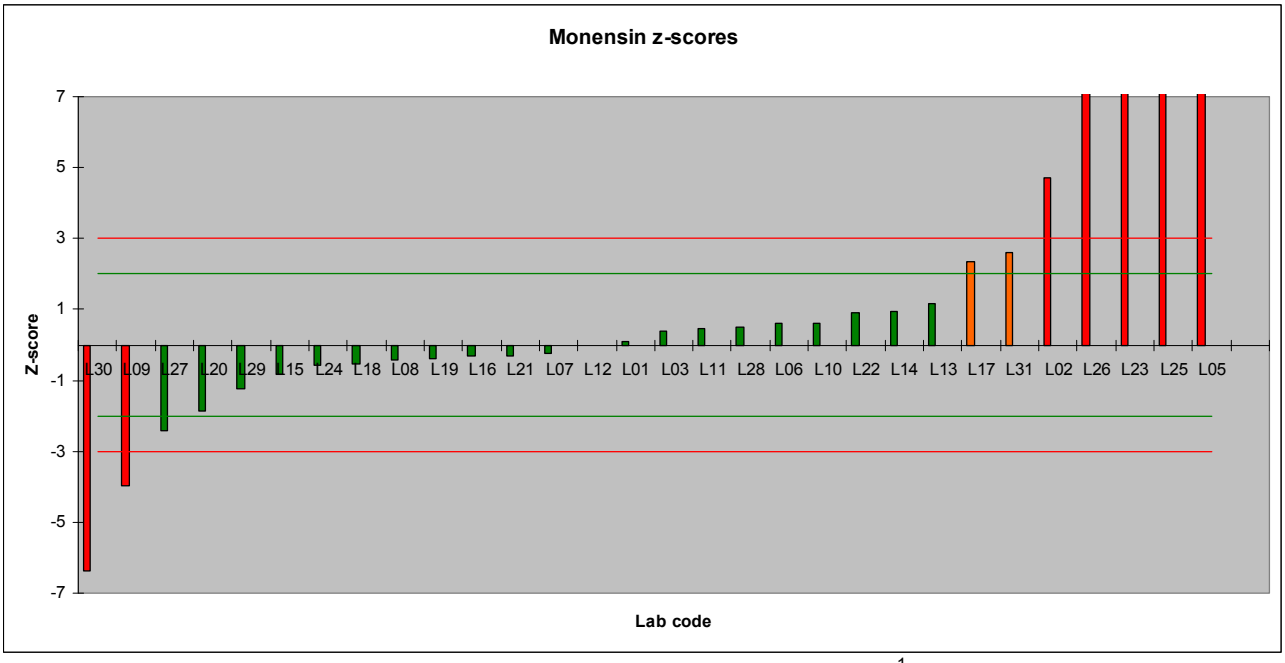


Figure 3: Z-scores for the determination of monensin (x_a: 1.094 mg kg^{-1}) for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

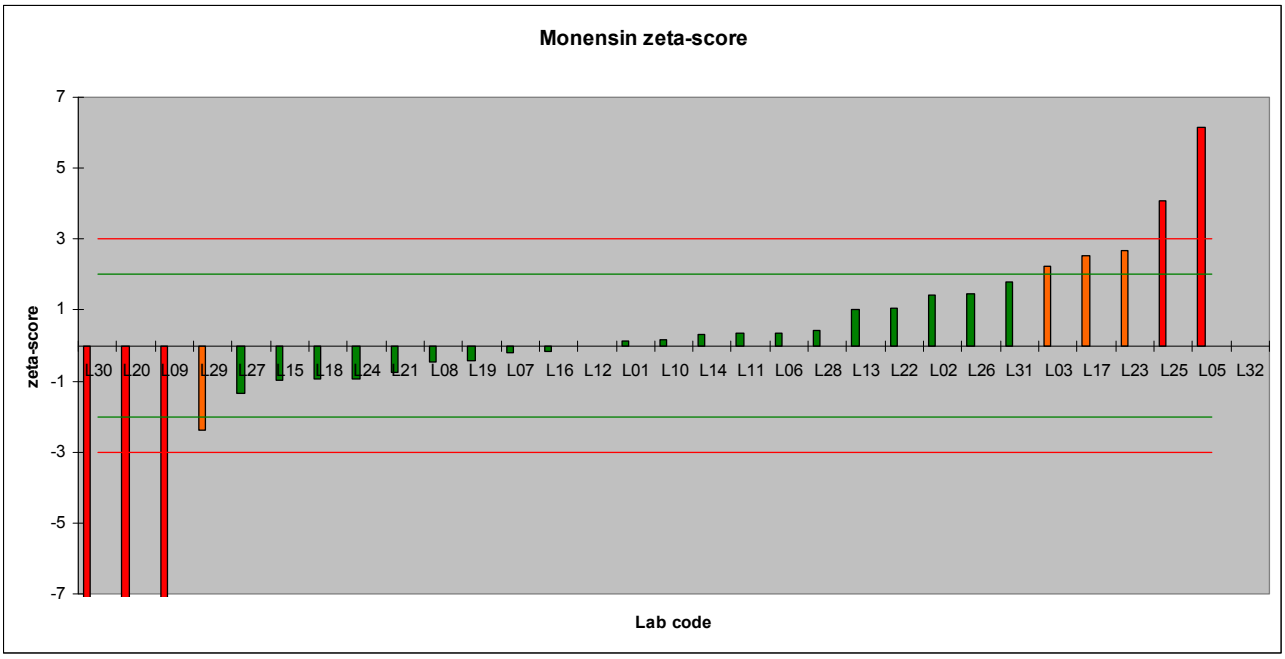


Figure 4: ζ-scores for the determination of monensin for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

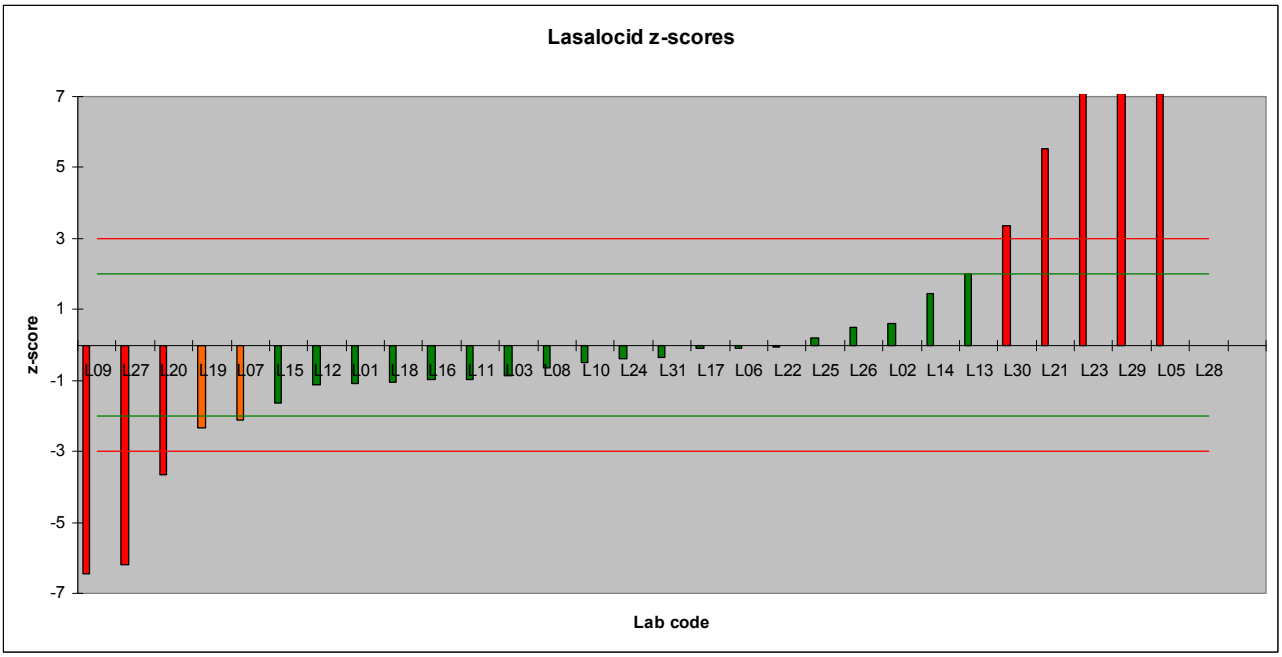


Figure 5: Z-scores for the determination of lasalocid (x_a: 1.191 mg kg^{-1}) for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

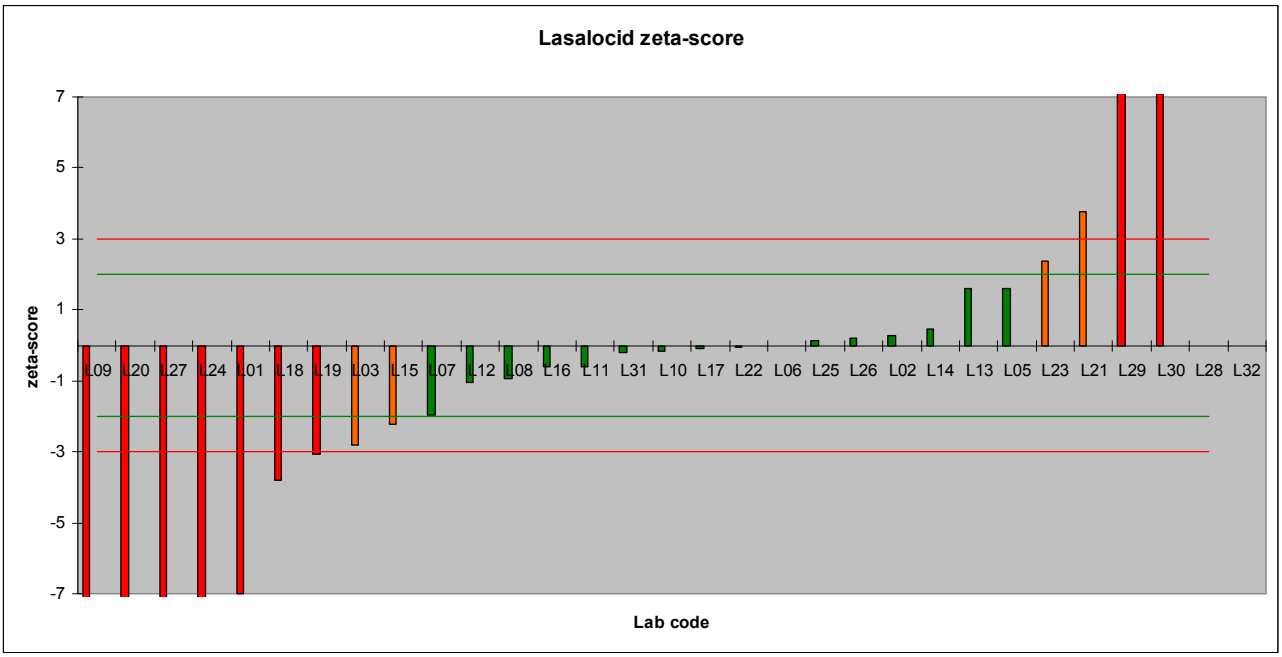


Figure 6: ζ-scores for the determination of lasalocid for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

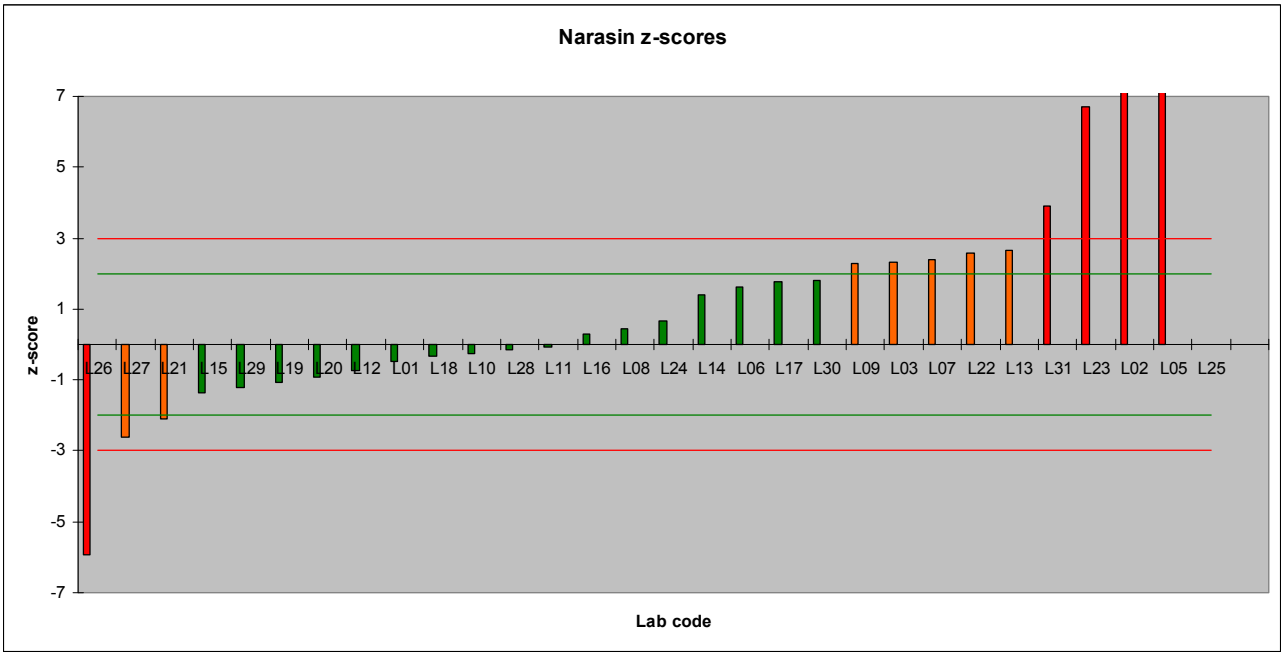


Figure 7: Z-scores for the determination of narasin (x_a: 0.678 mg kg^{-1}) for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

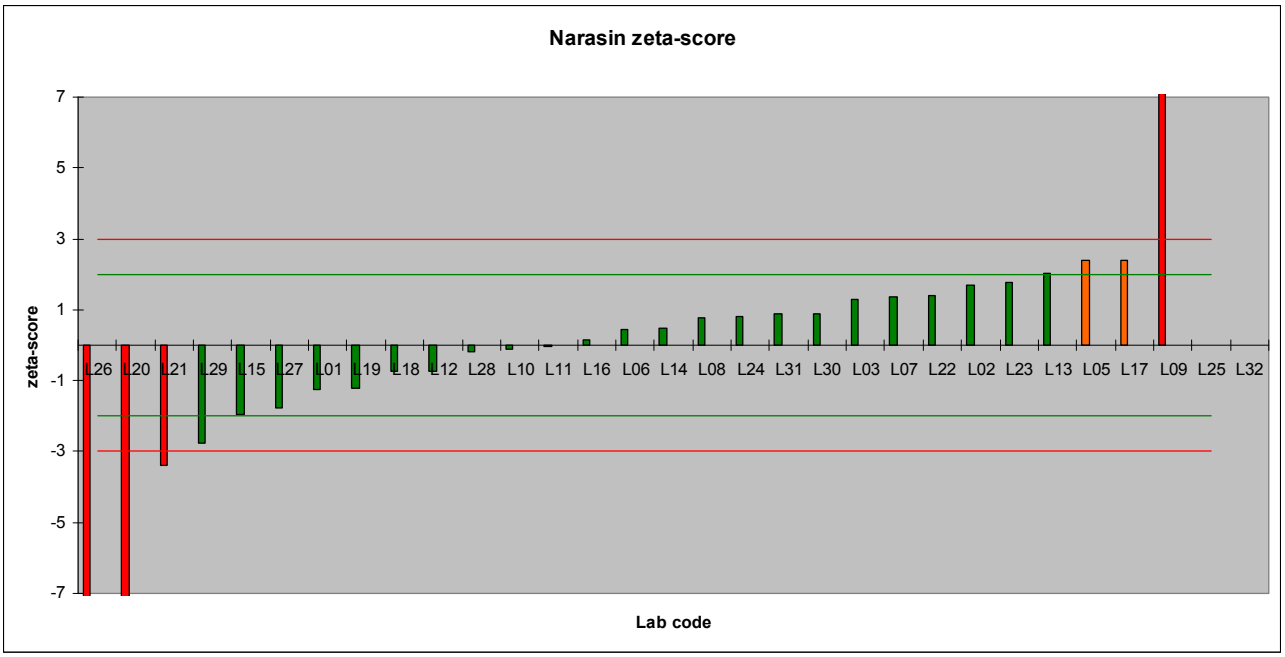


Figure 8: ζ-scores for the determination of narasin for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

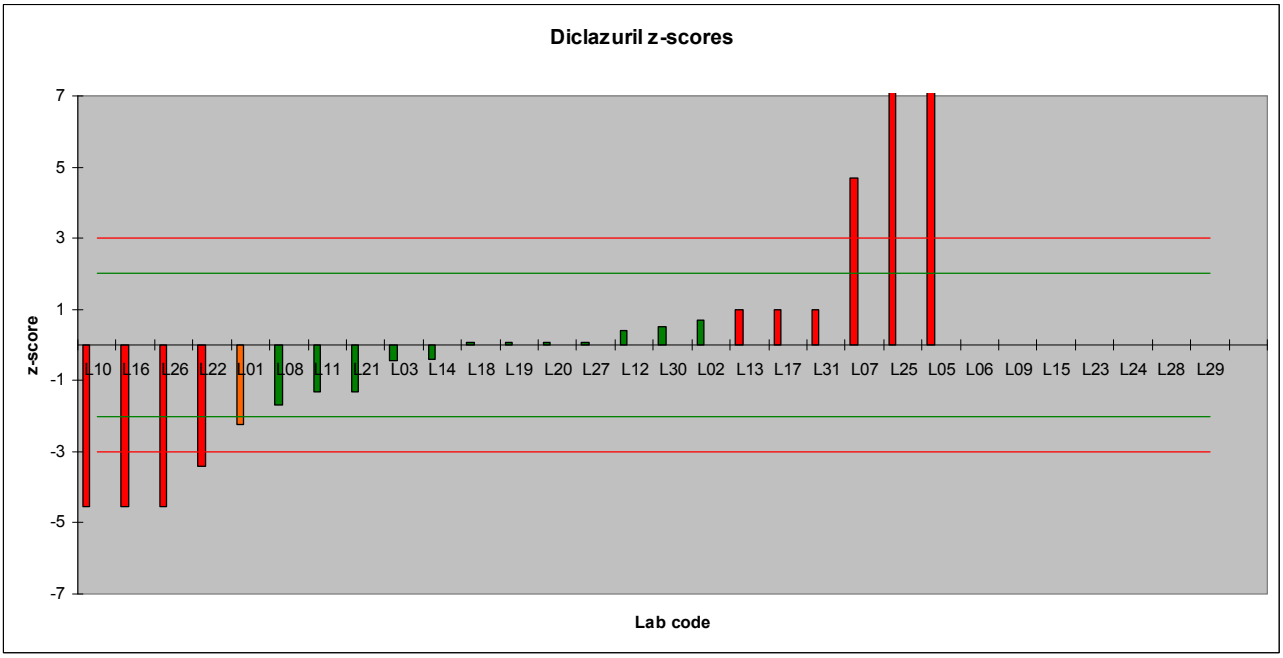


Figure 9: Z-scores for the determination of diclazuril (x_a: 0.010 mg kg^{-1}) for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

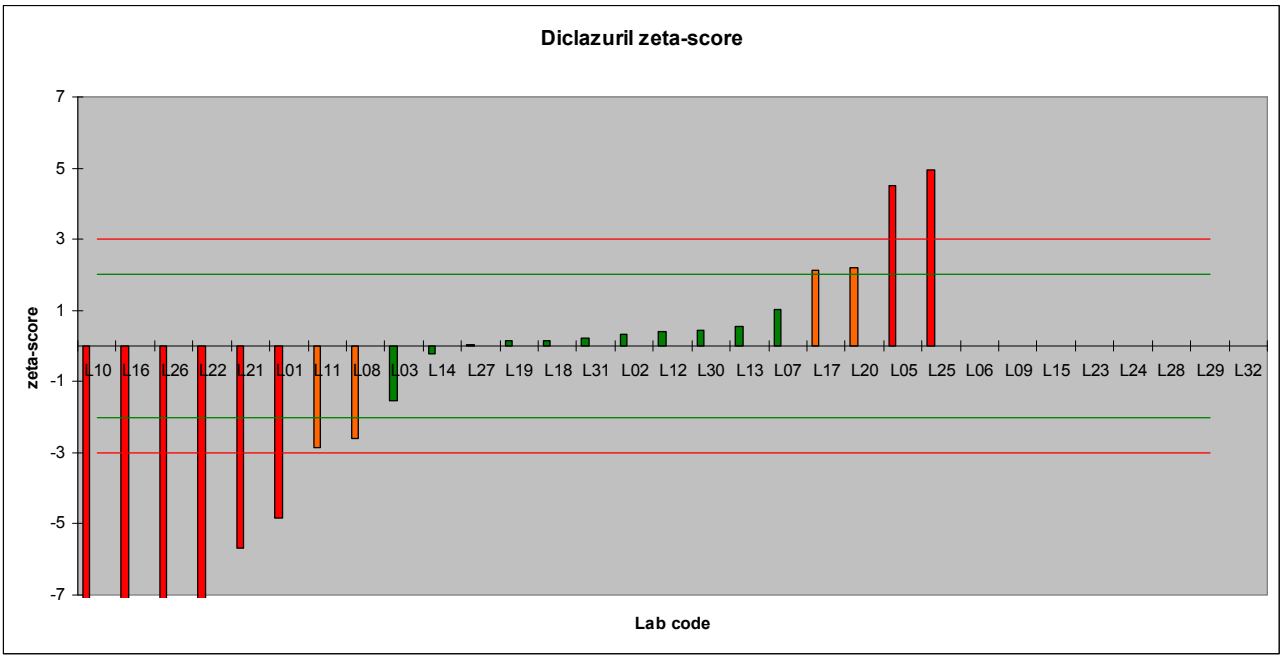


Figure 10: ζ-scores for the determination of diclazuril for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

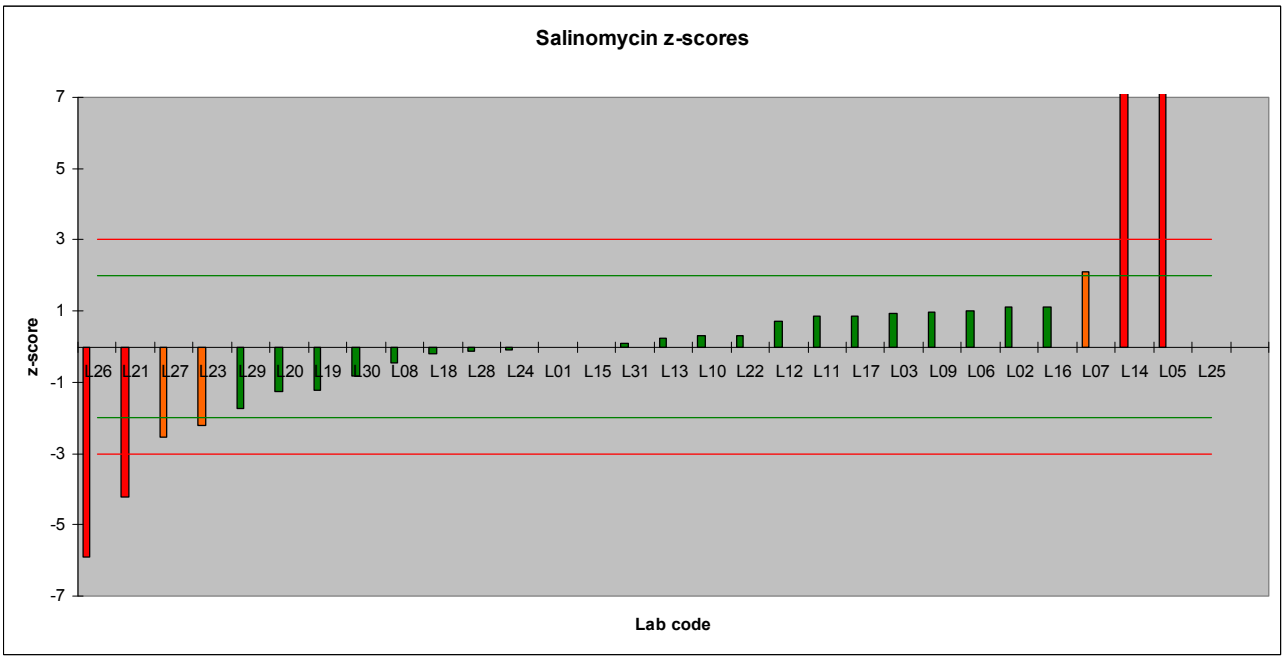


Figure 11: Z-scores for the determination of salinomycin (x_a: 0.663 mg kg^{-1}) for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

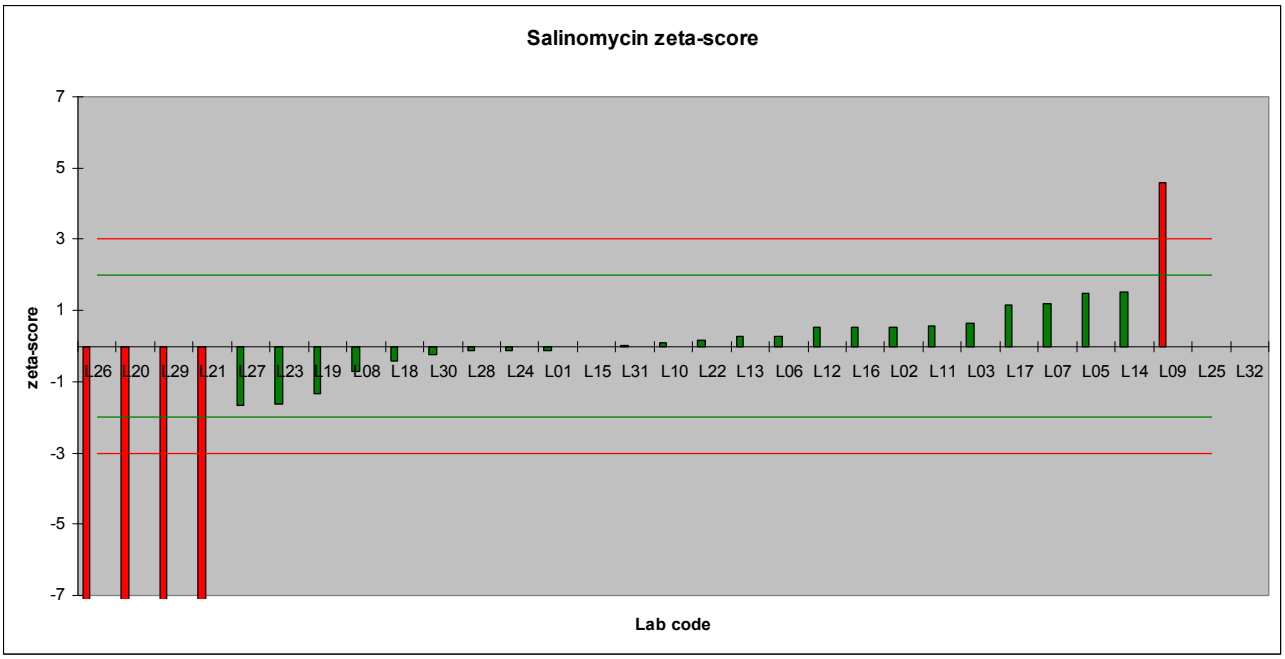


Figure 12: ζ-scores for the determination of salinomycin for the participating laboratories. The green line shows the limit for satisfactory and the red line for questionable performance.

8.3.2. False positives and false negatives

Another important aspect for official control is the percentage of false positive and false negative rates of the method used. For a screening method, it is of key importance that the rate of false negative is as low as possible whereas for a confirmatory method, the rate of false positives should be kept to a minimum. Ideally, both rates should be as low as possible but the necessary compromises are to be done depending on the scope of the method.

In this PT exercise, if the laboratory did not analyse for a particular analyte, it should not report any value and not fill in the respective area. If the sample was analysed for the selected analyte and the analyte was detected but lower than the LOQ of the method then the laboratory should report "not detected". If an analyte present in the sample is reported as "not detected" (concentration of 0) while the method used has a fit for purpose LOQ then the result is to be classified as (true) false negative. Alternatively, if the method used

is e.g. not sensitive enough for the scope of the analysis, the non detection of the analyte is obviously due to a lack of sensitivity and consequentely Z and ζ-scores are not calculated.

In the questionnaire to the participants, information on the limits of detection (LOD) and quantification (LOQ) was requested. Based on this information (Annex 7), the false negative results were therefore also discriminated during the evaluation.

For false positives, a result is considered as "false positive" if the laboratory detects and reports a numerical value (above the LOQ) for an analyte which is not present in the given sample.

Table 6 displays the percentages of false negatives and false positives in the study.

Table 6: False positive and false negative rates for each target coccidiostat.

	N	False positive rate %	False negative rate %
Monensin	30	3	3
Narasin	30	3	3
Lasalocid	29	7	3
Diclazuril	24	0	13
Salinomycin	30	0	3
Nicarbazin	26	0	0
Maduramicin	25	0	0
Decoquinate	21	0	0
Halofuginone	19	0	0
Robenidine	25	4	0
Semduramicin	19	0	0

N: number of reporting laboratories

8.4. Additional information extracted from the questionnaire

Additional information was gathered from the questionnaire filled in by the participants in Annex 9.

Table 7 gives an overview for each analyte of the information gathered regarding e.g. the sample treatment, the type of instrumentation used and the quantification method. Table 8 gives an overview of the information gathered, sorted by laboratory.

Details on the technique used are given, as reported by the participant in the Annex 9.

Table 9 displays the conditions used by the most critical laboratories as assessed in Table 4 for each analyte. On the whole no obvious analytical characteristics appear to explain the Z-scores above or below 2 obtained. However, the discussion on analytical techniques will certainly be an interesting discussion point in the final workshop.

Table 7: Overview of the additional information gathered for each analyte

							Sample Preparation				Elution Mode		Detection					Calibration		
	N	Nq	Acc. Meth.	Multi-analyte	Single analyte	Nq	LSE (clean-up)	Ultrasonic (clean-up)	Other (clean-up)	Nq	HPLC gradient	HPLC isocratic	Nq	MS	Fluo	UV-PCD	Nq	Ext (IS)	MM (IS)	St. Add (IS)
MON	30	30	17	29	0	30	26 (9)	2 (2)	2 (1)	28	23	5	30	26	0	4	28	6 (1)	14 (9)	8 (3)
NAR	30	28	17	28	0	28	25 (9)	2 (2)	1 (0)	27	23	4	27	24	0	3	26	5 (1)	15 (9)	6 (3)
LAS	29	28	14	27	1	27	23 (9)	3 (2)	2 (1)	27	23	4	27	24	3	0	26	5 (1)	14 (7)	7 (3)
DICL	24	24	13	23	1	24	21 (9)	2 (2)	1 (0)	23	22	1	24	23	0	1	21	2 (0)	14 (11)	6 (3)
SAL	28	28	17	28	0	28	25 (8)	2 (2)	1 (0)	26	22	4	28	25	0	3	26	5 (0)	15 (11)	6(3)

MON: monensin, NAR: narasin, LAS: lasalocid, DICL: diclazuril, SAL: salinomycin; N: number of reporting laboratories, Nq: number of laboratories answering the related question; Acc. Meth.: the method used is accredited; Multi-analyte: the method used is a multi-analyte method; Single analyte: the method used is a single-analyte method; LSE: liquid-solid extraction; Ultrasonic: the extraction is performed using an ultra-sonic bath; Other: the extraction is declared to be performed using something else than liquid-solid extraction or an ultra-sonic bath but is not always specified; clean-up: the extraction is followed by a clean-up; HPLC gradient: the chromatographic separation is performed using a gradient elution mode; HPLC isocratic: the chromatographic separation is performed using an isocratic elution mode; Triple quad: the detector is a mass spectrometer triple quadrupole; Qtrap: the detector is a mass spectrometer Qtrap (quadrupole/ion trap) technology; UV-PCD: the detector is a spectrophotometer UV coupled with post-column derivatisation; Ext: the calibration is performed against a standard external calibration curve; MM: the calibration is performed against a matrix-matched calibration curve; St. Add.: the calibration is performed using standard addition; IS: Internal Standard.

Table 8: Overview of the additional information gathered, sorted by laboratory

	Multi/Single	Sample preparation			HPLC	Detection					Quantification			
		Extraction	Dilution	Clean-up	Elution mode	UV-PCD	Fluorescence	Triple quadrupole	TOF	Qtrap	External calibration	Matrix-match calibration	Standard addition	Internal standard
L01														
MON, NAR, LAS, DICL, SAL	Multi	LSE	N	n-hexane	Gradient	-	-	Y	-	-	nr	nr	nr	N
L02														
MON, NAR, LAS, DICL, SAL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	-	Y	N
L03														
MON, NAR, LAS, SAL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	Y	-	Nigericin
DICL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	Y	-	Diclazuril-IS
L05														
MON, NAR	Multi	LSE	N	C1	Gradient	-	-	Y	-	-	-	Y	-	Nigericin
LAS	Multi	LSE	N	C1	Gradient	-	-	Y	-	-	-	Y	-	N
DICL	Multi	LSE	N	C1	Gradient	-	-	Y	-	-	-	Y	-	Nicarbazin-d8
SAL	Multi	LSE	N	C1	Gradient	-	-	Y	-	-	-	Y	-	? Nigericin
L06														
MON, NAR, LAS, DICL, SAL	Multi	LSE	Y	Centrifuge	Gradient	-	-	Y	-	-	Y	-	-	N
L07														
MON, NAR, LAS, DICL, SAL	Multi	LSE	Y	Quechers principle	Gradient	-	-	Y	-	-	-	Y	-	N
L08														
MON, NAR, LAS, SAL	Multi	LSE	N	SPE (Oasis HLB)	Gradient	-	-	Y	-	-	-	Y	-	Nigericin-Na
DICL	Multi	LSE	N	SPE (Oasis HLB)	Gradient	-	-	Y	-	-	-	Y	-	Methyl-diclazuril
L09														
MON, NAR, LAS, SAL	Multi	LSE	N	-	Isocratic	-	-	Y	-	-	-	Y	-	N
DICL	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L10														
MON, NAR, LAS, SAL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	Y	-	Nigericin
DICL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	Y	-	bis-diclazuril
L11														
MON, NAR, LAS, SAL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	Y	-	Nigericin-Na
DICL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	Y	-	bis-diclazuril
L12														
MON	nr	LSE	nr	-	Gradient	-	-	nr	-	-	-	-	Y	N
NAR, LAS, DICL, SAL	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13														
MON, NAR, LAS, DICL, SAL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	-	Y	N
L14														
MON, NAR, LAS, DICL, SAL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	-	Y	N
L15														
MON, NAR, LAS, DICL, SAL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	-	Y	Nigericin
L16														
MON, NAR, SAL	Multi	LSE	N	-	Isocratic	520	-	-	-	-	Y	-	-	N
LAS	Multi	LSE	N	-	Isocratic	-	310	-	-	-	Y	-	-	N
DICL	Multi	LSE	N	-	Isocratic	-	-	-	Y	-	Y	-	-	N
L17														
MON, NAR, LAS, SAL	Multi	LSE	Y	C2	Gradient	-	-	Y	-	-	-	Y	-	N
DICL	Multi	LSE	Y	C2	Gradient	-	-	Y	-	-	-	Y	-	bis-Diclazuril
L18														
MON, NAR, LAS, SAL	Multi	LSE	Y	SPE or dilution depending on expected concentration	Gradient	-	-	Y	-	-	-	Y	-	Nigericin
DICL	Multi	LSE	Y	SPE or dilution depending on expected concentration	Gradient	-	-	Y	-	-	-	Y	-	Diclazuril-bis
L19														
MON, NAR, LAS, SAL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	Y	-	Nigericin
DICL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	-	Y	-	DNC-d8
L20														
MON, NAR, LAS, SAL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	Y	-	Nigericin
DICL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	-	Y	-	Nicarbazin-d8
L21														
MON, NAR, LAS, SAL	Multi	Ultrasonic bath	N	Filtration	Gradient	-	-	Y	-	-	-	Y	-	IPZOH-D3
DICL	Multi	Ultrasonic bath	N	Filtration	Gradient	-	-	Y	-	-	-	Y	-	Methyldiclazuril
L22														
MON, NAR, LAS, DICL, SAL	Multi	LSE	N	C18 dispersive	nr	-	-	Y	-	-	-	Y	-	N
L23														
MON, NAR, LAS, SAL	Multi	LSE	N	-	Gradient	-	-	Y	-	-	Y	-	-	Nigericin
DICL	nr	nr	nr	nr	nr	nr	nr	Y	nr	nr	nr	nr	nr	nr
L24														
MON, NAR, SAL	Multi	LSE	N	-	Isocratic	520	-	-	-	-	Y	-	-	N
LAS	Multi	Other	N	-	Isocratic	-	214	-	-	-	Y	-	-	N
DICL	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L25														
MON, NAR, LAS, DICL, SAL	Multi	Other	Y	-	Gradient	-	-	Y	-	-	-	-	Y	MON, NAR, LAS, DICL, SAL
L26														
MON, NAR, LAS, DICL, SAL	Multi	Ultrasonic bath	N	Freezing	Gradient	-	-	Y	-	-	-	-	Y	Nigericin
L27														
MON, NAR, LAS, DICL, SAL	Multi	LSE	Y	-	Gradient	-	-	Y	-	-	nr	nr	nr	nr
L28														
MON	Multi	Other	N	SPE Silica	Isocratic	520	-	-	-	-	Y	-	-	N
NAR, LAS, DICL, SAL	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29														
MON, NAR, SAL	Multi	LSE	N	-	Isocratic	520	-	-	-	-	Y	-	-	N
LAS	Single	Ultrasonic bath	N	-	Isocratic	-	380	-	-	-	Y	-	-	N
DICL	Single	LSE	N	SPE	Gradient	280	-	-	-	-	Y	-	-	N
L30														
MON, NAR, LAS, SAL	Multi	LSE	Y	-	Gradient	-	-	-	-	Y	-	-	Y	N
DICL	Multi	LSE	Y	-	Gradient	-	-	-	-	Y	-	Y	-	Methyldiclazuril
L31														
MON, NAR, LAS, DICL, SAL	Multi	LSE	Y	SPE Silica	nr	-	-	Y	-	-	-	Y	-	nr

Table 9: Conditions used by the most critical laboratory, sorted by analyte
a - Monensin

	Sample preparation							HPLC - reverse phase							
	Acc. Meth.	**Multi/Single**	**Test portion (g)**	**Extraction**	**Dilution**	**Solvent**	**Clean-up**	**Guard column**	**Column**	**Elution mode**	**Mobile phase**	**flow rate (ml min^{-1})**	**inj. Vol. (µl)**	**Col. Temp. (°C)**	**Autosampler Temp. (°C)**
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8

	Detection													Quantification				Limits of detection & quantification	
	UV-PCD	**Fluorescence**		**Mass spectrometry**										**External calibration**	**Matrix-match calibration**	**Standard addition**	**Internal standard**	**LOD**	**LOQ**
	λ (nm)	**λexc. (nm)**	**λem. (nm)**	**Triple quadrupole**					**TOF**		**Qtrap**								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L05	-	-	-	50	50/38	693.40	461.50	675.50	-	-	-	-	-	-	Y	-	Nigericin	< ML (not determined)	< ML (not determined)
L09	-	-	-	55	33	693.6	675.1	461	-	-	-	-	-	-	Y	-	N	0.01	0.025
L23	-	-	-	250	39	693.4	675.4	461.3	-	-	-	-	-	Y	-	-	Nigericin	0.0074	0.0123
L25	-	-	-	4500/600	20	688.5	617.5	635.5	-	-	-	-	-	-	-	Y	Monensin	0.38	1.25
L26	-	-	-	62	52	693.5	479.3	461.3	-	-	-	-	-	-	-	Y	Nigericin	0.04	0.125
L27	-	-	-	20	54	693.2	461.3	479.2	-	-	-	-	-	nr	nr	nr	nr	0.1	0.1

b - Narasin

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	**Multi/Single**	**Test portion (g)**	**Extraction**	**Dilution**	**Solvent**	**Clean-up**	**Guard column**	**Column**	**Elution mode**	**Mobile phase**	**flow rate (ml min^{-1})**	**inj. Vol. (µl)**	**Col. Temp. (°C)**	**Autosampler Temp. (°C)**
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8

	Detection													Quantification				Limits of detection & quantification	
	UV-PCD	**Fluorescence**		**Mass spectrometry**										**External calibration**	**Matrix-match calibration**	**Standard addition**	**Internal standard**	**LOD**	**LOQ**
	λ (nm)	**λexc. (nm)**	**λem. (nm)**	**Triple quadrupole**					**TOF**		**Qtrap**								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L05	-	-	-	55	53/45	787,5	431,5	531,5	-	-	-	-	-	-	Y	-	Nigericin	< ML (not determined)	< ML (not determined)
L07	-	-	-	76	52/46	787.3	431.5	531.5	-	-	-	-	-	-	Y	-	N	0.01	0.03
L09	-	-	-	60	48	787.3	430.7	530.8	-	-	-	-	-	-	Y	-	N	0.01	0.025
L21	-	-	-	120	60	787.6	531	431	-	-	-	-	-	-	Y	-	IPZOH-D3	0.100	0.200
L23	-	-	-	45	59	787.4	431.3	531.3	-	-	-	-	-	Y	-	-	Nigericin	0.0046	0.0077
L25	-	-	-	4500/600	31	782.6	373.2	747.5	-	-	-	-	-	-	-	Y	Narasin	0.21	0.7
L26	-	-	-	74	52	787.7	431.3	531.4	-	-	-	-	-	-	-	Y	Nigericin	0.02	0.07
L27	-	-	-	60	100	787.5	431.2	531.2	-	-	-	-	-	nr	nr	nr	nr	0.1	0.1

c - Lasalocid

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	**Multi/Single**	**Test portion (g)**	**Extraction**	**Dilution**	**Solvent**	**Clean-up**	**Guard column**	**Column**	**Elution mode**	**Mobile phase**	**flow rate (ml min^{-1})**	**inj. Vol. (µl)**	**Col. Temp. (°C)**	**Autosampler Temp. (°C)**
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8

	Detection													Quantification				Limits of detection & quantification (mg	
	UV-PCD	**Fluorescence**		**Mass spectrometry**										**External calibration**	**Matrix-match calibration**	**Standard addition**	**Internal standard**	**LOD**	**LOQ**
	λ (nm)	**λexc. (nm)**	**λem. (nm)**	**Triple quadrupole**					**TOF**		**Qtrap**								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L05	-	-	-	55	35	613,3	377,5	577,5	-	-	-	-	-	-	Y	-	N	< ML (not determined)	< ML (not determined)
L07	-	-	-	54	38/28	613	377.4	577.5	-	-	-	-	-	-	Y	-	N	0.01	0.03
L09	-	-	-	40	35	612.98	376.9	358.9	-	-	-	-	-	-	Y	-	N	0.04	0.08
L21	-	-	-	90	45	613.4	377.4	595.3	-	-	-	-	-	-	Y	-	IPZOH-D3	0.250	0.500
L23	-	-	-	227	39	613.3	377.3	595.4	-	-	-	-	-	Y	-	-	Nigericin	0.0130	0.0217
L27	-	-	-	55	45	613.1	377.2	577.2	-	-	-	-	-	nr	nr	nr	nr	0.05	0.05

d - Diclazuril

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	**Multi/Single**	**Test portion (g)**	**Extraction**	**Dilution**	**Solvent**	**Clean-up**	**Guard column**	**Column**	**Elution mode**	**Mobile phase**	**flow rate (ml min^{-1})**	**inj. Vol. (µl)**	**Col. Temp. (°C)**	**Autosampler Temp. (°C)**
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature

	Detection													Quantification				Limits of detection & quantification	
	UV-PCD	**Fluorescence**		**Mass spectrometry**										**External calibration**	**Matrix-match calibration**	**Standard addition**	**Internal standard**	**LOD**	**LOQ**
	λ (nm)	**λexc. (nm)**	**λem. (nm)**	**Triple quadrupole**					**TOF**		**Qtrap**								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L05	-	-	-	25	17	404,8/406,8	334	336	-	-	-	-	-	-	Y	-	Nicarbazin d8	< ML (not determined)	< ML (not determined)
L07	-	-	-	-35	22/22	404.7/406.8	334	336.1	-	-	-	-	-	-	Y	-	N	0.01	0.03
L25	-	-	-	4500/600	12,0 V	403.4	332.1	333.4	-	-	-	-	-	-	-	Y	Diclazuril	0.003	0.01
L26	-	-	-	25	25	404.9	334	/	-	-	-	-	-	-	-	Y	Nigericin	0.002	0.005

e - Salinomycin

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	**Multi/Single**	**Test portion (g)**	**Extraction**	**Dilution**	**Solvent**	**Clean-up**	**Guard column**	**Column**	**Elution mode**	**Mobile phase**	**flow rate (ml min^{-1})**	**inj. Vol. (µl)**	**Col. Temp. (°C)**	**Autosampler Temp. (°C)**
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8

	Detection													Quantification				Limits of detection & quantification (mg	
	UV-PCD	**Fluorescence**		**Mass spectrometry**										**External calibration**	**Matrix-match calibration**	**Standard addition**	**Internal standard**	**LOD**	**LOQ**
	λ (nm)	**λexc. (nm)**	**λem. (nm)**	**Triple quadrupole**					**TOF**		**Qtrap**								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L05	-	-	-	55	50/55	773,3	431,40	531,3	-	-	-	-	-	-	Y	-	? Nigericin	< ML (not determined)	< ML (not determined)
L07	-	-	-	75	52/46	773.2	431.4	531.5	-	-	-	-	-	-	Y	-	N	0.01	0.03
L21	-	-	-	167	65	773.6	431.3	265.1	-	-	-	-	-	-	Y	-	IPZOH-D3	0.075	0.150
L23	-	-	-	270	55	773.5	431.3	531.3	-	-	-	-	-	Y	-	-	Nigericin	0.0006	0.0011
L25	-	-	-	4500/600	45 V	773.5	431.3	531.2	-	-	-	-	-	-	-	Y	Salinomycin	0.21	0.7
L26	-	-	-	72	50	773.6	431.3	531.3	-	-	-	-	-	-	-	Y	Nigericin	0.02	0.07
L27	-	-	-	60	54	773.2	265	431.1	-	-	-	-	-	nr	nr	nr	nr	0.02	0.07

9. Workshop

The 1st Workshop of the EURL-FA Control network was organised at the JRC-IRMM on November 13-14 2012. This 1st Workshop was the concluding event for the organisation, upon request of DG Health and Consumers, of this PT exercise for the determination of coccidiostats at cross-contamination level in feedingstuffs. A total of thirty-nine participants attended the event, representing 20 National Reference Laboratories (NRLs), 7 National Official Control Laboratories (OCLs), DG SANCO, (JRC-IRMM) IMEP and the EURL-FA.

The results from the PT exercise were presented as well as the "raison d'être" of the EURL-FA Control. Furthermore, group discussions allowed many fruitful exchange of information between the participants and gave an opportunity to also gather the needs and expectations of the experts in the field.

My last presentation on the work programme of the EURL-FA Control for next year suggested the direction the EURL-FA Control network should follow to enhance harmonization of measurements in EU in the field of coccidiostats feed additives.

The collected evaluation forms indicate the satisfaction of the participants and the areas to improve.

This 1st event should be followed by other successful ones and was the starting point for a constructive and fruitful collaboration among the network members for the years to come.

10. Conclusions

This proficiency test exercise is the first one of this kind organised, targeting authorised coccidiostats, at cross-contamination levels, in compound feed materials. The participating laboratories were requested to screen for the presence or absence of the 11 authorised coccidiostats and, if detected, to report the content of the detected coccidiostat(s) in mg kg^{-1} of feed with the associated uncertainty value. Three test materials were sent to the laboratories for analysis. One material contained 3 coccidiostats (monensin, lasalocid and salinomycin), one contained 2 coccidiostats (narasin and diclazuril) and the third material was a blank. The

proficiency of the laboratories in correctly determining the coccidiostats was assessed by the calculation of the z- and ζ-scores.

On the whole, between 62% and 73 % of the laboratories reported satisfactory results for four spiked coccidiostats, (monensin, lasalocid, diclazuril and salinomycin). For narasin, only 57 % of the laboratories submitted satisfactory results. The laboratories also reported qualitative results as regards the presence of one or more of the other authorised coccidiostats. The rate of false positive results was 3% for monensin and narasin, 4% for robenidine, 7% for lasalocid and 0% for all the others.

Regarding the reported uncertainties, the percentage of satisfactory ζ-score is higher than the same percentage for the z-score, indicating that in general, the evaluation of the uncertainties is satisfactory. However, many laboratories did not report any uncertainty associated with their results. The measurement uncertainty is of paramount importance in cases of litigation and so it is fundamental for control laboratories to be able to report a sound uncertainty.

11. Acknowledgements

The authors want to acknowledge the helpful advices provided by F. Serano for the preparation of the test materials. The EURL-FA Control wishes to thank Dr A. Boix, Dr A. Maquet, Dr F. Cordeiro and Dr P. Robouch for the fruitful discussions held for the evaluation of the results, their help in some of the statistical data treatments and/or the reviewing of this report.

All laboratories participating in this exercise and listed below are kindly acknowledged.

Institute name	Country
Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Inst.f.Tierernährung und Futtermittel/Abt. Zusatzstoffanalytik und Prüfung	Austria
Federal Laboratory for the Safety of the Food Chain (FLVVT)	Belgium
Scientific Institute of Public Health, Wetenschappelijk Instituut Volksgezondheid, Institut Scientifique de Santé publique	Belgium
National Reference Laboratory of the Central Institute for Supervising and Testing in Agriculture, Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha	Czech Republic
Danish Veterinary and Food Administration, Feed Laboratory	Denmark
Veterinary and Food Laboratory	Estonia
Research Department, Chemistry and Toxicology, Finnish Food Safety Authority Evira	Finland
Service Commun des Laboratoires des ministères du budget, des comptes publics, de la fonction publique et de la réforme de l'état (MBCPFPRE), de l'économie, des finances et de l'industrie (MEFI), Laboratoire de RENNES; SCL-L35, section Additifs de l'alimentation animale	France
Lebensmittel- und Futtermittelanalytik - LUFA-ITL GmbH	Germany
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft - BfUL	Germany
Thüringer Landesanstalt für Landwirtschaft (TLL)	Germany
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	Germany
LAVES, Futtermittelinstitut Stade	Germany
Federal Institute for Risk Assessment (BfR), National Reference Laboratory for Feed Additives Unit Contaminants Department Safety in the Food Chain	Germany
Central Agricultural Office, Food and Feed Safety Directorate, Feed Investigation National reference Laboratory	Hungary
The State Laboratory	Ireland
Centro Sviluppo e Validazione Metodi Analitici, Istituto Zooprofilattico Sperimentale, dell'Umbria e delle Marche	Italy
S.S. Ricerca Residui, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta	Italy

Institute name	Country
Instrumental Analysis Division, Institute of Food Safety, Animal Health and Environment (BIOR)	Latvia
National Food and Veterinary Risk Assessment Institute	Lithuania
Department of Pharmacology and Toxicology, National Veterinary Research Institute	Poland
National Research Institute of Animal Production, National Laboratory for Feedingstuffs	Poland
Laboratório de Controlo da Alimentação Animal, Unidade de Higiene Pública, INRB, I.P. - LNIV - Laboratório Nacional de Investigação Veterinária	Portugal
Central Control and Testing Institute of Agriculture (CCTIA) - Feedingstuffs and animal nutrition, Lab. of feedingstuff analysis	Slovakia
University in Ljubljana, Veterinary Faculty , National Veterinary Institute - Unit for Pathology of Animal Nutrition and Environmental Hygiene	Slovenia
Laboratori Agroalimentari de Cabrils	SPAIN
Statens Veterinärmedicinska Anstalt	Sweden
Station de recherche, Agroscope Liebefeld-Posieux ALP	Switzerland
RIKILT - Institute of Food Safety	The Netherlands
Laboratory of the Government Chemist (LGC)	United Kingdom

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13. Annexes

Annex 1 : Invitation letter sent to NRLs

Ref: ARES/2012/279351

Dr Ursula Vincent
Project Leader European Reference Laboratory for Feed Additives (control)
(EURL-FA control)
Joint Research Centre
Food Safety and Quality
Institute for Reference Materials and Measurements (IRMM)

08 March 2012

Call for participation in the inter-comparison study for the
determination of authorised coccidiostats in feed at cross-contamination levels

1. Introduction

Feed additives are authorised within the European Union according to Regulation (EC) No 1831/2003 requiring various criteria to be fulfilled including the need of providing suitable methods of analysis for official control in feedingstuffs. Coccidiosis is a major disease in poultry as well as in many other hosts. Coccidiostats are the only anti-bacterial substances still authorised as feed additives European Union (Regulation (EC) No 1831/2003, Community Register of Feed Additives pursuant to Regulation (EC) No 1831/2003) and constitute the main choice to fight against coccidiosis. This disease is caused by highly host-specific protozoan parasites belonging to the genera *Eimeria* in the class Sporozoa and it is considered the most important parasitic disease in poultry. The main strategy for controlling this disease is the combination of a good implementation of the prescribed hygiene requirements together with the addition of coccidiostats or histomonostats to the feed at the authorised level. The conditions of use are given in the respective Commission Regulations authorising the feed additive, specifying individually for each additive important aspects such as the target animal, the inclusion level of the active substance in the feed and – in the case of cocciodiostats – the duration of the period before slaughter (withdrawal period) when the use of these substances is prohibited.

It is well known that during the production of feed containing coccidiostats as feed additives, unavoidable carry-over of the coccidiostats from feed which is covered by the authorisation (target feed) to feed which must not contain coccidistats (non-target feed) occurs when the same production lines are used. A too high concentration of coccidiostats in non-target feed would harm non-target animal species. Additionally, as all anti-bacterial substances, coccidiostats may be a risk for human health because the presence of their residues in foodstuffs could cause toxic effects, directly in sensitive individuals and also indirectly because their widespread usage could be responsible for the promotion of resistant strains of bacteria. Recently, the EU legislation therefore addressed both concerns and established maximum limits for the unavoidable carry-over

of coccidiostats or histomonostats (Commission Regulation (EU) No 574/2011 amending Annex I to Directive 2002/32/EC). Enforcing the legislation and performing the compulsory monitoring requires the availability of reliable analytical methods. Many methods have been developed and single-house validated in this aim, mainly based on LC-MS/MS hyphenated techniques. However, little information is currently available about the proficiency of laboratories in determining the coccidiostats at these levels using these methods. In order to update on the reliability of analytical results delivered by laboratories in charge of ensuring the official control of coccidiostats in feed, the European Commission's Directorate-General Health and Consumers decided to organise an inter-comparison study for the determination of coccidiostats at cross-contamination levels in feed.

2. The organising team

The study will be conducted by the European Union Reference Laboratory for Feed Additives (Control) (EURL-FA (control)), hosted at the European's Commission Joint Research Centre Institute for Reference Materials and Measurements (IRMM).

3. Objective

The major objective of this study is to assess the proficiency of the participating laboratories to correctly determine coccidiostats in potentially contaminated feed samples. This aim will be achieved by conducting an inter-comparison study in which the laboratories analyse feed samples applying their own analytical method and reporting the results to the organiser of the study. The evaluation of the results will show which laboratories deliver acceptable results. The proficiency test also includes evaluating the capability of the laboratories to carry out the requested analysis within a defined time frame.

4. Test material

Samples will be prepared containing typical compound feed fortified with all or selected authorised coccidiostats at cross-contamination concentration level (see Annex 1). Each laboratory will have to quantitatively analyse 3 samples and to submit the results to the coordinator of the study.

Prior to sending out the samples to the participants the organising team will have demonstrated sufficient homogeneity of the test material by analysing randomly taken sub-samples.

5. General outline of the exercise

The participants are requested to report the results of the analyses together with the information about the analytical method applied. Each laboratory will be assigned a unique code and only the organiser of the study (EURL-FA (control)) knows the key to this code. The EURL-FA (control) will send out a report on the outcome of the study containing information about the score of the laboratories; the personal laboratory keys will be individually communicated to each participant.

Appropriate statistical tool for data evaluation will be used to investigate the proficiency of the laboratories. Statistical assessment of the proficiency of laboratories will be evaluated by calculating an individual dimensionless Z-score calculated according to ISO 13528.

$$z = \frac{(x - x_a)}{\sigma_p} \qquad \text{Eq. 1}$$

Where x = the value reported by the participant

x_a = the assigned value

σ_p = the target standard deviation

Participation in the inter-comparison study is free of charge.

The proficiency test is scheduled for the period from the middle of May 2012 to the middle of July 2012.

The exercise will be completed by the organisation of a workshop opened to all participants to the study. The workshop is free of charge. Travel and accommodation costs will be reimbursed for one representative of each National reference Laboratory for Feed Additives (control) defined according to Regulation (EC) No 882/2004.

6. Expression of interest

National Reference Laboratories for Feed Additives (control) defined according to Regulation (EC) No 882/2004 shall participate in the proficiency exercise organised by the EURL-FA (control) depending on their expertise.

If not yet done, the interested expert laboratories are kindly asked to confirm their interest in taking part in the exercise and their relevant expertise by returning the attached annex 1 by email to the functional mailbox JRC-IRMM-CRL-FEED-ADDITIVES-CONTROL@ec.europa.eu by **27 March 2012** at the latest.

Dr U. Vincent and Dr C. von Holst (EURL-FA operating manager)

ANNEX 1

Please indicate in the table which coccidiostat(s) your laboratory is able to determine and return this page to JRC-IRMM-CRL-FEED-ADDITIVES-CONTROL@ec.europa.eu by **27 March 2012**

Laboratory name :

Contact person :

Table 1: Authorised coccidiostats in feed and cross-contamination levels

Feed additive	Active substance	Cross-contamination concentration level in mg of active substance per kg of compound feed	Ability to determine
Coxidin Elancoban G100, 100, G200, 100/Elancogran 100	Monensin	1.25	☐
Deccox	Decoquinate	0.4	☐
Cycostat 66G	Robenidine	0.7	☐
Avatec 150G	Lasalocid A	1.25	☐
Halofuginone	Halofuginone	0.03	☐
Monteban, Monteban G100	Narasin	0.7	☐
Sacox 120G, 120µG Salinomax 120G Kokcisan 120G	Salinomycin	0.70	☐
Cygro 1%, Cygro 10G	Maduramicin	0.05	☐
Clinacox 0,5% Premix Clinacox 0,2% Premix	Diclazuril	0.01	☐
Maxiban G160	Narasin - Nicarbazin	0.7 – 1.25	☐
Nicarbazin 250 g/kg	Nicarbazin	1.25	☐
Aviax 5%	Semduramicin	0.25	☐

Regulation (EC) No 1831/2003; European Union Register of Feed Additives. European Union legislation on feed additives
http://ec.europa.eu/food/food/animalnutrition/feedadditives/legisl_en.htm
Commission Regulation (EU) no 574/2011, Amending annex I to Directive 2002/32/EC (values given for chickens reared for laying)

Important note: It is not compulsory to be able to determine ALL coccidiostats listed to take part in the study.

Annex 2 : Homogeneity of the materials

10 bags of each material were randomly selected. Two aliquots from each bag were extracted and further analysed in duplicate by HPLC-MS/MS. Since the blank material is available in the EURL-FA (control) laboratory, quantification of the target analytes was performed against matrix-matched calibration curves. The obtained concentrations were corrected for purity. The mean concentration values of the sub-sample duplicates were subjected to analysis of variance (ANOVA single factor – F-test). If the F-value obtained from the data set was lower than the F-critical, the material was considered adequately homogeneous for the related analyte. For diclazuril the F value was slightly higher than the F critical. However, as can be seen in the tables below, the detected between-sample standard deviation is very low and considered acceptable for the purpose of the study. Furthermore considering the low level of concentration and the high repeatability of the measurements, it appears that a slight and non significant difference in the concentration would lead to a "high" standard deviation which explains the result of the F test. It was therefore considered not relevant to give any allowance for the homogeneity contribution, i.e. the estimated uncertainty on the assigned value was not expanded with the uncertainty contribution arising from the homogeneity study and the material was therefore considered suitable to undergo the collaborative trial.

Monensin

Sacket	Homogeneity study Subsample 1	Subsample 2
1	1.03	1.02
2	1.03	1.05
3	1.02	1.08
4	1.04	1.09
5	1.06	1.09
6	1.09	1.11
7	1.11	1.07
8	1.08	1.02
9	1.02	1.07
10	1.06	1.05
	average:	**1.06**

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Row 1	2	2.05	1.02	5.33E-06
Row 2	2	2.08	1.04	2.26E-04
Row 3	2	2.10	1.05	1.50E-03
Row 4	2	2.13	1.06	9.37E-04
Row 5	2	2.16	1.08	5.48E-04
Row 6	2	2.20	1.10	9.64E-05
Row 7	2	2.19	1.09	9.26E-04
Row 8	2	2.10	1.05	1.58E-03
Row 9	2	2.09	1.05	1.11E-03
Row 10	2	2.10	1.05	2.32E-05

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.05E-02	9	1.16E-03	**1.67**	0.22	**3.02**
Within Groups	6.94E-03	10	6.94E-04			
Total	1.74E-02	19				

Standard deviation of the analytical procedure (mg/kg):	0.03
RSD (%):	2.49

Lasalocid

Sacket	Homogeneity study Subsample 1	Subsample 2
1	1.14	0.93
2	0.88	0.97
3	0.92	0.93
4	0.95	0.93
5	0.95	0.96
6	1.14	1.06
7	1.09	1.04
8	1.07	1.03
9	1.31	1.07
10	1.09	1.07
	average	**1.03**

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Row 1	2	2.07	1.03	2.19E-02
Row 2	2	1.85	0.92	3.79E-03
Row 3	2	1.85	0.92	4.06E-05
Row 4	2	1.87	0.94	2.40E-04
Row 5	2	1.91	0.95	1.68E-05
Row 6	2	2.20	1.10	2.78E-03
Row 7	2	2.13	1.06	1.57E-03
Row 8	2	2.10	1.05	5.69E-04
Row 9	2	2.39	1.19	2.98E-02
Row 10	2	2.16	1.08	3.35E-04

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.43E-01	9	1.59E-02	**2.61**	0.08	**3.02**
Within Groups	6.10E-02	10	6.10E-03			
Total	2.04E-01	19				

Standard deviation of the analytical procedure (mg/kg):	0.08
RSD (%):	7.61

Salinomycin

Homogeneity study

Sacket	Subsample 1	Subsample 2
1	0.58	0.59
2	0.59	0.61
3	0.59	0.59
4	0.60	0.63
5	0.58	0.63
6	0.62	0.58
7	0.61	0.59
8	0.59	0.57
9	0.56	0.59
10	0.60	0.58
	average	**0.59**

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Row 1	2	1.17	0.58	8.78E-05
Row 2	2	1.19	0.60	2.02E-04
Row 3	2	1.18	0.59	1.02E-05
Row 4	2	1.23	0.61	4.28E-04
Row 5	2	1.21	0.60	1.09E-03
Row 6	2	1.21	0.60	7.96E-04
Row 7	2	1.20	0.60	3.98E-04
Row 8	2	1.16	0.58	1.35E-04
Row 9	2	1.15	0.57	3.09E-04
Row 10	2	1.17	0.59	2.80E-04

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.92E-03	9	3.25E-04	**0.87**	0.58	**3.02**
Within Groups	3.74E-03	10	3.74E-04			
Total	6.66E-03	19				

Standard deviation of the analytical procedure (mg/kg): 0.02
RSD (%): 3.26

Diclazuril

Homogeneity study

Sacket	Subsample 1	Subsample 2
1	0.008	0.009
2	0.008	0.008
3	0.008	0.008
4	0.008	0.008
5	0.007	0.008
6	0.008	0.008
7	0.008	0.008
8	0.009	0.009
9	0.008	0.010
10	0.008	0.008
	average	**0.008**

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Row 1	2	0.017	0.009	2.463E-07
Row 2	2	0.016	0.008	6.114E-08
Row 3	2	0.016	0.008	6.279E-12
Row 4	2	0.015	0.008	4.026E-08
Row 5	2	0.015	0.007	3.887E-07
Row 6	2	0.017	0.008	9.682E-09
Row 7	2	0.016	0.008	1.098E-08
Row 8	2	0.018	0.009	7.410E-09
Row 9	2	0.018	0.009	1.144E-06
Row 10	2	0.015	0.008	1.692E-08

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.288E-06	9	6.987E-07	**3.629**	0.028	**3.020**
Within Groups	1.926E-06	10	1.926E-07			
Total	8.214E-06	19				

Standard deviation of the analytical procedure (mg/kg): 4.388E-04
Between-sample standard deviation (mg/kg): 5.03E-04
RSD (%): 5.403

Narasin

Sacket	Homogeneity study Subsample 1	Subsample 2
1	0.80	0.79
2	0.80	0.80
3	0.78	0.79
4	0.81	0.79
5	0.75	0.77
6	0.75	0.79
7	0.80	0.75
8	0.78	0.76
9	0.76	0.78
10	0.80	0.77
	average	**0.78**

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Row 1	2	1.59	0.80	1.50E-05
Row 2	2	1.60	0.80	2.25E-05
Row 3	2	1.58	0.79	2.16E-05
Row 4	2	1.61	0.80	2.09E-04
Row 5	2	1.53	0.76	2.63E-04
Row 6	2	1.53	0.77	7.49E-04
Row 7	2	1.56	0.78	1.35E-03
Row 8	2	1.54	0.77	9.79E-05
Row 9	2	1.54	0.77	1.78E-04
Row 10	2	1.56	0.78	4.86E-04

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.79E-03	9	4.21E-04	**1.24**	0.37	**3.02**
Within Groups	3.40E-03	10	3.40E-04			
Total	7.18E-03	19				

Standard deviation of the analytical procedure (mg/kg):	0.02
RSD (%):	2.36

Annex 3 : Stability of the materials

The stability was evaluated on two series of measurements of each analyte. The first serie was performed at T0, when the materials were produced and homogenised and the second at T12 weeks, after the completion of the proficiency exercise by all participants and reception of the results.

Three different bags for each material containing the selected coccidiostats were analysed in duplicate and each duplicate was injected 3 times.

A F-test one tailed showed that the variances of the two series were equal. The following t-test demonstrated that the mean value of the concentration obtained after 12 weeks was not significantly different to the mean value of the concentration at T0. The samples can therefore be considered as stable.

Note: the blank material is available in the EURL-FA (control) laboratory. The quantification was therefore performed in this case using matrix-matched calibration curves. The obtained concentrations were corrected for purity.

	T0	T 12 weeks	T0	T 12 weeks	T0	T 12 weeks	T0	T 12 weeks	T0	T 12 weeks
	Monensin mg kg^{-1}		Lasalocid mg kg^{-1}		Salinomycin mg kg^{-1}		Diclazuril mg kg^{-1}		Narasin mg kg^{-1}	
Bag 1 - replicate 1 - 3 injections	1.0604	1.0834	1.8119	1.2022	0.5695	0.5801	0.0086	0.0090	0.7866	0.7603
	0.9913	1.0243	1.1032	1.0981	0.5839	0.6378	0.0078	0.0079	0.8110	0.8053
	1.0329	0.9229	1.1379	1.1524	0.5813	0.5794	0.0090	0.0074	0.7839	0.8525
Bag 1 - replicate 2 - 3 injections	1.0022	1.0759	1.1407	1.2843	0.5867	0.6361	0.0084	0.0076	0.8157	0.8280
	1.0534	0.9200	1.1641	1.1668	0.5870	0.6021	0.0076	0.0081	0.7916	0.8204
	1.0632	0.9756	1.1981	1.1570	0.6323	0.6552	0.0077	0.0064	0.7924	0.8075
Bag 2 - replicate 1 - 3 injections	1.0320	0.8953	1.1964	1.1572	0.5923	0.5797	0.0080	0.0089	0.7908	0.7488
	1.0151	0.9192	1.0860	1.1624	0.5855	0.6464	0.0080	0.0093	0.7791	0.7810
	1.0824	0.9018	1.1718	1.1303	0.5981	0.5965	0.0077	0.0064	0.7786	0.8085
Bag 2 - replicate 2 - 3 injections	1.0056	0.9182	1.1706	1.1864	0.5908	0.5863	0.0088	0.0080	0.8002	0.7233
	1.0806	0.9770	1.1043	1.1656	0.6086	0.5927	0.0063	0.0076	0.8275	0.7665
	1.1209	0.8168	1.1908	1.0916	0.6266	0.6171	0.0081	0.0089	0.7960	0.7700
Bag 3 - replicate 1 - 3 injections	1.1027	0.8522	1.6831	1.0581	0.5814	0.5623	0.0070	0.0078	0.7639	0.7071
	1.0192	0.8252	1.1082	1.0568	0.5791	0.5581	0.0068	0.0070	0.7400	0.7367
	1.0597	0.8248	1.1618	1.0575	0.6284	0.5695	0.0084	0.0082	0.7810	0.7598
Bag 3 - replicate 2 - 3 injections	1.0511	0.8946	1.1488	1.1246	0.6106	0.5840	0.0089	0.0076	0.7457	0.7582
	1.1353	0.8740	1.1224	1.1025	0.6388	0.5725	0.0078	0.0069	0.7489	0.6992
	1.0966	0.8460	1.0730	1.2023	0.5896	0.6289	0.0090	0.0071	0.7733	0.7391
F value (one tail)	**0.009374**		**0.000007**		**0.128296**		**0.622251**		**0.018702**	
t value (one tail)	**0.0000001**		**0.0893529**		**0.4642599**		**0.2182676**		**0.1324609**	

degrees of freedom data set 1 =degrees of freedom data set 2	= 17
degrees of freedom total	= 34
Fcrit (P=0.05)	= 2.308
t_{30} at 95% (P=0.05)	= 2.04

Annex 4 : Dispatch of materials

From: JRC IRMM CRL FEED ADDITIVES CONTROL
Sent: 10 May 2012 18:17
To: JRC IRMM CRL FEED ADDITIVES CONTROL
Subject: Launch of proficiency test exercise on coccidiostats organised by the EURL for Feed additives (Control)

Dear participant,

I would first like to thank you for having registered for participating in the PT in subject.

We would like to inform you that the parcel containing the samples will be dispatched from IRMM (Geel, Belgium) as of Monday 14.05.2012. The parcel will contain:
- 3 samples to be analysed, packed in sealed bags; these bags have to be stored at -18°C / -20°C upon arrival and until one hour before the analysis
- an accompanying letter with the instructions for conducting the exercise
- a list of contents of the package (including your laboratory code) that we would ask you to complete and send back to us

The shipment will be done in dry ice by express courier.

The term of this exercise is set to 22.06.2012.
In a next step, you receive electronically the reporting form in which you will report your results, to be sent back to us.

Would you have any further questions or need for clarification, please do not hesitate to contact us through the functional mailbox jrc-irmm-crl-feed-additives-control@ec.europa.eu

Kind regards,

Ursula Vincent on behalf of the EURL-FA

Dr URSULA VINCENT
Project Leader European Reference Laboratory for Feed Additives (control)
(EURL-FA control)

European Commission
Joint Research Centre
Food Safety and Quality
Institute for Reference Materials and Measurements (IRMM)
Retieseweg 111, B-2440 Geel/Belgium
Tel: +32 14 571 207
Fax: +32 14 571 787
ursula.vincent@ec.europa.eu

http://www.irmm.jrc.be

Annex 5 : Accompanying letter and receipt form

14 May 2012

Proficiency Test exercise 2012

Determination of authorised coccidiostats at cross-contamination level in feedingstuffs

Instructions

The materials you received should be analysed in routine conditions, i.e. utilizing your usual method for control.
Please check the content of the package, fill in and send back the 'List of Contents of the Package' form as specified below.

All materials have been grinded and homogenised; no further pre-treatment is necessary before the analysis. The samples have to be left at room temperature for 1 h before the start of the analysis.
Analyse all materials for the presence and the content of the authorised coccidiostats. The content should be reported as the concentration of the detected coccidiostat in mg kg^{-1} of feed given with 3 decimals. The concentrations of the coccidiostats present in these materials are close to the cross-contamination levels as defined in Commission Regulation (EU) No 574/2011 amending Annex I to Directive 2002/32/EC.
Instructions on how to report your results and information on the method used will be sent to you in due time in the format of a digital form via an electronic message.

Please note that the ultimate deadline for reporting the results is **22 June 2012**.

Please remember that the major objective of this study is to assess the proficiency of the participating laboratories to correctly determine coccidiostats in potentially contaminated feed samples and report results in the specified units within a defined time frame.

Any deviation as regards the non-respect of the deadline and/or the specified units for reporting will lead to the exclusion of your results from the statistical evaluation.

Statistical evaluation

Appropriate statistical tool for data evaluation will be used to investigate the proficiency of the laboratories. Statistical assessment of the proficiency of laboratories will be evaluated by calculating an individual dimensionless Z-score calculated according to ISO 13528.

$$z = \frac{(x - x_a)}{\sigma_p} \qquad \text{Eq. 1}$$

Where x = the value reported by the participant

x_a = the assigned value, set as the nominal value calculated from the formulation (IUPAC guidelines)

σ_p = the target standard deviation, set using the modified Horwitz equation

In September 2012, a report including the statistical evaluation of all valid participant results will be issued and distributed to all reporting participants. The report will be confidential. The list of participating laboratories will be included but any result will be strictly linked only to the laboratory code. Each participant will be able to retrieve its results and calculated z-score using its individual confidential laboratory code communicated on the list of contents of the package form included in this letter.

Finally, a concluding workshop opened to all participants to the study will be organised to close the exercise in Autumn 2012. Participation to the PT and to the workshop is free of charge. However, travel and accommodation costs will only be reimbursed for one representative of each National reference Laboratory for Feed Additives (control) defined according to Regulation (EC) No 882/2004.

If you have any question, please contact the EURL-FA (Control), jrc-irmm-crl-feed-additives-control@ec.europa.eu.

Kind regards,

Dr Ursula VINCENT (Proficiency Test Coordinator)
On behalf of the EURL for Feed Additives

List of Contents of the Package[1]

Acknowledgement of Reception

Dear «TITLE» «First NAME»«SURNAME»,

please find below the list of contents for the proficiency test exercise related to the determination of authorised coccidiostats at cross-contamination level, organised by the EURL-Feed Additives (Control), on behalf of DG SANCO (European Commission).
Please check that the sample codes of the samples you received correspond to those declared on this list. The samples should **be stored at -18°C / -20°C** upon reception and until one hour before the analysis.

Your laboratory code is given below. This code will be applied to your laboratory for the whole PT exercise.

Your laboratory code is: **«Lab code»**

Number of samples in the package: 3

Sample code	Present Y/N
«sample 1 code»	
«sample 2 code»	
«sample 3 code»	

Samples received on (dd/mm/yyyy) :
Content checked on (dd/mm/yyyy) :
Comments (if applicable) :

Date: Signature:

[1] Form to fill in, sign and send back to the EURL-Feed additives (control) by electronic mail (jrc-irmm-crl-feed-additives-control@ec.europa.eu) or by fax (+32 14 571 787)

Annex 6 : Reporting sheet

This information will assist you in reporting your results

This excel workbook contains four worksheets and should be sent back when filled in.

In all worksheets, the accessible areas for writing are the dedicated empty **couloured areas**.

In the worksheet "**Method questionnaire**", enter the information related to the method used for the identification and quantification (if relevant) of the coccidiostats. Please use the "comments" text box for any additionl information you wish to provide and/or to specify an "other" answer.

"**Report form**" please fill in the results from each of the 3 unknown samples sent to your laboratory. Remember that only the empty **coloured areas** of the sheets are reserved for inserting results and other requested information. Since the assay most probably gives you both qualitative results and a value for the concentration, we ask you to report the result as "Detected" or "Not detected" and to report the concentration you determined in **mg kg^{-1}**.
Please note that an analyte for which the determined concentration would be lower than LOQ should be reported as "Not detected"

Reporting the results

The analysis should be conducted within 1 month from the date of reception of the samples.
The **deadline** for reporting the results is **22 June 2012**.
Please modify the name of the file by inserting the labcode. For instance: For laboratory with the labcode L04 the filename should be:
L04_PT_CCs_2012_Reporting_sheet.xls

What to report to the EURL-FA (control):
- the complete current file
- the signed and dated "r**esults to be faxed or emailed**" sheet

How to report to the EURL-FA (control):
- by e-mail to jrc-irmm-crl-feed-additives-control@ec.europa.eu
- by Fax: +32 14 571 787

In addition, please send to us the chromatograms from all analyses either by e-mail or by normal mail.

The information collected will be kept confidential and used for discussion and exchange during the final workshop.

Thank you and good luck.
Dr Ursula Vincent on behalf of the EURL-FA control

Results reporting form for the determination of coccidiostats in feedingstuffs

Name of laboratory:

Lab code: You will find the labcode on the document accompanying the shipment of the samples

☐ I confirm that I have read the information in the worksheet "Important information"

From | To

Period during which the analysis was conducted (dd/mm/yyyy - dd/mm/yyyy)

	Result of analysis	Result of analysis	Result of analysis
Labcode			
Sample Code			
Monensin			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Narasin			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Lasalocid			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Nicarbazin (DNC)			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Diclazuril			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Maduramicin			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Decoquinate			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Halofuginone			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Salinomycin			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			
Robenidine			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}	20	100	5
Semduramicin			
concentration in mg kg^{-1}			
expanded uncertainty in mg kg^{-1}			

Note: The major objective of this study is to assess the proficiency of the participating laboratories to correctly determine coccidiostats in potentially contaminated feed samples in the frame of the implementation of the legislation related to the unavoidable carry-over of coccidiostats or histomonostats (Commission Regulation (EU) No 574/2011 amending Annex I to Directive 2002/32/EC). The statistical evaluation of the results will therefore focus on the quantitative results delivered by the participating laboratories

Print this area for reporting the results

Date, sign and return to EURL-FA (control)

by fax:+32 14 571 787

by email: jrc-irmm-crl-feed-additives-control@ec.europa.eu

PT exercise 2012: COCCIDIOSTATS IN FEEDINGSTUFFS

Laboratory name: **0**

Laboratory code:

Sample code	0	0	0	0	0	0
Monensin	no result	0	no result	0	no result	0
		0		0		0
Narasin	no result	0	no result	0	no result	0
		0		0		0
Lasalocid	no result	0	no result	0	no result	0
		0		0		0
Nicarbazin (DNC)	no result	0	no result	0	no result	0
		0		0		0
Diclazuril	no result	0	no result	0	no result	0
		0		0		0
Maduramicin	no result	0	no result	0	no result	0
		0		0		0
Decoquinate	no result	0	no result	0	no result	0
		0		0		0
Halofuginone	no result	0	no result	0	no result	0
		0		0		0
Salinomycin	no result	0	no result	0	no result	0
		0		0		0
Robenidine	no result	0	no result	0	no result	0
		0		0		0
Semduramicin	no result	0	no result	0	no result	0
		0		0		0

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Signature (responsible of analysis): Date

Annex 7 : Reported concentration results with associated uncertainties and reported limits of detection and quantification, in mg kg^{-1} of feed

1) in the contaminated materials

(-: false negative due to lack of sensitivity; NR: not analysed)

	Monensin (MAT 2)				Narasin (MAT 1)			
$x_a \pm \sigma_p$	**1.094 ± 0.171**				**0.678 ± 0.114**			
LAB	**x_{lab}**	**u_{lab}**	**LOD**	**LOQ**	**x_{lab}**	**u_{lab}**	**LOD**	**LOQ**
L01	1.11	0.11	0.03	0.100	0.63	0.04	0.03	0.100
L02	1.90	0.57	0.013	0.025	1.64	0.57	0.013	0.025
L03	1.16	0.03	0.025	0,050 mg/kg	0.94	0.21	0.025	0.050
L05	2065.00	335.00	< ML (not determined)	< ML (not determined)	1721.00	723.00	< ML (not determined)	< ML (not determined)
L06	1.20	0.30	-	0.007	0.86	0.43	-	0.01
L07	1.05	0.20	0.01	0.03	0.95	0.20	0.01	0.03
L08	1.021	0.16	-	0.200	0.729	0.066	-	0.125
L09	0.41	0.03	0.01	0.025	0.94	0.01	0.01	0.025
L10	1.20	0.60	0.3	0.6	0.65	0.30	0.15	0.3
L11	1.18	0.24	0.332	0.894	0.67	0.12	0.133	0.363
L12	1.09	0.22	0.005	0.01	0.59	0.12	-	-
L13	1.29	0.19	0.004	0.01	0.98	0.15	0.004	0.01
L14	1.26	0.50	0.02	0.05	0.84	0.34	0.03	0.1
L15	0.95	0.29	0.049	156	0.52	0.16	0.041	131
L16	1.04	0.31	0.10	0.50	0.71	0.21	0.20	0.50
L17	1.49	0.31	0.274	0.830	0.88	0.17	0.127	0.384
L18	1.00	0.20	0.0032	0.0032	0.64	0.10	0.0032	0.0032
L19	1.03	0.16	-	0.25	0.56	0.10	-	0.25
L20	0.78	-	-	0.01	0.57	-	-	0.01
L21	1.04	0.14	0.025	0.500	0.44	0.14	0.100	0.200
L22	1.25	0.15	-	0.325	0.97	0.21	-	0.175
L23	3.68	0.97	0.0074	0.0123	1.44	0.44	0.0046	0.0077
L24	1.00	0.11	0.3	1.0	0.75	0.09	0.4	0.7
L25	5.99	2.40	0.38	1.25	-	-	0.21	0.7
L26	2.57	1.02	0.04	0.125	0.00	-	0.02	0.07
L27	0.68	0.31	0.1	0.1	0.38	0.17	0.1	0.1
L28	1.18	0.20	0.03	0.1	0.66	0.10	-	-
L29	0.88	0.09	=>LOQ/3	1	0.54	0.05	=>LOQ/3	2
L30	0.00	-	-	0.625	0.89	0.23	-	0.35
L31	1.54	0.25	-	0.625	1.12	0.51	-	0.35

	Lasalocid (MAT 2)				Diclazuril (MAT 1)			
$x_a \pm \sigma_p$	**1.191 ± 0.184**				**0.010 ± 0.002**			
LAB	x_{lab}	u_{lab}	**LOD**	**LOQ**	x_{lab}	u_{lab}	**LOD**	**LOQ**
L01	0.99	0.028	0.030	0.100	0.005	0.001	0.001	0.0033
L02	1.3	0.39	0.013	0.025	0.011	0.005	0.003	0.005
L03	1.035	0.055	0.025	0.050	0.009	0.001	0.0025	0.0050
L05	1181	733	< ML (not determined)	< ML (not determined)	4.500	1.000	< ML (not determined)	< ML (not determined)
L06	1.175	0.588	-	0.02	NR	NR	-	-
L07	0.8	0.2	0.01	0.03	0.020	0.010	0.01	0.03
L08	1.072	0.125	-	0.145	0.006	0.001	-	0.0015
L09	0.000	-	0.04	0.08	NR	NR	-	-
L10	1.1	0.5	0.3	0.6	0.000	-	0.005	0.01
L11	1.012	0.304	0.375	0.950	0.007	0.001	0.001	0.003
L12	0.986	0.197	-	-	0.011	0.002	-	-
L13	1.56	0.23	0.0004	0.001	0.012	0.004	0.0004	0.001
L14	1.457	0.583	0.02	0.05	0.009	0.004	0.01	0.025
L15	0.89	0.27	0.038	123	-	-	0.009	32
L16	1.01	0.3	0.10	0.50	0.000	-	0.004	0.01
L17	1.173	0.364	0.283	0.859	0.012	0.002	0.001	0.003
L18	1	0.1	0.032	0.032	0.010	0.002	0.0032	0.0032
L19	0.76	0.14	-	0.25	0.010	0.002	-	0.005
L20	0.516	0	-	0.01	0.010	-	-	0.01
L21	2.21	0.54	0.250	0.500	0.007	0.001	0.0025	0.005
L22	1.18	0.16	-	0.312	NR	NR	-	0.025
L23	3.296	0.884	0.0130	0.0217	NR	NR	-	-
L24	1.121	in validation	0.5	1.0	1.175	0.470	-	-
L25	1.227	0.4908	0.38	1.25	NR	NR	0.003	0.01
L26	1.283	0.48	0.04	0.125	0.000	-	0.002	0.005
L27	0.05	0.03	0.05	0.05	0.010	0.006	0.01	0.01
L28	NR	NR	-	-	NR	NR	-	-
L29	20.2	0.6	=>LOQ/3	1	NR	NR	=>LOQ/3	0.1
L30	1.809	-	-	0.625	0.011	0.003	-	0.005
L31	1.124	0.315	-	0.625	0.012	0.009	-	0.005

	Salinomycin (MAT 2)			
$x_a \pm \sigma_p$	**0.663 ± 0.112**			
LAB	**x_{lab}**	**u_{lab}**	**LOD**	**LOQ**
L01	0.66	0.028	0.030	0.100
L02	0.788	0.236	0.013	0.025
L03	0.769	0.168	0.025	0.050
L05	473	321	< ML (not determined)	< ML (not determined)
L06	0.776	0.388	-	0.01
L07	0.9	0.2	0.01	0.03
L08	0.611	0.074	-	0.140
L09	0.77	0.023	0.01	0.025
L10	0.7	0.35	0.15	0.3
L11	0.758	0.167	0.117	0.320
L12	0.741	0.148	-	-
L13	0.69	0.1	0.004	0.01
L14	1.654	0.661	0.03	0.1
L15	0.66	0.2	0.026	0.086
L16	0.79	0.24	0.20	0.50
L17	0.76	0.167	0.132	0.400
L18	0.64	0.11	0.0032	0.0032
L19	0.53	0.10	-	0.25
L20	0.52	-	-	0.01
L21	0.19	0.07	0.075	0.150
L22	0.7	0.23	-	0.175
L23	0.416	0.152	0.0006	0.0011
L24	0.651	0.095	0.4	0.7
L25	-	-	0.21	0.7
L26	0.000	-	0.02	0.07
L27	0.38	0.17	0.1	0.1
L28	0.65	0.1	-	-
L29	0.47	0.01	=>LOQ/3	1
L30	0.57	0.37	-	0.35
L31	0.674	0.532	-	0.35

Figures 1-5 to display the results as reported by the participants, in mg kg^{-1}; the error bars represent the reported uncertainties.

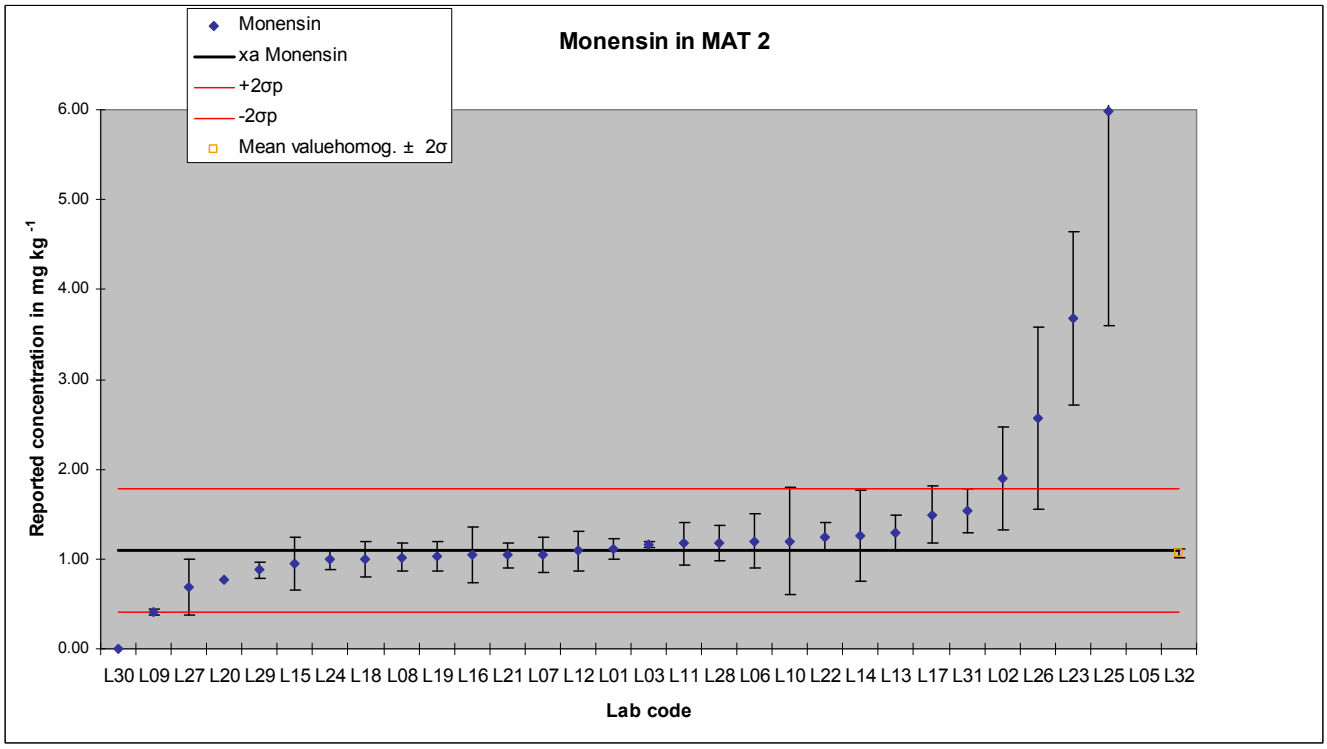


Figure 1: Reported concentrations for monensin in MAT 2 – the error bars are the associated uncertainties as reported by the participants. The estimation of the concentration from the homogeneity study is displayed as indicative value under the L32 label.

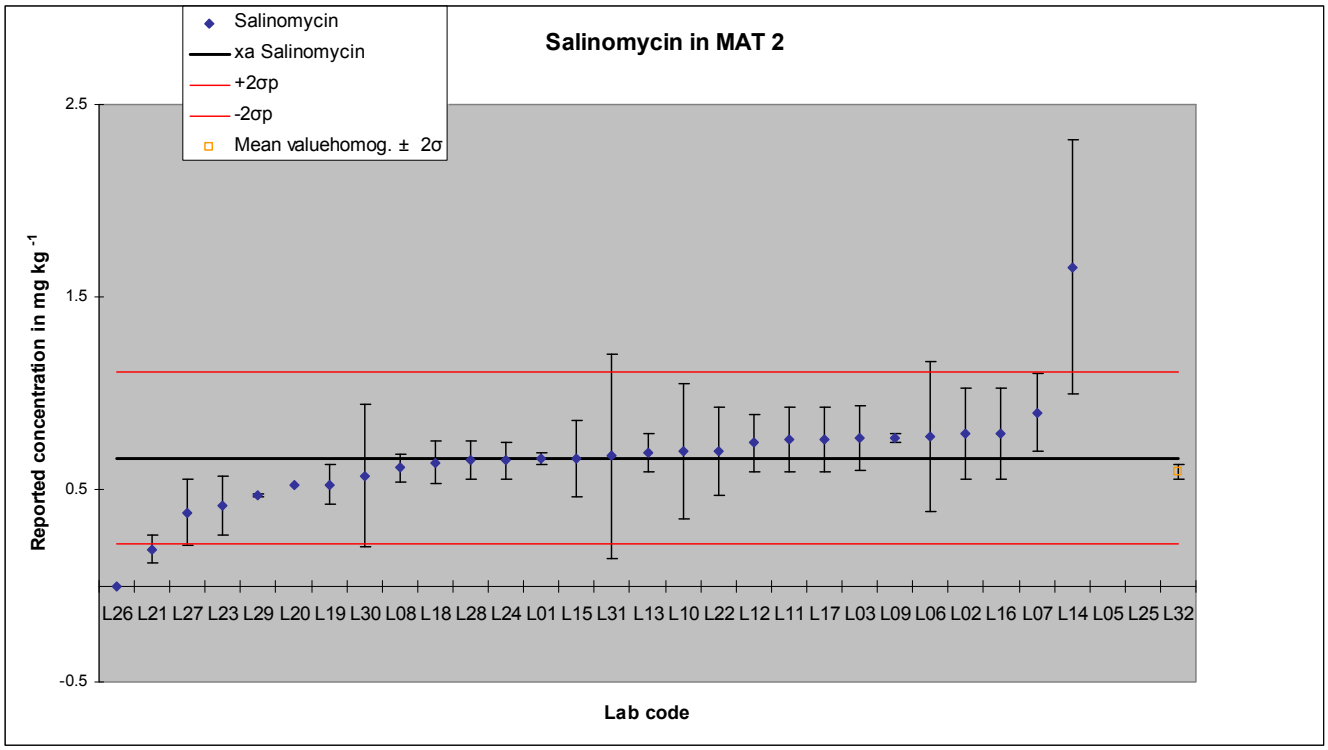
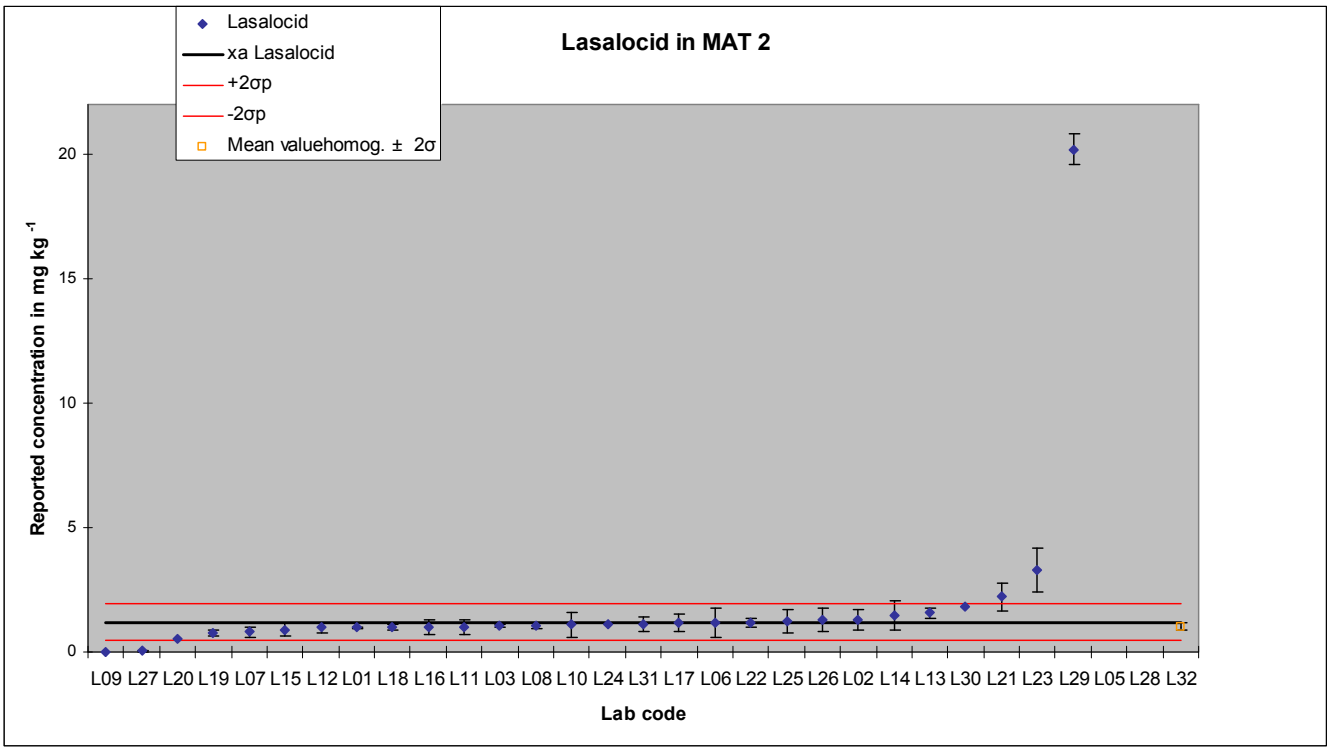


Figure 2: Reported concentrations for salinomycin in MAT 2 – the error bars are the associated uncertainties as reported by the participants. The estimation of the concentration from the homogeneity study is displayed as indicative value under the L32 label.



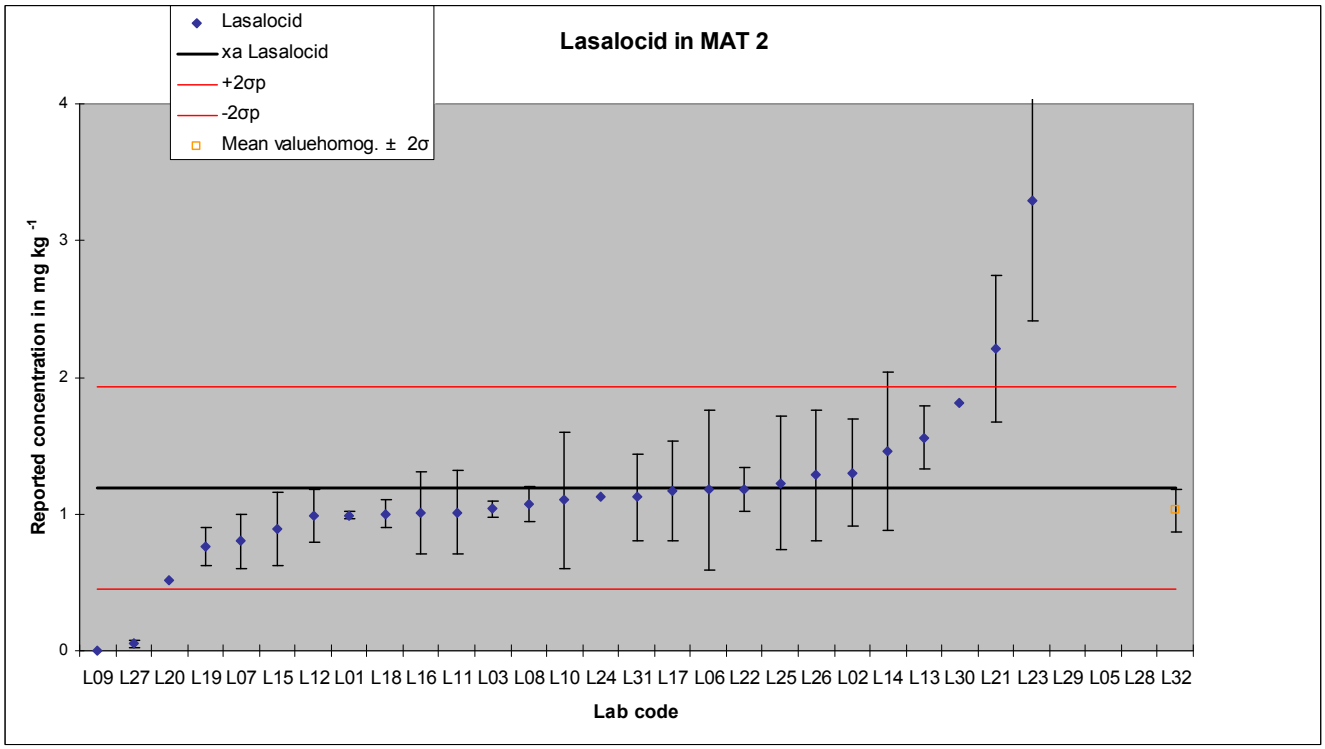


Figure 3: Reported concentrations for lasalocid in MAT 2 – the error bars are the associated uncertainties as reported by the participants. The estimation of the concentration from the homogeneity study is displayed as indicative value under the L32 label.

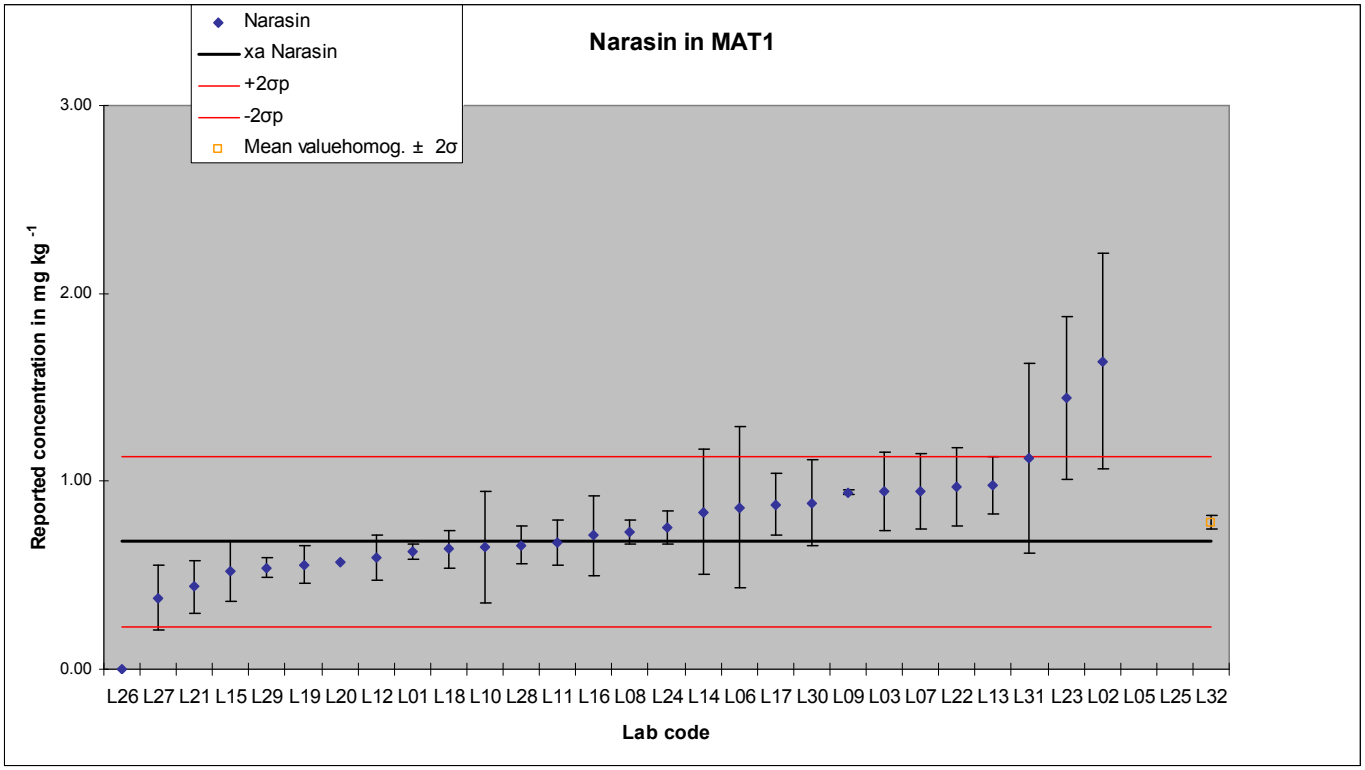


Figure 4: Reported concentrations for narasin in MAT 1 – the error bars are the associated uncertainties as reported by the participants. The estimation of the concentration from the homogeneity study is displayed as indicative value under the L32 label.

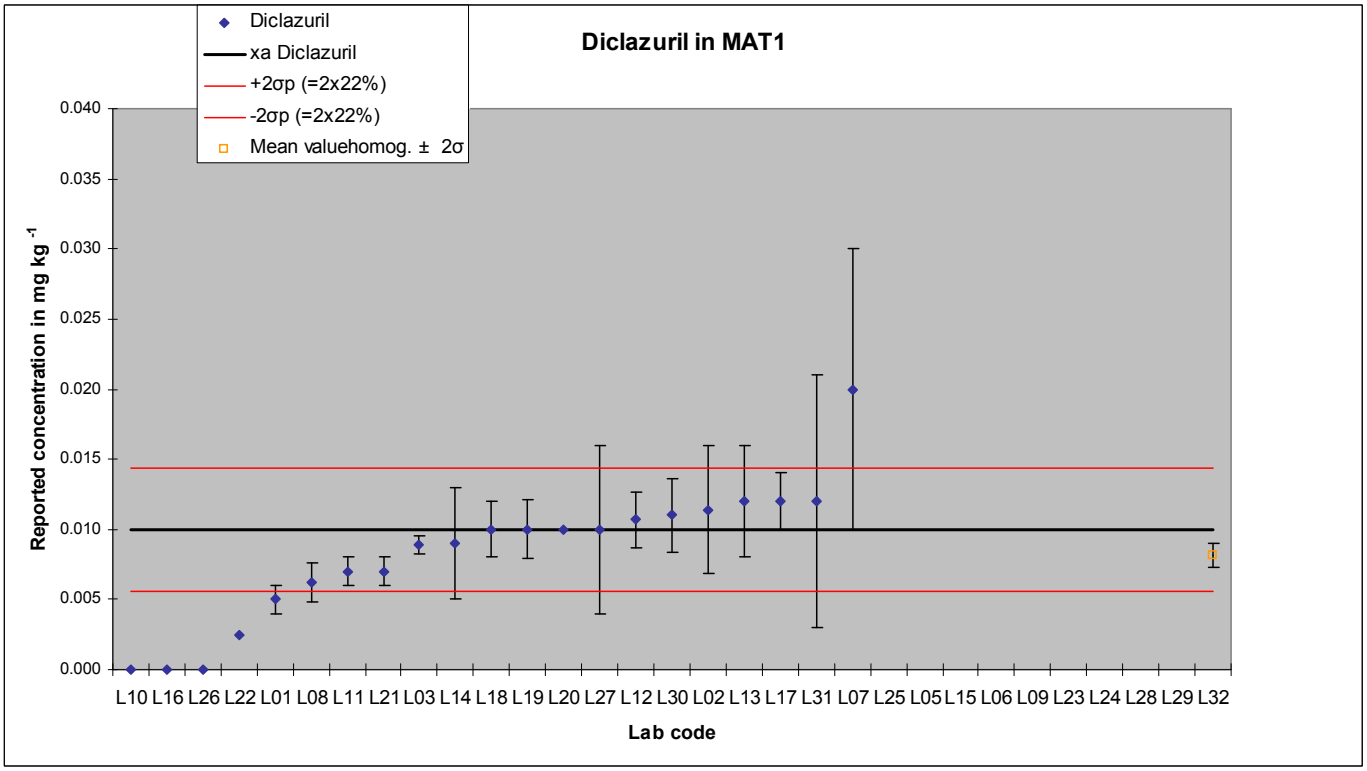


Figure 5: Reported concentrations for diclazuril in MAT 1 – the error bars are the associated uncertainties as reported by the participants. The estimation of the concentration from the homogeneity study is displayed as indicative value under the L32 label.

2) in the non-contaminated materials

	Monensin (MAT 1)		Narasin (MAT 2)		Lasalocid (MAT 1)		Lasalocid (MAT 3)	
$x_a \pm \sigma_p$	0.000 ± 0.000							
LAB	x_{lab}	u_{lab}	x_{lab}	u_{lab}	x_{lab}	u_{lab}	x_{lab}	u_{lab}
L05	0	0	**221.00**	43.00	0	0	0	0
L16	**0.14**	0	0.00	0.00	0	0	0	0
L20	0	0	**0.015**	0.00	0	0	0	0
L29	0	0	0.00	0.00	**17.4**	0.5	**19.4**	0.6

All laboratories correctly reported the absence of the 5 spiked measurands in all materials except for the laboratories displayed in the table above in the indicated materials.

For robenidine, one false positive was reported in MAT 3:

	Robenidine					
	MAT 1		MAT 2		MAT 3	
$x_a \pm \sigma_p$	0.000 ± 0.000					
LAB	x_{lab}	u_{lab}	x_{lab}	u_{lab}	x_{lab}	u_{lab}
L25	0	0	0	0	**23.32**	9.328

The limits of detection (LOD) and of quantification (LOQ), in mg kg^{-1}, as reported by the participants for the 6 remaining authorised coccidiostats are displayed in the following tables.

	Nicarbazin (DNC)		Maduramicin		Decoquinate	
LAB	**LOD**	**LOQ**	**LOD**	**LOQ**	**LOD**	**LOQ**
L01	0.030	0.100	0.030	0.100	0.0010	0.0033
L02	0.003	0.005	0.013	0.025	-	-
L03	0.025	0.050	0.025	0.050	0.0025	0.0050
L05	< ML (not determined)	< ML (not determined)	< ML (not determined)	< ML (not determined)	< ML (not determined)	< ML (not determined)
L06	-	0.5	-	0.01	-	-
L07	0.01	0.03	0.01	0.03	0.01	0.03
L08	-	0.200	-	0.020	-	0.080
L09	-	-	0.01	0.025	-	-
L10	0.1	0.2	0.01	0.02	0.1	0.2
L11	0.044	0.126	0.014	0.038	0.038	0.106
L12	-	-	-	-	-	-
L13	0.0004	0.001	0.004	0.01	0.0004	0.001
L14	0.03	0.1	0.03	0.1	0.02	0.1
L15	0.040	130	0.014	47	0.059	189
L16	0.10	0.50	0.01	0.03	0.10	0.30
L17	0.046	0.141	0.013	0.040	0.054	0.163
L18	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032
L19	-	0.25	-	0.025	-	-
L20	-	0.01	-	0.01	-	-
L21	0.250	0.500	-	-	0.050	0.100
L22	-	0.125	-	0.0125	-	0.1
L23	-	-	-	-	0.0013	0.0022
L24	0.7	1.0	-	-	-	-
L25	0.12	0.4	0.015	0.05	0.21	0.7
L26	0.02	0.05	0.002	0.005	0.01	0.04
L27	0.1	0.1	-	-	0.1	0.1
L28	-	-	-	-	-	-
L29	-	-	=>LOQ/3	1	-	-
L30	-	0.25	-	0.025	-	0.2
L31	-	0.625	-	0.025	-	-

	Halofuginone		Robenidine		Semduramicin	
LAB	**LOD**	**LOQ**	**LOD**	**LOQ**	**LOD**	**LOQ**
L01	0.0010 µg/kg	0.0033	0.0010	0.0033	0.0010	0.0033
L02	0.015	0.03	0.025	0.05	0.013	0.025
L03	0.0025	0.0050	0.025	0.050	0.025	0.050
L05	>ML (Not determined)	> ML (not determined)	< ML (not determined)	< ML (not determined)	< ML (not determined)	< ML (not determined)
L06	-	0.02	-	0.004	-	0.005
L07	-	-	0.01	0.03	-	-
L08	-	0.0125	-	0.235	-	0.100
L09	-	-	-	-	0.04	0.08
L10	0.015	0.03	0.2	0.4	0.05	0.1
L11	-	-	-	-	0.069	0.185
L12	-	-	0.05	0.1	-	-
L13	0.004	0.01	0.004	0.01	0.004	0.01
L14	0.003	0.007	0.05	0.1	0.01	0.025
L15	-	-	0.087	300	0.020	67
L16	0.01	0.03	0.10	0.50	0.05	0.20
L17	-	0.016	0.128	0.389	0.037	0.112
L18	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032
L19	-	-	-	0.25	-	-
L20	-	-	-	0.01	-	-
L21	0.005	0.015	0.100	0.200	-	-
L22	-	0.0075	-	0.175	-	0.00625
L23	-	-	-	-	-	-
L24	-	-	in validation	0.7	-	-
L25	0.009	0.03	0.21	0.7	0.075	0.25
L26	0.001	0.003	0.02	0.07	0.01	0.025
L27	-	-	-	-	0.1	0.1
L28	-	-	-	-	-	-
L29	-	-	=>LOQ/3	0.7	-	-
L30	-	0.015	-	0.35	-	0.125
L31	-	0.015	-	0.35	-	-

Annex 8 : Z- and ζ-scores calculated for each reporting laboratory

	Z-scores				
LAB	Monensin	Narasin	Lasalocid	Diclazuril	Salinomycin
Target value	**1.094**	**0.678**	**1.191**	**0.010**	**0.663**
L01	0.10	-0.46	-1.09	**-2.24**	-0.03
L02	**4.70**	**8.43**	0.59	0.71	1.11
L03	0.39	**2.33**	-0.85	-0.45	0.94
L05	**12039.36**	**15074.04**	**6400.55**	**2070.10**	**4215.44**
L06	0.61	1.61	-0.09		1.01
L07	-0.25	**2.39**	**-2.12**	**4.68**	**2.11**
L08	-0.42	0.45	-0.65	-1.69	-0.47
L09	**-3.99**	**2.30**	**-6.46**		0.95
L10	0.62	-0.24	-0.49	**-4.55**	0.33
L11	0.47	-0.06	-0.97	-1.32	0.85
L12	-0.02	-0.75	-1.11	0.39	0.69
L13	1.15	**2.65**	**2.00**	0.99	0.24
L14	0.95	1.40	1.44	-0.40	**8.84**
L15	-0.84	-1.38	-1.63		-0.03
L16	-0.31	0.28	-0.98	**-4.55**	1.13
L17	**2.34**	1.76	-0.10	0.99	0.86
L18	-0.55	-0.33	-1.04	0.06	-0.21
L19	-0.38	-1.07	**-2.33**	0.06	-1.23
L20	-1.86	-0.93	**-3.66**	0.06	-1.28
L21	-0.31	**-2.08**	**5.53**	-1.32	**-4.22**
L22	0.91	**2.56**	-0.06	**-3.39**	0.33
L23	**15.08**	**6.72**	**11.42**		**-2.21**
L24	-0.58	0.65	-0.38		-0.11
L25	**28.55**		0.19	**537.17**	
L26	**8.59**	**-5.94**	0.50	**-4.55**	**-5.92**
L27	**-2.41**	**-2.61**	**-6.19**	0.06	**-2.53**
L28	0.50	-0.15			-0.12
L29	-1.25	-1.21	**103.12**		-1.72
L30	**-6.38**	1.82	**3.35**	0.53	-0.83
L31	**2.60**	**3.91**	-0.36	0.99	0.10

	Zeta-scores				
LAB	Monensin	Narasin	Lasalocid	Diclazuril	Salinomycin
Target value	**1.094**	**0.678**	**1.191**	**0.010**	**0.663**
L01	0.14	-1.25	**-7.00**	**-4.85**	-0.11
L02	1.41	1.68	0.28	0.34	0.53
L03	**2.24**	1.27	**-2.82**	-1.52	0.63
L05	**6.16**	**2.38**	1.61	**4.49**	1.47
L06	0.35	0.43	-0.03		0.29
L07	-0.22	1.36	-1.95	1.01	1.18
L08	-0.46	0.78	-0.95	**-2.61**	-0.70
L09	**-21.67**	**19.65**	**-186.47**		**4.60**
L10	0.18	-0.09	-0.18	**-154.80**	0.11
L11	0.35	-0.05	-0.59	**-2.85**	0.57
L12	-0.02	-0.73	-1.04	0.42	0.53
L13	1.03	**2.02**	1.60	0.54	0.27
L14	0.32	0.48	0.46	-0.21	1.50
L15	-0.99	-1.97	**-2.23**		-0.03
L16	-0.17	0.15	-0.60	**-154.80**	0.53
L17	**2.55**	**2.40**	-0.10	**2.14**	1.16
L18	-0.94	-0.75	**-3.79**	0.14	-0.42
L19	-0.41	-1.23	**-3.06**	0.13	-1.33
L20	**-54.38**	**-34.87**	**-105.69**	**2.21**	**-45.21**
L21	-0.76	**-3.39**	**3.77**	**-5.67**	**-13.46**
L22	1.04	1.39	-0.07	**-115.55**	0.16
L23	**2.68**	1.76	**2.38**		-1.63
L24	-0.93	0.83	**-10.98**		-0.13
L25	**4.09**		0.15	**4.96**	
L26	1.44	**-223.65**	0.19	**-154.80**	**-209.43**
L27	-1.33	-1.75	**-37.20**	0.02	-1.67
L28	0.43	-0.18			-0.13
L29	**-2.37**	**-2.75**	**31.68**		**-18.42**
L30	**-186.62**	0.90	**96.73**	0.44	-0.25
L31	1.81	0.88	-0.21	0.24	0.02

Annex 9 : Questionnaire

Method used for Proficiency Test EURL-FA (Control) 2012

Sample code 1

	Monensin	Narasin	Lasalocid	Nicarbazin	Diclazuril	Maduramicin
Is the method used accredited?	Y	Y	Y	Y	N	Y
Is it a multi-analyte method or a single analyte one?*	multi-analyte	multi-analyte	multi-analyte	multi-analyte	multi-analyte	multi-analyte
Reference (Journal)						
Type of method	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC
Sample amount used for analysis (g)	1	1	1	1	1	1
Sample preparation						
type of extraction	Liquid-solid	Liquid-solid	Liquid-solid	Liquid-solid	Liquid-solid	Liquid-solid
dilution	N	N	N	N	N	N
solvents	acetonitrile+NH3	acetonitrile+NH3	acetonitrile+NH3	acetonitrile+NH3	acetonitrile+NH3	acetonitrile+NH3
Other	N	N	N	N	N	N
Specify clean-up	n-hexane	n-hexane	n-hexane	n-hexane	n-hexane	n-hexane
HPLC type	Reverse phase	Reverse phase	Reverse phase	Reverse phase	Reverse phase	Reverse phase
HPLC Guard column used?	N	N	N	N	N	N
HPLC column stationary phase	Xterra C18ec (50x2,1mm;	Xterra C18ec (50x2,1mm; 3,5µ)	Xterra C18ec (50x2,1mm;	Xterra C18ec (50x2,1mm; 3,5µ)	Xterra C18ec (50x2,1mm; 3,5µ)	Xterra C18ec (50x2,1mm;
Mobile phase programme	Gradient	Gradient	Gradient	Gradient	Gradient	Gradient
Mobile phase components	methanol, water, ammonium	methanol, water, ammonium	methanol, water, ammonium	methanol, water, ammonium	methanol, water, ammonium	methanol, water, ammonium
Mobile phase flow rate	0,3 ml/min	0,3 ml/min	0,3 ml/min	0,3 ml/min	0,3 ml/min	0,3 ml/min
HPLC injection volume	10 µl	11 µl	12 µl	13 µl	14 µl	15 µl
Column temperature	40°C	40°C	40°C	40°C	40°C	40°C
Autosampler temperature	20°C	20°C	20°C	20°C	20°C	20°C
UV Detection						
Wavelength (absorbance) (nm)						
Fluorescence detection						
Wavelength (excitation) (nm)						
Wavelength (emission) (nm)						
Mass spectrometry detection						
detector type	Triple quadrup	Triple quadrup	Triple quadrup	Triple quadrup	Triple quadrup	Triple quadrup
cone voltage (V)	5500	5500	5500	5500	5500	5500
collision energy (eV)	29	32	28	-34	-34	31
parent ion m/z	688	782	608	301	405	934
daughter ion 1 m/z	461	729	237	107	299	647
daughter ion 2 m/z	635	748	337	137	334	629
Quantification						
Use of an internal standard	N	N	N	N	N	N
Specify internal standard used						
Limit of detection of method used	30 µg/kg	30 µg/kg	30 µg/kg	30 µg/kg	1,0 µg/kg	30 µg/kg
Limit of quantification of method used	100 µg/kg	100 µg/kg	100 µg/kg	100 µg/kg	3,3 µg/kg	100 µg/kg
Was the same method used for the remaning 2 samples? If no (N), specify in the "Other comments" text box	Y	Y	Y	Y	Y	Y

* specify which analytes are determined by the same multi-analyte method

Other comments (optional)

Monensin

	Sample preparation							HPLC - reverse phase							
	Acc. Meth.	Multi/Single	Test portion (g)	Extraction	Dilution	Solvent	Clean-up	Guard column	Column	Elution mode	Mobile phase	flow rate (ml min^{-1})	inj. Vol. (µl)	Col. Temp. (°C)	Autosampler Temp. (°C)
L01	Y	Multi	1	LSE	N	ACN/NH_3	n-hexane	N	Xterra18ec 50x2.1mm, 3.5um	Gradient	MeOH/H_2O/CH_3COONH_4	0.3	10	40	20
L02	Y	Multi	2.5	LSE	N	MeOH/H_2O	-	Y	Phenomenex GEMINI 150*2 mm, 5 µm	Gradient	A: ACN/H20/NH4AC B. ACN/H2O/MeOH/THF/HCOOH	0.3	10	40	15
L03	Y	Multi	5	LSE	Y	MeOH	-	N	C18	Gradient	ACN, H2O, FA	0.2	10	35	off
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L06	Y	Multi	1	LSE	Y	MeOH/H_2O	Centrifuge	N	C18	Gradient	MeOH/H_2O	0.3	50	30	20
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L08	N	Multi	1	LSE	N	ACN	SPE (Oasis HLB)	N	UPLC BEH Phenyl	Gradient	HCOOH in H_2O; HCOOH in ACN	0.5	2-5	50	15
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L10	N	Multi	5	LSE	Y	ACN/MeOH	-	Y	C8,Symmetry	Gradient	H_2O/MeOH/ACN/HCOOH	0.35	10	25	4
L11	N	Multi	2.5	LSE	N	ACN	-	Y	C8	Gradient	ACN + 5 mM CH_3COONH_4 containing 0.5% CH_3COOH	0.2	5	50	room temperature
L12	nr	nr	2	LSE	nr	MeOH/H_2O	-	N	Zorbax C18	Gradient	MeOH/H_2O	0.5	50	room temperature	room temperature
L13	Y	Multi	nr	LSE	N	ACN/MeOH 90/10 v/v	-	Y	C18, 5µm, 110 A	Gradient	10mM CH_3COONH_4/ACN	0.3	5	25	room temperature
L14	Y	Multi	10	LSE	N	MeOH/H_2O 90/10	-	Y	Pursuit 3 C 18 150 x 2 mm	Gradient	ACN+HCOOH/H2O+HCOOH	0.25	5	40	15
L15	Y	Multi	1	LSE	Y	ACN	-	Y	C18	Gradient	H_2O/MeOH/HCOOH	0.35	5	40	4
L16	Y	Multi	5	LSE	N	MeOH/H_2O	-	Y	C18	Isocratic	CH_3COOH/MeOH	0.9	100	30	15
L17	Y	Multi	2.5	LSE	Y	H_2O, ACN	C2	Y	C8 (Symmetry Column, Waters)	Gradient	A: H_2O + 0.1% HCOOH, B: ACN + 0.1 % HCOOH	0.6	20	40	8
L18	Y	Multi	2 or 5 depending on expected concentration	LSE	Y	ACN	SPE or dilution depending on expected concentration	Y	C18	Gradient	A) ACN 20 µM CH_3COONa + 0.1% HCOOH; B) 0,1% HCOOH	0.25	5	40	16
L19	Y	Multi	2	LSE	Y	MeOH/H2O 90/10	-	Y	RP-C18	Gradient	A: HCOOH 0.1% in H_2O B: (HCOOH 0.1%+20 µM CH_3COONa) in CH3CN	0.2	5	40	15
L20	N	Multi	1	LSE	N	ACN	-	Y	nr	Gradient	H_2O, ACN, CH3COONH5, HCOOH	0.3	20	40	nr
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L22	N	Multi	5	LSE	N	ACN, MeOH	C18 dispersive	Y	Poroshell 120 EC-C18	nr	ACN, MeOH, $HCOONH_3$	0.3	5	50	room temperature
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L24	Y	Multi	5	LSE	N	MeOH/H2O 90/10	-	Y	C18	Isocratic	MeOH:H_2O:CH_3COOH 94:6:0.1	0.9	100	30	nr
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8
L28	Y	Multi	15	Other	N	$CHCl_3$	SPE Silica	Y	C18	Isocratic	MeOH:H_2O:CH_3COOH	0.5	100	40	nr
L29	Y	Multi	10	LSE	N	MeOH:H_2O (9:1)	-	Y	C18	Isocratic	MeOH-H2O (94:6) with 0.1% CH_3COOH	0.7	100	40	room temperature
L30	N	Multi	2	LSE	Y	MeOH/ACN/ NaOH	-	N	XDB-C8	Gradient	H_2O/ACN/CH_3COOH	0.4	40	40	nr
L31	Y	Multi	5	LSE	Y	0.4% DMF in ACN	SPE Silica	N	Varian, Pursuit XRs C8, 2.8 µm	nr	0.1% HCOOH in H_2O 0.1% HCOOH in ACN/0.1% HCOOH in ACN	0.3	15	55	4

	Detection													Quantification				Limits of detection & quantification	
	UV-PCD	Fluorescence		Mass spectrometry										External calibration	Matrix-match calibration	Standard addition	Internal standard	LOD	LOQ
	λ (nm)	λexc. (nm)	λem. (nm)	Triple quadrupole					TOF		Qtrap								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L01	-	-	-	5500	29	688	461	635	-	-	-	-	-	nr	nr	nr	N	0.03	0.100
L02	-	-	-	4500	35/35	688.4	6,5,5	461.3	-	-	-	-	-	-	-	Y	N	0.013	0.025
L03	-	-	-	134	50 - 52	693.4	461.1	479.2	-	-	-	-	-	-	Y	-	Nigericin	0.025	0.050
L05	-	-	-	50	50/38	693.40	461.50	675.50	-	-	-	-	-	-	Y	-	Nigericin	< ML (not determined)	< ML (not determined)
L06	-	-	-	35	50/55	693.25	461.3	479.3	-	-	-	-	-	Y	-	-	N	nr	0.007
L07	-	-	-	68	52/38	693.1	461.5	675.1	-	-	-	-	-	-	Y	-	N	0.01	0.03
L08	-	-	-	65	38; 50	693.3	461.2	675.2	-	-	-	-	-	-	Y	-	Nigericin-Na	nr	0.200
L09	-	-	-	55	33	693.6	675.1	461	-	-	-	-	-	-	Y	-	N	0.01	0.025
L10	-	-	-	111	51	693.32	461.3	479.3	-	-	-	-	-	-	Y	-	Nigericin	0.3	0.6
L11	-	-	-	+ 4500	25	688.4	635.4	461.3	-	-	-	-	-	-	Y	-	Nigericin-Na	0.332	0.894
L12	-	-	-	5500	nr	nr	nr	nr	-	-	-	-	-	-	-	Y	N	0.005	0.01
L13	-	-	-	nr	50/35	688.5	461.3	635.1	-	-	-	-	-	-	-	Y	N	0.004	0.01
L14	-	-	-	111	49	693.42	675.1	461.38	-	-	-	-	-	-	-	Y	N	0.02	0.05
L15	-	-	-	not applicable	54/34	693.4	461.2	675.4	-	-	-	-	-	-	-	Y	Nigericin	0.049	156
L16	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.10	0.50
L17	-	-	-	4500	41, 55	693.4	675.3	461.5	-	-	-	-	-	-	Y	-	N	0.274	0.830
L18	-	-	-	4200	51;52	693	501	479	-	-	-	-	-	-	Y	-	Nigericin	0.0032	0.0032
L19	-	-	-	4000V	34	693.3	501.805	675.597	-	-	-	-	-	-	Y	-	Nigericin	nr	0.25
L20	-	-	-	22	25	688.2	421	461	-	-	-	-	-	-	Y	-	Nigericin	nr	0.01
L21	-	-	-	67	40	693.5	461	675	-	-	-	-	-	-	Y	-	IPZOH-D3	0.250	0.500
L22	-	-	-	110	42/ 63	693.5	675.4	461.4	-	-	-	-	-	-	Y	-	N	nr	0.325
L23	-	-	-	250	39	693.4	675.4	461.3	-	-	-	-	-	Y	-	-	Nigericin	0.0074	0.0123
L24	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.3	1.0
L25	-	-	-	4500/600	20	688.5	617.5	635.5	-	-	-	-	-	-	-	Y	Monensin	0.38	1.25
L26	-	-	-	62	52	693.5	479.3	461.3	-	-	-	-	-	-	-	Y	Nigericin	0.04	0.125
L27	-	-	-	20	54	693.2	461.3	479.2	-	-	-	-	-	nr	nr	nr	nr	0.1	0.1
L28	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.03	0.1
L29	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	LOQ/3	1
L30	-	-	-	-	-	-	-	-	-	-	693.5	501.3	581.2	-	-	Y	N	nr	0.625
L31	-	-	-	35	38/49	693.57	675.17	461.21	-	-	-	-	-	-	Y	-	nr	nr	0.625

C1: Extraction + centrifugation + filtration on paper filters + filtration on Si cartridges + evaporation + reconstitution (ACN + 0.1% formic acid) + defatting with hexane (saturated with ACN)

C2: H2O, ACN extraction followed by addition of Magnesium Sulphate and Sodium Chloride, agitated and separated by centrifugation. Precipitation of impurities by freezing at -80 °C, extraction and further centrifugation to separate solids.

Narasin

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	Multi/Single	Test portion (g)	Extraction	Dilution	Solvent	Clean-up	Guard column	Column	Elution mode	Mobile phase	flow rate (ml min^{-1})	inj. Vol. (µl)	Col. Temp. (°C)	Autosampler Temp. (°C)
L01	Y	Multi	1	LSE	N	ACN/NH_3	n-hexane	N	Xterra18ec 50x2.1mm, 3.5um	Gradient	MeOH/H_2O/CH_3COONH_4	0.3	10	40	20
L02	Y	Multi	2.5	LSE	N	MeOH/H_2O	-	Y	Phenomenex GEMINI 150*2 mm, 5 µm	Gradient	A: ACN/H20/NH4AC B. ACN/H2O/MeOH/THF/HCOOH	0.3	10	40	15
L03	Y	Multi	5	LSE	Y	MeOH	-	N	C18	Gradient	ACN, H2O, FA	0.2	10	35	off
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B. H_2O + 1% HCOOH	0.35	40	30	10
L06	Y	Multi	1	LSE	Y	MeOH/H_2O	Centrifuge	N	C18	Gradient	MeOH/H_2O	0.3	50	30	20
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L08	N	Multi	1	LSE	N	ACN	SPE (Oasis HLB)	N	UPLC BEH Phenyl	Gradient	HCOOH in H_2O; HCOOH in ACN	0.5	2-5	50	15
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L10	N	Multi	5	LSE	Y	ACN/MeOH	-	Y	C8,Symmetry	Gradient	H_2O/MeOH/ACN/HCOOH	0.35	10	25	4
L11	N	Multi	2.5	LSE	N	ACN	-	Y	C8	Gradient	ACN + 5 mM CH_3COONH_4 containing 0.5% CH_3COOH	0.2	5	50	room temperature
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	Y	Multi	nr	LSE	N	ACN/MeOH 90/10 v/v	-	Y	C18, 5µm, 110 A	Gradient	10mM CH_3COONH_4/ACN	0.3	5	25	room temperature
L14	Y	Multi	10	LSE	N	MeOH/H_2O 90/10	-	Y	Pursuit 3 C 18 150 x 2 mm	Gradient	ACN+HCOOH/H2O+HCOOH	0.25	5	40	15
L15	Y	Multi	1	LSE	Y	ACN	-	Y	C18	Gradient	H_2O/MeOH/HCOOH	0.35	5	40	4
L16	Y	Multi	5	LSE	N	MeOH/H_2O	-	Y	C18	Isocratic	CH_3COOH/MeOH	0.9	100	30	15
L17	Y	Multi	2.5	LSE	Y	H_2O, ACN	C2	Y	nr	Gradient	nr	nr	nr	nr	nr
L18	Y	Multi	2 or 5 depending on expected concentration	LSE	Y	ACN	SPE or dilution depending on expected concentration	Y	C18	Gradient	A) ACN 20 µM CH_3COONa + 0.1% HCOOH; B) 0,1% HCOOH	0.25	5	40	16
L19	Y	Multi	2	LSE	Y	MeOH/H2O 90/10	-	Y	RP-C18	Gradient	A: HCOOH 0.1% in H_2O B: (HCOOH 0.1%+20 µM CH_3COONa) in CH3CN	0.2	5	40	15
L20	N	Multi	1	LSE	N	ACN	-	Y	nr	Gradient	H_2O, ACN, CH3COONH3, HCOOH	0.3	20	40	nr
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L22	N	Multi	5	LSE	N	ACN, MeOH	C18 dispersive	Y	Poroshell 120 EC-C18	nr	ACN, MeOH, HCOONH3	0.3	5	50	room temperature
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L24	Y	Multi	5	LSE	N	MeOH/H2O 90/10	-	Y	C18	Isocratic	MeOH:H_2O:CH_3COOH 94:6:0.1	0.9	100	30	nr
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	Y	Multi	10	LSE	N	MeOH:H_2O (9:1)	-	Y	C18	Isocratic	MeOH-H2O (94:6) with 0.1% CH_3COOH	0.7	100	40	room temperature
L30	Y	Multi	2	LSE	Y	MeOH/ACN/ NaOH	-	N	XDB-C8	Gradient	H_2O/ACN/CH_3COOH	0.4	40	40	nr
L31	Y	Multi	5	LSE	Y	0.4% DMF in ACN	SPE Silica	N	Varian, Pursuit XRs C8, 2.8 µm	Gradient	0.1% HCOOH in H_2O 0.1% HCOOH in ACN/0.1% HCOOH in ACN	0.3	15	55	4

	Detection														Quantification				Limits of detection & quantification	
	UV-PCD	Fluorescence		Mass spectrometry											External calibration	Matrix-match calibration	Standard addition	Internal standard	LOD	LOQ
	λ (nm)	λexc. (nm)	λem. (nm)	Triple quadrupole					TOF		Qtrap									
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z							
L01	-	-	-	5500	32	782	729	748	-	-	-	-	-	nr	nr	nr	N	0.03	0.100
L02	-	-	-	4500	29/41	782.4	747.5	726.9	-	-	-	-	-	-	-	Y	N	0.013	0.025
L03	-	-	-	170	42 - 53	787.5	431	279.3	-	-	-	-	-	-	Y	-	Nigericin	0.025	0.050
L05	-	-	-	55	53/45	787,5	431,5	531,5	-	-	-	-	-	-	Y	-	Nigericin	< ML (not determined)	< ML (not determined)
L06	-	-	-	35	50/55	787.6	431.3	279.1	-	-	-	-	-	Y	-	-	N	nr	0.01
L07	-	-	-	76	52/46	787.3	431.5	531.5	-	-	-	-	-	-	Y	-	N	0.01	0.03
L08	-	-	-	60	50; 45	787.2	431.1	531.1	-	-	-	-	-	-	Y	-	Nigericin-Na	nr	0.125
L09	-	-	-	60	48	787.3	430.7	530.8	-	-	-	-	-	-	Y	-	N	0.01	0.025
L10	-	-	-	121	57	787.39	431.3	531.4	-	-	-	-	-	-	Y	-	Nigericin	0.15	0.3
L11	-	-	-	+ 4500	29	782.6	747.4	729.5	-	-	-	-	-	-	Y	-	Nigericin-Na	0.133	0.363
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	-	-	-	nr	41/35	782.4	729.7	747	-	-	-	-	-	-	-	Y	N	0.004	0.01
L14	-	-	-	106	67	787.6	431.4	531.5	-	-	-	-	-	-	-	Y	N	0.03	0.1
L15	-	-	-	not applicable	52/52	787.5	431.2	531.2	-	-	-	-	-	-	-	Y	Nigericin	0.041	131
L16	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.20	0.50
L17	-	-	-	4500	50, 45	787.4	431.4	531.5	-	-	-	-	-	-	Y	-	N	0.127	0.384
L18	-	-	-	4200	46;51	787	531	431	-	-	-	-	-	-	Y	-	Nigericin	0.0032	0.0032
L19	-	-	-	4000V	47	787.5	413.332	431.293	-	-	-	-	-	-	Y	-	Nigericin	nr	0.25
L20	-	-	-	30	34	782.3	321	373	-	-	-	-	-	-	Y	-	Nigericin	nr	0.01
L21	-	-	-	120	60	787.6	531	431	-	-	-	-	-	-	Y	-	IPZOH-D3	0.100	0.200
L22	-	-	-	110	56/ 62	787.6	531.4	431.4	-	-	-	-	-	-	Y	-	N	nr	0.175
L23	-	-	-	45	59	787.4	431.3	531.3	-	-	-	-	-	Y	-	-	Nigericin	0.0046	0.0077
L24	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.4	0.7
L25	-	-	-	4500/600	31	782.6	373.2	747.5	-	-	-	-	-	-	-	Y	Narasin	0.21	0.7
L26	-	-	-	74	52	787.7	431.3	531.4	-	-	-	-	-	-	-	Y	Nigericin	0.02	0.07
L27	-	-	-	60	100	787.5	431.2	531.2	-	-	-	-	-	nr	nr	nr	nr	0.1	0.1
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	LOQ/3	2
L30	-	-	-	-	-	-	-	-	-	-	787.5	413.2	279.1	-	Y	-	N	nr	0.35
L31	-	-	-	35	50/43	787.68	431.32	531.31	-	-	-	-	-	-	Y	-	nr	nr	0.35

C1: Extraction + centrifugation + filtration on paper filters + filtration on Si cartridges + evaporation + reconstitution (ACN + 0.1% formic acid) + defatting with hexane (saturated with ACN)

C2: H2O, ACN extraction followed by addition of Magnesium Sulphate and Sodium Chloride, agitated and separated by centrifugation. Precipitation of impurities by freezing at -80 °C, extraction and further centrifugation to separate solids.

Lasalocid

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	Multi/Single	Test portion (g)	Extraction	Dilution	Solvent	Clean-up	Guard column	Column	Elution mode	Mobile phase	flow rate (ml min^{-1})	inj. Vol. (µl)	Col. Temp. (°C)	Autosampler Temp. (°C)
L01	Y	Multi	1	LSE	N	ACN/NH_3	n-hexane	N	Xterra18ec 50x2.1mm, 3.5µm	Gradient	MeOH/H_2O/CH_3COONH_4	0.3	10	40	20
L02	Y	Multi	2.5	LSE	N	MeOH/H_2O	-	Y	Phenomenex GEMINI 150*2 mm, 5 µm	Gradient	A: ACN/H20/NH4AC B. ACN/H2O/MeOH/THF/HCOOH	0.3	10	40	15
L03	Y	Multi	5	LSE	Y	MeOH	-	N	C18	Gradient	ACN, H2O, FA	0.2	10	35	off
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L06	Y	Multi	1	LSE	Y	MeOH/H_2O	Centrifuge	N	C18	Gradient	MeOH/H_2O	0.3	50	30	20
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L08	N	Multi	1	LSE	N	ACN	SPE (Oasis HLB)	N	UPLC BEH Phenyl	Gradient	HCOOH in H_2O; HCOOH in ACN	0.5	2	50	15
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L10	N	Multi	5	LSE	Y	ACN/MeOH	-	Y	C8,Symmetry	Gradient	H_2O/MeOH/ACN/HCOOH	0.35	10	25	4
L11	N	Multi	2.5	LSE	N	ACN	-	Y	C8	Gradient	ACN + 5 mM CH_3COONH_4 containing 0.5% CH_3COOH	0.2	5	50	room temperature
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	Y	Multi	nr	LSE	N	ACN/MeOH 90/10 v/v	-	Y	C18, 5µm, 110 A	Gradient	10mM CH_3COONH_4/ACN	0.3	5	25	room temperature
L14	Y	Multi	10	LSE	N	MeOH/H_2O 90/10	-	Y	Pursuit 3 C 18 150 x 2 mm	Gradient	ACN+HCOOH/$H_2$0+HCOOH	0.25	5	40	15
L15	Y	Multi	1	LSE	Y	ACN	-	Y	C18	Gradient	H_2O/MeOH/HCOOH	0.35	5	40	4
L16	Y	Multi	5	LSE	N	MeOH/H_2O	-	Y	C18	Isocratic	CH_3COOH/MeOH	0.9	100	30	15
L17	Y	Multi	2.5	LSE	Y	H_2O, ACN	C2	Y	nr	Gradient	nr	nr	nr	nr	nr
L18	Y	Multi	2 or 5 depending on expected concentration	LSE	Y	ACN	SPE or dilution depending on expected concentration	Y	C18	Gradient	A) ACN 20 µM CH_3COONa + 0.1% HCOOH; B) 0,1% HCOOH	0.25	5	40	16
L19	Y	Multi	2	LSE	Y	MeOH/H2O 90/10	-	Y	RP-C18	Gradient	A: HCOOH 0.1% in H_2O B: (HCOOH 0.1%+20 µM CH_3COONa) in CH3CN	0.2	5	40	15
L20	N	Multi	1	LSE	N	ACN	-	Y	nr	Gradient	H_2O, ACN, CH3COONH3, HCOOH	0.3	20	40	nr
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L22	N	Multi	5	LSE	N	ACN, MeOH	C18 dispersive	Y	Poroshell 120 EC-C18	nr	ACN, MeOH, $HCOONH_3$	0.3	5	50	room temperature
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L24	Y	Multi	5	Other	N	MeOH + 0.5% acid	-	Y	C18	Isocratic	ACN:CH3COONH3 buffer 90:10	1	20	25	nr
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	Y	Single	10	Ultrasonic bath	N	MeOH	-	Y	C18	Isocratic	MeOH-Phosphate buffer	0.6	20	40	room temperature
L30	N	Multi	2	LSE	Y	MeOH/ACN/ NaOH	-	N	XDB-C8	Gradient	H_2O/ACN/CH_3COOH	0.4	40	40	nr
L31	Y	Multi	5	LSE	Y	0.4% DMF in ACN	SPE Silica	N	Varian, Pursuit XRs C8, 2.8 µm	Gradient	0.1% HCOOH in H_2O 0.1% HCOOH in ACN/0.1% HCOOH in ACN	0.3	15	55	4

	Detection													Quantification				Limits of detection & quantification	
	UV-PCD	Fluorescence		Mass spectrometry										External calibration	Matrix-match calibration	Standard addition	Internal standard	LOD	LOQ
	λ (nm)	λexc. (nm)	λem. (nm)	Triple quadrupole					TOF		Qtrap								
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z						
L01	-	-	-	5500	28	608	237	337	-	-	-	-	-	nr	nr	nr	N	0.03	0.100
L02	-	-	-	-4500	-50/-20	-52/-20	589.4	235	173	-	-	-	-	-	-	Y	N	0.013	0.025
L03	-	-	-	108	41 - 32	613.3	377	577.1	-	-	-	-	-	-	Y	-	Nigericin	0.025	0.050
L05	-	-	-	55	35	613,3	377,5	577,5	-	-	-	-	-	-	Y	-	N	< ML (not determined)	< ML (not determined)
L06	-	-	-	50	33/37	613.2	377.2	577.4	-	-	-	-	-	Y	-	-	N	nr	0.02
L07	-	-	-	54	38/28	613	377.4	577.5	-	-	-	-	-	-	Y	-	N	0.01	0.03
L08	-	-	-	50	37; 29	613.2	377.2	595.2	-	-	-	-	-	-	Y	-	Nigericin-Na	nr	0.145
L09	-	-	-	40	35	612.98	376.9	358.9	-	-	-	-	-	-	Y	-	N	0.04	0.08
L10	-	-	-	81	69	613.3	377.2	359.2	-	-	-	-	-	-	Y	-	Nigericin	0.3	0.6
L11	-	-	-	+ 4500	17	608.5	591.4	573.4	-	-	-	-	-	-	Y	-	N	0.375	0.950
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	-	-	-	nr	-46	589.2	234.9	172.9	-	-	-	-	-	-	-	Y	N	0.0004	0.001
L14	-	-	-	106	47	613.4	337.4	577.4	-	-	-	-	-	-	-	Y	N	0.02	0.05
L15	-	-	-	not applicable	36/28	613.4	377.1	595.2	-	-	-	-	-	-	-	Y	Nigericin	0.038	123
L16	-	310	419	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.10	0.50
L17	-	-	-	4500	32, 32	613.3	377.2	577.2	-	-	-	-	-	-	Y	-	N	0.283	0.859
L18	-	-	-	4200	28;37	613	595	377	-	-	-	-	-	-	Y	-	Nigericin	0.0032	0.0032
L19	-	-	-	4000	34	613.4	359.465	377.37	-	-	-	-	-	-	Y	-	Nigericin	nr	0.25
L20	-	-	-	50	40	613	3559	377	-	-	-	-	-	-	Y	-	Nigericin	nr	0.01
L21	-	-	-	90	45	613.4	377.4	595.3	-	-	-	-	-	-	Y	-	IPZOH-D3	0.250	0.500
L22	-	-	-	110	55/66	613.5	577.8	557.4	-	-	-	-	-	-	Y	-	N	nr	0.312
L23	-	-	-	227	39	613.3	377.3	595.4	-	-	-	-	-	Y	-	-	Nigericin	0.0130	0.0217
L24	-	214	418	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.5	1.0
L25	-	-	-	4500/600	16.5	608.4	337.2	237.2	-	-	-	-	-	-	-	Y	Lasalocid	0.38	1.25
L26	-	-	-	56	34	613.5	577.3	377.3	-	-	-	-	-	-	-	Y	Nigericin	0.04	0.125
L27	-	-	-	55	45	613.1	377.2	577.2	-	-	-	-	-	nr	nr	nr	nr	0.05	0.05
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	-	380	420	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	LOQ/3	1
L30	-	-	-	-	-	-	-	-	-	-	613.4	377.3	577.3	-	-	Y	N	nr	0.625
L31	-	-	-	35	36/36	613.65	377.27	359.29	-	-	-	-	-	-	Y	-	nr	nr	0.625

C1: Extraction + centrifugation + filtration on paper filters + filtration on Si cartridges + evaporation + reconstitution (ACN + 0.1% formic acid) + defatting with hexane (saturated with ACN)

C2: H2O, ACN extraction followed by addition of Magnesium Sulphate and Sodium Chloride, agitated and separated by centrifugation. Precipitation of impurities by freezing at -80 °C, extraction and further centrifugation to separate solids.

Diclazuril

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	Multi/Single	Test portion (g)	Extraction	Dilution	Solvent	Clean-up	Guard column	Column	Elution mode	Mobile phase	flow rate (ml min^{-1})	inj. Vol. (µl)	Col. Temp. (°C)	Autosampler Temp. (°C)
L01	N	Multi	1	LSE	N	ACN/NH_3	n-hexane	N	Xterra18ec 50x2.1mm, 3.5µm	Gradient	MeOH/H_2O/CH_3COONH_4	0.3	10	40	20
L02	Y	Multi	2.5	LSE	N	MeOH/H_2O	-	Y	Phenomenex GEMINI 150*2 mm, 5 µm	Gradient	A: ACN/H20/NH4AC B. ACN/H2O/MeOH/THF/HCOOH	0.3	10	40	15
L03	Y	Multi	5	LSE	Y	MeOH	-	N	C18	Gradient	ACN, H2O, FA	0.2	10	35	off
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L06	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L08	N	Multi	1	LSE	N	ACN	SPE (Oasis HLB)	N	UPLC BEH Phenyl	Gradient	HCOOH in H_2O; HCOOH in ACN	0.5	5	50	15
L09	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L10	N	Multi	5	LSE	Y	ACN/MeOH	-	Y	C8,Symmetry	Gradient	H_2O/MeOH/ACN/HCOOH	0.35	10	25	4
L11	N	Multi	2.5	LSE	N	ACN	-	Y	C8	Gradient	ACN + 5 mM CH_3COONH_4 containing 0.5% CH_3COOH	0.2	5	50	room temperature
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	Y	Multi	nr	LSE	N	ACN/MeOH 90/10 v/v	-	Y	C18, 5µm, 110 A	Gradient	10mM CH_3COONH_4/ACN	0.3	5	25	room temperature
L14	Y	Multi	10	LSE	N	MeOH/H_2O 90/10	-	Y	Pursuit 3 C 18 150 x 2 mm	Gradient	ACN+HCOOH/H_2O+HCOOH	0.25	5	40	15
L15	Y	Multi	1	LSE	Y	ACN	-	Y	C18	Gradient	H_2O/MeOH/HCOOH	0.35	5	40	4
L16	Y	Multi	5	LSE	N	MeOH/H_2O	-	Y	C18	Isocratic	CH_3COOH/MeOH	0.9	100	30	15
L17	Y	Multi	2.5	LSE	Y	H_2O, ACN	C2	Y	nr	Gradient	nr	nr	nr	nr	nr
L18	Y	Multi	2 or 5 depending on expected concentration	LSE	Y	ACN	SPE or dilution depending on expected concentration	Y	C18	Gradient	A) ACN 20 µM CH_3COONa + 0.1% HCOOH; B) 0.1% HCOOH	0.25	5	40	16
L19	Y	Multi	2	LSE	Y	MeOH/H2O 90/10	-	Y	RP-C18	Gradient	A: HCOOH 0.1% in H_2O B: (HCOOH 0.1%+20 µM CH_3COONa) in CH3CN	0.2	5	40	15
L20	N	Multi	1	LSE	N	ACN	-	Y	nr	Gradient	H_2O, ACN, CH3COONH3, HCOOH	0.3	20	40	nr
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L22	N	Multi	5	LSE	N	ACN, MeOH	C18 dispersive	Y	Poroshell 120 EC-C18	nr	ACN, MeOH, $HCOONH_3$	0.3	5	50	room temperature
L23	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L24	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	MCIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	Y	Single	15	LSE	N	MeOH:HCl 25% 995:5	SPE	Y	C18	Gradient	H2O (buffer)/MeOH-ACN	1	20	40	room temperature
L30	Y	Multi	2	LSE	Y	MeOH/ACN/ NaOH	-	N	XDB-C8	Gradient	H_2O/ACN/CH_3COOH	0.4	40	40	nr
L31	Y	Multi	5	LSE	Y	0.4% DMF in ACN	SPE Silica	N	Varian, Pursuit XRs C8, 2.8 µm	Gradient	0.1% HCOOH in H_2O 0.1% HCOOH in ACN/0.1% HCOOH in ACN	0.3	15	55	4

	Detection														Quantification				Limits of detection & quantification	
	UV-PCD	Fluorescence		Mass spectrometry											External calibration	Matrix-match calibration	Standard addition	Internal standard	LOD	LOQ
	λ (nm)	λexc. (nm)	λem. (nm)	Triple quadrupole					TOF		Qtrap									
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z							
L01	-	-	-	5500	-34	405	299	334	-	-	-	-	-	nr	nr	nr	N	0.001	0.0033
L02	-	-	-	-4500	-28/-26	405/407	333.7	335.8	-	-	-	-	-	-	-	Y	N	0.003	0.005
L03	-	-	-	77	21 - 22	406.9	336	337.1	-	-	-	-	-	-	Y	-	Diclazuril-IS	0.0025	0.0050
L05	-	-	-	25	17	404,8/406,8	334	336	-	-	-	-	-	-	Y	-	Nicarbazin-d8	< ML (not determined)	< ML (not determined)
L06	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L07	-	-	-	-35	22/22	404.7/406.8	334	336.1	-	-	-	-	-	-	Y	-	N	0.01	0.03
L08	-	-	-	35	19	404,85; 406,85	333.9	335.9	-	-	-	-	-	-	Y	-	Methyl-diclazuril	nr	0.0015
L09	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L10	-	-	-	-75	-20	404.9	333.9	298.9	-	-	-	-	-	-	Y	-	bis-diclazuril	0.005	0.01
L11	-	-	-	-4500	- 28	407	335.6	nr	-	-	-	-	-	-	Y	-	bis-diclazuril	0.001	0.003
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	-	-	-	nr	-26	405.1	333.6	335.8	-	-	-	-	-	-	-	Y	N	0.0004	0.001
L14	-	-	-	-75	-26	404.865	333.9	335.8	-	-	-	-	-	-	-	Y	N	0.01	0.025
L15	-	-	-	not applicable	20/20	407/405	335.9	333.9	-	-	-	-	-	-	-	Y	Nigericin	0.009	32
L16	-	-	-	-	-	-	-	-	50	389.25	-	-	-	Y	-	-	N	0.004	0.01
L17	-	-	-	4500	22, 20	405, 407	333.9	335.9	-	-	-	-	-	-	Y	-	bis-Diclazuril	0.001	0.003
L18	-	-	-	3000	21;27	405	334	299	-	-	-	-	-	-	Y	-	Diclazuril-bis	0.0032	0.0032
L19	-	-	-	4000	25	405.98	334.155	336.173	-	-	-	-	-	-	Y	-	DNC-d8	nr	0.005
L20	-	-	-	35	21	405.2	334	336	-	-	-	-	-	-	Y	-	Nicarbazin-d8	nr	0.01
L21	-	-	-	-55	-20	407; 405.1	336	334.2	-	-	-	-	-	-	Y	-	Methyldiclazuril	0.0025	0.005
L22	-	-	-	-80	-25	407/ 405	336	334	-	-	-	-	-	-	Y	-	N	nr	0.025
L23	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L24	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L25	-	-	-	4500/600	12,0 V	403.4	332.1	333.4	-	-	-	-	-	-	-	Y	Diclazuril	0.003	0.01
L26	-	-	-	25	25	404.9	334	/	-	-	-	-	-	-	-	Y	Nigericin	0.002	0.005
L27	-	-	-	30	22	405,0-407,1	333.8	336.1	-	-	-	-	-	nr	nr	nr	nr	0.01	0.01
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	280	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	LOQ/3	0.1
L30	-	-	-	-	-	-	-	-	-	-	405	334	299.2	-	Y	-	Methyldiclazuril	nr	0.005
L31	-	-	-	35	26/25	404.92	334.07	336.09	-	-	-	-	-	-	Y	-	nr	nr	0.005

C1: Extraction + centrifugation + filtration on paper filters + filtration on Si cartridges + evaporation + reconstitution (ACN + 0.1% formic acid) + defatting with hexane (saturated with ACN)

C2: H2O, ACN extraction followed by addition of Magnesium Sulphate and Sodium Chloride, agitated and separated by centrifugation. Precipitation of impurities by freezing at -80 °C, extraction and further centrifugation to separate solids.

Salinomycin

			Sample preparation					HPLC - reverse phase							
	Acc. Meth.	Multi/Single	Test portion (g)	Extraction	Dilution	Solvent	Clean-up	Guard column	Column	Elution mode	Mobile phase	flow rate (ml min^{-1})	inj. Vol. (µl)	Col. Temp. (°C)	Autosampler Temp. (°C)
L01	Y	Multi	1	LSE	N	ACN/NH_3	n-hexane	N	Xterra18ec 50x2.1mm, 3.5µm	Gradient	MeOH/H_2O/CH_3COONH_4	0.3	10	40	20
L02	Y	Multi	2.5	LSE	N	MeOH/H_2O	-	Y	Phenomenex GEMINI 150*2 mm, 5 µm	nr	A: ACN/H20/NH4AC B. ACN/H2O/MeOH/THF/HCOOH	0.3	10	40	15
L03	Y	Multi	5	LSE	Y	MeOH	-	N	C18	Gradient	ACN, H2O, FA	0.2	10	35	off
L05	N	Multi	3	LSE	N	ACN/MeOH 90/10 %(v/v)	C1	Y	Symmetry C8, 3.5 µm, 2.1x50 mm	Gradient	A: ACN + 1% HCOOH; B: H_2O + 1% HCOOH	0.35	40	30	10
L06	Y	Multi	1	LSE	Y	MeOH/H_2O	Centrifuge	N	C18	Gradient	MeOH/H_2O	0.3	50	30	20
L07	N	Multi	2.5	LSE	Y	ACN	Quechers principle	N	UPLC BEH C18	Gradient	MeOH/H_2O	0.4	5	40	5
L08	N	Multi	1	LSE	N	ACN	SPE (Oasis HLB)	N	UPLC BEH Phenyl	Gradient	HCOOH in H_2O; HCOOH in ACN	0.5	2	50	15
L09	Y	Multi	5	LSE	N	ACN : H2O (84:16)	-	Y	C18	Isocratic	ACN : 2 % CH3COOH + 2 mM CH3COONH4 (95:5)	0.5	10	room temperature	room temperature
L10	N	Multi	5	LSE	Y	ACN/MeOH	-	Y	C8,Symmetry	Gradient	H_2O/MeOH/ACN/HCOOH	0.35	10	25	4
L11	N	Multi	2.5	LSE	N	ACN	-	Y	C8	Gradient	ACN + 5 mM CH_3COONH_4 containing 0.5% CH_3COOH	0.2	5	50	room temperature
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	Y	Multi	nr	LSE	N	ACN/MeOH 90/10 v/v	-	Y	C18, 5µm, 110 A	Gradient	10mM CH_3COONH_4/ACN	0.3	5	25	room temperature
L14	Y	Multi	10	LSE	N	MeOH/H_2O 90/10	-	Y	Pursuit 3 C 18 150 x 2 mm	Gradient	ACN+HCOOH/H_2O+HCOOH	0.25	5	40	15
L15	Y	Multi	1	LSE	Y	ACN	-	Y	C18	Gradient	H_2O/MeOH/HCOOH	0.35	5	40	4
L16	Y	Multi	5	LSE	N	MeOH/H_2O	-	Y	C18	Isocratic	CH_3COOH/MeOH	0.9	100	30	15
L17	Y	Multi	2.5	LSE	Y	H_2O, ACN	C2	Y	nr	Gradient	nr	nr	nr	nr	nr
L18	Y	Multi	2 or 5 depending on expected concentration	LSE	Y	ACN	SPE or dilution depending on expected concentration	Y	C18	Gradient	A) ACN 20 µM CH_3COONa + 0.1% HCOOH; B) 0,1% HCOOH	0.25	5	40	16
L19	Y	Multi	2	LSE	Y	MeOH/H2O 90/10	-	Y	RP-C18	Gradient	A: HCOOH 0.1% in H_2O B: (HCOOH 0.1%+20 µM CH_3COONa) in CH3CN	0.2	5	40	15
L20	N	Multi	1	LSE	N	ACN	-	Y	nr	Gradient	H_2O, ACN, CH3COONH3, HCOOH	0.3	20	40	nr
L21	N	Multi	2	Ultrasonic bath	N	ACN	Filtration	Y	C18	Gradient	MeOH/0.1% HCOOH	0.6	10	50	20
L22	N	Multi	5	LSE	N	ACN, MeOH	C18 dispersive	Y	Poroshell 120 EC-C18	nr	ACN, MeOH, $HCOONH_3$	0.3	5	50	room temperature
L23	N	Multi	5	LSE	N	ACN	-	Y	Poroshell 120 EC-C19	Gradient	0.1% HCOOH in H_2O / 0.1% HCOOH in ACN	1	10	25	25
L24	Y	Multi	5	LSE	N	MeOH/H2O 90/10	-	Y	C18	Isocratic	MeOH:H_2O:CH_3COOH 94:6:0.1	0.9	100	30	nr
L25	N	Multi	5	Other	Y	MeOH/H2O/ HCOOH	-	Y	C18	Gradient	MeOH/H_2O/ $HCOONH_3$ 10 mM	0.3	20	35	room temperature
L26	Y	Multi	5	Ultrasonic bath	N	ACN	Freezing	N	C18	Gradient	ACN, H_2O	0.5	10	30	room temperature
L27	N	Multi	4	LSE	Y	McIlvaine-ACN-MeOH-EDTA	-	Y	Kinetex C18	Gradient	HCOOH-ACN	0.5	8	35	8
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	Y	Multi	10	LSE	N	MeOH:H_2O (9:1)	-	Y	C18	Isocratic	MeOH-H2O (94:6) with 0.1% CH_3COOH	0.7	100	40	room temperature
L30	Y	Multi	2	LSE	Y	MeOH/ACN/ NaOH	-	N	XDB-C8	Gradient	H_2O/ACN/CH_3COOH	0.4	40	40	nr
L31	Y	Multi	5	LSE	Y	0.4% DMF in ACN	SPE Silica	N	Varian, Pursuit XRs C8, 2.8 µm	Gradient	0.1% HCOOH in H_2O 0.1% HCOOH in ACN/0.1% HCOOH in ACN	0.3	15	55	4

	Detection															Quantification				Limits of detection & quantification (mg	
	UV-PCD	Fluorescence		Mass spectrometry												External calibration	Matrix-match calibration	Standard addition	Internal standard	LOD	LOQ
	λ (nm)	λexc. (nm)	λem. (nm)	Triple quadrupole				TOF		Qtrap											
				Cone voltage (V)	Collision energy (eV)	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z	Cone voltage (V)	parent ion m/z	parent ion m/z	daughter ion 1 m/z	daughter ion 2 m/z								
L01	-	-	-	5500	32	768	715	734	-	-	-	-	-	nr	nr	nr	N	0.03	0.100
L02	-	-	-	4500	27/39	768.5	733.5	715.4	-	-	-	-	-	-	-	Y	N	0.013	0.025
L03	-	-	-	135	44 - 46	773.5	431	413	-	-	-	-	-	-	Y	-	Nigericin	0.025	0.050
L05	-	-	-	55	50/55	773,3	431,40	531,3	-	-	-	-	-	-	Y	-	? Nigericin	< ML (not determined)	< ML (not determined)
L06	-	-	-	35	50/55	773.3	431.3	265.15	-	-	-	-	-	Y	-	-	N	nr	0.01
L07	-	-	-	75	52/46	773.2	431.4	531.5	-	-	-	-	-	-	Y	-	N	0.01	0.03
L08	-	-	-	60	47; 48	773.3	431.2	413.1	-	-	-	-	-	-	Y	-	Nigericin-Na	nr	0.14
L09	-	-	-	58	50	773.1	430.7	530.8	-	-	-	-	-	-	Y	-	N	0.01	0.025
L10	-	-	-	111	81	773.37	431.3	531.3	-	-	-	-	-	-	Y	-	Nigericin	0.15	0.3
L11	-	-	-	+ 4500	27	768.5	733.4	715.4	-	-	-	-	-	-	Y	-	Nigericin-Na	0.117	0.320
L12	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L13	-	-	-	nr	37/27	768.9	715.6	733.6	-	-	-	-	-	-	-	Y	N	0.004	0.01
L14	-	-	-	96	69	773.4	431.3	531.4	-	-	-	-	-	-	-	Y	N	0.03	0.1
L15	-	-	-	not applicable	52/48	773.5	431.2	531.3	-	-	-	-	-	-	-	Y	Nigericin	0.026	86
L16	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.20	0.50
L17	-	-	-	4500	50, 45	773.5	431	531.4	-	-	-	-	-	-	Y	-	N	0.132	0.400
L18	-	-	-	4200	49;49	773	531	431	-	-	-	-	-	-	Y	-	Nigericin	0.0032	0.0032
L19	-	-	-	4000V	47	773.5	265.125	431.28	-	-	-	-	-	-	Y	-	Nigericin		0.25
L20	-	-	-	32	35	768	225	373	-	-	-	-	-	-	Y	-	Nigericin	-	0.01
L21	-	-	-	167	65	773.6	431.3	265.1	-	-	-	-	-	-	Y	-	IPZOH-D3	0.075	0.150
L22	-	-	-	110	55/ 61	773.6	531.4	431.4	-	-	-	-	-	-	Y	-	N	nr	0.175
L23	-	-	-	270	55	773.5	431.3	531.3	-	-	-	-	-	Y	-	-	Nigericin	0.0006	0.0011
L24	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	0.4	0.7
L25	-	-	-	4500/600	45 V	773.5	431.3	531.2	-	-	-	-	-	-	-	Y	Salinomycin	0.21	0.7
L26	-	-	-	72	50	773.6	431.3	531.3	-	-	-	-	-	-	-	Y	Nigericin	0.02	0.07
L27	-	-	-	60	54	773.2	265	431.1	-	-	-	-	-	nr	nr	nr	nr	0.02	0.07
L28	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
L29	520	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	N	LOQ/3	1
L30	-	-	-	-	-	-	-	-	-	-	773.5	431.3	531.3	-	Y	-	N	nr	0.35
L31	-	-	-	35	49/42	773.62	431.32	531.41	-	-	-	-	-	-	Y	-	nr	nr	0.35

C1: Extraction + centrifugation + filtration on paper filters + filtration on Si cartridges + evaporation + reconstitution (ACN + 0.1% formic acid) + defatting with hexane (saturated with ACN)

C2: H2O, ACN extraction followed by addition of Magnesium Sulphate and Sodium Chloride, agitated and separated by centrifugation. Precipitation of impurities by freezing at -80 °C, extraction and further centrifugation to separate solids.

European Commission
EUR 25633 EN – Joint Research Centre – Institute for Reference Materials and Measurements

Title: **Determination of authorised coccidiostats in poultry compound feed**

Author(s): Dr Ursula Vincent, Mostafa Chedin, Dr Christoph von Holst

Luxembourg: Publications Office of the European Union

2012 – 68 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online)

ISBN 978-92-79-27781-8 (pdf)

doi:10.2787/69470

Abstract

The European Union Reference Laboratory for Feed Additives Control (EURL-FA Control) has been mandated by the Directorate General for Health and Consumers (DG SANCO) to organise a Proficiency Test exercise (PT) among appointed National Reference Laboratories (NRLs) in order to assess their capacity to correctly determine the 11 authorised coccidiostats in feed matrices. This report presents the results of the PT. Thirty laboratories from 22 countries registered to the exercise.
Laboratory results were rated using z- and ζ-scores (zeta-scores). Between 62% and 73 % of the laboratories reported satisfactory results for four of the five spiked measurands, namely monensin, lasalocid, diclazuril and salinomycin. For the fifth spiked one, narasin, only 57 % of the laboratories submitted satisfactory results. The laboratories also reported qualitative results as regards to the presence of one or more of the other authorised coccidiostats. On the whole, the rate of false positive results was 3% for monensin and narasin, 4% for robenidine, 7% for lasalocid and 0% for all the others.

LA-NA-25633-EN-N

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Publications Office

ISBN 978-92-79-27781-8